Buoyant density of DNA and its relationship with mole (G+C) content in DNA

The most common methods for determination of guanine plus cytosine content of DNA (%GC; in mole percent) are buoyant density centrifugation, thermal denaturation, and high-performance liquid chromatography (HPLC) of DNA. Density gradient centrifugation is a common method of separating macromolecules, particularly nucleic acids, in solution. A cell extract is mixed with a solution of CsCl to a final density of about 1.7g/cm³ and centrifuged at high speed (40,000rpm, giving relative centrifugal forces of about 200000g). The biological macromolecules in the extract will move to equilibrium positions in the CsCl gradient that reflect their buoyant densities. A number of factors affect the buoyant density, such as:

- the nature of the caesium salt,
- the presence of heavy metals or DNA-binding dyes,
- the pH and
- the temperature.

Density gradient centrifugation

- Density gradient centrifugation can be used to isolate DNA from other macromolecules. The densities of DNAs are about the same as concentrated solutions of cesium chloride, CsCl (1.6 to 1.8 g/mL). Centrifugation of CsCl solutions at very high rotational speeds, where the centrifugal force becomes $10^5$ times stronger than the force of gravity, causes the formation of a density gradient within the solution.
- If DNA is present in the centrifuged CsCl solution, it moves to a position of equilibrium in the gradient equivalent to its buoyant density.
- The net movement of solute particles in an ultracentrifuge is the result of two processes: diffusion and sedimentation due to centrifugal force. A macromolecular species that has reached its position of equilibrium in isopycnic centrifugation has formed a concentrated band of material.
- Cesium chloride centrifugation is an excellent means of removing RNA and
proteins in the purification of DNA. The density of DNA is typically slightly greater than 1.7 g/cm\(^3\) (1.70\(\pm\) 0.01), while the density of RNA is more than 1.8 g/cm\(^3\), proteins have densities less than 1.3 g/cm\(^3\). In CsCl solutions of appropriate density, the DNA bands near the center of the tube, RNA pellets to the bottom, and the proteins float near the top. Hence, DNA can be separate from the macromolecular mixture. Further the via density gradient centrifugation the difference in the composition of DNA can be found out.

- Density of DNA is dependent on relative G: C content.
- G: C-rich DNA has a significantly higher density than A: T-rich DNA.
- Furthermore, a linear relationship exists between the buoyant densities of DNA from different sources and their G: C content.
- For every 10% increase in GC content, the density rises by 0.12 units
On the basis of their buoyant density the differentiation between the small size, medium and large DNA molecule can be done via this method more than that the variation in the DNA composition that is AT and GC content can be found out as shown in the above diagram with this method as AT rich DNA would be having DNA density band near the 1.65 gram/ml CsCl and GC rich DNA would be having density band near the 1.75 gram/ml CsCl.

Under constant conditions (usually 25°C in caesium chloride at neutral pH) the buoyant density of DNA is related to the GC content:

\[
\%G+C \text{ content} = \frac{\text{buoyant density (g cm}^{-3}\text{)} - 1.660}{0.098} \times 100
\]

DNAs with different base compositions can therefore be separated by this method. Figure 1 shows the fractionation of *Phaseolus aureus* DNA (buoyant density = 1.695) and *E. coli* DNA (buoyant density = 1.710) in caesium chloride.
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Figure 1 The separation of a mixture of *E. coli* and *P. aureus* DNA in caesium chloride.

Effect of methylation

- However, the presence of 5-methyl-cytosines serves to reduce the density slightly, thereby giving rise to an under-estimate of the GC content. In general, 1% methylation decreases the buoyant density by 1 mg cm$^{-3}$.

References:

- [https://publishing.cdlib.org/ucpressebooks/view?docId=ft796nb4n2&chunk.id=d0e12918&toc.depth=1&toc.id=d0e12859&brand=eschol](https://publishing.cdlib.org/ucpressebooks/view?docId=ft796nb4n2&chunk.id=d0e12918&toc.depth=1&toc.id=d0e12859&brand=eschol)