File name: iGEM2016_01_Hybrid_promoter_PHB_library.docx

iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic **Project title:** Additional promoter RBS biobricks **Sub project:** 1. K2018000 2. K2018038 & K2018039 3. K2018033, K2018034, K2018035 & K2018036	**Creation date:** 2016.09.15 **Written by:** Joel Mario Vej-Nielsen **Performed by:** The entire iGEM team

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
SOP0019 – plasmid miniprep
SOP0021 - Colony PCR with MyTag
SOP0023 - Ca^{2+} transformation

2. Purpose.

To create a library of k934001 with different additional promoter and RBS combinations.

3. Overview.

Day	SOPs	Experiments
1	SOP0009	TSB-transformation of K608002 & K934001 into E. coli.
2	SOP0021	Colony PCR on K608002 & K934001 containing cells.
	SOP0001	ON culture of k608002 & K934001 containing cells.
3	SOP0019	Plasmid MiniPrep of K608002 & K934001.
	SOP0017	Fast Digest of K608002 & K934001.
	SOP0014	Gel purification on K608002 & K934001.
	SOP0015	Ligation of K934001 insert with K608002 backbone (K2018000).

4	SOP0009	TSB-transformation of K2018000 ligations into E. coli.
5	SOP0021	Colony PCR to identify successful ligation.
6	SOP0001	ON culture of bacteria with K2018000
7	SOP0005	Freeze stock of bacteria containing K2018000.
8	**SOP0009**	TSB-transformation of J23106 & K934001 into E. coli.
9	SOP0001	ON culture of J23106 & K934001 containing cells.
10	SOP0019	Plasmid miniprep of J23106 & K934001.
	SOP0017	Fast Digest of J23106 & K934001.
	SOP0014	Gel purification of linearized J23106 & a K934001 insert.
11	**SOP0009**	TSB-transformation of J23104 into E. coli.
12	SOP0001	ON culture of J23104 containing cells.
13	SOP0019	Plasmid miniprep of J23104
	SOP0017	Fast Digest of J23104.
14	SOP0014	Gel purification of linearized J23104.
	SOP0015	Ligation of the K934001 insert into the J32104 (K2018038) and the J23106 (K2018039) backbone.
15	SOP0023	Ca^{2+} transformation of K2018038 ligation into E. coli.
16	SOP0001	ON cultures of K2018038 containing cells.
17	SOP0019	Miniprep på K2018038 containing cells.
18	SOP0023	Transformation of K2018039 into E. coli
19	SOP0001	ON culture of K2018039 containing cells.
20	SOP0019	Miniprep på K2018039 containing cells.
	SOP0017	Fast Digest of K2018038 & K2018039 in order to check if the size is correct, as colony PCR was giving two bands.
21	SOP0023	Ca^{2+} Transformation of J23104 & J23106 into E. coli.
22	SOP0001	ON Culture of K2018039 & K2018038 containing cells.
23	SOP0019	Miniprep on K2018038 & K2018039.
24	SOP0017	Fast digest of K2018038 & K2018039.
25	SOP0014	Ligation of K2018038 & K2018039 into pSB1C3.
26	SOP0023	Ca^{2+} transformation of K2018038 & K2018039 ligations into E. coli.
27	SOP0001	ON cultures of K2018038 & K2018039 containing cells.
28	SOP0005	Freeze stock of bacteria containing K2018038 & K2018039.
29	SOP0009	TSB-transformation of K608003, K608004, K880005 & K081005 into E. coli.
30	SOP0001	ON culture of K608003, K608004, K880005 & K081005 containing cells.
31	SOP0019	Plasmid miniprep of K608003, K608004, K880005 & K081005.
32	SOP0017	Fast Digest of K608003, K608004, K880005 & K081005.
33	SOP0014	Gel purification of linearized K608003, K608004, K880005, K081005 & a K934001 insert.
	SOP0015	Ligation of the K934001 into the K608003 backbone (K2018036), the K608004 (K2018035), the K081005 (K2018034) & the K880005 backbone (K2018033).

34	SOP0023	Ca^{2+} transformation of K2018033, K2018034, K2018035 & K2018036 ligations into E. coli.
35	SOP0001	ON cultures of K2018033, K2018034, K2018035 & K2018036 containing cells.
36	SOP0005	Freeze stock of bacteria containing K2018033, K2018034, K2018035 & K2018036.
37	SOP0005	Freeze stock of bacteria containing K2018038 & K2018039.

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
LB		Sigma?	Chem room	
LA		Sigma?	Chem room	
Chloramphenicol	30mg/ml			yes
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.

<table>
<tr><th>Date (YY.MM.DD)</th><th>SOPs</th><th>Alterations to SOPs and remarks to experiments</th></tr>
<tr><td>16.04.14</td><td>SOP0009 TSB transformation</td><td>Every biobrick in this SOP was worked with here, but only the work with K608002 was finished.</td></tr>
<tr><td>16.04.15</td><td>SOP0021
Colony PCR</td><td><u>Deviations:</u>
- PCR settings
<table><tr><td>Step 1</td><td>Initial duration</td><td>95 ℃</td></tr><tr><td>Step 2</td><td>Denaturation</td><td>95 ℃</td></tr><tr><td>Step 3</td><td>Annealing</td><td>60 ℃</td></tr><tr><td>Step 4</td><td>Extension/Elongation</td><td>72 ℃</td></tr><tr><td>Step 5</td><td>Repeat of step 2-4</td><td></td></tr><tr><td>Step 6</td><td>Extra Elongation</td><td>72 ℃</td></tr><tr><td>Step 7</td><td>Keep the samples cold</td><td>20 ℃</td></tr></table>- Another polymerase – Tag2x Mastermix RED AMPLIQON
- Another dilutions of master mix:
For one PCR sample:
Primers: VF2 and VR 1 µL of each
Tag 2x: 5µL
Water: 2.5 µL
Template sample: 0.5µL</td></tr>
<tr><td>16.04.15</td><td>SOP0001
ON culture</td><td></td></tr>
<tr><td>16.04.16</td><td>SOP0019
Plasmid miniprep</td><td>For K608002
After eluting DNA, solution was added to filter a second time, incubated for two minutes at room temperature and spun for two minutes at 14.000 rpm.</td></tr>
<tr><td>16.04.16</td><td>SOP0017
Fast digest</td><td>For K608002
Due to poor concentration the amount of buffer and the final volume of the reaction was doubled for PR4.
K934001 was digested with XbaI and PstI
K608002 was digested with SpeI and PstI</td></tr>
<tr><td>16.04.16</td><td>SOP0014</td><td>For K608002</td></tr>
</table>

	Gel purification	
16.04.16	SOP0015 T4 ligation	The amount of substance wasn't calculated, instead the two samples were added in a volume K608002:k934001 (k201800) of 1:0, 1:2 & 1:5.
16.04.19	SOP0009 TSB transformation	K2018000 into *E. coli.*
16.04.21	SOP0021 Colony PCR	<u>Deviations:</u> - PCR settings - Another polymerase – Tag2x Mastermix RED AMPLIQON - Another dilutions of master mix: For one PCR sample: Primers: VF2 and VR 1 µL of each Tag 2x: 5µL Water: 2.5 µL Template sample: 0.5µL
16.04.27	SOP0001 ON culture	For K2018000
16.04.28	SOP0005 Freeze stock	After the storage of this first biobrick, the other were created, but due to a mutation in K934001 and poor storage of DNA we had to start over with new bricks from the iGEM kit.
16.06.27	SOP0009 TSB transformation	DNA fresh from the iGEM kit. Again work was done with all the remaing bricks but failed for all but J23104 (K2018038) & J23106 (K2018039).
16.06.28	SOP0001 ON culture	For J23106
16.06.29	SOP0019 Plasmid miniprep	For J23106
16.06.29	SOP0017	For J23106 with SpeI and PstI

Step 1	Initial duration	95 °C
Step 2	Denaturation	95 °C
Step 3	Annealing	60 °C
Step 4	Extension/Elongation	72 °C
Step 5	Repeat of step 2-4	
Step 6	Extra Elongation	72 °C
Step 7	Keep the samples cold	20 °C

	Fast Digest	
16.06.29	SOP0014 Gel purification	Work was halted for the linearized J23106 until the rest of the DNA was ready to be ligated as well.
16.06.30	SOP0009 TSB transformation	Of J23104 into *E. coli.*
16.07.01	SOP0001 ON culture	of J23104 containing cells
16.07.02	SOP0019 Plasmid miniprep	of J23104
16.07.02	SOP0017 Fast digest	Fast digest of J23104 with SpeI and PstI
16.07.03	SOP0014 Gel purification	Purification of linearized J23104
16.07.03	SOP0015 T4 ligation	The amount of substance was not calculated, instead the two samples were added in a volume J23104:K934001 (K2018038) J23106:K934001 (K2018039) of 1:0, 1:2 & 1:5.
16.07.07	SOP0023 Ca^{2+} transformation	Transformation of K2018038 and K2018039 into *E. coli.*
16.07.08	SOP0001 ON culture	No growth on K2018039 containing cells. ON culture of K2018038 containing cells.
16.07.09	SOP0019 Plasmid miniprep	Miniprep of K2018038. This DNA was stored while the rest of the biobricks were assembled
16.07.15	SOP0023 Ca^{2+} transformation	Transformation of K2018039 into *E. coli.*
16.07.18	SOP0001 ON culture	ON culture of K2018039 containing cells
16.07.19	SOP0019 Plasmid miniprep	Plasmid miniprep of K2018039
16.07.19	SOP0017 Fast digest	Digestion of K2018038 & K2018039 with SpeI and PstI
16.07.20	SOP0014 Gel purification	Of linearized K2018038 & K2018039
16.07.27	SOP0015 T4 ligation	Ligation of K2018038 & K2018039 into pSB1C3. Ligated 1:1.
16.07.28	SOP0009 TSB transformationer	Transformation of K2018038 & K2018039 into *E. coli.* This time they are in pSB1C3.

16.07.29	SOP0001 ON culture	ON culture of cells containing K2018038 & K2018039.
16.07.30	SOP0005 Freeze stock	Freeze stock is made of K2018038 (J23104) & K2018039 (J23106).
16.08.29	SOP0009 TSB transformation	K608003, K608004 K081005 & K880005 from the iGEM kit.
16.08.30	SOP0001 ON culture	Of cells containing K608003, K608004 K081005 & K880005.
16.08.31	SOP0019 Plasmid miniprep	Plasmid purification of K608003, K608004 K081005 & K880005.
16.09.01	SOP0017 Fast digest	Digests of K608003, K608004 K081005 & K880005 With speI and PstI.
16.09.02	SOP0014 Gel purification	Gel purification of linearized K608003, K608004 K081005 & K880005.
16.09.02	SOP0015 T4 ligation	The amount of substance wasn't calculated, instead the two samples were added in a volume K608003:k934001 (K2018035) K608004:K934001 (K2018036) K081005:K934001 (k2018034) k880005:k934001 (k2018033) of 1:0, 1:2 & 1:5.
16.09.03	SOP0009 TSB transformation	Transformation of K2018033, K2018034, K2018035 & K2018036
16.09.04	SOP0001 ON culture	Ligation of K2018033, K2018034, K2018035 & K2018036
16.09.06	SOP0019 Plasmid miniprep	Plasmid purification of K2018033, K2018034, K2018035 & K2018036.
16.09.17	SOP0001 ON culture	ON culture of cells containing K2018033, K2018034, K2018035 & K2018036
16.09.18	SOP0005 Freeze stock	Freeze stock of cells containing K2018033, K2018034, K2018035 & K2018036

7. Sample specification.

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Sample name	Sample content	From	Used for / Saved where
PR4	BBa_K934001	2015 kit, plate 2, well 11C	Fast digest
PR5	BBa_K608002	2015 kit, plate 1, well 3O	Fast digest
PG7	BBa_K934001 Cut with XbaI & PstI	PR4	Ligation
PG3	BBa_K608002 cut with SpeI & Pst.	PR5	Ligation
PR10	BBa_k2018000	PG3 & PG7	Saved as #8
PR26	BBa_J23104	2014 kit, plate 4 well 17L	Fast digest
PG18	BBa_J23104	PR26	Ligation
PR3	BBa_J23106	2014 kit, plate 4, well 17P	Fast digest
PR22	BBa_K934001	From PR4	Fast digest
PG26	BBa_J23106 cut with SpeI & PstI	PR3	Ligation
PG27	BBa_K934001	PR22	Ligation
PR42	BBa_K2018038	PG18 & PG27	Fast digest
PR43	BBa_K2018039	PG26 & PG27	Fast digest
PG31	BBa_K2018038	PR42	Ligation
PG32	BBa_K2018039	PR43	Ligation
PR42	BBA_K2018038	PG31 & PG33	Saved as #64
PR43	BBa_K2018039	PG32 & PG33	Saved as #65
PR59	BBa_K880005	2013 kit, plate 2 well 3F	Fast digest
PR60	BBa_K081005	2013 kit, plate 3 well 15F	Fast Digest
PR61	BBa_K608003	2013 kit, plate 1 well 5A	Fast digest
PR62	BBa_K608004	2013 kit, plate 1 well 5C	Fast digest
PG48	BBa_K880005	PR59	Ligation
PG49	BBa_K081005	PR60	Ligation
PG50	BBa_K608003	PR61	Ligation
PG51	BBa_K608004	PR62	Ligation
PG53	BBa_K934001	PR65	Ligation
PR69	BBa_K2018033	PG48 & PG53	Stored as #91
PR70	BBa_K2018034	PG49 & PG53	Stored as #92
PR71	BBa_K2018036	PG51 & PG53	Stored as #94
PR72	BBa_K2018035	PG50 & PG53	Stored as #93

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8. Remarks on setup.

When K2018000 and K2018038 + K2018039 the remaining biobricks were also attempted to be synthesized. However, it failed and therefore the complete synthesize took three rounds.

9. Results and conclusions.

A library of BioBricks that can produce PHB.

10. Appendixes