Plasmid Extraction from *E. coli* - Alkaline Lysis Method

- Harvest 2-4 ml of cells in eppendorf (13,000rpm, 1 min) Decant supernatant (aspirate)
- Resuspend cells in 300 µl P1 buffer to a homogenous suspension
- Add 300 µl of lysis buffer (P2 buffer), invert about 6 times (not more!)
- Add 300 µl K-Ac/5% formic acid and invert tube approx 6 times. Should see a precipitate form
- Spin at 13,000 rpm for 10 min then transfer supernatant into new eppendorf
- Precipitate plasmid DNA in 0.7 vol (i.e. 630 µl) of room temperature isopropanol and invert about 6 times
- Spin at 13,000 rpm for 15mins and decant supernatant.
- Wash pellet in 70% ethanol (ca. 700 µl) and remove supernatant, spin again (2 min) if pellet becomes dislodged.
- Quick spin to remove final trace ethanol and allow pellet to air dry (approx 10-15 mins)
- Dissolve DNA in 50-100 µl of MQ H_20 (pH5.5) or 10 mM Tris/HCl (pH8.0).

Recipes:

P1 Buffer (Recipe from Qiagen kit) (store in fridge)

50mM Tris/HCl [pH 8]

10mM EDTA [pH 8]

Make up part of the final volume with the Tris/HCl and EDTA solutions with water.

100µg/ml DNase-free RNase (from 10 mg/ml stock)

Lysis Buffer (P2) (store at RT, but only make about 10 or 20 ml as it doesn't keep forever)

0.2M NaOH

1% SDS

K Acetate/5% formic acid (P3) (store at RT)

88.3g K-acetate

15ml Formic Acid

300ml volume with dH_20

# Preps	5	10	24	48	1
Vol. needed	1800 µl	3300 µl	7.5 ml	15 ml	300 µl
H_2O	1260 µl	2310 µl	5.25 ml	10.5 ml	210 µl
1 M NaOH	360 µl	660 µl	1.5 ml	3 ml	60 µl
10% SDS	180 µl	330 µl	750 µl	1.5 ml	30 µl

Protocol generously provided by the lab
Prof. Thorsten Mascher
Großhadernerstr. 2-4
82152 Planegg-Martinsried
www.syntheticmicrobe.bio.lmu.de