Protocol for inducing the AND gate system

Materials:

- Ligand solution of different concentrations, arabinose solution, salicylic acid 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 0.3.
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 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. The OD value of the culture when induced could influence the performance of AND gate system a lot, so it must be strictly controlled.
3. The maximum final concentration of arabinose is 1mM, and the maximum final concentration of salicylic acid is 0.1mM.