Chalmers University of Technology

Lab journal

A documentation of the work in the lab

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Supervisors:
Verena Siewers
Ivan Mijakovic
David Bergenholm
Raphael Ferreira

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Contents

1 Week 25

1.1 Tuesday 20/6

- Amplification of Olfr1258, RatI7 and STE2/STE3-gRNA constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 52 °C for the GPCRs and 65 °C for the gRNA.
  - ☐ Verification by gel electrophoresis
  - ☐ **RESULT:** The PCR worked and all bands were shown on the gel.
- Overnight culture of *E.coli* for amplification of the p413, p416 and Cas9 vector
  - ☐ preparation of LB+Ampicillin medium
  - ☐ Inoculation of *E.coli*
- DNA extraction of Olfr1258, RatI7 and STE2/gRNA constructs
  - ☐ Gel extraction of Olfr1258 and RatI7 using Thermo Scientific GeneJET Gel Extraction Kit
  - ☐ Purification of STE2/gRNA using Thermo Scientific GeneJET PCR Purification Kit
  - ☐ Concentration measurement using NanoDrop
  - ☐ **RESULT:** The final concentration of the constructs was quite low and the PCR should be repeated.

1.2 Wednesday 21/6

- Plasmid purification of p413, p416 and Cas9-vector using GeneJet plasmid miniprep kit
- Amplification of Olfr1258 and RatI7 constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 55 °C
- Digestion of Cas9 vector using FastDigest restriction enzymes

1.3 Thursday 22/6

- Amplification of Olfr1258 and RatI7 constructs, cont.
  - ☐ Verification by gel electrophoresis
  - ☐ Purification using Thermo Scientific GeneJET PCR Purification Kit
  - ☐ Concentration measurement using NanoDrop
  - ☐ **RESULT:** The PCR worked and all bands were shown on the gel. The concentrations for the Olfr1258 constructs were quite low and the PCR should be repeated.

- DNA fusion of STE2/STE3-gRNA constructs and Cas9 vector using Gibson Assembly® Master Mix
- Transformation of Gibson product in *E.coli* using Heat shock

1.4 Friday 23/6

- Take the transformed *E.coli* cells and put into the fridge (done by Raphael, supervisor)
- **RESULT:** The *E.coli* transformation was successful and approximately 40 colonies were shown on each plate.

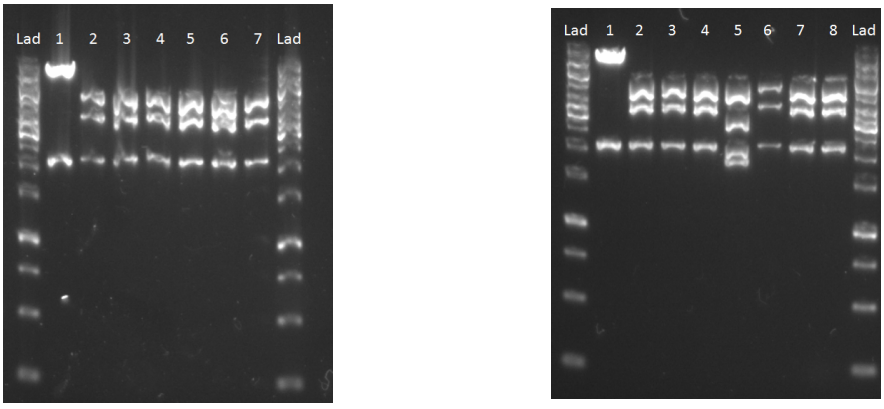
1.5 Sunday 25/6

- Inoculation of the transformed *E.coli* cells (done by Raphael, supervisor)

2 Week 26

2.1 Monday 26/6

- Verification of Gibson product (Cas9-STE2 and Cas9-STE3 vector)
  - ☐ Purification by Thermo Scientific GeneJET Plasmid Miniprep Kit
  - ☐ Concentration measurement using NanoDrop
  - ☐ Restriction analysis and Gel electrophoresis
  - ☐ **RESULT:** The concentration of the plasmids were sufficiently high and the result from the restriction analysis showed that all colonies except one had incorparated the plasmid correctly. The result from the restriction analysis is shown in Figure 1. The best samples were chosen to send in for sequencing.



(a) Cas9-STE2 vector (b) Cas9-STE3 vector

Figure 1: Result from restriction analysis of Cas9-STE2 and Cas9-STE3 vectors visualized with gel electrophoresis

- Yeast transformation of STE2-gRNA, STE3-gRNA and pCas9 with gRNA
  - ☐ Preparation of medium
  - ☐ Inoculation of the yeast cultures 11c and 61a respectively
- Amplification of Cre-Cas9 constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 60 °C.
  - ☐ Verification of PCR product using gel electrophorersis
  - ☐ Purification of PCR product using Thermo Scientific GeneJET PCR Purification Kit
  - ☐ Concentration measurement using NanoDrop
  - ☐ **RESULT:** Four of seven PCR products were shown on the gel. Those who worked were purified but only two of those gave sufficient DNA concentrations. Five out of seven constructs must therefore be amplified again.
- Water evaporation of GPCR constructs to increase the concentration

2.2 Tuesday 27/6

- Verification of Gibson product (Cas9-STE2-STE3 vector)
  - ☐ Sent for sequencing
- Yeast transformation of STE2gRNA, STE3gRNA and pCas9 with gRNA
  - ☐ Yeast transformation using LiAc protocol
- Purification of PCR fragments Cre-Cas9
  - ☐ Verification by Gel electrophoresis
  - ☐ Purification of some bands by Thermo Scientific GeneJET Gel Extraction Kit
  - ☐ Purification of som bands by Thermo Scientific GeneJET PCR Purification Kit
  - ☐ Concentration measurement using NanoDrop
  - ☐ **RESULT:** Three of five PCR products were shown on the gel. The one that was purified by Gel extraction had a very low concentration and was discarded. The two that was purified by PCR purification showed sufficiently high concentrations. Three of five constructs must therefore be amplified again.
- Amplification of Cre-Cas9 constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 60 °C.
  - ☐ Verification by Gel electrophoresis
  - ☐ Purification of some bands by Thermo Scientific GeneJET Gel Extraction Kit

- ☐ Concentration measurement using NanoDrop
- ☐ **RESULT:** Two of three PCR products were shown on the gel. Those two who was purified by gel extraction gave good concentrations.

■ Amplification of Cre-gRNA constructs

- ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 60 °C.
- ☐ Verification by Gel electrophoresis
- ☐ **RESULT:** Only one PCR product was shown on the gel and after purification the DNA concentration was too low. All constructs will be amplified again.

■ Amplification of Cre-gRNA constructs and one of Cre-Cas9 construct

- ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 60 °C
- ☐ Verification using Gel electrophoresis
- ☐ Purification of gRNA fragments using Thermo Scientific GeneJET PCR Purification Kit
- ☐ Concentration measurement using NanoDrop
- ☐ **RESULT:** Two of four PCR products was shown on the gel but gave low concentrations after purification. The amplification will be repeated.

■ Fusion PCR of Cre-Cas9 constructs

- ☐ Fusion PCR using Fusion PCR protocol
- ☐ Verification by Gel electrophoresis
- ☐ Purification of some constructs using Thermo Scientific GeneJET PCR Purification Kit
- ☐ Purification of some constructs using Thermo Scientific GeneJET Gel Extraction Kit
- ☐ Concentration measurement using NanoDrop

2.3 Wednesday 28/6

■ Fusion PCR of Cre-Cas9 constructs

- ☐ Fusion PCR round 1
- ☐ Verification using Gel electrophoresis
- ☐ Fusion PCR round 2

■ Amplification of Cre-gRNA constructs

- ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 55 °C

- ☐ Verification by Gel electrophoresis
- ☐ Purification of some constructs using Thermo Scientific GeneJET PCR Purification Kit
- ☐ Concentration measurement using NanoDrop
- ☐ **RESULT:** One of two PCR products was shown on the gel. The one that worked was purified and a sufficiently high concentration was obtained. The constructs that gave no band was amplified again.

■ Amplification of Cre-gRNA constructs

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 60 °C.
- ☐ Verification by Gel electrophoresis
- ☐ **RESULT:** Three of four PCR constructs was shown on the gel, as can be seen in Figure 2. These will be purified with Thermo Scientific GeneJET PCR Purification Kit.

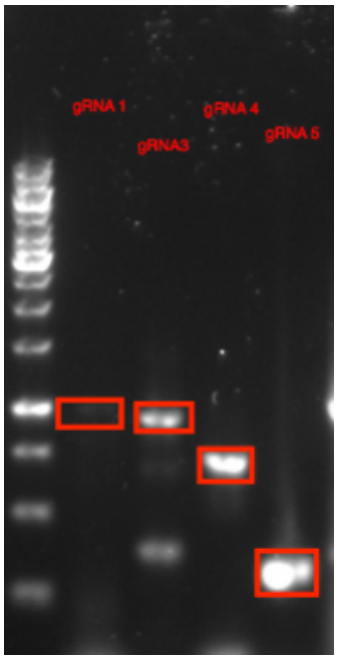


Figure 2: Result of PCR amplification of Cre-gRNA constructs visualized using gel electrophoresis

2.4 Thursday 28/6

■ Amplification of Cre-gRNA and Cas9 constructs

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 55 °C
- ☐ Verification by Gel electrophoresis
- ☐ **RESULT:** Only one of the PCR products gave a good band after gel electrophoresis and was purified. The rest was amplified again.

- Amplification of Cre-gRNA constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 58 °C.
  - ☐ Verification by Gel electrophoresis
  - ☐ **RESULT:** One of three PCR products showed a band on the gel.
- Fusion PCR of Cre-Cas9 constructs
  - ☐ Verification by Gel electrophoresis
- Assembly of Cas9 construct and p416 vector using Gibson Assembly® Master Mix
- Amplification of GPCR constructs
  - ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA Polymerase. Annealing temperature 52 °C.
- Yeast transformation of STE2gRNA, STE3gRNA and Raphaels Cas9 plasmid with gRNA, again
  - ☐ Inoculation of the yeast cultures 11c and 61a respectively

2.5 Friday 30/6

- Amplification of Cre-gRNA constructs, continued.
  - ☐ Purification using Thermo Scientific GeneJET Gel Extraction Kit
  - ☐ **RESULT:** The DNA concentration was quite low but since this is the first the construct has been successfully amplified and purified it was stored for further use.
- Amplification of GPCR constructs, continued
  - ☐ Verification by Gel electrophoresis
- Amplification of GPCR constructs again, toghether with a gRNA construct
  - ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 52 °C.
  - ☐ Verification by Gel electrophoresis
- Yeast transformation of STE2gRNA and STE3gRNA, and pCas9 with gRNA, continued
  - ☐ Yeast transformation using LiAc protocol
- Touchdown PCR of a gRNA construct
  - ☐ PCR using Touchdown protocol and Thermo Scientific Phusion High-Fidelity DNA Polymerase

3 Week 27

3.1 Monday 3/7

- Amplification of gRNA constructs 1 and 3
  - PCR using Thermo Scientific Phusion High-Fidelity DNA polymerase with touchdown protocol. For gRNA construct 1 the first annealing temperature was 74 °C and the second 64 °C. For gRNA construct 3 the first annealing temperature was 67 °C and the second 57 °C. as first annealing temperature.
  - Verification using gel electrophoresis.
  - **RESULT:** gRNA consturct 1 showed no bands and gRNA construct 3 showed three wrong bands. The PCR failed and should be repeated.
- Amplification of gRNA constructs 4.5 with and without DMSO, continued.
  - Verification using gel electrophoresis.
  - **RESULT:** Both worked but the sample with DMSO had higher concentration. Both were stored for later use.
- Digestion of p413 vector.
  - Digestion using EcoRI and BamHI.
  - Gel extraction of product using Thermo Scientific GeneJET Gel Extraction Kit.
  - Concentration measurement using NanoDrop.
  - **RESULT:** Low concentration of the product. Stored for later use.

3.2 Tuesday 4/7

- Amplification of GPCRs.
  - PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 52 °C.
  - Verification using gel electrophoresis.
  - Purification of all constructs using Thermo Scientific GeneJET PCR Purification Kit.
  - Concentration measurement using NanoDrop.
  - **RESULT:** Correct product but low concentration. Stored for later use.

3.3 Wednesday 5/7

- Amplification of gRNA construct 1.
  - PCR using Thermo Scientific Phusion High-Fidelity DNA polymerase with touchdown protocol. DMSO was used in the mixture.

- ☐ Verification using gel electrophoresis.
- ☐ **RESULT:** The gel electrophoresis showed no bands.

■ Amplification of GPCRs.

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 55 °C.
- ☐ Verification using gel electrophoresis.
- ☐ Purification of all constructs using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Concentration measurement using NanoDrop.
- ☐ Fusion PCR was performed to fuse together the RatI7 and Olfr 1258 respectively.
- ☐ Verification using gel electrophorsis.
- ☐ **RESULT:** The PCR products were correct and had high concentrations. The fusion PCR did not work.

■ Yeast transformation of GPCRs and markers for the mating test

- ☐ Overnight cultures of 11C and 61A was prepared.
- ☐ TE-buffer was prepared.

3.4 Thursday 6/7

■ Amplification of gRNA construct 1.

- ☐ PCR using Thermo Scientific Phusion High-Fidelity DNA polymerase with gradient. Mixtures was prepared with and without DMSO. Annealing temperatures were 65 °C, 64.4 °C, 62.6 °C and 61.3 °C.
- ☐ Verification using gel electrophoresis.
- ☐ **RESULT:** The gel electrophoresis showed no good bands, smeared bands for some samples.

■ Gibson and E. coli transformation of Cas9 constructs.

- ☐ Two mixtures of the Cas9 constructs were prepared. One that was evaporated and one that was not.
- ☐ The mixtures were transformed into *E. coli*.
- ☐ The cells were plated.
- ☐ **RESULT:** There were no colonies on the plates, probably the gibson assembly did not work.

■ Amplification of gRNA construct 1.

- ☐ PCR using Thermo Scientific DreamTaq DNA Polymerase. Annealing temperature was 55 °C.

- ☐ Verification using gel electrophoresis.
- ☐ **RESULT:** The gel electrophoresis showed no bands.

■ Amplification of RatI7 construct 2.

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 55 °C.
- ☐ Verification using gel electrophoresis.
- ☐ Purification of all constructs using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** The PCR products were correct but had low concentrations.

■ Yeast transformation of GPCRs and markers for the mating test, continued.

- ☐ Transformation of GPCRs using electroporation.
- ☐ Transformation of URA and HIS markers for the mating test using chemical transformation.

3.5 Friday 7/7

■ Amplification of gRNA construct 1.

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase.
- ☐ Verification using gel electrophoresis.
- ☐ **RESULT:** The gel electrophoresis showed no bands.
- ☐ PCR with gradient using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature between .
- ☐ Verification using gel electrophoresis.
- ☐ Purification of all constructs using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** The PCR products were correct and had good concentrations. The smeary bands from erlier was probably due to too high template concentration.

■ Amplification of all gRNA and Cas9 constructs.

- ☐ PCR using Takara PrimeSTAR HS DNA Polymerase. Annealing temperature 55 °C.
- ☐ Verification using gel electrophoresis.
- ☐ Purification of some products using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Gel extraction of some products using Thermo Scientific GeneJET Gel Extraction Kit.

- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** Varying results for different constructs. Cas9 construct 5 and gRNA constructs 2 and five had high concentrations.

3.6 Saturday 8/7

■ Yeast transformation of GPCRs and markers for the mating test, continued.

- ☐ 28 single colonines for each GPCR transformation was plated on new plates.
- ☐ **RESULT:** p416 seems to have been transformed correclty into 61A. 413 into 11C do not seem to have worked and will probably have to be redone.

4 Week 28

4.1 Monday 10/7

■ Amplification of Cas9 construct 1, 2, 3, 4, 4.5 and gRNA construct 4.

- ☐ PCR using PrimeSTAR polymerase with an annealing temperature of 65 °C for all constructs.
- ☐ Verification using gel electrophoresis.
- ☐ **RESULT:** Cas9 constructs 1, 4.5 and gRNA construct 4 produced correct bands, however all of them were weak.
- ☐ Purification of all constructs using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** Correct product but very low concentration. Samples were discarded.

■ Gel purification of Cas9 construct M and gRNA construct 3.

- ☐ Verification by Gel electrophoresis.
- ☐ Purification of correct bands using Thermo Scientific GeneJET Gel Extraction Kit.
- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** Both constructs gave high concentrations after purification.

■ Gel purification of Cas9 constructs 1, 2, 3, 4, 4.5 and gRNA construct 4.

- ☐ Verification by Gel electrophoresis.
- ☐ Purification of correct bands using Thermo Scientific GeneJET Gel Extraction Kit.
- ☐ **RESULT:** All constructs gave reasonably high concentrations except for 2 and 4.5.

■ Colony PCR of CENPK111-61A transformed with Olfr1258 and CENPK11-11C transformed with Ri7.

- ☐ DNA from the cells was extracted using the colony PCR protocol.
- ☐ PCR using DreamTaq and Ta=53 °C
- ☐ Verification by Gel electrophoresis.
- ☐ **RESULT:** All colonies produced the wrong bands.

■ Preparations of CENPK11-11C overnight cultures for transformation of gRNA construct, Cas9 Construct the plasmid p413

4.2 Tuesday 11/7

■ Electroporation transformation of gRNA construct, Cas9 Construct the plasmid p413 into CENPK11-11C

- ☐ The gRNA/Cas9 construct pieces with the highest concentrations were chosen for the transformation and assembly in yeast.
- ☐ These construct pieces along with the plasmid p413 was transformed into yeast using the electroporation protocol.

■ Colony PCR of CENPK111-61A transformed with Olfr1258 and CENPK11-11C transformed with RI7.

- ☐ DNA from the cells was extracted using the colony PCR protocol.
- ☐ Verification by Gel electrophoresis.
- ☐ **RESULT:** One colony produced the correct band which means that we have a working CENPK11-11CSTE2::RI7 strain.

4.3 Wednesday 12/7

■ CENPK11-11CSTE2::RI7 preparations.

- ☐ Distinct colonies were picked and restreaked to 2 plates: one with kanamycin, where the cells need the pCas9 plasmid to grow and one without selection pressure, where all can grow. This will show which cells have gotten rid of the plasmid.
- ☐ CENPK11-11CSTE2::RI7 was also cryopreserved at −80 °C

■ Colony PCR of CENPK111-61A transformed with Olfr1258.

- ☐ DNA from the cells was extracted using the colony PCR protocol.
- ☐ Verification by Gel electrophoresis.
- ☐ **RESULT:** No colonies produced correct bands.

■ PCR of CENPK11-11CSTE2::RI7 to check for mutations.

- ☐ DNA from the cells was extracted using the colony PCR protocol.
- ☐ PCR using PrimeSTAR polymerase with an annealing temperature of 55 °C.

- ☐ Verification by Gel electrophoresis.
- ☐ **RESULT:** No bands. PCR was redone.
- ☐ A gradient PCR using PrimeSTAR polymerase with an annealing temperature of between 48 °C-58 °C was done in seven tubes.
- ☐ Verification by Gel electrophoresis.
- ☐ **RESULT:** Two tubes produced correct bands.
- ☐ Purification using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ **RESULT:** Both samples gave high concentrations after purification.
- ☐ One of the constructs was sent in for sequencing.

4.4 Thursday 13/7

- ■ Preparations for gRNA construct transformation into yeast.
  - ☐ The vector p413 was digested by EcoRI and BamHI for 5 minutes.
  - ☐ Purification using Thermo Scientific GeneJET PCR Purification Kit.
  - ☐ PCR using PrimeSTAR polymerase with an annealing temperature of 55 °C was done on fragments 1 and 2 of the Olfr1258 receptor.
  - ☐ Verification by Gel electrophoresis.
  - ☐ **RESULT:** All samples produced correct bands.
  - ☐ Purification using Thermo Scientific GeneJET PCR Purification Kit.
  - ☐ Preparations of CENPK11-11C and CENPK111-61A overnight cultures for transformation of gRNA construct.

4.5 Friday 14/7

- ■ Colony PCR of CENPK11-11C with Cas9 construct.
  - ☐ DNA from the cells was extracted using the colony PCR protocol. 14 colonies were tested.
  - ☐ PCR using PrimeSTAR polymerase with an annealing temperature of 55 °C. The PCR was done using the primers for piece 2 of the Cas9 construct.
  - ☐ Verification by Gel electrophoresis.
  - ☐ **RESULT:** 9 out of 14 colonies showed correct bands.
- ■ Digestion and purification of pSense0 plasmid
  - ☐ The pSense0 plasmid was digested for 15 minutes.
  - ☐ Verification by Gel electrophoresis.

- ☐ **RESULT:** All bands were correct.
- ☐ Concentration measurement using NanoDrop.
- ☐ **RESULT:** Low concentrations, so the samples were evaporated.

■ Electroporation transformation of gRNA construct and p413 into CENPK11-11C, and Olfr1258 into CENPK111-61A.

- ☐ These construct pieces along with the plasmid p413 was transformed into yeast using the electroporation protocol.

■ Colony PCR of CENPK11-11C with Cas9 construct.

- ☐ PCR using PrimeSTAR polymerase with an annealing temperature of 55 °C. The previous DNA samples from the 14 colonies were used. However, this time the PCR was done using the end primers of the entire Cas9 construct (1F and 5R).
- ☐ A gel to check the results will be done on monday.

5 Week 29

5.1 Monday 17/7

■ Gel verification of colony PCR of whole Cas9 construct.
RESULT: The PCR did not work, probably since the construct was too big.

■ Extraction from CENPK_11-11c_ΔSTE2 and transformation in *E.coli* of Cas9 construct
RESULT: No colonies survived.

■ GPCR sensitivity.

- ☐ PCR with primeSTAR and Ta=55°C and gel verification of pFUS1.
- ☐ Digestion of pFUS1 using RE DpnI for 10 min, 37°C, followed by purification.
- ☐ PCR purification of cut pSense0 vector.
RESULT: The concentration was too low, new digestion needed.
- ☐ New digestion of pSense0 vector, using RE SmaI and SacI, and gel purification.
- ☐ Transformation of pSense0 vector in *E. coli*.

■ Preparation for Colony PCR of CENPK_111-61A transformed with Olfr1258 by restreaking 28 colonies each on two new plates.

■ Preparation for mating test by preparing an overnight culture in YPD of CENPK_11-11C.

■ Restreaking of 10 colonies of gRNA-transformed CENPK_11-11c_ΔSTE2 on new SD-His plates.

5.2 Tuesday 18/7

■ Transformation of Cas9 construct in *E.coli*.

- Transformation of p413 GDP plasmid in CENPK_11-11C using electroporation.
- Colony PCR of CENPK_111-61A transformed with Olfr1258.
  - ☐ PCR 1 using DreamTaq and Ta=53°C.
    RESULT: Most fragments not correct length but for some colonies, the PCR did not work at all. These should be done again.
  - ☐ PCR 2 of specific colonies using DreamTaq and Ta=53°C.
    RESULT: No PCR result. Might need to extract more DNA. New PCR done on Wednesday.
- Assembly of digested pSense0 and pFUS1 promoter.
  - ☐ Gibson assembly using 3:1 ration vector and insert with 50ng vector and 150ng insert. Incubation 1h, 50°C.
  - ☐ Transformation in *E.coli*.
- Preparation of overnight culture of CENPK_11-11C_ ΔSTE2::RatI7 for cryopreservation.
- Preparation of overnight culture of *E.coli* transformed with pSense0 with native promoter for extraction of plasmid tomorrow.

5.3 Wednesday 19/7

- Troubleshooting of gRNA construct since there has been trouble with this construct.
  - ☐ Doublechecking band sizes for fragments used earlier.
  - ☐ Digestion of p413TEF using RE EcoRI and BamHI.
    RESULT: It was realized that this vector could not work and RE were wrong and digestion was stopped.
- Inoculation of *E.coli* transformed with Cas9 construct for extraction of construct tomorrow.
- Plasmid MiniPrep to extract pSense0 from *E.coli*.
- New colony PCR of CENPK_111-61A transformed with Olfr1258 using the colonies that showed no result last time. A gradient PCR using PrimeSTAR and 2μl DNA template.
  RESULT: The gel electrophoresis showed no bands.
- Inoculation of transformed *E.coli* for extraction of pSense0+pFUS1 vector tomorrow.
- Cryopresevation of CENPK_11-11C_ΔSTE2::RatI7.

5.4 Thursday 20/7

- Extraction of pSense+pFUS1 vector.
  - ☐ Miniprep on *E.coli* to extract vector.

- ☐ Digestion of pSens0+pFUS1, using RE PstI to verify correct assembly of vector and insert. Incubation 5min, 37°C.
  RESULT: The gel electrophoresis, Fig 3 showed the correct bands. The plasmid can now be sent for sequencing.

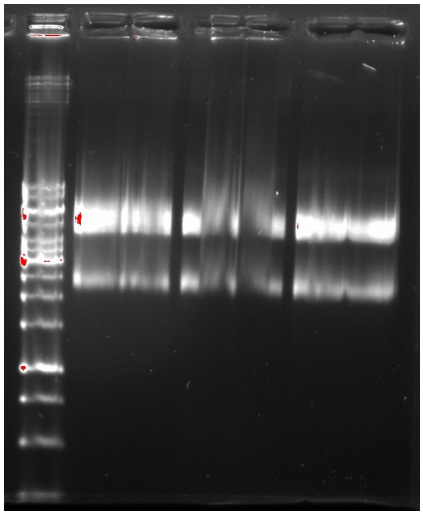


Figure 3: The gel electrophoresis show two bands which verify the correct assembly of vector and insert.

- ■ Overnight culture of CENPK_11-11C_ ΔSTE2::RatI7 for transformation of pSense0+pFUS1 and Cas9 construct tomorrow.
- ■ Fusion PCR with Olfr1258 fragments using PrimeSTAR and Ta=56°C.
  RESULT: Gel electrophoresis confirmed that the fusion PCR did not work. Redo PCR with the individual fragments.
- ■ Overnight PCR of individual Olfr1258 fragments using PrimeSTAR and Ta=55°C.
- ■ Extraction, digestion sequencing of Cas9 construct
  - ☐ Extraction of Cas9 construct from *E.coli.*
  - ☐ Digestion of plasmids using RE KpnI and XbaI, 5 min, 37°C for verification.
    RESULT: Almost all samples were correct. Three samples were picked to send for sequencing.
- ■ Amplification of ADE2gRNA constructs
  - ☐ Dilution of primers and g-blocks before use.
  - ☐ PCR using phusion polymerase and Ta=61°C.
    RESULT: Gel electrophoresis, Fig 4, showed correct sizes for three out of four samples.

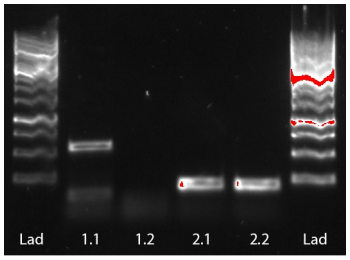


Figure 4: Fragments 1.1, 2.1 and 2.2 show the correct length which is 544 bp for fragment 1 and 197 bp for fragment 2. Fragment 1.2 show no bands at all.

- Mating test of CENPK_11-11C transformed with plasmid with HIS marker and CENPK_111-61A transformed with plasmid with URA marker. Both strains are plated on SD HIS URA plate.

5.5 Friday21/7

- Chemical transformation of pSense0+pFUS1 and Cas9 construct in CENPK_11-11C_ΔSTE2::RatI7 using 100ng DNA of pSense0+pFUS1.
- Assembly of Olfr1258 fragments, O1 and O2.
  - ☐ Gel electrophoresis of overnight PCR of Olfr1258 individual fragments from yesterday.
    RESULT: Smeary bands but correct size was obtained.
  - ☐ Gel purification of individual fragments O1 and O2.
    RESULT: The concentrations were very low; O1= 5,7ng/L, O2=46,8ng/L.
  - ☐ Gradient PCR of fragments O1 and O2 overnight using primeSTAR and Ta 51°C; 52°C; 53,2°C; 54,2°C; 55°C.
- GPCR-GFP fusion proteins
  - ☐ Dilution of primers for GPCR-GFP fusion proteins.
  - ☐ PCR using Phusion polymerase.
    RESULT: Four out of six fragments had correct lengths. The other two fragments look like they could have been mixed up.
  - ☐ PCR purification of the four samples that had correct lengths.
  - ☐ Digestion of the two potentially mixed up samples.
    RESULT: Gel electrophoresis showeid that the two samples were mixed up and hence the results from the PCR were correct.
- Assembly and transformation of ADE2gRNA in *E.coli*.
  - ☐ Digestion of Cas9 vector using BsiWI/Pfl2311, Incubated in 15 min at 37°C and inactivated in 5 min at 65°C. PCR purification.

- ☐ Gibson assembly of ADE2gRNA and digested pCas9 vector, using 3:1 ration insert and vector, 1h at 50°C.
- ☐ Transformation of ADE2gRNA in *E.coli*.

6 Week 30

6.1 Monday 24/7

- ■ Verification of mating test and transformations using microscopy
  - ☐ Checking mating test for CENPK_11-11C transformed with CENPK_111-61A in microscope.

    RESULT: Mating test worked.
  - ☐ Checking CENPK_11-11C with Cas9 vector in fluorescence microscope.

    RESULT: Transformation worked.
  - ☐ Checking CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct in microscope.

    RESULT: Contaminated.
- ■ Transformation of pSense0+P_{FUS1} in CENPK_11-11C_ ΔSTE2::RatI7 using electroporation.
- ■ Transformation of Cas9 vector in CENPK_11-11C_ ΔSTE2::RatI7 using electroporation.
- ■ Gibson Assembley of GPCR-GFP fusion proteins using p413TEF vector
  - ☐ Digestion of p413TEF vector using Xho1 and Xba1
  - ☐ Gel extraction of digested p413TEF vector using Thermo Scientific GeneJET Gel Extraction Kit.
  - ☐ Gibson assembley of RatI7-GFP fragment and p413TEF. Incubation 1h, 50 °C.
  - ☐ Gibson assembley of Olfr1258-GFP fragment and p413TEF. Incubation 1h, 50 °C.
  - ☐ Transformation of RatI7-GFP fusion vector in *E. coli*.
  - ☐ Transformation of Olfr1258-GFP fusion vector in *E. coli*.
- ■ Flow cytometry for CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct.
  - ☐ Preparing Delft+HIS media.
- ■ ADE2 deletion test
  - ☐ Six colonies containing Cas9 vector with ADE2-gRNA construct was inoculated in LB Amp.
- ■ Verification of PCR gradient for O1 and O2 fragments
  - ☐ Gel electrophoresis to verify that the fragments hade correct size.

- ☐ Purification of O1 and O2 fragments using Thermo Scientific GeneJET PCR Purification Kit.

■ Gibson Assembley of Olfr1258 fragments, O1 and O2, using 500 ng of each fragment. Incubation 1h, 50 °C.

- ☐ Gel extraction of the Gibson product using Thermo Fisher GeneJET Gel Extraction Kit.

■ PCR gradient from 50-60 °C of Gibson product using PrimeStar HS DNA Polymerase. 8 samples. Extension time 1 min, 30 cycles.

- ☐ Gel elextrophoresis on PCR product. No bands, therefore not successful.

■ Over night culture of CENPK_11-11C for transformation of Cre-gRNA construct.

6.2 Tuesday 25/7

■ ADE2 deletion test

- ☐ Cas9 vector with ADE2-gRNA construct was purified using Thermo Scientific GeneJET Plasmid Miniprep Kit.
- ☐ Digestion to verify that Cas9 vector with ADE2-gRNA construct was correct using Xba1.

  RESULT: Gel electrophoresis showed three band instead of two.
- ☐ Digestion to verify that Cas9 vector with ADE2-gRNA construct was correct using Nhe1 and Nco1.

  RESULT: Gel electrophoresis showed three band instead of two.

■ Gibson assembley of ADE2-gRNA construct and Cas9 vector.

- ☐ Digestion of Cas9 vector using BsiWI/Pfl2311, Incubated in 15 min at 37°C and inactivated in 5 min at 65°C.
- ☐ PCR purification on digested vector using Thermo Scientific GeneJET PCR Purification Kit.
- ☐ Gibson assembly of ADE2-gRNA and digested pCas9 vector, using 3:1 ration insert and vector, 1h at 50°C.
- ☐ Transformation of ADE2-gRNA Gibson product in *E.coli*.
- ☐ Electroporation of ADE2-gRNA Gibson product in CENPK_11-11C

■ Gibson Assembley of Olfr1258 fragments, O1 and O2, using 500 ng of each fragment. Incubation 1h, 50 °C.

■ PCR of Gibson Olfr1258 product.

- ☐ PCR gradient from 45-55 °C using Phusion High-Fidelty DNA Polymerase. 7 samples. Extension time 30 s.

  RESULT: Gel eletrophiresis of PCR product showed no bands.

- ☐ PCR gradient from 47-57 °C on Gibson product using PrimeStar HS DNA Polymerase. 7 samples. Extension time 1 min.

  RESULT: Gel electrophoresis on PCR product. Sample 5, 6 and 7 was successful.

- ☐ Gel extraction for PCR PrimeStar product 5, 6 and 7 using Thermo Scientific GeneJET Gel Extraction Kit.

■ Transformation of p413TEF (HIS marker) in CENPK_111-61A using "Quick and dirty" method.

■ Transformation of Cre-gRNA construct and p416TEF vector in CENPK_11-11C.

- ☐ Digestion of p416TEF using Sac1 and Kpn1.
- ☐ Gel extraction of p413TEF using Thermo Scientific GeneJET Gel Extraction Kit.
- ☐ Electroporation using 200 ng vector and 200 ng of each Cre-gRNa fragment.

■ Transformation of ADE2-gRNA Gibson product in CENPK_11-11C using electroporation.

■ Inoculation of RatI7-GFP fusion vector and Olfr1258-GFP fusion vector.

■ Over night cultures of CENPK_11-11C and CENPK_111-61A

6.3 Wednesday 26/7

■ Colony PCR of Cas9 vector with ADE2-gRNA fragment in *E. coli*.

RESULT: Two colonies was correct.

■ Inoculation of Cas9 vector with ADE2-gRNA fragment.

■ Verification of RatI7-GFP fusion vector and Olfr1258-GFP fusion vector.

- ☐ Miniprep using Thermo Scientific GeneJET Plasmid Miniprep Kit.
- ☐ Restriction analysis using NdeI.
- ☐ Four colonies sent for sequencing.

■ Transformation of RatI7-GFP fusion vector and Olfr1258-GFP fusion vector in CENPK_11-11C using electroporation.

■ Preparing Delft + URA media

■ Over night culture of CENPK_11-11C_ ΔSTE2::RatI7 with pSense0+P_{FUS1}.

■ Over night culture of CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct.

■ PCR of Olfr1258 fragment using PrimeStar HS DNA Polymerase. 47 °C. Extension time 1 min.

■ Over night culture of CENPK_111-61A.

6.4 Thursday 27/7

- Verification of cas9 vector woth ADE2-gRNA fragment.
  - ☐ Miniprep using Thermo Scientific GeneJET Plasmid Miniprep Kit.
  - ☐ Restriction analysis with NheI and EcoRI. **RESULT:** Two vectors were correct.
  - ☐ Send vector for sequencing
- Diluton of Octanal in DMSO to 1M, 1mM, 1μM and 1nM.
- Flow cytometry of CENPK_11-11C_ ΔSTE2::RatI7 with pSense0+P$_{FUS1}$.
  - ☐ Diluting OD to 0.1 in 10 ml Delft+URA media.
  - ☐ Induce with 1mM, 1μM and 1 nM Octanal. Incubate in shake incubator, 1 h in 30 °C.
  - ☐ Preparing cells for flow cytometry. Diluted to 0.02 OD in 200 μl Delft+URA media.
  - ☐ Flow cytometry measurements.

    RESULT: No GFP expressed.
- Inducing LoxP-flip for CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct
  - ☐ Diluting OD to 0.1 in 10 ml Delft+HIS media.
  - ☐ Induce with 10 mM, 1 mM and 1μM Octanal. Incubate in shake incubator over night in 30 °C.
- Transformation of Cas9 vector with ADE2-gRNA construct in CENPK_111-61A using electroporation. **RESULT:** The cells did not turn red.
- Transformation of p413GDP in CENPK_111-61A using electroporation.
- Re-streaking CENPK_11-11C with Cre-gRNA construct colonies.

6.5 Friday 28/7

- Inducing LoxP-flip for CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct
  - ☐ Observing induced cells in fluoroscence microscope. **RESULT:** The flip did not seem to work using these concentrations of Octanal.
- Flow cytometry of CENPK_11-11C_ ΔSTE2::RatI7 with pSense0+P$_{FUS1}$.
  - ☐ Diluting OD to 0.1 in 10 ml Delft+URA media.
  - ☐ Induce with 1mM, 1μM and 1 nM Octanal. Incubate in shake incubator, 3 h in 30 °C.
  - ☐ Preparing cells for flow cytometry. Diluted to 0.02 OD in 200 μl Delft+URA media.
  - ☐ Flow cytometry measurements.

    RESULT: No GFP expressed.

- Lethality test for Octanal
  - ☐ Inducing CENPK_11-11C cells with 10 mM Octanal. Incubate in shake incubator, 30 °C.
  - ☐ Measure OD every 2 hours.
- Inducing LoxP-flip for CENPK_11-11C_ ΔSTE2::RatI7 with Cas9 construct.
  - ☐ Diluting OD to 0.1 in 10 ml Delft+HIS media.
  - ☐ Induce with 10 mM Octanal. Incubate in shake incubator, 30 °C.
  - ☐ OD measurements every 2 hours.
  - ☐ Observing induced cells in fluorescence microscope.

    RESULT: The flip did not seem to work.
- Colony PCR of CENPK_11-11C with Cre-gRNA construct.

7 Week 31

7.1 Monday 31/7

- Preparing a O/N culture for lethality test of VOCs
- Overnight culture of 61A, 11C and 11C with P*FUS1* pSense0.
- Dilution of butanone with DMSO
  - ☐ Three concentration was prepared 1M, 1mM and 1μM

Test of the flipping of P*TEF1* with Cre-recombinase

- Cells from 61A(with *HIS* and 11C+Cra-Cas9 was crossed on a plate, to achive natural phermonones through mating to ativate the *FUS1* and reading of Cre-recombinase.
- Verification of Cre-gRNA transformation
  - ☐ Due to the failure colony-PCR 28/9, the PCR was runned twice again.
    Results: Even these two failed, and due to that a last try to were made the day after to purify and transform in to *E. coli*.
- Doublecheck the Cre-gRNA construct
  - ☐ All parts of the Cre-gRNA construct was run on a gel, to juste verify that all parts looks good.
    Results: 4.5b didn't show any band, due to low concentration. Rest of the band shows there they are supose to be. Some of the parts seems to be a bit of smeared.

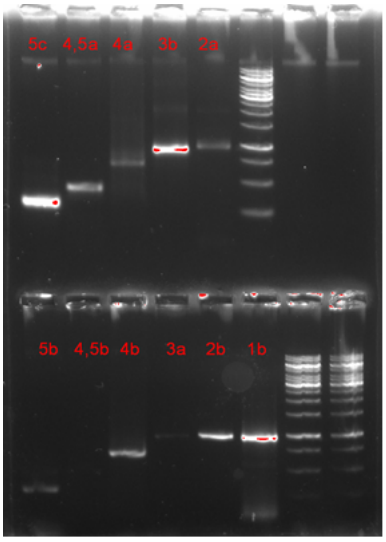


Figure 5: The number stands for which part it is in the construct, and the letter is an other copy of same part.

7.2 Tuesday 1/8

■ Lethality test VOCs

□ The final concentration VOCs in the flasks and the respective volumes added were:
10 mM - 100 μl VOC (1M)
1 mM - 10 μl VOC (1M)
100 μM - 1 ml VOC (1mM)
10 μM - 100 μl VOC (1mM)
1 μM - 10 μl VOC (1mM)
Two controls were prepared, one with 100 μM DMSO and one with only cells. OD measurements were performed at 12:00, 14:00 and 15:20.
Results: See table below

Conc.	OD 12:00		OD 14:00		OD 15:20	
	Butanone	Ocatanal	Butanone	Ocatanal	Butanone	Ocatanal
10 mM	0.202	0.162	0.394	0.166	0.542	0.166
1 mM	0.200	0.184	0.410	0.326	0.638	0.432
100 μM	0.136	0.144	0.172	0.180	0.184	0.196
10 μM	0.196	0.200	0.384	0.402	0.576	0.580
1 μM	0.196	0.206	0.412	0.414	0.617	0.592
Control with DMSO	0.162		0.218		0.236	
Control without DMSO	0.204		0.414		0.628	

■ Biolector

□ An assay with the RatI7 pSence0 + pFus culture with different concentrations of

octanal was performed in the biolector. The concentrations and the corresponding volume of octanal added were:

5mM - 5μl (1M)
1mM - 1μl (1M)
100 μM - 100 μl (1mM)
10 μM - 10μl (1mM)
1 μM - 1 μl (1mM)
500 μM - 0.5 μl (1M)
50 μM - 50 μl (1mM)
Control - 100 μl DMSO

- GPCR-GFP Microscope
  - The GPCR were checked under a flourecesnce microscope.
    Results: No GPF was observed for rat-GFP (perhaps because of the devation in the sequence). GFP was observed for the olf. But not concentrated in the membrane.
- PCR, for exchange gRNA in template STE3
  - A PCR with the new primers were done to exchange the old (not working) gRNA with a new gRNA.
- Verification of Cre-gRNA
  - Plasmid purification were done with the Zymoprep Yeast Plasmid Miniprep II protocol for the yeast cells which might contain the plasmid Cre-gRNA.
  - The purified plasmid was later transformed into *E. coli*.
    Results: No colonies was visible the next day. Due to this many failure we decided that the assembly didn't work. We will try another approach for the Cre-gRNA construct.
- Yeast transformation of the plasmid pCAS9-gRNA(ADE2)
  - Due to no red colonies was visible for the transformation last week, a new transformation was made. The transformation from last week were believed to have a a petite mutation according to microscopic picture. Both pCas9-gRNA(ADE2) plasmid were transformed this time.
    Results: After 1 week no red colonies were seen. The deliton of ADE2 seems not to work, perhaps something with the gRNA or the construct itself. A new approache was planned.

7.3 Wednesday 2/8

- Biolector continued
  - **Results:** The cells in the control had not grown, probably due to too high levels of DMSO. Therefore the control wells were emptied and filled with new controls without DMSO. Those had, as earlier, 0.05 OD and the volume 1ml.

■ Exchange of gRNA in template STE3

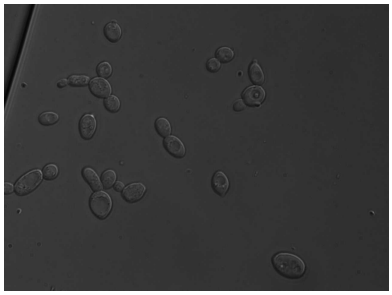
□ Gel electrophoresis was run for all PCR products, and purified.
Results: All temp succedded!
1.1 320 ng/μl
1.2 283 ng/μl
2.1 306 ng/μl
2.2 205 ng/μl

□ The PCR products was later assembly in to pCas9 by gibson.

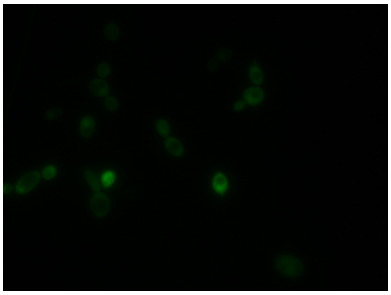
□ The gibson mix was later transformed into

■ Test of Cre-flip of the promotor *TEF1*

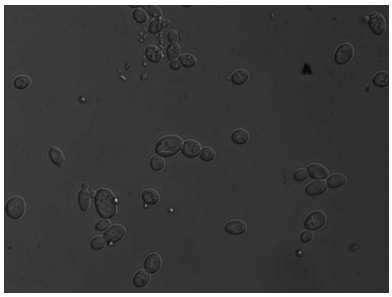
□ The colonies from the mating test in Mondays were watched under fluorescent microscopic, to see if the GFP expression hade decrease. **Results:** The fluorescence seems to have decrease/disappear in some of colonies according to the results. Out of 6 colonies, 3 seems to show a decrease in GFP expression.



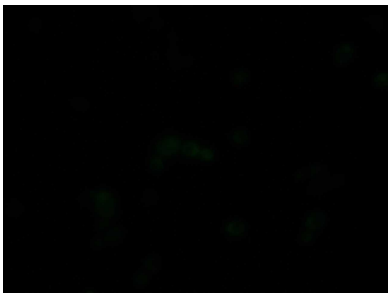
(a) Microcopic picture from one of the colonies.



(b) The fluorescent microscopic picture for the same colonies to the left.



(c) Microcopic picture from an other of the colonies.



(d) The fluorescent microscopic picture for the same colonies to the left.

7.4 Thursday 3/8

■ Exchange of gRNA in template STE3

□ Some colonies were seen from the *E. coli* transformation from the day before, and was inoculated over the night.

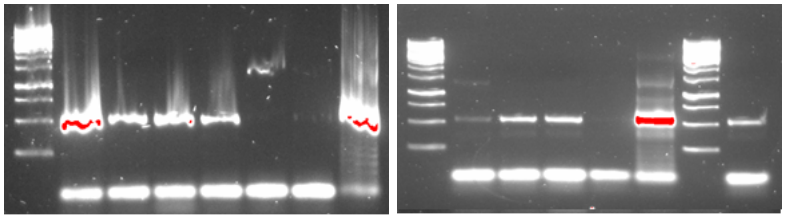
■ O/N culture of 61A

☐ A over night culture was prepared for 61A

■ Test of Cre-flip of the promotor *TEF1*

☐ The colonies were re-steaked out yesterday and were looked under microscope once again to make sure that the flip doen't flip back.
Results: The GFP expression showed a similare results as yesterday.

☐ To make sure on a sequence level of the construct that the flip worked, a colony-PCR were made.
Results: The results show that only colony 5 seems to have flipped. But due the bad/smeared band the colony-PCR was run again with lower concentration for the control (0,5 μL) and a higher temperature, 59.
The new result is shown to the right. It seems that the higher band for colony 5 is no longer visible. Might have beens some error, in further tries to accomplish the colony-PCR no band showed a success. And therefor a new approach was planned.



(a) First colony-PCR run. (b) Second colony-PCR run.

7.5 Friday 4/8

■ Exchange of gRNA in template STE3

☐ A mini-prep was done on the O/N culture from the *E. coli* transformation.
Results: See table below.

	STE3_1 [ng/μl]	STE3_2 [ng/μl]
1	366.9	421
2	140.8	439.9
3	321.4	392.4
4	425.5	473.6
5	458.1	426
6	381.8	418.4
7	419.3	393.3
8	378.8	411.4

☐ Restriction analysis was made on the plasmids, and cut with NheI and BamHI.
Results: The correct plasmids in the uppper run is 2, 6, 7, 1 and 2 and in the lower run, 1, 2, 3, 4, 5, 7 and 8.

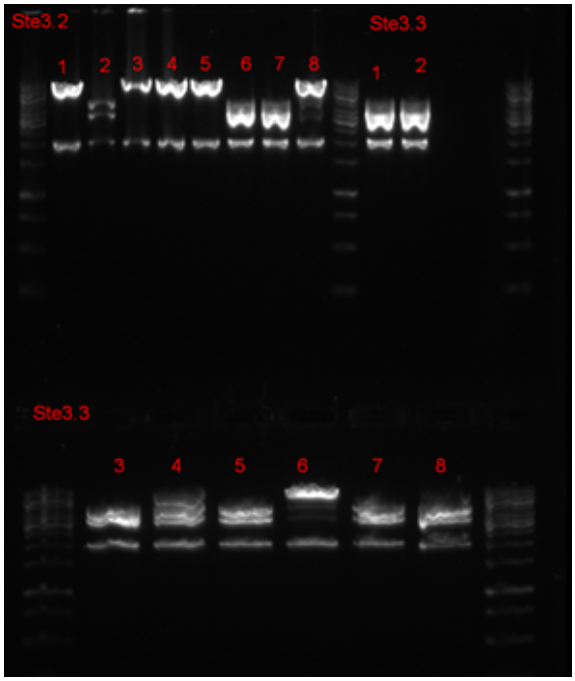


Figure 8: Gel electrophoresis for the restriction analysis.

- Transformation in 61A of Olfr1258, STE3_1 and STE3_2
  - ☐ Electroporation yeast transformation was used according to protocol.
- Test of Cre-flip of the promotor *TEF1*.
  - ☐ The colonies was watched a third time to make sure the flip doesn't flip back, and to see if it takes time for the GFP do degrade.
    Results: Just as the earlier tests, some colonies was still fluorescence and other not. And since the colony-PCR didn't give any clear results, no conclusion could be drawn.
  - ☐ Due to no clear conclusion can be drawn from the cre-flip test, a new mating tests were made, from diffrent colonies of 11C-Cre-Cas9.

8 Week 32

8.1 Monday 7/8

- Restreaction of colonies for colony PCR of transformed CENPK_111-61A with DNA for Olf1258
- Control of the flip by analyzing the mating between CENPK_11-11C strain with the Cas9 construct and the CENPK 111-61A strain
  - ☐ Colonies from plates Cas9_4, Cas9_5, Cas9_8, Cas9_9 and Cas9_10 restreaked on SD-URA-HIS plates

8.2 Tuesday 8/8

- Colony PCR of CENPK_111-61A transformed with Olf1258
  - Verification by using Dreamtaq PCR
  - **RESULT:** All visible bands had the lenght of approximatly 500 bp, where a successful transformation should show a band of approximately 1000 bp but an unsuccessful tranformation should give aband of approximately 700 bp. Some bands were missing; S2.2 : 1, 9; S2.6 : 1, 3, 6, 7; S3.4 : 4, 9. PCRs for these colonies will be remade!
- Fusion-PCR of Cre-gRNA for assembling of the Cre-gRNA constructs
  - Due to problems with assembling the Cre-gRNA construct fusion-PCR was tested for the six pieces. The pieces were put together two by two.
  - Verification with gel electrophoresis
  - **RESULT:** No sample showed any bands, there were only PCR mess at the end of the gel. Concluding that noone of the Fusion-PCRs had worked.
- Fluorescence microscopy of mating between CENPK_11-11C with Cas9-vector and CENPK_111-61A strain, restreaked on monday
  - Verification of activated flip where Cas9 should be expressed instead of GFP
  - **RESULT:** The cells of the colonies; Cas9_4, Cas9_10 and Cas9_9 did not show any green fluorescence and might show a working flip. To verify a working flip, colony-PCR had to be done.

8.3 Wednesday 9/8

- New PCR for colonies with missing bands from the colony PCR on tuesday
  - Verification by gel electrophoresis
  - **RESULT:** The gel showed no bands
- Colony PCR of Cas9_4, Cas9_10 and Cas9_9
  - Colony 1 and 2 from plate 11C Cas9_4 61A, colony 3 from plate 11C Cas9_9 61A and colony 2 from plate 11C Cas9_10 61A.
  - This was done to be able to verify the flip from GFP to Cas9.
  - Verification with gel electrophoresis
  - **RESULT:** The gel showed an unsuccessful result

8.4 Thursday 10/8

- Colony PCR of CENPK_111-61A transformed with Olf1258
  - Nanodroping the purified colonies to find the reason why there was the wrong size/no bands in the latest colony PCR

- □ **RESULT:** The concentrations were good and there should be no problem with runnuing PCRs on these concentrations.

■ Purifying untransformed CENPK_111-61A as control

- □ We need to have a control of untransformed 61A to see if also this gives a band of 500 bp. If so we know that this band also is present in the original strain and that this length of the band is to expect when the transformation have not succeded.
- □ Followed the colony PCR protocol

■ PCR of untransformed CENPK_111-61A and six transformed colonies

- □ Picked sample from six different colonies from the transformed cells, where the bands had showed clear bands.
- □ Verification using gel electrophoresis
- □ **RESULT:** The untransformed 61A yeast strain gave the same band length as the transformed yeast, thereby the conclusion that the colonies that have giving us these bands had not been successfully transformed with Olf1258.

■ New PCR of some colonies using only two primers to control that using three primers do not give unexpected fragments

- □ Primers that was used was Olf1258-R together with before overlap-primer and STE3-R together with before overlap-primer.
- □ **RESULT:** The PCR with the STE3-R together with before overlap-primer gave a band at approximately 750 bp and the PCR with Olf1258-R gave no bands. Concluding that there is no unspecific binding between the three primers when using all together and the Olf1258-R can not bind when the Olf1258-fragment is not present.

■ Overnight culture of CENPK_111-61A for transformation of Olf1258

■ New colony PCR of mating between CENPK_11-11C with Cas9-vector and CENPK_111-61A

- □ Two controls, the unmated haploid cells CENPK_11-11C Cas9 and the pure Cas9 plasmid.
- □ The annealing temperature was changed from 60C to 59C
- □ **RESULT:** The gel showed no bands at all

■ Examine G418 ADE2 pCas9 in microscope

- □ Contamination was suspected and to examine this a microscope was used
- □ **RESULT:** They looked like yeast and no signs of contamination

■ Extraction of Cre-plasmid with HIS-marker from E.coli

- □ Plasmid Miniprep Kit was used
- □ **RESULT:** They looked like yeast and no signs of contamination

■ Preparation for electroporation

- ☐ Overnight culture of CENPK_11-11C with Cas9-vector

8.5 Friday 11/8

- Transformation of pSH62 into CENPK_11-11C with Cas9-vector
- Transformation of Olf1258 into CENPK_111-61A with three gRNA plasmids; STE3_2_2, STE3_2_6 and STE3_3_4
  - ☐ Preparation of DNA for transformation of Olf1258 into CENPK_111-61A with three different gRNA plasmids; STE3_2_2, STE3_2_6 STE3_3_4
  - ☐ Transformed DNA; Ste3_2_2 with fragment O1 and O2, Ste3_2_6 with fragment O1 and O2, Ste3_3_4 with fragment O1 and O2. A control was used with only 61A cells that went through all steps in the transformation except the electroporation.
- Yeast electroporation protocol followed

9 Week 33

9.1 Monday 14/8

- Preperation of colony PCR for OLf1258 transformed into CENPK111-61A.
  - ☐ 14 colonyes were picked from the plates STE3_2.6, STE3_3.4, STE3_2.2 and put in a overnight culture.
- Preperation for observation in the flouroscence microscope.
  - ☐ The transformed pSH62 in CENPK_11-11c with Cas9 vector is reastreak into 6 colonies on a SD-URA-HIS plate. 6 colonies of the controll was restreaked on both SD-URA-HIS plate and SD-URA plate.

9.2 Tuesday 15/8

- Colony PCR of the OLf1258 transformed into CENPK111-61A.
- Gel electrophoresis of the DNA fragments from the colony PCR of OLf1258 transformed into CENPK111-61A
  - ☐ The gel showed that colonies 10, 11, 12 and 13 from plate 2.6 needs to be redone.
- Fluorescence microscopy of 11c-Cas9-pSH62
  - ☐ The cells studied do not look like yeast cells. The cells have been contanimated during transformation.
  - ☐ Transformation needs to be redone.
- Inoculation of CENPK_11-11c to prepair for transformation of pSH62 in CENPK_11-11c with Cas9 vector.

- A amplification PCR is done to creat the BioBrick of loxP-TEF from Cas9.
  - The DNA template used were Cas9 11.7 f. E.coli and it was amplified with phusion PCR
  - The PCR sample were run on a gel and a expected band at 500.
  - The sample was cleaned with a PCR purification kit.

9.3 Wednesday 16/8

- Transformation of pSH62 into CENPK_11-11C with Cas9-vector.
  - A control sample is done where the cell colonie is transformed without the plasmid pSH62.
- Gel of PCR products BB_gRNA_STE2 and Gel extraction
  - The concentration of the DNA was not high enough to use as a DNA primer, and was used as template istead.
- Phusion PCR and gel extraction was done to creat the gRNA STE2 biobrick. STE2 and STE3 was used as template.
  - Gel exstraction of the gRNA bio brick DNA sample. The DNA caoncentration was messured to 52.8 ng/ul
- A gel was run of the PCR product from the colony PCR och conlony 10 and 13 in the olfR1258.

  gel showed correct bands.
- Cell conlnys 10 and 13 from the olfR1258 were resctrect on YPD plates and grow in 30 degree room for 2 days. This was done to lose the plasmid.
- Amplification PCR of the RatI7 biobrick.
  - First a Phusion PCR are done with template from Rat colony 4. Anneling temperature 59 degrees.
  - A gel extraction was done to clean the PCR product.
  - A second PCR with colony a c-PCR and RatI7 F is done.

9.4 Thursday 17/8

- Fusion GPCRs prepare for microscope by adding YPD to the cell conlonies.
  - RatI7-GFP fusion vector and Olfr1258-GFP fusion vector in CENPK11-11C were the colonies prepared for mircroscope.
- RatI7-GFP and Olfr1258-GFP were studied in a microscope. The cells needed to grow more.
- Amplification PCR and gel verification was done to creat the gRNA STE3 biobrick.

- The PCR product from the RatI7 biobrick was run on a gel and then gel extraction was used.
  - ☐ The messured concentrations was following: 47.4 ng/uL, 18.9 ng/uL and 37.6 ng/uL
- Chloramphenicol stock solution and plates was made.

9.5 Friday 18/8

- The BB_Rati7, BB_loxP_TEF, BB_gRNA_StE2 and BB_gRNA_STE3 were send for sequencing.
- The biobrick vector pSB1C3 was transformed into E. coli in order to amplify it.
- Double check so the CENPK_11-11c with Cas9 vector is not contanimated in microscope.

9.6 Sunday 20/8

- Preparation for transformation of pSense0 into Olf1258.
  - ☐ Inoculation of STE3_2_6 col 10_5 in YPD over night.
  - ☐ The plate with colony 10 for STE3_2_6 where the los of plamid test was done were checked and colonie 5, 7 and 8 have lost there plamid.
- The Biobrick vector pSB1C3 was amplified in E coli, before extraction could be done.

10 Week 34

10.1 Monday 21/8

- Interlab study - Day 1
  - ☐ Transformation into *E.coli*
  - ☐ Performed calibration of the plate reader according to interlab protocol
  - ☐ Made 1 L of PBS
- Transformation of CENPK_111-61A_ ΔSTE3::Olfr1258 with pFUS1-GFP vector
  - ☐ To obtain a strain with both Olf incorporated as well as the plasmid containing GFP to be able to test activation of the receptor
  - ☐ Protocol for electroporation was followed
  - ☐ Plated on SD-HIS plates
- Amplification of Olf1258 receptor to send for sequencing
  - ☐ Incorporated Olfr1258 must be sent for sequencing and to do so it must be amplified by colony PCR.

- ☐ Protocol for colony PCR is followed.
- ☐ Gradient PCR (50-60 C) with 8 samples
- ☐ **RESULT:** No bands at 2000bp
- ☐ Redo with gradient 40-50 C and new primer dilutions from stock
- ☐ New gradient PCR for Olf sequencing

■ O/N culture of CreCas9 + pSH62 strain

- ☐ The gene expressing Cre is under an inducible promoter that activates expression at the presence of galactose. Therefore, a medium containing galactose was prepared and an overnight culture was made.
- ☐ Preparation of Delft+galactose medium
- ☐ Overnight culture of "SD-Ura-His Restreak of 11c-Cas9 col13-pSH62 iGEM 20/8"

■ Preparations for biobrick ligations

- ☐ The biobrick plasmid was inoculated the day before and extracted digested today. The inserts were also digested
- ☐ Extraction of pSB1C3 from *E. coli*
- ☐ The Plasmid Miniprep protocol was followed
- ☐ **RESULT:** concentration: 195,2 ng/uL
- ☐ Digestion with XbaI PstI
- ☐ **RESULT:** All concentrations were very low = 10 ng/uL
- ☐ This concentration is not enough for the next step and will have to be redone

10.2 Tuesday 22/8

■ Interlab study - Day 2

- ☐ Performed the standard curve measurements according to protocol
- ☐ **RESULT:** All plates had colonies except device 1
- ☐ Redo transformation for device 1

■ Microscopy, GPCR Fusion protein

- ☐ Preculture of fusion proteins and 11c
- ☐ **RESULT:** cells were spun down but no pellet was formed
- ☐ O/N culture rat+GFP and Olf+GFP
- ☐ 5ml Delft+URA and one colony

■ Olf for sequencing

- ☐ Gel electrophoresis of overnight gradient PCR

- ☐ **RESULT:** All samples have bands at 2 kb. Samples 1-4 have clearest bands so these are used for PCR purification. Concentration: 239.1 ng/l

■ Preparation of GPCR-GFP to send to sequencing

- ☐ V= 20 ul, final concentration 50 ng/ul
- ☐ **RESULT:** Sequences look ok

■ Verification of the loxP flip with pSH62

- ☐ Colony PCR, two diffrent PCR was made. 1st with 2 primers and 2end with 3 primers.
- ☐ The PCR with 2 primers should only give a band if the flip worked. The control should not give any band in this case.

  The PCR with 3 primers should give band at both the expected size for not flipped and flipped (539 and 1305).

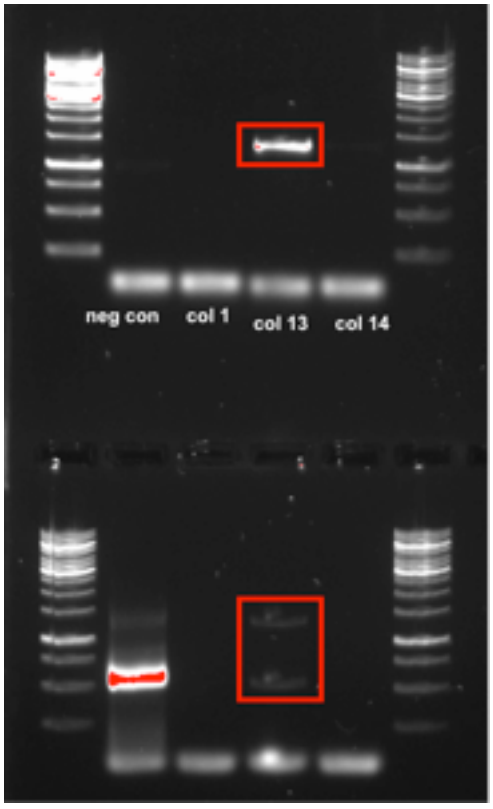


Figure 9: According to the results, the flip seems to worked on colony 13. No band was visible for 1 and 14 in the both cases, which indicate that the PCR failed for those.

■ Preparations for and performance of biobrick ligations

- ☐ Re-digestion with XbaI and PstI
- ☐ **RESULT:**

Table 1: Concentration (ng/uL) of the PCR products

RatI7	16.9
loxPTEF	11.7
gRNA Ste2	20.6
gRNA Ste3	16.0
pSB1C3	21.1

- Ligation of pSB1C3 and biobrick inserts
  - ☐ Biobrick protocols are followed. 50 ng of vector is used, the amount of insert was calculated by the NEB calculator.
- Transformation of ligated biobricks
  - ☐ Protocol for *E. coli* transformations was followed. 5 uL of the ligation products were used for each sample. The cells incubated 90 min before plating and were plated on Chloramphenicol plates.

10.3 Wednesday 23/8

- Microscopy, GPCR Fusion protein
  - ☐ Olfr1258 looks good
  - ☐ RatI7 looks bad
  - ☐ RatI7 barely fluoresce, a new culture is prepared which is covered with tinfoil since GFP might be sensitive to the light
- O/N culture Fusion protein RatI7+GFP
- Olfr1258/RatI7+ pSense0
  - ☐ The negative control have colonies!
- Preparation for Colony PCR of Olf+pSense0
  - ☐ 14 colonies picked and restreaked on numbered SD-HIS plates.
- Overnight cultures for Olf/Rat+pSense0
  - ☐ 5 ml Delft+URA and one colony each
- Verification of the flip with pSH62, microscopy
  - ☐ Flip is irreversible we checked if the ratio of cells expressing GFP is increasing, decreasing or stays the same
- Colony PCR of Cre-Cas9 + pSH62 and gel electrophoresis
  - ☐ The previous colony PCR gave no result so a new was made from scratch
  - ☐ no result
- Restreak of transformed biobricks new Chloramphenicol plates in order to avoid false positives

10.4 Thursday 24/8

- Inoculation of Biobrick colonies and colonies for Interlab study
  - Inoculated in 5ml LB + 5 l Chloramphenicol.
- Verification of the flip with pSH62
  - Colony PCR
  - **RESULT:** PCR did not succed.
- Colony PCR of olf + pSence0 + pFUS
  - Primers: pFUS1-F, pFUS1-R
  - **RESULT:** No visable band
- Media preparation of Delft URA+LEU
  - VOC dilution
  - Inoculation olf 10.5 was inoculated in 5 mL YPD O/N

10.5 Friday 25/8

- Verification of the flip with pSH62
  - Microscope footage

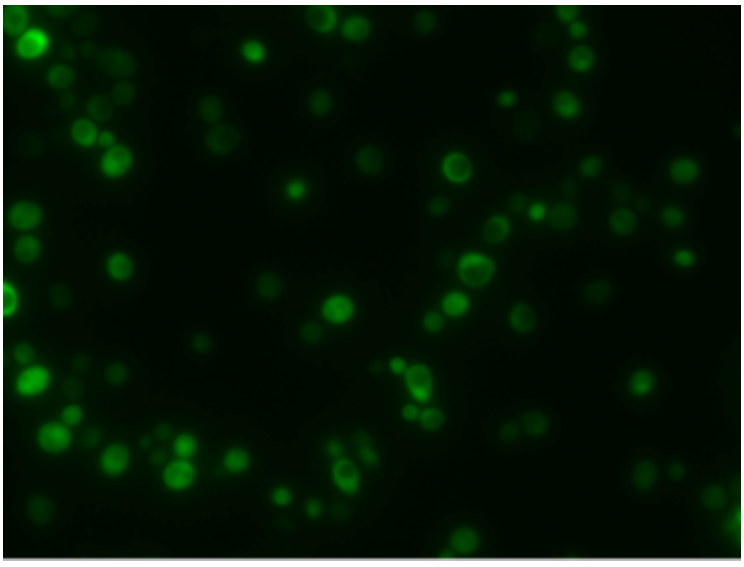


Figure 10: Negative control, colony 13

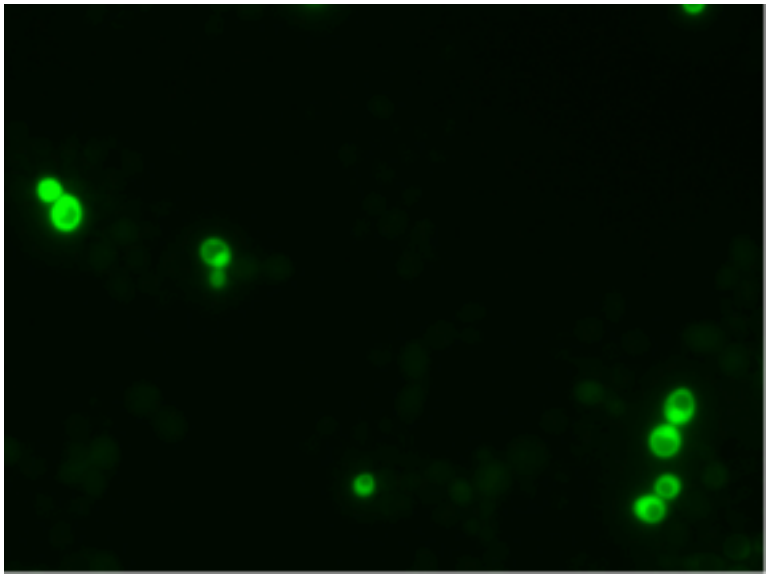


Figure 11: Colony 13, Day 1

- Transformation of olf1258 with pSence0 + pFUS1
  - ☐ Restreaked on SD-His plates

11 Week 35

11.1 Monday 28/8

- Sequencing of finalized constructs.
  - ☐ Verification of the constructs inserted into yeast.
- Over night cultures on CENPK_111-61A_ ΔSTE3::Olfr1258 transformed with pFUS1-GFP vector
  - ☐ One colony was inoculated in 5 μl of YPD.

11.2 Tuesday 29/8

- Colony PCR on CENPK_111-61A_ ΔSTE3::Olfr1258 transformed with pFUS1_GFP vector
  - ☐ Verification of if the transformation was successfull and the vector is incoorporated in the strain.
  - ☐ Verification by Gel electrophoresis
- Restriction verification of bio brick BB_RatI7
  - ☐ Verification of if the transformation was successfull and the vector is incoorporated in the strain.

- ☐ Verification by Gel electrophoresis

- ■ Restriction verification of bio brick BB_RatI7
  - ☐ Restriction enzymes used was PstI and XbaI.
  - ☐ Verification by Gel electrophoresis

12 Week 39

12.1 Tuesday 26/9

- ■ PCR 1 (creating megaprimer) of BB RatI7 fragment using Phusion HF DNA Polymerase protocol. Annealing temperature 59 °C, extension time 30s.
  - ☐ Verification of PCR product with gel electrophoresis.

    RESULT: PCR did not succeed.

12.2 Wednesday 27/9

- ■ PCR 1 (creating megaprimer) of BB RatI7 fragment using Phusion HF DNA Polymerase protocol. Annealing temperature 59 °C, extension time 30s.
  - ☐ Verification of PCR product with gel electrophoresis.

    RESULT: PCR did not succeed.

12.3 Thursday 28/9

- ■ PCR 1 (creating megaprimer) of BB RatI7 fragment using Phusion HF DNA Polymerase protocol. Annealing temperature 59 °C, extension time 45s.
  - ☐ Verification of PCR product with gel electrophoresis.

    RESULT: PCR did not succeed.

12.4 Friday 29/9

- ■ PCR 1 (creating megaprimer) of BB RatI7 fragment, using PrimeStar HS DNA Polymerase protocol. Gradient from 55 to 63 °C, extension time 45s.
  - ☐ Verification of PCR product with gel electrophoresis.

    RESULT: PCR succeeded.

- ■ Gel extraction of the BB RatI7 PCR fragment was performed using GeneJET Gel Extraction Kit.

- ■ PCR of BB Olfr1258 fragment using Phusion HF DNA Polymerase protocol. Annealing temperature 62 °C, extension time 1 min.

- □ Verification of PCR product with gel electrophoresis.

  RESULT: PCR succeeded.

- Gel extraction of the BB Olfr1258 PCR fragment was performed using GeneJET Gel Extraction Kit.
- Making LB + Chloramphenicol plates.

13 Week 40

13.1 Monday 2/10

- PCR 2 of BB RatI7, using megaprimer according to Phusion HF DNA Polymerase protocol. Annealing temperature 59 °C, extension time 1 min.

  - □ Verification of PCR product using gel electrophresis.

    RESULT: PCR did not succeed.

13.2 Tuesday 3/10

- Biolector with RatI7+pSense0+pFUS1 culture and Olfr1258+pSense0+pFUS1 culture, induced with different concentrations of octanal and butanone respectivley.

13.3 Sunday 8/10

- Digestion of BB Olfr1258 framgent with XbaI and PstI.
- Ligation of BB Olfr1258 framgent and vector pSBIC3.

14 Week 41

14.1 Tuesday 10/10

- Transformation of BB Olfr1258+pSBIC3 in to *E.coli*.