

Plasmid Mini Prep (Alkaline Lysis 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 bacterial pellet in 100ul Soln' I
- Add 200ul Soln' II (made fresh – 0.2N NaOH, 1% SDS)
- Pipette up and down several times (it's gonna be sticky and there's no need to be gentle)
- Add 150ul Soln' III
- Hold tubes in rack and gently rock tubes back and forth until a white fluffy precipitate is observed
- Spin 5 minutes in microfuge (high speed)
- Transfer supernatant to new eppendorf tube
- Add 300ul of phenol/CHCl₃, vortex well, spin (high speed for 5 minutes in microfuge)
- Transfer top aqueous layer to new tube
- Add 1ml EtOH, mix well, and spin for 20-30 minutes (high speed) in microfuge
- Take off (or aspirate) the EtOH
- Wash with 800ul 70% absolute 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 Resuspend 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
<u>H₂O</u>	<u>16ul</u>
Total	30ul

Note:

This method does not include an RNase digestion step, so 2ul of 10mg/ml RNase must be added during the restriction digestion analysis.

Solutions

<u>Soln' I</u>	<u>1 liter</u>
50mM Glucose	9g Glucose
10mM EDTA	20ml 0.5 M EDTA (pH 8.0)
25mM Tris (8.0)	25ml 1 M (Tris pH 8.0)

Soln' II
Made Fresh – see above

<u>Soln' III</u>	<u>1 liter</u>
KOAc	294g
Glacial Acetic Acid	115ml Glacial Acetic Acid

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.

<u>10X Low Restriction Buffer (10XMRB)</u>	<u>10ml of 10X LRB</u>
100 mM Tris-Cl (pH 7.5)	1ml 1M Tris (pH 7.5)
100mM MgCl ₂	1ml 1M MgCl ₂
10mM DTT	100ul 1M DTT

<u>10X Medium Restriction Buffer (10XMRB)</u>	<u>10ml of 10X MRB</u>
100 mM Tris-Cl (pH 7.5)	1ml 1M Tris (pH 7.5)
500 mM NaCl	1ml 5M NaCl
100mM MgCl ₂	1ml 1M MgCl ₂
10mM DTT	100ul 1M DTT

<u>10X High Restriction Buffer (10XMRB)</u>	<u>10ml of 10X HRB</u>
500 mM Tris-Cl (pH 7.5)	5ml 1M Tris (pH 7.5)
1 M NaCl	2ml 5M NaCl
100mM MgCl ₂	1ml 1M MgCl ₂
10mM DTT	100ul 1M DTT

<u>2xYT</u>	<u>1L</u>
tryptone	16g
yeast extract	10g
NaCl	5g