Plasmid Amplification

Plasmid DNA from **abm** inc. is supplied in 10mM Tris (unless otherwise requested) and intended for direct transformation into an authentic DH5a *E.coli* strain. To amplify any plasmid received from **abm**, transform into DH5a competent cells, pick a single colony and perform a mini-plasmid preparation as usual (it is better not to amplify the plasmid in protein expression strains of *E.coli,* since they are not always well suited to plasmid extraction and purification). Always check with the provider or the appropriate reference to determine the antibiotic selection for the plasmid. Resistance genes included are usually ampicillin, kanamycin, or spectinomycin, but can also include tetracycline or chloramphenicol in some cases. All of **abm**'s pLenti vectors are high copy plasmids.

Subcloning Efficiency DH5a competent *E.coli* cells

- Use **abm**'s ProClone™ Competent Cells (Cat. E003) for best results
- Cells will be provided in 4 X 1.25 ml aliquots and are stored at -80°C.
- The cells will need to be aliquoted to avoid freeze-thaw cycles.
- Thaw a vial on wet ice. Pre-chill the fresh tubes before adding cells to the aliquots.
- Mix the cells after thawing, by gentle inversion.
- Promptly aliquot into 50 µl aliquots and re-freeze in dry ice/ 95% ethanol bath.

Transformation Protocol

1. Thaw an aliquot of ProClone™ Competent DH5a cells on wet ice.
2. Add 1 µl of plasmid. If plasmid concentration is given, dilute to 10 ng/µl and use 1 µl. Mix by tapping the tube gently. Leave on ice for 30 mins.
3. Heat-shock for exactly 45 seconds in a 42°C water bath.
4. Place the tube back on ice for 2 mins.
5. Add 150 µl sterile LB broth, and recover for 1 hour in an incubated shaker set at 37°C, 240 rpm.
6. Spread entire volume of cells on an LB agar plate containing the appropriate antibiotic (see below for concentrations*).
7. Incubate at 37°C overnight (around16 hours) to allow colonies to form. If the colonies are too dense, plate 1µl cells in a 100 µl pool of LB on a fresh LB + antibiotic plate.
8. Inoculate 4-10 ml of LB broth containing the appropriate antibiotic with a single picked colony. Grow overnight (16-18 hours) in an incubated shaker set at 37°C, 240 rpm.
9. Isolate plasmid by a mini-prep protocol, as standard.

***Antibiotic Selection:**
KanR: 50 µg/ml Kanamycin
AmpR: 100 µg/ml Carbenicillin/Ampicillin
SpecR: 50 µg/ml Spectinomycin
TetR: 12.5 µg/ml Tetracycline
CamR: 25 to 34 µg/ml Chloramphenicol

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