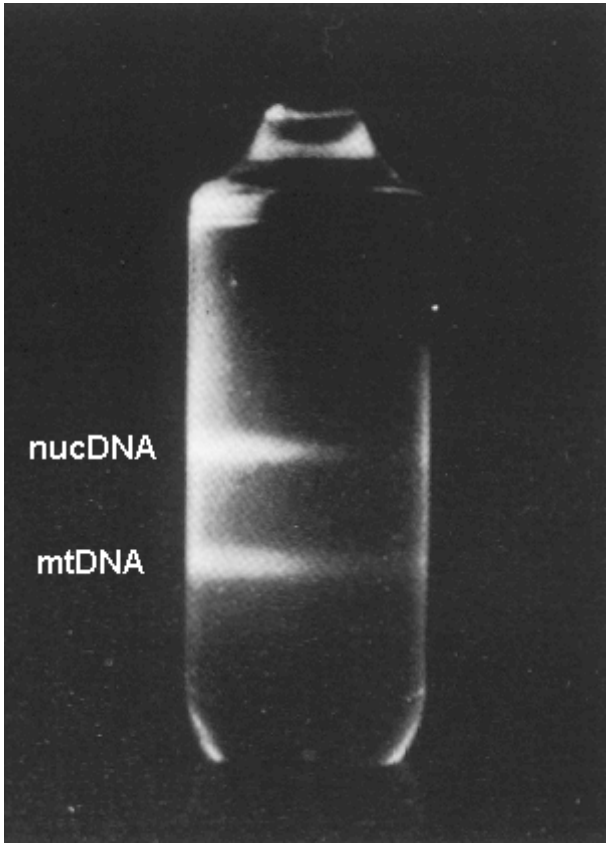

Preparative density-gradient ultracentrifugation of DNA
(SM Carr & OM Griffiths.1987. *Biochem Genet* 25:385-390)

Under high centrifugal force, a solution of **cesium chloride** (**CsCl**) molecules will dissociate.The heavy $\mathbf{Cs^+}$ atoms will be forced away from the center towards the outer end of the tube, but will at the same time *diffuse* back towards the top of the tube, thus forming a shallow density gradient. **DNA** molecules placed in this gradient will migrate to the point where they have the same density as the gradient (the **neutral buoyancy** or **isopycnic point**). The gradient is sufficient to separate types of **DNA** with slight differences in density due to differing **[G+C]** content, or physical form (*e.g.*, linear *versus* circular molecules).

In the experiment above, after centrifugation for 10 hrs at **100,000 rpm** (450,000 x ***g***), two distinct bands, corresponding to sheared **linear nuclear DNA** above and **circular mitochondrial DNA** below, are visible under ultraviolet light. The **DNA** has been mixed with the intercalating dye **ethidium bromide**, which enhances the density difference between the two forms and causes the **DNA** to fluoresce. The separate bands are

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collected by poking a hole in the bottom of the tube. The intact **mtDNA** is available for further biological analysis.

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