DEPARTMENT OF BIOTECHNOLOGY

"BASIC IDEA OF COT CURVES"

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- The *Cot* value is the product of *Co* (the initial concentration of DNA) and *t* (time in seconds), and a constant that depends on the concentration of cations in the buffer used.
- **Cot = DNA Concentration (Mole per L) x Renaturation time in seconds x Buffer factor** [Buffer factor accounts for the effects of cations on the speed of renaturation]

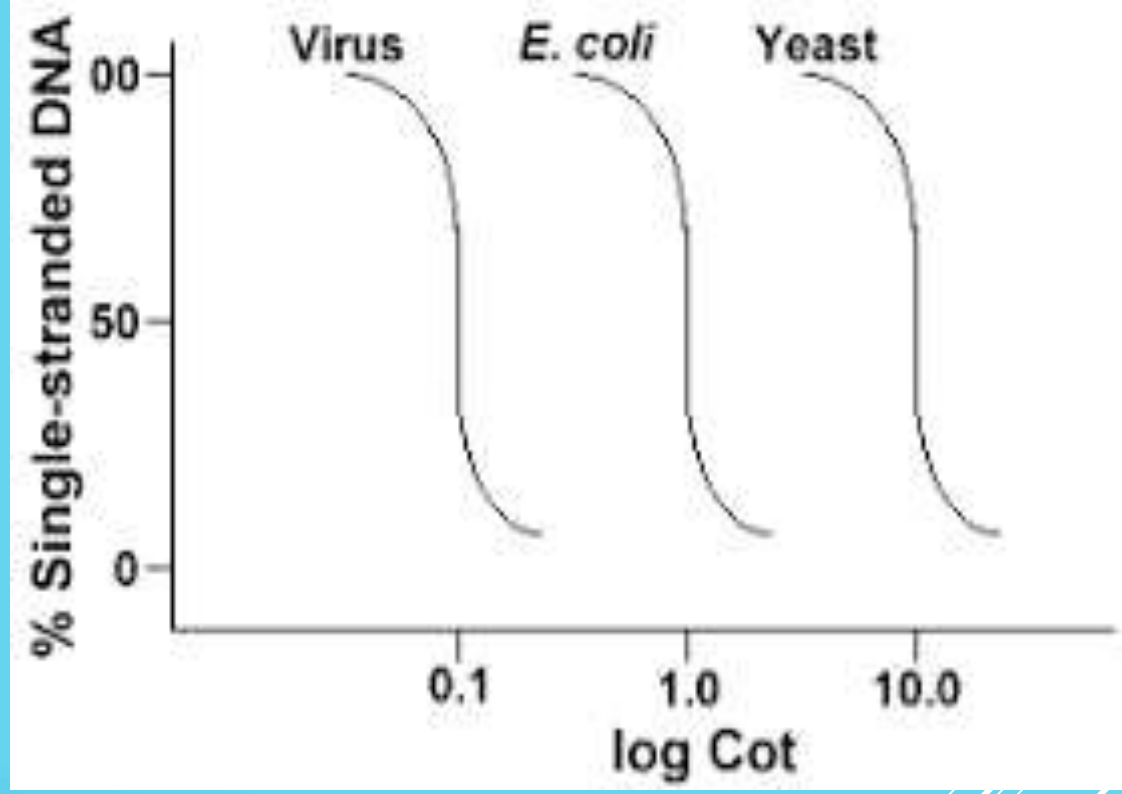
- Low *Cot* values indicate more number of repetitive sequences whereas
- High *Cot* values indicate more number of unique sequences or less number of repetitive sequences

➢ Examples :

Cot Curve is a curve (graphical presentation) that indicates the rate of DNA - DNA annealing (re-association or renaturation) as a function of DNA concentration and time

Renaturation often depends upon the factors like DNA concentration, type of DNA molecules, re-association temperature, cation concentration and viscosity.

If we plot the "Cot" curves of the genome of three species such as bacteriophage lambda, (it is not an organism though) E. coli and yeast we will see that they have the same shape, but the Cot1\2 of the yeast will be largest, E. coli next and lambda smallest. Physically, the larger genome size, the longer it will take for any one sequence to encounter its complementary sequence in the complementary sequences must species encounter each other before they can pair. The more complex the genome, that is the more unique sequences that are available, the longer it will take for any two complementary sequences to encounter each other and pair. Given similar concentrations in solution, it will then take a more complex species longer to reach Cot1/2.

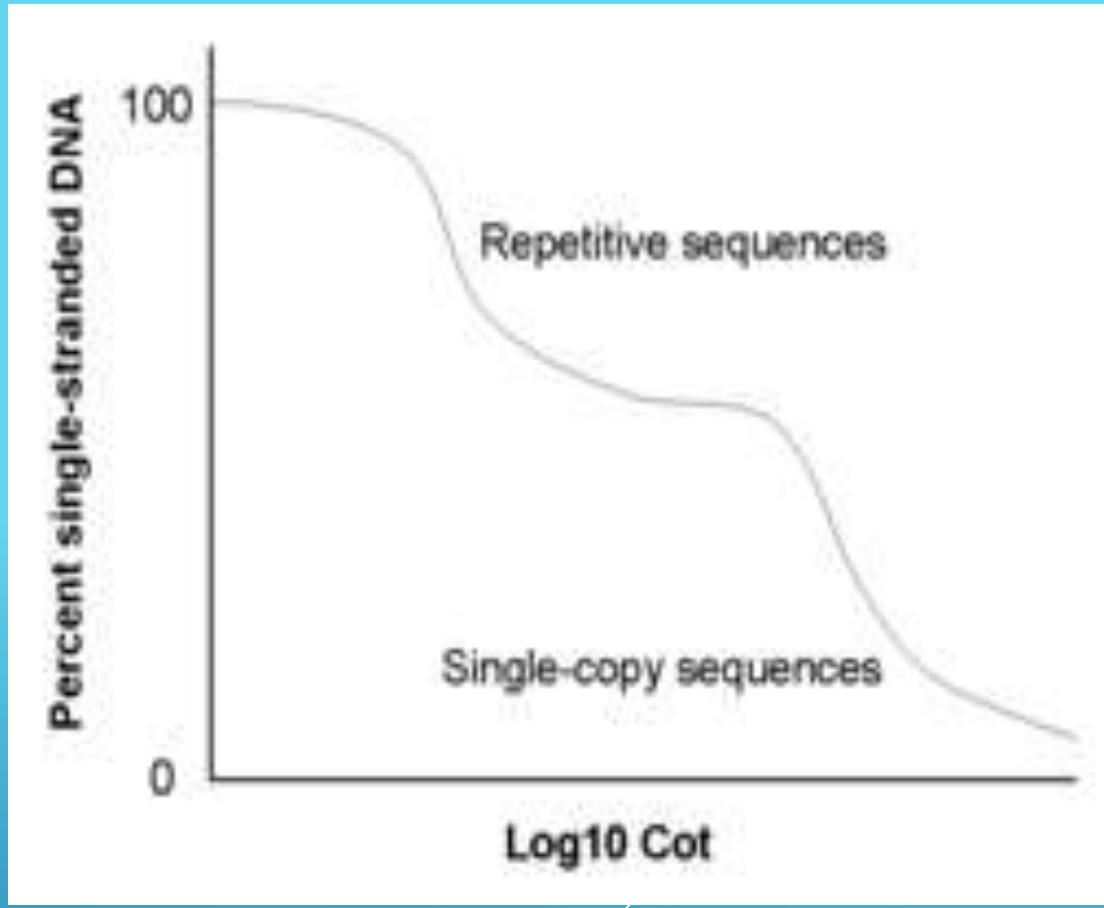


Repeated DNA sequences, DNA sequences that are found more than once in the genome of the species, have distinctive effects on "Cot" curves. If a specific sequence is represented twice in the genome it will have two complementary sequences pair with, and as such will have a Cot value half as large as a sequence represented only once in the genome.

- It is a common knowledge that the single-stranded DNA will take more time to reassociate or renature as it will need more time to find its complementary strand.
- On the contrary, the double-stranded DNA, after dissociation, will reassociate (renature or anneal) more rapidly than the single-stranded DNA as it can get its complementary strand faster.
- Further, the highly repetitive DNA sequences will reassociate much faster than even the double-stranded DNA.
- Common sequences will renature more rapidly than rare sequences (uncommon) e.g., single-copy functional DNA.
- Larger DNA strands will take more time to anneal as compared to the smaller ones.
- Thus we may conclude that the rate at which a sequence will reassociate is proportional to the number of copies of that sequence in the DNA sample.

Eukaryotic genomes usually have multiple components, which generate complex Cot curves.

Cot analysis is a technique based on the principles of DNA re-association **(renaturation or annealing)** kinetics. It is a biochemical technique that measures how much repetitive DNA is present in a DNA sample such as a genome. It is used to study genome structure and organization. It is also used to simplify the sequencing of genomes that contain large amounts of repetitive or satellite DNA sequences. It was first developed and used in 1960s by at lower Cot values than single-copy sequences. Roy Britten and Eric Davidson et al.



- This process involves heating a sample of genomic DNA so as to denature i.e. **(dissociate the double-stranded DNA into single-stranded molecules (Over 90°C).**
- It is followed by slow cooling so that the separated single strands can pair back.
- During this cooling the readings are taken as to how much of the original double stranded DNA is base-paired at different temperatures.
- The amount of single stranded DNA and the double stranded DNA is measured by rapidly diluting the sample, which slows down the re-association and then binding the DNA to a **hydroxyapatite (hydroxyl end member of the complex apatite group)** column.

The column is first washed with a low concentration of sodium phosphate buffer; this brings about **elution** (**the process of extracting one material from another by washing with a solvent**) of single stranded DNA; this is followed by high concentration of the buffer which helps **elute [remove an adsorbed substance by washing with a solvent]** the double stranded DNA.

- The amount of single stranded and double stranded DNA is measured **spectrophotometrically (An instrument used to determine the relative intensity of various wavelengths in a spectrum of light)** .
- Instead of simply measuring the percentage of double stranded DNA against time, the amount of **renaturation (the reconstruction of a protein or nucleic acid (such as DNA) to their original form especially after denaturation)** is measured relative to a Cot value.

Repetitive DNA will show fast renaturation because of the availability of numerous complimentary sequences. It will renature at low Cot values, while complex and unique DNA sequences will renature at high Cot values.

COT VALUE CAN BE CALCULATED AS UNDER WITH AN EXAMPLE :

- ***Cot* = DNA Concentration (Moles per L) x Renaturation time in seconds x Buffer factor** [Buffer factor accounts for the effects of cations on the speed of Renaturation].

Nucleotide Concentration = 0.050 M

Renaturation time = 344 seconds

Buffer Factor = 5.820

Therefore, **Cot value = 0.050 x 344 x 5.820**

= 100.104

APPLICATIONS OF COT CURVE ANALYSIS :

It is highly useful for the following :

Understanding Genome size and its complexity; Complexity of sequences; Relative proportion of single-copy and Repetitive sequences.

THANK YOU