Labbook

Project: iGEM 2022
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TUESDAY, 19/4/2022

Members: Timo, Melissa, Max

WEDNESDAY, 20/4/2022

Advisor: Vincent
Tasks: Setting up LB medium, LB medium (liquid), Azotobacter medium, Azotobacter medium (liquid)

LB medium (liquid) for E. coli

	A	B	C	D	E
1	Component	amount	unit	min	max
2	Water	500	ml	495	505
3	LB medium	10	g	9.9	10.1

LB medium for E. coli

	A	B	C	D	E
1	Component	amount	unit	min	max
2	Water	500	ml	495	505
3	LB medium	10	g	9.9	10.1
4	Agar	7.5	g	7.4	7.6

Instead of $CaCO_3$ we used $NaCO_3$ due to logistic problems.
Variation stronger than 1% were made with Na_2MoO_4.

Azotobacter medium

	A	B	C	D	E	F
1	1. Preparation					
2	1.1	All chemicals are available				
3	2. Glucose-mannitol solution (GMS)					
4	Component	amount	unit	min	max	
5	Glucose	5	g	4.95	5.05	
6				X		
7	Mannitol	5	g	4.95	5.05	
8				X		
9	Deltilled Water	50	ml	48.50	50.50	
10				X		
11	2.2	Sterilization				
12	3. Medium					
13	Component	amount	unit	amount to weight		
14	**CaCl2**	75.49	mg	74.74	76.25	
15				X		
16	**MgSO4**	48.84	mg	48.35	49.32	
17				X		
18	Na2MoO4	4.26	mg	4.21	4.31	
19				4.23 mg		
20	**K2HPO4**	900	mg	891	909	
21				X		
22	**KH2PO4**	100	mg	99	101	
23				X		
24	**FeSO4 x 7 H2O**	10	g	9.9	10.1	
25				X		
26	NaCO3	4.15	g	4.1	4.2	
27				X		
28	Agar	15	g	14.85	15.15	
29				X		
30	Distilled water	950	ml	940.5	959.5	
31				X		
32	3.1	Label (Burks medai for agar platesm, lot)		Burks media for agar plates		
33						
34	3.2	Autoclavation				

35	3.3	add GMS (Step 2)			
36	3.4	Further use	Storage	pour plates (3.5)	
37					
38	3.5	Pour plates			
39		the CaCo2 will settle in plates before the agar has setm producing an opaque layer in the bottom			
40	3.6	Label plates (Burk, lot)			

Azotobacter medium (liquid)

	A	B	C	D	E	F
1	1. Preparation					
2	1.1	All chemicals are available				
3	2. Glucose-mannitol solution (GMS)					
4	Component	amount	unit	min	max	
5	Glucose	5	g	4.95	5.05	
6				X		
7	Mannitol	5	g	4.95	5.05	
8				X		
9	Deltilled Water	50	ml	48.50	50.50	
10				X		
11	2.2	Sterilization				
12	3. Medium					
13	Component	amount	unit	amount to weight		
14	**CaCl2**	75.49	mg	74.74	76.25	
15				X		
16	**MgSO4**	48.84	mg	48.35	49.32	
17				X		
18	Na2MoO4	4.26	mg	4.21	4.31	
19				4.9 mg		
20	**K2HPO4**	900	mg	891	909	
21				X		
22	**KH2PO4**	100	mg	99	101	
23				X		
24	**FeSO4 x 7 H2O**	10	g	9.9	10.1	
25				X		
26	NaCO3	4.15	g	4.1	4.2	
27				X		
28	Agar	15	g	14.85	15.15	
29				X		
30	Distilled water	950	ml	940.5	959.5	
31				X		
32	3.1	Label (Burks medai for agar platesm, lot)				
33						
34	3.2	Autoclavation				

35	3.3	add GMS (Step 2)			
36	3.4	Further use	Storage	pour plates (3.5)	
37					
38	3.5	Pour plates			
39		the CaCo2 will settle in plates before the agar has setm producing an opaque layer in the bottom			
40	3.6	Label plates (Burk, lot)			

THURSDAY, 21/4/2022

Members: Jona, Olli

Advisor: Moritz

Tasks: resetting up the media: *A. vinelandii* media and *A. vinelandii* media liquid

Set up amounts for 1000mL, weighted with 1% deviation. 500mL for each media. Agar was just added to one of them. Glucose-Mannitol Soultion is ready from 19.04.22.

Azotobacter vinelandii media 1000mL

	A	B	C	D	E
1	Component	amount	unit	amount to weight	
2	**$CaCl_2$**	100	mg	99	101
3				X	
4	**$MgSO_4$**	100	mg	99	101
5				X	
6	Na_2MoO_4	5	mg	4.95	5.05
7				X	
8	**$K_2HPO_4 \times 3H_2O$**	1.18	g	1.168	1.192
9				X	
10	**KH_2PO_4**	100	mg	99	101
11				X	
12	**$FeSO_4 \times 7 H_2O$**	20	mg	19.8	20.2
13				X	
14	$CaCO_3$	5	g	4.95	5.05
15				X	
16	Agar	7.5	g	7.425	7.575
17				X	
18	Distilled water	950	ml	940.5	959.5
19				X	

After setting up the media, the pH was adjusted to 7.307 by adding 10% HCl.
Change in Volume negligible.

TUESDAY, 26/4/2022

Preparation of Medium III and LB plates and cultivation of A. vinelandii, distribution transformation

Introduction

Members: Alexa, Florian Advisor: Vincent

Materials

- Microwave
- Chloramphenicol
- Agar plates
- sterile work bench
- prepared Medium III + agar and LB agar medium
- Glucose-Mannitol-Solution (GMS)
- Chloramphenicol
- iGEM distribution kit plate 1
- *E. coli* cells

Procedure

- Preparation of AV medium plates

1. The AV+agar medium was liquified in the microwave (approx 3 minutes, 100% power with shaking and tempertaure controls). Then 25 mL GMS was added and the plates were poured under the sterile work bench. 20 plates were poured.

- Preparation of LB-medium plates

1. The agar medium was liquified in the microwave. Then the 0.5 mL chloramphenciol was added and the plates were poured under the sterile work bench. 16 plates were poured.

- Transformation of *E. coli* with distribution constructs

1. Three plasmids from the wells 1A, 2A, 3A from the iGEM distrubution kit were treated as in the following instruction for DNA Kit Plate Instructions:

- *Note: There is an estimated 2-3ng of DNA in each well. When following this protocol, assume that you are transforming with 200-300pg/µL*
- •With a pipette tip, punch a hole through the foil cover into the corresponding well of the part that you want. Make sure you have properly oriented the plate. Do not remove the foil cover, as it could lead to cross contamination between the wells.
- •Pipette 10µL of dH2O (distilled water) into the well. Pipette up and down a few times and let sit for 5 minutes to make sure the dried DNA is fully resuspended. The resuspension will be **red**, as the dried DNA has cresol red dye. We recommend that you do not use TE to resuspend the dried DNA.
- •Transform 1µL of the resuspended DNA into your desired competent cells, plate your transformation with the appropriate antibiotic* and grow overnight.
- •Pick a single colony and inoculate broth (again, with the correct antibiotic) and grow for 16 hours.
- •Use the resulting culture to miniprep the DNA AND make your own glycerol stock (for further instruction on making a glycerol see this page). We recommend using the miniprepped DNA to run QC tests, such as restriction digests and sequencing.

1. *E. coli* was heat shocked according to the protocol from Michaela Gerards, and plated out on LB-agar plates with chloramphenicol, and the plasmids were transferred. Plates were incubated at 37°C 1d.

- Inoculation of *Azotobacter vinelandii*

1. The dried culture of *Azotobacter vinelandii* strain DSM 576 from the DSMZ was opened, rehydrated and inoculated according to the delivered protocol. Half of the culture was incubated in a falcon tube with liquid AV medium, the other half was plated out on AV+agar plates. Both cultures were incubated at 30°C (liquid culture: 100rpm) 1d.

WEDNESDAY, 27/4/2022

Members: Florian, Max

Advisor: Vincent

Task: Cultivation of transformed E. coli, distribution transformationCultivation of transformed E. coli, distribution transformation

Materials

- Agar plates containing transformed *E. coli*
- Liquid LB-Medium
- Chloramphenicol
- Falcon tubes

Procedure

- Inoculation of transformed *E. coli*

1. Filling the Falcon tubes with 5ml liquid LB-medium each. Getting a probe of the colony by sticking the pipette tip into the colony. Adding 5µl Chloramphenicol to each culture.
2. Cultures were incubated at 30°C 1d.

- Transformation of *E. coli* with distribution constructs

1. The isolated and suspended DNA from the iGEM distribution kit 2021 (A1-A3) were used. We transformed 5µL of the resuspended DNA into our desired competent cells.
2. *E. coli* was heat shocked according to the protocol from Michaela Gerards, and plated out on LB-agar plates with chloramphenicol, and the plasmids were transferred. Plates were incubated at 37°C 1d.

THURSDAY, 28/4/2022

Member:

Advisor:

Task: MiniPrep of the Plasmids cloned in E.coli

Materials

- Liquid culture of transformed E.coli
- PureYield™ Plasmid Miniprep System (New England Biolabs)
- Pipetts
- Sterile workbench
- Nulease-free Water

Procedure

- Miniprep

1. 800µL of nuclease-free water + 100µL cell lyses buffer for 5µL cell culture each.
2. 350µL Neutralization Solution cooled in the fridge.
3. adding 2x 600 µL supernatant transfered in a microcentrifuge and centrifuged.
4. 200 µL Endotoxin removal wash.
5. 400 µL column wash solution, washed two times.
6. 30 µL nuclease free water.
7. for detailed procedure see PureYield™ Plasmid Miniprep System (New England Biolabs) protocoel.

Table 1: Measurements of the DNA-concentrations and contaminations of the plasmid DNA.

	piGEM003 (1)	piGEM003 (2)	piGEM001 (3)
concentration [ng/µl]	73.25	64.45	64.35
contamination: A260/A280	1.8	1.826	1.852
A260/A230	0.889	1.874	2.103

FRIDAY, 29/4/2022

Introduction

Member: Alexa, Jona (joined for the restriction digestion)

Advisor: Marjorie

Task: Restriction digest: calculating the dilution of the probes and water volume according to protocol

Materials

- Restriction digest
  - restriction enzymes :SpeI, XbaI
  - rCutSmart Buffer
  - mini preped plasmids from 28.04.2022 (Melissa and Max)
- Equipment for Gelelectrophoresis (GEP)
  - Agarose
  - GEP chamber and power supply
  - Gel loading dye purple 6X
  - 100 bp ladder
  - UVP ChemStudio Plus (Analytic Jena)
- Gel purification
  - QIAGEN (QiaQuick Gel extraction kit 250)
  - Machery Nagel (740609 250 Nucleo Soin Gel+PCR Clean up)

image.png

SpeI Order R0133

XbaI Order R0145

Restriction Enzyme Double Digestion

Steps

1. Set up reaction as follows:

COMPONENT	50 µl REACTION
DNA	1 µg
10X rCutSmart Buffer	5 µl (1X)
SpeI	1.0 µl (10 units)†
XbaI	1.0 µl (20 units)†
Nuclease-free Water	to 50 µl

2. Incubate at 37°C for 5-15 minutes as both enzymes are Time-Saver qualified.

Table1

	A	B	C	D	E
1	piGEM	ng/µl	µg/µl	µl needed to get 1µg	amount of nuclease free H2O
2	(1) 003	73,25	0,07325	13,65	29,35
3	(2)003	64,45	0,06445	15,52	27,48
4	(3)001	64,35	0,06435	15,54	27,46

perform the restriction digest at 20 min 37°C, then heat inactivation for 10 min 80°C

IMPORTANT the volumes of water and plasmid were accidentality exchanged for probe (1) 003 and probe (3) 001. After talking to the advisor it was agreed to go on with experiment, as the exchanged volumes weren't too high and probably wouldn't affect the digestion.

Preparation and performing of the GEP

50 ml 1% agarose gel was poured

all thre samples (60 µl -> 50µl sample + 10 µl Gel loading dye purple 6X), 4 µl 100 bp ladder were applied

Gel ran for 30 min, 85 V 200 mA

Gel was transefrred to UVP ChemStudio Plus (Analytic Jena) and analyzed with UV light

Note: 1 kb ladder would have been better

the band at 2000 bp was cut out with a scalpell and processed with the QIAQuick Kit

the gel fragment didn´t dissolve with the reagents provided in the kit. The supernateant was used for the following purification steps and the final concentration was measured with the IMPLEN NanoPhotomerter

Table2

	A	B
1	**piGEM**	**ng/µl**
2	(1) 003	1,35
3	(2)003	2,15
4	(3)001	1,1

As the concentrations were so low another gel extraction kit from Machery Nacgel was used, which successfully diluted the gel, folling purification steps were also performed.
All plasmids were labeled and stored in -20°C

Table3

	A	B
1	**piGEM**	**ng/µl**
2	(1) 003	6,4
3	(2)003	5,45
4	(3)001	2,25

Conclusion
the gel-dissolving with the QIAQuick didn´t work because one buffer was exchanged so that the components in the box could be used for a PCR Extraction. For further extractions another kit is recommended

TUESDAY, 10/5/2022

Burks cultures

Cultivating Azotobacter Vinelandii

Liquid Culture 1:
5mL Burksmedium according to Patricia C. Dos Santos (https://link.springer.com/protocol/10.1007/978-1-61779-194-9_6)
200 µl Liquid culture from A. Vinelandii from 26.04.2022

Liquid Culture 2:
5mL Burksmedium according to Patricia C. Dos Santos (https://link.springer.com/protocol/10.1007/978-1-61779-194-9_6)
Diffrent colonies from a A. vinelandii agar plate from 26.04.2022 brushed on a pipette tip.
Pipette tip dopped in a falcon tube with the medium.

Agar Plate 1:
Agar plate with the Burks medium according to Patricia C. Dos Santos
30 µl Liquid culture from A. Vinelandii from 26.04.2022 spread on the plate.

Additional actions:

The original plate and liquid culture from the 26.04.2022 were put on the working bench to prevent overgrowth.

THURSDAY, 12/5/2022

Members: Merve & Laura

Advisor: Carroll

Task: setting up Burks medium

A new Burks medium was made with the linked protocol.

Notes: Only half of the amount was made and water (from millipore) has to be added before autoclaving.

- Located at Grossmann lab (downstairs)

- Azotobacter plate and liquid cultures were put in the fridge (4 degrees)

MONDAY, 27/6/2022

Members: /

Advisor: Carroll

Task: Inoculating *A. vinelandii* for preparation of electrocompetent cells

A. vinelandii cultures from a propagation plate were inoculated in 250 ml flasks with 150 ml Burk's Medium .

Two cultures were prepared, with different amounts of inoculant - high and low (arbitrary).

The incubation at 30°C and 250 rpm started at 17:00.

FRIDAY, 8/7/2022

Members: Florian, Olli

Advisor: /

Task: measuring growth curve and taking samples for modeling; making *A. vinelandii* electrocompetent

The OD of the three cultures of *A. vinelandii* inoculated the day before by Alexa were measured at 620nm. From all three cultures were three samples of 1ml each taken for dry cell mass and alginate measurement and frozen at -20C. From each culture was one sample of 1ml taken for sucrose measurement, centrifuget at 15,000rpm for 5mins and the supernatant stored at room temperature.

From the 27.6. culture of *Azotobacter vinelandii* were ODs measured and to reach a cell density equivalent to approx. 0.5 the high inoculation was diluted x12 and the low inoculation diluted x10. Two times 25ml of each inoculation were prepared according to the Eppendorf Multiporator protocol, four times centrifuged at 4,500rpm for 10min and washed with glycerol in between. Aliquots were stored in liquid nitrogen at -70C.

TUESDAY, 12/7/2022

Members: Jasmin, Laura

Advisor: /

Task: Task: measuring growth curve and taking samples for modeling

Process acording to the protocol made by Alexa.

Data was saved in the "modeling tabel"

WEDNESDAY, 13/7/2022

Members: Max, Timo

Advisor: /

Task: measuring growth curve and taking samples for modeling

Process according to the protocol made by Alexa.

Data was saved in the "modeling tabel"

THURSDAY, 14/7/2022

Members: Max, Timo

Advisor: Caroll (Introduction)

Task: Cloning the Alg8-Construct

Primers oiGEM2022_001 and oiGEM2022_006 were diluted to a 100µM solution in their original tubes. They were stored at -20°C.
The alg8 gene block was centrifuged for 5 min at 3000g. By adding 100µL Nuclease free water, a 10ng/µL solution was created. After a briefly vortexing it was incubated at 39°C for 20 minutes.

The araBAD sequence was from an iGEM distribution kit (**BBa_K1321333**).
Retransformation was performed with heat shock in E.coli. They were placed on an agarplate with Chloramphenicol.

The PCR was performed under following conditions:

94°C for 1 min

-

98°C for 10 sec

61°C for 15 sec

68°C for 90sec

(30 cycles)

-

20°C

Table4

	A	B	C	D	E	F	G	H	I	J	K
1	pSB1C3	50 uL	Units		BBa_K1321333	50 uL	Units		alg8	50 uL	Units
2	10 uM oiGEM2022_001	2.5	uL		10 uM oiGEM2022_003	2.5	uL		10 uM oiGEM2022_005	2.5	uL
3	10 uM oiGEM2022_002	2.5	uL		10 uM oiGEM2022_004	2.5	uL		10 uM oiGEM2022_006	2.5	uL
4	Template (64,35 ng/uL)	0.16	uL		Template	1	uL		Template (10 ng/uL)	1	ng
5	Mastermix	25	uL		Mastermix	25	uL		Mastermix	25	uL
6	MQ	19.84	uL		MQ	19	uL		MQ	19	uL
7	Total Volume	50			Total Volume	50			Total Volume	50	

Three products were stored at -20°C in freezerbox 1

1: pSB1C3

2: BBa_K1321333

3: alg8

FRIDAY, 15/7/2022

Members: Olli, Timo, Vallery
Advisor: /
Task: measuring growth curve and taking samples for modelling
Process according to the protocol in drive made by Alexa.
Data was saved in the "modelling table".

Table5

	A	Measurement 1	Measurement 2	Measurement 3	Dilution factor
1	culture 1	0,489	0,501	0,519	8
2	culture 2	0,382	0,337	0,368	8
3	culture 3	0,366	0,365	0,363	8

Tuesday, 7/26/22

Members: Alexa, Melissa
Advisor: /
Task: gelelectrophoresis of the PCR products from 14.7.2022

preparation of 1% agarose gel
-> applying all samples with ladders

for oIGEm2022_0005/0006 and oIGEm2022_00001 1 kb ladder was used

for oIGEm2022_0003/0004 100 bp ladder was used

gel was loaded:

empty-1kb ladder-sample1-sample3-empty-100bp ladder-sample2-empty...

sample 1 pSB1C3 oIGEM2022_01/02 2110 bp
sample 2 BBa_K1321333 oIGEM2022_03/04 210 bp
sample 3 alg8 oIGEM2022_05/06 1520 bp

gel was run at 85V 200 mA for 20 minutes

MONDAY, 15/8/2022

Members: Max
Advisor:

Task: Cloning the Alg8-Construct

The PCR was performed under following conditions:

94°C for 1 min

-

98°C for 10 sec

61°C for 15 sec

68°C for 90sec

(32 cycles)

-

20°C

PCR alg8 blocks

	A	B	C	D	E	F	G	H	I	J	K
1	pSB1C3	50 uL	Units		BBa_K1321333	50 uL	Units		alg8	50 uL	Units
2	10 uM oiGEM2022_001	2.5	uL		10 uM oiGEM2022_003	2.5	uL		10 uM oiGEM2022_005	2.5	uL
3	10 uM oiGEM2022_002	2.5	uL		10 uM oiGEM2022_004	2.5	uL		10 uM oiGEM2022_006	2.5	uL
4	Template (64,35 ng/uL)	0.16	uL		Template	1	uL		Template (10 ng/uL)	1	uL
5	Mastermix	25	uL		Mastermix	25	-		Mastermix	25	uL
6	MQ	19.84	uL		MQ	19	uL		MQ	19	uL
7	Total Volume	50			Total Volume	50			Total Volume	50	

The first PCR strip was not pippeted correctly (more than 50 uL in each tube), so a second PCR was additonaly performed.

1. PCR2.1 - too much volume
2. PCR2.2 - green

The second PCR strip is green to differenciate them.

mastermix:

https://www.takarabio.com/assets/a/207545

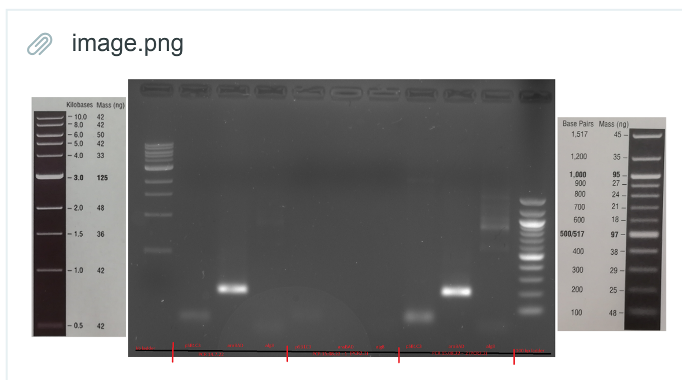
Electrophoreses was performed under following conditions:

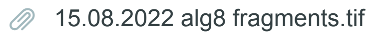
-1% Agarose

-200 ma

-100 V

image.png

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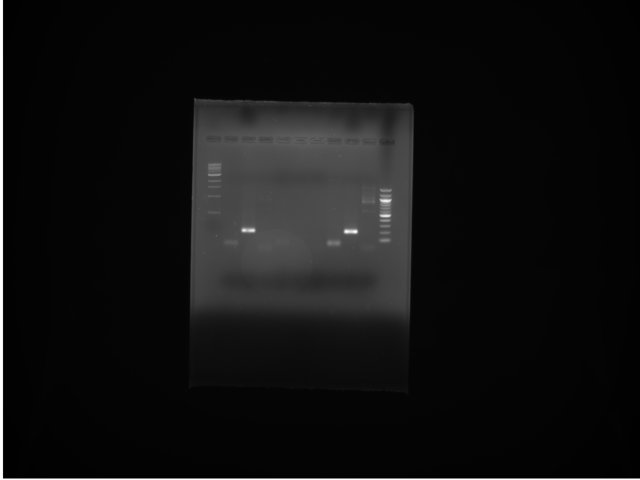
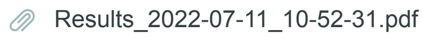



Results_2022-07-11_10-52-31.pdf

TUESDAY, 16/8/2022

Members: Merve and Laura

Advisor: Carroll and Vincent

Task: Amplification of the Alg8-Construct

Gradient PCR was formed under following conditions

94° for 1 minute

98° 10 seconds

56-61° 15 seconds

68° 1:30 min

68° 5 min

(30 cycles)

22°

Table6

	A	B	C	D	E	F	G	H	I	J	K
1	pSB1C3 (2110bp)	50 uL	Units		BBa_K1321333 (210 bp)	50 uL	Units		alg8 (1500bp)	50 uL	Units
2	10 uM oiGEM2022_001 (3.)	2.5	uL		10 uM oiGEM2022_003	2.5	uL		10 uM oiGEM2022_005	2.5	uL
3	10 uM oiGEM2022_002 (2.)	2.5	uL		10 uM oiGEM2022_004	2.5	uL		10 uM oiGEM2022_006	2.5	uL
4	Template (64,35 ng/uL) (4.)	0,25 (0,5/1)	uL		Template	1	uL		Template (10 ng/uL)	2	uL
5	Mastermix (5.)	25	uL		Mastermix	25	uL		Mastermix	25	uL
6	MQ (1.)	19.84	uL		MQ	19	uL		MQ	19	uL
7	Total Volume	50			Total Volume	50			Total Volume	50	

5 Tubes per 50uL Template-mix for gradient PCR
Blocks of Gradient PCR: 56°, 58°, 59.5°, 60.5°, 61°

Electrophoreses was performed under following conditions:

For BBa_K1321333:
-1% Agarose (0,5 g Agarose + 50ml TEA)
-200 ma
-100 V

For pSB1C3 and alg8
-1,5% Agarose (0,75 g Agarose + 50ml TEA)
-200 ma
-100 V

side note: we used 2uL Ladder, Michalea says we should use at least 5uL

both 15 min.

29AEE68E-527A-43E9-ADE1-F4558BDE24EF.jpeg

PCR didn´t work

WEDNESDAY, 17/8/2022

Members: Florian and Jona

Advisor: Carroll

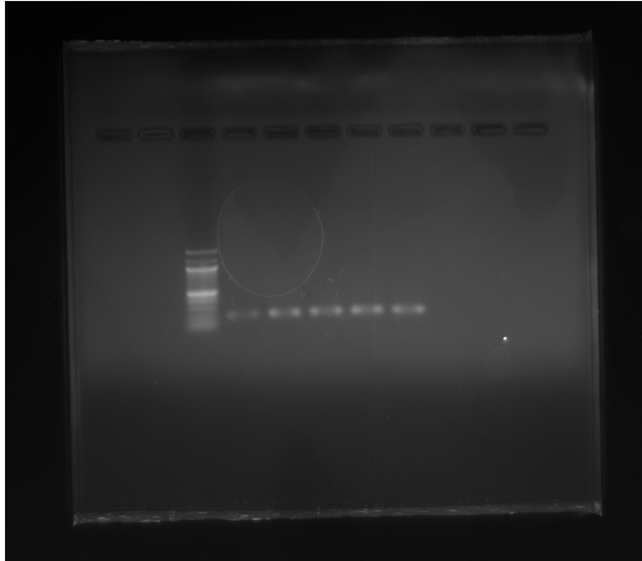
Task: amplification of the alg8-construct parts

Gradient PCR with five tubes of each of the three parts pSB1C3, BBa_K1321333 and alg8 was prepared according to Table6 and performed under the same conditions as on 16th August.

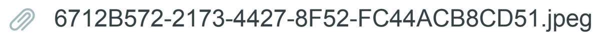
Electrophoreses of the resulting solutions was performed under the same conditions.

5µl ladder and 2µl samples were used for each approach.

A517E25A-C12D-4FDD-98B8-B39BFDF66F3A.jpeg



Amplification of the araBAD promotor (BBa_K1321333) worked and a clear band at a little over 200bp was observed.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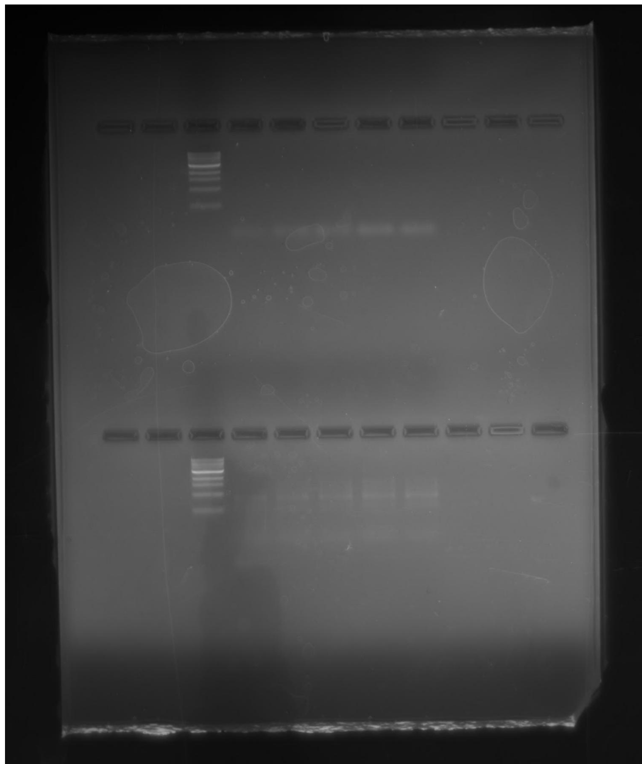


Amplification of the pSB1C3 failed, only small fragments were obtained. Amplification of alg8 partially worked, but only a smear at around 1.5kbp was obtained.

TUESDAY, 23/8/2022

Members: Merve and Yasemin

Task: Amplification the Alg8-Construct + Alliquots of the Cellulose Primers

Gradient PCR was formed under following conditions
94° for 1 minute
98° 10 seconds
56-61° 15 seconds
68° 2 min
68° 5 min
(30 cycles)

22°

Table7

	A	B	C
1	alg8 (1500bp)	50 uL	Units
2	10 uM oiGEM2022_005	2.5	uL
3	10 uM oiGEM2022_006	2.5	uL
4	Template (10 ng/uL)	2	uL
5	Mastermix MgCl2 5mM	25	uL
6	MQ	19	uL
7	Total Volume	50	

The Concentration of $MgCl_2$ was raised from 2mM to 5mM in an Eppi and was stored at -20° C

5 Tubes per 50uL Template-mix for gradient PCR:
Blocks of Gradient PCR: 56°, 58°, 59.5°, 60.5°, 61°

Electrophoresis was performed under following conditions:

-1,5% Agarose (0,75 g Agarose + 50ml TEA)
-Roti GelStain 2,5uL
-200 ma
-100 V

Ladder:
1 Kb 10uL
40 min.

The gel electrophoresis showed no results at all.

Primers oiGem2022_13 - oiGem 2022_20 were diluted to a 100 uM solution in their original tubes. They were stored at -20°C.

WEDNESDAY, 24/8/2022

Members: Olli, Robin, Merve
Task: Retransformtion of Cellulose Parts + Amplification of the Alg8- Construct

Gradient PCR with five tubes of each of alg8 was prepared according to Table7 and performed under the same conditions as on 23rd August.
Electrophoreses of the resulting solutions was performed under the same conditions but with a 5ul ladder.

1. The competent E.coli cells were left on ice to defrost
2. The Distribution Kit from 2019 was used. 10 ul of dH20 were added into the well (17L and 17 N)and up and down pippeted. The DNA was left to rest for 5 minutes.
3. 1ul of DNA was added to the cells on ice and then incubated for 30 min on the ice
4. The cells were heat shocked for 45 sec with 42°.
5. It was left to chill on ice for 2 minutes.
6. 0,75 ml LB medium was added and then incubated for 1 hour at 37°
7. The cells were collected by centrifuging for 1 min at 13000 rpm.
8. The supernatant was removed an the resuspended cell in the remaining medium was pipetted up an down.
9. 10 ul of the cells were plated on a plate with chloramphenicol and incubated over night at 37°.

THURSDAY, 25/8/2022

Members: Melissa, Timo
Task: Setting up LB Medium

Prepared LB Medium for plates and put it in the autoclave.

Table8

	A	B	C
1	Component	amount	unit
2	Water	400	mL
3	LB medium	8	mg
4	Agar	6	mg

FRIDAY, 26/8/2022

Members: Robin, Melissa

Task: pouring plates and repeat retransformation of cellulose parts

Poured plates with LB Medium and chloramphenicol (only plates which are marked contain chloramphenicol).

Retransformation of cellulose parts:

1. Defrosted the competent *E.coli* on ice.
2. Pre-chilled two Eppendorf tubes and added 20 µL of cells in each tube.
3. The Distribution Kit from 2019 was used. 10 ul of dH20 were added into the well (17L and 17 N)and up and down pippeted. The DNA was left to rest for 5 minutes.
4. Added 0.5 µL DNA to the cells and incubated it for 30 min. on ice.
5. The cells were heatshocked for 45 sec. with 42°C.
6. It was left to chill on ice for 2 min.
7. 1 mL LB Medium was added and incubated for 1 h at 37°C.
8. The cells were collected by centrifuging them for 1 min. at 13000 rpm.
9. The supernatant was removed and the the cells were resuspended in the remaining medium by carefully pipetting up and down.
10. 5 µL of the cells were plated on each plate (we prepared two plates which contain chloramohenicol and two plates without any antibiotic).
11. The plates were incubated over night at 37°C.

THURSDAY, 1/9/2022

Members: Olli, Max

Task: PCR alg8 with corrected Primer and PCR alg8 without overhangs

Table9

	A	B	C	D	E	F	G	H
1	Construct:	alg8 no overhangs	1479 bp	tm		94°C	60 s	
2						98°C	10 s	
3	primer names	oIGEM2022_025	ATGGACAGGCTTAAGCATGCTCTT	61°		61°C	15 s	32 cycles
4		oIGEM2022_026	TCAGACCGACAGCATCAGCAC	61°		68°C	50 s	
5								
6								
7	Construct:	alg8	1520 bp			94°C	60 s	
8						98°C	10 s	
9	primer names	oIGEM2022_005	CTCCATAAAGAGGAGAAAAAGCTTATGGACAGGCTTAAGCATGC	55°		61°C	15 s	32 cycles
10		oIGEM2022_006_corr	TCAGTGATGGTGATGGTGATGGACCGACAGCATCAGCACAG	59°		68°C	50 s	
11								
12								

Material: 2.5 ul primer (10mM)
1ul template (alg8, ng/ul)

Gelelectrophoresis was performed under following conditions:
-85V
-1,5% Agarose (0,75 g Agarose + 50ml TEA)
-Roti GelStain 2,5uL
-1kb Ladder 10uL
-Wells filled with 10uL DNA

Notes:
New freezer box with all PCR stripes. (Orange Freezerbox2- PCR)

TUESDAY, 6/9/2022

Members: Jasmin, Laura
Task: gradient PCR of alg8 without overhangs and positve control

Table10

	A	B	C
1	alg8	50 uL	Units
2	10 uM oiGEM2022_025	2.5	uL
3	10 uM oiGEM2022_026	2.5	uL
4	Template (10 ng/uL)	1	uL
5	Mastermix (q5 polymerase)	12,5	uL
6	MQ	32,5	uL
7	Total Volume	50	

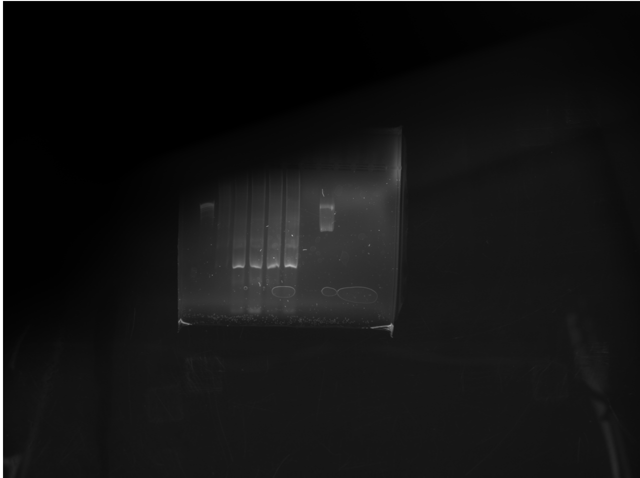
5 Tubes per 10uL Template-mix for gradient PCR + positive control:
Blocks of Gradient PCR: 66°,67°, 68°, 69°, 70°

1:00 98°
0:15 98°
0:15
0:50 72°
5:00 72°
20°

Gelelectrophoresis was performed under following conditions:
-100V
-1,5% Agarose (0,75 g Agarose + 50ml TEA)
-Roti GelStain 2,5uL
-1kb Ladder 5uL
-Wells filled with 10uL DNA

Picture is on our USB-Stick

PCR alg 8 no overhangs_IGEM_06.09.22.tif



TUESDAY, 13/9/2022

Members: Merve, Timo

Retransformation of cellulose parts:

1. Defrosted the competent *E.coli* on ice.
2. Pre-chilled two Eppendorf tubes and added 20 µL of cells in each tube.
3. The Distribution Kit from 2021(? unclear from which Distribution kit it was taken from) was used. 10 ul of dH20 were added into the well (17L and 17 N)and up and down pippeted. The DNA was left to rest for 5 minutes.
4. Added 0.5 µL DNA to the cells, vortexed it and incubated it for 30 min. on ice.
5. The cells were heatshocked for 45 sec. with 42°C.
6. It was left to chill on ice for 2 min.
7. 1 mL LB Medium was added and incubated for 1 h on the shaker at 700 RPM at 37°C.
8. The cells were collected by centrifuging them for 1 min. at 13000 rpm.
9. The supernatant was removed and the the cells were resuspended in the remaining medium by carefully pipetting up and down.
10. 5 µL of the cells were plated on each plate (we prepared two plates which contain chloramphenicol and two plates without any antibiotic).
11. The plates were incubated over night at 37°C.

WEDNESDAY, 14/9/2022

Members: Melissa, Laura
Task: Pouring plates and Gelelectrophoresis

8 plates with Chloramphenicol and LB Media
stored at freezer room

Gelelectrophoresis

70% Gel

AcsAB, AcsCD, alg8

200 mA

100 V

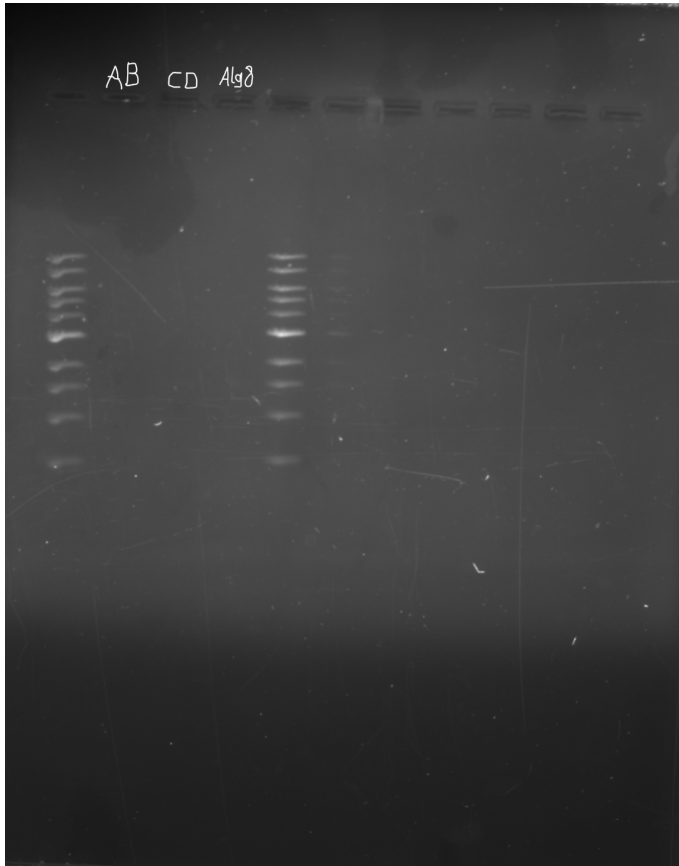
40 min

1kb ladder

AcsAB and AcsCD were used from the Distribution kit from 2019, 4 uL of each stored (freezerbox 1)

Result Gel-Electrophoresis:

Gel Alg8 and cellulose parts 14.09.22 (2).tif



The Gel-Electrophoresis did not work and only the ladders were visible.

TUESDAY, 20/9/2022

Member: Yassy, Robin, Timo

100 mL Methanol and 2g of SodiumSulfate for the sucrose measurement were brought from the Zurbriggen lab (Debbie). They are on the left side of the lab bench in the Grossmann lab labeled with whats in them.
Freezer box 1 60-62 Cellulose parts, 63 competent *E. coli*

WEDNESDAY, 21/9/2022

Member: Yassy, Robin

Robin/ Modelling:

Abcam Sucrose Assay Kit: Used for measurement of sucrose levels in A.vinelandii

1. The samples had been diluted (100.000x) with superfiltred water -> (2x 100:1 + 1x 10:1) because the sucrose levels of pur samples were to high for the measurement.
2. Standard curve with different dillution Levels were prepared (0/2/4/6/8/10 as ist Stands in the Protocol)
3. Because the Kit run low on his contents only the Standard curve of the sucrose was setted and measured. The diluted samples were stored in the freezer for a next run.
4. Result:

210922.xlsx

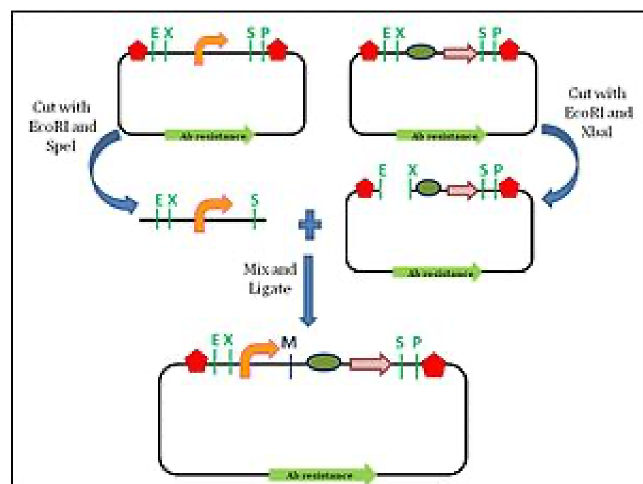
Yassy/ PCR:

Working on BBa_K1321336 we need to assemble Promotor BBa_K1321333 and BBa_K1321334

BBa_K1321333 from the distrubiution Kit 2021 D21 with 10ul of water --> freezer Box 1

BioBrick Cloning

image.png



To Do/ Workflow:

http://parts.igem.org/wiki/index.php?title=Part:BBa_K1321334

Step 1: measure DNA Concentration of BBa_K1321333 and BBa_K1321334

--> DNA concentration can be determined by **measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer using a quartz cuvette**.

Step 2: measur 1 ug minimum material Check if there is enough

Step 3.1: If there is not enough than replicade BBa_K1321333 and BBa_K1321334 look into a protocol --> PCR

Step 3.2: If there is enough than digest BBa_K1321334 with Xba1 and Pst1 1ug
and digest BBa_K1321333 with Spe1 and Pst1

Step 4: run both on a gel --> Seperation
Check the size BBa_K1321333 3280bp and BBa_K1321334 2070 bp (backbone) and 4684 bp --> keep always the largest one for both
Step 5: gel purification and quantifie the product
Step 6: Ligation (1:1) NEBio Calculator because of the diffrent sizes Vector 50ng BBa_K1321333 and 71,40 ng BBa_K1321334 insert --> https://nebiocalculator.neb.com/#!/ligation
Step 7: transfer into E. Coli and put it on a plate with Chloramphinicol

THURSDAY, 22/9/2022

Member: Florian,Robin

Retransformation **BBa_K1321335 and BBa_K1321334**

TUESDAY, 27/9/2022

Task: alg8 PCR
Members: Merve, Max
Advisor: /
Since the alg8 PCRs always showed a smear, we will try to amplify them in 2 smaller parts and assemble them later during the Gibson assembly in the vector. We will try the PCR with different combination of primers.

Table11

	A	B	C	D	E
1	Template	Primer 1	Primer 2	Produktlänge	Produkt
2	araBAD with alg8-overhang	oiGEM2022_029	oiGEM2022_030	166	araBAD without overhangs
3	alg8	oiGEM2022_05 corr	oiGEM2022_028	913	alg8_1_with araBAD + RBS-overhang
4	alg8	oiGEM2022_025	oiGEM2022_028	889	alg8_1 without overhang to araBAD
5	alg8	oiGEM2022_06 corr	oiGEM2022_027	638	alg8_2 with HisTag + pSB1C3-overhang
6	alg8	oiGEM2022_026	oiGEM2022_027	620	alg8_2 without overhang to vector

PCR:
Polymerase: Q5 (NEB)

image.png

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Q5 Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	< 1,000 ng
Q5 High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
5X Q5 High GC Enhancer (optional)	(5 µl)	(10 µl)	(1X)
Nuclease-Free Water	to 25 µl	to 50 µl	

https://www.neb.com/protocols/2012/12/07/protocol-for-q5-high-fidelity-2x-master-mix-m0492
https://international.neb.com/protocols/2012/12/07/protocol-for-q5-high-fidelity-2x-master-mix-m0492

2uL of tempate

tm for alg8: 65°C
tm for araBAD: 61.5 °C
10 seconds for araBAD
30 seconds for alg8

image.png

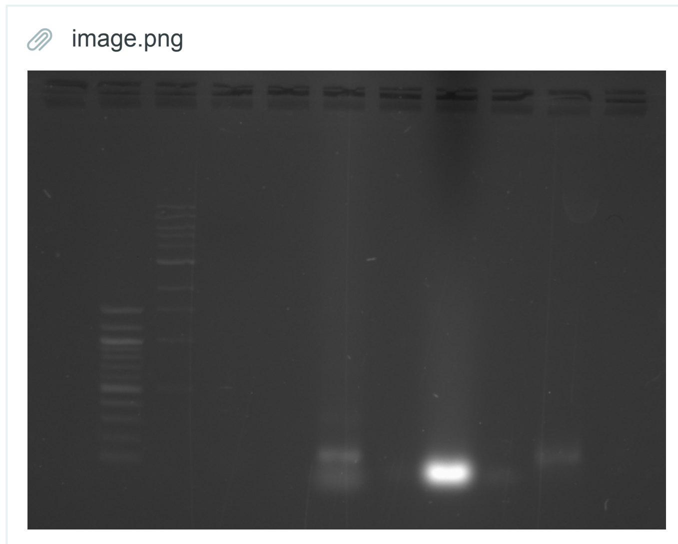
STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	*50–72°C	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

The gel electrophoresis was performed under following conditions:

Agarose content: 1%
Voltage: 100V
Ladder: 1kb / 100bp

Roti GelStain 2,5uL
Wells filled with 36 uL PCR-Product
Gel loading:

image.png



Conclusion:
probally forgot to add the water
PCR not clear. result has to be discussed, but we definitly have to repeat

acsCD liquid cultures
5 ml LB medium
1.75 uL ChlA (25mg/ml)
one colony each

WEDNESDAY, 28/9/2022

Members: Robin and Merve
Task: Miniprep of AcsCD + Retransformation of AcsAB

MiniPrep was performed according to the Protocol of Promega:

DNA Purification by Centrifugation

Prepare Lysate
1. Add 600µl of bacterial culture to a 1.5ml microcentrifuge tube.
Note: For higher yields and purity use the alternative protocol below to harvest and process up to 3ml of bacterial culture.
2. Add 100µl of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.
3. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.

4. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
5. Transfer the supernatant (~900µl) to a PureYieldTM Minicolumn without disturbing the cell debris pellet.
6. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.
7. Discard the flowthrough, and place the minicolumn into the same Collection Tube.

Wash
8. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds.
9. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.

Elute
10. Transfer the minicolumn to a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
11. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

Retransformation was performed under the following conditions:

Retrafo: 1ul
Heat shock: 60 sec 42 C
Incubation: 1h 15 min, 37C

FRIDAY, 30/9/2022

Task: alg8 PCR, alginate-modelling measurements, new measurement culture
Member: Alexa, Max
Advisor:

Table12

	A	B	C	D	E	F	G
1	Nr	Template	Primer 1	Primer 2	Product length	Product name	PCR strip
2	1	alg8	5	28	913	alg8_1_mit araBAD + RBS Überhang	2
3	2	alg8	25	28	889	alg8_1 ohne Überhang	2
4	3	alg8	6 corr	27	638	alg8_2 mit HisTag + pSB1C3 Überhang	2
5	4	alg8	26	27	620	alg8_2 ohne Überhänge	2
6	5	alg8	31	28	889	alg8_1 ohne Überhang	2
7	6	alg8	27	32	620	alg8_2 ohne Überhänge	2
8	7	alg8	31	32	1479	alg8 (komplett)	1
9							

Conditions:

- strip 1: tm = 62, time = 42
- strip 2: tm = 62.5, time = 30

image.png

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C *50–72°C 72°C	5–10 seconds 10–30 seconds 20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

image.png

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X Q5 Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	variable	variable	< 1,000 ng
Q5 High-Fidelity DNA Polymerase	0.25 µl	0.5 µl	0.02 U/µl
5X Q5 High GC Enhancer (optional)	(5 µl)	(10 µl)	(1X)
Nuclease-Free Water	to 25 µl	to 50 µl	

Measurements:

created stock solutions according to the Carbohydrate chapter from the protocol: https://www.mdpi.com/2227-9717/6/5/38/htm

2% sodium sulfate solution: 2 g Sodiumsulfate filled up to 100 ml

stored in our bench

75% sulfuric acid: (stock solution from the lab might be outdated, but experiment seemed to have worked)

118 ml 95 % sulfuric acid + 42 ml water

stored in acid cupboard

1 mg/ml glucosestandard 2 mg glucose + 2 ml water → heat up shortly in microwave to completely dissolve

used standars: 250, 200,150,100, 50, 10 µg/ml

stored in our bench (15 ml Falcon-tube)

Methanol is in the drawer under the laboratory fume hood (Abzug) behind our bench

Ethanol is in the cupboard for explosive goods

Anthrone is in the cupboard/drawer for hazardous chemicals, box A-C

NOTE: Anthrone is not hazardous, is just stored there because our lab has no safety-form for this chemical, an storing it with the hazardous chemicals is the easiest way to store it in the lab without the form

step with water bath: 2 beakers (which can hold all glasstubes) were filled with water and then microwaved till the water boiled. glasstube were put in the cooking water. After approx. 5 min the tubes were transferred into the other beaker with freh boiled up water, because the first one got to cold.

for next time follow the total carbohydrate measurment steps

for further storage information of the chmicals see the paper in the lab or ask Michaela

results:

the standard curve was not measurable on the implen, as the referece Anthrone solution was yellow, an the standards were blue → no reasonable measurement could be done

measured probes OD 625

15.7 cult. 2 - 0.162

17.7 cult. 3- 0.040

14.7 cult. 1 - 0.036

14.7 cult. 3 - 0.119

13.7 cult. 1 -

13.7 cult. 2 - 0.015

11.7 cult. 1 - 0.047

11.7 cult. 3 - 0.025

Experiment that has to be done: new measurment of the standard curve

1.new anthrone mix:

my suggestion for the volume: 30 mg anthrone + 750 µL fresh absolute ethanol (anthrone will partially dissolve), add 75% sulfuric acid (under the dedection, with protection goggles :)) to a final volume of 15 mL,

- mix via inverting the tube
- store in the cold (ice, freezer, don´t worry it won´t become solid)

when you use the mix it is kinda viskose, so make sure to pipette it slowly and carefully. Also there will be some "fragments" in the solution, which seems to be normal, as the protocol has a hint, that you don´t have to mix again.

2. make a new glucose standard solution 1 mg/ml

my suggestion 2 mg glucose + 2 ml water → in 15 ml falcon tube

- you have to quickly heat the solution up in the microwave (start with 5 s or so, when the solution is clear everything is soluted)
- I used a small beaker to keep the falcon vertical, as i was not sure if the falcon racks can stand the microwave

dilute the standard solution to 5-6 samples with anthrone mix to concentrations between 250 µg/ml and 10 µg/ml (my suggestion 250, 200, 150, 100, 50, 10 µg/ml)

if the standars look yellow-greenish measure the probes and blank with anthrone mixl :)

if the standards turn blue again you don´t have to go on, no reasonable values will be measured
→ then maybe the water in the standard solution is the problem, then you can try the following:

2 mg glucose + 2 ml anthrone solution

(I am not sure if you have to microwave this one. If it seems that the glucose is completly dissolved you don´t have to microwave it.

If the glucose isn´t dissolved, better talk to Michaela before microwaving to make sure there will be no reaction between the plastic and the sulfate acid, and to make sure if its safe to heat up acid. If this is not possible it´s ok)