Lazzara Lab
Alice M. Walsh
Modified by MJL & EKD June 6, 2017

Transforming competent cells

Before you begin: Be sure the water bath is turned on and set to 42°C.

1. On ice: thaw competent E. coli (kept in the -80°C freezer). If using a new 0.5 mL tube of E. coli (applies to DH5α or XL1 Blue), aliquot into 50 µL aliquots in 1.5 mL Eppendorf tubes. For each transformation, use 50 µL of DH5α, XL1 Blue, or Stbl3 cells. Add 1 - 5 µL DNA to the mixture. [Typically, this means somewhere in the range of 1-100 ng, depending on the source of the DNA, e.g., low yield extraction from filter paper or high concentration stock from a miniprep or maxiprep.] Be careful pipetting and mixing cells as they are fragile; do not pipet up and down.

2. Incubate the mixture of bacteria and DNA on ice with occasional swirling for 30 min.

3. Heat shock bacteria by floating tubes in a 42°C water bath for 40-45 s.

4. Incubate tubes on ice for 2 min.

5. Add 750 µL SOC medium per sample, place the Eppendorf tube inside a plastic 15 mL culture tube, and incubate for 1 hr with shaking (220 rpm) at 37°C. [If necessary, a 30 min minimum incubation is fine.] During the 1 hr incubation, put ampicillin LB agar plates into the 37°C dry air incubator to warm.

6. Spread 50-300 µL of bacteria onto a plate using a few glass beads (autoclaved) or a sterile pipet tip. [If needed to plate more cells, you can spread a larger volume of bacteria on the plate. To minimize the total volume of fluid added to the plate, you can spin down the bacteria and re-constitute in a lower volume.]

7. Incubate plates overnight at 37°C. Pick colonies about 12-16 hr later. [Plates with colonies can be kept in the cold room or in a refrigerator dedicated to microbiological work.]