Osmotic pressure testing

Aim

Show the function of the OmpR promoter.

Procedure

1. Prepare the solutions in appendix 1

2. Choose sample with similar fluorescent protein as a positive control and sample without fluorescent protein as a negative control.

3. Use only 0% and 15% sucrose nutrient broth for both the positive and the negative controls.

4. Prepare overnight culture of the bacteria in 10 mL osmo media in E-flasks (100 ml). Inoculate one fresh colony in each flask and incubate overnight at 37°C.

5. Do not forget to include negative and positive controls. Check the OD next morning and note it down.

6. Dilute the overnight culture using 1:20 dilutions (2 ml of overnight cultured bacteria into 38 mL of respective osmo media in E-flask) and let them grow in the incubator at 37°C.

7. At each 0.1 OD (OD 0.1, 0.2, 0.3, etc) take 1 ml of the culture and add Kanamycin 340 µg to 1 mL culture to inhibit protein synthesis and immediately put the sample in refrigerator. When all the samples are done, centrifuge all the samples and remove the media. Suspend the cell pellets in 1 mL of PBS and perform fluorescence analysis directly using plate reader. Replace Kanamycin with other antibiotics if your cells contain Kanamycin resistance gene.

8. Use excitation and emission length accordingly (depending on the fluorescent protein). An example that we used is the excitation length 580 nm ± 10 and emission length 627 nm ± 30 to measure RFP. Use the same settings for the control samples.

9. Add 100 µl of sample to a 96 well plate and measure in CLARIOstar. For each sample, 5 replicates were made and the average values of each sample were used.

10. Do not forget to include negative and positive control during measurement. Use only media as a blank.

11. Measure the fluorescence and compare with the controls and blank. Plot it as a graph to observe *relative* change with different osmo concentrations.

Appendix

DON'T FORGET TO ADD ANTIBIOTICS TO FLASKS

0% sucrose

For 10 ml:

5 ml nutrient broth

5 ml ddH_2O

Required for osmo test: 30 ml (3 samples)

5% sucrose

For 10 ml:

5 ml nutrient broth

5 ml 10% sucrose solution

Required for osmo test: 10 ml (1 sample)

10% sucrose

For 10 ml:

5 ml nutrient broth

5 ml 20% sucrose solution

Required for osmo test: 10 ml (1 sample)

15% sucrose

For 10 ml:

5 ml nutrient broth

5 ml 30% sucrose solution
Required for osmo test: 30 ml (3 samples)

Recipes for sucrose solutions from 30% stock solution

10% sucrose
For 10 ml:
3.33 ml 30% sucrose solution
7.67 ml ddH_2O

20% sucrose
For 10 ml:
6.67 ml 30% sucrose solution
3.33 ml ddH_2O

Kanamycin 340 µg/mL
For 20 ml:
136 µl kanamycin
19864 µl ddH2O
Required: 150 ml

Sources

iGEM Stockholm 2015
http://2015.igem.org/wiki/images/0/05/STHLM_Alternative_Osmolarity_protocol.pdf