Preparing electrocompetent cells.

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Day 1.
Start 3ml overnight culture.
Prepare 1L LB and 2.5L 10% (w/v) glycerol; autoclave. Place LB at 37˚C and glycerol at 4˚C.
Autoclave 4 x 250ml centrifuge bottles and 2 x 50ml centrifuge tubes.
Place centrifuge rotors and bottles in cold room (4˚C).

Day 2.
Inoculate LB; grow at 37˚C until OD ~0.6.

Cool culture in ice bath until cold. Pour into 250ml centrifuge bottles and spin, 5000 rpm, 8min., 4˚C

Pour off supernatant, put bottles on ice. Resuspend pellets in small volume glycerol soln.; fill bottles with glycerol soln. and spin again.

Repeat.

Resuspend pellets in ~30ml total of glycerol soln. Combine solutions and pour into small centrifuge tubes. Spin again, 5000rpm, 5min., 4˚C.

Pour off supernatant. Resuspend pellets in remaining supernatant and combine (~1-2 ml total). Dispense 50µl aliquots into eppendorf tubes and quick-freeze in ethanol/dry ice bath. Store at -80˚C.

**Cells are more competent if stored in 200µl aliquots, then separated into 50 µl aliquots when thawed.

Testing competence of cells

Transform using a known amount of high-copy-plasmid DNA (such as pUC19). 10-100 pg is a convenient amount.

Plate several dilutions of the transformation (100 µl each 1/10, $1/10^3$, $1/10^5$) on selective plates and incubate at 37˚C overnight.

Choose the plate with 20-200 colonies. Count the number of colonies and calculate the number of cfu (colony-forming units) per µg of plasmid DNA as follows:

$$\frac{\#\text{ colonies} \times \text{dilution}}{\mu\text{g DNA}}$$