Genkartierung
HHR 13.05.2003

Strukturelle Chromosomenaberrationen

Kopplungsanalyse, genetische Marker,
Single Nucleotide Polymorphisms (SNPs)

physikalische Kartierung:
Somazellhybride,
Fluoreszenz In Situ Hybridisierung (FISH),
Chromosomensortierung etc.

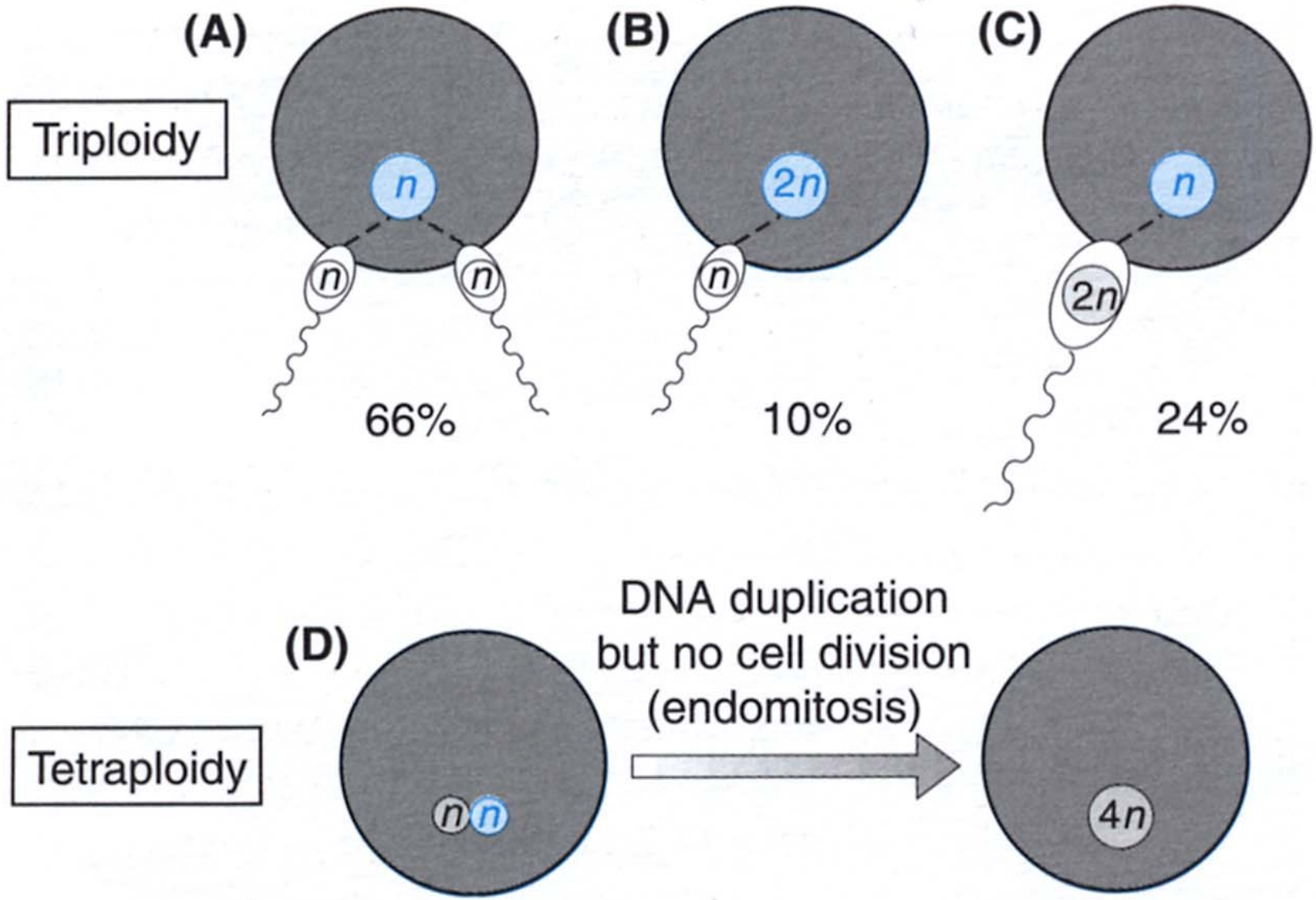

Figure 2.19: Origins of triploidy and tetraploidy.

Entstehung von Trisomien und Monosomien:

Chromosomenfehlverteilung während der Meiose
(--> Keimzellen mit einem Chromosom zu viel oder zu wenig)

Ursache von Mosaiken (z.B. 46,XX/45, X0 = Turner-Mosaik):
- Mitotische Chromosomenfehlverteilung während der frühen Keimesentwicklung ('somatic nondisjunction')
- oder 'Reparaturversuch' eines meiotischen Fehlers (Verlust eines überzähligen Chromosoms in einem Teil der Körperzellen bei Trisomie)

Sonderfall 'uniparentale Disomie': euploider Karyotyp (z.B. 46,XX), beide Chromosomen eines Chromosomenpaares stammen jedoch vom gleichen Elternteil (z.B. beide Chromosomen 15 von der Mutter --> Prader-Willi-Syndrom). Ursache meist wie oben (hier: Verlust des einzigen väterlichen #15 nach (letaler) Trisomie 15)

Table 2.5: Structural abnormalities resulting from misrepair of chromosome breaks or recombination between nonhomologous chromosomes

	One chromosome involved	Two chromosomes involved
One break	Terminal deletion (healed by adding telomere)	–
Two breaks	Interstitial deletion; Inversion; Ring chromosome (*Figure 2.20*) Duplication or deletion by unequal sister-chromatid exchange (*Figure 9.7*)	Reciprocal translocation (*Figure 2.21*) Robertsonian translocation (*Figure 2.21*) Duplication or deletion by unequal recombination (*Figure 9.7*)
Three breaks	Various rearrangements, e.g. inversion with deletion, intrachromosomal insertion	Interchromosomal insertion (direct or inverted)

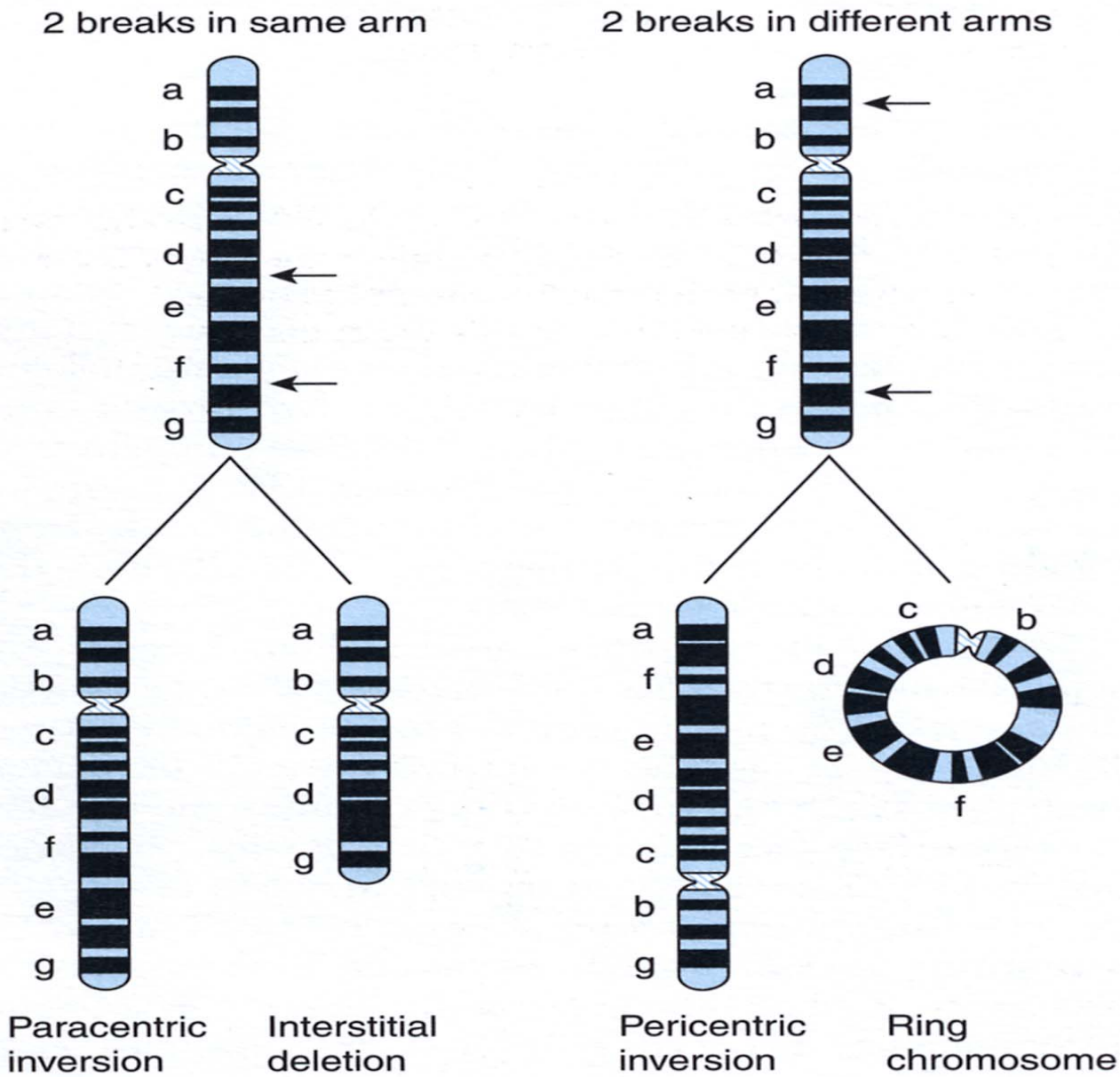

Figure 2.20: Possible stable results of two breaks on a single chromosome.

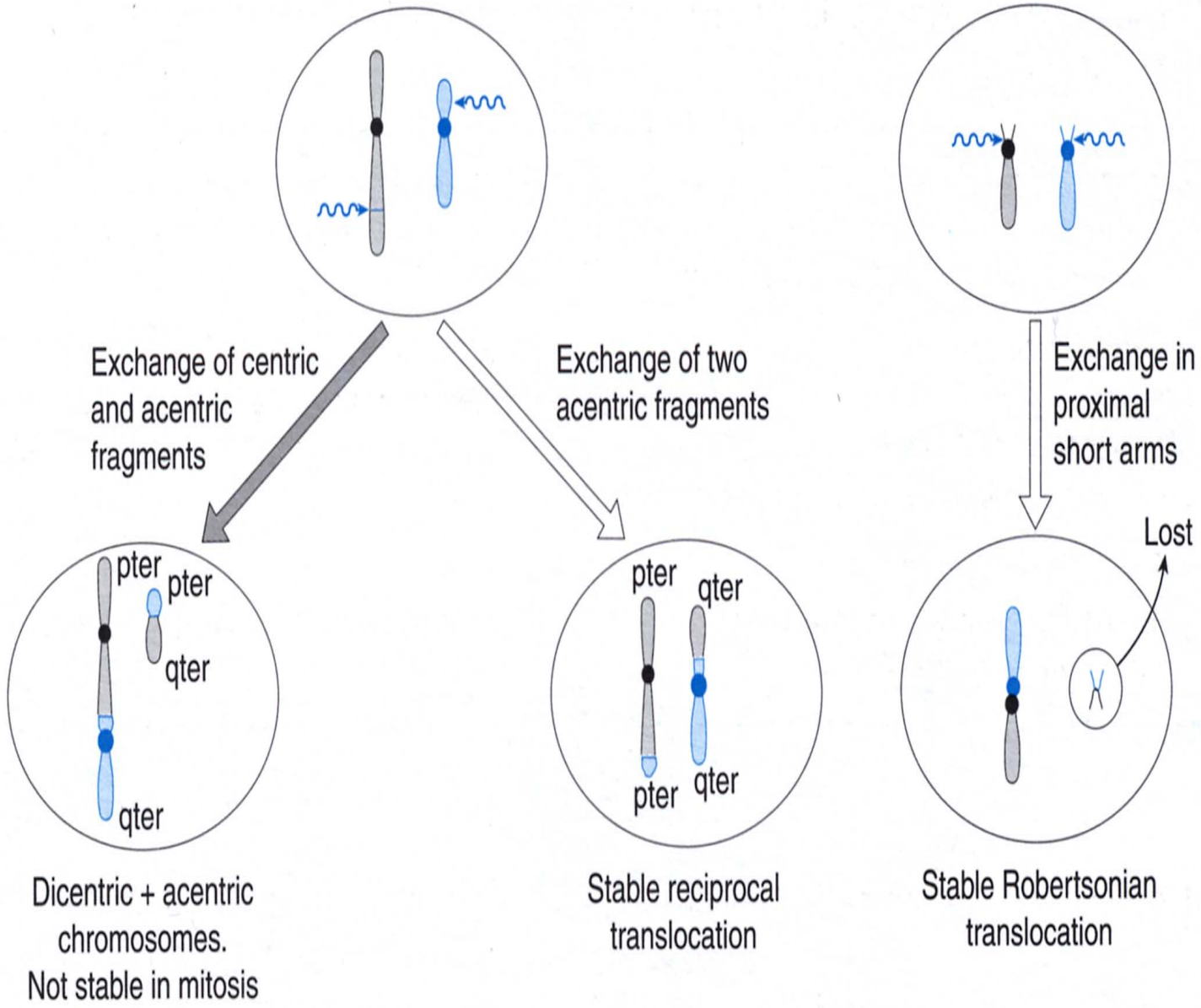

Figure 2.21: Origins of translocations.

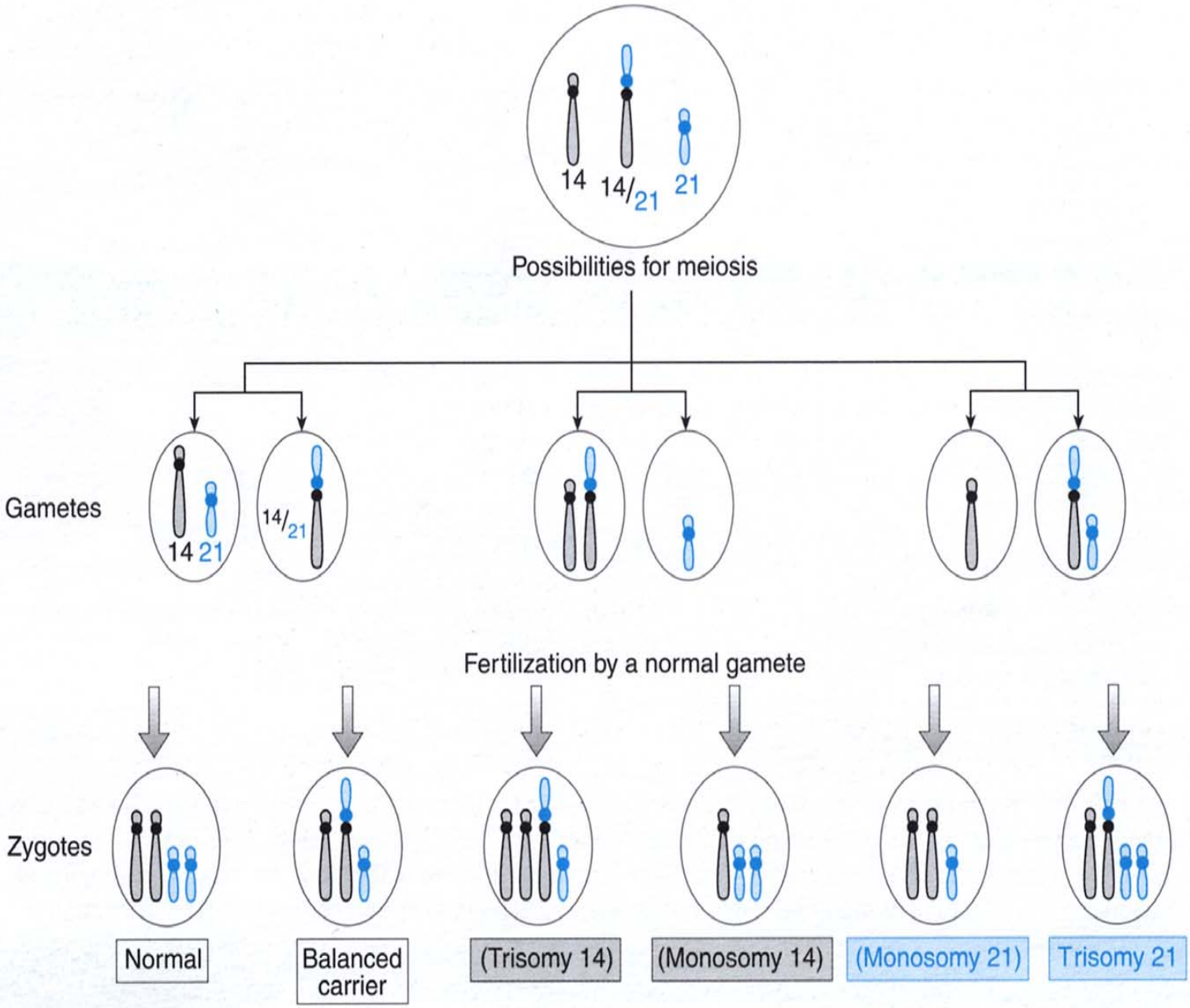

Figure 2.23: Results of meiosis in a carrier of a Robertsonian translocation.

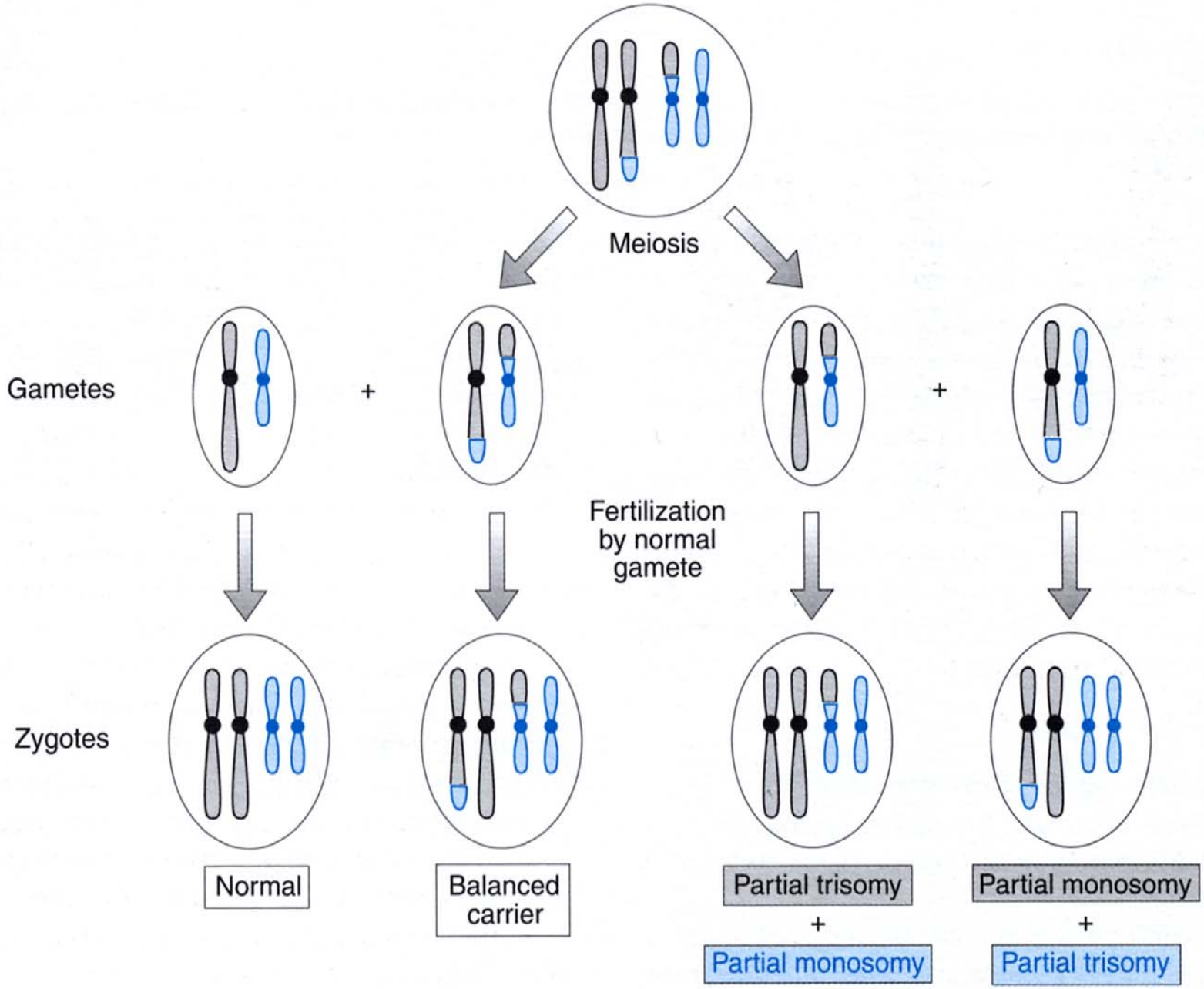

Figure 2.22: Results of meiosis in a carrier of a balanced reciprocal translocation.

Table 2.5: Structural abnormalities resulting from misrepair of chromosome breaks or recombination between nonhomologous chromosomes

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Two breaks	Interstitial deletion; Inversion; Ring chromosome (*Figure 2.20*) Duplication or deletion by unequal sister-chromatid exchange (*Figure 9.7*)	Reciprocal translocation (*Figure 2.21*) Robertsonian translocation (*Figure 2.21*) Duplication or deletion by unequal recombination (*Figure 9.7*)
Three breaks	Various rearrangements, e.g. inversion with deletion, intrachromosomal insertion	Interchromosomal insertion (direct or inverted)

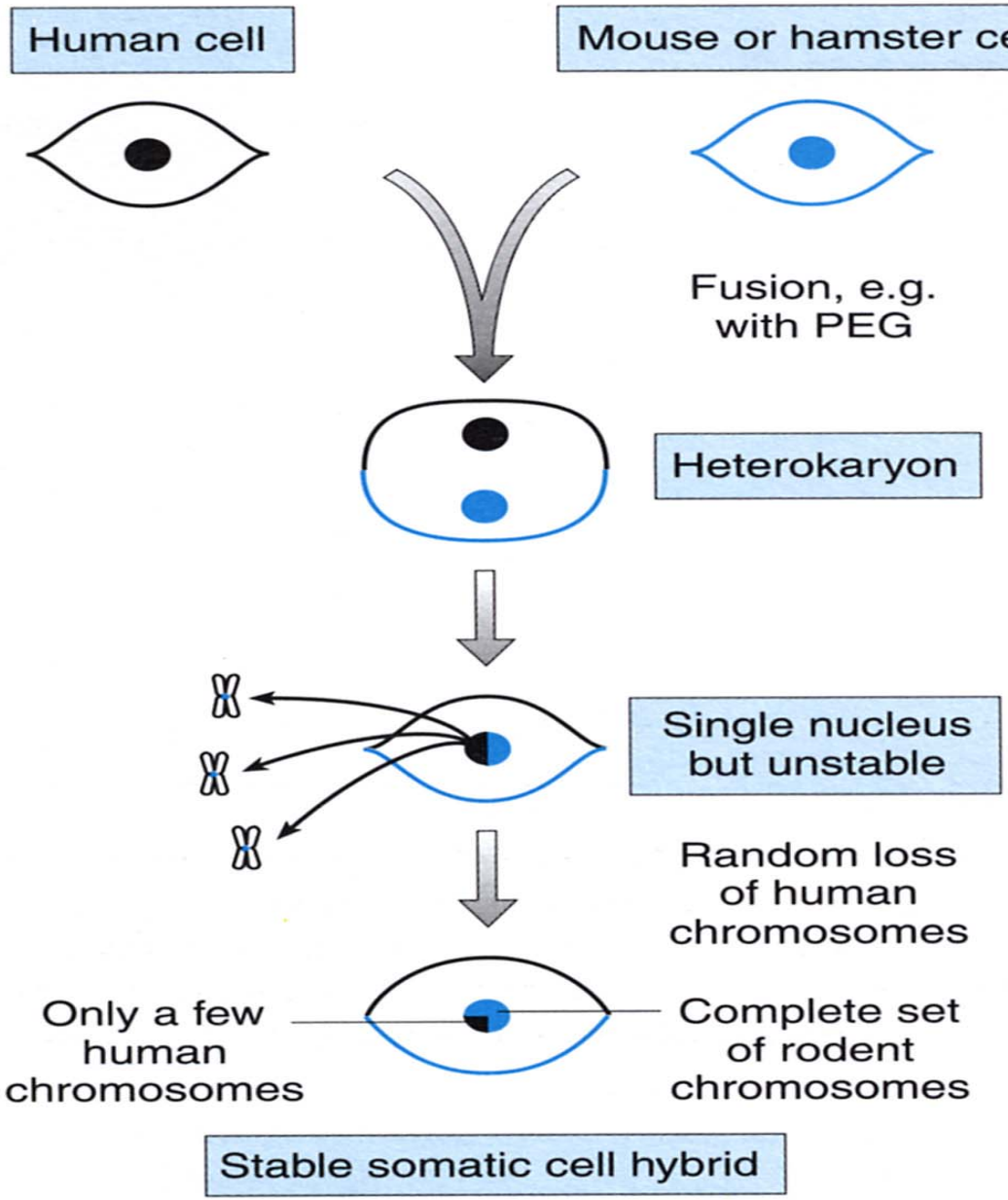


Figure 10.1: Fusion of cells from different species can result in stable somatic cell hybrids.

Box 10.1

Selecting for the chromosome contents of hybrids

Hybrids can be selected for retention of a given human chromosome or chromosome fragment if it corrects an otherwise lethal abnormality in the rodent cell. Frequently used systems include :

- **HAT selection**. Somatic cell hybrids can be forced to retain human chromosome 17 by using thymidine kinase deficient (TK^-) rodent cells and growing the hybrids in *HAT* (hypoxanthine-aminopterin-thymidine) medium. TK^- cells are killed in HAT medium, but are rescued by the human TK gene on chromosome 17.
- **G418 selection**. Hybrids can be selected for the presence of a particular human chromosome segment if it has been tagged by incorporation of a neomycin resistance (neo^R) gene. The neomycin analog G418 kills nonresistant cells. Neo^R is a typical example of a dominant selectable marker.

Table 10.1: Mapping of a gene for microfibril-associated glycoprotein (MAGP) to human chromosome 1 using a panel of 16 somatic cell hybrids

	Human chromosome																						
MAGP/chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant hybrids																							
+/+	7	3	4	3	2	5	0	6	4	1	2	5	2	6	4	6	2	6	6	3	6	7	2
−/−	9	8	3	6	6	6	7	6	4	9	4	6	3	3	4	6	9	5	5	4	6	5	3
Discordant hybrids																							
+/−	0	3	2	2	5	1	5	1	4	6	2	2	5	1	3	1	5	1	0	4	0	0	0
−/+	0	2	7	3	3	3	2	4	3	1	6	4	6	5	6	3	1	5	5	6	4	4	2
Total discordant hybrids	0	5	9	5	8	4	7	5	7	7	8	6	11	6	9	4	6	6	5	10	4	4	2
Total informative hybrids[a]	16	16	16	14	16	15	14	17	15	17	14	17	16	15	17	16	17	17	16	17	16	16	7
Percentage discordant hybrids	0	31	56	36	50	27	50	29	47	41	57	35	69	40	53	25	35	35	31	59	25	25	29

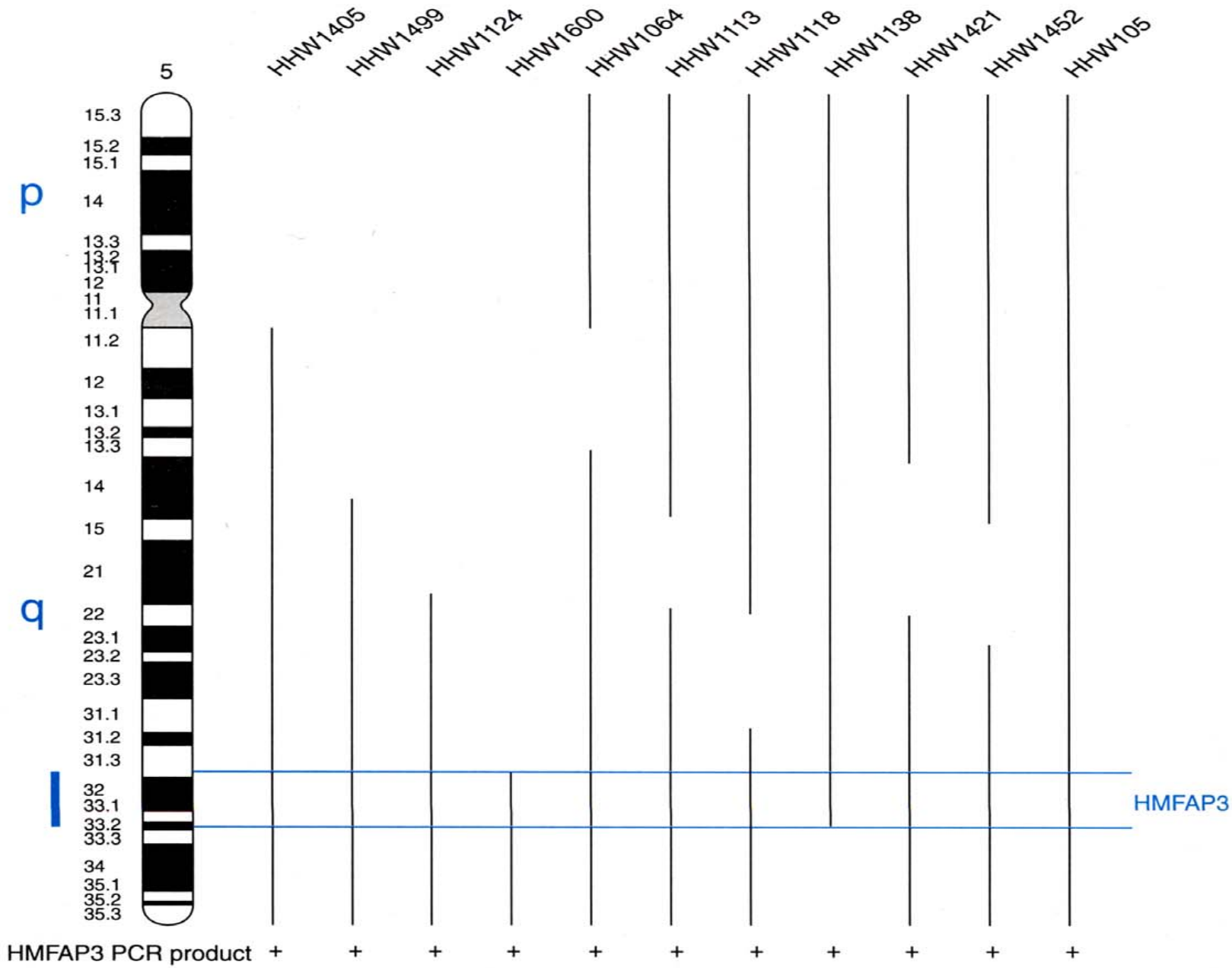


Figure 10.2: Subchromosomal localization can be achieved by mapping against a panel of hybrid cells containing translocation or deletion chromosomes.

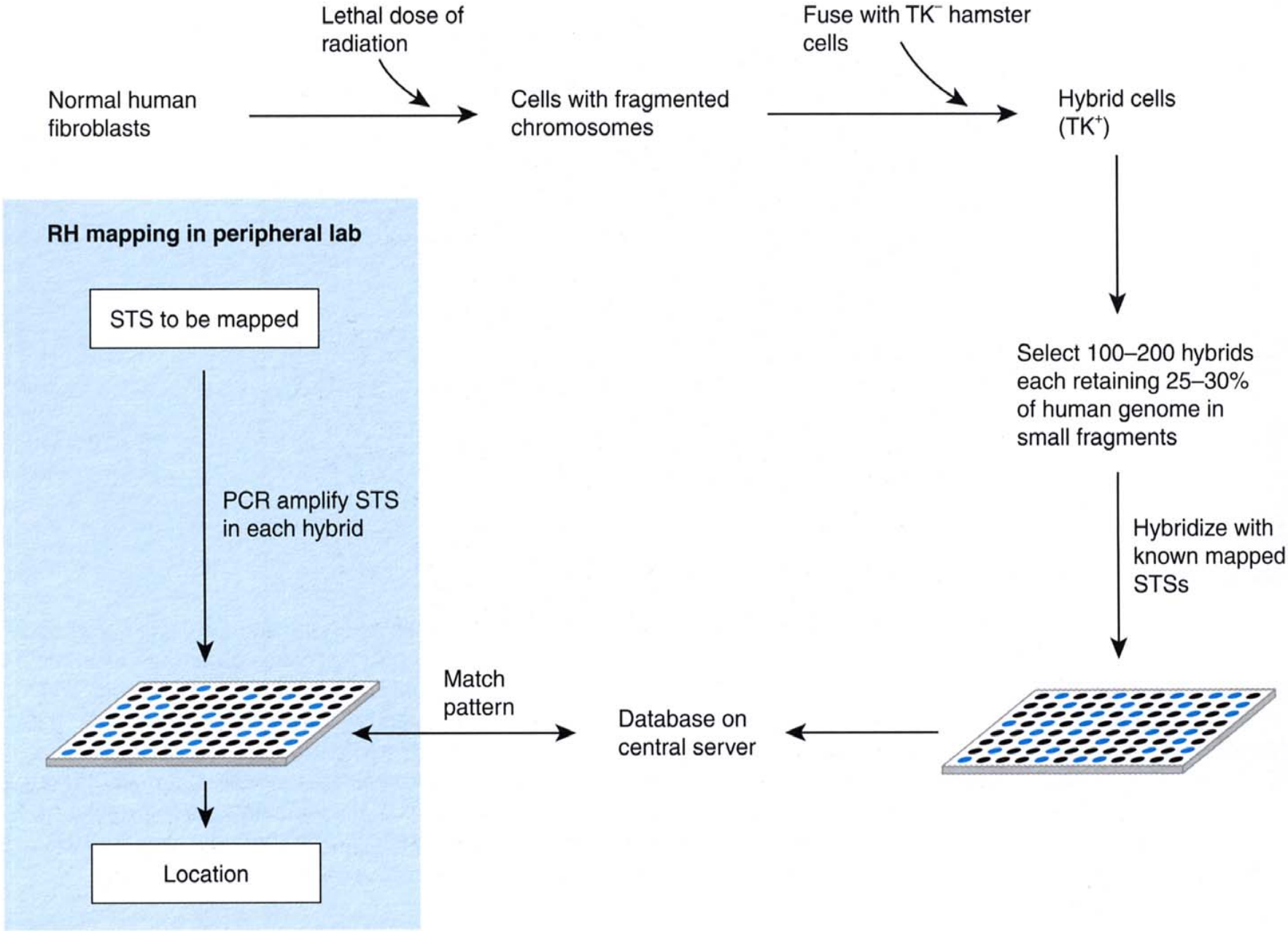


Figure 10.4: Use of the Genebridge 4 radiation hybrid panel for physical mapping.

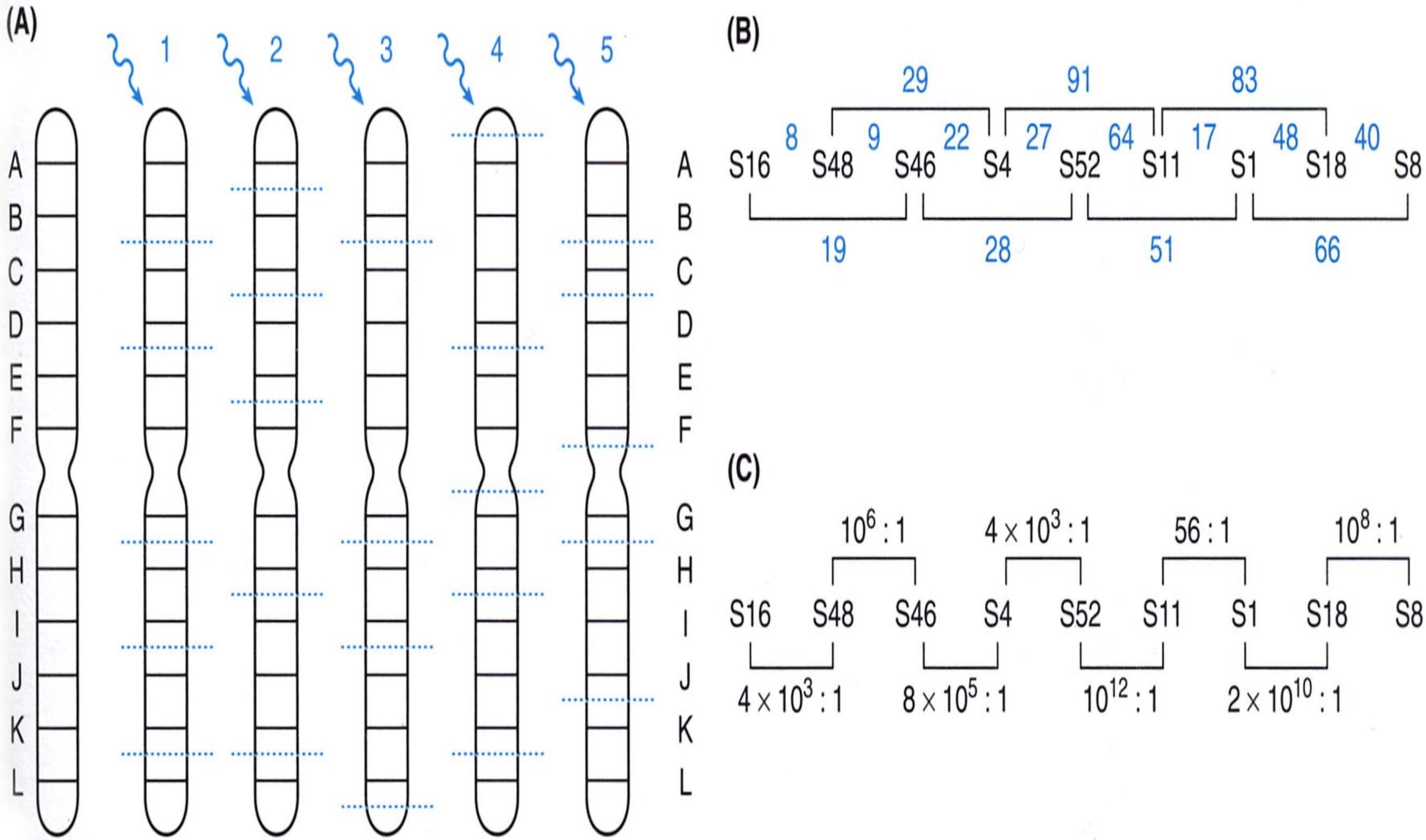
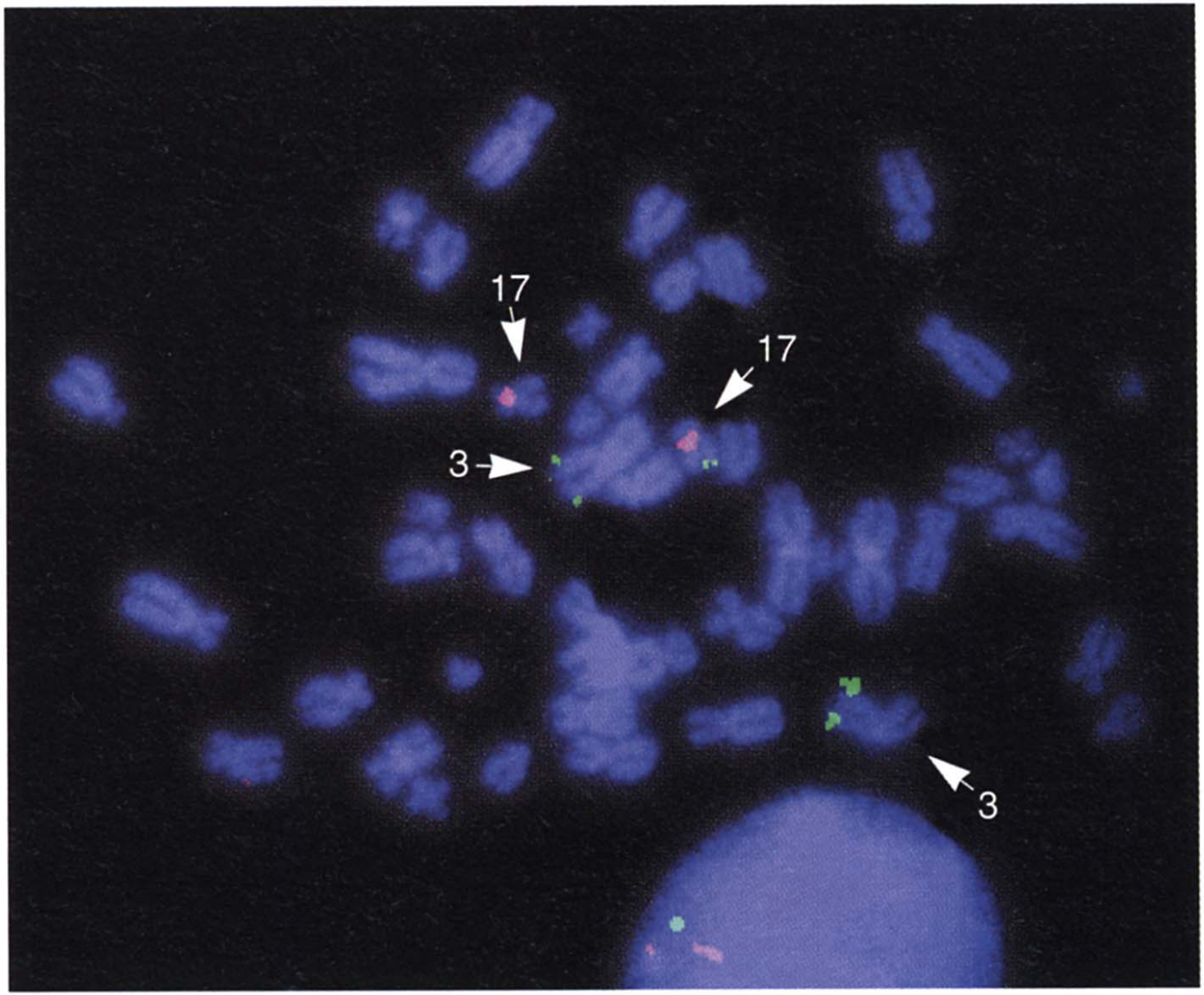


Figure 10.3: Constructing radiation hybrid maps.



Chromosome FISH (fluorescence *in situ* hybridization).

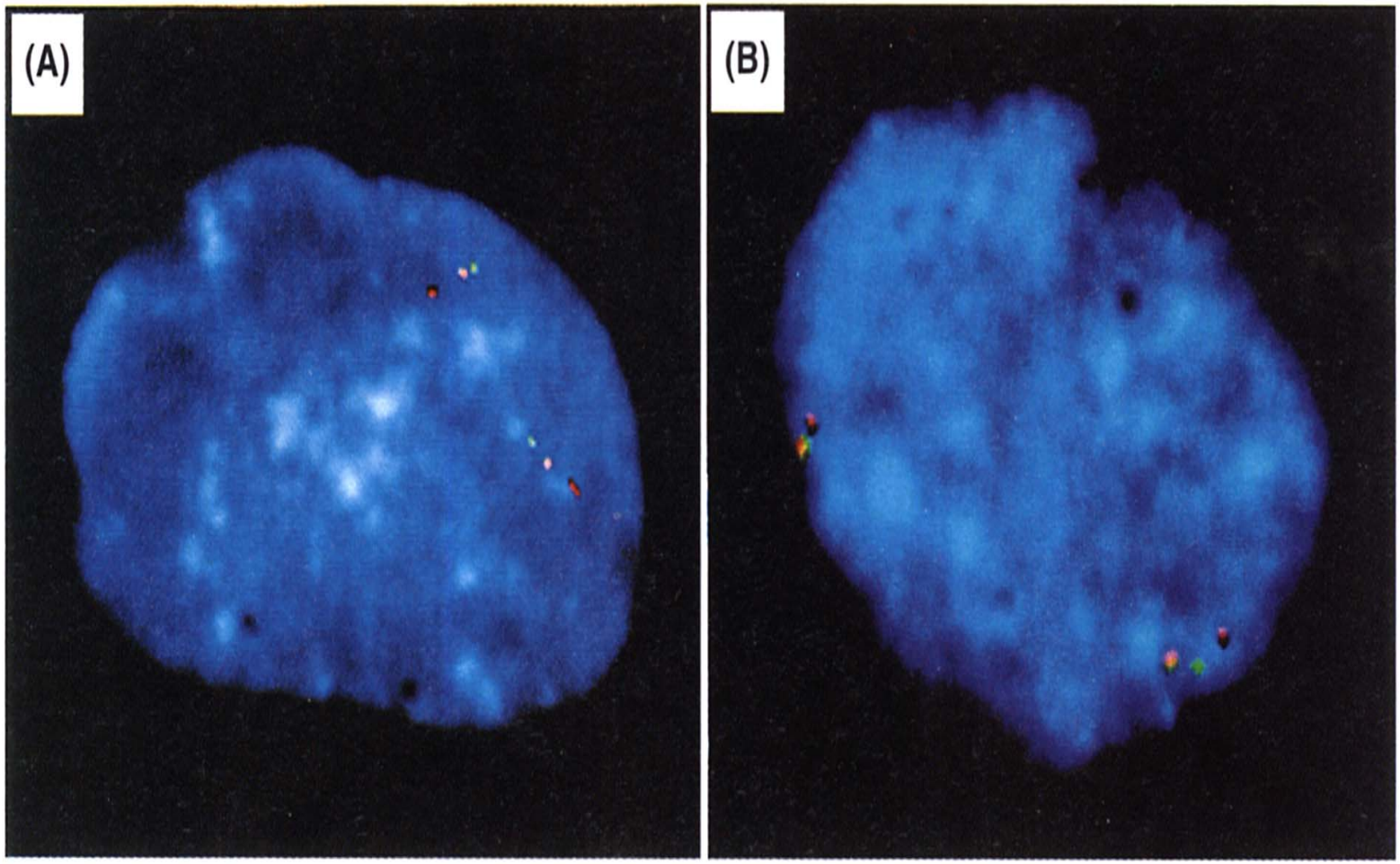


Figure 10.8: Determining the map order of syntenic DNA clones by three-color interphase FISH.

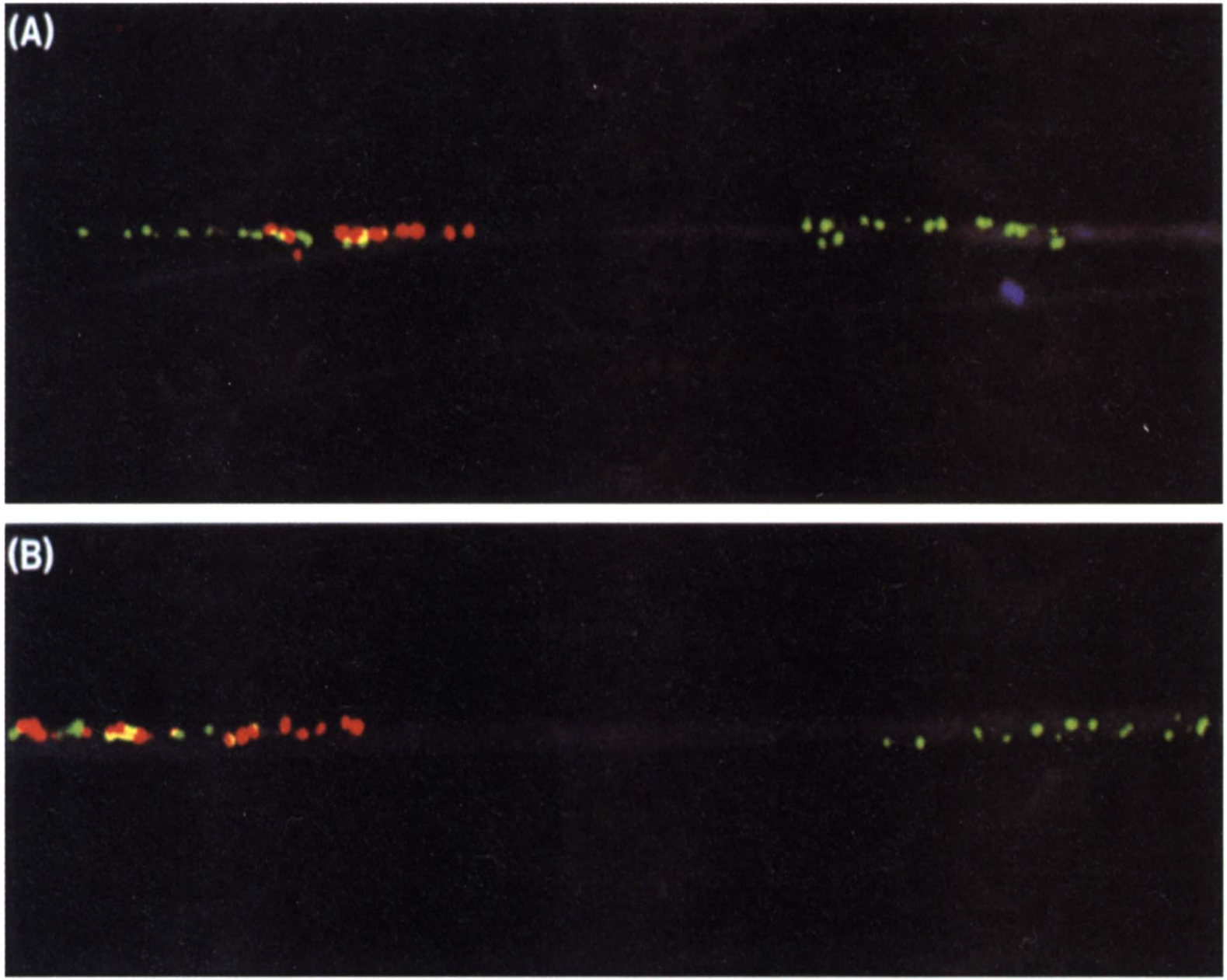


Figure 10.9: Extended chromatin fiber (ECF) FISH.

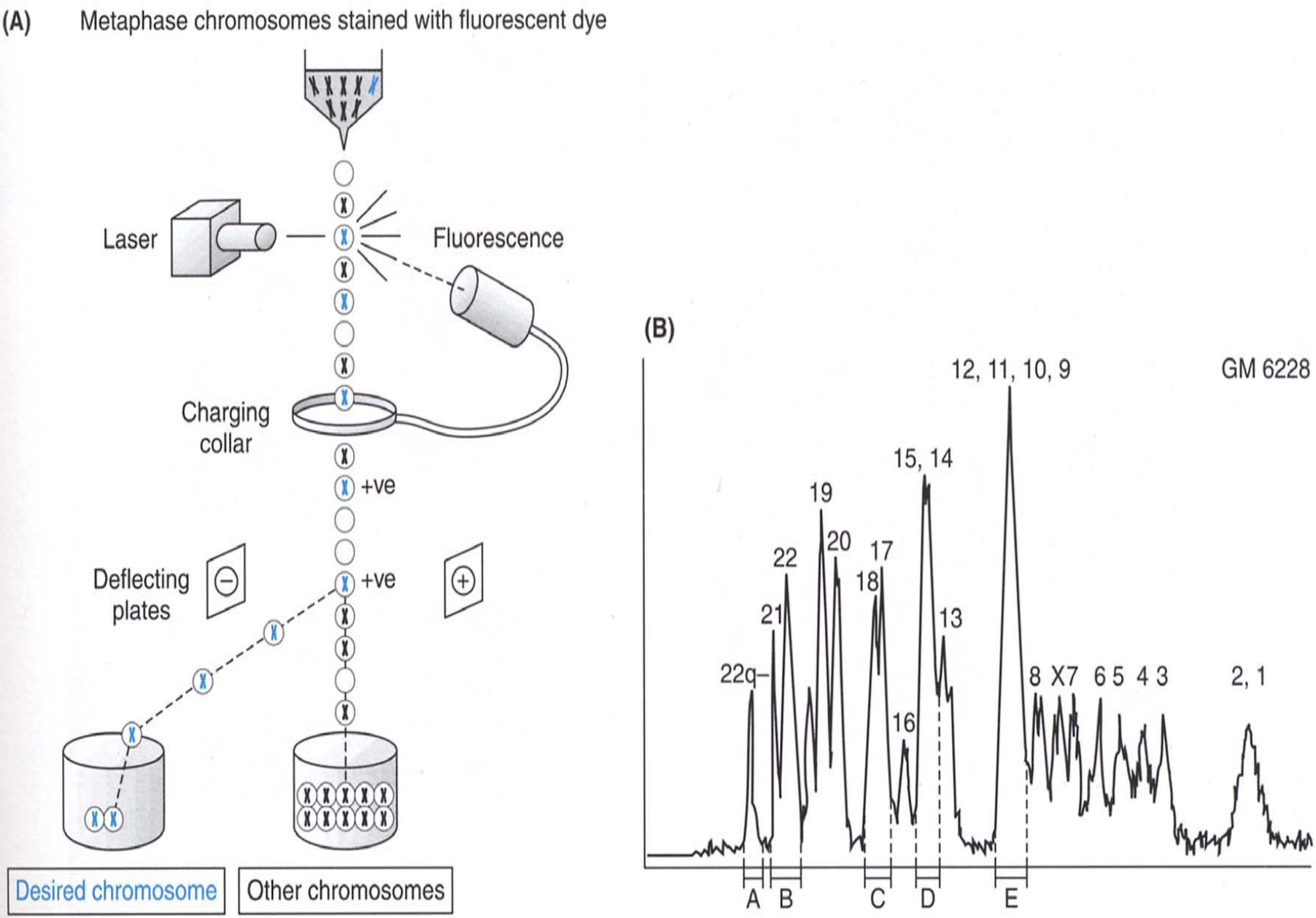


Figure 10.7: Fractionating chromosomes in a flow cytometer.

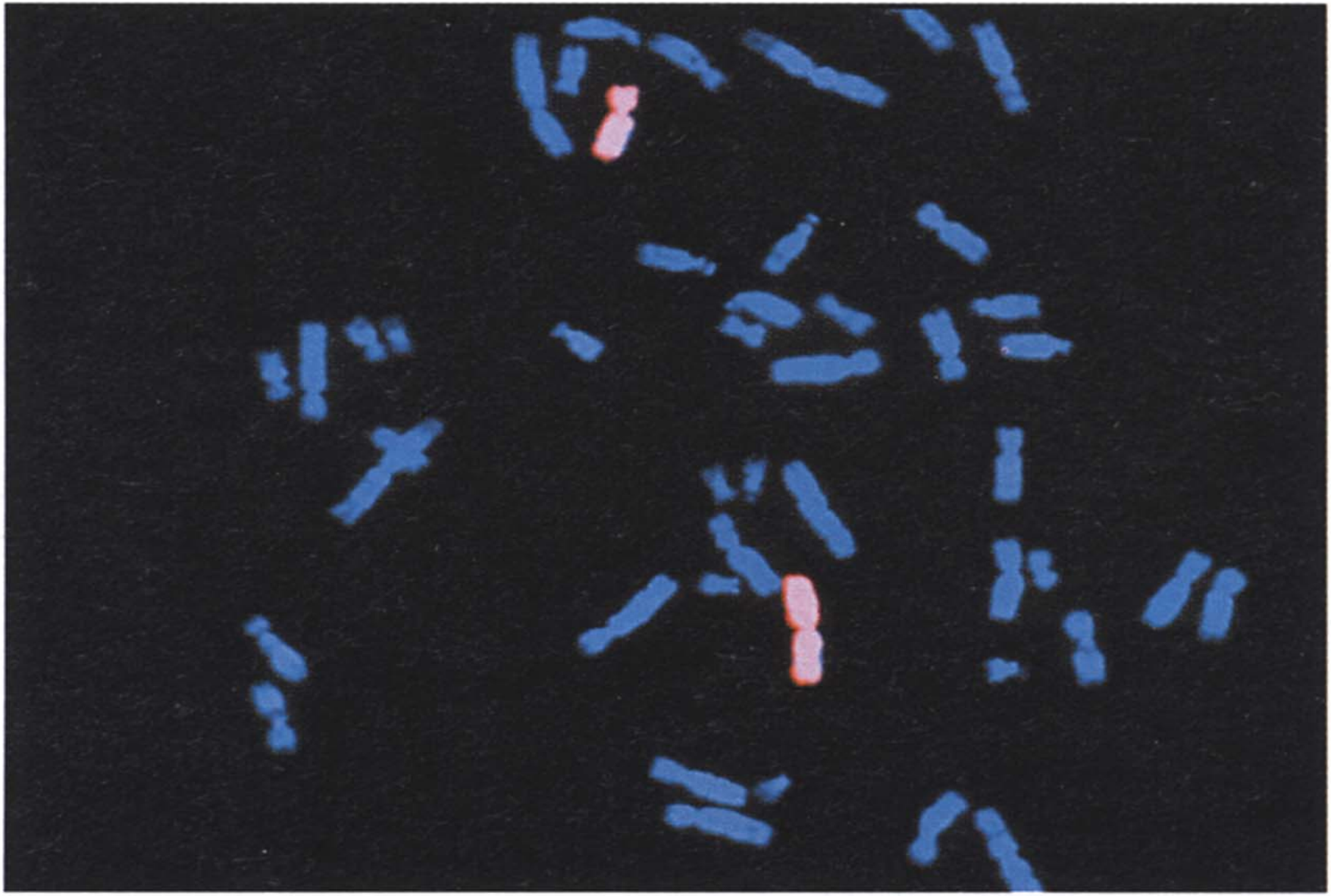


Figure 10.6: Chromosome painting can be used to define chromosome rearrangements.

Box 10.2

Chromosome painting

A special application of FISH has been the use of DNA probes where the starting DNA is composed of a large collection of different DNA fragments from a single type of chromosome. Such probes can be prepared by combining all human DNA inserts in a chromosome-specific DNA library (see Section 10.1.5). The resulting hybridization signal represents the combined contributions of many loci spanning a whole chromosome and causes whole chromosomes to fluoresce (chromosome painting; Ried *et al.*, 1998).

Chromosome painting was initially limited by the relatively small number of differently colored fluorescent dyes (fluorophores) one could use to distinguish different chromosomes. To increase the number of different targets that can be detected beyond the number of available differently colored fluorophores, two approaches have been used: (i) combinatorial labeling involves labeling individual probes with more than one type of fluorophore; and (ii) ratio labeling uses a combination of different fluorophores but also in different ratios (Lichter, 1997). The mixed colors are not detected by standard fluorescence microscopy using appropriate filters (see *Figure 5.5*). Instead, automated digital image analysis is preferred by which various combinations of fluorophores are assigned artificial pseudocolors. The long awaited goal of simultaneously visualizing all 24 different human chromosomes was achieved using two approaches:

- **Multiplex FISH (M-FISH)**. This approach reported by Speicher *et al.* (1996) uses digital images acquired separately for each of five different fluorophores using a CCD (charge coupled device) camera. The images are analyzed by a software package which generates a composite image in which each chromosome is given a different pseudocolor depending on the fluorophore composition (see *Figure 18.6*).
- **Spectral karyotyping (SKY)**. Schrock *et al.* (1996) reported this approach, in which CCD imaging is combined with Fourier spectroscopy. The spectrum of fluorescent wavelengths for each pixel (picture element) is assessed using an interferometer and a dedicated computer program assigns a specific pseudocolor depending on the particular fluorescence spectrum identified.

Chromosome painting has found increasing applications in defining *de novo* rearrangements and marker chromosomes (see *Box 2.6*) in clinical and cancer cytogenetics (see *Figure 10.6* for an example). It is particularly helpful in cancer cytogenetics for two reasons. Chromosome preparations from tumors are often of poor quality, but information can often be obtained with the aid of chromosome painting. Additionally, complex chromosome rearrangements are particularly frequent in tumor samples and chromosome paints can be used in combination with standard probes to help recognize particular chromosome segments (see *Figure 18.6*).

Box 10.4

Commonly used methods for identifying genes in cloned DNA

Method	Comments
Zoo blotting	A DNA clone is hybridized at reduced hybridization stringency against a Southern blot of genomic DNA samples from a variety of animal species, a **zoo blot**. Depends on coding DNA being more strongly conserved in evolution than non-coding DNA (*Figure 10.21*).
CpG island identification	Many vertebrate genes have associated **CpG islands**, hypomethylated GC-rich sequences usually having multiple rare-cutter restriction sites (Cross and Bird, 1995). ■ **Identification by restriction mapping**. DNA clones are usually hybridized against Southern blots of genomic DNA cut with *Sac*II, *Eag*I or *BssH*II to identify clustering of rare-cutter sites (*Figure 10.22*). ■ **Island-rescue PCR**. This is a way of isolating CpG island sequences from YACs by amplifying sequences between islands and neighbouring *Alu* repeats.
Hybridization	A genomic DNA clone can be hybridized against a Northern to mRNA/cDNA blot of mRNA from a panel of culture cell lines, or against appropriate cDNA libraries.
Exon trapping	This is essentially an artificial RNA splicing assay (see *Figure 10.23*). It relies on the observation that the vast majority of mammalian genes contain multiple exons which need to be spliced together at the RNA level.
cDNA selection or capture	These techniques involve repeated purification of a subset of genomic DNA clones which hybridize to a given cDNA population (see *Figure 10.24*).
Computer analysis of DNA sequence	■ **Homology searches**. Any DNA sequence obtained from a genomic clone can be compared against all other sequences in sequence data-bases. Significant homology to known coding DNA or gene-associated sequences may indicate a gene (see Section 20.1.4) ■ **Gene searching algorithms**. A variety of computer programs have been developed to search sequences for exons and other gene-associated motifs (see *Figure 10.25* and Section 20.1.4).

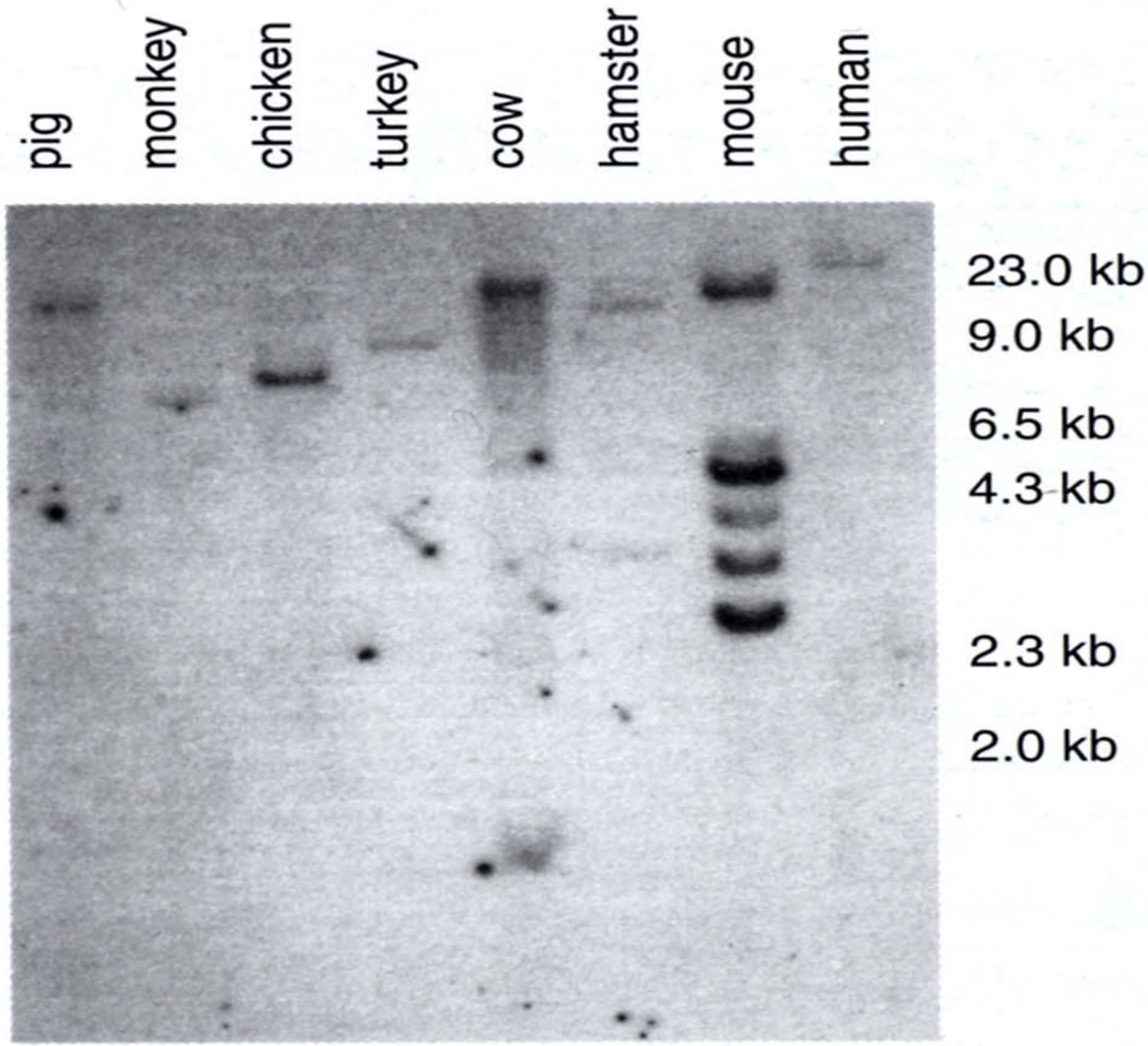


Figure 10.21: Zoo blot hybridization is an assay for DNA sequences that are highly conserved between species.

UCSC Genome Browser on Human April 2003 Freeze

move <<< << < > >> >>> zoom in 1.5x 3x 10x zoom out 1.5x 3x 10x

position chr7:78951600-79479699 size 528,100 image width 610 jump

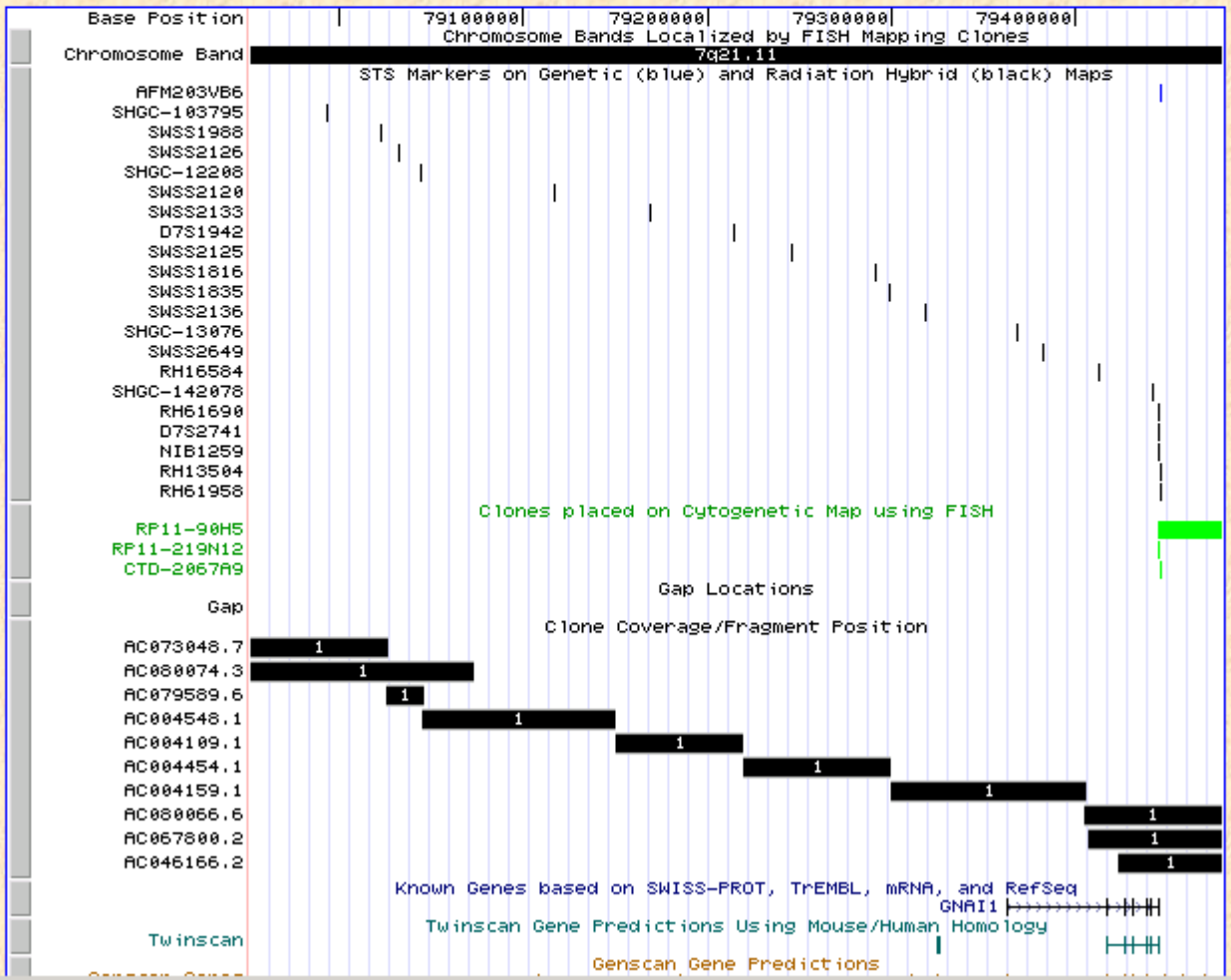


Table 11.1 Gene ordering by three-point crosses

Class of offspring	Position of recombination (x)	Number
ABC/abc abc/abc	Nonrecombinant	853
ABc/abc abC/abc	(A, B)–x–C	5
Abc/abc aBC/abc	A–x–(B, C)	47
AbC/abc aBc/abc	B–x–(A, C)	95

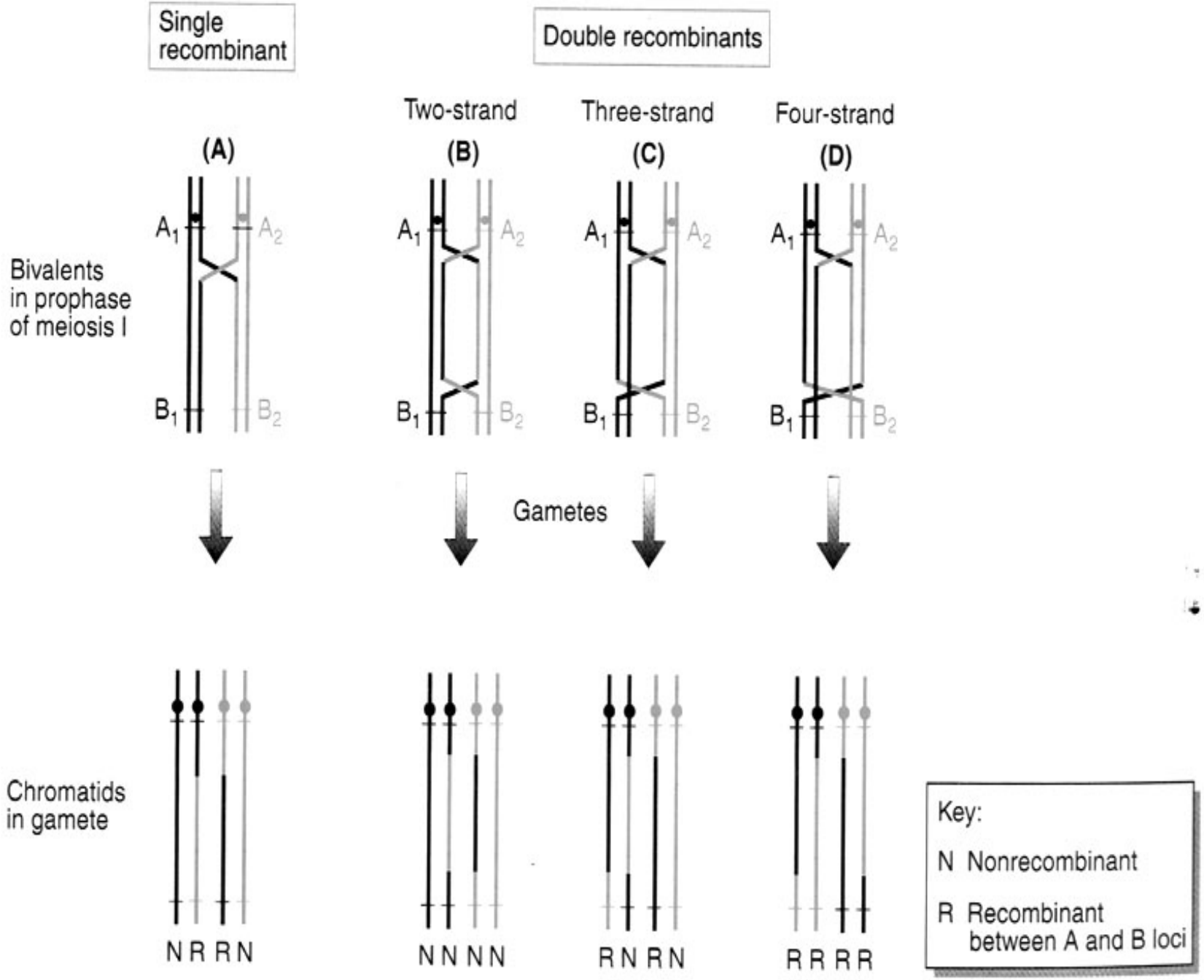


Figure 11.2: Single and double recombinants.

tion. If crossovers occurred at random along a bivalent and had no influence on one another, the appropriate mapping function would be Haldane's function:

$$w = -\tfrac{1}{2} \ln(1 - 2\theta)$$

or

$$\theta = \tfrac{1}{2} [1 - \exp(-2w)]$$

where w is the map distance and θ the recombination fraction; as usual ln means logarithm to the base e, and exp means 'e to the power of'. However, we know that crossovers do not occur at random. The presence of one chiasma inhibits formation of a second chiasma nearby. This phenomenon is called **interference**. A variety of mapping functions exist that allow for varying degrees of interference. A widely used function for human mapping is Kosambi's function:

$$w = \tfrac{1}{4} \ln [(1 + 2\theta) / (1 - 2\theta)]$$

or

$$\theta = \tfrac{1}{2} [\exp(4w) - 1] / [\exp (4w) + 1]$$

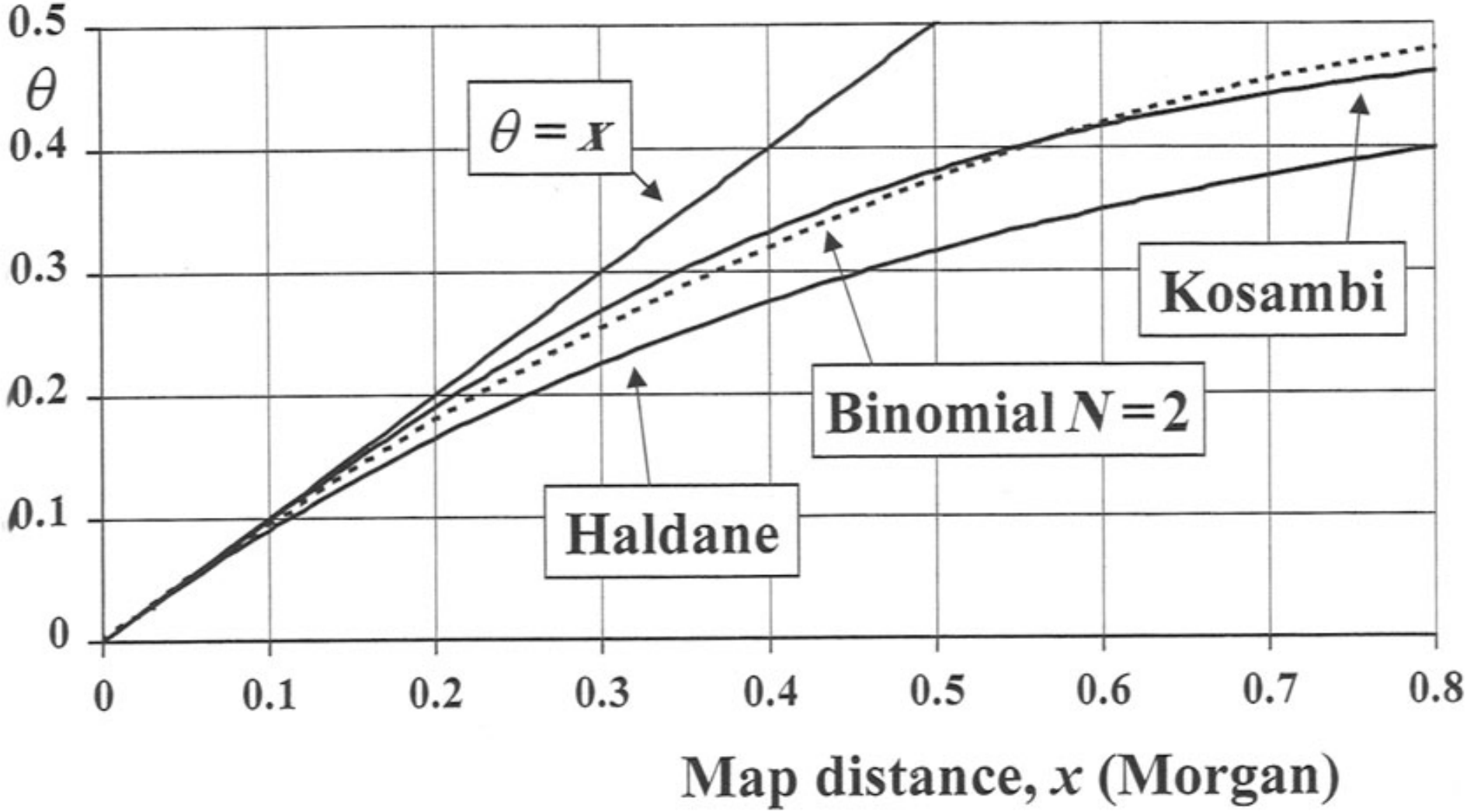


Figure 1.4. Graphs of several map functions.

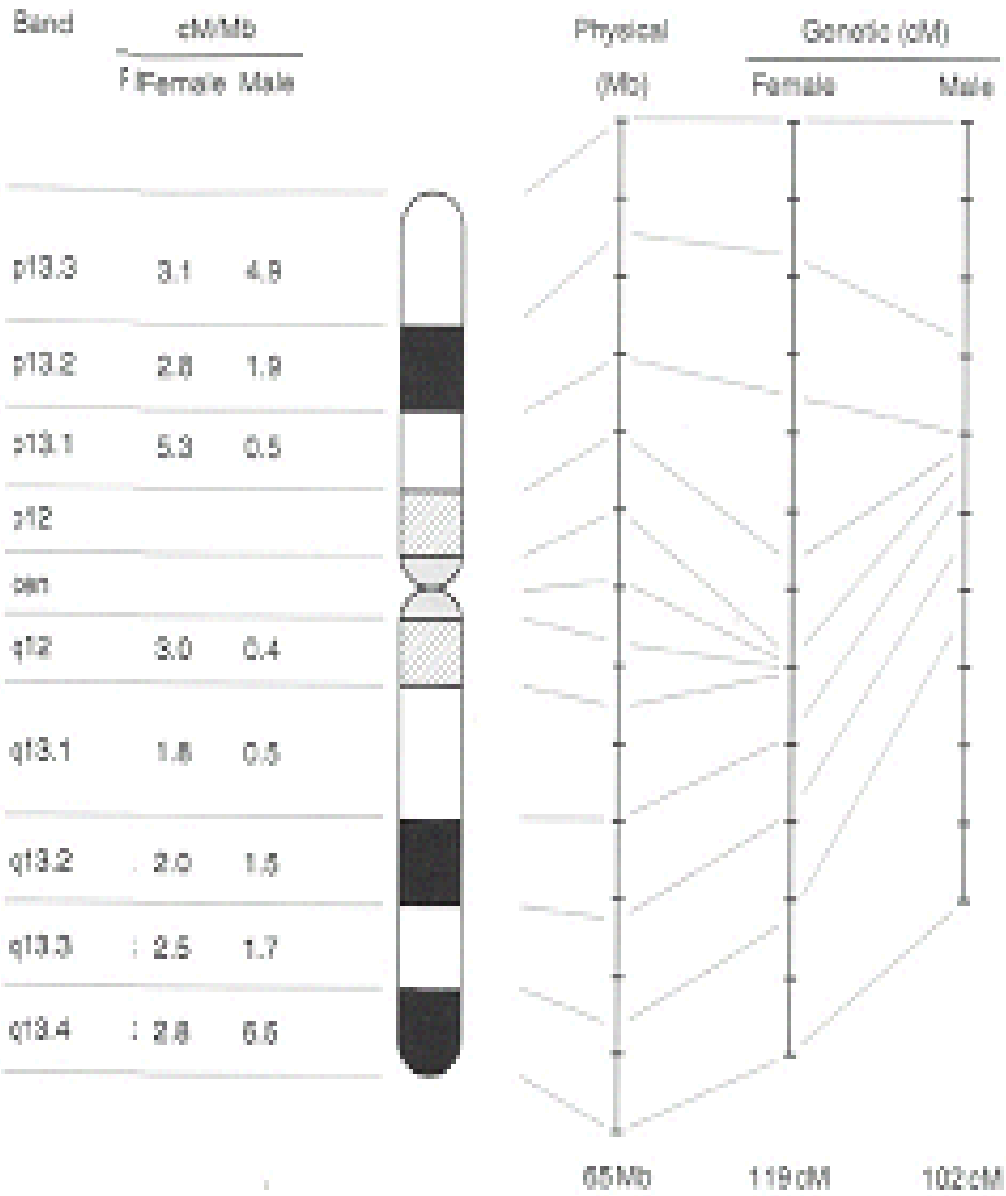


Figure 11.3: Relation of physical and genetic maps of chromosome 19.

Box 11.1

The development of human genetic markers

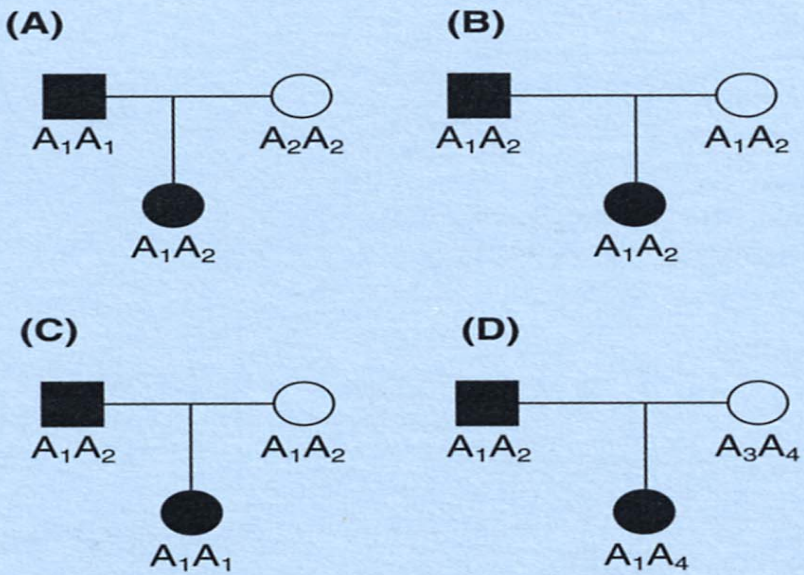
Type of marker	No. of loci	Features
Blood groups 1910–1960	~20	May need fresh blood, rare antisera Genotype cannot always be inferred from phenotype because of dominance No easy physical localization
Electrophoretic mobility variants of serum proteins 1960–1975	~30	May need fresh serum, specialized assays No easy physical localization Often limited polymorphism
HLA tissue types 1970–	1 (haplotype)	One linked set Highly informative Can only test for linkage to 6p21.3
DNA RFLPs 1975–	$>10^5$ (potentially)	Two allele markers, maximum heterozygosity 0.5 Initially required Southern blotting, now PCR Easy physical localization
DNA VNTRs (minisatellites) 1985–	$>10^4$ (potentially)	Many alleles, highly informative Type by Southern blotting Easy physical localization Tend to cluster near ends of chromosomes
DNA VNTRs (microsatellites) (di-, tri- and tetranucleotide repeats) 1989–	$>10^5$ (potentially)	Many alleles, highly informative Can type by automated multiplex PCR Easy physical localization Distributed throughout genome
DNA SNPs (single nucleotide polymorphisms) 1998–	$>10^6$ (potentially)	Less informative than microsatellites Can be typed on a very large scale by automated equipment without gel electrophoresis

VNTR, variable number of tandem repeats

Box 11.2

Informative and uninformative meioses

A meiosis is informative for linkage when we can identify whether or not the gamete is recombinant. Consider the male meiosis which produced the paternal contribution to the child in the four pedigrees below. We assume that the father has a dominant condition that he inherited along with marker allele A_1.

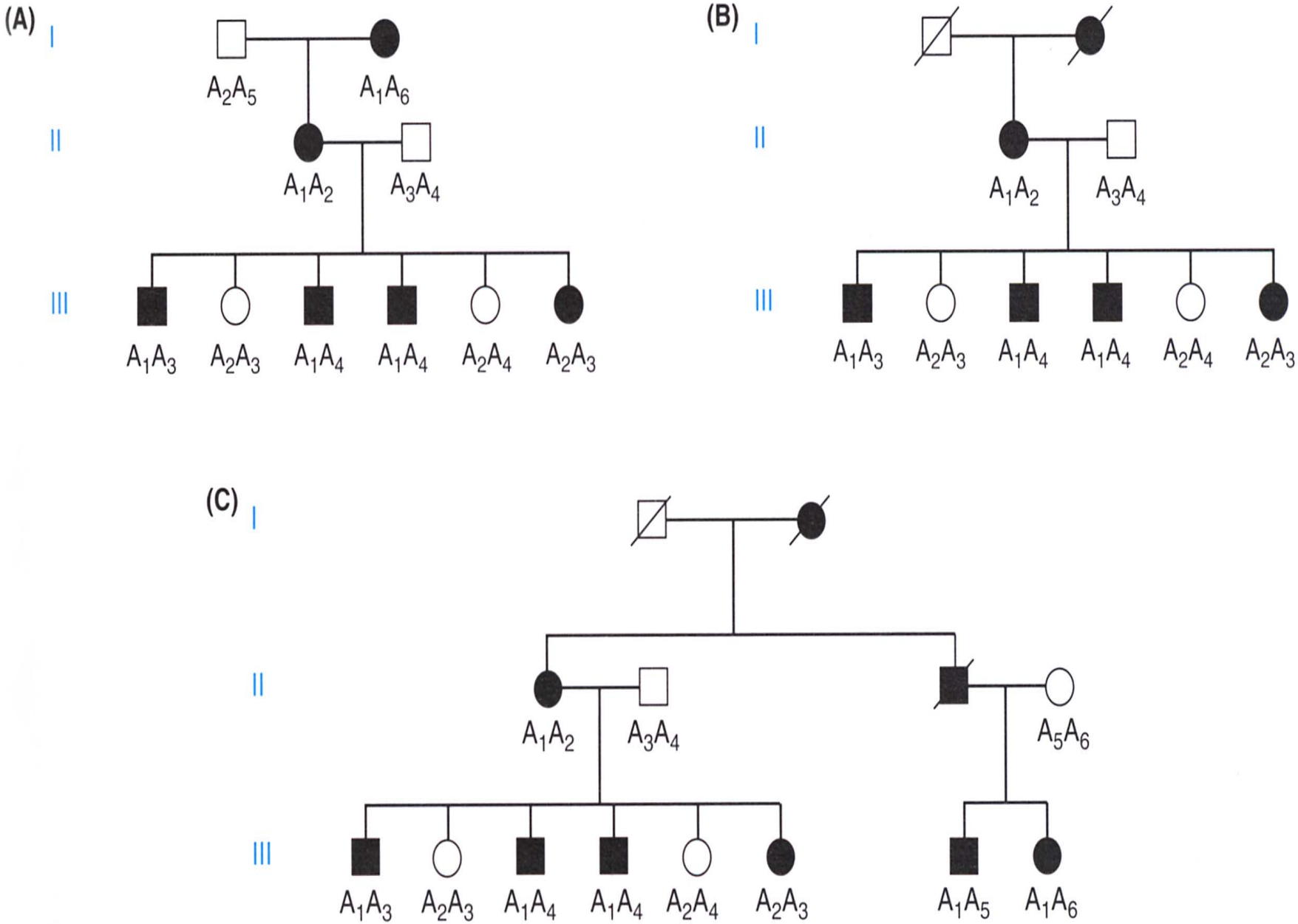


(A) This meiosis is uninformative: the marker alleles in the homozygous father cannot be distinguished.

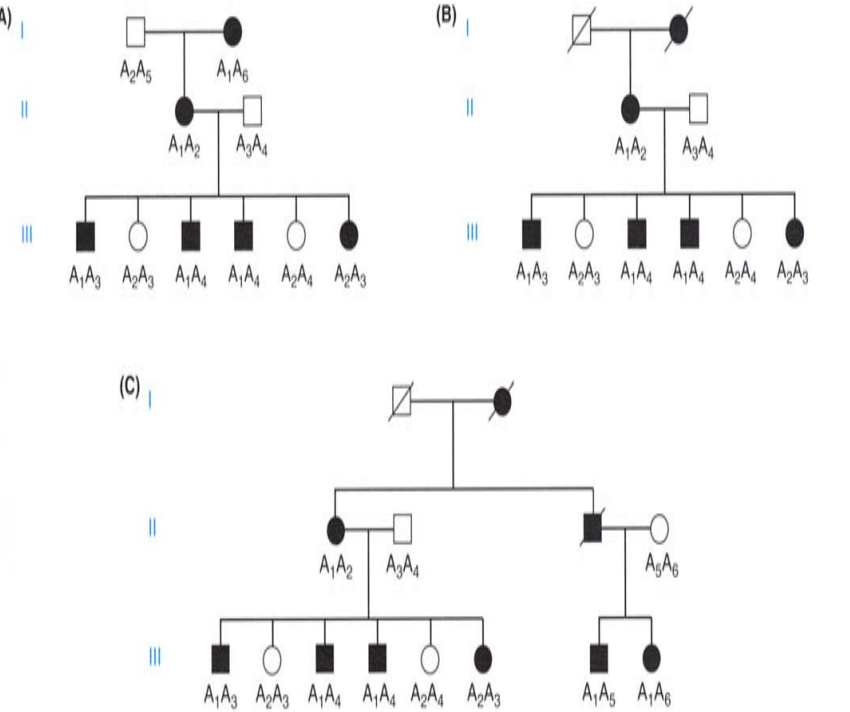
(B) This meiosis is uninformative: the child could have inherited A_1 from father and A_2 from mother, or vice versa.

(C) This meiosis is informative: the child inherited A_1 from the father.

(D) This meiosis is informative: the child inherited A_1 from the father.



Recognizing recombinants: three versions of a family with an autosomal dominant disease, typed for a marker A.



Recognizing recombinants: three versions of a family with an autosomal dominant disease, typed for a marker A.

Calculation of lod scores for the families in *Figure 11.4*

- Given that the loci are truly linked, with recombination fraction θ, the likelihood of a meiosis being non-recombinant is $1 - \theta$ and the likelihood of it being recombinant is θ.
- If the loci are in fact unlinked, the likelihood of a meiosis being either recombinant or nonrecombinant is 1/2.

Family A

There are five recombinants and one nonrecombinant.
The overall likelihood, given linkage, is $(1 - \theta)^5.\theta$
The likelihood given no linkage is $(1/2)^6$
The likelihood ratio is $(1 - \theta)^5.\theta / (1/2)^6$
The lod score, Z, is the logarithm of the likelihood ratio.

θ	0	0.1	0.2	0.3	0.4	0.5
Z	− infinity	0.577	0.623	0.509	0.299	0

Family B

II_1 is phase-unknown.
If she inherited A_1 with the disease, there are five non-recombinants and one recombinant.
If she inherited A_2 with the disease, there are five recombinants and one nonrecombinant.
The overall likelihood is $\frac{1}{2}[(1 - \theta)^5.\theta / (1/2)^6] + \frac{1}{2}[(1 - \theta).\theta^5 / (1/2)^6]$. This allows for either possible phase, with equal prior probability.
The lod score, Z, is the logarithm of the likelihood ratio.

θ	0	0.1	0.2	0.3	0.4	0.5
Z	− infinity	0.276	0.323	0.222	0.076	0

Family C

At this point nonmasochists turn to the computer.

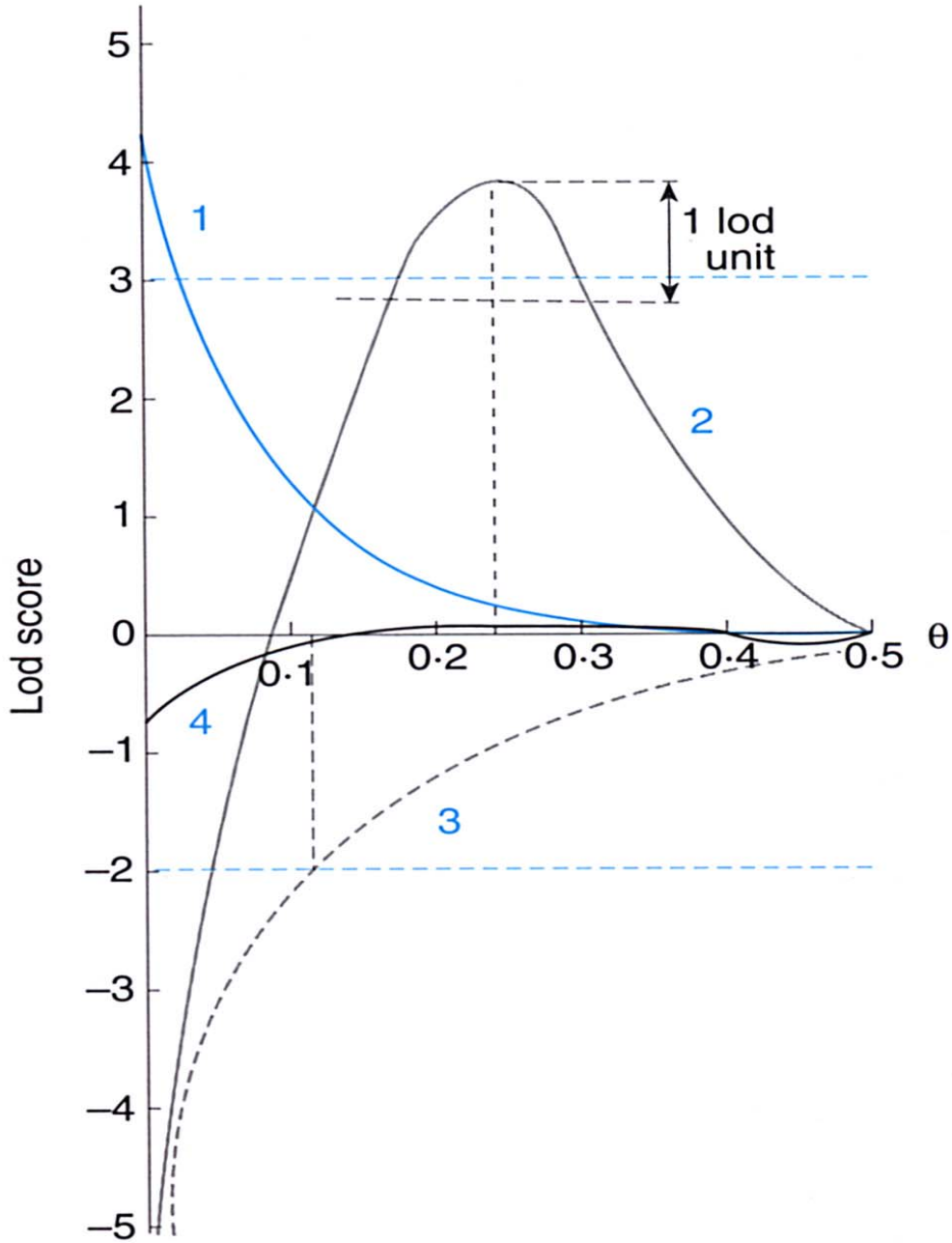


Figure 11.5: Lod score curves.

Box 11.4

Bayesian calculation of linkage threshold

The likelihood that two loci should be linked (the prior probability of linkage) has been argued over, but estimates of about one in 50 are widely accepted.

Hypothesis	Loci are linked (recombination fraction = θ)	Loci are not linked (recombination fraction = 0.5)
Prior probability	1/50	49/50
Conditional probability: 1000 : 1 odds of linkage (lod score $Z(\theta) = 3.0$)	1000	1
Joint probability (prior × conditional)	20	~1

Because of the low prior probability that two randomly chosen loci should be linked, evidence giving 1000 : 1 odds in favor of linkage is required in order to give overall 20 : 1 odds in favor of linkage. This corresponds to the conventional $p = 0.05$ threshold of statistical significance. The calculation is an example of the use of Bayes' formula to combine probabilities (see *Box 17.1* and *Figure 17.14*). See text for description of the lod score.

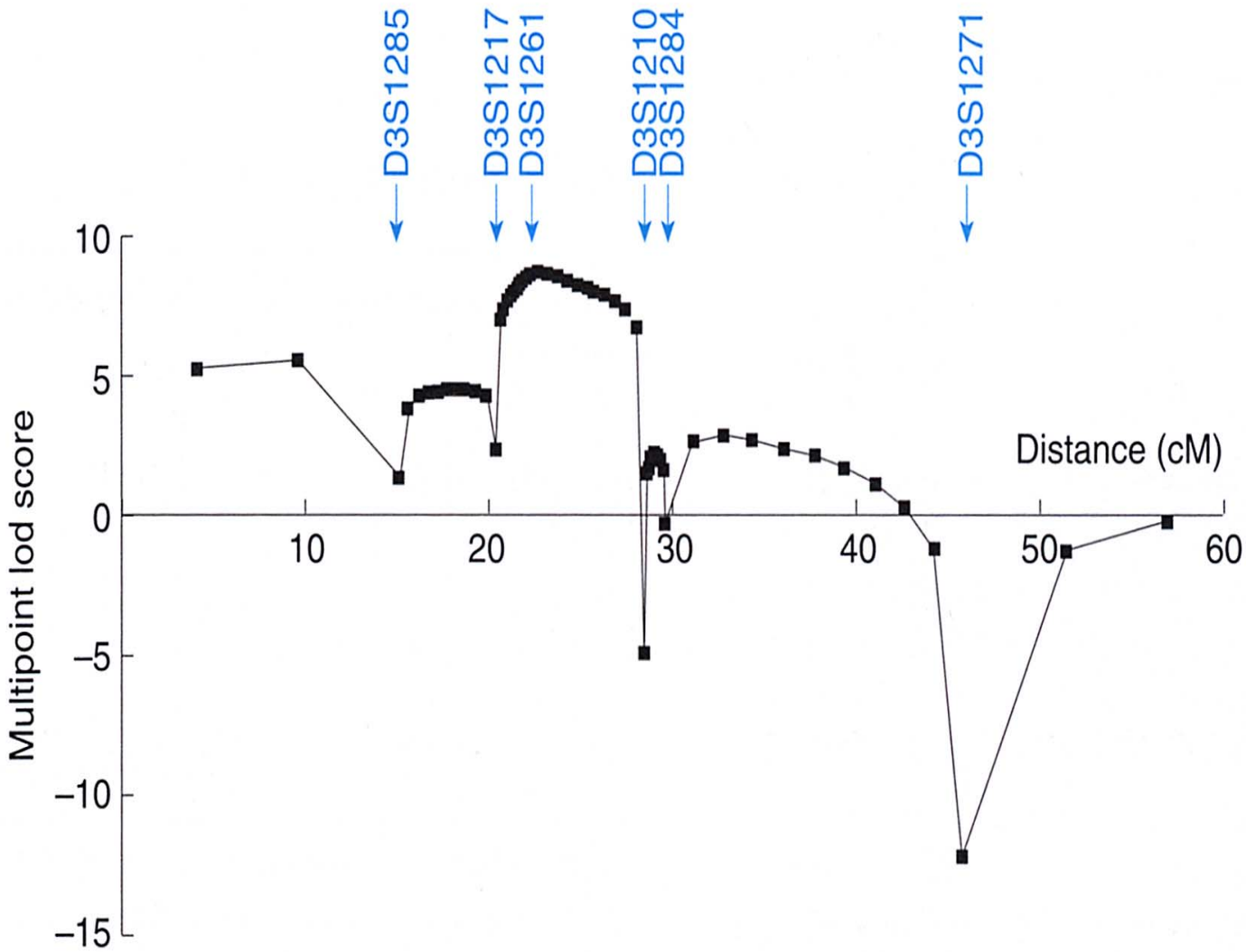


Figure 11.6: Multipoint mapping in man.

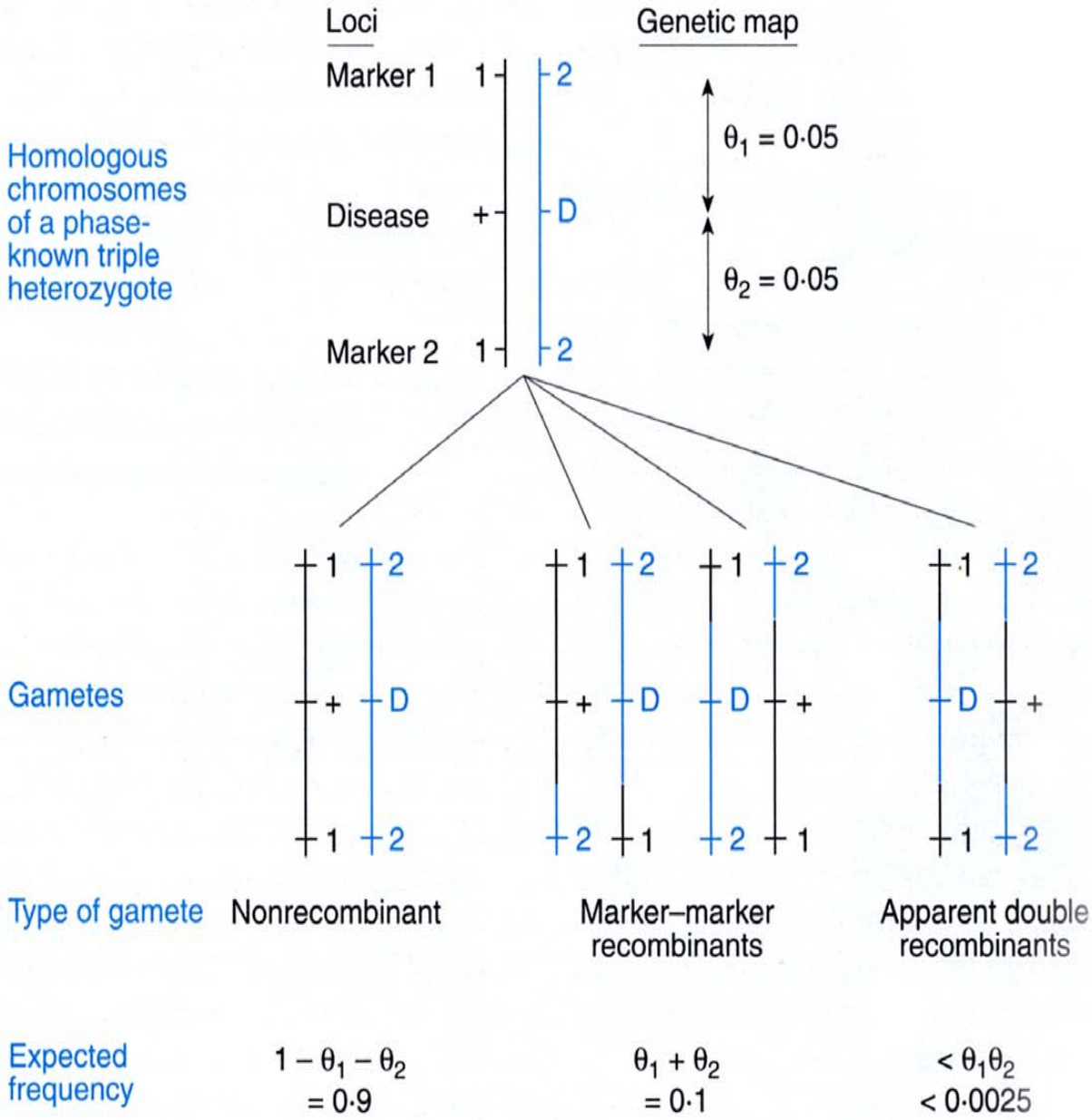


Figure 11.7: Apparent double recombinants suggest errors in the data.

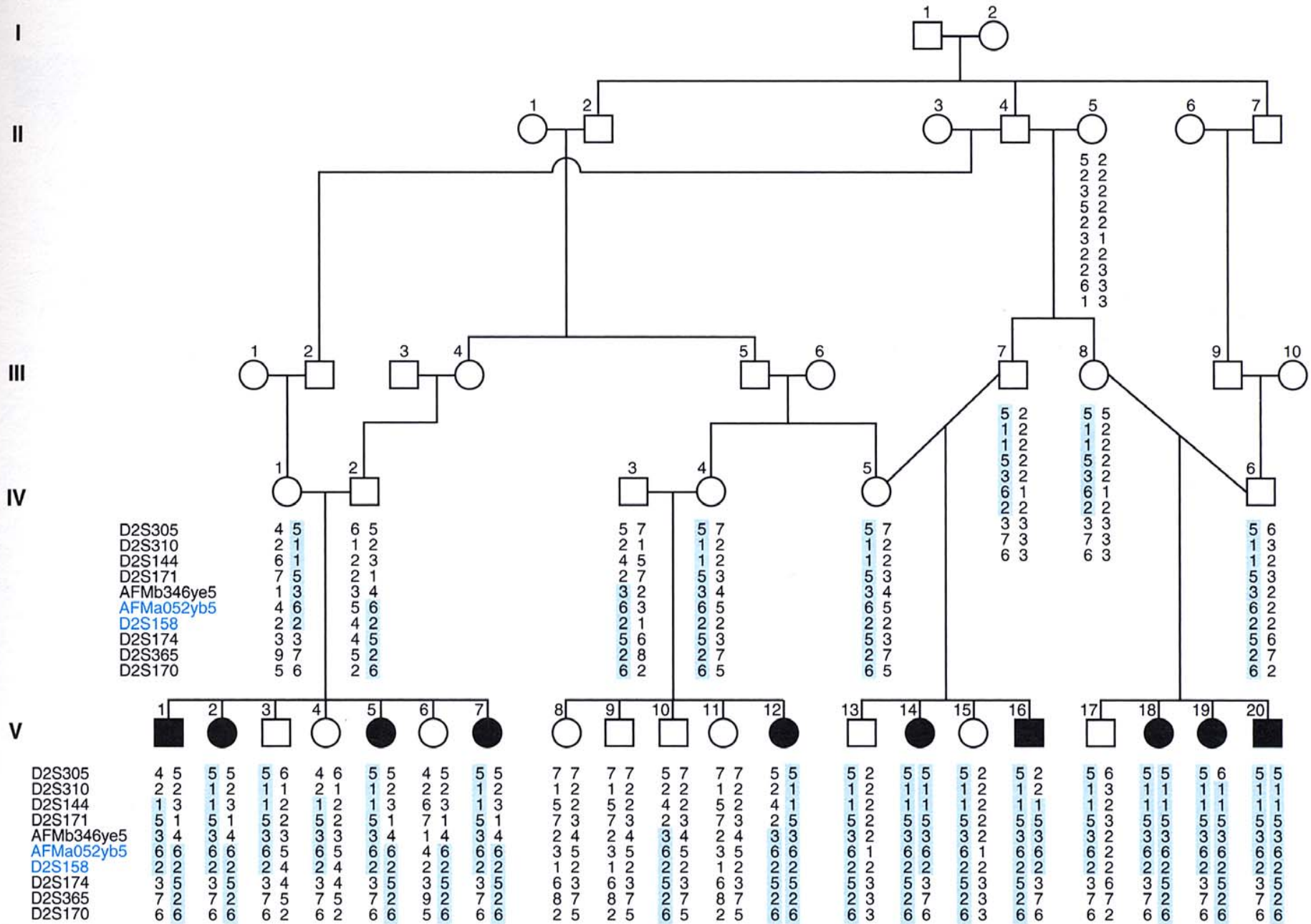


Figure 11.8 Autozygosity mapping.