CarH system kinetics

Introduction

Protocol for measuring growth kinetics of the CarH system, desgned by Vilnius-Lithuania 2019 iGEM team

Materials

- Sterile standard M9-CA broth
- Cyanocobalamin (vitamin B12)
- Syringe and sterile syringe filters (optional)
- TG1 chemocompetent cells
- Polycystronic CarH system plasmid with mRFP as a reporter
- A red light source
- A green or white light source
- Saline solution (0.9 NaCl)
- Chloramphenicol

Procedure

Experiment plan:

1. Transform the CarH plasmid plasmid to TG1 cells and plate on LB agar plates with Ampicillin
2. Next day, inoculate the bacteria in LB liquid media.
3. During the time of bacteria growth make a stock 1mM cyanocobalamin solution and sterilize it with a filter syringe or autoclave. IMPORTANT: cobalamin is LIGHT SENSITIVE, only work under RED LIGHT.
4. Prepare 8 sterile 5 mL tubes with LB with 10μM of cobalamin and Ampicillin.
5. Transfer 50μL of the inoculate (1:100) to the prepered tubes. Wrap 8 of them in foil – this will protect them from light. To keep the growing conditions the same, it is best to put the light source and an appropriate shaker inside a thermostat, set the temperature to 30 degrees Celsius (light source can create extra heat, therefore, it is not advised to grow the bacteria at 37 degrees celsius).
6. Let the bacteria grow for an hour and then every hour take 180μL out of every tube and mix it with 20μL of 25 ng/ml chloramphenicol solution and keep the in the bacteria cold (on ice or in a refrigerator). Everytime time when taking a sample, remove the foil from a single tube and let it to continue growing in the light.
7. Stop the experiment when you have to take the foil of the last tube (9 hours).

8. Centrifuge every sample, discard the supertenant, wash with and resuspend in 100 uL of saline solution with chloramphenicol (this is done because cyanocobalamin is light sensitive, therefore, there will be different concentrations of it and there will be no way to get a blank)

9. Fill a microplate with the samples and measure with a microplate reader. Microplate reader settings:

Table1

	A	B	C	D	E	F	G	H
1	Plate Number	Plate 1						
2	Date	2019-10-17						
3	Time	5:21:01 pop.						
4	Reader Type:	Synergy H4						
5	Reader Serial Number:	257149						
6	Reading Type	Reader						
7								
8	**Procedure Details**							
9	Plate Type	96 WELL PLATE						
10	Read	Absorbance Endpoint						
11		Full Plate						
12		Wavelengths: 600						
13		Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 8						
14	Set Temperature	Incubator off						
15	Read	Fluorescence Endpoint						
16		Full Plate						
17		Filter Set 1						
18		Excitation: 565/20.0, Emission: 606/20.0						
19		Optics: Top, Gain: 100						
20		Light Source: Xenon Flash						
21		Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10						
22		Read Height: 8 mm						

10. When analyzing data, divide the sample's fluorescence by its OD