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iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic **Project title:** Optimizing and characterizing production of plastic **Sub project:** Creating BioBricks that contain the secretion system	**Creation date:** 2016.08.24 **Written by:** Joel Vej-Nielsen **Performed by:** Joel Mario Vej-Nielsen & Jakob Rønning

1. SOPs in use.

SOP0001 – ON culture
SOP0005 – Freeze stock
SOP0007 - LA plates with antibiotics - (SOP is not mentioned in protocol.)
SOP0009 – TSB transformation
SOP0014 – Gel purification
SOP0015 - Ligation
SOP0017 – Fast Digest
SOP0019 – plasmid miniprep

2. Purpose.

Assembly of K2018030 and incorporation of this part into phaCAB BioBricks.

3. Overview.

Day	SOPs	Experiments
1	SOP0009_v1	TSB transformation
2	SOP0001_v1	ON culture
3	SOP0019_v1	Plasmid Miniprep
4	SOP0017_v1	Fast digest
5	SOP0014_v1	Gel purification
6	SOP0015_v1	T4 igation
7	SOP0009_v1	TSB Transformation
8	SOP0019_v1	Plasmid miniprep
9	SOP0017_v1	Fast digest

10	SOP0014	Gel purification.
11	SOP0015	T4 Ligation
12	SOP0009	TSB transformation
13	SOP0001	
14	SOP0005	Freeze stock of ligation
	SOP0019	Miniprep on K2018030
15	SOP0017	Fast digest
16	SOP0015	T4 ligation
17	SOP0009	TSB transformation
18	SOP0001	ON culture
19	SOP0019	Miniprep
	SOP0017	Fast digest
	SOP0005	Freeze stock
	SOP0009	TSB transformation
21	SOP0009	TSB transformation
22	SOP0001	ON culture
23	SOP0019	Miniprep
	SOP0017	Fast digest
	SOP0005	Freeze stock
24	SOP0015	T4 ligation
25	SOP0009	TSB transformation
26	SOP0001	ON culture
27	SOP0019	Miniprep
	SOP0017	Fast digest
	SOP0005	Freeze stock

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
LB		Sigma-Aldrich	Chem room	
LA		Sigma-Aldrich	Chem room	
Ampicillin	100mg/ml			
Miniprep plasmid kit		Thermo Fischer	iGEM work station	
Gel purification kit		Thermo Fischer	iGEM work station	
Fasdigest enzymes: EcoRI, XbaI, SpeI & PstI		Agilent Technologies	iGEM freezer	
Ligase			iGEM freezer	
Fast digest buffer		Agilent Technologies	iGEM freezer	
Ligase buffer		Agilent Technologies	iGEM freezer	
Glycerol	50%			
Taq DNA polymerase		Ampliqon	iGEM freezer	
Polyethylene glycol	3,350	Sigma Aldrich	Chem room	
Dimethyl sulfoxide		Sigma Aldrich		Fuming hood
Magnesium Chloride	1M			

5. Other

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
16.08.15	SOP0009_v1 TSB transformation	Transformation of K2018024, K2018027 & K2018029. Bacteria with amp resistance, were plated directly after heat shock Bacteria with chloramphenicol resistance were give 40- 60 min. of phenotypical expression
16.08.16	SOP0001_v1 ON culture	ON culture of Top10 containing K2018024, K2018027 & K2018029.
16.08.17	SOP0019_v1 Plasmid miniprep	Miniprep of K2018024, K2018027 & K2018029. 30-50µl H_2O was used, instead of elution buffer and the flow through was run through twice.
16.08.18	SOP0017_v1 Fast digest	Fast digest of K2018024 with SpeI & PstI, K2018027 with XbaI and SpeI & K2018029 with XbaI and PstI.
16.08.19	SOP0014_v1 Gel purification	300µl binding buffer was used. The sample was washed twice. 30-50µl H_2O was used, instead of elution buffer and the sample was run through the filter twice in order to increase the concentration of the purified DNA.
16.08.22	SOP0015_v1 Ligation	The ligation in this protocol was only used as part of finding the biobrick of correct length. So 17µl of linearized DNA was ligated with 2µl ligase buffer and 1µl T4 ligase.
16.08.23	SOP0009_v1 TSB transformation	Transformation of ligated hlyABD (K2018030). Bacteria with amp resistance, were plated directly after heat shock Bacteria with chloramphenicol resistance were give 40- 60 min. of phenotypical expression
16.08.27	SOP0019_v1 Plasmid miniprep	The transformation from the 23. Was incubated for 48 hours at 37℃ and with a pipette tip several colonies were scooped up and performed miniprep on. 30-50µl H_2O was used, instead of elution buffer and the flow through was run through twice.
16.08.27	SOP0017_v1 Fast Digest	Digestion with EcoRI The mixed DNA was digested with EcoRI. Three parts of DNA was ligated together at once. hlyA/phaP, hlyB and hlyD.
16.08.27	SOP0014_v1 Gel purification	300µl binding buffer was used. The sample was washed twice. 30-50µl H_2O was used, instead of elution buffer and the sample was run through the filter twice in order to increase the concentration of the purified DNA. Most of the assembled DNA didn't include the middle part (hlyB) so only the band at the right length was purified from the gel.

16.08.27	SOP0015_v1 Ligation	The ligation in this protocol was only used as part of finding the biobrick of correct length. So 17µl of linearized DNA was ligated with 2µl ligase buffer and 1µl T4 ligase. The linearized DNA was ligated back together.
16.08.28	SOP0009_v1 TSB transformation	TSB transformation of K2018030.
16.08.29	SOP0001_v1 ON culture	
16.08.30	SOP0005_v1 Freeze stock	
16.08.30	SOP0019_v1 Plasmid miniprep	30-50µl H_2O was used, instead of elution buffer and the flow through was run through twice.
16.10.01	SOP0017_v1 Fast digest	The miniprep was performed on K2018030 with XbaI and PstI. The miniprep was performed on K2018030 with XbaI and SpeI. The miniprep was performed on K2018029 with XbaI and PstI. The miniprep was performed on K2018036 with EcoRI and SpeI.
16.10.03	SOP0015_v1 T4 ligation	Ligation between K2018036, K2018030 & K2018021 (K2018050), between K2018036 & K2018021 (K2018048) and between K2018036 & K2018030 (K2018049).
16.10.05	SOP0009_v1 TSB transformation	Transformation of new BioBricks, K2018048 & K2018050. Also K2018049
16.10.06	SOP0001_v1 ON culture	ON cultures of *E. coli* containing K2018048 & K2018050. The transformation of K2018049 provided no colonies.
16.10.08	SOP0019_v1 miniprep	Miniprep and fast digestion was done to verify the size of the BioBricks
16.10.08	SOP0017_v1 Fast digest	Miniprep and fast digestion was done to verify the size of the BioBricks Fast digestion was performed with EcoRI and then the digests were run on a gel.
16.10.08	SOP0005_v1 Freeze stock	The *E. coli* containing the correct BioBricks were stored.
16.10.08	SOP0009_v1 TSB transformation	Transformation of BioBrick K2018049 into top10.
16.10.09	SOP0001_v1 ON culture	ON cultues of cells containing K2018049.
16.10.10	SOP0019_v1 miniprep	Miniprep and fast digestion was done to verify the size of the BioBricks
16.10.10	SOP0017_v1 Fast digest	Miniprep and fast digestion was done to verify the size of the BioBricks
16.10.10	SOP0005_v1 Freeze stock	The *E. coli* containing the correct BioBricks were stored.
16.10.10	SOP0015_v1 T4 Ligation	Ligation of K2018030 into pSB1C3.
16.10.11	SOP0009_v1 TSB transformation	Of K2018030 into top10.

16.10.12	SOP0001_v1 ON culture	Of cells containing K2018030.
16.10.15	SOP0019_v1 miniprep	Miniprep and fast digestion was done to verify the size of the BioBricks
16.10.15	SOP0017_v1 Fast digest	Miniprep and fast digestion was done to verify the size of the BioBricks
16.10.15	SOP0005_v1 Freeze stock	The *E. coli* containing the correct BioBricks were stored.

7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
PR46	K2018028+K2018029	Synthesized from Genscript	Digestion
PR47	K2018026+K2018027	Synthesized from Genscript	Digestion
PR48	K2018024	Synthesized from Genscript	Digestion
PG42	K2018028+K2018029	PR46	Ligation
PG41	K2018026+K2018027	PR47	Ligation
PG44	K2018024	PR48	Ligation
PR58	K2018030	PG41, PG42 & PG44	Digestion
PR49	K2018021	Genscript	
PS1	K2018021	PR49	Ligation
PS2	K2018030	PR58	Ligation
PS3	K2018030	PR58	Ligation
PR71	K2018036	Synthesized from iGEM kit	Digestion
PS4	K2018036	PR71	Ligation
PL1	K2018050	PS1, PS2 & PS4.	Saved as #103
PL2	K2018048	PS1 & PS4.	Saved as #104
PL3	K2018049	PS3 & PS4.	Saved as 3-2

8. Remarks on setup.

9. Results and conclusions.

The Biobricks were all constructed successfully and tested. Furthermore, the secretion system works!

10. Appendixes