MAY

THURSDAY 20/05/2021

Today's Team: Justus Arndt, Khadija Rombi, Mohammad Nour

I. Preparation of primers

First autoclaved milliQ H_2O added until a concentration of 100 µM (table 200521-1)

Table 200521-1: Dilutions of the primers for storage.

Number	Order Name	H_2O added [µL]
1	iGEM_OleT-BM3R-Fusion_pSB1C3_FWD	363.4
2	iGEM_OleT-BM3R-Fusion_pSB1C3_REV	287.0
3	iGEM_OleT-BM3R-Fusion_OleT_FWD	545.4
4	iGEM_OleT-BM3R-Fusion_OleT_REV	424.0
5	iGEM_OleT-BM3R-Fusion_BM3R _FWD	353.5
6	iGEM_OleT-BM3R-Fusion_BM3R_REV	524.1
7	iGEM_CYP1A1-CPR-Fusion_pSB1C3_FWD	292.2
8	iGEM_CYP1A1-CPR-Fusion_pSB1C3_REV	349.5
9	iGEM_CYP1A1-CPR-FusionRATCYP1A1_FWD	513.2
10	iGEM_CYP1A1-CPR-FusionRATCYP1A1_REV	562.5
11	iGEM_CYP1A1-CPR-Fusion_RAT-CPR_FWD	579.0
12	iGEM_CYP1A1-CPR-Fusion_RAT-CPR_REV	583.0
13	iGEM_OleT+CYP1A1KontrollepSB1C3_FWD	296.1
14	iGEM_OleT+CYP1A1KontrollepSB1C3_REV	340.3
15	iGEM_OleT+CYP1A1-Kontrolle_CYP_FWD	533.0
16	iGEM_OleT+CYP1A1-Kontrolle_CYP_REV	602.2
17	iGEM_OleT+CYP1A1-Kontrolle_CPR_FWD	332.0
18	iGEM_OleT+CYP1A1-Kontrolle_CPR_REV	567.3

The primer solutions were then diluted even further (1:10) for work ready primers and they were stored a -20°C.

Table 202521-2: Overview of planned PCR for specific Template/Primer combinations.

	Template	Primer	Construct
PCR 1	pSB1C3	1+2	**OleT-BM3R Fusion**
PCR 2	OleT	3+4	
PCR 3	BM3R	5+6	
PCR 4	pSB1C3	7+8	**CYP-CPR-Fusion**
PCR 5	CYP1A1	9+10	
PCR 6	CPR	11+12	
PCR 7	pSB1C3	13+14	**OleT-BM3R-Control**
PCR 8	OleT	15+16	
PCR 9	BM3R	17+18	

	Template	Primer	Construct
PCR 10	pSB1C3	13+14	**CYP-CPR-Control**
PCR 11	CYP1A1	15+16	
PCR 12	CPR	17+18	

II. PCR for amplification of OleT (PCR2)

The first PCR for the OleT-Bm3R-Fusion was conducted:

Table 200521-3: Mastermix recipe for PCR 2.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (3)	2.5 µl	0.5 µM
10 µM Reverse Primer (4)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-4.

Table 200521-4: PCR-Program used for the PCR´s 2,3,6.

98 °C	0:00:30
98°C	0:00:10
63°C	0:00:30
72°C	0:00:38
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The expected length of the PCR product was ~1355 bp and the gel electrophoresis (figure 1) showed that the PCR amplified correctly.

III. PCR for amplification of BM3R (PCR3)

The PCR for the OleT-Bm3R-Fusion was conducted:

Table 200521-5: Mastermix recipe for PCR 3.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (5)	2.5 µl	0.5 µM
10 µM Reverse Primer (6)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-4.

The expected length of the PCR product was ~ 1932 bp, the gel (figure 1) however showed no amplicons, thus the PCR was repeated.

IV. PCR for amplification of CPR (PCR6)

The PCR for the CYP1A1-CPR-Fusion was conducted:

Table 200521-6: Mastermix recipe for PCR 6.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (11)	2.5 µl	0.5 µM
10 µM Reverse Primer (12)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-4.

The expected length of the product was ~2178 bp, the gel (figure 1) however showed no amplicons, thus the PCR was repeated.

V. PCR for amplification of OleT (PCR 8)

The PCR for the OleT-Bm3R-Control was conducted:

Table 200521-7: Mastermix recipe for PCR 8.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (15)	2.5 µl	0.5 µM
10 µM Reverse Primer (16)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-8.

Table 200521-8: PCR-Program used for the PCR´s 8,9,11.

98 °C	0:00:30
98°C	0:00:10
61°C	0:00:30
72°C	0:00:35
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The expected length of the PCR product was ~1465 bp, the gel (figure 1) however showed no amplicons, thus the PCR was repeated.

VI. PCR for amplification of BM3R (PCR 9)

The PCR for the OleT-Bm3R-Control(PCR 9) was conducted:

Table 200521-9: Mastermix recipe for PCR 9.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (17)	2.5 µl	0.5 µM
10 µM Reverse Primer (18)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-8.

The expected length of the PCR product was ~ 1986 bp, the gel (figure 1) however showed no amplicons, thus the PCR was repeated.

VII. PCR for amplification of CYP1A1 (PCR 11)

The PCR for the CYP1A1-CPR-Fusion was conducted:

Table 200521-7: Mastermix recipe for PCR 11.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (15)	2.5 µl	0.5 µM
10 µM Reverse Primer (16)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

Then IDT OleT was added, and the OCR was conducted with the settings seen in table 200521-8.

The expected length of the PCR product was ~1776 bp, the gel (figure 1) however showed a potential low concentration, thus the concentration will be measured.

VIII. Purification of PCR-Products

The PCR products were all purified using Monarch PCR 4 DNA Cleanup Kit. 4 volumes of Ethanol were added, and the instructions were followed as provided. A fifth step of respinning for 30 seconds was added and in the sixth step H_2O was used 50°C, 20 µL

PCR-Products of PCR 2, 3, 8, 9, 11: bb: 2:1; 100:50 µL

FRIDAY 21/05/21

Today's Team: Ben Bimberg, Justus Arndt, Khadija Rombi

I. Preparation of backbone pSB1C3

The pSB1C3 backbone was diluted 1:10 and the concentration was quantified via Nanodrop.

Table 210520-1: Quantification of the plasmid concentration of pSB1C3.

Concentration	260/230	260/280
21.8 ng/µL	2.23	1.95

II. PCR for amplification of pSB1C3 (PCR 1)

The PCR for the OleT-Bm3R-Fusion was conducted.

Table 210520-2: Mastermix recipe for PCR 1.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (1)	2.5 µl	0.5 µM
10 µM Reverse Primer (2)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	18.8 µL	

30 ng worth of plasmid was added, and the PCR was conducted with the settings seen in table 210520-3.

Table 210520-3: PCR-Program used for the PCR´s 1,4,5.

98 °C	0:00:30
98°C	0:00:10
68°C	0:00:15
72°C	0:00:38
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The gel (figure 1) however showed no amplicons, thus the PCR was repeated.

III. PCR for amplification of pSB1C3 (PCR 4)

The PCR for the CYP1A1-CPR-Fusion was conducted.

Table 210520-4: Mastermix recipe for PCR 4.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (7)	2.5 µl	0.5 µM
10 µM Reverse Primer (8)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	18.8 µL	

30 ng worth of plasmid was added, and the PCR was conducted with the settings seen in table 210520-3.

The gel (figure 1) however showed no amplicons, thus the PCR was repeated.

IV. PCR for amplification of CYP1A1 (PCR 5)

The PCR was conducted for the CYP1A1-CPR-Fusion.

Table 210520-5: Mastermix recipe for PCR 5.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (09)	2.5 µl	0.5 µM
10 µM Reverse Primer (10)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

30 ng worth of plasmid was added, and the PCR was conducted with the settings seen in table 210520-3.
The gel (figure 1) showed an amplicon, thus the concentration will be measured.

V. PCR for amplification of pSB1C3 (PCR 7)

The PCR was conducted for the OleT-Bm3R-Control.

Table 210520-6: Mastermix recipe for PCR 7.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (13)	2.5 µl	0.5 µM
10 µM Reverse Primer (14)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	18.8 µL	

30 ng worth of plasmid was added, and the PCR was conducted with the settings shown in table 210520-7.

Table 210520-7: PCR-Programm used for the PCR´s 7,10.

98 °C	0:00:30
98°C	0:00:10
71°C	0:00:15
72°C	0:00:40
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The gel (figure 1) however showed no amplicons, thus the PCR was repeated.

VI. PCR for amplification of pSB1C3 (PCR 10)

The PCR was conducted for the CYP1A1-CPR Control.

Table 210520-8: Mastermix recipe for PCR 10.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (13)	2.5 µl	0.5 µM
10 µM Reverse Primer (14)	2.5 µl	0.5 µM

Component	50 µl Reaction	Final Concentration
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	18.8 µL	

30 ng worth of plasmid was added, and the PCR was conducted with the settings shown in table 210520-7.
The gel (figure 1) however showed no amplicons, thus the PCR was repeated.

VII. PCR for amplification of CPR (PCR 12)

The PCR was conducted for the CYP1A1-CPR Control.

Table 210520-9: Mastermix recipe for PCR 12.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (17)	2.5 µl	0.5 µM
10 µM Reverse Primer (18)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (IDT) - 25 ng (Plasmid)
Nuclease-Free Water	19.5 µL	

IDT CPR was added, and the PCR was conducted with the settings seen in table 210520-10.

Table 210520-10: PCR-Programm used for the PCR 12.

98 °C	0:00:30
98°C	0:00:10
61°C	0:00:15
72°C	0:00:45
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The gel (figure 1) however showed no amplicons, thus the PCR was repeated, as the PCR cycler failed at an undefined point, the PCR was redone and the insert was correctly amplified, its concentration will be measured.

VIII. Gel-Electrophoresis of PCR-Products

1 µL of Loading Dye 6x was added to 5 µL of the cleaned-up PCR-Products and the 1% agarose gel was run at 120 V, 400 mA for 70 min. A Quickload purple 1 kb Plus DNA Ladder from NEB as used.

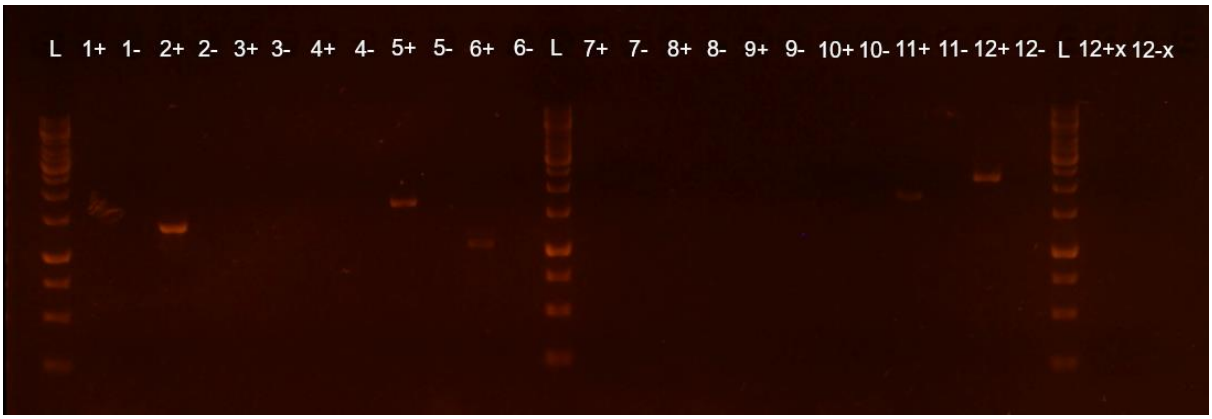

Figure 1: Gel-Electrophoresis of the PCR products in ascending order, each sample followed by a negative control.

TUESDAY 25/05/2021

Today´s Team: Mohammad Nour, Marius Heitmann, Marie Eichholtz

I. Repetition of PCR 1, 4, 7, 10

The PCR´s 1, 4, 7, 10 were repeated with the different backbones MH01 and LA01 (both pSB1C3 from last year) and a negative control without the template. For each PCR 50 µL of backbone was used.

Table 250521-1: Mastermix recipe for PCR 1 with template MH01 and LA01.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (1)	2.5 µl	0.5 µM
10 µM Reverse Primer (2)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-2: Mastermix recipe for PCR 4 with template MH01 and LA01.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (7)	2.5 µl	0.5 µM
10 µM Reverse Primer (8)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-3: Mastermix recipe for PCR 7 with template MH01 and LA01.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (13)	2.5 µl	0.5 µM
10 µM Reverse Primer (14)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-4: Mastermix recipe for PCR 10 with template MH01 and LA01.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (13)	2.5 µl	0.5 µM
10 µM Reverse Primer (14)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-5: PCR-Programm used for the PCR´s 1,4,7,10.

98 °C	0:00:30
98°C	0:00:10
68°C	0:00:15
72°C	0:00:40
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

II. Gradient PCR of PCR 3, 6, 8, 9 (analytical)

Table 250521-6: Mastermix recipe for PCR 3 with template BM3R.

Component	10 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	5 µl	1X
10 µM Forward Primer (5)	0.5 µl	0.5 µM

10 µM Reverse Primer (6)	0.5 µl	0.5 µM
Template DNA	0.1 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-7: Mastermix recipe for PCR 6 with template CPR.

Component	10 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	5 µl	1X
10 µM Forward Primer (11)	0.5 µl	0.5 µM
10 µM Reverse Primer (12)	0.5 µl	0.5 µM
Template DNA	0.1 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-8: Mastermix recipe for PCR 8 with template OleT.

Component	10 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	5 µl	1X
10 µM Forward Primer (15)	0.5 µl	0.5 µM
10 µM Reverse Primer (16)	0.5 µl	0.5 µM
Template DNA	0.1 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-9: Mastermix recipe for PCR 9 with template BM3R.

Component	10 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	5 µl	1X
10 µM Forward Primer (17)	0.5 µl	0.5 µM
10 µM Reverse Primer (18)	0.5 µl	0.5 µM
Template DNA	0.1 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 250521-10: PCR-Programm used for the PCR´s 3,6,8,9.

98 °C	0:00:30
98°C	0:00:10
63 - 51 °C	0:00:15
72°C	0:00:40
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

Table 250521-11: Temperature gradients used for the PCR´s 3,6,8,9.

Gradient PCR 3	Gradient PCR 6	Gradient PCR 8	Gradient PCR 9
51.8 °C	52.9 °C	51.0 °C	51.0 °C
54.3 °C	55.8 °C	52.9 °C	52.9 °C
55.8 °C	57.5 °C	55.8 °C	55.8 °C
57.5 °C	59.2 °C	57.5 °C	57.5 °C
60.7 °C	60.7 °C	59.2 °C	59.2 °C
62 °C	62.9 °C	60.7 °C	60.7 °C

III. Cleanup of PCR 5, 12

The PCR products were all purified using Monarch PCR 4 DNA Cleanup Kit. 4 volumes of Ethanol were added, and the instructions were followed as provided. A fifth step of respinning for 30 seconds was added and in the sixth step H_2O was used 50°C, 20 µL.

- PCR5: bb 5:1 PCR-product (1671 bp)
- PCR12: bb 2:1 PCR-product (2226 bp)

- in step 6 20 µL H_2O (50°C) were used to elute the DNA

Table 250521-12: Quantification of PCR products of PCR 5 and 12.

	c [ng/µL]	OD260/OD230 []	OD260/OD280 []
PCR 5	121,9	1,6	1,77
PCR 12	49,4	1,56	1,69

WEDNESDAY 26/05/2021

Today's Team: Mohammad Nour, Marius Heitmann, Marie Eichholtz

I. Gel Electrophoresis of PCR-Products 1, 4, 7, 10

1 µL of Loading Dye 6x was added to 5 µL of the cleaned-up PCR-Products and the gel was run at 120 V, 400 mA for 60 min. The gel had an agar concentration of 1 %. A Quickload purple 1 kb Plus DNA Ladder from NEB as used.

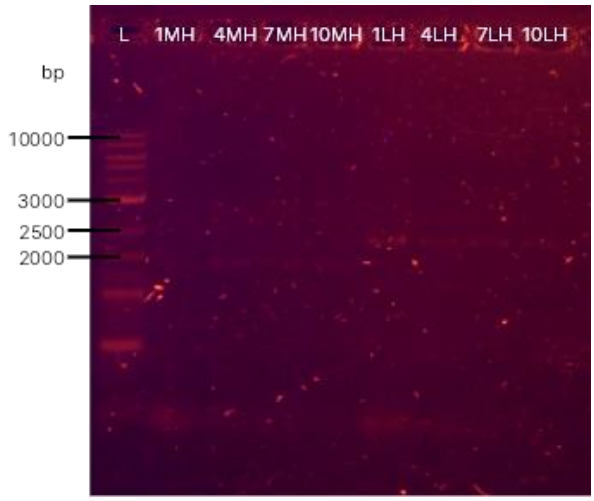

Figure 1: Gel-Electrophoresis of the PCR-Products of the LA and MH backbones (pSB1C3).

All PCRs seemed to work, however with a low concentration. Additionally, they differed in length, the expected length however was 2046 bp for both inserts.

II. Results of Gradient PCR 3, 6, 8, 9 (analytical)

1 µL of Loading Dye 6x was added to 5 µL of the cleaned-up PCR-Products and the gel was run at 120 V, 400 mA for 60 min. The gel had an agar concentration of 1 %. A Quickload purple 1 kb Plus DNA Ladder from NEB as used.

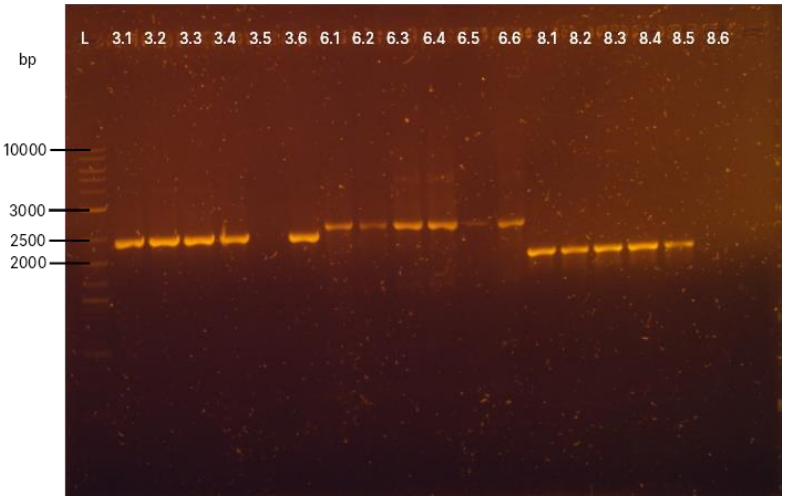

Figure 2: Gel-Electrophoresis on a 1% agarose gel of the PCR-Products of the PCRs 3, 6, 8, 9 (pSB1C3).

The gradient PCRs 3, 6 and 8 proved to be successful (except for 3.5 and 8.6). The PCR 9 didn't seem successful and needs to be repeated.

III. Clean-up of PCR 1, 4, 7, 10

The PCR products were all purified using Monarch PCR 4 DNA Cleanup Kit. 4 volumes of Ethanol were added, and the instructions were followed as provided. A fifth step of respinning for 30 seconds was added and in the sixth step H_2O was used 50°C, 20 µL.

Table 260521-1: Quantification of PCR products of PCR 1, 4, 7 and 10.

PCR	Backbone	c [ng/µL]	OD260/OD230 []	OD260/OD280 []
1	MH01	44,150	1,20	1,78
1	LA01	45,531	1,24	1,78
4	MH01	40,604	1,41	1,79
4	LA01	50,600	0,90	1,70
7	MH01	51,372	0,84	1,63
7	LA01	47,522	0,76	1,66
10	MH01	22,800	1,11	1,83
10	LA01	31,767	0,85	1,77

IV. PCR 3, 6, 9 (quantitative)

Table 260521-2: Mastermix recipe for PCR 3 with template BM3R.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (5)	2.5 µl	0.5 µM
10 µM Reverse Primer (6)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 260521-3: Mastermix recipe for PCR 6 with template CPR.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (11)	2.5 µl	0.5 µM
10 µM Reverse Primer (12)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 260521-4: Mastermix recipe for PCR 8 with template OleT.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (15)	2.5 µl	0.5 µM
10 µM Reverse Primer (16)	2.5 µl	0.5 µM
Template DNA	1.2 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 260521-5: PCR-Programm used for the PCR´s 3,6,8.

98 °C	0:00:30
98°C	0:00:10
57°C	0:00:15
72°C	0:00:40
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

V. Repetition of Gradient PCR 9

Table 260521-2: Mastermix recipe for PCR 9 with template BM3R.

Component	10 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	5 µl	1X
10 µM Forward Primer (17)	0.5 µl	0.5 µM
10 µM Reverse Primer (18)	0.5 µl	0.5 µM
Template DNA	0.1 µL	5 ng (IDT) - 25 ng (Plasmid)

Table 260521-5: PCR-Programm used for the PCR 9.

98 °C	0:00:30
98°C	0:00:10
47 - 63.5 °C	0:00:15
72°C	0:00:50
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

Table 260521-6: Temperature gradients used for the PCR 9.

Gradient PCR 9
47.0 °C
49.5 °C
51.3 °C
53.4 °C
55.6 °C
67.8 °C
61.6 °C
63.5 °C

FRIDAY 28/05/2021

Today's Team: Lukas Schulte, Justus Arndt, Khadija Rombi

I. Gel-Electrophoresis of PCR-Products from PCR 3, 6, 8, 9.

1 µL of Loading Dye 6x was added to 5 µL of the cleaned-up PCR-Products and the gel was run in a 1% agarose gel at 120 V, 400 mA for 70 min. A Quickload purple 1 kb Plus DNA Ladder from NEB as used.

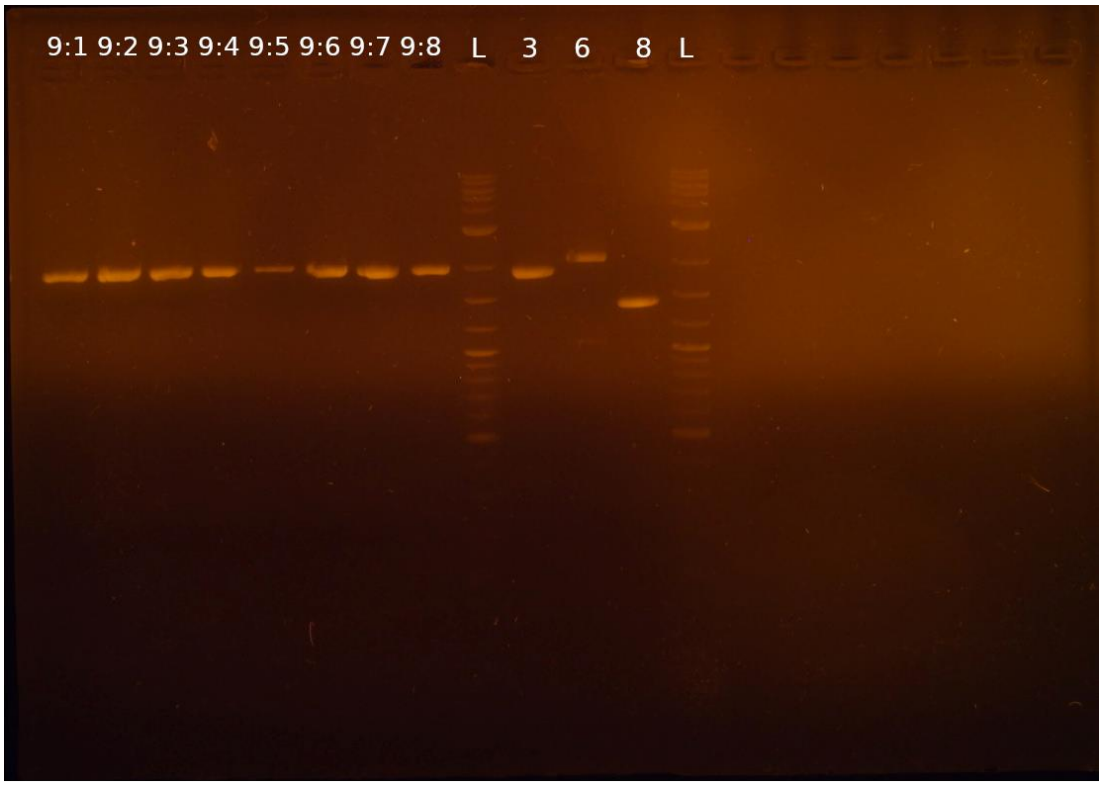

Figure 3: Gel electrophoresis of the PCR products from PCR 3, 6, 8 and 9.

All PCRs proved to be successful and a quantitative PCR of PCR 9 and GGAs 1, 2 and 4 can be performed.

II. Purification of PCR-Products

The PCR products were all purified using Monarch PCR 4 DNA Cleanup Kit. 4 volumes of Ethanol were added, and the instructions were followed as provided. A fifth step of respinning for 30 seconds was added and in the sixth step H_2O was used 50°C, 20 µL.

III. PCR for amplification of BM3R (PCR9)

The PCR was performed to introduce GGA overhangs into BM3R for the assembly of OleT-BM3R fusion.

Table 280521-1: Mastermix recipe for PCR 9 with template BM3R.

Component	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	25 µl	1X
10 µM Forward Primer (17)	2.5 µl	0.5 µM
10 µM Reverse Primer (18)	2.5 µl	0.5 µM
Template DNA	0.5 µL	5 ng (BM3R gBlock)
Nuclease-Free Water	19.5 µL	

Table 280521-2: PCR-Programm used for the PCR 9.

98 °C	0:00:30
98°C	0:00:10
55°C	0:00:15
72°C	0:00:50
Go to step 2 25x	
72°C	0:02:00
4 °C	hold

The expected length for the PCR product was 1968 bp.

IV. Golden Gate Assemblies of OleT-BM3R fusion (1), CYP1A1-CPR fusion (2), CYP1A1-CPR control (3)

Table 280521-3: Preparation of the Assembly Solutions for the GGAs.

Component	**GGA 1**	**GGA 2**	**GGA 4**
T4-Ligase Buffer 10X	2 µl		
GGA-Enzyme Mix	1 µL		
Nuclease-Free Water	13.4 µl	14.32 µL	11.6 µL
Template DNA 1	PCR 1, 1.65 µL	PCR 4, 1.48 µL	PCR 10, 2.36 µL
Template DNA 2	PCR 2, 0.98 µL	PCR 5, 0.49 µL	PCR 11, 1.4 µL
Template DNA 3	PCR 3, 0.97 µL	PCR 6, 0.71 µL	PCR 12, 1.61 µL

Table 280521-4: Program for the GGAs.

42 °C	1 min
16 °C	1 min
Go to step 1; 25x	
60 °C	5 min
4°C	∞

V. Heat Shock Transformation of GGAs 1, 2 and 4

1. NEB 10-beta competent E. coli were thawed on ice.
2. Three 50 µL aliquots of competent cells were aliquotted.
3. 2 µL of Assembly product (respectively GGA 1, 2 or 4 see table 280521-3) were added and incubated on ice for 30 minutes.
4. Heat shock was performed at 42 °C for 30 seconds.
5. Cells were incubated on ice for 5 minutes.
6. 950 µL of NEB-beta/stable outgrowth medium were added and incubated at 37 °C and 300 rpm for

VI. Purification of PCR-Products

The PCR products were all purified using Monarch PCR 4 DNA Cleanup Kit. 4 volumes of Ethanol were added, and the instructions were followed as provided. A fifth step of re-spinning for 30 seconds was added and in the sixth step H_2O was used 50°C, 20 µL.

MONDAY 31/05/2021

Today's Team: Marcel, Nico, Vanessa

The plates with the GGA transformed bacteria showed no growth → were incubated at 37°C again for longer.

I. New Transformation protocol based on "How to GGA" with NEB 10-beta *E. coli*

1. Cells were thawed for 15 minutes on ice
2. 5 µL GGA solution was added to 100 µL *E. coli*
3. incubated for 30 minutes on ice
4. 30 s at 42°C
5. incubated for 5 Min on ice
6. 950 µL NEB growth medium was added
7. 1h at 37°C and 300 rpm
8. centrifuged for 2 minutes at 4500 rcf
9. on agar plate

II. Testing of the competent cells

The protocol is identical to the new transformation protocol except that instead of NEB medium, LB medium was used.

- E200220LA04 was used as a plasmid

Table 310521-1: tested E. coli and calculated transformation efficiency.

	tested E. coli	**Efficiency**
old	DH5α 12	4*10^4 F4 ng^-1 ml^-1
old	DH5α 10	x
old	DH5α comp. (red)	1,2*10^5 F4 ng^-1 ml^-1
new	DH5α	1,2*10^5 F4 ng^-1 ml^-1
new	BL21 DE3	x
old	BL21 DE3	x

The plasmid was diluted to 10ng/mL and 10 ng wer used per transformation.

The old DH5α comp. (red) and the new DH5α cells had a suitable transformation efficiency. The BL21 cells showed no colonies, thus an overnight colony was prepared for making them competent.