Chapter 10

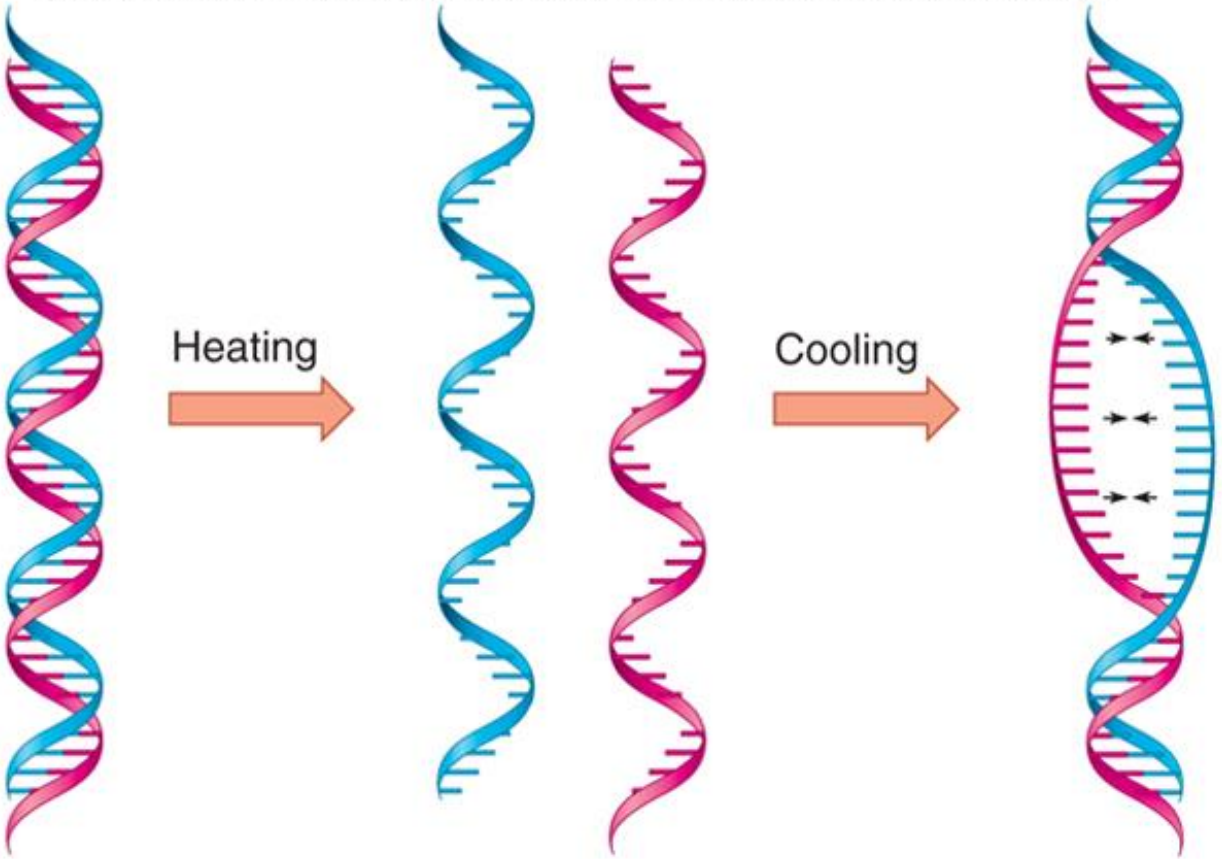
Genetic Engineering: *A Revolution in Molecular Biology*

Genetic Engineering

- Direct, deliberate modification of an organism's genome
  - bioengineering
- **Biotechnology** – use of an organism's biochemical and metabolic pathways for industrial production

Practical Properties of DNA

- Intrinsic properties of DNA hold true even in a test tube.
- DNA heated from 90°C to 95°C; the two strands separate. The nucleotides can be identified, replicated, or transcribed.
- Slowly cooling the DNA allows complementary nucleotides to hydrogen bond and the DNA will regain double-stranded form.



(a) DNA heating and cooling. When heated, the two strands of DNA separate as the hydrogen bonds between paired nucleotides are broken. When cooled, single strands of DNA will rejoin as complementary nucleotides once again bind to one another. The new annealed strands of DNA need not be from the same organism as long as they have complementary DNA sequences.

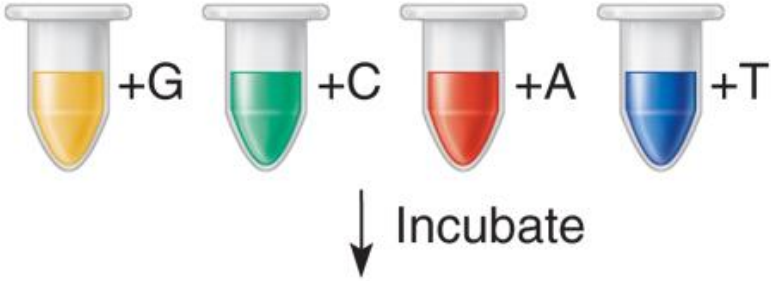
Enzymes for Dicing, Splicing, and Reversing Nucleic Acids

restriction endonucleases – recognize specific sequences of DNA and break phosphodiester bonds between adjacent nucleotides

- The enzymes can be used to cleave DNA at desired sites.
- Recognize and clip the DNA at **palindrome** base sequences.
- Used in the lab to cut DNA into smaller pieces – **restriction fragments**
- Restriction fragments of varying lengths are called restriction fragment length polymorphisms (RFLPs).

(2) DNA is denatured to produce single template strand.

(4) DNA polymerase and all four nucleotides (ATP, CTP, GTP, TTP) are added to each tube. Each tube also contains a small amount of a single type (A or C or T or G) of dideoxy nucleotide which will stop the chain lengthening reaction when it is incorporated into the growing DNA strand. The dideoxy nucleotides each have a unique fluorescent color which allows them to be identified.



Enzymes for Dicing, Splicing, and Reversing Nucleic Acids

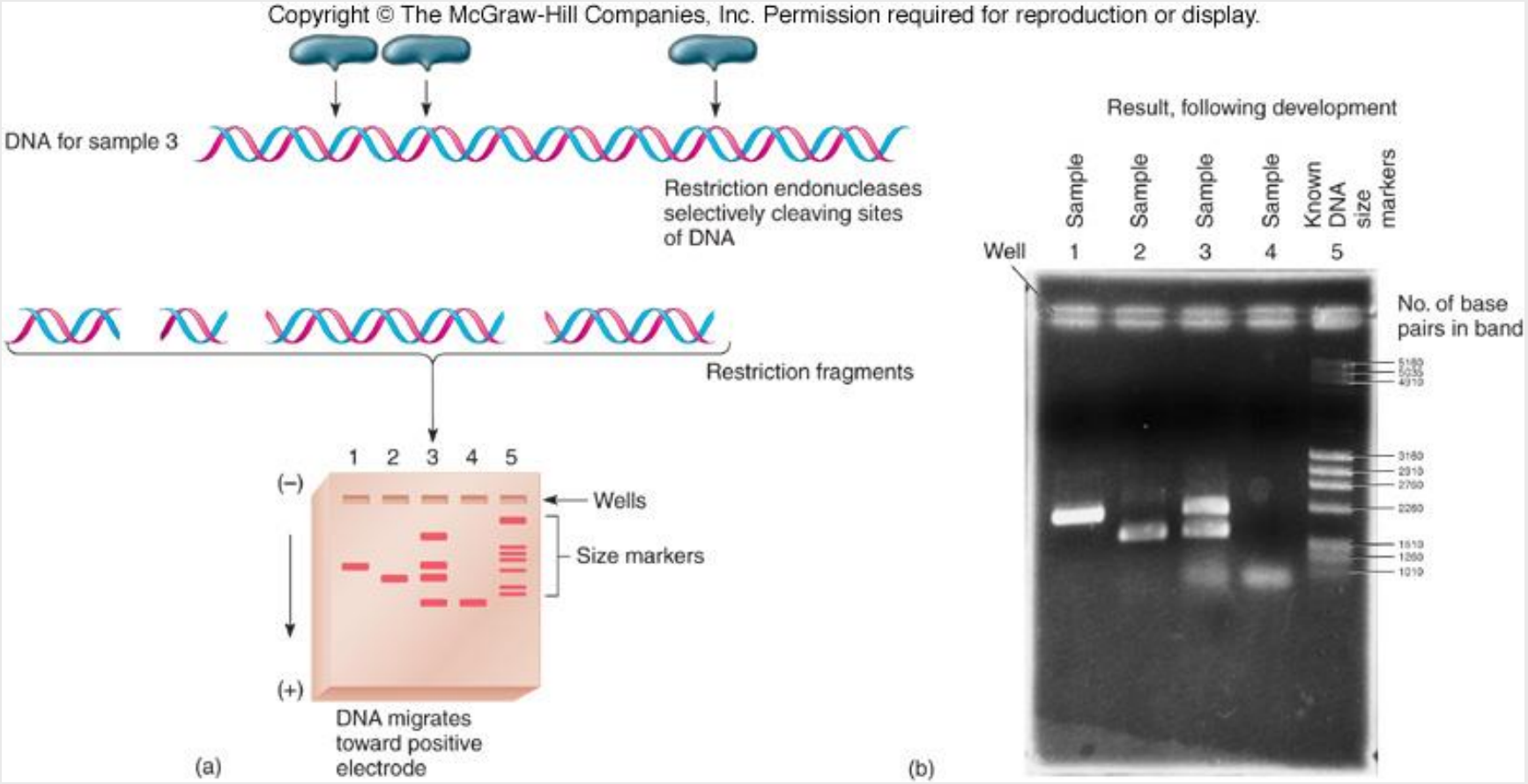
- **Ligase** – rejoins phosphate-sugar bonds (sticky ends) cut by endonucleases
- Used for final splicing of genes into plasmids and chromosomes

Enzymes for Dicing, Splicing, and Reversing Nucleic Acids

- **Reverse transcriptase** – makes a DNA copy of RNA – cDNA
- cDNA can be made from mRNA, tRNA, or rRNA
- Provides a means of synthesizing eucaryotic genes from mRNA transcripts – synthesized gene is free of introns

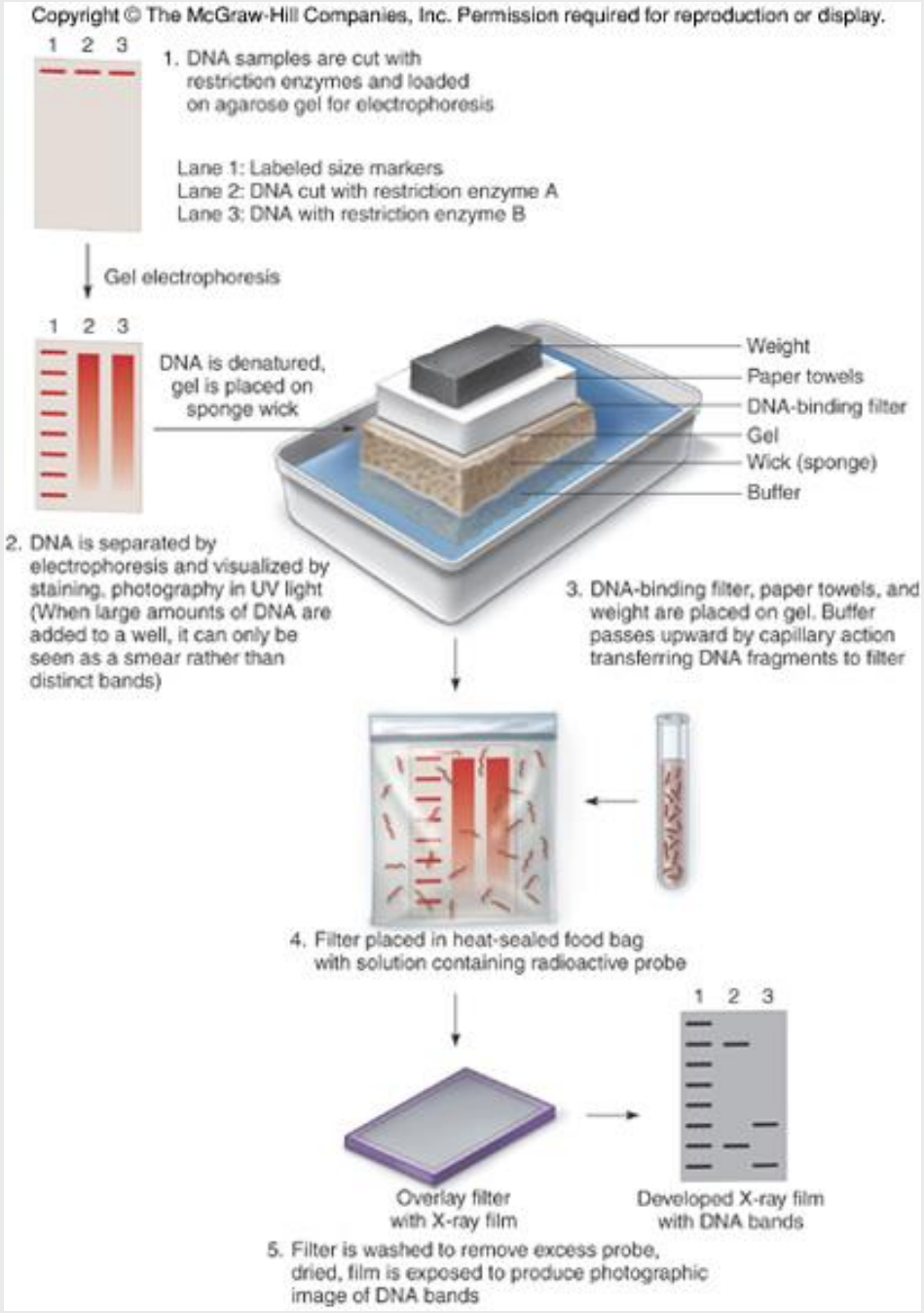
Methods for Analysis of DNA

- **Gel electrophoresis -** separates DNA fragments based on size
  - DNA samples are placed on soft agar gel and subjected to an electric current.
  - Negative charge of molecule causes DNA to move toward positive pole.
  - Rate of movement is dependent on size of fragment – larger fragments move more slowly.
  - Fragments are stained for observation.
  - Useful in characterizing DNA fragments and comparing for genetic similarities

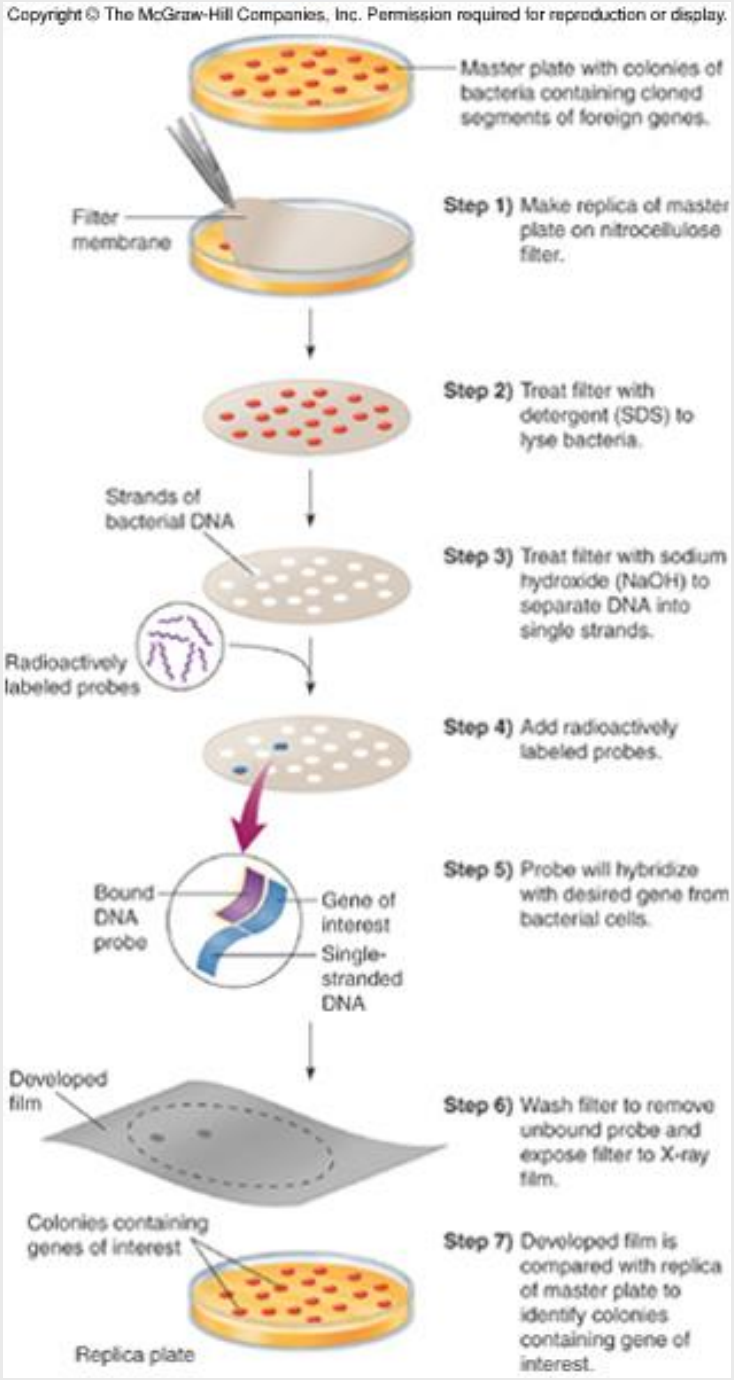


Methods for Analysis of DNA

- **Nucleic acid hybridization and probes** –
- Single-stranded DNA can unite with other single-stranded DNA, or RNA can unite with other RNA – **hybridization**
- Foundation for **gene probes** – short fragments of DNA of a known sequence that will base-pair with a stretch of DNA with a complementary sequence, if one exists in the sample
- Useful in detecting specific nucleotide sequences in unknown samples
  - **Southern blot** method – DNA fragments are separated by electrophoresis, denatured and then incubated with DNA probes. Probes will attach to a complementary segment if present.
  - isolate fragments from a mix of fragments and find specific gene sequences



- Hybridization test – used for diagnosing cause of infection and identifying unknown bacterium or virus
  - DNA from test sample is isolated, denatured, placed on filter, and combined with microbe-specific probe
  - commercially available diagnostic kits



Methods Used to Size, Synthesize, and Sequence DNA

- **DNA sequencing** – determining the actual order and type of bases for all types of DNA
- Most common sequencing technique is Sanger technique
  - Test strands are denatured to serve as a template to synthesize complementary strands.
  - Fragments are divided into tubes that contain primers, DNA polymerase, all 4 nucleotides, and fluorescent labeled dideoxynucleotide.

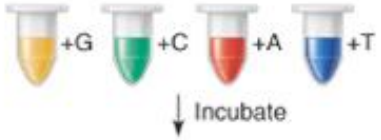
(1) Isolated unknown DNA fragment.

Original DNA to be sequenced

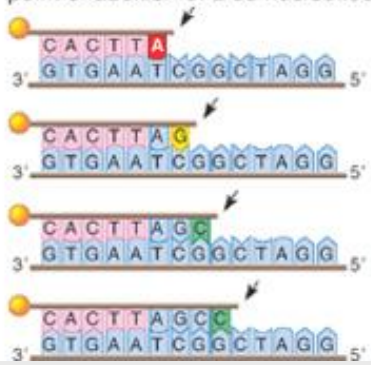
(2) DNA is denatured to produce single template strand.

(3) Strand is labeled with specific primer molecule.

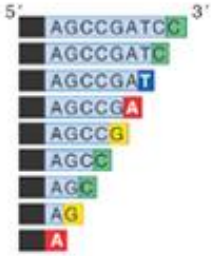
(4) DNA polymerase and all four nucleotides (ATP, CTP, GTP, TTP) are added to each tube. Each tube also contains a small amount of a single type (A or C or T or G) of dideoxy nucleotide which will stop the chain lengthening reaction when it is incorporated into the growing DNA strand. The dideoxy nucleotides each have a unique fluorescent color which allows them to be identified.



(5) Newly replicated strands are terminated at the point of addition of a dd nucleotide.



(6) A schematic view illustrating how each fragment will end with a labeled dideoxy nucleotide, after all four tubes are mixed.



(7) A gel showing the results of a sequencing run for six different strands of DNA. The location and color of the band provide the correct identity and order of the bases.

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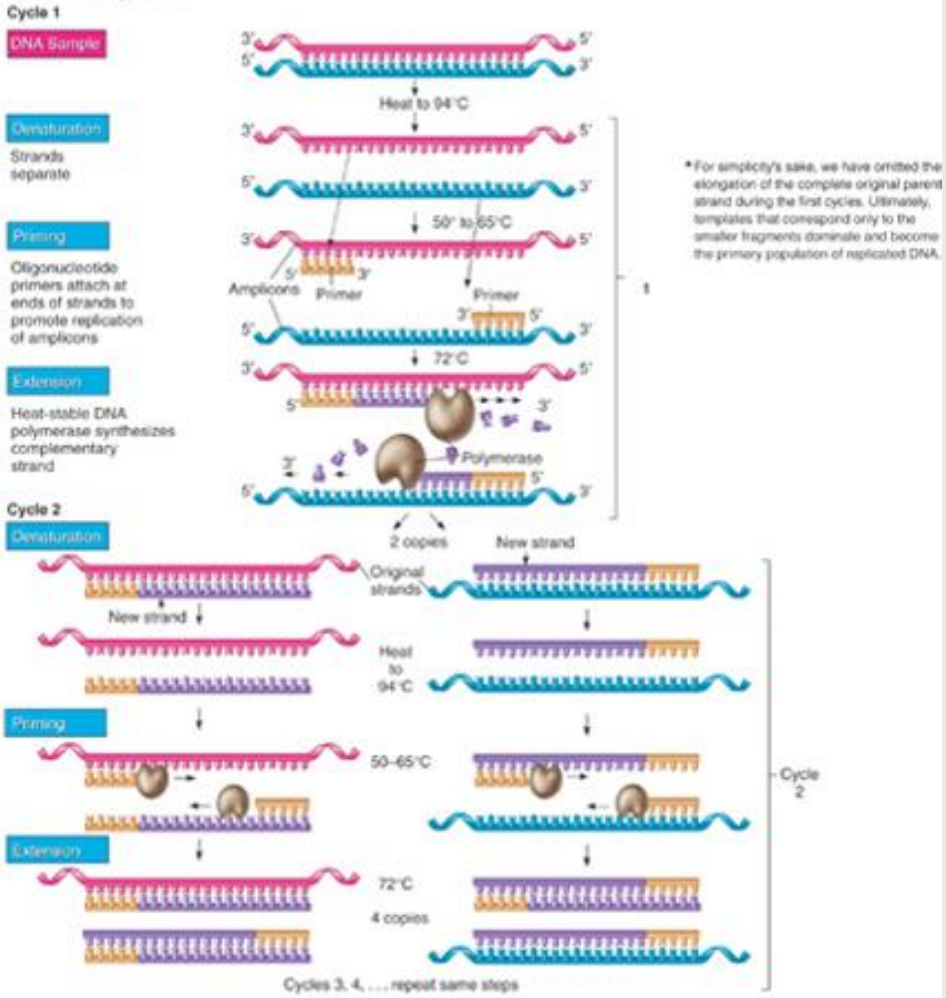
Methods Used to Size, Synthesize, and Sequence DNA

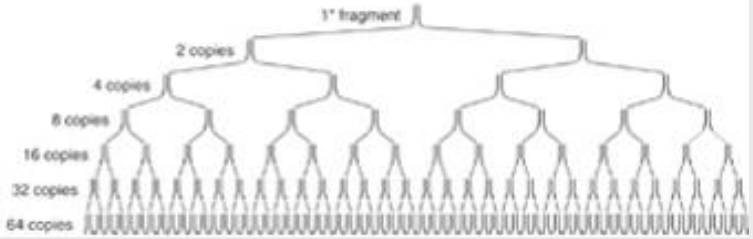
- **Polymerase Chain Reaction** (PCR)– method to amplify DNA; rapidly increases the amount of DNA in a sample
  - Primers of known sequence are added, to indicate where amplification will begin, along with special heat tolerant DNA polymerase and nucleotides.
  - repetitively cycled through denaturation, priming, and extension
  - Each subsequent cycle doubles the number of copies for analysis.
  - essentially important in gene mapping, the study of genetic defects and cancer, forensics, taxonomy, and evolutionary studies

(a) In cycle 1, the DNA to be amplified is denatured, primed, and replicated by a polymerase that can function at high temperature. The two resulting strands then serve as templates for a second cycle of denaturation, priming, and synthesis.*



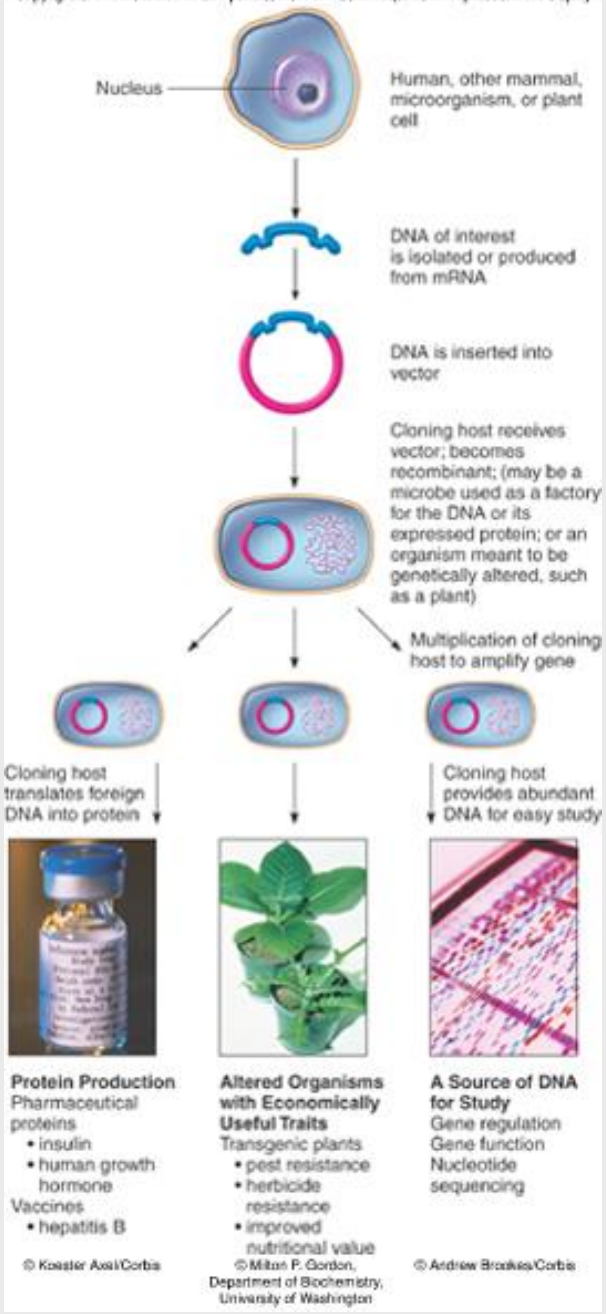
*For simplicity's sake, we have omitted the elongation of the complete original parent strand during the first cycles. Ultimately, templates that correspond only to the smaller fragments dominate and become the primary population of replicated DNA.

(b) A view of the process after 6 cycles, with 64 copies of amplified DNA. Continuing this process for 20 to 40 cycles can produce millions of identical DNA molecules.



Methods in Recombinant DNA Technology

- **Recombinant DNA technology** – the intentional removal of genetic material from one organism and combining it with that of a different organism
  - Objective of recombinant technology is **cloning** which requires that the desired donor gene be selected, excised by restriction endonucleases, and isolated.
  - The gene is inserted into a **vector** (plasmid, virus, cosmids) that will insert the DNA into a **cloning host**.
  - Cloning host is usually bacterium or yeast that can replicate the gene and translate it into a protein product.



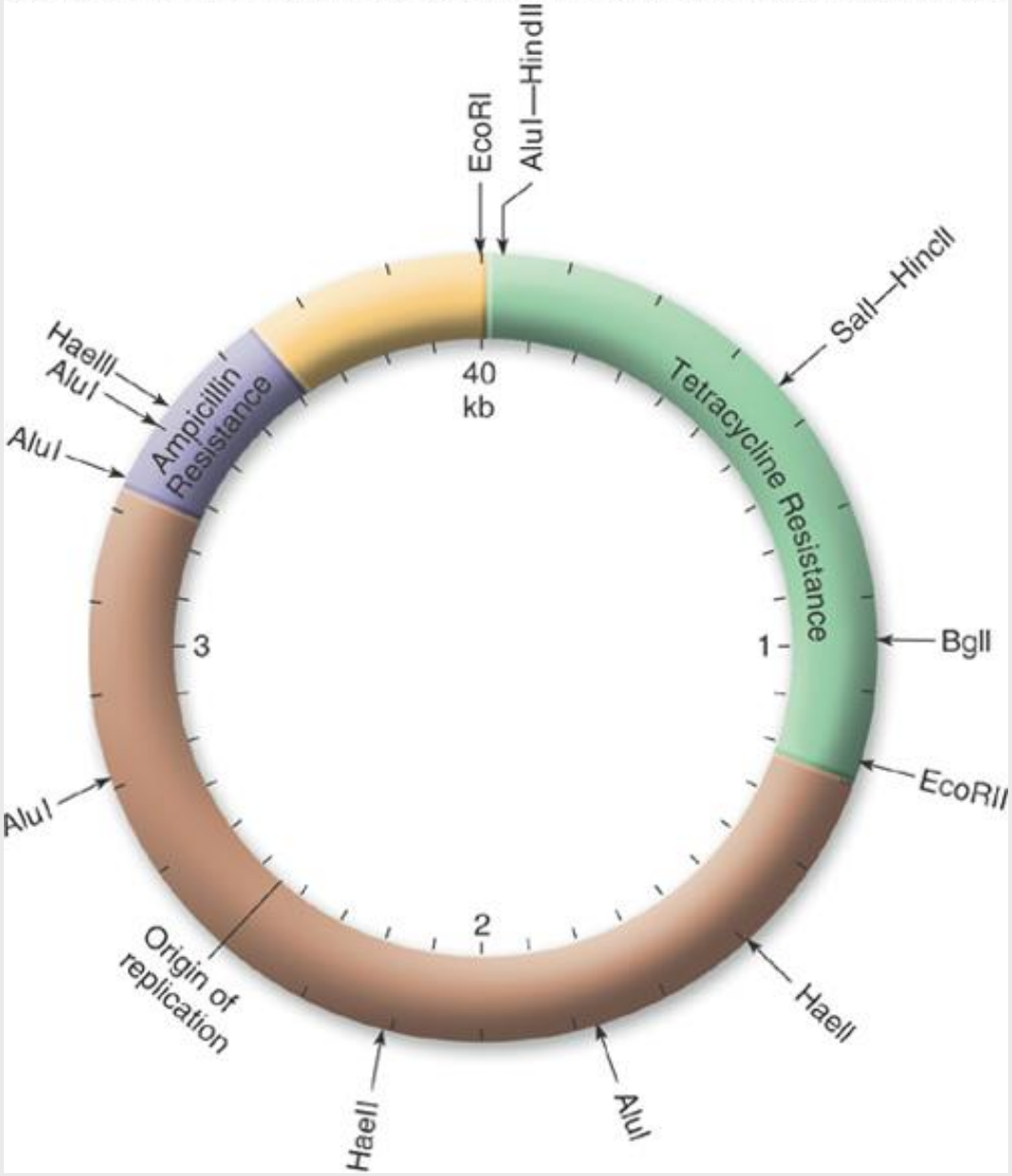
Characteristics of Cloning Vectors

- Must be capable of carrying a significant piece of donor DNA
- Must be readily accepted by the cloning host

- **Plasmids** – small, well characterized, easy to manipulate and can be transferred into appropriate host cells through transformation
- **Bacteriophages** – have the natural ability to inject their DNA into bacterial hosts through transduction

Vector Considerations

- Origin of replication is needed so it will be replicated.
- Vector must accept DNA of the desired size.
- Gene which confers drug resistance to their cloning host

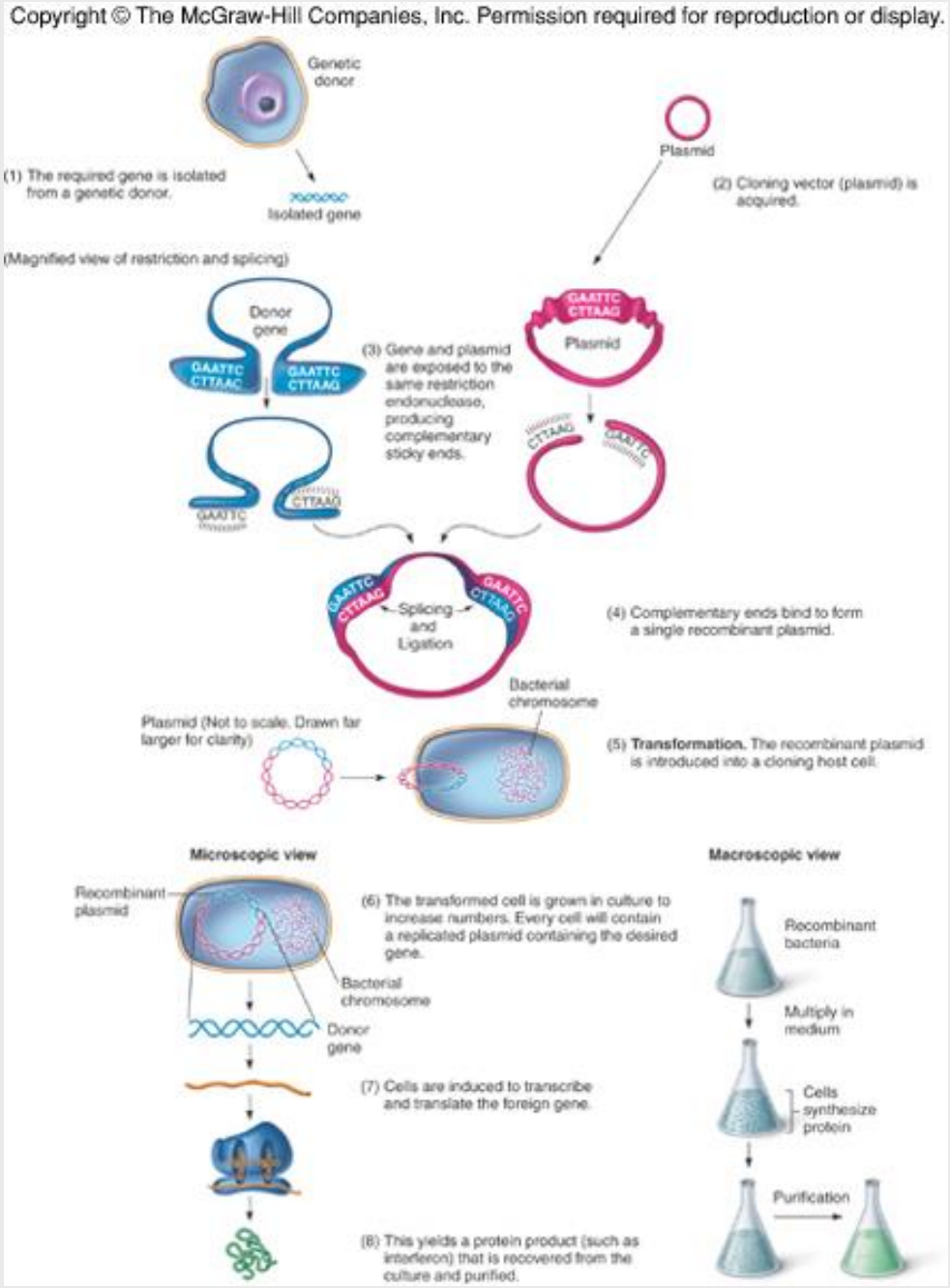


Characteristics of Cloning Hosts

1. Rapid overturn, fast growth rate
2. Can be grown in large quantities using ordinary culture methods
3. Nonpathogenic
4. Genome that is well delineated
5. Capable of accepting plasmid or bacteriophage vectors
6. Maintains foreign genes through multiple generations
7. Will secrete a high yield of proteins from expressed foreign genes

Construction of a Recombinant, Insertion, and Genetic Expression

- Prepare the isolated genes for splicing into a vector by digesting the gene and the plasmid with the same restriction endonuclease enzymes creating complementary sticky ends on both the vector and insert DNA.
- The gene and plasmid are placed together, their free ends base-pair, and ligase joins them.
- The gene and plasmid combination is a **recombination**.
- The recombinant is introduced into a cloning host.

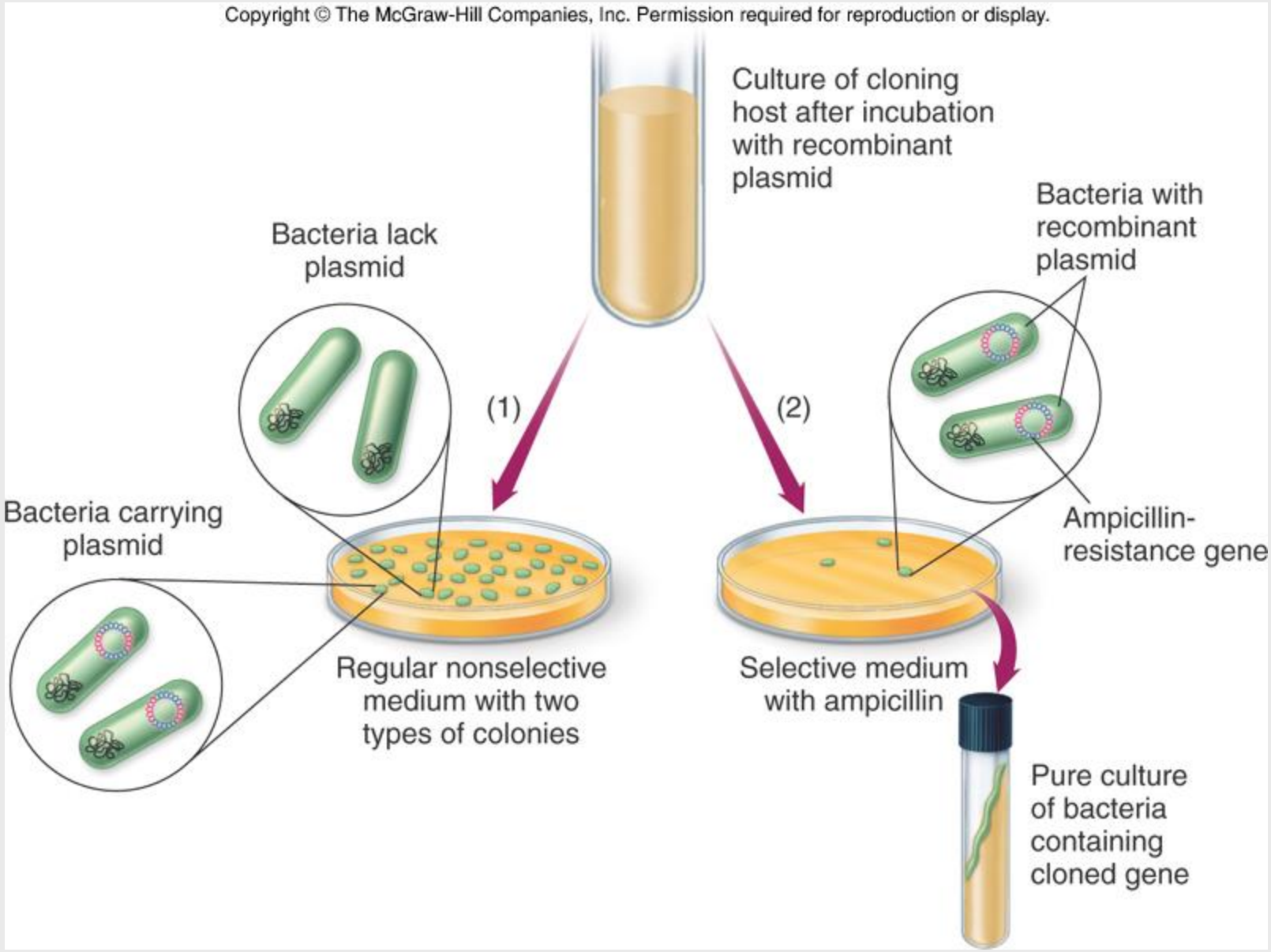


Biochemical Products of Recombinant DNA Technology

- Enables large scale manufacturing of life-saving hormones, enzymes, vaccines
  - insulin for diabetes
  - human growth hormone for dwarfism
  - erythropoietin for anemia
  - Factor VIII for hemophilia
  - HBV vaccine

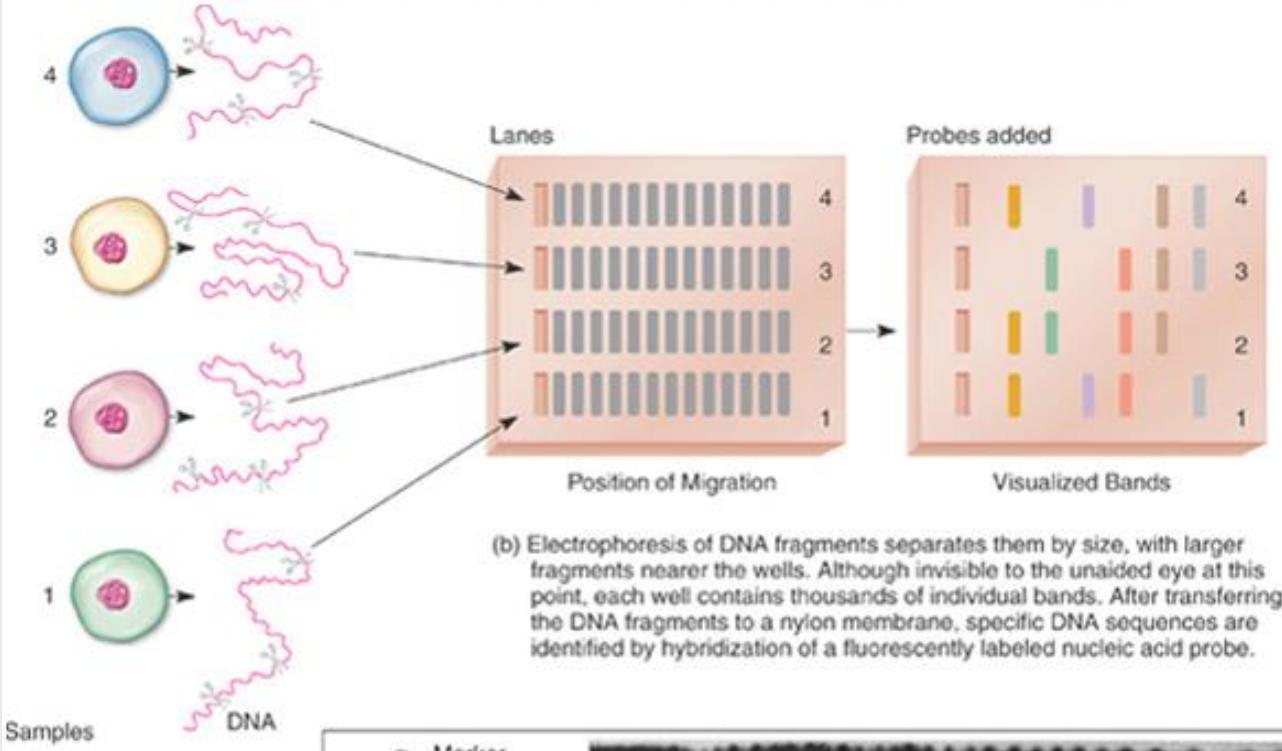
Genetically Modified Organisms (GMO)

- Recombinant microbes
  - *Pseudomonas syringae* – prevents ice crystals
  - *Bacillus thuringienisis* –encodes an insecticide
- Transgenic plants
  - rice that makes beta-carotene
  - tobacco resistant to herbicides
  - peas resistant to weevils
- Transgenic animals
  - mouse models for CF, Alzheimer's, sickle cell anemia
  - sheep or goats that make medicine in their milk semen



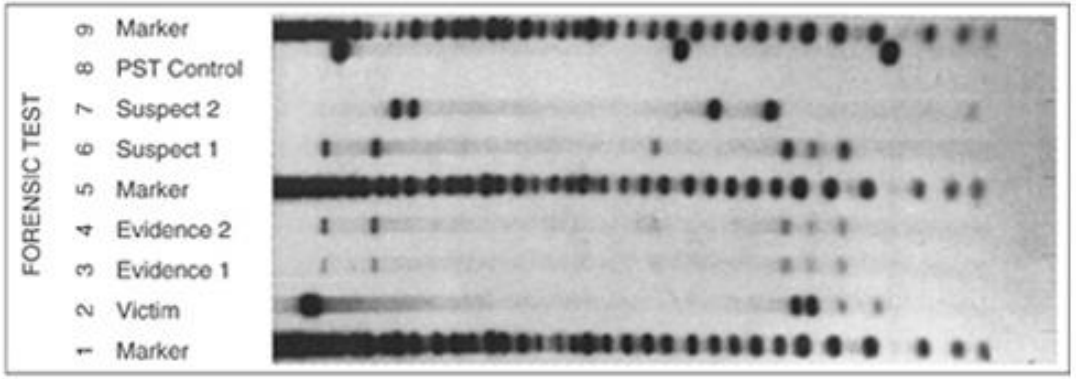
Genome Analysis

- **DNA Fingerprinting –**
  - Every individual has a unique sequence of DNA.
  - used to:
    - identify hereditary relationships
    - study inheritance of patterns of diseases
    - study human evolution
    - identify criminals or victims of disaster
- Analysis of mitochondrial DNA is used to trace evolutionary origins.
- microarray analysis – track the expression of genes; used to identify and devise treatments for diseases based on the genetic profile of the disease



(a) Cells from different samples are processed to isolate their DNA. The DNA samples are exposed to endonucleases which snip them at specific sites into a series of different fragments.

(b) Electrophoresis of DNA fragments separates them by size, with larger fragments nearer the wells. Although invisible to the unaided eye at this point, each well contains thousands of individual bands. After transferring the DNA fragments to a nylon membrane, specific DNA sequences are identified by hybridization of a fluorescently labeled nucleic acid probe.



(c) An actual DNA fingerprint used in a rape trial. Control lanes with known markers are in lanes 1, 5, 8, and 9. The second lane contains a sample of DNA from the victim's blood. Evidence samples 1 and 2 (lanes 3 and 4) contain semen samples taken from the victim. Suspects 1 and 2 (lanes 6 and 7) were tested. Can you tell by comparing evidence and suspect lanes which individual committed the rape?

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