

# pFruB Characterization

---

**Project:** Sequencing

**Authors:** Gourav Saha

**Created at:** 2020-07-03T05:13:02.626891+00:00

FRIDAY, 3/7/2020

---

## Aim:

To check pFruB activity in the presence and absence of D-Fructose in the medium; To characterise the change in fluorescence at different concentrations of D-Fructose.

## Principle:

Through literature survey, we identified the maximum and minimum concentrations of fructose inside various genotypes of sugarcane

The minimum conc, at 0.5% is .277 mM = 277 microM

The maximum conc at 1.7% is .944 mM = 944 microM

Hence, we take the range of Fructose concentrations from 200 microM to 1000 microM to cover that range of concentrations.

This can then be measured alongside a blank LB medium in a fluorospectrometer. This will tell us how the promoter behaves in different concentrations.

## Materials Required:

- 1 L flask
- 96 well plates
- LB media
- Fructose
- Distilled water
- Cloning kit

## Procedure:

- Clone 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).
- Prepare 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 (company) in 10 ml of distilled autoclaved water.
- Use that to prepare solutions ranging from 200  $\mu$ M to 1000  $\mu$ M.

**Table1** ^

	A	B	C	D	E	F
1	<b>Sample No.</b>	<b>[Fructose] (in <math>\mu\text{M}</math>)</b>	<b>Volume of stock to be added (in <math>\mu\text{l}</math>)</b>	<b>Volume of water to be added (in ml)</b>	<b>Total Volume (in ml)</b>	<b>Amount of culture to be added (in ml)</b>
2	Blank	0.00	0.00	Only LB medium	1.50	0.00
3	Control	0.00	0.00	0.50	1.50	1.00
4	1	200.00	30.00	0.47	1.50	1.00
5	2	300.00	45.00	0.46	1.50	1.00
6	3	400.00	60.00	0.44	1.50	1.00
7	4	500.00	75.00	0.43	1.50	1.00
8	5	600.00	90.00	0.41	1.50	1.00
9	6	700.00	105.00	0.40	1.50	1.00
10	7	800.00	120.00	0.38	1.50	1.00
11	8	900.00	135.00	0.37	1.50	1.00
12	9	1000.00	150.00	0.35	1.50	1.00
13	<b>Total</b>		810.00	4.19	16.50	10.00

- 96 wells plate is prepared with all the different samples prepared using Table1
- Fluorescence is detected by the ELISA machine or the fluorospectrometer after 5 hours of incubation.

## Hypothesis:

An increase in fluorescence is expected with an increase in fructose concentration.