Denaturation and Renaturation Of DNA
Cot Curves, Hypochromaticity and Hyperchromaticity of DNA

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To understand the functional significance of nucleic acids we must understand its chemical properties. We know that DNA is the store house of genetic information and this role can be partly due to its inherent stability. So, what makes this DNA a stable molecule? Actually DNA undergoes chemical transformation very slowly. Although the changes acquired are very slow but get accumulated over a period of time. For example, ageing where slowly accumulated changes over the years result in irreversible modifications in DNA. Here we are not considering mutations. Mutations are sudden, heritable changes in the genetic material. It may be spontaneous or induced.

However, certain transient and non destructive alterations also occur in DNA, like strand separation, which is very much needed during replication and transcription. Moreover, these alterations are reversible and transient in nature.

Nucleic acid absorbs UV light at 260 nm. For example, if we have three solutions (i) double-stranded DNA, (ii) single stranded DNA, and (iii) free bases, each at 50µg/ml, absorption values at 260nm (A_{260}) are like this:

Double stranded DNA	$A_{260} = 1.00$
Single stranded DNA	$A_{260} = 1.37$
Free bases	$A_{260} = 1.60$

The question which comes to our minds is why so much variation in the absorption values when concentration is same? This can be very well understood in terms of denaturation and renaturation properties of nucleic acids.

Denaturation is the separation of the two component strands of a double stranded DNA in a solution by increasing the temperature (above 80°C) or decreasing the salt concentration (alteration of pH). During nucleic acid denaturation, hydrogen bonds between the paired bases are disrupted causing unwinding of the double helix resulting in two single strands (Fig. 14.1). You must note that during denaturation process **no covalent bonds in the nucleic acids are broken**. When a nucleic acid molecule undergoes denaturation, its viscosity decreases sharply, indicating that the DNA has undergone a physical change.

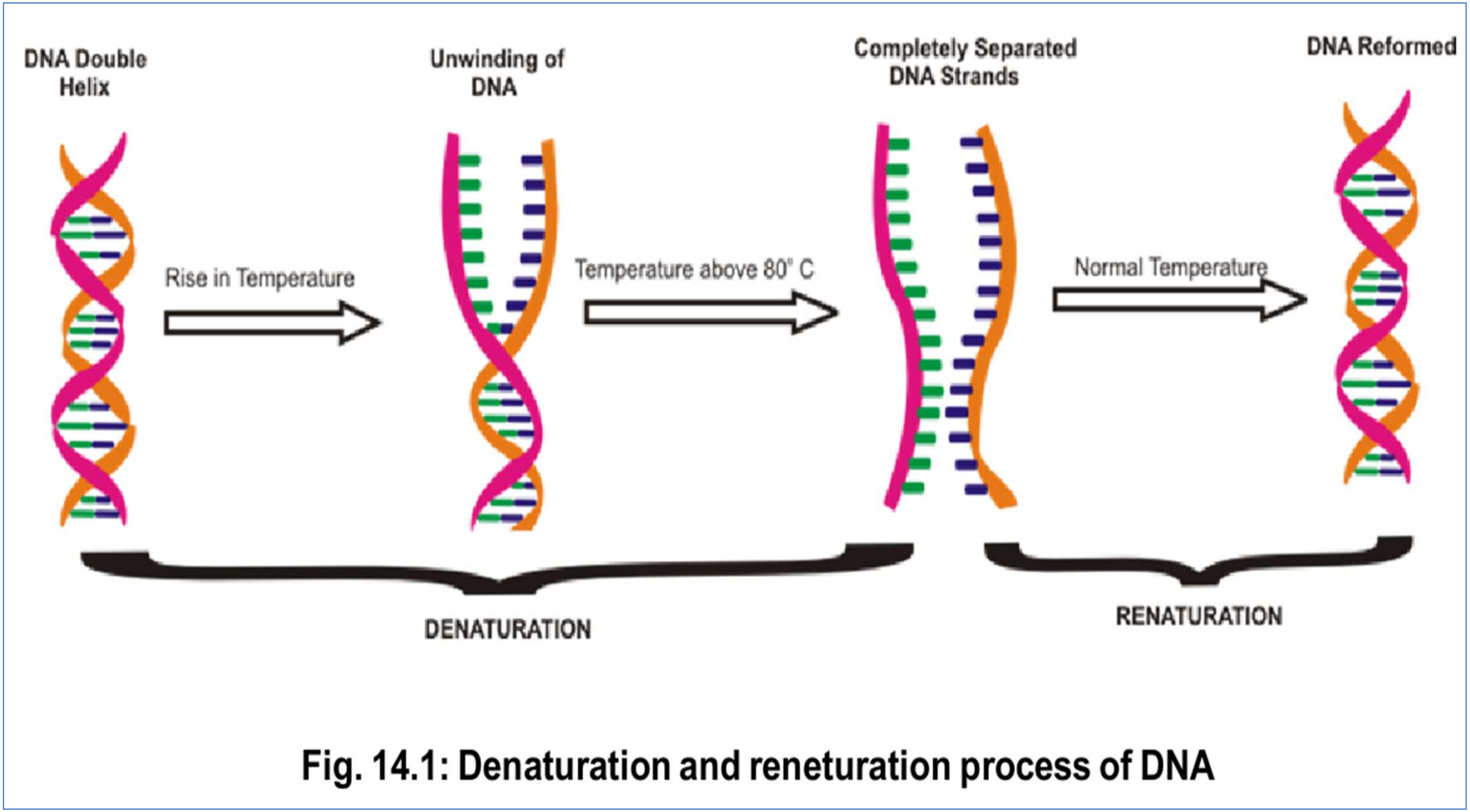


Fig. 14.1: Denaturation and reneturation process of DNA

Denaturation of the DNA molecule results in the **increase of the optical absorbance** of the purine and pyrimidine bases - a phenomenon known as **hyperchromicity**. Under physiological conditions, interaction between stacked bases in a nucleic acid leads to the decrease in the absorption of UV light relative to that of a solution with the same concentration of free nucleotides. The absorption of UV light is decreased further when two complementary nucleic acid strands are paired and this is known as **hypochromic effect**. This is why absorption values of free bases > single stranded DNA > double stranded DNA.

Thus, transition from double strand to single strand i.e. denaturation can be detected by monitoring the absorption of the UV light.

Renaturation or annealing is a process during which the separated strands of DNA join together under appropriate physiological temperature and salt conditions. It is a spontaneous, rapid and one step process. The unwound segments of the two strands spontaneously rewind/anneal, to yield the double helix. But, if the two strands are completely separated, renaturation occurs in two steps. First step is relatively slow; the two strands "find" each other by random collisions and form a short segment of complementary double helix (Fig. 14.1). The second step is much faster where the remaining unpaired bases successively pair themselves together to form the double helix.

Denaturation and renaturation are the basis of **hybridization**. This technique is a powerful tool useful in determining identical or related sequences of DNA or RNA among two different species or within the genome of a single species.

The ability of DNA to anneal has been very beautifully used for investigating genetic similarity between pools of DNA sequences. Closely related sequences form base pairs are said to be complementary. The complementarity of sequences reveals genetic closeness. When genome of several species is compared, genetic distance between two species can be determined. The more closely related the two species are, lesser is the mis-pairing in hybridization (Fig. 14.2).

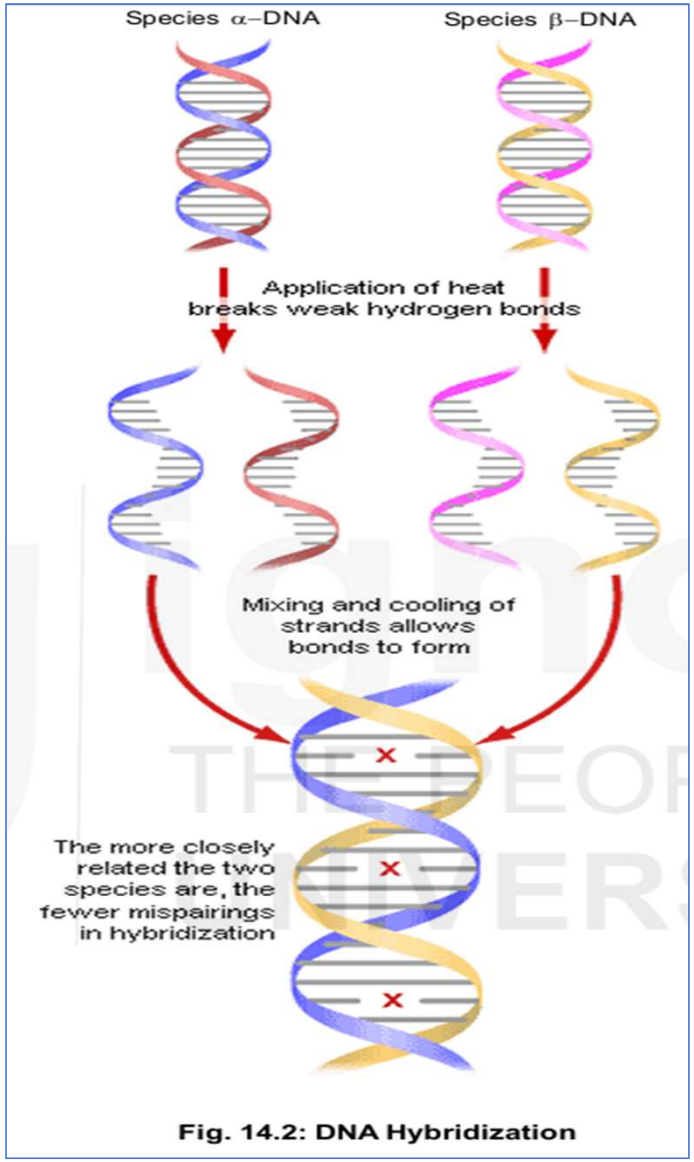


Fig. 14.2: DNA Hybridization

Hyperchromicity / Hyperchromic effect

1. Hyperchromicity is the increase of absorbance (optical density) of a material.
2. The most famous example is the hyperchromicity of DNA that occurs when the DNA duplex is denatured.
3. The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level.
4. Heat denaturation of DNA, also called melting, causes the double helix structure to unwind to form single stranded DNA.
5. When DNA in solution is heated above its melting temperature (usually more than 80 °C), the double stranded DNA unwinds to form single-stranded DNA.
6. The bases become unstacked and can thus absorb more light.
7. In their native state, the bases of DNA absorb light in the 260-nm wavelength region.

8. When the bases become unstacked, the wavelength of maximum absorbance does not change, but the amount absorbed increases by 37%.

9. A double stranded DNA strand dissociating to two single strands produces a sharp cooperative transition.

10. The phenomenon of UV absorbance increasing as DNA is denatured is known as the hyperchromic shift.

11. Hyperchromicity can be used to track the condition of DNA as temperature changes.

12. The transition/melting temperature (Tm) is the temperature where the absorbance of UV light is 50% between the maximum and minimum, i.e. where 50% of the DNA is denatured. A ten fold increase of monovalent cation concentration increases the temperature by 16.6 °C.

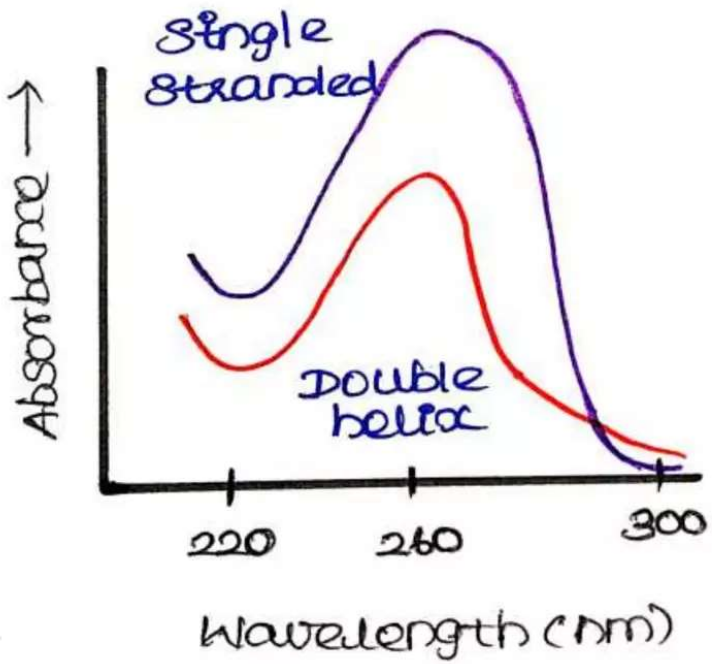


Fig.2.The absorbance spectra of a DNA in the solution at 260nm and pH 7.

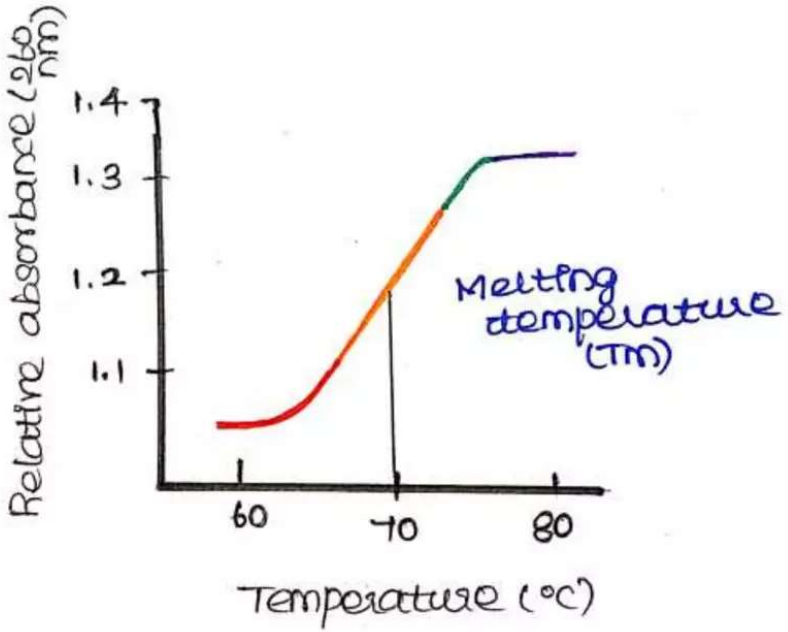


Fig.3.DNA melting curve

Hypochromicity

- Hypochromicity describes a material's decreasing ability to absorb light.

Hypochromic effects

1. The Hypochromic Effect describes the decrease in the absorbance of ultraviolet light in a double stranded DNA compared to its single stranded counterpart.
2. Compared to a single stranded DNA, a double stranded DNA consists of stacked bases that contribute to the stability and the hypochromicity of the DNA.
3. When a double stranded DNA is denatured, the stacked bases break apart an thus becomes less stable.
4. It also absorbs more ultraviolet light since the bases no longer forms hydrogens bonds and therefore are free to absorb light.

5. Ways to denature DNA include high temperature, addition of denaturant, and increasing the pH level.

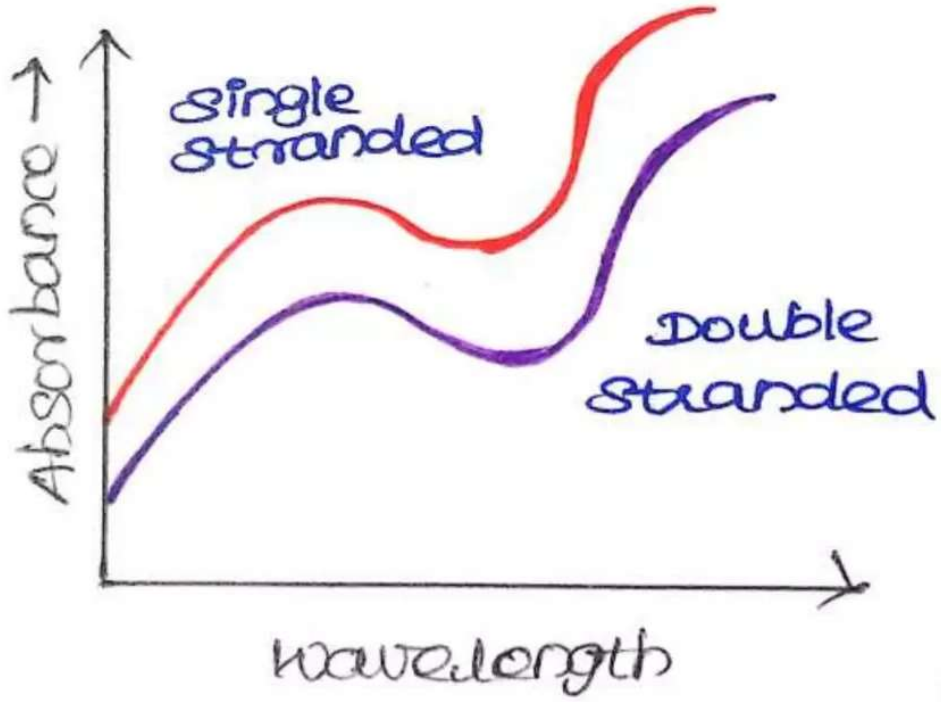


Fig.4. Hypochromic effect of DNA

Importance of hypochromic effect

1. The measurement of absorption of light is important in monitoring the melting and annealing of DNA.
2. At the melting temperature (Tm), the DNA is half denatured and half double stranded.
3. By lowering the temperature below the Tm, the denatured DNA strands would anneal back into a double stranded DNA. When temperature is above the Tm, the DNA is denatured.

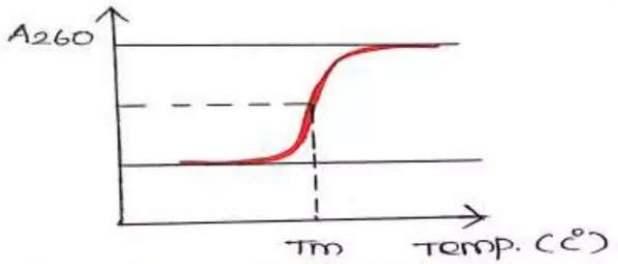


Fig.5. Nucleic acid melting curve showing hyperchromicity as a function of temperature

4. Because melting occurs almost instantly at a certain temperature, monitoring the absorbance of the DNA at various temperature would indicate the melting temperature.

5. By being able to find the temperature at which DNA melted and annealed, scientists are able to separate DNA strands and anneal them with other DNA strands.

6. This is important in creating hybrid DNAs, which consists of two DNA strands from different sources.

7. Since DNA strands can only anneal if they are similar, the creation of hybrid DNAs can indicate similarities between genomes of different organisms.

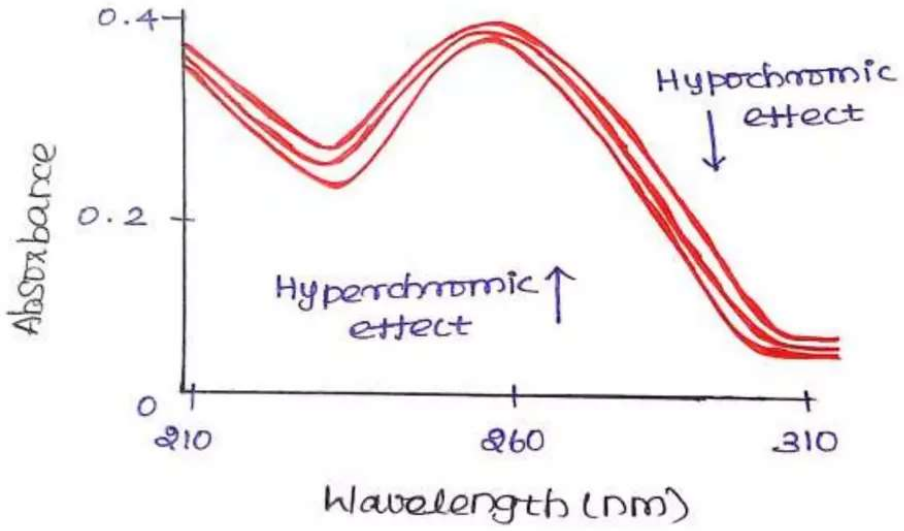


Fig.6 Hypo and Hyperchromic effect of DNA

Cot curve

History

- Cot analysis was first developed and utilized in the mid 1960s by Roy Britten, Eric Davidson, and associates.

Cot analysis

- It is based upon the principles of DNA renaturation kinetics.

DNA Renaturation Kinetics

- The rate at which heat-denatured DNA sequences in solution will renature is dependent on DNA concentration, reassociation temperature, cation concentration, and viscosity (usually not a factor if the DNA is free of contaminants).

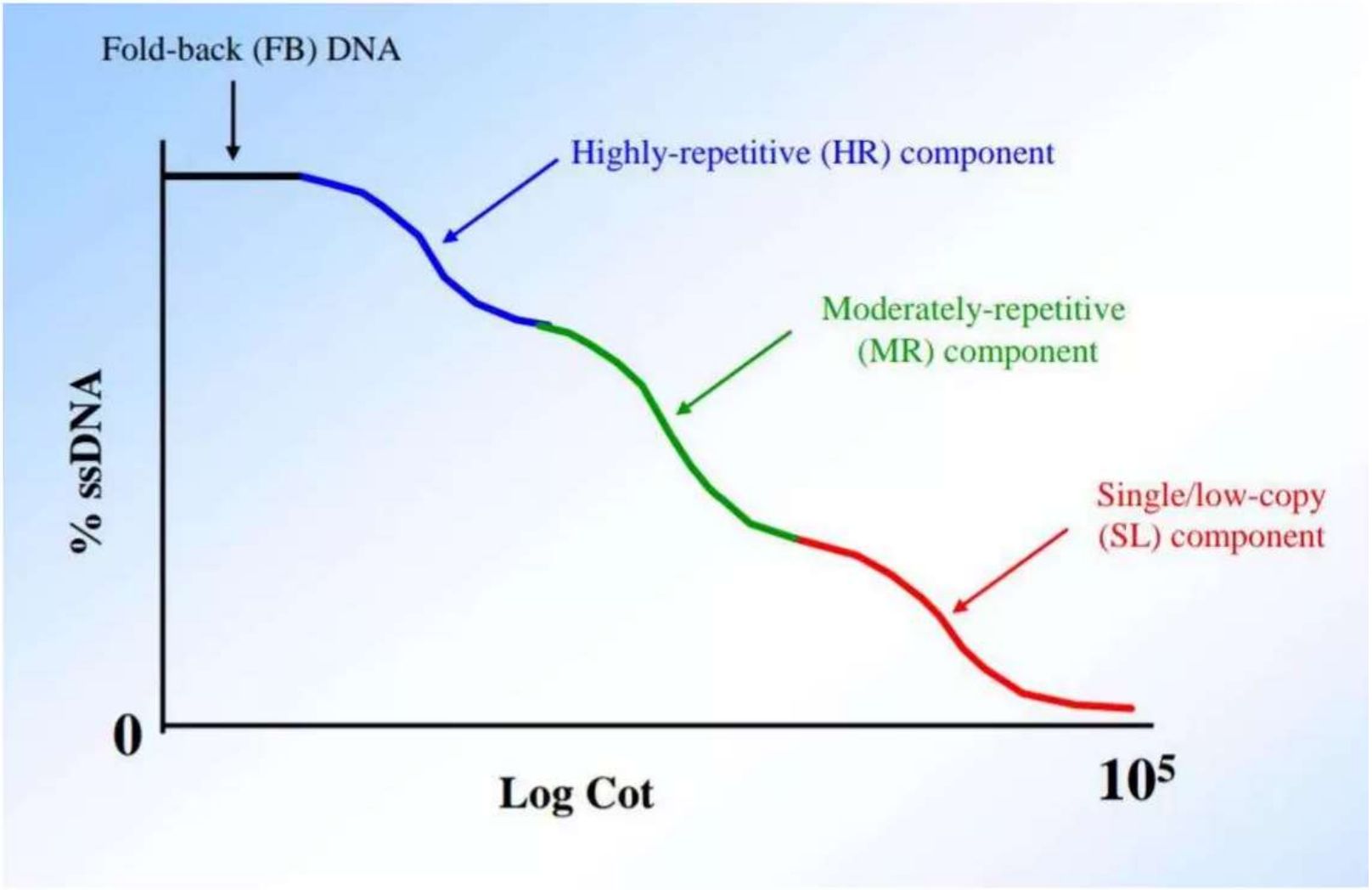
• Cot = DNA conc. (mol/L) x renaturation time in sec x a buffer factor that accounts for the effect of cations on the speed of renaturation

Procedure

1. The procedure involves heating a sample of genomic DNA until it denature into the single stranded-form, and then slowly cooling it, so the strands can pair back together.
2. While the sample is cooling, measurements are taken of how much of the DNA is base paired at each temperature.
3. The amount of single and double-stranded DNA is measured by rapidly diluting the sample, which slows reassociation, and then binding the DNA to a hydroxylapatite column.
4. The column is first washed with a low concentration of sodium phosphate buffer, which elutes the single-stranded DNA, and then with high concentrations of phosphate, which elutes the double stranded DNA.
5. The amount of DNA in these two solutions is then measured using a spectrophotometer.

Analysis

1. Since a sequence of single-stranded DNA needs to find its complementar strand to reform a double helix, common sequences renature more rapidly than rare sequences.
2. Indeed, the rate at which a sequence will reassociate is proportional to the number of copies of that sequence in the DNA sample.
3. A sample with a highly-repetitive sequence will renature rapidly, while complex sequences will renature slowly.
4. However, instead of simply measuring the percentage of double-stranded DNA versus time, the amount of renaturation is measured relative to a C_0t value.
5. The C_0t value is the product of C_0 (the initial concentration of DNA), t (time in seconds), and a constant that depends on the concentration of cations in the buffer.
6. Reptitive DNA will renature at low C_0t values, while complex and unique DNA sequences will renature at high C_0t values.
7. The fast renaturation of the repetitive DNA is because of the availability of numerous complementary sequences.


Fold-back (FB) DNA
Highly-repetitive (HR) component
Moderately-repetitive
(MR) component
Single/low-copy
(SL) component
% ssDNA
0
Log Cot
10^5

Application to genome sequencing

1. C_0t filtration is a technique that uses the principles of DNA renaturation kinetics to separate the repitative DNA sequence that dominate many eukaryotic genome from "gene-rich" single/low-copy sequences
2. This allows DNA sequencing to concentrate on the parts of the genome that are most informative and interesting, which will speed up the discovery of new genes and make the process more efficient.


The future belongs to those who believe in the beauty of their dreams.
-Eleanor Roosevelt