MAKING ELECTROCOMPETENT BACTERIA

Day 1

1) Inoculate 4 mls of 2xYT (or LB) with a fresh bacterial colony. Or take 5 µl from an aliquot of competent cells (thawed on ice) and add to 5 mls of LB.
2) Grow in shaker-incubator overnight at 37ºC.
3) Place 1 liter of sterile deionized water; 500 mls of sterile 15% glycerol, and centrifuge rotors at 4ºC to chill.

Day 2

4) Inoculate 400 mls of 2xYT (or LB) with the overnight culture.
5) Grow in shaker-incubator at 37ºC till OD_{600} ~ 0.5 (~2 hrs).
6) Meanwhile, fill several ice buckets, and use them to prechill conical tubes, sterile deionized water (diw), and sterile 15% glycerol. Also fill 1 ice bucket with dry ice, and place a rotor in the centrifuge.
7) When bacteria have reached the appropriate optical density, chill the flasks for 15 min on ice, then pour the bacteria into chilled conical tubes. **From this point on, it is essential to keep the cells and everything that comes in contact with them ice cold.**
8) Spin at 5000 rpm for 5 min to pellet the bacteria.
9) Pour off the medium.
10) Resuspend the cells in 200 mls of cold diw.
11) Spin at 5000 rpm for 5 min to pellet the bacteria.
12) Pour off the diw.
13) Repeat steps 10.-12. **2** times, making sure to keep everything cold.
14) Repeat steps 10.-12. using 15% glycerol instead of diw.
15) Resuspend the bacteria in 2 mls of 15% glycerol. Gently tap sides of tube to break up the pellet.
16) Save an aliquot to calculate the efficiency of the batch.
17) Freeze bacteria in 100 µl aliquots in dry ice/ethanol bath.
18) Store at -80ºC till ready to use for transformations.

Calculating Transformation Efficiency

Transformation efficiency is the number of transformed cells (transformants) generated by 1 µg of supercoiled plasmid DNA in a transformation reaction. A known quantity of pUC19 DNA is typically used as the control.

Transformation efficiency (transformants/µg) is calculated as follows:

 # colonies on plate/ng of DNA plated X 1000 ng/µg

Perform serial dilutions:

0.1 ng of control DNA (1 µL of 0.1 ng/µl, freshly diluted) is added to 100 µL of competent cells. 900 µL of SOC medium is added prior to expression. 10 µL (equivalent to 0.001 ng DNA) is then diluted in 990 µL SOC and 100 µL is plated (equivalent to 0.0001 ng DNA).

If **100 colonies** are counted on the plate, the transformation efficiency is:
100 transformants / 0.0001 ng X 1000 ng/µg = 1 x 10^9 transformants/µg.

Factors That Affect Transformation Efficiency

1. DNA

Actual DNA Concentration

The amount of pUC19 DNA in the control tube included with commercial competent cells is carefully quantitated. However, some vendors may have different amounts of plasmid DNA in their preparations.

Forms of DNA

Linear and single-stranded DNA transforms <1% as efficiently as supercoiled DNA.

Purity of DNA

- **For electroporation**, never use more than 1 µl plasmid DNA per transformation. The salts contributed by the preparation can cause low transformation efficiencies.
- **Column-purified DNA** is generally free of contaminants that would interfere with chemical transformation.
- **Contaminants in miniprep DNA** can interfere with transformation. Limit the amount of miniprep DNA in a transformation as much as possible and never use more than 5 µl per 50-µl reaction.

- **Ligation mixtures** inhibit transformation. Ligase strongly inhibits electroporation, but this effect can be limited by heat inactivating the ligase in the ligation mixture (65℃ for 5 min) prior to adding DNA to the cells.