Chapter 20: Terms to Know

1. Genetic engineering
2. Biotechnology
3. Recombinant DNA
4. Gene cloning
5. Restriction enzymes
6. Sticky ends
7. DNA ligase
8. Cloning vector
9. Nucleic acid hybridization
10. Genomic library
11. cDNA library
12. PCR
13. Gel electrophoresis
14. Southern blotting
15. DNA microarray assays
16. SNPs
17. RFLPs
18. Stem cells
19. Gene therapy
20. GMO (genetically modified organism)

What You Must Know:

- The terminology of biotechnology.
- The steps in gene cloning with special attention to the biotechnology tools that make cloning possible.
- The key ideas that make PCR possible.
- How gel electrophoresis can be used to separate DNA fragments or protein molecules.

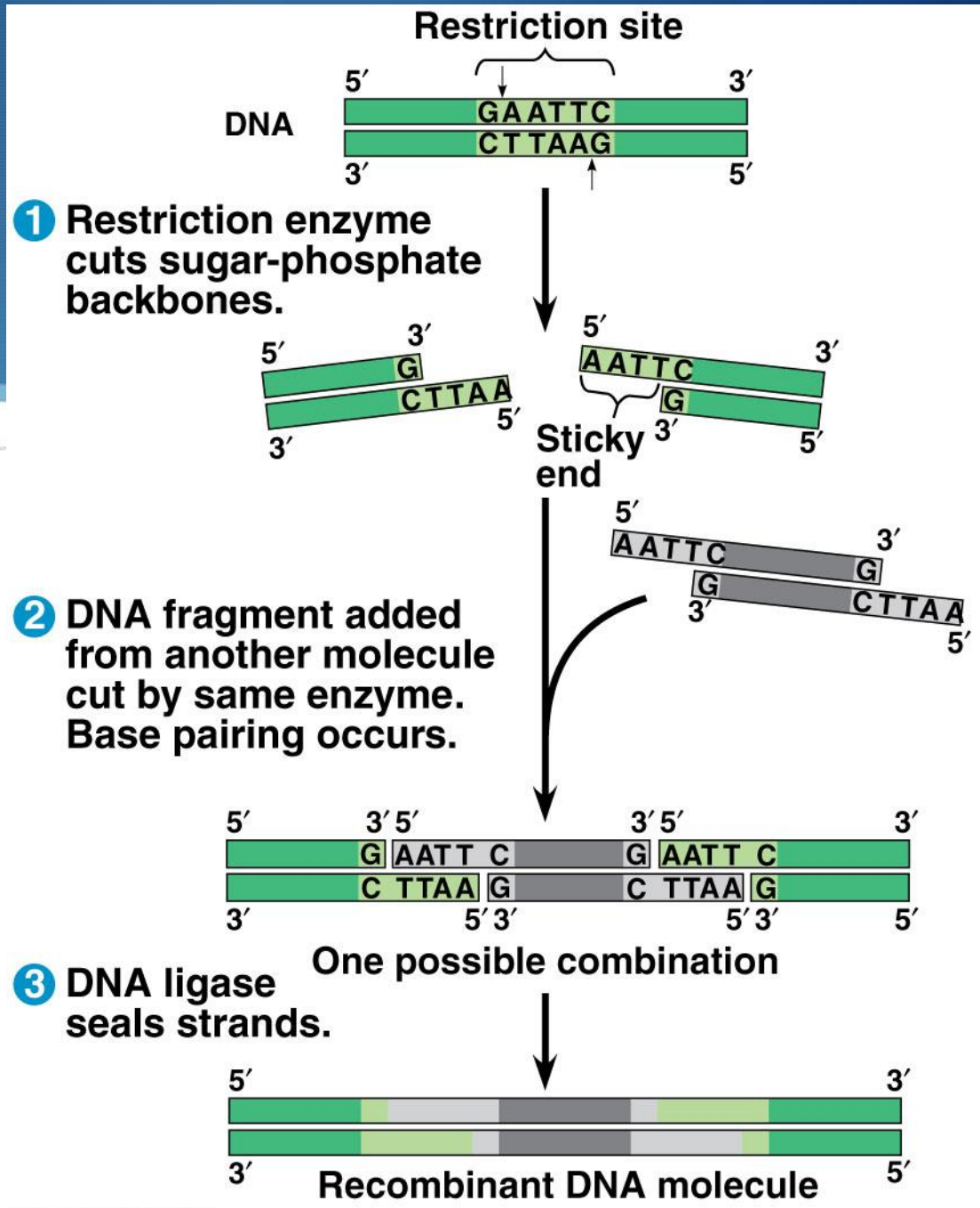
- **Genetic Engineering**: process of manipulating genes and genomes
- **Biotechnology**: process of manipulating organisms or their components for the purpose of making useful products.

- **Recombinant DNA**: DNA that has been artificially made, using DNA from different sources
  - eg. Human gene inserted into E.coli
- **Gene cloning**: process by which scientists can product multiple copies of specific segments of DNA that they can then work with in the lab

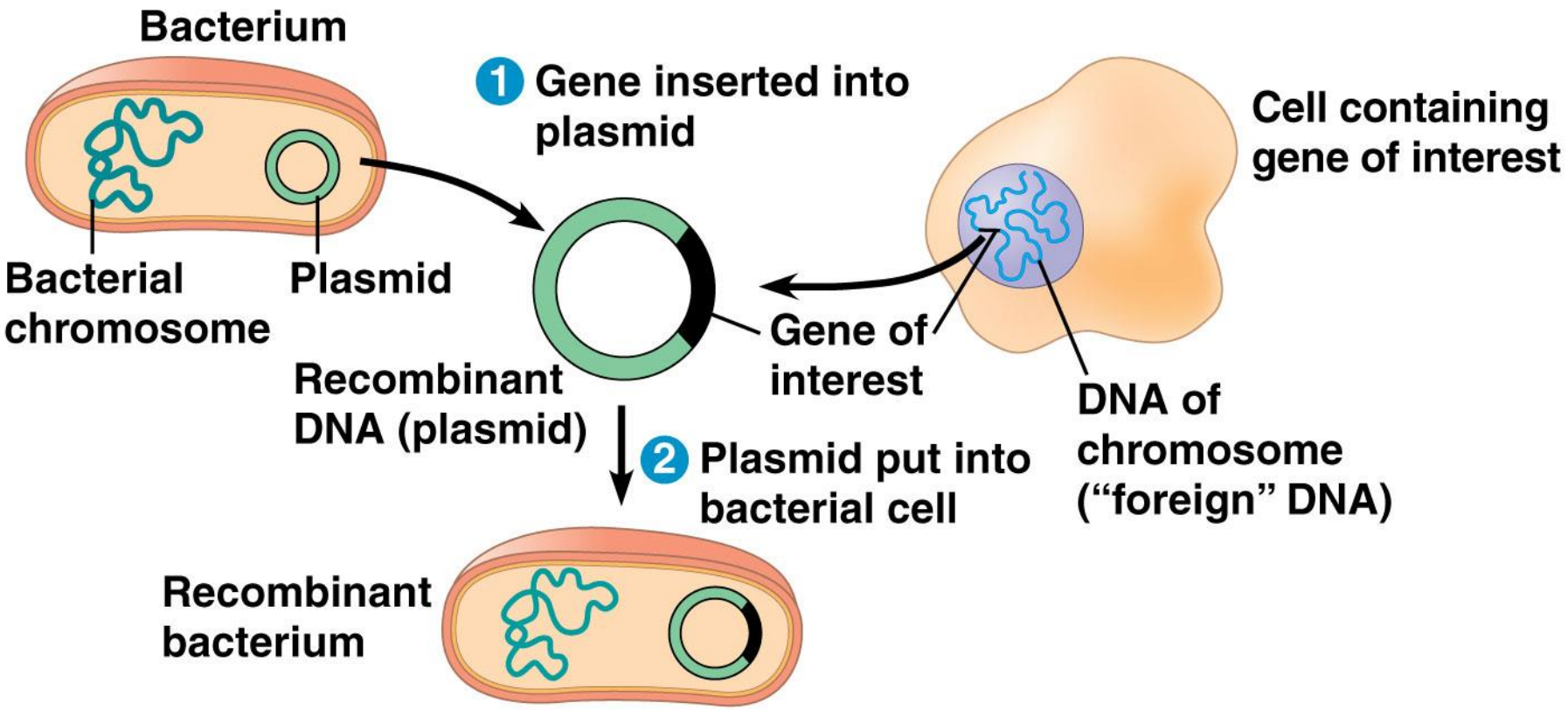
Tools of Genetic Engineering

- **Restriction enzymes** (restriction endonucleases): used to cut strands of DNA at specific locations (restriction sites)
  - **Restriction Fragments:** have at least 1 **sticky end** (single-stranded end)
- **DNA ligase**: joins DNA fragments
- **Cloning vector**: carries the DNA sequence to be cloned (eg. bacterial plasmid)

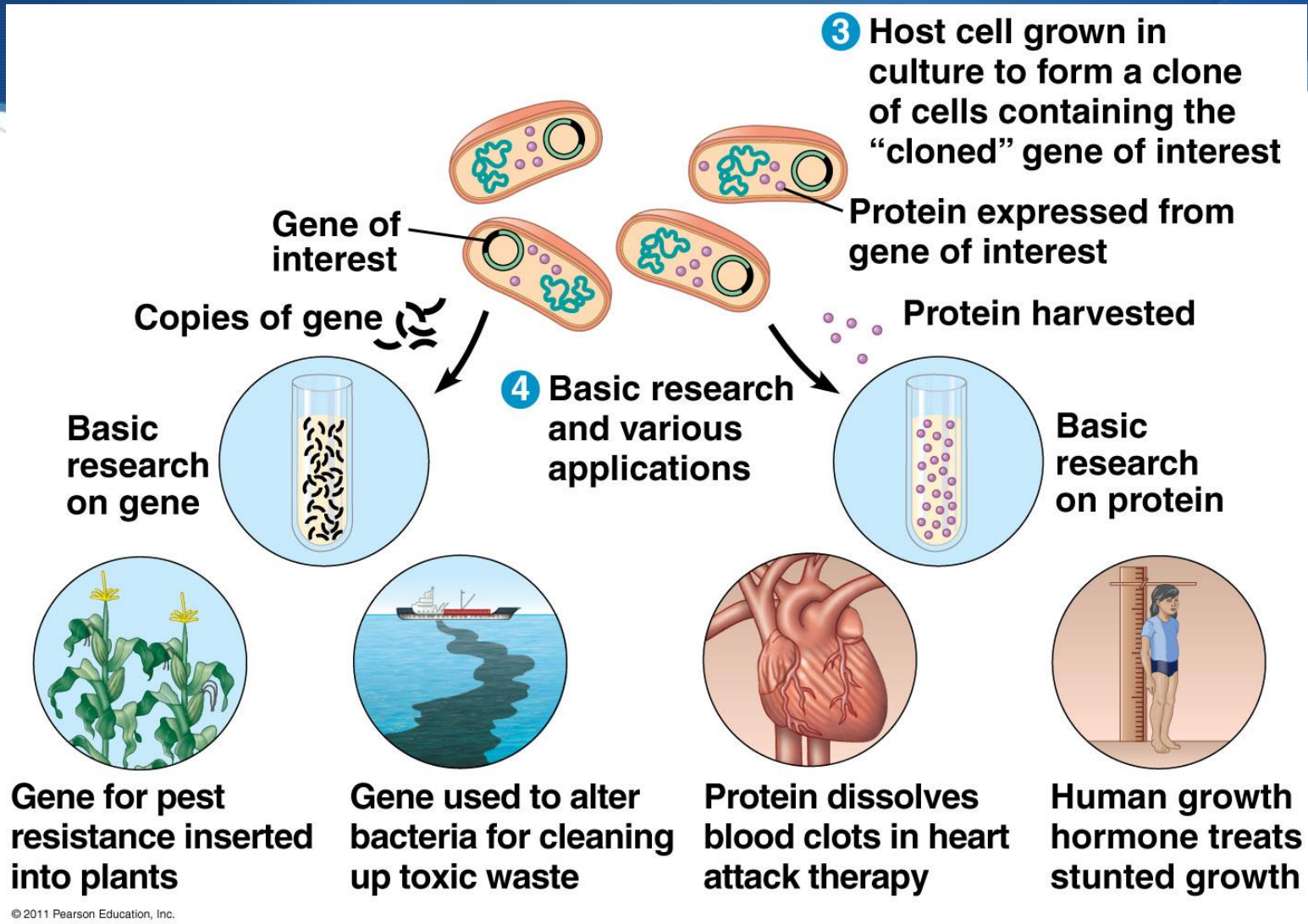
Using a restriction enzyme (RE) and DNA ligase to make recombinant DNA



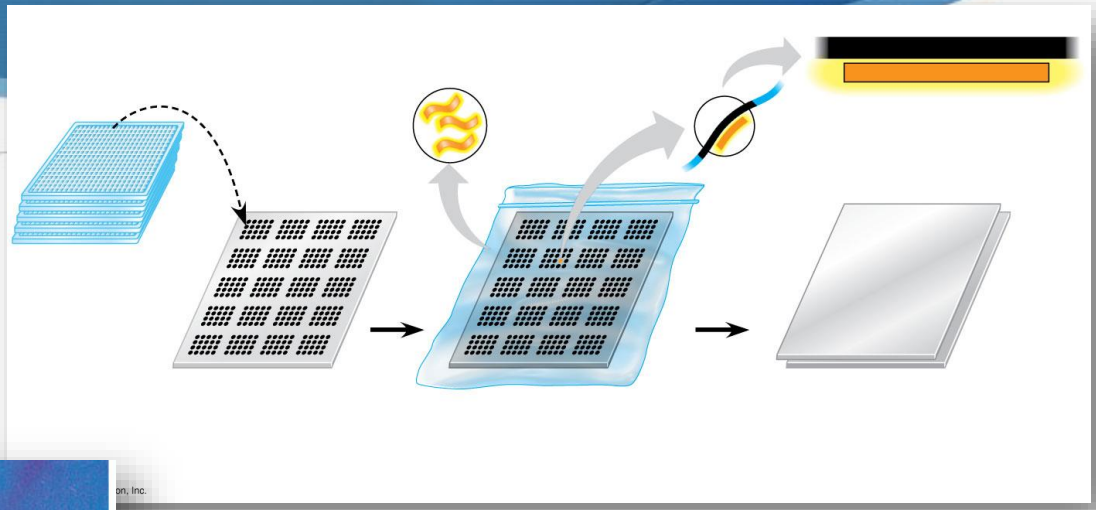
Gene Cloning

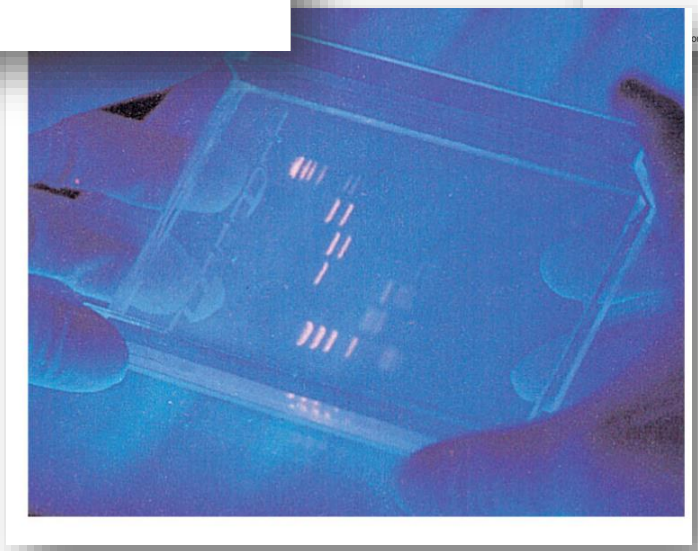


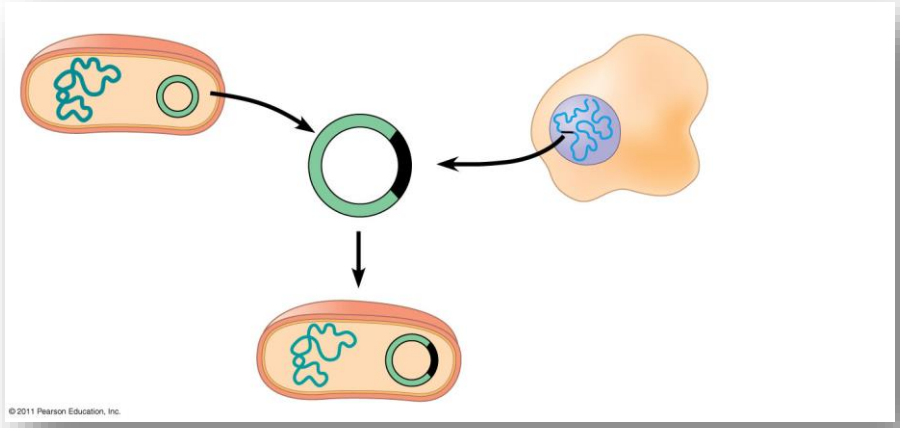
Applications of Gene Cloning

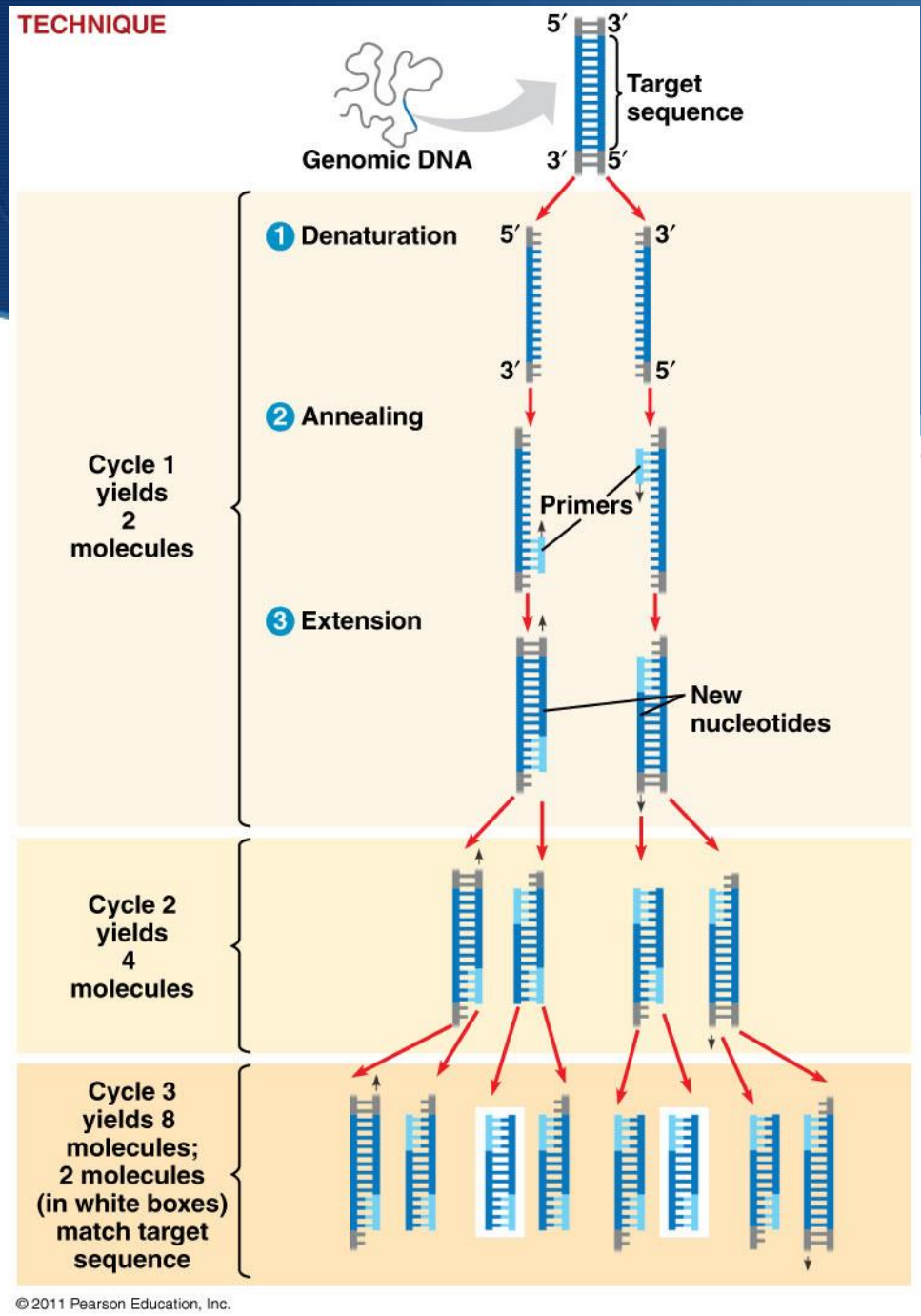


Techniques of Genetic Engineering



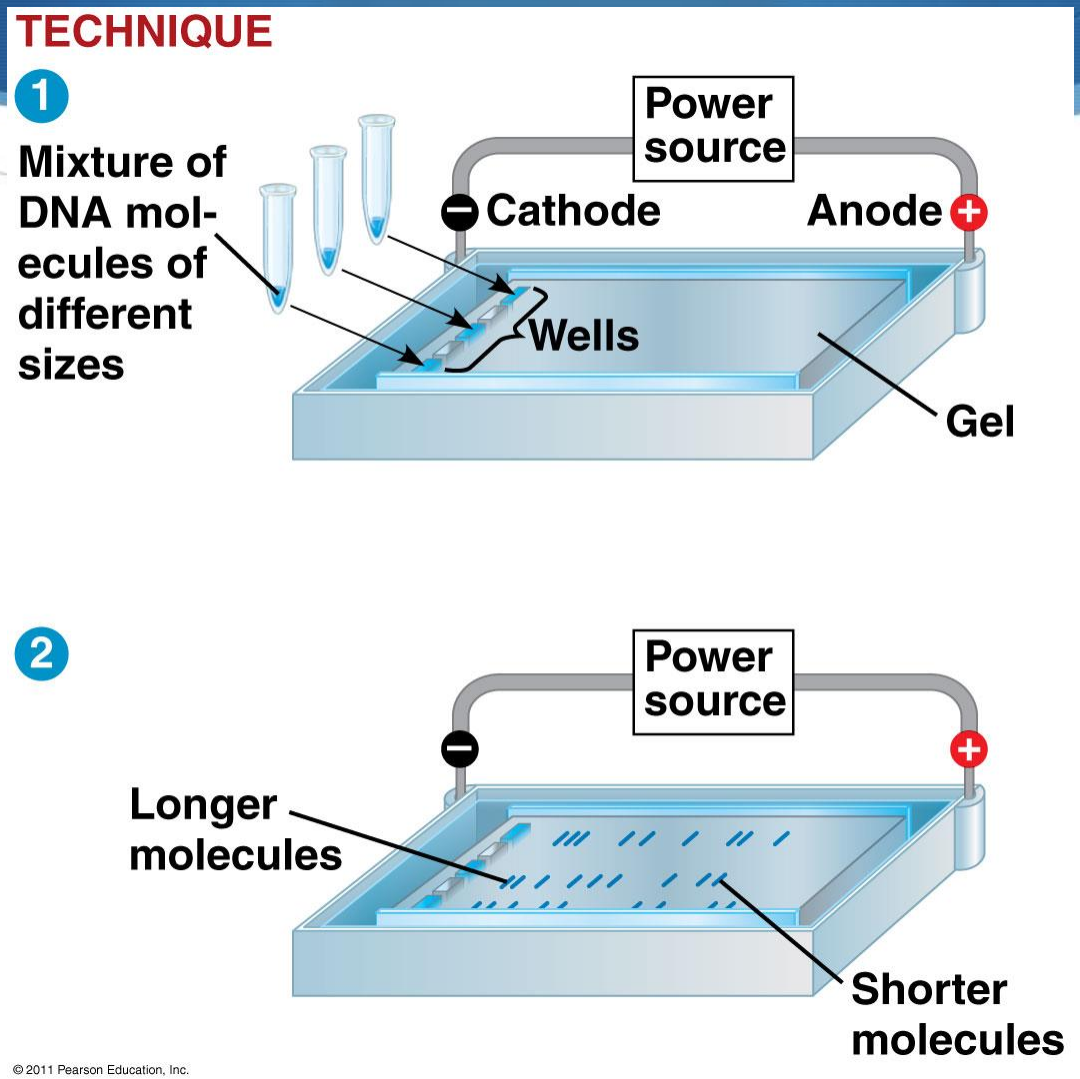






PCR (Polymerase Chain Reaction): amplify (copy) piece of DNA without use of cells

Gel Electrophoresis: used to separate DNA molecules on basis of size and charge using an electrical current (DNA → + pole)



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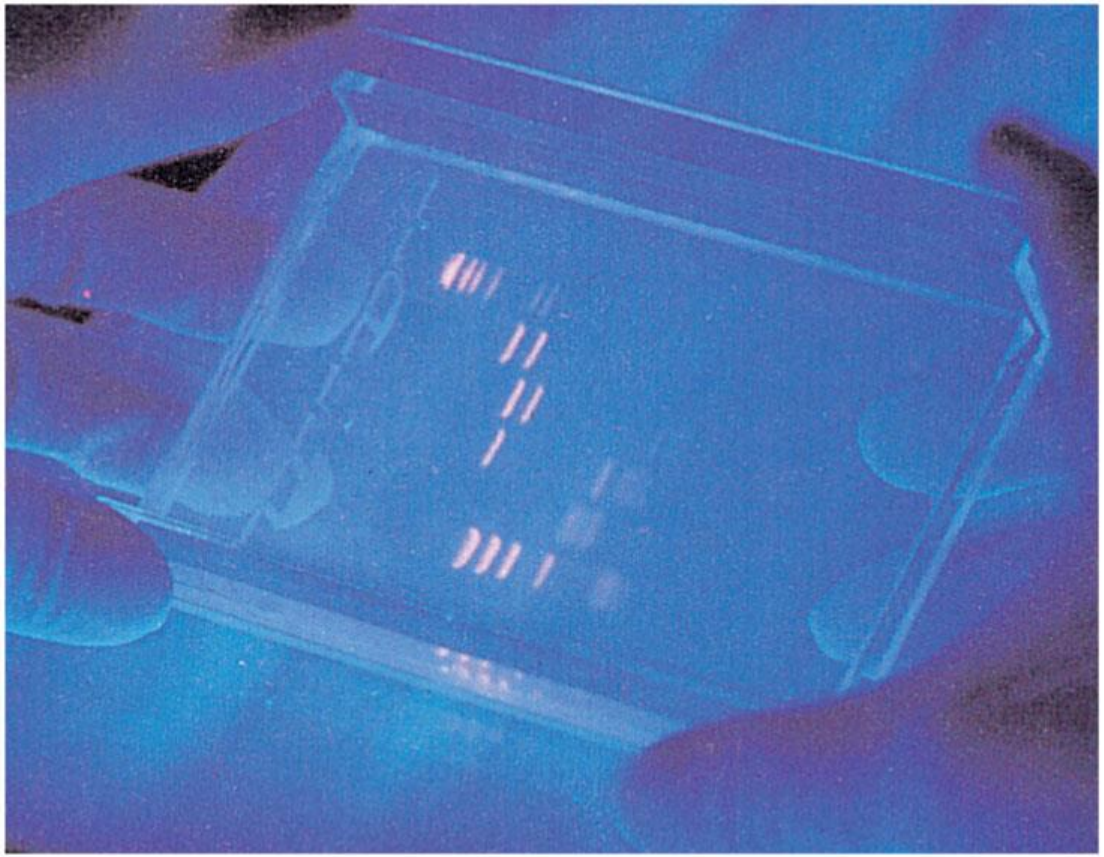
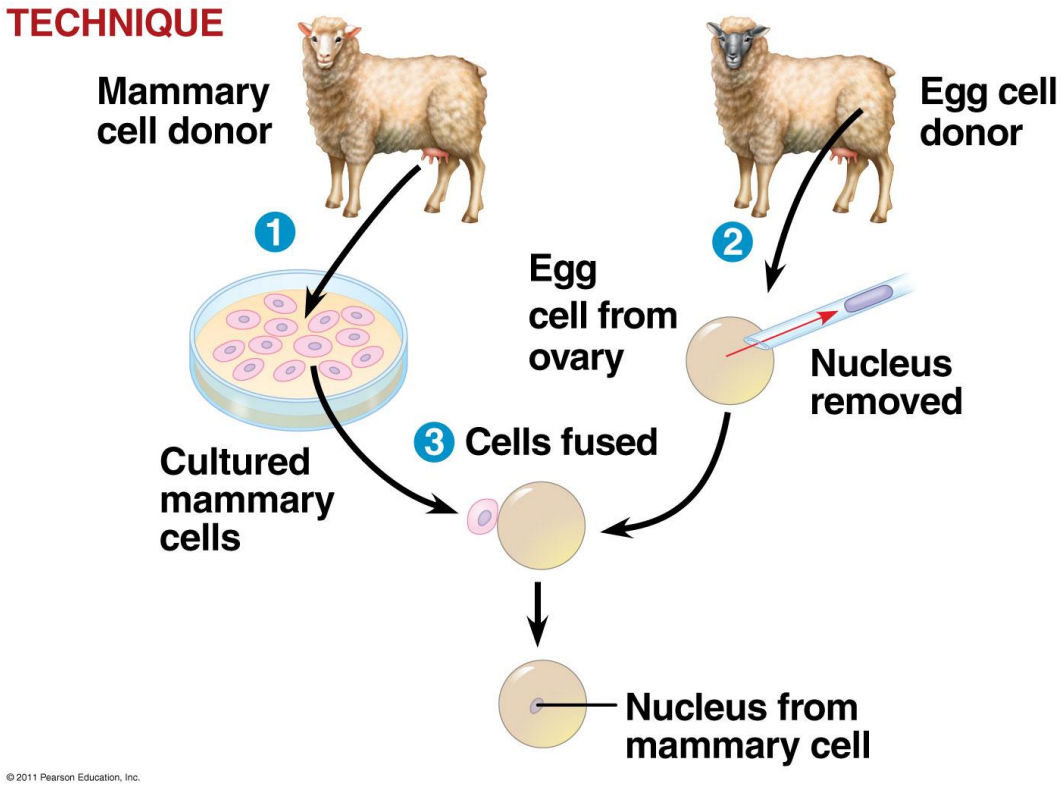
RESULTS

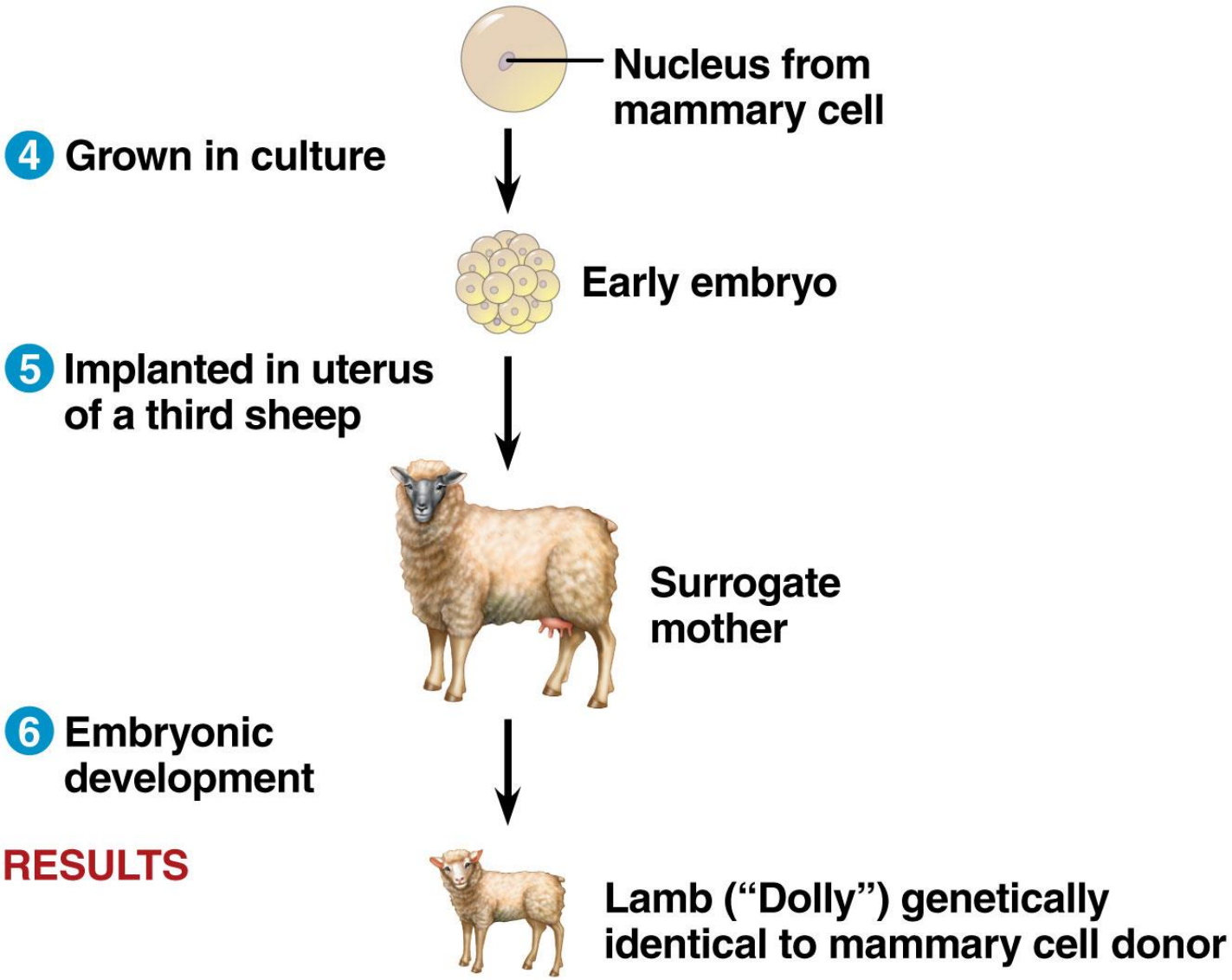


Cloning Organisms

- **Nuclear transplantation**: nucleus of egg is removed and replaced with nucleus of body cell



Nuclear Transplantation



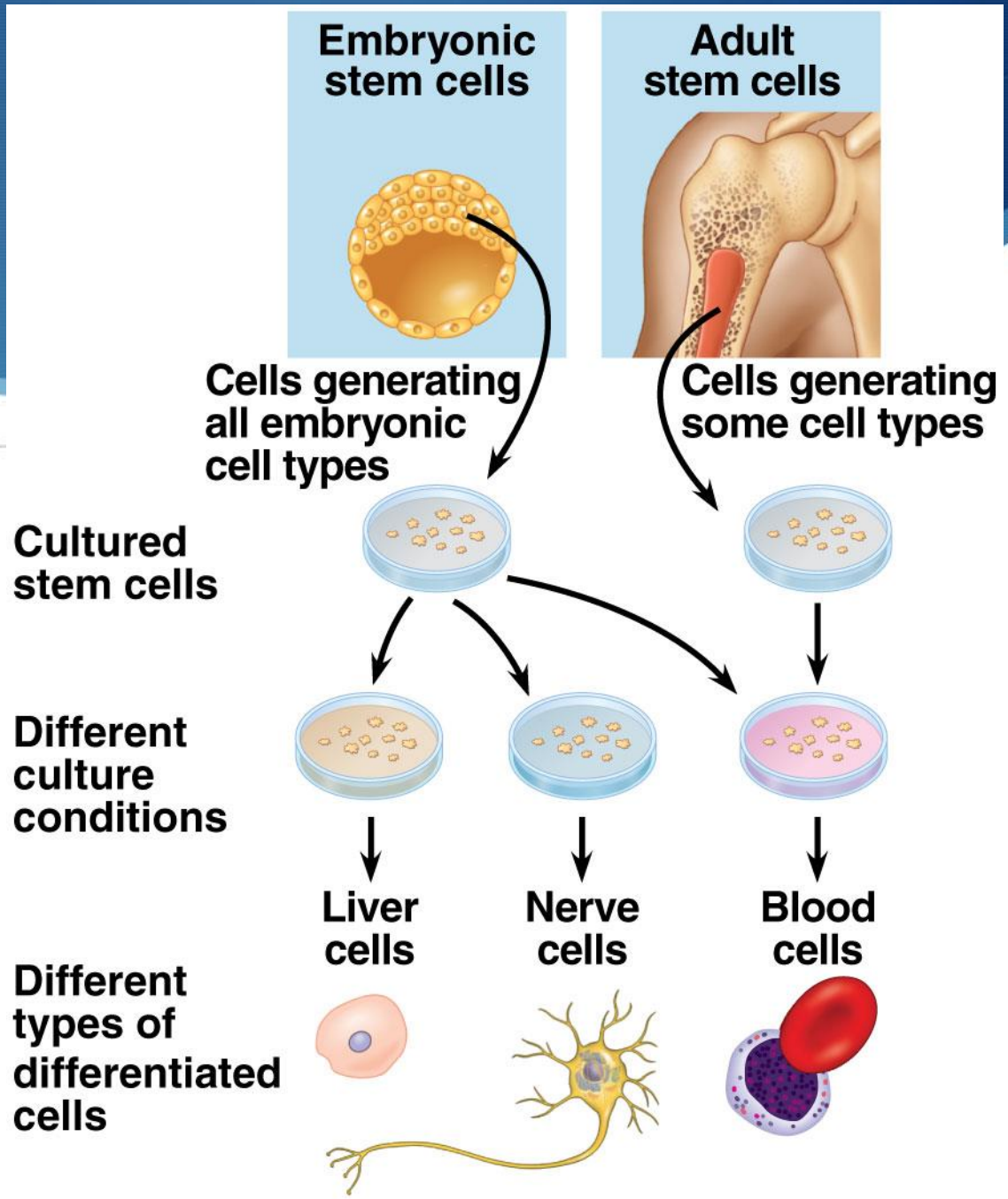
Problems with Reproductive Cloning

- Cloned embryos exhibited various defects
- DNA of fully differentiated cell have **epigenetic changes**

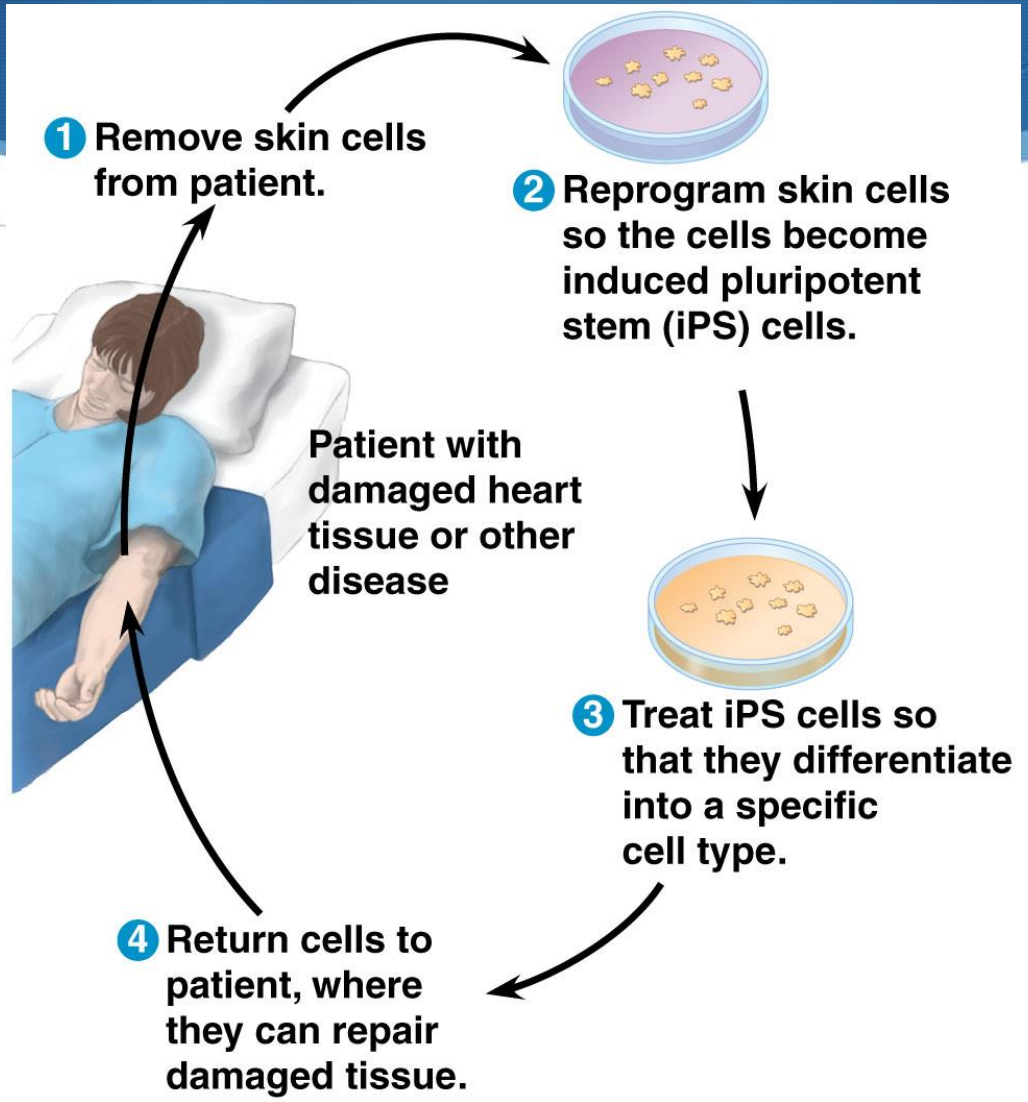
Stem Cells

- **Stem cells**: can reproduce itself indefinitely and produce other specialized cells
  - Zygote = totipotent (*any* type of cell)
  - Embryonic stem cells = pluripotent (*many* cell types)
  - Adult stem cells = multipotent (a *few* cell types) or induced pluripotent, iPS (forced to be pluripotent)

Embryonic vs. Adult stem cells



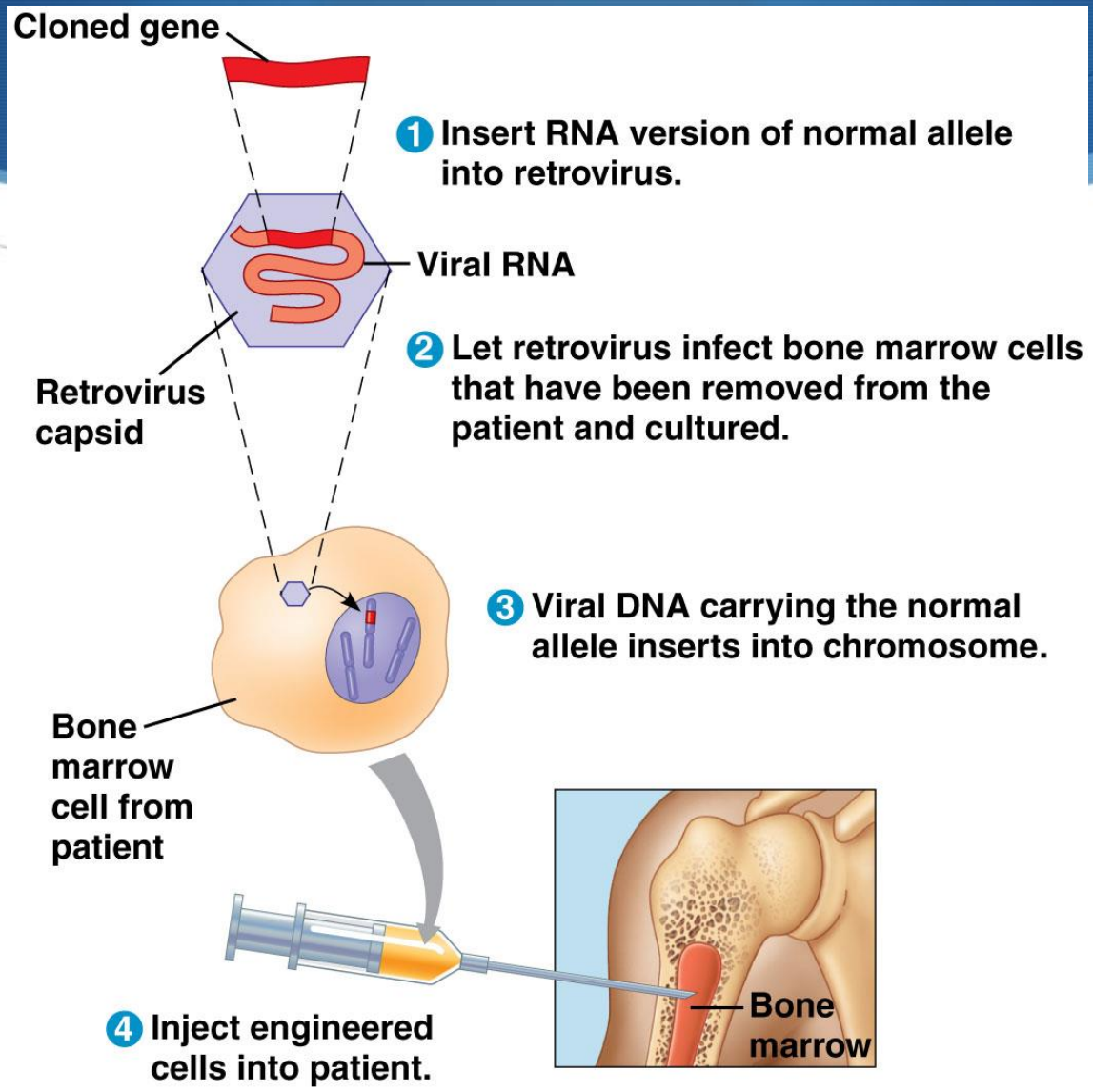
Using stem cells for disease treatment



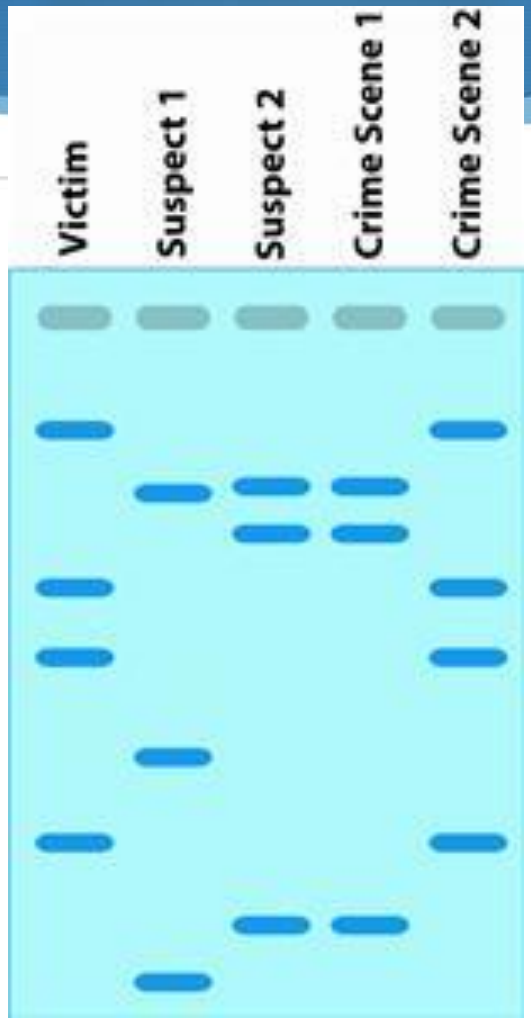
Applications of DNA Technology

1. Diagnosis of disease – identify alleles, viral DNA
2. Gene therapy – alter afflicted genes
3. Production of pharmaceuticals
4. Forensic applications – DNA profiling
5. Environmental cleanup – use microorganisms
6. Agricultural applications - GMOs

Gene therapy using a retroviral vector



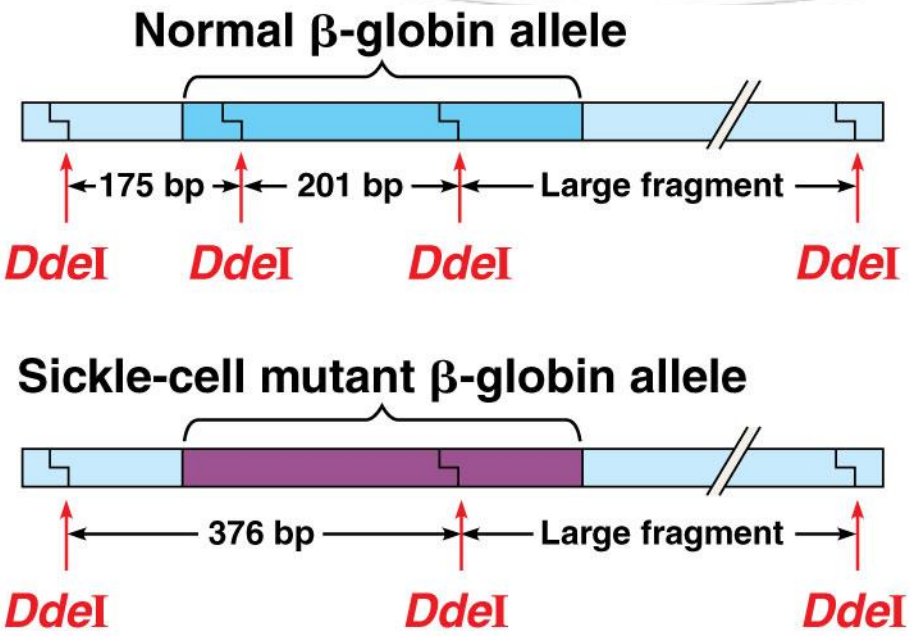
DNA Fingerprinting



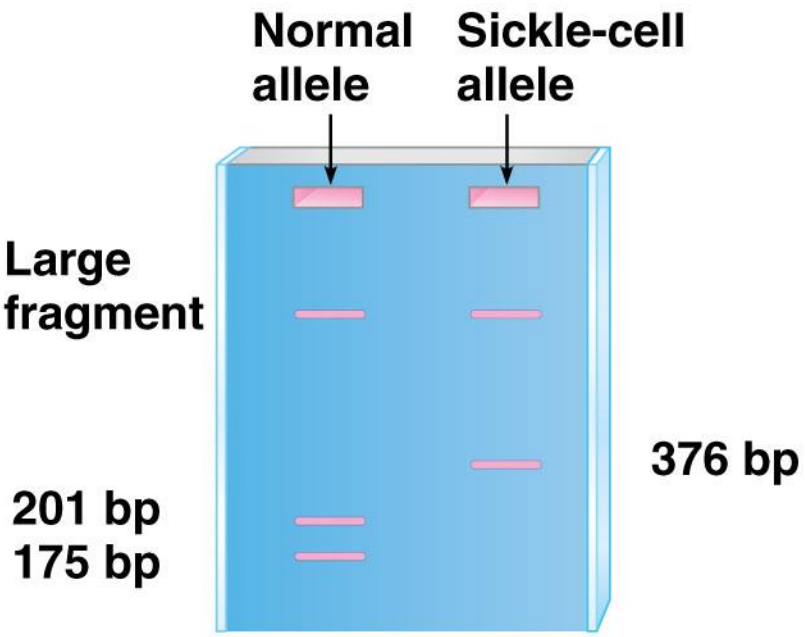
RFLPs ("rif-lips")

- Restriction Fragment Length Polymorphism
- Cut DNA with different restriction enzymes
- Each person has different #s of DNA fragments created
- Analyze DNA samples on a gel for disease diagnosis
- Outdated method of DNA profiling (required a quarter-sized sample of blood)

RFLPs – Disease Diagnosis



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

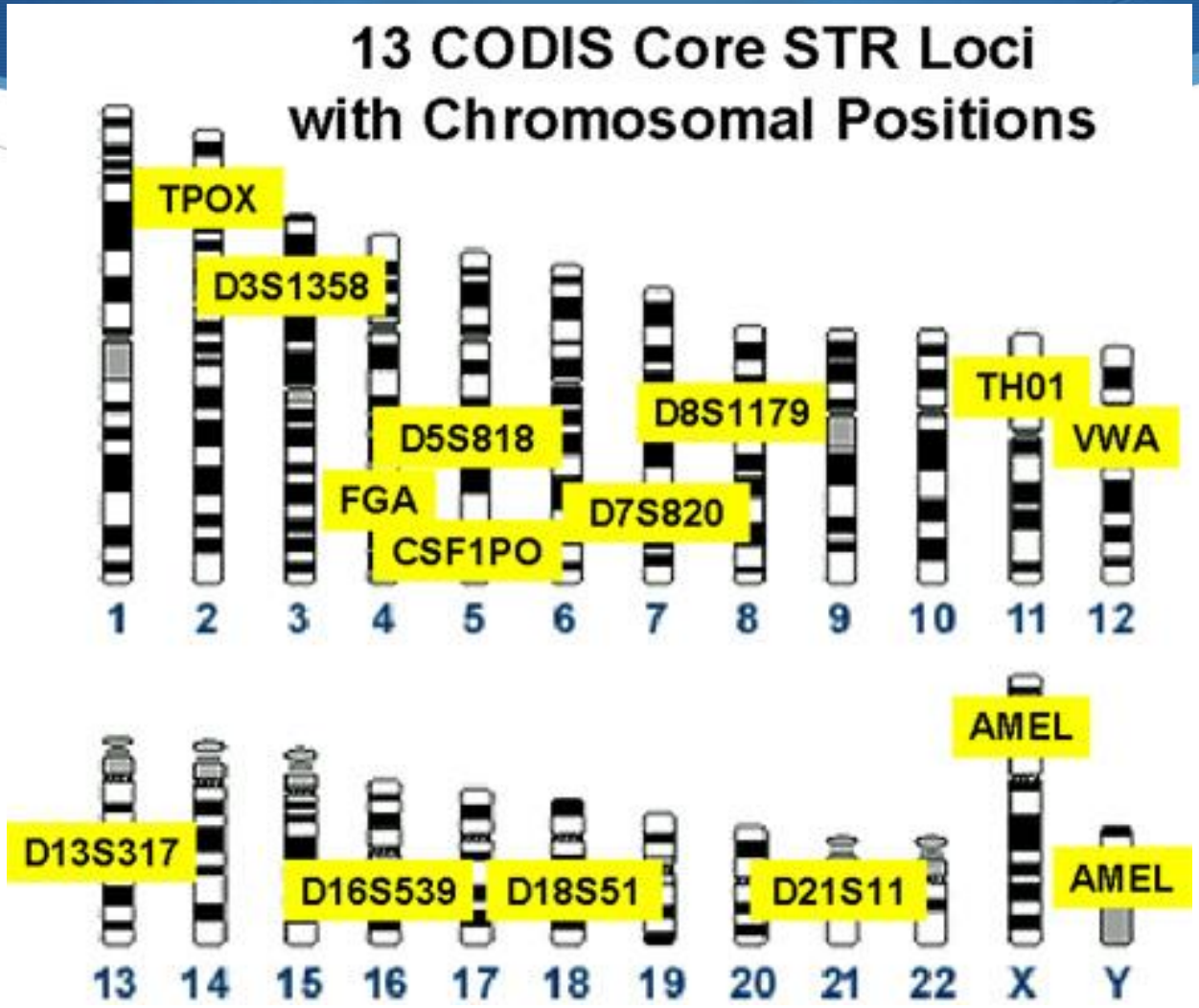


(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

STR Analysis

- STR = Short Tandem Repeats
- Non-coding DNA has regions with sequences (2-5 base length) that are repeated
- Each person has different # of repeats at different locations (loci)
- Current method of DNA fingerprinting used – only need 20 cells for analysis

STR Analysis



STR Analysis