

## Protocol: Small-scale electrocompetent cells

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This protocol is useful when you need to move a supercoiled plasmid or two into a strain that you don't transform very often. It works well with both *E. coli* and *Salmonella* but it is **not** useful for transforming ligations, which should be transformed into highly competent "cloning strains"—*e.g.* *E. coli* that are *recA*<sup>-</sup>. Before you start, make sure you have cold, sterile, purified water *or* cold, sterile 10% glycerol (it's handy to always have a bottle of sterile water or 10% glycerol in the fridge for this purpose). You should try to keep the cells cold (*e.g.* on ice) as much as possible for the entire procedure. Don't vortex the cells at any point—use a pipettor to resuspend the pellets.

- Subculture an overnight culture of the recipient strain 1:100 in a rich medium with appropriate antibiotics. You will need ~1 ml of culture for every transformation you do, but it's wise to have a bit extra.
- Grow at 37°C (or temperature appropriate for the strain) with shaking for 2-4 h, until the culture is partly turbid but still somewhat see-through. The cells should be in "mid-log phase" at this point. The OD<sub>600</sub> will be ~0.2-0.6 but there's no need to take a reading for this protocol.
- For each transformation, pellet 1 ml of culture in a microcentrifuge tube, preferably in a refrigerated microcentrifuge. If you don't have access to a refrigerated microcentrifuge, limit the time the cells spend in the centrifuge, which gets quite hot. It only takes ~30 sec to pellet 1 ml of cells at maximum speed. Put the tubes back on ice as soon as the 30 sec spin is finished.
- Resuspend pellet in 1 ml cold sterile water or 10% glycerol and keep tubes on ice while working with other tubes.
- Pellet and repeat with another 1 ml of water or 10% glycerol and another pellet step.
- Resuspend pellet in ~50 µl cold sterile water or 10% glycerol. The amount of liquid added depends on how dry you got the pellet. You want 50-70 µl of cell slurry in the end
- Your cells are ready for electroporation. Don't forget to de-salt your DNA (*e.g.* by dialysis) and keep those cuvettes chilled!

### To Transform Electrocompetent Cells:

- Mix an aliquot (~50-70 µl) of cells with 1-2 µl DNA in a low ionic strength buffer/water (dialyze or EtOH-precipitate ligations etc.)
- Place in pre-chilled cuvette
- Electroporate (2.5 V for 2 mm gap or 1.8 V for 1 mm gap; 200 Ω, 25 µF), Time constant should be ≥4 msec
- *Immediately* add 1 ml of SOC or LB to cell/DNA mix and place in test tube
- Grow at 37°C for one hour (or other temperature appropriate for plasmid)

- Plate onto selective media (*e.g.* use ~100  $\mu$ l of reaction on one plate, then spin down rest, resuspend in 100  $\mu$ l and plate all of that onto another plate)
  - Grow overnight at 37°C (or other temperature appropriate for plasmid)
- Pick colonies (hopefully)