University of St Andrews iGEM Team
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pBAD Strong/Protegrin-1 'Drug Delivery' Protocol

Requirements:

1x Tecan Infinite 200 Pro Microplate Reader
1x eppendorf Centrifuge 5424
3x HisLink Protein Purification columns
1x agar plate with *E. coli* (DH5α) that has been transformed with both K628006 and {J23101 + B00032 + E0040 + his-tag + B0015}. This plate should be 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
50 μL filter-sterilzed 10% arabinose solution (1g of arabinose : 10 mL water)
30 μL 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 μL chloramphenciol and a single DH5α colony that has been transformed with the Bba_K628006 biobrick and the {J23101 + B00032 + E0040 + his-tag + B0015} biobrick into one tube, labeled Sample 1.

Add a single DH5α colony that has been transformed with ONLY {J23101 + B00032 + E0040 + his-tag + B0015} into the other tube, labled Sample 2.

Incubate at 37 °C, shaking at 210 rpm for 12-16 hours and then remove to room temperature.

Step 2

Add 500 μL DH5α from Sample 1 to a 15 mL falcon tube with 4500 μL luria broth, labeled tube A.

Add 500 μL DH5α from Sample 2 into two separate 15 mL falcon tubes. Add to each tube 4500 μL luria broth, and label the tubes B and C.

Step 3

To Tube A, add 50 μL of sterilized water.
To Tube B, add 30 μL of gomesin and 20 μL sterilized water.
To Tube C, add 50 μL of the 10% arabinose solution

Incubate these tubes for 90 minutes at 37 °C, shaking at 210 rpm.

Step 4

Remove 1 mL from each tube and place them in labeled 1.5 mL microcentrifuge tubes. Spin these tubes down in a centrifuge at 10,000* rpm for 1 minute.

Step 5

Pipette the GFP-containing supernatant from each tube and run it through separate columns set up for metal ion affinity chromatography.

For more information on metal ion affinity chromatography, please consult: http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Bulletins/101/HisLink%20Protein%20Purification%20Resin%20Protocol.ashx

Step 6

Elute the GFP from each column and separately measure their fluorescence under a microplate reader.

* It is important not to centrifuge faster than this speed, as cells damaged via sheer force damage will release their GFP contents, skewing the final results of the experiment.