pFruB Binary Sugar combination

Project: Sequencing
Authors: Suhas Badadal
Created at: 2020-07-03T14:12:11.049690+00:00

FRIDAY, 3/7/2020

AIM:

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

PRINCIPLE:

One blank with just autoclaved LB
One negative control with sugar absent
One positive control with only fructose and another with only glucose
One solution with both Fructose and Glucose
Try with different conc of glucose
Fluoroscence

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 biosensor into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Also mention the antibiotic used)
- 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 (company) in 10 ml of distilled autoclaved water. The same is repeated for glucose (company).

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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

- To prepare the Glucose+Fructose solution the sugar concentration is effectively doubled. W
- Fluoroscence is measured using the (fluorometer?) starting after 5 hours, 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 (how change? affinity??)
The solution with both sugars should show a similar rise in fluoroscence to the Fructose solution.

PCR

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

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Procedure

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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 concnetrations 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.
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Materials Required:

- 1 L flask
- 96 well plates (company) or eppendorfs
- LB media
- Fructose
- Distilled water
- Cloning kit (company)

Protocols

Procedure:

- Cloned the fructose biosensor into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Also mention the antibiotic used)
- 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 (company) in 10 ml of distilled autoclaved water.
- Use that to prepare solutions ranging from 200 microM to 1000 microM

Table1

	A	B	C	D	E	F
1	**Sample No.**	**[Fructose] (in µM)**	**Volume of 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	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	**Total**		810.00	4.19	16.50	10.00

- 96 wells plate is prepared with all the different samples.
- Induction period of 5 hours.
- Fluoroscence is detected by the ELISA machine or the fluorospectrometer.

Hypothesis:

An increase in fluoroscence is expected with an increase in fructose concentration, which can be characterized.
Talk about the sensitivity and expected
Look at various other promoters to use downstream and make a cascade

mCherry Characterization

Project: Sequencing
Authors: naman choudhary
Created at: 2020-07-09T08:28:07.770037+00:00

THURSDAY, 9/7/2020

Aim:

To characterize the mCherry and check the stability of mCherry in sunlight and at different pH.

Principle:

Proteins are stable at a particular temperature and pH.

Materials Required:

cloning kit
LB media
His-purification kit
buffers with different pH

Procedure:

- Cloned the mCherry gene into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Also mention the antibiotic used).
- Prepared culture using LB media as directed by the LB Medium Preparation protocol.
- After cultivation and cell lysis protein was purified using His-purification kit from .....
- Then we run the protein on SDS-PAGE to check the purity.
- Then we measure its emission and excitation spectra.
- To check light tolerance, expose the protein in sun light at room temperature and check the fluorescence intensity after every 20 minutes.
- To test pH sensitivity, measure the fluoroscence intensity after incubation it with buffers of different pH.

Hypothesis

Anti-Invertase Assay

Project: Sequencing
Authors: Suhas Badadal
Created at: 2020-07-09T14:08:01.665323+00:00

THURSDAY, 9/7/2020

Aim:

To verify the action of anti-invertase on invertase, and determine the concentration needed to inhibit invertase activity

Principle:

Materials Required:

- 96 well plate (company) or Eppendorfs
- 100 ml conical flask
- LB media
- Cloning kit
- Glucose stock
- Sucrose stock
- Pipettes
- 10 µl microtips
- 100 µl microtips

Procedure:

- Cloned the fructose biosensor into E.coli K-12 using pET28-a backbone as directed by the Cloning protocol, and plates are prepared (Also mention the antibiotic used)
- Prepared (1L)? culture using LB media as directed by the LB Medium Preparation protocol
- Anti-invertase protein extracted as directed by the [His-tag Antibody protein purification] protocol and stored at an appropriate temp(?) 1 L culture is expected to give around 2-3 mg, 4 mg at best, while a 10 L culture can give 25 mg.

- A commercial **invertase assay kit** employing yeast invertase (Cat.MAK118, Sigma Aldrich, USA) was used as per the manufacturer's protocol to examine the potency of recombinant ShINH1.
  https://www.sigmaaldrich.com/catalog/product/sigma/mak118?lang=en&region=IN

- 40 µl of reaction volume containing different concentrations of ShINH1, from 0.1 µM to 0.5 µM, in increments of 0.1 µM are pre-incubated in a 96-well plate with commercial acid invertase at 37°C for 30 min.
- Glucose standards (40 µl volume, 0–100 µM glucose) are added to separate wells of the plate. The same volume of reaction buffer was used as the assay blank in separate wells.
- Substrate was added to each well 5 µl of 20 mM sucrose followed by incubation for 20 min at room temperature.

- After incubation, the reaction mixture containing 95 µl of reaction buffer, 1 µl of enzyme mix and 1 µl of dye reagent (all supplied with the kit) was prepared and 90 µl of reaction mix was added to each of the blank, sample, and standard wells followed by incubation for 20 min at room temperature in darkness.
- The amount of glucose liberated was calculated from the glucose standard curve.
- The specific activity of enzyme was calculated and expressed as µmoles of glucose formed per milligram of protein per minute.

Procedure

	A	B	C	D	E	F	G
1	**Sample**	**[Anti-Invertase Protein] (in µM)**	**Reaction volume of Anti-Inv (in µl)**	**[Sucrose] (in mM)**	**Volume of [Sucrose] stock to be added (in µl)**	**Volume of reaction mix to be added (in µl)**	**Total Volume (in ml)**
2	Blank	0.00	40	20.00	5.00	90.00	0.135
3	1	0.10	40	20.00	5.00	90.00	0.135
4	2	0.20	40	20.00	5.00	90.00	0.135
5	3	0.30	40	20.00	5.00	90.00	0.135
6	4	0.40	40	20.00	5.00	90.00	0.135
7	5	0.50	40	20.00	5.00	90.00	0.135
8	**Total**	1.50	240	120.00	30.00	540.00	0.81

Hypothesis