

Lecture 9: Mapping a gene defined by the mutation

I. Classical mapping

II. Molecular mapping

III. Positional cloning

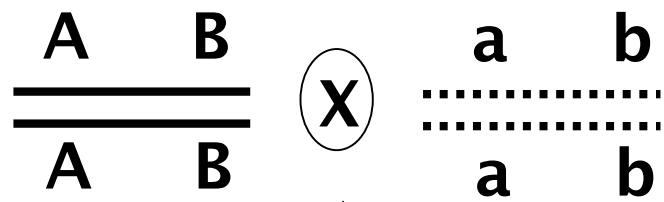
Read 387-398

Fig. 11.17; 11.19; 11.20; 11.21; 11.22

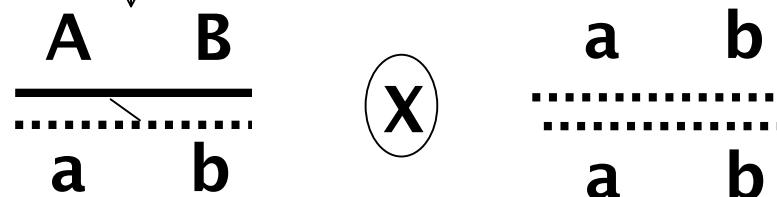
Self-reviewing of classical genetics

Read: 114-117; 123-127

Fig. 5.2; 5.3; 5.4; 5.5; 5.10; 5.12



F1:



F2:

A B/a b	<hr/>	90
a b/a b	<hr/>	90
A b/a b	<hr/>	10
a B/a b	<hr/>	10

$$\frac{\text{Ab} + \text{aB}}{\text{total}} = \text{rec, freq.}$$

$$\frac{10 + 10}{200} = 0.1 = 10\%$$

P

Fig. 5.2

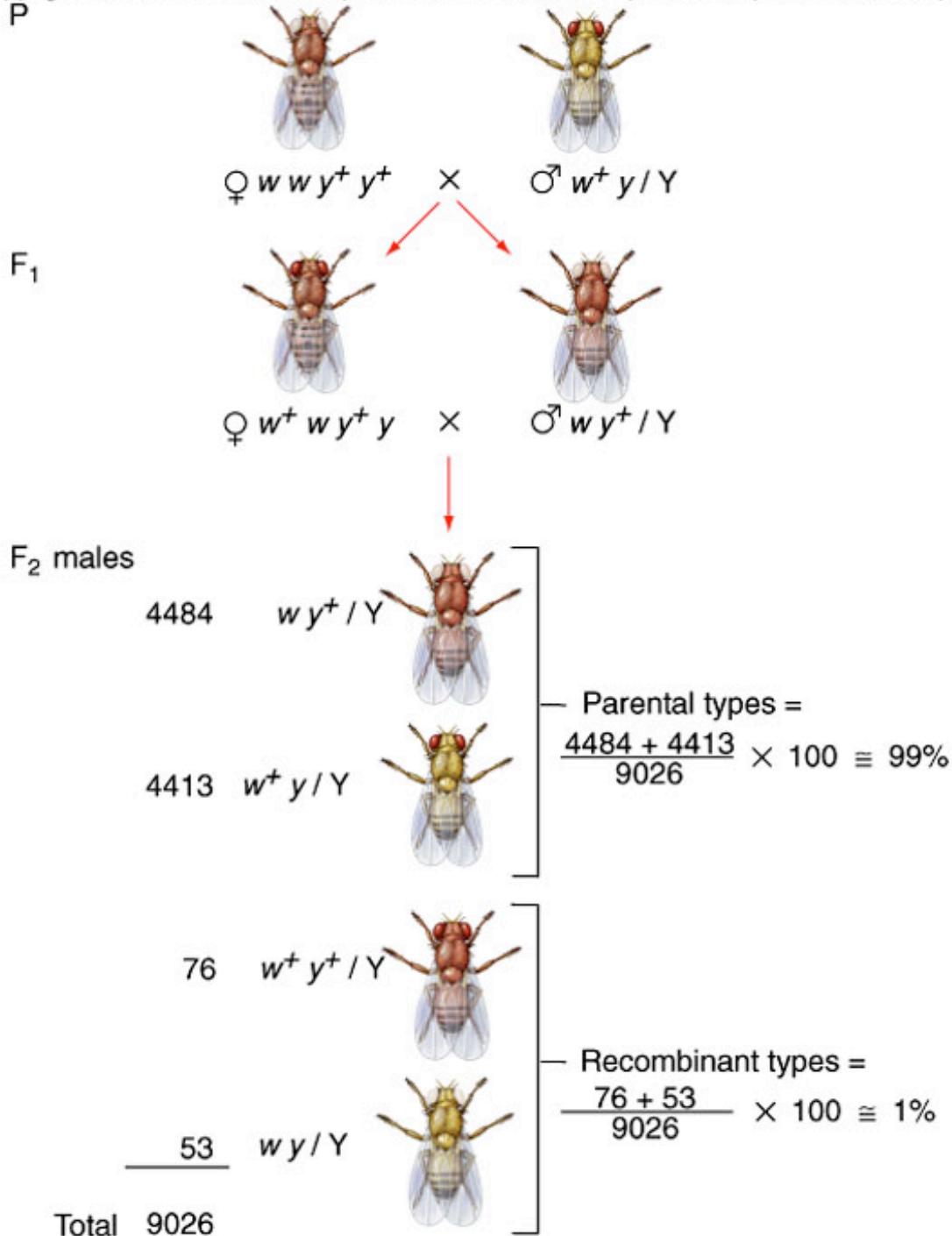


Fig. 5.4

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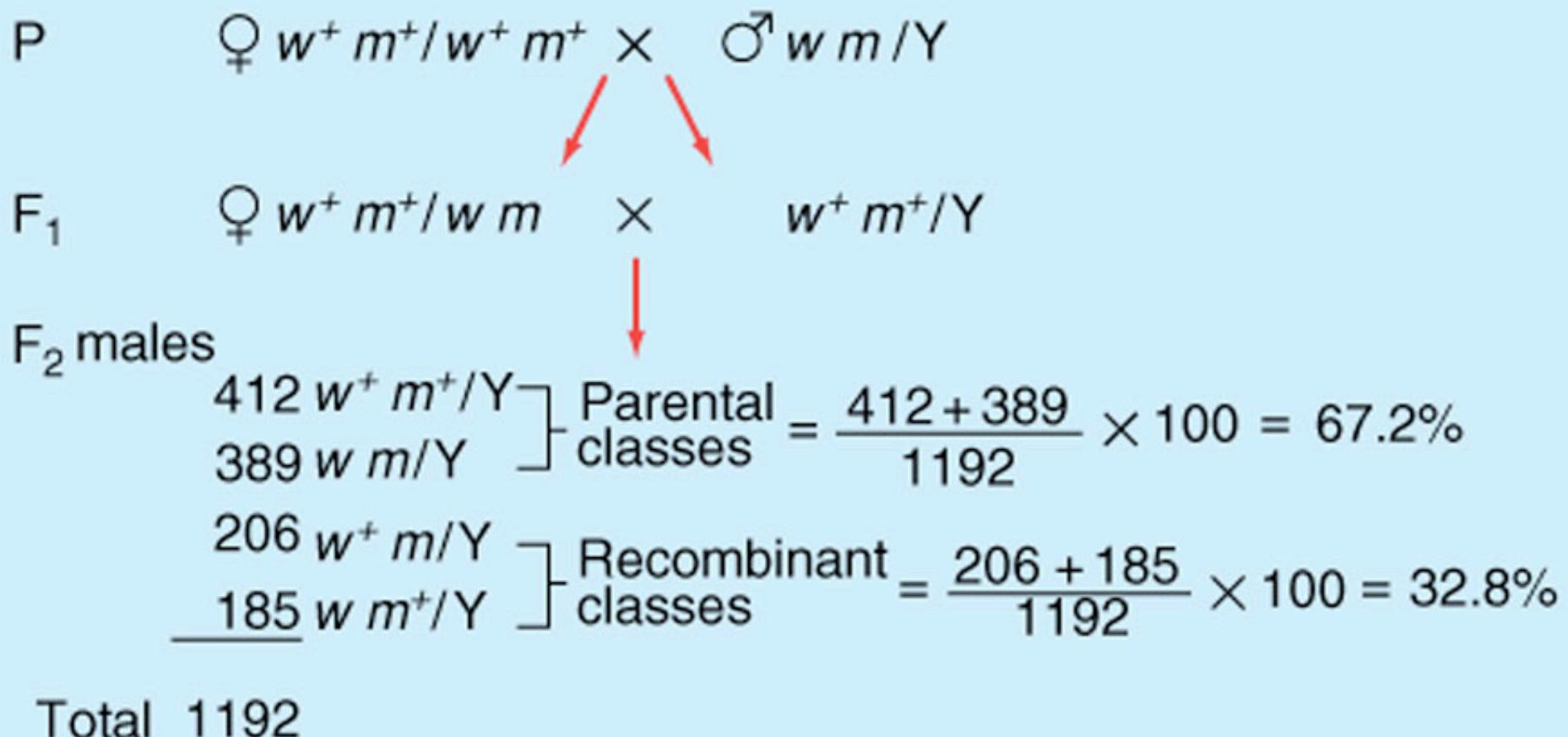


Fig. 5.5

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 $\text{♀ } b c^+ / b c^+ \times \text{♂ } b^+ c / b^+ c$  F_1 (all identical) $b c^+ / b^+ c$ 

Testcross

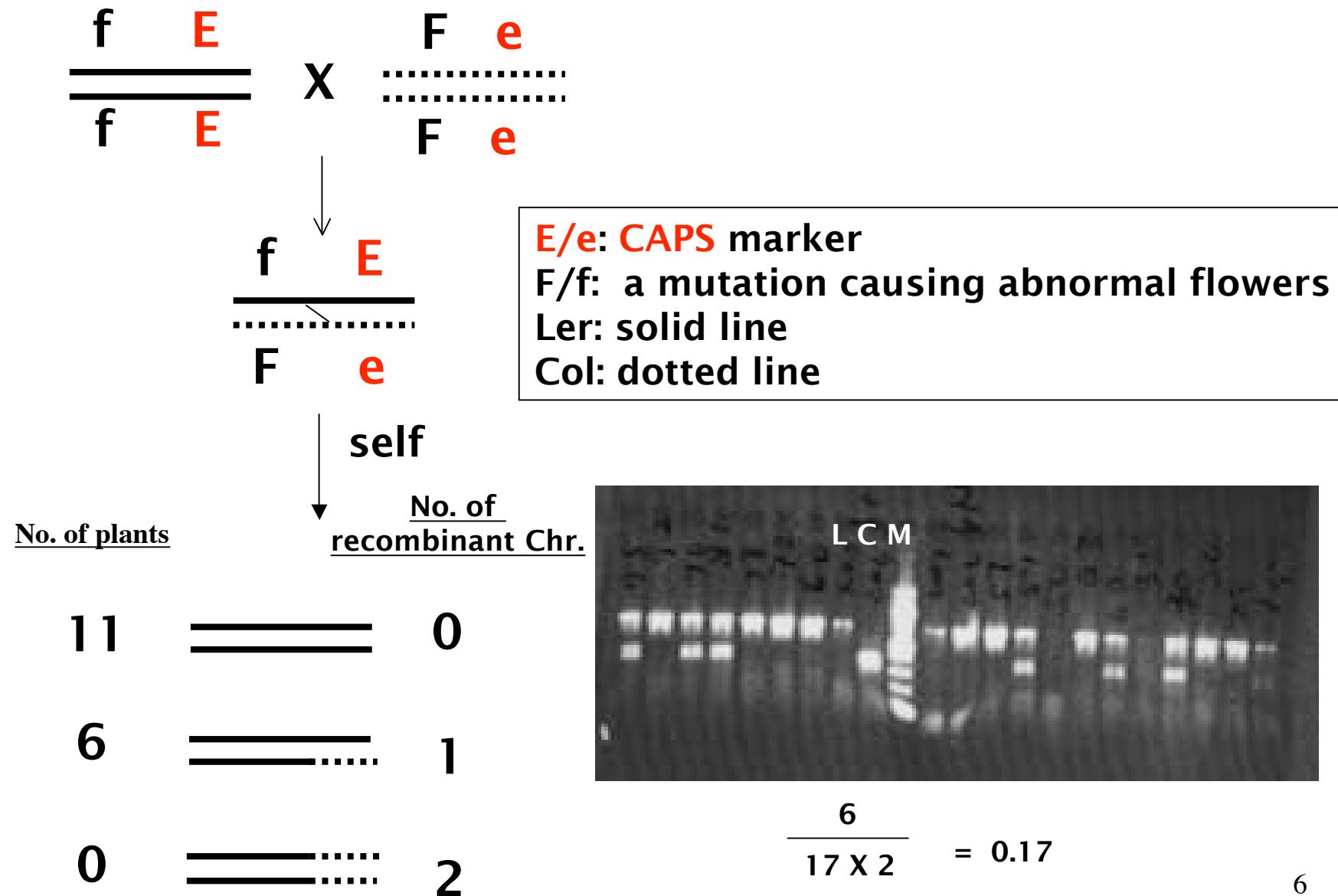
 $\text{♀ } b c^+ / b^+ c \times \text{♂ } b c / b c$ 

Testcross progeny $2934 \ b \ c^+ / b \ c$ $2768 \ b^+ \ c / b \ c$ Parental classes = $\frac{2934 + 2768}{7419} \times 100 = 77\%$

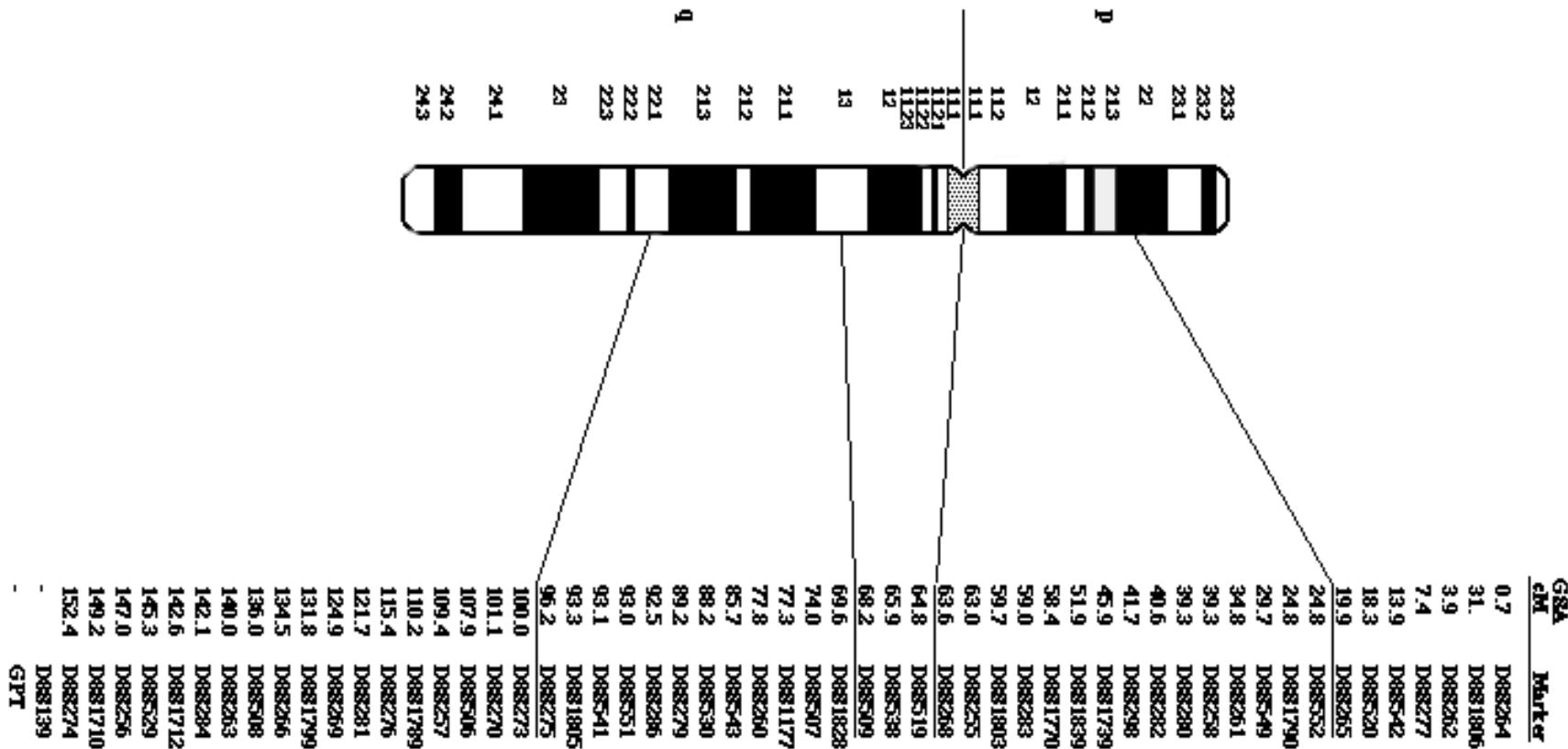
$871 \ b \ c / b \ c$ $846 \ b^+ \ c^+ / b \ c$ Recombinant classes = $\frac{871 + 846}{7419} \times 100 = 23\%$

Total 7419

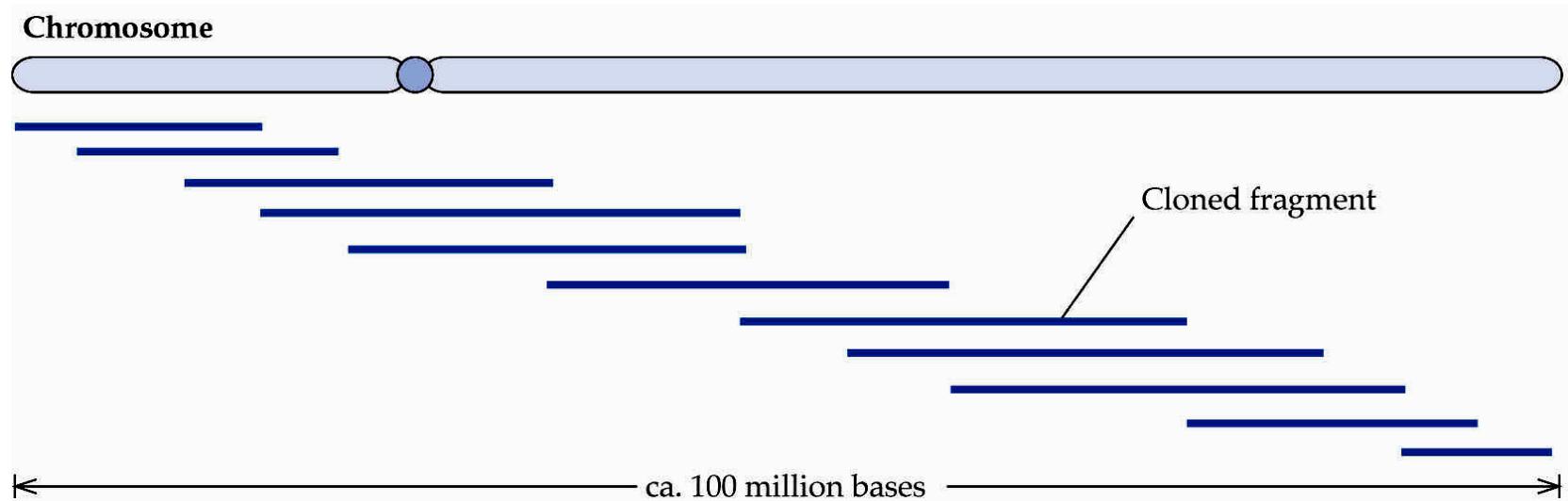
Mapping with a CAPS marker



A genetic map of DNA markers

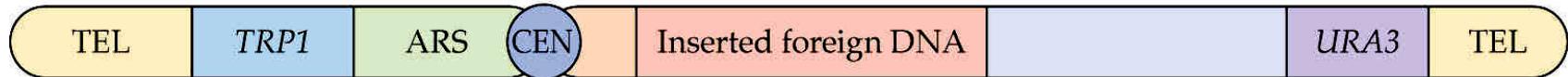


Physical map



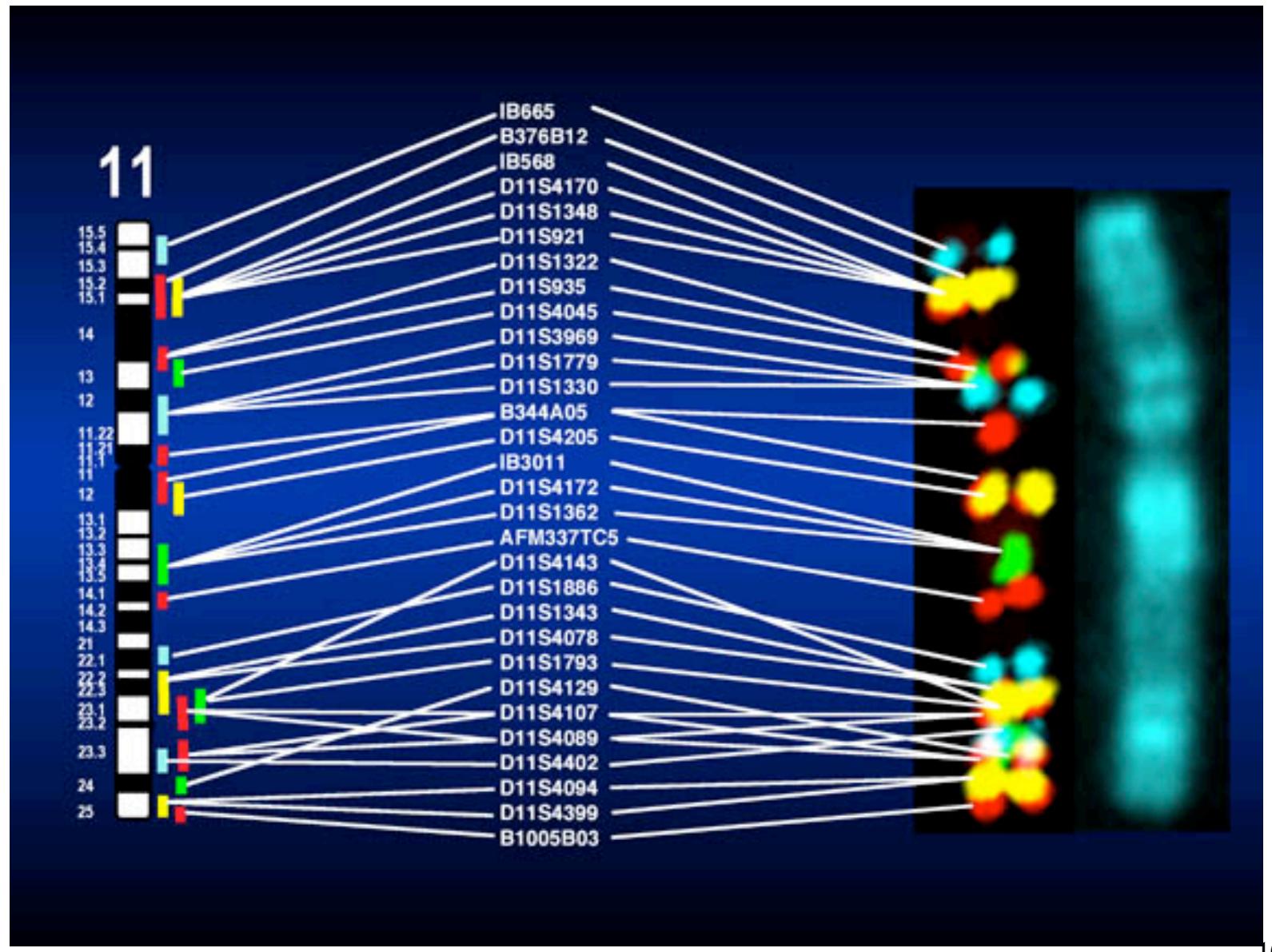
In Arabidopsis: 1 cM = about 200 kb (50 genes)
In human 1cM = 1000 kb (~17 genes)

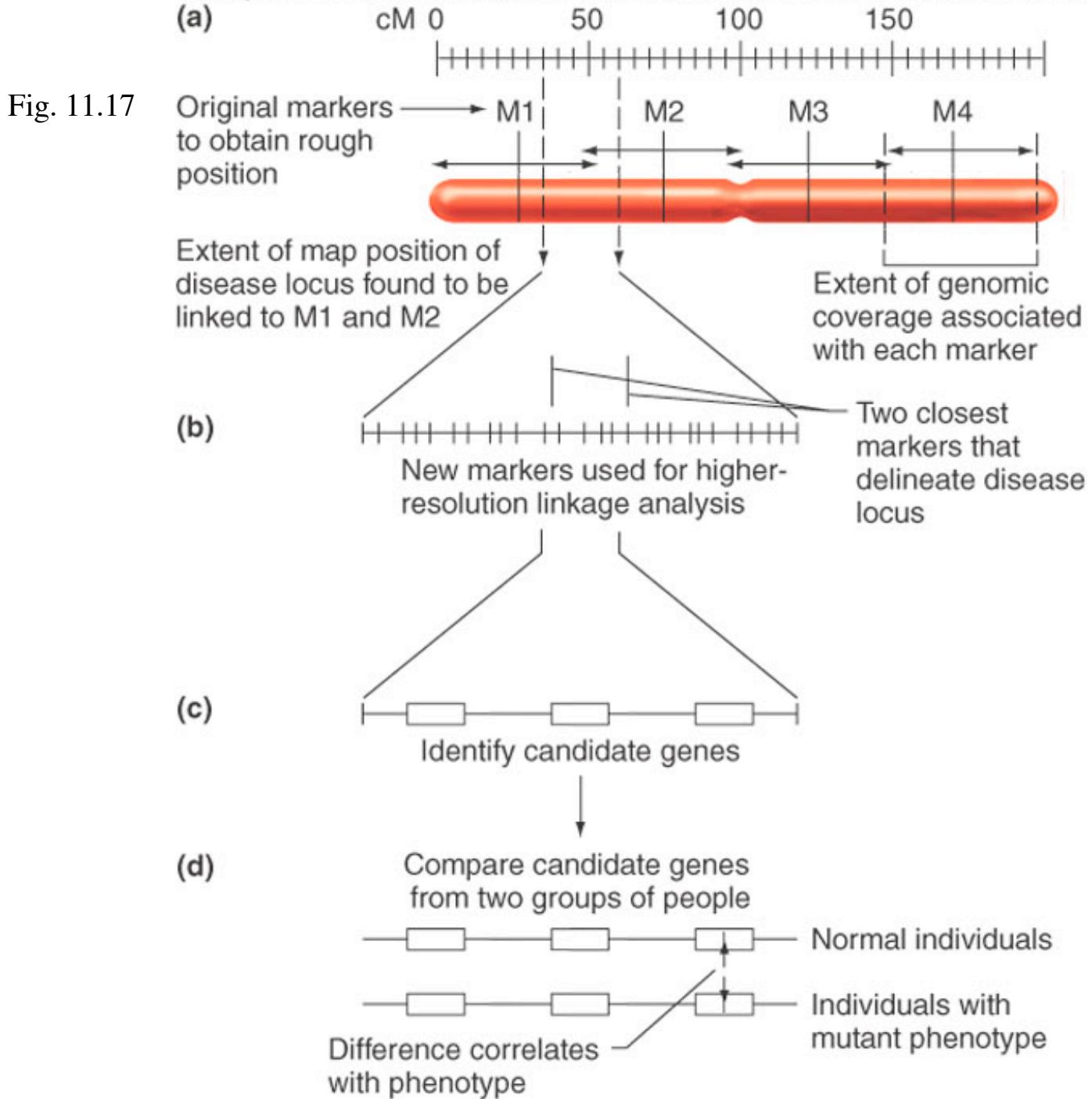
Yeast Artificial Chromosomes YAC



<u>Vector</u>	<u>insert size</u>	<u>Host</u>
YAC:	100-1000kb	yeast
BAC:	80-300kb	bacterium
Cosmid:	20-50 kb	bacterium
Lamda:	10-20kb	bacterium
Plasmid:	0.2-15kb	bacterium

A PHYSICAL MAP OF HUMAN CHROMOSOME 11





Steps in identifying the gene in a chromosome region

Step1: Categorize genes in the region

Step2: Determine mRNA expression pattern

EST

Northern hybridization

Step3: Determine changes between wt and mut

Expression pattern

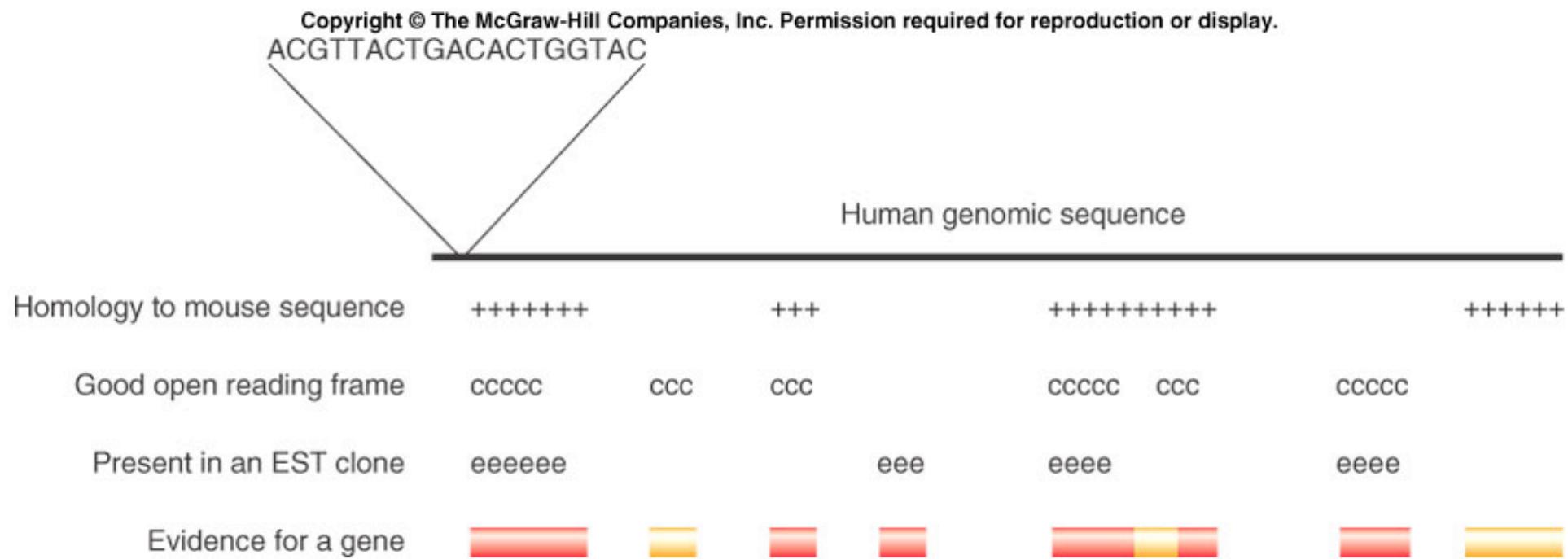
DNA sequence

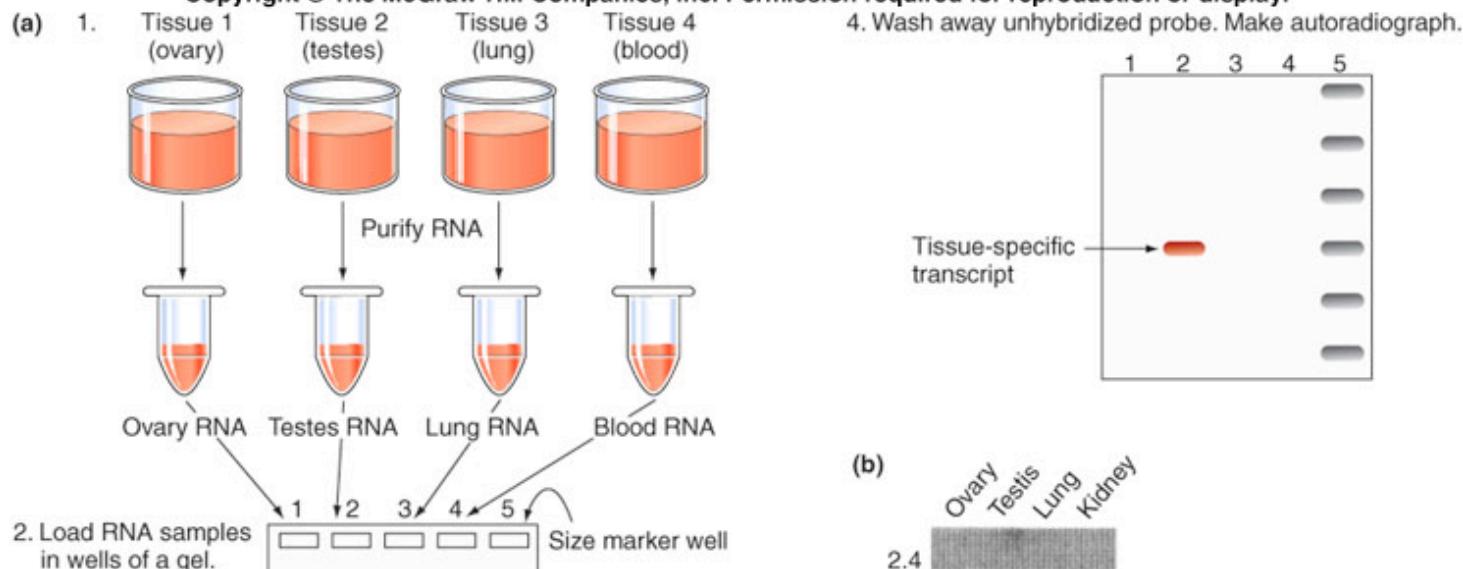
Step4: Transgenic studies (SRY example)

Case study: Cystic fibrosis

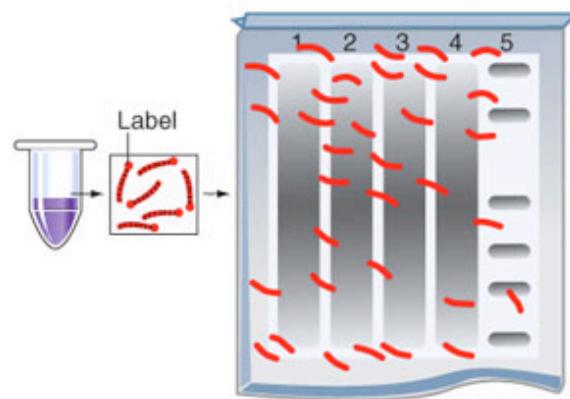
CFTR (Cystic fibrosis transmembrane conductance regulator)

Fig. 11.19

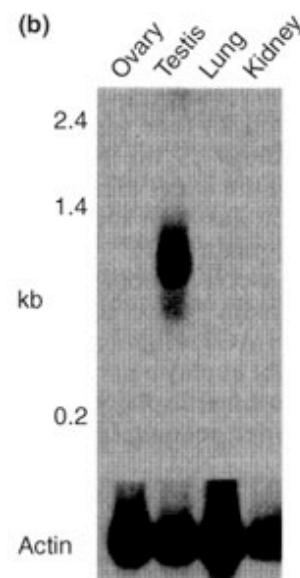
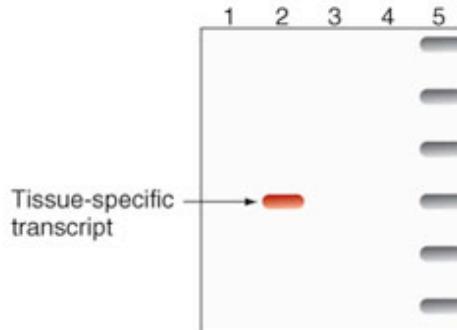




3. Separate RNA samples by gel electrophoresis.
Blot onto filter. Expose filter to labeled hybridization probe.



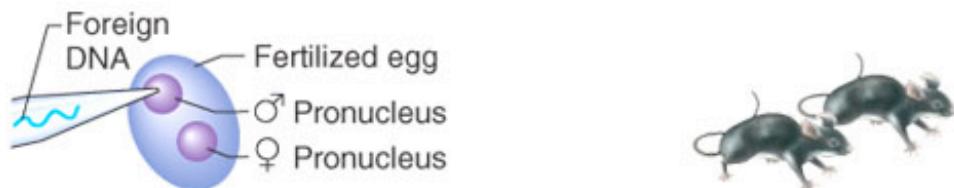
4. Wash away unhybridized probe. Make autoradiograph.



Testes-determining factor (TDF)

Fig. 11.20

Fig. 11.21

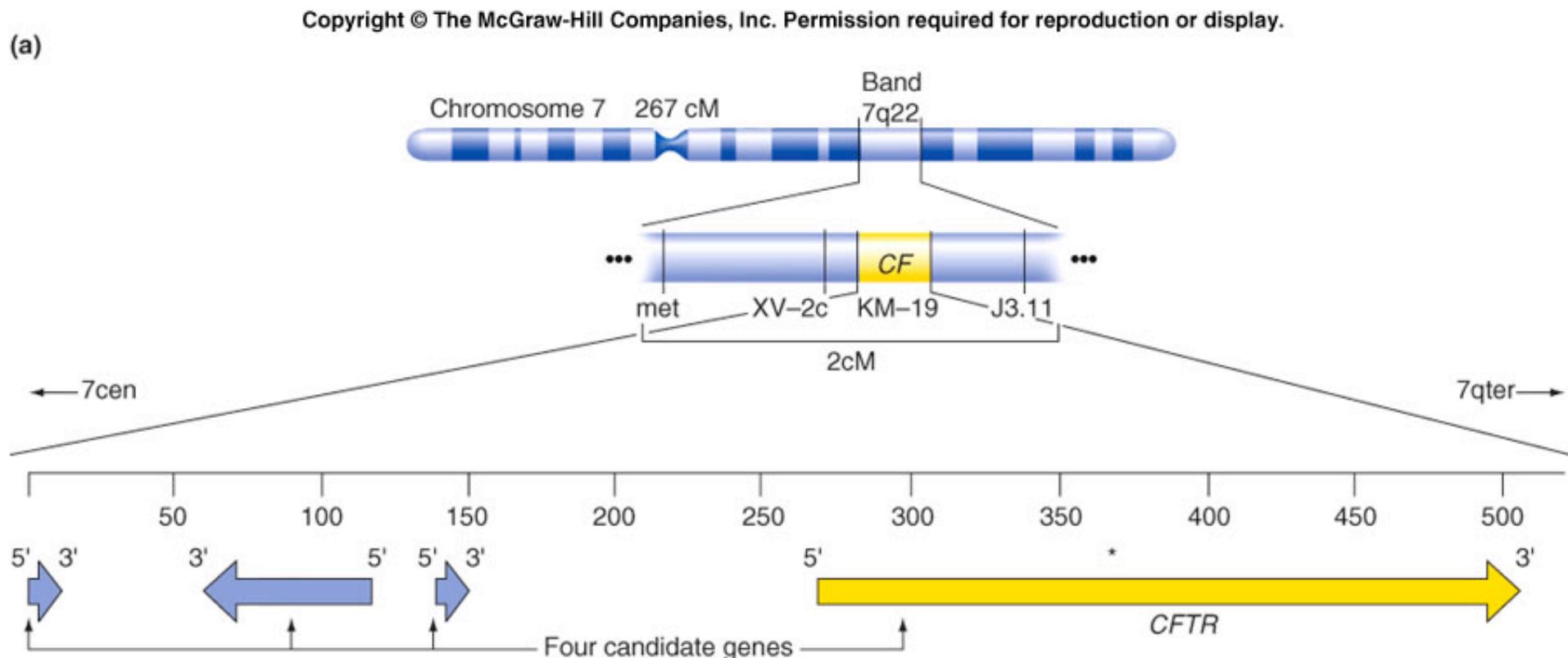


Foreign DNA injected into male pronucleus of newly fertilized egg. → Injected eggs surgically implanted into uterus of "foster" mother and allowed to develop. → Mice are born with foreign DNA in every cell nucleus.



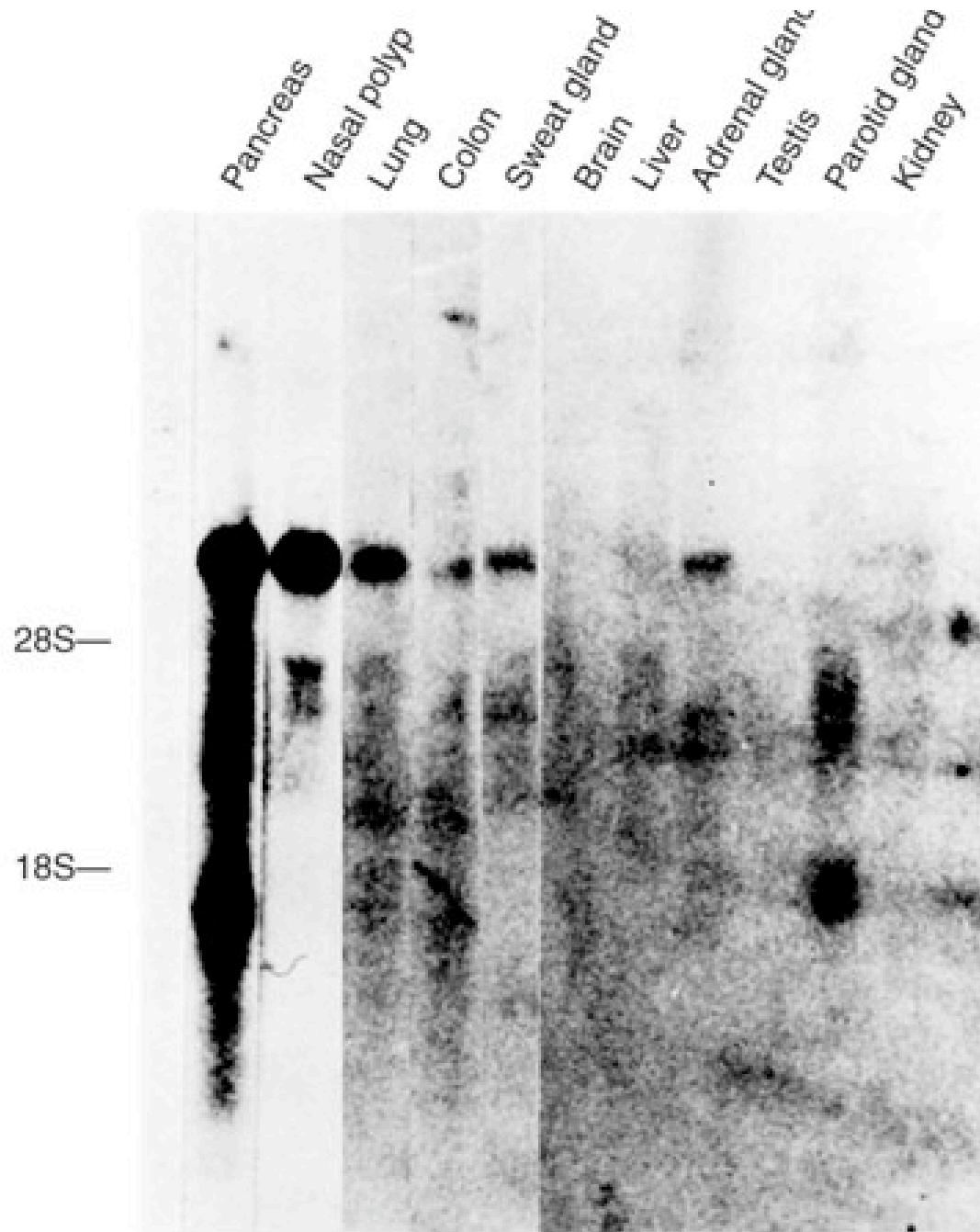
Cloning cystic fibrosis transmembrane conductance regulator (CFTR)

Fig. 11.22a



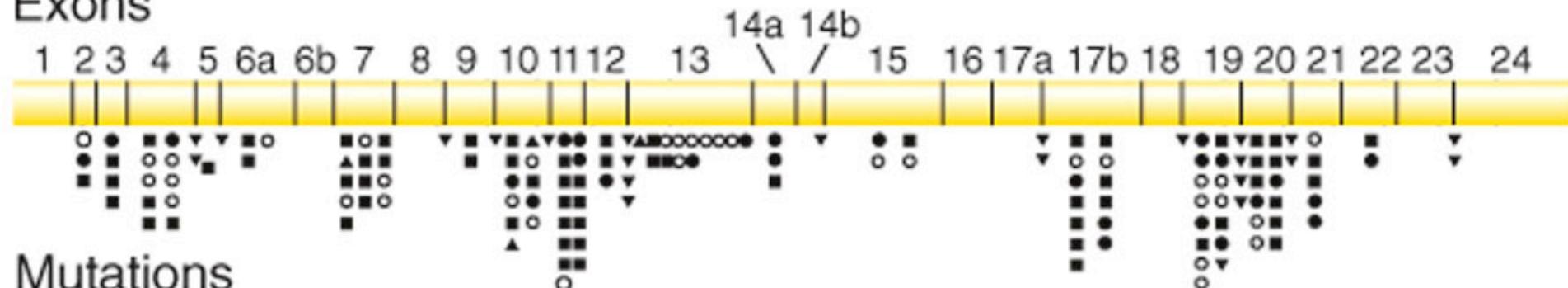
Northern blots help eliminate other 3 candidate genes

Fig. 11.22b



(c)

Exons



Mutations

(d)

Corresponding wild-type polypeptide structure

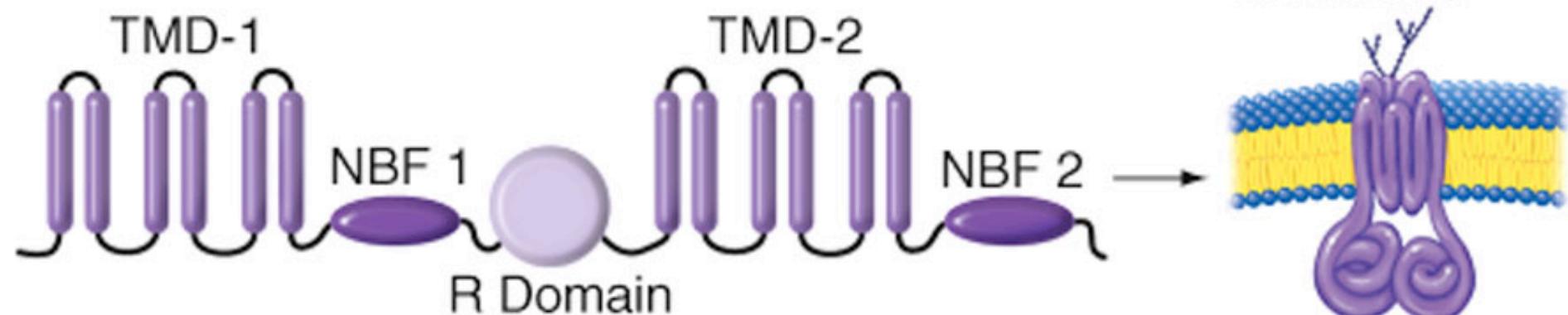


Fig. 11.22cd

CFTR

