Notebook week 17/6-23/6

Project: iGEM uppsala 2019
Authors: Sofia Larsson

MONDAY, 17/6/2019

#1 Preparing PBS buffer

Aim: Create buffer for later use

Experimentator: Sofia and Tereza
Sample: -
Protocol: PBS

Table1

	A	B
1	**Chemical**	**Amount (g)**
2	NaCl	4.0
3	KCl	0.100
4	Na2HPO4 (hydrated)	1.359
5	KH2PO4	0.120
6	pH adjustment with HCl	

Result: 2x500 mL were successfully prepared according to standard protocol. pH 7.4 in both solutions. Stored?

#2 Preparing Tris-HCl buffers

Aim: Create buffer for later use

Experimentator: Yannick and Hugo
Sample: -
Protocol:

Table7

	A	B	C	D
1	Solution	Amount	Calculation	Needed Tris Base
2	0,5 M Tris-HCl (pH=6,8)	250 mL	0,25 L * 0,5 mol/L * 121,14 g/mol =	15,14 g
3	1,5 M Tris-HCl (pH=8,8)	250 mL	0,25 L * 1,5 mol/L * 121,14 g/mol =	45,43 g

The needed amount of Tris base was dissolved in ~half the final volume of water, pH adjusted with 12% HCl and somewhat concentrated NaOH, diluted to almost the total volume and the pH adjusted once more.

#3 Preparing YPD Agar

Aim: Creating YPD agar plates for later use.

Experimentator: Yannick (Plates poured by all Yeast Priests)
Sample: -
Protocol:

Table6

	A	B
1	Chemical	Weight
2	Yeast extract	5g
3	Peptone	10g
4	Dextrose	10g
5	Agar	10g

2x500 mL YPD medium with agar was made by adding the above ingredients to each of 2 1L bottles, dissolving them in dest. water and autoclaving the filled bottles. After autoclaving the YPD agar was cast into petri dishes by most team members so that it covered the bottom of the dish.

#4 Preparing YPD-medium

Aim: Prepare YPD-medium for general use

Experimenter: Hugo Grounes
Sample: -
Protocol:

Table8

	A	B
1	Chemical	Weight
2	Yeast extract	8g
3	Peptone	16g
4	Dextrose	16g

800 ml YPD-medium was made by adding ca 200 ml deionized water to a 1 L glass bottle. 8 g of yeast extract, 16 g of dextros and 16 g of peptone was added to the solution. Everything was measured through differential weighing. The solution was diluted to a total of 800 mL. The YPD-medium was autoclaved.

Results: The medium was prepared successfully. Stored in the cold room.

#5 Preparation of 10x Running Buffer pH = 8.3

Aim: Prepare a 10x Running Buffer solution

Experimenter: Hugo Grounes
Sample: -
Protocol:

Table9

	A	B
1	Chemical	Weight
2	Tris base	30 g
3	Glycine	144 g
4	SDS	10 g

Ca 200 mL deionized water was added to a 1 L measuring cylinder. The chemicals were added to the cylinder and 750 mL water was added to the solution. The pH was adjusted using concentrated HCl to a final pH of 8.3. Water was added to the final volume of 1 L.

Results: The buffer was prepared successfully. Stored at room temperature.

Casting YPD+Agar plates
An autoclaved YPD+Agar solution (prepared by Yannick) was casted into plates (the bottom was covered).

#6 Liquid cultures of *P. pastoris*

Aim: create liquid cultures for further experiments

Experimentator: Jonas
Sample: plates with *P. pastoris* X-33 and KM71H provided by Topi Haataja (SLU)

Protocol: colonies were picked with a loop and transferred into 10 mL YPD medium.
Culture was incubated at 30 °C overnight.

Results:

#7 Restreaks of *P. pastoris* strains

Aim: generate backup plates

Experimentator: Jonas
Sample: plates with *P. pastoris* X-33 and KM71H provided by Topi Haataja (SLU)

Protocol: resuspend picked colony of *P. pastoris* in 1 mL 0.9 % NaCl
plate with loop for dilution streaking on YPD plate
incubate 30 °C for 2 days

Results: No visible colonies after one day.

#8 Transformation of *E. coli*

Aim: tranform plasmid into *E. coli* to try out the protocol

Experimentator: Tereza and Sofia (start), Jonas plating
Sample: BBa_K592009 (Cm^R)

Protocol: Synthetic Biology - A Lab Manual, Protocol 6 (page 113) was followed with these deviations: 1uL of plasmid BBa_K592009 (Cm^R) was used instead of 5 uL. LB media was used instead of SOB. Step 8 was 1h on, no mixing done.

Result: No transfromants found in the morning next day. During the day small colonies developed.

TUESDAY, 18/6/2019

#1 Restreaks of *P. pastoris* strains

Aim: generate back-up plates with single colonies

Experimentator: Tereza, Hugo, Sofia and Yannick
Sample: Liquid culture #6 17.06.19

Protocol: Yeasts were restreaked on YPD plates with loop next to Bunsen burner.
3 plates with *P. pastoris* X-33 and 2 plates *with P. pastoris* KM71H were prepared and incubated at 30°C.

Results: Some single colonies.

#2 Sorbitol freezing buffer preparation

Aim: Prepare sorbitol buffer for freezing electroporation-competent yeast cells

Experimentator: Tereza and Sofia

Sample: -
Protocol: Sorbitol buffer

Table2

	A	B
1	**Chemical**	**Amount (g)**
2	Sorbitol (143 mL solution was prepared separatly)	72.83
3	CaCl2	0.1110
4	HePeS	0.2383
5	NaOH and HCl	A few drops for pH adjustment

Result: A solution of $CaCl_2$ and HePeS was prepared and autoclaved. Flask contains 50 mL of solution (for final volume 100 mL) with pH 7.5. Sorbitol not added. Stored on shelf over lab-bench. The sorbitol was prepared separatly and sterile filtered. Amount 143 mL. Concentration 2.797 M.

#3 Preparing 25% NaOH solution

Aim: Prepare strong base for adjusting pH in sorbitol buffer

Experimentator: Tereza and Sofia
Sample: -
Protocol: -

Table3

	A	B
1	**Chemical**	**Amount**
2	Water	10 mL
3	NaOH (s)	2.5 g

Result: 10mL of 25% NaCl stored in falcon tube the room with chemicals, next to pH meter.

#4 Lithium Acetate 1M stock solution

Aim: Prepare Lithium Acetate stock solution for yeast transformation protocol

Experimentator: Tereza
Sample: -
Protocol: 1M stock solution of LiAc prepared with ddH_2O
Result: 10ml 1M stock, autoclaved, stored in RT

#5 *P. pastoris* OD measurement

Aim: estimate the growth rate of both *P. pastoris* strains

Experimentator: Jonas start, then Tereza and Sofia
Sample: *P. pastoris* X-33 and KM71H liquid cultures #6 17.08.19

Protocol: OD_{600} was measured after 16 hrs and 21 hrs, cultures were grown in incubator 30°C anaerobically.

Result:

Table4

	A	B	C
1	strain / time	16 h	21 h
2	X33	0.370	0.980
3	KM7HM	1.364	1.05

#6 SOB-Medium

Aim: get SOB for future trafos

Experimentator: Jonas
Sample: -
Protocol:

Table5

	A	B
1	Compound	weight (g)
2	yeast extract	
3	tryptone	
4	NaCl	
5	KCl	
6	MgSO4	
7	adjust ph to 7.5 with 1M NaOH	

pH was not adjusted

#7 Overnight cultures

Aim: cultures for mini-prep the next day

Experimentator: Jonas, Irina
Sample: Trafos from 17.06.19, all labgroups (8 plates)
Protocol: 40 mL LB was mixed with Chloramphenicol (40 uL)
aliquoted 5 mL in 15 mL falcons
colonies picked and incubated at 37 °C shaking o/N

#8 Preparing 1 M salt solutions

Aim: Prepare 200 ml 1 M solutions of KCl, $MgSO_4$ and $CaCl_2$

Experimentator: Hugo, Yannick
Protocol: -

Table10

	A	B	C
1	Chemical	Calculation	Weight
2	MgSO4	0,2 mol * 120,36 g/mol =	24,7 g
3	KCl	0,2 mol * 74,55 g/mol =	14,9 g
4	CaCl2	0,2 mol * 110.98 g/mol =	22,2 g

The different salts were dissolved in ca 150 ml water and were then diluted to a final volume of 200 ml with water.
Results. The preparation was successfull. Stored in room temperature.

#9 Making *E. Coli* DH5α $CaCl_2$ competent cells

Aim: Create 100 mL of competent cells for later transformations

Experimenter: Yannick and Hugo
sample: -
protocol: 5. Preparation of competent *E. Coli* cells using $CaCl_2$

Table11

	A	B
1	Material	Amount
2	LB medium	100 mL
3	O/N DH5α culture	1 mL
4	0,1 M CaCl2	30,2 mL
5	0,1 M CaCl2 20% glycerol	4 mL
6	Liquid nitrogen	

1. 2x0,5 mL O/N DH5α culture was inoculated into 2x50mL autoclaved LB Medium and grown pooled into 100 mL until OD=0,4 at 37 °C with shaking.
2. The culture was chilled on ice for 15 min., then poured into 2x50mL falcon tubes and centrifuged at 3500 rpm for 5 min. at 4 °C.
3. As much supernatant as possible was removed and the pellet resuspended in 2x100 µL ice-cold 0,1M $CaCl_2$. 2x15mL ice-cold 0,1M $CaCl_2$ was added and mixed by pipetting
4. The cells were incubated on ice for 30 min.

Result: The next steps were left to Karthik: Resuspending in 0,1M $CaCl_2$ 20% glycerol, then aliquoting 50 µL samples into tubes and snapfreezing. The competent cells were stored at 80°C in the freezer downstairs.

#10 Sorbitol solution, 0.95 M

Aim: Prepare 1M sorbitol

Experimentator: Tereza, Sofia
Sample: -
Protocol: Correct amount of sorbitol was dissolved in 100mL ddH_2O, which resulted in different final concentration.
Result: 2 x 50mL of 0.95 M sorbitol, sterile filtered, stored in the fridge

#11 Sterile filtered Glucose, 0.95 M

Aim: Prepare 1M sterile filtered glucose

Experimentator: Tereza, Sofia
Sample: -
Protocol: Correct amount of sorbitol was dissolved in 100mL ddH_2O, which resulted in different final concentration.
Result: 2 x 50mL of 0.95 M glucose, sterile filtered, stored in the fridge

WEDNESDAY, 19/6/2019

#1 Sorbitol buffer

Aim: Prepare buffer for making competent cells for electroporation transformation

Experimentator: Sofia and Tereza
Sample: #2 from 2019-06-18
Protocol: Sorbitol buffer
Mixing 50ml sorbitol 2.787 M solution with 50ml $CaCl_2$ and HePeS.
Result: 100mL of freezing sorbitol buffer stored in the fridge.

Table13

	A	B
1	Compound	Concentration
2	HePeS	10mM
3	CaCl2	10mM
4	Sorbitol	~1.4 M

#2 PeG 4000 solution

Aim: Prepare a 50% (w/v) PeG solution for electroporation transformation

Experimenter: Hugo
Sample: -
Protocol: 5 g PeG 4000 was diluted with water to a final volume of 10 mL and was thereafter autoclaved.
Result: The solution was prepared successfully. Stored in a glass bottle in room temperature.

#3 1M sorbitol stock solution

Aim: Prepare sorbitol stock solution for electroporation transformation

Experimentator: Sofia
Sample: -
Protocol: Measuring 18.22 g of sorbitol and add water to 100 mL. Sterile filter.
Result: 1M sorbitol solution was prepared succesfully.

#4 Mini-prep

Aim: Prepare DNA-plasmid for digestion

Experimenter: Sofia, Tereza, Yannick and Hugo
Sample: E.coli with plasmid: pSB1C3. Part: BBa_K592009.
Protocol: Sigma mini-prep kit protocol (look up the exact name).
Result:

#5 Digestion of plasmid

Aim: Digest DNA-plasmid

Experimentator: Sofia, Tereza, Yannick and Hugo
Sample: Plasmid from #4 20190619
Protocol: Digestion of plasmid
Result: Sample with digested DNA stored on ice.

#6 Preparation of 1% agarose gel

Aim: Preparation of agarose gel for running DNA

Experimentator: Sofia and Jinwen
Sample: -
Protocol: 1% agarose gel preparation
Result:

#7 Sterile filtered glucose, 1M

Aim: Preparation of 100mL sterile filtered 1M glucose

Experimentator: Tereza
Sample: -

Protocol: 18.04g glucose dissolved in 50mL ddH2O and filled up till 100mL. Solutions were sterilized by filtration.
Result: 2 x 50mL sterile filtered 1M glucose stored in the fridge

#8 Overnight cultures of *P. pastoris*

Aim: Set up overnight cultures of both strains

Experimentator: Tereza
Sample: liquid cultures #6 2019-06-17
Protocol: 20mL YPD inoculated by 10uL of P. pastoris X33 and KM71H liquid cultures (2 000 x diluted) and cultivated aerobically on the shaker in 28°C water bath.

Result: Overnight cultures set up at 16:30.

THURSDAY, 20/6/2019

#1 DH5α transformation

Aim: Transforming DH5α with the plasmid isolated in the miniprep

Experimentator: Yannick and Hugo
Sample: PPIC RV1284 plasmid from miniprep 19.6.19
Protocol: 6. Transformation of $CaCl_2$ -competent *E. coli* cells

Table12

	A	B
1	Material	Amount
2	DH5α CaCl2 -competent cells	2x50 µL
3	ddH20	~4 µL
4	Low salt LB medium	2x950 µL
5	Plasmid: PPIC RV1284	0,25 µL of 39,6 ng/µL (~10ng)
6	Low-Salt LB zeocin plates	3

1. DH5α $CaCl_2$ -competent cells were fetched from the -80°C freezer and allowed to thaw on ice for 15 min.
2. 2x50 µL aliqouts of the cells were pipetted to 2 eppendorf tubes. To one tube, the transformation tube, 0,25 µL plasmid DNA and 1,75 µL water was added. To the other tube, the negative control, 2 µL water was added. Worked around a flame and sterile.
3. Tubes incubated on ice for 30 min.
4. Heat shocked at 42 °C for 45s
5. Tubes incubated on ice for 5 min.
6. Pipetted 950 µL of Low-Salt LB medium to both tubes. (the negative control got slightly less than 950 µL)
7. Incubated at 37 °C for 75 min. Tubes were inverted for mixing 3 times during this time.
8. Spun down cells at 4000 rpm for 5 min., discarded 800 µL of the ~950 µL supernatant without disturbing the pellet. Resuspended the pellet by pipetting.
9. Plated 100 µL and 10 µL of cells transformed with the PPIC RV1284 plasmid on 2 seperate plates and 100 µL of cells tranformed with the negative control (water) onto the third plate.
10. Incubated at 37°C O/N

Result: 2 plates with DH5α transformed with the PPIC RV1284 plasmid and 1 negative control incubating in the 37°C incubation room.

#2 Cell count of *P. pastoris*

Aim: Determine the number of cells/mL in overnight cultures. If cell count is $1x10^7$, competent cells for electroporation might be prepared.

Experimentator: Tereza, Sofia
Sample: Liquid ON cultures #8 2019-06-19
Protocol: *P. pastoris* X33 culture was diluted 10 times and cells were counted in Bürken chamber in 1 big square

Result: Cell density is roughly $9.6x10^8$, too high for competent cell preparation.

#2 Overnight cultures of *P. pastoris*

Aim: Get cultures of $1x10^7$ cells/mL for competent cells preparation.

Experimentator: Tereza
Sample: Liguid ON cultures #8 2019-06-19
Protocol: 30mL of YPD was inoculated at 11:30 by 10uL of *P. pastoris* X33 and KM71H liquid ON cultures from previous day (3 000 x diluted). Shaked in 28°C water.

Result: Two overnight cultures, but growing too slow. Too small inoculum.

#3 OD measurement of *P. pastoris*

Aim: Estimate growth rate and check relationship between OD and cell count of both strains.

Experimentator: Tereza
Sample: Liquid ON cultures #2 2019-06-20
Protocol: OD measured 3 times
Result:

Table14

	A	B	C	D
1		start	2h	5h
2	X33	0.002	0.003	0.005
3	KM71H	0.001	0.001	0.006

#4 Restreaking yeast strains

Aim: Dilute and get new colonies of X-33 and KM71M

Experimentator: Sofia
Sample: #1 from 20190618

Protocol: Restreaking of single colonies from previous plates with X-33 and KM71M. Colonies taken from the plates and suspended in 500 µL of 0.9% NaCl before streaking on new YPD plate. Incubated at 28℃. 4 plates in total. 2 of the plates seeled with parafilm.

Result: Colonies appered on all plates but the agar had dryed on the unseeled plates.

Example Entry

Project: iGEM uppsala 2019
Authors: Jonas Gockel

MONDAY, 17/6/2019

#1 Preparing solutions for SDS-PAGE Gels

Aim: create buffers to use in SDS-PAGE gel electrophoresis

experimentator: Jonas
sample: -
protocol: 10x Running buffer

Table1

	A	B
1	chemical	amount (g)
2	tris-base	30
3	glycine	144
4	SDS	10
5	no pH adjustment	fill up to 1 L

Buffer X blabla

Result: Buffers were sucesfully created after standard protocols. 10x Running buffer was stored at RT, Buffer X was stored at 4 °C in the cold room.

#2 Casting SDS-PAGE gels

blabla see up there you figured it

Notebook week 24/6-30/6

Project: iGEM uppsala 2019
Authors: Yannick Hajee

MONDAY, 24/6/2019

#1 Preparing 2xYPD medium

Aim: Making 500mL 2x YPD medium

Experimenter: Sofia
Sample: -
Protocol:

Table1

	A	B	C
1	**Chemical**	**Amount (g)**	
2	Yeast extract	10	
3	Peptone	20	
4	+Add 40% dextrose solution	50 mL	

Yeast extract and peptone mixed with sterile water to a volume of 450 mL. Autoclaved.
50 mL of 40% dextrose solution prepared by mixing 20 g of glucose (dextrose) and adding sterile water to 50 mL. The solution was sterile filtered and then added to the autoclaved solution.

Result: Media prepared and stored in cold room.

#2 Preparing YPD medium and agar

Aim: Making 1000 mL of YPD medium+agar

Experimenter: Yannick
Sample: -
Protocol: Made two batches of 500mL of YPD agar according to the same recipe as #3 on monday 17/6/2019. Autoclaved and poured plates later.

Result: 1 liters worth of YPD agar plates stored in the cold room.

#3 Restreaking yeast strains

Aim: Dilute and get new colonies of X-33 and KM71M

Experimentator: Sofia
Sample: #4 from 20190620
Protocol: Restreaking of single colonies from previous plates with X-33 and KM71M. Colonies taken from the plates and suspended in 500 µL of 0.9% NaCl before streaking on new YPD plate. Incubated at 28°C. 4 plates in total, seeled with parafilm.
Result: Plates stored in 28°C. Sample transfered to yeast fridge.

#3.5 O/N cultures of transformed *E. coli*

Aim: To create 6 O/N cultures from 6 seperate single colonies from the PPIC RV1284 transformed DH5α

Sample: 10 µL plate with transformed pPICZαA with RV1284 in DH5α from #1 Thursday 20190620
Protocol: 8 µL of 100 mg/mL zeocin stock was added to 32 mL LSLB for an effective 25 µg/mL concentration of zeocin. 6x 15mL falcon tubes were filles with 5 mL each and inoculated with pipette tips with different single colonies from the 10 µL DH5α pPICZαA RV1284 plate (stored in fridge). Colonies labeled I-VI
Result: 6x 5mL O/N cultures incubating O/N at 37°C with shaking in incubation room (falcon tube lids NOT tightly closed)

#4 O/N culture *P. pastoris* for chemical transformation

Aim: Create a O/N culture for chemical transformation

Experimentator: Sofia
Sample: #4 from 20190620. Colonies III from both plates.
Protocol: 5 mL of 2xYPD media added to a 20 mL e-flask. Colonie III from Sofias plate from 20190620 taken and diluted in the media. Culture started at 17.30, incubated in 28°C on shaker.
Result: Inoculated culture of X-33 and KM71M in 28°C on shaker.

#5 YPDS agar plates with Zeocin 100ug/ml

Aim: Prepare YPD media with sorbitol, add Zeocin and pour plates for selection of yeast tranformants

Experimentator: Tereza
Sample: -
Protocol:

Table4

	A	B
1	yeast extract	5g
2	peptone	10g
3	sorbitol	91.1g
4	agar	10g

After the autoclaving, 50 mL of (20% dextrose) and 500uL of 100mg/mL Zeocin was added.

Result: Approximatelly 25 of plates of YPDS with 100 ug/ml Zeocin stored in cold room.

#6 Preparation of competent *P. pastoris X33* and *KM71H* for Suga and Hatakeyama electroporation

Aim: Prepare frozen competent *P. pastoris* cells for electroporation according to Suga and Hatakeyama protocol

Experimentator: Tereza, Sofia
Sample: #2 2019-06-20
Protocol:

At 11:20, 20 mL of YPD inoculated by 250uL of *P. pastoris* cultures #2 2019-06-20 and incubated at 28°C on shaker **(diluted 80x)**.

At 12:20 OD_{600} was 0.38 and 0.41 for X33 and KM71H.

Cells were put 15min on ice, then harvested by centrifugation (10min 2 701g + 5min 3 844g) and washed 3x by ice-cold sterile water (5ml, 5min, 3 844g). Cells were resuspended in sorbitol freezing buffer (#2 2019-06-18), 760uL for X-33 and 820uL for KM71H.

Volume calculation: For P. pastoris applies that

OD_{600}=1 ~ 5 x 10^7 cells/mL

OD_{600}=100 ~ 5 x 10^9 cells/mL

(EasySelectTM Pichia Expression Kit - Invitrogen)

Volumes counted so as to reach *5 x 10^8 cells/mL for both cultures.*

100 uL were aliquoted into microtubes and put into -80°C.

Result:

Competent cells *P. pastoris* for electroporation according to Suga and Hatakeyama protocol stored in -80°C, "Cell and Glycerol stock" box. Aliquotes: 7x100uL + 50uL X33 and 9x100uL + 20uL of KM71H.

TUESDAY, 25/6/2019

#1 Chemical transformation

Aim: Create competent cells for chemical transformation

Experimentator: Sofia and Tereza
Sample: O/N culture #4 from 20190624
Cut plasmid DNA from colony V from #3 from 20190625
Protocol: Protocol for the LiAc/single-stranded carrier DNA/PeG method developed by Gietz and Woods

Step 1: #4 20190626 and 2 flasks of 50 mL, 2xYPD preheated to 28℃.

Step 2: KM inoculation left in incubation, did not proceed with this strain. Proceed with X-33.

Calculations:

X-33: 4.6*10^7*5=2.3*10^8 cells

KM: 1.0*10^7*5=5*10^7 cells

Step 3: The total sample (#4 20190624, 5 mL) transfered to preheated 2xYPD. Cell concentration 4.2*10^6 cells/mL.

Step 4: Wanted cell amount met. Cells harvested by centrufugation at 3500 rpm for 10 min in 4℃.

Calculations:

2.8*10^7 cells/mL*55mL=1.54*10^9 cells.

Step 5

Step 6: Transformation mix with cut plasmid DNA #3 20190625. In total 3 transformations made. Sample V, 1 transformaion with HindI and 2 with BamH.

Transformation mix for plasmid DNA BamH, 20 µL of plasmid DNA and 48 µL water.

Transformation mix for plasmid DNA HindI, 20 µL of plasmid DNA and 14 µL water.

Step 7 Incubated 40 minutes at 42°C.

Step 8 Microcentrifuged 30s 16 100g, supernatant discarded and dissolved in 1 mL of sterile water, vortexed. One of the samples was left dry for 30 minutes before adding water.

Step 9 After approximately 30 minutes, 100uL of suspension was plated on YPDS+Zeocin (100ug/uL) agar plates

Result:

Result of OD_{600} measurement in step 1:

Table2

	A	B	C	D	E	F	G	H	I	J	K
1	**OD600 Step 1**	At 9.45	Number of cells	At 10.55	Number of cells	At 11.45	Number of cells	At 13.15	Number of cells	At 14.50	Number of cells
2	X-33	4.6	4.6*10*10^6*5= 2.3*10^8 cells	-	-	-	-	-	-	-	-
3	KM71M	1.0	1.0*10*10^6*5= 5*10^7 cells	1.7	1.7*10*10^6*5 =8.5*10^7 cells	2.5	2.5*10*10^6*5 =1.25*10^8 cells	3.4	3.4*10*10^6*5 =1.7*10^8 cells	5.8	5.8*10*10^6*5 =2.9*10^8 cells

Result of OD_{600} measurement in step 4:

0.028 abs (dilution 1:100), gives 2.8 abs undiluted.

No colonies the next day. No colonies after 9 days. Yeast were not given the time to express resistance genes.

#2 Miniprep & Measurement of DNA conc.

Aim: Isolate plasmid DNA from each of the 6 O/N cultures of E.coli DH5α PPIC RV1284 and measure the concentrations of the isolated DNA

Experimenter: Yannick

Sample: O/N culture I-VI from #3.5 from Monday 24/6/2019

Protocol: Miniprep kit manual

Did exactly as per protocol, harvesting cells from 4 mL of each O/N culture, skipping the optional washing step and eluting with 100µL of elution buffer.

Then measured the DNA concentrations by photospectroscopy. Cleaned the machine with 1,0 µL of water and a tissue. Set the blank to 1,0 µL of elution buffer. Pipetted 1,0 µL of the measured sample of the machine, closed the lid and ran the program. Cleaned the lense with a tissue betweeen measurements. Measured sample nr. III multiple times because the first results were completely outside of the normal measurement range.

Result: Isolated DNA from O/N cultures I-VI and measured very low DNA concentrations. Should use 50 µL of elution buffer next time and also do the optional washing step because there may have been some RNA contamination in the Miniprep. Measured the following DNA concentrations:

Table5

	A	B
1	Sample	Dna conc. (ng/µL)
2	I	30,0
3	II	29,1
4	III	27,3 (average of 28,7;26,2;27,1)
5	IV	31,6
6	V	33,6
7	VI	27,7

#3 Digests

Aim: Linearize the PPIC RV1284 plasmid for transformation into Pichia.

Experimenter: Yannick
Sample: Miniprepped PPIC RV1284 from colony V, from #2 20190625
Protocol: 2 tubes with 200 ng of plasmid DNA were cut with restriction enzymes BAMH1 and HIND3 respectively. 200ng/33.6 (ng/µL)=5.95µL of plasmid DNA solution was needed. The following were added together in that order for a final volume of 20 µL:

Table3

	A	B
1	Material	Amount
2	Water	11.25 µL
3	Plasmid solution	5.95 µL
4	Reaction buffer (specific to either HIND3 or BAMH1)	2 µL
5	Restriction enzyme (HIND3 or BAMH1 respectively)	0.8 µL

The tubes were on ice while the reaction buffer and ezymes were added. The tubes were then incubated at 37°C for ~40 min., heat inactivated at 80°C for ~20 min.

Results: PPIC RV1284 plasmid from colony V digested with HIND3 or BAMH1 respectively. Went to Sofia for transformation into yeast at #1 20190625

#4 Agarose gel electrophoresis

Aim: Perform agarose gel electrophoresis on the undigested plasmid DNA from miniprep to see which colonies are best to use for transformation

Experimenter: Yannick
Sample: Miniprepped plasmid DNA from colonies I-VI from miniprep #2 20190625
Protocol: Dissolved 0.5 g agarose in 50 mL TBE by microwaving it. Added 5 µL 10000x Sybr Safe stain and cast the agarose gel in a mold with a comb. Removed an air bubble with a pipette tip.

Loading samples of 200ng of the miniprepped plasmid DNA were made by mixing the following to a final volume of 24 µL:

Table8

	A	B	C	D
1	Plasmid DNA from colony	Plasmid solution	Water	6x loading dye
2	I	6.7 µL	13.3 µL	4.0 µL
3	II	6.9 µL	13.1 µL	4.0 µL
4	III	7.3 µL	12.7 µL	4.0 µL
5	IV	6.3 µL	13.7 µL	4.0 µL
6	V	6.0 µL	14.0 µL	4.0 µL
7	VI	7.2 µL	12.8 µL	4.0 µL

The gel was placed in the electrophoresis machine and covered in running buffer. 20 µL of each of the samples and twice 5 µL of purple ladder was loaded in the following order:

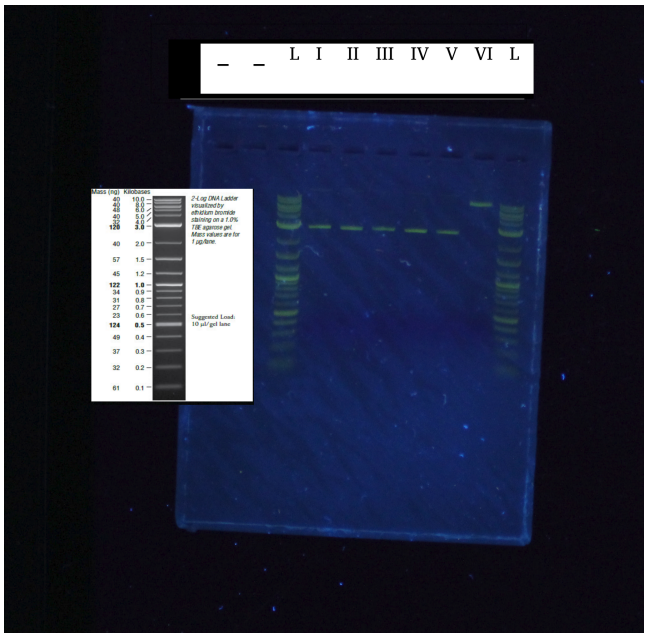
Table9

	A	B	C	D	E	F	G	H	I	J	K
1	Empty	Empty	Empty	Ladder	I	II	III	IV	V	VI	Ladder

The electrophoresis was run at 100V between 16.15 and 16.30 and the voltage was then turned up to 120V. After ~1.5 hours of run time the markers had almost reached the middle cut in the plastic cast and the machine was turned off. The gel was transported on a paper towel and photographed with UV.

Results:

20190625 Miniprep undigested gel (PPIC RV128A I-VI) Yannick Edited.png



The plasmids obtained from colonies I-V showed a band at ~3kb and the plasmids obtained from colony VI showed a band at ~10kb

#5 O/N cultures of *E. coli*

Aim: Preparation for Miniprep of pPICZα with RV1284 construct in DH5α *E. coli*

Experimenter: Teresa & Yannick
Sample: Colonies VII-X from the 10uL plate from #1 Thursday 20/6/2019

Protocol:

Zeocin was added to 20mL of Low Salt LB media (final concentration 25ug/ml). 5mL of this media was inoculated by colonies VII-X from #1 Thursday 20/6/2019, incubated in 37°C on shaker with not tigthly closed lid of 20ml Falcon tubes for aeration of media.

Results: 4 x 5ml O/N cultures of *E.coli* DH5α carrying plasmid pPICZα containing RV1284 construct.

WEDNESDAY, 26/6/2019

#1 Miniprep & Measurement of DNA conc.

Aim: Isolate plasmid DNA from each of the 4 O/N cultures of E.coli DH5α PPIC RV1284 and measure the concentrations of the isolated DNA

Experimentators: Sofia, Yannick, Jonas and Tereza
Sample: O/N culture VII-X from #5 from 20190625
Protocol: Miniprep kit manual

Did exactly as per protocol, harvesting cells from 4 mL of each O/N culture, performing the optional washing step and eluting with 100µL of elution buffer. Jonas and Yannick made a mistake by adding the lysed, neutralised solution to the miniprep column while the column preparation solution was still in there. This may have decreased yield.
Then measured the DNA concentrations by photospectroscopy. Cleaned the machine with 1,0 µL of water and a tissue. Set the blank to 1,0 µL of elution buffer. Pipetted 1,0 µL of the measured sample of the machine, closed the lid and ran the program. Cleaned the lense with a tissue betweeen measurements.

Result: Isolated pPICZpA DNA from O/N cultures VII-X and measured very low DNA concentrations. Measured the following DNA concentrations:

Table6

	A	B	C	D
1	**Sample**	**Dna conc. (ng/µL)**	**260/280**	**260/230**
2	VII			
3	VIII	35.1	1.87	2.96
4	IX	30.1	1.83	1.75
5	X	31.1	1.89	1.64

#2 Preparing 1xYPD medium

Aim: Making 800mL 1x YPD medium

Experimenter: Sofia
Sample: -
Protocol:

Table7

	A	B	C
1	**Chemical**	**Amount (g)**	
2	Yeast extract	8	
3	Peptone	16	
4	+Add 20% dextrose solution	80 mL	

Yeast extract and peptone mixed with sterile water to a volume of 720 mL. Autoclaved.
Result: Stored in cold room. Not autoclaved correctly for 1 week, should consider if to use.

#3 Preparing Tris-HCl buffer

Aim: Making 1 M Tris-HCl buffer, pH 6.8 for SDS-Page gel electrophoreses

Experimenter: Sofia
Sample: -
Protocol: Mixing 60,56 g of Tris-base with water and adjusting pH to 6.8 with HCl to a final volume of 500 mL.
Result: A 1 M Tris-HCl buffer was prepared successfully with pH 6.8. Stored on shelf over lab-bench.

THURSDAY, 27/6/2019

#1 Preparing samples for SDS-Page

Aim: Preparing samples of supernatant and pellet of cultures of X-33 and KM71M for analyzing protein content with SDS-Page

Experimenter: Sofia, Tereza and Yannick
Sample: O/N culture #2 from 20190620
Protocol: 1. Transfering 100 µL of cultures into tubes.
2. Centrifugation at 17 000 g for 2 min.
3. Separate supernatant and pellet into different tubes.
4. Mixing 80 µL of supernatant with 20 µL of loading sample buffer.
5. Discard all remaing liquid from the pellet and resuspend it with 30 µL of loading sample buffer.
6. Boil in 95°C for 10 min.
Result: 3 samples of supernatant and 3 samples of pellet for both X-33 and KM71M were prepared and are stored on lab bench.

#2 Preparing 10% SDS-PAGE gel

Aim: Preparing SDS-PAGE gel for analyzing protein content of X-33 and KM71M

Experimenter: Sofia, Tereza, Yannick and Jonas
Sample: -
Protocol:

10% Separation Gel - 10mL (too much)

Table10

	A	B
1	H20	4.15 mL
2	Acrylamide/Bisacrylamide !fill in which exactly!!	3.3 mL
3	Tris-HCl 1.5M pH 8.8	2.5 mL
4	SDS 20%	50 µL
5	Amonnium persulfate (APS)	100 µL
6	TEMED (NNN'N'-tetramethylene-diamine)	10 µL
7		

Covered with isopropanol and left 30min to polymerize. Chamber was leaky a bit.

4% Stacking Gel - 10mL (way too much)

Table11

	A	B
1	H20	6.1 mL
2	Acrylamide/Bisacrylamide !fill in which exactly!!	1.3 mL
3	Tris-HCl 1.5M pH 8.8	2.5 mL
4	SDS 20%	50 µL
5	Amonnium persulfate (APS)	100 µL
6	TEMED (NNN'N'-tetramethylene-diamine)	10 µL

Result: Volume 10mL was too much for separation gel and way too much for stacking gel.

#3 Running SDS-Page gel

Aim: Analyzing protein content of supernatant and cells of X-33 and KM71M to be able to compare when starting to express HRP, AAO and GOD

Experimenter: Sofia, Tereza, Yannick and Jonas

Sample: Samples #1 20190627
Gel #2 20190627

Protocol: Running conditions: 80V after 30min 120V and after 1h 130V

Result:

FRIDAY, 28/6/2019

SATURDAY, 29/6/2019

SUNDAY, 30/6/2019

#1 Restreaks of X33 and KMK71H

Aim: fresh single colonies

Experimenter: Jonas

Sample:

Protocol:

Results:

Notebook week 1/7-7/7

Project: iGEM uppsala 2019
Authors: Sofia Larsson

MONDAY, 1/7/2019

#1 Restriction digestion for analyzation

Aim: Digest pPICV1284 to analyze plasmids from different cultures of DH5α.

Experimenter: Sofia and Yannick
Sample: Miniprepped pPIC RV1284 from colony VII-X, from #1 20190626
Protocol: Digestion of DNA-plasmid
Final volume 20 µL.
The following were added together in that order for a final volume of 20 µL:

Table3

	A	B	C	D	E
1	**Material**	**VII**	**VIII**	**IX**	**X**
2	Water (µL)	9.29	11.5	10.56	10.77
3	Plasmid solution (µL, 200 ng plasmid)	200/25.3=7.91	200/35.1=5.70	200/30.1=6.64	200/31.1=6.43
4	Reaction buffer (Buffer 2.1, biolabs, 10x, #B7202S) (µL)	2	2	2	2
5	HIND3	0.4	0.4	0.4	0.4
6	BAMH1	0.4	0.4	0.4	0.4

The tubes were on ice while the reaction buffer and enzymes were added. The tubes were then incubated at 37°C for 30 min., heat inactivated at 80°C for ~20 min.

Results: pPIC RV1284 plasmid from colony V digested with HIND3 or BAMH1 respectively. Loaded onto a agarose gel for analyzation.

#2 Agarose gel for analyzation of digestion

Aim: Analyze the digested plasmids of pPIC RV1284 from different cultures of DH5α.

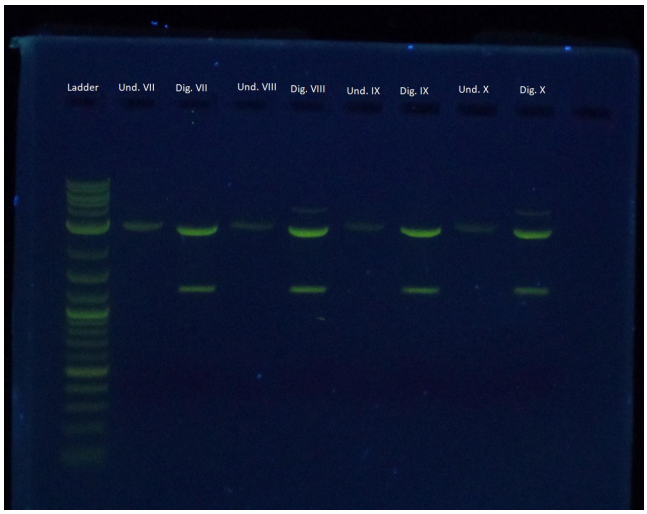
Experimenter: Sofia and Yannick
Sample: Digested pPIC RV1284 from colony VII-X, from #1 20190701, and the same undigested plasmids pPIC RV1284 from colony VII-X from #1 20190626
Protocol: 1% agarose gel preparation
4µL 6x purple loading dye #B7025S was added to each of the 20µL digested samples and to 20 µL samples of 200ng undigested plasmid in water (see #1 table 3 for the amounts of plasmid solution corresponding to 200 ng)
Gel put in electrophoses machine and ladder and samples loaded. Run at 120 Volt, 100 milliampere for about 1 h.
Samples loaded as follows:

Table2

	A	B	C	D	E	F	G	H	I
1	Ladder	VII undigested	VII digested	VIII und.	VIII dig.	IX undig.	IX dig.	X und.	X dig.

Results:

20190701_pPIC RV1284 analyticdig bamhi hindiii, undig,dig.jpg



The digested DNA from colonies VII and IX show 2 bands, from colonies VIII and X show 3 bands. VII and IX were restreaked (#4) in order to get both types of plasmids.

#3 Restriction digestion for electroporation transformation

Aim: Linarization of pPIC RV1284 plasmids for electroporation transformation of competent X-33 yeast cells.

Experimenter: Sofia
Sample: Miniprepped pPIC RV1284 from colony VII-X, from #1 20190626
Protocol: Digestion of DNA-plasmid
Final volume 50 µL, enzyme Sac1
The following were added together in that order for a final volume of 50 µL:

Table1

	A	B	C	D	E
1	**Material**	**VII**	**VIII**	**IX**	**X**
2	Water (µL)	23.24	28.75	26.39	26.92
3	Plasmid solution (µL, 500 ng plasmid)	500/25.3=19.76	500/35.1=14.25	500/30.1=16.61	500/31.1=26.92
4	Reaction buffer (for Sac1) (µL)	5	5	5	5
5	Sac1	2	2	2	2

The tubes were on ice while the reaction buffer and enzymes were added. The tubes were then incubated at 37°C for 30 min., heat inactivated at 80°C for ~20 min.

Results: Stored in frige.

#4 Restreaking of analyzed colonies

Aim: Getting plates with only single colonies of colonies VII and X analyzed in #1 and #2 20190701

Experimenter: Yannick

Sample: Liquid cultures VII and X from #5 from Tuesday 25/6/2019

Protocol:

#5 Preparation of sodium acetate

Aim: Preparing 3 M sodium acetate for electroporation transformation protocol

Experimenter: Sofia

Sample: -

Protocol: 2.46 g of sodium acetate mixed with deionized water to a final volume of 10 mL.

Calculation: M=82.02 g/mol

3 M*0.010 L=0.03 mol

82.03 g/mol*0.03 mol=2.4609 g

Result: 3 M sodium acetate prepared and stored on shelf over lab bench.

#6 YPD media for EasySelect Electroporation

Aim: Prepare 800mL YPD media with dextrose autoclaved separately and added after cooling down the media

Experimenter: Tereza

Sample: -

Protocol:

8g yeast extract

16g peptone

in 720mL

80mL 20% dextrose added later

Result: 800 mL YPD with autoclaved glucose added *ex post.*

#7 O/N culture *P. pastoris* X33 for EasySelect Electroporation

Aim: Prepare *P. pastoris* X33 for electroporation

Experimenter: Tereza

Sample: O/N culture from Sunday 30th June [missing reference in benchling]

Protocol: At 16:25 500mL YPD inoculated by 250 uL of O/N culture, OD_{600} of inoculum high ~ 20.6, incubated in 28°C while shaking

Result:

TUESDAY, 2/7/2019

#1 Restriction digestion for electroporation transformation

Aim: Linarization of pPIC RV1284 plasmids for electroporation transformation of competent X-33 yeast cells.

Experimenter: Sofia

Sample: Miniprepped pPIC RV1284 from colony VII-X, from #1 20190626

Protocol: Digestion of DNA-plasmid

Final volume 50 µL, enzyme Sac1

The following were added together in that order for a final volume of 50 µL:

Table4

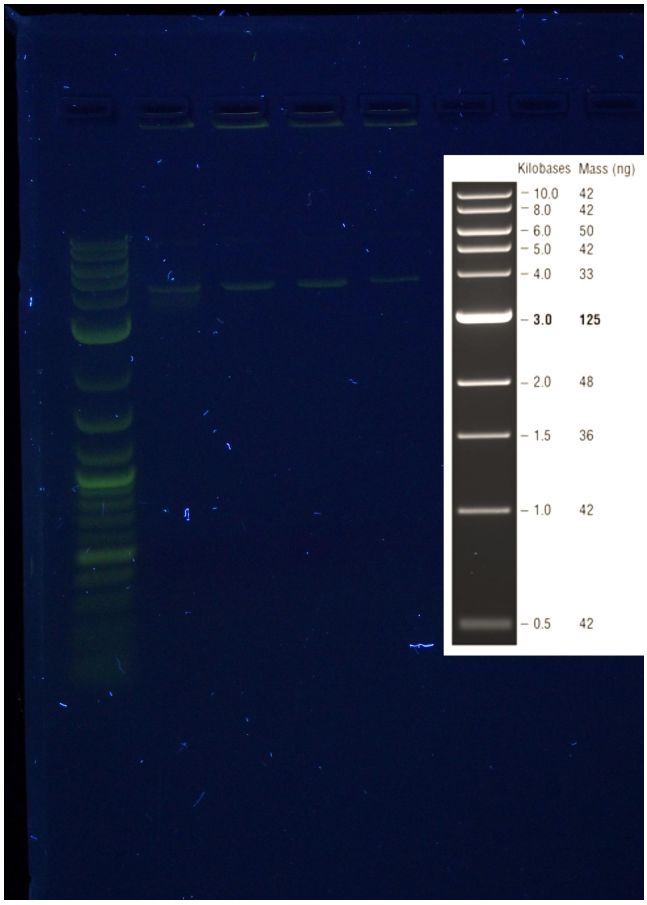
	A	B	C	D	E
1	**Material**	**VII**	**VIII**	**IX**	**X**
2	Water (µL)	23.24	28.75	26.39	26.92
3	Plasmid solution (µL, 500 ng plasmid)	500/25.3=19.76	500/35.1=14.25	500/30.1=16.61	500/31.1=26.92
4	Reaction buffer (for Sac1) (µL)	5	5	5	5
5	Sac1	2	2	2	2

The tubes were on ice while the reaction buffer and enzymes were added. The tubes were then incubated at 37°C for 30 min., heat inactivated at 80°C for ~20 min.

Analyzation on agarose gel.

Results: Stored in frige. Agarose gel shows that the plasmid was not completely digested, increase incubation time to 45 min for next digestion with SacI.

20190702 agarose gel SacI.jpg



#2 Preparation of DNA for electroporation transformation

Aim: Preparing linearized DNA for EasySelect electroporation of competent X-33 yeast cells.

Experimenter: Sofia
Sample: Miniprepped pPIC RV1284 from colony VII-X, from #1 20190626
Protocol: DNA preparation using phenol/chloroform
Results: 10ul, unknown cncentration

#3 Analytical double digest of colonies I-IV

Aim: Digest pPIC RV1284 with BAMH1 & HIND3 to analyze plasmids from different cultures of DH5α (Colonies I-VI)

Experimenter: Yannick & Sofia
Sample: Miniprepped pPIC RV1284 from colony I-VI, from miniprep #2 20190625
Protocol: Digestion of DNA-plasmids I-VI by adding together the following in that order for a final volume of 20 µL:

Table5

	A	B	C	D	E	F	G
1	**Material**	**I**	**II**	**III**	**IV**	V	VI
2	Water (µL)	10.53	10.33	9.88	10.87	11.25	12.78
3	Plasmid solution (µL, 200 ng plasmid)	6.67	6.87	7.32	6.33	5.95	7.22
4	Reaction buffer (Buffer 2.1, biolabs, 10x, #B7202S) (µL)	2	2	2	2	2	2
5	HIND3	0.4	0.4	0.4	0.4	0.4	0.4
6	BAMH1	0.4	0.4	0.4	0.4	0.4	0.4

The tubes were on ice while the reaction buffer and enzymes were added. The tubes were then incubated at 37°C for 30 min., heat inactivated at 80°C for ~20 min.

Results: Doubly digested pPIC RV1284 plasmids from colonies I-VI that were then analysed on an agarose gel at #4 20190702

#4 O/N culture *P. pastoris* KM71H

Aim: Create a O/N culture for EasySelect electroporation

Experimentator: Sofia
Sample: Colonie I from #3 20190624 KM71H.
Protocol: 5 mL of 1xYPD media added to a 50 mL e-flask. Colonie I from Sofias plate from 20190624 taken and diluted in the media. Culture started at 16.45, incubated in 28℃ on shaker.
Result: Inoculated culture of KM71H in 28℃ on shaker.

#5 O/N culture *DH5α* pPCI RV1284

Aim: Create a O/N culture for miniprep 20190703

Experimentator: Sofia
Sample: Colonie I from X #4 20190701 *DH5α* pPCI RV1284.
Protocol: 20 mL of LS-LB media with Zeocin.
Colonie I from Sofias plate from 20190624 taken and diluted in the media. Culture started at 17.30, incubated in 37℃ on shaker.
Zeocin calculation: Wanted concentration: 25 µg/mL. Concentration in stock 100 mg/mL.
20mL*25µg/mL=500µg
0.500mg/100mg/mL=0.005 mL=5 µL
Result: Inoculated culture of *DH5α* pPCI RV1284 in 37℃ on shaker.

#6 Preparation of competent cells *P. pastoris* X33 for EasyMan Electroporation

Aim: Prepare *P. pastoris* X33 for electroporation
Experimenter: Tereza

Sample: O/N culture #7 20190701

Protocol:

At 8:45 OD_{600} reached 1.6, working on ice.

1. Cells centrifuged at 1,500 × g for 5 minutes at 4°C. Pellet resuspended with 500 ml of ice-cold, sterile water.
2. Cells centrifuged as in Step 1. Pellet resuspended with 250 ml of ice- cold, sterile water.
3. Cells centrifuged as in Step 1. Pellet resuspended in 20 ml of ice-cold 1 M sorbitol.
4. Cells centrifuged as in Step 1. Pellet resuspended in 1 ml of ice-cold 1 M sorbitol.
5. Aliquot the cells into 80 uL.

Result:

Cells ready for EasyMan electroporation.

#7 EasyMan Electroporation of *P. pastoris* X33

Aim: *P. pastoris* X33 electroporation

Experimenter: Tereza, Yannick, Sofia, Jonas

Sample: Competent cells #6 20190702, Plasmid DNA (10ul, unknown concentration) #2 20190702

Protocol: 0.2 cuvette, U = 1.5 kV, C = 25uF, R = 200 ohm

Result:

Electroporation time constants:

Table9

	A	B
1	time constant [ms]	
2	4.9	
3	4.7	
4	4.7	
5	4.7	
6	4.5	
7	4.5	
8	4.4	
9	4.4	
10	4.0	negative control

No transformants.

#8 Preparing 1xYPD medium

Aim: Making 800mL 1x YPD medium

Experimenter: Sofia

Sample: -

Protocol:

Table7

	A	B	C
1	**Chemical**	**Amount (g)**	
2	Yeast extract	8	
3	Peptone	16	
4	+Add 20% dextrose solution	80 mL	

Yeast extract and peptone mixed with sterile water to a volume of 720 mL. Autoclaved. Dextorse solution added.

Result: Stored in cold room.

#9 Agarose gel electrophoresis analysis of double digest

Aim: Analyze the undigested and doubly digested pPIC RV1284 plasmids from DH5α Colonies I-VI on an agarose gel.

Experimenter: Yannick & Sofia

Sample: Miniprepped pPIC RV1284 from colony I-VI, from miniprep #2 20190625, and doubly digested pPIC RV1284 from colony I-VI from digest #3 20190702

Protocol:

Three 1% agarose gels were prepared (0.5 g agarose in 50 mL 1x TBE solution, heated to boiling in microwave. 5 µL of 10,000x SybrSafe was added and the gels poured into clean molds when the gel was ~<70°C) Bubbles were removed with pipette tips and a comb was inserted to form the loading wells.

4µL 6x purple loading dye #B7025S was added to each of the 20µL digested samples and to 20 µL samples of 200ng undigested plasmid in water (see #3 table 5 for the amounts of plasmid solution corresponding to 200 ng). Two gels were put in electrophoresis machine and 5µL ladder and ~20 µL samples loaded. Run at 120 Volt, 100 milliampere for about 1 h.

Samples loaded as follows onto the two gels:

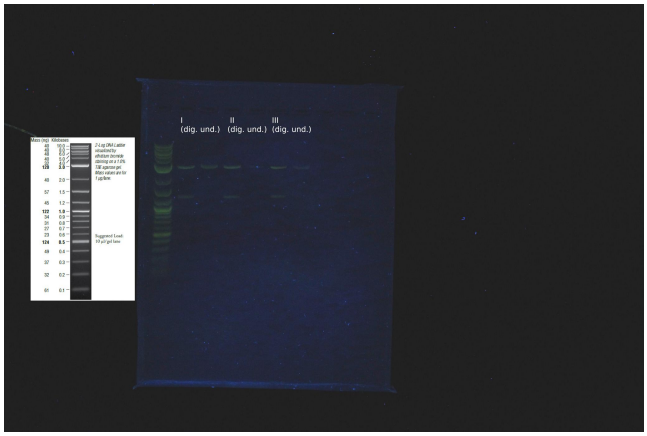
Table6

	A	B	C	D	E	F	G	H	I	J
1	Gel 1									
2	Ladder	I (digested)	I (undigested)	II (dig.)	II (undig.)	III (dig.)	III (undig.)	empty	empty	empty
3	Gel 2									
4	empty	IV (digested)	IV (undigested)	V (dig.)	V (undig.)	VI (dig.)	VI (undig.)	Ladder	empty	empty

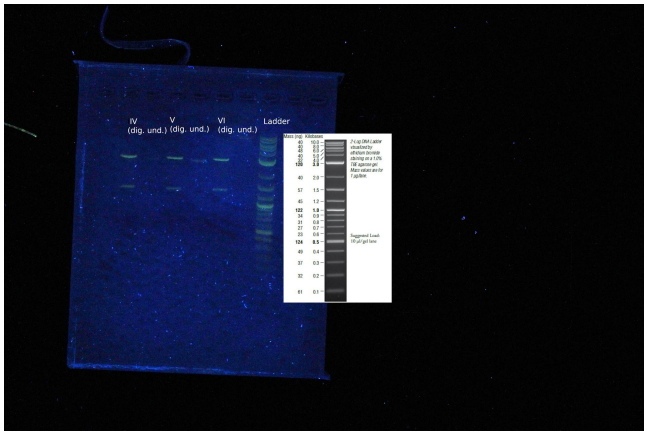
The gel was then run @120V, 100A for ~1.5 hours. Then the gels were taken out, transported to the picture room on a paper towel. A picture was taken of each of the gels with UV backlight.

Results:

20190702 PPIC RV1284 I-III Digest BamH1+HIND3 Yannick (edited).jpeg



20190702 PPIC RV1284 IV-VI Digest BamH1+HIND3 Yannick (edited2).jpeg



All of the colonies I-VI showed the same results: 2 bands at ~3.0 kb and ~1.3 kb for the plasmids digested with BAMH1 and HIND3. And only 1 band at ~3.0 kb for the undigested plasmids.

WEDNESDAY, 3/7/2019

#1 Preparation of various solutions

Aim: Prepare the following solutions:

-500mL 1M Tris-HCl pH 8.0 (autoclaved)
-100mL 0.2 M NaOH 1% SDS
-50mL 3M Sodium Acetate solution pH 5.3
-100mL 0.5M EDTA pH 8.0 (autoclaved)
-200mL TE buffer

Experimenter: Yannick & Sofia
Sample: -
Protocol:
100mL 0.2 M NaOH 1% SDS was prepared from earlier stock solution by 20mL 1M NaOH + 5 mL 20% SDS + 75mL destilled water.

500mL 1M Tris-HCl pH 8.0 (autoclaved) was prepared by dissolving 1M*0.5L*121.14 g/mol = 60.57 g Tris Base into ~350mL of water, adjusting the pH to 8.0 with 10% HCl, diluting to a final volume of 500mL and doing a final pH adjustment with 10%HCl to pH 8.0. It was then autoclaved.
100mL 0.5M EDTA pH 8.0 (autoclaved) was prepared by dissolving (0.5M*0.1L=0.05mol; 0.05mol*292.24g/mol=14.612g) 14.61 g of EDTA in 60 mL of water, adding NaOH pellets to raise the pH to ~8.0, diluting to a final volume of 100mL and doing a final pH adjustment with 10%HCl and 1M NaOH to pH 8.0. The EDTA was made to dissolve by the high pH and by heating the measuring cylinder with hot water streaming along the sides. The solution was then autoclaved.
50mL 3M Sodium Acetate solution pH 5.3 was prepared by dissolving (3M*0.05L=0.15mol; 0.15*82.02g/mol=12.3045g) 12.30 g of sodium acetate to 30 mL of water, adding 10% HCl to lower pH to ~5.3. Adding water to final volume of 50 mL, adjusting last pH with 10% HCl and 1 M NaOH.
200mL TE buffer was prepared by mixing 2 mL 1M Tris-HCl (pH 8.0) and 0.4 mL EDTA and adding water to the final volume of 200 mL. The solution was then autoclaved.

Result: These solutions were stored above the lab benches.

#2 Transformation of backbone pSB1A3 and pSB1K3

Aim: Transform *DH5α* to amplify backbone for later use

Experimenter: Sofia and Johanna
Sample: DNA from registry: Plate 6, 2G for pSB1A3 and, plate 6, 6A for pSB1k3.
Competent cells 13/09 form -80°C, 150 µL.
Protocol: Protocol 6 in Syntetic biology:
Step 3: 1 µL DNA/water used instead of 5 µL.
Result: Bacterial lain on all the ampicilin plates. Probably something wrong with the ampicilin stock. No growth on the Cm plates, because the wrong antibiotic was used for this plasmid. Plates discarded.

#3 Miniprep pPICZ*α* RV1284 from DH5*α* E. coli

Aim: Prepare plasmid DNA for EasyMan Electroporation

Experimenter: Tereza
Sample: #5 O/N 20190702
Protocol: 4 minipreps, 2 preparation kits were combined, Sigma Aldrich protocol followed until step 5, then Thermo scientific further on. Eluted in 50uL each and pooled tgether. Concentration measured by Nanodrop.
Result: 200uL, 55.1ng/uL, pPICZalfa RV1284

#4 Inoculation of 500 mL O/N culture

Aim: Prepare a 500mL O/N culture for EasySelect electroporation transformation

Experimenter: Yannick
Sample: Yesterday's 5mL O/N KM71H culture: #4 20190702
Protocol: Inoculate 500 ml of fresh YPD medium in a 2 liter flask with 0.25 ml of the 5mL O/N culture.
Result: The 500mL KM71H O/N culture was left to grow in the shaker at 28*C. The OD_{600} was 15.1 at 17.45. [check that this measurement was for this culture and not yesterdays smaller overnight culture with Tereza]

#5 Increase concentration of pPICZ*α* RV1284 Miniprep

Aim: Increase DNA concentration

Experimenter: Tereza
Sample: #3 O/N 20190703
Protocol:
200uL of plasmid

20 uL 3M sodium acetate pH 5.3
500 uL of ice-cold 100% ethanol
1h in 20°C, then centrifuged, no pellet observed, washed with 70% ethanol and dissolved in 10uL sterile water

Result: catastrophe - 9uL of 145.5ng/uL

#6 O/N culture *DH5α* with pPICZ*α* with RV1284

Aim: Create a O/N culture for miniprep 20190704

Experimentator: Tereza
Sample: Colonie I from #4 20190701 *DH5α* pPICZ*α* RV1284. Really? That is a reference to plates with only colonies VII and X on them.
Protocol: 10 mL of LS-LB media with Zeocin 25uL/mL inoculated from which plate. Cultivation started at ~ 19:00, incubated in 37°C on shaker. 2.5 uL Zeocin added after inoculation.
Result: 10ml culture of *DH5α* pPCIZ*α* RV1284 in 37°C on shaker.

THURSDAY, 4/7/2019

#1 Electrocompetent *P. pastoris* KM71H preparation

Aim: generate electrocompetent pichia cells for transformation
Experimenter: Jonas
Sample: o/N culture KM71H #4 03.07.19
Protocol: measure OD (got 1.22)
let grow for 30 more min
harvest cells by centrifugation 5 min 1500g 4 °C
resuspend pellets in 500 mL icecold sterile water
centrifuge as above
resuspend in 250 mL icecold sterile water
centrifuge as above
resuspend in 20 mL icecold 1M sorbitol
centrifuge as above
resuspend in 1 mL icecold 1M sorbitol
keep on ice at all time and use the same day
results: well we will see if that worked

#2 Linearising pPIC RV1284 for transformation

Aim: prepare vector for transformation
Experimenter: Jonas
Sample: #5 20190703
Protocol: all of the sample (10 uL with 1ug dna) was digested in 50 uL volume with 2 uL of SacI enzyme
digestion was done 2h on 37 °C and afterwards heat inactivated at 80 °C 20 min
Results: 5 uL were loaded on a 1% agarose gel to check if digest was efficient
no leftover plasmid was detected

#3 Miniprep pPIC RV1284

Aim: get dna for transformation

experimenter: Jonas
Sample: O/N culture #6 20190703
Protocol: according to manufactures instructions (thermo)
eluted in 50 uL ddH2O and both minipreps were pulled together

Results: 125,3 ng/uL concentration

#4 Linearising pPIC RV1284

Aim: prepare vector for transformation

Experimenter: Jonas
Sample: miniprep #3 04.07.2019
protocol: 40 uL DNA (5 ug)
5 uL buffer
2 uL SacI
3 uL H2O
37 °C for 1h 15 min
80°C 20 min
results: DNA was not analysed and directly used for transformation

#5 Transformation of KM71H

Aim: get colonies to express RV1284

Experimenter: Jonas
Sample: cells from #1 04.07.2019, DNA from #2 and #4 04.07.2019
Protocol: 90uL cells were mixed with the whole digest (ca. 40-50uL) or 40 uL ddH2O
cells were incubated in 0.2 cm electroporation cuvettes for 5 min
cells were shocked in electroporator with following parameters

Table8

	A	B
1	V	1.5 kV
2	C	25 uF
3	R	200 ohm

cells were resuspended immideatly with 1M icecold sorbitol 1 mL and transfered into 15 mL falcon
incubated at 28 °C for 1 h 15 min
plated on ypds + zeocin (100ug/mL) and incubated at 28 °C for several days

Result: Colonies appeared on 8th July (after 4 days). Massive growth on negative control plate however, the plate was destroyed bz the process of plating.

FRIDAY, 5/7/2019

#1 Transformation of backbone pSB1A3 and pSB1k3

Aim: Transform *DH5α* to amplify backbone for later use

Experimenter: Sofia

Sample: DNA from registry: Plate 6, 2G for pSB1A3 and, plate 6, 6A for pSB1k3.
Competent cells 13/09 2018 from -80°C, 150 µL.

Protocol: Protocol 6 in Syntetic biology:
Step 3: 1 µL DNA+4 µL water used.

Result:

SUNDAY, 7/7/2019

#1 Linearising pPIC RV1284

Aim: Making a SacI digest of 5µg of pPIC RV1284 for later transformation

Experimenter: Yannick

Sample: miniprep #3 04.07.2019

Protocol: protocol: 40 µL DNA (5 µg)
5 µL buffer
3 µL H2O
2 µL SacI
37 °C for 30 min.
80°C 20 min.

Results: DNA was not analysed and frozen in the yiest priest box in the second drawer of the freezer.

#2 Two minipreps of pSB1K3-RFP

Aim:

Experimenter: Teresa & Yannick

Sample: *DH5α* pSB1K3 culture from #1 friday 05.07.2019 [edit when Jonas writes an entry on the O/N culture he made on saturday]

Protocol: Thermo scientific kit used, protocol followed. Resuspension solution from Sigma used. Eluted in 50uL of Thermo elution buffer.

Results: 2 minipreps, stored in the -20°C freezer yeast-priest box.

Table10

	A	B	C
1	c [ng/uL]	260/280	260/230
2	290.2	1.90	2.23
3	133.5	1.92	2.18

#3 Phenol/chloroform extraction of the SacI digest

Aim: Extract the linearized pPIC RV1284 DNA from the digest for later use in transformation.

Experimenter: Yannick

Sample: Digested pPIC RV1284 from #1 07.07.2019

Protocol: Added 50 µL of 25:24:1 Phenol:Chloroform:Isoamyl alcohol to the 50 µL of linearized pPIC RV1284.
Vortexed ~20 seconds
Centrifuged 5 min. at 16.100 g
Transfered the upper aqueous phase (~35 µL) to a fresh tube.

Added 0.1 volume 3M sodium acetate (3.5 µL) and 2.5 volume 100% ethanol (87.5 µL). Mixed by pipetting and stored in the freezer O/N

Results: Left the DNA to precipitate in the freezer O/N. Stored in the 2nd drawer of the freezer in the yeast-priest box.

#4 Overnight culture X-33

Aim: get culture for electrocompetent cells

Experimenter: Jonas

Sample: o/N culture from x-33 day before

protocol: 500 mL YPD inoculated with 200 uL preculture

incubated 28 °C shaking o/N

Notebook week 8/7-14/7

Project: iGEM uppsala 2019
Authors: Sofia Larsson

MONDAY, 8/7/2019

#1 Preparing YPDS+Zeocin Agar

Aim: Creating YPDS agar plates for later use.

Experimentator: Sofia
Sample: -
Protocol:

Table6

	A	B
1	**Chemical**	**Amount**
2	Yeast extract	8 g
3	Peptone	16 g
4	Sorbitol	145,8 g
5	Agar	20 g
6	Dextrose 20%	80 mL

Yeast extract, peptone and sorbitol solved in ddH_2O to a final volume of 720 mL. Agar added. Solution autoclaved. Destrose stock-solution added.
Result: Plates stored in cold room.

#2 Transformation of *DH5α* with pSB1A3

Aim: Transformation for amplifying backbone pSB1A3.

Experimentator: Sofia
Sample: Competent cells *DH5α* form 190618
DNA from registry: Plate 6, 2G for pSB1A3.
Protocol: Protocol 6 in Synthetic biology labmanual.
Step 3: 1 µL DNA+4 µL water used.
Result: Red colonies on plate with concentrated cell plated. A few colonies on the non concentrated plate and no colonies on negative control.

#3 Harvested precipitated pPIC RV1284

Aim: Getting the precipitated SacI linearized pPIC RV1284 into a concentrated water solution for later transformation.

Experimenter: Yannick
Sample: The precipitated DNA from sunday 190707 #3
Protocol: The solution was centrifuged @max (16100 g) for 5 minutes, and then an additional 30 min @max
There was no visible pellet but knowing where the pellet should be the supernatant was taken away

The pellet was washed with 200 µL 80% ethanol
Almost all of the ethanol was taken away and the pellet was left to air-dry for ~20 min.
Vortexed and spun down after resuspending in 10 µL of water. Stored in fridge.
The concentration was measured to 182.0 ng/µL. Recovered almost the entire 1 µL used for measuring the DNA concentration so there is a total of around 10 µL * 182.0 ng/µL = 1.82 µg of DNA in the tube.

Results: <10 µL of 182.0 ng/µL pPIC RV1284 in water (Total 1.82 µg of DNA) to be used for transformation (#7 190708, today)

#4 Preparing YPD Agar + zeocin plates

Aim: Preparing YPD Agar + zeocin plates for later use.

Experimenter: Yannick

Sample: -

Protocol:

The following ingredients were dissolved into water to make 500 mL final solution and autoclaved.

Table1

	A	B
1	Compound	Amount
2	Yeast extract	5g
3	Peptone	10g
4	Dextrose	10g
5	Agar	10g

When the autoclaved YPD Agar solution had cooled down significantly 500 µL of 100mg/mL zeocin stock was added to the 500 mL of medium to give a zeocin concentration of 100 µg/mL. Plates were poured around a flame.

Results: A little over 20 plates of YPD Agar + zeocin plates stored in the cold room.

#5 Measuring OD_{600} for O/N X33

Aim: Determining whether the culture of X33 has grown enough to start the EasySelect Competent Cell preparation protocol

Experimenter: Hugo

Sample: *P. Pastoris*, X33 O/N culture from sunday 7/7 #4

Protocol: 100 µL of the culture (in YPD-media) was diluted to a total volume of 1 mL with water. A negative test was done with 1:10 diluted YPD-media. The tests were transfered to cuvettes and was analyzed with the spectrofotometer.

Results: Abs=0.138 and the OD was ca 1.38.

#6 Making competent cells, X33

Aim: Preparing the X33 culture for electrophoration

Experimenter: Hugo

Sample: P. Pastoris, X33 O/N culture from sunday 7/7 #4

Protocol: Preparing Pichia for Electroporation p.27 in EasySelect Pichia Expression Kit User Manual

1. Grow 5 ml of your Pichia pastoris strain in YPD in a 50 ml conical at
30°C overnight.

2. Inoculate 500 ml of fresh medium in a 2 liter flask with 0.1–0.5 ml of the overnight culture. Grow overnight again to an OD_{600}= 1.3–1.5.
3. Centrifuge the cells at 1,500×g for 5 minutes at 4°C. Resuspend the pellet with 500 ml of ice-cold, sterile water.
4. Centrifuge the cells as in Step 3, then resuspend the pellet with 250 ml of ice-cold, sterile water.
5. Centrifuge the cells as in Step 3, then resuspend the pellet in 20 ml of ice-cold 1 M sorbitol.
6. Centrifuge the cells as in Step 3, then resuspend the pellet in 1 ml of ice-cold 1 M sorbitol for a final volume of approximately 1.5 ml. Keep the cells on ice and use that day. Do not store cells.

Instead of using x1 500 mL centrifuge tube x10 50 mL Falcon tubes were used.

Results:

#7 Transformation of X33 with pPICZ RV1284

Aim: To try out the EasySelect electroporation protocol for X33

Experimenter: Tereza
Sample: linearized pPIC RV1284 #3 20190708, *P. Pastoris*, X33 #6 20190708
Protocol: Transformation by EasySelect Electroporation p.28 in EasySelect Pichia Expression Kit User Manual

1. Mix 80 µl of the cells from Step 6 (previous page) with 5–10 µg of linearized DNA (in 5–10 µl sterile water) and transfer them to an ice-cold 0.2 cm electroporation cuvette.
Note: For circular DNA, use 50–100 µg.
2. Incubate the cuvette with the cells on ice for 5 minutes.
3. Pulse the cells using the manufacturer's instructions for *Saccharomyces cerevisiae.*
4. Immediately add 1 ml of ice-cold 1 M sorbitol to the cuvette. Transfer the cuvette contents to a sterile 15-ml tube and incubate at 30°C without shaking for 1 to 2 hours.
5. Spread 10, 25, 50, 100, and 200 µl each on separate, labeled YPDS plates containing 100 µg/ml Zeocin™. Plating at low cell densities favors efficient Zeocin™ selection.
6. Incubate plates from 3–10 days at 28°C until colonies form.
7. Pick 10–20 colonies and purify (streak for single colonies) on fresh YPD or YPDS plates containing 100 µg/ml Zeocin™

Deviations from protocol: Ca 12 µL of the linearized DNA was used (all of it). The instrument settings for the electroporation machine was V = 1.5 kV; C=25 µF; R=200 Ω; t=4-10 ms. In step 4 the culture was incubated for ca 2 hours. Plating was done as indicated, for negative control (consisting of competent cells and water instead plasmid DNA) 50 µl was plated. Cells incubated without shaking in 28°C.

Results: Time constants 4.7 ms for sample and 4.6 for negative control. Thursday 20190711 only 1 single colony formed and restreaked .

#8 Restriction double digestion of pSB1K3-RFP plasmid

Aim: We need to amplify pSB1K3 backbone. Cleave pSB1K3 backbone and later isolate it from the agarose gel.

Experimenter: Tereza
Sample: 2 Minipreps #2 20190707
Protocol: 500 ng of plasmid pSB1K3 DNA, 1uL of enzymes EcoRI and PstI, 5uL 10X Buffer O and sterile ddH2O till final volume 50uL. Gently mixed by tapping on the tube, spinned down. 45min 37°C incubation, 20min 80°C heat inactivation. Then stored on bench and on ice, then -20°C freezer yiest-priest box.
Results: 2 x 50uL of psB1K3

#9 agarose gels 1%, 3 pieces

Aim: Prepare agarose gel for DNA gel purification and 2 gels for use by anybody

Experimenter: Tereza

Sample: -

Protocol:

- 50 mL 1x TBE buffer
- 0.5 g agarose
- 5 µL SYBR safe

Agarose dissolved in TBE by heating up in microvawe, carefully cooled down in cold tap-water before SYBR safe was added and gels casted. One gel was prepared in sterile flask and using sterile measuring cilinder.

Results: 1 gel used by other group, 2 gels stored in cold-room (before they were few hours uncovered on the bench).

#10 Prepared 1M sorbitol

Aim: Prepared 100 mL of sterile 1M sorbitol solution for use in electroporation transformation protocol

Experimenter: Yannick

Sample: -

Protocol: Sorbitol molar weight = 182.172 g/mol so needed 0.1L*1M*182.172 g/mol = 18.2127g of sorbitol
Dissolved 18.21 g of sorbitol into water to get 100 mL of final solution.
Sterile-filtered into two 50mL falcons.

Results: 2 50mL falcons with 1M sorbitol stored in the lab fridge.

#11 O/N culture of DH5α pPICZα RV1284 and DH5α pPICZα

Aim: Making 5 mL O/N culture of DH5α pPIC RV1284 and DH5α pPICZα for minipreps and glycerol stocks tomorrow

Experimenter: Yannick & Sofia

Sample: [insert exakt reference: DH5α pPICZα from plates that Lilly made? Ask Johanna]. DH5α pPIC RV1284 colony 1 from the plate from #1 20.6.2019

Protocol: Needed a 25 µg/mL concentration of zeocin in the media and the stock solution had a concentration of 100mg/mL so added (10mL*25µg/mL)/100,000µg/mL = 2.5 µL zeocin to 10 mL of LSLB.
Divided the 10mL of LSLB + zeocin into two 5mL samples. Inoculated them with DH5α pPIC RV1284 and DH5α pPICZα respectively.

Results: Two different O/N cultures (DH5α pPICZα RV1284 and DH5α pPICZα) on the shaker in the 37°C

#12 Analyzation of transformed KM71H on plates

Aim: Analyze result of transformation

Experimenter: Sofia (Johanna, Hugo)

Sample: Plates from 20190704 #5

Protocol:

Results:

Table2

	A	B
1	**Plate**	**Number of colonies**
2	10 uL	11
3	25 uL	Gel dried out, no colonies
4	50 uL (1 ug)	Smeere and lump in gel
5	50 uL (5 ug)	99
6	100 uL	147
7	200 uL	488
8	Neg. control 100 uL	Smeere

TUESDAY, 9/7/2019

#1 Prepared 50% glycerol

Aim: Make 50% glycerol stock for making glycerol stocks of bacteria.

Experimenter: Yannick

Sample: -

Protocol: Want to make 30mL of 50% glycerol out of 85% glycerol so need 50/85 *30mL=17.65 mL.

Dissolved ~17.7mL of 85% glycerol in water to a final volume of 30mL (rinsed the measuring cilinder for 85% glycerol with the water used for diluting it)

Autoclaved the 50% glycerol solution.

Results: 30mL of 50% glycerol stored on the shelf above Yannick's bench

#2 Two minipreps of pPICZα RV1284 an pPICZα

Aim: Prepare DNA for analyzation (compare with midiprep) and prepare plasmid for cloning-groups.

Experimenter: Hugo and Sofia

Sample: #11 O/N culture of DH5α pPICZα RV1284 and DH5α pPICZα

Protocol: Thermo scientific kit used, protocol followed. Resuspension solution from Sigma used. Eluted in 50 uL of sterile water.

Results: 2 minipreps, stored in the yeast fridge with concentrations:

Table3

	A	B	C
1		**pPICZa**	**RV1284**
2	**Concentration**	307.0 ng/uL	191,4 ng/uL
3	**260/280**	1,90	1,89
4	**260/230**	2,23	1,98

#3 Made glycerol stocks

Aim: Make glycerol stocks of DH5α with plasmids pPIC RV1284 and pPICZα

Experimenter: Yannick

Sample: 600 µL of each of the O/N cultures from #11 2019.07.08 and sterile 50% glycerol from #1 2019.07.09

Protocol: Added 400 µL of 50% glycerol to each of the 600 µL cultures and mixed by pipetting. Worked around flame.

Results: Two glycerol stocks of DH5α with plasmids pPIC RV1284 and pPICZα stored in the -80°C freezer in the box of bacterial glycerol stocks of iGEM 2019

#4 Concentration measurements of double digested pSB1K3 RFP

Aim: Measure DNA concentration for gel purification

Experimenter: Sofia

Sample: Double digested pSB1K3 RFP #8 20190708

Protocol: Nanodrop used. Blanked with elution buffer from Sigma.

Results:

Table4

	A	B	C
1		**#1**	**#2**
2	**Concentration**	26,4 ng/uL	24,9 ng/uL
3	**260/280**	1,04	1,02
4	**260/230**	0,29	0,29
5	**Total amount in tube (50 uL)**	1320 ng	1245 ng

#5 O/N culture of DH5α pSB1A3

Aim: Make 2 5mL cultures for glycerol stock and minipreps (to give the cloning groups more backbone)

Experimenter: Yannick

Sample: DH5α pSB1A3 from the 'centrifuged' plate from #2 monday 8.7.2019

Protocol: Inoculated 2 15mL falcons with 5mL of LB + Ampicillin (from the new ampicillin stock) with colonies I and II from the DH5α pSB1A3 'centrifuged' plate using pipette tips

Results: Incubated the tubes with somewhat unscrewed caps with shaking in the 37°C room

Saw the next day that two of the tips ended up in only 1 of the O/N culture tubes, so we had 1 culture without any bacteria and 1 5mL culture inoculated with 2 different colonies from the same DH5α pSB1A3 plate.

#6 O/N culture of DH5α pPICZα RV1284

Aim: Make a 50 mL O/N culture for midiprep tomorrow

Experimenter: Yannick

Sample: DH5α pPIC RV1284 colony I from the plate from #1 20.6.2019

Protocol: (made a mistake and only inoculated 5 mL of medium):

Needed a 25 µg/mL concentration of zeocin in the media and the stock solution had a concentration of 100mg/mL so added (5mL*25µg/mL)/100,000µg/mL = 1.25 µL zeocin to 5 mL of LSLB.

Inoculated with a pipette tip with colony I from the plate mentioned above.

Results: A 5mL O/N culture of DH5α pPIC RV1284 colony I incubating in the 37°C room with shaking with a slightly unscrewed lid.

#7 O/N culture of X-33

Aim: Preparation for transformation

Experimenter: Hugo

Sample: X-33 colony V from one of the plates done in #3 monday 20190624

Protocol: The colony was inoculated in 5 ml YPD

Results:

WEDNESDAY, 10/7/2019

#1 Miniprep of pPICZα RV1284 and pSB1A3

Aim: Harvesting the ?

Experimenter: Yannick & Hugo

Sample: 5 mL O/N cultures from #5 and #6 from tuesday 9.7.2019

Protocol: Followed miniprep protocol from Thermo Scientific miniprep kit. Used 4 mL of each culture. The resuspension buffer came from the other miniprep kit, lysed for ~4 min. and centrifuged at 16,100 g at most steps. ddH_2O was used instead of Elution buffer.

Measured the concentration and the purity of the DNA mixtures.

Results:

Table5

	A	B	C	D
1	Miniprep	Concentration (ng/µL)	A260/280	A260/230
2	pPICZα RV1284 (colony I)	114.8	1.89	2.04
3	pSB1A3 (colony I+II)	82.2	1.83	1.68

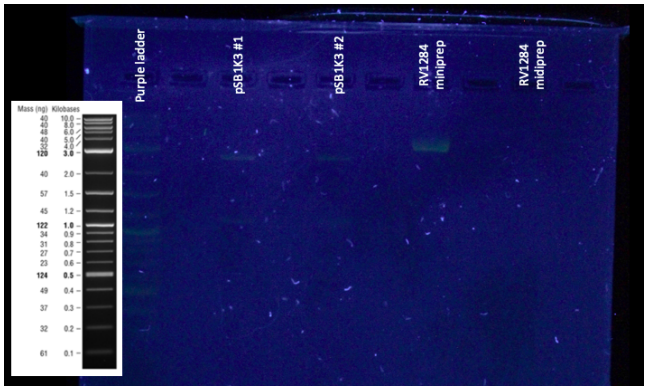
#2 Gel Extraction of pSB1K3 and pPICZα RV1284 DNA

Aim: To get clean backobone pSB1K3 DNA and clean plasmid pPICZα RV1284 DNA from Midiprep

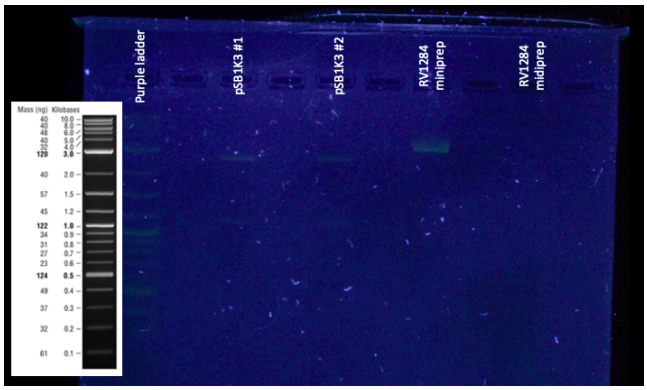
Experimenter: Tereza, Sofia, Yannick, Hugo

Sample:

Screenshot 2019-07-10 16.43.45.png



Screenshot 2019-07-10 16.43.45.png



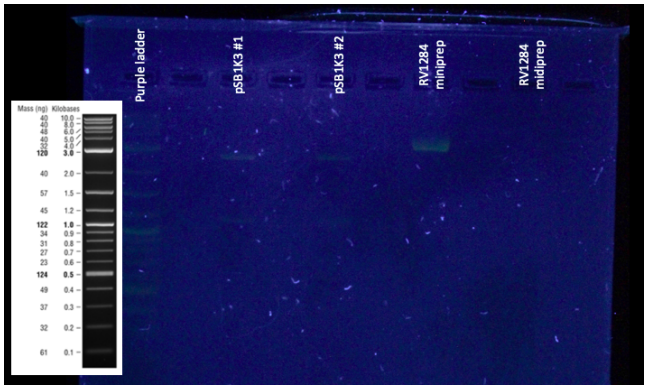
Protocol:

1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 mL tube and weigh. Record the weight of the gel slice. *Note: If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure. Minimize UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV illumination*.
2. Add 1:1 volume of Binding Buffer to the gel slice (volume: weight) (e.g., add 100 µL of Binding Buffer for every 100 mg of agarose gel). *Note: For gels with an agarose content greater than 2%, add 2:1 volumes of Binding Buffer to the gel slice*
3. Incubate the gel mixture at 50-60 °C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved. Vortex the gel mixture briefly before loading on the column. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
4. Optional: use this step only when DNA fragment is ≤500 bp or >10 kb long. *If the DNA fragment is ≤500 bp, add 1 gel volume of 100% isopropanol to the solubilized gel solution (e.g. 100 µL of isopropanol should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer). Mix thoroughly. If the DNA fragment is >10 kb, add 1 gel volume of water to the solubilized gel solution (e.g. 100 µL of water should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer). Mix thoroughly.*
5. Transfer up to 800 µL of the solubilized gel solution (from step 3 or 4) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube. *If the total volume exceeds 800 µL, the solution can be added to the column in stages. After each application, centrifuge the column for 30-60 s and discard the flow-through after each spin. Repeat until the entire volume has been applied to the column membrane. Do not exceed 1 g of total agarose gel per column.* Close the bag with GeneJET Purification Columns tightly after each use!

6. Optional: use this additional binding step only if the purified DNA will be used for sequencing. Add 100 µL of Binding Buffer to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
7. Add 700 µL of Wash Buffer (diluted with ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
8. Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove residual wash buffer. *Note. This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.*
9. Transfer the GeneJET purification column into a clean 1.5 mL microcentrifuge tube (not included). Add 50 µL of Elution Buffer to the center of the purification column membrane. Centrifuge for 1 min.
   Note.
   For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended.
   If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column.
   If the elution volume is 10 µL and DNA amount is ≤ 5 µg, incubate column for 1 min at room temperature before centrifugation.
10. Discard the GeneJET purification column and store the purified DNA at -20 °C.

Results:

Screenshot 2019-07-10 16.43.45.png



#3 250 mL O/N of DH5α pPIC RV1284

Aim: Create a culture for midi-prep

Experimenter: Sofia?

Sample: DH5α pPIC RV1284 colony ??? from the plate from #1 20.6.2019

Protocol:

Results:

#4 Double digest on PSBA3 with EcoRI and PstI

Aim: To prepare backbone for later cloning

Experimenter: Hugo

Sample: PSB1A3 extracted in #1 10/7/2019

Protocol: Four double digests were preformed. For 1 double digest: 6.1 uL DNA was diluted in 37 uL ddH2O. 5 uL reaction buffer, 1 uL EcoRI and PstI were added to the solution. The solution was incubated at 37 C for 45 min. The solution was then heat inactivated at 80 C for 20 min. Stored in 4 C in yeast fridge.

Results: 200 ul of double digested PSBA3 with a concentration of ca 0.01 ug/uL.

#5 Big O/N culture of X-33

Aim: Make a 500 mL O/N culture of X-33 in preparation for transformation

Experimenter: Yannick

Sample: Previous small X-33 O/N culture from 20190710 #7.

Protocol: Inoculated 500mL of YPD with 300µL of yesterdays O/N culture.

Results: 500 mL O/N X-33 being on circular shaking at 28°C

#6 Restreak of KM71H pPIC RV1284

Aim: Keeping the KM71H transformants fresh by restreaking

Experimenter: Yannick & Hugo

Sample: Plates with KM71H pPIC RV1284 transformants from 20190704 #5.

Protocol: Restreaked single colonies from plates marked 5uL and 100uL onto YPD + Zeocin plates. Took colonies #4 and #5 and #6 from the 5uL plate and colony #1 from the 100 uL plate. Gave the new plates names #1.1, 4.1, 5.1 and 6.1 to be able to track where they came from.

Results: Restreaked KM71H colonies, wrapped the plates with parafilm and incubated at 28°C

#7 O/N of KM71H pPIC RV1284

Aim: In preperation for making glycerol stock

Experimenter: Hugo

Sample: Plates with KM71H pPIC RV1284 transformants from 20190704 #5 colony 1

Protocol: 1 uL zeocin was diluted with 10 ml YPD and the culture was inoculated. Stored at 28°C and shaken.

Results: Discarded the 7/11 because nothing had grown

#8 2x5mL O/N of DH5α pPIC RV1284

Aim: Make two O/N cultures for miniprep tomorrow, to have DNA for X-33 transformation.

Experimenter: Yannick & Sofia

Sample: DH5α pPIC RV1284 colony XI and XII from the plate from #1 20.6.2019 (new colonies)

Protocol: 4x5mL of LSLB + zeocin (final conc. 25 µg/mL) were made. Two of these were inoculated with new colonies from our standard plate. The other two were saved for later.

Results: The inoculated tubes were incubated O/N @37°C and shaking. We found out the next day that the lids were not unscrewed. Less oxygen probably led to sub-optimal growth.

THURSDAY, 11/7/2019

#1 Midi-prep of pPIC RV1284

Aim: Prepare DNA for transformation into yeast

Experimenter: Sofia

Sample: O/N DH5α pPIC RV1284 from 20190710 #3

Protocol: Midiprep bitesizebio.com

- Step 2: Centrifugation made twice, 3000 rpm, 5 min, 10°C.
- Step 5: Centrifugation made twice, 4000 rpm, 15 min, 20°C.
- Step 7: Missed step (did not wash with ethanol).
- Step 9: Phenol extraction (according to Thermofisher)

i) ~600 µL
ii) 16 100 g, 5 min centrifugation
Extraction made twice.

Step 10: Ethanol precipitation (accordning to easyselect-man protocol)
i) 1/10 volume of 3 M sodium acetate (45 µL) and 2.5 volume of 100% ethanol (1125 µL) added to eht extraction.
ii) Centrifuge 16 100 g, 5 min. Wash the pellet with 80% ethanol (100 µL). Airdry ~20 min.
iii) Resuspend in 200 µL sterile water.

Results: Stored in fridge. Run on gel #2 20190711. Concentration:

Table7

	A	B
1	Concentration:	20 610 ng/µL
2	260/280	1.71
3	230/280	2.10

#2 Agarose gel analysis

Aim: Analyze different samples

Experimenter: Sofia

Sample: 2. ? Miniprep digested with SacI
3. 20190710 #1 Miniprep
4. 20190709 #2 Miniprep
5. 20190704 #4
6. #8 20190710 XI
7. #8 20190710 XII
8-10. 20190711 #1 Midiprep

Protocol: Samples loaded as followed in 0.7% agarose gel. Run on 80 V for about 1 h.

Table8

	A	B	C	D
1	**Well**	**DNA (µL)**	**Water (µL)**	**Loading dye (µL)**
2	1	Ladder: 1 µL Gene Ruler	4	1
3	2	0.603	9.4	2
4	3	0.697 (80 ng)	9.30	2
5	4	0.418 (80 ng)	9.58	2
6	5	0.638 (80 ng)	9.36	2
7	6	1.6 (80 ng)	8.4	2
8	7	1.41 (80 ng)	8.59	2
9	8	0.25 (5.15 µg)	9.75	2
10	9	0.75 (15.5 µg)	9.25	2
11	10	1.5 (30.9 µg)	8.5	2

Results: [Insert gel picture] Use more than 80 ng of DNA when loading a gel.

#3 Check red colonies

Aim: Compare the red colonies on plates from #2 20190708 with a picture taken yesterday to see if the small white colonies have turned red or if they might be cross-contamination.

Experimenter: Yeast priests except Jonas

Sample: Plates from #2 monday 8/7

Protocol: The plates were compared with a picture taken yesterday and very briefly checked for fluorescence under UV (~2 seconds)

Results: All the white colonies were still white and still smaller than the red colonies. Previous red colonies of the same size as these current white colonies had developed some red color by this point. So we suspect that these white colonies don't have the correct plasmid.

#4 Making competent cells, X33

Aim: Preparing the X33 culture for electrophoration

Experimenter: Yannick

Sample: P. Pastoris, X33 500 mL O/N 20190710 #5

Protocol: Preparing Pichia for Electroporation p.27 in EasySelect Pichia Expression Kit User Manual

Grow overnight again to an OD_{600}= 1.3–1.5. The OD_{600} was measured multiple times during the morning by taking a 1mL sample and measuring OD_{600} on 1/10 dilutions. Water was used as blank. At first the results were inconsistent and the OD_{600} of dilutions seemed to go down over time. This might have been because of sedimentation of the yeast cells. The results became consistent when the O/N culture was mixed well before taking a sample and the dilutions were made and mixed directly before measuring them.

At 12.55 the culture had OD_{600}= 1.5, the upper limit and the rest of the preperation was carried out:

3. Centrifuge the cells at 1,500×g for 5 minutes at 4°C. Resuspend the pellet with 500 ml of ice-cold, sterile water.

4. Centrifuge the cells as in Step 3, then resuspend the pellet with 250 ml of ice-cold, sterile water.

5. Centrifuge the cells as in Step 3, then resuspend the pellet in 20 ml of ice-cold 1 M sorbitol.
6. Centrifuge the cells as in Step 3, then resuspend the pellet in 1 ml of ice-cold 1 M sorbitol for a final volume of approximately 1.5 ml. Keep the cells on ice and use that day. Do not store cells.

Instead of using x1 500 mL centrifuge tube x10 50 mL Falcon tubes were used.

Results: ~1.5 mL of competent X-33 cells stored on ice and ready for electroporation transformation today at #6 20190711

#5 Prepare YPD + 15% glycerol

Aim: Prepare YPD + 15% glycerol solution for resuspending harvested Pichia cells in when making a glycerol stock

Experimenter: Yannick

Sample: -

Protocol: Working sterie mixed the following

Table9

	A	B	C
1	Chemical	Amount	Calculation
2	2x YPD	3 mL	
3	50% glycerol	1.8 mL	15%/50% * 6mL =1.8 mL
4	Sterile ddH2O	1.2 mL	(6-3-1.8)mL = 1.2 mL

Results: 6mL of YPD+15% glycerol solution stored in the yeast fridge.

#6 Electroporation transformation of X-33

Aim: Performing electroporation transformation of pPICZα RV1284 into X-33

Experimenter: Yannick & Tereza

Sample: X-33 from #4 20190711. SacI digested pPICZα RV1284 from #8 20190711

Protocol:

-In the lab mixed 90 µL of X-33 cells between 40 and 50 µL of SacI digested pPIC RV1284

-Also mixed 90 µL of X-33 cells and 50 µL of sterile water (negative control)

-Transfered these to eectroporation cuvettes. Incubated these for 10-15 min on ice. (protocol says 5 min. but we had to wait outside of the lab with the electroporator)

-Electroporated with settings U=1.5kV, C=25µF, R=200Ω. t=3.9 ms for actual transformation. t=4.8 ms for negative control.

-Immediately added 1 mL of ice-cold 1M sorbitol and transfered to 15mL falcon. Transported back to lab on ice and incubated at 30°C for 1-2 hours.

-Streaked out different amounts on YPDS + Zeocin plates

-Spread 10, 25, 50, 100, and 200 µl each on separate, labeled YPDS plates containing 100 µg/ml Zeocin. Negative control plated accordingly.

- Plates incubed at 28°C incubator.

Results: Colonies appeared on Sunday 14th (after 3 days), counted and put into fridge.

Table10

	A	B
1	Plate	# of colonies
2	- control	0
3	10ul	1
4	25ul	15
5	50ul	8
6	100ul	21
7	200ul	25
8	total number of colinies	

#7 Miniprep of pPICZα RV1284

Aim:

Experimenter: Tereza

Sample: 2x5mL O/N DH5α pPICZα RV1284 from #8 20190710

Protocol:

O/N cultures were not aerated.

5ml of clone XI and XII harvested

Manufacturers instruction followed with deviations:

- Resuspension solution from Sigma 250uL
- Centrifuged 20min after Neutralization
- Eluted in 50uL sterile ddH_2O, 1.5uL sample taken and 48.5 uL put again on column and centrifuged 2min on MAX

Results:

Table11

	A	B	C	D	E	F	G
1	Clone	1st elute concentration [ng/ul]	260/280	260/230	2nd elute concentration [ng/ul]	260/280	260/230
2	XI	47.9	1.88	2.07	50.0	1.90	2.04
3	XII	53.0	1.90	2.03	56.7	1.89	2.00

#8 SacI digest of pPICZα RV1284

Aim: Linearize pPICZα RV1284 for transformation into X-33 at #6 20190711

Experimenter: Tereza

Sample: Miniprepped pPICZα RV1284 from various minipreps combined to achieve sufficient DNA concentration (5-10 ug):

- 20 ul 191.4 ng/ul - #2 20190709
- 15 ul 125.3 ng/ul - #3 20190704
- 8 ul 114.8 ng/ul - #1 20190710

Expected amount of DNA 6 625 ng in 50 ul.

Protocol:

- 50ul reaction
- 43 ul DNA
- 5ul 10X Buffer ThermoFisher B26 Ecl136II, PacI, SacI
- 2 ul SacI

Incubated 1h 15min 37C, heat/inactivated 20min 80C

Results: DNA 6 625 ng in 50 ul

#9 Restreaks from glycerol stocks

Aim: Test how good the glycerol stocks are for restreaking

Experimenter: Yannick

Sample: Glycerol stocks of DH5α pSB1A3 (), pPICZα RV1284 () and pPICZα ()

Protocol:

Transported the glycerol stocks from the -80°C freezer to the lab on ice. Not great because they thawed while on ice in the lab. Might want to restreak in the freezer room next time or transport the glycerol stocks on dry ice or in liquid nitrogen?

Restreaked DH5α pSB1A3 on LB+Amp, DH5α pPICZα RV1284 and DH5α pPICZα RV1284 on LSLB+zeocin.

Results: Restreaked plates incubating in the corner of the 37°C room. There was growth the next day so the glycerol stocks worked. They might not work as well next time because they were thawed and refrozen but it shouldn't matter too much.

#10 Restreaks of *P. pastoris* X33 transformed by pPICZα RV1284

Aim: Verify that transformant is Zeocin^R

Experimenter: Tereza

Sample: #7 20190708

Protocol: restreak colony #1 from plates #7 20190708 on YPD + zeocin and incubate in 28C

Results: Restreaked to single colonies, fridge

#11 Restreaks *DH5α* with pSB1A3-RFP Red and White colonies

Aim: See if both colours of colonies are Ampicilin^R and grow

Experimenter: Tereza

Sample: #2 20190708

Protocol: Restreak one red and one white colony on ampicilin LB plates

Results: Red grows and white does not on 20190712, no growth on Saturday 20190713, put into fridge

#12 Making a glycerol stock

Aim: Make a glycerol stock of DH5α with plasmid psB1A3

Experimenter: Hugo

Sample: The O/N culture of DH5α pSB1A3 from #5 2019.07.09

Protocol: Added 400 µL of 50% glycerol to the 600 µL culture and mixed by pipetting. Worked around flame.

Results: A DH5α with the plasmid pSB1A3 stored in the -80°C freezer in the box of bacterial glycerol stocks of iGEM 2019

#13 Measuring OD_{600}

Aim: Preparation for glycerol stock

Experimenter: Hugo

Sample: KM71H RV1284 from #7 2019.07.10

Protocol: 100 µL of the culture (in YPD-media) was diluted to a total volume of 1 mL with water. A negative test was done with 1:10 diluted YPD-media. The tests were transfered to cuvettes and was analyzed with the spectrofotometer.

Results: The diluted value was OD_{600}=0.006 and the non-diluted value would be 0.06, however it apeared like it hadn't grown during the night and that the absorption value instead could be the diluted media and not the bacteria. Therefore it was discarded.

#14 O/N of KM71H pPIC RV1284

Aim: In preperation for making glycerol stock

Experimenter: Hugo

Sample: Plates with KM71H pPIC RV1284 transformants from 20190704 #5 colony 4

Protocol: 1 uL zeocin was diluted with 10 ml YPD and the culture was inoculated. Stored at 28°C and shaken.

Results: At friday the 7/12 nothing had grown and it was therefore left over the weekend but for some reason the shaker had been turned off. It was discarded the 15/11 for this reason.

#1 Restreaks of *P. pastoris* KM71H transformed by pPICZα RV1284

FRIDAY, 12/7/2019

Aim: Prepare fresher colonies for inoculation for Expression of secreted protein RV1284 in *P. psatoris* KM71H on Tuesday 16th July #1 20190716

Experimenter: Tereza

Sample: #5 20190704 (transformation) and missing reference 20190708 restreaks #1-6 of transformation #5 20190704

Protocol: Clones #7 #8 #9 #10 #11 #12 from #5 20190704 (transformation) and #1.2 #4.2 #5.2 #6.3 from missing reference 20190708 restreaks #1-6 of transformation #5 20190704 restreaked on YPD + Zeocin plates. Clones #1.2 #5.2 #8 and #9 additionally restreaked on YPD without Zeocin. Almost all YPD+Zeocin plates were wet, be sure to dry them properly!

Results: Single colonies used for inoculation

#2 Measuring OD_{600}

Aim: Preparation for glycerol stock

Experimenter: Hugo

Sample: KM71H RV1284 from #14 2019.07.11

Protocol: 100 µL of the culture (in YPD-media) was diluted to a total volume of 1 mL with water. A negative test was done with 1:10 diluted YPD-media. The tests were transfered to cuvettes and was analyzed with the spectrofotometer.

Results: The diluted value was OD_{600}=0.005 and the non-diluted value would be 0.05, however it apeared like it hadn't grown during the night and that the absorption value instead could be the diluted media and not the bacteria. Therefore it was it was put on the shaker over the weekend.

SATURDAY, 13/7/2019

SUNDAY, 14/7/2019

Working Space if Benchling is full of people

Project: iGEM uppsala 2019
Authors: Tereza Hubáčková

MONDAY, 8/7/2019

#5 Restriction double digestion of psB1A3-RFP plasmid

Aim: We need to amplify psB1A3 backbone. Cleave out psB1A3 backbone and later isolate it from the agarose gel.

Experimenter: Tereza
Sample: 2 Minipreps #2 20190707
Protocol: 500 ng of plasmid DNA, 1uL of enzymes EcoRI and PstI, 5uL 10X Buffer O and sterile ddH2O till final volume 50uL. Gently mixed by tapping on the tube, spinned down. 45min 37°C incubation, 20min 80°C heat inactivation. Then stored on bench and on ice.
Results: 2 x 50uL of psB1A3

Inoculation for expression of secreted protein RV1284 in *P. psatoris* KM71H

Aim: Try small-scale expression of a secreted protein in *P. pastoris* KM71H. Sampling of supernatant and pellet will be done during the process to determine the effectivity of secretion and the best time for protein harvesting. We test scaling down the protocol as well.

Experimenter: Tereza, Sofia, Yannick, Hugo
Sample: Restreaks from Friday 14th Hugo + Tereza
Protocol:

- Using a single colony, twice 100 ml of BMGY (for induced culture and control culture) was inoculated in a 1 liter flask by clones 6.3.1 and 5.2.1. Glass beads for possible improvement of aeration were added to 6.3.1.
- To test scaling down: twice 50 ml of BMGY was inoculated in a 0.5 liter flask by clone 5.2.1.
- Twice 50 ml of BMGY was inoculated in a 1 liter flask was inoculated by clone 5.2.1.
- Inoculation was done by pipette tip which was left inside of flask. Cultures grown at 28°C in shaking water-bath incubator (100 RPM) until the culture reached an OD_{600} = 2–6.

Results:

#5 Mut^S - Mut^+ phenotype testing of *P. pastoris* X33

Aim: We want to verify that our transformants are Mut^+. If they are Mut^S, the integration into host chromosome interrupted the AOX1 gene.

Experimenter: Tereza
Sample: X33 transformations
Protocol: will be copied from EasySelect
Results: Since both plates were MM (minimal methanol), no valuable results.

#1 X33 expression - Inoculation

Aim: Inoculate 2 *P. pastoris* X33 cultures for expression of RV1284. We want to reach even inoculation which will reach OD_{600}=5 at 8am 20190719.

Experimenter: Tereza

Sample: #8 20190715 plates #5 and #25 (#8 lost during this process)

Protocol: All the biomass from plate was picked by a sterile toothpick and resuspended in 50ul of BMGY. OD_{600} was measured.

#5 - OD_{600} = 397

#25 - OD_{600} = 421

With estimated doubling time 2.5hrs, 2hrs time reserve for lag phase, if we inoculate to starting OD_{600} = 0.08 after 17hrs the OD_{600}~5. To reach starting OD_{600} = 0.08 in 25ml culture, 5ul of #5 and 4.75uL of #25 was used.

Results:

#2

Aim:

Experimenter:

Sample:

Protocol:

Results:

Notebook week 15/7-21/7

Project: iGEM uppsala 2019
Authors: Sofia Larsson

MONDAY, 15/7/2019

#1 Preparing 13.4% YNB (10x)

Aim: Preparing YNB stock solution for media used for expression

Experimentator: Sofia
Sample: -
Protocol: 134 g of yeast nitrogen base with ammonium sulfate, without amino acids mixed with water to final volume 1000 mL. Sterile filtered with 0.45 µm filter.
Result: Solution stored in cold room.

#2 Preparing 5% methanol stock

Aim: Preparing 250 mL of 5% (10x) methanol stock

Experimenter: Yannick
Sample: -
Protocol: Diluted 12.5 mL 100% methanol to 250 mL with sterile water.
Results: 5% methanol stock stored above lab bench.

#3 Preparing 0.02% biotin (500x)

Aim: Prepare 20mL of 0.02% (500x) biotin stock solution.

Experimenter: Yannick
Sample: -
Protocol: Dissolved 0.0040 g biotin in 20,0 mL of water and sterile-filtered this into a 50mL falcon.
Results: Stored in yeast-fridge

#4 Preparing 1.6L of YPD

Aim: Preparing 2 bottles of 800mL YPD

Experimenter: Yannick
Sample: -
Protocol: Mixed the following into 720mL water twice:

Table1

	A	B
1	Material	Amount
2	Yeast extract	8g
3	Peptone	16g

Autoclaved and mixed in 80mL 20% Dextrose (10x) into each of the bottles.

Results: 1.6L of sterile YPD stored in the cold room.

#5 Mut^S - Mut$^+$ phenotype testing of *P. pastoris* X33

Aim: We want to verify that our transformants are Mut$^+$. If they are Mut^S, the integration into host chromosome interrupted the AOX1 gene.

Experimenter: Tereza

Sample: Restreak #10 20190711 (which is colony #1 from transformation #7 20190708) and transformation #6 20190711 colonies #1-#35,#55-56

Protocol: #Mut^S / Mut$^+$ phenotype testing

Results: By accident both plates were MM (minimal methanol), no comparison with MD (glucose minimal) possible so no valuable results.

#6 Made MM and MD plates

Aim: Make 500mL of minimal methanol and minimal dextrose plates for Mut phenotype testing and culturing prior to expression

Experimenter: Yannick & Hugo

Sample: Stock solutions from #1,2,3 20190715 (today)

Protocol:

Autoclaved two bottles of 400mL water + 7.5g agar. Added to these bottles the following

Table2

	A	B	C
1	Material	MM plates	MD plates
2	10x YNB	50mL	50mL
3	10x MeOH	50mL	-
4	10x Dextrose	-	50mL
5	500x Biotin	1mL	1mL

Poured the plates and let them dry around a flame with the lids slightly opened for a while before closing the lids when the agar had solidified.

Results: Around 30 MM and MD plates stored in the cold room.

#7 Made BMGY and BMMY

Aim: Make complex buffered minimal dextrose and minimal methanol media.

Experimenter: Yannick

Sample: Stock solutions from #1,2,3 20190715 (today)

Protocol:

The following were added together. The peptone and yeast extract were added to the 700mL and autoclaved, then the rest of the stock solutions were added.

Table4

	A	B	C
1	Material	BMGY	BMMY
2	ddH20	700mL	700mL
3	1M KH2PO4 (pH 6.0)	100mL	100mL
4	10x YNB (13.4%)	100mL	100mL
5	500x Biotin (0.02%)	2mL	2mL
6	10x MeOH (5%)	-	100mL
7	50% glycerol	20mL	-
8	Sterile ddH2O	80mL	-

Results: 1L each of BMGY and BMMY medium stored in the cold room, to be used for expression.

#8 Restreaked X-33 pPICZα RV1284

Aim: To have fresh X33 transfromants to inoculate for expression 20190718

Experimenter: Yannick

Sample: Plates with X-33 pPICZα RV1284 transformants from transformation at #6 20190711

Protocol: Restreaked colonies #1,5,8 and 25 on YPD + zeocin and incubated at 28°C

Results: See protocol^

#9 Discarding the overnight culture of KM71H pPIC RV1284

Aim: The shaker/incubator had been turned off over the weekend and although a lot had grown the culture might not have been fresh and it was therefore not suitable to use as glycerol stock.

Experimenter: Hugo

Sample: The O/N culture of KM71H pPIC RV1284 from 20190711 #14

Protocol: -

Results: It was discarded

#10 Preparing a 1 M; pH 6; 1 L KH_2PO_4 solution (not enough K_2HPO_4 in storage)

Aim: Prepare 1 L of 1 M; pH 6 potassium phosphate stock solution

Experimenter: Hugo

Sample: -

Protocol: 136.1 g KH_2PO_4 was diluted in ca 900 mL ddH_2O. pH was adjusted using 5M NaOH to a final pH of 6 and the final volume was 1 L.

Results: The stocksolution was prepared and autoclaved, stored in room temperature above Yannick's bench.

TUESDAY, 16/7/2019

#1 Inoculation for Expression of secreted protein RV1284 in *P. pastoris* KM71H

Aim: Try small-scale expression of a secreted protein in *P. pastoris* KM71H. Sampling of supernatant and pellet will be done during the process to determine the effectivity of secretion and the best time for protein harvesting. We test scaling down the expression protocol as well, for the possibility of testing multiple recombinants in smaller volume.

Experimenter: Tereza, Sofia, Yannick, Hugo
Sample: Restreaks #1 20190712 done by Hugo and Tereza
Protocol:

- Using a single colony picked by pipette tip, twice 100 ml of BMGY (1 for induced culture and 1 control culture) was inoculated in a 1 liter flasks by clones 6.3.1 and 5.2.1. Glass beads for possible improvement of aeration were added to 6.3.1 culture.
- To test scaling down: twice 50 ml of BMGY was inoculated in a 0.5 liter flask by clone 5.2.1 and 9.1.
- Inoculation was done by pipette tip which was left inside the flask. Cultures were grown at 28°C in shaking water-bath incubator (100 RPM) until the culture reached an OD_{600} = 2–6.

Results: OD_{600} was low. Cultures were inoculated very unevenly. Next time we should resuspend colonies in the sterile media to create an inoculum, measure OD_{600} of inoculum and estimate the needed inoculation volume. On wednesday 20190717 the unharvested expression cultures (all but #9.1) were moved from the shakers to the fridge for 5-10 min worrying that they would grow too much O/N but they were then moved back to the shakers again for incubation @28°C O/N.

#2 Make 20% Dextrose stock

Aim: Prepare 800mL of sterile 20% dextrose stock solution
Experimenter: Yannick
Sample: -
Protocol: Dissolved 160g dextrose in water to a final volume of 800mL. Autoclaved.
Results: 800mL 20% dextrose stock (labeled glucose) stored above lab bench.

#3 Mut^S - Mut^+ phenotype testing of *P. pastoris* X33

Aim: Verify that the transformants are Mut^+. If they are Mut^S, the integration into host chromosome interrupted the AOX1 gene.
Experimenter: Hugo
Sample: Restreak #10 20190711 (which is colony #1 from transformation #7 20190708) and transformation #6 20190711 colonies #1-#33, #40-41, #55-56
Protocol: Protocol: See EasySelect manual p.34:
1. Using a sterile toothpick, pick one colony and streak or patch one Zeo^R transformant in a regular pattern on both an MMH plate and an MDH plate, making sure to patch the MMH plate first.
2. Use a new toothpick for each transformant and continue until 10 transformants have been patched (1 plate).
3. To differentiate Mut^+ from Mut^S, make one patch for each of the controls (GS115/MutS Albumin and GS115/pPICZ/lacZ Mut+) onto the MDH and MMH plates.
4. Incubate the plates at 30°C for 2 days.
5. After 2 days or longer at 30°C, score the plates. Mut+ strains will grow normally on both plates, while Mut Sstrains will grow normally on the MDH plate but show little or no growth on the MMH plate.

Differences from the protocol: Instead of using MDH and MMH plates, MM and MD plates were used. Instead of using toothpicks in step 1 new yellow pipettips were used. Also 37 samples were done.

Results: No Mut^S identified.

WEDNESDAY, 17/7/2019

#1 OD_{600}-measurements of KM71H

Aim: Measure optical density of inoculation of KM71H to see if ready for induction

Experimentator: Sofia
Sample: #1 from 20190716
Protocol: Diluted 1:10 in sterile water. Blanked with 1:10 BMGY and sterile water.
Result:

Table3

	A	B	C	D	E	F	G	H	I
1	**OD600**	**#5.2.1.L induced**	**#5.2.1.L uninduced**	**#5.2.1.s induced**	**#5.2.1.s uninduced**	**#6.3.1 induced**	**#6.3.1 uninduced**	**#9.1 induced**	**#9.1 uninduced**
2	8.15	1.52	-		-	-	-	3.91	3.89
3	9.30	-	-	0.03	0.09	-	-	-	-
4	10.00	0.14	0.17	-	-	0.28	0.29	-	-
5	12.00	0.28	0.29	0.07	0.21	0.21	0.13	-	-
6	15.45	1.15	0.96	0.25	0.83	0.72	0.50	-	-

Both #9.1 inoculations harvested at 8.40, further in #4 20190717. Harvested by centrifugation 2500 g for 5 min in room temperature.

#2 Expression of X33 - preparation of fresh restreaks

Aim: Restreak suspected Mut$^+$, which will be verified on 20190718
Experimenter: Tereza
Sample: colonies #2, #11, #12, #40 from transformation #6 20190711
Protocol: Restreaked by flame-sterilized metal loop on YPD plates without Zeocin. Incubated at 28°C.
Results: Not used for expression.

#3 50% glycerol stock

Aim: Make 100mL of 50% glycerol stock
Experimenter: Hugo
Sample: -
Protocol: Diluted 50%/85%*100mL = 58,8 mL 85% glycerol to a volume of 100 mL with ddH_2O
Results: 100 mL of 50% glycerol stored at room temperature above lab bench.

#4 Resuspension of KM71H

Aim: Resuspending the harvested KM71H pPICZα RV1284 for expression
Experimenter: Yannick
Sample: Pelleted cells from #9.1 inoculation harvested in #1 20190717
Protocol: The supernatant was poured off.

#9.1 induced and uninduced were resuspended in 10mL BMMY and BMGY respecitvely (20% of the volume before harvesting).

This was done around 10.00-10.30 probably so extra methanol should be added to the induced culture 10.30 each day.

Results: Resuspended cultures #9.1 induced & uninduced (control) were incubated at 28°C with shaking.

#5 O/N KM71H pPICZα RV1284

Aim: Making a 10mL culture in preparation for a glycerol stock (again)

Experimenter: Yannick

Sample: Restreaks #1 20190712 done by Hugo and Tereza

Protocol: Inoculated 10mL of YPD + 100µg/mL zeocin (10µL of zeocin stock) with colony #9.1. This is the same colony as is used for expression

Results: 10 mL O/N culture on 28°C shaker.

#6 Taking samples from KM71H pPICZα RV1284

Aim: To later evaluate the expression through SDS-PAGE

Experimenter: Hugo

Sample: The KM71H culture of clone #9.1 from #4 2019/7/17

Protocol: 1 mL of the induced and uninduced cultures were transfered to 1.5 ml cent. tubes and centrifuged for 2.5 min at the highest speed at tabletop centirfuge (16:35). The supernatants were transfered to other tubes and all tubes were snapped freezed in $N_{2(l)}$ for ca 5 minutes and were then stored at -80 °C in a box called KM71H Yeast Priests.

Results:

#7 Making 1 L BMGY-medium

Aim: Make complex buffered minimal dextrose media.

Experimenter: Hugo

Sample: Stock solutions from #1,2,3 20190715 (today)

Protocol: The following were added together. The peptone and yeast extract were added to the 700mL and autoclaved, then the rest of the stock solutions were added.

Table5

	A	B	C	D
1	Material	BMGY		
2	ddH20	700mL		
3	1M KH2PO4 (pH 6.0)	100mL		
4	10x YNB (13.4%)	100mL		
5	500x Biotin (0.02%)	2mL		
6	50% glycerol	20mL		
7	Sterile ddH2O	80mL		

Results:

THURSDAY, 18/7/2019

#1 RV1284 expression in X33 - Inoculation

Aim: Inoculate 2 *P. pastoris* X33 cultures for expression of RV1284. We want to reach even inoculation which will reach OD_{600}=5 at 8am 20190719.

Experimenter: Tereza

Sample: #8 20190715 plates #5 and #25 (#8 lost during this process)

Protocol: Almost all of the biomass from plate was picked by a sterile toothpick and resuspended in 50ul of BMGY. OD_{600} was measured.

#5 - OD_{600} = 397

#25 - OD_{600} = 421

With estimated doubling time 2.5hrs, 2hrs time reserve for lag phase, if we inoculate to starting OD_{600} = 0.08 after 17hrs the OD_{600}~5. To reach starting OD_{600} = 0.08 in 25ml culture, 5ul of #5 and 4.75uL of #25 was used.

Results: Succesful transfer to BMMY media between 8 and 9 am Friday 20190719.

#2 OD_{600}-measurements of KM71H

Aim: To measure OD of culture for glycerol stock

Experimenter: Hugo

Sample: O/N KM71H pPICZα RV1284 from 17/07/2019 #4

Protocol: 500 µL of the culture (in YPD-media) was diluted to a total volume of 1 mL with water. A negative test was done with ddH_2O. The tests were transfered to cuvettes and was analyzed with the spectrofotometer.

Results: The diluted value was OD_{600}=0.022 and the non-diluted value would be 0.044, however it appeared like it hadn't grown during the night and that the absorption value instead could be the diluted media and not the yeast. The OD was too low to use for glycerol stock. Incubated to friday.

#3 Adding methanol to cultures KM71H #9.1

Aim: Add methanol to maintain induction of AOX promoter

Experimenter: Hugo

Sample: Culture 9.1 from #1 16/7/2019

Protocol: Methanol added to final 0.5% concentration

Results: Methanol added, hopefully induction maintained.

#4 OD_{600}-measurements of KM71H expression inoculation

Aim: Measure optical density of inoculation of KM71H to see if ready for induction

Experimenter: Yannick

Sample: #1 from 20190716

Protocol: Diluted 1:10 in sterile water. Blanked with 1:10 BMGY and sterile water.

Result: Measured the follwoing OD-600 values between 8.20 and 8.40

Table6

	A	B	C	D	E	F	G
1		**#5.2.1.L induced**	**#5.2.1.L uninduced**	**#5.2.1.s induced**	**#5.2.1.s uninduced**	**#6.3.1 induced**	**#6.3.1 uninduced**
2	**OD600**	0.710	0.764	0.740	0.804	0.847	0.809

Slightly overshot the target of OD_{600} 2-6. Went ahead with harvesting of the cells and resuspension anyway in 20190718 #5.

#5 Resuspension of KM71H

Aim: Resuspending the harvested KM71H pPICZα RV1284 for expression

Experimenter: Yannick (and Sofia did the last 6 resuspensions)

Sample: The induced and uninduced versions of cultures #5.2.1s, #5.2.1L and #6.3.1 from #1 20190716 (OD_{600} measured in #4 20190718)

Protocol: Harvested cells by centrifugation at 2500 g for 5 min in room temperature.

The supernatant was poured off.

The induced and uninduced cells were resuspended by vortexing in BMMY and BMGY respecitvely (20% of the volume before harvesting, so 10mL for 5.2.1s and 20mL for 5.2.1L and 6.3.1)

This was done around 10.40 so extra methanol should be added to the induced culture ~10.30 each day.

The cultures were moved to 100mL and 200 mL e-flasks (10x culture volume) and 1.0 mL samples were taken from each.

This is sample time 0. They will be sampled at the same time every day, see the "sampling of KM71H during expression".

Results: Resuspended cultures #5.2.1s, #5.2.1L and #6.3.1 induced & uninduced (control) were incubated at 28°C with shaking. Samples will be taken every day.

#6 Adding methanol to plates

Aim: Keeping the selectivity of the MM plates by adding methanol

Experimenter: Yannick

Sample: All of the previous MM plates

Protocol+Results: Added 100 uL of 100% MeOH to each of the lids of the previous MM plates

FRIDAY, 19/7/2019

#1 Adding methanol to cultures KM71H #9.1 #5.2.1L, #5.2.1S, #6.3.1

Aim: Add methanol to maintain induction of AOX promoter

Experimenter: Hugo

Sample: Cultures 5.2.1L, 5.2.1S, 6.3.1 and 9.1 from #1 16/7/2019

Protocol: Methanol added to final 0.5% concentration

5.2.1L - 90 ul

5.2.1S - 40 ul

6.3.1 - 90 ul

9.1 - 35 uL

Results: Methanol added, hopefully induction maintained.

#2 Took samples of all expression cultures

Aim: Take 1 mL samples of all expression cultures and store at -80

Experimenter: Yannick

Sample: KM71H cultures resuspended at #1 20190716 and X-33 cultures resuspended at #3 20190719

Protocol & Results: See "Sampling of KM71H/X-33 during expression"

#3 Resuspending X-33 expression cultures in Inducing Media

Aim: Transfer espresssion culture from BMGY media to media with inducer - methanol BMMY.

Experimenter: Tereza

Sample: #1 20190718

Protocol: OD_{600} was measured and new media were inoculation to starting OD_{600} = 1 (different volumes between 100-200mL depending on measuredOD_{600}) in 1L flasks. For “induced“ BMMY was used and for control BMGY.
Results: Expression analysed by SDS-PAGE 20190729.

#4 Prepared labeled tubes for the weekend samples

Aim: -
Experimenter: Sofia, Yannick, Hugo
Sample: -
Protocol: See "Sampling of KM71H/X-33 during expression"
Results: -

#5 Took samples of X-33 expression cultures

Aim: Take 1 mL samples of X-33 cultures and store at -80 (done at 18.40)
Experimenter: Hugo
Sample: X-33 cultures resuspended in #3 20190719
Protocol & Results: See "Sampling of KM71H/X-33 during expression"

SATURDAY, 20/7/2019

#1 Sampling of X-33 and KM71H expression cultures

Aim: Take 1 mL samples of X-33 and KM71H cultures and store at -80 (done at 10.00)
Experimenter: Tereza
Sample: 20190719 #3, 20190717 #4, 20190718 #5
Protocol & Results: At 10.00, See "Sampling of KM71H/X-33 during expression"

#2 Sampling of X-33 expression cultures

Aim: Take 1 mL samples of X-33 and KM71H cultures and store at -80 (done at 21.00)
Experimenter: Tereza, Hugo
Sample: 20190719 #3, 20190717 #4, 20190718 #5
Protocol & Results: Around 21.00, See "Sampling of KM71H/X-33 during expression"

SUNDAY, 21/7/2019

#1 Took samples of all expression cultures

Aim: Take 1 mL samples of all expression cultures and store at -80 (done at 11.30)
Experimenter: Hugo
Sample: KM71H cultures resuspended at #1 20190716 and X-33 cultures resuspended at #3 20190719
Protocol & Results: See "Sampling of KM71H/X-33 during expression"

#2 Adding methanol to cultures KM71H and X-33

Aim: Add methanol to maintain induction of AOX promoter (done at 12.15)
Experimenter: Hugo
Sample: Cultures KM71H 5.2.1L, 5.2.1S, 6.3.1 and 9.1 from #1 16/7/2019 and X-33 5 and 25 from #3 20190719
Protocol: Methanol added to final 0.5% concentration

KM 9.1 - 25 uL

KM 5.2.1L - 80 ul
KM 5.2.1S - 30 ul
KM 6.3.1 - 80 ul
X-33 5 - 825 uL
X-33 25 - 630 uL

Results: Methanol added, hopefully induction maintained.

SDS-PAGE results for RV1284

Project: iGEM uppsala 2019

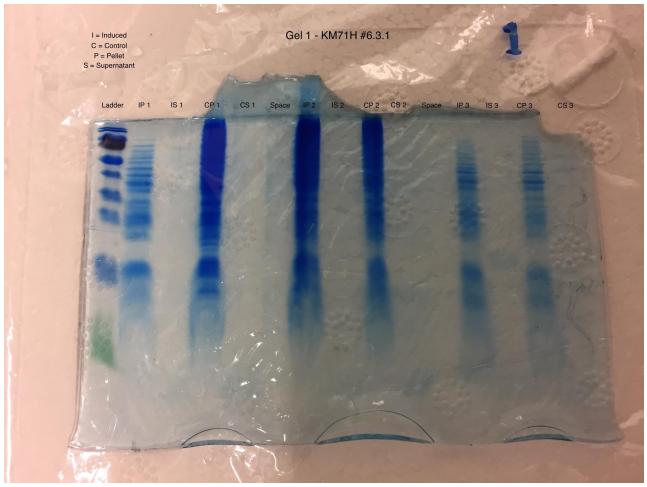
Authors: Hugo Grounes

THURSDAY, 18/7/2019

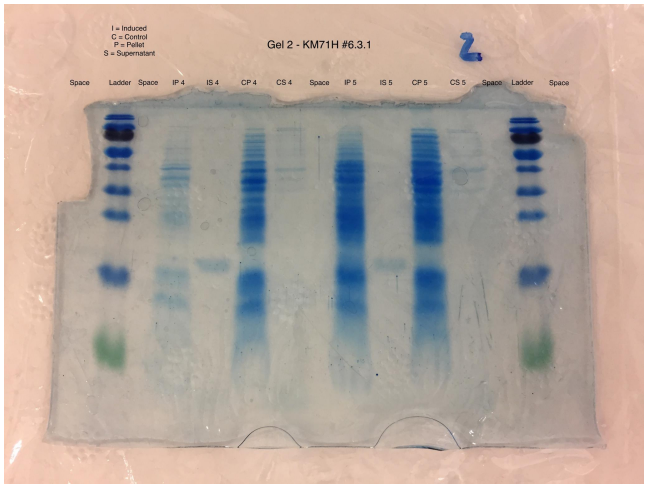
This powerpoint summarizes results of RV1284 expression in KM71H and X-33.

RV1284_Expression_squares.pptx

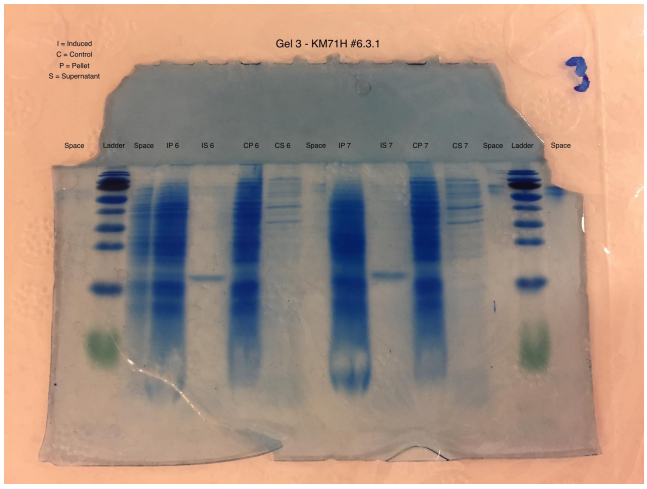
IMG_2984.jpg



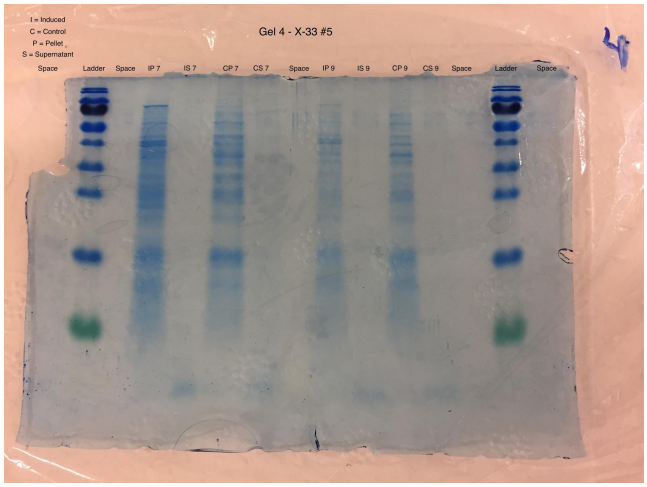
IMG_2985.jpg



IMG_2986.jpg



IMG_2989.jpg



Sampling of KM71H during expression

Project: iGEM uppsala 2019

Authors: Sofia Larsson

THURSDAY, 18/7/2019

Sampling

- 1 mL of sample transfered to a 1.5 mL ependorf tube. Centrifuged 16 100 g for 3 min in room temperature.
- Supernatant transfered to different tube.
- Snap frozen with liquid nitrogen.
- Stored in -80°C.

Freezer system

Table1

	A	B	C	D	E	F	G	H	I	J
1			#9.1		#5.2.1.s		#5.2.1.L		#6.3.1	
2			Induced	Uninduced	Induced	Uninduced	Induced	Uninduced	Induced	Uninduced
3	Timepoint 0	Pellet	20190717, HG	20190717, HG	20190718, SL	20190718, SL	20190718, SL	20190718, SL	20190718, SL	20190718, SL
4		Supernatant	20190717, HG	20190717, HG	20190718, SL	20190718, SL	20190718, SL	20190718, SL	20190718, SL	20190718, SL
5	Timepoint 24	Pellet	20190718, TH	20190718, TH	20190719, YH	20190719, YH	20190719, YH	20190719, YH	20190719, YH	20190719, YH
6		Supernatant	20190718, TH	20190718, TH	20190719, YH	20190719, YH	20190719, YH	20190719, YH	20190719, YH	20190719, YH
7	Timepoint 48	Pellet	20190719, YH	20190719, YH						
8		Supernatant	20190719, YH	20190719, YH						
9	Timepoint 72	Pellet								
10		Supernatant								
11	Timepoint 96	Pellet								
12		Supernatant								
13	Timepoint 120	Pellet								
14		Supernatant								
15	Timepoint 144	Pellet								
16		Supernatant								

The #9.1 20190718 sample was taken by Yannick but snapfrozen and stored by Teresa probably

Sampling of X33 during expression

Project: iGEM uppsala 2019
Authors: Tereza Hubáčková

FRIDAY, 19/7/2019

Table1

	A	B	C	D	E	F	G	H	I	J	K
1			#5		#5			#25		#25	
2			Induced	Uninduced	Induced	Uninduced		Induced	Uninduced	Induced	Uninduced
3	Timepoint 0	Pellet	20190719, YH	20190719, YH						20190719, YH	20190719, YH
4		Supernatant	20190719, YH	20190719, YH						20190719, YH	20190719, YH
5	Timepoint 6	Pellet	Hugo								
6		Supernatant									
7	Timepoint 12	Pellet	20190719, 20.45, TH	20190719, 20.45, TH						20190719, 20.45, TH	20190719, 20.45, TH
8		Supernatant	20190719, 20.45, TH	20190719, 20.45, TH						20190719, 20.45, TH	20190719, 20.45, TH
9	Timepoint 24	Pellet									
10		Supernatant									
11	Timepoint 96	Pellet									
12		Supernatant									
13	Timepoint 120	Pellet									
14		Supernatant									
15	Timepoint 144	Pellet									
16		Supernatant									

Notebook week 22/7-28/7

Project: iGEM uppsala 2019
Authors: Tereza Hubáčková

MONDAY, 22/7/2019

#1 Sampling of KM71H cultures and preparations of samples for SDS-PAGE

Aim: Take 1ml samples of all cultures in a sterile way. Boil 100ul of these samples in Sample Loading Buffer to prepare them for SDS-PAGE. (done at 11.40)

Experimenter: Tereza, Hugo
Sample: Cultures 5.2.1L, 5.2.1S, 6.3.1, 9.1 #1 16/7/2019
Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80 ul of pellet transferred to the new tube
then all tubes snap frozen and put to -80˚C

Results: Samples were boiled with Sample Loading Buffer and analyzed.

#2 1.25X Sample Loading Buffer (SLB) with 0.8% Triton

Aim: Prepare 5 times 2ml aliquots of buffer for boiling pellets for SDS-PAGE

Experimenter: Tereza, Hugo
Sample: -
Protocol: For 2ml

500ul 5X SLB
16ul Triton X-100, 100%
1 484ul ddH_2O

Results: 5 aliquots in the -20 SDS-PAGE box

#3 Sampling of X-33 cultures #5, #25 and preparations of samples for SDS-PAGE

Aim: Take 1ml samples of both cultures in a sterile way. Boil these samples in Sample Loading Buffer to prepare them for SDS-PAGE. (Done at 16.46)

Experimenter: Tereza, Hugo
Sample: X-33 culture 5 and 25 from #3 20190719
Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and put to -20˚C

Results: Samples were boiled with Sample Loading Buffer

#4 Boiling of suprnatants X-33 #5, sapling timepoints 0,1,2,3,4,5,6,7

Aim: Boil supernatant samples in Sample Loading Buffer to prepare them for SDS-PAGE.

Experimenter: Tereza, Hugo

Sample:

Protocol:

80ul of supernatant transferred to a new tube
mixed with 20ul 5XSLB
boiled on heating block for 10 min, 95˚C
tubes with 900ul of culture snap frozen and put to -80˚C

Results: Analyzed by SDS-PAGE.

#5 Adding methanol to cultures KM71H and X-33

Aim: Add methanol to maintain induction of AOX promoter (done at 11.44)

Experimenter: Hugo

Sample: Cultures KM71H 5.2.1L, 5.2.1S, 6.3.1 and 9.1 from #1 16/7/2019 and X-33 5 and 25 from #3 20190719

Protocol: Methanol added to final 0.5% concentration

KM 9.1 - 2 uL
KM 5.2.1L - 75 ul
KM 5.2.1S - 25 ul
KM 6.3.1 - 75 ul
X-33 5 - 815 uL
X-33 25 - 620 uL

Results: Methanol added, hopefully induction maintained.

#6 Took samples of X-33 expression cultures

Aim: Take 1 mL samples of X-33 cultures and boiled (done at 16.46)

Experimenter: Hugo

Sample: X-33 cultures resuspended in #3 20190719

Protocol & Results:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and stored in fridge

TUESDAY, 23/7/2019

#1 Adding methanol to cultures KM71H #5.2.1L, #5.2.1S, #6.3.1

Aim: Add methanol to maintain induction of AOX promoter

Experimenter: Tereza

Sample: Cultures 5.2.1L, 5.2.1S, 6.3.1 from #1 16/7/2019
Protocol: Methanol added to final 0.5% concentration

5.2.1L - 75ul
5.2.1S - 25ul
6.3.1 - 75ul

Results: Methanol added, hopefully induction maintained.

#2 Sampling of X-33 cultures #5, #25 and KM71H #5.2.1L, #5.2.1S, #6.3.1 and preparations of these samples for SDS-PAGE.

Aim: Take 1ml samples from all cultures in a sterile way. Boil these samples in Sample Loading Buffer 1X to prepare them for SDS-PAGE. Use Triton for pellets.

Experimenter: Tereza, Hugo
Sample: Cultures KM71H #5.2.1L, #5.2.1S, #6.3.1, #9.1 from #1 16/7/2019 and X-33 #5, #25 from #3 20190719
Protocol:

taken 1ml, working on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
all samples were shortly vortexed and spinned down
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of leftover culture snap frozen and stored in fridge

Results: Samples are stored in the fridge. By accident culture #25 samples were boiled twice. New samples should be prepared from frozen stocks.

#3 SDS-PAGE preparation for all #6.3.1 samples

Aim: Boil the samples in Sample Loading Buffer 1X to prepare them for SDS-PAGE. Use Triton for pellets.

Experimenter: Tereza, Hugo
Sample: KM71H culture #6.3.1 sampled at #2 20190719, #1 20190720, #? 20190721, #1 20190722
Protocol:
For supernatant.

Samples were thawed
80ul of the supernatant was transferred to a new tube and mixed with 20ul 5XSLB

For pellet.

Samples were thawed
The pellet was resuspended in 800 uL PBS
80 uL was taken and spinned down at 3min, 17 000 x g and 60 uL supernatant was removed
20ul pellet mixed with 80uL 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of leftover culture snap frozen and put to -20˚C

Results: Samples are stored in the fridge.

WEDNESDAY, 24/7/2019

#1 Sampling of KM71H cultures #6.3.1, #5.2.1 L, #5.2.1. S

Aim:

Experimenter: Tereza, Hugo

Sample: Cultures KM71H #5.2.1L, #5.2.1S, #6.3.1

taken 1ml, working on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
all samples were shortly vortexed and spinned down
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of leftover culture snap frozen and stored in fridge

Results:

#2 SDS-PAGE 15% gel analysis of RV1284 expression of KM71H 6.3.1

Aim: Analyse expression of X-33

Experimenter: Tereza, Hugo

Sample: 4x 15% Separation gel

Table2

	A	B
1	Component	Volume
2	H2O	1.41 mL
3	Acrylamide-Bis 30 % *Note	3 mL
4	Tris HCl 1.5 M pH 8.8	1.5 mL
5	SDS 20%	30 uL
6	APS 10%	60 ul
7	TEMED	6 ul
8	Total volume	6 mL
9	*note: 29:1 Acrylamide was used instead of 37.5:1 in orignial protocol.	

4x 4% Stacking gels

Table3

	A	B
1	Component	Volume
2	H2O	3.075 mL
3	Acrylamide-Bis 30 % *Note	0.65 mL
4	Tris HCl 0.5 M pH 6.8	1.25 mL
5	SDS 20%	25 uL
6	APS 10%	50 ul
7	TEMED	5 ul
8	Total volume	5 mL
9	*note: 29:1 Acrylamide was used instead of 37.5:1 in orignial protocol.	

Protocol: Running conditions: 85 V for ca 20 min and 130 V for 65 min.
Staining: The gels were placed in Coomassie Brilliant Blue (CBB) and shaken for 30 min, 40 s microwaved then incubated in CBB for 10 min. Rinsed in ddH2O twice and O/N in destaining solution
Results: See the results in #3 for 20190729

THURSDAY, 25/7/2019

#1 SDS-PAGE sample preparation - X33 #5

Aim: Prepare samples for analysis of RV1284 expression in X-33 strain #5

Experimenter: Tereza, Hugo
Sample: X-33 cultures #5 and #25 from #3 20190719. Sample 3,4,5 for #5 and 4,7 for #25
Protocol:
For supernatant.

Samples were thawed
80ul of the supernatant was transfered to a new tube and mixed with 20ul 5XSLB

For pellet.

Samples were thawed
The pellet was resuspended in 800 uL PBS
80 uL was taken and spinned down at 3min, 17 000 x g and 60 uL supernatant was removed
20ul pellet mixed with 80uL 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of leftover culture snap frozen and put to -20˚C

Results: Samples are stored in the fridge.

#2 SDS-PAGE 15% gel analysis of RV1284 expression of KM71H 6.3.1

Aim: Analyse expression of X-33

Experimenter: Tereza, Hugo

Sample: 4x 15% Separation gel

Table4

	A	B
1	Component	Volume
2	H2O	1.41 mL
3	Acrylamide-Bis 30 % *Note	3 mL
4	Tris HCl 1.5 M pH 8.8	1.5 mL
5	SDS 20%	30 uL
6	APS 10%	60 ul
7	TEMED	6 ul
8	Total volume	6 mL
9	*note: 29:1 Acrylamide was used instead of 37.5:1 in orignial protocol.	

4x 4% Stacking gels

Table5

	A	B
1	Component	Volume
2	H2O	3.075 mL
3	Acrylamide-Bis 30 % *Note	0.65 mL
4	Tris HCl 0.5 M pH 6.8	1.25 mL
5	SDS 20%	25 uL
6	APS 10%	50 ul
7	TEMED	5 ul
8	Total volume	5 mL
9	*note: 29:1 Acrylamide was used instead of 37.5:1 in orignial protocol.	

Protocol: Running conditions: 80 V for ca 20 min and 130 V for 70 min.

Staining: The gels were placed in Coomassie Brilliant Blue (CBB) and shaken for 1h, rinsed in ddH2O twice and O/N in destaining solution.
Results: See the results in #3 for 20190729

FRIDAY, 26/7/2019

#1 Fresh restreaks of X33 and KM71H for transformation

Aim: Prepare freah restreaks of colonies for transformation of constructs from cloning groups sometime next week
Experimenter: Tereza
Sample: #1 20190630
Protocol: YPD plates, sterile conditions using flame and metal loop
Results: Small single colonies observed on Monday 20190729, smaller for KM71H

#2 Gibson assembly of AAO-2A-HRP

Aim: Insert AAO-2A-HRP peptide into pPICZα-B *E. coli* - yeast shuttle vector
Experimenter: Tereza
Sample: -
Protocol: 2:1 ration used, NEBioCalculator used for counting
10 ng/ul diluted construct
13.6 ng/ul vector

Table1

	A	B
1	**Final volume**	**24 ul**
2	Master Mix (2X)	12 ul
3	pPICZα-B	3.68 ul
4	AAO-2A-HRP	8.33 ul

there has been a delay before the incubation started, 50°C, 15min
Results: Failure

SATURDAY, 27/7/2019

#

Aim:
Experimenter:
Sample:
Protocol:
Results:

SUNDAY, 28/7/2019

#

Aim:

Experimenter:

Sample:

Protocol:

Results:

Ethanol precipitation Easyselect-man

Introduction

Ethanol precipitation after phenol extraction of DNA according to Easyman-select protocol p.26.

Materials

- 3M Sodium acetate
- 100% ethanol
- 80% ethanol
- Sterile deionized water

Procedure

- [x] 1. Add 1/10 volume of 3M sodium acetate to the upper aquatious phase and 2.5 volume of 100% ethanol.
- [x] 2. Centrifuge the solution to pellet DNA, wash the pellet with 80% ethanol, air-dry, and resuspend in 10 µL sterile, deionized water.
- [x] 3. Use immidiately or store at -20°C.

Notebook week 29/7-4/8

Project: iGEM uppsala 2019
Authors: Jonas Gockel

MONDAY, 29/7/2019

#1 preculture BL21

Aim: preculture for competent cells

Experimentator: Jonas
sample: plate BL21 from may
Protocol: 5mL LB + colony
incubated 37 °C shaking o/N
results: preculture

#2 PCR of gibson minipreps

Aim: analyse if gibson assembly worked

Experimentator: Jonas
Sample: various minipreps from qiang
protocol: MasterMix was prepared for 13 reactions according to following recipy

Table1

	A	B
1	H2O	497.25 uL
2	10x Dreamtag buffer	65 uL
3	dNTPs 2mM	65 uL
4	Fwd primer 100 uM	3.25 uL
5	rev primer 100 uM	3.25 uL
6	DreamTaq	3.25 uL

1 uL of the different minipreps was used as template for PCR

PCR program was as following

Table2

	A	B
1	95 °C	3:00
2	95 °C	0:30
3	55 °C	0:30
4	72 °C	1:30
5	goto step 2 x25	
6	72 °C	7:00
7	4 °C	unlimited

Samples were loaded on 1 % agarose gel after run

Results: ADD GEL PIC

Glox 3, MnP 1, MnP 2 and MnP 3 were postive with a band at the correct height

#3 Complete analysis of RV1284 expression in *P. pastoris* by SDS-PAGE

Aim: Analyze expression of X-33 #5

Experimenter: Tereza

Sample: 20190718 #1 X-33 expression culture, 20190716 #1 KM71H expression culture

Protocol: -

Results: Attached powerpoint

RV1284_Expression_squares.pptx

#4 YPD cultures of the 5 expression colonies

Aim: Make 20mL YPD cultures of each of the colonies used for expression, for later PCR analysis

Experimenter: Yannick

Sample: Colonies X-33 pPIC RV1284 #5 and #25, KM71H pPIC RV1284 #5.2.1, #6.3.1, #9.2 (#9.1 had too little material and #9.2 should be the same) from the same plates as were used for the expression: Restreaks from #1 20190712 and transformation plates from #6 20190711

Protocol: Aliquoted 20mL of YPD to each of 5 sterile 100mL e-flasks. Inoculated with the appropriate colonies with a pipette tip.

Results: 20mL cultures of X-33 pPIC RV1284 #5, #25, KM71H pPIC RV1284 #5.2.1, #6.3.1, #9.2 being incubated at 28°C and shaking from ~12.00

TUESDAY, 30/7/2019

#1 Preparation *of P. pastoris* X-33 and KM71H for electroporation - small cultures

Aim: To have competent cells on Thursday 20190801 morning

Experimenter: Tereza

Sample: restreaks #1 20190726, colonies #1 KM71H, #2 X33.

Protocol: At 17:00, 5ml of YPD inoculated by sterile metal loop, incubated at 28C shaking 120 rpm.

Results: Continued with KM71H and X-33 #1 20190731.

#2 Colony PCR transformed pichia cells

Aim: analyse presence of Rv1284

Experimenter: Jonas

Sample: liquid cultures inoculated by yannik the day before

Protocol: DNA was prepared by spinning down 100 uL of culture and resuspending the supernatant in 100 uL of 0.2 M LiOAc +1% SDS

incubated at 70 °C for 5 min

add 300 uL EtOh absolut

spin down max speed 3 min

wash pellet with 70 % EtOH

dissolve pellet in 100 uL H2O

spin down debris 1 min max speed

1 uL used in PCR reaction

Master mix for 7.5 reactions was made

Table4

	A	B
1	Buffer	37,5 ul
2	dNTPS 2mM	37.5
3	fwd prim	1.875
4	rev prim	1.875
5	polymerase	1.875
6	h2o	286.875

Following pcr program was used

Table5

	A	B
1	95 °C	5:00
2	95 °C	0:30
3	55 °C	1:00
4	72 °C	1:30
5	goto step 2 x30	
6	72 °C	7:00
7	4 °C	unlimited

Results: Bands visible in all samples except in KM71H pPIC-Rv1284 #9.2

-> fake resistance against zeocin and no Rv1284

#3 Colony PCR pPICzaB

Aim: determine if any gibson was successful

Experimenter: Jonas

Sample: plate from Jinwen

Protocol:

colony picked and resuspended in 30 uL h20 with sterile yellow pipet tip

master mix was prepared as follows for 12.5 reaxtions volume 50 uL

Table6

	A	B
1	Buffer	62.5
2	dNTPS 2mM	62.5
3	fwd prim	3.125
4	rev prim	3.125
5	polymerase	3.125
6	h2o	487.125

PCR program was as follows

Table7

	A	B
1	95 °C	5:00
2	95 °C	1:00
3	55 °C	1:00
4	72 °C	1:30
5	goto step 2 x30	
6	72 °C	7:00
7	4 °C	unlimited

Results:

#4 Preparation of SDS-PAGE samples X-33 #25,

Aim: Prepare expression samples for SDS-PAGE gel analysis

Experimenter: Yannick

Sample: Samples taken of X-33 #25[most days], KM71H #5.2.1s[4-5], #5.2.1L[1-5], #9.1 [5]ip and up

Protocol: The samples were taken in a few different ways and therefore needed to be prepared in a few different ways.

Samples [1-4] were prepared as follows:

For supernatant.

Samples were thawed

80ul of the supernatant was transfered to a new tube and mixed with 20ul 5XSLB

For pellet.

Samples were thawed

The pellet was resuspended in 800 uL PBS

80 uL was taken and spun down at 3min, 17 000 x g and 60 uL supernatant was removed

20ul pellet mixed with 80uL 1.25SLB + 0.8% Triton X-100

samples boiled on heating block for 10 min at 95˚C

tubes with 900ul of leftover culture snap frozen and put to -20˚C

Samples [5] were prepared just by adding the 5x SLB and 1.25SLB + 0.8% Triton X-100 to the supernatant and pellet respectively in the same way as the above, and then boiling in the same way.

Results: Processed samples ready for loading into SDS-PAGE gels.

WEDNESDAY, 31/7/2019

#1 Preparation of *P. pastoris* X-33 and KM71H for electroporation - big cultures

Aim: Inoculate so that the big cultures reach OD_{600} between on Thursday 20190801 1.4 tomorrow at 9:30, so that they can be harvested and we have competent cells

Experimenter: Tereza

Sample: small cultures #1 20190730

Protocol:

OD measurement of small culture

X-33 OD_{600}= 6.91

KM71H OD_{600}= 18.54

Calculation of desired starting OD_{600} and volumes of inoculums

- How many generation between 17:30 20190731 and 9:30 20190801?

16 hrs ~ considering 2hrs generation time and no lag ~ **8 generations**

To reach OD_{final}= 1.4 $\mathbf{2^8 \times OD_{starting} = OD_{final}}$

$OD_{starting} = 1.4 / 2^8$

$OD_{starting} = 0.00547$

- Volume of inoculum ?

$\mathbf{V_{culture} \times OD_{starting} = V_{inoculum}\ OD_{inoculum}}$

$V_{inoculum\ X\text{-}33}$ = 396 ul

$V_{inoculum\ KM71H}$ = 148 ul

Inocultaion

X-33 inculated by 400 ul of culture and KM71H by 150ul.

Results: Successfully reached proper OD_{600} of KM71H cuture, but not of X-33 #1 20190801.

#2 Running SDS-PAGE gels with #9.1, #5.2.1s, #5.2.1L This day or tuesday???

Aim: Analyzing the expression samples #9.1[5-7], #5.2.1s[4-7], #5.2.1L[1-4]

Experimenter: Yannick

Sample: Samples prepared in 20190730 #4 and previously prepared samples.

Protocol:

In table 8 is the planned loading pattern. #9.1[5]up is empty because that sample was nowhere to be found. The empty cells were loaded with 1x SLB to give a consistent osmotic pressure. L stands for ladder. 10 uL was loaded each time.

Table8

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	#5.2.1L (gel 5)		[1]				[2]				[3]						
2		L	ip	is	up	us	ip	is	up	us	ip	is	up	us	empty	empty	
3	#5.2.1L & #5.2.1s (gel 6)		#5.2.1L [4]								#5.2.1s [4]						
4		empty	ip	is	up	us	empty	empty	empty	empty	ip	is	up	us	L	empty	
5	#5.2.1s (gel 20)		[5]				[6]				[7]						
6		empty	ip	is	up	us	ip	is	up	us	ip	is	up	us	L	empty	
7	#9.1 (gel 21)			[5]				[6]				[7]					
8		empty	L	ip	is	empty	us	ip	is	up	us	ip	is	up	us	empty	

Some small mistakes were made during the loading. The last empty slots in gels 5 and 21 were not empty (marked green) so another sample must have been loaded twice in that gel.

Some of #5.2.1s [7]is spilled into [7]up.

The gels were then run at 80V for ~15 min, put och 130V after that.

1.5 hours later the gels were taken out, stacking gel removed, put into stain on 50 rev/min. shaking until tomorrow.

Results: After destaining Tereza imaged the gels. [insert pictures]

THURSDAY, 1/8/2019

#1 OD measurement of cultures for competent cells

Aim: Harvest at correct OD_{600} between 1.3 - 1.5

Experimenter: Yannick & Tereza

Sample: big cultures #1 20190731

Protocol: Measure on spectrophotometer, different dilutions combined

Table3

	A	B	C	D	E	F	G
1	Ttime / Strain	KM71H			X-33		
2		measured	dilution	real OD	measured	dilution	real OD
3	9:30	0.024	10X	0.233	0.109	10X	1.073
4	9:30	0.045	5X		0.211	5X	
5	10:50	0.131	10X	1.330	-	-	-
6	10:50	0.135	10X		-	-	
7	11:30	-	-	-	0.084	5X	0.429
8		-	-		0.146	3X	
9	*time when the growth was interrupted						

Results: KM71H harvested at 11:00 #2 20190801, X-33 is following doubling time 2hrs when measures at 9:30 and 11:30. Thus it could be ready for harvesting between 14:00 -14:30, busdt it is too late so the culture was discarded.

#2 Harvesting KM71H competent cells

Aim: Competent cells for transformation today

Experimenter: Yannick

Sample: #1 20190801

Protocol: -Centrifuged 500 mL culture at 4°C, 1500 g for 5 min., resuspend pellet in 500mL ice-cold sterile water

-Centrifuge the same way, resuspend in 250 mL ice-cold sterile water.

-Centrifuge, resuspend in 20 mL ice-cold 1M sorbitol

-Centrifuge, resuspend in 1 mL ice-cold 1M sorbitol by pipetting instead of the vortexing used in the previous steps.

Results: -Stored on ice until use.

#3 Transformation

Aim:

Experimenter: Jonas

Sample: Competent cells from 20190801 #2, DNA from ???

Protocol:

Results:

#4 Plating of trafos

Aim: Plating the transformations

Experimenter: Yannick & Johan

Sample: Tranformants from 20190801 #3

Protocol: Had both new and old YPDS+zeocin plates. Plated the most important dilutions on new plates and the less important ones on older plates.
Results:

FRIDAY, 2/8/2019

#1 Preparation of: Expression media BMGY and BMMY, YPDS plates + Zeocin™, YPD medium

Aim: Prepare for transformations and following expression
Experimenter: Tereza, Yannick
Sample: -
Protocol: Standard `Easy select protocol followed. Media completed 20190805 Hugo
Results:

4L BMGY
1l BMMY
2 x 500mL YPD
500mL YPDS + Zaocin™ -> plates

SATURDAY, 3/8/2019

#1 O/N culture for trafo X33

Aim:
Experimenter: Jonas
Sample:
Protocol:
Results:

SUNDAY, 4/8/2019

#1 Big culture for trafo X33

Aim:
Experimenter: Jonas
Sample:
Protocol:
Results:

Notebook week 5/8-11/8

Project: iGEM uppsala 2019
Authors: Tereza Hubáčková

MONDAY, 5/8/2019

#1 Preparation of SDS-PAGE gels

Aim: Remove proteins from linearized plasmid for transformation. We hope that it might improve transformation efficiency.

Experimenter: Tereza
Sample: Jonas - HRP1, HRP2, HRP3, MnP1, MnP2, MnP3, Glox3 5ug of DNA in 25ul volume
Protocol:

Phenol Chloroform Extraction

1. Add 1 volume of Phenol : Chloroform : Isoamylalcohol (25:24:1)
2. vortexed for ~20sec
3. centrifuged 5min, 16 000g, RT - repeated for some samples, because of difficulties with phases separation
4. Aqueous phase transferred to the new tube

Ethanol precipitation

1. 1/10 volume of 3M sodium acetate pH 5.3 added, RT (2ul)
2. 2.5 volumes of 100% ethanol (50ul) added
3. centrifuged to pellet DNA - 10min, 17 000g, RT
4. 300ul of 70% ethanol added to wash, air dried for 15min
5. resuspended in 10ul sterile ddH_20
6. left -20˚C, 3 hours

Results:

#2 Competent *P. pastoris* big O/N culture

Aim: To reach OD_{600} = 1.3 - 1.5 tomorrow 20190806, to harvest competent cells for transformation of GLOX, HRP, MnP

Experimenter: Tereza
Sample: #1 Big culture for trafo 20190804, Jonas
Protocol: 500 ml YPD inoculated by 150ul of culture from the big culture for transformation, instead of standard inoculation from small culture
Results: Cells hravested and electroporated on Tuesday 20190806

#3 Competent *P. pastoris* small O/N culture X33

Aim: To have backup small culture for compretent cells preparation, if big culture #2 20190805 fails
Experimenter: Tereza
Sample: #1 20190726
Protocol: 5ml of preheated YPD inoculated by sterile loop from the plate from the fridge
Results: Culture not used.

#4 Linearization of pPICZαB HRP-2A-AAO

Aim: Linearize pPICZαB HRP-2A-AAO

Experimenter: Sofia

Sample: Miniprep Jonas 20190804 pick I and II [Not yet in benshling]

Protocol: 50 µL total volume, 10 µg DNA.

Volume of DNA: Pick I 388.0 ng/µL --> 10000ng/388.0 ng/µL=25.8 µL

Pick II 465.7 ng/µL --> 10000ng/465.7ng/µL=21.5 µL

1. Add water: 17.2 µL for pick I and 21.5 µL for pick II
2. Add 5 µL of reaction buffer
3. Add 10 ng of DNA: 25.8 µL for pick I and 21.5 µL for pick II
4. Add 2 µL of SacI
5. Incubate at 37°C for 2 h.
6. Left on ice for about 2 h.
7. Heat inactivation 20 min at 85°C.

Results: Stored in -20°C.

#5 PCR for analyzing construct

Aim: Amplifying pPICZαB HRP-2A-AAO to analyze the construct

Experimenter: Sofia

Sample: Miniprep pPICZαB HRP-2A-AAO Jonas 20190804 pick I and II [Not yet in benshling]

Protocol:

Table1

	A	B	C
1	Chemical	In 1 tube (µL)	Master mix (4.5) (µL)
2	dNTPs	5	22.5
3	Forward primer	0.25	1.125
4	Reverse primer	0.25	1.125
5	Buffer	5	22.5
6	Taq polymerase	0.25	1.125
7	Template	1	-
8	H20	38.25	172.125

1. Make a mastermix with the chemicals stated above.
2. Add DNA/water to the tubes. Transfer 49 µL master mix to the 4 different tubes.
3. PCR made according to instuctions for pPICZaB.

Results: Later run on agarose gel #12 20190805.

#6 OD_{600} measurement of big X-33 culture (for making competent cells)

Aim: To estimate whether the culture should be used to make competent cells or not

Experimenter: Hugo

Sample: #1 Big culture for trafo 20190804, Jonas

Protocol: Absorption was measured at 600 nm for a 1:10 diluted culture. The diluted OD=0.250 and the non-diluted OD=2.50

Results: The culture was used to inoculate a new culture in #2 20190805

#7 Preparing BMGY and BMMY media

Aim: To prepare media for further use during expression

Experimenter: Hugo

Sample: Solutions prepared in #1 20190802

Protocol: Standard Easy select protocol followed.

Table4

	A	B	C
1	Material	BMGY	BMMY
2	ddH20	700mL	700mL
3	1M KH2PO4 (pH 6.0)	100mL	100mL
4	10x YNB (13.4%)	100mL	100mL
5	500x Biotin (0.02%)	2mL	2mL
6	10x MeOH (5%)	-	100mL
7	50% glycerol	20mL	-
8	Sterile ddH2O	80mL	-

Results: BMGY- and BMMY medium ready to use, stored in coldroom at 4 °C

#8 Making 1 M H_2KPO_4 pH 6 and 5% methanol solution

Aim: Preparing H_2KPO_4 and methanol to use in BMGY and BMMY

Experimenter: Hugo

Sample: -

Protocol: 108.87g H_2KPO_4 was dissolved in ca 0.5L ddH_2O and the pH was adjusted to pH=6 using 5M NaOH. The solution was diluted to a final concentration of 800mL, the solution was autoclaved.

10 mL 100% methanol was diluted to a final volume of 200 mL.

Table3

	A	B	C	D
1	Chemical	Molecular weight	Mass added to 0.8 L	Volume of 100% methanol added to solution
2	KH2PO4	M(H2KPO4) = 136.09 g/mol	1M*0.8L*136.09g/mol= 108.872g	
3	Methanol			5%*200mL/100%=10mL

Results: Both solutions stored in room temperature above Yannicks bench.

#9 Linearization of pPICZaB HRP3 #1 and pPICZaB MnP3 #1 midipreps

Aim: Linearize for transformation

Experimentator: Sofia
Sample: Midiprep of pPICZaB HRP3 #1 and pPICZaB MnP3 #1
Protocol: 1. Water: 9.23 µL for HRP and 18.53 µL for MnP
2. 5 µL buffer for SacI
3. 10 µL of DNA. 33.77 µL for HRP and 24.47 µL for MnP.
4. 2 µL of SacI
5. Incubated in 37°C for 2 h.
6. Heat inactivated 20 min in 85°C.
Result: Used for transformation.

#10 Phenol/Choroform extraction of pPICZαB HRP-2A-AAO

Aim: Extract DNA for concentration

Experimentator: Sofia
Sample: Digested pPICZαB HRP-2A-AAO #4 Sofia 20190805 pick I and II
Protocol: #Phenol/chloroform extraction of DNA
Result: Extracted DNA used in ethanol precipitation #11 20190805

#11 Ethanol precipitation of pPICZαB HRP-2A-AAO

Aim: Precipitate DNA for concentration

Experimentator: Sofia
Sample: Extracted pPICZαB HRP-2A-AAO #10 Sofia 20190805 pick I and II
Protocol: #Ethanol precipitation openwetware
Step 1 and 2 preformed this day. Left in -20°C over night.
Result:

#12 1% Agarose gel and analysation of run gel of PCR result pPICZαB HRP-2A-AAO

Aim: Prepare and run an agarose gel of pPICZαB HRP-2A-AAO

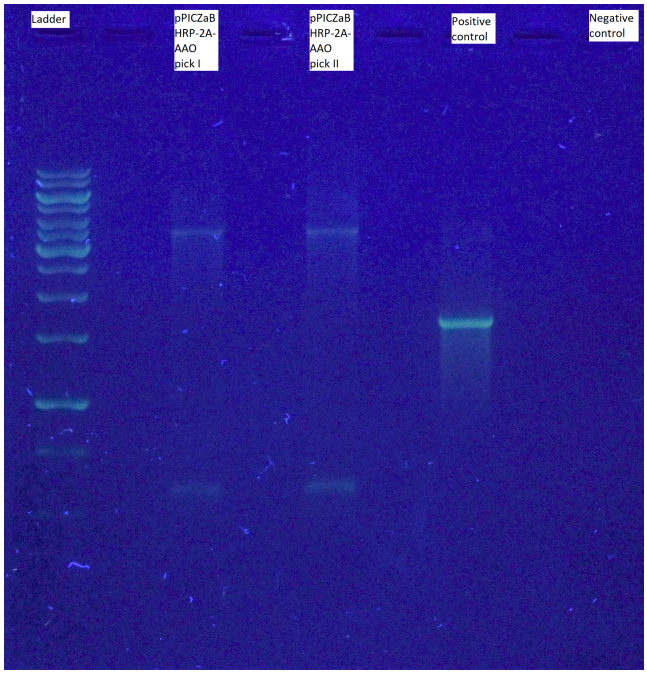
Experimentator: Sofia
Sample: PCR of pPICZαB HRP-2A-AAO #5 Sofia 20190805
Protocol: #1% agarose gel preparation

Table5

	A	B	C	D	E	F	G	H	I	J
1	1	2	3	4	5	6	7	8	9	10
2	Ladder	X	pPICZαB HRP-2A-AAO pick I	X	pPICZαB HRP-2A-AAO pick II	X	Positive	X	Negative	X

Result:

20190805_pcr_HRP_2a_AAO construct test 1.tif



#13 O/N cultures

Aim: Create O/N cultures of different strains and cultures

Experimentator: Sofia and Tereza
Sample: 1. Restreak form 20190630 --> 20190726 KM71H Tereza colony #2
2. [Tereza insert]
3. Glycerol stock of Glox3 in DH5a

4. Restreak from 20190630 --> 20190726 x-33 colony #2

Protocol: 1. 5 mL YPD. Inoculated at 17.30
2. 500 mL YPD
3. 50 mL LSLB+Zeocin. Inoculated at 17.45.
4. 5 mL YPD. Inoculated at 18.00.

Result:

TUESDAY, 6/8/2019

#1 Transformation of X33 by HRP, GLOX, MnP, HRP-2A-AAO #1 and #2

Aim: Transform constructs and express genes in *P. pastoris* X33

Experimenter: Tereza, Sofia

Sample: cells #2 20190805, DNA?

Protocol: Transformation by adjusted EasySelect Electroporation p.28 in EasySelect Pichia Expression Kit User Manual

When culture 2 #13 20190805 reach OD_{600} 1.3-1.5, cells harvested according to EasySelect Pichia Expression Kit User Manual.

1. Mix 80 µl of the cells with 5–10 µg of linearized DNA (in 5–10 µl sterile water) and transfer them to an ice-cold 0.2 cm electroporation cuvette.
2. Incubate the cuvette with the cells on ice for 5 minutes.
3. Pulse the cells
4. Immediately add 1 ml of ice-cold 1 M sorbitol to the cuvette. Transfer the cuvette contents to a sterile 15-ml tube and incubate at 30°C without shaking for 1-2 hours
5. Spreaded **25, 50, 100 µl each** on separate, labeled YPDS plates containing 100 µg/ml Zeocin™, also negative control.
6. Incubate plates from 3–10 days at 30°C until colonies form.

Deviations from protocol:

- The instrument settings for the electroporation machine was V = 1.5 kV; C=25 µF; R=200 Ω; t=4-10 ms.
- In step 4 the culture was incubated for ca 2 hours.
- Plating was done as indicated, for negative control (consisting of competent cells and water instead plasmid DNA) 100 µl was plated.
- Cells incubated without shaking in 28°C.

Results:

#2 Midiprep of pPICzαB-GLOX III

Aim: To extract pPICzαB-GLOX and further use in transformation of yeast

Experimenter: Hugo

Sample: Either from Qian or Jinwen

Protocol: 50 mL of the culture was centrifuged for 15 min at 4000 rpm. The pellet was resuspended in in 3 mL Resuspension sulution. 3 mL Lysis solution was added and turned 10x times. After 3 min 5 mL Neutralization buffert solution was added and turned 10x times. The mixture was transferred to an Ependorf-tube and centrifuged for 15 min at 15000 g

Results:

#3 Preparing 1xYPD medium

Aim: Making 800mL 1x YPD medium

Experimenter: Sofia

Sample: -

Protocol:

Table7

	A	B	C
1	**Chemical**	**Amount (g)**	
2	Yeast extract	8	
3	Peptone	16	
4	+Add 20% dextrose solution	80 mL	

Yeast extract and peptone mixed with sterile water to a volume of 720 mL. Autoclaved. Dextorse solution added.

Result: Stored in cold room.

#4 Ethanol precipitation (continuation #11 Ethanol precipitation of pPICZαB HRP-2A-AAO 20190805)

Aim: Precipitate DNA for concentration

Experimentator: Sofia

Sample: Extracted pPICZαB HRP-2A-AAO #10 Sofia 20190805 pick I and II

Protocol: #Ethanol precipitation openwetware

Continued after overnight storage in -20°C.

Step 3: Centrifuge at full speed (16 000 g) for 30 min in room temperature (4°C in protocol).

Step 4: Decnat the supernatant

Step 5: Airdry for 30 min (15 min accordning to the protocol).

Step 6: Resuspen in 10 µL water. Vortex, spin down.

Result: Concentration 1556,5 ng/µL

An additional peak at ~225 nm

#4 Linearization of pPICZαB HRP-2A-AAO

Aim: Linearize pPICZαB HRP-2A-AAO

Experimenter: Sofia

Sample: Miniprep Jonas 20190804 pick I and II [Not yet in benshling]

Protocol: 50 µL total volume, 10 µg DNA.

Volume of DNA: Pick I 388.0 ng/µL --> 10000ng/388.0 ng/µL=25.8 µL

Pick II 465.7 ng/µL --> 10000ng/465.7ng/µL=21.5 µL

1. Add water: 17.2 µL for pick I and 21.5 µL for pick II
2. Add 5 µL of reaction buffer
3. Add 10 ng of DNA: 25.8 µL for pick I and 21.5 µL for pick II
4. Add 2 µL of SacI
5. Incubate at 37°C for 2 h.
6. Left on ice for about 2 h.
7. Heat inactivation 20 min at 85°C.

Results: Stored in -20°C.

WEDNESDAY, 7/8/2019

#1 O/N culture DH5α pPICZαB

Aim: To midiprep plasmid DNA and use it as empty-vector control during yeast expression of target proteins.

Experimenter: Tereza

Sample: colony #5 plate Lilli 20190620

Protocol: 50ml of LSLB + Zeocin inoculated via sterile loop by colony #5

Results: Midiprep 20190808

#2 Streaking of transformation

Aim: Streaking transformed cells on YPDS+zeocin plates

Experimentator: Sofia, Hugo, Yannick, Tereza

Sample: Transformation 20190807 [Not yet in benshling]

Protocol: Streaking of 10, 25, 50, 100 and 200 µL of transformed cells for those stroken on 5 plates:
AAO 2, Glox3
Streacking of 25, 50, 100 and 200 µL of transformed cells for those stroken on 4 plates:
AAO 1, HRP #1

Result:

THURSDAY, 8/8/2019

#1 OD measurement for transfer of expression cultures to Inducing Media

Aim: Transfer cells to medium containing methanol and start expression.

Experimenter: Tereza

Sample: Expression culture HUgo 20190807

Protocol: Standard OD_{600} measurement at 9:30 (time of sampling = growth interrupted)

Table2

	A	B	C	D
1	Culture	1:5	1:3	Indiluted
2	MnP3 #3	0.020	0.038	0.099
3	MnP3 #4	0	0.009	0.039
4	HRP3	0.022	0.043	
5	HRP	0.002	0.004	

Results:

#2 Midiprep of empty pPICZaB

Aim: Extract DNA for transformation

Experimentator: Sofia

Sample: 50 mL #1 O/N culture DH5α pPICZαB 20190807
Protocol: #PureYield™ Plasmid Midiprep System
In step 1-12 centrifugation at 4000 rpm, 2 min uesd instead of vacuum.
Result: Concentration 381,4 ng/µL

#3 Analytical digestion of pPICZaB

Aim: Digest pPICZaB and later run on agarose gel to see if the construct match the expected sizes.

Experimentator: Sofia
Sample: Midiprep of empty pPICZaB #2 20190808.
Protocol: BamHI: Cut at bp 1682
SacI: Cut at bp 209
--> Expected fragments: 1473 bp and 2124 bp.

20 µL reaction volume, 200 ng DNA.
--> 0.524 µL of sample.

1. Water: 16.676 µL
2. 5 µL buffer for SacI
3. 200 ng of DNA. 0.524 µL of sample.
4. 0.4 µL of SacI and 0.4 µL of BamHI
5. Incubated in 37°C for 30 h.
6. Heat inactivated 20 min in 80°C.

Result: Run on agarose gel #5 20190808

#4 OD-measurement of KM71H-cultures for expression

Aim: Measure optical density to see if the cultures are ready to be induced

Experimenter: Sofia
Sample:
Protocol: Samples of the cultures diluted 1:5 and 1:3 in BMGY before measuring the OD.
Results:

Table6

	A	B	C	D	E
1		1:5	Undiluted	1:3	Undiluted
2	MnP #3	0.110	0.55	0.188	0.564
3	MnP #4	0.015	0.075	0.027	0.081
4	HRP c1	0.135	0.675	0.226	0.678
5	HRP c2	0.021	0.105	0.033	0.099

Cultures not ready to be induced. Left in incubation for longer time.

#5 1% Agarose gel and analysation of gel of pPICZaB

Aim: Prepare and run an agarose gel of digested pPICZαB

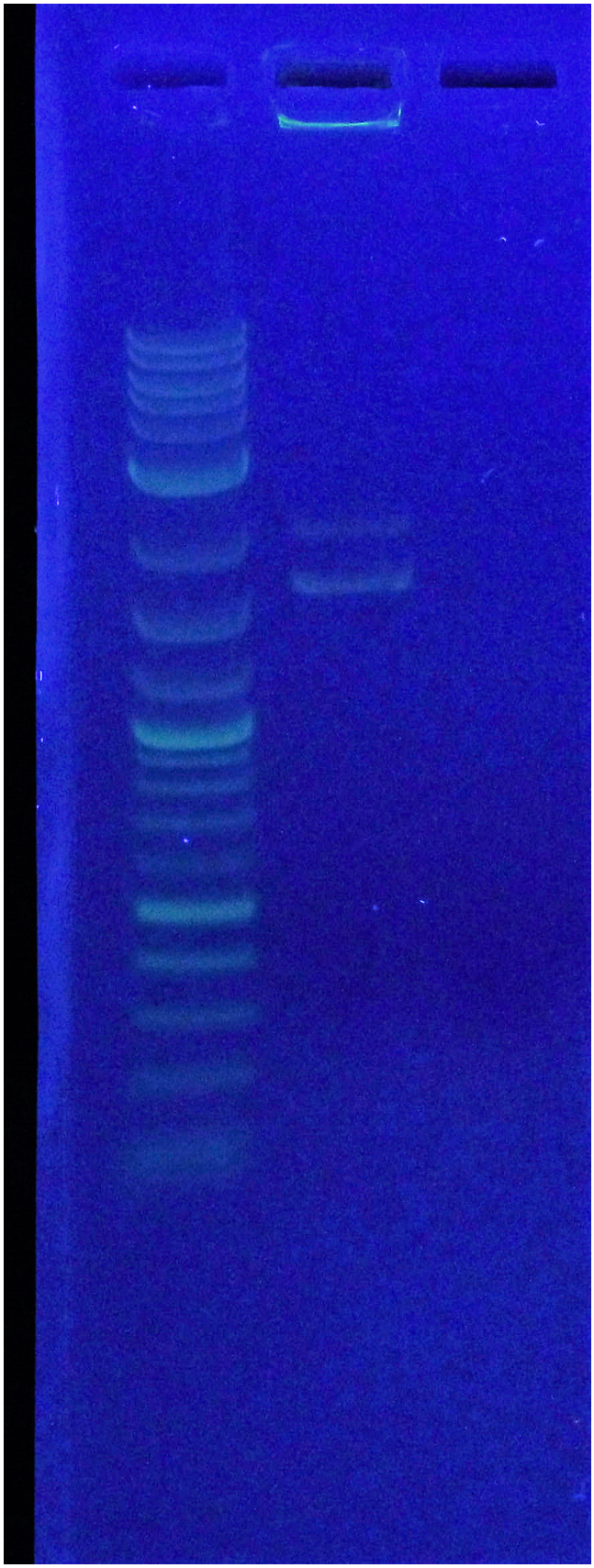
Experimentator: Sofia
Sample: Analytical digestion of pPICZαB #2 Sofia 20190808
Protocol: #1% agarose gel preparation

Table8

	A	B	C
1	1	2	3
2	Ladder	Digested pPICZaB	Negative control

Result: Band match the expected sizes.

20190808 rest_dig_pPICZaB 1.tif



#6 50 mM phosphate buffer with 1 M ammonium sulphate, pH 7.5

Aim: Prepare buffer for enzymatic assay group

Experimenter: Sofia
Sample:
Protocol: Chemicals for 50 mL of buffer:

Table9

	A	B	C
1	Chemical	Amount	
2	Sodium (Hydrogen) Phospate 7-hydrate	0.505 g	
3	Sodium phosphate monobasic monohydrate	0.085 g	--> 0.739 g sodium phosphate monobasic
4	Ammonium sulphate	6.757 g	

Deionized water added to final concentration of 50 mL. Sterile filtered.
Results: Stored by enzymatic assay group.

FRIDAY, 9/8/2019

#1 OD measurement of HRP colony 1 control for induction

Aim: Measuring OD for changing media for expression

Experimenter: Sofia
Sample:
Protocol: Measuring OD after dilution of sample 1:5 and 1:3 in BMGY. Done at ~7.30
Results: 1:5: 1.260 --> 6.3 undiluted
1:3: 1,565 --> 4.695 undiluted
The culture is ready to be harvested.

#2 Harvest HRP colony 1 control

Aim: Harvest and change media of the control culture for expression

Experimenter: Sofia
Sample:
Protocol: Culture transfered into 2 falcon tubes. Centrifuge at 3000 g for 5 min in roomtemperature.
First sample for SDS taken at 8.20.
Results:

#3 Colony PCR of HRP c1 and c2 and MnP #3 and #4

Aim: Amplify the construct in transformed KM71H cells for analyzation

Experimenter: Sofia and Hugo
Sample: KM71H expression cultures: HRP c1, HRP c2, MnP #3, MnP #4
Protocol: #EXTRACTION OF GENOMIC DNA FROM YEASTS FOR PCR-BASED APPLICATIONS
Results: 1 µL of supernatant used for PCR #4 20190809.

#4 Colony PCR of HRP c1 and c2 and MnP #3 and #4 continuation

Aim: Amplify the construct in transformed KM71H cells for analyzation

Experimenter: Sofia
Sample: #3 Colony PCR of HRP c1 and c2 and MnP #3 and #4 20190809
Protocol:

Table10

	A	B	C
1	Chemical	In 1 tube (µL)	Master mix (x7) (µL)
2	dNTPs	5	35
3	Forward primer	0.25	1.75
4	Reverse primer	0.25	1.75
5	Buffer	5	35
6	Taq polymerase	0.25	1.75
7	Template	1	-
8	H20	38.25	267.75

1. Make a mastermix with the chemicals stated above.
2. Add DNA/water to the tubes. Transfer 49 µL master mix to the 6 different tubes.
3. PCR made according to instuctions for pPICZaB in P. Pastoris:
    a. 95°C 5 min.
    b. 95°C 30 s
    c. 55°C 1 min
    d. 72°C 1 min 30 s
    e. Goto step 2 X30
    f. 72°C 7 min
    g. 4°C ∞

Results: Later run on agarose gel #5 20190809.

#5 1% Agarose gel and analysation of gel of colony PCR

Aim: Prepare and run an agarose gel of colony PCR

Experimentator: Sofia

Sample: Colony PCR #4 20190809
Protocol: #1% agarose gel preparation

Table11

	A	B	C	D	E	F	G
1	1	2	3	4	5	6	7
2	Ladder	HRP c1	HRP c2	MnP #3	MnP #4	Positive control	Negative control

Result: [Jonas insert result]

#

Aim:

Experimenter:
Sample:
Protocol:
Results:

SATURDAY, 10/8/2019

#6

Aim:

Experimenter:
Sample:
Protocol:
Results:

SUNDAY, 11/8/2019

#6

Aim:

Experimenter:
Sample:
Protocol:
Results:

Notebook week 12/8-18/8

Project: iGEM uppsala 2019
Authors: Yannick Hajee

MONDAY, 12/8/2019

#1 YPD+Zeocin and YPDS+Zeocin plates

Aim: Prepare YPD media with and without sorbitol, add Zeocin and pour plates for selection of yeast tranformants

Experimentator: Sofia
Sample: -
Protocol:

Table4

	A	B	C
1	Ingredients	YPD+Zeocin (500 mL)	YPDS+Zeocin (500 mL)
2	yeast extract	5g	5g
3	peptone	10g	10g
4	sorbitol	-	91.1g
5	agar	10g	10 g
6	Dextrose (20%)	50mL	50mL
7	Zeocin	500uL	500uL

After the autoclaving, 50 mL of (20% dextrose) and 500uL of 100mg/mL Zeocin was added.

Result: Plates of YPD and YPDS with 100 ug/ml Zeocin stored in cold room.

#2 PCR purification

Aim: Purify DNA to get rid of all primers etc to be able to send the samples for sequencing

Experimenter: Sofia
Sample: PCR from 20190809 #4: pPICZaB HRP colony 1 and 2, pPICZaB MnP #3 and #4
Protocol: #GeneJET PCR Purification Kit
Results: Concentrations measured but the curve showed that the samples didn't look like DNA. PCR re-done later.

#3 Colony PCR of pPICZaB HRP colony 1 and 2, pPICZaB MnP #3 and #4

Aim: Amplify the construct in transformed KM71H cells for analyzation

Experimenter: Sofia
Sample: Restreak of trafo 4 HRP colony 1 och 2 5.8.19, Restreak of trafo 3 MnP #3 and #4 5.8.19.
Protocol: 1. #EXTRACTION OF GENOMIC DNA FROM YEASTS FOR PCR-BASED APPLICATIONS
2:

Table10

	A	B	C
1	Chemical	In 1 tube (µL)	Master mix (x7) (µL)
2	dNTPs	5	35
3	Forward primer	0.25	1.75
4	Reverse primer	0.25	1.75
5	Buffer	5	35
6	Taq polymerase	0.25	1.75
7	Template	1	-
8	H20	38.25	267.75

a. Make a mastermix with the chemicals stated above.
b. Add DNA/water to the tubes. Transfer 49 µL master mix to the 6 different tubes.
c. PCR made according to instuctions for pPICZaB in P. Pastoris:
d. 95°C 5 min.
e. 95°C 30 s
f. 55°C 1 min
g. 72°C 1 min 30 s
h. Goto step 2 X30
i. 72°C 7 min
j. 4°C ∞

Results: Later run on agarose gel and sent for sequencing.

#4 Streaking plates

Aim:

Experimenter: Sofia

Sample:

Protocol:

Results:

TUESDAY, 13/8/2019

#1 OD_{600} measurement for transfer of expression cultures to Inducing Media (Erik + Yannick)

Aim: Checking OD Transfer cells to medium containing methanol and start expression.

Sample:

See table below

Protocol:

Standard OD_{600} measurement at 17.00 (time of sampling = growth interrupted)

Yannick put OD BELOW

1 to 10 dilution

Approximately 100 ul are taken from the yeast culture and placed into an eppendorf tube

100 ul is pipetted to a cuvette, where it is suspended in 900 ul of BMGY

OD Measurements 600 nm at 23.00

	A	B	C	D
1	Culture	OD	Undiluted OD	
2	PBS control induced	0.005	0.05	
3	PBS control non-induced	0.006	0,06	
4	X-33 induced culture V pick XIV HRP 2A AAO	0,344	3,44	
5	X-33 control culture V pick XIV HRP 2A AAO	0,525	5,25	
6	X-33 induced mutS MnP Pick 1 VII	0,144	1,44	
7	X-33 mutS MnP control Pick 1 VII	0,219	2,19	
8	Blank (BMGY)	0,09		

OD Measurements at 1.50 with 600 nm wavelength

	A	B	C
1	Culture	OD	Undiluted OD
2	X-33 induced mutS MnP Pick 1 VII	0,342	3,42

Observations Notes:

-The blank was approximately what previous values had been for absorbance at 600 nm for BMGY

-X-33 induced mutS MnP Pick 1 VII was not ready to be induced. Therefore, the OD was taken again at 1.50 AM

Culture

Aim:

Experimenter:

Sample:

Protocol:

#2 Inducing X-33 mut+ and mut S Strains (Erik+ Yannick)

Aim:

To induce the cultures in order to induce methanol promotor and thus express protein of interest.:

Sample:

PBS control/induced, X-33 induced/control culture V pick XIV HRP 2A AAO, X-33 induced/control mutS MnP Pick 1 VII

Protocol:

Different protocol depending on the if the samples were mut+ or mut ^{S}

Mut+:

1. Harvest cells by centrifuging 2500 x g for 5 minutes at room temperature
2. Supernatant decanted and cell pellet is resupsended in BMGY for control and BMMY for induced using 10ml of the solution

Mut S:

1. Harvest cells by centrifuging 2500 x g for 5 minutes at room temperature
2. Decant supernatant and resuspend cell pellet to an OD600 of 1.0 in MMH, BMMH, or BMMY medium to induce expression

Calculations:

To obtain OD of about 1.0 for mut S strains

X-33 induced/control culture V pick XIV HRP 2A AAO

24x 3,44= 83 ml of suspension needed

X-33 induced/control mutS MnP Pick 1 VII

24 x5, 25= 126 ml of suspension needed

Results:

#3 1% Agarose gel and analysation of run gel of PCR result pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4

Aim: Prepare and run an agarose gel of pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4

Experimentator: Sofia

Sample: PCR of pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4, #3 Sofia 20190812

Protocol: #1% agarose gel preparation

10 uL of sample loaded.

Table5

	A	B	C	D	E	F	G	H	I	J
1	1	2	3	4	5	6	7	8	9	10
2	Ladder	HRP c1	HRP c2	MnP #3	MnP #4	Positive control	Negative control	X	X	X

Result: No bands shown on the gel except for the positive control. Use more sample next time.

#4 50 mM phosphate buffer with 1 M ammonium sulphate, pH 7.5

Aim: Prepare buffer for Enzymatic assay group

Experimenter: Sofia

Sample: -

Protocol: For 1 L:

Table1

	A	B
1	Chemical	Amount (g)
2	Sodium (hydrogen) phosphate 7-hydrate	10.1
3	Sodium phosphate monobasic	14.78
4	Ammonium sulphate	135.14

Sterile filtrated.

Results: Buffer given to enzymetic assay group.

#5 1% Agarose gel and analysation of run gel of PCR result pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4

Aim: Prepare and run an agarose gel of pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4

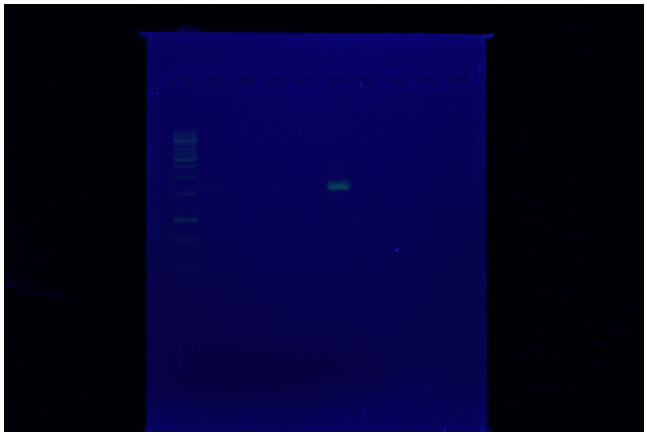
Experimentator: Sofia
Sample: PCR of pPICZαB HRP colony 1 and 2 and pPICZαB MnP #3 and #4, #3 Sofia 20190812
Protocol: #1% agarose gel preparation
20 uL of sample loaded.

Table3

	A	B	C	D	E	F	G	H	I	J
1	1	2	3	4	5	6	7	8	9	10
2	Ladder	HRP c1	HRP c2	MnP #3	MnP #4	Positive control	Negative control	X	X	X

Result:

20190813 colony PCR HRP MnP TRY2.tif



WEDNESDAY, 14/8/2019

1. Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE (Erik+Sofia+???)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.

Time of sampling :

18.00?

Aug 13th/14th Sampling of X-33 Induction

Aug 15th Sampling of Km Induction

#2 YNB preparation

Aim: Prepare YNB for BMMY and BMGY

Experimenter: Sofia

Sample: -

Protocol: 67 g of yeast nitrogen base with ammonium sulphate without amino acids solved in sterile water to a final volume of 500 mL. Sterile filtered.

Results: Stored in cold room.

#3 Inoculation of pre-culture for expression

Aim: Create pre-cultures for expression if KM71H with different constructs

Experimenter: Sofia

Sample: I: KM71H HRP-2A-AAO Restreak #2 200 uL colony I from 12.8.19

II: KM71H HRP-2A-AAO Restreak #2 100 mL colony II from 12.8.19

III: KM71H HRP-2A-AAO Restreak #2 50 mL colony I from 12.8.19
IV: KM71H HRP-2A-AAO Restreak #1 50 uL colony I from 12.8.19

Protocol: 100 mL BMGY used for all pre-cultures. Induced and control-cultures divided later.
Results: Left over night in shaker at 28°C.

#4 OD-measurement of pre-expression cultures #3

Aim: Measure OD to make sure that the cultures don't overshoot over the night

Experimenter: Sofia
Sample: #3 20190814
Protocol: Diluted 1:5 in BMGY
Results:

Table6

	A	B
1	Culture	OD
2	I	0.050
3	II	0.040
4	III	0.050
5	IV	0.045

The cultures will not overshoot over night.

#5 Inducing cultures

Aim: Adding methanol, trace metal mix and vitamin b1 to induced cultures

Experimenter: Sofia
Sample: Culture I-VII (more information...?)
Protocol:

Table7

	A	B	C	D
1	Culture	Methanol (uL)	Trace metal mix (uL)	Vitamin b1 (10 uL)
2	I	617	61.7	X
3	II	433	43.3	X
4	III	267	26.7	-
5	IV	293	29.3	X
6	V	277	27.7	X
7	VI	167	16.7	X
8	VII	33.3	3.33	-

Results: -

Lab 83: 10% SDS Page Gel (Erik Palm)

Aim:

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel asa support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

Method:

Refer to the SDS Page Gel Protocol below. Four 15% SDS gels were made.

Deviations from protocol:

1. SDS Page gel preparation largely failed because of leakage. Water had been used to test leakage, however, Erik maybe have messed with the chambers, resulting in three of the four chambers not retaining enough of the separation gel.
2. Therefore, one chamber could be used to add stacking gel, but the rest could not.
3. The stacking had already been prepared without APS and TEMED. Therefore, 2 aliquots (1,25 ml) were taken, each with the amount of stacking gel needed for 1 gel.
4. The stacking gel was placed in the first gel and allowed to congeal
5. One additional separation gel was made (only one was made because APS ran out). The TEMED and APS was added.
6. The stacking gel was added to the second gel.
7. Two gels were placed in the 4 degree fridge.

SDS Page Gel (10 and 15%)

THURSDAY, 15/8/2019

1. 10% SDS Page Gel (Erik Palm)

Aim:

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel asa support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

Method:

Refer to the SDS Page Gel Protocol below. Four 15% SDS gels were made.

SDS Page Gel (10 and 15%)

2. #2 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE (Erik+???+???)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.

Time of sampling :

15.30

Aug 13th/14th Sampling of X-33 Induction

Aug 15th Sampling of Km Induction

3. OD_{600} measurement for transfer of expression cultures to Inducing Media (Erik and ???

Aim:

Measure yeast growth in order to transfer cells to medium containing methanol and start expression.

Experimenter: Erik and ?????

Sample:

I Km HRP-2A-AAO, II Km HRP-2A-AAO, III Km HRP-2A-AAO, IV Km HRP-2A-AAO

Protocol:

Standard OD_{600} measurement at 17.00 (time of sampling = growth interrupted)

Table2

	A	B	C
1	Culture	OD	Undiluted OD
2	I Km HRP-2A-AAO	0,117	1,17
3	II Km HRP-2A-AAO	0,231	2,31
4	III Km HRP-2A-AAO	0,090	0,90
5	IV Km HRP-2A-AAO	0,371	3,71
6	First blank (BMGY)	0,215	
7	Second Blank (BMGY)	0,09	

Observations:

-The first blank seemed abnormally high compared to previous readings of only BMGY. A new aliquot of BMGY was made and used as the blank, and this second blank is what was used for measuring the OD

Results:

-Cultures II Km HRP-2A-AAO and IV Km HRP-2A-AAO were induced directly as the cultures reached the desired range of OD between 2 and 6. Since all of the cultures had the same genes being expressed, the other two were discarded due to low growth levels

#4 Checking for contamination in microscope

Aim: Checking in microscope for contamination for culture I-VII control and induced cultures

Experimenter: Sofia

Sample: Culture I-VII
Protocol: A drop from each culture placed on a microscopy glass and then checking for contamination.
Results:

Table8

	A	B	C
1	Culture	Control	Induced
2	I	No contamination	Some contamination
3	II	Some contamination, still manly yeast cells	More contamination
4	III	No contamination	No contamination
5	IV	No contamination	No contamination
6	V	No contamination	No contamination
7	VI	No contamination	No contamination
8	VII	No contamination	No contamination

#5 Inducing cultures

Aim: Adding methanol, trace metal mix and vitamin b1 to induced cultures

Experimenter: Sofia
Sample: Culture I-VII (more information...?)
Protocol:

Table9

	A	B	C	D
1	Culture	Methanol (uL)	Trace metal mix (uL)	Vitamin b1 (5 uL)
2	I	867	86.7	X
3	II	609	60.9	X
4	III	375	37.5	-
5	IV	413	41.3	X
6	V	389	38.9	X
7	VI	234	23.4	X
8	VII	46.9	4.69	-

Results: Induction done at 16.00.

FRIDAY, 16/8/2019

#1 Restreaks of X33 and KM71H for transformation (Erik+Yannick+Hugo)

Aim:

Prepare fresh restreaks of colonies for transformation of constructs from cloning groups sometime next week.

Protocol:

YPD plates, sterile conditions using flame and metal loop

Restreaks

	A	B	C
1	Culture	Number of colonies restreaked	Concentration
2	HRP 2A eGFP	1	200ul
3	HRP 2A eGFP	1	50ul
4	HRP 2A eGFP	1	100ul
5	AAO- 2A eGFP	2	200ul
6	AAO 2A eGFP	1	25ul

#2 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE (Erik+???+???)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.

Time of sampling :

15.00

Aug 13th/14th Sampling of X-33 Induction

Aug 15th Sampling of Km Induction

Deviations/ Observations:

+ KM IV HRP-2A-AA0 control looked a little bit strange. It had a different color compared to the rest of the sample plus buffer solutions. One mistake may have been using adding 5x SLB to the pellet instead of the buffer. Only the pellet eppendorf tube looks strange.

#3 Concentration measurement of colony PCR

Aim: Measure concentration of colony PCR

Experimenter: Sofia
Sample: Colony PCR of X-33 culture I-VII 20190815 by Yannick
Protocol: 1 uL of sample pipettet onto the nonodrop. Nuclease free water used as blank.
Results:

Table11

	A	B
1	Culture	Concentration (ng/uL)
2	I	511.1
3	II	593.8
4	III	484.0
5	IV	488.3
6	V	493.4
7	VI	516.3
8	VII	120.5

#4 Samples for agarose gel

Aim: Measure concentration of colony PCR

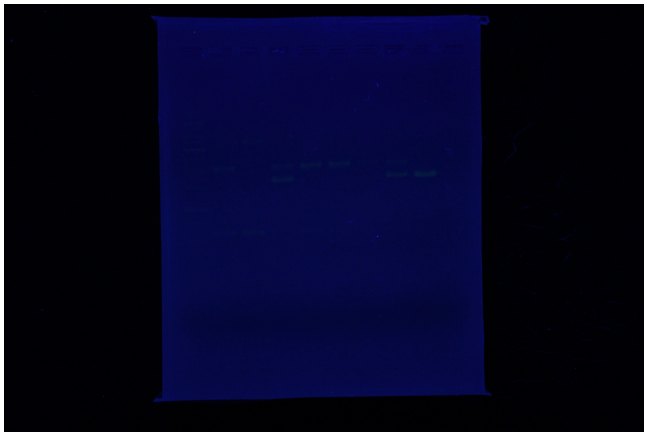
Experimenter: Sofia
Sample: Colony PCR of X-33 culture I-VII, positive and negative control 20190815 by Yannick
Protocol: 5 uL of sample, 1 uL loading dye. Ladder: 1 uL 1kB ladder, 1 uL loading dye, 4 uL water. Loading sheme:

Table12

	A	B	C	D	E	F	G	H	I	J
1	Well 1	2	3	4	5	6	7	8	9	10
2	Ladder	I	II	III	IV	V	VI	VII	+	-

Results:

20190816 colony PCR X-33.tif



#5 Sending colony PCR for sequencing

Aim: Prepare samples for sanger sequencing using provided kit

Experimenter: Sofia

Sample: Colony PCR of X-33 culture I-VII 20190815 by Yannick, HRP colony 1 and 2 and MnP #3 and #4

Protocol: Wanted DNA concentration: 10 ng/uL

Table13

	A	B	C	D
1	Sample	DNA	Water	Tube
2	I	1.95	13.05	EF30500581
3	II	0.675	14.325	EF30500582
4	III	0.767	14.233	EF30500587
5	IV	1.31	13.69	EF30500588
6	V	1.52	13.48	EF30500589
7	VI	0.872	14.128	EF30500590
8	VII	1.45	13.55	EF30500591
9	c1	15	0	EF30500592
10	c2	15	0	EF30500593
11	#3	2.96	12.04	EF30500595
12	#4	15	0	EF30500596

2 uL of primer added to every tube and then the mixes wére transfered to provided tubes in the kit.

Result: [Jonas insert result]

SATURDAY, 17/8/2019

#1 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE (Erik+Sofia)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colony.

Time of sampling :

16.00

Aug 13th/14th Sampling of X-33 Induction

Aug 15th Sampling of Km Induction

Deviations from the protocol:

-We did not have enough 1,25 x buffer. As a result, we froze the yeast pellets at 80 degrees celsius after centrifugation. There was enough 5x buffer to add the buffer to the supernatent, which was then stored in the freezer.

#2 Inducing cultures

Aim: Adding methanol, trace metal mix and vitamin b1 to induced cultures

Experimenter: Sofia

Sample: Culture I-VII (more information...?)

Protocol:

Table14

	A	B	C	D
1	Culture	Methanol (uL)	Trace metal mix (uL)	Vitamin b1 (5 uL)
2	I	925	92.5	X
3	II	650	65.0	X
4	III	400	-	-
5	IV	440	44	X
6	V	415	41.5	X
7	VI	250	25	X
8	VII	50	-	-
9	KM	50	5	X

Results: Induction done at 16.00.

SUNDAY, 18/8/2019

#1 Samples for SDS

Aim: Prepare samples SDS-Page

Experimenter: Sofia

Sample: KM71H HRP-2A-AAO culture II and IV

Protocol: 100 uL of sample centrifuged at 7000 g for 3 min. 80 uL of supernatant transfered to another tube, and the rest of the supernatant discarded. Pellet resuspended with 80 uL of 1.25x SB and the supernatant mixed with 20 uL of 5xSB. All samples boiled for 10 min at 95°C. Freezed in -20°C.

Result: Samples stored in -20°C.

#2 Inducing cultures

Aim: Adding methanol, trace metal mix and vitamin b1 to induced cultures

Experimenter: Sofia

Sample: Culture I-VII (more information...?)

Protocol:

Table15

	A	B	C	D
1	Culture	Methanol (uL)	Trace metal mix (uL)	Vitamin b1 (5 uL)
2	I	925	92.5	X
3	II	650	65.0	X
4	III	400	-	-
5	IV	440	44	X
6	V	415	41.5	X
7	VI	250	25	X
8	VII	50	-	-
9	KM	50	5	X

Results: Induction done at 16.00.

Empty

Project: iGEM uppsala 2019

Authors: Erik Palm

THURSDAY, 15/8/2019

Notebook week 19/8-25/8

Project: iGEM uppsala 2019
Authors: Yannick Hajee

MONDAY, 19/8/2019

#1 Preparation of YPD Agar + Zeocin Plates and YPD Solution (Erik+Yannick)

Aim:

Preparation of media and agar for future restreaks and transformation.

-500ml of YPD Agar +Zeocin was made
-800ml of YPD was made

Preparation of YPD Media

	A	B	C	D	E	F
1		Yeast	Dextros	Peptone	Agar	Water
2	500ml YPD Agar+ Zeocin	5	10	10	10	500
3	800ml YPD	8	16	16	---------	800

YPD Medium/Agar

Sampling of Km II and IV and preparations of Samples for SDS-Page (Erik)

Aim:
To prepare samples at set intervals to run on on SDS-PAGE.
Protocol:
Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.
Time of sampling :
15.00

Aug 15th Sampling of Km Induction

#2 OD for expression

Aim: Measure OD of inoculated cultures to see if they have grown enougth to be induced

Experimenter: Sofia
Sample: X33: AAO, AAO-2A-eGFP, HRP-2A-eGFP
KM71H HRP-2A-eGFP
Protocol: -
Result: All X33-cultures over shoot, thrown away. KM culture induced at 15.

Table1

	A	B	C
1	Strain	Diluted(1:10)	Undiluted
2	X33 AAO	0.955	9.55
3	X33 AAO-2A-eGFP	0.923	9.23
4	X33 HRP-2A-eGFP	1.114	11.14
5	KM71H HRP-2A-eGFP	0.053	0.53

#3 Inoculate new cultures for expression

Aim: Inoculate new cultures to be able to induce expression because old cultures over shoot

Experimenter: Sofia

Sample: X33 pPICZaB AAO colony 26, X33 pPICZaB HRP-2A-eGFP colony 27, X33 pPICZaB AAO-2A-eGFP colony 13 old liquid cultures.

Protocol: 25 mL BMGY was inoculated with 10 uL of old culture at 14.30.

Result: Left over night.

TUESDAY, 20/8/2019

#1 Sampling of Km II and IV and preparations of Samples for SDS-Page (Erik)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.

Time of sampling :

15.00

Aug 15th Sampling of Km Induction

#2 OD for expression

Aim: Measure OD of inoculated cultures to see if they have grown enougth to be induced

Experimenter: Sofia

Sample: X33: AAO, AAO-2A-eGFP, HRP-2A-eGFP

Protocol: -

Result: All X33-cultures over shoot, thrown away. KM culture induced at 15.

Table2

	A	B	C
1	Strain	Diluted(1:10)	Undiluted
2	X33 AAO	0.290	2.9
3	X33 AAO-2A-eGFP	0.348	3.48
4	X33 HRP-2A-eGFP	0.313	3.13

#2 10x running buffer for SDS

Aim: Prepare running buffer for SDS

Experimenter: Sofia

Sample: -

Protocol: 30 g tris base, 144 g glycin, 10 g SDS mixed with water to a final volume of 1 L.

Result:

#2 OD for expression

Aim: Measure OD of inoculated cultures to see if they have grown enougth to be induced

Experimenter: Sofia

Sample: X33: AAO, AAO-2A-eGFP, HRP-2A-eGFP

Protocol: -

Result:

Table3

	A	B	C
1	Strain	Diluted(1:10)	Undiluted
2	X33 AAO	0.534	5.34
3	X33 AAO-2A-eGFP	0.622	6.22
4	X33 HRP-2A-eGFP	0.600	6

Cultures splited and induced at 11.00

#3 Induction of X33 cultures

Aim: Inducing cultures for expression

Experimenter: Sofia

Sample: X33: AAO, AAO-2A-eGFP, HRP-2A-eGFP

Protocol: Pre-cultures splited into 2 tubes and then harvested by centrifugation at 2500 g for 5 min at room temperature. Resuspended to OD=1.

Table4

	A	B	C	D
1	Strain	OD in 25 mL (precultures)	OD=1 in new media	Media for splitted cultures
2	I: X33 AAO-2A-eGFP	5.34	133.5	67
3	II: X33 HRP-2A-eGFP	6.22	155.5	78
4	III: X33 AAO	6.00	150	75

Result: Induced at 14.30. Trace metal mix and vitamin b1 added.

#4 Harvest expression cultures

Aim: Old expression cultures harvested

Experimenter: Sofia

Sample: ?

Protocol: Cultures diveded into falcon tubes and then harvested by centrifugation at 2500 g for 5 min in room temperature. 2 mL of supernatant snap frozen and stored in -80°C. Supernatant and pellet divided and the rest stored in -20°C.

Result: Harvested cultures stored in -80°C and -20°C.

WEDNESDAY, 21/8/2019

#1 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE (Erik+Hugo)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE.

Protocol:

Below are the two different sampling data tables that are being used. Time and date are present for each culture and for each induced and noninduced colonies. All of the below colonies were sampled.

Time of sampling :

15.00

Aug 13th/14th Sampling of X-33 Induction

Aug 15th Sampling of Km Induction

Deviations from protocol.

-200ul of sample in order to check contamination

#2 Native SDS-Page gel preparation

Aim: Create native 10% SDS-page gel

Experimenter: Sofia

Sample: -
Protocol: #Native SDS-Page gel, 10% casting gel
Result:

#3 SDS-Page gel preparation

Aim: Create native 10% SDS-page gel

Experimenter: Sofia
Sample: -
Protocol: #Native SDS-Page gel, 10% casting gel
Result:

#4 Harvest expression cultures

Aim: Old expression cultures harvested

Experimenter: Sofia
Sample: KM71H HRP-2A-AAO Restreak #1 colony I 50 uL 12/8 I, KM71H HRP-2A-AAO Restreak #2 colony II 100 mL 12/8 IV
Protocol: Cultures diveded into falcon tubes and then harvested by centrifugation at 2500 g for 5 min in room temperature. 2 mL of supernatant snap frozen and stored in -80°C. Supernatant and pellet divided and the rest stored in -20°C.
Result: Harvested cultures stored in -80°C and -20°C.

THURSDAY, 22/8/2019

#1 Colony PCR of pPICZaB MnP pick II 9.8.19 used for culture III (induced 14.8.19)

Aim: Amplify the construct in transformed cells for analyzation

Experimenter: Sofia
Sample: Restreak of MnP pick II 9.8.19 used for culture III (induced 14.8.19)
Protocol: 1. #EXTRACTION OF GENOMIC DNA FROM YEASTS FOR PCR-BASED APPLICATIONS
2:

Table10

	A	B	C
1	Chemical	In 1 tube (µL)	Master mix (x7) (µL)
2	dNTPs	5	35
3	Forward primer	0.25	1.75
4	Reverse primer	0.25	1.75
5	Buffer	5	35
6	Taq polymerase	0.25	1.75
7	Template	1	-
8	H20	38.25	267.75

a. Make a mastermix with the chemicals stated above.
b. Add DNA/water to the tubes. Transfer 49 µL master mix to the 6 different tubes.

c. PCR made according to instuctions for pPICZaB in P. Pastoris:
d. 95°C 5 min.
e. 95°C 30 s
f. 55°C 1 min
g. 72°C 1 min 30 s
h. Goto step 2 X30
i. 72°C 7 min
j. 4°C ∞

Results: Later run on agarose gel. No bands shown on the gel.

#2 Preparing BMMY agar

Aim: To prepare plates for screening for expressing colonies

Experimenter: Sofia
Sample: Solutions prepared in #1 20190802 and #2 20190814
Protocol: Standard Easy select protocol followed. For 100 mL:

Table5

	A	B
1	Material	
2	Yeast extract	1 g
3	Peptone	2 g
4	Agar	2 g
5	ddH20	up to 70mL
6	1M KH2PO4 (pH 6.0)	10mL
7	10x YNB (13.4%)	10mL
8	500x Biotin (0.02%)	0.2mL
9	10x MeOH (5%)	10mL

Results: 7 plates made, stored in coldroom at 4 °C

#3 Loadning SDS-gel

Aim: Load SDS-gel for analyzation

Experimenter: Sofia
Sample: 9: Gel #20 X-33 IV 139 h - 163 h, V 0 h - 14 h (up | us | ip | is)
10: Gel #21 X-33 V 14 h-85 h (ip | is | up | us | ip | is ...)
11: Gel #22 X-33 V 109 h - 157 h (up | us | ip | is), boiled and unboiled sample from Jinwen
12: Gel #23 X-33 0 h - 67 h (up | us | ip | is)
Protocol:
Results: [Insert gel picture]

#4 Colony PCR of pPICZaB MnP pick II 9.8.19 used for culture III (induced 14.8.19)

Aim: Redoing the colony PCR because no bands were shown on the agarose gel

Experimenter: Sofia

Sample: Restreak of MnP pick II 9.8.19 used for culture III (induced 14.8.19)

Protocol: 1. #1 20190822

2: New tube with NF water used.

Table6

	A	B	C
1	Chemical	In 1 tube (µL)	Master mix (x7) (µL)
2	dNTPs	5	35
3	Forward primer	0.25	1.75
4	Reverse primer	0.25	1.75
5	Buffer	5	35
6	Taq polymerase	0.25	1.75
7	Template	1	-
8	H20	38.25	267.75

a. Make a mastermix with the chemicals stated above.
b. Add DNA/water to the tubes. Transfer 49 µL master mix to the 6 different tubes.
c. PCR made according to instuctions for pPICZaB in P. Pastoris:
d. 95°C 5 min.
e. 95°C 30 s
f. 55°C 1 min
g. 72°C 1 min 30 s
h. Goto step 2 X30
i. 72°C 7 min
j. 4°C ∞

Results: Later run on agarose gel #1 20190823.

#5 Restreaks of eGFP constructs fron transformation 12.8.19 on BMMY plates

Aim: Restreak colonies from the transformation to be able to screen for colonies that express the GFP.

Experimenter: Sofia

Sample: Colonies from all plated with colonies contain eGFP from transformation 12.8.19

Protocol: -

Result: ?

FRIDAY, 23/8/2019

#1 Agarose gel of colony PCR #2 20190822

Aim: Analyze the result of the colony PCR

Experimenter: Sofia
Sample: Colony PCR #2 20190822
Protocol: #1% agarose gel preparation
Gel loaded as followed:

Table7

	A	B	C	D
1	Well 1	Well 2	Well 3	Well 4
2	Ladder	MnP III	Positive control	Negative control

Result: [Hugo insert result]

#2 Restreak of X-33

Aim: Restreak the colony that was used for culture III (MnP) that has shown activity

Experimenter: Sofia
Sample: X-33 pPICZaB MnP pick II colony I 9.8.19 (plate marked culture III)
Protocol: -
Result: Plate put in incubator (28°C).

SATURDAY, 24/8/2019

SUNDAY, 25/8/2019

#1 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE and adding methanol, trace metals, and Vitamin B (Erik+Katherin)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE as well as continued induction of samples with appropriate raw materials needed to create active enzymes.

Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and put to -20˚C

Time of sampling :

15.00

Table of Substances added to Cultures after samples had been tak…

	A	B	C	D
1	Culture	u/i	added	
2	X-33 pPICzaB	u	----	
3		i	50 ul MeOH	
4	Km V pPICZaB HRP-2A-eGFP	u	5ul trace metals, 5ul Vit B, 15ul AA	
5		i	5ul trace metals, 5ul Vit B, 15ul AA 50 ul MeOH	

Notebook week 26/8-1/9

Project: iGEM uppsala 2019
Authors: Yannick Hajee

MONDAY, 26/8/2019

#

Aim:
Experimenter:
Sample:
Protocol:
Results:

TUESDAY, 27/8/2019

#1 SDS-PAGE analysis of HRP-2A-AAO expression in *P. pastoris (gels 24-25)*

Aim: See the secretion of protein of appropriate size at different times after expression
Experimenter: Tereza
Sample:
Protocol:

loaded volumes: 5ul of ladder and pellets, 10ul of superntants
pellets heated up for ~5min in 95°C
gel scheme:

Table2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
1	lad.	cp 0h	cs 0h	i p 0h	i s 0h	cp 25h	cs 25h	i p 14h	i s 14h	cp 49h	cs 49h	i p 37 h	i s 37h	cp 73h	cs 73h	gel 24	X33-HI AAO-V
2	lad.	i p 61h	i s 61h	cp 97h	cs 97h	i p 85h	i s 85h	cp 121h	cs 121h	i p 133h	i s 133h	cp 145h	cs 145h	i p 157h	i s 157h	gel 25	X33-HI AAO-V

Results:

WEDNESDAY, 28/8/2019

#1 SDS-PAGE analysis of MnP and HRP-2A-AAO expression in *P. pastoris (gels 26-29)*

Aim: See the secretion of protein of appropriate size at different times after expression
Experimenter: Tereza
Sample:
Protocol:

loaded volumes: 5ul of ladder and pellets, 10ul of superntants
pellets heated up for ~5min in 95°C
gel scheme:

Loading

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	lad.	cp	cs	ip	is	cp	cs	ip	is	cp	cs	ip	is	cp	cs	gel 26	X33-MnP-VII	0h till 37h control		
2	lad.	ip	is	cp	cs	ip	is	cp	cs	ip	is	cp	cs	ip	is	gel 27	X33-MnP-VII	37h induced till 109h induced		
3	lad.	cp	cs	ip	is	cp	cs	ip	is	cp	cs	ip	is	cp	cs	gel 28	X33-MnP-VII	till 137h induced	KM71H-H2A-II	0h till 2 control
4	lad.	ip	is	cp	ip	cs	is	cp	cs	ip	is	cp	cs	ip	is	gel 29			KM71H-H2A-II	21h ind 93h co

Results:

#2 4x10% SDS Page Gels (Erik Palm)

Aim: Gels for espression analysis

Experimenter: Tereza

Sample: -

Protocol: Premixes for 4 gels

Results: Bad gels because stacking gel was mixed with isopropanol

THURSDAY, 29/8/2019

#2 4x10% SDS Page Gels (Erik Palm)

Aim: Gels for espression analysis

Experimenter: Tereza

Sample: -

Protocol:

Results: Stacking gels takes so long to polzmerize, over 1.5hrs

SUNDAY, 1/9/2019

#1 Sampling of KM71H and X-33 Cultures and preparations of samples for SDS-PAGE and adding methanol, trace metals, and Vitamin B (Erik+Tereza)

Aim:

To prepare samples at set intervals to run on on SDS-PAGE as well as continued induction of samples with appropriate raw materials needed to create active enzymes.

Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and put to -20˚C

Time of sampling :

15.00

Table1

	A	B	C	D
1	Culture	u/i	added	
2	Km VI pPICZaB AAO-2A-eGFP	u	-----	
3		i	50 ul MeOH	
4	Km VII pPICZaB AAO-2A-eGFP	u	----	
5		i	50 ul MeOH	
6	Km VIII pPICZaB HRP-2A-eGFP	u	5ul trace metals, 5ul Vit B, 15ul AA	
7		i	5ul trace metals, 5ul Vit B, 15ul AA 50 ul MeOH	
8	X33 Culture 0 pPICzaB only vector	u	----	
9		i	360ul MeOH	

SATURDAY, 28/9/2019

SUNDAY, 29/9/2019

Notebook week 2/9-9/9

Project: iGEM uppsala 2019
Authors: Erik Palm

SATURDAY, 7/9/2019

#1 Sampling of KM71H empty vector and preparations of samples for SDS-PAGE and adding methanol (Erik+Tereza)

Aim:
To prepare samples at set intervals to run on on SDS-PAGE as well as continued induction of samples with appropriate raw materials needed to create active enzymes.

Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube
80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and put to -20˚C

Time of sampling :
15.00

Table1

	A	B	C	D
1	Culture	u/i	added	
2	Km VI pPICZaB	u	-----	
3		i	50 ul MeOH	

SUNDAY, 8/9/2019

#1 Sampling of KM71H empty vector and preparations of samples for SDS-PAGE and adding methanol (Erik+Tereza)

Aim:
To prepare samples at set intervals to run on on SDS-PAGE as well as continued induction of samples with appropriate raw materials needed to create active enzymes.

Protocol:

taken 1ml, on ice by glass pipette
100ul separated
spinned donwn 3min, 17 000 x g
80ul of supernatant transferred to a new tube

80ul of supernatant mixed with 20ul 5XSLB
20ul pellet mixed with 1.25SLB + 0.8% Triton X-100
samples boiled on heating block for 10 min at 95˚C
tubes with 900ul of culture snap frozen and put to -20˚C

Time of sampling :

15.00

Table3

	A	B	C	D
1	Culture	u/i	added	
2	Km VI pPICZaB	u	-----	
3		i	50 ul MeOH	