SOP Name: *E. coli* Transformation Procedure
Date: 09/05/17
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Source(s): N/a
Time Required: Up to 2.5 hours

Notes: Carry out all steps under sterile conditions. Keep everything on ice unless specified otherwise. Handle the cells with care as competent cells can be very fragile. This protocol is for one transformation – remember to perform controls.

Materials:

- 50 µL competent *E. coli* cells
- 950 µL LB or SOB media
- Sterile glass beads (~ 5)
- Agar plates (+ antibiotic if required)

Procedure:

1. Thaw 50 µL competent cells on ice (~30 mins)
2. Add 1-2µL plasmid DNA to the cells
3. GENTLY mix the cells and DNA by pipetting up and down
4. Incubate the mixture on ice for a further 30 mins
5. Heat shock the cells by placing the tubes in a float and leaving in a 42°C water bath for 1.5 mins
6. Immediately incubate on ice for 5 mins
7. Add 950 µL of SOB or LB media to the cells and incubate at 250 RPM and 37°C for 60 mins
8. Label an agar plate (containing the correct antibiotic, if required) with: initials, cell strain, plasmid name, antibiotic, date, and any other necessary information required for identification
9. Warm the agar plates in a 37°C static incubator for ~ 30-60 mins before plating
10. Pellet the cell mixture at 4,000 RPM for 5 mins and remove 700 µL of the supernatant
11. GENTLY resuspend the pellet in the remaining ~300 µL by pipetting up and down
12. Plate the entire mixture onto an agar plate by pipetting the mixture into the centre of the agar, adding ~5 sterile glass beads, and swirling the plate so that the beads spread the mixture over the entire surface
13. Leave for a few minutes and then turn the plate agar-side down, remove the beads from the lid, and incubate overnight in a static incubator at 37°C