iGEM 2015 – Microbiology – BMB – SDU

Project type: Biobrick creation **Project title:** Construction of two hybrid system **Sub project:** CyaA controlled by lacZ promoter activated by dimerization.	**Creation date:** 31.03.2015 **Written by:** Elise, Christian and Mette **Performed by:** iGEM 2015

1. SOPs in use

All SOP's can be found in: iGEM SOP's
iGEM2014_SOP0010_v01_Phusion PCR
iGEM2013_SOP0016_v01_Making_LB_and_LA_medium
iGEM2014_SOP0014_v01_Gelpurification
iGEM2014_SOP0017_v01_ Fast digest
iGEM2014_SOP0015_v02_Ligation
iGEM2013_SOP009_v01_TSB_transformation
iGEM2013_SOP0021_v01_TK_Colony_PCR_with_MyTaq
iGEM 2014 SOP0019 - Miniprep

2. Purpose

To construct two bricks that interact with each other allowing CyaA activation. The activation only occurs if the proteins bound to CyaA interact with each other. This interaction allows CyaA to produce cAMP from ATP.

3. Overview

Date (YY.MM.DD)	**Person(s) (initials)**	**Experiments**	**SOPs**
15.03.31	EMT;CEM ;MMWN	Phusion PCR Gel Purification Phusion PCR Gel Purification	iGEM2014_SOP0010_v01 iGEM2014_SOP0014_v01 iGEM2014_SOP0010_v01 iGEM2014_SOP0014_v01
15.04.01	EMT;CEM ;MMWN	Phusion PCR Gel Purification Miniprep DNA/gel band	iGEM2014_SOP0010_v01 iGEM2014_SOP0014_v01 no SOP

		Phusion PCR Gel Purification	iGEM2014_SOP0010_v01 iGEM2014_SOP0014_v01
15.04.02	EMT;CEM ;MMWN	Phusion PCR Gel Purification Miniprep DNA/gel band	iGEM2014_SOP0010_v01 iGEM2014_SOP0014_v01 no SOP
15.04.11	TBA, JSP, AC	Fast Digest Gel purification Ligation Gel purification	iGEM2014_SOP0017_v01 iGEM2014_SOP0014_v01 iGEM2014_SOP0015_v02 iGEM2014_SOP0014_v01
15.04.12	AC, MMWN	Transformation	iGEM2013_SOP009_v01
15.05.04	ADK	Fast Digest	iGEM2014_SOP0017_v01
15.05.05	AC	Gel purification of fast digest	iGEM2014_SOP0014_v01_G elpurification
15-05-06	AC	Ligation of fast digest	iGEM2014_SOP0015_v02_Li gation
15-05-07	AC	Transformation	iGEM2013_SOP009_v01_TS B_transformation
15-05-08	AC	Cell incubation for miniprep	
15-05-08	AC	Colony PCR	iGEM2013_SOP0021_v01_T K_Colony_PCR_with_MyTaq
15-05-08	AC	PCR	iGEM2014_SOP0010_v01_P husion PCR
15.05.12	ADK, JSP	Fast digestion	iGEM2013_SOP0017
15.05.12	ADK, JSP	Ligation	iGEM2014_SOP0015
15.05.13	Kat	Tranformation	iGEM2013_SOP0009_v01
15.05.14	ADK, JSP,	Colony PCR with MyTaq	iGEM2013_SOP0021_v01
15.05.19	EMT, Thea,JSP	Colony PCR with MyTaq	iGEM2013_SOP0021_v01_T K_Colony_PCR_with_MyTaq

15.05.22	ADK,TBA, AC, JSP	Colony PCR with MyTaq	iGEM2013_SOP0021_v01_TK_Colony_PCR_with_MyTaq
15.05.25	ADK, TBA	Ligation	iGEM2014_SOP0015_v02
15.05.26	EMT, AC, CEM	Transformation	SOP0009_v01
15-07-			
15-07-06	AC	Fast digest	iGEM2014_SOP0017_v01_ Fast digest
15-07-06	AC	Ligation	iGEM2014_SOP0015_v02_Ligation
15-07-07	AC	Transformation	iGEM2013_SOP009_v01_TSB_transformation
15-07-08	AC	Colony-PCR	iGEM2013_SOP0021_v01_TK_Colony_PCR_with_MyTaq
15-07-10	AC	Fast digest	iGEM2014_SOP0017_v01_ Fast digest
15-07-11	AC	Ligation	iGEM2014_SOP0015_v02_Ligation
15-07-14	AC	Transformation	iGEM2013_SOP009_v01_TSB_transformation
15-07-15	AC	Miniprep	iGEM 2014 SOP0019 - Miniprep
15-07-15	AC	Fast digest	iGEM2014_SOP0017_v01_ Fast digest
15-07-15	AC	Ligation	iGEM2014_SOP0015_v02_Ligation
16-07-15	AC	Colony PCR	iGEM2013_SOP0021_v01_TK_Colony_PCR_with_MyTaq

16-07-15	AC	Miniprep (Low concentration)	iGEM 2014 SOP0019 - Miniprep
16-07-15	AC	Fast digest (Cut with EcoRI)	iGEM2014_SOP0017_v01_ Fast digest
17-07-15	AC	Miniprep	iGEM 2014 SOP0019 - Miniprep
17-07-15	AC	Fast digest	iGEM2014_SOP0017_v01_ Fast digest
17-07-15	AC	Transformation (into Delta_Cya)	iGEM2013_SOP009_v01_TSB_transformation
20-07-15	ADK	Miniprep of PSB1C3-RFP-UT18-LeuZ and PSB1C3-RFP-KT25-LeuZ, both transformed into top10	ge healthcare miniprep kit

4. Materials required.

Materials in use

Name	**Components (Concentrations)**	**Manufacturer / Cat. #**	**Room**	**Safety considerations**
Primer	pKT25_BB_F			
Primer	pUT18C_BB_R			
Strain	MG1655			

5. Experiment history

Date (YY.MM.DD)	Person(s) (initials)	SOPs	Alterations to SOPs and remarks to experiments
15.03.31	EMT;CEM; MMWN	Phusion PCR: iGEM2014_SOP0010_v01	Everything was done according to SOP. Enzyme: HF Template: pUt18C + pKT25 Primers 18: pKT25_BB_F (001F) and pUT18C_BB_R (003F). Dilution factor 1:10
15.03.31		iGEM2014_SOP0014_v01 Gel purification	Primers 25: pKT25_BB_F (001F) and pKT25_BB_R (002F). Dilution factor 1:10
15.03.31		iGEM2014_SOP0010_v01 Phusion PCR	Temperature gradient [^{0}C]: 55-63.9 Number of bp: ca. 1000 => 30 sec. at 72^{0}C UT18 bp: 759 KT25 bp: 891
15.03.31		iGEM2014_SOP0014_v01 Gel purification	Everything was done according to SOP. Enzyme: HF Template: pUt18 + pKT25 Primers 18: pKT25_BB_F (001F) and pUT18C_BB_R (003F). Dilution factor 1:10 Primers 25: pKT25_BB_F (001F) and pKT25_BB_R (002F). Dilution factor 1:10 Optimised Temperature gradient [^{0}C]: 57.1-60.6 pUt18 61.8-65.6 pKT25 Number of bp: 1000 => 30 sec. at

			72^0C UT18 bp: 759 KT25 bp: 891
15.04.01 15.04.01 15.04.01 15.04.01 15.04.01	EMT;CEM;MMWN	iGEM2014_SOP0010_v0 Phusion PCR iGEM2014_SOP0014_v01 Gel purification No SOP Miniprep DNA/gel band iGEM2014_SOP0010_v0 Phusion PCR iGEM2014_SOP0014_v01 Gel purification	Everything was done according to SOP. Enzyme: HF og GC Template: pUt18 + pKT25 Primers 18: pKT25_BB_F (001F) and pUT18C_BB_R (003F). Dilution factor 1:10 Primers 25: pKT25_BB_F (001F) and pKT25_BB_R (002F). Dilution factor 1:10 Optimised Temperature gradient [$_0$C]: 58.1-65.6 pUt18 and pKT25 Number of bp: 1000 => 30 sec. at 72^0C UT18 bp: 759 KT25 bp: 891 Everything was done according to instruction pamphlet Elution in 10 µl Product from Ut18C Labelled G1 and G2 Everything was done according to SOP. Enzyme: HF og GC Template: pKT25 Primers 25: pKT25_BB_F (001F) and pKT25_BB_R (002F). Dilution factor 1:10 Optimised Temperature gradient [^{0}C]: 58.1-65.6 pKT25 Number of bp: 1000 => 30 sec. at 72^0C KT25 bp: 891
15.04.02	EMT;CEM;MMWN	iGEM2014_SOP0010_v01 Phusion PCR	Everything was done according to SOP. Enzyme: HF Template: pKT25

15.04.02 15.04.02		iGEM2014_SOP0014_v01 Gel purification no SOP miniprep DNA/gel band iGEM2014_SOP0017_v01	Primers 25: pKT25_BB_F (001F) and pKT25_BB_R (002F). Dilution factor 1:10 Optimised Temperature gradient [^{0}C]: 61.8-64.9 pKT25 DMSO gradient conc.: 0; 0.5; 1.5 Number of bp: 1000 => 30 sec. at 72^{0}C KT25 bp: 891 Everything was done according to instruction pamphlet Elution in 30 ^{0}C Product from KT25 Labelled G3 Everything was done according to SOP.
15.04.03	CEM EMT MMWN CEM MMWN	iGEM2014_SOP0015_v01 iGEM2014_SOP0009_v01	Everything was done according to SOP. 15 mu\L was transferred into the Ependorff tubes. Everything else was done according to SOP. The plates incubated overnight.
15.04.04	ADK, AC	iGEM2013_SOP0021_v01 Colony PCR with My Tag	Master mix: 150µl MyTaq, 30µl VF2 primer, 30µl VR2 primer, 90µl H_2O

15.04.11	TBA, JSP, AC	iGEM2014_SOP0017_v01_ Fast digest	Done according to SOP, R11: pSB1C3_UT18C cut with BamH1 and Pst, stored as Y7 R12: pSB1C3_KT25 cut with BamH1 and Pst, stores as Y8 R13: pSB1K3_UT18C cut with BamH1 and Pst1, stored as Y9 R14: pSB1K3_KT25 cut with BamH1 and Pst1, stored as Y10
15.04.11	TBA, JSP, AC	iGEM2014_SOP0014_v01_Gelpurification	Done according to SOP pSB1C3_UT18C: Y7 = 4,7 ng/µL pSB1C3_KT25: Y8 = 12,9 ng/ µL pSB1K3_UT18C: Y9 = 9,6 ng/ µL pSB1K3_KT25: Y10 = 11,5 ng/µL
15.04.11	TBA, JSP, AC	iGEM2014_SOP0015_v02_Ligation	Volumes used for 10fmol plasmid: Y7: pSB1C3_UT18C: 3,9 µL Y8: pSB1C3_KT25: 1,5 µL Y9: pSB1K3_UT18C: 1,9 µL Y10: pSB1K3_KT25: 1,7 µL Volumes used from G9: linker-GFP (see protocol for Linker-GFP): inadequete amount of G9, unable to make ligation with both 20 fmol and 50 fmol. Only ligations with 0 fmol and 20 fmol were preformed. for 20 fmol 3,5 µL linker-GFP were used.
15.04.11	TBA, JSP, AC	iGEM2014_SOP0014_v01_Gelpurification	
15.04.12	JSP, AC, MMWN	iGEM2013_SOP009_v01_TSB_transformation	done according to SOP, but plates left in fridge..
15.04.13	AC, TBA	no SOP	moving plates from fridge to 37 ^{0}C growth chamber
15,04.14	AC, TBA	no SOP	No colonies, transformation unsuccessful.

15-04-30	AC	SOP0015_v02	Ligering af Y7, Y8, Y9 og Y10, med Liker-GFP (Y12, Y13 og Y14)
15-05-01	AC	iGEM2014_SOP0009_v01	Transformering af ligeringer fra 15-04-30. Transformeret ind i TOP10
15-05-01	AC, TBA	Udpladning	Udpladning af transformerede celler.
15-05-02	MM, AD		
15-05-03	AC	Miniprep	Ekstraheret DNA fra overnight kultur, lavet fra freeze stock. Navne kommer senere.
15.05.04	ADK	Fast digest	cut with BamhI and PstI

15-05-05	AC	Gel extraction	T18 and T25 extracted
15-05-06	AC	Ligation	T18 ligated with GFP, and T25 ligated with GFP
15-05-07	AC	Transformation	Transformation af T18 og T25, samt udpladning
15-05-08	AC	Colony PCR	Colony PCR og T18 and T25
15-05-08	AC	Opformering af celler	Transformation for T18-GFP og T25-GFP med chloramphenicol resistens succesfuld. Kolonier udtaget og placeret i LB.
15-05-08.	AC	PCR	1µL of each primer were used. Experiment were executed with both verification primers,

			and leucine zipper primers
15-05-09	AC	Miniprep	Miniprep of psB1C3-puT18-GFP koloni 1 og 3, and psB1C3-kT25-GFP
15-05-11	AC	Sent to sequencing	R28:608F, 609R. R29:610F, 611R. R30:612F, 613R. R31:614F, 615R.
15.05.12	ADK, JSP	iGEM2013_SOP0017	Fast digestion. Used restrictions enzymes: BamH1 and Pst1, incubated for 15min at 37 degree
15.05.12	ADK, JSP	iGEM2014_SOP0015	Ligation. Used 10fmol plasmid, and 0 fmol, 20fmol and 50fmol of; psB1C3-T18, psB1C3-T25, psB1K3-T18 and psB1K3-T25 all cut with BamHI and PstI (+FastAP) - Ligation overnight at 16 degree celsius.
15.05.13	Kat	iGEM2013_SOP0009_v01	Tranformation
15.05.14	ADK	iGEM2013_SOP0021_v01	Colony PCR woth MyTaq:Used 5µl MyTaq, 2x1 primer V4 and R5, and 3µl water, pr reaction
15.05.14	JSP Thea	iGEM2013_SOP0021_v01	Colony PCR with MyTaq: Used 5 µl

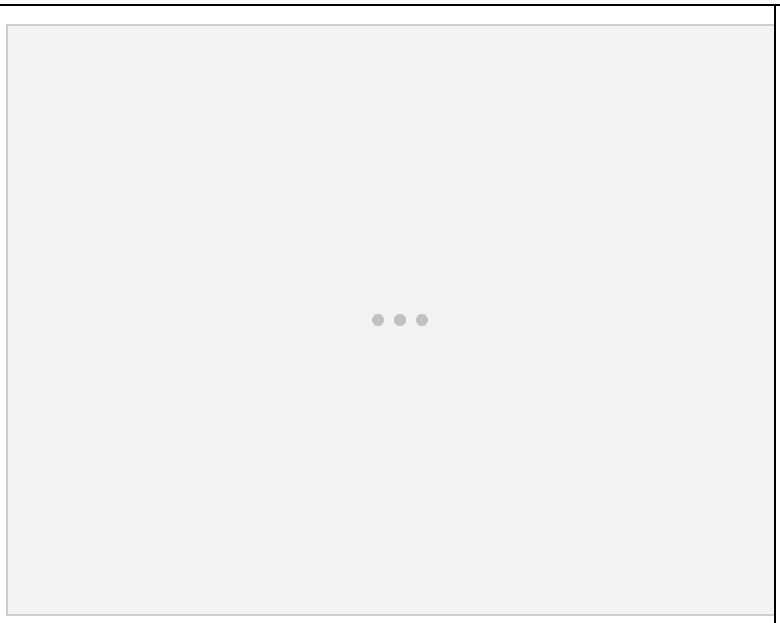
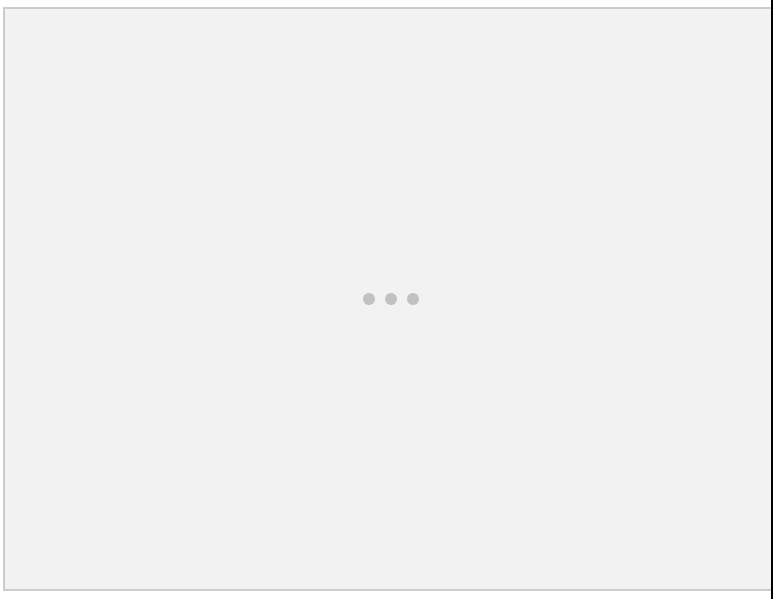
			MyTaq, 2*1 µl primer (VF + VR) and 2,5 µl H2O, 0,5 µl template suspension, pr reaction/pcr-tube
15-05-16	AC, ADK	Mininprep of T18/T25, with leucin zipper	
15-05-16	AC, ADK	sent to Sequencing	R34:626R, 627F. R35:628R, 629F. R36: 630R, 631F. R37: 632R, 633F.
15.05.16	AC, ADK	Fast digest iGEM2013_SOP0017_v01	Digests of psB1K3-UT18c-leuZ, psB1C3-UT18c-leuZ, psB1K3-KT25-LeuZ, psB1C3-KT25-LeuZ and psB1C3-RFP. T18/T25 + Leu Z cut with EcoRI and XbaI. psB1C3-RFP cut with EcoRI and SpeI.
15.05.16	AC	Ligation	Ligation of psB1C3-uT18-LeuZ and RFP indicator, and psB1K3-kT25-Leuz and RFP indicator.
15.05.17	AC	Transformation	Transformation of ligation from previous step, into Top10.
15.05.18	EMT, Thea, JSP	Colony-PCR	Colony PCR on 4 colonies from each of: psB1C3-mRFP-uT18-LeuZ

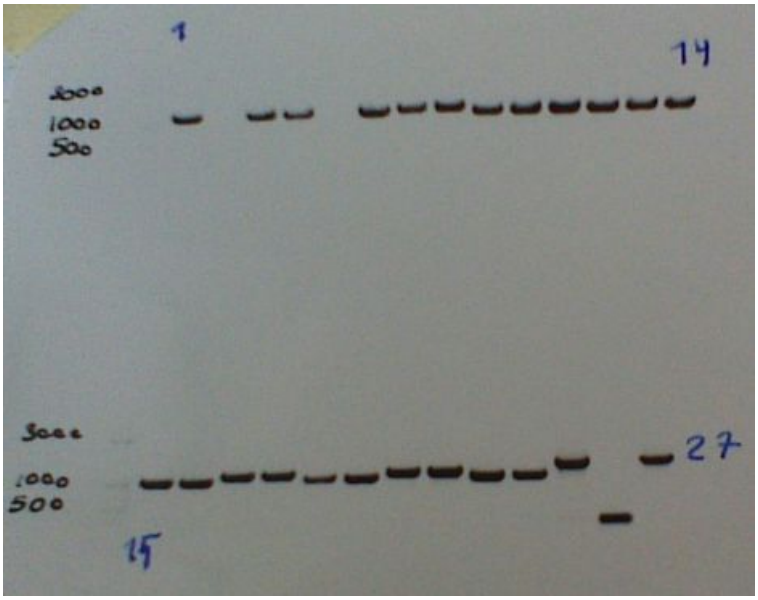
			psB1C3-mRFP-kT25-LeuZ psB1K3-mRFP-uT18-LeuZ psB1K3-mRFP-kT25-LeuZ Colony samples transferred to agar-plates
15-05-21	AC	Ligation	Ligation with correctly cut RFP, and T18/T25
15-05-22	ADK,TBA,AC,JSP	Colony PCR with MyTaq	Colony PCR on 4 colonies from each of: psB1C3-mRFP-uT18-LeuZ psB1C3-mRFP-kT25-LeuZ psB1K3-mRFP-uT18-LeuZ psB1K3-mRFP-kT25-LeuZ -psB1K3-T25-linkerGFP -psB1C3-mRFP Colony samples transferred to agar-plates + 2 colonies from -psB1C3-T25-linkerGFP
15-05-24	AC	iGEM2013_SOP0017_v01 Fast Digest	Fast digest of R16 R17 R18 and R19. Cut with XbaI, Eco and P

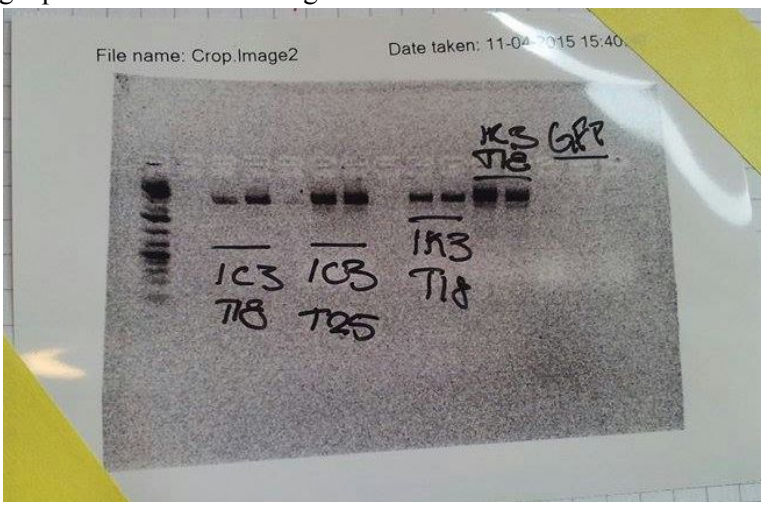
15-05-24	AC	Colony PCR	PCR with colonies 4, 15 and 16
15-05-25	AC, ADK	Colony PCR	PCR with colonies 4, 15 and 16, using VF2 and LeuZR.
15-05-25	AC, ADK	iGEM2013_SOP0017_v01 Fast digest	Fast digest of R16 R17 R18 and R19. Cut with XbaI, Eco and P. R32 (RFP/cAMP indicator) cut with Eco and SpeI.
15-05-25	AC, ADK	Gel Extraction	Gel extraction of DNA from 24-25.

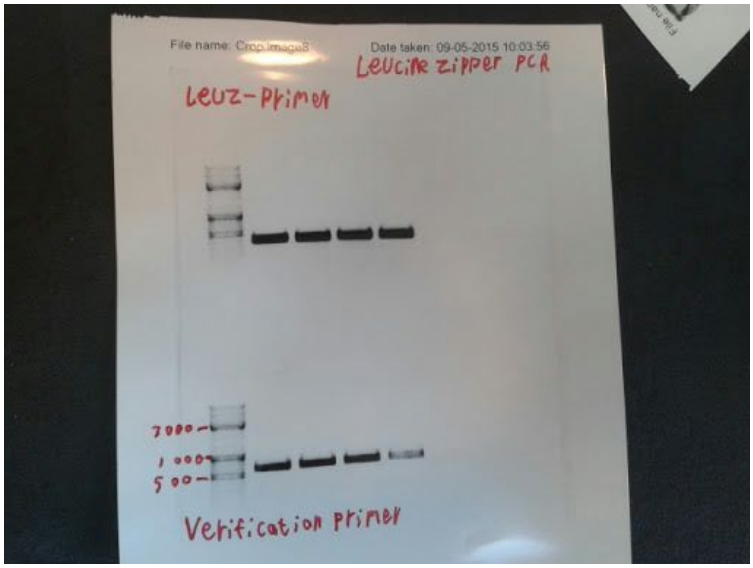
6. Results

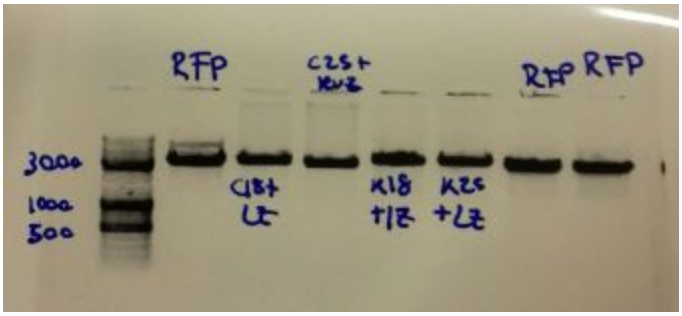
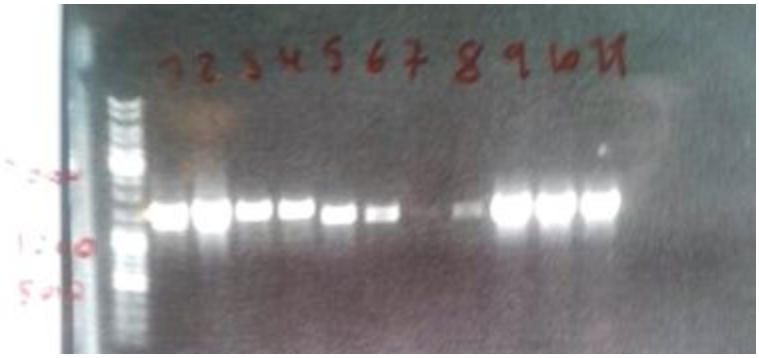
Date (YY.MM. DD)	**Picture**	**Comments**

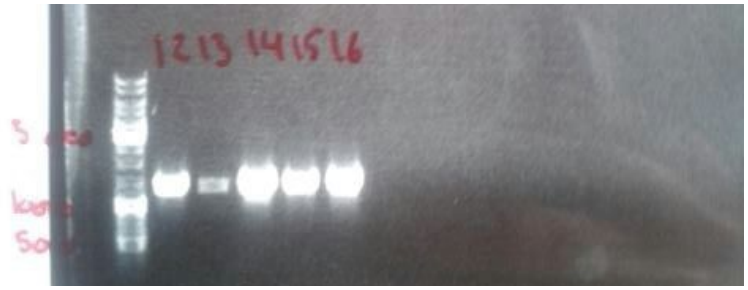
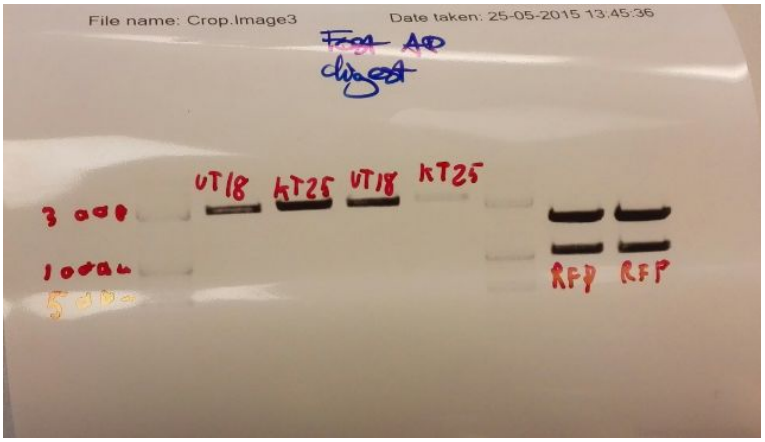
15.03.31		Results were unclear, PCR was therefore rerun. Though product was seen for UT18C at 59.1 ^{0}C and for KT25 at 63.9 ^{0}C. PCR unsuccessful and rerun
15.03.31		
15.04.01		Miniprep DNA/gel band concentration found using nanodrop: UT18C= Gel purification performed for PCR product only pKT25. Unsuccessful.
15.04.01		

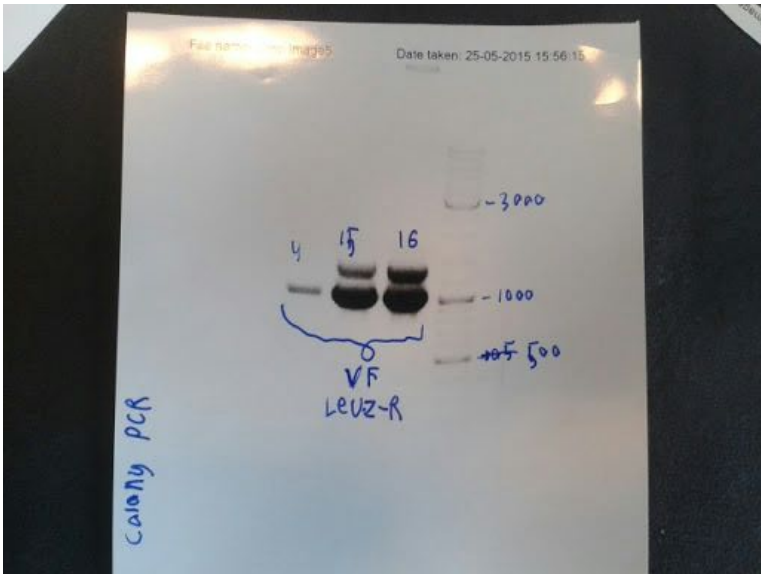
15.04.02		Gel purification KT25, successful for DMSO 1.5 at all temperatures, cut.
15.04.04		Miniprep DNA/gel band concentration found using nanodrop: KT25= 36.6^{0}C
15.04.04		- 1-4: 30^{y}Cml, PLAS:Y1, INS Y3, Conc 50 - 5-8: 30^{y}Cml, PLAS:Y1, INS Y3, Conc 20 - 9-12: 30^{y}Cml, PLAS:Y1, INS Y2, Conc 20 - 13-16: 30^{y}Cml, PLAS:Y1, INS Y2, Conc 50 - 17-18: 30^{y}Kan, PLAS:Y4, INS Y3, Conc 50 - 19-20: 30^{y}Kan, PLAS:Y4, INS Y2, Conc 20

		- 21-22: 30ɣKan, PLAS:Y4, INS Y3, Conc 20 - 23-24: 30ɣKan, PLAS:Y4, INS Y2, Conc 50 - 25 (0-sampl): 30ɣCml, PLAS:Y1, Conc 0 - 26 (0-sample, white): 30ɣKan, PLAS:Y4, Conc 0 - 27 (0-sample, red): 30ɣKan, PLAS:Y4, Conc 0
15.04.11	gel purifictaion of Fast Digest: 	plasmids successfully cut. last two wells are fast digest of linker-GFP, unsuccessful (see linker-GFP protocol)
15.04-11		Plasmids successfully cut

	RESULTATER MANGLER!!!	
15-05-08		Colony PCR confirmed
15.05.09		Every temperature 58 through 62 were succescull
15.05.12		Fast digestion of leucine Zipper: We purified the sample and got the concentration 5,9ng/µl.

15.05.13		We have colonies on the plates
15.05.14		Wrong primers were used in 1st colony PCR. Gel electrophoresis showed only primers. In 2nd colony PCR right primers were used. Gel electrophoresis gave the right bond lenghts (see picture). (approx. 1200 nt (+-))
15.05.16		Succsful, Fast digest. Make gel purifaction.
15-05-18		Cultures visible on petri dishes.
15-05-19		The inserts was expected to be approx. 2100 nt. As the bonds is only around 1200-1500 nt., it doesn't seem like ligation was executed properly. The colonies was red though, which we wouldn't have expected, if the ligation wasn't executed properly.

		
15.05.22		
15-05-22		Cut plasmid in gel ready for purification
15-05-22		Results need verification
15-05-25		Fast digest

		
15-05-26		
15-05-27		

7. Appendices