MiniPlasmid DNA Micropreps

Experimental considerations

- This method is useful for screening large numbers of clones by restriction analysis. It is not suitable for sequencing analysis and we haven't tested the DNA for cloning experiments.
- The protocol outlined below is a modified version of that described by Shepard and Rae [we use less overnight culture volume (200ul) which allows the use of a 96 well format (Microtest III U bottom 96 well flexible plates, Becton/Dickinson Cat#353911-plate- #3913-lid-). This makes the protocol easier and faster when a large number of samples are being processed.
- This microprep procedure was originally described by A.R. Shepard and J.L. Rae (BioTechniques 1999,26:868-870). The method is rapid (it takes 2 hours) and cheap (only 1 U of enzyme per sample is added) and yields good quality DNA which is adequate for restriction analysis. Shepard and Rae tested DH10B cells and two restriction enzymes (Sal I and Not I). We have had excellent success using DH5 cells and several different restriction enzymes. We have only used high copy number plasmids, pBlueScript, pcDNA3. We have not tested the use of this protocol for analysis of low copy number plasmids – however, in this case, larger culture volumes (i.e. 500 ul) of overnight culture are recommended.

Protocol

- Inoculate 3 ml 2xYT media containing antibiotic (100-200 ug/ml Ampicillin) with a bacterial colony and incubate over night on a shaker or rotator a 37^oC.
- Transfer 200 ul of each overnight culture into wells of a 96 well microtest plate and spin down for 1 min at 1,400xg (2,500-3,000 rpm).
- Remove media completely with an aspirator.
- Resuspend bacterial pellet in 20 ul (19 ul H_2O + 1 ul RNase 10mg/ml) by pipetting up and down several times (at least 15 times – avoid making bubbles).
- Place microtest plate in low boiling water (80^oC) for 1 minute.
- Spin down 96 well plate for 1 min at 1400xg (2500-3000 rpm) to pellet cell debris.
- Transfer 15 ul of supernatant to a new well.
- Add 5 ul of "digestion solution" (see below) per sample (mix completely by pipetting up and down a couple of times).
- Incubate reactions for 45 minutes to 1 hr at restriction enzyme incubation temperature.
- Add 4 ul 6x loading buffer and load onto 1% Agarose gel.
- Clones found to be positive can be growth up using remaining overnight culture.

Solutions

<u>Digestion Solution</u>

2 ul 10x Restriction buffer
1 Unit Restriction enzyme **
Add H_2O up to 5 ul

** Most of the commercially available enzymes contain between 20-400 Units per ul. The use of only 1 unit per reaction works well and makes this protocol very cheap.

Restriction Buffers

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

10X Low Restriction Buffer (10XLRB)	10ml of 10X LRB
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

10X Medium Restriction Buffer (10XMRB)	10ml of 10X MRB
100 mM Tris-Cl (pH 7.5)	1ml 1M Tris (pH 7.5)
500 mM NaCl	1ml 5M NaCl
100mM MgCl	1ml 1M $MgCl_2$
10mM DTT	100ul 1M DTT

10X High Restriction Buffer (10XHRB)	10ml of 10X HRB
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

50X TAE
1 L
242g Tris Base
57.1 ml Glacial Acetic Acid
100 ml 0.5M EDTA (pH 8.0)

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