

## **Plasmid DNA Preparation (Teaching Edition)**

*Ancient and FYI as many new technologies have made this somewhat outdated.*

Review of some of the many applications of DNA technology.

Using cloning of plasmid DNA to amplify a desired fragment of DNA has been largely replaced by PCR techniques (although is still used for DNA libraries and larger DNA fragments.)

**Cultivation of DNA: Sequential** inoculation. Clones into vials, and vials into bottles. Samples agitated and incubated (@ 37 °C) in large flasks to permit gas exchange.

After grow out, Chloroamphenicol (ribosome inhibitor) is added, which stops bacterial growth, but plasmid amplification still occurs.

Spin down sample @ 4 °C, drain excess media, add sucrose and re-suspend pellet.

**Lysis of bacteria:** Put pellet into 50 ml conical bottom tube and add Lysozyme, Triton 100-X, incubate and spin down (8,100 G's) and cell debris will pellet.

Technical note: Viscous solution is due too long strands of genomic DNA, not the short plasmidal DNA, so as long as viscous, genomic DNA is present.

**Removal of bacterial debris:** After incubation, sample is boiled and flocculent forms (unwanted DNA and proteins) and is removed. Plasmid is in solution.

**Formation of density gradient:**

**Removal of plasmid DNA:**

Optional: Addition of Et-Br (keep out of light and is a very messy step.)

Plasmid DNA is super-coiled and has a greater density than genomic DNA. Also the intercalation (wedging in-between) of the Et-Br in between the base pairs alters the density even further.

When placed onto CeCl gradient, the sample will stratify. From top down: mineral oil, protein, genomic DNA, super coiled DNA and RNA pellet.

Note that the centrifuge tubes are sealable and are spun for incredibly long periods of time at very high RPMs (this is one of the reasons that this particular technique has fallen out of favor.)

Sample is removed by forcing syringe into vial and aspirating fraction out.

**Removal of Et-Br:** Collected fraction is subjected to butanol extraction and Et-Br goes into butanol and DNA is in aqueous phase.

**Purify plasmid DNA: Et-OH/salt ppt and spin down plasmidal DNA.**

**Follow-up gel analysis to confirm desired insert, DNA seq. etc...**

