PROTOCOL: Cultivation experiment

Preparation of modified 2x Mal medium

Material and methods:

Check if you have a sufficient amount of maltose!

For 1 l of this medium:

Yeast extract	2%	20 g	▢
Tryptone peptone	1%	10 g	▢
NaCl	1%	10 g	▢
dH_2O	solvent	1 l (but count with addition of maltose and sulfate)	▢
Now autoclave (15 min, 121 °C)			▢
maltose hydrate	7,5%	75 g	▢
$MgSO_4.7H_2O$	7,5 µg/ml of media	9,8 g	▢

Workflow:

1. Weight yeast extract, tryptone peptone and NaCl to the glass flask and add appropriate amounts of distilled water. Now sterilize the medium.

2. Dilute maltose in 100 ml of distilled water and use a 0.2 nm filter for sterilization. You can not autoclave maltose in medium - the medium would caramelize. The same way is used for sterilisation of sulfate - only diluted in 50 ml of water. If you use this method of sterilization of maltose and sulfate, then use only 850 ml of water to the autoclavable media. This way, you will avoid precipitation.

3. Let autoclavable media cool a little bit and mix all sterilized components together.

4. Adjust pH to 7. And store at 4 °C.

Cultivation experiment

Material and chemicals:

Expression and control strains of *Bacillus subtilis*
LB medium
Appropriate antibiotic
modified 2x Mal medium
spectrophotometer and cuvettes
orbital shaker with adjustable temperature

Workflow:

1. Read Notes before proceeding.

2. Prepare overnight (ON) cultures of expression and control strains in 10 ml of liquid LB medium with appropriate antibiotics. Use a test tube, Erlenmayer flask or Falcon tube with volume at least 50 ml.

3. Incubate at 37 °C with vigorous shaking no longer than 16 hours.

4. Measure OD_{600} of every sample.

5. Calculate the amount of ON culture you need to inoculate to 20 ml of fresh LB medium to get the starting OD_{600} = 0.05. Do this for every sample. Use this formula:

$$V_{(ON)} = \frac{V_{(medium)} \times OD(desired)}{OD(actual)}$$

6. Inoculate the calculated volume of the ON culture to 20 ml of liquid LB medium with appropriate antibiotics. Use a test tube, Erlenmayer flask or Falcon tube with volume at least 100 ml.

7. Incubate at 37 °C with vigorous shaking.

8. Measure OD_{600} of each sample every 30 minutes.

9. Stop the cultivation when the slowest growing strain reaches OD_{600} = 1.2.
10. Calculate the amount of LB culture you need to inoculate to 50 ml of modified 2x Mal medium to get the starting OD_{600} = 0.0048. Do this for every sample. Use the above mentioned formula.

11. Inoculate the calculated volume of the LB culture to 50 ml of modified 2x Mal medium with appropriate antibiotics. Use a test tube, Erlenmayer flask or Falcon tube with volume at least 250 ml.

12. Incubate at 37 °C with vigorous shaking.

13. Measure OD_{600} of each sample every 30 minutes.

14. Stop the cultivation when the slowest growing culture reaches OD_{600} = 1.5.

15. Fresh 50 ml of modified 2x Mal medium for each strain were inoculated from this culture to the starting OD_{600} = 0.0025. The culture was grown for 14 hours and then used for further experiments.

16. Calculate the amount of modified 2x Mal culture you need to inoculate to 50 ml of fresh modified 2x Mal medium to get the starting OD_{600} = 0.0025. Do this for every sample. Use the above mentioned formula.

17. Inoculate the calculated volume of the modified 2x Mal culture to 50 ml of fresh modified 2x Mal medium with appropriate antibiotics. Use a test tube, Erlenmayer flask or Falcon tube with volume at least 250 ml.

18. Incubate at 37 °C with vigorous shaking for 14 hours.

19. Use the cells for further experiments.

Notes:

- This is not an ideal cultivation experiment. We will optimize it in iGEM 2021.
- When measuring the OD_{600}, use the growth medium as blank.
- When measuring the OD_{600}, when it is higher than 0.8 you have to dilute the sample with LB. We recommend the 10x dilution where you mix the growth medium and cell culture 9:1 and the OD_{600} shown by the measuring device multiply by 10.
- During each cultivation, create a growth curve in your PC so that you can check the growth phases in which your cultures currently are.