AMPLIFICATION OF HIGH-COPY NUMBER PLASMIDS

Day 1

Materials

1. Sterile pipet tips.

2. Sterile 1.5 ml Eppendorf tubes.

3. Plasmids to amplify.

4. DH5alpha or XL-1 blue E. coli, transformation competent, ~1 x 10^6 /ml.
Stored at -80^o C. Thaw rapidly immediately before use and keep on ice.

5. Agar plates, with appropriate antibiotics. Leave on room temperature bench for 1-2 days to get a semi-dry surface. Protect from light. Need one per plasmid plus a no-DNA control.

6. LB medium, 1 ml.

7. SOC medium, 1 ml (optional, for high efficiency).

8. Water bath, 42^o C.

Procedure

1. Mix 0.5 - 1 ul plasmids with 0.1 ml competent E. coli in a sterile Eppendorf tube on ice. Mix gently by tapping. Also prepare a no DNA control tube.

2. Incubate on ice for 20 min.

3. Incubate at 42^o for exactly 2 min (alternative procedure – 30 seconds). Use floater in a circulating water bath.

4. Incubate on ice for 1-2 minutes.

5. Add 1 ml SOC or LB medium, mix by gentle tumbling (optional, for high efficiency transformation to increase the number of transformants).

6. Incubate for 30-60 min at 37^o C.

7. Pipet 10 - 300 ul onto an agar plate. For larger volumes, break them into multiple small puddles. Tilt the plate around to make even coverage.

8. Smear the E. coli gently with sterile glass rod (ethanol soaked and flamed) or pipets across the agar surface. Hold the lid over the surface with one hand and rotate the plate to smear in multiple directions.

9. Incubate the plate overnight at 37^{o} C, place upside down to avoid condensation problem.

Day 2 on

Materials

1. Sterile tooth picks.

2. LB or 2XYT with antibiotics (e.g. 40 μg/ml kanomycin or 50 μg/ml ampicillin), need 100 ml per colony.

3. Antibiotics, stock solution or solid. To use solid, weigh antibiotics on "clean" weighing paper (middle of the pile); wear gloves. Transfer to a sterile bottle and add appropriate volume of LB in the sterile hood. To prepare stock solution, make 20 mg/ml kanamycin or 25 mg/ml ampicillin in distilled water and pass through 0.22 μm syringe filter. Store at -20^{o}C. Dilute 1:500 into medium.

4. 30% glycerol in water, autoclaved.

5. 15 ml sterile conical tubes.

6. Qiagen Maxi or Mini plasmid preparation kit.

7. Sterile 30 ml Kimax Sorvall tubes, one per plasmid; 50 ml centrifuge tubes with caps for spinning down E. coli, 3 per plasmid.

8. Sterile 500 ml flasks, one per plasmid.

9. Sterile 1.5 ml Eppendorf tubes.

10. TE buffer, 1 mM EDTA, 10 mM Tris-Cl, pH 8.0. Need 0.5 ml per plasmid.

Procedure

1. Add 3 ml of LB with antibiotics to a 15 ml conical tube.

2. Select a well-formed, smooth-edged, isolated colony on the agar plate. Do not use colonies with tiny dots around (these may be non-transformed E. coli growing in regions of depleted antibiotics).

3. Pick E. coli from the colony with a sterile toothpick. Dip the tip into the LB medium.

4. Place on a shaking incubator at 37^{o} C, 200-250 rpm for 4- 8 hours. The medium should turn cloudy. Store the tube overnight at 4^{o} C if necessary.

5. Mix 0.5 ml with 0.5 ml 30% sterile glycerol in sterile 1.5 ml Eppendorf tube, freeze at -80^{o} C. To use frozen stock, dip sterile toothpick into the solution and dip the tip into the LB medium as in step 3.

6. To amplify the plasmid, add 0.2 ml E. coli to 100 ml LB with antibiotics in a 500 ml sterile flask. Cover with foil and incubate overnight on a shaking incubator at 37^{o} C. Follow directions of Qiagen-tip 500 kit for the preparation of plasmids. Do two 5 ml ethanol washes at the end. Air dry and resuspend by gentle swirling in 300 ul TE buffer. Do not vortex or pipet.

7. Determine DNA concentration by OD260, after 1:100 dilution. The microcuvette holds 100 µl. 1 OD_{260} = 50 µg/ml. OD_{280} should be 50% of OD_{260}. Store at 4^{o}C or -20^{o}C.