Genetic Information: DNA Structure and Function

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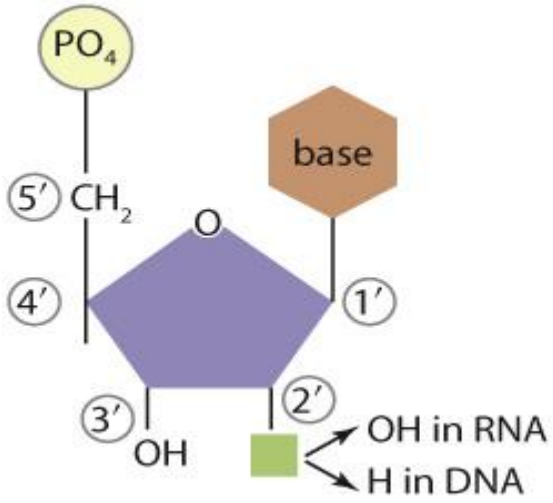
Determining the Chemical Composition and Structure of DNA

- DNA was discovered in 1869 by Fredrich Miescher. By isolating the nuclei of white blood cells, he extracted an acidic molecule he called *nuclein*.
- Nucleotide contains :

nitrogenous base

pentose sugar

phosphate group.



What is DNA?

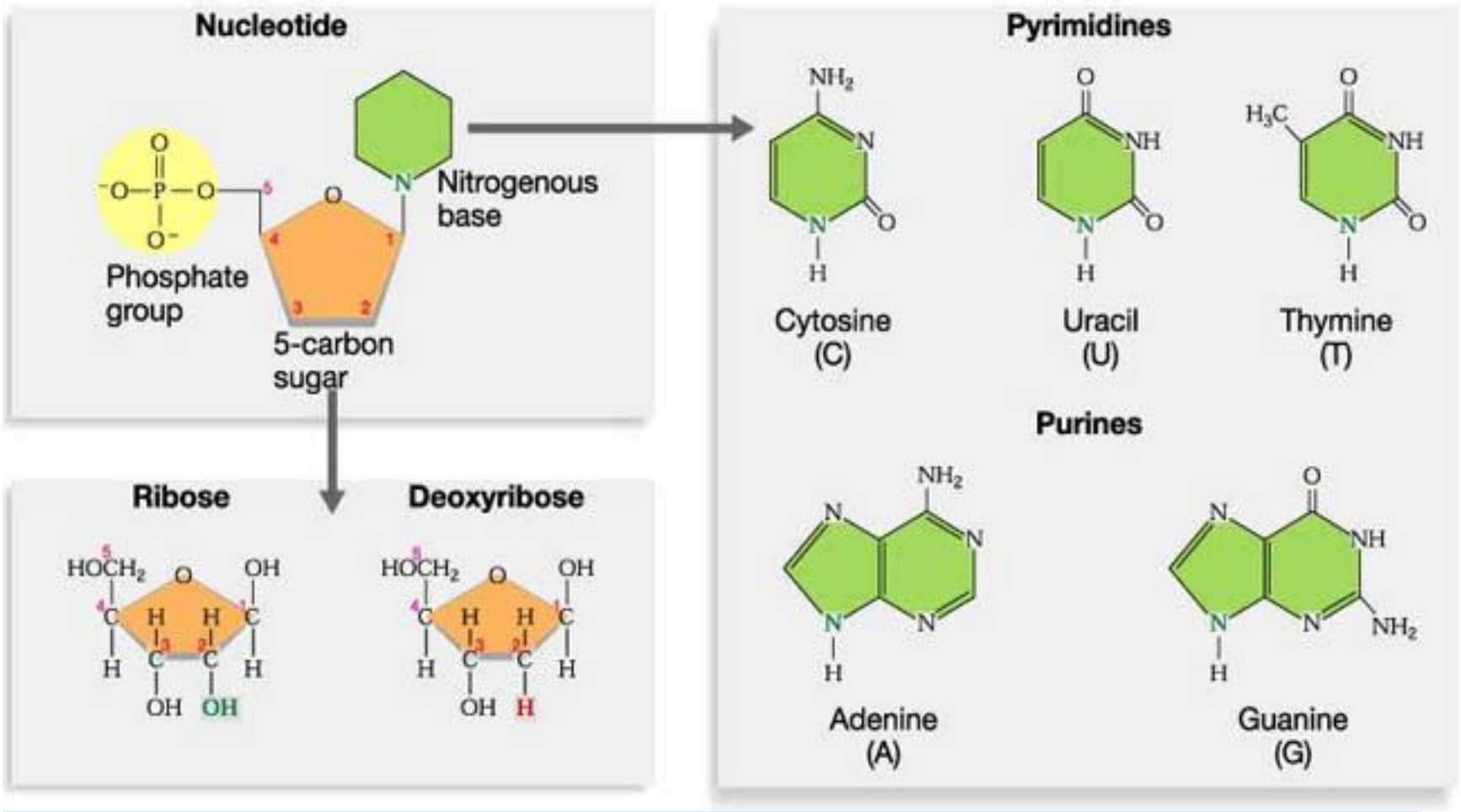
- DNA is a **Nucleic Acid**
- Each nucleotide consists of
  - Deoxyribose (5-carbon sugar)
  - Phosphate group
  - A nitrogen-containing base
- Four bases
  - Adenine, Guanine, Thymine, Cytosine

✖There are two kinds of nitrogenous bases

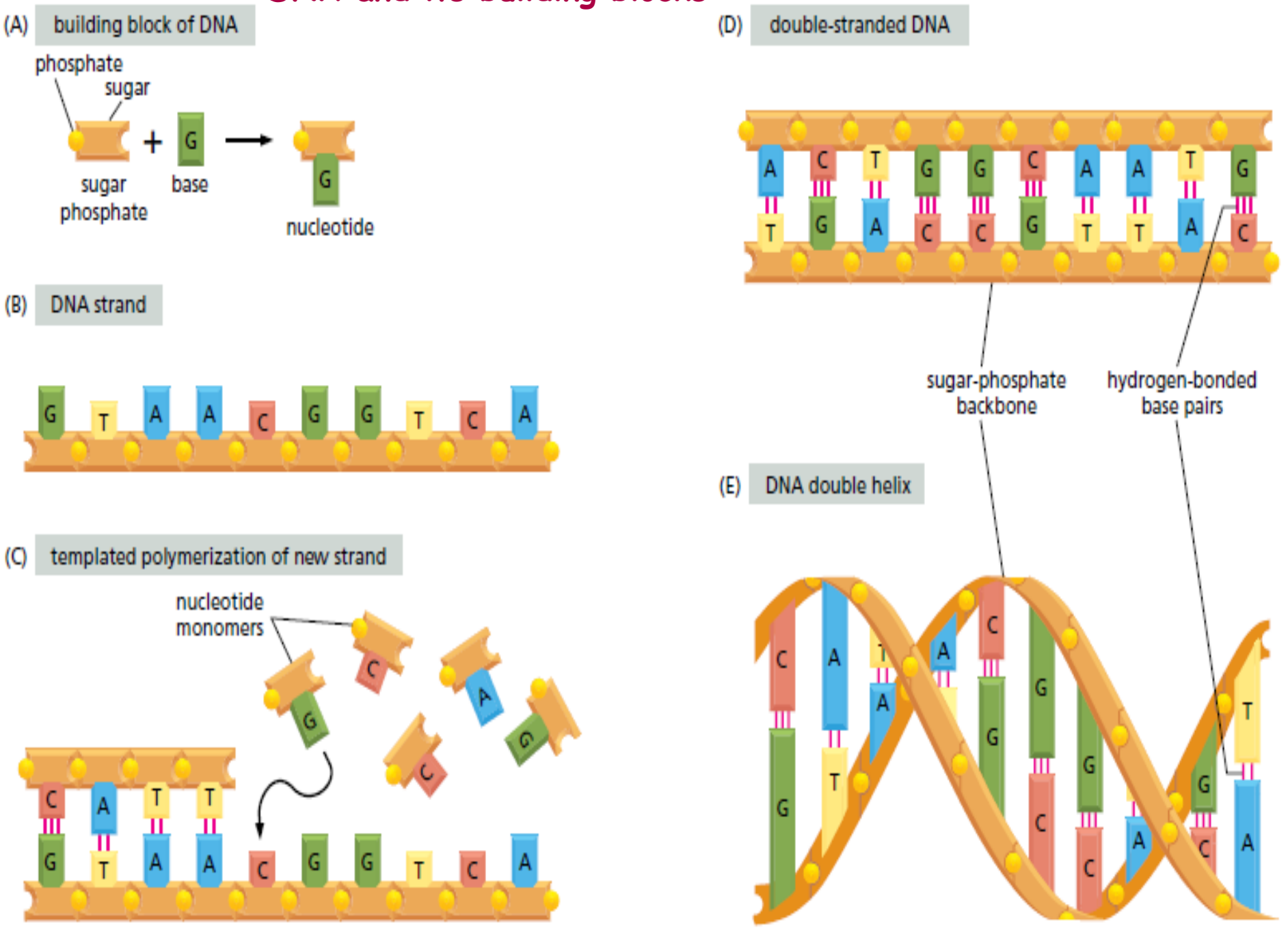
+Nine-member double ring purines (A,G)

+Six member single ring pyrimidines (C,T,U)

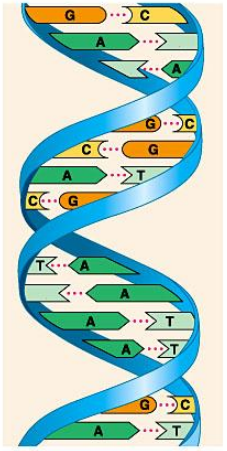
Nucleotide Structure

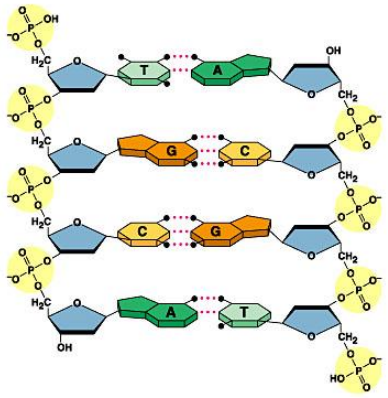


DNA and its building blocks



Base-pairing rule





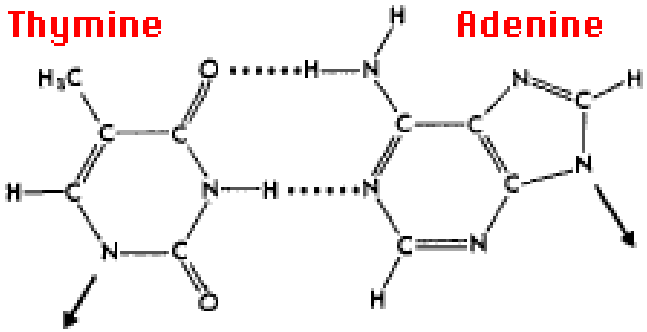
The four bases of DNA are:

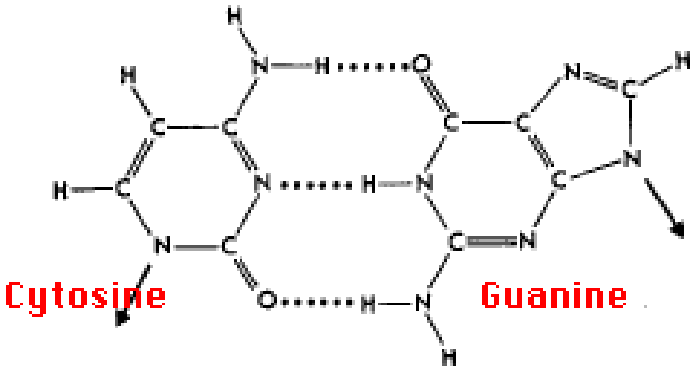
Adenine (A) Guanine (G) Thymine (T) Cytosine (C)

Adenine always hydrogen bonds with Thymine (A-T)

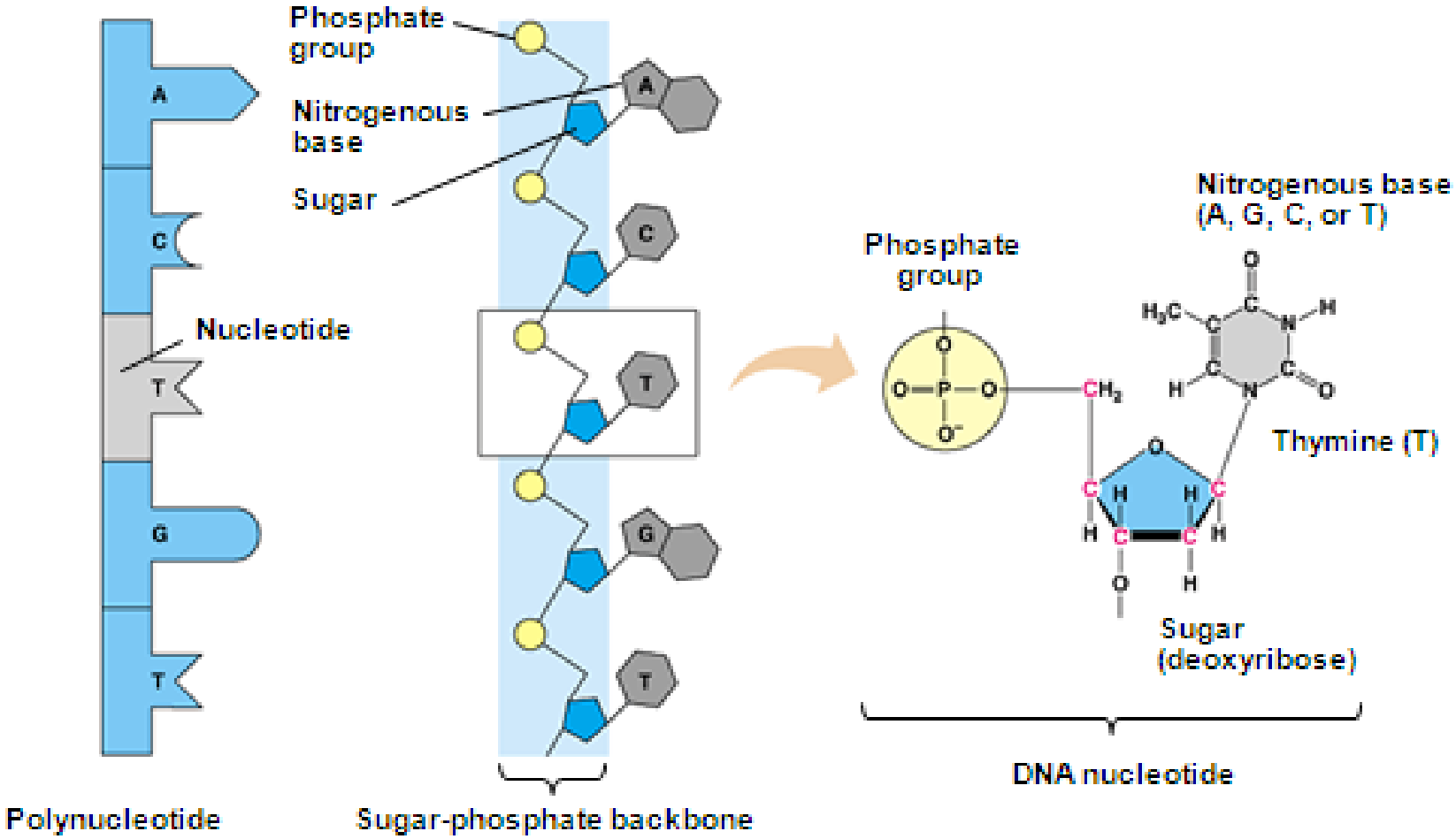
Guanine always hydrogen bonds with Cytosine (G-C)

These bonding patterns are called **base pairings (bp)**





- DNA is a nucleic acid, made of long chains of nucleotides

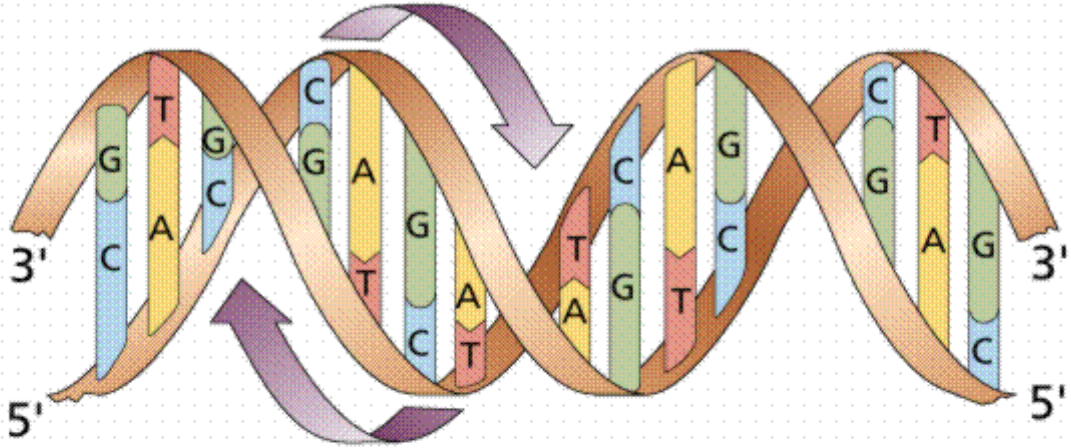


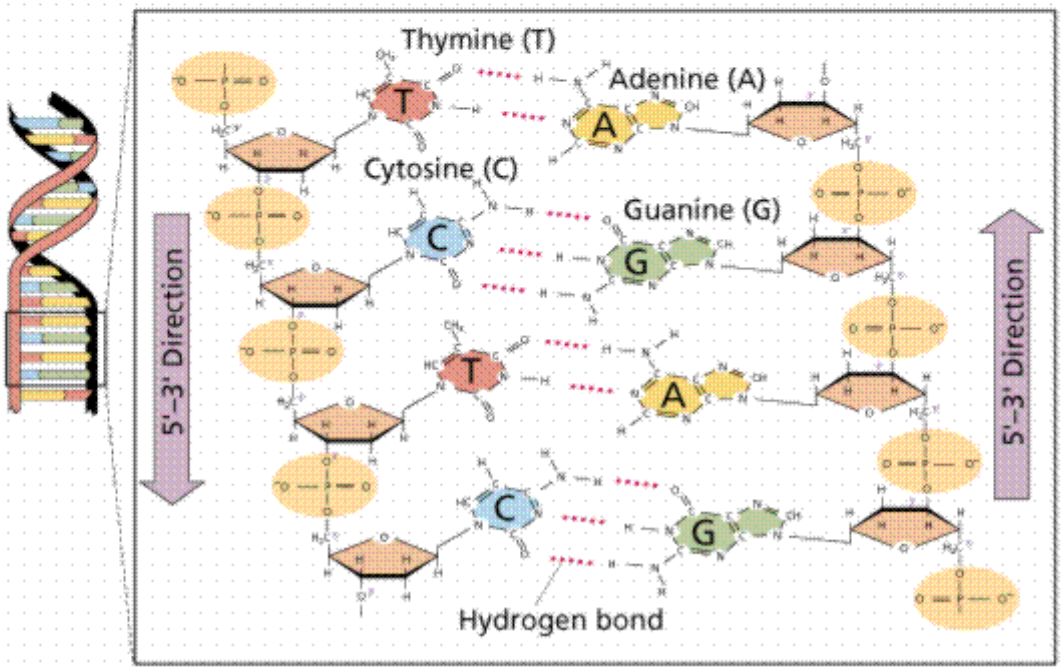
- The pattern of base pairing is the mechanism by which DNA holds information.
- Humans have a > 6 billion of these base pairings
- Less than 5% of our DNA actually forms genes
- There about 30,000 genes encoded in our DNA, nearly half of these genes either have yet to be discovered or their function is unknown
- DNA is written out like this:
- CTCGAGGGGCCTAGACATTGCCCTCCAGAGAGAGCACCCAACA CCCTCCAGGCTTGACCGGCCAGGGTGTCCCCTTCCTACCTTGG AGAGAGCAGCCCCAGGGCATCCTGCAGGGGGTGCTGGGACACC AGCTGGCCTTCAAGGTCTCTGCCTCCCTCCAGCCACCCCACTA CACGCTGCTGGGATCCTGGA

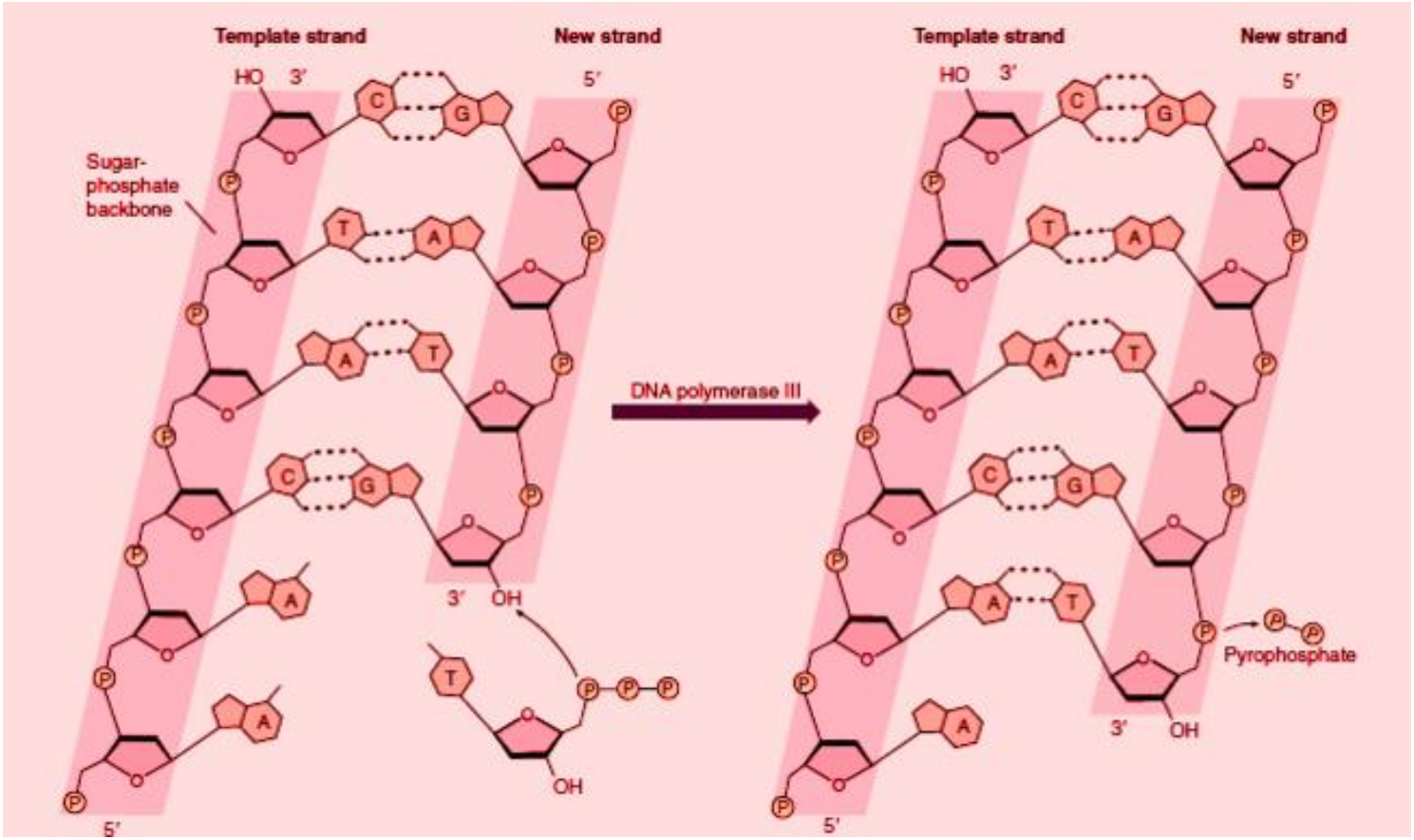
- Base + sugar → nucleoside
  Example
  - Adenine + ribose = Adenosine
  - Adenine + deoxyribose = Deoxyadenosine

- Base + sugar + phosphate(s) → nucleotide
  Example
  - Adenosine monophosphate (AMP)
  - Adenosine diphosphate (ADP)
  - Adenosine triphosphate (ATP)

- Nucleotides are covalently linked together by phosphodiester bonds
  - A phosphate connects the 5′ carbon of one nucleotide to the 3′ carbon of another
- Therefore the strand has directionality
  - 5′ to 3′
  - In a strand, all sugar molecules are oriented in the same direction
- The phosphates and sugar molecules form the backbone of the nucleic acid strand
  - The bases project from the backbone



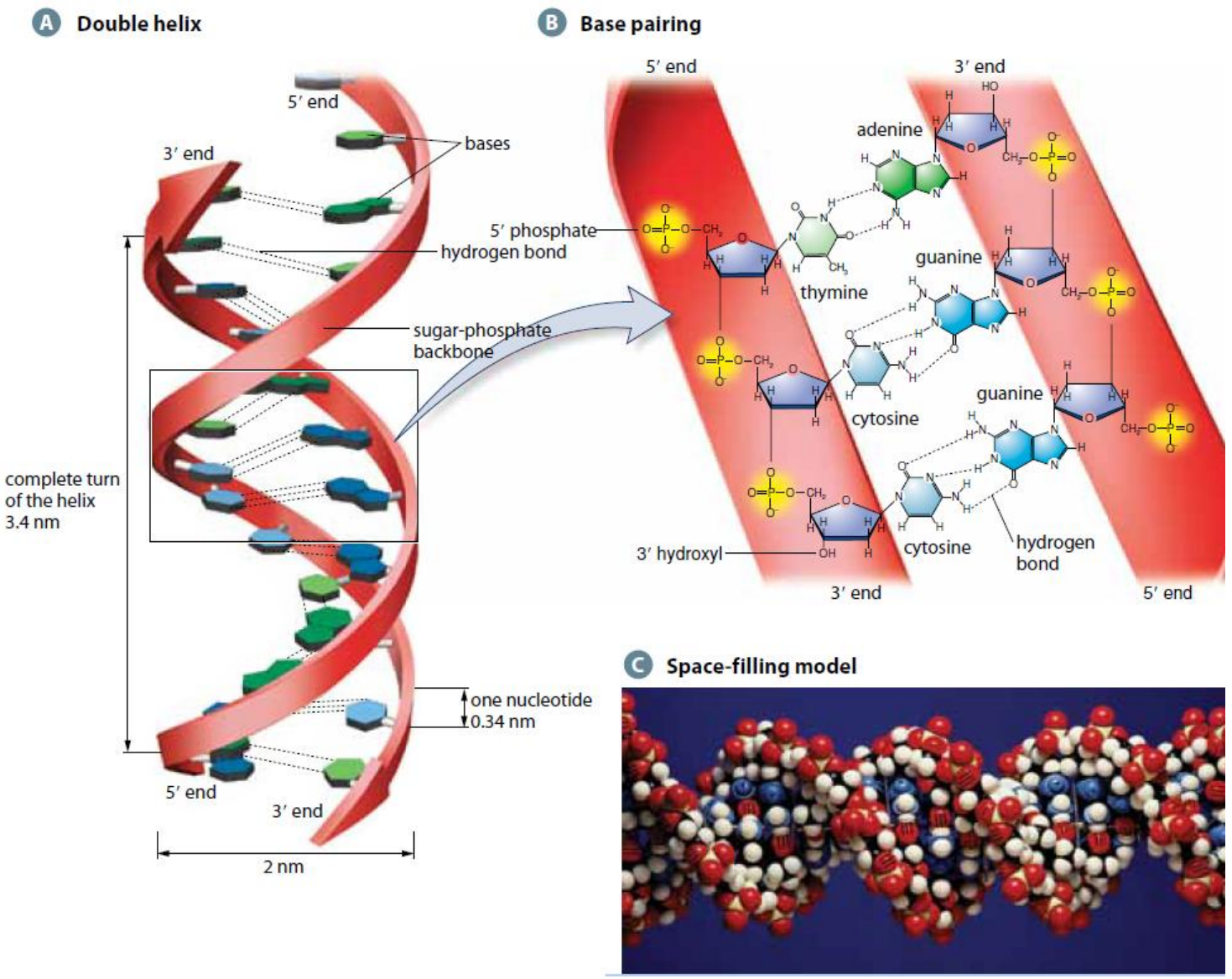




- Addition of nucleotides to the 3'-OH terminus of a growing strand.

- The recognition stepis shown as the formation of hydrogen bonds between the A and the T.
- The chemical reaction is that the 3'-OH group of the 3' end of the growing chain attacks the innermost phosphate group of the incoming trinucleotide.

The DNA Double Helix

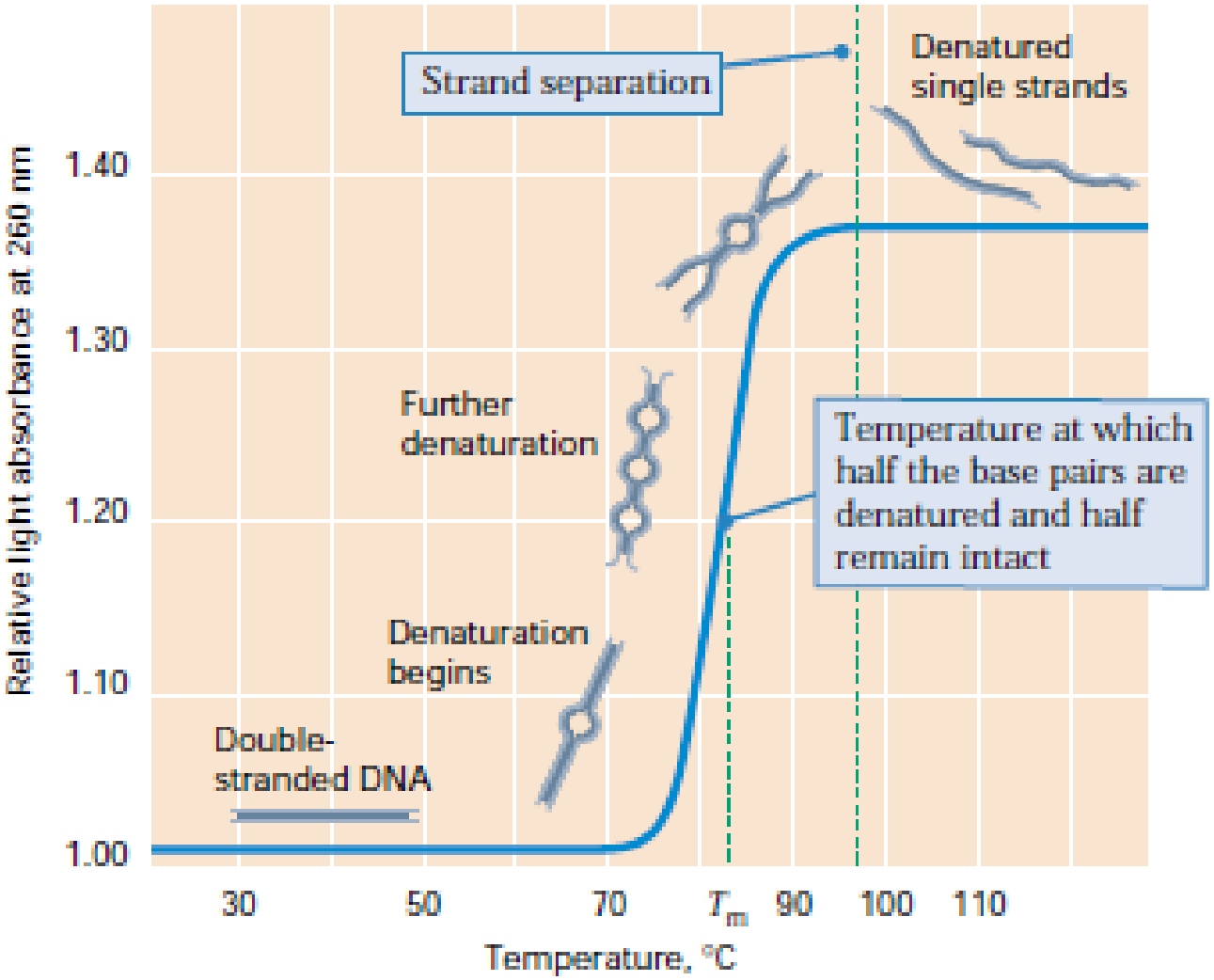


Advantages to Double Helix

- Stability---protects bases from attack by H_2O soluble compounds and H_2O itself.
- Provides easy mechanism for replication

Chemical Properties of DNA

- Factors that affect DNA structure:
  - Temperature: denaturation (can be reversible)
  - pH: high pH can denature DNA
  - Salt concentration: lowering salt concentration can denature DNA
  - Chemicals: sodium hydroxide, formamide can also denature DNA



Mechanism of denaturation of DNA by heat. The temperature at which 50 percent of the base pairs are denatured is the *melting temperature,* symbolized *Tm.*

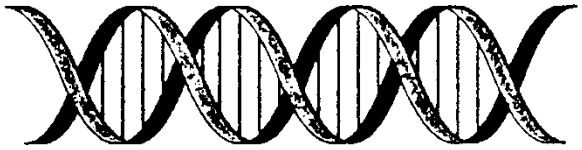
Denaturation of Nucleic Acids

- Denaturation involves the breaking of hydrogen bonds
  - Disrupts the base stacking in the helix and lead to increased absorbance at 260 nm

- By increasing temperature slowly and measuring absorbance at 260 nm as melting profile can be generated
  - Temperature for midpoint of denaturation is called the T_m

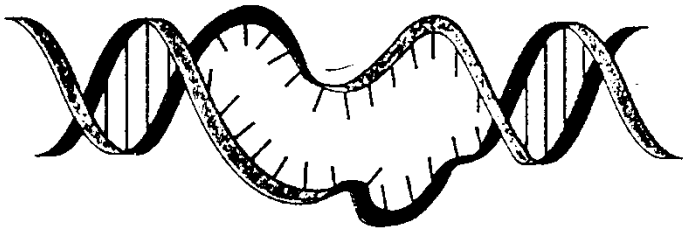
Denaturation of DNA

Double-stranded DNA

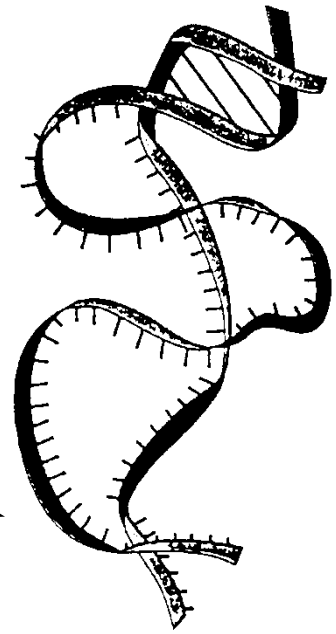


Extremes in pH or high temperature

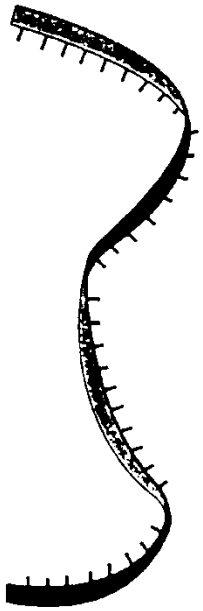
A-T rich regions denature first



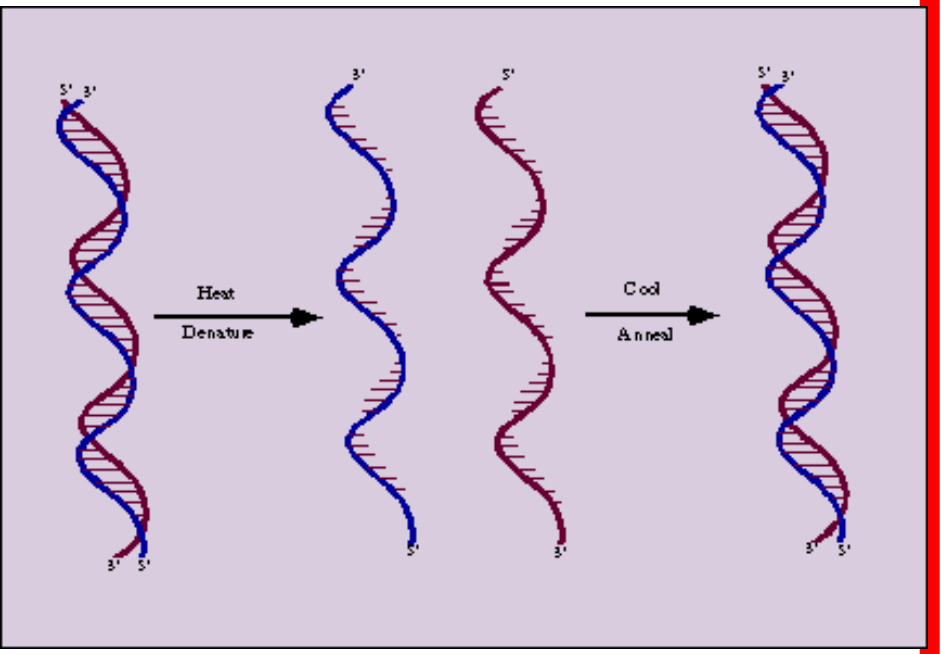
Cooperative unwinding of the DNA strands



Strand separation and formation of single-stranded random coils



Denaturation of DNA



- Denaturation by heating.
- How observed?
  - A_{260}
  - For dsDNA,
    A_{260}=1.0 for 50 µg/ml
  - For ssDNA and RNA
    A_{260}=1.0 for 38 µg/ml
  - For ss oligos
    A_{260}=1.0 for 33 µg/ml
  - Hyperchromic shift

The T at which $\frac{1}{2}$ the DNA sample is denatured is called the melting temperature (T_m)

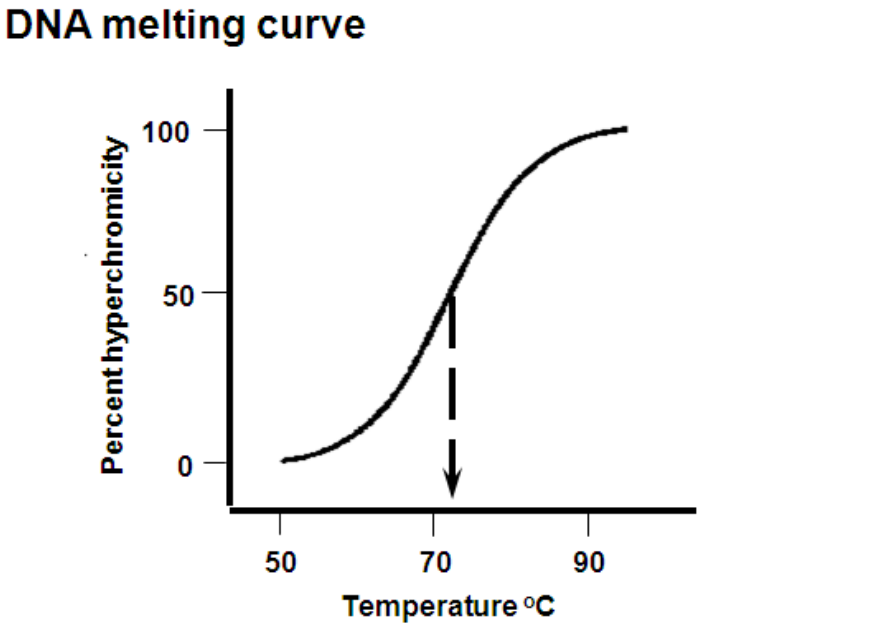
https://seqcore.brcf.med.umich.edu/doc/educ/dnapr/pg2.html

Importance of T_m

- Critical importance in any technique that relies on complementary base pairing
  - Designing PCR primers
  - Southern blots
  - Northern blots

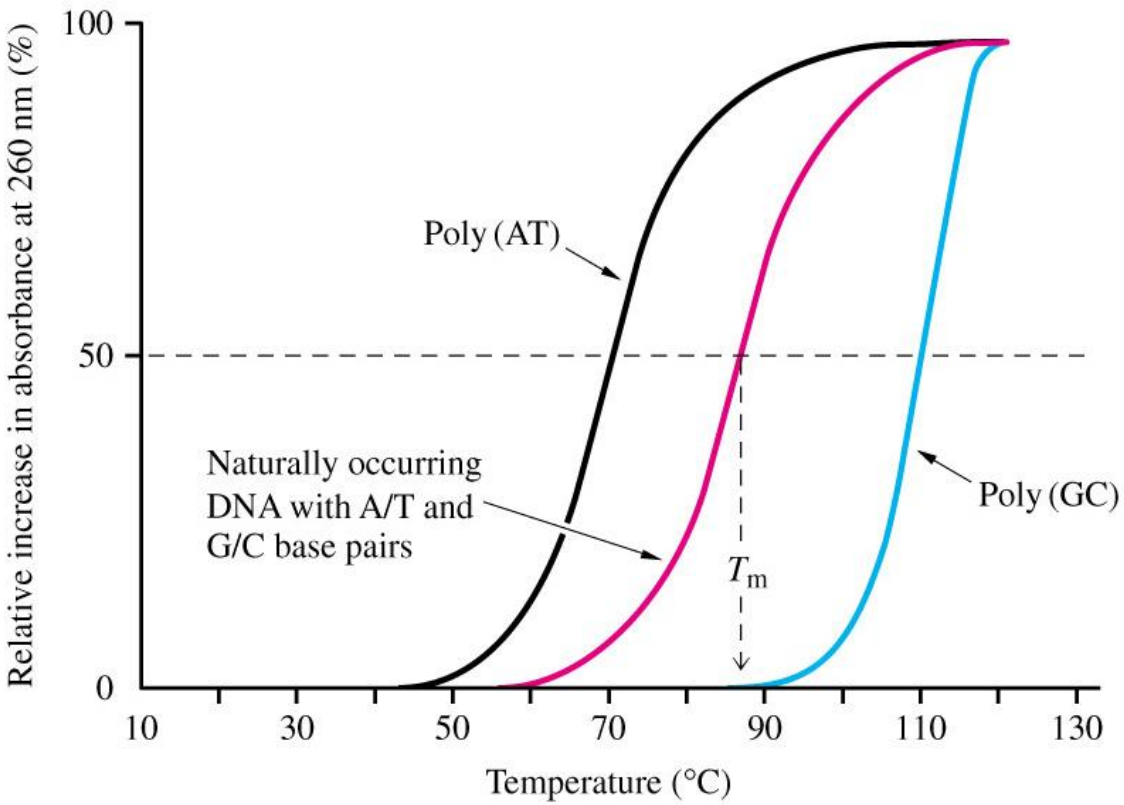
Factors Affecting T_m

- G-C content of sample
- Presence of intercalating agents (anything that disrupts H-bonds or base stacking)
- Salt concentration
- pH
- Length



DNA sequence Determines Melting Point

- Melting temperature related to G:C and A:T content.
- 3 H-bonds of G:C pair require higher temperatures to denture than 2 H-bonds of A:T pair



DNA Denaturation and Renaturation and the Role of Hydrogen Bonds and Stacking Interactions,2007

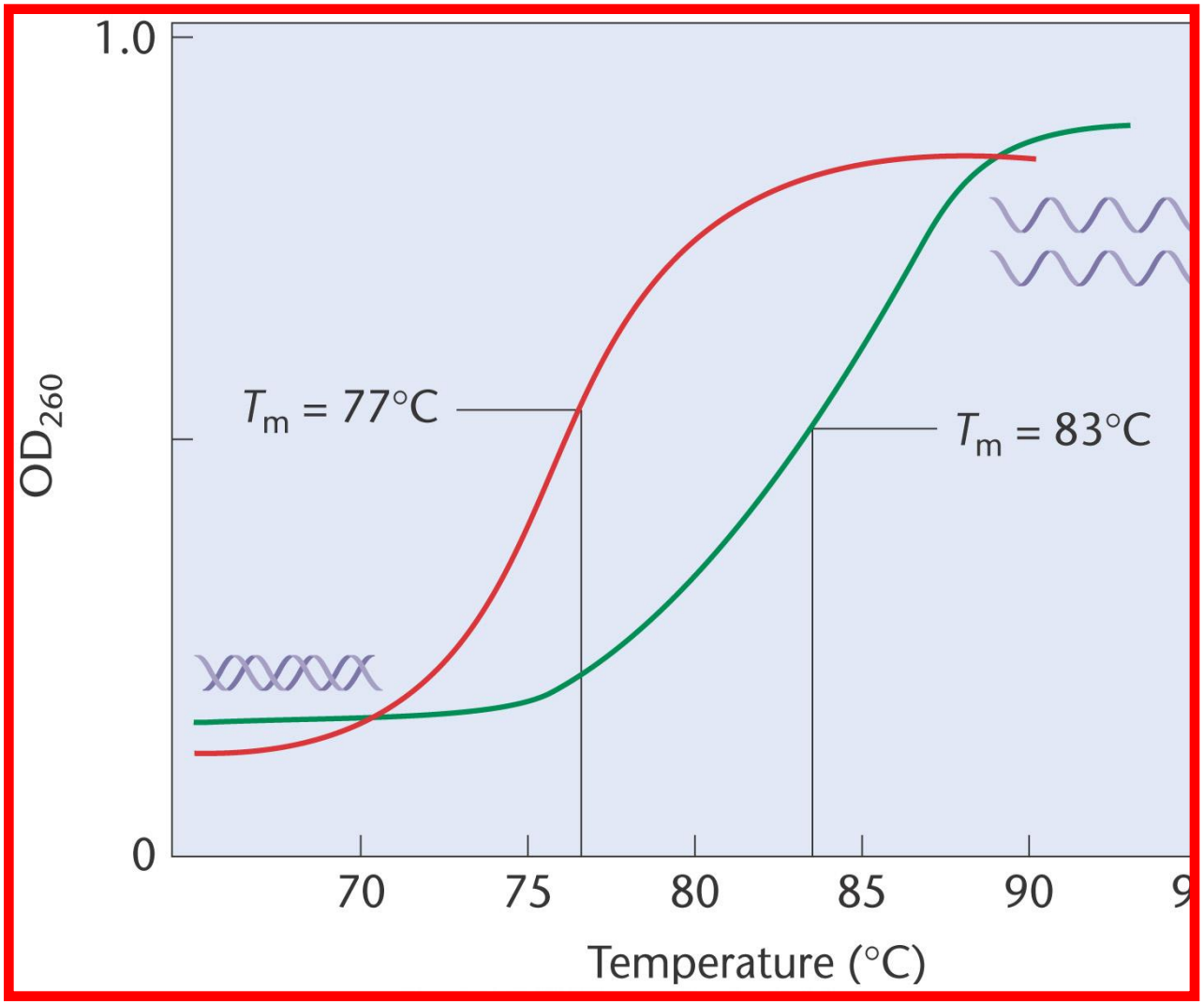
Renaturation

- Strands can be induced to renature (anneal) under proper conditions.

Factors to consider:

- Temperature
- Salt concentration
- DNA concentration
- Time

Thermal Denaturation

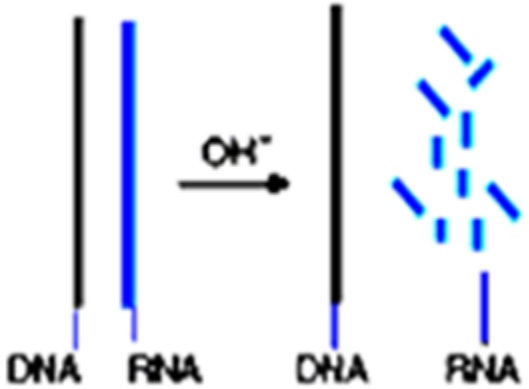


- Increased G+C gives increased T_m
  - 3 vs. 2 hydrogen bonds
- Increased ionic strength also increases T_m

http://bio3400.nicerweb.com/Locked/media/ch10/DNA_denaturation.html

- Alkali → seperation of the DNA strands

- Alkali → cleavage of phosphodiester bonds in RNA



- "Alkali are used to purify DNA strands from RNA"

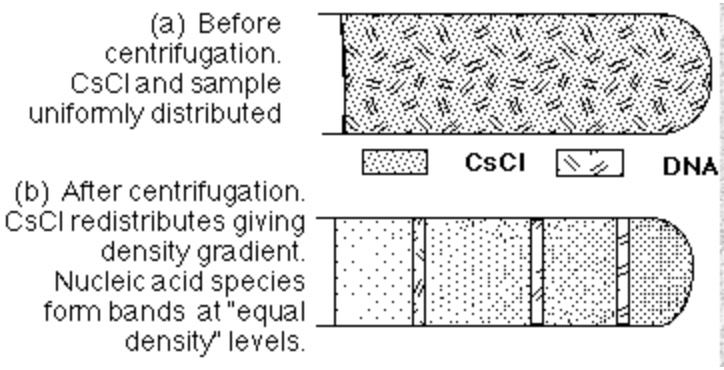
Forces affecting the stability of DNA

- hydrophobic interactions – stabilize
  - The hydrophobic environment inside with the bases and the hydrophilic environment outside with the sugar phosphate backbone
- stacking interactions – stabilize
  - relatively weak but additive van der Waals forces
- hydrogen bonding – stabilize
  - relatively weak but additive and facilitates the stacking of the bases
- electrostatic interactions – destabilize
  - contributed primarily by the (negative) phosphates
  - affect intrastrand and interstrand interactions
  - repulsion can be neutralized with positive charges

  (e.g., positively charged Na^+ ions or proteins)

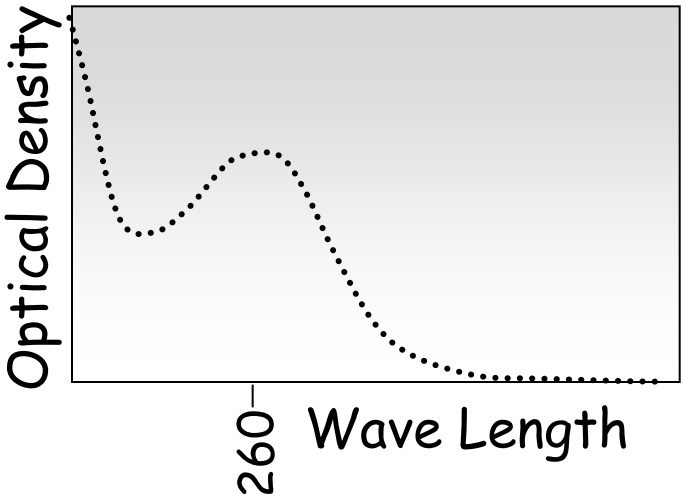
Nucleic Acid Characterization

- Absorption Spectra
  - Absorb light in ultraviolet range, most strongly in the 254-260 nm range
    - Due to the purine and pyrimidine bases
    - Useful for localization, characterization and quantification of samples
- Sedimentation and density
  - Can be characterized by sedimentation velocity (Svedberg coefficient, S)
    - Sedimentation velocity centrifugation
    - Related to MW and shape
  - Or by buoyant density
    - CsCl (DNA) or $CsSO_4$ for RNA
    - Sedimentation equilibrium centrifuc



USING SPECTROSCOPY TO ANALYZE DNA

DNA absorbs UV light with a major peak at 260 nm (proteins 280 nm)

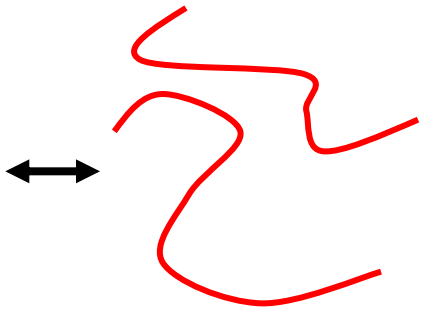


This absorption is useful because it varies with the structure of DNA *(&RNA)*

i.e. extinction coefficient depends on the structure

dsDNA

Low extinction coefficient



ssDNA

Higher extinction coefficient

What are Spectroscopy and Spectrophotometry??

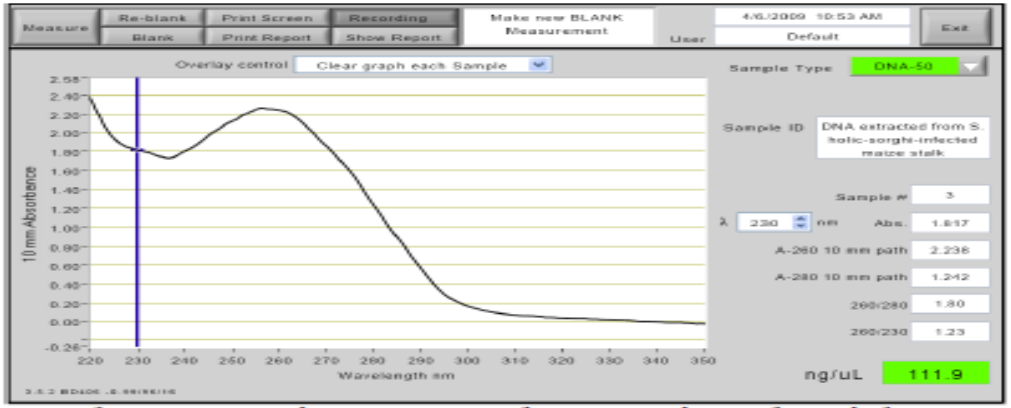
- Light can either be *transmitted* or *absorbed* by dissolved substances
- Presence & concentration of dissolved substances is analyzed by passing light through the sample
- Spectroscopes measure electromagnetic ***emission***
- Spectrophotometers measure electromagnetic ***absorption***

Evaluation of Nucleic Acids

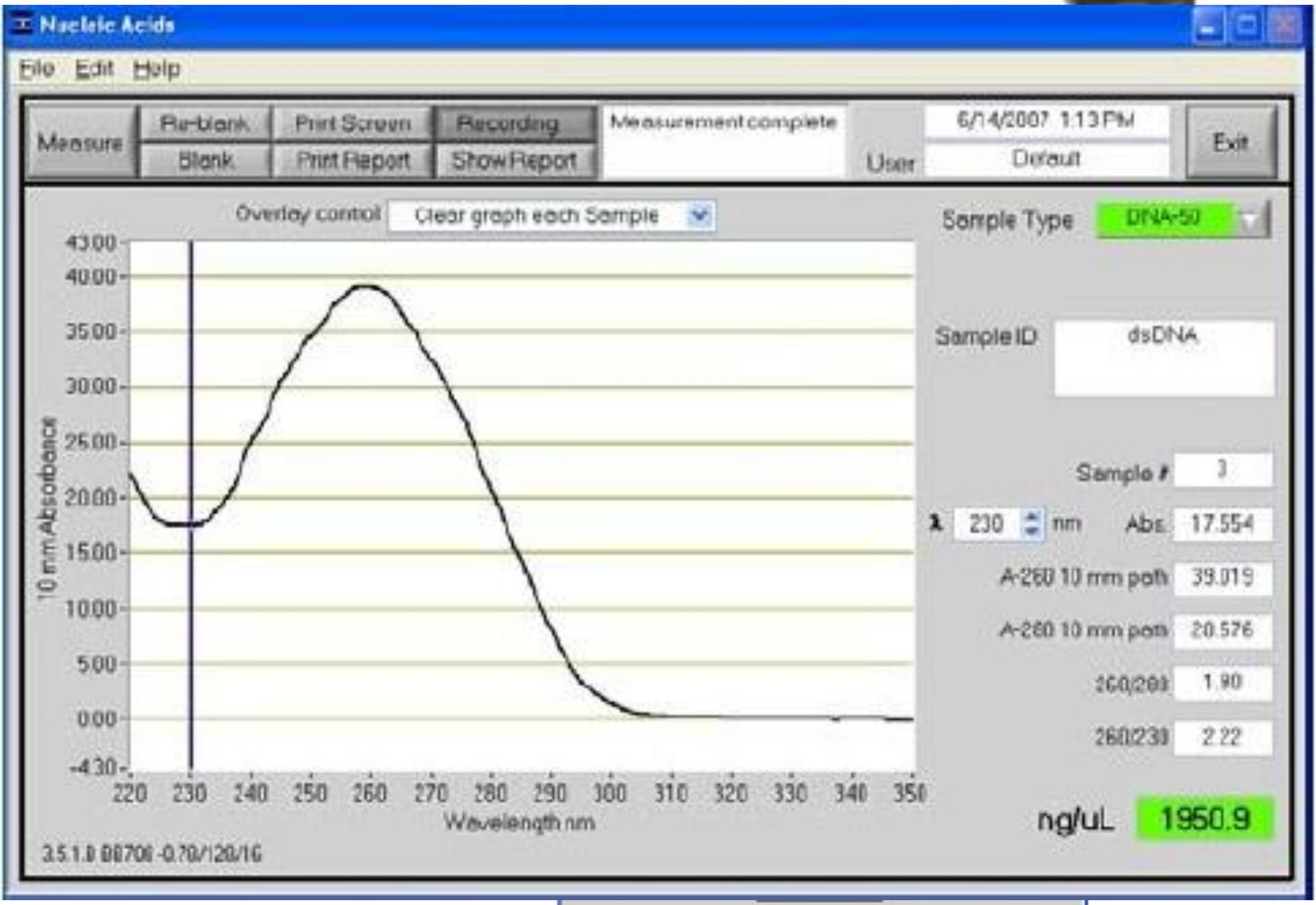
- spectrophotometrically
  - quantity
  - quality
- fluorescent dyes
  - gel electrophoresis

DNA	A_{260}	1.0 ≈ 50 μg/ml
	A_{260}/A_{280}	1.6 - 1.8
RNA	A_{260}	1.0 ≈ 40 μg/ml
	A_{260}/A_{280}	~2.0

Spectrophotometric estimation of DNA



Quantity and quality of total genomic DNA determined with an NanoDrop-1000


Nucleic Acids
File Edit Help
Measure
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Print Screen
Print Report
Recording
Show Report
Measurement complete
6/14/2007 1:13 PM
User
Default
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Sample Type
DNA-50
Sample ID
dsDNA
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3
λ 230 nm
Abs 17.554
A-260 10 mm path 39.019
A-260 10 mm path 20.576
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260/230 2.22
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220 230 240 250 260 270 280 290 300 310 320 330 340 350
Wavelength nm

Flow Cytometry

- fluorescence-activated cell sorter or FAC
- flow cytometer is a fluorescence microscope which analyses moving particles in a suspension.

- These are excited by a source of light (U.V. or laser) and in turn emit an epi-fluorescence which is filtered through a series of dichroic mirrors .

- in-built programme of the equipment converts these signals into a graph plotting the intensity of the epi-fluorescence emitted against the count of cells emitting it at a time given.

- Flow cytometer consists of fluidics, optics and electronics, as it measures cells in suspension that flow in single-file through an illuminated volume where they scatter light and emit a fluorescence that is collected, filtered and converted to digital values for storage on a computer.

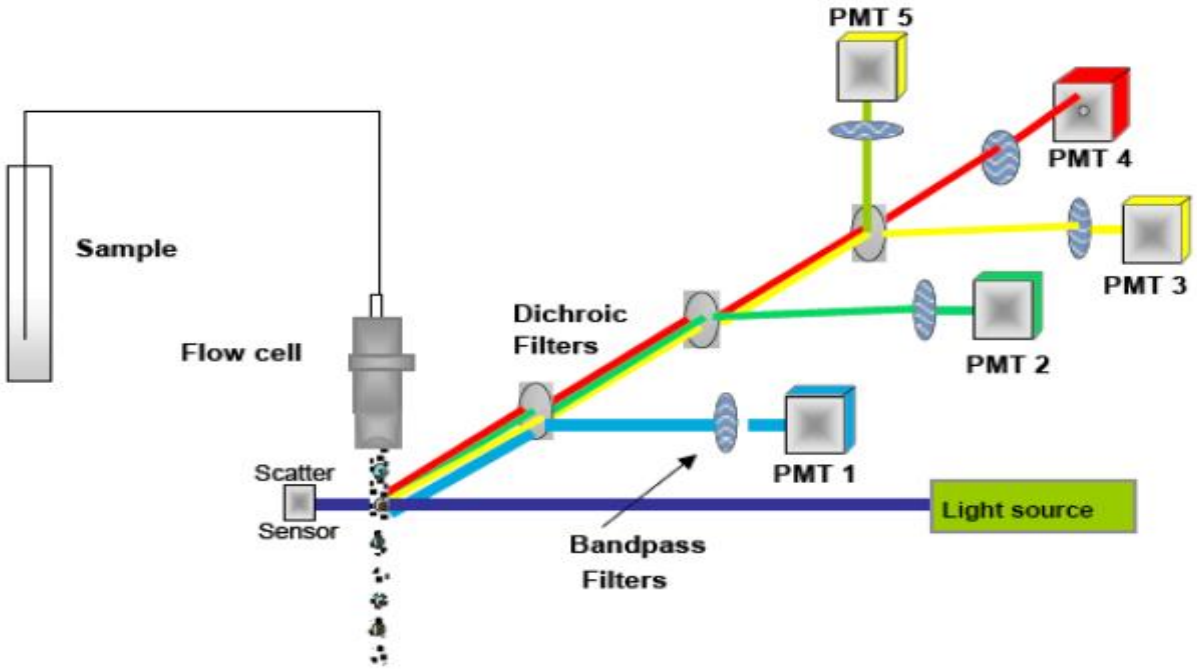


Figure 1. Simplified scheme of a flow cytometer (from Murphy, 2006).

Flow Cytometry

- Using appropriate dye to label DNA and then measuring the fluorescence by flow cytometry.

- DNA dyes using on flow cytometry:

*PropidiumIodide (PI) *DAPI

*VybrantDyeCycle *Hoechst 33342, 33258

*7-AAD *AcridineOrange (AO)

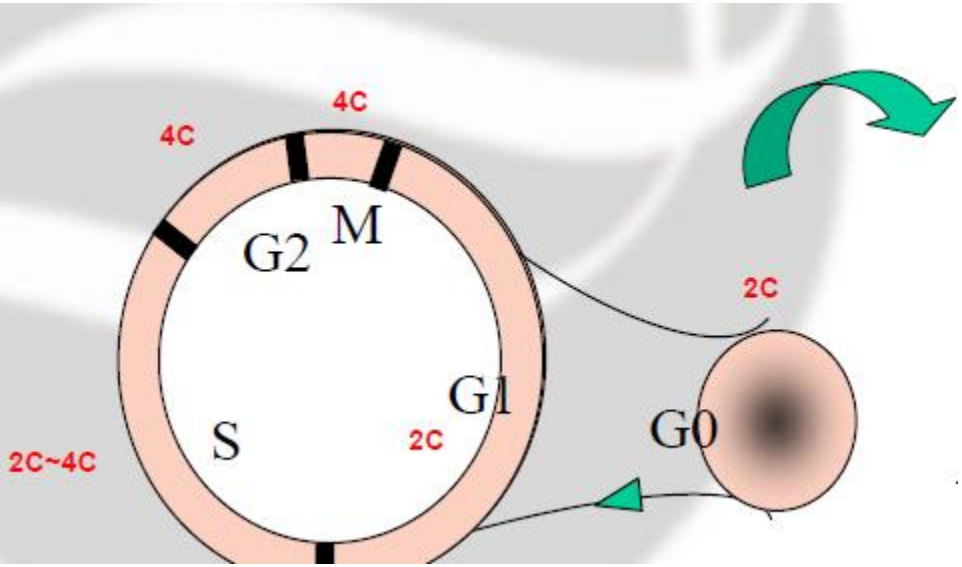
Common DNA Probes

Probe	Excitation	Emission
Propidium Iodide	536 nm (488 nm laser)	623 nm
DAPI	359 nm (UV laser)	461 nm
DRAQ5	650 nm (488 nm or 633 nm laser)	680 nm
Hoescht	346 nm (UV laser)	460 nm

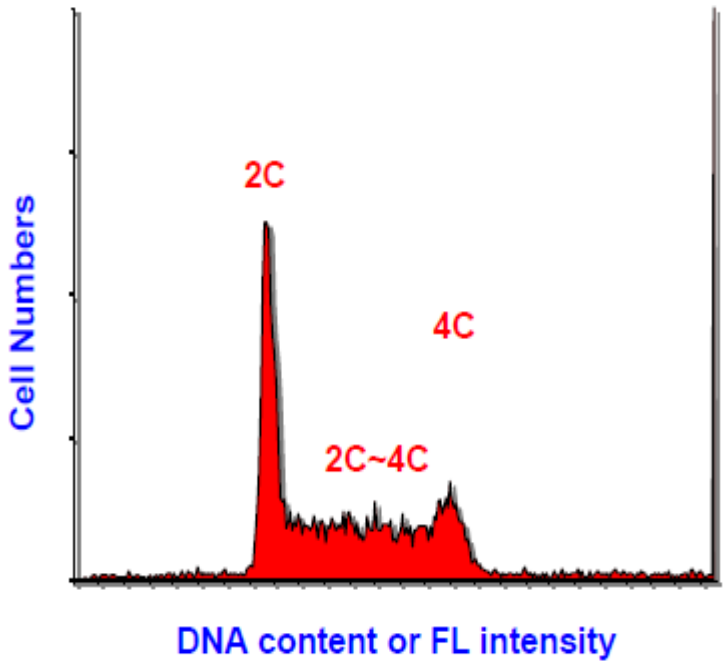
- Familiar applications using this technology:

-Cell cycle analysis
-Ploidyanalysis
-DNA index analysis
-Apoptosis sub-G1 analysis

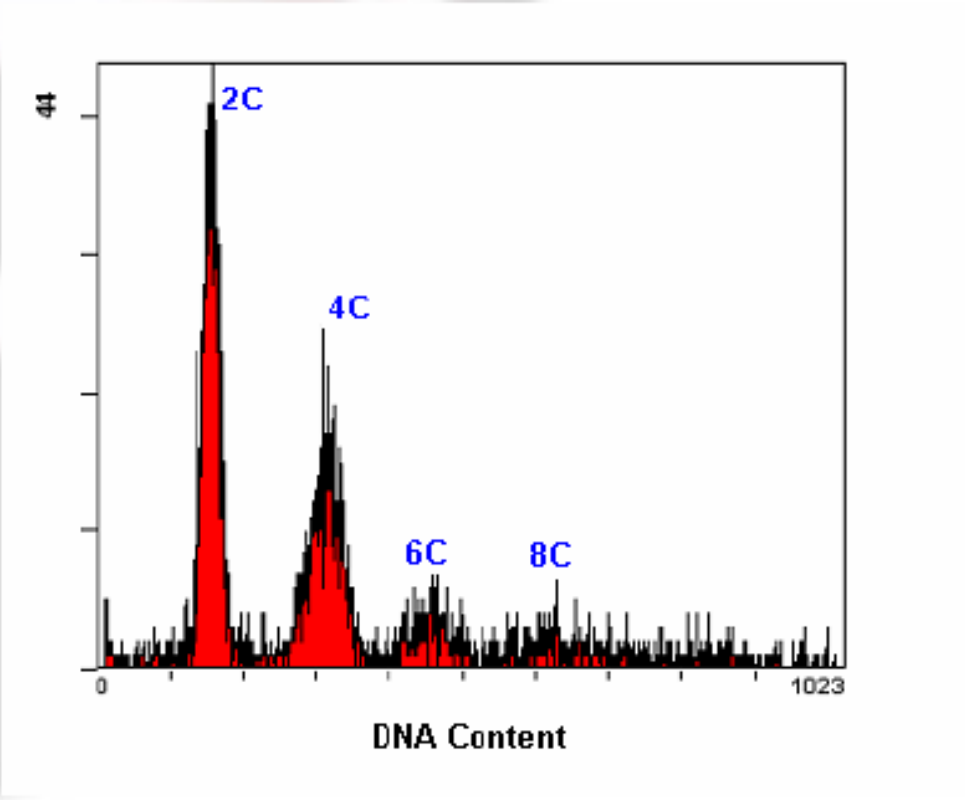
Application 1: cell cycle analysis



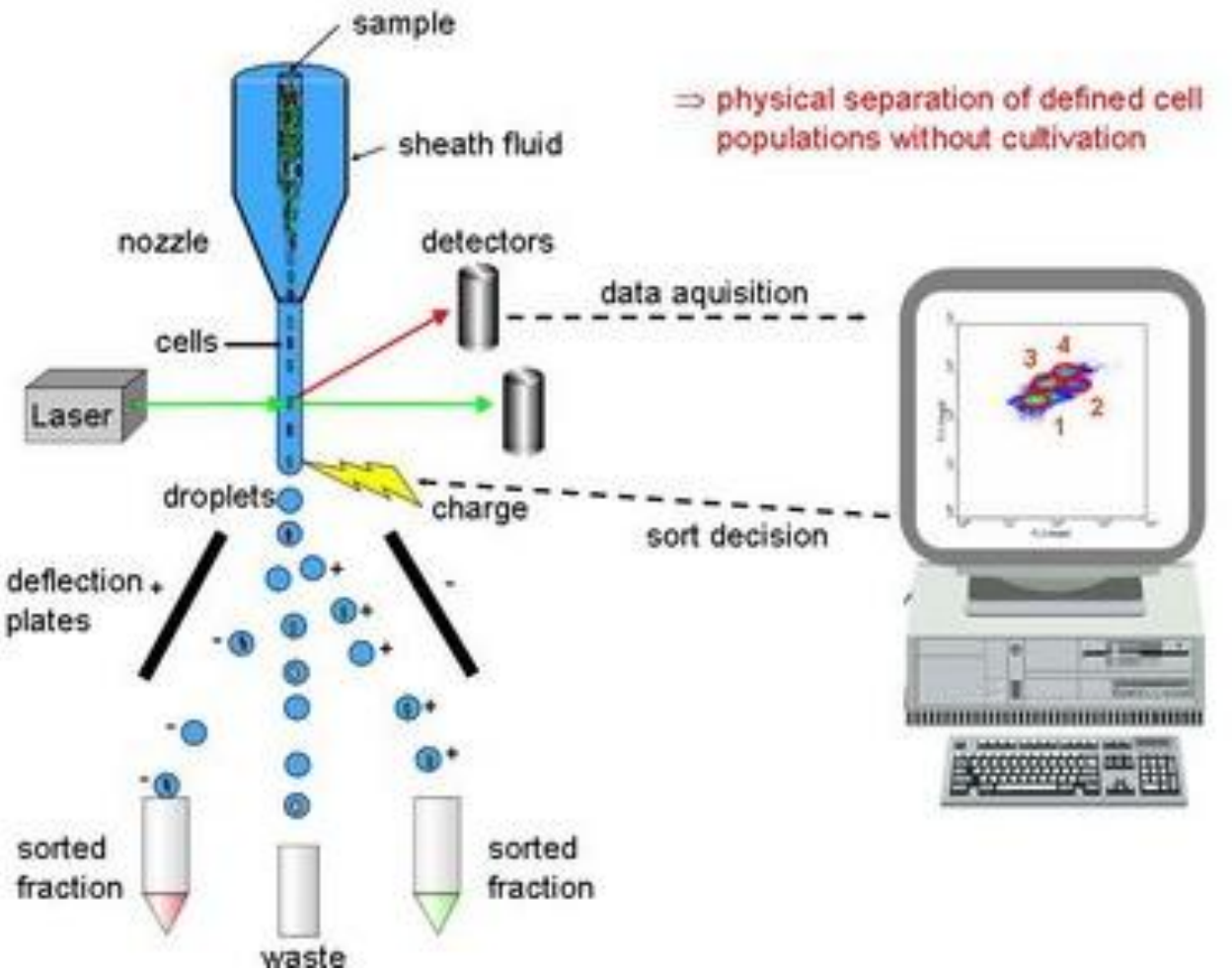
Stained with PI,
analyzed by Flow



Application 2: ploidy analysis



- *Hyperdiploid*: greater than the normal 2n number of chromosomes
- *Hypodiploid*: Less than the normal 2n number of chromosomes
- *Tetraploidy*: Containing double the number of chromosomes


sample
sheath fluid
⇒ physical separation of defined cell populations without cultivation
nozzle
detectors
data aquisition
cells
Laser
droplets
charge
sort decision
deflection plates
sorted fraction
waste
sorted fraction
1
2
3
4