

**GENETIC CONTROL OF TUMOUR SUSCEPTIBILITY  
IN MOUSE SKIN CARCINOGENESIS**

**XUEMEI WU**

**This thesis is submitted to the University of Glasgow in part fulfilment  
of the degree of Doctor of Philosophy**

**Beatson Institute for Cancer Research  
&  
CRC Department of Medical Oncology  
Faculty of Medicine  
University of Glasgow  
January, 2000**

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*To my parents and my husband*

1991

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## ABBREVIATIONS

cM	centimorgans
DEN	N,N-diethyl-nitrosamine
DMBA	7,12-dimethyl-benzanthracene
DMH	1,2-dimethyl-hydrazine
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
EDTA	ethylene diamine tetra-acetic acid
ENU	ethylnitrosourea
FVB6F1	(FVB/N x C57BL/6J)F1
FVB6F2	(FVB/N x C57BL/6J)F2
F1BX	(FVB/N x C57BL/6J)F1 x FVB/N
LOD	logarithm of the odds
LOH	loss of heterozygosity
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MNU	N-methyl-N-nitrosourea
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
QTL	quantitative trait locus
RB	retinoblastoma
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
SSCP	single strand conformation polymorphism
SSLP	simple sequence length polymorphism
SSR	simple sequence repeat
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
TSG	tumour suppressor gene
UV	ultraviolet
VNTR	variable number of tandem repeats
w/v	weight/volume

## **ACKNOWLEDGEMENTS**

I would like to take this opportunity to give my regards to my supervisors, Allan Balmain and Ken Brown, for their continuous enthusiasm towards my work. I am grateful to Cancer Research Campaign (CRC) for providing the fund which enable me to carry out this work in Beatson Laboratories. I appreciate greatly the assistance of all members of R7, especially Sheila, Hiroki and Mao, who gave me enormous help to my research work. Many thanks also go to my colleagues in Beatson who gave me help and pleasure in the last few years.

Special thanks go to my parents for their support and encouragement. Finally, I would like to thank my husband, Ming, who gave his constant supports and essential help, and accompany me passing through the difficulties during my study.

## ABSTRACT

The development of tumours in mouse, as in humans, is a multiple genetic process. The molecular analysis of somatic mutations in tumours has led to the association of many oncogenes and tumour suppresser genes with a particular genetic events in tumourigenesis. However, the genes that control susceptibility to tumour formation are largely unknown. To identify these tumour susceptibility genes, genetic linkage analysis was carried out using DMBA-TPA two stage mouse skin carcinogenesis system.

Linkage analysis was first carried out in the F1 backcross mice (FVB/N x C57BL/6J)F1 x FVB/N). At least seven loci on chromosomes 1, 4, 6, 7, 9, 10 and 12 were found to be involved in skin tumour development. Particularly, the locus D4Mit126 was associated with papilloma, while the locus D9Mit269 with both papilloma and carcinoma. Substantial contribution to tumour susceptibility also came from locus-locus interaction. The papilloma formation was influenced by the interaction of D4Mit126-D12Mit203 and D6Mit14-D9Mit269, and the carcinoma formation was affected by the interaction of D7Mit83-D10Mit134.

To confirm the loci identified in the backcross analysis, a further intensive studies on (FVB/N x C57BL/6J)F2 cross was carried out. In addition to the loci on chromosomes 4, 6, 9 and 12, seven new loci on chromosomes 3, 5, 10, 11, 15 and 16 were identified to be associated with papilloma formation. Significant contributions came from loci D6Mit14, D10Mit248 and D12Mit68, as well as the interactions of D11Mit99-D3Mit49, D10Mit248-D16Mit51 and D16Mit64-D15Mit189. The loci linked to carcinoma formation were found at loci D3Mit46, D8Mit211 and D12Nds2.

The results of these two linkage studies demonstrate that the susceptibility to skin tumour development is influenced by multiple genetic loci and their interactions. The fact, that the loci identified to be associated with papilloma and carcinoma are mostly different, implies that the development of papillomas and carcinomas is under different genetic controls.

# **CHAPTER 1**

## **INTRODUCTION**



## 1.1 Cancer as a multistep genetic process

A cancer manifests as a group of cells that proliferate outside of the normal framework of growth control. The familial nature of certain cancers (Ponder, 1990) and the mutagenic capability of many carcinogens (McCann *et al.*, 1975; Ames, 1979) all support the view that cancer is, in essence, a genetic disease. However, the conversion of a normal cell to malignant cancer seldom occurs in a single step, and can not be attributed solely to the mutation of a single gene. Rather, there must be a series of changes in the properties of the collection of cells which make up the developing tumour (Bishop, 1988; Weinberg, 1989). The evidence for multiple steps in carcinogenesis comes from a range of observations, including clinical, epidemiological and laboratory experiments. That tumours evolve towards a more malignant phenotype is common clinical experience; the discrete morphological and histological stages of many cancers provide direct evidence for a multistep process towards malignancy, as does the successive emergence of more aneuploid subclones during tumour development (Heim *et al.*, 1988). Studies of chemical carcinogenesis of mouse skin have established a three stage process consisting of initiation, promotion and progression towards tumour development (Slaga *et al.*, 1986; DiGiovanni, 1992; Yuspa, 1994). Knudson, Miller and others even proposed a three to seven 'hits' models after a thorough statistical study of the relationship of cancer incidence with age (Knudson, 1971; Miller, 1980). These 'hits' represent sequential mutations of key growth-regulatory genes in a single cell and its progeny.

Over the past decade, studies of human and animal tumours have added a new dimension. Much of the research has focused on dissection of the process and characterisation of the mutations, and led to the discovery of two distinctive classes of genes: proto-oncogenes and tumour suppressor genes. Both classes are implicated in growth control, but contribute to malignancy in separate ways. The positively acting oncogenes can induce cell growth and are found to be activated in tumours, frequently as a result of mutation, gene amplification or chromosomal translocation. Tumour suppressor genes, in contrast, are associated with the negative regulation of cellular proliferation, and are frequently functionally inactivated or deleted in a wide variety of human and animal tumours.

## 1.2 Oncogenes

### 1.2.1 The identification of oncogenes

The discovery of retroviral oncogenes is the first step towards understanding the molecular events underlying tumourigenesis and the function of genes crucial to these processes. Retroviruses are small viruses that carry up to 10 genes in a genome comprised of single-stranded RNA. They replicate through a proviral DNA intermediate which is integrated into chromosomal DNA of the infected host cells. The acutely transforming retroviruses, the prototype of which is Rous sarcoma virus (RSV), can rapidly induce tumours in animals and efficiently transform cells in culture. Analyses of these viruses lead to the identification of the very first oncogene, *v-src*, the transforming gene of the RSV virus (Varmus *et al.*, 1972). Since then, more than 20 different oncogenes (Table 1.1A) have been identified in the genomes of more than forty acutely transforming viruses isolated from chickens, turkeys, mice, rats, cats and monkeys (Cooper, 1995).

However, detailed studies suggest these viral oncogenes are only responsible for cell transformation but play no part in retrovirus replication. In deed, most of these highly oncogenic virus strains are defective for replication and must be propagated by a helper virus that contains functional replicative genes (Varmus, 1988). The presence of oncogenes in a viral genome seems paradoxical. The answer to this puzzle lead to a significant discovery that these viral oncogenes have normal cellular counterparts: proto-oncogenes (Stehelin *et al.*, 1976). Elucidation of the life cycle of the retroviruses shows that it requires a reverse transcription step in which the viral RNA is transcribed into a proviral DNA by a RNA-dependent DNA polymerase, reverse transcriptase. The proviral DNA then integrates into the host genome (Baltimore, 1970; Temin and Mizutani, 1970). The proto-oncogene is captured by the retrovirus as a result of the inclusion of cellular RNA within a viral particle. This leads to the incorporation of the transduced cellular genes into viral genome and delivery of this novel unit into the host genome. These transduced cellular genes may either be placed under virally determined transcriptional control such as the viral long terminal repeat (LTR), and/or sustain critical mutations such that the structure and function of the proteins are changed. As a consequence of such alterations, the transduced genes have acquired a new biological

activity, the ability to induce cell transformation (Weiss *et al.*, 1985). Subsequent studies have demonstrated that nearly all retroviral oncogenes are altered versions of cellular proto-oncogenes.

The life cycle of retrovirus suggests that the site of provirus integration can be vital to tumourigenesis. The provirus can act as a mutagen whose insertion disrupts host gene expression in that region. Hayward *et al.* (1981) provided the first evidence to substantiate this mechanism of insertional mutagenesis. They demonstrated that the *c-myc* proto-oncogene was activated in over 80% of ALV-induced chicken bursal lymphomas. The powerful LTRs lead to elevated *c-myc* transcripts by acting as an enhancer. Insertional mutagenesis has been used prospectively to identify proto-oncogenes that are reproducible targets for proviral insertion in neoplasm (Nusse and Varmus, 1982). Some of these oncogenes have also been found in acutely transforming retroviruses or encode known growth factors, whereas others are novel oncogenes (Table 1.1B).

While research into the retroviral oncogenes continued, more direct methods of identifying oncogenic sequences in human genome were also developed. Gene transfer experiments show that about 10% to 20% of human tumours possess DNA sequences with transforming ability (Shih *et al.*, 1981), indicating the presence of biologically activated cellular oncogenes. Since there is little evidence that acutely transforming retroviruses isolated from animal tumours are involved in human neoplasms, gene transfer provides a new approach to detect active oncogenes in human tumours. Most oncogenes identified in this fashion are novel genes and often belong to *ras* and *src* families, though some of them, such as H-*ras*, *src* and *fos*, are already known from their homology with retroviral oncogenes (Table 1.1C). Characterisation of these oncogenes shows that they are activated either by point mutations or DNA rearrangements that occur spontaneously or in the process of gene transfer (Taparowsky *et al.*, 1982; Santos *et al.*, 1982).

Oncogenes have also been identified through their association with chromosomal abnormalities. Consistent chromosome translocations have been recognised in a number of neoplastic diseases, particularly cancers of the haemopoietic system (Solomon *et al.*, 1991). The reproducibility of tumour-specific genetic alterations suggests a new

---

**A) Retroviral oncogenes**

*abl, erbA, fos, jun, myb, myc, raf/mil, H-ras, K-ras, src*

**B) Oncogenes activated by retroviral integration**

*fit-1, int-1, int-2, lck, pim-1, wnt-1, wnt-3, pvt/mis*

**C) Oncogenes identified by gene transfer**

*met, neu, N-ras, ret, cot, dbl, fgf-5, hst, mas, B-raf*

**D) Oncogenes identified by their association with chromosome abnormality****i) Oncogenes identified by chromosome translocation**

*bcl-2, bcl-6, tal-1, bcr/abl*

**ii) Oncogenes identified by gene amplification**

*N-myc, L-myc, erbB-2*

---

**Table 1.1** The identification of oncogenes (See *The Oncogene and Tumour Suppressor Gene Factsbook*, Hesketh, 1997 for the majority of entries).

mechanism for oncogene activation: Chromosome translocation can lead to DNA rearrangements that result in inappropriate expression of the oncogenes. A well known example is Burkitt's lymphoma. Translocations between chromosome 8 and chromosome 14 results in constitutive expression of *c-myc*, which is sufficient to activate *c-myc* as an oncogene (Adams *et al.*, 1983). Another well-known translocation is the formation of the Philadelphia chromosome in chronic myelocytic leukaemia. The translocation between chromosome 9 and 22 creates a BCR-ABL fusion protein, in which the tyrosine kinase activity of the viral oncogene protein ABL is enhanced and its substrate specificity altered (Shtivelman *et al.*, 1985; Lugo *et al.*, 1990). Continuing analyses of translocation breakpoints have lead to many additional oncogenes that are activated in human neoplasm, particularly leukaemia and lymphomas (Table 1.1D).

Another type of chromosome abnormality observed in tumours is related to gene amplification (Alitalo and Schwab, 1986). There are two different types of chromosome amplification: homogeneously staining region (HSR), which occurs as a contiguous element in a chromosome, and double minute chromosomes (DM), which are additional mini-chromosomes. Amplification of the copy number of a proto-oncogene would be

expected to increase its expression and possibly activate it as an oncogene. Although some oncogenes detected by this approach such as *c-myc* had previously been identified (Collins and Groudine, 1982), two other members of the *myc* gene family, L- and N-*myc* were first identified (Table 1.1D) (Schwab *et al.*, 1983; Nau *et al.*, 1985).

### 1.2.2 The function of oncogenes

Proto-oncogenes encode proteins that are involved in the regulation of cell proliferation and differentiation. They appear to function at various levels in signalling from the extracellular compartment to the nucleus. Oncogenes are classified primarily according to their functional role and position in pathways of signal transduction and subcategorised as growth factors, receptor or non-receptor tyrosine kinases, GTP-binding proteins, serine/threonine kinases, and nuclear proteins /transcription factors (Table 1.2). Through a cascade of direct interaction and phosphorylation events, an external signal is transmitted via specific receptor–ligand interactions and relayed through the cytoplasm to the nucleus, where transcription factors elicit a response by modulating gene expression (Tonks and Neel, 1996).

The involvement of proto-oncogenes in differentiation has also been well documented. During mouse embryonic and fetal development, some proto-oncogenes are expressed at high levels, whereas others appear and disappear in a temporal manner (Slamon and Cline, 1984). When individual mouse tissues are evaluated in a stage-specific manner, differential expression is even apparent. Expression of N-*myc* (Mugrauer *et al.*, 1988) is detected in the embryonic kidney specially in cells undergoing tubulogenesis or branching morphogenesis and expression of *ret* is limited to the branching epithelium (Schuchardt *et al.*, 1994). Thus, appropriate expression of a proto-oncogene during development can be critical to normal tissue differentiation.

In addition, the regulatory importance of the proto-oncogenes is also inferred from their conservation in evolution. Homologous *ras* sequences have been identified in *Drosophila*, yeast, mouse and human, and apparently function similarly as well (Santos and Nebreda, 1989). The redundancy of function found in families of certain oncogenes also provides assurance that their regulatory capabilities will be maintained within a cell. For example, the *ras* gene family is represented by three separate forms: H-*ras*, K-

---

**A) Growth factors**

*sis/PDGFR, int-2/FGF3, hst/FGF4, WNT1/WNT3*

**B) Tyrosine kinases****i) Receptor-like tyrosine kinases**

*erbB/EGFR, kit, met, neu/HER2, ret*

**ii) Non-receptor tyrosine kinases**

Membrane associated: *src, fgr, fyn, hck, tkl*

Cytoplasmic: *abl, sck, fak, fes/fps*

**C) GTP-binding proteins**

*H-ras, K-ras, N-ras, gsp, gip*

**D) Serine/threonine kinases**

*bcr, raf/mil, mos, pim-1*

**E) Transcription factors**

*erbA/THR, fos, jun, myb, myc, tal-1*

---

**Table 1.2** Classifications of proto-oncogenes/oncogenes according to their functional roles in signal transduction (See *The Oncogene and Tumour suppressor Gene Factsbook*, Hesketh, 1997 for the majority of entries).

*ras* and *N-ras* which differ significantly in their introns or noncoding regions, but differ little in the coding regions.

### 1.3 Tumour suppressor genes

Tumour development is a multi-stage process which requires multiple genetic alterations. Many of these changes lead to activation of the oncogenes. The activated oncogene generally behaves in a dominant manner, its growth promoting effects can be maintained in the presence of a normal proto-oncogene allele. However, given the fact that most genetic changes are actually deleterious, it is possible that loss of function may be more important to the development of malignancy than oncogene activation. These

genes make up another distinctive class of genes, tumour suppressor gene, which functions as a negative regulator of tumour development.

Evidence for existence of tumour suppressor genes is derived from somatic cell hybridisation experiments (Harris *et al.*, 1969). Extensive studies have established that most hybrids between normal and malignant cells are no longer tumourigenic. Such suppression implies normal cells contain genes that act as negative regulators of the neoplastic phenotype. This interpretation is strengthened by the fact that such hybrids frequently revert to the tumourigenic phenotype following loss of specific chromosomes of the normal cell (Evans *et al.*, 1982; Benedict *et al.*, 1984). Furthermore, for some tumours, malignancy can be suppressed by the introduction of a normal chromosome to replace the lost parts (Tanaka *et al.*, 1991). A second piece of evidence comes from studies of familial cancers. Many forms of cancer have a higher incidence in relatives of cancer patients than in the general population, suggesting the existence of an inherited component in their aetiology. Some familial cancers even show a pattern of Mendelian inheritance, the commonest pattern of autosomal dominant transmission (Ponder, 1990).

In fact, tumour suppressor genes were first identified in inherited cancer syndromes, primarily through studies of rare familial cancers such as hereditary retinoblastoma and Wilms' tumour (Knudson, 1993). Since the isolation of the retinoblastoma (*Rb*) gene (Friend *et al.*, 1986; Fung *et al.*, 1987; Lee *et al.*, 1987a), many more have been cloned (see Table 1.3). The family of tumour suppressor genes is composed of genes that encode proteins that are localised to different subcellular compartments and involved in a diverse functions including cell cycle regulation, check point control, transcriptional repression, signal transduction modulation, DNA repair, and apoptosis (see review in Knudson, 1993; Hind and Weinberg, 1994; Fearon, 1997; Ghebranious and Donehower, 1998). The studies on tumour suppressor genes demonstrate that loss of their functions cause disturbance in cell proliferation, genomic stability, and cell death and lead to tumour development.

### **1.3.1 Tumour suppressor gene alterations in human cancers —The 'two-hit' model**

The information presented in many comprehensive reviews of tumour suppressor genes clearly established that germ line mutation of tumour suppressor genes is associated

Gene	Chromosome location	Proposed function of gene product	Inherited syndromes	Sporadic cancers with mutations
RB	13q14	Cell cycle and transcriptional regulation	Familial retinoblastoma	Retinoblastoma, osteosarcoma, sarcoma, lung, breast and bladder carcinoma
p53	17p13	Transcriptional factor; apoptosis	Li-Fraumeni syndrome	Multiple tumours
WT1	11p13	Transcriptional regulation; interacts with splicing factors	Wilm's tumour	Nephroblastoma
VHL	3p25	Interacts with elongation factors	von Hippel-Lindau disease	Renal cell carcinoma
DPC4	18q21.1	Signalling molecule in TGF- $\beta$ pathway	Peutz-Jeghers syndrome (PJS)	Pancreatic carcinoma
INK4A/MTS1	9p21	Cyclin-dependent kinase (CDK) inhibitor	Familial melanoma	Melanoma, pancreatic carcinoma
APC	5q21	Regulation of $\beta$ -catenin	Familial adenomatous polyposis	Colon and stomach carcinoma
NF1	17q11	GAP for p21ras protein	Neurofibromatosis type 1	Schwannoma
NF2	22q12	Membrane cytoskeletal attachment	Neurofibromatosis type 2	Schwannoma and meningioma
PTEN/MMAC1	10q23	Dual-specificity phosphatase with similarity to tensin	Cowden disease	Multiple tumours
DCC	18q21	N-CAM homology	Juvenile polyposis syndrome (JPS)	Colon and stomach carcinoma
PTCH	9q22	Transmembrane receptor for hedgehog signalling molecule	Gorlin's syndrome	Basal cell carcinoma
ATM	11q21	DNA repair; induction of p53	Ataxia telangiectasis	lymphoma and breast cancer
BRCA1	17q21	Interacts with Rad51 protein; repair of double-strand breaks	Familial breast cancer	ovarian cancer
BRCA2	13q13	Interacts with Rad51 protein; repair of double-strand breaks	Familial breast cancer	Breast and ovarian cancer, pancreatic cancer
MLH1,MSH2 PMS1,PMS2	3p21,2p16 2q31,7p22	DNA mismatch repair	Hereditary non-polyposis colon cancer (HNPCC)	Colon tumour, ovarian cancer

**Table 1.3** Tumour suppressor genes and their involvement in inherited cancer syndromes and sporadic tumours (See Fearon, 1997 and Jacks, 1996 for the majority of entries).



with an inherited predisposition to a familial cancer syndromes, but somatic mutations are detected in more common sporadic forms of cancer. Knudson addressed the question of the relationship between the hereditary and sporadic forms of these tumours (Knudson, 1971). Based on statistical analysis of the frequency and age of tumour development of inherited and sporadic cases of retinoblastoma, he proposed that the development of retinoblastoma involves recessive genetic events causing inactivation of the same gene, the retinoblastoma (*Rb*) gene, in both hereditary and sporadic forms of disease. Since there are two copies of this gene in the normal cells, he reasoned that in hereditary cases, a mutated *Rb* allele is inherited, and consequently present in all cells of a carrier, inactivation of the remaining allele in a single cell of the retina would give rise to retinoblastoma. In the sporadic cases, two mutations in both alleles were proposed to occur sequentially in the somatic cells of the retina. By this scenario, cancer in affected families is inherited as a dominant trait because, having inherited one abnormal gene, the probability of a second event occurring is sufficiently higher. However, the disease itself at cellular level is recessive, with a single functional allele at a tumour suppressor locus such as *Rb* being sufficient at single cell level to prevent tumour development.

Knudson's now famous 'two-hit' model has become engraved in the annals of cancer genetics. The model applies not only to retinoblastoma but also fits the epidemiological data for other familial cancers such as neuroblastoma, pheochromocytoma and Wilms' tumour (Knudson and Strong, 1972a and 1972b). A broader version of this model can also be applied to cancers which require the accumulation of several more mutations before expression of the fully malignant phenotype. Colorectal carcinoma occurs in both familial and sporadic forms, but the epidemiological data suggest that accumulation of approximately six independent mutational events is required (Armitage and Doll, 1954). Fearon and Vogelstein (1990) demonstrated that an early, probably initial, requirement for the development of colon cancer in both familial and sporadic cases was inactivation of both copies of the *APC* gene. Despite the requirement for further somatic mutations to occur stochastically before the emergence of colon carcinoma, inheritance of a single inactivated copy of *APC* gene is sufficient to confer an increased risk of developing the disease, by initiating a chain of tumourigenic events (Kinzler and Vogelstein, 1996).

### **1.3.2 Genetic mechanisms of tumour suppressor gene loss**

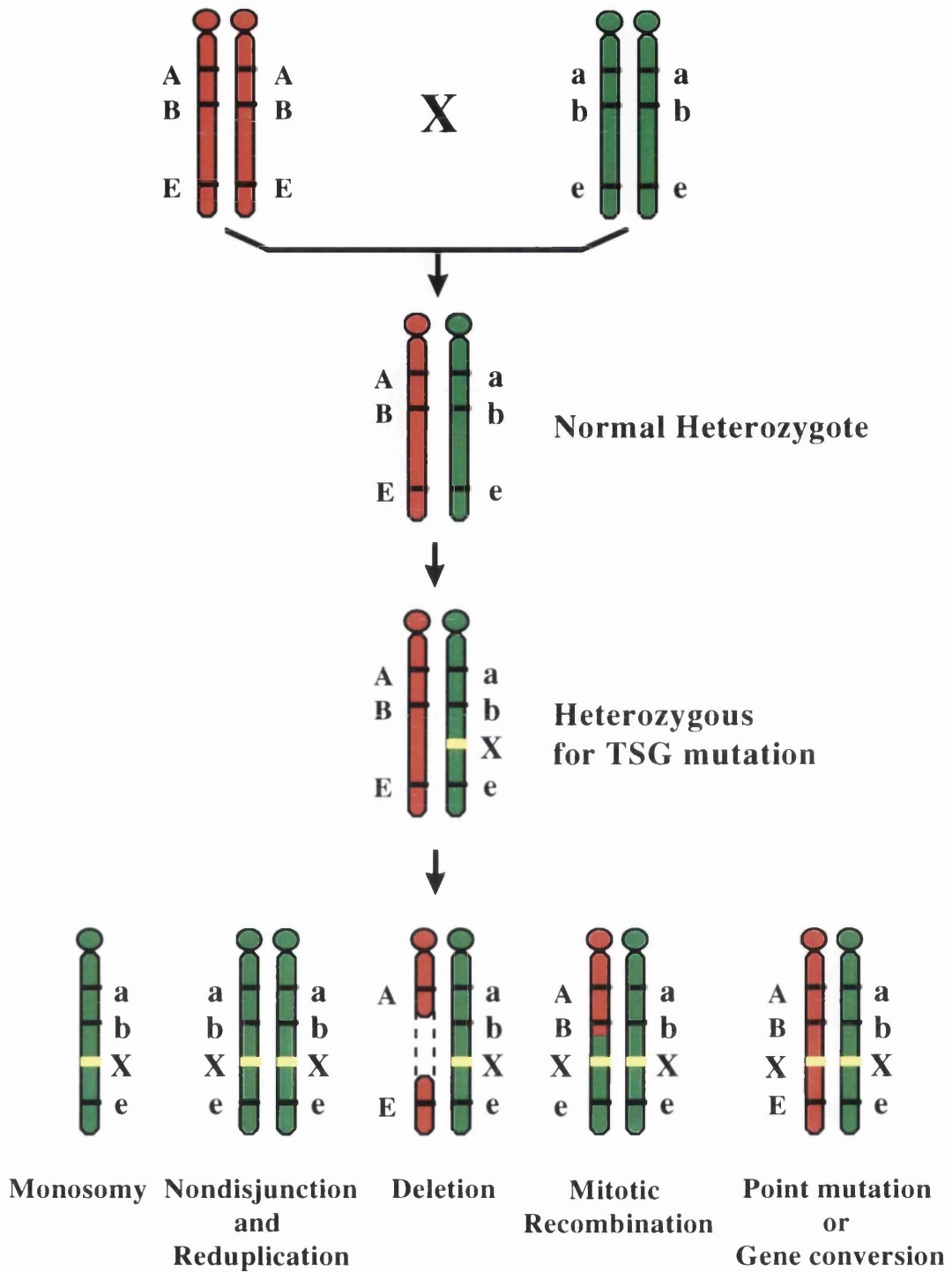
Tumour suppressor genes are grouped together because they are all mutated to an inactive form in the course of tumourigenesis. There are many ways by which a gene can be rendered functionally silent. These include: 1) single point mutations which introduce stop codon, thus preventing translation of a full length protein; 2) mutations which affect promoter activity or cause aberrant splicing of the pre-mRNA; 3) methylation of the gene, preventing its recognition by the transcriptional machinery; and 4) removal of all or part of the gene by chromosomal mechanisms such as non-disjunction, mitotic recombination or deletion. For the complete loss of tumour suppressor gene function, both copies present in diploid cells have to be inactivated, and this can occur by sequential events within developing tumour cells as shown in Figure 1.1 (Brown *et al.*, 1993). Of the mechanisms shown, loss by mitotic recombination or deletion has proved the most useful for detection of potential tumour suppressor genes in the genome, since comparison of the loss of heterozygosity (LOH) intervals in different tumours can narrow down the region of interest to a size where positional cloning approach can be attempted to isolate the gene (Collins, 1991). The application of these techniques has revealed the presence of putative tumour suppressor gene loci on almost every human chromosome (Lasko *et al.*, 1991).

### **1.3.3 Tumour suppressor genes in inherited cancer syndromes**

In addition to retinoblastoma, there are some other rare inherited cancer syndromes which have been the subject of intensive study. By a combination of linkage analysis in members of cancer families and LOH analysis in tumours of affected members, many of the genes responsible for these cancers have been located to a particular chromosomes and an increasing number of tumour suppressor genes have now been cloned (Table 1.3).

#### **1.3.3.1 *Rb* and retinoblastoma**

One of the genetic highlights of the last decade is the identification of a gene involved in the formation of retinoblastoma. Retinoblastoma is a rare ocular tumour that affects 1 in 20,000 children. There are two forms of the disease present: inherited and sporadic. In the former, tumours are seen affecting both eyes, where the latter is characterised by the appearance of single eye lesions. About 40% of all cases of retinoblastoma occur as a



**Figure 1.1** Genetic alteration leading to loss of heterozygosity at tumour suppressor gene loci. Vertical columns represent mouse chromosomes with the centromere at the top. Letters A (a), B (b) and E (e) represent alleles at different genomic loci. Those derived from the mother are in red and indicated in capital letters; those derived from the father are in green and indicated in small letters. X denotes a mutation in the tumour suppressor gene (TSG) which can become homozygous by the mechanisms indicated.

clear-cut, dominantly inherited Mendelian disorder, the remainder are sporadic (Knudson, 1993).

Knudson (1971) proposed that retinoblastoma was caused by two mutations. In inherited case, the first mutation is present in the germ line and the second mutation occurs somatically, resulting in tumour development. Although his hypothesis was based upon statistical modelling, it has received supports from cytogenetic and molecular studies which have subsequently led to the cloning of the *Rb* gene. It was first observed that about 5-10% of individuals who inherit retinoblastoma posed a constitutional deletion of part or all of chromosome 13q14 (Yunis and Ramsay, 1978). This finding was strengthened by the finding that the esterase D (ESD) gene, which had been independently mapped to the same chromosome locus, was also deleted in both hereditary and sporadic retinoblastoma (Lee *et al.*, 1987b ). Such deletion suggests that the first 'hit' produced a loss-of-function mutation of a gene located within the deleted regions. The presumption that the second hit would eliminate the other functional copy of the underlying gene was later confirmed by the use of restriction fragment-length polymorphisms (RFLPs) markers (Cavenee *et al.*, 1983). Further studies by three independent groups eventually led to the identification of the first human tumour suppressor gene, the retinoblastoma gene (Friend *et al.*, 1986; Fung *et al.*, 1987; Lee *et al.*, 1987a).

The *Rb* gene itself has been the subject of intense investigation since its discovery. The *Rb* gene encodes a 105 kD nuclear phosphoprotein ubiquitously expressed throughout the body, suggesting that RB play an important role in the maintenance of a broad range of tissues (see review in Weinberg, 1995). From biochemical studies, RB appears to function as an important regulator of the G1 checkpoint. It binds to members of the E2F family, transcription factors needed for transcription of S phase early gene, and prevent S phase entry. Hyper-phosphorylation of RB by G1/S cyclin-dependent kinases (CDKs) releases E2F from RB-mediated repression and enables progression of the cell into S phase. RB mutations occur at high frequency in a variety of tumour types. In addition to retinoblastomas (Horowitz *et al.*, 1989), RB mutations have been detected in many other sporadically arising tumours, including osteosarcomas, soft tissue sarcomas and carcinomas of the breast, lung, bladder and prostate, and some forms of leukaemia. (Lee *et al.*, 1988; Bookstein *et al.*, 1990; Ishikawa *et al.*, 1991; Chen *et al.*, 1990). Frameshift

and chain termination mutations, deletions of entire exons, and point mutations are common forms of mutation that result in loss of function of RB.

*Rb*-deficient mice, on the other hand, revealed previously unknown cellular functions (Lee *et al.*, 1992; Jacks *et al.*, 1992; Clarke *et al.*, 1992). *Rb*-deficient mice die at the 15th day of gestation largely because of the defects in hepatic erythropoiesis. In addition, widespread apoptosis and aberrant S phase entry is also observed in cells in the central and peripheral nervous system (Morgenbesser, 1994). Further studies demonstrate that this apoptosis is mediated largely by E2F-1 overexpression (Tsai *et al.*, 1998). Mice that are heterozygous for the *Rb* gene show no signs of retinoblastoma or precursor lesions, but they can develop pituitary adenomas (Jacks *et al.*, 1992; Hu *et al.*, 1994). These tumours consistently shows loss of heterozygosity of the wild-type *Rb* allele, consistent with Knudson's 'two hit' hypothesis (Hu *et al.*, 1994).

### **1.3.3.2 *p53* and Li-Fraumeni syndrome**

Li-Fraumeni syndrome is a rare familial cancer syndrome with an incidence estimated at 2-4 per 100 000 population. Families with this syndrome are characterised by the high incidence of a spectrum of tumours, including breast cancer, soft tissue sarcomas, brain tumours, leukaemia, and adrenocortical carcinomas (Knudson, 1993). It has been demonstrated that inactivating mutations of *p53* gene are associated with a similar broad spectrum of non-inherited cancers (Nigro *et al.*, 1989), suggesting that *p53* gene might be responsible for this inherited cancer susceptibility. This was confirmed by the finding of germline *p53* mutation in Li-Fraumeni syndrome families (Malkin *et al.*, 1990; Srivastava *et al.*, 1990).

Interestingly, the *p53* gene was originally discovered by its association with SV40 T antigen in SV40 transformed cells (Lane and Crawford, 1979; Sarnow *et al.*, 1982). The very early studies suggested that *p53* could act primarily as a dominant oncogene (Eliyahu *et al.*, 1984). It was only in the late 1980s that investigators established that the transforming ability of *p53* was resulted from mutations which behaved in a dominant negative manner by forming inactive oligomeric complexes between mutant and wild-type proteins (Hinds *et al.*, 1989; Herskowitz, 1987), suggesting the role of *p53* as a tumour suppressor gene rather than an oncogene (Finlay *et al.*, 1989; Eliyahu *et al.*,

1989). Subsequent studies have shown that deletions and inactivating mutations of the *p53* gene is frequently deleted in a wide range of human tumours (Hollstein *et al.*, 1991). In fact, the mutations of the *p53* gene contribute to approximately 50% of all human cancers as well as inherited Li-Fraumeni syndrome, making *p53* the most common target for genetic alterations leading to human tumour development.

*p53* is a ubiquitously expressed transcription factor which appears to be involved in diverse range of cellular functions and responses, including both activation and repression of transcription, regulation of protein translation, inhibition of DNA and RNA helicase activity, DNA repair, cell cycle arrest, and apoptosis (see review in Ko and Prives, 1996; Levine, 1997). Therefore, *p53* may suppress tumourigenesis through multiple mechanisms. It has been shown that various cellular stresses, including DNA damage, hypoxia, ribonucleotide depletion, serum starvation, and aberrant oncogene activation, may induce stabilisation of *p53* through phosphorylation, preventing it from binding to the *mdm2* which facilitates its degradation (Prives, 1998). Stabilised *p53* can induce either cell cycle arrest or apoptosis (Lane, 1992). The decision by *p53* about whether to initiate an arrest or apoptotic program can be influenced by a variety of factors such as cell type, cytokine or growth factor concentrations, levels of *p53*, or the extent of DNA damage (Ko and Prives, 1996). The end result of the choice is to prevent the damaged DNA being replicated thus eliminate the potential oncogenic DNA lesions.

Given the importance of the *p53* gene in maintaining genomic integrity, not surprisingly, in the absence of *p53*-mediated growth arrest and apoptosis genetic lesions can more readily lead to neoplastic transformation. The *p53*-deficient mice develop tumours at a very young age, and the *p53* heterozygous mice are susceptible to tumour development (Donehower *et al.*, 1992; Kemp *et al.*, 1994). Thymic T-cell lymphomas are the most frequently arising tumours in the *p53*-deficient mice, but lymphomas of B-cell origin, soft tissue sarcomas, osteosarcomas, testicular teratomas, and other forms of tumour are also observed (Donehower *et al.*, 1992; Purdie *et al.*, 1994; Jacks *et al.*, 1994). These tumour types observed in mice are signature tumours often seen in Li-Fraumeni families (Malkin, 1994).

### 1.3.4 Caretaker and Gatekeeper genes

The notion that specific tumours of a familial cancer syndromes share mutations of critical genes with their sporadic forms was established decades ago. Most of the tumour suppressor genes are indeed mutated in both hereditary and sporadic tumours. However, recent examples of tumour suppressor gene mutations such as *BRCA1* and *BRCA2* in inherited breast cancers are absent in sporadic cases has seriously shaken this concept. In addition, some tumour suppressor genes such as *p53*, *RB*, and *APC* are widely expressed and found mutated in many types of tumour, but predispose most strongly to only a limited spectrum of tumour types. Kinzler and Vogelstein (1997) addressed this issue with the classification of tumour suppressor genes as ‘gatekeepers’ and ‘caretakers’, a timely paradigm that is likely to guide the field of tumour suppressor genes as much as Knudson’s ‘two-hit’ hypothesis.

The concept of gatekeeper and caretaker genes is characterised by their distinct functions towards tumour suppression. Gatekeeper genes control cellular proliferation directly by inhibiting cell growth or promoting cell death. Inactivation of a gatekeeper gene manifests itself directly as a growth advantage to the affected cell. Each cell type has only one or few gatekeeper genes, and inactivation of a gatekeeper gene may be necessary for passing the genetic threshold of the neoplastic process in a given tissue. Therefore, inactivation of gatekeepers is rate-limiting for the initiation of a tumour, and both the maternal and paternal copies must be altered for tumour development. Examples of gatekeeper genes include *APC* and  $\beta$ -catenin in colon epithelial cells, *Rb* in retinal epithelial cells, *NFI* in Schwann cells, and *VHL* in kidney cells.

In contrast, caretaker genes maintain the genomic integrity after DNA damage and recombination. Inactivation of a caretaker gene does not result directly in tumour initiation, but rather lead to genomic instabilities which enhance the probability of mutation in all genes, specially those in the gatekeeper pathway. Once such a tumour is initiated by inactivation of a gatekeeper gene, it may progress rapidly due to an accelerated rate of mutation in other genes that directly control cell growth or death. Mismatch DNA repair genes, such as *MSH2* and *MLH1*, are caretaker genes, and abnormalities in these genes enhance genome instability and increase the risk of human colon cancer (Fishel *et al.*, 1993; Bronner *et al.*, 1994). More recently, breast cancer susceptibility genes, *BRCA1* and *BRCA2*, have also been included in the list of caretaker

genes due to recent findings of their involvement in DNA repair pathways via interaction with Rad51 (Sharan *et al.*, 1997; Scully *et al.*, 1997).

The interpretation of gatekeeper and caretaker is consistent with the finding that three or more additional somatic mutations are required to initiate neoplasia in the caretaker pathway, whereas only one additional somatic mutation is required to initiate neoplasia in the gatekeeper pathways. Because in inherited cancer syndromes of the caretaker type, in addition to the mutant caretaker gene inherited from an affected parent, the predisposed individuals still need at least three subsequent somatic mutations to initiate cancer: mutation of the normal caretaker allele followed by mutation of both gatekeeper genes. Thus, a predisposed individual with an inherited mutated allele of a caretaker gene is at lower risk of cancer when compared with an individual with a mutated gatekeeper allele. Importantly, mutations in caretaker genes would not be expected to occur in sporadic tumours since a single cell would need to acquire four independent mutations (two caretaker alleles plus two gatekeeper alleles) to become initiated. The odds of acquiring even three somatic mutations before a cell undergoes malignancy are slight, which probably explains why germ line mutations of *BRCA* genes account for almost 90% of inherited breast cancer cases, but somatic mutations of these genes are only a rare event.

More recently, Kinzler and Vogelstein (1998) added one more category of tumour suppressor genes defect to their new theory, the 'Landscape' defect. The concept of landscape defect was introduced by recent studies on Juvenile polyposis syndrome (JPS) and ulcerative colitis (UC). Patients with JPS and UC develop hamartomatous polyps in which the proliferation of defective cells appear to be derived from the stroma. Consequently, the epithelial cells associated with polyps are more likely to undergo neoplastic transformation as a result of an altered terrain for epithelial cell growth. It has been demonstrated that germline mutations in *PTEN* or *SMAD4* can lead to the development of the hamartomatous polyps (Howe *et al.*, 1998; Olschwang *et al.*, 1998). However, somatic mutations of these two genes in colon cancer are infrequent events, the mutations more commonly occur in brain and pancreatic cancer.



### 1.3.5 Identification of tumour susceptibility genes

The past decade has seen rapid progress towards the identification of genes involved in inherited predisposition to cancer. The genetic linkage analysis, by which one compares the inheritance pattern of a trait with the inheritance patterns of chromosomal regions, has been the mainstay of efforts although in some cases there are also contribution of cytogenetic clues. Major advances have been made in identifying genes which control familial predisposition to the development of certain tumour type in humans, these studies have been limited to relatively rare cases where susceptible individuals have inherited high penetrance genes (such as tumour suppressor genes) conferring substantial probability of diseases development. However, theoretical considerations have indicated that the majority of sporadic human cancers may also have an important hereditary component and much of the cancer incidence may be attributable to high frequency, low penetrance genes (such as the tumour modifier genes) tumour susceptibility gene present within the population. Because of the lack of clear cut familial inheritance pattern, and due to the complexities implicit in the genetic-environmental interactions which may lead to tumour development in predisposed individual, the identification of tumour susceptibility genes may be extremely difficult solely on the basis of human genetic studies.

Rodent systems offer a number of distinctive advantages for the detection of tumour susceptibility loci. A large number of inbred mouse strains are available which show enormous variation in susceptibility to the development of tumours of the lung, colon, skin, liver and lymphoid system after exposure to a variety of environmental agents, including chemicals, radiation and viruses (Demant, 1992). Selective breeding has consequently resulted in homozygosity at a number of tumour susceptibility or tumour resistance loci which are randomly distributed between different strains of mice. Classical genetic approaches can therefore be used to analyse segregation of such predisposition loci in backcross or intercross between susceptible and resistant strains. The development of large panel of microsatellite markers has further facilitated the identification of tumour susceptibility genes. Importantly, the multistage process of tumour development in mouse carcinogenesis is very similar to that seen in humans, and the genetic alterations detected in mouse tumours involve genes, such as *ras*, *Rb*, *p53* and *p16*, that are also the most commonly altered genes in human tumours. This underlying similarity in the biology of carcinogenesis in mice and in humans implies

that the genes that control susceptibility to mouse tumour development will also be relevant to the human situation.

Another major advance is the development of statistical methods that take account the fact that multiple genes make different quantitative contributions to the phenotype. The identification of the critical gene within a QTL is greatly facilitated by the availability of unlimited size of 'families' for linkage analysis, the use of selective mouse breeding to identify recombinant animals which may be used for further phenotypic analysis, and the development of congenic mice (Moen *et al.*, 1991). Thus far, a large number of mouse tumour susceptibility loci have been mapped that control the size, growth rate, or number of lesions that develop, or that can independently influence early or late stages of tumourigenesis (Table 1.4). The mapping may be further refined if the QTL harbours a tumour suppressor gene which exhibits LOH in tumours. Positional cloning and candidate gene approaches can then be used to identify the target genes.

## **1.4 Quantitative trait loci linkage mapping of cancer predisposition genes**

The concept of genetic linkage was first recognised by Mendel, who noted that certain characteristics of his experimental plant tended to be co-inherited. The explanation for this phenomenon became clear once it was recognised that chromosomes contain the genetic material. Two traits were linked if the corresponding genes reside close together on the same chromosome. This leads to the idea of linkage analysis — to trace and measure the co-segregation of disease allele in a family with genetic markers. If the marker and disease gene are linked, then a particular allele at the marker locus is likely to travel with the disease in a special family. In a different family, a different allele from the same marker locus may segregate with the disease gene. The distance between the marker locus and the disease gene can then be inferred by using information from putative recombination events during meiosis. Genetic distance is generally measured in centi-Morgans (cM), where 1 cM is approximately the distance between two loci that on average show 1% recombination ( $\theta=0.01$ ). The recombination fraction ( $\theta$ ) refers to the probability that a gamete produced by a parent is a recombinant between two loci (in this case, the marker locus and the disease gene). The extent of linkage is measured by

Tumour	Induction	Locus	Chromosome location (cM)	Reference		
Colon cancer	APC mutation DMH and ENU	Mom1	4 (66.6)	Dietrich <i>et al.</i> , 1993		
		Scc3	1 (101.5)	van Wezel <i>et al.</i> , 1996		
		Scc4	17 (47.4)	van Wezel <i>et al.</i> , 1996		
		Scc5	18 (25.0)	van Wezel <i>et al.</i> , 1996		
		Scc1	2 (45.0)	Moen <i>et al.</i> , 1992		
	DMH	Scc2	2 (32.5)	Moen <i>et al.</i> , 1996		
		Ccs1	12 (38.0)	Jacoby <i>et al.</i> , 1994		
		Small intestine tumour Hepatomas	ENU	Ssic1	4 (64.0)	Remond <i>et al.</i> , 1995
			DEN	Hcf1	17 (19.0)	Poole <i>et al.</i> , 1996
				Hcf2	1 (82.0)	Poole <i>et al.</i> , 1996
Hcr1	4 (44.5)	Lee <i>et al.</i> , 1995				
Hcr2	10 (25.1)	Lee <i>et al.</i> , 1995				
Urethane	Hcs4	Hcs4	2 (99.0)	Manenti <i>et al.</i> , 1994		
		Hcs5	5 (49.0)	Manenti <i>et al.</i> , 1994		
	Hcs1	Hcs1	7 (24.0)	Gariboldi <i>et al.</i> , 1993b		
		Hcs2	8 (56.0)	Gariboldi <i>et al.</i> , 1993b		
	Hcs3	Hcs3	12 (59.0)	Gariboldi <i>et al.</i> , 1993b		
		Hcs6	19 (40.0)	Manenti <i>et al.</i> , 1994		
	Lung cancer	ENU	Sluc2	2 (38.0)	Fijneman <i>et al.</i> , 1996	
			Sluc3	6 (57.0)	Fijneman <i>et al.</i> , 1996	
			Sluc4	11 (40.0)	Fijneman <i>et al.</i> , 1996	
			Lts1	17 (19.0)	Fijneman <i>et al.</i> , 1995	
Pas3			19 (5.0)	Devereux <i>et al.</i> , 1994		
Urethane		Sluc1	19 (47.0)	Fijneman <i>et al.</i> , 1996		
		Papg1	4 (49.6)	Manenti <i>et al.</i> , 1997		
		Par4	6 (3.0)	Manenti <i>et al.</i> , 1997		
		Pas1	6 (79.0)	Gariboldi <i>et al.</i> , 1993a		
		Pas4	9 (48.0)	Festing <i>et al.</i> , 1994		
Plasmacytoma	Pristane	Par1	11 (53.5)	Manenti <i>et al.</i> , 1996		
		Par3	12 (30.5)	Pataer <i>et al.</i> , 1997		
		Pas2	17 (19.1)	Festing <i>et al.</i> , 1994		
		Par2	18 (40.0)	Manenti <i>et al.</i> , 1997		
		Pas3	19 (15.0)	Festing <i>et al.</i> , 1994		
		Pctm	1 (80.0)	Mock <i>et al.</i> , 1993		
		Pcts	4 (60.5)	Mock <i>et al.</i> , 1993		
		Pctr1	4 (46.0)	Potter <i>et al.</i> , 1994		
		Pctr2	4 (76.5)	Potter <i>et al.</i> , 1994		
		Pre B-cell Lymphoma	Endogenous virus	Foc1	4 (38.9)	Yamada <i>et al.</i> , 1994
Esl1	17 (47.4)			Yamada <i>et al.</i> , 1994		
Msmr1	17 (22.0)			Pataer <i>et al.</i> , 1996		
Msmr2	18 (32.0)			Pataer <i>et al.</i> , 1996		
Thymic lymphoma	MNU	Tlag1	7 (47.0)	Angel <i>et al.</i> , 1993		
	Endogenous virus	Tlsm1	7 (66.0)	Yamada <i>et al.</i> , 1994		
Skin tumour	DMBA-TPA	Skts4	5 (64.0)	Mock <i>et al.</i> , 1998		
		Spr3	5 (24.0)	Nagase <i>et al.</i> , 1995		
		Spr1	7 (27.0)	Nagase <i>et al.</i> , 1995		
		Spr2	7 (64.0)	Nagase <i>et al.</i> , 1995		
	MNNG-TPA	Psl1	9 (51.0)	Angel <i>et al.</i> , 1997		

**Table 1.4** Mouse tumour susceptibility loci.

Abbreviation: DEN: N,N-diethylnitrosamine; DMBA: 7,12-dimethyl-benzanthracene; DMH: 1,2-dimethylhydrazine; ENU: ethylnitrosourea; MNU: N-methyl-N-nitrosourea; MNNG: N-methyl-N'-nitro-N-nitrosoguanidine; TPA: 12-O-tetradecanoyl-phorbol-13-acetate

formulation a 'logarithm of the odds' (LOD) scores:  $LOD(\theta) = \log_{10}[L(\theta)/L(1/2)]$ . The  $LOD(\theta)$  is the logarithm (base 10) of the likelihood ratio of the probability of the observed data given linkage at a certain recombination fraction  $\theta$  to the corresponding probability in the absence of linkage. The closer the marker is to the disease gene, the greater the extent of co-segregation (smaller  $\theta$ ) and the bigger the LOD score. A LOD score of 3 or more implies at least a 95% posterior probability of linkage (Ott, 1991).

#### **1.4.1 Genetic markers and DNA polymorphism**

As mentioned in previous section, linkage analysis test for co-segregation of disease trait within a family with a random marker. Ideally, the marker should be (i) highly polymorphic, so that pairs of individuals are likely to carry different alleles at each locus; (ii) abundant, so comprehensive marker coverage of the genome is achieved; (iii) neutral, with respect to the phenotypic effect of disease trait and reproductive fitness; and (iv) co-dominant, so all possible genotypes at a marker locus can be identified.

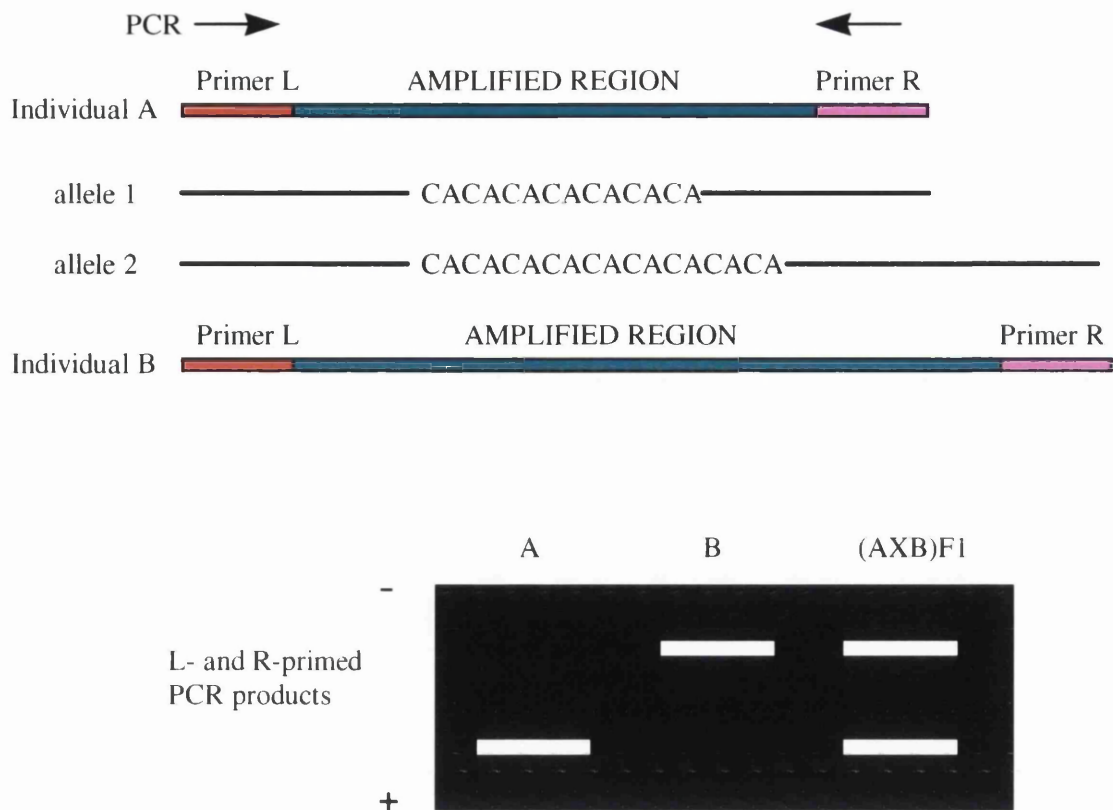
For many years, linkage mapping was seriously hampered by the lack of suitable markers. Cryptic protein variation, such as blood group antigens and electrophoretically distinguishable enzyme alleles, often satisfied the criteria of neutrality and co-dominance, but is neither sufficiently polymorphic nor abundantly to mark the entire genome. The most important breakthrough came in the late 1970s with the realisation that single base pair DNA polymorphisms could be recognised by restriction enzymes and resolved by electrophoresis, using a technique known as Southern blotting (Southern, 1975). This revolutionised the field by providing a much larger class of polymorphisms which were numerous throughout the genome and satisfied all essential criteria outlined above. These restriction fragment length polymorphisms (RFLPs) became the basis of many successful linkage studies in Mendelian disorders, including cancer syndromes such as retinoblastoma and familial adenomatous polyposis (Botstein *et al.*, 1980).

Another major advance has been the development of DNA polymorphism based on repetitive sequences. These were first recognised by Jeffreys *et al.* (1986), who noted that certain short DNA sequences were randomly repeated and that the number of repeats were often highly variable between individuals. The variation in these

minisatellite polymorphism (also known as variable number of tandem repeat or VNTRs) can also be detected by electrophoresis. The identification of microsatellite polymorphisms by Weber and May (1989). Microsatellites (also known as SSRs or SSLPs) are small blocks of randomly repeated DNA, in which the repeated element is usually a di-, tri-, or tetra- nucleotide sequence (e.g. [CA]<sub>n</sub>, [CAG]<sub>n</sub>, [AGAT]<sub>n</sub>) (Weber and May, 1989; Litt and Luty, 1989). The number of repeat elements in these block is often highly polymorphic, and shows simple Mendelian inheritance. Specific microsatellite loci can easily be defined by polymerase chain reaction (PCR) using oligonucleotide primers to the conserved sequences flanking the repeats, and length polymorphisms among individuals are identified by electrophoresis of the amplified products on agarose or polyacrylamide gels (Figure 1.2). Microsatellites are abundant and almost uniformly dispersed throughout the mammalian genome, thereby providing an enormous pool from which to derive markers. Their detection using PCR means linkage maps can be constructed more rapidly and efficiently than is possible using RFLP markers. Primarily through the efforts of a group of scientists at MIT and at Genethon, several thousand microsatellite markers have thus far been identified and mapped to the mouse and human genomes, respectively (Dietrich *et al.*, 1994a and 1996; Copeland *et al.*, 1993; Dib *et al.*, 1996). The availability of complete genome maps opens up the opportunity for new statistical approaches for detecting poly-genes in complex diseases.

#### **1.4.2 Linkage mapping of quantitative trait loci**

The past decade has seen rapid progress towards the genetics of inherited predisposition to cancer. A number of genes, whose germline mutations cause a highly penetrant familial predisposition to cancer have been identified via linkage analysis approaches (Fearon, 1997); the majority of these are genes involved in monogenic Mendelian diseases with simple patterns of inheritance (Lander and Schork, 1994). However, a complicating factor in genetic studies is that cancer is not a single disease, even when it arises in the same organ site. Rather, it is a collection of many diseases, some of which may even be multifactorial diseases. Most of them are influenced by more than one gene as well as environmental factor and thus do not exhibit a simple mode of inheritance (e.g. dominant, recessive, sex linked). The genetic dissection of such complex traits is much more difficult than the analysis of monogenic diseases because the genes



**Figure 1.2** Gene mapping by analysis of microsatellite polymorphisms. Length polymorphism of a microsatellite marker, between individual A and B in this figure, is detected using polymerase chain reaction (PCR) with a set of primers to the conserved flanking region (primer L and R). The size and number of products identifies the genotype of individual. In this example, the PCR products of allele 1 are larger than allele 2, since the former has two additional CA repeats than the latter. Thus, individual A and B are homozygous for the allele 1 and 2, respectively. (AXB)F1, which carries both A and B chromosomes, displays both parental PCR products.

influencing the multifactorial diseases may interact with each other and with environmental factors to create a statistical susceptibility (as opposed to conferring strict determination) to the disease, and these genes may neither be necessary nor sufficient for disease expression. Therefore, identifying and characterising these genes requires substantial resources, including very large collections of family data, highly informative genetic markers that span the genome and specifically developed statistical approaches that deal with complex traits. An effective approach for studying complex and polygenic form of disease is known as *Quantitative trait loci (QTL)* mapping.

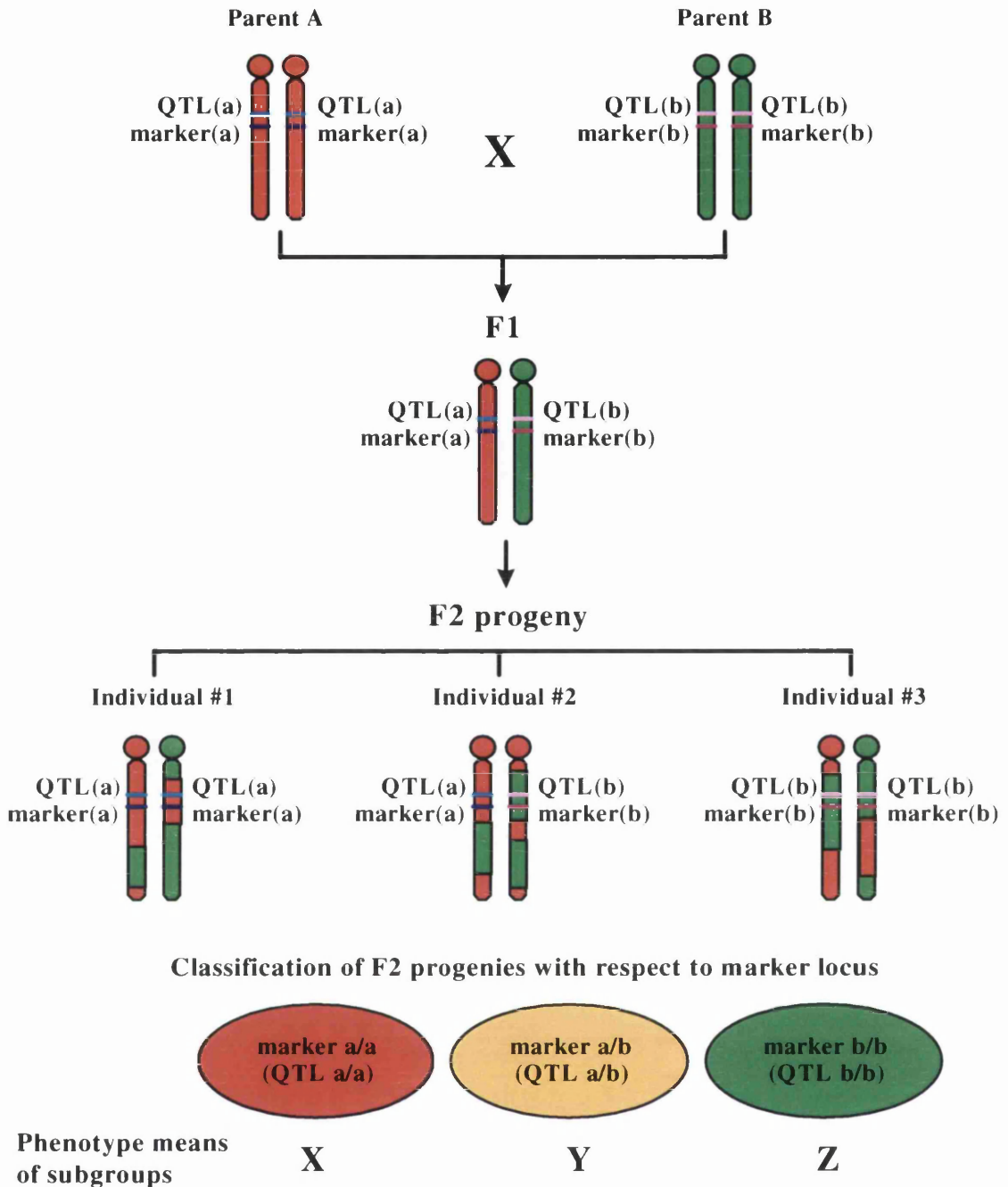
### **1.4.2.1 The general principles of QTL linkage analysis**

The theory of QTL analysis was first described in 1923 by Sax (Sax, 1923), when he noted that seed size in bean (a complex trait) was associated with seed coat colour (a simple monogenic trait). This concept was further elaborated by Thoday (Thoday, 1961), who suggested that if the segregation of simple inherited monogenes could be used to detect their linked QTLs, then it should eventually be possible to map and characterise all the QTLs involved in complex traits. Modern QTL mapping is essentially the fulfilment of this idea, with the key innovation being that defined sequence of DNA act as the linked monogenic markers. With the development of comprehensive DNA marker map (Dib *et al.*, 1996; Dietrich *et al.*, 1996), it is now possible to search for QTLs throughout the genomes. This has had the profound effect of moving the focus in studies of polygenic traits to questions about the chromosomal locations, gene actions, and biological roles of specific loci involved in complex phenotypes (Tanksley, 1993).

QTL analysis involves testing DNA markers throughout a genome for the likelihood they are associated with a QTL. Individuals are scored for their genotype at marker locus, and their phenotype for the quantitative trait of interest. For each marker locus, the individuals are split into classes according to the genotype. Mean and variance parameters are calculated and compared among classes. If there is a significant difference in phenotype among genotype classes, then we can infer the presence of a QTL linked to the marker (Figure 1.3).

### **1.4.2.2 Single marker analysis**

The simplest approach for detecting QTL is to analyse one marker at a time. If a marker is tightly linked to the target gene, the marker and QTL alleles will be associated and as a consequence the genotypic means of the marker will be different. As the single marker analysis does not require a complete molecular marker map, it is often first employed in a molecular marker/quantitative genetic study (Edwards *et al.*, 1987; Winkelman and Hodgetts, 1992). The disadvantage of single marker analysis are: (i) The further a QTL is from the marker gene, the less likely it is to be detected statistically due to crossover events between the marker and QTL that result in misclassification. (ii) The magnitude of the effect of any detected QTL will normally be underestimated, due also to



\* The QTL is detected if a significant difference is observed among the means of subpopulations X, Y and Z

**Figure 1.3** Marker-aided detection of a QTL in a theoretical F2 population segregating for quantitative trait. Top portion of figure depicts region of chromosomes in parent A (in red) and parent B (in green) that contains a marker and a linked QTL. F1 hybrid is heterozygous at both marker and QTL. F2 progeny segregate for recombinant chromosomes, but in most instances, genotype of linked marker accurately predicts the genotype for the QTL. Statistical comparisons of phenotypic means of the sub-populations (comprised of each of the three possible marker genotypes — a/a, a/b, b/b) can permit detection of the linked QTL.



recombination between the marker locus and the QTL. Both problems can be minimised when a large number of segregating markers which cover the entire genome are used. Under this condition, any potential QTL would be closely linked to at least one marker.

### **1.4.2.3 Interval mapping analysis**

The effect of a QTL and its distance from the proxy marker are inter-related. A QTL of small effect lying close to the proxy marker may appear similar to that of a QTL of large effect located further from the marker, as judged by the phenotypic differences between marker genotype classes. With only a single marker, we can not use this approach to determine both the contribution of a QTL and its position. The difficulty of separately estimating the effect of a QTL and its position is resolved by the availability of complete marker maps and the use of analysis by *interval mapping* (Lander and Botstein, 1989). Instead of analysing the population one marker at a time, sets of linked markers are analysed simultaneously with regard to their relative position and their effects on quantitative traits. By using linked markers for analysis, it is possible to compensate for recombination between the markers and the QTL, increasing the probability of statistically detecting the QTL and also providing an unbiased estimate of the QTL effect on the character.

In many available software packages, MapMaker/QTL is the most widely used software (Lander *et al.*, 1987). This method is usually implemented by choosing a given point in the genome and predicting the effect of a QTL at that position. The process is repeated at fixed positions through a chromosome and the position at which a QTL would explain most of the variance between marker classes is identified. The point is then the estimated position of the QTL, and the effect of the QTL is that estimated for this position.

The interval mapping method can work well when there is only one QTL on a chromosome. When there are several QTLs involved, however, the results can be misleading. The number of QTLs detected by linkage with markers is always an underestimate of the number of loci because QTLs identified by linkage to marker loci are not loci in the usually genetic sense but rather effective factors and may contain several loci affecting the trait. Therefore when a test reveals a QTL linked to a marker,

the effect observed may be contributed by two or more close linked loci, not just one locus. The effect observed is, thus, the aggregate of the effects of these loci. Furthermore, the alleles at the linked loci may be in association or in dispersion. Consequently, the effects of each individual loci may be smaller (in case of association) or larger (in case of dispersion) than the observed aggregate effects. In extreme cases, two QTLs closely linked to each other may not be detected at all if in dispersion. The complete solution to this problem has yet to be resolved, but it can at least be partially ameliorated by explicitly testing alternative models which assume multiple QTLs.

#### **1.4.2.4 Multiple QTLs mapping**

Given the multistage nature of cancer, tumour development is likely to involve the contribution of multiple genetic loci. Some loci may be closely linked by location or by function. Therefore, two methods have been developed to fit the multiple-locus model, namely MQM (multiple QTL model or marker-QTL-marker) mapping and CIM (composite interval mapping) (Jansen 1993; Jiang and Zeng, 1995). The genetic concepts of these two methods are more or less identical, combining the interval mapping with regression analysis to dissect the effects of two or more linked QTLs. Like simple interval mapping, the test evaluates the possibility of a target QTL at multiple analysis points across each inter-marker interval. However, at each point it also includes the effects of other one or more background markers. The inclusion of the background markers makes the analysis more sensitive to the presence of a QTL in the target interval and help to separate the target QTL from other linked QTLs (Zeng, 1994).

#### **1.4.3 Parametric and nonparametric analysis for QTL mapping**

The use of markers to detect individual loci responsible for a QTL trait has been a powerful tool for the study of genetic variation. Many statistical methods have been developed to analyse mapping data for QTL traits. In principle, there are two linkage analysis methods, one is termed as the parametric method which involves testing whether the inheritance pattern fits a specific model for a trait-causing gene, and the other is the non-parametric method which involves testing whether the inheritance pattern deviates from expectation under independent assortment.

All the parametric analysis methods share a common assumption that the phenotype follows a normal distribution with equal variance in both parental strains. However, many phenotypes of interest are, often, not normally distributed. Examples include counts generated by a Poisson process (such as number of tumours, which in many cases follows a negative binomial distribution) (Drinkwater and Klotz, 1981), truncated data (such as survival times in an experiment of limited duration), probabilities (such as chance of an epileptic seizure in a given trail), and qualitative data (such as severity grade assigned upon histological examination). Traditional QTL mapping methods can not be directly applied in such cases. The solution to this problem is to find a mathematical transformation that will convert the phenotypic data into an approximately normal distribution. An alternative approach is to apply non-parametric statistic test, a measure based on the rank of each trait value rather than the trait values itself (Kruglyak and Lander, 1995). This approach is a model free method and can be applied to any phenotypic distributions.

#### **1.4.4 Power and precision of QTL mapping**

Each QTL-detection experiment provides an estimate of the strength of a QTL. In some experiments, the QTL will be overestimated, in others underestimated. The power of a QTL-detection experiment, defined as the probability of detecting a QTL at a given level of statistical significance, depends on the strength of the QTL and the number of progeny in the population. If we consider the strength of the QTL in terms of fraction of the total trait variance that it explains, we can define three categories of QTLs. Those which explain over 20% of the variance are strong QTLs. Traits controlled by such QTLs can be considered almost Mendelian. Strong QTLs can be detected with a power greater than 80% even with the AxB/BxA set of recombinant inbred strains. At the other extreme, weak QTLs, which explain 1% or less of the trait variance, require at least a thousand progeny to detect them with high power. Detection of such QTLs is not routinely feasible. Between these extremes are moderate QTLs, which can be detected with crosses of reasonable size, but not necessarily at high power (Manly and Olson, 1999).

Since moderate QTLs span a wide range of strength, the interpretation of any experiment designed to detect such QTLs should include an estimate of the power of the

experiment. Rebai *et al.* (1995), Darvasi (1998), and Lynch and Walsh (1998) provide estimates of power for QTL detection. The number of progeny required to detect a QTL is, generally speaking, proportional to the variance of the nongenetic (environmental) contributions and inversely proportional to the square of the strength of the QTL. In addition to the strength of the QTL and the size of the population, the power of a QTL-detection experiment depends on the type of cross, the dominance of the QTL alleles and marker spacing.

The ultimate goal of linkage mapping is to find the location of QTLs. Lander and Botstein (1989) proposed a simple rule for constructing confidence intervals for QTL position. This method use the LOD score for a QTL. According to them, the position of the maximum LOD score is taken as the position of the QTL, and the region in which the LOD score is within one LOD unit of the maximum is taken as a 96.8% confidence interval. The size of a confidence interval is expected to be inversely proportional to the number of progeny in the mapping population and inversely proportional to the square of the strength of the QTL (Darvasi, 1998). Therefore, the strength of the QTL is critical to establishing location. Strong QTLs can be located by a large backcross or intercross in small confidence interval; weak QTLs can be assigned to a chromosome but not located with much more precision (Darvasi *et al.*, 1993). For moderate QTLs, precision is limited by the size of the population and the proportion of variance explained (Darvasi, 1998). Corresponding to the wide range of strength expected among QTLs, Lander and Kruglyak (1995) have proposed standards for detection that cover a wide range of significance. The suggestive level is defined as that a false positive result would be expected to occur one time at random in a genome scan; the significant level corresponds to a probability of 0.05 times in a genome scan. In the case of backcross studies, the LOD scores of the two threshold levels (suggestive and significant) would be 1.9 and 3.3; In the case of intercross studies, the LOD scores would be 2.0 and 3.4.

#### **1.4.5 From QTL to gene**

The ultimate achievement of QTL mapping will be the molecular cloning of the underlying genes. Once a QTL implicated in a cancer predisposition syndrome has been mapped by linkage analysis, candidate genes from the region can be identified by positional cloning strategies (Collins, 1991). Positional cloning usually requires that

map position of the locus of interest is known to within 1 cM interval, which is approximately the size of genomic inserts that can be contained in currently available cloning vectors. Thus high resolution QTL mapping is needed to refine the region. Linkage disequilibrium and cytogenetic analysis, as well as LOH and homozygous deletions studies, can then be used to locate the candidate locus more precisely (Goddard *et al.*, 1996; Fearon *et al.*, 1990). Ultimately, sequence based analysis of the candidate genes remains the gold standard for identifying mutant alleles. To establish the authenticity of the candidate gene, the mutant alleles must be shown not only to segregate with cancer predisposition but to be causally involved in cancer development through functional studies. Analysis of LOH and homozygous deletions can also be used independently to identify tumour suppressor gene loci.

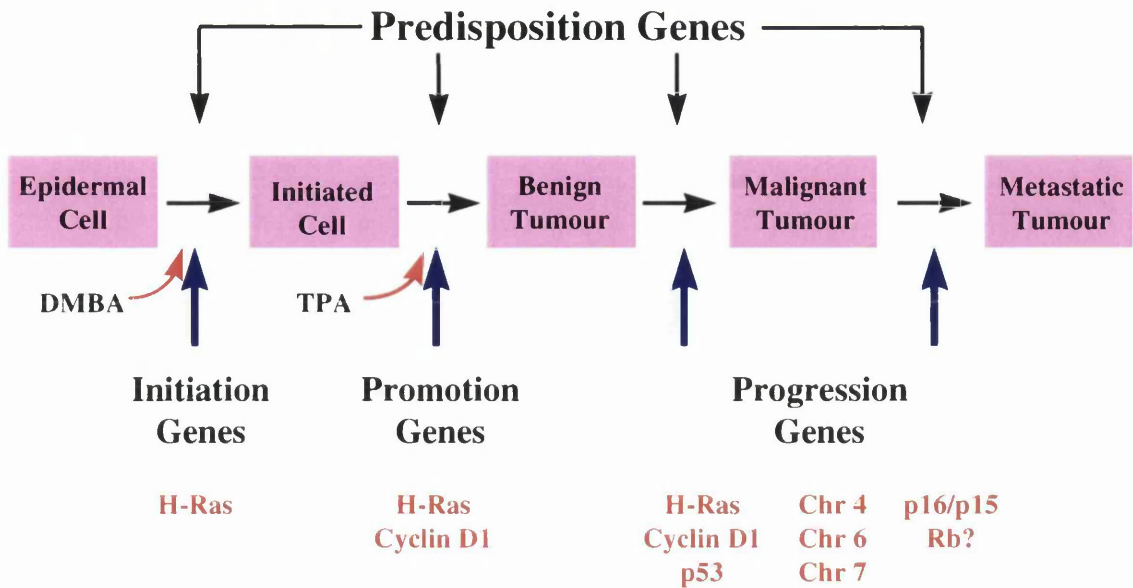
In addition to positional cloning strategies, other investigative approaches have also been applied successfully to the search for cancer genes. The most common one is the candidate gene approach. This approach depends on linkage analysis for gene mapping and subsequently sequence analysis for detection of germline mutations in known oncogenes, tumour suppressor genes, or genes that involves in critical cellular pathways, in the implicated chromosomal region (Mulligan *et al.*, 1993; Hofstra *et al.*, 1994; Fishel *et al.*, 1993; Hussussian *et al.*, 1994).

## **1.5 Mouse skin carcinogenesis**

### **1.5.1 Mouse skin carcinogenesis — a multistage process**

Chemical carcinogenesis in mouse skin has been extensively studied and represents one of the most well-defined *in vivo* models of experimental carcinogenesis. It has contributed significantly to our current understanding of the multistage nature of carcinogenesis. Studies on this model have led to the operational definitions of initiation, promotion and progression (Figure 1.4) (Slaga, 1989; DiGiovanni, 1992; Yuspa, 1994 and 1998).

The sequential application of a sub-threshold dose of a carcinogen (initiation stage) followed by repetitive treatment with a non-carcinogenic promoter (promotion stage) will effectively induce skin tumours. A proportion of these papillomas then undergo



**Figure 1.4** Summary of genetic events and candidate genes in multistage skin carcinogenesis based on a linear malignant progression model.

further genetic changes and progress to squamous carcinomas. The final event in this model system involves conversion of squamous cell carcinomas to highly invasive undifferentiated spindle carcinomas (Klein-Szanto *et al.*, 1989).

### 1.5.2 Genetic events in mouse skin carcinogenesis

The genetic studies on the molecular level have led to the identification of a number of sequential genetic alterations that are associated with initiation, promotion and progression (Brown *et al.*, 1993).

#### 1.5.2.1 Initiation

Initiation is generally accomplished by topical application of a single sub-carcinogenic dose of a skin carcinogen, such as 7,12-dimethyl-benzanthracene (DMBA). The treatment produces a subtle change in the phenotype of keratinocyte although it is unrecognisable in the context of the intact epidermis. These initiated cells can remain dormant in the skin for a considerable period until activated by the promoter, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA). This indicates that the alterations caused by initiators are persistent and irreversible (Loehrke *et al.*, 1983).

Important insights into the genetic alterations associated with the tumour initiation have emerged from the identification of mutations in the *H-ras* oncogene in skin tumours (Balmain *et al.*, 1984; Harper *et al.*, 1987). It has been shown that nearly all benign papillomas and malignant carcinomas induced by DMBA initiation and TPA promotion exhibit mutational activation of *H-ras* proto-oncogene by an A to T transversion at codon 61 (Quintanilla *et al.*, 1986). Subsequent studies demonstrated that the type of mutation was dependent on the chemical initiator and independent of the promoter, suggesting that the initiator has a direct effect on *H-ras* (Bizub *et al.*, 1986; Brown *et al.*, 1990). Nelson *et al.* (1992) proved later that the mutation of *H-ras* oncogene can be detected before the emergence of visible tumours. Furthermore, infection of mouse skin by an activated viral *H-ras* oncogene can serve as the initiating event in two stage carcinogenesis (Brown *et al.*, 1986).

### **1.5.2.2 Promotion**

The promotion stage is characterised by selective and sustained hyperplasia leading to the specific expansion of the initiated cells into benign papillomas (Yuspa and Poirier, 1988; Slaga, 1989; DiGiovanni, 1992). It is well known that most tumour promoters do not bind covalently to DNA and are not mutagenic, but they bring about a number of important epigenetic changes (Yuspa *et al.*, 1982). However, the range of cellular and biochemical changes which tumour promoters can induce is so large that it is difficult to identify those which are responsible for their ability to promote tumour formation. Nevertheless, it is possible that the clonal outgrowth of initiated cells presumably reflects some subtle alteration in their response to tumour promoters.

The most potent promoters of mouse skin are the phorbol esters such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA). The transcription of many genes can be affected by TPA, several of which have obvious connection with the regulation of growth and differentiation. It has been known that TPA can activate protein kinase C (PKC) and induce differentiation of normal keratinocytes (Yuspa *et al.*, 1980 and 1982; Dlugosz and Yuspa, 1993). Initiated keratinocytes are resistant to terminal differentiation induced by activators of PKC (Hennings *et al.*, 1987; Yuspa *et al.*, 1983; Hennings *et al.*, 1990a), and the differential response of normal and initiated cells favours the growth of the neoplastic subpopulation, enhancing clonal outgrowth and

producing papillomas. The ornithine decarboxylase gene (ODC) which is a growth related enzyme is also upregulated in epidermis upon TPA treatment (Imamoto *et al.*, 1992; Lichti *et al.*, 1981; Verma *et al.*, 1988). Mice overexpressing a human ODC gene were shown to be sensitive to two stage skin carcinogenesis (Halmekyto *et al.*, 1992). TPA can also induce transforming growth factor  $\alpha$  (TGF- $\alpha$ ) mRNA and protein expression (Imamoto *et al.*, 1991). TGF- $\alpha$  is a major autocrine controlling growth in epidermal cells, and elevated levels of TGF- $\alpha$  have been detected in squamous tumours from human and mouse (Kiguchi *et al.*, 1995; Reiss *et al.*, 1991). TGF- $\alpha$  has also been shown to synergise with activated H-*ras* in tumourigenicity studies (Finzi *et al.*, 1988). The expression of *c-fos*, *c-myc* and *c-jun* proto-oncogene, which modulates transcription of genes involved in cell proliferation, is also found elevated following administration of TPA (Lamph *et al.*, 1988; Skouv *et al.*, 1986).

### 1.5.2.3 Progression

The skin tumour progression stage is characterised by a high level of genetic instability that leads to a number of chromosomal alterations. This is generally a spontaneous process that is not enhanced by most exogenous tumour promoters, but can be enhanced and accelerated by exposing animals bearing papillomas to a mutagen (Hennings *et al.*, 1983; Hennings *et al.*, 1990b; O'Connell *et al.*, 1986).

The progression stage can be further subdivided into premalignant progression and malignant conversion. Premalignant progression of a papilloma involves repeat episodes of selection and clonal outgrowth of cells which have acquired a growth advantage, usually as a result of further genetic changes (Yuspa, 1994). A number of genetic changes have been found associated with premalignant progression of mouse skin papillomas. Cytogenetic and molecular studies have demonstrated frequent trisomy of chromosome 7 and chromosome 6 in papillomas (Aldaz *et al.*, 1989; Kemp *et al.*, 1993). The critical gene on mouse chromosome 7 appears to be the H-*ras* gene itself, since the trisomy invariably duplicated the chromosome carrying the mutant H-*ras* allele (Kemp *et al.*, 1993; Bremner and Balmain, 1990). Cyclin D1, which is also located in the distal region of mouse chromosome 7, is another gene found amplified in chemically induced mouse skin tumours (Bianchi *et al.*, 1993). Recently, Robles *et al.* (1998) have shown that cyclin D1 is a critical target for oncogenic *ras* in mouse skin. The



development of papillomas is significantly reduced in cyclin D1-deficient mice. The target gene on chromosome 6 is unknown, but several candidate genes have been identified, including *raf-1*, TGF- $\alpha$  and *K-ras*.

Malignant conversion of benign tumours is a relatively rare event with around 5-10% of papillomas progressing to carcinomas. However, this transition can be increased by treating papillomas with mutagens (Hennings *et al.*, 1983; O'Connell *et al.*, 1986). Several studies have shown that spontaneous malignant conversion does not appear to be stochastic in nature, and that not all papillomas have an equal probability of progressing to carcinomas. Discrete subsets of papillomas have been identified which display an increased risk of progression (Brown *et al.*, 1990; Hennings *et al.*, 1985). More recently, differences in phenotypic markers such as TGF- $\beta$ ,  $\alpha_6\beta_4$  integrin and keratin 13 expression have been used to distinguish between high and low risk of papillomas at early stage (Glick *et al.*, 1993; Tennenbaum *et al.*, 1993; Gimenez-Conti *et al.*, 1990). Several genetic alterations have been associated with malignant conversion. Loss of the normal *H-ras* allele is often observed in carcinomas which contain a mutant *H-ras* gene (Bremner and Balmain, 1990). Mutations in the *p53* tumour suppressor gene, which are rarely found in papillomas, are frequently detected in carcinomas (Burn *et al.*, 1991; Ruggeri *et al.*, 1991). Loss of heterozygosity (LOH) on mouse chromosome 11, on which the tumour suppressor gene *p53* is located, is also detected in malignant skin tumours (Burns *et al.*, 1991). Mice lack of functional *p53* do not show any alteration in the incidence or growth rate of benign papillomas, but tumours have a greatly elevated rate of malignant progression. This suggests that *p53* play an important role in late tumour progression, rather than early initiation or promotion stage (Kemp *et al.*, 1993).

A further advanced stage in mouse skin carcinogenesis is the progression of squamous carcinoma to spindle carcinoma, in which the markers of epithelial differentiation are lost (Klein-Szanto *et al.* 1989; Navarro *et al.*, 1991; Diaz-Guerra *et al.*, 1992). The squamous to spindle conversion is associated with an increase in the ratio of mutant to normal *H-ras* gene, and has been demonstrated, in cell fusion experiment, to be a recessive event (Buchman *et al.*, 1991). LOH on mouse chromosomes 4, on which *p16INK4a* (MTS1) tumour suppressor gene is located, is also associated with late progression stage (Linardopoulos *et al.*, 1995).

### 1.5.3 Genetic susceptibility in mouse skin carcinogenesis

The genetic susceptibility to chemical carcinogens has been studied in the mouse skin model for many years. Early work by Boutwell *et al.* (1974) showed that the response of the skin to chemical carcinogens is strongly influenced by the genetic background of the host. For example, SENCAR, FVB and DBA/2 are relatively susceptible to two-stage carcinogenesis, whereas C57BL/6 and BALB/c are relatively resistant (Naito and DiGiovanni, 1989; Ashman *et al.*, 1982; Hennings *et al.*, 1993). Although the differences in susceptibility among various stocks and strains of mice is related to the carcinogens and promoters used, as well as the protocols for treatment, the mechanisms involved in predisposition are not fully understood. Studies carried out by Naito and DiGiovanni (1989) showed that although some differences can be attributed to initiation, the major contribution to tumour susceptibility appears to be at the level of tumour promotion. Using a cross between DBA/2, a strain susceptible to promotion with phorbol esters, and C56BL/6J which is resistant, they established that susceptibility to promotion in the model is inherited as an autosomal incomplete dominance (Naito *et al.*, 1988). Further studies using recombinant inbred strains have better defined their genetic model postulating that a minimum of three loci, two dominant and one recessive, control susceptibility to tumour promotion (DiGiovanni *et al.*, 1991; DiGiovanni *et al.*, 1992). Using intraspecific crosses between outbred *Mus spretus* and inbred *Mus musculus*, Nagase *et al.* (1995) have identified three susceptibility loci (*Spr1-3*), two on chromosome 7 that control the development of skin tumours at promotion stage and one on chromosome 5 that takes effect at both promotion and progression stages. Recently, two loci which involve in skin tumour development have also been identified in two inbred strains (*Skts4* in SENCARA/Pt and *Ps11* in DBA/2) by two separate groups, they are located on chromosome 5 and 9, respectively (Angel *et al.*, 1997; Mock *et al.*, 1998).

### 1.6 Aim--linkage analysis of predisposition loci to mouse skin tumour

The rodent model we have chosen for our studies on tumour predisposition is the classical two-stage (DMBA-TPA) mouse skin chemical carcinogenesis system which represents a realistic model for cancer development in humans (Yuspa, 1994). To date, genetic loci identified in different crosses are mostly different, implying the presence of

different genetic control in different mouse strains. As we know that different strains of mice share the same biological pathways as well as genetic alterations during skin tumour development (Yuspa, 1994; Naito and DiGiovanni, 1989), do they have common genetic loci, susceptible or resistant, that control the predisposition to tumour? To address this issue, we have conducted linkage analysis in genetic crosses between two inbred strains, the sensitive FVB/N mice and resistant C57BL/6J mice (Hennings *et al.*, 1993; Naito *et al.*, 1988; DiGiovanni, 1992).

The FVB/N strain was developed in the 1970s by inbreeding the Fv-1<sup>b</sup> allele for sensitivity to the B strain of Friend leukaemia virus. Because of its superfecundity, large prominent pronuclei in fertilised zygotes and large litter size, FVB/N mouse has been widely utilised for transgenic analysis (Taketo *et al.*, 1991). The widespread use of FVB/N mice for the establishment of transgenic lines containing active oncogenes make it an important host for study of carcinogenesis. In addition, FVB/N mice are not only highly susceptible to carcinogen induced skin tumours, but also have higher malignant conversion rate than any other mouse strain (Hennings *et al.*, 1993). In contrast, C57BL/6 mice, which are the most widely used inbred mouse strain for chemical induced carcinogenesis studies, are relatively resistant to skin tumour development by the DMBA-TPA model of two stage carcinogenesis (Naito *et al.*, 1988).

To detect the genetic loci that control the skin tumour development in mice, particularly those involved in malignancy progression, we carried out two-stage carcinogenesis in mice from FVB6F1XFVB/N backcross (F1BX) and FVB6F1 intercross (FVB6F2). The whole genome scan was then performed with mouse microsatellite markers to examine the genotypes of experiment mice. The linkage of genetic loci to tumour susceptibility were detected by QTL linkage analysis at three different levels. The association of these loci with tumour development were further investigate by analysing their genetic alteration in tumours derived from FVB6F1 mice.

# **CHAPTER 2**

## **MATERIALS AND METHODS**

## 2.1 Materials

### 2.1.1 Chemicals, reagents and enzymes

All chemicals were of AnalaR grade and were obtained from BDH chemicals Ltd., Poole, Dorset or Sigma Chemical Co. Ltd., Poole, Dorset except those obtained from the suppliers listed below.

#### Bioline, London

BioTaq DNA Polymerase and buffer

#### Boehringer Mannheim UK Ltd., Lewes, East Sussex

Proteinase K

Molecular weight DNA marker VIII

#### J. Burrough (FAD) Ltd., Witham, Essex

Ethanol

#### FMC, Rockland, ME

NuSieve 3:1 agarose

MetaPhor agarose

#### Life Technologies Ltd., Paisley

Restriction Enzymes

Proteinase K

Taq DNA polymerase

100bp DNA ladder

Phenol:chloroform:isoamyl alcohol (25:24:1, v/v )

#### Pharmacia Ltd., Milton Keynes, Buckinghamshire

Ultrapure dNTP set 2' Deoxynucleoside 5' Triphosphate

Promega, Madison, WI

Taq DNA polymerase

dNTP set (dGTP, dATP, dTTP and dCTP)

Novex Experimental Technology, San Diego, CA

20% TBE precast polyacrylamide gel

Severn Biotech, Kidderminster

Acrylamide (40% stock, 19:1)

**2.1.2 General plasticware**

Advanced Biotechnologies Ltd., Surrey

PCR reaction tubes

96-well Thermo-Fast PCR plate

48-well Thermo-Fast PCR plate

Adhesive sealing film for microtiter plate

Beckman Instrument Inc., Fullerton, CA

96-well deep well plate

Adhesive aluminium sealing film for 96-well plate

Griener Labortechnik Ltd., Dursley

Eppendorf tube

Filter pipette tip

Labsystems, Basingstoke

Pipette tip

Aerosol-resistant filter tip

**2.1.3 Websites for the databases**

Genome Data Base (GDB):

<http://www.gdb.org>

Mouse Genome Informatics (MGI), including Mouse Genome Database (MGB) and Gene Expression Database (GXD):

<http://www.informatics.jax.org>

National Center for Biotechnology Information (NCBI):

<http://www.ncbi.nlm.nih.gov>

National Human Genome Research Institute (NHGRI):

<http://www.nhgri.nih.gov>

Whitehead Institute/MIT Center for Genome research:

<http://www-genome.wi.mit.edu>

#### **2.1.4 Mouse microsatellite marker**

##### Research Genetics, Huntsville, AL

Mouse MapPairs<sup>TM</sup> microsatellite markers

Total of 247 mouse microsatellite markers used for genotyping of the mice, as well as the tumours from the F1 mice. The list of these markers and their chromosome locations are shown in Table A1.

## **2.2 Animals**

### **2.2.1 Sources**

Two inbred mouse strains, FVB/N and C57BL/6J, were used in the animal experiment. They were originally obtained from Harlan UK Limited. Mice were bred and maintained in Beatson Animal Resource Facility for the experiments. All experiments were carried out following the UKCCCR Guidelines, Animal Scientific Procedure Act (1986), and local Beatson Animal Welfare Committee Guidelines for use of animals in neoplasia. In general, mice were kept in the metal-wired cages with free access to food (Teklad rodent diet, Harlan UK Ltd.) and drinking water in an air-conditioned room ( $24\pm 2^{\circ}\text{C}$ ) with 12 hour light/dark cycles.

### **2.2.2 Tumour induction**

The mice used for tumour induction were generally eight weeks old. The back of the mice were carefully shaved with a surgical clipper 24 hours before each chemical treatment. All the chemicals were applied topically to the shaved area in 0.2 ml acetone. Mice were initiated with 25µg of DMBA (9,10-dimethyl-1,2-benzanthracene) and promoted with 20 nmol (12.5 µg) TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) one week later. Promotion was conducted twice weekly for 20 weeks. Mouse skin tumours were evaluated and scored once a week for the presence of papillomas and carcinomas.

### **2.2.3 Tumour data score and record**

The papilloma is a cauliflower-like structure with either a narrow or broad base consisting of a series of folds united to the underlying skin by one or few common stalks. When a papilloma of diameter > 1 mm was noted and found to be persistent for one week, the first date of observation was recorded and the actual number of papillomas were recorded as the papilloma data.

The carcinoma is usually an endophytic growth of atypical epithelial cells which invades the dermis and subcutaneous tissue. Most frequently, an induration of the subepidermal tissues with a swelling appearing on a limited area of the base of a papilloma or a ulcerated lesion showing a thickening and swelling of the edge of the ulcer indicate the formation of a carcinoma. A carcinoma was recorded according to their morphological changes, and subsequently confirmed by the histological examination. A dichotomous score system was used to record the carcinoma data. When one or more carcinomas were diagnostic, it was scored one; otherwise it was scored zero.

The dorsal skin of mice was inspected for the presence of papillomas and carcinomas visually once a week for 60 weeks period. The mouse was removed from the experiment when the total papilloma burden exceeded 10% of its body weight or when the diameter of a carcinoma exceeded 10 mm. The mouse was then sacrificed and all papillomas and carcinomas were collected. A small section of the tumour was fixed in 5% formalin and processed for H&E staining while the rest was frozen for further molecular analysis.



## **2.3 Histology**

Tumour sections were fixed in 5% formalin at 4°C for 24 hours before processed for conventional paraffin sections and haematoxylin-eosin (H&E) staining. The stained specimens were then examined under light microscopy. Generally, in animal cells, nuclei stained blue and cytoplasm stained pink or red.

## **2.4 DNA preparation**

### **2.4.1 Preparation of genomic DNA from mouse tail**

Mouse tail biopsy of no longer than 1 cm was cut and transferred to a 1.5 ml eppendorf tube containing 500 µl lysis buffer (100 mM Tris-HCl pH 8.5, 5mM EDTA, 200 mM NaCl, 0.2% SDS, and 100 µg/ml proteinase K). The tail was then digested at 55°C for several hours or overnight. Following lysis completion, the sample was spun in an eppendorf centrifuge for 10 minutes to remove hair and tissue residue. The supernatant containing genomic DNA was then transferred to a fresh tube. One volume of isopropanol was added and the sample was mixed gently until viscosity completely disappeared. The sample was kept at -20°C for 10 minutes to allow the precipitation completed. The sample was then spun again for 5 minutes. The DNA pellets were briefly air-dried and resuspended in 200 µl TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5). The DNA sample was stored at 4°C.

### **2.4.2 Preparation of genomic DNA from frozen tumour or tissue**

Frozen tumour or tissue sample was ground to powder using a mortar and pestle, which was pre-cooled with liquid nitrogen (N<sub>2</sub>). The sample was transferred to a 2 ml eppendorf tube containing 500 µl lysis buffer (as described in 2.4.1.), and digested overnight at 55°C on a rotating tube rack. The cell lysate was mixed with one volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v) and rotated for 15 minutes at room temperature, and then centrifuged at 2000 rpm for 10 minutes to separate the aqueous and organic phases. The top aqueous layer which contained genomic DNA was transferred into a fresh tube. The phenol/chloroform extraction procedure was repeated once to obtain cleaner supernatant. The genomic DNA was precipitated with one

volume of isopropanol, and spooled onto a disposable pipette tip. The DNA was rinsed in 70% v/v ethanol and air-dried briefly before dispersed into 500  $\mu\text{l}$  TE pH 7.5. The DNA sample was stored at 4°C.

### **2.4.3 Quantitation of nucleic acid concentrations**

Nucleic acids were quantified by spectrophotometric determination of their UV light absorbency. 5  $\mu\text{l}$  sample was added to 495  $\mu\text{l}$  de-ionised water and the absorbency of the solution measured at 260 nm and 280 nm in a quartz cuvette, using de-ionised water as a blank control. The concentration of the solution was calculated using de Beer's law on the basis that an optical density of 1.0 at 260 nm corresponds to a concentration of 50  $\mu\text{g/ml}$  for double-stranded DNA, 40  $\mu\text{g/ml}$  for RNA and 33  $\mu\text{g/ml}$  for single-stranded oligonucleotides. Pure preparation of DNA and RNA has a ratio of  $A_{260}/A_{280}$  reading between 1.8 and 2.0.

## **2.5 Polymerase chain reaction (PCR)**

### **2.5.1 General PCR**

PCR reactions were performed in 50  $\mu\text{l}$  reaction mixture containing 100 ng template DNA, 0.2  $\mu\text{M}$  forward and reverse PCR primers, 200  $\mu\text{M}$  4dNTPs, 10 mM Tris-HCl pH8.8, 50 mM KCl, 1.5 mM  $\text{MgCl}_2$  and 1 unit of Taq Polymerase. All PCR reactions were carried out on an automatic thermocycler (Perkin Elmer 9600, Applied Biosystem or PTC-200, MJ Research) using the following cycling conditions: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 40 seconds. A final extension step was added at 72°C for 3 minutes. PCR products were separated on either agarose or polyacrylamide gel.

### **2.5.2 Genotyping using mouse tail DNA**

Genotyping was conducted by mouse microsatellite PCR using mouse tail DNA. PCR reactions were carried out as described in 2.5.1 with slight modification. The PCR reactions were performed in individual tubes on 96-well or 48-well thermo-fast

microtiter plates. 50 ng of tail DNA and 0.2uM of Mouse MapPairs™ were used in 15 µl reaction mixture. To achieve the best condition of PCR amplification, the concentration of MgCl<sub>2</sub> was ranged from 1.5 mM to 3 mM, and the annealing temperature varied from 50°C to 60°C according to the T<sub>m</sub> values of paired PCR primers. 35 cycles of PCR amplification programme was generally applied.

The PCR products were analysed by agarose or polyacrylamide gel electrophoresis depending on their size difference. Generally, the PCR products were between 100-300 bp in size. When the difference was more than 8 bp, 4% (w/v) NuSieve 3:1 agarose gel was used for the analysis. When size difference was less than 8 bp, higher resolution was needed, thus 3-4% (w/v) MetaPhor agarose gel or 12-15% (w/v) non-denaturing polyacrylamide gel were used.

### **2.5.3 Genotyping using tumour DNA**

50 ng template DNA from paired normal tissue (spleen or kidney) and tumour (papilloma and carcinoma) was used in each PCR reaction with standard reaction conditions (see section 2.5.1 and 2.5.2). 25-30 cycles of PCR amplification was determined to be in the linear part of the amplification process (i.e. before product saturation), permitting the assumption that the ratio of the optical densities arising from two allele would be the same for both normal and tumour DNA samples if no LOH occurs. PCR products were separated on either 4% NuSieve 3:1 agarose gel, or 12-15% (w/v) non-denaturing polyacrylamide gel. Allele loss was determined by computed density analysis.

## **2.6 Nuclei acid analysis**

### **2.6.1 Digestion of DNA with restriction endonucleases**

DNA was digested using restriction enzymes in the appropriate reaction buffers for 1-3 hours under conditions specified by the suppliers. Generally, 1 µg DNA was digested in a total volume of 20 µl reaction mixture with 1-5 units of restriction endonucleases.

### **2.6.2 Agarose gel electrophoresis**

Agarose gel electrophoresis was performed using a horizontal apparatus. Gel for analysing PCR products and restriction enzyme digestion fragments was made from 1-2% (w/v) agarose and cast in 1X TAE buffer (40 mM Tris-HCl pH 7.8, 20 mM sodium acetate, 1 mM EDTA). Gel for genotyping and tumour LOH studies was made from 4% (w/v) NuSieve 3:1 agarose and cast in 0.5X TBE buffer (90 mM Tris-HCl, 90 mM boric acid, 2.5 mM EDTA, pH 8.3).

Gel was submerged in 1X TAE (or 0.5X TBE) buffer and DNA samples were loaded with gel loading buffer (0.25% w/v bromophenol blue, 0.25% w/v xylene cyanol FF, 30% v/v glycerol) into the wells. . The appropriate molecular weight DNA markers were also loaded into wells. TAE gel was run at 2V/cm, low melting point agarose gel at 40-50 volts and TBE gel at 5V/cm until the bands were sufficiently resolved. Ethidium bromide was added to the gel at a final concentration of 0.5 µg/ml. DNA was stained with ethidium bromide and visualised under ultraviolet light.

### **2.6.3 Fast running protocols for high resolution in MetaPhor agarose gels**

For typical genotyping, the 4% (w/v) NuSieve 3:1 agarose gels were electrophoresed for 3-4 hours to obtain a resolution of 8% size difference with DNA between 100 and 500 bp. To decrease the electrophoretic time and achieve higher resolution (4%), MetaPhor agarose gel was used in the fast running method.

Fast running protocol was performed using a horizontal submarine gel apparatus with recirculator-chiller water bath. 4% w/v MetaPhor agarose gel was cast in 1X TBE buffer. The solidified gel was kept at 4°C for 30 minutes before submerged in 0.5 X TBE buffer at 20°C. The DNA samples were loaded with gel loading buffer and separated at the voltage of 17V/cm. The electrophoresis was run at constant 20°C for 1-1.5 hours with running buffer in circulation.

### **2.6.4 Non-denaturing polyacrylamide gel electrophoresis**

For non-denaturing polyacrylamide gel, a 40% w/v stock solution of acrylamide (ratio of acrylamide to N,N'methyl-bisacrylamide of 29:1) was diluted to give a 12% or 15% w/v gel forming solution in 1 X TBE buffer. The solution was polymerised by the addition

of 240 µl 10% (w/v) ammonium persulphate and 24 µl TEMED per 40 ml of gel. The solution was poured between two glass plates separated by 1 mm spacers and allowed to set at room temperature for at least 1 hour. The DNA samples were then loaded with gel loading buffer and the electrophoresis was run at a constant 125 volts for 16-20 hours at room temperature. The DNA bands were visualised with silver staining.

### **2.6.5 SSCP-mutation analysis**

20% TBE precast polyacrylamide gel (Novex Experimental Technology) was used for SSCP-mutation analysis. 50 ng of DNA from tumour or normal tissue was subject to PCR reaction as described above (section 2.5.3). 5 µl PCR product was mixed with stop buffer (95% v/v formamide, 200 mM EDTA pH 8.0, 0.01% w/v bromphenol blue and xylene cyanol FF) and denatured at 95°C for 5 minutes. The denatured DNA sample was loaded into cold 20% precast polyacrylamide gel and the electrophoresis was run at a constant 200 volts for 18 hours with running buffer (1.25X TBE) in circulation at 8°C. The DNA fragments were visualised with silver staining.

### **2.6.6 Silver staining of polyacrylamide gels**

After electrophoresis, polyacrylamide gel was merged in fixation buffer (10% v/v ethanol, 0.5% v/v acetic acid) for 10 minutes with gentle shaking at room temperature, and then merged in 6 mM AgNO<sub>3</sub> for 15 minutes. The gel was rinsed twice with distilled water and then merged in the developer buffer (375 mM NaOH, 0.15% formaldehyde v/v). When the DNA bands were clearly visible, the gel was transferred to stop solution (70 mM Na<sub>2</sub>CO<sub>3</sub>) for 10 minutes. After the silver staining procedure, the polyacrylamide gel was dried under vacuum or sealed in a plastic bag. The gel was then kept in the dark for long-term storage.

## **2.7 Methods for statistical and QTL linkage analysis**

### **2.7.1 The tumour data analysis**

The tumour response was measured in terms of latency, tumour incidence, and papilloma frequency and distribution.

Tumour latency represents the time (weeks) between the start of chemical treatment and the appearance of the first papilloma. It was plotted with the cumulative percentage of mice with papillomas versus different time points (week) after the initiation treatment. Most of the papillomas were developed during the first 20 weeks. After week 25, only a few papillomas were developed. Thus, the time point was cut at week 25.

Tumour incidence represents the number of tumours developed on a mouse. The papilloma incidence was plotted with the average number of papillomas per mouse versus different time points (weeks) after the initiation treatment. The time point was cut at week 30 because by this time point the development of papillomas had reached the peak. Afterwards, the average number of papillomas starts to drop artificially due to the loss of mice with higher number of papillomas. Because of the application of the dichotomous score system, the carcinoma incidence was represented by the cumulative percentage of mice with carcinomas instead of the average number of carcinomas.

The frequency of papillomas indicates the percentage of mice with the same number of papillomas. The distribution of papillomas was the spectrum of the frequency of different number of papillomas.

## **2.7.2 Methods for QTL linkage analysis**

### **2.7.2.1 Single marker analysis**

Single marker analysis tests the association between genotypes and phenotypes at each marker locus. The incidences of mouse skin tumour in each genotypic groups of mice (homozygous and heterozygous) were calculated and the differences between each groups analysed by statistical methods. When the difference is proved statistically significant ( $p < 0.05$ ), the linkage of marker locus to tumour development is established.

The papilloma incidence at week 19 was chosen as the papilloma phenotypic data. In F1BX backcross, the Mann-Whitney U test was used to compare the differences of the average number of papillomas in the homozygous and heterozygous groups. In FVB6F2 cross, a 2x3 contingency of crosstable and the chi-square test were used for the comparison of the differences of the frequency of three genotypic subpopulations

(homozygous for FVB/N, homozygous for C57BL/6J and heterozygous) in the resistant and sensitive groups.

For the analysis of carcinoma data, the Kaplan-Meier test was used to compare the differences of the carcinoma incidence in the two (F1BX) or three (FVB6F2) genotypic subpopulations.

### **2.7.2.2 Interval mapping analysis**

The interval mapping analysis was performed with MapMaker program package. The genetic map was constructed using the MapMaker/Exp program (Lander *et al.*, 1987; Lincoln *et al.*, 1992a). The marker locus order was determined by minimising the number of recombination events among the allele distribution patterns of microsatellite markers across the chromosome. Genetic distances between marker loci were computed using Haldane's mapping function.

The linkage between skin tumour susceptibility (considered a quantitative genetic trait) and genetic loci was detected using MapMaker/QTL program (Lincoln *et al.* 1992b). The extent of linkage was measured by a three point interval analysis formulating a LOD score. The LOD score was the logarithm of the likelihood ratio comparing the hypothesis of linkage (the maximum likelihood estimates of recombination probabilities  $\theta = \text{recombinants } r / \text{total number } n$ ) with the hypothesis of free recombination ( $\theta=0.5$ ),  $\text{LOD}(\theta) = \log_{10} [L(\theta)/L(1/2)]$ . A  $\text{LOD}>1.9$  is widely accepted as indicating of a suggestive linkage, whilst a  $\text{LOD}>3.3$  indicates a significant linkage.

The same as the single marker analysis, the papilloma incidence at week 19 was chosen as the papilloma data. The carcinoma incidence at week 46 was chosen as the carcinoma data. Since it has been shown that the skin tumour data often follows a negative binomial distribution (Drinkwater and Klotz, 1981), the tumour data was transformed with a root square mathematical conversion to improve the fit to a normal distribution, or alternatively was analysed utilising a non-parametric test, for the linkage analysis.

### 2.7.2.3 Multiple stepwise regression analysis

In F1BX, the number of papillomas was regressed against genotypes using linear model

$$y = \mu_0 + \sum_{i=1}^n \beta_i x_i + e$$

where  $y$  is the number of papillomas,  $\mu_0$  is the mean of the papilloma number,  $x_i$  is the vector of linear predictors such as sex, genotype and interactions with other markers at the  $i$ th marker,  $\beta_i$  is the vector of coefficient to be estimated, and  $e$  represents a random environmental deviation. The genotype took the value 1 for homozygous for FVB/N and 0 for heterozygous. Sex took the value 1 for a male and 0 for a female. The interaction of homozygote took the value 1 and that of heterozygote took the value 2.

In FVB6F2, the number of papillomas was regressed against genotypes using logistic model

$$\ln [p/(1-p)] = \sum_{i=1}^n \beta_i x_i + e$$

where  $p$  is the probability of being resistant or sensitive (groups). The genotype took the value 1 for homozygous for FVB/N, 0 for heterozygous, and -1 for homozygous for C57BL/6J. The phenotype took the value 0 for resistant group and -1 for the sensitive group.

The genetic loci involved in carcinoma development was analysed using Cox regression. This is a multivariate analysis method that considers the influence of carcinoma latency and the survival time, in addition to the effects of sex, genotype of the marker and the interactions between markers.



# **CHAPTER 3**

**GENETIC CONTROL OF SKIN  
TUMOUR SUSCEPTIBILITY IN  
(FVB/N x C57BL/6J)F1 x FVB/N  
BACKCROSS MICE**

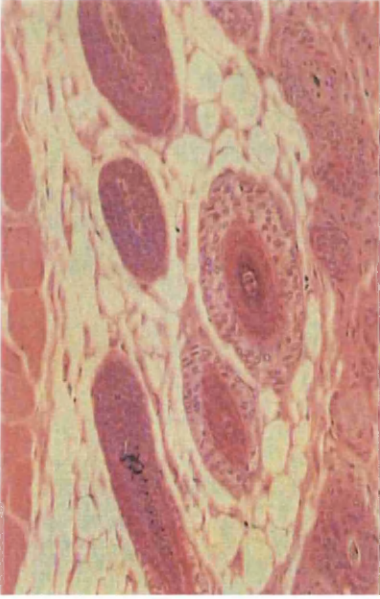
Carcinogenesis in mouse skin has been studied for many years and represents a good model system for the identification of genetic loci predisposing to tumour development. A large number of inbred mouse strains are available that show enormous variation in their susceptibility to the development of skin tumour after exposure to a variety of environmental agents, including chemicals, radiation and viruses (Demant, 1992). Specific crosses between susceptible and resistant mouse strains have consequently resulted in the segregation and recombination of a number of tumour susceptibility or resistance loci that are randomly distributed between different inbred strains. Classical genetic approaches, therefore, can be used to identify and map these predisposition loci. In addition, the development of a large panel of microsatellite markers and major advances in methodology of statistical analysis have further facilitated the linkage mapping of tumour susceptibility genes in the mouse.

The inbred strains FVB/N and C57BL/6J have extreme opposite responses to carcinogen induced skin tumours. FVB/N mice are highly sensitive (Hennings *et al.*, 1993), whereas C57BL/6J mice are very resistant (Naito *et al.*, 1988). Previous experiments have shown large variation in response to carcinogen treatment in their F1 hybrids and F2 offspring, indicating that the tumour susceptibility loci are well segregated between these two parental strains. Thus, FVB/N and C57BL/6J strains have been chosen for study of genetic linkage to tumour predisposition. (FVB/N x C57BL/6J)F1 x FVB/N backcross (F1BX) and (FVB/N x C57BL/6J)F1 intercross (FVB6F2) mice were generated and used in a DMBA-TPA two stage carcinogen experiment. The development of skin tumours was observed and segregation of genetic loci subsequently mapped with mouse microsatellite markers. Statistical analysis was then used to identify the genetic loci linked to the development of skin tumour.

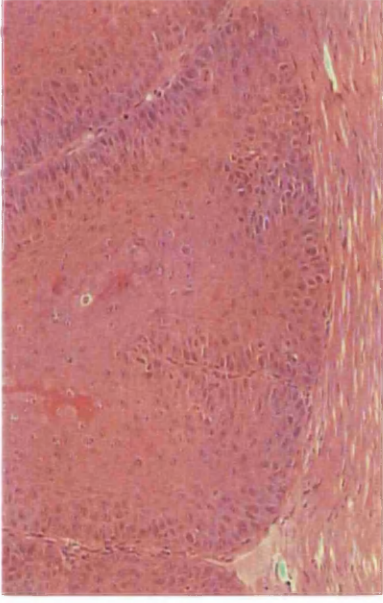
### **3.1 Initiation and progression of mouse skin tumour**

Carcinogenesis experiments were carried out on eight-week old mice. Mice were initiated with one dose of 25 µg DMBA and one week later promoted with 20 nmol TPA twice a week for 20 consecutive weeks. The development of skin tumours was observed and scored weekly for 60 weeks. Papillomas started to appear on the surface of the skin as a small pink wart, which was usually soft and had a diameter of 1-2 mm in

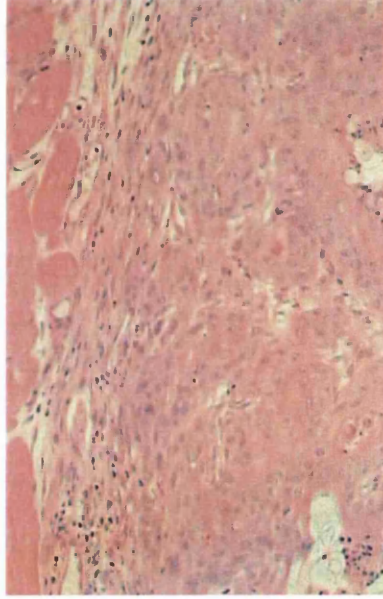
**A**



**B**



**C**



**D**

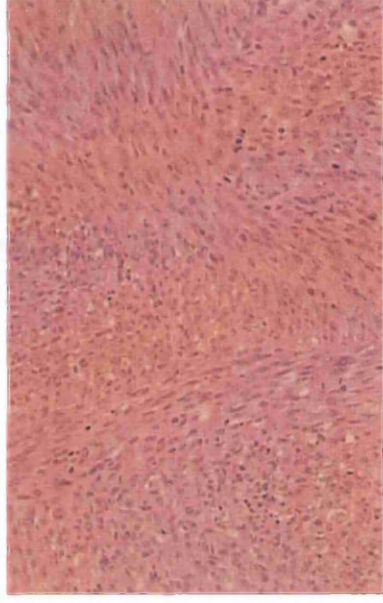


Figure 3.1 Histology of mouse normal skin and skin tumours. A, normal skin (40x); B, papilloma (20x); C, squamous carcinoma (40x); D, spindle carcinoma (20x).

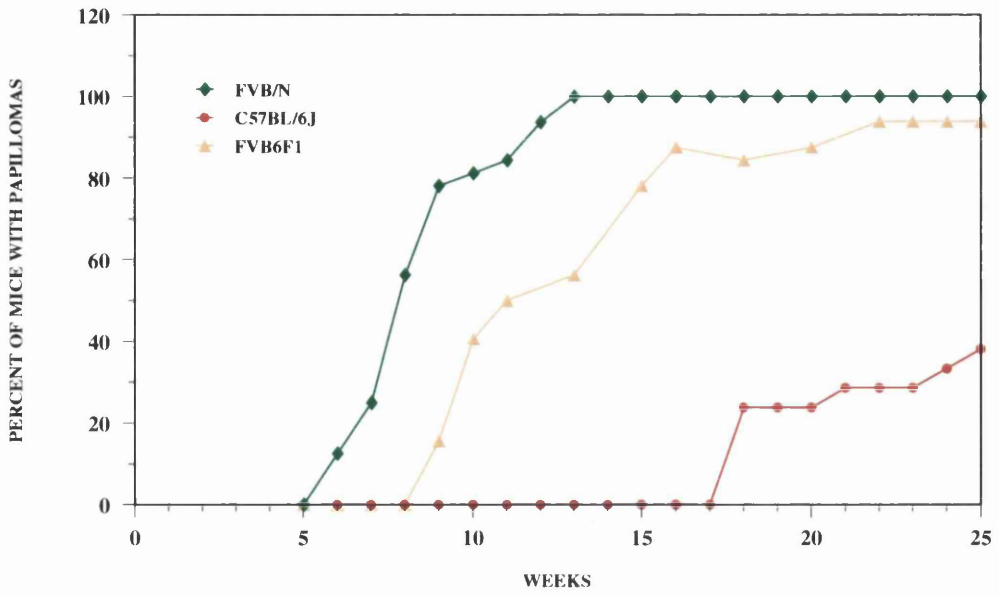
size. Some warts remained soft, others hardened, the apex becoming horny and usually consisting of minute excrescence. Some warts continued to grow and became pedunculated papillomas; others had a broader base and became less protruding flat papillomas. At this stage, the growing papilloma was still a benign tumour with an intact basal layer (Figure 3.1B). Some of the papillomas continued to progress and converted to malignant squamous cell carcinomas with clear changes in morphology (usually one-sided hyperaemic swelling at the base of the tumour), as well as in histology (Figure 3.1C). Occasionally, squamous cell carcinomas progressed further to invasive spindle cell carcinomas (Figure 3.1D).

### **3.2 Papilloma development in FVB/N, C57BL/6J and their F1 hybrids**

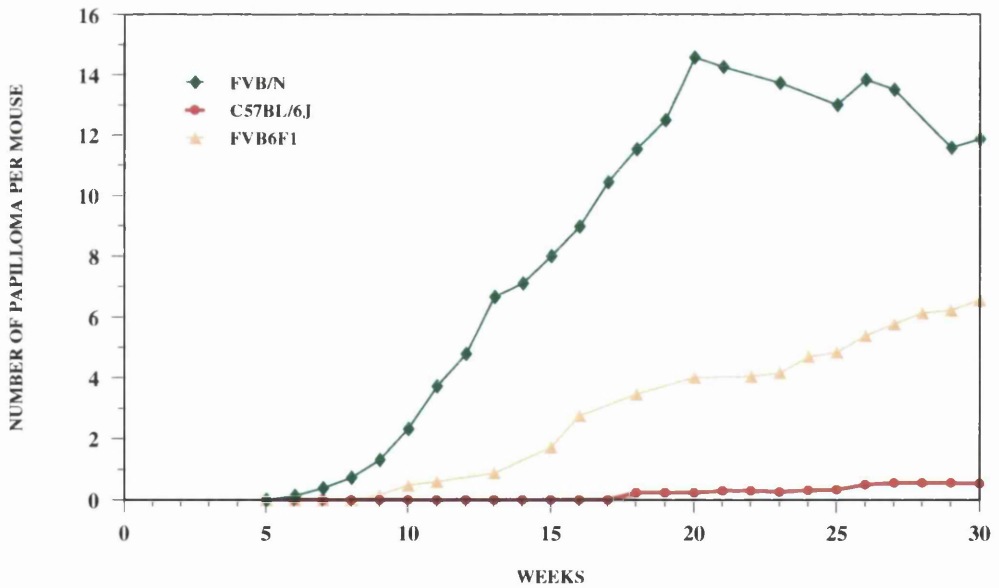
An initial carcinogenesis experiment was carried out on the two parental strains FVB/N and C57BL/6J and their F1 hybrids. 32 female FVB/N, 21 female C57BL/6J and 32 female (FVB/N x C57BL/6J)F1 mice (FVB6F1) were treated with DMBA and TPA as described in 2.2.2. The development of papillomas was observed, the actual papilloma number was recorded weekly for 60 weeks and presented in Table A2 (FVB/N), A3 (C57BL/6J) and A4 (FVB6F1) (see Appendix).

FVB/N mice were highly susceptible to carcinogen treatment. The first papilloma was observed only six weeks after the start of treatment. By week 13, all mice had developed papillomas (Figure 3.2). The papilloma number reached maximum at week 20 with  $14.6 \pm 1.0$  papillomas per mouse (21 surviving mice) (Figure 3.3). Afterwards, the number decreased due to the sacrifice of mice with high yield of papillomas (Section 2.2.2). The profile of papilloma distribution indicated that all FVB/N mice had more than two papillomas, with 23 papillomas as the highest number (Figure 3.4A). Moreover, 42% of mice developed between 12 and 15 papillomas, 23% of mice had more than 16 papillomas and only 6% of mice developed less than 5 papillomas (Figure 3.4A).

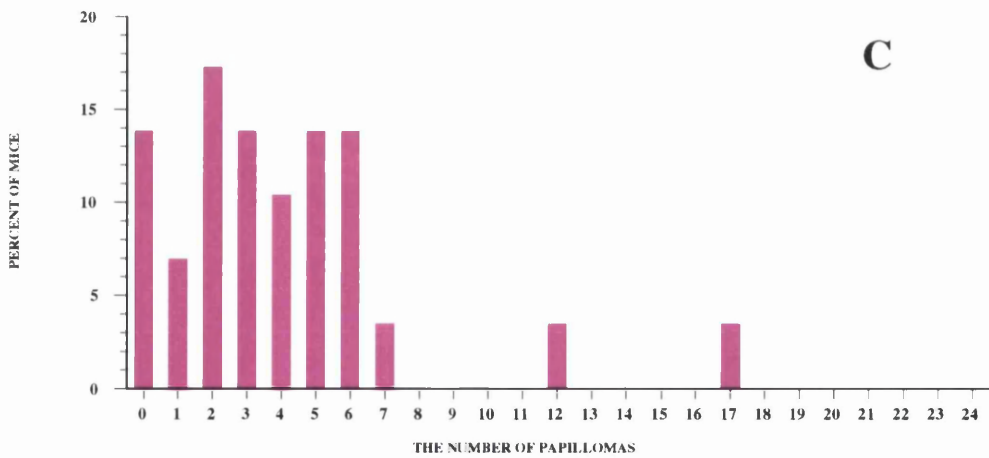
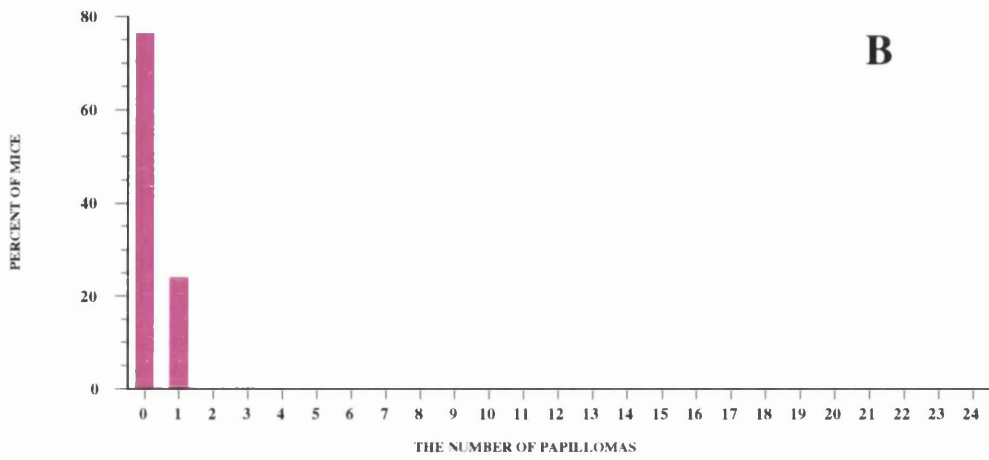
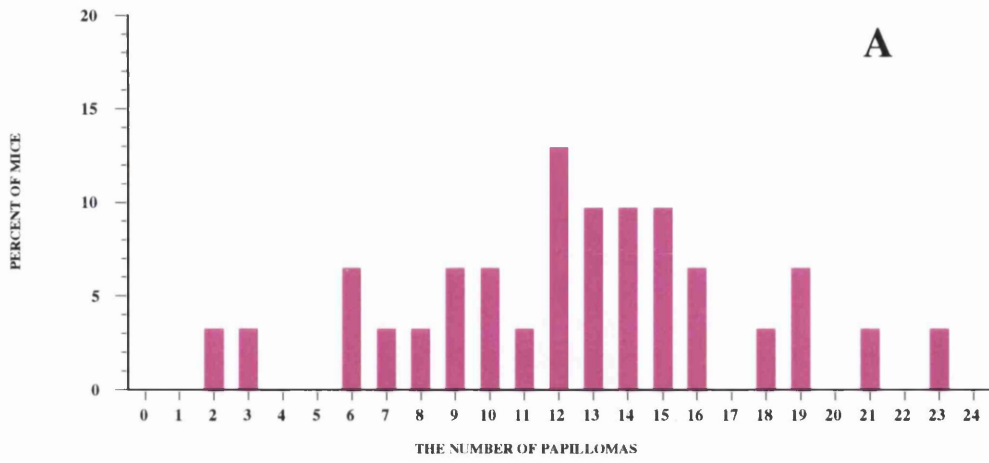
In contrast, C57BL/6J mice displayed a marked resistance to tumour development. The first papilloma did not appear until week 18 (Figure 3.2). Over 76% of mice were completely free of papillomas (Figure 3.2), whilst the remainder developed only a single



**Figure 3.2** The latency of papilloma development in FVB/N, C57BL/6J and FVB6F1 mice. The cumulative percentage of mice with papillomas is plotted versus time (weeks) after initiation.



**Figure 3.3** The average number of papillomas per mouse in FVB/N, C57BL/6J and FVB6F1 mice. Due to sacrifice of mice, the average number of papillomas per mouse showed a decrease at certain time points.



**Figure 3.4** The distribution and frequency of papillomas at week 20 in FVB/N (A), C57BL/6J (B) and FVB6F1 mice (C).

papilloma (Figure 3.4B). The average papilloma number per mouse was  $0.56 \pm 0.15$  (at week 27, 18 surviving mice) (Figure 3.3).

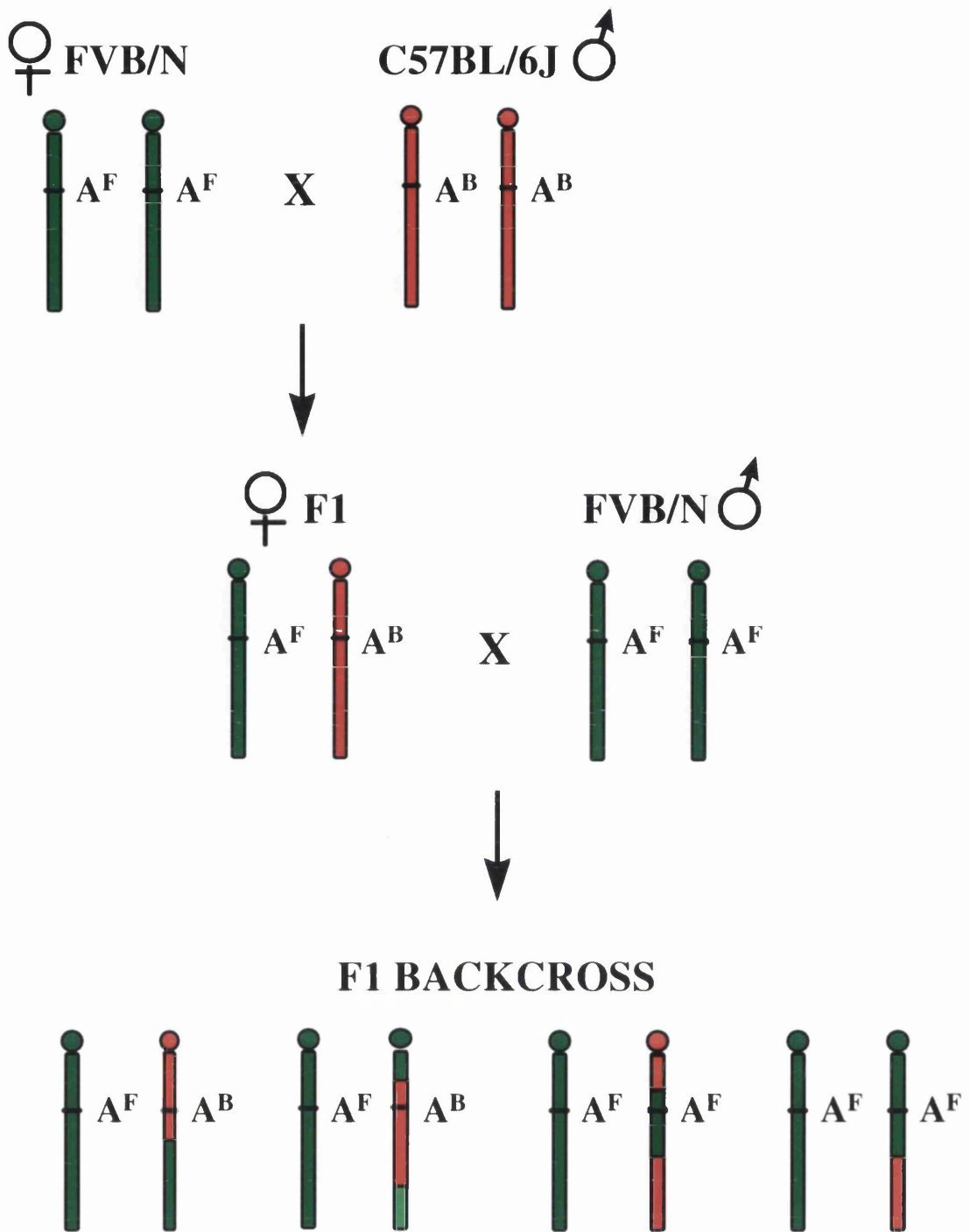
Compared with two progenitor strains, F1 hybrid mice exhibited an intermediate response. With regards to latency of papilloma formation, F1 hybrid mice demonstrated a phenotype closer to that of the FVB/N parent. The first papilloma occurred at week 9, only three weeks later than FVB/N mice but nine weeks earlier than C57BL/6J mice (Figure 3.2). By week 22, the papilloma positive population reached to the highest level at 94 percent (Figure 3.2). In terms of papilloma incidence, however, F1 hybrids displayed an intermediate phenotype (Figure 3.3). The highest papilloma number per mouse was  $6.5 \pm 0.7$  (at week 31, 23 surviving mice), compared with  $14.6 \pm 1.0$  in FVB/N mice and  $0.56 \pm 0.15$  in C57BL/6J mice (Figure 3.3). 69% of F1 hybrids had between 2 and 7 papillomas, whereas only 7% of mice were as sensitive as FVB/N with more than 12 papillomas and 21% of mice behaved like C57BL/6J with less than one papilloma (Figure 3.4C).

Taken together, these results clearly demonstrate the relative sensitivity of the FVB/N strain and resistance of the C57BL/6J strain to DMBA-TPA induced skin tumours. Analysis of papilloma development in F1 hybrids indicated that the latency of papilloma was inherited as an incomplete dominant trait. The papilloma incidence, however, was inherited in a co-dominant manner. This result suggests that the papilloma latency and incidence are likely to be under different genetic controls.

### **3.3 Skin tumour development in (FVB/N x C57BL/6J)F1 x FVB/N backcross mice**

#### **3.3.1 Mouse breeding strategy**

The breeding scheme for (FVB/N x C57BL/6J)F1 x FVB/N backcross (F1BX) mice was illustrated in Figure 3.5. In brief, four mating pairs were set up for the generation of F1 hybrids (FVB/N x C57BL/6J and their reciprocal C57BL/6J x FVB/N). Eight female FVB6F1 hybrids (four from each group) were then mated with male FVB/N mice, the sensitive parental strain, to produce the F1BX mice. A total of 34 female and 31 male mice were produced and subsequently used for the carcinogenesis experiment.



**Figure 3.5** Breeding schemes used to generate (FVB/N x C57BL/6J)F1 x FVB/N backcross mice (F1BX). For comparison, only one pair of chromosomes (i.e. chromosome 7) are shown for each parent. FVB/N alleles are indicated by green; C57BL/6J alleles are indicated by red. Note that there are two different subtypes of genotypes, homozygous to FVB/N allele and heterozygous, in F1BX mice.



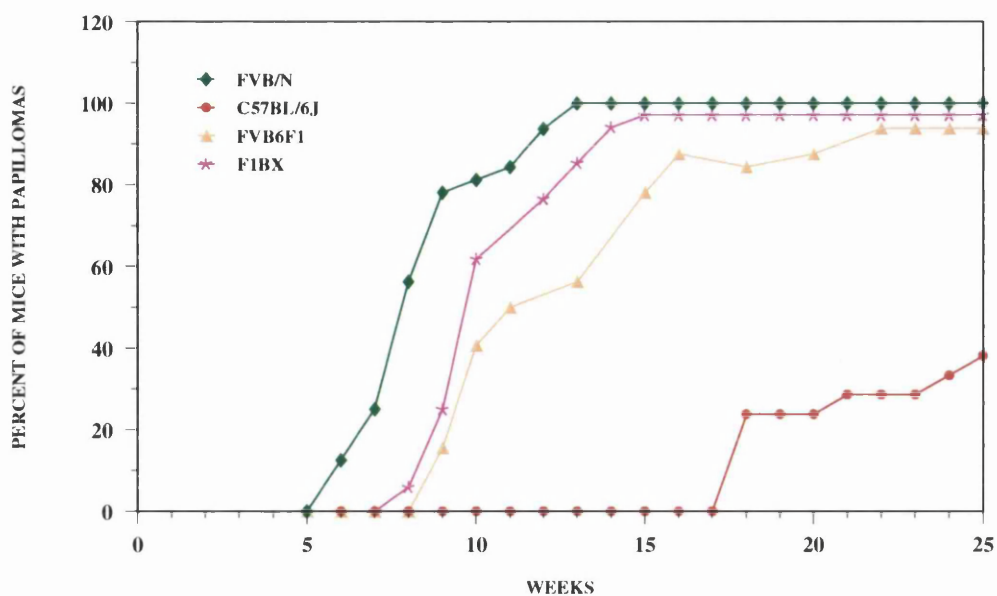
### 3.3.2 Tumour latency

Carcinogenesis experiments were carried out on a total of 65 F1BX mice as described in Section 2.2.2. The development of papillomas was observed and the papilloma incidence was recorded once a week over a 60 weeks period.

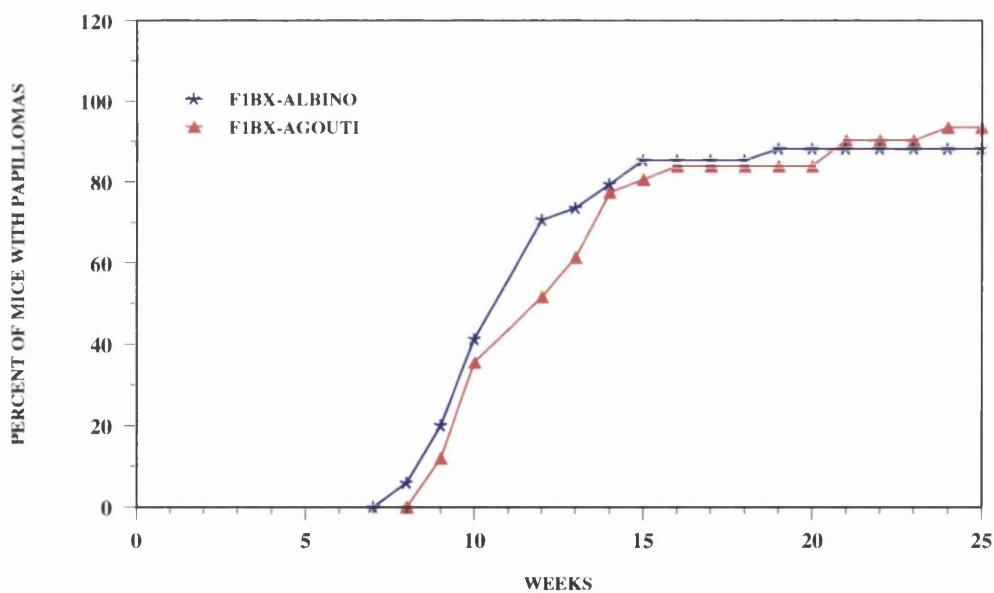
The female F1BX mice had a tumour latency intermediate to that of the FVB/N and F1 hybrid mice (Figure 3.6). The first papilloma occurred at week 8 in F1BX mice, one week earlier than in F1 hybrids but two weeks later than in FVB/N parental mice. The tumour positive population in F1BX increased very quickly and reached the maximum within eight weeks. A total of 97% of female F1BX mice, compared with 94% in F1 hybrids and 100% in FVB/N mice developed skin tumours during the 60 weeks observation period. Thus, it seems that the increased genetic composition of FVB/N strain in F1BX mice did reduce the latency of tumour formation. This result implies that susceptibility to tumour latency in F1BX is inherited as an incomplete dominant trait.

Previous data has suggested that the *c* locus on chromosome 7, where the coat colour associated gene *tyrosinase* is located, is linked to the development of mouse skin tumour (Nagase *et al.*, 1995). Therefore, analysis was also carried out to examine the linkage between coat colour and tumour susceptibility. The FVB/N mice are albino, the C57BL/6J mice are black, and the F1 hybrids are agouti in colour. The F1BX mice, therefore, can have two different coat colours, albino (homozygous for FVB/N allele at *c* locus) and agouti (heterozygous at *c* locus). Analysis of coat colour versus tumour latency indicated that there was little difference between agouti and albino F1BX mice (Figure 3.7). The first tumour in agouti mice appeared at week 9, just one week later than in albino mice. Their tumour positive populations increased at similar rates and reached the maximum at similar time points. A total of 94% agouti mice developed skin tumour, slightly higher than in albino mice which was 88%. These results suggest that there is no association between tumour latency and coat colour in F1BX mice.

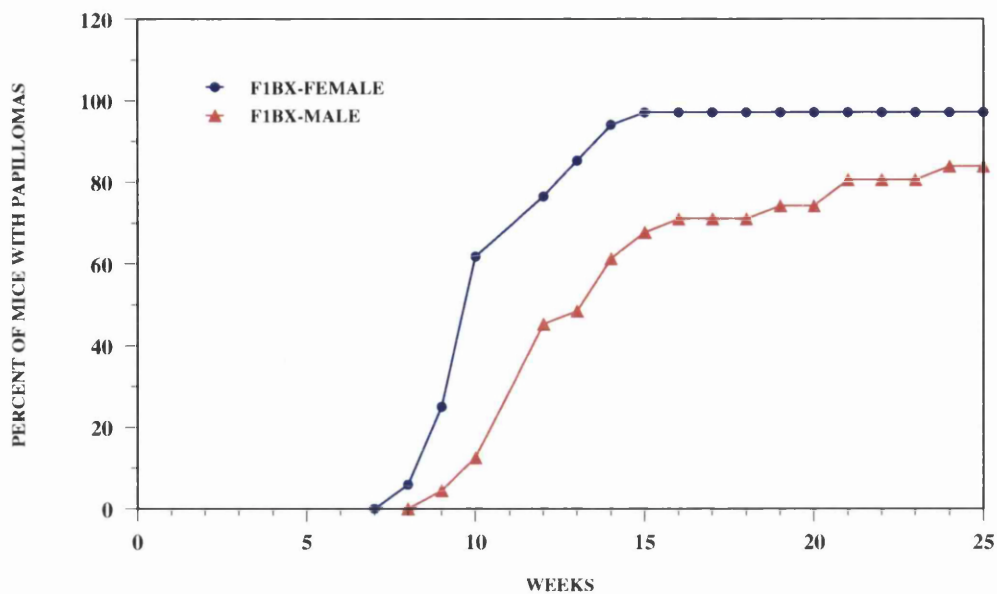
However, the tumour latency was associated with gender factor. Female F1BX mice were more sensitive to tumour induction than the males (Figure 3.8). Although the first tumour in female mice occurred just one week earlier than in male mice, the tumour positive population of female mice increased more rapidly than male mice. At week 15,



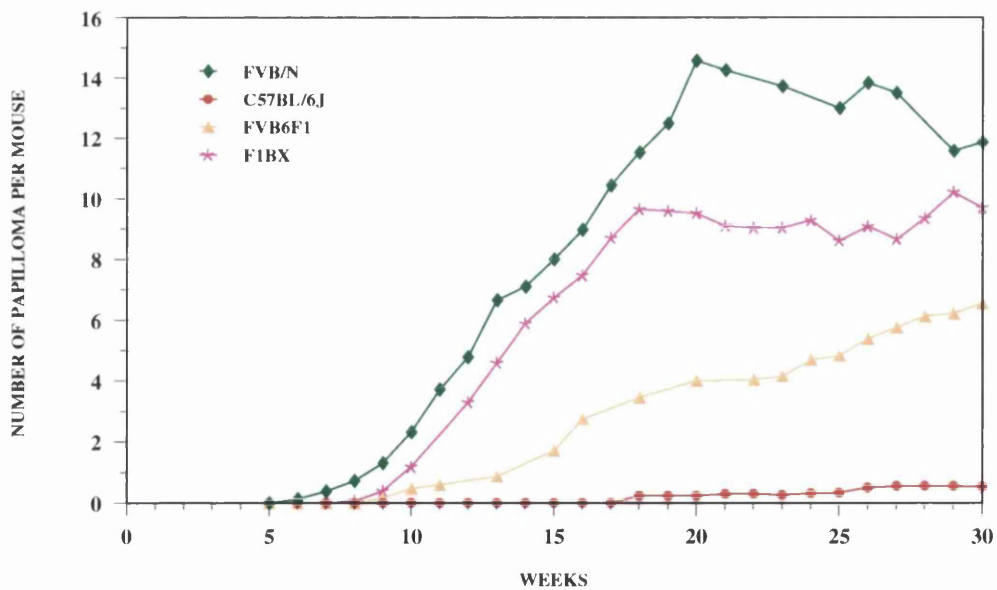
**Figure 3.6** The tumour latency in FVB/N, C57BL/6J, FVB6F1 and F1BX mice. Only data of female F1BX mice were used because all the parental and F1 mice were female. The cumulative percentage of mice with skin tumour is plotted versus time (weeks) after initiation.



**Figure 3.7** Comparison of tumour latency of agouti and albino coat colour groups in F1BX mice. The cumulative percentage of mice with skin tumour is plotted versus time (weeks) after initiation.



**Figure 3.8** Comparison of tumour latency between female and male F1BX mice. The cumulative percentage of mice with skin tumour is plotted versus time (weeks) after initiation.



**Figure 3.9** The average number of papillomas per mouse in FVB/N, C57BL/6J, FVB6F1 and F1BX mice. Only data of female F1BX mice were used because all the parental and F1 mice were female. Due to sacrifice of mice with higher number of papillomas, the average numbers of papilloma showed a decrease at certain time points.

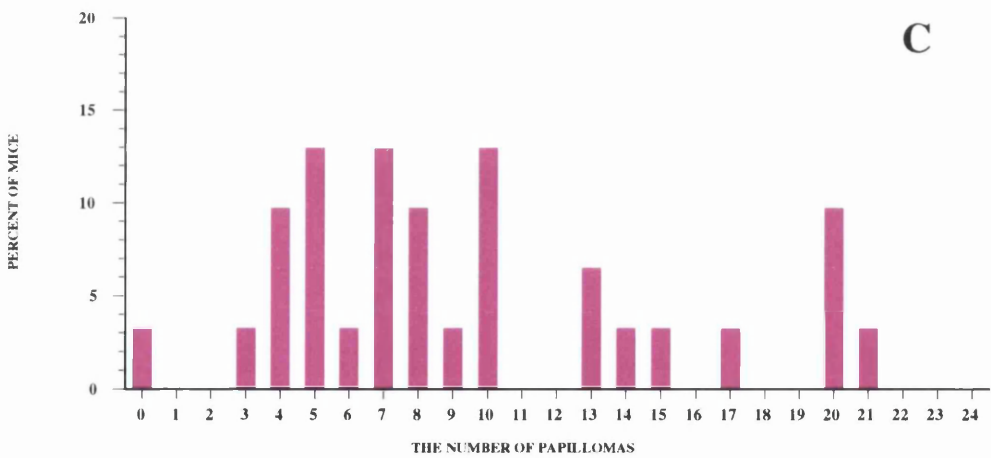
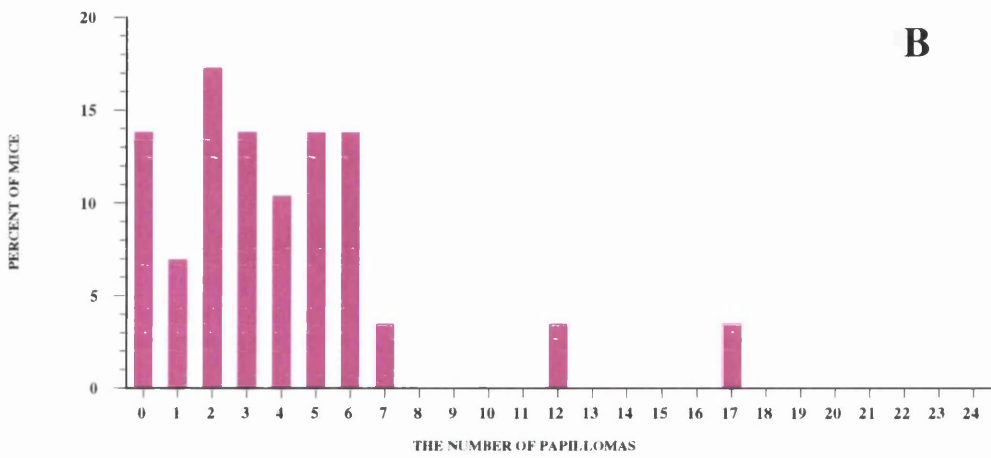
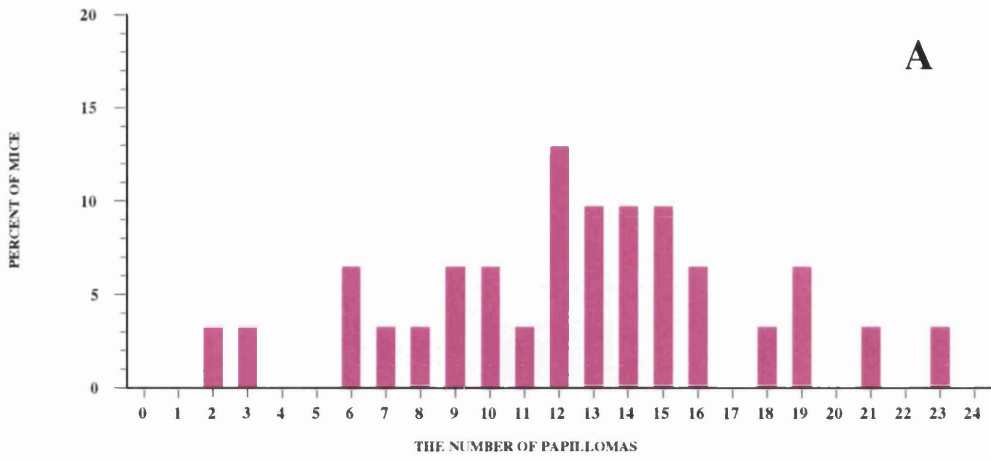
more than 97% of female mice had already developed skin tumours, while only 68% of male mice were tumour positive. Even though the figure eventually reached 84% in male mice at week 24, this was still lower than that in the females, indicating that that gender factor did have a significant influence on tumour latency.

### 3.3.3 Papilloma incidence

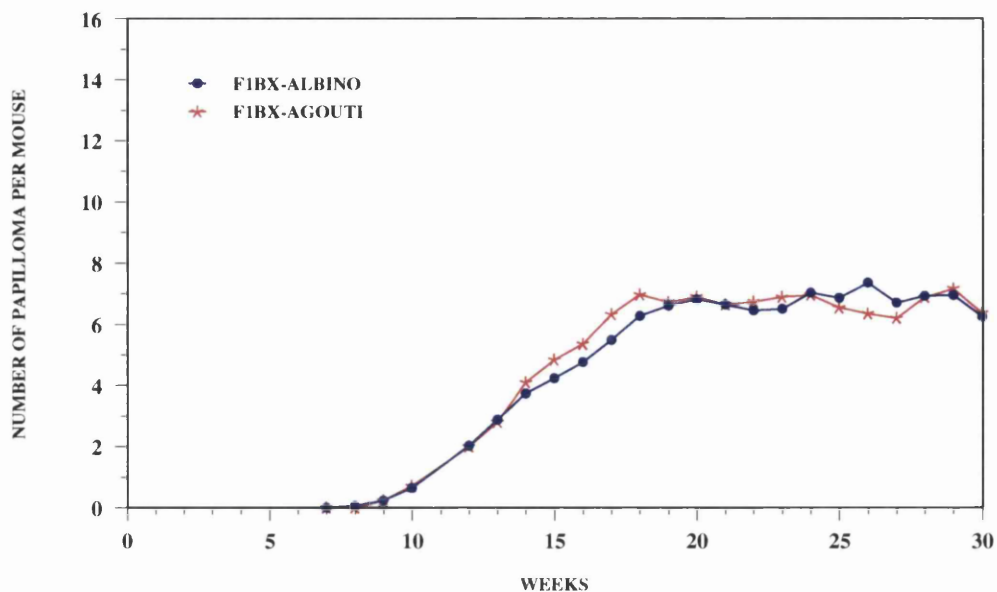
The actual number of papillomas for each individual F1BX mouse was scored once a week for 60 weeks. The data presented here is the calculated papilloma incidence whilst the raw data is presented in Table A5 (see Appendix).

As shown in Figure 3.9, the female F1BX mice had an intermediate phenotype, the papilloma incidence was higher than the F1 hybrids but lower than the FVB/N parental mice. At week 30, the average number of papilloma in F1BX was  $9.7 \pm 0.5$ , compared to  $6.5 \pm 0.7$  in F1 and  $11.9 \pm 0.7$  in FVB/N mice. But, before week 18 the response of F1BX mice were so close to the parental FVB/N mice that the average papilloma numbers in these two groups increased at a similar rate. However analysis of papilloma distribution revealed that they were actually quite different (Figure 3.10). In F1BX more than 50% of mice had between 4 to 10 papillomas, while the majority of FVB/N mice had more than 12 papillomas. Compared to F1 and FVB/N mice, it is evident that as the genomic makeup of sensitive parent FVB/N increased the papilloma incidence in F1BX mice increased as well. This indicates that the development of papillomas in F1BX is transmitted in a co-dominant manner.

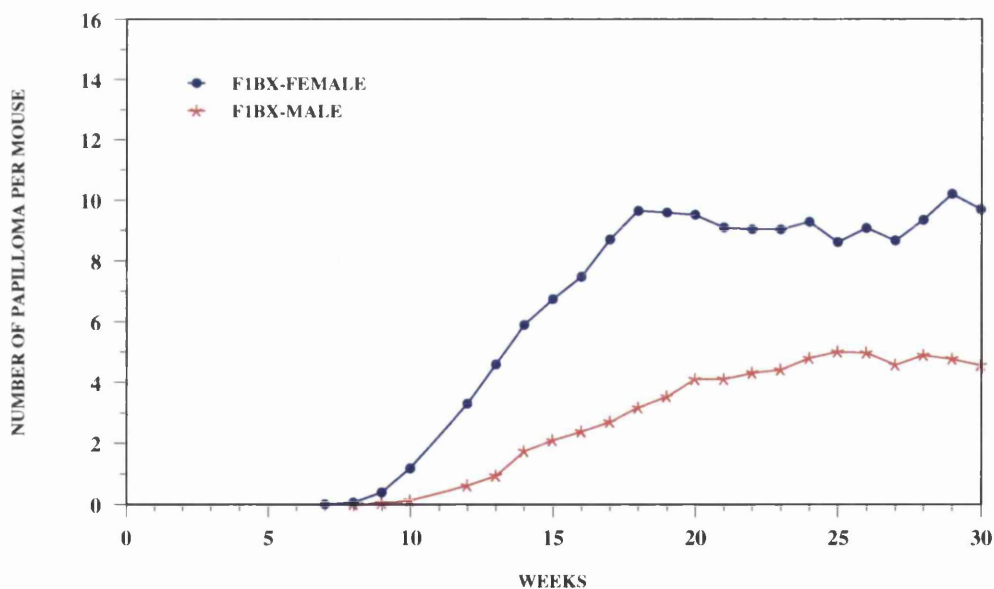
The association of papilloma incidence with coat colour and gender was also examined. Analysis of coat colour indicated that there was little difference between agouti and albino F1BX mice (Figure 3.11). Their profiles nearly overlapped. However, a gender difference was detected. The female F1BX mice were more sensitive to tumour induction than the males. At week 20, the average papilloma number was  $9.5 \pm 1.0$  in female mice, twice that of the male mice ( $4.1 \pm 0.8$ ) (Figure 3.12). Detailed analysis suggested that the gender differences were more significant in agouti mice ( $p=0.0002$ ) than in albino mice ( $p=0.1$ ). The agouti male mice were most resistant to carcinogenesis with a mean of  $2.9 \pm 0.9$  papillomas (15 survival mice), whilst the agouti female mice were most susceptible ( $11.1 \pm 1.6$  papillomas per mouse, 14 survival mice) (data not



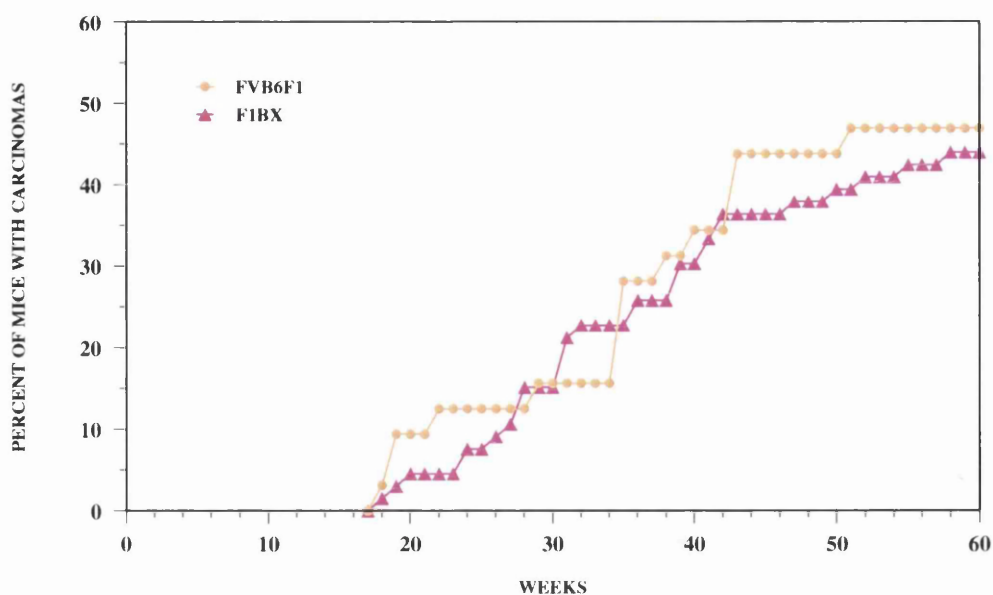
**Figure 3.10** The distribution and frequency of papillomas at week 20 in female FVB/N (A), FVB6F1 (B), and F1BX (C) mice.



**Figure 3.11** Comparison of the average number of papillomas per mouse between F1BX mice with agouti and albino coat colours. Because of the loss of mice, the average number of papillomas decreased at certain time points.



**Figure 3.12** Comparison of the average number of papillomas per mouse between male and female F1BX mice. Due to the loss of mice, the average number of papillomas decreased at certain time points.



**Figure 3.13** Carcinoma incidence in FVB6F1 and F1BX mice. Because carcinomas were scored as a dichotomous trait, the percentage of mice with carcinomas instead of the average number of carcinomas was used.

shown), indicating that the papilloma incidence was influenced by gender, as well as coat colour related genetic loci.

### 3.3.4 Carcinoma incidence

As indicated in chapter 2 (section 2.2.2), a dichotomous system was applied in scoring carcinomas regardless of the actual number of carcinomas that each individual mouse developed. Therefore, the carcinoma incidence in F1BX mice was presented as the number of carcinoma positive mice over time points.

The carcinoma incidence in F1BX mice was very similar to that in F1 hybrids (Figure 3.13). In both groups, the first carcinoma was recorded at week 18 and their carcinoma positive populations increased at a similar rate. Thus, despite a reduced C57BL/6J:FVB/N (resistance versus sensitivity) ratio in the genomic makeup, the altered genomic composition had little effect on the carcinoma incidence, implying that the susceptibility to carcinomas was inherited as an dominant trait.

The association of carcinoma development with coat colour and gender was also examined. Significant difference in the carcinoma incidence was detected between albino and agouti mice ( $p=0.005$ ) (Figure 3.14). Around 51.4% of albino mice developed carcinomas, compared with 38.7% in agouti mice, suggesting that the carcinoma development was linked to coat colour. However, no gender difference was observed in carcinoma formation. Considering the marked gender difference in papilloma incidence, it was surprising that the carcinoma incidence was so similar in female and male F1BX mice (41.2% and 46.9%, respectively). These results imply that the development of papilloma and carcinoma are likely affected by different genetic loci. While the susceptibility to papillomas was influenced by gender factor, the susceptibility to carcinomas was inherited as an autosomal trait.

### **3.4 Genomic mapping of F1 backcross mice**

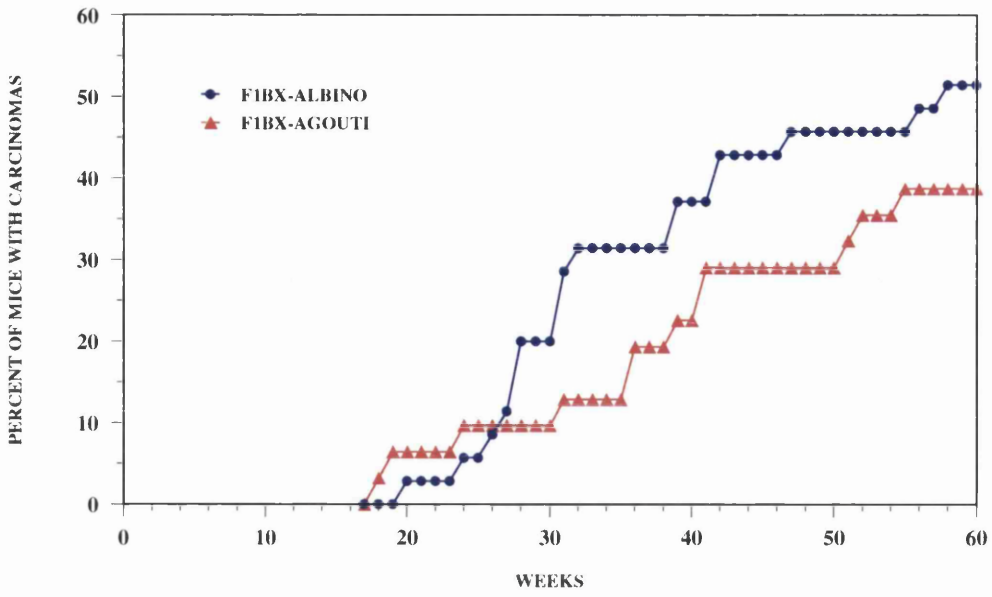
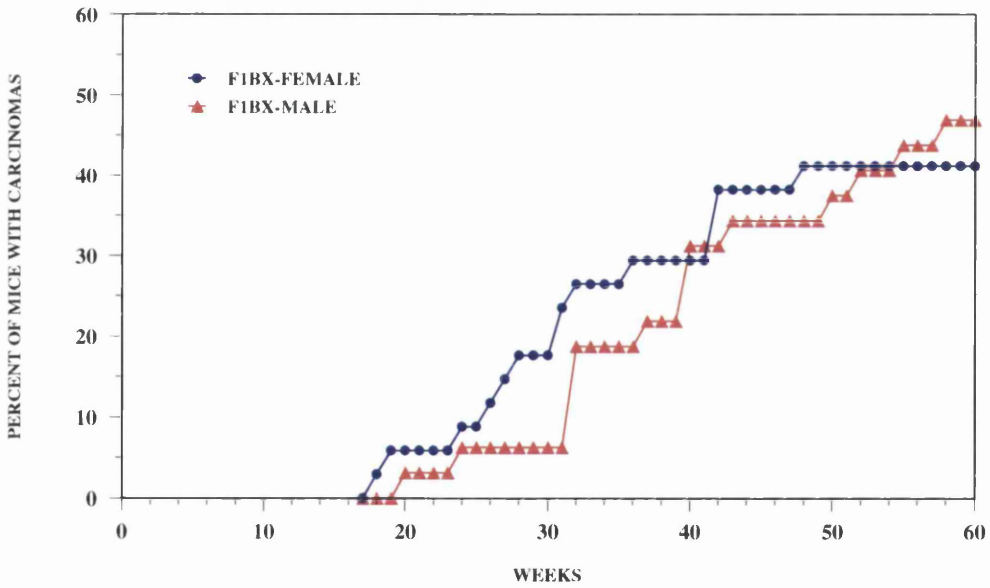
#### **3.4.1 Polymorphism of microsatellite markers**

As one of the most widely studied strains, many microsatellite loci have been characterised for the C57BL/6J strain. However, little information is available for the FVB/N strain. Thus, it was necessary to obtain a panel of microsatellite markers that are polymorphic between FVB/N and C57BL/6J strains and distributed throughout the genome. Microsatellite markers that are polymorphic for most of the inbred strains were selected from the mouse genome database and the size of the microsatellite PCR products for the FVB/N and C57BL/6J strains were determined.

An example of a test PCR result is shown in Figure 3.15. Four markers, D4Mit59, D4Mit61, D4Mit48 and D4Mit63, were tested using genomic DNA isolated from FVB/N, C57BL/6J and F1 hybrid mice. Size differences between two parental PCR products were detected at markers D4Mit59 and D4Mit48. Therefore, these two markers were polymorphic and suitable for genomic mapping.

In total, more than 700 mouse microsatellite markers were examined. 17% were found to be polymorphic between the FVB/N and C57BL/6J strains using high resolution agarose gels capable of separating DNA fragment with more than 6bp difference. When polyacrylamide gels which separate DNA fragments with 1bp difference were used the



**A****B**

**Figure 3.14** Comparison of carcinoma development by coat colour (A) and gender (B) in FIBX mice.

percentage of polymorphic markers increased to 41%. These polymorphic markers were well distributed throughout all 19 autosomes as well as X-chromosome, and subsequently used for genomic mapping (see Table A1).

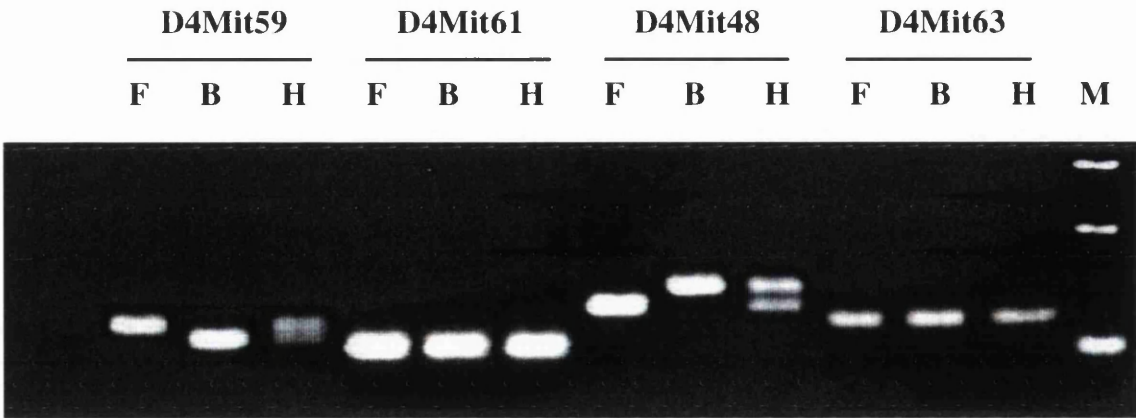
### **3.4.2 Genomic mapping of F1 backcross mice**

Genomic mapping was carried out to examine the genotype of individual F1BX mice. Theoretically, there are only two genotypes of F1BX mice. Homozygous mice have two copies of FVB/N allele, therefore produce only one PCR product; Heterozygous mice have one FVB/N allele and one C57BL/6J allele, thus two different sizes of PCR products will be amplified. Examples of genotyping with markers D15Mit26 and D11Mit288 are presented in Figure 3.16. At locus D15Mit26, 10 mice showed single PCR band, therefore were homozygous; the other 6 mice displayed two PCR bands, thus were heterozygous. Similarly, at locus D11Mit288, 15 mice were homozygous with two lower PCR bands and 11 mice were heterozygous with four PCR bands.

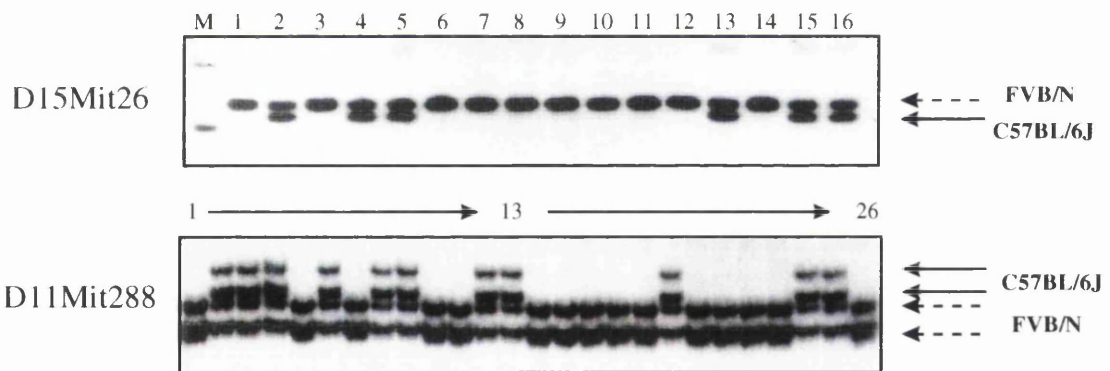
Genomic mapping was performed in two steps. First, 45 F1BX mice were mapped with 150 markers and the data analysed to detect markers showing association with skin tumours. Then, the chromosomes with potential linkage were mapped more intensively. A total of 204 microsatellite markers were used in the genomic mapping. These markers constructed a genetic map spanning 1314.0 centimorgans (cM) and covered 96.2% of the genome, with an average distance of 8.7 cM between markers. The result of more than 20,000 genotypes of F1BX mice are listed in Table A6 (see Appendix).

## **3.5 Genetic linkage of susceptibility loci to skin tumour in F1 backcross mice**

Genetic linkage to skin tumour formation was analysed at three levels using three different methods. The linkage analysis started with single marker analysis which tests the association between phenotype and the genotype of each individual marker locus. Interval mapping is the second level of linkage analysis (Lander and Botstein, 1989). This method evaluates the association between phenotype and the expected contribution of a target QTL at multiple analysis point between each pair of adjacent marker loci. It



**Figure 3.15** PCR analysis of polymorphic microsatellite markers on chromosome 4 with genomic DNA isolated from two parental strains of mice, FVB/N (F) and C57BL/6J (B), and their FVB6F1 hybrid (H). M: 100bp ladder DNA molecular weight marker.



**Figure 3.16** Microsatellite PCR analysis of genotypes of F1BX mice. The gel shows representative examples of genetic mapping in F1BX mice. There are two subtypes of genotypes, homozygous with two FVB/N alleles (indicated by dashed arrow) and heterozygous with one FVB/N allele and one C57BL/6J allele (indicated by solid arrow). M: 100 bp ladder DNA molecular weight marker. For marker D15Mit26, PCR products were examined by 4% NuSeive agarose gel; for marker D11Mit288, PCR products were separated by 6% polyacrylamide gel, thus multiple PCR bands were detected.

require prior construction of a marker genetic map. The third level of analysis, multi-locus regression, estimates the possibility of a target QTL at multiple analysis points across each inter-marker interval, including the effect of their interactions.

### **3.5.1 Single marker linkage analysis**

Single marker analysis tests the association between phenotype and the genotype of marker locus. Since this test considers each marker locus separately, it does not require the marker loci to be mapped relative to each other. The incidences of mouse skin tumours in two genotypic groups mice (homozygous and heterozygous) were calculated and the differences analysed by statistical methods. When a statistically significant difference is detected, the linkage of marker locus to tumour development is established.

#### **3.5.1.1 Genetic linkage of susceptibility loci to papilloma development**

The average papilloma number was calculated in the two groups of F1BX mice (homozygous and heterozygous) for each marker. The difference between these two groups was then compared using the Mann-Whitney U test with 95% confidence interval.

The papilloma development was found to link to markers on chromosomes 4, 6, 9, and 12 (Table 3.1). The most significant linkage was detected in the central and distal region of chromosome 4 between D4MIT45 and D4MIT42, particularly at D4Mit126 ( $p=0.00025$ ), D4MIT205, D4MIT14, D4MIT33 and D4MIT42 ( $p=0.004$ ). At these loci, heterozygous mice had lower papilloma incidence than mice homozygous for FVB/N allele, indicating that the C57BL/6J allele functioned as a resistant allele to papilloma formation. Significant association with papilloma formation was also detected in the central region of chromosome 9, especially at loci D9MIT269 ( $p=0.005$ ) and D9MIT196 ( $p=0.008$ ). Mice heterozygous at these loci had fewer papillomas than homozygous mice, suggesting that the resistance was inherited from C57BL/6J allele. Similarly, C57BL/6J allele on the proximal (D12Mit 10 and D12Nds11) and central regions of chromosome 12 (between D12Mit203 and D12Mit231) was associated with resistant to papilloma formation. In contrast, the presence of C57BL/6J alleles on chromosome 6 was linked to increased papilloma susceptibility. Mice heterozygous at loci D6Mit59 and D6Mit14 developed more papillomas than homozygous mice. In addition, the

Marker	Locus (cM)	Number of mice		Number of papillomas per mouse		<i>p</i> (M-W)
		BF	FF	BF	FF	
D4MIT45	42.50	29	36	4.59	8.06	0.026
D4MIT175	49.60	29	36	4.76	7.92	0.036
D4NDS2	55.60	29	36	4.55	8.08	0.029
D4MIT12	57.50	29	36	4.55	8.08	0.029
D4MIT40	59.00	32	33	4.88	8.09	0.016
D4MIT16	59.10	30	35	4.60	8.14	0.019
D4MIT72	59.90	30	35	4.60	8.14	0.019
D4MIT203	60.00	29	36	4.59	8.06	0.033
D4MIT148	66.00	27	38	4.70	7.79	0.024
D4MIT170	66.60	27	38	4.63	7.84	0.021
<b>D4MIT126</b>	<b>71.00</b>	<b>28</b>	<b>37</b>	<b>4.79</b>	<b>7.81</b>	<b>0.00025</b>
<b>D4MIT205</b>	<b>76.60</b>	<b>31</b>	<b>34</b>	<b>5.68</b>	<b>7.26</b>	<b>0.004</b>
<b>D4MIT14</b>	<b>78.50</b>	<b>31</b>	<b>34</b>	<b>5.68</b>	<b>7.26</b>	<b>0.004</b>
<b>D4MIT33</b>	<b>79.00</b>	<b>31</b>	<b>34</b>	<b>5.68</b>	<b>7.26</b>	<b>0.004</b>
<b>D4MIT42</b>	<b>81.00</b>	<b>31</b>	<b>34</b>	<b>5.68</b>	<b>7.26</b>	<b>0.004</b>
D9MIT163	33.00	27	38	5.63	7.13	0.022
D9MIT74	41.00	28	37	5.71	7.11	0.022
<b>D9MIT269</b>	<b>43.00</b>	<b>28</b>	<b>37</b>	<b>5.25</b>	<b>7.46</b>	<b>0.005</b>
<b>D9MIT196</b>	<b>48.00</b>	<b>30</b>	<b>34</b>	<b>5.60</b>	<b>7.29</b>	<b>0.008</b>
D9MIT182	55.00	30	35	5.67	7.23	0.027
D9MIT19	71.00	32	33	5.56	7.42	0.020
D12MIT10	6.00	17	26	5.29	7.27	0.033
D12NDS11	6.00	26	39	4.77	7.67	0.019
D12MIT20	37.00	23	42	5.22	7.21	0.015
D12MIT23	48.00	26	39	5.81	6.97	0.043
D6MIT59	67.00	32	33	7.28	5.76	0.048
<b>D6MIT14</b>	<b>74.00</b>	<b>29</b>	<b>36</b>	<b>7.90</b>	<b>5.39</b>	<b>0.010</b>
SEX		<b>Male</b>	<b>Female</b>	<b>Male</b>	<b>Female</b>	$1.2 \times 10^{-6}$
		31	34	3.06	9.65	

**Table 3.1** The result of single marker linkage analysis of susceptibility to papilloma development in F1BX mice. Locations of marker locus in cM are the relative map locations from the centromere and map distances are determined from the report of mouse chromosome committee in the mouse genome database. BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N allele. The number of mice and average number of papillomas for each genotype were compared and analysed with Mann-Whitney U test with significant level at  $p=0.05$ . Of 204 markers analysed only markers with  $p$  value less than 0.05 were listed. Markers with  $p$  value less than 0.01 were highlighted and considered as the location of genes that influence susceptibility to the development of papillomas.

gender difference in papilloma formation was indeed significant ( $p=1.2\times 10^{-6}$ ). Female mice had three times the number of papillomas than male mice.

Taken together, the inheritance of C57BL/6J alleles on chromosome 4, 9 and 12 was linked to resistance to papilloma formation, however the presence of C57BL/6J alleles on chromosome 6 was associated with increased papilloma incidence. The segregation of these counter-acting C57BL/6J alleles in F1BX mice, therefore, resulted in variation of the papilloma incidence in individual F1BX mice. The fact that the F1BX mice exhibited an intermediate phenotype between the two parental strains implies that none of these loci has a prominent effects on tumour development. Moreover, consistent with the earlier analysis, papilloma development in F1BX mice was also associated with gender.

### **3.5.1.2 Genetic linkage of susceptibility loci to carcinoma development**

The method used to analyse genetic linkage to carcinoma development differed from that used to analyse papilloma formation because a dichotomous trait rather than a linear related trait was used to score carcinomas. Differences in carcinoma incidences between two genotypic groups was compared using the Kaplan-Meier test with 95% confidence intervals.

The carcinoma development in F1BX mice was linked to markers on chromosomes 3, 7, 9 and 10 (Table 3.2). The most significant linkage was detected at loci on the central region of chromosome 9 between D9MIT97 and D9MIT196, particularly at D9Mit163 ( $p=0.008$ ), D9MIT74 ( $p=0.009$ ), and D9MIT269 ( $p=0.0002$ ). Mice heterozygous at these loci had lower carcinoma incidence than homozygous mice, indicating that the C57BL/6J alleles carried loci resistant to carcinoma formation. Significant linkage was also obtained at loci D3MIT46 ( $p=0.006$ ), D10MIT134 ( $p=0.008$ ), as well as the central region of chromosome 7 (between loci D7Mit83 and D7Mit220). Mice that inherited the C57BL/6J alleles at any of these loci developed fewer carcinomas. Hence, the presence of C57BL/6J alleles at loci on chromosome 3, 7, 9, and 10 all conferred resistance to carcinoma development in F1BX mice.

Marker	Locus (cM)	Percentage of mice with carcinomas		<i>p</i> (K-M)
		BF	FF	
<b>D3MIT46</b>	<b>13.80</b>	<b>44.1</b>	<b>74.2</b>	<b>0.006</b>
D7MIT83	26.50	48.4	67.6	0.016
D7MIT319	37.00	51.6	67.6	0.045
D7MIT32	46.40	48.4	67.6	0.045
D7MIT96	50.30	46.7	68.6	0.019
D7MIT281	52.00	47.6	70.8	0.015
D7MIT220	52.40	47.6	70.8	0.015
D9MIT97	29.00	52.4	66.7	0.025
<b>D9MIT163</b>	<b>33.00</b>	<b>48.1</b>	<b>65.8</b>	<b>0.008</b>
D9MIT31	35.00	52.4	66.7	0.025
D9MIT259	38.00	54.5	65.2	0.042
<b>D9MIT74</b>	<b>41.00</b>	<b>50.0</b>	<b>64.9</b>	<b>0.009</b>
<b>D9MIT269</b>	<b>43.00</b>	<b>46.4</b>	<b>67.6</b>	<b>0.0002</b>
D9MIT196	48.00	50.0	65.7	0.020
<b>D10MIT134</b>	<b>59.00</b>	<b>55.6</b>	<b>62.1</b>	<b>0.008</b>
		Agouti	Albino	
COAT COLOUR		48.4	67.6	0.019
		Male	Female	
SEX		64.5	52.9	0.028

**Table 3.2** The result of single marker linkage analysis of susceptibility to carcinoma development in F1BX mice. Locations of marker locus in cM are the relative map locations from the centromere and map distances are determined from the report of mouse chromosome committee in the mouse genome database. BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N allele. The number of mice with and without carcinomas for each genotype were compared using Kaplan-Meier test with significant level of  $p=0.05$ . Of 204 markers analysed only markers with  $p$  value less than 0.05 are listed. Markers with  $p$  value less than 0.01 are highlighted and considered as the location of genes that influence susceptibility to the development of carcinomas.

In addition, the association of carcinoma incidence with coat colour and gender in F1BX mice was also examined. Consistent with earlier analysis, albino mice were more sensitive than agouti mice ( $p=0.019$ ), and male mice had higher carcinoma incidence than female mice ( $p=0.028$ ) (Table 3.2). Thus, carcinoma formation was also linked to the coat colour and gender.

### **3.5.2 Interval mapping linkage analysis**

The most widely used interval mapping method is MapMaker, a computer program package designed specially for genetic linkage detection (Lander and Botstein, 1989). It consists of two programs. MapMaker/EXP constructs a marker genetic map and calculates the distance between two adjacent marker loci on the chromosome; and MapMaker/QTL evaluates the effect of marker locus and examines the linkage by a three point interval analysis. The result is presented in the form of LOD (logarithm of the odds) score. A  $\text{LOD} \geq 1.9$  is considered to indicate a suggestive linkage and  $\text{LOD} \geq 3.3$  a for significant linkage.

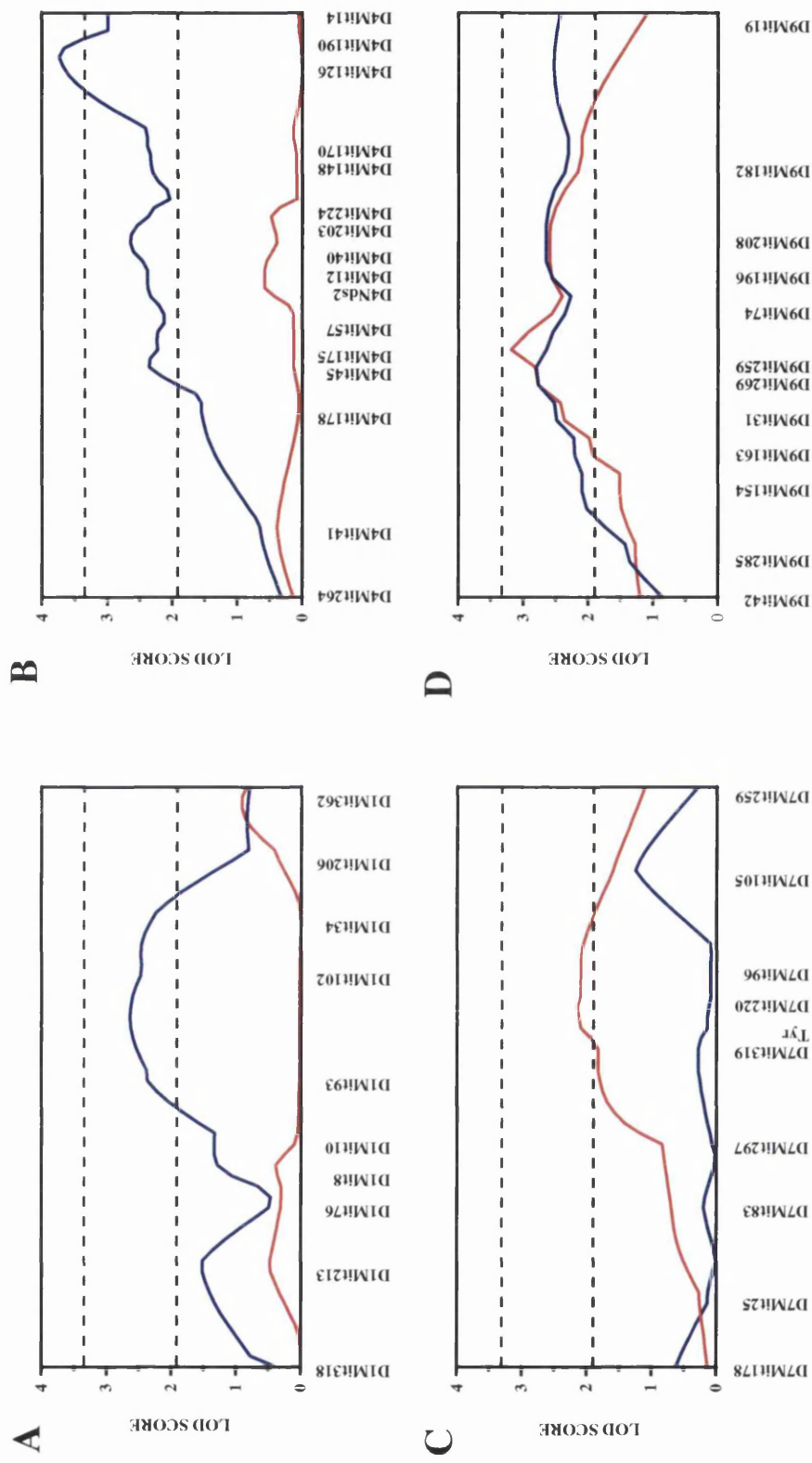
#### **3.5.2.1 Genetic linkage to papilloma development**

The result of Mapmaker/QTL analysis shows that the loci on chromosomes 1, 4 and 9 were linked to papilloma formation (Figure 3.17). Two adjacent regions on the distal and central part of chromosome 4 were associated with papilloma formation. The major region resided in a 12cM interval between D4Mit170 and D4Mit190 with a peak LOD score of 3.7 at D4Mit126. The minor region was a 18cM interval between D4Mit45 and D4Mit224 with 2.6 as the highest  $\text{LOD}=2.6$  score at D4Mit40. Linkage to papilloma formation was also detected on the central region of chromosome 9. In this broad area which spanned nearly 40cM from D9Mit154 to D9Mit19, two peak LOD scores were evaluated as 2.8 at D9Mit269 and 2.6 at D9Mit196. The distal region of chromosome 1 was also linked to papilloma development. In a 20cM interval between D1Mit93 and D1Mit 34, the highest LOD score of 2.63 was obtained at D1Mit102.

#### **3.5.2.2 Genetic linkage to carcinoma development**

The linkage analysis revealed that carcinoma development was linked to two regions on chromosomes 7 and 9, respectively (Figure 3.17). The region on chromosome 9 resided in a 22cM interval between D9Mit163 and D9Mit182. The highest LOD score was 3.18 at D9Mit259. Interestingly the same region was also associated with papilloma development (Section 3.5.2.1). Carcinoma formation was also linked to the distal region of chromosome 7. In a 20cM interval between D7Mit319 and D7Mit105, the highest LOD score of 2.14 was obtained at D7Mit220. Therefore, it appears that the development of carcinomas is also controlled by more than one genetic loci.





**Figure 3.17** LOD score map from interval mapping analysis using MapMaker program in F1BX mice. The papilloma LOD score is shown in blue and carcinoma in red. LOD score bigger than 1.9 was considered suggestive linkage; LOD score bigger than 3.3 was considered significant linkage. A: Chromosome 1; B: Chromosome 4; C: Chromosome 7; D: Chromosome 9.

### 3.5.3 Multiple loci stepwise regression analysis

Data from earlier linkage analysis shows that the tumour development is associated with multiple genetic loci. It is possible that the interactions between these loci are also likely to have a significant effect to tumour formation. Thus, it is necessary to include the contributions of locus-locus interactions in the linkage analysis. Multi-locus stepwise regression analysis was used to estimate the possibility of a target QTL at multiple analysis points across each inter-marker interval inclusion of the effect of their interactions. The data was analysed in two steps. The first step was to evaluate the individual effect of each marker locus. The second step was to estimate the overall contribution of every locus including the interactive effects.

#### 3.5.3.1 Linear regression analysis of genetic linkage to papilloma development

Genetic linkage to papilloma formation in F1BX mice was analysed by the linear regression method. As shown in Table 3.3, a gender factor exerted profound influences on papilloma formation ( $p < 1.0 \times 10^{-16}$ ). Female mice had three times the papilloma incidence of male mice (Table 3.3).

When the individual effects of marker locus was evaluated, significant linkage was found at D4Mit126 ( $p = 8.2 \times 10^{-6}$ ), D9Mit269 ( $p = 7.4 \times 10^{-5}$ ), and D1Mit318 ( $p = 2.9 \times 10^{-4}$ ) (Table 3.3A). Mice carrying the C57BL/6J alleles at loci D4Mit126 and D9Mit269 were more resistant to papilloma formation. However, the inheritance of the C57BL/6J allele at D1Mit318 increased susceptibility to papilloma induction in F1BX mice.

When the contribution of interactions were taken into account, two more marker loci, D12Mit203 and D6Mit14, were identified (Table 3.3B). Their influences on papilloma formation were highly significant when they interacted with other loci. The major interactions were between D12Mit203 and D4Mit126 ( $p = 6.9 \times 10^{-10}$ ), D6Mit14 and D9Mit269 ( $p = 1.0 \times 10^{-9}$ ), and D6Mit14 and D1Mit318 ( $p = 5.0 \times 10^{-5}$ ). Within an interaction the effect of one locus depends on the genotype of the other locus. The effect of D4Mit126 was small when its interacting locus D12Mit203 was heterozygous, but increased when homozygous for FVB/N allele (Table 3.3B). Thus, mice homozygous for FVB/N allele at both loci were more sensitive to papilloma induction than any other combination. Similarly, the interaction between two sensitive C57BL/6J alleles at loci

**A**

MARKER	LOCUS (cM)	PAPILLOMA INCIDENCE*		<i>p</i> **
		BF	FF	
SEX		3.06/31M	9.65/34F	$< 1.0 \times 10^{-16}$
D4MIT126	71.0	4.79/28	7.81/37	$8.2 \times 10^{-6}$
D9MIT269	43.0	5.25/28	7.46/37	$7.4 \times 10^{-5}$
D1MIT318	18.5	6.86/36	6.07/29	$2.9 \times 10^{-4}$

**B**

MARKER		PAPILLOMA INCIDENCE*		<i>p</i> **
SEX		3.06/31M	9.65/34F	
		D12MIT203 (37.0 cM)		$6.9 \times 10^{-10}$
		BF	FF	
D4MIT126 (71.0 cM)	BF	5.80/10	4.22/18	
	FF	4.77/13	<b>9.46/24</b>	
		D6MIT14 (74.0 cM)		$1.0 \times 10^{-9}$
		BF	FF	
D9MIT269 (43.0 cM)	BF	7.17/12	<b>3.81/16</b>	
	FF	8.41/17	6.65/20	
		D6MIT14 (74.0 cM)		$5.0 \times 10^{-5}$
		BF	FF	
D1MIT318 (18.5 cM)	BF	<b>9.11/18</b>	4.61/18	
	FF	5.91/11	6.17/18	

\*Number of papillomas per mouse/Total number of mice. BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N allele.

\*\* Multi-point significant levels.

**Table 3.3** The result of multi-locus linear regression analysis of linkage to papilloma development in F1BX mice. Only markers with significant linkage are listed in the table. A: Multi-locus stepwise linear regression without locus-locus interactions. B: Multi-locus stepwise linear regression with locus-locus interaction. Figures in bold indicate the effect of interactions on a particular genotypes at two interacting loci.

D6Mit14 and D1Mit318 resulted in super-sensitivity, as mice heterozygous at both loci had the highest papilloma incidence. The interaction between D9Mit269 and D6Mit14 was, however, different (Table 3.3B). Mice heterozygous at D9Mit269 but homozygous at D6Mit14 were more resistant to papilloma development compared with mice of the other three genotypes. Therefore, the interacting alleles switched from inter-strain (between two FVB alleles or two C57BL/6J alleles) to intra-strain (between one FVB/N allele and one C57BL/6J allele). The interaction between resistant C57BL/6J allele at D9Mit269 and resistant FVB/N allele at D6Mit14 produced super-resistance.

### 3.5.3.2 Cox regression analysis of genetic linkage to carcinoma development

The Cox regression was used to detect the linkage to carcinoma development because of the application of a dichotomous trait in carcinomas incidence. Significant linkage was detected in three loci, D9Mit269 ( $p=6.0\times 10^{-4}$ ), D10Mit134 ( $p=4.0\times 10^{-3}$ ) and D7Mit83 ( $p=5.5\times 10^{-3}$ ) (Table 3.4). Mice carrying C57BL/6J allele at these loci developed fewer carcinomas. When the contribution of loci interactions was included in linkage analysis, no additional markers were identified (Table 3.4B). The D9Mit269 locus appeared to affect carcinoma incidence independently, whilst D7Mit83 was found to interact with D10Mit134, and the interaction between them showed significant effect on carcinoma formation ( $p=6.9\times 10^{-5}$ ). Mice heterozygous at both loci were more resistant than the other three genotypes. Hence, the interaction between two resistant C57BL/6J alleles at both loci resulted in super-resistance to carcinoma development.

Due to the difference in detecting power, different markers were detected when using different linkage analysis methods. However, this does not mean that a different genetic loci was detected. For example, loci D9Mit196 and D9Mit269 were both linked to papilloma formation when they were analysed using the single marker method and the interval MapMaker method. Since they are located within 5 cM distance, it is possible that they refer to the same target gene. Therefore, when data was analysed using the regression method, only marker D9Mit269 was detected. Similarly, the marker loci D9Mit163 and D9Mit259 detected in carcinoma linkage analysis also refer to the same locus as D9Mit269, as well as the loci D7Mit220 and D7Mit83. However, the distance between loci D1Mit318 and D1Mit102 are more than 40 cM. Therefore, they are unlikely to correspond to the same gene.

### 3.5.4 Summary of genetic loci susceptible to skin tumour in F1BX mice

The genetic linkage of susceptibility to skin tumour in (FVB x C57BL)F1 x FVB backcross mice (F1BX) was analysed with three different methods at three different levels. In addition to gender factor, the most significant contribution to the variation of papilloma incidence was detected at loci D4Mit126 and D9Mit269. Three more loci on chromosomes 4, 6, and 12 were also linked to papilloma development. The most significant linkage to carcinoma formation was detected at locus D9Mit269, while two more loci on chromosomes 7 and 10 were also found to be associated with carcinoma

**A**

MARKER	LOCUS (cM)	CARCINOMA INCIDENCE*		<i>p</i> **
		BF	FF	
D9MIT269	43.0	13/28 (46.4)	25/37 (67.6)	$6.0 \times 10^{-4}$
D10MIT134	59.0	20/36 (55.6)	18/29 (62.1)	$4.0 \times 10^{-3}$
D7MIT83	26.5	15/31 (48.4)	23/34 (67.6)	$5.5 \times 10^{-3}$

**B**

MARKER		CARCINOMA INCIDENCE*		<i>p</i> **
D9MIT269 (43.0 cM)		BF	FF	
		13/28 (46.4)	25/37 (67.6)	
		D10MIT134 (59.0 cM)		$6.9 \times 10^{-5}$
		BF	FF	
D7MIT83 (26.5 cM)	BF	7/18 (38.9)	8/13 (61.5)	
	FF	13/18 (72.2)	10/16 (62.5)	

\*Number of mice with carcinomas/Total number of mice (per cent of carcinoma incidence). BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N allele.

\*\* Multi-point significant levels.

**Table 3.4** The result of multi-locus cox regression analysis of linkage to carcinoma development in F1BX mice. Only markers with significant linkage are listed in the table. A: Multi-locus stepwise Cox regression without locus-locus interactions. B: Multi-locus stepwise Cox regression with locus-locus interaction.

development. Locus-locus interactions also exerted profound effects on tumour formation. The interactions of D4Mit126-D12Mit203, D9Mit269-D6Mit14, and D1Mit318-D6Mit14 had major influences on papilloma formation, whereas the interaction of D7Mit83-D10Mit134 made significant contribution to carcinoma formation. These results indicate that the development of papilloma and carcinoma are affected by multiple genetic loci. It is particularly interesting to note that, whilst most loci were associated to either papillomas or carcinomas, only locus D9Mit269 was involved in both stages. This suggests that although there are some common regulating factors, the development of papillomas and carcinomas is largely under different genetic control.

# **CHAPTER 4**

## **GENETIC CONTROL OF SKIN TUMOUR SUSCEPTIBILITY IN (FVB/N x C57BL/6J)F2 MICE**

In (FVB/N x C57BL/6J)F1 x FVB backcross mice, the C57BL/6J allele is segregated between different loci, whereas the FVB/N allele is present at every genetic loci. Thus, the variation in tumour incidence reflects only the effects of C57BL/6J allele at different loci. It is impossible to evaluate the association of FVB/N alleles with tumour formation. To be able to examine the effects of both FVB/N and C57BL/6J alleles, segregation of both the FVB/N and C57BL/6J alleles is required. Therefore, the linkage study was expanded to include (FVB/N x C57BL/6J)F1 intercross (FVB6F2) mice.

## **4.1 Mouse breeding strategy**

The breeding scheme for F2 mice is illustrated in Figure 4.1. In brief, four female FVB/N mice and male C57BL/6J mice were mated to produce FVB6F1 hybrid mice. Ten mating pairs of F1 hybrids were then set up for continuous breeding to generate FVB6F2 mice. A total of 367 female FVB6F2 mice were raised and subsequently used for the carcinogenesis study.

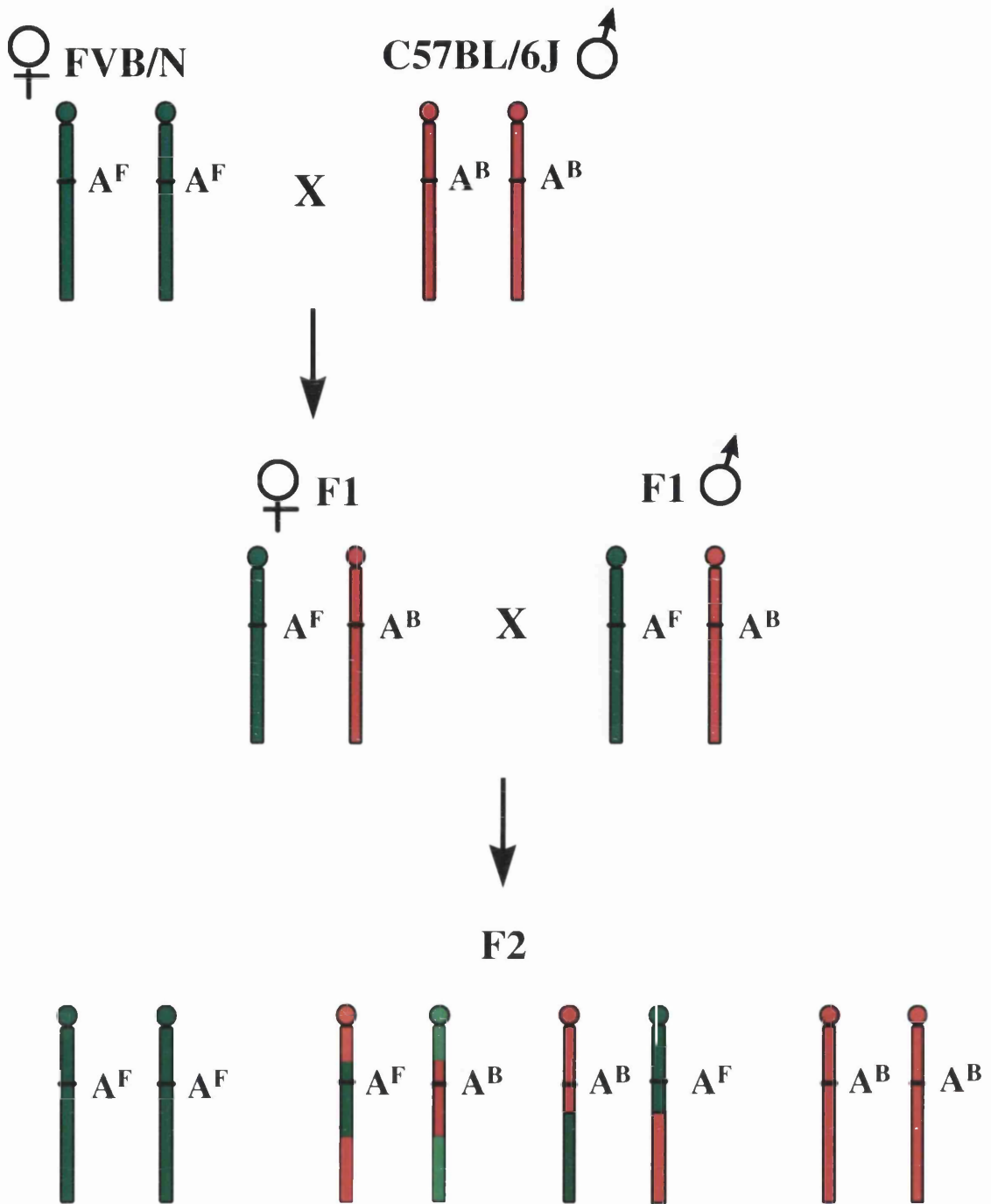
## **4.2 Mouse skin tumour development in (FVB/N x C57BL/6J)F2 mice**

### **4.2.1 Mouse skin tumour induction**

The tumour induction experiment was carried out as described in Section 2.2.2. In brief, FVB6F2 mice were treated with a single dose of 25 mg DMBA and one week later with 20 nmol TPA twice a week for 20 consecutive weeks. The development of papillomas and carcinomas was observed and scored once a week for 60 weeks. The tumour data is presented in Table A7 (see Appendix).

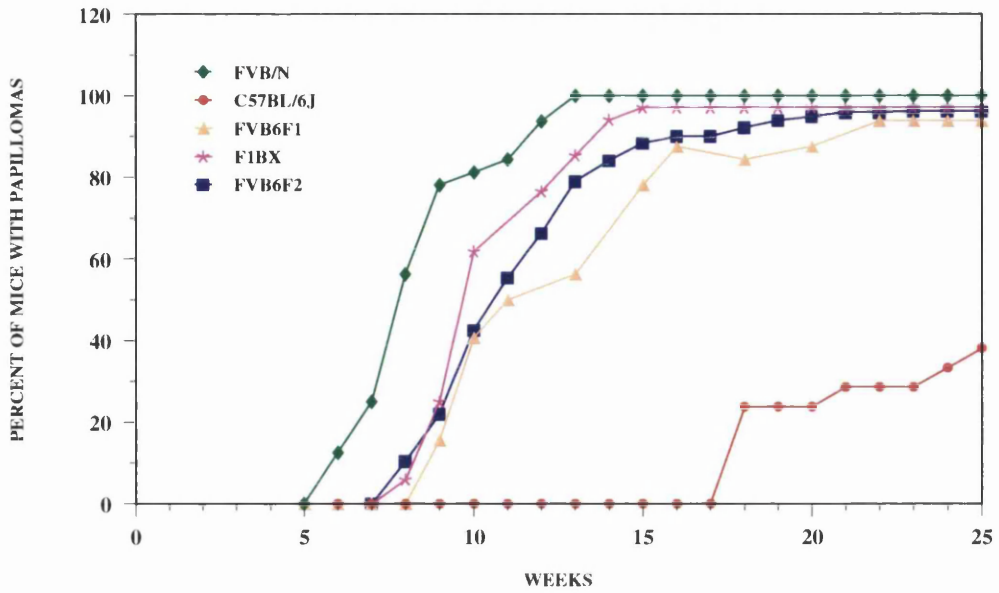
### **4.2.2 Tumour latency**

The tumour latency in FVB6F2 mice is quite similar to that in F1 and F1BX mice (Figure 4.2). The first tumour (papilloma) in FVB6F2 mice was observed at week 8. Three weeks later (at week 11), more than half of the mice had developed skin tumours. The tumour positive population reached a maximum of 96% at week 23. Compared with

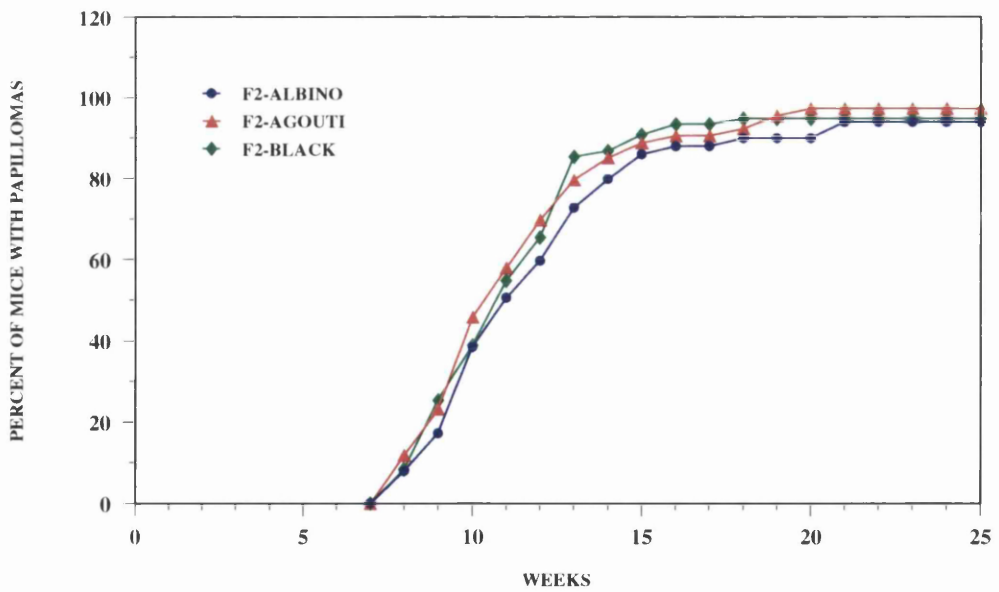


**Figure 4.1** Breeding schemes used to generate FVB6F2 mice. For comparison, only one pair of chromosomes (i.e. chromosome 7) is shown for each parent. FVB/N alleles are indicated by green; C57BL/6J alleles are indicated by red. Note that there are three different subtypes of genotype in F2 mice.





**Figure 4.2** The latency of skin tumour development in two parental strains, FVB/N and C56BL/6J, and their FVB6F1, F1BX and FVB6F2 mice. Only data of female F1BX mice were used because all the other four groups of mice are female.



**Figure 4.3** Comparison of the tumour latency of three coat colour groups in FVB6F2 mice, albino, agouti and black.

the two parental strains, FVB6F2 mice behaved similarly to the sensitive FVB/N parent, indicating the dominance of FVB/N alleles in tumour latency in FVB6F2 mice.

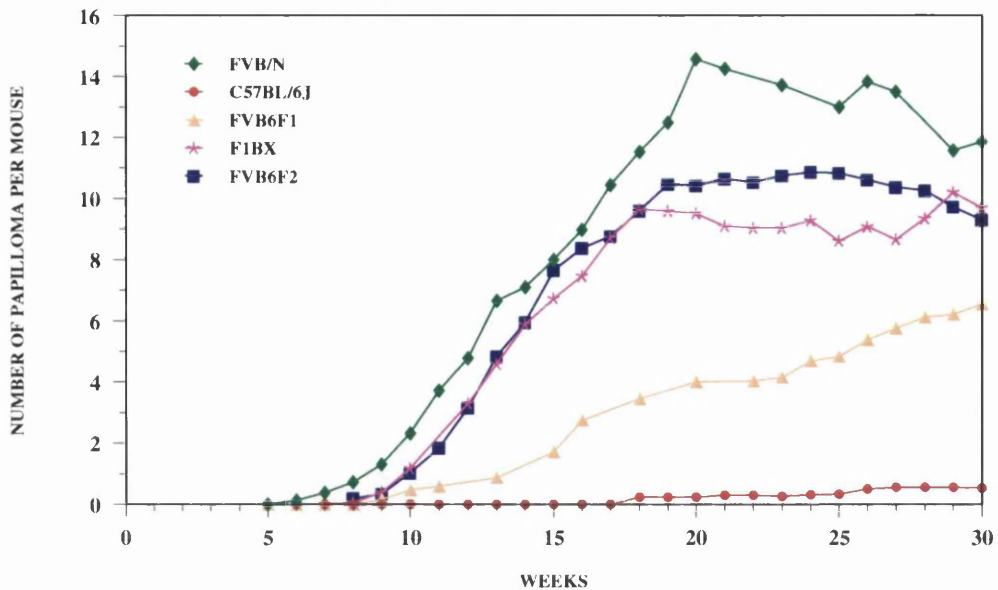
Further analysis was carried out to examine the association of coat colour with tumour latency. There were three coat colours in F2 progeny, albino, agouti, and black. Thus, mice were divided into three colour groups and tumour latency of each groups examined (Figure 4.3). As in F1BX study, all three colour groups of FVB6F2 mice also had very similar tumour latency. The first tumour appeared at the same time (week 8). Their tumour positive populations increased at similar rates and reached the maximum at similar time points. The highest level was 94% in albino mice, 97% in agouti mice and 95% in black mice. These results suggest that loci associated with coat colours had little influence on the tumour latency in FVB6F2 mice.

#### **4.2.3 Papilloma incidence**

The average papilloma number in FVB6F2 mice at different time points is displayed in Figure 4.4. The papilloma incidence in FVB6F2 mice was rather similar to that in the female F1BX mice, much higher than that in F1 mice. And the same as the F1BX mice, it was in fact very close to the sensitive parental FVB/N mice, particularly during the chemical treatment period. At week 20, the papilloma development reached a maximum with an average of  $10.4 \pm 0.4$  papillomas (330 surviving mice). The average numbers decreased about ten weeks later due to the sacrifice of mice with high yield of papillomas (see section 2.2.2).

However, analysis of papilloma distribution revealed the differences between these three groups of mice (Figure 4.5). The distribution of papilloma number in FVB6F2 was the broadest of all different groups, including the parental FVB/N mice, ranging from 0 to as many as 42 papillomas. 12% of mice were, as C57BL/6J, highly resistant with less than one papilloma; another 12% of mice were even more sensitive than FVB/N, responding with more than 23 papillomas; and 32% of mice behaved like F1 hybrids with less than 5 papillomas.

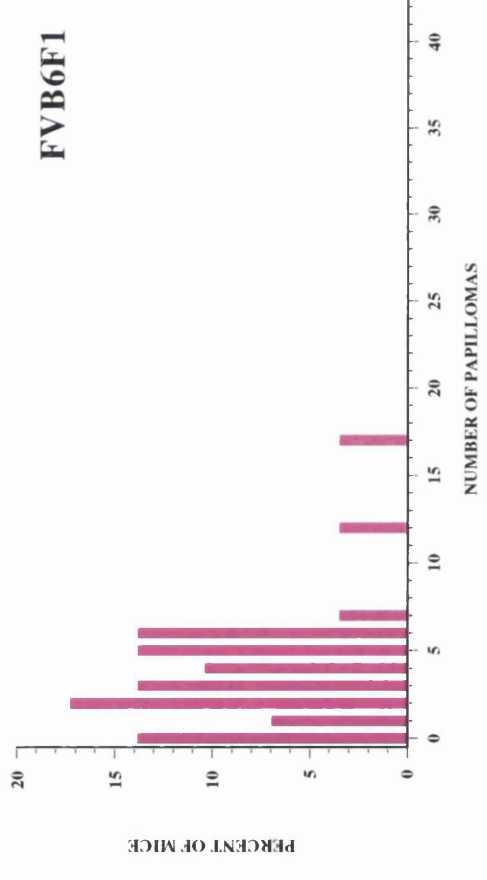
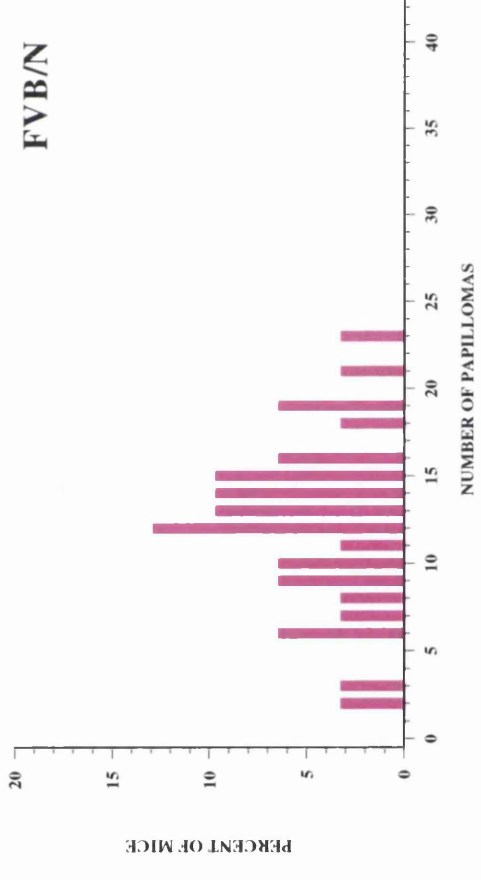
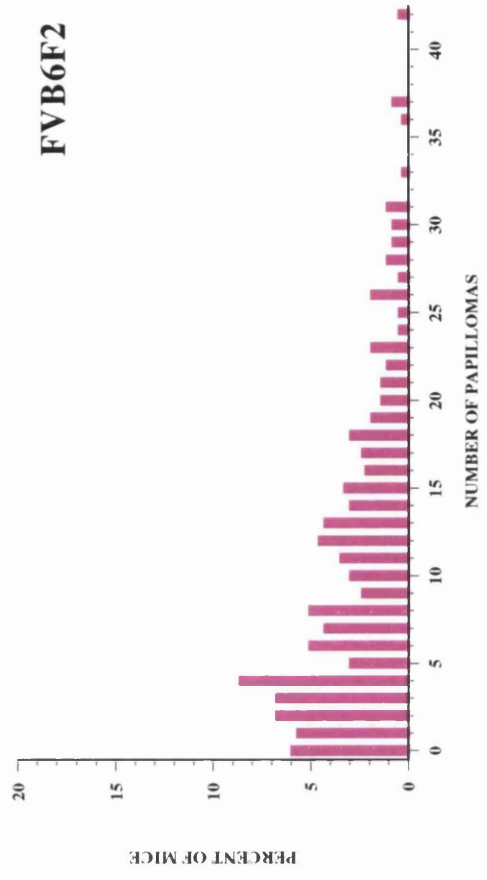
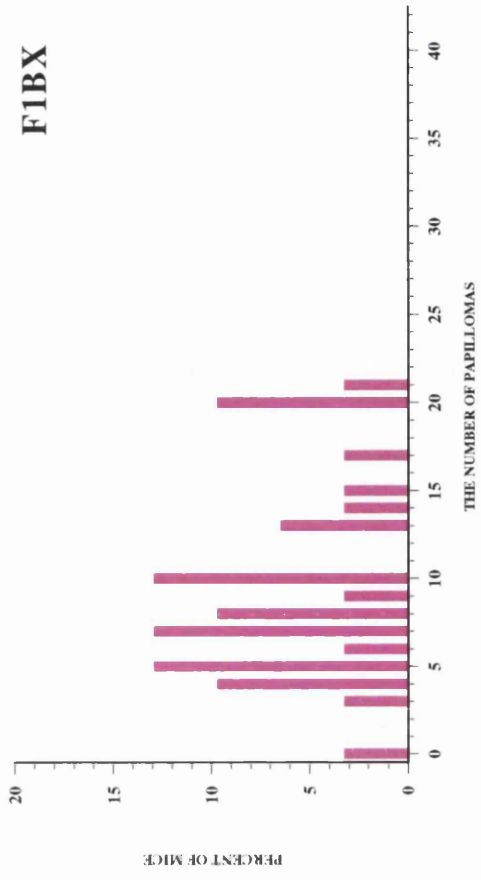
Taken together, although the overall genomic makeup of the susceptible FVB/N alleles is higher in F1BX mice than in FVB6F2 mice, the FVB6F2 mice exhibited a phenotype



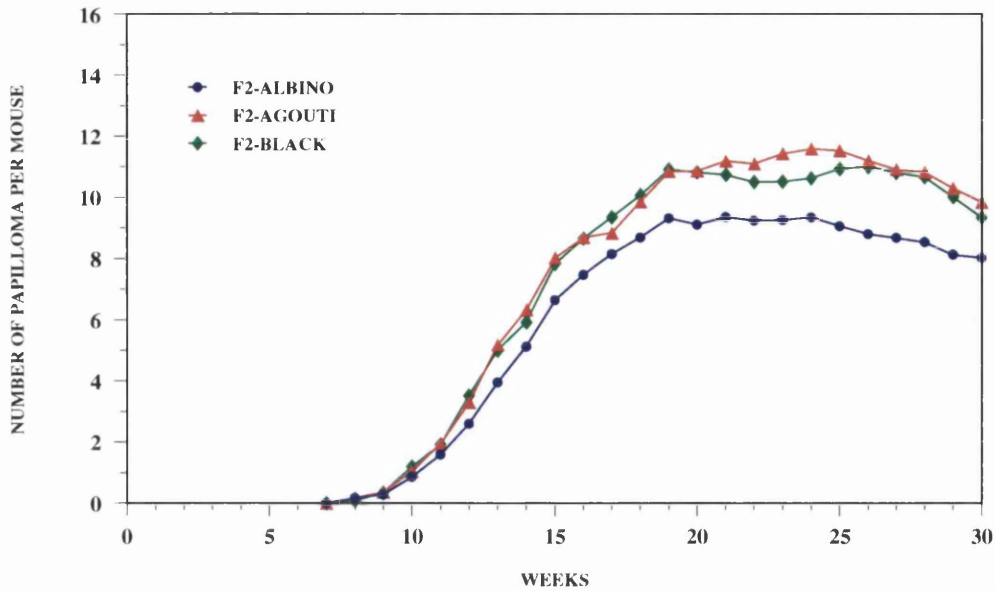
**Figure 4.4** The average number of papillomas per mouse in parental mice FVB/N and C57BL/6J, and their FVB6F1, F1BX and FVB6F2 mice. All data were from female mice.

similar to that in F1BX mice. When compared to the FVB6F1 mice which had exactly the same ratio of FVB/N:C57BL/6J in the genome, the FVB6F2 mice, however, had higher papilloma incidence. One possible explanation is the existence of recessive C57BL/6J susceptibility loci. In the C57BL/6J parental mice, the effects of papilloma resistant loci are larger, thus the overall phenotype is resistant. However, in the absence of stronger dominant resistant loci as a result of the segregation events that occurred in FVB6F2 mice, the effects of the C57BL/6J susceptibility loci, in addition to the FVB/N susceptibility loci, made some FVB6F2 mice even more susceptible than the FVB/N parental mice.

The influence of coat colour in papilloma formation was also examined. As shown in Figure 4.6, all three colour groups had a similar pattern of papilloma development. The number of papilloma increased quickly at early stages until they reached the maximum level at around week 20. However, the average papilloma number in albino mice was clearly less than that in agouti and black mice which had a similar papilloma incidence.

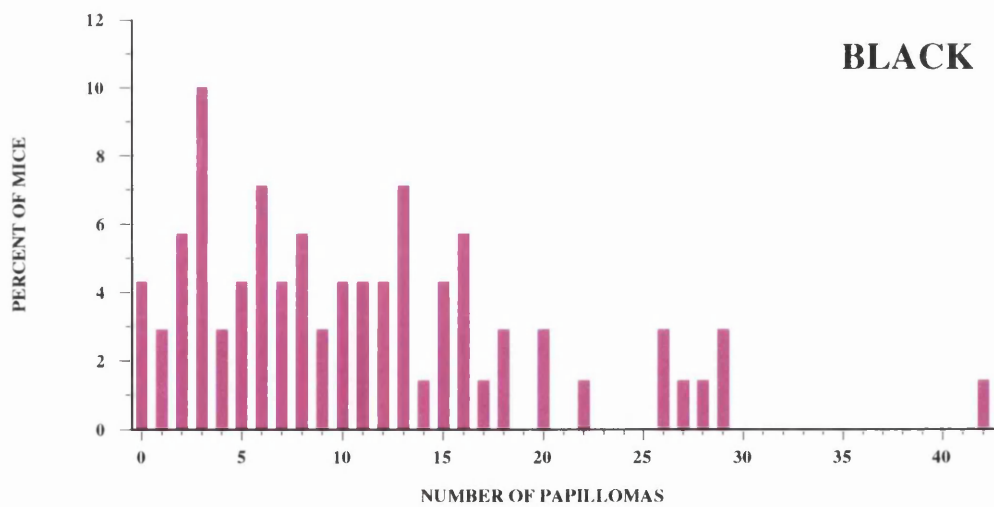
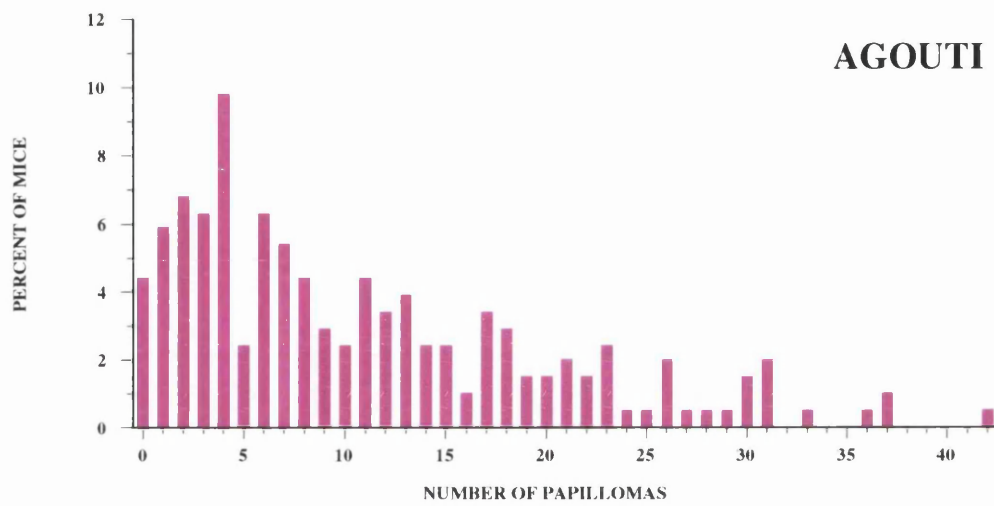
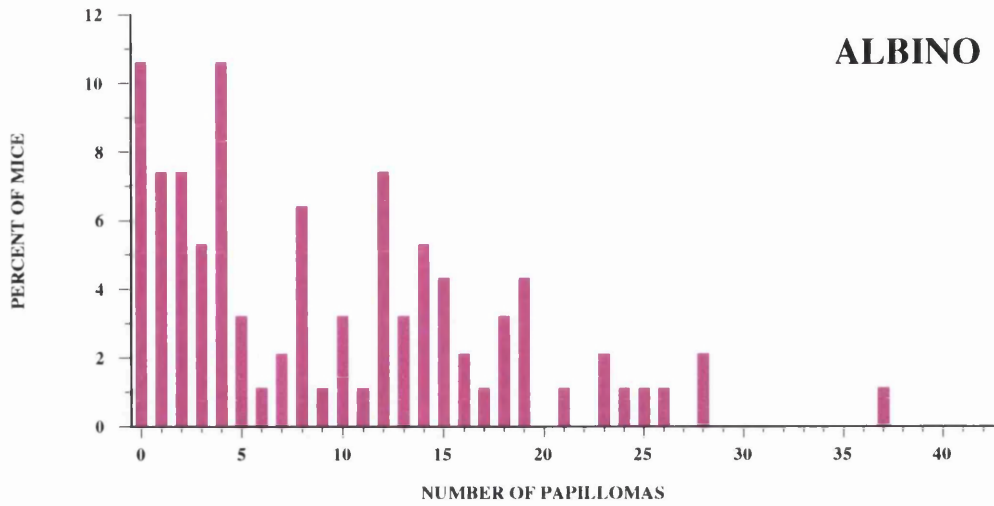


**Figure 4.5** The distribution and frequency of papillomas at week 20 in FVB/N, FVB6F1, F1BX and FVB6 F2 mice. All data are from female mice.



**Figure 4.6** Comparison of average papilloma numbers of three different coat colours in FVB6F2 mice.

At week 20, the average papilloma number was  $9.5 \pm 0.9$  (78 surviving mice) in albino mice, compared with  $11.2 \pm 0.6$  (189 surviving mice) in agouti mice and  $11.0 \pm 1.0$  (63 surviving mice) in black mice. The distribution and frequency of papillomas showed that the albino group had a narrower spectrum than the other two colour groups (Figure 4.7). Only 8.6% of albino mice had more than 23 papillomas, while this was 13% in agouti mice and 10% in black mice. Moreover, the majority of albino mice had 0-5 papillomas, compared with 3-8 papillomas in agouti and black mice. These results suggest that the albino FVB6F2 mice were more resistant to papilloma induction than the agouti and black mice. It is in consistent with the data of FIBX backcross. Although the overall papilloma incidence was similar between agouti and albino FIBX mice, the female albino FIBX mice were more resistant than the agouti female mice. Taken together, these results imply that papilloma development in the (FVB/N x C57BL/6J) crosses is associated with coat colour related genetic loci.



**Figure 4.7** Comparison of the distribution and frequency of papillomas at week 20 by different coat colours in FVB6F2 mice.

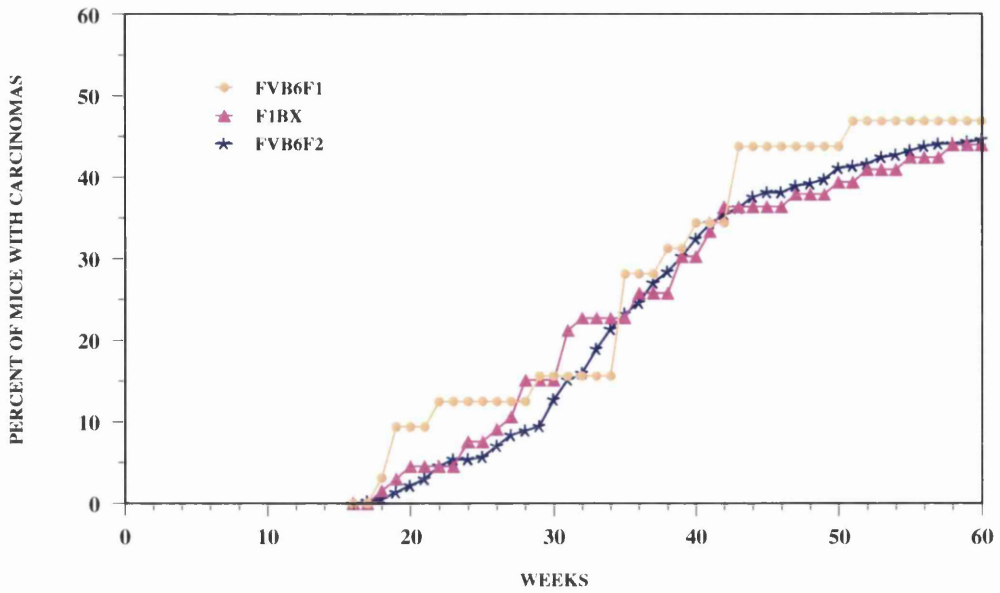
#### 4.2.4 Carcinoma incidence

Due to the application of a dichotomous scoring system, the carcinoma incidence in F2 mice, as in F1BX mice, was presented in format of carcinoma positive population, regardless of the actual number of carcinomas that each individual mice developed.

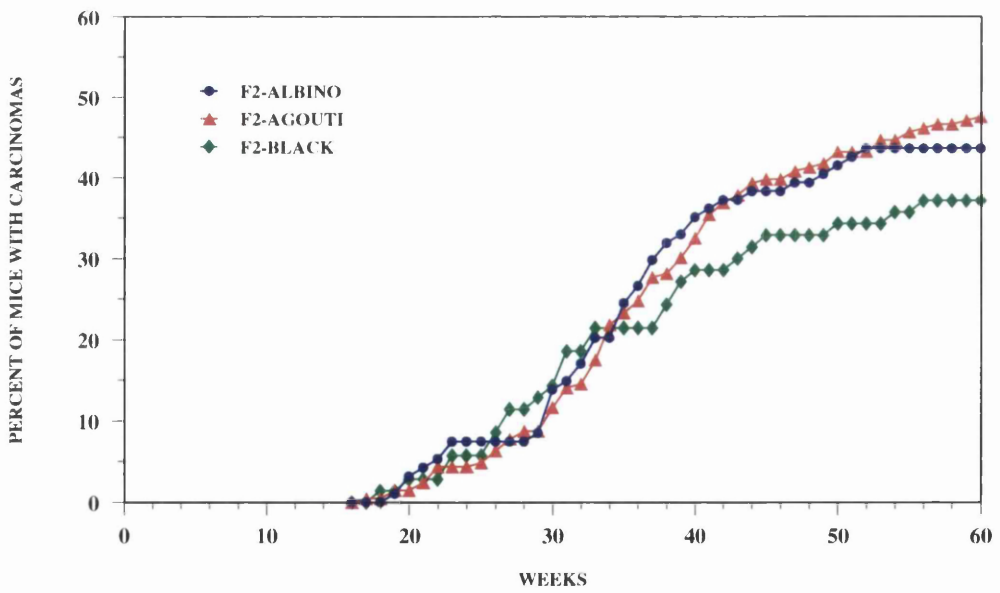
As shown in Figure 4.8, the carcinoma formation in F2 mice was similar to that in F1BX mice. The first carcinoma in F2 mice was observed at week 17, one week earlier than that in F1BX mice. The population of carcinoma positive mice increased steadily until the end of the 60 week observation period. A maximum of 45% of F2 mice, compared with 44% in F1BX, developed carcinomas. However, there were some differences between F2 and F1 hybrid mice. Particularly during week 19-26 and week 43-55, fewer F2 mice than F1 hybrid mice developed carcinomas, though the difference is not significant. This result indicates that the carcinoma development is likely to be inherited as a dominant trait.

The association of coat colour with carcinoma incidence was also examined (Figure 4.9). At early stages, the carcinoma positive populations in all three colour groups increased steadily to about 20% and little difference was observed. After week 35, however, differences began to appear and became greater towards the end of observation period. It appeared that black mice were more resistant to carcinoma formation than agouti and albino mice. At week 50, 34% of black mice developed carcinomas, compared with 41% in albino and 43% in agouti mice. These results suggest that carcinoma development in F2 mice is also associated with coat colours.

Taken together, the data shows that F2 mice exhibit different responses to papilloma and carcinoma formation. Compared to F1 mice, the F2 mice had a higher papilloma incidence, but a similar carcinoma incidence. This implies that the development of papillomas and carcinomas are probably under different genetic control. Moreover, the fact that albino mice were the most resistant to papillomas whilst black mice were the most resistant to carcinomas further confirms the involvement of different genetic loci in the development of papillomas and carcinomas. It is possible that some loci are involved in the development of both papilloma and carcinomas, whilst others are only associated with either papilloma or carcinoma formation.



**Figure 4.8** Carcinoma incidence in FVB6F1, F1BX and FVB6F2 mice.



**Figure 4.9** Comparison of carcinoma incidence in three coat colour groups (albino, agouti and black) in FVB6F2 mice.



### **4.3 Genetic mapping of (FVB/N x C57BL/6J)F2 mice**

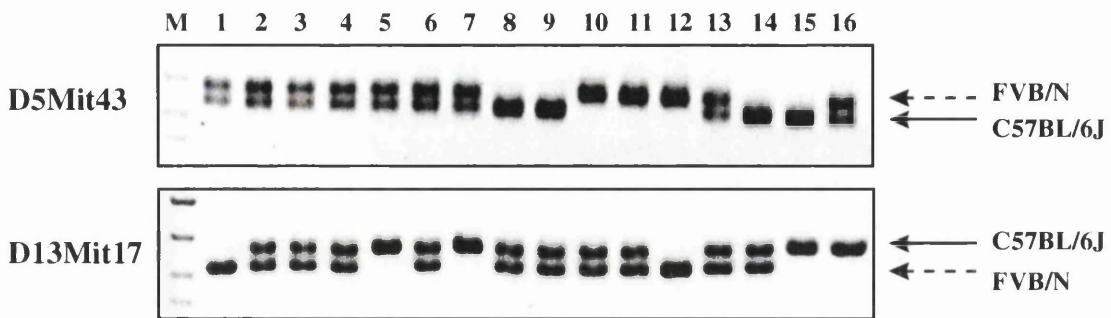
Genetic mapping was carried out to examine the genotypes of individual F2 mice. There are total three possible genotypes: homozygous for FVB/N alleles, homozygous for C57BL/6J alleles and heterozygous with one FVB/N allele and one C57BL/6J allele. The heterozygous mice will have two PCR products of different sizes, whereas mice homozygous for FVB/N or C57BL/6J with two identical alleles will only have one PCR product amplified. Examples of genotyping of 16 F2 mice at loci D5Mit43 and D13Mit17 are shown in Figure 4.10.

Despite technological improvement in the speed and accuracy with which molecular markers can be assayed, it can still be expensive and time consuming to analyse a large population. Lander and Botstein have shown that, in the context of a cross between divergent lines, most of the evidence on the existence of QTLs for a trait comes from the highest and lowest performing individuals (Lander and Botstein 1989). Therefore, to obtain the maximum information but reduce the number of individuals needed to be genotyped in marker-QTL linkage analysis, a modified approach termed 'selective genotyping' was used in genomic mapping of FVB6F2 mice (Darvasi and Soller, 1992; Risch and Zang, 1995). F2 mice were separated into three groups, resistant, mediate and sensitive groups. The resistant group consisted of 147 mice which had less than 7 papillomas; the sensitive group was made up by 125 mice which had more than 12 papillomas; the remaining mice became the mediate groups. Only mice belonging to the two extreme groups were genotyped. A panel of 128 microsatellite markers and more than 45,000 PCR reactions were used in the genomic mapping. They constructed a genetic linkage map spanning 1213.8 cM and covered 88.9% of the genome, the average distance between markers being 9.4 cM. The genotypes of F2 mice is presented in Table A8 (see Appendix).

### **4.4 Linkage of genetic loci to skin tumour in FVB6F2 mice**

The linkage analysis in F2 mice was also performed using three different methods, namely single marker analysis, interval mapping and multi-locus regression analysis. Since only mice from two extreme groups were genotyped in the selective genotyping

approach, non-parametric statistical methods were applied to analyse the difference in allele frequency between two groups.



**Figure 4.10** PCR microsatellite analysis of genotypes of FVB6F2 mice with marker D5Mit43 and D13Mit17. The gels show representative examples of genetic mapping in 16 F2 mice. There are three subtypes of genotypes, homozygous with two FVB/N alleles (indicated with discontinued arrow), homozygous with two C57BL/6J alleles (indicated with solid arrow) and heterozygous with one FVB/N allele and one C57BL/6J allele. Therefore, the homozygous mice will have one DNA band and the heterozygous mice will have two DNA bands. M: DNA molecular weight marker VI.

#### 4.4.1 Single marker linkage analysis

As one of the simplest approach in linkage analysis, the single marker method was first used to detect QTLs. The allele frequencies of the sensitive and resistant groups at each marker locus were calculated and the differences were compared using a non-parametric statistical method. If the difference is statistically significant, it is inferred that a QTL controlling the character of interest is located near the marker.

##### 4.4.1.1 Linkage to papilloma development

Genetic association with papilloma development was examined. The allele frequency of the resistant and sensitive groups was analysed using a crosstable (2X3 contingency) method and the difference was measured by the chi-square test with 95% confidence intervals.

Marker	Locus (cM)	Number of mice (resistant group)			Number of mice (sensitive group)			<i>p</i>
		BB	BF	FF	BB	BF	FF	
D6MIT30	48.50	24	85	38	34	73	18	0.0179
D6MIT254	57.10	30	79	38	39	66	20	0.0454
D6MIT59	67.00	30	79	38	42	63	20	0.0217
<b>D6MIT14</b>	<b>74.00</b>	<b>29</b>	<b>77</b>	<b>41</b>	<b>44</b>	<b>62</b>	<b>19</b>	<b>0.0040</b>
<b>D10MIT248</b>	<b>7.00</b>	<b>50</b>	<b>77</b>	<b>20</b>	<b>25</b>	<b>60</b>	<b>40</b>	<b>0.0005</b>
<b>D11MIT217</b>	<b>19.00</b>	<b>47</b>	<b>78</b>	<b>22</b>	<b>21</b>	<b>66</b>	<b>38</b>	<b>0.0012</b>
D11MIT23	28.10	46	75	26	24	64	37	0.0185
D11MIT30	39.80	53	72	22	29	60	36	0.0075
D11MIT38	49.00	51	73	23	29	59	37	0.0107
<b>D11MIT99</b>	<b>65.00</b>	<b>54</b>	<b>72</b>	<b>21</b>	<b>27</b>	<b>64</b>	<b>34</b>	<b>0.0044</b>
D11MIT254	71.00	49	79	19	32	58	35	0.0074
D12NDS11	6.00	50	70	27	28	60	37	0.0333
D12MIT2	19.00	53	71	23	27	63	35	0.0079
<b>D12MIT68</b>	<b>28.00</b>	<b>54</b>	<b>69</b>	<b>24</b>	<b>22</b>	<b>74</b>	<b>29</b>	<b>0.0020</b>
D12MIT149	37.00	50	69	28	22	74	29	0.0093
<b>D16MIT110</b>	<b>21.00</b>	<b>42</b>	<b>72</b>	<b>33</b>	<b>22</b>	<b>53</b>	<b>50</b>	<b>0.0043</b>
<b>D16MIT4</b>	<b>27.30</b>	<b>38</b>	<b>77</b>	<b>32</b>	<b>19</b>	<b>56</b>	<b>50</b>	<b>0.0026</b>
<b>D16MIT64</b>	<b>38.00</b>	<b>45</b>	<b>67</b>	<b>35</b>	<b>23</b>	<b>51</b>	<b>51</b>	<b>0.0051</b>
D16MIT50	53.50	43	72	32	27	51	47	0.0153

**Table 4.1** The result of single marker linkage analysis of susceptibility to papilloma development in FVB6F2 mice. Locations of marker locus in cM are the relative map locations from the centromere and map distances are determined from the report of mouse chromosome committee in the mouse genome database. BB, homozygous with two copies of C57BL/6J alleles; BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N allele. The number of mice of each genotype in resistant and sensitive groups were compared and analysed by chi-square test with significant level at  $p=0.05$ . Of 128 markers analysed only markers with  $p$  value less than 0.05 were listed. Markers with  $p$  value less than 0.01 were highlighted and considered as the location of genes that influence the susceptibility to the development of papillomas.

The papilloma development was linked to markers on chromosomes 6, 10, 11, 12 and 16 (Table 4.1). The most significant linkage was detected at locus D10Mit248 ( $p=0.0005$ ). The frequency of FVB/N homozygous alleles was higher in the sensitive group but lower in the resistant group. Similarly, the frequency of C57BL/6J homozygous alleles was lower in the sensitive group but higher in the resistant group. Thus, at locus D10Mit248, the FVB/N allele was susceptible and the C57BL/6J was

resistant to papilloma formation. The linkage was also detected in the central region of chromosome 16 between D16Mit110 and D16Mit64, particularly at D16Mit110 ( $p=0.0043$ ), D16Mit4 ( $p=0.0026$ ) and D16Mit64 ( $p=0.0051$ ), and the central region of chromosome 12 between D12Mit2 and D12Mit149 especially at locus D12Mit68 ( $p=0.002$ ). Furthermore, nearly the whole of chromosome 11 was connected to papilloma formation, with the most significant linkage measured at D11Mit217 ( $p=0.0012$ ) and D11Mit99 ( $p=0.0044$ ). At these loci, the FVB/N allele was associated with susceptibility and the C57BL/6J allele was linked to tumour resistance. However, the effects of the two parental alleles at the distal region of chromosome 6 between D6Mit30 and D6Mit14 were reversed. At D6Mit14 ( $p=0.004$ ), there were more FVB/N homozygous mice in the resistant group and more C57BL/6J homozygous mice in the sensitive group. Thus, the presence of the FVB/N allele on chromosome 6 was linked to resistance to papillomas, while the inheritance of the C57BL/6J allele was associated with susceptibility to papilloma development.

#### **4.4.1.2 Linkage to carcinoma development**

The method used for carcinoma linkage analysis differed from that of papilloma linkage analysis because a dichotomous trait rather than a linear related trait was used to score carcinomas. Difference in carcinoma formation of each genotypes was analysed using the Kaplan-Meier test with 95% confidence intervals.

The carcinoma development in F2 mice was linked to markers on chromosomes 3, 6, 8, 10, and 16 (Table 4.2). The most significant linkage was detected at locus D3Mit62 ( $p=0.0003$ ). At this locus, 54.1% of C57BL/6J homozygous mice developed carcinomas, compared with 45.7% in FVB/N homozygous mice and 47.6% in heterozygous mice. Thus, mice carrying a FVB/N allele, including homozygotes with two copies of FVB/N alleles and heterozygotes with one FVB/N allele and one C57BL/6J allele, were more resistant to carcinoma formation than mice homozygous for C57BL/6J allele. Similarly, FVB/N allele on the distal region of chromosome 6, particularly at locus D6Mit14 ( $p=0.006$ ), was linked to carcinoma resistance. A resistant locus was also identified on chromosome 16 between D16Mit64 and D16Mit50. However, it was the presence of C57BL/6J alleles at these loci that reduced the probability of developing carcinomas.

Marker	Locus (cM)	Percentage of mice with carcinomas			<i>p</i> (K-M)
		BB	FB	FF	
<b>D3MIT62</b>	<b>4.60</b>	<b>54.1</b>	<b>47.6</b>	<b>45.7</b>	<b>0.0003</b>
D6MIT59	67.00	57.7	43.8	47.5	0.022
<b>D6MIT14</b>	<b>74.00</b>	<b>58.4</b>	<b>44.8</b>	<b>44.4</b>	<b>0.006</b>
D16MIT64	38.00	43.8	48.0	52.9	0.028
D16MIT50	53.50	44.0	46.2	56.3	0.017
<b>D8MIT211</b>	<b>49.00</b>	<b>37.1</b>	<b>52.0</b>	<b>56.2</b>	<b>0.003</b>
<b>D8MIT215</b>	<b>59.00</b>	<b>35.4</b>	<b>53.4</b>	<b>53.3</b>	<b>0.006</b>
D10MIT42	44.00	41.6	49.3	54.4	0.048
D10MIT134	59.00	43.2	50.3	50.0	0.019

**Table 4.2** The result of single marker linkage analysis of susceptibility to carcinoma development in FVB6F2 mice. Locations of marker locus in cM are the relative map locations from the centromere and map distances are determined from the report of mouse chromosome committee in the mouse genome database. BB, homozygous with two copies of C57BL/6J alleles; BF, heterozygous with one C57BL/6J allele and one FVB/N allele; FF, homozygous with two copies of FVB/N alleles. The percentage of mice with carcinomas for each genotype were compared and analysed using Kaplan-Meier test with significant level at  $p=0.05$ . Of 128 markers analysed only markers with  $p$  value less than 0.05 were listed. Markers with  $p$  value less than 0.01 were highlighted and considered as the location of genes that influence the susceptibility to the development of carcinomas.

Significant linkage was also detected at D8Mit211 ( $p=0.0003$ ) and D8Mit215 ( $p=0.0006$ ). Mice inheriting the FVB/N allele at these two loci were more sensitive to carcinoma formation than mice homozygous for the C57BL/6J allele. Similarly, the FVB/N allele at loci D10Mit42 and D10Mit134 were also linked to higher carcinoma incidence. Hence, the presence of the FVB/N alleles on chromosomes 8 and 10 were associated with susceptibility to carcinoma development.

#### 4.4.2 Interval mapping analysis

Once a potential linkage was detected in the single marker analysis, more loci on the same chromosome were genotyped, and the data was analysed using the interval mapping method to obtain a more accurate evaluation of the position and the effect of underlying QTL. The MapMaker program package was used for an automatic analysis. MapMaker/EXP was used to construct a genetic map and calculate the distance of two

adjacent marker loci; MapMaker/QTL was used to detect the linkage by a three point interval analysis and evaluate the effect of each loci. The results are presented in the format of LOD score. A  $\text{LOD} \geq 1.9$  is widely accepted as indicating a suggestive linkage and  $\text{LOD} \geq 3.3$  as indicating a significant linkage.

#### **4.4.2.1 Linkage to papilloma development**

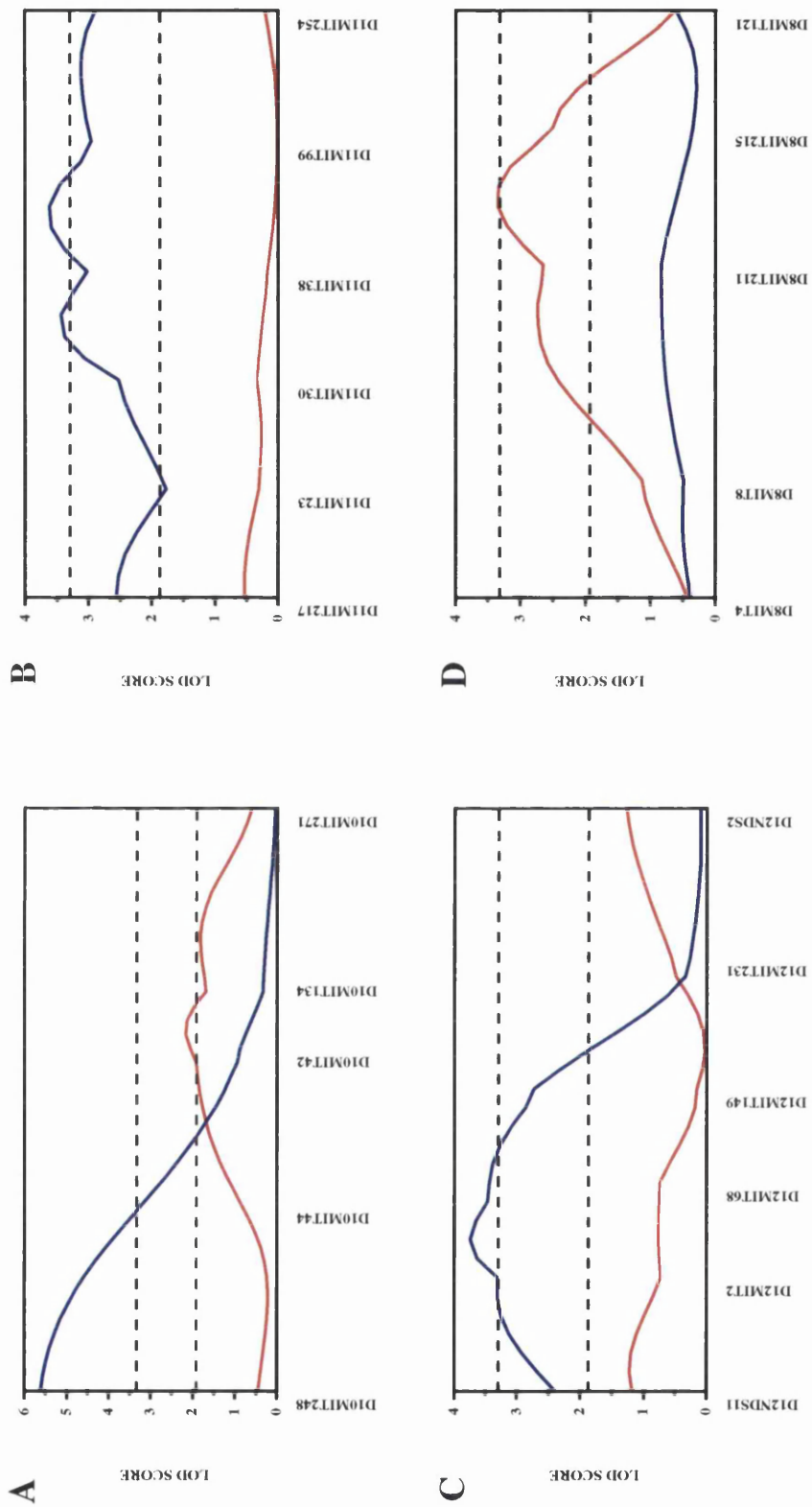
Linkage to papilloma development was detected on chromosomes 10, 11 and 12 (Figure 4.11). The most significant linkage was detected on the proximal region of chromosome 10 in a 20 cM interval between D10Mit248 and D10Mit44. The highest LOD score was 5.61 at D10Mit248 (Figure 4.11A). A second region linked to papilloma development was found in a 30 cM interval from D12Nds11 to D12Mit149 with a peak LOD score of 3.74 at D12Mit68 (Figure 4.11B). Linkage to papilloma development was also obtained on the central-distal region of chromosome 11 between D11Mit30 and D11Mit254. Two highest LOD scores were measured 3.62 between D11Mit38 and D11Mit99 and 3.43 between D11Mit30 and D11Mit38, all reached to the significant levels (Figure 4.11C).

#### **4.4.2.2 Linkage analysis of carcinoma development**

The most significant linkage to carcinoma development was detected on the central region of chromosome 8 (Figure 4.11D). In a 27 cM interval between D8Mit8 and D8Mit215, the highest LOD score of 3.35 was obtained between D8Mit211 and D8Mit215, just above the significant levels.

#### **4.4.3 Multi-locus stepwise regression analysis**

As we mentioned earlier, the tumour development is likely to involve the contribution of multiple genetic loci, and many of these loci are interactive. Therefore, a third level analysis was necessary to detect the contribution of locus-locus interactions. There are mainly two classes of interactions, dominant and additive. When the phenotype of heterozygote is the same as that of one of the homozygotes, it is characterised as a dominant gene action; when the phenotype of heterozygote is exactly intermediate between that of two homozygotes, it is characterised as an additive gene action. In addition to interactions between loci, the interactions between two parental alleles can also be measured to specify the degree of dominance because the presence of all three



**Figure 4.11** LOD map of chromosomes with linkage to skin tumour formation in FVB6F2 mice. The LOD scores of papilloma is shown in blue and carcinoma in red. The dashed lines represent the Lander-Kruglyak thresholds for significant linkage (LOD=3.3) and suggestive linkage (LOD=1.9).

genotypes at each locus in a F2 cross. At locus underlying quantitative variation, the gene action could range from completely recessive to completely dominance, even over-dominance (heterozygotes exceed either of parental homozygotes).

The regression analysis was performed in two steps. The first step was to evaluate the effect of each individual locus. The second step was to estimate the contributions of every individual locus as well as the interaction between loci and alleles. A  $p$  value of  $1.0 \times 10^{-4}$  is generally considered as indicating a significant linkage and a  $p$  value of  $3.4 \times 10^{-3}$  as indicating a suggestive linkage.

#### **4.4.3.1 Logistic regression analysis of linkage to papilloma development**

The logistic regression test was used to examine the allele frequency within and between two extreme groups and evaluate the effect of individual locus as well as the contribution of interactions to the development of papillomas in FVB6F2 mice.

The most significant linkage to papilloma development was detected at locus D12Mit68 ( $p=0.000034$ ). The C57BL/6J allele at this locus conferred resistance to papillomas. Six more markers were also found to be significantly associated with papilloma formation when the contribution of individual loci was evaluated (Table 4.3A). Linkage of papillomas resistance from C57BL/6J alleles was identified at loci D4Mit175 ( $p=0.0014$ ), D10Mit248 ( $p=0.0002$ ), D11Mit99 ( $p=0.0007$ ), and D16Mit64 ( $p=0.0008$ ). Loci D5Mit233 ( $p=0.0013$ ) and D6Mit14 ( $p=0.0017$ ) were also associated with the development of papillomas. However, the resistant effects were contributed from FVB/N alleles at these two loci.

When locus-locus interactions were taken into account, locus D6Mit14 was the only locus that acted independently and the resistant effect was contributed from the FVB/N allele. Moreover, nine pair of interacting loci were also detected to have a significant effect on papilloma formation (Table 4.3B). The most significant contribution came from D11Mit99 x D3Mit49 ( $p=3.6 \times 10^{-6}$ ), D10Mit248 x D16Mit51 ( $p=1.9 \times 10^{-5}$ ), and D16Mit64 x D15Mit189 ( $p=4.3 \times 10^{-6}$ ). The interactions between two parental alleles at the paired loci resulted in the unusually high or low number of mice. For example, (34 mice) which were homozygous for C57BL/6J at D10Mit248 and heterozygous at



A

Markers	Locus (cM)	Coefficient	<i>p</i>
D4MIT175	49.60	-0.656	1.4×10 <sup>-3</sup>
D10MIT248	7.00	-0.804	2.0×10 <sup>-4</sup>
D11MIT99	65.00	-0.803	7.0×10 <sup>-4</sup>
D12MIT68	28.00	-1.058	3.4×10 <sup>-5</sup>
D16MIT64	38.00	-0.649	8.0×10 <sup>-4</sup>
D5MIT233	29.00	0.816	1.3×10 <sup>-3</sup>
D6MIT14	74.00	0.744	1.7×10 <sup>-3</sup>

B

Marker	Interactions	Coefficient	<i>p</i>
D6MIT14		2.501	1.0×10 <sup>-4</sup>
D12MIT68 X D6MIT59	Hom x Het	-3.140	1.0×10 <sup>-4</sup>
D12MIT2 X D9MIT207	Het x Het	-2.075	4.0×10 <sup>-4</sup>
D11MIT99 X D3MIT49	Het x Het	-3.019	3.6×10 <sup>-6</sup>
D10MIT248 X D15MIT26	Hom x Hom	-3.864	2.0×10 <sup>-4</sup>
D10MIT248 X D16MIT51	Het x Hom	-3.290	1.9×10 <sup>-5</sup>
D16MIT64 X D15MIT56	Hom x Het	-5.099	1.0×10 <sup>-4</sup>
D16MIT64 X D15MIT189	Het x Het	-5.117	4.3×10 <sup>-6</sup>
D4MIT175 X D5MIT43	Hom x Het	2.824	1.0×10 <sup>-4</sup>
	Het x Hom	2.948	1.0×10 <sup>-4</sup>

**Table 4.3** Multi-locus stepwise logistic regression analysis of linkage to papilloma development in FVB6F2 mice. A. Multi-locus stepwise logistic regression analysis excluding any interactions B. Multi-locus stepwise logistic regression analysis including interaction between loci and alleles. The coefficient value indicated the degree of interaction between two parental alleles at individual locus or two alleles from different loci. A positive coefficient value indicates a resistant effect; a negative coefficient value implicates susceptibility to papilloma formation.

	D16MIT51				
		BB	FB	FF	Total
Resistant group D10MIT248	BB	10	<b>34</b>	6	50
	FB	23	37	17	77
	FF	4	9	7	20
	Total	37	80	30	147
Sensitive group D10MIT248	BB	6	11	8	25
	FB	11	26	<b>23</b>	60
	BB	12	23	5	40
	Total	29	60	36	125

**Table 4.4** The effect of interaction between loci D10Mit248 and D16Mit51 in FVB6F2 mice. The number in bold indicates the genotypes of abnormal allele frequency.

D16Mit51 in resistant groups, and a high number of mice (23 mice) which were heterozygous at D10Mit248 and homozygous for FVB/N at D16Mit51 in sensitive groups (Table 4.4). Similarly, unusual higher or lower number of mice of particular genotype were also discovered due to the interactions between two parental alleles at two interactive loci in the other eight pairs. Interestingly, neither loci D9Mit207 nor D12Mit2 (Table 4.3B) had profound contribution to papilloma formation when they acted alone. However, the synergistic interactions between parental alleles at the two loci had significant influence on papilloma development.

#### **4.4.3.2 Cox regression analysis of susceptibility to carcinoma development**

Multi-locus cox regression method was used to detect the genetic linkage to carcinoma formation in F2 mice. The results are presented in Table 4.5. Three loci, D3Mit46 ( $p=0.0001$ ), D8Mit211 ( $p=0.000029$ ) and D12Nds2 ( $p=0.0001$ ) were linked to carcinoma development when the locus-locus interactions were not considered. The FVB/N alleles at loci D3Mit46 and D12Nds2 were associated with resistance to carcinoma, while the C57BL/6J allele at locus D8Mit211 was the resistance allele. When the locus-locus interactions were included in the analysis, however, none of the contribution from interaction were statistically significant.

#### **4.4.4 Summary of genetic linkage to skin tumour development**

The genetic linkage to skin tumour formation in F2 was analysed with three different methods, namely single marker analysis, interval QTL analysis and multi-locus regression analysis. Consistent with the analysis in F1BX, both papilloma and carcinoma development were indeed controlled by multiple genetic loci from both parental strains.

The data from all three analysis showed that papilloma development was linked to loci on chromosomes 6, 10, 11, 12 and 16. While the FVB/N allele on chromosome 6 was associated with lower papilloma incidence, its presence on the other four chromosomes increased the probability of papilloma formation. In another words, C57Bl/6J alleles on chromosomes 10, 11, 12 and 16 were the resistant alleles. Moreover, the results of regression analysis indicated that loci on chromosomes 3, 4, 5, 9, and 15 were also involved in papilloma formation in means of interactions with other loci.

<b>Markers</b>	<b>Locus (cM)</b>	<b>Coefficient</b>	<b>(p)</b>
D3MIT46	13.80	0.669	$1.0 \times 10^{-4}$
D12NDS2	55.00	0.571	$1.0 \times 10^{-4}$
D8MIT211	49.00	-0.637	$2.9 \times 10^{-5}$

**Table 4.5** Multi-locus stepwise Cox regression analysis of linkage to carcinoma development in FVB6F2 mice. The coefficient value indicated the degree of interactions between two parental alleles at individual locus. A positive coefficient value indicated resistant effect; A negative coefficient value implicated susceptibility to papilloma formation.

The carcinoma development in F2 mice was linked to loci on chromosomes 3 and 8. Mice carrying the FVB/N allele on chromosome 3 had lower carcinoma incidence, while those mice inheriting the FVB/N allele on chromosome 8 were susceptible to carcinoma formation. The resistant allele on chromosome 8 was C57BL/6J allele. Loci on chromosomes 6, 10, 12 and 16 may also involved in carcinoma formation even though they were only detected in one of the analysis methods. However, no significant locus-locus interactions were identified.

# **CHAPTER 5**

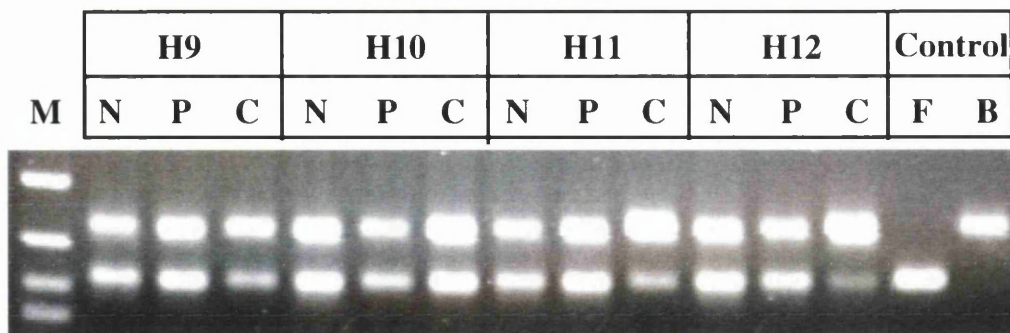
**THE LOH STUDY OF SKIN  
TUMOURS IN  
(FVB/N x C57BL/6J)F1 MICE**

The development of highly polymorphic genetic markers and progress in sophisticated statistical methods have made genetic linkage analysis a popular approach to identify the genetic loci that underlie complex traits. In addition, the advances of mapping techniques make it possible to detect genetic alterations which take place during tumour development in somatic cells and also germline mutations which can cause predisposition to the development of tumours (Knudson, 1993). This tumour mapping approach is particularly efficient in identifying loci that harbour genes conferring resistance to tumour development because they are likely to be deleted or mutated in most tumours.

## 5.1 Genomic mapping of skin tumours

PCR reactions were carried out using genomic DNA from papillomas (and carcinomas), in parallel with their corresponding tail DNA as controls. Most polymorphic microsatellite markers used in the linkage mapping were also used in the tumour mapping. A total of 16 papillomas and 12 carcinomas from 16 FVB6F1 mice (namely H1-H16) were used in the analysis. Marker loci with higher percentage of allelic losses or amplifications were considered as the location of genetic loci (genes) involved in the development of skin tumours.

Figure 5.1 shows an example of allelic analysis of tumours (papillomas and carcinomas) from four FVB6F1 mice (H9-H12) at locus D4Mit124. Compared with the two parental allelic amplifications in normal tissue, all four papilloma samples exhibited two equally amplified PCR products. But, unbalanced amplifications were observed in all four carcinoma samples. While the upper band (corespondent to C57BL/6J allele) of carcinoma H9 and H10 was only slightly stronger than the lower band (corespondent to FVB/N allele), the C57BL/6J band of carcinoma H11 and H12 was far stronger than the FVB/N band. There are two possible explanations for the cause of allelic imbalance. One is that the difference in the quantity of amplified PCR products reflects the difference in the copy numbers of parental alleles resulted from the genetic alteration events taking place in the tumours. In another words, the allelic imbalance reflects an equal amplification of unequal copy number of parental alleles, thus is *bona fide* allelic imbalance. The reduplication could be just a part of the chromosome, or the



**Figure 5.1** Allelotype analysis of papillomas and carcinomas from four FVB6F1 mice (H9-H12) at marker locus D4Mit124. PCR analysis were performed using the genomic DNAs isolated from the tails (N), papillomas (P) and carcinomas (C). The control samples were from FVB/N (F) and C57BL/6J (B) which showed the sizes of the PCR products of the two parental alleles.

whole chromosome which gave rise to the formation of chromosome trisomy (Aldaz *et al.*, 1989; Bremner and Balmain, 1990). Under the former circumstance, the allelic imbalance would only be shown at limited regional markers loci; under the latter circumstance, the allelic imbalance would be detected at marker loci throughout the whole chromosome. The allelic imbalance may also be interpreted as loss of heterozygosity (LOH) but obscured by the heterogeneity of tumour cells (thus uncompleted losses of FVB/N allele) or, in some cases, contamination with DNA from normal tissue. In both cases, the amplification of one allele would be far less than another. In the case genotyping at locus D4Mit124, the allelic imbalances shown in carcinomas H9 and H10 probably reflect reduplication of the C57BL/6J allele, while the allelic imbalances shown in carcinomas H11 and H12 are likely due to the loss of the FVB/N allele in these tumours.

## 5.2 Genomic alterations on chromosome 4

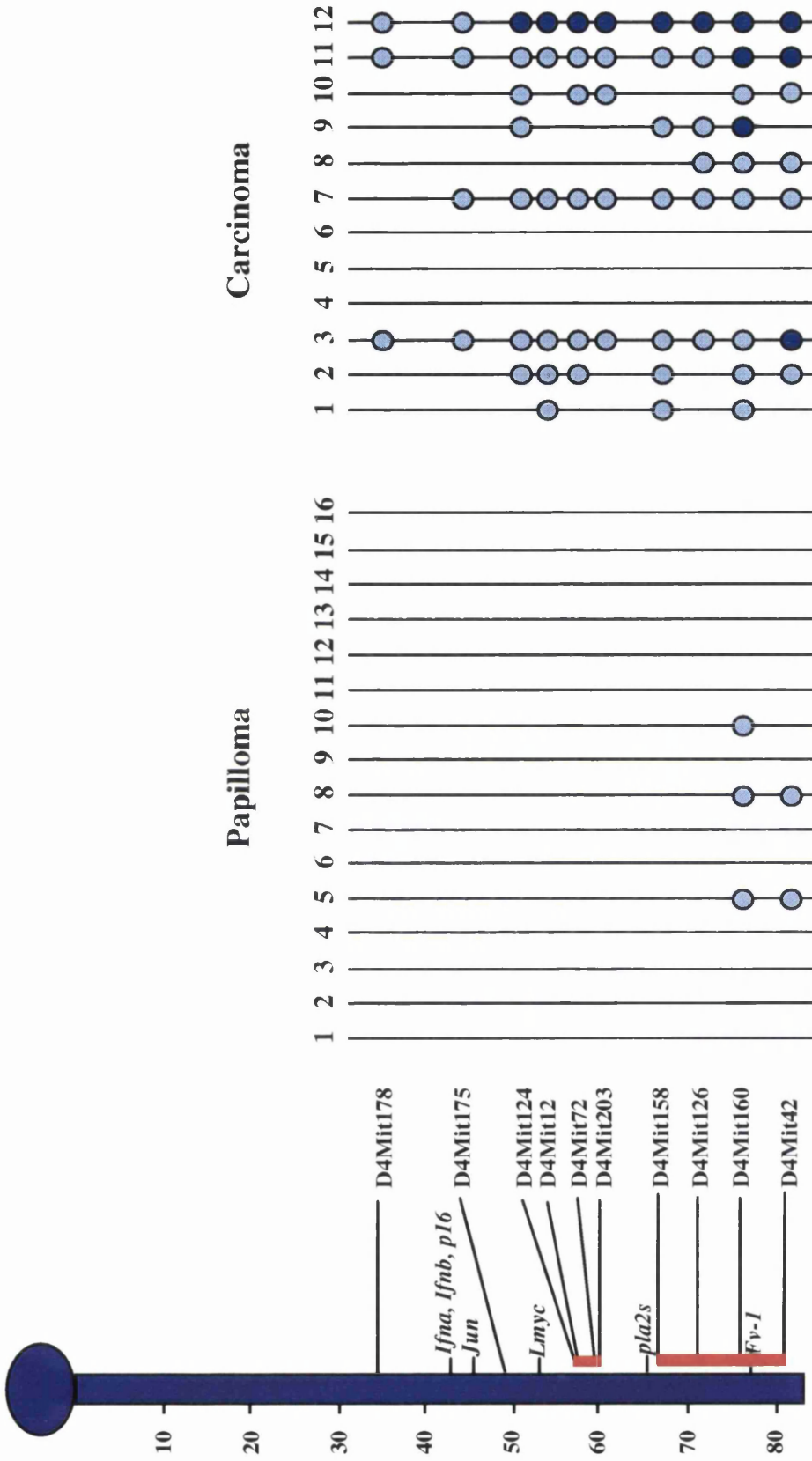
Mouse chromosome 4 has figured prominently in the findings of several mouse QTL linkage mapping and tumour LOH studies based upon a variety of established carcinogen induced tumour models, and a number of candidate tumour suppressor loci have been localised on it (Herzog *et al.*, 1994; Santos *et al.*, 1996; Aldaz *et al.*, 1996).

LOH of chromosome 4 has already been detected in spindle carcinomas from the Spr/NIH hybrid mice (Kemp *et al.*, 1993), implying that alteration of this chromosome is an important genetic event in the advantage stage of skin tumour development. To investigate the involvement of chromosome 4 in the skin tumours in FVB6F1 mice, the genomic DNA of papilloma and carcinoma samples were genotyped using ten markers on chromosome 4 to examine the genomic changes on this chromosome (Figure 5.2).

Of sixteen papillomas examined, only three showed allelic imbalance at loci on the distal region of chromosome 4. Two papillomas (H5 and H8) showed preferential amplification of C57BL/6J allele at both loci D4Mit160 and D4Mit42, whereas one papilloma H10 displayed allelic imbalance at locus D4Mit160, also in favour of C57BL/6J allele. Since the other eight loci on chromosome 4 all had normal amplification, it was unlikely that there was any major alteration affecting the whole chromosome. It is possible that only the distal part of C57Bl/6J chromosome was reduplicated in some of the papilloma cells.

In contrast, genetic alteration happened more frequently in carcinomas (Figure 5.2). Nine carcinoma samples exhibited some degree of LOH and allelic imbalance on chromosome 4, and as in papillomas all the alterations in carcinomas were in favour of retention of the C57BL/6J allele. Compared with the allelic amplification in normal tissues, five carcinoma samples (H1, 2, 8, 9, 10) showed reduplication of the distal part of C57BL/6J alleles, whilst allelic imbalances occurred at all ten loci in carcinomas H3, H11 and H12 and at nine loci in carcinoma H7. As the genomic amplification was consistent throughout chromosome 4, it is likely that the whole chromosome from the C57BL/6J parent was reduplicated and gave rise to chromosome 4 trisomy in tumour cells. Moreover, complete or partial loss of the FVB/N alleles was also observed. Extensive LOH was detected in carcinoma H12, the FVB/N allele was deleted at eight distal loci on chromosome 4, though in carcinomas H3, H9 and H11 LOH was restricted to the distal loci, D4Mit160 and D4Mit42, while. Of ten loci examined, marker locus D4Mit160 had the highest incidence of allelic alteration, preferential amplification was observed in 75% of carcinomas. Other most frequently altered loci were D4Mit124, D4Mit158 and D4Mit42, 58% of carcinomas showed allelic imbalances and LOH.

## Mouse Ch. 4



**Figure 5.2** Allelic loss and imbalance on chromosome 4 in papillomas and carcinomas of FVB/6F1 mice. The numbers on the top of each line represent the mouse that the tumour sample came from. The blue stippled and solid circle represents preferential amplification of the C57BL/6J allele and loss of the FVB/N allele, respectively. The red bars indicate the regions that are identified to be associated with tumour development in the linkage analysis.



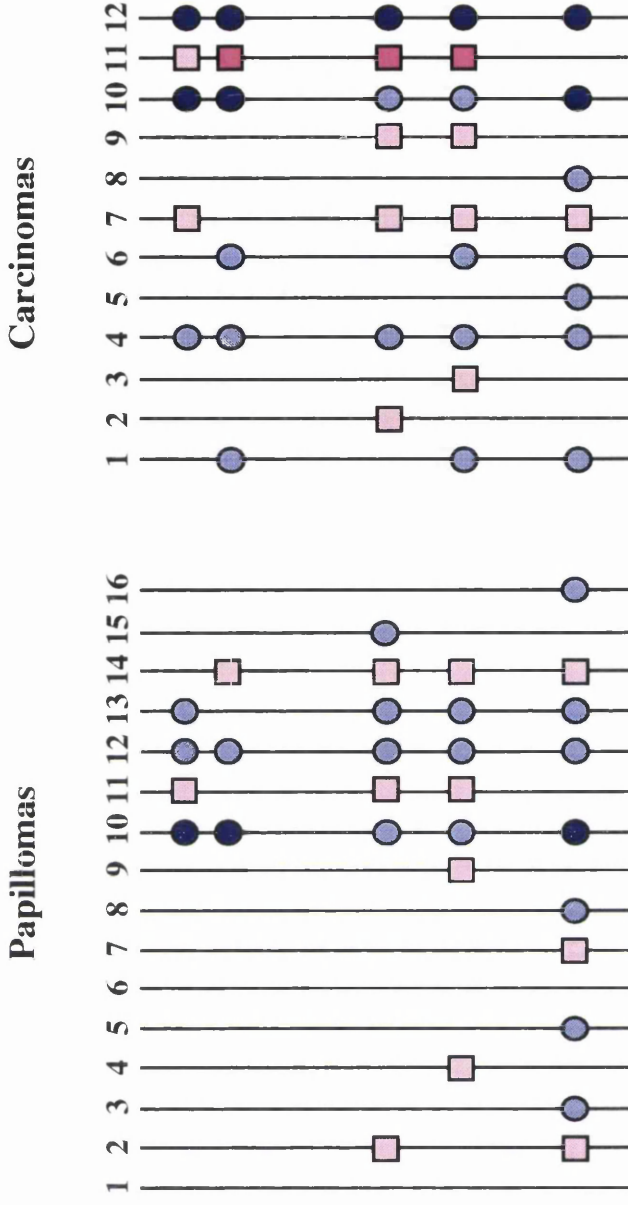
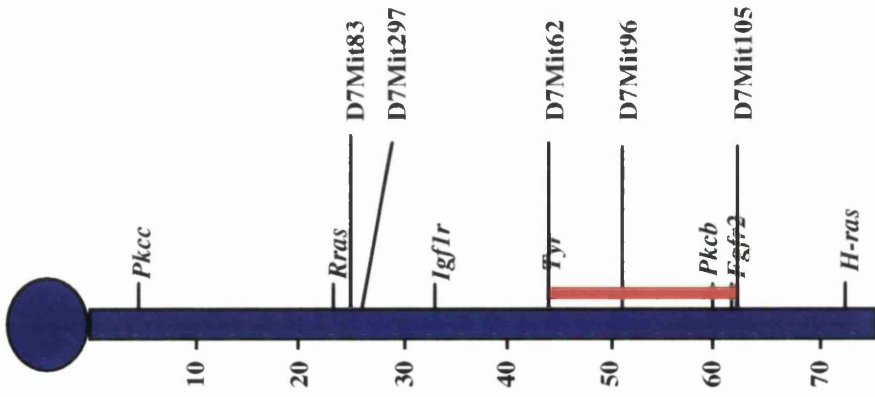
The data from tumour genotyping showed that genetic alterations in papillomas were limited to the distal part of chromosome 4. As papillomas progressed to carcinomas, it became more frequent and extended to the central region and beyond. Moreover, genetic events were also intensified from allelic imbalance in papillomas to LOH in carcinomas, particularly in the distal region of chromosome 4. Therefore, it seems a reasonable proposition that genetic loci on the distal region of chromosome 4 are involved throughout the whole process of skin tumour development, from the early papilloma stages to the late carcinoma stages. Furthermore, the fact that a higher incidence of genetic alterations on the central region of chromosome 4 was only observed in carcinoma suggests that genetic loci in this region were only involved in advanced carcinoma stages. These results are in consistent with the data of earlier QTL linkage analysis in which the same two regions were identified although they were mainly linked to the development of papillomas.

### **5.3 Genomic alterations on chromosome 7**

The involvement of mouse chromosome 7 in skin tumour was proposed when studies by two groups showed that trisomy of chromosome 7 frequently occurred in both papillomas and carcinomas (Aldaz *et al.*, 1989; Bremner and Balmain, 1990). Further analysis has demonstrated that loss of heterozygosity (LOH) of mouse chromosome 7 is also a consistent feature in malignant carcinomas (Bremner and Balmain, 1990; Bianchi *et al.*, 1990 and 1991). To investigate that contribution of chromosome 7 to the development of skin tumours in FVB6F1 mice, genotyping was carried out using genomic DNA extracted from 16 papillomas and 12 carcinomas of 16 FVB6F1 mice (H1-H16), in parallel with their corresponding tail DNA samples.

As shown in Figure 5.3, 88% of the papillomas showed allelic imbalances and LOH at loci on chromosome 7. Of fourteen papillomas which showed allelic imbalance, six had preferential amplification of the FVB/N allele and the other eight were in favour of the C57BL/6J allele amplification. While most papillomas displayed allelic imbalance at just one or two loci, extensive genetic alteration was also observed in four of fourteen papillomas. Papilloma H13 and H14 had allelic imbalance at four loci, and papillomas H10 and H12 at all five loci examined. The co-ordinated preferential amplification of

## Mouse Ch. 7



**Figure 5.3** Allelic loss and imbalance on chromosome 7 in papillomas and carcinomas of FVB6F1 mice. The numbers on the top of each line represent the mouse that the tumour sample came from. The blue stippled and solid circle represents the preferential amplification of the C57BL/6J allele and loss of the FVB/N allele, respectively. The pink stippled and solid square represents preferential amplification of the FVB/N allele and loss of the C57BL/6J allele, respectively. The red bar indicates the region which is identified involving in tumour development in the linkage analysis.

one particular allele throughout the whole chromosome and the ratio between FVB/N and C57BL/6J in these four papilloma samples indicated that chromosome 7 was trisomic in these papillomas. Most of the allelic imbalances took place in the central part of chromosome 7, with the highest incidence, 63%, observed at locus D7Mit105, followed by 44% at loci D7Mit96 and D7Mit62, implying that genetic loci from this region were probably involved in the papilloma formation. LOH (loss of FVB/N allele) was also observed in one papilloma, H10.

Consistent with previous reports (Bremner and Balmain, 1990; Bianchi *et al.*, 1990 and 1991), allelic imbalances and LOH were also detected in all carcinoma samples throughout chromosome 7. Five carcinomas showed FVB/N allele amplification and C57BL/6J allele loss; the other seven displayed amplification of the C57BL/6J allele and loss of the FVB/N allele. The co-ordination of preferential allele amplification in these tumours also indicated the presence of chromosome 7 trisomy in carcinomas. Moreover, seven of twelve of carcinomas exhibited extensive genetic alterations (at more than three loci). The highest incidence of allele imbalances, 75%, was observed at locus D7Mit96, followed by 67% at D7Mit105 and 58% at D7Mit62. Even the least frequent genetic alterations had reached 42% at locus D7Mit83.

The data presented here indicates that a broad region of the central part of chromosome 7 is involved in the whole process of skin tumour development. As papillomas progress to carcinomas, the incidence of genetic alterations increased. It is interesting to notice that, in papillomas and carcinomas, any genetic events at two proximal loci D7Mit83 and D7Mit297 were always coupled with alterations at the other three distal loci D7Mit62, D7Mit96 and D7Mit105, but not vice versa. This result implies that the genes close to the latter three loci may play more important roles in the tumour formation. Moreover these three loci had also been identified in the earlier linkage analysis, thus further confirming their association to the skin tumour development.

It has been shown that H-ras plays an important role in the development of skin tumours. Nearly all papillomas and carcinomas induced by DMBA-TPA exhibit mutation of H-ras gene by an A to T transversion at codon 61 (Quintanilla *et al.*, 1986). Moreover, reduplication of chromosome 7 does not take place randomly, it is always the one carrying the mutated H-ras gene that is duplicated (Bremner and Balmain, 1990;

Bianchi *et al.*, 1990). Unfortunately, due to the lack of polymorphic markers at the *H-ras* gene locus, we were unable to differentiate the two parental alleles (FVB/N and C57BL/6J). Therefore, we did not know whether the amplified chromosome was the one that carried the mutated *H-ras* gene. However, we did detect a mutation of the *H-ras* gene at codon 61 of in all the skin tumour samples.

#### **5.4 Genetic alteration on other chromosomes**

Genetic alterations in skin tumours induced by DMBA-TPA were also examined with microsatellite markers on other autosomal chromosomes. 16 papillomas and 12 carcinomas from 16 FVB6F1 mice were used to examine the allelic alteration. Of 8,000 genotyping with more than 100 microsatellite markers, only the marker loci with consistent genetic alteration are summarised in Table 5.1.

In general, allelic reduplication was the most frequent genetic events that took place in the skin tumours, whereas LOH rarely happened and was mostly detected in the carcinomas. Moreover, the frequency of genetic alterations was higher in carcinomas than in papillomas. The highest percentage of allelic imbalance in papillomas, 25%, was observed at locus D6Mit268, while it was 75% in carcinomas which was obtained at D12Mit231. Higher incidence of allelic imbalance was also observed on other chromosomes. On chromosome 6 (D6Mit14), chromosome 9 (D9Mit269), chromosome 10 (D10Mit248), and chromosome 16 (D16Mit64), more than 12.5% of papillomas had genomic alterations; on Chromosome 1 (D1Mit318), chromosome 12 (D12Mit231), chromosome 15 (D15Mit189), and chromosome 18 (D18Mit94), more than 40% of carcinomas showed allelic imbalances. At locus D6Mit268 on chromosome 6, higher incidence of allelic imbalance was found both in papillomas and carcinomas.

#### **5.5 Summary of tumour LOH analysis**

Genetic alterations in papillomas and carcinomas induced by carcinogen DMBA-TPA were examined with microsatellite markers spanning all autosomal chromosomes. The data presented here shows that the frequency of genetic alterations was higher in

Markers	Locus (cM)	Incidence of allelic imbalances *	
		Papillomas	Carcinomas
<b>D1Mit318</b>	<b>18.50</b>	1/16 ( 6.3%)	<b>5/12 (41.7%)</b>
D3Mit62	4.60	1/16 ( 6.3%)	2/12 (16.7%)
<b>D6Mit268</b>	<b>15.50</b>	<b>4/16 (25.0%)</b>	<b>6/12 (50.0%)</b>
D6Mit14	74.00	2/16 (12.5%)	4/12 (33.3%)
D9Mit269	43.00	2/16 (12.5%)	3/12 (25.0%)
<b>D10Mit248</b>	<b>7.00</b>	<b>3/16 (18.8%)</b>	4/12 (33.3%)
D11Mit99	65.00	1/16 ( 6.3%)	2/12 (16.7%)
<b>D12Mit231</b>	<b>48.00</b>	1/16 ( 6.3%)	<b>9/12 (75.0%)</b>
<b>D15Mit189</b>	<b>48.50</b>	1/16 ( 6.3%)	<b>5/12 (41.7%)</b>
D16Mit64	38.00	2/16 (12.5%)	2/12 (16.7%)
D17Mit176	22.50	1/16 ( 6.3%)	2/12 (16.7%)
<b>D18Mit94</b>	<b>17.00</b>	1/16 ( 6.3%)	<b>6/12 (50.0%)</b>

\* Number of samples with allelic imbalances/total number of samples (percentage of incidence)

**Table 5.1** The result of allelic analysis of skin tumours in FVB6F1 mice. The location of markers is the distance from centromere according to Mouse Genome Database. Markers with higher incidence of allelic imbalances were printed in bold. Note only marker locus with genetic alterations are listed in the table.

carcinomas than in papillomas. The most frequent genetic event that took place in papillomas and carcinomas was allelic reduplication. It was observed on nearly all chromosomes, particularly on chromosomes 4, 6, 7 and 10 in papillomas and chromosomes 1, 4, 6, 7, 12, 15, and 18 in carcinomas higher incidence of allelic imbalance was observed. This result is in consistent with the data discussed in previous two chapters, thus, further confirming the results of genetic linkage analysis. Moreover, consistent with previous studies, our data also demonstrated the presence of trisomies of chromosomes 4 and 7 in the papillomas and carcinomas. Loss of heterozygosity (LOH) was a rare event and mostly took place in carcinomas. High incidence of extensive LOH was only detected on two chromosomes, 4 and 7. Moreover, extensive allelic imbalance was also detected on these two chromosomes in both papillomas and carcinomas. These results suggest that genes on these two chromosomes may play important roles in the development of skin tumours.

# **CHAPTER 6**

## **DISCUSSION**

The aim of this study is to define the genetic basis of tumour susceptibility. We have chosen mouse skin carcinogenesis as the model system and utilised the genetic mapping approach to identify the genetic loci that affect susceptibility to skin tumours. The results of QTL linkage analysis in the F1BX and FVB6F2 crosses between the sensitive FVB/N and resistant C57BL/6J strains shows that the genetic loci on several mouse chromosomes, particularly chromosomes 4, 6, 9, 12, are associated with skin tumour formation. Confirmation of the involvement of these loci in tumour development is demonstrated in studies of tumours derived from the FVB6F1 mice.

## **6.1 Mouse as model organism for study of tumour susceptibility genes**

Cancer is a genetic disease which requires the involvement of multiple genetic factors. Linkage analysis has been the mainstay of efforts to identify the genes that can affect susceptibility to tumour development. As this approach usually requires large multi-generation families with clear inheritance pattern, studies are mainly limited to the relatively rare cancer families in which multiple members develop a particular form of tumour. Most genes identified by this approach are highly penetrant, such that individuals carrying mutated alleles through inheritance have a substantial probability of disease development. However, for the high frequency, low penetrance genes that are thought to affect the development of the vast majority of sporadic human cancers, it is extremely difficult to identify these genes by the classic human linkage analysis due to the lack of clear cut inheritance pattern.

The mouse offers distinctive advantages as a model system for identification of these low penetrance tumour susceptibility genes. A large number of mouse strains are available that, as a result of inbreeding or selective breeding, show enormous variation in their susceptibility to tumour development in certain tissues. Crossing of resistant and sensitive strains can give valuable information about the pattern of inheritance, as well as providing indications of the number of genes involved and their approximate locations in the genome. The unlimited number of 'family members' available for linkage analysis and advanced QTL linkage analysis methods greatly enhance the probability of finding multiple loci associated with a particular tumour. Moreover, the breakthrough in molecular genetic techniques has enable us to manipulate the mouse

germline to generate transgenic and knockout mice, thus, further facilitating the study of tumour susceptibility genes. Importantly, mice exposed to carcinogens develop tumours by a multistep process very similar to that seen in humans. The genetic alterations detected in mouse tumours involve genes such as *Ras*, *Rb*, *p53* and *p16Ink4a*, that are also the most commonly altered genes in human tumours (Knudson, 1993; Jacks, 1996; Ghebranious and Donehower, 1998). This underlying similarity in the biology of carcinogenesis in mouse and human implies that the genes which control susceptibility to mouse tumour development are also likely to be relevant to humans.

## 6.2 Multiple genetic loci control in skin tumour development

Analysis of the crosses between the sensitive FVB/N and resistant C57BL/6J strains showed that the F1 mice displayed a phenotype almost intermediate between the two parental strains. This indicates that the susceptibility to papillomas is inherited in a co-dominant manner. A similar inheritance pattern has also been observed by another group in the study of a cross between BABL/c (resistant) and SENCAR (sensitive) strains (Stern *et al.*, 1995). However, data from other genetic crosses suggest a different patterns of inheritance. In the study of the cross between *Mus spretus* (resistant) and NIH (sensitive) strains, the F1 mice were similar to the resistant parental *Mus spretus* mice and completely resistant to papillomas, indicating that the resistance was inherited as a dominant trait (Nagase *et al.*, 1995). The susceptibility to papillomas can also be inherited in an incomplete dominant pattern, as Naito observed in the F1 cross between C57BL/6 (resistant) and DBA/2 (sensitive) strains (Naito *et al.*, 1988). Such variation in inheritance patterns implies that the genes conferring the resistance (or susceptibility) in these resistant (or sensitive) strains are likely to be different. In different crosses the major dominant or recessive loci responsible for the phenotype of the F1 mice are likely to vary, resulting in different inheritance patterns. Hence, the susceptibility to the development of skin tumours in mice are likely to be controlled by multiple genetic loci.

Evidence of the involvement of multiple genetic loci also came from the studies of the F1BX and FVB6F2 mice. In terms of papilloma incidence, the phenotypes were clearly different in these two groups as the average number of papillomas was higher in the FVB6F2 mice than in the F1BX mice. In terms of the latency of papillomas, the



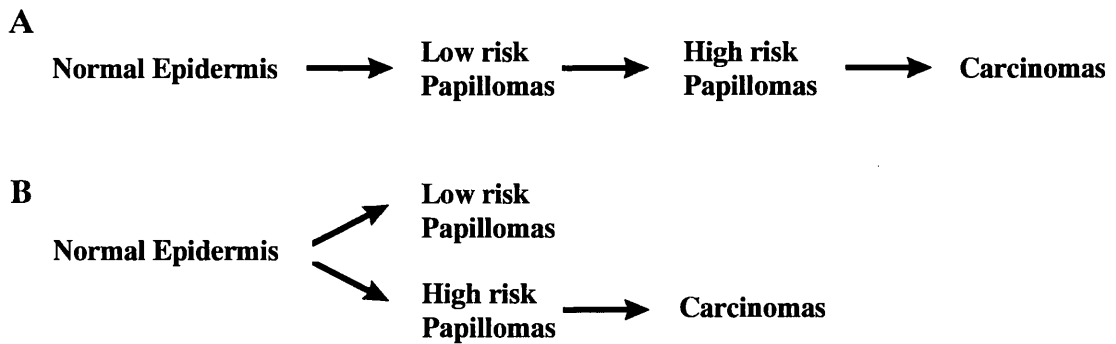
responses of the two groups of mice were, however, very similar despite the differences in their genomic background. Thus, it appears that different loci control the latency and incidence of the papillomas. Furthermore, data from the FVB6F2 cross shows that more than 15% of the mice developed extremely large numbers of papillomas (see Figure 4.5). Such high tumour incidence implies that susceptibility alleles are not only present in the sensitive strain (FVB/N), but are also likely to exist in the resistant strain (C57BL/6J). In the parental mice, the effects of susceptibility alleles in the resistant strain can not be observed because of the dominant effects of the resistant genes. However, in some of the F2 mice, these susceptibility alleles are segregated from the resistant genes and combined with the susceptibility alleles from the FVB/N strain, thus, increasing the overall sensitivity to papilloma formation. In a similar fashion, it is also possible that there are some FVB/N resistant alleles conferring resistance in the FVB6F2 mice. However, it is impossible to examine the additive effects of these resistant genes as resistant mice do not develop any papillomas.

The strongest evidence to support the involvement of multiple loci in skin tumour formation comes from linkage analysis. Previous studies of skin tumourigenesis by other groups have identified five loci that are associated with tumour development (Nagase *et al.*, 1995; Angel *et al.*, 1997; Mock *et al.*, 1998). Several more loci, from both the FVB/N and C57BL/6J alleles, have been identified in our linkage studies. Analysis of the F1BX cross shows that there are at least five loci involved in papilloma development. The C57BL/6J alleles at the loci D4Mit126 and D9Mit269, and D1Mit318 and D12Mit203 are associated with resistance, while the locus D6Mit14 is associated with sensitivity, to papilloma development. Analysis of the FVB6F2 cross has also identified several loci, particularly D6Mit14, D10Mit248, D11Mit99, D12Mit68, D15Mit189 and D16Mit64. The FVB/N alleles at D6Mit14 locus confers resistance, whereas the FVB/N alleles at the other loci confer susceptibility to tumour development mostly through interactions with other loci. The data from carcinomas further confirms this notion that skin tumour development is controlled by multiple genetic loci. Analysis of the F1BX cross shows that three loci, D7Mi83, D9Mit269 and D10Mit134, are associated with carcinoma formation. The F1BX mice carrying C57BL/6J alleles at these loci are resistant. In the FVB6F2 two more loci are identified. The FVB/N alleles at locus D3Mit46 confers resistance and locus D8Mit211 susceptibility to the development of carcinomas.

Cancer is a genetic disease arising from an accumulation of genetic alterations that may facilitate the cell transformation and outgrowth. Therefore, the notion of multiple genetic control discussed above should not be restricted to skin tumours. Indeed, linkage studies of carcinogenesis in other mouse models such as colon, liver, lung, and lymphomas have also identified many loci which affect tumour development (see Table 1.4 for loci and references). Among these loci, some are only found in a particular type of tumour, therefore, may act in a tissue-specific manner; others are often detected in multiple tumour models, suggesting their involvement in tumour development through a common mechanism.

### **6.3 The development of papillomas and carcinomas are under different genetic control**

Skin tumour progression takes place in a number of steps, passing through benign papillomas to malignant carcinomas. However, not all papillomas will progress to carcinomas, some papillomas remain at the benign tumour stage. Based on this observation, Yuspa (1994) has categorised the papillomas into two classes: 'low risk' papillomas which are likely to remain as a benign tumour, and 'high risk' papillomas which have a high probability of undergoing malignant progression and becoming carcinomas (Figure 6.1). It is, however, not clear whether the high risk papillomas are developed from the low risk papillomas or under an independent genetic pathway. If the progression from low risk papillomas to high risk papillomas and then carcinomas is purely linear, a gene which influences papilloma formation should also affect carcinoma formation. Our data suggest that this is not the case. Data from tumour incidence shows that in the three crosses (FVB6F1, F1BX and FVB6F2) the papilloma incidences are different but the carcinoma incidences are surprisingly similar. Furthermore, mice with large numbers of papillomas did not have a higher probability of developing carcinomas. Hence, our data support the notion that the development of papillomas and carcinomas are regulated by different genetic pathways. Further evidence comes from studies of the SENCAR strain and its derived inbred lines SSIN in which the genetic elements controlling the development of papillomas and carcinomas can be segregated during selection and inbreeding (Gimenez-Conti *et al.*, 1992).



**Figure 6.1** Genetic models for tumour development in multi-step skin carcinogenesis. Model A: Linear model; Model B: divergent pathway model.

This notion is also supported by the linkage studies. If the progression pathway is linear, it would be expected that any locus that confers resistance to papillomas should also inhibit the development of carcinomas. The loci that were detected, however, appear to exert their effects predominantly at one stage of carcinogenesis. Analyses of the F1BX and FVB6F2 crosses show that the loci identified can be divided into three groups: 1) loci that are only associated with papilloma formation; 2) loci that are only associated with carcinoma formation; and 3) loci that are involved in the development of both papillomas and carcinomas. A similar conclusion has also been formulated by Nagase *et al.* (1995). Using crosses between *Mus spretus* (resistant) and NIH (sensitive) strains they have identified three susceptibility loci, two on chromosome 7 that appear to control papilloma formation and one on chromosome 5 that affects the development of papillomas and carcinomas. Hence, we conclude that the development of benign and malignant tumours are largely under independent genetic control.

#### **6.4 Different response of male and female mice to skin tumour induction**

In our carcinogenesis studies, we found that the female F1BX mice developed nearly twice more papillomas with shorter latency than the male mice. A similar response has also been observed in the FVB6F2 cross (data not shown). Since these experiments were not originally designed to study the sex difference, only female FVB/N, C57BL/6J and F1 mice were used in the experiments. It is, unfortunately, not clear if such a

phenomenon is also present in the F1 and two parental strains of mice. Nevertheless, this study clearly demonstrates a significant sex difference in skin tumour development.

A substantial amount of effort has been expended by many research groups in an attempt to understand the genetic basis of strain variation in the sensitivity to skin tumours induced by carcinogens (Naito and DiGiovanni, 1989; DiGiovanni, 1995; Yuspa, 1994). However, none of the studies has focused on a sex difference. The genes responsible for the sex difference is still unknown. The previous study by Nagase *et al.* has detected a locus on chromosome 7 at which the *Mus spretus* allele confers resistant to papilloma development in female but not in male mice. However, the absence of a sex difference in papilloma incidence in their study implies that it may not be the locus responsible for the sex difference, or this locus may confer a sex difference but its effect may not be sufficient to result in a significant sex difference. Nevertheless, the fact that this locus is not identified in our linkage analysis means it is not responsible for the sex difference in our crosses. Moreover, as *Mus spretus* separate from *Mus musculus* approximately a million years ago, it is possible that this locus is specific to *Mus spretus*, no allelic variation is present in the two parental strains.

As the linkage analysis failed to detect any loci on the X chromosome, it is likely that the gene(s) conferring sex difference is an autosomal gene(s) which is regulated differentially in male and female mice. Among the genes known to be involved in mouse skin carcinogenesis, the Glutathione S-transferase (GST) pi gene has been found to have a sexually dimorphic expression pattern in the mouse (Hatayama *et al.*, 1993; Bammler *et al.*, 1994). It is transcribed at significantly higher levels in male mice than in females. GSTs are a superfamily of enzymes, responsible for the detoxification of a wide range of environmental chemicals and carcinogens, including the carcinogenic metabolite of polycyclic aromatic hydrocarbons such as DMBA (Hayes and Pulford, 1995; Romert *et al.*, 1989). Elevated levels of GSTs have been associated with malignant transformation and with experimental drug resistance, especially pi-class GSTs (Schechter *et al.*, 1992; Hayes and Pulford, 1995). Over-expression of GST pi has been associated with carcinogenesis and the development of many different human tumours, including lung, colon, testis, ovary, bladder, oral and kidney (Strange *et al.*, 1998; Henderson *et al.*, 1998a). Studies of lung tumour carcinogenesis have demonstrated that the GST pi protein is responsible for the sex difference in

susceptibility to tumour formation in the CD-1 mice (Sharma *et al.*, 1997). Recently, Henderson *et al.* (1998b) have found that GST pi-deficient mice have increased papilloma incidence, indicating that the GST pi protein plays an important role in resistance to skin tumourigenesis. It is however unclear whether the GST pi protein confers sex difference in FVB6F2 and F1BX mice. Further analysis is required to investigate if altered expression levels of GST pi protein could influence the susceptibility to papilloma development.

Although the locus where the GST pi genes located is not identified in our linkage analysis, we have detect a locus (the *Skt4* locus) on the distal region of chromosome 9 on which the alpha class of GST proteins is localised. Although the majority of human tumours and tumour cell lines express significant amounts of class pi GSTs, overexpression of class alpha isoenzymes is also often observed (Morel *et al.*, 1994; Eickelmann *et al.*, 1995; Den Boer *et al.*, 1999). Therefore, the alpha class GST proteins may, similarly to the pi class GSTs, also have a resistant effect on skin tumour development. Further studies are certainly needed to understand the roles that GST alpha may play in skin carcinogenesis.

## **6.5 Selective QTL mapping in the FVB6F2 mice**

The detection of QTLs requires a large sample size to attain reasonable power (Soller and Genizi, 1967). Despite technological improvement in the speed and accuracy with which molecular markers can be assayed, it can still be expensive and time consuming to analyse a large population. Lander and Botstein (1989) point out that, in the context of a cross between divergent lines, the most of the evidence on the existence of QTLs for a trait comes from the highest and lowest performing individuals. Genotyping only 50% of the population (the top and bottom 25%) can give more than 90% of the information that would be obtained from genotyping the whole population. To reduce the number of individuals needed to be genotyped in a QTL linkage study, a modified approach termed *selective genotyping* has been proposed (Lander and Botstein, 1989; Darvasi and Soller, 1992; Risch and Zhang, 1995). This approach starts with a large segregation population, but only the individuals from the high and low phenotypic extremes are analysed. Obviously, whole genome scanning of all mice (367 FVB6F2

mice in total) needs enormous time and effort. Because the majority of these mice exhibit an intermediate phenotype, we employed a selective mapping approach to analyse those mice that were highly sensitive or highly resistant to tumour induction.

The major benefit of the selective mapping approach lies in the saving of time and resources in genomic mapping. Given the same number of individuals genotyped in total population analysis versus selective analysis, the statistical power of QTL detection will be greater for the latter (Lander and Botstein, 1989). However, while it is more efficient at detecting linkage between marker loci and QTLs, it is less efficient in determining individual QTL effects. As individuals with extreme phenotypes tend to have either a large number of positive or negative alleles at all QTLs, there is a deficiency of individuals with a mixture of positive and negative alleles, which confounds the ability to individually measure the effects of QTLs (Allison *et al.*, 1998).

## **6.6 The advantage of multiple linkage analyses**

The genetic mapping approach has proven to be a powerful tool in the identification of tumour susceptibility loci which affect the development of tumours of the lung, colon, skin, liver and lymphoid system (Balmain and Nagase, 1998). Many statistical methods have been developed to extract all available inheritance information from experimental data and to test for inheritance of chromosomal regions with QTL traits. To obtain an unbiased and relatively complete result, we have chosen three different methods, namely single marker analysis, interval mapping and multi-locus regression analysis, to analyse the mapping data at three levels of increasing complexity.

All three methods have unique strengths or situations in which they are particularly useful in detecting and localising loci that contribute to quantitative traits. The single marker method is generally considered the very first step in data analysis because it tests each marker locus separately and is very sensitive in detecting the presence of QTLs with major or dominant effects on tumour incidence. However, the effect of a QTL and its distance from the proxy marker locus are inter-related. A QTL of small effect lying close to the proxy marker may appear similar to that of a QTL of large effect located further from the marker, as judged by the phenotypic differences between marker

genotype classes. With only a single marker, it is impossible to determine both the contribution of a QTL and its position. This problem can be resolved by the availability of complete marker maps and analysis by the interval mapping method (Lander and Botstein, 1989). This method gives much more accurate parameter estimates of the effect of a target QTL than the single marker analysis, although it requires prior construction of a marker genetic map, and the genotyping markers have to be well distributed throughout the chromosomes and the whole genome.

The first two methods work well when there is only a single QTL with a considerable contribution. However, it can be misleading when several QTLs are involved, particularly when the effect of a QTL depends on the phenotypes of its interacting QTL. Therefore, a third level of linkage analysis, termed multiple regression analysis, is required. The inclusion of the background markers makes the analysis more sensitive to the presence of a QTL in the target interval and helps to separate the target QTL from other linked QTLs (Jansen and Stam, 1994; Zeng, 1994). For instance, regression analysis shows that D12Mit203 can interact with D4Mit126. The effect of D4Mit126 is significant whatever the phenotype of D12Mit203, thus, D4Mit126 is detected by all three methods. The contribution of D12Mit203 is, however, largely dependent on the phenotype of D4Mit126. It can only reach to the significant level when the D4Mit126 locus is homozygous for the FVB/N alleles. When the effect of D12Mit203 was measured independently, it did not reach the significant level required for the interval mapping method, thus, was not detected. However, it can be detected by the more sensitive single marker analysis method. Therefore, we are certain that D12Mit203 is involved in papilloma development.

It is not surprising to note that linkage mapping in the F1BX and FVB6F2 crosses detected distinct set of QTLs. Some loci are the same or closely located, whilst others are completely different. The development of skin tumours requires the involvement of multiple genetic loci, and each individual locus is different in its strength and dominance. As every linkage analysis method has its limitation in its ability to locate and estimate the value of QTLs, it is impossible to detect all QTLs involved in one experiment. Moreover, the power of a QTL-detection experiment is also affected by many factors, including the strength of the QTL, the dominance of the QTL alleles, the type of cross, the size of the population, and the marker spacing (Lynch and Walsh,

1998). For instance, for additive QTLs, an F2 is more powerful than a backcross, but for dominant QTLs a backcross can be twice as powerful as an F2 (Darvasi, 1998). Therefore, it is quite possible that distinct sets of QTLs are detected in the F1BX and FVB6F2 crosses because each analysis will detect a different proportion of total QTLs. Even for the QTLs that are detected in both crosses, the differences in precision of location could result in the highest LOD scores being assigned to different markers. The linkage analysis data shows that D12Mit203 is involved in papilloma formation in the F1BX mice, whereas in the FVB6F2 mice the D12Mit68 locus is detected. Because the D12Mit203 locus is about 9 cM distal to the D12Mit68 locus and within the suggestive interval, it is likely that both loci correspond to the same gene.

## **6.7 Candidate genes for the susceptibility/resistance loci in skin carcinogenesis**

### **6.7.1 Candidate genes on chromosome 4**

The involvement of mouse chromosome 4 in mouse skin carcinogenesis was first highlighted during a genotype analysis of skin tumours from F1 hybrid mice (Kemp *et al.*, 1993). The loss of all or part of the *Mus spretus* chromosome 4 was detected in carcinomas. Analysis of three (one squamous and two spindle) carcinoma cell lines derived from (Spr x CBA) F1 mice showed that they were all trisomic for chromosome 4 (Liddell, 1995). Further analysis of carcinoma cell lines has also demonstrated LOH on the central region of chromosome 4 (Linardopoulos *et al.*, 1995). The data presented here has enable us to confirm the presence of two putative tumour susceptibility loci, *Skt1* and *Skt2*, on chromosome 4 that confer the resistance to mouse skin carcinogenesis.

*Skt1* resides on the central region of mouse chromosome 4 with D4Mit175 (49.60 cM) as the closet marker. Mice carrying the C57BL/6J alleles at this locus are more resistant to papillomas formation. Evidence for a tumour suppressor gene located in this region has also been indicated by studies of many other types of mouse tumours, including liver tumour (Lee GH *et al.*, 1995), plasmacytoma (Potter *et al.*, 1994), lung cancer (Hegi *et al.*, 1994; Herzog *et al.*, 1994), and thymic lymphoma (Santos *et al.*, 1996; Zhuang *et al.*, 1996). Moreover, the human syntenic regions to *Skt1*, chromosome 9p21-



22 and 1p31-32 (Abbott *et al.*, 1992), are also frequently deleted in head and neck carcinomas (van der Riet *et al.*, 1994), melanomas (Fountain *et al.*, 1992), bladder carcinomas (Cairns *et al.*, 1995), lung carcinomas (Olopade *et al.*, 1993), acute lymphocytic leukaemia (Iolascon *et al.*, 1997), and breast cancer (Bieche *et al.*, 1994).

Many cancer related genes, including the interferon genes and the oncogenes *Jun* and *Myc* (Ceci *et al.*, 1989; Abbott *et al.*, 1992), are located within this region. Thus it is difficult to propose a candidate tumour suppressor gene for this locus. Recently, the *p18* (*Cdkn2c*) gene, which encodes a protein belonging to a family of cyclin-dependent kinase (CDK) inhibitors, has been mapped to human chromosome 1p32 (Guan *et al.*, 1994; Hirai *et al.*, 1995). *p18* protein binds directly to the CDK4 or CDK6 and inhibits their promotion effects on the transition from G1 to S phases of the cell cycle, thus is a good candidate for the *Skt1* locus. Interestingly, the other two members of the same CDK inhibitor family, *p16* (*INK4a/Cdkn2a*) and *p15* (*INK4b/Cdkn2b*) genes, are located just 7 cM proximal to the *Skt1* region (Serrano *et al.*, 1993; Hannon and Beach, 1994; Quelle *et al.*, 1995). They, too, play an important role in the regulation of cell cycle (Serrano *et al.*, 1996; Sherr and Roberts, 1995). It has been demonstrated that *p15* and *p16* genes are frequently inactivated and deleted in a wide variety of human tumours (Kamb *et al.*, 1994; Nobori *et al.*, 1994; Hebert *et al.*, 1994), as well as mouse tumours including mouse skin tumours (Gause *et al.*, 1997; Linardopoulos *et al.*, 1995; Obata *et al.*, 1997). Therefore, *Skt1* could be the domain of three structurally related mouse cell-cycle regulatory genes *p15*, *p16* and *p18*. Extensive deletions of the *Skt1* region on chromosome 4 may cause the functional defect of multiple CDK inhibitors and lead to deregulated cell proliferation and transformation.

The second tumour susceptibility locus on chromosome 4, *Skt2*, is localised to the distal part of the chromosome. The most closely linked marker is D4Mit126 (71.0 cM). Linkage analysis in the F1BX cross shows that the *Skt2* locus has a major effect on papilloma development. Mice carrying C56BL/6J alleles were much more resistant than mice homozygous for FVB/N alleles. Further analysis of the FVB6F2 data indicates that it also has a profound effect on mice with high papilloma incidence. Genetic alterations in tumours from the FVB6F1 mice were also detected in this region although it was more frequent in carcinomas than in papillomas. This region is syntenic to human chromosome 1p35-36, which is often deleted in many different types of tumours

including colon cancer (Bardi *et al.*, 1993), breast cancer (Nagai *et al.*, 1995), hepatoma (Yeh *et al.*, 1994), and neuroblastoma (White *et al.*, 1995).

Linkage analysis in other mouse tumour models has also identified several tumour susceptibility loci close to the *Sk2* region, such as *Mom1* (Dietrich *et al.*, 1993) and *Ssic1* (Remond *et al.*, 1995) in colon cancer, *Pctr2* in plasmacytoma (Potter *et al.*, 1994), and loci in thymic lymphoma (Santos *et al.*, 1996; Zhuang *et al.*, 1996). The *Mom1* locus which is just 5 cM proximal of the *Sk2* locus is particularly interesting. The *Mom1* (Modifier of Min) locus was the first modifier locus identified which affects intestinal neoplasia in the study of genetic background effects on *Min*-mice (multiple intestinal neoplasia). A positional cloning study suggested a secretory phospholipase 2a (*Pla2g2a*) as the candidate gene for this locus (Dietrich *et al.*, 1993; MacPhee *et al.*, 1995). Confirmation of the *Pla2g2a* gene as the *Mom1* gene came from the demonstration that all sensitive strains had a mutation in the *Pla2g2a* gene while the gene was intact in resistant strains (Cormier *et al.*, 1997). Since there are some similarities in the process of intestinal and skin tumour development (Yuspa, 1994), it might be possible that *Mom1* also affects the development of skin tumours, and the mice susceptible to skin tumourigenesis should be expected to carry a mutated *Pla2g2a* gene. However, our data show that this is not the case. We have found that C57BL/6J mice, which are resistant to skin tumour formation, actually carry a mutated *Pla2g2a* gene, while the sensitive FVB/N mice, however, have a normal *Pla2g2a* gene (data not shown). Therefore, the *Pla2g2a* gene is unlikely to be the candidate gene for the *Sk2* locus.

There are many other genes located in the *Sk2* region that can be considered a potential candidate. One is the *p73* tumour suppressor gene which is localised to human chromosome 1p36, a syntenic with the *Sk2* region (Kaghad *et al.*, 1997; Jost *et al.*, 1997). The *p73* protein shows significant amino acid sequence and functional similarities to *p53*. It can activate the transcription of *p53*-responsive genes and inhibit cell growth in a *p53*-like manner by inducing cell cycle arrest and apoptosis (Jost *et al.*, 1997). Loss of the *p53* gene and normal *p53* function has been widely reported in mouse and human skin tumours (Burn *et al.*, 1991; Kemp *et al.*, 1993; Basset-Seguin *et al.*, 1994). Deletions of the *p73* gene have also been reported in many human cancers (Ichimiya *et al.*, 1999; Mai *et al.*, 1998).

Several more cancer related genes have also been mapped to the distal region of chromosome 4 (Mock *et al.*, 1997). The CDC2L1 gene, the mouse homologue of human p34cdc2-related PITSLRE protein kinase gene complex, shows a function consistent with those of tumour suppressors (Lahti *et al.*, 1995; Nelson *et al.*, 1999; Dave *et al.*, 1999). The RIZ gene encodes a zinc finger protein that can bind to the RB tumour suppressor and function as a negative regulator of tumourigenesis in breast cancer, neuroblastoma and lung cancer (Xie *et al.*, 1997; He *et al.*, 1998). They are also the potential candidates for the *Skf2* locus.

### 6.7.2 Candidate genes on mouse chromosome 7

The involvement of mouse chromosome 7 in skin tumours was first proposed in 1989. Studies from two groups have shown that trisomy of chromosome 7 is a frequent event at both the papilloma and carcinoma stages (Aldaz *et al.*, 1989; Bremner and Balmain, 1990). Moreover, the duplication of chromosome 7 is not random, but always involves the one which carries the mutated *H-ras* gene (Bremner and Balmain, 1990; Bianchi *et al.*, 1990), indicating that signal transduction through the *H-ras* pathway plays an important role in the development of skin tumours. Further analysis has demonstrated that LOH of chromosome 7 is also a consistent feature in malignant carcinomas (Bremner and Balmain, 1990; Bianchi *et al.*, 1990 and 1991).

Consistent with previous results, chromosome 7 trisomy, as well as LOH, was also demonstrated in our tumour studies. However, due to the lack of polymorphism in the *H-ras* gene locus between the two parental strains, it was impossible to identify which parental allele was duplicated. Nevertheless, the mutation of *H-ras* gene has been detected in all papillomas and carcinomas (data not shown). Linkage analysis has localised a carcinoma resistance locus *Skf3* to the distal region of chromosome 7, where most of the genetic alterations are found.

Interestingly, a previous study by Nagase *et al.* (1995) using (Spr x NIH) cross has already identified two resistance loci, *Spr1* and *Spr2*, on chromosome 7. These two loci are located in the two border regions (proximal and distal, respectively) of the *Skf3* locus. However, in contrast to *Skf3* which influences the carcinoma development, both

*Spr* loci appear to confer resistance to papilloma development. Therefore, they may represent different genes with effects on different stages of skin tumour formation.

The localisation of the *Skt3* locus to a region near the *Hbb* locus is particularly intriguing because this region of mouse chromosome 7 (Brilliant *et al.*, 1997) shows conservation of synteny with the human chromosome 11p15 region, which putatively contains a tumour suppressor affecting Wilm's tumour, rhabdomyosarcomas, gliomas and neuroectodermal tumour, (Besnard-Guerin *et al.*, 1996; Sonoda *et al.*, 1995; Fults *et al.*, 1992). Several other loci that influence tumour susceptibility to oral cavity tumours, hepatoma and thymic lymphoma in mice are also located in the same region on chromosome 7 (Held *et al.*, 1994; Angel *et al.*, 1993; Yuan *et al.*, 1997).

Several genes residing in this region can be considered as potential candidates for the *Skt3* locus. One possible gene is the p57(Kip2) gene, a member of cyclin-dependent kinase (CDK) inhibitor family (Brilliant *et al.*, 1997; Lee MH *et al.*, 1995). It has been demonstrated that mouse p57(Kip2) can control the cell cycle progression from G1 to S phase by functioning as a strong inhibitor of several G1 cyclin/CDK complexes (Matsuoka *et al.*, 1995; Lee MH *et al.*, 1995). Recently, studies carried out by Rodriguez-Puebla *et al.* (1998a and 1998b) indicate that p57Kip2 plays a key role in regulating proliferation in the epidermis. Another gene of interest encodes the protein kinase C (PKC), which is involved in skin tumour promotion. PKC plays an important role in the regulation of keratinocyte differentiation (Nishizuka, 1986; Yuspa, 1994). The phorbol ester promoters such as TPA can activate PKC and accelerate differentiation of normal keratinocytes, but not carcinogen-initiated keratinocytes (Hennings *et al.*, 1987; Dlugosz and Yuspa, 1993). Hence, alterations in this enzyme family are likely to contribute to skin tumourigenesis.

Several more genes which play an important role in skin carcinogenesis have also been mapped to chromosome 7, particularly *H-ras* and cyclin D1 (Brilliant *et al.*, 1997). Mutation of the *H-ras* gene has been identified as the initiation event (Balmain and Brown, 1988). The *H-ras* mutation can be detected in almost all papillomas and carcinomas, even in initiated skin prior to the emergence of tumours (Nelson *et al.*, 1992). Amplification or over-expression of the mutated *H-ras* gene is also associated with tumour promotion and progression (Quintanilla *et al.*, 1986; Bizub *et al.*, 1986).

The Cyclin D1 protein plays an important role in cell cycle regulation. Overexpression of the cyclin D1 protein has been observed in most advanced papillomas and carcinomas, as well as in some early papillomas (Bianchi *et al.*, 1993; Robles and Conti, 1995). The deficiency of cyclin D1 in mice, on the other hand, can result in up to an 80% decrease in the development of squamous tumours (Robles *et al.*, 1998). This is consistent with the observation that amplification of chromosome 7 is detected in most papillomas and carcinomas whereas loss of chromosome 7 is mainly seen in malignant carcinomas.

### 6.7.3 Mouse chromosome 9

Evidence of tumour suppressor loci on mouse chromosome 9 first came from the studies of tumours in transgenic mice (Dietrich *et al.*, 1994b). A genome-wide LOH analysis of end-stage insulinomas and metastatic carcinoid tumours led to the identification of the Loh-1 locus on the central region of chromosome 9. Further analysis of islet cell tumours at different stages from the same transgenic model has also demonstrated the loss of the central region of chromosome 9 in advanced carcinomas (Parangi *et al.*, 1995). This region shares synteny with human chromosome 3p21, which is also a hot spot for LOH in a wide variety of human tumour types (Lasko *et al.*, 1991). Moreover, linkage studies using recombinant congenic mouse strains has detected the Rapop2 locus, proximal to the Loh-1 locus, which is associated with radiation-induced apoptosis in the thymus and colon (Mori *et al.*, 1995 and 1998). The same region has also been found to be associated with skin tumour susceptibility in SENCAR/Pt and DBA/2 mouse strains (*Psl1* locus) (Angel *et al.*, 1997; Mock *et al.*, 1998). The *Skt4* locus, identified in our linkage study on the central region of mouse chromosome 9, confers resistance to the development of papillomas and carcinomas. D9Mit269 (43.0 cM) is the central marker for this locus.

Several interesting genes can be considered as the candidate genes, of which the genes encoding alpha class glutathione S-transferases (GST) is particularly interesting (Imai, 1997). As mentioned earlier, the GST pi proteins have been shown to confer resistance to skin tumourigenesis (Henderson *et al.*, 1998b). As the expression of GST alpha is also altered in a variety of human tumours and tumour cell lines (Eickelmann *et al.*, 1995; Den Boer *et al.*, 1999), it may also play an important role in skin tumourigenesis.

Another gene, the transforming growth factor  $\beta$  type II receptor (Tgfbr2), is also noteworthy. TGF $\beta$  plays an important role in differentiation processes and in the regulation of cell proliferation (Roberts *et al.*, 1990; Alexandrow and Moses, 1995). TGF $\beta$  is implicated in epithelial carcinogenesis due to its various effects on epithelial cells. It is a potent epithelial growth inhibitor and can alter the differentiative properties of keratinocytes (Masui *et al.*, 1986; Sellheyer *et al.*, 1993). The expression pattern of TGF $\beta$  in various epithelial derived tumours has been implicated as having a negative or positive effect on tumourigenesis (Cui *et al.*, 1996). The expression of TGF $\beta$  is elevated in response to the tumour promoter TPA (Akhurst *et al.*, 1988; Fowlis *et al.*, 1992), but disappears during the progression to carcinomas due to its growth inhibitory activities (Glick *et al.*, 1993; Cui *et al.*, 1994). But TGF $\beta$  can also stimulate malignant progression by its immunosuppressive activities or by enhancing angiogenesis (Glick *et al.*, 1994; Welch *et al.*, 1990). TGF $\beta$  conveys its signals via two TGF $\beta$  receptors, type I and type II (Derynck, 1994). Mutations of the receptors can interrupt the signal transduction pathways (Carcamo *et al.*, 1995). For example, inactivation or altered expression of TGF $\beta$  receptor II have been reported in some human tumours and tumour cell lines, as well as in mouse skin tumours (Markowitz, *et al.*, 1995; Garrigue-Antar *et al.*, 1995). The altered expression of TGF $\beta$  receptor II is also detected in skin tumourigenesis (Cui *et al.*, 1995).

The mouse homologue for the human ATM gene, located just proximal to the Skt4 locus, could also be a good candidate gene. ATM, the gene mutated in the inherited human disease ataxia telangiectasia, is a member of a family of kinases involved in DNA metabolism and cell-cycle checkpoint control (Hoekstra, 1997). It is a key regulator of multiple signalling cascades which respond to DNA strand breaks induced by damaging agents or by normal processes, such as meiosis and recombination. These responses involve the activation of cell cycle checkpoints, DNA repair and apoptosis (Xu *et al.*, 1996; Rotman and Shiloh, 1998). The roles of ATM in maintaining the integrity of the genome also make it a potential candidate gene.

#### **6.7.4 Mouse chromosome 8**

*Skt5*, an 10cM interval in the distal region of mouse chromosome 8, shows significant linkage to carcinoma development in the FVB6F2. Mice with FVB/N alleles at

D8Mit211 had higher carcinoma incidence than C57BL/6J homozygous mice. Tumour studies also detected LOH at the *Skf5* in carcinomas, but not in papillomas, suggesting that the gene at the *Skf5* locus is involved in the late stage of carcinoma development. The same region is also the location of the genetic loci detected in the liver tumours (Gariboldi *et al.*, 1993b; Davis *et al.*, 1994). In addition, its human syntenic region, chromosome 16q22-24, is often deleted in many types of tumour, such as prostate cancer, breast cancer and ovarian cancer (Carter *et al.*, 1990; Sato *et al.*, 1990 and 1991).

The genes of interest in this locus are the cluster of cadherin genes, particularly the E-cadherin (*Cdh1*) gene (Ceci and Mills, 1997). E-cadherin is a  $\text{Ca}^{2+}$ -dependent intercellular adhesion molecule which plays a central role in the regulation of keratinocyte intercellular contact junctions as well as in the epidermal morphogenesis and maintenance of skin structure (Wheelock and Jensen, 1992). Down-regulation of E-cadherin has been demonstrated during tumour progression in a wide variety of epithelial carcinomas (Birchmeier and Behrens, 1994; Takeichi, 1993), including mouse skin carcinomas (Navarro *et al.*, 1991; Ruggeri *et al.*, 1992). The loss of E-cadherin can cause the impairment of junction integrity of epithelial cells and lead to the loss of cell-cell adhesion. As a consequence, tumour cells obtain increased mobility and invasiveness (Birchmeier and Behrens, 1994).

### **6.7.5 Mouse chromosome 6**

It is well known that the trisomy of chromosomes 6 and 7 are a consistent feature at the early stage of mouse skin carcinogenesis (Aldaz *et al.*, 1989; Kemp *et al.*, 1993). Although it is clear that the duplication of chromosome 7 is to amplify the mutated *H-ras* gene, the reason behind chromosome 6 trisomy is still unknown. Although previous studies in mouse liver and lung tumours have identified several loci on the proximal and distal regions of mouse chromosome 6 (Zenklusen *et al.*, 1997; Fijneman *et al.*, 1996; Gariboldi *et al.*, 1993a), no locus has yet been found in skin tumours. The data presented here enable us to demonstrate the presence of a susceptibility locus, *Skf6*, on mouse chromosome 6 that is involved in the development of papillomas. This locus is located on the distal region of chromosome 6 (D6Mit14, 74.0 cM), overlapping the lung cancer susceptibility loci (Gariboldi *et al.*, 1993a).

Several genes located in this region are involved in the regulation of cell cycle and signal transduction pathway, such as K-ras2, cyclin D2 and fibroblast growth factor 6 (Fgf6), thus can be considered as the candidate genes (Elliott and Moore, 1997). Recently, the p27Kip1 gene (*cdn1b*) has been mapped to human chromosome 12p13 (Polyak *et al.*, 1994a; Martin *et al.*, 1995), which is syntenic to the *Skf6* locus and often deleted in ovarian cancer and leukaemia (Cave *et al.*, 1995; Hatta *et al.*, 1997). The p27Kip1 gene encodes a cyclin-dependent kinase inhibitor which binds to and inhibits the activity of cyclin-CDK complexes, thus blocks cell cycle progression (Polyak *et al.*, 1994b). Its importance in regulating cell growth is emphasised by the fact that p27Kip1 activity responds to a variety of growth inhibitory signals, including cell-cell contact, treatment with TGF $\beta$ , mitogens, cyclic AMP, and the growth-inhibitory drug rapamycin (Sherr and Robert, 1995). Abnormal low levels of expression and inactivating mutations of the p27Kip1 gene are frequently detected in many human tumours (Esposito *et al.*, 1997; Tsihlias *et al.*, 1998). The fact that p27 nullizygous and p27 heterozygous mice are predisposed to tumours in multiple tissues when treated with gamma-irradiation or carcinogens has also confirmed its roles in tumour suppression (Fero *et al.*, 1996 and 1998). p27Kip1 deficiency also resulted in an increased growth rate of benign papillomas and slightly accelerated conversion to carcinomas during skin carcinogenesis (Philipp *et al.*, 1999). Therefore, p27Kip1 is also a good candidate gene for the *Skf6* locus on chromosome 6.

## 6.8 Final thoughts

Using two-stage mouse skin carcinogenesis as a model system, we have carried out genetic linkage studies to identify the genetic loci that control susceptibility to skin tumour development. To our knowledge, it is the first time that two different crosses, a backcross (F1BX) and an intercross (FVB6F2), have been used in the same linkage study. The combined detecting powers of both crosses give a more accurate result, because the intercross is more powerful for additive loci detection while the backcross is better for detecting the C57BL/6J dominant loci (Darvasi, 1998). Ideally, a further third cross of that backcrosses the F1 to the resistant strain C57BL/6J ( (FVB/N x C57BL/6J)F1 x C57BL/6J) would give a better estimation of the FVB/N dominant loci, thus generate a complete set of genetic loci which affect tumour formation with different



patterns of dominance. In addition, we have also employed three different statistical methods to analyse the mapping data at three different levels. The combined analyses effectively compensate for the limitations and eliminate the systematic errors in each individual analysis, and give an unbiased and accurate result.

The genetic linkage approaches employed in this study have permitted the identification of the loci that influence susceptibility to skin tumour development. Most loci detected are new in skin carcinogenesis, though others have already been identified in other forms of cancers and some have been detected previously in skin tumourigenesis in other crosses. Points that can be draw from this study are: 1) the genetic linkage mapping approach can be a powerful tool in identifying the genes involved in the development of skin tumours; 2) the fact that several loci are identified in many types of tumours indicates that the genes identified via linkage approach are in some respects different from the known tumour suppressor genes. The germline mutations of these genes can influence the development of many types of tumour but can not be the determinative factor, whilst the germline mutation of tumour suppressor genes can cause tumour development but only limited to certain forms of hereditary cancer; 3) the genes which are detected in many types of tumours are likely to be involved in a common mechanism controlling the cell growth and differentiation. Genes of this type may include those associated with cell cycle control, signal transduction pathways, or apoptosis. Any mutation in these genes may alter the control of cell proliferation and could result in cell transformation or malignant conversion. Identification of these genes can greatly help understanding the genetic mechanism of tumour susceptibility.

The ultimate aim of such studies is to clone the gene and characterise its function in mouse skin tumour development. Once the position of a locus is determined, candidate gene from this region can be identified by positional cloning (Collins, 1991). However, the interval of genetic loci identified in our linkage study is relatively large and needs to be refined to less than 1 cM before a positional cloning approach can be attempted. The skin tumour samples from the F1, F1BX and F2 mice in our linkage study can certainly provide much valuable information. LOH and homozygous deletion studies as well as cytogenetic analysis can all be used to define the minimal deleted regions that contains the gene conferring resistance to tumour formation. Mutational analysis and newly developed microarray techniques can be used to examine mutations or differential

expression of known genes within the interval of interest, in tumours and normal tissue, and regard it as a starting point to determine the candidate gene and its roles in tumourigenesis (Skena *et al.*, 1995; DeRisi *et al.*, 1996). Moreover, the linkage mapping approach can also be used to obtain a refined and more precise location of the target locus. Higher resolution can also be achieved by using a larger population and a higher density of genetic markers around the tumour susceptibility locus, or selectively establishing inbred recombinant congenic lines (Moen *et al.*, 1991) or advanced intercross lines (Darvasi and Soller, 1995).

# REFERENCES

- Abbott, C. M., Blank, R., Eppig, J. T., Friedman, J. M., Huppi, K. E., Jackson, I., Mock, B. A., Stoye, J., and Wiseman, R. (1992). Mouse chromosome 4. *Mamm Genome 3 Spec No*, S55-64.
- Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M., and Cory, S. (1983). Cellular myc oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. *Proc Natl Acad Sci U S A 80*, 1982-6.
- Akhurst, R. J., Fee, F., and Balmain, A. (1988). Localized production of TGF-beta mRNA in tumour promoter-stimulated mouse epidermis. *Nature 331*, 363-5.
- Aldaz, C. M., Liao, Q. Y., Paladugu, A., Rehm, S., and Wang, H. (1996). Allelotypic and cytogenetic characterization of chemically induced mouse mammary tumors: high frequency of chromosome 4 loss of heterozygosity at advanced stages of progression. *Mol Carcinog 17*, 126-33.
- Aldaz, C. M., Trono, D., Larcher, F., Slaga, T. J., and Conti, C. J. (1989). Sequential trisomization of chromosomes 6 and 7 in mouse skin premalignant lesions. *Mol Carcinog 2*, 22-6.
- Alexandrow, M. G., and Moses, H. L. (1995). Transforming growth factor beta and cell cycle regulation. *Cancer Research 55*, 1452-7.
- Alitalo, K., and Schwab, M. (1986). Oncogene amplification in tumor cells. *Adv Cancer Res 47*, 235-81.
- Allison, D. B., Heo, M., Schork, N. J., Wong, S. L., and Elston, R. C. (1998). Extreme selection strategies in gene mapping studies of oligogenic quantitative traits do not always increase power. *Hum Hered 48*, 97-107.
- Ames, B. N. (1979). Identifying environmental chemicals causing mutations and cancer. *Science 204*, 587-93.
- Angel, J. M., Beltran, L., Minda, K., Rupp, T., and DiGiovanni, J. (1997). Association of a murine chromosome 9 locus (Psl1) with susceptibility to mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. *Mol Carcinog 20*, 162-7.
- Angel, J. M., Morizot, D. C., and Richie, E. R. (1993). Localization of a novel chromosome 7 locus that suppresses development of N-Methyl-N-nitrosourea-induced murine thymic lymphomas. *Mol Carcinog 7*, 151-6.
- Armitage, P., and Doll, R. (1954). The age distribution of cancer and a multi-stage theory of carcinogenesis. *Br J Cancer 8*, 1-12.
- Ashman, L. K., Murray, A. W., Cook, M. G., and Kotlarski, I. (1982). Two-stage skin carcinogenesis in sensitive and resistant mouse strains. *Carcinogenesis 3*, 99-102.
- Balmain, A., and Brown, K. (1988). Oncogene activation in chemical carcinogenesis. *Adv Cancer Res 51*, 147-82.

- Balmain, A., and Nagase, H. (1998). Cancer resistance genes in mice: models for the study of tumour modifiers. *Trends Genet* 14, 139-44.
- Balmain, A., Ramsden, M., Bowden, G. T., and Smith, J. (1984). Activation of the mouse cellular Harvey-ras gene in chemically induced benign skin papillomas. *Nature* 307, 658-60.
- Baltimore, D. (1970). RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 226, 1209-11.
- Bammler, T. K., Smith, C. A., and Wolf, C. R. (1994). Isolation and characterization of two mouse Pi-class glutathione S-transferase genes. *Biochem J* 298, 385-90.
- Bardi, G., Pandis, N., Fenger, C., Kronborg, O., Bomme, L., and Heim, S. (1993). Deletion of 1p36 as a primary chromosomal aberration in intestinal tumorigenesis. *Cancer Res* 53, 1895-8.
- Basset-Seguin, N., Moles, J. P., Mils, V., Dereure, O., and Guilhou, J. J. (1994). TP53 tumor suppressor gene and skin carcinogenesis. *J Invest Dermatol* 103, 102S-106S.
- Benedict, W. F., Weissman, B. E., Mark, C., and Stanbridge, E. J. (1984). Tumorigenicity of human HT1080 fibrosarcoma X normal fibroblast hybrids: chromosome dosage dependency. *Cancer Res* 44, 3471-9.
- Besnard-Guerin, C., Newsham, I., Winqvist, R., and Cavenee, W. K. (1996). A common region of loss of heterozygosity in Wilms' tumor and embryonal rhabdomyosarcoma distal to the D11S988 locus on chromosome 11p15.5. *Hum Genet* 97, 163-70.
- Bianchi, A. B., Aldaz, C. M., and Conti, C. J. (1990). Nonrandom duplication of the chromosome bearing a mutated Ha-ras-1 allele in mouse skin tumors. *Proc Natl Acad Sci U S A* 87, 6902-6.
- Bianchi, A. B., Fischer, S. M., Robles, A. I., Rinchik, E. M., and Conti, C. J. (1993). Overexpression of cyclin D1 in mouse skin carcinogenesis. *Oncogene* 8, 1127-33.
- Bianchi, A. B., Navone, N. M., Aldaz, C. M., and Conti, C. J. (1991). Overlapping loss of heterozygosity by mitotic recombination on mouse chromosome 7F1-ter in skin carcinogenesis. *Proc Natl Acad Sci U S A* 88, 7590-4.
- Bieche, I., Champeme, M. H., and Lidereau, R. (1994). A tumor suppressor gene on chromosome 1p32-pter controls the amplification of MYC family genes in breast cancer. *Cancer Res* 54, 4274-6.
- Birchmeier, W., and Behrens, J. (1994). Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta* 1198, 11-26.
- Bishop, J. M. (1988). The molecular genetics of cancer: 1988. *Leukemia* 2, 199-208.

- Bizub, D., Wood, A. W., and Skalka, A. M. (1986). Mutagenesis of the Ha-ras oncogene in mouse skin tumors induced by polycyclic aromatic hydrocarbons. *Proc Natl Acad Sci U S A* 83, 6048-52.
- Bookstein, R., Shew, J. Y., Chen, P. L., Scully, P., and Lee, W. H. (1990). Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated RB gene. *Science* 247, 712-5.
- Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32, 314-31.
- Boutwell, R. K. (1974). The function and mechanism of promoters of carcinogenesis. *Crit Rev Toxicol* 2, 419-443.
- Bremner, R., and Balmain, A. (1990). Genetic changes in skin tumor progression: correlation between presence of a mutant ras gene and loss of heterozygosity on mouse chromosome 7. *Cell* 61, 407-17.
- Brilliant, M. H., Williams, R. W., Holdener, B. C., Angel, J. M., Stern, M., and Hunter, K. (1997). Mouse Chromosome 7. *Mamm Genome* 7, 121-42.
- Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., and et al. (1994). Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 368, 258-61.
- Brown, K., Buchmann, A., and Balmain, A. (1990). Carcinogen-induced mutations in the mouse c-Ha-ras gene provide evidence of multiple pathways for tumor progression. *Proc Natl Acad Sci U S A* 87, 538-42.
- Brown, K., Kemp, C. J., Burns, P. A., Stoler, A. B., Fowles, D. J., Akhurst, R. J., and Balmain, A. (1993). Positive and negative growth control in multistage skin carcinogenesis. *Recent Results Cancer Res* 128, 309-21.
- Brown, K., Quintanilla, M., Ramsden, M., Kerr, I. B., Young, S., and Balmain, A. (1986). v-ras genes from Harvey and BALB murine sarcoma viruses can act as initiators of two-stage mouse skin carcinogenesis. *Cell* 46, 447-56.
- Buchmann, A., Ruggeri, B., Klein-Szanto, A. J., and Balmain, A. (1991). Progression of squamous carcinoma cells to spindle carcinomas of mouse skin is associated with an imbalance of H-ras alleles on chromosome 7. *Cancer Res* 51, 4097-101.
- Burns, P. A., Kemp, C. J., Gannon, J. V., Lane, D. P., Bremner, R., and Balmain, A. (1991). Loss of heterozygosity and mutational alterations of the p53 gene in skin tumours of interspecific hybrid mice. *Oncogene* 6, 2363-9.
- Cairns, P., Polascik, T. J., Eby, Y., Tokino, K., Califano, J., Merlo, A., Mao, L., Herath, J., Jenkins, R., Westra, W., and et al. (1995). Frequency of homozygous deletion at p16/CDKN2 in primary human tumours. *Nat Genet* 11, 210-2.

Cárcamo, J., Zentella, A., and Massagué, J. (1995). Disruption of transforming growth factor beta signaling by a mutation that prevents transphosphorylation within the receptor complex. *Molecular And Cellular Biology* 15, 1573-81.

Carter, B. S., Ewing, C. M., Ward, W. S., Treiger, B. F., Aalders, T. W., Schalken, J. A., Epstein, J. I., and Isaacs, W. B. (1990). Allelic loss of chromosomes 16q and 10q in human prostate cancer. *Proc Natl Acad Sci U S A* 87, 8751-5.

Cave, H., Gerard, B., Martin, E., Guidal, C., Devaux, I., Weissenbach, J., Elion, J., Vilmer, E., and Grandchamp, B. (1995). Loss of heterozygosity in the chromosomal region 12p12-13 is very common in childhood acute lymphoblastic leukemia and permits the precise localization of a tumor-suppressor gene distinct from p27KIP1. *Blood* 86, 3869-75.

Cavenee, W. K., Dryja, T. P., Phillips, R. A., Benedict, W. F., Godbout, R., Gallie, B. L., Murphree, A. L., Strong, L. C., and White, R. L. (1983). Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* 305, 779-84.

Ceci, J. D., and Mills, K. A. (1997). Mouse Chromosome 8. *Mamm Genome* 7, 143-58.

Ceci, J. D., Siracusa, L. D., Jenkins, N. A., and Copeland, N. G. (1989). A molecular genetic linkage map of mouse chromosome 4 including the localization of several proto-oncogenes. *Genomics* 5, 699-709.

Chen, Y. C., Chen, P. J., Yeh, S. H., Tien, H. F., Wang, C. H., Tang, J. L., and Hong, R. L. (1990). Deletion of the human retinoblastoma gene in primary leukemias. *Blood* 76, 2060-4.

Clarke, A. R., Maandag, E. R., van Roon, M., van der Lugt, N. M., van der Valk, M., Hooper, M. L., Berns, A., and te Riele, H. (1992). Requirement for a functional Rb-1 gene in murine development. *Nature* 359, 328-30.

Collins, F. S. (1991). Of needles and haystacks: finding human disease genes by positional cloning. *Clin Res* 39, 615-23.

Collins, S., and Groudine, M. (1982). Amplification of endogenous myc-related DNA sequences in a human myeloid leukaemia cell line. *Nature* 298, 679-81.

Cooper, G. M. (1995). *Oncogene*, 2nd Edition (London: Jones and Bartlett Publishers).

Copeland, N. G., Jenkins, N. A., Gilbert, D. J., Eppig, J. T., Maltais, L. J., Miller, J. C., Dietrich, W. F., and et al. (1993). A genetic linkage map of the mouse: current applications and future prospects. *Science* 262, 57-66.

Cormier, R. T., Hong, K. H., Halberg, R. B., Hawkins, T. L., Richardson, P., Mulherkar, R., Dove, W. F., and Lander, E. S. (1997). Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. *Nat Genet* 17, 88-91.

Cui, W., Fowles, D. J., Bryson, S., Duffie, E., Ireland, H., Balmain, A., and Akhurst, R. J. (1996). TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* 86, 531-42.

Cui, W., Fowles, D. J., Cousins, F. M., Duffie, E., Bryson, S., Balmain, A., and Akhurst, R. J. (1995). Concerted action of TGF-beta 1 and its type II receptor in control of epidermal homeostasis in transgenic mice. *Genes Dev* 9, 945-55.

Cui, W., Kemp, C. J., Duffie, E., Balmain, A., and Akhurst, R. J. (1994). Lack of transforming growth factor-beta 1 expression in benign skin tumors of p53null mice is prognostic for a high risk of malignant conversion. *Cancer Res* 54, 5831-6.

Darvasi, A. (1998). Experimental strategies for the genetic dissection of complex traits in animal models. *Nat Genet* 18, 19-24.

Darvasi, A., and Soller, M. (1995). Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* 141, 1199-207.

Darvasi, A., and Soller, M. (1992). Selective genotyping for determination of linkage between a marker locus and a quantitative trait loci. *Theor Appl Genet* 85, 353-359.

Darvasi, A., Weinreb, A., Minke, V., Weller, J. I., and Soller, M. (1993). Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics* 134, 943-51.

Dave, B. J., Pickering, D. L., Hess, M. M., Weisenburger, D. D., Armitage, J. O., and Sanger, W. G. (1999). Deletion of cell division cycle 2-like 1 gene locus on 1p36 in non-Hodgkin lymphoma. *Cancer Genet Cytogenet* 108, 120-6.

Davis, L. M., Caspary, W. J., Sakallah, S. A., Maronpot, R., Wiseman, R., Barrett, J. C., Elliott, R., and Hozier, J. C. (1994). Loss of heterozygosity in spontaneous and chemically induced tumors of the B6C3F1 mouse. *Carcinogenesis* 15, 1637-45.

Demant, P. (1992). Genetic resolution of susceptibility to cancer--new perspectives. *Semin Cancer Biol* 3, 159-66.

Den Boer, M. L., Pieters, R., Kazemier, K. M., Janka-Schaub, G. E., Henze, G., Creutzig, U., Kaspers, G. J., Kearns, P. R., Hall, A. G., Pearson, A. D., and Veerman, A. J. (1999). Different expression of glutathione S-transferase alpha, mu and pi in childhood acute lymphoblastic and myeloid leukaemia. *Br J Haematol* 104, 321-7.

DeRisi, J., Penland, L., Brown, P. O., Bittner, M. L., Meltzer, P. S., Ray, M., Chen, Y., Su, Y. A., and Trent, J. M. (1996). Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nature Genetics* 14, 457-60.

Derynck, R. (1994). TGF-beta-receptor-mediated signaling. *Trends In Biochemical Sciences* 19, 548-53.

Devereux, T. R., Wiseman, R. W., Kaplan, N., Garren, S., Foley, J. F., White, C. M., Anna, C., Watson, M. A., Patel, A., Jarchow, S., and et al. (1994). Assignment of a locus for mouse lung tumor susceptibility to proximal chromosome 19. *Mamm Genome* 5, 749-55.



- Diaz-Guerra, M., Haddow, S., Bauluz, C., Jorcano, J. L., Cano, A., Balmain, A., and Quintanilla, M. (1992). Expression of simple epithelial cytokeratins in mouse epidermal keratinocytes harboring Harvey ras gene alterations. *Cancer Res* 52, 680-7.
- Dib, C., Faure, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J., and Weissenbach, J. (1996). A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380, 152-4.
- Dietrich, W. F., Lander, E. S., Smith, J. S., Moser, A. R., Gould, K. A., Luongo, C., Borenstein, N., and Dove, W. (1993). Genetic identification of Mom-1, a major modifier locus affecting Min- induced intestinal neoplasia in the mouse. *Cell* 75, 631-9.
- Dietrich, W. F., Miller, J., Steen, R., Merchant, M. A., Damron-Boles, D., Husain, Z., and et al. (1996). A comprehensive genetic map of the mouse genome. *Nature* 380, 149-52.
- Dietrich, W. F., Miller, J. C., Steen, R. G., Merchant, M., Damron, D., Nahf, R., and et al. (1994a). A genetic map of the mouse with 4,006 simple sequence length polymorphisms. *Nat Genet* 7, 220-45.
- Dietrich, W. F., Radany, E. H., Smith, J. S., Bishop, J. M., Hanahan, D., and Lander, E. S. (1994b). Genome-wide search for loss of heterozygosity in transgenic mouse tumors reveals candidate tumor suppressor genes on chromosomes 9 and 16. *Proc Natl Acad Sci U S A* 91, 9451-5.
- DiGiovanni, J. (1995). Genetic factors controlling responsiveness to skin tumor promotion in mice. *Prog Clin Biol Res* 391, 195-212.
- DiGiovanni, J. (1992). Multistage carcinogenesis in mouse skin. *Pharmacol Ther* 54, 63-128.
- DiGiovanni, J., Imamoto, A., Naito, M., Walker, S. E., Beltran, L., Chenicek, K. J., and Skow, L. (1992). Further genetic analyses of skin tumor promoter susceptibility using inbred and recombinant inbred mice. *Carcinogenesis* 13, 525-31.
- DiGiovanni, J., Walker, S. C., Beltran, L., Naito, M., and Eastin, W. C., Jr. (1991). Evidence for a common genetic pathway controlling susceptibility to mouse skin tumor promotion by diverse classes of promoting agents. *Cancer Res* 51, 1398-405.
- Dlugosz, A. A., and Yuspa, S. H. (1993). Coordinate changes in gene expression which mark the spinous to granular cell transition in epidermis are regulated by protein kinase C. *J Cell Biol* 120, 217-25.
- Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 356, 215-21.
- Drinkwater, N. R., and Klotz, J. H. (1981). Statistical methods for the analysis of tumor multiplicity data. *Cancer Res* 41, 113-9.

- Edwards, M. D., Stuber, C. W., and Wendel, J. F. (1987). Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* 116, 113-25.
- Eickelmann, P., Morel, F., Schulz, W. A., and Sies, H. (1995). Turnover of glutathione S-transferase alpha mRNAs is accelerated by 12- O-tetradecanoyl phorbol-13-acetate in human hepatoma and colon carcinoma cell lines. *Eur J Biochem* 229, 21-6.
- Eliyahu, D., Michalovitz, D., Eliyahu, S., Pinhasi-Kimhi, O., and Oren, M. (1989). Wild-type p53 can inhibit oncogene-mediated focus formation. *Proc Natl Acad Sci U S A* 86, 8763-7.
- Eliyahu, D., Raz, A., Gruss, P., Givol, D., and Oren, M. (1984). Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* 312, 646-9.
- Elliott, R. W., and Moore, K. J. (1997). Mouse chromosome 6. *Mamm Genome* 7, S100-20.
- Esposito, V., Baldi, A., De Luca, A., Groger, A. M., Loda, M., Giordano, G. G., Caputi, M., Baldi, F., Pagano, M., and Giordano, A. (1997). Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. *Cancer Research* 57, 3381-5.
- Evans, E. P., Burtenshaw, M. D., Brown, B. B., Hennion, R., and Harris, H. (1982). The analysis of malignancy by cell fusion. IX. Re-examination and clarification of the cytogenetic problem. *J Cell Sci* 56, 113-30.
- Fearon, E. R. (1997). Human cancer syndromes: clues to the origin and nature of cancer. *Science* 278, 1043-50.
- Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., and et al. (1990). Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 247, 49-56.
- Fearon, E. R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759-67.
- Fero, M. L., Randel, E., Gurley, K. E., Roberts, J. M., and Kemp, C. J. (1998). The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature* 396, 177-80.
- Fero, M. L., Rivkin, M., Tasch, M., Porter, P., Carow, C. E., Firpo, E., Polyak, K., Tsai, L. H., Broudy, V., Perlmutter, R. M., Kaushansky, K., and Roberts, J. M. (1996). A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. *Cell* 85, 733-44.
- Festing, M. F., Lin, L., Devereux, T. R., Gao, F., Yang, A., Anna, C. H., White, C. M., Malkinson, A. M., and You, M. (1998). At least four loci and gender are associated with susceptibility to the chemical induction of lung adenomas in A/J x BALB/c mice. *Genomics* 53, 129-36.

Festing, M. F., Yang, A., and Malkinson, A. M. (1994). At least four genes and sex are associated with susceptibility to urethane-induced pulmonary adenomas in mice. *Genet Res* 64, 99-106.

Fijneman, R. J., de Vries, S. S., Jansen, R. C., and Demant, P. (1996). Complex interactions of new quantitative trait loci, Sluc1, Sluc2, Sluc3, and Sluc4, that influence the susceptibility to lung cancer in the mouse [see comments]. *Nat Genet* 14, 465-7.

Fijneman, R. J., Oomen, L. C., Snoek, M., and Demant, P. (1995). A susceptibility gene for alveolar lung tumors in the mouse maps between Hsp70.3 and G7 within the H2 complex. *Immunogenetics* 41, 106-9.

Finlay, C. A., Hinds, P. W., and Levine, A. J. (1989). The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 57, 1083-93.

Finzi, E., Kilkenny, A., Strickland, J. E., Balaschak, M., Bringman, T., Derynck, R., Aaronson, S., and Yuspa, S. H. (1988). TGF alpha stimulates growth of skin papillomas by autocrine and paracrine mechanisms but does not cause neoplastic progression. *Mol Carcinog* 1, 7-12.

Fishel, R., Lescoe, M. K., Rao, M. R., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1993). The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75, 1027-38.

Fountain, J. W., Karayiorgou, M., Ernstoff, M. S., Kirkwood, J. M., Vlock, D. R., Titus-Ernstoff, L., Bouchard, B., Vijayasaradhi, S., Houghton, A. N., Lahti, J., and et al. (1992). Homozygous deletions within human chromosome band 9p21 in melanoma. *Proc Natl Acad Sci U S A* 89, 10557-61.

Fowlis, D. J., Flanders, K. C., Duffie, E., Balmain, A., and Akhurst, R. J. (1992). Discordant transforming growth factor beta 1 RNA and protein localization during chemical carcinogenesis of the skin. *Cell Growth Differ* 3, 81-91.

Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M., and Dryja, T. P. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323, 643-6.

Fults, D., Petronio, J., Noblett, B. D., and Pedone, C. A. (1992). Chromosome 11p15 deletions in human malignant astrocytomas and primitive neuroectodermal tumors. *Genomics* 14, 799-801.

Fung, Y. K., Murphree, A. L., T'Ang, A., Qian, J., Hinrichs, S. H., and Benedict, W. F. (1987). Structural evidence for the authenticity of the human retinoblastoma gene. *Science* 236, 1657-61.

Gariboldi, M., Manenti, G., Canzian, F., Falvella, F. S., Pierotti, M. A., Della Porta, G., Binelli, G., and Dragani, T. A. (1993b). Chromosome mapping of murine susceptibility loci to liver carcinogenesis. *Cancer Res* 53, 209-11.

Gariboldi, M., Manenti, G., Canzian, F., Falvella, F. S., Radice, M. T., Pierotti, M. A., Della Porta, G., Binelli, G., and Dragani, T. A. (1993a). A major susceptibility locus to murine lung carcinogenesis maps on chromosome 6. *Nat Genet* 3, 132-6.

Garrigue-Antar, L., Muñoz-Antonia, T., Antonia, S. J., Gesmonde, J., Vellucci, V. F., and Reiss, M. (1995). Missense mutations of the transforming growth factor beta type II receptor in human head and neck squamous carcinoma cells. *Cancer Research* 55, 3982-7.

Gause, P. R., Lluria-Prevatt, M., Keith, W. N., Balmain, A., Linardopolous, S., Warneke, J., and Powell, M. B. (1997). Chromosomal and genetic alterations of 7,12-dimethylbenz[a]anthracene- induced melanoma from TP-ras transgenic mice. *Mol Carcinog* 20, 78-87.

Ghebranious, N., and Donehower, L. A. (1998). Mouse models in tumor suppression. *Oncogene* 17, 3385-400.

Gimenez-Conti, I., Aldaz, C. M., Bianchi, A. B., Roop, D. R., Slaga, T. J., and Conti, C. J. (1990). Early expression of type I K13 keratin in the progression of mouse skin papillomas. *Carcinogenesis* 11, 1995-9.

Gimenez-Conti, I. B., Bianchi, A. B., Fischer, S. M., Reiners, J. J., Jr., Conti, C. J., and Slaga, T. J. (1992). Dissociation of sensitivities to tumor promotion and progression in outbred and inbred SENCAR mice. *Cancer Res* 52, 3432-5.

Glick, A. B., Kulkarni, A. B., Tennenbaum, T., Hennings, H., Flanders, K. C., O'Reilly, M., Sporn, M. B., Karlsson, S., and Yuspa, S. H. (1993). Loss of expression of transforming growth factor beta in skin and skin tumors is associated with hyperproliferation and a high risk for malignant conversion. *Proc Natl Acad Sci U S A* 90, 6076-80.

Glick, A. B., Lee, M. M., Darwiche, N., Kulkarni, A. B., Karlsson, S., and Yuspa, S. H. (1994). Targeted deletion of the TGF-beta 1 gene causes rapid progression to squamous cell carcinoma. *Genes Dev* 8, 2429-40.

Goddard, K. A., Yu, C. E., Oshima, J., Miki, T., Nakura, J., Piussan, C., Martin, G. M., Schellenberg, G. D., and Wijsman, E. M. (1996). Toward localization of the Werner syndrome gene by linkage disequilibrium and ancestral haplotyping: lessons learned from analysis of 35 chromosome 8p11.1-21.1 markers. *Am J Hum Genet* 58, 1286-302.

Guan, K. L., Jenkins, C. W., Li, Y., Nichols, M. A., Wu, X., O'Keefe, C. L., Matera, A. G., and Xiong, Y. (1994). Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. *Genes Dev* 8, 2939-52.

Halmekyto, M., Syrjanen, K., Janne, J., and Alhonen, L. (1992). Enhanced papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene. *Biochem Biophys Res Commun* 187, 493-7.

Hannon, G. J., and Beach, D. (1994). p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 371, 257-61.

Harper, J. R., Reynolds, S. H., Greenhalgh, D. A., Strickland, J. E., Lical, J. C., and Yuspa, S. H. (1987). Analysis of the rasH oncogene and its p21 product in chemically induced skin tumors and tumor-derived cell lines. *Carcinogenesis* 8, 1821-5.

Harris, H., Miller, O. J., Klein, G., Worst, P., and Tachibana, T. (1969). Suppression of malignancy by cell fusion. *Nature* 223, 363-368.

Hatayama, I., Nishimura, S., Narita, T., and Sato, K. (1993). Sex-dependent expression of class pi glutathione S-transferase during chemical hepatocarcinogenesis in B6C3F1 mice. *Carcinogenesis* 14, 537-8.

Hatta, Y., Takeuchi, S., Yokota, J., and Koeffler, H. P. (1997). Ovarian cancer has frequent loss of heterozygosity at chromosome 12p12.3-13.1 (region of TEL and Kip1 loci) and chromosome 12q23-ter: evidence for two new tumour-suppressor genes. *Br J Cancer* 75, 1256-62.

Hayes, J. D., and Pulford, D. J. (1995). The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 30, 445-600.

Hayward, W. S., Neel, B. G., and Astrin, S. M. (1981). Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukemia. *Nature* 290, 475-80.

He, L., Yu, J. X., Liu, L., Buyse, I. M., Wang, M. S., Yang, Q. C., Nakagawara, A., Brodeur, G. M., Shi, Y. E., and Huang, S. (1998). RIZ1, but not the alternative RIZ2 product of the same gene, is underexpressed in breast cancer, and forced RIZ1 expression causes G2-M cell cycle arrest and/or apoptosis. *Cancer Res* 58, 4238-44.

Hebert, J., Cayuela, J. M., Berkeley, J., and Sigaux, F. (1994). Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) display frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. *Blood* 84, 4038-44.

Hegi, M. E., Devereux, T. R., Dietrich, W. F., Cochran, C. J., Lander, E. S., Foley, J. F., Maronpot, R. R., Anderson, M. W., and Wiseman, R. W. (1994). Allelotype analysis of mouse lung carcinomas reveals frequent allelic losses on chromosome 4 and an association between allelic imbalances on chromosome 6 and K-ras activation. *Cancer Res* 54, 6257-64.

Heim, S., Mandahl, N., and Mitelman, F. (1988). Genetic convergence and divergence in tumor progression. *Cancer Res* 48, 5911-6.

Held, W. A., Pazik, J., O'Brien, J. G., Kerns, K., Gobey, M., Meis, R., Kenny, L., and Rustum, Y. (1994). Genetic analysis of liver tumorigenesis in SV40 T antigen transgenic mice implies a role for imprinted genes. *Cancer Res* 54, 6489-95.

Henderson, C. J., McLaren, A. W., Moffat, G. J., Bacon, E. J., and Wolf, C. R. (1998a). Pi-class glutathione S-transferase: regulation and function. *Chem Biol Interact* 111-112, 69-82.

- Henderson, C. J., Smith, A. G., Ure, J., Brown, K., Bacon, E. J., and Wolf, C. R. (1998b). Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. *Proc Natl Acad Sci U S A* 95, 5275-80.
- Hennings, H., Glick, A. B., Lowry, D. T., Krsmanovic, L. S., Sly, L. M., and Yuspa, S. H. (1993). FVB/N mice: an inbred strain sensitive to the chemical induction of squamous cell carcinomas in the skin. *Carcinogenesis* 14, 2353-8.
- Hennings, H., Michael, D., Lichti, U., and Yuspa, S. H. (1987). Response of carcinogen-altered mouse epidermal cells to phorbol ester tumor promoters and calcium. *J Invest Dermatol* 88, 60-5.
- Hennings, H., Robinson, V. A., Michael, D. M., Pettit, G. R., Jung, R., and Yuspa, S. H. (1990a). Development of an in vitro analogue of initiated mouse epidermis to study tumor promoters and antipromoters. *Cancer Res* 50, 4794-800.
- Hennings, H., Shores, R., Balaschak, M., and Yuspa, S. H. (1990b). Sensitivity of subpopulations of mouse skin papillomas to malignant conversion by urethane or 4-nitroquinoline N-oxide. *Cancer Res* 50, 653-7.
- Hennings, H., Shores, R., Mitchell, P., Spangler, E. F., and Yuspa, S. H. (1985). Induction of papillomas with a high probability of conversion to malignancy. *Carcinogenesis* 6, 1607-10.
- Hennings, H., Shores, R., Wenk, M. L., Spangler, E. F., Tarone, R., and Yuspa, S. H. (1983). Malignant conversion of mouse skin tumours is increased by tumour initiators and unaffected by tumour promoters. *Nature* 304, 67-9.
- Herskowitz, I. (1987). Functional inactivation of genes by dominant negative mutations. *Nature* 329, 219-22.
- Herzog, C. R., Wiseman, R. W., and You, M. (1994). Deletion mapping of a putative tumor suppressor gene on chromosome 4 in mouse lung tumors. *Cancer Res* 54, 4007-10.
- Hesketh, R. (1997). *The Oncogene and Tumour Suppressor Gene Factsbook*, 2nd Edition (San Diego London: Academic Press).
- Hinds, P., Finlay, C., and Levine, A. J. (1989). Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. *J Virol* 63, 739-46.
- Hinds, P. W., and Weinberg, R. A. (1994). Tumor suppressor genes. *Curr Opin Genet Dev* 4, 135-41.
- Hirai, H., Roussel, M. F., Kato, J. Y., Ashmun, R. A., and Sherr, C. J. (1995). Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. *Mol Cell Biol* 15, 2672-81.
- Hoekstra, M. F. (1997). Responses to DNA damage and regulation of cell cycle checkpoints by the ATM protein kinase family. *Current Opinion In Genetics And Development* 7, 170-5.

Hofstra, R. M., Landsvater, R. M., Ceccherini, I., Stulp, R. P., Stelwagen, T., and et al. (1994). A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature* 367, 375-6.

Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991). p53 mutations in human cancers. *Science* 253, 49-53.

Horowitz, J. M., Yandell, D. W., Park, S. H., Canning, S., Whyte, P., Buchkovich, K., Harlow, E., Weinberg, R. A., and Dryja, T. P. (1989). Point mutational inactivation of the retinoblastoma antioncogene. *Science* 243, 937-40.

Howe, J. R., Roth, S., Ringold, J. C., Summers, R. W., Jarvinen, H. J., Sistonen, P., Tomlinson, I. P., Houlston, R. S., Bevan, S., Mitros, F. A., Stone, E. M., and Aaltonen, L. A. (1998). Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 280, 1086-8.

Hu, N., Gutschmann, A., Herbert, D. C., Bradley, A., Lee, W. H., and Lee, E. Y. (1994). Heterozygous Rb-1 delta 20/+mice are predisposed to tumors of the pituitary gland with a nearly complete penetrance. *Oncogene* 9, 1021-7.

Hussussian, C. J., Struewing, J. P., Goldstein, A. M., Higgins, P. A., Ally, D. S., Sheahan, M. D., Clark, W. H., Jr., Tucker, M. A., and Dracopoli, N. C. (1994). Germline p16 mutations in familial melanoma. *Nat Genet* 8, 15-21.

Ichimiya, S., Nimura, Y., Kageyama, H., Takada, N., Sunahara, M., Shishikura, T., Nakamura, Y., Sakiyama, S., Seki, N., Ohira, M., Kaneko, Y., McKeon, F., Caput, D., and Nakagawara, A. (1999). p73 at chromosome 1p36.3 is lost in advanced stage neuroblastoma but its mutation is infrequent. *Oncogene* 18, 1061-6.

Imai, K. (1997). Mouse Chromosome 9. *Mamm Genome* 7, 159-75.

Imamoto, A., Beltran, L. M., and DiGiovanni, J. (1991). Evidence for autocrine/paracrine growth stimulation by transforming growth factor-alpha during the process of skin tumor promotion. *Mol Carcinog* 4, 52-60.

Imamoto, A., Beltran, L. M., Fujiki, H., Chenicek, K. J., and DiGiovanni, J. (1992). Enhanced induction of epidermal ornithine decarboxylase activity in C57BL/6 compared to DBA/2 mice by protein kinase C-activating skin tumor promoters: relevance to genetically mediated differences in promotion susceptibility. *Carcinogenesis* 13, 177-82.

Iolascon, A., Faienza, M. F., Coppola, B., Moretti, A., Basso, G., Amaru, R., Vigano, G., and Biondi, A. (1997). Frequent clonal loss of heterozygosity (LOH) in the chromosomal region 1p32 occurs in childhood T cell acute lymphoblastic leukemia (T-ALL) carrying rearrangements of the TAL1 gene. *Leukemia* 11, 359-63.

Ishikawa, J., Xu, H. J., Hu, S. X., Yandell, D. W., Maeda, S., Kamidono, S., Benedict, W. F., and Takahashi, R. (1991). Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Res* 51, 5736-43.

- Jacks, T. (1996). Tumor suppressor gene mutations in mice. *Annu Rev Genet* 30, 603-36.
- Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A., and Weinberg, R. A. (1992). Effects of an Rb mutation in the mouse. *Nature* 359, 295-300.
- Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T., and Weinberg, R. A. (1994). Tumor spectrum analysis in p53-mutant mice. *Curr Biol* 4, 1-7.
- Jacoby, R. F., Hohman, C., Marshall, D. J., Frick, T. J., Schlack, S., Broda, M., Smutko, J., and Elliott, R. W. (1994). Genetic analysis of colon cancer susceptibility in mice. *Genomics* 22, 381-7.
- Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics* 135, 205-11.
- Jansen, R. C., and Stam, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136, 1447-55.
- Jeffreys, A. J., Wilson, V., Thein, S. L., Weatherall, D. J., and Ponder, B. A. (1986). DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees. *Am J Hum Genet* 39, 11-24.
- Jiang, C., and Zeng, Z. B. (1995). Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* 140, 1111-27.
- Jost, C. A., Marin, M. C., and Kaelin, W. G., Jr. (1997). p73 is a human p53-related protein that can induce apoptosis. *Nature* 389, 191-4.
- Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Lelias, J. M., Dumont, X., Ferrara, P., McKeon, F., and Caput, D. (1997). Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 90, 809-19.
- Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavitgian, S. V., Stockert, E., Day, R. S., 3rd, Johnson, B. E., and Skolnick, M. H. (1994). A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264, 436-40.
- Kemp, C. J., Fee, F., and Balmain, A. (1993). Allelotype analysis of mouse skin tumors using polymorphic microsatellites: sequential genetic alterations on chromosomes 6, 7, and 11. *Cancer Res* 53, 6022-7.
- Kemp, C. J., Wheldon, T., and Balmain, A. (1994). p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. *Nat Genet* 8, 66-9.
- Kiguchi, K., Beltran, L. M., You, J., Rho, O., and DiGiovanni, J. (1995). Elevation of transforming growth factor-alpha mRNA and protein expression by diverse tumor promoters in SENCAR mouse epidermis. *Mol Carcinog* 12, 225-35.



- Kinzler, K. W., and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 386, 761, 763.
- Kinzler, K. W., and Vogelstein, B. (1998). Landscaping the cancer terrain. *Science* 280, 1036-7.
- Kinzler, K. W., and Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell* 87, 159-70.
- Klein-Szanto, A. J., Larcher, F., Bonfil, R. D., and Conti, C. J. (1989). Multistage chemical carcinogenesis protocols produce spindle cell carcinomas of the mouse skin. *Carcinogenesis* 10, 2169-72.
- Knudson, A. G. (1993). Antioncogenes and human cancer. *Proc Natl Acad Sci U S A* 90, 10914-21.
- Knudson, A. G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 68, 820-3.
- Knudson, A. G., Jr., and Strong, L. C. (1972a). Mutation and cancer: a model for Wilms' tumor of the kidney. *J Natl Cancer Inst* 48, 313-24.
- Knudson, A. G., Jr., and Strong, L. C. (1972b). Mutation and cancer: neuroblastoma and pheochromocytoma. *Am J Hum Genet* 24, 514-32.
- Ko, L. J., and Prives, C. (1996). p53: puzzle and paradigm. *Genes Dev* 10, 1054-72.
- Kruglyak, L., and Lander, E. S. (1995). Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57, 439-54.
- Lahti, J. M., Xiang, J., and Kidd, V. J. (1995). The PITSLRE protein kinase family. *Prog Cell Cycle Res* 1, 329-38.
- Lamph, W. W., Wamsley, P., Sassone-Corsi, P., and Verma, I. M. (1988). Induction of proto-oncogene JUN/AP-1 by serum and TPA. *Nature* 334, 629-31.
- Lander, E., and Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11, 241-7.
- Lander, E. S., and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121, 185-99.
- Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1, 174-81.
- Lander, E. S., and Schork, N. J. (1994). Genetic dissection of complex traits. *Science* 265, 2037-48.
- Lane, D. P. (1992). Cancer. p53, guardian of the genome. *Nature* 358, 15-6.

- Lane, D. P., and Crawford, L. V. (1979). T antigen is bound to a host protein in SV40-transformed cells. *Nature* 278, 261-3.
- Lasko, D., Cavenee, W., and Nordenskjold, M. (1991). Loss of constitutional heterozygosity in human cancer. *Annu Rev Genet* 25, 281-314.
- Lee, E. Y., Chang, C. Y., Hu, N., Wang, Y. C., Lai, C. C., Herrup, K., Lee, W. H., and Bradley, A. (1992). Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature* 359, 288-94.
- Lee, E. Y., To, H., Shew, J. Y., Bookstein, R., Scully, P., and Lee, W. H. (1988). Inactivation of the retinoblastoma susceptibility gene in human breast cancers. *Science* 241, 218-21.
- Lee, G. H., Bennett, L. M., Carabeo, R. A., and Drinkwater, N. R. (1995). Identification of hepatocarcinogen-resistance genes in DBA/2 mice. *Genetics* 139, 387-95.
- Lee, M. H., Reynisdottir, I., and Massague, J. (1995). Cloning of p57KIP2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev* 9, 639-49.
- Lee, W. H., Bookstein, R., Hong, F., Young, L. J., Shew, J. Y., and Lee, E. Y. (1987a). Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* 235, 1394-9.
- Lee, W. H., Bookstein, R., Wheatley, W., Benedict, W. F., and Lee, E. Y. (1987b). A null allele of esterase D is a marker for genetic events in retinoblastoma formation. *Hum Genet* 76, 33-6.
- Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-31.
- Lichti, U., Patterson, E., Hennings, H., and Yuspa, S. H. (1981). The tumor promoter 12-O-tetradecanoylphorbol-13-acetate induces ornithine decarboxylase in proliferating basal cells but not in differentiating cells from mouse epidermis. *J Cell Physiol* 107, 261-7.
- Liddell, J. (1995). Genetic and biochemical factors involved in the progression of mouse skin tumours: University of Glasgow).
- Linardopoulos, S., Street, A. J., Quelle, D. E., Parry, D., Peters, G., Sherr, C. J., and Balmain, A. (1995). Deletion and altered regulation of p16INK4a and p15INK4b in undifferentiated mouse skin tumors. *Cancer Res* 55, 5168-72.
- Lincoln, S. E., Daly, M., and Lander, E. S. (1992a). Constructing genetic maps with MAPMAKER/EXP 3.0., 3rd Edition.
- Lincoln, S. E., Daly, M., and Lander, E. S. (1992b). Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1, 2nd Edition.

- Litt, M., and Luty, J. A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet* 44, 397-401.
- Loehrke, H., Schweizer, J., Dederer, E., Hesse, B., Rosenkranz, G., and Goertler, K. (1983). On the persistence of tumor initiation in two-stage carcinogenesis on mouse skin. *Carcinogenesis* 4, 771-5.
- Lugo, T. G., Pendergast, A. M., Muller, A. J., and Witte, O. N. (1990). Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 247, 1079-82.
- Lynch, M., and Walsh, B. (1998). *Genetics and analysis of quantitative traits* (Sunderland, Mass.: Sinauer Association, Inc).
- MacPhee, M., Chepenik, K. P., Liddell, R. A., Nelson, K. K., Siracusa, L. D., and Buchberg, A. M. (1995). The secretory phospholipase A2 gene is a candidate for the Mom1 locus, a major modifier of ApcMin-induced intestinal neoplasia. *Cell* 81, 957-66.
- Mai, M., Qian, C., Yokomizo, A., Tindall, D. J., Bostwick, D., Polychronakos, C., Smith, D. I., and Liu, W. (1998). Loss of imprinting and allele switching of p73 in renal cell carcinoma. *Oncogene* 17, 1739-41.
- Malkin, D. (1994). Germline p53 mutations and heritable cancer. *Annu Rev Genet* 28, 443-65.
- Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., and et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250, 1233-8.
- Manenti, G., Binelli, G., Gariboldi, M., Canzian, F., De Gregorio, L., Falvella, F. S., Dragani, T. A., and Pierotti, M. A. (1994). Multiple loci affect genetic predisposition to hepatocarcinogenesis in mice. *Genomics* 23, 118-24.
- Manenti, G., Gariboldi, M., Elango, R., Fiorino, A., De Gregorio, L., Falvella, F. S., Hunter, K., Housman, D., Pierotti, M. A., and Dragani, T. A. (1996). Genetic mapping of a pulmonary adenoma resistance (Par1) in mouse. *Nat Genet* 12, 455-7.
- Manenti, G., Gariboldi, M., Fiorino, A., Zanesi, N., Pierotti, M. A., and Dragani, T. A. (1997). Genetic mapping of lung cancer modifier loci specifically affecting tumor initiation and progression. *Cancer Res* 57, 4164-6.
- Manly, K. F., and Olson, J. M. (1999). Overview of QTL mapping software and introduction to map manager QT. *Mamm Genome* 4, 327-34.
- Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., and al, e. (1995). Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268, 1336-8.

- Martin, E., Cacheux, V., Cave, H., Lapierre, J. M., Le Paslier, D., and Grandchamp, B. (1995). Localization of the CDKN4/p27Kip1 gene to human chromosome 12p12.3. *Hum Genet* 96, 668-70.
- Masui, T., Wakefield, L. M., Lechner, J. F., LaVeck, M. A., Sporn, M. B., and Harris, C. C. (1986). Type beta transforming growth factor is the primary differentiation-inducing serum factor for normal human bronchial epithelial cells. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 83, 2438-42.
- Matsuoka, S., Edwards, M. C., Bai, C., Parker, S., Zhang, P., Baldini, A., Harper, J. W., and Elledge, S. J. (1995). p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev* 9, 650-62.
- McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. *Proc Natl Acad Sci U S A* 72, 5135-9.
- Miller, D. G. (1980). On the nature of susceptibility to cancer. The presidential address. *Cancer* 46, 1307-18.
- Mock, B. A., Krall, M. M., and Dosik, J. K. (1993). Genetic mapping of tumor susceptibility genes involved in mouse plasmacytogenesis. *Proc Natl Acad Sci U S A* 90, 9499-503.
- Mock, B. A., Lowry, D. T., Rehman, I., Padlan, C., Yuspa, S. H., and Hennings, H. (1998). Multigenic control of skin tumor susceptibility in SENCARA/Pt mice. *Carcinogenesis* 19, 1109-15.
- Mock, B. A., Neumann, P. E., and Fiedorek, F. T., Jr. (1997). Mouse chromosome 4. *Mamm Genome* 7, S60-79.
- Moen, C. J., Groot, P. C., Hart, A. A., Snoek, M., and Demant, P. (1996). Fine mapping of colon tumor susceptibility (Scc) genes in the mouse, different from the genes known to be somatically mutated in colon cancer. *Proc Natl Acad Sci U S A* 93, 1082-6.
- Moen, C. J., Snoek, M., Hart, A. A., and Demant, P. (1992). Scc-1, a novel colon cancer susceptibility gene in the mouse: linkage to CD44 (Ly-24, Pgp-1) on chromosome 2. *Oncogene* 7, 563-6.
- Moen, C. J., van der Valk, M. A., Snoek, M., van Zutphen, B. F., von Deimling, O., Hart, A. A., and Demant, P. (1991). The recombinant congenic strains--a novel genetic tool applied to the study of colon tumor development in the mouse. *Mamm Genome* 1, 217-27.
- Morel, F., Schulz, W. A., and Sies, H. (1994). Gene structure and regulation of expression of human glutathione S-transferases alpha. *Biol Chem Hoppe Seyler* 375, 641-9.
- Morgenbesser, S. D., Williams, B. O., Jacks, T., and DePinho, R. A. (1994). p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. *Nature* 371, 72-4.

- Mori, N., Okumoto, M., Hart, A. A., and Demant, P. (1995). Apoptosis susceptibility genes on mouse chromosome 9 (Rapop2) and chromosome 3 (Rapop3). *Genomics* 30, 553-7.
- Mori, N., van Wezel, T., van der Valk, M., Yamate, J., Sakuma, S., Okumoto, M., and Demant, P. (1998). Genetics of susceptibility to radiation-induced apoptosis in colon: two loci on chromosomes 9 and 16. *Mamm Genome* 9, 377-80.
- Mugrauer, G., Alt, F. W., and Ekblom, P. (1988). N-myc proto-oncogene expression during organogenesis in the developing mouse as revealed by in situ hybridization. *J Cell Biol* 107, 1325-35.
- Mulligan, L. M., Kwok, J. B., Healey, C. S., Elsdon, M. J., Eng, C., Gardner, E., and et al. (1993). Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 363, 458-60.
- Nagai, H., Negrini, M., Carter, S. L., Gillum, D. R., Rosenberg, A. L., Schwartz, G. F., and Croce, C. M. (1995). Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. *Cancer Res* 55, 1752-7.
- Nagase, H., Bryson, S., Cordell, H., Kemp, C. J., Fee, F., and Balmain, A. (1995). Distinct genetic loci control development of benign and malignant skin tumours in mice. *Nat Genet* 10, 424-9.
- Naito, M., Chenicek, K. J., Naito, Y., and DiGiovanni, J. (1988). Susceptibility to phorbol ester skin tumor promotion in (C57BL/6 x DBA/2) F1 mice is inherited as an incomplete dominant trait: evidence for multi-locus involvement. *Carcinogenesis* 9, 639-45.
- Naito, M., and DiGiovanni, J. (1989). Genetic background and development of skin tumors. *Carcinog Compr Surv* 11, 187-212.
- Nau, M. M., Brooks, B. J., Battley, J., Sausville, E., Gazdar, A. F., Kirsch, I. R., McBride, O. W., Bertness, V., Hollis, G. F., and Minna, J. D. (1985). L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature* 318, 69-73.
- Navarro, P., Gomez, M., Pizarro, A., Gamallo, C., Quintanilla, M., and Cano, A. (1991). A role for the E-cadherin cell-cell adhesion molecule during tumor progression of mouse epidermal carcinogenesis. *J Cell Biol* 115, 517-33.
- Nelson, M. A., Ariza, M. E., Yang, J. M., Thompson, F. H., Taetle, R., Trent, J. M., Wymer, J., Massey-Brown, K., Broome-Powell, M., Easton, J., Lahti, J. M., and Kidd, V. J. (1999). Abnormalities in the p34cdc2-related PITSLRE protein kinase gene complex (CDC2L) on chromosome band 1p36 in melanoma. *Cancer Genet Cytogenet* 108, 91-9.
- Nelson, M. A., Futscher, B. W., Kinsella, T., Wymer, J., and Bowden, G. T. (1992). Detection of mutant Ha-ras genes in chemically initiated mouse skin epidermis before the development of benign tumors. *Proc Natl Acad Sci U S A* 89, 6398-402.

Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., and et al. (1989). Mutations in the p53 gene occur in diverse human tumour types. *Nature* 342, 705-8.

Nishizuka, Y. (1986). Studies and perspectives of protein kinase C. *Science* 233, 305-12.

Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K., and Carson, D. A. (1994). Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature* 368, 753-6.

Nusse, R., and Varmus, H. E. (1982). Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 31, 99-109.

Obata, M., Lee, G. H., Kanda, H., Kitagawa, T., and Ogawa, K. (1997). Loss of heterozygosity at loci on chromosome 4, a common genetic event during the spontaneous immortalization of mouse embryonic fibroblasts. *Mol Carcinog* 19, 17-24.

O'Connell, J. F., Klein-Szanto, A. J., Digiovanni, D. M., Fries, J. W., and Slaga, T. J. (1986). Malignant progression of mouse skin papillomas treated with ethylnitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine, or 12-O-tetradecanoylphorbol-13-acetate. *Cancer Lett* 30, 269-74.

Olopade, O. I., Buchhagen, D. L., Malik, K., Sherman, J., Nobori, T., Bader, S., Nau, M. M., Gazdar, A. F., Minna, J. D., and Diaz, M. O. (1993). Homozygous loss of the interferon genes defines the critical region on 9p that is deleted in lung cancers. *Cancer Res* 53, 2410-5.

Olschwang, S., Serova-Sinilnikova, O. M., Lenoir, G. M., and Thomas, G. (1998). PTEN germ-line mutations in juvenile polyposis coli. *Nat Genet* 18, 12-4.

Ott, J. (1991). *Analysis of Human Geneti Linkage* (Baltimore and London: The Jhon Hopkins University Press).

Parangi, S., Dietrich, W., Christofori, G., Lander, E. S., and Hanahan, D. (1995). Tumor suppressor loci on mouse chromosomes 9 and 16 are lost at distinct stages of tumorigenesis in a transgenic model of islet cell carcinoma. *Cancer Res* 55, 6071-6.

Pataer, A., Kamoto, T., Lu, L. M., Yamada, Y., and Hiai, H. (1996). Two dominant host resistance genes to pre-B lymphoma in wild-derived inbred mouse strain MSM/Ms. *Cancer Res* 56, 3716-20.

Pataer, A., Nishimura, M., Kamoto, T., Ichioka, K., Sato, M., and Hiai, H. (1997). Genetic resistance to urethan-induced pulmonary adenomas in SMXA recombinant inbred mouse strains. *Cancer Res* 57, 2904-8.

Philipp, J., Vo, K., Gurley, K. E., Seidel, K., and Kemp, C. J. (1999). Tumor suppression by p27Kip1 and p21Cip1 during chemically induced skin carcinogenesis. *Oncogene* 18, 4689-4698.

- Polyak, K., Kato, J. Y., Solomon, M. J., Sherr, C. J., Massague, J., Roberts, J. M., and Koff, A. (1994b). p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 8, 9-22.
- Polyak, K., Lee, M. H., Erdjument-Bromage, H., Koff, A., Roberts, J. M., Tempst, P., and Massague, J. (1994a). Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 78, 59-66.
- Ponder, B. A. (1990). Inherited predisposition to cancer. *Trends Genet* 6, 213-8.
- Poole, T. M., and Drinkwater, N. R. (1996). Two genes abrogate the inhibition of murine hepatocarcinogenesis by ovarian hormones. *Proc Natl Acad Sci U S A* 93, 5848-53.
- Potter, M., Mushinski, E. B., Wax, J. S., Hartley, J., and Mock, B. A. (1994). Identification of two genes on chromosome 4 that determine resistance to plasmacytoma induction in mice. *Cancer Res* 54, 969-75.
- Prives, C. (1998). Signaling to p53: breaking the MDM2-p53 circuit. *Cell* 95, 5-8.
- Purdie, C. A., Harrison, D. J., Peter, A., Dobbie, L., White, S., Howie, S. E., and al., e. (1994). Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. *Oncogene* 2, 603-9.
- Quelle, D. E., Ashmun, R. A., Hannon, G. J., Rehberger, P. A., Trono, D., Richter, K. H., Walker, C., Beach, D., Sherr, C. J., and Serrano, M. (1995). Cloning and characterization of murine p16INK4a and p15INK4b genes. *Oncogene* 11, 635-45.
- Quintanilla, M., Brown, K., Ramsden, M., and Balmain, A. (1986). Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature* 322, 78-80.
- Rebai, A., Goffinet, B., and Mangin, B. (1995). Comparing power of different methods for QTL detection. *Biometrics* 1, 87-99.
- Reiss, M., Stash, E. B., Vellucci, V. F., and Zhou, Z. L. (1991). Activation of the autocrine transforming growth factor alpha pathway in human squamous carcinoma cells. *Cancer Res* 51, 6254-62.
- Remond, J. A., Fijneman, R. J., and Demant, P. (1995). A gene for susceptibility to small intestinal cancer, *ssic1*, maps to the distal part of mouse chromosome 4. *Cancer Res* 55, 3179-82.
- Risch, N., and Zhang, H. (1995). Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science* 268, 1584-9.
- Roberts, A. B., Flanders, K. C., Heine, U. I., Jakowlew, S., Kondaiah, P., Kim, S. J., and Sporn, M. B. (1990). Transforming growth factor-beta: multifunctional regulator of differentiation and development. *Philosophical Transactions Of The Royal Society Of London. Series B: Biological Sciences* 327, 145-54.

Robles, A. I., and Conti, C. J. (1995). Early overexpression of cyclin D1 protein in mouse skin carcinogenesis. *Carcinogenesis* *16*, 781-6.

Robles, A. I., Rodriguez-Puebla, M. L., Glick, A. B., Trempus, C., Hansen, L., Sicinski, P., Tennant, R. W., Weinberg, R. A., Yuspa, S. H., and Conti, C. J. (1998). Reduced skin tumor development in cyclin D1-deficient mice highlights the oncogenic ras pathway in vivo. *Genes Dev* *12*, 2469-74.

Rodriguez-Puebla, M. L., LaCava, M., Gimenez-Conti, I. B., Johnson, D. G., and Conti, C. J. (1998a). Deregulated expression of cell-cycle proteins during premalignant progression in SENCAR mouse skin. *Oncogene* *17*, 2251-8.

Rodriguez-Puebla, M. L., Robles, A. I., Johnson, D. G., LaCava, M., and Conti, C. J. (1998b). Synchronized proliferation induced by 12-O-tetradecanoylphorbol-13- acetate treatment of mouse skin: an in vivo model for cell cycle regulation. *Cell Growth Differ* *9*, 31-9.

Romert, L., Dock, L., Jensen, D., and Jernstrom, B. (1989). Effects of glutathione transferase activity on benzo[a]pyrene 7,8- dihydrodiol metabolism and mutagenesis studied in a mammalian cell co- cultivation assay. *Carcinogenesis* *10*, 1701-7.

Rotman, G., and Shiloh, Y. (1998). ATM: from gene to function. *Hum Mol Genet* *7*, 1555-63.

Ruggeri, B., Caamano, J., Goodrow, T., DiRado, M., Bianchi, A., Trono, D., Conti, C. J., and Klein-Szanto, A. J. (1991). Alterations of the p53 tumor suppressor gene during mouse skin tumor progression. *Cancer Res* *51*, 6615-21.

Ruggeri, B., Caamano, J., Slaga, T. J., Conti, C. J., Nelson, W. J., and Klein-Szanto, A. J. (1992). Alterations in the expression of uvomorulin and Na<sup>+</sup>,K<sup>+</sup>-adenosine triphosphatase during mouse skin tumor progression. *Am J Pathol* *140*, 1179-85.

Santos, E., and Nebreda, A. R. (1989). Structural and functional properties of ras proteins. *Faseb J* *3*, 2151-63.

Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S., and Barbacid, M. (1982). T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. *Nature* *298*, 343-7.

Santos, J., Perez de Castro, I., Herranz, M., Pellicer, A., and Fernandez-Piqueras, J. (1996). Allelic losses on chromosome 4 suggest the existence of a candidate tumor suppressor gene region of about 0.6 cM in gamma-radiation-induced mouse primary thymic lymphomas. *Oncogene* *12*, 669-76.

Sarnow, P., Ho, Y. S., Williams, J., and Levine, A. J. (1982). Adenovirus E1b-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. *Cell* *28*, 387-94.

Sato, T., Saito, H., Morita, R., Koi, S., Lee, J. H., and Nakamura, Y. (1991). Allelotype of human ovarian cancer. *Cancer Res* *51*, 5118-22.



- Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G., and Nakamura, Y. (1990). Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res* 50, 7184-9.
- Sax, K. (1923). The association of size difference with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8, 552-60.
- Schechter, R. L., Alaoui-Jamali, M. A., and Batist, G. (1992). Glutathione S-transferase in chemotherapy resistance and in carcinogenesis. *Biochem Cell Biol* 70, 349-53.
- Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467-70.
- Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., and Pachnis, V. (1994). Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature* 367, 380-3.
- Schwab, M., Alitalo, K., Klempnauer, K. H., Varmus, H. E., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. (1983). Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* 305, 245-8.
- Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 88, 265-75.
- Sellheyer, K., Bickenbach, J. R., Rothnagel, J. A., Bundman, D., Longley, M. A., Krieg, T., Roche, N. S., Roberts, A. B., and Roop, D. R. (1993). Inhibition of skin development by overexpression of transforming growth factor beta 1 in the epidermis of transgenic mice. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 90, 5237-41.
- Serrano, M., Hannon, G. J., and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366, 704-7.
- Serrano, M., Lee, H., Chin, L., Cordon-Cardo, C., Beach, D., and DePinho, R. A. (1996). Role of the INK4a locus in tumor suppression and cell mortality. *Cell* 85, 27-37.
- Sharan, S. K., Morimatsu, M., Albrecht, U., Lim, D. S., Regel, E., Dinh, C., Sands, A., Eichele, G., Hasty, P., and Bradley, A. (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 386, 804-10.
- Sharma, R., Haque, A. K., Awasthi, S., Singh, S. V., Piper, J. T., and Awasthi, Y. C. (1997). Differential carcinogenicity of benzo-a-pyrene in male and female CD-1 mouse lung. *J Toxicol Environ Health* 52, 45-62.
- Sherr, C. J., and Roberts, J. M. (1995). Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 9, 1149-63.

- Shih, C., Padhy, L. C., Murray, M., and Weinberg, R. A. (1981). Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. *Nature* 290, 261-4.
- Shtivelman, E., Lifshitz, B., Gale, R. P., and Canaani, E. (1985). Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature* 315, 550-4.
- Skouv, J., Christensen, B., Skibshoj, I., and Autrup, H. (1986). The skin tumor-promoter 12-O-tetradecanoylphorbol-13-acetate induces transcription of the c-fos proto-oncogene in human bladder epithelial cells. *Carcinogenesis* 7, 331-3.
- Slaga, T. J. (1989). Cellular and molecular mechanisms involved in multistage skin carcinogenesis. *Carcinog Compr Surv* 11, 1-18.
- Slaga, T. J., O'Connell, J., Rotstein, J., Patskan, G., Morris, R., Aldaz, C. M., and Conti, C. J. (1986). Critical genetic determinants and molecular events in multistage skin carcinogenesis. *Symp Fundam Cancer Res* 39, 31-44.
- Slamon, D. J., and Cline, M. J. (1984). Expression of cellular oncogenes during embryonic and fetal development of the mouse. *Proc Natl Acad Sci U S A* 81, 7141-5.
- Soller, M., and Genizi, A. (1967). Optimum experimental designs for realized heritability estimates. *Biometrics* 23, 361-5.
- Solomon, E., Borrow, J., and Goddard, A. D. (1991). Chromosome aberrations and cancer. *Science* 254, 1153-60.
- Sonoda, Y., Iizuka, M., Yasuda, J., Makino, R., Ono, T., Kayama, T., Yoshimoto, T., and Sekiya, T. (1995). Loss of heterozygosity at 11p15 in malignant glioma. *Cancer Res* 55, 2166-8.
- Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98, 503-17.
- Srivastava, S., Zou, Z. Q., Pirolo, K., Blattner, W., and Chang, E. H. (1990). Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature* 348, 747-9.
- Stehelin, D., Varmus, H. E., Bishop, J. M., and Vogt, P. K. (1976). DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260, 170-3.
- Stern, M. C., Gimenez-Conti, I. B., and Conti, C. J. (1995). Genetic susceptibility to papilloma progression in SENCAR mice. *Carcinogenesis* 16, 1947-53.
- Strange, R. C., Lear, J. T., and Fryer, A. A. (1998). Polymorphism in glutathione S-transferase loci as a risk factor for common cancers. *Arch Toxicol Suppl* 20, 419-28.
- Takeichi, M. (1993). Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 5, 806-11.

Taketo, M., Schroeder, A. C., Mobraaten, L. E., Gunning, K. B., Hanten, G., Fox, R. R., Roderick, T. H., Stewart, C. L., Lilly, F., Hansen, C. T., and et al. (1991). FVB/N: an inbred mouse strain preferable for transgenic analyses. *Proc Natl Acad Sci U S A* 88, 2065-9.

Tanaka, K., Oshimura, M., Kikuchi, R., Seki, M., Hayashi, T., and Miyaki, M. (1991). Suppression of tumorigenicity in human colon carcinoma cells by introduction of normal chromosome 5 or 18. *Nature* 349, 340-2.

Tanksley, S. D. (1993). Mapping polygenes. *Annu Rev Genet* 27, 205-233.

Taparowsky, E., Suard, Y., Fasano, O., Shimizu, K., Goldfarb, M., and Wigler, M. (1982). Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature* 300, 762-5.

Temin, H. M., and Mizutani, S. (1970). RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* 226, 1211-3.

Tennenbaum, T., Weiner, A. K., Belanger, A. J., Glick, A. B., Hennings, H., and Yuspa, S. H. (1993). The suprabasal expression of alpha 6 beta 4 integrin is associated with a high risk for malignant progression in mouse skin carcinogenesis. *Cancer Res* 53, 4803-10.

Thoday, J. M. (1961). Location of polygenes. *Nature* 191, 368-70.

Tonks, N. K., and Neel, B. G. (1996). From form to function: signaling by protein tyrosine phosphatases. *Cell* 87, 365-8.

Tsai, K. Y., Hu, Y., Macleod, K. F., Crowley, D., Yamasaki, L., and Jacks, T. (1998). Mutation of E2f-1 suppresses apoptosis and inappropriate S phase entry and extends survival of Rb-deficient mouse embryos. *Mol Cell* 2, 293-304.

Tsihlias, J., Kapusta, L. R., DeBoer, G., Morava-Protzner, I., Zbieranowski, I., Bhattacharya, N., Catzavelos, G. C., Klotz, L. H., and Slingerland, J. M. (1998). Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. *Cancer Research* 58, 542-8.

van der Riet, P., Nawroz, H., Hruban, R. H., Corio, R., Tokino, K., Koch, W., and Sidransky, D. (1994). Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. *Cancer Res* 54, 1156-8.

van Wezel, T., Stassen, A. P., Moen, C. J., Hart, A. A., van der Valk, M. A., and Demant, P. (1996). Gene interaction and single gene effects in colon tumour susceptibility in mice. *Nat Genet* 14, 468-70.

Varmus, H. (1988). Retroviruses. *Science* 240, 1427-35.

Varmus, H. E., Weiss, R. A., Friis, R. R., Levinson, W., and Bishop, J. M. (1972). Detection of avian tumor virus-specific nucleotide sequences in avian cell DNAs. *Proc Natl Acad Sci U S A* 69, 20-4.

- Verma, A. K., Hsieh, J. T., and Pong, R. C. (1988). Mechanisms involved in ornithine decarboxylase induction by 12-O- tetradecanoylphorbol-13-acetate, a potent mouse skin tumor promoter and an activator of protein kinase C. *Adv Exp Med Biol* 250, 273-90.
- Weber, J. L., and May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 44, 388-96.
- Weinberg, R. A. (1989). Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. *Cancer Res* 49, 3713-21.
- Weinberg, R. A. (1995). The retinoblastoma protein and cell cycle control. *Cell* 81, 323-30.
- Weiss, S. H., Teich, N., Varmus, H., and Coffin, J. (1985). *Molecular biology of tumor viruses: RNA tumor viruses*, 2nd Edition (New York: Cold Spring Harbor).
- Welch, D. R., Fabra, A., and Nakajima, M. (1990). Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc Natl Acad Sci U S A* 87, 7678-82.
- Wheelock, M. J., and Jensen, P. J. (1992). Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. *J Cell Biol* 117, 415-25.
- White, P. S., Maris, J. M., Beltinger, C., Sulman, E., Marshall, H. N., Fujimori, M., Kaufman, B. A., Biegel, J. A., Allen, C., Hilliard, C., and et al. (1995). A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3. *Proc Natl Acad Sci U S A* 92, 5520-4.
- Winkelman, D. C., and Hodgetts, R. B. (1992). RFLPs for somatotropic genes identify quantitative trait loci for growth in mice. *Genetics* 131, 929-37.
- Xie, M., Shao, G., Buysse, I. M., and Huang, S. (1997). Transcriptional repression mediated by the PR domain zinc finger gene RIZ. *J Biol Chem* 272, 26360-6.
- Xu, Y., Ashley, T., Brainerd, E. E., Bronson, R. T., Meyn, M. S., and Baltimore, D. (1996). Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma [see comments]. *Genes Dev* 10, 2411-22.
- Yamada, Y., Matsushiro, H., Ogawa, M. S., Okamoto, K., Nakakuki, Y., Toyokuni, S., Fukumoto, M., and Hiai, H. (1994). Genetic predisposition to pre-B lymphomas in SL/Kh strain mice. *Cancer Res* 54, 403-7.
- Yeh, S. H., Chen, P. J., Chen, H. L., Lai, M. Y., Wang, C. C., and Chen, D. S. (1994). Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. *Cancer Res* 54, 4188-92.
- Yuan, B., Hu, L. H., Lentsch, E. M., Shum-Siu, A., and Hendler, F. J. (1997). Consistent allelic loss on mouse chromosome 7 distal to tyrosinase in 4- nitroquinoline-

1-oxide-induced oral cavity tumors with loss of heterozygosity at Ha-ras-1. *Mol Carcinog* 19, 8-16.

Yunis, J. J., and Ramsay, N. (1978). Retinoblastoma and subband deletion of chromosome 13. *Am J Dis Child* 132, 161-3.

Yuspa, S. H. (1998). The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J Dermatol Sci* 17, 1-7.

Yuspa, S. H. (1994). The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis--thirty-third G. H. A. Clowes Memorial Award Lecture. *Cancer Res* 54, 1178-89.

Yuspa, S. H., Ben, T., and Hennings, H. (1983). The induction of epidermal transglutaminase and terminal differentiation by tumor promoters in cultured epidermal cells. *Carcinogenesis* 4, 1413-8.

Yuspa, S. H., Ben, T., Hennings, H., and Lichti, U. (1982). Divergent responses in epidermal basal cells exposed to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* 42, 2344-9.

Yuspa, S. H., Ben, T., Hennings, H., and Lichti, U. (1980). Phorbol ester tumor promoters induce epidermal transglutaminase activity. *Biochem Biophys Res Commun* 97, 700-8.

Yuspa, S. H., and Poirier, M. C. (1988). Chemical carcinogenesis: from animal models to molecular models in one decade. *Adv Cancer Res* 50, 25-70.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics* 136, 1457-68.

Zenklusen, J. C., Hodges, L. C., and Conti, C. J. (1997). Loss of heterozygosity on murine chromosome 6 in two-stage carcinogenesis: evidence for a conserved tumor suppressor gene. *Oncogene* 14, 109-14.

Zhuang, S. M., Eklund, L. K., Cochran, C., Rao, G. N., Wiseman, R. W., and Soderkvist, P. (1996). Allelotype analysis of 2',3'-dideoxycytidine- and 1,3-butadiene-induced lymphomas in B6C3F1 mice. *Cancer Res* 56, 3338-43.

**Table A1** Mouse microsatellite markers used in the genetic linkage mapping.

MARKER	LOCUS	MARKER	LOCUS	MARKER	LOCUS	MARKER	LOCUS
D1MIT3	11.0	D4MIT126	71.0	D8MIT121	67.0	D13MIT17	8.0
D1MIT318	18.0	D4MIT160	76.0	D9MIT42	8.0	D13MIT16	10.0
D1MIT213	25.0	D4MIT205	76.0	D9MIT205	18.0	D13MIT117	19.0
D1MIT76	32.0	D4MIT14	78.0	D9MIT285	21.0	D13MIT10	31.0
D1MIT303	34.0	D4MIT226	78.0	D9MIT328	23.0	D13MIT41	43.0
D1MIT46	43.0	D4MIT59	78.0	D9MIT191	26.0	D13MIT193	47.0
D1MIT8	52.0	D4MIT190	79.0	D9MIT154	27.0	D13MIT75	59.0
D1MIT10	56.0	D4MIT33	79.0	D9MIT97	29.0	D13MIT151	71.0
D1MIT93	64.0	D4MIT42	81.0	D9MIT162	30.0	D14MIT109	3.0
D1MIT30	70.0	D5MIT346	1.0	D9MIT102	31.0	D14MIT133	10.0
D1MIT102	73.0	D5MIT73	11.0	D9MIT163	33.0	D14MIT101	17.0
D1MIT34	81.0	D5MIT76	20.0	D9MIT207	33.0	D14MIT203	28.0
D1MIT36	92.0	D5MIT233	29.0	D9MIT31	35.0	D14MIT160	40.0
D1MIT206	95.0	D5MIT113	42.0	D9MIT208	36.0	D14MIT75	54.0
D1MIT362	106.0	D5MIT7	45.0	D9MIT259	38.0	D15MIT11	10.0
D2MIT6	9.0	D5MIT23	54.0	D9MIT74	41.0	D15MIT56	14.0
D2MIT7	28.0	D5MIT24	60.0	D9MIT269	43.0	D15MIT24	15.0
D2MIT14	48.0	D5MIT370	70.0	D9MIT11	48.0	D15MIT26	29.0
D2MIT15	50.0	D5MIT168	80.0	D9MIT196	48.0	D15MIT92	35.0
D2MIT252	53.0	D5MIT43	89.0	D9MIT10	49.0	D15MIT3	39.0
D2MIT58	60.0	D6MIT138	0.68	D9MIT182	55.0	D15MIT189	48.0
D2MIT398	61.0	D6MIT268	15.0	D9MIT347	56.0	D15MIT171	54.0
D2MIT260	83.0	D6MIT274	20.5	D9MIT116	61.0	D15MIT217	57.0
D2MIT285	86.0	D6MIT74	20.5	D9MIT212	61.0	D15MIT35	61.0
D2MIT343	86.0	D6MIT277	27.0	D9MIT18	71.0	D15MIT15	64.0
D2MIT48	87.0	D6MIT188	32.0	D9MIT19	71.0	D16MIT154	3.0
D2MIT148	105.0	D6MIT70	34.0	D10MIT248	7.0	D16MIT87	4.0
D3MIT62	4.0	D6MIT261	37.0	D10MIT17	16.0	D16MIT110	21.0
D3MIT46	13.0	D6MIT67	41.0	D10MIT44	27.0	D16MIT4	27.0
D3MIT63	22.0	D6MIT36	46.0	D10MIT15	32.0	D16MIT171	36.0
D3MIT6	23.0	D6NDS5	46.0	D10MIT42	44.0	D16MIT64	38.0
D3MIT209	33.0	D6MIT30	48.0	D10MIT11	48.0	D16MIT114	41.0
D3MIT49	41.0	D6MIT254	57.0	D10MIT134	59.0	D16MIT50	53.0
D3MIT77	49.0	D6MIT25	65.0	D10MIT233	62.0	D16MIT189	55.0
D3MIT107	55.0	D6MIT59	67.0	D10MIT271	70.0	D16MIT70	57.0
D3MIT14	64.0	D6MIT14	74.0	D11MIT150	2.0	D16MIT51	66.0
D3MIT17	71.0	D7MIT178	0.0	D11MIT306	12.0	D17MIT197	9.65
D3MIT44	78.0	D7MIT57	4.0	D11MIT217	19.0	D17MIT13	18.95
D3MIT116	84.0	D7MIT25	16.0	D11MIT130	20.0	D17MIT176	22.0
D3MIT19	87.0	D7MIT83	26.0	D11MIT23	28.0	D17MIT10	24.0
D4MIT264	1.0	D7MIT297	27.0	D11MIT30	39.0	D17MIT7	32.0
D4MIT41	10.0	D7MIT181	37.0	D11MIT116	44.0	D17MIT119	38.0
D4MIT17	31.0	D7MIT319	37.0	D11MIT38	49.0	D17MIT38	45.0
D4MIT178	35.0	D7MIT234	44.0	D11MIT288	55.0	D17MIT128	48.0
D4MIT45	42.0	D7MIT62	44.0	D11MIT99	65.0	D17MIT56	54.0
D4MIT175	49.0	D7MIT32	46.0	D11MIT254	71.0	D18MIT94	17.0
D4NDS2	55.0	D7MIT321	48.0	D12MIT10	6.0	D18MIT177	20.0
D4MIT57	56.0	D7MIT96	50.0	D12NDS11	6.0	D18MIT58	24.0
D4MIT37	56.0	D7MIT281	52.0	D12MIT136	13.0	D18MIT124	32.0
D4MIT124	57.0	D7MIT220	52.0	D12MIT46	16.0	D18MIT50	41.0
D4MIT12	57.0	D7MIT284	57.0	D12MIT2	19.0	D18MIT7	47.0
D4MIT40	59.0	D7MIT8	60.0	D12MIT112	22.0	D18MIT44	55.0
D4MIT16	59.0	D7MIT105	63.0	D12MIT68	28.0	D19MIT29	4.0
D4MIT72	59.0	D7MIT259	72.0	D12MIT52	32.0	D19MIT41	16.0
D4MIT203	60.0	D8MIT95	8.0	D12MIT149	37.0	D19MIT46	24.0
D4MIT224	60.0	D8MIT4	14.0	D12MIT203	37.0	D19MIT13	33.0
D4MIT71	61.0	D8MIT190	21.0	D12MIT231	48.0	D19MIT53	43.0
D4MIT148	66.0	D8MIT8	32.0	D12MIT233	52.0	D19MIT10	47.0
D4MIT54	66.0	D8MIT45	40.0	D12NDS2	55.0	D19MIT29	51.0
D4MIT170	66.0	D8MIT211	49.0	DXMIT114	0.0	D19MIT1	52.0
D4MIT158	67.0	D8MIT186	59.0	DXMIT166	16.0	D19MIT71	54.0
D4MIT312	69.0	D8MIT215	59.0	DXMIT186	73.0		

NT: The location of each marker (the distance from the centromere in cM) is from chromosome committee report in the database of Mouse Genome Informatics (<http://www.informatics.jax.org>).

**Table A2** The raw data of papilloma incidence in female FVB/N mice. The data recording terminated when the mouse was removed from the experiment.

MOUSE ID	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30
F1	0	0	1	1	2	3	4	4	4	6	6	8	12	13	17										
F2	1	1	2	3	5	6	6	8	8	8	8	10	10	10	12										
F3	0	1	1	2	3	8	7	8	8	8	8	9	9	9	9										
F4	0	0	1	1	1	1	1	3	4	6	8	11	11	12	15										
F5	1	1	1	3	5	5	5	7	7	7	7	7	7	12	14										
F6	1	2	2	3	6	9	9	9	9	8	8	9	12	12	15										
F7	0	0	0	0	2	2	2	3	3	4	4	8	8	10	10	10	11								
F8	0	0	0	2	3	4	7	9	10	12	12	20	20	23	23	24									
F9	1	3	3	4	6	11	12	16	16	18	18	18	18	16	15	14	14	14	16	15	15	13	14	15	16
F10	0	0	0	0	0	0	3	2	2	3	3	5	4	6	5	5	8	8	9	8	8	7	7	7	8
F11	0	1	1	2	5	6	7	7	7	9	11	14	21												
F12	0	0	0	0	0	0	3	6	6	8	9	9	10	13	14										
F13	0	2	2	3	6	6	6	6	7	9	11	11	15	15	15										
F14	0	0	1	1	1	3	4	6	8	9	11	11	11	11	11										
F15	0	1	1	2	4	6	8	12	14	17	18	18	18	12	12										
F16	0	0	1	1	2	5	5	6	6	8	8	12	12	13	13										
F17	0	0	0	1	1	3	6	8	8	8	8	12	12	14	13	4									
F18	0	0	1	1	1	2	4	5	6	8	9	11	11	14	14	17									
F19	0	0	0	1	1	3	5	6	6	8	9	9	18	18	16	16	16	21	21	20	20	19			
F20	0	0	0	1	1	2	2	3	3	3	5	9	13	19	24	24	23	23	20	20	21				
F21	0	0	1	1	2	6	6	11	11	12	14	20	20	21	23	22	22	23	23	21	21				
F22	0	0	1	1	1	3	4	6	6	7	9	10	12	16	16	19	21								
F23	0	0	0	1	3	4	5	6	7	16	6	7	9	9	-	13	-	13	-	16	17	17	-	15	15
F24	0	0	0	0	0	0	2	3	2	4	6	5	6	8	-	10	-	13	-	14	17	16	-	17	17
F25	0	0	0	0	0	0	0	3	3	4	2	1	1	3	-	7	-	6	-	6	7	7	-	4	4
F26	0	0	0	1	4	4	5	8	8	12	11	14	13	14	-	19	-	14	-	17	18	17	-	16	16
F27	0	0	0	0	0	0	0	2	3	10	4	3	2	2	-	5	-	7	-	4	6	6	-	7	7
F28	0	0	1	1	3	5	7	12	13	6	12	14	16	19	-	17									
F29	0	0	0	0	2	2	3	3	3	2	6	2	6	6	-	9	-	11	-	15	15	19			
F30	0	0	1	1	1	1	1	4	5	2	10	8	9	7	-	8	-	9	-	11	12	14			
F31	0	0	0	1	2	6	9	11	13	11	16	16	10	15	-	16	-	12	-	9	10				
F32	0	0	1	3	3	3	5	10	11	3	10	13	15	15	-	20	-	18	-						

- missing data

**Table A3** The raw data of papilloma incidence in female C57BL/6J mice.

MOUSE ID	W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	W34	W35	W36	W37	W38	W39
B1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B5	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
B6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B7	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
B8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B9	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	2
B10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B11	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B12	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B16	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

MOUSE ID	W40	W41	W42	W43	W44	W45	W46	W47	W48	W49	W50	W51	W52	W53	W54	W55	W56	W57	W58	W59	W60
B1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B2	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B3	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B4	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B8	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
B9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
B10	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
B11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B16	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B21	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



**Table A4** The raw data of papilloma incidence in female (FVB/N x C57BL/6J)F1 mice.

MOUSEID	W9	W10	W11	W13	W15	W16	W18	W20	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W33	W35	W36	W38	W41	W45	W47	W48	
H1	0	0	1	1	3	4	6	6	8	8	8																
H2	0	0	1	2	3	6	9	12																			
H3	1	1	1	2	4	5	7	7	7	7	9	9	9	11	12	12	12	11	9	10	10	10	10				
H4	0	0	1	1	1	2	2	3	3	2	3	4	4	4	4	4	5	6	6	6	6	6	7	3			
H5	0	1	1	1	1	2	4	4	4	4	3	3	5	5	5	7	9	11	6	6	6	6	3	2	2	2	
H6	1	1	1	1	2	3	3	3	3	3	3	5	5	7	8	7	7	6									
H7	0	0	0	1	1	2	2	3	3	3	3	4	5	5	5	5	6	6	7	7	7	6	6	3	3	1	
H8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
H9	0	0	0	0	1	2	2																				
H10	0	0	0	0	2	4	4	5	6	6	6	5	5	6	6	7	6	6	3								
H11	0	1	1	1	2	3	4																				
H12	0	0	0	0	0	2	2	2	4	4	4	5	5	6	6	7	7	8	8	8	8	6					
H13	1	1	2	2	3	3	4	6	9	10	10	11	11	12	12	12	12										
H14	0	0	0	0	0	0	0	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	
H15	0	1	1	1	2	3	4	5	5	5	5	5	5	5	5	5	6	6	6	6	7	7	7	5			
H16	0	0	0	1	2	3	3	3	2	2	1	1	1	2	2	1	1	1	1	1	2	2	2	2	2	1	
H17	0	0	0	0	1	4	4	4	5	5	5	6	7	10	10	10	10	12	12	12	15						
H18	0	0	0	0	1	1	2	2	2	2	3	3	5	5	5	5	6	6	6	6	6	6	6				
H19	0	2	2	4	8	12	16	17																			
H20	0	1	1	2	3	5	5	5	3	3	4																
H21	0	0	0	0	0	1	1	1	3	3	4	5	7	7	7	6	6	6	6	6	6						
H22	0	0	0	0	0	0	0	0	2	2	3	2	3	3	4	5	5	5	7	7	7	8	7	8			
H23	1	1	1	2	4	5	6	6	9	10	12	12	12	12	12	13	14	14	14								
H24	0	1	1	1	1	1	0	0	0	0	0	0	1	1	2	2	2	2	2	2							
H25	0	1	1	1	1	1	1	2	3	3	3	3	5	5	5	6	7	7	7	7	7	6	6				
H26	0	0	0	0	0	1	1	1	5	5	5	5	5	5	7	6	6	7	5	4	4	4	3	3	2	2	
H27	0	0	0	0	0	0	0	2	3	3	4	5	5	5	6	6	6	7	7	7	9						
H28	1	1	1	1	1	2	3	2	2																		
H29	0	2	2	3	5	5																					
H30	0	0	0	0	1	3	3	4	4	4	6	7	5	5	6	5	6	7	7	7	7	7	7	7	7	7	
H31	0	0	0	0	1	2	5	6	7	7	7	7	7	7	7	7	8	8	8	6	4	4	4	5			
H32	0	0	0	0	1	2	4	5	6	6	8	8	8	8	8	8	8	8	8	7	7	7	7	7	7	7	7

**Table A5** The raw data of papilloma incidence in F1BX mice.

MOUSE ID	COLOUR*	SEX**	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18
BX1	A	M	0	0	0	-	0	0	1	1	1	1	1
BX2	W	M	0	0	0	-	0	3	5	6	6	6	6
BX3	W	M	0	0	0	-	1	1	2	3	3	3	4
BX4	W	M	0	0	0	-	0	0	1	1	1	1	1
BX5	A	F	0	0	7	-	9	13	13	14	17	24	24
BX6	A	F	0	0	1	-	3	6	7	7	7	9	11
BX7	W	F	0	2	3	-	4	8	7	10	12	15	15
BX8	W	F	1	1	1	-	2	2	3	4	4	6	7
BX9	A	F	0	0	0	-	4	8	7	12	12	14	18
BX10	A	M	0	0	0	-	0	0	1	1	1	1	1
BX11	A	M	0	0	0	-	0	0	1	2	2	2	2
BX12	A	M	0	0	0	-	0	0	0	1	1	1	1
BX13	W	M	0	1	1	-	1	1	2	2	2	2	2
BX14	W	M	0	0	0	-	0	0	0	1	2	4	4
BX15	W	M	0	0	0	-	2	3	6	6	6	6	6
BX16	W	M	0	0	0	-	1	1	1	1	2	4	7
BX18	A	F	0	0	0	-	0	1	4	4	4	4	4
BX19	W	F	0	0	0	-	0	0	0	0	0	0	0
BX20	W	F	0	0	2	-	2	3	5	7	7	7	9
BX21	A	M	0	0	0	-	1	2	5	5	6	6	7
BX22	A	M	0	0	1	-	2	4	8	8	8	8	8
BX23	A	M	0	0	0	-	0	0	0	0	1	1	2
BX24	A	M	0	0	0	-	0	0	0	0	0	0	0
BX25	W	M	0	0	0	-	2	2	4	6	6	5	7
BX26	W	M	0	0	0	-	0	0	0	0	0	0	0
BX27	A	F	0	0	0	-	0	5	12	14	14	14	15
BX28	A	F	0	0	0	-	0	0	3	3	3	3	3
BX29	W	F	0	0	0	-	0	0	0	1	2	4	6
BX30	W	F	0	1	1	-	6	6	8	8	8	9	11
BX31	W	F	0	0	1	-	8	10	10	10	10	10	10
BX32	A	M	0	0	0	-	0	0	0	0	0	0	0
BX34	W	M	0	0	0	-	1	2	4	4	5	7	9
BX36	A	F	0	0	0	-	0	4	7	8	9	12	16
BX37	A	F	0	1	1	-	1	1	4	4	6	9	9
BX38	A	F	0	4	5	-	7	7	7	7	7	7	9
BX39	A	F	0	0	0	-	0	0	1	2	2	2	3
BX40	W	F	0	0	0	-	1	2	6	7	7	6	9
BX41	W	F	0	0	0	-	2	3	4	4	5	5	5
BX42	A	M	0	0	1	-	1	1	1	1	1	1	1
BX44	W	M	0	0	0	-	0	0	0	0	0	0	0
BX46	W	M	0	0	0	-	1	2	4	4	4	5	9
BX47	A	F	0	0	0	-	7	7	10	10	10	11	12
BX49	A	F	0	1	1	-	6	6	8	9	11	15	15
BX50	W	F	0	0	0	-	0	0	2	2	2	4	6
BX51	W	F	0	0	1	-	2	6	7	7	7	7	7
BX52	A	M	0	0	0	-	0	0	0	0	0	0	0
BX57	A	F	0	0	1	-	4	4	5	7	7	10	14
BX58	A	F	0	0	2	-	2	2	5	5	6	9	8
BX59	W	F	1	1	1	-	3	3	4	4	4	5	5
BX60	W	F	0	0	2	-	3	3	5	6	6	6	5
BX61	W	F	0	0	0	-	1	3	3	3	5	8	8
BX63	A	M	0	-	0	0	0	0	0	0	0	0	0
BX64	A	M	0	-	0	0	0	0	0	0	0	0	0
BX65	W	M	0	-	0	0	0	0	0	0	0	0	0
BX66	W	M	0	-	0	0	0	0	0	0	0	0	0
BX67	W	M	0	-	1	1	1	1	1	1	1	1	1
BX68	W	M	0	-	0	0	0	0	0	0	0	0	0
BX69	W	M	0	-	0	0	1	1	1	1	2	2	2
BX70	A	F	0	-	1	4	9	9	9	12	14	14	13
BX71	A	F	0	-	1	1	2	2	2	3	3	4	5
BX72	W	F	0	-	1	4	4	10	10	11	16	16	18
BX73	W	F	0	-	1	1	4	4	4	4	4	4	5
BX74	W	F	0	-	2	2	8	7	6	5	6	6	6
BX75	W	F	0	-	4	4	8	11	12	15	17	17	17
BX76	A	M	0	-	0	2	2	3	4	6	6	7	7
BX77	A	M	0	-	0	0	2	2	2	4	7	7	7

MOUSE ID	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32
BX1	2	2	2	3	3	4	4	4	4	4	4	4	4	4
BX2	8	11	12	11	10	11	11	11	11	11	11	11	9	8
BX3	4	5	5	5	4	4	2	3	3	4	4	4	5	7
BX4	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BX5														
BX6	11	10	9	10	10	11	11	13						
BX7	16	21	20	20	19	19	19	19	19	20	19	17	14	13
BX8	7	7	7	7	6	9	11	14	8	5	3			
BX9	20	20	20	19	17	16	15	16	18	19	19	19	20	20
BX10	0	0	1	1	1	1	1	1	1	1	1	1	1	1
BX11	2	4	5	6	7	8	9	2	3	6	6	6	4	
BX12	1	1	1	1	1	1	1	1	1	2	2	2	3	3
BX13	4	4	4	4	4	5	5	4	4	2	2	2	2	1
BX14	4	4	4	4	4	3	4	5	5	5	5	4	4	5
BX15	8	8	8	8	7	9	8	8	8	8				
BX16	7	9	9	9	8	10	11	13	13	15	15	14	13	
BX18	4	5	5	5	4	6	6	6	7	8	9			
BX19	0	0	0	0	0	0	0	0	0	1	1			
BX20	9	10	10	11	12	14	13	14	14	14	17			
BX21	7	7	7	7	8	7	10	9	9	10	10	10	10	10
BX22	8	9	8	8	7	8	8	7	6	6				
BX23	2	2	2	2	2	2	2	3	1	2	2	2	2	
BX24	0	0	1	1	1	1	2	3	2	2	2	2	3	3
BX25	9	8												
BX26														
BX27	15	15												
BX28	5	4	4	4	5	4	4	4	4	5	5	5	5	6
BX29	6	7	8	8	8	8	7	8	7					
BX30	13	13	12											
BX31	10	8	8		6	7	8	7	7	8				
BX32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX34	9	15	14	15	16	16	18	20	20	21	20	18	16	16
BX36	20	20	20	22	25	23	22	19	21	24	27			
BX37	9	7	7	8	9	8	8	8	8	9	9	9	9	10
BX38	9	10	10											
BX39	4	3	3	3	2	2	1	2	2	4	4	5	6	6
BX40	8	7	7	7	7	7	6	7	5	5	5	3	3	3
BX41	5	4	4	5	7	7	7							
BX42	1	1	1	1	1	1	1	1	1	0	0	0	0	0
BX44	0	0	0	0	0	0	0	1	2	3	3	2	2	3
BX46	10	10	11	13	14	15	15	15						
BX47														
BX49	19	20	20	20	21	20								
BX50	7	8	9	9	9	9	9	11	8	9	9			
BX51	7	6	7	7	6	4	4	4	4	6	6	4	5	
BX52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX57	14	14	14	14	12	14	14	16	16	16	16	15	15	17
BX58	9	10	11	12	13	13	13	13	13	13	13	13	13	16
BX59	6	8	7	7	6	6	7	7	7	7				
BX60	5	5	3	4	5	6	7	7	7	7	7	7	8	
BX61	8	9	8	8	6	4	5	6	6	6				
BX63	0	0	0	0	0	1	1	1	1	2	2	2	3	3
BX64	1	1	2	3	3	3	3	5	6	7	9	11	11	11
BX65														
BX66	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BX67	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BX68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX69	2	2	2	2	5	7	7	8	8	7	7			
BX70	13	13	12	10	10	10	10	10	10	10	10	10	12	12
BX71	5	5	4	4	5	5	5	5	5	6	6	8	8	8
BX72	17	17	15	11	12	16								
BX73	5	5	5	5	6	7	7	8	8	9	9	11	11	11
BX74	4	4	4	4	5	5	5	3	4	4				
BX75	17													
BX76	7	9	9	9	10	10	10	8	8	8	8	8		
BX77	7	8	8	9	9	9	9	8	8	8	8	8	8	6

MOUSE ID	W33	W34	W35	W36	W37	W38	W39	W40	W41	W42	W43	W44	W45	W46
BX1	4	4	3	3	3	3	4	4	4	4	4	4	5	4
BX2	8													
BX3														
BX4	1	1	1	1	1	1	1	2	2	2	2	2	2	3
BX5														
BX6														
BX7														
BX8														
BX9	22	20	19	17	17									
BX10	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BX11														
BX12	3	3	3	3	4	4	3	3	4	4	3	4	3	3
BX13	1	1	1	1	1	1	1							
BX14	5	5	4	4	6	6	7	7	5	7	6	6	7	7
BX15														
BX16														
BX18														
BX19														
BX20														
BX21	10	9	8	8										
BX22														
BX23														
BX24	3	3	4	4	5	5	5	6	4	2	2	1	1	
BX25														
BX26														
BX27														
BX28	6													
BX29														
BX30														
BX31														
BX32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX34	16	16	16	16	16	20	21							
BX36														
BX37	10	10	9	9	9	8	7	6	5	5	4	3	3	3
BX38														
BX39	6	5	3	3	3	3	3							
BX40	4	4	3	3	3	2	3	3	3	2				
BX41														
BX42	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX44	3	3	2	2	3	3	3	3	3	3	3	4	4	
BX46														
BX47														
BX49														
BX50														
BX51														
BX52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX57	17	17	14	14	12	11	14	13	11					
BX58	16	14	14	14	16	15	14	14	13	9				
BX59														
BX60														
BX61														
BX63	3	2	2	3	2	2	3	3	5	5	6	6	6	7
BX64	10	10	9	9	8	7	8	8	9	9	7	8	10	8
BX65														
BX66	1	1	1	1	1	1	1							
BX67	1	1	1	1	1	1	1	1	1	1	1			
BX68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX69														
BX70	10	10	11	8	8									
BX71	6	6	6	6	6	8	8	9						
BX72														
BX73	9	11	12	11	11	11	12	12	11	11	11	11	10	12
BX74														
BX75														
BX76														
BX77	6	6	7	7	6	6	5							

MOUSE ID	W47	W48	W49	W50	W51	W52	W53	W54	W55	W56	W57	W58	W59	W60
BX1	4	4	4	4	4	4	4	4	4	4				
BX2														
BX3														
BX4	3	3	3	3	3	3	3	3	3	3	3	3	2	
BX5														
BX6														
BX7														
BX8														
BX9														
BX10	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BX11														
BX12	3	3	2	2	2	2	2	2	2	2	2	2	2	
BX13														
BX14	7													
BX15														
BX16														
BX18														
BX19														
BX20														
BX21														
BX22														
BX23														
BX24														
BX25														
BX26														
BX27														
BX28														
BX29														
BX30														
BX31														
BX32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX34														
BX36														
BX37	3	4	3	3	3									
BX38														
BX39														
BX40														
BX41														
BX42	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX44														
BX46														
BX47														
BX49														
BX50														
BX51														
BX52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX57														
BX58														
BX59														
BX60														
BX61														
BX63	9	8	8	9	9									
BX64	9	8	9	7	5	5	4	4						
BX65														
BX66														
BX67														
BX68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BX69														
BX70														
BX71														
BX72														
BX73	10	9	8											
BX74														
BX75														
BX76														
BX77														

\*Mouse coat colour. A is agouti and W is albino; \*\* F is female and M is male; - missing data

**Table A6** The genotypes of F1BX mice. A, homozygous to FVB/N alleles; H, heterozygous.

MARKERS	BX1	BX2	BX3	BX4	BX5	BX6	BX7	BX8	BX9	BX10	BX11	BX12	BX13	BX14	BX15	BX16	BX18
D1MIT10	H	H	A	A	A	A	H	H	H	A	A	H	A	A	H	H	H
D1MIT102	A	A	H	A	H	H	H	H	H	A	A	H	A	A	A	H	H
D1MIT206																	
D1MIT213	H	H	A	H	A	A	A	H	H	A	A	A	H	A	H	H	H
D1MIT3	H	H	A	H	A	A	A	H	H	H	H	A	H	H	H	H	A
D1MIT318	H	H	A	H	A	A	A	H	H	H	H	A	H	H	H	H	A
D1MIT34																	
D1MIT362	A	A	H	A	H	H	H	H	H	H	A	H	A	A	A	H	H
D1MIT46	H	H	A	H	A	A	A	H	H	A	A	A	A	A	H	H	H
D1MIT76	H	H	A	H	A	A	A	H	H	A	A	A	H	A	H	H	H
D1MIT8	H	H	A	H	A	A	H	H	H	A	A	A	A	A	H	H	H
D1MIT93	H	H	A	A	H	H	H	H	H	A	A	H	A	A	A	H	H
D2MIT14	H	A	H	A	H	A	H	H	A	A	A	A	A	H	H	A	A
D2MIT148	A	H	H	H	H	A	A	H	A	H	A	H	H	A	H	A	A
D2MIT15	H	A	H	A	H	A	H	H	A	A	A	A	A	H	H	A	A
D2MIT252																	
D2MIT260																	
D2MIT285	H	H	H	H	H	A	H	H	A	A	A	A	H	A	H	A	A
D2MIT343																	
D2MIT398																	
D2MIT58	H	A	H	H	H	A	H	H	A	A	A	A	H	A	H	A	A
D2MIT6	H	H	H	A	A	A	H	A	A	A	A	A	A	H	H	A	H
D2MIT7	H	H	H	A	A	A	H	A	A	A	A	A	A	H	H	A	H
D3MIT107																	
D3MIT116	H	H	H	A	A	H	A	H	A	H	A	H	H	H	H	A	A
D3MIT14	H	H	H	A	A	H	A	H	H	H	A	H	H	H	A	A	A
D3MIT17																	
D3MIT19	H	H	H	A	A	H	A	H	A	H	A	H	H	H	H	A	A
D3MIT209																	
D3MIT44	H	H	H	A	A	H	A	H	H	H	A	H	H	H	H	A	A
D3MIT46	H	H	A	A	H	H	A	A	H	H	A	H	H	A	A	A	A
D3MIT6																	
D3MIT62	H	H	H	A	H	H	A	A	H	H	A	H	H	A	H	A	A
D3MIT77																	
D4MIT12	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D4MIT124																	
D4MIT126	H	A	A	A	A	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT14	H	A	H	A	H	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT148	H	A	A	A	A	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT16	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D4MIT17	H	A	A	A	A	H	A	H	H	A	H	H	A	H	H	H	H
D4MIT170	H	A	A	A	A	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT175	H	A	A	A	A	A	A	H	H	A	H	H	A	A	H	H	H
D4MIT178	H	A	A	A	A	H	A	H	H	A	H	H	A	H	H	H	H
D4MIT190																	
D4MIT203	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D4MIT205	H	A	H	A	H	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT224	H	A	A	A	A	A	A	A	H	A	H	A	A	A	A	H	H
D4MIT226																	
D4MIT264	H	A	A	H	A	H	H	H	H	H	A	A	A	H	H	H	H
D4MIT33	H	A	H	A	H	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT37	H	A	A	A	A	A	A	A	H	A	H	H	A	A	H	H	H
D4MIT40	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D4MIT41	H	A	A	H	A	H	H	H	H	H	A	H	A	H	H	H	H
D4MIT42	H	A	H	A	H	A	A	A	A	A	H	A	A	A	A	H	H
D4MIT45	H	A	A	A	A	H	A	H	H	A	H	H	A	A	H	H	H
D4MIT54																	
D4MIT57	H	A	A	A	A	A	A	A	H	A	H	H	A	A	H	H	H
D4MIT72	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D4NDS2	H	A	A	A	A	A	A	A	H	A	H	H	A	A	A	H	H
D5MIT113																	
D5MIT168																	
D5MIT23	H	H	A	H	H	A	H	A	A	H	H	A	A	A	A	A	H
D5MIT233	H	H	A	A	H	A	H	A	A	H	H	A	A	A	A	A	H
D5MIT346	H	H	H	A	H	A	A	A	H	H	H	A	A	A	A	H	H
D5MIT370	H	H	A	H	H	A	H	H	A	A	H	A	A	A	A	A	H
D5MIT7	H	H	A	H	H	A	H	A	A	H	H	A	A	A	A	A	H

D5MIT73	H	H	H	A	H	A	H	A	H	H	H	A	A	A	A	H	H
D5MIT76	H	H	H	A	H	A	H	A	A	H	H	A	A	A	A	A	H
D6MIT138	H	H	A	H	A	H	H	A	H	H	A	H	A	A	H	A	A
D6MIT14	A	H	H	A	A	H	A	A	H	A	H	A	A	A	H	H	A
D6MIT25																	
D6MIT254																	
D6MIT261																	
D6MIT268	H	H	H	H	A	H	H	A	H	H	H	H	H	H	H	A	H
D6MIT274																	
D6MIT277																	
D6MIT30	A	H	H	A	A	H	A	A	H	H	H	A	H	H	H	A	H
D6MIT59	A	H	H	A	A	H	A	A	H	H	H	A	A	H	H	H	A
D6MIT67																	
D6MIT70																	
D6NDS5	A	H	H	A	A	H	A	A	A	H	H	A	H	H	H	A	H
D7MIT105	A	A	H	A	H	H	A	A	H	H	H	A	A	A	A	A	H
D7MIT178	H	H	A	A	H	H	H	A	H	H	A	H	A	H	A	H	H
D7MIT181																	
D7MIT220																	
D7MIT234																	
D7MIT25	H	H	A	A	H	H	A	A	H	H	A	H	A	H	A	A	H
D7MIT259																	
D7MIT281																	
D7MIT284	A	H	H	A	A	H	H	A	A	H	H	H	H	H	H	A	H
D7MIT297																	
D7MIT319	H	H	A	A	H	H	A	A	H	H	H	H	A	A	A	A	H
D7MIT32	A	H	A	A	H	H	A	A	H	H	H	H	A	A	A	A	H
D7MIT321																	
D7MIT57																	
D7MIT8	A	H	H	A	H	H	A	A	H	H	H	H	A	A	A	A	H
D7MIT83	A	H	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D7MIT96	A	H	A	A	H	H	A	A	H	H	H	H	A	A	A	A	H
D8MIT121	A	A	H	A	A	H	H	A	H	A	H	A	H	H	A	H	H
D8MIT186																	
D8MIT190																	
D8MIT211	A	A	H	A	A	H	H	A	A	A	A	A	H	A	A	H	H
D8MIT4	A	H	H	H	A	H	A	A	A	A	A	H	H	A	H	H	H
D8MIT45																	
D8MIT8	A	A	H	H	A	H	A	A	A	A	A	H	H	A	H	H	H
D8MIT95																	
D9MIT10																	
D9MIT116																	
D9MIT154																	
D9MIT163	A	A	A	H	A	A	A	A	H	A	A	H	A	A	A	A	A
D9MIT182	H	A	H	H	A	A	A	A	H	H	A	H	A	A	A	A	A
D9MIT19	H	A	H	H	A	A	H	A	A	H	A	H	A	H	A	A	A
D9MIT191																	
D9MIT196	H	A	A	H	A	A	A	A	H	H	A	H	A	A	A	A	A
D9MIT205	A	H	A	H	A	A	A	A	H	A	H	H	H	H	A	A	A
D9MIT208																	
D9MIT259																	
D9MIT269	H	A	A	H	A	A	A	A	H	H	A	H	A	A	A	A	A
D9MIT285																	
D9MIT31																	
D9MIT42																	
D9MIT74	H	A	A	H	A	A	A	A	H	H	A	H	A	A	A	A	A
D9MIT97																	
D10MIT11																	
D10MIT134	H	H	H	H	H	A	H	A	H	H	A	H	H	H	A	A	H
D10MIT15																	
D10MIT17																	
D10MIT248	A	A	H	H	A	H	H	A	H	A	A	H	H	H	A	A	H
D10MIT271	H	H	H	H	H	A	H	A	H	H	A	H	H	H	A	A	H
D10MIT42	H	H	H	H	H	A	H	A	H	A	A	H	H	H	A	A	H
D10MIT44																	
D11MIT116																	
D11MIT130																	
D11MIT150																	
D11MIT23	H	H	H	A	H	A	A	H	H	A	H	H	H	A	A	A	H
D11MIT254	H	H	A	A	H	A	A	H	A	A	H	H	H	A	A	H	A

D11MIT288	H	H	A	A	H	A	A	H	A	A	H	H	H	A	A	H	A
D11MIT30																	
D11MIT306																	
D11MIT99	H	H	A	A	H	A	A	H	A	A	H	H	H	A	A	H	A
D12MIT10																	
D12MIT112	A	A	A	H	A	A	A	A	A	H	H	A	A	A	H	A	H
D12MIT136	A	A	A	H	A	A	A	A	A	H	H	A	A	A	H	A	H
D12MIT203	A	A	A	H	A	A	A	A	A	H	A	A	A	A	H	A	H
D12MIT231	A	A	H	H	H	A	A	A	A	H	A	A	A	A	H	A	H
D12MIT233																	
D12MIT46																	
D12MIT52																	
D12MIT68	A	A	A	H	A	A	A	A	A	H	H	A	A	A	H	A	H
D12NDS11	H	A	A	H	A	A	A	A	A	H	H	A	A	A	H	A	H
D12NDS2																	
D13MIT10																	
D13MIT117	A	A	A	A	H	H	A	H	H	A	H	A	H	H	H	A	A
D13MIT151	H	H	H	A	H	H	A	H	A	H	A	A	H	H	H	A	A
D13MIT16																	
D13MIT193																	
D13MIT41	A	H	A	A	H	H	A	H	H	H	H	A	H	H	H	A	A
D13MIT75	H	H	A	A	H	H	A	H	H	H	A	A	H	H	H	A	A
D14MIT101																	
D14MIT109																	
D14MIT133																	
D14MIT160																	
D14MIT203	A	A	A	A	H	H	A	H	H	H	A	A	A	H	A	H	H
D14MIT75	A	A	A	A	H	H	A	H	H	H	A	A	A	A	A	H	H
D15MIT11	A	A	A	A	A	A	H	H	A	H	H	A	H	A	H	H	A
D15MIT171																	
D15MIT189																	
D15MIT24																	
D15MIT26	A	A	A	A	H	A	H	A	H	H	H	A	A	A	H	A	A
D15MIT3																	
D15MIT35	A	H	A	H	H	H	A	A	H	H	A	A	A	A	H	A	H
D16MIT110	A	A	H	H	A	A	A	H	H	H	A	H	H	A	H	H	H
D16MIT154																	
D16MIT171	H	A	H	H	A	A	A	H	H	A	A	H	A	A	A	H	A
D16MIT189	H	A	H	H	A	A	A	H	H	A	A	H	A	H	A	H	A
D16MIT4	A	A	H	H	A	A	A	H	H	H	A	H	H	A	H	H	H
D16MIT51																	
D16MIT87																	
D17MIT119																	
D17MIT128																	
D17MIT13																	
D17MIT176	A	H	A	A	A	H	H	H	A	A	H	A	H	H	A	A	A
D17MIT197	H	H	H	A	A	H	H	H	A	H	H	H	H	H	A	A	A
D17MIT38																	
D17MIT56																	
D17MIT7																	
D18MIT124																	
D18MIT177																	
D18MIT44	H	A	H	A	H	H	H	H	A	H	A	A	H	A	H	A	H
D18MIT50																	
D18MIT58																	
D18MIT7	H	A	H	A	H	H	H	H	A	H	A	A	H	A	H	A	H
D18MIT94																	
D19MIT1																	
D19MIT10	A	A	A	H	A	A	A	A	H	H	H	A	A	A	H	A	A
D19MIT29	A	A	A	H	A	H	H	A	A	H	H	A	A	A	H	A	H
D19MIT41	A	A	A	H	A	H	H	A	A	H	H	A	A	A	H	A	A
D19MIT46A																	
D19MIT46B																	
D19MIT53																	
D19MIT71	A	H	A	H	A	A	A	H	H	H	H	A	A	H	A	H	A
DXMIT114	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
DXMIT166	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
DXMIT186	B	B	A	B	H	A	H	H	H	A	A	A	A	B	A	B	A



MARKERS	BX19	BX20	BX21	BX22	BX23	BX24	BX25	BX27	BX28	BX29	BX30	BX31	BX32	BX34	BX36	BX37
D1MIT10	H	A	A	A	A	H	H	A	A	A	H	H	A	H	A	A
D1MIT102	H	H	A	A	A	A	H	H	A	A	H	H	A	H	A	A
D1MIT206				A	A	A	A	H	A	A	H	A	A	H	A	A
D1MIT213	A	H	A	A	A	H	H	H	A	A	A	H	A	H	A	A
D1MIT3	A	H	A	A	A	H	H	H	A	A	A	H	H	H	H	A
D1MIT318	A	H	A	A	A	H	H	H	A	A	A	H	H	H	H	A
D1MIT34				A	A	A	H	H		A	H	A	A	H	A	A
D1MIT362	H	H	A	A	A	A	A	H	A	A	H	A	H	H	H	A
D1MIT46	H	A	A	A	A	H	H	A	A	A	A	H	A	H	A	A
D1MIT76	H	A	A	A	A	H	H	A	A	A	A	H	A	H	A	A
D1MIT8	H	A	A	A	A	H	H	A	A	A	H	H	A	H	A	A
D1MIT93	H	A	A	A	A	H	H	A	A	A	H	H	A	H	A	A
D2MIT14	H	H	A	A	A	A	A	A	A	A	H	A	H	H	A	A
D2MIT148	A	H	H	H	A	H	A	H	H	H	A	A	H	H	A	H
D2MIT15	H	H	A	A	A	A	A	A	A	A	H	A	H	H	A	A
D2MIT252				A	A	A	A	A	A	A	H	A	H	H	A	A
D2MIT260				A	A	H	A	A	H	A	A	A	H	H	A	H
D2MIT285	A	H	A	A	A	H	A	H	H	A	A	A	H	H	A	H
D2MIT343				H	A	H	A	H	H	A	A	A	H	H	A	H
D2MIT398				A	A	A	A	A		A	H	A	H	H	A	A
D2MIT58	H	H	A	A	A	A	A	A	A	A	H	A	H	H	A	A
D2MIT6	H	H	A	A	A	A	A	A	A	A	H	H	H	H	A	A
D2MIT7	H	H	A	A	A	A	A	A	A	A	H	H	H	H	A	A
D3MIT107				H	H	A	A	H	H	A	H	A	H	H	H	A
D3MIT116	H	A	H	H	H	A	A	H	H	A	H	A	H	H	H	A
D3MIT14	H	A	H	H	H	A	A	H	H	A	H	A	H	H	H	A
D3MIT17				H	H	A	A	H	H	A	H	A	H	H	H	H
D3MIT19	H	A	H	H	H	A	A	H	H	A	H	A	H	H	H	A
D3MIT209				H	H	H	A	H	H	H	H	H	H	H	H	H
D3MIT44	H	A	H	H	H	A	A	H	H	A	H	A	H	H	H	A
D3MIT46	H	H	A	H	H	H	A	H	A	H	H	H	H	H	H	H
D3MIT6				H	H	H	A	H	A	H	H	H	H	H	H	H
D3MIT62	H	H	A	H	H	H	A	A	A	H	H	H	A	H	H	H
D3MIT77				H	H	A	A	H	H	H	H	A	H	H	H	H
D4MIT12	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT124				A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT126	H	A	A	A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT14	H	A	A	A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT148	H	A	A	A	A	H	A	A	A	H	A	A	H	H	H	A
D4MIT16	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT17	H	A	A	A	H	A	A	H	A	H	H	A	H	H	A	A
D4MIT170	H	A	A	A	A	H	A	A	H	H	A	A	H	H	H	A
D4MIT175	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT178	H	A	A	A	H	A	A	H	A	H	H	A	H	H	A	A
D4MIT190				A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT203	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT205	H	A	A	A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT224	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT226				A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT264	A	A	A	A	A	A	A	H	A	H	H	H	H	A	A	A
D4MIT33	H	A	A	A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT37	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT40	H	A	A	A	H	H	A	H	H	H	A	A	H	H	H	A
D4MIT41	A	A	A	A	H	A	A	H	A	H	H	H	H	A	A	A
D4MIT42	H	A	A	A	A	H	A	A	H	H	A	A	H	A	H	A
D4MIT45	H	A	A	A	H	H	A	A	A	H	A	A	H	H	A	A
D4MIT54				A	A	H	A	A	A	H	A	A	H	H	H	A
D4MIT57	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4MIT72	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D4NDS2	H	A	A	A	H	H	A	A	A	H	A	A	H	H	H	A
D5MIT113				H	H	H	A	A	A	A	A	H	A	H	H	H
D5MIT168				A	H	H	A	A	A	A	A	H	A	H	H	H
D5MIT23	A	A	H	A	H	H	A	A	A	A	A	H	A	H	H	H
D5MIT233	A	A	H	H	H	H	A	H	A	H	A	H	A	H	H	H
D5MIT346	A	A	H	H	H	H	A	H	A	H	A	H	A	A	H	A
D5MIT370	A	A	H	A	H	H	A	A	A	A	A	H	A	H	H	H
D5MIT7	A	A	H	A	H	H	A	A	A	A	A	H	A	H	H	H

D5MIT73	A	A	H	H	H	H	A	H	A	H	A	H	A	H	H	A
D5MIT76	A	A	H	H	H	H	A	H	A	H	A	H	A	H	H	H
D6MIT138	A	A	A	H	H	H	A	A	A	H	A	H	H	H	H	H
D6MIT14	H	H	A	H	H	A	H	H	A	A	H	A	H	H	H	A
D6MIT25				H	H	H	H	H	A	A	H	A	H	H	H	A
D6MIT254				H	H	H	H	H	A	A	H	A	H	H	H	A
D6MIT261				H	H	H	A	H	A	H	H	H	H	H	H	H
D6MIT268	A	A	A	H	H	H	A	H	A	H	H	H	H	H	H	H
D6MIT274				H	H	H	A	H	A	H	H	A	H	H	H	H
D6MIT277				H	H	H	A	H	A	H	H	H	H	H	H	H
D6MIT30	H	H	H	H	H	H	H	H	A	A	H	A	H	H	H	A
D6MIT59	H	H	A	H	H	H	H	H	A	A	H	A	H	H	H	A
D6MIT67				H	H	H	A	H	A	H	H	A	H	H	H	H
D6MIT70				H	H	H	A	H	A	H	H	H	H	H	H	H
D6NDS5	H	H	H	H	H	H	H	H	A	H	H	A	H	H	H	H
D7MIT105	A	A	H	H	H	H	A	H	H	A	A	H	H	A	A	H
D7MIT178	H	A	A	H	H	A	A	A	A	A	H	A	A	A	H	H
D7MIT181				H	H	H	A	H	A	A	A	A	H	A	H	H
D7MIT220				H	H	H	A	H	H	A	A	A	H	A	H	H
D7MIT234				H	H	H	A	H	A	A	A	A	H	A	H	H
D7MIT25	A	A	A	H	H	H	A	A	A	A	A	A	A	A	H	H
D7MIT259				H	H	H	H	H	H	H	H	H	H	A	A	H
D7MIT281				H	H	H	A	H	H	A	A	A	H	A	H	H
D7MIT284	A	H	H	H	H	H	A	H	A	H	H	H	H	H	H	H
D7MIT297				H	H	H	A	A	A	A	A	A	H	A	H	H
D7MIT319	A	A	H	H	H	H	A	H	A	A	A	A	H	A	H	H
D7MIT32	A	A	H	H	H	H	A	H	H	A	A	A	H	A	H	H
D7MIT321				H	H	H	A	H	H	A	A	A	H	A	H	H
D7MIT57				H	H	A	A	A	A	A	H	A	A	A	H	H
D7MIT8	A	A	H	H	H	H	A	H	H	A	A	H	H	A	A	H
D7MIT83	A	A	A	H	H	H	A	A	A	A	A	A	H	A	H	H
D7MIT96	A	A	H	H	H	H	A	H	H	A	A	A	H	A	H	H
D8MIT121	H	H	A	H	A	H	A	H	H	H	H	A	A	A	A	A
D8MIT186				H	A	H	A	H	H	H	H	A	A	A	A	A
D8MIT190				H	H	H	H	H	A	H	A	A	A	H	H	H
D8MIT211	H	H	A	H	H	H	A	H	H	H	A	A	A	A	A	H
D8MIT4	H	H	A	H	H	H	H	H	A	H	A	A	A	H	H	H
D8MIT45				H	H	H	H	H	H	H	A	A	A	H	A	H
D8MIT8	H	H	A	H	H	H	H	H	H	H	A	A	A	A	H	H
D8MIT95				H	H	H	H	H	A	H	A	A	A	H	H	H
D9MIT10				A	A	H	H	H	H	A	A	A	A	A	A	H
D9MIT116				A	A	A	H	H	H	A	A	A	A	A	A	A
D9MIT154				A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT163	H	H	H	A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT182	H	H	A	A	A	A	H	H	H	A	A	A	A	A	A	A
D9MIT19	H	H	A	A	A	A	H	H	H	A	A	A	A	A	A	A
D9MIT191				A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT196	H	H	A	A	A	H	H	H	H	A	A	A	A	A	A	H
D9MIT205	H	H	H	A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT208				A	A	H	H	H	H	A	A	A	A	A	A	H
D9MIT259				A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT269	H	H	A	A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT285				A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT31				A	A	H	H	H	H	A	A	A	H	A	A	H
D9MIT42				A	A	H	H	H	H	A	A	A	H	A	H	H
D9MIT74	H	H	A	A	A	H	H	H	H	A	A	A	A	A	A	H
D9MIT97				A	A	H	H	H	H	A	A	A	H	A	A	H
D10MIT11				A	H	H	A	A	H	H	H	A	A	H	H	A
D10MIT134	A	A	H	A	H	H	A	A	H	H	H	A	A	H	H	H
D10MIT15				A	H	H	A	A	H	H	H	A	A	H	A	A
D10MIT17				A	H	H	A	A	H	H	H	A	A	H	H	A
D10MIT248	A	A	H	A	H	H	A	A	H	H	H	A	A	A	A	A
D10MIT271	A	A	A	A	H	H	A	H	H	H	H	A	A	H	H	H
D10MIT42	A	A	H	A	H	H	A	A	H	H	H	A	A	H	A	A
D10MIT44				A	A	H	H	H	A	A	A	H	H	H	H	A
D11MIT116				A	H	H	H	A	A	A	H	A	A	H	H	A
D11MIT130				A	H	H	A	A	A	A	H	H	A	H	A	H
D11MIT150				A	H	H	A	A	A	A	H	A	A	A	A	H
D11MIT23	H	H	A	A	H	H	A	A	A	A	H	H	A	H	H	H
D11MIT254	A	H	H	A	H	H	H	H	A	H	H	A	A	H	H	A

D11MIT288	A	H	H	A	H	H	H	H	A	H	H	A	A	H	H	A
D11MIT30				A	H	H	H	A	A	A	H	H	A	H	H	A
D11MIT306				A	H	H	A	A	A	A	H	A	A	A	A	H
D11MIT99	A	H	H	A	H	H	H	H	A	H	H	A	A	H	H	A
D12MIT10				A	A	H	A	A	A	A	A	H	H		A	H
D12MIT112	A	A	H	A	A	H	A	A	A	A	A	H	A	A	A	H
D12MIT136	A	A	H	A	A	H	A	A	A	A	A	H	A	A	A	H
D12MIT203	A	A	H	A	A	H	A	A	A	H	A	H	A	A	A	A
D12MIT231	A	A	H	A	A	H	A	A	A	H	A	H	A	A	A	A
D12MIT233				A	A		A	H	H	H		H	A	A	A	A
D12MIT46				A	A	H	A	A	A	A	A	H	A	A	A	H
D12MIT52				A	A	H	A	A	A	A	A	H	A	A	A	A
D12MIT68	A	A	H	A	A	H	A	A	A	A	A	H	A	A	A	A
D12NDS11	A	A	H	A	A	H	A	A	A	A	A	H	H	A	A	H
D12NDS2				A	A	H	A	H	H	H	A	A	A	A	A	A
D13MIT10				H	H	H	H	A	H	H	H	H	H	A	H	A
D13MIT117	H	A	H	H	H	H	H	A	H	H	H	H	H	A	H	A
D13MIT151	H	A	A	H	H	H	A	H	H	H	A	H	H	A	A	A
D13MIT16				H	H	H	H	A	H	H		H	H	A	H	A
D13MIT193				H		H	H	H		H	H	H	H	A	H	A
D13MIT41	H	A	H	H	H	H	H	A	H	H	H	H	H	A	H	A
D13MIT75	H	A	A	H	H	H	A	H	H	H	A	H	H	A	A	A
D14MIT101				H	A	A	A	A	A	A	H	A	A	H	H	A
D14MIT109				H	A	A	A	A	H	H	H	H	H	H	H	H
D14MIT133				H	A	A	A	A	A	H	H	A	H	H	H	H
D14MIT160				H	A	A	A	H	A	H	A	A	H	A	A	H
D14MIT203	H	H	A	H	A	A	A	H	A	H	A	A	H	A	A	H
D14MIT75	H	A	H	H	A	H	H	H	A	A	A	A	H	A	A	H
D15MIT11	A	H	A	A	H	H	A	A	A	H	A	H	A	A	H	H
D15MIT171				A	H	H	A	A	A	A	A	A	A	H	H	A
D15MIT189				A	H	H	A	A	A	A	A	A	A	H	H	H
D15MIT24				A	H	H	A	A	A	A	A	A	A	H	A	H
D15MIT26	A	H	A	A	H	H	A	A	A	A	A	A	H	A	H	H
D15MIT3				A	H	H	A	A	A	A	A	A	H	H	H	H
D15MIT35	H	A	A	A	A	A	H	H	A	A	A	A	H	H	H	A
D16MIT110	H	H	H	A	H	H	A	A	A	H	H	H	H	A	A	H
D16MIT154				A	H	H	A	A	H	A	H	H	H	H	A	H
D16MIT171	A	H	H	A	H	H	A	A	A	A	A	H	H	A	A	H
D16MIT189	A	A	H	A	H	A	A	A	A	A	A	H	A	A	A	H
D16MIT4	A	H	H	A	H	H	A	A	A	A	A	H	H	A	A	H
D16MIT51				A	H	A	H	A	A	A	A	H	A	A	A	H
D16MIT87				A	H	H	A	A	H	A	H	H	H	H	A	H
D17MIT119				A	H	A	A	A	A	H	A	A	A	H	H	A
D17MIT128				A	H	A	A	A	A	H	A	A	H	H	H	A
D17MIT13				A	H	A	A	A	A	H	A	A	H	H	H	A
D17MIT176	A	H	A	A	H	A	A	A	H	A	A	H	H	H	H	A
D17MIT197	A	H	A	A	H	A	A	A	A	H	A	A	H	H	H	H
D17MIT38				A	A	A	A	A	A	H	A	A	H	H	H	A
D17MIT56				A	A	A	H	A	A	H	A	A	A	H	H	A
D17MIT7				A	H	A	A	A	A	H	A	A	A	H	H	A
D18MIT124				A	A	A	A	A	H	H	H	A	H	H	A	A
D18MIT177				H	A	A	A	A	H	H	H	A	H	H	A	H
D18MIT44	A	A	H	A	A	A	A	A	H	A	H	A	H	A	A	A
D18MIT50				A	A	A	A	A	H	H	H	A	H	H	A	A
D18MIT58				H	A	A	A	A	H	H	H	A	H	H	A	H
D18MIT7	A	A	H	A	A	A	A	A	H	A	H	A	H	H	A	A
D18MIT94				H	A	A	H	A	H	H	H	H	H	H	A	H
D19MIT1				A	H	A	H	A	A	H	H	A	H	H	A	H
D19MIT10	H	A	A	A	A	A	H	A	A	H	H	A	H	H	A	H
D19MIT29	H	A	A	H	A	H	H	A	H	A	A	H	A	A	H	A
D19MIT41	H	A	A	A	A	A	H	A	H	A	H	H	A	H	H	A
D19MIT46A				A	A	A	H	A	H	H	H	A	A	H	H	H
D19MIT46B				A	H	A	A	H	A	A	A	A	H	A	H	A
D19MIT53				A	A	A	H	A	A	H	H	A	A	H	A	H
D19MIT71	A	A	A	A	H	A	H	A	A	H	H	A	H	H	A	H
DXMIT114	H	A	A	B	A	A	B	A	H	H	A	H	B	B	H	H
DXMIT166	H	A	A	B	A	B	B	H	H	H	A	H	B	B	H	A
DXMIT186	H	H	B	A	A	A	A	A	A	H	A	H	A	A	H	H

MARKERS	BX38	BX39	BX40	BX41	BX42	BX44	BX46	BX47	BX49	BX50	BX51	BX52	BX57	BX58	BX59	BX60
D1MIT10	H	H	A	H	H	A	A	A	A	A	A	A	H	H	A	A
D1MIT102	H	A	A	A	H	A	A	A	A	A	A	A	A	H	A	A
D1MIT206	H	A	A	A	H	H	H	A	A	H	A	H	A	H	A	A
D1MIT213	H	H	A	H	H	A	H	A	A	A	A	A	H	H	A	H
D1MIT3	H	A	A	H	H	A	H	A	A	A	A	A	H	H	A	H
D1MIT318	H	A	A	H	H	A	H	A	A	A	A	A	H	H	A	H
D1MIT34	H	A	A	A	H	A	H	A	A	H	A	A	A	H	A	A
D1MIT362	H	A	A	A	A	H	H	A	A	H	A	H	A	A	A	H
D1MIT46	H	H	A	H	H	A	A	A	A	A	A	A	H	H	A	A
D1MIT76	H	H	A	H	H	A	A	A	A	A	A	A	H	H	A	A
D1MIT8	H	H	A	H	H	A	A	A	A	A	A	A	H	H	A	A
D1MIT93	H	A	A	H	H	A	A	A	A	A	A	A	H	H	A	A
D2MIT14	A	A	H	H	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT148	A	A	A	A	A	H	A	A	A	A	H	A	A	A	A	A
D2MIT15	A	A	H	H	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT252	H	A	H	H	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT260	H	A	A	A	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT285	H	A	A	A	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT343	H	A	A	A	A	H	A	A	A	A	H	A	A	A	A	A
D2MIT398	H	A	A	A	A	A	H	H	A	H	A	H	A	A	A	A
D2MIT58	H	A	H	H	A	A	H	H	H	A	H	A	H	A	A	A
D2MIT6	A	H	H	A	A	A	H	A	H	A	H	H	H	A	A	A
D2MIT7	A	H	H	A	A	A	H	A	H	A	H	H	H	A	A	A
D3MIT107	A	H	H	A	H	A	H	A	A	A	H	A	A	A	A	H
D3MIT116	A	H	H	H	H	H	H	A	A	A	A	A	A	A	A	H
D3MIT14	A	H	H	A	H	H	H	A	A	A	A	A	A	A	A	H
D3MIT17	A	H	H	A	H	H	H	A	A	A	A	A	A	A	A	H
D3MIT19	A	H	H	H	H	H	H	A	A	A	A	A	A	A	A	H
D3MIT209	A	H	A	A	H	A	H	H	A	A	H	H	A	A	A	A
D3MIT44	A	H	H	H	H	H	H	A	A	A	A	A	A	A	A	H
D3MIT46	A	A	A	A	H	A	H	A	A	A	H	H	A	A	A	A
D3MIT6	A	A	A	A	H	A	H	H	A	A	H	H	A	A	A	A
D3MIT62	A	A	A	H	H	A	H	A	A	A	H	A	A	A	H	A
D3MIT77	A	H	H	A	H	A	H	H	A	A	H	A	A	A	A	H
D4MIT12	A	A	A	H	H	H	H	H	A	A	A	H	A	A	H	H
D4MIT124	A	A	A	H	H	H	H	H	A	A	A	H	A	A	H	H
D4MIT126	A	A	A	H	H	A	A	H	H	H	H	A	A	A	A	H
D4MIT14	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT148	A	A	A	H	H	A	H	H	H	H	A	H	A	A	A	H
D4MIT16	A	A	A	H	H	H	H	H	A	H	A	H	A	A	H	H
D4MIT17	A	A	A	H	A	H	H	A	A	A	A	H	A	H	H	H
D4MIT170	A	A	A	H	H	A	H	H	H	H	A	H	A	A	A	H
D4MIT175	A	A	A	H	A	H	H	A	A	A	A	H	A	H	H	H
D4MIT178	A	A	A	H	A	H	H	A	A	A	A	H	A	H	H	H
D4MIT190	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT203	A	A	A	H	H	H	H	H	A	H	A	H	A	A	A	H
D4MIT205	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT224	A	A	A	H	H	H	H	H	H	H	A	H	A	A	A	H
D4MIT226	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT264	H	H	A	A	A	A	H	H	A	H	A	H	A	H	H	H
D4MIT33	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT37	A	A	A	H	A	H	H	A	A	A	A	H	A	A	H	H
D4MIT40	A	A	A	H	H	H	H	H	A	H	A	H	A	A	H	H
D4MIT41	H	A	A	H	A	H	H	A	A	H	A	H	A	H	H	H
D4MIT42	A	A	A	H	H	A	A	H	H	H	H	A	H	A	A	H
D4MIT45	A	A	A	H	A	H	H	A	A	A	A	H	A	H	H	H
D4MIT54	A	A	A	H	H	A	H	H	H	H	A	H	A	A	A	H
D4MIT57	A	A	A	H	A	H	H	A	A	A	A	H	A	A	H	H
D4MIT72	A	A	A	H	H	H	H	H	A	H	A	H	A	A	H	H
D4NDS2	A	A	A	H	H	H	H	H	A	A	A	H	A	A	H	H
D5MIT113	A	A	H	A	A	A	H	H	A	A	H	H	H	H	H	H
D5MIT168	A	H	H	H	A	A	H	A	A	A	H	H	A	H	A	H
D5MIT23	A	A	H	A	A	A	H	H	A	A	H	H	H	H	A	H
D5MIT233	A	A	H	A	A	A	A	H	A	A	H	H	H	H	H	H
D5MIT346	A	H	A	A	A	H	A	A	H	A	A	H	H	A	H	A
D5MIT370	A	A	H	H	A	A	H	H	A	A	H	H	A	H	A	H
D5MIT7	A	A	H	A	A	A	H	H	A	A	H	H	H	H	A	H

D5MIT73	A	A	A	A	A	A	A	H	H	A	A	H	H	H	H	A
D5MIT76	A	A	A	A	A	A	A	H	A	A	A	H	H	H	H	A
D6MIT138	A	A	A	A	H	A	A	H	H	H	H	A	H	H	A	A
D6MIT14	A	A	A	A	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT25	A	A	A	A	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT254	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT261	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT268	A	A	A	H	H	A	A	H	H	H	H	A	H	H	A	A
D6MIT274	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT277	A	A	A	H	H	H	A	A	H	H	H	A	H	H	H	A
D6MIT30	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT59	A	A	A	A	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT67	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D6MIT70	A	A	A	H	A	H	A	A	H	A	A	A	A	H	A	A
D6NDS5	A	A	A	H	A	H	A	A	H	A	A	H	A	H	A	A
D7MIT105	A	A	A	A	H	H	A	A	A	A	A	H	H	H	A	A
D7MIT178	H	H	H	H	H	H	H	H	A	A	H	A	H	A	H	A
D7MIT181	H	H	A	A	H	A	A	A	H	A	A	H	H	H	A	A
D7MIT220	H	H	A	A	H	A	A	A	H	A	A	H	H	H	A	A
D7MIT234	H	H	A	A	H	A	A	H	H	A	A	H	H	H	A	A
D7MIT25	H	H	H	H	H	H	H	H	A	A	H	A	H	A	H	A
D7MIT259	A	A	A	A	H	A	A	A	A	A	A	H	H	H	A	A
D7MIT281	H	H	A	A	H	A	A	A	H	A	A	H	H	H	A	A
D7MIT284	A	A	A	H	H	H	A	A	H	H	H	A	A	H	A	A
D7MIT297	H	H	H	A	H	A	A	H	H	A	A	A	A	H	H	A
D7MIT319	H	H	A	A	H	A	A	H	H	A	A	H	H	H	A	A
D7MIT32	H	H	A	A	H	A	A	H	H	A	A	H	H	H	A	A
D7MIT321	H	H	A	A	H	A	A	A	H	A	A	H	H	H	A	A
D7MIT57	H	H	H	H	H	H	H	H	A	A	H	A	H	A	H	A
D7MIT8	A	A	A	A	H	A	A	A	A	A	A	H	H	H	A	A
D7MIT83	H	H	H	A	H	H	A	H	A	A	H	A	H	H	H	A
D7MIT96	H	H	A	A	H	A	A	A	H	A	A	H	H	H	A	A
D8MIT121	A	H	H	A	H	A	A	A	H	H	A	H	A	H	A	H
D8MIT186	A	H	H	A	H	A	A	H	H	H	A	H	H	H	A	H
D8MIT190	A	A	H	H	H	H	A	H	H	H	H	H	H	H	A	A
D8MIT211	A	H	H	A	H	H	A	H	H	H	A	H	H	H	A	H
D8MIT4	A	A	H	H	H	H	A	H	H	H	H	H	H	H	A	A
D8MIT45	A	H	H	H	H	H	A	H	H	H	A	H	H	H	A	H
D8MIT8	A	A	H	H	H	H	A	H	H	H	H	H	H	H	A	A
D8MIT95	A	A	H	H	H	H	A	H	H	H	H	H	H	H	A	A
D9MIT10	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	H
D9MIT116	A	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H
D9MIT154	A	A	A	H	A	H	H	A	A	H	A	H	H	H	H	A
D9MIT163	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	A
D9MIT182	A	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H
D9MIT19	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H	H
D9MIT191	A	A	A	H	A	H	H	A	A	H	A	H	H	H	H	A
D9MIT196	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	H
D9MIT205	A	A	A	A	A	H	H	A	A	H	A	H	H	H	H	A
D9MIT208	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	H
D9MIT259	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	A
D9MIT269	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	A
D9MIT285	A	A	A	A	A	H	H	A	A	H	A	H	H	H	H	A
D9MIT31	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	A
D9MIT42	A	A	A	A	A	H	H	A	A	H	A	H	H	H	H	A
D9MIT74	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	H
D9MIT97	A	A	A	H	H	H	H	A	A	H	A	H	H	H	H	A
D10MIT11	A	A	H	A	H	H	H	H	A	A	H	A	A	H	A	H
D10MIT134	A	A	H	A	H	H	H	H	A	A	A	H	A	A	H	A
D10MIT15	A	A	H	A	H	H	A	A	A	A	H	A	A	H	A	H
D10MIT17	A	A	H	A	H	H	A	A	A	A	H	A	A	H	A	H
D10MIT248	A	H	H	A	A	H	A	A	A	A	A	H	A	H	A	H
D10MIT271	A	A	H	A	H	A	H	H	A	A	H	A	A	H	A	H
D10MIT42	A	A	H	A	H	H	H	H	A	A	H	A	A	H	A	H
D10MIT44	H	A	A	H	H	A	H	A	A	A	A	A	H	H	A	H
D11MIT116	A	A	A	A	H	A	A	A	A	H	H	A	H	A	A	A
D11MIT130	A	H	A	A	H	A	A	A	A	H	H	H	H	A	A	A
D11MIT150	A	H	H	H	H	A	A	A	H	H	H	H	H	H	A	A
D11MIT23	A	H	A	A	H	A	A	A	A	H	H	A	H	A	A	A
D11MIT254	A	A	A	H	H	H	A	A	A	H	H	A	H	A	A	A

D11MIT288	A	A	A	A	H	A	A	A	A	H	H	A	H	A	A	A
D11MIT30	A	A	A	A	H	A	A	A	A	H	H	A	H	A	A	A
D11MIT306	A	H	H	A	H	A	A	A	H	H	H	H	H	H	A	A
D11MIT99	A	A	A	A	H	H	A	A	A	H	H	A	H	A	A	A
D12MIT10	A	H	H	H	A	A	A	A	H	A	A	H	A	H	H	H
D12MIT112	A	H	H	H	A	A	A	H	H	A	A	H	A	A	H	H
D12MIT136	A	H	H	H	A	A	A	A	H	A	A	H	A	H	H	H
D12MIT203	A	H	H	H	A	H	A	H	H	A	A	H	A	A	H	A
D12MIT231	A	H	H	H	A	H	A	H	H	A	A	H	A	H	H	A
D12MIT233	A	H	A	H	A	H	A	H	H	A	A	H	A	A	H	A
D12MIT46	A	H	H	H	A	A	A	H	H	A	A	H	A	A	H	H
D12MIT52	A	H	H	H	A	H	A	H	H	A	A	H	A	A	H	A
D12MIT68	A	H	H	H	A	H	A	H	H	A	A	H	A	A	H	A
D12NDS11	A	H	H	H	A	A	A	A	H	A	A	H	A	H	H	H
D12NDS2	A	H	A	H	A	H	H	H	H	A	A	H	A	A	H	A
D13MIT10	A	A	A	A	H	A	A	A	A	A	A	A	A	A	H	A
D13MIT117	A	A	A	A	H	A	A	A	A	A	A	A	A	A	H	A
D13MIT151	H	H	A	A	H	A	A	A	H	A	H	A	A	A	H	A
D13MIT16	A	A	A	A	H	A	A	A	A	H	A	H	A	H	A	A
D13MIT193	H	A	A	A	H	A	A	A	H	A	H	A	A	A	H	A
D13MIT41	H	A	A	A	H	A	A	A	H	A	H	A	A	A	H	A
D13MIT75	H	A	A	A	H	A	A	A	H	A	H	A	A	A	H	A
D14MIT101	H	H	A	A	H	H	A	A	A	A	A	A	A	A	H	A
D14MIT109	A	H	A	A	H	H	A	A	A	A	H	H	A	A	H	A
D14MIT133	H	H	A	A	H	H	A	A	A	A	H	A	A	A	H	A
D14MIT160	H	A	A	A	H	H	A	A	A	A	H	A	A	A	A	A
D14MIT203	H	A	A	A	H	H	A	A	A	A	A	A	A	A	H	A
D14MIT75	H	A	A	A	H	H	A	A	A	A	H	A	A	A	A	A
D15MIT11	H	A	H	A	A	A	A	A	H	H	H	A	H	H	H	H
D15MIT171	H	A	H	A	A	A	A	A	H	H	A	H	A	A	H	H
D15MIT189	H	A	H	A	A	A	A	A	H	H	A	H	A	A	H	H
D15MIT24	H	A	H	A	A	A	A	A	H	A	H	A	H	H	H	H
D15MIT26	H	A	H	A	A	A	A	A	H	A	H	A	H	H	H	H
D15MIT3	H	A	H	A	A	A	A	A	H	A	A	A	A	A	H	H
D15MIT35	H	H	A	A	A	A	A	A	H	H	A	H	A	A	H	H
D16MIT110	A	H	H	A	A	A	H	A	A	H	H	A	A	A	H	A
D16MIT154	A	H	H	A	H	A	A	A	H	H	H	A	A	A	H	A
D16MIT171	A	H	H	A	H	A	H	A	A	H	A	A	A	H	A	A
D16MIT189	A	H	H	A	H	A	H	A	A	H	A	A	A	A	H	A
D16MIT4	A	H	H	A	A	A	H	A	A	H	H	A	A	H	A	A
D16MIT51	H	H	H	A	H	A	H	A	A	H	A	A	H	A	H	H
D16MIT87	A	H	H	A	H	A	A	A	H	H	H	A	A	H	A	A
D17MIT119	H	H	A	H	A	A	H	H	H	H	A	A	H	H	A	A
D17MIT128	H	H	A	H	H	A	H	H	H	H	A	A	A	H	A	A
D17MIT13	H	H	A	H	H	A	H	H	H	H	A	A	A	H	A	A
D17MIT176	H	H	A	H	H	A	H	H	H	H	A	A	A	H	A	A
D17MIT197	H	H	A	A	H	A	H	H	H	H	H	A	A	H	A	A
D17MIT38	H	H	A	H	A	A	H	H	H	H	A	A	H	H	A	A
D17MIT56	H	H	A	H	A	A	H	A	H	H	A	A	H	H	H	A
D17MIT7	H	H	A	H	A	A	H	H	H	H	A	A	H	H	A	A
D18MIT124	A	H	H	A	H	A	H	H	H	H	A	H	H	H	A	A
D18MIT177	A	H	H	H	H	A	H	H	H	H	A	H	H	H	A	H
D18MIT44	A	H	H	A	H	A	H	A	A	H	A	H	H	H	A	A
D18MIT50	A	H	H	A	H	A	H	H	H	H				H	A	A
D18MIT58	A	H	H	A	H	A	H	H	H	H	A	H	H	H	A	H
D18MIT7	A	H	H	A	H	A	H	H	A	H	A	H	H	H	A	A
D18MIT94	A	H	H	H	H	A	H	H	H	H	A	H	H	H	A	H
D19MIT1	A	H	A	A	H	A	H	A	H	H	H	A	A	A	H	A
D19MIT10	A	H	A	A	H	A	H	A	H	H	H	A	A	A	A	A
D19MIT29	A	H	A	H	A	A	A	H	H	A	H	H	A	H	A	A
D19MIT41	A	H	A	H	A	A	H	H	H	H	H	H	A	H	A	A
D19MIT46A	A	H	A	A	H	A	H	A	H	H	H	A	A	H	A	A
D19MIT46B	A	H	H	H	H	A	H	A	A	A	H	H	A	H	A	A
D19MIT53	A	H	A	A	H	A	H	A	H	H	H	A	A	A	A	A
D19MIT71	A	H	H	A	H	A	H	A	H	H	H	A	A	A	H	A
DXMIT114	H	H	H	A	A	B	B	H	H	A	H	A	H	H	H	A
DXMIT166	H	H	H	H	A	B	A	H	H	A	H	A	A	H	A	A
DXMIT186	A	H	A	A	A	A	B	H	H	A	A	A	H	H	H	H

MARKERS	BX61	BX63	BX64	BX65	BX66	BX67	BX68	BX69	BX70	BX71	BX72	BX73	BX74	BX75	BX76	BX77
D1MIT10	H	A	A	A	A	A	H	H	H	A	H	H	A	H	A	H
D1MIT102	H	A	A	A	H	A	A	H	H	H	H	H	A	A	A	H
D1MIT206	H	A	H	A	H	A	A	A	H	H	A	H	A	A	A	H
D1MIT213	H	A	A	A	A	A	H	H	H	A	H	H	H	H	H	H
D1MIT3	H	A	A	A	A	A	H	H	A	H	A	H	H	H	H	H
D1MIT318	H	A	A	A	A	A	H	H	A	H	H	H	H	H	H	H
D1MIT34	H	A	A	A	H	A	A	A	A	H	H	H	A	A	A	H
D1MIT362	H	A	H	A	H	A	A	A	H	H	A	H	A	A	H	A
D1MIT46	H	A	A	A	A	A	H	H	H	A	H	H	H	H	A	H
D1MIT76	H	A	A	A	A	A	H	H	H	A	H	H	H	H	A	H
D1MIT8	H	A	A	A	A	A	H	H	H	A	H	H	H	H	A	H
D1MIT93	H	A	A	A	A	A	A	H	H	H	H	H	A	H	A	H
D2MIT14	A	H	H	H	A	A	A	H	H	A	A	H	H	A	A	A
D2MIT148	H	H	A	A	H	A	H	A	H	H	A	H	A	A	A	A
D2MIT15	A	H	H	H	A	A	A	H	H	A	A	H	A	H	A	A
D2MIT252	A	H	H		A	A	A	H	H	H	A	H	H	A	A	A
D2MIT260	A	H	H	A	H	A	A	A	H	H	A	H	A	A	A	A
D2MIT285	H	H	H	A	H	A	A	A	H	H	A	H	A	A	A	A
D2MIT343	H	H	H	A	H	A	H	A	H	H	A	H	A	A	A	A
D2MIT398	A	H	H	A	A	A		A		H	A	H	H	A	A	A
D2MIT58	A	H	H	A	A	A	A	H	H	H	A	H	H	A	A	A
D2MIT6	A	H	A	H	H	H	H	H	H	A	A	A	A	A	A	A
D2MIT7	A	H	H	H	A	H	H	H	H	A	A	A	A	A	A	A
D3MIT107	A	H	A	H	A	H	A	H	H	A	H	A	H	H	A	A
D3MIT116	A	A	A	H	A	H	H	H	A	H	H	A	H	H	A	H
D3MIT14	A	A	A	H	A	H	A	H	H	H	H	A	H	H	A	H
D3MIT17	A	A	A	H	A	H	H	H	A	H	H	A	A	H	A	H
D3MIT19	A	A	H	H	A	H	H	H	A	H	H	A	H	H	A	H
D3MIT209	A	H	A	H	H	H	A	H	H	A	H	A	A	H	A	A
D3MIT44	A	A	A	H	A	H	H	H	H	H	H	A	H	H	A	H
D3MIT46	A	H	A	H	H	H	H	A	H	H	H	A	A	H	A	A
D3MIT6	A	H	A	H	H	H	H	A	H	A	H	A	A	H	A	A
D3MIT62	A	H	A	H	A	H	A	A	H	H	H	A	A	H	A	A
D3MIT77	A	H	A	H	H	H	A	H	H	A	H	A	H	H	A	A
D4MIT12	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT124	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT126	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT14	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT148	H	A	H	H	A	H	H	H	A	A	A	H	H	A	A	H
D4MIT16	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT17	H	A	H	H	A	H	A	A	A	A	A	H	H	A	A	H
D4MIT170	H	A	H	H	A	H	H	H	A	A	A	A	H	A	A	H
D4MIT175	H	A	H	H	A	H	A	A	A	A	A	H	H	A	A	H
D4MIT178	H	A	H	H	A	H	A	A	A	A	A	H	H	A	A	H
D4MIT190	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT203	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT205	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT224	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT226	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT264	A	A	H	A	A	H	A	A	A	H	H	A	A	A	A	A
D4MIT33	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT37	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT40	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT41	A	A	H	A	A	H	A	A	A	A	H	H	A	A	A	H
D4MIT42	H	A	H	H	H	H	H	H	A	H	A	H	H	H	A	A
D4MIT45	H	A	H	H	A	H	A	A	A	A	A	H	H	A	A	H
D4MIT54	H	A	H	H	A	H	H	H	A	A	A	H	H	A	A	H
D4MIT57	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4MIT72	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D4NDS2	H	A	H	H	A	H	A	H	A	A	A	H	H	A	A	H
D5MIT113	A	H	H	A	A	A	H	H	H	A	A	A	A	H	H	A
D5MIT168	H	A	A	A	H	A	A	H	H	H	A	H	H	H	H	A
D5MIT23	A	H	H	A	A	A	A	H	H	H	A	A	A	H	H	A
D5MIT233	A	H	H	A	A	A	H	H	H	A	A	A	A	H	H	A
D5MIT346	A	H	H	H	A	A	H	H	H	A	A	A	A	A	A	H
D5MIT370	H	H	H	A	A	A	A	H	H	H	A	A	A	H	H	A
D5MIT7	A	H	H	A	A	A	H	H	H	A	A	A	A	H	H	A

D5MIT73	A	H	H	H	A	A	H	H	H	A	A	A	A	H	A	A
D5MIT76	A	H	H	H	A	A	H	H	H	A	A	A	A	H	A	A
D6MIT138	H	H	A	H	A	H	A	A	H	H	H	A	H	A	A	A
D6MIT14	H	A	A	A	A	H	A	A	H	H	H	A	H	H	A	H
D6MIT25	H	A	A	A	H		A		H		H	A	H	H	A	H
D6MIT254	H	A	A	H	H	A	A	A	H	H	H	A	H	H	A	H
D6MIT261	H	H	H	H	H	H	A	A	H	H	H	A	H	H	A	A
D6MIT268	H	H	H	H	H	H	A	A	H	H	H	A	H	H	A	A
D6MIT274	H	H	A	H	A	A	A	A	H	H	H	A	H	H	A	A
D6MIT277	H	H	H	H	H	H	A	A	A	H	H	A	H	H	A	A
D6MIT30	H	A	A	H	H	A	A	A	H	H	H	A	H	H	A	H
D6MIT59	H	A	A	A	H	A	A	A	H	H	H	A	H	H	A	H
D6MIT67	H	H	A	H		A	A	A	H	H	H	A	H	H	A	A
D6MIT70	H	H	H	H	H	H	A	A	H	H	H	A	H	H	A	A
D6NDS5	H	A	A	H	H	A	A	A	H	H	H	A	H	H	A	H
D7MIT105	A	H	H	A	H	A	A	A	H	H	A	A	A	H	A	H
D7MIT178	A	H	H	A	H	A	H	A	A	H	H	A	H	H	A	A
D7MIT181	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT220	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT234	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT25	A	H	H	A	H	A	A	A	A	H	H	H	A	H	A	A
D7MIT259	A	H	A	A	H	A	A	A	H	H	A	A	H	A	H	H
D7MIT281	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT284	H	H	H	H	H	H	A	A	H	H	H	A	H	H	A	A
D7MIT297	A	H	H	A	A	A	A	A	A	H	A	H	A	A	H	H
D7MIT319	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT32	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT321	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D7MIT57	A	H	H	A	H	A	A	A	A	H	H	H	H	H	A	A
D7MIT8	A	H	H	A	H	A	A	A	H	H	A	A	A	A	H	H
D7MIT83	A	H	H	A	A	A	A	A	A	H	A	H	A	H	A	H
D7MIT96	A	H	H	A	A	A	A	A	H	H	A	A	A	A	H	H
D8MIT121	A	A	A	H	H	A	H	H	H	A	A	A	H	A	H	A
D8MIT186	A	A	A	H	H	A	H	H	H	A	A	A	H	A	H	A
D8MIT190	A	H	A	H	H	A	H	H	H	A	A	A	A	A	H	A
D8MIT211	A	A	A	H	H	A	H	H	H	A	A	A	A	A	H	A
D8MIT4	A	H	A	H	H	A	H	H	H	A	A	A	A	A	H	A
D8MIT45	A	A	A	H	H	A	H	H	H	A	A	A	A	A	H	A
D8MIT8	A	H	A	H	H	A	H	H	H	A	A	A	A	A	H	A
D8MIT95	A	H	A	H	H	A	H	A	H	A	A	A	H	A	H	A
D9MIT10	H	A	A	H	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT116	H	A	A	H	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT154	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D9MIT163	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D9MIT182	H	A	A	H	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT19	H	H	A	H	H	A	A	A	H	H	A	A	H	A	A	A
D9MIT191	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D9MIT196	H	A	A	H	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT205	A	A	A	A	A	H	A	A	A	H	H	H	H	A	A	A
D9MIT208	H	A	A	H	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT259	A	A	A	A	H	A	H	A	H	H	H	H	H	A	A	A
D9MIT269	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D9MIT285	A	A	A	A	A	A	H	A	A	H	H	H	H	A	A	A
D9MIT31	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D9MIT42	A	A	A	A	A	A	H	A	A	H	H	H	H	H	A	A
D9MIT74	A	A	A	A	H	A	H	A	H	H	H	A	H	A	A	A
D9MIT97	A	A	A	A	H	A	H	A	A	H	H	H	H	A	A	A
D10MIT11	H	A	A		H	H	A		A	H	H	A	A	H	A	H
D10MIT134	H	H	A	A	H	H	H	A	A	A	H	H	A	A	H	A
D10MIT15	A	A	A	H	H	H	A	A	A	H	H	A	A	H	A	H
D10MIT17	H	A	A	H	H	H	A	A	A	H	H	A	A	H	A	H
D10MIT248	A	H	A	H	A	H	A	A	A	H	H	A	A	A	H	H
D10MIT271	H	H	A	H	H	H	A	A	A	H	H	A	H	H	A	A
D10MIT42	A	A	A	H	H	H	A	A	A	H	H	A	A	H	A	H
D10MIT44	H	A	A	A	A	A	H	H	A	H	A	H	H	H	H	H
D11MIT116	A	A	H	A	H	A	H	H	H	A	H	H	H	H	A	H
D11MIT130	A	A	A	A	A	A	H	H	H	A	H	H	H	H	H	H
D11MIT150	A	A	A	A	A	A	H	H	H	A	H	H	A	H	H	H
D11MIT23	A	A	A	A	H	A	H	H	H	A	H	H	H	H	H	H
D11MIT254	H	A	H	A	H	H	H	H	A	A	A	H	H	H	A	H



D11MIT288	A	A	H	A	H	H	H	H	A	A	H	H	H	H	A	H
D11MIT30	A	A	A	A	H	A	H	H	H	A	H	H	H	H	A	H
D11MIT306	A	A	A	A	A	A	H	H	H	A	H	H	A	H	H	H
D11MIT99	A	A	H	A	H	H	H	H	A	A	H	H	H	H	A	H
D12MIT10	A	A	A	H	H	A	A	H	H		A	A	H	A	A	A
D12MIT112	A	A	A	H	H	A	A	H	H	H	A	A	H	A	A	H
D12MIT136	A	A	A	H	H	A	A	H	H	H	A	A	H	A	A	H
D12MIT203	A	H	A	H	A	A	A	A	H	H	A	H	H	A	A	H
D12MIT231	H	H	A	H	A	A	A	A	A	H	A	H	H	A	A	H
D12MIT233	H	H	A	H	A	A	A	A	A	H	A	H	H	A	A	H
D12MIT46	A	A	A	H	H	A	A	A	H	H	A	A	A	H	A	H
D12MIT52	A	H	A	H	A	A	A	H	H	H	A	H	H	A	A	H
D12MIT68	A	A	A	H	A	A	A	H	H	H	A	A	H	A	A	H
D12NDS11	A	A	A	H	H	A	A	H	H	H	A	A	H	A	A	H
D12NDS2	H	H	A	A	A	A	A	A	A	H	A	H	H	A	A	H
D13MIT10	H	A	H	A	H	H	A	A	H	H	H	A	A	H	A	A
D13MIT117	H	A	H	A	H	H	A	H	H	H	H	A	A	H	A	A
D13MIT151	H	A	H	H	A	H	A	A	H	A	H	H	A	A	H	A
D13MIT16	A	A	H	A	H	H	A	H	H	H	H	A	A	H	A	H
D13MIT193	H	H	H	H	A	H	A	A	H	A	H	H	A	A	H	A
D13MIT41	H	H	H	A	H	H	A	A	H	H	H	A	A	H	H	A
D13MIT75	H	A	H	H	A	H	A	A	H	A	H	H	A	A	H	A
D14MIT101	H	A	H	H	H	A	H	A	H	H	H	H	A	H	A	H
D14MIT109	H	H	H	H	H	A	H	A	H	A	H	A	H	H	A	H
D14MIT133	H	A	H	H	H	A	H	A	H	A	H	H	A	H	A	H
D14MIT160	H	A	H	A	H	A	H	H	A	H	H	H	A	H	A	A
D14MIT203	H	A	H	H	H	A	H	H	A	H	H	H	A	H	A	H
D14MIT75	H	A	H	A	H	A	H	H	A	H	H	H	A	H	H	A
D15MIT11	H	A	H	H	A	H	H	H	H	H	H	H	H	H	H	H
D15MIT171	A	H	A	A	A	A	A	H	H	A	H	H	A	A	H	A
D15MIT189	A	H	A	H	A	A	A	H	H	A	H	H	A	A	H	A
D15MIT24	H	H	H	H	A	A	H	H	H	A	H	H	A	A	H	H
D15MIT26	H	H	H	H	A	A	H	H	H	A	H	H	A	A	H	H
D15MIT3	H	H	H	H	A	A	H	H	H	A	H	H	A	A	H	H
D15MIT35	A	H	A	A	A	A	A	A	H	A	H	A	H	A	A	A
D16MIT110	A	A	H	A	H	A	A	H	H	A	A	A	H	A	A	A
D16MIT154	A	H	H	H	H	H	A	H	H	A	A	A	A	H	A	H
D16MIT171	A	A	H	A	H	A	A	H	H	A	A	A	H	A	H	A
D16MIT189	H	A	H	A	H	A	A	A	A	A	A	A	H	A	H	A
D16MIT4	A	A	H	A	H	A	A	H	H	A	A	A	A	H	A	H
D16MIT51	H	A	H	A	H	A	A	A	A	A	A	A	H	A	H	A
D16MIT87	A	H	H	H	H	H	A	H	H	A	A	A	A	H	A	H
D17MIT119	A	A	H	H	H	A	H	H	A	H	A	A	A	A	A	H
D17MIT128	A	A	H	H	H	H	H	H	A	H	H	A	A	A	A	A
D17MIT13	A	A	H	H	H	H	H	H	A	H	H	A	A	A	A	A
D17MIT176	A	A	H	H	H	H	H	H	A	H	H	A	A	A	A	A
D17MIT197	A	A	A	H	A	H	H	H	H	H	H	A	A	A	A	A
D17MIT38	A	A	H	H	H	A	H	A	A	H	A	A	H	A	A	H
D17MIT56	A	A	H	H	A	A	H	A	A	H	A	A	H	A	A	H
D17MIT7	A	A	H	H	H	A	H	H	A	H	H	A	A	A	A	A
D18MIT124	H	H	H	A	A	H	H	H	A	H	H	A	A	A	H	A
D18MIT177	H	A	H	A	A	H	H	H	A	H	H	A	A	A	H	A
D18MIT44	H	H	H	A	A	H	H	H	A	H	H	A	A	A	A	H
D18MIT50	H	H	H	A	A	H	H	H	A	H	A	A	A	A	A	A
D18MIT58	H	H	H	A	A	H	H	H	A	H	H	A	A			A
D18MIT7	H	H	H	A	A	H	H	H	A	H	H	A	A	A	A	A
D18MIT94	H	A	H	A	A	H	H	H	A	H	H	A	A	A	H	A
D19MIT1	H	A	A	H	A	H	H	H	H	H	A	A	A	H	A	H
D19MIT10	H	A	A	H	A	H	H	H	A	H	A	H	A	H	A	H
D19MIT29	A	H	A	H	H	H	H	A	A	H	A	A	A	H	A	A
D19MIT41	A	A	A	H	A	H	H	A	A	H	A	H	A	H	A	H
D19MIT46A	A	A	A	H	A	H	H	H	A	H	A	H	A	H	A	H
D19MIT46B	H	A	A	A	H	A	A	H	H	H	A	H	H	H	H	A
D19MIT53	H	A	A	H	A	H	H	H	A	H	A	H	A	H	A	H
D19MIT71	H	A	A	H	A	H	H	H	H	H	A	A	A	A	A	H
DXMIT114	A	B	A	B	A	A	A	A	A	A	H	A	H	H	A	A
DXMIT166	H	A	B	A	B	A	A	B	A	A	H	H	H	H	A	A
DXMIT186	A	B	A	B	A	A	B	A	H	H	H	A	A	H	B	A

**Table A7** The raw data of papilloma incidence in FVB6F2 mice.

M.ID	*CC	**SEX	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	W23
FB1	A	F	0	1	1	5	6	9	14	16	16	16	15	16	21	20	18	15
FB2	W	F	0	0	0	1	2	4	5	6	5	4	8	6	8	8	8	6
FB3	A	F	1	2	2	2	4	6	11	16	16	19	18	19	21	24	24	
FB4	B	F	0	0	0	0	0	0	1	5	5	6	3	0	0	0	0	
FB5	A	F	0	1	1	1	3	5	9	11	12	15	18	22	21	21	19	
FB6	A	F	0	0	0	0	1	2	3	3	3	3	4	6	6	6	5	
FB7	A	F	0	0	0	0	0	0	0	0	0	0	2	2	3	3	2	
FB8	A	F	0	0	0	0	0	1	1	1	1	3	3	2	3	2	3	
FB9	A	F	2	1	4	8	11	10	18	25	25	31	33	32	23	21	18	
FB10	A	F	0	0	0	0	0	1	1	1	1	1	4	5	7	8	8	
FB11	A	F	0	0	0	0	2	2	2	2	3	3	4	5	5	5	5	
FB13	A	F	3	3	6	6	16	20	26	26	29	29	31	30	30	28	26	
FB14	A	F	0	0	0	0	2	4	3	7	8	6	7	9	8	8	6	
FB15	B	F	0	0	0	1	5	4	9	9	11	10	8	10	9	9	9	
FB16	A	F	0	0	0	0	4	4	5	3	3	3	4	3	3	4	4	
FB17	W	F	0	2	2	3	4	7	10	12	12	17	19	20	24	27	27	
FB18	A	F	0	0	0	0	5	5	5	8	7	8	12	12	12	13	15	
FB20	A	F	0	0	0	0	5	5	13	17	17	22	31	32	32	32	32	
FB21	A	F	0	0	0	0	0	0	0	1	1	1	2	2	2	2	2	
FB23	A	F	1	0	1	1	1	2	8	7	7	9	9	8	10	8	8	
FB24	A	F	0	0	0	1	4	3	3	6	7	8	11	12	11	12	14	
FB25	W	F	1	1	1	1	2	2	6	7	7	7	15	17	12	13	16	
FB26	B	F	0	2	2	3	3	3	6	6	9	10	16	17	18	18	18	
FB27	B	F	0	0	0	1	1	1	3	3	4	7	7	8	5	5	5	
FB28	A	F	0	0	0	0	2	1	3	4	5	5	6	6	6	9	11	
FB29	A	F	0	0	0	0	0	1	2	1	1	2	2	2	8	8	8	
FB30	B	F	1	1	1	1	2	2	6	7	7	11	12	14	16	15	15	
FB31	A	F	0	1	2	4	13	18	18	21	26	29	29	29	21	18	16	
FB32	B	F	0	0	0	0	0	0	0	0	0	1	1	1	3	4	4	
FB34	A	F	0	0	0	0	2	2	3	2	4	4	4	4	3	2	1	
FB35	A	F	0	0	0	1	3	2	6	8	8	12	17	14	14	15	15	
FB37	A	F	0	0	0	0	4	4	5	5	6	5	8	7	6	6	8	
FB38	A	F	0	0	0	3	6	10	17	18	19	20	21	18	22	21	16	
FB39	B	F	0	1	1	4	10	10	11	18	25	24	26	26	16	12	11	
FB41	B	F	0	0	0	0	2	1	3	3	5	4	3	3	5	6	5	
FB42	B	F	1	1	2	2	4	6	12	13	16	20	27	27	26	22	24	
FB43	A	F	0	0	1	1	4	6	11	10	14	14	26	25	28	31	35	
FB44	B	F	0	0	0	0	1	4	10	7	12	11	10	12	8	4	4	
FB45	B	F	0	0	0	0	1	1	1	2	2	2	4	4	5	5	5	
FB46	A	F	0	0	0	0	5	5	12	15	15	15	17	16	19	19	19	
FB47	A	F	2	5	7	9	20	24	34	36	39	39	36	29	25			
FB48	W	F	0	0	0	0	0	0	2	2	1	1	1	2	3	2	3	
FB49	A	F	0	0	0	0	6	6	17	18	17	20	21	21	26	24	22	
FB50	B	F	0	0	0	0	0	0	0	1	1	1	1	0	2	2	2	
FB51	W	F	0	2	2	2	18	25										
FB52	A	F	0	0	0	0	0	0	1	1	3	4	9	8	3	1	1	
FB53	A	F	1	1	2	2	1	0	2	3	3	3	4	4	3	4	3	
FB54	W	F	0	1	1	1	3	4	10	13	16	18	23	23	26	26	26	
FB55	W	F	1	0	0	0	4	2	7	7	7	7	7	3	3	3	3	
FB56	A	F	1	1	1	1	2	2	3	5	5	6	9	8	5	5	5	
FB57	B	F	0	0	0	0	2	2	5	5	9	10	13	12	11	13	12	
FB58	A	F	0	0	1	1	4	4	3	3	3	1	2	1	1	1	0	
FB59	B	F	0	0	0	0	0	2	6	6	6	11	9	11	10	10	11	
FB60	W	F	1	1	1	1	2	2	4	4	4	6	8	5	4	3	2	
FB61	B	F	0	0	0	0	0	0	1	3	3	3	10	10	13	10	13	
FB62	B	F	0	0	0	0	0	0										
FB63	A	F	1	3	4	5	12	17	33	34	34	34	37	33	33	31	29	
FB64	B	F	0	0	0	0	2	2	7	6	4	4	3	3	5	1	1	
FB65	A	F	0	0	2	2	3	5	15	15	18	22	24	25	24	22	20	
FB66	A	F	0	0	0	0	8	8	12	12	11	11	11	10	10	6	7	
FB67	A	F	0	0	0	2	2	3	10	9	7	9	13	11	12	9	9	
FB68	W	F	0	0	0	0	0	0	0	0	2	4	8	8	10	10	12	
FB69	A	F	0	0	0	8	1	1	2	2	2	4	6	6	7	7	7	
FB70	B	F	0	0	1	1	2	2	4	4	5	5	5	5	8	8	9	
FB71	W	F	0	0	0	1	3	6	13	21	21	24	19	22	13	17	20	
FB72	A	F	0	0	0	3	6	6	9	10	10	10	13	13	17	17	18	
FB73	A	F	0	0	0	0	0	0	3	4	4	7	8	6	9	9	9	
FB74	A	F	2	2	2	2	7	7	13	13	19	20	26	27	31	31	28	
FB75	A	F	0	0	0	0	0	0	2	4	4	5	6	6	4	3	6	
FB76	B	F	0	0	0	0	2	2	2	6	8	6	3	3	2	2	2	
FB77	W	F	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	
FB78	B	F	1	2	1	1	3	4	11	14	13	11	9	9	10	7	8	

FB79	A	F	0	0	0	0	0	0	0	0	3	4	5	6	6	7
FB80	A	F	1	2	5	5	15	21	31	39	42	44	42	39	36	32
FB81	B	F	0	0	0	0	2	4	6	7	6	7	8	6	8	9
FB82	A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB83	A	F	1	2	2	3	3	4	10	8	9	15	15	15	18	18
FB84	A	F	0	0	0	0	2	6	14	13	13	16	14	14	16	15
FB85	W	F	0	0	0	0	2	2	2	2	0	2	2	1	1	0
FB86	W	F	0	0	0	1	3	3	7	10	8	10	12	11	11	10
FB87	W	F	0	1	2	3	6	6	6	8	14	12	12	8	7	6
FB88	A	F	0	1	2	2	5	5	7	9	10	11	14	15	17	22
FB89	A	F	0	0	0	1	2	4	9	9	9	11	11	10	12	15
FB90	W	F	0	1	2	3	9	9	25	25	24	25	28	24	23	21
FB91	W	F	0	0	0	0	0	0	1	1	1	1	2	2	2	3
FB92	A	F	0	1	2	5	10	10	14	13	13	14	13	11	14	16
FB93	W	F	0	0	0	0	3	5	10	11	14	17	19	16	10	15
FB94	W	F	0	1	2	2	2	7	20	20	21	19	18	14	16	12
FB95	W	F	0	0	0	0	0	0	0	1	0	0	0	0	0	0
FB96	B	F	1	1	1	4	11	12	21	19	24	25	29	28	23	20
FB97	W	F	0	0	0	0	1	1	1	0	0	1	1	1	1	1
FB98	A	F	0	1	1	2	6	6	11	10	12	15	18	25	23	22
FB99	W	F	0	0	0	0	3	4	6	5	5	7	8	5	8	8
FB100	A	F	0	0	1	2	1	1	2	2	3	3	4	3	3	4
FB101	W	F	0	1	2	3	5	6	12	13	12	15	12	11	11	12
FB102	B	F	1	1	1	1	3	3	6	6	5	5	5	5	5	5
FB103	W	F	0	0	1	1	1	1	2	3	3	3	4	4	5	5
FB104	A	F	1	1	2	2	8	10	14	16	16	17	17	17	19	21
FB105	W	F	0	0	0	0	0	2	3	5	8	7	10	9	10	8
FB106	A	F	1	1	1	2	6	7	11	12	12	14	17	16	16	16
FB107	A	F	0	0	0	1	3	3	10	14	12	12	12	17	16	14
FB109	A	F	0	0	1	2	3	4	9	12	12	12	14	12	15	16
FB110	B	F	0	0	0	1	4	5	12	12	13	10	10	11	11	11
FB111	A	F	0	0	0	0	0	0	0	0	0	0	1	1	0	1
FB112	B	F	0	0	0	0	2	2	2	3	3	3	6	7	6	4
FB113	A	F	0	0	0	0	0	1	0	1	2	0	0	2	1	2
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FB115	A	F	1	3	2	6	6	15	15	15	15	15	15	15	15	15
FB116	A	F	0	0	0	0	0	1	2	2	2	3	6	7	7	7
FB117	A	F	0	0	0	0	1	1	3	5	4	7	11	15	17	17
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FB119	A	F	0	0	1	2	2	6	8	8	8	8	8	8	8	8
FB120	A	F	0	0	2	4	3	5	6	5	5	7	9	10	10	15
FB121	A	F	0	0	0	0	2	3	7	7	6	7	6	6	5	7
FB122	W	F	0	1	1	4	5	6	6	7	7	11	10	13	14	14
FB123	A	F	0	0	0	0	0	4	4	5	9	8	10	7	9	10
FB124	A	F	0	0	2	4	6	6	7	10	12	11	11	10	8	7
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FB126	A	F	0	0	3	8	8	19	19	19	18	14	17	20	18	16
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FB129	W	F	0	0	1	1	1	3	3	3	2	2	2	3	3	4
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FB131	B	F	0	0	1	4	5	8	8	11	10	11	13	15	12	14
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FB133	A	F	0	0	1	2	5	9	9	9	14	15	19	23	20	19
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FB137	A	F	0	1	2	2	4	5	6	8	13	13	15	19	17	11
FB138	A	F	0	0	0	0	0	1	0	2	2	2	2	3	3	4
FB139	A	F	0	1	1	6	10	26	32	38	38	38	38	38	38	38
FB140	W	F	0	1	1	1	1	4	7	8	10	16	16	12	12	12
FB141	B	F	0	0	0	0	1	3	3	5	5	6	5	2	3	3
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FB143	A	F	0	0	0	1	1	2	2	4	5	6	6	10	11	11
FB144	A	F	0	0	2	6	5	13	18	22	24	24	31	31	34	32
FB145	A	F	0	1	1	1	2	3	3	3	3	3	3	3	3	3
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FB148	A	F	0	0	0	0	0	7	8	8	9	8	8	12	12	9
FB149	W	F	0	0	0	2	3	5	9	11	17	21	22	21	23	20
FB150	W	F	0	0	0	0	1	1	1	1	1	1	1	1	1	1
FB151	W	F	0	0	1	1	5	6	6	6	7	13	14	15	13	17
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FB154	A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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FB156	A	F	0	0	2	6	11	11	12	12	9	13	12	6	6	6	6
FB157	A	F	0	3	2	2	4	4	4	5	3	4	3	2	2	1	1
FB159	W	F	0	1	1	1	1	1	5	5	3	3	1	3	3	2	2
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FB162	B	F	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
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FB164	A	F	0	0	0	0	0	0	2	1	1	0	0	2	2	3	2
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FB166	A	F	1	1	3	2	7	8	10	10	10						
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FB168	W	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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FB170	W	F	0	1	2	3	10	15	13	17	28	30	37	33	33	33	
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FB173	A	F	0	3	3	3	8	10	10	10	11	11	9	9	7	5	
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FB176	A	F	0	1	1	5	12	14	16	18	19	21	20	21	17	13	10
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FB178	W	F	0	0	0	0	0	0	1	1	2	2	2	2	3	3	3
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FB182	B	F	0	0	0	2	1	1	3	7	11	9	13	14	17	21	21
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FB185	W	F	0	0	1	1	3	9	12	15	20	23	23	23	23	20	18
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FB187	B	F	0	0	1	1	1	1	1	2	3	3	3	3	4	4	3
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FB189	W	F	0	0	1	1	2										
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FB191	A	F	0	0	1	2	2	5	7	4	6	7	7	8	8	9	9
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FB200	W	F	0	0	0	0	0	0	0	1	1	1	2	2	4	4	4
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FB202	B	F	0	0	1	1	1	4	5	6	6	6	6	6	6	8	8
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FB204	A	F	3	5	6	5	5	3	3	7	8	8	8	7			
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FB206	A	F	0	0	0	1	1	1	2	3	7	8	7	8	8	9	9
FB207	A	F	0	0	0	0	0	0	0	0	0	1	1	1	5	5	5
FB208	W	F	1	1	1	1	3	5	5	6	5	7	5	5	7	9	9
FB209	W	F	0	2	3	3	6	9	9	17	12	13	13	13	13	8	
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FB213	B	F	0	0	1	1	2	3	3	3	3	3	7	8	13	16	20
FB214	A	F	0	0	1	0	0	1	0	1	1	1	0	0	2	2	2
FB216	A	F	0	0	0	1	1	1	2	2	3	3	2	2	2	2	2
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FB218	A	F	0	1	2	2	2	5	5	9	8	10	13	14	15	13	12
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FB220	A	F	1	4	10	10	10	8	8	8	5	5	4	4	4	3	
FB221	W	F	0	0	0	0	0	1	1	1	1	1	3	4	7	8	8
FB222	B	F	0	1	1	3	4	5	8	11	12	13	13	13	16	16	14
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FB227	A	F	1	3	3	5	7	8	10	15	12	12	12	12	11	9	
FB228	W	F	0	0	2	2	2	3	7	8	8	9	10	10	10	11	12
FB229	W	F	1	4	5	5	6	7	7	6	4	4	2	2	3	3	3
FB230	B	F	0	2	2	1	1	3	3	8	9	7	8	8	7	6	6
FB231	A	F	0	3	5	4	6	9	9	9	9	8	11	13	11	13	13
FB233	A	F	0	3	0	4	5	6	15	18	16	18	20	21	21	21	21
FB234	A	F	0	1	1	1	1	1	2	2	3	3	4	4	7	7	7
FB235	A	F	0	0	0	0	0	0	0	0	0	0	3	2	2	3	3
FB236	A	F	0	0	0	1	1	3	3	3	5	8	8	11	15	17	17
FB237	A	F	0	0	0	0	0	1	3	2	3	4	3	3			

FB238	W	F	0	0	0	0	2	2	5	8	10	8	8	8	8	9
FB239	W	F	1	3	3	4	5	11	13	13	17	17	16	17	17	16
FB240	B	F	0	0	0	0	0	0	0	0	0	0	0	0	1	1
FB242	A	F	0	2	3	5	7	9	11	12	10	13	13	12	7	8
FB243	A	F	0	1	1	3	4	3	9	9	9	11	11	11	11	9
FB245	A	F	0	0	0	1	1	1	1	0	0	0	0	0	0	1
FB246	W	F	0	1	1	2	6	13	18	19	22	26	26	26	25	20
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FB248	W	F	0	0	1	2	2	6	12	14	16	18	19	20	20	21
FB249	W	F	0	4	4	4	4	8	13	10	13	14	14	14		
FB251	W	F	0	0	0	0	1	2	3	2	3	4	4	4	5	5
FB252	B	F	0	0	0	1	2	8	10	11	16	15	17	20	20	21
FB253	A	F	1	3	4	11	12	16	22	22	15	15	10	9	5	3
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FB256	A	F	0	0	0	0	0	0	0	0	0	0	0	1	1	1
FB257	W	F	0	0	0	1	3	6	9	11	10	11	10	10	14	12
FB258	W	F	0	0	2	8	9	17	19	19	17	22	24			
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FB260	B	F	0	0	2	3	3	11	16	14	14	19	15	15	10	9
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FB265	A	F	0	0	0	0	0	0	3	6	5	6	6	6	7	8
FB266	A	F	0	2	3	6	5	7	8	10	11	7	7	4	2	3
FB267	W	F	0	0	0	0	1	1	1	3	4	5	6	7	7	8
FB268	W	F	1	5	9	9	6	9	17	15	13	10	12	10	10	10
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FB270	B	F	1	5	10	12	14	19	17	15	13	13	13	14	15	11
FB271	B	F	0	0	0	0	0	0	0	0	2	4	5	5	6	8
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FB273	A	F	0	1	4	6	4	12	8	2	1	3	1	1		
FB274	W	F	0	0	0	0	2	4	6	6	8	9	8	8	8	9
FB275	W	F	0	0	0	0	1	5	8	8	9	6	4	2	4	4
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FB281	A	F	6	7	8	7	16	19	21	16	16	18	18	18	16	16
FB282	A	F	0	0	1	3	3	5	9	9	10	12	14	17	18	23
FB283	A	F	1	2	4	8	13	14	16	18	20	22	22	25	25	22
FB284	A	F	0	0	1	1	1	2	3	3	4	4	4	4	3	4
FB285	A	F	1	0	0	0	1	1	3	3	5	6	6	6	6	
FB286	W	F	0	0	0	0	0	0	1	0	0	0	0	0	1	1
FB287	B	F	0	1	1	3	4	5	6	10	14	14	14	16	16	18
FB288	A	F	0	0	1	1	8	15	23	23	25	26	29	31	31	31
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FB291	A	F	0	1	6	9	10	16	16	20	22					
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FB293	A	F	0	0	1	2	1	2	2	2	2					
FB294	A	F	0	0	0	1	2	3	5	5	5	6	7	7	6	6
FB295	W	F	0	0	2	4	9	11	11	11	11	12	7	4	2	2
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FB300	A	F	0	0	0	1	1	2	3	4	4	5	5	6	6	9
FB301	A	F	0	0	0	0	0	0	2	2	2	2	2	2	2	1
FB302	A	F	0	0	3	5	8	9	12	12	12	12	12	13	12	14
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FB304	W	F	0	0	4	6	7	12	7	7	10	10	10	14	12	15
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FB306	W	F	0	0	0	0	0	0	1	1	1	1	2	3	3	3
FB307	A	F	0	0	2	5	5	7	8	9	12	14	14	15	17	15
FB308	A	F	0	0	0	0	1	1	1	2	4	5	8			
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FB310	A	F	1	2	2	8	10	11	14	14	14					
FB312	B	F	0	1	12	18	31	30	30	30	30	29	29	26	23	18
FB313	B	F	0	0	0	2	2	2	2	2	2	3	3	3	3	3
FB314	W	F	0	1	2	3	6	9	12	12	12	12	13	15	15	15
FB315	W	F	3	3	8	13	21	31	31	31	31					
FB316	B	F	0	0	0	0	0	3	5	4	5	4	5	8	4	6
FB317	B	F	1	1	2	2	2	6	6	7	7	8	8	11		

FB318	B	F	0	0	0	0	0	1	2	7	7	7	8	12	12	15	16	18
FB320	A	F	0	1	6	10	10	18	17	18	18	15	12					
FB321	A	F	1	2	3	3	2	5	6	6	7	7	9	9	9	12	12	14
FB322	A	F	0	0	2	2	1	1	1	1	1	1	1	1	1	1	1	1
FB323	A	F	1	1	2	3	8	9	11	15	16	18	20	23	23	23	20	20
FB324	A	F	2	3	4	7	15	13	18	20	22	21	21	22	18	13	14	14
FB325	W	F	0	0	3	3	5	8	9	9	13	18	22	25	25	28	28	28
FB326	A	F	0	0	0	0	0	0	1	3	3	3	4	6	7	9	8	8
FB327	A	F	0	0	7	7	14	17	17	27	27	27	27	14	14	13	12	10
FB328	A	F	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
FB329	A	F	0	0	0	0	6	8	11	13	16	18	18	21	21	21	19	19
FB330	A	F	0	0	1	3	8	6	6	7	8	8	8	10	8	10	8	8
FB331	A	F	0	1	1	2	0	2	3	2	4	6	6	7	8	8	8	9
FB332	A	F	0	1	1	7	13	19	18	23	24	24	25	30	30	33	30	30
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FB334	W	F	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2
FB335	W	F	0	0	2	5	10	8	9	7	6	7	4	4				
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FB337	B	F	1	3	4	5	15	18	20	20	20	21	21	20	18	18	20	20
FB338	A	F	0	0	1	2	5	5	5	6	6	6	7	11	11	9	9	9
FB339	B	F	0	1	6	8	13	18	16	17	17	17	15	15				
FB340	B	F	0	1	2	3	9	9	13	16	16	16	18	18	16	13	8	7
FB341	A	F	0	1	6	11	19	20	27	27	27	27	27	26	23	26	26	26
FB344	B	F	0	0	0	1	3	1	0	1	0	0	0	0				
FB345	W	F	0	0	0	4	4	4	8	9	8	11	11	12	12	12		
FB346	W	F	1	2	3	5	5	11	6	9	10	10	10	12	12	12	12	12
FB347	A	F	0	0	0	0	1	3	2	3	3	4	4	4	3	2	1	1
FB348	A	F	0	1	0	1	4	3	3	2	2	2	3	3	3	3	3	3
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FB350	A	F	0	0	0	0	0	7	8	9	8	8	8	8	8			
FB351	A	F	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
FB352	W	F	0	0	2	3	8	7	9	13	12	11	10	9				
FB353	A	F	0	0	2	3	6	14	15	17	17	18	20	22	22	20	21	21
FB354	W	F	0	0	0	1	1	1	1	1	1							
FB355	W	F	0	2	4	5	9	8	7	7	6	4	4					
FB356	A	F	0	1	3	6	17	18	25	28								
FB357	A	F	0	0	0	1	4	4	2	3	3	3	3	3				
FB358	A	F	0	2	1	4	7	12	7	13	16	16	16	21	21	21	21	21
FB359	A	F	0	0	4	6	13	13	21	22	22	20	20	18	16	16	16	15
FB360	A	F	0	1	7	14	16	19	23	23	23	21	21	19	15	13	13	13
FB361	W	F	0	0	0	2	4	4	4	8	10	14	14	14	12	12		
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FB365	W	F	0	2	2	4	5	7	7	11	11	11	11	14	14	14	14	14
FB366	B	F	0	1	8	13	16	12	8	10	7							
FB367	A	F	1	3	4	10	16	18	24	25	25	24	27	30	30	30	30	29
FB368	B	F	0	0	1	1	1	2	3	3	4	6	6	7	7	9	7	7
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FB371	W	F	0	0	0	0	2	3	3	3	3	1	0	0				
FB372	A	F	0	0	0	0	1	4	4	4	5	5	5	5	6	6	5	4
FB373	A	F	0	0	3	2	6	6	9	9	11	12	14	15	14	14	15	15
FB374	W	F	0	3	4	6	9	12	13	13	13	15	16	16	15	13	13	12
FB376	A	F	0	0	0	3	3	7	8	15	15	15	15	17	17	17	17	17
FB377	A	F	0	0	0	0	0	0	0	0	0	2	3	5	5	5	5	6
FB378	W	M	0	0	2	4	6	6	7	10	12	11	12	7	7	7	7	5
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FB380	W	M	0	0	0	0	0	0	0	2	2	1	1	1	2	2	2	1
FB381	W	M	0	0	0	0	2	2	2	3	3	4	4	5	5	5	4	5
FB382	W	M	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
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FB385	A	M	0	1	1	1	2	2	2	3	4	6	6	6	6	8	11	11
FB386	A	M	0	0	0	0	0	0	0	0	0	0	1	1	1	2	3	3
FB387	A	M	1	1	0	1	3	3	2	3	4	4	4	6	8	8	9	9
FB388	W	M	0	3	2	10	23	23	24	21	21	24	28	28	30	31	34	34
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FB390	W	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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FB392	A	M	0	0	0	0	0	0	2	2	2	2	2	3	7	9	9	8
FB393	A	M	0	0	0	2	3	3	3	2	2	1						
FB394	A	M	0	0	1	5	7	9	9	9	9	7	6	6				
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FB396	A	M	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2

FB397	A	M	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	
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FB402	A	M	0	0	0	0	0	0	0	0	0	0	2	2	5	5	5	4
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FB406	A	M	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
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FB417	A	F	0	0	0	1	1	0	0	2	1	1	3	3	4	5	5	
FB418	B	F	2	5	5	12	14	14	18	21								
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M.ID	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	W34	W35	W36	W37	W38	W39	W40	W41	W42
FB1	19	18	18	18	18	18	18	17	17	17	17	16	16	16					
FB2	7	6	6	6	4	4	4	4	4	4	4	5	5	5	5	5	5	4	4
FB3	24	24	24	20	23	23													
FB4																			
FB5	21	21	21	21	21	19	19	17	17	17	16	16	16						
FB6	5	5	5	4	5	5	5	5	5	5	5	5	3	4	4	4	4	4	4
FB7	2	2	2	2	2	2													
FB8	3	3	3	3	4	3	2	2	1	1	1	1	1	1	1	1	1	1	2
FB9	17	13	10																
FB10	9	9	9	9	9	8	8	10	9	9	9	9	10	10	12	12	12	12	10
FB11	5	5	4	4	4	4	3	3	4	4	4	2	2	1	1	0	1	0	0
FB13	27	29	28	28	27	29	24	23	23	22									
FB14	5	5	4	4	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
FB15	9	6	5	4	4	4	4	4	4	4	4	4	3	3	4	3	3	3	3
FB16	4	4	3	3	3	3	3	4	3	3	3								
FB17	26	25	23	20	17	16	16	15	15	15	14	15	14	15	15	14	15	15	13
FB18	15	16	16	14	13	11	9	8	5	4	4	3	2	4					
FB20	32	31	30	29	27	25	26	25	25	25	24	24	24						
FB21	2	2	2	2	2	3	3	3	3	3	3	3	2	2	2	2	2	2	2
FB23	8	8	7																
FB24	14	14	12	11	10	7	6	6	6	6	4	4	2	1	1	0	0	0	0
FB25	16	16	16	16	16	16	16	17	16	16	16	15	14						
FB26	16	15	15	15	15	13	13	12	11	11	10	11							
FB27	6	7	7	9	8	8	8	8	8	8	8	8	6	3	3	5	5	5	5
FB28	10	12	12	11	12	12	12	12	11	11	9	9	8	9	9	10	9	9	9
FB29	8	8	8	9	8	8	8	9	9	9	8	8	8	10	9	7	8	7	7
FB30	15	15	13	13	12	10	9	9	7	7	6	6	7	7	7	7	7	7	7
FB31	16	16	17	16	15	13	12												
FB32	3	3																	
FB34	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB35	15	16	16	15	15	15	15	11	13	14	11								
FB37	7	7	7	8	8	8	8	8	8	8	8	9	8	8					
FB38	18	18	18	18	17	15	15	17	16	15	15	15	15						
FB39	11	10																	
FB41	6	6	6	8	8	7	6	7	6	6	6	6	5	4	4	4	4	4	5
FB42	25	25	25	25	25	23	24	23	23	23	21	20	17	15	15	15	15	13	13
FB43	35	35																	
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FB46	19	21	21	21	21	19	21	21	22	22	19	16	16						
FB47																			
FB48	3	6	6	6	6	5	4	4	3	3	3	2	2	3	3	3	3	2	2
FB49	19	19	16																
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FB51																			
FB52	1	1	1	1															
FB53	3																		
FB54	28	28																	
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<b>FB62</b>																			
<b>FB63</b>	29	29	29	29	29	29	29												
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<b>FB66</b>	3	2	2	2	3	3													
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<b>FB70</b>	9	9	9	9	9	9	7												
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<b>FB73</b>	9	9	9	9	8	8	8	8	8	8	7	6	5						
<b>FB74</b>	30	29	30	29	30	30	30	30	30	30	30	30	30						
<b>FB75</b>	6	6	6	6	6	5	4	4	3	3	3	3	3	2	2	2	2	2	
<b>FB76</b>	2	2																	
<b>FB77</b>	1	1	1	0															
<b>FB78</b>	7	8	8	7	6	5	5	4	3	3									
<b>FB79</b>	7	7	7	9	9	11	11	11	11	11	10	9	9	9					
<b>FB80</b>	33	31	31	27	26	24													
<b>FB81</b>	11	10	12	13	15	15													
<b>FB82</b>																			
<b>FB83</b>	20	20	19	19	17	16	16	16	16	16	16	16	16	13	10				
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<b>FB85</b>	2	2	1	0															
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<b>FB87</b>	7	6	6																
<b>FB88</b>	22	22	22	22	22	22													
<b>FB89</b>	18	18	18	16	16	14	13	13	13	13	13	12	10	8	8				
<b>FB90</b>	27	27	27	27	27	24	28	28	26	25	25	25	25	25	25	25			
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<b>FB92</b>	14	14	14	14	14	16	15	15	14	15	14	14	14						
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<b>FB94</b>																			
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<b>FB96</b>	18	20	20	18	17	17													
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<b>FB99</b>	8	7	7	7	7	7	7	7	7	7	7	7	7	8	7	7	7	7	7
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<b>FB102</b>																			
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<b>FB104</b>	17	17	16	15	13														
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<b>FB111</b>	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<b>FB112</b>	8	12	12	15	15	15	15	14	14	14	15	13	11	11	11	9	9	9	9
<b>FB113</b>	4	4	4	2	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3
<b>FB114</b>	34	36	39	38	38	39	39	39	37	34	38	35	35	35	35	35	34	30	28
<b>FB115</b>																			
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<b>FB117</b>	16	13	14	14	14														
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<b>FB119</b>																			
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<b>FB121</b>	3	2																	
<b>FB122</b>	18	18	17	18	18	17	17	17	15	15	15	15	15	15	12	14	14	14	15
<b>FB123</b>	12	11	10	10	10	8	9	8	8	8	7	9	10	10	6	6	5	5	5
<b>FB124</b>	6	6	6	6	6	6	6	6	6	6	6	5	6	5	5				
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<b>FB126</b>	17	16	16	15	14	14	14	14	14										
<b>FB127</b>	18	18	17																
<b>FB128</b>	26	26	26	24	26	18	16	16	20	20	18	18	18	15	10	7			
<b>FB129</b>	5	5	5	5	4	4	4	4	3	2	2	2	2	1	1	1	1	1	1
<b>FB130</b>	7	6	4	3	4	4	4	4	4	2	3	3	2						
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FB139																			
FB140																			
FB141																			
FB142	9	8	8	8	7	4	2	2	2	2	2	2	2	2	2	2	2	2	
FB143	13	14	14	13	13	13	15	15	15	16	16	14	14	14	14				
FB144	30	29	28	28	28	28	28	28											
FB145																			
FB146	22	22	22	22	22	23	23	23	21	21	19	15	15	13	12	12			
FB147	29	29	29	29	29	26	21	21	12	11	11								
FB148	8																		
FB149	17																		
FB150																			
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FB157	1																		
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FB160	0	0																	
FB161	0	0																	
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FB163																			
FB164	2	2	2	2	3	3	3	3	3	3	3	3	3						
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FB166																			
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FB168																			
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FB170																			
FB171																			
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FB173																			
FB174	5	6																	
FB176																			
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FB179	12	11	11	9	9	9	9	9	9	9	9	9							
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FB181	35	34	34	34	32	30	30	29	27										
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FB183	5	4	5	5	5	5	4	4	3	3	3	3	3						
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FB185	19	15	15	15	15	14	15	12	12										
FB186	26	26	26	24	24	25	24	20	19	17	17	18	18	18	15	17	17	17	
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FB188	4	4	4	4	4	4	2	3	2	2	1	2	2	2	2	2	1	1	1
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FB191	13	12	12	13	13	12	11	11	12	12	12	11	10	8	8	8	7		
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FB194	10	10	8	8	8	8	8	7	6	6	6	6	5	5	5	5	5	5	5
FB195	12	13	12	12	11	12	12	12	12	10	10	8	7	8	8	8	9	8	8
FB196	3	3	3	3	2	2	2	2	2	2	2	3	3	3	2	2	2	2	2
FB199	7	7	7	7	7	7	7	7	8	7	9	8	8	8	8	8	8	8	8
FB200	4	4	4	4	4	4	4	4	4	4									
FB201	13	13	12	12	12	11	11	11	11	11									
FB202	8	8	5	5	5	5	5	3	3	4	3	3	2	1					
FB203	12	17	12	10	8	8													
FB204																			
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FB207	5	5	4	4	4	4	5	5	2	2	2	2	2	2	2	2	2	2	2
FB208	8	8	9	9	10	10	9	9	9	7	8	6	6	5	6	5	4	4	4
FB209																			
FB211																			
FB212	25	25	25	25	25	26	26	24	24	25									
FB213	20	23	23	23	23	23	22	21	20	18	16	13	14	13	13	13	13	13	11
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FB217	8	8	7	7	7	9	9	6	6	4	4	5	4	4	4	4	4	4
FB218	12	12	10	10	9	9	8	7	7	6	6	6	6	5	5	5	5	5
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FB220																		
FB221	8	8	8	8	8	8	6	6	6									
FB222	13	14	13	12	12	11	10	12	12									
FB223	7	7	5	6	6	6	6	6	7	6	7	7	8	8	8	8	8	7
FB224	4	5	4	4	4	5	5	4	4	4	4	4	4	3	3	3	3	3
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FB227																		
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FB230	6	6	5	5	5	5												
FB231	15	14	12	14	15	14	15	13	11	10	11	11	11	12	12	12	8	9
FB233	21	21	21	21	21	22	22	22	22	20	18	17	17	17	14	12	12	11
FB234	7	5	6	6	6	6	6	6	6	6								
FB235	4	4	4	4	4	4	5	4	4									
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FB237																		
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FB239	15																	
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FB242																		
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FB251																		
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FB255	15	12	12	13	13	13	11	13	11	11								
FB256																		
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FB261	3	5	5	3														
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FB268																		
FB269	4	4	4	4	4	4	4	4	4	4								
FB270	11																	
FB271	9	9	8	8	8	8	8	8	8									
FB272	15	15	14	13	13	13	13	13	14	14	12	11	11	11	10	11	11	8
FB273																		
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FB275	4	5	2	2	2	2	2	3	3	3								
FB276	5	5	3	3	3	2	3	4	2	2	2	2	2					
FB277	20	20	19	18	18	19	18	18	19	19	19	19	19	19	20	20	20	
FB278	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
FB279																		
FB280																		
FB281	16	13	10	8	7													
FB282	23	23	23	23	19	15	14	14	14	14	14	12	12	12	12	12	12	11
FB283	21																	9
FB284	5	5	6	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4
FB285																		
FB286	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1
FB287	19	19	18	16	13	11	11	11	12	11	10	5	7	6	6	6	6	7
FB288	31	27	25	22	22	22	22	22	20	15	12	14						
FB289	27	27																
FB290	10	10	10	12	12	12	12	12	12	12	12	12	12	10	10	11	11	11
FB291																		
FB292																		
FB293																		
FB294	6	6	9	7	9	8	7	7	7	7	7	6	3	1	1			

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<b>FB296</b>	19	18	20	19	19	17	14	10	11	5									
<b>FB297</b>	20	19	19	15	14														
<b>FB299</b>	12	12	11	11	8	8	8	8	8	8									
<b>FB300</b>	9	11	11	11	11	9	10	11	11	10	7	7	6	4	2				
<b>FB301</b>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>FB302</b>	11	11	10	10	10	10	8	10	9	8	8	8	8	11	10	7	7	7	7
<b>FB303</b>	16	16	17	17	17	17	14	14	14	14	14	14	11	11	6	5	7	7	
<b>FB304</b>	16	19	19	21	18	19	19	19											
<b>FB305</b>	20	19	20	20	18	18	18	16	12	12	12	14	13	12					
<b>FB306</b>	4	4	5	5	5	4	3	3	3	3	3	3	3	3	3	3	3	3	3
<b>FB307</b>	16	16	16	16	16	16	14	14	13	12	10	9							
<b>FB308</b>																			
<b>FB309</b>																			
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<b>FB312</b>	16	16	15	16	16	16	16	13	14	13	13	13	13	8	7	7			
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<b>FB316</b>	6	5	5	4	4	4	4	3	3	4	3	3							
<b>FB317</b>																			
<b>FB318</b>	18	19	20	20	20														
<b>FB320</b>																			
<b>FB321</b>	14	15	15	16	14	11	8	9	8	6									
<b>FB322</b>	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
<b>FB323</b>	19	17	19	18	13	14	13												
<b>FB324</b>	13	14	14	14	14	15	14	16	14	16	16								
<b>FB325</b>	29	29	29	29	25	25	25	25	23	19	19	20	19	19	19				
<b>FB326</b>	8	8	8	9	9	7	7	7	7	7	7	7	6	6	6	6	5	6	6
<b>FB327</b>	7																		
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<b>FB329</b>	18	18	19	19	15	14	12	11	13	12	10	8	5	5	1	1			
<b>FB330</b>	8	5	5	4	5	5	4	4	4	3	3	2	3	3	3	2	2	2	2
<b>FB331</b>	9	13	13	13	14	14	14	12	12	12	12	9	9	8					
<b>FB332</b>	29	29	27	24	24	21	19	19	18	16	13	11							
<b>FB333</b>	6	6	4	5	5														
<b>FB334</b>	2	3	3	2	2	2	2	3	3	3	3	3	3	3	3				
<b>FB335</b>																			
<b>FB336</b>																			
<b>FB337</b>	20	21	21	21	21	19	19	19	16	14	12	13	11	11	10	9	5	8	8
<b>FB338</b>	9	9	8	8	7	7	6	6	6	7	6	5	6	6	6	6	6	6	6
<b>FB339</b>																			
<b>FB340</b>	7	7	6	7	7	6	4	4	3	4	2	2	2	2	2	1	1	1	2
<b>FB341</b>	26	26	24	24	19	15	18	18	17	15	16	15	14	14	14	14	14		
<b>FB344</b>																			
<b>FB345</b>																			
<b>FB346</b>	11	12	9	10	10	10	10	10	8	8	6	6	6	5	5				
<b>FB347</b>																			
<b>FB348</b>	3	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
<b>FB349</b>	12	12	12	11	11	12	12	7	4	3	2								
<b>FB350</b>																			
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<b>FB352</b>																			
<b>FB353</b>	22	22	24	24	24														
<b>FB354</b>																			
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<b>FB356</b>																			
<b>FB357</b>																			
<b>FB358</b>																			
<b>FB359</b>	15	13	12	11	12	11	11	11	10										
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<b>FB361</b>																			
<b>FB362</b>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<b>FB363</b>	10	10	10	10	10	8	8	7	7	7	7	8	8	7	7	7	5	7	6
<b>FB364</b>	13	16	15	16	16	15	12	12	12	10	10	10	10	10	10	9	9	10	10
<b>FB365</b>	14	12	12	12	10	10	9												
<b>FB366</b>																			
<b>FB367</b>	29	27	23	21	21														
<b>FB368</b>	7	7	7	7	7	7	6	6	6	6	6	6	6	6	6	6	6	6	6
<b>FB369</b>																			
<b>FB370</b>																			
<b>FB371</b>																			
<b>FB372</b>	4	3	2	2	3	3	3	3											
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<b>FB374</b>	12	12	10	10	8	6	7	6	5	5	6	3	3	4	4	4	4	3	4

FB376	17	15	17	17	17	17	14	13	14	12	13	12	12	12	11				
FB377	6	7	7	7	7	7	8	7	6	6	6	4	6	6	4	4	4	4	5
FB378	5	3																	
FB379	13	11	10	10	10	10	9	9	10	9	9	9	9	9	10	10			
FB380	1	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
FB381	3	4	2	2	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1
FB382	1	1	1	1	3	3	2	2	1	1	1	1	1	0	0	1	1	1	1
FB383																			
FB384	3	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
FB385	11	11	11	11	11	10	12	11	11	11	11	10	10						
FB386	4	2	4	4	4	3	2	4	3	2	2	2	2	1	1	1	1	1	1
FB387	9	7	8	9	9	9	11	11	11	10	9	7	6	6	6	6	6		
FB388	35	33	30	25	23	17	14	9	10	7	4	4	4	4	3	4	3		
FB389	3	2	2	2	3	3	2	2	1	0	1	1	1	1	1	0	1	0	0
FB390	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB391	4	1	1	2	2	1	1	2	1	1	1	1	1	1	1	0	0	0	0
FB392	6	5	5	5	4	5	3	3	4	4	3	4	4	4	4	4	4	4	4
FB393																			
FB394																			
FB395	8	6	5	5	4	2	2	2	2	1	2	1	1	1	1	1	1	1	1
FB396	2	1	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0
FB397	2	3	3	3	7	6	6	5	4	4	4	4	4	3					
FB398	8	8	7	7	8	9	9	9	8	6	7	7	7	7	6				
FB399	3	2																	
FB401	3	2	2	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
FB402	3	2	2	1	3	4	3	3	2	2	3	3	3	3	3	2	1	0	0
FB403	11	8	5	6	6	6	6	5	5	4	5	5	5	5	5	3	2	2	2
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FB405	4	4	3	3	3	3	5	4	5	5	7	7	6	6	5				
FB406	1	0	0	0	0	1	1	1	0	0	2	2	2	2	1	1	1	2	2
FB407	1																		
FB408	4	4	3	4	4	4	5	5	4	4	4	4	4	4	4	4	4	3	3
FB409	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB410	12	12	12	12	12	13	13	12	13	13	13	12	12	12	10	10	10	11	11
FB411	7	9	9	9	9	8	9	9	9	9	9	8	8	9	8	8	8	6	6
FB412	12	11	11	11															
FB413	22	22	22	21	21	23	23	23	23	23	23	23	23	20	20	17	17	16	16
FB414	15	15	14	14	14	12	10	10	10	9	9	9	10	9	8	9			
FB415																			
FB416																			
FB417																			
FB418																			
FB419	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
FB420																			

M.ID	W43	W44	W45	W46	W47	W48	W49	W50	W51	W52	W53	W54	W55	W56	W57	W58	W59	W60	
FB1																			
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FB3																			
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FB5																			
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FB7																			
FB8	2	2																	
FB9																			
FB10	11	8	7	6	5	6	6	5	6	5	5	5	5						
FB11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB13																			
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FB16																			
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FB28	9	9	9	9	9	8	8												
FB29																			
FB30	6	6	6	6	6	6	6	5	6	5	5	5	6	6	6				
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FB38																	
FB39																	
FB41	5	5															
FB42	12																
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FB45																	
FB46																	
FB47																	
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FB49																	
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FB69																	
FB70																	
FB71																	
FB72																	
FB73																	
FB74																	
FB75	2	2	2	2	2	2	2	2									
FB76																	
FB77																	
FB78																	
FB79																	
FB80																	
FB81																	
FB82																	
FB83																	
FB84																	
FB85																	
FB86	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB87																	
FB88																	
FB89																	
FB90																	
FB91	2	2	2	2	2	1	2										
FB92																	
FB93																	
FB94																	
FB95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB96																	
FB97	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB98																	
FB99	7	7	7	7													
FB100																	
FB101																	
FB102																	
FB103																	
FB104																	
FB105	5	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	2
FB106																	
FB107																	
FB109																	
FB110	5	6	6	6	5	6	6	5	5								
FB111	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB112	9	9	8	8	6	3	3	4									

FB113	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
FB114	26	24	23														
FB115																	
FB116																	
FB117																	
FB118	16	16	16	16	16	14	15										
FB119																	
FB120																	
FB121																	
FB122	15	15	15	15	15	14	13	11	12	10							
FB123	5	5	5	4	4	4	3										
FB124																	
FB125	3	3	3	3	3	3	3										
FB126																	
FB127																	
FB128																	
FB129	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
FB130																	
FB131																	
FB132	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0
FB133																	
FB134	2	2	2	2	2	2	2	2									
FB135	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB136																	
FB137																	
FB138	2	2	2	2	2	1											
FB139																	
FB140																	
FB141																	
FB142	2	2	2	2	2	2	2	2	2	2	2	2	2	4	4	3	2
FB143																	
FB144																	
FB145																	
FB146																	
FB147																	
FB148																	
FB149																	
FB150																	
FB151																	
FB153																	
FB154	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB155																	
FB156																	
FB157																	
FB159	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
FB160																	
FB161																	
FB162																	
FB163																	
FB164																	
FB165																	
FB166																	
FB167																	
FB168																	
FB169	3	3	2	2	2	2	1	1	2	2	2	2	2	2	2	2	2
FB170																	
FB171																	
FB172	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
FB173																	
FB174																	
FB176																	
FB177																	
FB178	6	6	6														
FB179																	
FB180																	
FB181																	
FB182																	
FB183																	
FB184																	
FB185																	
FB186																	
FB187	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB188	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB189																	
FB190																	

FB191																		
FB192																		
FB193	3	3	3	3	3	3	3	3										
FB194	5	5	5	5	5	5	5	6	5	5	5	5	5					
FB195	8	8	8	8	8	8	8	8	6	6	6	6						
FB196	2	2	2	2	1	1	1	1	1	1	1	1	1					
FB199																		
FB200																		
FB201																		
FB202																		
FB203																		
FB204																		
FB205	2	2	2	2	3	3	2											
FB206																		
FB207	2	2	3	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4
FB208																		
FB209																		
FB211																		
FB212																		
FB213	11	11	9	9	8	8	8	8	8	8	8	6	6	5	6			
FB214	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB216	1	1	1	1														
FB217	4	3	4	4	3	3	3	3	3	3	3	2	2	2	2			
FB218	5	5	5	6														
FB219	3	3	3	3	3	2	2	2	2	2	2	3						
FB220																		
FB221																		
FB222																		
FB223	7	7	7	7	7	7	6											
FB224	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
FB225	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
FB226	3	3	3	3	3	3												
FB227																		
FB228																		
FB229	2																	
FB230																		
FB231	7																	
FB233	11																	
FB234																		
FB235																		
FB236	9	9	10	8	8	9	10	10	9	8	7	6						
FB237																		
FB238	3	5	4	4	4	4	5	5										
FB239																		
FB240	2	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
FB242																		
FB243	1	2	2	2														
FB245																		
FB246																		
FB247																		
FB248																		
FB249																		
FB251																		
FB252																		
FB253																		
FB254																		
FB255																		
FB256																		
FB257																		
FB258																		
FB259	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB260	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB261																		
FB262																		
FB263																		
FB264																		
FB265																		
FB266																		
FB267																		
FB268																		
FB269																		
FB270																		
FB271																		
FB272																		
FB273																		

FB274	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3
FB275																		
FB276																		
FB277																		
FB278	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB279																		
FB280																		
FB281																		
FB282	10	10	10	10														
FB283																		
FB284	4	4	4	4	4	3	4	3	2									
FB285																		
FB286	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB287	7	7	7	6	6	6	6	6	4	6	6	4	2					
FB288																		
FB289																		
FB290																		
FB291																		
FB292																		
FB293																		
FB294																		
FB295	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
FB296																		
FB297																		
FB299																		
FB300																		
FB301	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB302	8	8	8	8	8	8	7	6										
FB303																		
FB304																		
FB305																		
FB306	3	3	3	3	3	3	3	3	3	3								
FB307																		
FB308																		
FB309																		
FB310																		
FB312																		
FB313	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB314																		
FB315																		
FB316																		
FB317																		
FB318																		
FB320																		
FB321																		
FB322	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB323																		
FB324																		
FB325																		
FB326	6	6	6	6	6	6	6	6	6	6	6	7	6					
FB327																		
FB328	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB329																		
FB330	2	2	2	2	2	2	1	1	2	2	2							
FB331																		
FB332																		
FB333																		
FB334																		
FB335																		
FB336																		
FB337	8	10	10	8	7	6	7	7	6	6	6	6	6	6	5	5		
FB338	6	6	6	6	6	6	6	6	6	6	5	6	6	3	3			
FB339																		
FB340	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
FB341																		
FB344																		
FB345																		
FB346																		
FB347																		
FB348	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB349																		
FB350																		
FB351	2	2	3	2	2	1												
FB352																		
FB353																		



FB354																		
FB355																		
FB356																		
FB357																		
FB358																		
FB359																		
FB360																		
FB361																		
FB362	1	1	1	1														
FB363	6	6	6	6	6	6	6	6	6	5	5	4	4	4	3	3		
FB364																		
FB365																		
FB366																		
FB367																		
FB368	6	6	6	6	6	6	6	6	6	6	7	7	7					
FB369																		
FB370																		
FB371																		
FB372																		
FB373																		
FB374	4	4	4	3	3	4	4	3	2	2	3	2	1					
FB376																		
FB377	4	3	3	4	4	4	4	4	4	4								
FB378																		
FB379																		
FB380	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB381	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
FB382	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
FB383																		
FB384	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB385																		
FB386	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
FB387																		
FB388																		
FB389	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1
FB390	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB391	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
FB392	4	4	4	4	4	3	4	4	4	3	4	4	4	4	4	4	4	4
FB393																		
FB394																		
FB395	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	4	4	4
FB396	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
FB397																		
FB398																		
FB399																		
FB401	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB402	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FB403	2	2	1	0	0	0	0	0	0									
FB404	1	1	1	2	2	2	2	1	1	1	1	2	2	2	3	2	2	2
FB405																		
FB406	1	1	2	2	2	2	2	2	2	2	3	3	3					
FB407																		
FB408	3																	
FB409	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
FB410	9	8																
FB411	6	5	5	5	5	5	5	5	5	5	5	4	3	3	3			
FB412																		
FB413	14	14	14															
FB414																		
FB415																		
FB416																		
FB417																		
FB418																		
FB419	3	2	2	3														
FB420																		

\* Mouse coat colour. A for agouti, B for black and W for albino; \*\* F is female and M is male

**Table A8** The genotypes of FVB6F2 mice. A, homozygous to FVB/N alleles; B, homozygous to C57BL/6J alleles; H, heterozygous.

MARKERS	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30				
D10MIT134	A	H	B	H	H	B	H	B	H	A	B	A	H	H	H	H	H	H	H	H	B	H	H	H	H	B	A	H	A	B	A			
D10MIT248	H	B	B	B	B	B	A	H	H	H	B	H	B	H	B	H	H	H	H	H	H	H	B	H	H	B	A	B	A	B	H	H		
D10MIT271	A	H	B	A	H	B	H	H	H	A	B	A	B	H	H	H	H	H	H	H	B	H	H	H	A	H	A	H	A	H	H	H		
D10MIT42	A	H	B	H	H	B	H	B	H	B	B	A	H	B	H	B	H	H	H	H	B	B	H	H	B	A	B	A	B	A	B	A		
D10MIT44	H	B	B	H	H	B	A	A	H	B	B	B	H	H	B	H	H	H	B	A	A	H	B	B	H	H	H	H	H	H	B	B		
D11MIT217	H	A	B	A	H	B	B	B	B	A	B	A	A	B	B	B	H	H	H	B	A	B	B	H	H	A	A	H	A	H	A	A		
D11MIT23	H	A	B	A	H	H	B	B	B	A	B	A	A	H	B	B	H	B	B	B	A	A	B	B	H	H	H	H	A	H	A	A		
D11MIT254	H	H	A	B	H	H	H	B	B	A	B	A	A	H	H	H	H	B	B	B	H	H	B	H	H	H	H	B	H	H	H	H		
D11MIT30	H	A	B	H	H	H	B	B	B	A	B	A	A	H	B	B	H	B	B	B	A	B	B	H	B	H	H	B	H	H	A	A		
D11MIT38	H	H	A	H	H	H	B	B	B	A	B	A	A	H	B	H	H	B	B	B	A	B	B	B	B	H	B	B	H	H	A	A		
D11MIT99	H	H	A	H	H	H	H	B	B	A	B	A	A	H	B	H	H	B	B	B	H	H	B	B	H	B	H	A	B	H	H	H		
D12MIT149	B	B	H	A	H	H	H	B	B	B	B	H	A	A	H	A	B	B	A	A	H	H	A	B	H	A	B	H	A	H	H	B	B	
D12MIT2	B	B	H	A	H	H	H	B	B	B	B	H	A	A	H	A	B	B	A	A	B	B	A	B	A	B	A	B	H	H	H	B	B	
D12MIT231	B	B	H	A	H	H	H	B	B	B	B	H	A	A	H	A	B	B	A	A	H	H	A	B	H	A	H	H	A	H	H	B	B	
D12MIT68	B	B	H	A	H	H	H	B	B	B	B	H	A	A	H	A	B	B	A	A	H	H	A	B	A	B	A	B	H	H	H	B	B	
D12NDS11	B	B	B	A	B	H	H	B	B	H	B	H	H	H	H	A	B	H	A	B	H	A	B	B	A	B	A	B	B	H	H	H	B	
D12NDS2	H	B	H	A	H	B	H	H	B	H	A	H	H	B	H	A	H	B	A	A	B	A	H	H	H	H	H	H	A	H	H	B	B	
D13MIT117	H	H	H	A	H	H	A	A	H	H	H	A	A	A	H	H	H	A	A	H	H	A	H	H	H	A	A	A	H	B	B	A	A	
D13MIT17	H	H	H	A	H	H	A	A	H	H	H	A	A	A	H	H	H	A	A	B	H	H	A	H	H	A	A	H	B	B	B	A	A	
D13MIT41	H	H	H	B	H	H	H	B	A	H	H	A	B	H	H	H	H	H	H	H	B	B	A	H	A	A	H	A	B	B	B	A	A	
D13MIT75	H	B	H	H	H	A	H	B	H	H	B	H	B	B	B	H	H	H	H	B	B	B	A	B	H	A	H	A	B	H	H	A	A	
D14MIT133	B	A	B	B	H	H	B	H	H	H	B	H	H	B	H	H	H	H	H	H	H	H	H	A	B	B	B	H	B	H	H	H	H	
D14MIT203	H	A	B	B	H	H	B	A	B	H	B	H	H	B	H	H	H	H	A	A	H	H	A	A	A	A	H	H	B	A	H	H	H	
D14MIT75	H	A	H	B	H	H	B	A	H	H	B	A	A	A	B	H	H	H	H	H	B	B	A	A	H	A	A	H	B	A	A	H	H	
D15MIT11	B	H	H	H	H	H	H	H	B	H	H	B	A	A	B	A	H	A	A	H	B	H	B	B	H	B	H	B	A	H	A	H	A	
D15MIT15	B	H	B	B	B	H	A	B	H	H	H	H	H	H	H	H	H	A	A	A	H	B	H	B	B	B	H	B	B	B	H	H	A	
D15MIT171	B	H	H	H	B	B	H	B	H	A	A	H	H	H	H	A	A	A	H	H	B	H	H	A	A	A	H	A	A	H	H	A	A	
D15MIT189	B	H	H	H	B	B	H	B	H	H	H	H	H	H	H	A	A	A	H	B	B	H	H	A	A	H	H	A	A	H	H	A	A	
D15MIT217	B	H	H	H	B	B	H	B	H	A	A	H	H	H	H	A	A	A	H	H	B	H	H	A	A	A	H	A	A	H	H	A	A	
D15MIT26	B	H	H	H	H	B	H	H	B	H	H	B	A	H	B	A	H	H	H	B	B	H	H	A	A	B	H	H	A	A	H	A	A	
D15MIT35	B	H	B	H	H	B	H	H	B	H	H	H	H	H	H	A	H	H	H	B	B	H	B	B	A	B	H	H	A	H	H	A	A	
D15MIT56	B	H	H	H	H	B	H	H	H	H	H	B	A	A	H	H	H	H	H	B	B	H	B	B	H	A	H	H	A	H	H	A	A	
D16MIT110	H	A	H	H	H	H	H	B	H	H	A	H	H	B	A	H	A	H	H	B	B	H	H	A	B	H	H	A	H	A	H	A	A	
D16MIT4	H	A	H	H	H	H	H	B	H	H	A	H	H	B	A	H	A	H	H	B	B	H	H	A	B	H	H	A	H	B	B	B	B	
D16MIT50	H	H	H	H	H	H	H	B	A	H	H	A	A	H	H	H	B	H	H	B	B	H	H	A	B	B	H	H	A	H	H	H	B	B



D3MIT62  
D4MIT12  
D4MIT126  
D4MIT148  
D4MIT160  
D4MIT170  
D4MIT175  
D4MIT178  
D4MIT205  
D4MIT312  
D4MIT41  
D4MIT42  
D4MIT59  
D4MIT72  
D5MIT113  
D5MIT23  
D5MIT233  
D5MIT370  
D5MIT43  
D5MIT73  
D5MIT76  
D6MIT14  
D6MIT188  
D6MIT254  
D6MIT261  
D6MIT268  
D6MIT274  
D6MIT30  
D6MIT59  
D7MIT105  
D7MIT25  
D7MIT259  
D7MIT284  
D7MIT297  
D7MIT319  
D7MIT57  
D7MIT83  
D7MIT96



D13MIT117  
D13MIT17  
D13MIT41  
D13MIT75  
D14MIT133  
D14MIT203  
D14MIT75  
D15MIT11  
D15MIT15  
D15MIT171  
D15MIT189  
D15MIT217  
D15MIT26  
D15MIT35  
D15MIT56  
D16MIT110  
D16MIT4  
D16MIT50  
D16MIT51  
D16MIT64  
D16MIT70  
D17MIT176  
D17MIT197  
D17MIT38  
D17MIT7  
D18MIT124  
D18MIT50  
D18MIT58  
D18MIT94  
D19MIT10  
D19MIT13  
D19MIT41  
D19MIT71  
D1MIT102  
D1MIT213  
D1MIT3  
D1MIT303  
D1MIT318

D1MIT34  
D1MIT36  
D1MIT362  
D1MIT8  
D1MIT93  
D2MIT148  
D2MIT15  
D2MIT285  
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D4MIT126  
D4MIT148  
D4MIT160  
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D5MIT113  
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D5MIT370  
D5MIT43  
D5MIT73





MARKERS	R61	R62	R63	R64	R65	R66	R67	R68	R69	R70	R71	R72	R73	R74	R75	R76	R77	R78	R79	R80	R81	R82	R83	R84	R85	R86	R87	R88	R89	R90
D10MIT134	B	B	B	B	A	A	H	H	H	H	H	H	B	B	H	H	B	B	B	B	H	A	B	H	B	B	B	B	H	
D10MIT248	B	B	H	B	B	B	B	B	H	B	B	B	B	B	H	H	H	H	H	H	H	H	B	B	B	H	H	B	B	H
D10MIT271	B	H	B	B	A	A	A	H	A	H	H	H	B	B	H	A	H	H	B	B	B	A	B	B	H	H	H	H	B	H
D10MIT42	B	H	H	B	H	B	A	B	H	B	B	B	B	B	B	B	H	H	B	B	B	A	H	B	A	H	A	H	H	B
D10MIT44	B	H	H	H	H	H	B	H	A	H	B	B	B	B	H	B	H	H	B	B	A	B	H	H	H	A	A	A	H	H
D11MIT217	B	B	H	H	H	H	B	H	A	H	B	B	B	B	H	B	H	H	B	B	A	B	H	H	H	A	A	A	A	H
D11MIT23	B	B	H	H	H	H	B	H	A	H	B	B	B	B	H	B	H	H	B	B	A	B	H	H	H	A	A	A	A	B
D11MIT254	H	H	H	H	H	H	H	H	A	B	H	A	H	H	B	H	H	A	H	B	B	H	H	H	B	A	H	H	B	B
D11MIT30	B	B	H	H	H	H	A	A	A	H	H	A	H	H	B	B	B	B	B	B	B	B	B	B	A	A	A	H	B	B
D11MIT38	B	B	H	H	H	H	H	A	A	H	H	A	H	H	B	B	B	B	B	B	B	B	B	B	A	A	A	H	B	B
D11MIT99	B	H	H	H	H	H	H	H	A	B	H	A	H	H	B	B	B	B	B	B	B	B	B	B	A	A	H	H	B	B
D12MIT149	H	A	B	H	B	B	H	H	A	B	H	B	H	H	B	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B
D12MIT2	B	A	B	H	B	B	H	H	A	H	H	B	B	B	H	B	B	B	B	B	A	B	B	B	B	B	B	B	B	H
D12MIT231	H	H	B	B	H	H	H	H	A	B	H	B	B	B	H	H	H	H	B	B	B	A	B	B	B	B	B	B	B	H
D12MIT68	B	A	B	H	B	B	H	H	A	B	H	B	B	B	H	B	H	H	B	B	B	B	B	B	B	B	B	B	B	H
D12NDS11	H	A	B	H	B	B	H	H	A	H	H	H	H	H	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	H
D12NDS2	H	H	H	B	B	B	H	H	A	H	H	B	A	H	H	A	H	H	B	B	B	B	B	B	B	B	B	B	B	H
D13MIT117	H	A	H	B	B	A	H	H	B	B	B	B	B	B	H	B	A	H	H	B	A	B	B	B	B	B	B	B	B	H
D13MIT17	H	H	H	H	H	A	H	H	B	B	B	B	B	B	H	B	A	H	H	B	A	B	B	B	B	B	B	B	B	H
D13MIT41	H	H	H	A	B	A	H	H	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	H
D13MIT75	H	A	H	A	B	A	A	H	B	B	H	H	H	H	H	B	B	B	B	B	A	A	A	H	H	H	B	A	H	H
D14MIT133	H	B	B	B	B	A	H	B	B	B	H	H	A	A	B	H	H	A	B	A	A	A	A	A	H	B	A	H	B	A
D14MIT203	H	B	H	A	H	H	B	H	H	B	B	B	B	B	H	H	A	B	B	B	B	B	B	B	B	B	B	B	B	H
D14MIT75	B	B	H	A	A	H	B	H	H	B	B	B	B	B	A	H	A	B	B	B	B	B	B	B	B	B	B	B	B	H
D15MIT11	H	H	B	A	A	H	B	A	B	A	B	B	B	B	H	H	A	H	A	A	A	B	B	B	B	B	B	B	B	H
D15MIT15	A	H	A	A	A	B	H	B	B	H	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT171	B	H	H	A	A	H	H	B	B	A	B	B	B	B	H	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT189	B	H	H	A	H	H	H	B	B	A	B	B	B	B	H	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT217	B	H	H	A	H	H	H	B	B	A	B	B	B	B	H	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT26	H	H	H	A	H	H	A	B	A	A	B	B	B	B	H	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT35	H	A	H	A	A	B	H	B	B	A	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D15MIT56	H	H	H	A	H	H	H	B	B	A	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	A
D16MIT110	A	H	A	H	B	H	H	B	H	H	H	H	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	H
D16MIT4	A	H	A	H	B	H	H	B	H	H	H	H	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	H
D16MIT50	H	B	A	B	B	B	H	B	B	A	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	H
D16MIT51	H	B	H	B	H	B	H	H	H	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	H
D16MIT64	A	B	A	B	B	B	H	B	H	A	A	A	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	H

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MARKERS	R121	R122	R123	R124	R125	R126	R127	R128	R129	R130	R131	R132	R133	R134	R135	R136	R137	R138	R139	R140	R141	R142	R143	R144	R145	R146	R147	R148	R149	R150	
D10MIT134	H	H	B	H	B	H	H	B	B	A	H	H	B	A	A	H	H	H	B	A	B	A	H	A	A	H	H	H	A	A	
D10MIT248	H	H	B	H	A	A	H	A	H	A	H	H	B	A	A	H	H	H	B	H	H	H	H	H	A	A	H	B	H	H	
D10MIT271	A	H	H	A	H	H	B	H	B	A	H	H	B	A	A	H	H	H	B	A	H	A	H	A	A	H	H	H	H	A	A
D10MIT42	H	B	H	A	B	A	H	B	B	A	H	H	H	A	A	H	H	H	A	A	A	H	A	H	H	A	H	B	B	B	
D10MIT44	H	H	H	H	H	H	H	B	H	A	B	B	H	A	B	H	B	B	H	H	B	A	H	H	H	H	H	H	A	B	B
D11MIT217	H	H	H	H	H	H	H	B	H	B	A	B	B	A	A	H	A	B	B	H	H	A	A	H	H	A	A	H	A	B	B
D11MIT23	H	H	H	H	H	H	H	B	H	B	A	B	B	A	A	H	A	B	B	H	H	A	A	H	H	A	A	H	A	B	B
D11MIT254	H	A	B	H	H	H	H	B	B	B	A	B	B	A	A	H	A	B	B	H	H	A	A	H	A	B	B	H	H	A	A
D11MIT30	H	H	H	H	H	H	H	B	H	B	A	B	B	A	A	H	A	B	B	H	H	A	A	H	H	A	A	H	A	H	A
D11MIT38	H	H	B	H	H	H	H	B	H	B	A	B	B	A	A	H	A	B	B	H	H	A	A	H	H	B	B	A	H	A	H
D11MIT99	B	A	B	B	H	B	H	B	H	B	A	B	B	A	A	H	A	B	B	H	A	A	B	A	B	B	B	A	H	A	A
D12MIT149	H	H	B	H	A	B	B	H	H	B	B	A	A	A	H	B	B	B	A	A	B	H	A	B	H	H	B	B	H	H	H
D12MIT2	H	H	A	H	H	A	B	A	B	B	H	A	A	A	H	B	B	B	A	A	B	A	B	H	H	H	B	B	H	H	H
D12MIT231	H	A	H	H	H	A	B	A	B	B	A	A	A	A	H	H	B	B	A	H	B	B	B	B	H	H	B	B	H	H	H
D12MIT68	H	H	B	H	A	B	H	H	H	B	B	A	A	A	H	H	B	B	A	H	B	B	B	B	A	B	B	H	H	H	H
D12NDS11	H	H	H	H	A	B	H	B	H	H	A	A	A	A	H	H	B	B	A	H	B	B	B	B	A	B	B	H	H	H	H
D12NDS2	H	H	A	H	H	A	H	A	B	B	B	H	H	H	A	A	B	A	A	H	B	A	H	H	H	H	H	H	H	H	H
D13MIT117	B	H	H	B	H	A	H	H	H	H	B	B	H	H	H	B	B	H	H	H	H	H	H	A	A	A	B	H	H	H	H
D13MIT17	B	A	H	B	H	A	H	H	H	H	B	B	H	H	H	B	B	H	H	H	H	A	H	A	A	A	H	H	A	A	A
D13MIT41	B	H	A	B	H	H	H	A	B	B	B	H	H	H	H	H	B	B	H	H	B	B	H	A	B	H	B	B	H	H	A
D13MIT75	B	H	A	H	H	H	B	B	A	H	H	H	A	A	H	H	H	H	H	H	B	B	B	H	A	H	B	B	H	H	A
D14MIT133	B	H	A	H	A	H	H	H	B	A	B	A	B	H	H	A	H	H	H	A	A	A	B	H	H	B	B	H	H	H	H
D14MIT203	B	A	A	H	A	B	A	H	H	A	H	H	H	A	B	A	A	A	B	H	A	A	A	H	H	B	B	H	H	H	H
D14MIT75	H	A	A	H	A	A	H	A	H	A	H	H	H	A	B	A	A	H	B	H	A	A	A	H	A	B	B	H	H	H	H
D15MIT11	A	H	H	H	A	A	H	A	A	B	B	H	H	A	H	H	H	H	H	H	H	A	H	H	B	A	B	B	H	H	H
D15MIT15	B	H	A	H	H	H	H	A	A	H	H	A	H	H	A	A	A	A	H	H	A	B	B	H	H	H	H	H	H	H	H
D15MIT171	B	H	A	H	A	H	B	A	A	B	H	H	H	A	H	A	A	A	H	H	A	B	B	H	A	A	A	B	B	H	H
D15MIT189	B	H	A	B	A	A	B	A	A	B	H	H	H	A	H	A	A	A	H	H	B	A	H	B	H	A	A	B	B	H	H
D15MIT217	B	H	A	H	A	H	A	B	A	B	H	H	H	A	H	A	A	A	H	H	A	B	B	H	A	H	H	B	B	H	H
D15MIT26	B	H	H	H	A	H	H	A	A	B	H	H	H	A	H	H	A	A	H	H	H	A	B	B	H	A	H	B	B	H	H
D15MIT35	B	H	A	H	H	H	H	A	A	H	H	A	H	H	A	A	A	A	H	H	A	H	H	H	H	H	H	B	B	H	H
D15MIT56	B	H	A	B	A	A	B	A	A	B	H	H	H	A	H	H	A	A	H	B	H	A	H	H	H	H	H	B	B	H	H
D16MIT110	A	H	H	B	H	H	A	H	B	H	H	B	H	A	B	B	B	H	H	H	H	A	H	A	A	H	H	B	B	H	H
D16MIT4	A	H	H	B	H	H	A	H	B	H	H	B	H	A	B	B	B	H	H	H	H	B	H	A	A	H	H	B	B	H	H
D16MIT50	A	H	H	H	H	H	H	B	B	H	B	B	H	A	B	B	B	A	B	H	H	B	H	A	A	H	H	B	B	H	H
D16MIT51	A	H	H	A	H	H	A	H	B	H	B	B	H	A	B	B	H	A	B	H	H	B	H	A	A	H	H	B	B	H	H





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D8MIT211	A	H	B	B	B	A	B	B	B	H	B	A	B	H	H	H	H	H	A	A	B	B	A	A	A	A	A	A	H	B	B	B		
D8MIT215	B	H	B	B	B	A	B	B	B	B	B	H	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	
D8MIT4	H	H	H	B	B	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	
D8MIT8	H	H	B	B	B	H	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT154	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT18	B	H	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT182	B	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT196	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT205	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT207	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT212	B	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT259	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT269	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
D9MIT42	H	B	B	B	B	A	B	B	B	H	B	B	B	B	B	B	B	B	B	H	A	H	B	B	A	A	A	A	A	H	B	B	B	B
DXMIT166	A	H	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	H	A	A	A	A
DXMIT186	A	A	A	A	A	A	H	H	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	H	A	A	A	A

MARKERS	RI51	RI52	RI53	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27						
D10MIT134	H	B	B	A	H	A	H	H	A	H	A	H	B	A	H	H	H	H	H	B	A	H	H	H	H	H	H	H	H	H	H	A				
D10MIT248	A	B	H	A	H	H	A	H	A	B	H	H	B	A	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H	H	B	A	A			
D10MIT271	H	B	H	A	H	H	H	H	A	B	H	H	B	A	H	H	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	A	A			
D10MIT42	A	H	H	B	B	H	A	H	H	B	H	A	H	A	H	B	H	B	H	H	H	B	H	B	A	A	A	A	A	H	H	H	H	A		
D10MIT44	B	A	B	H	H	A	A	B	H	H	A	B	A	A	H	H	H	A	A	H	H	H	H	B	A	A	A	A	A	H	H	H	H	H		
D11MIT217	B	A	B	H	H	A	A	B	H	H	A	H	A	A	H	H	H	A	A	H	B	H	H	B	A	A	A	A	A	H	H	H	H	H		
D11MIT23	B	A	B	H	H	A	A	B	H	H	A	H	A	A	H	H	H	A	A	H	B	H	H	B	A	A	A	A	A	H	H	H	H	H		
D11MIT254	H	A	H	B	A	H	H	H	A	A	H	H	A	A	H	B	B	A	A	H	B	B	H	H	H	H	H	H	H	H	H	H	B	B		
D11MIT30	B	A	B	H	H	H	H	H	A	H	H	H	A	A	H	H	H	A	A	H	B	B	H	B	A	A	A	A	A	H	H	H	H	H		
D11MIT38	B	A	B	H	H	H	H	H	A	H	H	H	A	A	H	B	B	A	A	H	B	B	H	B	A	A	A	A	A	H	H	H	H	H		
D11MIT99	H	A	B	H	H	H	H	H	A	A	H	H	A	A	H	B	B	A	A	H	B	B	H	H	H	H	H	H	H	H	H	H	B	B		
D12MIT149	H	H	H	H	H	H	H	H	A	H	H	H	A	A	H	H	H	A	A	H	B	B	H	H	H	H	H	H	H	H	H	H	B	B		
D12MIT2	B	H	H	H	H	A	H	B	A	A	A	B	A	A	H	H	H	A	A	H	B	B	H	H	H	H	H	H	H	H	H	H	B	B		
D12MIT231	A	H	H	H	H	H	B	H	A	H	H	A	A	H	H	H	H	A	A	H	B	B	H	H	A	A	A	A	A	H	H	H	H	A		
D12MIT68	H	H	H	H	H	H	B	H	A	A	H	H	A	A	H	H	H	A	A	H	B	B	H	H	A	A	A	A	A	H	H	H	H	B	A	
D12NDS11	B	H	H	H	H	A	H	B	A	A	A	B	A	A	A	A	B	A	A	H	B	B	H	H	H	H	H	H	H	H	H	H	H	A	A	
D12NDS2	A	B	H	H	A	H	B	H	A	H	H	A	H	H	A	A	H	H	A	A	H	H	H	A	A	A	A	A	A	A	A	A	A	A	H	H



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MARKERS	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57		
D10MIT134	H	H	H	A	H	H	B	H	B	H	A	H	H	B	A	A	H	H	H	H	B	B	A	H	H	B	H	H	H	B		
D10MIT248	A	B	H	A	A	B	H	A	A	A	A	A	H	B	H	H	A	A	A	H	A	A	H	A	A	A	B	H	H	A	H	
D10MIT271	B	A	H	H	H	H	H	H	H	H	A	B	H	B	A	A	A	A	H	A	B	B	H	H	H	B	H	H	H	H	H	
D10MIT42	A	H	H	A	H	H	B	H	B	H	A	H	H	B	A	H	H	A	H	A	H	H	A	H	H	A	H	H	H	H	B	
D10MIT44	B	B	A	A	A	H	H	H	H	A	A	B	H	A	A	B	H	A	B	A	A	A	A	H	A	B	H	H	A	H	H	
D11MIT217	H	A	H	A	B	H	A	H	H	A	B	H	B	H	A	B	H	A	A	A	A	A	A	H	A	B	H	H	B	B	A	
D11MIT23	A	A	H	A	B	H	A	H	B	A	B	B	B	H	A	B	H	A	A	A	A	A	A	H	A	B	B	H	H	B	A	
D11MIT254	B	A	H	A	H	H	H	A	H	H	H	B	H	B	A	H	H	H	H	B	A	A	B	H	H	B	B	A	A	B	H	
D11MIT30	H	A	H	A	B	H	A	H	B	H	H	B	B	H	A	B	H	A	A	A	A	A	A	H	H	B	B	A	A	B	A	
D11MIT38	H	A	H	A	H	H	A	H	H	H	H	B	B	H	A	H	H	H	A	A	A	A	A	H	H	B	B	A	A	B	A	
D11MIT99	H	A	H	A	H	H	A	H	H	H	H	B	B	H	A	H	H	H	A	A	A	A	A	H	H	B	B	A	A	B	H	
D12MIT149	H	H	B	A	B	B	A	B	A	B	A	H	B	H	H	B	B	H	A	A	A	A	A	H	A	H	A	A	B	A	H	
D12MIT2	H	H	B	A	B	B	B	H	B	H	A	B	H	H	H	B	B	H	H	H	B	A	A	H	A	H	A	A	B	A	H	
D12MIT231	B	H	B	A	B	B	B	A	B	H	A	H	B	H	H	H	B	B	H	A	H	H	H	A	H	A	B	B	A	A	H	
D12MIT68	H	H	B	A	B	B	B	A	B	H	A	B	H	H	H	B	B	B	H	H	B	A	A	H	H	A	A	H	A	A	B	
D12NDS11	H	B	B	H	H	B	H	H	B	H	A	B	H	H	H	B	B	H	H	H	H	H	H	H	B	A	A	B	A	H	H	
D12NDS2	B	H	H	A	B	H	A	A	B	H	A	H	B	B	A	A	A	H	H	H	A	H	H	B	A	B	A	B	A	A	H	H
D13MIT117	H	B	A	A	B	B	A	A	B	H	H	B	B	A	A	A	A	H	H	H	A	A	A	A	B	A	B	A	B	A	B	H
D13MIT17	H	B	A	A	B	B	H	A	B	B	A	B	B	A	A	A	A	H	H	H	B	B	B	A	B	A	B	A	B	A	B	H
D13MIT41	H	B	A	H	H	H	A	A	H	H	H	B	H	A	A	A	A	H	H	A	B	A	B	B	A	B	B	A	B	B	B	H
D13MIT75	H	B	A	H	H	H	A	A	B	H	A	B	A	A	H	B	B	B	B	A	B	A	A	H	H	B	B	A	B	B	B	H
D14MIT133	A	A	A	B	B	B	H	B	A	H	H	B	A	B	B	B	B	B	B	B	B	B	A	H	H	B	B	H	A	B	H	H
D14MIT203	H	A	H	B	B	H	A	H	H	B	H	B	B	A	B	H	H	H	H	B	B	B	B	H	H	B	B	H	A	B	H	H
D14MIT75	H	H	B	H	H	A	H	H	H	A	B	B	A	B	B	H	H	B	B	B	B	B	B	B	H	H	B	B	A	B	H	H
D15MIT11	B	H	A	A	A	A	H	A	H	H	B	B	A	H	H	H	B	B	A	B	A	A	A	H	H	B	B	H	A	B	A	B
D15MIT15	B	H	H	A	B	H	A	H	H	H	B	B	H	H	H	A	H	H	A	H	A	A	A	H	A	A	H	H	A	B	H	H
D15MIT171	B	H	H	A	B	H	A	H	H	H	B	B	H	H	A	A	A	B	B	A	H	H	B	B	A	A	A	H	A	B	B	B
D15MIT189	B	H	H	A	H	H	A	H	H	H	B	B	A	H	A	A	A	B	B	A	H	H	B	B	A	A	H	H	A	B	B	B
D15MIT217	B	H	H	A	B	H	A	H	H	H	B	B	H	H	A	A	A	H	H	A	H	H	B	B	A	A	H	H	A	B	B	B
D15MIT26	B	H	H	A	A	H	A	H	H	H	B	B	H	H	A	A	A	B	B	A	B	A	H	H	A	A	H	H	A	B	B	B
D15MIT35	B	H	H	A	B	H	A	H	H	H	B	B	H	H	A	A	A	H	H	A	B	A	H	H	A	A	H	H	A	B	B	H
D15MIT56	B	H	H	A	A	H	A	H	H	H	B	B	H	H	A	A	A	B	B	A	B	A	H	H	A	A	H	H	A	B	B	B
D16MIT110	A	B	H	H	A	A	H	H	A	H	B	B	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B
D16MIT4	A	B	H	H	A	A	H	H	A	H	B	B	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A
D16MIT50	A	B	H	H	A	A	H	H	A	H	B	B	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	A	B	A	H	A
D16MIT51	H	B	H	A	B	H	H	B	B	H	H	B	H	H	A	B	B	H	A	A	A	A	B	B	A	H	H	A	B	H	H	H

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MARKERS	S88	S89	S90	S91	S92	S93	S94	S95	S96	S97	S98	S99	S100	S101	S102	S103	S104	S105	S106	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117	
D10MIT134	A	H	H	B	A	H	H	H	A	H	H	H	H	A	B	A	B	A	A	B	B	H	H	A	A	H	H	B	H	H	A
D10MIT248	H	B	H	A	A	H	A	A	H	H	A	A	H	H	B	B	H	A	A	A	B	H	H	A	A	H	H	H	H	A	A
D10MIT271	H	H	A	B	A	H	H	H	H	H	H	H	H	A	B	A	H	A	A	B	B	H	H	A	A	H	B	B	H	H	A
D10MIT42	A	H	H	H	A	B	H	H	H	H	H	H	A	A	B	H	B	A	A	A	B	H	A	A	A	H	H	H	H	H	A
D10MIT44	A	B	H	H	A	H	H	B	H	H	H	B	H	B	H	H	A	A	A	H	B	A	H	H	H	H	H	H	H	B	B
D11MIT217	B	A	H	B	A	H	A	H	A	B	H	H	B	H	H	H	A	A	A	A	H	B	A	H	H	H	H	H	A	H	H
D11MIT23	B	A	H	B	A	H	H	A	A	B	H	H	H	H	H	H	A	A	A	A	H	H	A	H	H	H	H	B	H	H	H
D11MIT254	H	H	B	B	B	H	B	H	H	B	B	A	A	A	H	A	H	A	A	H	H	H	A	H	H	H	H	A	H	B	B
D11MIT30	H	A	B	B	A	H	H	A	A	B	H	H	H	H	H	H	A	A	A	A	H	A	B	H	H	H	B	B	H	H	A
D11MIT38	H	H	B	B	H	H	H	A	A	B	B	H	H	H	A	A	H	A	A	A	A	H	A	B	H	H	B	H	H	H	A
D11MIT99	H	H	B	B	H	H	B	H	A	B	B	H	H	A	A	H	H	A	A	H	H	H	A	B	H	H	H	H	H	H	H
D12MIT149	H	A	H	B	B	H	B	B	A	H	H	A	A	H	B	H	H	A	A	H	H	A	H	H	A	H	H	H	B	B	H
D12MIT2	H	A	H	B	B	H	B	B	H	H	A	A	H	H	B	H	H	A	A	H	H	A	H	H	A	A	A	A	B	B	A
D12MIT231	H	H	A	H	A	H	B	B	A	H	H	A	H	H	B	H	H	A	A	H	H	A	H	H	A	H	H	B	H	B	B
D12MIT68	H	A	H	B	H	H	B	B	A	H	H	A	A	H	B	B	H	A	A	H	H	A	H	H	A	H	H	B	B	H	H
D12NDS11	H	A	H	H	H	H	H	H	H	H	A	A	H	B	H	H	H	A	A	H	H	H	B	H	A	H	A	A	B	B	A
D12NDS2	H	H	A	H	A	H	B	A	H	H	H	H	H	H	H	A	H	A	A	H	H	A	A	H	A	H	A	B	H	B	B
D13MIT117	H	H	B	B	H	B	H	B	A	H	H	B	A	B	A	B	B	B	B	A	H	A	B	H	H	H	H	A	B	A	B
D13MIT17	H	H	H	H	H	B	H	B	H	H	H	B	A	H	H	B	B	A	A	H	A	A	H	B	H	H	H	A	B	A	B
D13MIT41	H	B	B	B	H	A	H	B	A	H	H	B	A	B	A	B	B	B	H	H	A	A	B	H	H	H	H	A	B	H	B
D13MIT75	A	B	B	B	A	H	B	A	H	H	H	B	A	H	H	B	B	H	H	H	A	A	B	H	H	H	H	A	B	B	B
D14MIT133	H	H	H	A	H	A	H	H	H	H	H	H	H	B	B	H	H	B	B	B	A	B	H	H	A	A	H	H	B	B	A
D14MIT203	H	H	H	H	H	A	H	H	H	H	H	B	B	B	B	H	A	H	B	B	H	A	H	A	A	A	H	H	H	H	A
D14MIT75	H	B	H	B	A	A	A	A	B	H	B	A	B	B	H	H	A	H	A	H	A	H	A	A	A	A	A	H	H	H	A
D15MIT11	H	H	A	A	B	A	H	H	A	A	A	B	B	H	H	H	A	A	A	A	B	A	H	H	B	B	B	B	B	A	A
D15MIT15	A	B	H	B	A	H	H	B	H	H	B	B	B	H	A	B	A	A	A	A	B	B	H	H	B	B	H	B	B	H	H
D15MIT171	A	B	H	A	A	H	H	H	A	B	B	B	B	H	A	A	A	A	A	A	H	A	H	H	H	H	B	H	B	H	H
D15MIT189	A	H	H	A	A	H	H	A	A	A	H	B	B	B	B	A	A	A	A	A	A	H	H	B	B	B	B	H	B	B	H
D15MIT217	A	B	H	A	A	H	H	H	H	A	B	B	B	H	A	A	A	A	A	A	A	H	H	B	B	B	B	H	B	B	H
D15MIT26	H	H	A	A	H	A	H	A	A	A	A	B	H	B	A	A	H	H	H	H	H	H	B	H	B	B	B	H	B	B	H
D15MIT35	A	B	H	H	A	H	B	B	H	A	B	B	B	H	A	A	A	A	A	A	B	A	H	H	B	B	H	H	H	H	H
D15MIT56	A	H	A	A	A	A	H	A	A	A	H	B	B	H	A	A	A	A	A	A	A	H	H	B	B	B	H	H	H	B	B
D16MIT110	B	H	A	A	H	B	H	A	A	A	H	B	B	B	A	A	H	A	A	A	A	B	B	B	B	B	H	H	A	A	H
D16MIT4	B	H	A	H	B	H	A	H	A	A	A	B	B	B	A	A	A	A	A	A	A	B	B	B	B	B	B	H	B	B	B
D16MIT50	H	B	A	H	H	H	A	H	A	A	A	B	B	B	A	H	A	A	A	A	A	H	B	B	B	B	A	A	A	H	H
D16MIT51	H	B	A	H	H	H	A	H	A	A	A	B	B	B	A	H	A	A	A	A	A	H	B	B	B	B	A	A	A	H	H



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