Molecular Techniques II

1. Expression Library

2. Hybridization

    Southern and Northern blots

    Colony hybridization

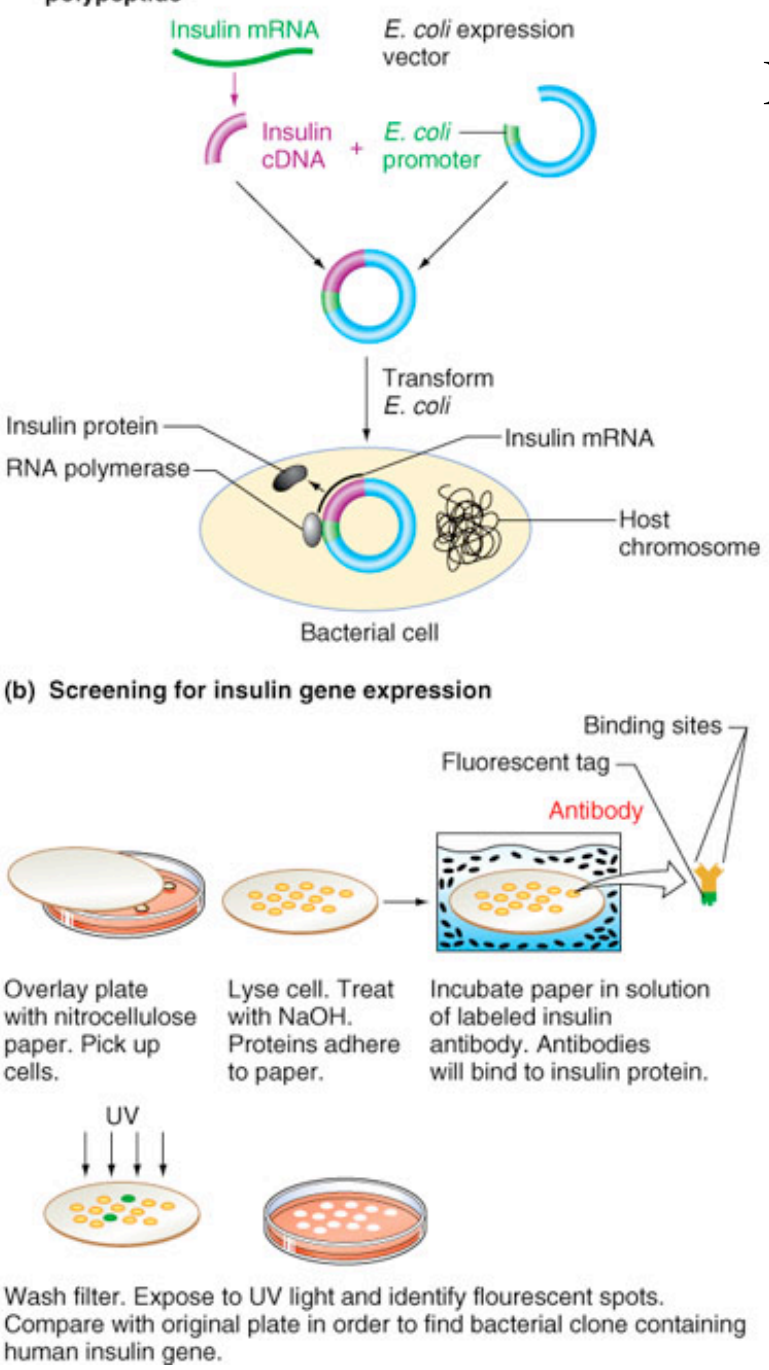
3. PCR (Polymerase Chain Reaction)

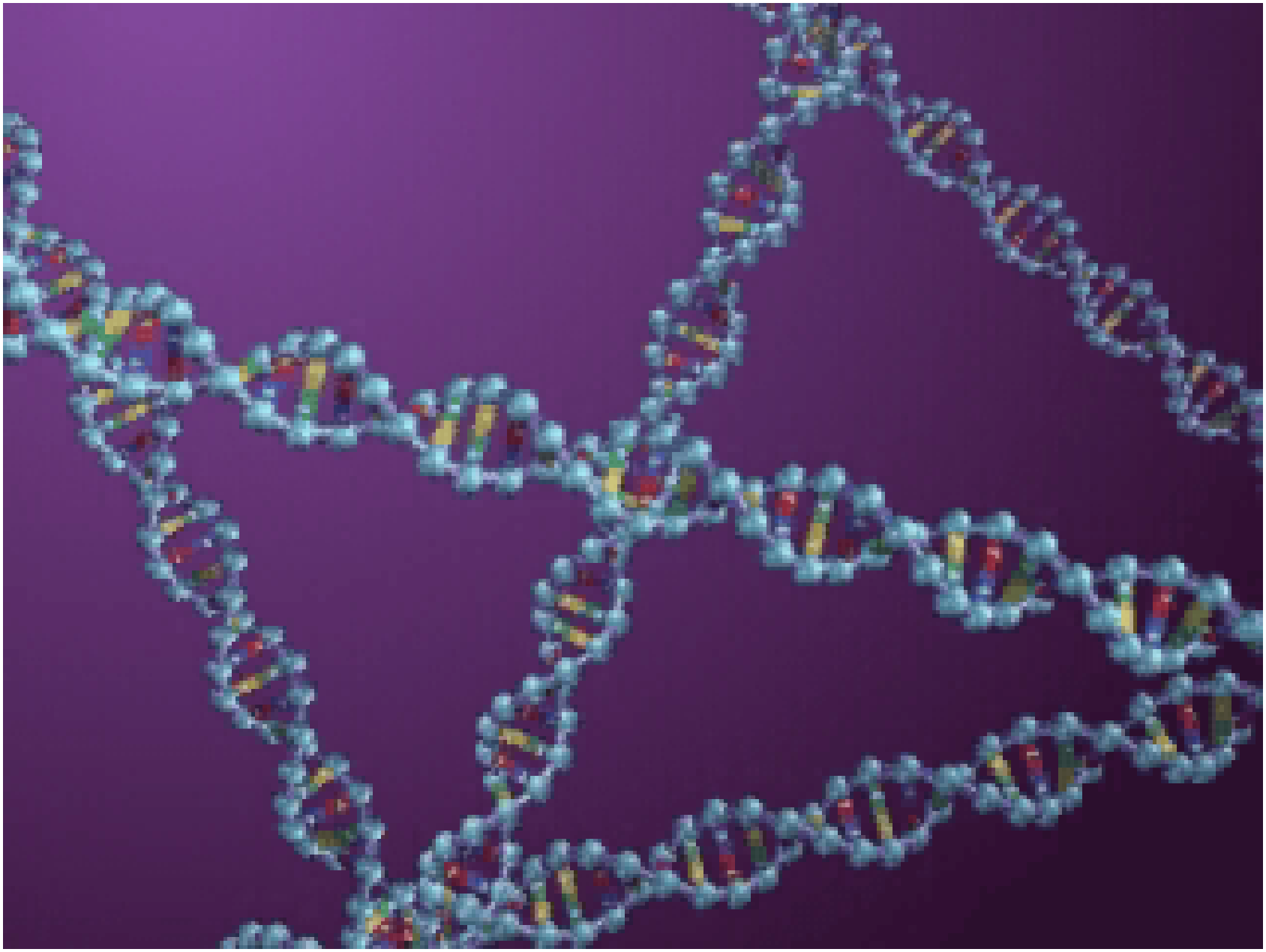
4. DNA sequencing

Read 58-62; 64-68; 241-245
Fig. 2.14-16; 2.19-20; 6.23-26

Fig. 9.12

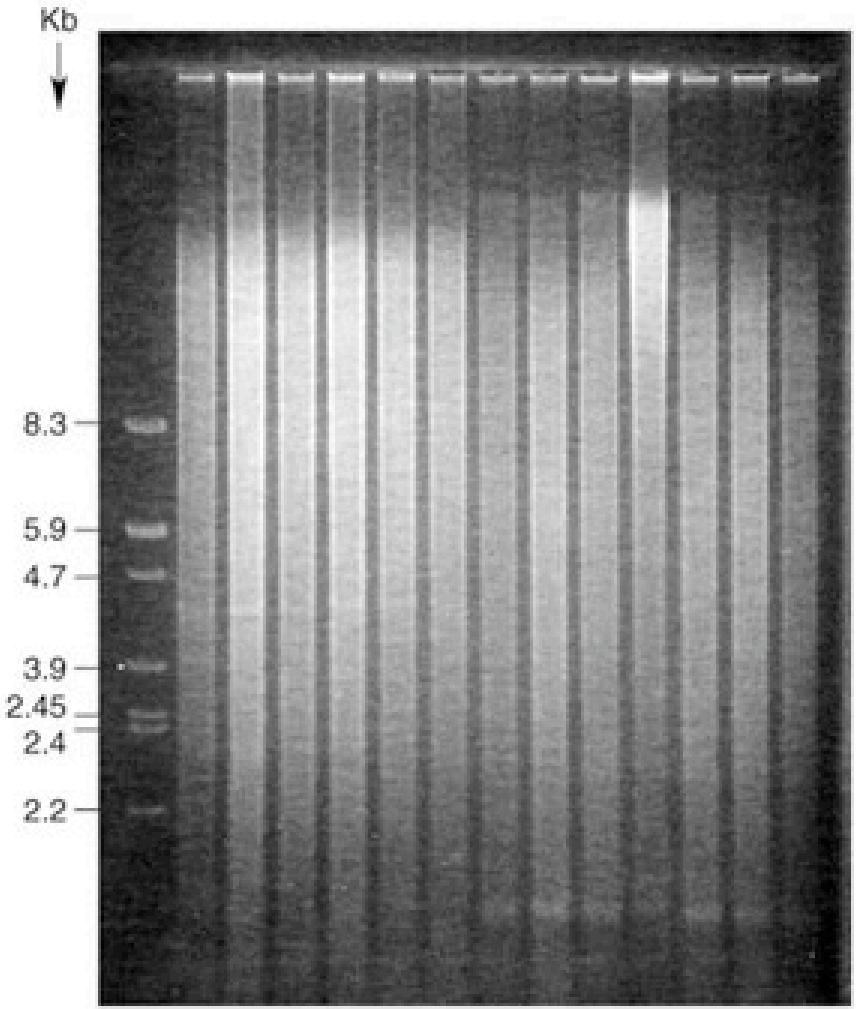
Expression Library





Hybridization-probes

Fi



Southern Blot

Fig. 9.15b

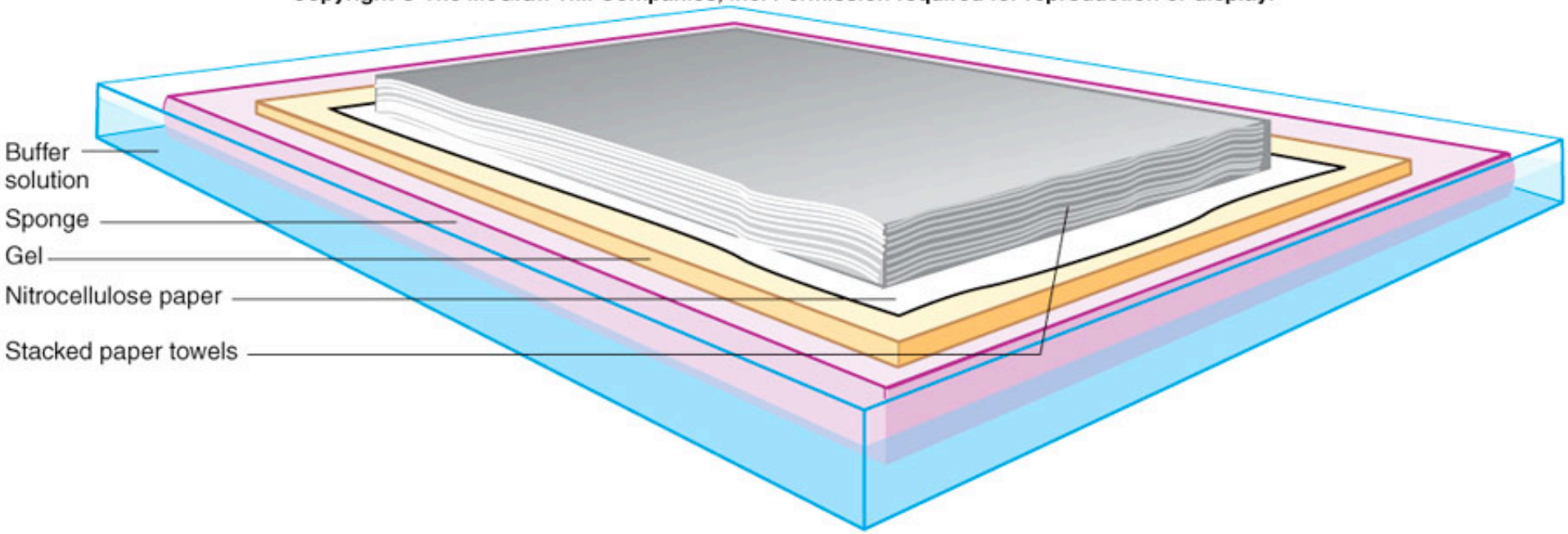
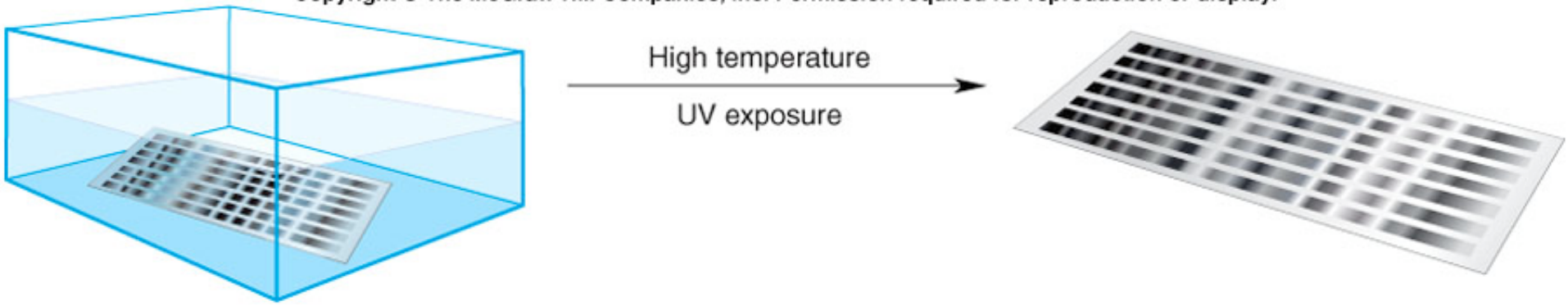
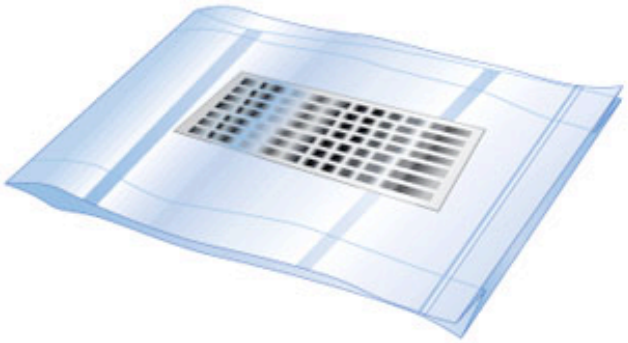


Fig. 9.15c

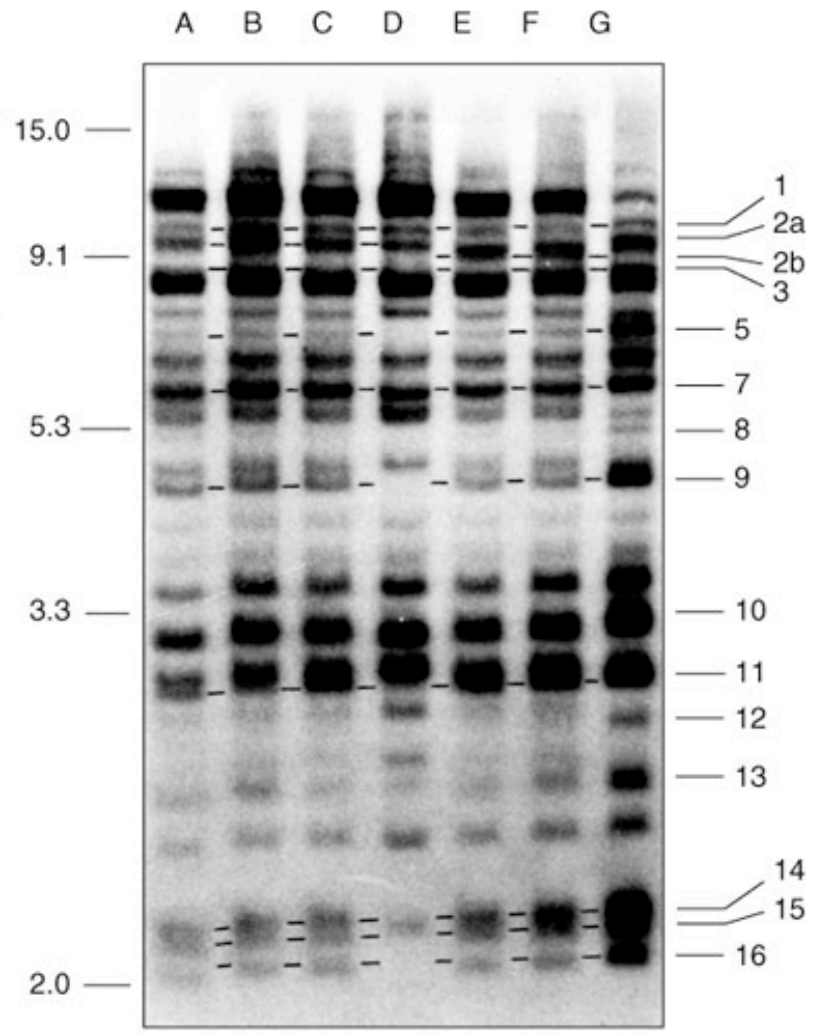




Blot is removed,

washed, and exposed
to X-ray film.

Autoradiograph



In each genomic DNA sample, the *H2K* probe hybridizes to all 20–30 related major histocompatability genes present within the mouse genome.

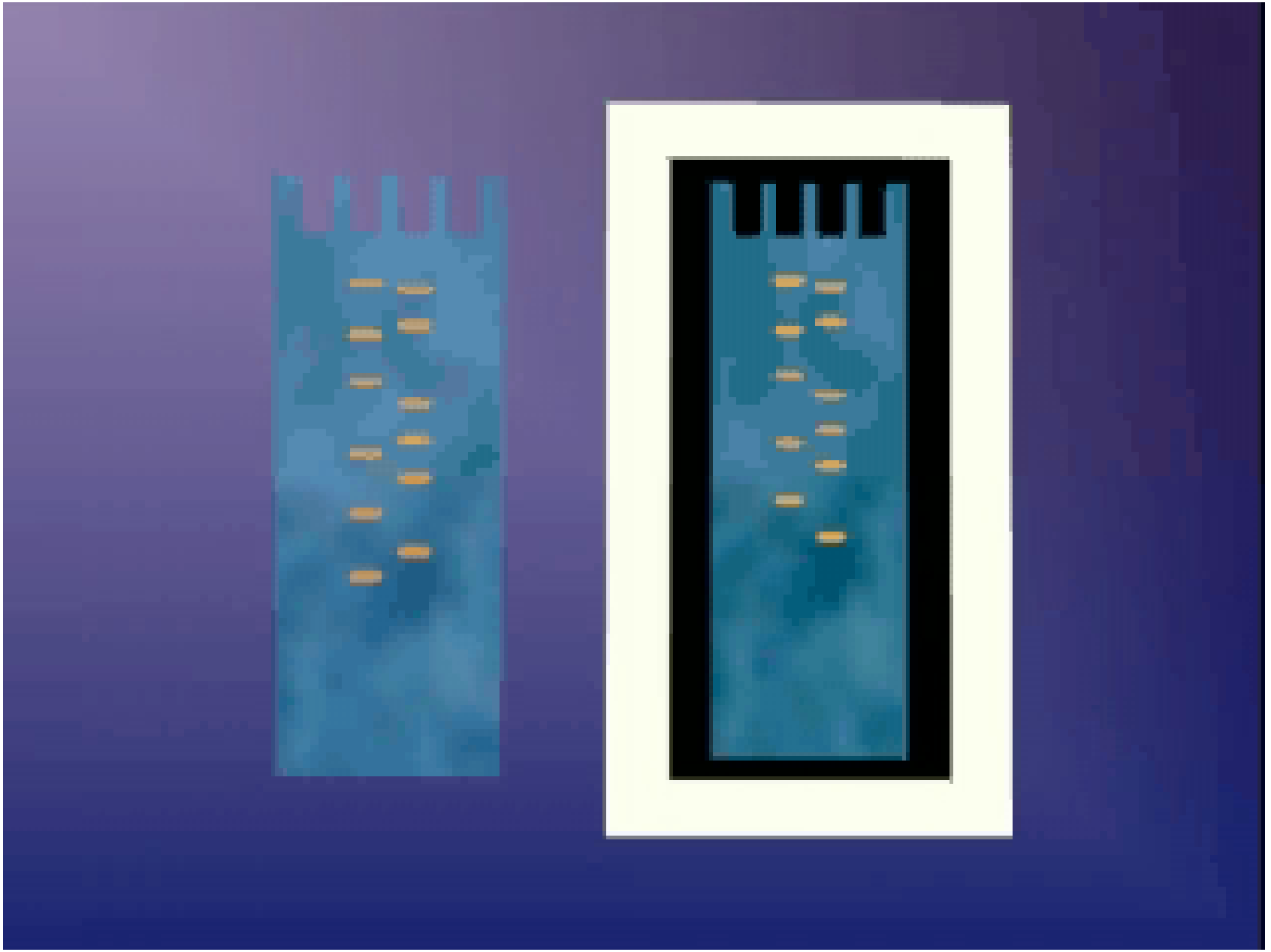
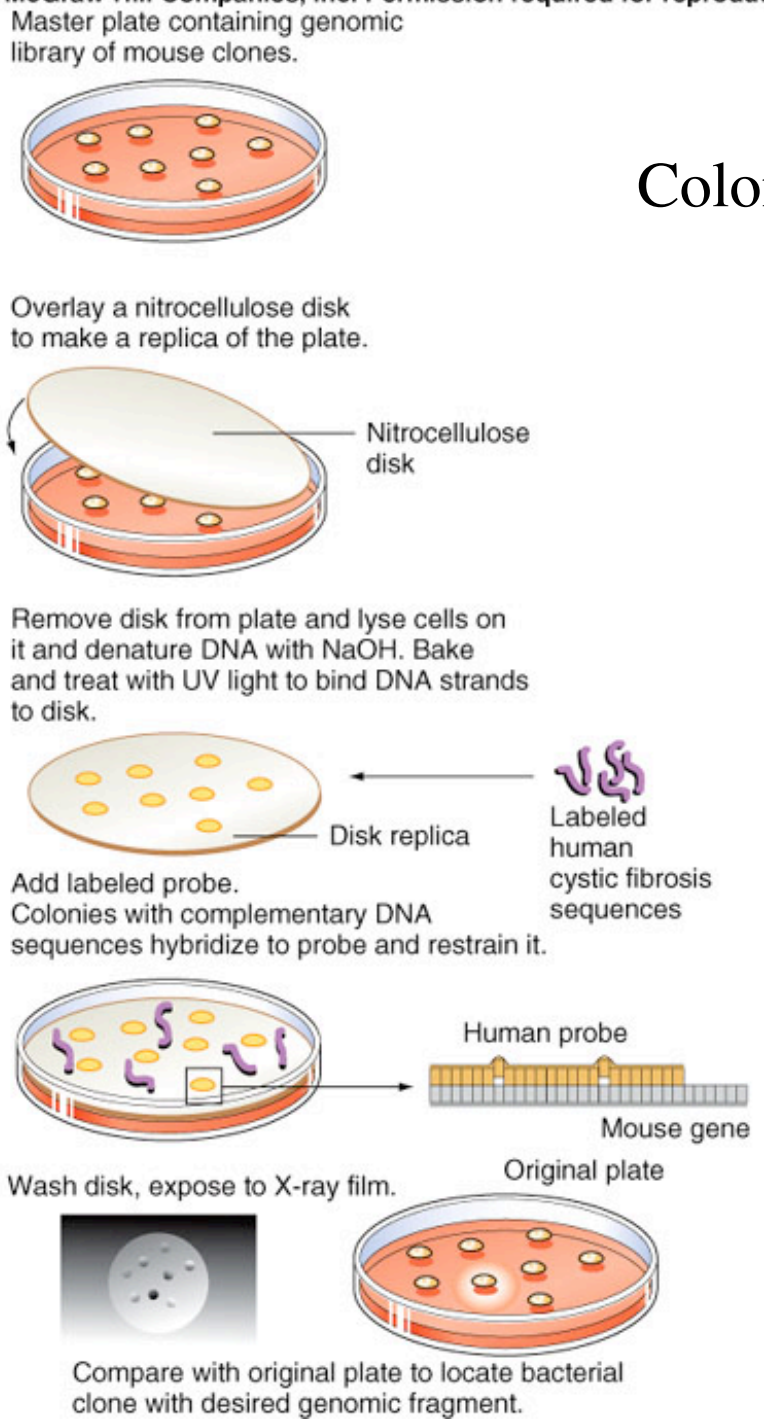
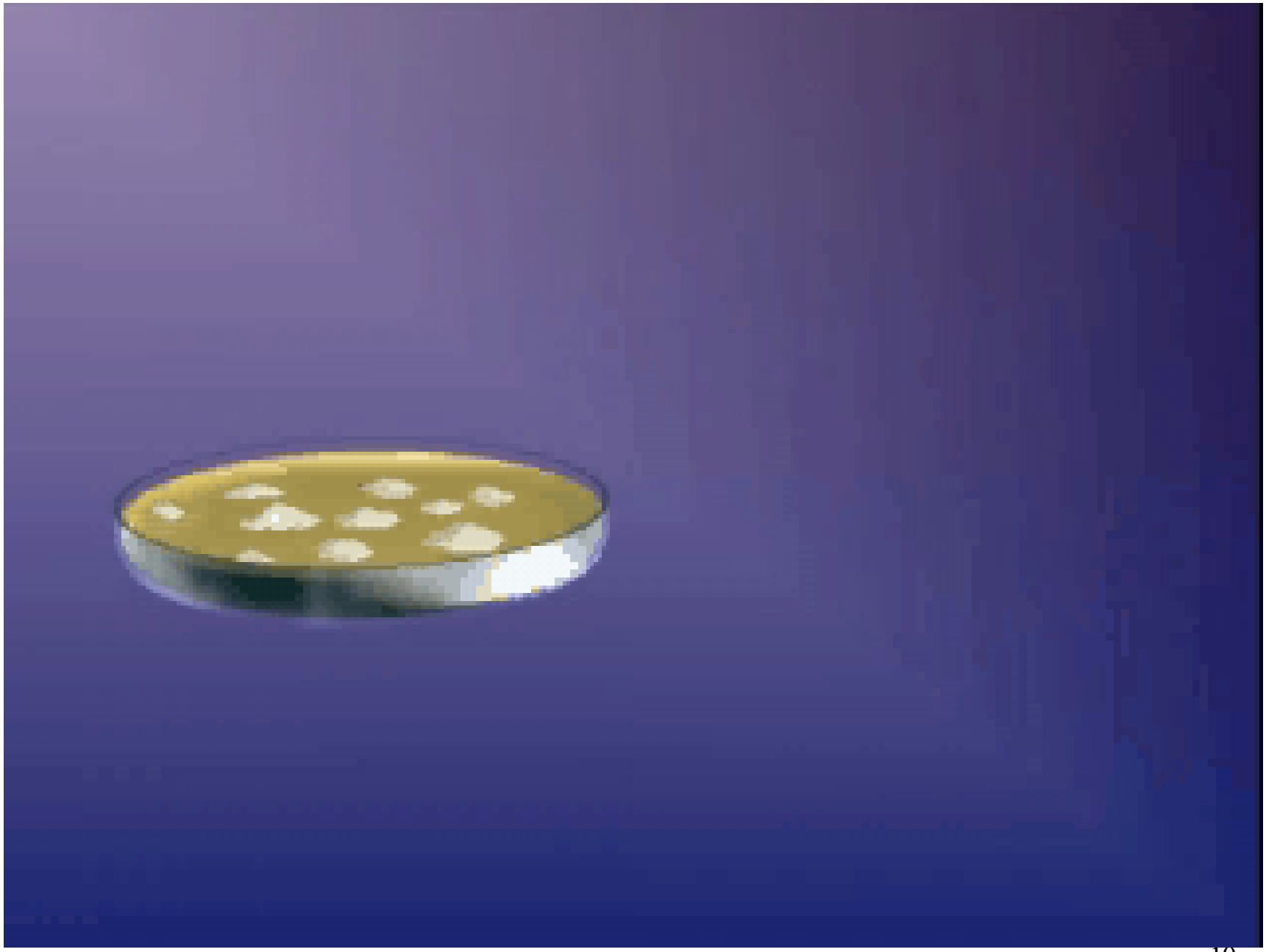


Fig. 9.13

Colony hybridization





Colony hybridization

Fig. 9.16

The Polymerase Chain Reaction (PCR)

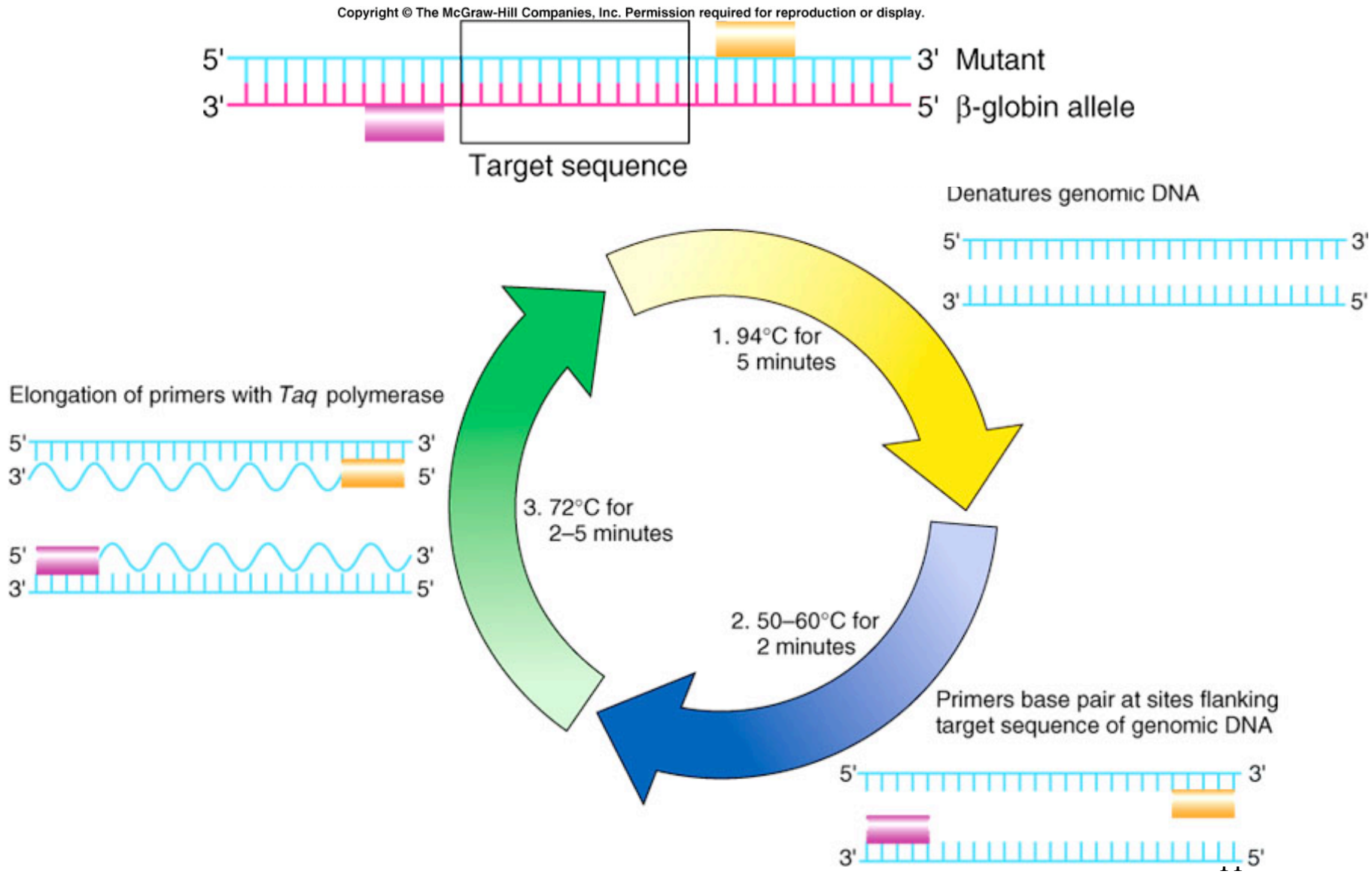
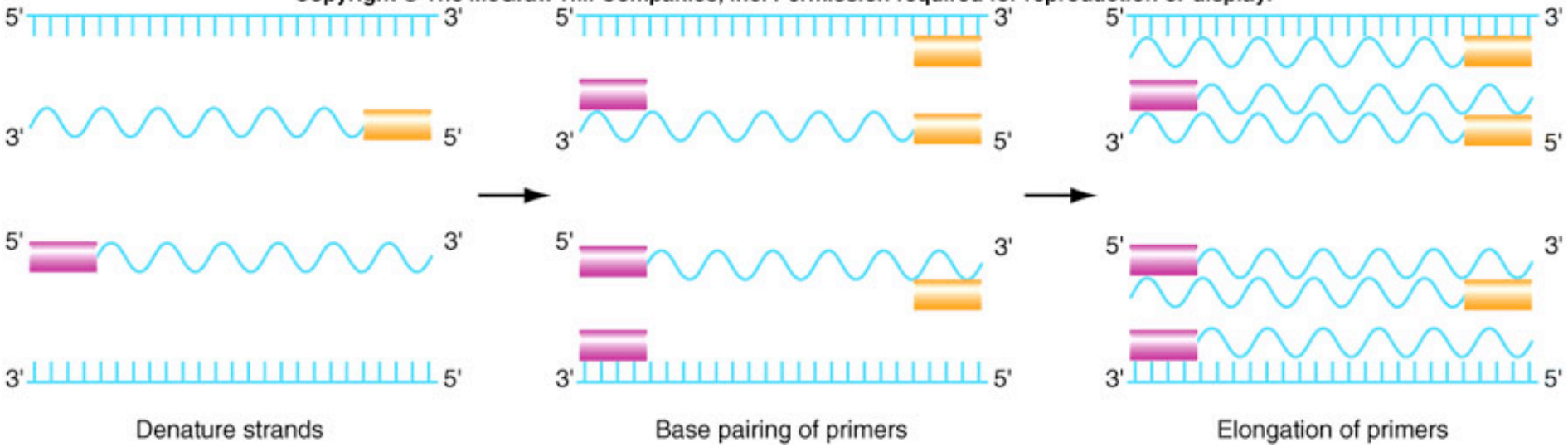
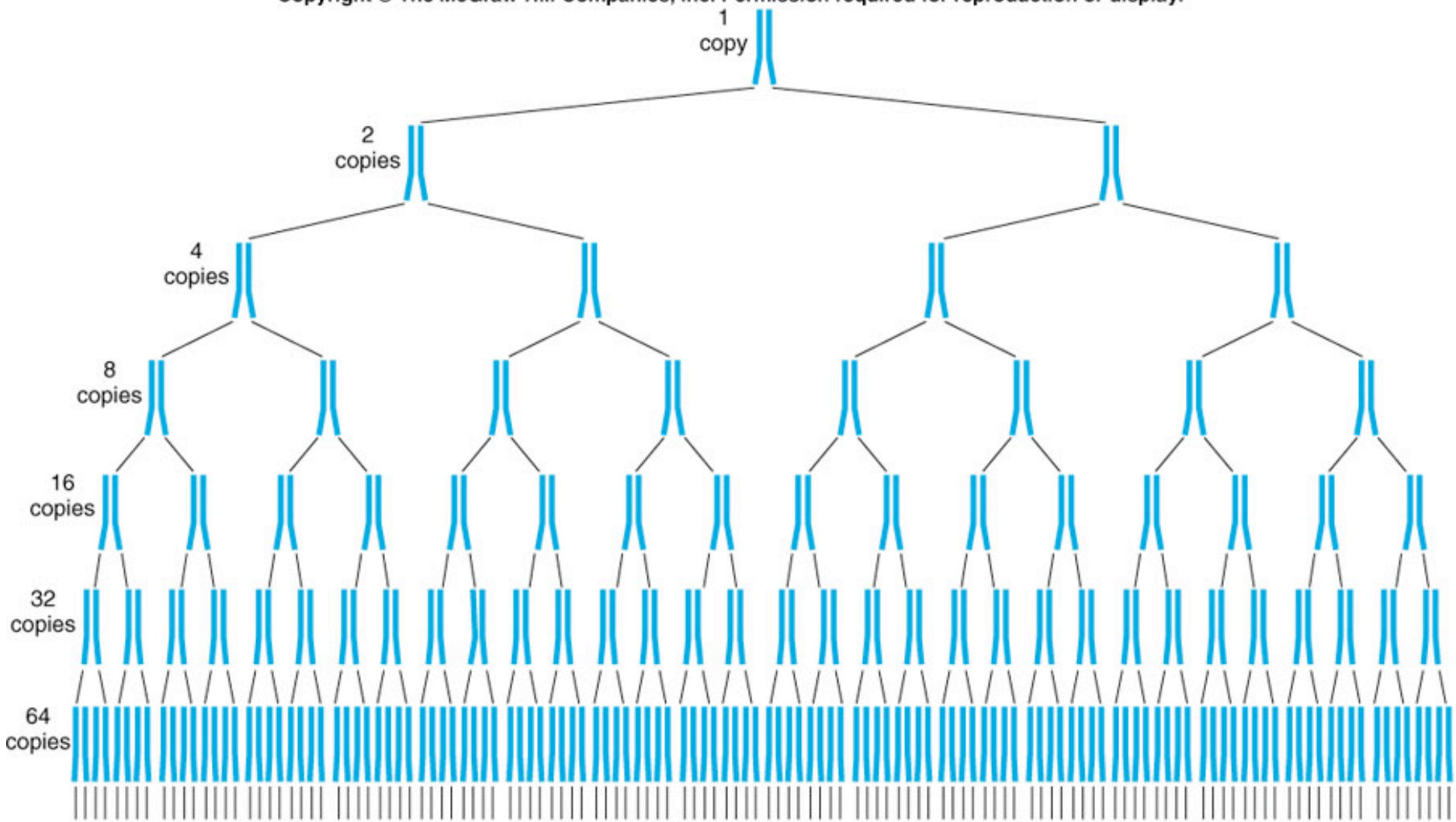
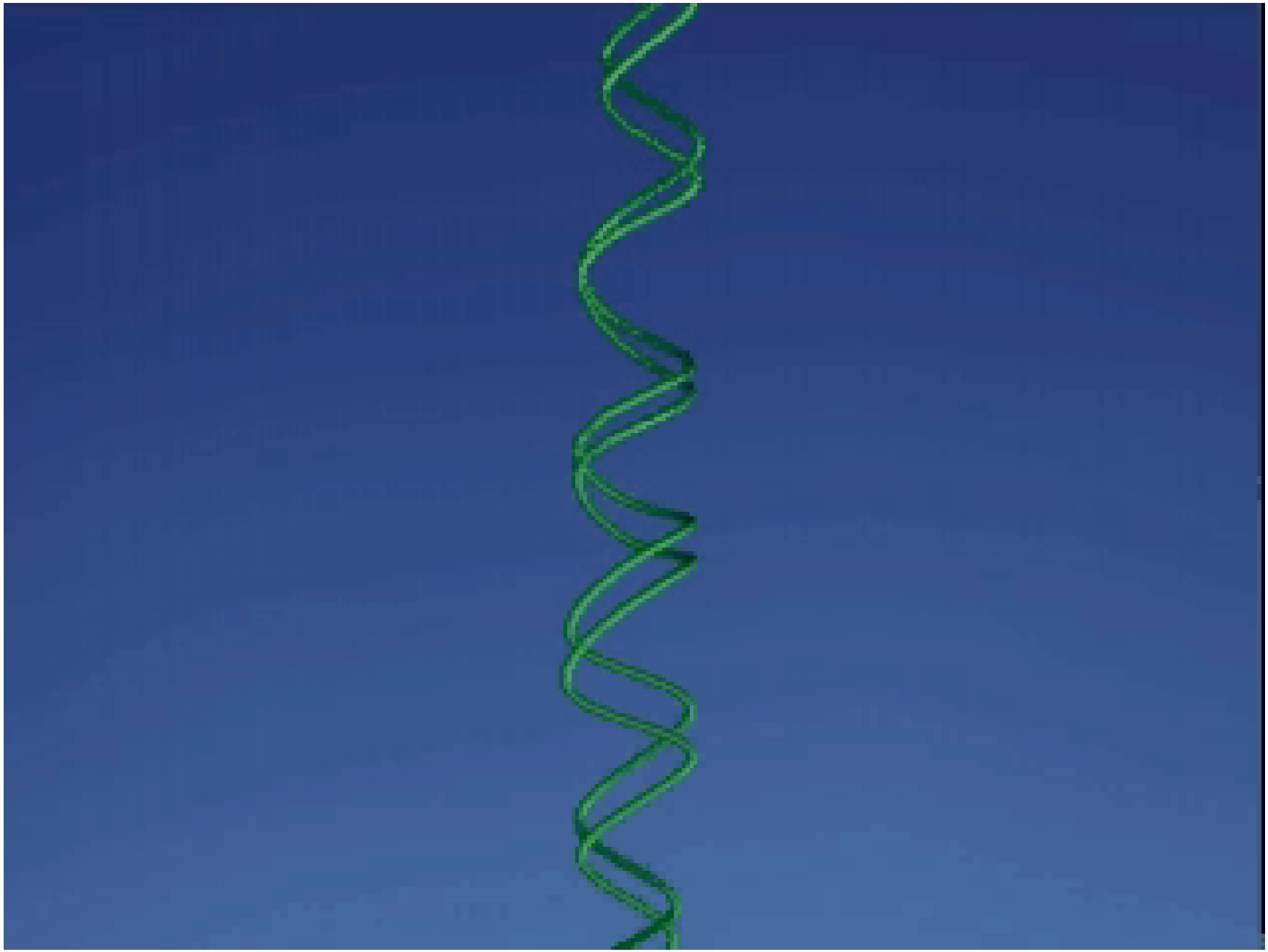


Fig. 9.16c

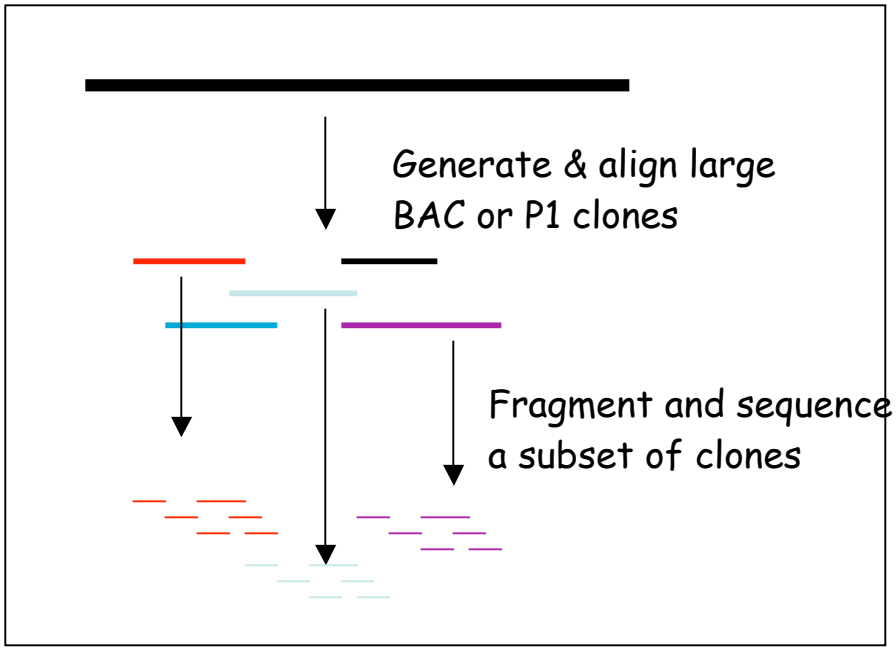




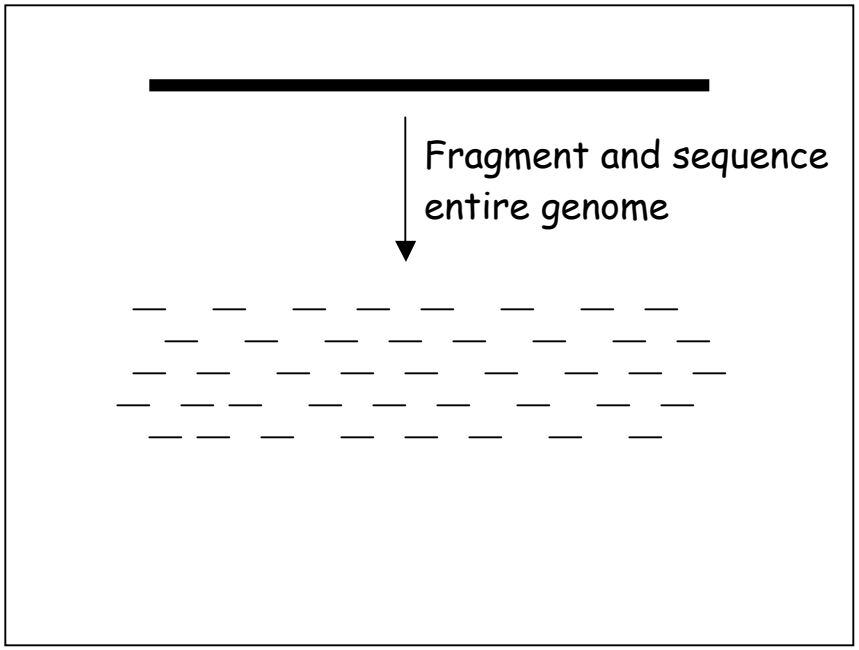


PCR movie

Hierarchical sequencing



Shotgun sequencing



DNA sequencing

Adapted from Fig. 2.7 Gibson and Muse

Sanger Sequencing

(chain termination with a specific ddNTP (dideoxynucleotides)

Fig. 9.17a

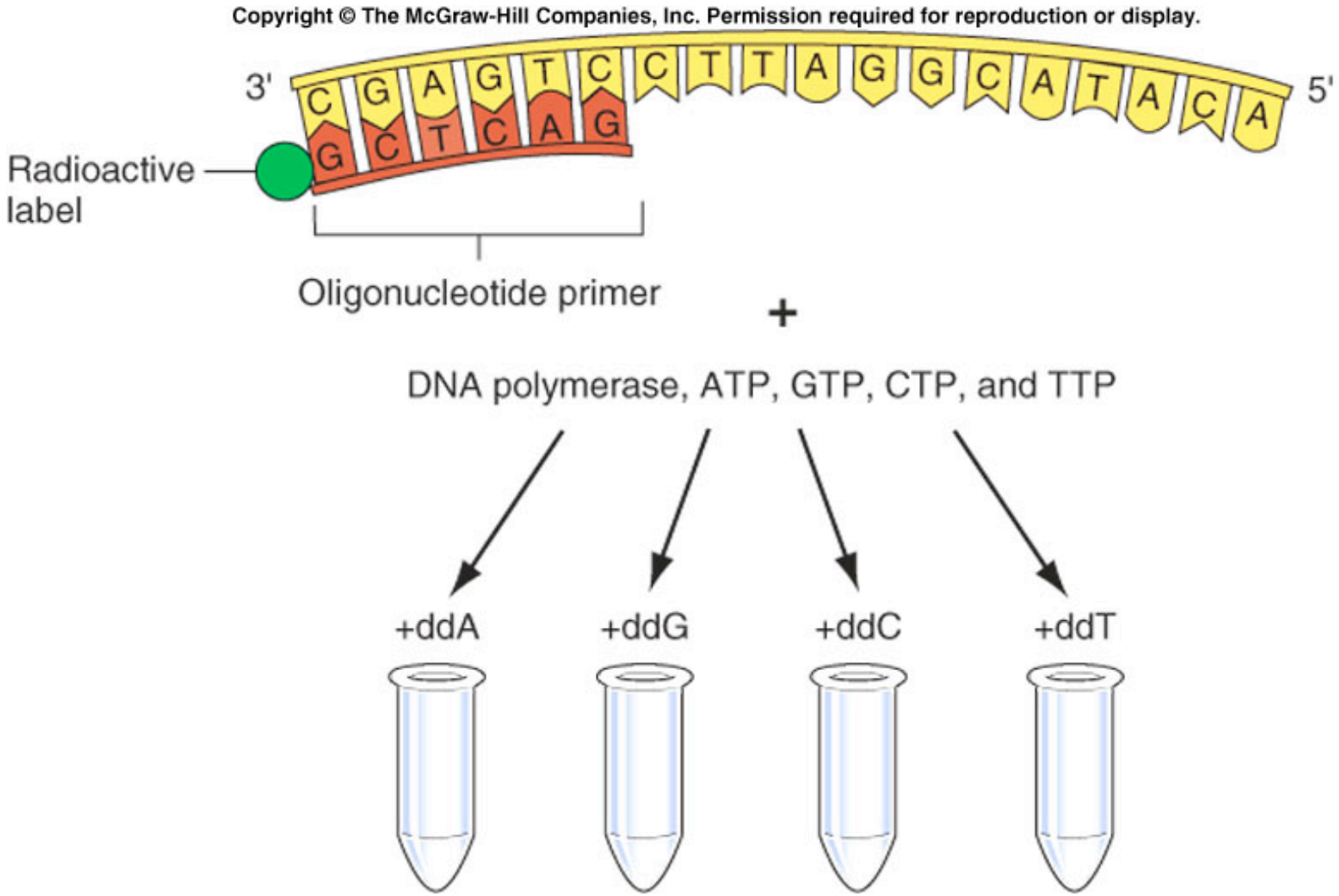
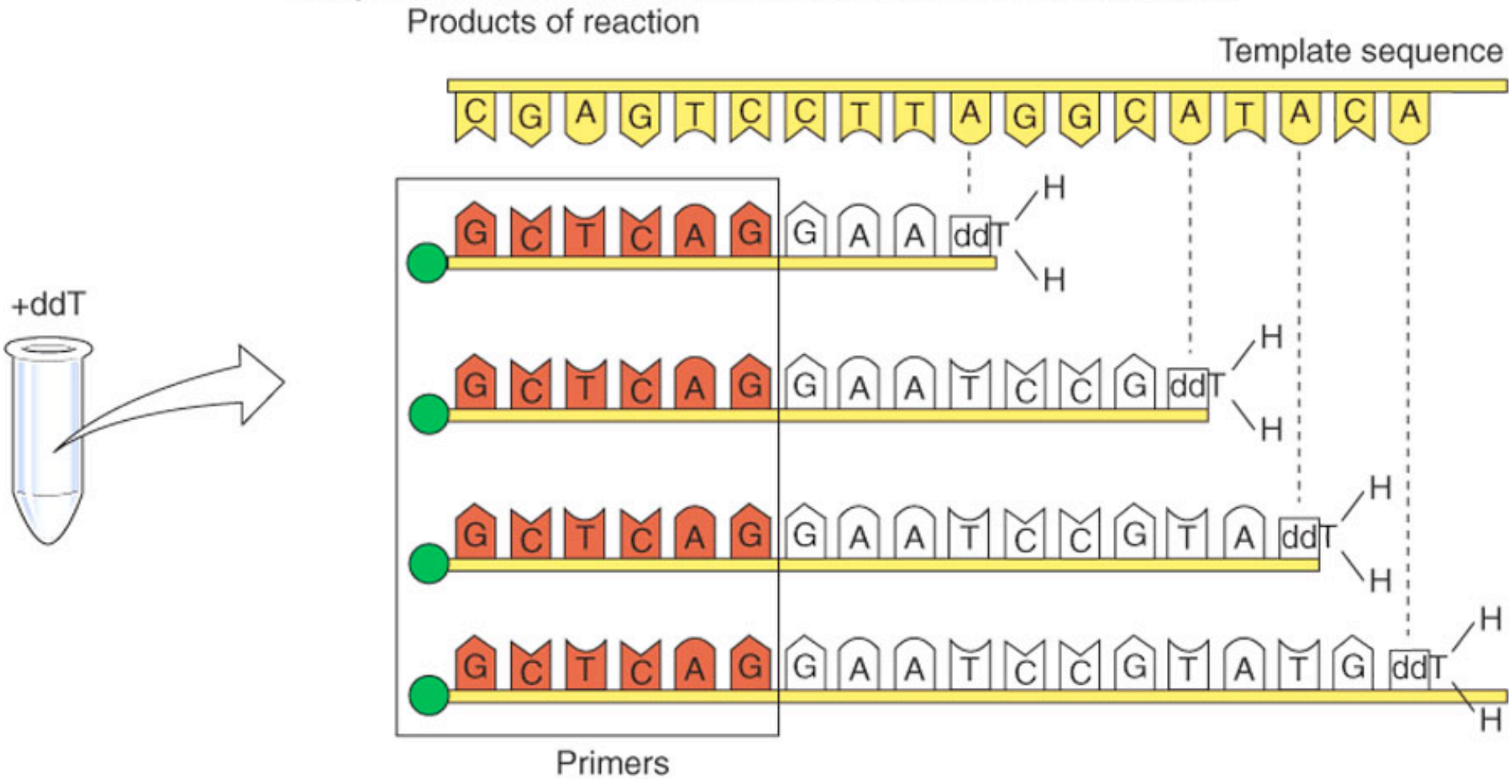
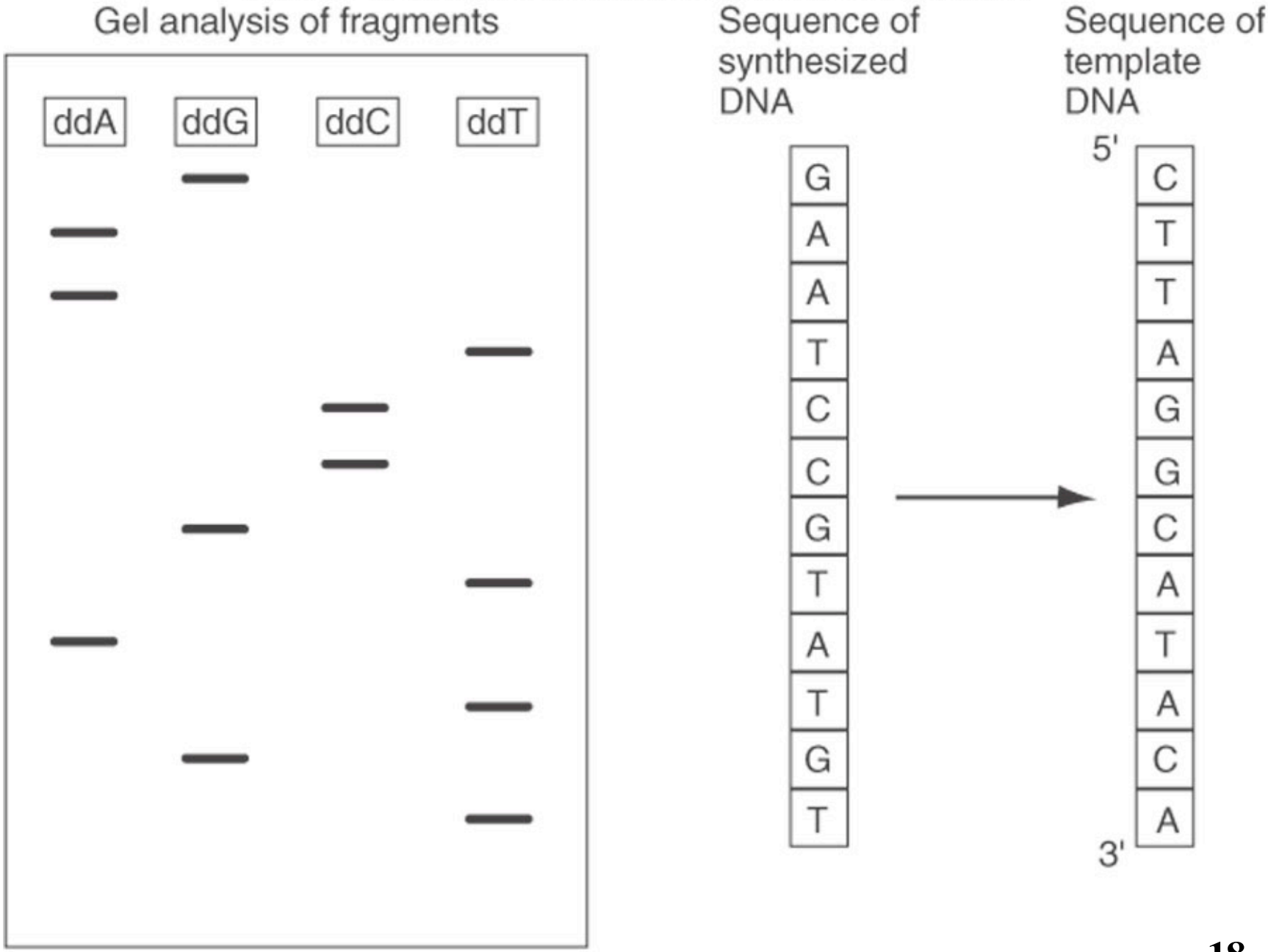


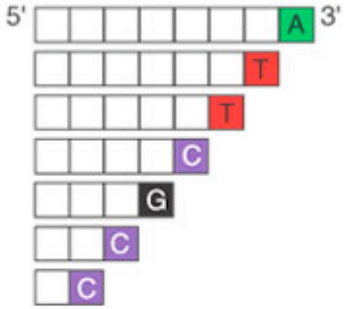
Fig. 9.17b





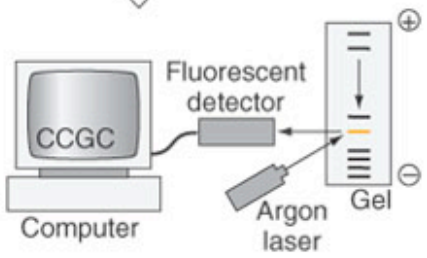
(a) Automated sequencing

1. Generate nested array of fragments; each with a fluorescent label corresponding to the terminating 3' base.



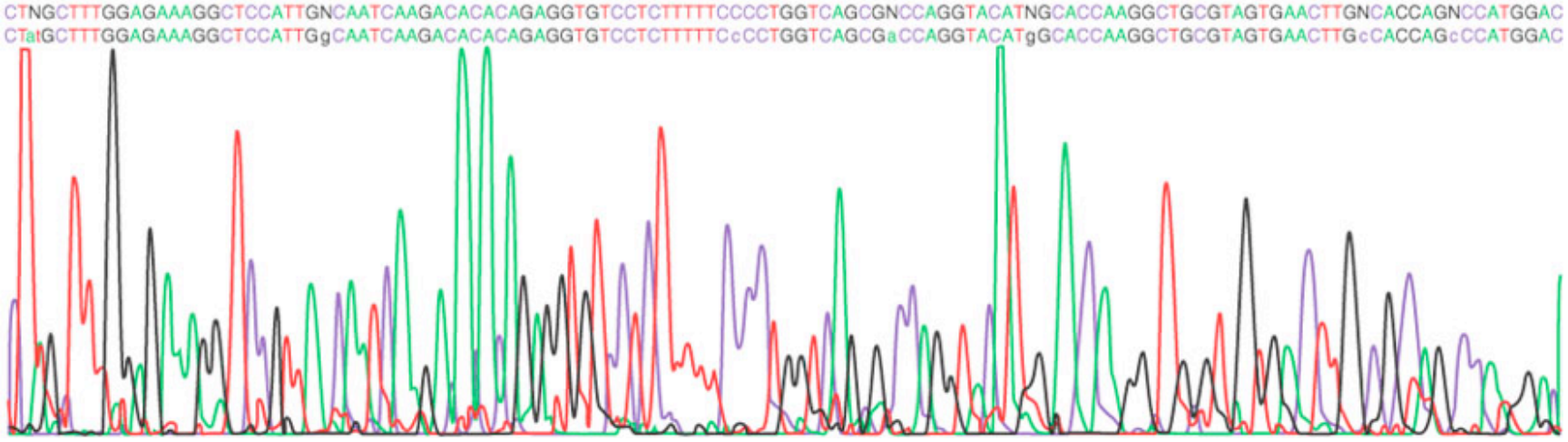
2. Fragments separated by electrophoresis in a single vertical gel lane.

3. As migrating fragments pass through the scanning laser, they fluoresce. A fluorescent detector records the color order of the passing bands. That order is translated into sequence data by a computer.

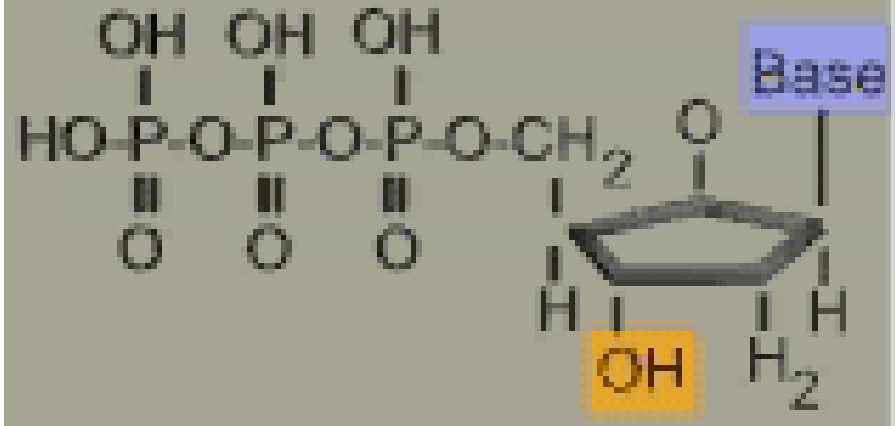


(b)

(c)



Deoxyribonucleotide



Dideoxyribonucleotide

Personal Genome Project (PGP)

Goal: sequencing full genome of individuals at $1000

Human Genome Project (HGP) (total 3 billion $) :
Motivated 100X reduction in cost (10 $ per base to 10 base per 1$.)

Personal Genome Project(PGP: has motivated the development of Ultra-Low-Cost-Sequencing (ULCS).