iGEM 2015 – Microbiology – BMB – SDU

Project type: Screening **Project title:** Peptide aptamer screening	**Creation date:** **Written by:** TBA, EMT, CEM **Performed by:**TBA, JSP, AC and EMT

1. SOPs in use

iGEM2014_SOP0017_v01_ Fast digest
iGEM2014_SOP015_v02_ligation
iGEM2013_SOP0009_v01_TSB transformation
iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
iGEM 2014 SOP0019 - Miniprep

2. Purpose

To screen for peptide aptamers using the two-hybrid system.

3. Overview

Date (DD.MM.YY)	**Person(s) (initials)**	**Experiments**	**SOPs**
11.08.15	TBA, JSP	Fast Digest of pSB1C3 and pSB1K3 with Eco + Pst and Xba + Pst. Fast Digest of "Scaffold-3xFLAG" with Eco + Pst Fast Digest of "Intein" with Xba + Pst	iGEM2014_SOP0017_v01_ Fast digest

		Ligation of Scaffold and Intein with the two backbone. Ligation left overnigth	iGEM2014_SOP015_v02_ligation
12. 08.15	TBA	Transformations of Ligations into MG1655	iGEM2013_SOP0009_v01_TSB transformation
13.08.15	TBA, JSP	Redone transformation from ligation-leftovers	iGEM2013_SOP0009_v01_TSB transformation
14.08.15	JSP	New ligation of Scaffold and Intein in pSB1C3 + pSB1K3	iGEM2014_SOP015_v02_ligation
15.08.15	ADK	Transformation of ligations	iGEM2013_SOP0009_v01_TSB transformation
16.08.15	TBA	Colony-PCR	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
17.08.2015	ADK, TBA	Miniprep on overnight cultures Freeze stock preparation	Bio-Rad Quantum Prep: plasmid miniprep Kit
17.08.15	TBA, JSP	Fast digest of received targets proteins and appropiate backbones.	iGEM2014_SOP0017_v01_ Fast digest
17.08.15	ADK, TBA	Ligations of target proteins, linker-initin-scaffold (LIS) and into different backbones	iGEM2014_SOP015_v02_ligation
18.08.15	ADK	Transformation of ligations from yesterday	iGEM2013_SOP0009_v01_TSB transformation
24.08.15	JSP	Ligation of PcstA-RFP(Y79) and T25-IL2 (Y80) and T25-Hfq (Y83).	iGEM2014_SOP015_v02_ligation

25.08.15	JSP	Transformation of ligations from *24.08.2015*.	iGEM2013_SOP0009_v01_TSB transformation
26.08.15	JSP	Colony-PCR on transformations from 25.08.2015	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
27.08.15	JSP	Miniprep on PcstA-RFP-T25-Hfq	Bio-Rad Quantum Prep: plasmid miniprep Kit
29.08.15	AC TBA	Fast Digest of pSB1C3-T18-LIS. Cut with EcoI + PstI. Stored as Y105 Ligation of digestion into pSB1K3-backbone	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation
30-08.2015	EMT	Transformation	iGEM2013_SOP0009_v01_TSB transformation
31.08.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI + PstI. Ligation of digestion into pSB1K3-backbone Transformation	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
01.09.2015	EMT	Ligation of digestion from 31.08.2015 into pSB1K3-backbone Transformation	iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation

2.09.2015	EMT	Kolony PCR on pSB1K3-T18-LIS pSB1K3-LSx3FLAG	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
3.9.2015	EMT	Miniprep of overnight Cultures	iGEM 2014 SOP0019 - Miniprep
5.08-2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with XhoI. Fast Digest of Library with WhoI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library Transformation	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
06.08.2015	EMT	Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library Transformation	iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
07.08.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with XhoI. Fast Digest of Library with XhoI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library Transformation	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation

08.09.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI and PstI. Fast Digest of pSB1A2 with EcoI and PstI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with pSB1A2 Transformation	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02 _ligation iGEM2013_SOP0009_v0 1_TSB transformation
09.09.2015	EMT	Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with pSB1A2 Transformation	iGEM2014_SOP015_v02 _ligation iGEM2013_SOP0009_v0 1_TSB transformation
10.09.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI and PstI. Fast Digest of pSB1A2 with EcoI and PstI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with pSB1A2 Transformation Koloni PCR on transformation from 09.09.2015	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02 _ligation iGEM2013_SOP0009_v0 1_TSB transformation iGEM2013_SOP0021_v0 1_ Colony PCR with MyTaq

11.09.2015	EMT	Koloni PCR on transformation from 10.09.2015	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
12.09.2015	EMT, AC	Restriction Blot, done on pSB1A2-T18-LSx3FLAG, cuts made; -EcorI -EcorI+PstI -XhoI and uncut pSB1A2 Miniprep on T18-LSx3FLAG Fast Digest on pSB1A2-T18-LSx3FLAG with XhoI Ligation of digestion with Library Transformation of targets (T25-linker-CRSTA) into BTH101	 iGEM 2014 SOP0019 - Miniprep iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations

5. Experiment history

Date (DD.MM.YY)	Person(s) (initials)	Alterations to SOPs and remarks to experiments	SOPs
11.08.2015	TBA, JSP	Fast digest of Scaffold (using 15 µL of IDT-stock) with Eco + Pist. Stored as Y68 Fast Digest of Intein (using 15 µL of IDT-stock) with Xba + Pst. Stored as Y69 Fast digest of pSB1K3 (R71 using 10 µL) with Eco + Pst and Xba + Pst. Stored as Y70 and Y71, respectively. Fast digest of pSB1C3 (R72 using 5 µL) with Eco + Pst and Xba + Pst. Stored as Y72 and Y73 respectively. Backbone digestions run on gel, Scaffold and Intein purified using coloumbs.	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02 _ligation

		Otherwise done according to SOP. **<u>Ligation:</u>** <u>Backbones to obtain 10 fmol</u> Y70: 1,6 µL was used Y71: 1,8 µL was used Y72: 1,33 µL was used Y73: 1,15 µL was used <u>Scaffold (Y68)</u>, for 20 fmol 1,4 µL was used. for the 50 fmol ligation 3,5 µL was used. <u>Intein (Y69)</u>, for 20 fmol 1,5 µL was used. for 50 fmol 4,5 µL was used Ligations left for ~20 hours	
12.08.2015	TBA	Done according to SOP.	iGEM2013_SOP0009_v01_TSB transformation
13.08.2015	TBA, JSP	Previous transformation failed, possibly due to mess up during plating. Transformation redone	iGEM2013_SOP0009_v01_TSB transformation
14.08.2015	JSP	Ligation of scaffold (Y68) and intein (Y69) in pSB1C3 (Y72+Y73) and pSB1K3 (Y70+Y71). The amount of digested insert used: <u>Scaffold:</u> 20 fmol: 1,6 µl 50 fmol: 4 µl <u>Intein</u>	iGEM2014_SOP015_v02_ligation

		20 fmol: 1,8 µl 50 fmol 4,5 µl <u>Backbones (10 fmol):</u> Y70: 1,6 µl Y71: 1,8 µl Y72: 1,33 µl Y73: 1,2 µl	
15.08.2015	ADK	Transformation	iGEM2013_SOP0009_v01_TSB transformation
16.08.2015	TBA	Colony-PCR on 3 colonies from each of the transformations. Colony-PCR showed appropiate bands for all except one, which was discarded. One successful colony of Intein and Scaffold in both backbones (4 in total) was selected for overnight culture. From Y70, 50 fmol colony 2 was selected for overnight culture. From Y71, 50 fmol colony 2 was selected for overnight culture. From Y72, 50 fmol colony 1 was selected for overnight culture. From Y73, 20 fmol colony 1 was selected for overnight culture.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
17.08.2015	TBA, ADK	Miniprep on the overnight Cultures and freezestock preparation. Miniprep stored as: - R83: pSB1C3-initin - R84: pSB1C3-T18-linker-Scaffold-3xFlag - R85: pSB1K3-initin	iGEM 2014 SOP0019 - Miniprep

		- R86: pSB1K3-T18-linker-scaffold-3xFlag	
17.08.2015	JSP, TBA	Received Target proteins (5 in total) from IDT along with Linker-Intein-Scaffold = LIS. Fast Digest of targets and LIS and appropiate backbones. Targets are linked to KT25, and were all digested with Xba1 + Pst1. Targets include; IL-2, Ybh, YbeY, Hfq and CsrA. LIS were digested with BamH1 + Pst1. Backbones were digestions of R32 (PcstA-RFP to fit targets) with Spe1 + Pst1 and R16 and R18 (to fit LIS) were digested with BamH1 + Pst1. Digestions were stored as: KT25_IL-2 = Y80 KT25_Ybh = Y81 KT25_YbeY = Y82 KT25_Hfq = Y83 KT25_CsrA = Y84 LIS = Y85 R32 (PcstA-RFP) = Y79 R16 (pSB1C3-UT18) = Y86 R18 (pSB1K3-UT18) = Y89	iGEM2014_SOP0017_v01_ Fast digest
17.08.2015	ADK, TBA	Ligations of the following: -LIS (Y85)/pSB1C3-UT18(Y86) -leuZ(Y63)/ pSB1C3-UT18(Y86) -leuZ(Y63)/pSB1C3-KT25(Y87) -leuZ(Y63)/pSB1K3-UT18(Y89) -leuZ(Y63)/pSB1K3-KT25 (Y88) -LIS(Y85)/pSB1K3-UT18(Y89)	iGEM2014_SOP015_v02_ligation

		-T25_CsrA(Y84)/pSB1C3-PcstA(Y79) -T25_Hfq(Y38)/pSB1C3-PcstA(Y79) -T25_ybeY(Y82)/pSB1C3-PcstA(Y79) -T25_yhbJ(Y81)/pSB1C3-PcstA(Y79) -T25_iL2(Y80)/pSB1C3-PcstA(Y79) - Everything done according to the SOP	
18.08.15	ADK	Transformation of ligations from yesterday, done according to the SOP	iGEM2013_SOP0009_v01_TSB transformation
24.08.15	JSP	Ligation of PcstA-RFP(Y79) and T25-IL2 (Y80) and T25-Hfq (Y83). 0, 20 and 50 fmol insert used. No alterations to SOP.	iGEM2014_SOP015_v02_ligation
25.08.15	JSP	Transformation of ligations from *24.08.2015*.	iGEM2013_SOP0009_v01_TSB transformation
26.08.15	JSP	Colony-PCR on transformations from 25.08.2015. No alteration to SOP.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
27.08.15	JSP	Miniprep on PcstA-RFP-T25-Hfq	Bio-Rad Quantum Prep: plasmid miniprep Kit
29.08.15	AC TBA	Fast Digest of pSB1C3-T18-LIS. Cut with EcoI + PstI. Stored as Y105 Ligation of Y105 into pSB1K3-backbone (Y104). Done	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation

		according to SOP and left overnight	
30-08.2015	EMT	Transformation done according to SOP	iGEM2013_SOP0009_v01_TSB transformation
31.08.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI + PstI. Ligation of digestion into pSB1K3-backbone done with 0, 20, and 50 fmol insert to 10 fmol backbone Transformation	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
01.09.2015	EMT	Ligation of digestion from 31.08.2015 into pSB1K3-backbone done according to SOP Transformation done according to SOP	iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
2.09.2015	EMT	Kolony PCR on pSB1K3-T18-LIS pSB1K3-LSx3FLAG done according to SOP	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
3.9.2015	EMT	Miniprep of overnight Cultures done according to manufacturer's instructions	iGEM 2014 SOP0019 - Miniprep
5.08-2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with XhoI. 100ng	iGEM2014_SOP0017_v01_ Fast digest

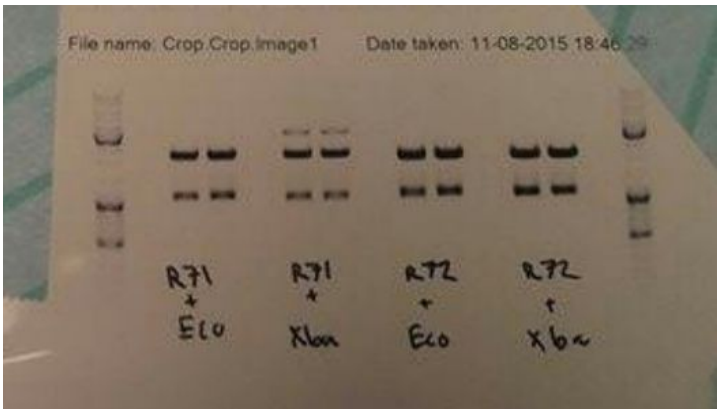
		done according to SOP Fast Digest of Library with WhoI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library with a 1:10 scale (100fmol backbone to 1000fmol insert) Transformation done according to SOP	iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
06.08.2015	EMT	Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library (100fmol backbone to 1000fmol insert) Transformation: pSB1C3-T18-LSx3FLAG + Library pSB1C3-T18-LIS + Library done according to SOP	iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation
07.08.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with XhoI. Fast Digest of Library 200ng with XhoI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with Library Transformation pSB1C3-T18-LSx3FLAG + Library	iGEM2014_SOP0017_v01_Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation

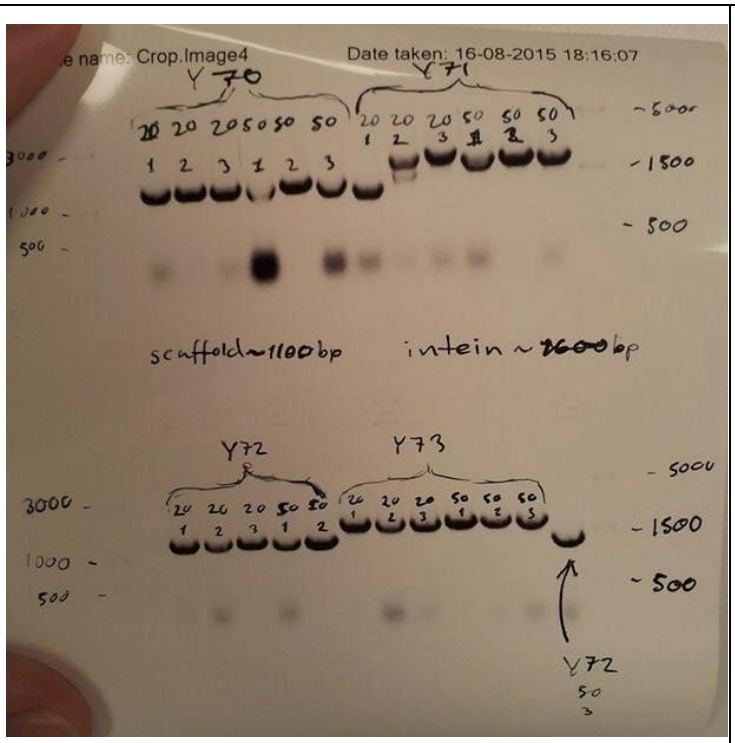
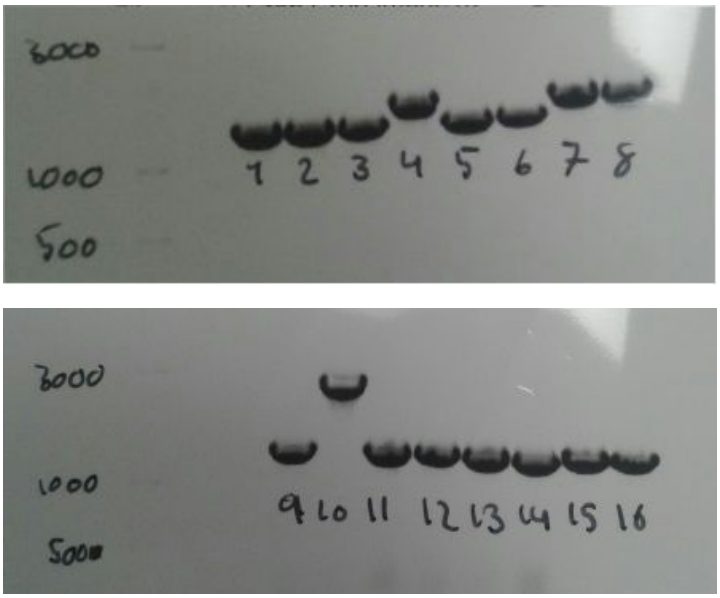
		pSB1C3-T18-LIS + Library done according to SOP	
08.09.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI and PstI. Fast Digest of pSB1A2 with EcoI and PstI Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with pSB1A2 (0, 20 and 50fmol insert to 10fmol backbone) Transformation; pSB1A2 + T18-LIS pSB1A2 +T18-LSx3FLAG	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02 _ligation iGEM2013_SOP0009_v0 1_TSB transformation
09.09.2015	EMT	Ligation of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG with pSB1A2 Transformation pSB1A2 + T18-LIS pSB1A2 +T18-LSx3FLAG	iGEM2014_SOP015_v02 _ligation iGEM2013_SOP0009_v0 1_TSB transformation
10.09.2015	EMT	Fast Digest of pSB1C3-T18-LIS and pSB1C3-T18-LSx3FLAG. Cut with EcoI and PstI. Fast Digest of pSB1A2 with EcoI and PstI Ligation of T18-LIS and T18-LSx3FLAG with pSB1A2 Transformation; pSB1A2 + T18-LIS	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02 _ligation

		pSB1A2 +T18-LSx3FLAG Koloni PCR on transformation from 09.09.2015	iGEM2013_SOP0009_v01_TSB transformation iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
11.09.2015	EMT	Koloni PCR on transformation from 10.09.2015	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
12.09.2015	EMT, AC	Restriction Blot, done on pSB1A2-T18-LSx3FLAG, cuts made; -EcorI -EcorI+PstI -XhoI and uncut pSB1A2 Miniprep on T18-LSx3FLAG Fast Digest on pSB1A2-T18-LSx3FLAG with XhoI Ligation of digestion with Library Transformation of targets (T25-linker-CRSTA) into BTH101	 iGEM 2014 SOP0019 - Miniprep iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation iGEM2013_SOP0009_v01_TSB transformation

6. Results

Date (DD.MM.YY)	**Picture**	**Comments**

11.08.2015		R71 and R72 both contained BBa_J04450, which is the band visible at ~1000 bp. Bands just under 3000 was exercised from gel.
12.08.2015		
13.08.2015		Yesterdays tranformations failed. might be due to mess up during plating out tranformations
14.08.15		
15.08.2015		

16.08.2015		Colony-PCR. Y71, 20 fmol colony 1 do not show appropiate band. This colony was discarded. Y71, 20 fmol colony 2 might have contained two colonies, one of which might have contained RFP instead of Intein. The second band corresponds to the lenght of RFP.
26.08.15		Only ligation of PcstA-RFP+T25-Hfq was succesful (#10). This was expected to be around 2700 nt. One of the bands showed a length of approx. 2700 nt. PcstA-RFP+T25-IL2 was

		expected be approx. 2600 nt, but were only approx. 1300 and 1600 nt. 1-4: Y80-20 5-8: Y80-50 9-12: Y83-20 1-16: Y83-50
30.08.2015		Transformation Failed
02.09.2015		Transformation of T18-LIS and T18-LSx3FLAG suddeced. tjeked with kolony PCR
10.09.2015		Colony PCR was inconslusive
11.09.2015		Colony PCR showed that ligation of pSB1A2-T18-LSx3FLAG was succesful, and pSB1A2-T18-LIS failed

7. Appendices