

Molecular Techniques II

1. Expression Library

2. Hybridization

Southern and Northern blots

Colony hybridization

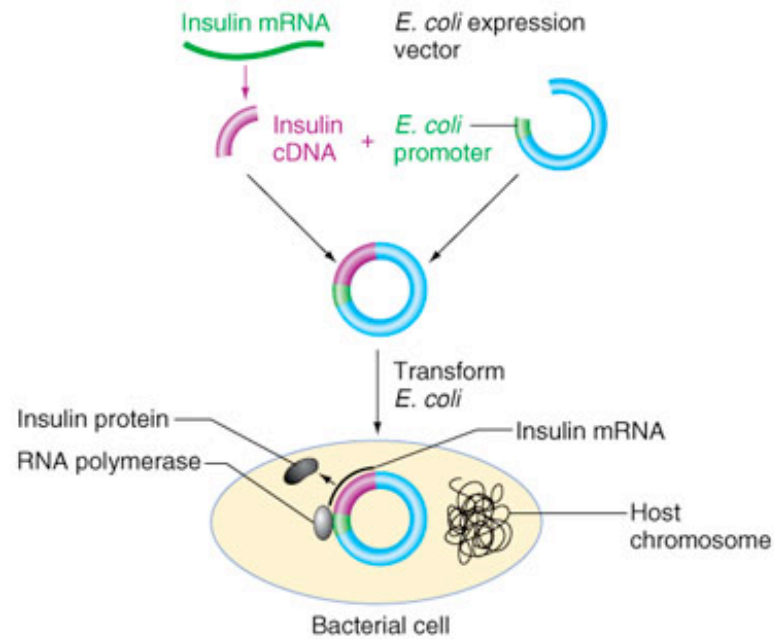
3. PCR (Polymerase Chain Reaction)

4. DNA sequencing

Read 58-62; 64-68; 241-245

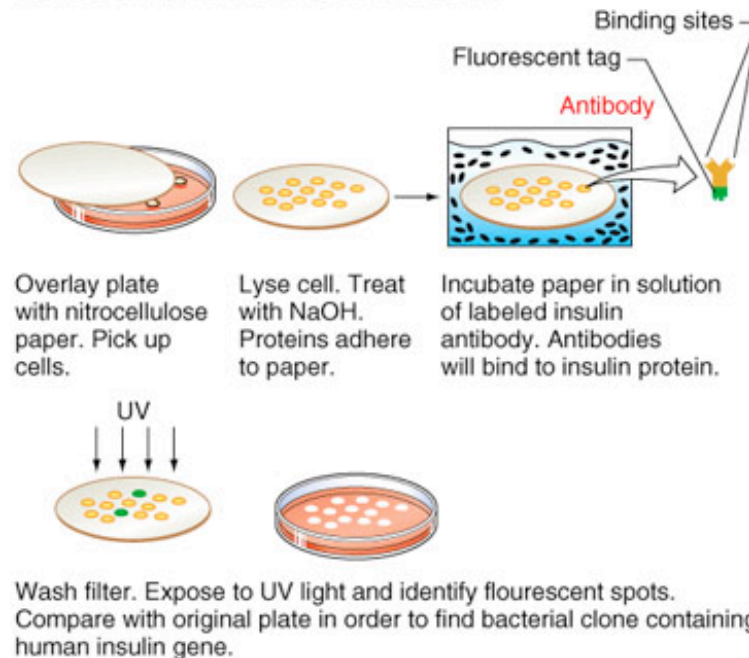
Fig. 2.14-16; 2.19-20; 6.23-26

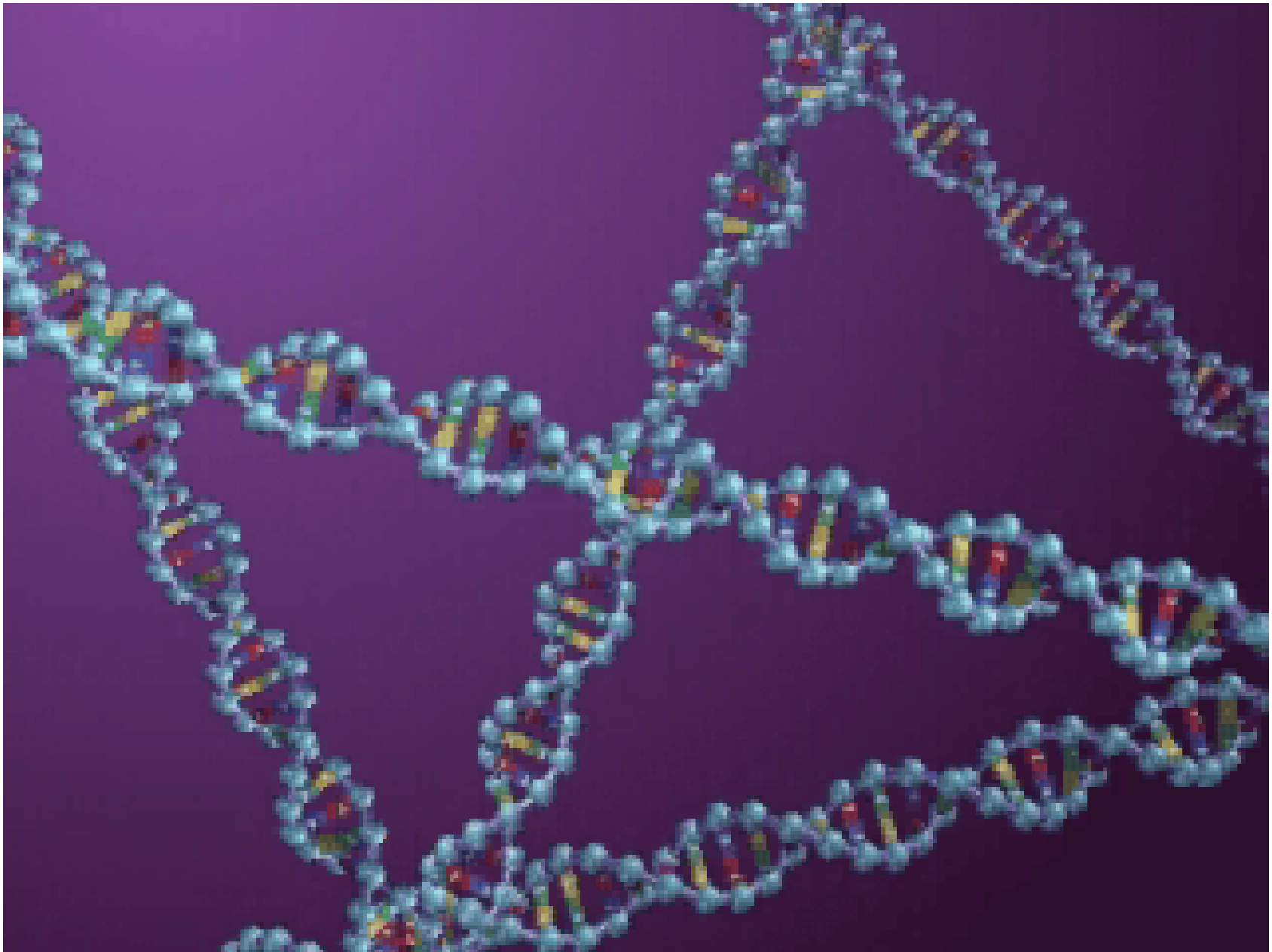
(a) An expression vector allows production of specific polypeptide



Expression Library

(b) Screening for insulin gene expression

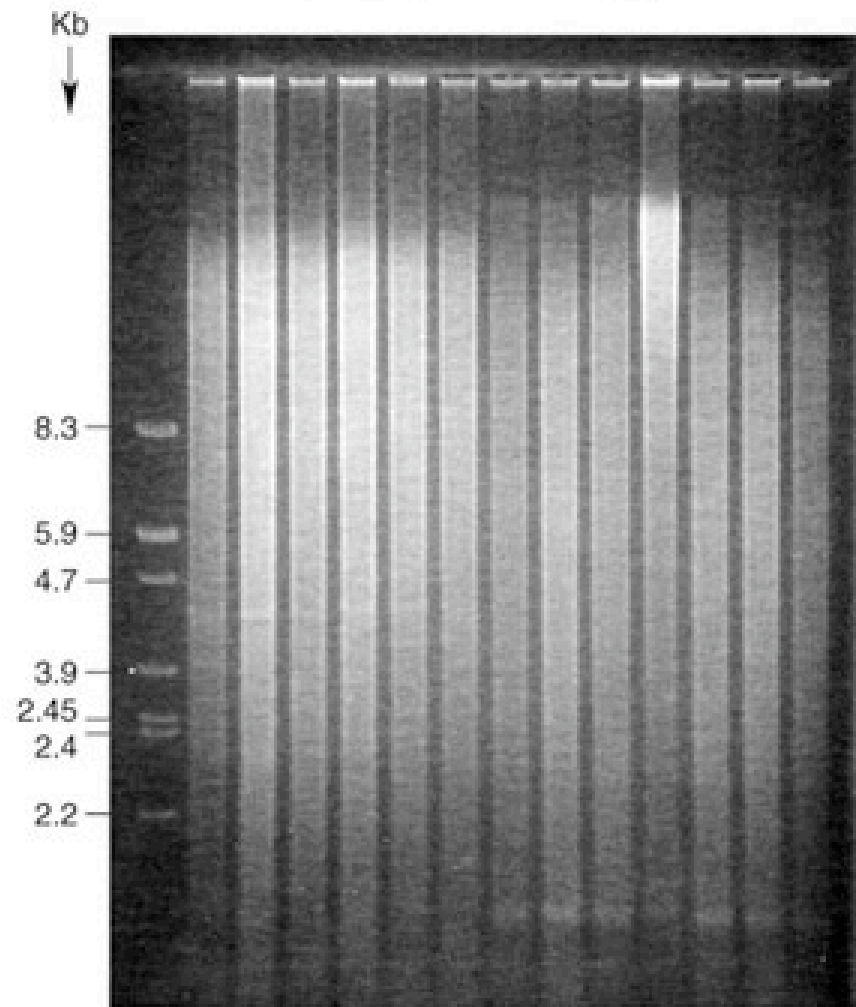




3

Hybridization-probes

Fj



Southern Blot

Fig. 9.15b

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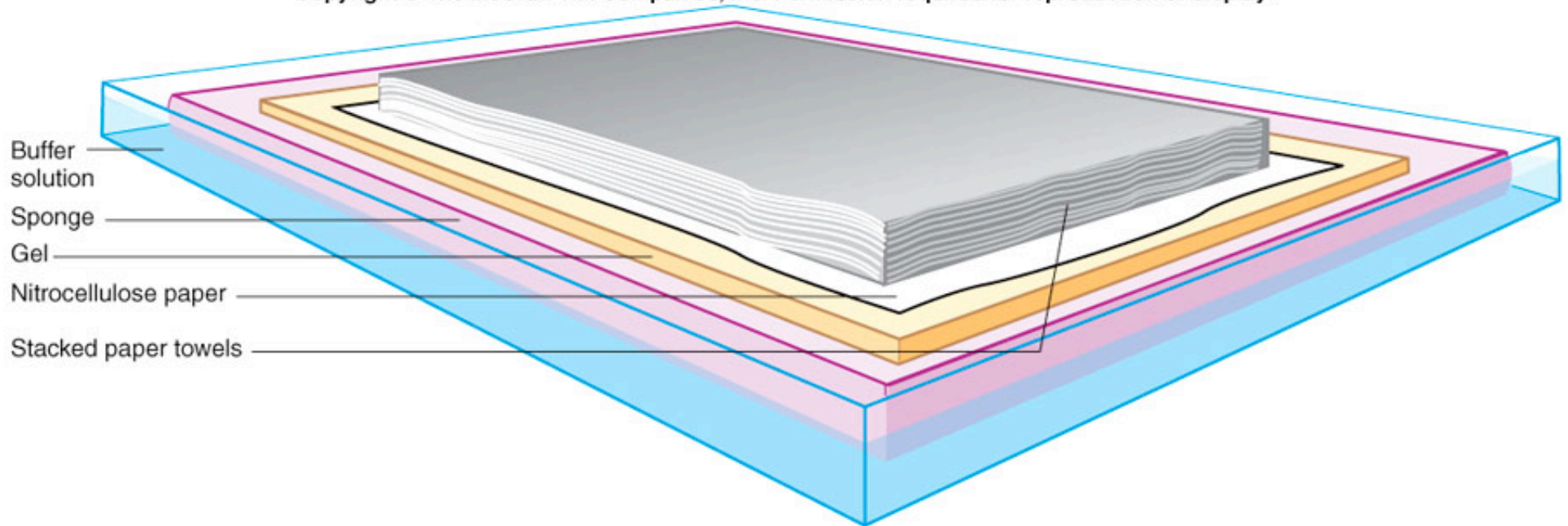
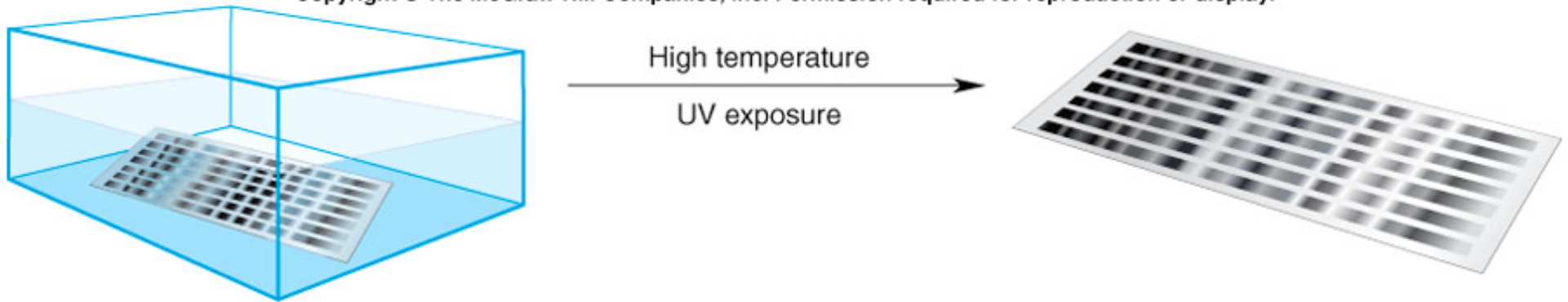


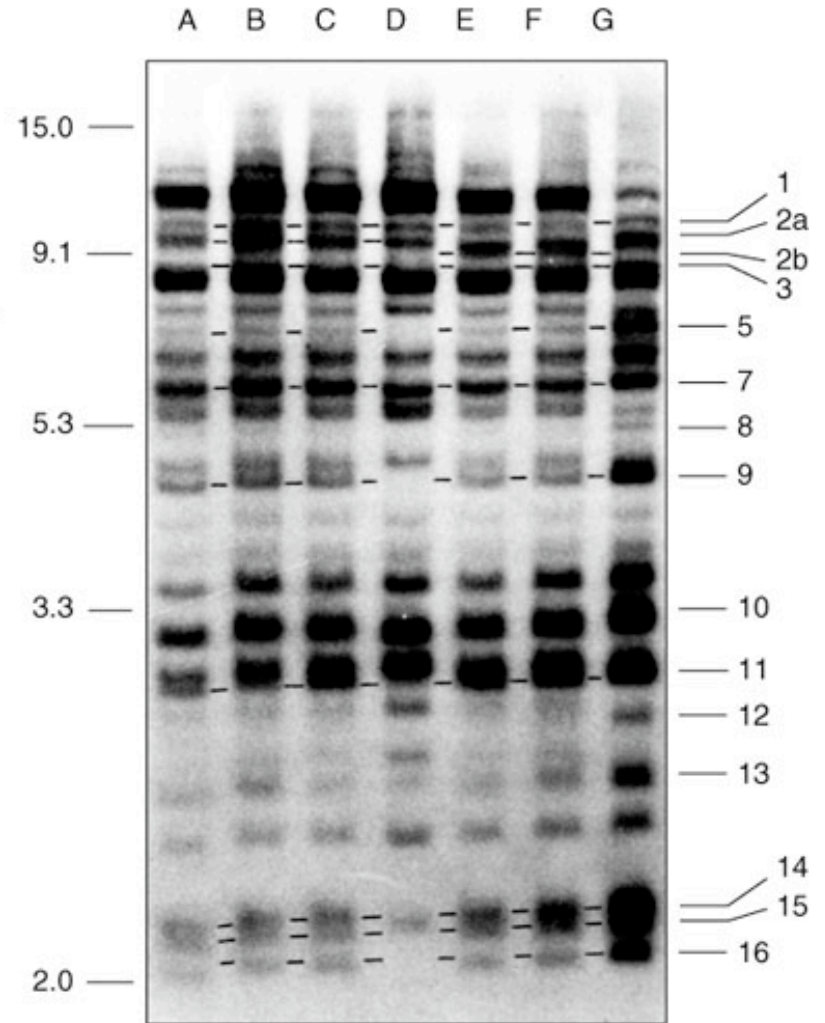
Fig. 9.15c

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Blot is removed,
washed, and exposed
to X-ray film.



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

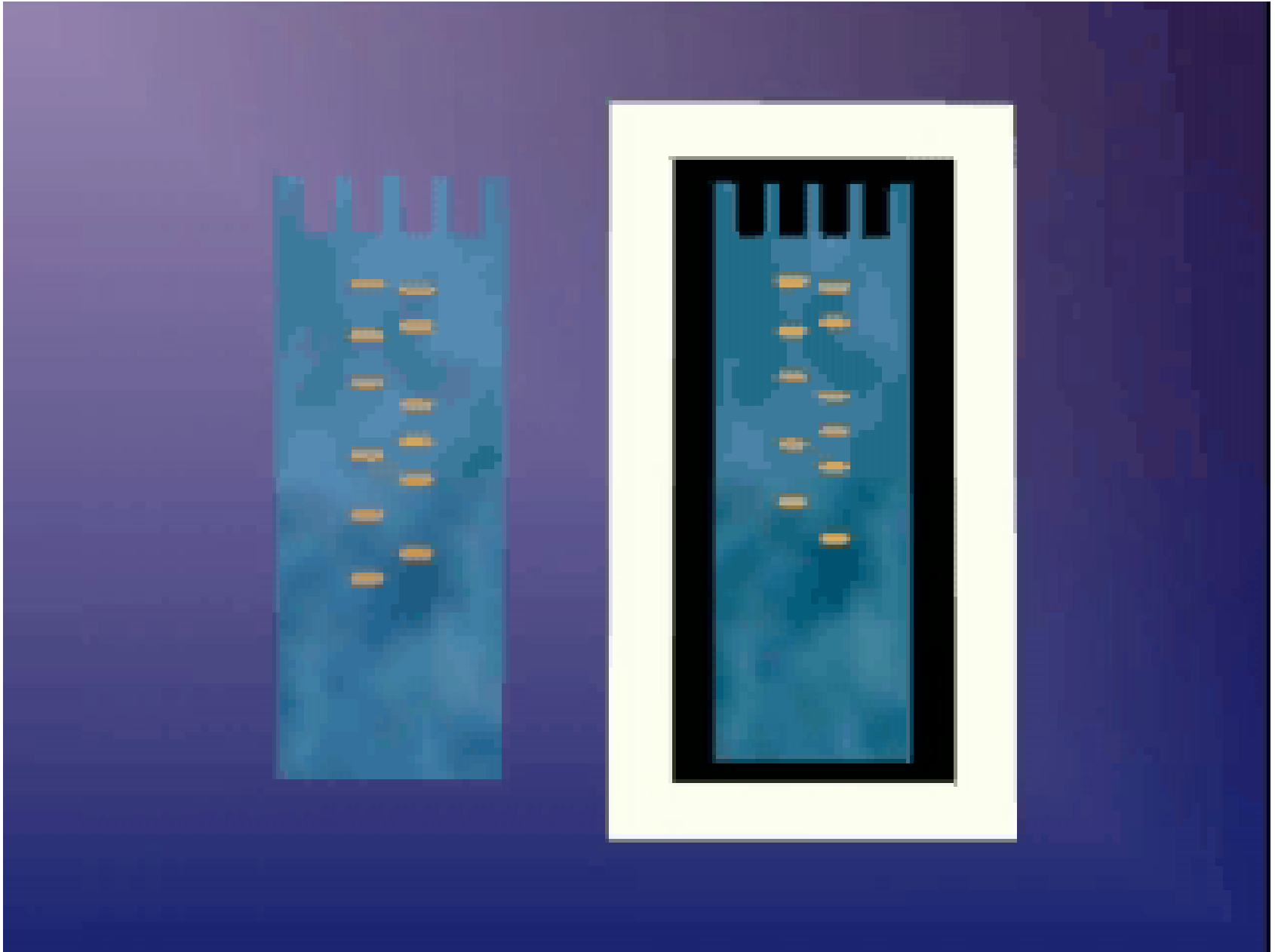
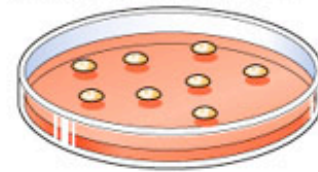
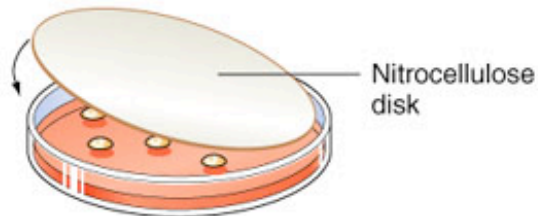


Fig. 9.13

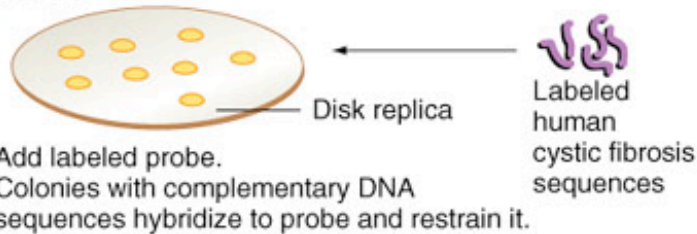
Colony hybridization



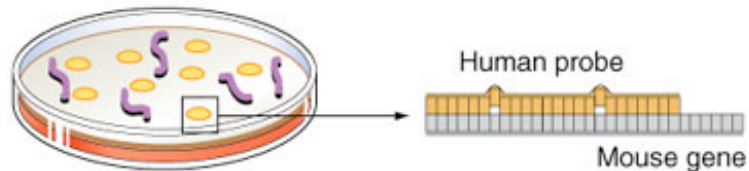
Overlay a nitrocellulose disk to make a replica of the plate.



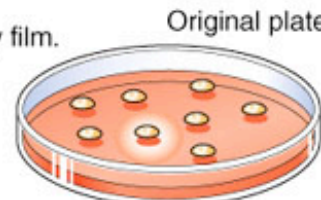
Remove disk from plate and lyse cells on it and denature DNA with NaOH. Bake and treat with UV light to bind DNA strands to disk.



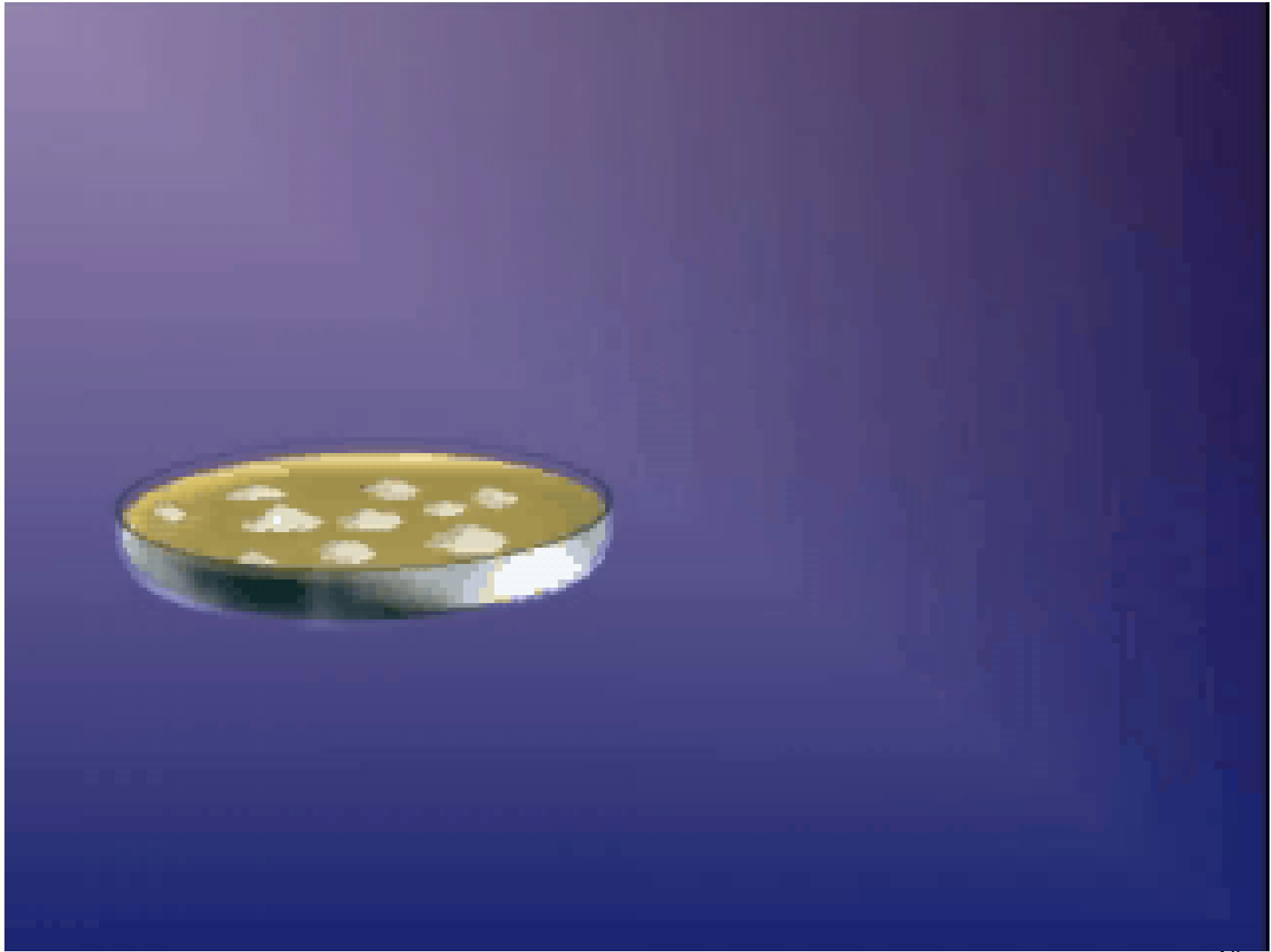
Add labeled probe.
Colonies with complementary DNA sequences hybridize to probe and restrain it.



Wash disk, expose to X-ray film.



Compare with original plate to locate bacterial clone with desired genomic fragment.



Colony hybridization

Fig. 9.16

The Polymerase Chain Reaction (PCR)

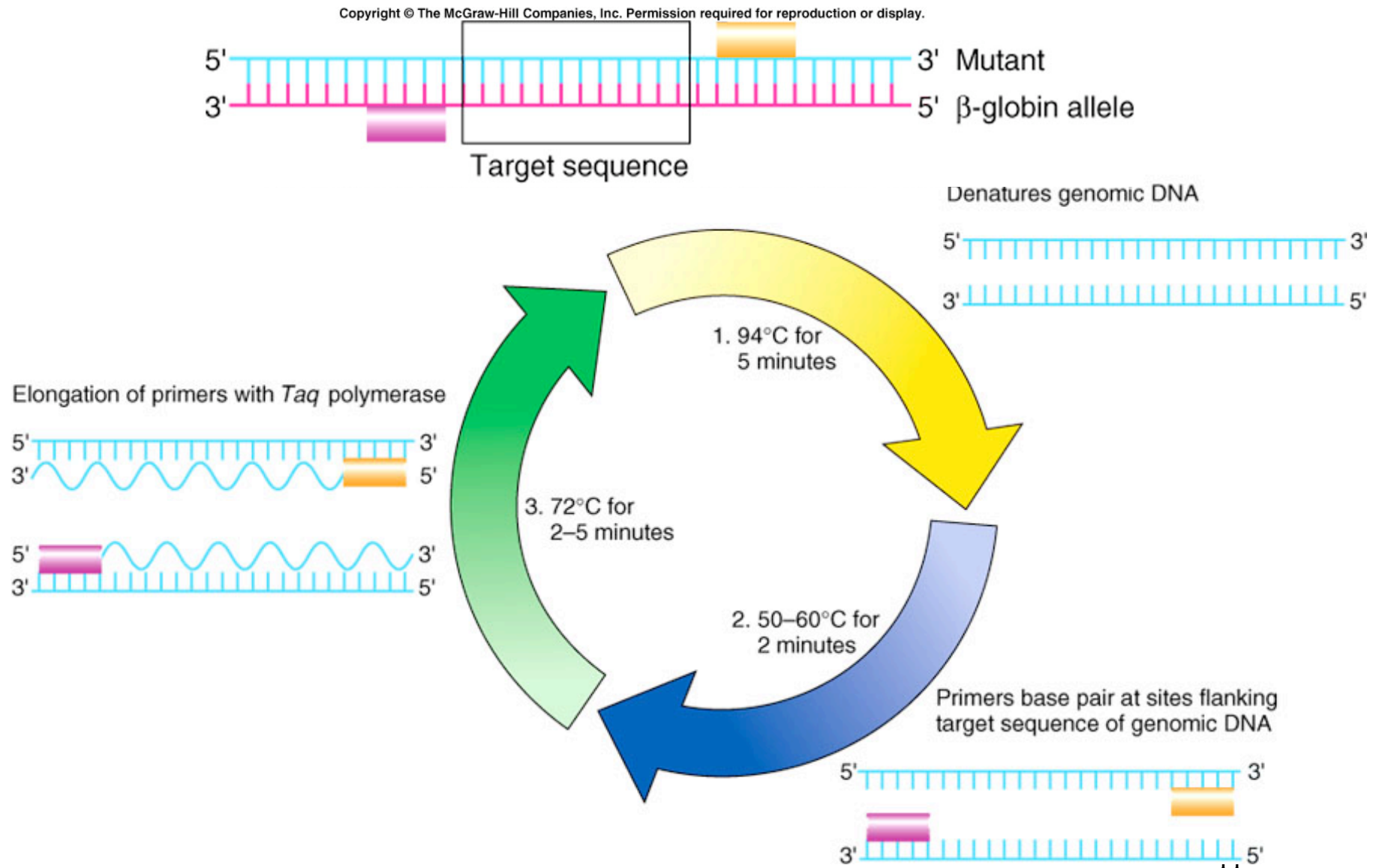
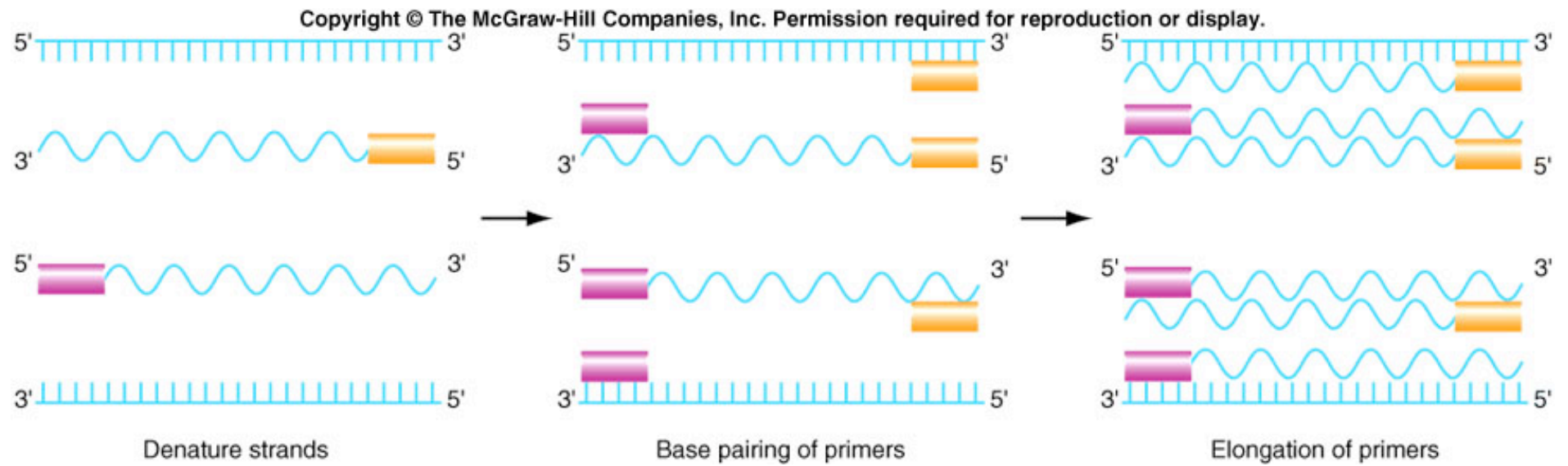
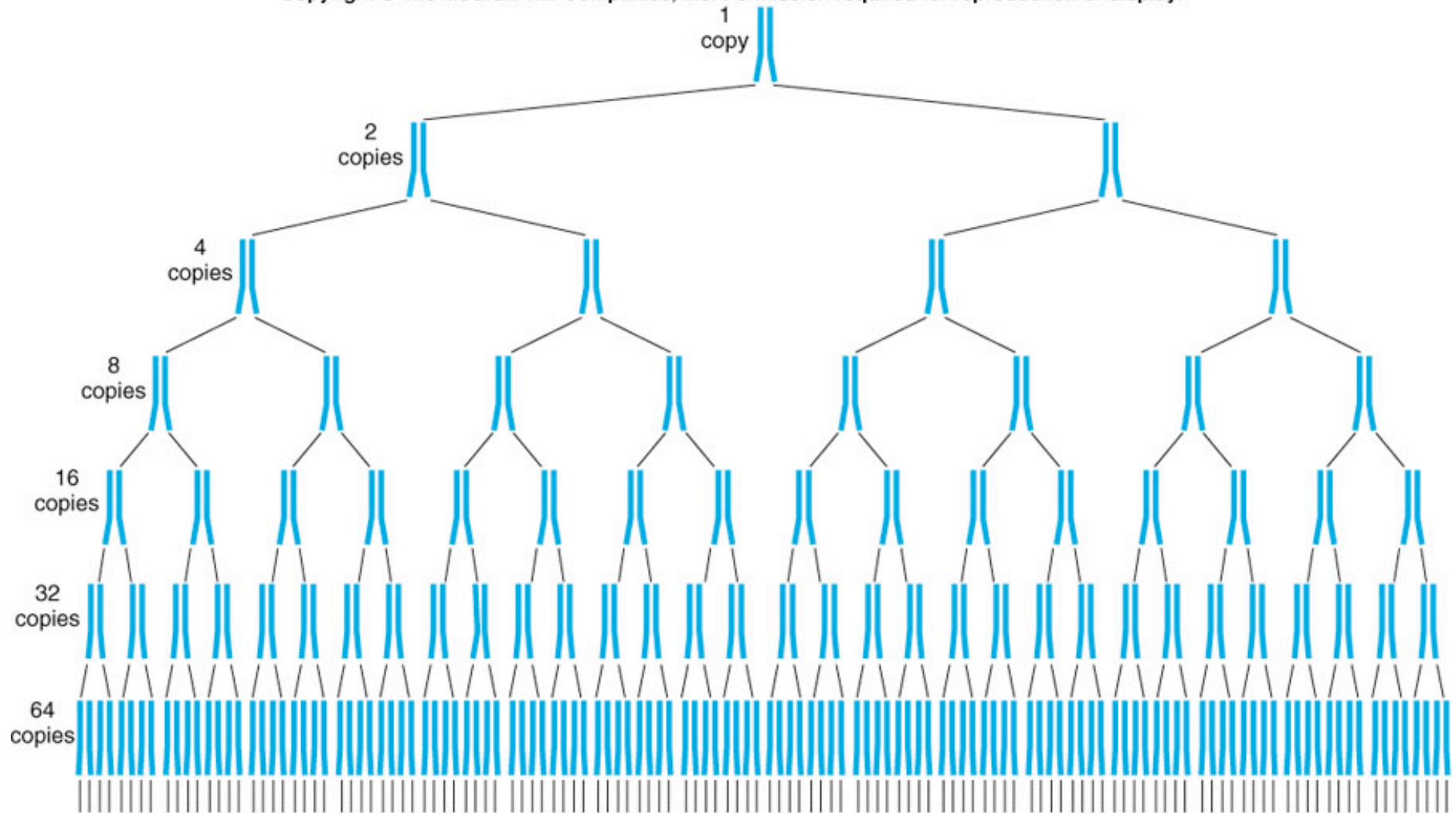
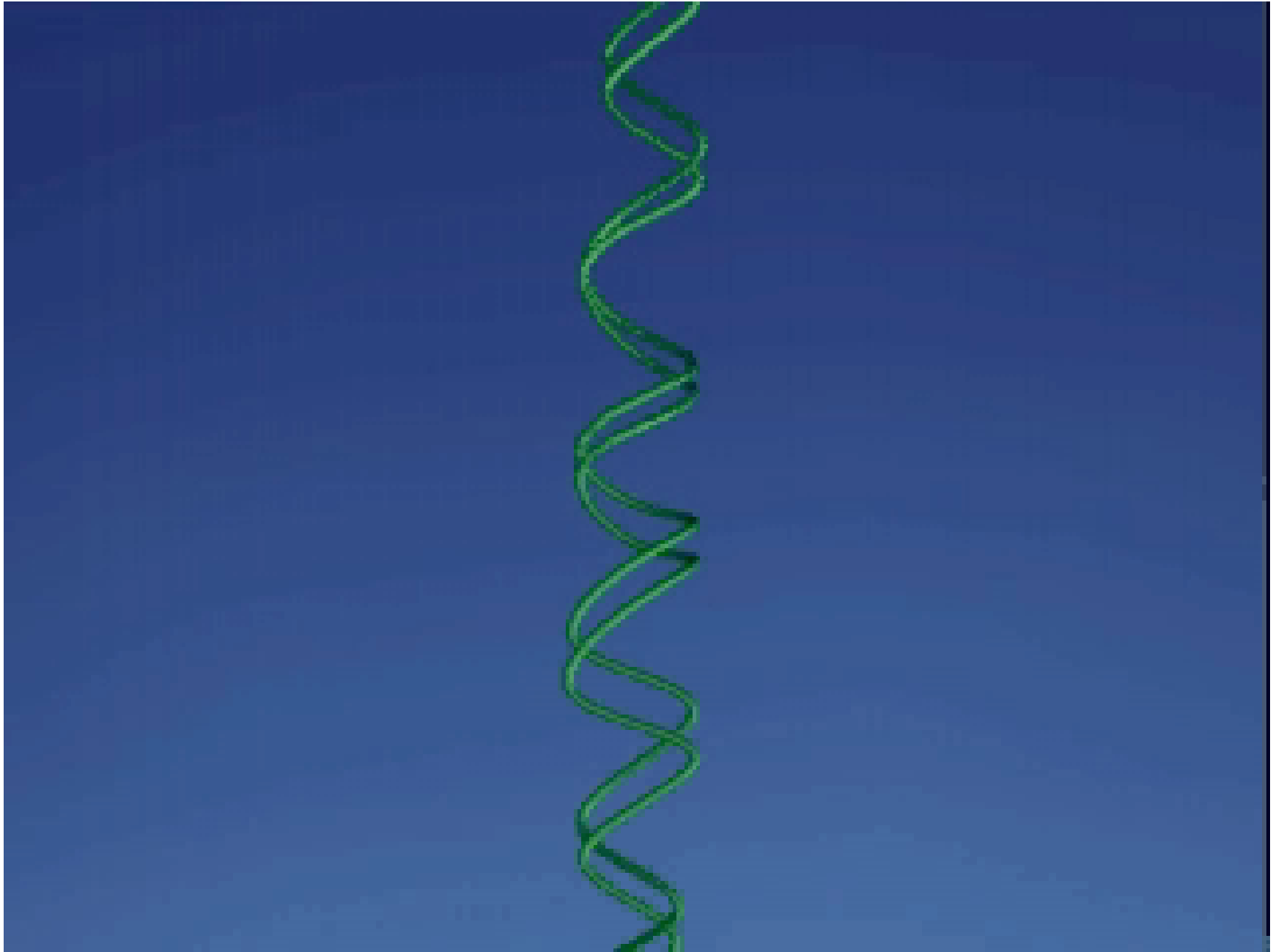


Fig. 9.16c

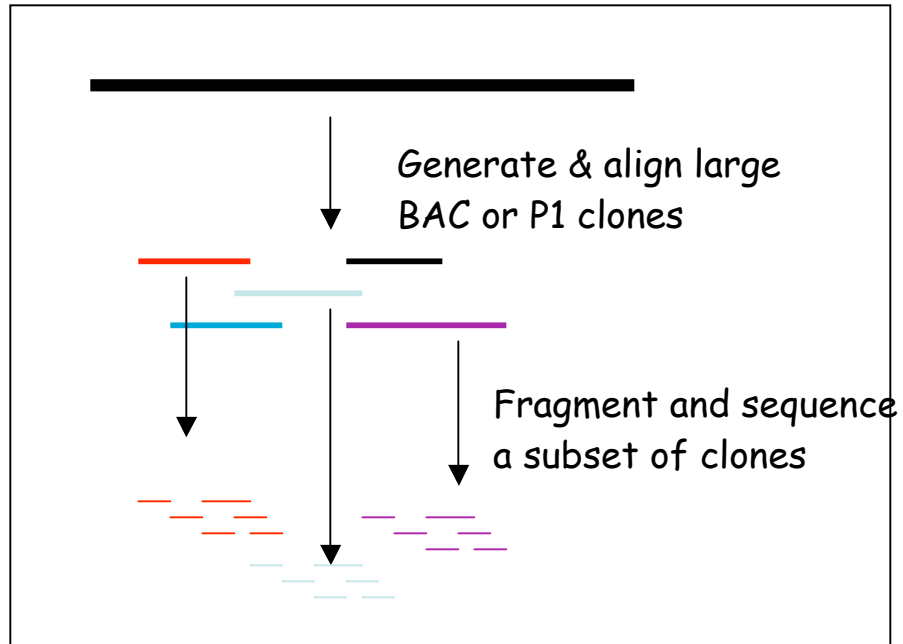




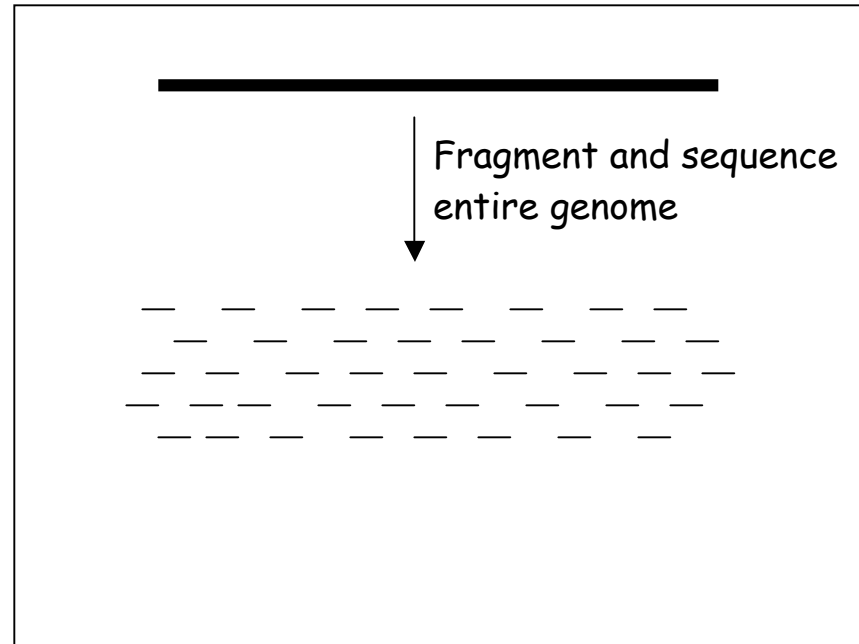


PCR movie

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

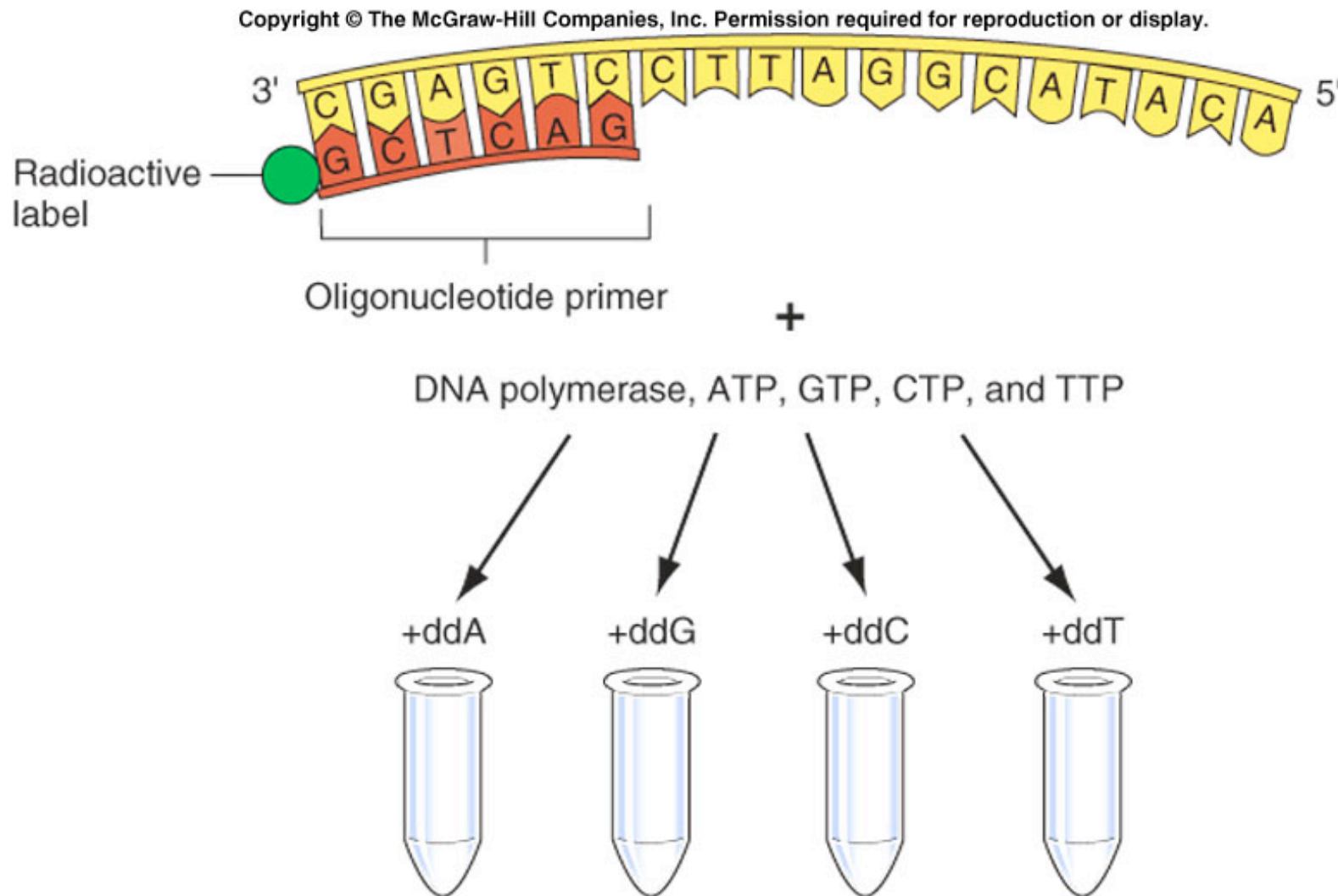
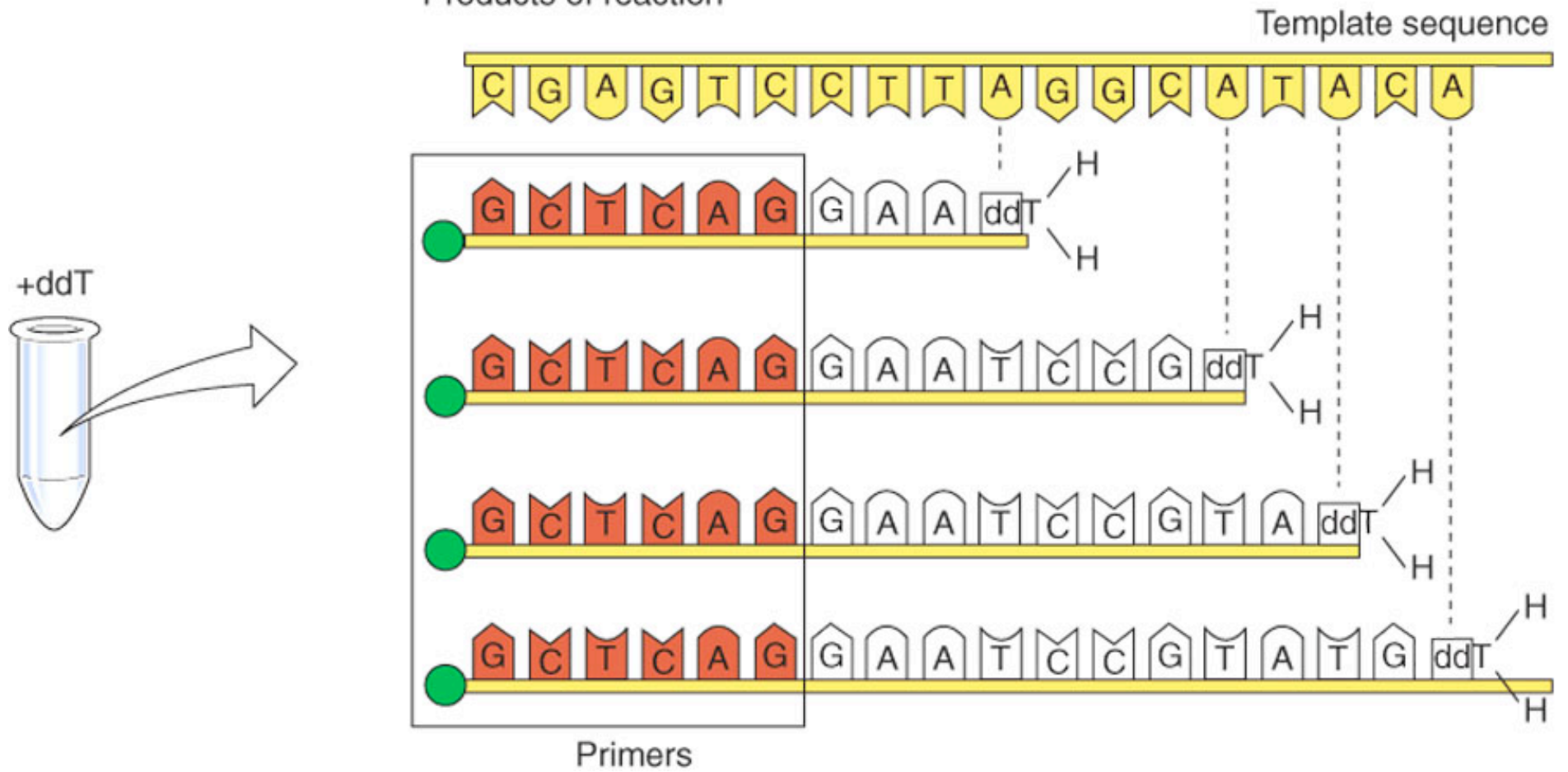


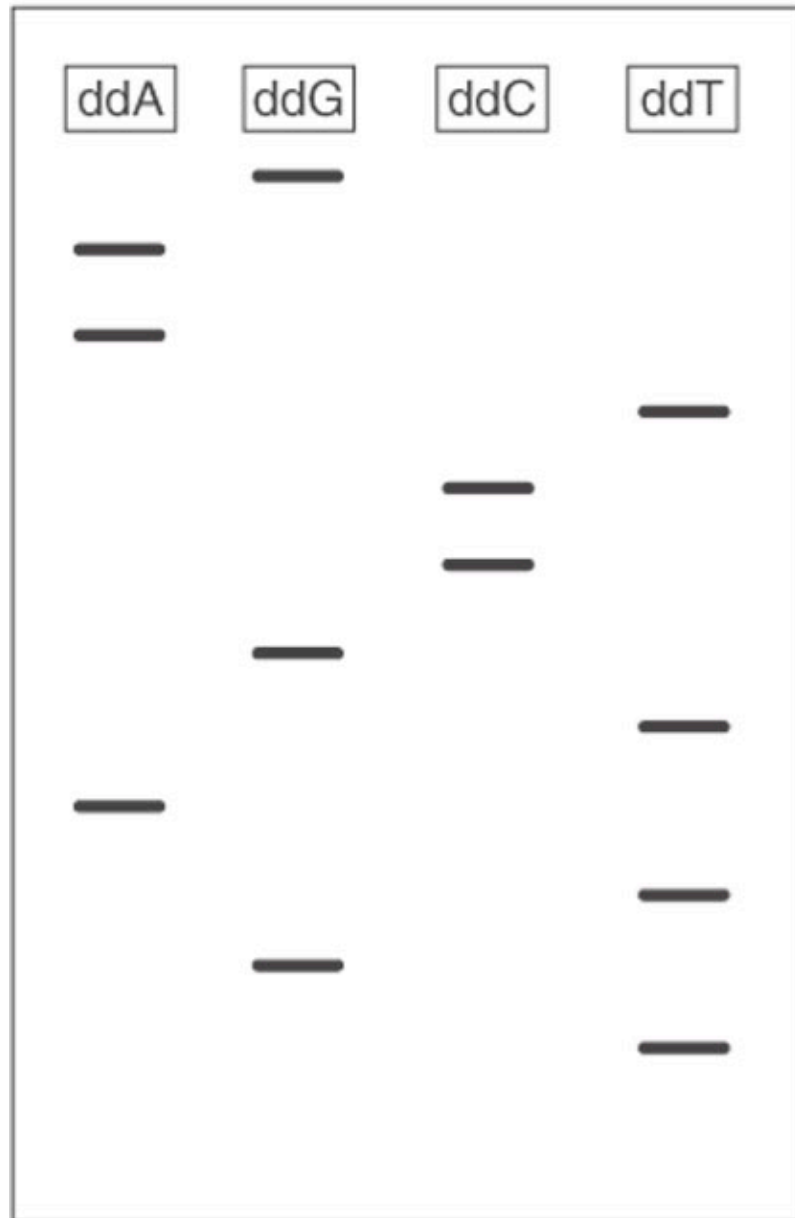
Fig. 9.17b

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Products of reaction



Gel analysis of fragments



Sequence of synthesized DNA

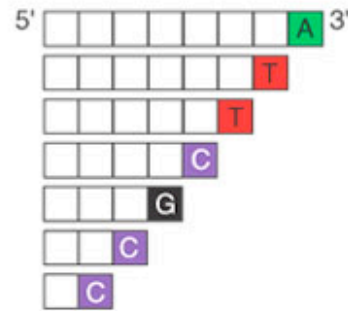
G
A
A
T
C
C
G
T
A
T
G
T

Sequence of template DNA

5' C
T
T
A
G
G
C
A
T
A
C
A
3'

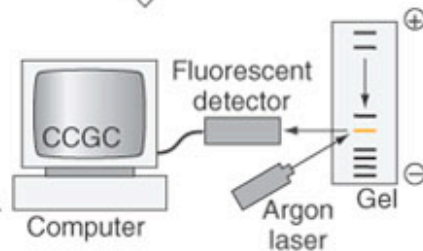
(a) Automated sequencing

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.

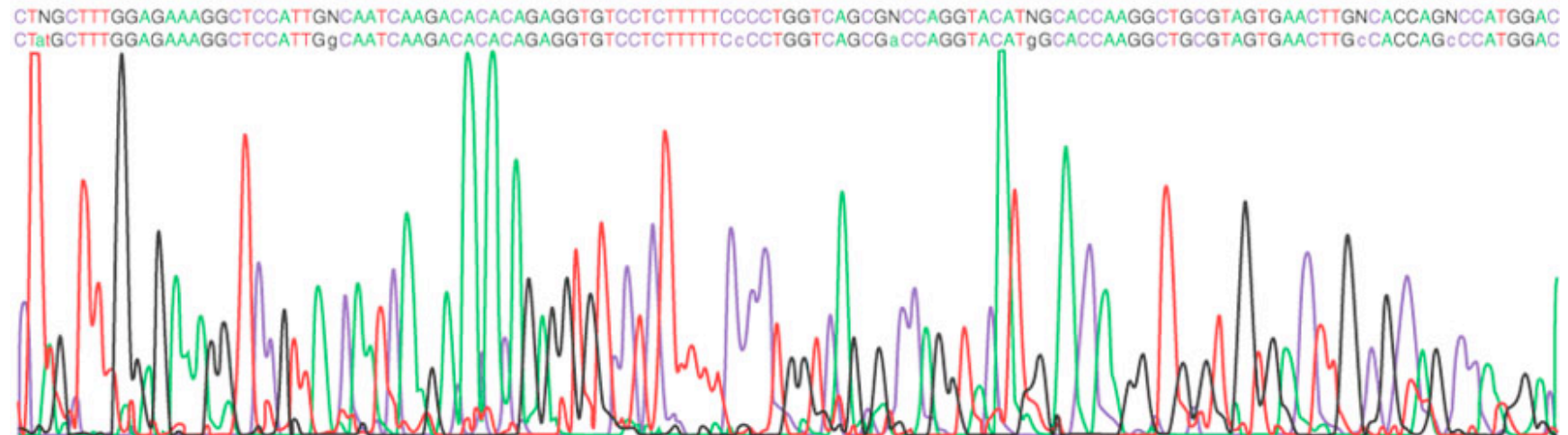
3. As migrating fragments pass through the scanning laser, they fluoresce. A fluorescent detector records the color order of the passing bands. That order is translated into sequence data by a computer.



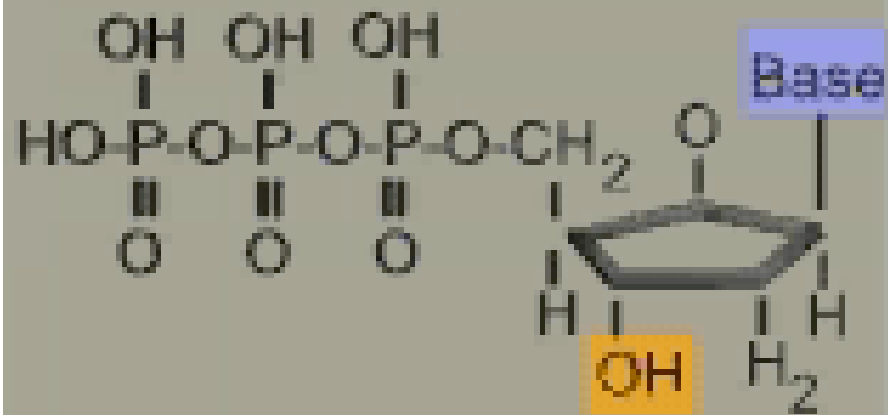
(b)



(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).

