Molecular Biology (1)

DNA structure and basic applications

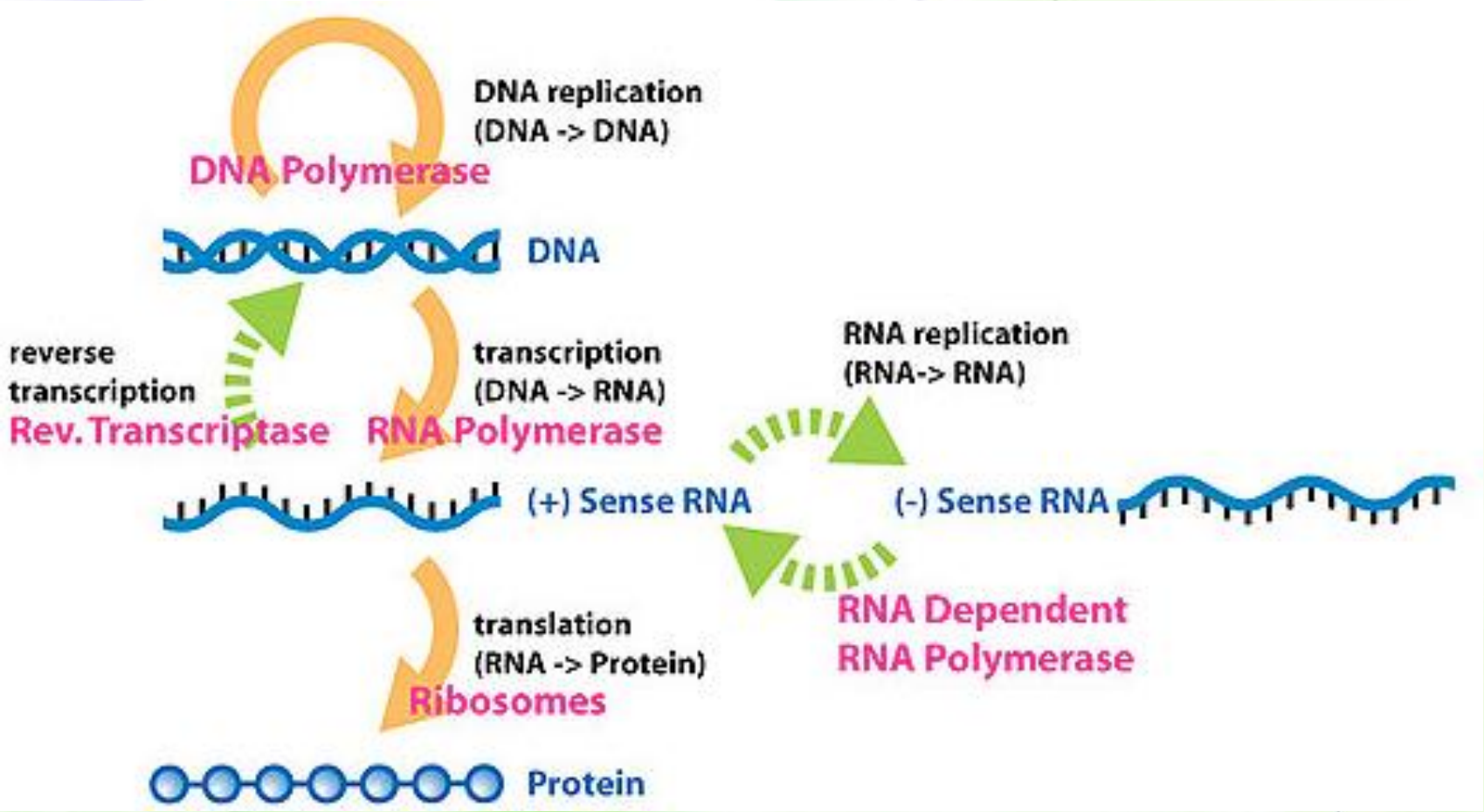
Mamoun Ahram, PhD
Second semester, 2018-2019

Resources

- This lecture
- Cooper, pp. 49-52, 118-119, 130

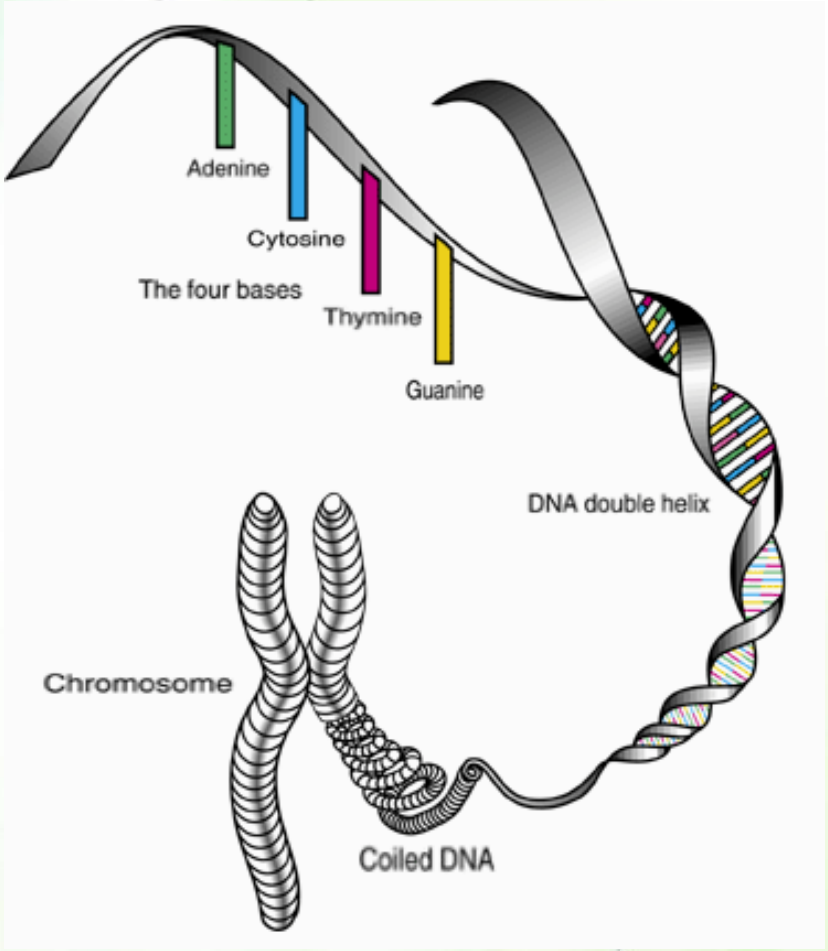
What is molecular biology?

Central dogma of molecular biology

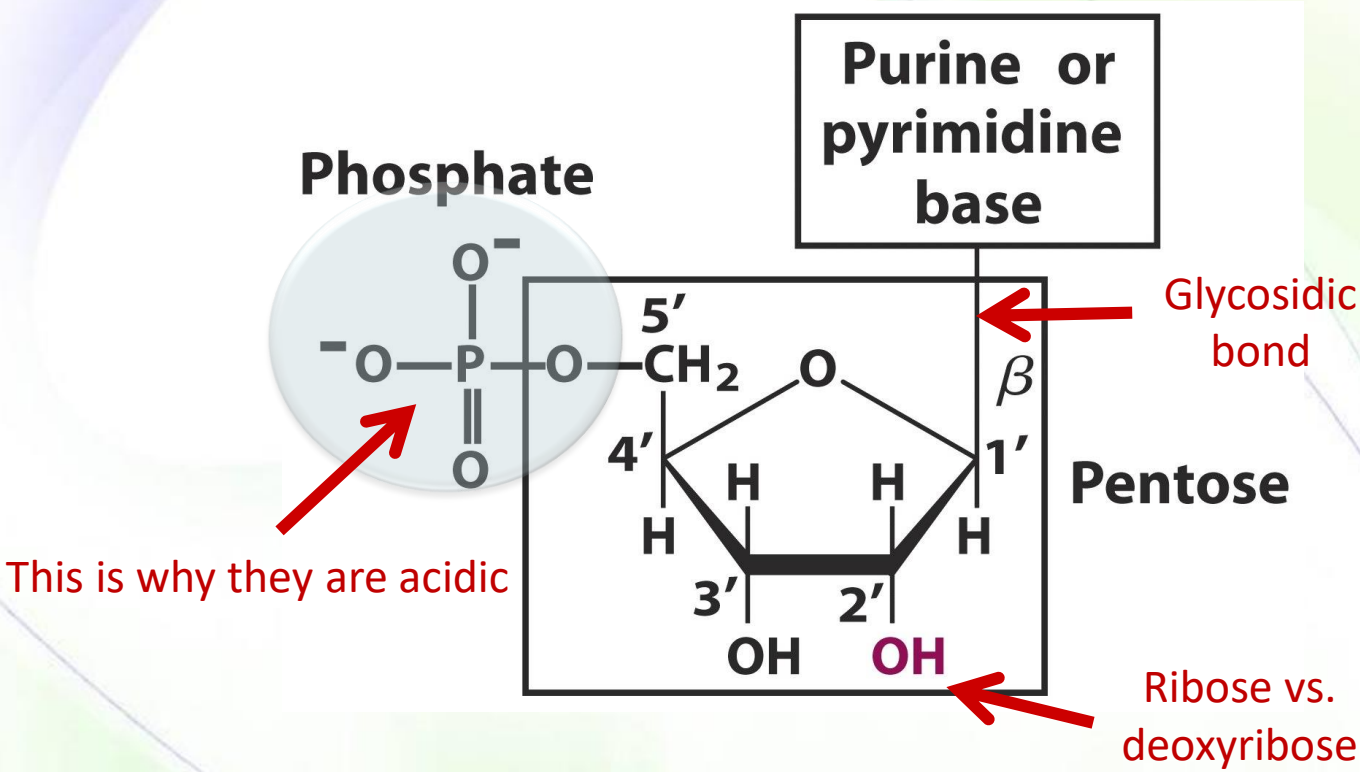


Nucleic acids

- The primary structure of nucleic acids is linear polymers of nucleotides (monomers) bound to each other via phosphodiester bonds.
- DNA is coiled and can be associated with proteins forming chromosomes.

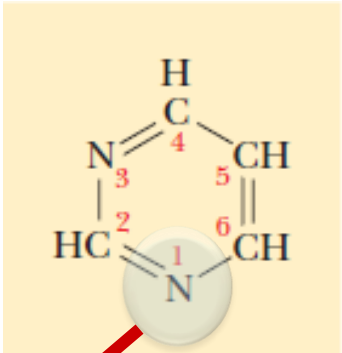


Chemical composition and bonds



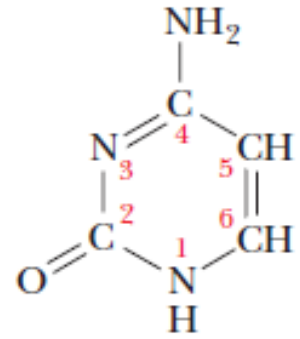
- Positively charged ions (Na+ or Mg2+) and peptides with positively charged side chains can associate with DNA
- Eukaryotic DNA, for example, is complexed with histones, which are positively charged proteins, in the cell nucleus.

Nitrogenous bases

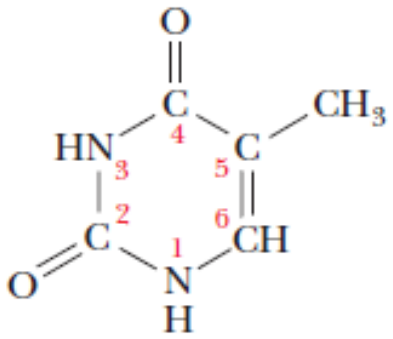


Pyrimidine

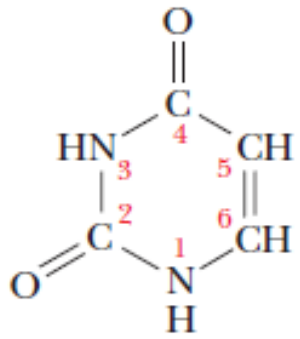
Glycosidic bond



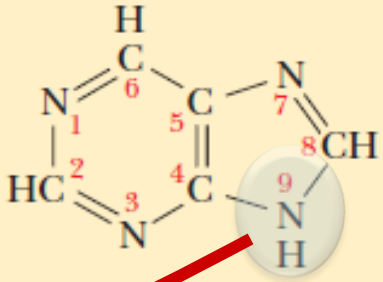
Cytosine
(in DNA & RNA)



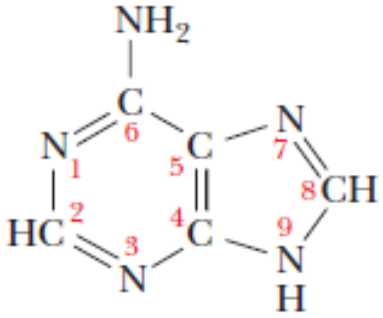
Thymine
(in DNA & some RNA)



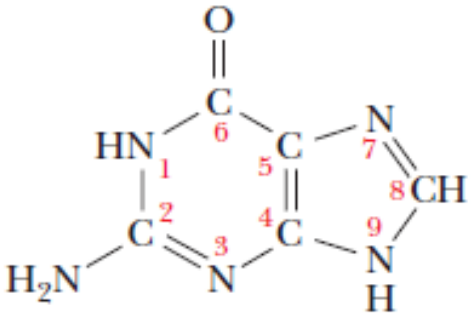
Uracil
(in RNA)



Purine

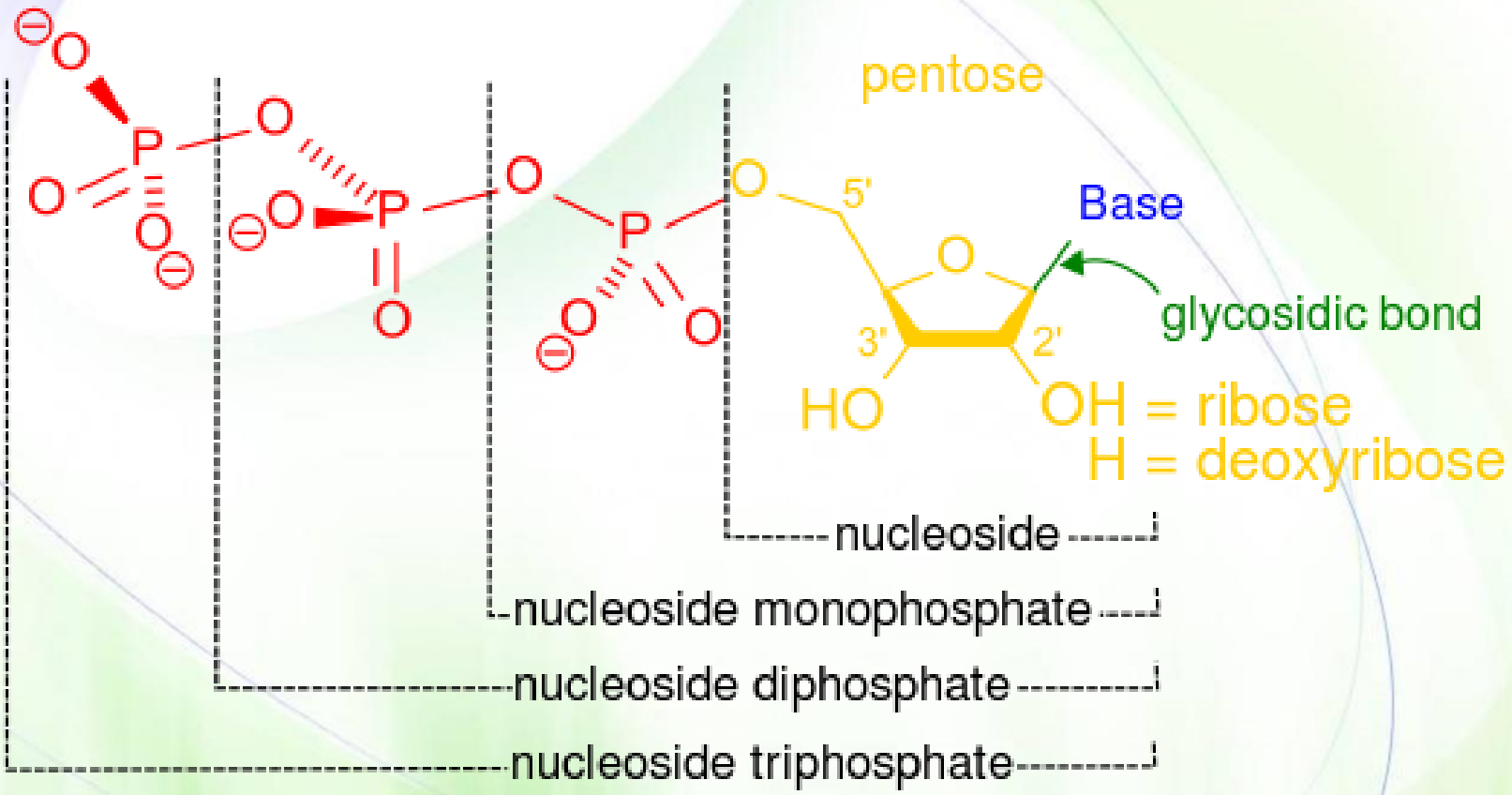


Adenine
(in DNA & RNA)

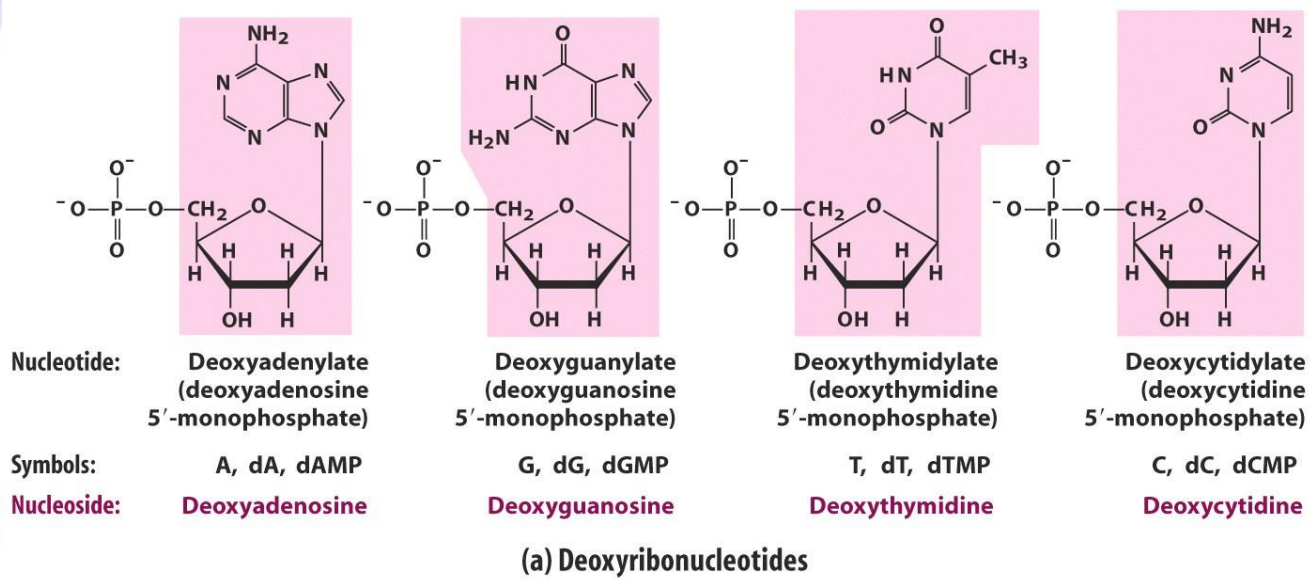


Guanine
(in DNA & RNA)

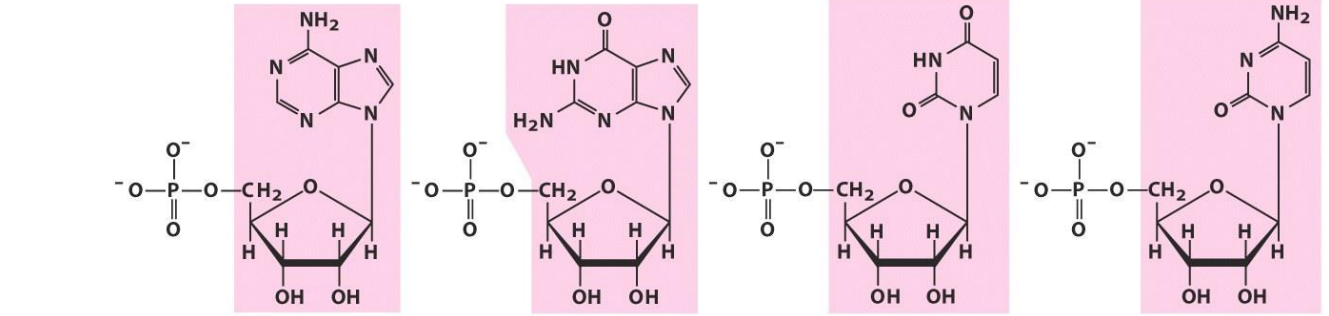
Nucleotides vs. Nucleosides



Nucleotides vs. Nucleosides

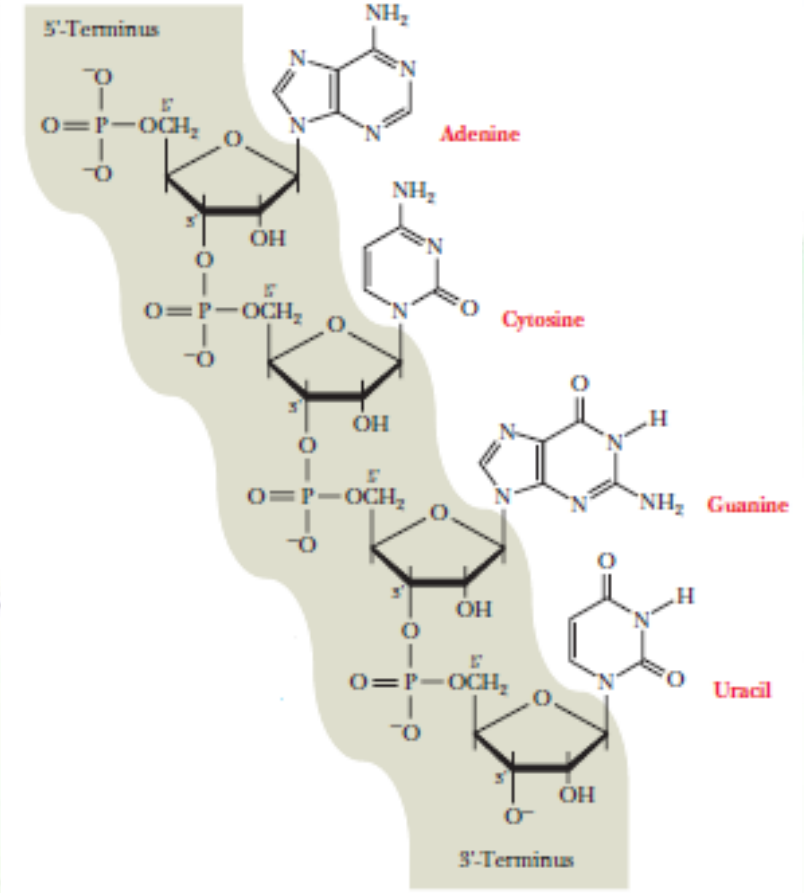


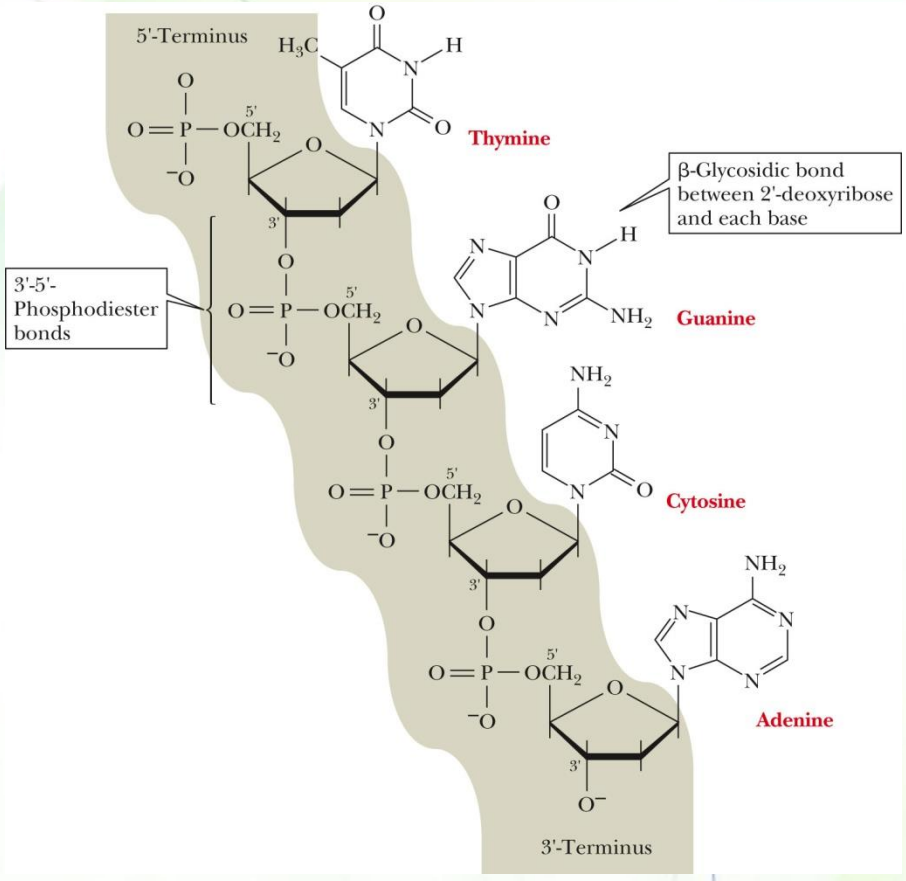
(a) Deoxyribonucleotides



(b) Ribonucleotides

Nucleic acid polymers

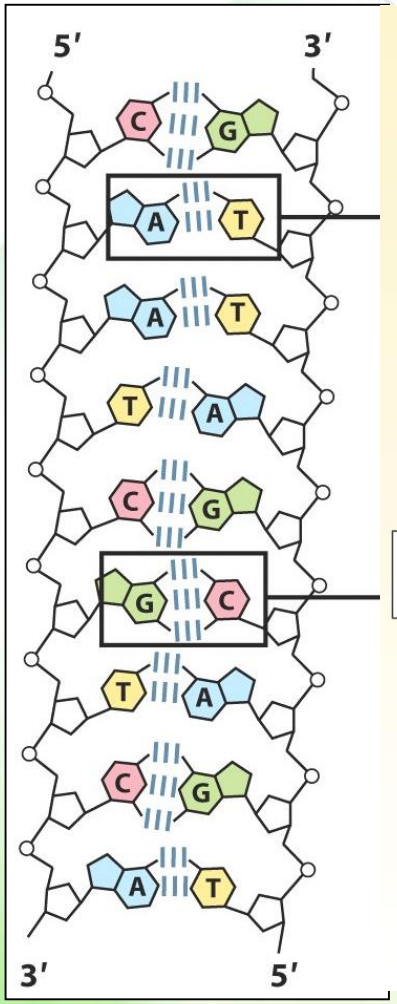


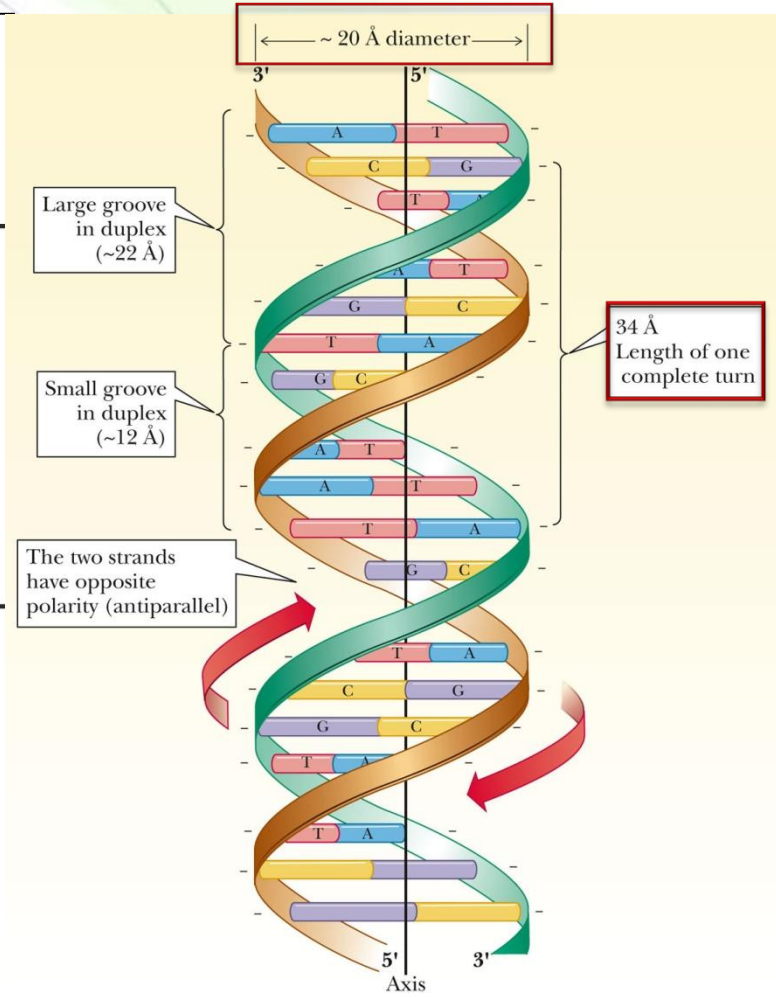


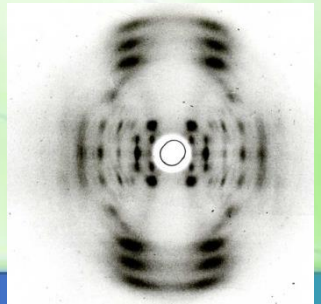
- A letter d can be added to indicate a deoxyribonucleotide residue.
  - for example, dG is substituted for G.
  - The deoxy analogue of a ribooligonucleotide would be d(GACAT).

DNA structure

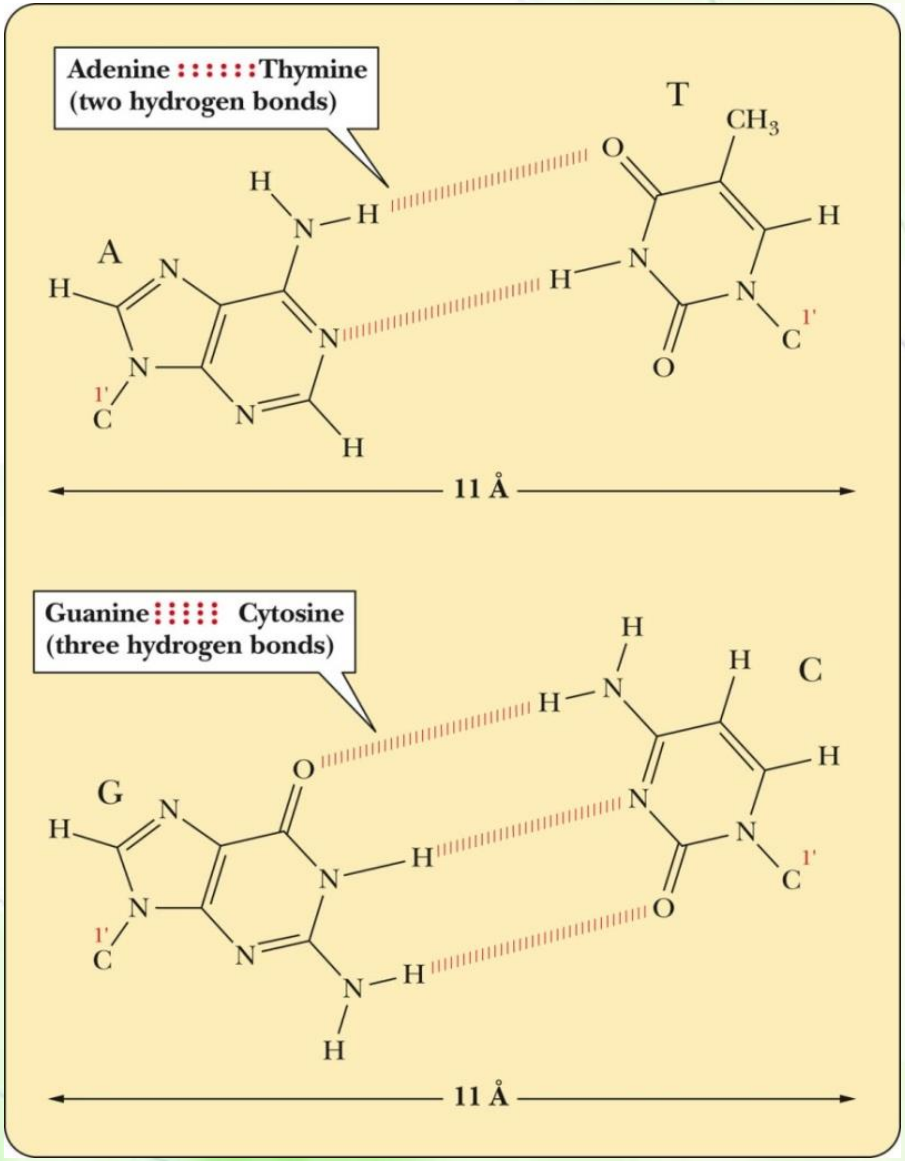
- Specific base-pairing
  - A = T; G = C; Pur = pyr
- Complementary
- A double helix
- Backbone vs. side chains
- Antiparallel
- Stable
- Flexible
- Groovings
- Stability vs. flexibility





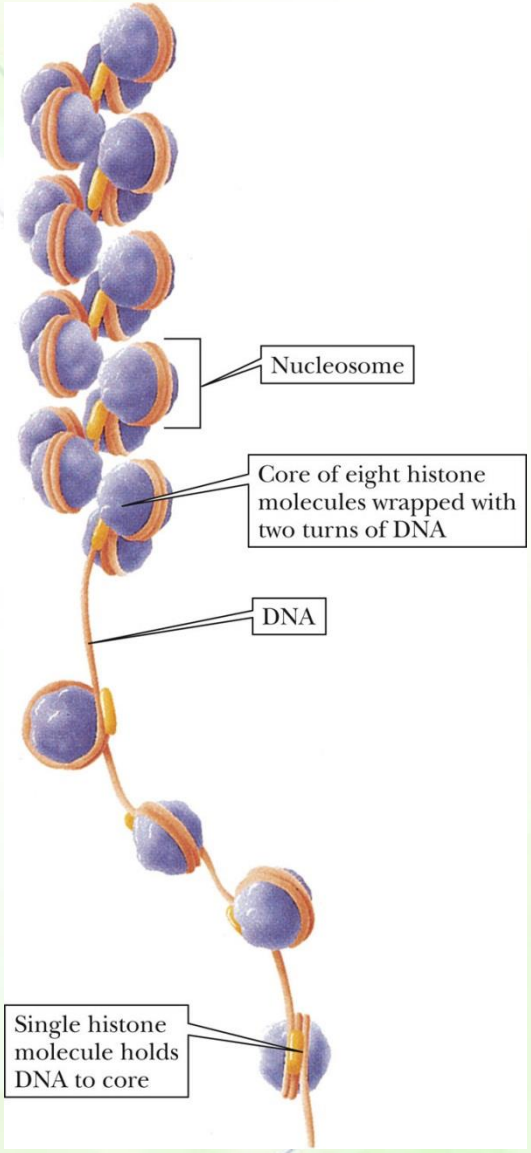


Base pairing



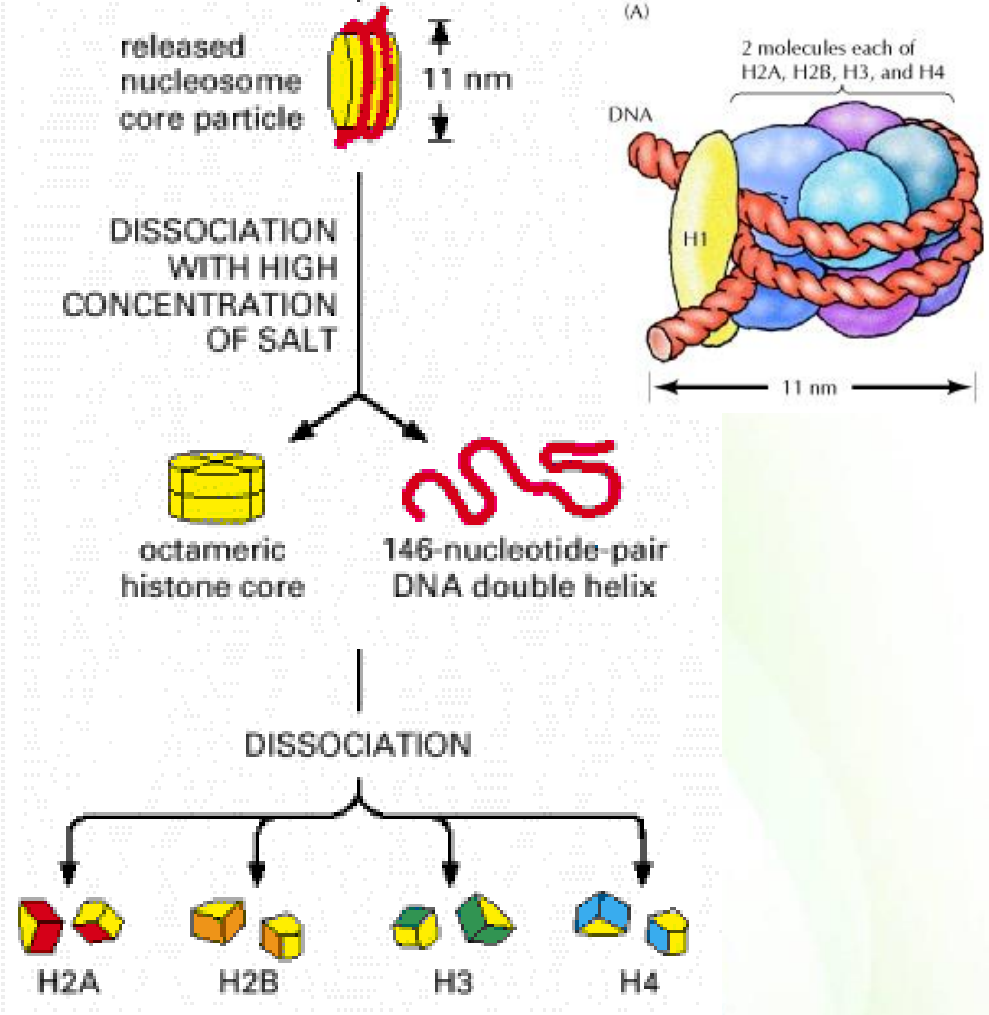
In eukaryotes…

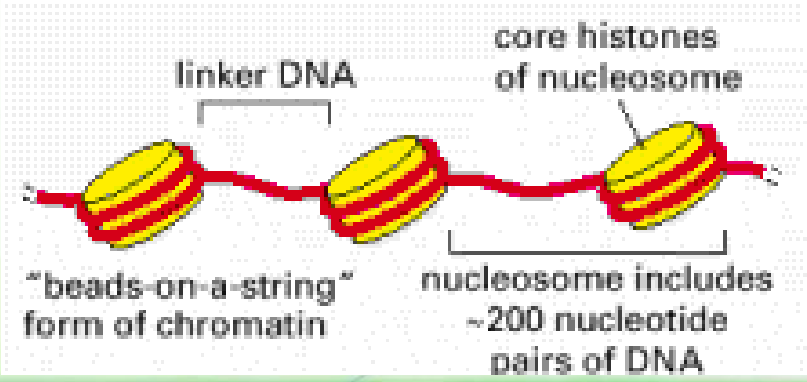
- In eukaryotes, DNA is coiled to package the large DNA and regulate gene activity.
- Eukaryotic DNA is complexed with a number of proteins, principally histones, which are surrounded by DNA.
- Chromatin = DNA molecule + proteins.



Nucleosomes

- The histone protein core is an octamer (two molecules of histones H2A, H2B, H3, and H4).
- A linker DNA/spacer region connects the octamer-DNA complexes.
- A nucleosome consists of DNA wrapped around a histone core.
- H1 is bound to the the octamer and wrapped DNA (a chromatosome).
- Histones are positively charged facilitating DNA interaction and charge neutralization.





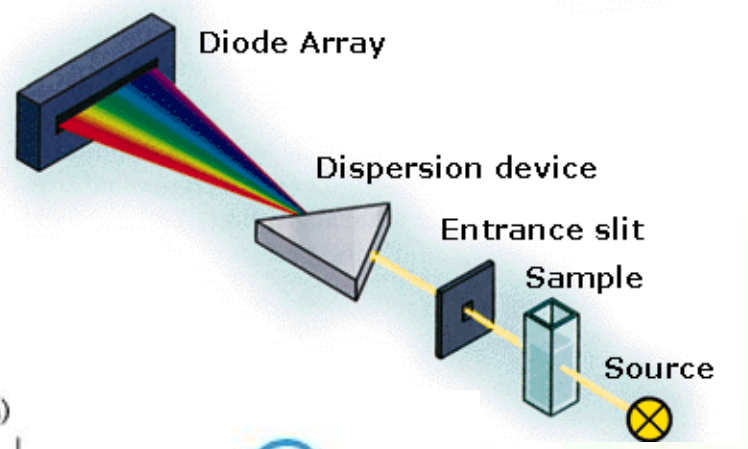
Light absorbance of nucleic acids

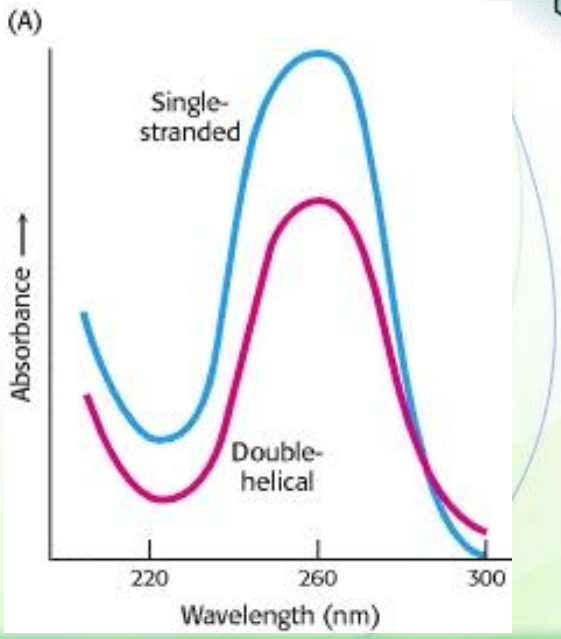
- Aromatic pyrimidines and purines can absorb UV light
- The peak absorbance is at 260 nm wavelength

- The absorbance of nucleic acids at 260 nm (A260) is constant
  - dsDNA: A260 of 1.0 = 50 ug/ml
  - ssDNA: A260 of 1.0 = 30 ug/ml
  - ssRNA: A260 of 1.0 =40 ug/ml

Reason for ss vs. ds absorbance:

- Unstacked bases vs. stacked bases





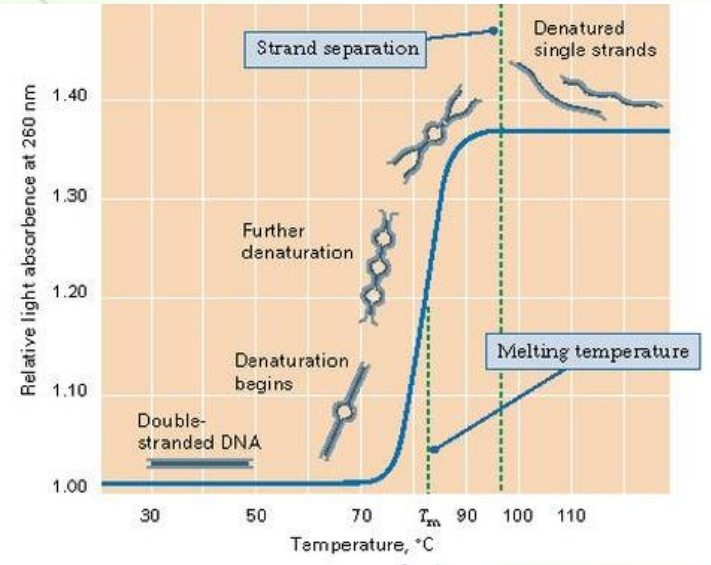
What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

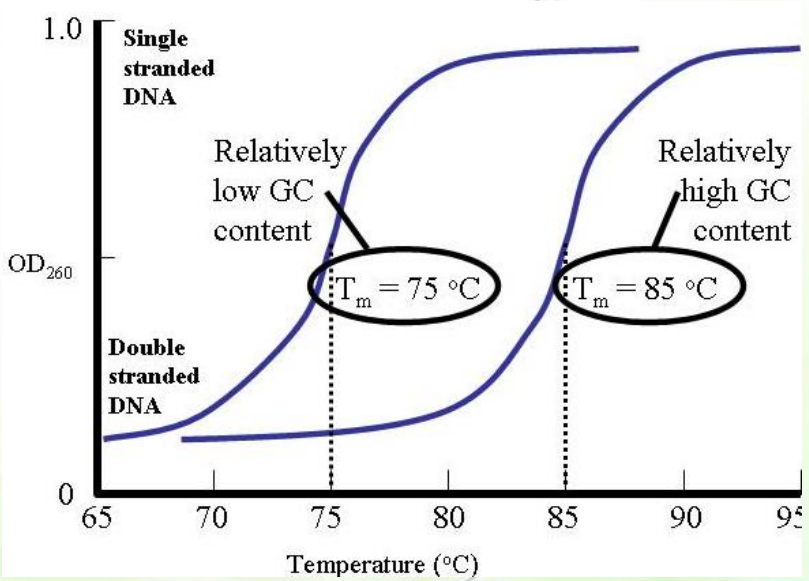
DNA concentration = 0.1 x 10 x 50 µg/ml

= 50 µg /ml

Observation of denaturation

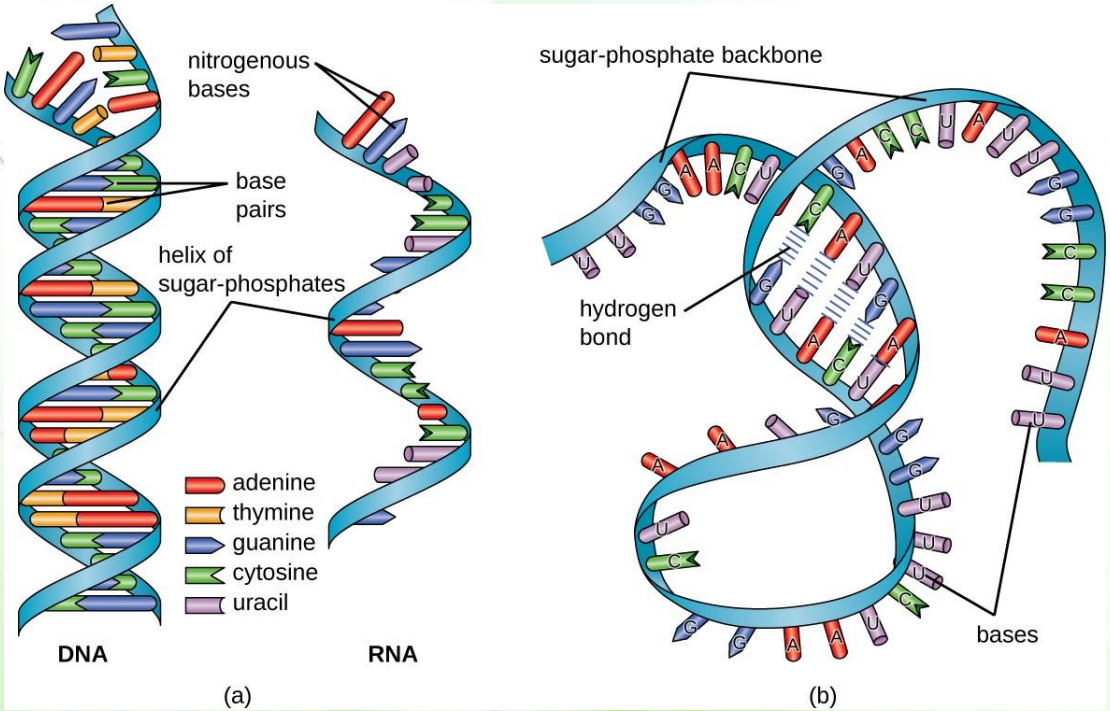
- The transition temperature, or melting temperature (Tm).
- Factors influencing Tm
  - Length
  - G·C pairs
    - Hydrogen bonds
    - Base stacking
  - pH
  - Salts and ions
  - Destabilizing agents (alkaline solutions, formamide, urea)





RNA

- It consists of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3'-OH of one pentose and the 5'-PO_4^- of the next.
- The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA).
- The pyrimidine bases are uracil and cytosine (thymine and cytosine in DNA).
- In general, RNA is single stranded (DNA is double stranded).



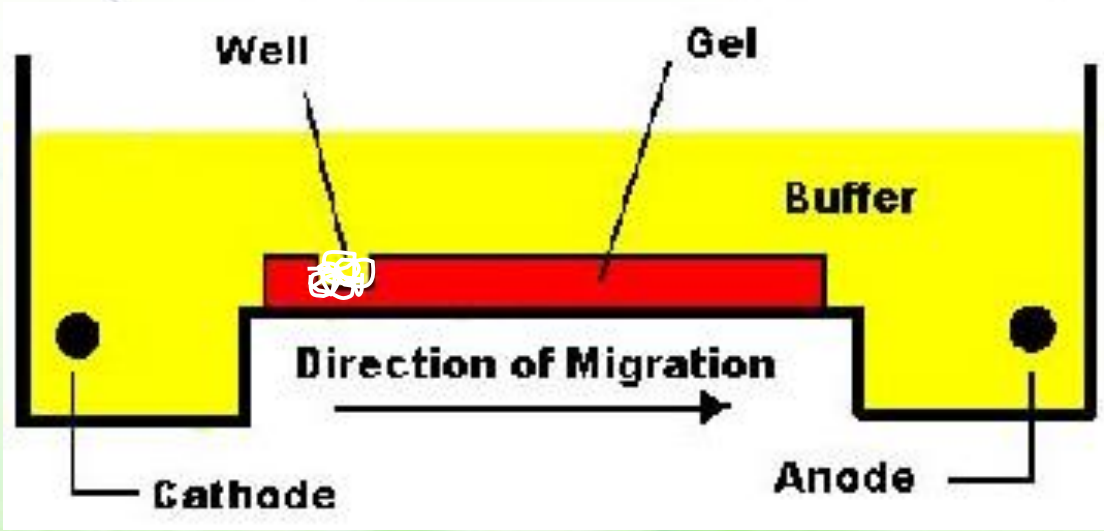
RNA does not have a precise structure, but it can fold on itself forming hydrogen bonds within the same molecule.

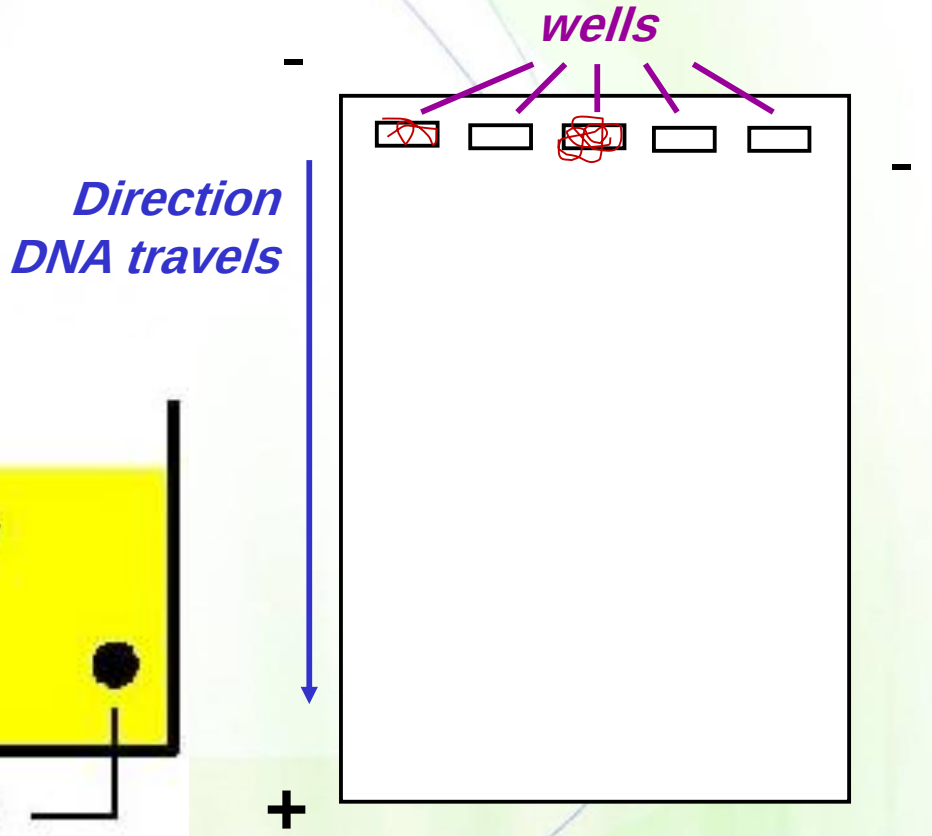
Types of RNA

Non-coding RNA		Length (nt)	Species	Function
Ribosomal RNA (rRNA)		120~4700	All	Translation
Transfer RNA (tRNA)		70~100	All	Translation
Small nuclear RNA (snRNA)		70~350	Eukaryote	Splicing, mRNA processing
Small nucleolar RNA (snoRNA)		70~300	Eukaryote, archaea	**RNA modification, rRNA processing**
miRNA	Small ncRNA	21~25	Eukaryote	Translational regulation
siRNA		21~25	Eukaryote	Protection against viral infection
piRNA		24~30	Eukaryote	Genome stabilization
Long ncRNA		several hundreds~ several hundred thousands	Eukaryote	Transcription, splicing, transport regulation

Gel electrophoresis

- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.





Resources

- http://www.personal.psu.edu/pzb4/electrophoresis.swf
- http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html
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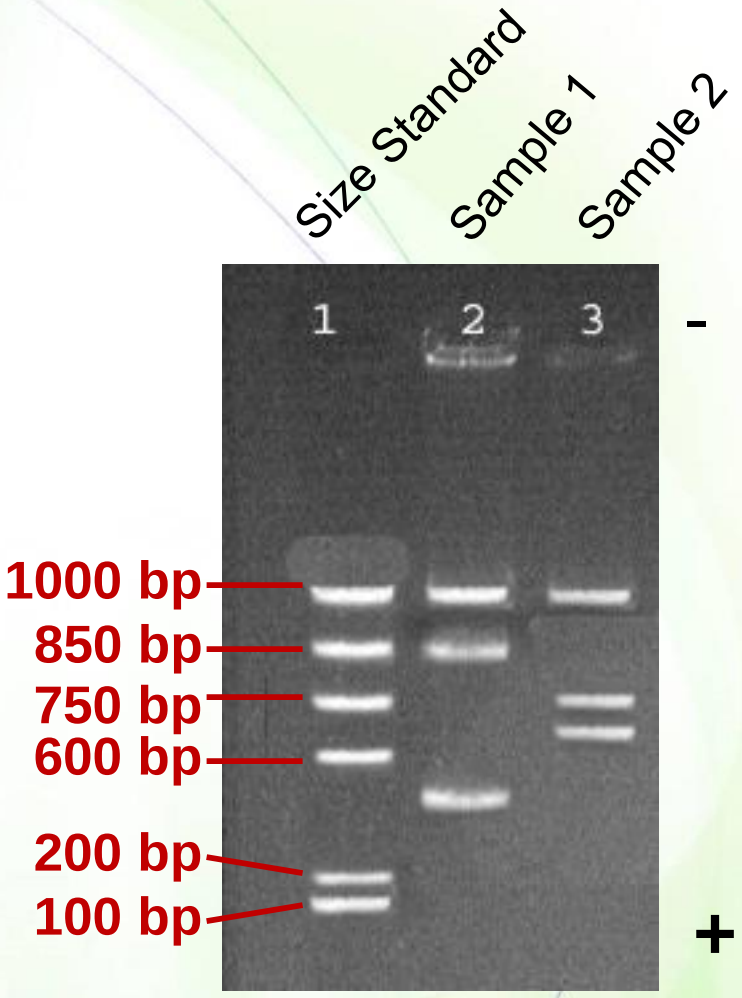
Detection

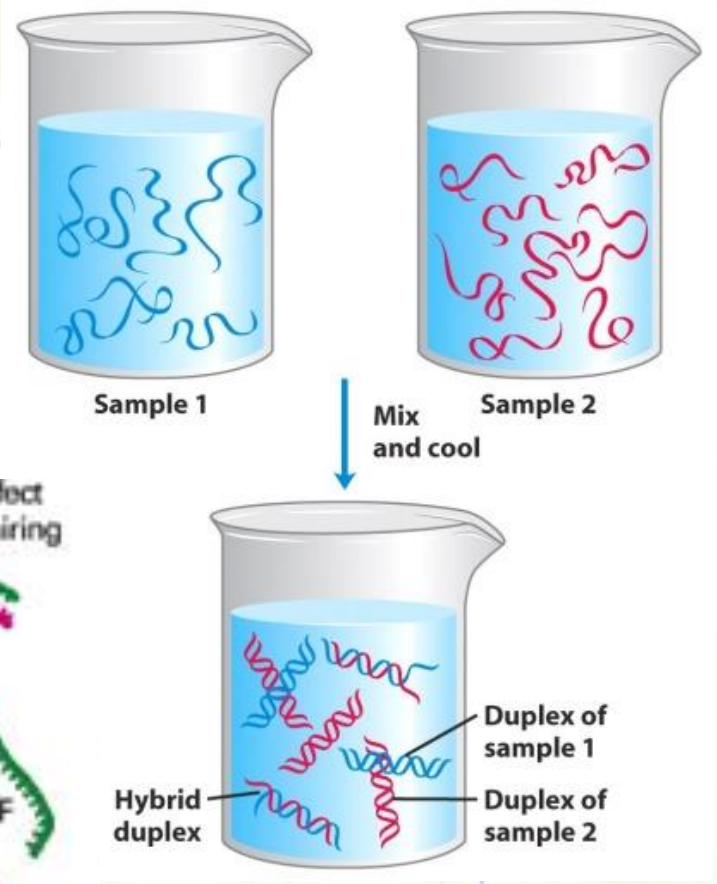
- The DNA molecules of different lengths will run as "bands".
- Each bands contains thousands to millions of copies of DNA fragments of the same length. They can be of same or different type (not one DNA molecule).
- DNA is stained (that is, colored) with a dye (ethidium bromide) or radioactively labeled (^{32}P).
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.

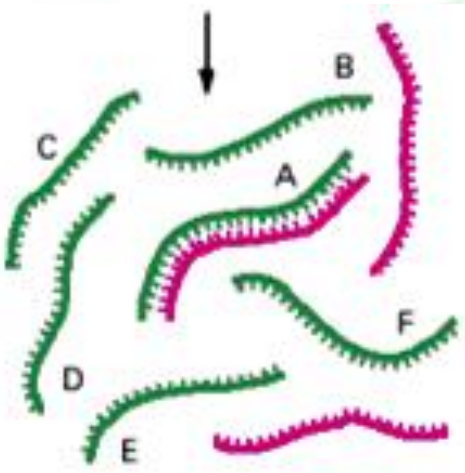


bp: base pair

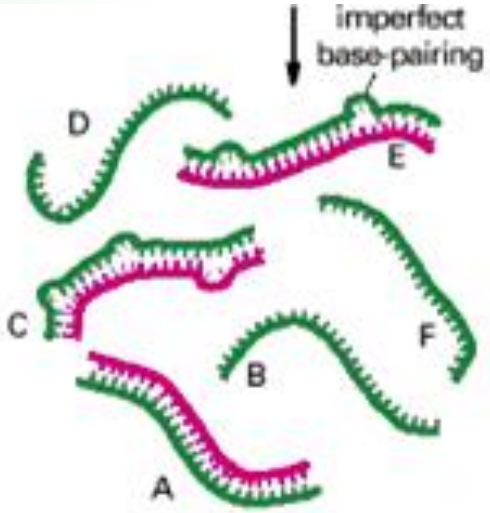
Hybridization

- DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA).
- Hybridization can be imperfect.





only A forms stable double helix



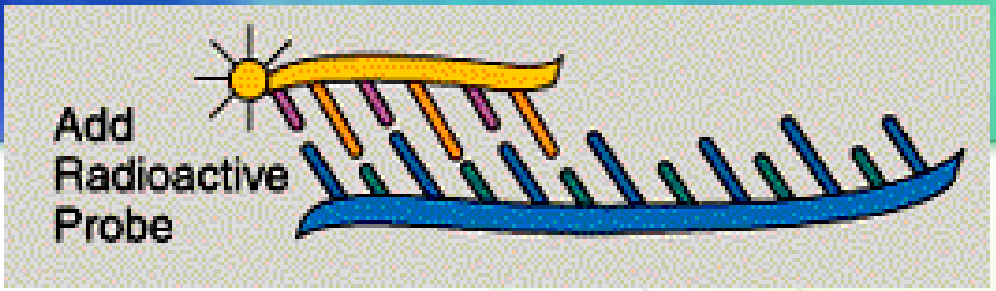
A, C, and E all form stable double helices

Hybridization techniques

- Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences

- Hybridization reactions are used to detect and characterize specific nucleotide sequences

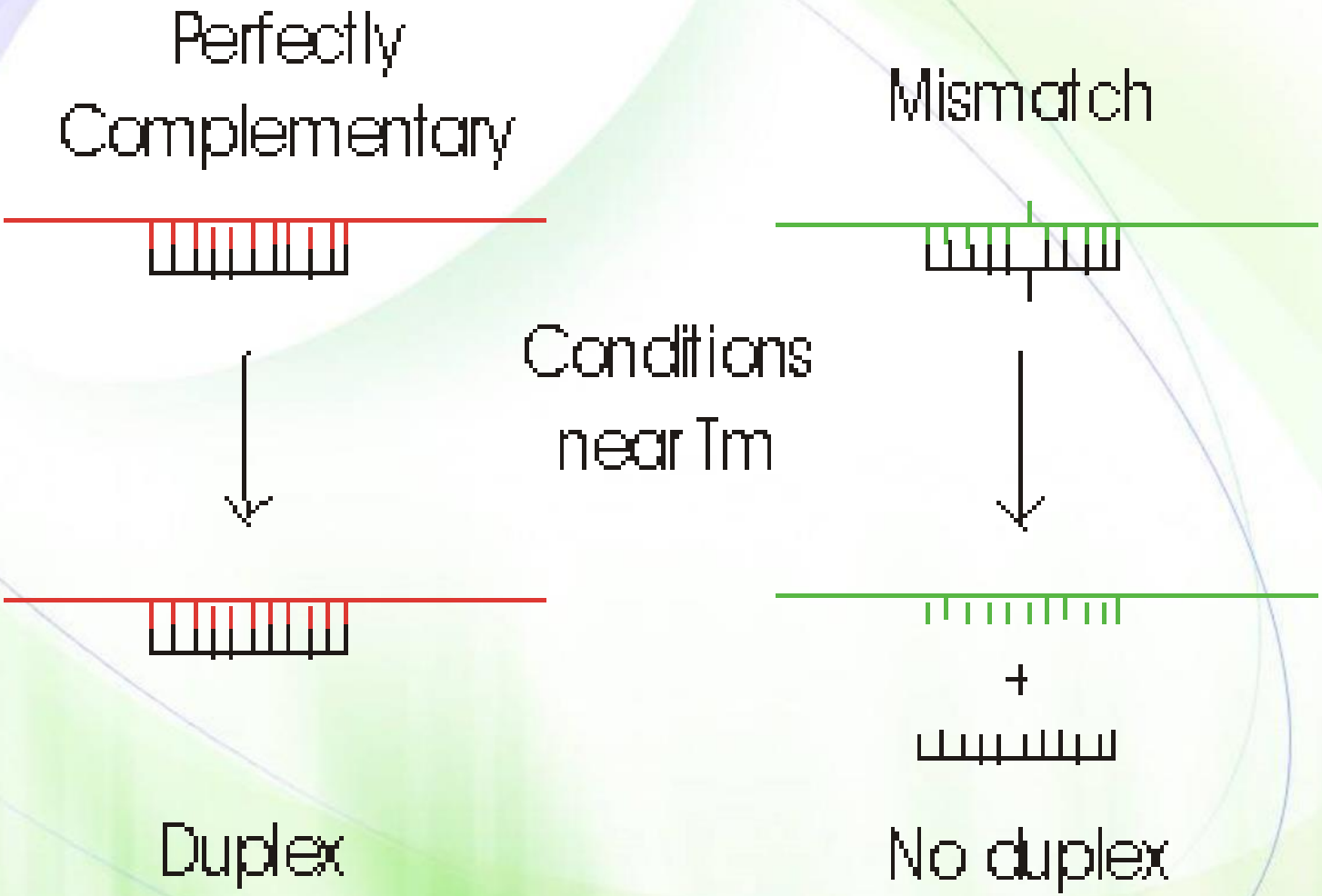
Probes



- A probes is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.

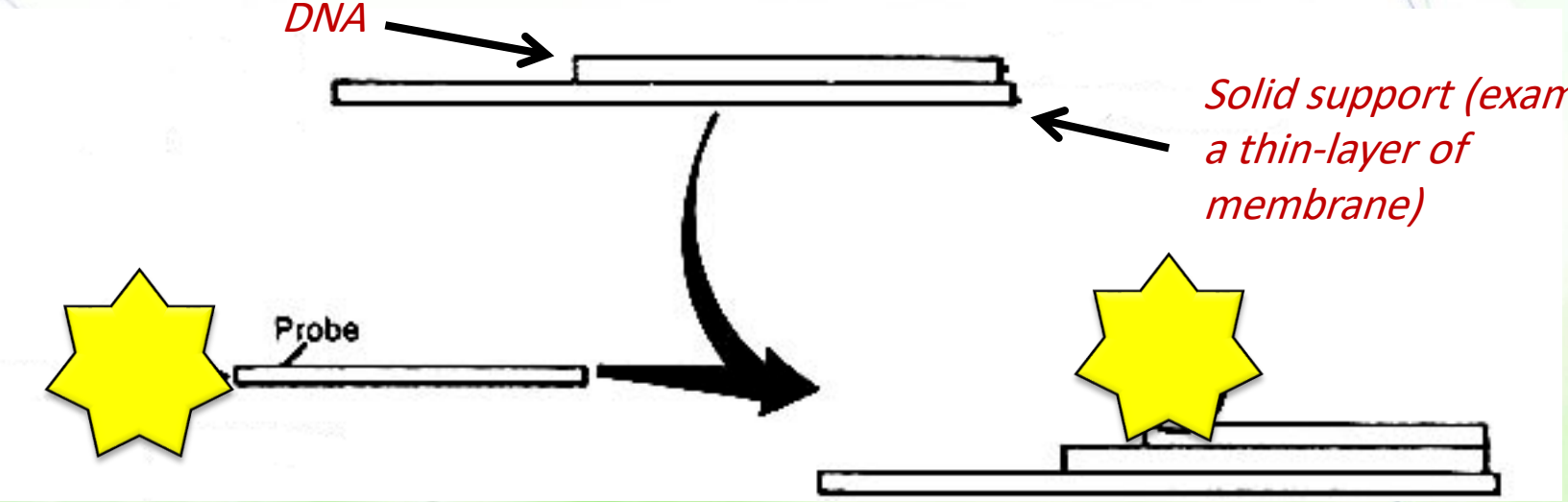
- Hybridization reactions use labeled DNA probes to detect larger DNA fragments.

Hybridization can be (non)specific



Dot blot

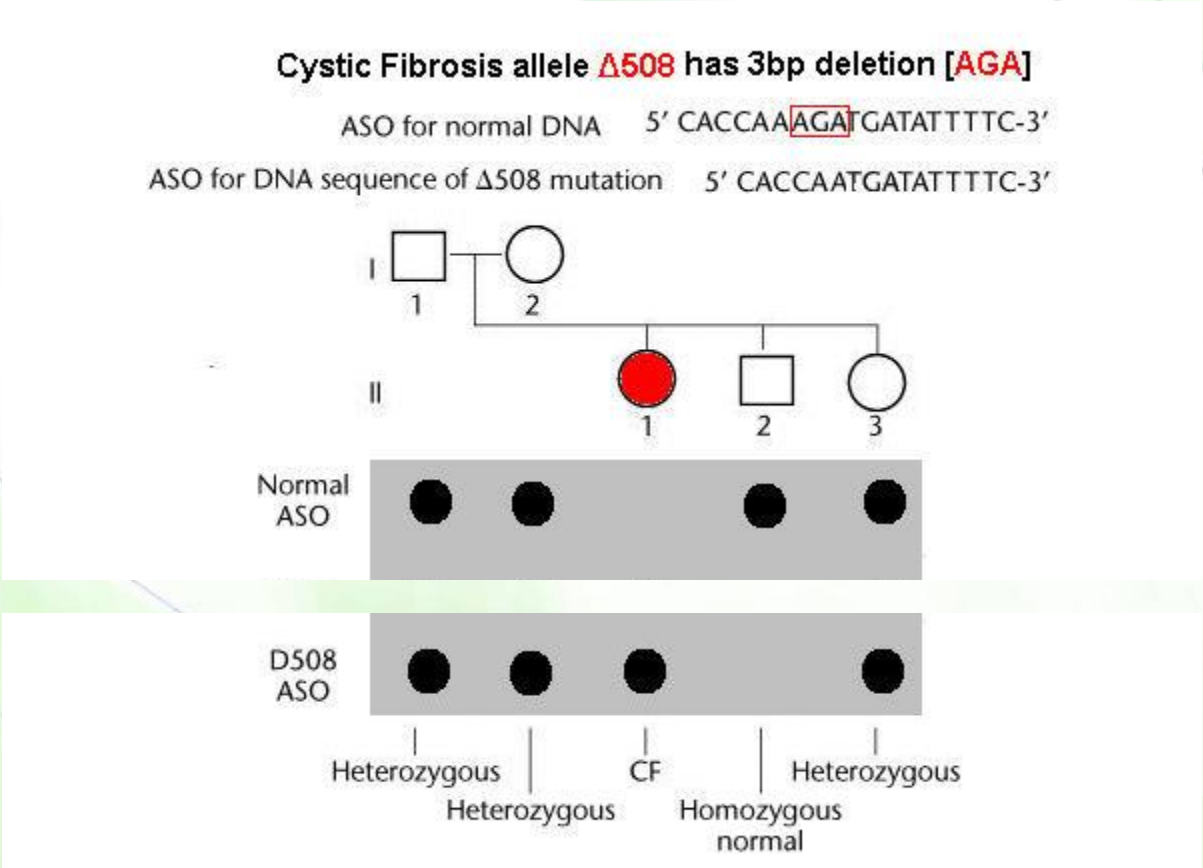
- This is a technique that informs us if a specific sequence that is complementary to a probe of a known sequence exists in a larger DNA.
- DNA is bound to a solid support and a labeled probe is added. If binding occurs, the sequence exists.



Disease detection by ASO (Cystic fibrosis)

ASO: Allele-specific oligonucleotide

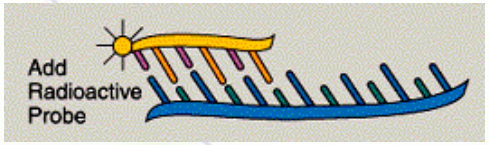
The whole genomic DNA is spotted on a solid support (like a nylon membrane) and hybridized with two ASO's, one at a time.

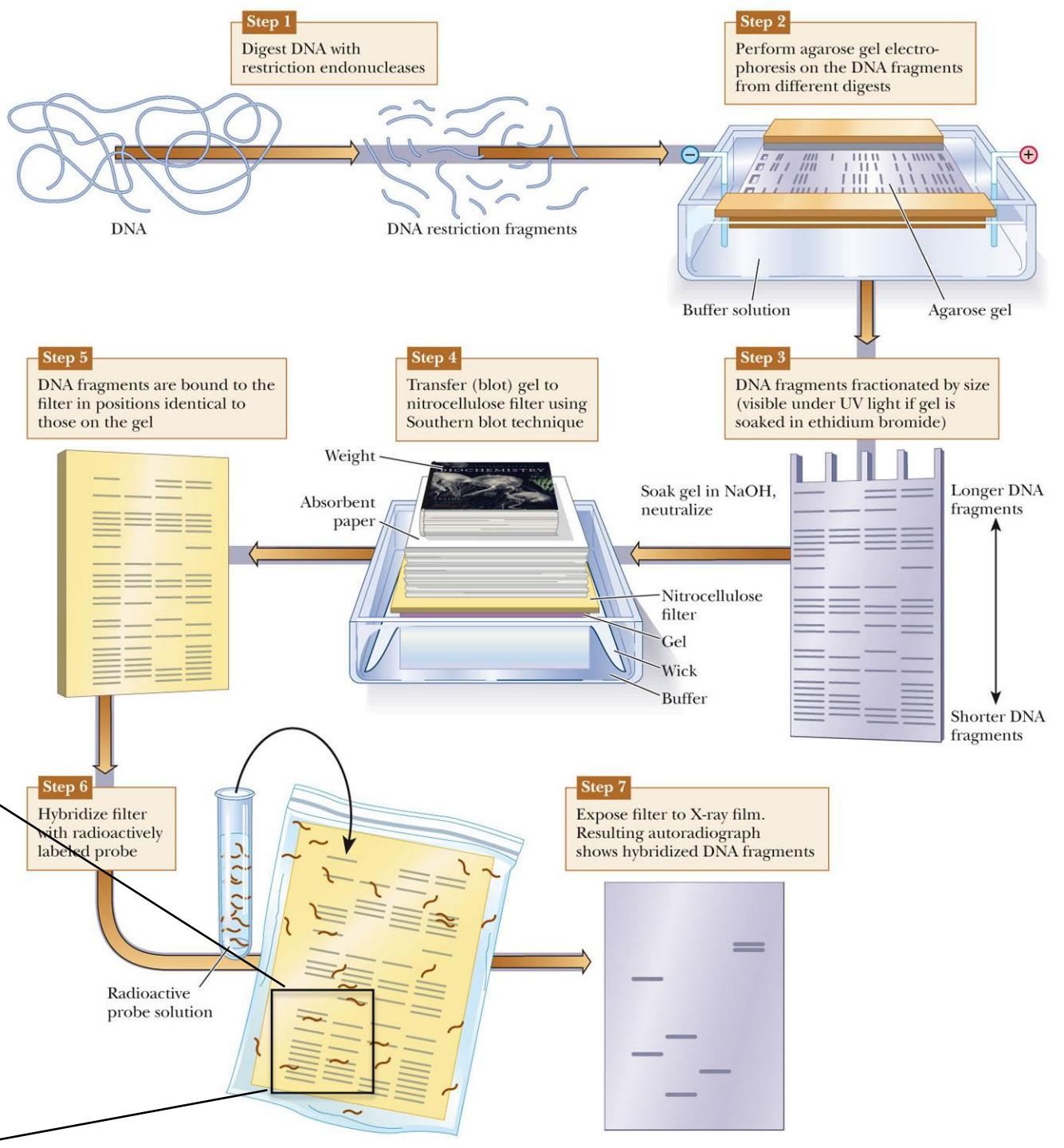


Southern blotting

- This technique is a combination of DNA gel electrophoresis and hybridization

- Used to detect:
  - the presence of a DNA segment complementary to the probe
  - the size of the DNA fragment

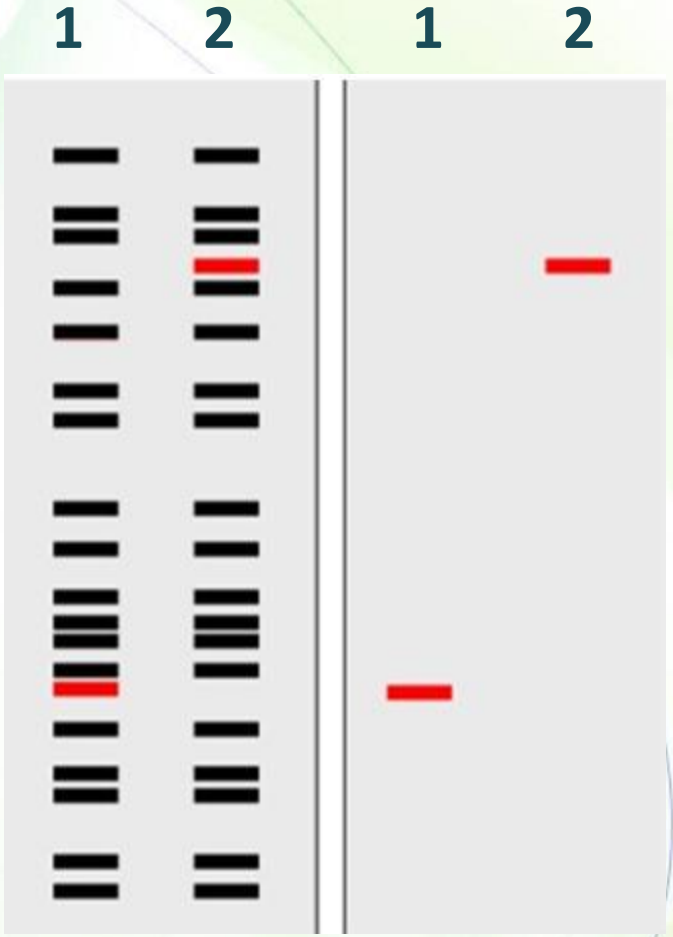

Add
Radioactive
Probe


Step 1
Digest DNA with restriction endonucleases
Step 2
Perform agarose gel electrophoresis on the DNA fragments from different digests
DNA
DNA restriction fragments
Buffer solution
Agarose gel
Step 3
DNA fragments fractionated by size (visible under UV light if gel is soaked in ethidium bromide)
Step 4
Transfer (blot) gel to nitrocellulose filter using Southern blot technique
Step 5
DNA fragments are bound to the filter in positions identical to those on the gel
Soak gel in NaOH, neutralize
Longer DNA fragments
Shorter DNA fragments
Weight
Absorbent paper
Nitrocellulose filter
Gel
Wick
Buffer
Step 6
Hybridize filter with radioactively labeled probe
Radioactive probe solution
Step 7
Expose filter to X-ray film. Resulting autoradiograph shows hybridized DNA fragments

1

2

: probe



Electrophoresis Southern blot