05 May 2007
Typed by C. Conrad

# BRIJ LYSIS PLASMID PREP

- Start with a fresh plate of cells on LBamp. Inoculate a single colony onto 5 ml LBAMP (100µg/ml) in 16x100mm culture tube and grow overnight, 37ºC on tissue culture wheel. Use this starter culture to inoculate appropriate volume of LBamp for plasmid prep.

| | **Large-scale prep** | **Midi-prep** | **Mini-Prep** |
|---|---|---|---|
| | 1L cells in Fernbach flasks | 250ml cells in 1L flask | 100ml cells in 250ml flask |

- Grow Cells overnight to stationary phase.

- Harvest Cells 6,400xg for 10 min. Resuspend in tris-sucrose solution by banging bottle on stack of paper towels. Be sure cells are uniformly resuspended with no clumps.

| | | | |
|---|---|---|---|
| | 10ml | 2ml | 1ml |

- Add freshly prepared lysozyme solution, incubate 10 minutes on ice with occasional swirling.

| | | | |
|---|---|---|---|
| | 2ml | 0.4ml | 0.2ml |

- Add EDTA solution. Ice 5 minutes.

| | | | |
|---|---|---|---|
| | 5ml | 1ml | 0.5ml |

- Slowly add Brij solution while swirling. Transfer samples to capped autoclaved Oakridge centrifuge tubes.

| | | | |
|---|---|---|---|
| | 16ml | 3.2ml | 1.6ml |

- Centrifuge at 17,000rpm, 90 min.

- Transfer to 50 ml (large scale) or 15 ml blue capped tubes, carefully avoiding white pellet and viscous material. Add solid CsCl and TE (50/10) to indicated final volume. Mix gently. Add ethidium bromide (and 10% Triton X-100 for midi and mini preps) mix and check refractive index. Should be 1.386 (1.55g/ml). See lab manual for conversion chart for CsCl conc. and refractive index.

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|---|---|---|---|
| CsCl: | 35.2g | 9.9g | 3.3g |
| TE to: | 42.5ml | 12ml | 4ml |

| | | | |
|---|---|---|---|
| EtBr: | 1ml | 0.25ml | 80µl |
| Triton: | none | 120µl | 40µl |

- Transfer to quick-seal tubes. Add CsCl solution to neck. There are special funnels for this. Balance to 0.05g with TE (50/10). Seal, mix well, check seals. Ask for help with sealer if you have not previously used.

| | | | |
|---|---|---|---|
| Tube: | 342414 | 342413 | 361082 |
| Rotor: | VTi50 (Weiss Lab) | NVT65 | TLN120 |
| Centrifuge: | LE-80 | LE-80 | TLX |
| Speed (rpm): | 45K | 62K | 120K |
| Time: | 24 hr | 6 hr | 1.5 hr |

- Centrifuge at indicated at 15ºC (Tmax = 30ºC). If you use quick seal tubes, you need to put spacer caps. Please read rotor manual. If you have not used ultra, please read ultra manual too, especially the part about CsCl solubility and temperature and centrifugal force relationship.

- Pull plasmid band with 18 gauge needle and appropriate size syringe. Remember to make an air hole at the top.

- If you want to run 2$^{nd}$ gradient, add additional EtBr and add 828mg/ml CsCl solution to appropriate volume. Load into quick-seal tubes. Fill to neck with CsCl solution and balance with TE. Seal, mix well, check seals. Centrifuge as indicated 15ºC.

| | | | |
|---|---|---|---|
| EtBr: | 0.3ml | 0.3ml | 80µl |
| CsCl sol'n to: | 12 ml | 12ml | 1ml |
| Rotor: | NVT65 | NVT65 | TLN120 |
| Centrifuge: | LE-80 | LE-80 | TLX |
| Speed (rpm): | 65K | 65K | 120K |
| Time: | 5hr | 5hr | 1.5 hr |

- If you do not run second gradient, continue from here. Pull plasmid band and extract EtBr with n-butanol (pre-equilibriated with 82.8% CsCl) in Sarstedt tubes.

- Do not skip dialysis steps. It is important for removing CsCl bound to DNA.

- Dialyze overnight against TE (50/10) containing 0.5M NaCl. The NaCl will displace CsCl bound to DNA.

- Dialyze overnight at 4ºC against TE (10/1) with one change. This step remove excess NaCl.

- Phenol extract, chloroform extract, and enthanol precipitate (in Sarstedt tubes). Resuspend in $H_20$ to 1ml/ml.

Solutions:

50mM tris-HCl pH = 8.0 + 25%w/v sucrose. Make fresh.

20mg/ml lysozyme in $H_20$. Make fresh, keep on ice. If it does not dissolve, add some HCl.

0.25M EDTA, pH = 8.0

Brij Lysis buffer: 50mM tris-HCl, pH=8.0
62.5 mM EDTA
0.4% sodium deoxycholate
1% Brij58

10mg/ml ethidium bromide in $H_20$.

TE (50/10): 50mM tris-HCl, pH=8.0
10mM EDTA

TE (10/1): 10mM tris-HCl, pH=8.0
1 mM EDTA

82.8% w/v (final) CsCl in TE (50/10)

J. Quinn 8/99, modified by JK & SM 5/2007