Week 26

** Template → More detail written how to do stuff (for example the first time we do it)

Monday 26/6

Date: 26/6	Performed by: Alex & Angelo
What was done	**Observations/comments**

Verification of Cas9-STE2-STE3 vector

Purification

The 6 tubes from each strain (STE2 and STE3) was purified according to the purification protocol (plasmid miniprep kit). The following concentration was observed after the purification.

The colonies from STE2 was marked with X.2, where X is the colony, and STE3 was marked with X.3.

Those which are marked in red are those which were sent in for sequencing. Based on the concentration and which were clearest to see on the gel. See next day (27/6) for more information about sequencing.

Concentration:

Tube	conc. [ng/µL]
1.2	208,5
2.2	295,1
2.3	272,3
4.2	**357,7**
5.2	399,7
6.2	**273,9**

Tube	conc. [ng/µL]
1.3	369,5
2.3	290,1
3.3	335,8

	4.3 — 483,9 **5.3 — 360,9** **6.3 — 365,6**

Restriction analysis

The digestions were done according to the FastDigest protocol. A master mix was made (x15)

- 210 µL Water
- 30 µL FastDigest buffer
- 15 µL NheI
- 15 µL BamHI

18 µL of the master mix was mixed with 2 µL DNA (the samples).

2 µL DNA was calculated from 4.3. The max amount of DNA should be < 1 µg. So the concentration for 4.3 is 483,9 ng/µL → 1000 ng / 483,9 ng/µL = 2 µL. The same volume was used for all digestions.

2 µL was loaded in every well (The bands became messy) 0,5 µL of ladder was added

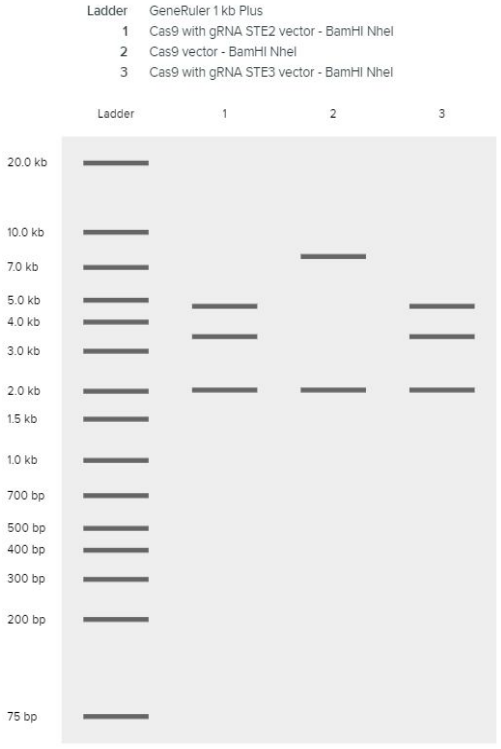
1 µL was loaded in the second try, and the bands became much clearer.

In the second try a misload appeared for colony 5, and was loaded twice.

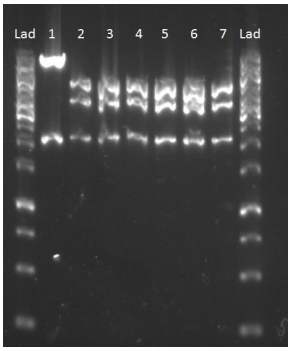
The samples sent for sequencing were 4.2 and 6.2 for STE2 and for STE3 5.3 and 6.3. See text below!

Expected results for the restriction analysis can be seen below

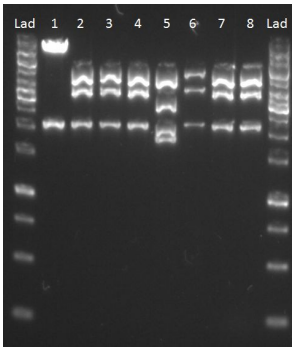
The expected result is based on the program Benchling's approximation for digestion. The control (backbone plasmid Cas9-vector) is supposed the give two bands. If the insert had been successfully inserted in the backbone plasmid three bands should be visible.



The results from restriction analysis is shown to the left. The Lad (Ladder) used was GeneRuler 1 kb. Well 1 is the control (backbone plasmid cut with RE) and well 2-7 is our samples (the different colonies). All of the colonies for STE2 showed results according to the prediction, and for STE3 all except one (colony 4 seemed a bit off) produced expected results. Based on this and the concentration shown in Verification (27/6), purification step above and those which looked best on the gel sent for sequencing.



STE2



STE3

Date: 26/6	Performed by: Alex & Angelo
What was done	**Observations/comments**

Yeast transformation STE2gRNA and STE3gRNA, and Raphaels Cas9 plasmid with gRNA

Preparation of medium and O/N-culture

Medium that's need to be autoclaved:
Sorbitol 1M
Preparation of 50 mL:
50 mL MQ + 9,1 g Sorbitol
LiAc 1M
Preparation of 50 mL:
50 mL MQ + 5,101 g LiAc
PEG 50% w/v
Preparation of 50 mL:
50 mL MQ + 25 g PEG

O/N-culture:
2 yeast culture was inoculated in 5 mL YPD along with a negative control (falcon 50 mL tubes). One colony was picked from each strain. The O/N-culture were put in a shake room 30°C overnight.

Yeast strain	**deletion**	**Mating type**
11C	Δ*HIS,* Δ*URA*	a
61A	Δ*HIS,* Δ*URA,* Δ*LEU*	**α**

OBS! Important to remember which is a and **α**!!

Date: 26/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-Cas9 constructs	
Preparations and PCR	
IDT blocks and primers for the Cre-Cas9 constucts and Cre-gRNA were diluted 1:10 in their original tubes. The constructs and primers for the Cre-Cas9 was diluted again before the PCR. The PCR was done according to the standard Phusion protocol with an annealing temperature at 60°. The constructs used were the Cas9 constructs 1, 2, 3, 4, 4.5, 5 and “middle”. Middle was the long Cas9 part and used a plasmid as template.	Note: Some of the constructs had a original amount of 500 ng and therefore the dilution became 5 ng/µl before this was discovered. This is noted on the tubes! The second dilutions were all made correctly so that the concentrations was 1 ng/µl.
Gel electrophoresis	
A gel electrophoresis was performed. The constructs loaded were in the order: 1, 2, 3, 4, 4.5, 5 and “middle”.	1 - wrong - remake 2 - ok - PCR purification 3 - wrong - remake 4 - ok - PCR purification 4.5 - ok - PCR purification 5 - wrong - remake middle - ok?? - digested - PCR purification
PCR purification	
PCR purification was performed with the GeneJET purification kit. Samples were the gRNA constructs 2, 4, 4.5 & middle. Concentrations were then checked with nano drop.	Concentrations were: 2: 0,3 ng/µl. - remake 4: 4,8 ng/µl. - remake 4.5: 21,7 ng/µl - ok! middle: 21 ng/µl - ok!

Date: 26/6	Performed by: Group 1
What was done	**Observations/comments**
Water evaporation of GPCRs	
Evaporation for increasing the concentration of the GPCRs	
Two sampels each of the constructs O1 (Olfr1258 part 1) O2, R1 (RatI7 part 1) and R2 were mixed and evaporated together in the evaporation machine. After the evaporation the DNA from O2, R1 and R2 were resuspended in 20 µl water. O2 wasn't fully evaporated. The concentrations were measured with nano drop.	The volumes of the samples after the resuspension and after nanodrop was: O1: 16 µl O2: 19 µl R1: 19 µl R2: 19 µl The concentrations were: O1: 126,3 ng/µl O2: 152,9 ng/µl R1: 199,6 ng/µl R2: 80,8 ng/µl Total milligram: O1: 2,02 O2: 2,91 R1: 3,79 R2: 1,54

Tuesday 27/6

Date: 27/6	Performed by: Alex & Angelo
What was done	**Observations/comments**
Verification of Cas9-STE2-STE3 vector	
Sequencing	
The total volume, V_{Total} should be 15 µL, and the concentration, c_{DNA} of DNA 50 ng/µL. c1*V1=c2*V2 $c_{DNA} \times V_{Total\ (15\ \mu L)} = c_{sample} \times V_{Need\ to\ be\ added}$	$\frac{50\frac{ng}{\mu L} \times 15 \mu L}{c} = V_{Need\ to\ be\ added}$ The volume MQ needed was calculated by $V_{Total} - V_{Need\ to\ be\ added}$

With this equation the volume needed of our purified plasmids was calculated as following:

Sample	Conc. [ng/µL]	Volume DNA	Volume MQ
STE2 colony 4 (4.2)	**357,7**	**2,7**	**12,3**
STE2 colony 6 (6.2)	273,9	2,1	12,9
STE3 colony 5 (5.3)	360,9	2,1	12,9
STE3 colony 6 (6.3)	**365,6**	**2,1**	**12,9**

4 tubes were prepared. 4 stickers with following labelled was taken and match up
AAA0085 073 4.2
AAA0085 074 4.6
AAA0085 075 5.3
AAA0085 076 6.3
AAA0085 077 Primer

The primers are designed on the computer later on when you will sent in the order. A supervisor will help you with this step.

Fast way to calculate is always to take 750 divided by the concentration of the plasmid or construct yo will sent in (15 * 50 = 750).

Keep in mind that the following with highest concentration were chosen in the transformation, marked in red.

You will find the stickers in a folder in the large lab on the other side of the MQ-water tap, across the refrigerator. Were the risk declaration will be handed in. Write down which stickers you take (for example AAA0085 73-77) and sign.

Date: 27/6	Performed by: Alex & Angelo
What was done	**Observations/comments**

Yeast transformation STE2gRNA and STE3gRNA, and Raphaels Cas9 plasmid with gRNA

Transformation

The two O/N-cultures was diluted 10x (45 mL YPD was added to the 50 mL falcon tubes and then transferred to a shake flask, one for each strain). A OD measurement were made at 8:15 for a 100x dilution. The shake flasks was put in shaker room for 2 h 20 min. →

Following OD was measured

	100x	10x
11C	~ 0,03	→ 0,3
61A	~ 0,02	→ 0,2

→ This may differ, see protocol for how long. It depends on OD.

After the 2 h 20 min the LiAc protocol was followed for the whole transformation. After the heat shock the tubes were centrifuge and the supernatant was discarded. A recovery step was added: 500 mL Sorbitol and 500 mL YPD was added. The tubes were put in incubation at 30℃ for 1 h. Then the protocol was followed again.	NOTE! Some steps were made different. When the PEG, LiAC and SS-DNA was added the order was not the same as the protocol said. And the incubation for 30℃ was skipped. Some other rfc was used than what the protocol says and different times. The steps that we skipped might be bad (have not seen the results yet) but follow the protocol instead for skipping steps and so on to be sure. The recovery step was good due!

Date: 27/6	Performed by: Group 1
What was done	**Observations/comments**
Gel electrophoresis and purification of PCR fragments Cre-Cas9	
Gel electrophoresis	
The fragments were loaded in the following order: 1, 2, 3, 4, 5. The gel was run for 35 min at 75 V, 160 mA. A picture was taken on the gel with UV-light. _____ GEL EXTRACTION First the sample was prepared for gel extraction. 8 µl loading dye was added to 48 µl PCR-product. It was then loaded on the gel. Gel extraction was done on Cre-Cas9 fragment 1 using the GeneJet Gel extraction Protocol _____ PCR PURIFICATION Purification was done on Cre-Cas9 fragment 2 and 4 using PCR purification protocol. Concentration was measured with Nanodrop.	The following was observed in each well 1: OK -> gel electrophoresis 2: OK-> PCR purification 3: no band. -> redo with annealing T 60 C. 4: OK -> PCR purification 5: no band -> redo with annealing T 60 C. _____ Concentration after gel extraction of Cre-Cas9 fragment 1 was: c(cre-cas9 1)= 0.3 ng/µl. We decided to discard it and redo the PCR for fragment 1. _____ Concentration after PCR purification c(cre-cas9 2)=23.6 ng/µl c(cre-cas9 4)=31,8 ng/µl Good concentrations!

Date: 27/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-Cas9 constructs	
Preparation and PCR	
PCR mix was done for fragments 1, 3 and 5 using Phusion PCR ‘protocol. The PCR was done using 60 C as annealing temperature and 1 min elongation.	
Gel electrophoresis	
The fragments were loaded on a gel in following order: 1, 3, 5. the gel was run for 35 min av 75 V, 160 mA. Picture was taken.	The following was observed in each well: 1: OK -> Gel extraction 3: OK -> Gel extraction 5: No band → redo
Gel extraction	
First the sample was prepared for gel extraction. 8 µl loading dye was added to 48 µl PCR-product. It was then loaded on the gel. Gel extraction was done on Cre-Cas9 fragment 1 and 3 using the GeneJet Gel extraction Protocol The concentrations were then measured with Nanodrop	Concentrations for Cre-Cas9 fragment 1 and 3. c(Cas9_1)=23.2 ng/µl c(Cas9_3)=11,2 ng/µl Both was stored in the freezer! :)

Date: 27/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-gRNA constructs	
Preparation and PCR	
PCR mix was done for fragments and 1, 3, 4 and	

5 using Phusion PCR ‘protocol. The PCR was done using 60 C as annealing temperature and 1 min extension.	
Gel electrophoresis	
PCR . the gel was run for 35 min av 75 V, 160 mA. Picture was taken.	Follwoing was observed for each fragmentl: gRNA 1: wrong -> redo gRNA3: two bands -> gel extraction gRNA 4: wrong ->redo gRNA5: wrong -> redo After gel extraction the concentration för gRNA 3 was too low. c(gRNA_3)=1.6 ng/µl

Date: 27/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-gRNA constructs and Cre-Cas9_5 construct	
Preparation and PCR	
PCR mix was done for gRNA fragments and 1, 4 and 5 and also Cas9_5 using Phusion PCR protocol. The PCR was run over night. The PCR was done using 60 C as annealing temperature and 1 min extension.	
Gel electrophoresis	
The day after the gel was run for 40 min av 75 V, 160 mA. Picture was taken.	Following was observed. gRNA 1: weak band at ~1000 bp -> PCR purification gRNA 4: no band -> redo gRNA 5: weak band at ~400 bp -> PCR purification Cas9_5: no band. -> troubleshoot and redo!
PCR purification of gRNA fragments 1 and 5.	
PCR purification was done according to protocol.	Concentrations was:

After the purification the concentrations were low and we decided to redo the PCR again.	c(gRNA1)=3,2 ng/µl c(gRNA5)=2,0 ng/µl

Date: 27/6	Performed by: Group 1
What was done	**Observations/comments**

Fusion PCR of CreCas9s construct

PCR

The fusion PCR were done according to the Fusion PCR protocol.

Mixture	size	Total ng
4.5+m		
4+4.5+m		

Mixture	Sample 4 [ng]	Samp 4.5 [ng]	Sample m [ng]	
4.5+m				
4+4.5+m				

Mixture	4 [µl]	4.5 [µl]	m [µl]	

It was then loaded on a gel.

The gel picture was unclear and smudgy but this is what we got from it:

They seemed to work. _____ PCR purification and gel extraction was done on the Fusion PCR fragments. The concentration for 4+4.5+m after gel extraction was 2.4 ng/µl. The concentration for 4.5+m after PCR purification was 16 ng/µl.	4.5+m seems to have a clear band at 4300 bp. -> PCR purification 4+4.5+m had one strong band at 4800 bp and one weak at 1500 bp -> gel extraction _____ c(Cas9_4+4.5+m)=2.4 ng/µl Low, but we could use this and amplify this fragment!! c(Cas9_4.5+m)=16 ng/µl Ok concentration. Could use this and amplify this fragment! We will do one last gel electrophoresis to really confirm that the length of each fragment is correct!! This is done on thursday 29/6.

Wednesday 28/6

Date: 28/6	Performed by: Group 1
What was done	**Observations/comments**
Fusion PCR of CreCas9s construct	
PCR	
The fusion PCR were done according to the Fusion PCR protocol. In the table below is the fragments (Cas9_1-Cas9_4) that will be fused together, and the size of them, and the total amount loaded.	Amanda did a new protocol, which is much easier to follow. If something is unclear talk to her.

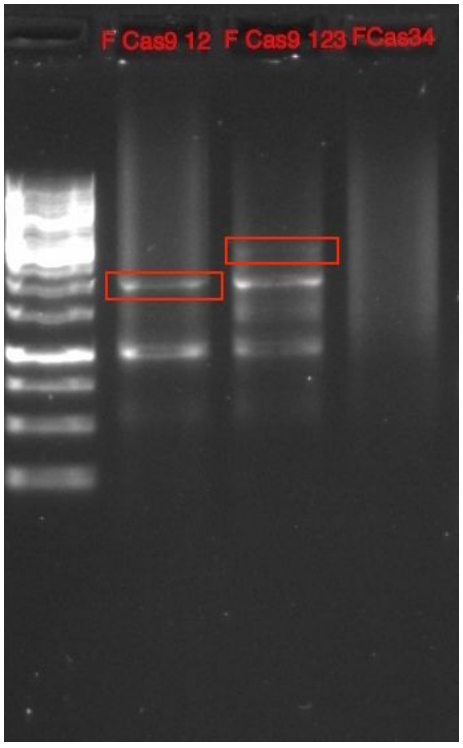
Mixture	size [kb]	Total ng
1 & 2	2	100
1 & 2 & 3	3	150
3 & 4	1,5	95

Mixture	Sample 1 [ng]	Samp 2 [ng]	Samp 3 [ng]	Samp 4 [ng]
1 & 2	50	50	-	-
1 & 2 & 3	30	90	30	-

3 & 4	-	-	35	35

Mixture	Sample 1 [µl]	Sample 2 [µl]	Sample3 [µl]	Sample 4 [µl]
1 & 2	2,2	1,95	-	-
1 & 2 & 3	1,3	3,8	2,7	-
3 & 4	-	-	3	1

The gel results from first round fusion PCR:



The fragments 1&2 seems to have fused correctly

Fragments 1&2&3 produced a faint band at the correct position when fused

Fragments 3&4 did not produce a band after the first round of fusion PCR

Second round fusion PCR of Cas9 fragments

All Cas9 fragments (12, 123, 34) were amplified again using their respective end primers. The second round PCR was done according to the fusion PCR protocol Amanda made.

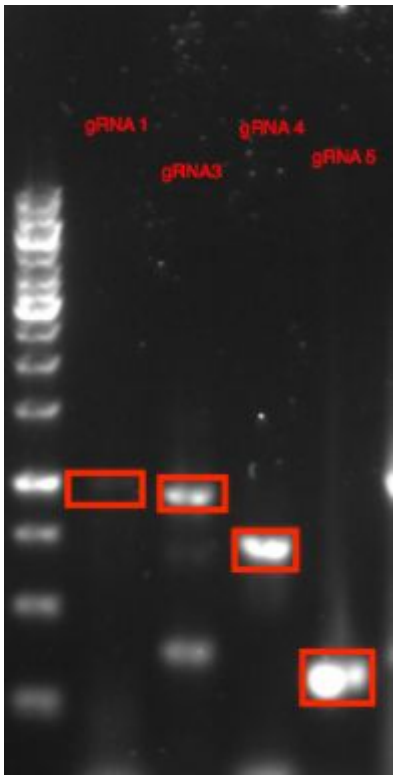
Fragment 12 used the primers 1F and 2R
Fragment 123 used the primers 1F and 3R

Fragment 34 used the primers 3F and 4R

PCR was left overnight

Date: 26/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-gRNA constructs	
Preparation and PCR	
PCR mix was done for gRNA fragments and 2 and 4.5 and also Cas9_5 using Phusion PCR protocol. The PCR was run over night. The PCR was done using 55 C as annealing temperature and 1 min extension.	
Gel electrophoresis	
The the gel was run for 40 min av 75 V, 160 mA. Picture was taken. gRNA_2 was okay! :D	Following was observed. gRNA 2: band at ~1000 bp -> PCR purification gRNA 4.5: no band -> redo using PrimeSTAR. If PrimeSTAR does not work, we could try to to a Touchdown PCR.
PCR Purification of gRNA_2	
Purification was done according to protocol. The concentrations was good, the fragment could be used for Fusion!	 c(gRNA_2)=15.3 ng/µl

Date: 26/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-gRNA constructs	
Preparation and PCR	
PCR mix was done for gRNA fragments1, 3, 4 and 5 and also Cas9_5 using PrimeSTAR PCR protocol.	Apperently when you are using PrimeSTAR Polymerase you should use 55 C annealing temperature. That is the optimal temperature!!!

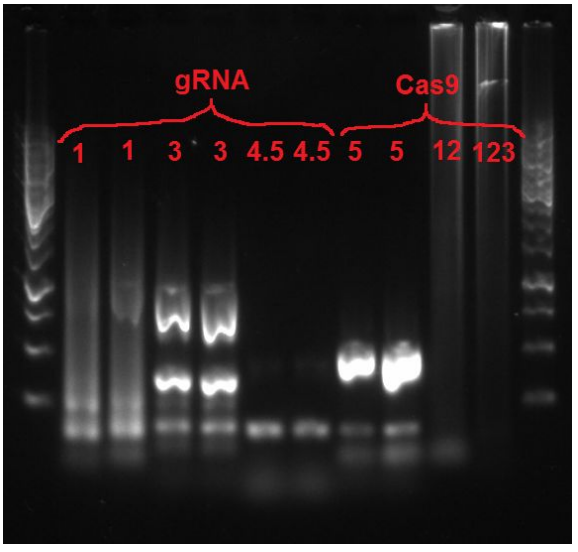
The PCR was done using 60 C as annealing temperature and 1 min extension.	And also respect the extension time!
Gel electrophoresis	
The fragments were loaded on a gel! 75 V, 40 min, 160 mA.  	Observed: gRNA1 had a very faint band but at the correct length. Because the band was so faint no purification was done. We decided to redo the PCR. gRNA3 had a band at ~900, correct. PCR purification. gRNA4 had a band ~700, correct. PCR purification. gRNA5 had a band at ~300, correct. PCR purification

Thursday 29/6

Date: 29/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of Cre-gRNA constructs 1,3 and 4.5 and Cre-Cas9 construct 5	
Preparations and PCR	
The PCR mixture was prepared using PrimeSTAR PCR Protocol for gRNA fragments 1, 3 and 4.5 and Cas9 fragment 5.	

The annealing temperature was 55 C and the extension time was 1 min.	

Gel electrophoresis

A gel electrophoresis was done with gRNA fragments 1, 3 and 4.5 and Cas9 fragment 5 from the previous PCR. We also loaded the Cas9 fusion PCR fragments 12 and 123 from the 28/6 to see if the gel loaded correctly.	gRNA fragment 1 produced a very weak fragment around the expected 1kb mark. Too weak and unspecific ⇒ redo! gRNA fragment 3 produced correct bands at 1kb, however the yield was very low compared to the other smaller sized bands ⇒ redo! gRNA fragment 4.5 produced no band. Cas9 fragment 5 produced a very strong band at about 500kb which is the expected size! It was PCR purified at a concentration of 141 ng/µl. The fusion PCR Cas9 fragments 12 and 123 produced no band. 

Date: 29/6	Performed by: Group 1
What was done	**Observations/comments**

PCR amplification of Cre-gRNA constructs 1,3 and 4.5

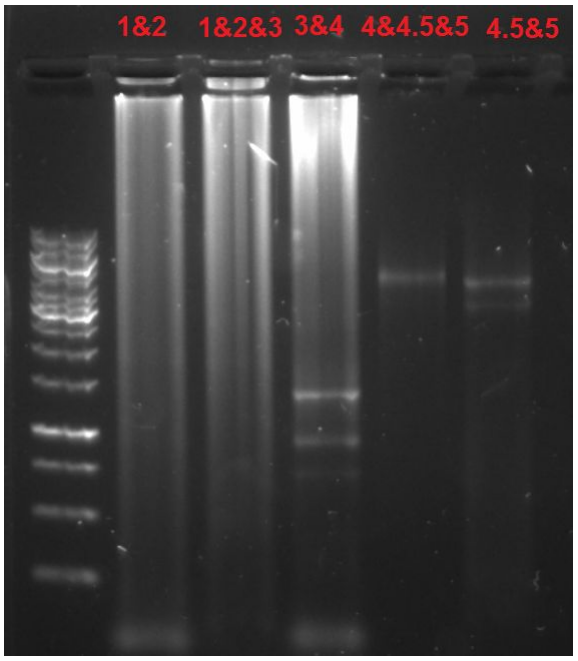
Preparations and PCR	
The PCR mixture was prepared using Phusion PCR Protocol for gRNA fragments 1, 3 and 4.5. The annealing temperature was 58 C and the extension time was 1 min.	
Gel electrophoresis	
A gel electrophoresis was done on gRNA fragments 1, 3 and 4.5 @75V for 45mins. No gel image available, however the results are as following: 1: no band 3: multiple bands, one at correct position ⇒ gel extraction! 4.5: no band	

Date: 29/6	Performed by: Group 1
What was done	**Observations/comments**
Fusion PCR of CreCas9s construct	
Gel electrophoresis	

A gel was loaded with our Cas9 fusion constructs from the 28/6 and 27/6.

These fused constructs are 1&2, 1&2&3, 3&4, 4&4.5&m, 4.5&m

Gel pictures after second round PCR:



Fragments 12 and 123 did not produce any band

Fragments 34 produced a correct band @1.3kb when fused ⇒ gel extraction!!

4.5&5 produced 2 bands, however 4&4.5&5 produced a single band ⇒ PCR purication was done on that band!

Date: 29/6	Performed by: Alex & Angelo
What was done	**Observations/comments**

Gibson assembly for Cas9 with p416 vector

The Plasmid p416 was cut between H/R regions and gel purified before the gibson assembly.
The concentration of the cut plasmid was 12.9 ng/µl.

The DNA amounts(µl) for each Cas9 fragment:

Fragment	volume (µl)
1	1.8
2	1.6

Due to the difficulty of fusing our fragments using fusion PCR before performing the Gibson assembly, we opted to just performing it without fusion PCR.

This involves combining our 7 fragments and our plasmid vector all at once with the Gibson mastermix. It should theoretically work to combine these many fragments, as other researchers have been able to combine even more at a reasonable yield.

3	3.5
4	0.7
5	0.5
4.5	1.9
M	6.2
Vector	3.9
Total	20.1

This was added to an eppendorf tube with 10µl of Gibson mastermix.

It should be noted that the protocol calls for a 1:1 volume ratio between Gibson mastermix and DNA. We used a 1:2 ratio

Date: 29/6	Performed by: Group 1
What was done	**Observations/comments**

PCR amplification of repair fragments O1, O2, R1 and R2

Preparations and PCR

The PCR mixture was prepared using Phusion PCR Protocol for O1, O2, R1, R2.
These correspond to the following tubes from IDT:

upStr2_ptef1_ratI7--------->R_1
RatI7_CYCTer_dnSTE2--------->R_2
ste3up_olfr1258--------->O_1
Olfr1258_ste3dwn--------->O_2

The annealing temperature was 52 C and the extension time was 1 min.

The PCR was left to run overnight.

Date: 29/6	Performed by: Alex
What was done	**Observations/comments**

Yeast transformation of STE2gRNA and STE3gRNA, and Raphaels Cas9 plasmid with gRNA

O/N-culture

2 yeast culture was inoculated in 5 mL YPD along with a negative control (falcon 50 mL tubes). One colony was picked from each strain. The O/N-culture were put in a shake room 30℃ overnight.

Yeast strain	deletion	Mating type
11C	Δ*HIS,* Δ*URA*	a
61A	Δ*HIS,* Δ*URA,* Δ*LEU*	**α**

Friday 30/6

Date: 30/6	Performed by: Group 1
What was done	**Observations/comments**

Gel Extraction of gRNA fragment 3

mass of eppendorf tube = 1.00199g mass of tube with gel = 1.4114g ⇒ ⇒ 410 µl binding buffer The final concentration was 3,2 ng/µl.	Low final concentration, but we are saving this! If we get low concentration a lot of times on this fragment we can evaporate them and dilute, and add to each other.

Date: 30/6	Performed by: Group 1
What was done	**Observations/comments**

Gel electrophoresis of PCR products O1, O2, R1 and R2

SHOW RESULTS!	

Date: 30/6	Performed by: Group 1
What was done	**Observations/comments**
PCR amplification of repair fragments O1, O2, R1 and R2 as well as gRNA fragment 4.5	
A PCR was prepared using the PrimeSTAR protocol. The fragments used were O1, O2, R1 and R2 as well as gRNA fragment 4.5. To the 4.5 fragment, DMSO (1.5 µl) was added, according to protocol. The PCR was run at an annealing temp of 52C and an extension time of 1min.	
Gel electrophoresis on GPCRs and gRNA 4.5	
The gel was added in the order: O1 O2 R1 R2 gRNA_4.5 gRNA_3	gRNA fragment 3 was loaded to double check that we have the right fragment!

Date: 30/6	Performed by: Alex
What was done	**Observations/comments**
Yeast transformation of STE2gRNA and STE3gRNA, and Raphaels Cas9 plasmid with gRNA	
Transformation	
Since the first transformation didn't seems to work, a new one were made (No colonies after 3	Following OD was measured after 3 h. (Note that the OD measurement were done after the 3 h

days now).

The two O/N-cultures was diluted 10x (45 mL YPD was added to the 50 mL falcon tubes and then transferred to a shake flask, one for each strain). The shake flasks was put in shaker room for 3 h. →

After the 3 h the LiAc protocol was followed for the whole transformation.

Amount of DNA (GPCRs) added:

New sample	conc. [ng/µL]	V [µL]
O1	12,3	50
O2	22,2	27,7
R1	30,6	50
R2	19,0	31,6

Old sample	conc. [ng/µL]	V [µL]
O1	126,3	10
O2	153	8,26
R1	199	4
R2	80,8	10

4 µL of plasmids were added (360 ng/µL → 1500 ng) According to the protocol.

After the heat shock the tubes were centrifuge and the supernatant was discard. A recovery step was added: 500 mL Sorbitol and 500 mL YPD was added. The tubes were put in incubation at 30℃ for 1 h. Then the protocol was followed again.

instead of the beginning of the day. This was done because Raphael told us to do so).

100x 10x
11C ~ 0,096 → 0,96
61A ~ 0,063 → 0,63

Note! This time the protocol was followed except one step. The amount of DNA added was different. More DNA was added due to the low concentration and Raphael told us)

The amount of DNA was calculated so the ratio between O1 and O2 should be 1:1 and same for R1 and R2. Max volume of the lowest concentration was added (50 µL for the new sample and 10 µL for the old sample)

Exempel calculation:
12,3 ng/µL x 50 µL = 615 ng
615 ng of O1 is added, then 615 ng of O2 should be added as well.
615 ng / 22,2 ng/µL = 27,7 µL

Date: 30/6	Performed by: Group 1
What was done	**Observations/comments**

Touchdown PCR of gRNA 4.5

Preparations and PCR

PCR was done on gRNA 4.5 using Touchdown Protocol! Phusion Polymerase was used.

Two tubes were prepared, one with DMSO and one without DMSO.
Following was mixed:

30 µl MQ
10 µl Phusion buffer
1.5 MgCl2
1 µl dNTP
3 µl forward primer
3 µl reverse primer
1 µl template
0.5 Phusion polymerase

In the tube with DMSO:
2 µl DMSO (3 %)
28 µl MQ

PCR machine was set on the touchdown protocol! Annealing T was first set on 65, den it decrease on degree for every cycle. We had 12 cycles.

The protocol is now in our iGEM folder on the PCR computer!

The original protocol is also in a drawer in our lab bench. The annealing T is first
™+10
and then
™
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