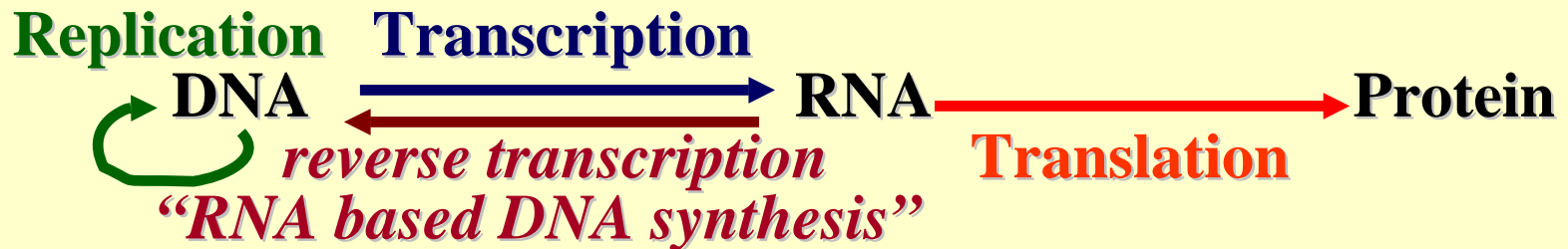


3. Basics of Biomolecules & Genetics

a. The central dogma – universality



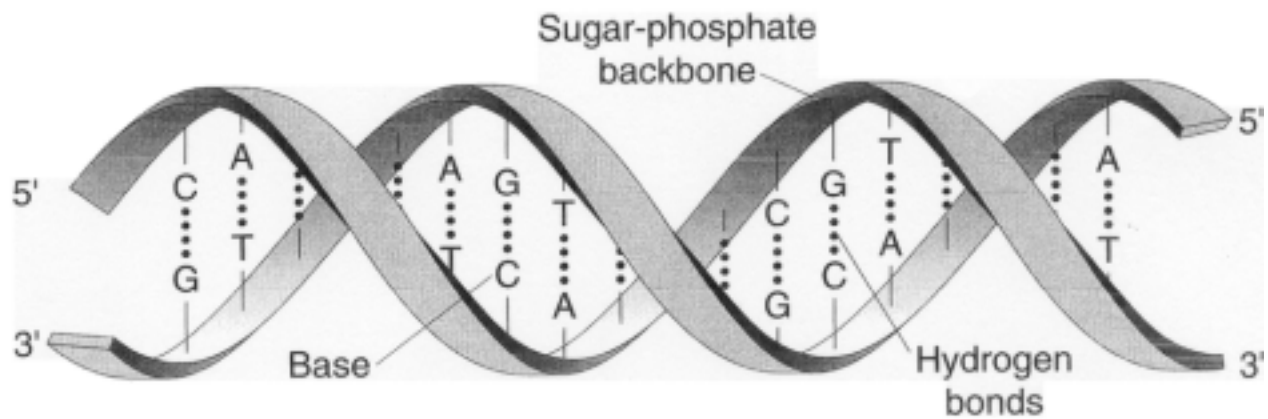
- Language for information storage and exchange
 - letters: a four-letter alphabet (made-up of the nucleotides), A, T, C, G.
 - words: only three letters long (*codons*), represent a particular amino acids or “stop” symbol of protein synthesis.
 - Universality: bacterial cells to Homo sapiens.

1. Structure of Nucleic Acids

Genetic Materials:

- 1) A specific form that can be copied extremely accurately – information can be transmitted from cell to cell/ generation to generation.**
 - 2) It can be translated.**
- a. Structure of DNA: made of only three components*
- i) Sugar molecule (deoxyribose or Ribose)**
 - ii) A phosphate group**
 - iii) four different nitrogen-containing bases: Adenine, guanine, cytosine, thymine**

Ribbon model of DNA



b. DNA Replication

- **enzymes: DNA polymerases for covalently linking the monomers.**
- **activated monomers: nucleoside triphosphates.**
- **Template.**

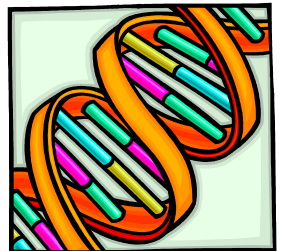
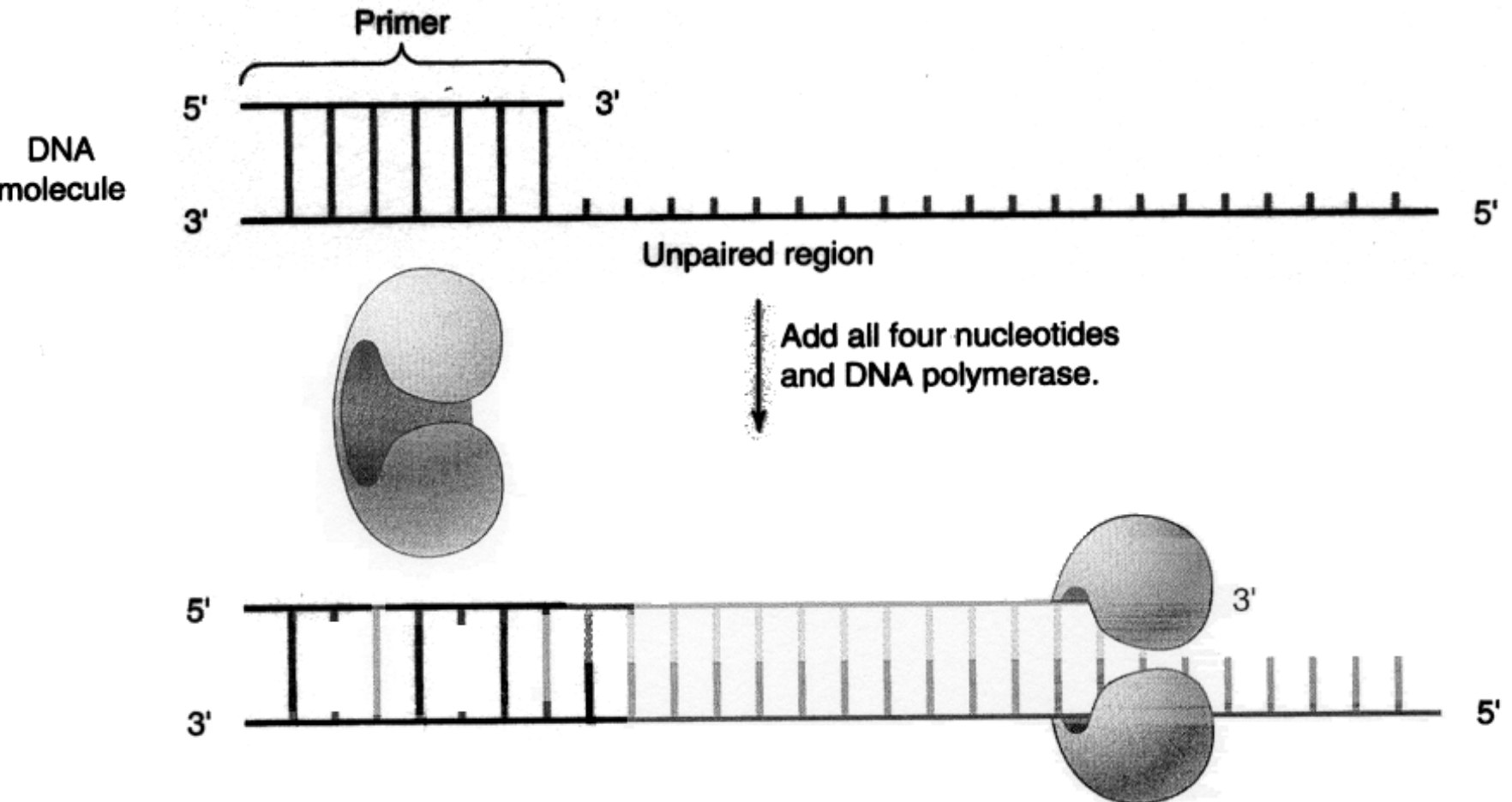


Figure 5.2 Activity of DNA polymerase.



DNA polymerase synthesizes a new complementary strand (in yellow) by adding nucleotides to the 3' end of the primer.

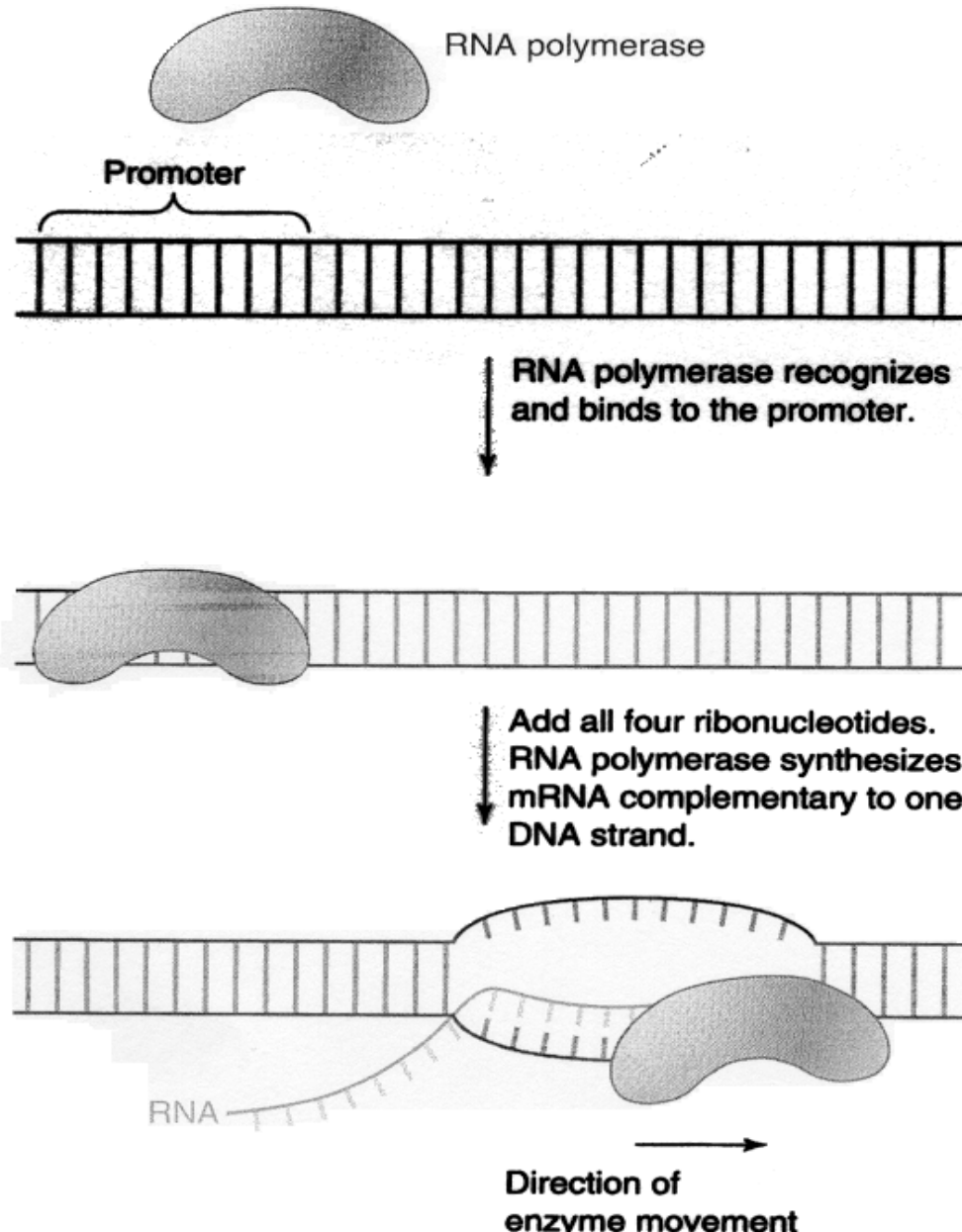
c. Transcription: message transfer (DNA→RNA)

Products: m-RNA, t-RNA, r-RNA

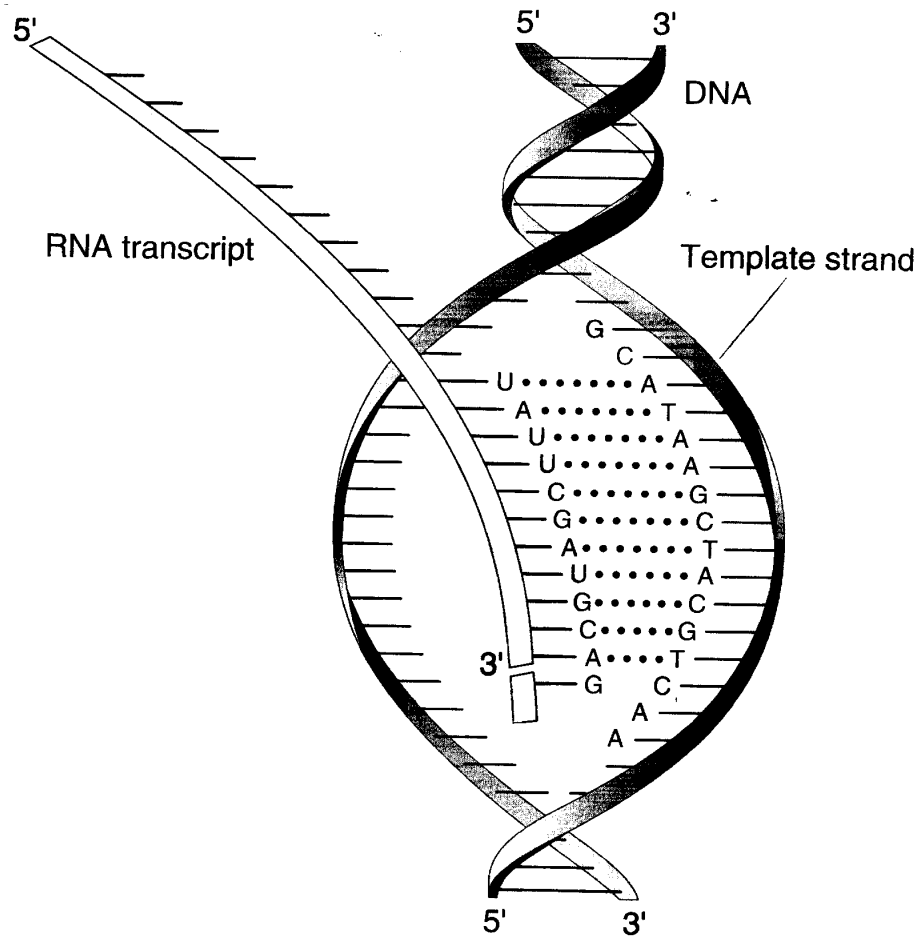
- rate of synthesis of RNAs: critical capacity to make proteins.
- Enzymes: RNA polymerase (holoenzyme)



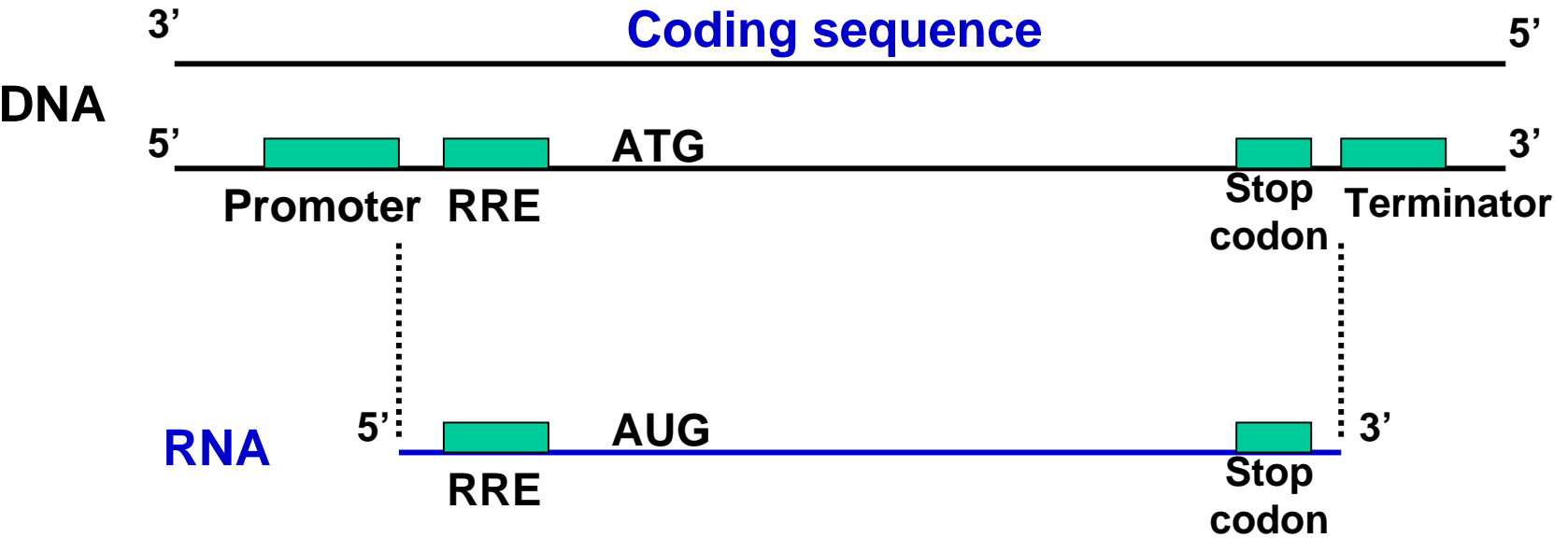
Figure 5.3 Activity of RNA polymerase.



Transcription



Transcription Products

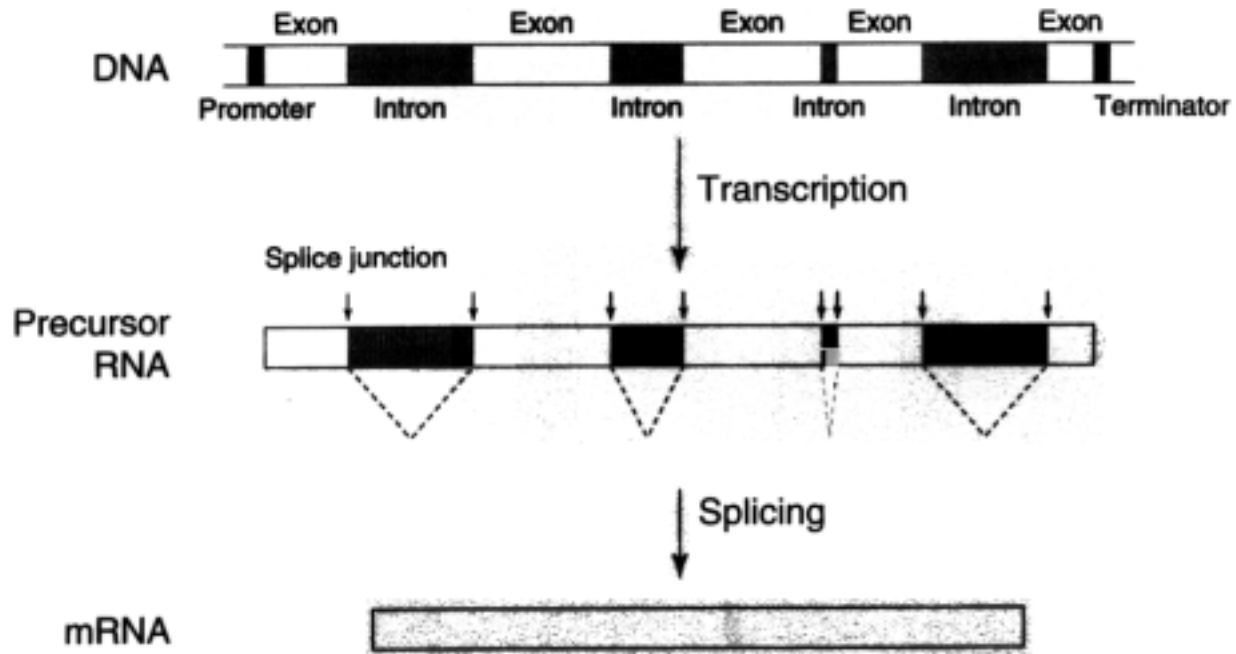


In Bacteria

Splicing of precursor

In Eukaryotic Cells

Transcription of Eukaryotic Genes – no operons, Exon and Introns



D. Transcription

- i) **Initiation:**
 - **Sigma factors:** recognize the vast majority of promoters, special sigma factors under unusual growth conditions/ stress.
- ii) **Elongation:** RNTPs required for energy supply
- iii) **Termination:** a stop signal or transcription terminator.
 - **RNA pol. Dissociates from the DNA templates**
 - **RNA transcript is released.**

iv) Transcripts

- **Stable: r-RNA, t-RNA**
- **Unstable: m-RNA (highly unstable) ~ a 1 min. half-life for a typical *E. coli***
- **Special Notes: in prokaryotic cells, polygenic**

- **Prokaryotic cells: no physical separation of the chromosome from the cytoplasm & ribosome; m-RNA bind and begin translation even while part of it is still being transcribed.**

- **Eukaryotic cells:**
 - + **separation of chromosome & ribosomes by the nuclear membrane.**
 - + **intron can be transcribed (nonsense DNA part – no specific sequence for specific amino acid: this fact make it difficult to express eukaryotic genes in *E. coli*).**
 - + **m-RNA splicing should occur.**



e. Translation

1) Genetic code: universal message (blueprint)

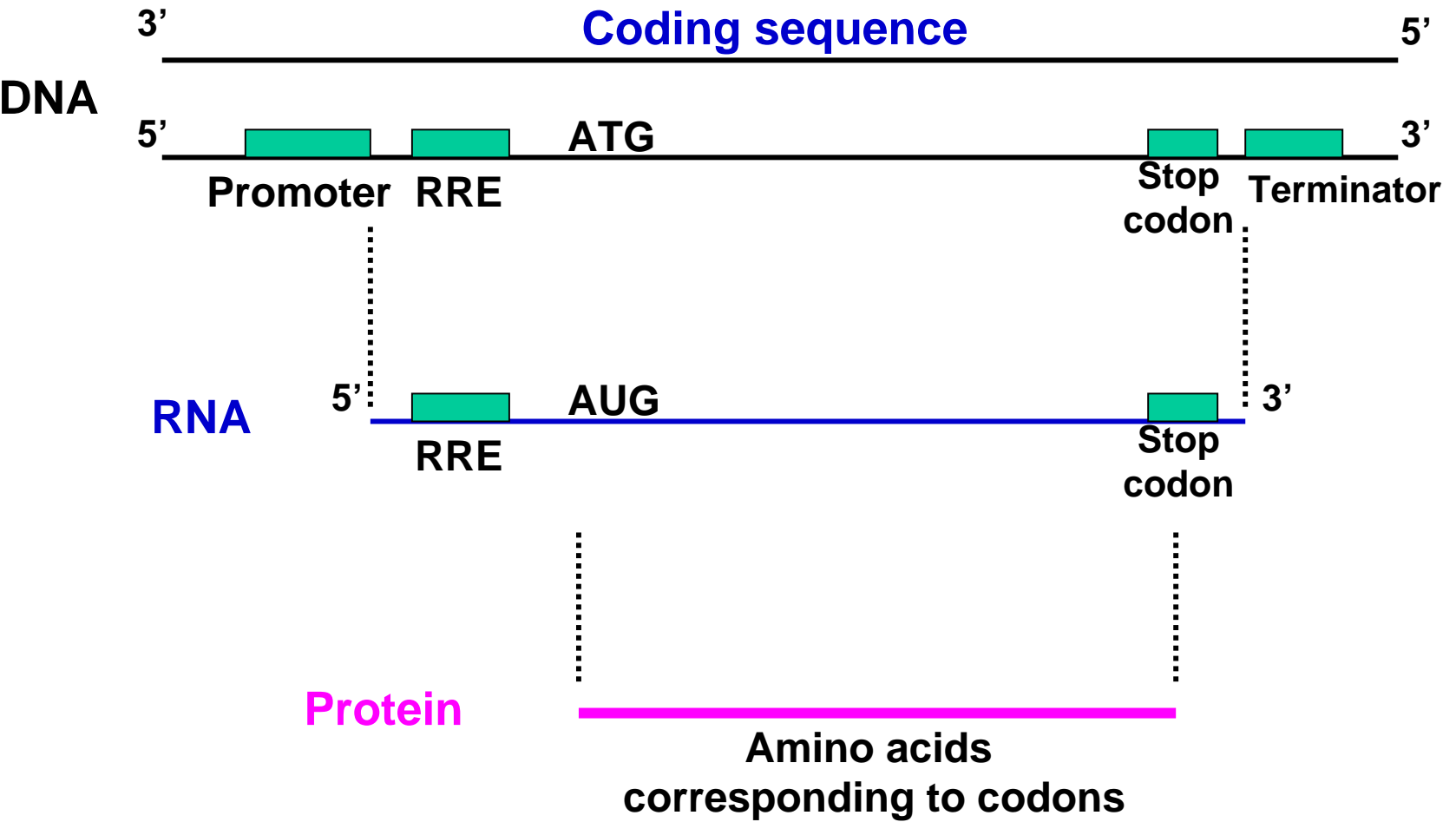
- **sixty-four words are possible (many redundant!):**
 $nPr = 4P3 = n^r = 4^3 = 64$
(permutation with repetition of objects)
- **UCU, UCC, UCA, & UCG – “serine”**
- **UAA, UAG, UGA – “nonsense codon” (not for amino acids), “stop point” in translation at the end of message.**

2) Translation – initiation, elongation, termination

i) Initiation:

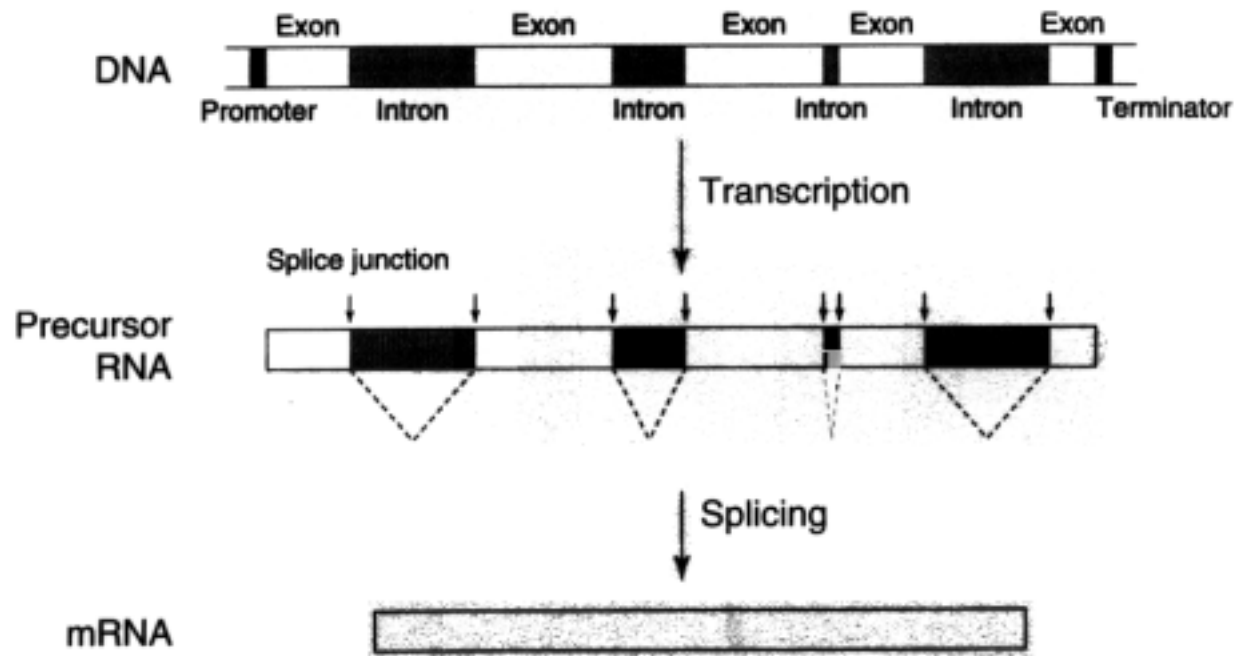
- **m-RNA binds to the ribosome at the ribosome binding site (Shine-Delgarno box).**
- **AUG or GUG (modified methionine; N-formylmethionine) codon at the early site of mRNA: starting codon for protein synthesis**

Major genetic traffic signals

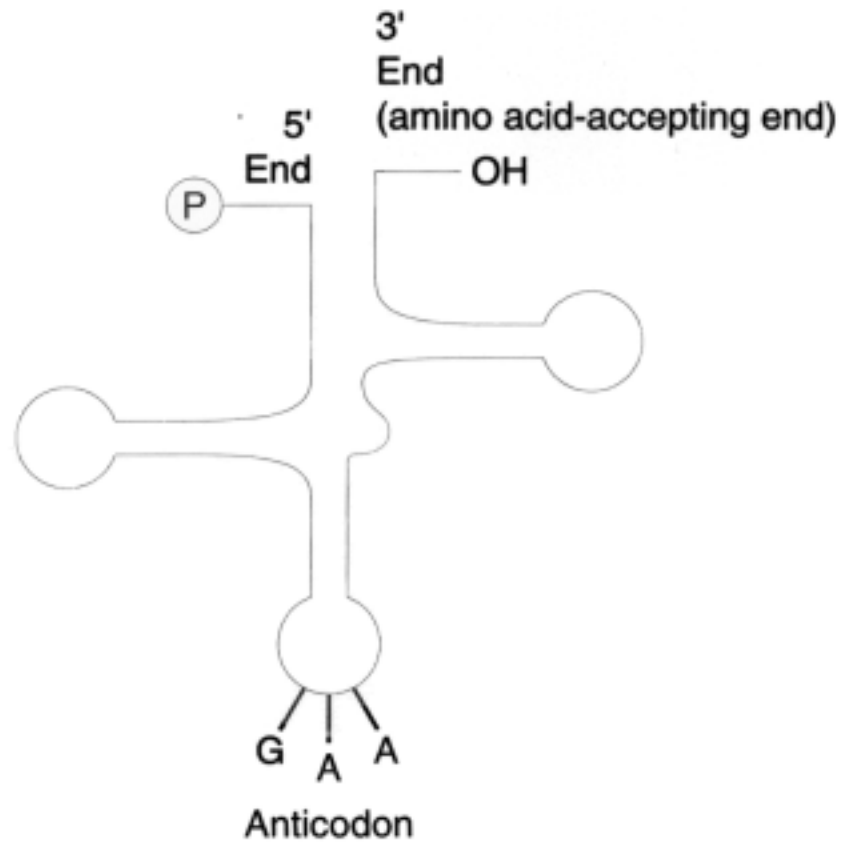


In Bacteria

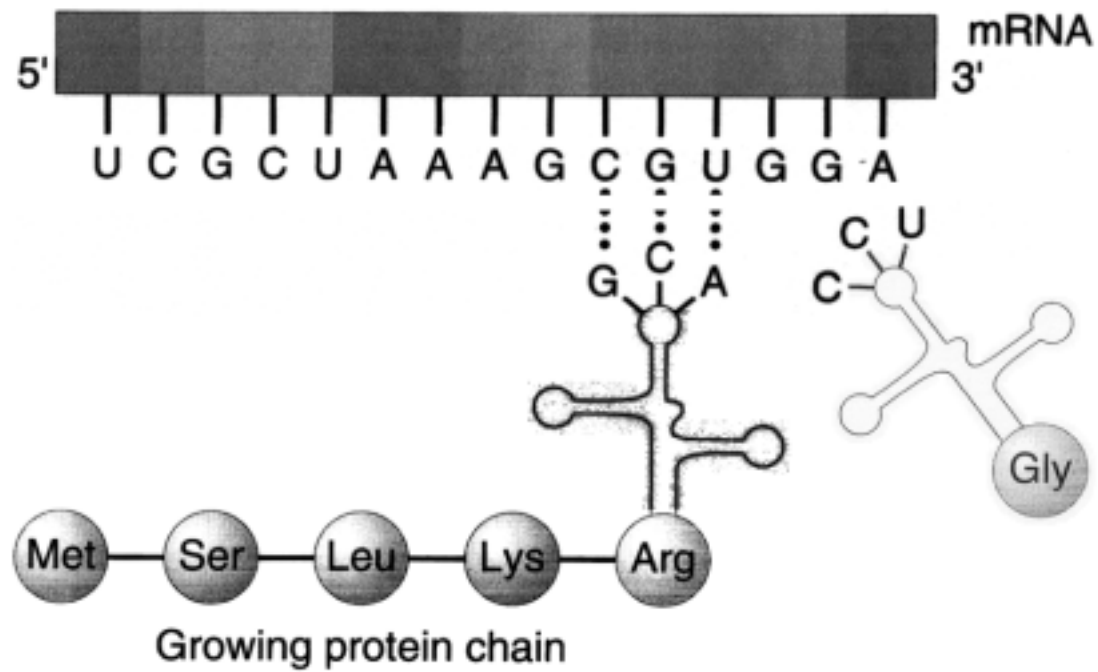
Splicing of precursor



tRNA molecule



Translation



- **AUG at the middle of m-RNA: codon for methionine.**
- **Initiation complex should be formed for initiation of polymerization.**
 - **30S ribosomal unit with N-formylmethionine bound**
 - **50S ribosomal unit**
 - **three proteins (initiation factors: IF1, IF2, IF3)**
 - **GTP**

ii) Elongation : t-RNA as decoders

- **anticodon: complementary to the codon on the m-RNA.**
- **Charged t-RNA: t-RNA carrying an amino acid.**
- **Energy obtained from phosphate bonds.**
- **Energy required to add one amino acid to a growing polypeptide: two for charging t-RNA, two for elongation process.**

iii) Termination

- **protein released from the ribosome with the aid of release factor (RF)**
- **Dissociation of 70S ribosome into 30S and 50s subunits.**
- **About 10-20 ribosomes can bind to the mRNA at once.**

Ways to manipulate DNA

Cellular Enzymes

- DNA polymerase:

Ex) bacteria that live at very high temp. (in hot springs or thermal vents in the ocean floor) – enzyme for PCR reaction.

- RNA polymerase: Promoter, no primer required.
- Reverse Trnascriptase: read an RNA sequence and synthesize a complementary DNA (cDNA) sequence. Made by RNA viruses.

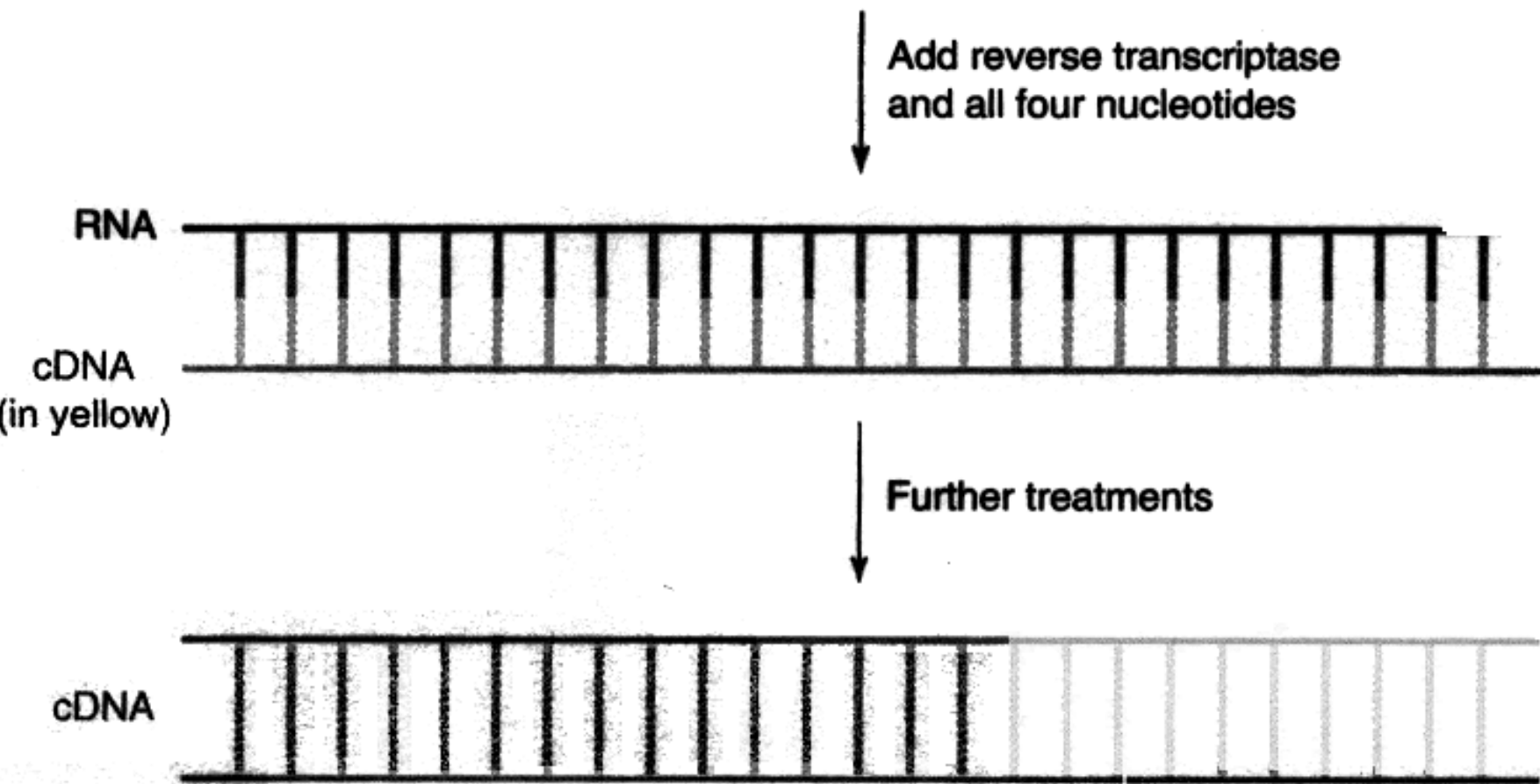


Figure 5.5 Activity of reverse transcriptase.

Ways to manipulate DNA

Restriction Enzymes

- **A nuclease: any enzyme that cuts the phosphodiester bonds of the DNA backbone**
- **Endonuclease: cuts somewhere within a DNA molecule.**
- **Exonuclease: cuts phosphodiester bonds by starting from a free end of the DNA and working inward.**

DNA Polymerase

- **DNA dependent- DNA synthetase**
- **5' to 3' direction**
- **substrates: dNTPs (A, C, G, T)**

Reverse Transcriptase

- **RNA dependent- DNA synthetase**
- **5' to 3' direction**
- **substrates: dNTPs (A, C, G, U)**

Terminal Trnasferase

- **No template required**
- **addition of nucleotides to 3' end of DNA fragments**
- **used for tagging fluorescence or radiation reporters.**

Nuclease

- **Restriction enzyme for digesting single strands of DNA or RNA**
- **used for S1 mapping in intron confirmation and digestion of hairpin loop strands.**

- DNA Restriction Enzymes..continued

Ex) 5'AGCT3'
3'TCGA5' --- DNA palindrome (*AluI*)

cut sites: 5'AG CT3'
3'TC GA5' (blunt end)

Ex) 5'CCCGGG3'
3'GGGCC5' --- DNA palindrome (*Sma I*)

cut sites: 5'CCC GGG3'
3'GGG CCC5' (blunt end)

Ex) 5'GCGC3'
3'CGCG5' --- DNA palindrome (*Hba I*)

cut sites: 5'GCG C3'
3'C GCG5' (sticky end)

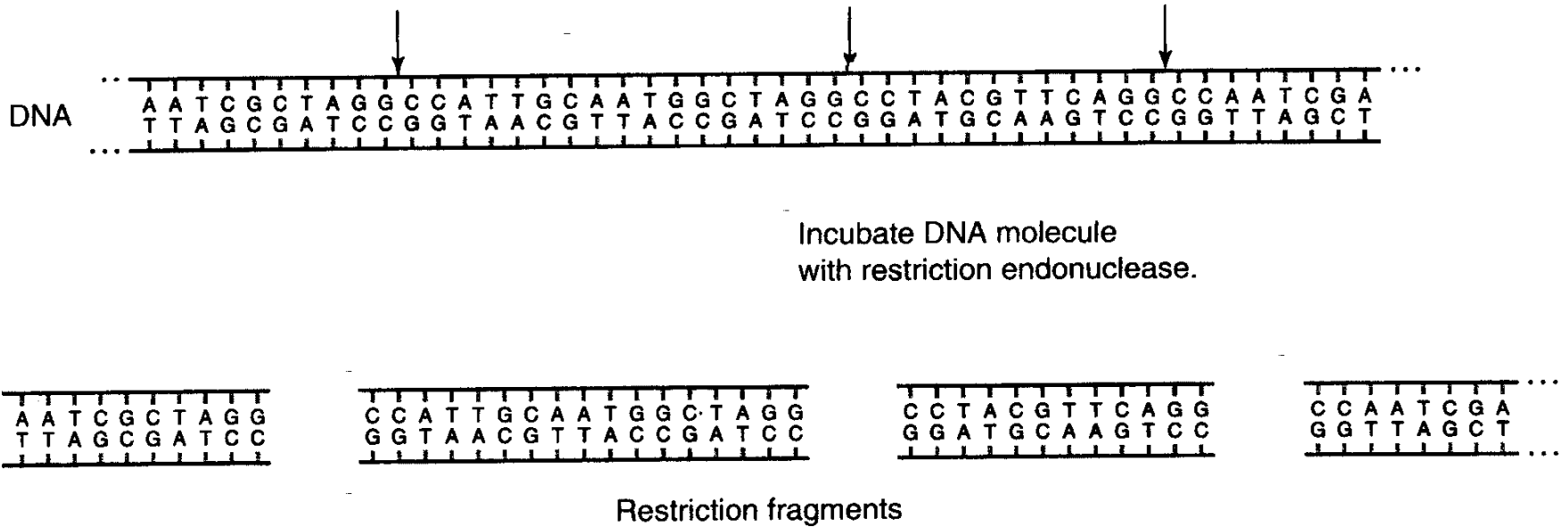


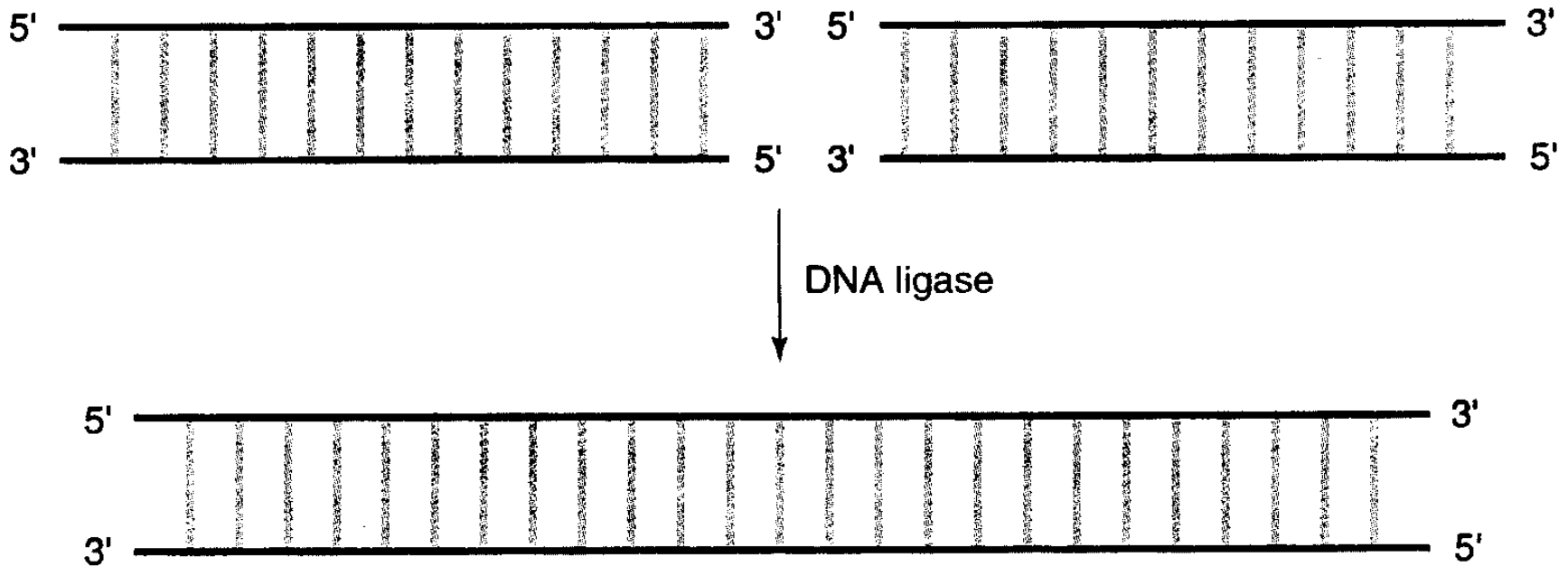
Figure 5.1 Restriction endonucleases recognize and cut specific sites in a DNA molecule. The arrows indicate the cleavage sites of one such endonuclease.

- Palindromic Tetra and Hexa-nucleotide Recognition**
- Palindromic Penta-Nucleotide Recognition**
- Specificities greater than 6 bases.**
- Multiple Recognition Sequences.**
- Nonpalindromic Sequences.**

DNA Ligases (Glues)

- Join piece of DNA (or RNA) together by forming new phosphodiester bonds between the pieces.**
- Sticky ends > blunt ends : easier to ligate. (hybridization between the single-stranded regions holds the fragments together in the proper position for ligation)**

Figure 5.4 Activity of DNA ligase.



Gel Electrophoresis – separation

- **Agarose:** a polysaccharide that dissolve in boiling water and then gels as it cools.
- **DNA is highly negatively charged in the normal circumstances (because of phosphate group):** it is attracted to positive electrode – thus, DNA migrate.
- **Smaller fragments faster (for linear molecules):**

The rate of migration of linear DNA fragment is proportional to $1/[\log (M.W.)]$.
- **The migration rate = f [the shape of the molecules, concentration of agarose, buffers, voltages (5 V/cm)]**
- **Buffers:** necessary for preventing anode to become alkaline and the cathode acidic during electrophoresis.

Electrophoresis (protocols)

- Agarose preparation
- DNA loading and electric voltage applied under buffered conditions
- DNA fragments are separated.
- Gel Staining (staining solutions: methylene blue, ethidium bromide, or fluorescence tags)
- Recording data under UV exposure. (Photography)

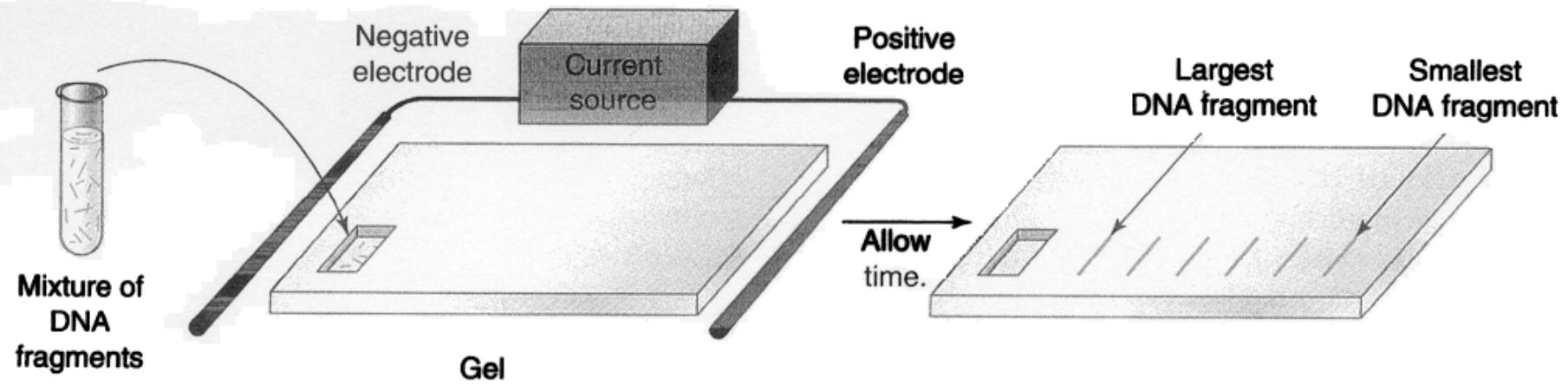


Figure 5.6 Gel electrophoresis of DNA fragments.

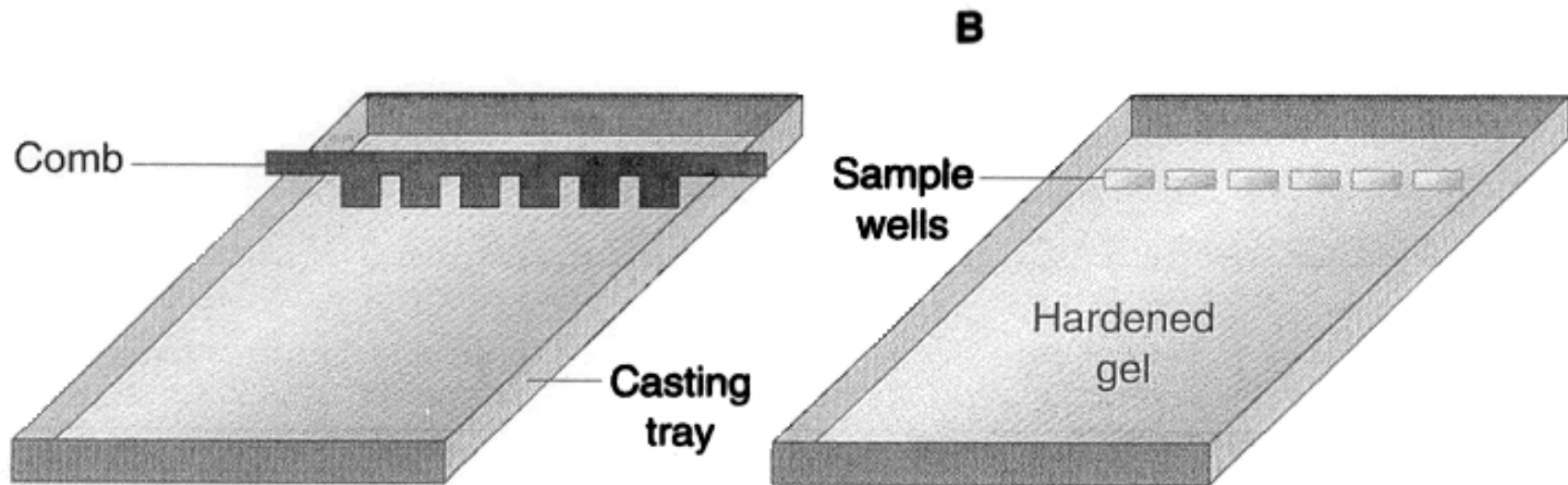


Figure 12.1 Casting an agarose gel. (A) To make a gel, hot liquid agarose solution is poured into a casting tray (any shallow container), and the comb is put in place. (B) After the agarose cools and hardens, the comb is removed, leaving behind pits in the gel called sample wells. Samples are loaded into the wells prior to electrophoresis.

Figure 12.2 In electrophoresis, the gel is placed in a tank of salt solution, and an electric current is applied. The DNA migrates toward the positive pole.

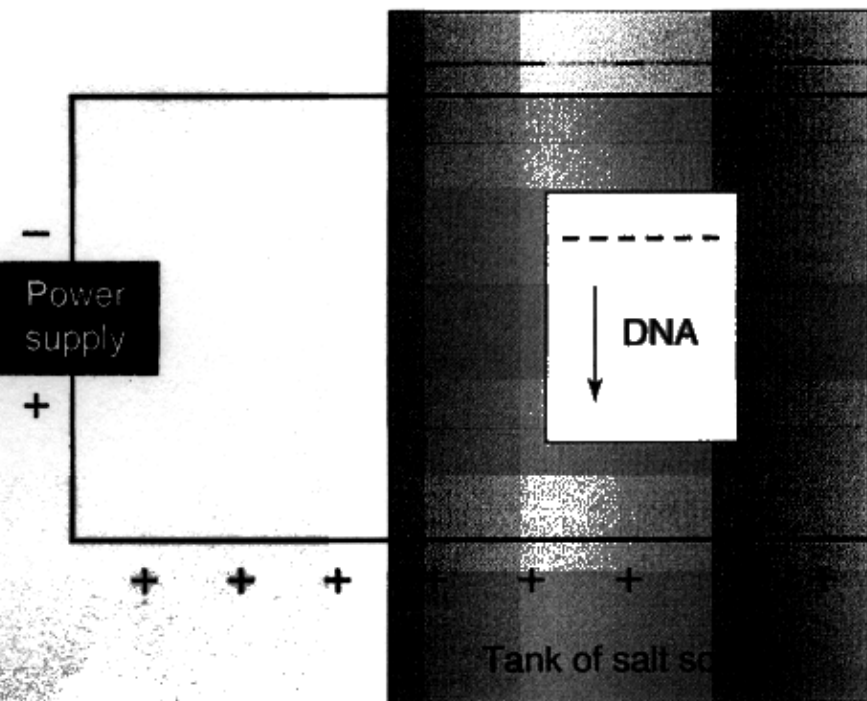


Figure 12.3 A scientist is using a micropipette to load DNA sample into an agarose gel. The gel is in an electrophoresis chamber full of buffer. The power supply for the chamber is on the laboratory bench behind the chamber.



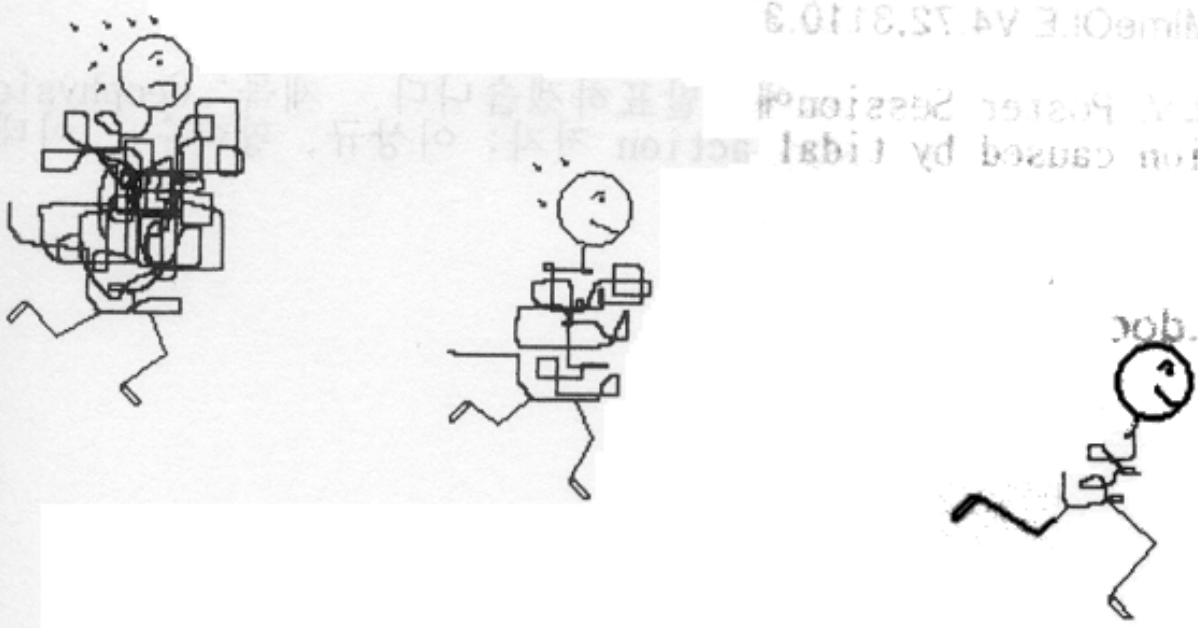
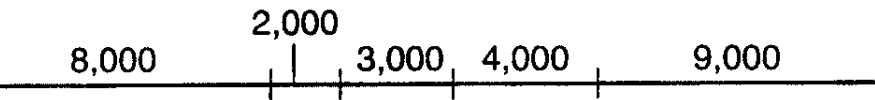


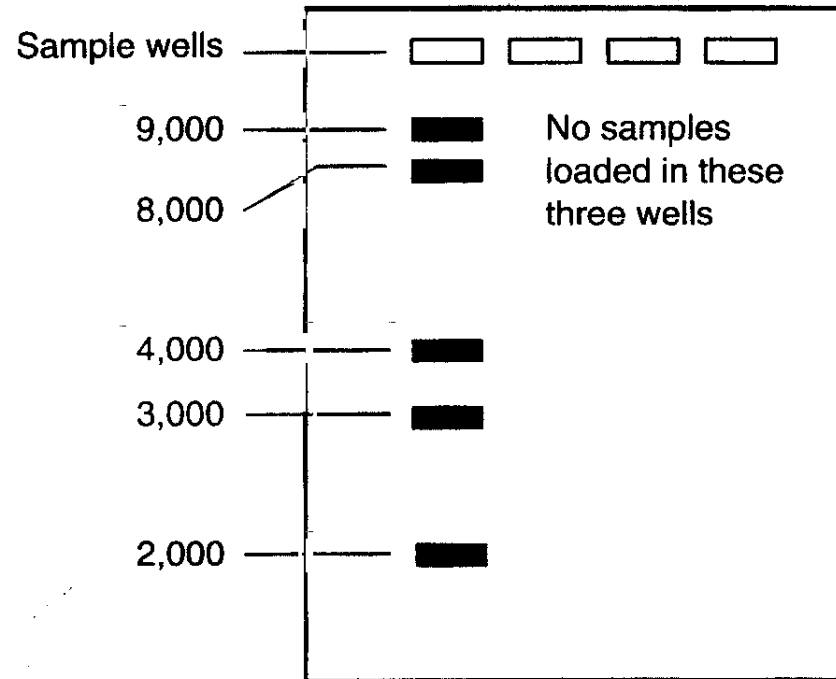
Figure 12.4 In electrophoresis races, the small DNA always wins!

Figure 12.5 Gel electrophoresis is used to separate products of restriction digestion. (A) Restriction map, with fragment sizes in base pairs; (B) view of gel after electrophoresis.



1. Digest with restriction enzyme.
2. Load solution into sample well and perform electrophoresis.
3. Stain gel and view DNA bands.

B



Polyacrylamide Gel Electrophoresis

- **For Smaller Molecule Separation (more tighter mesh than agarose, a higher resolving power)**
- **Proteins separated (proteins much smaller molecules than the DNA fragments)**
- **Size differences:**
 - A base pair DNA: ~ 660 Da**
 - 60,000 Da (fairly average-size protein) ~ 91 b.p. DNA fragment**
 - 53,000 Da protein ~ 83 b.p. DNA fragment**
- **60,000 Da vs. 53,000 Da : clearly separated in polyacrylamide gel but difficult in agarose gel.**

Hybridization

- Hybridization is a technique that takes advantages of the specificity of DNA base pairing for the detection of specific DNA sequences in a mixed sample.
- Restriction digestion/ Electrophoresis/ Staining techniques: information about the sizes of DNA fragments
- What about sequence of the DNA fragments?

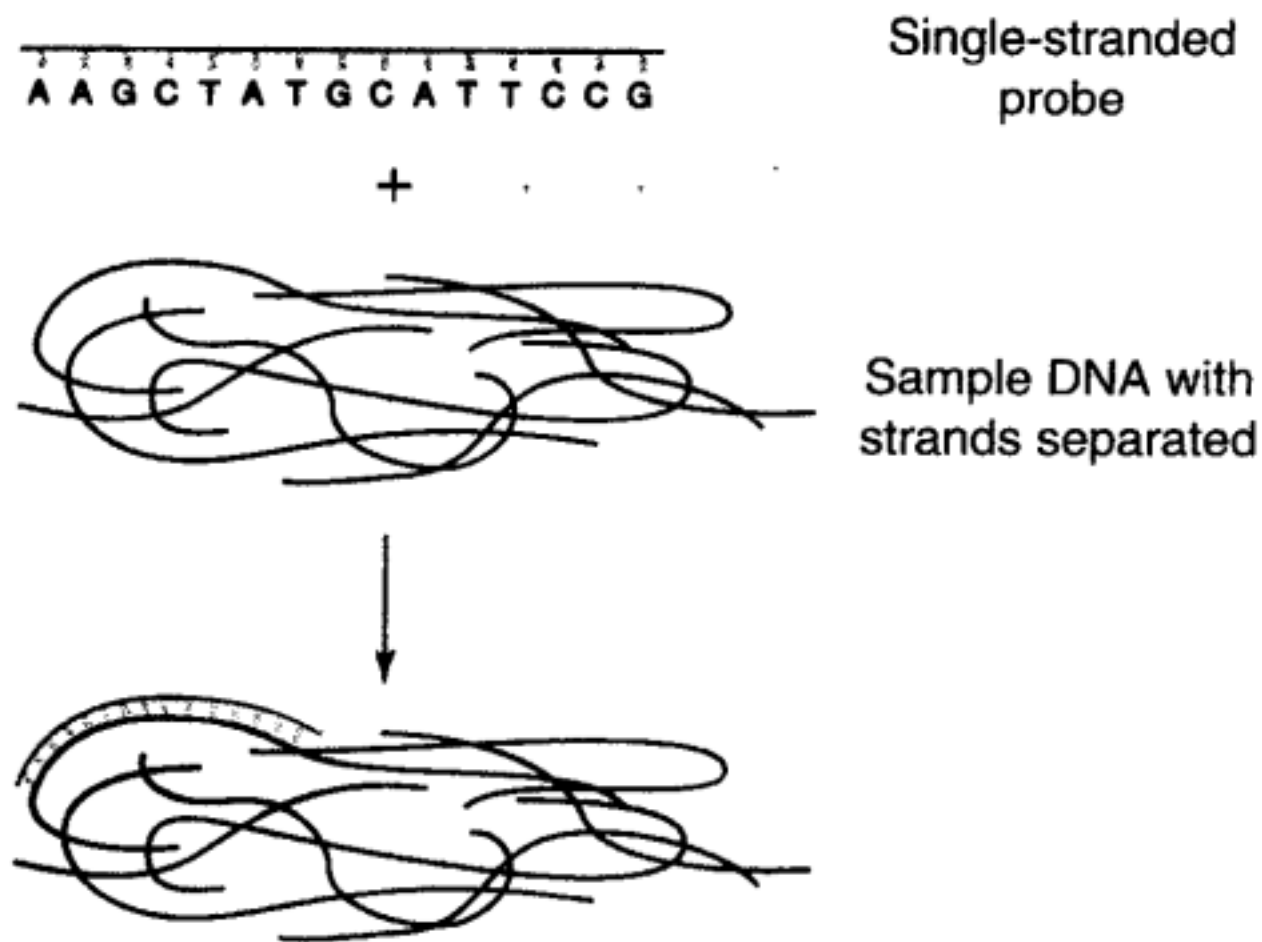
Important:

- to know whether a specific DNA base sequence is present in a sample.
- to determine whether a certain gene has been introduced into an organism.
- to analyze the structure of a certain region of DNA.

Hybridization

- **Hybridization (also called annealing or renaturation) is the term used to describe the process in which two single DNA strands with complementary base sequences stick together to form a correctly base-paired double-stranded molecule.**
- **It occurs spontaneously if two complementary single DNA strands are mixed together and left alone, they will hybridize.**
- **The time it takes for hybridization is proportional to the length of the DNA sequence.**
- **Usages:**
 - 1) a way to look for a specific DNA sequence (as a probe)**
 - 2) a starting place for additional techniques. (ex, DNA chip)**

Figure 5.7 Hybridization analysis.



If sample DNA contains the base sequence complementary to the probe sequence, the probe will form base pairs with the sample DNA, or hybridize to the sample, and physically stick to it

Hybridization

- Steps:

- 1) separating the strands of the DNA molecules
- 2) mixing those separated strands with many copies of ssDNAs or RNAs (It is called a “probe”)
- 3) Hydrogen bonds formed between the probe and its complementary single strands
- 4) The sample is rinsed to remove unhybridized probes

Hybridized probe indicates the presence of the DNA sequence of interest.

Hybridization

- Denaturation:

- 1) For breaking the hydrogen bonds between the base pairs**
- 2) The extremity of the conditions: G***C/ A**T, higher temperature or pH required for GC than AT**
- 3) The melting temperature required to denature a given DNA molecule is predicted on the basis of its G-C content. (~ 95 – 100 C)**
- 4) For probe hybridization, the melting temperature in consideration.**

Hybridization

- Hybridization:

- 1) Solution allowed to cool**
- 2) The strand reannealed and the double helix formed**
- 3) Annealing occurred between any complementary single strands of DNA; no enzyme required**
- 4) Short complementary molecules > longer one – for facilitate hybridization: the sample DNA transferred to a membrane (nylon or nitrocellulose)**

Hybridization

- Probes:

1) Short synthetic oligonucleotides of 15-30 bases/ purified restriction fragments/ whole linearized plasmids/ products of in vitro enzymatic DNA or RNA synthesis used as Probes

**2) - Radioactive labeling: synthesis in the presence of radioactive nucleotides or attachment of a radioactive terminal phosphate group by polynucleotide kinase.
- colored or luminescent product formation through a detection reaction (usually enzymatic one).**

3) The critical aspect to probe selection: the probe will hybridize only to the DNA of interest

4) The chance of random occurrence: 1 in 4^n , where n is the number of bases in the sequence.

Hybridization

- Probes:

- 5) The chance of random hybridization decrease dramatically as the probe becomes longer: but possible hybridization between somewhat mismatched two ss molecules.
- 6) Less stable base pairing for mismatched molecules (no hydrogen bonds between mismatched bases)
- 7) By adjusting hybridization temperature, the mismatched annealing permitted or blocked.

Hybridization .. continue

- **Southern Hybridization:** a procedure used to detect a specific sequence of nucleotides among fragment of DNA which have been separated by gel electrophoresis.

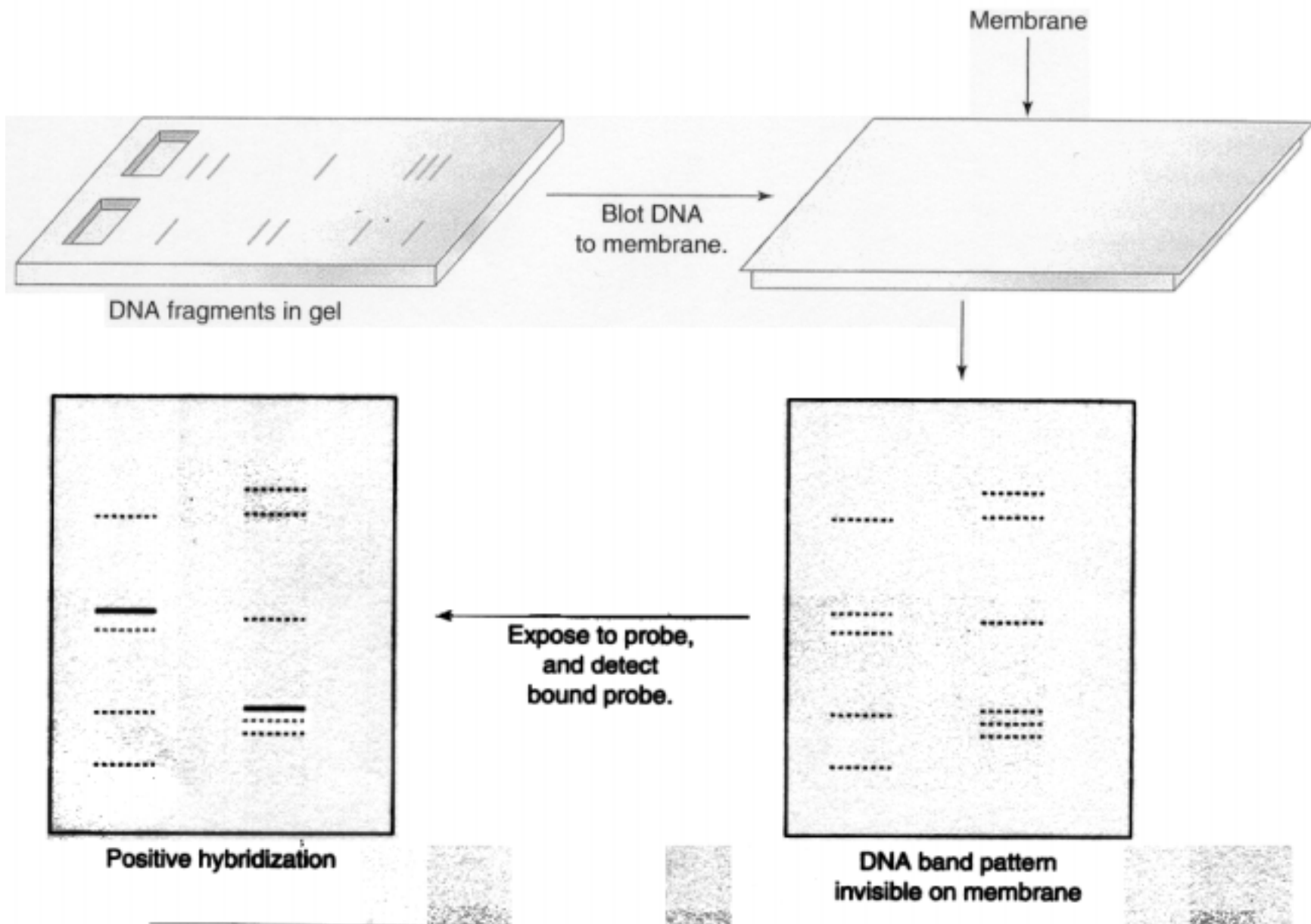
- 1) ss forms of the fragment bound to a matrix
- 2) the matrix exposed to a labelled probe complementary to the sequence of interest
- 3) hybridization between the probe and the specific region of the blotted DNA.

Blotting

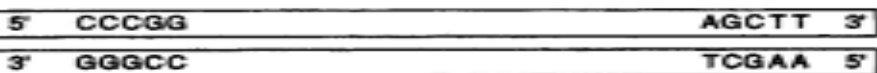
Picking up a specific DNA sequence using so called “a probe” through transfer of DNAs to membrane (or papers) [blotting] and DNA hybridization

- 1) Southern blotting: DNA hybridized in paper membrane
- 2) Northern blotting: RNA transferred & hybridized
- 3) Western blotting: Protein transferred & hybridized
- 4) No eastern blotting!!

Figure 5.8 Blotting and hybridization analysis. DNA fragments separated by gel electrophoresis are transferred from the gel to a membrane. The membrane is then exposed to a probe to test for the presence of specific DNA sequences.

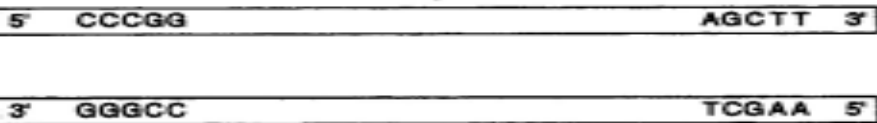


Double-stranded parental DNA

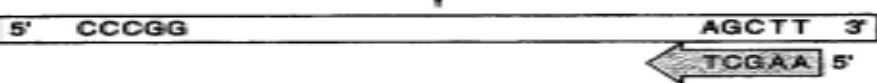


Single-stranded primers

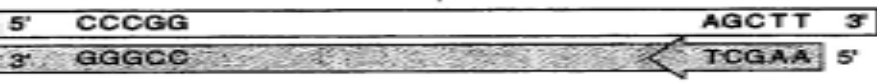
Denaturation



Hybridization



DNA synthesis

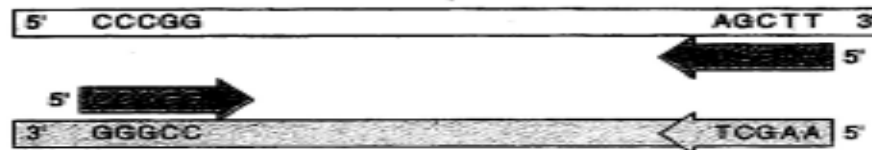


Round 2 is shown in the next column.

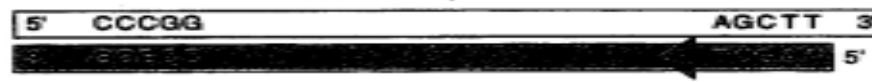
Denaturation



Hybridization



DNA synthesis



etc.

Figure 5.9 PCR makes many copies of a DNA segment lying between and including the sequences at which two single-stranded primers hybridize to the substrate DNA molecule. The primers are usually synthetic oligonucleotides.

