File name: iGEM2016_01_HNMR.docx

iGEM2016 – Microbiology – BMB – SDU

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

1. SOPs in use.

SOP0031_v1 – TB media
SOP0001_v1 - ON culure of *E. coli*
SOP0032_v1 – Ethyl acetate PHB recovery
SOP0033_v1 – Hypochlorite and triton x-100 PHB recovery
SOP0034_v1 – Chloroform PHB recovery
SOP0044_v1 – Hypochlorite PHB recovery

2. Purpose.

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

3. Overview.

Day	SOPs	Experiments
1	SOP0031	TB media
2	SOP0001	ON culture
3	SOP0034	Chloroform PHB recovery
	SOP0033	Hypochlorite and triton x-100 PHB recovery
	SOP0032	Ethyl acetate PHB recovery
4	SOP0044	Hypochlorite PHB recovery

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Ethyl acetate		AppliChem	Anne Mette, RT	Handle in fume closet
Destilled water			Micro storage	
50 mL falcon tubes		Contact Lab-manager	Micro storage	
Acetone				Handle in fume closet
100 – 200 mL flask			Micro storage	
250 or 500 mL centrifuge tubes		Incubation room (class 1) – V18-404b-0		
Triton X-100	1% v/v in PBS		Micro storage	
PBS				
Natriumhypoclorit	13% v/v with pH of 12,3		Micro storage	
Destilled H_2O				
Ethanol	70 %			
Acetone		AppliChem	Anne Mette, RT	Handle in fume closet
chloroform		Contact Lab-manager	Micro storage	Handle in fume closet
Freezing tubes		Contact Lab-manager	Micro storage	
Distilled water				
Tryptone			Chemical room	
Yeast extract			Chemical room	
Glycerol			Chemical room	
1L media bottle			Micro storage	
10 x TB phosphate	2.31 g KH_2PO_4 and 12.54 g K_2HPO_4 in 90 ml H_2O, adjust to 100 ml with H_2O.		Chemical room	

5. Other

Primer to PCR dilution:

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
16.09.27	SOP00031_v1 TB media	1 L of TB was produced
16.09.27	SOP0001 ON culture	Three times 250 mL TB was inoculated with K2018000
16.09.30	SOP0034 Chloroform PHB recovery	The purified material was dissolved in 1 mL of chloroform
16.09.30	SOP0033 Hypochlorite and triton x-100 PHB extraction	The purified material was dissolved in 1 mL of chloroform
16.09.30	SOP0032 Ethyl acetate PHB recovery	The purified material was dissolved in 1 mL of chloroform
16.10.10	ON culture	250 mL TB was inoculated with K2018000
16.10.13	SOP0044 Hypochlorite PHB extraction	The purified material was dissolved in 1 mL of chloroform
16.10.15		HNMR measurements where made 600Mhz with cdcd13 solvent 0.1 s delay then a 0.3 µs pulse

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	Sample tested containing pantothenate kinase II

8. Remarks on setup

9. Results and conclusions.

All extraction methods provide PHB. Purity is varying.

10. Appendixes

Setup:

File name: iGEM2016_01_HNMR.docx

ACQUISITION		TRANSMITTER		SPECIAL		PROCESSING	
seqfil	s2pul	tn	H1	temp	25.0	lb	not used
sw	9615.4	sfrq	599.685	spin	0	sb	not used
at	4.000	tof	254.4	gain	20	gf	1.200
np	76924	tpwr	59	hst	0.008	gfs	not used
fb	4000	pw	8.300	pw90	8.300	awc	not used
bs	32	DECOUPLER		alfa	10.000	lsfid	not used
dl	1.000	dn	C13	PRESATURATION		fn	262144
nt	4	dof	0	satmode	n	FLAGS	
ct	4	dm	nnn	wet	n	il	n
SAMPLE		decwave	W40_HCN5mm	Details in dgss		in	n
date	Oct 15 2016	dpwr	40			dp	y
solvent	cdcl3	dmf	35088			hs	nn
sample							

AXIAL SHIMS		NON AXIAL SHIMS		NON AXIAL SHIMS		AUTOMATION	
z1	-15409	x1	-2048	z2x2y2	-5339	method	/home/pcs/v
z2	-4443	y1	-260	z2xy	-3722	rmrsys/shimmethods	
z3	2189	xz	-2436	zx3	-2150		/x_ym
z4	1063	yz	-3794	zy3	3149	wshim	n
z5	1906	xy	-96	z4x	14219	load	n
z6	270	x2y2	3726	z4y	10504		
z7	2710	x3	-14655			spin	0
		y3	14372			gain	20
		xz2	7138			alock	n
		yz2	10307			loc	not used
		zxy	-73			SPECIAL	
		zx2y2	-5620			temp	25
		z3x	-4301				
		z3y	4489				

DISPLAY		CHART		FLAGS		INTEGRAL	
sp	3107.3	sc	127	ai	ph	intmod	off
wp	81.1	wc	81		FID	is	27706.30
vs	809	hzmm	1.00	sf	0	ins	10.000
REFERENCE		vp	18	wf	4.000	io	10
rfl	1554.8	axis	p	vf	2345		
rfp	0	pltmod	fixed			lvl	0.156
cr	3107.5					tlt	0.004
delta	218.1	th	3				
PHASE							
lp	-1.3	ho	-51.15				
rp	90.8	vo	0				

SPIN SYSTEM		CHEM SHIFTS		COUPLING CONSTANTS	
spinsys	AB	A	500.00	JAB	5.00
sminf	0	B	700.00		
smaxf	6497.73				
sth	0.0100				
slw	0.500				
niter	0				
iterate					
svs	0.994				