Bacterial Transformation

(Chemically Competent Cells)

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1) Retrieve the required number of tubes of competent cells and place on wet ice. Our lab stock is XL1-Blue MRF' and is packaged in 400 ul aliquots stored at –80^{o}C. You will need 100 ul of competent cells for each transformation. Once thawed, aliquots cannot be re-frozen, so discard extra cell volume if you are only transforming 1 or 2 samples.

2) Thaw the competent cells on ice. You can accelerate the thawing by briefly warming the tubes in your hands.

3) Once competent cells are thawed, dispense 100 ul of competent cells into each sample, consisting of either 5 ul of a ligation or 0.5 ul of a pure plasmid to be retransformed. Flick tube gently to mix DNA + Competent Cells.

4) Incubate DNA + Competent Cells on wet ice for 20'-30'.

5) Heat shock at 42^{o}C for EXACTLY 45". Do not exceed 45".

6) Allow cells to recover on wet ice for 2 minutes.

7) Add 200 ul of SOC to cells and incubate at 37^{o}C for 20'-30'.

8) Briefly spin cells down in capsulefuge, discard all but ~100 ul of supernatant, resuspend cells and plate entire volume on LB plates including appropriate antibiotic. Invert plates and incubate overnight at 37^{o}C.