# #1 Preperation of solutions and LB-media

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 17/6/2019

Experimenter: Vamsi, Karthik and Johanna
Aim: To prepare solutions for the LB-media
Protocol: Lab Manual Synthetic Biology, LB medium page 98

600 ml of LB medium was prepared with:
NaCl 0,17 M which is 5,96 grams
1% (w/v) Bacto Tryptone which is 6 grams
0,5% yeast extract- 3 grams
dd$H_2O$- 600 ml
5M NaOH- 100ml (prepared from another group)
Autoclave

Result: 600 ml LB media

MONDAY, 8/7/2019

Experimenter: Johanna and Gabriel

Aim: To prepare an LB-media in order to use it for further lab-use

Protocol: Lab Manual, Synthetic Biology, LB medium p. 98
600ml of LB-medium was prepared with:
0,17 M (5,96 g) NaCl
1% (w/v) (6 g) Bacto Tryptone
0,5% (3g) yeast extract
600 ml dd$H_2O$
5M 100 µl NaOH
Autoclave

Result: 600 ml LB-media

# #4 Making of 0,9% NaCl

**Project:** iGEM uppsala 2019

**Authors:** Johanna Cederblad

MONDAY, 17/6/2019

Experimenter: Vamsi, Karthik and Johanna

Protocol: Lab Manual, Synthetic Biology, Making of 0,9% NaCl page 91

Aim: making the 0,9% of NaCl solution

0,9% NaCl-10ml

0,09 grams NaCl

9,91ml dd$H_2O$

Result: 10 ml of 0,9% NaCl Solution

# #3 Making of the 1 M CaCl2

**Project:** iGEM uppsala 2019

**Authors:** Johanna Cederblad

MONDAY, 17/6/2019

Experimenter: Vamsi, Karthik and Johanna

Aim: Makingof the $CaCl_2$ 1M solution

Protocol: Lab Manual Synthetic Biology, Preparation of Solution $CaCl_2$ page 93

dd$H_2O$ to the 10 ml mark

1,1098 gram $CaCl_2$

Result: 1 M $CaCl_2$

1M $CaCl_2$ - 10 ml

# #2 Preparation of LB medium + Agar + Kanamycin for 500 ml

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 17/6/2019

Experimenter: Vamsi, Karthik and Johanna
Aim: to prepare LB medium with agar to be able to make agar plates for growth of bacteria.
Protocol: Lab Manual Synthetic Biology, LB Agar Plates and addition of Antibiotics

NaCl 0,17 M- 4,967 grams
1% Bacto Tryptone- 5 grams
0,5% yeast extract- 2,5 grams
dd$H_2O$- 500 ml
5 M NaOH- 83,33 microlitre
Agar- 7,5 gram
Autoclave

Result:
Error: instead of agar we added agarose which made the LB medium agar unusuable, therfore we are not continuing on making the agar plates.

# #5Preparation of competent cells (DH5α) using CaCl2

**Project:** iGEM uppsala 2019

**Authors:** Vamsi Krishna Polavarapu

TUESDAY, 18/6/2019

Experimenter: Vamsi, Karthik and Johanna

Protocol: Lab Manual, Synthetic Biology, Page .no 111

Diluted the overnight culture of DH5α 500µl to 50ml L.B medium and kept in 37 °C room

Measured the $OD_{600}$ till it reach 0.4.

Centrifuged the 50ml of the inoculum at 3500rpm for 5min at 4°C and decanted the supernatent.

Pellet was suspended in 100µl of icecold $CaCl_2$ and mixed well .Then added 15ml of icecold $CaCl_2$ ,left for 30mins incubation.

Again centrifuged at 3500rpm for 5min at 4°C and decanted the supernatent .

Added 2ml of 0.1 Micecold $CaCl_2$ mixed with 20% of glycerol and incubated it for 45 mins in Ice.

Prepared aliquotes of 50µl in 1.5 eppendorf tube.By putting them in Liquid $No_2$ ,snap freezed it.

Competent cell vials are stored in -80°C.

# #6 Transformation of CaCl2 competent E.Coli cells

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 19/6/2019

Experimenter: Johanna, Vamsi, Karthik

Aim: to transform the competent cells preparade from protocol #5 and to also plate them on agar plates.
Sample: Competent cells DH5α 18.06.19 #5
Protocol: Lab Manual, Synthetic Biology, Transformation of $CaCl_2$-Competent E.Coli cells p. 113-115

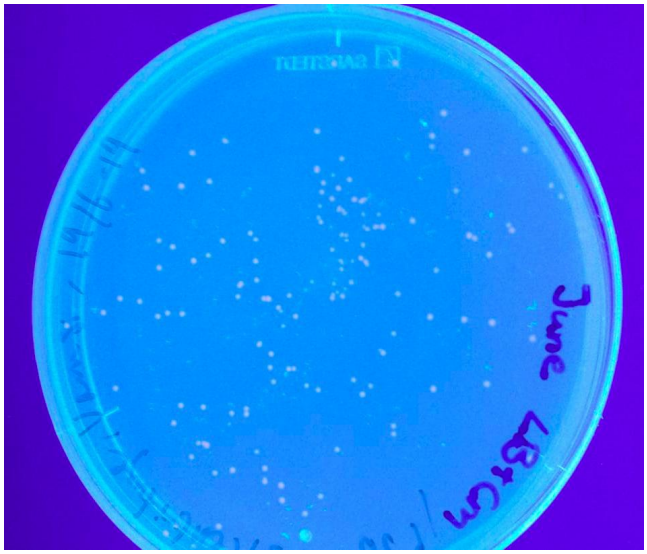
Material:
5 ng PSB-1C3
50 µl deionized water

5 µl PSB-1C3 plasmid diluted 1:10 from stocksolution that was 5 ng diluted with 50 µl deionized water.
50 µl competent cells snap frozen
50 µl competent cells stored over night in cold room
950µl LB media
Agar plates

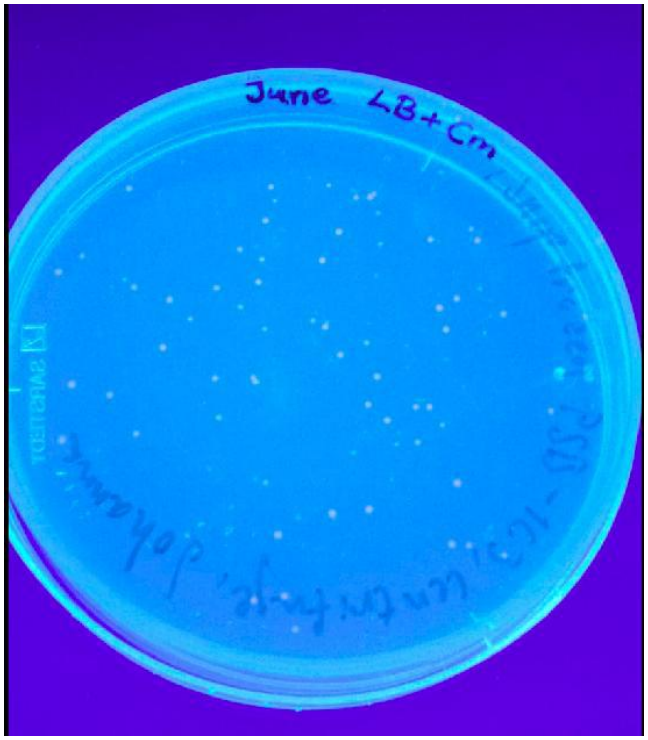
Stock solution of PSB-1C3 was diluted with 1 µl stocksolution and 9 µl deionized water. This was then used with the competent cells.

Result: Agar plate with competent cells.

PSB-1C3_NOT_SNAP_Frozen.jpg



PSB-1C3_SNAP_frozen.jpg




**INSERT PHOTO OF COLONIES**

DNA-plasmid PSB-1C3
Agar plate incubating in 37 degree celsius room.

WEDNESDAY, 3/7/2019

Experimenter: Johanna and Sofia (group 2)

Aim: To transform the two plasmids PSB1A3 and PSB1K3 into competent cells of DH5α E.coli strains.

Protocol: Lab Manual, Synthetic biology, protocol #6 page 113
1 µl PSB1A3
1 µl PSB1K3
50 µl DH5α
3x 950 µl SOB media
1 µl $ddH_2O$

Sample: Escherichia Coli DH5α from 13/9-2018
BBa- PSB1A3
BBa- PSB1K3

Result: 6 agar plates, 3 with Amp and 3 with Cm (wrong penicillin, so the plates did not grow).
The Amp grew to much, the plan is to re-do the plates with new ampicellin.

Stored: 37°C room for growth

MONDAY, 8/7/2019

Experimenter: Sofia and Johanna
Aim: To transform the wanted DNA into competent cells
Protocol: Lab manual synthetic biology, Protocol #6
Sample: PSB1A3
DH5αs
Result: Agar plates with transformed
Stored:

# #7 Overnight Culture of K5-00000 and PSB-1C3 with chloramphenicol

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

THURSDAY, 20/6/2019

Experimenter: Johanna, Louise
Aim: To make overnight cultures with DH5α bacteria with the plasmids K5-00000 and PSB-1C3.

Sample: DNA plasmid PSB-1C3 from 18.06.2019 protocol #6 from Victors labgroup
DNA plasmid K5-00000 protocol # 6 from Victors labgroup (19.06.2019)
LB medium
Chloramphenicol 1 µl/ml

Protocol: Lab Manual, Synthetic Biology, Overnight Cultures with Antibiotics page 102-103
Different from the protocol: we mixed 12 ml LB media with 12 µl chloramphenicol and then mixed it gently. This was then aliquoted into two 5 ml solutions for the K5-00000 colonies and 2 ml for the PSB-1C3 colonies.
The colonies were picked up with the tip of a pipet and then put into the falcontube for the corresponding colonie, the pipet was left over night with the solution.

Results: 3 tubes with colonies with LB medium + chloramphenicol + colonies from agar plates and was left for an overnight growth.

K5-00000 falcon tube stored in the 37 degree celsius room
PSB-1C3 falcon tube stored in the 37 degree celsius room.

# # 8 Ligation of 3A-Assembly

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 24/6/2019

Experimenter: Johanna and Louise

Aim: To ligate the plasmids with the prevailiong promotor(KB0-8006) with the ribosome bindingsite, coding sequence (lignin peroxidase) and the backbone with AmpR.

Protocol:  Lab Manual, Synthetic Biology 3A Assembly p.107

Material:
2 µl of each (the three) digestion mixures
11 µl dd$H_2O$
2µl 10x reaction buffer (for T4 DNA ligase)
1 µl T4 DNA ligase

Sample: 3A Assembly

Result:  Eppendorf tube with the samples.

Stored: used directly for transformation see protocol #10.

# #11 GenElute Plasmid- Mini prep kit

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 24/6/2019

Experimenter: Johanna and Louise

Aim: To concentrate DNA from the samples prepared with the 3A assembly from protocol #8 (from Viktors group)

Protocol: GenElute Plasmid mini prep kit protocol.

Sample: K5-00000 in LB media O/N culture with 1:100 chloramphenicol

Result: 2 eppendorf tubes with concentrated DNA. The tube with sample #1 had 89,5 ng/µl and the tube with sample #2 had 113,5 ng/µl amount of DNA lignin peroxidase (K5-00000).

Stored: in -20°C freezer in two eppendorf tubes.

# #10 Transformation of 3A assembly into DH5α

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 24/6/2019

Experimenter: Johanna and Louise

Aim: To transform the ligated 3A assembly into DH5α bacteria.

Protocol: Protocol #6

Material:
5 µl ligation mixture (from protocol #8)
50 µl DH5α
950 µl LB medium

Sample: 3A assembly from protocol #8 and from group 5s 3A assembly

Results: 3 Agar plates with AmpR, one with $ddH_2O$, one with non centrifuged bacteria and centrifuged ones.

Stored: in 37°C room for growth.

# #9 Measurement of concentration from O/N culture from protocol #7

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 24/6/2019

Experimenter: Johanna and Louise

Aim: To measure the concentration of the O/N culture from protocol #7

Protocol: The nano-drop was cleaned with deionized water. The elution buffer was used as a blank for the samples. 2 µl of the samples with K5-00000 was put on the measurer and then closed, the measuring was started and the values noted.

Material:
2µl dd$H_2O$
2 µl Elute buffer
2 µl Sample of K5-00000

Sample: K5-00000 from overnight culture from protocol #7

Results: The samples were tested for the concentration and sample #1 prepared by Johanna showed 89,5 ng/µl, sample #2 prepared by Louise showed 113,5 ng/µl.

Stored: Sample #1 and sample #2 was saved in a -20 °C freezer. The samples were marked with Lignation Peroxidase and the corresponding number.

# #12 Make bacteria colonies on agar plate for PSB1A3 with K608006 from O/N 25.6.19

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 26/6/2019

Experimenter: Johanna and Vanja

Aim: To investigate whether the overnight culture had the relevant bacteria

Protocol: Lab Manual, Synthetic biology, protocol #6 Transformation of $CaCl_2$ competent E.coli cells from p.114 step 11-12
1 Agar plate
1 Coloni

Sample: PSB1A3, K608006 +Amp, colony #4 from 25.06.19

Result: Two agar plates with 100 µl each of the O/N cultures spread on Agar plates

Stored: 37°C room for growth.

# #13 Overnight Culture with Ampicillin

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 26/6/2019

Experimenter: Johanna and Vanja

Aim: To make O/N culture from colonies on agarplate from 25/6-19

Protocol: Lab manual, Synthetic biology, protocol #2 Overnight Culture with antibiotic p.102.
Gloves were not used when the first coloni was taken, gloves was used for the second coloni.
5 µl ampicillin
1 coloni
5 ml LB media

Sample: PSB1A3, K608006 + Amp (Lignin peroxidase)

Result: 5 falcon tubes with one coloni each and: ampicillin, LB media.

Stored: 37°C room on shake tray, left over night.

# #14 3A Assembly, Digestion of promotor and RBS

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 26/6/2019

Experimenter: Johanna and Vanja

Aim: To digest the promotor and restriction enzymes in order to have one the plasmids needed for the assembly.

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly
14µl K608006
29µl dd $H_2O$
5µl 10x reaction buffer
1µl EcoR1
1µl Spe1

Sample: K608006 + EcoR1 + Spe1

Result: One eppedorf tube with a plasmid with the promotor and RBS

Stored: in -20°C fridge (in lab)

# #15 Running the gel

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 26/6/2019

Experimenter: Johanna and Vanja

Aim: To verify that the digestion from notebook #14 was succesful

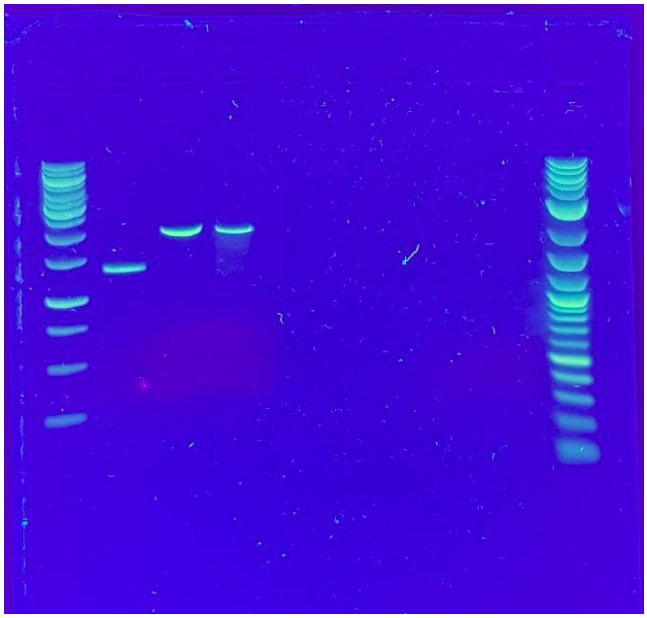
Protocol: Lab Manual, Synthetic Biology, Protocol #3 Biobrik 3A assembly and gel analysis and Protocol #4 Agarose Gelelectrophores
The gel was running with 120 V
well #1: 1µl ladder + 1µl loading dye + 4µl $ddH_2O$
well #2: 10 µl uncut plasmid + 10 µl $ddH_2O$ + 4µl loading dye
well #3: 20 µl sample from group 5 (--.06.19) + 4µl loading dye
well #4: 20 µl sample from 26.06.19 + 4 µl loading dye

Sample: 3A assembly 26.06.19 from note#14 and 3A assembly from group 5 (--.06-19)

Result: A gel with clear lines of the samples. (insert photo)

Stored: Gel discarded

Gel_26-06-19.jpg



# #17 Transformation of 3A Assembly

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

THURSDAY, 27/6/2019

Experimenter: Johanna and Vanja
Aim: to transform the earlier ligated 3A Assembly
Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation
5 µl ligation mixure
50 µl competent cells, DH5α
950 µl LB medium

Sample: PSB1A3 + K68006 + Amp + Competent cells from iGEM 2018
Result: 3 Agar plates with the prevailing solutions
Stored: 37°C room for growth

Error: The incubation for 1-1,5 h was made with LB media and ligation mixture from entry #14, the error was detected when the agar plates where being plated. Therefore the incubation on ice lasted not for 30 minutes but for approxiamtly 3,5 h before the cells were heat shocked and the incubation on 37°C and the protocol was continued

# #18 Thermo scientific GenJet plasmid miniprep kit, from O/N culture 26.06.19

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

THURSDAY, 27/6/2019

Experimenter: Johanna and Vanja
AIm: To concentrate the DNA from the overnight culture
Protocol: Thermo Scientific GenJet plasmid miniprep kit
Sample: O/N PSB1A3 from 26.06.19
Result: 4 eppendorf tubes with the different colonies for O/N cultures.
Stored: Discarded

Nanodrop:
Experimenter:
Aim: To examine the concentration of the DNA in the solution
Protocol: Blank with elution buffer, and then measure 2 µl of sample
Sample: PSB1A3
Result:
#6 had -3,8 ng/µl DNA
#8 had 7,5 ng/µl DNA
#9 had 27,5 ng/µl DNA
#10 had 6,9 ng/µl DNA

Stored: Discarded, since there were to little DNA in the samples.

# #16 Ligation for 3A assembly from entry #14

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

THURSDAY, 27/6/2019

Experimenter: Johanna and Vanja

Aim: To ligate the three digestion mixture into one plasmid

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick assembly ligation
2 µl of each digestion mixture + 11 µl dd$H_2O$
2 µl 10x reaction buffer for T4 DNA ligase
1 µl T4 DNA ligase

Sample: Sample #2 from 20.06.19 (group 5) and sample #3 from 20.06.19(group 5) and sample #1 from entry #14

Result: A sample with, hopefully, the three digestion mixtures ligated together

Stored: Used directly for transformation

# Enzymatic Assay- Piercy Quantitative Peroxide Assay Kit [23280]

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

MONDAY, 1/7/2019

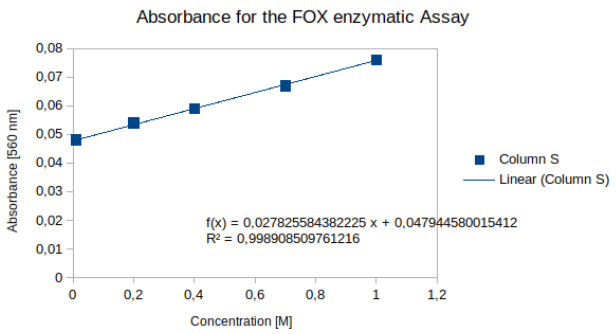
Experimenter: Johanna and Vamsi
Aim: To investigate whether the kit that had arrived would work with our enzymes and to test the kit
Protocol: Piercy Quantitative Peroxide Assay Kit, the protocol for the aquaeous solution.
A stock solution of $H_2O_2$ was preparade. $H_2O_2$ was diluted from a 35% solution to a 1 M solution. It was done by mixing 9,7 µl of the 35% solution with 99,99 µl of dd$H_2O$.
The 1 M solution was diluted to a:
1 mM solution: 0,9 ml $H_2O_2$ and 0,1 ml $H_2O$
0,9 Msolution: 0,8 ml $H_2O_2$ and 0,2 ml $H_2O$
0,8 M solution: 0,7 ml $H_2O_2$ and 0,3 ml $H_2O$
0,7 M solution: 0,6 ml $H_2O_2$ and 0,4 ml $H_2O$
0,5 M solution: 0,5 ml $H_2O_2$ and 0,5 ml $H_2O$
0,4 M solution: 0,4 ml $H_2O_2$ and 0,6 ml $H_2O$
0,3 M solution: 0,3 ml $H_2O_2$ and 0,7 ml $H_2O$
0,2 M solution: 0,2 ml $H_2O_2$ and 0,8 ml $H_2O$
0,1 M solution: 0,1 ml $H_2O_2$ and 0,9 ml $H_2O$
1 µM solution: 0,001 ml $H_2O_2$ and 9,999 ml $H_2O$

Result:
The table are from a spectrophotometry with the absorbance measured at 560 nm

**Table1**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Samples micro molar | Spec #1 [15 min incubation] | Spec #2 [20+ min incubation] | Spec #3 [30 min incubation] |
| 2 | 1000 | 0,078 | 0,078 | 0,009 |
| 3 | 700 | 0,067 | 0,071 | 0,002 |
| 4 | 400 | 0,059 | 0,062 | -0,011 |
| 5 | 200 | 0,054 | 0,058 | -0,016 |
| 6 | 10 | 0,048 | 0,053 | -0,018 |

image.png




Stored: The not used concentrations were left in the fridge along with 1M stock solution.

TUESDAY, 2/7/2019

Experimenter:Johanna ,Gaberial and Vamsi

Aim:To perform the enzymatic assay by using pierce quantitative peroxide assay kit
Procedure: Followed the protocol provided in the kit (Thermo fisher - protocol 23280)

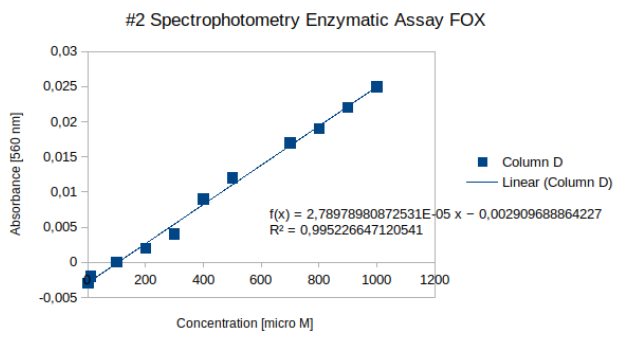
From the stock solutoon 35% of H2O2 we prepared 1M and 1mM stock solutions.
We later diluted the 1mM H2O2 stock solutions to 900µM, 800cM,700µM,500µM,400µM,300µM,200µM,100µM,10µM and 1µM concentarations.
By mixing 120ul of reagent A in 12 ml of reagent B on ice we prepared working reagent for 12 samples.
We mixed 100ul of H2O2 sample and 1ml of working reagent .we did it for all 11 samples.
Afer 15 minutes of incubation in 37 °C, we measured the OD at 590nm.

**Table2**

| | A | B | C |
|---|---|---|---|
| 1 | Samples | Time 15 mins | Time 25 mins |
| 2 | 1000µM | 0.025 | 0.009 |
| 3 | 900µM | 0.022 | 0.004 |
| 4 | 800µM | 0.019 | 0.003 |
| 5 | 700µM | 0.017 | 0.001 |
| 6 | 500µM | 0.012 | -0.005 |
| 7 | 400µM | 0.009 | -0.008 |
| 8 | 300µM | 0.004 | -0.011 |
| 9 | 200µM | 0.002 | -0.015 |
| 10 | 100µM | 0.000 | -0.016 |
| 11 | 10µM | -0.002 | -0.017 |
| 12 | 1µM | -0.003 | -0.019 |

A graph from the 11 samples.

image.png



# Enzymatic Assay with Platereader Tecan

**Project:** iGEM uppsala 2019

**Authors:** Johanna Cederblad

TUESDAY, 9/7/2019

Experimeter: Johanna and Gabriel

Aim: To investigate whether the kit that was ordered worked on platereaders as well.

Protocol: Piercy Quantitative Peroxide Assay Kit

Concentrations:

1000 µM

700 µM

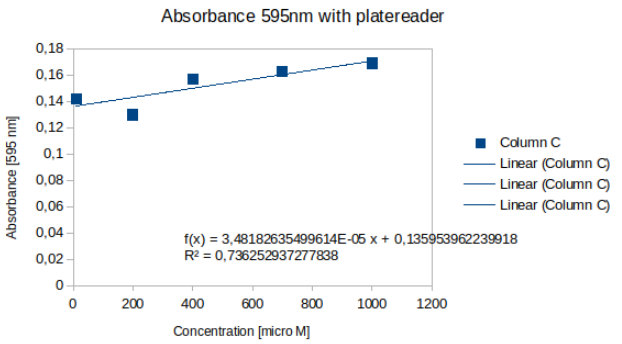
400 µM

200 µM

10 µM

Result:

image.png




#1_Microplate.png

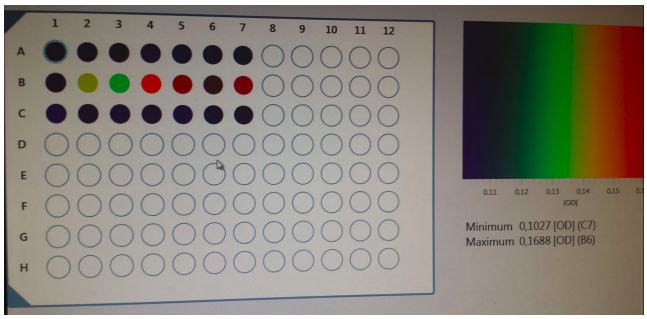


**Table1**

| | A | B | C |
|---|---|---|---|
| 1 | Sample | Concentration [µM] | Absorbance |
| 2 | 1 | 1000 | 0,1688 |
| 3 | 2 | 700 | 0,1628 |
| 4 | 3 | 400 | 0,157 |
| 5 | 4 | 200 | 0,1296 |
| 6 | | 10 | 0,142 |

# Biobrick Assembly

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

## WEDNESDAY, 10/7/2019

Experimenter: Johanna and Gabriel
Aim: To get the desired DNA from the iGEM plates and directory.
Protocol:
10 µl $ddH_2O$
2-3 pg/µl DNA from plates
Punch hole on the aluminum cover, discard first pipettip. Take the $ddH_2O$ and pipet up and down in the well with DNA. Let sit for 5 min. Take the sample from the well and put it in eppendorf tubes.

Sample:
asPink (BBa_K1033933)- plate 4, well 21C
amilCp -blur/purple chromoprotein (BBa_K103930)- plate 6, well 9L
Promotor (BBa_J23119)- plate 2, well 18P
Promotor and RBS (BBa_K081005)- plat 3, well 5E

Result: 4 tubes of the collected DNA, asPink (BBa_K1033933), amilCp-blue/purple chromoprotein (BBa_K103930), Promotor for ribosome standby site (BBa_J23119), promotor and RBS (BBa_K081005)

Stored: in 4°C fridge

## THURSDAY, 11/7/2019

**Transformation of the plasmids from 10/7-19**
Experimenter: Johanna, Gabriel and Vamsi

Aim: To transform the appropriate plasmids into E.Coli DH5α

Protocol: Lab Manual, Synthetic Biology, protocol #6
instead of adding 5 µl of plasmid we used 1 µl of the plasmid from the samples aquired yesterday from the iGEM registry.

Sample:
300 µl E.Coli, DH5α
1 µl asPink (BBa_K1033933)
1 µl amilCp -blur/purple chromoprotein (BBa_K103930)
1 µl Promotor (BBa_J23119)
1 µl Promotor and RBS (BBa_K081005)

Result: 10 agar plates with chlorampicellin, 5 were with the samples and a negative control that was not centrifuged and 5 were the samples and negative control but centrifuged.

Stored: in 37°C room for growth

**Making of LB plates with chlorampicelin**
Experimenter:
Aim: To make agar plated with the correct antibiotic

Protocol: Lab manual, Synthetic Biology protocol #1 page 100, LB agar plates and addition of antibiotics.
Result: Agar plates with chlorampicellin
Stored: stacked together in the 4°C fridge

**Preparation of competent *E.coli* (BL21)**
Experimenter: Gabriel, Johanna

Aim: Start making competent cells.

Protocol: Lab manual, Synthetic Biology protocol #5 page 111. One colony of BL21 was taken from plate and innoculated in 5 mL SOB. Incubated at 37° with shaking.

Result: Overnight culture was started.

Stored: in 37°C room for growth.

FRIDAY, 12/7/2019

**Prepare 0,1 M $CaCl_2$ and 0,1 $CaCl_2$ with 20% glycerol**
Experimenter: Johanna
Aim: To prepare the correct amount for the different solutions in order to use them for competent cells
Protocol: Lab manual, Synthetic Biology protocol # 1 page 92-93
Result:

**Table1**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Sample | 1 M CaCl2 | 50% glycerol | ddH2O | Total Volume |
| 2 | 0,1 M CaCl2 | 4 ml | - | 36 ml | 40 ml |
| 3 | 0,1 M CaCl2 + 20% glycerol | 0,6 ml | 2,4 ml | 3 ml | 6 ml |

**Making competent cells of E.Coli BL21**
Experimenter: Johanna, Vamsi and Gabriel
Aim: To make competent cells with BL21 to be able to use it for future transformation
Protocol: Lab Manual, Synthetic biology protocol#5, page 111
Sample: BL21 from may from the overnight culture
Result:
Stored: In -80° freezer

**Prepare ON culture of transformed cells**
Experimenter: Johanna, Vamsi and Gabriel

Protocol: Start overnight culture by innoculating colonies into 5mL of SOB media with shaking at 37°C

Sample: Plates with transformed cells of:
1 µl asPink (BBa_K1033933)
1 µl amilCp -blur/purple chromoprotein (BBa_K103930)

1 µl Promotor (BBa_J23119)
1 µl Promotor and RBS (BBa_K081005)

Result: 4 falcon tubes tubes with 5mL of SOB containing one of the transformed cells.

Stored: In 37°C room for growth.

SATURDAY, 13/7/2019

**Plasmid purification - GeneJET plasmid Miniprep**
Experimenter: Gabriel, Johanna

Aim: To concentrate plasmid DNA from transformed cells

Protocol: GeneJET plasmid Miniprep kit X2

Sample: 4 O/N culture in LB media of cells with one of following plasmids each:
asPink (BBa_K1033933)
amilCp -blur/purple chromoprotein (BBa_K103930)
Promotor (BBa_J23119)
Promotor and RBS (BBa_K081005)

Result: 2 eppendorfs with purified plasmid of each plasmid. 8 tubes in total.
Gabriels samples:
46,7 ng/µl asPink (BBa_K1033933)
42,6 ng/µl amilCp -blur/purple chromoprotein (BBa_K103930)
30,1 ng/µl Promotor (BBa_J23119)
38,5 ng/µl Promotor and RBS (BBa_K081005)
Johannas samples:
53,7 ng/µl asPink (BBa_K1033933)
39,1 ng/µl amilCp -blur/purple chromoprotein (BBa_K103930)
35,9 ng/µl Promotor (BBa_J23119)
37,8 ng/µl Promotor and RBS (BBa_K081005)

Stored: in -20°C freezer in 4x2 eppendorf tubes.

TUESDAY, 16/7/2019

Begin 3A assembly

**Digestion**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly.
Chromoproteins digested with E and X
Promotors digested with E and S
0,7 agarose gel was used

Sample:

Gabriels samples:
46,7 ng/µl asPink (BBa_K1033933)
42,6 ng/µl amilCp -blur/purple chromoprotein (BBa_K103930)
30,1 ng/µl Promotor (BBa_J23119)
38,5 ng/µl Promotor and RBS (BBa_K081005)

Result:
3 eppendorfs with 30 µl of 100 ng/µl (300 ng total)of digested plasmids:
asPink (BBa_K1033933)
amilCp -blur/purple chromoprotein (BBa_K103930)
Promotor (BBa_J23119)

Stored: In -20° freezer.

**Digestion 2**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly.
Promotor and RBS (BBa_K081005) digested with S+P and E+S
Codon optimized AsPink digested with E+S

Sample:
38,5 ng/µl Promotor and RBS (BBa_K081005)
Codon optimized AsPink (optimized BBa_K1033933)

Result:
Succesfull digestion according to gel.
3 eppendorfs
(1) Promotor and RBS (BBa_K081005) digested with S+P
(2) Promotor and RBS (BBa_K081005) digested with E+S
(3) Codon optimized AsPink digested with X+P

Stored: In -20° freezer.

**Ligation**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly
AmilCP(Digested with E+X) + Promotor(no RBS)(Digested with E+S)
AsPink (non-optimized)(Digested with E+X) + Promotor-RBS(digested with E+S)(2)
AsPink (optimized)(Digested with X+P) + Promotor-RBS(digested with S+P)(1)

Sample:
Digested plasmids in eppendorf:
AmilCP (Digested with E+X)
AsPink (non-optimized) (Digested with E+X)
AsPink (optimized) (Digested with X+P)
Promotor(no RBS) (Digested with E+S)
Promotor-RBS(digested with E+S) (2)
Promotor-RBS(digested with S+P) (1)

Result: 3 eppendorfs with 20µl of ligated products.

Stored: In -20° freezer

WEDNESDAY, 17/7/2019

**Transformation using ligated products**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells (X2)

Sample: Ligated products in eppendorfs:
-AmilCP(Digested with E+X) + Promotor(no RBS)(Digested with E+S)
-AsPink (non-optimized)(Digested with E+X) + Promotor-RBS(digested with E+S)(2)
-AsPink (optimized)(Digested with X+P) + Promotor-RBS(digested with S+P)(1)
-Competent DH5-alpha *E.coli*

Result: 7 LB plates (Each ligation X2 + 1 negative control)

Stored: In 37° room

THURSDAY, 18/7/2019

**Digestion 3**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly
Digest "asPink (BBa_K1033933)" and "amilCp -blur/purple chromoprotein (BBa_K103930)" with X+P.

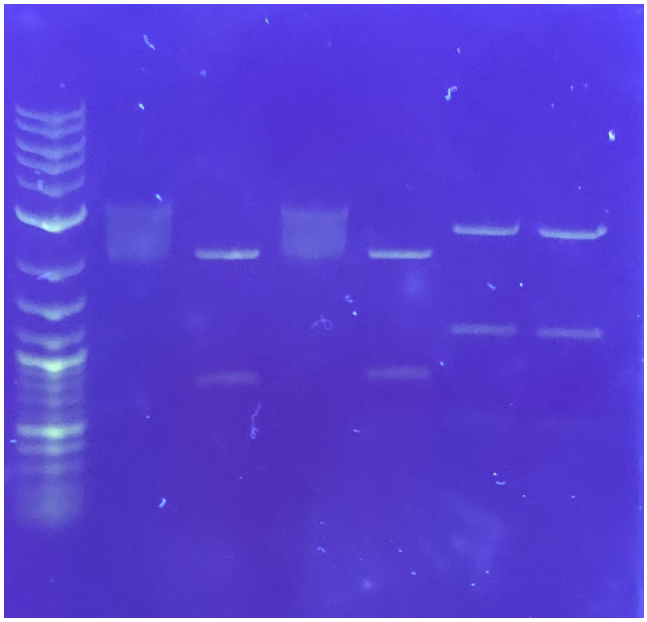
Sample: Purified plasmids
Johannas samples:
53,7 ng/µl asPink (BBa_K1033933)
39,1 ng/µl amilCp -blur/purple chromoprotein (BBa_K103930)

Result:
Digested: "asPink (BBa_K1033933)" and "amilCp (BBa_K103930)". In eppendorfs with concentration of 10 ng/µl.

Stored: In -20° freezer

GEL
6 samples+ ladder.
Ladder, asPink(control+digest), amilCp(control+digest), Two different PSB1A3 with RFP (digested).

Digestion 19:07-19.JPG



**Phosphorylation of primers(amilCP)**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #10 Inverse PCR mutagenesis

Sample:
FWD primer
REV primer

Result: Phosphorylated primers in eppendorfs with primer concentration of 5 µM. 20µl total volume.

Stored: In -20° freezer

FRIDAY, 19/7/2019

**Ligation**

Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly
AmilCP(Digested with X+P) + Promotor(no RBS)(Digested with E+S) + PSB1A3 with RFP (digested with E+P)
AsPink (non-optimized)(Digested with X+P) + Promotor-RBS(digested with E+S)(2) + PSB1A3 with RFP (digested with E+P)
AsPink (optimized)(Digested with X+P) + Promotor-RBS(digested with E+S)(1) + PSB1A3 with RFP (digested with E+P)

Sample:
Digested plasmids in eppendorf:
AmilCP (Digested with X+P)
AsPink (non-optimized) (Digested with X+P)
AsPink (optimized) (Digested with X+P)

Promotor(no RBS) (Digested with E+S)
Promotor-RBS(digested with E+S) (2)
PSB1A3 with RFP (digested with E+P)

Result: 3 eppendorfs with 20µl of ligated products

Stored: -20° freezer

**Transformation**
Experimenter: Gabriel, Katherin

Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells
Number of plates prepared of which sample. Total of 8 plates:
3x AsPink (optimized)
2x AsPink (non-optimized)
2 AmilCP
1x $H_2O$ (control)

Sample: Ligated products in eppendorfs:
1. AmilCP(Digested with X+P) + Promotor(no RBS)(Digested with E+S) + PSB1A3 with RFP (digested with E+P)
2. AsPink (non-optimized)(Digested with X+P) + Promotor-RBS(digested with E+S)(2) + PSB1A3 with RFP (digested with E+P)
3. AsPink (optimized)(Digested with X+P) + Promotor-RBS(digested with E+S)(1) + PSB1A3 with RFP (digested with E+P)

Result: 8 plated amp plates

Stored: 37°room

WEDNESDAY, 24/7/2019

**Transformation**
Experimenter: Gabriel, Katherin

Aim: Insert plasmids into competent *E.coli*

Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells
Number of plates prepared of which sample. Total of 3 plates:

Sample: pSB1C3 with GFP, pSB1C3 with asPink, competent DH5α *E.coli*

Result: No growth on plates

**Making competent cells of *E.coli* DH5α**
Experimenter: Gabriel, Katherin
Aim: To make competent cells with DH3to be able to use it for future transformation
Protocol: Lab Manual, Synthetic biology protocol#5, page 111
Sample: plate with DH5α from fridge
Result: ON prepared
Stored: 37°room

THURSDAY, 25/7/2019

**Transformation**
Experimenter: Gabriel, Katherin, Qian

Aim: Insert plasmids into competent *E.coli*

Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells

Sample: pSB1C3 with GFP, pSB1C3 with asPink, competent DH5α *E.coli*

Result: 6 plates, 2x GFP, 2x asPink, 1x $H_2O$

Stored: 37°room

**Digestion with DpnI**
Experimenter: Gabriel, Katherin
Aim: Remove plasmid template, from PCR product.
Protocol: Lab Manual, Synthetic biology page 129
Sample: Purified product of amilCP PCR
Result: Digestion performed on PCR product sample
Stored: -20° freezer

**Making competent cells of *E.coli* DH5α**
Experimenter: Gabriel, Katherin
Aim: To make competent cells with DH3to be able to use it for future transformation
Protocol: Lab Manual, Synthetic biology protocol#5, page 111
Sample: ON culture
Result: Eppendorfs with 100µl of competent cells
Stored: -80° freezer

FRIDAY, 26/7/2019

**Transformation**
Experimenter: Gabriel, Katherin
Aim: Insert plasmid pSB1C3 with cjBlue(BBa_k1073026) into competent DH5α *E.coli*
Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells
Sample: pSB1C3 with cjBlue, 2 different batches of competent DH5α *E.coli (Gabriel and Irina)*
Result: 4 plates: 2x cjBlue(Gabriel and Irinas E.coli), 2x $H_2O$ (Gabriel and Irinas E.coli)
Stored: 37°room

MONDAY, 29/7/2019

Ligation
**Transformation**
Experimenter: Gabriel, Katherin, Johanna, Jonas
Aim: Insert plasmid pSB1C3 with cjBlue(BBa_k1073026) into competent DH5α *E.coli*
Protocol: Lab Manual, Synthetic Biology, protocol #6 Transformation of $CaCl_2$-competent *E.coli* cells
Sample: pSB1C3 with cjBlue, 2 different batches of competent DH5α *E.coli (Gabriel and Irina)*
Result: 4 plates: 2x cjBlue(Gabriel and Irinas E.coli), 2x $H_2O$ (Gabriel and Irinas E.coli)
Stored: 37°room

**Ligation**

Experimenter: Gabriel and Johanna

Aim: To ligate the PCR sequences in order to make them circular (plasmid)

Protocol: Lab Manual, Synthetic Biology, protocol #10 page 130-131

one tube had both p1 and p2 in it and where therefore doubled in volume since the concentration would otherwise be to high. Tubes with just p1 and p2 where also preparade.

**Table2**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Chemical | DpnI p1 | DpnI p2 | DpnI p1 and p2 mix |
| 2 | DpnI p1 | 2 µl | - | 2 µl |
| 3 | DpnI p2 | - | 2 µl | 2 µl |
| 4 | T4 ligation buffer | 5 µl | 5 µl | 10 µl |
| 5 | T4 ligation | 1 µl | 1 µl | 2 µl |
| 6 | ddH2O | 42 µl | 42 µl | 84 µl |

Sample: DpnI p1 and p2 from group 5, the samples are the same sequence but labeled different.

Result: One tube with DpnI p1, one tube with DpnI p2 and one tube with the mix of DpnI p1 and p2

Concentration of DpnI p1 was 24,0 ng/µl

Concentration of DpnI p2 was 24,9 ng/µl

Stored: -20° freezer in the group 4 box.

**O/N Culture of cjBlue from 26/7-19**

Experimenter: Johanna

Aim: To make an O/N culture to make a miniprep the next day in order to concentrate the DNA

Protocol: Lab Manual Synthetic Biology, protocol #2 page 102

5 ml LB media

5 µl Chloroampicellin

1 colony from plate

Sample: cjBlue from transformation 26/7-19

Result: Two falcon tubes with one colony each

Stored: 37°C room overnight (the room is at 35°C)

TUESDAY, 30/7/2019

**Miniprep of the transformed cjBlue from 29/7-19**
Experimenter: Johanna
Aim: To concntrate the DNA of cjBlue
Protocol: Thermo Scientific GenJET Plasmid miniprep Kit
Sample: cjBlue from transformation 29/7-19
Result: 4 eppendorf tubes 2 different colonies.
cjBlue #1: 29,9 ng/µl
cjBlue #1 40,9 ng/µl
cjBlue #2 50,1 ng/µl
cjBlue #2 46,0 ng/µl

Stored: -20°C freezer

**Transformation**
Experimenter: Johanna and Gabriel
Aim: To transform cjBlue into *E.coli* BL21
Protocol: Lab Manual, Synthetic Biology, protocol #6
Sample: cjBlue from miniprep made 30/7-19
Result: 4 agar plates that were spread with cjBlue
Stored: 37°C

**Making of Agar plates**
Experimenter: Johanna and Gabriel
Aim: To make agar plates with chloramphenicol for future use
Protocol: Lab manual, Synthetic Biology protocol #1 page 100, LB agar plates and addition of antibiotics.
Result: 23 plates of agar with chloramphenicol
Stored: 4°C

**Overnight Culture**
Experimenter: Johanna
AIm: To make an overnight culture in order to do a miniprep the following day.
Protocol: Lab Manual Synthetic Biology, protocol #2 page 102
Sample: cjBlue, DpnI p1 and DpnI p1+p2
Result: 3 falcon tubes with O/N cultures
Stored: 37°C

WEDNESDAY, 31/7/2019

**Miniprep**
Experimenter: Gabriel
Aim: To concentrate the DNA of cjBlue, P1 and P1+P2 mix
Protocol: GeneJET plasmid Miniprep kit. Cells were harvested from 1 ml of ON culture. Concentration measured with NanoDrop.
Sample: Overnight cultures of transformed cells with cjBlue, DpnI p1 and DpnI p1+p2
Result:
P1+P2 mix 53,6 ng/µl
cjBlue: 37,6 ng/µl
P1: 47,3 ng/µl

Stored: -20° freezer in group 4 box

**Miniprep**
Experimenter: Gabriel, Johanna
Aim: To concentrate the DNA of cjBlue, P1 and P1+P2 mix, harvesting cells from 2ml instead of 1ml as used earlier this day.
Protocol: GeneJET plasmid Miniprep kit. Cells were harvested from 2 ml of ON culture. Concentration measured with NanoDrop.
Sample: Overnight cultures of transformed cells with cjBlue, DpnI p1 and DpnI p1+p2

Result:
P1: 124 ng/µl
P1+P2: 143,4 ng/µl
cjBlue: 91,1 ng/µl

Stored: -20° freezer in group 4 box

**Preparation of sample (P1) for sequencing**
Experimenter: Gabriel, Johanna
AIm: Prepare sample for sequencing
Protocol: Followed protocol on package of Mix2Seq from Eurofins.
Primers used: VR, VF2
Sample: Concentrated P1 from Miniprep (124 ng/µl)
Result: One tube ready for sequencing
Stored: Sent for sequencing

**Restreak of transformed E. coli**
Experimenter: Johanna
Aim: Too visually observe the rate at which cells express cjBlue.
Protocol: Picked 1 colony
Sample: cjBlue colonies from transformation performed on friday 26/7.
Result: 1 plate with 1 streaked colony
Stored: 37°C room

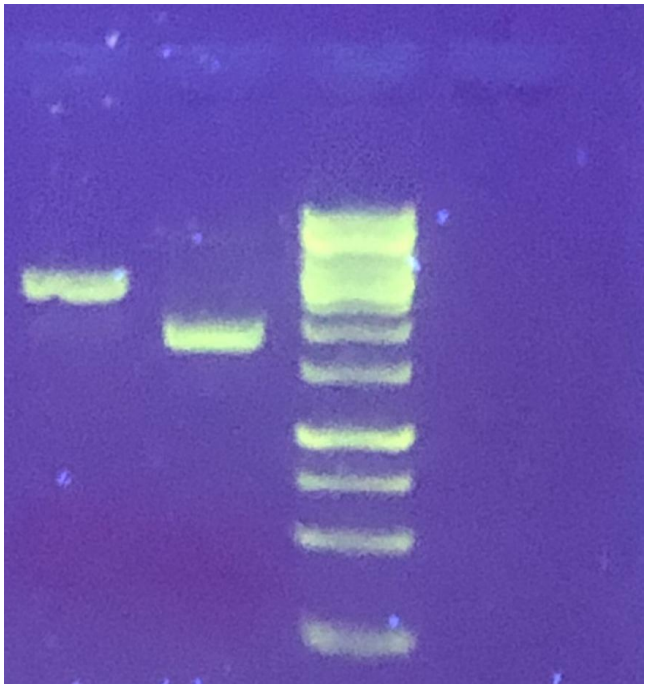
**Overnight Culture**
Experimenter: Johanna
AIm: To make an overnight culture in order to observe rate of color expression.
Protocol: Lab Manual Synthetic Biology, protocol #2 page 102
Sample: cjBlue colonies from transformation performed on friday 26/7.
Result: 1 falcon tube with O/N cultures
Stored: 37°C room

THURSDAY, 1/8/2019

**Digestion of P1(amilCP with RSS) with P and X**
Experimenter: Gabriel, Johanna
Aim: Digest P1 for ligation with PSB1C3 and Promotor (BBa_J23119).
Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly.
Sample: Eppendorf with P1 with concentration of 124 ng/µl.

Result: Succesfull digestion according to gel.
From left: Digested P1, undigested plasmid, ladder.

IMG_1584.JPG



Stored: In -20° freezer.

**Ligation**
Experimenter: Gabriel, Johanna
AIm: To ligate digested P1, PSB1C3 and Promotor (BBa_J23119).
Protocol: Lab Manual, Synthetic Biology, protocol #3 Biobrick, 3A assembly.
Sample: digested P1, PSB1C3 and Promotor (BBa_J23119).
Result: Eppendorf with ligation mixture.
Stored: In -20° freezer.

**Transformation**
Experimenter: Gabriel, Johanna
AIm: To transform ligation mixture of P1, PSB1C3 and Promotor (BBa_J23119) into DH5α
Also transform eforRed (BBa_K864402) into DH5α
Protocol: Lab Manual, Synthetic Biology, protocol #6
Sample: eforRed, ligation mixture of P1, PSB1C3 and Promotor. Competent DH5α, 3 amp plates, 3 cloramphenicol plates.
Result: 6 plates: 2 ligation mixture, 2 eforRed, 2 controls (one for each type of plate).
Stored: 37°C room

FRIDAY, 2/8/2019

**Transformation**
Experimenter: Gabriel and Johanna
Aim: To transform eforRed, amilCp and asPink into DH5α
Protocol: Lab manual, Synthetic Biology protocol #6
Sample: eforRed, amilCp and asPink on chloramphenicol plates
Result: 3 agar plates with the corresponding plasmids
Stored: 37°C room

**Sequencing**

Experimenter: Gabriel and Johanna
Aim: To sequence the plasmid
Protocol: Mix2Seq Kit
Sample: DpnI p1 (amilCp)
Result: One tube of 15 µl DNA plasmid and 2µl VF2 primer.
A sequence was returned that showed that our overhang was succselful in the insertion. One nucleotide was missing for the Xba1 restriction site which means that we have to order new primers and redo a PCR in order to submit the biobrick to iGEM.

MONDAY, 5/8/2019

**Restreak**
Experimenter: Johanna and Gabriel
Aim: To restreak the colonies that should have colour, since they are a high number of colonies on the plate.
Protocol: Agar plates with chloramphenicol
one colonie was taken from each agar plate with colonies and then restreaked onto the new agar plates.
Sample: amilCp and asPink
Result: two agar plates with restreaking of colonies

**Overnight culture**
Experimenter: Johanna and Gabriel
Aim: to make overnight cultures
Protocol: Lab Manual Synthetic Biology, protocol #2 page 102
Sample: asPink and amilCp from transformation
Result: two overnight cultures with SOB media. one with asPink and the other with amilCp
Stored: 37°C room

TUESDAY, 6/8/2019

**Miniprep. Thermo Scientific GeneJET plasmid miniprep Kit**
Experimenter: Johanna
Aim: to concentrate DNA
Protocol: Thermo Scientific GeneJET plasmid miniprep kit
Sample: O/N culture from 5/8-19
Result: two eppendorf tubes with concentrated DNA.
asPink: 65,5 ng/µl
amilCp: 84,4 ng/µl
Stored: -20°C freezer

WEDNESDAY, 7/8/2019

**Ligation**
Experimenter: Gabriel and Johanna
Aim: to ligate different genes that are desired to be in one plasmid
Protocol: Lab manual, Synthetic Biology, protocol #3 page 107
Sample:
amilCp
asPink
Promotor
Promotor + RBS
Result: 2 eppendorf tubes with ligation mixtures of asPink and amilCp

Stored: -20°C freezer

**Transformation**

Experimenter:

Aim: To transformed the ligation mixtures that was

Protocol: lab manual, Synthetic Biology, protocol #6

Sample: Ligation mixtures of asPink and amilCp

Result: 2 agar plates with ampicellin, one with amilCp and one with asPink (one negative as well).. No plate had any colonies, we suspect the plates

Stored:37°C room

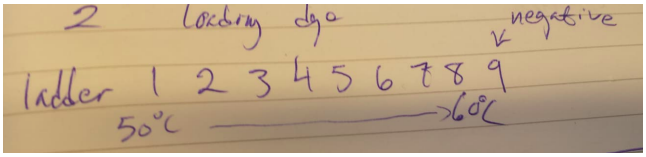
# PCR

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

TUESDAY, 30/7/2019

Experimenter: Johanna
Aim: To multiply DNA
Protocol: Thermo Scientifiq DreamTaq DNA polymerase
A temperature gradient was used for the samples (50-60°C)

Loading_the_PCR.png




Sample: pPicz
Result: 8 tubes into the PCR machine
Stored: Left overnight in the PCR machine and put in a gel the day after

WEDNESDAY, 31/7/2019

**Agarose gel to detect the DNA from PCR**
Experimenter: Johanna
Aim: To detect whether the PCR was succesful and if the temperature gradient was useful
Protocol: Lab manual, Synthetic Biology protocol #4 page 108-112
Sample: the pPICz from the PCR done 30/7-19
Result: Nothing showed on the gel, due to probable mix-up with the tubes

Stored: Discarded

# Degrading of Lignin- Proof of concept

**Project:** iGEM uppsala 2019
**Authors:** Johanna Cederblad

WEDNESDAY, 7/8/2019

**Fractionating lignin**

Drag and drop a file from the sidebar to attach or

Upload a File

Experimenter: Johanna and Gabriel
Aim: to collect fractions of the lignin in order to degrade it further in an easier way.
Protocol:
Methanol fraction:
2 gram lignin and 20 ml methanol was stirred for 2 h (500 varv/min) and then filtered with level 1 filterpaper, the supernatant was saved.
2 gram lignin and 20 ml methanol was stirred for 2 h (500 varv/min) and then centrifuged for 5 min in 4000 rpm. the supernatant was saved.

Aceton fraction:
the pellet from the filter fraction was mixed with 20 ml aceton and then stirred for 2h (500 varv/min) and then filtered through a level 3 filter paper.
the pellet from the centrifuged samples was mixed with 20 ml aceton and then stirred for 2h (500 varv/min) and then centrifuged for 10 min in 4000 rpm. supernatant and pellet was saved seperatly.
Sample: Lineo Prime W
Result:
Methanol:
One falcon tube with filtered supernatant
one falcon tube with centrifuged supernatant
Acetone:
one falcon tube with filtered supernatant
one falcon tube with centrifuged supernatant
one falcon tube with the pellet from the centrifuged sample.
two eppendorf tubes with the scraped pellet from the filterpaper.
Stored: room temperature

THURSDAY, 8/8/2019

**Native-PAGE**

Experimenter: Johanna and Gabriel

Aim: To detect whether the lignin is detectable in a gel.

Protocol:

Sample buffer(2X):

49µl 1,5 M tris HCl, pH 8,8

50µl 25% glycerol

1µl 1% bromophenol Blue

All was mixed into eppendorf tube.

Running buffer(10X):

275 ml dd$H_2O$

125 ml Tris HCl pH8,8

100 ml glycerol (1,92M)

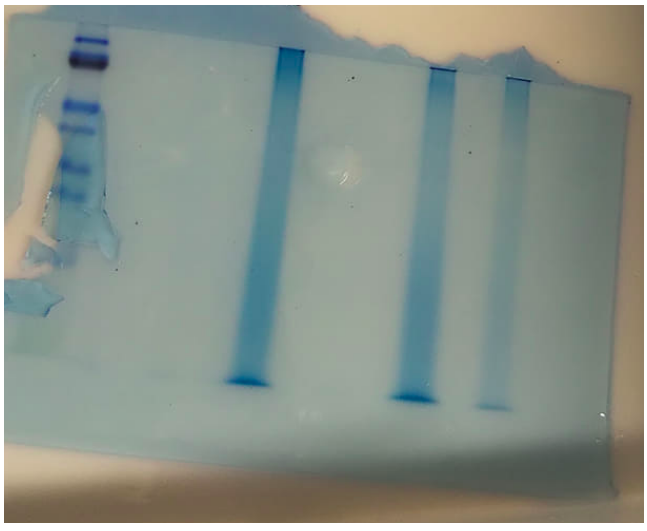
All was mixed an diluted to 1X when need to run the gel.

For running the gel 10µl sample was mixed with 10µl sample buffer

Sample: Filtered MeOH, Filtered Acetone, Centrifuge MeOH, Centrifuged Acetone, Original Lignin, Lignin pellet from Acetone

Result:

Native-PAGE.png



**SDS-PAGE 20% separation gel, 4% stacking gel**

Experimenter: Johanna and Gabriel

Aim: To see if lignin is visible on an SDS-PAGE

Protocol:

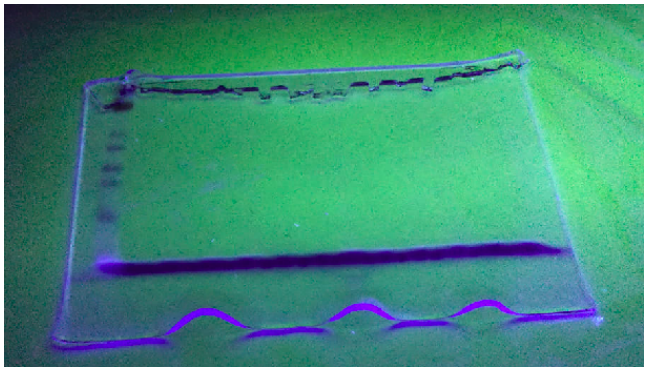
Separation gel:

0,59 ml dd$H_2O$

6,7 ml acrylamid/bis (30%)
2,5 ml Tris-HCl (1,5 M, pH 8,8)
100 µl SDS (10%)
10 µl TEMED
100 µl APS

Stacking gel:
6,1 ml dd$H_2O$
1,3 ml Acrylamide/bis (30%)
Tris-HCl (0,5M pH6,8)
Sample:
Result:

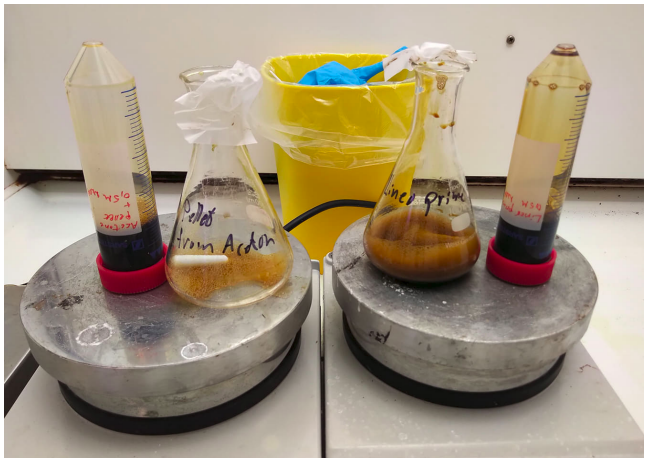
Screenshot_#1_20%_SDS-PAGE.png



MONDAY, 12/8/2019

**Fractionating of lignin in high pH**
Experimenter: Johanna and Gabriel
Aim: To make the kraft lignin (Lineo Prime) soluble with the help of high pH
Protocol: 2 gram of Lineo Prime was mixed with 20 ml of 0,5M NaOH and stirred over night
a lump of pellet from the aceton fraction was mixed with 20 ml of 0,5 M NaOH
Sample: Lineo Prime W
Result: Two 50 ml falcon tubes with soluble lignin.
Stored: On bench in room temperature

Lignin_NaOH.png



TUESDAY, 13/8/2019

**Degrade Lignin with HRP in Phosphate buffer**

Experimenter: Johanna and Gabriel

Aim: To degrade lignin with HRP and $H_2O_2$ in phosphate buffer

Protocol: Lignin blandades i olika lösning (se tabell) and were tested wth a nanodrop.

**Table2**

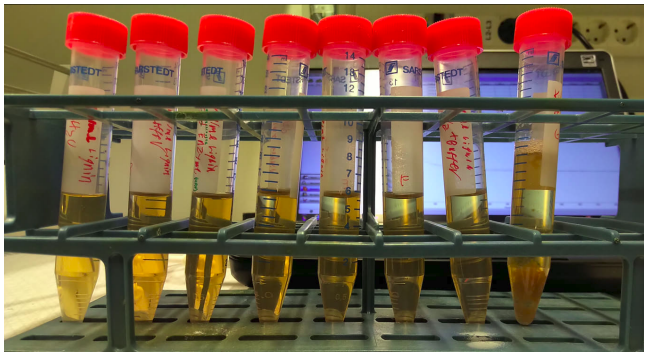
| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Sample | Lignin | H2O | Phosphate buffer | H2O2 | Heat-inactivated enzyme | ICA HRP |
| 2 | A | 100 µl | 9,9 ml | - | - | - | - |
| 3 | B | 100 µl | | 9,9 ml | - | - | - |
| 4 | C | 100 µl | | 9,74 ml | - | 0,16 ml | - |
| 5 | D | 100 µl | | 9,74 ml | - | - | 0,16 ml |
| 6 | E | 100 µl | | 9,41 ml | 0,33 ml | - | 0,16 ml |
| 7 | F | 100 µl | | 9,41 ml | 0,33 ml | 0,16 ml | - |
| 8 | G | 100 µl | | 9, 57 ml | 0,33 ml | - | - |

Sample: Sulfonated lignin

Result: Pellet in the samples that had the ICA HRP, both in the denaturated HRP and the regular one. The denaturated lignin might not be completly inactive.

samples were taken every hour. These were loaded on an SDS-PAGE later.

Screenshot_Lignin_samples_phosphate_buffer.png



**Run of SDS-PAGE of lignin samples 15%**

Experimenter: Johanna and Gabriel

Aim: To see if any pattern of lignin could be detected on the gel.

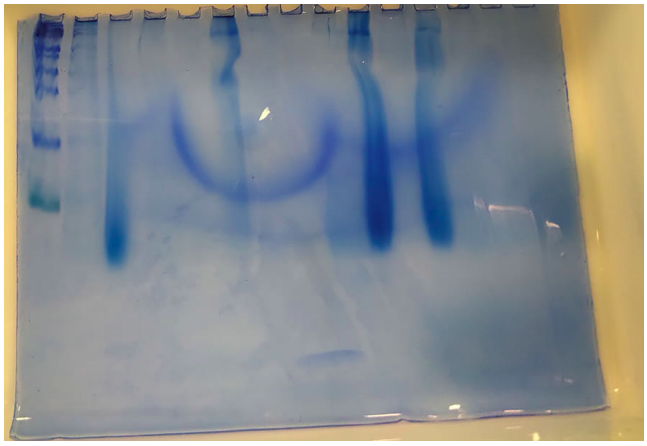
Protocol: The samples were mixed with running dye and then loaded into the wells of the gel. The gel was started at 80 V and then increased to 130 V after the samples had run down the gel.

Sample: The samples from the degrading of lignin.

Result: a gel se picture

[Ladder: E0:E1:E2:E4:kill_it_0:kill_it_1:A:B:C:D:F:G]

Screenshot_#2_15%_SDS-PAGE.png



WEDNESDAY, 14/8/2019

**SDS-PAGE #3 of lignin samples 15%**

Experimenter: Johanna and Gabriel

Aim: To detect difference in the gel and whether the lignin has been degraded.

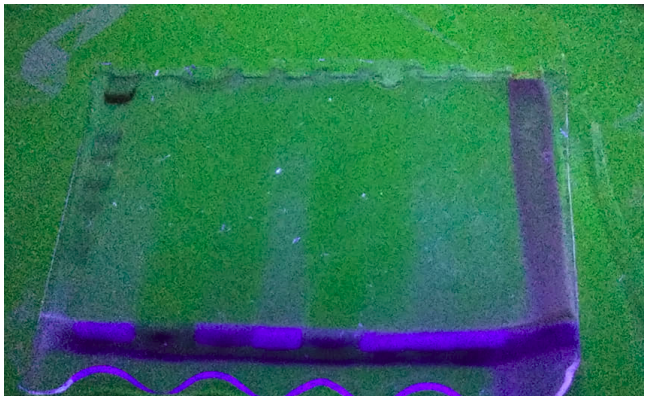
Protocol: The samples were mixed with running dye and then loaded into the wells of the gel. The gel was started at 80 V and then increased to 130 V after the samples had run down the gel.

Sample: from lignin degradation earlier

Result:

[ladder: centrifuged sonicated aceton: centrifuged sonicated MeOH: Filter MeOH:Filter Aceton: centrifuge MeOH: bubble: centrifuge aceton: original Lineo prime: Original sulfonated]

Screenshot_#3_15%_SDS-PAGE.png



**Sonication of lignin**

Experimenter: Johanna

Aim: To degrade the lignin with sonication

Protocol: 50% amplitude, 5 seconds puls 5 seconds rest, for 30 min

Sample: sulfonated lignin

Result: a falcon tube with sonicated lignin

THURSDAY, 15/8/2019

**Degrade lignin with HRP in Tris-HCl buffer**

Experimenter: Johanna and Gabriel

Aim: To degrade lignin with HRP

Protocol: lignin was mixed with different solutions in order to see if any degradation would occur.

**Table3**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | Sample | Tris-HCl | ICA HRP | Sigma HRP | Heat-inactivated ICA | Heat-inactivated Sigma | H2O2 | Lignin |
| 2 | A | 9,74 ml | - | 160 µl | - | - | - | 100 µl |
| 3 | B | 9,74 ml | 160 µl | - | - | - | - | 100 µl |
| 4 | C | 9,41 ml | - | 160 µl | - | - | 330 µl | 100 µl |
| 5 | D | 9,41 ml | 160 µl | - | - | - | 330 µl | 100 µl |
| 6 | E | 9,41 ml | - | - | - | 160 µl | 330 µl | 100 µl |
| 7 | F | 9,41 ml | - | - | 160 µl | - | 330 µl | 100 µl |
| 8 | G | 9,90 ml | - | - | - | - | - | 100 µl |

Sample: sulfonated lignin

Result: Pellet and sign of change in the ICA HRP, otherwise some decrese in curves.

**Prepare 0,1 M tris-buffer**

Experimenter: Johanna

Aim: To get the correct M of the buffer

Protocol: diluted a 1 M tris-buffer to a 0,1 M tris-buffer

Sample: 1 M tris- buffer from group 2
Result: a 0,1 M tris-buffer

**SDS-PAGE 18%**
Experimenter: Johan Erkers
Aim: To make SDS-plate for analysis of lignin samples.
Protocol:
The following protocoll was used:

SDS Page Gel (10 and 15%)

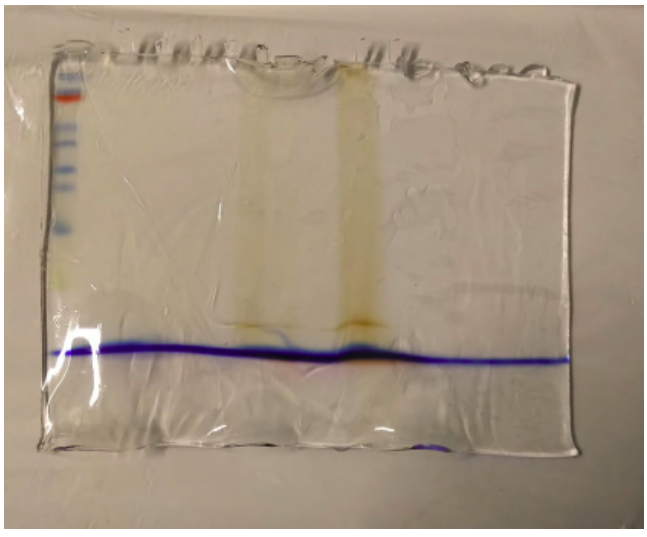
The ammount of sample used was:

**Table1**

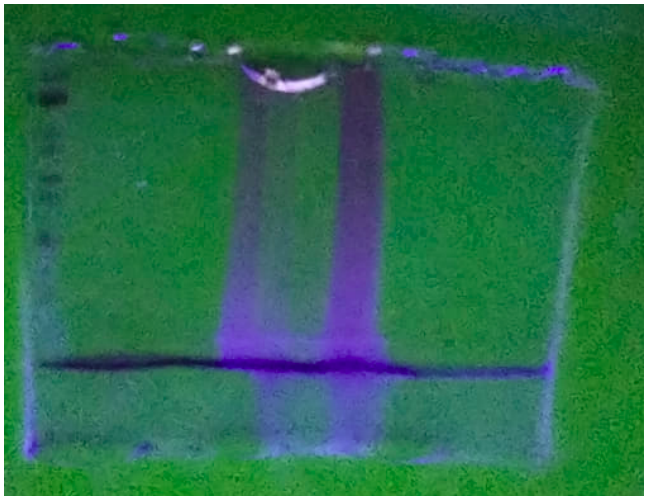
| | A | B |
|---|---|---|
| 1 | Material | Quantities for 2 Gels |
| 2 | water | 1.334ml |
| 3 | Akrylamide | 6.066ml |
| 4 | 1,5 M Tris pH 8.8 | 2,5ml |
| 5 | SDS 10% | 100 ul |
| 6 | APS 10% | 100 ul |
| 7 | TEMED | 10ul |

Sample: See protocoll
Result: One plate was succesfully made

Screenshot_#4_18%_SDS-PAGE.png



Screenshot_#4_TLC_SDS-PAGE.png



**Platereader with lignin samples**

Experimenter: Johanna and Gabriel

Aim: To examine several different samples at the same time

Protocol:

Sample:

Result:

SUNDAY, 18/8/2019

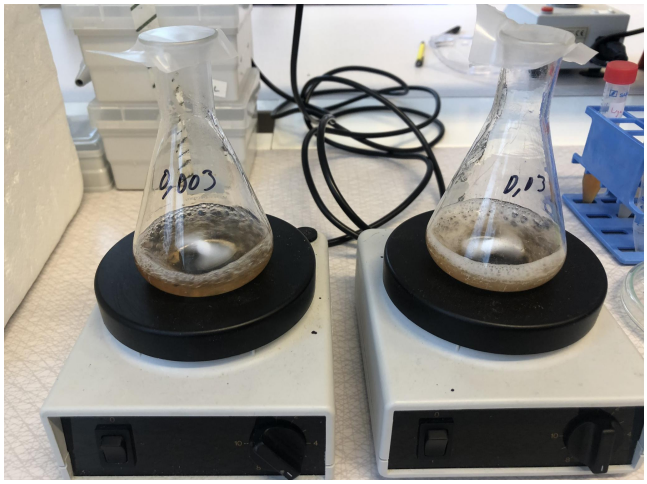
**Degradation of lignin with HRP with continous addition of hydrogen peroxide**

Experimenter: Johanna, Gabriel

Aim: To degrade lignin with HRP and measure changes in nanodrop by full spektrum analysis

Protocol: The reactions were performed in room temperature and the final volume was 10ml. Lignin (1 mg/ml) was solved in deionized water and stirred. HRP was added to a concentration of 0,03mg/ml or 0,003mg/ml, and hydrogen peroxide (90 µM) was added every 120 minutes. These two samples were stirred for 4 hours******

IMG_4549.jpg




Sample: Lignosulfanate lignin, Horse radish peroxidase (thermo fischer), hydrogen peroxide, deionized water.

Result: Peaks that showed a decrease of lignin on the nano-drop

MONDAY, 19/8/2019

**SDS-PAGE #5**

Experimenter: Johanna and Gabriel

Aim: To see a change of the different treated lignin running on the gel.

Protocol: 10 µl Sample were mixed with 10 µl loading dye and then loaded into the wells of the SDS-PAGE and then run from 80V and later increased to 120V

Sample: [1mg/ml lignin: 0,03_0h_HRP: 0,03_2h_HRP: 0,03_4h_HRP: sonicated sulfonated lignin 20mg/m: sonicated MeOH lignin 20mg/ml: soniated acetone lignin 20mg/ml: sulfonated lignin 20mg/ml: Pellet_kill_it_4h: Kill_it_1_ : kill_it_2: kill_it_3:]

Result:

**Degrading lignin with HRP**

Experimenter: Johanna and Gabriel

AIm: to degrade lignin with enzyme

Protocol: : The reactions were performed in room temperature and the final volume was 10ml. Lignin (1 mg/ml) was solved in deionized water and stirred. HRP was added to a concentration of 0,03mg/ml or 0,003mg/ml, and hydrogen peroxide (90 µM) was added every 120 minutes. These two samples were stirred for 4 hours

Sample:Sulfonated lignin

Result:

**Making of 50mM sodium acetate**

Experimenter: Gabriel and Vamsi

AIm:

Protocol:

Sample:

Result:

TUESDAY, 20/8/2019

**Degrading lignin with HRP**

Experimenter: Johanna and Gabriel

Aim: to degrade lignin with HRP

Protocol: : The reactions were performed in room temperature and the final volume was 10ml. Lignin (1 mg/ml) was solved in deionized water and stirred. HRP was added to a concentration of 0,03mg/ml or 0,003mg/ml, and hydrogen peroxide (90 µM) was added every 120 minutes. These two samples were stirred for 4 hours

Sample: Sulfonated lignin

Result:

**Bradford test**

Experimenter: Johanna and Gabriel

Aim: To investigate what concentration the MnP from Sigma has

Protocol: BSA(Bovine Serum Albumin) was added in different concentration and checked with a spectrophotometer. The MnP was then tested with the same wavelength (595 nm), and later ajdusted to the curve from the BSA

Sample: MnP from SIgma

Result: the enzyme concentration was around 0,005 mg/ml

**run of SDS-PAGE #6**

Experimenter: Johanna and Gabriel

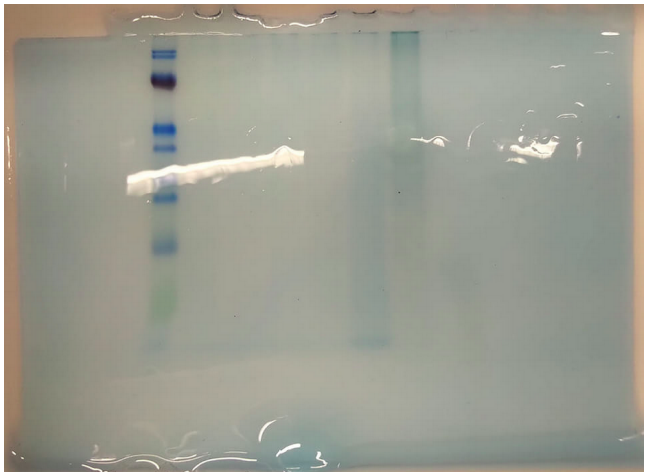
Aim: to see a change of the degrading of lignin on SDS-PAGE

Protocol: 10 µl Sample were mixed with 10 µl loading dye and then loaded into the wells of the SDS-PAGE and then run from 80V and later increased to 120V

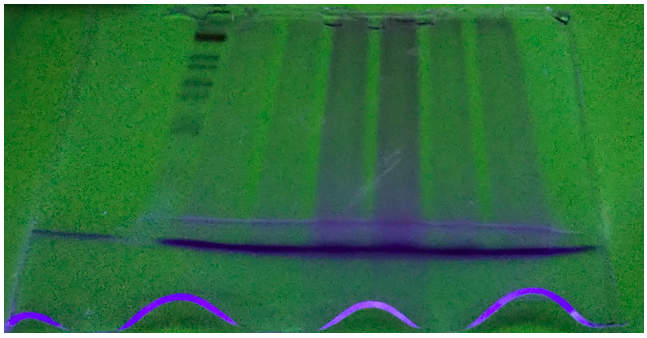
Sample: [20mg/ml sulfonated lignin: 20mg/ml sonicated lignin: 50 mg/ml sulfonated lignin: 50 mg/ml boiled sulfonated lignin: 20 mg/ml boiled sulfonated lignin: 20 mg/ml boiled sonicated lignin]

Result:

Screenshot_#6_SDS-PAGE.png



Screenshot_#6_SDS-PAGE_TLC.png



WEDNESDAY, 21/8/2019

**Degrading lignin with HRP with continous addition of hydrogen peroxide**

Experimenter: Johanna and Johan

Aim: To degrade lignin with HRP and measure changes in nanodrop by full spektrum analysis

Protocol: The reactions were performed in room temperature and the final volume was 10ml. Lignin (1 mg/ml) was solved in deionized water and stirred. HRP was added to a concentration of 0,03mg/ml or 0,003mg/ml, and hydrogen peroxide 33  was added every 120 minutes. These two samples were stirred for 4 hours******

Sample: Lignosulfanate lignin, Horse radish peroxidase (thermo fischer), hydrogen peroxide, deionized water.

Result:

THURSDAY, 22/8/2019

**Degrading lignin with HRP**

Experimenter: Johanna and Johan

Aim: To degrade lignin with HRP and $H_2O_2$ and to measure the changes

Protocol:

**Table4**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Sample | Kraft Lignin | HRP | H2O2 | ddH2O |
| 2 | A | 0,02 g | - | 33 ul | 9,67 ml |
| 3 | B | 0,02 g | 250 ul | 33 ul | 9,717 ml |
| 4 | C | 0,02 g | - | - | 10 ml |
| 5 | D | 0,02 g | 250 ul | - | 9,75 ml |
| 6 | | | | | |

Sample: Kraft Lignin and sulfonated lignin

Result:

**SDS-PAGE #7 from lignin degrading samples**

Experimenter: Johanna and Johan

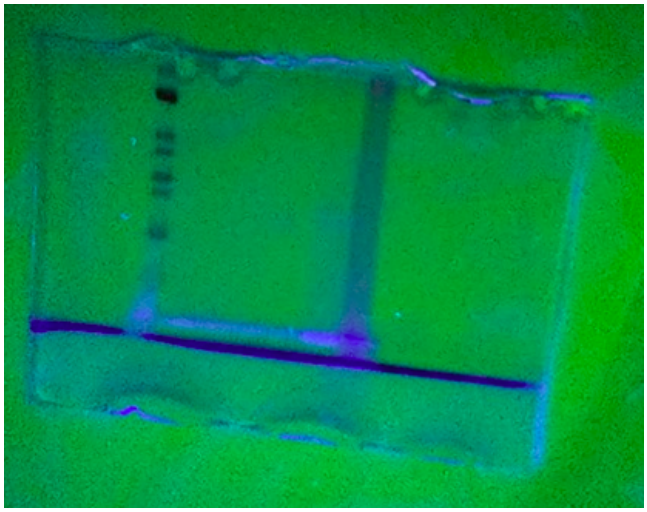
Aim: to see a change in the degraded lignin, on the gel.

Protocol: 10 µl Sample were mixed with 10 µl loading dye and then loaded into the wells of the SDS-PAGE and then run from 80V and later increased to 120V

Sample: [B_0h: B_2h: B_4h: B_16h: C : sulfonated lignin 20 mg/ml]

Result:

Screenshot_#7_SDS-PAGE_TLC.png



WEDNESDAY, 28/8/2019

**Degrading Lignin with HRP and higher amounts of lignin**

**Making 18% SDS_PAGE with 4% stacking gel**

Experimenter: Johanna

Aim: To make a gel in order to run the lignin on it

Protocol:

Sample:

THURSDAY, 29/8/2019

**Degrading Lignin with HRP and higher amounts of lignin**

Experimenter: Gabriel and Johanna

Aim: Add HRP and hydrogen peroxide to break down lignin.

Protocol: The reactions were performed in room temperature and the final volume was 10ml. Lignin (Lineo prime, wet) (0,02 mg/ml) was solved in deionized water and stirred. HRP was added to a concentration of **0,03mg/ml or 0,003mg/ml, and hydrogen peroxide (90 µM)*****  was added at time 0. Samples were taken and put on ice at minute: 1, 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90.

Sample:

Results:

**Making 18% SDS_PAGE with 4% stacking gel**

Experimenter: Gabriel

Aim: To make a gel in order to run the lignin on it

Protocol:

**4% stacking gel**

| | A | B | C |
|---|---|---|---|
| 1 | What | Quantity | |
| 2 | Water | 6,1 ml | |
| 3 | Akrylamide/Bis 30% | 1.3 ml | |
| 4 | Tris HCl 0,5M pH 6.8 | 2. 5 ml | |
| 5 | SDS 10% | 100 ul | |
| 6 | APS 10% | 100 ul | |
| 7 | Temed | 10 ul | |

**18% resolving gel**

| | A | B | C |
|---|---|---|---|
| 1 | What | Quantity | |
| 2 | Water | 1,334 | |
| 3 | Akrylamide/Bis 30% | 6,066 | |
| 4 | Tris HCl 1,5M pH 8,8 | 2. 5 ml | |
| 5 | SDS 10% | 100 ul | |
| 6 | APS 10% | 100 ul | |
| 7 | Temed | 10 ul | |

Sample:
Results:

**Sonication of yeast pellet**
Experimenter: Johanna, Gabriel
Aim: To lyse cells before testing samples on enzymatic assay.
Protocol: Cells were sonicated for 30 minutes.
Sample: Pellet of "X-33 HRP-2A-AAO pick XV VI (induced + uninduced)".
Results: Two falcon tubes with sonicated pellets suspension.

**Gel with lignin**
Experimenter: Johanna, Gabriel
Aim: To observe potential breakdown of lignin on SDS gel.
Protocol: 1, 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90.
Sample:
Results:

**Enzymatic assay on sonicated pellet of (AAO, induced+uninduced)**
Experimenter: Johanna ,Gabriel
Aim: To perform enzymatic assay with sonicated pellet of (X-33 AAO, induced+uninduced)*** using pierce quantitative peroxide assay kit.
Procedure: Followed the protocol provided in the kit (Thermo fisher - protocol 23280)
Induced and uninduced samples were tested, and each sample was tested with both reagent A which was made in the lab and reagent A that came with the kit. A total of four samples.
1mL of working reagent was added to 1,5mL cuvettes, the sample was added and mixed. After 15 minutes of incubation at room temperature, the OD was measured at 560nm.
Sample: Induced and uninduced pellet of "X-33 AAO"
Results: Sedimentation occured in the induced samples when added to the working reagent

IMG_4792.JPG

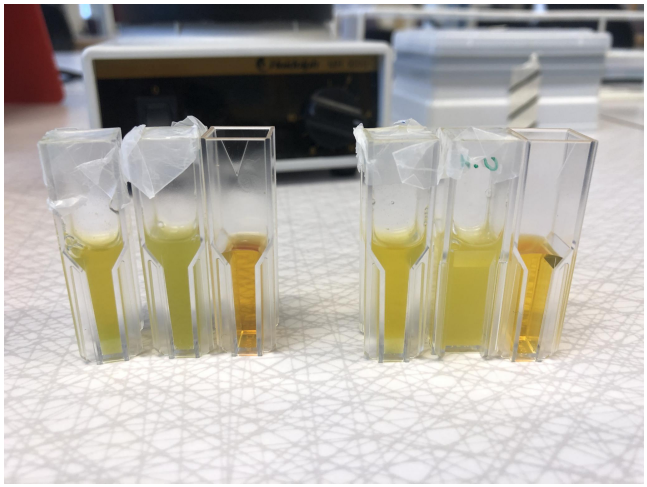


Figure 1. Samples in working reagent after 15 minutes at room temperature.  Order from left: reagent A from kit (Induced, uninduced, blank), self made reagent A (induced, uninduced, blank).

**Table 1**

| | A | B |
|---|---|---|
| 1 | Table 1. Absorbance measurments of induced and uninduced samples at 560nm | |
| 2 | Sample | abs (560nm) |
| 3 | Original: induced | 1,12 |
| 4 | Original: uninduced | 1,356 |
| 5 | Self made: induced | 0,773 |
| 6 | Self made: uninduced | 1,828 |

# Enzymatic assay of Peroxidase using ABTS as substrate

**Project:** iGEM uppsala 2019

**Authors:** Gabriel Torres Muñoz

FRIDAY, 9/8/2019

Experimenter: Gabriel, Johanna

Aim: To test if the assay works for HRP by using HRP extracted from horseradish. Obtain the $\Delta A_{405\ nm}$/minute for the maximum linear rate for both the Test and Blank to calculate Units/mg solid.

Protocol: Enzymatic Assay of Peroxidase (EC 1.11.1.7) 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) as a Substrate (CR SOP-DEK-ENZ42). Two different dilusions were tested: 0,005 and 0,0005.

Sample: HRP derived from horseradish, substrates needed according to instructions

Result:. When using 0,005, the increase in absorbtion was beyond the maximum readings of the machines (>3). The maximum linear rate of the 0,0005 reaction was used to calculate the units/mg solid:***

image.png

$$\text{Units/mg solid} = \frac{(\Delta A_{405\ nm}/\text{min(test)} - \Delta A_{405\ nm}/\text{min(blank)}) * 3.05 * DF}{36.8 * 0.05}$$

units/mg solid =

**Table1**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Table 1. Absorbance values obtained from two different dilutions of HRP and test with Bovine serum albumin | | | |
| 2 | **Time (s)** | **Test (abs)** | **Dilute 0,005 (abs)** | **Dilute 0,0005 (abs)** |
| 3 | 0 | -0,193 | 0,298 | -0,19 |
| 4 | 10 | -0,214 | 0,501 | -0,18 |
| 5 | 20 | -0,222 | 0,732 | -0,171 |
| 6 | 30 | -0,222 | 0,966 | **-0,173** |
| 7 | 40 | -0,22 | 1,211 | -0,166 |
| 8 | 50 | -0,221 | 1,459 | -0,151 |
| 9 | 60 | -0,221 | 1,738 | -0,134 |
| 10 | 70 | -0,219 | 2,06 | -0,117 |
| 11 | 80 | -0,219 | 2,45 | -0,098 |
| 12 | 90 | -0,219 | >3 | -0,081 |
| 13 | 100 | -0,219 | >3 | -0,064 |
| 14 | 110 | -0,219 | >3 | -0,047 |
| 15 | 120 | -0,218 | >3 | -0,028 |
| 16 | 130 | -0,218 | >3 | -0,011 |
| 17 | 140 | -0,218 | >3 | 0,007 |
| 18 | 150 | -0,218 | >3 | 0,023 |
| 19 | 160 | -0,217 | >3 | 0,042 |
| 20 | 170 | -0,217 | >3 | 0,058 |
| 21 | 180 | -0,217 | >3 | 0,077 |

image.png

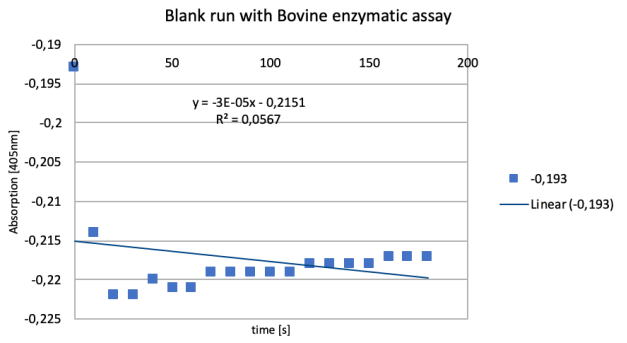


image.png

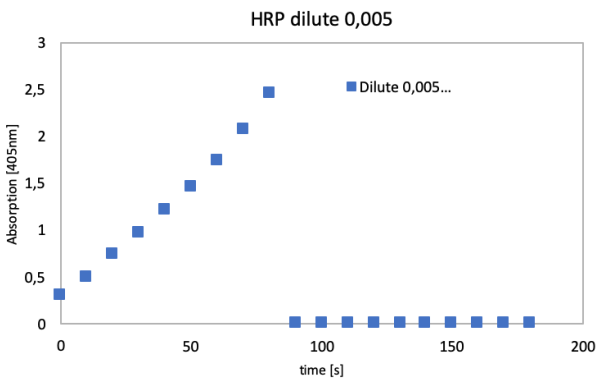
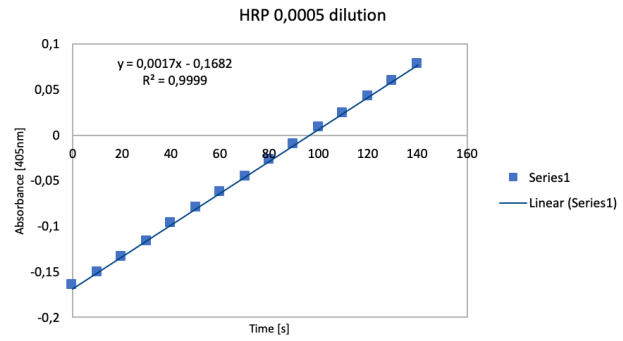



image.png




MONDAY, 12/8/2019

Experimenter: Gabriel, Johanna

Aim: To test again if the assay works for HRP by using HRP extracted from horseradish. Obtain the $\Delta A_{405\ nm}$/minute for the maximum linear rate for both the Test and Blank to calculate Units/mg solid.

Protocol: Enzymatic Assay of Peroxidase (EC 1.11.1.7) 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) as a Substrate (CR SOP-DEK-ENZ42). A dilution of 0,0005 was used.

Sample: HRP derived from horseradish. Substrates used according to instructions.

Result: Maximum linear rate for 0,0005 was 0,007, and for the test eith ovine serum albumin it was 0,0001. In this test the absorbance values were positive as ecpected, compared to earlier test.

The maximum linear rate of the 0,0005 reaction was used to calculate the units/mg solid ***

image.png

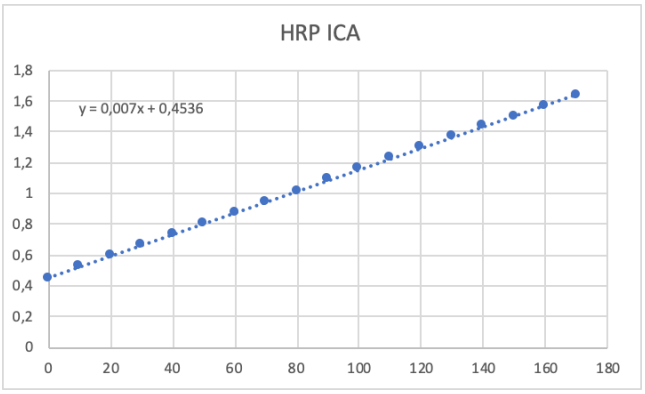



image.png

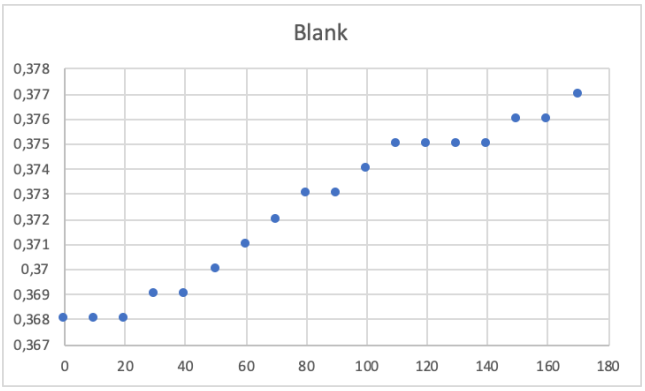
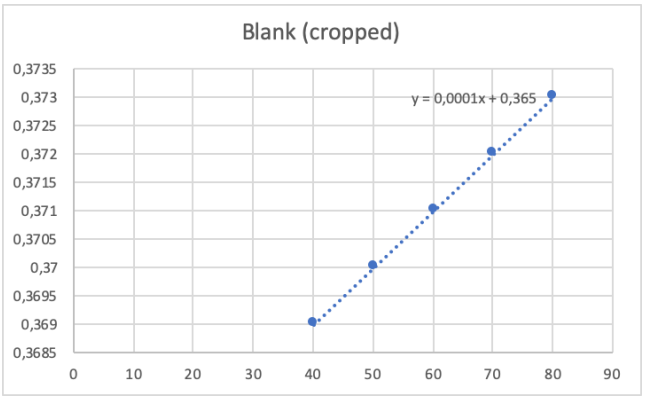



image.png




TUESDAY, 13/8/2019

Experimenter: Gabriel, Johanna

Aim: To observe the activity of the HRP obtained from Thermo Fischer

assay works for HRP by using HRP extracted from horseradish. Obtain the $\Delta A_{405\ nm}$/minute for the maximum linear rate for both the Test and Blank to calculate Units/mg solid.

Protocol:

Sample:

Results: