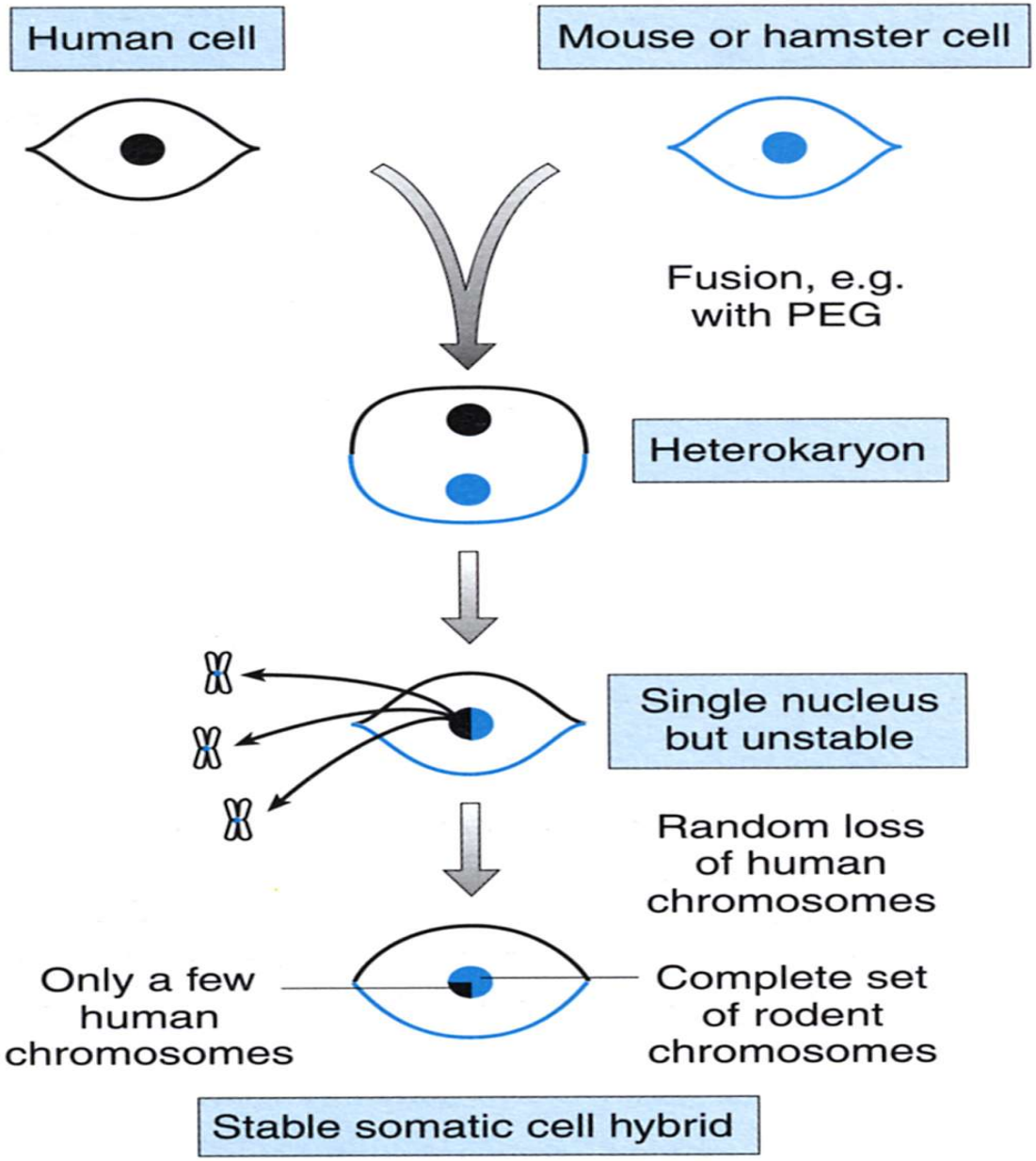



**Figure 10.1:** Fusion of cells from different species can result in stable somatic cell hybrids.

## 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 |

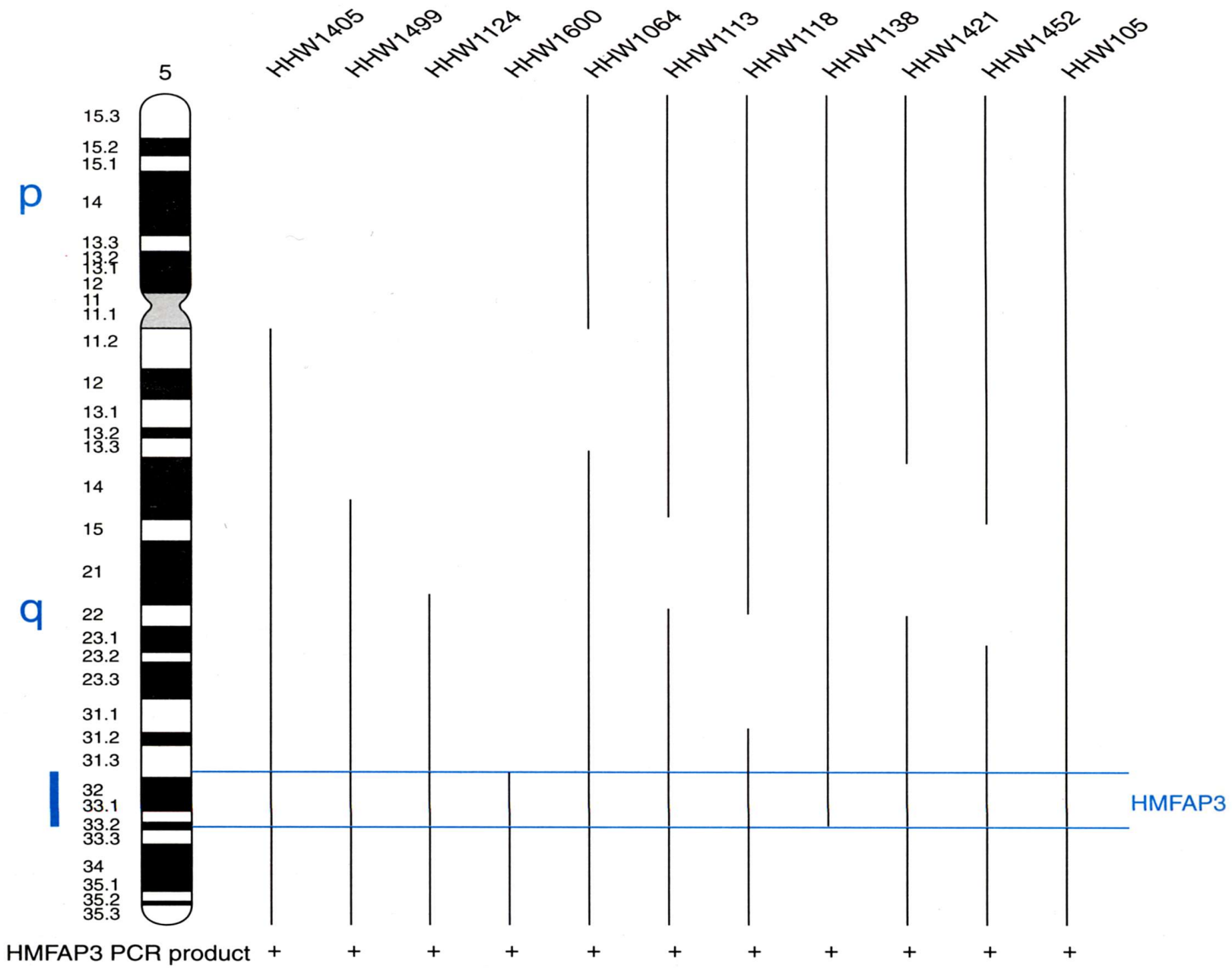



**Figure 10.2:** Subchromosomal localization can be achieved by mapping against a panel of hybrid cells containing translocation or deletion chromosomes.

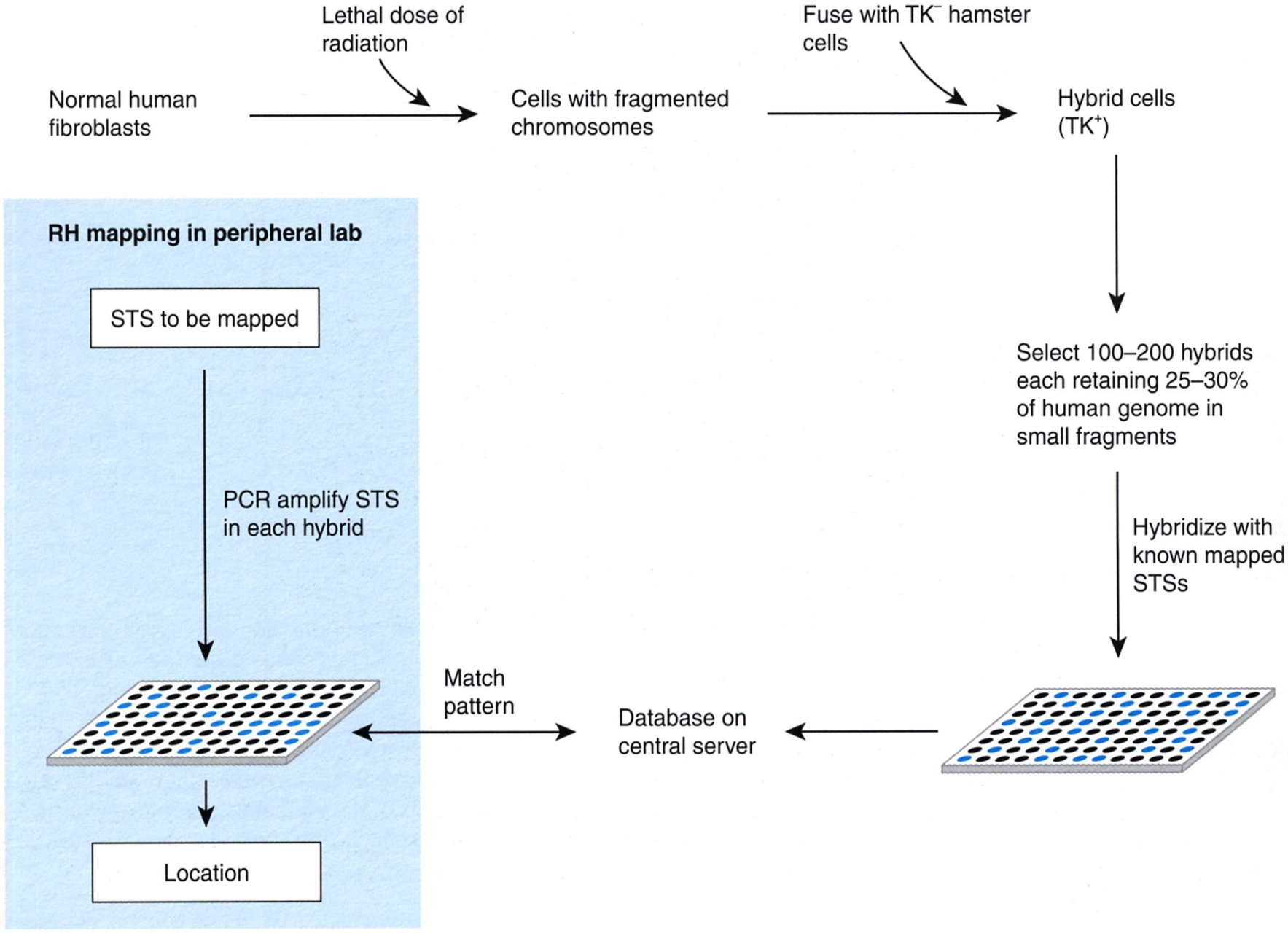



**Figure 10.4:** Use of the Genebridge 4 radiation hybrid panel for physical mapping.

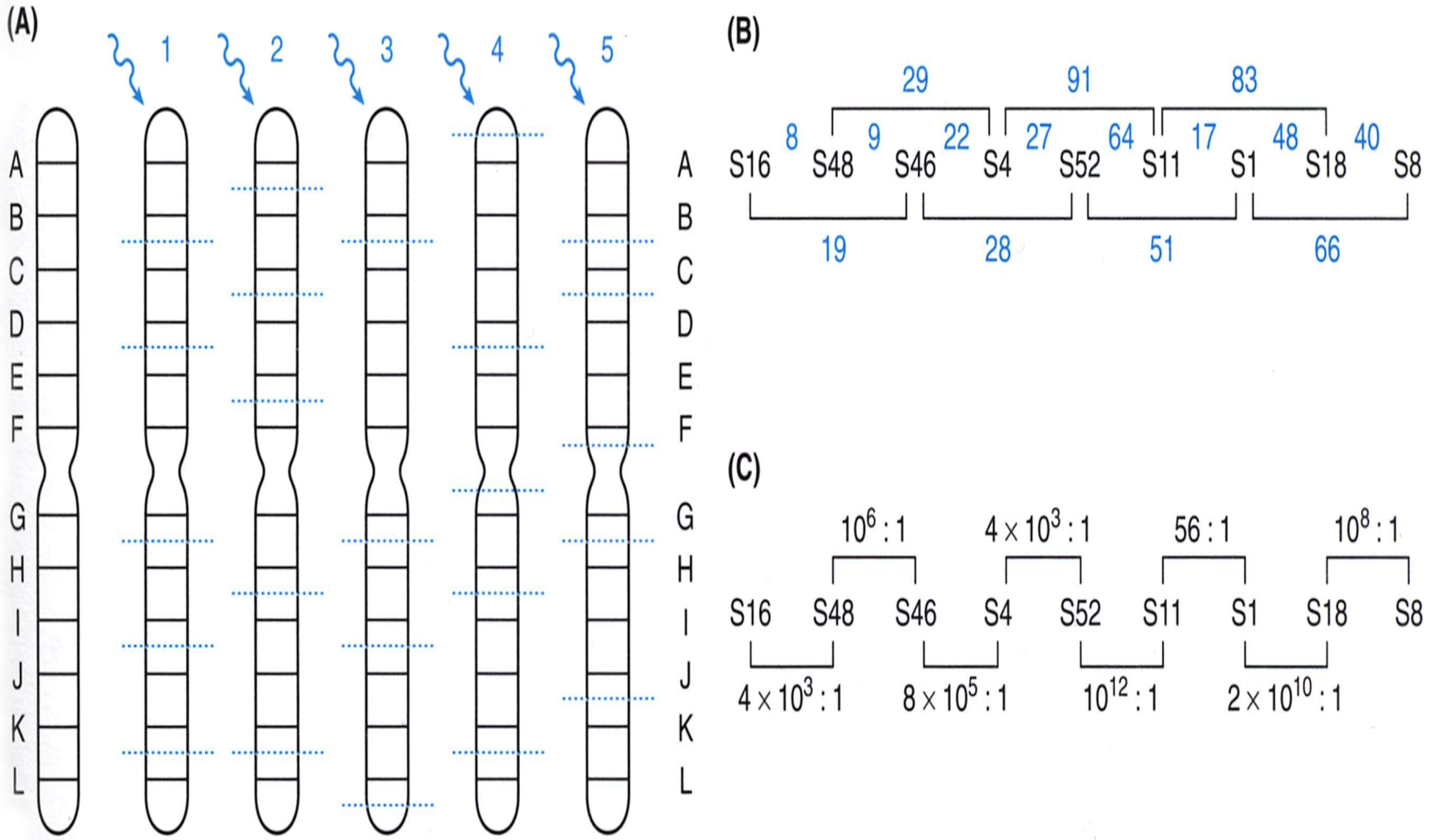
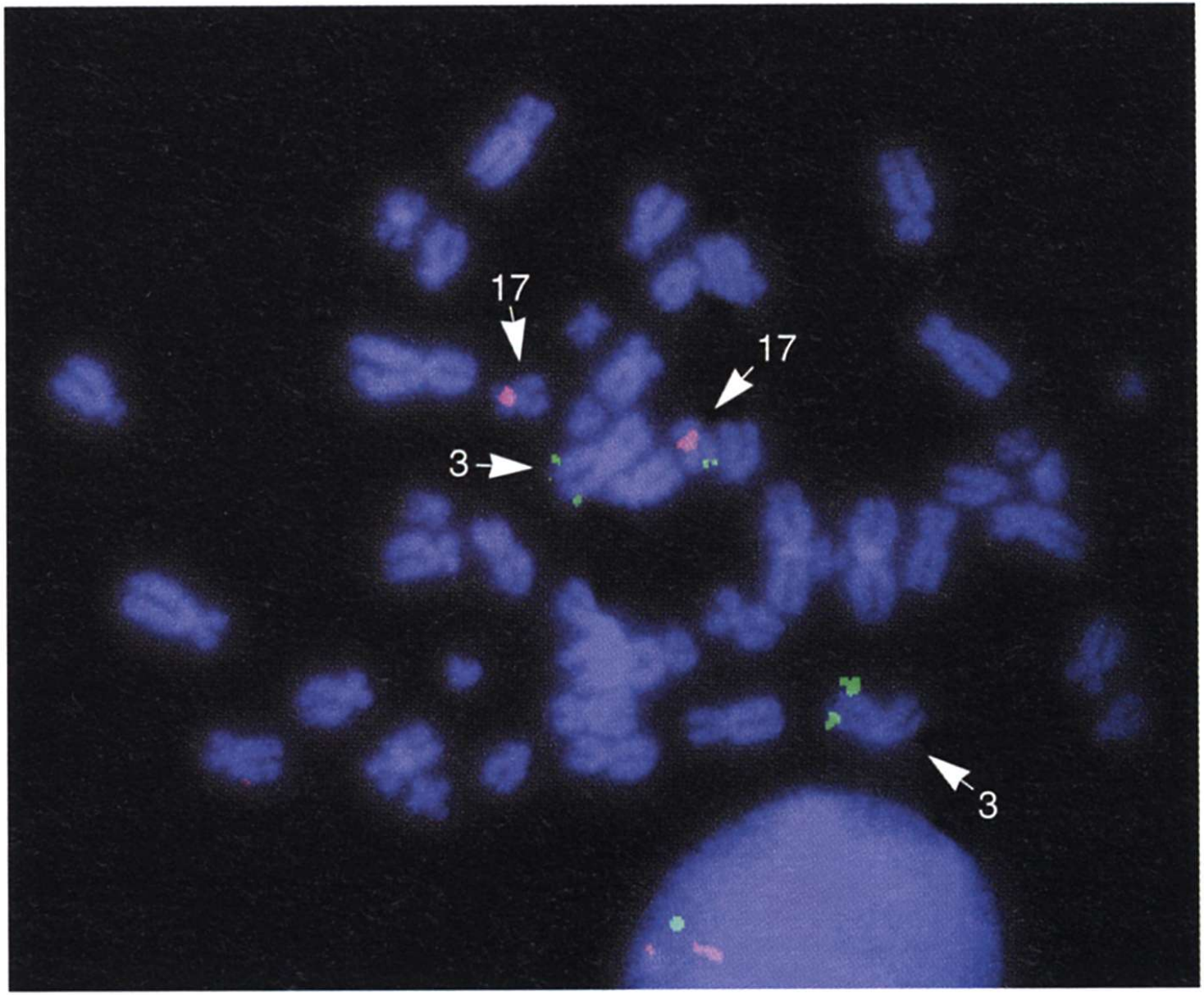



**Figure 10.3:** Constructing radiation hybrid maps.




Chromosome FISH (fluorescence *in situ* hybridization).

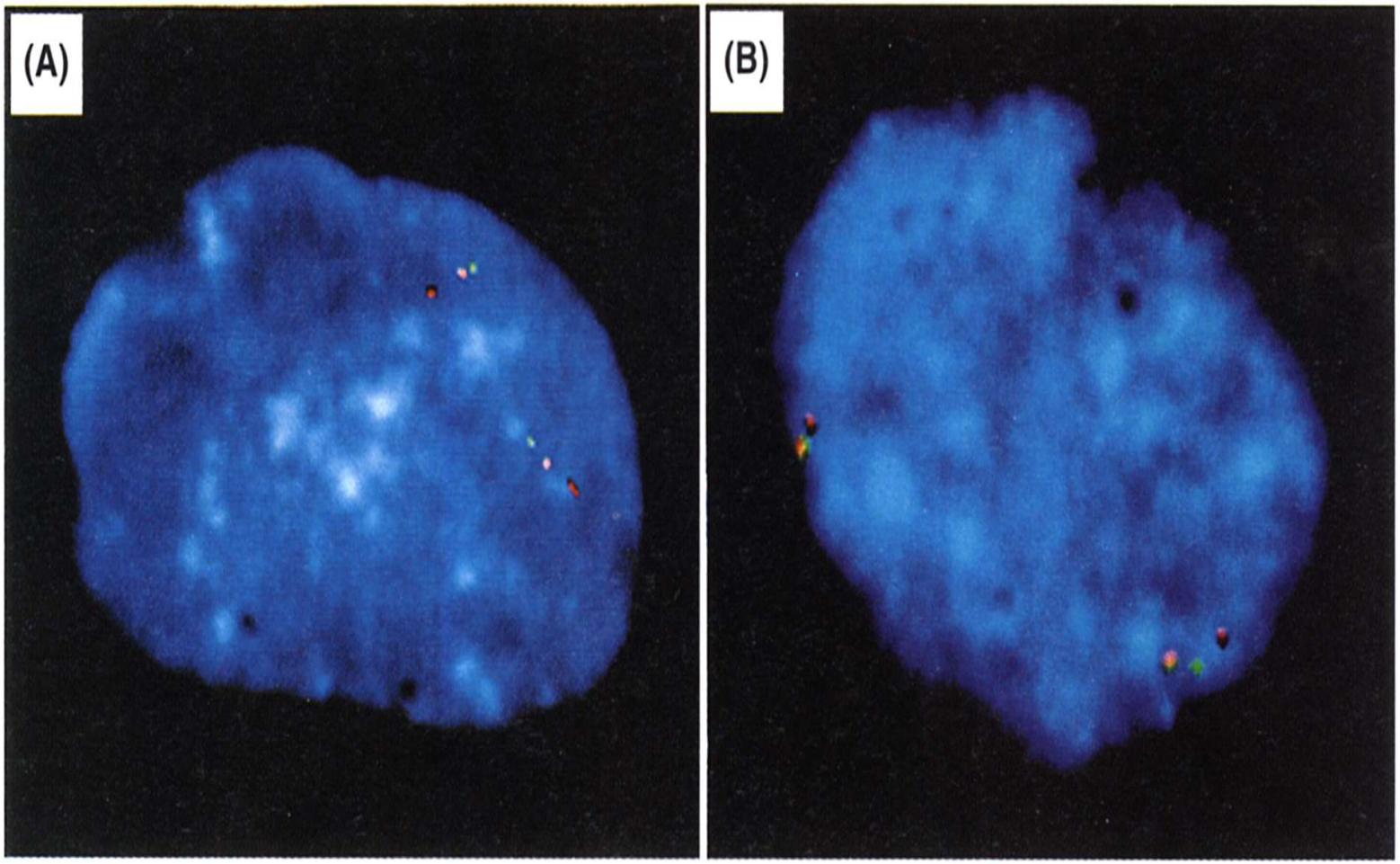



**Figure 10.8:** Determining the map order of syntenic DNA clones by three-color interphase FISH.

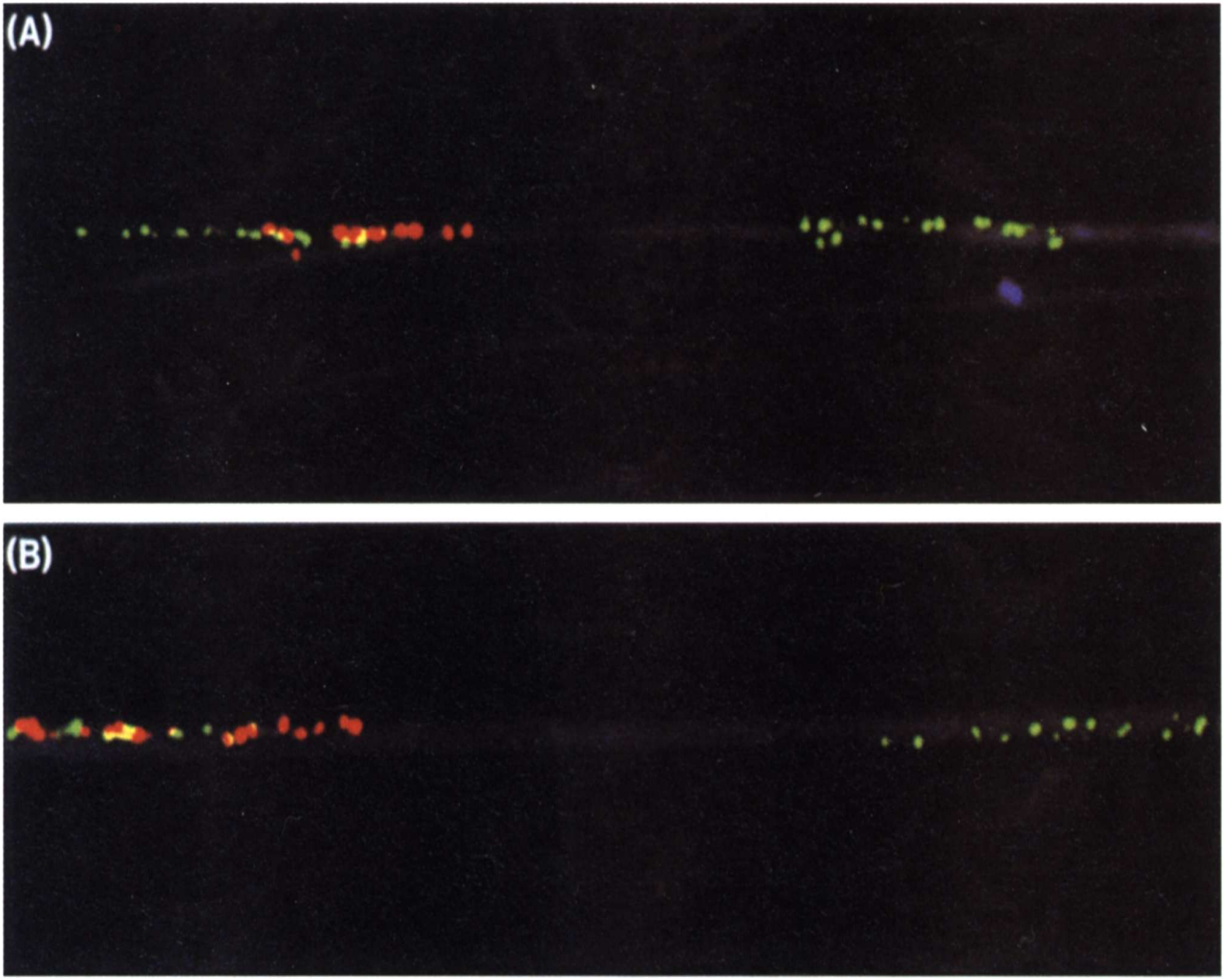



**Figure 10.9:** Extended chromatin fiber (ECF) FISH.

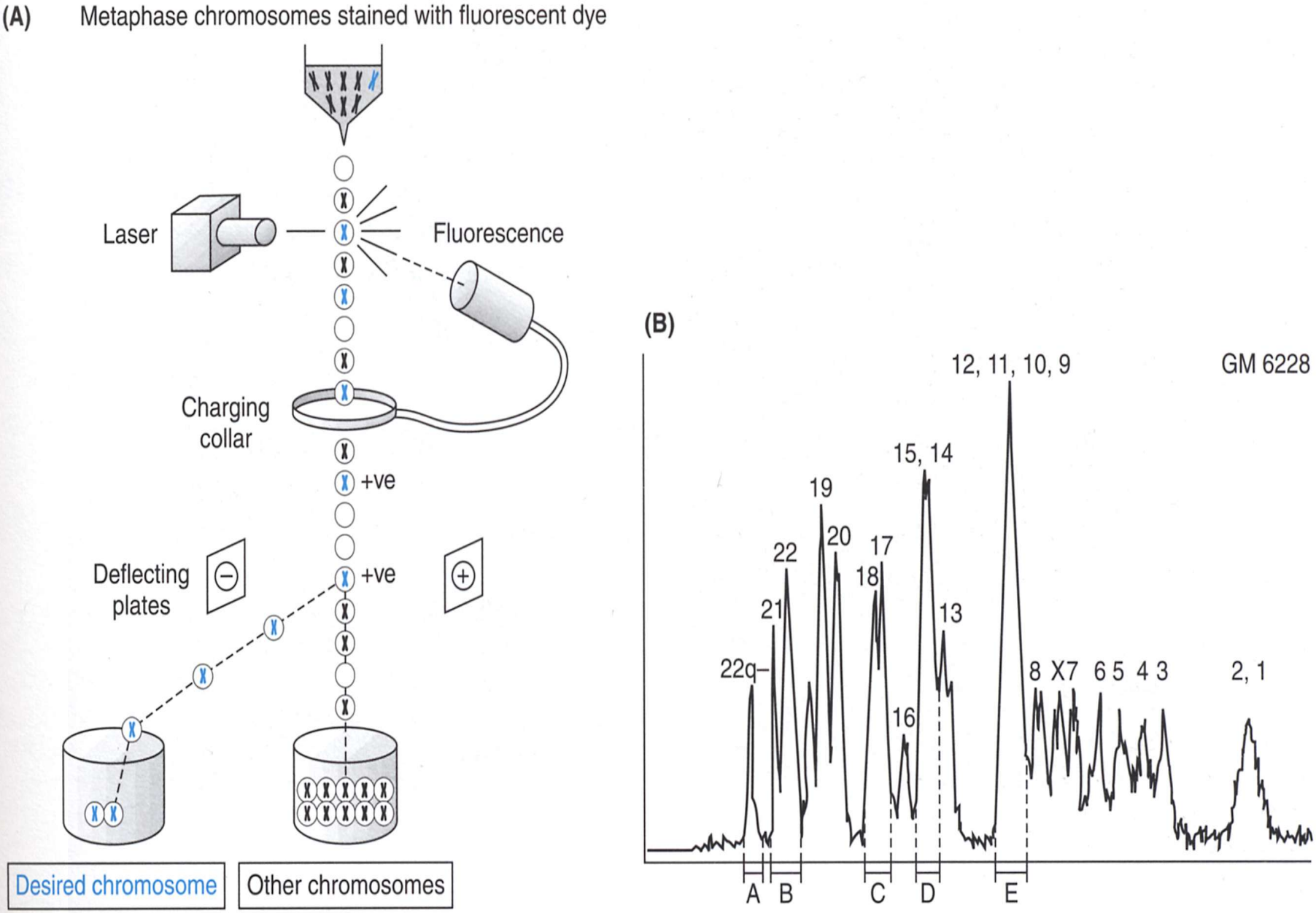



**Figure 10.7:** Fractionating chromosomes in a flow cytometer.

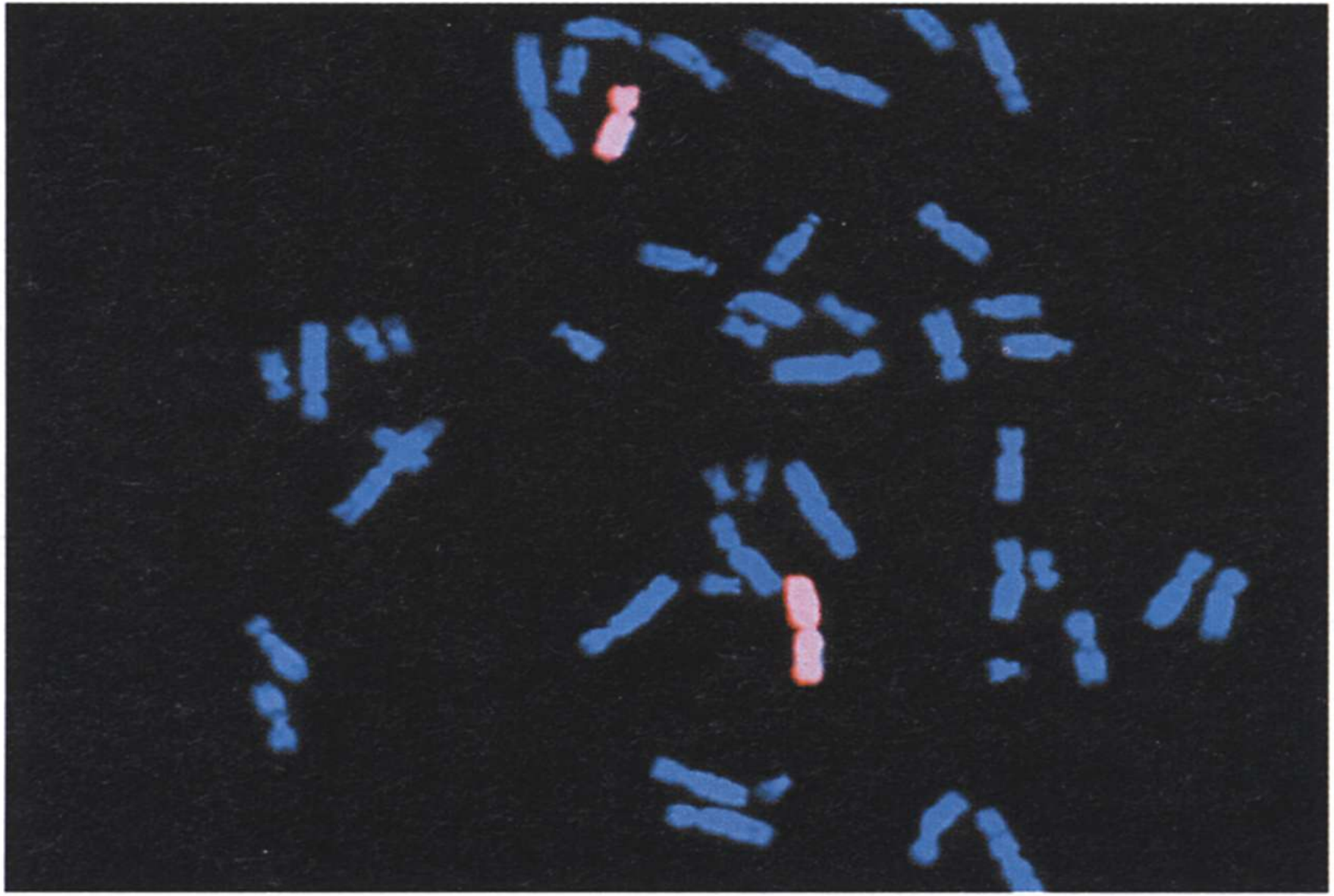
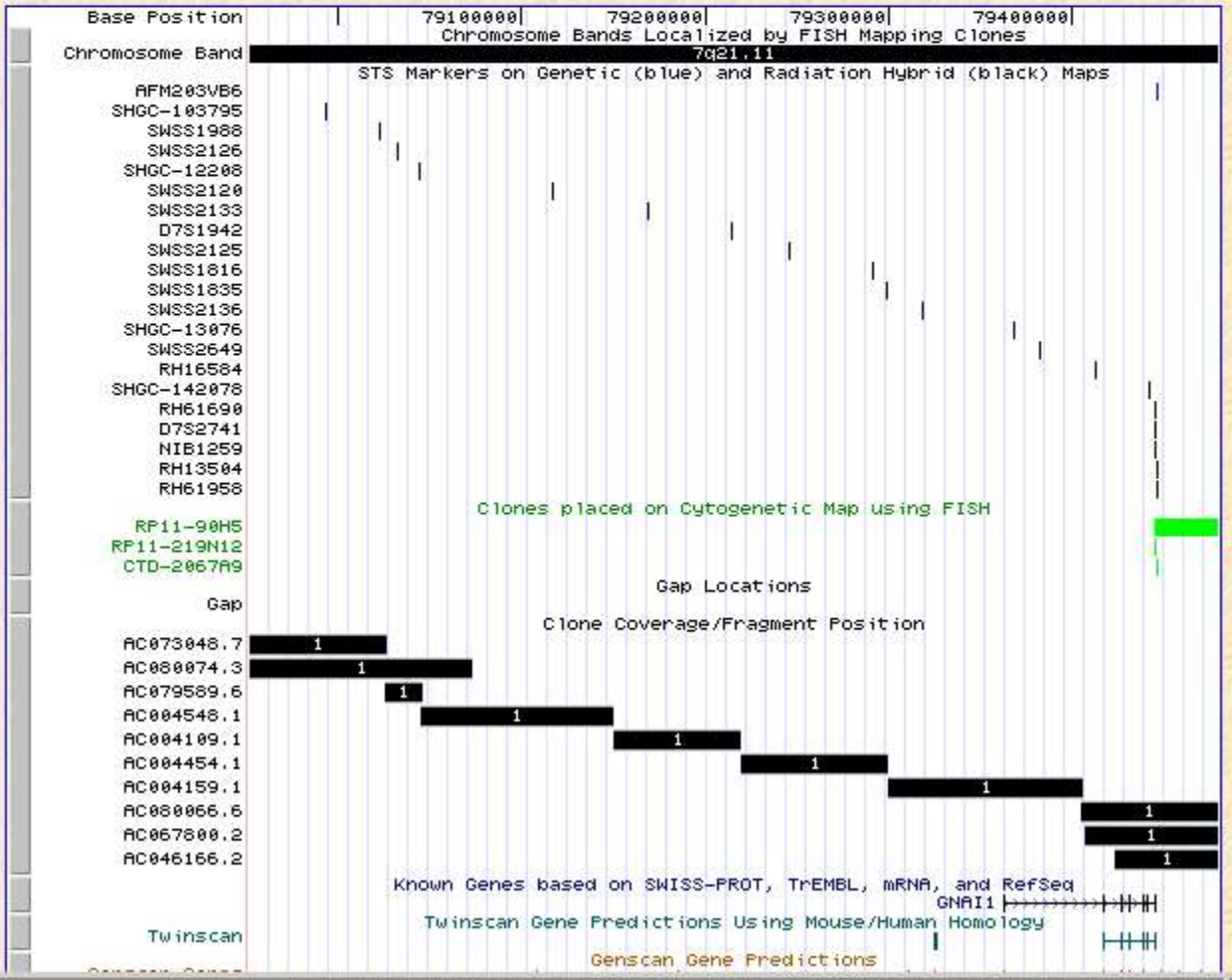


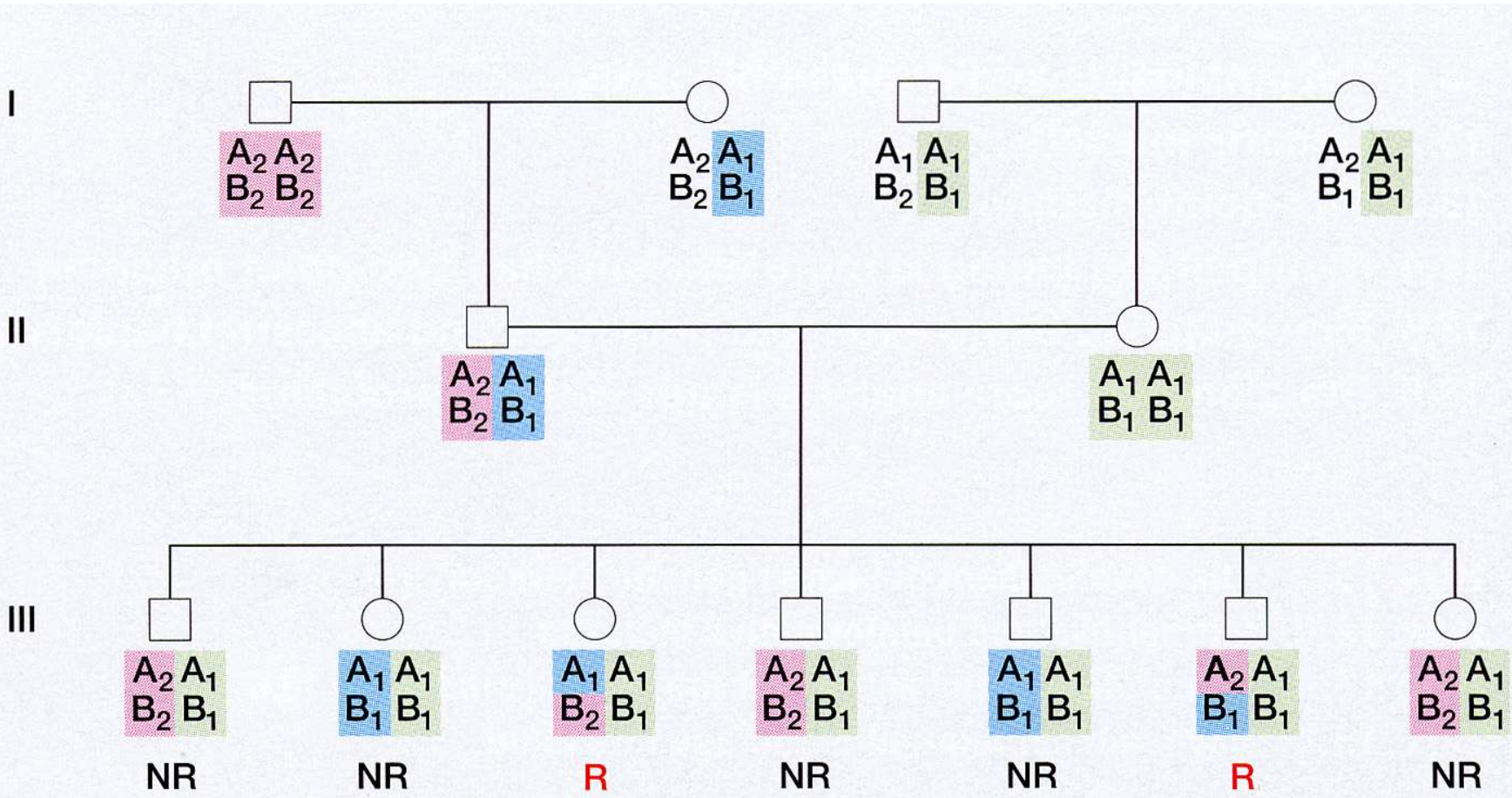
**Figure 10.6:** Chromosome painting can be used to define chromosome rearrangements.

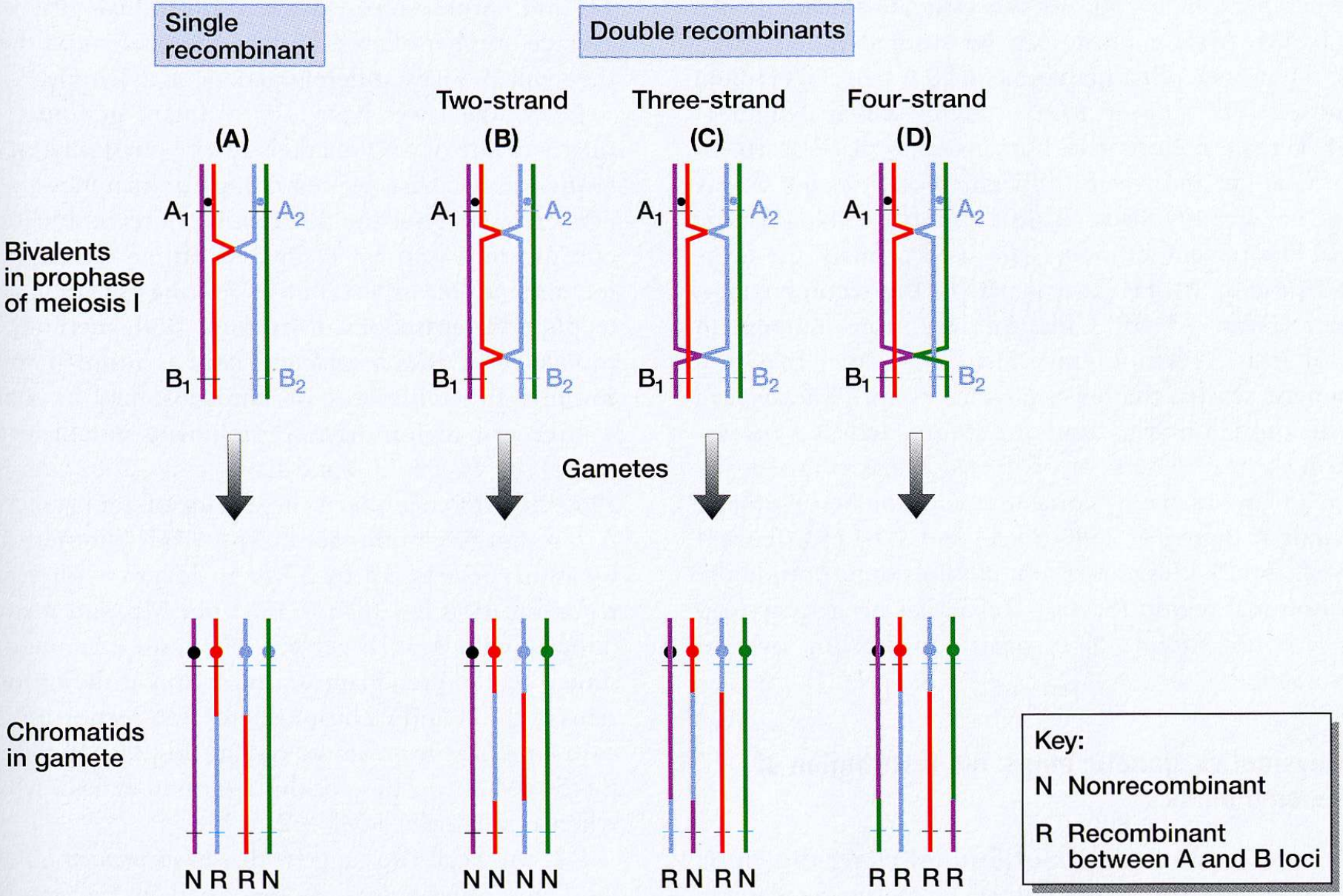
# UCSC Genome Browser on Human April 2003 Freeze

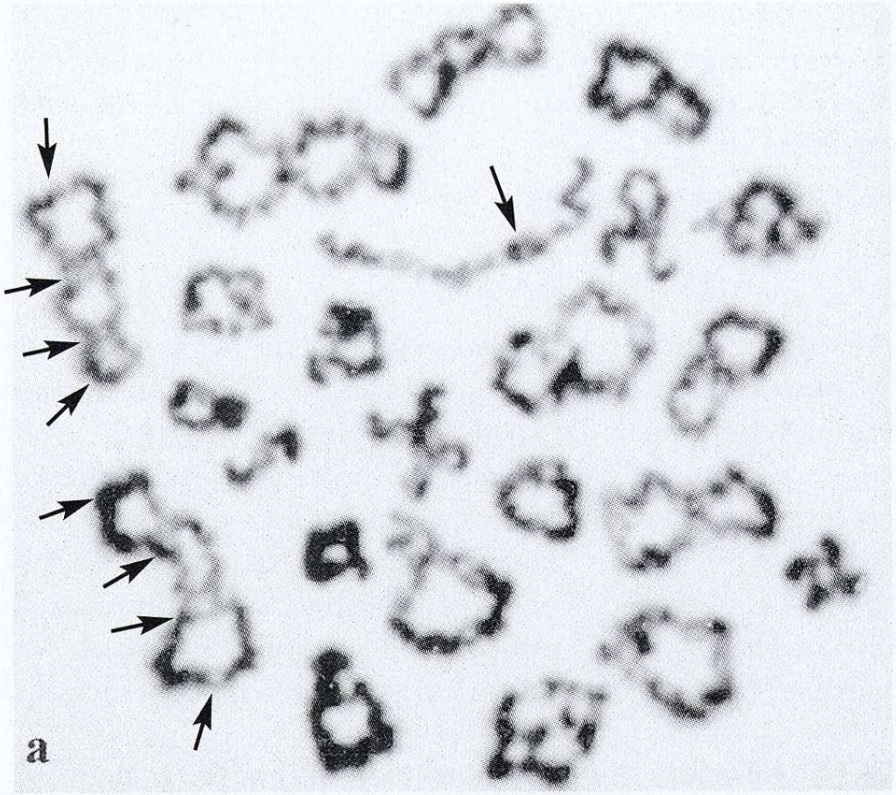
move <<< << < > >> >>> zoom in 1.5x 3x 10x zoom out 1.5x 3x 10x

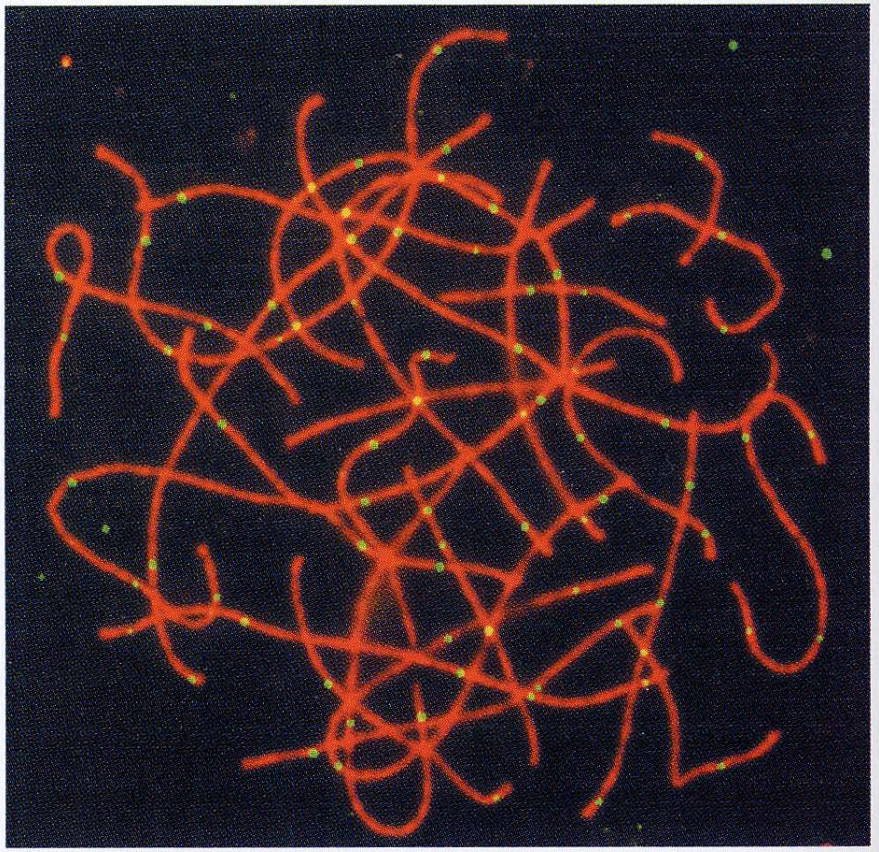
position chr7:78951600-79479699 size 528,100 image width 610 jump



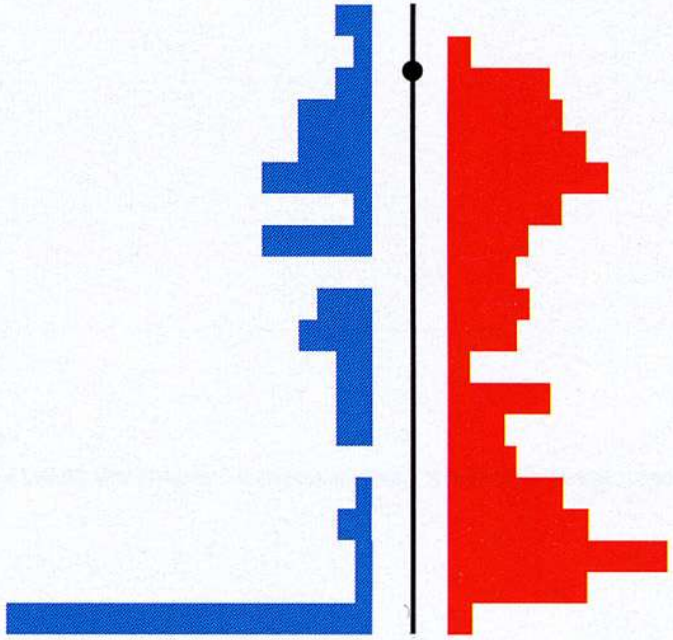

I
II
III
A2 A2 B2 B2
A2 A1 B2 B1
A1 A1 B2 B1
A2 A1 B1 B1
A2 A1 B2 B1
A1 A1 B1 B1
A2 A1 B2 B1
A1 A1 B1 B1
A1 A1 B2 B1
A2 A1 B2 B1
A1 A1 B1 B1
A2 A1 B1 B1
A2 A1 B2 B1
NR
NR
R
NR
NR
R
NR


Single recombinant
Double recombinants
Two-strand
Three-strand
Four-strand
(A)
(B)
(C)
(D)
Bivalents in prophase of meiosis I
A1
A2
B1
B2
Gametes
Chromatids in gamete
N R R N
N N N N
R N R N
R R R R
Key:
N Nonrecombinant
R Recombinant between A and B loci

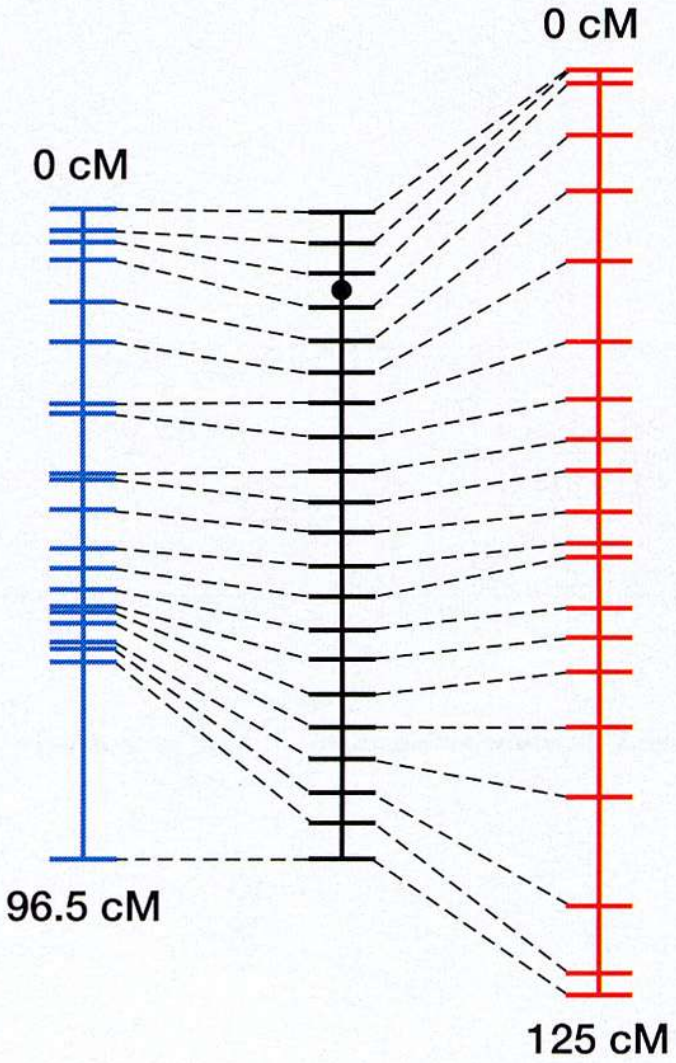

a



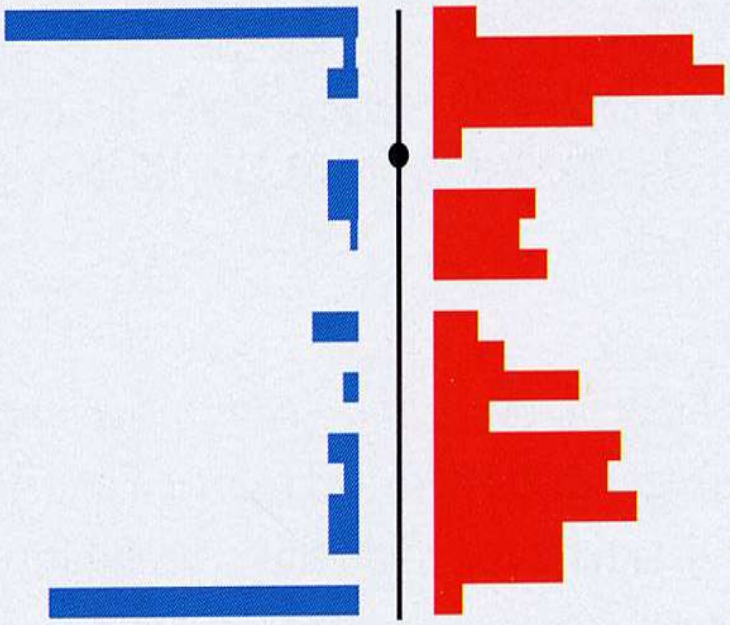
(A)



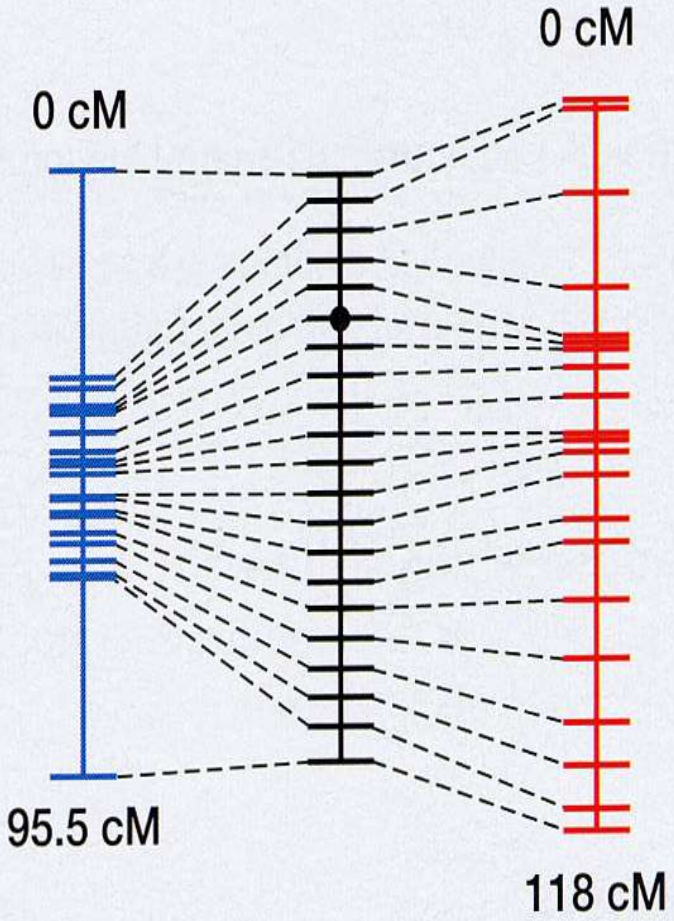
Chromosome 13



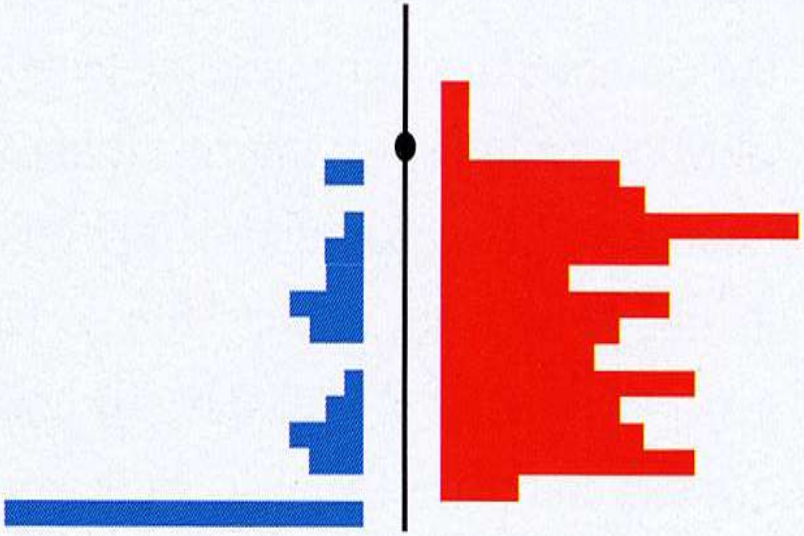
(B)



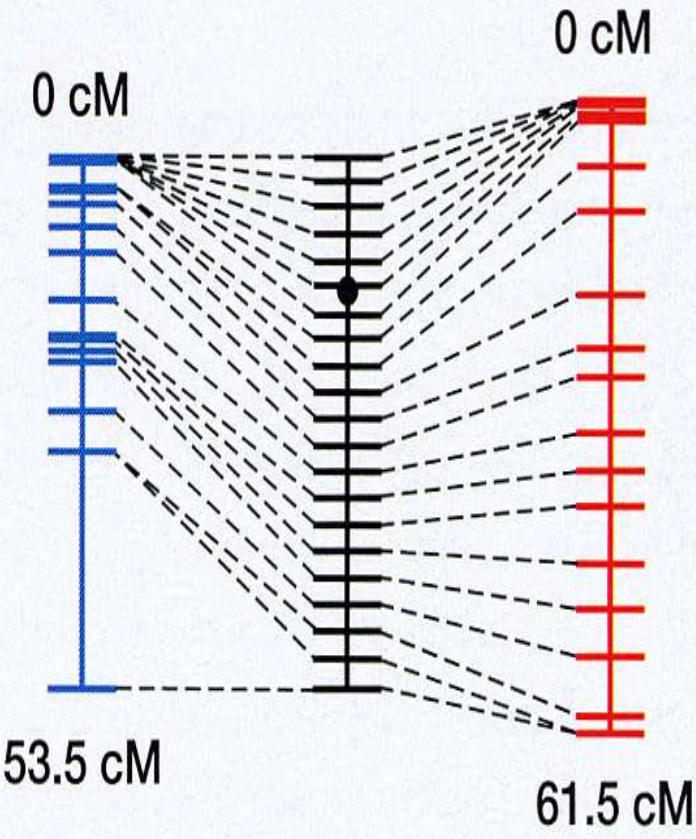
Chromosome 18

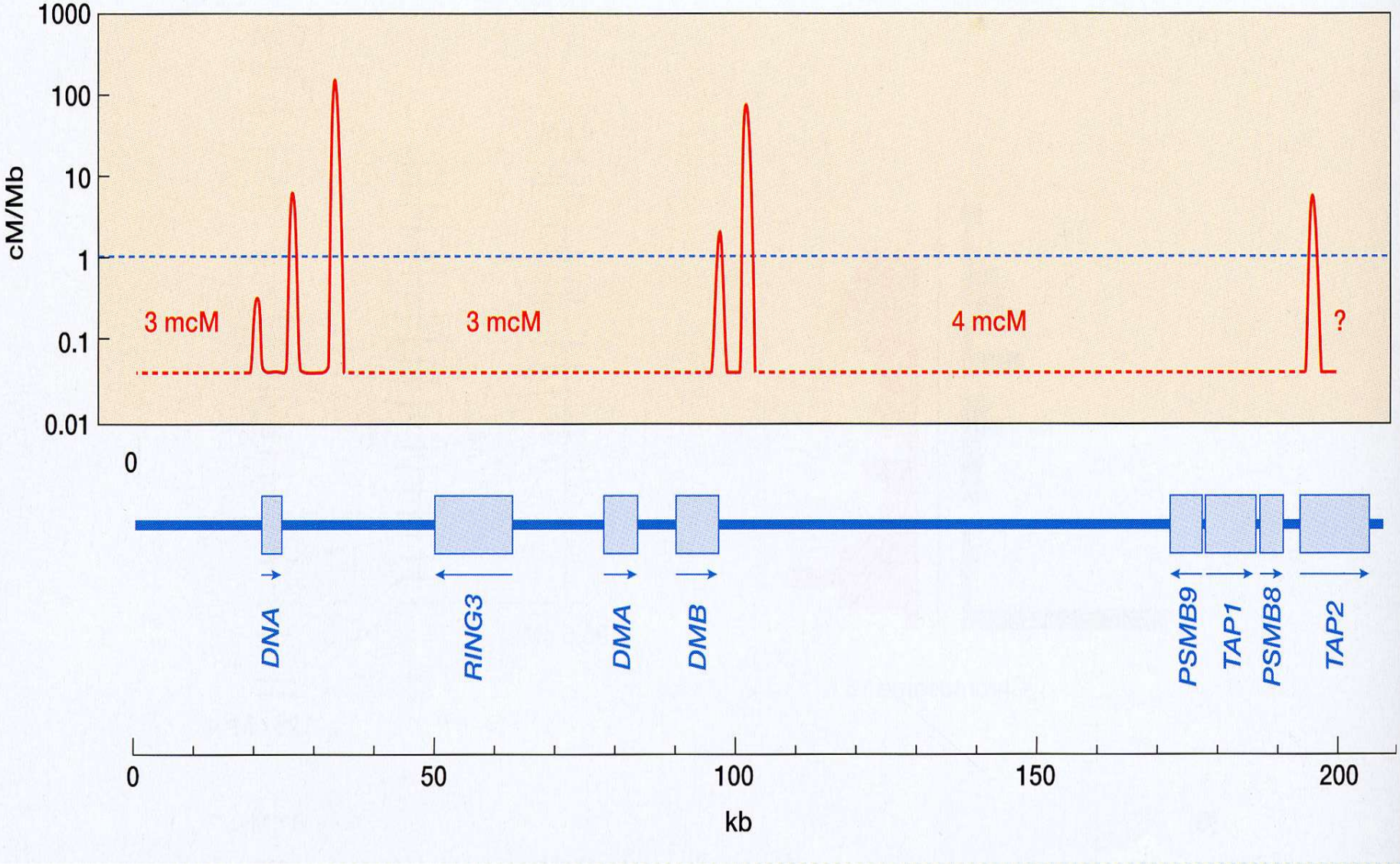


(C)



Chromosome 21




1000
100
10
1
0.1
0.01
cM/Mb
3 mcM
3 mcM
4 mcM
?
0
DNA
RING3
DMA
DMB
PSMB9
TAP1
PSMB8
TAP2
0
50
100
150
200
kb

**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 $\theta$ 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

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

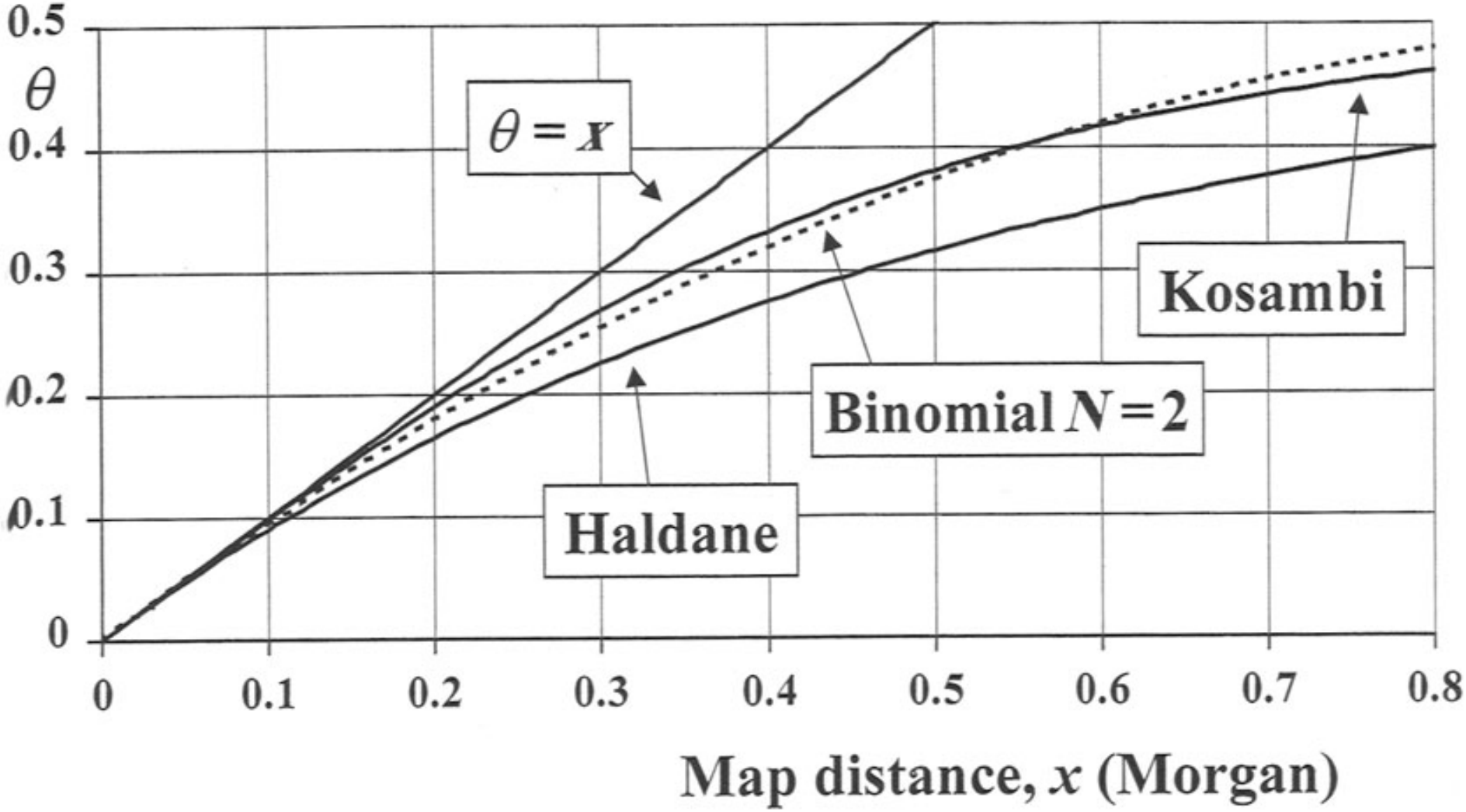



**Figure 1.4.** Graphs of several map functions.

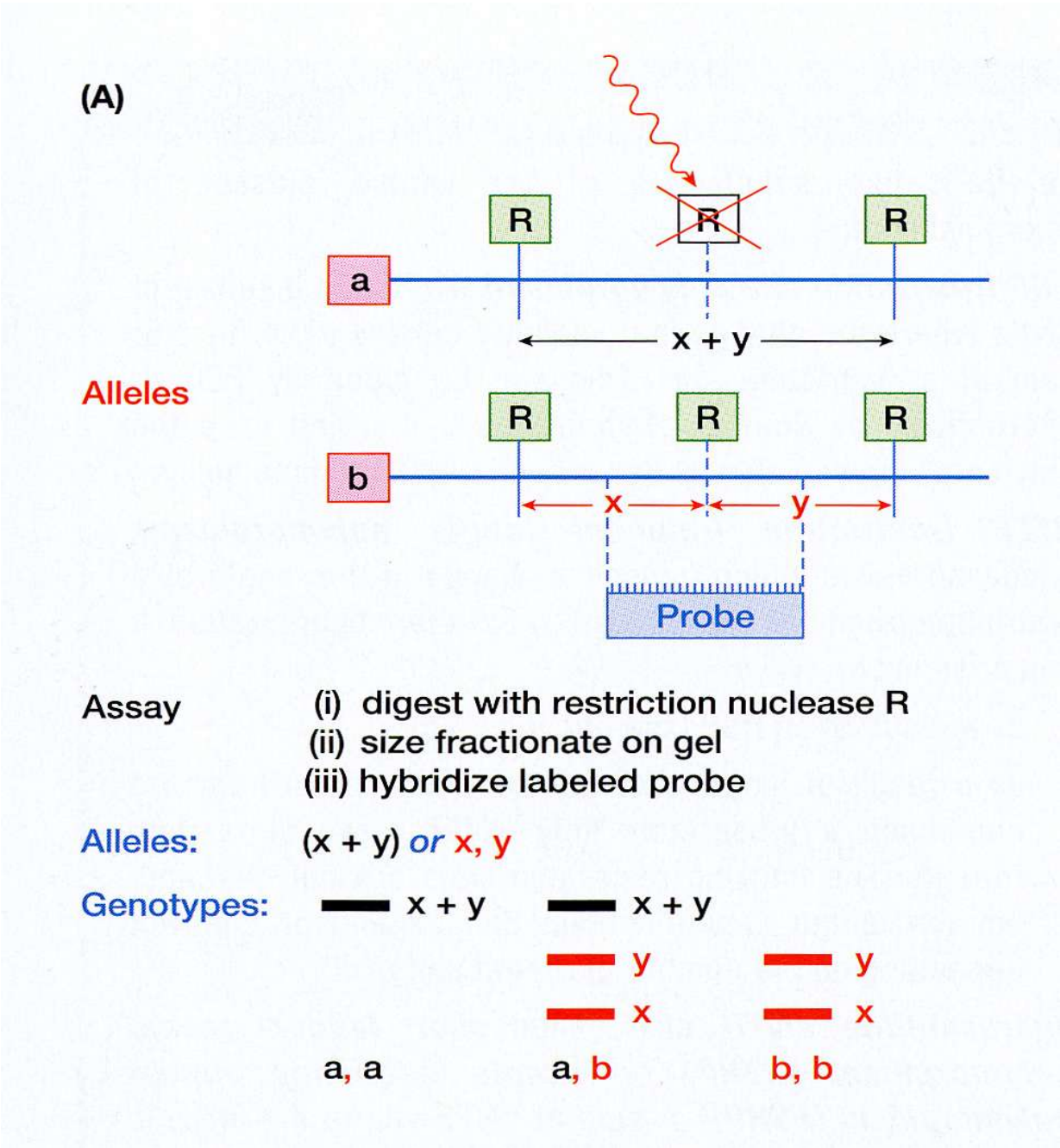
**Box 11.1**

## The development of human genetic markers

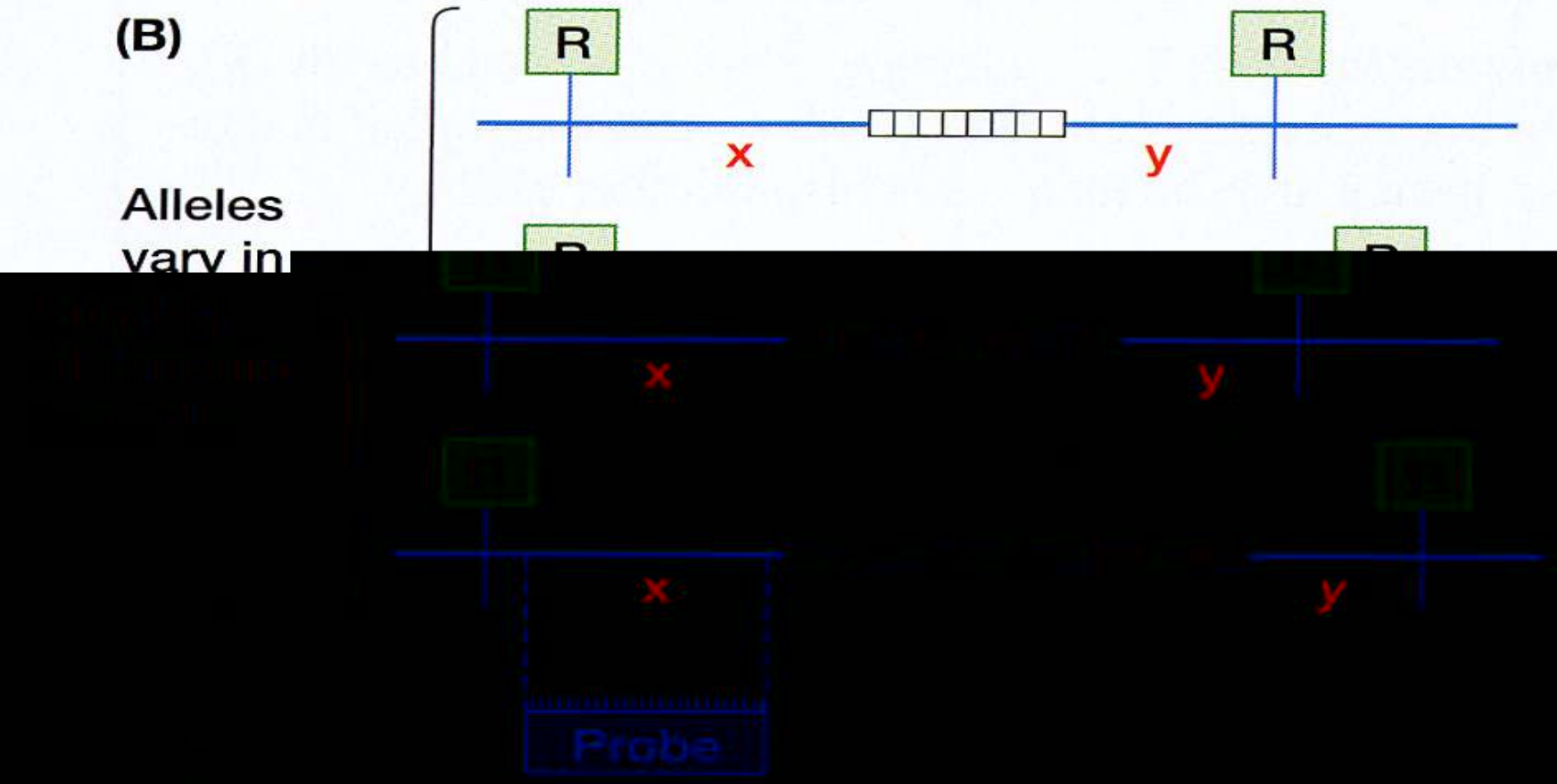
| Type of marker | No. of loci | Features |
|---|---|---|
| Blood groups<br>1910–1960 | ~20 | May need fresh blood, rare antisera<br>Genotype cannot always be inferred from phenotype because of dominance<br>No easy physical localization |
| Electrophoretic mobility variants of serum proteins<br>1960–1975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

VNTR, variable number of tandem repeats

# Genetic markers:Restriction fragment length polymorphisms (RFLPs)

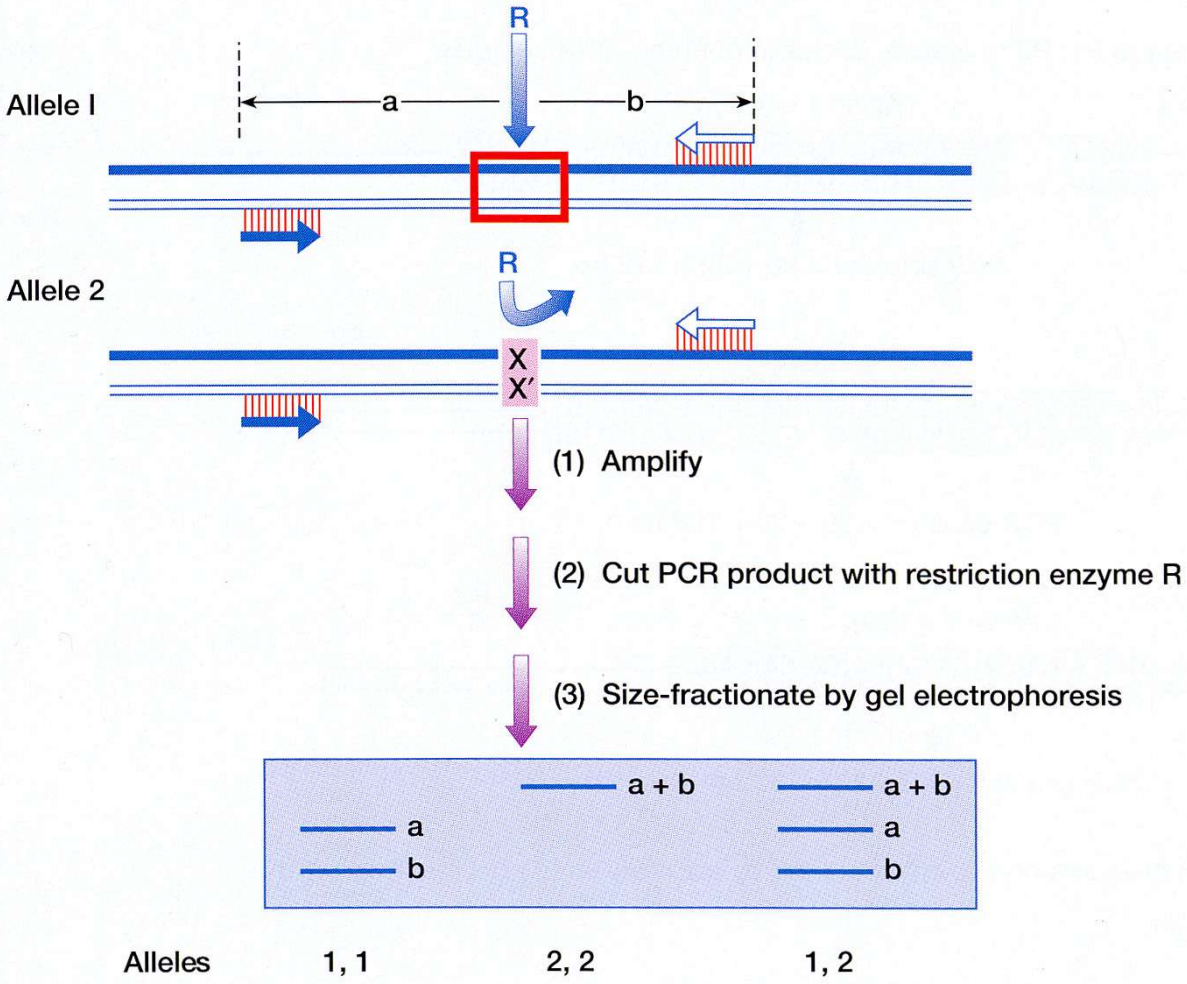


# Genetic markers: Variable Numbers of Tandem Repeats (VNTRs), e.g. ‚minisatellites‘ or ‚microsatellites‘

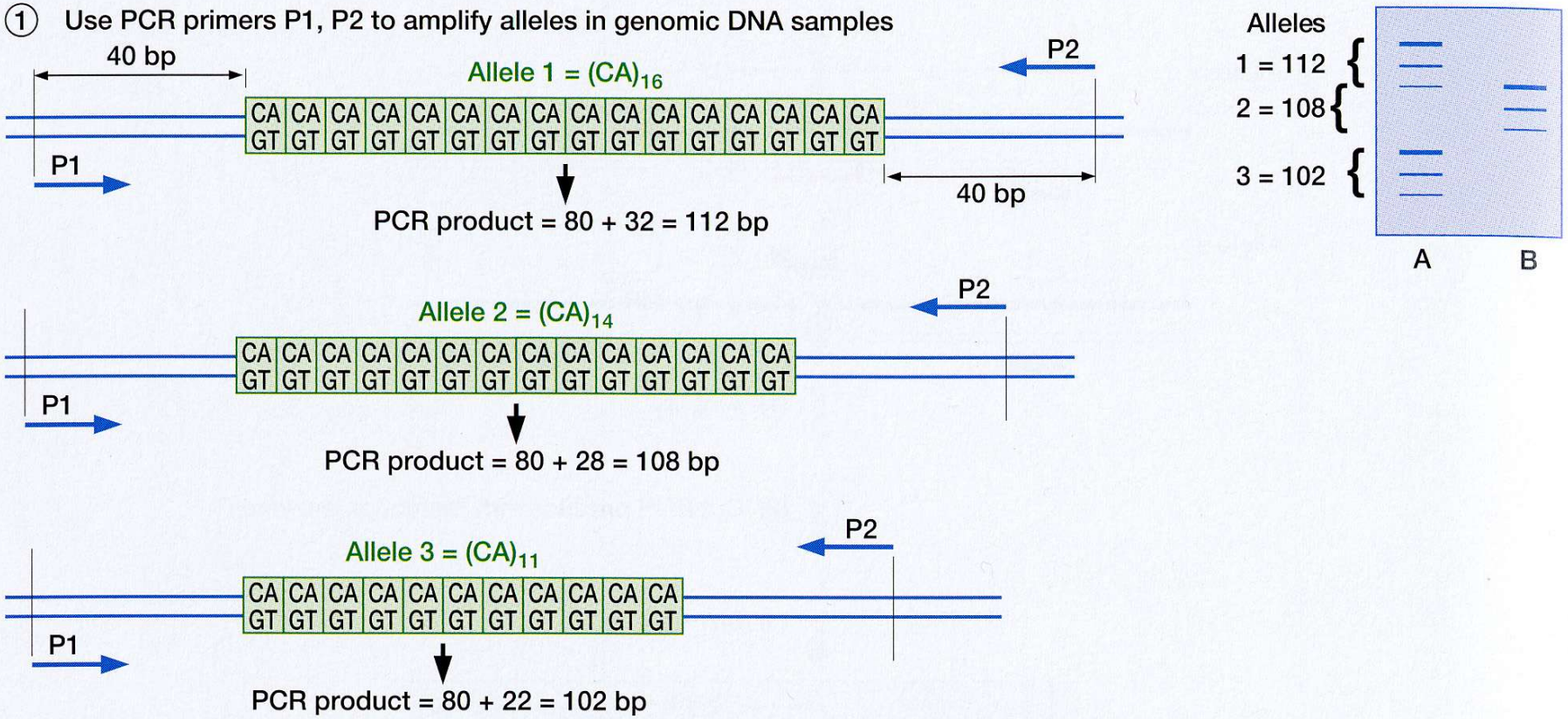



Allele sizes: x + y + (*n* × repeats) where *n* is variable

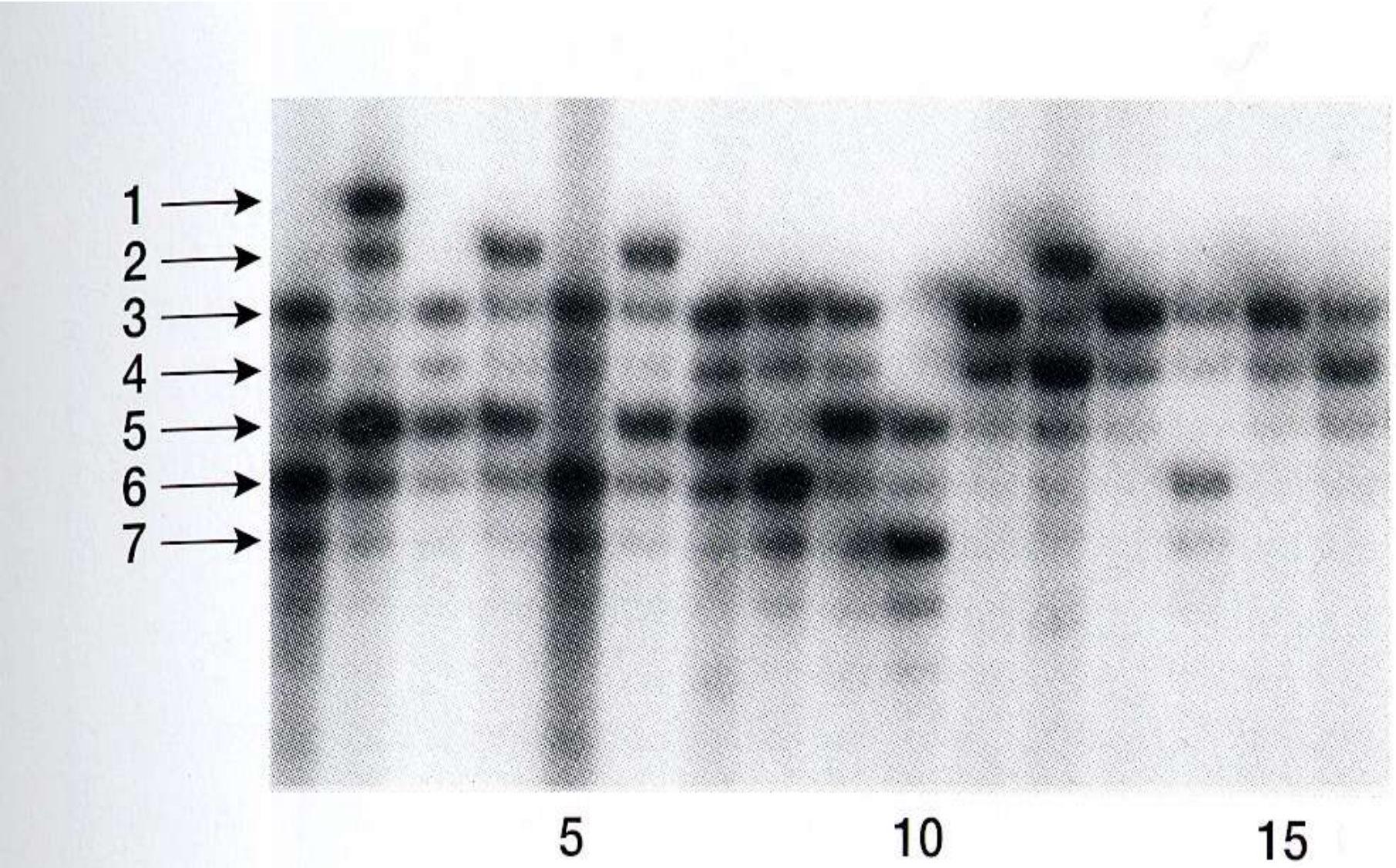
# Simplified RFLP typing by polymerase chain reaction (PCR)



# Genetic markers: di- to tetranucleotide repeats
## (e.g., CA repeats and other microsatellite markers)



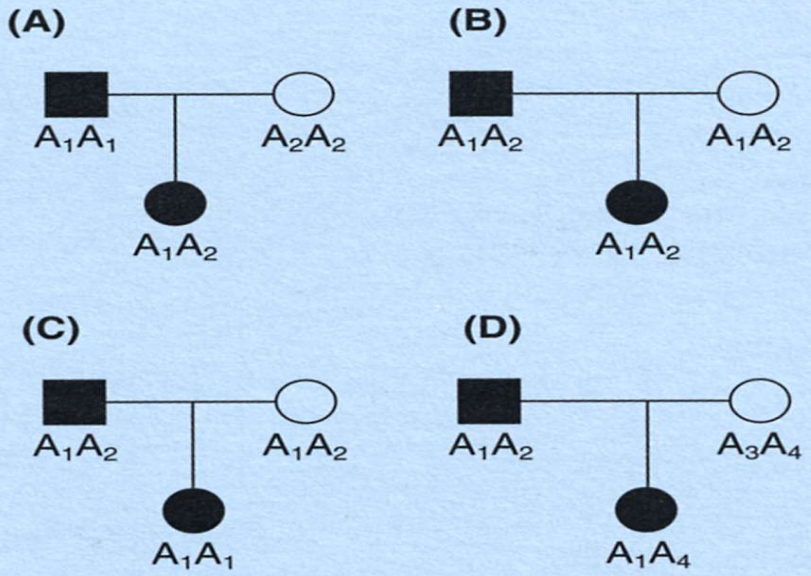
# Typing of microsatellite markers



**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 $A_1$.

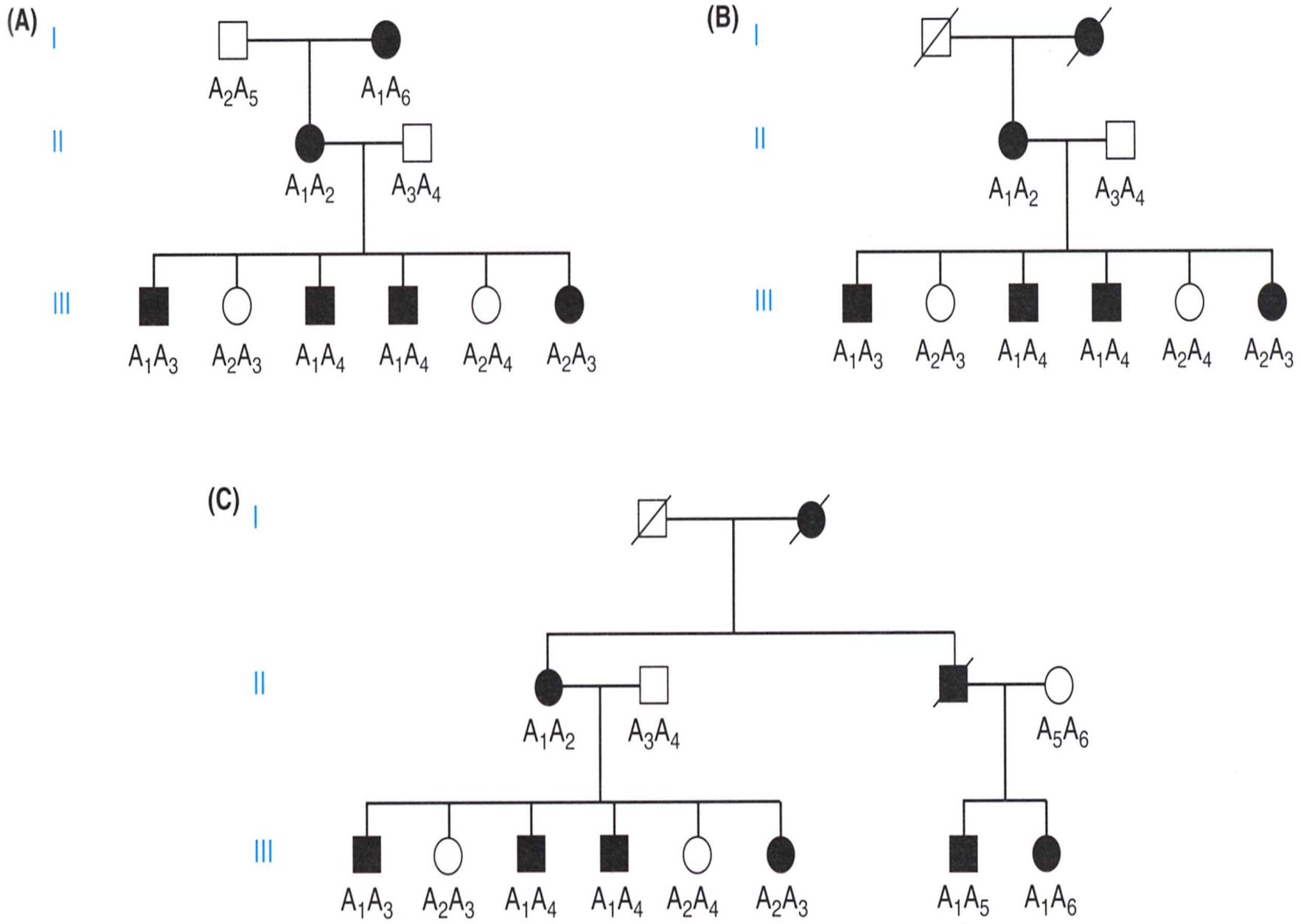



**(A)** This meiosis is uninformative: the marker alleles in the homozygous father cannot be distinguished.

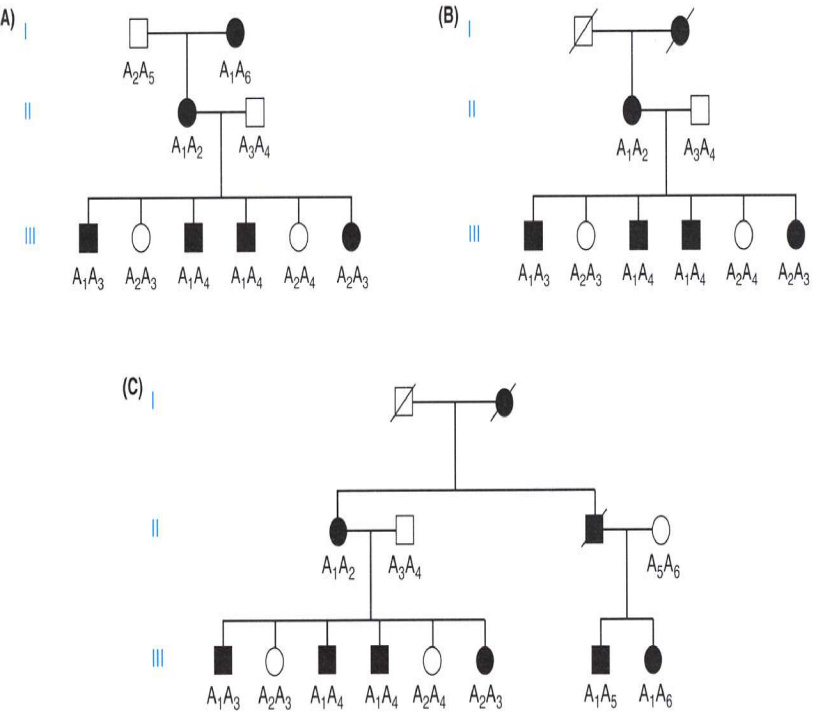
**(B)** This meiosis is uninformative: the child could have inherited $A_1$ from father and $A_2$ from mother, or vice versa.

**(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 $\theta$, the likelihood of a meiosis being non-recombinant is $1 - \theta$ and the likelihood of it being recombinant is $\theta$.
- 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.

| $\theta$ | 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.

| $\theta$ | 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.

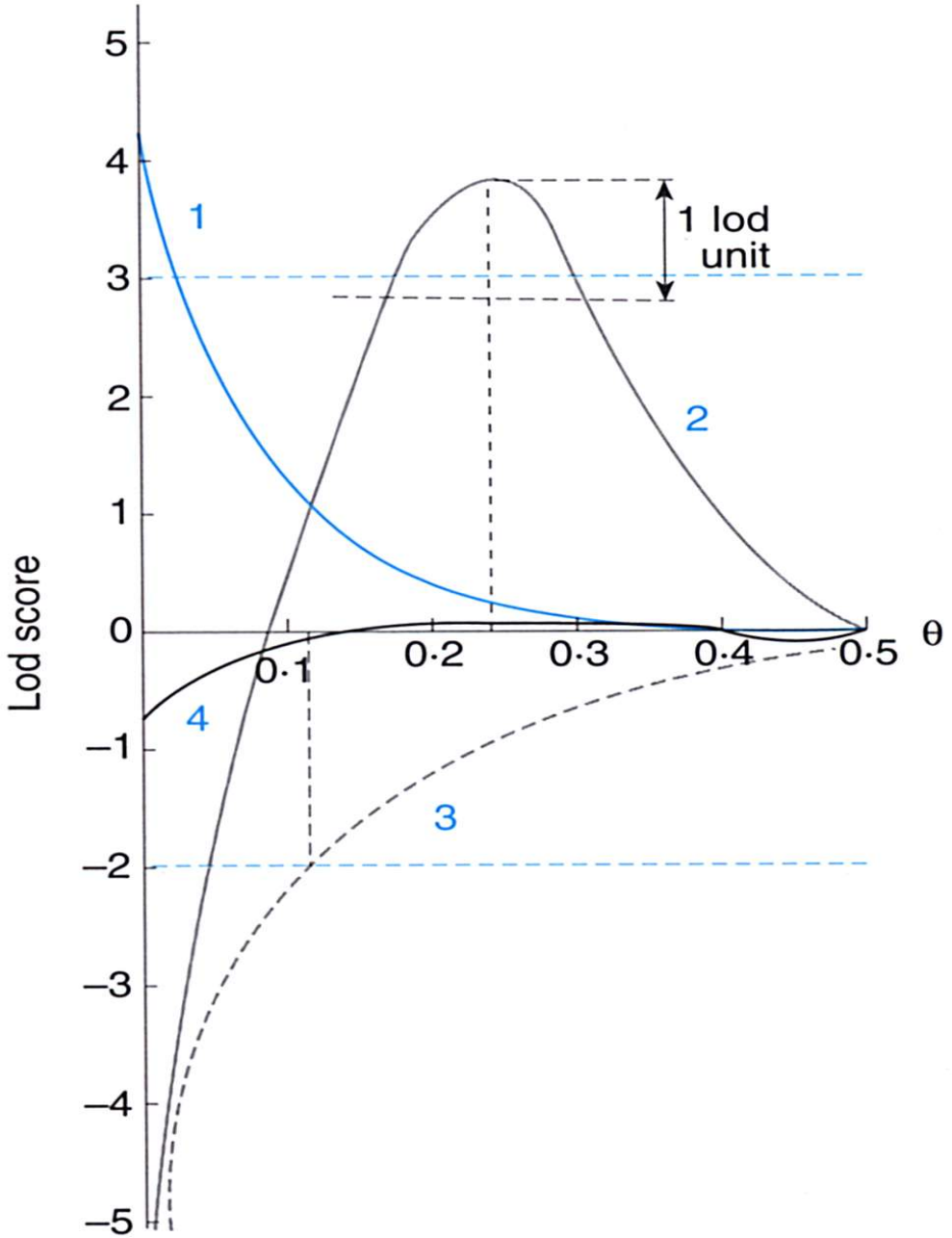



**Figure 11.5:** Lod score curves.

## 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 = $\theta$) | 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.

**Table 11.1** Gene ordering by three-point crosses

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

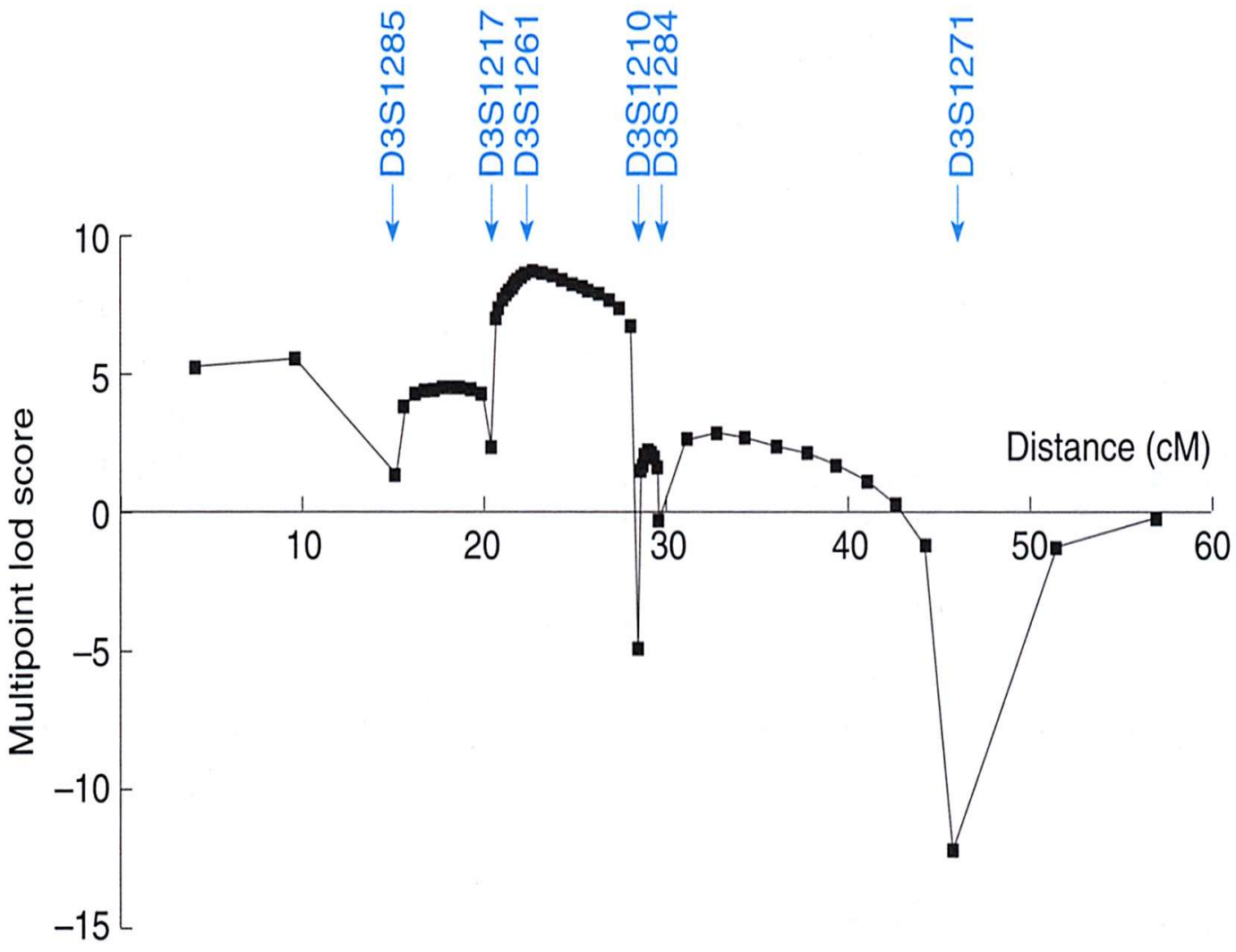



**Figure 11.6:** Multipoint mapping in man.

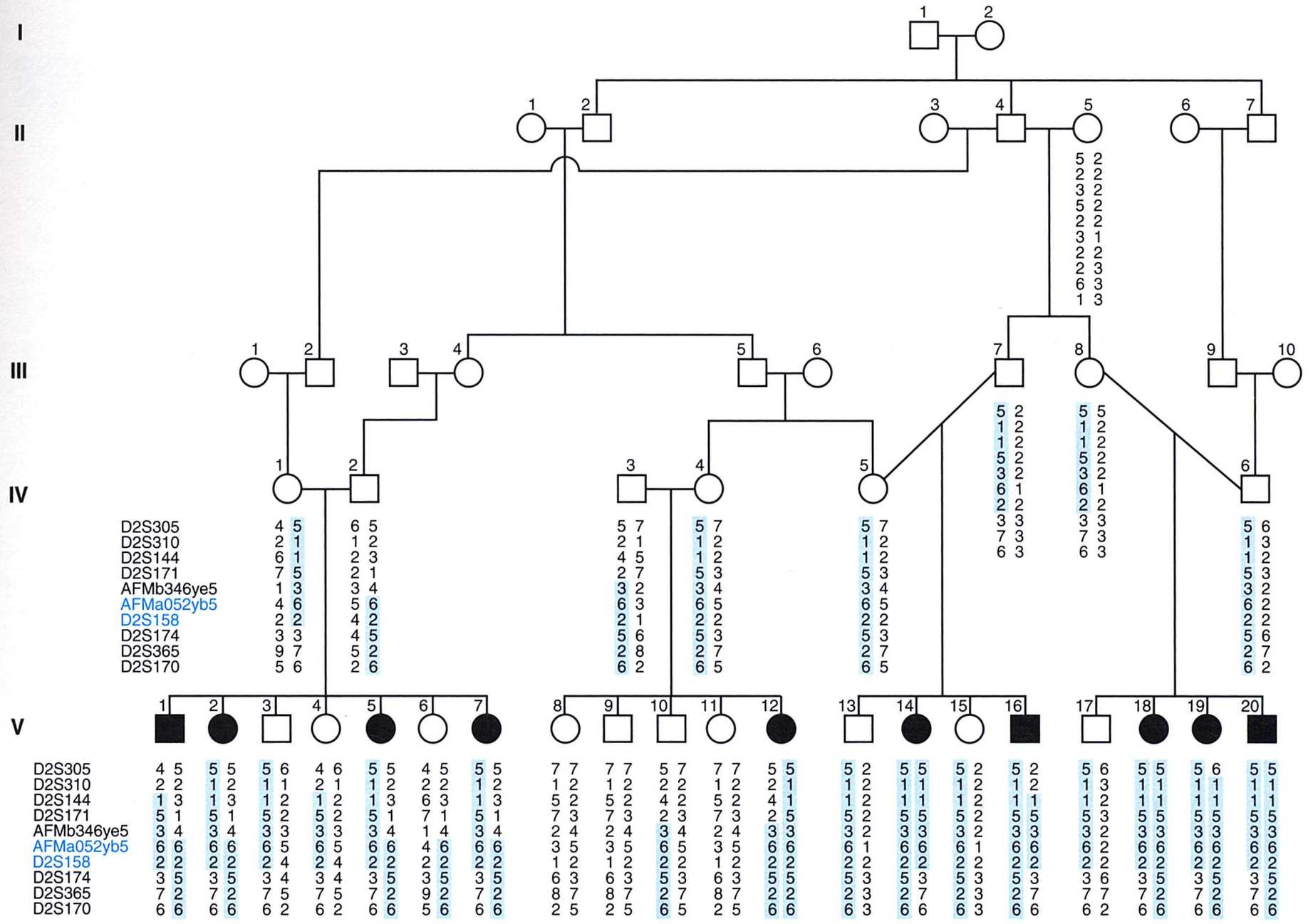
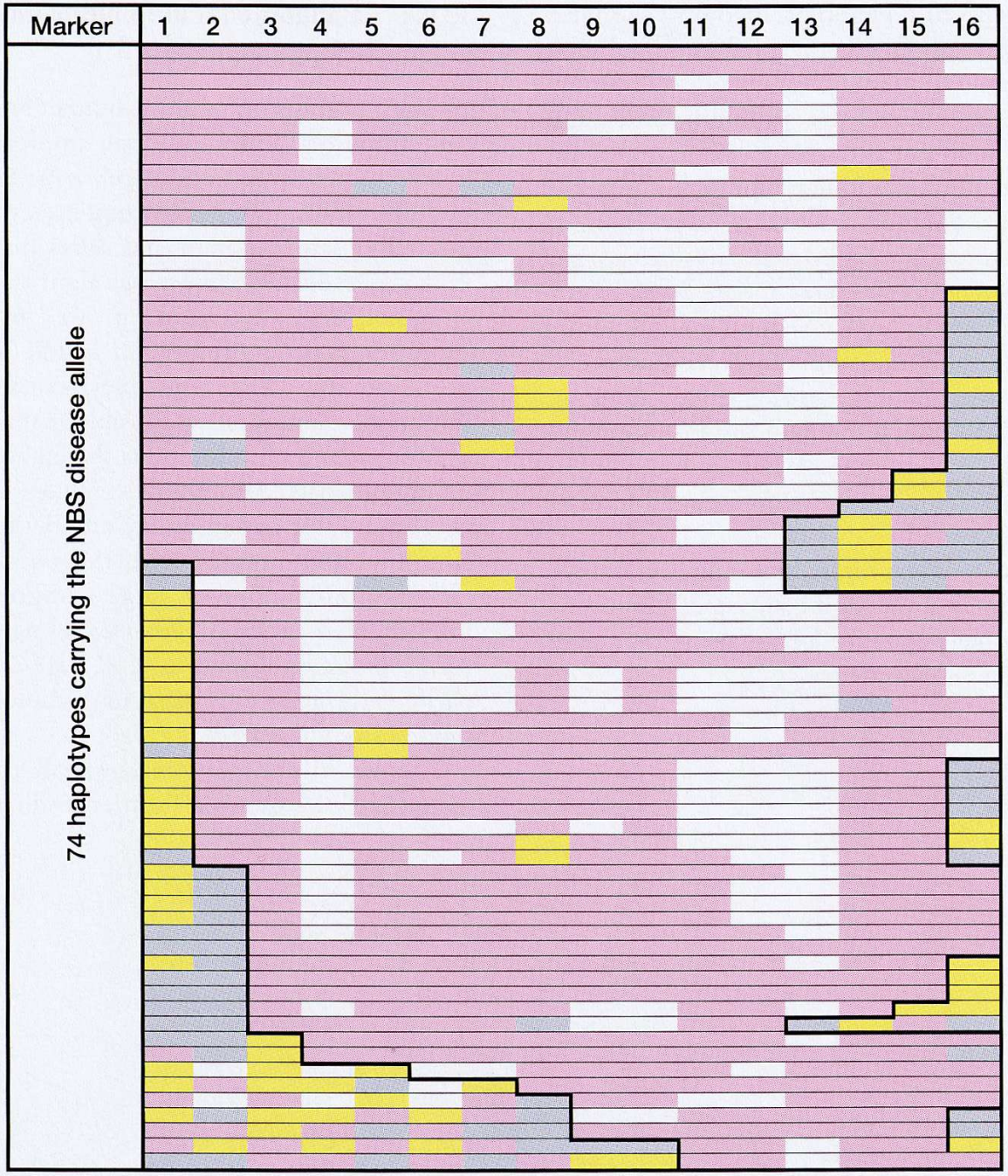



**Figure 11.8** Autozygosity mapping.


Marker
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
74 haplotypes carrying the NBS disease allele

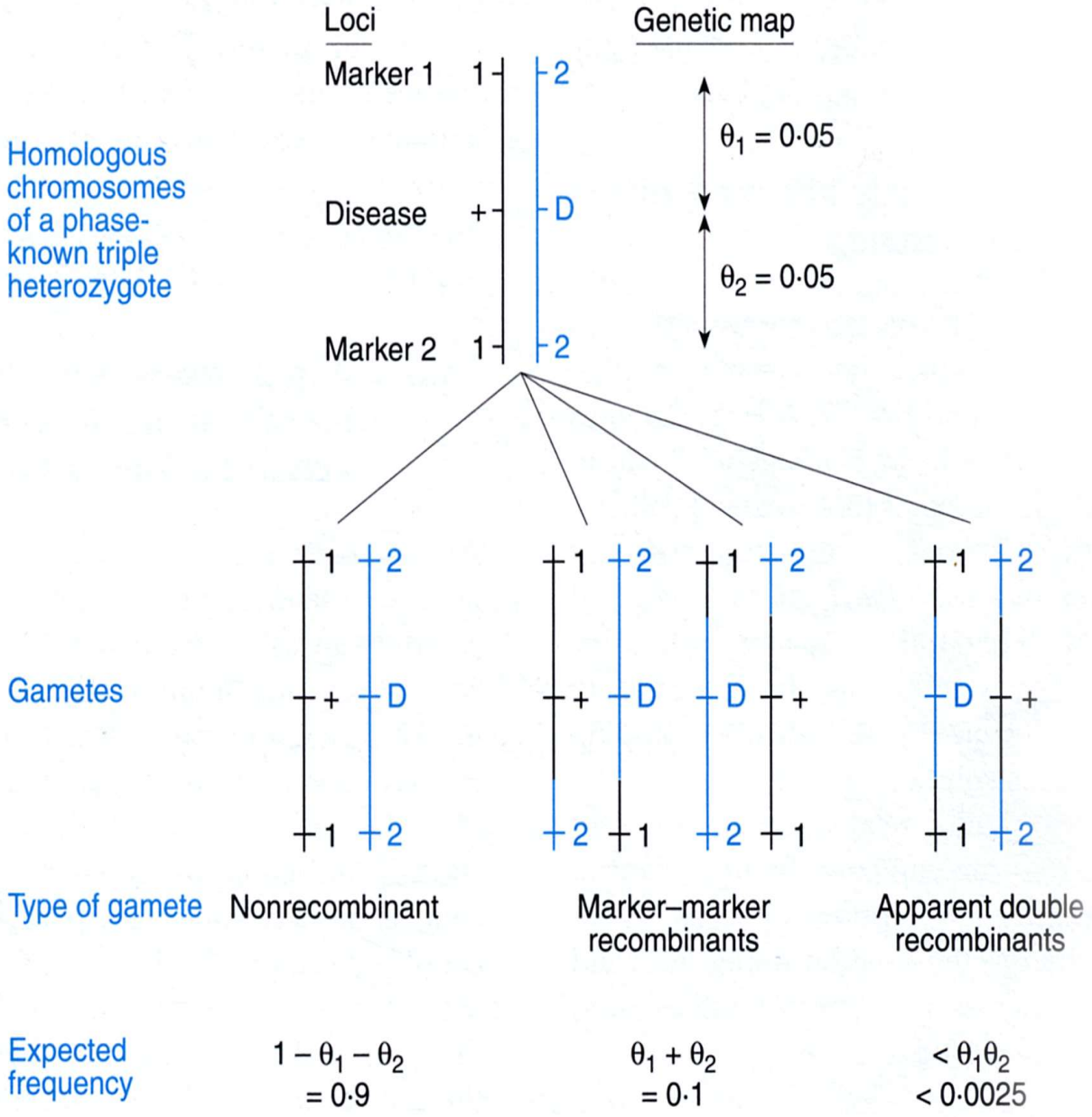



**Figure 11.7:** Apparent double recombinants suggest errors in the data.