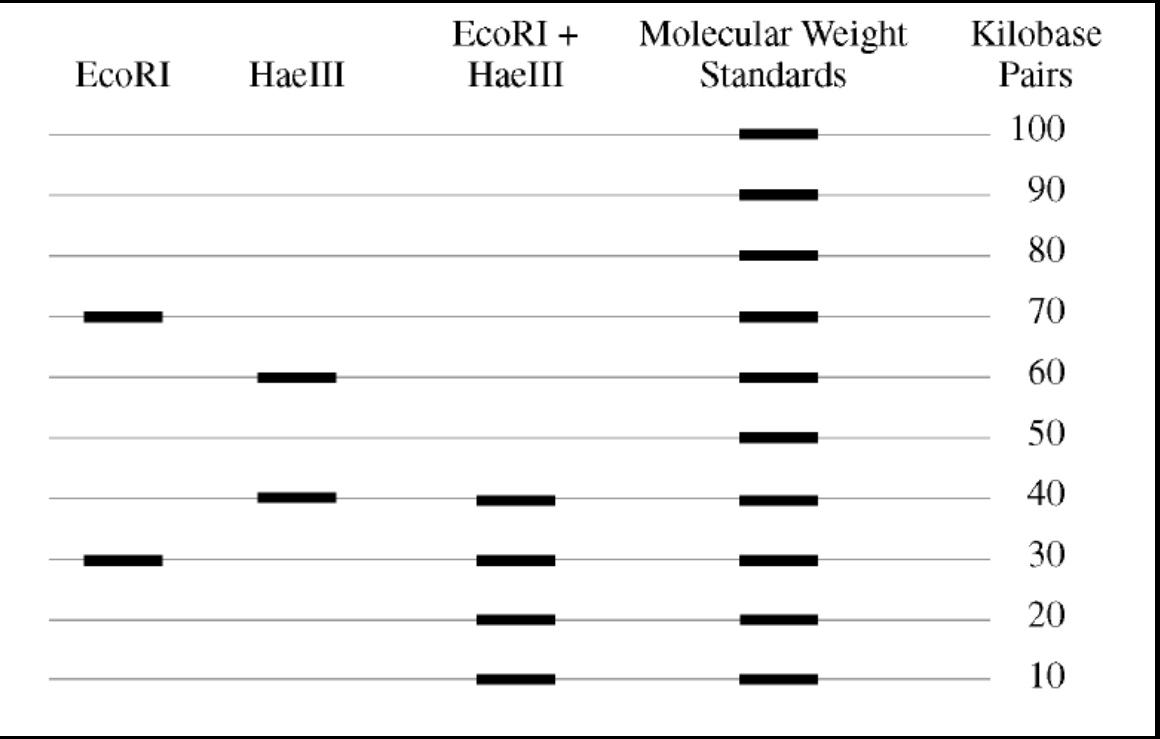
Biotechnology warm-up:

Use your textbook to answer the following review questions.

1. What is recombinant DNA?
2. What are plasmids?
3. What are restriction enzymes (RE)?
4. When DNA is cut using an RE, describe the ends of the DNA fragments.

Warm-up

A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with 2 restriction enzymes in 3 separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double-digest). The fragments were separated by gel electrophoresis below.



Draw a circle to represent the plasmid. On the circle, **construct** a labeled diagram of the restriction map of the plasmid.

Warm-up

1. Describe how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid.
2. How can a genetically modified organism provide a benefit for humans and at the same time pose a threat to a population or ecosystem?

BIOTECHNOLOGY

WHAT YOU MUST KNOW:

- The terminology of biotechnology.
- How plasmids are used in bacterial transformation to clone genes.
- The key ideas that make PCR possible and applications of this technology.
- How gel electrophoresis can be used to separate DNA fragments or protein molecules.
- Information that can be determined from DNA gel results, such as fragment sizes and RFLP analysis.

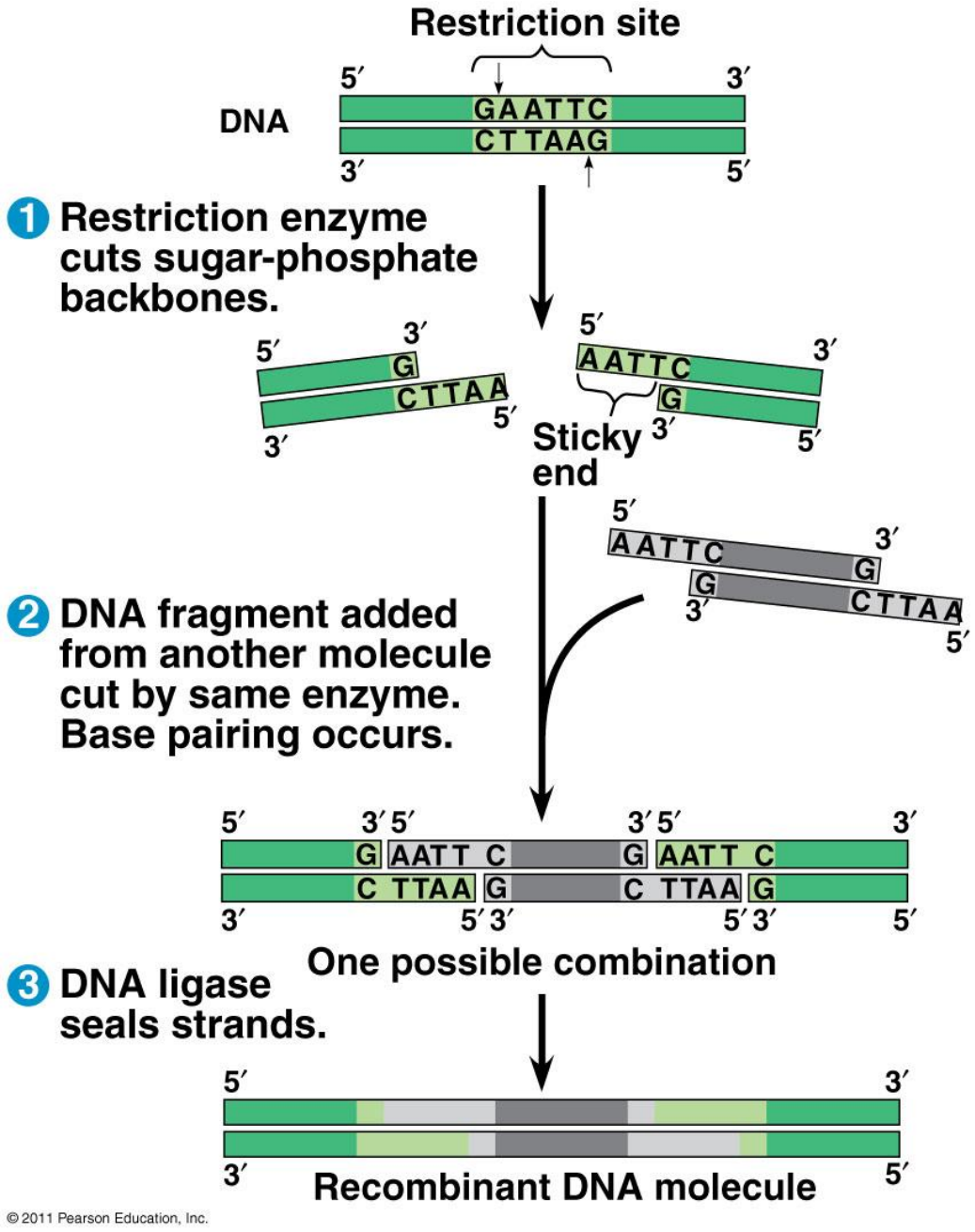
- **Genetic Engineering**: process of manipulating genes and genomes
- **Biotechnology**: process of manipulating organisms or their components for the purpose of making useful products.

- **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

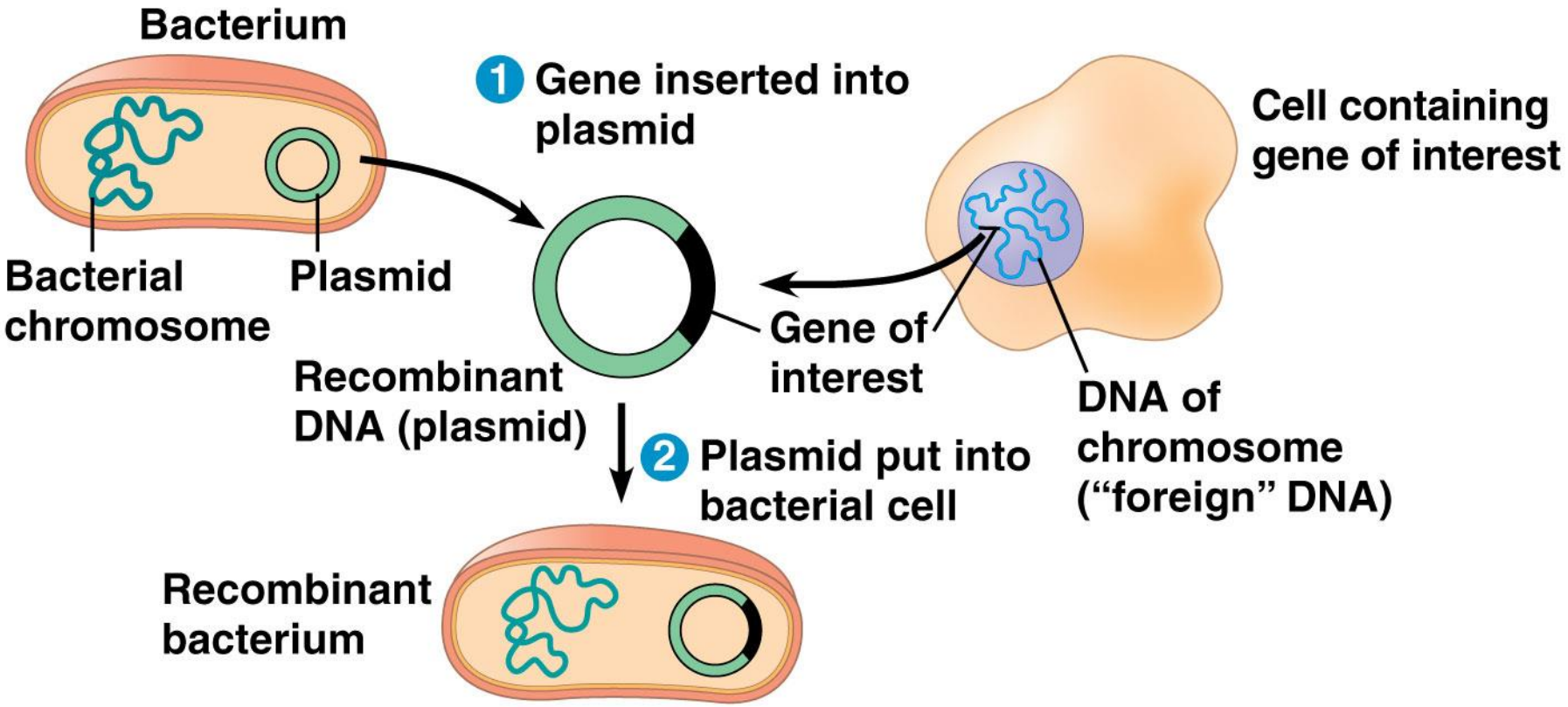
TOOLS OF GENETIC ENGINEERING

- **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)

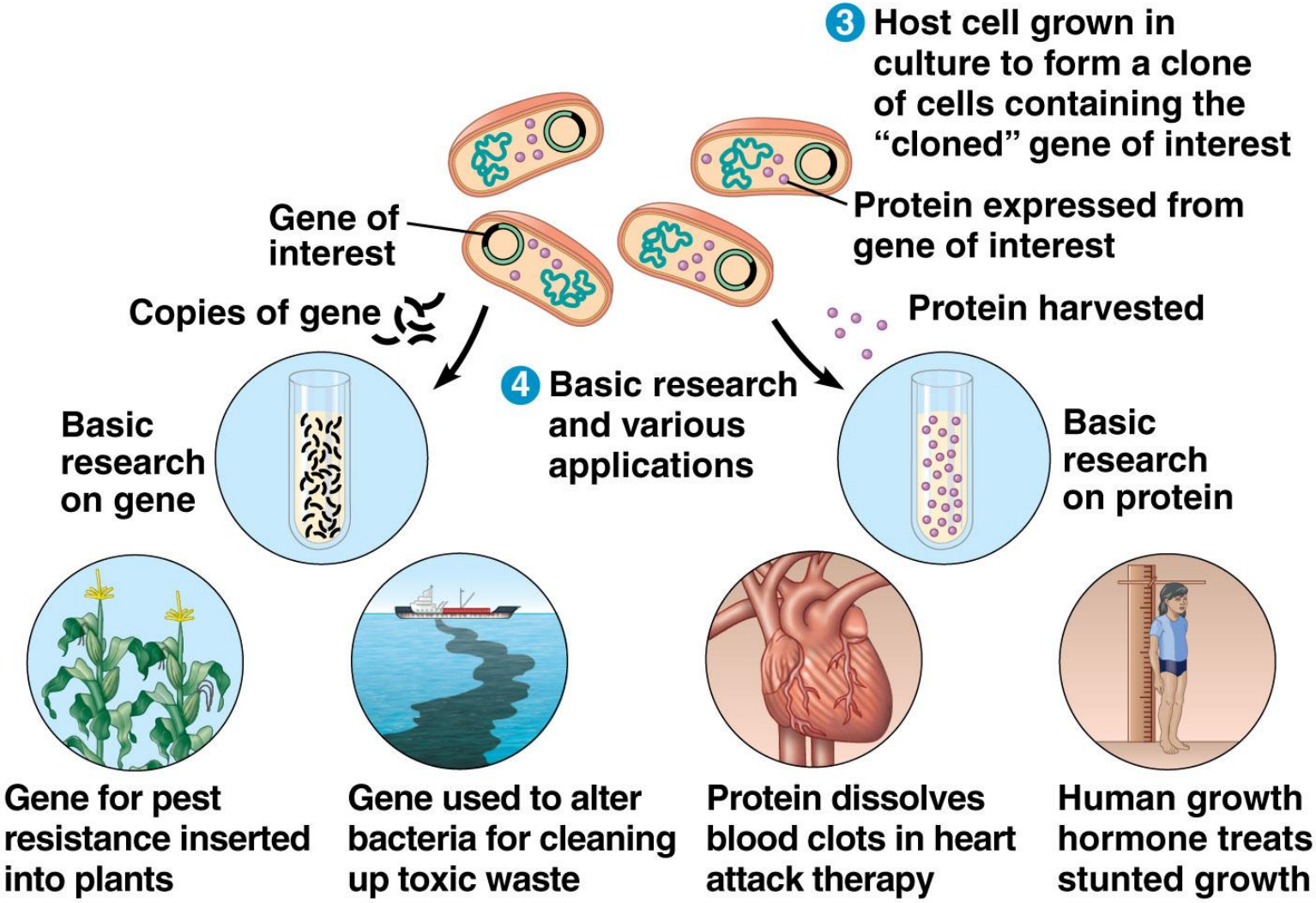
Using a restriction enzyme (RE) and DNA ligase to make recombinant DNA



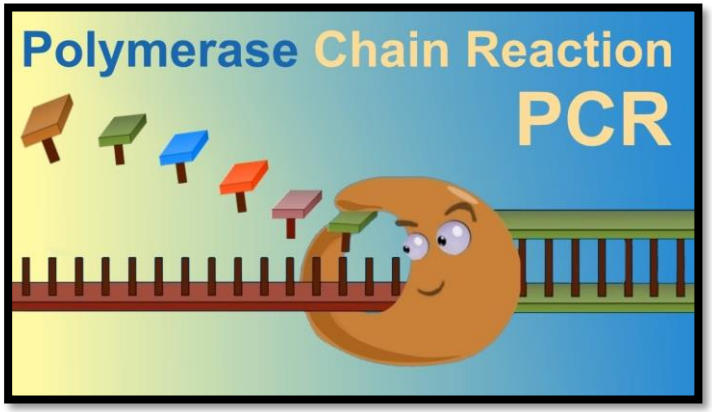
Gene Cloning

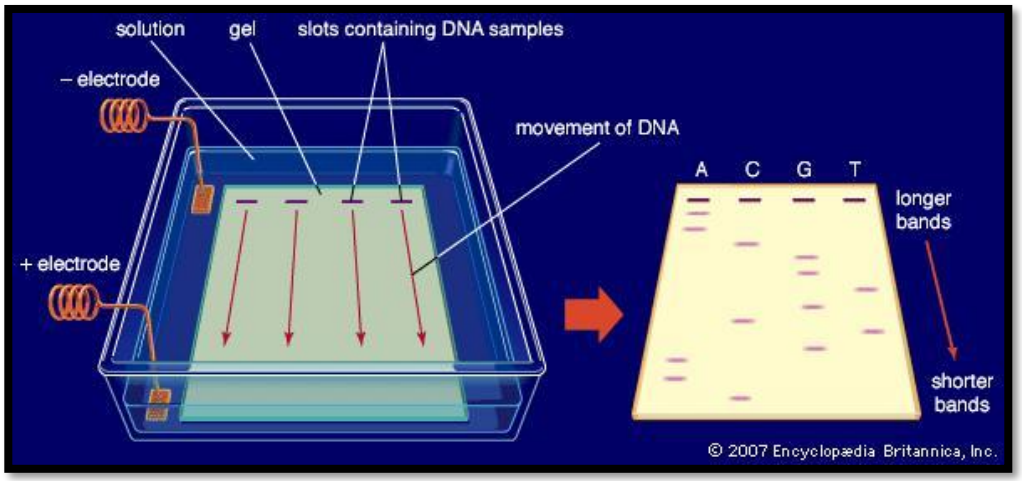


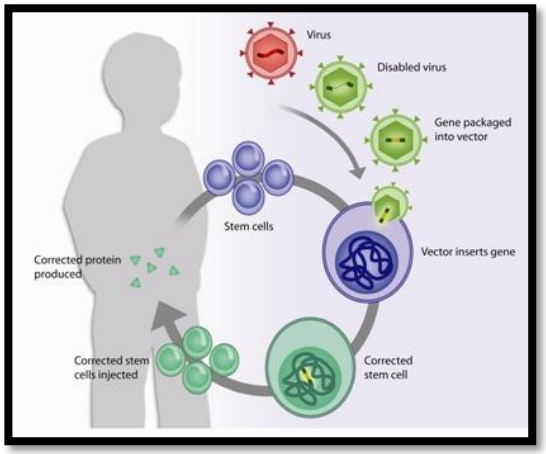
Applications of Gene Cloning



Techniques of Genetic Engineering

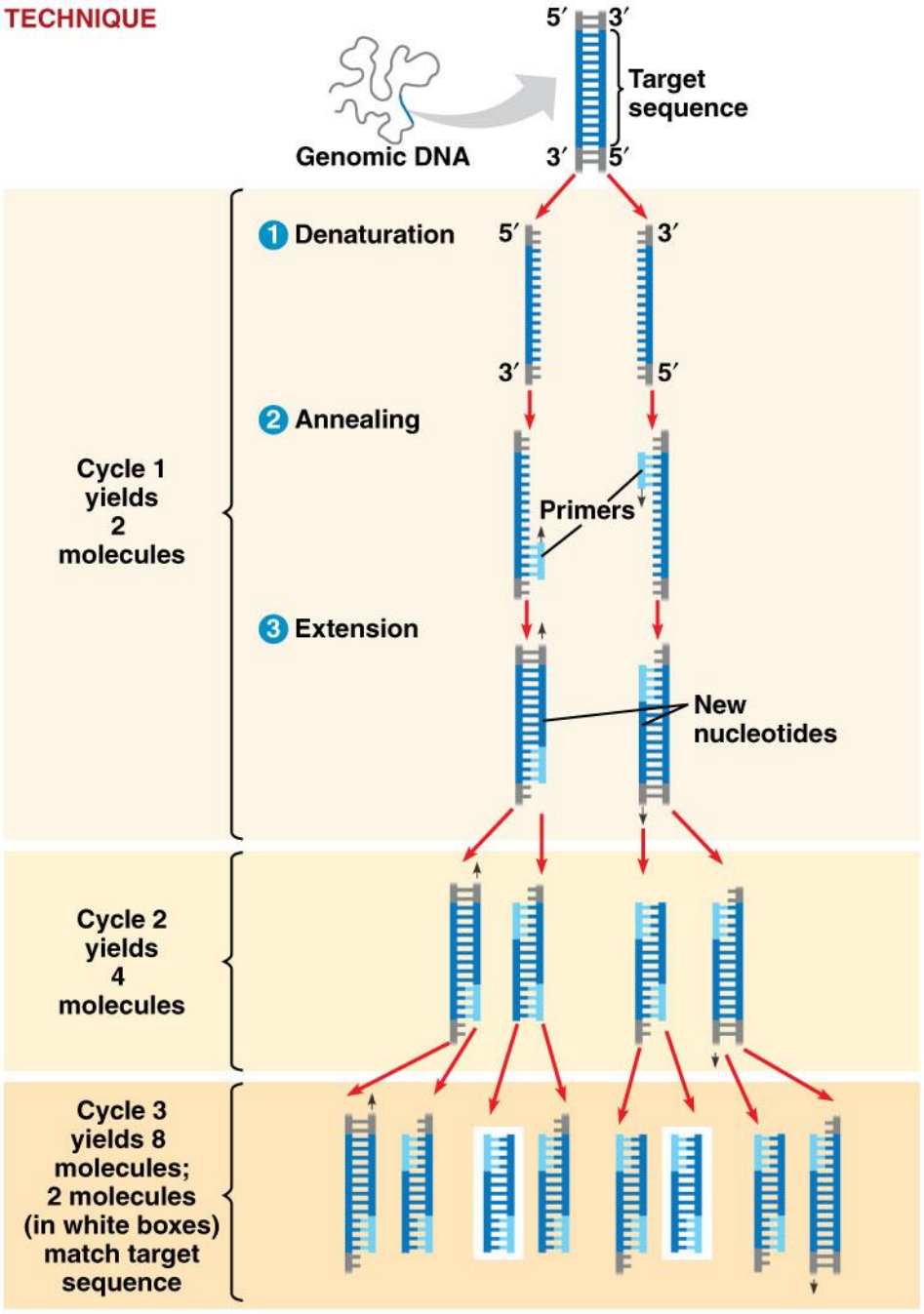






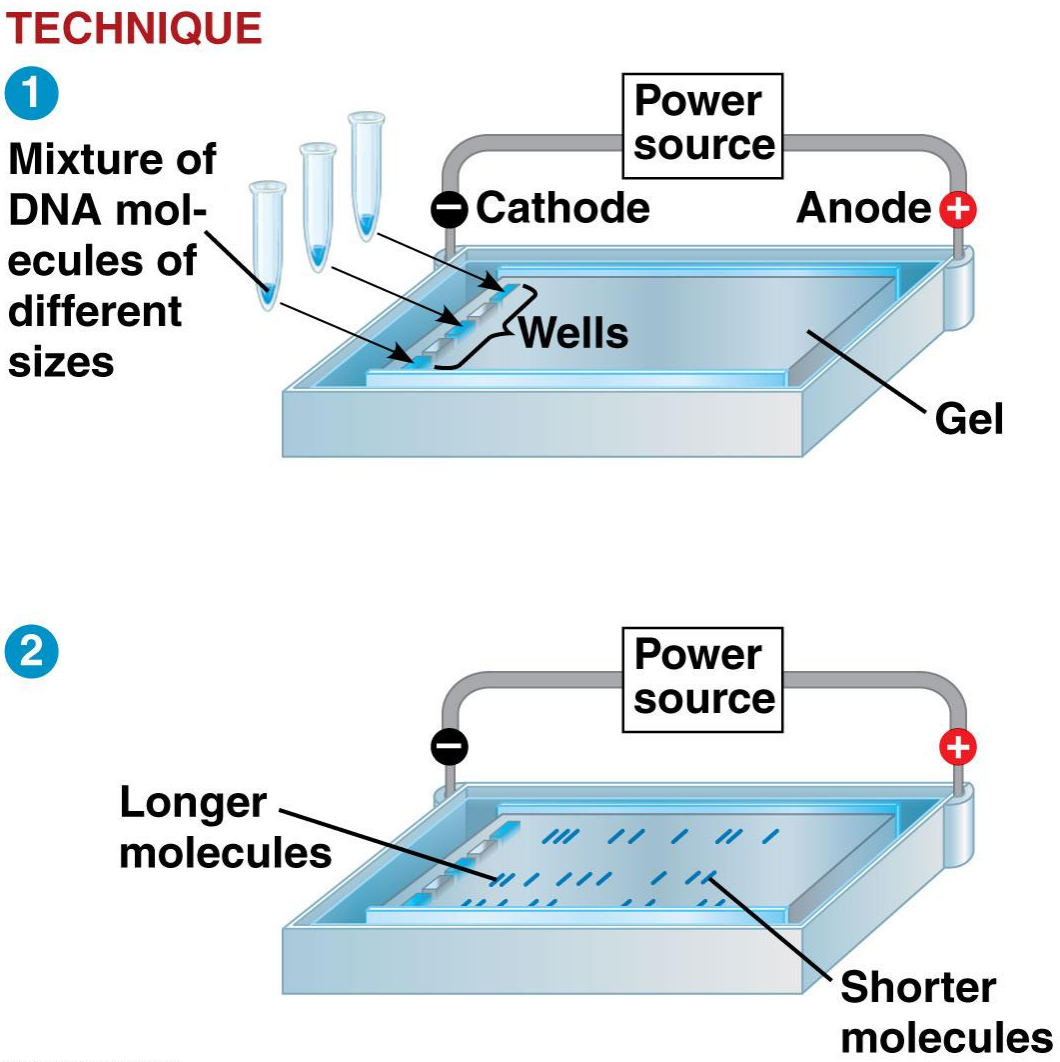
TECHNIQUES OF GENETIC ENGINEERING

- **Transformation**: bacteria takes up plasmid (w/gene of interest)
- **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)
- **DNA microarray assays**: study many genes at same time



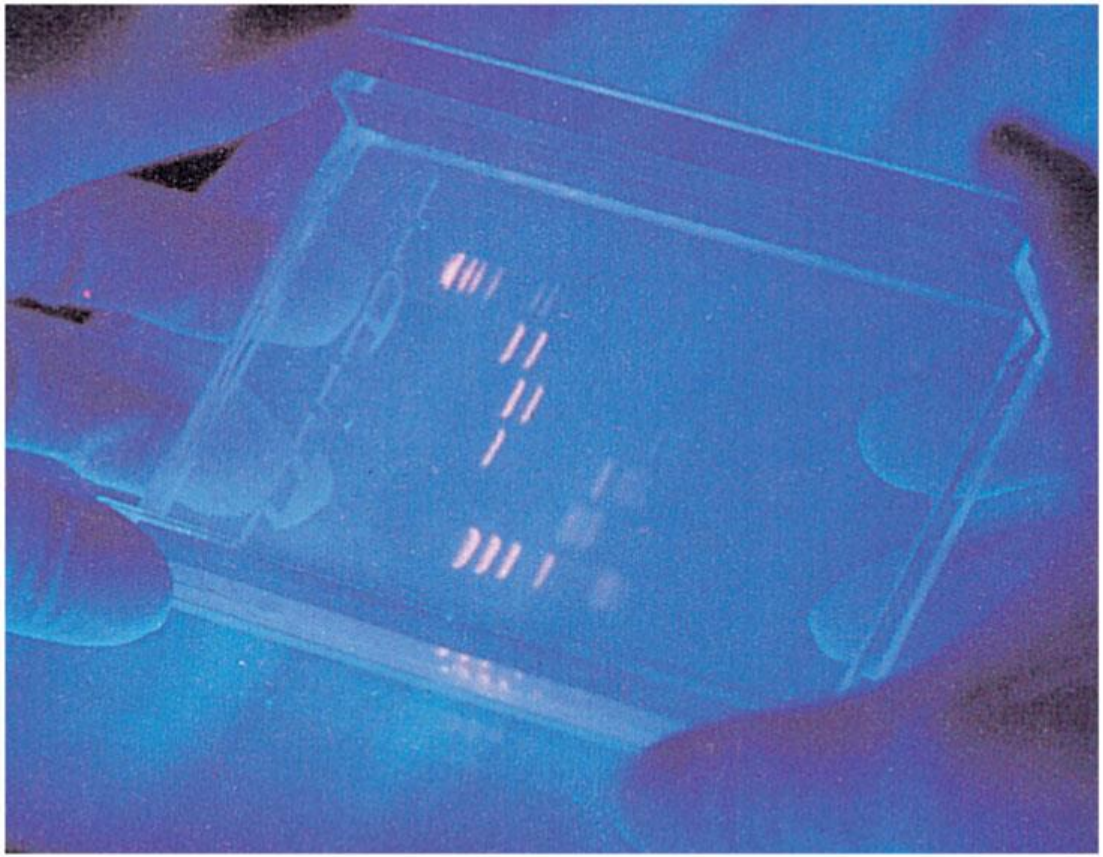
PCR (Polymerase Chain Reaction): amplify (copy) piece of DNA without use of cells

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

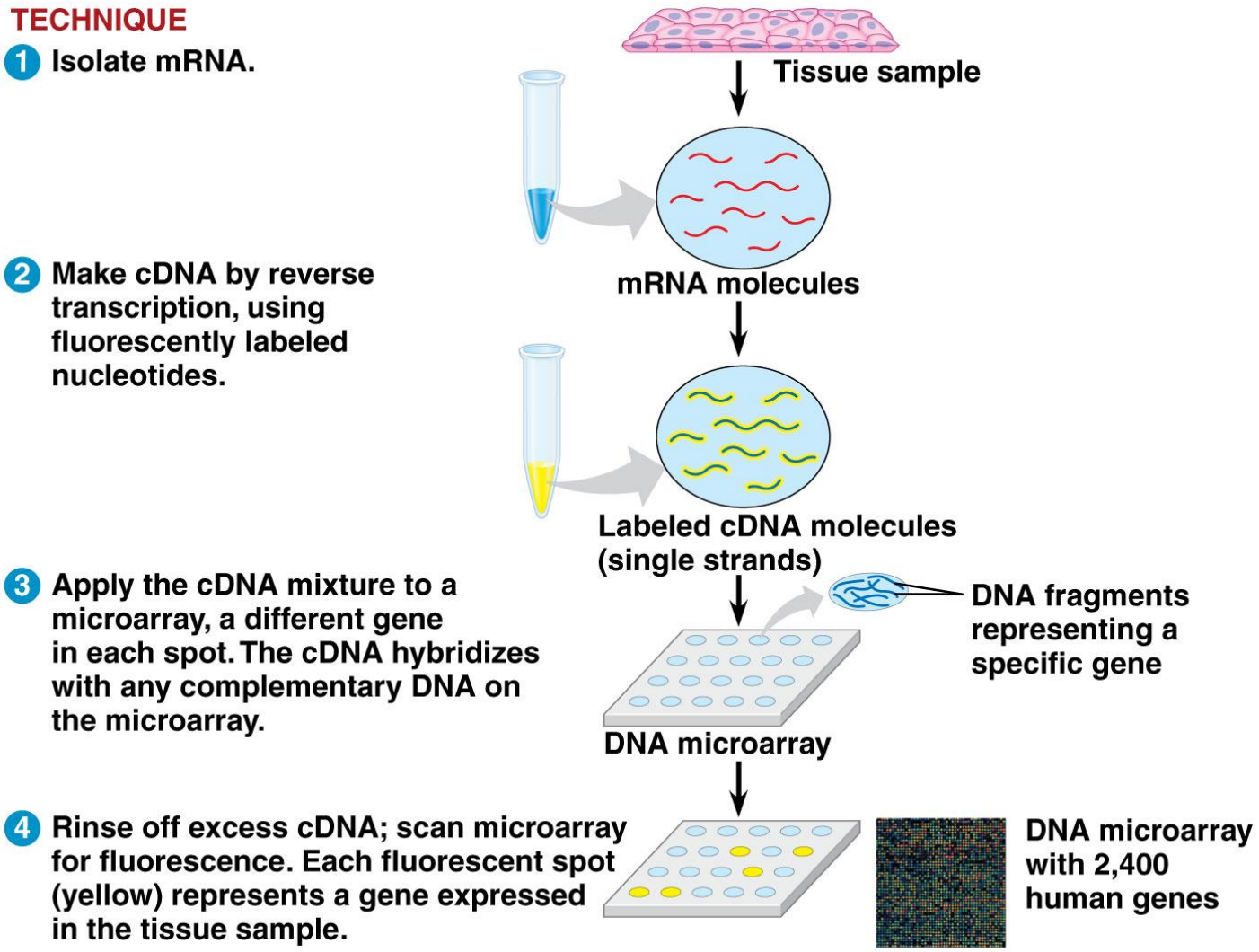


Gel Electrophoresis

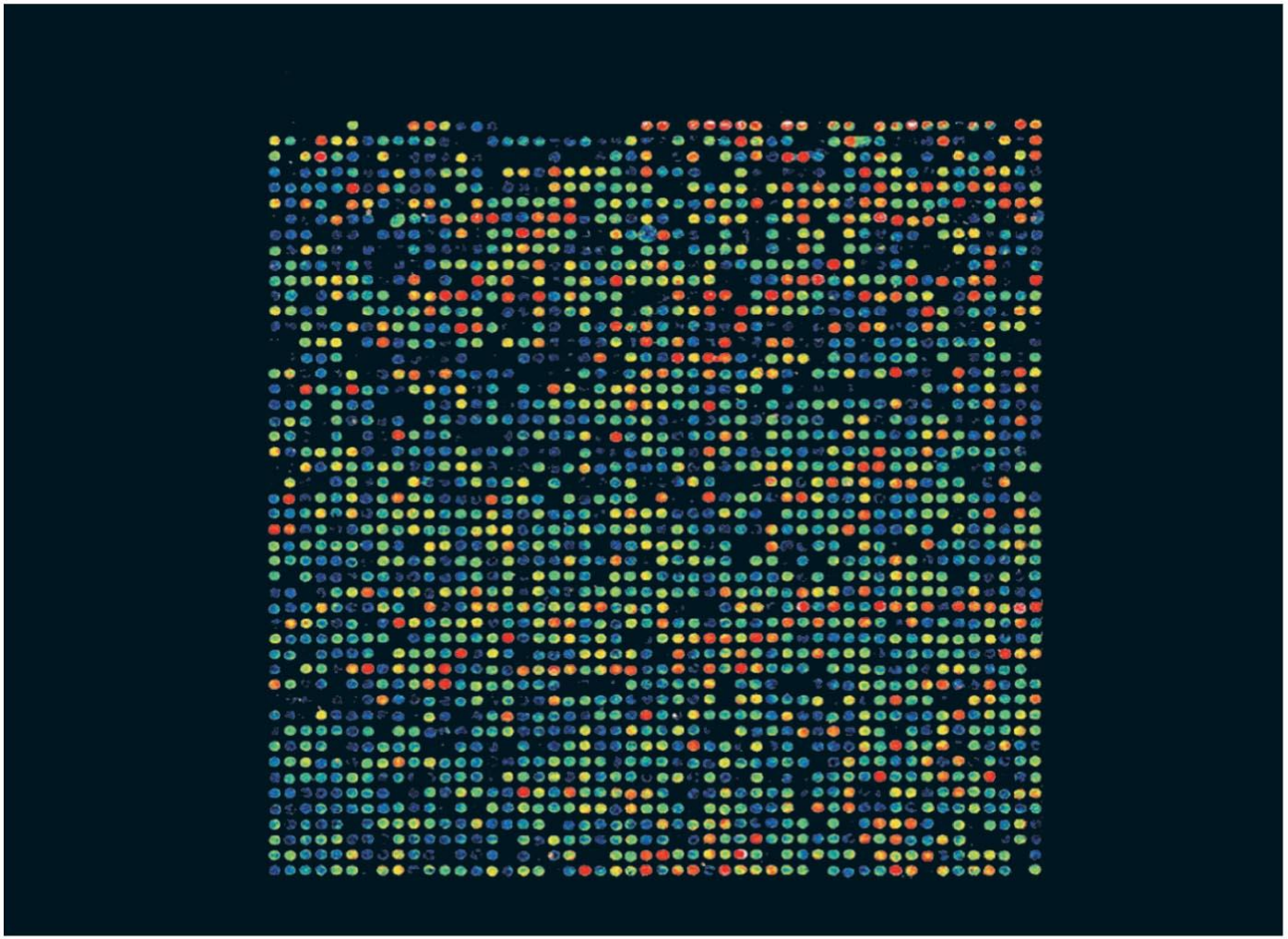
RESULTS



Microarray Assay: used to study gene expression of many different genes

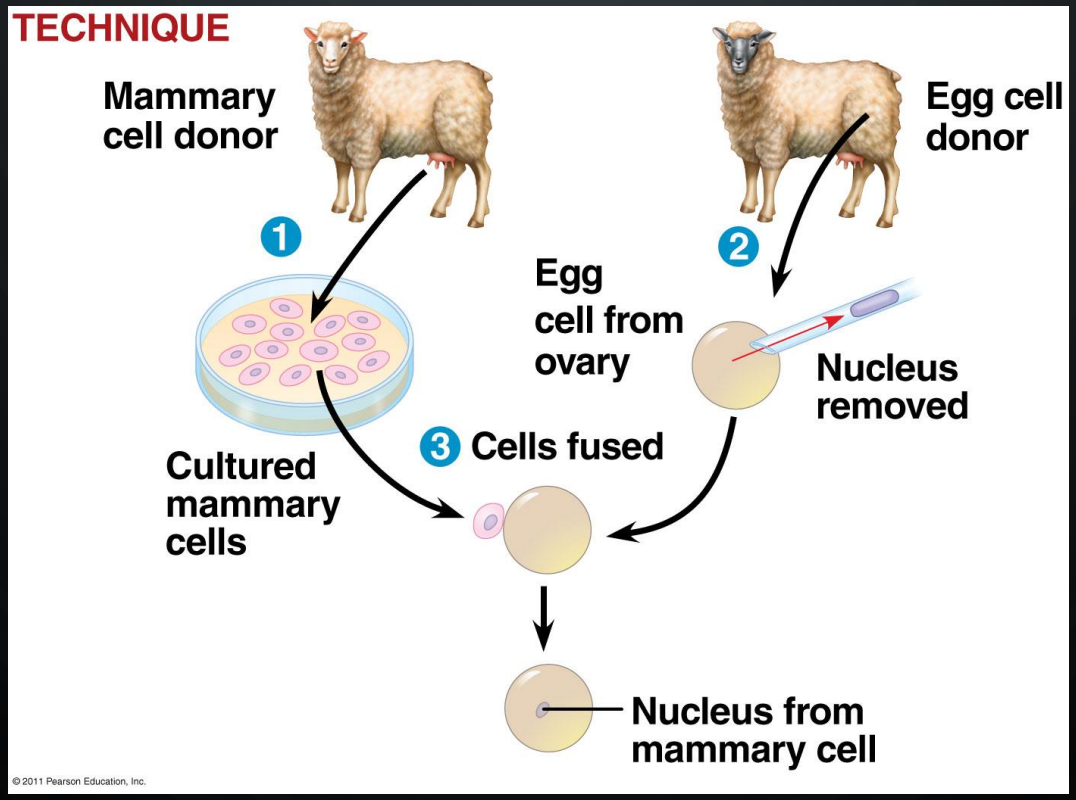


DNA microarray that reveals expression levels of 2,400 human genes

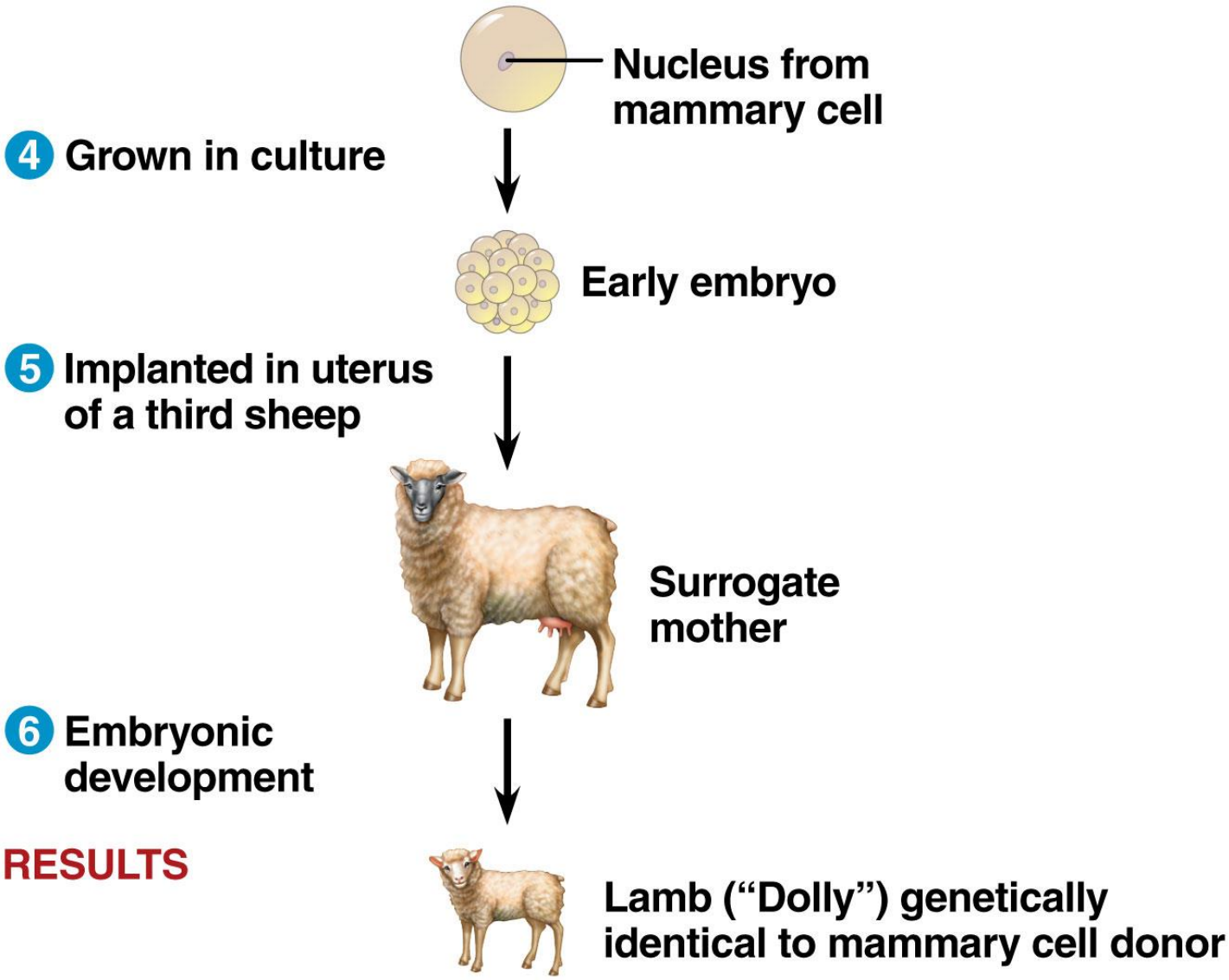


CLONING ORGANISMS

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



Nuclear Transplantation



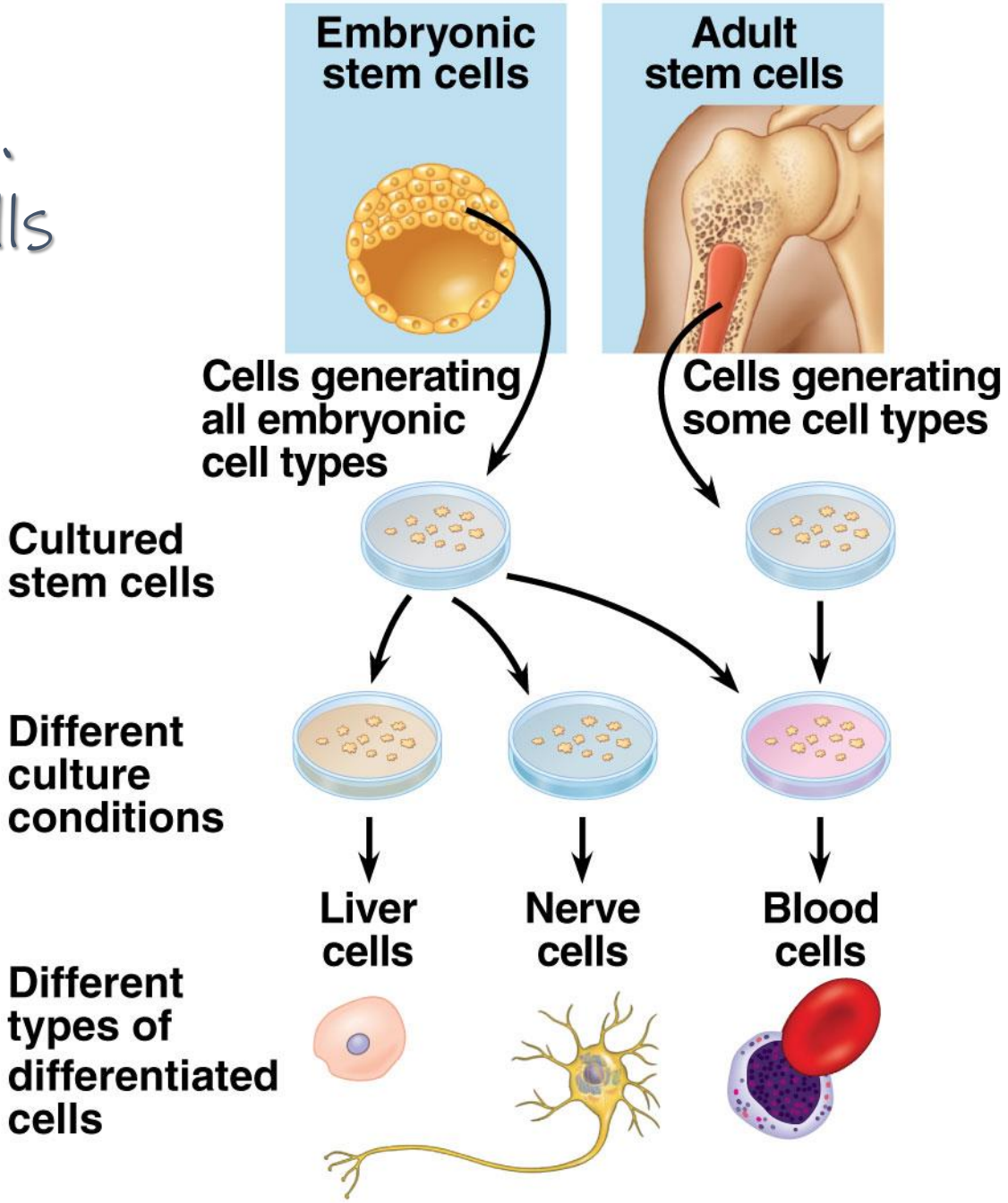
Problems with Reproductive Cloning

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

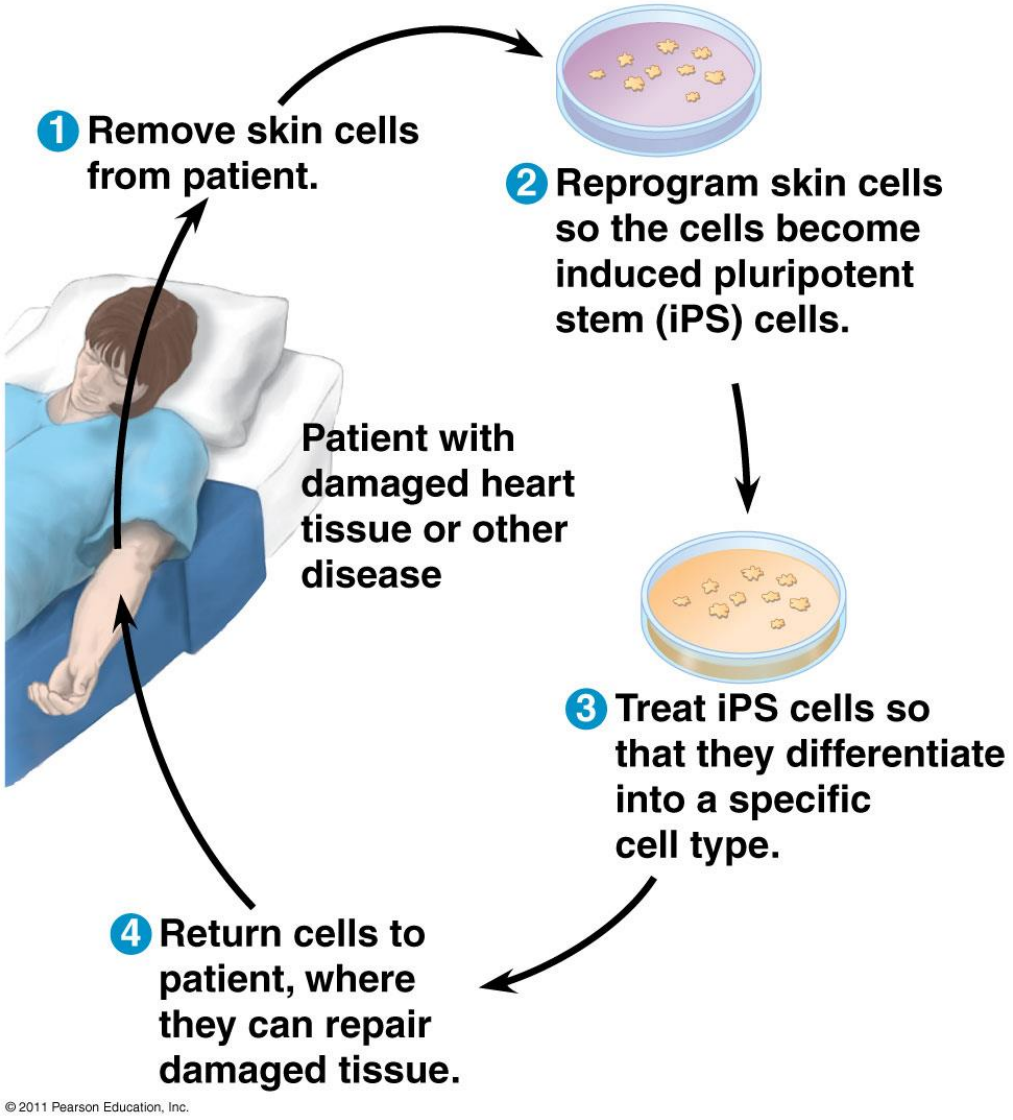
STEM CELLS

- **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



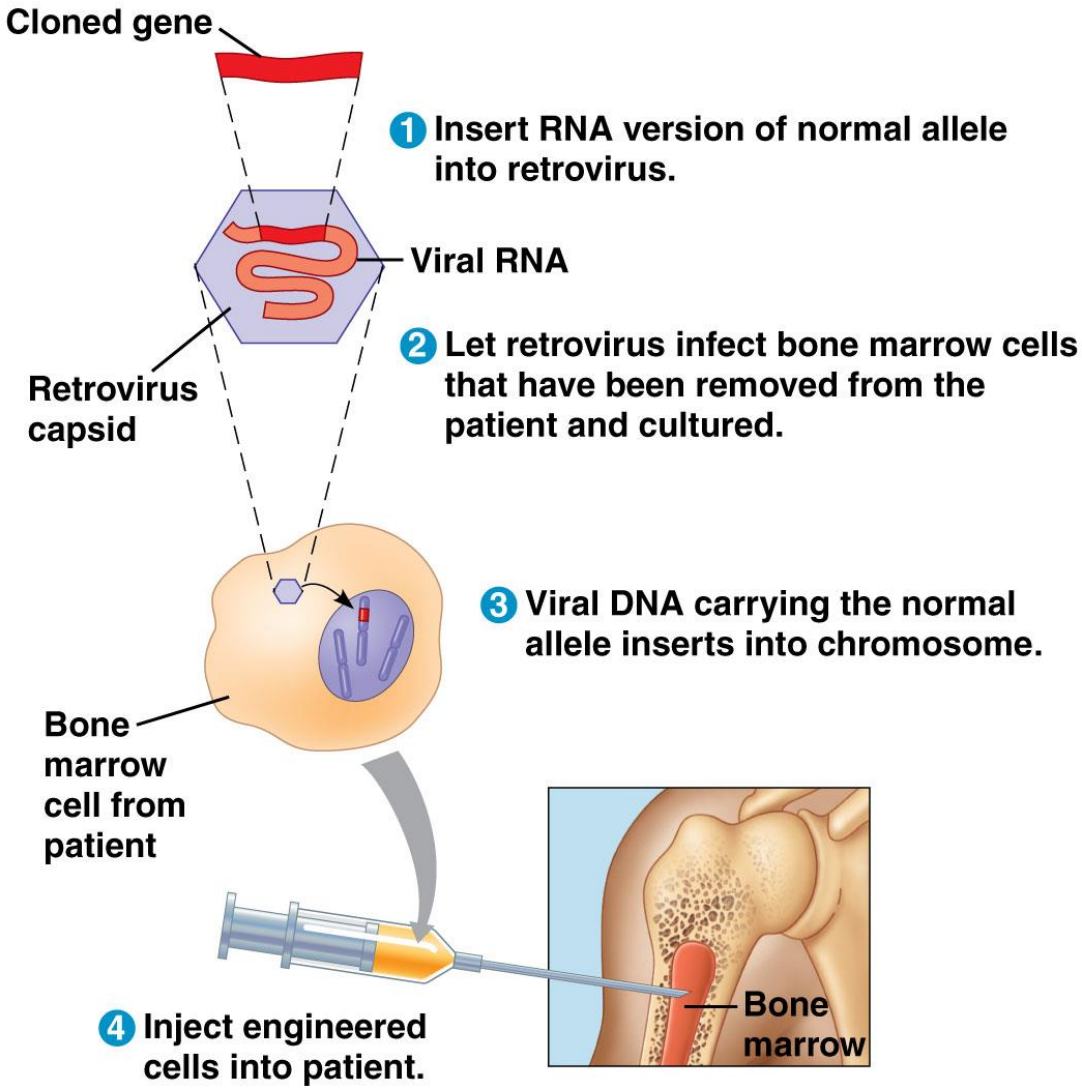
Using stem cells for disease treatment



APPLICATIONS OF DNA TECHNOLOGY

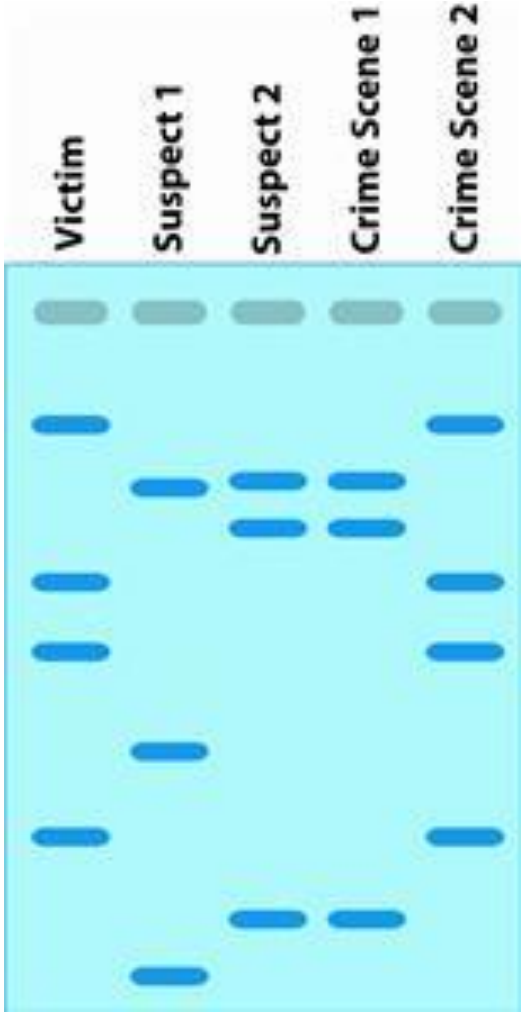
1. Diagnosis of disease – identify alleles, viral DNA
2. Gene therapy – alter afflicted genes
3. Production of pharmaceuticals
4. Forensic applications – DNA profiling
5. Environmental cleanup – use microorganisms
6. Agricultural applications - GMOs

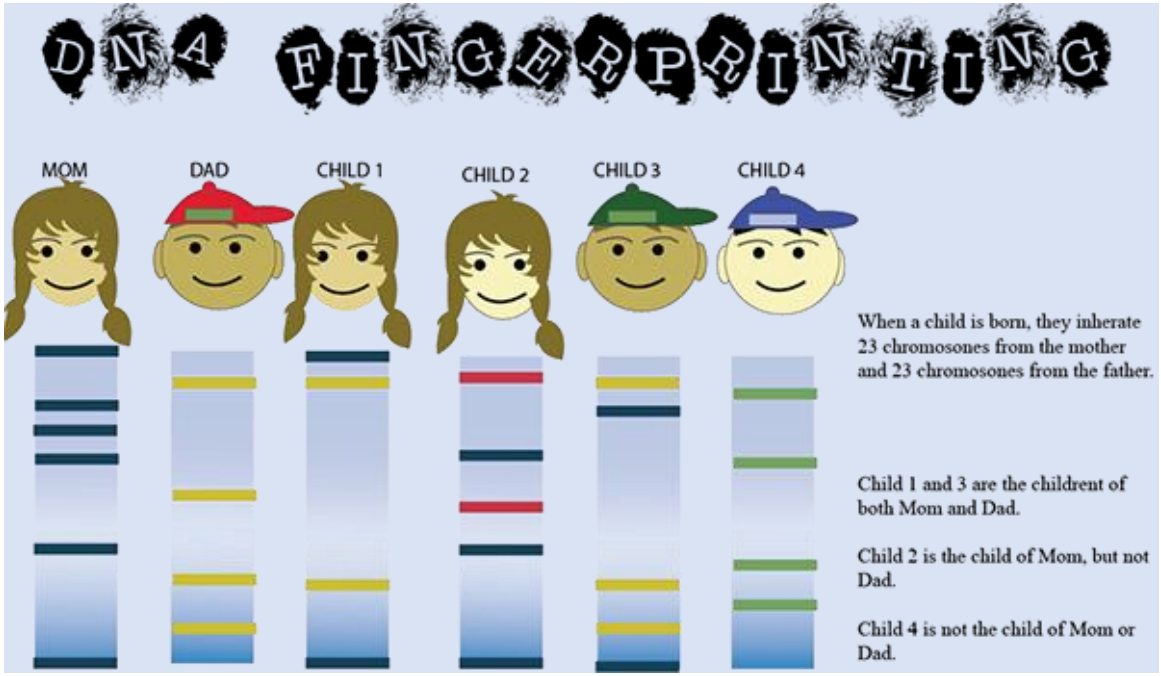
Gene therapy using a retroviral vector



"Pharm" animal: produce human protein secreted in milk for medical use

DNA Fingerprinting

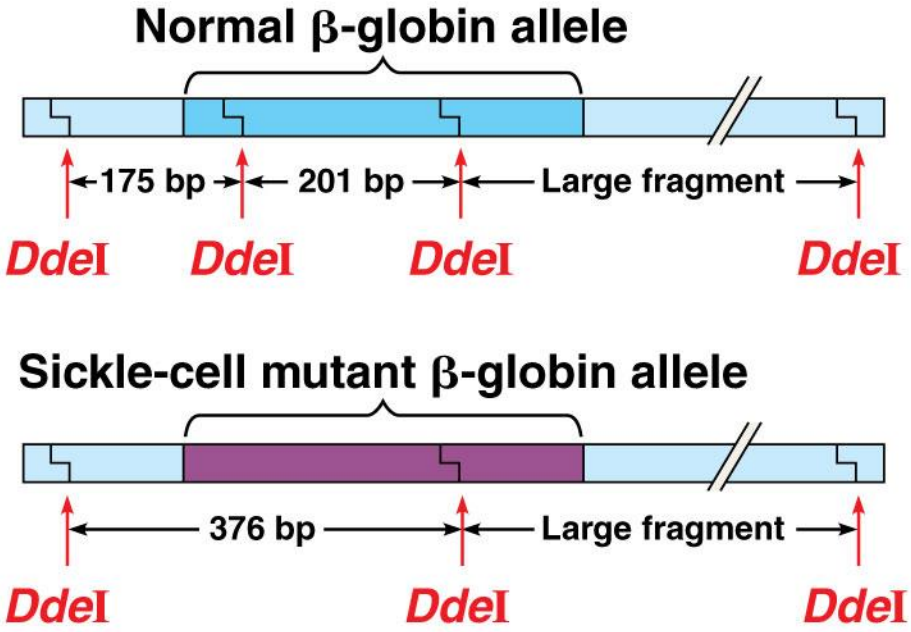




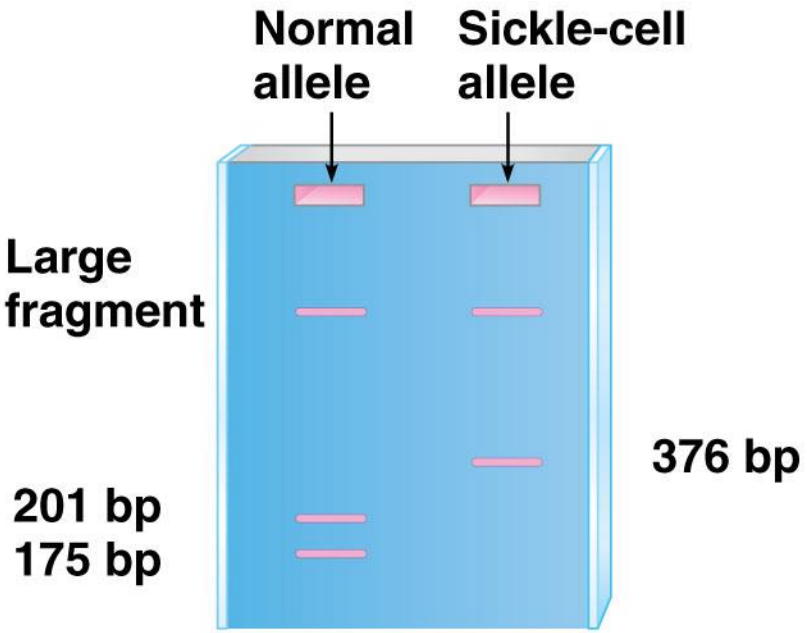
RFLPS ("RIF-LIPS")

- 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

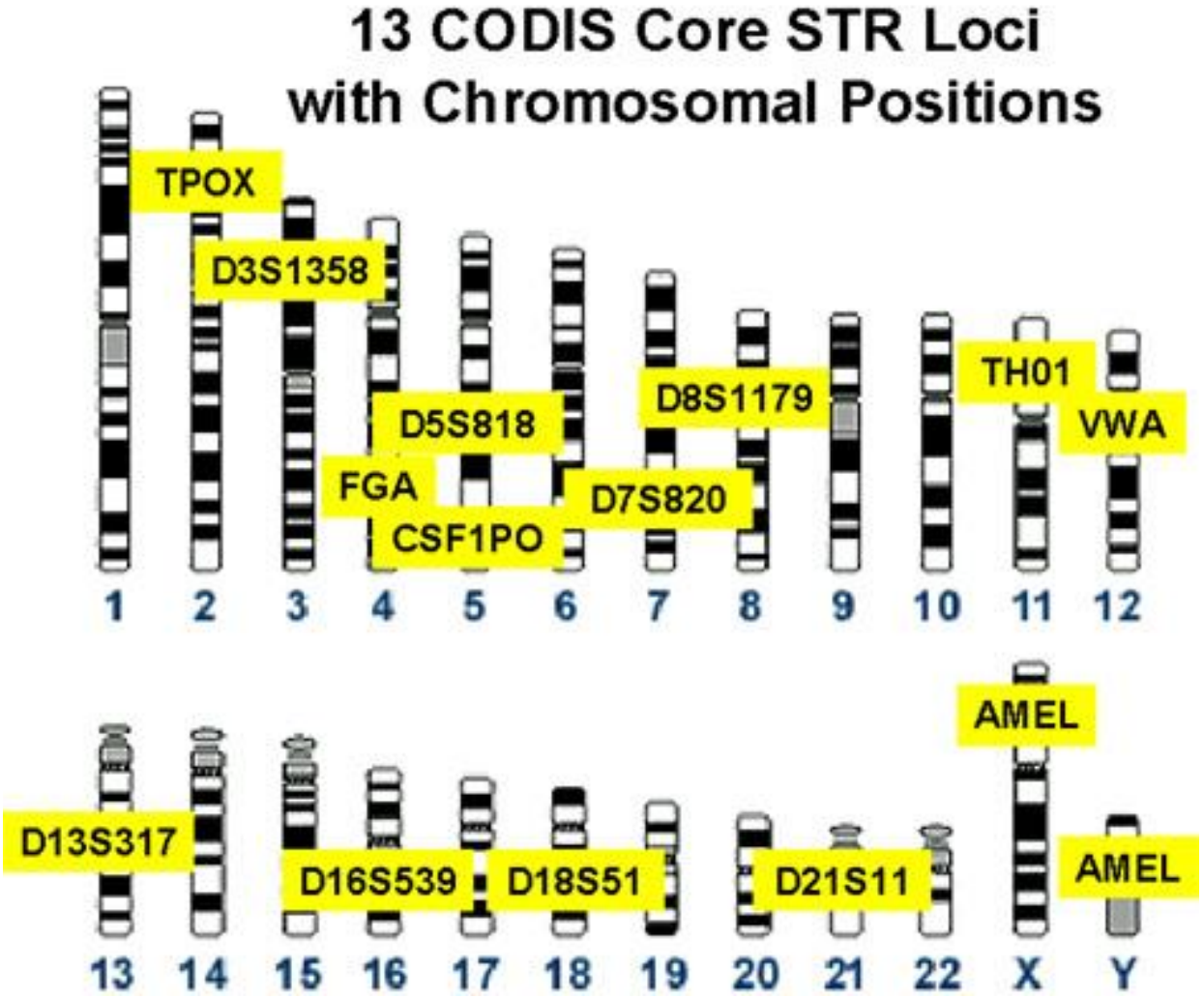
VIDEO: INTRODUCTION TO DNA FINGERPRINTING

NAKED SCIENCE SCRAPBOOK

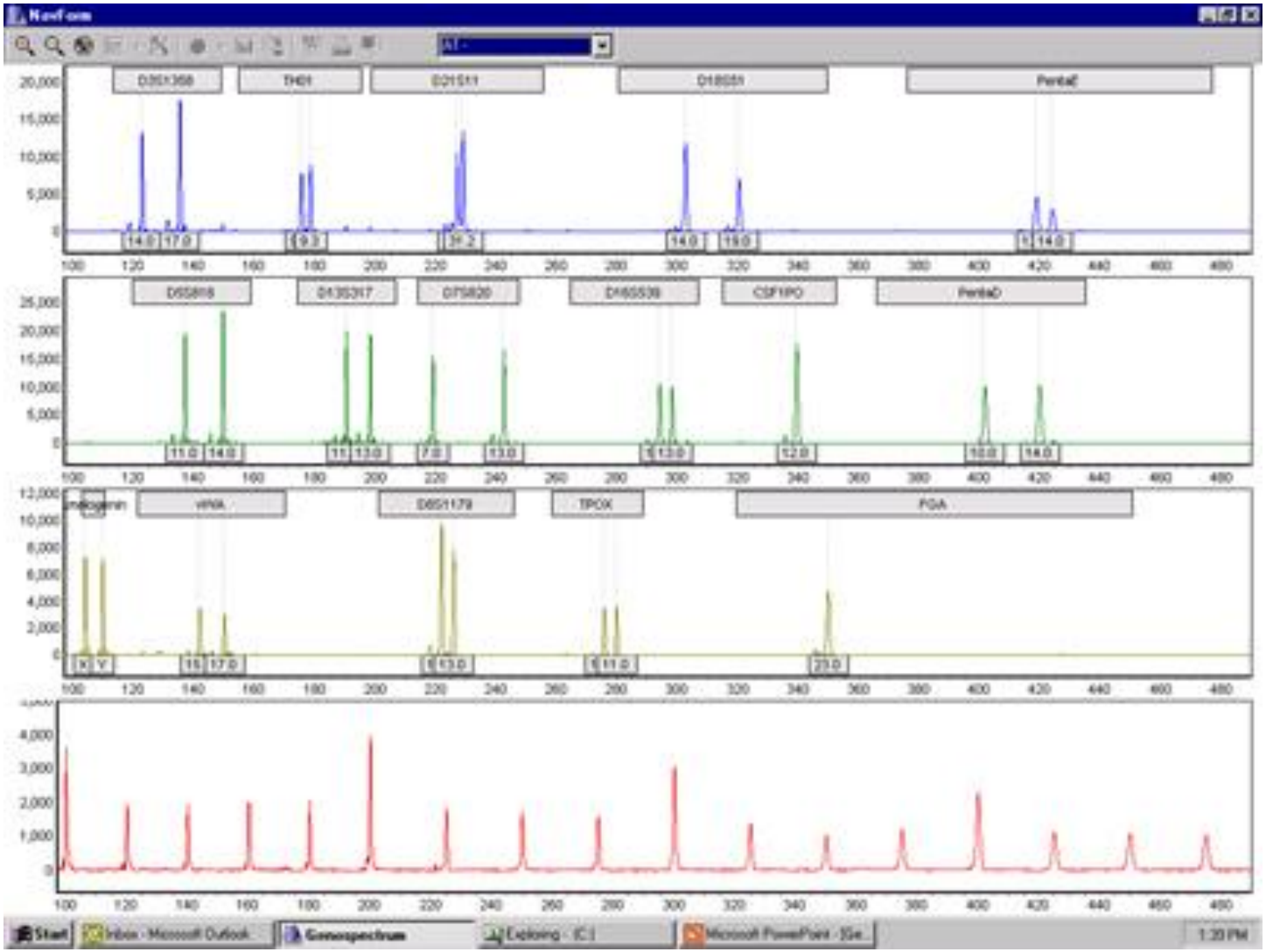
STR ANALYSIS

- 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



GENETICALLY MODIFIED (GM) ORGANISMS

- Organisms altered through recombinant DNA technology
- Insert foreign DNA into genome or combine DNA from different genomes

Splicing Genes Together

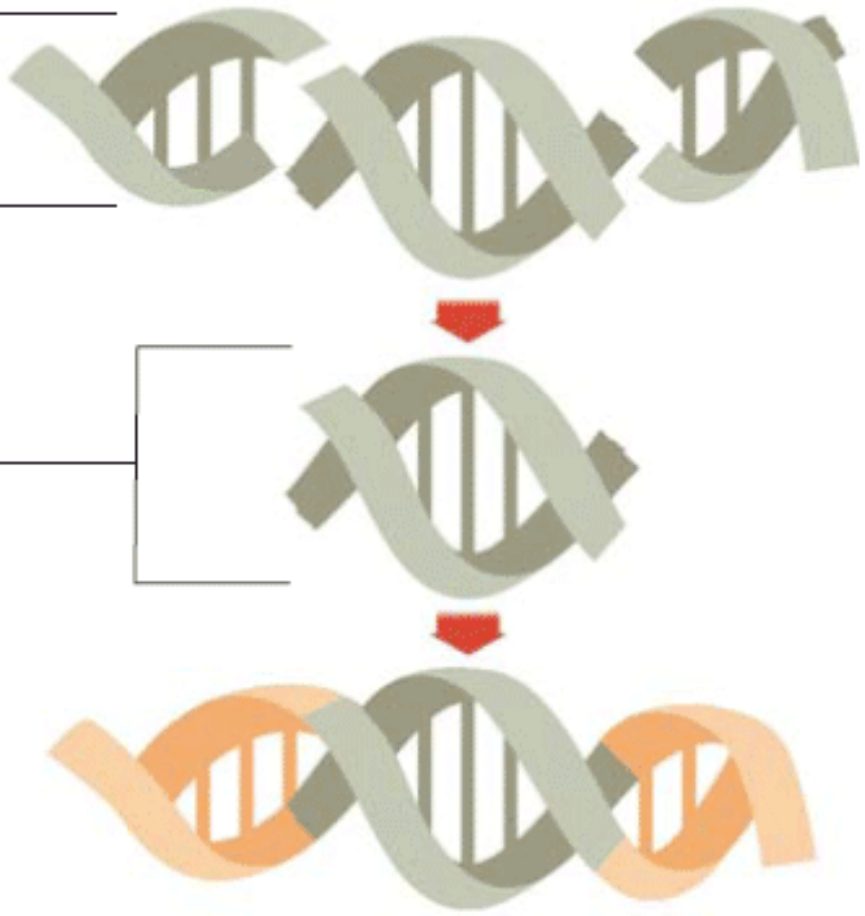
Employing genetic engineering, researchers can take certain genes from a source organism and put them into another plant or animal.

An Example of Genetic Engineering:

1 Scientists take *Bacillus thuringiensis*, a commonly occurring soil bacteria…

2 …and use enzymes to remove from it the Bt gene, which produces a protein that turns toxic in the digestive tract of caterpillars.

3 The Bt gene is then incorporated into the chromosomes of cotton and corn, killing caterpillars that feed upon these plants.



SOURCE: North Carolina State University, College of Agriculture and Life Sciences

Top 10 Genetically Modified Foods

Corn

Soy

Cotton

Papaya

Rice

Rapeseed (Canola)

Potatoes

Tomatoes

Dairy products

Peas