Transformation protocol

Materials:

- Plasmid samples or ligation product;
- Commercially competent cells;
- LB non-antibiotic liquid meium;
- LB antibiotic agar plates

Procedure:

1. Get the competent cells from −70 degree, and wait for its fusion. 30–50 μl of competent E.*coli* cells for each sample. Put microcentrifuge tubes to chill on ice for at least 2 min.

2. Add 2 – 3 ul of each plasmid sample or all the ligation product into the competent cells in the microcentrifuge tubes. Mix and incubate on ice for 30 min.

3. Heat pulse for 90 sec, at 42 degree. Put back to ice and incubate for 5 min.

4. Add 200 uL LB non-antibiotic liquid medium into each microcentrifuge tube. Shake the microcentrifuge tubes in shaker, at 37 degree, for 30 min to recover.

5. Plate 150 uL of the liquid medium with transformed cells immediately, on prewarmed LB antibiotic agar plates. Incubate overnight at 37°C for 10–14h.

Tips:

- All procedures are performed on ice.
- Make sure the cells are not left at ambient temperature for more than 5

min as thiswill significa ntly decrease the transformation efficiency.

- When got out from the shaker, the competent cells ma y form pellet in the microcentrifuge tubes. You need to resuspend the cells before plating.

References:

*Current protocols in molecular biology.