Methods to study the denaturation and re-association of DNA strands

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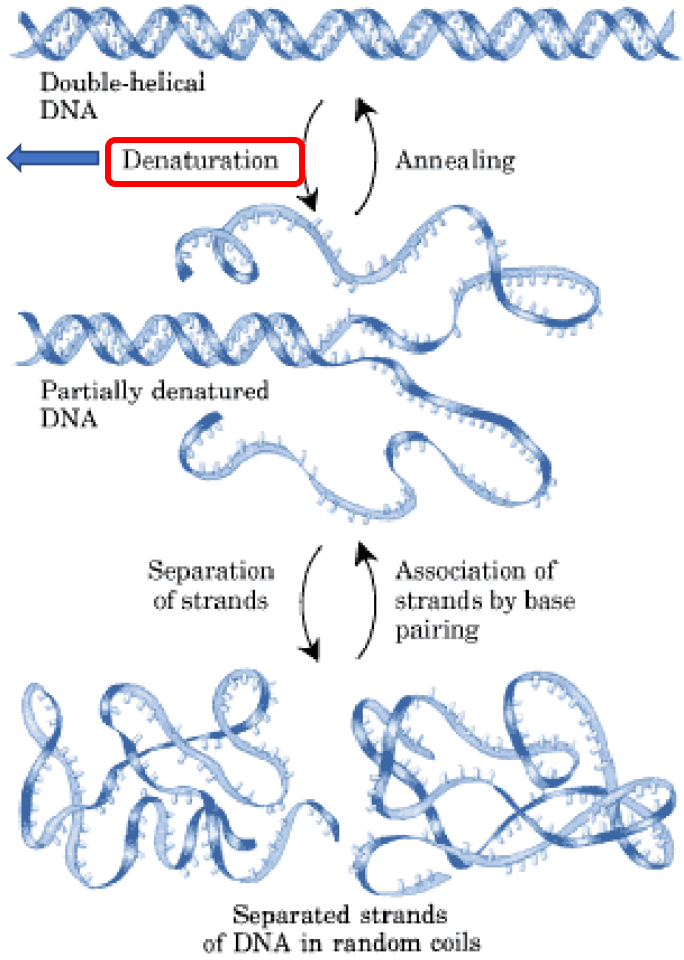
Depends on the amount of hydrogen bonds between nitrogenous bases

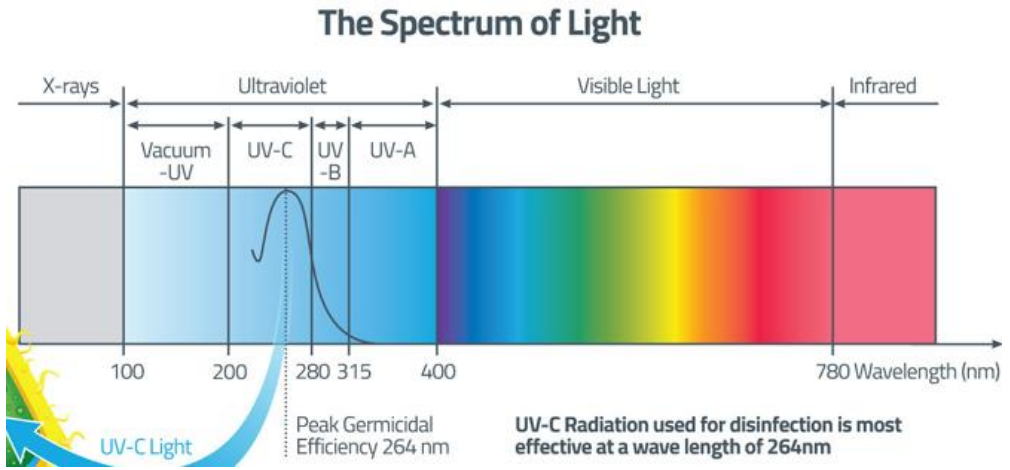
The hydrogen bonds can be destabilized after different treatments:

- Temperature
- High pH
- Chemical reagents e.g. UREA

***Reversible** event*

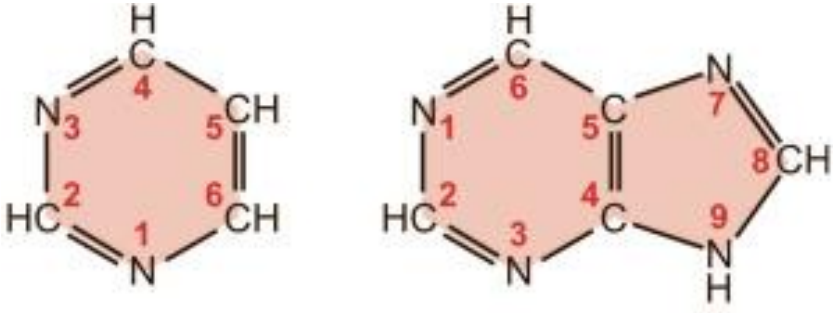


DNA absorbs at 260nm due to the heterocyclic rings of bases

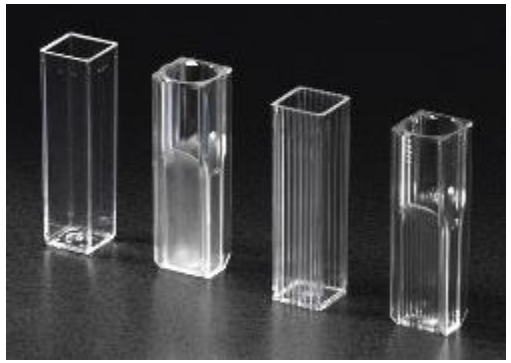


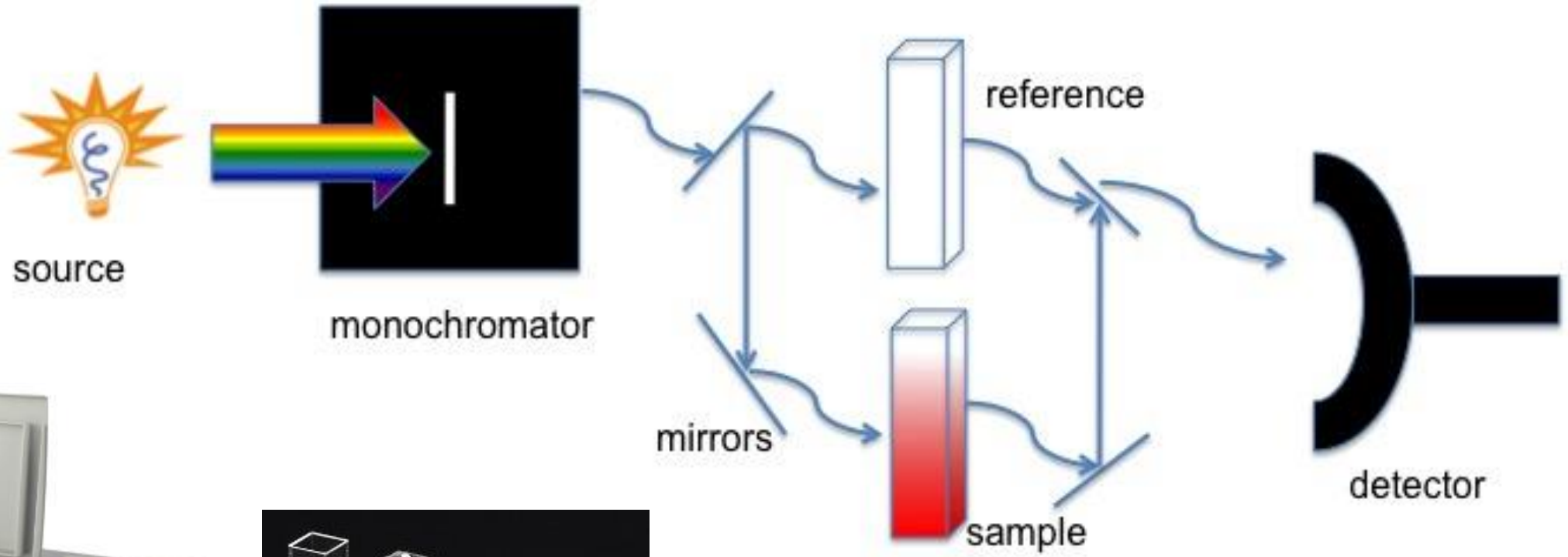
Nucleic acids **absorb ultraviolet (UV) light due to the heterocyclic rings of the nucleotides**; the sugar-phosphate backbone does not contribute to absorption. The wavelength of maximum absorption for both DNA and RNA is 260nm (**λmax = 260nm**) with a characteristic value for each base.

Base	*pH*	*λmax nm*
Adenine	1	262.5
	7	260.5
	12	269
Cytosine	1	276
	7	267
	14	282
Guanine	1	276
	7	276
	11	274
Thymine	4	264
	7	264
	12	291

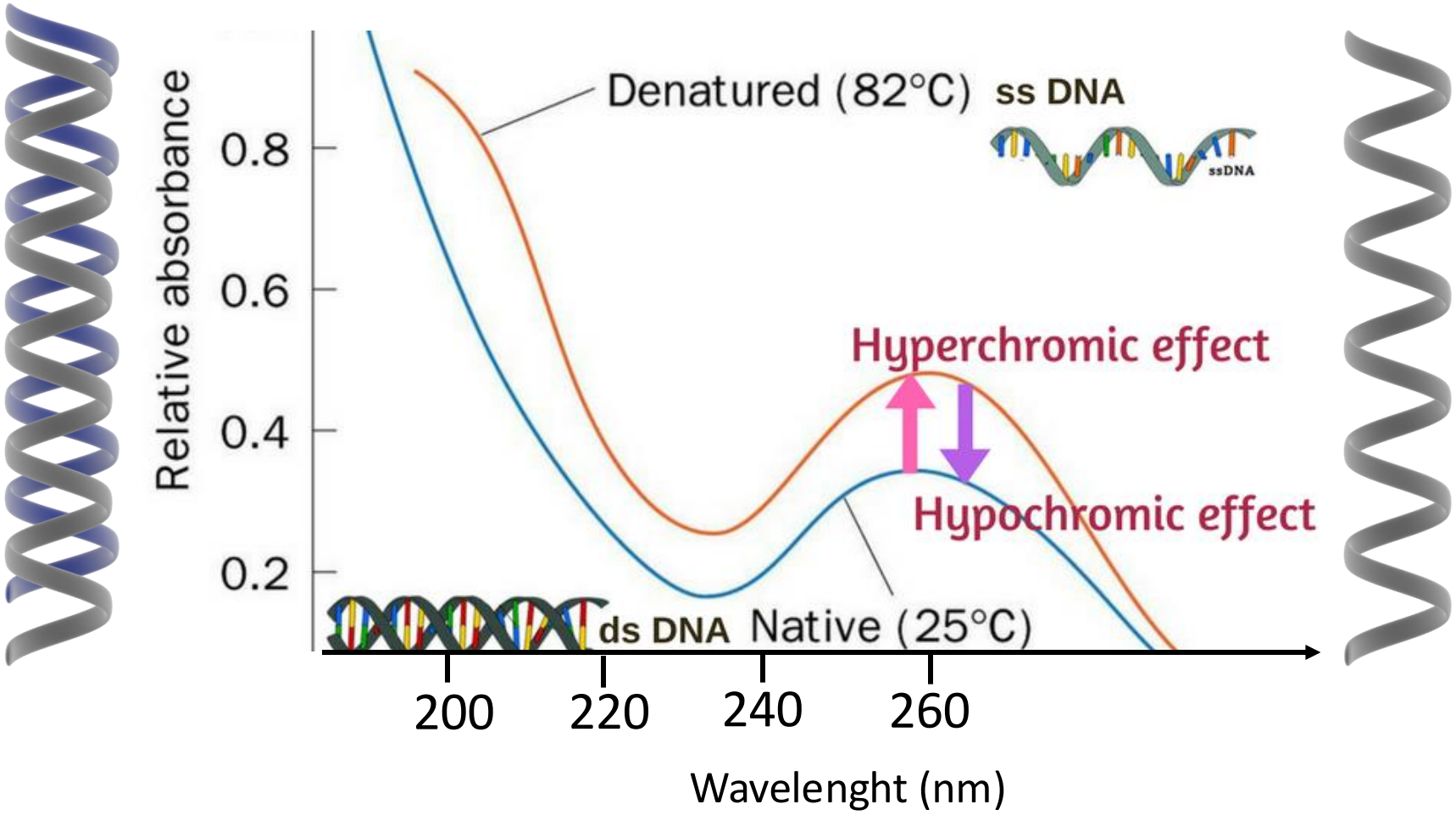


DNA state by spectrophotometry





DNA denaturation state by spectrophotometry



Hypochromicity describes decreasing ability to absorb light: NATIVE DNA

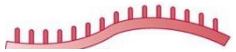
Hyperchromicity is the increasing ability to absorb light: DENATURATED DNA (more exposed nitrogenous bases).

DNA A_{260nm} → DNA concentration

It has been determined that **Absorbance** **(A) of 1** corresponds to a concentration of **50 µg/ml** for double-stranded DNA.

Single stranded DNA shows a higher Absorbance value (as described in previous slide)

1 O.D. at 260 nm for **double-strand DNA** = 50 ug/ml of dsDNA 

1 O.D. at 260 nm for **single-strand DNA** = 20-33 ug/ml of ssDNA 

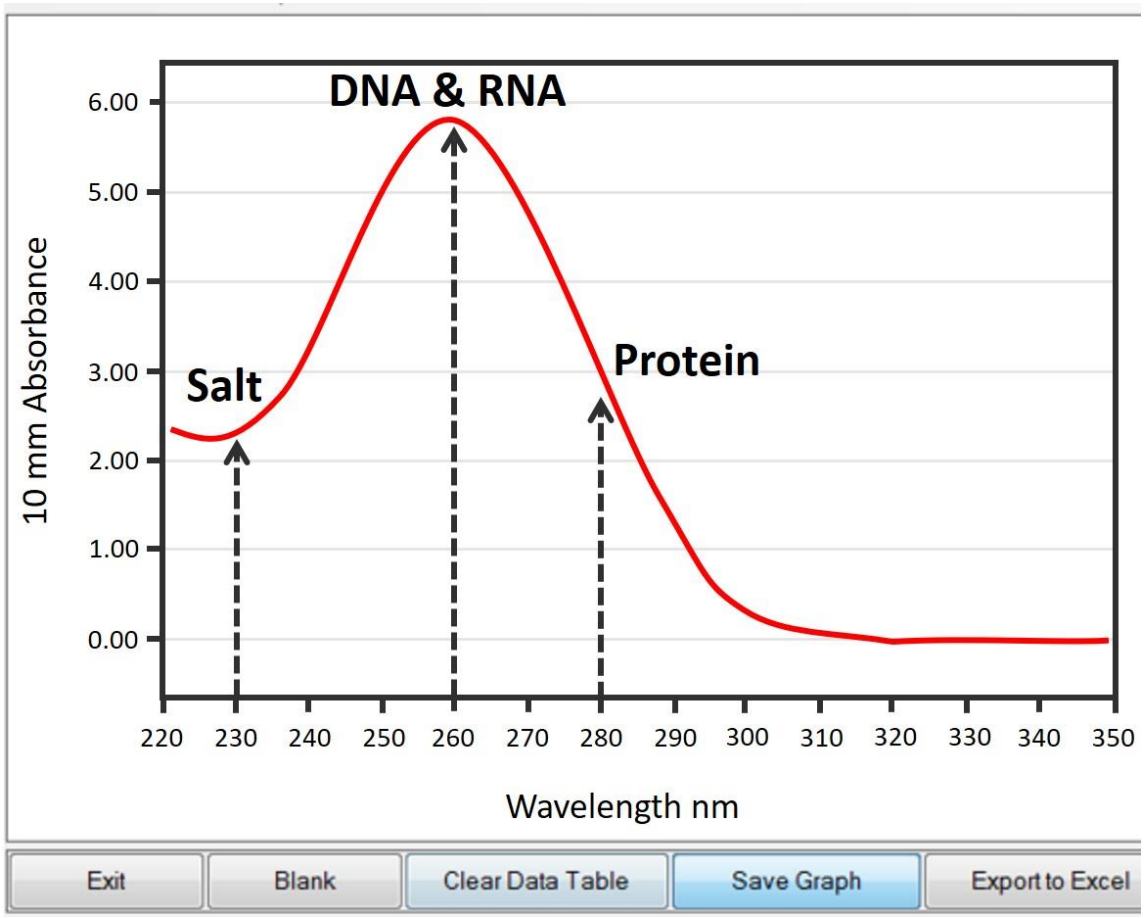
1 O.D. at 260 nm for **RNA molecules** = 40 ug/ml of RNA

(O.D.; Optic Density)

So **typically, dilute sample 1 ul in 100 ul** of water, so the dilution factor is 100.
Put whole 100 ul in spectrophotometer cuvette or 1 drop in nano-spectrophotometer.
The **DNA concentration** will then be**: OD260 * 50 ug/ml * dilution factor**

For example, if have OD260 = 1.6. Then the concentration is: 1.6 * 50 ug/ml * 100 = 8000 ug/ml or 8 ug/ul.

DNA purity



Contamination with proteins

DNA A260/ A280 = **1,8**

RNA A260/ A280 = **2**

If there is **contamination** with protein, this ratio will be significantly less than the values given above, and accurate quantitation of the amount of nucleic acid will not be possible.

Other contaminants

A230: Some compound can absorbe at 230nm (SALTS, phenol, urea, etc..), so correction at this wavelenght avoid contamination problems.

A260/ A230 value **lower than 2 indicates contamination**

A320 Background correction

Nanodrop: small volumes spectrophotometer

Concentration 10.7 µg/ml

A230	**A260**	**A280**
9.27	0.244	0.129

A260/A280	**A260/A230**
2.162	0.026

Concentration 11,1 µg/ml

A230	**A260**	**A280**
0,09	0.385	0.206

A260/A280	**A260/A230**
1.86	4.27

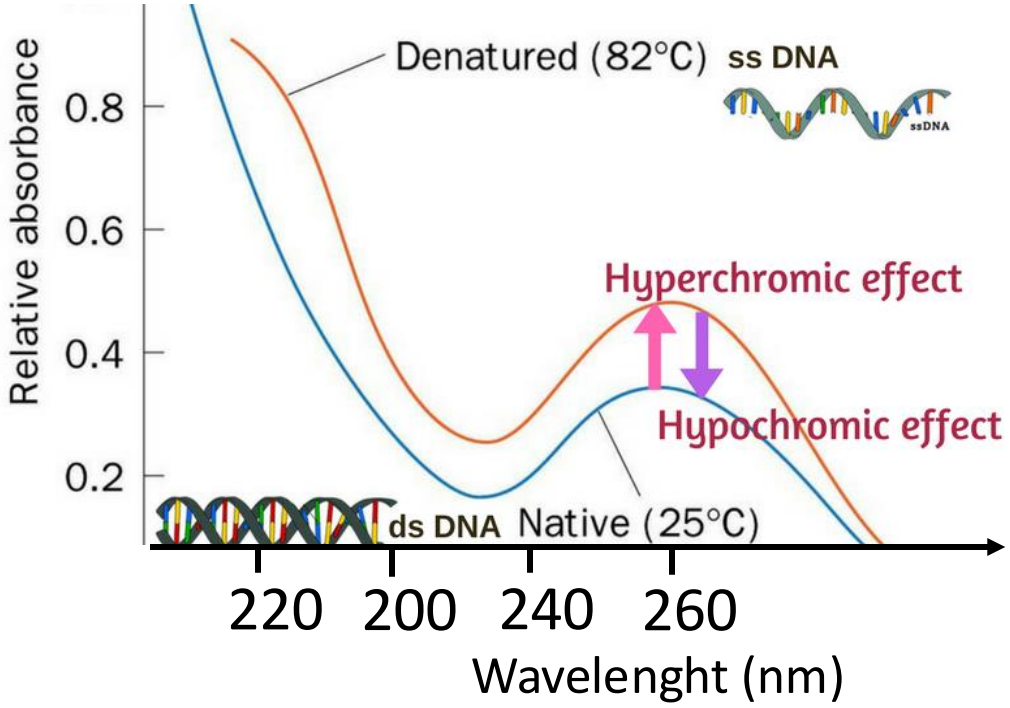
Concentration 11,1 µg/ml

A230	**A260**	**A280**
9.33	0.323	0.226

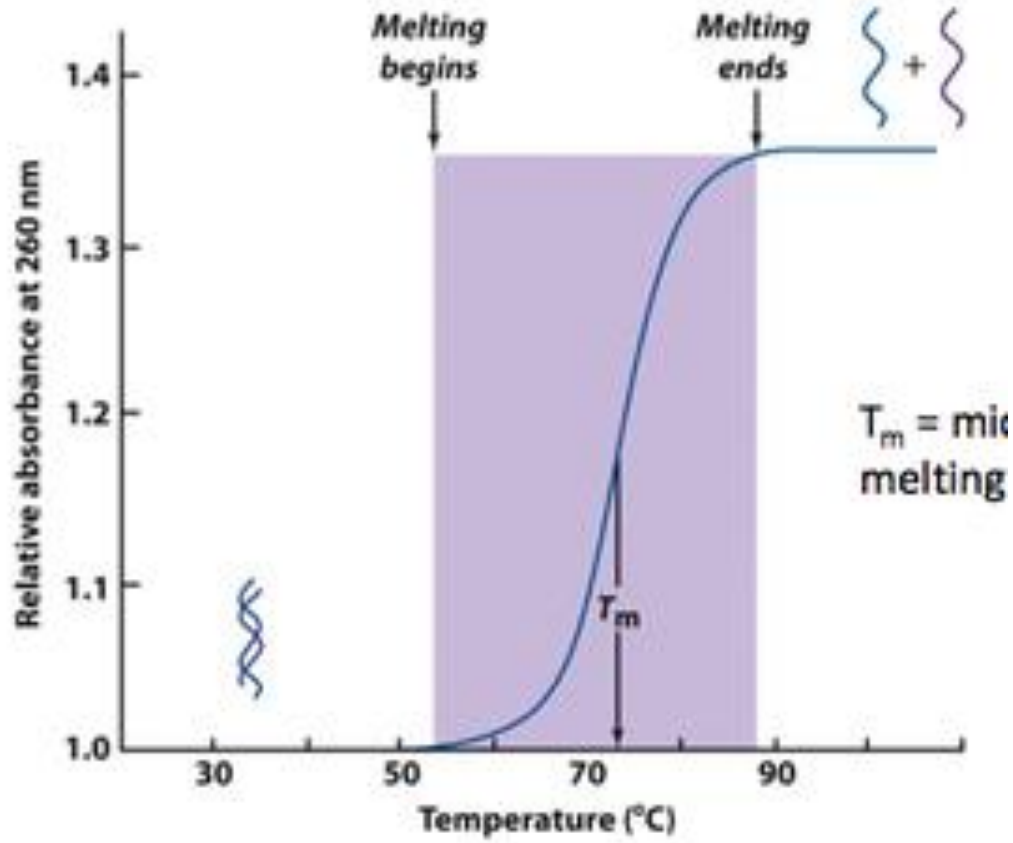
A260/A280	**A260/A230**
1,429	2.024

DNA denaturation state

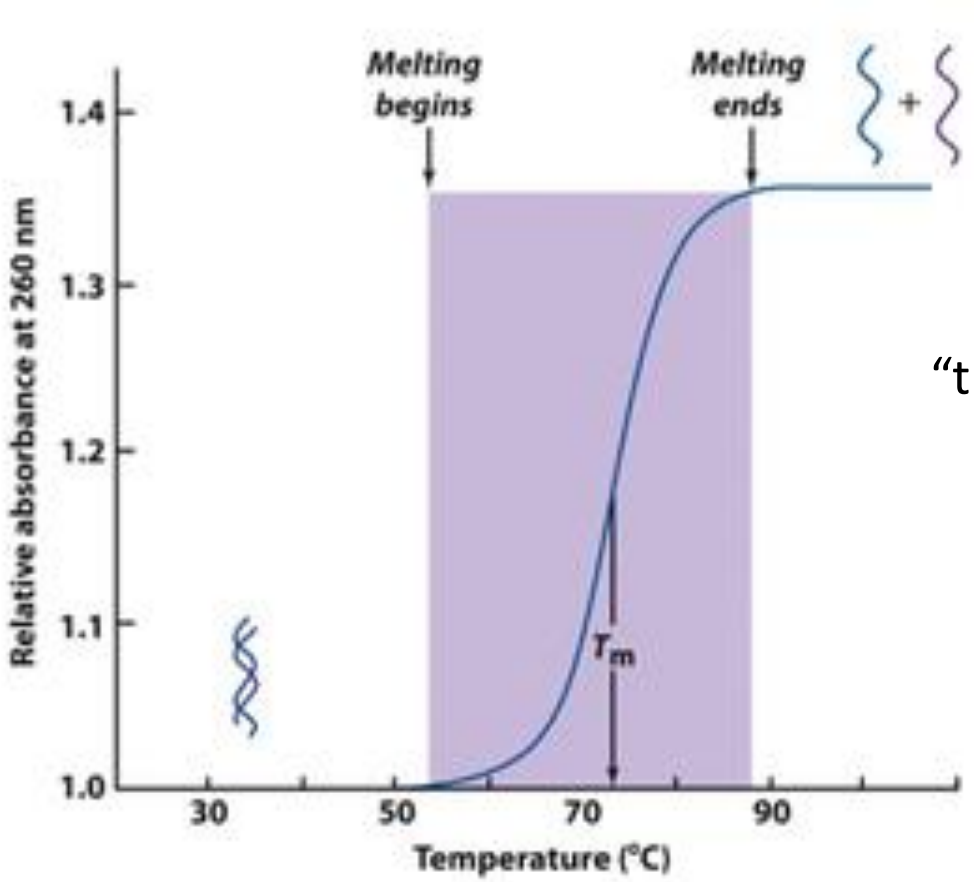
The hyperchromic effect allows to study the denaturation state of DNA. This can be done by measuring A260 in a cuvette while increasing the temperature of the solution containing the DNA.



Absorbance 260
(relative increase)



Melting Temperature

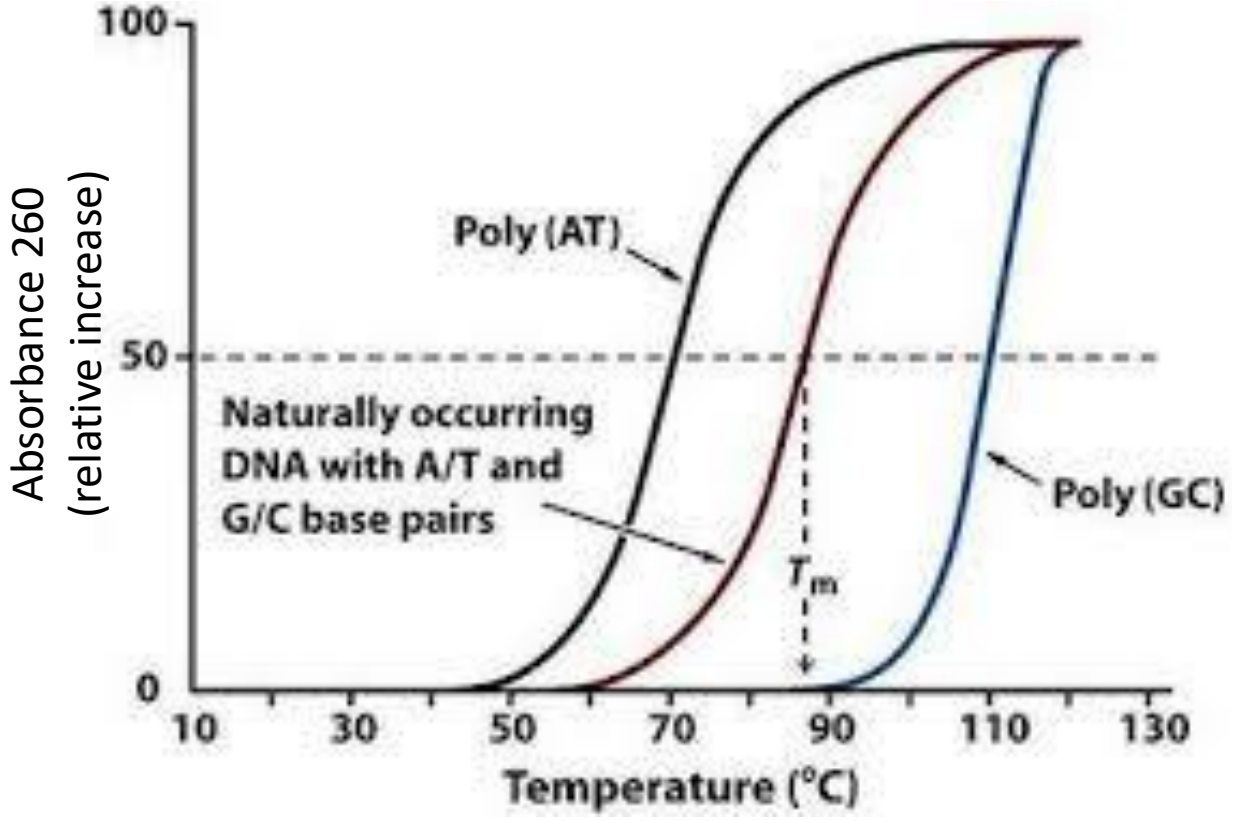


DNA denaturation, or DNA melting, is the process by which double-stranded DNA unwinds and separates into single-stranded strands through the breaking of hydrogen bonds between the bases.

Tm: melting temperature

"the temperature at which half of helix structure is denaturated "

Melting Temperature



Tm: melting temperature

"the temperature at which half of helix structure is denaturated"

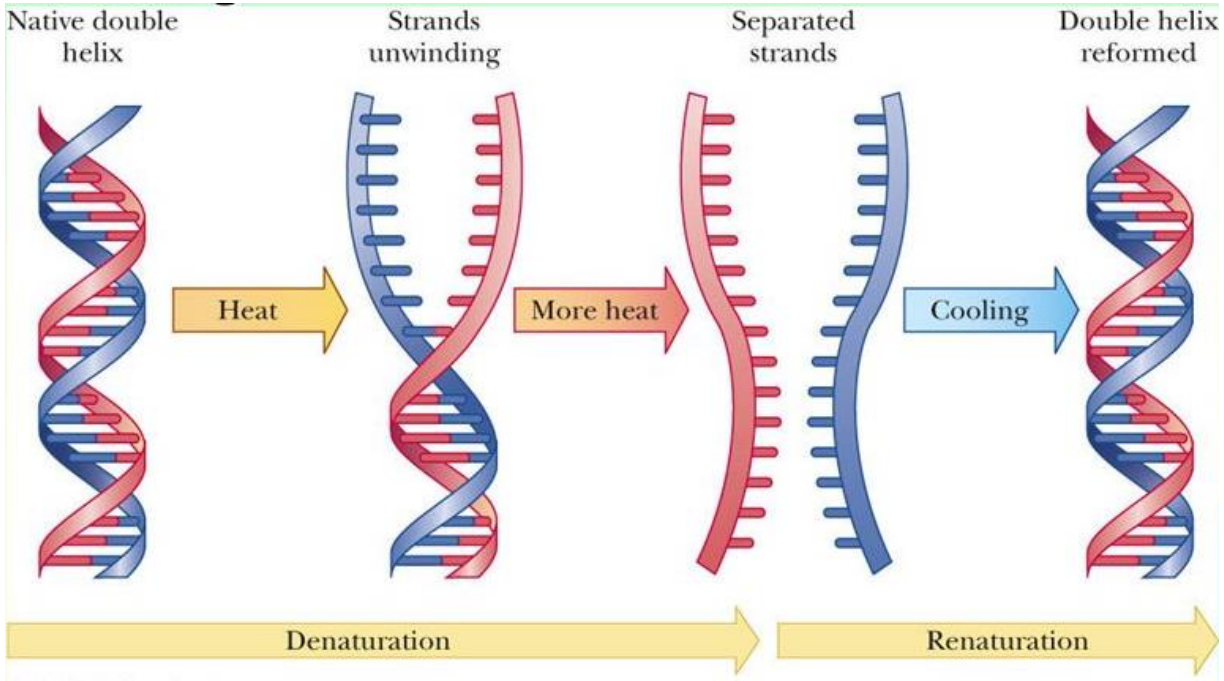
The melting temperature depends on:

- the length of the DNA
- the nucleotide sequence composition, higher GC content higher Tm

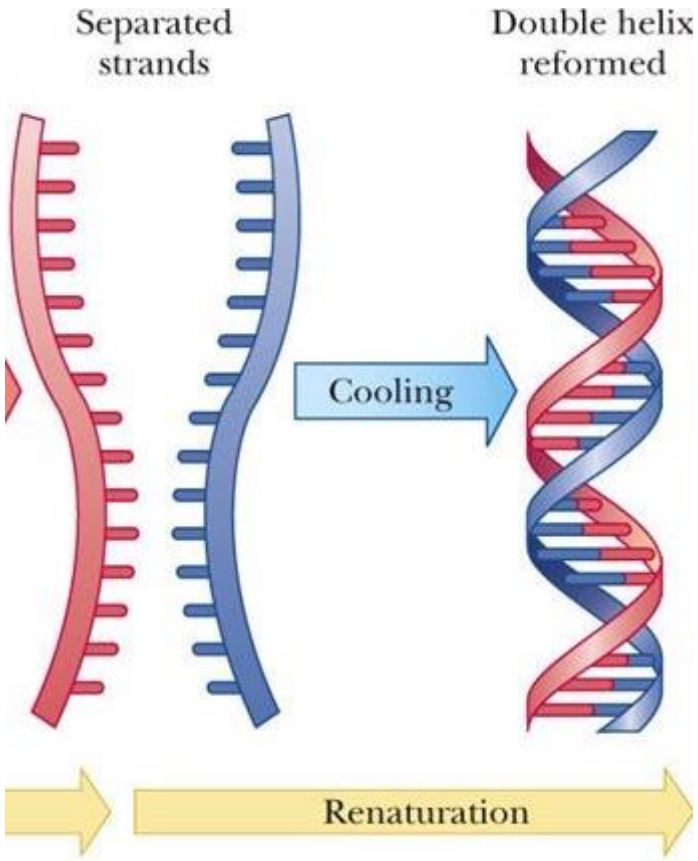
Also, the ionic force or pH of the buffer in which the DNA is resuspended influences the denaturation curve. Therefore, to compare results from different labs the buffer must be standard

DNA Reassociation or Annealing

After denaturation, the two DNA filaments can reassociate based on the nucleotide sequence. This event is known as **Annealing**



DNA annealing Temperature



- The **annealing temperature** determines the specificity of the annealing.
- At a T° close to the Tm there will be a high specificity for complementation, riassociating only nucleotide chains with the exact complementary sequences.
- Lower temperatures allow a more efficient but less specific annealing.