Miniprep of plasmid DNA

> **NOTE 1:** Use Qiagen mini-prep (or suchlike) columns if at all possible. I hate this protocol – takes longer and DNA quality isn't as good. It should NEVER be used to prepare a vector for digestion to clone with – many restriction enzymes cut with <100% efficiency giving horrendous backgrounds.

Microfuge 6000rpm, 5 min, RT^{o}) 1.5 ml of a fresh overnight culture. Aspirate the supernatant.

Resuspend pellet in 100µl of Solution I

Add 200µl of Solution II. Invert x 10 or vortex lightly to lyse. Mixture should become viscous.

Add 150µl of Solution III. Invert x 10 or vortex lightly: not too much or you will release the genomic DNA. A white, fluffy precipitate should form.

Microfuge 10min, 14000rpm, 4^{o}C.

Transfer supernatant to a new ependorff.

Add 300 µl of PCI (25:24:1 Phenol : Chloroform : Isoamyl Alcohol). Vortex well to mix.

Microfuge 10min, 14000rpm, 4^{o}C.

Transfer upper aqueous phase to a new ependorff.

Add 2.5vol of 100% EtOH (-20^{o}C). Vortex to mix and precipitate DNA: -80^{o}C, 60min.

Microfuge 10min, 14000rpm, 4^{o}C. A small white pellet should be visible.

Discard supernatant. Add 500µl 70% ethanol (-20^{o}C). Vortex and microfuge: 10min, 14000rpm, 4^{o}C). pour off supernatant.

Discard supernatant and airdry pellet ≥10 min on bench

Resuspend pellet in 30µl of TER. A typical restriction digest uses 3-5 µl.

Solution I: 20% glucose, 50 mM Tris pH 7.9, 10 mM EDTA

Solution II: 0.2 N NaOH, 1 % SDS

Solution III: 3M KAc pH 5.2 (Add Glacial acetic Acid to 3M KAc to pH)

TER: 10mM Tris pH 8.0, 1mM EDTA pH8.0 (5µl 10mg/ml RNAseA / ml)