

Cot Analysis: An Overview



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

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

The same Cot value can be reached in a variety of ways

For example:

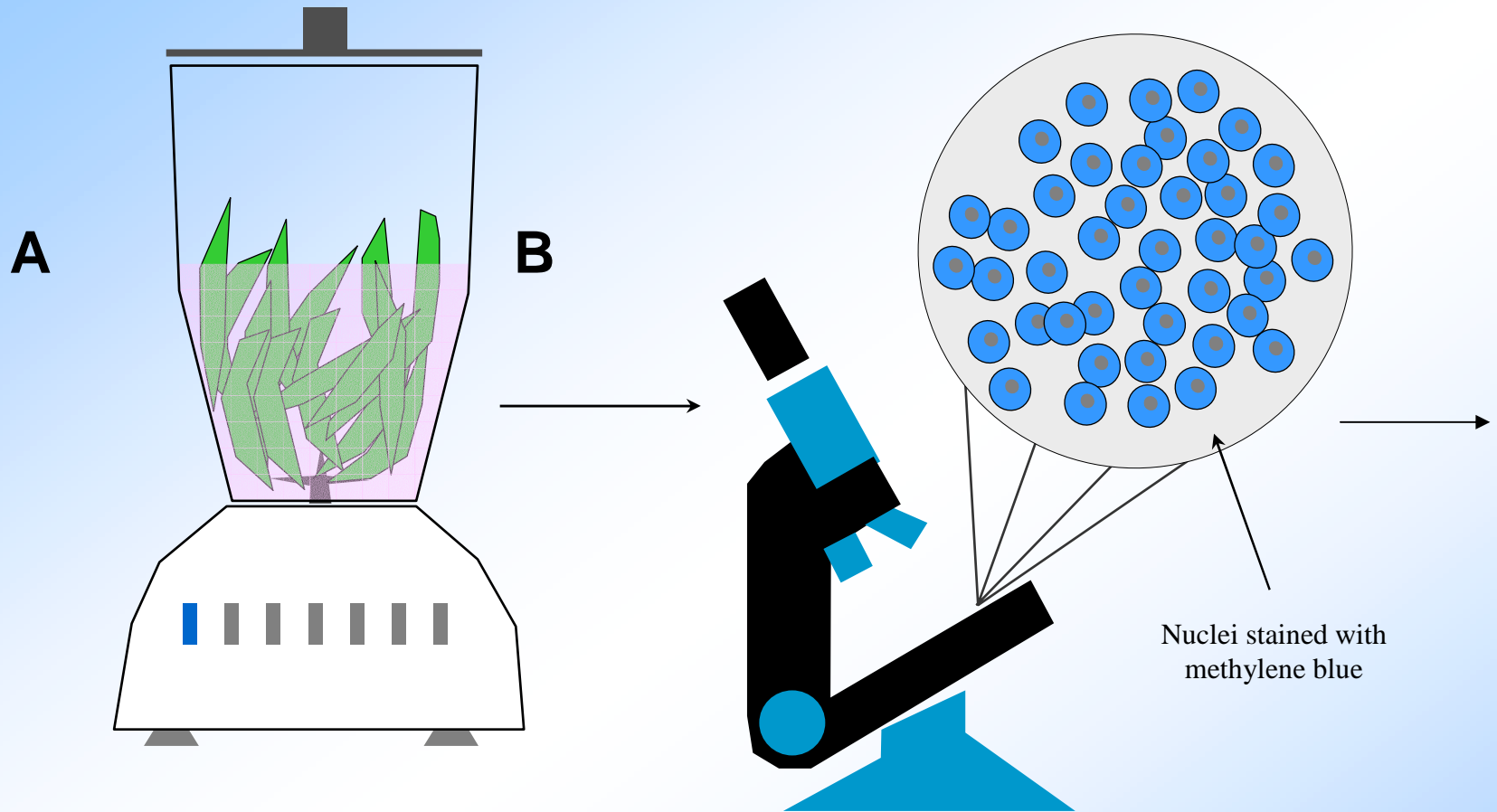
Nucleotide concentration	=	0.050 M
Renaturation time	=	X 344.000 sec
Buffer factor, 0.5 M SPB	=	<u>5.820</u>
		Cot 100.000

Nucleotide concentration	=	0.002 M
Renaturation time	=	X 50,000.000 sec
Buffer factor, 0.12 M SPB	=	<u>1.000</u>
		Cot 100.000

The total amount of reassociation for a given Cot value does not change.

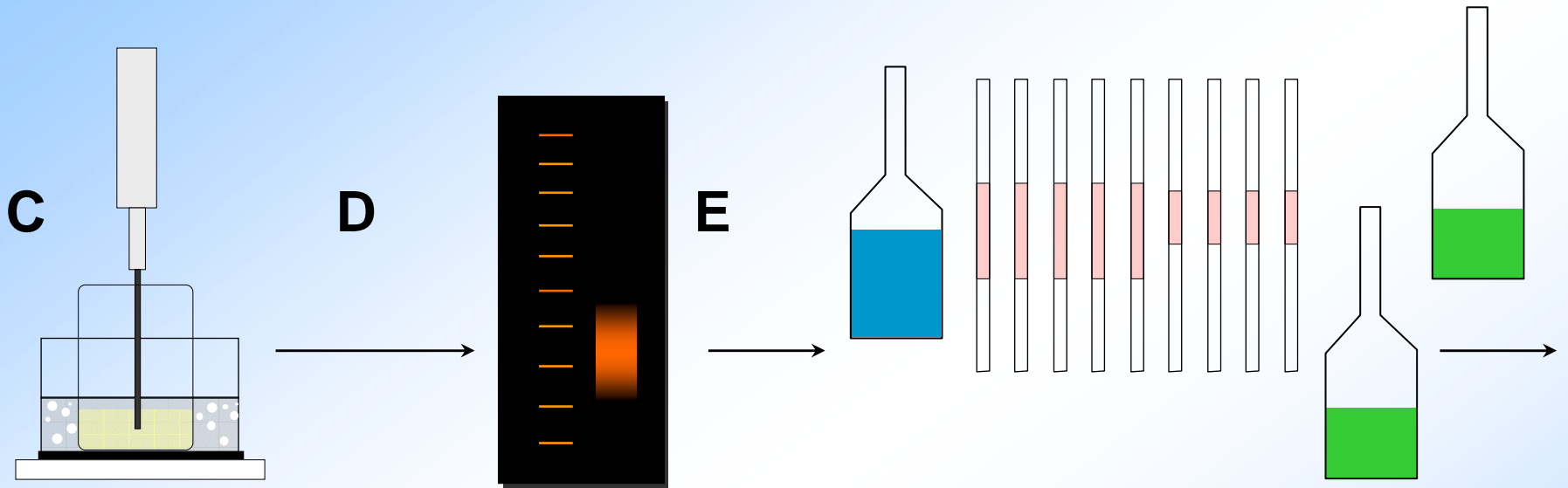
- **The rate at which a particular sequence will reassociate is proportional to the number of times it is found in the genome.**
- **Given enough time, nearly all of the DNA in a heat-denatured DNA sample will reassociate.**

The steps in a Cot analysis



(A) Tissue from the organism of interest is placed in an antioxidant medium and homogenized in a blender. The homogenate is filtered, plastids are preferentially lysed, and nuclei are pelleted by centrifugation.

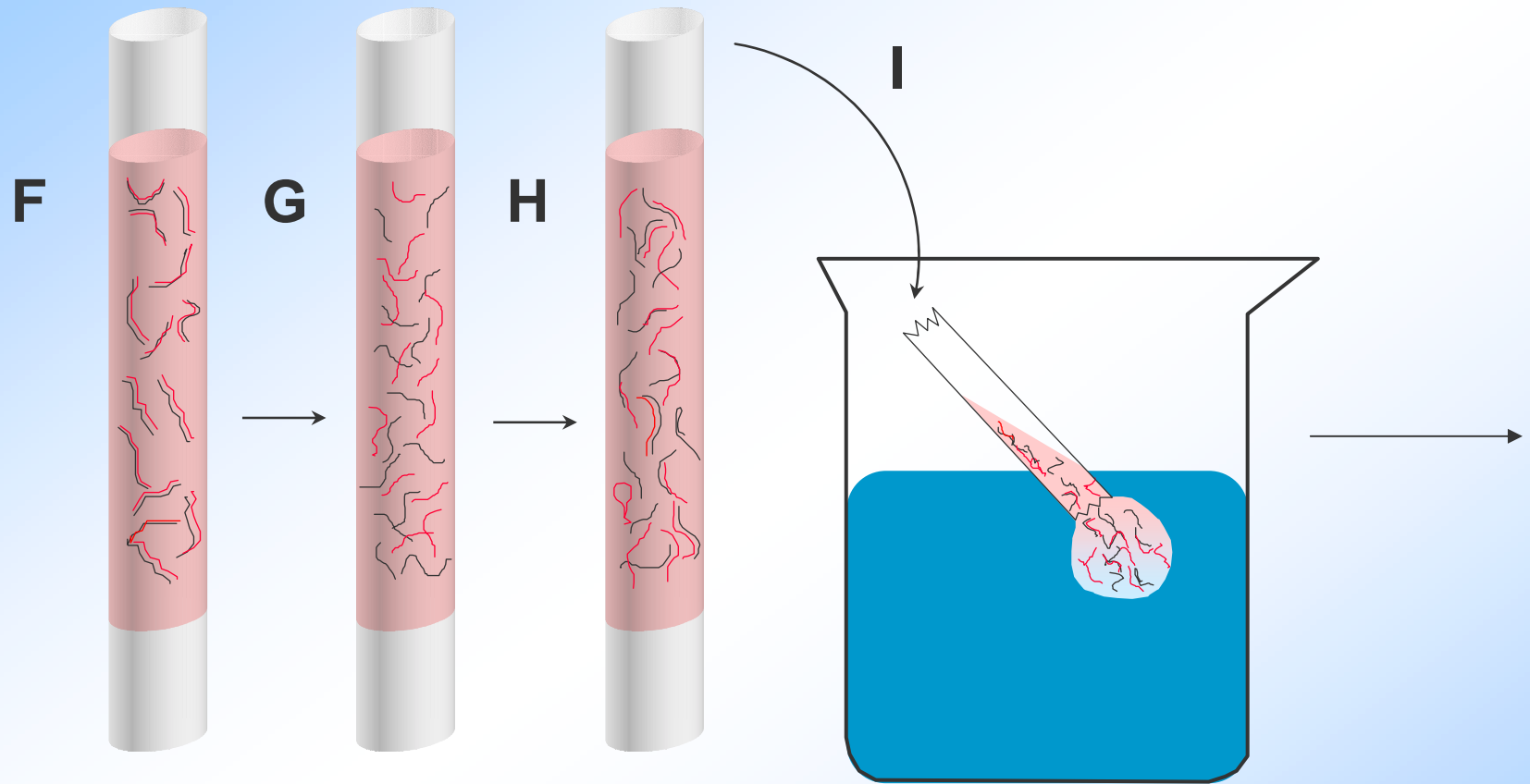
(B) The pellet should be free of contaminating organelles (as determined by phase-contrast microscopy). DNA is isolated from purified nuclei using phenol/chloroform extractions coupled with proteinase and RNase digestions.



(C) The DNA is cut into pieces of about 450 bp by sonication or high-speed blending.

(D) Fragment size is checked by agarose gel electrophoresis. Sheared DNA is precipitated, and aliquots of the DNA are dissolved in 0.03 M, 0.12 M, and 0.50 M sodium phosphate buffer (SPB) to produce solutions of known concentrations.

(E) The DNA solutions are distributed into glass microcapillary tubes or glass ampoules so that each tube/ampoule contains 100 μ g of DNA. The ends of the tubes/ampoules are sealed.

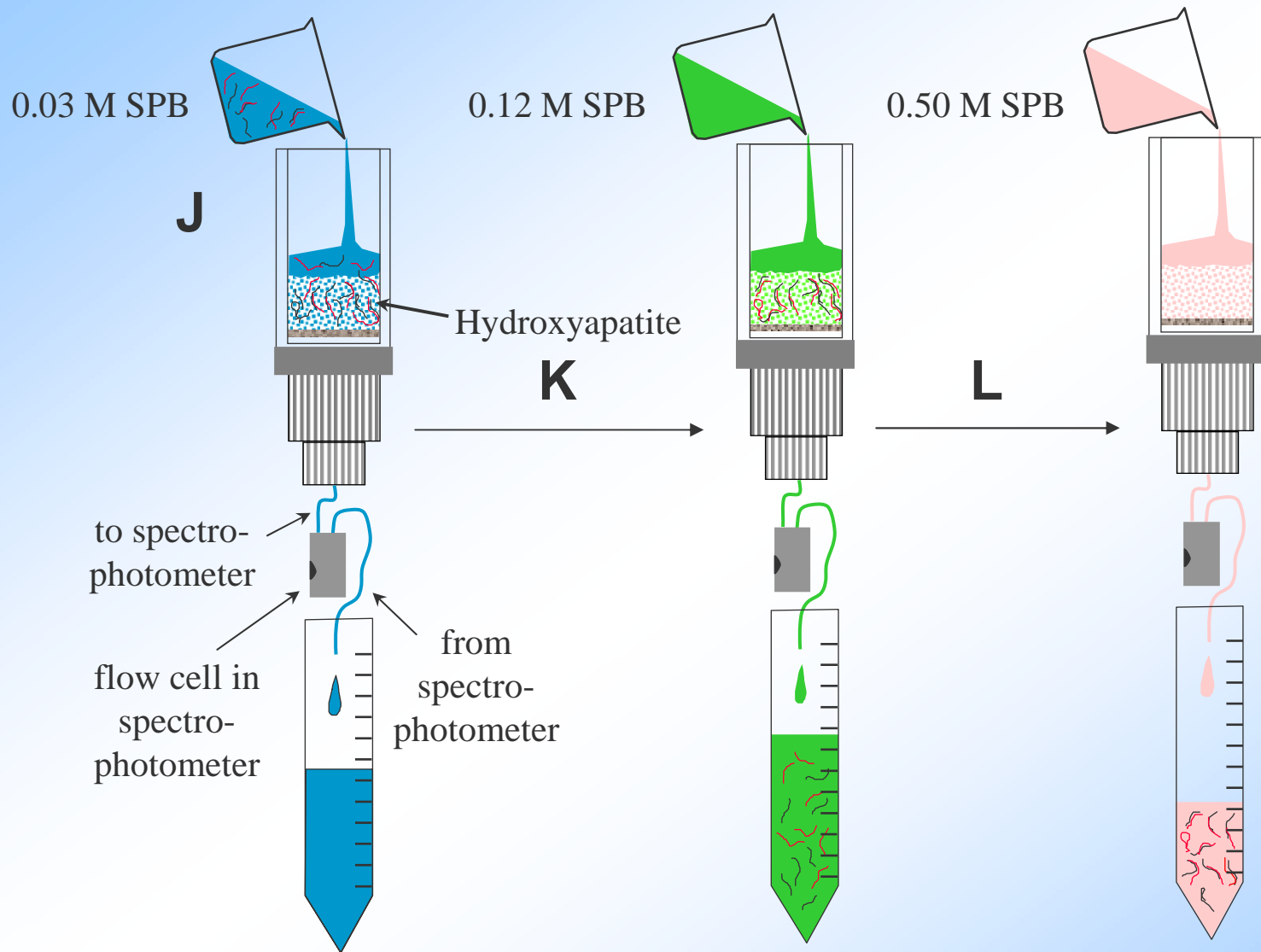


(F) One of the tubes containing a known concentration of DNA is selected.

(G) The tube is placed in boiling water to denature DNA duplexes.

(H) The tube then placed in a water bath set at the sample's 'criterion' (i.e., $T_m - 25^\circ\text{C}$). Renaturation is allowed to occur until the sample reaches a specific Cot value [Cot value = the product of the sample's nucleotide concentration (moles of nucleotides per liter), its reassociation time in seconds, and an appropriate buffer factor based upon cation concentration].

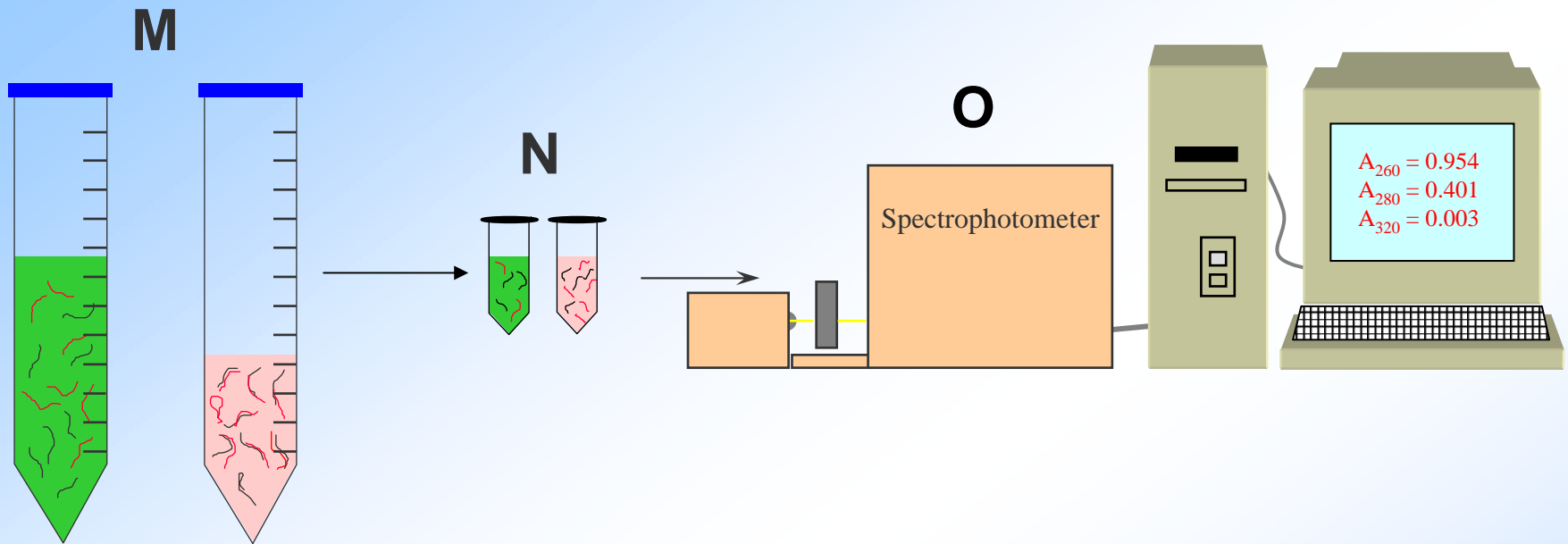
(I) Once the sample has reached the desired Cot value, the end is broken off of the tube/ampoule, and the solution is blown into a 100-fold excess of 0.03 M SPB.



(J) The diluted sample quickly is loaded onto a hydroxyapatite (HAP) column equilibrated with 0.03 M SPB. At this buffer concentration, all DNA binds to the HAP.

(K) Once all of the solution has entered the HAP, 0.12 M SPB is added causing single-stranded DNA (ssDNA) to elute. Eluant containing ssDNA is collected in a graduated polypropylene tube.

(L) After the ssDNA had been collected, 0.50 M SPB is added to the column to elute double-stranded DNA (dsDNA).



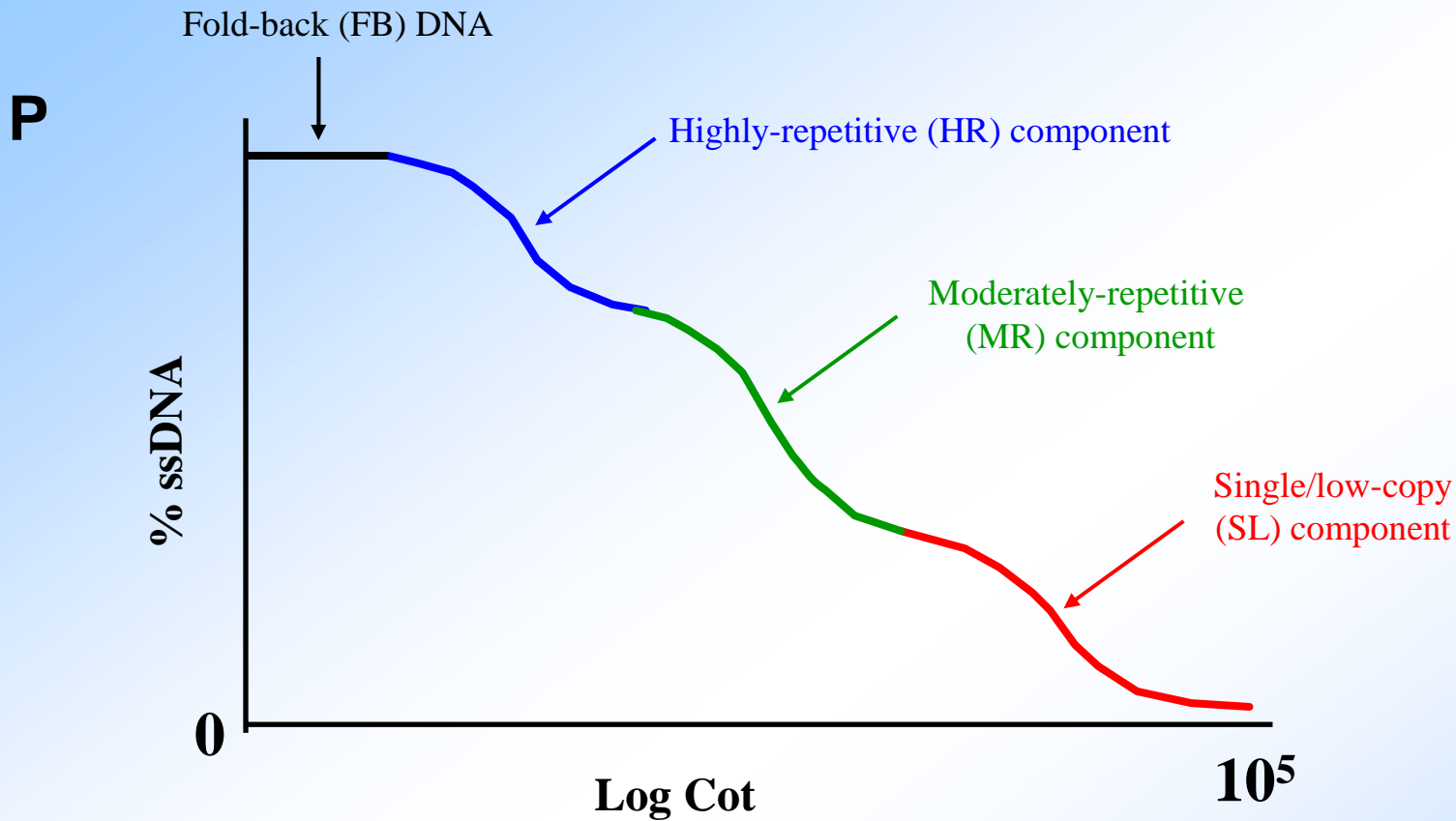
(M) The volumes of the ssDNA eluant and the dsDNA eluant are determined.

(N) Exactly 0.9 ml of the centrifuged ssDNA eluant is mixed with 0.1 ml of aqueous 10 N KOH to denature any DNA duplexes. A sample of the dsDNA eluant is likewise denatured.

(O) The A_{260} values (adjusted for light scatter at 320 nm) of the ssDNA/KOH mixture and the dsDNA/KOH mixture are determined. For a particular Cot value, the percentage of ssDNA (% ssDNA) is calculated as follows:

$$[(V_{ss} \times A_{ss}) \times 100] \div [(V_{ss} \times A_{ss}) + (V_{ds} \times A_{ds})] = \% \text{ ssDNA}$$

where V_{ss} = total volume of single-strand fraction, V_{ds} = total volume of double-strand fraction, $A_{ss} = A_{260}$ (adjusted for light scatter) for the KOH-denatured single-strand fraction, and $A_{ds} = A_{260}$ (adjusted for light scatter) for the KOH-denatured double-strand fraction.



(P) The logarithms of Cot values ranging from essentially no renaturation to nearly complete renaturation are plotted against corresponding % ssDNA values to yield Cot points. A graph of Cot points ranging from little or no reassociation until reassociation approaches completion is called a Cot curve. Because a DNA sequence reassociates at a rate that is directly proportional to the number of times it occurs in the genome, sequences that occur more than once in a genome (repetitive DNA) reassociate at lower Cot values than sequences found only once per genome (single-copy DNA). From an analysis of a Cot curve one can determine genome size, relative proportions of single-copy and repetitive sequences, the fraction of the genome occupied by each frequency component, and the mean kinetic complexity of the sequences in each frequency component.