# Electro-transformation of E. coli with a psgRNA library

## Competent cells

When introducing a guide RNA library into a strain it is vital not to introduce any bottleneck. We typically aim to obtain a minimum of 100x more colonies than the number of unique guides in the library. We provide here a protocol that has given reliable results to prepare competent cells and electroporate them.

This protocol starts from 300ml of culture to obtain ~400-500ul of cells which should be enough for ~20 electroporations.

1. Dilute 3 ml of overnight culture into 300 ml of LB.
2. Incubate at 37 degree to OD 600 of 1 (2.2-2.5 hrs).
3. Split culture into six 50 ml falcon tubes, chill tubes on ice, 5-10 min.
4. Spin cells at 1000 g for 20 min (4°C).
5. Discard the supernatant by inverting the tube, and allow the tube to stand upside-down without a cap on a tissue for a few seconds (5-10 s)
6. Perform the following steps twice:
	1. Put all the tubes on ice, pipette 2 ml chilled ddH2O to each tube, completely resuspend cells by pipetting gently.
	2. Pool all cells into two 50 ml falcon tubes (6 ml for each tube), adjust the volume to 50 ml (for each tube) by adding chilled ddH2O. Let the tubes stand in ice for 5-10 minutes.
	3. Spin cells at 1000 g for 20 min (4°C).
	4. Discard the supernatant by inverting the tube, and allow the tube to stand upside-down without a cap on a tissue for a few seconds (5-10 s).
7. Put all the tubes on ice, pipette 2 ml chilled 10% Glycerol (in ddH2O) to each tube, completely resuspend cells by pipetting gently.
8. Pool all resuspended cells into one 50 ml falcon tube, adjust the volume to 50 ml by adding chilled 10% Glycerol (in ddH2O). Let the tubes stand in ice for 5-10 minutes
9. Spin cells at 1000 g for 20 min (4°C), then 4000 g for 5 min (4°C).
10. Discard the supernatant by inverting the tube, and allow the tube to stand upside-down without a cap on a tissue for a few seconds (5-10 s)
11. Put all the tubes on ice, pipette 200 ul chilled 10% Glycerol (in ddH2O) to the tube, completely resuspend cells by pipetting very gently.
12. The final volume could be between 400 ul and 500 ul of cells in 10% Glycerol
13. Prepare aliquots of 20ul into pre-chilled eppendorf tubes and keep @ -80.

Notes:

1. We have observed that the optimal OD600 to grow the cells depends on the specific strain that you are using and might require some optimization. In our hands OD600 of 1.0 gave good results for E. coli MG1655 while OD600 of 0.4 seemed optimal for DH5a cells.
2. Freezing electro-competent cells will decrease competence substantially. We recommend to use fresh electro-competent cells to maximize coverage.

## Library Transformation

**Before getting started:**

1. Warm 3 ml of recovery medium (LB or SOC) at 37°C until usage.
2. Warm 10 square (12cm x 12cm) LB-kanamycin agar plates for plating the library.
3. Place electroporation cuvettes (1mm) on ice.
4. Desalt the DNA to be electroporated by dialysis on a 0.025um membrane filter (VSWP). Alternatively very small volumes of non-desalted DNA can be used (<0.3ul)
5. Thaw 1 vial of electro-competent cells and rest on ice until complete thawed. (The cell should be the host cell, where you want to do your screen )

**Electroporation protocol:**

1. Add ~ 50ng of plasmid (maximum 2ul) to the bottom of a cold 1.5 ml Eppendorf tube.
2. Add 20 ul of thawed electro-competent cells.
3. Transfer 20 µL of the bacteria/plasmid mixture into 1 mm electroporation cuvettes.
4. Electroporate with the following settings: 1.8 kV, 25uF, 200Ω
5. **Note:** if an arch occurs, try to add 10ul of cold ddH2O to a new tube of competent cells to dilute any leftover salts and try again.
6. After the pulse, quickly add 980 µL of the Recovery Medium into the cuvette. Pipet up and down three times to re-suspend the cells and transfer mixture to a 1.5 mL Eppendorf tube.
7. Place the tube in a shaking incubator for 1 hour at 37°C.
8. Serially dilute 10 µL of the transformation mixture in recovery medium 6 times, using a dilution factor of 1:10 at each step. Spot 10 µL of each dilution onto a round 10cm LB-kanamycin plate. This plate will be used to quantify the number of transformants obtained.
9. Resuspend the cells in 10ml of recovery medium and immediately plate 1ml on each of the square plates
10. Incubate plates at 37°C for 4-5 hours.
11. Pipette 10ml of LB on each plate and pool the colonies with a cell spreader. Collect the liquid in a tube.
12. You can save your pooled cells at -80°C with Glycerol, or proceed to library screening directly.