TRANSFORMATION OF BRL SUBCLONING EFFICIENCY
DH5a COMPETENT CELLS

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Subcloning efficiency DH5a competent cells are prepared by a procedure developed at BRL. These cells are recommended for routine subcloning into plasmid vectors and are not suitable for the generation of cDNA libraries. DH5a is capable of being transformed efficiently with large plasmids, and can also serve as a host for the M13 mp cloning vectors if a lawn of JM101 or JM107 is provided to allow plaque formation.

TRANSFORMATION PROCEDURE OF DH5a COMPETENT CELLS

TO ALIQUOT:

1. Prepare a dry ice/ethanol bath and maintain at -70°C.
2. Remove competent cells from -70°C freezer; thaw on wet ice.
   Place required number of 1.5 ml microcentrifuge tubes on wet ice.
3. Gently mix cells, then aliquot 50 µl competent cells into chilled microcentrifuge tubes.
4. Re-freeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer.

TO TRANSFORM:

1. Thaw 50 µl aliquot from -70°C freezer on wet ice 10 min.
2. Add 2 ng DNA. Mix well.
3. Ice 30 min.
4. Heat shock cells 20 seconds at 37°C.
5. Ice 2 min.
6. Add 950 µl of room temperature LB.
7. Incubate 37°C for 1 hour.
8. Spread on desired agar plates volumes of:
   50 µl
   150 µl
   250 µl

9. Incubate plates at 37°C overnight.

FOR M13 CLONING IN DH5-a CELLS

1. Perform transformation as above but omit steps 6-8.

2. To two 13x100 mm test tubes, add:
   50 µl 2% x-gal or Bluo-gal
   10 µl 100 mM isopropyl- D-thiogalactopyranoside (IPTG)

3. Add lawn cells (JM101 or JM107).
   To one tube add 25 µl lawn cells; to the second add 200 µl.

4. To each tube add 3 µl top agar (LB or YT) at 55°C.

5. Add transformed cells to the top agar:
   5 µl (10%) of cells to tube containing 25 µl of lawn cells.
   45 µl (90%) of cells to tube containing 200 µl of lawn cells.
   Mix contents well.

6. Immediately pour top agar on YT or LB plates.

7. Incubate at 37°C overnight.

References:

1. King, P.V. and Blakesley, R. (1986) FOCUS 8:1, 1.
2. FOCUS 8:3, 13.
3. Miller, J. (1972) EXPERIMENTS IN MOLECULAR GENETICS, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
4. Hanahan, D. (1983) J. Mol. Biol. 166:557.