pFruB Specificity Experiment

Project: Sequencing
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AIM:

To analyse the activity (specificity) of pFruB-FruR system in the presence of 2 sugars at a time: it's own specific sugar (Fructose) and another (Glucose).

PRINCIPLE:

The sugarcane internal environment has comparable concentrations of fructose and glucose. Hence, it is important for us to determine the pFruB-FruR system's specificity to Fructose.

MATERIALS REQUIRED

- 5 x 5 ml Eppendorfs
- 100 ml conical flask
- LB media
- Cloning kit
- Fructose powder
- Glucose powder
- Distilled water
- Measuring cylinder
- Pipettes
- 1 mL microtips

PROCEDURE:

- Cloned the Fructose regulated anti-invertase construct into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Kanamycin).
- 50 mL of prepared culture using LB media as directed by the LB Medium Preparation protocol.
- Fructose medium is prepared at a stock solution of 10mM. That would require 0.18g of fructose powder in 10 ml of distilled autoclaved water. The same is repeated for glucose.
- To prepare the Glucose+Fructose solution the sugar concentration is effectively doubled.
- Table1 lists the varying concentrations and volumes to be followed.

Table1

	A	B	C	D	E	F	G	H
1	**Sample**	**[Fructose] (in µM)**	**[Glucose] (in µM)**	**Volume of [Fructose] stock to be added (in µl)**	**Volume of [Fructose] stock to be added (in µl)**	**Volume of water to be added (in ml)**	**Total Volume (in ml)**	**Amount of culture to be added (in ml)**
2	Blank	0.00	0.00	0.00	0.00	Only LB medium	1.50	
3	Control	1000.00	0.00	150.00	0.00	0.85	1.50	1.00
4	1	0.00	1000.00	0.00	150.00	0.85	1.50	1.00
5	2	1000.00	1000.00	150.00	150.00	0.70	1.50	1.00
6	3	1000.00	800.00	150.00	120.00	0.73	1.50	1.00
7	4	1000.00	600.00	150.00	90.00	0.76	1.50	1.00
8	5	1000.00	400.00	150.00	60.00	0.79	1.50	1.00
9	6	1000.00	200.00	150.00	30.00	0.82	1.50	1.00
10	**Total**			900.00	600.00	5.50	12.00	7.00

- Fluoroscence is measured after 5 hours of incubation, in 2 hour intervals for a total duration of 12 hours

HYPOTHESIS:

Negative control (Blank) should show basal level of fluoroscence.
Among the positive controls, the Fructose solution should give a rise in Fluoroscence while the Glucose solution should not.
The solution with both sugars should show a similar rise in fluoroscence to the Fructose solution.