Protocol for Bistable Switch Characterization

Materials:

- Bistable switch plasmid
- Commercial competent cells
- LB non-antibiotic liquid medium
- LB or M9 antibiotic agar plates with appropriate concentration of ligand

Procedure:

1. Add 2ul of each plasmid into 50 µ l of competent *E. coli* cells in the microcentrifuge tubes.
2. Mix and incubate on ice for 30 min.
3. Water bath the tubes at 42 degrees for 90 sec. Then put them back on ice and incubate for 5 min.
4. Add 200 uL LB non-antibiotic liquid medium into each microcentrifuge tube.

Incubate the microcentrifuge tubes at 37 degrees at 200 r.p.m. for 40 min.

5. Plate 150 uL of the culture on prewarmed LB or M9 agar plates with appropriate concentration of ligand. Incubate overnight at 37 degrees.
6. Count the number of green or red colonies using fluorescence microscopy and calculate the ratio of green colonies to red ones.

Note:

1. All procedures are performed on ice.
2. Make sure the cells are not left at ambient temperature for more than 5 min as this will significantly decrease the transformation efficiency.
3. When got out from the shaker, the competent cells may form pellet in the microcentrifuge tubes. You need to resuspend the cells before plating.