Ligation and Transformation Procedure (E.coli)

1. Create table a la Eli that outlines the amounts of things to be put together. Generally, it’s water plus buffer, ATP, digested vector, digested insert, and the ligase.
2. Put together the components, starting with water and ending with ligase. Others can be in whatever order.
3. Spin at low speed (~5000 RPM) briefly to bring contents to bottom of tube
4. Let ligation mixture incubate at room temperature for 20 minutes. In the meantime, heat up the dry incubator to 75°C.
5. Incubate ligation mixture at 75°C for 15 minutes. Transfer to bench to cool to room temperature for ~15 min or so, though longer is fine. Pulse spin the mixture to gather contents at the bottom of the tube. Set it down on the bench
6. Take 1 tube of ultra-competent XL-10 *E.coli* and place directly on ice, along with the accompanying tube of 2-beta-mercaptoethanol (2-BME).
7. Take 2 autoclaved round-bottomed tubes and place them on ice as well. Wait about 10-15 minutes for them to chill and for the other 2 entities to thaw.
8. Add 70 uL of cells to each tube, followed by 2.8 uL of 2-BME. Swirl to mix and incubate on ice for 10 minutes
9. Add 2 uL of ligation mixture to each tube of cells. Swirl to mix and incubate on ice for 30 minutes. In the meantime, heat up the dry incubator to 42°C. Take sterile SAC media, unscrew the cap, and place in the 37°C bath to heat up.
10. Bring tubes on ice to dry incubator. Heat shock them for exactly 30 seconds.
11. Plunge tubes into ice and allow to sit for 2 minutes or so.
12. Take tubes off ice and into a rack. Take rack to bath and, with sterile technique, place 0.9 mL of SAC media into each tube
13. Place tubes into shake incubator and allow to grow for 1 hour.
14. Bring out tubes and place on rack. Bring out appropriate number of plates per tube.
15. For each plate, start by pouring out about a dozen glass beads into the plate. Add cells (usually 30 uL) to the plate, place on the lid, and use beads to streak the plate.
16. Dump beads into a beaker, cover and invert the plate, and label the plate itself. Continue with other plates.
17. Once finished, place the plates upside-down in the incubator in a stack and allow to grow overnight. Place the beads into the jar of EtOH.