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iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic **Project title:** characterizing and optimizing PHB productin in *E. coli.* **Sub project:** 1.	**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*
SOP0037_v1 - iTRAQ sample preparation
SOP0038_v1 - Qubit® Protein Assay Kits
SOP0039_v1 – C2 and C3 columns
SOP0040_v1 - iTRAQ labelling
SOP0041_v1 - TiO_2 purification

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	SOP0037_v1 SOP0038_v1	iTRAQ sample preparation Qubit® Protein Assay Kits
3	SOP0039_v1 SOP0038_v1 SOP0040_v1	C2 and C3 columns Qubit® Protein Assay Kits iTRAQ labelling
4	SOP0041_v1 SOP0039_v1	TiO_2 purification C2 and C3 columns
5	SOP0041_v1 SOP0039_v1	TiO_2 purification C2 and C3 columns

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
PBS				
Lysis buffer	93,8 µl Urea (6M) thiourea (2M) solution		Protein research group	
	1 µl 1M DTT (10mM)		Stored in freezer	Handle in fume closet
	3 µl phosSTOP phosphatase inhibitor	Sigma Aldrich	Stored in freezer	
	1,2 µl complete protease inhibitor		Stored in freezer	
	2 µl benzonase		Stored in freezer	
Triethyla-mmonium bicarbonate (TEAB)	20mM, pH 7,5		Protein research group	
Iodoacetamide			Protein research group	
Trypsin			Stored in freezer	
Qubit® Reagant		ThermoFischer Scientific	Protein research group	
Qubit® Buffer		ThermoFischer Scientific	Protein research group	
Qubit® Standards		ThermoFischer Scientific	Protein research group	
Qubit® Assay tubes		ThermoFischer Scientific	Protein research group	
0,5 ml PCR tubes		ThermoFischer Scientific	Protein research group	
iTRAQ Reagant 114		Sciex	Stored in freezer	
iTRAQ Reagant 115		Sciex	Stored in freezer	
iTRAQ Reagant 116		Sciex	Stored in freezer	
iTRAQ Reagant 117		Sciex	Stored in freezer	
ethanol		Sciex	Stored in freezer	
Low binding eppendorf tubes			Protein research group	
Loading Buffer	80% Acetonitril, 5% TFA & 1M glycolytic acid (76 mg/ml)		Protein research group	

Washing Buffer 1	80% Acetonitril & 1% TFA	Protein research group
Washing Buffer 2	10% Acetonitril & 0,1% TFA	Protein research group
Elution Buffer	60µl ammonia solution in 940µl H_2O (pH 11,3)	Protein research group
TFA	0,1%	Protein research group
TFA	10%	Protein research group
TFA	100%	Protein research group
Acetonitril	30%	Protein research group
HCl	12M	Protein research group
HCl	1M	Protein research group
PNGase F		Protein research group
Sialidase A		Protein research group
Formic acid	100%	Protein research group
Low binding eppendorftubes		Protein research group
$\boldsymbol{TiO_2}$ beads		Protein research group

5. Other

Primer to PCR dilution:

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments

16.10.03	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.
16.10.05	SOP0037_v1 iTRAQ sample preparation	
16.10.05	SOP0038_v1 Qubit® Protein Assay Kits	Concentrations were: Sample 1 (panK) – 4.38 mg/ml Sample 2 (panK) – 4.24 mg/ml Sample 2 (control) – 5.2 mg/ml Sample 4 (control) – 5.48 mg/ml
16.10.06	SOP0039_v1 C2 and C3 columns	Samples were run through a C2 column and then a C3 column
16.10.06	SOP0038_v1 Qubit® Protein Assay Kits	Concentrations were: 2.5 mg/ml 2.5 mg/ml 2.0 mg/ml 2.8 mg/ml The samples were analyzed by MALDI – MS In order to determine why concentrations were so low.
16.10.06	SOP0040_v1 iTRAQ labelling	As there was no 4plex available, we used 8plex chemicals instead. However, they are of a different compositions and are should be suspended in isopropanol not ethanol. We didn't know this and this has effected the labelling. Therefore, instead of pooling the entirety of each sample, 5 µl of each sample were kept separately.
16.10.07	SOP0041_v1 TiO_2 purification	The purification was performed on the pooled peptides.
16.10.07	SOP0039_v1 C2 and C3 columns	Afterwards LC-MS/MS were performed on the samples in order to retrieve data on the bacterial proteome.
16.10.07	SOP0039_v1 C2 and C3 columns	The purification was repeated, due to visible impurities in the dried down sample.
16.10.08	SOP0041_v1 TiO_2 purification	The purification was performed on each of the remaining 5 µl that had not been pooled.
16.10.08	SOP0041_v1 SOP0039_v1	TiO_2 purification C2 and C3 columns

7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
#102-1	Top10 containing K2018021	Assembled from genscript genes and iGEM	Sample tested containing pantothenate kinase II
#102-2	Top10 containing K2018021	Assembled from genscript genes and iGEM	Sample tested containing pantothenate kinase II
#56-1	Top10 containing K608004	From iGEM kit	Control sample
#56-2	Top10 containing K608004	From iGEM kit	Control sample

8. Remarks on setup.

9. Results and conclusions.

10. Appendixes