Plasmid Mini Prep Method (Boiling Method)

Protocol

- Inoculate 5ml 2XYT media containing 100-200ug/ml Ampicillin with a bacterial colony and incubate over night on a shaker or rotator at 37°C
- Transfer 1.5ml of over night culture into an Eppendorf tube and spin at high speed in microfuge for 2 minutes. Store remaining culture at 4°C until needed
- Remove media using pipetman or aspirator
- Resuspend cells in 350ul of Boiling Prep Buffer using 1ml pipetman (pipette up and down)
- Add 25ul of freshly prepared solution of lysozyme (10mg/ml - 100mg in 10ml H_2O) and vortex briefly
- Place tube in boiling water bath for 90 seconds
- Spin immediately after boiling (30 minutes, high speed in microfuge)
- Remove pellet with toothpick or yellow pipette tip
- Add 160ul 5M NH_4OAc
- Add 300 ul of phenol/$CHCl_3$, vortex well, spin (high speed for 5 minutes in microfuge)
- Transfer top aqueous later to new tube
- Repeat above Phenol/$CHCl_3$ extraction
- Add 800 ul EtOH, mix well, and spin for 20-30 minutes (high speed) in microfuge
- Take off (or aspirate) the EtOH
- Wash with 800 ul 70% EtOH (stored at –20°C)[i.e. add 70% EtOH, vortex, spin 5 minutes (high speed in microfuge) and take off EtOH].
- Dry in speedy vac and suspend DNA in 30ul TE

Typical Mini Prep Restriction Digest

DNA	5ul
10XRB (usually 10X HRB)	3ul
Enzyme 1	1.5ul
Enzyme 2	1.5ul
RNase	3ul
H_2O	16ul
Total	30ul

Notes:

1) This method does not include an RNase digestion step, so 2ul of 10mg/ml RNase must be added during the restriction digestion analysis.
2) Good quality for sequencing. Denaturation of plasmid by high temperature alkaline treatment effectively degrades RNA

Buffers

Boiling Prep Buffer
8% Sucrose
0.5% Triton X-100
50mM EDTA (pH 8.0)
10mM Tris-Cl (pH 8.0)

TE
10mM Tris (pH 7.6)
1mM EDTA (pH 8.0)

We find that the standard restriction buffers described in Maniatis give more consistent results than those supplied by vendor.

10X Low Restriction Buffer (10XMRB)
100 mM Tris-Cl (pH 7.5)
100mM $MgCl_2$
10mM DTT

10ml of 10X LRB
1ml 1M Tris (pH 7.5)
1ml 1M $MgCl_2$
100ul 1M DTT

10X Medium Restriction Buffer (10XMRB)
100 mM Tris-Cl (pH 7.5)
500 mM NaCl
100mM $MgCl_2$
10mM DTT

10ml of 10X MRB
1ml 1M Tris (pH 7.5)
1ml 5M NaCl
1ml 1M $MgCl_2$
100ul 1M DTT

10X High Restriction Buffer (10XMRB)
500 mM Tris-Cl (pH 7.5)
1 M NaCl
100mM $MgCl_2$
10mM DTT

10ml of 10X HRB
5ml 1M Tris (pH 7.5)
2ml 5M NaCl
1ml 1M $MgCl_2$
100ul 1M DTT

2xYT	1L
tryptone	16g
yeast extract	10g
NaCl	5g