University of St Andrews iGEM Team
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K628006 Characterization Experiment Protocol

Requirements:

1x Tecan Infinite 200 Pro Microplate Reader
1x Corning 96-Well plate
1x Baclight LIVE/DEAD Viability kit (series 7012)
1x agar plate with K628006-transformed (PSB1C3 backbone) *E. coli* (DH5α) that has been streaked for single colonies
1x agar plate with non-transformed supercompetent *E. coli* (DH5α) that has been streaked for single colonies

10 mL sterile luria broth solution
10 μL chloramphenicol
2 mL sterile water
1 mL filter-sterilized 10% arabinose (1g : 10 mL water)
1 mL filter-sterilized 1% arabinose (0.1g : 10 mL water)
1 mL filter-sterilized 0.1% arabinose (0.01g : 10 mL water)
1 mL filter-sterilized 0.01% arabinose (0.001g : 10 mL water)

1.5 ml microcentrifuge tubes
15 mL falcon tubes

Protocol:

Step 1

Add 5 mL of luria broth to two sterile 15 mL falcon tubes. Mix 10 μL chloramphenciol and a single DH5α colony that has been transformed with the BBa_K628006 biobrick into one tube. Add a single non-transformed supercompetent colony into the other tube. Incubate at 37 °C, shaking at 210 rpm for 12-16 hours and then remove to room temperature.

Step 2*

From the Baclight LIVE/DEAD Viability kit, pipette 6 μL of the SYO9 dye and add it to 6 μL of the propidium iodine dye in a 1.5 mL microcentrifuge tube. Pipette up and down several times to mix.

Step 3

Add 2 mL sterile water to a 15 mL falcon tube. Pipette the 12 μL dye solution from Step 2 and mix it into the water.

Step 4*

Pipette 1 mL of transformed DH5α into a 15 mL falcon tube labeled Tube 1. Pipette 1 mL of non-transformed DH5α into a separate 15 mL tube labeled Tube 2. Add 1 mL of the dye solution (at a 1:1 ratio) to each tube and mix gently. Let the tubes sit in a dark area for 15 minutes at room temperature while the dye incubates.

Step 5

During the 15-minute incubation period, pipette the following into separate wells of a Corning 96-Well Flat plate:

1 μL of 0.01% arabinose solution
2.5 μL of 0.01% arabinose solution
2.5 μL of 0.1% arabinose solution
2.5 μL of 1% arabinose solution
2.5 μL of 10% arabinose solution

Leave the next well empty of arabinose, totaling six wells.

Repeat this process in a different row of wells, totaling twelve used wells.

Step 6

After the 15-minute incubation period, pipette 200 μL of the Tube 1 solution into the first row of six wells. Pipette up and down several times to mix.

Pipette 200 μL of the Tube 2 solution into the second row of six wells. Pipette up and down several times to mix.

The concentration of arabinose in each set of wells should be:

1 μL of 0.01% arabinose solution ..... 2.96 μM
2.5 μL of 0.01% arabinose solution .....7.4 μM
2.5 μL of 0.1% arabinose solution .....74 μM
2.5 μL of 1% arabinose solution .....740 μM
2.5 μL of 10% arabinose solution .....7400 μM
0 μL of arabinose .....0 μM

Step 7*

Load the plate into the 200 Pro Microplate Reader. Set the fluorescence options to produce exciting light at 485 nm and read at 600 nm. Take fluorescence readings in a 4x4 'circular filled' pattern (at radius of 500) every 5 minutes for 90 minutes.

*Consult the Baclight LIVE/DEAD Viability Kit Protocol (7012) for more information on these steps.
http://probes.invitrogen.com/media/pis/mp07007.pdf