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General Chemical Transformation Protocol

1. Remove tube of frozen competent cells from -70°C and place on ice. Allow cells to thaw.

   Note: Keep cells chilled on ice to ensure high transformation efficiency.

2. Mix cells by flicking the tube gently, then remove 100 µl per transformation into a sterile pre-chilled (on ice) Eppendorf tube. Transfer into thin-walled tubes to increase transformation efficiency.

3. Add 1-50ng of DNA (in a volume no greater than 10ul) per 100 µl cells. Quickly flick the tube several times to ensure the even distribution of DNA. To determine the transformation efficiency, add 1-10 µl (0.1-1ng, depending on how competent the cells are) of a control plasmid.

4. Immediately place tubes on ice for at least 10 minutes.

5. Heat-shock the cells for 45 seconds in water bath at **exactly** 42°C. Do not shake.

6. Immediately, place tubes on ice for 2 minutes.

7. Add 900 µl of room temp (or 37°C) SOC or LB medium and incubate for 1 hour at 37°C with shaking at ~225 rpm.

8. Plate 100-200 µl of the transformation mix or an appropriate dilution onto antibiotic plates. You may have to plate all of the transformation mix if you know your cells or DNA produce low transformation efficiencies (common with plasmid mini-preps); the cells may be pelleted by centrifugation at 1000 x g for 1 minute, then the cells can be resuspended in 50-200 µl of SOC or LB medium and plated. (The maximum amount of solution that may be spread on a plate is ~200 µl).

   Note: For the positive control DNA, a 1:100 to 1:1000 dilutions are recommended for plating on LB plates.

9. Place plates in the 37°C incubator and grow overnight 14-18 hrs depending on the cell growth rate.

10. Use following calculations to determine the transformation efficiency (colony forming units=cfu):

    Transformation efficiency (cfu/ug DNA) =

    [(cfu on control plate)/(ng of control vector DNA used)] x ($1x10^3$ ng/µg) x (dilution plated)

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