iGEM 2015 – Microbiology – BMB – SDU

Project type: Cloning **Project title:** Control with Leucin Zipper	**Creation date:** **Written by: ADK, TBA and JSP** **Performed by: ADK, TBA, EMT, AC and JSP**

1. SOPs in use

iGEM2014_SOP0010_v01_Phusion PCR
iGEM2014_SOP0017_v01_ Fast digest
iGEM2014_SOP015_v02_ligation
iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
iGEM2013_SOP0009_v01_TSB transformation

2. Purpose

To conjugate a leucin zipper to the T18 and T25 domain as used in the bacterial two-hybrid system and to furthermore verify that the bacterial two-hybrid system works.

3. Overview

Date (DD.MM.YY)	**Person(s) (initials)**	**Experiments**	**SOPs**
28.07.15	JSP	Phusion PCR, amplification of Leucine Zipper gene.	iGEM2014_SOP0010_v01_ Phusion PCR
29.07.15	JSP	FastDigest	iGEM2014_SOP0017_v01_ Fast digest
29.07.15	ADK	Phusion PCR, amplification of Leucine Zipper gene.	iGEM2014_SOP0010_v01_ Phusion PCR

30.07.15	JSP	FastDigest (Leucin Zipper)	iGEM2014_SOP0017_v01_ Fast digest
30.07.15	JSP	FastDigest (backbones)	iGEM2014_SOP0017_v01_ Fast digest
31.07.15	ADK	Miniprep of (Top10) pSB1C3-UT18-LeuZ, and (Top10) pSB1K3-KT25-LeuZ	Bio-Rad Quantum Prep: plasmid miniprep Kit
31.07.15	ADK	Fast digest of pcr product leuZ (G23)	iGEM2014_SOP0010_v01_ Phusion PCR
31.07.15	ADK	Fast digest of R63 (pSB1C3-UT18-LeuZ) and R64 (pSB1K3-KT25-LeuZ), with BamH1 and Pst1 to cut of the wrong LeuZ	iGEM2014_SOP0010_v01_ Phusion PCR
31.07.15	TBA, JSP	Ligation of Leucine zipper into backbones: pSB1K3-RFP-T25 pSB1C3-RFP-T18 pSB1C3-T18 pSB1K3-T25	iGEM2014_SOP015_v02 _ligation
31.07.15	JSP	Phusion PCR, amplification of Leucine Zipper gene.	iGEM2014_SOP0010_v01_ Phusion PCR
01.08.15	ADK	Transformation of pSB1K3-RFP-T25(Y57) pSB1C3-RFP-T18 (Y58) pSB1C3-T18 (Y59), pSB1K3-T25 (Y60), ligated with leuzine zipper (Y63) into *E. coli* Top10	iGEM2013_SOP0009_v0 1_TSB transformation
03.08.15	ADK	Colony PCR with MyTaq of first draw of Y59 and Y58	iGEM2013_SOP0021_v0 1_ Colony PCR with MyTaq
04.08.15	ADK	Colony PCR with MyTaq of first draw of Y59 and Y58	iGEM2013_SOP0021_v0 1_ Colony PCR with

			MyTaq
04.08.15	ADK	Transformation of pSB1K3 into Top10	iGEM2013_SOP0009_v01_TSB transformation
05.08.15	ADK	Cheeked if our Freeze stocks, contains the right plasmids	
07.08.15	ADK	Miniprep of: #6: MG1655/ pSB1C3-KT25 #7: MG1655/pSB1K3-UT18C #22: pSB1C3-KT25-LeuZ-RFP #23: pSB1K3-UT18-LeuZ-RFP	Bio-Rad Quantum Prep: plasmid miniprep Kit
08.08.15	ADK	Miniprep and freeze stock of pSB1K3 (R71) and pSB1C3 (R72)	Bio-Rad Quantum Prep: plasmid miniprep Kit
08.08.15	ADK, TBA	Fast digest of: R67: MG1655/ pSB1C3-KT25 R68: MG1655/pSB1K3-UT18C R69: pSB1C3-KT25-LeuZ-RFP R70: pSB1K3-UT18-LeuZ-RFP With BamH1 and Pst1	iGEM2014_SOP0017_v01_Fast digest
08.08.2015	ADK	Ligation of cut R67, R68, R69, R70 with leuZ (Y63)	iGEM2014_SOP015_v02_ligation
09.08.2015	ADK	Transformation of R67, R68, R69, R70 with leuZ (Y63) into *E. coli* MG1655	iGEM2013_SOP0009_v01_TSB transformation
10.08.2015	JSP	Transformation of R54 & R55 (suspect we used backbones with error in sequences)	iGEM2013_SOP0009_v01_TSB transformation

11.08.2015	ADK	Miniprep of transformations from yesterday, stored as R77 and R78 respectively.	Bio-Rad Quantum Prep: plasmid miniprep Kit
12.08.2015	ADK, TBA	Fast digest of R77, R78, R63 and R64, using BamH1 and Pst1. PCR of G24 (true leucin zipper), stored as G43.	iGEM2014_SOP0017_v01_ Fast digest
13.08.2015	ADK	PCR of G24, stored as G44 and G45 Fast Digest of G45 using BamH1 and Pst1	iGEM2014_SOP0010_v01_ Phusion PCR iGEM2014_SOP0017_v01_ Fast digest
13.08.15	JSP	Ligation of Leucine zipper into backbones: pSB1K3-RFP-T25 pSB1C3-RFP-T18 pSB1C3-T18 pSB1K3-T25	iGEM2014_SOP015_v02 _ligation
14.08.15	ADK	Transformation of yesterday's ligations into MG1655	iGEM2013_SOP0009_v0 1_TSB transformation
15.08.2015	JSP	Colony PCR on pSB1K3-RFP-T25 and pSB1C3-RFP-T18 (Y76+Y77) ligated with leucin zipper (Y63)	iGEM2013_SOP0021_v0 1_ Colony PCR with MyTaq
15.08.15	JSP	Ligation of Leucine zipper into backbones: pSB1C3-T18 pSB1K3-T25 (Y74+Y75)	iGEM2014_SOP015_v02 _ligation
16.08.2015	JSP	Transformation of ligation from 15.08.2015 (Y63+Y74/Y75)	iGEM2013_SOP0009_v0 1_TSB transformation
17.08.2015	TBA, JSP.	Fast Digest of R16, R17, R18	iGEM2014_SOP0017_v01_

	TBA, ADK	& R19 with BamH1 + Pst1. Stored as Y86, Y87, Y89 & Y88 respectively. Ligation of Y86, Y87, Y88 & Y89 with Y63 (Leucinzipper)	Fast digest iGEM2014_SOP015_v02_ligation
18.08.2015	ADK	Transformation of yesterdays ligations	iGEM2013_SOP0009_v01_TSB transformation
20.08.2015	JSP	FastDigest of pSB1C3-T25 (R17) and pSB1K3-T18 (R18), and Leucine Zipper (G46). Ligation of pSB1C3-T25 (R17) and pSB1K3-T18 (R18), and Leucine Zipper (G46).	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation
21.08.2015	JSP	Transformation of ligation from *20.08.2015*	iGEM2013_SOP0009_v01_TSB transformation
21.08.2015	JSP	Mini-prep on pSB1C3-T18+T25 and pSB1K3-T18+T25 from freeze-stock #5-#8 - stored as R89-R92	Bio-Rad Quantum Prep: plasmid miniprep Kit
22.08.2015	AC	Colony-PCR on transformation from 21.08.2015	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
25.08.2015	JSP	FastDigest on R32 (PcstA-RFP) and R89-92 (pSB1C3-T18+T25 and pSB1K3-T18+T25) Ligation of the above digested	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation

		products.	
26.08.2015	TBA	Transformation of ligations from 25th.	
27.08.2015	JSP	Colony-PCR on 4 selected colonies from each transformation.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
30.08.2015	TBA ETM	Miniprep from #52 and #53, stored as R110 and R111 Transformation (test of the two-hybrid system)	iGEM2013_SOP0009_v01_TSB transformation
31.08.2015		Colony PCR	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
01.09.2015	EMT, JSP	PCR on plasmids	iGEM2014_SOP0010_v01_ Phusion PCR
02.09.2015	TBA	Transformation (test of the two-hybrid system) into *Δ*cyaA	iGEM2013_SOP0009_v01_TSB transformation
04.09.2015	JSP	Transformation (test of the two-hybrid system) into *Δ*cyaA	iGEM2013_SOP0009_v01_TSB transformation
05.09.2015	JSP	Colony-PCR with specific primers and on wt *Δ*cyaA + BTH101	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
06.09.2015	JSP	Transformation (test of the two-hybrid system) into *Δ*cyaA	iGEM2013_SOP0009_v01_TSB transformation
06.09.2015	JSP	Colony-PCR on transformation from *05.09.2015*	iGEM2013_SOP0021_v01_ Colony PCR with

			MyTaq
06.09.2015	JSP	Transformation (preparation for test of the two-hybrid system) into BTH101	iGEM2013_SOP0009_v01_TSB transformation
07.09.2015	JSP	Transformation (test of the two-hybrid system) into BTH101	iGEM2013_SOP0009_v01_TSB transformation
08.09.2015	JSP	Colony-PCR on transformation from *07.09.2015*	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq

4. Materials required.

Materials in use

Name	**Components (Concentrations)**	**Manufacturer / Cat. #**	**Room**	**Safety considerations**
Bio-Rad Quantum Prep: plasmid miniprep Kit				

5. Experiment history

Date (DD.MM.YY)	**Person(s) (initials)**	**Alterations to SOPs and remarks to experiments**	**SOPs**
28.07.15	JSP	Primers in use: Zip_F - 019 cgctTCTAGaGGGATCCgaaaatttgtattttcaatctggtatgaaacagctggaagacaaagttga Zip_R - 020 atatCTGCAGCggccgctACTAGTaacgttcaccaaccagttttttcaga Two PCR was done; one with normal volume of primers (2,5 µl) and one with half amount (1,25 µl). HF buffer used in both occations.	iGEM2014_SOP0010_v01_ Phusion PCR
29.07.15	JSP	Restriction enzymes used: BamHI and PstI Incubated for 30 min + 15 min in ice afterwards. 10 µl of PCR product used (7,4 ng/µl)	iGEM2014_SOP0017_v01_ Fast digest
29.07.15	ADK	Pcr produkt of G20 (leuZ) used HF buffer and CG buffer, 0,5µl primers: Zip_F - 019 and Zip_R - 020.	iGEM2014_SOP0010_v01_ Phusion PCR
30.07.15	JSP, TBA	For the FastDigest 7 µl (approx. 0,21 µg) PCR product	iGEM2014_SOP0017_v01_ Fast digest

		was used (G23)	
30.07.15	JSP, TBA	FastDigest was done on the following backbones: pSB1K3-RFP-KT25-LeuZ (R54): 10 µl pSB1C3-RFP-UT18-LeuZ (R55): 48 µl pSB1K3-KT25-LeuZ (R62): 30 µl pSB1C3-UT18-LeuZ (R61): 30 µl For R55, R61, R62: 6 µl 10x FastDigest buffer, 2 µl enzyme (of both FasAP and FD enzymes) (in a total volume of 60 µl)	iGEM2014_SOP0017_v01_ Fast digest
31.07.2015	ADK	Miniprep of (Top10) pSB1C3-UT18-LeuZ, and (Top10) pSB1K3-KT25-LeuZ - Done according to the SOP	GE healthcare kit
31.07.2015	ADK	(Attempt 1) Fast digest of pcr product leuZ (G23): 2µl 10x FD buffer, 1µl BamH1, 1µlPst1, 16µl G23	iGEM2014_SOP0017_v01_ Fast digest
31.07.15	ADK	Fast digest of R63 (pSB1C3-UT18-LeuZ) and R64 (pSB1K3-KT25-LeuZ), with BamH1 and Pst1. Made two mix: 8µl R63/10µl R64, 3µl 10x FD buffer, 1µl BamH1, 1µl Pst1, 1µl phosphate FD, and micro H_2O to a total volume of	iGEM2014_SOP0010_v01_ Phusion PCR

		30µl. (Attempt 2) Fast digest of pcr product leuZ, used the last of leuZ product from G21, G22 (approx. 70µl in total),2µl 10x FD buffer, 1µl BamH1, 1µlPst1	
31.07.15	JSP	PCR on leucin zipper from R27. Both HF an GC was tested. Half amount of primers used (1,25 µl). A sample with no template was also tested. PCR was made with temperature gradient.	iGEM2014_SOP0010_v01_ Phusion PCR
31.07.15	TBA, JSP	For ligation approx. 0, 20 and 50 fmol was used of digested leucin zipper(Y63) (0, 0,2 µl + 0,4 µl) Digested backbones used for ligation: Y57, Y58, Y59 and Y60 Ligation was set to overnight.	iGEM2014_SOP015_v02 _ligation
01.08.2015	ADK	Transformation of pSB1K3-RFP-T25(Y57) pSB1C3-RFP-T18 (Y58) pSB1C3-T18 (Y59), pSB1K3-T25 (Y60), ligated with leuzine zipper (Y63) into *E. coli* Top10 - Done according to the SOP - plated out on 25ɣCML and 25ɣ, according to the plasmid resistance	iGEM2013_SOP0009_v0 1_TSB transformation
03.08.2015	ADK	Colony PCR with MyTaq of	iGEM2013_SOP0021_v0

		first draw of Y59 and Y58. Annealing temp. 55^0	1_ Colony PCR with MyTaq
04.08.15	ADK	Colony PCR with MyTaq of first draw of Y59 and Y58. Annealing temp. 60^0. 5µl MyTaq, 1µl VF primer, 1µl VR primer, 3µl water pr. reaction and 0,5µl boiled culture (boiled in 50µl water)	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
04.08.15	ADK	Transformation of pSB1K3 into Top10 - because we have a suspicion that we might have swapped up the two plasmids - plated out on 25ɣ kan plates	iGEM2013_SOP0009_v01_TSB transformation
05.08.15	ADK	Checked if our freeze stocks contains the right plasmids according to our freeze stock book, by plaiting them out on plates according to their resistance, and to see if those containing RFP turned red. We checked the following: #5: MG1655/pSB1C3-UT18C #6: MG1655/ pSB1C3-KT25 #7: MG1655/pSB1K3-UT18C #8: MG1655/pSB1K3-KT25 #17: TOP10/pSB1C3-UT18C-LeuZ #20: Top10/pSB1K3-KT25-LeuZ #22: pSB1C3-KT25-LeuZ-RFP #23: pSB1K3-UT18-LeuZ-RFP #33: TOP10/pSB1K3-RFP-	

		KT25-LeuZ	
07.08.15	ADK	Miniprep of #6, #7, #22, #23 - done according to the SOP in the Kit	Bio-Rad Quantum Prep: plasmid miniprep Kit
08.08.15	ADK	Miniprep and freeze stock of pSB1K3 (R71) and pSB1C3 (R72) Freezestock 700µl overnight culture, and 300µl glycerol	Bio-Rad Quantum Prep: plasmid miniprep Kit
		Fast digest of: R67: MG1655/ pSB1C3-KT25 R68: MG1655/pSB1K3-UT18C R69: pSB1C3-KT25-LeuZ-RFP R70: pSB1K3-UT18-LeuZ-RFP With BamH1 and Pst1 - done according to SOP	iGEM2014_SOP0017_v01_ Fast digest
08.08.2015	ADK	Ligation of cut R67, R68, R69, R70 with leuZ (Y63), overnight. Done according to the SOP, inserted 0Fmol, 20Fmol, 50Fmol.	iGEM2014_SOP015_v02 _ligation
09.08.2015	ADK	Transformation of R67, R68, R69, R70 with leuZ (Y63) into *E. coli* MG1655. Done according to the SOP, and plated out on CML and KAN LA-plates according the backbones resistance	iGEM2013_SOP0009_v0 1_TSB transformation

10.08.2015	JSP	Transformation of R54 and R55	iGEM2013_SOP0009_v01_TSB transformation
11.08.2015	ADK	Miniprep of transformations from yesterday, stored as R77 and R78	Bio-Rad Quantum Prep: plasmid miniprep Kit
12.08.2015	ADK, TBA ADK	Fastdigest of R63, R64, R77, R78. stored Y74, Y75, Y76 and Y77 respectively PCR of leuZ G24 - two attempts. Second attempt added 4µl $MgCl_2$	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP0010_v01_ Phusion PCR
13.08.2015	ADK	PCR of leuZ G24, didn't add $MgCl_2$, used half amount of primers , HF buffer. stored as G45, G44 FastDigest of G45 using BamH1 and Pst1. Left for ~ 45 min. Ligation of Y74, Y75, Y76 and Y77 with digested G45	iGEM2014_SOP0010_v01_ Phusion PCR
13.08.2015	JSP	For ligation approx. 0, 20 and 50 fmol was used of digested leucin zipper(Y78) (0, 0,4 µl + 0,9 µl) Digested backbones used for ligation: Y74, Y75, Y76 and Y77 - approx. 10 µl backbone was used to each ligation. Ligation was set to overnight.	iGEM2014_SOP015_v02_ligation

14.08.15	ADK	Transformation of yesterday's ligations into MG1655 - Done according to SOP	iGEM2013_SOP0009_v01_TSB transformation
15.08.2015	JSP	Colony PCR on Y76 and Y77 ligated with Y63. No real alterations to SOP. Colonies were streaked out on agar plates and added directly to MyTaq solutions.	
15.08.2015	JSP	For ligation approx. 0, 20 and 50 fmol was used of digested leucin zipper(Y63) (0, 0,25 µl + 0,5 µl) Digested backbones used for ligation: Y74 and Y75- approx. 10 µl backbone was used to each ligation. Ligation was set to overnight.	iGEM2014_SOP015_v02_ligation
16.08.2015	JSP	Transformation of ligation from 15.08.2015 (Y63+Y74/Y75): no alterations to SOP	iGEM2013_SOP0009_v01_TSB transformation
17.08.2015	TBA, JSP	Fast Digest of R16 (pSB1C3-UT18), R17 (pSB1C3-KT25), R18 (pSB1K3-UT18) and R19 (pSB1K3-KT25) with BamH1 + Pst1. By error, 2 µL of each restriction enzymes was added instead of 1 µL. Incubated for approx. 50 min. Digested R16 stored as Y86, digested R17 stored as Y87, digested R18 stored as Y89 and	iGEM2014_SOP0017_v01_Fast digest

	TBA, ADK	digested R19 stored as Y88. Ligation of Y86 - Y89 with Y63 (digested Leucin zipper). Possible contamination of Y63. Ligations left overnight at 16 degrees.	iGEM2014_SOP015_v02_ligation
18.08.2015	ADK	Transformation of yesterdays ligations	iGEM2013_SOP0009_v01_TSB transformation
20.08.2015	JSP	FastDigest of pSB1C3-T25 (R17) and pSB1K3-T18 (R18), and Leucine Zipper (G46). All cut with BamHI and PstI - digestions was given approx. 2 hours. Ligation of pSB1C3-T25 (R17) and pSB1K3-T18 (R18), and Leucine Zipper (G46). Instead of using 30 and 50 fmol inserts, 60 and 90 fmol inserts were used. Ligations were left overnight.	iGEM2014_SOP0017_v01_Fast digest iGEM2014_SOP015_v02_ligation
21.08.2015	JSP	Transformation of ligation from *20.08.2015* pSB1C3-T25 + Leucine Zip (Y91+Y90) pSB1K3-T18 + Leucine Zip (Y92+Y90) Strain used: MG1655 When streaked out on agar plates, Y91 was added to	iGEM2013_SOP0009_v01_TSB transformation

		kanamycin plates, and Y92 chloramphenicol plates. This should have been the other way around(!). As 10 µl (max.) was still left in the eppendorf tubes, these left overs was streaked out on the appropriate agar plates.	
21.08.2015	JSP	Mini-prep on pSB1C3-T18+T25 and pSB1K3-T18+T25 from freeze-stock #5-#8 - stored as R89-R92 - only 1,5 mL of liquid culture used for miniprep (for higher concentration, next time use all 5 mL) - No alteration to protocol.	Bio-Rad Quantum Prep: plasmid miniprep Kit
22.08.2015	AC	Colony-PCR on transformation from 21.08.2015	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
25.08.2015	JSP	FastDigest on R32 (PcstA-RFP) and R89-92 (pSB1C3-T18+T25 and pSB1K3-T18+T25) R32 cut with SpeI+PstI R89-92 cut with XbaI+PstI Stored as Y93(PcstA-RFP), Y94(T18), Y95 (T25) Ligation of the above digested products. No alteration to SOP.	iGEM2014_SOP0017_v01_ Fast digest iGEM2014_SOP015_v02_ligation

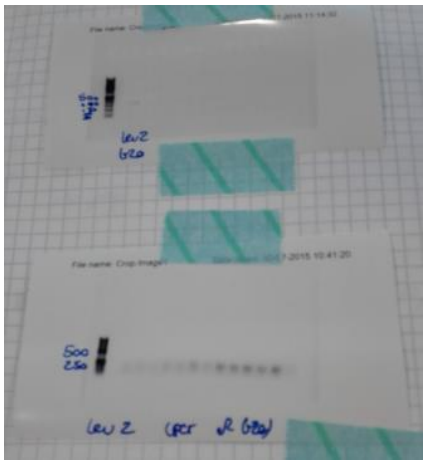
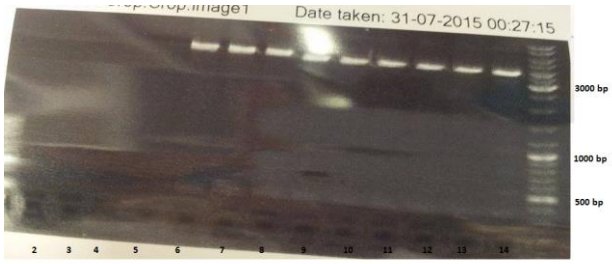
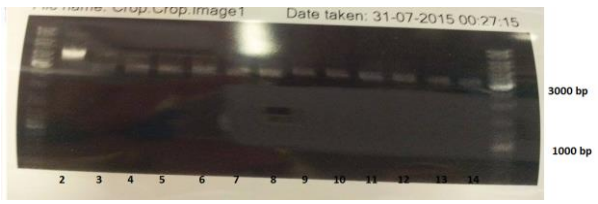
26.08.2015	TBA	Transformation of yesterdays overnight ligations into MG1655.	
27.08.2015	JSP	Colony-PCR. No alteration to SOP.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
30.08.2015	TBA EMT	Miniprep of overnight cultures of #52 and #53. stored as R110 and R111, respectively. Transformation of 8 plasmid combinations to test the two-hybrid system	iGEM2013_SOP0009_v01_TSB transformation
31.08.2015		Results inconclusive. colony PCR on colonies from selected plates.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
01.09.2015	EMT, JSP	PCR of plasmids in use to check if lenght of content matches what they supposedly contain	iGEM2014_SOP0010_v01_ Phusion PCR
02.09.2015	TBA	Transformation of different Rxx+Rxx, Rxx+Rxx	iGEM2013_SOP0009_v01_TSB transformation
04.09.2015	JSP	Transformation (test of the two-hybrid system) into *Δ*cyaA. The following combinations was used: R111+R113 (T18-Zip+25-Zip) (R113 transformed into R111	iGEM2013_SOP0009_v01_TSB transformation

		containing *Δ*cyaA strain.) R111+R104 (T18-Zip+T25) (R104 transformed into R111 containing *Δ*cyaA strain.) R107+R113 (T18+T25-Zip) (R113 transformed into R107 containing *Δ*cyaA strain.) R108+R103 (T18+T25) (R103 transformed into R108 containing *Δ*cyaA strain.) R110 R111 R108 R107	
05.09.2015	JSP	Colony-PCR on transformation from *04.09.2015* - No changes to SOP	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
06.09.2015	JSP	Transformation (test of the two-hybrid system) into *Δ*cyaA	iGEM2013_SOP0009_v01_TSB transformation
06.09.2015	JSP	Colony-PCR with specific primers and on wt *Δ*cyaA + BTH101	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
06.09.2015	JSP	Transformation (preparation for test of the two-hybrid system) into BTH101: R103, R104, R107, R108, R112 and R113 were transformed.	iGEM2013_SOP0009_v01_TSB transformation
07.09.2015	JSP	Transformation (test of the two-hybrid system) into	iGEM2013_SOP0009_v01_TSB transformation

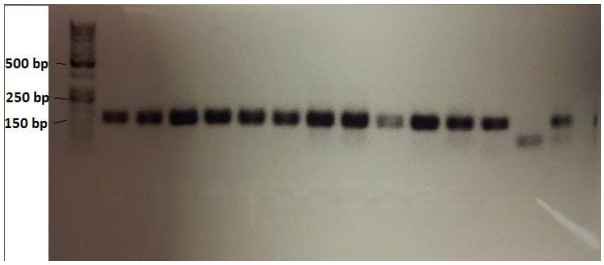
		BTH101. R102+R98 were transformed into BTH101 strain containing R103. R112 was transformed into BTH101 containing R104. R113 was transformed into BTH101 containing R111. These transformation were also made: R97+R98 (double-plasmid transformation) R97 R98 R112 R113	
08.09.2015	JSP	Colony-PCR on transformation from *06.09.2015*	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
08.09.2015	JSP	Colony-PCR on transformation from *07.09.2015*	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq

6. Results

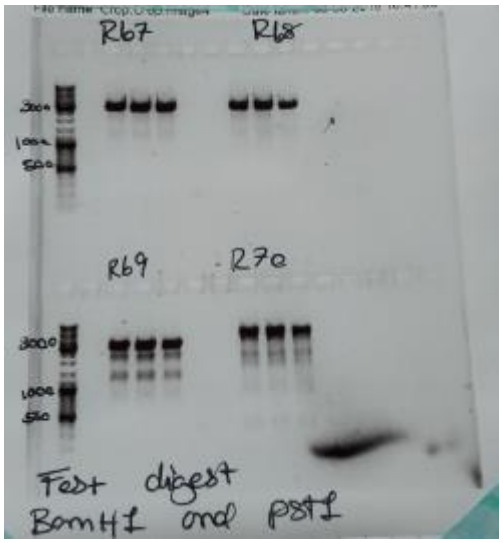
Date (DD.MM.YY)	**Picture**	**Comments**
28.08.15		PCR on leucin-zipper from R49: Strong bonds at approx. 100-200 bp (difficult to distinguish between the bonds in the ladder) Conc. from

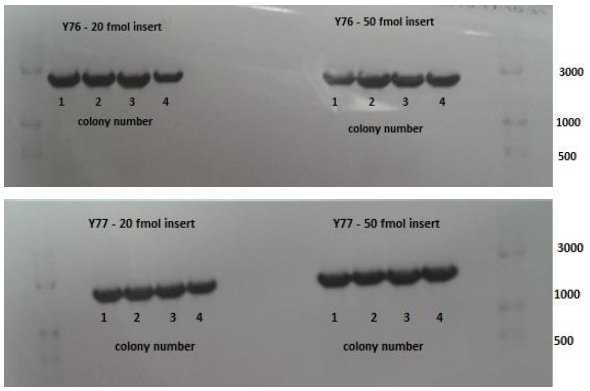
		purification: 7,6 ng/µl (labeled G20)
29.07.15	no picture	No bonds on gel after FastDigest.
29.07.15		Pcr of G20: Both buffers worke, got to concentrations: G21=12,88ng/µl and G22=10,79ng/µl. Did also purified G20: 20µl G20(pcr product of leuZ), 300µl binding buffer, 300µl isopropanol - ran it on the column and washed with washing buffer. Got the concentration G23=30,18ng/µl
30.07.15	**Row 1**  **Row 2** 	<u>Row 1:</u> 2-5: G23 6-8: R54 9-14: R55 <u>Row 2:</u> 2: R55 3-8: R61 9-14: R62 R54 + R55 was expected to be around 4000 bp. This is consistent with the bands shown. R61+R62 was expected to be around 3000 bp. This is also consistent with the bands shown. G23 was

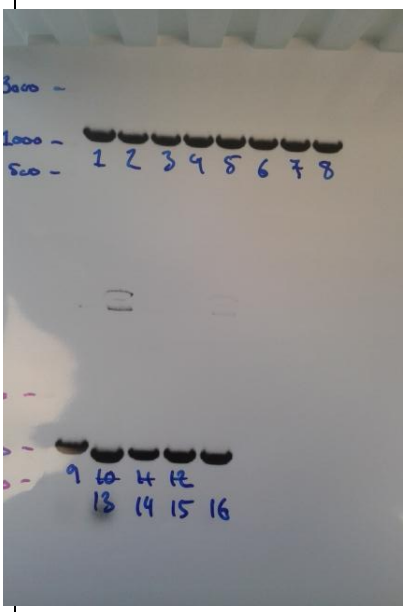
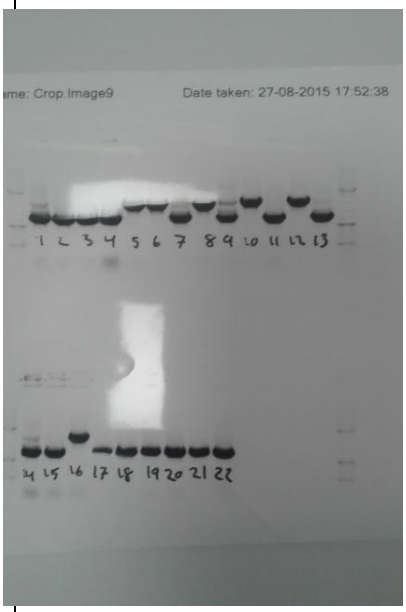
		expected to around 160 bp. No bands shown can be explained by a low concentration.
31.07.15		Got following concentration: R63 (pSB1C3-UT18-LeuZ)=121,57ng/µl, and R64 (pSB1K3-KT25-LeuZ)=83,86ng/µl
31.07.15		Fast digested leuZ: Got no bands on the gel
31.07.2015		Fast digest of pSB1C3-UT18-LeuZ (R63) and pSB1K3-KT25-LeuZ (R64), with BamH1 and Pst1 - Got the following concentration: pSB1C3-UT18-LeuZ (Y61):7,96ng/µl, and pSB1K3-KT25-LeuZ (Y62): 5,81ng/µl

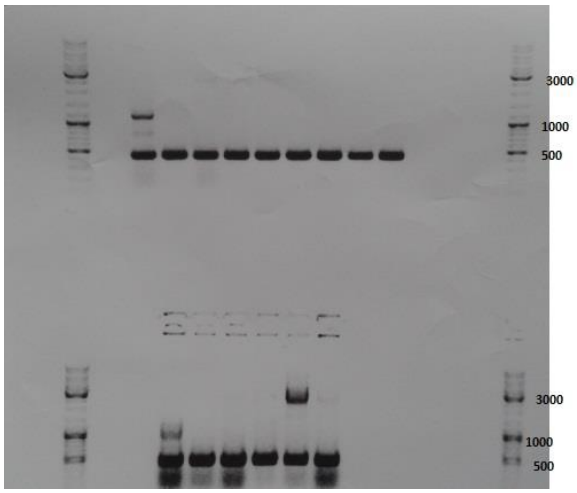
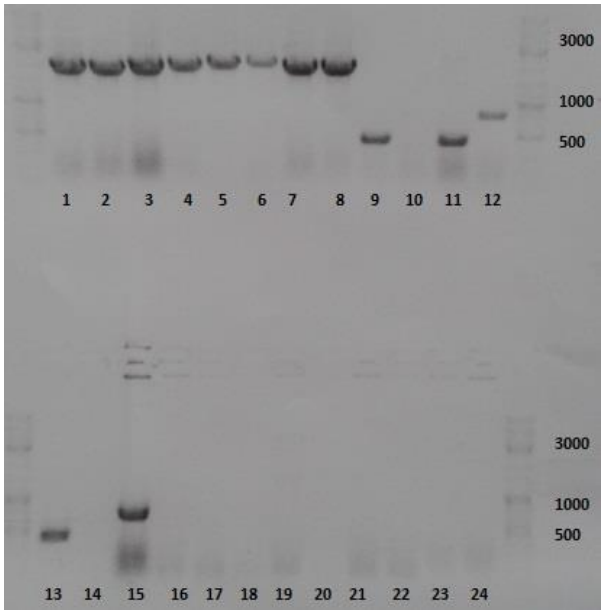
31.07.2015		(Attempt 2) Fast digest of pcr product leuZ. Got two very unclear bands - Measured the concentration of the digested leuZ (Y63) on the nanodrop, Y63=13,8ng/µl
31.07.15	**Row 1**  **Row 2** 	<u>Row 1</u> 2-11: PCR with GC buffer 12-15: PCR with HF buffer <u>Row 2</u> 2-7: PCR with HF buffer 8-12: No template For those PCR reactions with DNA template, the bands were as expected around 160 bp (with some exceptions: row 1, #14 and row 2, #5). The bands from the PCR reactions with no template were as expected much lower: approx. 50 bp.

03.08.2015		Transformation: got colonies of the Top10 with Y58(pSB1C3-RFP-T18-leuZ) and Y59(pSB1C3-T18-leuZ) on the CML plates, but unfortunately, we didn't got any colonies on the Kan plates with Y57 (pSB1K3-RFP-T25), and Y60 (pSB1K3-T25) - We try to plate these out on Cml plates, which gave colonies
03.08.2015		Colony PCR failed, no bands - will try again tomorrow, with 10µl MyTaq, 1µl VF primer, 1µl VR primer, 8µl water pr. reaktion. I
05.08.2015		Transformation failed
06.08.2015		Check of freeze stock: It looks like all sample contains the right plasmids. We made overnight culture of #6, #7, #22, #23.
07.08.2015		Miniprep: - R67 (#6: MG1655/pSB1C3-KT25)=96,8ng/µl -R68 (#7 MG1655/pSB1K3-

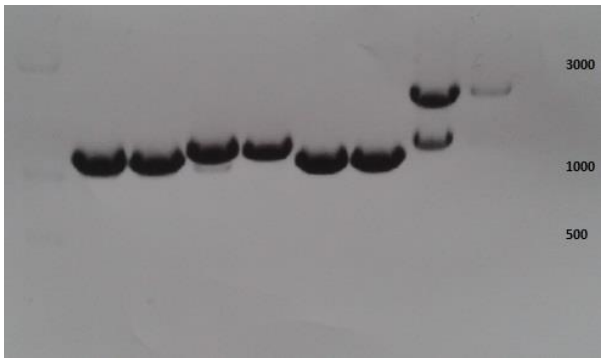
		UT18C)=58,7ng/µl - R69 (#22 pSB1C3-KT25-LeuZ-RFP)=212,9ng/µl -R70 (#23: pSB1K3-UT18-LeuZ-RFP) =116,7ng/µl
08.08.15		Miniprep: pSB1K3 (R71)=103,4ng/µl pSB1C3 (R72)=198,04ng/µl
08.08.15		Fastdigest: we are a little uncertain of why we get so many bands. Have decided to purified the clear bands, and ligate these backbones eith Y63 (leuZ cut with BamH1 and Pst1)
09.08.2015		
10.08.2015		Yesterdays transformations failed. all of them
11. 08.2015		Transformations with correct plasmid backbones from the 10th succeeded
12.08-2015		First PCR G24 (true leucin zipper) attempt

		with both MyTaq and phusion (with extra MgCl2) polymerase failed due to wrong primers…. second attempt using MyTaq yielded only poor concentrations. Fast Digests was succesful, but due to insuccicient amount of G24, ligation was postponed.
13.08.2015		Phusion PCR of G24 without extra MgCl2 yielded better concentrations. Fast digest of G45
14.08.15		Transformation. Colonies of MG1655/Y76 and MG1655/Y77 - MG1655/Y74 and MG1655/Y75 failed
15.08.2015		Colony PCR showed bands at approx. 2000-2500 nt. For Y76 the band should be around 2200 nt and for Y77 the band should be around 2300 nt.
17.08.2015		Transformation from

		16.08.2015 failed. No colonies.
22.08.2015		Transformation was succesful, even though only 10 µl instead of approx. 100 µl was streaked out. The results from colony-PCR confirmed the succesful ligation+transformation (the bands should be approx. 1200-1300 nt) 1-8: Y91 9-16: Y92
27.08.2015		Transformation was succesful (#5, #6, #8, #10, #12, #16). Bands were expected to around 2100-2200 nt long. 1-4: Y94-20 (PcstA-RFP-T18) 5-8: Y94-50 (PcstA-RFP-T18) 9-12: Y95-20 (PcstA-RFP-T25) 13-16: Y95-50 (PcstA-RFP-T25)

05.09.2015		Not succesful. We would have expected Two bands in each of them (one at approx. 1000 bp and one at 2000 bp.) Instead we got a band at approx. 450 bp. Probably a contaminant.
06.09.2015		What we expected: 1+2: R107 (approx. 2100) 3+4: R108 (approx. 2100) 5+6: R110 (approx. 2100) 7+8: R111 (approx. 2100) 9+10: R111 (approx. 2100 (VF/VR-primers) or 750 bp (001+003 primers)) 11+12:R107 (approx. 2100 (VF/VR-primers) or 750 bp (001+003 primers)) 13+14:R108 (approx. 2100 (VF/VR-primers) or 850 bp (001+003 primers)) 15+16: R108+R103 (two bands at approx. 750 and 850 bp 001+002/3 primers used) 17+18:R111+R104 (two bands at approx. 750 and 850 bp 001+002/3 primers used) 19+20:R107+R113 (two

		bands at approx. 750 and 850 bp 001+002/3 primers used) 21+22:R113+R111 (two bands at approx. 750 and 850 bp 001+002/3 primers used) 23: 'clean' *Δ*cyaA: no band 24: 'clean' BTH101: no band **Conclusion:** From these results it can be concluded that only colony 1-8+23+24 were correct (and partially 12)
07.09.2015		What was expected: 1+2: R103 (1050 bp) 3+4: R104 (1150 bp) 5+6: R107 (approx 2100 bp) 7+8: R108 (approx 2100 bp) 9+10:R111 (approx 2100 bp) **Conclusion:** Transformations were succesful

08.09.2015		What was expected: 1+2: R103+R102 (1050-1150 bp) 3+4: R103+R98 (1050-1300 bp) 5+6: R104+R112 (approx 1100 bp) 7+8: R111+R113 (1300+2100 bp) **Conclusion:** Transformations were succesful This was also confirmed by the presence of blue colonies at the plate with colonies transformed with R111+R113 (T18-zip+T25-zip)

7. Appendices