Transformation of Electrocompetent *E. coli* with Blue/White selection

**Procedure**

1. Desalt DNA template by EtOH precipitation in NaOAc followed by at least 2x washes with 70% EtOH. Resuspend in 5 - 15 µL of sterile H₂O.
2. Rinse cuvettes (if they have been used before) 5x with deionied H₂O, and place them on ice. This is sufficient to avoid background growth in most cases.
3. Set a BioRad MicroPulser to "Ec1" for 1 mm cuvettes, or "Ec 2" for 2 mm cuvettes.
4. Electroporate the DNA into the bacteria:
   1. Add 5 µL of DNA to 50 µL of bacteria and mix by pipetting.
   2. Transfer bacteria/DNA mix to a cold cuvette, and immediately pulse in the electroporator.
   3. Quickly add 1 mL of r.t. L.B. to the bacteria in the cuvette. Use a sterile Pasteur pipette to transfer the suspension to a bacteria tube.
   4. Repeat for additional DNA samples and control DNA.
5. Rotate at 37°C x30 min. to allow the bacteria to recover.
6. Meanwhile plate LB/Amp plates with IPTG + X-gal if blue/white selection of colonies will be performed, as follows:
   1. Mix 100 µL X-gal + 20 µL of IPTG per plate.
   2. Spread X-gal/IPTG mix across surface of plate with a sterile glass spreader and a plate spinner.
   3. Allow the mix to infiltration the media for 20 min.
7. Perform two 10-fold serial dilutions of the LB/bacteria suspension into fresh sterile L.B. Concentrate the remaining bacteria by spinning 1 min. at 5k rpm in a sterile microcentrifuge tube, and then resuspend pellet in 0.1 mL of L.B.
8. Plate 100 µL of the various concentrations of bacteria onto LB/Amp (+ X-gal/IPTG as necessary), and spread with a sterile spreader and plate spinner to evenly coat the plate. Record the DNA construct and the Bacteria Dilution Factor (9x, 1x, 1/10x, 1/100x) on each plate.
9. Incubate the plates inverted at 37°C o.n. They may need to be placed in a container if the humidity of the incubator is so low that it causes the agar to dry out.
10. Remove the plates when the colonies are ~1mm in diameter. The color development on X-gal treated plates will continue to occur after the plates are removed from the incubator.
11. Pick white colonies (also with no central blue coloration) and restreak on L.B./Amp plates to ensure that pure clones are obtained before performing plasmid preps.
12. Store at 4°C for at least 1 hr. (to firm up the agar) if colony hybridation will be performed. Seal edges of plates with parafilm for storage up to 1 week.
13. Count the numbers of colonies on the plate with control DNA to determine the efficiency of competent cells:
   1. Efficiency (Transformants/µg) = colonies/plate x (Bacterial dilution factor) x 20,000
   2. Highly competent cells should have ~50 colonies on the plate with a 1/10 dilution of the control DNA's bacteria suspension.

**Materials**

- Control DNA (e.g. pBS) diluted to 1 pg/µL in sterile H₂O, on ice.
- Electrocompetent bacteria, frozen (50 - 100 µL aliquots), on ice.
- BioRad cuvettes (1 mm gap), on ice.
- Sterile L-Broth (L.B.)
- Bacteria culture tubes
- P1000, P200, P20 Pipetman and sterile tips
- Sterile Pasteur pipets and bulb
- 37°C incubator and rotating wheel
- L.B./Amp bacterial culture plates (or other suitable selection media)
- X-gal 20 mg/mL in DMF (dimethylformamide), store at -20°C
- IPTG 0.2 g/mL in H₂O, store at -20°C