

Protocol for ligand inducible expression of GFP

Materials:

- Ligand solution of Different concentrations, inducing solution
- Overnight bacterial culture or bacterial colonies
- Phosphate buffered solution (PBS)

Procedure:

1. Add 20 μ l of the overnight bacteria l culture or pick a colony to 5 ml of M9 minimal antibiotic medium, and incubate at 37 degrees in a shaker till the OD600 value reaches 0.4-0.5.
2. Prepare the induction system with ligand concentration gradient by adding appropriate volume of inducing solution and ligand solution into 0.5 mL of the fresh bacterial culture.
3. Place the induction system at 37 degrees in a shaker for 6 hours.
4. Pellet bacterial cells by 10 min centrifugation at 3000 rpm, discard the supernatant.
5. Resuspend the pelleted cells in 0.5mL of PBS.
6. Transfer 0.1mL of the solution into each well of 96-well plate to test the expression of GFP and OD value by Microplate Reader .

Note:

1. To guarantee that the medium doesn't contain the ligand, all of our inducible medium is M9 minimal medium that all of the components are known, and it also requires longer time to induce the expression of proteins.
2. If desired, time sequential expression of GFP could also be tested, through verifying the incubating time of induction system at 37 degrees.