

pBAD Strong/Protegrin-1 Experiment Protocol

Requirements:

1x Tecan Infinite 200 Pro Microplate Reader
1x Corning 96-Well plate
1x agar plate with K628006-transformed (in the PSB1C3 plasmid) *E. coli* (DH5 α) that has been streaked for single colonies
1x agar plate with non-transformed supercompetent *E. coli* (DH5 α) that has been streaked for single colonies

10 mL sterile luria broth solution
10 μ L chloramphenicol
50 mL filter-sterilized 10% arabinose solution (1g of arabinose : 10 mL water)
100 μ L gomesin at 8 mg/mL

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 μ L chloramphenicol and a single DH5 α 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

Add 500 μ L non-transformed DH5 α to two separate 15 mL falcon tubes. Add to each tube 4500 μ L luria broth, and label the tubes as Tube 1 and Tube 2.

Add 500 μ L transformed DH5 α to a 15 mL falcon tube with 4500 μ L luria broth, labeled Tube 3.

Step 3

To Tube 2, add 30 μ L of gomesin. Pipette up and down several times to mix.

To Tube 3, add 50 μ L of the 10% arabinose solution. Pipette up and down several times to mix.

Step 4

Remove 200 uL of liquid from Tubes 1, 2, and 3, and pipette the solutions into separate wells of a Corning 96-Well plate. Place the tubes in an incubator at 37 °C shaking at 210 rpm.

Step 5

Load the plate into the 200 Pro Microplate Reader. Set the reader to produce measures of absorbance. Take absorbance readings in a 4x4 'circular filled' pattern (at radius of 500).

Step 6

Repeat Step 4 - 5 every 10 minutes for 120 minutes.