Author: Kai Huß
Entry 1/192: Plating of _C. glutamicum_
In Project: Level_2
With tags: Cultivation, Corynebacterium, glutamicum

created: 10.04.2018 20:20
updated: 11.04.2018 08:59

Single *Corynebactericum glutamicum* WT strain #385 (Feldi intern number UMa2540) from GlyciStock on YT plate

Incubate at 28°C over Night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 12.04.2018 19:05
updated: 20.04.2018 18:12
Entry 2/192: Kryo C. glutamicum Z. mobilis
In Project: Level_2
With tags: Corynebacterium, glutamicum, Zymomonas, mobilis, gDNA, Kryo

Preparation of Kryostocks for -80 storage

We used: 800 µl overnight culture

fill up to 1 ml with glycerin (Sterile)

Storage at -80°C freezer in Box 1 for:

Zymomonas mobils (2iGEM0099)

Corynebacterium glutamicum WT strain #385 (2iGEM0100)

Number in Kryostock: #99 #100

After creating stocks:

cell-harvesting for isolation of gDNA with the GENEKAM DNA ISOLATION KIT

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 12.04.2018 19:18
updated: 12.04.2018 19:24

Entry 3/192: cell-harvesting
In Project: Level_2
With tags: Corynebacterium, gDNA, glutamicum, mobilis, Zymomonas, harvesting, -80

Overnight-cultur of *Zymomonas mobilis* and *Corynebacterium glutamicum* were centrifuged (4000 rpm, 10 min.). The supernatant was discarded. The cell-pellet is stored at -80°C.

Further steps:

Isolation of gDNA with the GENEKAM DNA ISOLATION KIT

Measure the concentrations of the DNA (same day)

Storage of gDNA at -20°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 13.04.2018 22:53
updated: 13.04.2018 23:54
Entry 4/192: Preparation of gDNA
In Project: Level_2
With tags: Corynebacterium, gDNA, glutamicum, mobilis, Zymomonas, -20, genekam

Preparation of genomic DNA of *Zymomonas mobilis* and *Corynebacterium glutamicum*

Used Kit: GENEKAM DNA ISOLATION KIT

(0. Preheated heat block at 88°C.)

1. Preparing of solution Z (solution C (270 µl) + solution A (30 µl))

2. Added 100 µl of solution Z to each cell pellet and resuspended it.

3. Transfered pellet suspension into a sterile 1.5 ml reaction tube (eppi).

4. Added 50 µl Lysozym (concentration) and xg of glass beads (size?) to each reaction.

5. Shaked the reaction on the heat block at 2000 rpm and 88°C for 1 minute.

6. Added 100 µl B (vortex!) and 200 µl C solution.

7. Centrifuged samples at 11000 x g for 5 min.

8. Took off clear supernatant and stored it in -20°C freezer.

Protocol based on c) Isolation from tissue samples, changes were marked *italic*.

SB0071-74_DNA-Isolation-kit_V_2016-1.pdf

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 17.04.2018 12:11
updated: 17.04.2018 12:19
Entry 5/192: measurement nucleic acid concentration
In Project: Level_2
With tags: Corynebacterium, gDNA, glutamicum, mobilis, genekam, Zymomonas, nucleic acid, nanodrop

The concentration gDNA of *Corynebacterium gutamicum* and *Zymomonas mobilis* were measured by nanodrop.

Corynebacterium glutamicum: 251,35 ng/µl

Zymomonas mobilis: 662,25 ng/µl

In both samples seems to be a higher amount of contamination of organic substances, as RNA or single Nucleotides.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
created: 17.04.2018 17:55
updated: 18.04.2018 10:06
Entry 7/192: PCR: ddh (from C. glutamicum)
In Project: Level_2
No tags associated

PCR: ddh (from *C. glutamicum*) note: *C. glutanicum*

test digest:

we used from Duber Toolbox these Plasmids:

Number:	Concentration:		used µl from DNA	Puffer[µl]	MilliQ[µl]	Enzym[µl]	Σ in total[µl]
0011	128,1	ng/µl	4	2,5	18	0,5	25
0012	93,1	ng/µl	5,5	2,5	16,5	0,5	25
0018	113,5	ng/µl	4,5	2,5	17,5	0,5	25
0019	175,6	ng/µl	3	2,5	19	0,5	25
0020	168,7	ng/µl	3	2,5	19	0,5	25
0067	86,4	ng/µl	6	2,5	16	0,5	25

first we filled our eppis with the MilliQ Water. After we filled our DNA fragments into the water. Now we put in the Buffer (cutsmart 10x).

We used Bsa1 (enzyme) to cut our DNA.

We incubated for 1 hour with 37 °C without rotation.

after we stopped the enzyme reaction with 65°C for 20 minutes.

Then we put loading dye in our solution.

For the gelelectrophoresis we used 1% agarose (1x tae based) . 40 ml gel with 2 µl Gelred.

the Electrophoresis ran with 100 V for 30 minutes

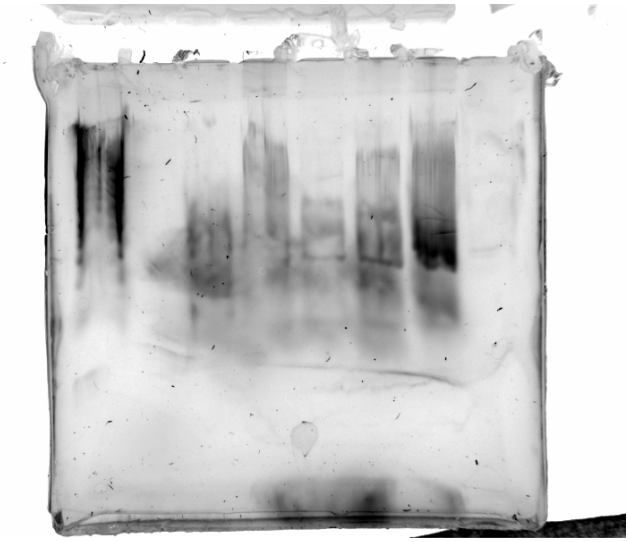
the rows are :

1	Ladder
2	gDNA from *corynebacterium glutanicum* (for testing) 2µl ca 500 ng
3	0011
4	0012
5	0018
6	0019
7	0020
8	0067

the 2 nd gelbad is empty, it shouldnt be.

we couldnt indentificate the 2 visible bands because the ladder is smeared.

Gel_2018-04-18_09hr_12min_Testrestriction_Duber_coryne.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 8/192: Miniprep p2iGEM0075 (DUEBER 75)
In Project: Level_2
No tags associated

created: 26.04.2018 14:36
updated: 26.04.2018 15:06

Mini prep of the Plasmid **p2iGEM0075** with the following Protocoll:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- --> Repeat step 1 and 2 if necessary
- Add supernatant (~ 800 µl) to column
- centrifuge 15 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 15 Sek. max rpm
- For elution use new tube+ 30 µl 37°C prewarmed water on column
- Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

Container: 2x 1.5ml Eppies. White box in -20°C

Concentrations: **247,85 ng/l, 250,57 ng/l**

Performed by Lev on 26.04.2018

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 9/192: Cryoculture 2iGEM0075
In Project: Level_2
No tags associated

created: 26.04.2018 15:09
updated: 26.04.2018 15:16

Cryoculture 2iGEM0075 has been made with 730µl of culture, containing p2iGEM0075, and 270µl Glycerin (100%).
Container: Cryotube; White box in -80°C.

Performed by Lev on 26.04.2018

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
created: 30.04.2018 14:53
updated: 30.04.2018 15:06
Entry 10/192: PCR pYTK075_LEU2
In Project: Level_2
With tags: pcr, plasmid

PROTOCOL PCR;

LVL2PCR of the p2iGEM0075 plasmid from DUEBER toolbox (pYTK075_LEU2) to amplify leu2 gene using the following protocol:

Primers used:

O_iGEM18_0005

O_iGEM18_0006

Add into PCR-tube(25 µl total each PCR-tube):

5 µl high GC enhancer

5 µl Q5 Buffer

12,75 µl MilliQ-water

0,5 µl Template

0,5 µl dNTPs

1 µl of each Primer

0,25 µl Q5 polymerase (add this at last, on ice!)

95°C 2min

then starting the cycle with:

95°C 15 sec

72°C 15 sec (depending on the anealing temperature of the Primer)

72°C 30 secrepeating this 28x (depending on the situation 28-30)

72°C 2min5°C HOLD

Performed by Lev on 27th of April

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 11/192: plasmid stock
In Project: Level_2
No tags associated

created: 01.05.2018 23:39
updated: 02.05.2018 00:03

Yesterday we inoculated needed DUEBER and CIDAR parts for future GoldenGate cloning:

We inoculated with matching antibiotics:

Cryo 2iGEM:

001, 009 - 027, 75, 104, 105, 106, 113, 116, 117, 121, 124, 125, 128, 129, 132, 133, 136, 153, 167, 171, 178, 200, 204

Today we isolated the plasmids from these inoculated cultures:

Plasmids p2iGEM:

001, 009 - 027, 75, 97, 98, 99, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 149, 164, 167, 174, 196, 200

- 600 µl culture + 100 µl Lysis buffer --> invert 6 - 8x + 350 µl Neutralisation buffer (cold) --> invert 6 - 8x
- centrifuge at 3 min max rpm
- Add supernatant (~800 µl) to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 30 sec max rpm
- add 400 µl column wash
- 30 sec max rpm
- For elution use new tube + 30 µl elution buffer
- Let incubate for 5 min at RT. Centrifuge for 30 Sek. max

lvl 2 stock: https://docs.google.com/spreadsheets/d/1P6CL1MdZHOVDJ37rQv5djjiXNmT-FkjLEDNZbB7P4Ck/edit#gid=0

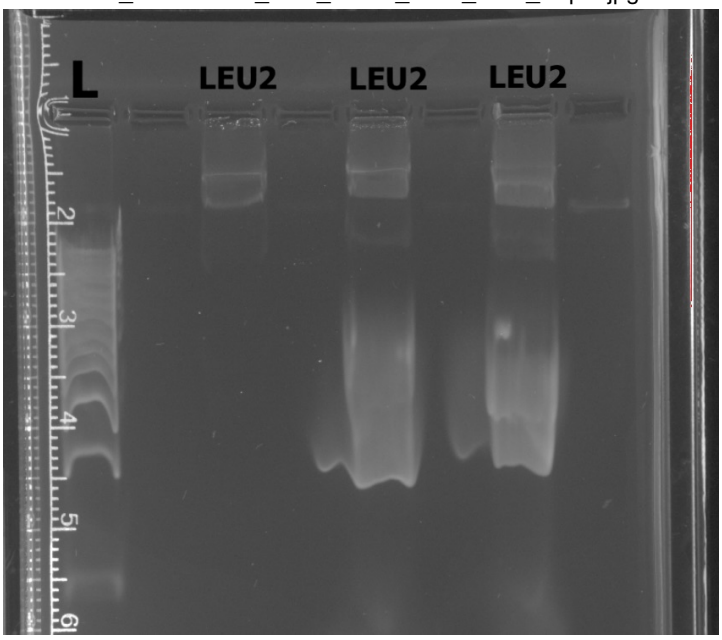
Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 12/192: PCR result LEU2
In Project: Level_2
No tags associated

created: 02.05.2018 00:06
updated: 02.05.2018 00:21

We applied the PCR product *LEU2 (+ 5µl 6x loading dye)* on a 1% agarose gel, stained with GelRed, for 45 min at 100 V (5 V/cm). As seen in the picture there is no clear band (*LEU2* with overhangs: 1146 bp). Our PCR was not successful.

Gel_2018-04-30_17hr_40min_Leu2_PCR_Kopie.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
created: 02.05.2018 15:38
updated: 03.05.2018 12:04
Entry 13/192: PCR pYTK075_LEU2
In Project: Level_2
No tags associated

PROTOCOL PCR;

LVL2PCR of the p2iGEM0075 plasmid from DUEBER toolbox (pYTK075_LEU2) to amplify leu2 gene using the following protocol:

Primers used:

O_iGEM18_0005

O_iGEM18_0006

Add into PCR-tube (25 µl total each PCR-tube):

1) two samples contained:

5 µl high GC enhancer

5 µl Q5 Buffer8,

25 µl MilliQ-water

1 µl Template

0,5 µl dNTPs

2,5 µl of each Primer

0,25 µl Q5 polymerase2)

The other two contained:

5 µl high GC enhancer

5 µl Q5 Buffer

11,75 µl MilliQ-water

0,5 µl Template

0,5 µl dNTPs

1 µl of each Primer

0,25 µl Q5 polymerase

98°C - 30 sec.

then starting the cycle with:

95°C - 10 sec.

72°C - 40 sec.

(1334 - 2000 kb)

repeating this 30x72°C - 2 min. 5°C - HOLD

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 16/192: Gel electrophoresis LEU2
In Project: Level_2
No tags associated

created: 02.05.2018 23:09
updated: 02.05.2018 23:20

We loaded our PCR products with 5 µl 6x Purple Loading Dye and applied them on a 1% agarose gel stained with GelRed. The loaded gel was run at 80 V (4 V/cm for 80 min with 1x TAE buffer.

Gel image comes tomorrow.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 17/192: DNA Gel Extraction
In Project: Level_2
No tags associated

created: 02.05.2018 23:21
updated: 02.05.2018 23:59

We cut our LEU2 bands out of our gel and put it in a 2 ml reaction tube. Then we used the Wizard® SV Gel and PCR Clean-Up System from Promega:

We added 1 ml of Membrane Binding Solution to the gel slices and incubated it for 10 min. at 60°C until the gel slices dissolved.

Then we loaded 350 µl of the gel solution into the SV minicolumn with collection tube, let it incubate for 1 min at RT and centrifuge it for 1 min at 16.000 x g. We discarded the supernatant. These steps were repeated until the whole gel solution was filtered through the column membrane.

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min. We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°) in these steps:

-adding 30 µl 37°C warm MilliQ to the membrane.

-incubate membrane at 37°C shook at 300 rpm.

-centrifuge for 1 min at 50 x g

-centrifuge for 1 min at 16.000 x g

Later we measured the concentration of our LEU2 PCR product residue: 2,4 ng/µl

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Nicholas Schmitt
Entry 18/192: Leu 2 PCR
In Project: Level_2
No tags associated

created: 03.05.2018 15:34
updated: 03.05.2018 15:34

PROTOCOL PCR;

LVL2PCR of the p2iGEM0075 plasmid from DUEBER toolbox (pYTK075_LEU2) to amplify leu2 gene using the following protocol:

Primers used:

- O_iGEM18_0005
- O_iGEM18_0006

Add into PCR-tube (25 µl total each PCR-tube):

All 7 samples contained:

- 5 µl high GC enhancer
- 5 µl Q5 Buffer8,
- 8,25 µl MilliQ-water
- 1 µl Template
- 0,5 µl dNTPs
- 2,5 µl of each Primer
- 0,25 µl Q5 polymerase2)

98°C - 30 sec.

then starting the cycle with:

- 95°C - 10 sec.
- 72°C - 40 sec.
- (1334 - 2000 kb)

repeating this 30x72°C - 2 min. 5°C - HOLD

After that 5µl Loading Dye were added and the samples were loaded into an 1% Agarose Gel and underwent Gelelectrophorsis for 80 min at 80V

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Nicholas Schmitt
Entry 19/192: No entry title yet
In Project: Level_2
No tags associated

created: 03.05.2018 15:38
updated: 03.05.2018 15:40

GoGate Assembly of Leu2 with Entry-Vector for the DUEBER Toolbox

Mix:

- 0.75 µl T4 Ligase
- 3 µl T7 Buffer (10x)
- 0.75 µl BsaI
- 0.75 µl each:
  - p2iGEM0001
  - The PCR Product of LEU2/p2iGEM0075
- 22.5 µl H_2O

used PCR program: "GoGate BsaI"

Thermocycler (Programm GoGate BasI)

- 60 cycles of digestionand ligation (37°C for 2 min, 16 °C for 5 min),
- final digestion step (60 °C for 10 min)
- a heat inactivation step (80 °C for 10 min).
- Hold at 4°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Nicholas Schmitt
Entry 20/192: DNA Gel Extraction
In Project: Level_2
No tags associated

created: 03.05.2018 17:01
updated: 03.05.2018 17:51

We cut our LEU2 bands out of our gel and put it in a 2 ml reaction tube. Then we used the Wizard® SV Gel and PCR Clean-Up System from Promega:

We added 1 ml of Membrane Binding Solution to the gel slices and incubated it for 10 min. at 60°C until the gel slices dissolved.

Then we loaded 350 µl of the gel solution into the SV minicolumn with collection tube, let it incubate for 1 min at RT and centrifuge it for 1 min at 16.000 x g. We discarded the supernatant. These steps were repeated until the whole gel solution was filtered through the column membrane.

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min. We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°) in these steps:

-adding 30 µl 37°C warm MilliQ to the membrane.

-incubate membrane at 37°C shook at 300 rpm.

-centrifuge for 1 min at 50 x g

-centrifuge for 1 min at 16.000 x g

The concentrations will have to be measured tomorrow

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
Entry 21/192: transformation Leu2
In Project: Level_2
No tags associated

created: 04.05.2018 12:47
updated: 04.05.2018 12:55

We did a transformation with a CamR Resistance in E.coli DH5α

the new Kryo is named **2iGEM0227**

1 Control with : 1 µl plasmid

15 µl competent cells

First we incubated the competent cells 10 minutes on ice

then we put 1 µl plasmid **(p2iGEM0209**) into 15 µl competent cells and incubated 5 minutes on ice.

Now we heatshocked them for 45 seconds on 42°C

Then we incubated for 2 minutes on ice

We put 300 µl LB in the culture and incubated for one hour on 37°C with 300 rpm

we plated out the bacteria on a Agarplate with chloramphenikol and incubated them over night without rotation on 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
Entry 22/192: Inoculation 051
In Project: Level_2
No tags associated

created: 06.05.2018 17:07
updated: 06.05.2018 17:49

inokulate Kryo pYTK051_tENO1

3 ml LB with

2,04 µl CamR

incubate with 37 °C

(tomorrow to prep for goGate)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 23/192: Mini prep p2iGEM0209
In Project: Level_2
No tags associated

created: 06.05.2018 18:40
updated: 06.05.2018 18:43

Mini prep of the Plasmid **p2iGEM0209** with the following Protocoll:

- 600µl culture+100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- --> Repeat step 1 and 2 if necessary
- Add supernatant (~ 800 µl) to column
- centrifuge 15 Sek. max rpm --> discard supernatant
- + 400µl Column Wash
- 15 Sek. max rpm
- For elution use new tube+ 30 µl 37°C prewarmed water on column
- Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

Container: 1.5ml Eppie. White box in -120°C

Concentrations: 239.3 ng/µl

Performed by Lev on 06.05.2018

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 24/192: Kryoculture 2iGEM0227
In Project: Level_2
No tags associated

created: 06.05.2018 18:44
updated: 06.05.2018 18:47

A **Kryoculture 2iGEM0227** has been prepared with the following methodology: 700 µl culture, 300 µl Glycerol.

Performed by Lev on 06.05.2018

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 25/192: p2iGEM0209 Transformation in E. coli
In Project: Level_2
No tags associated

created: 06.05.2018 18:48
updated: 06.05.2018 18:53

A **transformation** has been performed with **p2iGEM0209** in E.coli:

- Incubate competent cells 10 minutes on ice
- Add 10-15 µl cells + 1 µl plasmids in Eppis (sterile)
- Incubate 30 min on ice
- 42°C, 45 sec heatshock
- Incubate 5 minutes on ice
- + 300 µl LB without antibiotic *STERILE*
- 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
- cells were plated out --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
created: 07.05.2018 10:52
updated: 07.05.2018 11:26
Entry 26/192: minipräp p2iGEM0051
In Project: Level_2
No tags associated

minipräp p2iGEM0051 (DUEBER 51, Kryo 2iGEM0051)

first we centrifugate the cultures with max rpm for 6 minutes.

we removed supernatant and resuspended the pellet and put all in an empyt eppi.

100 µl Lysisbuffer were added and inverted.

350 µl neutralsation buffer were added (fast after lysisbuffer) and inverted.

now we centrifugated with max rpm for 3 minutes.

Then we put the supernatant in a column and centrifugated with max rpm for 30 seconds

the flow was disposed.

400 µl column wash were added in the column and centrifugated with max rpm for 30 seconds.

the flow was disposed and we repeated the step with the column wash

then we used a new eppi under the column and put 30 µl MilliQ (37°C) into the column

we waited 5 minutes (in heatblock 37°C and 300 rpm) and centrifugated first for 2 minutes with 50 g (rcf) and then with max rpm for 1 minute.

we messed a concentration of 457,5 ng/µl

The Plasmid is in the level 2 Box "Team Jenny"

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
Entry 27/192: Inoculation Kryos
In Project: Level_2
No tags associated

created: 07.05.2018 14:56
updated: 07.05.2018 15:00

inokulate these Kryos in 3 ml LB

Kryo	Plasmid	Antibiotikum	µl an Antibiotikum in 3 µl	In E. coli Stamm:
2iGEM0106	p2iGEM0099	KanR	15	T10
2iGEM0218	p2iGEM0100	AmpR	30	DH5a
2iGEM0101	p2iGEM0101	AmpR	30	T10
2iGEM0102	p2iGEM0102	AmpR	30	T10
2iGEM0103	p2iGEM0103	AmpR	30	T10
2iGEM0108	p2iGEM0104	AmpR	30	T10
2iGEM0109	p2iGEM0105	AmpR	30	T10
2iGEM0110	p2iGEM0106	AmpR	30	T10
2iGEM0111	p2iGEM0107	AmpR	30	T10
2iGEM0112	p2iGEM0108	AmpR	30	T10
2iGEM0113	p2iGEM0109	AmpR	30	T10
2iGEM0116	p2iGEM0112	AmpR	30	T10
2iGEM0117	p2iGEM0113	AmpR	30	T10
2iGEM0120	p2iGEM0116	AmpR	30	T10
2iGEM0121	p2iGEM0117	AmpR	30	T10
2iGEM0124	p2iGEM0120	AmpR	30	T10
2iGEM0125	p2iGEM0121	AmpR	30	T10
2iGEM0128	p2iGEM0124	AmpR	30	T10
2iGEM0129	p2iGEM0125	AmpR	30	T10
2iGEM0132	p2iGEM0128	AmpR	30	T10
2iGEM0133	p2iGEM0129	AmpR	30	T10

incubate with 37 °C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
Entry 28/192: Inoculation p2iGem209
In Project: Level_2
No tags associated

created: 07.05.2018 15:00
updated: 07.05.2018 15:05

inokulate p2iGem0209 | iGem0227

3 ml LB with

2,04 µl CamR

incubate with 37 °C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
Entry 30/192: No entry title yet
In Project: Level_2
No tags associated

created: 08.05.2018 20:22
updated: 08.05.2018 20:44

Testgel with gDNA from *C.glutanicum*

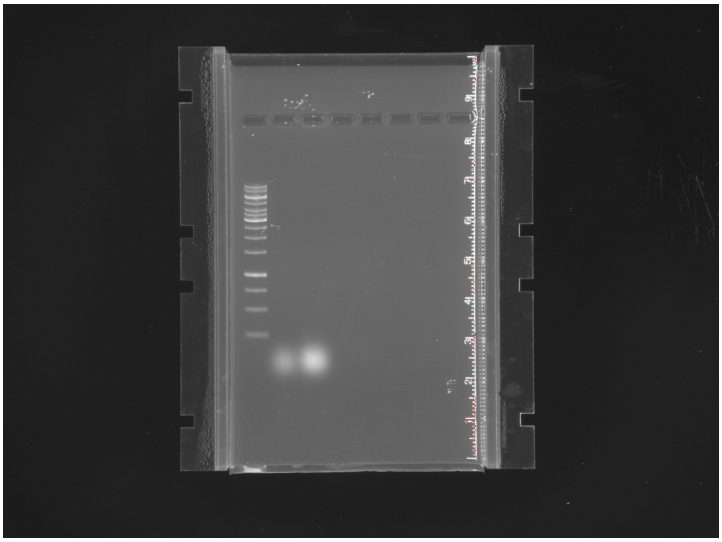
We filled 2 eppis with :

	MQ in µl	loading dye in µl	gDNA in µl
Eppi 1/Line 2	14	1	5
Eppi 2/Line 3	8	2	10

we let our gel run with 90 v for 60 minutes.

Here is our gel:

Gel_2018-05-08_17hr_47min_Testgel_gDNA_Corynebacterium_glutanicum.png



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 09.05.2018 11:53
updated: 10.05.2018 00:47
Entry 31/192: test restriction of p2iGEM0209
In Project: Level_2
No tags associated

Restriction of 6x **p2iGEM0209 with BbsI**

3 µl p2iGEM0209 DNA

2 µl Cutsmart

1 µl BbsI

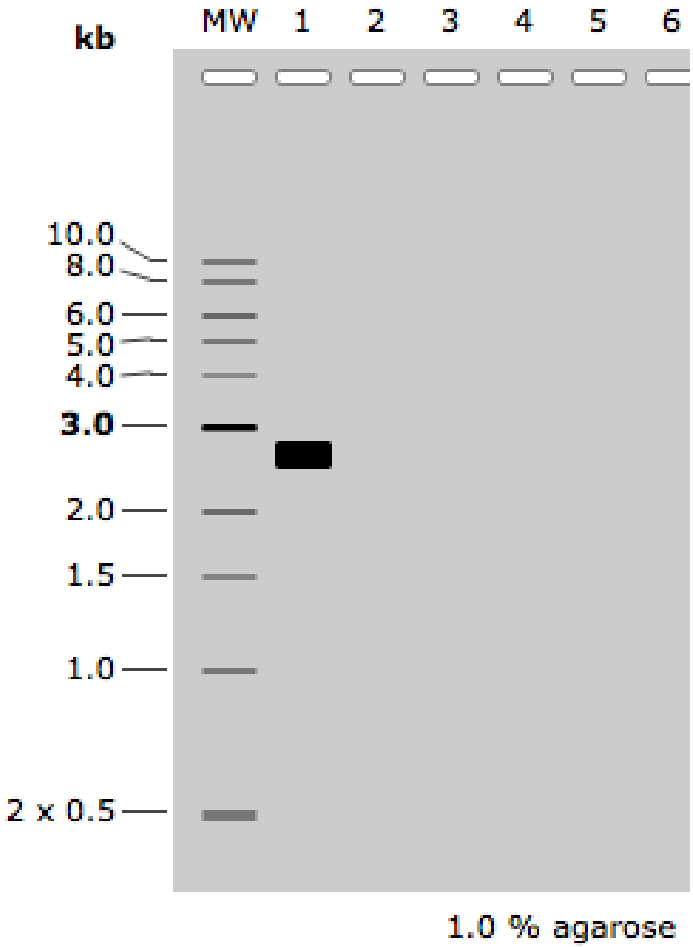
14 µl Millipore water

per reaction

Result:

Expected:

Gel_Image_0209_cut_with_BbsI.png



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Lev Petrov
Entry 32/192: Mini prep
In Project: Level_2
No tags associated

created: 09.05.2018 16:34
updated: 09.05.2018 16:37

Mini prep of the plasmids:

p2iGEM0099-0109, 0112, 0113, 0116, 0117, 0120, 0121, 0124, 0125, 0128, 0129 x1 each

p2iGEM0209 x5

with the following Protocoll:

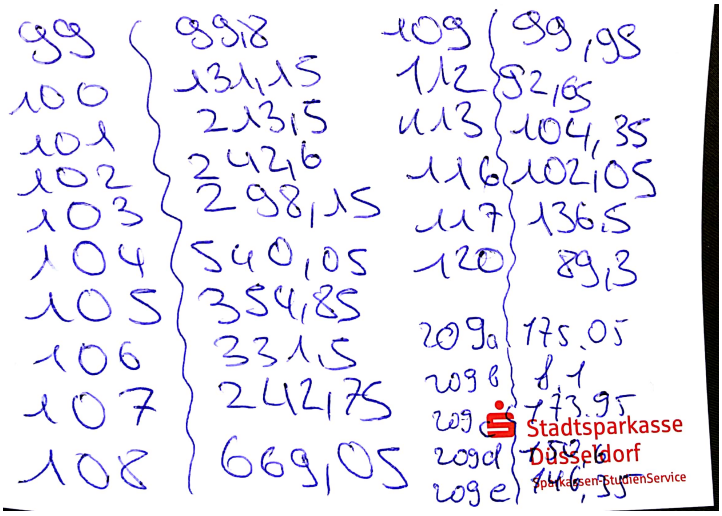
- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- --> Repeat step 1 and 2 if necessary
- Add supernatant (~ 800 µl) to column
- centrifuge 15 Sek. max rpm --> discard supernatant
- + 400µl Column Wash
- 15 Sek. max rpm
- For elution use new tube+ 30 µl 37°C prewarmed water on column
- Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

Container: 1.5ml Eppie. White box in -20°C

Concentrations: see picture

Performed by Lev, Salima on 06.05.2018

Konz_Prep_08.05.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Matthias Willmann
created: 10.05.2018 00:00
updated: 10.05.2018 00:27
Entry 33/192: transformation Promotor
In Project: Level_2
No tags associated

We did a transformation with a Amp Resistance in E.coli DH5α

the new Kryos are named p**2iGEM0243- p2iGEM0260**

in total 19 new Kryos

each reactiontube contains:

25 µl competent cells

2 µl Plasmid

we did these kryos to test 19 different Promotors from the DUBER Toolbox with the Plasmid **p2iGEM0223 (2iGEM241)**

New Kryo	Tribe	Origin	Plasmidname

2iGEM0242	E.coli DH5a	cassetteGOI2_1_pYTK009_LEU2_pYTK051	p2iGEM0224
2iGEM0243	E.coli DH5a	cassetteGOI2_1_pYTK010_LEU2_pYTK051	p2iGEM0225
2iGEM0244	E.coli DH5a	cassetteGOI2_1_pYTK011_LEU2_pYTK051	p2iGEM0226
2iGEM0245	E.coli DH5a	cassetteGOI2_1_pYTK012_LEU2_pYTK051	p2iGEM0227
2iGEM0246	E.coli DH5a	cassetteGOI2_1_pYTK013_LEU2_pYTK051	p2iGEM0228
2iGEM0247	E.coli DH5a	cassetteGOI2_1_pYTK014_LEU2_pYTK051	p2iGEM0229
2iGEM0248	E.coli DH5a	cassetteGOI2_1_pYTK015_LEU2_pYTK051	p2iGEM0230
2iGEM0249	E.coli DH5a	cassetteGOI2_1_pYTK016_LEU2_pYTK051	p2iGEM0231
2iGEM0250	E.coli DH5a	cassetteGOI2_1_pYTK017_LEU2_pYTK051	p2iGEM0232
2iGEM0251	E.coli DH5a	cassetteGOI2_1_pYTK018_LEU2_pYTK051	p2iGEM0233
2iGEM0252	E.coli DH5a	cassetteGOI2_1_pYTK019_LEU2_pYTK051	p2iGEM0234
2iGEM0253	E.coli DH5a	cassetteGOI2_1_pYTK020_LEU2_pYTK051	p2iGEM0235
2iGEM0254	E.coli DH5a	cassetteGOI2_1_pYTK021_LEU2_pYTK051	p2iGEM0236
2iGEM0255	E.coli DH5a	cassetteGOI2_1_pYTK022_LEU2_pYTK051	p2iGEM0237
2iGEM0256	E.coli DH5a	cassetteGOI2_1_pYTK023_LEU2_pYTK051	p2iGEM0238
2iGEM0257	E.coli DH5a	cassetteGOI2_1_pYTK024_LEU2_pYTK051	p2iGEM0239
2iGEM0258	E.coli DH5a	cassetteGOI2_1_pYTK025_LEU2_pYTK051	p2iGEM0240
2iGEM0259	E.coli DH5a	cassetteGOI2_1_pYTK026_LEU2_pYTK051	p2iGEM0241
2iGEM0260	E.coli DH5a	cassetteGOI2_1_pYTK027_LEU2_pYTK051	p2iGEM0242

First we incubated the competent cells 20 minutes on ice

then we put 2 µl plasmid (from the golden gate 8.5.18) into 25 µl competent cells and incubated 30 minutes on ice.

Now we heatshocked them for 45 seconds on 42°C

Then we incubated for 5 minutes on ice

We put 700 µl LB in the culture and incubated for two hours on 37°C with 300 rpm

we plated out the bacteria on a Agarplate with Amp and incubated them over night without rotation on 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 10.05.2018 00:24
updated: 10.05.2018 01:03
Entry 34/192: GoGate p2iGEM0098_00xx_0209_0051
In Project: Level_2
No tags associated

GoGate Assembly of **p2iGEM0224 to p2iGEM0242** (just differing in promoter) (08.05.18/09.05.18)

Mastermix:

- 0.75 µl T4 Ligase
- 3 µl T7 Buffer (10x)
- 0.75 µl BsaI
- 0.75 µl each:
  - p2iGEM0209
  - p2iGEM0051
  - p2iGEM0097
- 22.5 µl H_2O

into PCR tube:

- 0.75 µl either:
  - p2iGEM0224
  - p2iGEM0225
  - p2iGEM0...
  - p2iGEM0242

used PCR program: "GoGate BsaI", overnight

Thermocycler (Programm GoGate BasI)

- 60 cycles of digestion and ligation (37°C for 2 min, 16 °C for 5 min),
- final digestion step (60 °C for 10 min)
- a heat inactivation step (80 °C for 10 min).
- Hold at 4°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 35/192: Saving of lysC synthesis
In Project: Level_2
No tags associated

created: 10.05.2018 00:25
updated: 10.05.2018 01:08

Transformation of "pUC57_*lysC*":

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 2 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 10.05.2018 18:41
updated: 11.05.2018 14:57
Entry 36/192: Saving of lysC (PCR)
In Project: Level_2
With tags: pcr, gBlocks, q5, cidar, lysC

To save our lysC synthesis, we amplified it with a PCR:

Per reaction we used:

- 5.0 µl Q5 reaction buffer (5x)
- 5.0 µl Q5 GC Enhancer
- 2.5 µl Primer, forward, O_iGEM18_0013_DVK_AE_LysC_fwd
- 2.5 µl Primer, reverse, O_iGEM18_0014_DVK_AE_LysC_rev
- 0.5 µl dNTPs
- 0.2 µl template (*lysC*)
- 0.25 µl Q5 polymerase
- 8.75 µl Millipore water

Because of our Annealing temperature of 72°C, we used a two-step PCR:

98°C - 30 sec.

Start cycle

98°C - 10 sec.

72°C - 40 sec. (20-30 sec. Per kb)

End cycle

(30x)

72°C - 2 min.

4°C Hold

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 10.05.2018 18:44
updated: 10.05.2018 19:27
Entry 37/192: Phenol/Chloroform gDNA isolation of C. glutamicum
In Project: Level_2
No tags associated

We extracted the gDNA from *Corynebacterium glutamicum:*

- yesterday we started overnight cultures of *C. glutamicum*
- we centrifuged them at 4000 rpm for 10 minutes
- removed the supernatant from our pellet
- washed the pellet with 400 µL STE buffer (pH=8.2)
- centrifuged the solution at 8000 rpm for 2 Minutes
- washed again with 400 µL STE buffer (pH=8.2)
- centrifuged again at 8000 rpm for 2 minutes
- resuspended the pellet in 200 µL TE buffer (pH=8.2)
- added 100 µL Tris-saturated phenol
- vortexed them for 60 seconds
- centrifuged at 13000 rpm for 5 minutes at 4 °C
- transferred 140 µL (as much as possible) of the upper aqueous phase to a clean 1.5 mL tube
- added 40 µL TE buffer and 100 µL chloroform
- mixed the samples and centrifuged at 13000 rpm for 5 minutes at 4 °C
- purified our lysate by chloroform extraction until a white interface is no longer present
- again transferred 140 µL (as much as possible) of the upper aqueous phase to a clean 1.5 mL tube
- added 40 µL TE buffer an 5 µL RNase A (10 mg/mL)
- incubated at 37 °C for 10 minutes
- added 100 µL chloroform and mixed
- centrifuged at 13000 rpm for 5 minutes
- transferred 130 µL (as much as possible) of the upper aqueous phase to a clean 1.5 mL tube

After we were done we measured the DNA concentration:

1) Corynebacterium glutamicum: 1404,4 ng/µL

2) *Corynebacterium glutamicum*: 1208,6 ng/µl

The concentrations were very high and our samples were phenol-contaminated. So we decided to purify them:

- (we added 1/10 Vol. (10 µL) 3 M NaOAc and 3 Vol. (300 µL) 100% isopropanol)
- now the samples are stored at -20 °C until Friday.

On Monday we will continue the purification.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 10.05.2018 18:55
updated: 10.05.2018 19:01
Entry 38/192: gDNA isolation of C. glutamicum (kit)
In Project: Level_2
No tags associated

Preparation of genomic DNA of *Corynebacterium glutamicum*

Used Kit: GENEKAM DNA ISOLATION KIT

(0. Preheated heat block at 88°C.)

1. Preparing of solution Z (solution C (450 µl) + solution A (50 µl))

2. Added 100 µl of solution Z to each cell pellet and resuspended it.

3. Transferred pellet suspension into a sterile 1.5 ml reaction tube.

4. Added 100 µl Lysozym (concentration) and xg of glass beads (size?) to each reaction.

5. Shaked the reaction on the heat block at 2000 rpm and 88°C for 7 minutes.

6. Added 100 µl B (vortex!) and 200 µl C solution.

7. Kept the reaction tubes overnight at 4°C.

Next step:

8. Took off clear supernatant and stored it in -20°C freezer.

Protocol based on b) Isolation from blood samples, changes were marked *italic*.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 39/192: Overnight cultures
In Project: Level_2
With tags: overnight

created: 10.05.2018 22:44
updated: 10.05.2018 22:57

Today we prepared overnight cultures of the stains with 19 different Promotors from the DUBER Toolbox. 2iGEM0242-2iGEM260

- 57 culture tubes were filled with 3ml LB-medium (sterile)(each strain type 3x)
- 2,04 µl ampicillin were added from a 10mg/ml stock (sterile)
- non fluorescent colonies were picked and added to the culture tube
- overnight incubation by 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 40/192: lysC cloning into Topo backbone
In Project: Level_2
With tags: lysC, topo, topo kit

created: 11.05.2018 16:42
updated: 01.08.2018 19:20

Cloning of *lysC* synthesis into Topo-plasmid with Topo TA Cloning Kit (Invitrogen)

For the cloning following the protocole for the Kit

The input pc template was 0,7 µl

The transformation was performed after cloning and 100% of the cells were plated

Incubation overnight at 37°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 41/192: Miniprep
In Project: Level_2
With tags: miniprep

created: 11.05.2018 21:51
updated: 11.05.2018 22:31

We checked wether fluorescence was present in the overnight cultures. Fluorescent cultures were sorted out

The remaining cultures were:

strain	amount	plasmid
2iGEM0242	3x	p2iGEM0224
2iGEM0243	1x	p2iGEM0225
2iGEM0244	1x	p2iGEM0226
2iGEM0245	2x	p2iGEM0227
2iGEM0248	1x	p2iGEM0230
2iGEM0249	1x	p2iGEM0231
2iGEM0250	1x	p2iGEM0232
2iGEM0251	1x	p2iGEM0233
2iGEM0252	2x	p2iGEM0234
2iGEM0253	1x	p2iGEM0235
2iGEM0255	2x	p2iGEM0237
2iGEM0257	2x	p2iGEM0239
2iGEM0258	2x	p2iGEM0240
2iGEM0259	2x	p2iGEM0241

Mini prep of the Plasmids p2iGEM0209, p2iGEM0224, p2iGEM0225,p2iGEM0226, p2iGEM0227, p2iGEM0230-p2iGEM0235, p2iGEM0237, p2iGEM0239-p2iGEM0241 with the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- + 200 µl endotoxin removal wash
- centrifuge 30 sec max rpm -> discard supernatant
- add 400µl column wash
- For elution use new tube
- 30 µl elution buffer on column
- Let incubate for 5 Min at RT
- Centrifuge for 15 Sek. max

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 42/192: Testdigetsion
In Project: Level_2
With tags: digest, enzyme

created: 12.05.2018 12:30
updated: 12.05.2018 12:30

testdigestion with following protocol:

Mastermix:

- 9,6 µl EcoRI Enzyme (from NEB)
- 21 µl Cutsmart buffer
- 159µl milli Q water

in each tube 1µl Plasmid and 9µl Mastermix

in total 22 tubes with Plasmids of different colonies of p2iGEM0209, p2iGEM0224, p2iGEM0225,p2iGEM0226, p2iGEM0227, p2iGEM0230-p2iGEM0235, p2iGEM0237, p2iGEM0239-p2iGEM0241

Digestion with these temperatures:

- 60min 37°C
- 20min 65°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 43/192: Inoculation of blue white screening
In Project: Level_2
With tags: screening

created: 12.05.2018 12:55
updated: 01.08.2018 19:21

Picking 5 white clones (blue white screening)

Topo Kit cloning seems not succesful: 1 large blue and 7 small white colonies were oberved on plate. Plate stored again into the inubator (37 °C)

5 clones are now in:

3ml LB-Amp (100 µg/ml)

After 2 h: 1 µl og x-GAL was added to the culture.

Cultures are stored at 37 °C 220rpm over night.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 44/192: New GoGate product transformation
In Project: Level_2
With tags: dueber, dh5a, LEU2, amp, E.coli, goldenGate, transformation, trafo

created: 15.05.2018 10:58
updated: 15.05.2018 11:27

Because of the fact that the harvesting of our GoGate products was not successful, we retried to transform our them into *E. coli*.

Transformation of p2iGEM0224, p2iGEM0226 - 0242 (2iGEM0242, 2iGEM0244 - 0260):

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 2 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 15.05.2018 11:14
updated: 15.05.2018 11:26
Entry 45/192: Topo cloning and transformation
In Project: Level_2
With tags: topo, cloning, lysC, trafo, transformation, gBlocks, dh5a, pdna, plasmid, vector

Yesterday we cloned our lysC into a TOPO vector with the TOPO TA cloning kit from Invitrogen (p2iGEM0241, 2iGEM0243)

Reagent	Amount
gBlock gene fragment	1 µl
Taq DNA Pol. 10x Rxn buffer	1 µl
dNTP	0.4 µl
Taq DNA pol.	2 µl
H20	5,6 µl

The samples are incubated for 30 min at 72°C.

3. TOPO cloning reaction (TOPO TA cloning kit, Invitrogen)

Reagent	Amount
gBlock product from Step 2	2,5 µl
Salt solution	1 µl
water	1,5 µl
TOPO vector	1 µl

The reagents are added to PCR tubes, mixed gently and incubated at room temperature for 30 minutes.

4. Transformation into E.coli DH5a according to the following protocol

- competent cells are thawed on ice for 5-10 min

- 2 µl from Step 3 incubation are used for transformation

- incubation for 30 min on ice

- heat shock: 45 sec 42°C

- incubation on ice for 5 min

- addition of 300 µl LB (sterile) and incubated at 37°C at 300rpm for 1 h

- centrifuge cells down, remove excessive supernatant, resuspend cells in remaining supernatant and spread on plate (LB Kan / x-Gal in DMSO (20mg/ml))

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 16.05.2018 16:10
updated: 17.05.2018 09:39
Entry 47/192: Overnight cultures
In Project: Level_2
With tags: incubation, blue white screening, lysC, promoter, fluorescent plasmid

Green-white screening

The the colonies with the different promoters were screened for the GFP dropout under the bluelight table

Overnight cultures were made with non fluorescent colonies: 2iGEM0242,2iGEM0244,2iGEM0245(2x),2iGEM0248,2iGEM0250, 2iGEM0251,2iGEM0252(2x),2iGEM0253(2x),2iGEM0255,2iGEM0256,2iGEM0257,2iGEM0258,2iGEM0259

- 17 culture tubes were filled with 3ml LB-medium (sterile)
- 30 µl ampicillin were added from a 10mg/ml stock (sterile)
- non fluorescent colonies were picked and added to the culture tube
- overnight incubation by 37°C

Overnight cultures of the strains with a topo LysC plasmid p2iGEM0241

Blue white screening.(Only the white coloniues were picked)

- 12 culture tubes were filled with 3ml LB-medium (sterile)
- 15 µl kamamycin were added from a 10mg/ml stock (sterile)
- white colonies were picked and added to the culture tube
- overnight incubation by 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert

created: 17.05.2018 15:13
updated: 17.05.2018 20:13

Entry 48/192: Testrestriction and pure yield miniprep

In Project: Level_2

With tags: Pure yield miniprep, miniprep, restriction, bsai, bsmi, Topo

Mini prep of the Plasmids p2iGEM0243(12x), p2iGEM0233 and p2iHEM0239 with the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 400µl column wash
- centrifuge 30 sec max rpm -> discard supernatant
- add 400µl column wash
- For elution use new tube
- 30 µl elution buffer on column
- Let incubate for 5 Min at RT
- Centrifuge for 15 Sek. max

Measured concentrations

p2iGEM02431	366.45 ng/µl
p2iGEM02432	641.35 ng/µl
p2iGEM02433	469.75 ng/µl
p2iGEM02434	641.7 ng/µl
p2iGEM02435	660.85 ng/µl
p2iGEM02436	750.85 ng/µl
p2iGEM02437	688 ng/µl
p2iGEM02438	531.6 ng/µl
p2iGEM02439	92,65 ng/µl
p2iGEM024310	223,6 ng/µl
p2iGEM024311	261.05 ng/µl
p2iGEM024312	358.96 ng/µl
p2iGEM0233	223,1 ng/µl
p2iGEM0239	409.15 ng/µl

Testrestriction of our topo-plasmids and the promotor-plasmids

p2iGEM0233,p2iGEM0239,p2iGEM0243

Master mix preparation

- 234µl MiliQ
- 30µl Cutsamart
- 6µl EcorI

The PCR tubes were prepared with 2µl of the Plasmids

Add 18µl of the mastermix to the PCR-tube

Restriction of the entry verctor of the dueber-toolbox(p2iGEM0001) and of p2iGEM0097 and p2iGEM0098

p2iGEM0001 restriction(2 tubes)

- 0,5 µl BsmbI
- 15,5µl MiliQ
- 2µl cutsmart
- 1µl template(500ng)

p2iGEM0097(3x) and p2iGEM0098(7x) restriction

Mastermix

- 0,5 µl BsaI
- 16,5µl MiliQ
- 2µl cutsmart
- 2µl template(500ng)

The PCR tubes were prepared with 2µl of the Plasmids

Add 18µl of the mastermix to the PCR-tube

Incubation: 37°C for 7h, 65°C for 20 min, 4° hold

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 49/192: Kryoculture
In Project: Level_2
With tags: Kryo

created: 18.05.2018 19:54
updated: 18.05.2018 19:55

Kryoculture of 2iGEM241

- 300µl glycerin **sterile**
- 700µl culture **sterile**

Storage -80°C in Box4

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 50/192: Inoculation for plasmid isolation
In Project: Level_2
With tags: inoculation

created: 20.05.2018 12:31
updated: 20.05.2018 14:55

Inoculation for plasmid isolation

Inoculated in 3ml LB with antibiotic treatment (Amp = 100 µg/ml; Kan 50 µg/ml)

Cultures were incubated overnight at 37°C, 220rpm.

2iGEM0125	p2iGEM0121
2iGEM0128	p2iGEM0124
2iGEM0129	p2iGEM0125
2iGEM0133	p2iGEM0129
2iGEM0178	p2iGEM0174
2iGEM0200	p2iGEM0196
2iGEM0204	p2iGEM0200

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 20.05.2018 14:41
updated: 23.05.2018 10:41
Entry 51/192: Plasmidisolation for Lvl2 stock
In Project: Level_2
With tags: Plasmid prep, miniprep, promega miniprep, miniprep kit

Plasmidisolation with Promega PureYield Plasmid Miniprep System

3 ml cultures were pulled & resuspended in 600 ul

100 ul Lysis Buffer was added (shaked)

350 ul Neutralization Buffer was added (shaked)

centrifuge for 3 min at max rpm

900 ul of supernatend filled into a prepared collumn

centrifugation 30 sec at max rpm

wash 2 times with 350 ul Column Wash Buffer (centrifugation 30 sec at max rpm)

centrifuge empty column 30 sec

Column into a new tube

Add 30 ul Elution Buffer & incubate for 3 min

centrifuge 30 sec at max rpm

measure the concentration

Storage: -20 °C (Lvl2)

Isolation for Lvl2 stock

p2iGEM0124	26,35 ng/ul
p2iGEM0129	26,2 ng/ul
p2iGEM0125	40 ng/ul
p2iGEM0121	47,9 ng/ul
p2iGEM0196	161,2 ng/ul
p2iGEM0200	203,1 ng/ul
p2iGEM0174	103,3 ng/ul

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 52/192: gel electrophoresis for testing and purification
In Project: Level_2
With tags: restriction, cassettes, backbone, gelelution

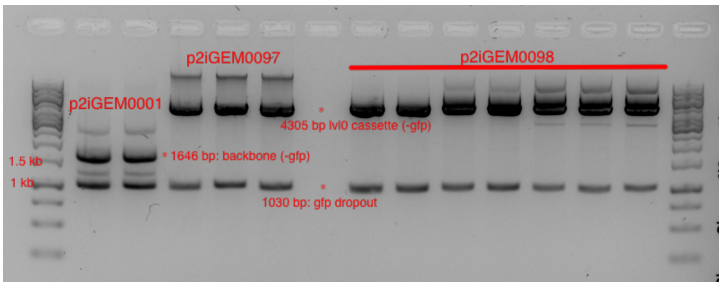
created: 20.05.2018 16:31
updated: 24.05.2018 16:54

1% agarose gel:

- 200 ml 1x TAE
- 2 g agarose
- 10 µl GelRed

Digested p2iGEM0001 (entry vector) and p2iGEM0097/0098 (lvl1 cassette) were applied on a gel and run at 80 V for 80 min.

Gel_2018-05-18_17hr_40min_Vorverdau_Entry_&_cassetten.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 53/192: PCR on lysC (psiGEM00243)
In Project: Level_2
With tags: 2step PCR, pcr, 2 step

created: 20.05.2018 19:47
updated: 20.05.2018 20:10

PCR on p2iGEM0243 (pTopo_*lysC*) to amplify *lysC*

lysC is needed for cloning into CIDAR Toolbox

Mastermix 10X!

50 ul	Q5 Buffer (5x)
50 ul	Q5 Enhancer
25 ul	Primer fwd (O_iGEM_013)
25 ul	Primer rev (O_iGEM_014)
5 ul	dNTPs
2 ul	Template DNA (Plasmid p2iGEM0243)
2,1 ul	Q5 Polymerase
15 ul	DMSO
72,5 ul	Milli Q Water

Mastermix was prepares in ice. 25 ul used per reaction

Template was pipettet NOT into the Mastermix but later on into the PCR tube.

PCR Protocol (2 step PCR)

98°C	30 sec	
98°C	10 sec	
72°C	35 sec	Repeat 25 cycles
72°C	2 min	
4°C	Hold	

PCR runs overnight.

25 cycles used to test if the run is more successful with less cycles.

35 sec elongation to test if the shorter elongation time is more successful.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 54/192: Inoculation for plasmid isolation
In Project: Level_2
With tags: inoculation

created: 21.05.2018 15:13
updated: 21.05.2018 15:18

Inoculation of Kryos for plasmidisolation

CIDAR plasmids needed for cloning with CIDAR toolbox

3 ml LB with antibiotic treatment: Amp (100 ug/ml) or Kan (50 ug/ml)

Incubation:

37°C, 220 rpm overnight

2iGEM0178	p2iGEM0174
2iGEM0200	p2iGEM0196
2iGEM0204	p2iGEM0200

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 55/192: Gelelectrophoresis: lysC & LEU2 (Lvl2)
In Project: Level_2
With tags: gelelectrophoresis

created: 21.05.2018 15:43
updated: 21.05.2018 15:53

Gelelctrophoresis of: PCR of *lysC* (p2iGEM0243) & *LEU2* (p2iGEM0075)

150 ml 1% agarose with 7 ul GelRed was used for gelelectrophoresis.

5 ul Ladder was loaded

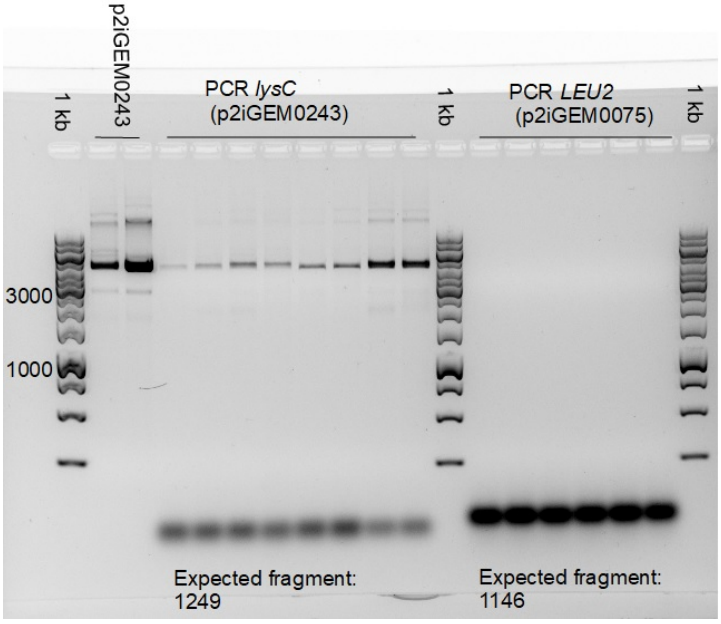
3 ul of loading dye added to PCR salmples (28 ul in total were loaded to gel / sample)

Programm:

100 min at 95V.

PCR was not successful.

Gel_2018-05-21_PCR_lysC_&_PCRLEU2_(Geldoc_Jenny)_LABELED.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter

created: 23.05.2018 10:36

updated: 23.05.2018 10:41

Entry 56/192: Isolation of plasmids (Kit)

In Project: Level_2

With tags: Plasmid prep, plasmid isolation, promega miniprep, miniprep kit

Plasmidisolation with Promega PureYield Plasmid Miniprep System

3 ml cultures were pulled & resuspended in 600 ul

100 ul Lysis Buffer was added (shaked)

350 ul Neutralization Buffer was added (shaked)

centrifuge for 3 min at max rpm

900 ul of supernatend filled into a prepared collumn

centrifugation 30 sec at max rpm

wash 2 times with 350 ul Column Wash Buffer (centrifugation 30 sec at max rpm)

centrifuge empty column 30 sec

Column into a new tube

Add 30 ul Elution Buffer & incubate for 3 min

centrifuge 30 sec at max rpm

measure the concentration

Storage: -20 °C (Lvl2)

Isolation of

p2iGEM0174	2iGEM0178
p2iGEM0196	2iGEM0200
p2iGEM0200	2iGEM0204

Concentrations were labled on the tubes.

Isolation was done in duplicates

Plasmids stored at -20°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 57/192: 3 step PCR of lysC
In Project: Level_2
With tags: 3 step PCR, pcr, amplification, PCR programm, 10X mastermix

created: 23.05.2018 10:41
updated: 23.05.2018 10:48

PCR on p2iGEM0243 (pTopo_*lysC*) to amplify *lysC*

lysC is needed for cloning into CIDAR Toolbox

Mastermix 10X!

50 ul	Q5 Buffer (5x)
50 ul	Q5 Enhancer
25 ul	Primer fwd (O_iGEM_013)
25 ul	Primer rev (O_iGEM_014)
5 ul	dNTPs
2 ul	Template DNA (Plasmid p2iGEM0243)
2,1 ul	Q5 Polymerase
15 ul	DMSO
72,5 ul	Milli Q Water

Mastermix was prepared on ice.

Template was added directly into the mastermix.

Programm:

98°C	30 sec	
98°C	10 sec	
60°C	20 sec	
72°C	40 sec	30 cycles
72°C	2 min	
4°C	Hold	

Tubes were stored at 4°C after the PCR.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 58/192: PCR
In Project: Level_2
With tags: pcr, ddh

created: 23.05.2018 14:02
updated: 24.05.2018 17:27

PCR on gDNA from *C. glutamicum* to amplify ddh

Template preparation:

Two template dilution were made from the stock(191 ng/µl)

- 1:10 dilution
- 1:100 dilution

Mastermix 11X!

55 ul	Q5 Buffer (5x)
55 ul	Q5 Enhancer
17 ul	Primer fwd (O_iGEM_001)
17 ul	Primer rev (O_iGEM_002)
5.5 ul	dNTPs
2.2 ul	Template DNA (Plasmid p2iGEM0243)
2.75 ul	Q5 Polymerase
16.5 ul	DMSO
103 ul	Milli Q Water

Mastermix was prepared on ice.

Template was added directly into the mastermix after it was splitted in half.

PCR tubes were filled with 25µl.

Programm:

98°C	30 sec	
98°C	10 sec	
59°C	20 sec	
72°C	30 sec	35 cycles
72°C	2 min	
4°C	Hold	

Tubes were stored at 4°C after the PCR.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 23.05.2018 15:45
updated: 23.05.2018 15:53
Entry 59/192: Gelelectrophoresis lysC
In Project: Level_2
With tags: gelelectrophoresis, amplification, lysC

Gelelectrophoresis of PCR on *lysC* (out of p2iGEM0243)

Gelectrophoresis performed on the 3 step PCR of lysC

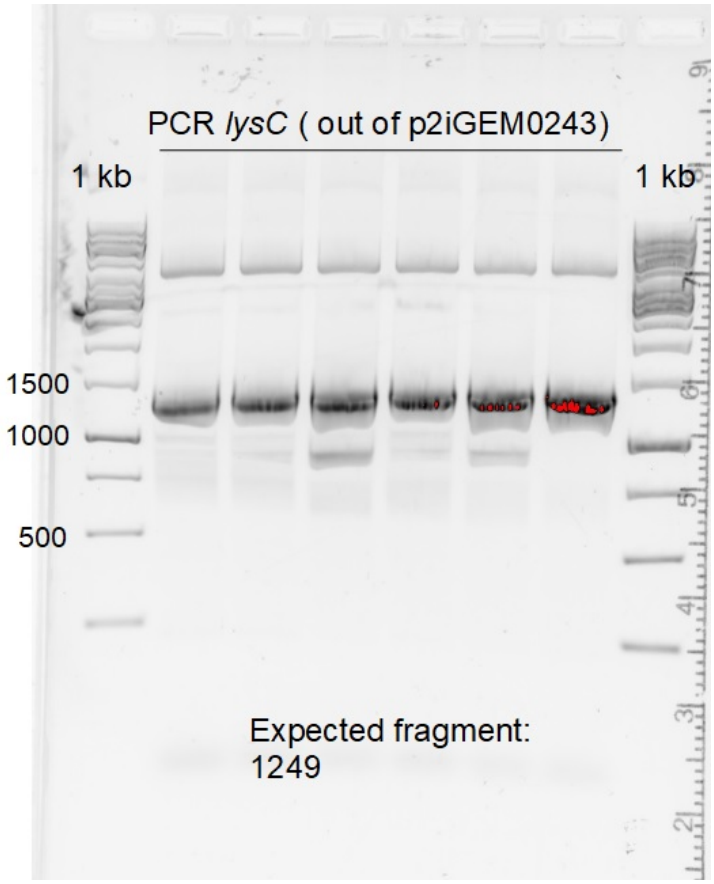
Samples were loaded with 3 ul loading dye (Thermo Fischer Scientific)

1 kb ladder was used (Thermos Fischer Scientific)

Gel conditions: 35 ml 1 % agarose gel with 2 ul GelRed

Gel run at 100V for 55 min.

Gel_2018-05-23_PCR_lysC_(out_of_topo_plasmid)_for_Gel_elution_LABELED.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 24.05.2018 16:16
updated: 24.05.2018 16:27
Entry 60/192: Gelelution of lysC
In Project: Level_2
With tags: gel elution, gelelution, elution, gel purification

Gel elution with the promega Kit to purify *lysC* gene

The bandy were cuttet with UV light conditions and the weight measured

Per 10 mg of gel 10 µl of Membrane binding buffer were added to the tube.

Incubation at 55 °C till the gel was diluted in the buffer (around 7 min)

Fill diluted gel into the column (incubate at room temperature for 1 minute)

centrifuge at 16000 g für 1 min -> discart the flow through

add 700 µl washing buffer and centrifuge again -> discart the flow through

add 400 µl washing buffer and centrifuge 5 min --> discart the flow through

centrifuge empty column for 1 min

Put column into a new tube

Add 30 µl of nuclease free water (from the kit) and elute for 1 h at room temperature

Centrifuge 1 min.

Measure the concentration

Store DNA at -20°C.

(In this case 2 of 6 bands were eluted. 4 Samples are frozen as gel (-20°C). 1 eluted sample was needed completely for an restriction as preparation for MoClo).

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter

created: 24.05.2018 16:31
updated: 24.05.2018 16:50

Entry 61/192: Restriction of lysC (gene) and DVA_CD (CIDAR Entry)

In Project: Level_2

With tags: restriction, restriction script

Restriction of lysC and p2iGEM0174 with BbsI as preparation for MoClo into CIDAR Toolbox.

3x restriction of p2iGEM0174 (equivalent to DVA_CD od CIDAR toolbox)

1x restriction of purified l*ysC* gene

1x restriction protocole:

1 µg	Template (DNA)
5 µl	Buffer (10x)
1 µl (10 units)	Enzyme
Fill up to 50 µl	Milli Q Water

Restriction with BbsI was done at 37°C for 1,5h

The reaction was inactivated at 62 °C for 15 minutes.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 24.05.2018 17:12
updated: 24.05.2018 18:16
Entry 62/192: Gelelution and -electrophoresis
In Project: Level_2
With tags: gel electrophoresis, ddh, gelelution, gel

The PCR products of yesterday were loaded on a 1% agarose gel

Agarose gel preparation:

- 150 ml 1x TAE buffer
- 1,5 g agarose

To the 25µl of the PCR product 3µl 6x-loading dye(Thermo Fischer) was added

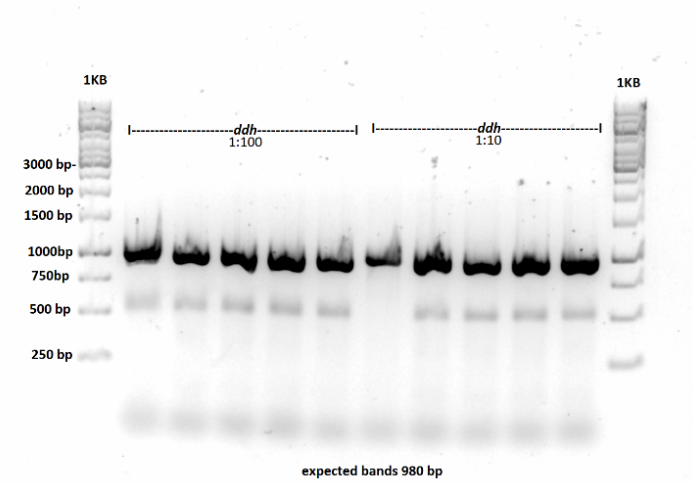
2µl of 1 kb ladder(Thermo Fischer) was used

On the right site the pcrproduct with 1:10 template dilution was added and on the left the 1:100 template dilution

The gel ran on 80V for 140 min

The band were cut afterwards to make a gel elution

Gel_2018-05-24_14hr_30min_ddh_aus_gDNA_85V_140_min_bearbeitet.tif



Gel elution with the promega Kit to purify *ddh* and *Leu2* gene

- The bandy were cut with UV light conditions and the weight measured
- Per 10 mg of gel 10 µl of Membrane binding buffer were added to the tube.
- Incubation at 55 °C till the gel was diluted in the buffer (around 7 min)
- Fill diluted gel into the column (incubate at room temperature for 1 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min --> discart the flow through
- add 500 µl washing buffer and centrifuge 5 min --> discart the flow through
- centrifuge empty column for 1 min
- Put column into a new tube
- Add 30 µl of nuclease free water (from the kit) and elute for 1 h at room temperature
- Centrifuge 1 min.
- Store DNA at -20°C.

Measured concentrations

ddh 1	20.1 ng/µl
ddh 2	15.4 ng/µl
ddh 3	13.9 ng/µl
ddh 4	19.05 ng/µl
ddh 5	23.45 ng/µl
Leu2 1	1.6 ng/µl
Leu2 2	2.6 ng/µl

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 63/192: No entry title yet
In Project: Level_2
With tags: Topo, taq-polymerase

created: 25.05.2018 14:24
updated: 25.05.2018 14:27

2. Elongation of *ddh* with taq-polymerase (according to IDT protocol) (Only the first two gBlocks)

Reagent	**Amount**
gene fragment	2 µl
Taq DNA Pol. 10x Rxn buffer	1 µl
dNTP	0.4 µl
Taq DNA pol.	1 µl
H20	5,6 µl

The samples are incubated for 30 min at 72°C.

3. TOPO cloning reaction (TOPO TA cloning kit, Invitrogen)

Reagent	**Amount**
gene product from Step 2	2,5 µl
Salt solution	1 µl
water	1,5 µl
TOPO vector	1 µl

The reagents are added to PCR tubes, mixed gently and incubated at room temperature (22°C-23°C) for 5 minutes. For longer products (>1kb) increase incubation time to up to 30 min.

After incubation place reaction on ice, store at -20°C or use immediately.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 26.05.2018 10:44
updated: 26.05.2018 11:05

Entry 64/192: CIDAR cloning: lysC in DVA_CD
In Project: Level_2
With tags: Cidar, CIDAR cloning, cloning

MoClo: lysC into DVA_CD

New plasmid is named: **p2iGEM0272**

Pipetting advice for 1 sample:

amount (µl)	Substance
0,5	Template (gBlock)
10ng	Entry vector
1	T4 Ligase
1,5	T4 Ligase Buffer
0,75	BsaI Enzyme
1	CutSmart Buffer (10x)
Fill to 15	Milli Q H2O

For the template the pre-cutted gene lysC was used.

cloning was performed with following protocole:

Temperature	Time	
37°C	20 min	
37°C	1,5 min	
16 °C	3 min	25X
16°C	1 h	
50°C	5 min	
80°C	10 min	
4°C	Hold	

Protocole run over night.

The samples will be used for a transformation.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 65/192: Topo transformation
In Project: Level_2
With tags: Topo, Transformation, X-gal

created: 26.05.2018 10:59
updated: 26.05.2018 12:25

Tranformation of p2iGEM0271, p2iGEM0272 and p2iGEM0047 into E.coli Top 10 according to the following protocol

- competent cells are thawed on ice for 5-10 min

- 1.5 µl from Step 3 incubation are used for transformation

- incubation for 30 min on ice

- heat shock: 45sec 42°C

- incubation on ice for 5 min

- addition of 300 µl LB (sterile) and incubated at 37°C at 300rpm for 1 h

- centrifuge cells down, remove excessive supernatant, resupend cells in remaining supernatant and spread on plate (LB Kan or Amp/ x-Gal in DMF (20mg/ml))

- incubate over night at 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 66/192: Overnightcultures
In Project: Level_2
With tags: blue white screening, ddh, Topo, overnight

created: 27.05.2018 12:43
updated: 27.05.2018 12:46

Blue white screening of 2iGEM0303(5 colonies were picked)

Overnightculture of 2iGEM0304

- preparation of 47ml LB with amp(100µg/ml)
- 15 culture tubes were filled with 3ml LB-medium (sterile)
- overnight incubation by 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 67/192: Plasmidprep & Testdigestion
In Project: Level_2
With tags: Plasmid prep, test restriction

created: 28.05.2018 13:34
updated: 28.05.2018 16:14

Mini prep of the Plasmids p2iGEM0271 and p2iGEM0272 with the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- + 200 µl endotoxin removal wash
- centrifuge 30 sec max rpm -> discard supernatant
- add 400µl column wash
- For elution use new tube
- add 30 µl 37°C miliQ-water on column
- Let incubate for 1h at RT
- Centrifuge for 15 Sek. max

Measured concentrations of the plasmids in ng/µl

p2iGEM0271 1	513.15
p2iGEM0271 2	508.95
p2iGEM0271 3	746.65
p2iGEM0271 4	847.05
p2iGEM0271 5	753.15
p2iGEM0272 1	220.45
p2iGEM0272 2	379.85
p2iGEM0272 3	343.90
p2iGEM0272 4	187.55
p2iGEM0272 5	209.10
p2iGEM0272 6	151.25
p2iGEM0272 7	358.15
p2iGEM0272 8	247.00
p2iGEM0272 9	381.25
p2iGEM0272 10	281.05

Testdigestion with the following protocol:

Mastermix:

- 2 µl *EcoRI* hf Enzyme (from NEB)
- 5 µl Cutsmart buffer
- 38 µl milli Q water

each tube was filled with 1µl Plasmid and 9µl Mastermix

in total 5 tubes were prepared with Plasmid 2iGEM0271

Digestion togehter with Ylenjas samples with these temperatures:

- 7h 37°C
- 20min 65°C

Storage at -20°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 68/192: No entry title yet
In Project: Level_2
No tags associated

created: 29.05.2018 13:57
updated: 30.05.2018 14:18

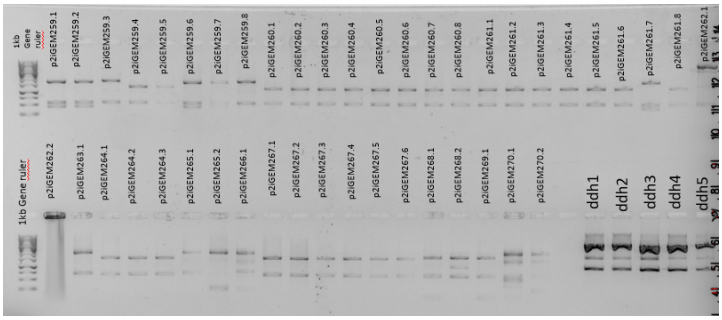
Gel electrophoresis of p2iGEM0271

- 10µl with 2µl loading dye were added to the gel
- 2µl 1 kb ladder(thermo fisher) was used

The gel was prepared together with Ylenja

Two bands were at the expected location, but an additional third one appeared which should not be there. It is possible that the enzyme cut unspecific because after the lobg incubation. Therefore the restriction was repeated a second time.

LAB.PNG



Second testrestriction with p2iGEM0271

Testdigestion with the following protocol:

Mastermix:

- 2 µl *EcoRI* hf Enzyme (from NEB)
- 5 µl Cutsmart buffer
- 38 µl milli Q water

each tube was filled with 1µl Plasmid and 9µl Mastermix

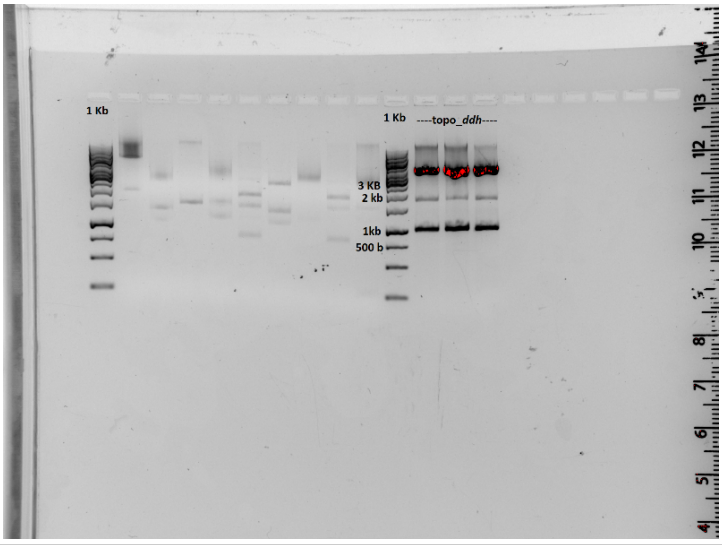
in total 5 tubes were prepared with Plasmid 2iGEM0271

Digestion togehter with Ylenjas samples with these temperatures:

- 1h 37°C
- 20min 65°C

The samples were afterwards loaded on a Gel

ddh_topo_2te.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 69/192: Testrestriction & Kryo
In Project: Level_2
With tags: test restriction, ddh, gel electrophoresis, Kryo

created: 30.05.2018 14:08
updated: 30.05.2018 16:00

Third testrestriction with p2iGEM0271 because the ladder did not separte properly on the second.

Testdigestion with the following protocol:

Mastermix:

- 2 µl *EcoRI* hf Enzyme (from NEB)
- 5 µl Cutsmart buffer
- 38 µl milli Q water

each tube was filled with 1µl Plasmid and 9µl Mastermix

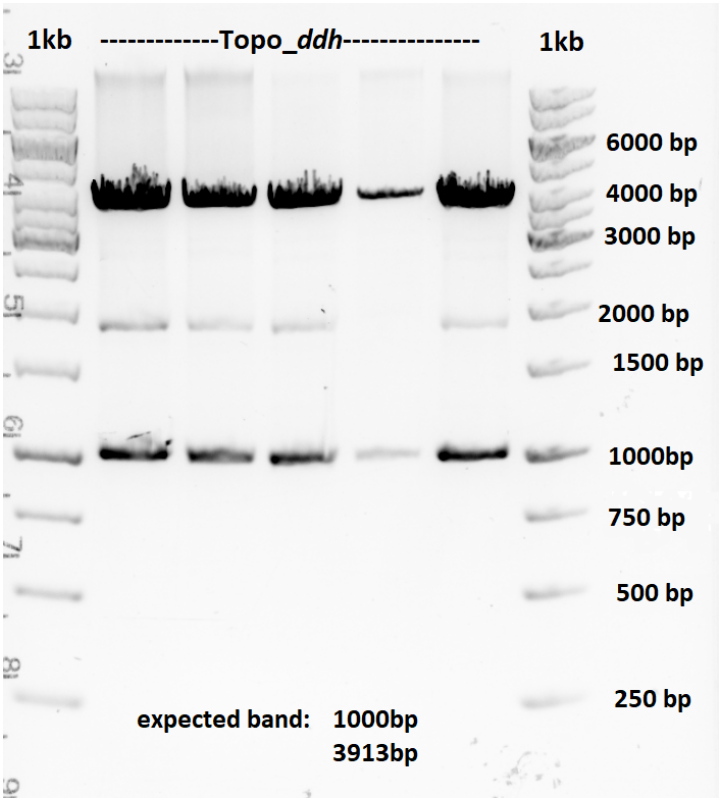
in total 5 tubes were prepared with Plasmid 2iGEM0271

- 1h 37°C
- 20min 65°C

The samples were afterwards loaded on a Gel with less volt than before

- 80V 75 min

Gel_2018-05-30_14hr_30min_topo_ddh_80V_70min_bearbeitet.tif



Kryoculture of 2iGEM0303 (the 4. one)

Add to a kryotube:

- 200µl glycerine (**sterile**)
- 800µl culture (**sterile**)

Storage at -80°C (Box4)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger

created: 01.06.2018 17:51
updated: 01.06.2018 18:28

Entry 70/192: Touchup PCR LEU2

In Project: Level_2

With tags: pcr, touchup, LEU2, agarose gel, dueber, q5

Mastermix 6X

30 µl	Q5 Buffer (5x)
30 µl	Q5 Enhancer
7.5 µl	Primer fwd (O_iGEM_005)
7.5 µl	Primer rev (O_iGEM_006)
3 µl	dNTPs
1 µl for each reaction	Template DNA (Plasmid p2iGEM0243)
1.5 ul	Q5 Polymerase
9 µl	DMSO
55.5 ul	MilliQ Water

Mastermix was prepared on ice.

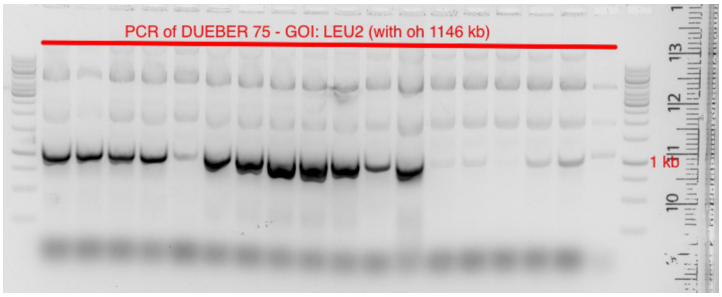
The template was added directly into the mastermix after it was split in half.

PCR tubes were filled with 25µl.

Programm:

98°C	30 sec	
98°C	10 sec	10 cycles
54 - 62°C (+0.8°C per cycle)	20 sec	
72°C	40 sec	
98°C	10 sec	25 cycles
62°C	20 sec	
72°C	40 sec	
72°C	2 min	
4°C	Hold	

Gel_2018-05-27_13hr_28min_LEU2_PCR_touchup,_90_V,_80_min.jpg



The PCR products were applied on a gel (1% agarose, 1x TAE, 90 V, 80 min., 1 kb Generuler from Thermo Scientific) and later purified.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 71/192: DNA Gel Extraction
In Project: Level_2
With tags: gel purification, gelelution, LEU2, dueber

created: 01.06.2018 18:29
updated: 01.06.2018 18:37

We cut our LEU2 bands out of our gel and put it in a 2 ml reaction tube. Then we used the Wizard® SV Gel and PCR Clean-Up System from Promega:

We added 1 ml of Membrane Binding Solution to the gel slices and incubated it for 10 min. at 60°C until the gel slices dissolved.

Then we loaded 350 µl of the gel solution into the SV minicolumn with collection tube, let it incubate for 1 min at RT and centrifuge it for 1 min at 16.000 x g. We discarded the supernatant. These steps were repeated until the whole gel solution was filtered through the column membrane.

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min. We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°) in these steps:

-adding 30 µl 37°C warm MilliQ to the membrane.

-incubate membrane at 37°C shook at 300 rpm.

-centrifuge for 1 min at 50 x g

-centrifuge for 1 min at 16.000 x g

Later we measured the concentration of our LEU2 PCR product residue: 165,7 ng/µl

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 01.06.2018 18:37
updated: 01.06.2018 18:57
Entry 72/192: GoGate pYTK001_LEU2
In Project: Level_2
With tags: LEU2, backbone, cloning, goldenGate, golden gate

GoGate Assembly of **p2iGEM0209**

Mastermix (6x):

- 4.5 µl T4 Ligase
- 18 µl T7 Buffer (10x)
- 4.5 µl BsmBI
- 3 µl PCR LEU2_oh (~75 ng)
- 7.2 µl p2iGEM0001 (digested! ~7,5 ng)
- 142.8 µl H_2O

-per reaction 30 µl.

-used PCR program: "GoGate BsmBI", overnight

Thermocycler (Programm GoGate BsmBI)

- 60 cycles of digestion and ligation (42°C for 2 min, 16 °C for 5 min),
- final digestion step (60 °C for 10 min)
- a heat inactivation step (80 °C for 10 min).
- Hold at 4°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 73/192: transformation pYTK001_LEU2
In Project: Level_2
With tags: LEU2, dueber, trafo, transformation

created: 01.06.2018 18:57
updated: 01.06.2018 19:00

Transformation of p2iGEM0209

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 74/192: Test Digest p2iGEM0209
In Project: Level_2
With tags: dueber, agarose gel, gel, electrophoresis, gel electrophoresis, gelelectrophoresis, restriction, digest, LEU2

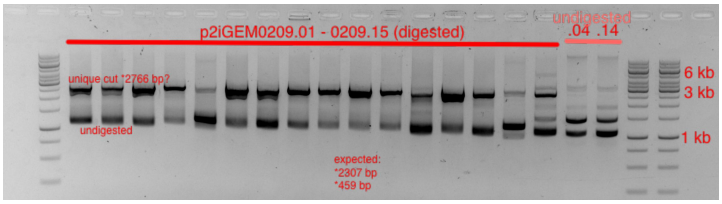
created: 01.06.2018 19:00
updated: 01.06.2018 19:52

Mastermix (16x):

- 2 µl template per reaction
- 8 µl EcoRI
- 8 µl BamHI
- 32 µl µl Cutsmart
- 240 µl MilliQ

20 µl per reaction.

Gel_2018-06-01_19hr_18min_p2iGEM0209_Test_digest_90V,_80_min.jpg



1% agarose gel, 1x TAE, GelRed, 90V, 80 min., 1kb GeneRuler Thermo Scientific, p2iGEM0209, digested and undigested.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 04.06.2018 15:03
updated: 04.06.2018 15:21

Entry 75/192: bsaI recognition site mutagenisis of ddh
In Project: Level_2
With tags: Mutagenesis, gradient pcr, ddh

Mutagenesis of p2iGEM0271(topo_ddh) to remove the recognitionsite of BsaI in the gene of *ddh*

6x Mastermix

- 12µl plasmid(10ng)
- 12 µl primer Mutagenic-Primer_ddh_BsaI
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 189 µl miliQ(RNAse free water)

6 tubes werefilled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - gradient 71°C - 65°C 30 sec
  - 72 °C 4 min
  - repeat this 30 times
- 72°C 5 min
- hold 4°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 04.06.2018 16:01
updated: 01.08.2018 19:24
Entry 76/192: Test PCR on p2iGEM0272 (lysC in DVA_CD)
In Project: Level_2
With tags: pcr, cidar, lvl0

PCR of lysC in p2iGEM0272 (DVA_CD_*lysC*)

Used primers: O_iGEM0013 & O_iGEM0014 (*lysC* fwd. & lysC rev)

PCR was performed with Q5 polymerase and a 3 step PCR protocole

12 times for 10 samples & 1 controle

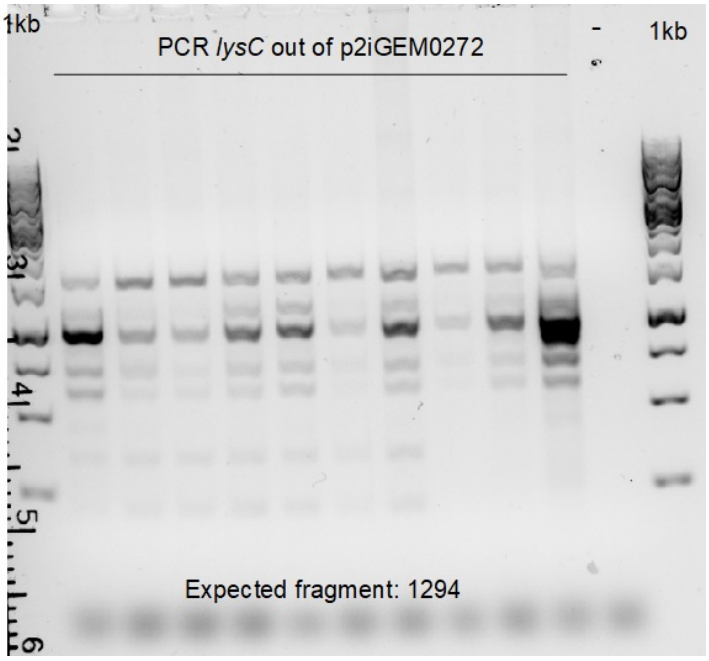
60 µl	Buffer (Q5)
60 µl	Enhancer (Q5)
20 µl	Primer fwd
20 µl	Primer rev
6 µl	dNTPs
3 µl	Q5 Polymerase
105 µl	Milli Q Water
20µl	DMSO

Following protocole was used:

Temperature	Time	
98°C	30 sec	
98°C	10 sec	
60°C	20 sec	
72°C	40 sec	Repeat 30 times
72°C	2 min	
4°C	Hold	

Afer PCR a gelelectrophoresis showed the amplifyed fragments.

Geldoc_Image_PCR_lysC_out_of_p2iGEM0272.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 77/192: CIDAR lvl0 cloning
In Project: Level_2
With tags: gogate, Cidar

created: 04.06.2018 16:15
updated: 01.08.2018 19:25

CIDAR cloning of lysC into DVA_CD (plasmidname: p2iGEM0272)

The cloning into the CIDAR entry was repeated. The cloning is running over night with follwing protocole:

Temperature	Time	
37°C	20 min	
37°C	1,5 min	
16 °C	3 min	25X
16°C	1 h	
50°C	5 min	
80°C	10 min	
4°C	Hold	

The pipetting scheme PER SAMPLE was:

amount (µl)	Substance
0,5	Template (gBlock)
10ng	Entry vector
1	T4 Ligase
1,5	T4 Ligase Buffer
0,75	BsaI Enzyme
1	CutSmart Buffer (10x)
Fill to 15	Milli Q H2O

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 78/192: Trafo of mutagenised Plasmids
In Project: Level_2
With tags: Transformation

created: 05.06.2018 15:44
updated: 05.06.2018 20:52

To the mutagenetic products of yesterday 1µl dnpI was added

- the products were filled in a new tube
- 1µl DnpI was added
- incubation 1h at 37°C

Trafo of the mutagenised Plasmid

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 2 h at 37°C and 300 rpm
- cells were plated out (LB+Kab+X-gal) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 81/192: Overnightculture
In Project: Level_2
No tags associated

created: 06.06.2018 21:59
updated: 06.06.2018 22:01

Overnightculture of 2iGEM0307

- 3ml LB
- 15µl Kan

Incubation over night at 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 82/192: Mutagenesis & Miniprep
In Project: Level_2
With tags: Mutagenesis, ddh, Pure yield miniprep, miniprep

created: 07.06.2018 10:27
updated: 07.06.2018 14:23

Mutagenesis of p2iGEM0271(topo_ddh) to remove the recognitionsite of BsaI in the gene of *ddh*

6x Mastermix

- 12µl plasmid(10ng)
- 12 µl primer Mutagenic-Primer_ddh_BsaI
- 12 µl primer Mutagenic-Primer_ddh_BsaI_rv
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 177 µl miliQ(RNAse free water)

6 tubes werefilled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - gradient 71°C - 65°C 30 sec
  - 72 °C 4 min
  - repeat this 30 times
- 72°C 5 min
- hold 4°C

To the mutagenic products 0.5 µl dnpI was added

- the products were filled in a new tube
- 0.5µl DnpI was added
- incubation 1h at 37°C

Miniprep of the mutagenic plamsid p2iGEM0290 from the overnight cultures according to the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 400µl column wash
- centrifuge 30 sec max rpm -> discard supernatant
- add 400µl column wash
- For elution use new tube
- 30 µl elution buffer on column
- Let incubate for 1h Min at RT
- Centrifuge for 15 Sek. max

Measured concentrations:

Plasmid	Concentration in ng/µl
p2iGEM0290 sample 1	360.55
p2iGEM0290 sample 2	623.55
p2iGEM0290 sample 3	831.85
p2iGEM0290 sample 4	478.85

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 07.06.2018 13:36
updated: 01.08.2018 19:25
Entry 83/192: Heat shock transformation of CIDAR cloning
In Project: Level_2
With tags: transformation, cidar

Transformation of CIDAR cloning samples into *E. coli* Top_10

Transformation was performed:

15 min incubation of competent cells on ice.

2 µl of cloning samples were added to 15 µl of competent cells and incubated on ice for 30 min

heatshock at 42°C for 40 sec

add 350 µl LB (without antibiotic treatment)

Incubation at 37°C at 300 rpm for 1,5 h

100% of the cells were plated at LB_Amp (100 µg/ml)

Incubation at 37°C over night

On the next day 10 cultures were inoculated in 3 ml LB_Amp (100 µg/ml) and incubated over night at 37 °C, 220 rpm

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 07.06.2018 13:46
updated: 01.08.2018 19:26
Entry 84/192: Plasmidisolation of p2iGEM0272
In Project: Level_2
With tags: miniprep

Plasmid isolation out of 2iGEM0304 (p2iGEM0272)

The plasmids were isolated to check further the success of the cloning.

sample 1	103 ng/µl
sample 2	77 ng/µl
sample 3	115,7 ng/µl
sample 4	195,6 ng/µl
sample 5	117 ng/µl
sample 6	155,7 ng/µl
sample 7	102,8 ng/µl
sample 8	118,7 ng/µl
sample 9	188,2 ng/µl
sample10	118,5 ng/µl

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 85/192: test digest p2iGEM0209
In Project: Level_2
With tags: agarose gel, restriction, dueber, digest, gel, gelelectrophoresis, electrophoresis, gel electrophoresis, LEU2

created: 08.06.2018 11:08
updated: 08.06.2018 11:30

p2iGEM0209:

Mastermix (15x):

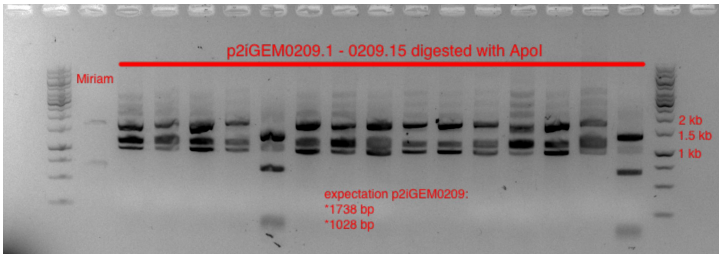
- 1 µl template per reaction
- 7.5 µl ApoI
- 30 µl µl Cutsmart
- 247,5 µl MilliQ

20 µl per reaction.

After reaction 4 µl Loading Dye (6x) was added.

Miriam: 3 µL overlap PCR NSIa + ori, 1 yL 6x LD

Gel_2018-06-04_22hr_47min_p2iGEM0209_ApoI.jpg



The shown gel contains 1% agarose, 1x TAE, 10 µl GelRed, 1kb GeneRuler Thermo Scientific, p2iGEM0209 (1-15), digested with ApoI. The gel run took 90V and 80 min.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 86/192: Inoculation of culture 2iGEM0178
In Project: Level_2
With tags: inoculation

created: 08.06.2018 18:11
updated: 01.08.2018 19:29

Inoculation of 2iGEM0178 to isolate p2iGEM0174

3ml LB with 100 µg/ml Amp

incutabte at 37°C over night (07.06.2018 - 08.06.2018)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter Entry 87/192: No entry title yet In Project: Level_2 No tags associated	created: 08.06.2018 18:13 updated: 08.06.2018 18:13
Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 88/192: Trafo
In Project: Level_2
With tags: Transformation

created: 09.06.2018 18:33
updated: 09.06.2018 18:38

Trafo of p2iGEM0290 in E.coliTOP10

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 2 h at 37°C and 300 rpm
- cells were centrifuged at 6000 rpm for 5 min (no visible pellet. Maybe the competent cells didn't work)
- remove 200µl supernatant
- cells were plated out (LB+Amp) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 11.06.2018 13:01
updated: 12.06.2018 11:05

Entry 89/192: Testrestriction & gel electrophoresis
In Project: Level_2
With tags: gel electrophoresis, ddh, test restriction

Testrestriction to check if the mutagenisis of 2iGEM0271 was successful

4x Mastermix

- 30.4 µl MiliQ-water
- 4 µl Cutsmart
- 1.6 µl BsaI

4 PCR-tubes were filled with 1µl template and 9µl of the mastermix

Incubation for 2h at 37°C

Directly after the incubation the samples are loaded on an agarose gel

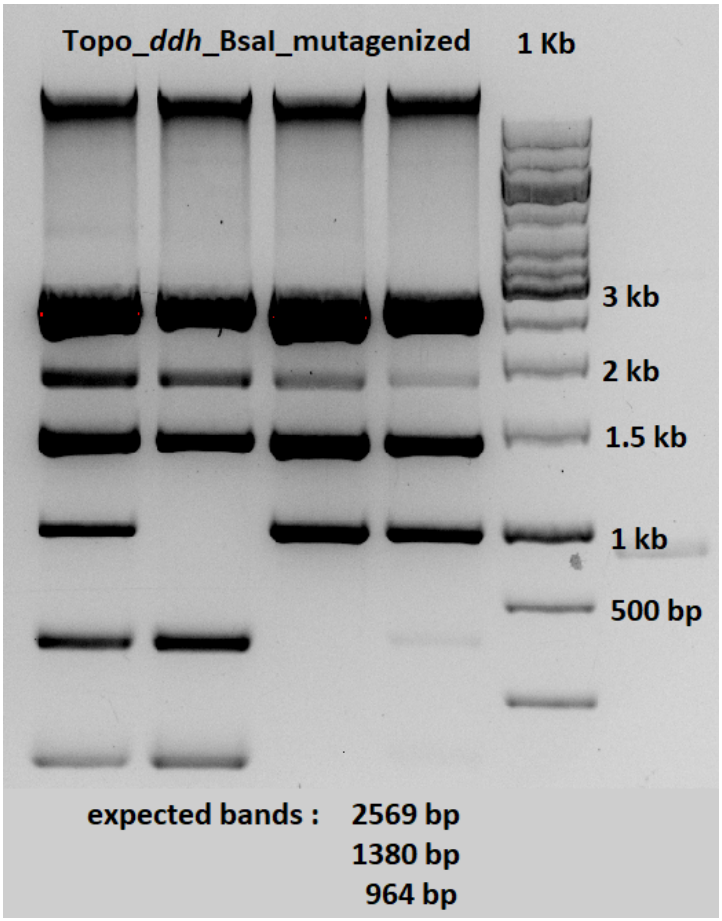
Gel electrophoresis of the samples loaded on the gel togehter with Miriams PCR product

the samples were loaded with 1µl 6xloading buffer(Thermo Fischer)

2µl 1kb genruler ladder was added (Thermo Fischer)

The gel ran on 80V for 1h

Gel_2018-06-11_16hr_26min_80V_60_min_ddh_mutageniesiert_&_touchdown_von_Miriam_bearbeitet.bmp



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 90/192: Restriction test
In Project: Level_2
With tags: agarose gel, LEU2, digest, dueber, electrophoresis, gel, gel electrophoresis, gelelectrophoresis, restriction

created: 12.06.2018 14:50
updated: 13.06.2018 23:37

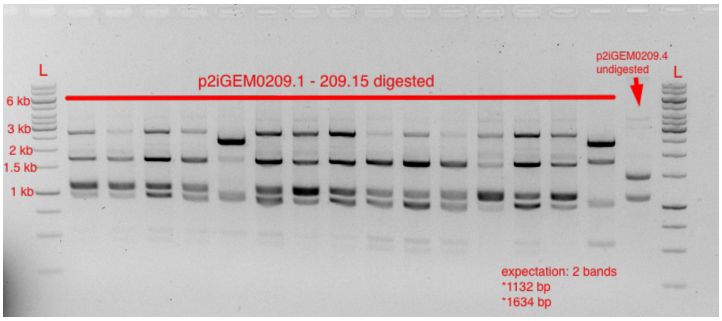
Mastermix (15x):

- 1 µl template per reaction
- 7.5 µl EcoRI
- 7.5 µl PvuII
- 30 µl µl Cutsmart
- 225 µl MilliQ

20 µl per reaction.

After reaction 4 µl Loading Dye (6x) were added.

Gel_2018-06-06_15hr_51min_p2iGEM0209_cut_with_EcoRI_and_PvuII.jpg



The shown gel contains 1% agarose, 1x TAE, 8 µl GelRed, 1kb GeneRuler Thermo Scientific, p2iGEM0209 (1-15), digested with ApoI and one sample of undigested p2iGEM0209.4. The gel run took 90V and 80 min.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 91/192: Last test restriction for p2iGEM0209
In Project: Level_2
With tags: LEU2, agarose gel, digest, dueber, electrophoresis, gel, gel electrophoresis, gelelectrophoresis, restriction

created: 12.06.2018 14:50
updated: 14.06.2018 00:04

p2iGEM0209 (no. 2 or no. 13):

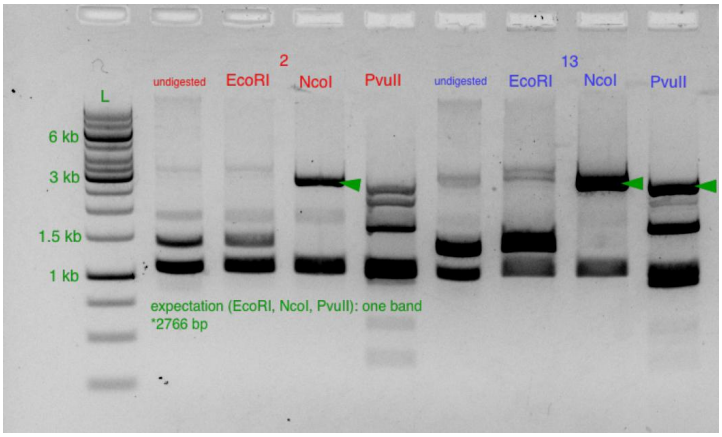
Each reaction contained:

- 1 µl template
- 0.5 µl restriction enzyme
- 2 µl µl Cutsmart
- 16,5 µl MilliQ

20 µl per reaction.

After reaction 4 µl Loading Dye (6x) were added.

Gel_2018-06-12_20hr_12min_Salima._80V,_80_min,_p2iGEM0209_digested.jpg



The shown gel contains 1% agarose, 1x TAE, 8 µl GelRed, 1kb GeneRuler Thermo Scientific, p2iGEM0209 (2 & 13), undigested and digested with EcoRI, NcoI, PvuII. The gel run took 80V and 80 min. (EcoRI: GOI, NcoI/PvuII: Backbone)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 13.06.2018 00:23
updated: 01.08.2018 19:30

Entry 92/192: PCR on p2iGEM0174 to check for contaminations
In Project: Level_2
With tags: cidar, Q5 PCR, 3 step PCR

Isolation of p2iGEM0174 out of the Kryo Stock Nr. 2iGEM0178

PCR with 4 samples of p2iGEM0174 with Q5 Polymerase

1 Reaction	
µl	Substance
5	Q5 Reaction Buffer (5x)
5	Q5 Enhancer
2,5	Primer fwd (10 µM stock)
2,5	Primer rev (10 µM Stock)
0,5	dNTPs
0,2	Template DNA (dependent on concentration)
0,25	Q5 Polymerase
7,75	Milli Q H2O
1	DMSO

The PCR was performed with 4 samples, 1 Water control and 1 Mastermix control.

Conditions:

Programm			
Temperature	Time		
98°C	30 sec		
98°C	10 sec		
61°C	20 sec		
72°C	35 sec	Repeat 30 times	
72°C	2 min	*Programm for lysC	
4°C	Hold		

Gelelectrophoresis was performed direktly after the PCR.

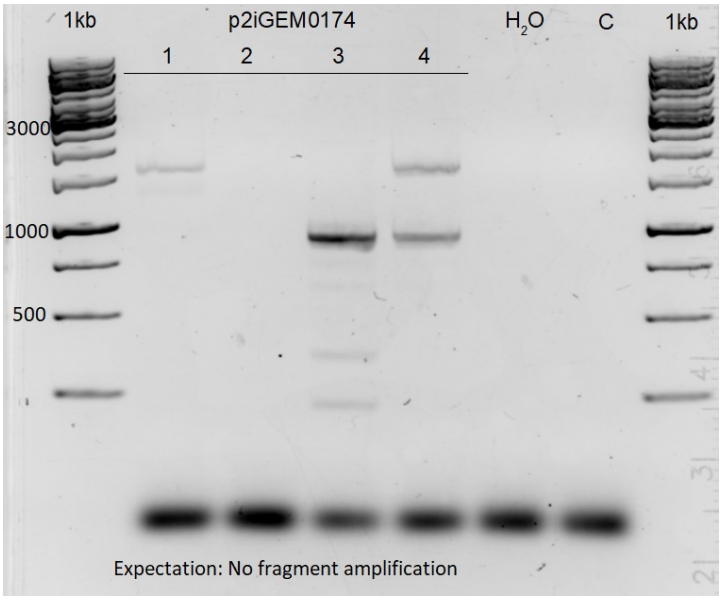
This experiment was performed to check the CIDAR Entry vector, bacause we think the Kryo Stock could be contaminated.

The Gelelectrophoresis shewed amplified fragments which were unexpected.

Just the sample Nr. 2 showed no amplification.

This sample will be transformed and checked then further to gain a new not contaminated Kryo-culture.

Check_p2iGEM0174_for_contaminations.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 93/192: Trnasformation
In Project: Level_2
With tags: transformation

created: 13.06.2018 00:44
updated: 13.06.2018 00:48

Transformation of the not contaminated sample of p2iGEM0174 (CIDAR Entry) into E. coli T 10.

The heat shock transformation was used.

After 80 min incubation at 37 °C at 300rpm the cells were plated 100% on LB_Amp and incubated o/n at 37 °C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 13.06.2018 10:16
updated: 13.06.2018 10:48
Entry 94/192: Testrestriction & gelelctrophoresis
In Project: Level_2
With tags: test restriction, gel electrophoresis

(12.06.2018)

Because an unexpected band appeared at 2 kb in the last testrestriction, another one was made in order to see ich this could be the undigested coiled plamsid.

This time the plasmid concentration was diluted by the factor 10. In addition undigetsted plasmid were filled on the gel to compare those bands.

Samples 1 are from the same plasmidtemplate and the samples 2 are the same plasmidtemplate

Testrestriction:

4x Mastermix

- 30.4 µl MiliQ-water
- 4 µl Cutsmart
- 1.6 µl BsaI

4 PCR-tubes were filled with 1µl template(1:10) and 9µl of the mastermix

Incubation for 5h at 37°C

Directly after the incubation the samples are loaded on an agarose gel

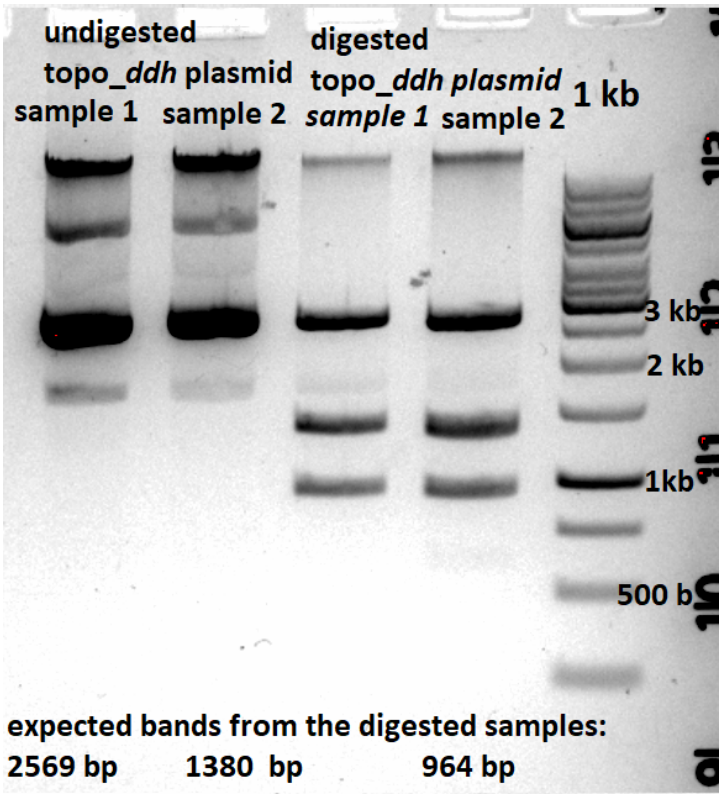
Gel electrophoresis of the samples loaded on the gel togehter with Salima's PCR product

the samples were loaded with 1µl 6xloading buffer(Thermo Fischer)

2µl 1kb genruler ladder was added (Thermo Fischer)

The gel ran on 80V for 90 min

Gel_2018-06-12_20hr_12min_Sarah_Salima._80V,_80_min,_p2iGEM0209_digested_bearbeitet.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 14.06.2018 00:05
updated: 14.06.2018 00:14
Entry 95/192: Golden Gate
In Project: Level_2
With tags: LEU2, golden gate, goldenGate, dueber, BsmbI

Gogate reaction: LEU2 (PCR p2iGEM0075) + Entry (p2iGEM0001) = p2iGEM0209

This was done according to the following protocol:

- 100 ng GOI (PCR LEU2) -> 0.6 µl
- 10 ng pre-digested Entry vector (p2iGEM0001) -> 1.5 µl
- 1 µl T7 ligase
- 1.5 µl T4 ligase buffer
- 0.75 µl BsmbI
- water up to 15µl (9.65 µl).

Reaction conditions:

1) 55°C --> 2 min

2) 16°C --> 5 min

1+2: 65 cycles

3) 60°C --> 10 min

4) 80°C --> 10 min

5) 4°C --> hold

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 14.06.2018 00:21
updated: 14.06.2018 00:22
Entry 96/192: Testing of pre-digested Entry/Cassettes
In Project: Level_2
With tags: backbone, cassette, test, entry, entry vector, LEU2, dueber

5 µl of each sample was applied to a 1% agarose gel.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 14.06.2018 16:08
updated: 14.06.2018 17:18

Entry 97/192: Colony PCR with Taq polymerase
In Project: Level_2
With tags: Colony PCR, Taq colony pcr

After the transformation (2iGEM0178) a ColonyPCR with 8 clones was performed.

Protocole for 1x Colony PCR

1 Reaction	
µl	Substance
2,5	10X Buffer
0,5	dNTPs
0,5	Primer fwd
0,5	Primer rev
-	DNA Template
0,125	Taq-Polymerase
20,5	Milli Q Water
0,5	DMSO

Samples were picked and plated onto a new plate. The rest of the sample was used for the ColonyPCR.

Plate was incubated at 37 °C.

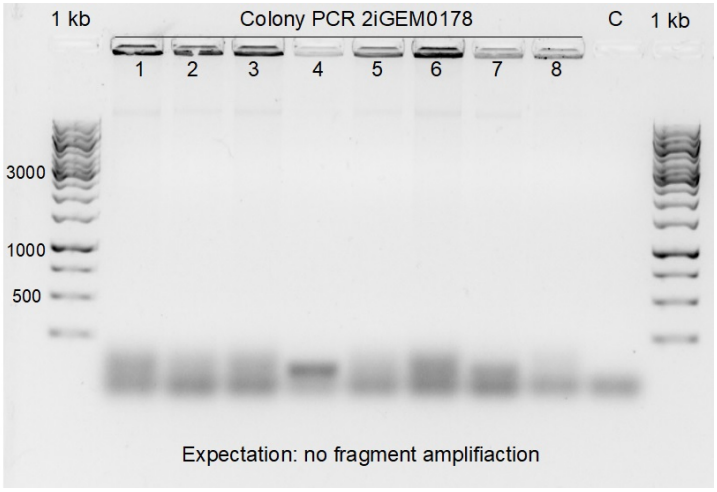
The PCR programm

Colony PCR (Taq Polymerase)			
Programm			
Temperature	Time		
98	10 min		
98	20 sec		
60 (58-68)	25 sec		
68	80 sec	20-30 cycles	
68	5 min		
4	Hold		

Here 25 cycles were used.

After the PCR gelelectrophoresis showed the results.

Colony_PCR_Jenny_p2iGEM0174_Beschriftet.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 98/192: Transformation p2iGEM0209
In Project: Level_2
With tags: LEU2, trafo, transformation, lvl0, dueber

created: 14.06.2018 16:36
updated: 14.06.2018 20:30

Transformation of p2iGEM0209 (GoGate 14.06.2018)

- Thaw competent cells 10 minutes on ice
- Add 3 µl plasmid (p2iGEM0209) in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 45 min. at 37°C and 300 rpm
- cells were plated out (LB+Cam) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 99/192: Inoculation in LB
In Project: Level_2
With tags: inoculation

created: 14.06.2018 17:11
updated: 01.08.2018 19:32

Inoculation of clone 4 of the ColonyPCR.

3 ml LB with 100 µg/ml Amp

Incubation o/n 37 °C, 220 rpm.

Plasmidisolation will follow with another test PCR.

If the plasmid is not contaminated a new 2iGEM0178 will be prepared.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 100/192: Sequencing order for p2iGEM0174 & p2iGEM0272
In Project: Level_2
With tags: sequencing

created: 14.06.2018 17:14
updated: 01.08.2018 19:33

Sequencing order for plasmids

p2iGEM0174 (2 samples) with primers: O_iGEM0068 & O_iGEM0069

p2iGEM0273 (2 samples) with primers: O_iGEM 0013 & O_iGEM0014

The p2iGEM0272 was orderd to check if the cloning worked - even the gel showed unexpected fragments.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 18.06.2018 12:54
updated: 01.08.2018 19:34
Entry 101/192: Test PCR on p2iGEM0174 (to check for contamination)
In Project: Level_2
With tags: taq pcr, taq polymerase, pcr

To test the p2iGEM0174 plasmid 2 different PCRs were performed.

2 times 4 PCRs with Taq polymerase.

One time primers 68 / 69 and one time 13 / 14.

2 Mastermixes (4x Mastermix) were prepared

PCR Taq Polymerase	
µl	Substance
2,5	10X Buffer
0,5	dNTPs
0,5	Primer fwd (10 µM stock)
0,5	Primer rev (10 µM Stock)
-	Template DNA (under 1 µg)
0,125	Taq Polymerase
fill up to 15	Milli Q Water

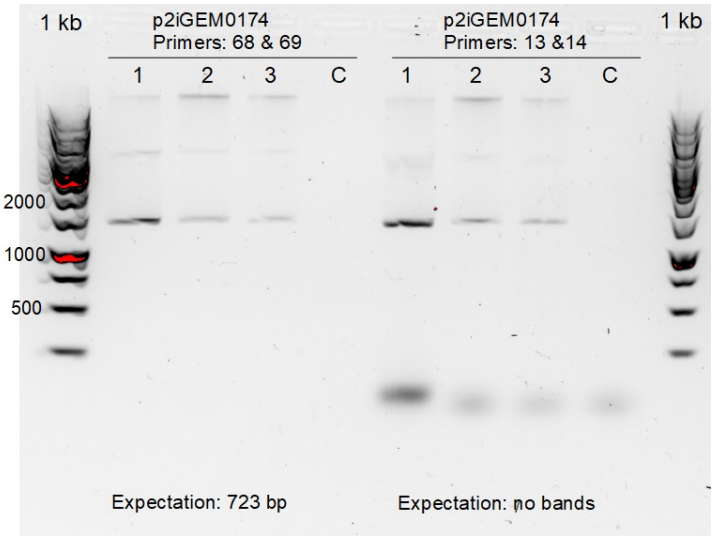
The Programm was:

PCR Taq Polymerase		
Temperature	Time	
98	30 sec	
98	15 sec	
58-68	20 sec	
68	1 min /kb	25 cycles
68	2 min	
4	Hold	

Here the elongationtime was 1 minute.

The longest expectet fragment was around 730 bases.

p2iGEM0174,_different_primers.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 102/192: No entry title yet
In Project: Level_2
No tags associated

created: 18.06.2018 13:37
updated: 20.06.2018 18:39

(15.06.2018)

Mutagenesis of p2iGEM0271(topo_ddh) to remove the recognitionsite of BbsI in the gene of *ddh*

6x Mastermix

- 12µl plasmid(10ng)
- 12 µl primer Mutagenic-Primer_ddh_BbsI
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 177 µl miliQ(RNAse free water)

6 tubes werefilled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - gradient 75°C - 65°C 30 sec
  - 72 °C 4 min
  - repeat this 30 times
- 72°C 5 min
- hold 4°C

To the mutagenic products 0.5 µl dnpI was added

- the products were filled in a new tube
- 0.5µl DnpI was added
- incubation 1h at 37°

Trafo of the mutagenised Plasmid

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 2 h at 37°C and 300 rpm
- cells were plated out (LB+Kan) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 18.06.2018 16:50
updated: 01.08.2018 19:35

Entry 103/192: Sequencing order for p2iGEM0174
In Project: Level_2
With tags: sequencing

To check the p2iGEM0174 (CIDAR Entry) the sequencing of the plasmid was ordered.

Two primer pairs were used for sequencing reactions:

1) O_iGEM0013 + O_iGEM0014 (normally no binding at all)

2) O_iGEM0068 + O_iGEM0069 (Binding expected.

Sequencing data will show if the sequence of the plasmid is as expected.

10 µl in total per reaction.

2.5 µl of primer + 500 ng of plasmid. Filled up to 10 µl with Milli Q Water.

4 reactions in total for 2 primer pairs.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 104/192: Miniprep & testristriction
In Project: Level_2
With tags: ddh, miniprep, test restriction

created: 20.06.2018 18:37
updated: 20.06.2018 18:51

(19.6)

Overnightculture

- From the plates one colonie for each retransormed culture was picked
- 3ml LB + 15 µl Kan

Miniprep of the BbsI mutagenic plamsid p2iGEM0293 from the overnight cultures according to the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 400µl column wash
- centrifuge 30 sec max rpm -> discard supernatant
- For elution use new tube
- 30 µl elution buffer on column
- Let incubate for 1h Min at RT
- Centrifuge for 15 Sek. max

Plasmid	Concentration in ng /µl
p2iGEM0293 1	610.45
p2iGEM0293 3	783.25
p2iGEM0293 5	760.10
p2iGEM0293 7	701.00
p2iGEM0293 9	611.00
p2iGEM0293 11	701.09

Testdigesion of the isolated Plasmid p2iGEM0293

6 x Mastermix

- 52,2 µl MiliQ-water
- 6 µl cutsmart
- 2 µl BbsI

6 PCRtubes were filled with 1 µl of 1:10 diluted plasmids

Add 9µl of the mastermix

incubate at 37°C overnight

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 105/192: No entry title yet
In Project: Level_2
No tags associated

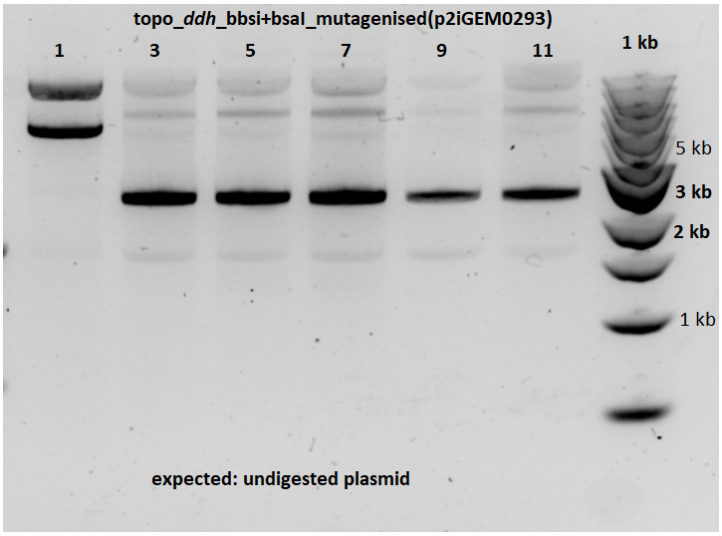
created: 21.06.2018 14:03
updated: 21.06.2018 14:13

Gelelctrophoresis of testdigested 2iGEM0293(Topo_*ddh*_BsaI+BbsI_mutagenised)

- 2µl purple loading dye (New England Biolabs) were added to the templates
- 2µl 1 kb ladder (New England Biolabs) wereloaded on the gel
- 12µl of the samples were loaded on the gel
- the gel ran on 80V for 65min

If the mutagenesis was successful, bands of an undigested plasmid should occur on the gelpicture like the bands from the gelelctrophoresis from 12.06.2018
If the mutagenesis was not successful and BbsI has cut, a band at 4916 bp should be visible.

testverdau_bbsI_bearbeitet.png



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 106/192: Inoculation p2iGEM0209
In Project: Level_2
With tags: LEU2, inoculation, dueber, lvl0

created: 23.06.2018 09:32
updated: 23.06.2018 09:37

due date: 18.06.2018

Three Colonies of 2iGEM0227 (p2iGEM0209) were inoculated in liquid LB-Cam (3ml LB + 2,04 µl) and grew overnight at 37°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 23.06.2018 09:40
updated: 23.06.2018 10:04

Entry 107/192: isolation p2iGEM0209
In Project: Level_2
With tags: miniprep, miniprep kit, LEU2, lvl0, isolation, plasmid, dueber

Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 900 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 400µl column wash
- centrifuge 30 sec max rpm -> discard supernatant
- For elution use new tube
- 30 µl Millipure water on column (a 37°C)
- Let incubate for 1h Min at RT
- Centrifuge for 30 Sek. max

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 108/192: Cryo 2iGEM0227
In Project: Level_2
With tags: cryogenic culture, LEU2, lvl0, dueber

created: 23.06.2018 10:05
updated: 25.06.2018 14:09

cryogenic culture of p2iGEM0227 (p2iGEM0209):

- 300 µl glycerol
- 700 µl culture

stored at -80°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 109/192: Blue white screening
In Project: Level_2
With tags: screening, blue white screening

created: 24.06.2018 15:40
updated: 01.08.2018 19:35

Blue white screening after CIDAR cloning showed just blue colinies.
Cloning were prepared again but the cycler broke in progress.

The 2 samples were discarted.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 24.06.2018 15:41
updated: 01.08.2018 19:36
Entry 110/192: CIDAR cloning (lysC into DVA_CD)
In Project: Level_2
With tags: cidar

CIDAR cloning was performed with following conditions:

Plasmid: CIDAR Entry (p2iGEM0174), PCR product *lysC* gene

Pipetting advice	
amount (µl)	Substance
0,5	Template (gBlock)
10ng	Entry vector
1	T4 Ligase
1,5	T4 Ligase Buffer
0,75	BbsI Enzyme
Fill to 15	Milli Q H2O

Protocole		
Temperature	Time	
37°C	20 min	
37°C	1,5 min	
16 °C	3 min	25X
16°C	1 h	
50°C	5 min	
80°C	10 min	
4°C	Hold	

2 samples were prepared.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 24.06.2018 15:51
updated: 24.06.2018 16:03

Entry 111/192: PCR with Taq polymerase
In Project: Level_2
With tags: taq pcr, taq protocole, pcr, taq polymerase

In order to make sure it was able to isolate the correct lysC gene I reperformed the PCR to amplify lysC out of the topo plasmid (p2iGEM0243).

Conditions:

PCR Taq Polymerase	
µl	Substance
2,5	10X Buffer
0,5	dNTPs
0,5	Primer fwd (10 µM stock)
0,5	Primer rev (10 µM Stock)
-	Template DNA (under 1 µg)
0,125	Taq Polymerase
fill up to 25	Milli Q Water

A 5X Mastermix was prepared for 4 PCR samples.

2 different plasmid aliquoted were used. The interted DNA was under 800ng per sample.

PCR Taq Polymerase		
Temperature	Time	
98	30 sec	
98	15 sec	
58-68	20 sec	
68	1 min /kb	25 cycles
68	2 min	
4	Hold	

The expected fragment is 1249 bases. **The elongation time was set as 85 sec**.

Gelelectrophoresis will be performed the next day.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 112/192: PCR of lysC and gel purification of lysC
In Project: Level_2
With tags: gel purification, elution

created: 24.06.2018 16:04
updated: 01.08.2018 19:37

The PCR to control the ability to amplify showed an expected band on the gelelectrophoresis.

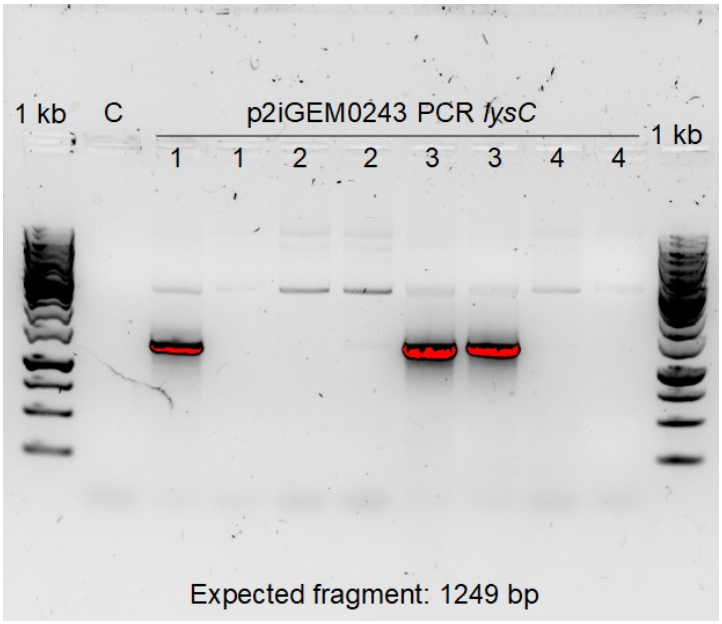
The samples which showed the expected band were purified and used for another CIDAR cloning.

For the purification the gel and PCR purification Kit of Promega was used and performed with the provided protocol.

One of the samples was later used for the pre restriction of the *lysC* gene. For the restriction in total 1 µg DNA was used.

Restriction was performed with BbsI restriction enzyme.

PCR_lysC_Test_PCR_-_Aufreinigung_von_Sample_1_und_Sample_3.jpg

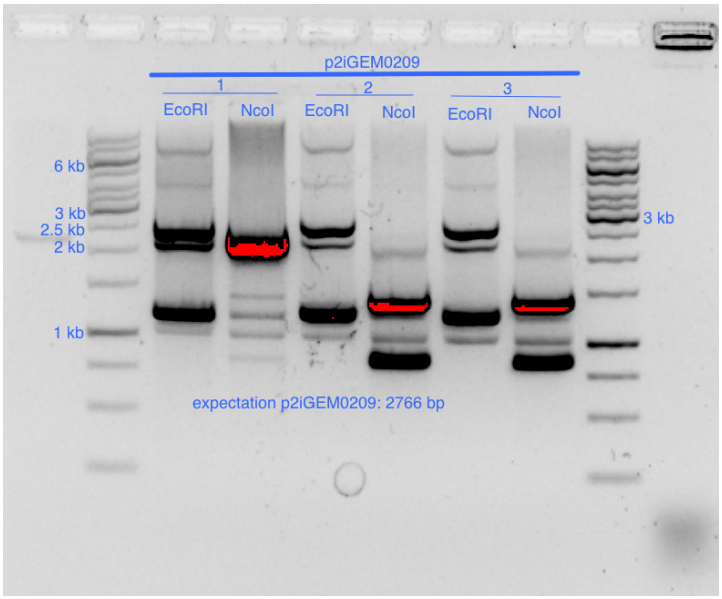


Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 113/192: restriction test p2iGEM0209
In Project: Level_2
With tags: LEU2, dueber, restriction, lvl0

created: 25.06.2018 14:13
updated: 25.06.2018 14:49

Gel_2018-06-22_16hr_09min_Ylenia;Salima,Thomas,Susanne.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 114/192: Sequencing
In Project: Level_2
With tags: sequencing

created: 25.06.2018 17:29
updated: 25.06.2018 17:33

To check if the mutagenisis of p2iGEM0293 was succesfull the sample 7 of the plasmid is beeng sequenced.

Plamid: p2iGEM0293

Primer: M13 primer fw (TA Topo-cloning kit)

M13 primer rv (TA Topo-cloning kit)

- 10 µl in total per reaction
- 2µl primer + 500 ng of plasmid (0,66µl)
- Filled up to 10 µl with Milli Q Water

2 reactions in total for 1 primer pair

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger Entry 115/192: No entry title yet In Project: Level_2 No tags associated	created: 28.06.2018 11:32 updated: 28.06.2018 11:32
Add text here	
Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 116/192: Sequencing
In Project: Level_2
With tags: sequencing

created: 28.06.2018 16:45
updated: 28.06.2018 16:56

The sequencing to test if the mutagenisation of p2iGEM0293 were succesfull showed a mutagenisation in the gene *ddh* which would change an aminoacid.

Now the two preplasmids p2iGEM0271 and p2iGEM0290 were also sequenced to see if the mutagenisis is there as well

Plamid: p2iGEM0290

Primer: M13 primer fw (TA Topo-cloning kit)

M13 primer rv (TA Topo-cloning kit)

- 10 µl in total per reaction
- 1µl primer + 500 ng of plasmid (0,64µl)
- Filled up to 10 µl with Milli Q Water

Plamid: p2iGEM0271

Primer: M13 primer fw (TA Topo-cloning kit)

M13 primer rv (TA Topo-cloning kit)

- 10 µl in total per reaction
- 1µl primer + 500 ng of plasmid (0,59µl)
- Filled up to 10 µl with Milli Q Water

4 reactions in total for 1 primer pair

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 01.07.2018 13:21
updated: 01.08.2018 19:38
Entry 117/192: CIDAR cloning (lysC into DVA_CD with different combinations)
In Project: Level_2
With tags: CIDAR cloning

Transformation of CIDAR cloning (2 samples) from the 24th June had no success. In total no colonies grew on the plate with amp and XGAL.

Another CIDAR cloning was performed. This time 6 samples were done:

1) Not pre restricted backbone (p2iGEM0174) with old pre restricted *lysC*.

2) Not pre restricted backbone (p2iGEM0174) with new, not pre restricted *lysC*.

3)Not pre restricted backbone (p2iGEM0174) with new, pre restricted *lysC*.

4) Pre restricted backbone (p2iGEM0174) with old pre restricted *lysC*.

5) Pre restricted backbone (p2iGEM0174) with new, not pre restricted *lysC*.

6) Pre restricted backbone (p2iGEM0174) with new, pre restricted *lysC*.

The protocol was:

Pipetting advice	
amount (µl)	Substance
0,5	Template (gBlock)
10ng	Entry vector
1	T4 Ligase
1,5	T4 Ligase Buffer
0,75	BbsI Enzyme
1	CutSmart Buffer (10x)
Fill to 15	Milli Q H2O

The cycler programm was:

Protocole		
Temperature	Time	
37°C	20 min	
37°C	1,5 min	
16 °C	3 min	25X
16°C	1 h	
50°C	5 min	
80°C	10 min	
12°C	Hold	

On the next day the samples were transformed into competens E. coli T10 cells. Here the heatshock method was used.

Cells were plated on LB_Amp with XGAL and incubated over night at 37 °C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 118/192: No entry title yet
In Project: Level_2
With tags: ddh, bsai, digest

created: 04.07.2018 16:42
updated: 06.07.2018 10:43

Predigestion of p2iGEM0174 and p2iGEM0290

p2iGEM0174 was digested with BbsI

p2iGEM0290 was digested wit BsaI

- 2µl enzyme
- 5µl cutsmart buffer
- 5µl template
- 38µl MiliQ Water

Incubation at 37°C for 3h

Touch up PCR with Salima's samples

Mastermix:

- 9µl Q5-Buffer
- 9µl GC enhancer
- 3,75 µl Primer OiGEM0006
- 3,75 µl Primer OiGEM0005
- 1,5 µl dNTP
- 0,75 µl Q5-polymerase
- 1,5 µl template
- 11,25 µl MiliQ-water

PCR programm

1. 98°C for 30 sec
2. 98°C for 10 sec
3. 54°C for 20 sec (for each cycle increase temperature by 0,8°C)
4. 72°C for 40 sec
5. repeat 2 to 4 10 times
6. 98°C for 10 sec
7. 62°C for 20 sec
8. 72°C for 40 sec
9. repeat 6 to 8 25 times
10. hold at 12°C

Additinally a clean up from a testrestriction with EcoRI and NcoI was made

Add in a tube

- 25µl template
- 1µl T5 exonuclease
- 3 µl 4 buffer

Let it incubate for 30 min

Then 30µl Membrane binding solution were added and the liquid was transfered into a collum

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min. We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°) in these steps:

-adding 30 µl 37°C warm MilliQ to the membrane.

-incubate membrane at 37°C shook at 300 rpm.

-centrifuge for 1 min at 50 x g

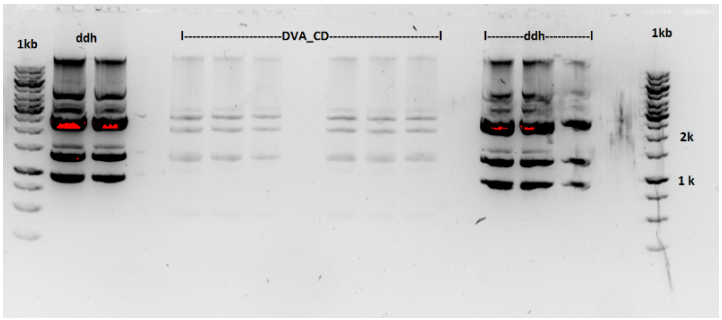
-centrifuge for 1 min at 16.000 x g

Gelelctrophoresis with the predigested Plamsid for gelpurification

- 4,5 µl purple loading dye(New Englanfd bio labs) were added to 25µl sample
- 2µl 1kb ladder (Thermo fisher)

The gel ran at 95V for 65 min

Gel_2018-07-04_17hr_46min_Vorverdau_Topo_und_DVA_95V_65_min_bearbeitet.tif



Because the bands on the gel did not look good the predigestion was repeated

Predigestion of p2iGEM0174 and p2iGEM0290

p2iGEM0174 was digested with BbsI

p2iGEM0290 was digested wit BsaI

- 2µl enzyme
- 5µl cutsmart buffer
- 5µl template
- 38µl MiliQ Water

Incubation at 37°C overnight (16h)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 119/192: Gelelution, T4 ligation
In Project: Level_2
With tags: T4 ligase, gel electrophoresis, gelelution

created: 06.07.2018 10:17
updated: 06.07.2018 15:27

(05.07.2018)

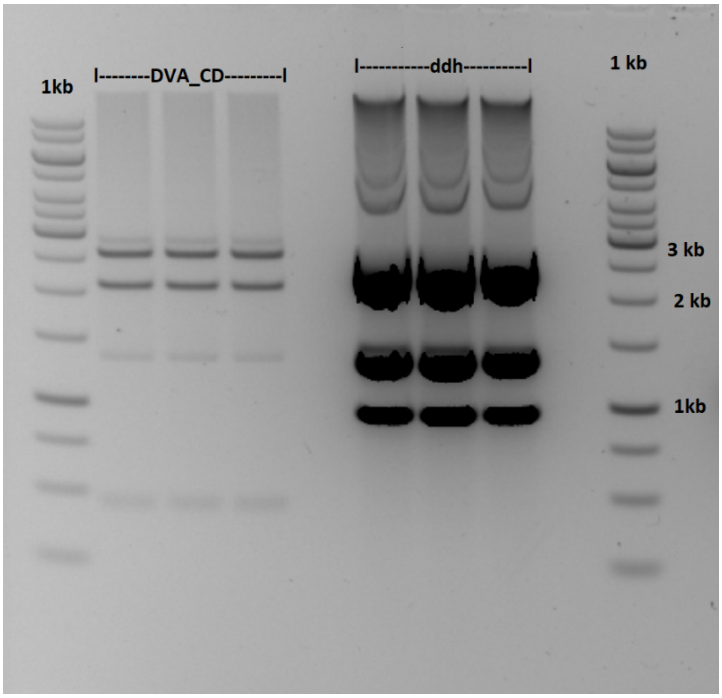
The predigestion was filled on a gel along with Salimas sampled

- 10µl loading was addded to my samples(50µl)
- 1µl 1 kb ladder was used

The gel ran on 80V for 150 min

The 1kb bands of *ddh* and the 2 kb of DVA_CD were cut out for a gelpurification

Gel_2018-07-05_19hr_31min_150_min_80_V_Salima_&_Sarah_predigestion_bearbeitet.tif



Ligation of *ddh* and CIDAR entry vector CD (p2iGEM0174)

- 2µl T4 ligation buffer
- 0,5µl T4 ligation
- 5µl entyvector
- 5µl *ddh*
- fill up to 20µl

Incubation at roomtemparture for 30 min

Gelelution of *ddh* and DVA_CD as well as Salimas samples

We added 1 ml of Membrane Binding Solution to the gel slices and incubated it for 10 min. at 60°C until the gel slices dissolved.

Then we loaded 350 µl of the gel solution into the SV minicolumn with collection tube, let it incubate for 1 min at RT and centrifuge it for 1 min at 16.000 x g. We discarded the supernatant. These steps were repeated until the whole gel solution was filtered through the column membrane.

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min.
We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°)

adding 30 µl 37°C warm MilliQ to the membrane.

incubate membrane for 2h

centrifuge for 1 min at 16.000 x g

Trafo of ligated *ddh*_DVA plasmid

Transformation

- thaw competent cells (DH5a or Top10) 5-10 min on ice
- add 5µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42 °C
- place on ice for 5min
- Pipette 300µl LB without Antibiothica (sterile)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica and X-gal->ampicilin
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 06.07.2018 15:44
updated: 01.08.2018 19:39

Entry 120/192: Plasmidisolation and sequencing order after CIDAR cloning
In Project: Level_2
With tags: sequencing, cidar

The colony PCR of the 6 cloning samples showed no results. all lanes were empty.

Genes and backbones were load on the gel to check their bands and fragments.

Just to check if the cloning worked at any point the Colonies 1 & 10 were inoculated in 3 ml LB_Amp (100 µg/ml).

Samples incubated over night at 37 °c 220rpm.

The next day the plasmids were isolated and sent to sequencing with Primers Nr. 13 & Nr. 14.

Sequencing

p2iGEM0272 (sample 1)

Primer 13: 82EC74

Primer 14: 83EC73

p2iGEM0272 (Sample 10)

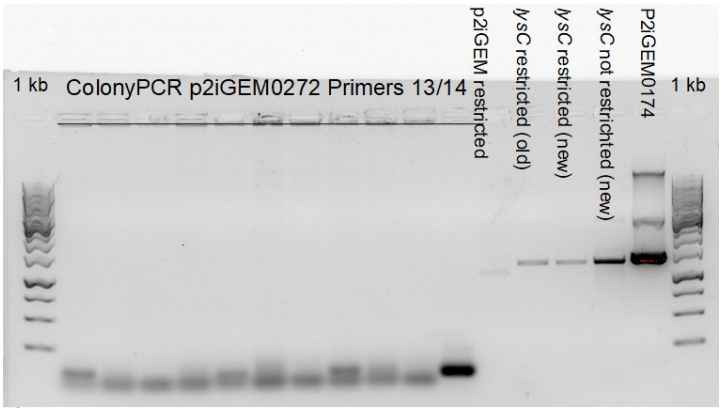
Primer 13: 82EC72

Primer 14: 82EC77

Sequncing showed a point mutation in the gene in Sample 1 - sample were discated.

Sequencing for sample 10 showed unclear ends & beginnings of the gene - plasmid will be sequences again also with backbone primers.

ColonyPCR_p2iGEM0272_(CIDAR_Entry_+_lysC).jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 121/192: Transformation & cloning into Cidar DVA
In Project: Level_2
With tags: T4 ligase, ddh, Transformation, X-gal

created: 10.07.2018 13:57
updated: 10.07.2018 14:00

Ligation of *ddh* and CIDAR entry vector CD (p2iGEM0174)

- 2µl T4 ligation buffer
- 0,5µl T4 ligation
- 10µl entyvector
- 10µl *ddh*
- fill up to 30µl

Incubation at roomtemparture for 15 min

Trafo of ligated *ddh*_DVA plasmid and p2iGEM0174

Transformation

- thaw competent cells (DH5a or Top10) 5-10 min on ice
- add 5µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42 °C
- place on ice for 5min
- Pipette 300µl LB without Antibiothica (sterile)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica and X-gal->ampicilin
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 122/192: Miniprep
In Project: Level_2
With tags: Plasmid prep, Pure yield miniprep

created: 12.07.2018 11:47
updated: 12.07.2018 15:48

p2iGEM0174 were miniprepped according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µ colum wash
- add 400µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

Because the conentrations of the digested fragments after the gelelution were so small the experiment was repeated with higher plasmid concentrationsPredigestion of p2iGEM0174 and p2iGEM0290

p2iGEM0174 was digested with BbsI

p2iGEM0290 was digested wit BsaI

- 2µl enzyme
- 5µl cutsmart buffer
- 5µl template
- 38µl MiliQ Water

Incubation at 37°C for 2h

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 123/192: gelelctrophoresis
In Project: Level_2
With tags: gel electrophoresis

created: 12.07.2018 15:48
updated: 12.07.2018 15:51

Gel electrophoresis together with Ylenjas samples

- 20µl of the digested plasmids(p2iGEM0174 and p2iGEM0290) with 4 µl pruple loading dye were loaded on the gel
- 5µl of Salimas 1kb ladder were loaded on the gel
- the gel ran on V for h

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 124/192: restriction
In Project: Level_2
With tags: digest

created: 13.07.2018 10:48
updated: 13.07.2018 10:49

Because the restriction of the Cidarentryvector(p2iGEM0174) did not work properly it was repeated.

- 1µl enzyme
- 15µl template
- 2µl cutsmart
- 2µl MiliQ water

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 125/192: Results of sequncing of p2iGEM0272
In Project: Level_2
With tags: sequencing

created: 13.07.2018 22:27
updated: 01.08.2018 19:39

The sequencing order of 6th July 2018 showed no successfeull cloned plasmid.

It was decided to perform a new cloning try.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 13.07.2018 22:52
updated: 15.07.2018 19:07

Entry 126/192: Restriction & Ligation cloning
In Project: Level_2
With tags: cidar, sequencing, cloning

To try the cloning of lysC into the CIDAR Entry Vector another method was done because the GoGate cloning showed no success several times.

Here a simple restriction & ligation were done.

A new restriction of p2iGEM0174 and a restriction of PCR purified *lysC* were performed with the BbsI enzyme.

The restrictions run for 1,5 h at 37 °C and stopped at 66 °C for 20 min.

Afterwards a ligation at roomtemperature with the T4 ligase was performed with both pre-restricted fragments. Ligation run for 10 min.

The sample were stored on ice and 1,5 µl used for a transformation into *E. coli* T 10.

The transformateion was plated on LB_Amp and incubated o/n at 37 °C.

A ColonyPCR of picked white colonies showed no amplification of the expected fragment at 1249 bp.

2 clones were inoculated randomly for a plasmid isolation and sent for sequencing.

Sequencing was ordered with 4 primers per plasmid (8 samples in total)

Sequencing:

p2iGEM0272 (colne 2)

Primer 13 82EC78

Primer 14 82EC83

Primer 68 82EC82

Primer 69 82EC81

--> Success

p2iGEM0272 (clone 13)

Primer 13 82EC47

Primer 14 82EC46

Primer 68 82EC45

Primer 69 82EC50

--> No success

The RESULT of the sequncing showed:

Plasmid of clone 2 hat a point mutation which NOT leads to a aminoacidexchange and is successfully clones into CIDAR entry Vector.

With this cloning p2iGEM0272 was successfully colned.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 14.07.2018 11:56
updated: 15.07.2018 10:19
Entry 127/192: No entry title yet
In Project: Level_2
With tags: ligation, ddh, gelelution, Transformation, Pure yield miniprep, Plasmid prep

We added 1 ml of Membrane Binding Solution to the gel slices and incubated it for 5min. at 60°C until the gel slices dissolved.

Then we loaded the gel solution into the SV minicolumn with collection tube, let it incubate for 1 min at RT and centrifuge it for 1 min at 16.000 x g. We discarded the supernatant. These steps were repeated until the whole gel solution was filtered through the column membrane.

After that, we washed the membrane with 700 µl Membrane Wash Solution and centrifuged again at 16.000 x g for 1 min. We repeated this step with 500 µl Membrane Wash Solution and centrifuged 5 min. To get rid of ethanol residues, we centrifuged the empty column at 16.000 x g for 1 min. Then we put the column in a sterile 1,5 µl reaction tube and eluted with 30 µl warmed up MilliQ water (37°) in these steps:

-adding 30 µl 37°C warm MilliQ to the membrane.

-incubate membrane at 37°C shook at 300 rpm.

-centrifuge for 1 min at 50 x g

-centrifuge for 1 min at 16.000 x g

Ligation of *ddh* and CIDAR entry vector CD (p2iGEM0174)

- 1µl T4 ligation buffer
- 0,5µl T7 ligation
- 1µl entyvector
- 3µl *ddh*
- fill up to 10µl

Incubation at roomtemparture for 15 min

Trafo of ligated *ddh*_DVA plasmid and p2iGEM0174

Transformation

- thaw competent cells (Top10) 10 min on ice
- add 10µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42 °C
- place on ice for 5min
- Pipette 300µl LB without Antibiothica (sterile)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica and X-gal->ampicilin
- incubate at 37°C over night

Isolation of plasmid p2iGEM0304 according to the PureYield Miniprep Plasmid Kit from Promega. .

1. Centrifugation of culture, afterwards a bit of supernatant was discarded
2. Resuspendation of culture in 2ml eppi
3. Addition of 100 µl Lysisbuffer --> mix
4. Addition of 350 µl Neutralisation buffer (cold) --> mix
5. Centrifugation for 3 min max rpm
6. Addition of supernatant (~ 800 µl) to column
7. centrifuge 15 Sek. max rpm --> supernatant was discarded
8. 15sec max rpm
9. Addition of 400µl Column Wash
10. 15 sec max rpm
11. Elution: new tube + 30 µl 37°C prewarmed water on column
12. Incubation at 37°C for
13. Centrifugation for 15 Sek. max

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 128/192: Colony PCR
In Project: Level_2
With tags: Colony PCR

created: 15.07.2018 11:54
updated: 15.07.2018 12:46

ColonyPCR of *E.coli* T10 (50 samples, 5 per transformation plate) with the Cidarentryvector + *ddh* (p2iGEMusing following protocol:

White colonies were picked and plated on a new LB-Amp plate. Small amount of the colony was added to the PCR tube for the reaction.

- 2,5 µl 10xPuffer
- 0,5 µl dNTP
- 0.5 µl Primer fwd (O_iGEM18_0080)
- 0.5 µl Primer rev (O_iGEM18_0081)
- 0,12 µl Taq
- 20.5 µl MilliQ
- 0.5 DMSO
- 1colony

PCR program:

- 98°C 10 min
  - 98°C 10 sec
  - 65°C 15 sec
  - 68°C 1min--> repeat this cycle 30 times
- 68°C 5 min
- 12°C HOLD

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 129/192: Gel electrophoresis
In Project: Level_2
With tags: gel electrophoresis

created: 16.07.2018 11:09
updated: 16.07.2018 11:14

The samples were loaded on a gel to see if the colony PCR was succesfull

- 2µl 1 kb ladder(Thermo Fischer) were used
- 2µl purple loading dye were added to the PCR samples

The gel ran on 85 V for 1h

The expected fragement size is 982bp

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 130/192: Miniprep
In Project: Level_2
With tags: Pure yield miniprep, ddh

created: 17.07.2018 15:51
updated: 17.07.2018 15:52

Plasmid Miniprep p2iGEM0310

Kit: PureYield^{TM} Plasmid Miniprep System from Promega

culture was concentrated in 600µl

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 700 µl) to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 30 sec max rpm
- For eluation use new tube + 30 µl MilliQ
- incubate for 2 h at room temperature

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 131/192: PCR
In Project: Level_2
With tags: pcr, ddh

created: 18.07.2018 11:39
updated: 18.07.2018 11:51

PCR of *ddh* in Cidar_CD to check if the ligation was successfull

7x mastermix was prepared

- 1.75µl Q5-polymerase
- 35 µl Q5 buffer
- 35µl GC enhancer
- 10.5 µl primer fw
- 10.5 µl primer rv
- 10.5 µl DMSO
- 3.5 µl dNTPs
- 64.75 µl MiliQ

Mastermix was prepared on ice.

6 PCR tubes were filled with 50µl.

Programm:

98°C	30 sec	
98°C	10 sec	
59°C	20 sec	
72°C	30 sec	35 cycles
72°C	2 min	
12°C	Hold	

Tubes were stored at 4°C after the PCR.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 132/192: Mutagenesis
In Project: Level_2
With tags: ddh, Mutagenesis, gradient pcr

created: 23.07.2018 11:31
updated: 31.07.2018 10:54

A dotmutation appeared in *ddh*. Therefore it had to be mutagenized

Mutagenesis of p2iGEM0310

6x Mastermix

- 5µl plasmid(60ng)
- 12 µl primer fw
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 196 µl miliQ

6 tubes were filled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - gradient 72°C - 65°C 30 sec
  - 72 °C 2:30 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

After the PCR the samples were digested with DpnI

- fill the samples into a new tube
- add 1µl DpnI
- incubate at 37°C for 1h

Trafo of with mutagenized plasmids

- thraw competent cells (*T10*) 5-10 min on ice
- add 5 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 20 min
- heatshock for 45 sec on 42°C
- place on ice for 5 min
- Pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- 1 min 6000 rpm discard 180 µl supernatant
- plate them out on a agarplate with the fitting antibiotica (Amp)
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 133/192: Overnightculture
In Project: Level_2
With tags: overnight

created: 24.07.2018 15:41
updated: 24.07.2018 15:45

Overnight culture of 2iGEM0361

- 3ml LB
- 30µl ampicillin

Incubation over night at 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 134/192: Plasmidprep of ddh in Cidar
In Project: Level_2
With tags: Plasmid prep, miniprep, ddh

created: 25.07.2018 10:16
updated: 26.07.2018 11:03

Mini prep of the Plasmids p2iGEM0327 with the following Protocoll of the Pure Yield Miniprep:

- 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- + 200 µl endotoxin removal wash
- centrifuge 30 sec max rpm -> discard supernatant
- add 400µl column wash
- For elution use new tube
- add 30 µl 37°C miliQ-water on column
- Let incubate for 1h at RT
- Centrifuge for 15 Sek. max

(26.07)
The three samples were send to sequencing

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 25.07.2018 17:38
updated: 27.07.2018 11:56
Entry 135/192: Enter 2iGEM0304 to Kryo stock, enter 2iGEM0272 to plasmid stock
In Project: Level_2
With tags: Keyo culture, plasmid isolation, miniprep

Inoculation of 2iGEM0304 from plate (including p2iGEM0272) 4 times.

3 ml LB with Amp (100 µg/ml)

picked from plate

Incubation over night at 37 °C, 220 rpm.

Plasmidisolation and preparation of kryo stock:

700 µl of ONE of the samples

300 µl Glycerin 98%

--> mix and store at -80 °C (kryo Box 4)

The rest of the culture and the 3 other cultures were used for a plasmid isolation with the promega plasmid isolation kit.

Plasmids are stored at -20 °C. (Cloning Box Jenny - so far).

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 25.07.2018 18:02
updated: 27.07.2018 12:21
Entry 136/192: Pre restriction for CIDAR lvl1 cloning (BsaI)
In Project: Level_2
With tags: restriction, bsai

Pre restriction of CIDAR lvl1 cloning parts.

Restrictions were done with **BsaI & Cutsmart Buffer**.

Of each plasmid **1 ng was used** for restriction.

p2iGEM0196	DVK_AE (CIDAR lvl1 backbone)
p2iGEM0109	Promoter J23100
p2iGEM0113	Promoter J23102
p2iGEM0117	Promoter J23103
p2iGEM0121	Promoter J23106
p2iGEM0125	Promoter J23107
p2iGEM0129	Promoter J23116
p2iGEM0149	RBS B0032
p2iGEM0164	Terminator B0015
p2iGEM0272	DVA_CD_*lysC* (lvl0 cloning)

Restriction run for **2h h at 37 °C**.

Inactivation was at **66 °C for 20 min**.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 138/192: Mutagenisis of p2iGEM0310
In Project: Level_2
With tags: Mutagenesis

created: 31.07.2018 13:34
updated: 02.08.2018 13:34

Sequencing showed that the mutagenisis of p2iGEM0310 was not succesfull. Therefore it was repeated with a slightly different protocol

Mutagenesis of p2iGEM0310

- 0,55µl plasmid(10ng)
- 2 µl primer fw
- 10 µl Q5-Buffer
- 10 µl GC enhancer
- 1 µl dNTP
- 0,5 µl Q5-polymerase(at last on ice!)
- 2µl DMSO
- 24 µl miliQ

In total 50µl were in the PCR-tube

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 72°C 20 sec
  - 72 °C 2:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 139/192: Ligation of CIDAR lvl1 plasmids
In Project: Level_2
With tags: cidar, ligation

created: 31.07.2018 14:13
updated: 01.08.2018 18:35

Ligation of pre restricted parts with T4 ligase.

The plasmids p2iGEM0321, p2iGEM0322 & p2iGEM0323 was ligated.

1 µl of each pre restricted sample was used for each ligation.

1 µl of T4 ligase was used

2 µl of T4 ligase buffer was used

the samples were filled up to 20 µl with Milli Q Water.

The ligation run at room temperature for around 15 min and afterwards inactivated at 66 °C for 25 min.

Samples were stored at -20 °C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 31.07.2018 15:37
updated: 01.08.2018 18:35

Entry 140/192: Transformation of CIDAR cloned plasmids
In Project: Level_2
With tags: inoculation, transformation

Transformtion of ligated p2iGEM 0321, p2iGEM0322 & p2iGEM00323 into E. coli DH5a.

Method: Heat shock transformation.

Samples were plated on LB_Kanamycin (50 µg/ml).

Plates were prepared with IPTG (50 µl) & XGAL (60 µl).

Plates were incubated at 37 °C over night.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 01.08.2018 18:36
updated: 01.08.2018 19:10

Entry 141/192: ColonyPCR & gelelectrophoresis of CIDAR lvl1 cloning
In Project: Level_2
With tags: Colony PCR, Cidar

To check the success of the ligation of CIDAR lvl1 parts a ColonyPCR war performed.

For the PCR the Taq - Polymerase was used.

The plates showed mostly blue colonies. All white colonies were picked for the ColonyPCR.

Picked colonies were plated onto a nes plate of LB_Kan (50 µg/ml) and incubated at 37 °C.

2iGEM0354 --> 4 white colonies

2iGEM0355 --> 6 white colonies

2iGEM0356 --> 3 white colonies

For the PCR a 14X mastermix was prepared

1x mastermix

2,5 µl Buffer

0,5 µl dNTPs

0,5 µl Primer fwd (O_iGEM_0013)

0,5 µl Primer rev (O_iGEM_0014)

0,14 µ Taq-polymerase

20,5 µl Milli Q Water

0,5 µl DMSO

Tip with the rest of colony

PCR programm was with following conditions

95 °C	10 min	
95 °C	20 sec	
61 °C	20 sec	
68 °C	90 sec	30 cycles
68 °C	4 min	
12 °C	Hold	

After the PCR 3 µl of Purple Loading Dye were addes to each sample.

The whole sample was loaded onto an 1 % Gel (documentation below).

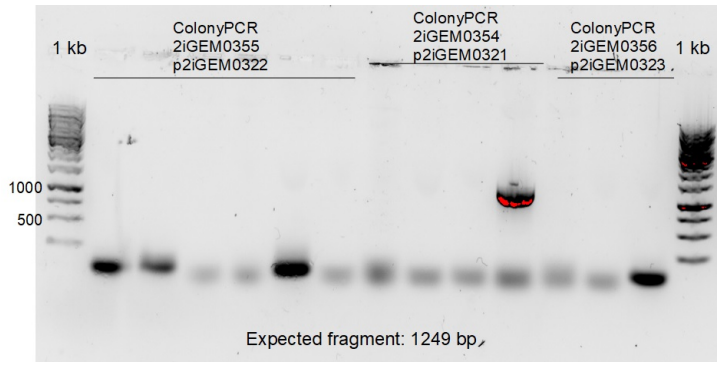
The gel run 27 min at 95 V.

One sample showed an fragmend of the expected size. This sample was inoculated in 3 ml LB with kanamycin (50 µg/ml).

Of the other strains 1 colony was inoculated randonly with the same conditions.

All 3 samples were placed into the incubator at 37 °C, 220 rpm o/n.

ColonyPCR_lvl_1_cloning_lysC_J23100-J23103.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 02.08.2018 11:30
updated: 02.08.2018 16:53

Entry 142/192: Transformation p2iGEM0209
In Project: Level_2
With tags: cloning, dh5a, dueber, E.coli, LEU2, lvl0, plasmid, trafo, transformation

Transformation of p2iGEM0209 (6x, 4-6 with cloned with a predigested backbone)

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Cam) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 143/192: Mutagenesis
In Project: Level_2
With tags: ddh, Mutagenesis

created: 02.08.2018 14:30
updated: 02.08.2018 16:51

Sequencing showed that the mutagenisis of p2iGEM0310 was not succesfull. Therefore it was repeated with a slightly different protocol

Mutagenesis of p2iGEM0310

- 0,55µl plasmid(10ng)
- 2 µl primer fw
- 10 µl Q5-Buffer
- 10 µl GC enhancer
- 1 µl dNTP
- 0,5 µl Q5-polymerase(at last on ice!)
- 2µl DMSO
- 24 µl miliQ

In total 50µl were in the PCR-tube

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 78°C 20 sec
  - 72 °C 2:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

stored at -20°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 02.08.2018 16:52
updated: 02.08.2018 22:28
Entry 144/192: Golden Gate Assembly
In Project: Level_2
With tags: LEU2, gogate, goldenGate, golden gate, dueber, lvl0

Golden Gate Assembly of p2iGEM0209 (12x):

Master Mix:

- 1 µl of p2iGEM0099 (ca. 4 ng)
- 1 µl of LEU2 samples (ca. 40 ng)
- 0,5 µl BsmBI
- 1 µl T4 DNA Ligase Buffer
- 0,5 µl T7 DNA Ligase
- up to 15 µl water (11 µl)

Thermocycler (Programm GoGate BasI)

- 65 cycles of digestion and ligation (37°C for 2 min, 16 °C for 5 min),
- 16 °C for 60 min
- a heat inactivation step (80 °C for 30 min).
- Hold at 12°C

**LEU2 samples: predigested with BsaI before PCR, digested with DpnI after PCR, new and old PCR samples*

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 02.08.2018 22:21
updated: 02.08.2018 22:47

Entry 145/192: Plasmidisolation and sequencing order (CIDAR lvl1 lysC)
In Project: Level_2
With tags: miniprep, sequencing, cidar, lvl1 cloning

Plasmids of inoculated samples of the day before were isolated with the Promega isolation kit.

3 different plasmids were isolated

p2iGEM0321	237 ng/µl
p2iGEM0322	295 ng/µl
p2iGEM0323	214 ng/µl

Each plasmid was sent for sequencing with the primers

0_iGEM_0013, 0_iGEM_0014 which bind at the beginning of the *lysC* gene and

0_iGEM_0070, 0_iGEM0071 which bind in the backbone of the DVK_AE backbone

Sequencing will show if the lvl1 cloning was successful.

Data of orders:

p2iGEM0321	#13	82EE76
	#14	82EF07
	#70	82EF08
	#71	82EF09
p2iGEM00322	#13	82EF10
	#14	82EF11
	#70	82EF12
	#71	82EF13
p2iGEM0323	#13	82EF14
	#14	82EF15
	#70	82EF16
	#71	82EF17

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 02.08.2018 22:50
updated: 02.08.2018 22:55
Entry 146/192: Ligation of CIDAR lvl1 plasmids (lysC)
In Project: Level_2
No tags associated

Pre restrictet parts of the CIDAR lvl1 cloning ware ligated in order to create an expression plasmid with the GOI *lysC*.

3 Plasmids were ligated:

p2iGEM0324 --> J23106

p2iGEM0325 --> J23107

p2iGEM0326 --> J23116

The plasmids are the same except the promoters.

Ligation protocole:

12 µl Milli Q Water

1 µl of each part was used (make sure to use nearly the same concentrations)

2 µl Reaction Buffer

1 µl Ligase

In this case the T4 ligase was used.

Reaction run for 10 min at RT and was inactivated at 66 °C for 25 min.

Samples were stored at -20 °C (Jennys cloning box) till next use.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 147/192: DpnI digest
In Project: Level_2
With tags: digest

created: 03.08.2018 11:17
updated: 03.08.2018 13:53

DpnI digest with p2iGEM0327

- digest at 37°C for 1 h

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 148/192: Transformation p2iGEM0209
In Project: Level_2
With tags: trafo, transformation, LEU2, lvl0, dueber

created: 03.08.2018 13:30
updated: 03.08.2018 13:35

Transformation of p2iGEM0209 (12x, 7-12 cloned with a predigested backbone)

- Thaw competent cells 10 minutes on ice
- Add 1 µl plasmid in reaction tubes
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 700 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Cam) --> Overnight 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 149/192: Transformation of ligated CIDAR lvl 1 ligations
In Project: Level_2
With tags: transformation, cidar

created: 06.08.2018 20:34
updated: 06.08.2018 20:43

The ligated lvl 1 CIDAR plasmids with *lysC* as GOI were transformed into *E. coli* DH5alpha

3 samples were transformed:

p2iGEM0324 (J23106)

p2iGEM0325 (J23107)

p2iGEM0326 (J23116)

Ligated constructs were transformed with the heat shock method.

Cells were incubated for 1 h at 37 °c at 220 rpm.

Cells were plated 100% on LB_Kan medium and incubated at 37 °C o/n.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 150/192: Inoculation from plate
In Project: Level_2
With tags: inoculation

created: 06.08.2018 20:43
updated: 06.08.2018 20:45

Inoculation of samples for sequencing.

Samples were picked from plate.

2iGEM0354 (1X)

2iGEM0355 (2X)

2iGEM0356 (1X)

Cultures were incubated at 37 °C, 220rpm o/n.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 07.08.2018 20:57
updated: 07.08.2018 21:25
Entry 151/192: Sequencing orders for CIDAR lvl1 cloning (lysC)
In Project: Level_2
With tags: sequencing

Inoculated an o/n incubates cultures were used for plasmid isolation.

Isolation was performed with Promega plasmid isolation kit.

Isolated plasmids were sent to sequencing.

All samples were sent with 4 different primers (#13/#14) & (#70/#71)

Primers #13 / #14 bind at the beginning and the end of the GOI

Primers #70 / #71 bind in the backbone

p2iGEM321	#13	82EF24
	#14	82EF29
	#70	82EF28
	#71	82EF27
p2iGEM0321 (same sample as before again)	#13	23GH75
	#13	23GH74
	#70	23GH73
	#71	23GH72
p2iGEM0322 (sample 4)	#13	82EF32
	#14	82EF31
	#70	83EF30
	#71	82EF33
p2iGEM0322 (Sample 5)	#13	23GH83
	#14	23GH82
	#70	23GH81
	#71	23GH80
p2iGEM0323	#13	23GH79
	#14	23GH78
	#70	23GH77
	#71	23GH76

Except 1 sample all plasmids are new ones.

One sequencing will be repeated bacause of bad results.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 09.08.2018 11:22
updated: 13.08.2018 12:55
Entry 152/192: Mutagenisis of pwiGEM0310
In Project: Level_2
With tags: touchdown pcr, Mutagenesis

Sequencing showed that the mutagenisis of p2iGEM0310 was not succesfull. Therefore it was repeated with a slightly different protocol

Mutagenesis of p2iGEM0310

3x Mastermix

- 30 µl Q5-Buffer
- 30 µl GC enhancer
- 3 µl dNTP
- 1,5 µl Q5-polymerase(at last on ice!)
- 15 µl DMSO
- 62 µl miliQ

45µl of the Mastermix and 0,55µl plasmid(10ng) were filled in the Tube

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 72°C 20 sec
  - 72 °C 3 min
  - repeat this 30 times
- 72°C 2.40 min
- hold 12°C

After the PCR the samples were digested with DpnI

- fill the samples into a new tube
- add 1µl DpnI
- incubate at 37°C for 2h

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 153/192: Miniprep & Trafo
In Project: Level_2
With tags: Pure yield miniprep, miniprep, Plasmid prep

created: 10.08.2018 11:07
updated: 10.08.2018 16:55

Miniprep of p2iGEM0327 and p2iGEM0258(plasmid from Carina and Susanne lvl1) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

Transformation of 2iGEM0361

- thraw competent cells (*T10*) 5-10 min on ice
- add 5 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- 3 min 4000 rpm discard 180 µl supernatant
- plate them out on a agarplate with the fitting antibiotica (Amp)
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger Entry 154/192: Inoculation In Project: Level_2 With tags: inoculation, LEU2	created: 10.08.2018 16:21 updated: 10.08.2018 16:23

Inoculation of 3x pYTK0075 ((p)2iGEM0075) in LB+Cam.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 155/192: Retransformation of PET GFP and mCherry in DH5alpha
In Project: Level_2
With tags: retrafo, Retransformation

created: 10.08.2018 16:23
updated: 11.08.2018 20:24

Retransformation of 2iGEM0383 and 2iGEM0384

- 1 µl Plasmid + 15 µl DH5alpha Compis
- 5 min Incubation on ice
- heat shock for 45 sec at 42 °C
- 15 min Incubation on ice
- added 300 µl LB
- 60 min Incubation at 37 °C while shaking
- transferred to culture tube with LB and chloramphenicol
- Incubation at 37°C overnight

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
created: 10.08.2018 16:36
updated: 10.08.2018 18:18
Entry 156/192: Miniprep p2iGEM0209
In Project: Level_2
With tags: LEU2, miniprep, miniprep kit, dueber

Miniprep:

- 600µl culture+ 100 µl Lysis buffer --> mix +350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 900 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 200µl column wash
- centrifuge 30 sec max rpm --> discard supernatant
- For elution use a new tube
- 30 µl Millipore water on column (at 37°C)
- Let incubate for 1h Min at RT
- Centrifuge for 30 Sek. max

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 10.08.2018 16:53
updated: 10.08.2018 17:08

Entry 157/192: Ligation of CIDAR lvl1 constructs
In Project: Level_2
No tags associated

CIDAR lvl1 constructs (6 samples) with different promoters were ligated with T7 Ligase.

Ligated construcs:

p2iGEM 0321

p2iGEM 0322

p2iGEM 0323

p2iGEM 0324

p2iGEM 0325

p2iGEM 0326

Ligation protocole:

12 µl Milli Q Water

2 µl Ligase Buffer

1 µl DNA of each part (pre digested parts, different promoters in different samples)

1 µl T7 Ligase

Incubation at RT for 30 min

Heat inactivation at 60°C for 30 min.

Ligated constructs were stored at -20 °C afterwards (Jennys Cloning Box).

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger

created: 10.08.2018 18:18
updated: 10.08.2018 18:20

Entry 158/192: miniprep pYTK0075 (LEU2) and fluorescence plasmid p2iGEM0383 & 0384

In Project: Level_2

No tags associated

Miniprep:

- 600µl culture+ 100 µl Lysis buffer --> mix +350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 900 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 200µl column wash
- centrifuge 30 sec max rpm --> discard supernatant
- For elution use a new tube
- 30 µl Millipore water on column (at 37°C)
- Let incubate for 1h Min at RT
- Centrifuge for 30 Sek. max

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 159/192: Restriction of pYTK0075 with BsaI
In Project: Level_2
With tags: restriction, digest, digestion, LEU2

created: 10.08.2018 18:20
updated: 11.08.2018 20:33

DNA: 1000 ng (x µl)

Cutsmart: 2 µl

BsaI: 1 µl

H_2O: fill up to 15 µl

incubated at 37°C ON.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Salima Rüdiger
Entry 160/192: PCR LEU2
In Project: Level_2
No tags associated

created: 11.08.2018 20:24
updated: 15.08.2018 19:24

Mastermix 32X

90 µl	Q5 Buffer (5x)
90 µl	Q5 Enhancer (5x)
37.5 µl	Primer fwd (O_iGEM_005)
37.5 µl	Primer rev (O_iGEM_006)
16 µl	dNTPs
1 ng for each reaction	Template DNA (Plasmid p2iGEM0243)
7.5 ul	Q5 Polymerase
45 µl	DMSO
97.5 ul	MilliQ Water

Mastermix was prepared on ice.

The template was added directly into the mastermix.

PCR tubes were filled with 15µl.

Programm:

98°C	30 sec	
98°C	10 sec	10 cycles
54 - 62°C (+0.8°C per cycle)	20 sec	
72°C	40 sec	
98°C	10 sec	25 cycles
62°C	20 sec	
72°C	40 sec	
72°C	2 min	
12°C	Hold	

After that, an enzyme reaction with DpnI took place to get rid of backbone residue. (0,2 µl each reaction, 37°C ON)

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 161/192: Transformation of ligated CIDAR lvl 1 plasmids
In Project: Level_2
With tags: transformation

created: 12.08.2018 13:46
updated: 15.08.2018 13:27

6 different CIDAR clonings were transformed into *E. coli* T10.

60 µl of XGAL was plated on LB-kanamycin and dryed dor 20 min under sterile conditions.

after transformation 100% of the cells were plated and incubated o/n at 37 °C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 13.08.2018 15:40
updated: 13.08.2018 15:48
Entry 162/192: Mutagenesis of p2iGEM0308+0310 & Trafo of p2iGEM0361
In Project: Level_2
With tags: Mutagenesis, touchdown pcr, ddh, pcr, Transformation

Mutagenesis of p2iGEM0310 and p2iGEM0308(lvl1)

Add in a PCR tube

- 10µl Q5-buffer
- 5µl DMSO
- 1µl dNTP
- 2µl primer fw
- 2µl primer rv(only for p2iGEM0308)
- 10ng plasmid
- 1µl Q5 polymerase
- fill up to 50µl with miliQ water

PCR- Programm for p2iGEM0310 mutagenesis

- 95°C 3 min
  - 95°C 30 sec
  - 78°C 20 sec(decrease of 0.3°C every cycle)
  - 72 °C 2 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

PCR- Programm for p2iGEM0308 mutagenesis

- 95°C 3 min
  - 95°C 30 sec
  - 78°C 20 sec(decrease of 0.2°C every cycle)
  - 72 °C 3 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Transformation of 2iGEM0361

- thraw competent cells (*T10*) 5-10 min on ice
- add 5 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- 3 min 4000 rpm discard 180 µl supernatant
- plate them out on a agarplate with the fitting antibiotica (Amp)
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 163/192: Trafo of ddh mutagenised
In Project: Level_2
With tags: Transformation

created: 14.08.2018 14:29
updated: 14.08.2018 15:06

Transformation of p2iGEM0361 and p2iGEM0332 (lvl1)

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) and stored in the 37°C incubator

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 164/192: Plasmidprep
In Project: Level_2
With tags: Plasmid prep, Pure yield miniprep

created: 15.08.2018 11:14
updated: 27.09.2018 15:31

Miniprep of p2iGEM0327(6x) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

The six samples were send to sequencing, to see if the mutagenesis was successfull

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 15.08.2018 16:11
updated: 15.08.2018 17:26
Entry 165/192: Inoculation & plasmid isolation of blue white screened colonies
In Project: Level_2
With tags: plasmid isolation

Transformed cells were incubated o/n and white colonies picked the next day.

Picked cells were inoculated directly in 3 ml LB with kanamycin treatment.

Inoculated colonies were incubaten o/n at 37 °C and 220 rpm.

10 Samples of 2iGEM0354 (p2iGEM0321 - J23100)

1 sample of 2igEM 0357 (p2iGEM0324 - J23106)

1 sample of 2iGEM0358 (p2iGEM0325 - J23107)

2 samples of 2iGEM0359 (p2iGEM0326 - J23116)

The strains 2iGEM0355 , 2iGEM0356 showed no white colonies at all so no cells were picked for this samples.

On the other transformation plates all white colonies were inoculated.

On the next day (15.08.2018) all inoculated cultures were used for a plasmid isolation.

Isolated plasmids are stored at -20 °C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 166/192: Mutagensis
In Project: Level_2
With tags: Mutagenesis

created: 18.08.2018 13:47
updated: 18.08.2018 13:52

Mutagenesis of p2iGEM0271

- 0,5µl plasmid
- 1 µl primer fw
- 10 µl Q5-Buffer
- 1 µl dNTP
- 0.5µl Q5-polymerase(at last on ice!)
- 5µl DMSO
- 21 µl miliQ

6 tubes were filled with 50µl of the mastermix

Touch up PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - Tuch-up 70°C (increase of 0.3°C every cycle)
  - 72 °C 3:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 167/192: Test restriction of CIDAR lvl1 cloning (lysC)
In Project: Level_2
With tags: Cidar, restriction, gelelectrophoresis, fail

created: 19.08.2018 13:20
updated: 19.08.2018 13:25

After cloning and transformation of CIDAR lvl 1 cloning with lysC, white colonies were picked and inoculated.

Thereafter plasmids were isplated with the Promega Pure Yiels Miniprep Kit.

Isolated plasmids were restricted each with EcoRI & PstI

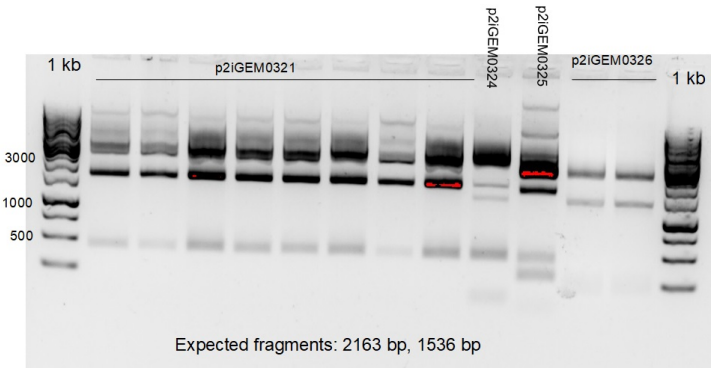
Restriction run at 37 °C for 1,5 h.

Reaction was inactivated at 75 °C for 20 min.

A gelelectrophoresis showed the fragments.

Several different bands appeared. So it was decided to send some random samples to sequencing.

test_restriction_CIDAR_lvl1_lysC.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 168/192: No entry title yet
In Project: Level_2
With tags: ligation

created: 20.08.2018 11:19
updated: 20.08.2018 14:03

DpnI digest of p2iGEM0327

- transfer plasmid into a new tube
- add 1µl DpnI
- incubate at 37 °C for 1h

Ligation of ddh into the level 1 Cidar vector (6 different promoters)

p2iGEM0347
p2iGEM0348
p2iGEM0349
p2iGEM0350
p2iGEM0351
p2iGEM0352

- 1 µl of each pre restricted sample was used for each ligation.
- 1 µl of T4 ligase was used
- 2 µl of T4 ligase buffer was used
- the samples were filled up to 20 µl with Milli Q Water.
- The ligation run at room temperature for around 15 min and afterwards inactivated at 66 °C for 25 min.
- Samples were stored at -20 °C.

Trafo of mutagenized topo_ddh plasmid and ligated plasmids (p2iGEM0347,p2iGEM0348,p2iGEM0349,p2iGEM0350,p2iGEM0351, p2iGEM0352)

- Thaw competent cells 10 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Kan+X-gal) and stored in the 37°C incubator

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert

created: 21.08.2018 17:06
updated: 21.08.2018 17:09

Entry 169/192: Amplification of ddh & overnightculutures of level 1 ligation
In Project: Level_2
With tags: ddh, pcr

Amplification of ddh from *C. glutamicum* gDNA in order to see if the point mutation is from the genome

Add in a tube

- 2µl Q5 buffer
- 2µl GC enhancer
- 2µl DMSO
- 12µl MiliQ
- 1µl primer fw (O_iGEM18_0001)
- 1µl primer rv(O_iGEM18_0002)
- 1µl template
- 0.5µl Q5 polymerase

Programm:

98°C	30 sec	
98°C	10 sec	
59°C	20 sec	
72°C	30 sec	35 cycles
72°C	2 min	
4°C	Hold	

Tubes were stored at 4°C after the PCR.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 23.08.2018 10:42
updated: 23.08.2018 16:17
Entry 170/192: Miniprep, Restriction with EcoRI and PstI & mutagenesis
In Project: Level_2
With tags: Pure yield miniprep, Plasmid prep, digest, Mutagenesis, ddh, gel electrophoresis

(22.8.2018)

Miniprep of p2iGEM0348, 0350, 0351, 0351 according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

Digest of p2iGEM0348, 0350, 0351, 0352 with PstI and EcoRI

18xMastermix

- 225µl MiliQ
- 36µl cutsmart
- 4.5µl EcoRI
- 4.5µl PstI

16 PCR tubes were filled with 15µl mastermix and 5µl DNA template

Incubation at 37°C for 2 h

Sequencing showed that the mutagenisis of p2iGEM0310 was not succesfull. Therefore it was repeated with a different primer

3Mastermix

- 6 µl primer fw
- 30 µl Q5-Buffer
- 30 µl GC enhancer
- 3 µl dNTP
- 0.75 µl Q5-polymerase(at last on ice!)
- 6µl DMSO
- 71.5 µl miliQ

Fill in each tube

- 49.45µl Mastermix
- 0.55µl template(10ng)

Touchdown PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 70°C 20 sec (decraease of -0.3°C every cycle)
  - 72 °C 2:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Gelelctrophoresis of digested plasmids

5µl purple loading dye was added to the samples

The gel ran on 90V for 60 min

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 23.08.2018 13:05
updated: 23.08.2018 13:11
Entry 171/192: Sequencing of CIDAR lvl 1 plasmids
In Project: Level_2
With tags: sequencing, cloning, Cidar

For p2iGEM0321 4 samples were sent to sequnecing

sample 1

sample 3

sample 4

sample 7

p2iGEM0324 was sent.

p2iGEM0325 sample 1 was sent

p2iGEM0326 smple 1 was sent

The sequencing of p2iGEM0324, p2iGEM0325 & p2iGEM0326 showed no positive results --> not insertes in the backbone or the sequencing looked unsecure at all.

p2iGEM0321 sample 1 showed one point mutation which causes a aminoacid switch: **Serin^{357} is switched to Glycin**.

The other samples of p2iGEM0321 showed the same point mutation and in addition also other ones.

The sample 1 of p2iGEM0321 was stroed further. The other samples were discarded.

The cloning will be repeated.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 172/192: Ligation and transformation of CIDAR lvl1 cloning
In Project: Level_2
No tags associated

created: 23.08.2018 13:12
updated: 29.08.2018 11:17

Try the ligation of CIDAR lvl 1 plasmids again

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 173/192: Transformation of mutagenesis
In Project: Level_2
With tags: Transformation

created: 28.08.2018 14:54
updated: 28.08.2018 14:57

p2iGEM0348, p2iGEM0350, p2iGEM0351, p2iGEM0352 and ddh were send to sequencing

Transformation of mutagenic ddh level 0 plasmid

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) and stored in the 37°C incubator

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
Entry 174/192: Inoculation of white CIDAR lvl1 colonies
In Project: Level_2
With tags: cidar, lvl1, Colony PCR

created: 29.08.2018 11:16
updated: 31.08.2018 11:10

From the transformants white colonies were picked and inoculated in 3 ml LB with kanamycine.

Cultures were incubated o/n at 37 °C, 220 rpm.

At the next day plasmids were isolated out of the culture.

Isolates plasmids were used for test PCRs.

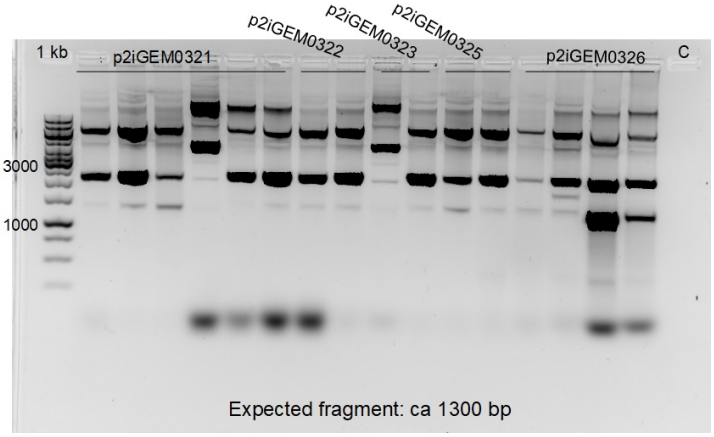
For the PCR Taq Polymerase was used.

The expected fragment is around 1300 bp and the PCR products were observed with gelelectrophoresis.

Nearly all products show the expected fragment.

One sample will be sent to sequencing for each plasmid.

CIDAR_lvl1_Try_6.jpg



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 175/192: Mutagenesis & overnight cultures
In Project: Level_2
With tags: Mutagenesis, overnight

created: 29.08.2018 16:13
updated: 29.08.2018 16:19

Sequencing showed that the dotmutation is not in the original genome. Therfore the side directed mutagenisis is repeated

6x Mastermix

- 3.15µl plasmid(10ng)
- 12 µl primer
- 60 µl GC enhancer
- 60 µl Q5-Buffer
- 6 µl dNTP
- 1.5µl Q5-polymerase(at last on ice!)
- 12µl DMSO
- 144 µl miliQ(RNAse free water)

6 tubes werefilled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - gradient 71°C - 65°C 30 sec
  - 72 °C 2:20 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Overnightculture of 2iGEM0361 (x6)

- 6 culture tubes were filled with 3ml LB-medium +amp (sterile)
- overnight incubation by 37°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert

created: 30.08.2018 13:48
updated: 30.08.2018 13:53

Entry 176/192: Trafo & Plasmidprep & Sequencing
In Project: Level_2
With tags: Plasmid prep, Pure yield miniprep, sequencing, Transformation, digest

Miniprep of p2iGEM0327(x6) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

DpnI digest of p2iGEM0327

- fill plasmid into a new tube
- add 1µl DpnI
- incubate at 37°C for 1h

Transformation of p2iGEM0327

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp) and stored in the 37°C incubator

Six samples of p2iGEM0327 were sent to sequencing

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 177/192: BsaI digest
In Project: Level_2
With tags: bsai, digest

created: 31.08.2018 14:31
updated: 31.08.2018 14:34

BsaI digest of p2iGEM0109, p2iGEM0113, p2iGEM0117, p2iGEM0121, p2iGEM0125, p2iGEM0129, p2iGEM0149, p2iGEM0164, p2iGEM0196 and p2iGEM0327

11x mastermix was prepared

- 22µl cutsmart
- 162.25µl MiliQ
- 2.75µl BsaI

Ech tube was filled with 18µl mastermix and 2µl template

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 178/192: Cidar level 1 ligation & Trafo
In Project: Level_2
With tags: ligation, T4 ligase, ddh, Transformation

created: 02.09.2018 14:31
updated: 02.09.2018 14:34

Ligation of ddh into the level 1 Cidar vector (6 different promoters)

p2iGEM0347
p2iGEM0348
p2iGEM0349
p2iGEM0350
p2iGEM0351
p2iGEM0352

- 1 µl of each pre restricted sample was used for each ligation.
- 0.5 µl of T4 ligase was used
- 2 µl of T4 ligase buffer was used
- the samples were filled up to 20 µl with Milli Q Water.
- The ligation run at room temperature for around 15 min and afterwards inactivated at 66 °C for 25 min.
- Samples were stored at -20 °C.

Transformation of ddh into level 1 plasmid as well as a retrafo of p2iGEM0327

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp or LB+Kan) and stored in the 37°C incubator

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 04.09.2018 17:39
updated: 14.09.2018 13:40
Entry 179/192: Tochdown PCR, Overnightculture, Digest & miniprep
In Project: Level_2
With tags: cryogenic culture, Knock out, pcr, touchdown pcr, restriction, Pure yield miniprep, Plasmid prep, miniprep, ddh, digest

(03.09.2018)

Picking 24 overnight cultures (2iGEM0394(4x), 2iGEM0395(5x), 2iGEM0396(2x), 2iGEM0397(3x), 2iGEM0398(4x), 2iGEM0399(5x) and 2iGEM0361(1x))

- culture was picked and inocculated in 3ml LB + kan or amp
- incubation overnight at 37°C

Miniprep of p2iGEM0347(4x), p2iGEM0348(5x), p2iGEM0349(2x), p2iGEM0350(3x), p2iGEM0351(4x), p2iGEM0352(5x) (23 plasmids in total) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

BsaI digest of p2iGEM0109, p2iGEM0113, p2iGEM0117, p2iGEM0121, p2iGEM0125, p2iGEM0129, p2iGEM0149, p2iGEM0164, p2iGEM0196 and p2iGEM0327

26x mastermix was prepared

- 325µl MiliQ
- 52µl Cutsmart
- 6.5µl PstI
- 6.5 µl EcoRI

Ech tube was filled with 18µl mastermix and 2µl template

Colony PCR to get Donor sequences for lysine and leucine knock out

4xMastermix:

- 1µl Q5 polymerase
- 10µl DMSO
- 40µl Q5Buffer
- 40µl GC enhancer
- 83 µl MiliQ water
- 4µl dNTPs

Each tube was filled with:

- 47µl mastermix
- 1µl primer fw
- 1µl primer rv
- 1µl DNA template

Tochdown PCR programm:

- 98°C 3 min
  - 98°C 30 sec
  - 64°C 20 sec (drecrease of 0.3 °C every cycle)
  - 72 °C 1 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Preparation of cryogenic culture 2iGEM0361

Fill in a cryogenic tube

- 300µl glycerine
- 700µl culture
- store at -80°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 180/192: Gelelctrophoresis and - elution
In Project: Level_2
With tags: gel electrophoresis, gelelution, ddh

created: 05.09.2018 16:30
updated: 17.09.2018 15:40

Gelelctrophoresis with digested plasmids

4µl purple loading was added to the samples

1µl 1kb ladder was used

The gel ran on 105V for 80 min

Gel purifcation

The bandy were cutted with blue light conditions and the weight measured

Per 10 mg of gel 10 µl of Membrane binding buffer were added to the tube.

Incubation at 55 °C till the gel was diluted in the buffer (around 7 min)

Fill diluted gel into the column (incubate at room temperature for 1 minute)

centrifuge at 16000 g für 1 min -> discart the flow through

add 700 µl washing buffer and centrifuge again -> discart the flow through

add 400 µl washing buffer and centrifuge 5 min --> discart the flow through

centrifuge empty column for 1 min

Put column into a new tube

Add 30 µl of nuclease free water (from the kit) and elute for 1 h at room temperature

Centrifuge 1 min.

Measure the concentration

Store DNA at -20°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 181/192: Go-gate & PCR
In Project: Level_2
With tags: pcr, golden gate

created: 08.09.2018 17:20
updated: 11.09.2018 13:52

The golden Gate of the Level 1 plasmids

The following reaction conditions were applied:

Amounts:

1.5µl CutSmart

1µl Plasmid (p2iGEM0113, p2iGEM0117, p2iGEM0121, p2iGEM0125 or p2iGEM0129),

1µl plasmid p2iGEM0149, p2iGEM0164, p2iGEM0196 and p2iGEM0327

10ng Backbone

1µl T4 ligase

1.5 µl T4 ligase buffer

1µl BsaI

water up to 15µl

Reaction conditions:

1.37°C 20 min

2.37°C 1.5min

3.16°C 3 min

2+3:50x

4.50°C 5min

6. 80°C 10min

7.4°C hold

Colony PCR to get Donor sequences for lysine and leucine knock out

3xMastermix:

- 0.75µl Q5 polymerase
- 7.5µl DMSO
- 15µl Q5Buffer
- 15µl GC enhancer
- 24.75 µl MiliQ water
- 1.5 µl dNTPs

Each tube was filled with:

- 23µl mastermix
- 1µl primer fw
- 1µl primer rv
- 1µl DNA template

PCR programm:

- 98°C 3 min
  - 98°C 30 sec
  - 55°C 20 sec
  - 72 °C 1 min
  - repeat this 10 times
  - 98°C 30 sec
  - 67°C 20 sec
  - 72 °C 1 min
  - repeat this 20 times
- 72°C 5 min
- hold 12°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 182/192: No entry title yet
In Project: Level_2
No tags associated

created: 11.09.2018 13:52
updated: 11.09.2018 17:27

Transformation of CIDAR level 1 constructs (10.9.18)

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid
- Incubate 30 min on ice
- 42°C, 45 sec heat shock
- Incubate 5 minutes on ice
- add 300 µl LB without antibiotic
- 1 h at 37°C and 300 rpm
- cells were plated out (LB+Amp or LB+Kan) and stored in the 37°C incubator

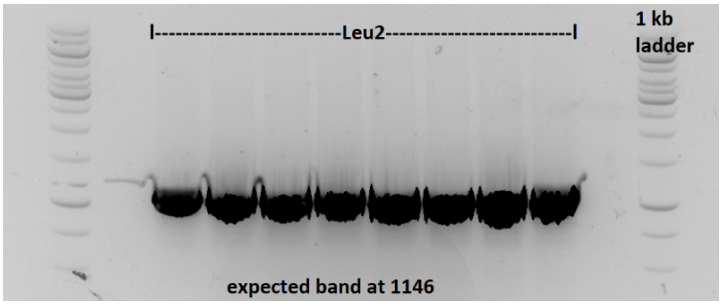
Gelelctrophoresis of Leu2

- 5µl were added to the sample
- 1µl 1kb ladder was used

Gel ran on 90V for 75min

The bands were cuuted out for further gel elution

Gel_2018-09-11_15hr_33min_Salima's_Leu2_75min_90V_bearbeitet.tif



Gel elution with the promega Kit to purify ori

- The bandy were cut with UV light conditions and the weight measured
- Per 10 mg of gel 10 µl of Membrane binding buffer were added to the tube.
- Incubation at 55 °C till the gel was diluted in the buffer (around 7 min)
- Fill diluted gel into the column (incubate at room temperature for 1 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min --> discart the flow through
- add 400 µl washing buffer and centrifuge 5 min --> discart the flow through
- centrifuge empty column for 1 min
- Put column into a new tube
- Add 30 µl of nuclease free water and elute at room temperature
- Centrifuge 1 min.
- Store DNA at -20°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 183/192: Plasmidprep
In Project: Level_2
With tags: Plasmid prep, Pure yield miniprep

created: 13.09.2018 11:22
updated: 13.09.2018 19:29

Miniprep of redCas9 and pGRB according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

PCR of pRGB with sgDNA for *LeuB* and *LysC* with the follwing protocol:

sample:

- 5 µl Q5 Buffer
- 0,5 µl Template
- 0,5 µl dNTP
- 1 µl Primer fwd
- 1 µl Primer rev
- 0,52 µl Q5 Polymerase
- 16.75 µl Milli Q

PCR programm:

- 95°C 5 min
  - 95°C 20 sec
  - 53°C 20 sec
  - 72 °C 1:30 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 184/192: No entry title yet
In Project: Level_2
With tags: gelelution, Pure yield miniprep, Plasmid prep

created: 14.09.2018 13:24
updated: 14.09.2018 17:10

Miniprep of p2iGEM0348, p2iGEM0349(5x),p2iGEM0350(5x) and p2iGEM0351(5x) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

Gel Elution with the Wizard® SV Gel and PCR Clean-Up System and the following protocol:

- add 10 µl Membrane binding solution per 10 mg gel
- incubate at 65 °C at 700 rpm
- Fill gel mixture in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g for 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000xg -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000xg -> discard the flow through
- centrifuge empty column with out lid for 1 min 16000g to allow for evaporation
- Put column into a new tube
- Add 30 µl of nuclease free water and elute for several hours at room temperature
- Centrifuge 1 min 16000g
- Put column into a new tube and add 20 µl nuclease free water and incubate for 10 min
- store at -20°C.

Transformation of pRGB with the sgDNA from *LeuB* and *LysC*

- thraw competent cells (*Top 10*) 5-10 min on ice
- add 4 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for environ 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- centrifuge with 6000 rpm for 2 min, decate 200 µl supernatant (sterile!), resuspend the rest (100 µl)
- plate 100 µl on LB amp
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 185/192: Gelelectrophoresis & Gelelution
In Project: Level_2
With tags: gelelution, gel electrophoresis

created: 15.09.2018 10:52
updated: 15.09.2018 12:11

Gelelctrophoresis of digested level 1 CIDAR plasmids with *ddh*

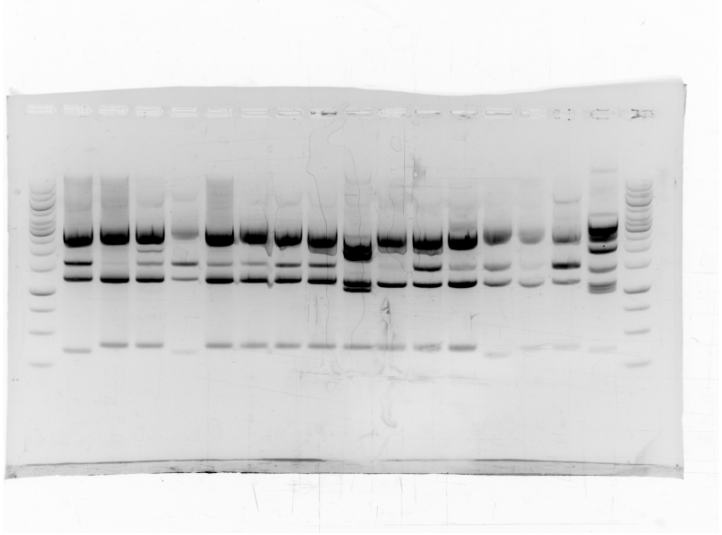
- 4µl purple loading dye(NEB) was added to the samples
- 1µl 1kb ladder(Thermo Fischer) was used

Gel ran on 90 V for 1h

Gel Elution of level 1 CIDAR plasmids with the Wizard® SV Gel and PCR Clean-Up System and the following protocol:

- add 10 µl Membrane binding solution per 10 mg gel
- incubate at 65 °C at 700 rpm
- Fill gel mixture in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g for 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000xg -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000xg -> discard the flow through
- centrifuge empty column with out lid for 1 min 16000g to allow for evaporation
- Put column into a new tube
- Add 30 µl of nuclease free water and elute for several hours at room temperature
- Centrifuge 1 min 16000g
- Put column into a new tube and add 30 µl nuclease free water and incubate for 10 min
- store at -20°C.

Gel_2018-09-15_11hr_42min_ddh_level1_testrestriction_EcoRI,_PstI.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Thomas Bick

created: 15.09.2018 15:05
updated: 15.09.2018 16:07

Entry 186/192: Gradient PCR for amplifing Donorsequencens for CRISPR Knockout from coli DH5a gDNA
In Project: Level_2
With tags: gel electrophoresis, pcr

Gradient PCR for amplifing Donorsequencens for CRISPR Knockout from coli DH5a gDNA:

LysC KO: Primer: O_iGEM_0109

O_iGEM_0110

LeuB KO: Primer: O_iGEM_0111

O_iGEM_0112

O_iGEM_0113

O_iGEM_0114

LysC KI: Primer: O_iGEM_0111

O_iGEM_0115

O_iGEM_0114

O_iGEM_0117

5X Q5 Reaction Buffer	10 µl
10 mM dNTPs	1 µl
10 µM Forward Primer	2.5 µl
10 µM Reverse Primer	2.5 µl
Template DNA	1 µl
Q5 High-Fidelity DNA Polymerase	0.5 µl
Nuclease-Free Water	to 50 µl (32.5 µl)

divide into 5 samples each 10 µl

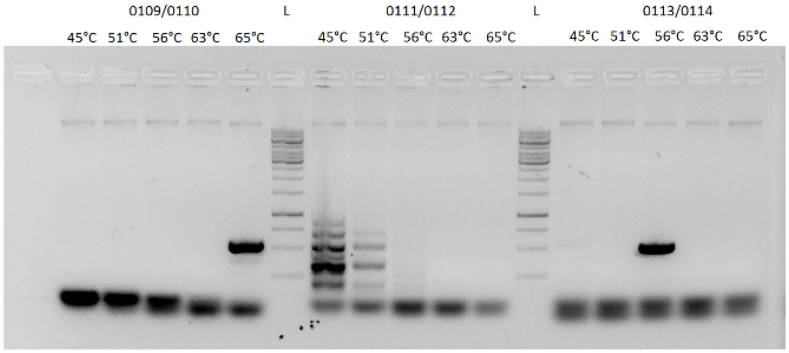
use one sample for each temperatur:

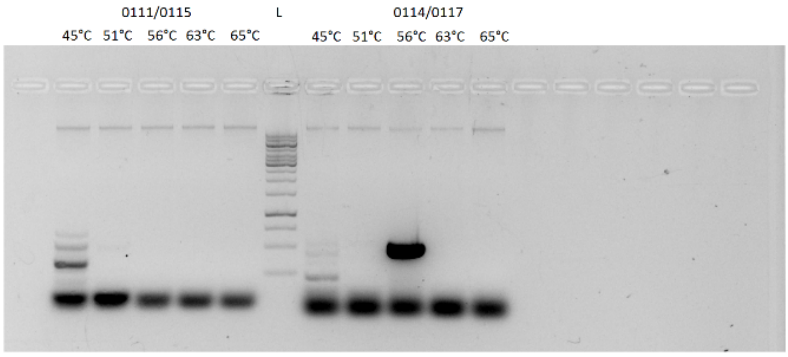
PCR programm:

- 98°C 2 min
  - 98°C 10 sec
  - gradient: 45°C, 51°C, 56°C, 63°C, 65°C 30 sec
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 10 min
- hold 12°C

1% Agarose Gel:

Gel_2018-09-15_16hr_00min_gradient_Donor_coli_DH5a.tif





Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 187/192: Plasmid prep of pRGB
In Project: Level_2
With tags: Plasmid prep, Pure yield miniprep

created: 16.09.2018 12:21
updated: 16.09.2018 12:25

Miniprep of pGRB with sgDNa of *LeuB* and *LysC* according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl elution buffer and centrifuge down for 1 min.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
created: 18.09.2018 16:06
updated: 18.09.2018 16:13
Entry 188/192: PCR of donor sequences for knockouts
In Project: Level_2
With tags: touchdown pcr, gradient pcr, pcr, Transformation

Gradient PCR for amplifing Donorsequencens for CRISPR Knockout from coli DH5a gDNA:

LysC KO: Primer: O_iGEM_0109

O_iGEM_0110

LeuB KO: Primer: O_iGEM_0111

O_iGEM_0112

O_iGEM_0113

O_iGEM_0114

LysC KI: Primer: O_iGEM_0111

O_iGEM_0115

O_iGEM_0114

O_iGEM_0117

5X Q5 Reaction Buffer	5 µl
10 mM dNTPs	0.5 µl
10 µM Forward Primer	1.25 µl
10 µM Reverse Primer	1.25µl
Template DNA	0.5 µl
Q5 High-Fidelity DNA Polymerase	0.25 µl
Nuclease-Free Water	to 25 µl

Gradient PCR programm:

- 98°C 2 min
  - 98°C 10 sec
  - gradient: 56°C(113+114, 114+117) 65°C(109+110)
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 10 min
- hold 12°C

Touchdown PCR programm:

- 98°C 2 min
  - 98°C 10 sec
  - 51°C(-0.3°C decrease every cycle) 111+115,111+112
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 10 min
- hold 12°C

Transformation of pSHDY in *E. coli* C43

- thraw competent cells 5-10 min on ice
- add 4 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for environ 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- centrifuge with 6000 rpm for 2 min, decate 200 µl supernatant (sterile!), resuspend the rest (100 µl)
- plate 100 µl on LB SpeR
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 189/192: No entry title yet
In Project: Level_2
No tags associated

created: 19.09.2018 15:23
updated: 19.09.2018 15:29

Gelelctrophoresis of Knockout PCR products

2µl(4µl) of the sample were added on the gel

2µl purple loading dye and 8µl MiliQ water were added to the sample

Gel ran on 100V for 90 min

Gelelution of two samples from the gel

Gel Elution of level 1 CIDAR plasmids with the Wizard® SV Gel and PCR Clean-Up System and the following protocol:

- add 10 µl Membrane binding solution per 10 mg gel
- incubate at 65 °C at 700 rpm
- Fill gel mixture in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g for 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000xg -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000xg -> discard the flow through
- centrifuge empty column with out lid for 1 min 16000g to allow for evaporation
- Put column into a new tube
- Add 30 µl of nuclease free water and elute for several hours at room temperature
- Centrifuge 1 min 16000g
- Put column into a new tube and add 30 µl nuclease free water and incubate for 10 min
- store at -20°C.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Sarah Seyffert
Entry 190/192: No entry title yet
In Project: Level_2
No tags associated

created: 27.09.2018 10:40
updated: 27.09.2018 10:45

(25.09.2018)

Miniprep of p2iGEM0404(10x), p2iGEM0405(10x) and p2iGEM0406(10x) according to the following protocol from pure yield miniprep:

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

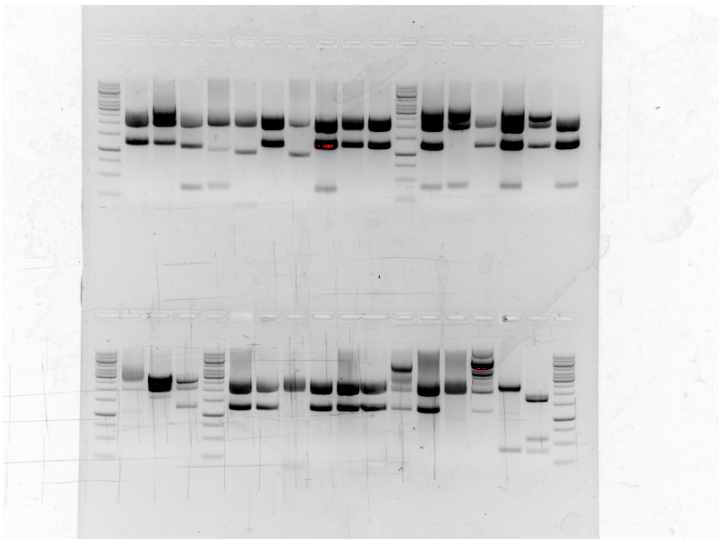
(26.09.2018)

Testrestriction of p2iGEM0404(10x), p2iGEM0405(10x) and p2iGEM0406(10x) with PstI and EcoRI

fill in each tube

- 0.2 µl EcorI
- 0.2 µl PstI
- 1µl Cutsmart
- 1.2 µl template
- fill up to 10µl with MiliQ

Gel_2018-09-26_17hr_58min_iGEM_backbone_cloning_ddh_level_1;_test_kryo_p2iGEM0392_and_p2iGEM0001;_1kb_ladder_90V_60_min.tif



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Jennifer Denter
created: 28.09.2018 14:19
updated: 28.09.2018 14:58
Entry 191/192: Growth experiment of E. coli C43 in M2 with sucrose
In Project: Level_2
With tags: growth, E. coli C43, sucrose, Glucose, growth experiment

Transformation of E. coli C43 with p2iGEM0321 (2iGEM0443)

Transformation with heat shock method.

Cells were incubated 50% on plate and 50% in liquid conditions.

37°C for both and 220rpm for the liquid tube.

On the next day the growth of the cells in both conditions was controlled.

Liquid culture was discarded afterwards.

A colony was picked from the plate and incubated in LB with kanamycine treatment o/n at 37 °C.

Kryo culture was prepared the next das with following solutions:

700 µl of culture

300 µl of 99% Gylcerin

-> Kryo was done under sterile conditions and stored at -80 °C in Kryo Box 4.

To test the grwoth of the cells in M2 media several inoculations were done with different conditions ans treatments:

1) M2 medium

2) M2 medium with 1 % Sucrose

3) M2 medium with 1,5 % Sucrose

4) M2 medium with 2 % Sucrose

5) M2 medium with 10 % Sucrose

6) M2 medium with 1,5 % Sucrose and 120 µl ammonumsulfate (50 mg/ml)

7) M2 medium with 0,5 % Glucose

8) M2 medium with 1% Glucose

9) M2 medium with 1,5 % Glucose

10) M2 medium with 1,5 % Glucose and 120µl ammoniumsulfate (50 mg/ml)

11) Positive controle in LB

--> All tubes contained knamycine treatment

Cultures incubates for 24 h at 37 °C, 220 rpm.

In M2 with Sucrose, no growth could be detected. With Glucose the cells grew.

It was decided that for the growth experiment glucose will be used as carbon source.

Also a additional nitrogene source will be added.

Cultivation experiments will be performed in 50 ml volume in 200 ml flasks.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 192/192: Lysine availability experiment
In Project: Level_2
With tags: lysine, E.coli, yeast

created: 03.10.2018 19:09
updated: 03.10.2018 19:19

Today an experiment was performed, where the main idea came from reading a paper from Liu and colleagues (https://msphere.asm.org/content/3/1/e00586-17). Here they spread different types of microorganisms horizontally and vertically on a plate and checked the impact that both organisms had on each other.

In this case, E.coli cells producing lysine, as well as S.cerevisiae cells and the respective E.coli BL21 (DE3) C43 cells were spread in a similar manner to check impact.

The following different plates were poured:

- M2 + Lys+ Leu+Ura+His+ Kan (2 plates w/o Kan)

- M2+ Lys+Leu+Ura+ His+ 1,5% glucose+ Kan (2 plates w/o Kan)

- M2+ Lys+Leu+Ura+ His+ 1,5% sucrose+ Kan (2 plates w/o Kan)

- M2+ Leu+Ura+ His+ 1,5% glucose+ Kan (2 plates w/o Kan)

- M2+Leu+Ura+ His+ 1,5% sucrose+ Kan (2 plates w/o Kan)

E.coli wild type cells were spread on plates without antibiotics.

The following combinations were plated out:

- E.coli lysC

- S.cerevisiae BY4742

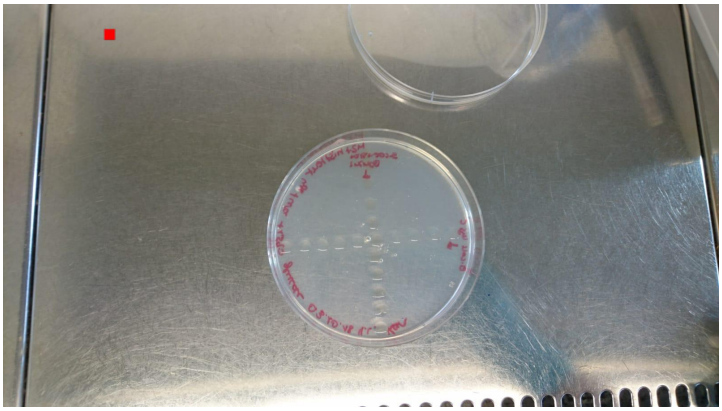
- E.coli BL21 (DE3) C43

- E.coli BL21(DE3) C43+ S.cerevisiae BY4742

- E.coli lysC + S.cerevisiae BY4742

An example of how the plates were spread can be seen in the Figure below.

Plate_experiment.jpeg



Date:	Signed and understood by:
Date:	Witnessed and understood by: