E. coli Transformation

1. **Take 1 tube of competent cell (100μl), thaw it on ice.**

2. **Add 7μl of a ligation product or 2μl BP/LR *in vitro* recombination product to the competent cell and incubate the sample on ice for 30 min. During the incubation, check that a water bath or heat block is ready at 42°C. In addition, take an LB plate containing the right antibiotic out of the refrigerator, and keep it at room temperature.**

3. **Heat shock the cells at 42 °C for 90 seconds; put the tube on ice immediately and incubate it on ice for 2 min.**

4. **Add 500μl LB (without antibiotic) and incubate the sample at 30 °C or 37 °C for 1 hour with shaking or rotating. During this time, place the LB plate in a 37 ℃ incubator.**

5. **Place 15-20 autoclaved glass beads in the LB plate. Plate 200ul cells in the LB plate and spread the bacteria be gentle shaking. Incubate at 37 °C at least 12 hours. Store the leftover culture in a refrigerator till you know your plate is fine the next day.**