# Alkaline Lysis

Alkaline lysis is the method of choice for isolating circular plasmid DNA, or even RNA, from bacterial cells. Alkaline lysis depends on a unique property of plasmid DNA: it is able to rapidly anneal following denaturation. This is what allows the plasmid DNA to be separated from the bacterial chromosome.

Spin 1.5 ml of fresh overnight culture cells for 5 minutes at 20,000 x g.

Discard the supernatant.

Add 100 µl of buffer TEG and resuspend your cells.

Add 200 µl of 0.2 M NaOH, 1% SDS and mix GENTLY by inversion 3 times. Leave on ice for 5 minutes.

Add 150 µl of cold 5 M KOAc and mix GENTLY by inversion 3 times. Leave on ice for 5 minutes.

Add 2 volumes of EtOH. After 5 minutes, spin for 5 minutes at 12,000 rpm.

Remove and discard supernatant. Add 500 µl of 70% EtOH to pellet and vortex briefly. Spin for 2 minutes at 12,000 rpm.

Remove and discard the supernatant and allow the pellet to dry at room temperature, with the tube open upside-down on a paper towel. Resuspend in 50 µl of TE (Tris-EDTA Buffer).

**Materials necessary**

- TEG Buffer
- Fresh overnight cultures
- 0.2 M NaOH
- 1% SDS
- 5M Potassium acetate (KOAc)
- 100% Ethanol
- 70% Ethanol
- TE