Course: Fundamentals of Biochemistry
Unit: 2

TOPICS

Nucleic Acids: Structure

Purines and pyrimidines

Nucleosides & Nucleotides

Types of DNA and RNA, Complementarity of DNA.

Nucleic acids Cot Curves: Base pairing, Denaturation and Renaturation of DNA.

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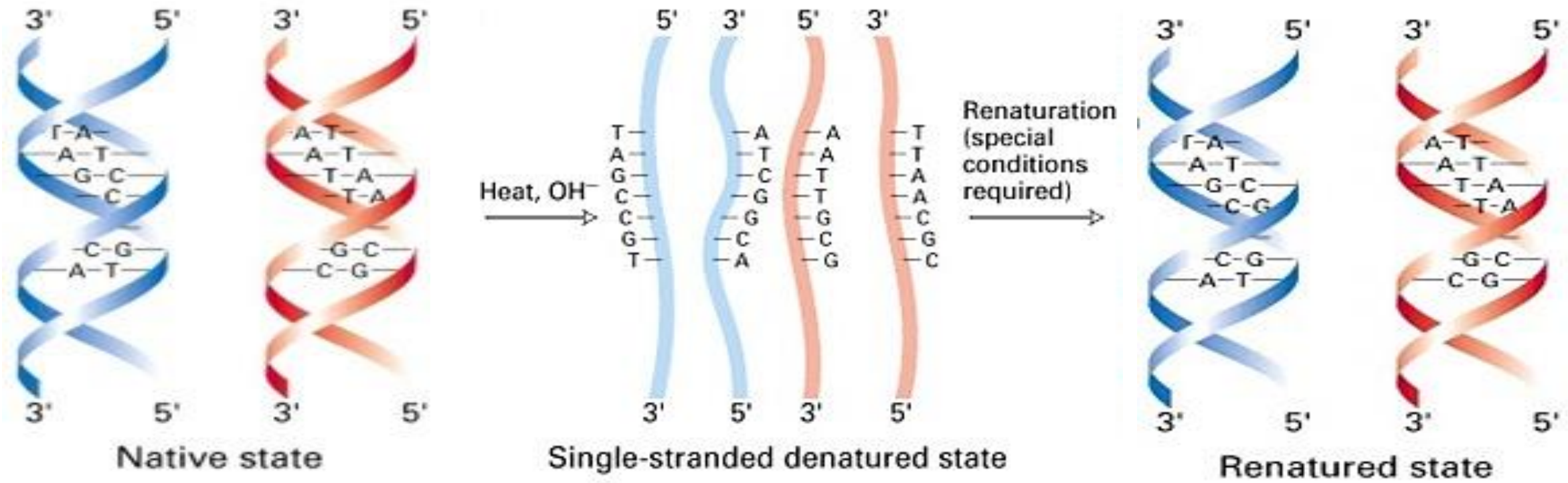
DNA Denaturation and Renaturation, Cot curves

Denaturation and Renaturation of DNA



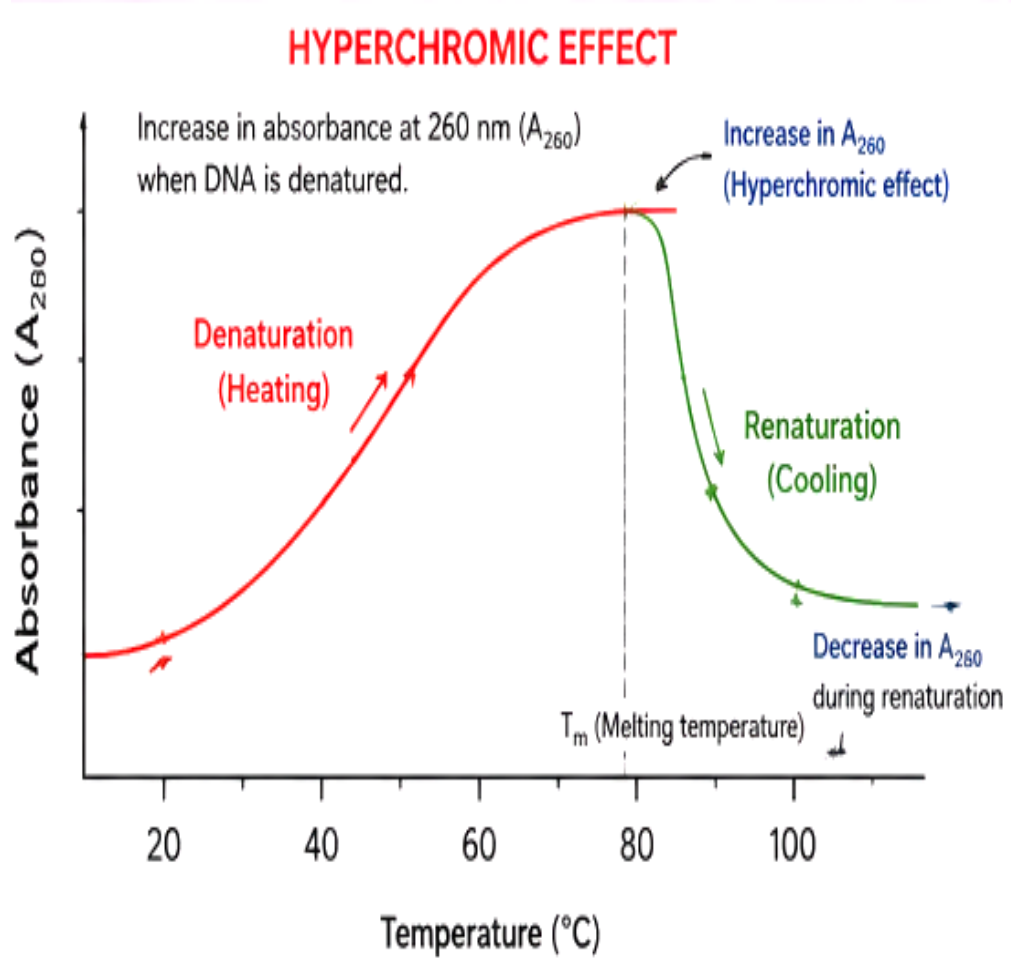
1. Denaturation : The process of formation of single stranded DNA from double stranded helical DNA by heating or by denaturating agents is called denaturation of DNA.

2. Renaturation : The process of formation of double stranded DNA (reannealing) from single stranded DNA upon cooling is called renaturation of DNA.

Absorption of UV light

- Nucleic acids absork ultraviolet (UV) light. This is because of the conjugated double bonds and the ring structure of purines and pyrimidines.
- The more ordered the structure, the less light is absorbed.
- So, free nucleotides absorb more light than single-stranded DNA or RNA, and they absorb more light than double-stranded DNA.
- Maximum absorption is at 260 nm (A_{260}) and minimum absorption is at 230 nm.
- Absorption is directly proportional to the concentration of the molecule. Value: 0.02 units per µg DNA per ml.
- Example: Three solutions of DNA and free bases at 50 µg/ml have these A_{260} values:
  - Double-stranded DNA $A_{260} = 1.00$
  - Single-stranded DNA $A_{260} = 1.37$
  - Free bases $A_{260} = 1.60$

Therefore, double-stranded DNA is hypochromic and the bases are hyperchromic.



Denaturation of DNA Molecules

1. In nature, DNA usually exists in an ordered, twisted form called the native form.

2. The two strands of DNA are held together by hydrogen bonds between the base pairs.
When we heat DNA or add acid/alkali, these bonds break.
The strands separate. This process is called melting.
The change from native to denatured state is called denaturation.

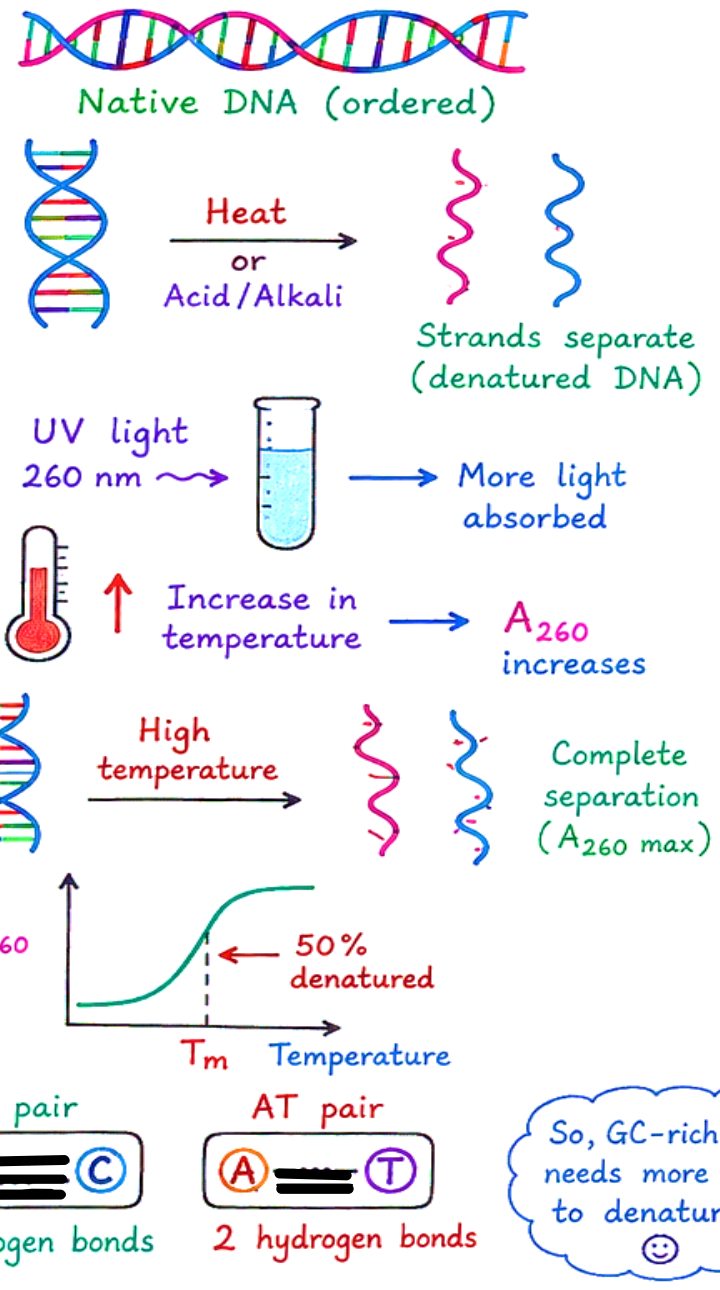
3. We can study denaturation by measuring how much UV light DNA absorbs at 260 nm.

4. When temperature increases, DNA absorbs more UV light.
So, the absorbance at 260 nm (A_{260}) goes up.

5. At a certain high temperature, the two strands are completely separated. At this point, the absorbance is maximum. This value is called $A_{260\ max}$ and shows complete denaturation.

6. The temperature at which half of the DNA is denatured (50% strands separated) is called the melting temperature (T_m).
It is a useful value to study melting.

7. DNA with more GC base pairs has a higher T_m than DNA with more AT base pairs.
This is because G-C pairs are held by 3 hydrogen bonds (stronger), while A-T pairs have 2 hydrogen bonds (weaker).



How can we achieve Denaturation

→ There are several ways (called denaturants) to make DNA strands separate.

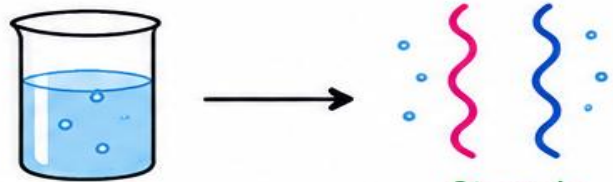
→ Different agents (heat, chemicals, extreme pH) act in different ways but all weaken the forces that hold the DNA helix together.

A. Denaturation by Temperature

- Heating DNA to high temperature (~90°C) gives enough energy to break hydrogen bonds and base-stacking interactions.
- This change from double-stranded DNA to single strands happens quickly over a small temperature range.
- Chemicals like urea or formamide make this happen even at lower temperature (faster).
- This separation of the double helix is called "melting".

B. Denaturation by Chemical Agents

- Certain chemicals like urea and formamide disrupt hydrogen bonds between base pairs.
- These chemicals pull out bases and mix with water, weakening base pairing.
- Urea weakens hydrogen bonds, so less heat is needed.
- DNA can be fully denatured by ~95% formamide at room temperature (even without heating).



C. Effect of pH on DNA

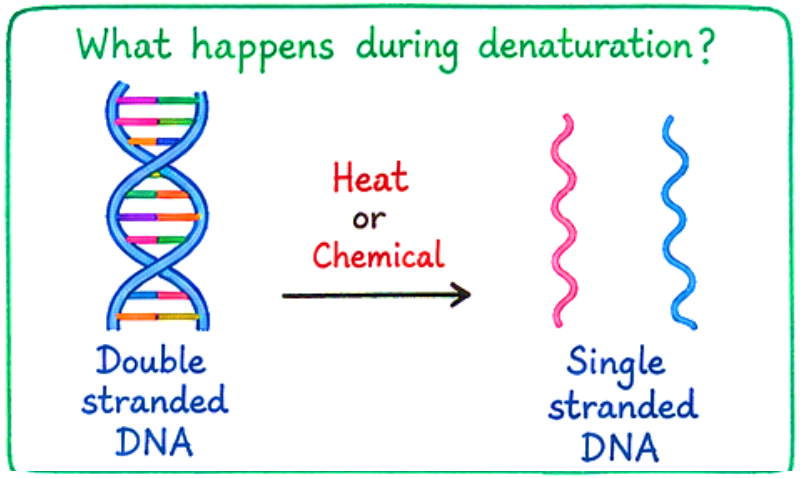
At very low pH (pH 2-3), the solution becomes acidic (more H^+).

- The amino group ($-NH_2$) in the base reacts with extra H^+ ions.
- It changes from $-NH_2$ to $-NH_3^+$ (positively charged).
- This change alters the shape and charge of the base, so it can't form correct hydrogen bonds with its partner.
- If enough bases are altered, the DNA double helix falls apart (denatures).

- At very high pH (~12), the solution is alkaline (basic).
- There are many OH^- ions and very few H^+ ions.
- OH^- ions pull H^+ from $-OH$ group → changes the group from $-OH$ (neutral) to $-O^-$ (negative).
- This also breaks hydrogen bonds → strands separate (denature).

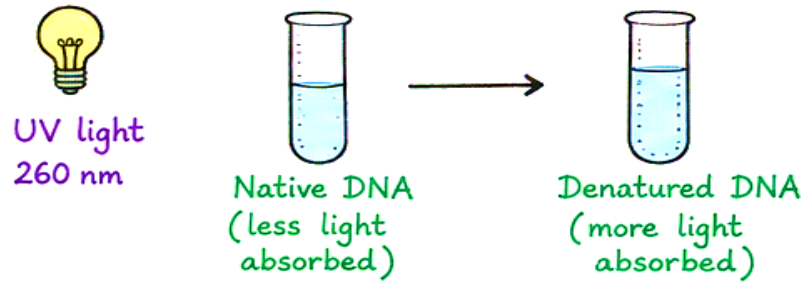
Denaturation involves changes

- Denaturation means changing the firm, double-stranded (helical) DNA into a loose, single-stranded form.
- The two strands separate because the hydrogen bonds between the bases are weak. These bonds are weaker than the bonds that hold the base to the sugar-phosphate backbone.

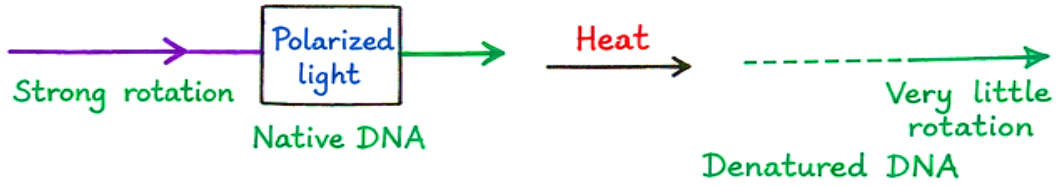


Denaturation involves the following changes:

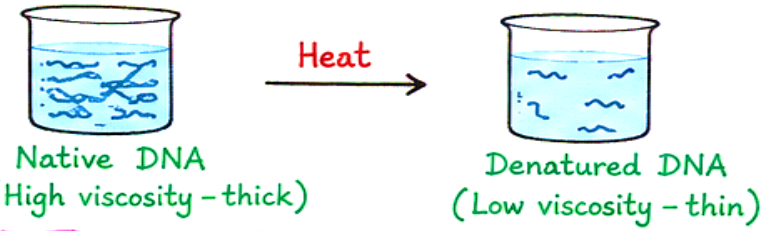
1. **Increase in absorption of ultraviolet (UV) light:**
   All bases in nucleic acids absorb UV light.
   They show maximum absorption at 260 nm.
   When DNA is denatured, the absorption of UV light increases a lot. This is called hyperchromicity.



2. **Decrease in specific optical rotation:** Native DNA rotates plane-polarized light in a strong positive direction.
   When DNA is denatured, this rotation decreases greatly.
   (Same as in proteins)



3. **Decrease in viscosity:** Native DNA solutions are thick (high viscosity) because DNA is long, rigid and double stranded.
   When DNA is denatured, the strands separate and the structure becomes loose and flexible. This causes a big decrease in viscosity.



In short:

- Denaturation = Double-stranded DNA $\xrightarrow{\text{Heat/Chemical}}$ Single-stranded DNA
- It increases UV absorption (at 260 nm).
- It decreases optical rotation.

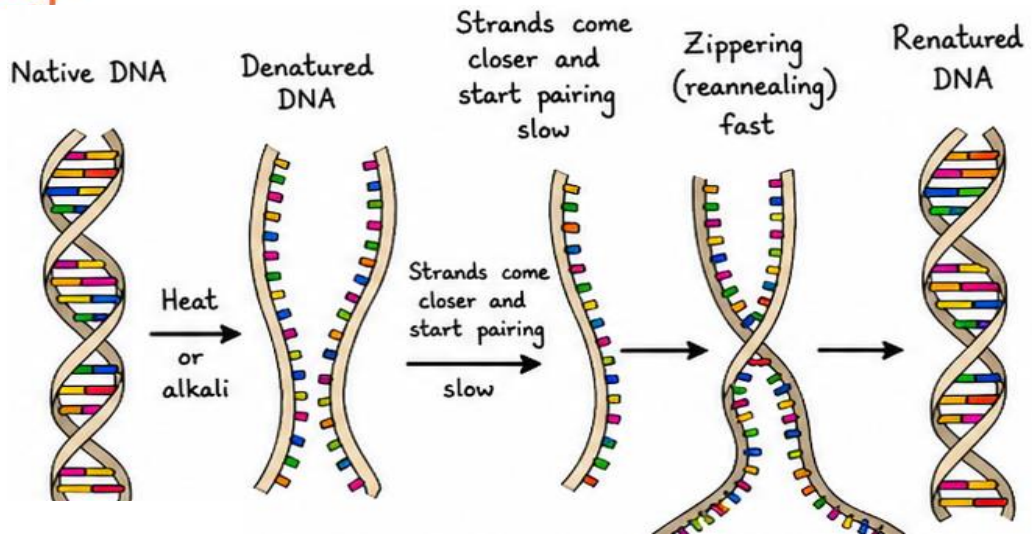
★ All these changes happen because the hydrogen bonds between the bases break during denaturation.

DNA Renaturation

- Denatured DNA will renature to re-form the duplex structure if the denaturing conditions are removed (that is, if the solution is cooled, the pH is returned to neutrality, or the denaturants are diluted out).
- Renaturation requires re-association of the DNA strands into a double helix, a process termed reannealing.
- For this to occur:

(1) The two strands must line up with each other so that their complementary bases can match again (this is called the nucleation process).

(2) The strands can twist around each other to form the double helix again.



- Renaturation is dependent on DNA concentration and time. Many of the realignments are imperfect, and thus the strands must dissociate again to allow for proper pairings to be formed.
- The process occurs more quickly if the temperature is warm enough. Warm temperature helps the large DNA molecules move (diffuse) faster in solution, so they can find each other and pair. But if the temperature is too high, the strands may separate again instead of joining.

In simple words: Warm temperature helps DNA strands meet and join faster, but not too warm, otherwise they won't stay together.

NUCLEIC ACIDS

Cot CURVES

DNA is made of two strands.

If you heat DNA, the two strands separate (this is called **denaturation**).

If you cool it back in the right conditions, the matching strands come back together (**renaturation**).

C_0t curve is just a graph that tells us:

How quickly different parts of the DNA find their partners and stick back together.

Nucleic Acid Cot Curves (C_0t Analysis)

A Cot curve is a graph used to study the complexity and sequence organization of DNA based on the rate at which single-stranded DNA reassociates (renatures) after being denatured.

① What does Cot mean?

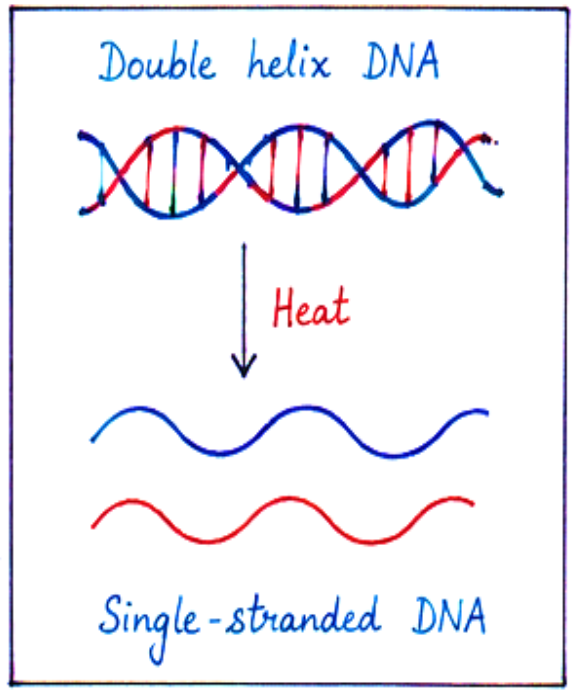
$$Cot = C_0 \times t$$

Where,

C_0 : Initial concentration of single-stranded DNA

t : Time allowed for reassociation

Thus, C_0t represents the concentration of DNA x reassociation time.



The basic principle is :

The more complex a DNA sequence is, the slower it reassociates.

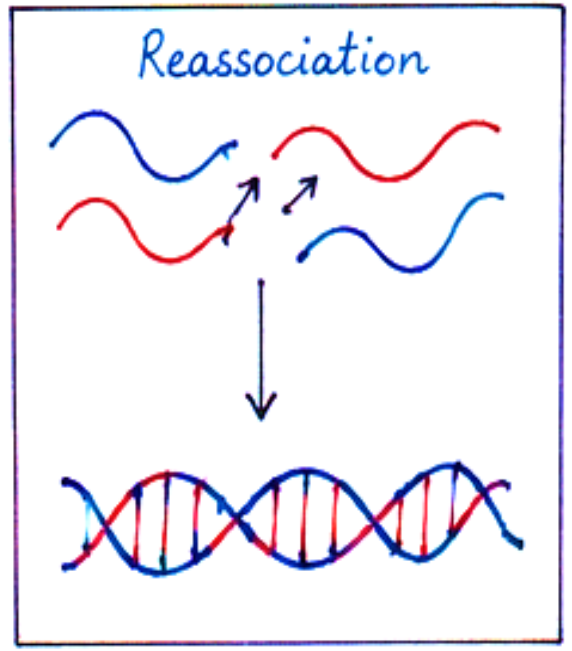
② Principle of Cot analysis

DNA is first :

Double-stranded DNA → (Heat) → Single-stranded DNA

Then the temperature is lowered so complementary strands can find each other and reassociate :

Single-stranded DNA → (Reassociation) → Double-stranded DNA



The reassociation rate depends mainly on :

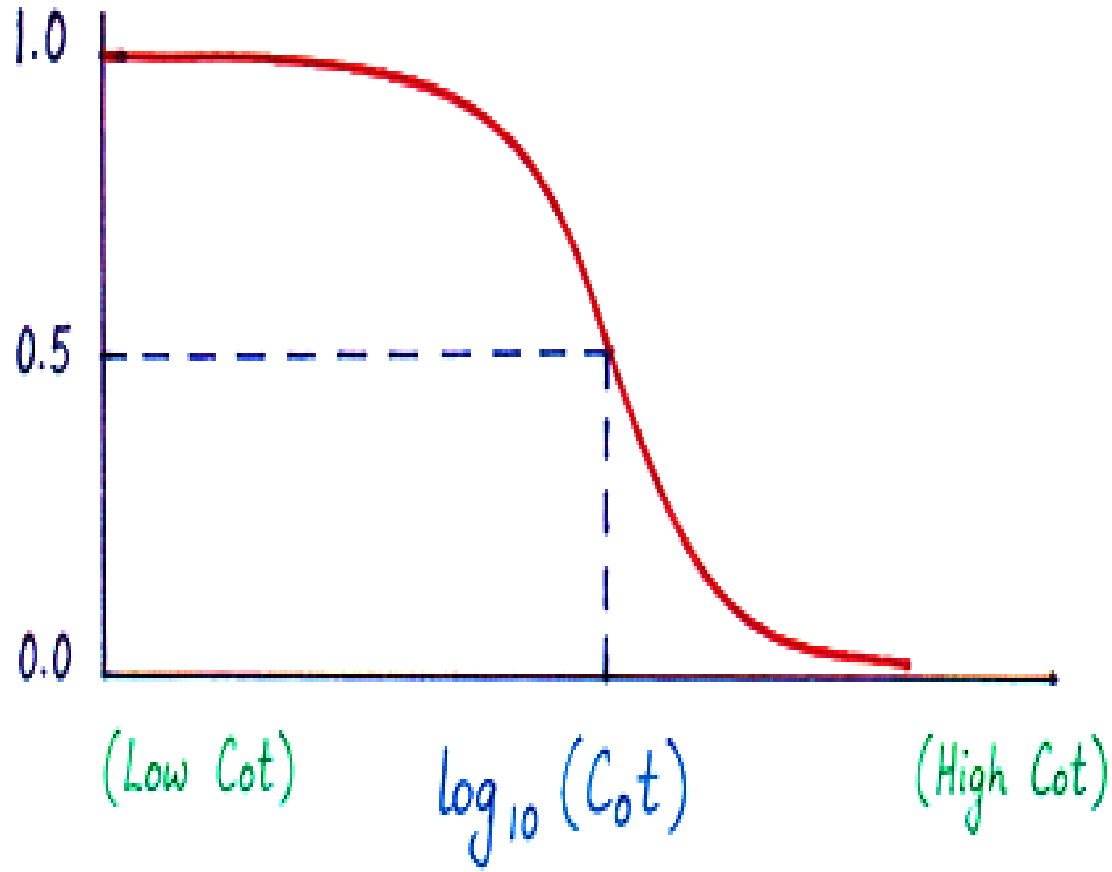
1. DNA concentration
2. Sequence complexity
3. Presence of repetitive sequences

} Highly repetitive DNA finds its complementary sequence quickly, whereas unique sequences take much longer.

Cot curve

A typical Cot curve plots :

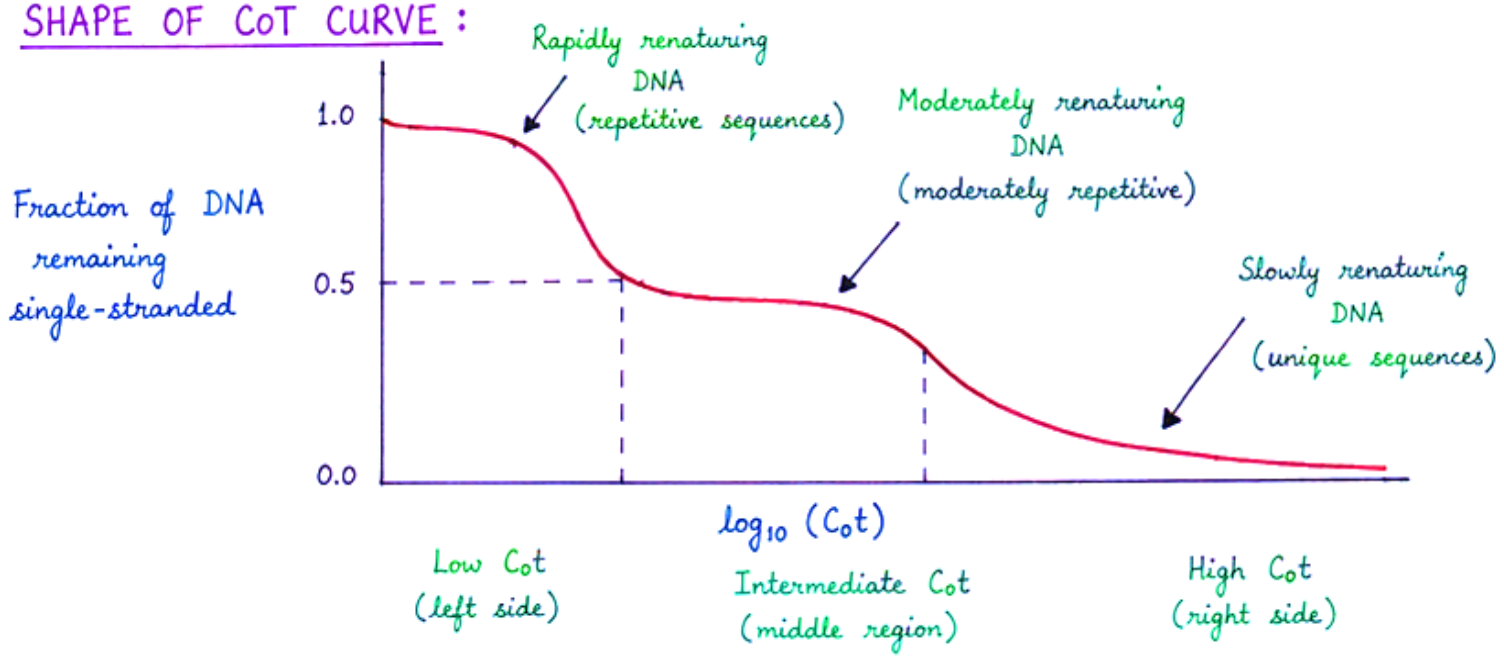
- X-axis : $\log_{10}(C_0t)$
- Y-axis : fraction of DNA remaining single-stranded



COMPONENTS OF A GENOME :

Component	Reassociation rate	Cot value
A. Highly repetitive DNA	Very fast (reassociates quickly)	Low Cot (left side)
B. Moderately repetitive DNA	Intermediate (reassociates at moderate rate)	Intermediate Cot (middle region)
C. Unique or single-copy DNA	Very slow (takes long time to reassociate)	High Cot (right side)

SHAPE OF CoT CURVE :



AS CoT INCREASES (→), MORE DNA REASSOCIATES, SO THE FRACTION REMAINING SINGLE-STRANDED DECREASES.

The curve has two (sometimes three) stages:

A. Rapidly renaturing DNA (low C_0t, left side of the curve)

- These are **repetitive sequences** — they appear many times in the genome.
- Because there are many identical copies, they find a partner very fast.
- Example: Satellite DNA, rRNA gene repeats.

B. Slowly renaturing DNA (high C_0t, right side of the curve)

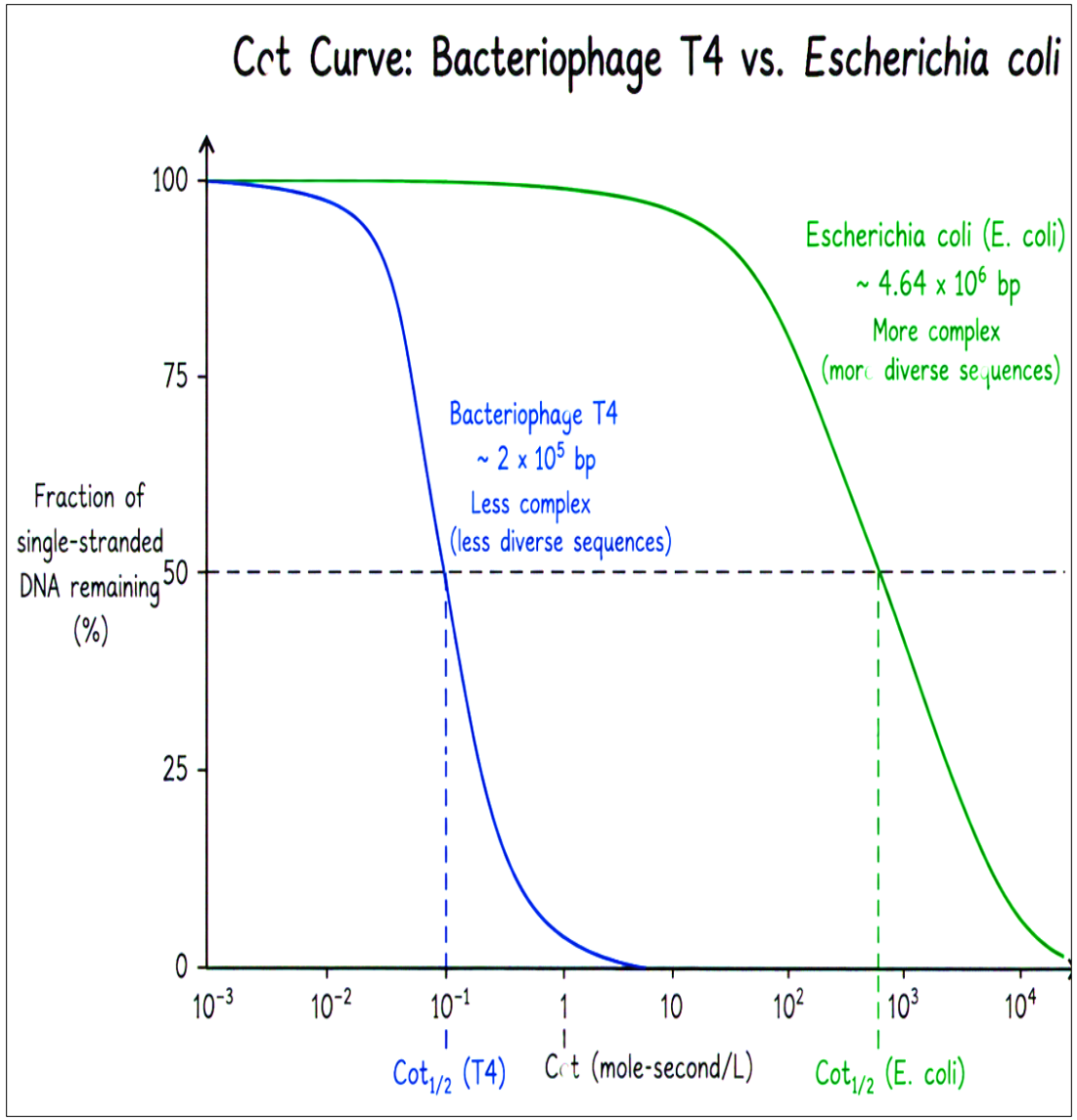
- These are **single-copy sequences** — each piece is unique.
- They have only one correct partner, so finding it takes much longer.
- Example: Most protein-coding genes.

WHAT THE SHAPE MEANS ?

- Left side (low Cot): Rapid drop → many strands find partners quickly → highly repetitive DNA.
- Middle region : Gradual drop → moderately repetitive sequences.
- Right side (high Cot): Slow drop → unique sequences need much larger Cot to reassociate.

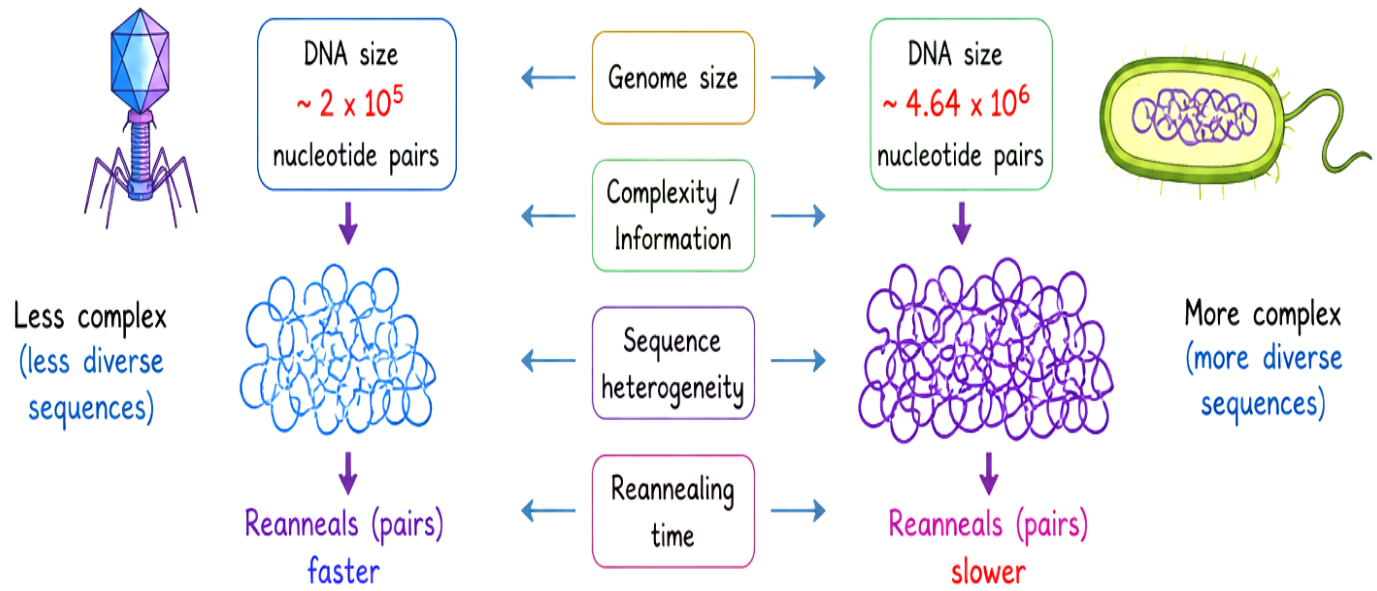
IMPORTANCE :

Cot analysis helps to estimate the complexity of genome and the proportion of repetitive vs unique DNA.



- Bacteriophage T4 has a relatively small DNA genome containing about 2×10^5 nucleotide pairs.
- Escherichia coli (E. coli) has a much larger and more complex DNA genome containing about 4.64×10^6 nucleotide pairs.
- Because E. coli DNA is more complex, it carries more genetic information.
- For the same amount of DNA (in grams), the sequences in E. coli are more heterogeneous (more different from each other) than the sequences in T4 DNA.
- Therefore, it takes E. coli DNA strands longer to find their complementary partners and reanneal (pair again) compared to T4 DNA.
- This whole situation can be studied and measured quantitatively.

Diagrammatic Representation



In short: E. coli DNA is bigger, more complex, and more diverse than T4 DNA. So, for the same amount of DNA, E. coli takes longer to reanneal.

$Cot_1/_2$ value

An important parameter is **$Cot_1/_2$**.
$Cot_1/_2$ = Cot value at which 50% of the DNA has reassociated

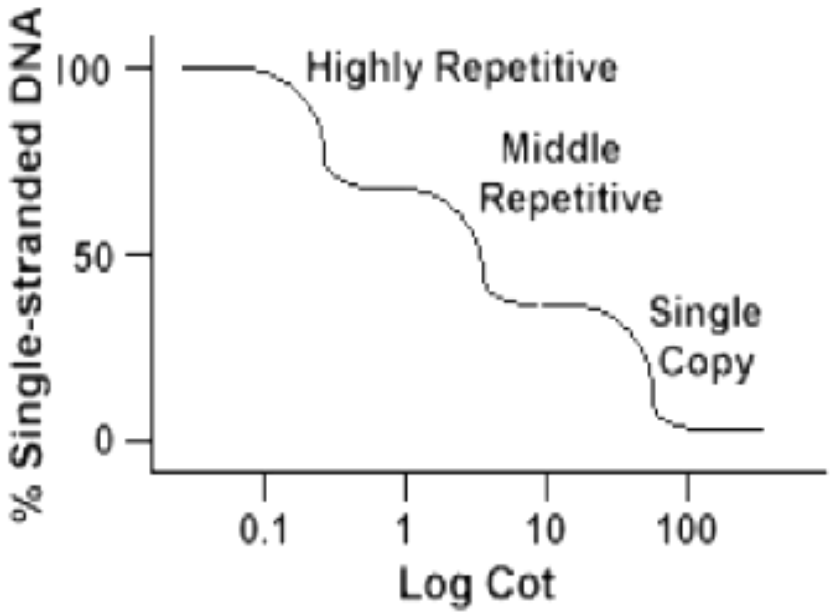
It is useful for comparing the **sequence complexity** of DNA.

Generally:
Low $Cot_1/_2$ → repetitive DNA
High $Cot_1/_2$ → unique/complex DNA

Relationship between Cot value and genome complexity

DNA type	Copy number	Reassociation	Cot value
Highly repetitive	Very high	Very fast	Low
Moderately repetitive	Intermediate	Intermediate	Intermediate
Unique DNA	Low	Slow	High

Applications of Cot curve analysis



Cot curve

Understanding genome size and complexity

Understanding complexity of sequences

Understanding relative proportion of single copy and repetitive sequences

Cot analysis has been particularly useful for understanding **eukaryotic genomes**.

It helped demonstrate that many eukaryotic genomes contain substantial amounts of:

- Repetitive DNA
- Moderately repetitive DNA
- Unique DNA

It can also help compare genome organization between organisms.

- So, Cot analysis tells us

how "repetitive" or "unique" a genome is by observing how quickly its DNA strands find and reassociate with their complementary sequences.

SEPARATION OF REPETITIVE DNA AND UNIQUE DNA

(Using Hydroxyapatite Column after Cot Analysis)

We want to separate repetitive DNA (fast-renaturing) from unique DNA (slow-renaturing) after heating and cooling the DNA.

The key property

- Hydroxyapatite (a calcium phosphate mineral) sticks to double-stranded DNA.
- Single-stranded DNA does not stick and just flows through.

= Double-stranded DNA (sticks to column)

= Single-stranded DNA (flows through)

★ Why this works

The timing is important:

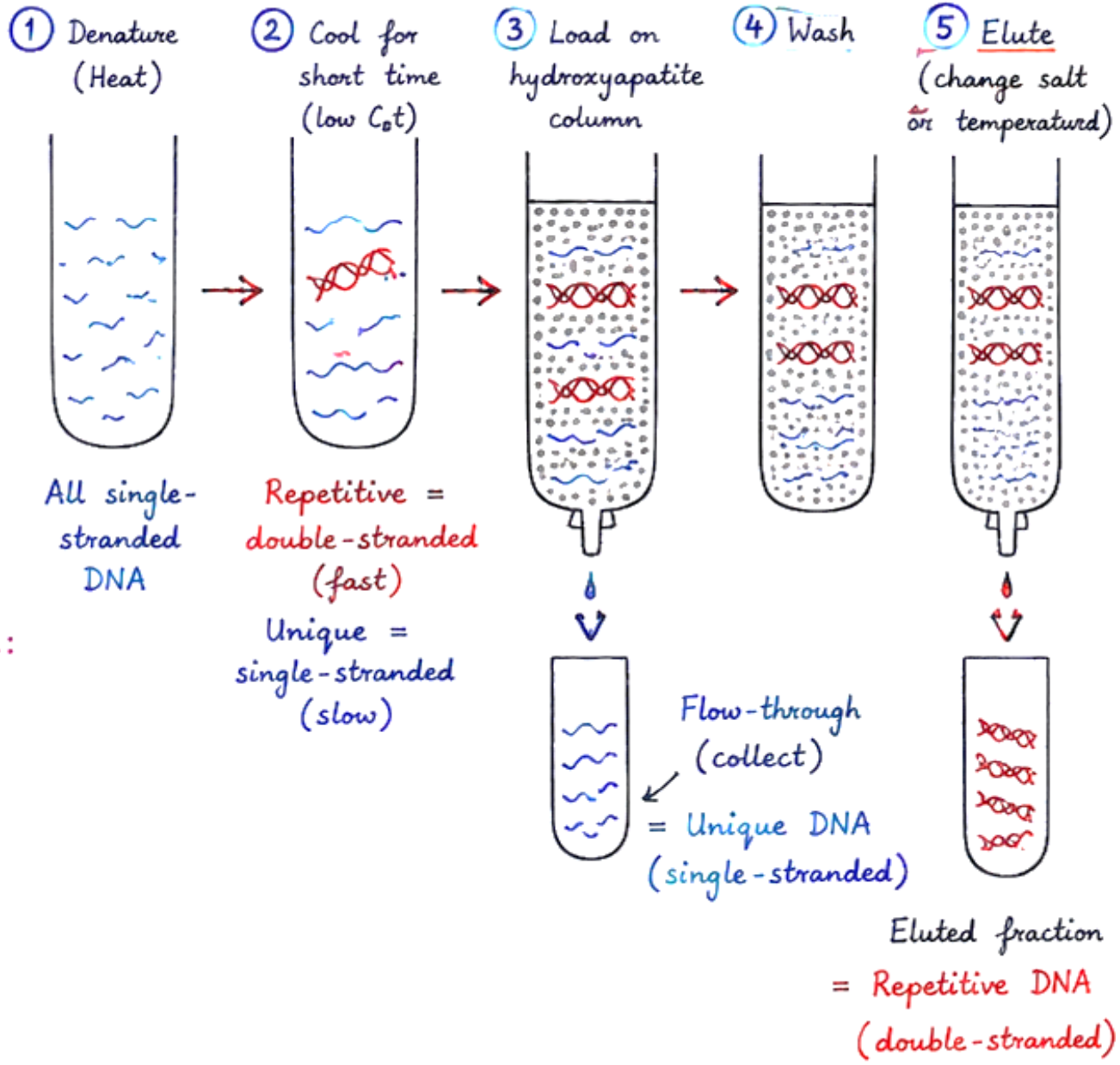
- If you let renaturation go on too long, even unique sequences will find their partners and you won't be able to separate them.
- By stopping early, only repetitive DNA has reannealed.

Low C_0t → only repetitive DNA reanneals.

★ The process

1. Start with fragmented genomic DNA (short pieces so they can't re-form loops within the same strand).
2. Heat the DNA to make it single-stranded (denaturation). Now all DNA — repetitive and unique — is separated.
3. Cool the DNA for a short time (low C_0t value).
   - Repetitive sequences: many copies → they find partners quickly → become double-stranded.
   - Unique sequences: only one copy → still searching → remain single-stranded.
4. Pass the sample through the hydroxyapatite column:
   - Double-stranded DNA (repetitive fraction) sticks to the column.
   - Single-stranded DNA (unique fraction) passes through and is collected.
5. Elute (wash off) the double-stranded DNA later by changing salt or temperature — now you have the repetitive fraction separated.

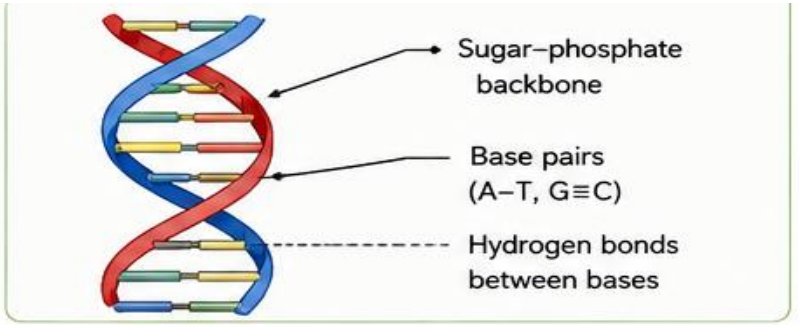
Steps shown in a column

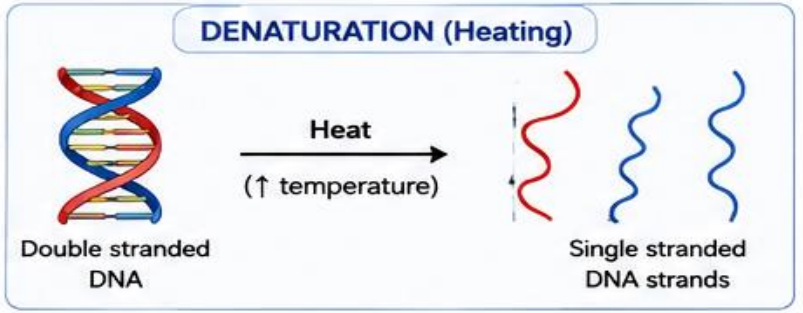


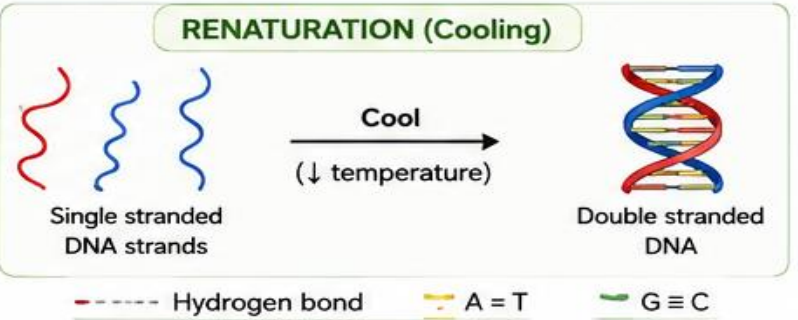
DIFFERENCES BETWEEN DENATURATION AND RENATURATION OF DNA

	DENATURATION	RENATURATION (ANNEALING)
1	Denaturation is the process of formation of **single stranded DNA** from double stranded helical DNA.	Renaturation is the process of formation of **double stranded DNA** from single stranded DNA strands.
2	It is effected by **heating**.	It is effected by **cooling**.
3	It involves **breakage of hydrogen bonds** between complementary base pairs.	It involves **reannealling or formation of hydrogen bonds** between complementary base pairs.
4	The **increase** in absorbance (A_{260}) upon denaturation is called as **hyperchromic effect**.	There is a **decrease** in absorbance (A_{260}) upon renaturation.
5	The rate of increase in absorbance is **directly proportional** to the rate of denaturation.	The rate of renaturation is **directly proportional** to the **concentration of complementary sequences**.
6	Upon denaturation, **viscosity decreases**.	Upon renaturation, **viscosity increases**.

Denaturation = Heat → Break H-bonds → ssDNA → ↑A_{260} → ↓Viscosity | Renaturation = Cool → Form H-bonds → dsDNA → ↓A_{260} → ↑Viscosity







IMPORTANT CONSEQUENCES

- Denaturation causes strand separation, loss of biological activity (e.g., loss of ability to replicate).
- Renaturation restores double helix and biological activity.
- **Hyperchromic effect** is widely used to monitor DNA denaturation and renaturation.
- **Hydrogen bonds** are the key forces broken during denaturation and reformed during renaturation.