Molecular Biology

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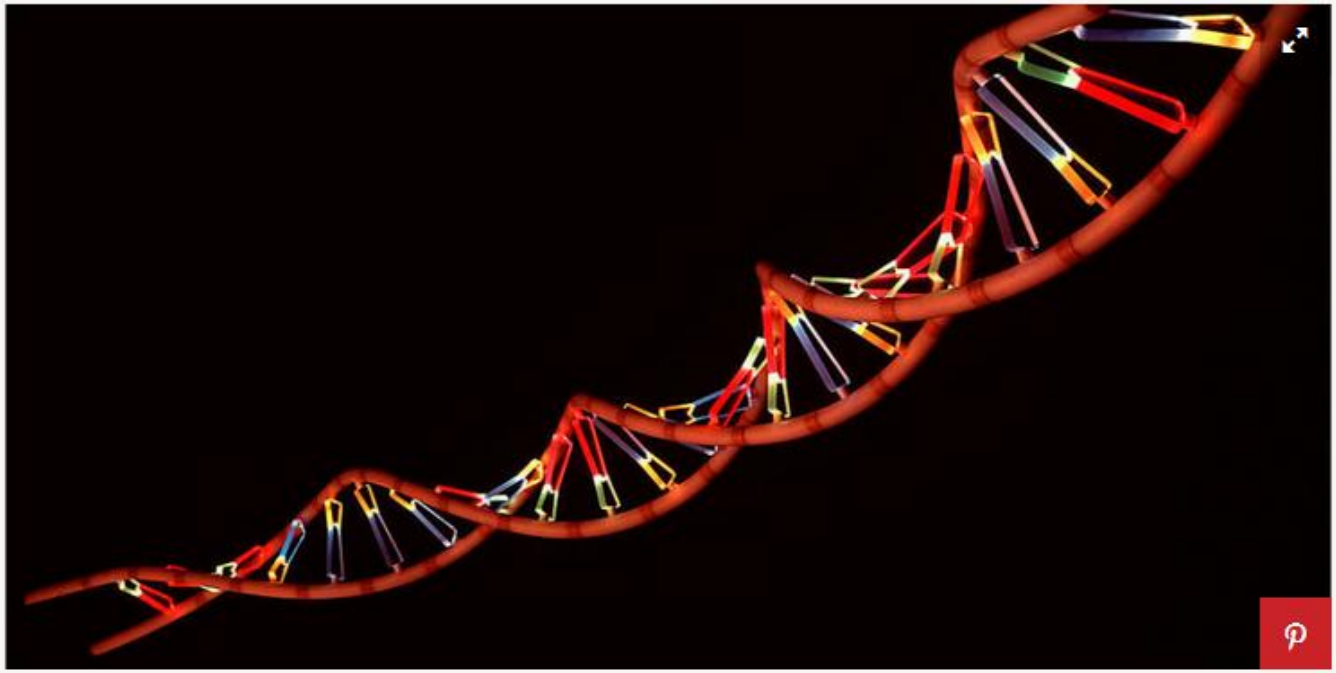
Second semester, 2016-2017

الأخبار > علوم وتكنولوجيا

العلماء يثبتون إمكانية بناء حاسوب "دي أن أي"

Scientists Build New Computer Made of DNA

The computer can copy itself many times over, making calculations much faster.

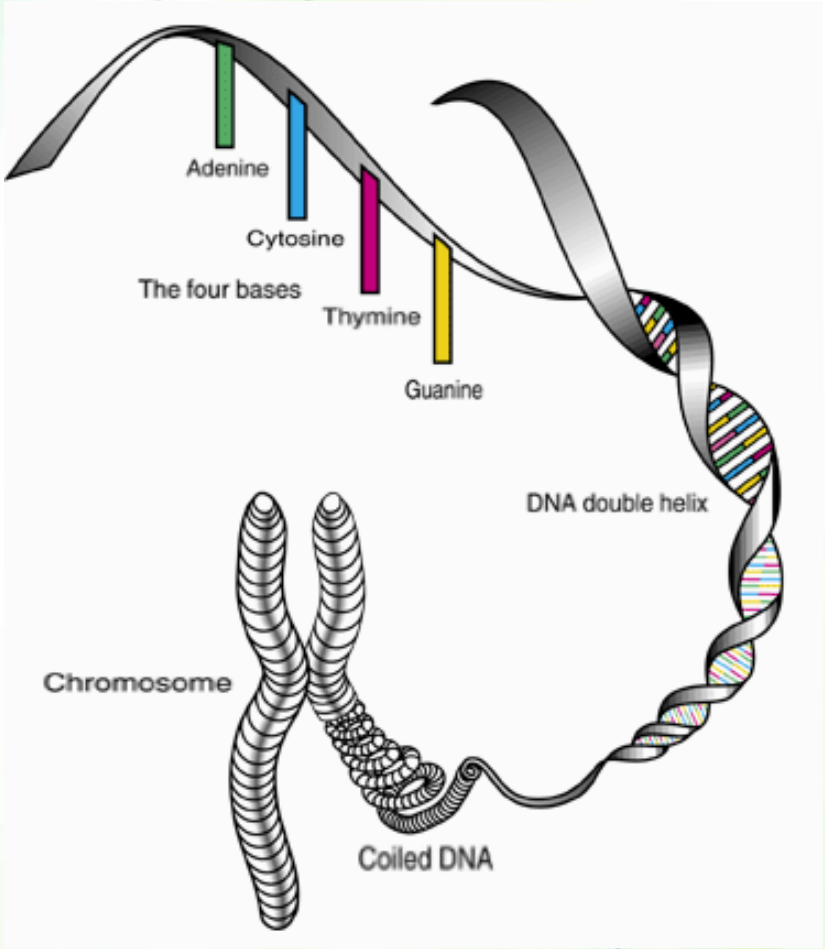


Resources

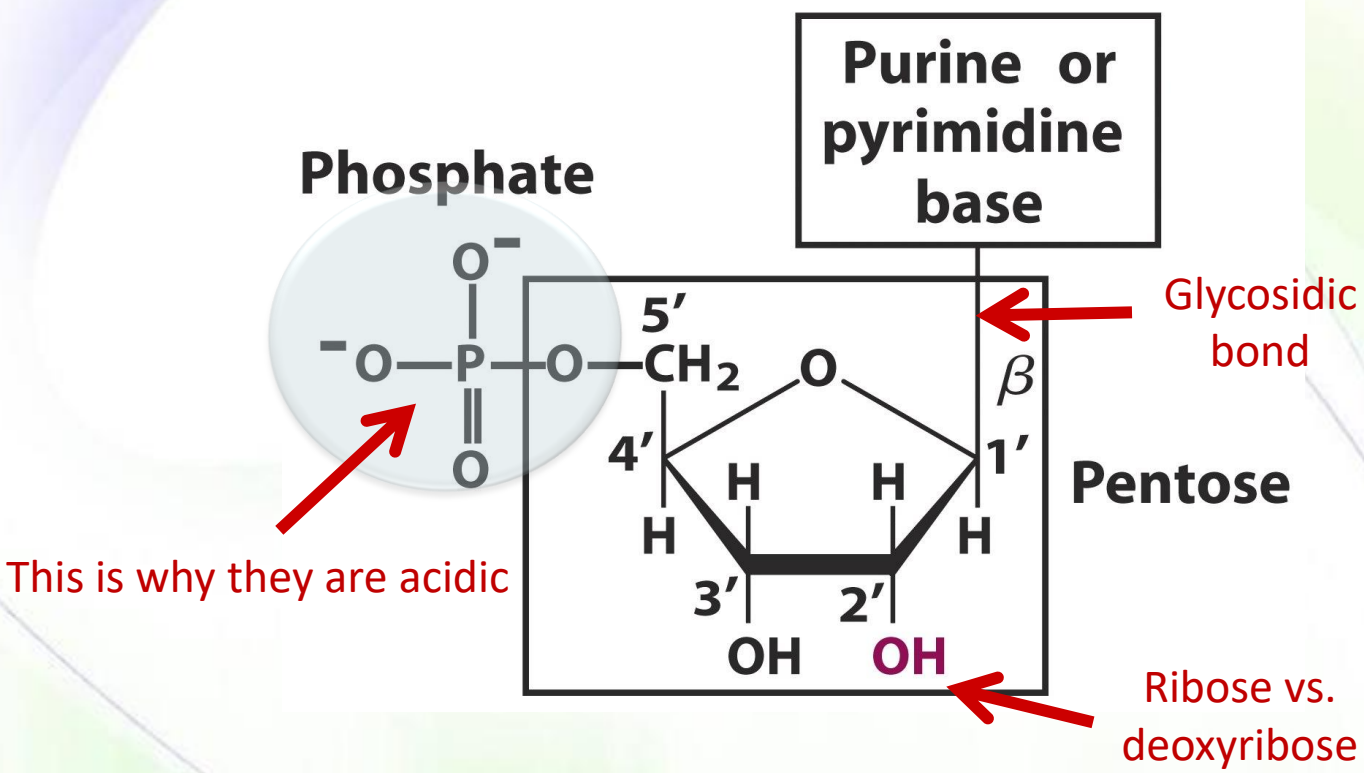
- This lecture
- Cooper, pp. 49-52, 118-119, 130

Nucleic acids

- The primary structure of nucleic acids is the order of bases in the polynucleotide sequence.
- The secondary structure is the three-dimensional conformation of the backbone.
- The tertiary structure is specifically the supercoiling of the molecule.

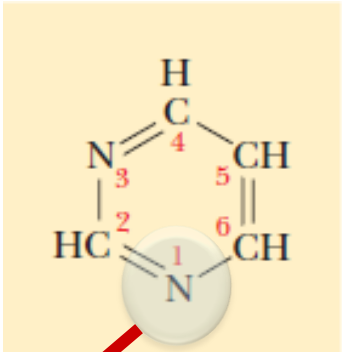


Chemical composition and bonds



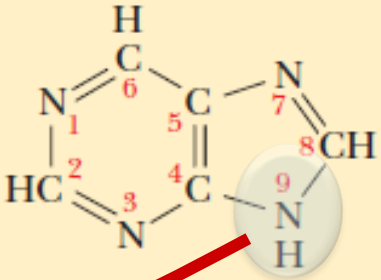
- Positively charged ions (Na+ or Mg2+) and peptides with positively charged side chains can associate with DNA
- Eukaryotic DNA, for example, is complexed with histones, which are positively charged proteins, in the cell nucleus.

Nitrogenous bases

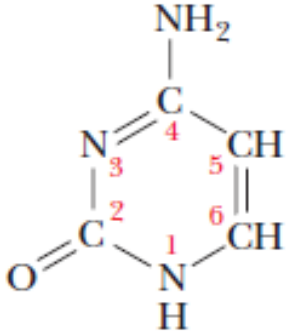


Pyrimidine

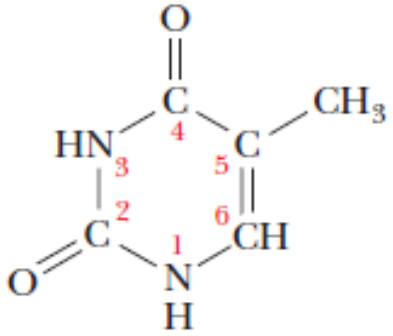
Glycosidic bond



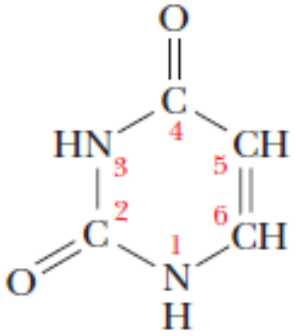
Purine



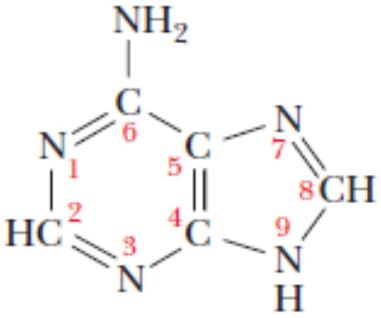
Cytosine
(in DNA & RNA)



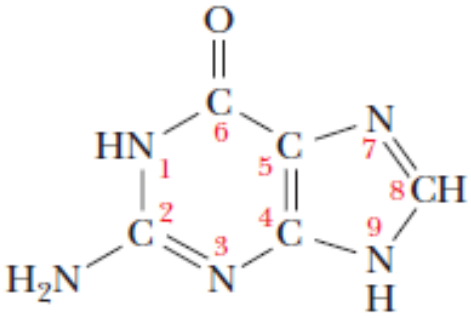
Thymine
(in DNA & some RNA)



Uracil
(in RNA)

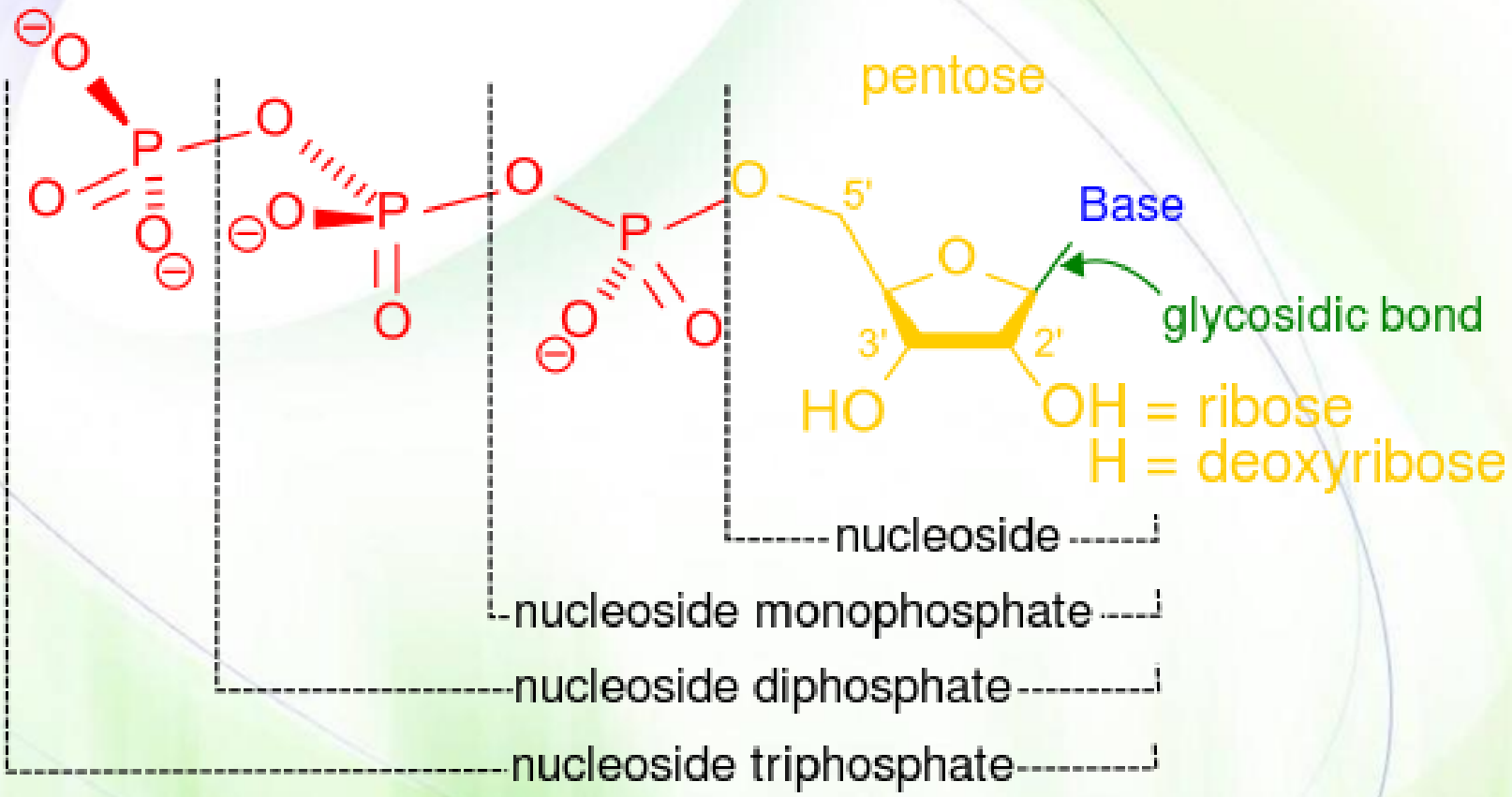


Adenine
(in DNA & RNA)

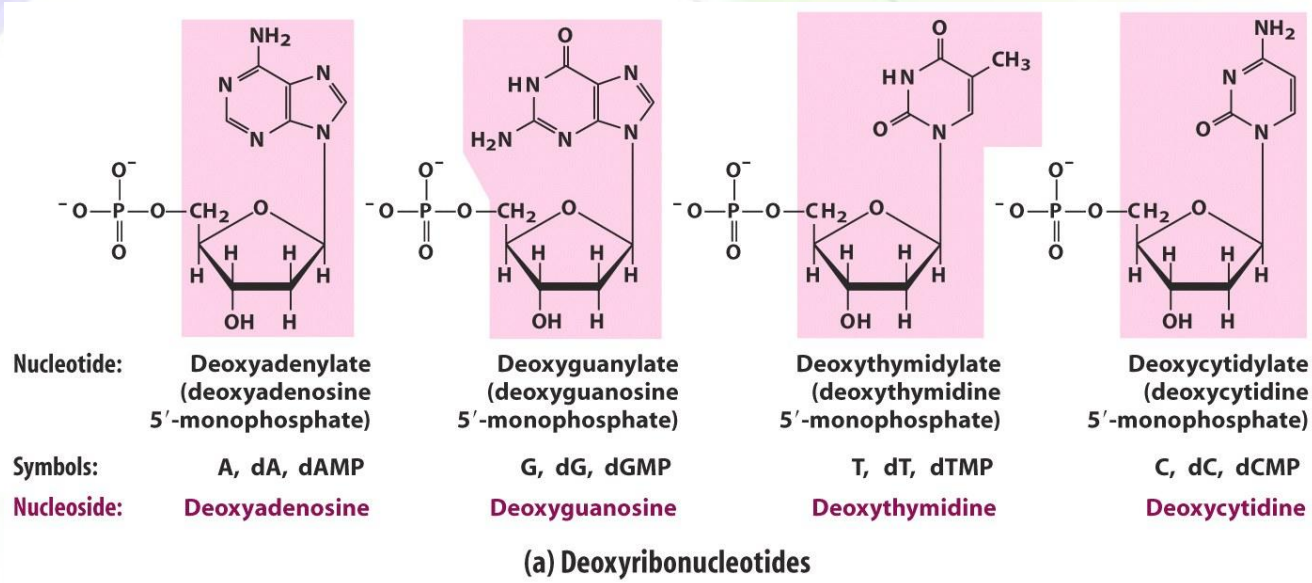


Guanine
(in DNA & RNA)

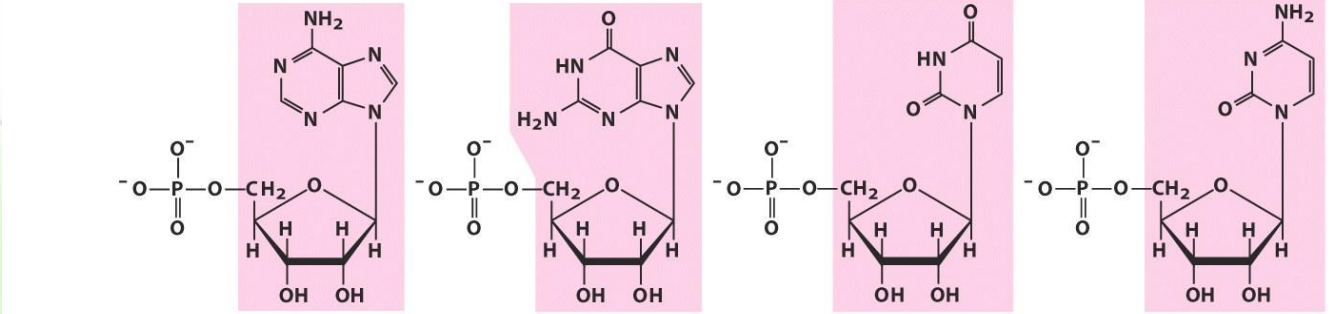
Nucleotides vs. Nucleosides



Nucleotides vs. Nucleosides

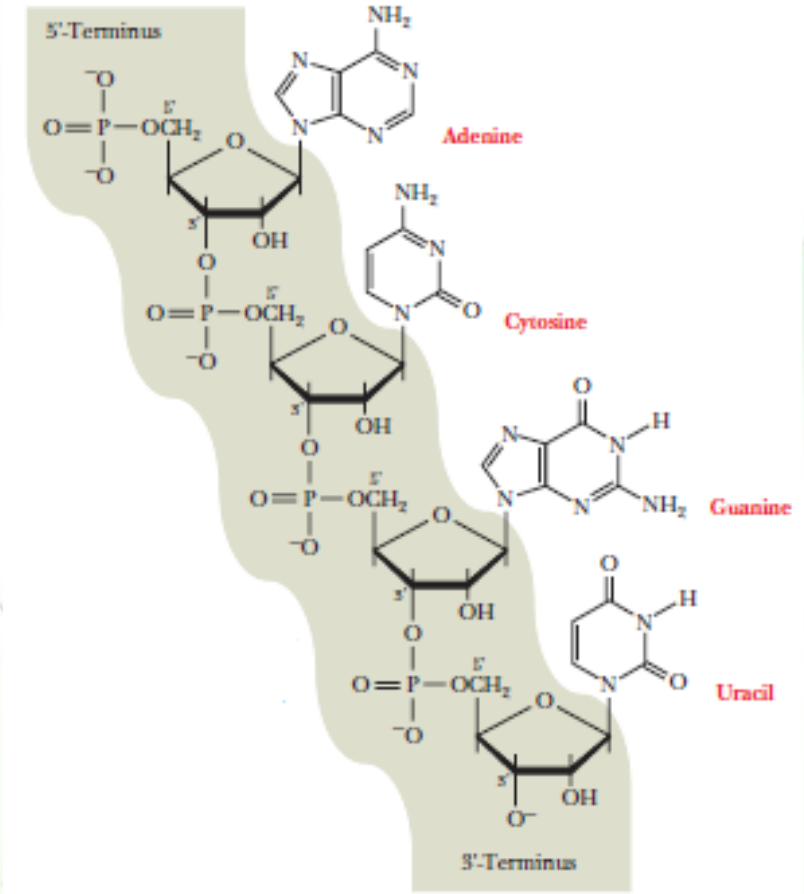


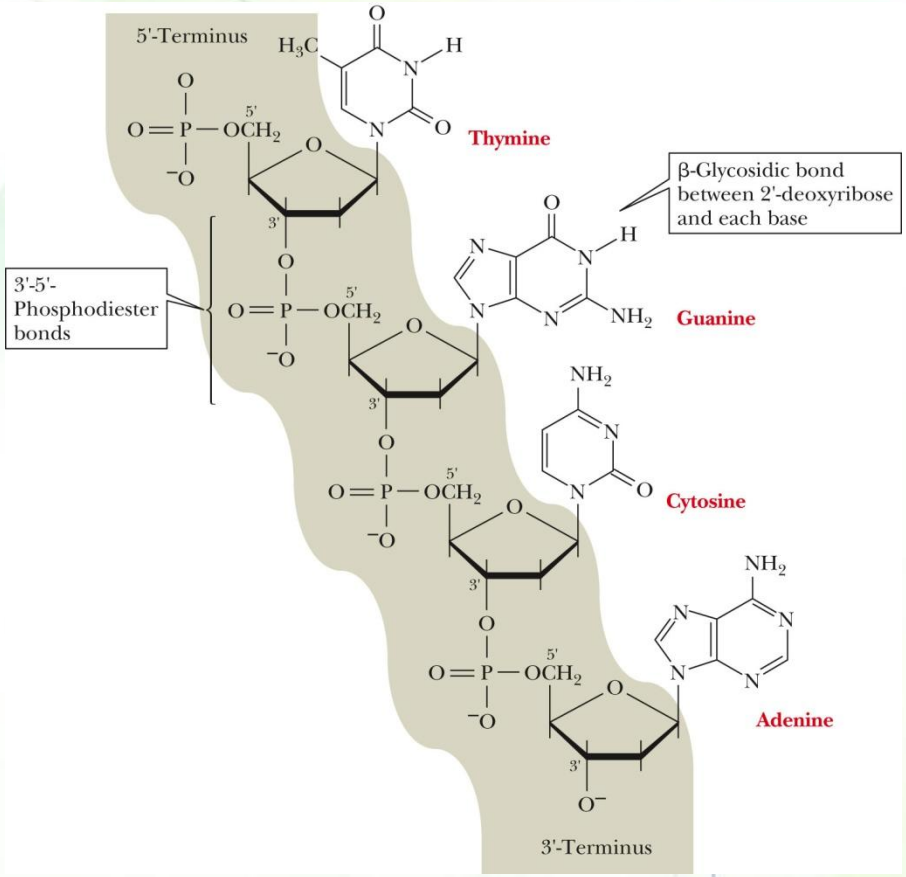
(a) Deoxyribonucleotides



(b) Ribonucleotides

Nucleic acid polymer

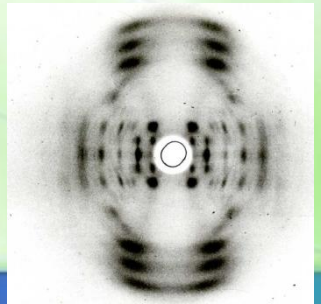


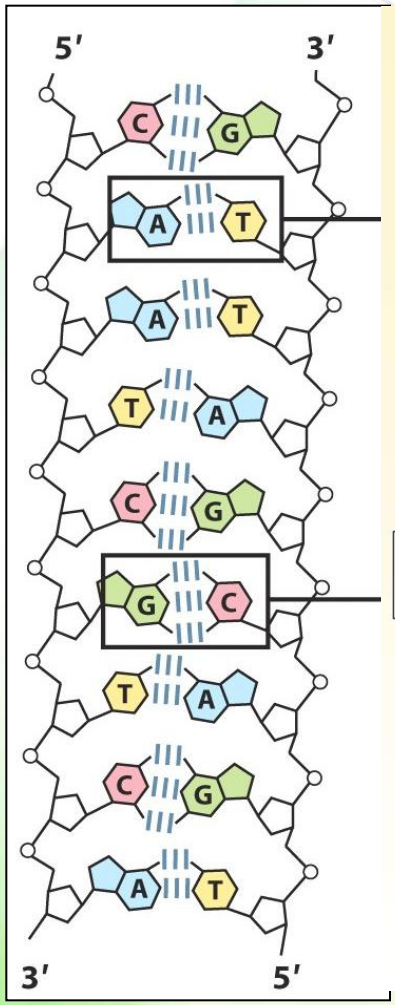


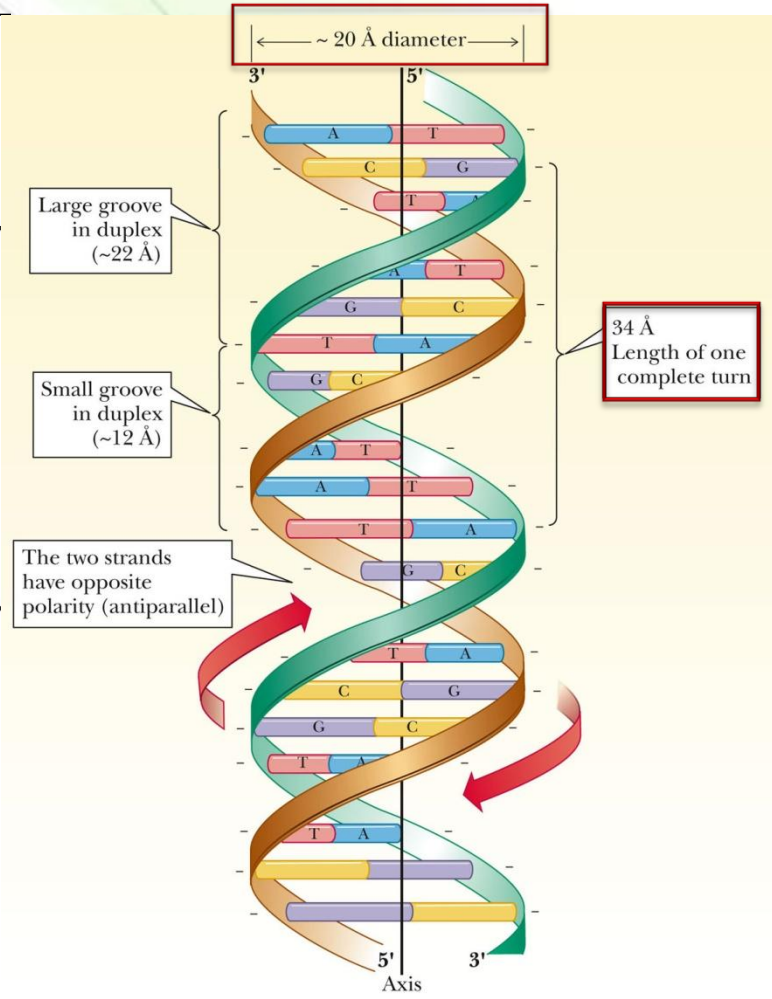
- A letter d can be added to indicate a deoxyribonucleotide residue.
  - for example, dG is substituted for G.
  - The deoxy analogue of a ribooligonucleotide would be d(GACAT).

DNA structure

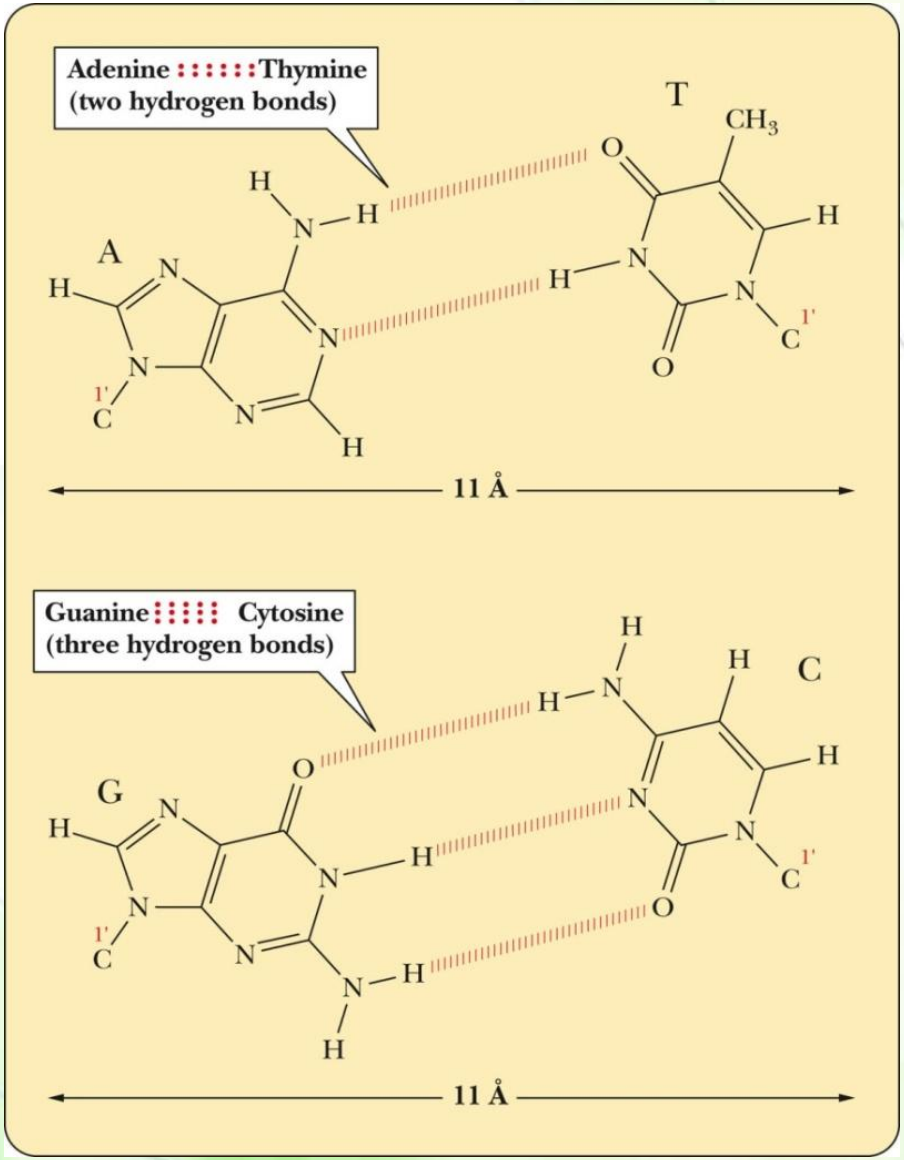
- Specific base-pairing
  - A = T; G = C; Pur = pyr
- Complementary
- A double helix
- Backbone vs. side chains
- Antiparallel
- Stable
- Flexible
- Groovings
- Stability vs. flexibility





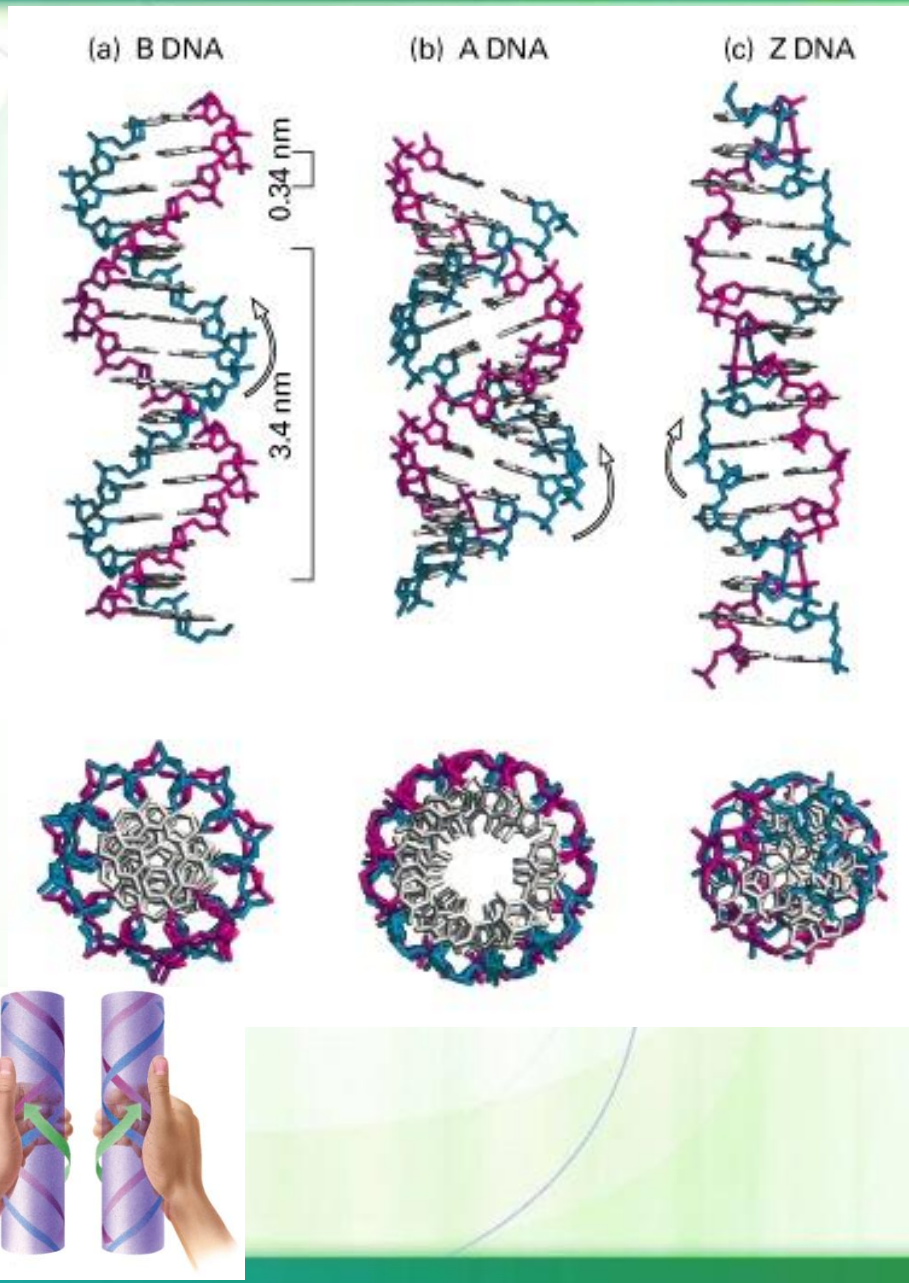


Base pairing



DNA forms

- **B-DNA**
  - The principal form of DNA.
  - Right-handed; 10 bp/turn
  - Base pairs are perpendicular.
- **A-DNA**
  - 11 base pairs per turn
  - Base pairs lie at an angle
  - Right-handed.
  - Wider than B-DNA
- **Z-DNA**
  - Left-handed
  - Occurs when alternating purine–pyrimidine and sequences with methylated C
  - Narrower than B-DNA



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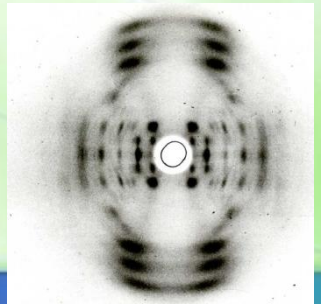
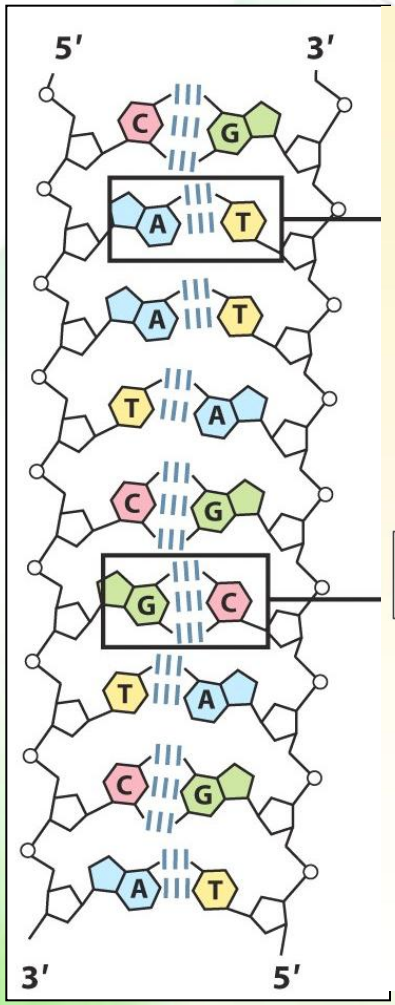
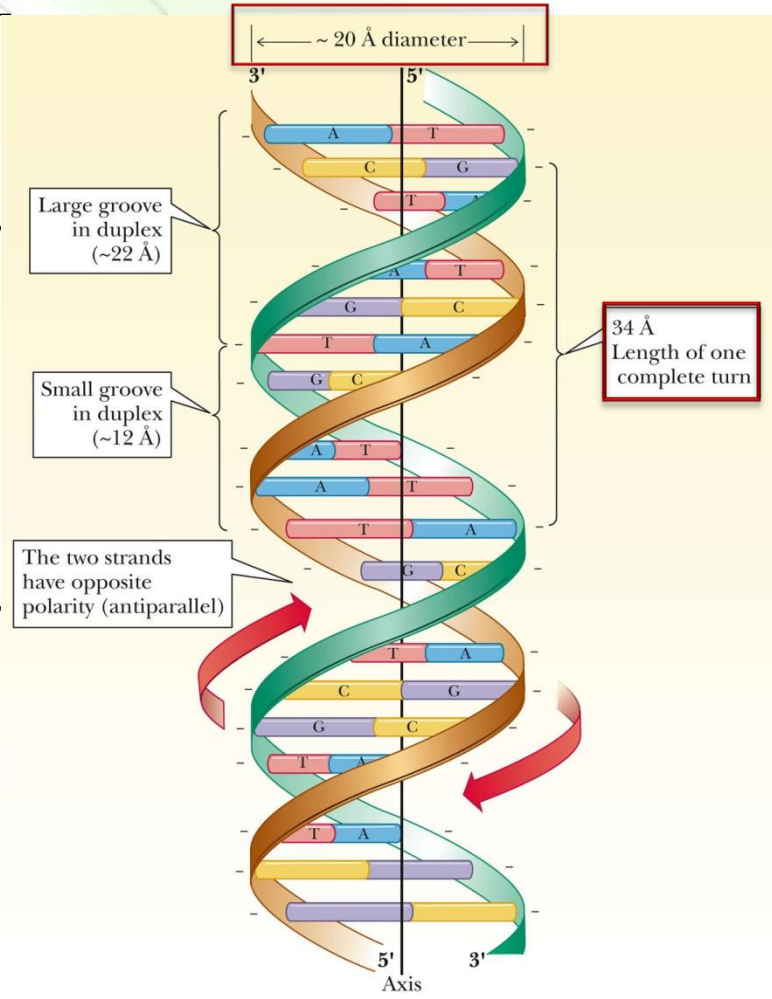
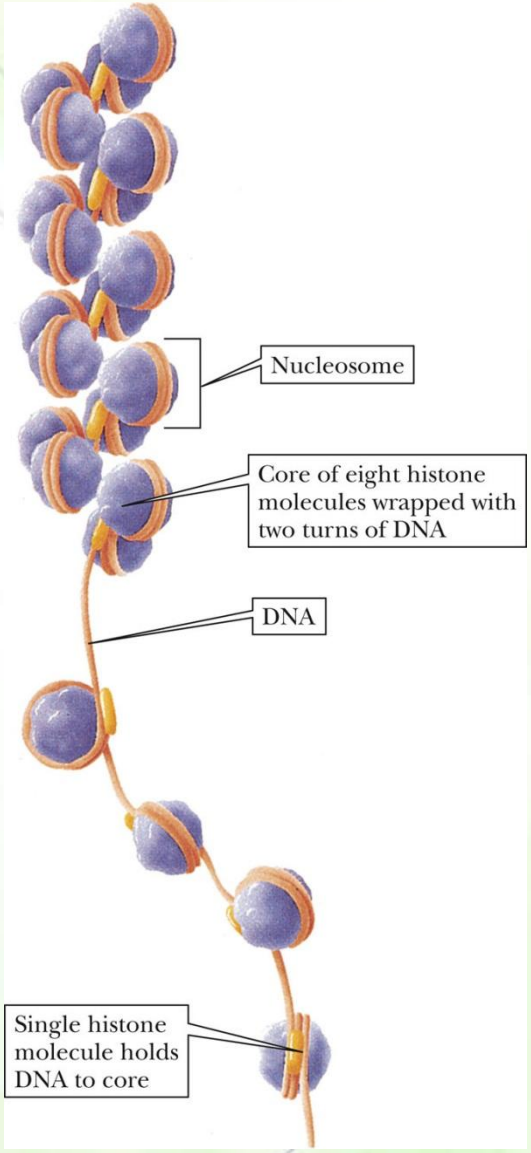






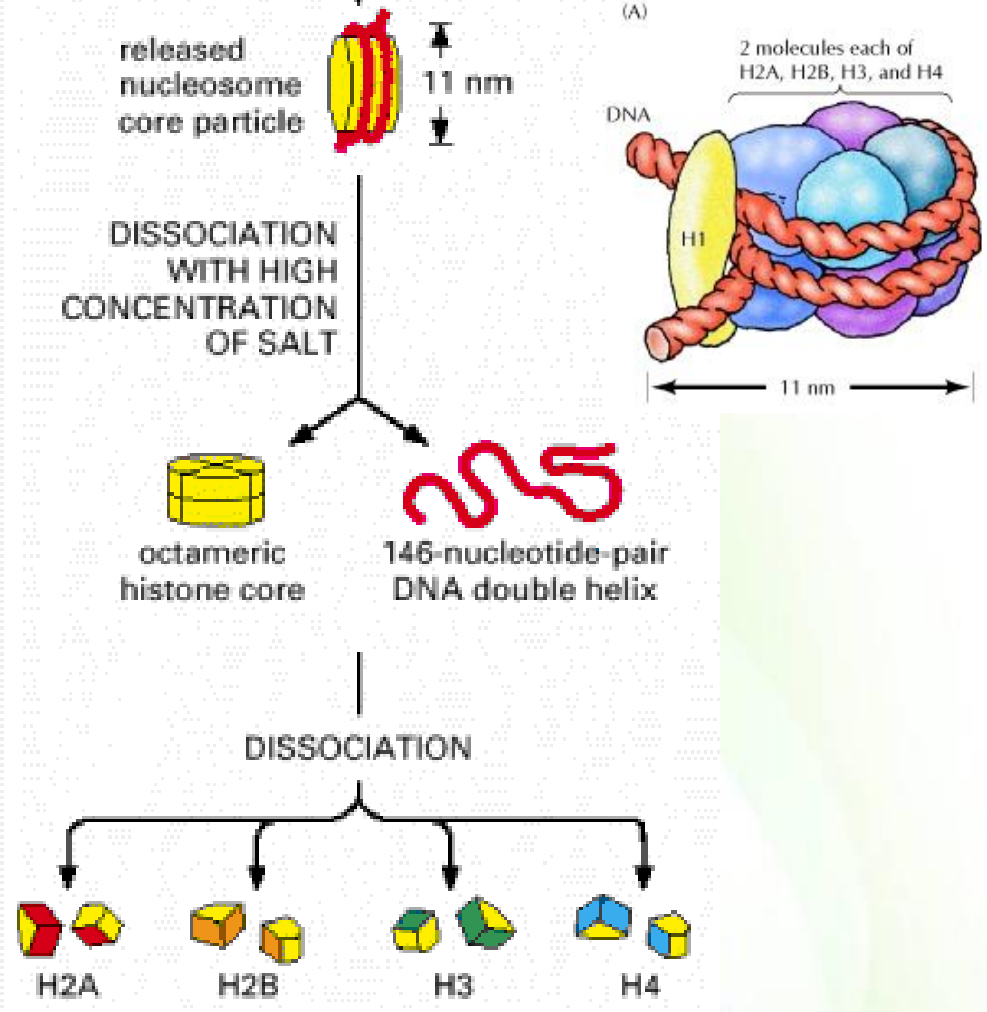
In eukaryotes…

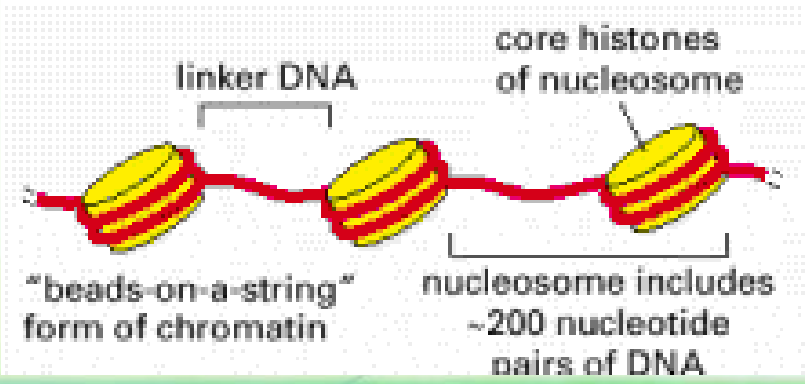
- In eukaryotes, DNA is coiled to package the large DNA and regulate gene activity.
- Eukaryotic DNA is complexed with a number of proteins, principally histones, which are surrounded by DNA.
- Chromatin = DNA molecule + proteins.



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.





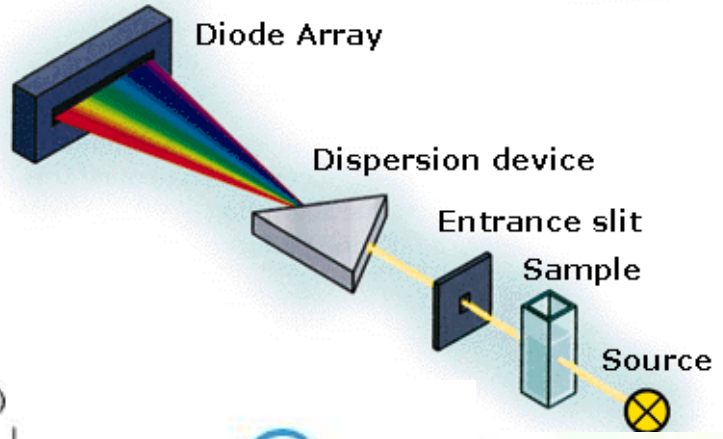
Light absorbance of nucleic acids

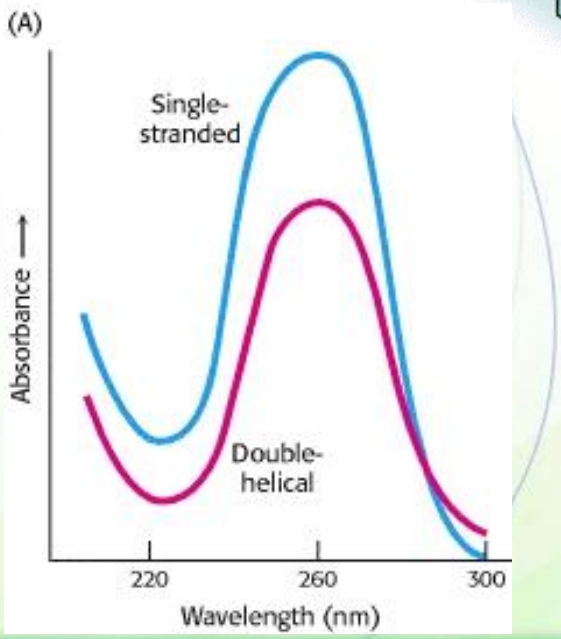
- Aromatic pyrimidines and purines can absorb UV light
- The peak absorbance is at 260 nm wavelength

- 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:

- Stacked bases, vs. unstacked bases





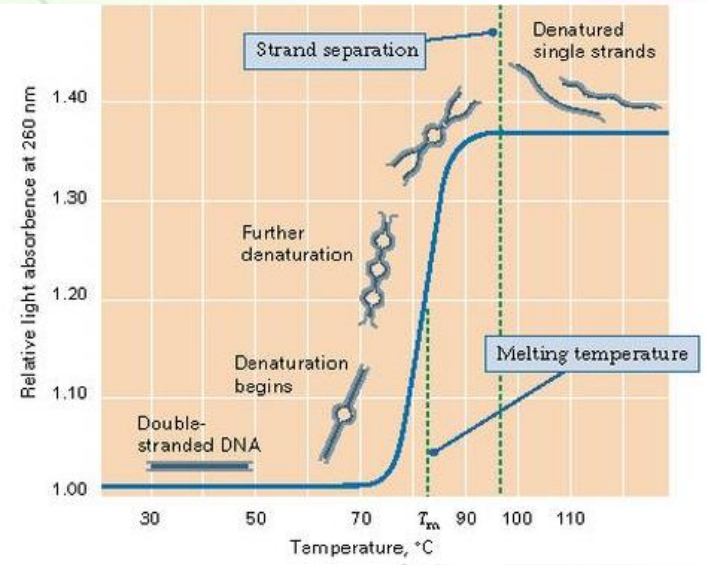
What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

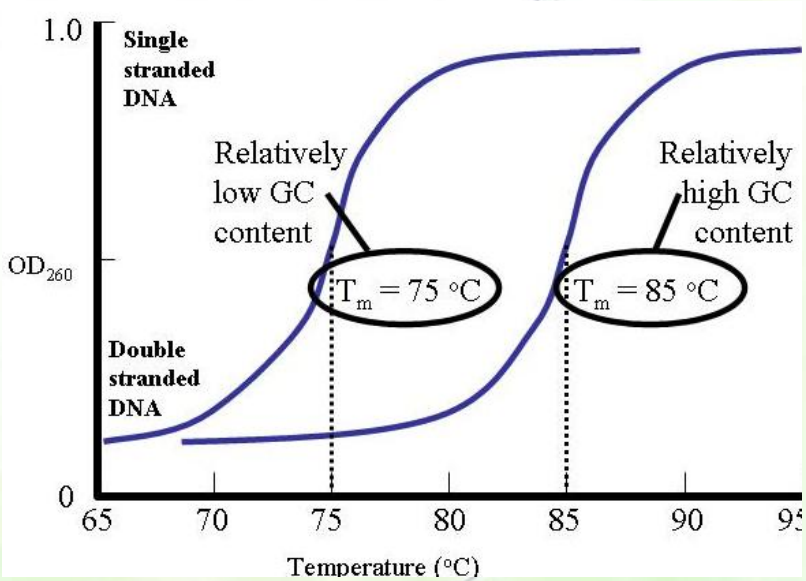
DNA concentration = 0.1 x 10 x 50 µg/ml

= 50 µg /ml

Observation of denaturation

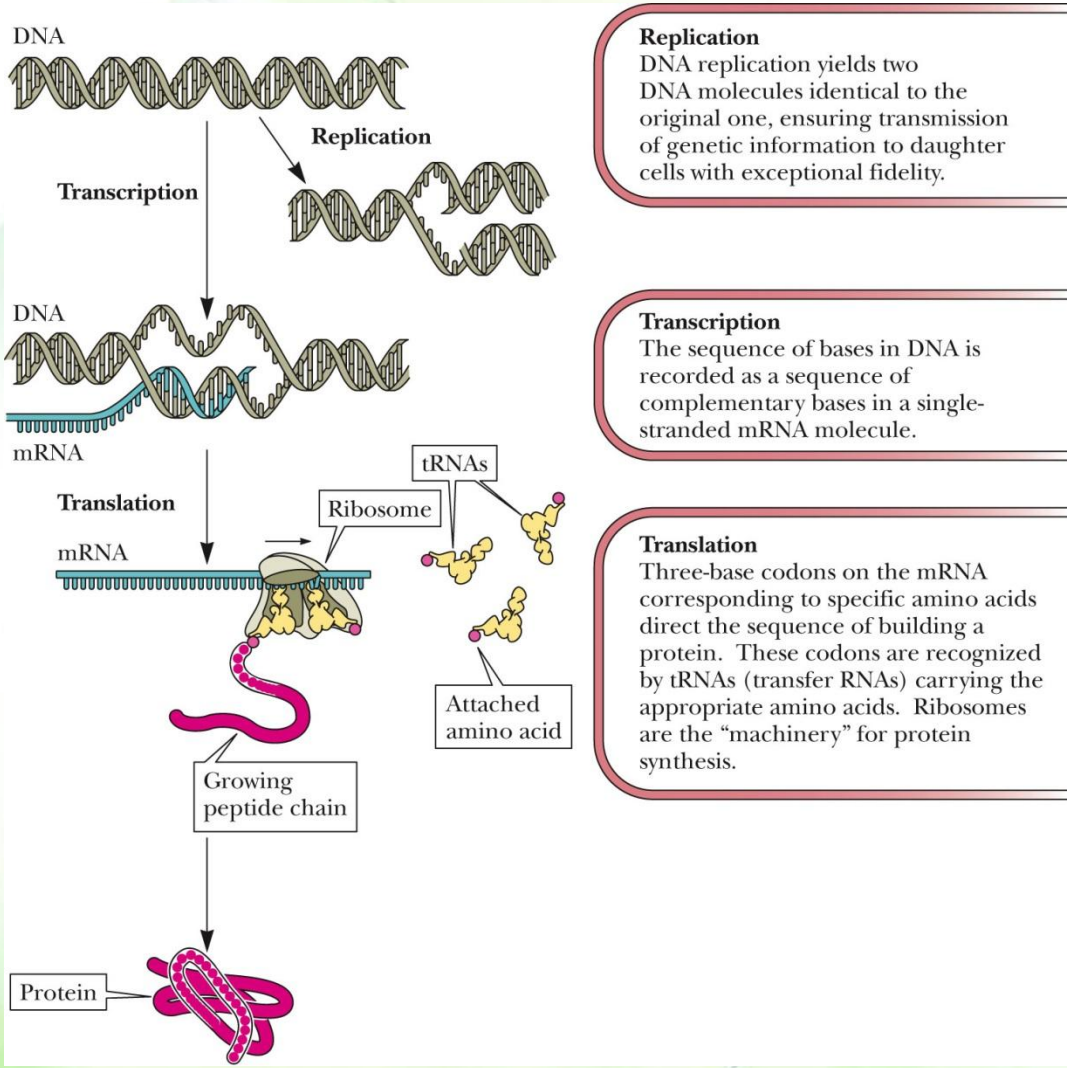
- The transition temperature, or melting temperature (Tm).
- Factors influencing Tm
  - G·C pairs
    - Hydrogen bonds
    - Base stacking
  - pH
  - Salt and ion concentration
  - Destabilizing agents (alkaline solutions, formamide, urea)





Central dogma of biology

- Genetic information must be preserved via DNA replication.
- Information must be translated into action makers (proteins) via transcription and translation.
- RNA Sequence is dictated by DNA sequence.



RNA

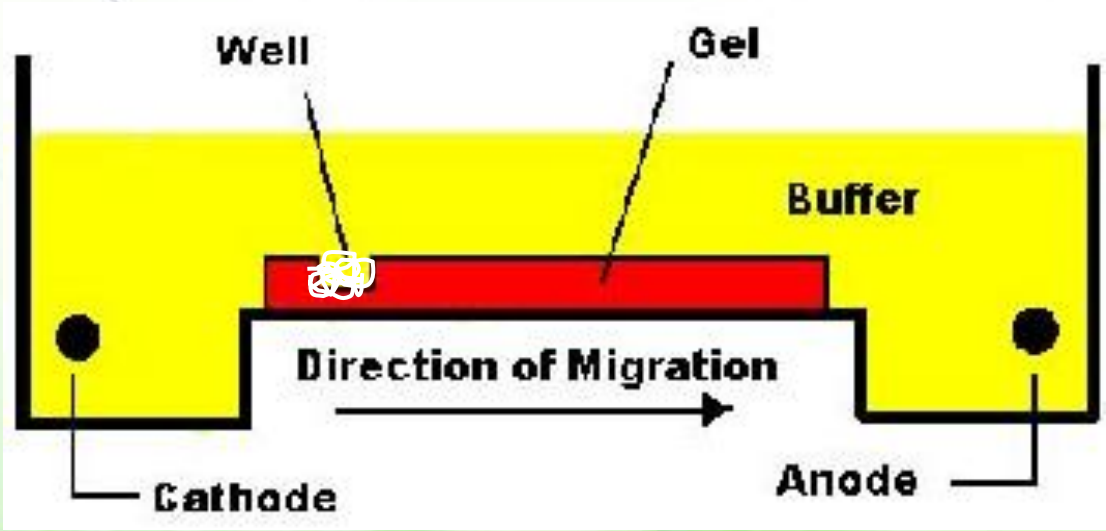
- Consist of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3’-OH of one pentose and the 5’-OH of the next
- The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA)
- The pyrimidine bases are uracil and cytosine (they are thymine and cytosine in DNA)
- In general,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

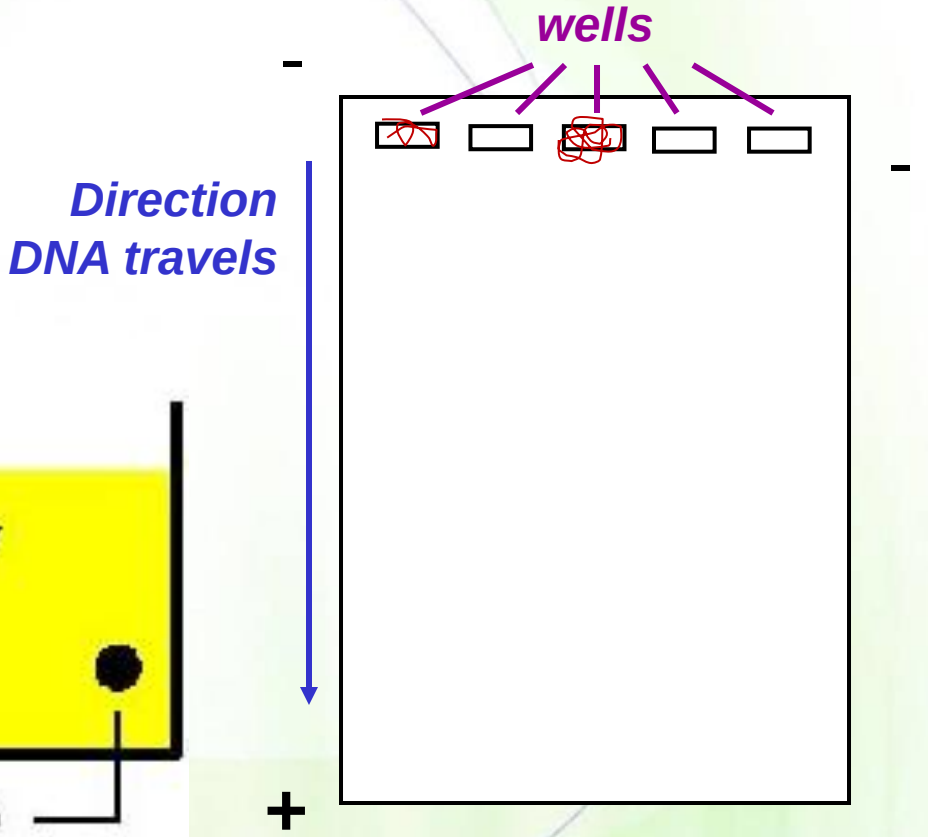
Types of RNA

The Roles of Different Kinds of RNA		
RNA Type	**Size**	**Function**
Transfer RNA	Small	Transports amino acids to site of protein synthesis
Ribosomal RNA	Several kinds—variable in size	Combines with proteins to form ribosomes, the site of protein synthesis
Messenger RNA	Variable	Directs amino acid sequence of proteins
Small nuclear RNA	Small	Processes initial mRNA to its mature form in eukaryotes
Small interfering RNA	Small	Affects gene expression; used by scientists to knock out a gene being studied
Micro RNA	Small	Affects gene expression; important in growth and development

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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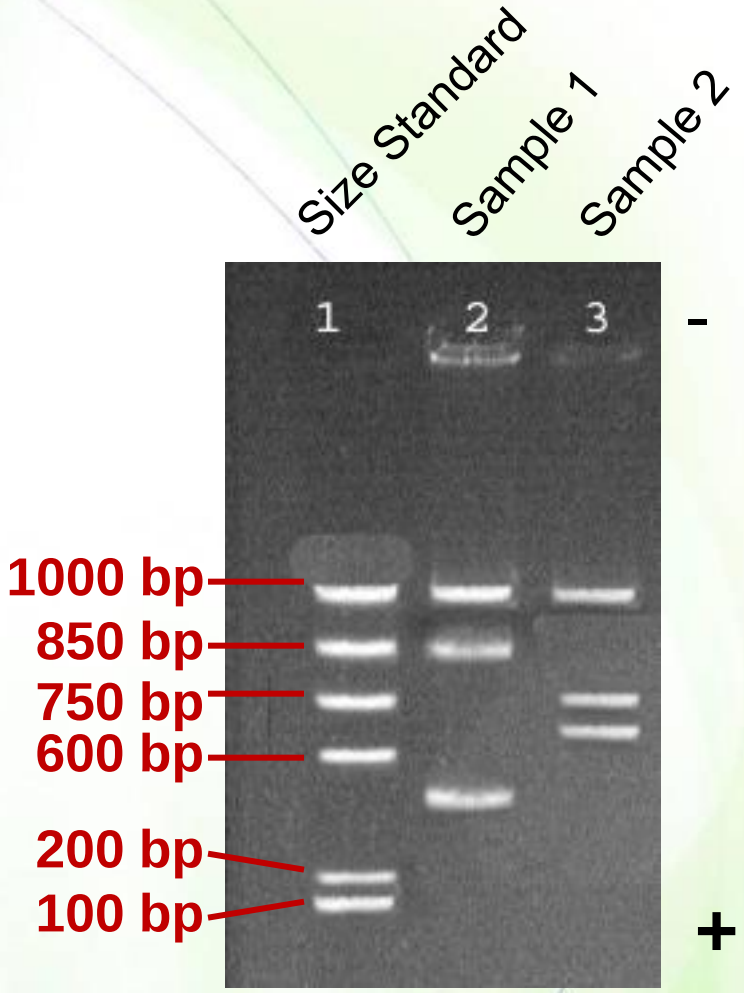
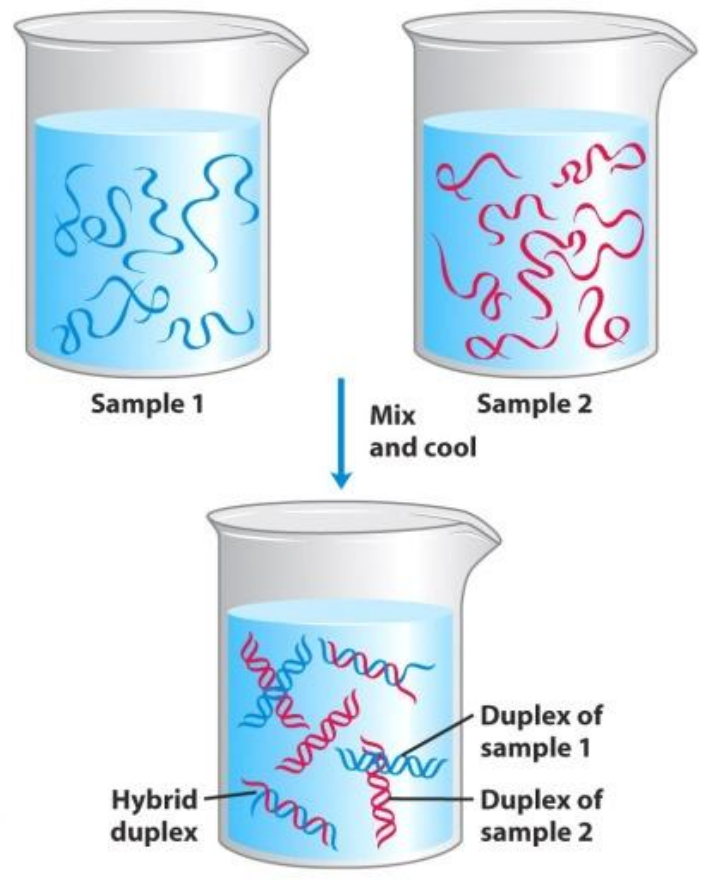
Detection

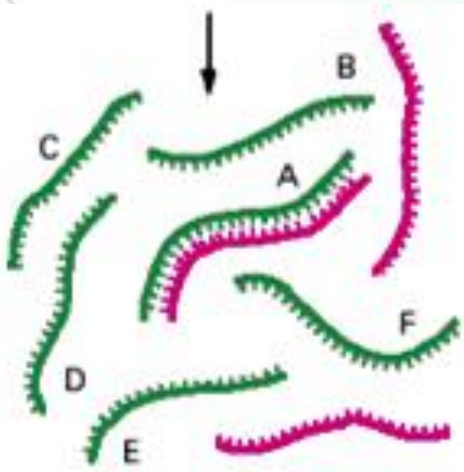
- The DNA molecules of different lengths will run as "bands".
- Each contains thousands or millions of copies of the DNA of the same length that can be of same or different type (not one DNA molecule).
- DNA is stained (that is, colored) with a dye (ethidium bromide) or radioactively labeled (^{32}P).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



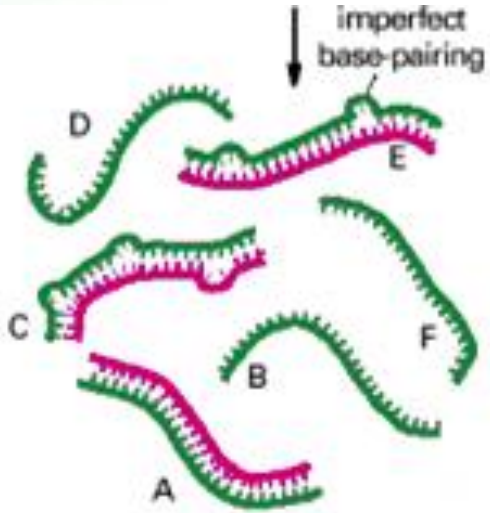
Hybridization

- 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



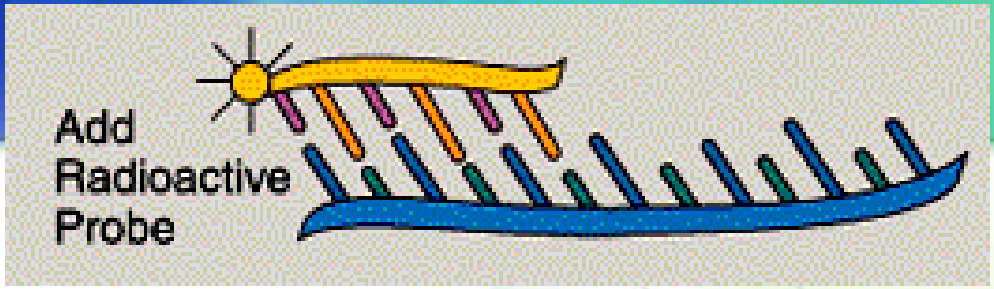
A, C, and E all form stable double helices

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



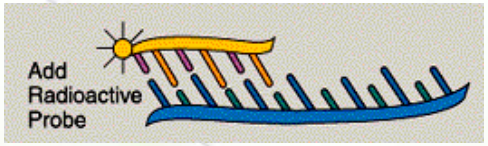
- A probes is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence

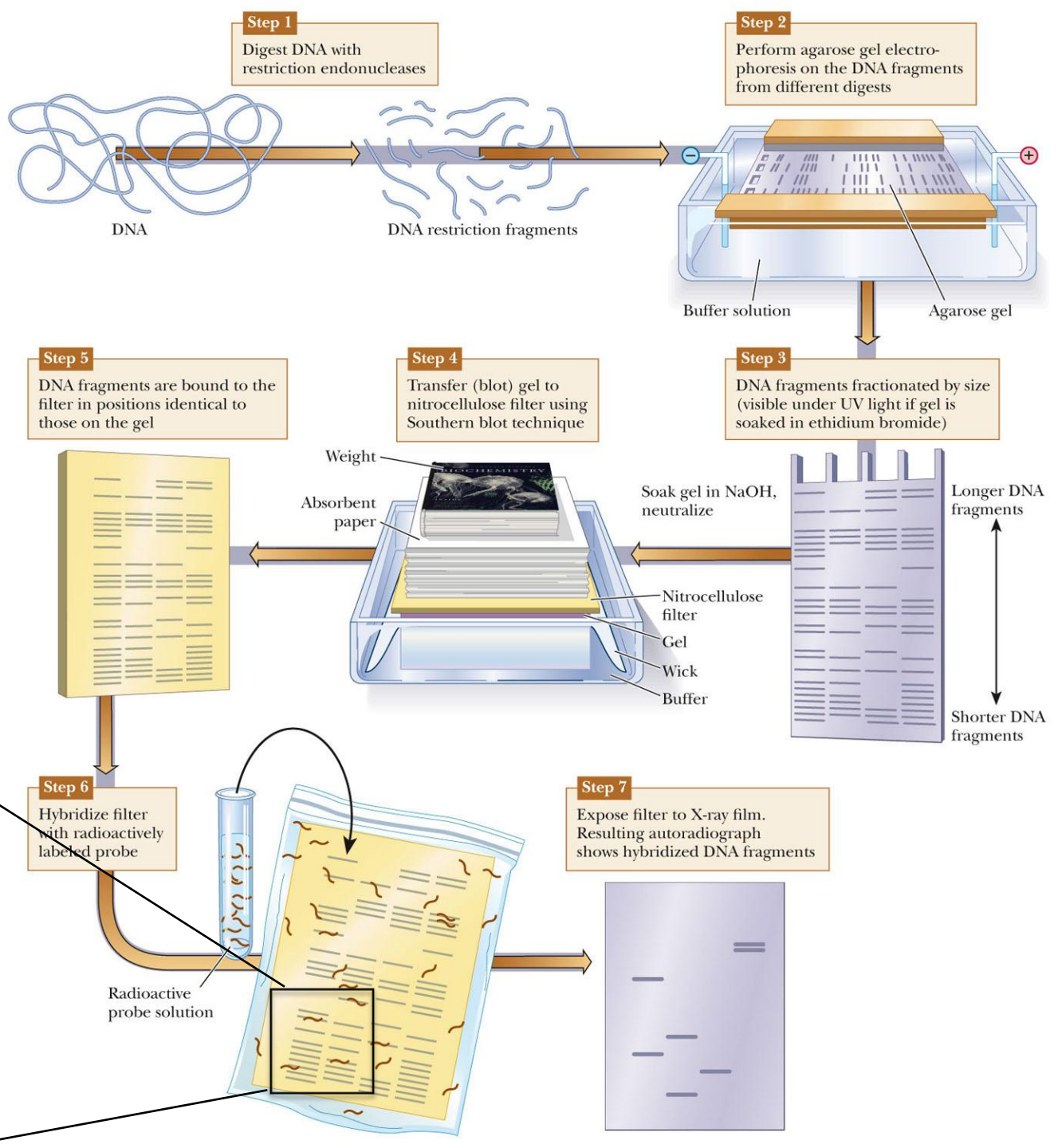
- Hybridization reactions use labeled DNA probes to detect larger DNA fragments

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
Weight
Absorbent paper
Soak gel in NaOH, neutralize
Nitrocellulose filter
Gel
Wick
Buffer
Longer DNA fragments
Shorter DNA fragments
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