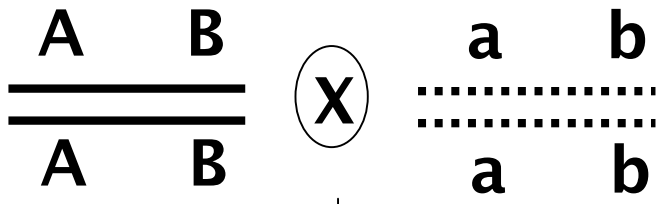
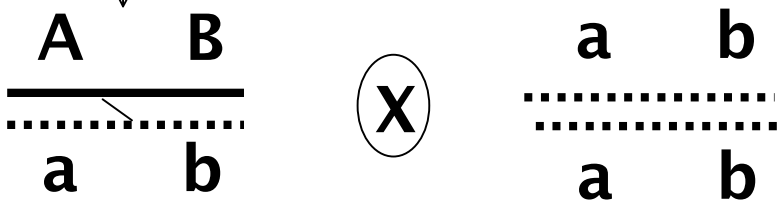
Lecture 9: Mapping a gene defined by the mutation

I. Classical mapping

II. Molecular mapping

III. Positional cloning





F2:

A B/a b		90
a b/a b		90
A b/a b		10
a B/a b		10

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

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

Fig. 5.4

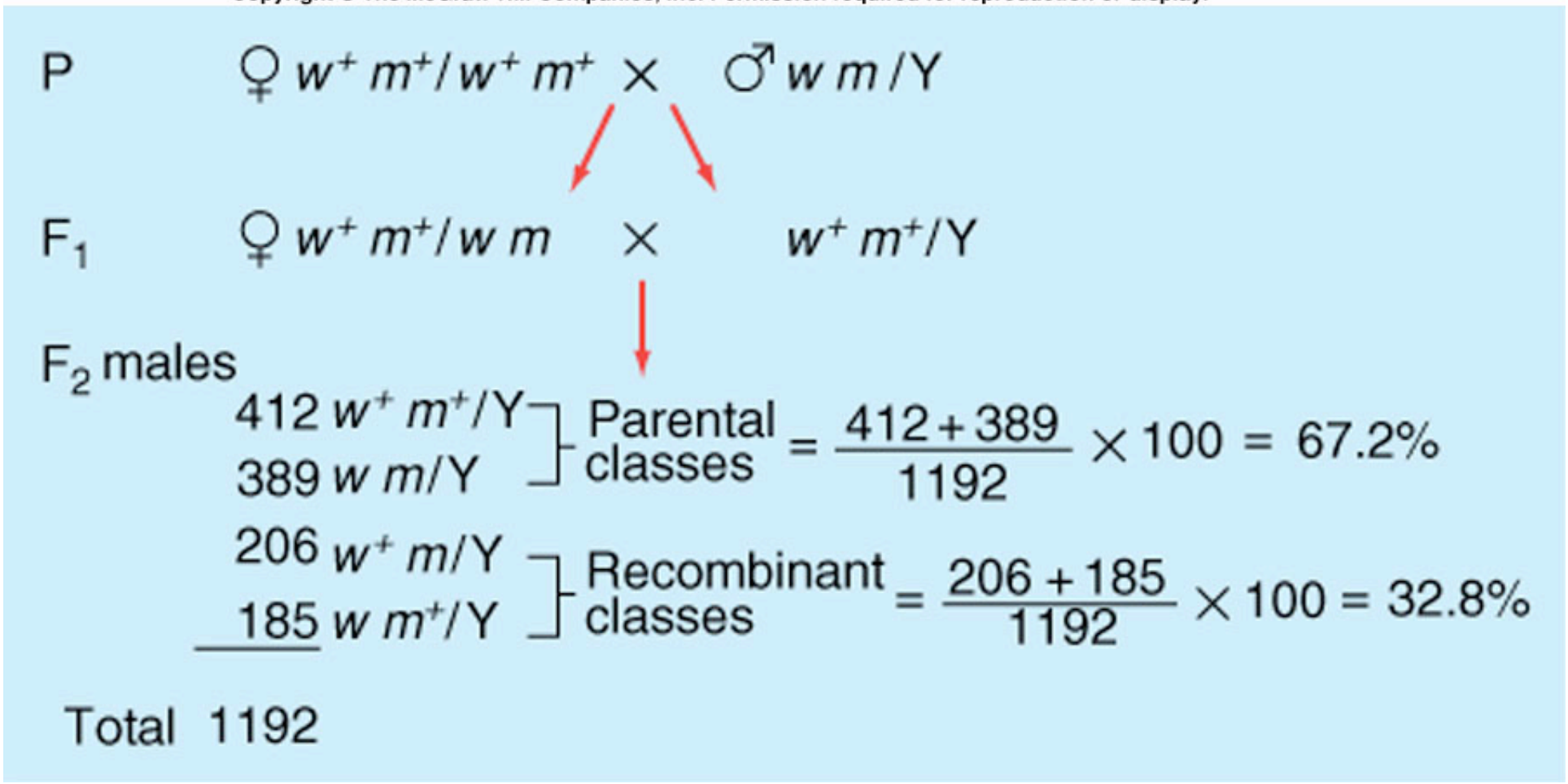
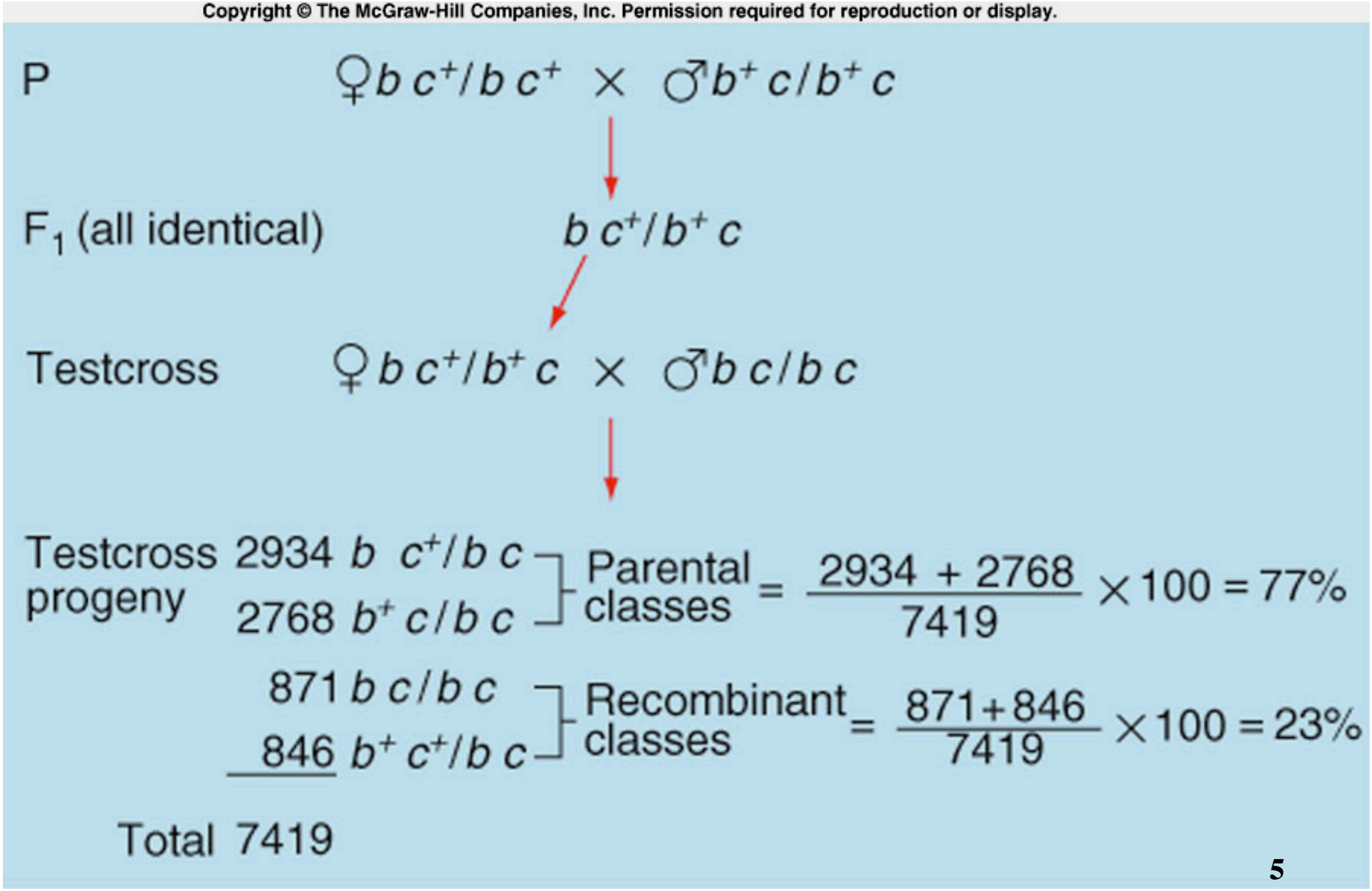
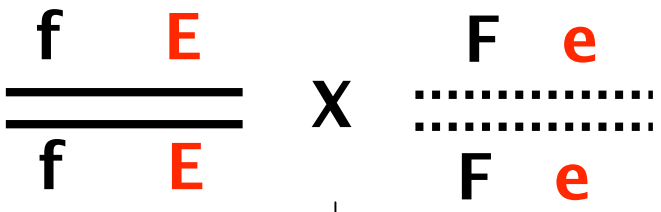
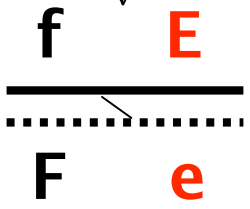


Fig. 5.5



Mapping with a CAPS marker

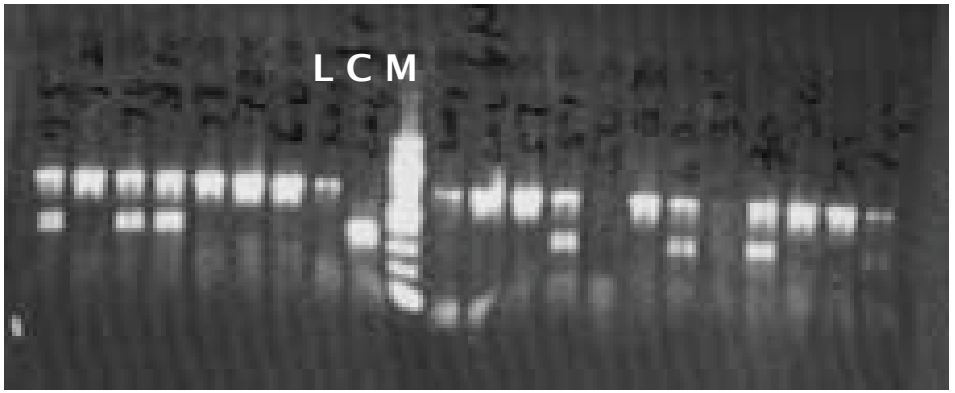




E/e: CAPS marker
F/f: a mutation causing abnormal flowers
Ler: solid line
Col: dotted line

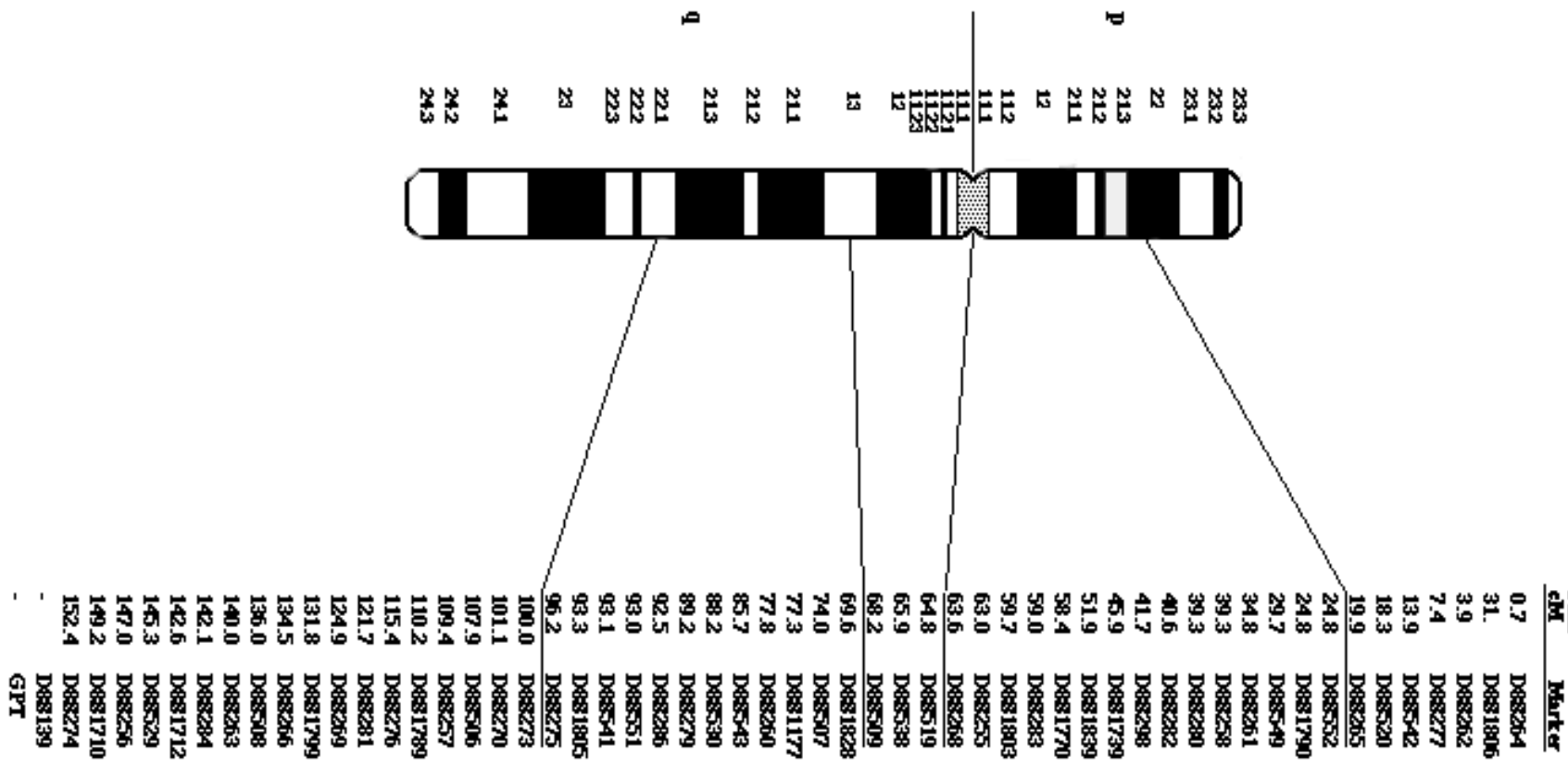
self

No. of plants		No. of recombinant Chr.
11		0
6		1
0		2

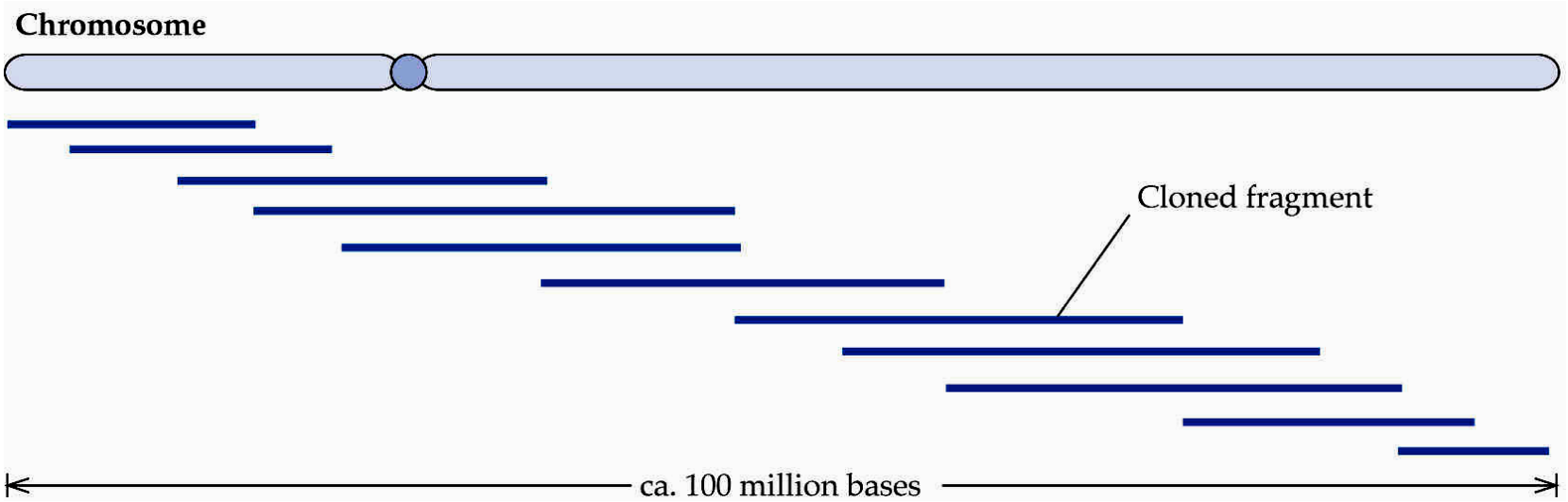


$$\frac{6}{17 \times 2} = 0.17$$

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

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

A MAP OF HUMAN CHROMOSOME 1

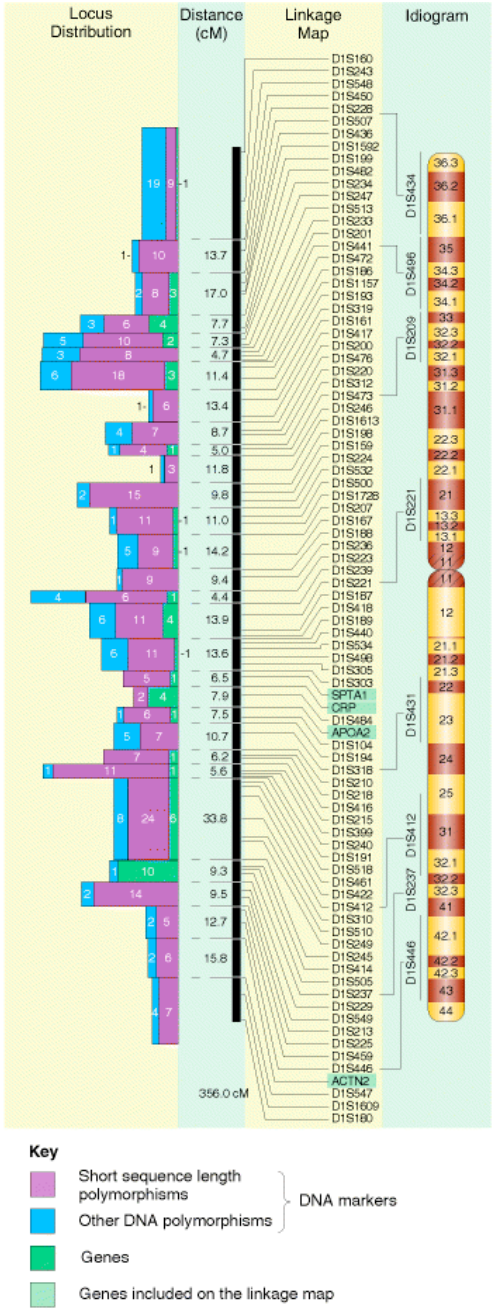


Figure 14-7. Linkage map of human chromosome 1, correlated with chromosome banding pattern. The histogram shows the distribution of all markers available for chromosome 1. Some markers are genes of known phenotype, but most are DNA markers based on neutral sequence variation. A linkage map, based on recombinant frequency analyses of the type described in this chapter, is in the center of the illustration. It shows only some of the markers available. Map distances are shown in centimorgans (cM, or m.u.). The total length of the chromosome 1 map is 356 cM; it is the longest human chromosome. The positions of some markers are cross-referenced to a diagram of subregions of chromosome 1 based on a standard banding pattern (such a diagram is called an idiogram). These kinds of correlations can be made only by using cytogenetic analysis (Chapter 17) and in situ hybridization. Most of the markers shown on the map are molecular, but several genes (highlighted in light green) also are included.

(Griffiths et al (Introduction to genetic analyses)

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

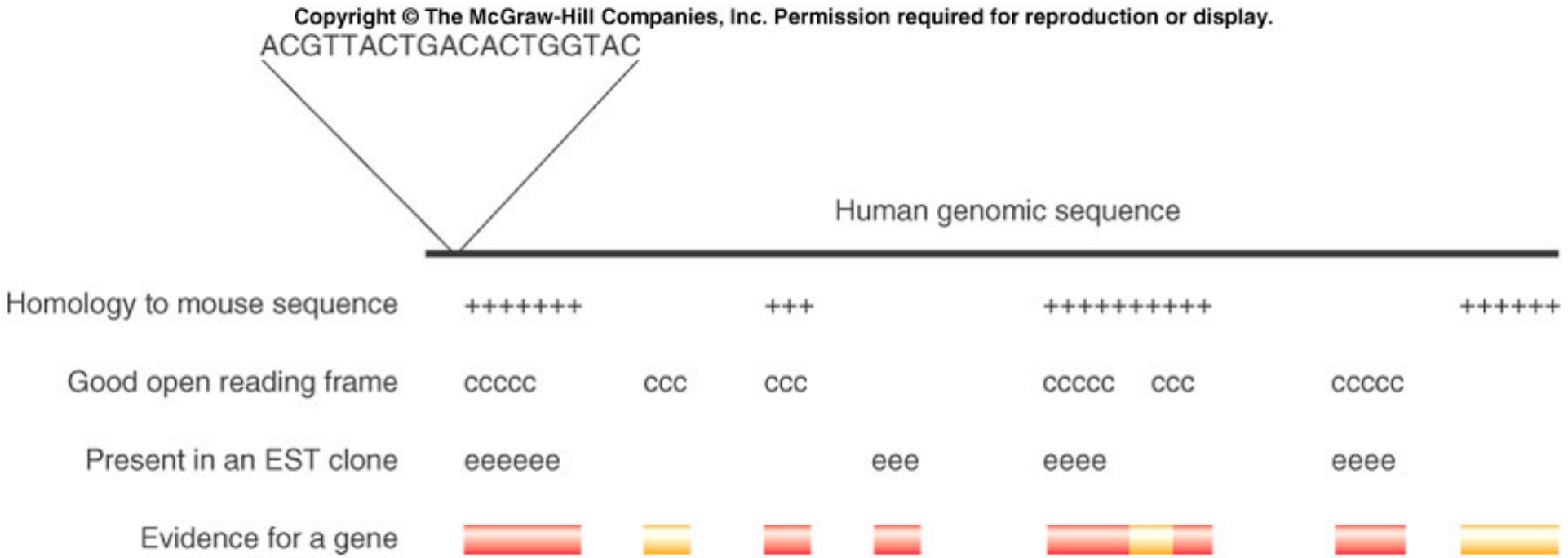
Step4: Transgenic studies (SRY example)

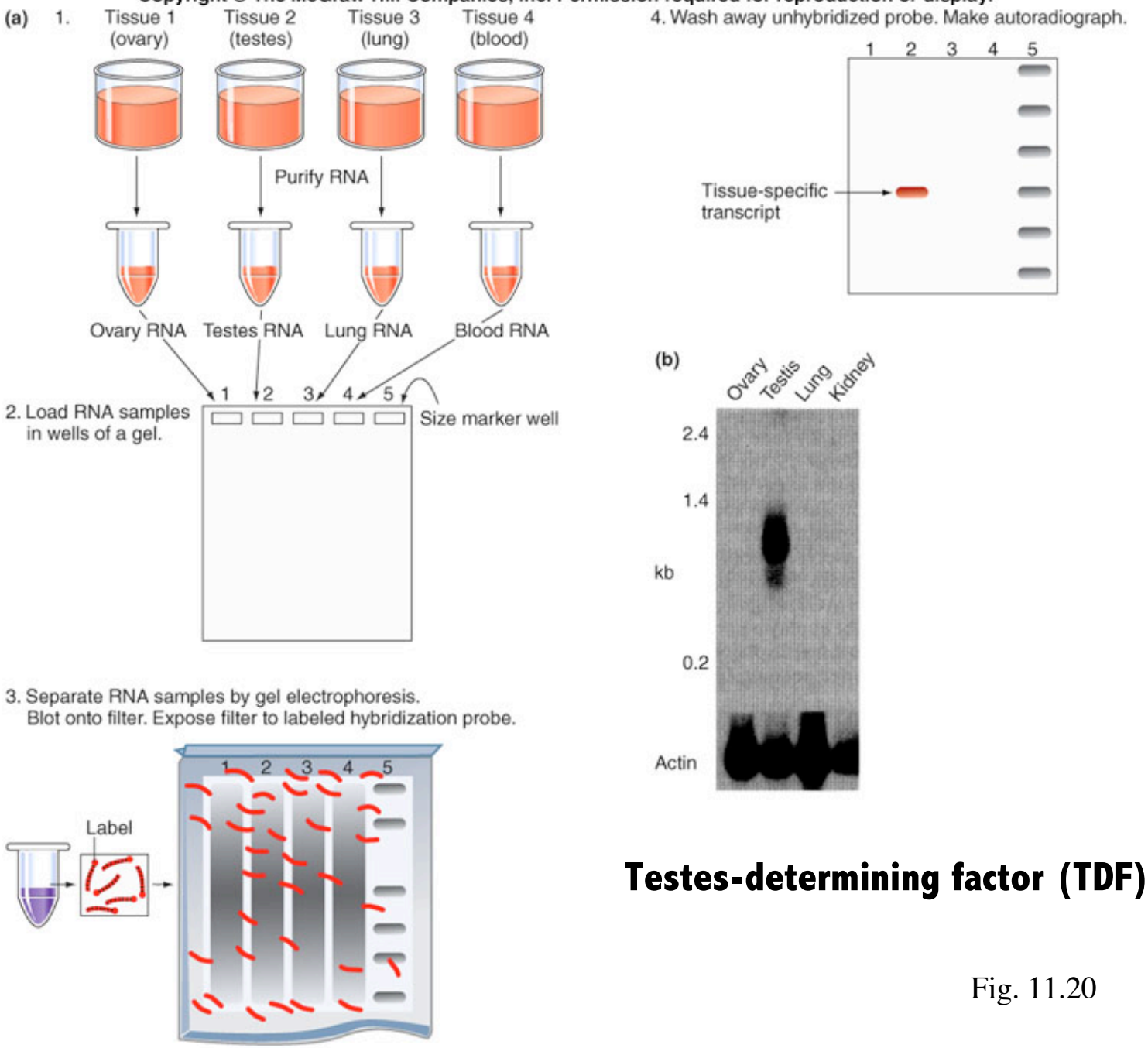
Case study: Cystic fibrosis

CFTR (Cystic fibrosis transmembrane conductance regulator)

How to determine where the genes are in a segment of DNA?

Fig. 11.19

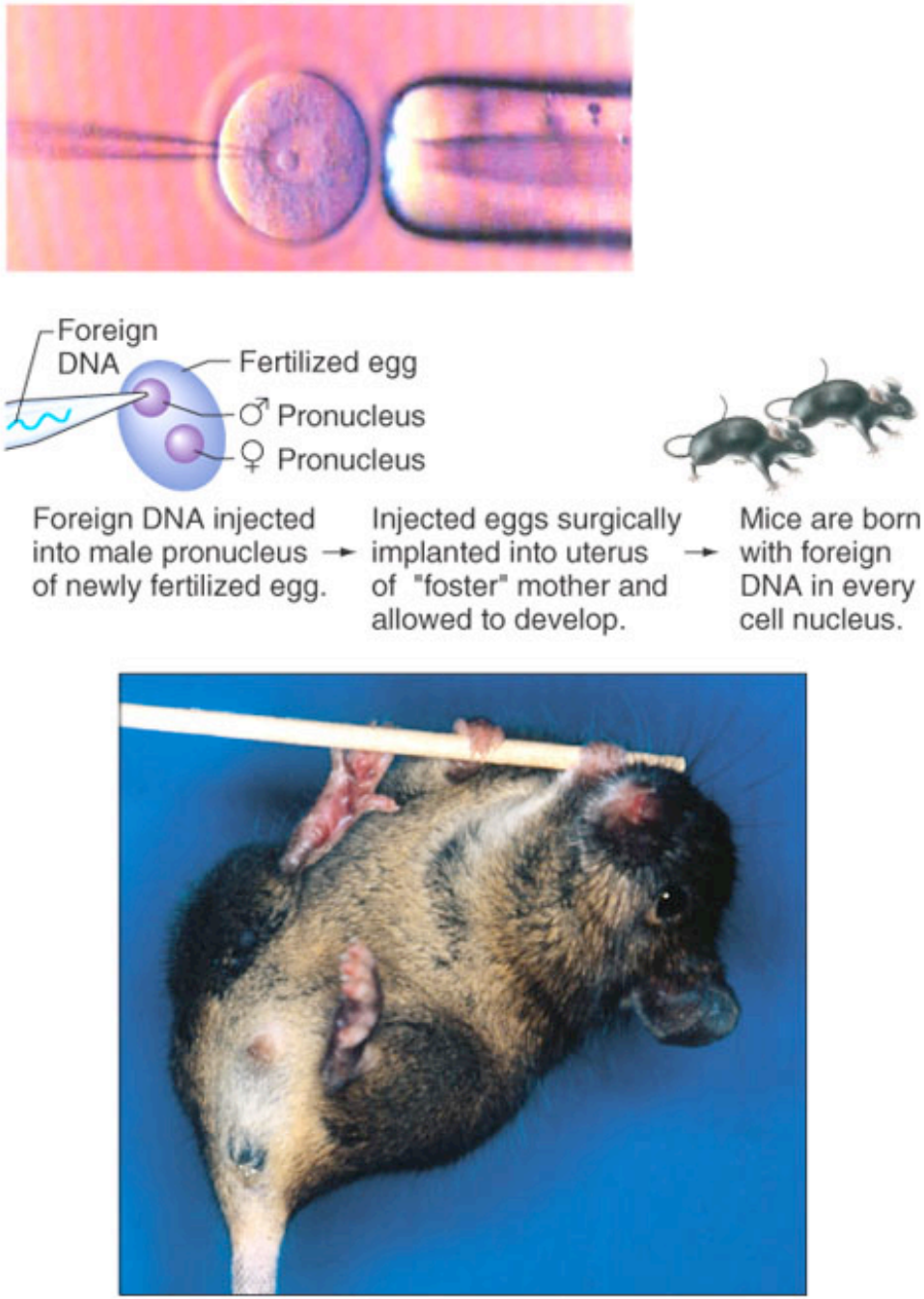




Testes-determining factor (TDF)

Fig. 11.20

Fig. 11.21

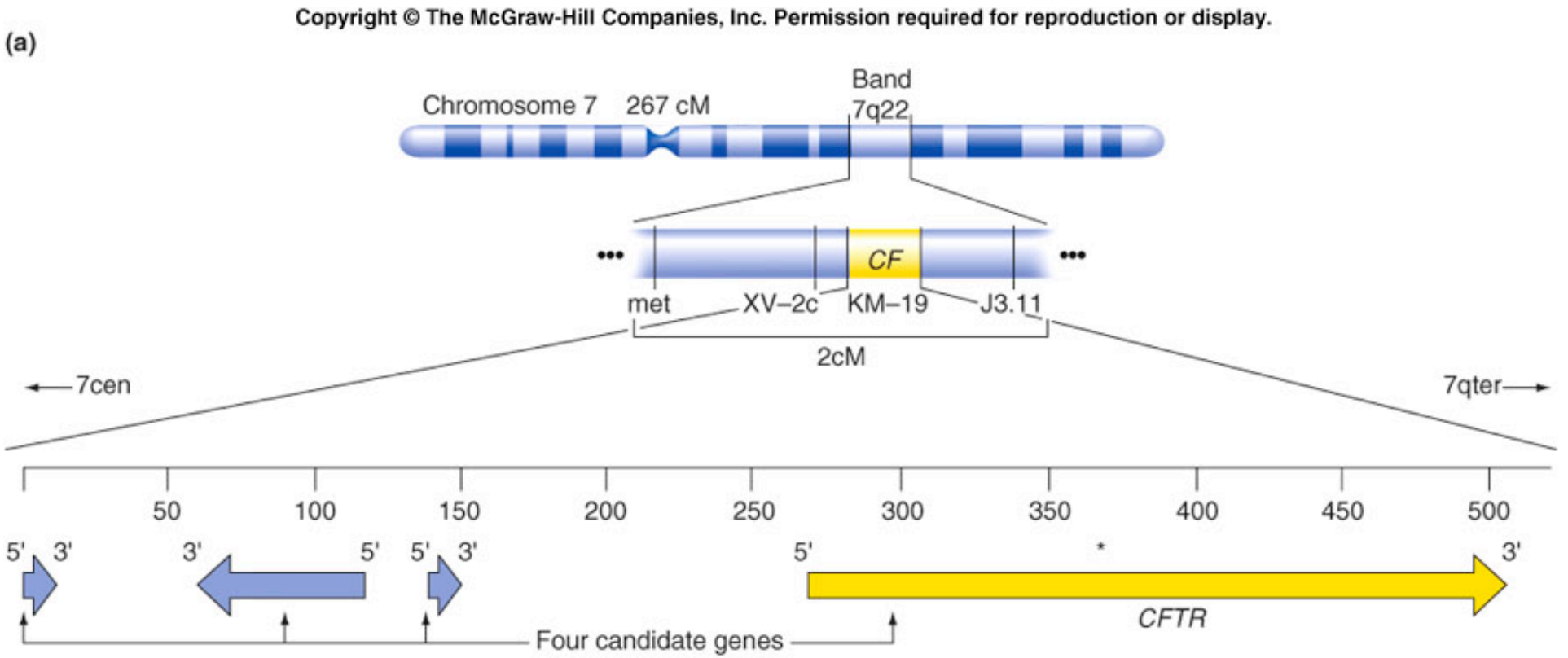


Cystic fibrosis: a case study

Cystic fibrosis is an autosomal recessive disorder that results in defective transport of chloride ions through epithelial cells, and results from mutations in a gene, CFTR, which encodes a cAMP-regulated chloride channel. The primary expression of the defect is in the lungs: a sticky mucus secretion accumulates which is prone to chronic infections. Because there are no methods to culture lung cells routinely in the laboratory, in vivo gene therapy approaches have been adopted. As respiratory epithelial cells are differentiated, retroviral vectors cannot be used. Instead, gene therapy trials have used adenovirus vectors or liposomes to transfer a suitably sized CFTR minigene, either through a bronchoscope or through the nasal cavity.

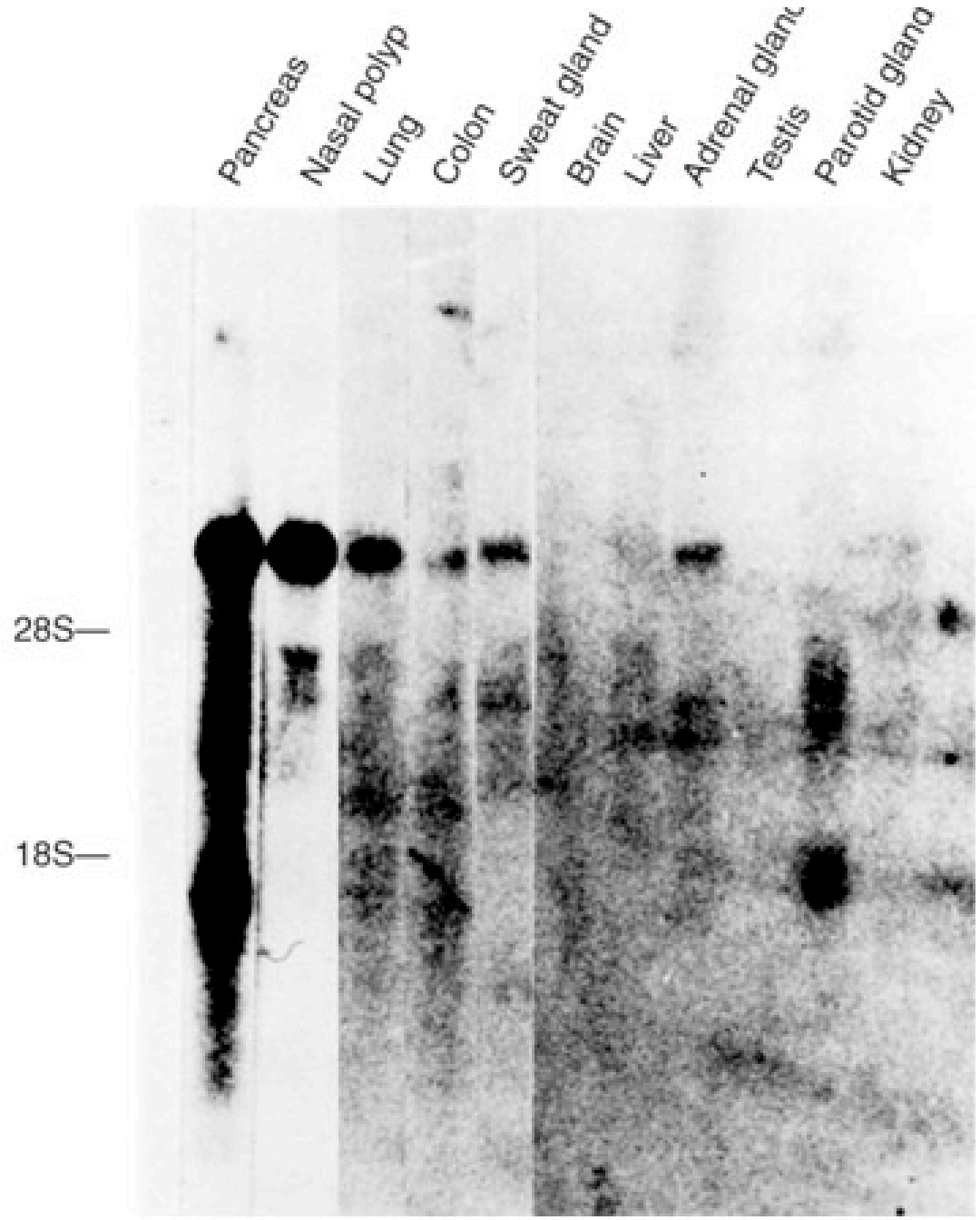
Cloning cystic fibrosis transmembrane conductance regulator (CFTR)

Fig. 11.22a



Northern blots help eliminate other 3 candidate genes

Fig. 11.22b



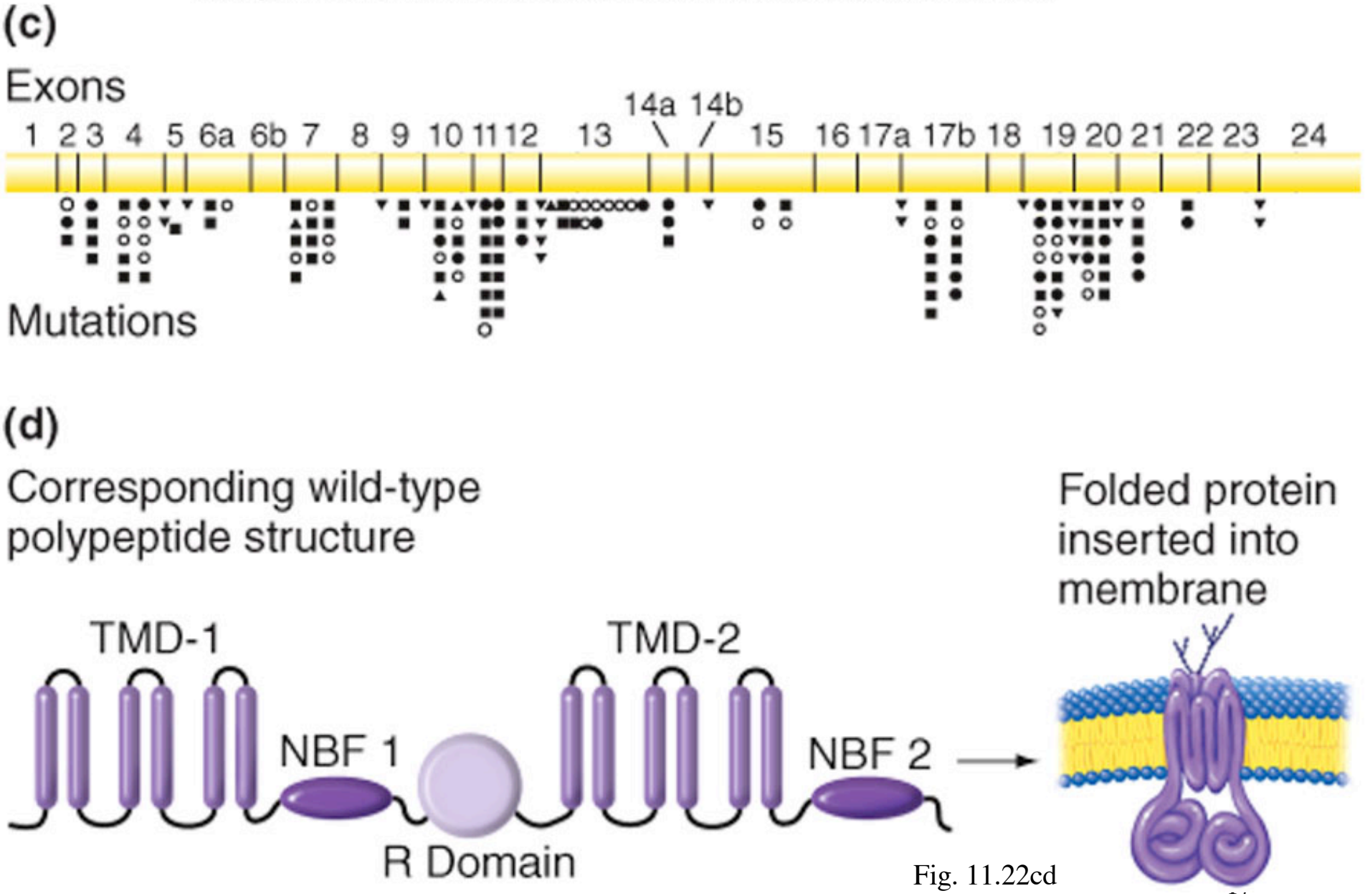
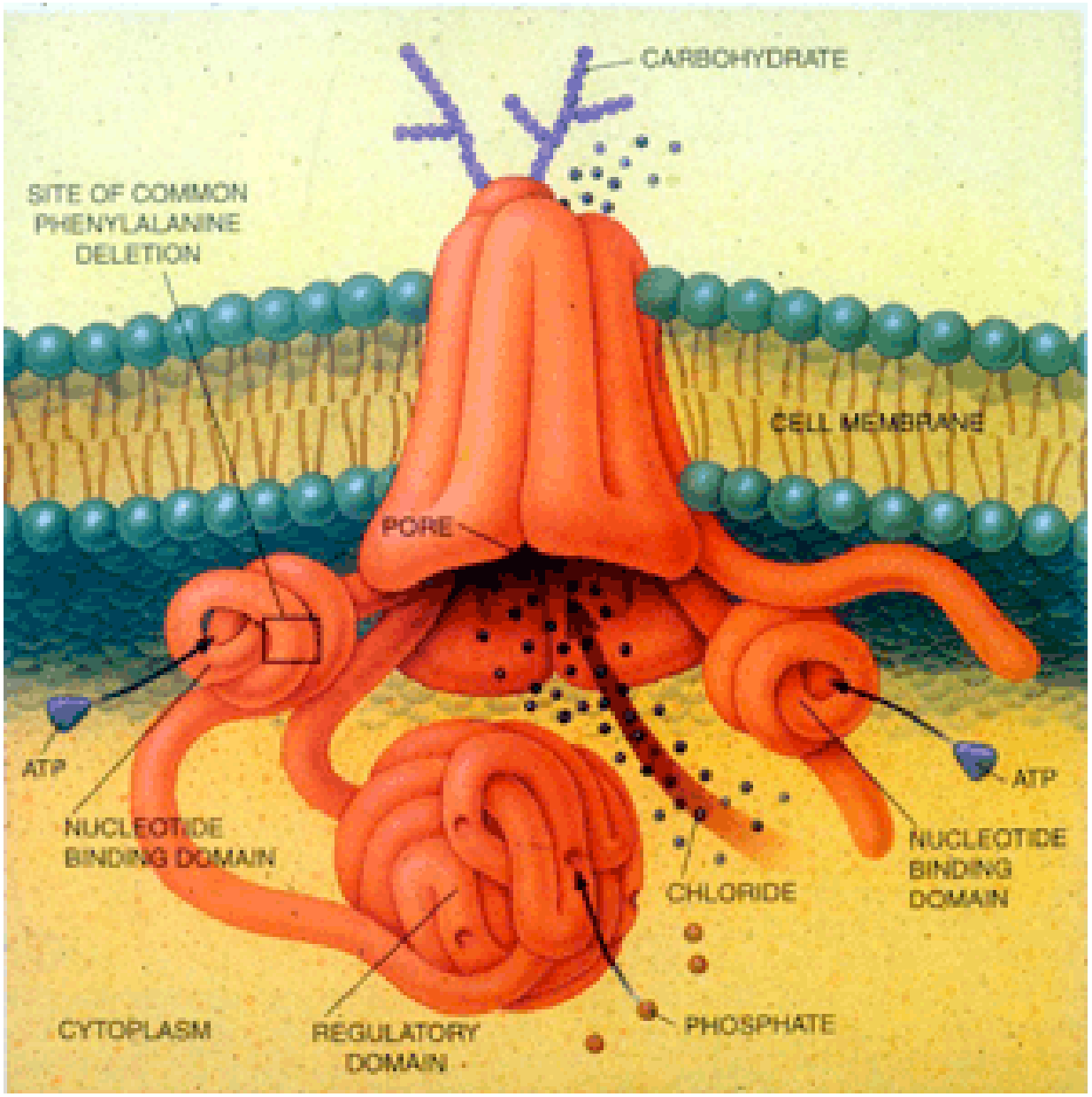


Fig. 11.22cd

CFTR


CARBOHYDRATE
SITE OF COMMON PHENYLALANINE DELETION
CELL MEMBRANE
PORE
ATP
NUCLEOTIDE BINDING DOMAIN
CHLORIDE
ATP
NUCLEOTIDE BINDING DOMAIN
CYTOPLASM
REGULATORY DOMAIN
PHOSPHATE