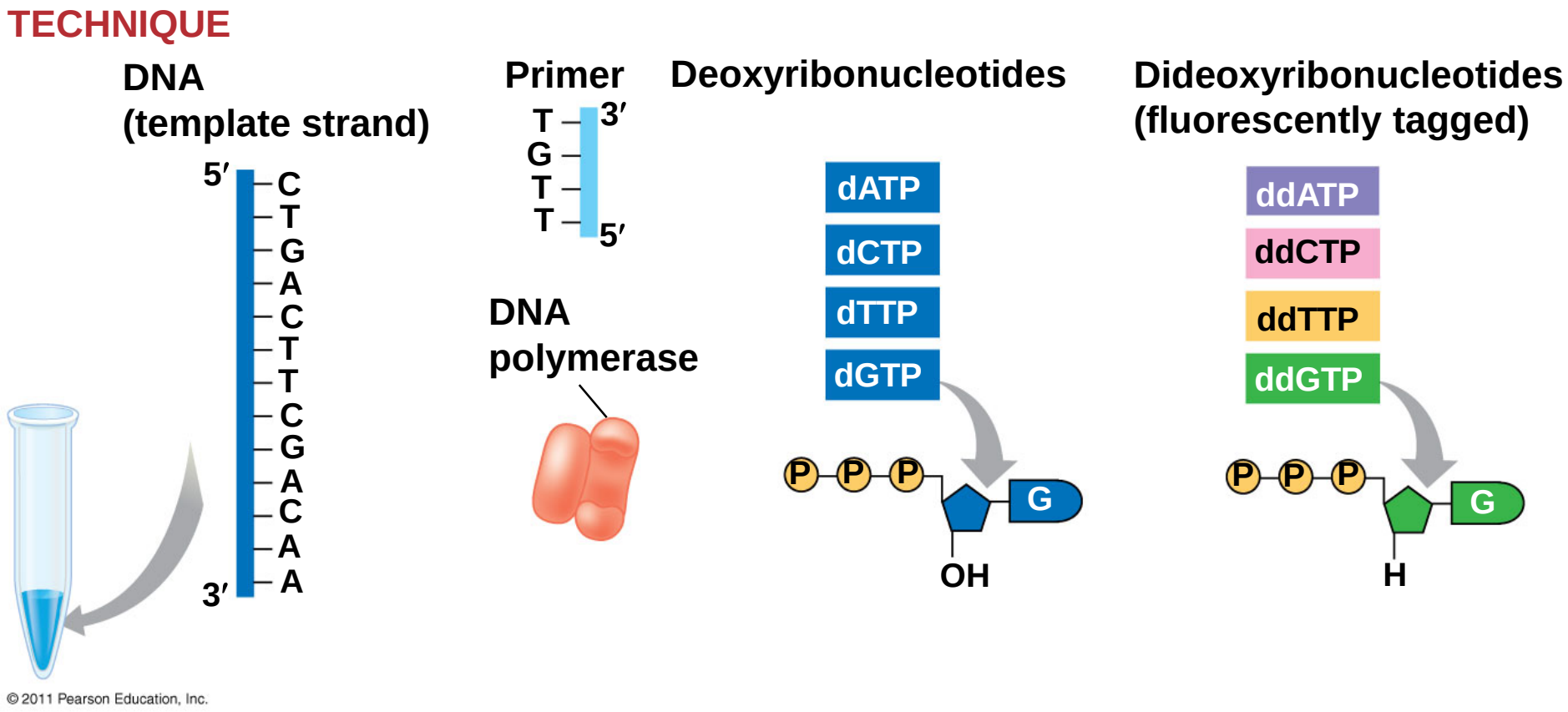
# Chapter 20: Biotechnology

1. DNA Sequencing
2. DNA Cloning
3. Studying Gene Expression
4. Manipulating Genomes
5. Therapeutic & Diagnostic Techniques

# 1. DNA Sequencing

**Chapter Reading – pp. 409-412**

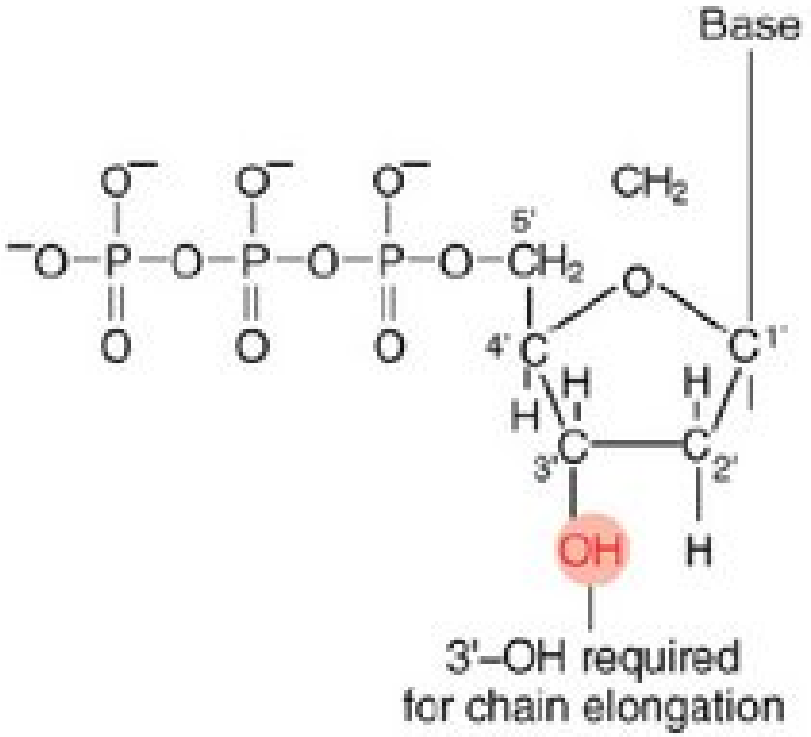
# DNA Sequencing…



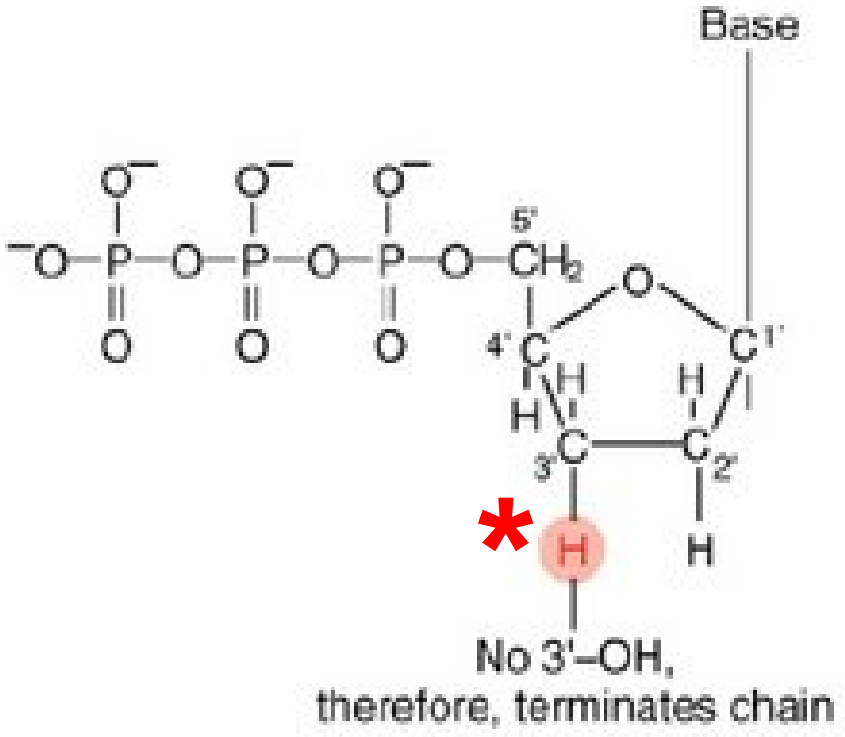

- DNA replication *in vitro* using 1 of 4 different "chain-terminating" <u>dideoxy</u>nucleotides (ddNTPs)
- results in a set of DNA fragments ending in all positions with A, C, G, or T that can be resolve by length on a gel

# DNA Sequencing uses Chain Terminators

**Normal nucleotide (dNTP)**




**Dideoxynucleotide (ddNTP)**



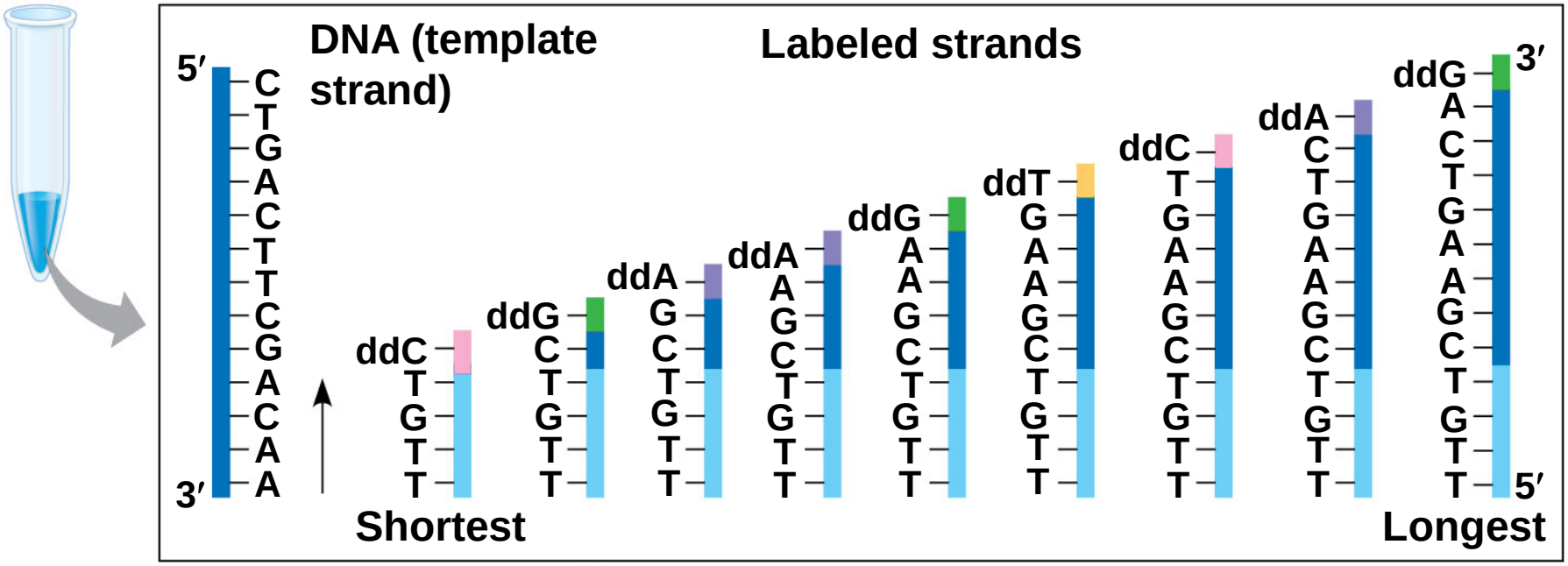
# DNA synthesis is carried out in reactions containing the following:

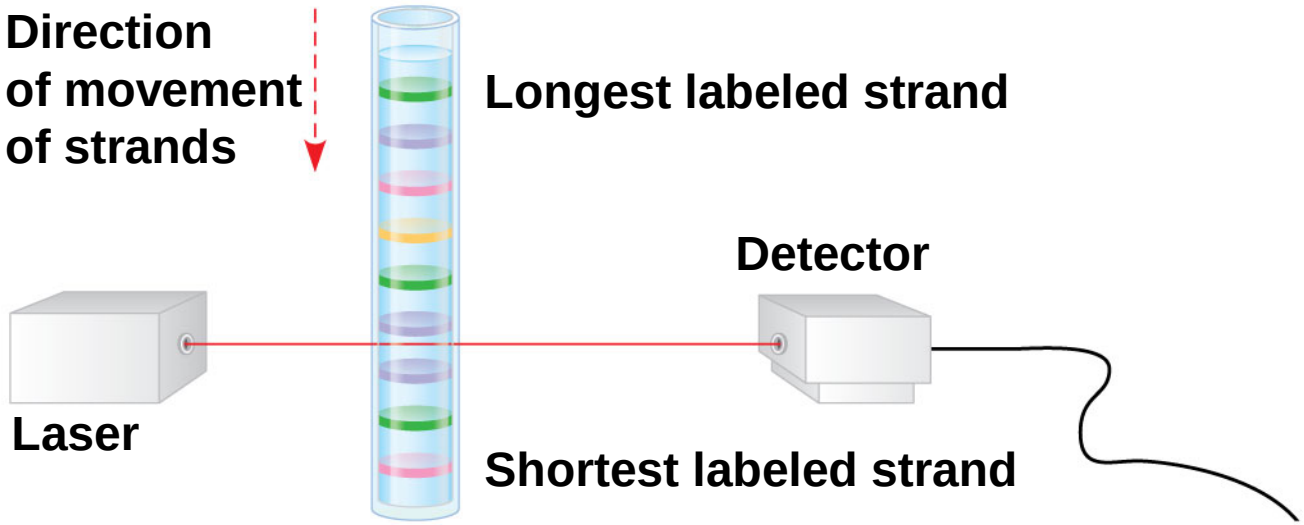
- DNA template to be sequenced
- dNTP's
- DNA primer
- DNA polymerase
- ddATP, ddCTP, ddGTP or ddTTP
  - each ddNTP is labeled with a different fluorescent tag

**The color reveals the identity of the ddNTP that terminated DNA synthesis at each position, thus revealing the sequence!**

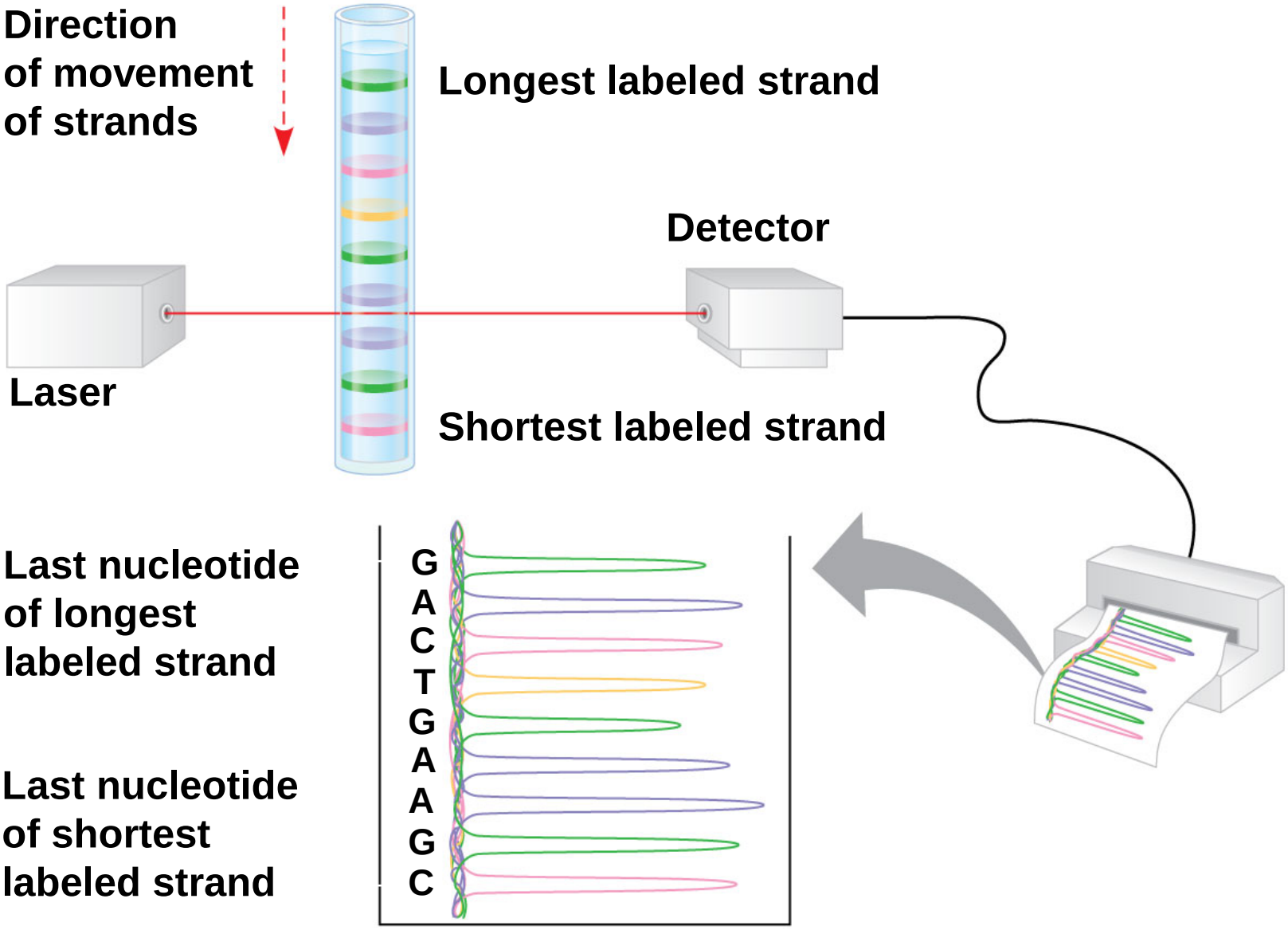
# …DNA Sequencing…

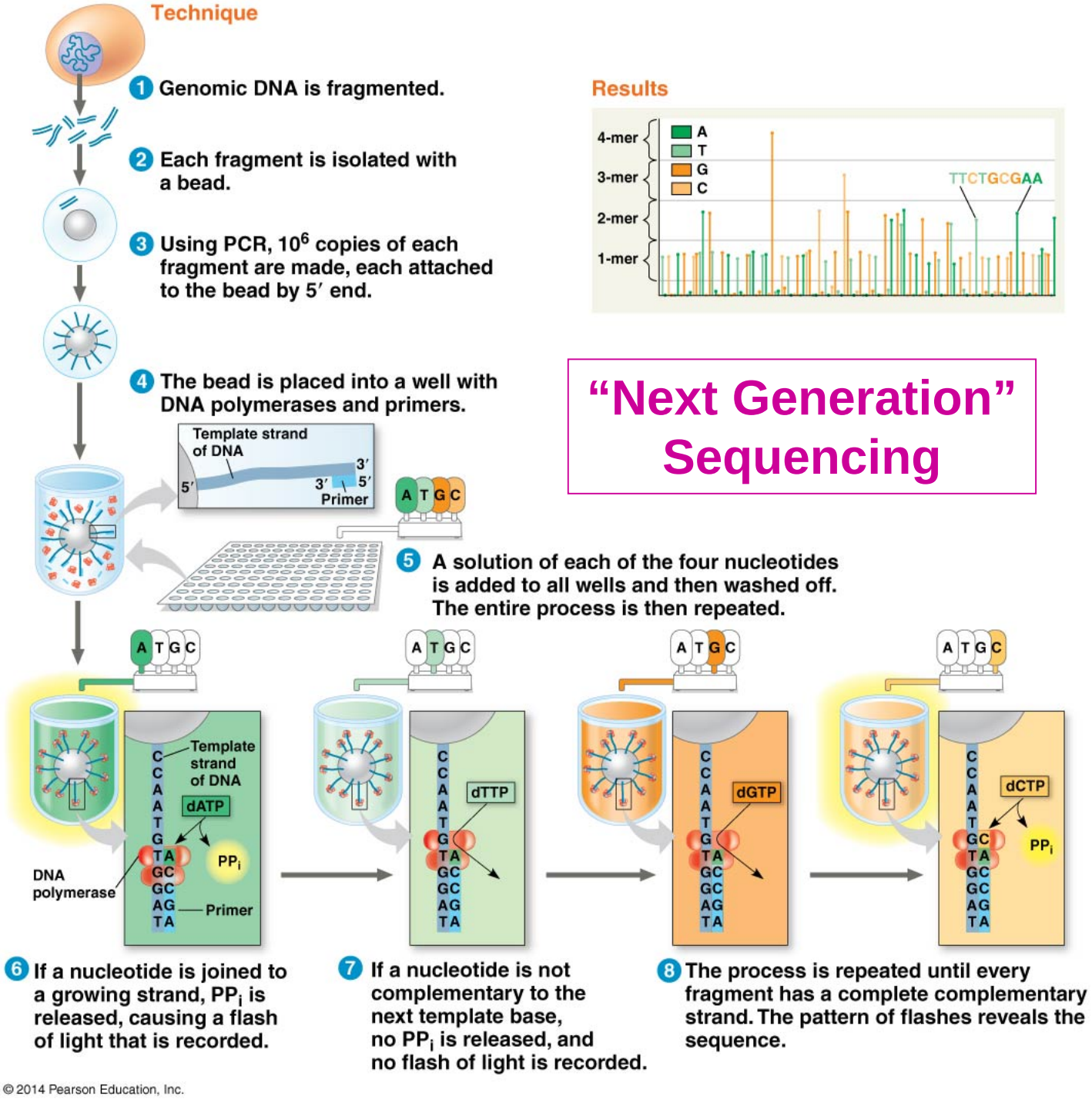
## TECHNIQUE (continued)



# …DNA Sequencing






**"Next Generation" Sequencing**

# 2. DNA Cloning

**Chapter Reading – pp. 409-417**

# What is “Recombinant DNA”?

**The joining of DNA from different sources.**

**This can happen in nature (*in vivo*)…**

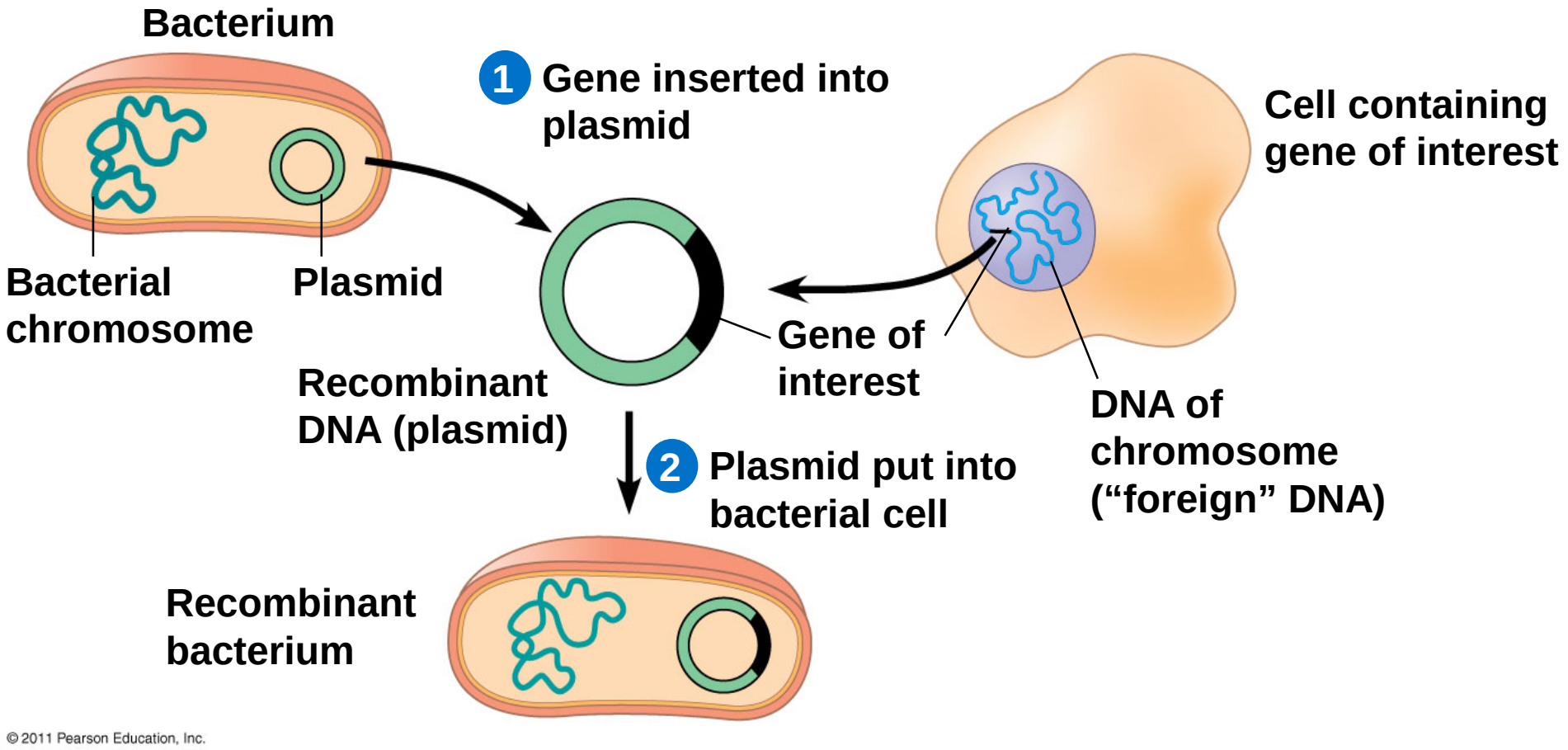
- **the transfer of DNA involving bacteria or viruses**

**…or in the laboratory (*in vitro*)**

- **the cutting & splicing of DNA fragments by molecular biologists**

**The term “recombinant DNA” generally refers to the in vitro kind which is commonly called “gene cloning”…**

# Gene Cloning…






- gene of interest is inserted into a plasmid vector
- bacterial host transformed with recombinant plasmid
- bacterial clone with recombinant plasmid is identified

# …Gene Cloning

**Cloned gene can then be used in a variety of ways.**

3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest

Protein expressed from gene of interest

Gene of interest

Copies of gene

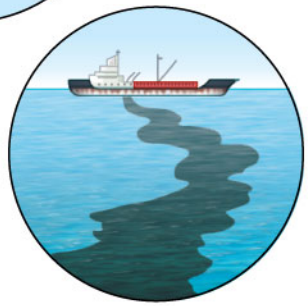
Protein harvested

4 Basic research and various applications

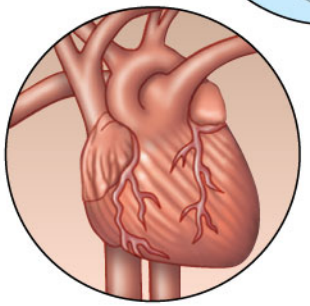
Basic research on gene

Basic research on protein

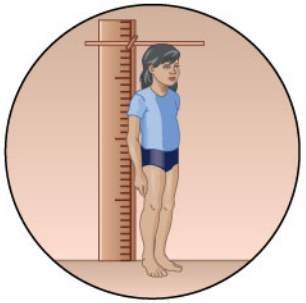
Gene for pest resistance inserted into plants



Gene used to alter bacteria for cleaning up toxic waste

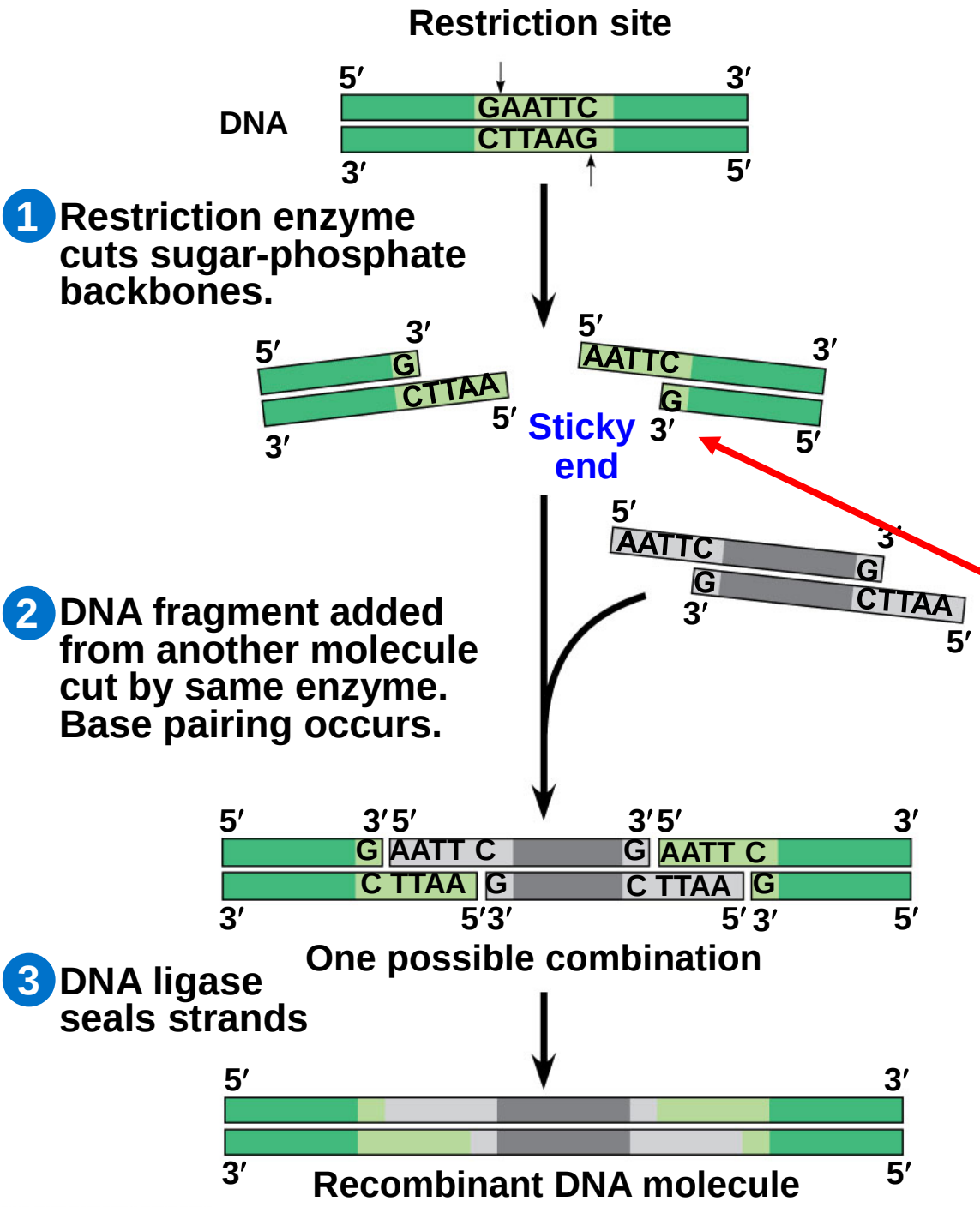


Protein dissolves blood clots in heart attack therapy



Human growth hormone treats stunted growth

# Restriction Enzymes



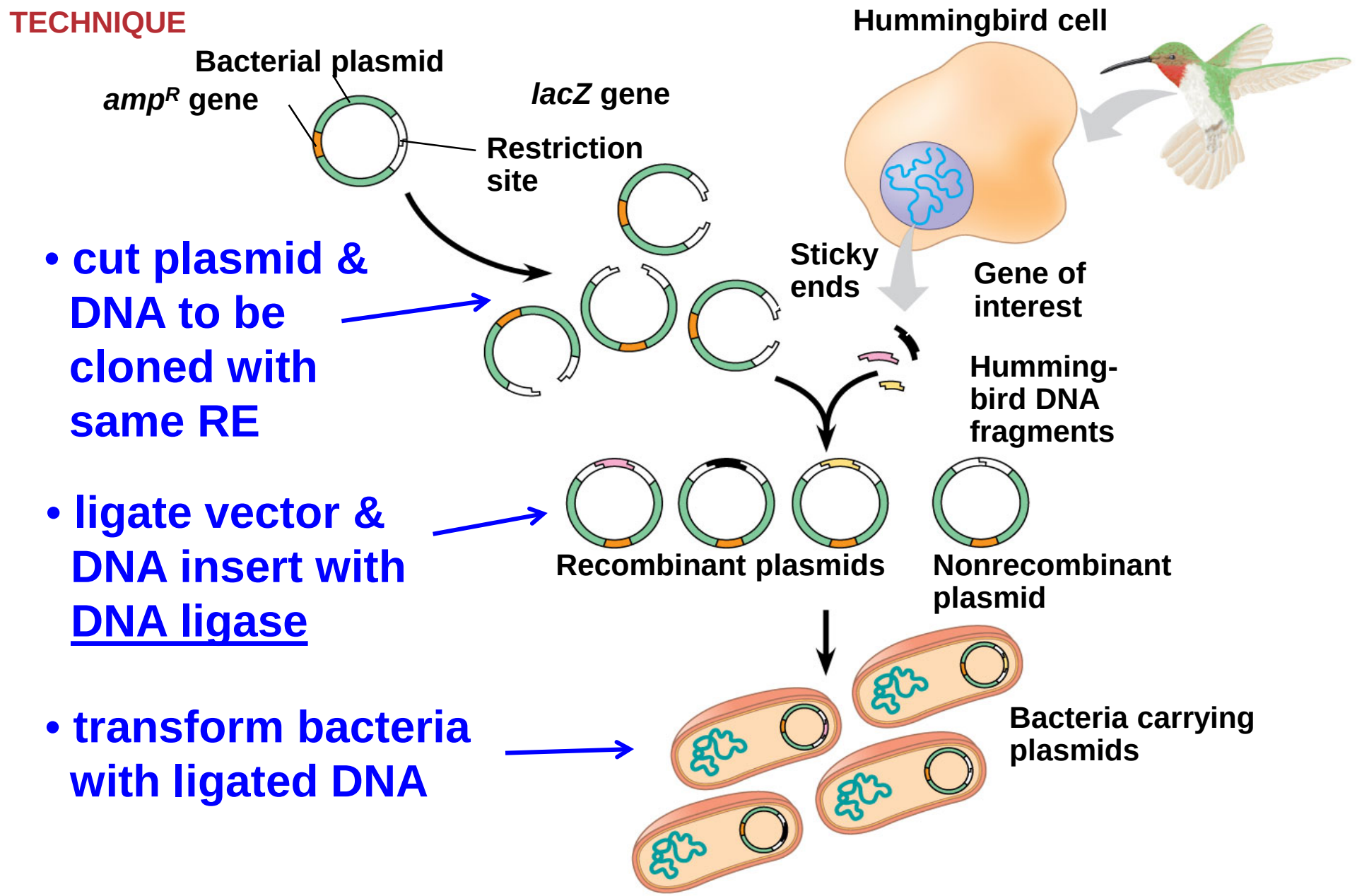

Gene cloning usually involves the use of <u>restriction enzymes</u> that cut DNA at very specific sequences:

e.g. EcoRI cuts at:
..GAATTC..
..CTTAAG..

There are many different restriction enzymes, each cutting a different sequence

# The Gene Cloning Technique

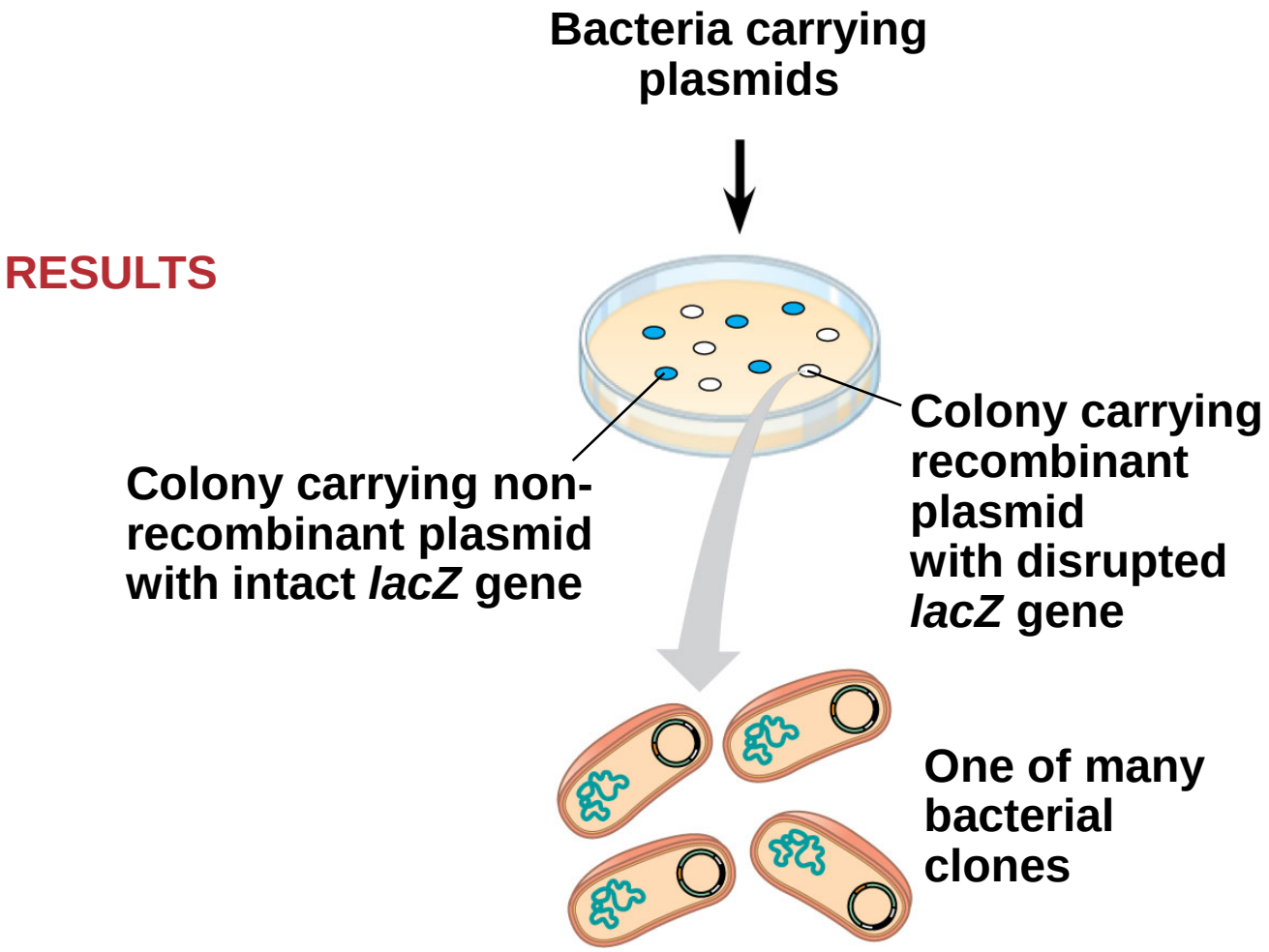
**TECHNIQUE**




- cut plasmid & DNA to be cloned with same RE
- ligate vector & DNA insert with <u>DNA ligase</u>
- transform bacteria with ligated DNA

# Selection for Bacterial Clones

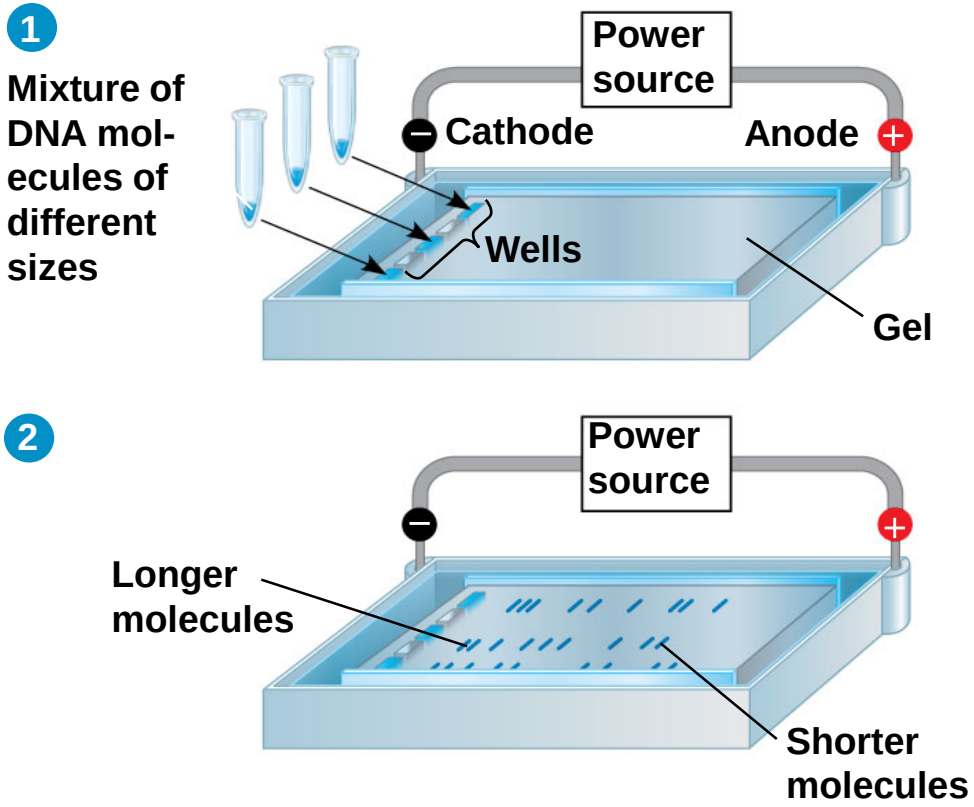
**Antibiotic resistance allows selection for bacterial clones containing the plasmid vector.**



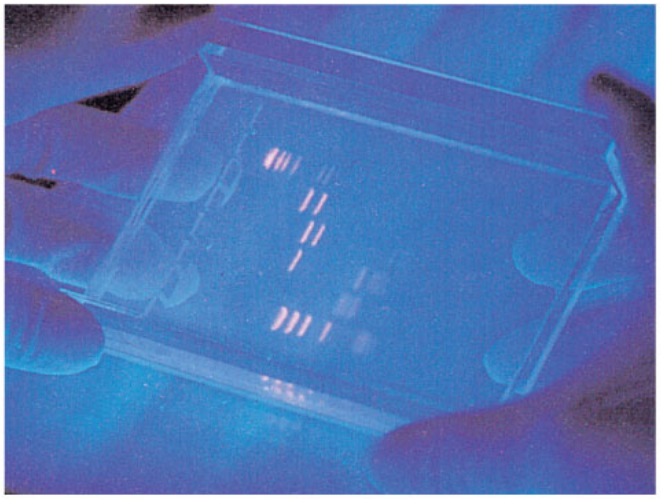

**Blue/white selection via *lacZ* gene disruption allows identification of clones containing a DNA insert.**

# Gel Electrophoresis

TECHNIQUE




RESULTS



**Separation of DNA fragments through a porous gel matrix:**

- gel is either agarose or polyacrylamide
- electric current pulls negatively charged DNA toward the positive pole
- rate of movement is inversely proportional to DNA fragment size

# DNA Libraries…

**A collection of cloned genes from an organism is called a DNA library.**

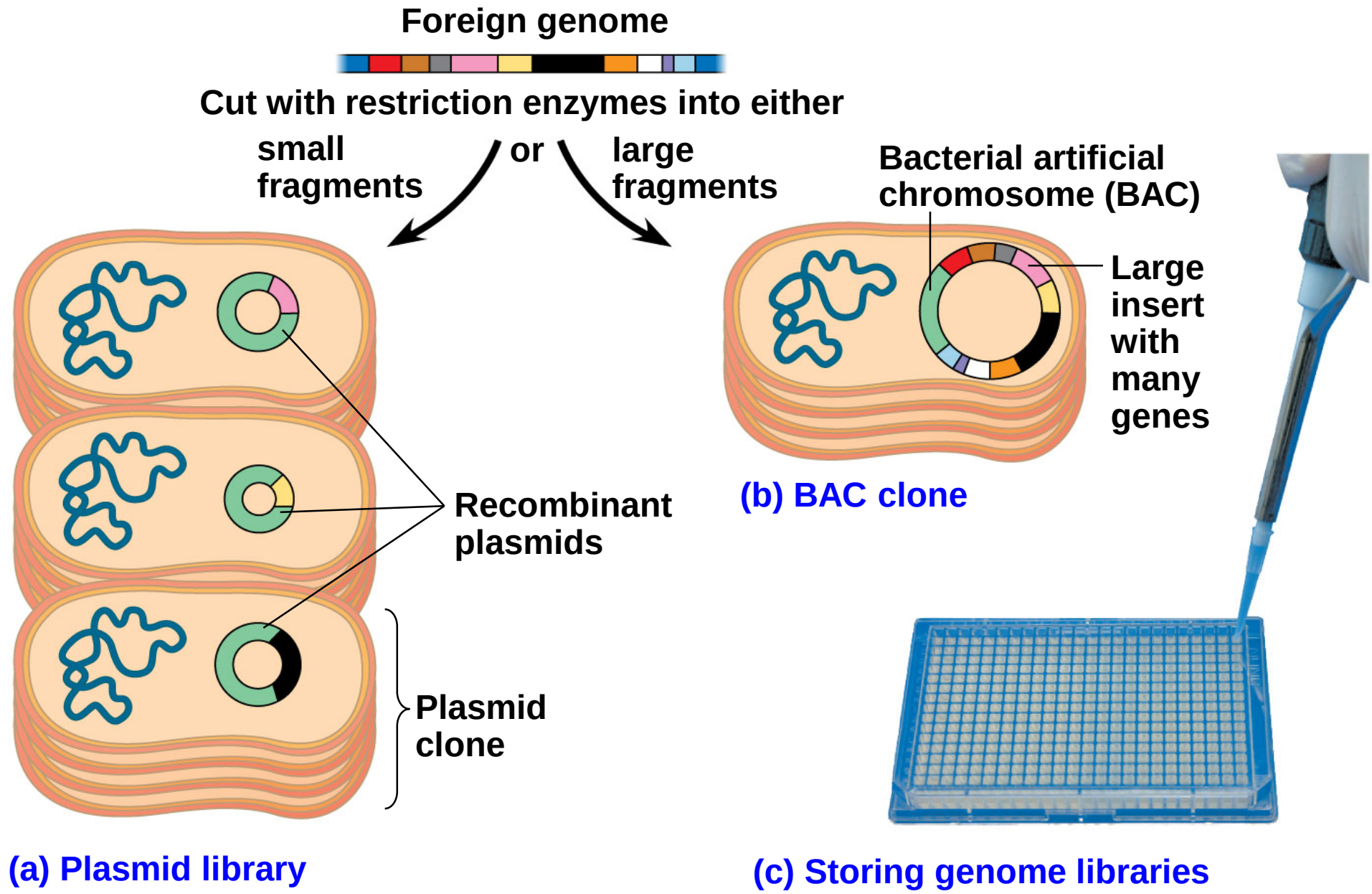
## Genomic DNA Library

- **a collection of chromosomal DNA fragments cloned into a particular vector**
- **essentially cloned pieces of the organism's genome**

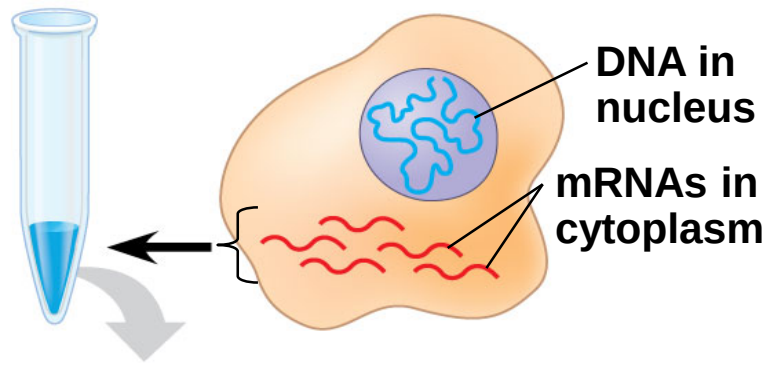
## cDNA Library

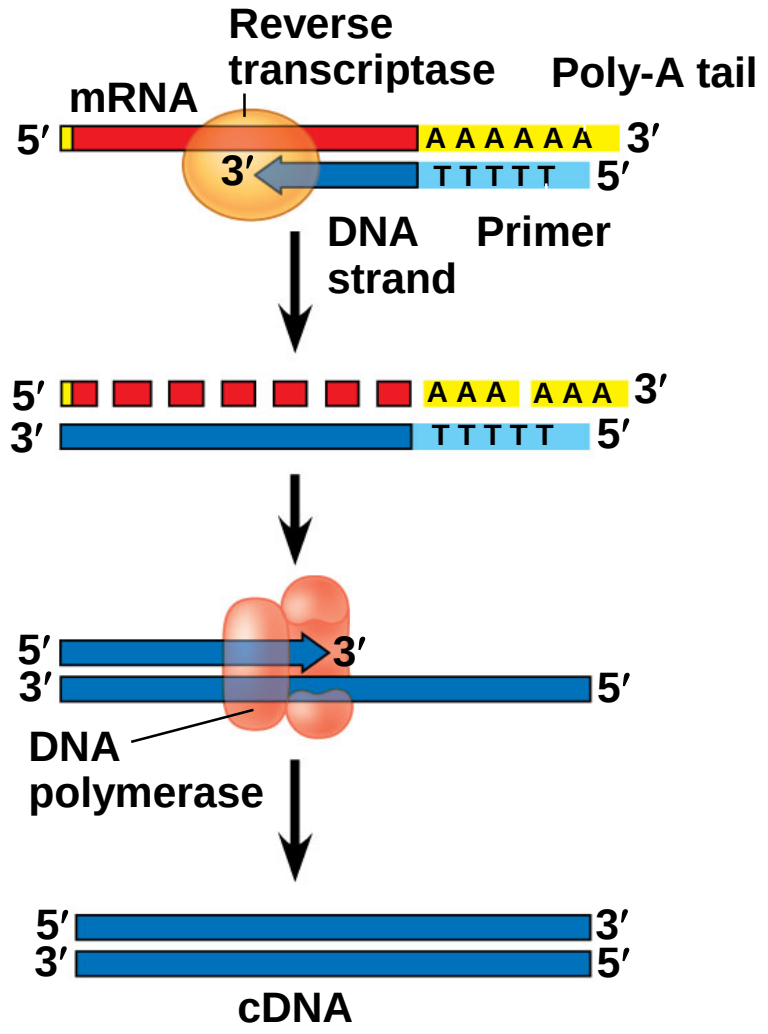
- **a collection of DNA fragments produced from messenger RNA (mRNA) cloned into a vector**
- **produced from mRNA by reverse transcriptase**
- **a collection of expressed genes with NO intron DNA**

# …DNA Libraries



# Producing cDNA



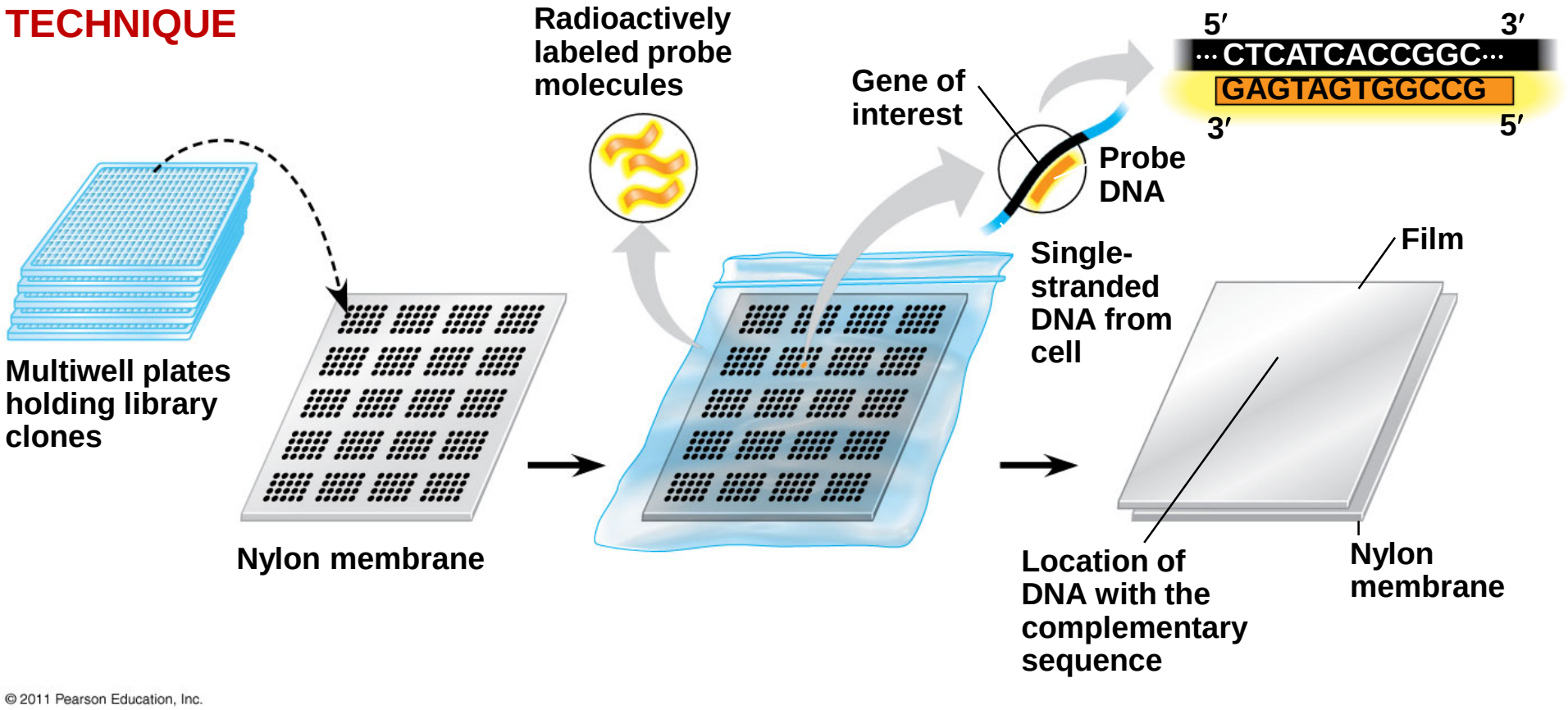



**cDNA (DNA complementary to mRNA) is produced as follows:**

- purify mRNA fr. desired cell type
- treat with reverse transcriptase and oligo-dT primer
- results in double-stranded DNA copies of all mRNA from the cell
- clone cDNA fragments into vector such as a plasmid
- transform bacterial hosts

**Results in a collection of cloned cDNA corresponding to coding sequences of all expressed proteins**

# Screening DNA Libraries



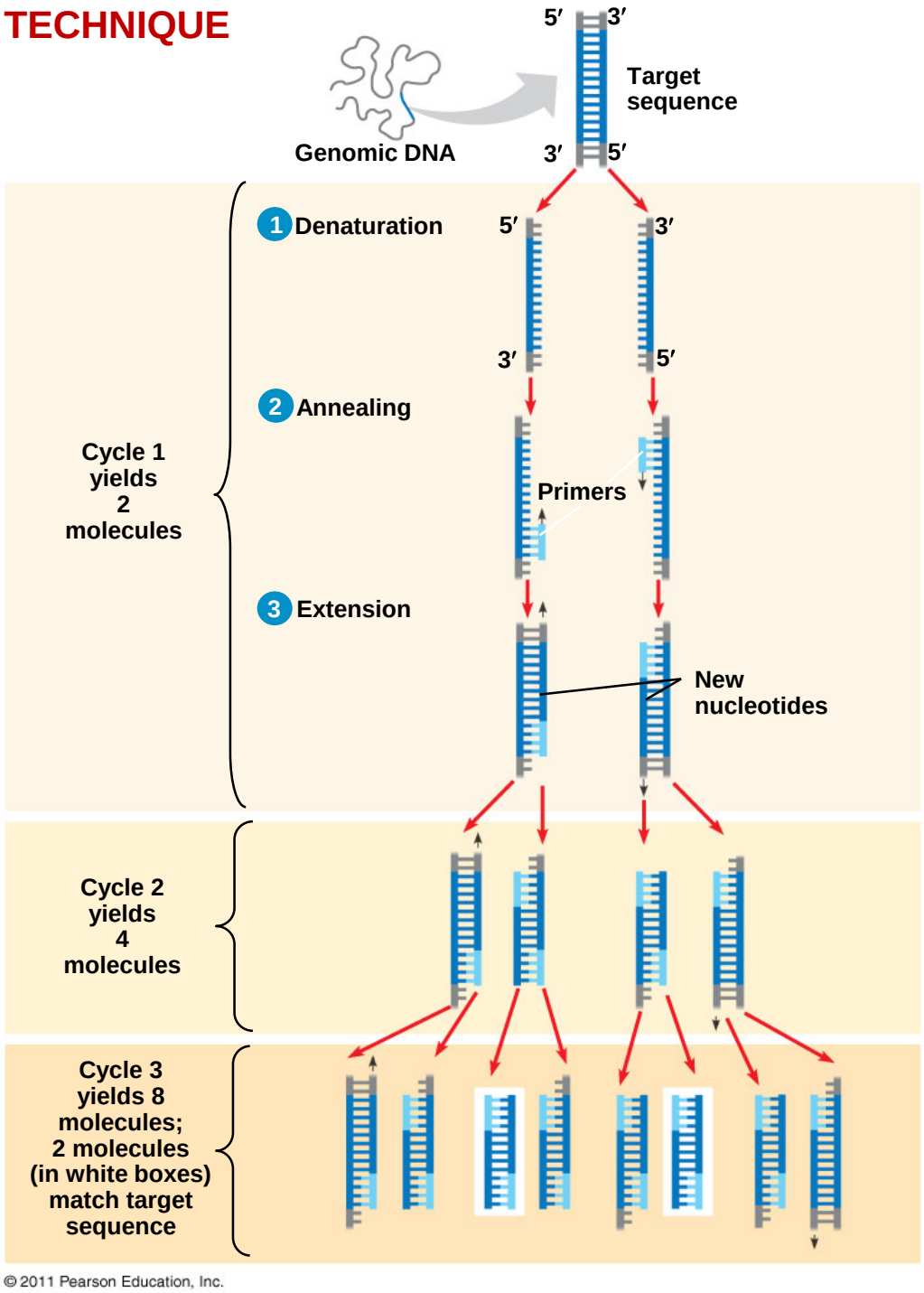



- DNA from library clones immobilized on a membrane
- radioactively labeled DNA similar in sequence to desired clone is added as a probe
- hybridization of probe to complementary DNA IDs clone

# PCR

**The Polymerase Chain Reaction is a technique to selectively amplify a desired DNA sequence:**

- **essentially DNA replication *in vitro***
- **results in exponential amplification of target DNA sequence**
- **artificial primers specific for target DNA sequence limit replication to DNA complementary to primers**

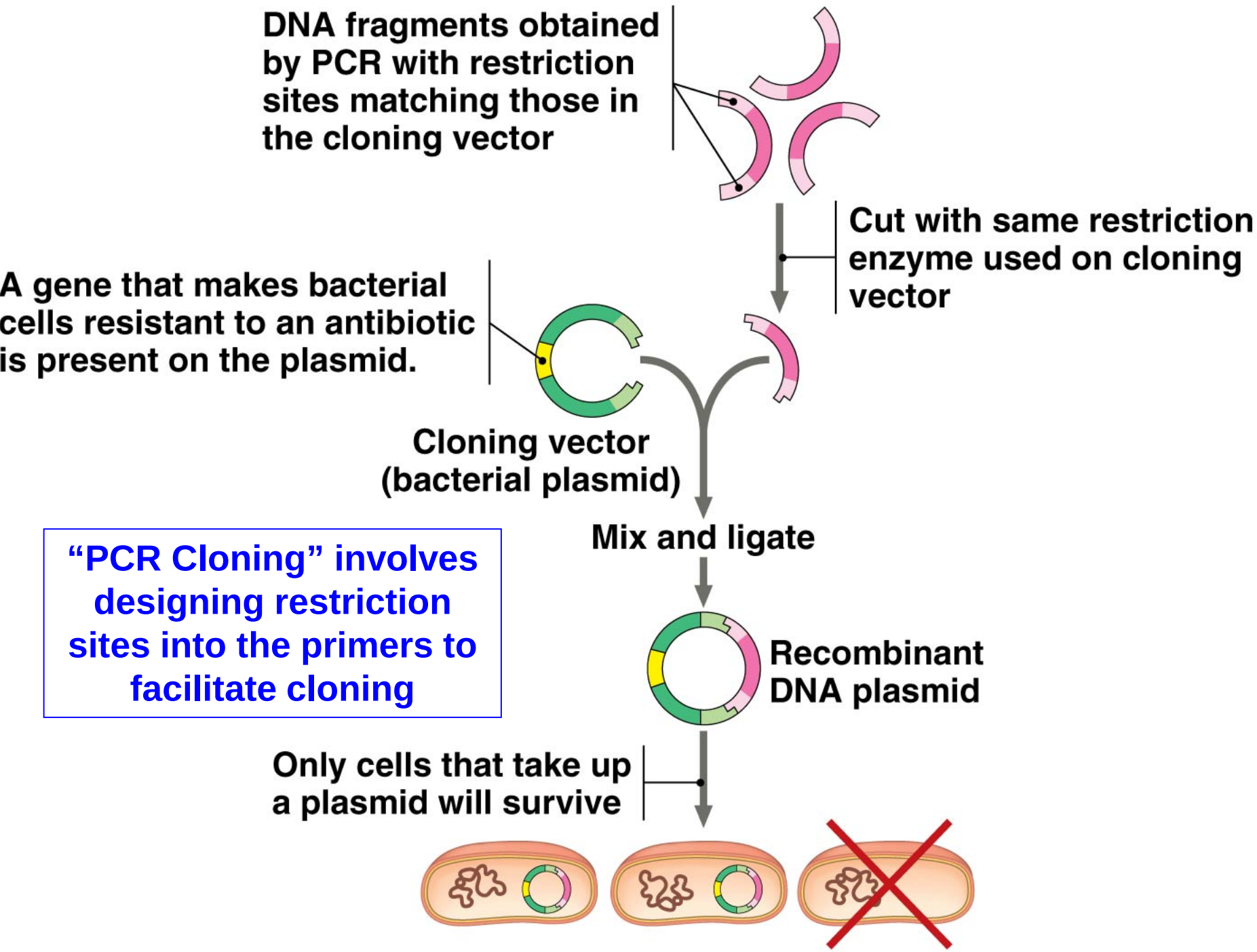


# Overview of PCR

**Every PCR reaction requires the following:**

1) **source of target DNA template**
2) **artificial primers "flanking" DNA of interest**
3) **heat-stable DNA polymerase (from hyperthermophile)**
4) **dNTP's**
5) **automated thermocycler to facilitate repeated:**
   - **denaturation of DNA (separating the 2 strands)**
   - **hybridization of primers to template**
   - **DNA synthesis**

**Resulting PCR products can then be analyzed by gel electrophoresis.**




"PCR Cloning" involves designing restriction sites into the primers to facilitate cloning

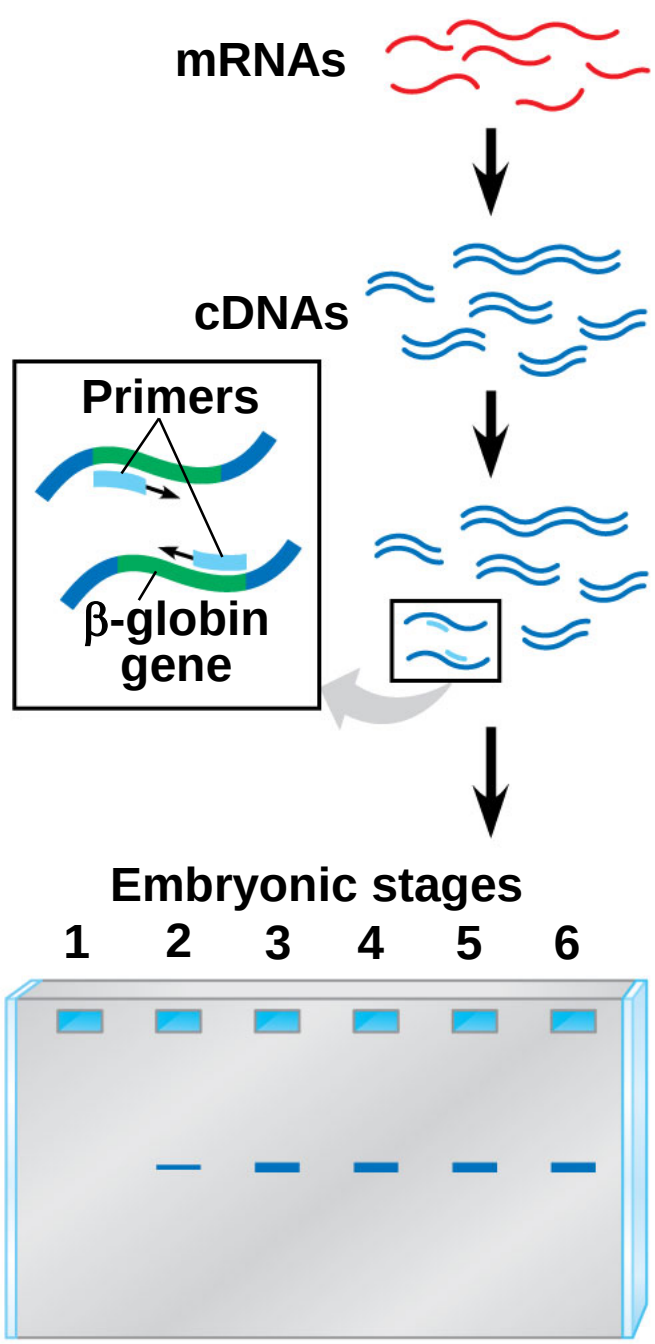
# 3. Studying Gene Expression

**Chapter Reading – pp. 417-419, 421-422**

# RT-PCR

**TECHNIQUE**

1. cDNA synthesis
2. PCR amplification
3. Gel electrophoresis

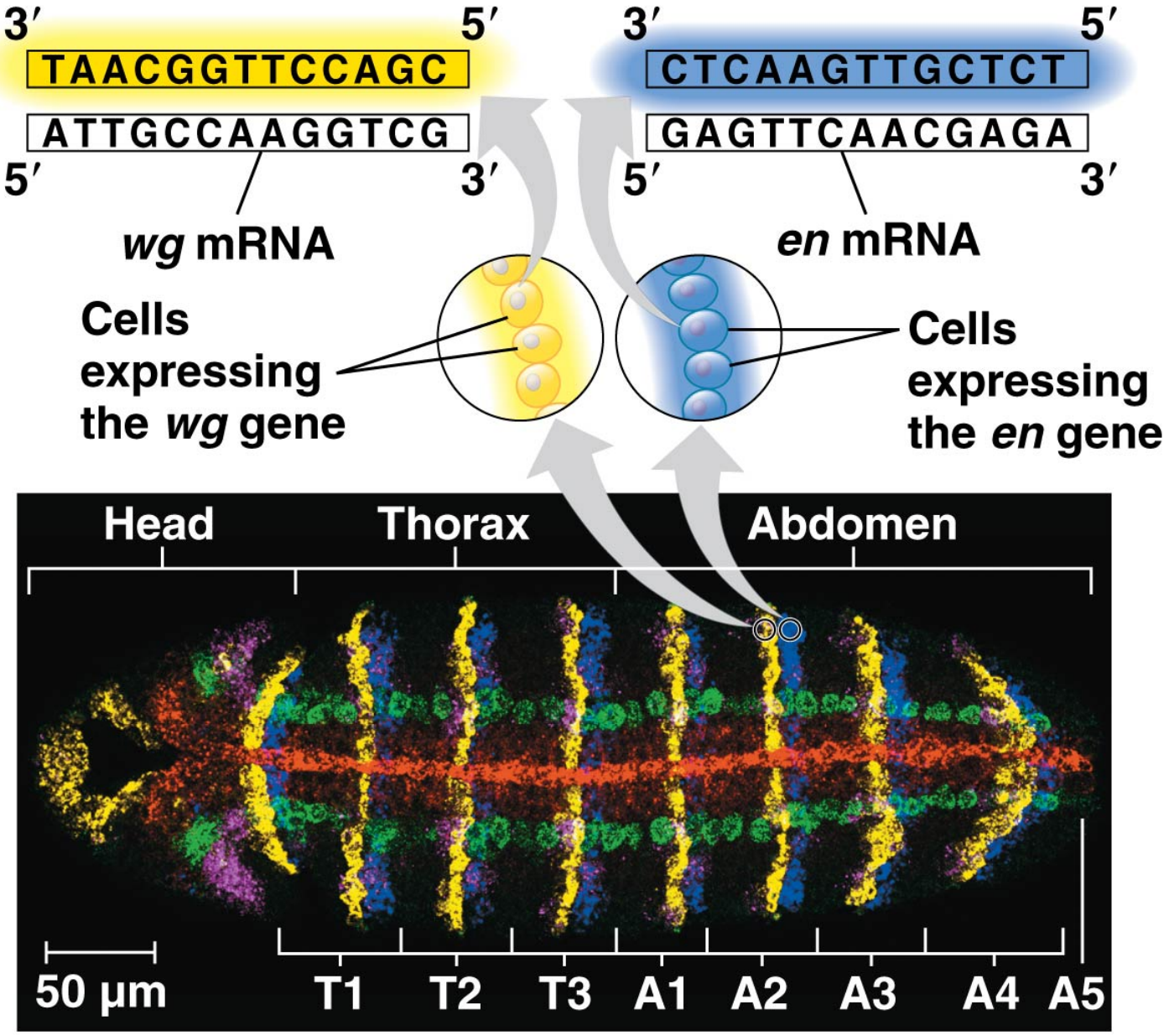



- produce cDNA with reverse transcriptase
- carry out PCR using specific primers for desired gene
- gel electrophoresis of resulting PCR fragments

**RESULTS**

**Amount of DNA reflects amount of original mRNA sequence.**

# *in situ* Hybridization



# …*in situ* Hybridization

## How is *in situ* hybridization carried out?

- **fluorescently labeled anti-sense RNA probes added to tissue sample**
- **hybridization with complementary mRNA sequences**
- **location of specific gene expression within the tissue (i.e., *in situ*) is revealed**

# DNA Microarrays

**A DNA microarray is a solid surface containing a precise array of single-stranded DNA sequences from 1000s of different genes in an organism.**

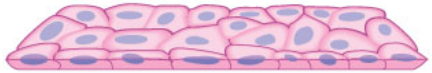
- **labeled cDNA is produced from test cells and allowed to hybridize with sequences in the array**
- **intensity of signals reveal expression of specific genes within the test cells.**

**When cDNA from different sources is labeled differently, gene expression from each source can be compared in a single microarray (as shown on the slide after next).**

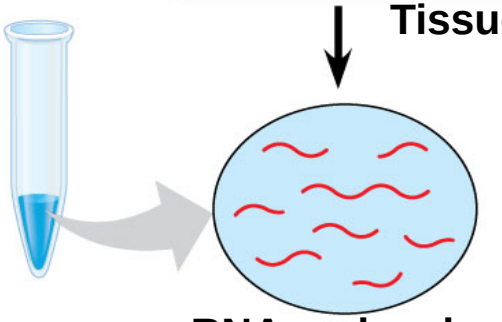
TECHNIQUE

# Analyzing gene expression using a DNA microarray.

1. Isolate mRNA.

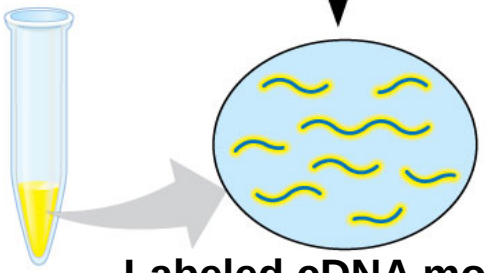


Tissue sample



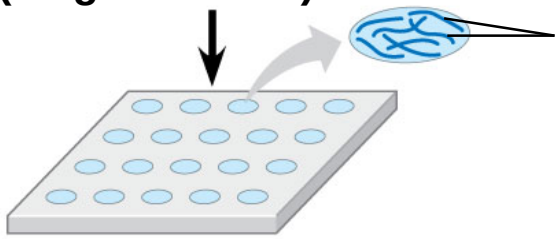
mRNA molecules

2. Make cDNA by reverse transcription, using fluorescently labeled nucleotides.



Labeled cDNA molecules (single strands)

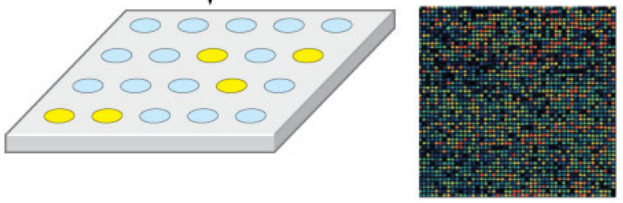
3. Apply the cDNA mixture to a microarray, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.



DNA fragments representing a specific gene

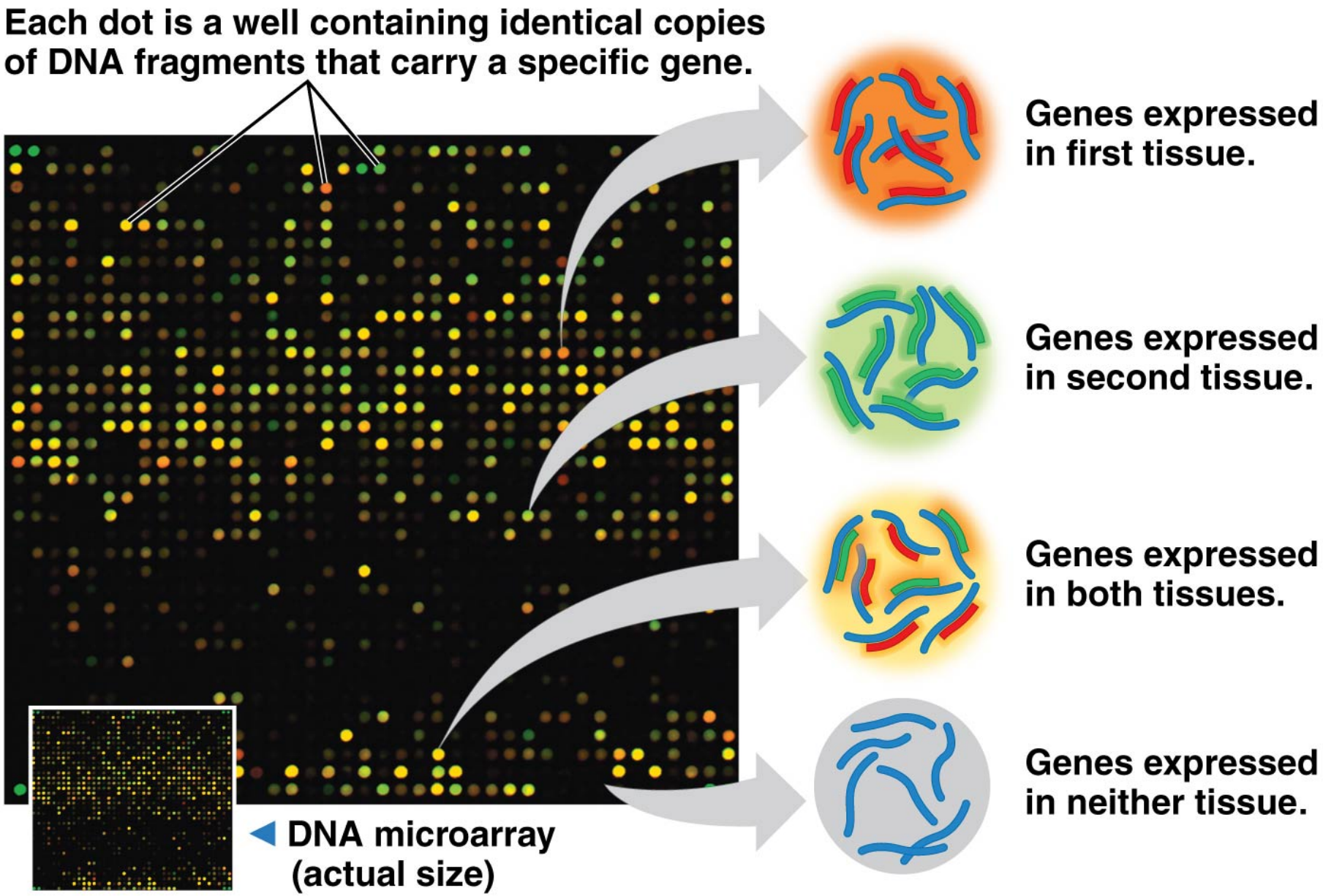
DNA microarray

4. Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot (yellow) represents a gene expressed in the tissue sample.



DNA microarray with 2,400 human genes

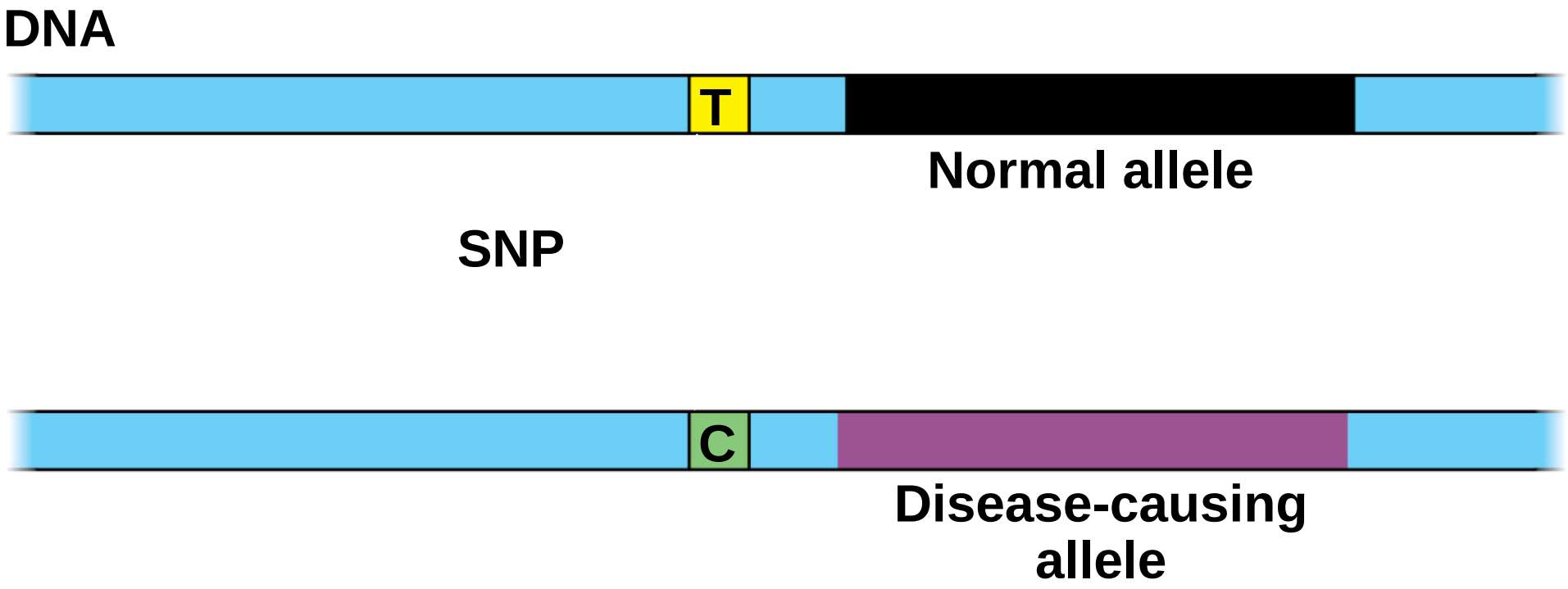
# Comparing Gene Expression



# Single Nucleotide Polymorphisms (SNPs)

**SNPs are single nucleotide differences that correspond with specific disease-causing alleles.**

**PCR and hybridization techniques (e.g., microarrays) can reveal the presence of such alleles (genetic testing).**

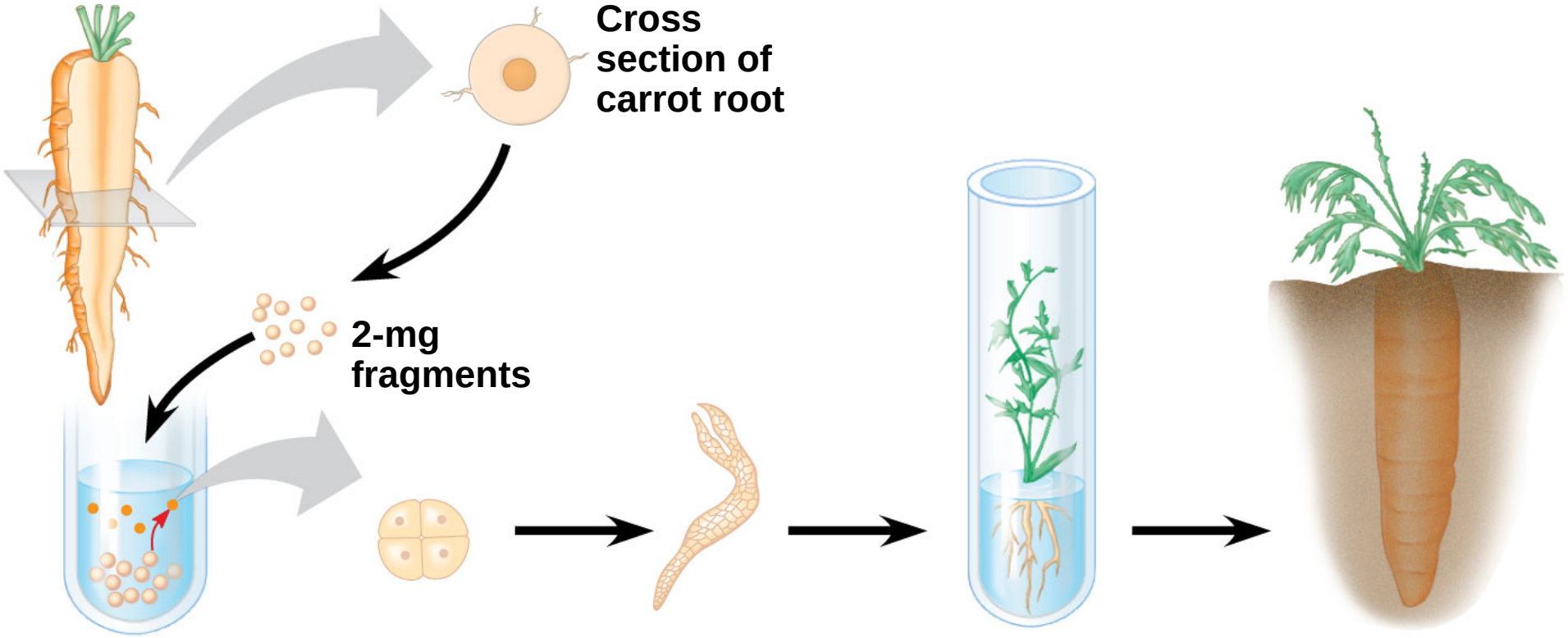


# 4. Manipulating Genomes

**Chapter Reading – pp. 422-425, 429-430**

# Cloning Plants

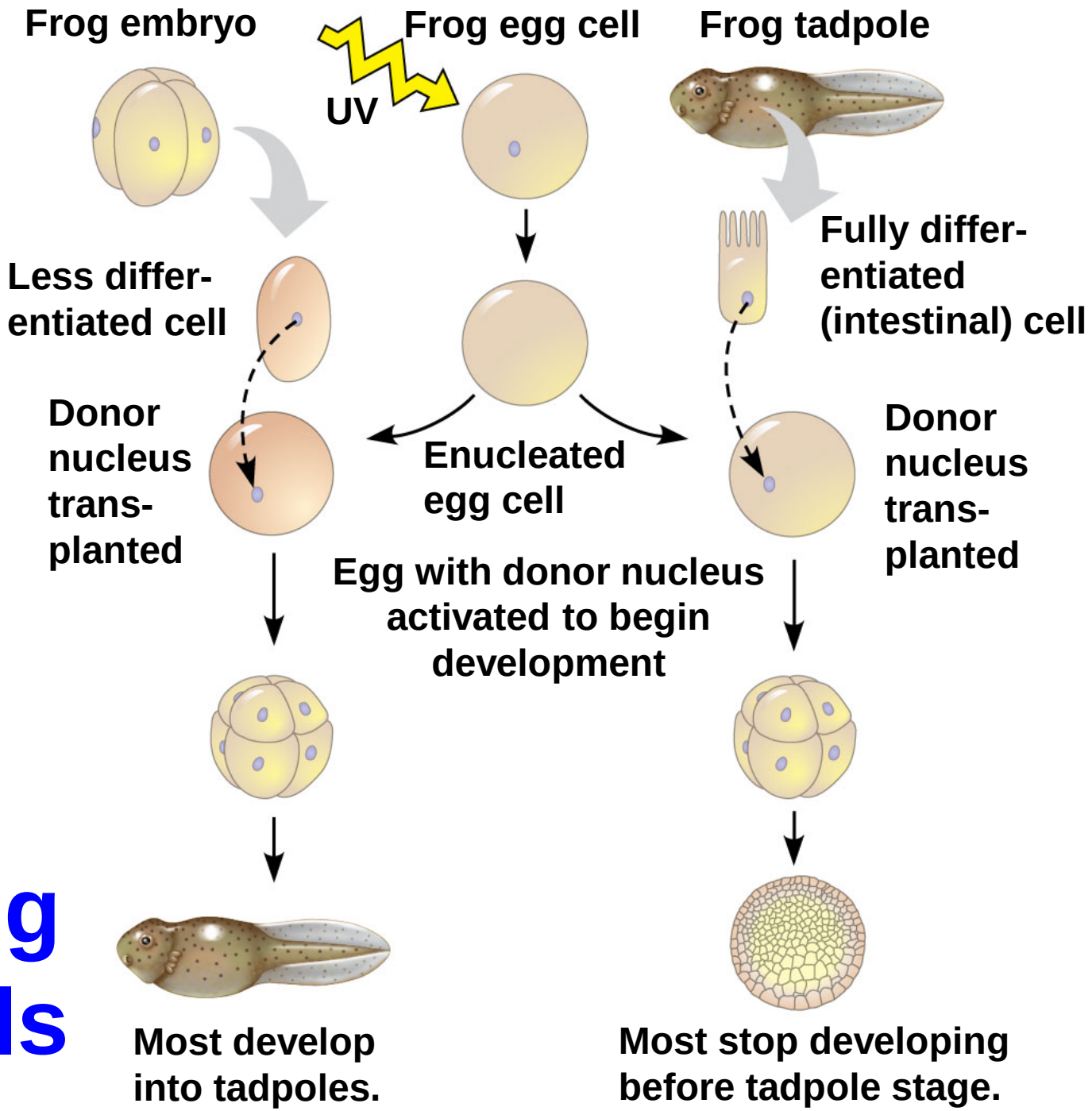
**Some plants can be cloned from a single adult plant cell.**



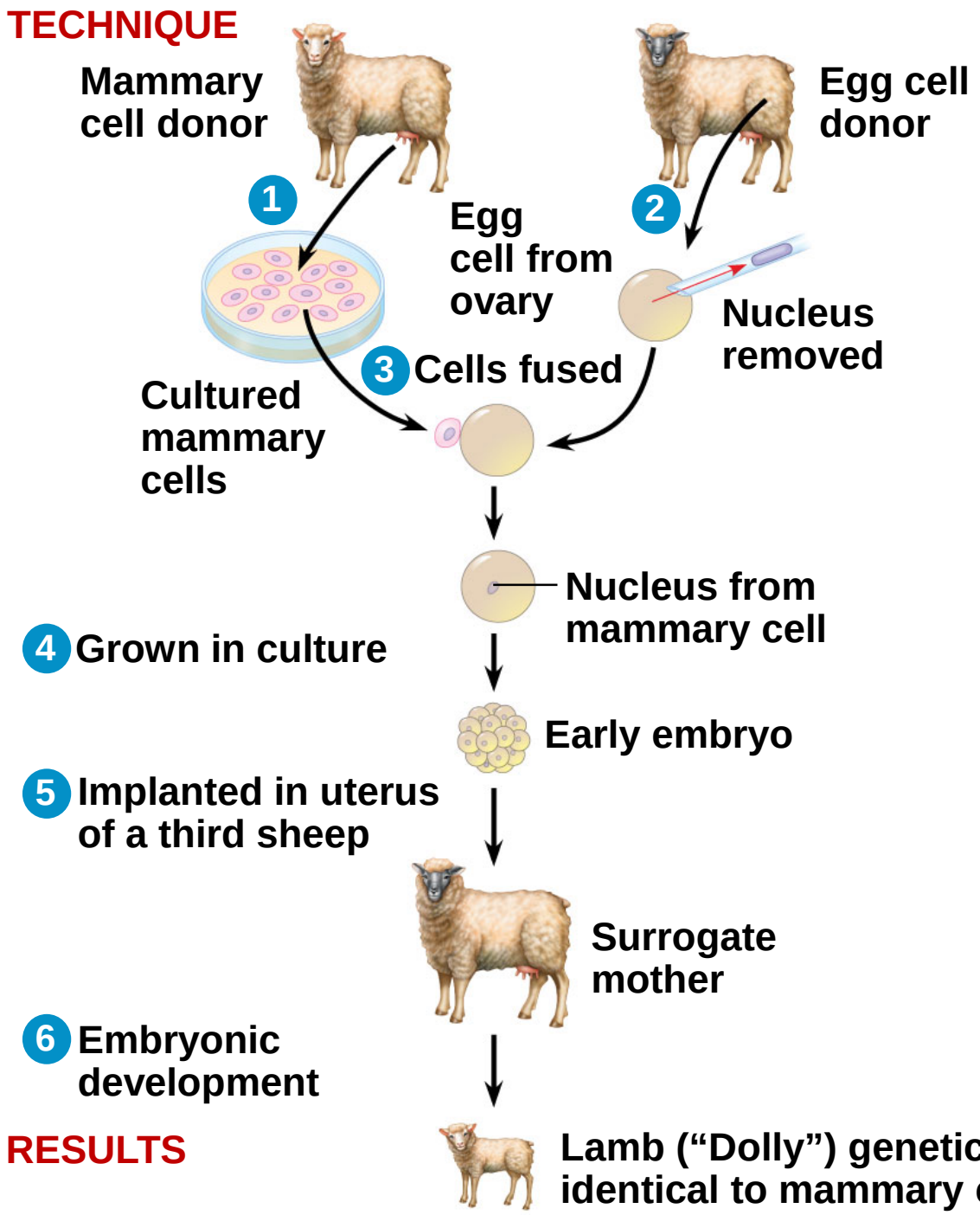
# Cloning Animals

**EXPERIMENT**

This experiment produced the first artificially cloned animal.




**RESULTS**




**The first cloned mammal, "Dolly the sheep", was cloned by this method.**

**Other mammals such as cats have since been cloned and presumably humans could be cloned this way as well.**

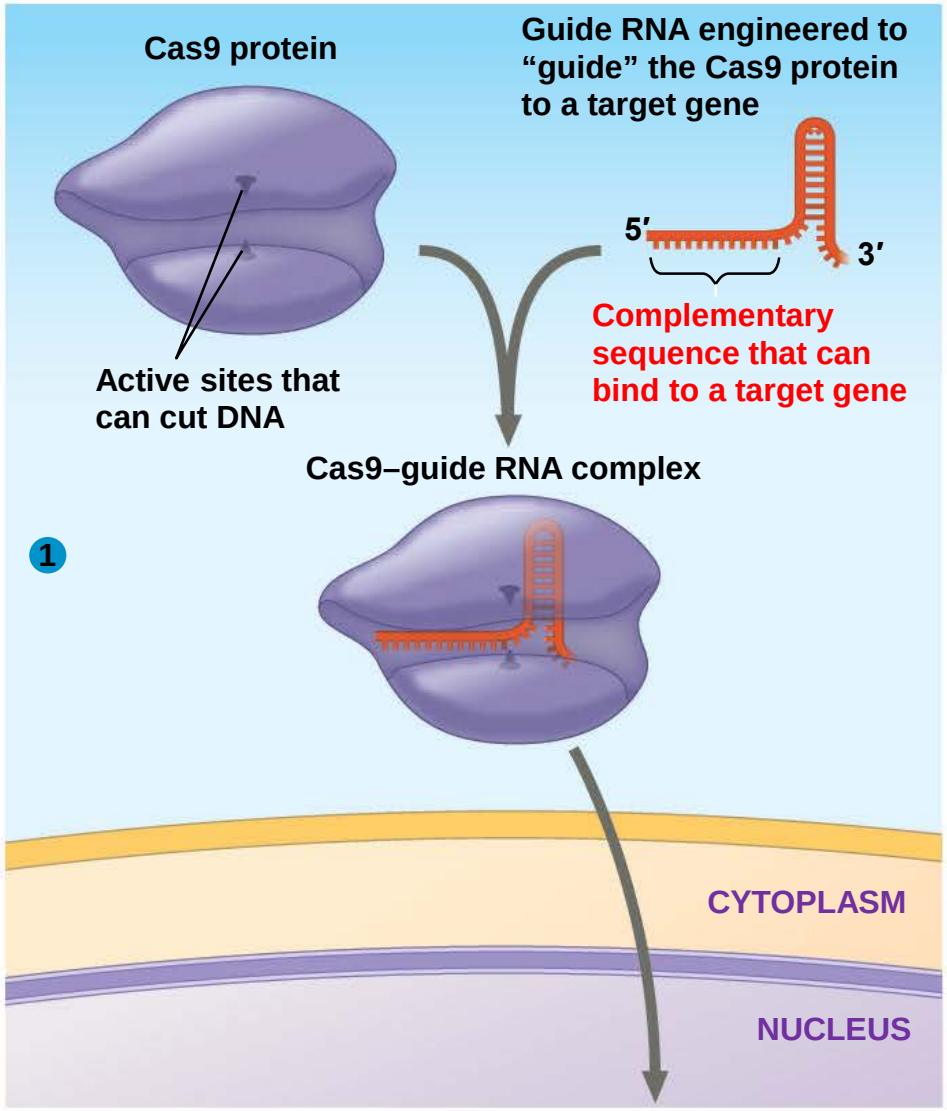
# Transgenic Organisms

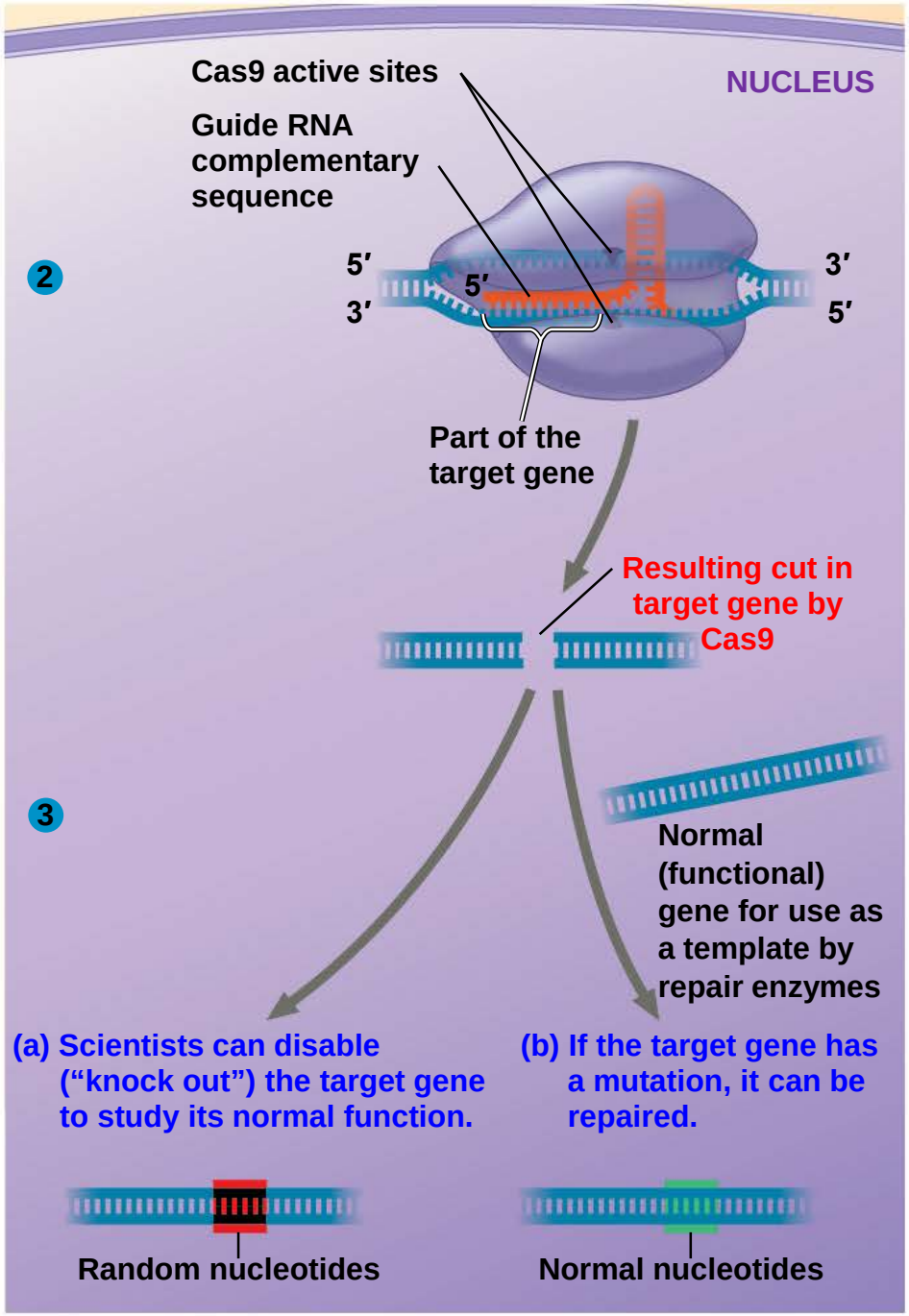
**Transgenic organisms have a foreign gene (e.g. from another species) inserted into their genome.**

- **also known as “genetically modified organisms” (GMOs)**

# CRISPR-Cas9

- a new method to modify genomes

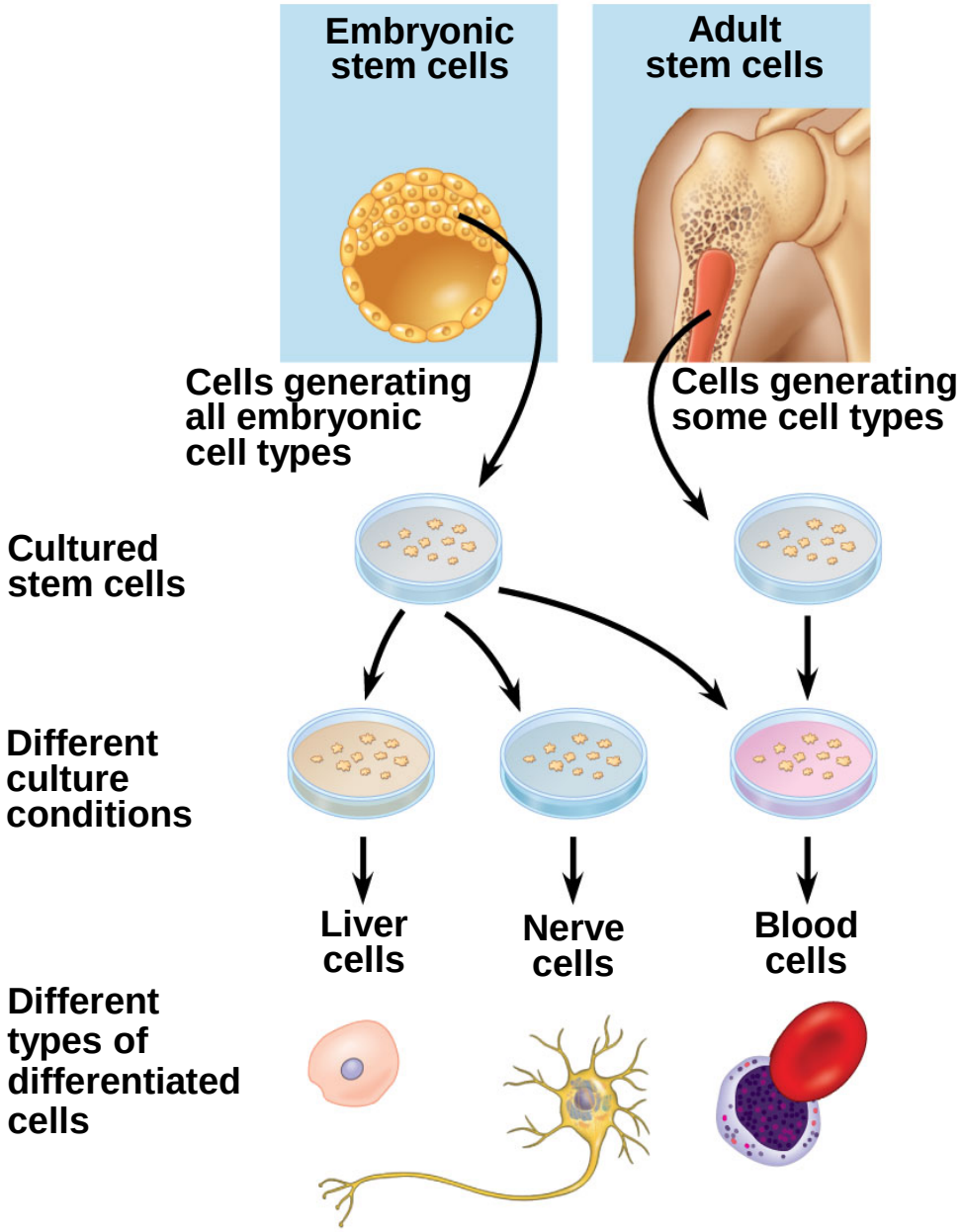


# 5. Therapeutic & Diagnostic Techniques

**Chapter Reading – pp. 425-431**

# Stem Cells



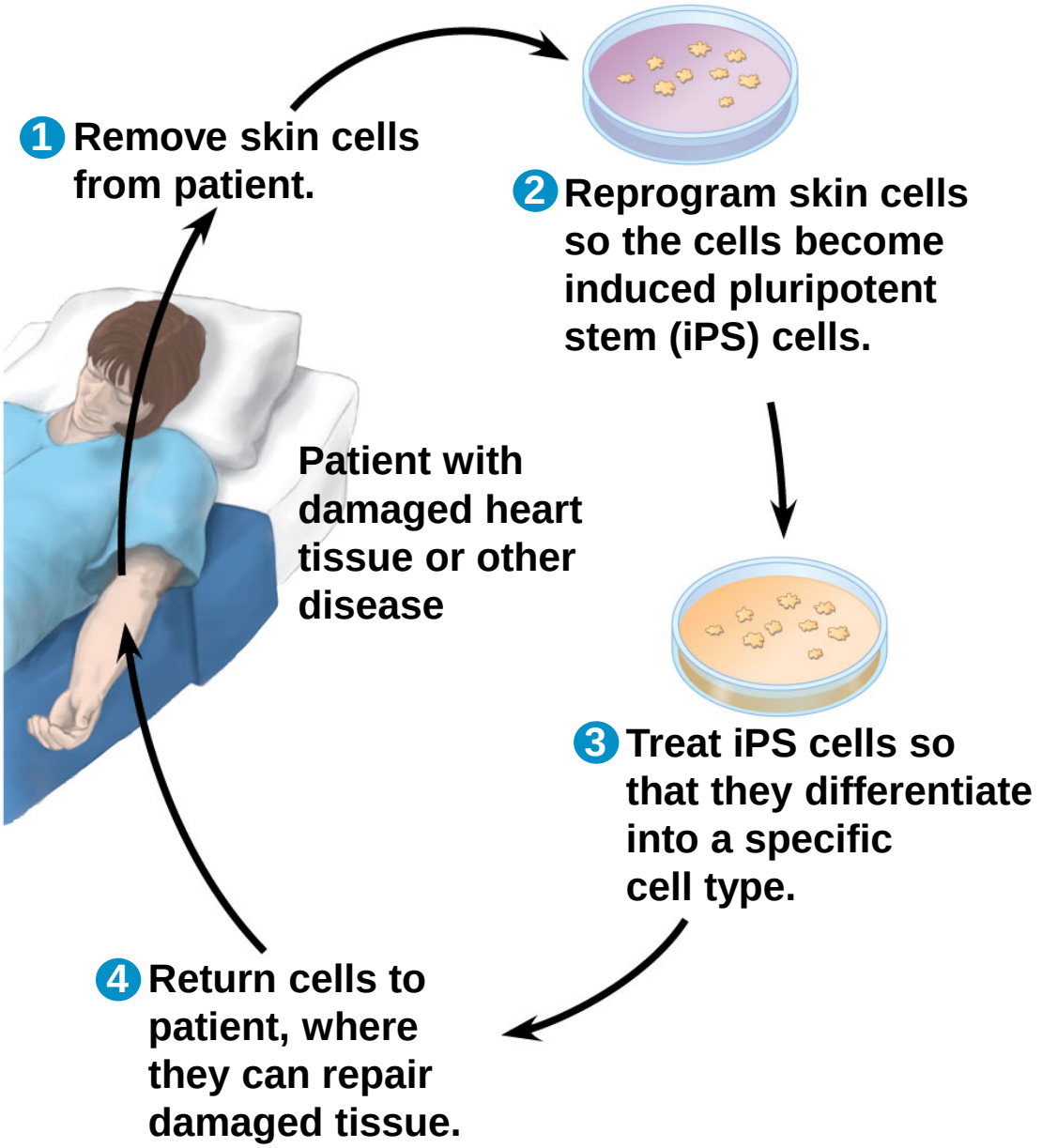



**Stem cells are undifferentiated cells that can give rise to multiple differentiated cell types.**

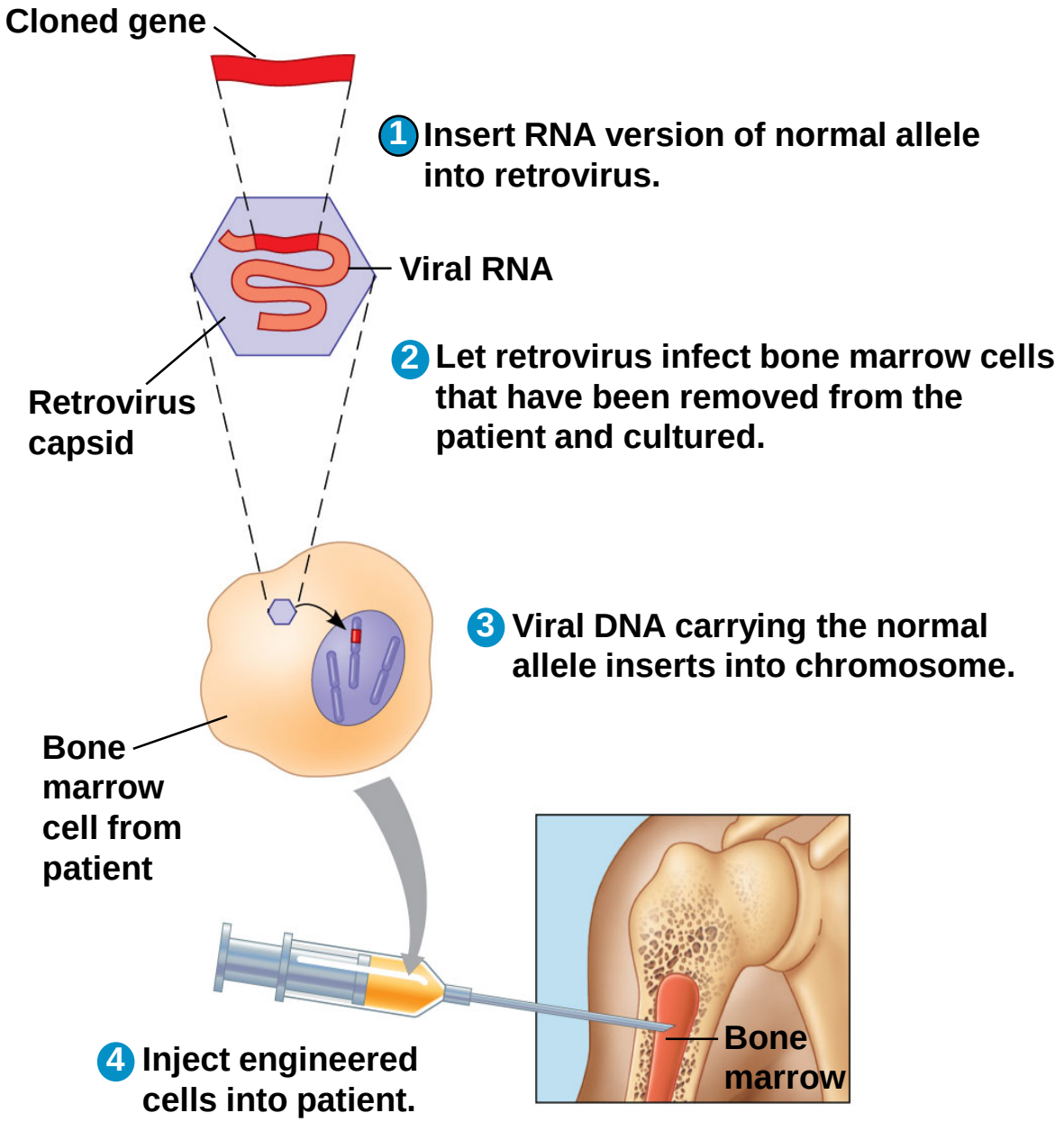
**Embryonic stem cells are considered more pluripotent than adult stem cells, though techniques are being developed to expand the potential of adult stem cell.**

# Stem Cell Therapy

**Techniques are being developed to treat a person's own cells *in vitro* to produce a needed cell type which is then reintroduce into the patient to repair damaged tissue.**

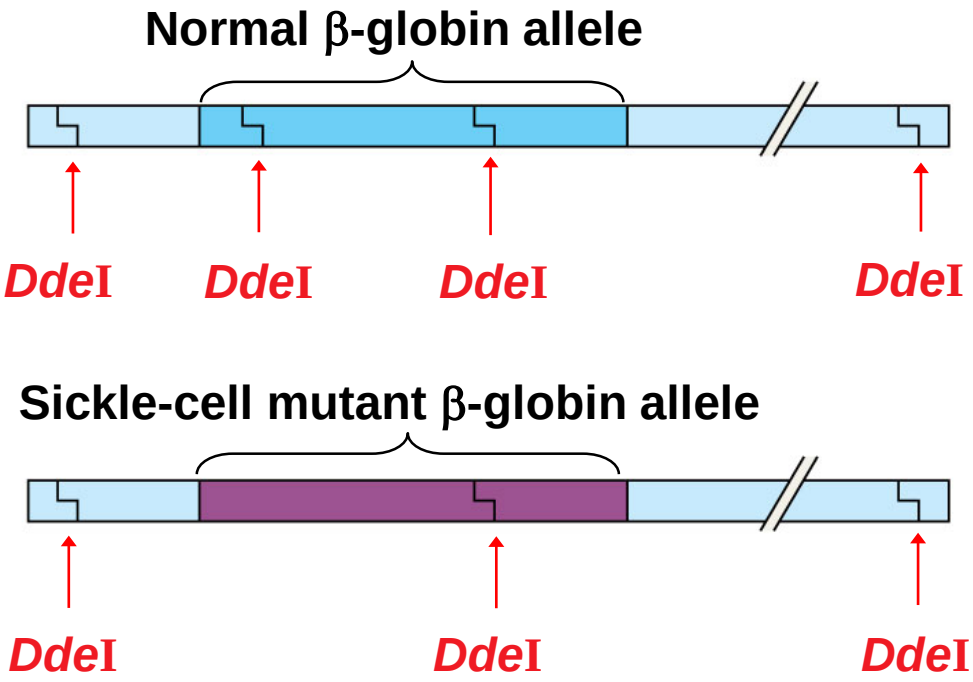


# Gene Therapy

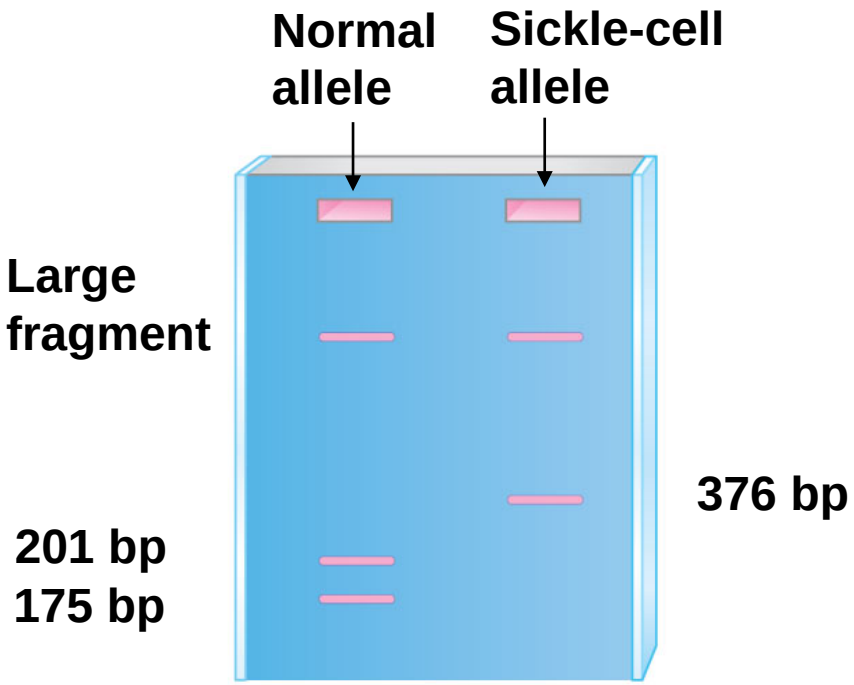



**Bone marrow stem cells can be genetically modified *in vitro* to fix a genetic defect and then reintroduced back into the patient.**

# Restriction Fragment Length Polymorphisms (RFLPs)




(a) *DdeI* restriction sites in normal and sickle-cell alleles of the β-globin gene




(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles



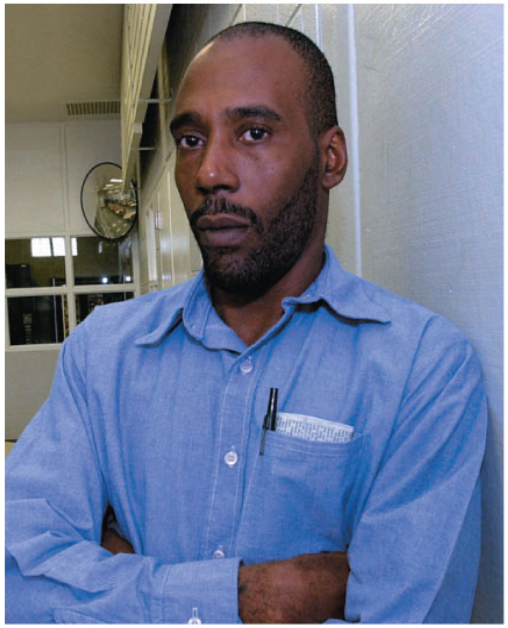
- DNA from different sources cut with the same RE will result in different size DNA fragments (RFLPs)

# Short Tandem Repeat Analysis

**Many parts of the human genome contain short DNA sequences repeated many times in a row (in tandem)**

- **the number of repeats in these regions is highly variable from person to person**
- **RFLP analysis can produce a DNA fingerprint that is unique for each individual**

(a) This photo shows Washington just before his release in 2001, after 17 years in prison.



| Source of sample | STR marker 1 | STR marker 2 | STR marker 3 |
|---|---|---|---|
| Semen on victim | 17,19 | 13,16 | 12,12 |
| Earl Washington | 16,18 | 14,15 | 11,12 |
| Kenneth Tinsley | 17,19 | 13,16 | 12,12 |

(b) These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

# Southern Blotting

## TECHNIQUE

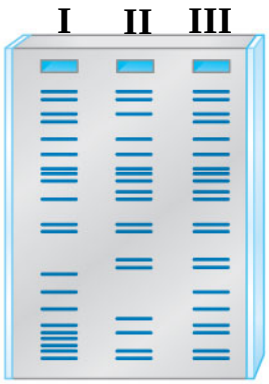
DNA + restriction enzyme

I Normal β-globin allele

II Sickle-cell allele

III Heterozygote

Restriction fragments




Nitrocellulose membrane (blot)

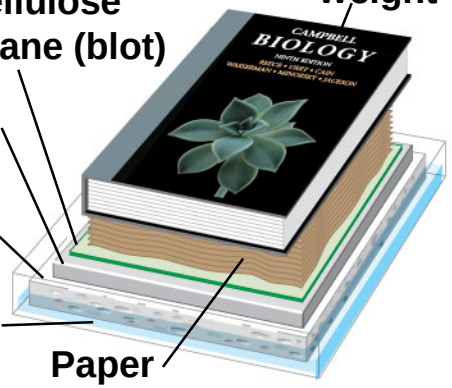
Heavy weight

Gel

Sponge

Alkaline solution

Paper towels

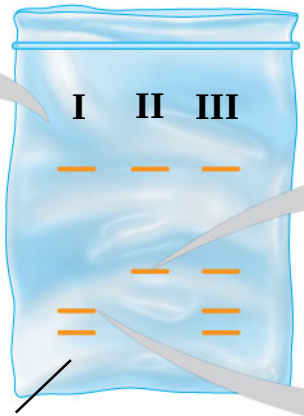



1 Preparation of restriction fragments

2 Gel electrophoresis

3 DNA transfer (blotting)

Radioactively labeled probe for β-globin gene

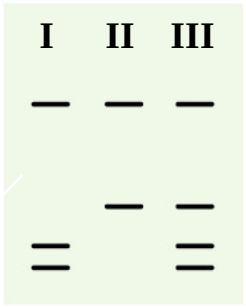



Nitrocellulose blot

Probe base-pairs with fragments

Fragment from sickle-cell β-globin allele

Fragment from normal β- globin allele




Film over blot

4 Hybridization with labeled probe

5 Probe detection

# Key Terms for Chapter 20

- recombinant DNA, gene cloning, plasmid vector
- transformation, ligation, restriction enzymes
- genomic & cDNA libraries, hybridization, probe
- gel electrophoresis, PCR, RT-PCR
- dideoxynucleotide, RFLP, STR, SNP
- in situ hybridization, DNA microarrays
- transgenic, stem cell, southern blot, CRISPR-Cas9
- in vivo, in vitro, in situ

**Relevant Chapter Questions**

**1-10, 12**