File name: iGEM2016_01_Gas_chromatography.docx

iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic **Project title:** Characterizing and optimizing PHB production **Sub project:** 1. Determining the intracellular amount of PHB	**Creation date:** 2016.09.26 **Written by:** Joel **Performed by:** Joel Mario Vej-Nielsen & Jakob Rønning

1. SOPs in use.

SOP0001_v1 – ON culure of *E. coli*
SOP0042_v1 - Gas chromatography

2. Purpose.

Proteomics analysis of Staphylococcal pantothenate kinase II in E. coli.

3. Overview.

Day	SOPs	Experiments
1	SOP0001_v1	ON cultures of E. coli.
2	SOP0042_v1	Gas chromatography
	SOP0042_v1	Gas chromatography

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4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Benzoic acid		Sigma Aldrich	Chemical room	
PHB	pure	Sigma Aldrich		
				Handle in fume closet
Methanol		Sigma Aldrich	FKF	
H_2SO_4	100%		Chemical room	
Chloroform		Sigma Aldrich	Chemical room	
Distilled water				
LB media		Sigma Aldrich	Chemical room	

5. Other

Primer to PCR dilution:

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
16.10.10	SOP0001_v1 ON culture	50 ml of ON culture was prepared for two biological replicates of bacteria expressing pantothenate kinase II and two biological replicates of *E. coli* with pSB1C3 alone. Samples were grown for 72 hours, then lyophilized and kept on ice.
16.10.13	SOP0042_v1 Gas chromatography	The injection split ratio was 120:1. The injection port and the detector temperatures were set at 180°C and 200°C, respectively. The initial oven temperature was constant for one minute at 90°C.

		Then increased at 3.8$\frac{℃}{minute}$ to a final temperature of 150°C. The temperature was maintained for 5 min. The flow rate of the helium carrier gas was 1 mL/minute. First the analysis was performed with different concentrations of PHB and benzoic acid, in order to determine appropriate values. Also Top 10 was analyzed as a negative control
16.10.13	SOP0042_v1 Gas chromatography	The injection split ratio was 120:1. The injection port and the detector temperatures were set at 180°C and 200°C, respectively. The initial oven temperature was constant for one minute at 90°C. Then increased at 3.8$\frac{℃}{minute}$ to a final temperature of 150°C. The temperature was maintained for 5 min. The flow rate of the helium carrier gas was 1 mL/minute.

7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
#94	Top10 containing K2018036	Assembled from genscript genes and iGEM	Sample tested containing pantothenate kinase II
#9	Top10	Assembled from genscript genes and iGEM	Sample tested containing pantothenate kinase II

8. Remarks on setup.

9. Results and conclusions.

10. Appendixes