Author: Svenja Hermanns
created: 12.04.2018 20:50
updated: 10.05.2018 17:14
Entry 1/259: Extraction of gDNA
In Project: Level_1
With tags: gDNA, Corynebacterium, Synechococcus, Zymomonas

We extracted the gDNA from *Synechococcus elongatus*, *Zymomonas mobilis* and *Corynebacterium glutamicum:*

- yesterday we started overnight cultures of all three organisms and they grew good enough to use today
- 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
- transfered 160 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 cholorform
- 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 transfered 160 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
- transfered 150 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:

Zymomonas mobilis : 1401,0 ng/µL

Synechococcus elongatis: 467,2 ng/µL

Corynebacterium glutamicum: 481,6 ng/µL

We noticed the concentrations were very high and our samples were not very pure. So we decided to purify them some more:

- we added 1/10 Vol. (10 µL) 3 M NaOAc and 3 Vol. (300 µL) 100% ethanol

- now the samples are stored at -20 °C until monday

On Monday we will continue the purification.

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

Author: Susanne Vollmer
created: 18.04.2018 17:30
updated: 18.04.2018 18:45
Entry 2/259: Organism for gDNA isolation
In Project: Level_1
No tags associated

P. putida strain KT2440 was spread on a LB plate and incubated on 30°C

Compis of *E.coli* strain (T10) DH5a on a LB plate incubate on 30°C

LB-plates without antibiotic were poured

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

Author: Thomas Bick
Entry 3/259: No entry title yet
In Project: Level_1
No tags associated

created: 20.04.2018 20:10
updated: 20.04.2018 20:17

Transformations of p2iGEM0009, p2iGEM0010, p2iGEM0011, p2iGEM0012, p2iGEM0013, p2iGEM0014, p2iGEM0015, p2iGEM0016, p2iGEM0017, p2iGEM0018, p2iGEM0019, p2iGEM0020, p2iGEM0021, p2iGEM0022, p2iGEM0023 in to competent Top10 E. coli for Mini plasmid isolation

- Incubate competent cells 10 minutes on ice
- Add 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 300rpm (incubator)
- inoculation of transformed coli in 3ml LB+ Cam
- inkubation over night at 37°C

Inoculation of wildtype E. coli DH5a and P. putida KT2440 in 3ml LB for gDNA isolation over night at 37°C (coli) and 30°C (putida)

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

Author: Susanne Vollmer

created: 20.04.2018 20:31
updated: 27.04.2018 10:03

Entry 4/259: Plasmid-prep and gDNA preperations

In Project: Level_1

No tags associated

Mini prep of the Plasmids p2iGEM0009-p2IGEM0023 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

Because there were 3ml of culture I made the first five steps with each 800µl culture and 133µlLysisbuffer and 467µl Neutralisationbuffer and repeat this four times.

The concentration of the isolated Plasmids:

Plasmidnumber:	Concentration [ng/µl]:
p2iGEM0009	198,30
p2iGEM0010	328,45
p2iGEM0011	263,85
p2iGEM0012	371,25
p2iGEM0013	341,45
p2iGEM0014	220,15
p2iGEM0015	372,75
p2iGEM0016	300,10
p2iGEM0017	217,55
p2iGEM0018	246,90
p2iGEM0019	180,55
p2iGEM0020	199,60
p2iGEM0021	307,15
p2iGEM0022	211,25
p2iGEM0023	232,95

From the *P.putida* culture which were inoculated yesterday, a Cryo with 750 µl culture and 250 µl glyceron was taken and is now in the -80 °C freezer. The rest and the inoculated *E.coli* culture were centrifuged, the supernatant was removed and the pellet stored by -20° C as preperation for the gDNA isolation.

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

Author: Svenja Hermanns
created: 20.04.2018 22:08
updated: 22.04.2018 13:27
Entry 5/259: Extraction of gDNA
In Project: Level_1
With tags: Corynebacterium, gDNA, Synechococcus, Zymomonas

Yesterday we finished our purification steps from gDNA extraction of *S.elongatus, Z.mobilis* and *C.glutamicum*

- the samples were stored at - 20°C
- 30 min. max rpm at 4 °C
- gently remove supernatant
- added 100 µl 70% ethanol --> don´t resuspend the pellet
- 15 min. max rpm at 4°C
- gently remove supernatant
- added 100 µl 70% ethanol --> don´t resuspend the pellet
- 15 min. max rpm at 4°C
- gently remove supernatant
- let the samples dry under the steril bench for about 5 min. (not to long or the pellet woun´t resuspend later)
- resuspend the pellet in 40 µl milliQ water

gDNA concentrations:

S.elongatus 247,3 ng/µl

Z.mobilis 489,15 ng/µl

C.glutamicum 16.35 ng/µl

Today in the morning we set up an liqid culture from *Z.mobilis* and *C.glutamicum*

In the afternoon we used the Quick-DNA Miniprep Plus Kit to extract gDNA from *S.elongatus, Z.mobilis* and *C.glutamicum*

- centrifuged the cultur and removed the supernatant
- resuspended our pellet in 200 µl DNA elution buffer
- transfered 200 µl of the solution to a new tube and added 200 µl BioFluid & Cell Buffer and 20 µl Proteinkinase K
- vortexed and incubated at 55 °C for 10 min.
- added 1 volume Genomic Binding Buffer to the tube and vortex
- transfered to a column in a collection tube and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 400 µl DNA Pre Wash Buffer and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 700 µl g-DNA Wash Buffer and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 200 µl g-DNA Wash Buffer and centrifuged for 1 min. at max. rpm
- dicarded the collection tube
- added the column to a new tube and added DNA Elution Buffer (50 µl for *S.elongatus* and 40 µl for *C.glutamicum and Z.mobilis*)
- incubated for 5 min. and centrifuged after that for 1 min. at max. rpm

gDNA concentrations:

S.elongatus 30,40 ng/µl

Z.mobilis 306,25 ng/µl

C.glutamicum 9,50 ng/µl

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

Author: Katharina Polzen
created: 23.04.2018 15:23
updated: 23.04.2018 15:39
Entry 6/259: gDNAisolation
In Project: Level_1
With tags: gDNA, isolation, E.coli, P. putida

gDNA isolation from the pellets from *E.coli* DH5a and *P.putida* KT2440 from Friday, with the Quick-DNA Miniprep Plus Kit and the following protocol:

- centrifuged the culture and removed the supernatant
- resuspended our pellet in 200 µl DNA elution buffer
- transffered 200 µl of the solution to a new tube and added 200 µl BioFluid & Cell Buffer and 20 µl Proteinkinase K
- vortexed and incubated at 55 °C for 10 min.
- added 1 volume Genomic Binding Buffer to the tube and vortex
- transfered to a column in a collection tube and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 400 µl DNA Pre Wash Buffer and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 700 µl g-DNA Wash Buffer and centrifuged for 1 min. at max. rpm
- discarded the flow through
- added 200 µl g-DNA Wash Buffer and centrifuged for 1 min. at max. rpm
- dicarded the collection tube
- added the column to a new tube and added DNA Elution Buffer (50 µl for *S.elongatus* and 40 µl for *C.glutamicum and Z.mobilis*)
- incubated for 5 min. and centrifuged after that for 1 min. at max. rpm

deviation: without Step 8,9

Concentration:

E.coli DH5a: 46,600ng/µl

P.putida KT2440: 234,35ng/µl

Stored at -20 °C

done by Susanne

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

Author: Susanne Vollmer
Entry 7/259: PCR ptxD amplification
In Project: Level_1
No tags associated

created: 24.04.2018 14:31
updated: 24.04.2018 14:59

PCR of the gDNA from *P. putida* to amplify ptxD using the following protocol:

Primer used:

O_iGEM0007_ptxD_lvl0_p

O_iGEM0008_ptxD_lvl0_p

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 sec

repeating this 28x (depending on the situation 28-30)

72°C 2min

5°C HOLD

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

Author: Sarah Seyffert
created: 24.04.2018 17:08
updated: 24.04.2018 18:29
Entry 8/259: Touchdown PCR & normal PCR
In Project: Level_1
With tags: pcr, tochdown pcr

Preparation:

- add in a PCR-tube:
- 250ng template(for S.elongatus 1µl(243,3ng/µl stock) and for Z.mobilis 0,5µl(489,16ng/µl stock))
- 10µl 5xBuffer
- dNTP 1,25µl (10mM stock)
- Primer foward 5 µl
- Primer reverse 5µl
- Q5-Polymerase 0,5µl (at last on ice!)
- fill to 50µl with miliQ

Touchdown PCR with those fragments:

- **glf** from Zymomonas mobilis gDNA; **primer**: iGEM027(fw) ; iGEM029(rv)
- **glf**(tagged) from Zymomonas mobilis gDNA; **primer** : iGEM022(rv) ; iGEM027(fw)
- **invA** from Zymomonas mobilis gDNA; **primer**: iGEM023(fw); iGEM029(rev)
- **invA**(tagged) from Zymomonas mobilis gDNA; **primer** iGEM026(fw); iGEM029(rv)
- **NISb** from Synechococcus elongatus sp. PCC 7942 gDNA; **primer** iGEM028(fw); iGEM031(rv)

Program of the Touchdown-PCR

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 60°C for 45 sec(annealing)
    3. 72°C for 1min(elongation)
    4. repeat 35 times -> each time the annealing temperature decreases by 0,77°C
3. 72°C for 10 min
4. Hold 4°c

Normal PCR

- **NSIa** from Synechococcus elongatus sp. PCC 7942 gDNA; **primer**: iGEM032(fw) ;iGEM017(rv)

Program of the PCR:

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec
    2. 56°C for 45 sec
    3. 72°C for 1min
    4. reapeat 35 times
3. 72°C for 10 min
4. Hold 4°c

Annotation: We adjust the annealing time depending of our fragment size instead of the elongation time. The elongation time should be the one adjusted

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

Author: Sarah Seyffert
Entry 9/259: Touchdown PCR & Retrafo
In Project: Level_1
With tags: tochdown pcr, Transformation

created: 25.04.2018 17:31
updated: 25.04.2018 22:29

Preparation:

- add in a PCR-tube:
- 1µl template
- 10µl 5xBuffer
- dNTP 1,25µl (10mM stock)
- Primer foward 5 µl
- Primer reverse 5µl
- Q5-Polymerase 0,5µl (at last on ice!)
- fill to 50µl with miliQ

Touchdown PCR with those fragments:

- **smR promotor**(Pcat) from NSI_TetR_LacA; **primer**: iGEM016(fw), iGEM019(rv)
- **SmR** from pSHDY; **primer** : iGEM018(fw), iGEM020(rv)
- **mVenus Tag** from pSHDY H56-3 mVenus; **primer**: iGEM021(fw), iGEM025(rv)
- **ori** from NSI_TetR_LacA; **primer**: iGEM030(fw), iGEM033(rv)

Program of the Touchdown-PCR

1. 98°C for 2 min
2. cicle with
    1. 98°C for 30 sec(denaturation)
    2. 60°C for 30 sec(annealing)
    3. 72°C for 30 sec(elongation)
    4. repeat 35 times -> each time the elongation temperature decreases by 0,6°C
3. 72°C for 10 min
4. Hold 4°c

Retransformation of

1. Add 10 µl cells + 1 µl plasmids in Eppis
2. Incubate 2 min on ice
3. 42°C, 45 sec heatshock (not longer)
4. Incubate 2 minutes on ice
5. + 300 µl LB without antibiotic **STERILE**
6. 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
7. 100 µl cells + 3 mL LB with antibiotic --> Overnight 37°C 200 rpm

The antibiotics were

- Spectinomycin, Kanamycin: psHD4
- Spectinomycin: pSHDY H56-3 mVenus
- Chloranphicol: NSI_TetR_LacA

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

Author: Miriam Dreesbach
Entry 10/259: Gel electrophoresis PCR 24.04.2018
In Project: Level_1
With tags: pcr, agarose gel, gel electrophoresis

created: 25.04.2018 21:12
updated: 25.04.2018 21:41

We loaded the PCR samples from yesterdays' PCR of the following constructs on an already prepared agarose gel by Susanne:

- NSIa 1.5 kb
- NSIb 1.5 kb
- glf 1.422 kb
- glf tagged 1.422 kb
- invA 1.583 kb
- invA tagged 1.583 kb

Therefore, we mixed 10 µL of each sample with 2 µL 6x Loading Dye (Thermo Fisher).

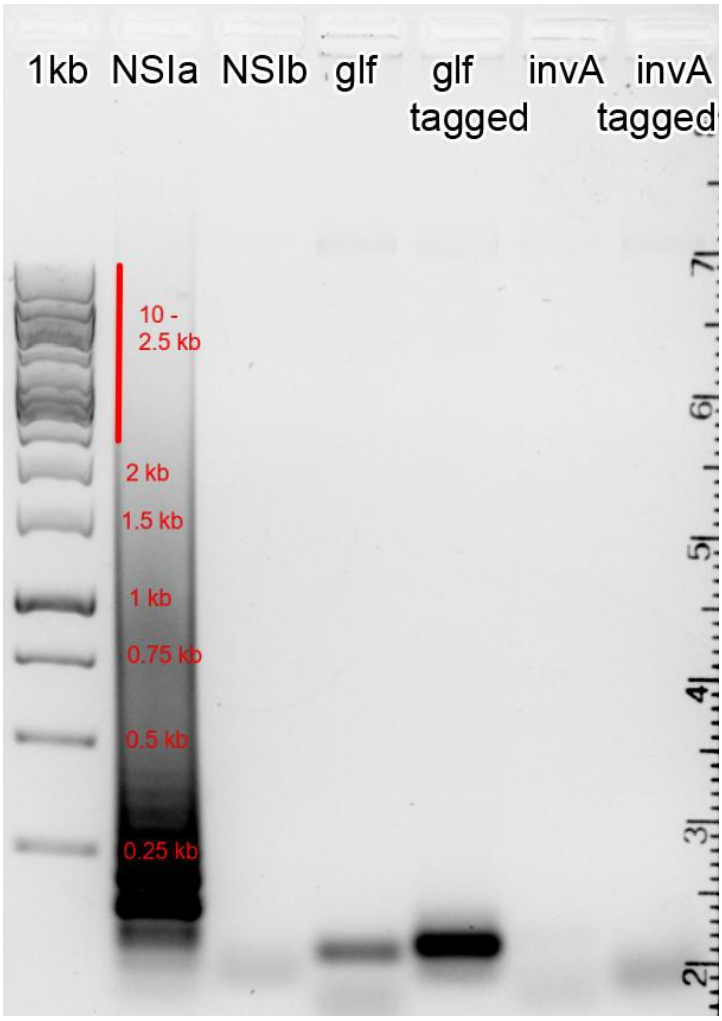
Afterwards we loaded the samples on the agarose gel.

The gel ran on 90 V for 1 hour.

After 1 hour, we visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

The visualized gel showed **no expected bands** (mostly primer dimer).

PCR20180425.jpg



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

Author: Susanne Vollmer
Entry 11/259: Gel of the PCR product ptxD
In Project: Level_1
No tags associated

created: 25.04.2018 21:14
updated: 25.04.2018 22:11

loding of the PCR product (ptxD amplified from *P.putida* gDNA) on gel:

using 1% agarose (0,5g Agarose and 50ml TAE_Buffer) with 2,5 µl gel red

in the gel pocket:

5µl of the PCR product

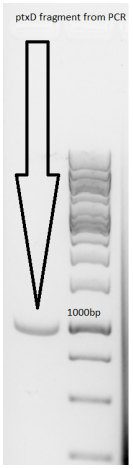
3µl water

2µl londing die 6x concentratet

using the 1kb ladder

the gel runns 1 hour with 90V in 1x TAE-Buffer

Gel_2018-04-25_16hr_21min_Testgel_1._ptxD_P._putida_2._1_kb_ladder_1_h_90_V_Ausschnitt.png



Isolation of the band using the following protocoll:

1. run gel
2. excise the band
3. but it into a 1,5 ml eppi
4. weight it
5. add Membrane solution at a Ratio of 10µl of solution per 10mg of agarose gel slice
6. vortex and incubate at 50-65°C for 10 min or until the gel is completly dissolved. vortex it every few minuts
7. put the solution on a colum in a collectiontube, incubate 1 min at roomtemperature
8. centrifuge at 16000g for 1 min
9. discard the liquid in the Collection tube
10. add 700µl Membrane wash solution
11. centrifuge at 16000g for 1 min
12. discard the liquid in the Collection tube
13. add 500µl of the Membrane wash solution
14. centrifuge at 16000 for 5 min
15. discard the liquid in the Collection tube
16. put the colum on a new eppi
17. add 50µl nuclease free water and put it on 37°C with 300rpm for 2-3 min
18. centrifuge 1min 50rcf
19. centrifuge 1-2min by max rpm

the measured concentration: 3,1500ng/µl

inoculate 2 culturetubes with 3ml LB and chlorampenicol with the cryo of 2iGEM0001, incubate at 37°C

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

Author: Susanne Vollmer
created: 26.04.2018 08:32
updated: 26.04.2018 19:35
Entry 12/259: PCR for GuaD amplification i gDNA of E.Coli
In Project: Level_1
No tags associated

PCR of the gDNA from *E.coli* DH5a to amplify guaD using the following protocol:

Primer used:

O_iGEM18_0047

O_iGEM18_0048

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

59°C 15 sec (depending on the anealing temperature of the Primer)

72°C 1min

repeating this 28x (depending on the situation 28-30)

72°C 2min

5°C HOLD

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

Author: Susanne Vollmer
Entry 13/259: Goldengate clonig of ptxD
In Project: Level_1
No tags associated

created: 26.04.2018 19:35
updated: 26.04.2018 19:54

Golden Gate cloning of the ptxD gen into the PYTK001 (p2iGEM0001) using the following protocol:

Mastermix for 3 tubes:

1,5µl p2iGEM0001

1,5µl Bsmb1

1,5µl T4 DNA Ligase

1,5µl T4 ligase Buffer

21µl milli Q water

from that put 9,5µl into each PCR-tube

in one PCR-tube: 0,5µl from the ptxD PCR product

in the other PCR-tube: 0,5µl from the ptxD Gel eluation

put the tubs in the cycler and start the following protocol:

2 min 37°C

5min 16°C

repeating that 60

10min 60°C

10 min 80°C

4°C HOLD

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

Author: Svenja Hermanns
Entry 14/259: Retrafo
In Project: Level_1
With tags: NSI, Zymomonas

created: 26.04.2018 21:36
updated: 15.05.2018 20:38

Retransformation of 2iGEM0208 (NSIa-TetR-lacA)

- 1 µl Plasmid in an eppi with small amount of Compis
- 5 min. Incubation on ice
- heatshock for 45 sec on 42 °C
- 15 min Incubation on ice
- added 300 µl LB
- 60 min Incubation on 37 °C while shaking
- transfered to chloramphenicol agar plate
- Incubstion at 37°C over night

Overnight culture of 2iGEM0099 (*Z. mobilis* WT)

- 3 ml LB with one scratch of agar culture
- Incubation overnight at 37°C while shaking

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

Author: Sarah Seyffert
created: 26.04.2018 21:54
updated: 26.04.2018 23:04
Entry 15/259: Gelelectrophoresis, cryoculutures an islolation of pcr fragment
In Project: Level_1
With tags: gel electrophoresis, cryogenic culture

Cryoculture was made with the strain 2iGEM0207

- 800µl culture + 200µlGlycerin **sterile**
- stored in -80°C freezer

Preperation of the agarose gel 1%

- 0,4g Agarose in 40 ml 1x TEA
- heating until the solution is clear
- add 2µl GelRed after cooling

We loaded the PCR samples from yesterdays' PCR of the following constructs and gDNA on an agarose gel:

- ori 589 bp
- mVenus 750 bp
- SmR 792 bp
- Pcat 205 bp
- gDNA of C.glutamicum 2x
- gDNA of Z. mobilis 2x
- gDNA of S. elongatus 2x

Therefore, we mixed 10 µL of each sample with 2 µL 6x Loading Dye (Thermo Fisher).

Afterwards we loaded the samples on the agarose gel.

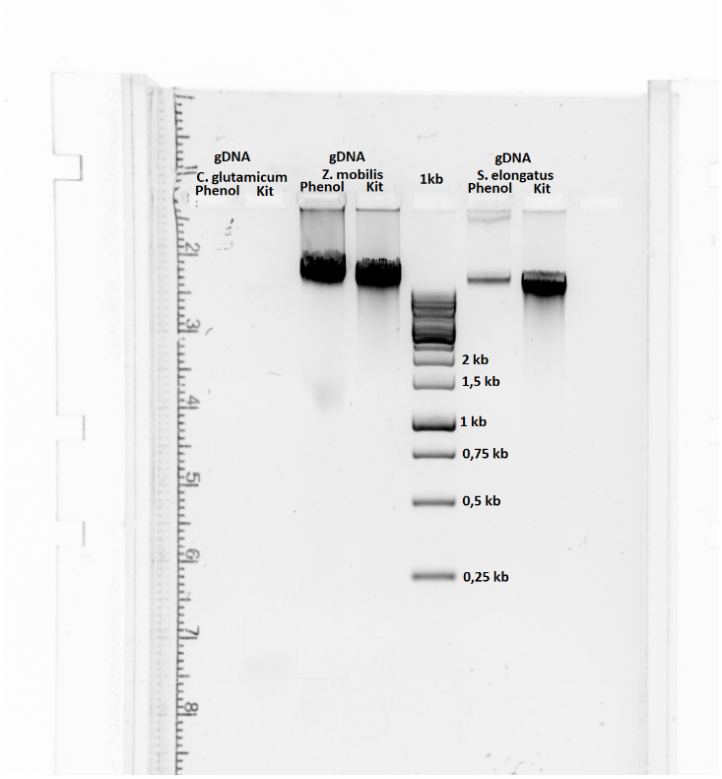
The gel ran on 100 V for 1 hour.

After 1 hour, we visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

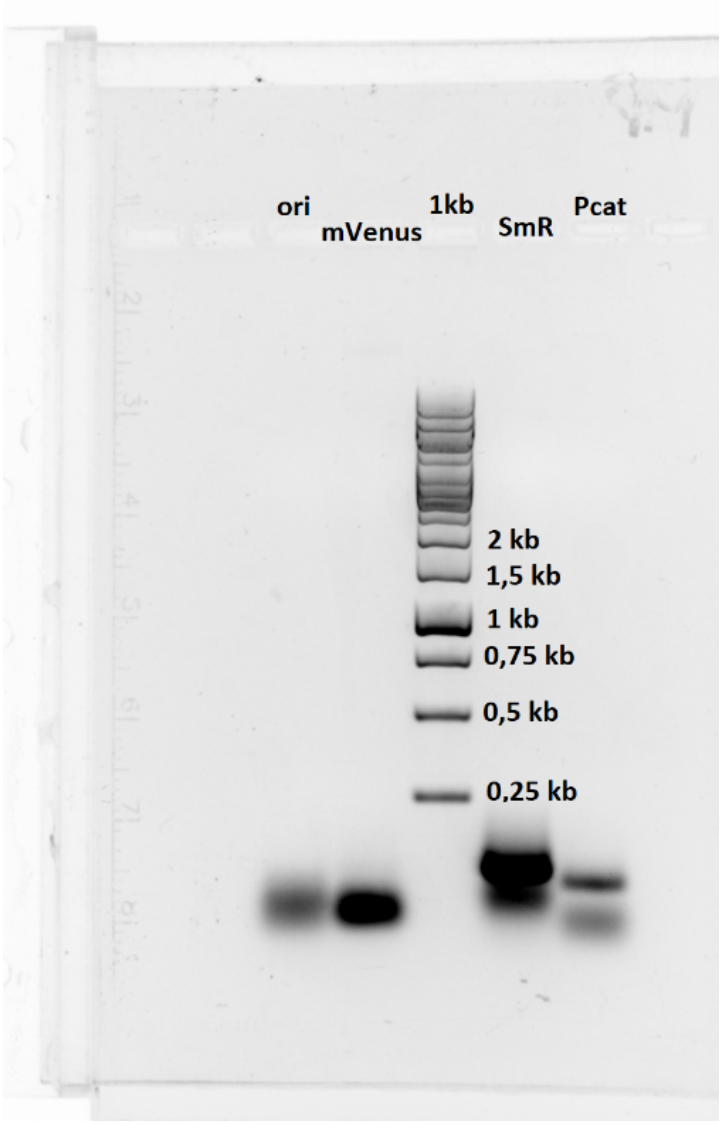
The visualized gel showed mostly **no expected bands** for the PCR fragments (mostly primer dimer) with the exeption of the Pcat band

The visualized gel showed **no band** for gDNA of C.glutamicum

Gel_2018-04-25_16hr_21min_Testgel_gDNA_links_PCR_Produkte_25.04.2018_rechts_1_kb_ladder_MD_1h_100V_gDNA.tif



Gel_2018-04-25_16hr_21min_Testgel_gDNA_links_PCR_Produkte_25.04.2018_rechts_1_kb_ladder_MD_1h_100V_pcr_fragmente.tif



isolation of the upper Pcat band using the following protocoll:

1. run gel
2. excise the band
3. but it into a 1,5 ml eppi
4. weight it ->0,1g
5. add Membrane solution at a Ratio of 10µl of solution per 10mg of agarose gel slice -> 100µl
6. vortex and incubate at 50-65°C for 10 min or until the gel is completly dissolved. vortex it every few minutes
7. put the solution on a colum in a collectiontube, incubate 1 min at roomtemperature
8. centrifuge at 16000g for 1 min
9. discard the liquid in the Collection tube
10. add 700µl Membrane wash solution
11. centrifuge at 16000g for 1 min
12. discard the liquid in the Collection tube
13. add 500µl of the Membrane wash solution
14. centrifuge at 16000g for 5 min
15. discard the liquid in the Collection tube
16. put the colum on a new eppi
17. add 50µl nuclease free water
18. centrifuge 16000g for 1 min

the measured concentration: 2,14ng/µl

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

Author: Susanne Vollmer
created: 27.04.2018 09:56
updated: 27.04.2018 10:24

Entry 16/259: Miniprep fromp2IGEM0001 (PKTY001)
In Project: Level_1
No tags associated

yesterday the 26.4.18 before the golden gate cloning, I did a miniprep with follwing protocol:

Mini prep of two tubes of the plasmid p2IGEM0001 with the following protocol:

- 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 15 Sek. max rpm --> discard flowtrough
- repeat the steps if ist to much liquid for the collum
- + 200 µl Endotoxin removal wash
- 15 sec max rpm->discard flowtrought
- 400µl Collum wash
- 30 sec cetrifuge by 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

measured concentration:

in one tube:120ng/µl

in the other:170ng/µl

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

Author: Susanne Vollmer

created: 27.04.2018 15:57
updated: 04.05.2018 16:15

Entry 17/259: transformation of E.coli DH5a with p2iGEM0001 and ptxD

In Project: Level_1

No tags associated

Transformation of *E.coli* DH5a with p2iGEM0001 (pYTK001) and the gen ptxD with the following protocol:

1. thaw competent cells 5-10 min on ice
2. add 1µl of the Plasmid DNA on the competent cells
3. flick the tube 3-4 times. Do not vortex
4. incubate on ice for 30 min
5. heatshock for 30 sec on 42 °C
6. place on ice for 5min
7. Pipette 300µl LB without Antibiothica (steriale)
8. place at 37°c for 60min, shake 300rpm
9. centrifuge the cells for 5min at 6000rcf, decant the supernatante and resuspend the cells in the rest(100-200µl)
10. screat the Suspension on a agarplate with the Fitting antibiothica (i used chloramphenicol)
11. incubate at 37°C

Gel of the PCR product from 26.4.18 the guaD from *E.Coli:*

Gel preparation:

150ml TAE and1,5g Agarose

1x TAE buffer

the sample:

5µl of the PCR product

3µl H2O

2µl loading dye 6x

using the 1kb ladder

the gel runs 1 hour with 90V in 1x TAE-Buffer:

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

Author: Sarah Seyffert
created: 27.04.2018 17:06
updated: 27.04.2018 17:14
Entry 18/259: Cryoculture and overnightculture
In Project: Level_1
With tags: cryogenic culture, overnight cultures

Cryoculture was made with the strain 2iGEM0206

- 800µl culture + 200µlGlycerin **sterile**
- stored in -80°C freezer

Overnight culture of 2iGEM0206

- 3 ml LB with 2,1µl chloramphenicol **sterile**
- Incubation overnight at 37°C while shaking

Z. moblis culture on plate agar without antibiotics

- add 300µl culture on plate **sterile**
- spread it over the plate

Aliquots of gDNA of Z.mobilis and S.elongatus

- add in a tube 9µl miliq water
- add 1µl gDNA

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

Author: Thomas Bick
Entry 19/259: No entry title yet
In Project: Level_1
No tags associated

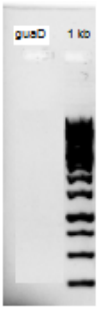
created: 28.04.2018 12:22
updated: 28.04.2018 12:27

1% Agarose Gel of guaD PCR from 26.04.18:

90 V, 1 1/2 h

left: PCR, right: 1kb ladder

guaD_Gel.png



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

Author: Thomas Bick
Entry 21/259: No entry title yet
In Project: Level_1
No tags associated

created: 28.04.2018 12:28
updated: 28.04.2018 12:32

Inoculation of 2iGEM0051 in 3 ml LB+Cm over night.

Miniprep with Kit:

- 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 15 Sek. max rpm --> discard flowtrough
- repeat the steps if ist to much liquid for the collum
- + 200 µl Endotoxin removal wash
- 15 sec max rpm->discard flowtrought
- 400µl Collum wash
- 30 sec cetrifuge by 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

Yield: 223 ng/mµl

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

Author: Sarah Seyffert
Entry 22/259: Miniprep & Cryogenic culture
In Project: Level_1
With tags: Pure yield miniprep, cryogenic culture

created: 28.04.2018 13:19
updated: 31.07.2018 16:22

Cryoculture was made with the strain 2iGEM0208

- 800µl culture + 200µlGlycerin **sterile**
- stored in -80°C freezer

Mini prep of plasmid p2iGEM202-0204 with 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 15 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 15 sec max rpm
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl elution buffer
- Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

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

Author: Susanne Vollmer
created: 30.04.2018 21:21
updated: 30.04.2018 21:50

Entry 23/259: 2. Try of the PCR of guaD from E.coli and Gel
In Project: Level_1
No tags associated

Because the gel show that the first PCR doesn't work, I try it again with the same protocol:

But in diffrence I take a anealing temperature of 55°C:

PCR of the gDNA from *E.coli* DH5a to amplify guaD using the following protocol:

Primer used:

O_iGEM18_0047

O_iGEM18_0048

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

59°C 15 sec (depending on the anealing temperature of the Primer)-> **changed to 55°C**

72°C 1min

repeating this 28x (depending on the situation 28-30)

72°C 2min

5°C HOLD

gel electrophoresis with the PCR product:

0,5g Agarose+50ml 1x TAE fot the gel (+2,5µl of GelRed after it was boiled up and already cooled a bit down)

add TAE until the chamber is full

in the gel pocket:

1.pocket: 5µ DNA 1kb ladder

2.pocket: 5µl of the PCR product

3µl water

2µl londing die 6x concentratet

3.pocket: 5µl of the gDNA of *E.coli* DH5a

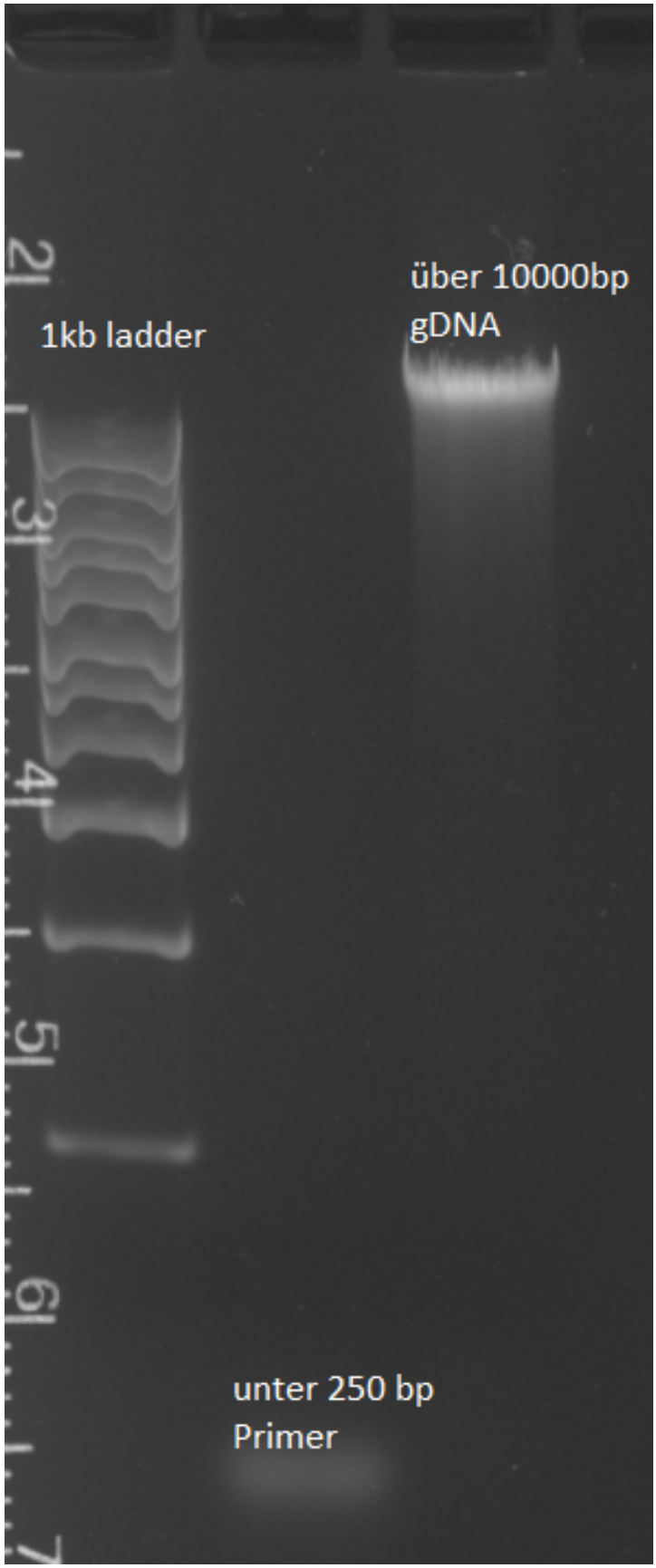
3µl water

2µl londing die 6x concentratet

run the gel 50 min by 90V

the image below show the gel, the gDNA is ok. But the PCR of guaD again failed and must be repeated.

Gel_2018-04-30_18hr_42min_Lvl1_gDNa&PCRguaD(2)_50min_90V_1kb_ladder,_final.png



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

Author: Susanne Vollmer

created: 01.05.2018 16:55
updated: 01.05.2018 17:18

Entry 24/259: inoculation of p2iGEM0208; trafo from p2iGEM0097-0108; Miniprep of p2iGEM0097 and 98

In Project: Level_1

No tags associated

selection of 5 white colonies of the icoculated plate with p2iGEM0208 (because of the GFP Dropout, then there must be the gen ptxD in, otherwise the colonie ist green under UV-light)

inoculation in 3ml LB-medium with Chloramphenicol

Transformation of the p2iGEM0097-p2iGEM0108 in *E.coli* DH5a with the following protocol:

- thaw competent cells 5-10 min on ice
- add 1µl 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 30 sec on 42 °C
- place on ice for 5min
- Pipette 300µl LB without Antibiothica (sterile)
- place at 37°c for 60min, shake 300rpm
- inoculation of transformed coli in 3ml LB+ Cam
- inkubation over night at 37°C

Miniprep of the Plasmids p2iGEM0097 and p2iGEM0098 with the following protocol (one tube each for level1 and one tube each for level2):

- 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 15 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 15 sec max rpm
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl elution buffer
- Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

concentration:

plasmidnumber	level1 tube	level2 tube
p2iGEM0097	119,15 ng/µl	241,20 ng/µl
p2iGEM0098	115,20 ng/µl	246,65 ng/µl

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

Author: Susanne Vollmer
created: 02.05.2018 18:55
updated: 02.05.2018 19:56

Entry 25/259: Cryo of p2iGEM0208 and p2iGEM0097-108 miniprep of them and testdigest of the 5 preps of p2iGEM0208
In Project: Level_1
No tags associated

Cryos from 2iGEM0212 and from 2iGEM0215-2iGEM0226 with the followig protocol:

300µl Glycerin

700µl Culture

placed in kryo 2 box except number 0212, it is the Level 1 box

Plasmidnumber	Cryonumber
0208	0212
0097	0215
0098	0216
0099	0217
0100	0218
0101	0219
0103	0221
0104	0222
0105	0223
0106	0224
0107	0225
0108	0226

Miniprep of the cultures with the following protocol:

culture was concentrated in 600µl LB and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 15 sec max rpm
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl eluation buffer
- incubate for 5 Min at RT. Centrifuge for 15 Sek. max

concentrations:

tube	concentration in ng/µl
0208 1	67,350
0208 2	67,600
0208 3	49,100
0208 4	46,350
0208 5	33,300
0097	203,65
0098	187,40
0099	97,300
0100	220,15

tube	concentration in ng/µl
0101	148,35
0103	127,15
0104	166,00
0105	203,25
0106	250,80
0107	153,45
0108	205,35

Testdigestion with NdeI of the five different isolations of p2iGEM0208 and as a control p2iGEM0001:

1µl plasmid

1µl cutsmart buffer

7µl Milli Q water

1µl NdeI enzyme

digestion with the following protocol:

60 min 37°C

20 min 65°C

stored in freezer over night

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

Author: Sarah Seyffert

created: 02.05.2018 20:55
updated: 02.05.2018 21:08

Entry 26/259: Gradient PCR
In Project: Level_1
With tags: gradient pcr, pcr

Preparation:

- add in a PCR-tube:
- 1µl template
- 10µl 5xBuffer
- dNTP 1,25µl (10mM stock)
- Primer foward 5 µl
- Primer reverse 5µl
- Q5-Polymerase 0,25µl (at last on ice!)
- 3% DMSO 1,5 µl (to avoid primer dimer)
- fill to 50µl with miliQ

Gradient PCR with this fragment:

- **NISb** from Synechococcus elongatus sp. PCC 7942 gDNA; **primer** iGEM028(fw); iGEM031(rv)

Program of the gradient PCR

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient between 69-61°C for 45 sec(annealing) ->each row for the trubes has another temperature
    3. 72°C for 45sec(elongation)
    4. repeat 35 times
3. 72°C for 10 min
4. Hold 4°c

Gradient PCR with

- **NSIa** from Synechococcus elongatus sp. PCC 7942 gDNA; **primer**: iGEM032(fw) ;iGEM017(rv)

Program of the PCR:

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec
    2. gradient between 61-59°C for 45 sec ->each row for the trubes has another temperature
    3. 72°C for 45 sec
    4. reapeat 35 times
3. 72°C for 10 min
4. Hold 4°c

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

Author: Svenja Hermanns
Entry 27/259: Retrafo
In Project: Level_1
With tags: mVenus, NSI, pSHDY

created: 02.05.2018 21:05
updated: 15.05.2018 20:35

Today we made a retransformation of p2iGEM0202 (pSHDY_SmR) , p2iGEM0203 (pSHDY_mVenus) and p2iGEM204 (NSI_TetR_LacA_Pcat)

- mix 1 µl DNA with 15 µl compis carefully

- incubate on ice for 5 min

- heatshock for 45 seconds on 42 °C

- incubate on ice for 2 min

- add 300 µl LB

- incubate for 1 hour on 37°C

- put 100 µl culture on plates with right antibiotica (spec+kan and kan plates we made new ones oureself)

- incubate over night at 37°C

p2iGEM0202 --> 2iGEM0206

p2iGEM0203 --> 2iGEM0207

p2iGEM0204 --> 2iGEM0208 (Retransfromation was made twice)

We set up an overnight cuture from *Zymomonas mobilis* WT in 3 ml LB

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

Author: Susanne Vollmer
Entry 28/259: gel of testdigestion
In Project: Level_1
No tags associated

created: 03.05.2018 20:11
updated: 03.05.2018 20:21

gel of the digstion

1% agarose gel

1xTAE

90V 50min

in the gel pocket:

1kb ladder

1: testdigestion of DNA of colonie1 (10µl from the Digestion and 3µl 6xloading dye)

2: testdigestion of DNA of colonie2 (10µl from the Digestion and 3µl 6xloading dye)

3: testdigestion of DNA of colonie3 (10µl from the Digestion and 3µl 6xloading dye)

4: testdigestion of DNA of colonie4 (10µl from the Digestion and 3µl 6xloading dye)

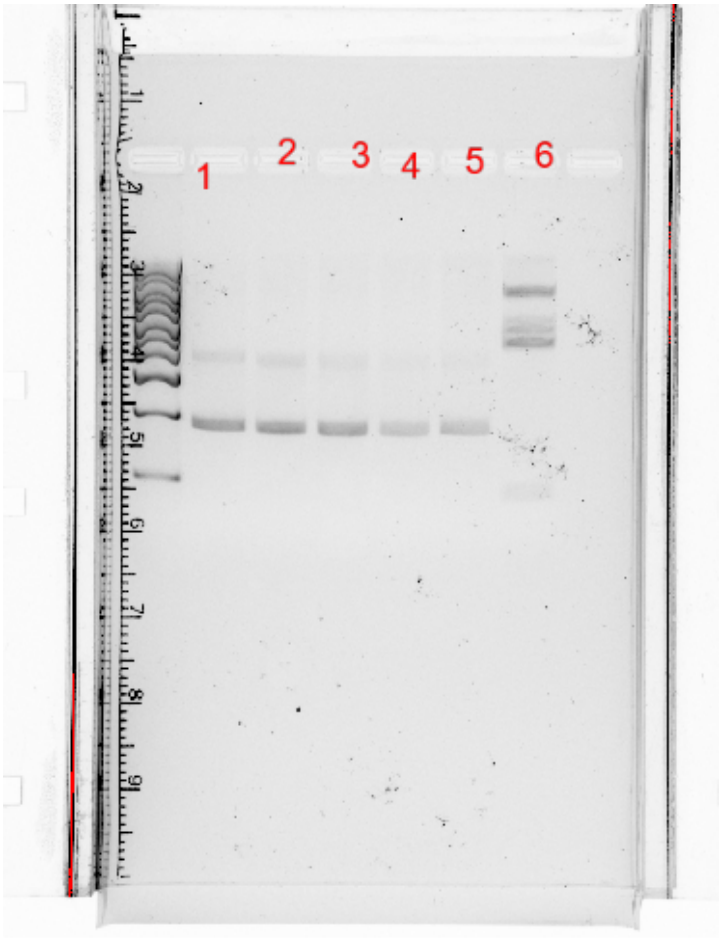
5: testdigestion of DNA of colonie5 (10µl from the Digestion and 3µl 6xloading dye)

6: testdigestion of p2iGEM0001 (entry vector)(10µl from the Digestion and 3µl 6xloading dye)

The Image of the gel:

it doesn't work, maybe because the enzyme didn't cut.

Gel.PNG



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

Author: Svenja Hermanns
Entry 29/259: Retrafo
In Project: Level_1
With tags: mVenus, Zymomonas

created: 03.05.2018 21:08
updated: 15.05.2018 20:37

Today we made a retransformation of p2iGEM0203 (pSHDY_mVenus) because the one

from yesterday was not successful:

- mix 1 µl DNA with 15 µl compis carefully

- incubate on ice for 5 min

- heatshock for 45 seconds on 42 °C

- incubate on ice for 2 min

- add 300 µl LB

- incubate for 30 min on 37°C

- take 100 µl for the overnight liqid culture

we set up overnight liquid cultur of:

- 2iGEM0206 with 15 µl Spec (20 mg/ml) and 15 µl Kan (10 mg/ml) in 3 ml LB

- 2iGEM0207 with 15 µl Spec (20 mg/ml) in 3 ml LB

- 2iGEM0208 with 2,04 µl Cam (50mg/ml) in 3 ml LB

We put 100 µl of the overnight cuture from *Zymomonas mobilis* WT on an agar plate

and incubate them overnight on 30 °C

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

Author: Sarah Seyffert
Entry 30/259: gradient PCR
In Project: Level_1
With tags: gradient pcr

created: 03.05.2018 23:05
updated: 31.07.2018 16:23

Preparation:

- Mastermix with
  - 28µl Q5-Buffer
  - 1,4µl Template
  - 3,5µl dNTP
  - 14µl Primer fw
  - 14µl Primer rv
  - 0,7µl Q5-polymerase
  - 4,2 DMSO
  - 74,2µl miliq-water
- fill 6 PCR tubes with 20µl of the mastermix

Gradient PCR with this fragments:

- **glf** from Zymomonas mobilis gDNA; **primer**: iGEM027(fw) ; iGEM029(rv)
- **glf**(tagged) from Zymomonas mobilis gDNA; **primer** : iGEM022(rv) ; iGEM027(fw)

Program of the gradient PCR

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient between 60-50°C for 45 sec(annealing) ->each row for the trubes has another temperature
    3. 72°C for 45sec(elongation)
    4. repeat 35 times
3. 72°C for 10 min
4. Hold 4°c

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

Author: Susanne Vollmer

created: 04.05.2018 15:24
updated: 04.05.2018 16:09

Entry 31/259: repetition of testdigestion and gel of p2iGEM0208 and p2iGEM0001
In Project: Level_1
No tags associated

testdigestion with following protocol:

Mastermix:

7µl NdeI Enzyme (from thermofisher)

7µl Cutsmart buffer

49µl milli Q water

in each tube 1µl Plasmid and 9µl Mastermix were addit

in total five tubes with Plasmids of diffrent colonies of p2iGEM0208 and one with p2iGEM0001

60min 37°C

20min 65°C

gel with the Digestion product:

0,5g agarose with 50ml 1xTAE and 2,5 GelRed

1xTAE buffer

70min 90V

gel pockets:

1kb ladder

1: testdigestion of DNA of colonie1 (10µl from the Digestion and 3µl 6x loading dye)

2: testdigestion of DNA of colonie2 (10µl from the Digestion and 3µl 6x loading dye)

3: testdigestion of DNA of colonie3 (10µl from the Digestion and 3µl 6x loading dye)

4: testdigestion of DNA of colonie4 (10µl from the Digestion and 3µl 6x loading dye)

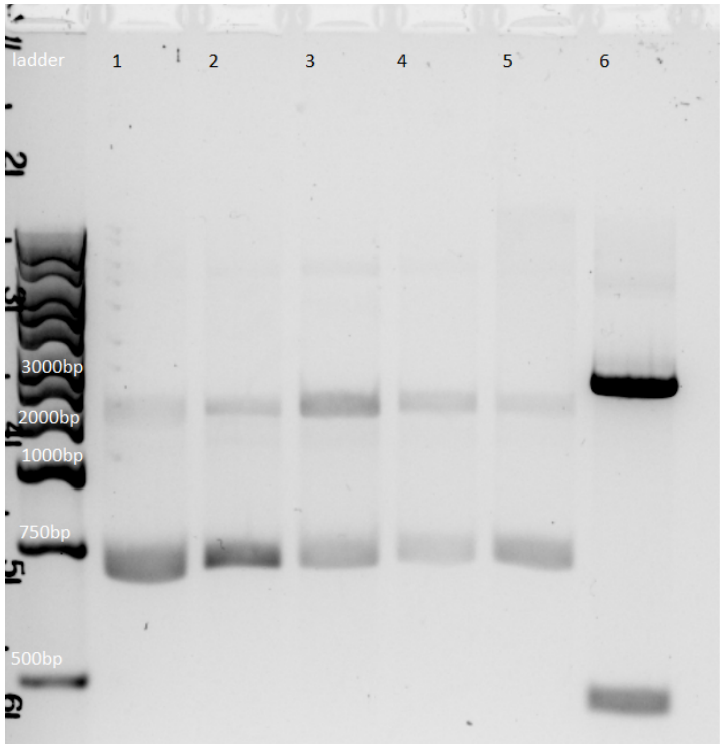
5: testdigestion of DNA of colonie5 (10µl from the Digestion and 3µl 6x loading dye)

6: testdigestion of p2iGEM0001 (entry vector)(10µl from the Digestion and 3µl 6x loading dye)

Gel Image:

the entry vector p2iGEM0001 was like expected, the others from p2iGEM0208 not

Gel_2018-05-04_13hr_43min_90V_70min_Testrestriktion_2_bearbeitung.png



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

Author: Sarah Seyffert
Entry 32/259: Gradient PCR
In Project: Level_1
With tags: gradient pcr

created: 04.05.2018 19:28
updated: 04.05.2018 19:32

Preparation:

- Mastermix with
  - 28µl Q5-Buffer
  - 1,4µl Template
  - 3,5µl dNTP
  - 14µl Primer fw
  - 14µl Primer rv
  - 0,7µl Q5-polymerase
  - 4,2 DMSO
  - 74,2µl miliq-water
- fill 6 PCR tubes with 20µl of the mastermix

Gradient PCR with this fragments:

- **invA** from Zymomonas mobilis gDNA; **primer**: iGEM021(fw); iGEM027(rev)
- **invA**(tagged) from Zymomonas mobilis gDNA; **primer** iGEM024(fw); iGEM027(rv)

Program of the gradient PCR

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient between 58-50°C for 45 sec(annealing) ->each row for the tubes has another temperature
    3. 72°C for 45sec(elongation)
    4. repeat 35 times
3. 72°C for 10 min
4. Hold 4°c

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

Author: Sarah Seyffert
created: 05.05.2018 20:07
updated: 06.05.2018 22:36
Entry 33/259: gradient PCR & gel electrophoresis
In Project: Level_1
With tags: gradient pcr, gel electrophoresis

Preparation:

- Mastermix with
  - 28µl Q5-Buffer
  - 0,5µl Template(15ng)
  - 3,5µl dNTP(0,25mM)
  - 14µl Primer fw(1 µM)
  - 14µl Primer rv(1 µM)
  - 0,7µl Q5-polymerase
  - 4,2 DMSO (3%)
  - 75,1µl miliq-water
- fill 6 PCR tubes with 20µl of the mastermix
-

Gradient PCR with this fragments:

- **SmR** from pSHDY; **primer** : O_iGEM18_0016(fw), O_iGEM18_0018(rv); **annealing temperature**: 57,7°C, 56,4°C, 54,8°C, 53,6°C, 52,7°C and 52°C
- **ori** from NSI_TetR_LacA; **primer**: O_iGEM18_0028(fw), O_iGEM18_0031(rv); **annealing temperature**: 59°C 58,5°C, 57,7°C, 56,4°C, 54,8°C and 53,6°C

Program of the gradient PCR

1. 98°C for 2 min
2. cicle with
   1. 98°C for 15 sec(denaturation)
   2. X°C for 30 sec(annealing) (X =varying annealing temperature as shown above)
   3. 72°C for 45sec(elongation)
   4. repeat 35 times
3. 72°C for 10 min
4. Hold 4°c

Preparation of the 1% agarose gel

- 300ml 1x TAE-buffer
- 3 g agarose
- 15µl Gel RED

We loaded the PCR samples from yesterdays' PCR of the following constructs and gDNA on an agarose gel:

- NSIa 1500 kb
- NSIb 1500 kb
- Glf 1422 kb
- Glf-tagge 1422 kb
- invA 1583 kb
- invA-tagg 1583 kb

Therefore, we mixed 3 µL of each PCR sample with 1 µL 6x Loading Dye (Thermo Fisher).

Afterwards we loaded the samples on the agarose gel along with 5 µl 1kb ladder.

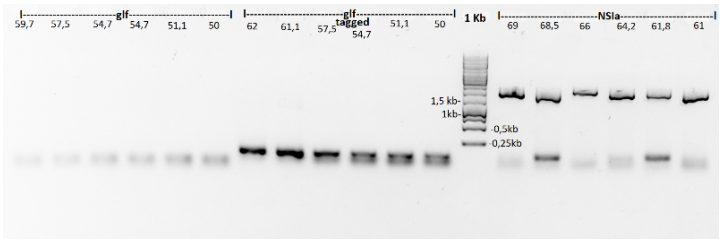
The gel ran on 120 V for 40 min and was then visualized with Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

After this the gel ran on 130V for another 20 min and was visualzied.

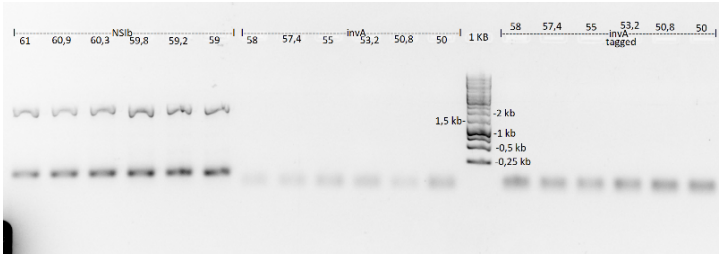
The visualized gel showed mostly **no expected bands** for the PCR fragments with the exeption of the NSIb-band

Pictures after the first 40 min of the gel are attached

Gel_2018-05-05_15hr_44min_glf_glftagged_NSIa_bearbeitet.tif



Gel_2018-05-05_15hr_44min_NSIb_invA_invAtagged_MD_120_V_40_min_1_kb_ladder_bearbeitet.tif



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

Author: Miriam Dreesbach
Entry 34/259: Gradient PCR of NSIa, NSIb, glf, glf tagged
In Project: Level_1
With tags: pcr, gradient pcr

created: 06.05.2018 11:56
updated: 13.05.2018 13:54

To amplify the fragments **NSIa, NSIb, glf and glf tagged**, we ran four different gradient three-step PCRs:

First, we created four Mastermixes for 6 (+1) PCR approaches (each) containing (final concentration):

- 28 µL 5x Q5 Reaction Buffer
- 0,5 µL Template (12.5 ng)
- 3,5 µL dNTP-mix (0.25 mM)
- 14 µL Primer fw (1 µM)
- 14 µL Primer rv (1 µM)
- 0,7 µL Q5 High-Fidelity DNA Polymerase
- 4,2 sterile DMSO (3 %)
- 75,1 µL nuclease-free miliQ water

Afterwards, we aliquoted 20 µL of each mastermix into 6 PCR tubes.

With these PCR tubes, gradient PCRs of the following constructs and varying annealing temperatures and extension time (fragment length) were run:

- **NSIa** from gDNA *S. elongatus* Phenol; **primer** : O_iGEM2018_0015(fw), O_iGEM2018_0030(rv); **61 °C; 60.9 °C, 60.3 °C, 59.8 °C, 59.2 °C, 59 °C**; **45 sec** (1500 bp).
- **NSIb** from gDNA *S. elongatus* Phenol; **primer**: O_iGEM2018_0026(fw), O_iGEM2018_0029(rv); **69 °C, 68.5 °C, 66 °C, 64.2 °C, 61.8 °C, 61 °C**; **45 sec** (1500 bp).
- **glf** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0025(fw), O_iGEM2018_0027(rv); **59.7 °C, 57.5 °C, 54.7 °C, 52.7 °C, 51.1 °C, 50 °C**; **45 sec** (1583 bp).
- **glf tagged** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0020(fw), O_iGEM2018_0025(rv); **62 °C, 61.1 °C, 57.5 °C, 54.7 °C, 51.1 °C, 50 °C**; **45 sec** (1583 bp).

Program of the gradient PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: X °C: 30 sec (X = varying annealing temperatures as shown above)
4. Extension: 72 °C: Y sec (Y = varying extension time as shown above)
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

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

Author: Susanne Vollmer
Entry 35/259: PCR of p2iGEM0208 to test if ptxd is in
In Project: Level_1
No tags associated

created: 07.05.2018 13:58
updated: 07.05.2018 14:52

PCR of the Plasmids of the five colonies of p2iGEM0208 with the O_igem07 and O_igem08 to test if ptxD was right inserted

following protocol wa used:

Primer used:

O_iGEM0007_ptxD_lvl0_p

O_iGEM0008_ptxD_lvl0_p

Mastermix:

30 µl high GC enhancer

30 µl Q5 Buffer

76,5 µl MilliQ-water

3 µl dNTPs

6 µl of O_iGEM07

6µl of O_iGEM08

1,5 µl Q5 polymerase (add this at last, on ice!)

0,5µl of the template in the tubes (colinies1-5)

25,6µl MasterMix

95°C 2min

then starting the cycle with:

95°C 15 sec

55°C 15 sec (depending on the anealing temperature of the Primer)

72°C 30 sec

repeating this 28x (depending on the situation 28-30)

72°C 2min

5°C HOLD

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

Author: Susanne Vollmer Entry 36/259: refill of 1xTAE In Project: Level_1 No tags associated	created: 08.05.2018 13:55 updated: 08.05.2018 13:56

refilled 3 Liter of 1xTAE with each 20ml of 50xTAE and 980ml Milli Q

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

Author: Susanne Vollmer
created: 08.05.2018 13:57
updated: 08.05.2018 16:14

Entry 37/259: Gel of the PCR product ptxD from p2iGEM0208
In Project: Level_1
No tags associated

1% agarose gel (0,5g Agarose, 50ml 1xTAE)

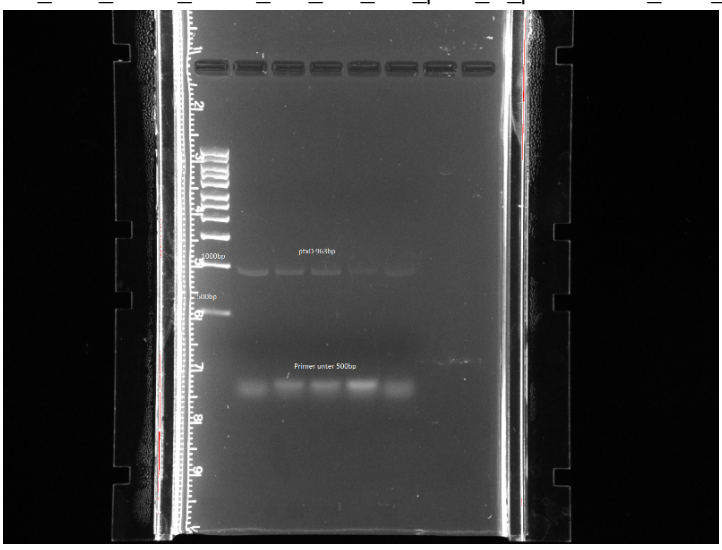
90V 60min

Gel Picture below it Looks like ptxD is there but not so much

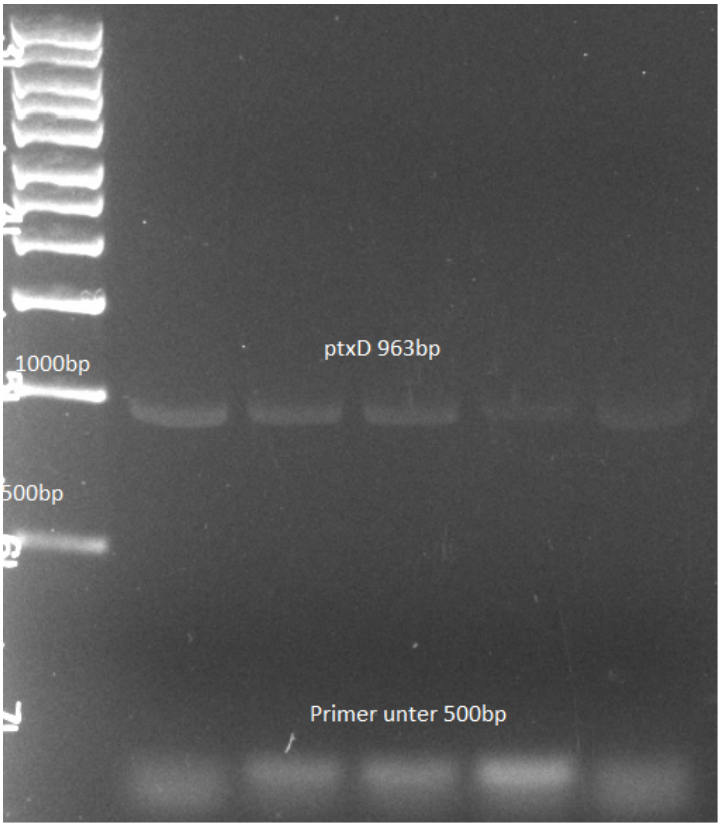
in the gel pockets:

1	2	3	4	5	6
1kb ladder	PCR product of colonie 1 from p2iGEM02028	PCR product of colonie 2 from p2iGEM02028	PCR product of colonie 3 from p2iGEM02028	PCR product of colonie 4 from p2iGEM02028	PCR product of colonie 5 from p2iGEM02028
2µl 1kb ladder genruler	5µl PCR product2µl loading Dye3µl milli Q	5µl PCR product2µl loading Dye3µl milli Q	5µl PCR product2µl loading Dye3µl milli Q	5µl PCR product2µl loading Dye3µl milli Q	5µl PCR product2µl loading Dye3µl milli Q

Gel_2018-05-08_15hr_53min_60min_90v_1kb_test_ptxD_in_p2iGEM208_PCR_bearbeitet.tif



gel_von_8.5_PCR_von_p2iGEM0208_für_ptxD.png



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

Author: Sarah Seyffert
created: 08.05.2018 14:44
updated: 08.05.2018 14:55
Entry 38/259: Touch down PCR
In Project: Level_1
With tags: pcr, tochdown pcr

To amplify the fragments **invA, invA tagged, glf and glf tagged**, we ran four different touchdown PCRs:

First, we created four Mastermixes for 5 PCR approaches (each) containing (final concentration):

- 20 µL 5x Q5 Reaction Buffer
- 01 µL Template (12.5 ng)
- 2.5 µL dNTP-mix (0.25 mM)
- 0.5 µL Q5 High-Fidelity DNA Polymerase
- 53 µL nuclease-free miliQ water

Afterwards, we aliquoted 20 µL of each mastermix into 4 PCR tubes.

Additionally, we added

- 2 µL Primer fw (1 µM)
- 2 µL Primer rv (1 µM)
- 0.6 sterile DMSO (3 %)

To each PCR tube.

With these PCR tubes, gradient PCRs of the following constructs and varying annealing temperatures(range); temperature decrease per step; extension time (fragment length) were run:

- **invA** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0021(fw), O_iGEM2018_0027(rv); **72 °C -> 58°C; -0,4°C**; **45 sec** (1583 bp).
- **invA tagged** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0024(fw), O_iGEM2018_0027(rv); **72 °C -> 58°C; -0,4°C**; **45 sec** (1583 bp).
- **glf** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0025(fw), O_iGEM2018_0022(rv); **72 °C -> 58°C; -0,4°C**; **45 sec** (1583 bp).
- **glf tagged** from gDNA *Z. mobilis* Phenol; **primer**: O_iGEM2018_0020(rv), O_iGEM2018_0025(fw); **72 °C -> 50°C; -0,6°C**; **45 sec** (1583 bp).

Program of the gradient PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: X °C: 30 sec (X = varying annealing temperatures as shown above)
4. Extension: 72 °C: Y sec (Y = varying extension time as shown above)
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

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

Author: Susanne Vollmer
Entry 39/259: 3.try of PCR of guaD from gDNA E.Coli DH5a
In Project: Level_1
No tags associated

created: 09.05.2018 16:00
updated: 09.05.2018 16:35

3.try of PCR of the gDNA from *E.coli* DH5a to amplify guaD using the following protocol:

two tubes with same Content

Primer used:

O_iGEM18_0047

O_iGEM18_0048

using a Mastermix:

15 µl high GC enhancer

15 µl Q5 Buffer

38,25 µl MilliQ-water

1,5 µl dNTPs

3µl O_iGEM18_0047

3µl O_iGEM18_0048

0,75 µl Q5 polymerase (add this at last, on ice!)

in the tubes each 1 µl Template gDNA from *E.coli* DH5a and 25,5µl Mastermix

95°C 2min

then starting the cycle with:

95°C 15 sec

53°C 15 sec (depending on the anealing temperature of the Primer)

72°C 40sec

repeating this 28x (depending on the situation 28-30)

72°C 2min

5°C HOLD

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

Author: Susanne Vollmer
created: 09.05.2018 21:02
updated: 09.05.2018 21:15

Entry 40/259: Gel of 3.PCR of guaD from gDNA from E.coli DH5a
In Project: Level_1
No tags associated

Gel of the 3. try of the PCR to amplify guaD from gDNA *E.coli* DH5a

1% Agarose (1,8g araose, 180ml 1xTAE)

60 V 2 stunden

in the gelpocket :

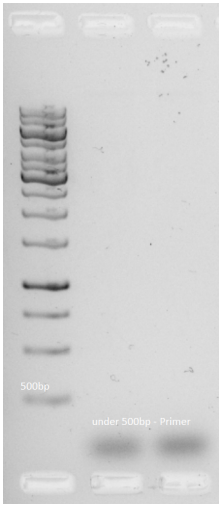
1: 2µl DNA 1kb ladder

2: 5µl PCR product tube 1, 3 milli Q, 2 loading Dye

3: 5µl PCR product tube 2, 3 milli Q, 2 loading Dye

Gelpicture shows only primers, no gDNA, no guaD fragment

Gel_2018-05-09_20hr_41min_SV_130h_60V_links_1_kb_ladder_2_yL_GuaD1_GuaD2_rechts_bearbeitet.tif



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

Author: Susanne Vollmer

created: 09.05.2018 21:15

updated: 09.05.2018 21:32

Entry 41/259: Golden Gate for the expression Plasmid for ptxD

In Project: Level_1

No tags associated

Golden Gate for the cloning of the expression plasmids for ptxD for promoter testing of p2iGEM0009-p2iGEM0017

Backbone: p2iGEM0097 (ura casette)

Promoter:p2iGEM0009-p2iGEM0017

Terminator: p2iGEM0051

gen of interest in lvl0 plasmid: p2iGEM0208

Mastermix:

- 60µl Milli Q water
- 10µ T4 ligase Buffer
- 5µl p2iGEM0208
- 5µl p2iGEM0051
- 5µl p2iGEM0097
- 5µl T4 ligase (add last->on ice)
- 5µl BsaI (add last->on ice)

in the tubes 9-17

each 9,5µl Mastermix

add the promoter p2iGEM0009-0017 in the fitting tube (p2iGEM0009 only in tube 9)

Cycle:

2 min 37°C

5min 16°C

repeating that 60

10min 60°C

10 min 80°C

4°C HOLD

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

Author: Miriam Dreesbach
created: 09.05.2018 22:25
updated: 11.05.2018 11:23
Entry 42/259: Gel electrophoresis PCR
In Project: Level_1
With tags: gel electrophoresis, agarose gel, gradient pcr, pcr, touchdown pcr

To check the amplified fragments of this weeks' PCRS of NSIa, NSIb, glf, glf tagged, invA, invA tagged, ori, SmR, we loaded the PCR samples from yesterdays' PCR of the following on a 1 % agarose gel.

Therefore, we weighted 1,8 g agarose, poured it into a 500 mL bottle and filled it up with 1xTAE to 500 mL. Afterwards, it was heated in the microwave until the agarose was completely dissolved and the solution was clear. The agarose gel was cooled until it was touchable with the bare hand. Afterwards, we added 6 µL GelRed, swinged the solution and poured the gel on a 15 x 15 cm gel carrier. After the gel was colpletely hardened, we mixed 3 µL of each PCR sample with 1 µL 6x Loading Dye (Thermo Fisher):

- NSIa 1.5 kbp (normal PCR, 61 °C annealing temperature)
- NSIb 1.5 kbp (normal PCR, 61 °C annealing temperature)
- glf 1.422 kbp (Touchdown PCR, 72 °C -> 58 °C annealing temperature)
- glf tagged 1.422 kbp (Touchdown PCR, 72 °C -> 50 °C annealing temperature)
- invA 1.583 kbp (Touchdown PCR, 72 °C -> 58 °C annealing temperature)
- invA tagged 1.583 kbp (Touchdown PCR, 72 °C -> 58 °C annealing temperature)
- ori 589 bp (Gradient PCR, 59 °C, 58.5 °C, 57.7 °C, 56.4 °C, 54.8 °C, 53.6 °C annealing temperature)
- SmR 792 bp (Gradient PCR, 57.7 °C, 56.4 °C, 54.8 °C, 53.6 °C, 52.7 °C, 52 °C annealing temperature)

Afterwards we loaded the samples on the agarose gel.

The gel ran on 60 V for 130 minutes.

After 130 minutes, we visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

The visualized gel showed **no expected bands of the following fragments** (mostly primer dimer):

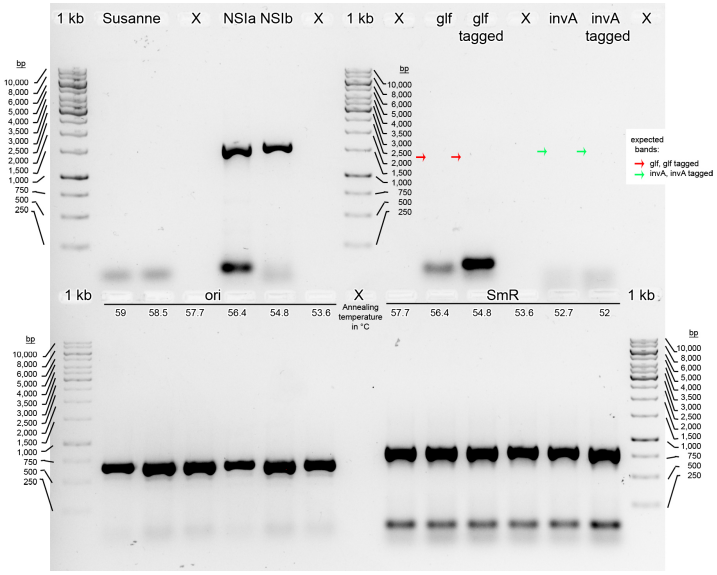
- glf
- glf tagged
- invA
- invA tagged

Plan for the following days:

We will load the PCR products of the following fragments on another gel to cut out the appropriate bands:

- NSIa (remaining 47 µL)
- NSIb (remaining 47 µL)
- ori (all annealing temperatures, 1/2 of all PCR products --> 51 µL in one well)
- SmR (all annealing temperatures, 1/2 of all PCR products --> 51 µL in one well)

Gel_2018-05-09_20hr_41min_MD_130h_60V_links_oben_1_kb_ladder_2_yL_GuaD1_GuaD2_Susanne_1_kb_ladder_1_yL_glf_glftagged_invA_invA_links_unten_1_kb_ladder_0.5_yL_0.5_yL_6xLD_0.5_yL_milliQ_6xori_6xSmR_1kb_ladder_1_yL_1_yL_milliQ_bearbeitet_Kopie.jpg



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

Author: Miriam Dreesbach
created: 09.05.2018 23:00
updated: 09.05.2018 23:10
Entry 43/259: PCR of NSIa and NSIb
In Project: Level_1
With tags: pcr

To amplify the fragments **NSIa and NSIb,** we ran two PCRs:

First, we created four Mastermixes for two PCR approaches (each) containing (final concentration):

- 30 µL 5x Q5 Reaction Buffer
- 3 µL Template (12.5 ng)
- 3.75 µL dNTP-mix (0.25 mM)
- 1.5 µL Q5 High-Fidelity DNA Polymerase
- 79.5 µL nuclease-free miliQ water

Afterwards, we aliquoted 38.5 µL of each mastermix into 2 PCR tubes, in which the following ingredients were prepared before:

- 5 µL Primer fw (1 µM)
- 5 µL Primer rv (1 µM)
- 1.5 µL sterile DMSO (3 %)

On these PCR tubes, PCRs of the following constructs (fragment length) were run:

- **NSIa** from gDNA *S. elongatus* Phenol; **primer** : O_iGEM2018_0015(fw), O_iGEM2018_0030(rv); (1500 bp).
- **NSIb** from gDNA *S. elongatus* Phenol; **primer**: O_iGEM2018_0026(fw), O_iGEM2018_0029(rv); (1500 bp).

Program of the gradient PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: 61 °C: 30 sec
4. Extension: 72 °C: 45 sec
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

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

Author: Susanne Vollmer

created: 10.05.2018 21:04
updated: 10.05.2018 21:21

Entry 44/259: Transformation of E.coli DH5a with p2iGEM0244-52
In Project: Level_1
No tags associated

Transformation of E.coli DH5a with the Plasmids of the over night Golden gate (for the promoter testing with promoter p2igem0009-17) with the Plasmids p2iGEM0244-p2iGEM0252 using following protocol:

- thaw competent cells 5-10 min on ice
- add 1µl 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 30 sec on 42 °C
- place on ice for 5min
- Pipette 300µl LB without Antibiothica (steriale)
- place at 37°c for 60min, shake 300rpm
- centrifuge the cells for 5min at 6000rcf, decant the supernatante and resuspend the cells in the rest(100-200µl),(if you decant to much, fill with 50µl LB-medium)
- screat the Suspension on a agarplate with the Fitting antibiothica (i used ampicillin)
- incubate at 37°C

The transformants are the expressionplasmids of ptxD with different Promoters, they are named 2iGEM0261-2iGEM0269

promoternumber	plasmidnumber	Cryonumber
09	p2iGEM0244	2iGEM0261
10	p2iGEM0245	2iGEM0262
11	p2iGEM0246	2iGEM0263
12	p2iGEM0247	2iGEM0264
13	p2iGEM0248	2iGEM0265
14	p2iGEM0249	2iGEM0266
15	p2iGEM0250	2iGEM0267
16	p2iGEM0251	2iGEM0268
17	p2iGEM0252	2iGEM0269

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

Author: Miriam Dreesbach
created: 11.05.2018 11:06
updated: 11.05.2018 20:58
Entry 45/259: PCR NSIa, NSIb, ori, SmR; Isolation of appropriate bands
In Project: Level_1
With tags: agarose gel, gel electrophoresis

To isolated the amplified fragments of this weeks' PCRS of NSIa, NSIb, ori and SmR, I loaded the PCR samples on a 1 % agarose gel.

Therefore, I weighted 0.43 g agarose, poured it into a 250 mL bottle and filled it up with 1xTAE to 43.2 mL. Afterwards, it was heated in the microwave until the agarose was completely dissolved and the solution was clear. The agarose gel was cooled until it was touchable with the bare hand. Afterwards, I added 2 µL GelRed, swinged the solution and poured the gel on a 10*6 cm gel carrier. After the gel was colpletely hardened, I mixed x µL of each PCR sample with y µL 6x Loading Dye (Thermo Fisher), z µL were poured on the gel:

- NSIa 1.5 kbp (normal PCR, 61 °C annealing temperature); x = 28.5 µL; y = 5 µL; z = 20 µL
- NSIb 1.5 kbp (normal PCR, 61 °C annealing temperature); x = 28.5 µL; y = 5 µL; z = 20 µL
- ori 589 bp (Gradient PCR, 59 °C, 58.5 °C, 57.7 °C, 56.4 °C, 54.8 °C, 53.6 °C annealing temperature); 8.5 µL of each PCR => x = 51 µL; y = 9 µL; z = 2*20 µL
- SmR 792 bp (Gradient PCR, 57.7 °C, 56.4 °C, 54.8 °C, 53.6 °C, 52.7 °C, 52 °C annealing temperature); 8.5 µL of each PCR => x = 51 µL; y = 9 µL; z = 2*20 µL

To identify the right bands, I used the following ladders:

- GeneRuler 1 kb DNA ladder (Thermo Scientific) --> 1 µL ladder, 1 µL 6x LD (Thermo Scientific), 1 µL nuclease-free water (milliQ)
  - to identify NSIa and NSIb
  - to compare two different ladders
- GeneRuler 100 bp plus DNA ladder (Thermo Scientific) --> 1 µL
  - to identify SmR and ori more precisely (troubleshooting)
  - 1 kb DNA ladder only distinguish fragments lower than 1000 bp in three steps: 250 bp, 500 bp, 700 bp.

The gel ran on 120 V for 60 minutes.

After 60 minutes, I visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

The ladders behaved very different. The 100 bp plus DNA ladder showed the right appropriate bands, the 1 kb DNA ladder showed the appropriate bands higher than the samples and the 100 bp plus ladder.

The gel revealed that the 100 bp plus DNA ladder is better for fragments smaller than 1000 bp.

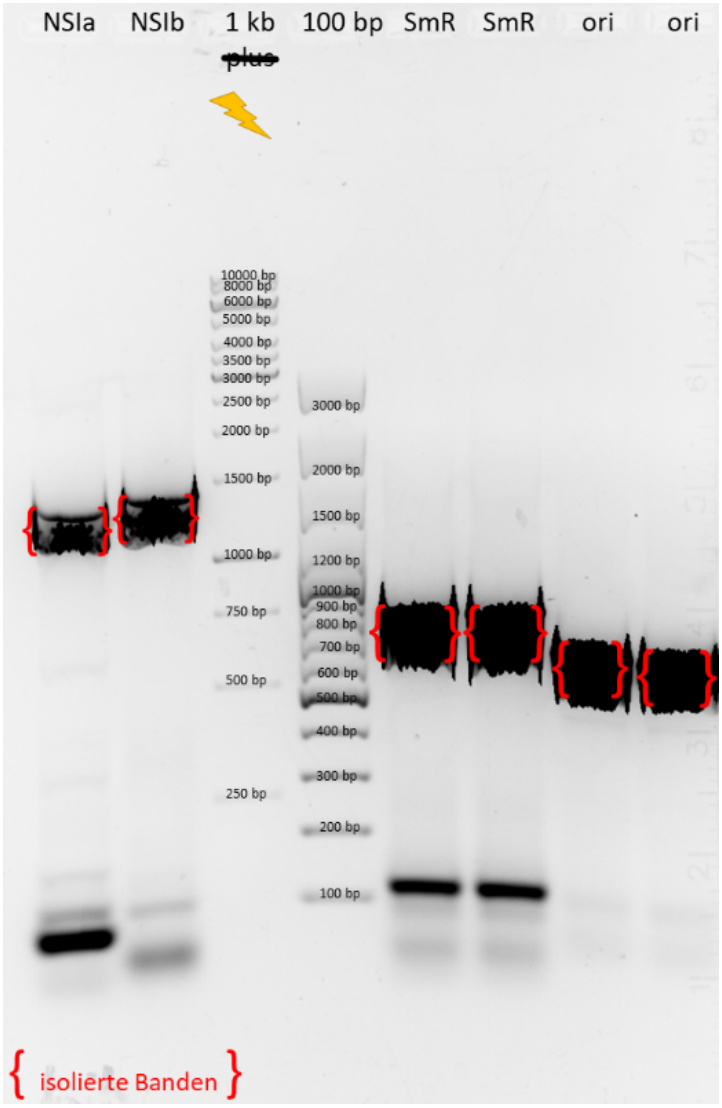
Afterwards, I cut the following bands out of the gel and loaded them into a 1.5 mL Eppi under blue light:

- NSIa 1.5 kp
- NSIb 1.5 kp
- ori 589 kp (2x)
- SmR 792 kp (2x)

Plan for the following days:

- Isolate the fragments out of the agarose gel
- Measure concentration

Gel_2018-05-10_18hr_16min_120_v_60_min_MD_nsia_nsib_1_kb_100_bp_plus_smr_smr_ori_ori_bearbeitet.tif



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

Author: Susanne Vollmer
created: 11.05.2018 17:52
updated: 11.05.2018 17:56
Entry 46/259: inoculation of the transformants 2iGEM0261-69
In Project: Level_1
No tags associated

selection of 1-4 colonies of the each plate depanding how much White colonies there are (Screening for the white colonies not the green, because the Bachbone has a GFP Dropout, using a bluelight tabel).

inoculation of 3ml LB-mediu with 30µl Amphicillin with the selectet colonies

Incubation over night on 37°C

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

Author: Miriam Dreesbach
created: 11.05.2018 20:59
updated: 11.05.2018 21:31
Entry 47/259: Inoculation of bacterial cultures for plasmid isolation
In Project: Level_1
With tags: inoculation, incubation

To isolate the plasmids p2iGEM0202 (pSHDY_SmR) of the strain 2iGEM0206 and the plasmid p2iGEM0204 (NSI_TetR_LacA_Pcat) from the strain 2iGEM0208, I prepared 3 mL of LB with the appropriate antibiotics:

- p2iGEM0202: 15 µL Spectinomycin
- p2iGEM0204: 2.04 µL Chloramphenicol

Afterwards, I inoculated the media with the appropriate colonies and incubated them overnight at 37 °C.

Plan for the following days:

- Isolate the plasmids from the incubated cultures.

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

Author: Miriam Dreesbach

created: 11.05.2018 21:33
updated: 11.05.2018 21:51

Entry 48/259: Gel isolation of PCR product

In Project: Level_1

With tags: agarose gel, isolation

To isolate the bands out of yesterdays' agarose gel (gel electrophoresis), I used the Wizard SV Gel and PCR Clean-Up System Kit (Promega).

First, I weighted the bands I cut out of the gel yesterday:

- SmR: 0.41 g
- ori: 0.42 g
- NSIa: 0.21 g
- NSib: 0.20 g

Afterwards, I added 10 µL membrane binding solution to 10 mg agarose gel bands. Therefore I added the following volume to the samples:

- SmR: 0.41 mL
- ori: 0.42 mL
- NSIa: 0.21 mL
- NSib: 0.20 mL

To dissolve the agarose gel in the membrane binding solution, I incubated the samples on 50 °C and vortexed from time to time until the solution was completely clear.

After incubation, I loaded max. 500 µL of the sample to a column in a collection tube. After 1 minute incubation at room temperature, I centrifuged the samples for 1 minute at 16,000 rpm. I discarded the flow-through.

For SmR and ori I repeated the last four steps (loading, incubation, centrifugation, discard flow-through) twice with the rest of the dissolved gel sample. After that, I added 700 µL membrane wash solution on the column and centrifugated the sample at 16,000 rpm for 1 minute. I discarded the flow-though and added 500 µL membrane wash solution and centrifuged the sample at 16,000 rpm for 1 minute, again. I discarded the flow-through carefully and centrifuged the samples with an open rotor cap. After centrifugation, I removed the collection tube carefully and put the column on a sterile 1.5 mL Eppi. I added 50 µL nuclease-free water on the column membrane and incubated it at room temperature for 5 minutes. Afterwards, I centrifuged the samples for the last time and measured the resulting concentrations of the samples:

- SmR: 27.5 ng/µL
- ori: 27.0 ng/µL
- NSIa: 4.5 ng/µL
- NSib: 8.35 ng/µL

Plan for the following weeks:

- Overlap extension PCR of NSIa, ori and NSIb.

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

Author: Susanne Vollmer
created: 12.05.2018 15:01
updated: 12.05.2018 15:30
Entry 50/259: miniprep of the inoculated colonies of p2iGEM0244-52
In Project: Level_1
No tags associated

miniprep of the cultures from the selectet colonies, which yesterday were inoculated, using following protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- + 200 µl Endotoxin removal wash
- 15 sec max rpm
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl eluation buffer
- incubate for 5 Min at RT. Centrifuge for 15 Sek. max

"- " = not enought white colonies on plate

Plasmid number	colonie 1 concentration	colonie 2 concentration	colonie 3 concentration	colonie 4 concentration	cryo number
p2iGEM0244	nicht angewachsen	nicht angewachsen	-	-	2iGEM0261
p2iGEM0245	97,850ng/µl	144,05ng/µl	-	-	2iGEM0262
p2iGEM0246	96,000ng/µl	-	-	-	2iGEM0263
p2iGEM0247	126,70ng/µl	204,70ng/µl	94,200ng/µl	83,300ng/µl	2iGEM0264
p2iGEM0248	78,950ng/µl	188,35ng/µl	195,15ng/µl	96,450ng/µl	2iGEM0265
p2iGEM0249	195,15ng/µl	189,80ng/µl	-	-	2iGEM0266
p2iGEM0250	79,800ng/µl	88,300ng/µl	79,550ng/µl	84,850ng/µl	2iGEM0267
p2iGEM0251	kein Medium	74,300ng/µl	-	-	2iGEM0268
p2iGEM0252	nicht angewachsen	leuchtet grün	-	-	2iGEM0269

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

Author: Miriam Dreesbach
created: 13.05.2018 13:51
updated: 13.05.2018 14:04
Entry 51/259: PCR Dur1,2 p1, Dur1,2 p2
In Project: Level_1
With tags: pcr, three-step-pcr

To amplify the synthesises of Dur1,2_gene_p1 (S_iGEM18_0016) and Dur1,2_gene_p2 (S_iGEM18_0017), I ran two different three-step PCRs with the following approach (20 µL):

- 2 µL 5x Q5 Reaction Buffer
- 0.4 µL Template (1 ng)
- 0.4 dNTP-mix (0.2 mM)
- 1 µL Primer fw (0.5 µM)
- 1 µL Primer rv (0.5 µM)
- 0,2 µL Q5 High-Fidelity DNA Polymerase
- 0.6 sterile DMSO (3 %)
- 14.4 µL nuclease-free miliQ water

I prepared two PCR tubes and ran two three-step PCRs of the following constructs and varying annealing temperatures (X) and extension time (Y) (fragment length) were run:

- **Dur1,2_gene_p1**; **primer** : O_iGEM2018_0036(fw), O_iGEM2018_0059(rv); **66.6 °C**; **85 sec** (2818 bp).
- **Dur1,2_gene_p2**; **primer**: O_iGEM2018_0060(fw), O_iGEM2018_0061(rv); **63.5 °C**; **90 sec** (2955 bp).

Troubleshooting: The PCR approach of **Dur1,2_gene_p2** may contain less volume than 20 µL.

Program of the three-step PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: X °C: 30 sec (X = varying annealing temperatures as shown above)
4. Extension: 72 °C: Y sec (Y = varying extension time as shown above)
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

Plan for the following days:

- Load 2µL of the PCR products on a gel to check if I gained the right PCR products.

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

Author: Miriam Dreesbach
Entry 52/259: Plasmid isolation SmR, Pcat
In Project: Level_1
With tags: plasmid, isolation

created: 13.05.2018 14:05
updated: 13.05.2018 14:16

To isolate the plasmids pSHDY(p2iGEM0202, 2iGEM0206) and NSI_TetR_LacA_Pcat(p2iGEM0204, 2iGEM0208) from overnight cultures (they incubated for 30 hours),

I added 600 µL of each culture to a sterile Eppi. Afterwards, I added 100 µL lysis buffer and inverted the Eppi for 6-8 times. After that, I added 350 µL neutralization buffer (4 °C storage)

and inverted the Eppi for another 6-8 times.

I centrifuged the samples for 3 minutes at max rpm and pipetted 900 µL of the supernatant on a column on a collection tube.

I centrifuged the samples for 30 seconds at max rpm and discarded the flow-through.

After that, I addded 400 µL column wash, centrifuged the samples for 30 seconds at max rpm and discarded the flow-through.

I put the column on a fresh, sterile Eppi and added 30 µL 37 °C nuclease-free water (milliQ) to the column.

I incubated the samples for 1 minute at room temperature and centrifuged the samples for 30 seconds at max rpm.

Afterwards, I measured the concentration of the gained isolated plasmids:

p2iGEM0202: 15.4 ng / µL

p2iGEM0204: 12.0 ng / µL

After that, I stored the private stock plasmids at -20 °C in the Level 1 box.

Troubleshooting: Add a second step of 400 µL column wash and centrifugation before the elution of the plasmids.

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

Author: Susanne Vollmer

created: 14.05.2018 16:37
updated: 14.05.2018 21:57

Entry 53/259: Miniprep of the remaining culture and testdigestion of all the plasmids of the ptxD.ExPlas (p2iGEM0244-52)

In Project: Level_1

No tags associated

Miniprep of the remaning culture p2iGEM0251 colonie 1 using the following protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 5 Min at RT. Centrifuge for 15 Sek. max

concentration:

89,950ng/µl

Digest with NotI for testing if the ptxD Expression Plasmids (p2iGEM0244-p2iGEM0252) were right cloned, unsing the following protocol:

Mastermix:

- 150µl milli Q water
- 20µl Cutsmart
- 10µl NotI-HF Enzyme--> there was a litle bit less than 10µl left in the stock

in each tube:

- 1µl Plasmid
- 9µl Mastermix (0,5µl Enzyme; 1µl cutsmart; 7,5µl milli Q)

in total 19 tubes, each colonie one tube:

- p2iGEM0245 colonies 1-2
- p2iGEM0246 colonie 1
- p2iGEM0247 colonies 1-4
- p2iGEM0248 colonies 1-4
- p2iGEM0249 colonies 1-2
- p2iGEM0250 colonies 1-4
- p2iGEM0251 colonies 1-2

Cycle:

- 60:00 min at 37°C
- 20:00 min at 65°C
- HOLD at 4°C

Test with a gel with following Parameters:

-1% agrose gel (200ml, 2.0g argarose)

-1xTAE one time used

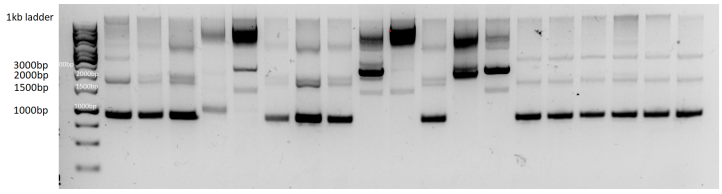
60V 100min

gelpicture with 1kb ladder below, pockets in the order as it been shown befor

expectet bands:

- nearly 2760bp
- nearly 1860bp
- nearly 1400bp

bearbeitet_gel_test_ptxd_exp._plas_14.5.18.png



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

Author: Susanne Vollmer
created: 15.05.2018 16:20
updated: 15.05.2018 16:34
Entry 54/259: 4.Try of the PCR for guaD from gDNA of E.coli
In Project: Level_1
No tags associated

this time using a overhnag PCR with the following protocol:

Mastermix:

- 4,5µl gDNA form E.coli DH5a
- 15µl high GC enhancer
- 15µl Q5 Reaktion buffer
- 4,5 µl dNTP
- 3µl primer O_iGEM18_0047
- 3µl primer O_iGEM18_0048
- 29,25µl Milli Q
- 0,75µl Q5

in three tubes each 25 µl Master mix, in each tube is nearly the same

using this program:

95°C 2 min

- 95°C 15 sec
- 53°C 15 sec
- 72°C 40 sec
- ->repeating 4 times

- 95°C 15 sec
- 67°C 15 sec
- 72°C 40 sec
- ->repeating 24 times

72°C 2 min

4°C HOLD

after the product is stored in the 4°C fridge

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

Author: Miriam Dreesbach

created: 16.05.2018 11:53
updated: 16.05.2018 11:57

Entry 55/259: 1 % agarose storage gel
In Project: Level_1
With tags: agarose gel

To prepare a storage agarose gel, I weighted 0.4 g agarose, poured it into a 100 mL bottle and filled it up with 1xTAE to 40 mL. Afterwards, it was heated in the microwave until the agarose was completely dissolved and the solution was clear. The agarose gel was cooled until it was touchable with the bare hand. Afterwards, I added 2 µL GelRed, swinged the solution and poured the gel on a 6*6 cm gel carrier. After the gel was colpletely hardened, I took it out of the gel carrier and stored it with 5 mL 1xTAE in a autoclave bag at 4 °C.

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

Author: Miriam Dreesbach
created: 16.05.2018 11:58
updated: 16.05.2018 20:58
Entry 56/259: Gel electrophoresis PCR, guaD, Dur1,2_gene_p1 & p2
In Project: Level_1
With tags: gel electrophoresis

To check the amplified fragments of the PCRS of **Dur1,2_gene_p1 and Dur1,2_gene_p2,** I loaded the PCR samples from sunday on a 1 % agarose gel I prepared yesterday and stored at 4 °C.

I sliced a part of the gel with 3 1/2 slots, put it on a gel carrier and mixed 3 µL of each PCR sample with 1 µL 6x Loading Dye (Thermo Fisher):

- **Dur1,2_gene_p1**; **primer** : normal PCR, **66.6 °C** annealing temperature; **85 sec** (2818 bp).
- **Dur1,2_gene_p2**; **primer**: normal PCR; **63.5 °C** annealing temperature; **90 sec** (2955 bp).

Afterwards I loaded the samples and 2 µL ladder (1 µL 1 kb DNA ladder (Thermo Fisher) mixed with 1 µL nuclease-free water(milliQ)) on the agarose gel.

The gel ran on 100 V for 90 minutes.

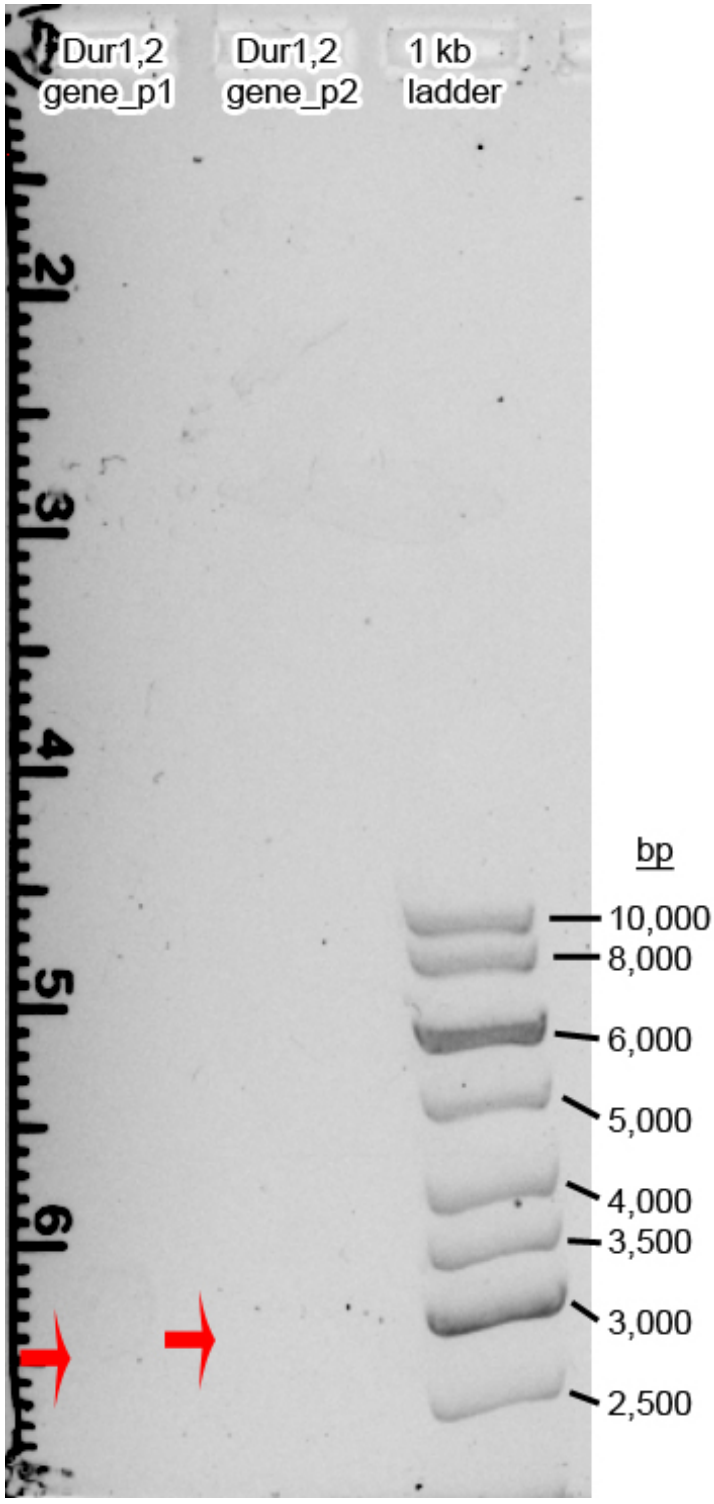
After 90 minutes, I visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

The gel leaked and the visualized gel showed **no expected bands of the following fragments**:

- Dur1,2_gene_p1
- Dur1,2_gene_p2

Therefore, the gel electrophoresis has to be repeated.

Gel_2018-05-16_13hr_33min_dur1.2_p1_p2_1_kb_ladder_90_min_100_v_MD_bearbeitet.jpg



To repeat the check check of the amplified fragments of the PCRS of **Dur1,2_gene_p1 and Dur1,2_gene_p2 (my PCRs) and guaD tube 1-3 (Susannes PCRs),** Susanne loaded my PCR samples from sunday on a 1 % agarose gel, she prepared today. Therefore, she weighted 0.5 g Agarose in a 100 mL bottle, filled it with 50 mL 1xTAE and heated it up in the microwave until the solution was clear and the agarose was completely dissolved. The gel cooled down until it was lukewarm. Afterwards, 10 µL GelRed (Troubleshooting: Too much GelRed), were added and put on a 10*6 cm gel carrier. After the gel was hardened, she mixed 5 µL of each of her PCR samples from 15.05.2018 with 2 µL 6x Loading Dye (Thermo Fisher) and 3 µL nuclease-free water (milliQ):

- **guaD tube 1**
- **guaD tube 2**
- **guaD tube 3**

Additionally, she mixed 3 µL of each of my PCR samples with 1 µL 6x Loading Dye (Thermo Fisher):

- **Dur1,2_gene_p1**; **primer** : normal PCR, **66.6 °C** annealing temperature; **85 sec** (2818 bp).
- **Dur1,2_gene_p2**; **primer**: normal PCR; **63.5 °C** annealing temperature; **90 sec** (2955 bp).

Afterwards she loaded the samples with a 1 kb DNA ladder (Thermo Fisher= on the agarose gel.

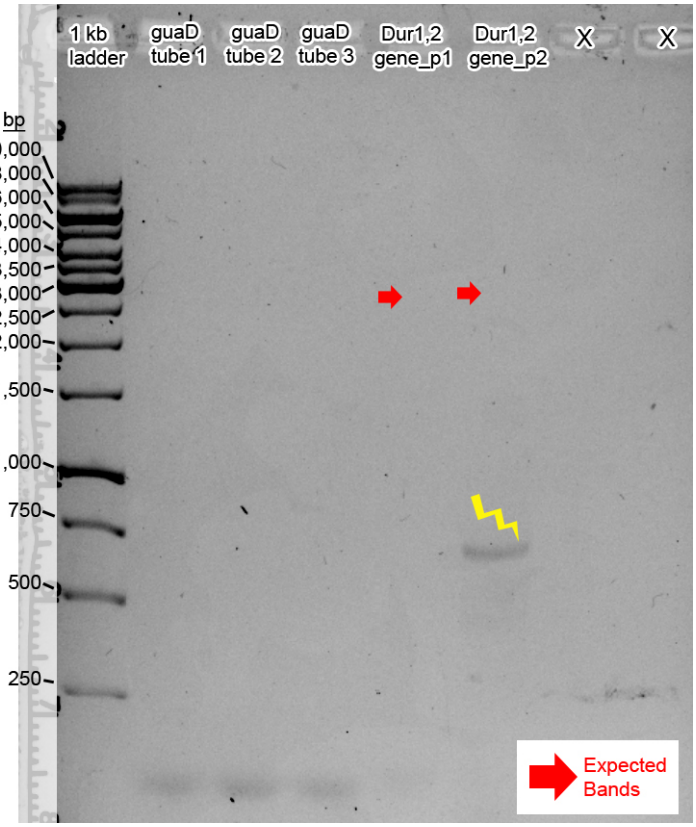
The gel ran on 60 V for 150 minutes.

After 150 minutes, we visualized the gel with the Nucleic Acids -> GelRed channel with the ChemyDoc MP System of Bio-Rad and the Image Lab software.

The visualized gel showed **no expected bands of the following fragments**:

- guaD (each tube) (Primer Dimer)
- Dur1,2_gene_p1 (Primer Dimer?)
- Dur1,2_gene_p2 (Wrong fragment size)

Gel_2018-05-16_19hr_15min_1_kb_ladder_gblocks_3xSV_2xMD_150_min_60_V_dark_mit_SV_bearbeitet_Kopie.jpg



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

Author: Miriam Dreesbach
created: 16.05.2018 20:25
updated: 16.05.2018 21:00
Entry 58/259: pJET blunt-end cloning of TrzE, Dur1,2 p1, Dur1,2 p2, trzC, atzD, triA
In Project: Level_1
With tags: pJET, blunt-end cloning, T4 ligase

Blunt-End Cloning Protocol with pJET (Thermo Scientific)

We (Susanne & Miriam) cloned the following synthesises into pJET1.2:

S_iGEM18_0015 (TrzE gene) (c = 25 ng/µL)

S_iGEM18_0016 (Dur1,2_gene_p1) (c = 10 ng/µL)

S_iGEM18_0017 (Dur1,2_gene_p2) (c = 10 ng/µL)

S_iGEM18_0019 (trzC-E-coli-codon-opt) (c = 25 ng/µL)

S_iGEM18_0020 (atzD-E-coli-codon-opt) (c = 10 ng/µL)

S_iGEM18_0021 (triA-E-coli-codon-opt) (c = 25 ng/µL)

1. **We set up the ligation reaction on ice**:

2X Reaction Buffer: 10 µL

Synthesis **fragment**: 0.5 µL

pJET1.2/blunt Cloning Vector (50ng/L): 1 µL (0.05 pmol ends)

Water, nuclease-free (Edit Mimi: milliQ): 7.5 µL

T4 DNA Ligase: 1 µL

Total volume: 20 µL

Afterwards, we **vortexed briefly** and **centrifuged for 3-5 s**.

2. We **incubated** the ligation mixture at **room temperature** (22°C) for **30 min**.

3. We **stored the ligation at - 20 °C** for further transformation.

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

Author: Susanne Vollmer
created: 17.05.2018 10:50
updated: 17.05.2018 20:28

Entry 59/259: Transformation of E.coli T10 with the pJET cloning Plasmids
In Project: Level_1
No tags associated

Transformations of the pJET Plasmids with the syntesis gens triA (Plasmisnumber:p2iGEM0253, Cryonumber:2iGEM0271), trzC (p2iGEM02054, 2iGEM0272), atzD (p2iGEM0255, 2iGEM0273) using the following protocol:

- thaw competent cells 5-10 min on ice
- add 1µl 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- centrifuge the cells for 5min at 6000rcf, decant the supernatante and resuspend the cells in the rest(100-200µl),(if you decant to much, fill with 50µl LB-medium)
- screat the Suspension on a agarplate with the Fitting antibiothica (i used ampicillin)
- incubate at 37°C

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

Author: Miriam Dreesbach

created: 17.05.2018 19:05
updated: 17.05.2018 19:28

Entry 60/259: Transformation of pJET ligations of TrzE_gene, Dur1,2_gene_p1 and Dur1,2_gene_p2
In Project: Level_1
With tags: Transformation, pJET

To transform pJET1.2 into DH5α, I pipetted 5 µL of yesterdays' pJET ligation mixture of the following synthesises to 50 µL of competent cells.

- TrzE_gene (S_iGEM18_0015)
- Dur1,2_gene_p1 (S_iGEM18_0016)
- Dur1,2_gene_p2 (S_iGEM18_0017)

Afterwards, it incubated on ice for 5 minutes.

After the incubation on ice, I heat shocked the cells accidently for 2 minutes at 42 °C.

Troubleshooting: Too long heat shock.

After the heat shock, I incubated the cells for 45 minutes on ice.

After incubation, I added 300 µL LB without any antibiotics to the cells and incubated them for 1 hour at 37 °C and 300 rpm.

Due to the ampicillin resistance of pJET1.2, I plated 100 µL the cells (without any centrifuging) on agar plates with 100 µg ampicillin / mL, which were prepared by Susanne today.

The cells incubated overnight at 37 °C.

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

Author: Miriam Dreesbach
Entry 61/259: No entry title yet
In Project: Level_1
No tags associated

created: 20.05.2018 18:01
updated: 20.05.2018 18:37

To prepare further cryo cultures and plasmid isolation of transformed pJET ligations, I inoculated 3 mL LB (+ 30 µL ampicillin) with the following colonies:

- pJET_TrzC
- pJET_TriA
- pJET_Dur1,2_gene_p2

Afterwards, I incubated the cultures overnight at 37 °C and after incubation I stored them at 4 °C for further preparation.

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

Author: Susanne Vollmer
created: 21.05.2018 11:54
updated: 21.05.2018 13:18
Entry 62/259: Miniprep and Cryo of 2iGEM0271,72,78
In Project: Level_1
No tags associated

Cryos of the pJET cloning Plasmids triA (p2iGEM0253, 2iGEM0271), trzC (p2iGEM0254, 2iGEM0272), Dur1,2_gene_p2 (p2iGEM0258, 2iGEM0278):

300µl Glycerin 99,5% und 700 culture (stored -80°C in box Level 1)

Miniprep from the rest unsing the following protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, decant the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 5 Min at RT. Centrifuge for 15 Sek. max
- (-> made a mistake, elution in the collumn wash, centrifuge it again trough the Membran and wash with 200 Collum wash again und then elut again with milli Q 37°C and incubate it on 37°C with 300rpm for 40 min, than centrifuge)

concentration:

p2iGEM0253: 281,6 ng1/µl

p2iGEM0254: 114,1 ng/µl

p2iGEM0258: 312,3 ng/µl

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

Author: Susanne Vollmer

created: 22.05.2018 16:54
updated: 24.05.2018 11:28

Entry 63/259: repetion of the pJET transformation, digestion of p2iGEM0097

In Project: Level_1

No tags associated

Transformation of the Plasmids p2iGEM0255 from two equal clonings using following protocol:

- thaw competent cells 5-10 min on ice
- add 1µl 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
- take 30µl and delute it with 100µ milli Q water
- centrifuge the rest for 30sec at 10000rpm,decant the supernatante and resuspend the cells in the rest(100-200µl),(if you decant to much, fill with 50µl LB-medium)
- screat the Suspension both on a agarplate each with the Fitting antibiothica (i used ampicillin)
- incubate at 37°C

predigestion of the Backbone p2iGEM0097 with bsaI for the cloning later, using following protocol:

- 1,5µl cutsmart
- 0,5µl BsaI (last on ice!)
- 1µl p2iGEM0097
- 12µl milli Q water

program:

- 7h 37°C
- 20min 65°C
- HOLD 4°C

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

Author: Susanne Vollmer

created: 23.05.2018 17:42
updated: 23.05.2018 20:49

Entry 64/259: inoculation of different 2iGEM0253 colonies, repetition of the golden Gate for the plasmids p2iGEM0244-52

In Project: Level_1

No tags associated

inoculation of 3ml LB with 30µl amicilin with 14 different colonies from the secundary try od the pJET cloning of atzD and 2 from the first try (16 in total)

repetiton of the Golden Gate for the Expression Plasmids of ptxD (p2iGEM0244-252)

in the tubes each:

- 3µl milli Q
- 1µl T4 ligation buffer
- 0,5µl T4 Ligase
- 0,5µl BsaI
- 0,5µl Terminator p2iGEM0051
- 2µl Backbone p2iGEM0097
- 2µl gen of interesst (ptxD) p2iGEM0208
- 0,5µl promoter p2iGEM0009-17

plasmidnumber	promoter	backbone	crynumber
p2iGEM0244	p2iGEM0009	predigested	2iGEM0261
p2iGEM0245	p2iGEM0010	no predigested	2iGEM0262
p2iGEM0246	p2iGEM0011	no predigested	2iGEM0263
p2iGEM0247	p2iGEM0012	no predigested	2iGEM0264
p2iGEM0248	p2iGEM0013	no predigested	2iGEM0265
p2iGEM0249	p2iGEM0014	no predigested	2iGEM0266
p2iGEM0250	p2iGEM0015	no predigested	2iGEM0267
p2iGEM0251	p2iGEM0016	no predigested	2iGEM0268
p2iGEM0252	p2iGEM0017	no predigested	2iGEM0269

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

Author: Miriam Dreesbach

created: 24.05.2018 10:34
updated: 24.05.2018 10:54

Entry 65/259: Inoculation of pJET tranformations of TrzE_gene and Dur1,2_gene_p1

In Project: Level_1

With tags: inoculation, pJET, Transformation, cryogenic culture, isolation, plasmid

As the plated transformations of pJET ligations of TrzE_gene (**2iGEM0276**) and Dur1,2_gene_p1 (**2iGEM0277**) from thursday (17.05.2018) did not show any colonies, I centrifuged the remaining 200 µL of the cultures which were stored at 4 °C at 2000 rpm for 2 minutes. Afterwards I discarded the supernatant and resuspended and plated the remaining 100 µL of the cultures on LB agar plates with ampicillin which were prepared by Susanne. Afterwards, I incubated them overnight at 37 °C.

On the following day the plates showed single big colonies with satellite colonies.

To make sure the big colonies harbour ampicillin resistance, I inoculated 6x 3 mL of LB with 30 µL ampicillin with 3 big colonies of each plate and incubated them overnight at 37 °C.

Each culture showed growth in ampicillin media. Therefore, I chose the first inoculated colony of each plate to prepare cryogenic cultures and plasmid isolation.

Therefore, I mixed 200 µL of each culture with 800 µL glycerol and stored them at - 80 °C.

To isolate the plasmid (**p2iGEM0256; p2iGEM0257**) of the cultures, I centrifuged the cultures at 4000 rpm for 5 minutes.

Afterwards, I poured off the supernatant and resuspended the pellet in the remaining media. I added 100 µL of cell lysis buffer, inverted the eppi 8 times, added 350 µL neutralization buffer and inverted the eppi for another 8 times. The solutions were centrifuged for 3 minutes at maximum rpm. I loaded 900 µL of the supernatant on a column and centrifuged it for 30 seconds at max. rpm. Afterwards, I discarded the flow-through, added 400 µL column wash buffer and centrifuged it for 30 seconds at max. rpm. I repeated the column wash step and eluted the remaining plasmid on the column with 30 µL 37 °C nuclease-free milliQ water for one minute. Afterwards, I centrifuged it for 30 seconds at max. rpm and measured the concentrations:

pJET_TrzE_gene (p2iGEM0256): 350.9 ng / µL

pJET_Dur1,2_gene_p1 (p2iGEM0257) : 315.95 ng / µL

I stored the plasmids in the level 1 box at - 20 °C.

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

Author: Miriam Dreesbach
Entry 66/259: Overlap PCR NSIa, ori, NSIb
In Project: Level_1
With tags: overlap pcr, pcr, three-step-pcr

created: 24.05.2018 11:07
updated: 30.05.2018 09:39

To create an extensive fragment of the single fragments NSIa (gDNA *Synechococcus elongatus* sp. PCC 7942), ori (NSI_TetR_LacI), NSIb (gDNA *S. elongatus*), I ran a overlap PCR. To calculate the appropriate amount of amplified fragments, I used the NEBioCalculator calculator. Therefore I pipetted the following ingredients:

5x Q5 reaction buffer (NEB): 5 µL

High GC enhancer (NEB): 5 µL

dNTPs (NEB): 0.4 µL

Fragment 1 (NSIa): 4.7 ng - 1 µL (4.7 ng / µL

Fragment 2 (ori): 1.8 ng - 1.7 µL (diluted 1:10 from 27.0 ng / µL)

Fragment 3 (NSIb): 4.7 ng - 0.5 µL (8.35 ng / µL)

Q5 high-fidelity polymerase: 0.5 µL

nuclease-free water (milliQ): fill up tp 25 µL.

All fragments were taken from the PCR from 06.05.2018).

As the annealing temperature of the homologous regions is 71-72 °C, I ran the PCR with the following protocol on **our left Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: 71 °C: 30 sec

4. Extension: 72 °C: 60 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 35 times. (Troubleshooting: Annas overlap PCR protocol recommends 15 cycles.)

Afterwards, I stored the PCR product at - 20 °C.

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

Author: Susanne Vollmer
created: 24.05.2018 11:20
updated: 24.05.2018 13:55
Entry 67/259: trafo of T10 with p2iGEM0244-52
In Project: Level_1
No tags associated

Transformation of *E.coli* T10 with the Plasmid of the 2.try of the Golden Gate for the Expression Plasmids of ptxD (p2iGEM0244-p2iGEM0252) using the following protocol:

- thaw competent cells 5-10 min on ice
- add 1µl 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
- take 30µl and delute it with 100µ milli Q water
- centrifuge the rest for 30sec at 10000rpm,decant the supernatante and resuspend the cells in the rest(100-200µl),(if you decant to much, fill with 50µl LB-medium)
- screat the Suspension both on a agarplate each with the Fitting antibiothica (i used ampicillin) for number 9 and 10 i concentrat ist, for the rest of the Plasmids only 100µl of all after a i suspensate it again
- incubate at 37°C

Plasmid number	p2iGEM0244	p2iGEM0245	p2iGEM0246	p2iGEM0247	p2iGEM0248	p2iGEM0249	p2iGEM0250	p2iGEM
organismnumber	2iGEM0291	2iGEM0292	2iGEM0293	2iGEM0294	2iGEM0295	2iGEM0296	2iGEM0297	2iGEM0

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

Author: Miriam Dreesbach

created: 24.05.2018 11:21
updated: 25.05.2018 19:12

Entry 68/259: Touchdown PCR of Dur1,2_gene_p1 and Dur1,2_gene_p2
In Project: Level_1
With tags: touchdown pcr, synthesises, gBlocks

To amplify the synthesises of Dur1,2_gene_p1 (S_iGEM18_0016) and Dur1,2_gene_p2 (S_iGEM18_0017), I ran two touchdown PCRs with the following approach (20 µL):

- 4 µL 5x Q5 Reaction Buffer
- 1 µL Template (1 ng)
- 0.5 dNTP-mix (0.2 mM)
- 1 µL Primer fw (0.5 µM)
- 1 µL Primer rv (0.5 µM)
- 0.5 µL Q5 High-Fidelity DNA Polymerase
- 0.6 µL sterile DMSO (3 %)
- 14.4 µL nuclease-free miliQ water

I prepared two PCR tubes and ran two three-step PCRs of the following constructs (fragment length) were run on **our left Eppendorf cycler**:

- **Dur1,2_gene_p1**; **primer** : O_iGEM2018_0036(fw), O_iGEM2018_0059(rv); (2818 bp).
- **Dur1,2_gene_p2**; **primer**: O_iGEM2018_0060(fw), O_iGEM2018_0061(rv); (2955 bp).

Program of the three-step PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: 72 °C: 30 sec (each step -0.6 °C)
4. Extension: 72 °C: 90 sec
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

Afterwards, I stored the PCR products at -20 °C.

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

Author: Miriam Dreesbach
created: 24.05.2018 11:25
updated: 25.05.2018 19:12

Entry 69/259: Three-step PCR of TrzE_gene
In Project: Level_1
With tags: three-step-pcr, synthesises, gBlocks

To amplify the synthesises of TrzE_gene (S_iGEM18_0015), I ran a three-step PCR with the following approach (20 µL):

- 4 µL 5x Q5 Reaction Buffer
- 1 µL Template (1 ng)
- 0.5 dNTP-mix (0.2 mM)
- 1 µL Primer fw (0.5 µM)
- 1 µL Primer rv (0.5 µM)
- 0.5 µL Q5 High-Fidelity DNA Polymerase
- 0.6 µL sterile DMSO (3 %)
- 14.4 µL nuclease-free miliQ water

I prepared a PCR tube and ran a three-step PCRs of the following constructs (fragment length) on the **left Bio-Rad cycler** (Pauly Lab, Felix Roth):

- **TrzE_gene** ; **primer**: O_iGEM2018_0034(fw), O_iGEM2018_0035(rv); (882 bp).

Program of the three-step PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: 67.5 °C: 30 sec
4. Extension: 72 °C: 30 sec
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

Afterwards, I stored the PCR product at -20 °C.

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

Author: Miriam Dreesbach

created: 24.05.2018 16:32
updated: 24.05.2018 18:59

Entry 70/259: Gel electrophoresis of PCR products of overlap PCR, Dur1,2_gene_p1, Dur1,2_gene_p2, TrzE_gene

In Project: Level_1

With tags: gel electrophoresis, overlap pcr, three-step-pcr, synthesises, gBlocks

To prepare a small 1 % agarose gel, I weigthed 0.4 g Agarose and solved it in 40 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 2 µL GelRed and loaded the gel. After it was hardened, I loaded the following onstructs on the gel:

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

4 µL yesterdays' overlap PCR product of NSIa, ori, NSIb

4 µL yesterdays' PCR product of Dur1,2_gene_p1

4 µL yesterdays' PCR product of Dur1,2_gene_p2

4 µL yesterdays' PCR product of TrzE_gene

I ran the gel electrophoresis on 100 V for 1 h.

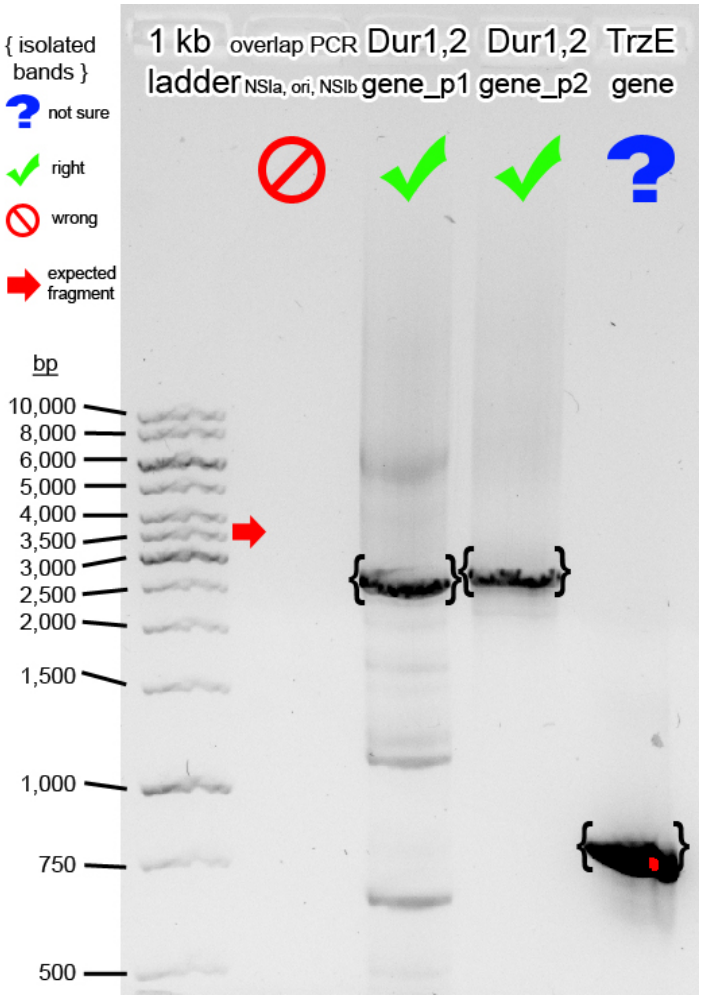
Afterwards, I visualized the gel.

Dur1,2_gene_p1 and Dur1,2_gene_p2 showed the right bands on the gel. Therefore, the appropriate bands were cut out.

TrzE_gene may show the right band. Therefore, the appropriate band was cut out, too.

The overlap PCR of NSIa, ori and NSIb did not show any band on the agarose gel. Therefore, the gel electrophoresis has to be repeated.

Gel_2018-05-24_16hr_56min_1_kb_ladder_overlap_PCR_Dur12_gene_p1_Dur12_gene_p2_TrzE_MD_100_V_1_h.jpg



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

Author: Miriam Dreesbach

created: 25.05.2018 19:06
updated: 25.05.2018 19:11

Entry 71/259: Touchdown PCR of Dur1,2_gene_p1 and Dur1,2_gene_p2; Three-step PCR of TrzE_gene

In Project: Level_1

With tags: pcr, three-step-pcr, touchdown pcr, gBlocks, synthesises

To amplify and isolate the fragments of the synthesis of TrzE_gene (S_iGEM18_0015), I ran a three-step PCR with the following approach (50 µL):

- 10 µL 5x Q5 Reaction Buffer
- 2.5 µL Template (1 ng)
- 1 dNTP-mix (0.2 mM)
- 2.5 µL Primer fw (0.5 µM)
- 2.5 µL Primer rv (0.5 µM)
- 0.5 µL Q5 High-Fidelity DNA Polymerase
- 1.5 µL sterile DMSO (3 %)
- 29.5 µL nuclease-free miliQ water

I prepared a PCR tube and ran a three-step PCRs of the following constructs (fragment length) on the **right Bio-Rad cycler** (Pauly Lab, Felix Roth):

- **TrzE_gene** ; **primer**: O_iGEM2018_0034(fw), O_iGEM2018_0035(rv); (882 bp).

Program of the three-step PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: 67.5 °C: 30 sec
4. Extension: 72 °C: 30 sec
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

Afterwards, I stored the PCR product at -20 °C.

To amplify and isolate the fragments of the synthesises of Dur1,2_gene_p1 (S_iGEM18_0016) and Dur1,2_gene_p2 (S_iGEM18_0017), I ran two touchdown PCRs with the following approach (50 µL):

- 10 µL 5x Q5 Reaction Buffer
- 2.5 µL Template (1 ng)
- 1 dNTP-mix (0.2 mM)
- 2.5 µL Primer fw (0.5 µM)
- 2.5 µL Primer rv (0.5 µM)
- 0.5 µL Q5 High-Fidelity DNA Polymerase
- 1.5 µL sterile DMSO (3 %)
- 29.5 µL nuclease-free miliQ water

I prepared two PCR tubes and ran two three-step PCRs of the following constructs (fragment length) were run on **our left Eppendorf cycler**:

- **Dur1,2_gene_p1**; **primer** : O_iGEM2018_0036(fw), O_iGEM2018_0059(rv); (2818 bp).
- **Dur1,2_gene_p2**; **primer**: O_iGEM2018_0060(fw), O_iGEM2018_0061(rv); (2955 bp).

Program of the three-step PCRs:

1. Initial denaturation: 98 °C: 2 min
2. Denaturation: 98 °C: 30 sec
3. Annealing: 72 °C: 30 sec (each step -0.6 °C)
4. Extension: 72 °C: 90 sec
5. Final Extension: 72 °C: 10 min
6. Hold: 4 °C

Step 2 to 4 were repeated for 34 times.

Afterwards, I stored the PCR products at -20 °C.

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

Author: Susanne Vollmer

created: 25.05.2018 19:12
updated: 25.05.2018 20:25

Entry 72/259: miniprep of the pJET atzD cloning, inoculation of the selected golden Gate colonies, repetions of the spreatout of the transformants of the Golden Gate, test PCR of the pJET cloning

In Project: Level_1

With tags: golden gate, inoculation, miniprep, nanodrop, PCR programm, Transformation, green-white-screening, synthesises, pJET

Miniprep of 14 cultures from different colonies of the second try of the pJET cloning of atzD and 1 one from the first try (all is the Plasmid p2iGEM0255 correspond to 2iGEM0273), using follwing protocol:

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

culture was centrifuged and the supernatate was decant, than stored over night at -20°C and at the next day resuspend in 600µl LB

- 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 15 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for nearly 3h at 37°C and 300rpm. Centrifuge for 15 Sek. max

content	concentration
p2iGEM02551	361,35 ng/µl
p2iGEM02552	311,60 ng/µl
p2iGEM02553	387,10 ng/µl
p2iGEM02554	440,05 ng/µl
p2iGEM02555	505,95 ng/µl
p2iGEM02556	480,65 ng/µl
p2iGEM02557	291,70 ng/µl
p2iGEM02558	427,30 ng/µl
p2iGEM02559	206,05 ng/µl
p2iGEM025510	160,90 ng/µl
p2iGEM025511	241,55 ng/µl
p2iGEM025512	359,30 ng/µl
p2iGEM025513	311,90 ng/µl
p2iGEM025514	344,75 ng/µl
p2iGEM025515/1	351,25 ng/µl

selection of the White colonies of the transfomration of the 2. try golden gate Plasmids p2iGEM0244-p2iGEM0252 wich correspond to 2iGEM0291-2iGEM0299:

- under blue light one colonie of 2iGEM0292 and two of 2iGEM0293 are White and one of each were selected
- 3ml LB with 30µl ampicilin where used to inoculate
- incubate over night on 37°C

due to the less colonies and very less White one, 125µl of the rest of the cultures of the Transformation, which were stored at 4°C over night were spread out on ampicilin plates (except for 2iGEM0291 and 2iGEM0292, there was no rest, all was spread out the first time). inoculation over night on 37°C

TestPCR of the pJET cloning Plasmids p2iGEM0253 (2iGEM0271), p2iGEM0254 (2iGEM0272), p2iGEM0255 (2iGEM0273) using the following protocol:

test of 5 different colonies of Plasmid p2iGEM0253 (4 from the 2.try and one of the 1.try), one of p2iGEM054 and one of p2iGEM0255

in each tube:

- 1µl primer fwd
- 1µl primer rwd --> both from the pJET cloning kit from Themo Scientific
- 0,5µl dNTP
- 0,5µl Q5 polymerase (ad last on ice!)
- 5µl Q5 reaction buffer
- 5µl high GC enhancer
- 11µl milli Q water
- 1µl of the tested Plasmids

using a Mastermix (10µl primer fwd, 10µl primer rwd, 5µl dNTP, 5µl Q5 polymerase (ad last on ice!), 50µl Q5 reaction buffer, 50µl high GC enhancer, 110µl milli Q water)

then add 24µl of the Mastermix and 1µl Plasmid in each tube

PCR program:

95°C 2min

then starting the cycle with:

95°C 15 sec

61°C 30 sec

72°C 40 sec

repeating this 30x

72°C 2min

5°C HOLD

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

Author: Miriam Dreesbach

created: 26.05.2018 11:05
updated: 28.05.2018 00:16

Entry 73/259: Gel electrophoresis of Dur1,2_gene_p1, Dur1,2_gene_p2, TrzE_gene for fragment isolation

In Project: Level_1

With tags: agarose gel, gBlocks, gel electrophoresis, pcr, three-step-pcr, touchdown pcr, synthesises

To prepare a small 10*6 cm 1 % agarose gel, I weigthed 0.48 g Agarose and solved it in 48 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 2.4 µL GelRed and loaded the gel. After it was hardened, I loaded the following onstructs on the gel:

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

12.5 µL yesterdays' PCR product of Dur1,2_gene_p1 + 2.5 µL 6x LD

12.5 µL yesterdays' PCR product of Dur1,2_gene_p1 + 2.5 µL 6x LD

12.5 µL yesterdays' PCR product of Dur1,2_gene_p2 + 2.5 µL 6x LD

12.5 µL yesterdays' PCR product of Dur1,2_gene_p2 + 2.5 µL 6x LD

12.5 µL yesterdays' PCR product of TrzE_gene + 2.5 µL 6x LD

12.5 µL yesterdays' PCR product of TrzE_gene + 2.5 µL 6x LD

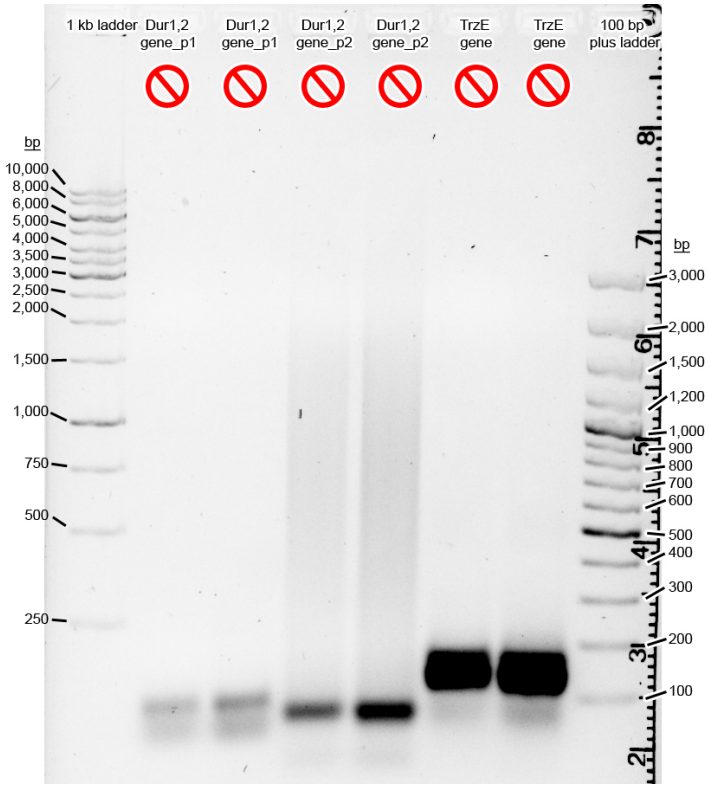
1 µL 100 bp plus DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

I ran the gel electrophoresis on 100 V for 1 h.

Afterwards, I visualized the gel.

Dur1,2_gene_p1, Dur1,2_gene_p2 and TrzE_gene showed no right bands on the gel.

Gel_2018-05-26_12hr_33min_1_kb_ladder-_Dur1-2_gene_p1-_Dur1-2_gene_p1-_Dur1-2_gene_p2-_Dur1-2_gene_p2-_TrzE_gene-_TrzE_gene-_100_bp_plus_MD_100_V_1_h_Kopie.jpg



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

Author: Susanne Vollmer

created: 28.05.2018 19:45
updated: 29.05.2018 16:21

Entry 74/259: Gel of the test PCR of pJET plasmids, icoculation of the selected colonies of p2iGEM244-52

In Project: Level_1

With tags: inoculation, pJET, golden gate, agarose gel, ampicillin, electrophoresis, gel electrophoresis

1% agarosegel: 200ml 1x TAE buffer and 2g agarose

90V 1h 30min

in 1x TAE buffer

Gel pockets:

1: 1kb ladder

2: PCR of 1 colonie of the 2. try of pJET cloning of atzD p2iGEM0255 ->1211bp(expected band)

3: PCR of 2 colonie of the 2. try of pJET cloning of atzD p2iGEM0255 ->1211bp

4: PCR of 3 colonie of the 2. try of pJET cloning of atzD p2iGEM025 ->1211bp

5: PCR of 4 colonie of the 2. try of pJET cloning of atzD p2iGEM025 ->1211bp

6: PCR of 5 colonie of the 2. try of pJET cloning of atzD p2iGEM025 ->1211bp

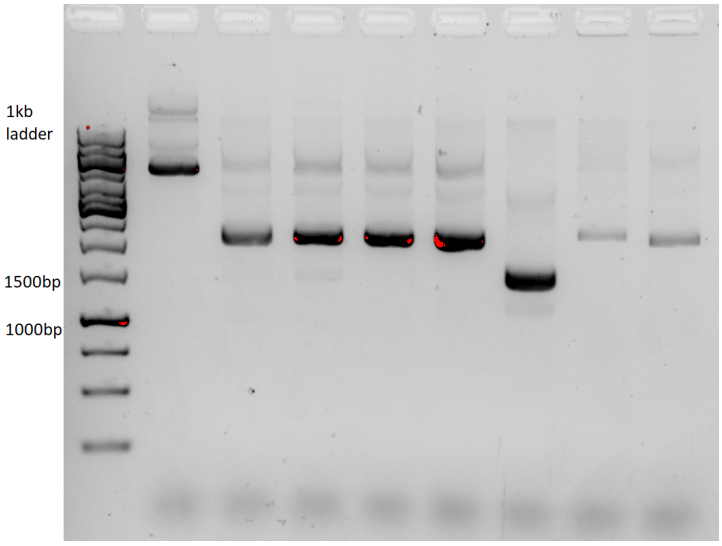
7: PCR of 1 colonie of the 1. try of pJET cloning of atzD p2iGEM025 -> 1211bp - only one that maybe works

8: PCR of pJET cloning of trzC p2IGEM0254->1358bp

9: PCR of pJET cloning of triA p2iGEM0253->1544bp

gel Picture below, in General not Show the expectet bands

pJET_test_PCR_gel_bearbeitet.png



inoculation of 3ml LB and 30µl ampicilin with the White colonies from the Transformation of the 2. try golden gate ptxD expresssion Plasmids

selection of the white colonies from 2iGEM0293-2iGEM0299 in total 8 cultures

incubation over night at 37°C

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

Author: Susanne Vollmer
created: 29.05.2018 16:21
updated: 29.05.2018 16:57

Entry 75/259: miniprep of the inoculated colonies of p2iGEM0244-52 from the 2. try golden gate
In Project: Level_1
With tags: miniprep, golden gate, dueber, E.coli, plasmid, promoter

miniprep of the inoculated selected colonies of p2iGEM291-2iGEM0299, with the Plasmids p2iGEM0244-p2iGEM0252, using the following protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 20 Min at 37°C and 300prm. Centrifuge for 15 Sek. max

plasmidnumber	cryonumber	colonie	concentration
p2iGEM0246	2iGEM0293	1	503,20 ng/µl
p2iGEM0247	2iGEM0294	1	257,50 ng/µl
p2iGEM0249	2iGEM0296	1	285,10 ng/µl
p2iGEM0249	2iGEM0296	2	299,55 ng/µl
p2iGEM0249	2iGEM0296	3	398,55 ng/µl
p2iGEM0249	2iGEM0296	4	326,40 ng/µl
p2iGEM0252	2iGEM0299	1	279,70 ng/µl
p2iGEM0252	2iGEM0299	2	334,00 ng/µl

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

Author: Miriam Dreesbach
created: 30.05.2018 09:35
updated: 30.05.2018 09:45
Entry 76/259: Overlap PCR NSIa & ori and gel electrophoresis
In Project: Level_1
With tags: overlap pcr, three-step-pcr, gel electrophoresis, agarose gel

To create an extensive fragment of the single fragments NSIa (gDNA *Synechococcus elongatus* sp. PCC 7942) and ori (NSI_TetR_LacI), I ran a overlap PCR. To calculate the appropriate amount of amplified fragments, I used the NEBioCalculator calculator. Therefore I pipetted the following ingredients:

5x Q5 reaction buffer (NEB): 5 µL

High GC enhancer (NEB): 5 µL

dNTPs (NEB): 0.4 µL

Fragment 1 (NSIa): 23.5 ng - 5 µL (4.7 ng / µL)

Fragment 2 (ori): 9 ng - 8.5 µL (diluted 1:10 from 27.0 ng / µL)

nuclease-free water (milliQ): 0.5 µL

Q5 high-fidelity polymerase: 0.5 µL

All fragments were taken from the PCR from 06.05.2018).

As the annealing temperature of the homologous regions is 71 °C, I ran the PCR with the following protocol on **our right Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: 71 °C: 30 sec

4. Extension: 72 °C: 60 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 35 times. (Troubleshooting: Annas overlap PCR protocol recommends 15 cycles.)

Afterwards, I loaded 3 µL of the PCR product on a 1 % agarose gel for further gel electrophoresis and stored the remaining 20 µL of the PCR product at - 20 °C.

To identify if the overlap PCR product is correct, I ran a gel electrophoresis with a 1 % agarose gel, which was stored at 4 °C.

Therefore, I loaded

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

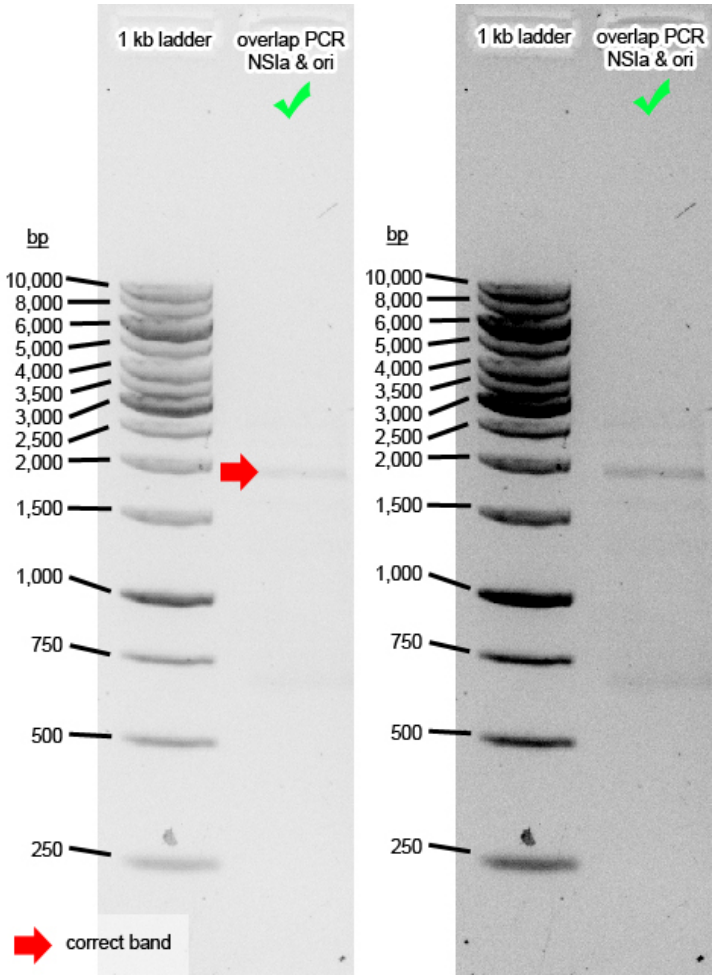
and 3 µL of the overlap PCR product + 1 µL 6x LD (Thermo Fisher) on the gel.

I ran the gel electrophoresis on 80 V for 1 h.

Afterwards, I visualized the gel.

The overlap PCR showed the correct fragment size (2125 bp) in a low concentration on the gel (left side: bright contrast; right side: low brightness).

Gel_2018-05-29_14hr_57min_1kb_dna_ladder._NSIa_ori_overlap_PCR._45_min_80_v_MD_dark_and_bright_Kopie.jpg



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

Author: Miriam Dreesbach
created: 30.05.2018 09:46
updated: 01.06.2018 12:16
Entry 77/259: Gelelectrophoresis and isolation of appropriate correct bands
In Project: Level_1
With tags: gel electrophoresis, agarose gel, gBlocks, synthesises, isolation

To isolate the appropriate fragments from an agarose gel, I prepared a small 6*6 cm 1 % agarose gel, weigthed 0.4 g Agarose and solved it in 40 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 2 µL GelRed and loaded the gel. After it was hardened, I loaded the following onstructs on the gel:

12.5 µL PCR product (23.05.2018) of Dur1,2_gene_p1 + 2.5 µL 6x LD

12.5 µL PCR product (23.05.2018) of Dur1,2_gene_p2 + 2.5 µL 6x LD

12.5 µL PCR product (23.05.2018) of TrzE_gene + 2.5 µL 6x LD

12.5 µL PCR product (25.05.2018 *) of Dur1,2_gene_p1 + 2.5 µL 6x LD

12.5 µL PCR product (25.05.2018 *) of Dur1,2_gene_p2 + 2.5 µL 6x LD

12.5 µL PCR product (25.05.2018 *) of TrzE_gene + 2.5 µL 6x LD

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

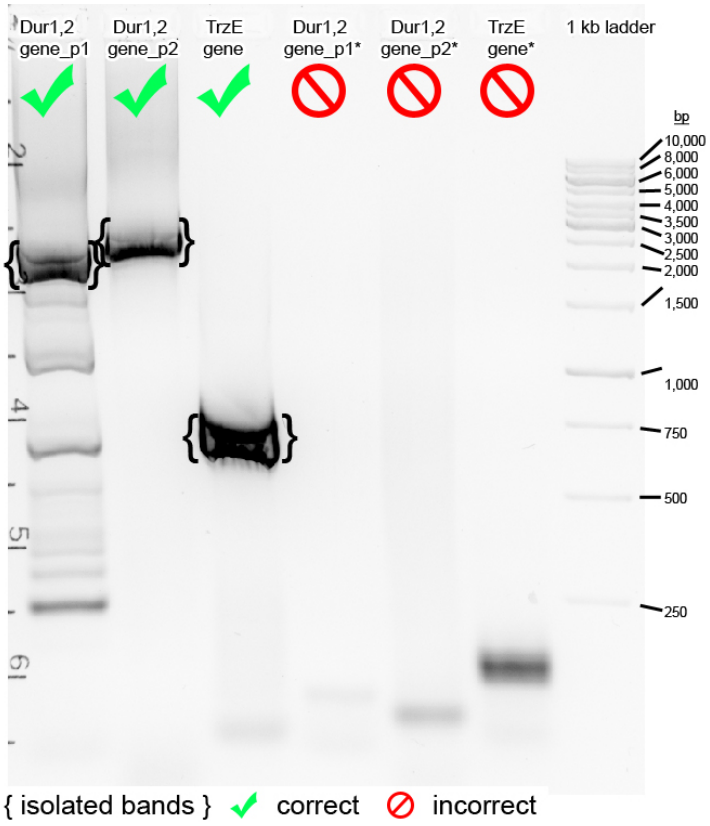
6 µL test digest 262 1 (Ylenia Longo) -> unknown amount of sample & 6x LD

I ran the gel electrophoresis on 80 V for 1 h.

Afterwards, I visualized the gel.

The PCR products of Dur1,2_gene_p1, Dur1,2_gene_p2 and TrzE_gene from the PCR of the 23th of may showed the right bands on the gel. Therefore, I isolated the appropriate bands under blue light.

Gel_2018-05-29_13hr_48min_Dur1.2_p1._Dur1.2_p2._TrzE._Dur1.2_p1._Dur1.2_p2._TrzE.
_1kb_dna_ladder_80_V_1_h_MD_bearbeitet_Kopie.jpg



To isolate the bands out of todays' and the 24th of may' agarose gel (gel electrophoresis), I used the Wizard SV Gel and PCR Clean-Up System Kit (Promega).

First, I weighted the bands I cut out of the gel yesterday:

- Dur1,2_gene_p1 (gel electrophoresis 24.05.2018): 0,24 g
- Dur1,2_gene_p2 (gel electrophoresis 24.05.2018): 0.33 g
- TrzE_gene (gel electrophoresis 24.05.2018): 0.33 g
- Dur1,2_gene_p1 (gel electrophoresis 29.05.2018): 0.28 g
- Dur1,2_gene_p2 (gel electrophoresis 29.05.2018): 0.14 g
- TrzE_gene (gel electrophoresis 29.05.2018): 0.17 g

Afterwards, I added 10 µL membrane binding solution to 10 mg agarose gel bands. Therefore I added the following volume to the samples:

- Dur1,2_gene_p1 (gel electrophoresis 24.05.2018): 0,24 mL
- Dur1,2_gene_p2 (gel electrophoresis 24.05.2018): 0.33 mL
- TrzE_gene (gel electrophoresis 24.05.2018): 0.33 mL
- Dur1,2_gene_p1 (gel electrophoresis 29.05.2018): 0.28 mL
- Dur1,2_gene_p2 (gel electrophoresis 29.05.2018): 0.14 mL
- TrzE_gene (gel electrophoresis 29.05.2018): 0.17 mL

To dissolve the agarose gel in the membrane binding solution, I incubated the samples on 50 °C and vortexed from time to time until the solution was completely clear.

After incubation, I loaded max. 500 µL of the sample to a column in a collection tube. After 1 minute incubation at room temperature, I centrifuged the samples for 1 minute at 16,000 rpm. I discarded the flow-through. After that, I added 700 µL membrane wash solution on the column and centrifugated the sample at 16,000 rpm for 1 minute. Afterwards, I added 500 µL Membrane binding solution (wrong!), centrifuged the samples for 1 minute at 16,000 rpm. I discarded the flow-through. After that, I added 700 µL membrane wash solution on the column and centrifugated the sample at 16,000 rpm for 1 minute. I discarded the flow-though and added 500 µL membrane wash solution and centrifuged the sample at 16,000 rpm for 1 minute, again. I discarded the flow-through carefully and centrifuged the samples with an open rotor cap. After centrifugation, I removed the collection tube carefully and put the column on a sterile 1.5 mL Eppi. I added 50 µL nuclease-free water (37 °C) on the column membrane and incubated it at room temperature for 5 minutes. Afterwards, I centrifuged the samples for the last time and stored them on - 20°C.

On the following day I measured the concentrations of the isolated fragments:

- Dur1,2_gene_p1 (gel electrophoresis 24.05.2018): 3.15 ng / µL
- Dur1,2_gene_p2 (gel electrophoresis 24.05.2018): 10.05 ng / µL
- TrzE_gene (gel electrophoresis 24.05.2018): 2.55 ng / µL
- Dur1,2_gene_p1 (gel electrophoresis 29.05.2018): 4.15 ng / µL
- Dur1,2_gene_p2 (gel electrophoresis 29.05.2018): 4.6 ng / µL
- TrzE_gene (gel electrophoresis 29.05.2018): 20.8 ng / µL

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

Author: Susanne Vollmer

created: 30.05.2018 17:38
updated: 30.05.2018 18:23

Entry 78/259: Testriction of the 2. try Golde Gaten of the plasmids p2iGEM0244-p2iGEM0252

In Project: Level_1

With tags: PCR programm, promoter, golden gate, overnight, test restriction

testristriction of the 2.try of the Golden Gaten of the Expression Plasmids p2iGEM0244-p2iGEM0252:

tested Plasmids, colony:

- p2iGEM0245, 1
- p2iGEM0246, 1
- p2iGEM0246, 2
- p2iGEM0247, 1
- p2iGEM0249, 1
- p2iGEM0249, 2
- p2iGEM0249, 3
- p2iGEM0249, 4
- p2iGEM0252, 1
- p2iGEM0252, 2

Mastermix (x11):

- 2,2µl NotI restriction Enzyme
- 11µl Cutsmart
- 91,3µl milli Q

in each tube 9,5µl mastermix and 0,5µl plasmid

cycler program:

37°C 8h

65°C 20min

4°C HOLD

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

Author: Susanne Vollmer

created: 31.05.2018 12:46
updated: 04.06.2018 20:24

Entry 79/259: gel of the testrestriction of the 2. Try of the Golden Gate of p2IGEM0244-52

In Project: Level_1

With tags: agarose gel, dueber, electrophoresis, golden gate, test restriction, cloning, ladder

gel of the testrestriction of the Plasmids of the selected colonies of the Golden Gate cloning of the ptxD Expression Plasmids (p2iGEM0244-p2iGEM0252) using:

200ml 1%agarose gel (2g agarose and 200ml 1xTAE)

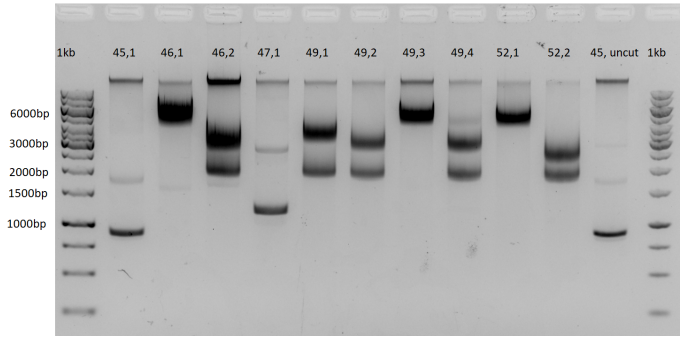
90V

gel Picture below:

Gel pockets:

gel pocket	1	2	3	4	5	6	7	8	
Content	2µl 1kb ladder	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	10µl from the Testrestriction 2µl 6x loading Dye	
Plasmid, colony	-	p2iGEM0245, 1	p2iGEM0246, 1	p2iGEM0246, 2	p2iGEM0247, 1	p2iGEM0249, 1	p2iGEM0249, 2	p2iGEM0249, 3	

Gel_2018-05-31_14hr_22min_90V_2h_NotI_Verdau_GoGate_Lvl1_bearbeitet.png



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

Author: Susanne Vollmer

created: 01.06.2018 09:33
updated: 01.06.2018 16:25

Entry 80/259: repetition of the testrestriction and gel of some plasmids of the 1.try of the golden gate for the ptxD expresstionplasmids

In Project: Level_1

With tags: cloning, dueber, golden gate, test restriction, cassettes, plasmid, promoter

Repetition of the testristriction of Plasmids from the 1. try golden Gate (p2iGEM0244-p2iGEM0252)

using Mastermix(x15):

- 3µl restriction Enzyme NotI (TF)
- 15µl Cutsmart
- 124,5µl milli Q

in each tube 9,5µl Mastermix and 0,5µl Plasmid, exept the one that shout not be digested

following Plasmids are testet:

- p2iGEM0245 colonies 1-2
- p2iGEM0246 coloniy 1
- p2iGEM0247 colonies 1-2
- p2iGEM0248 colonies 1-2
- p2iGEM0249 colonies 1-2
- p2iGEM0250 colonies 1-2
- p2iGEM0251 colonies 1-2
- p2iGEM0245 colony 1 uncut, so only 1µl Cutsmart and 8,5µl milli Q
- p2iGEM0097
- p2iGEM0250 colonies 3-4

cycler program:

- 37°C 4h
- 65°C 20min
- 4°C HOLD

200ml 1% agarose gel (200ml 1xTAE, 2g agarose)

90V

110min

1kb ladder

in each gelpocket 2µl loading dye and 10µl of the Digest, exept the first and last there 2µl of the genruler 1kb ladder

gelpockets:

- 1kb ladder
- p2iGEM0245 colonies 1-2
- p2iGEM0246 coloniy 1
- p2iGEM0247 colonies 1-2
- p2iGEM0248 colonies 1-2
- p2iGEM0249 colonies 1-2
- p2iGEM0250 colonies 1-2
- p2iGEM0251 colonies 1-2
- p2iGEM0245 colony 1 uncut, so only 1µl Cutsmart and 8,5µl milli Q
- p2iGEM0097
- p2iGEM0250 colonies 3-4
- 1kb ladder

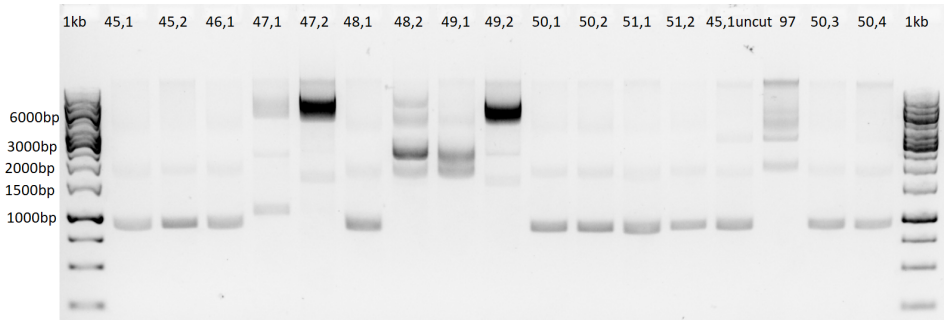
Gelpicture below:

expected band:

- nearly 2760bp
- nearly 1860bp
- nearly 1400bp

so the most of the Plasmids wasn't digested

Gel_2018-06-01_15hr_52min_90V_110min_1kb_ladder_2.testrestriktion_notI_of_1.try_goGate_lvl1,_ladder._bearbeitet.png



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

Author: Thomas Bick

created: 05.06.2018 12:59
updated: 06.06.2018 12:03

Entry 81/259: Sequencing of pJET clones (p2iGEM253, p2iGEM0254, p2iGEM0255)
In Project: Level_1
With tags: pJET, sequencing

Sequencing of p2iGEM0253 (pJET_triA), p2iGEM0254 (pJET_trzC) and p2iGEM0255 (pJET_atzD) by GATC Biotech.

Barcode	**Template**	**Oligo**
ID 63BC89	p2iGEM0253	pJET1.2_fw
ID 63BC88	p2iGEM0253	pJET1.2_rv
ID 63BC87	p2iGEM0254	pJET1.2_fw
ID 63BC86	p2iGEM0254	pJET1.2_rv
ID 63BC92	p2iGEM0255 (colony5)	pJET1.2_fw
ID 63BC91	p2iGEM0255 (colony5)	pJET1.2_rv
ID 63BC90	p2iGEM0255 (colony7)	pJET1.2_fw
ID 63BC95	p2iGEM0255 (colony7)	pJET1.2_rv

Every sequencing sample was prepared as followed:

500 ng Template

10 mM Oligo

ad 10 µl H20

(Each sample was prepared in a 1.5 ml reaction tube and all together handed in in a plastic bag.)

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

Author: Susanne Vollmer

created: 05.06.2018 17:27
updated: 05.06.2018 17:44

Entry 82/259: Yeast transformation of p2iGEM0245-51
In Project: Level_1
With tags: FROZEN EZ Yeast Transformation kit, overnight, Transformation

Transformation of the possible positiv Plasmids of the of cloning Expression Plasmids of ptxD (p2IGEM0244-p2iGEM0252) in Yeast BY4742:

using the FROZEN EZ Yeast Transformation kit and following protocol:

- incubate on until the cells are defrosted
- put 500ng Plasmid DNA on 50µl competent cells
- add 400µl EZ3 solution
- incubate 45min at 30°C
- screat 125µl on a plate
- incubate at 30°C overnight 2-4 days

used Plasmids:

- p2iGEM0245,1
- p2iGEM0246,2
- p2iGEM0248,2
- p2iGEM0249,2
- p2iGEM0250,2
- p2iGEM0251,2

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

Author: Thomas Bick
created: 06.06.2018 11:53
updated: 08.06.2018 12:40
Entry 83/259: triA insertion into pJET bluntend-cloning vektor
In Project: Level_1
With tags: amp, ampicillin, blunt-end cloning, cloning, gBlocks, pJET, T4 ligase

Pouring new LB-Amp plates.

1. Blunt-end ligation of *triA* in pJET blunt-end cloning Vector (Thermo Fisher):

2x reaction buffer : 10 µL

triA gBlock : 0.5 µL

pJET1.2/blunt Cloning Vector : 1 µL

Water, nuclease-free : 7.5 µL

T4 DNA Ligase: 1 µL

Total volume: 20 µL

Vortex briefly and centrifuge for 3-5 s.

2. Incubate the ligation mixture at room temperature (22°C) for 30 min.

3. Stored at -20°C until transformation

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

Author: Susanne Vollmer
created: 07.06.2018 09:11
updated: 08.06.2018 10:43
Entry 84/259: Transformation of the 2. try pJET cloninig for triA
In Project: Level_1
With tags: pJET, Transformation, Top10

Transformations of the 2.Try of the cloning of pJET Plasmids with the synthesis gens triA (Plasmisnumber:p2iGEM0253, Cryonumber: 2iGEM0271), using the following protocol:

- thaw competent cells (T10) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- take 40µl and add 80 ->10%culture
- centrifuge the cells for 30 sec at 7000rpm decant the supernatante and resuspend the cells in the rest(100-200µl)
- screat each (nearly 120µl) on a agarplate with the Fitting antibiotica (i used ampicillin)
- incubate at 37°C

Inoculation of 3 ml LB with 30 µl amp or 15 µl kan depending on the Plasmid, inoculation of the following Plasmids:

Shaking at 37°C over night.

CIDER Part	iGEM cryo	Selective marker
DVA_CD	2iGEM0178	Amp
DVK_AE	2iGEM0200	Kan
DVK_EF	2iGEM0196	Kan
DVK_FG	2iGEM0203	Kan
DVK_GH	2iGEM0204	Kan
DVA_AH	2iGEM0176	Amp
J23100_AB	2iGEM0113	Amp
J23100_EB	2iGEM0114	Amp
J23100_FB	2iGEM0115	Amp
J23100_GB	2iGEM0116	Amp
B0034m_BC	2iGEM0155	Amp
B0015_DE	2iGEM0168	Amp
B0015_DF	2iGEM0169	Amp
B0015_DG	2iGEM0170	Amp
B0015_DH	2iGEM0171	Amp

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

Author: Susanne Vollmer

created: 08.06.2018 14:04

updated: 08.06.2018 17:19

Entry 85/259: miniprep of the the needed plasmids from the CIDAR Toolbox

In Project: Level_1

No tags associated

Miniprep of CIDAR Toolbox Plasmids, using the follwing protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 30 Min at 37°C and 300prm. Centrifuge for 15 Sek. max
- because the values were extremly bad, but again on Collum incubate an RT and centrifuge two times and than measure again with the following concentration

CIDAR part	Plasmidnumber	Cryonumber	Concentration
DVA_CD	2iGEM0174	2iGEM0178	85,900 ng/µl
DVK_AE	2iGEM0196	2iGEM0200	97,200 ng/µl
pJ02B2Gm_AE	2iGEM0192	2iGEM0196	39,350 ng/µl
DVK_FG	2iGEM0199	2iGEM0203	38,800 ng/µl
DVK_GH	2iGEM0200	2iGEM0204	179,20 ng/µl curve bad
DVA_AH	2iGEM0172	2iGEM0176	31,450 ng/µl
J23100_AB	2iGEM0109	2iGEM0113	157,70 ng/µl curve bad
J23100_EB	2iGEM0110	2iGEM0114	65,250 ng/µl
J23100_FB	2iGEM0111	2iGEM0115	94,700 ng/µl curve not so good
J23100_GB	2iGEM0112	2iGEM0116	40,950 ng/µl
B0015_DE	2iGEM0164	2iGEM0168	223,80 ng/µl curve bad
B0015_DF	2iGEM0165	2iGEM0169	66,700 ng/µl
B0015_DG	2iGEM0166	2iGEM0170	52,800 ng/µl
B0015-DH	2iGEM0167	2iGEM0171	80,75 ng/µl

inoculation of each 3ml LB with 30µl ampicilin with 4 colonies of the 2. try pJET triA cloning

incubation at 37°C over night

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

Author: Susanne Vollmer
Entry 86/259: No entry title yet
In Project: Level_1
No tags associated

created: 09.06.2018 16:41
updated: 09.06.2018 18:28

Miniprep of 4 colonies of the 2.try pJET cloning of triA (p2iGEM0253) using following protocol:

bevor starting, put 700µl culture in a eppi and store at 4°C to keep some living organisms

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 200µ colum wash
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 1h at RT. Centrifuge for 15 Sek. max

cryonumber	colony	plasmidnumber	concentration
2iGEM0271	1	p2iGEM0253	195,45 ng/µl
2iGEM0271	2	p2iGEM0253	271,90 ng/µl
2iGEM0271	3	p2iGEM0253	376,25 ng/µl
2iGEM0271	4	p2iGEM0253	145,40 ng/µl double volume

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

Author: Miriam Dreesbach
created: 10.06.2018 21:40
updated: 11.06.2018 17:08
Entry 87/259: Overlap PCR TrzE_gene Dur1,2_gene_p1 & gel electrophoresis
In Project: Level_1
With tags: touchdown pcr, agarose gel, synthesises, gel electrophoresis, overlap pcr, gBlocks, three-step-pcr

To fuse TrzE_gene and Dur1,2_gene_p1 by overlap PCR, I calculated the following volumes:

Ratio vector:insert 1:3:

3.9 ng TrzE_gene (20.8 ng / µL) --> 0.2 µL

4.15 ng Dur1,2_gene_p1 (4.15 ng / µL) --> 1 µL

to fill a volume of 14 µL: 2.2 µL TrzE_gene & 11 µL Dur1,2_gene_p1

Afterwards, I pipetted the following ingredients:

- 5 µL Q5 reaction buffer
- 5 µL High GC buffer
- 0.5 µL dNTPs
- 2.2 µL TrzE_gene (PCR 29.05.2018)
- 11 µL Dur1,2_gene (PCR 29.05.2018)
- 0.5 µL Q5 polymerase
- 0.5 µL nuclease-free water (milliQ)

As the annealing temperature of the homologous regions is >65 °C, I ran the PCR with the following protocol on **our nexus gradient (previous right) Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: 66 °C: 30 sec

4. Extension: 72 °C: 90 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 15 times.

To prepare a small 6*6 cm 1 % agarose gel, I weigthed 0.4 g Agarose and solved it in 40 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 2 µL GelRed and loaded the gel. After it was hardened, I loaded the following onstructs on the gel:

3 µL yesterdays' overlap PCR product of TrzE & Dur1,2_gene_p1 + 1 µL 6x LD

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

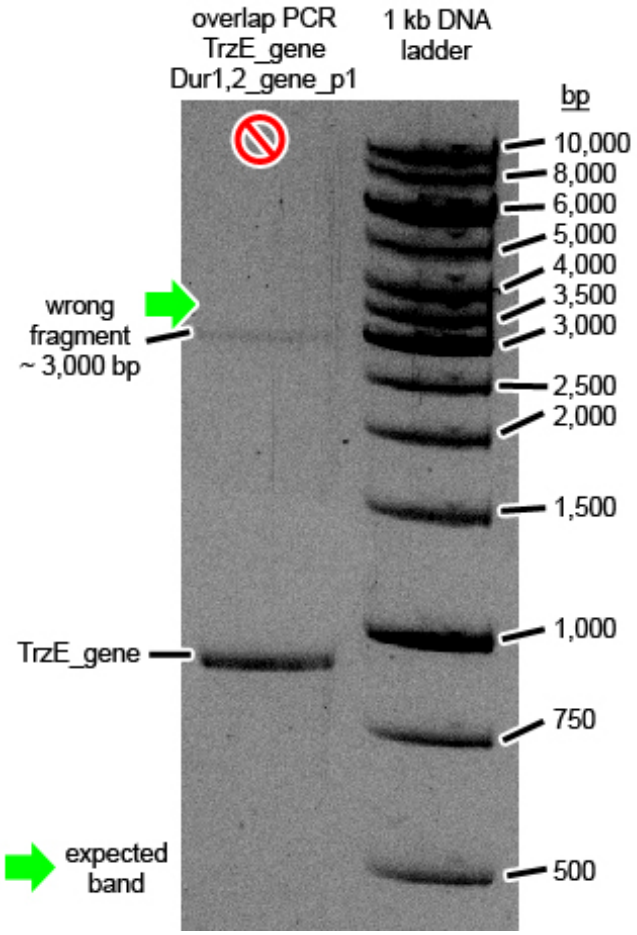
I ran the gel electrophoresis on 120 V for 30 and 40 min.

Afterwards, I visualized the gel.

As the amplified TrzE_gene fragment measures 882 bp and Dur1,2_gene_p1 2818 bp with 51 bp overlap, the right fragment should show at 3,649 bp.

Therefore, the gel didn't show the right bands.

Gel_2018-06-10_21hr_22min_Overlap_PCR_TrzE_Dur1-2_1_kb_ladder_MD_120_V_40_Min_bearbeitet_Kopie.jpg



To fuse TrzE_gene and Dur1,2_gene_p1 by overlap PCR, I calculated the following volumes:

Ratio vector:insert 1:2:

1.972 ng TrzE_gene (2.55 ng / µL) --> 0.8 µL

3.15 ng Dur1,2_gene_p1 (3.15 ng / µL) --> 1 µL

to fill a volume of 14 µL: 5.6 µL TrzE_gene & 7 µL Dur1,2_gene_p1

Afterwards, I pipetted the following ingredients:

- 5 µL Q5 reaction buffer
- 5 µL High GC buffer
- 0.5 µL dNTPs
- 5.6 µL TrzE_gene (PCR 24.05.2018)
- 7 µL Dur1,2_gene (PCR 24.05.2018)
- 0.5 µL Q5 polymerase
- 1.4 µL nuclease-free water (milliQ)

As the annealing temperature of the homologous regions is >65 °C, I ran the PCR with the following protocol on **our nexus gradient (previous right) Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: 66 °C: 30 sec

4. Extension: 72 °C: 90 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 15 times.

For gel electrophoresis, Thomas prepared a 10*6 cm 1 % agarose gel. After it was hardened, I loaded the following onstructs on the gel:

2 µL 1 kb DNA ladder (Thermo Fisher)

3 µL yesterdays' overlap PCR product of TrzE & Dur1,2_gene_p1 + 1 µL 6x LD

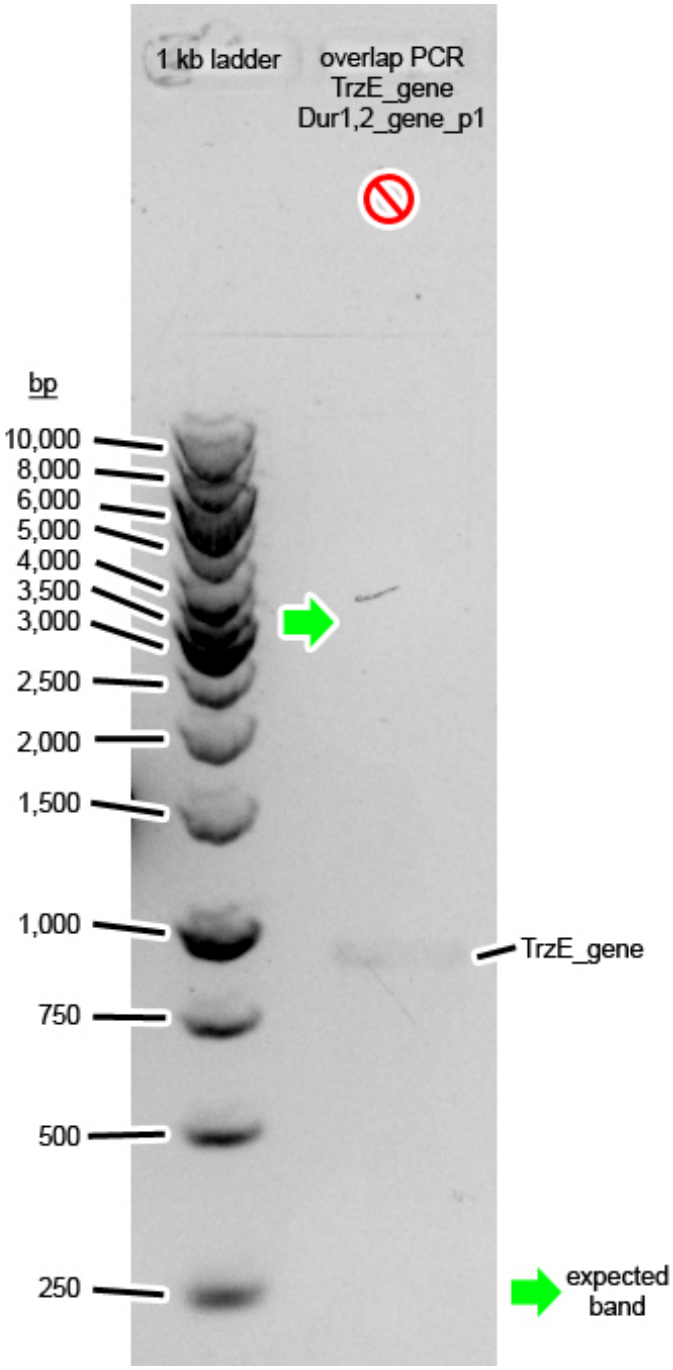
I ran the gel electrophoresis on 100 V for 56 min.

Afterwards, I visualized the gel.

As the amplified TrzE_gene fragment measures 882 bp and Dur1,2_gene_p1 2818 bp with 51 bp overlap, the right fragment should show at 3,649 bp.

Therefore, the gel didn't show the right bands.

Gel_2018-06-11_13hr_34min_90_v_56_min_1_kb_Ladder._Miriam_overlap_PCR_TrzE_gene_Dur1.2-p1_bearbeitet_Kopie.jpg



To fuse TrzE_gene and Dur1,2_gene_p1 by overlap PCR, I calculated the following volumes:

Ratio vector:insert 1:3:

3.9 ng TrzE_gene (20.8 ng / µL) --> 0.2 µL

4.15 ng Dur1,2_gene_p1 (4.15 ng / µL) --> 1 µL

to fill a volume of 14 µL: 2.2 µL TrzE_gene & 11 µL Dur1,2_gene_p1

Afterwards, I pipetted the following ingredients:

- 5 µL Q5 reaction buffer
- 5 µL High GC buffer
- 0.5 µL dNTPs
- 2.2 µL TrzE_gene (PCR 29.05.2018)
- 11 µL Dur1,2_gene (PCR 29.05.2018)
- 0.5 µL Q5 polymerase
- 0.5 µL nuclease-free water (milliQ)

As the annealing temperature of the homologous regions is >65 °C, I ran the PCR with the following protocol on **our nexus gradient (previous right) Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: X °C: 30 sec

4. Extension: 72 °C: 90 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 15 times.

The annealing temperature started at 72 °C and for each cycle the temperature was lowered by - 0.5 °C until 67.5 °C.

To prepare a small 6*6 cm 1 % agarose gel, I weigthed 0.4 g Agarose and solved it in 40 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 2 µL GelRed and loaded the gel. After it was hardened, Sarah loaded the following onstructs on the gel:

3 µL todays' overlap PCR product of TrzE & Dur1,2_gene_p1 + 1 µL 6x LD

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

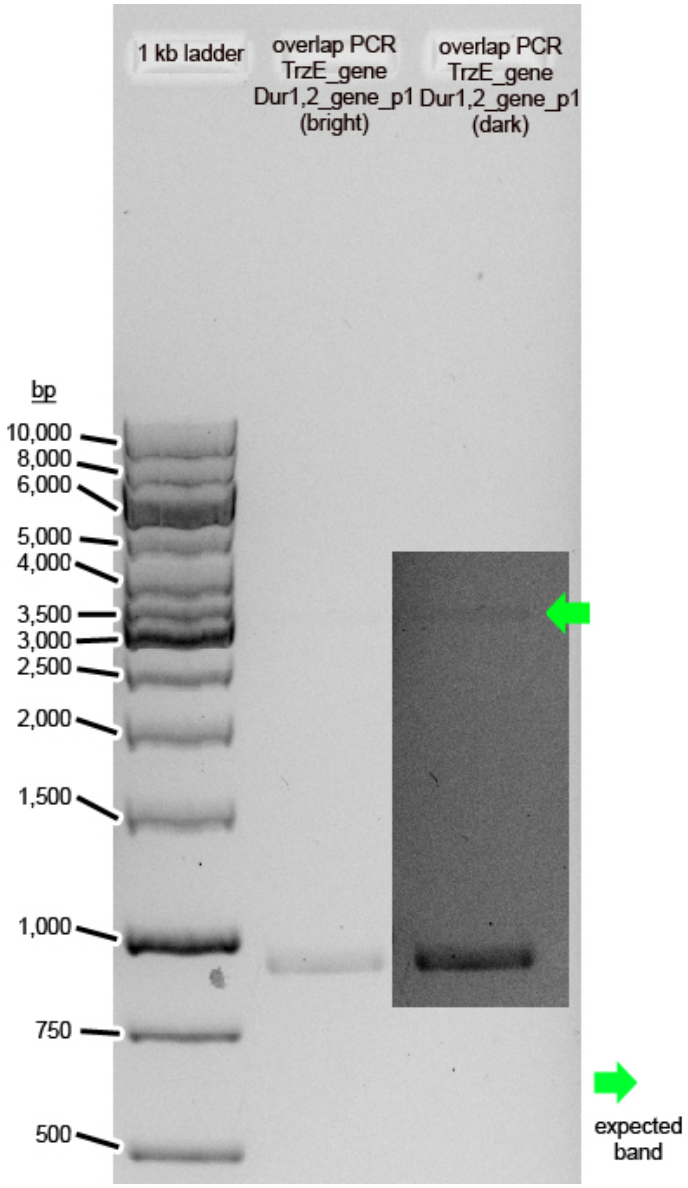
Sarah ran the gel electrophoresis on 80 V for 1 h.

Afterwards, Sarah visualized the gel.

As the amplified TrzE_gene fragment measures 882 bp and Dur1,2_gene_p1 2818 bp with 51 bp overlap, the right fragment should show at 3,649 bp.

The gel showed a band above 3,500 bp. Therefore, the PCR has to be repeated on a 50 µL approach and the fragment has to be isolated.

Gel_2018-06-11_16hr_26min_80V_60_min_ddh_mutageniesiert_&_touchdown_von_Miriam_bearbeitet_Kopie.jpg



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

Author: Thomas Bick
created: 11.06.2018 14:07
updated: 11.06.2018 14:22
Entry 88/259: Verification of p2iGEM0253 (pJET_triA)
In Project: Level_1
With tags: gel electrophoresis, sequencing, gBlocks, pJET

TestPCR of the pJET cloning Plasmids p2iGEM0253 (2iGEM0271) using the following protocol:

test of 4 different colonies of Plasmid p2iGEM0253 :

- 1µl primer fwd
- 1µl primer rwd --> both from the pJET cloning kit from Themo Scientific
- 0,5µl dNTP
- 0,5µl Q5 polymerase (ad last on ice!)
- 5µl Q5 reaction buffer
- 5µl high GC enhancer
- 11µl milli Q water
- 1µl of the tested Plasmids

PCR program:

95°C 2min

then starting the cycle with:

95°C 15 sec

61°C 30 sec

72°C 40 sec

repeating this 30x

72°C 2min

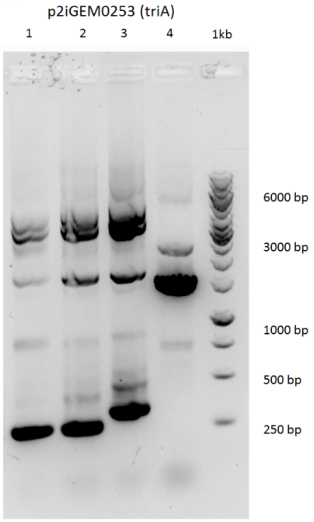
5°C HOLD

1% agarosegel: 50ml 1x TAE buffer and 0.5 g agarose

90 V 60 min

in 1x TAE buffer.

Gel_2018-06-11_13hr_34min_90_v_56_min_TB_pJET_TriA_2._Versuch_1_2_3_4._1_kb_Ladder._Miriam_overlap_PCR.tif



Sequencing of p2iGEM0253 (pJET_triA) by GATC Biotech.

Barcode	**Template**	**Oligo**
ID 63BC93	p2iGEM0253 (colony 4)	pJET1.2_fw
ID 63BC94	p2iGEM0253 (colony 4)	pJET1.2_rv
ID 63BC96	p2iGEM0253 (colony 2)	pJET1.2_fw
ID 63BC97	p2iGEM0253 (colony 2)	pJET1.2_rv

Every sequencing sample was prepared as followed:

500 ng Template

10 mM Oligo

ad 10 µl H20

(Each sample was prepared in a 1.5 ml reaction tube and all together handed in in a plastic bag.)

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

Author: Miriam Dreesbach
created: 12.06.2018 23:34
updated: 12.06.2018 23:38
Entry 89/259: Overlap PCR TrzE_gene Dur1,2_gene_p1 & gel electrophoresis
In Project: Level_1
With tags: touchdown pcr, synthesises, overlap pcr, gBlocks

To fuse TrzE_gene and Dur1,2_gene_p1 by overlap PCR for further isolation, I calculated the following volumes:

Ratio vector:insert 1:3:

3.9 ng TrzE_gene (20.8 ng / µL) --> 0.2 µL

4.15 ng Dur1,2_gene_p1 (4.15 ng / µL) --> 1 µL

to fill a volume of 28 µL: 4.6 µL TrzE_gene & 23 µL Dur1,2_gene_p1

Afterwards, I pipetted the following ingredients:

- 10 µL Q5 reaction buffer
- 10 µL High GC buffer
- 1 µL dNTPs
- 4.6 µL TrzE_gene (PCR 29.05.2018)
- 23 µL Dur1,2_gene (PCR 29.05.2018)
- 1 µL Q5 polymerase
- 0.5 µL nuclease-free water (milliQ)

As the annealing temperature of the homologous regions is >65 °C, I ran the PCR with the following protocol on **our nexus gradient (previous right) Eppendorf cycler**:

1. Initial denaturation: 98 °C: 2 min

2. Denaturation: 98 °C: 30 sec

3. Annealing: X °C: 30 sec

4. Extension: 72 °C: 90 sec

5. Final Extension: 72 °C: 10 min

6. Hold: 4 °C

I repeated step 2 to 4 for 15 times.

The annealing temperature started at 72 °C and for each cycle the temperature was lowered by - 0.5 °C until 67.5 °C.

Afterwards, the PCR product was stored at - 20 °C.

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

Author: Miriam Dreesbach
Entry 90/259: Gel electrophoresis overlap PCR NSI & ori
In Project: Level_1
With tags: gel electrophoresis, agarose gel

created: 12.06.2018 23:39
updated: 12.06.2018 23:58

To identify the difference between the further overlap PCRs of NSIa and ori I prepared a big 1 % agarose gel, I weigthed 1.6 g Agarose an solved it in 160 mL 1x TAE. Afterwards, I heated it up in the microwave until the agarose was completely dissolved. After it cooled down until it was lukewarm, I added 8 µL GelRed and loaded the gel. After it was hardened, Sarah loaded the following onstructs on the gel:

3 µL overlap PCR product of NSIa & ori + 2 µL 6x LD (20 µL approach)

3 µL overlap PCR product of NSIa & ori + 2 µL 6x LD (50 µL approach)

1 µL 1 kb DNA ladder (Thermo Fisher) + 1 µL nuclease-free milliQ water

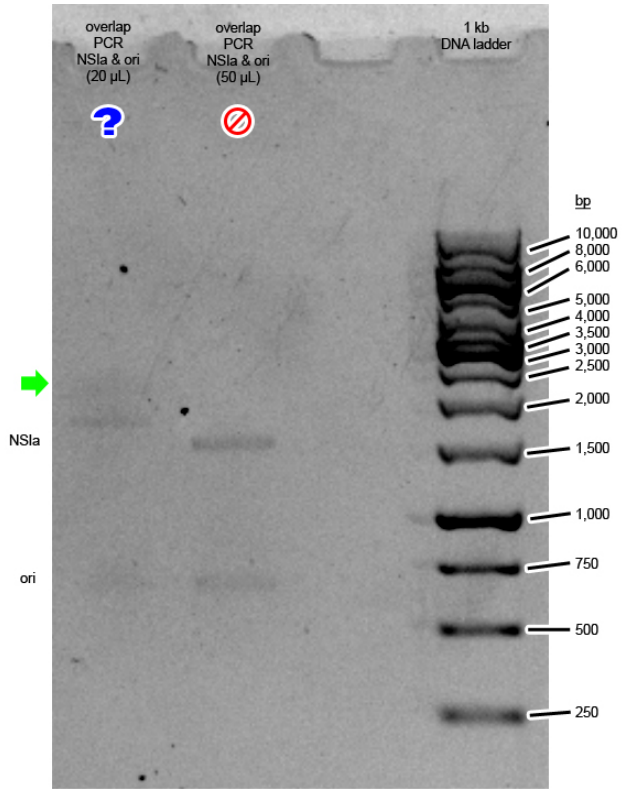
I ran the gel electrophoresis on 100 V for 45 min.

Afterwards, I visualized the gel.

As the amplified NSIa fragment measures 1,535 bp and ori 630 bp with 37 bp overlap, the right fragment should show at 2,128 bp.

The left overlap PCR product showed a band ~ 1,800 bp and a shadow near the wanted size, the second ~ 2,000 bp.

Gel_2018-06-12_18hr_00_min_100v_60_min_1kb_ladder._ylenia_und_käthe_links._rechts_unten_NSIa_ori_overlap_PCR_25yL_NSIa_ori_overlap_PCR_50_yL_1_kb_bearbeitet_Kopie.jpg



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

Author: Susanne Vollmer
created: 14.06.2018 13:18
updated: 14.06.2018 21:40
Entry 91/259: PCR atzD and trzC and sequencing of ptxD plasmids
In Project: Level_1
With tags: sequencing, Q5 PCR

PCR of the atzD syntesis in pJET (p2iGEM0254 and p2iGEM0255) with the primer for the CIDAR overhangs using follwing protocol:

Mastermix(3x):

- 5µl high GC enhancer
- 5µl Q5 polymerase
- 1,5µl dNTP
- 0,25 Q5 polymerase
- 10,75µl milli Q

in each tube 22,5µl Mastermix and 1µl each primer and 0,5µl template

atzD (p2iGEM0255): Primer O_iGEM18_0078 and O_iGEM18_0079

trzC (p2iGEM0254): Primer O_iGEM18_0080 and O_iGEM18_0081

PCR program:

- 98°C 30 sec
  - 98°C 10 sec
  - 60°C 15 sec
  - 72°C 35 sec--> repeat this cycle 30 times
- 72°C 2 min
- 4°C HOLD

sequencing of the Plasmids which were used for yeast Transformation:

add in a 1,5ml tube: the template (400-500ng) ad 2,5µl of the primer and milli Q up to 10µl, Label the tube only with the Barcode, put all in a bag and bring to the samplebox

seqID	template	which try of Golden Gate	Content template	primer
63BC98	p2iGEM0246,2	2.	0,7µl	O_iGEM18_0055
63BD01	p2iGEM0246,2	2.	0,7µl	O_iGEM18_0056
63BD00	p2iGEM0248,2	1.	2,6µl	O_iGEM18_0055
63BC99	p2iGEM0248,2	1.	2,6µl	O_iGEM18_0056
63BD34	p2iGEM0249,2	2.	1,6µl	O_iGEM18_0055
63BD33	p2iGEM0249,2	2.	1,6µl	O_iGEM18_0056
63BD32	p2iGEM0250,2	1.	5,6µl	O_iGEM18_0055
63BD37	p2iGEM0250,2	1.	5,6µl	O_iGEM18_0056

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

Author: Thomas Bick
created: 15.06.2018 15:18
updated: 15.06.2018 15:26

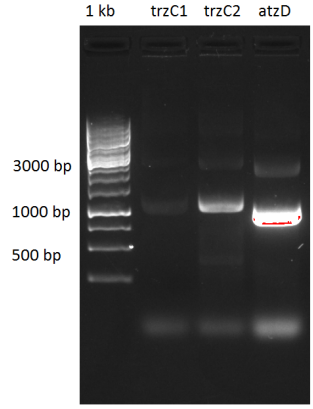
Entry 92/259: No entry title yet
In Project: Level_1
With tags: pJET, CIDAR cloning, pcr

1% agarose gel for PCR of atzD and trzC from 14.06.18 lvl1

5 µl PCR reaction + 2 µl loading dye

90 V 40 min, small gelchamber 50 ml

Gel_2018-06-15_atzD,_trzC_overhang_PCR.tif



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

Author: Thomas Bick

created: 15.06.2018 16:44
updated: 15.06.2018 17:00

Entry 93/259: lvl0 CIDER cloning atzD&trzC into DVA_CD
In Project: Level_1
With tags: CIDAR cloning, cloning, T4 ligase, digest, DVA, Lvl 0

GoGate lvl0 CIDER cloning: atzD & trzC into p2iGEM0174 (DVA_CD)

- 11µl Milli Q
- 1.5 µl T4 ligation buffer
- 0,5 µl T4 Ligase
- 0,5 µl BbsI
- 0,5 µl pJET_atzD / pJET_trzC
- 10 ng p2iGEM0174 (DVA_CD)

Cycler Program:

1. 20 min 37°C
2. 1.5 min 37°C |
3. 3 min 16°C | 2. & 3. 30 x
4. 60 min 16°C
5. 5 min 50°C
6. 10 min 80°C
7. HOLD 4°C

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

Author: Thomas Bick
Entry 94/259: No entry title yet
In Project: Level_1
No tags associated

created: 18.06.2018 13:05
updated: 18.06.2018 13:10

Transformation of DVA_CD_trzC (p2iGEM0294) and DVA_CD_atzD (p2iGEM0295) into competend E. coli DH5a:

- thaw competent cells (T10) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- take 40µl and add 80 ->10% culture
- plate on a agarplate with the Fitting antibiotica (normally also 60 µl XGAL for blue/white screening, which i did not)
- incubate at 37°C over night

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

Author: Thomas Bick
created: 19.06.2018 14:25
updated: 19.06.2018 14:34
Entry 95/259: No entry title yet
In Project: Level_1
With tags: miniprep, CIDAR cloning

Inoculation of 2iGEM0113, 2iGEM0115, 2iGEM0155, 2iGEM0168, 2iGEM0202, 2iGEM0204

in 3 ml LB + AB (Kan for 2iGEM0204, Amp for rest).

27°C over night

Miniprep of CIDAR Toolbox Plasmids, using the follwing protocol:

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 (~ 800 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 37°C warm MilliQ water
- incubate for 1 h at roomtemperature

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

Author: Susanne Vollmer
Entry 96/259: Sequencing of p2iGEM0208
In Project: Level_1
With tags: sequencing, dueber

created: 19.06.2018 16:18
updated: 19.06.2018 16:54

sequencing of the p2iGEM0208 using the GATC LIGHTrun tube NXP:

-add in a 1,5ml tube 7,5µl of the template (nearly 500ng) and 2,5µl primer, stick on the Barcode and throw in the letterbox

Barcode	Template	Primer
63BD74	p2iGEM0208,1	O_iGEM18_0082_pYTK001few
63BD75	p2iGEM0208,1	O_iGEM18_0083_pYTK001few

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

Author: Thomas Bick
created: 21.06.2018 03:53
updated: 21.06.2018 04:32
Entry 97/259: No entry title yet
In Project: Level_1
With tags: digest, gel electrophoresis, pcr, transformation

Pre-digestion of p2iGEM0001 with BsmBI:

10 µl MilliQ

2 µl cutsmart buffer

0.2 µl BsmBI

1 µl p2iGEM0001

incubate at 37°C over night

transformation of p2iGEM0294 & p2iGEM0295 in *E. coli* DH5a:

- thaw competent cells (DH5a) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica + XGAL
- incubate at 37°C over night

PCR of guaD from *E. coli* gDNA:

- 5 µl high GC enhancer
- 5 µl Q5 buffer
- 0.5 µl dNTP
- 0,2 µl Q5 polymerase
- 12 µl MillQ
- 1 µl O_iGEM18_0064
- 1 µl O_iGEM18_0065
- 0.25 µl DH5a gDNA

PCR program:

- 98°C 30 sec
  - 92°C 10 sec
  - 65°C 20 sec
  - 72°C 40 sec--> repeat this cycle 35 times
- 72°C 2 min
- 4°C HOLD

Test gel of guaD PCR:

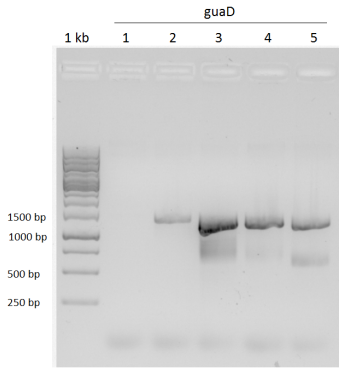
1% Agarose gel

90 V

30 min runtime

expected fragment with 1370 bp

Gel_2018-06-21_04hr_22min_guaD_CDoverhangs_PCR.tif



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

Author: Susanne Vollmer
created: 21.06.2018 09:55
updated: 21.06.2018 13:53
Entry 98/259: Golden Gate of guaD
In Project: Level_1
With tags: cloning, golden gate, Cidar, E.coli

inactivation of of the Restriktion Enzym (BsmBI) 20min on 65°C at 10 am.

Golden Gate of ptxD in the entryvector

Mastemix(2x):

- 2,5µl T4 Ligase b puffer
- 1,3µl BsmbI
- 2µl T4 LIgase
- 2µl p2iGEM001 predigested
- 8,2µl milli Q

- 2µl ptxD PCR product + 8,0µl Mastermix

rune one sample, discard the rest of the Mastermix

50°C 2min

16°C 5min

65mal beides

60°C 10min

80°C 10min

4°C HOLD

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

Author: Susanne Vollmer

created: 22.06.2018 08:04
updated: 06.07.2018 09:11

Entry 99/259: golden Gate of guaD and transformation of golden gate of ptxD and of pJET plasmids

In Project: Level_1

With tags: Cidar, golden gate, PCR programm, Transformation, DHSα, Top10

Golden Gate of guaD in the CIDAR entryvector (p2iGEM0174):

- 11µl Milli Q
- 1.5 µl T4 ligation buffer
- 0,5 µl T4 Ligase
- 0,5 µl BbsI
- 0,5 µl guaD
- 10 ng p2iGEM0174 (DVA_CD) -> 1:10 verdünnen ->1 µl

Cycler Program:

1. 20 min 37°C
2. 1.5 min 37°C |
3. 3 min 16°C | 2. & 3. 30 x
4. 60 min 16°C
5. 5 min 50°C
6. 10 min 80°C
7. HOLD 4°C

Transformation of the golden Gate of ptxD (p2iGEM0208, DH5a) and retrafo of the verified pJET Plasmids of atzD (colony 7, TOP10), triA (colony 4, TOP10):

- 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica->ampicilin
- incubate at 37°C over night

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

Author: Susanne Vollmer

created: 22.06.2018 14:13
updated: 22.06.2018 15:22

Entry 100/259: colony PCR of the Transformants of the atzD and trzC goldengates
In Project: Level_1
With tags: Colony PCR, gel, gelelectrophoresis

colony PCR of 5 colonies of each 2iGEM0235 (p2iGEM0294, trzC), 2iGEM0236 (p2iGEM0295, atzD), using follwing protocoll:

each:

- 2,5 µl 10xPuffer
- 0,5 µl dNTP
- 1 µl Primer fw (O_iGEM18_0068)
- 1 µl Primer rew (O_iGEM18_0069)
- 0,2 µl Taq
- 19,8 µl milli Q
- 1colony

used a 11x Mastermix

cycler program:

- 95°C 10 min
- 95°C 30 sec
- 49°C 20 sec
- 68°C 90sec
- 68°C 5min
- 4°C HOLD

1% agarose gel (300ml 1xTAE and 3g agarose)

in 1xTAE 100V 1kb ladder 90min

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

Author: Miriam Dreesbach
created: 25.06.2018 09:01
updated: 25.06.2018 09:09

Entry 101/259: Overnight culture inoculation, plasmid isolation, cryogenic cultures
In Project: Level_1
With tags: isolation, overnight cultures, inoculation, incubation, cryogenic culture, plasmid

To prepare overnight cultures for further cryogenic cultures and plasmid isolation, I inoculated bacteria colonies from agar plates in 3 mL LB with appropriate antibiotics.

2iGEM0325: 15 µL Kan

2iGEM0338: 15 µL Spec

2iGEM0339: 15 µL Kan

Afterwards, I incubated them at 37 °C overnight.

To prepare cryogenic cultures of 2iGEM0338 and 2iGEM0339, I mixed 800 µL autoclaved glycerol with 200 µL bacterial culture.

Afterwards, I stored them at - 80 °C.

To isolate the plasmids pSHDY mVenus and mCerulean (p2iGEM0306, 2iGEM0307) from overnight cultures,

I centrifuged the cultures for 10 minutes at 4000 rpm and dissolved the pellet in 100 µL lysis buffer into a sterile Eppi and inverted the Eppi for 6-8 times.

After that, I added 350 µL neutralization buffer (4 °C storage) and inverted the Eppi for another 6-8 times.

I centrifuged the samples for 3 minutes at max rpm and pipetted 900 µL of the supernatant on a column on a collection tube.

I centrifuged the samples for 30 seconds at max rpm and discarded the flow-through.

After that, I addded 400 µL column wash, centrifuged the samples for 30 seconds at max rpm and discarded the flow-through.

I put the column on a fresh, sterile Eppi and added 30 µL 37 °C nuclease-free water (milliQ) to the column.

I incubated the samples for 1 minute at room temperature and centrifuged the samples for 30 seconds at max rpm.

Afterwards, I measured the concentration of the gained isolated plasmids:

p2iGEM0306: 69.65 ng / µL

p2iGEM0307: 130.15 ng / µL

After that, I stored the private stock plasmids at -20 °C in the Level 1 box.

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

Author: Thomas Bick
Entry 102/259: Kryos and ColonyPCR
In Project: Level_1
With tags: CIDAR cloning, DVA, pcr, Kryo, Colony PCR

created: 25.06.2018 16:14
updated: 25.06.2018 17:33

Kryos of 2iGEM0271 and 2iGEM0273:

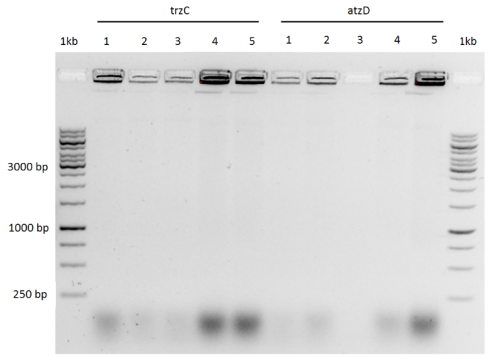
300 µl overnight culture

700 µl 99.5% Gycerol

stored at -80°C lvl1 Box

Agarose Gel of Colony PCR of p2iGEM0294 (DVA_trzC) and p2iGEM0295 (DVA_atzD)

Gel_2018-06-22_16hr_09min_ColonyPCR_trzC,_atzD_entry.tif



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

Author: Thomas Bick
created: 26.06.2018 16:37
updated: 26.06.2018 17:17

Entry 103/259: ColonyPCR of DVA_trzC, DVA_atzD and ptxD_Entry + Mutagenesis of triA

In Project: Level_1

With tags: Colony PCR, gel electrophoresis, Lvl 0, CIDAR cloning, Mutagenesis

colony PCR of 5 colonies of each 2iGEM0235 (p2iGEM0294, trzC), 2iGEM0236 (p2iGEM0295, atzD), and one colony 2iGEM0211 (p2iGEM0208, ptcS) using follwing protocoll:

each:

- 2,5 µl 10xPuffer
- 0,5 µl dNTP
- 1 µl Primer fw (O_iGEM18_0068)
- 1 µl Primer rew (O_iGEM18_0069)
- 0,2 µl Taq
- 19,8 µl milli Q
- 1colony --> single colony picked from agarplate and plated on a new fresh colony afterwards into the tube

used a 11x Mastermix

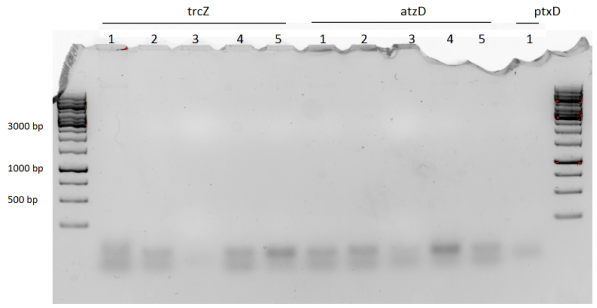
cycler program:

- 95°C 10 min
- 95°C 30 sec
- 49°C 20 sec
- 68°C 90 sec
- 68°C 5 min
- 4°C HOLD

1% agarose gel (150 ml 1xTAE and 1.5 g agarose)

in 1xTAE 100 V 1kb-ladder 90 min

Gel_2018-06-22_16hr_09min_ColonyPCR_trzC,_atzD_ptxD_entry.tif



mutagesis of p2iGEM0253 with O_iGEM18_0074 to mutagenise unwanted BbsI restriction side:

6x Mastermix

- 12µl plasmid (10ng/sample)
- 12 µl O_iGEM18_0074
- 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 20 sec
  - gradient 71°C - 65°C 30 sec
  - 72 °C 3.5 min
  - repeat this 30 times
- 72°C 5 min
- hold 4°C

DnpI digestion of mutagesis of p2iGEM0253 with O_iGEM18_0074

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

stored at -20°C

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

Author: Thomas Bick
Entry 104/259: No entry title yet
In Project: Level_1
No tags associated

created: 27.06.2018 11:25
updated: 27.06.2018 15:56

transformation of p2iGEM0308 (pJET_triA_BbsI_sdm) in *E. coli Top10*:

- thaw competent cells (*T10*) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica (Amp)
- incubate at 37°C over night

Miniprep of p2iGEM0294 and p2iGEM0295 from transformed *E. coli Top10*:

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 (~ 800 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 1 h at room temperature

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

Author: Susanne Vollmer
Entry 105/259: PCR of ptxD of the gDNA of p.putida
In Project: Level_1
With tags: gDNA, Q5 PCR, dueber

created: 27.06.2018 18:50
updated: 27.06.2018 19:01

Repetition of the PCR to amplify ptxD of the gDNA of *p.putida* with the followingprotocol:

Primer used:

O_iGEM0007_ptxD_lvl0_p

O_iGEM0008_ptxD_lvl0_p

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

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 (add this at last, on ice!)

samples: using a 5x mastermix, add 25µl in each tube, and additionally in two tubes each 0,5µl template more.

PCR program:

95°C 2min

then starting the cycle with:

95°C 15 sec

65°C 15 sec (depending on the anealing temperature of the Primer)

72°C 30 sec

repeating this 30

72°C 2min

4°C HOLD

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

Author: Susanne Vollmer

created: 29.06.2018 08:48
updated: 29.06.2018 13:31

Entry 106/259: Transformation of guaD (p2iGEM309) in E.coli
In Project: Level_1
With tags: Transformation, dueber, E.coli, Top10, X-gal

transformation of p2iGEM0309 (DVA_CD_guaD) in *E. coli Top10*:

- thraw competent cells (*T10*) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- plate on a agarplate with the fitting antibiotica (Amp)
- 30sec 5000rpm discard 180µl supernatant
- add 70µl IPTG and X-GAL ->mistake, Right: spreat it on the plate 30min bevor spreading out the cells
- incubate at 37°C over night

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

Author: Susanne Vollmer
created: 30.06.2018 12:13
updated: 02.07.2018 16:09
Entry 107/259: PCR of atzD, trzC (from pJET) and ptxD from gDNA
In Project: Level_1
With tags: gDNA, pJET, pcr, P. putida

repetition of the PCR of atzD from pJET (p2iGEM0255) and trzC from pJET (p2IGEM0254) and ptxD from gDNA of *p.putida* using the follwing protocols:

atzD:

each sample:

- 5 µl high GC enhancer
- 5 µl Q5 polymerase Buffer
- 0,2 µl Template (p2iGEM0255)
- 0,5 µl dNTPs
- 1,5 µl Primer fwd (O_iGEM18_0078)
- 1,5 µl Primer rv (O_iGEM18_0079)
- 0,25 µl Q5 polymerase
- 1,5 µl DMSO
- 9,6 µl milli Q
- -> 25,05 µl in total

PCR Programm:

95°C 5 min

- 95°C 20 sec
- 60°C 20 sec
- 72°C 40 sec
- ->repeat cycle 30x

72°C 2 min

4°C HOLD

trzC:

each sample, like atzD, except template and primers:

template: p2iGEM0254

Primer: O_iGEM18_0080 ; O_iGEM018_0081

PCR Programm:

95°C 5 min

- 95°C 20 sec
- 63°C 20 sec
- 72°C 45 sec
- ->repeat cycle 30x

72°C 2 min

4°C HOLD

ptxD:

each sample, like atzD, exept template and primers:

template: gDNA *P.putida*

Primer: O_iGEM18_0007 ; O_iGEM18_0008

using a gradient PCR:

PCR Programm:

95°C 5min

- 95°C 20 sec
- GRADIENT (60°C, 62°C, 64°C, 66°C, 68°C, 70°C) 20 sec
- 72°C 40 sec
- ->repeat cycle 30x

72°C 2 min

4°C HOLD

using each time a 6x mastermix for each 6 samples

gelelectrophoresis of the PCR

unsing 1% Agarose gel and 1xTAE buffer

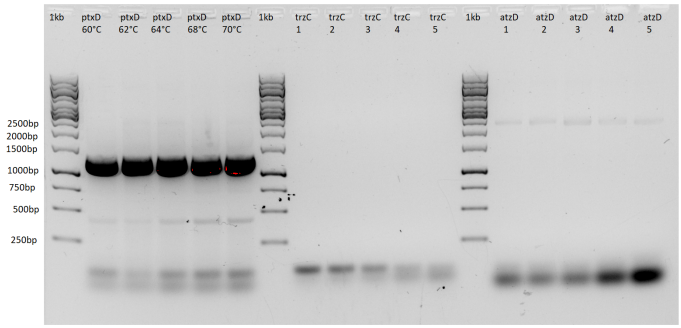
60min 90V

Gelpicture below:

1kb ladder	ptxD 60°C	ptxD 62°C	ptxD 64°C	ptxD 68°C	ptxD 70°C	1kb ladder	trzC 1	trzC 2	trzC 3	trzC 4	trzC 5	1kb ladder	atzD 1	atz
	1014bp	1014bp	1014bp	1014bp	1014bp		1263bp	1263bp	1263bp	1263bp	1263bp		1116bp	11

Maybe swaped the ptxD and trzC samples, see gel below

Gel_2018-06-30_15hr_28min,_60min_90v_1kbladder,_PCR_trzC,_atzD,_ptxD_bearbeitet.png



second 1%agarose gel for the rest of the samples

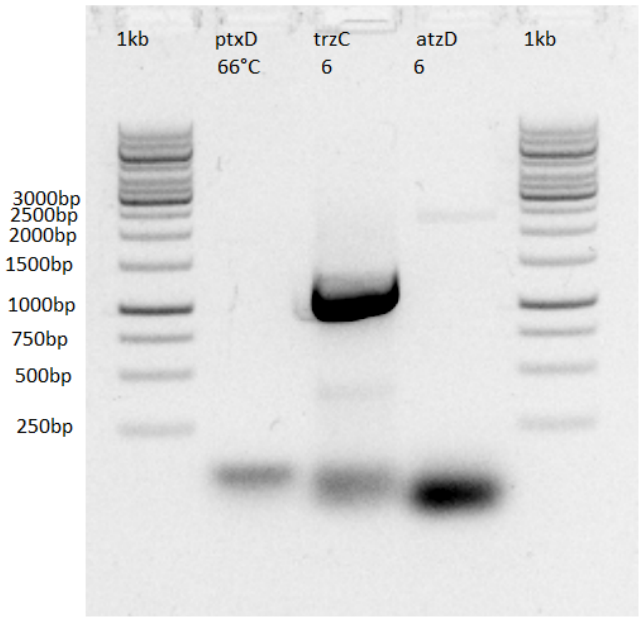
1x TAE

50min 100V 1kb ladder

Gelpicture below

1kb ladder	ptxD 66°C	trzC 6	atzD 6	1kb ladder
	1014bp	1263bp	1116bp	

Gel_2018-06-30_16hr_50min_50min_100V_1kb_ladder,_Testristrition;_PCR_ptxD_trzC_atzD,_heller_bearbeitet.png



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

Author: Susanne Vollmer

created: 02.07.2018 14:50
updated: 02.07.2018 17:57

Entry 109/259: Transformation of E.coli T10 with p2iGEM0294 and PCR of atzD and gel

In Project: Level_1

With tags: gel electrophoresis, X-gal, Transformation, Q5 PCR, amp, IPTG, Top10

Transformation of *E.coli* T10 (10 samples) with the repetion of golden Gate DVA_trzC (p2iGEM0294) using followinf protocol:

- thraw competent cells (*T10*) 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 (steriale)
- place at 37°c for 60min, shake 300rpm
- add 70µl IPTG and X-GAL on a plate 30min bevor spreat out the cells
- 1min 6000rpm discard 180µl supernatant
- spreat them out on a agarplate with the fitting antibiotica (Amp)
- incubate at 37°C over night

repetition of the PCR to amplify atzD from pJET_atzD (p2iGEM0255) with the following protocol:

each sample:

- 5 µl high GC enhancer
- 5 µl Q5 polymerase
- 1,5 µl dNTP
- 0,25 µl Q5 polymerase
- 10,75 µl milli Q
- 0,5 µl template (p2iGEM0255, 7)
- 1 µl Primer O_iGEM18_0078
- 1 µl Primer O_iGEM18_0079

using a 7x mastermix for 7 samples

PCR program:

- 98°C 30 sec
  - 98°C 10 sec
  - 60°C 15 sec
  - 72°C 35 sec--> repeat this cycle 30 times
- 72°C 2 min
- 4°C HOLD

1% agarose gel (50ml 1xTAE and 1g agarose) and 12,5µ GelRed

90V 25min 1kb ladder

Gel picture below

1kb ladder	trzC 1	trzC 1	trzC 3	trzC 4	trzC 5	trzC 6	trzC 7
	1263bp	1263bp	1263bp	1263bp	1263bp	1263bp	1263bp

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

Author: Thomas Bick
created: 03.07.2018 15:50
updated: 03.07.2018 16:28
Entry 110/259: ColonyPCR of DVA_trzC
In Project: Level_1
With tags: CIDAR cloning, Colony PCR, Lvl 0

ColonyPCR of *E.coli* T10 (50 samples, 5 per transformation plate) with the repetition of golden Gate DVA_trzC (p2iGEM0294) using 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

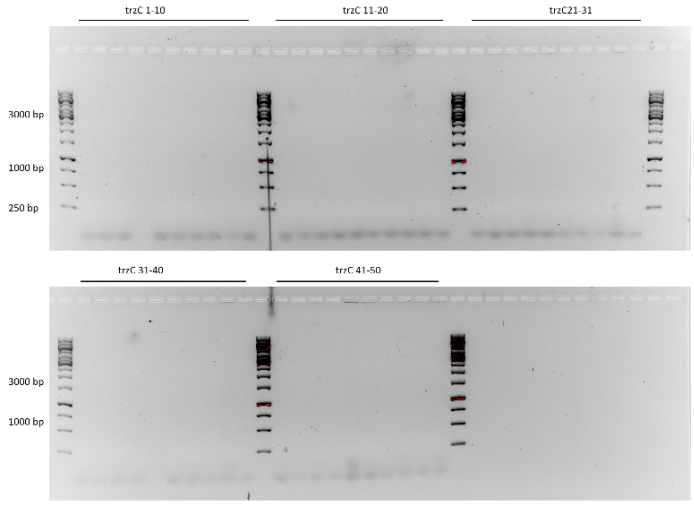
PCR program:

- 98°C 10 min
  - 98°C 10 sec
  - 63°C 15 sec
  - 68°C 1:30 min--> repeat this cycle 30 times
- 68°C 5 min
- 12°C HOLD

Inoculation of 2iGEM0001, 2iGEM0178, 2iGEM0271, 2iGEM0272 and 2iGEM0273 in 3 ml LB over night.

1% Agarose Gel of ColonyPCR of DVA_trzC

Gel_2018-07-03_16hr_16min_trzC_85min_120V_1kb_ladder_colony_PCR.tif



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

Author: Susanne Vollmer
created: 04.07.2018 15:03
updated: 04.07.2018 16:38
Entry 111/259: inoculation of 2iGEM0335, 36, 48
In Project: Level_1
With tags: incubation, amp, Top10, DHSα

inoculation of 3ml LB ampicilline with

- 2iGEM0335, 4
- 2iGEM0336, 1
- 2iGEM0336, 2
- 2iGEM0336, 3
- 2iGEM0336, 4
- 2iGEM0336, 5
- 2iGEM0348, 2.4
- 2iGEM0348, 4.4
- 2iGEM0348, 6.2
- 2iGEM0348, 7,2
- 2iGEM0348, 10.4

incubation at 37°C over night

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

Author: Thomas Bick
Entry 112/259: No entry title yet
In Project: Level_1
No tags associated

created: 04.07.2018 15:07
updated: 04.07.2018 16:13

Plasmid Miniprep of 2iGEM0001, 2iGEM0178, 2iGEM0271, 2iGEM0272 and 2iGEM0273 o/n cultures

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 x2
- 30 sec max rpm
- For eluation use new tube + 30 µl MilliQ
- incubate for 1 h at room temperature

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

Author: Susanne Vollmer

created: 05.07.2018 08:12

updated: 05.07.2018 18:47

Entry 113/259: Miniprep and sequencing of trzC anf atzD goldenGate plasmids

In Project: Level_1

With tags: miniprep, sequencing

Miniprep of of 6 colonies with the Plasmid p2iGEM0294 and 5 colonies with the Plasmid p2iGEM0295 and p2iGEM0272

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 200µ colum wash
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 1h at 37°C. Centrifuge for 15 Sek. max

plasmidnumber	colonie	concentration
p2iGEM0294	4	62,100 ng/µl
p2iGEM0294	2.4	132,45 ng/µl
p2iGEM0294	4.4	71,850 ng/µl
p2iGEM0294	6.2	91,050 ng/µl
p2iGEM0294	7.2	50,100 ng/µl
p2iGEM0294	10.4	59,150 ng/µl
p2iGEM0295	1	197,85 ng/µl
p2iGEM0295	2	193,50 ng/µl
p2iGEM0295	3	82,100 ng/µl
p2iGEM0295	4	78,350 ng/µl
p2iGEM0295	5	249,45 ng/µl
p2iGEM0272	1	61,350 ng/µl
p2iGEM0272	10	79,700 ng/µl

82sequencing with GATC LIGHTrun tube NXP

in each tube: 2,5 µl primer, 400-500ng template, and fill up to 10 µl with milli Q, put a Barcode on the tube, centrifuge short, collect the tubes in a bag and throw in the post box of GATC

Barcode	plasmidnumber	colony	Content of Plasmid in µl	primer with befor O_iGEM18_00
82EC56	p2IGEM0253	-	3,6	no iGEM prime pJET 1.2 few
82EC55	p2iGEM0253	-	3,6	no iGEM primer pJET 1.2 rev
82EC54	p2iGEM0254	-	3,25	no iGEM primer pJET1. 2 few
82EC59	p2iGEM0254	-	3,25	no iGEM primer pJET1. 2 rev
82EC58	p2iGEM0255	-	3,3	no iGEM primer pJET 1.2 few
82EC57	p2iGEM0255	-	3,3	no iGEM primer pJET 1.2 rew
82EC62	p2iGEM0294	4	7,5	68
82EC61	p2iGEM0294	4	7,5	69
82EC60	p2iGEM0294	2.4	3.75	68
82EC65	p2iGEM0294	2.4	3.75	69
82EC64	p2iGEM0294	6.2	5,5	68
82EC63	p2iGEM0294	6.2	5,5	69
82EC68	p2iGEM0295	1	2,5	68
82EC67	p2iGEM0295	1	2,5	69
82EC66	p2iGEM0295	2	2,55	68
82EC71	p2iGEM0295	2	2,55	69
82EC70	p2iGEM0295	5	2	68
82EC69	p2iGEM0295	5	2	69
82EC74	p2iGEM0272	1	7,5	13
82EC73	p2iGEM0272	1	7,5	14
82EC72	p2iGEM0272	10	6,3	13
82EC77	p2iGEM0272	10	6,3	14

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

Author: Susanne Vollmer
created: 06.07.2018 09:59
updated: 09.07.2018 14:35
Entry 114/259: mutagenesis of triA, digest and transformation
In Project: Level_1
With tags: Transformation, digest, Mutagenesis, Top10, PCR programm

mutagesis of p2iGEM0253 with O_iGEM18_0074 and O_iGEM18_0075 to mutagenise unwanted BbsI restriction side:

6x Mastermix

- 6 µl plasmid (of the 1:10 Dilution) (10ng/sample)
- 12 µl O_iGEM18_0074
- 12 µl O_iGEM18_0075
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 183 µl MiliQ

6 tubes were filled with 50µl of the mastermix

Gradient PCR programm

- 95°C 3 min
- 95°C 1min
  - 55°C 30 sec
  - gradient 71°C - 65°C 30 sec (65°C, 67°C, 68,5°C, 70°C, 71°C)
  - 72 °C 3min
  - repeat this 30 times
- 72°C 5 min
- 4°C HOLD

DpnI digestion of mutagesis of p2iGEM0253 with O_iGEM18_0074 and O_iGEM18_0075

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

Transformation of the mutagenesied Plasmid (p2iGEM0308, 2iGEM0346), with the following protocol:

- 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 (steriale)
- place at 37°C for 60 min, shake 300 rpm
- 1min 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: Susanne Vollmer

created: 09.07.2018 14:36
updated: 09.07.2018 16:54

Entry 115/259: inoculation of the transformants of the mutagenesis (2iGEM0246), retransformation of p2iGEM0254 and clean up of guaD PCR
In Project: Level_1
With tags: Top10, Transformation, inoculation, overnight, PCR Clean-Up

inoculation of 3 ml LB media with 30 µl Amp with 17 colonies of the mutagenesis 2iGEM0308 and p2iGEM0346 from two plates (13 from plate 4 and 4 from 5)

incubation on 37°C overnight

Retransformation of *E.coli* T10 with p2iGEM0272 and p2iGEM0254 with the following protocol for two tubes:

- thraw competent cells (*T10*) 5-10 min on ice
- add 1 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 15 min
- heatshock for 45 sec on 42°C
- place on ice for 5 min
- Pipette 300 µl LB without Antibiotica (steriale)
- place at 37°C for 30 min, shake 300 rpm
- add all to 3 ml LB + Amp
- incubate at 37°C over night

Clean up of the PCR product of guaD (sample 4 and 5) with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- add an equal volume of Membrane binding solution on the PCR sample
- put the solution on a colum in a collectiontube, incubate 10 min at roomtemperature
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 700µl Membrane wash solution
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 500µl of the Membrane wash solution
- centrifuge at 16000 rcf for 5 min
- discard the liquid in the Collection tube
- centrifuge at 16000 rcf for 1 min without the centrifugelid
- put the colum on a new eppi
- wait a few minuets to be sur that all alcohole is away
- add 50 µl nuclease free water
- incubate at room temperature 30 min
- centrifuge 1 min by max rpm

measured concentration:

sample 4: 91 ng/µl

sample 5: 86 ng/µl

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

Author: Susanne Vollmer
Entry 116/259: Miniprep of the triA mutagenesised plasmids and of trzC retrafo
In Project: Level_1
With tags: miniprep

created: 10.07.2018 16:01
updated: 10.07.2018 16:04

Miniprep of the 17 colonies of the mutagenesied triA (p2iGEM0346 and 2iGEM0308) with the following protocol:

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 (~ 800 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 45 min at room temperature

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

Author: Susanne Vollmer

created: 11.07.2018 15:08
updated: 17.07.2018 14:06

Entry 117/259: diggest, gel of p2iGEM0174 and golden gate with guaD

In Project: Level_1

With tags: gel electrophoresis, digest, golden gate, gelelution

Digest of p2iGEM0174 (CIDAR entry) with the follwing protocol:

- 5 µl cutsmart
- 500 ng DNA
- 0,5 µl BbsI
- ad mili Q up to 50 µl

Digest 2h at 37°C and than 20 min at 65°C for inactivation

1% Agarose gel

1xTAE

90V 90min

put all on gel with loading dye

cut out the right band

Gel Elution with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- add 10 µl Membrane binding solution per 10 mg of gel
- vortex and incubate at 50-65°C and Vortex until the gel is completly solved
- put the solution on a colum in a collectiontube, incubate 10 min at roomtemperature
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 700µl Membrane wash solution
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 500µl of the Membrane wash solution
- centrifuge at 16000 rcf for 5 min
- discard the liquid in the Collection tube
- centrifuge at 16000 rcf for 1 min without the centrifugelid
- put the colum on a new eppi
- wait a few minuets to be sur that all alcohole is away
- add 50 µl nuclease free water
- incubate at room temperature 30 min
- centrifuge 1 min by max rpm

Golden gate of guaD into the CIDAR entry Vector (p2iGEM0309) with the following protocol:

- 11µl Milli Q
- 1.5 µl T4 ligation buffer
- 0,5 µl T4 Ligase
- 0,5 µl BbsI
- 0,5 µl guaD
- 10 ng p2iGEM0174 (DVA_CD) -> predigested

Cycler Program:

1. 20 min 37°C
2. 1.5 min 37°C |
3. 3 min 16°C | 2. & 3. 50 x
4. 60 min 16°C
5. 5 min 50°C
6. 10 min 80°C
7. HOLD 4°C

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

Author: Susanne Vollmer

created: 11.07.2018 15:28
updated: 11.07.2018 15:58

Entry 118/259: Transformation of E.coli T10 with the p2iGEM0309, digest of the mutagenesist plasmids and sequencing of them and pJET trzC

In Project: Level_1

With tags: digest, transformation, gel electrophoresis, sequencing, Cidar

Transformation of *E.coli T10* with the golden Gate Plasmids (p2iGEM0308;2iGEM0347) with the following protocol:

- 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 (steriale)
- place at 37°C for 60 min, shake 300 rpm
- spreat out 60 µl X-Gal and 60 µl IPTG 30 min bevor spreat out the cells.
- 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

Digest of p2iGEM0308 to test the mutagenesis with the following protocol:

- 1 µl Cutsmart
- 0,2 µl BbsI
- 1 µl template (100ng) (p2iGEM0308)
- ad Milli Q up to 10 µl

incubate 2 h at 37°C than 65°C 20 min

gel electrophoresis with follwing protocol:

1% Agarose gel

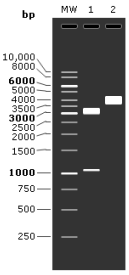
1x TAE

90V 90 min

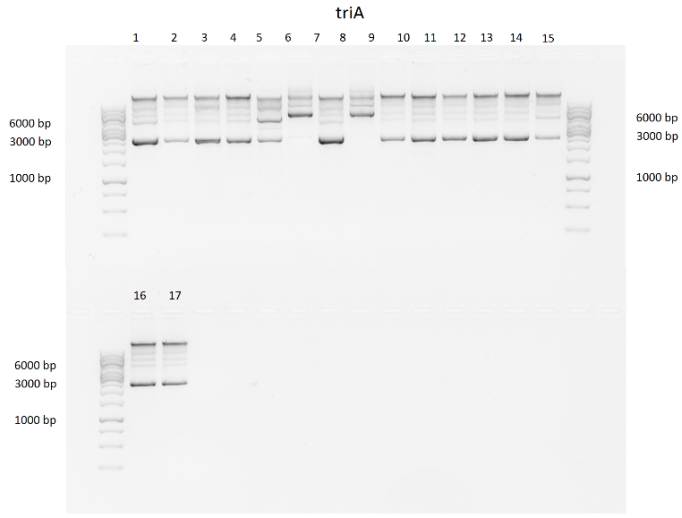
1kb ladder

Gel Picture and expected bands (1=not mutagenesised 2=mutagenesised) below

digest_tria_bbsI_sdm.png



Gel_2018-07-11_14hr_36min_triA_bbsI_SDM.tif



Sequencing of the possible positive Plasmids of the mutagesesis and of pJET trzC retrafo with the following protocol:

preparing the tubes as described in the table and add 2,5 µl primer. Put on the barcode, centrifuge short and put all in a tube and bring to the GATC box

Barcode	Template	Primer	Content Plasmid [µl]	contet milli Q [µl]
82EC85	p2iGEM0308 , 6	pJET sequencing Primer 1.2 fwd	2,3	5,2
82EC86	p2iGEM0308 , 6	pJET sequencing Primer 1.2 rev	2,3	5,2
82EC87	p2iGEM0308 , 8	pJET sequencing Primer 1.2 fwd	2,5	5
82EC88	p2iGEM0308 , 8	pJET sequencing Primer 1.2 rev	2,5	5
82EC89	p2iGEM0254 , 1	pJET sequencing Primer 1.2 fwd	2	5,5
82EC90	p2iGEM0254 , 1	pJET sequencing Primer 1.2 rev	2	5,5
82EC91	p2iGEM0254 , 2	pJET sequencing Primer 1.2 fwd	2,2	5,3
82EC92	p2iGEM0254 , 2	pJET sequencing Primer 1.2 rev	2,2	5,3

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

Author: Susanne Vollmer
created: 12.07.2018 11:35
updated: 12.07.2018 18:25

Entry 119/259: PCR and gelelectrophorese of tzrC and atzD (p2iGEM0254, 55)
In Project: Level_1
With tags: Q5 PCR, gel electrophoresis

PCR of the atzD syntesis in pJET (p2iGEM0254 and p2iGEM0255) with the primer for the CIDAR overhangs using follwing protocol:

Mastermix:

- 5 µl high GC enhancer
- 5 µl Q5 polymerase buffer
- 1,5 µl dNTP
- 0,25 µl Q5 polymerase
- 11 µl milli Q

in each tube 22,5µl Mastermix (using a 6x mastermix) and 1µl each primer and 0,25µl template (half trzC1 and half trzC2 from the retrafo)

6 samples for each template, 3 from them with an anealing temprature at 60,2°C and 3 at 62,8°C

atzD (p2iGEM0255): Primer O_iGEM18_0078 and O_iGEM18_0079

trzC (p2iGEM0254): Primer O_iGEM18_0080 and O_iGEM18_0081

PCR program:

- 98°C 2 min
  - 98°C 10 sec
  - 60°C/63°C 15 sec
  - 72°C 35 sec--> repeat this cycle 30 times
- 72°C 2 min
- 4°C HOLD

gelelectrophoresis of the PCR Fragments:

1%Agarose gel

1xTAE

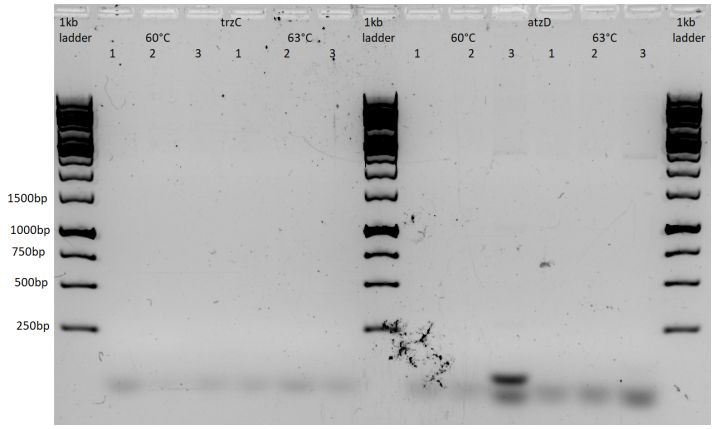
90V 80 min

1kb ladder

2 µl PCR, 2 µl loading dye, 8 µl milli Q each pocket

Gel Picture below (expected band for trzC 1263bp and for atzD 1116bp)

Gel_2018-07-12_14hr_08min_PCR_trzC_&_atzD_bearbeitet.png



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

Author: Thomas Bick
Entry 120/259: triA BbsI SDM sequencing
In Project: Level_1
With tags: Mutagenesis, sequencing

created: 12.07.2018 15:51
updated: 12.07.2018 16:03

Sequencing of p2iGEM0308 possible positive candidates: plasmid prep 13

500 ng template

2.5 µl 10 mM primer

ad. 10 µl

82EC93	p2iGEM0308	pJET1.2 fwd
82EC94	p2iGEM0308	pJET1.2 rev

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

Author: Susanne Vollmer
created: 13.07.2018 10:18
updated: 13.07.2018 17:06
Entry 121/259: Miniprep, PCR, gel electrophoresis of p2iGEM0309
In Project: Level_1
With tags: miniprep, taq pcr, gel electrophoresis

Miniprep of 10 colonies of 2iGEM0347 (p2iGEM0309) with the following protocol:

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

culture was concentrated in 600µl (centrifuges down by max rcf for 1 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 15 Sek. max rpm --> discard supernatant
- add 200µ colum wash
- add 400µl column wash
- 15 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 1h at RT. Centrifuge for 15 Sek. max

plasmidnumber and Goldengate sample . colony	concentration [ng/µl]
p2iGEM0309 1.1	88,35
p2iGEM0309 2.1	179,40
p2iGEM0309 2.2	231,10
p2iGEM0309 2.3	378,10
p2iGEM0309 2.4	223,35
p2iGEM0309 2.5	262,75
p2iGEM0309 3.1	380,25
p2iGEM0309 4.1	644,80
p2iGEM0309 5.1	471,10
p2iGEM0309 5.2	430,05

Taq PCR of the p2iGEM0309 to test if guaD is in the CIDAR emptyvektor, with the follwing protocol:

Mastermix:

- 19,875 µl Milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl O_iGEM18_0068
- 0,5 µl O_iGEM18_0069
- 0,125 µl Taq polymerase

use a 11x Mastermix and add than in each tube 24 µl Mastermix and 1 µl Template

PCR Programm:

98°C 30 sec

98°C 15 sec

47°C 20 sec

68°C 100 sec -> repeat 30x

68°C 2 min

4°C HOLD

Gel electrophorese with the following parameter:

1% Agarose gel

in each pocket: 2 µl PCR, 2 µl loading dye, and 8 µl milli Q

90V 50 min

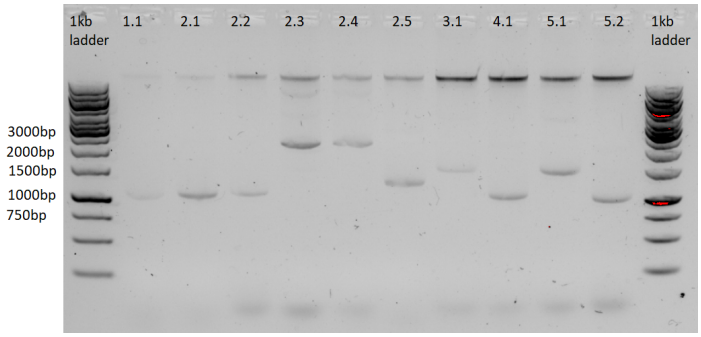
1kb ladder

gelpicture below

expected band: 1347bp

possible positive: 2.5, 3.1, 5.1

Gel_2018-07-13_16hr_52min_DVA_guaD_PCR_bearbeitet.png



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

Author: Thomas Bick
created: 16.07.2018 14:26
updated: 16.07.2018 14:43
Entry 122/259: No entry title yet
In Project: Level_1
No tags associated

Sequencing of p2iGEM0309 (DVA_guaD) possible positive candidates: 2.5 and. 5.1

500 ng template

2.5 µl 10 mM primer

ad. 10 µl

82EC95	p2iGEM0309	O_iGEM18_0068
82EC96	p2iGEM0309	O_iGEM18_0069
82EC97	p2iGEM0309	O_iGEM18_0068
82EC98	p2iGEM0309	O_iGEM18_0069

Predigest p2iGEM0174 with BbsI

500 ng template were digested

1 µl BbsI was used

incubatation 37°C over night

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

Author: Susanne Vollmer
created: 17.07.2018 10:40
updated: 13.08.2018 10:51

Entry 123/259: Gel electrophoresis and clean-up of the predigested p2iGEM0174 and golden gate with trzC and atzD

In Project: Level_1

With tags: gel electrophoresis, gel elution, Cidar, golden gate

1% Agarose gel (0,5 g Agarose and 50 ml 1x TAE)

run in 1xTAE 90V 45min

sample preparation:

1. inactivation of the enzyme: 20 min at 65°C
2. add 16 µl precutted plasmid and 4 µl loading dye in each tube

using 1kb ladder

cut out the 1500 bp band

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 of gel
- vortex and incubate at 50-65°C and Vortex until the gel is completly solved
- put the solution on a column in a Collection tube, incubate 5 min at roomtemperature
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 700 µl Membrane wash solution
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 500 µl of the Membrane wash solution
- centrifuge at 16000 rcf for 5 min
- discard the liquid in the Collection tube
- centrifuge at 16000 rcf for 1 min without the centrifuge lid
- put the column on a new eppi
- wait a few minutes (nearly 20 min) to be sure that all alcohol is away
- add 50 µl nuclease free water
- incubate at room temperature 45 min
- centrifuge 1 min at max rpm

measured concentration:

1: 5,8000 ng/µl

2: 6,0500 ng/µl

Golden Gate cloning of p2iGEM0294 (DVA_trzC) and p2iGEM0295 (DVA_atzD)

- 10µl MilliQ
- 1.5 µl T4 ligation buffer
- 0,5 µl T4 Ligase
- 0,5 µl BbsI
- 100 ng cleaned up template
- 10 ng p2iGEM0174 (DVA_CD) -> predigested and gel purified

Cycler Program:

1. 20 min 37°C
2. 1.5 min 37°C |
3. 3 min 16°C | 2. & 3. 50 x
4. 60 min 16°C
5. 5 min 50°C
6. 10 min 80°C
7. HOLD 4°C

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

Author: Thomas Bick
Entry 124/259: PCR purification atzD, trzC
In Project: Level_1
With tags: PCR Clean-Up

created: 17.07.2018 14:35
updated: 18.07.2018 12:29

PCR Clean Up with the Wizard® SV Gel and PCR Clean-Up System:

- add equal amount of Membrane binding solution as your PCR Volume
- put the solution on a colum in a collectiontube, incubate 10 min at roomtemperature
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 700 µl Membrane wash solution
- centrifuge at 16000 rcf for 1 min
- discard the liquid in the Collection tube
- add 500 µl of the Membrane wash solution
- centrifuge at 16000 rcf for 5 min
- discard the liquid in the Collection tube
- centrifuge at 16000 rcf for 1 min without the centrifuge lid
- put the column on a new eppi
- wait a few minutes to be sure that all alcohol has faded
- add 50 µl nuclease free water
- incubate at room temperature 30 min
- centrifuge 1 min at max rpm

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

Author: Sarah Seyffert
Entry 125/259: PCR
In Project: Level_1
With tags: gradient pcr, pcr

created: 17.07.2018 15:52
updated: 18.07.2018 12:03

PCR of the following genes ori,NSIa,NSIb, SmR, invA, glf, sps_T7

Preparation of a 7xmastermix

- 76,5 µl MiliQ
- 30 µl Q5-Buffer
- 30 µl GC enhancer
- 3µl dNTP
- 1,5 µl Q5-polymerase

Each tube was filled with

- 23,5µl mastermix
- 0,5 µl fw primer
- 0,5 µl rv primer
- 0,5 µl template

For NSIa,NSIb, invA, glf, sps_T7 we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient between 64-54°C for 15sec(annealing) -> NSIa + glf: 61,6°C; NSIb: 64°C; invA: 52,4°C; sps_T7: 56,7°C
    3. 72°C for 90sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

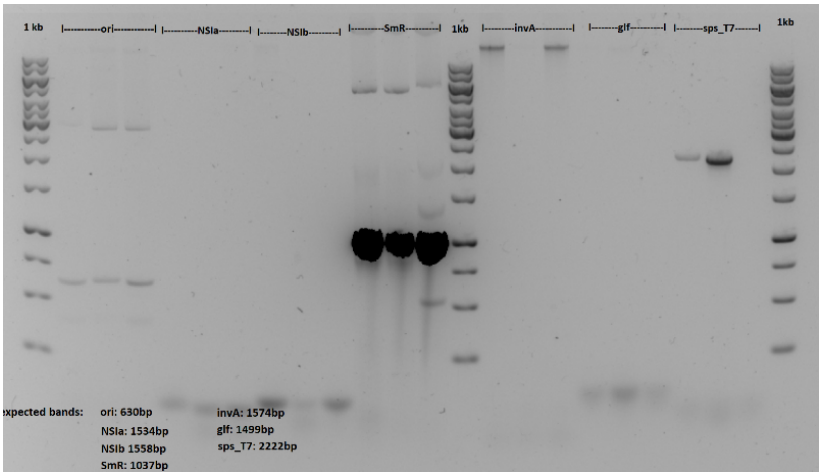
For ori and SmR we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 56°C for 15sec(annealing)
    3. 72°C for 40sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

To test if the amplification was successfull a gelelectrophoresis was done

- 5µl of those samples with1µl purple loading dye were loaded on a gel.
- 2µl 1kb was used
- The gel ran for 150 min on 90 V

Gel_2018-07-17_19hr_40min_150_min_90_V_cyano_gene(Jenny,Svenja,Sarah)bearbeitet.tif



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

Author: Sarah Seyffert
created: 18.07.2018 11:40
updated: 31.07.2018 10:51
Entry 126/259: Mutagenisis
In Project: Level_1
With tags: Mutagenesis, gradient pcr, gel electrophoresis, gel purification

Mutagenesis of p2iGEM0253

6x Mastermix

- 0,6µl plasmid(10ng)
- 12 µl primer fw
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 188.4 µl miliQ(RNAse free water)

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 70°C - 62°C 30 sec (sample 1: 70°C, sample 2: 68.5°C, sample 3: 67°C, sample 4: 65.2°C, sample 5: 62.8°C, sample 6: 62°C)
  - 72 °C 3:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

Preparation of the gel purification

The samples(20µl) were loaded on the gel together with 5µl purple loading dye

Additionally Carina's samples were added

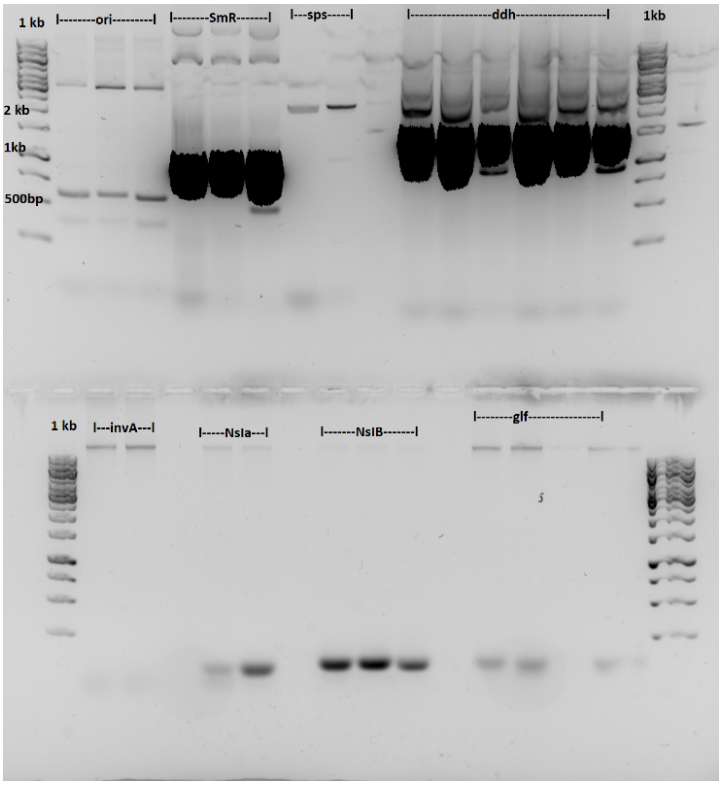
2µl 1kb ladder was used

The gel ran on 90V for 1h

The bands for *ori*, *SmR* and *sps* were cutted out under the blue light table.

Under this light the expeacted bands for *ddh* were visible at 950bp.

Gel_2018-07-18_15hr_35min_60_min_90_V_Cyano+ddh-bearbeitet.tif



Gelelution of *ori*, *SmR* and *sps*

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

Sadly only SmR did yield product. The other genes were not successfully purified.

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

Author: Carina Gude
created: 18.07.2018 12:13
updated: 18.07.2018 12:17
Entry 127/259: gradient PCR
In Project: Level_1
With tags: pcr, gradient pcr, Cyanobacteria

PCR of the following genes NSIa,NSIb, invA, glf

Preparation of a 11xmastermix

- 140,25 µl MiliQ
- 55 µl Q5-Buffer
- 55 µl GC enhancer
- 5,5µl dNTP
- 2,75 µl Q5-polymerase

Each tube was filled with

- 23,5µl mastermix
- 0,5 µl fw primer
- 0,5 µl rv primer
- 0,5 µl template

For NSIa,NSIb, invA, glf, sps_T7 we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient for 15 sec (annealing)
        1. NsIa: 61,6°C & 62,9°C
        2. NsIb: 62,9°C; 63,7°C & 64,0°C
        3. invA: 53,4°C & 55,1°C
        4. glf: 51,0°C; 51,3°C; 52,1°C
    3. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

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

Author: Thomas Bick
created: 18.07.2018 13:52
updated: 18.07.2018 16:45
Entry 128/259: testrestriction of p2iGEM 0308 & sequencing of p2iGEM0309
In Project: Level_1
With tags: DVA, digest, sequencing

Test digestion of p2iGEM0308 to test for positive SDM of BbsI restriction side

- 1 µl cutsmart
- 8.3 µl MilliQ
- 0.5 µl template
- 0.2 µl BbsI HF

incubate at 37°C for 2 h

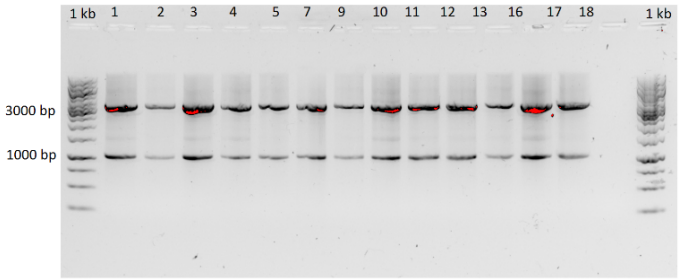
inactivation at 65°C for 20 min

Sequencing of p2iGEM0309 (DVA_guaD) possible positive candidates: 3.1 and 4.1

- 500 ng template
- 2.5 µl 10 mM primer
- ad. 10 µl

82ED63	p2iGEM0309 3.1	O_iGEM18_0068
82ED64	p2iGEM0309 3.1	O_iGEM18_0069
82ED65	p2iGEM0309 4.1	O_iGEM18_0068
82ED66	p2iGEM0309 4.1	O_iGEM18_0069

Gel_2018-07-18_16hr_36min_tria_sdm_restriktion_BbsI_repeat.tif



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

Author: Susanne Vollmer
Entry 129/259: DpnI digest of the mutagenesis
In Project: Level_1
No tags associated

created: 18.07.2018 17:25
updated: 18.07.2018 17:30

dpnI Digest of the mutagenesiesed Plasmids, with the follwing protocol:

fill it in a new 1,5 ml Eppendorf tube

add 1µl DpnI enzyme

incubation 2 h at 37 °C

inactivation 20 min at 80 °C

store at -20 °C

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

Author: Susanne Vollmer
created: 19.07.2018 10:28
updated: 19.07.2018 13:40

Entry 130/259: transformation of E.coli T10 with p2iGEM0294, 295, 254, 308
In Project: Level_1
With tags: Transformation, E.coli, Top10

Transformation of *E.coli* T10 with the Plasmids p2iGEM0294 (5 tubes, 2iGEM0348), p2iGEM0295 (5 tubes, 2iGEM0353), p2iGEM0254 (tube 2, 2iGEM0272) and p2iGEM0308 (6 tubes, 2iGEM0346) with the following protocol:

- 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 around 40 sec on 42°C
- place on ice for 5 min
- Pipette 300 µl LB without Antibiothica (steriale)
- place at 37°C for 60 min, shake 300 rpm
- spreat out 60 µl X-Gal and 60 µl IPTG 30 min bevor spreat out the cells.
- 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 131/259: PCR of ori
In Project: Level_1
With tags: pcr

created: 19.07.2018 11:43
updated: 23.07.2018 11:51

Because the gelelution of ori and sps was not succesfull they have to be repeated

PCR of ori

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 56°C for 15sec(annealing)
    3. 72°C for 20sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

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

Author: Carina Gude
created: 19.07.2018 11:57
updated: 19.07.2018 12:13
Entry 132/259: Touchdown PCR
In Project: Level_1
With tags: Q5 PCR, pcr, touchdown pcr, Cyanobacteria, NSI

Touchdown PCR of NSIa, NSIb genes

Preparation of a 7x mastermix

- 89,25 µl MiliQ
- 35 µl Q5-Buffer
- 35 µl GC enhancer
- 3,5 µl dNTP
- 1,75 µl Q5-polymerase

Each tube was filled with

- 23,5µl mastermix
- 0,5 µl fw primer
- 0,5 µl rv primer
- 0,5 µl template

For NSIa,NSIb we used this PCR-protocol

1. 98°C for 2 min
2. cycle with
    1. 98°C for 15 sec(denaturation)
    2. 67°C for 15 sec (annealing)
    3. repeat 30 times --> each time annealing temperature decreases by 0,3°C
3. 72°C for 1 min
4. Hold 10°C

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

Author: Susanne Vollmer
Entry 133/259: cryo from 2iGEM0272 and inoculation of the transformation colonies (p2iGEM0295, 294, 308)
In Project: Level_1
No tags associated

created: 20.07.2018 10:07
updated: 23.07.2018 13:08

Cryo from 2iGEM0272 (p2iGEM0254 in *E.coli* Top10) with:

700 µl over night culture and

300 µl 99,5 % Glycerol

stored in -80 °C in Level 1 box

inoculation of each 3ml LB media with ampicillin with:

plasmidnumber	**cryonumber**	**culternumber (Golden Gate/ mutagenese sample number . Colony number)**
p2iGEM0294	2iGEM0348	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4 4.1; 4.2; 4.3; 4.4; 4.5 5.1
p2iGEM0295	2iGEM0353	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4; 3.5 4.1; 4.2; 4.3; 4.4; 4.5 5.1; 5.2; 5.3; 5.4; 5.5
p2iGEM0308	2iGEM0346	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4; 3.5 4.1; 4.2; 4.3; 4.4; 4.5 5.1; 5.2; 5.3; 5.4; 5.5 6.1; 6.2; 6.3; 6.4; 6.5

75 samples in total

incubation over night at 37°C and 220-225 rpm

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

Author: Susanne Vollmer
created: 21.07.2018 14:48
updated: 23.07.2018 12:43
Entry 134/259: Miniprep of teh inoculated plasmids, p2iGEM0294, 295, 308
In Project: Level_1
With tags: miniprep

Miniprep of the inoculated culters, with the following protocol:

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

culture was concentrated in 600µl and used for plasmid isolation

- 600 µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 15 Sek. max rpm --> discard supernatant
- add 200 µl Endotoxin removal wash
- add 400 µl column wash
- 15 sec max rpm
- For elution use new tube + 30 µl autoclaved water
- incubate for 1 h at RT. centrifuge for 15 sek. max

samples:

plasmidnumber	**cryonumber**	**culternumber (Golden Gate/ mutagenese sample number . Colony number)**
p2iGEM0294	2iGEM0348	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4 4.1; 4.2; 4.3; 4.4; 4.5 5.1
p2iGEM0295	2iGEM0353	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4; 3.5 4.1; 4.2; 4.3; 4.4; 4.5 5.1; 5.2; 5.3; 5.4; 5.5
p2iGEM0308	2iGEM0346	1.1; 1.2; 1.3; 1.4; 1.5 2.1; 2.2; 2.3; 2.4; 2.5 3.1; 3.2; 3.3; 3.4; 3.5 4.1; 4.2; 4.3; 4.4; 4.5 5.1; 5.2; 5.3; 5.4; 5.5 6.1; 6.2; 6.3; 6.4; 6.5

75 samples in total

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

Author: Susanne Vollmer
created: 23.07.2018 11:25
updated: 23.07.2018 16:49

Entry 135/259: testrestriction of the mutagenesis p2iGEM0308 and gel, PCR of p2iGEM0294 and p2iGEM0295
In Project: Level_1
With tags: test restriction

testrestriction of the mutegenesised Plasmids of triA, p2iGEM0308, to test if the mutagenesis works, with the following protocol:

- 1 µl cutsmart
- 8.3 µl MilliQ
- 0.5 µl template
- 0.2 µl BbsI HF

incubate at 37°C for 2 h

inactivation at 65°C for 20 min

HOLD at 10°C

tested samples:

p2iGEM0308	2iGEM0346	1.1; 1.2; 1.3; 1.4; 1.5; 2.1; 2.2; 2.3 2.4; 2.5; 3.1; 3.2; 3.3; 3.4; 3.5; 4.1 4.2; 4.3; 4.4; 4.5; 5.1; 5.2; 5.3; 5.4 5.5; 6.1; 6.2; 6.3; 6.4; 6.5; pJET_triA

PCR to test if the Level 0 cloning was sucsessfully, with the following protocol:

Mastermix:

- 20,375 µl Milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl O_iGEM18_0068
- 0,5 µl O_iGEM18_0069
- 0,125 µl Taq polymerase

-add 24,5 µl mastermix in each tube and add 0,5 µl template

PCR Programm:

98°C 30 sec

98°C 15 sec

47°C 20 sec

68°C 100 sec -> repeat 30x-> for p2iGEM0295 1 min and 30 sec and for p2iGEM0294 1min and 45 sec

68°C 2 min

4°C HOLD

following templates were used:

p2iGEM0294	2iGEM0348	1.1; 1.2; 1.3; 1.4; 1.5; 2.1; 2.2; 2.3; 2.4; 2.5; 3.1; 3.2; 3.3; 3.4; 4.1; 4.2; 4.3; 4.4; 4.5; 5.1;
p2iGEM0295	2iGEM0353	1.1; 1.2; 1.3; 1.4; 1.5; 2.1; 2.2; 2.3; 2.4; 2.5; 3.1; 3.2; 3.3; 3.4; 3.5 4.1; 4.2; 4.3; 4.4; 4.5; 5.1; 5.2; 5.3; 5.4; p2iGEM0174; 5.5

150 ml 1% Agarose gel (1,5 g Agarose and 150 ml 1xTAE)

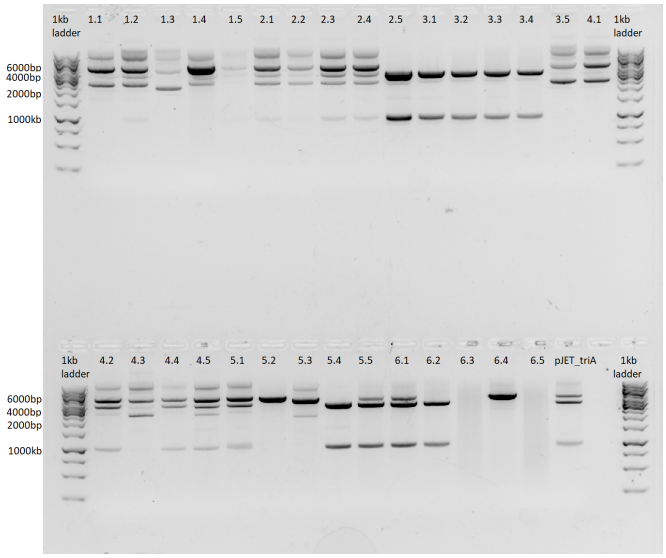
90V 1h 1kb ladder

in each pocket: PCR product and 2 µl loading Dye

gel Picture below

300 ml 1% Agarose gel (3g Agarose and 300 ml 1xTAE) stored at 4°C over night

Gel_2018-07-23_16hr_25min_testrestriction_triA_sdm_BbsI_bearbeitet.png



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

Author: Susanne Vollmer
created: 24.07.2018 12:00
updated: 25.07.2018 08:04

Entry 136/259: gel electrophoresis of the PCRs of p2iGEM0295 and p2iGEM0294
In Project: Level_1
With tags: gel electrophoresis, pcr

gel electrophoresis of the test PCR of p2iGEM0295 (DVA_atzD), p2iGEM0294 (DVA_trzC)

in each pocket 2 µl loading dye, 2 µl PCR product (see entry befor) and 8 µl MilliQ

100 V 60 min

1kb ladder from thermo fisher

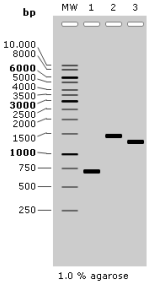
gel pictures below

1. expected bands see on the first picture: MW = 1kb ladder GeneRuler; 1 = p2iGEM0174 (DVA_CD); 2 = p2iGEM0294 (DVA_trzC); 3 = p2iGEM0295 (DVA_atzD)

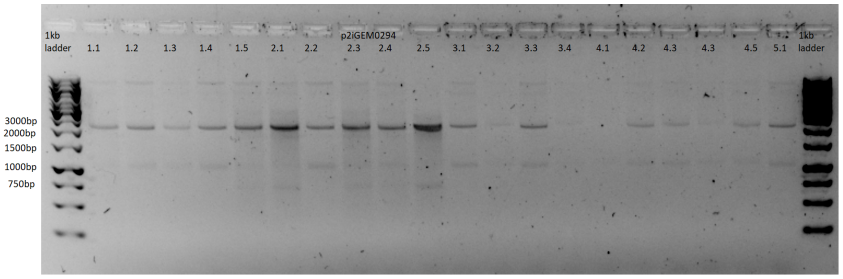
2. gel of p2iGEM0294 (DVA_trzC)

3. gel of p2iGEM0295 (DVA_atzD)

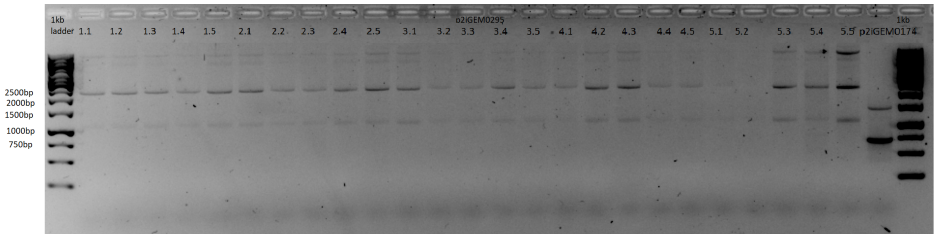
trzC_&_atzD_PCR_auf_DVA_CD.png



Gel_2018-07-24_13hr_26min_p2iGEM0294_PCR_bearbeitet.png



Gel_2018-07-24_13hr_26min_p2iGEM0295_PCR_bearbeitet.png



sequencing of p2iGEM0308

preparing the tubes with 400-500 ng template, add 2,5 µl primer and fill up milli Q up to 10 µl. Put on the barcode, centrifuge short and put all in a tube and bring to the GATC box

Barcode	Template	Primer	Content Plasmid [µl]	contet MilliQ [µl]
82EE13	p2iGEM0308 , 5.2	pJET sequencing Primer 1.2 fwd	1.8	5.7
82EE14	p2iGEM0308 , 5.2	pJET sequencing Primer 1.2 rev	1.8	5.7
82EE15	p2iGEM0308 , 5.3	pJET sequencing Primer 1.2 fwd	1.6	5.9
82EE16	p2iGEM0308 , 5.3	pJET sequencing Primer 1.2 rev	1.6	5.9
82EE17	p2iGEM0308 , 6.4	pJET sequencing Primer 1.2 fwd	1.8	5.7
82EE18	p2iGEM0308 , 6.4	pJET sequencing Primer 1.2 rev	1.8	5.7

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

Author: Sarah Seyffert
Entry 137/259: PCR of sps
In Project: Level_1
With tags: pcr, cryogenic culture

created: 24.07.2018 14:45
updated: 24.07.2018 15:33

Because the gelelution of sps did not work, the PCR was repeated with a larger amount of sample volume.

4xMastermix

- 100µl miliQ
- 20µl Q5 buffer
- 20µl GC-enhancer
- 2µl dNTP
- 2µl fwd primer
- 2µl rv primer
- 2µl template
- 1µl Q5-Polymerase

Each tube was filled with 50µl of the mastermix

For sps_T7 we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 56°C
    3. 72°C for 90sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

Preparation of the Cryoculture 2iGEM0314 (Pam's strain)

- 100µl DMSO
- 900µl Cyanoculture

Storage at -80°C

The plates from the S.elongatus wildtype and the cscb strain were plated out new from the old plates

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

Author: Susanne Vollmer
created: 25.07.2018 14:07
updated: 25.07.2018 15:14

Entry 139/259: restriction ligation of trzC, atzD, guaD in DVA_CD, p2iGEM0295, p2iGEM0294, p2iGM0309
In Project: Level_1
No tags associated

Restriction and Ligation of atzD, trzC and guaD in p2iGEM0174, with the following protocol,

mastermix for each sample:

- 200ng of the Backbone
- 2,5 µl CutSmart
- 0,5 µl BbsI enzyme
- add milli Q up to 25 µl, but don't forgett that you will add template

add to each sample 500 ng of the insert

incubate 6 h at 37°C

inactivation for 20 min at 65°C

for Ligation:

- 2 µl Buffer
- 1 µl T4 ligase
- 12 µl milli Q

add 2,5 µl from the restrictions, each from Backbone and from insert

than overnight (12 h) ligation at 16 °C

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

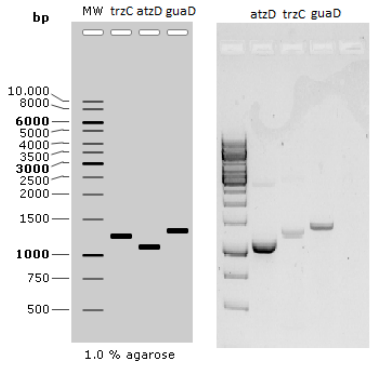
Author: Thomas Bick

created: 25.07.2018 14:08
updated: 25.07.2018 14:13

Entry 140/259: verification of trzC, atzD, guaD PCR
In Project: Level_1
No tags associated

1% Agarose Gel of cleaned up PCR Product of trzC, atzD and guaD

trzC,_atzD,_guaD_simulated.png



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

Author: Sarah Seyffert
Entry 141/259: PCR of NSIa, NSIb, invA and glf
In Project: Level_1
With tags: pcr, Colony PCR

created: 25.07.2018 17:38
updated: 09.08.2018 11:26

Because the gelelution of sps did not work, the PCR was repeated with a larger amount of sample volume.

6xMastermix

- 100µl miliQ
- 20µl Q5 buffer
- 20µl GC-enhancer
- 2µl dNTP
- 2µl template
- 1µl Q5-Polymerase

Each tube was filled with 23µl of the mastermix and 1µl Template as well as 0,5µl of both primers

For NSIa, NSIb, glf and invA we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. gradient between 57°C-64°C (NSIa: 61,8°C, NSIb: 64°C, invA:57°C, glf 61,8°C)
    3. 72°C for 90sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

For plac we used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 61°C
    3. 72°C for 20sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

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

Author: Svenja Hermanns
Entry 142/259: Trafo pSHDY*
In Project: Level_1
With tags: pSHDY, antibiotic, spectionmycin

created: 25.07.2018 20:11
updated: 25.07.2018 21:26

(23.07.18) Transformation of the pSHDY* plasmid (p2iGEM0360)

- thaw compenet cells (E. coli TOP 10) on ice for 10 minutes

- added competent cells to 3µl dried out pSHDY* plasmid

- incubated on ice for 5 minutes

- heatshock for 30 seconds at 42 °C

- incubated on ice for 2 minutes

- added 300µl LB sterile and incubated at 37 °C and 250 rpm for 1 hour

- the 300µl culture were added to 2,7 ml LB with 5µl Spectinomycin and 5µ Kanamycin

- incubated overnight at 37°C and 250 rpm

(24.07.18) On the next day

- a kryo with 700µl culture and 300µl glycerin was made

- 50 µl culture was spread on an LB agar plate with kanamycin

- from the remaining culture a miniprep was made with the PureYield MiniPrep Kit from Promega

  - liquid culture was centrifuged for 5 Minutes at 6000 rpm

  - pellet was resuspended in 600µl culture and transfered into a new eppi

  - 100 µl Lysis buffer and 350µl neutralisation buffer were added ad shaked

  - 900 µl supernatant were transfered to a column and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed

  - 400 µl column wash were added and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed

  - 200 µl endotoxin wash were added and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed

  - coulmn was transfered to a new eppi and 30 µl warm MilliQ was added and incubated for around 4 hours

  - centrifuged for 30 seconds at 15000 rpm and stored at -20°C

(25.07.18) On the next day the plate was grown very sadly so a new overnight culture was made

- one scratch from the plate in 3ml LB with 5µl Spectinomycin and 5 µl Kanamycin

- incubation overnight at 37°C and 250 rpm

a new spectinomycin stock was created

- 400 mg Spectinomycin powder filled up to 20 ml with MilliQ and was sterile filtered

- stored at -20°C in the antibiotic stock box

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

Author: Sarah Seyffert
Entry 143/259: Gelelution
In Project: Level_1
With tags: gel purification, gelelution, gel elution, pcr

created: 26.07.2018 13:59
updated: 26.07.2018 16:57

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 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 for 2 h at room temperature
- Centrifuge 1 min.
- Store DNA at -20°C.

Mastermix

- 102 µl MiliQ
- 40µl buffer
- 40µl High GC
- 4µl dNTP
- 2µl Q5-polymerase

Each PCR-tube was filled with 47µl Mastermix, 1µl Template, 1µl fw primer and 1µl rv primer

We used this PCR-protocol

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 61°C for Plac and 56°C ori
    3. 72°C for 20sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

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

Author: Susanne Vollmer

created: 26.07.2018 14:49
updated: 26.07.2018 17:08

Entry 144/259: Transformation of E.coli T10 with the ligation of atzD, trzC and guaD in DVA_CD

In Project: Level_1

With tags: E.coli, transformation

Transformation of *E.coli* T10 with the ligation of atzD (p2iGEM0295) , trzC (p2iGEM0294) and guaD (p2iGEM0309) in DVA_CD with the following protocol:

- 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 around 40 sec on 42°C
- place on ice for 5 min
- Pipette 300 µl LB without Antibiothica (steriale)
- place at 37°C for 60 min, shake 300 rpm
- spreat out 60 µl X-Gal and 60 µl IPTG 30 min bevor spreat out the cells.
- 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: Susanne Vollmer
created: 27.07.2018 11:18
updated: 27.07.2018 15:29

Entry 145/259: colony PCR and gel electrophoresis of the transformants of the Restriction ligation with atzD and trzC
In Project: Level_1
With tags: Colony PCR, gel electrophoresis

Colony PCR of *E.coli* T10 2iGEM0335 (p2iGEM0294, DVA_trzC) and 2iGEM0336 (p2iGEM0295, DVA_atzD) with the follwing protocol:

White colonies were picked and used für inoculate 3ml LB with 30 µl amp. Small amount of the colony was added to the PCR tube for the reaction.

PCR sample:

- 2,5 µl 10xPuffer
- 0,5 µl dNTP
- 0.5 µl Primer fwd (O_iGEM18_0068)
- 0.5 µl Primer rev (O_iGEM18_0069)
- 0,12 µl Taq
- 20.5 µl MilliQ
- 0.5 DMSO
- 1colony

PCR program:

- 98°C 10 min
  - 98°C 20 sec
  - 53°C 30 sec
  - 68°C 1min and 40 sec --> repeat this cycle 30 times
- 68°C 5 min
- 12°C HOLD

1% Agarose gel (1,5 g Agarose and 150 ml 1x TAE)

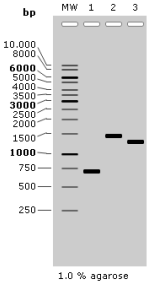
1kb ladder

90V 1h

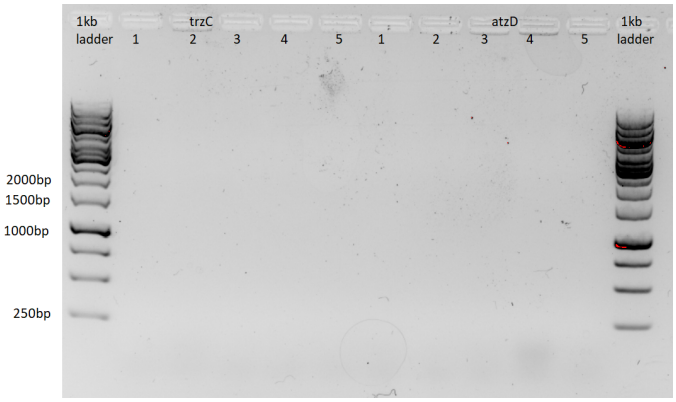
in each pocket: 5 µl PCR product, 5 µl milli Q and 2 µl loading Dye

gel picture below, expected band before

trzC_&_atzD_PCR_auf_DVA_CD.png



Gel_2018-07-27_15hr_17min_96_V_1h_colony_PCR_restriction_ligation_von_DVA_trzC_und_DVA_atzD_bearbeiten.png



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

Author: Jennifer Denter
Entry 146/259: PCR of Plac (p2iGEM0312)
In Project: Level_1
No tags associated

created: 27.07.2018 11:37
updated: 31.07.2018 13:35

To amplify Plac out of p2iGEM0312 a PCR was started.

PCR was done in triplets.

3X Mastermix

Amount (µl)	Solution
38,5	Milli Q Water
15	Enhancer
15	Buffer (10X)
1,5	dNTPs
1,5	Template DNA (p2iGEM0312)
1,5	Primer fwd (1043)
1,5	Primer rev (1044)

Devide 25 µl each into PCR tubes.

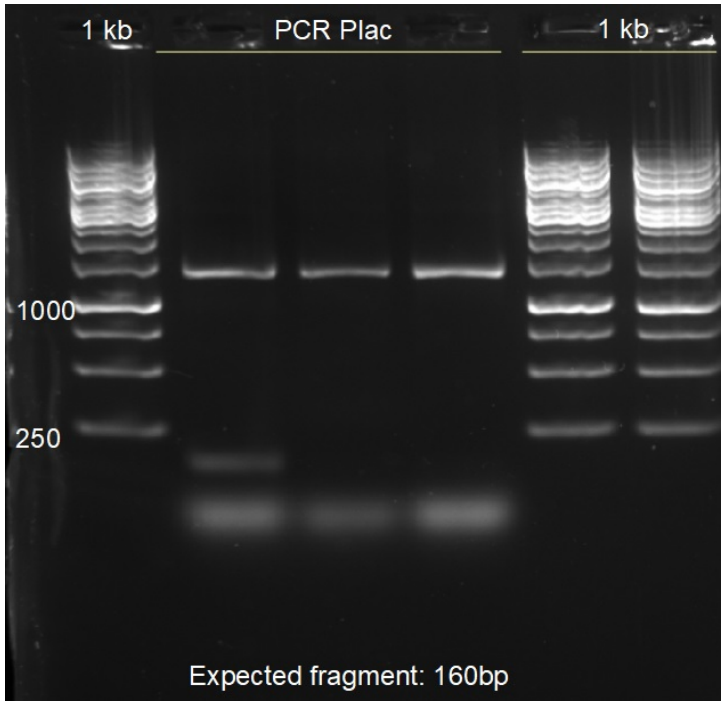
The PCR conditions were as follows

95 °C	100 sec	
95 °C	20 sec	
61 °C	20 sec	
72 °C	20 sec	30 cycles
72 °C	5 min	
12 °C	Hold	

Gelelectrophoresis showed an unexpected band with a size of 1500 bp. The expectet fragment was just amplifyed in one sample.

The band was cut from the gel and stored at -20 °C.

PCR_Plac_1._Versuch.jpg



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

Author: Thomas Bick
Entry 147/259: sequencing of p2iGEM0309
In Project: Level_1
With tags: sequencing, pJET

created: 30.07.2018 14:45
updated: 30.07.2018 14:51

sequencing of p2iGEM0308

12 µl Template (80ng/µl) + 3 µl Primer (10mM)

Barcode	Template	Primer
3717621	p2iGEM0308 , 5.2	pJET sequencing Primer 1.2 fwd
3717622	p2iGEM0308 , 5.2	pJET sequencing Primer 1.2 rev

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

Author: Thomas Bick
created: 31.07.2018 10:32
updated: 31.07.2018 15:37
Entry 148/259: verification of trzC, atzD PCR
In Project: Level_1
With tags: CIDAR cloning, gel electrophoresis, pcr

PCR to test if the Level 0 cloning was sucsessfully, with the following protocol:

Mastermix:

- 20,375 µl Milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl O_iGEM18_0068
- 0,5 µl O_iGEM18_0069
- 0,125 µl Taq polymerase

-add 24,5 µl mastermix in each tube and add 0,5 µl template

PCR Programm:

98°C 30 sec

98°C 15 sec

47°C 20 sec

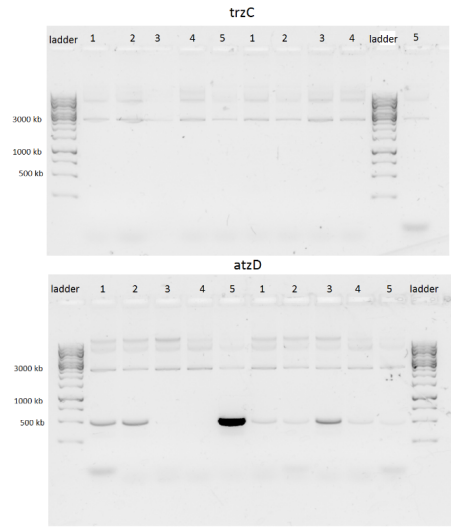
68°C 100 sec -> repeat 30x -> for p2iGEM0295 1 min and 30 sec and for p2iGEM0294 1min and 45 sec

68°C 5 min

10°C HOLD

1% Agarose Gel

Gel_2018-07-30_16hr_29min_96_V_1h_PCR_restriction_ligation_von_DVA_trzC_und_DVA_atzD.tif



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

Author: Jennifer Denter
Entry 149/259: Plac amplification & purification
In Project: Level_1
No tags associated

created: 31.07.2018 13:35
updated: 31.07.2018 13:42

In order to amplify Plac successfully a new PCR war performed with 0,2 µl more template in each sample compared to the PCR from 27th of July.

The Program was untouched and run under the same contitions.

The gelelectrophoresis was loaded with the 2 log Ladder by NEB and showed the expected fragment in every sample.

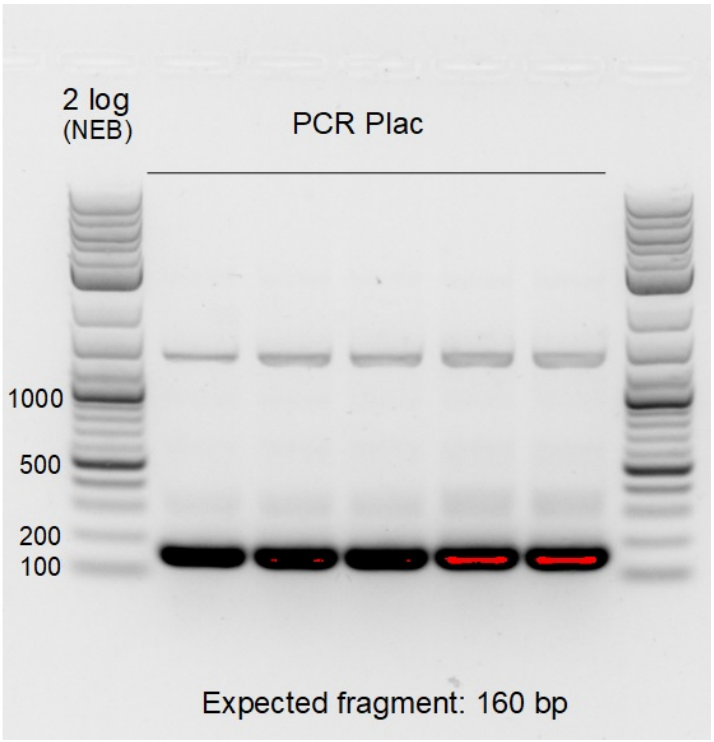
The bands were cut from gel and a gelelution was performed with the Promega Purification Kit.

The cutted sample oh the 27th was used for the purification as well.

All 3 samples were eluted in the same tube and with 30 µl each.

In total a tube with 90 µl and a final concentration of 29 ng/µl of Plac are stored at -20 °C.

UnbenanntPCR_Plac_Versuch_2.jpg



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

Author: Thomas Bick
created: 31.07.2018 15:37
updated: 31.07.2018 15:43
Entry 150/259: mutagenesis of p2iGEM0308
In Project: Level_1
With tags: Mutagenesis, digest

Mutagenesis of p2iGEM0308

6x Mastermix

- 0.6 µl plasmid(10ng)
- 12 µl primer fw (O-iGEM2018_0076)
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3 µl Q5-polymerase(at last on ice!)
- 18 µl DMSO
- 188.4 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 70°C - 62°C 30 sec (sample 1: 70°C, sample 2: 68.5°C, sample 3: 67°C, sample 4: 65.2°C, sample 5: 62.8°C, sample 6: 62°C)
  - 72 °C 3:40 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

DpnI digest of the mutagenesis:

0.5 µl DpnI added to each sample

incubation at 37°C for 2 h

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

Author: Sarah Seyffert
Entry 151/259: Predigestion of pSHDY
In Project: Level_1
With tags: digest, restriction

created: 31.07.2018 16:47
updated: 31.07.2018 16:49

Predigestion of pSHDY with EcoRI and PstI

- 0,5 µl EcoRI
- 0.5 µl PstI
- 5µl template
- 2µl cutsmart
- 12 µl MiliQ

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

Author: Sarah Seyffert
Entry 152/259: Gelectrophoresis digest
In Project: Level_1
With tags: gel electrophoresis, digest, Transformation

created: 01.08.2018 11:39
updated: 01.08.2018 17:49

Heat inactivation of the digestion of pSHDY(p2iGEM0311)

- inativation at 80°C for 20 min

Preparation of one agarose gel

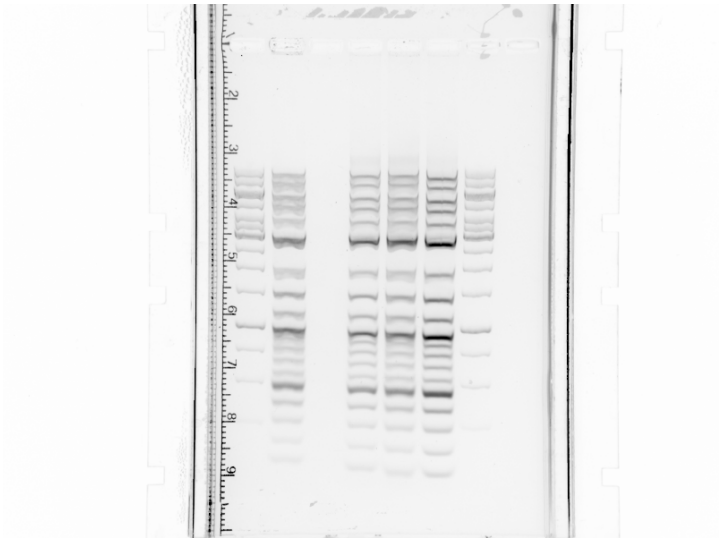
- 0.4g agarose
- 40ml 1x TAE-buffer
- 2µl Gelred(when the gel is cooled down)

Gelelctrophoresis of *ori* and digested pSHDY

- 7µl puple loading dye was added to the samples
- everything of the samples was loaded on the gel
- 2µl 1kb ladder was used

The gel ran on 90V for 1h

Gel_2018-07-24_13hr_26min_p2iGEM0294_PCR.tif



Repetition of digestion of pSHDY

- 7µl MiliQ
- 10µl template
- 2µl cutsmart
- 0.5µl EcoRI
- 0.5µl PstI

Incubate at 37°C for 2h

Transformation of 2iGEM0374, 2iGEM0361 and three samples of the ligated genes from Thomas Bick

- 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)(Kan)
- incubate at 37°C over night

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

Author: Thomas Bick
created: 01.08.2018 14:14
updated: 01.08.2018 15:05

Entry 153/259: Cryo of 2iGEM0373, Miniprep of 2iGEM0373, transformation of p2iGEM0332 into E. coli T10
In Project: Level_1
With tags: Kryo, transformation, miniprep

Preparation of the Cryoculture 2iGEM0373

- 700 µl overnight culture
- 300 µl Glycerin

Storage at -80°C

Miniprep of 2iGEM0373

- pellet was resuspended in 600 µl culture and transfered into a new eppi
- 100 µl Lysis buffer and 350µl neutralisation buffer were added ad shaked
- 900 µl supernatant were transfered to a column and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed
- 400 µl column wash were added and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed
- 200 µl endotoxin wash were added and centrifuged for 30 seconds at 15000 rpm -> supernatant was disposed
- coulmn was transfered to a new eppi and 30 µl MilliQ was added and incubated for around 3 hours
- centrifuged for 30 seconds at 15000 rpm and stored at -20°C

Transformation of *E.coli* T10 p2iGEM0332 with the following protocol:

- 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 (steriale)
- 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

Cider Lvl0 cloning:

- ligation of BbsI predigested DVA & atzD, trzC, guaD each
- 20 min incubation room temperature

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

Author: Jennifer Denter
Entry 154/259: Inoculation of 2iGEM0360 (p2iGEM0311)
In Project: Level_1
No tags associated

created: 01.08.2018 19:12
updated: 01.08.2018 19:15

2iGEM0360 was inoculated 3 times to prepare a plasmid isolation of p2iGEM0311 --> pSHDY*

p2iGEM0311 (pSHDY) is needed for further cloning steps with p2iGEM0311 as backbone.

Conditions:

3 ml LB with kan (50 µg/ml) and spec (100 µg/ml)

37 °C, 220 rpm o/n.

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

Author: Susanne Vollmer
created: 02.08.2018 14:28
updated: 02.08.2018 14:32
Entry 155/259: inoculation of 2iGEM0348 and 2iGEM0353
In Project: Level_1
With tags: inoculation, amp

inoculation of 3ml LB amp with:

p2iGEM0294 (2iGEM0348, DVA_trzC) Colony 1-7

p2iGEM0295 (2iGEM0353, DVA_atzD) Colony 1-7

so the 14 samples in total were incubated overnight at 37°C

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

Author: Sarah Seyffert
created: 02.08.2018 14:31
updated: 02.08.2018 17:01

Entry 156/259: gel electrophoresis & gelpurification
In Project: Level_1
With tags: gel electrophoresis, gel purification, gel elution, pcr, overhang pcr

Gelelectrophoresis of Ori(PCR done by Carina)

- 25µl PCR sample + 5µl purple loading dye were loaded on the gel
- 2 µl 1 kb ladder was applied on the gel

Gel ran at 90V for 1h

The 600 bands were cutted out for a gel purification

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 for 2 h at room temperature
- Centrifuge 1 min.
- Store DNA at -20°C.

Overhang PCR of *sps* and *Plac*

Two PCR tubes were filled with each containing

- 5µl Q5-Buffer
- 5µl GC enhancer
- 0.5µl dNTP
- 0.5µl Q5 polymerase
- 2 or 3 µl sps (2µl in tube 1 and 3µl in tube 2)
- 4 or 6 µl Plac (4µl in tube 1 and 6µl in tube 2)
- 8 or 5 µl MiliQ (8µl in tube 1 and 5µl in tube 2)

PCR- Programm for overlap

- 95°C 3 min
  - 95°C 30 sec
  - 63°C 20 sec
  - 72 °C 1:30 min
  - repeat this 10 times
- 72°C 5 min
- hold 12°C

stored at -20°C

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

Author: Carina Gude
Entry 157/259: PCR of ori
In Project: Level_1
With tags: pcr, Cyanobacteria

created: 02.08.2018 15:40
updated: 02.08.2018 15:43

Since the PCR was not successful it had to be repeated for ori on 01.08.2018

For each of five samples:

- 12,75µl H2O
- 5µl buffer
- 5µl High GC enhancer
- 0.5µl dNTPs
- 0.5µl Fwd primer (28)
- 0.5µl Rev primer (31)
- 0.5 µl Template
- 0.25 µl Q5 Polymerase

PCR steps:

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 70°C for 15sec(annealing)
    3. 72°C for 20sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

--> wrong annealing temperature was used!!

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

Author: Carina Gude
created: 02.08.2018 15:43
updated: 02.08.2018 15:44
Entry 158/259: PCR of ori
In Project: Level_1
With tags: pcr, Q5 PCR, Cyanobacteria

Since the last PCR was not successful due to the wrong annealing temperature it had to be repeated.

For each of five samples:

- 12,75µl H2O
- 5µl buffer
- 5µl High GC enhancer
- 0.5µl dNTPs
- 0.5µl Fwd primer (28)
- 0.5µl Rev primer (31)
- 0.5 µl Template
- 0.25 µl Q5 Polymerase

PCR steps:

1. 98°C for 2 min
2. cicle with
    1. 98°C for 15 sec(denaturation)
    2. 56°C for 15 sec(annealing)
    3. 72°C for 20sec(elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 10°C

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

Author: Jennifer Denter
created: 02.08.2018 21:52
updated: 02.08.2018 22:14

Entry 159/259: Isolation of p2iGEM0311 (pSHDY*)
In Project: Level_1
With tags: miniprep, plasmid, cyano

The plasmid p2iGEM0311 (pSHDY*) was isolated with the Promega plasmid isolation kit.

The inoculated cultures from the day before were used completely for plasmid isolation.

3 tubes in total are stored at -20 °C in 'cyano cloning box'

The plasmid is needed for restrictions and clonings.

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

Author: Sarah Seyffert
created: 03.08.2018 13:53
updated: 03.08.2018 17:33
Entry 160/259: pSHDY digest & extension PCR of overlap
In Project: Level_1
With tags: digest, pcr, overhang pcr

Repetition of digestion of pSHDY to get more yiel for the gelelution afterwards

- 7µl MiliQ
- 10µl template
- 2µl cutsmart
- 0.5µl EcoRI
- 0.5µl PstI

Incubate at 37°C for 3h

Inactivation at 80°C for 20 min

Extension PCR of yesterdays overlap PCR

To the PCR-tubes was added:

- 1µl Primer fw(1047)
- 1µl Primer rv (614)
- 0.5 Q5-polymerase

PCR programm:

1. 95°C 3 min
    1. 95°C 30 sec
    2. 65°C 20 sec
    3. 72 °C 1:30 min
    4. repeat this 30 times
2. 72°C 5 min
3. hold 12°C

stored at -20°C

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

Author: Susanne Vollmer
created: 03.08.2018 14:01
updated: 03.08.2018 17:48

Entry 161/259: testrestriction of teh retransformation of p2iGEM0308 and miniprep of the plasmids of p2iGEM0295 and p2iGEM0294
In Project: Level_1
With tags: miniprep, pcr, testrestriction, taq polymerase

testrestriction of p2iGEM0308 to test if the mutagenesis was sucsessfully, with the following protocol:

Sample:

- 1 µl Cutsmart
- 0,5 µl p2iGEM0308 (from the retransformation)
- 0,5 µl BbsI-HF
- 8 µl milli Q

incubation 3 h at 37°C

inactivation 20 min at 65°C

Miniprep of 7 colonies of each p2iGEM0294 (2iGEM0348, DVA_trzC) and p2iGEM0295 (2iGEM0353, DVA_atzD) with the follwing protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 Sek. max rpm --> discard supernatant
- add 200µ endotoxin removel wash
- add 400µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 45 min at RT. Centrifuge for 30 Sek. max

PCR of p2iGEM0295 and p2iGEM0294, each 7 colonies, to test if the Level 0 cloning was sucsessfully, with the following protocol:

Mastermix:

- 20,375 µl Milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl O_iGEM18_0068
- 0,5 µl O_iGEM18_0069
- 0,125 µl Taq polymerase

-add 24,5 µl mastermix in each tube and add 0,5 µl template

PCR Programm:

98°C 30 sec

98°C 15 sec

47°C 20 sec

68°C 100 sec -> repeat 30x -> for p2iGEM0295 1 min and 30 sec and for p2iGEM0294 1min and 45 sec

68°C 5 min

10°C HOLD

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

Author: Thomas Bick
Entry 162/259: verification of trzC, atzD PCR
In Project: Level_1
With tags: pcr, Lvl 0

created: 06.08.2018 14:53
updated: 06.08.2018 14:56

1% Agarose gel for test PCR of trzC and atzD:

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

Author: Carina Gude
Entry 163/259: Blunt end ligation of Dur1,2 p2 into pJET
In Project: Level_1
With tags: blunt-end cloning, Dur12, pJET, T4 ligase

created: 06.08.2018 16:22
updated: 06.08.2018 16:25

Blunt-end ligation of Dur,1,2 p2 in pJET blunt-end cloning Vector (Thermo Fisher):

2x reaction buffer : 10 µL

Dur1,2 p2 gBlock : 0.5 µL

pJET1.2/blunt Cloning Vector : 1 µL

Water, nuclease-free : 7.5 µL

T4 DNA Ligase: 1 µL

Total volume: 20 µL

Flick the tube briefly and centrifuge for 3-5 s.

Incubation of the ligation mixture NOT at room temperature (too hot), but at 16°C for 30 min.

Stored at -20°C until transformation.

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

Author: Jennifer Denter Entry 164/259: No entry title yet In Project: Level_1 No tags associated	created: 06.08.2018 20:45 updated: 06.08.2018 20:46
Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Thomas Bick

created: 07.08.2018 13:36
updated: 07.08.2018 18:13

Entry 165/259: mutagenesis of p2iGEM0308 and transformation into T10
In Project: Level_1
With tags: Mutagenesis, digest, transformation

mutagenesis of p2iGEM0308 with O_iGEM18_0076 and O_iGEM18_0077 to mutagenise unwanted pstI restriction side:

6x Mastermix

- 6 µl plasmid (of the 1:10 Dilution) (10ng/sample)
- 12 µl O_iGEM18_0074
- 12 µl O_iGEM18_0075
- 60 µl Q5-Buffer
- 6 µl dNTP
- 3µl Q5-polymerase(at last on ice!)
- 18µl DMSO
- 183 µl MiliQ77

6 tubes were filled with 50µl of the mastermix

Gradient PCR programm:

- 95°C 3 min
- 95°C 1min
  - 55°C 30 sec
  - gradient 71°C - 65°C 30 sec (65°C, 67°C, 68,5°C, 70°C, 71°C)
  - 72 °C 70 sec
  - repeat this 30 times
- 72°C 5 min
- 4°C HOLD

DpnI digestion of p2iGEM0332 (mutagesis of p2iGEM0308 with O_iGEM18_0076 and O_iGEM18_0077)

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

Transformation of p2iGEM0308 into *E. coli T10*: 2iGEM0373

- 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
- 1 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: Carina Gude
created: 07.08.2018 16:13
updated: 07.08.2018 16:23

Entry 166/259: Trafo of Dur1,2 p.2; ddh
In Project: Level_1
With tags: trafo, transformation, competent cells, ddh, Dur12, pJET

Transformation of p2iGEM0258 (5x) and p2iGEM0327 (2x)

- Thaw competent cells 15 minutes on ice
- Add 5 µl plasmid (for p2iGEM0258)/ 2µl (for p2iGEM0327) 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
- 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
created: 07.08.2018 21:28
updated: 09.08.2018 11:01
Entry 167/259: Pre-restriction of pSHDY
In Project: Level_1
No tags associated

Restriction of pSHDY with EcoRI & PstI

7,4 µl Plasmid (1µg)

5 µl Buffer (Cutsmart)

0,5 µl EcRI

0,5 µl PstI

36,6 µl Milli Q Water

Restriction run for 6 h at 37 °C.

Inactivation of the reaction for 25 min at 80 °C.

Afterwards the sample were stored at -20 °C.

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

Author: Sarah Seyffert

created: 07.08.2018 22:04

updated: 08.08.2018 00:38

Entry 168/259: Overhang PCR

In Project: Level_1

With tags: overhang pcr, gel purification, gel elution

Overhang PCR of *sps* and *Plac*

Two PCR tubes were filled with each containing

- 5µl Q5-Buffer
- 5µl GC enhancer
- 0.5µl dNTP
- 0.5µl Q5 polymerase
- 1 µl sps
- 5 µl Plac
- 8 µl MiliQ

PCR- Programm for overlap

- 95°C 3 min
  - 95°C 30 sec
  - 63°C 20 sec
  - 72 °C 1:30 min
  - repeat this 10 times
- 72°C 5 min
- hold 12°C

Extension PCR

To the PCR-tubes was added:

- 0.7µl Primer fw(1047)
- 0.7µl Primer rv (614)

PCR programm:

1. 95°C 3 min
    1. 95°C 30 sec
    2. 65°C 20 sec
    3. 72 °C 1:30 min
    4. repeat this 30 times
2. 72°C 5 min
3. hold 12°C

stored at -20°C

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 20 µl of nuclease free water and elute for 2 h at room temperature
- Centrifuge 1 min.
- Add 10 µl of nuclease free water and elute for 2 h at room temperature
- Store DNA at -20°C.

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

Author: Jennifer Denter
created: 09.08.2018 11:01
updated: 09.08.2018 11:02

Entry 169/259: Pre-restriction of pSHDY
In Project: Level_1
With tags: restriction, digestion

Restriction of pSHDY with EcoRI & PstI

7,4 µl	Plasmid (1µg)
5 µl	Buffer (Cutsmart)
0,5 µl	EcRI
0,5 µl	PstI
36,6 µl	Milli Q Water

Restriction run for 12 h at 37 °C.

Inactivation of the reaction for 25 min at 80 °C.

Afterwards the sample were stored at -20 °C.

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

Author: Jennifer Denter
Entry 170/259: Gelectrophoresis pSHDY (digested) & OH-PCR (Plac, sps)
In Project: Level_1
No tags associated

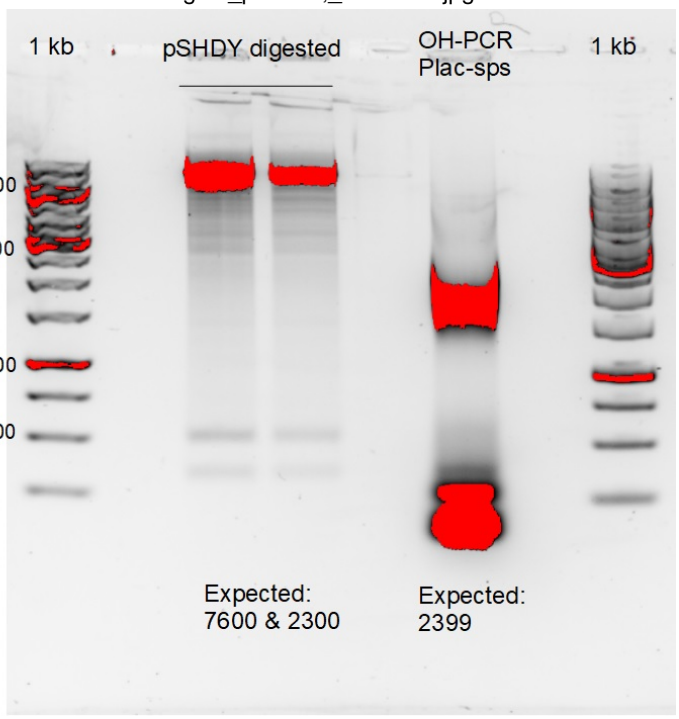
created: 09.08.2018 14:01
updated: 09.08.2018 14:07

Samples of pre digested pSHDY (p2iGEM0311) ans the OH-PCR of Plac & sps run in a 1% agarose gel.

70 min 90V.

Samples of the expected size will be used for gelelution in order to purify the DNA.

Digest_pSHDY,_OH-PCR.jpg



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

Author: Jennifer Denter Entry 171/259: No entry title yet In Project: Level_1 No tags associated	created: 09.08.2018 14:02 updated: 09.08.2018 14:02

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

Author: Carina Gude

created: 09.08.2018 14:15
updated: 13.08.2018 13:43

Entry 172/259: Gradient PCR of glf and invA of Pamelas strain

In Project: Level_1

With tags: gradient pcr, glf, invA, pcr, Q5 PCR

Since the last PCR did not work for glf and invA from Pamela's strain, a gradient PCR was done.

8x Mastermix:

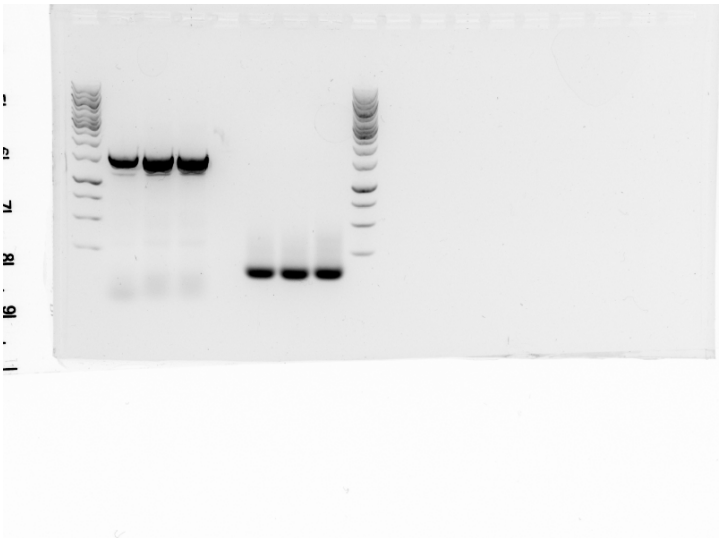
- 102 µl miliQ
- 40 µl Q5 buffer
- 40 µl GC-enhancer
- 4 µl dNTP
- 4 µl template (Pamela strain)
- 2 µl Q5-Polymerase

Each tube was filled with 24µl mastermix and 0,5µl of both primers

For glf and invA we used this gradient PCR-protocol

1. 98°C for 2 min
2. cycle with
    1. 98°C for 15 sec (denaturation)
    2. gradient between 56,4°C-62,4°C (glf: 62.4°C, 62°C, 61.3°C, 60.2°C; invA: 56.4°C; 57°C, 57.8°C, 58.8°C)
    3. 72°C for 60sec (elongation)
    4. repeat 30 times
3. 72°C for 1 min
4. Hold 12°C

glf_&_invA_Pam_PCR_for_gel_elution.tif



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

Author: Carina Gude
created: 09.08.2018 14:43
updated: 09.08.2018 14:47
Entry 173/259: Inoculation of Dur1,2 p2.
In Project: Level_1
With tags: inoculation, incubation, Dur12

After the transformation of E.coli DH5alpha with p2iGEM0258 (4x), 2 colonies were picked of each agar plate, inoculated in 3ml LB-amp and incubated over night.

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

Author: Sarah Seyffert
Entry 174/259: pSHDY digest
In Project: Level_1
With tags: digest, pSHDY

created: 09.08.2018 15:39
updated: 09.08.2018 15:40

Repetition of digestion of pSHDY

- 16µl MiliQ
- 10µl template
- 3µl cutsmart
- 0.5µl EcoRI
- 0.5µl PstI

Incubate at 37°C for 2h

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

Author: Susanne Vollmer
created: 09.08.2018 17:42
updated: 09.08.2018 18:42
Entry 175/259: PCR of guaD, trzC, atzD and gel electrophoresis
In Project: Level_1
With tags: pcr, gel electrophoresis

PCR of guaD, trzC and atzD with the follwing protocol:

sample:

- 5 µl Q5 Buffer
- 5 µl GC enhancer
- 0,2 µl Template
- 0,5 µl dNTP
- 1,5 µl Primer
- 1,5 µl Primer
- 0,2 µl Polymerase
- 1,5 µl DMSO
- 9,6 µl milli Q

Primer:

guaD: O_iGEM18_0064

O_iGEM18_0065

trzC: O_iGEM18_0080

O_iGEM18_0081

atzD: O_iGEM18_0064

O_iGEM18_0065

PCR programm:

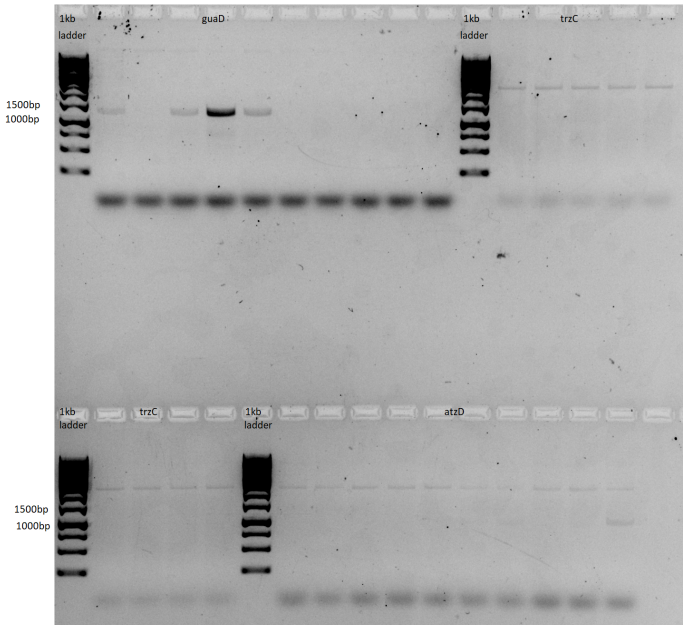
- 95°C 5 min
  - 95°C 20 sec
  - (66 (atzD), 65 (trzC, guaD))°C 20 sec
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 2 min
- hold 12°C

150ml 1% Agarose gel:

90V 45min

1kb ladder

Gel_2018-08-09_18hr_30min_CD_overhang_PCR_guaD,_trzC,_atzD_bearbeiten.png



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

Author: Susanne Vollmer

created: 10.08.2018 11:21
updated: 10.08.2018 18:50

Entry 176/259: PCR clean-up and gel from the PCR of atzD and guaD, test PCR of p2iGEM0258

In Project: Level_1

With tags: PCR Clean-Up, taq pcr

PCR clean-up of the PCR of guaD and atzD from the 9.8.18 with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- add a equal volume Membrane binding buffer to the tube.
- Fill it on the colum (incubate at room temperature for 5 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min 16000g --> discart the flow through
- add 500 µl washing buffer and centrifuge 5 min 16000g --> discart the flow through
- centrifuge empty column with out lid for 1 min 16000g
- Put column into a new tube
- incubate at roomtemperature until all the Ethanol is evaporated
- Add 30 µl of nuclease free water and elute for 1:30 h at room temperature
- Centrifuge 1 min 16000g.
- Store DNA at -20°C.

measured concentraition:

guaD: 63,250 ng/µl

atzD: 41,600 ng/µl

Taq PCR to test if the pJET cloning of Dur1,2 p2 was sucsessfully, with the following protocol:

Mastermix:

- 20,375 µl Milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl pJET 1.2 primer few
- 0,5 µl pJET 1.2 primer rev
- 0,125 µl Taq polymerase

-add 24,5 µl mastermix in each tube and add 0,5 µl template

PCR program:

98°C 30 sec

98°C 15 sec

57°C 20 sec

68°C 2min 15 sec -> repeat 30x

68°C 5 min

10°C HOLD

150ml 1% Agarose gel (7,5 µl GelRed)

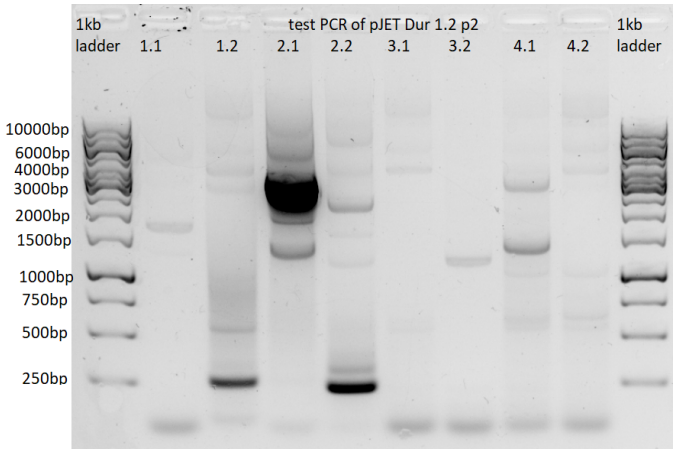
1kb ladder

90V

in each pocket: 5 µl sample, 5 µl milli Q, 2 µl loading dye

expectet band: 3000bp

Gel_2018-08-10_18hr_34min_90V_45_min_1kb,_PCR_pJET_Dur_1.2_p2;_infA;pSHDY_digest_bearbeitet.png



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

Author: Thomas Bick
created: 10.08.2018 13:37
updated: 10.08.2018 14:00
Entry 177/259: PCR atzD, trzC
In Project: Level_1
With tags: pcr, cloning

PCR of guaD, trzC and atzD with the follwing protocol:

sample:

- 5 µl Q5 Buffer
- 5 µl GC enhancer
- 0,2 µl Template
- 0,5 µl dNTP
- 1,5 µl Primer fwd
- 1,5 µl Primer rev
- 0,2 µl Q5 Polymerase
- 1,5 µl DMSO
- 9,6 µl Milli Q

Primer:

trzC: O_iGEM18_0080

O_iGEM18_0081

atzD: O_iGEM18_0064

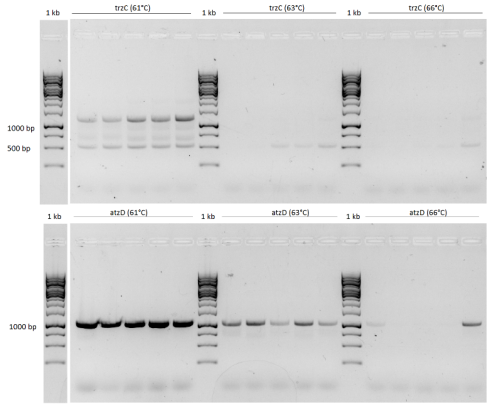
O_iGEM18_0065

PCR programm:

- 95°C 5 min
  - 95°C 20 sec
  - gradient: 61°C/63°C/66°C 20 sec
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 2 min
- hold 12°C

1% Agarose Gel of PCR atzD & trzC:

Gel_2018-08-10_13hr_33min_CD_overhang_PCR_trzC,_atzD.tif



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

Author: Sarah Seyffert
Entry 178/259: Touchdown PCR of invA
In Project: Level_1
With tags: tochdown pcr

created: 10.08.2018 14:24
updated: 10.08.2018 14:35

Touchdown PCR of invA from 2iGEM0314 gDNA

2x Mastermix

- 10µl Q5-buffer
- 10µl High GC
- 5µl DMSO
- 1µl dNTPs
- 1µl primer fw(1039)
- 1µl primer rv(1040)
- 0.5 µl template
- 0.5µl Q5-polymersae
- 20.5µl miliQ

25µl of the Mastermix were filled in the Tube

Touchdown PCR programm

- 95°C 5 min
  - 95°C 30 sec
  - 65°C 20 sec(decrease of -0.3°C every cycle)
  - 72 °C 1 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
created: 12.08.2018 13:47
updated: 12.08.2018 15:51
Entry 179/259: Restriction of pSHDY (p2iGEM0311)
In Project: Level_1
With tags: restriction, gelelectrophoresis, pSHDY

pSHDY (p2iGEM0311) was restricted with EcoRI & PstI fpr 2 h

Used:

500 ng of plasmid DNA

0.5 µl of each Enzyme

5 µl of 10X Buffer

Fill up to 50 µl with Milli Q Water

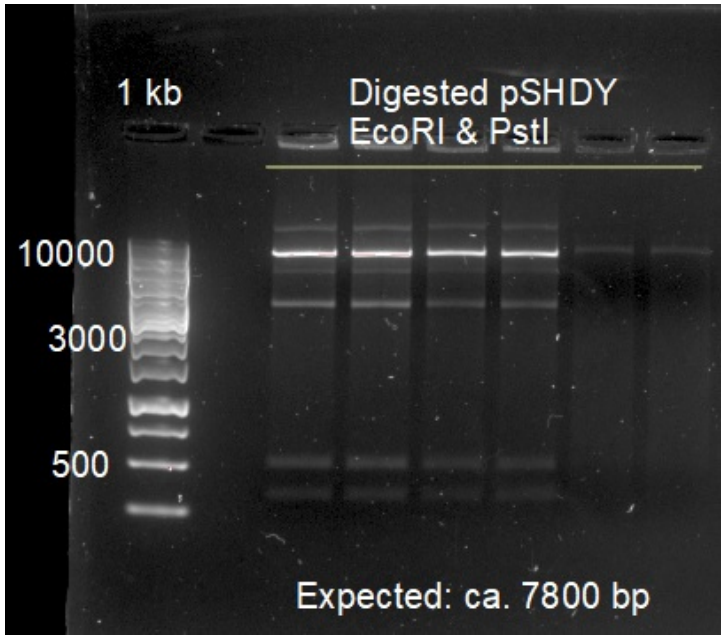
3 samples were restricted for 2 h at 37 °C.

Inactivation run for 30 min at 78 °C.

1% gelelectrophoresis was performed with the complete sample each in order to elute the correct fragment later on.

A small bnd of expected fragment was cut out of the gel and stored at -20 °C for a further gel purification.

digest_pSHADY_all_samples.jpg



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

Author: Carina Gude
created: 13.08.2018 13:21
updated: 13.08.2018 16:33
Entry 180/259: glf gel elution
In Project: Level_1
With tags: glf, gel elution

Gel elution with the promega Kit to purify glf PCR product

- Bands were cut under UV light and the weight measured (0,54g)
- Per 10 mg of gel 10 µl of Membrane binding buffer (540µl) was added to the tube.
- Incubation at 50 °C till the gel was dissolved in the buffer (around 7 min)
- Dissolved gel mixture was filled into the column, incubated at room temperature for 1 minute and centrifuged at 16000xg für 1 min --> flow through was discarded
- addition of 700 µl washing buffer and centrifuge 1 min --> discard flow through
- addition of 400 µl washing buffer and centrifuge 5 min --> discard flow through
- centrifuge empty column for 1 min with open lid to allow for evaporation
- Put column into a new 1,5 ml tube
- Add 30 µl of nuclease free water and incubate for 1 h at room temperature
- Centrifuge 1 min.
- Add 20 µl of nuclease free water and centrifuge 1min
- Store DNA at -20°C.

Concentration: 20,15 ng/µl

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

Author: Susanne Vollmer
created: 13.08.2018 13:31
updated: 14.08.2018 09:41

Entry 181/259: Gel elution of the PCR of trzC and PCR clean up from the PCR of atzD
In Project: Level_1
With tags: gel electrophoresis, gel elution, PCR Clean-Up

1% Agarose gel (50ml)

90V 70 min

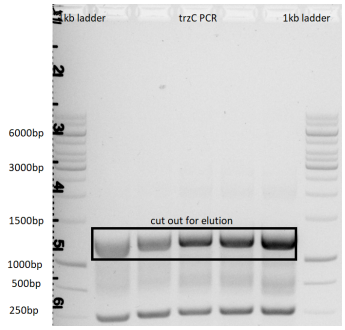
1kb ladder

in each pocket: 12 µl PCR sample and 3 µl loading Dye

cut out the 1300bp band

gel picture below

Gel_2018-08-13_15hr_48min_gel_elution_trzC_PCR_bearbeitet.png



Gel Elution and PCR clean-up with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- PCR:
  - add a equal volume Membrane binding buffer to the tube.
- Gel:
  - ad 10 µl Membran binding solution per each 10 mg gel
  - incubate at 65 °C and Vortex until the gel is completely deluted
- follow the rest of the protocol for both:
- Fill it on the colum (incubate at room temperature for 5 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min 16000g --> discart the flow through
- add 500 µl washing buffer and centrifuge 5 min 16000g --> discart the flow through
- centrifuge empty column with out lid for 1 min 16000g
- Put column into a new tube
- incubate at roomtemperature until all the Ethanol is evaporated
- Add 30 µl of nuclease free water and elute for 2:30 h at room temperature
- Centrifuge 1 min 16000g.
- cause only 15 µl are left-> again Elution with 20 µl 15 min incubation at RT, than again centrifuge
- Store DNA at -20°C.

measured concentraition: about 6 for the gel elution, about 70 ng/µl for the PCR clean-up

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

Author: Susanne Vollmer

created: 14.08.2018 09:39
updated: 14.08.2018 16:14

Entry 182/259: Digest of the CIDAR entry vector and gel elution, and dpnI digest of the mutagenesis of p2iGEM0308

In Project: Level_1

With tags: digest, gel elution

Digest of the CIDAR entry Vector for gel Elution:

- 200ng of the Backbone (p2iGEM0174)
- 2,5 µl CutSmart
- 0,5 µl BbsI enzyme
- add milli Q up to 25 µl

incubate at 37°C 2 h

inactivation at 65°C for 20 min

DpnI Digest of the PstI mutagenesis of p2iGEM0308:

Transfer the 50 µl in a Eppendorf tube and add 1 µl DpnI Enzyme

incubate at 37°C for 1.30 h

inactivation at 80°C for 20 min

1% agarose gel 50ml

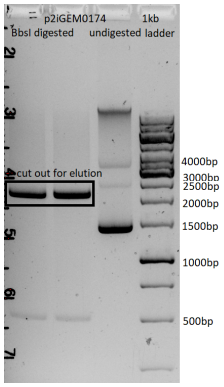
90V

1kb ladder

in the pocket: 1: 12 µl digested p2iGEM0174 and 3 µl loading dye 2: 12 µl digested p2iGEM0174 and 3 µl loading dye 3: 2 µl undigested p2iGEM0174 3 µl milli Q and 1 µl loading Dye

gelpicture below

Gel_2018-08-14_13hr_21min_digest_p2iGEM0174,_undigest_p2iGEM0174,_pSHDY_undigest,_pSHDY_digest_bearbeitet.png



Gel Elution with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- add 10 µl Membran binding solution per each 10 mg gel
- incubate at 65 °C and Vortex until the gel is completely deluted
- Fill it on the colum (incubate at room temperature for 5 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min 16000g --> discart the flow through
- add 500 µl washing buffer and centrifuge 5 min 16000g --> discart the flow through
- centrifuge empty column with out lid for 1 min 16000g
- Put column into a new tube
- incubate at roomtemperature until all the Ethanol is evaporated
- Add 30 µl of nuclease free water and elute for 45 min at room temperature
- Centrifuge 1 min 16000g.
- again Elution with 10 µl 10 min incubation at RT, than again centrifuge
- Store DNA at -20°C.

measured concentration: 4,600 ng/µl

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

Author: Sarah Seyffert
Entry 183/259: pSHDY digestion
In Project: Level_1
With tags: digest

created: 14.08.2018 10:04
updated: 14.08.2018 10:08

To test why the digestion of pSHDY is mostly unsuccessful we tried three different digestions.

One with EcoRI.

One with PstI.

One with both enzymes.

Fill in each tube

- 2µl template(ca 120 ng)
- 1µl cutsmart
- 0,5µl enzyme
- fill up to 10µl MiliQ

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

Author: Jennifer Denter
created: 14.08.2018 21:39
updated: 14.08.2018 21:52
Entry 184/259: Preparation of Gibson Assembly Mastermix
In Project: Level_1
With tags: Gibson, Gibson assembly mix, Gibson Asembly

Gibson Assembly Mastermix was prepared and thereafter stored in 15 µl aliquotes (PCR tubes) at -20 °C.

Mastermix was prepared on ice under sterile conditions.

PCR tubes were pre-cooled as well.

Following protocole was used:

Solution	Concentration	Amount
isothermal buffer	5 x	100 µl
Taq DNA Ligase	40 U/µl	50 µl
T5 Exonuclease	1 U/µl	2 µl
Q5 Hi-Fi Polymerase	2 U/µl	6,25 µl
Milli Q Water	-	216,75 µl

Full protocole is also available here:

https://www.protocols.io/view/homemade-gibson-mastermix-n9xdh7n

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

Author: Susanne Vollmer
created: 15.08.2018 09:43
updated: 15.08.2018 15:46
Entry 185/259: Restriction and ligation of guaD, trzC, atzD
In Project: Level_1
With tags: restriction, ligation

Restriction of the PCR clean-up of guaD and atzD and of the gel eluted trzC, with the following protocol:

guaD:

- 4,8 µl template
- 1,5 µl CutSmart
- 8,7 µl milli Q
- 0,2 µl BbsI-HF

trzC:

- About 39 µl template
- 5 µl CutSmart
- 5 µl milli Q
- 0,5 µl BbsI-HF

atzD:

- 4,5 µl template
- 1,5 µl CutSmart
- 9 µl milli Q
- 0,2 µl BbsI-HF

in General About 300 ng Template

incubate at 37°C for 1h

inactivation at 65°c for 20 min

Ligation of the guaD, atzD and trzC in the CIDAR entry vector with the follwing protocoll:

guaD

- 2,3 µl Backbone (gel eluted)
- 1 µl T4 Ligase
- 2 µ T4 Buffe
- 9,7 µl milli Q
- 5 µl from the guaD restriction

trzC:

- 2,3 µl Backbone (gel eluted)
- 1 µl T4 Ligase
- 2 µ T4 Buffe
- 22,3 µl milli Q
- 16 µl trzC from the trzC restriction

atzD:

- 2,3 µl Backbone (gel eluted)
- 1 µl T4 Ligase
- 2 µ T4 Buffe
- 9,7 µl milli Q
- 5 µl from the atzD restriction

in general a ratio of 10 backbone:100 insert

incubation at RT for about 1 h (over lunch)

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

Author: Carina Gude
created: 15.08.2018 10:19
updated: 15.08.2018 10:26
Entry 186/259: Retrafo p2iGEM2058 (pJET_Dur 1,2 p2)
In Project: Level_1
With tags: retrafo, Kryo, Retransformation, Dur12

14.08.2018

Retransformation of 2iGEM0278/p2iGEM0258

- 1 µl Plasmid + 15 µl DH5alpha competent cells
- 5 min incubation on ice
- heat shock for 45 sec at 42 °C
- 2 min Incubation on ice
- added 300 µl LB
- 15 min Incubation at 37 °C while shaking --> centrifugation 1min 6000rpm
- transferred to culture tube with 1ml LB + ampicillin
- Incubation at 37°C overnight

15.08.2018

Kryo preparation of 2iGEM0278

- 300µl Glycerin + 700µl overnight culture

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

Author: Carina Gude

created: 15.08.2018 11:58
updated: 15.08.2018 13:14

Entry 187/259: extension PCR of overlap products trzE and Dur1,2 p1
In Project: Level_1
With tags: Dur12, trz, extension PCR

extension PCR of overlap products trzE and Dur1,2 p1

Mastermix for 3 samples:

- 30 µl Milli Q
- 15 µl Q5 buffer
- 15 µl GC enhancer
- 4,5 µl DMSO
- 4,5 µl rev Primer
- 4,5 µl fwd Primer
- 0,6 µl Template (overhang prodct of trzE and Dur1,2 p1; 11.06.2018)
- 0,3 µl dNTP
- 0,6 Q5 Polymerase

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - 68°C 20 sec
  - 72°C 1 min 45 sec
- 72°C 2min
- hold on 4°C

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

Author: Sarah Seyffert
Entry 188/259: pSHDY digest
In Project: Level_1
With tags: digest

created: 15.08.2018 15:17
updated: 15.08.2018 15:18

Testdigestion of pSHDY to see if it really is pSHDY

- 6.5µl MiliQ
- 2µl template
- 1µl cutsmart
- 0.5µl BbsI

Incubate at 37°C for 2h

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

Author: Susanne Vollmer
Entry 189/259: gel electrophoresis of the extention PCR
In Project: Level_1
With tags: gel electrophoresis

created: 15.08.2018 15:46
updated: 15.08.2018 15:50

1% agarose gel

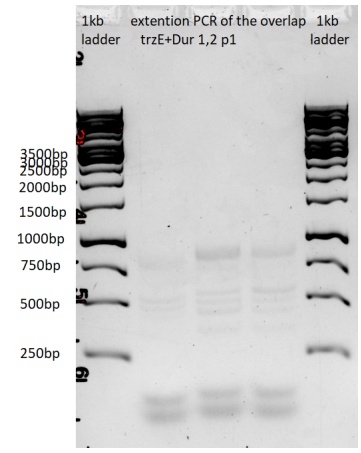
90 V 50 min

1kb ladder

in each pocket 3 µl PCR product, 1 µl loading Dye, 2 µl milli Q

gel picture below: expected Band: 3600 bp

Gel_2018-08-15_14hr_56min_90V_50min_1kb_ladder,_extention_PCR_of_overlap_trzE+Dur1,2_p1_bearbeitet.png



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

Author: Thomas Bick
created: 15.08.2018 15:49
updated: 15.08.2018 15:53

Entry 190/259: transformation of 2iGEM0347, 2iGEM0348, 2iGEM0353
In Project: Level_1
With tags: DVA, Lvl 0, transformation

transformation of 2iGEM0347, 2iGEM0348 and 2iGEM0353

3 transformations were done for each plasmid with 3 different ligation samples

- 5 µl Plasmid + *E. coli* T10 competent cells
- 10 min incubation on ice
- heat shock for 45 sec at 42 °C
- 30 min Incubation on ice
- added 300 µl LB
- 15 min incubation at 37 °C while shaking --> centrifugation 1min 4000rpm
- transfered to culture tube with 3ml LB + ampicillin
- Incubation at 37°C overnight

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

Author: Carina Gude

created: 16.08.2018 13:32
updated: 16.08.2018 16:11

Entry 191/259: Gradient extension PCR with gel electrophoresis of trzE-Dur1,2p1

In Project: Level_1

With tags: gel electrophoresis, extension PCR, gradient pcr, pcr, Dur12, trz, Q5 PCR

Since the last extension PCR did not show the expected bands on the gel, the extension PCR was repeated with more samples and in a gradient to test different temperatures for the primer.

gradient extension PCR:

Mastermix for 6 samples:

- 60 µl Milli Q
- 30 µl Q5 buffer
- 30 µl GC enhancer
- 9 µl DMSO
- 9 µl rev Primer (59)
- 9 µl fwd Primer (34)
- 1,2 µl Template (overhang prodct of trzE and Dur1,2 p1; 11.06.2018)
- 0,6 µl dNTP
- 1,2 µl Q5 Polymerase

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - gradient of 66°C, 66,9°C, 68,2°C, 68,8°C, 70,1°C, 71°C for 20 sec
  - 72°C 1 min 45 sec
- 72°C 2min
- hold on 4°C

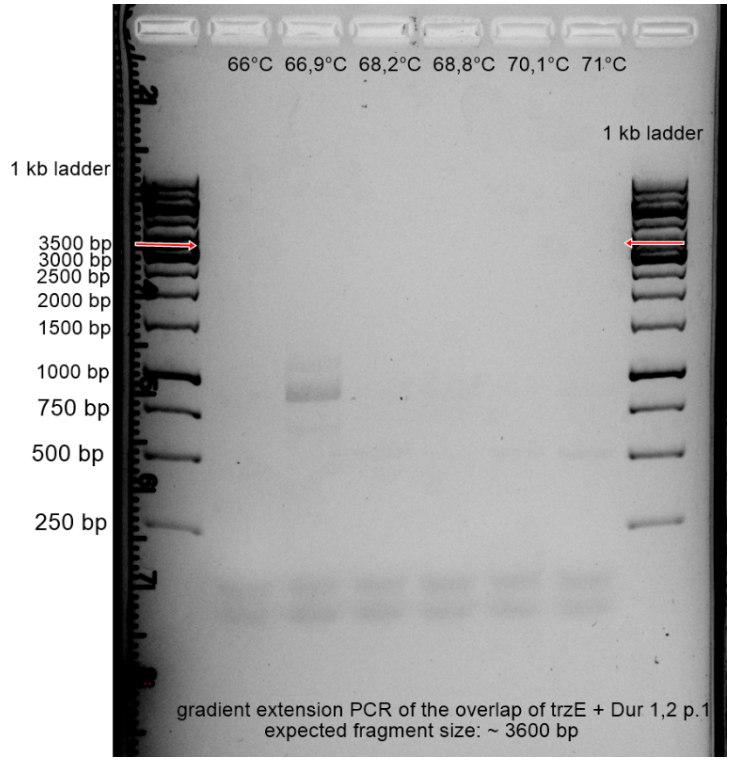
Gel electrophoresis:

90 V 50 min on 1% agarose gel with 1kb ladder

in each pocket 3 µl PCR product, 1 µl loading dye, 2 µl milli Q

gel picture below: expected Band ~ 3600 bp

trzE_Dur1,2_p1_overhang_extension_gradient_PCR.tif



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

Author: Thomas Bick
created: 16.08.2018 18:46
updated: 16.08.2018 18:51
Entry 192/259: pstI mutagenesis prep & test digest
In Project: Level_1
With tags: Mutagenesis, miniprep, gel electrophoresis, digest

Plasmid Miniprep p2iGEM0332

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

test digest with pstI:

2 µl plasmid

0.2 µl pstI-HF

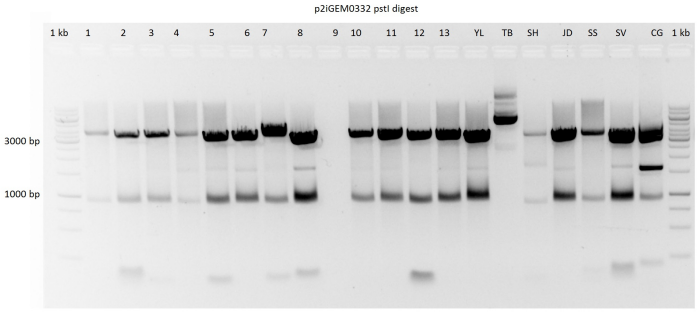
1 µl cutsmart

7 µl MilliQ

incubate 37°C 1 hour

1% Agarose Gel:

Gel_2018-08-16_18hr_05min_p2iGEM0332_pstI_sdm.jpg



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

Author: Jennifer Denter
created: 16.08.2018 20:15
updated: 16.08.2018 20:40
Entry 193/259: BG11 plates with 5% LB (for Cyano transformation)
In Project: Level_1
With tags: cyano, cyano transformation, bg11 plates

2 plates of BG11 media with 5% LB media were prepared.

Used were following solutions

2x BG11 Media

1,5% Wateragar

LB medium

For each plate a total volume of 25 ml was used.

2,5 ml LB medium was mixed with

23,75 ml of 2X BG11 and

23,75 ml of 1,5% wateragar (reheated / liquid)

unter totally sterile conditions.

25 ml of the solution was filled into each of 2 plates.

Plates dryed onter sterile conditions as well.

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

Author: Jennifer Denter
created: 16.08.2018 20:43
updated: 16.08.2018 21:16
Entry 194/259: Inoculation of Cyano cultures (preparation for Cyano transformation)
In Project: Level_1
With tags: cyano inoculation, cyano cultures, cyano transformation

OD of longtime liquid Cyano cultures were measured in a dilution of 1/10.

Undiluted:

Pams strain: OD_{750}=1,67

Danys strain: OD_{750}= 1,69

In 1 liter of 1xBG11 was filled:

1 ml Fe(III) chloride

200 µl $CuSo_4$

In total 2 flasks (300 ml) with in total 40 ml culture were prepared with an total OD_{750} = 0,4.

29 ml of fresh BG11 media with each 11 ml of long time culture.

Culrures were incubated ar 220 rpm, 30 °C.

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

Author: Jennifer Denter
created: 16.08.2018 21:28
updated: 16.08.2018 22:43
Entry 195/259: Inoculation of transformants of Gibson Assembly (p2iGEM0343)
In Project: Level_1
With tags: gibson assembly, Gibson, cyano

2 samples of Gibson assembly were done with the previous prepared mastermix.

2 aliquotes of mastermix were placed on ice.

Cutted and eluted pSHDY (p2iGEM0311) & eluted sample of Overhang PCR of sps and Plac were placed on ice as well.

Eluted samples were added in ratio 1/3 into the mastermix each.

Reaction run in a cycler with following conditions:

45 min 50 °C --> thereafter 9 °C.

Gibson Assemblys were transformed with the heat shock method in E. coli T10.

Cells of each transformation were plated 100% on LB-Kanamycin_Spectinomycin and incubated at 37 °C o/n.

6 colonies were picked and plated on new LB-kanamycine_spectinomycine media and as well inoculated in liqudi media with the same conditions.

plates were incubated at 37 °C o/n.

Liquid cultures incubatet at 37 °C, 220 rpm o/n.

Liquid samples will be used for plasmid isolation ans sending them for sequencing. Plates will be used as backup.

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

Author: Carina Gude
created: 17.08.2018 11:46
updated: 22.08.2018 10:13

Entry 196/259: PCRs of trzE, Dur1,2 p1; Dur1,2 p2; and extension gradient PCR of overhang_trzE,Dur1,2 p1
In Project: Level_1
With tags: extension PCR, gradient pcr, trz, Dur12, gel electrophoresis

PCRs of the following samples were done to repeat the previously failed extension gradient PCR, to prepare an overhang PCR (in case the overhang template is wrong) and to amplify the Dur1,2 p2 for further experiments in case the extension PCR is positive.

Mastermix for 10 samples:

- 100 µl Milli Q
- 50 µl Q5 buffer
- 50 µl GC enhancer
- 15 µl DMSO
- 1 µl dNTP
- 2 µl Q5 Polymerase

for trzE each sample:

- 21,8 µ Mastermix
- add 0,2 Template (p2iGEM0256)
- add 1,5 µl Primer O_iGEM18_0034
- add 1,5 µl Primer O_iGEM18_0035

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - 67°C for 20 sec
  - 72°C 15 sec elongation time
- 72°C 2min
- hold on 4°C

for Dur1,2 p1:

- 21,8 µ Mastermix
- add 0,2 Template (p2iGEM0257)
- add 1,5 µl Primer O_iGEM18_0036
- add 1,5 µl Primer O_iGEM18_0035

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - 66°C for 20 sec annealing
  - 72°C 35 sec elongation time
- 72°C 2min
- hold on 4°C

for Dur1,2 p2:

- 21,8 µ Mastermix
- add 0,2 Template (p2iGEM0258)
- add 1,5 µl Primer O_iGEM18_0060
- add 1,5 µl Primer O_iGEM18_0061
-

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec denaturation
  - 64°C for 20 sec annealing
  - 72°C 35sec elongation time
- 72°C 2min
- hold on 4°C

Mastermix for gradient PCR of overlap trzE+Dur1,2 p1

- 29,1µl Milli Q
- 15 µl Q5 buffer
- 15 µl GC enhancer
- 4,5 µl DMSO
- 0,3 µl dNTP
- 0,6 µl Q5 Polymerase
- 4,5 µl Primer O_iGEM18_0034
- 4,5 µl Primer O_iGEM18_0059
- 1,5 µl Template (overlap PCR from 11.6.18)

--> distributed in three tubes

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - gradient of 66°C, 66,9°C, 68,2°C for 20 sec
  - 72°C 1 min 45 sec
- 72°C 2min
- hold on 4°C

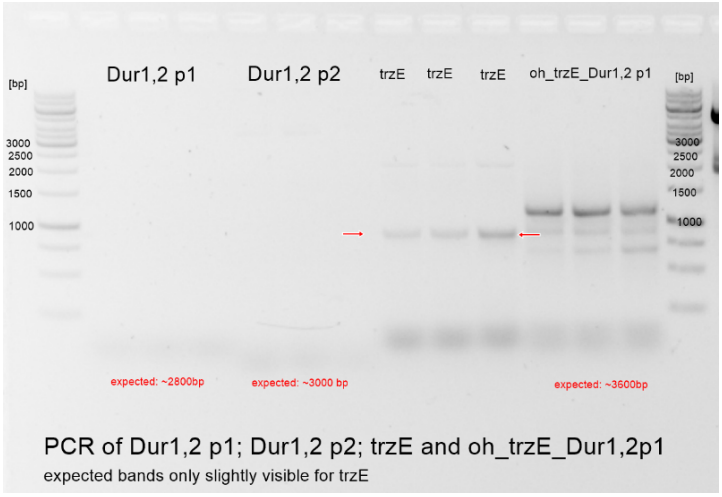
Gel electrophoresis:

90 V about 50 min on 1% agarose gel with 1 kb ladder

in each pocket 3 µl PCR product, 1 µl loading dye, 2 µl milli Q

--> negative except for trzE, which showed slightly visible expected bands

Gel_2018-08-17_16hr_55min_Ylenia,Carina,_Susanne.tif



PCR of Dur1,2 p1; Dur1,2 p2; trzE and oh_trzE_Dur1,2p1

expected bands only slightly visible for trzE

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

Author: Sarah Seyffert
created: 17.08.2018 18:29
updated: 17.08.2018 18:33
Entry 197/259: Transformation of gibsonassembly
In Project: Level_1
With tags: Transformation, pSHDY

Transformation of p2iGEM0343 plasmid in E.coli TOP10 (2iGEM0391)

- Thaw competent cells 15 minutes on ice
- Add 1.5 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+Spec) and stored in the 37°C incubator

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

Author: Jennifer Denter
Entry 198/259: Plasmidisolation & inoculation of transformants (Gibson assembly)
In Project: Level_1
With tags: cyano, conjugation, pSHDY, inoculation

created: 18.08.2018 10:37
updated: 18.08.2018 10:48

Plasmid DNA of all 6 pre-inoculated transformants (Gibson assembly p2iGEM0346, pSHDY_P_{lac}_*sps*) was isolated with the Promega Pure Yield Isolation Kit.

2 samples were sent randomly for sequencing (700 ng of DNA each).

Sample 2

Sample 4

As a preparation for the transformation into *Synechococcus elongatus* (strains of Dany Ducat and Pamela Silva) the clones 2 & 4 were inoculated from plate in 3 ml LB with antibiotic treatment.

Also 2 culture of *E. coli* RP4 was inoculated from plate in 3l ml LB with antibiotic treatment.

Cultures incubated o/n at 37 °C, 220 rpm.

Depending on the sequencing data the transformation will be performed with either transformant 2 or transformant 4.

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

Author: Jennifer Denter
created: 18.08.2018 10:48
updated: 18.08.2018 11:00

Entry 199/259: Transformation of Gibson Assembly (pSHDY_Plac_sps)
In Project: Level_1
With tags: trnasformation

In order to have a backup if further Gibson Assemblies failed a 3rd Assembly was performed.

1,5 µl of the cloning sample were transformed into *E. coli* T10 with the heat shock method.

Cells were platet to 100% on LB with antibiotic treatment: kanamycine & spectinomycine.

Plate incubatet o/n at 37 °C.

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

Author: Jennifer Denter
created: 18.08.2018 11:04
updated: 18.08.2018 11:46
Entry 200/259: Induction of cyano cultures
In Project: Level_1
With tags: induction, Cyanobacteria

Previous inoculated cultures of *Synechococcus elongatus* (Dany Ducats strain & Pamela Silvas strain) were induced to produce / export carbon.

Induction with NaCl and IPTG

- 150 mM CaCl

- 1mM IPTG

Directly before inoculation 1 ml of medium was taken and stored at -20 °C for HPLC analysis.

After induction of cultures flasks were incubated for 24 h.

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

Author: Jennifer Denter
created: 19.08.2018 13:03
updated: 19.08.2018 13:07

Entry 201/259: Test restriction p2iGEM0346 (pSHDY_plac_sps)
In Project: Level_1
With tags: pSHDY, restriction, cyano cloning

To check for a correct insertion of plac & sps into pre restricted pSHDY, test restriction was performed.

4 samples were cut each with EcoRI & PstI for 1,5 h at 37 °C.

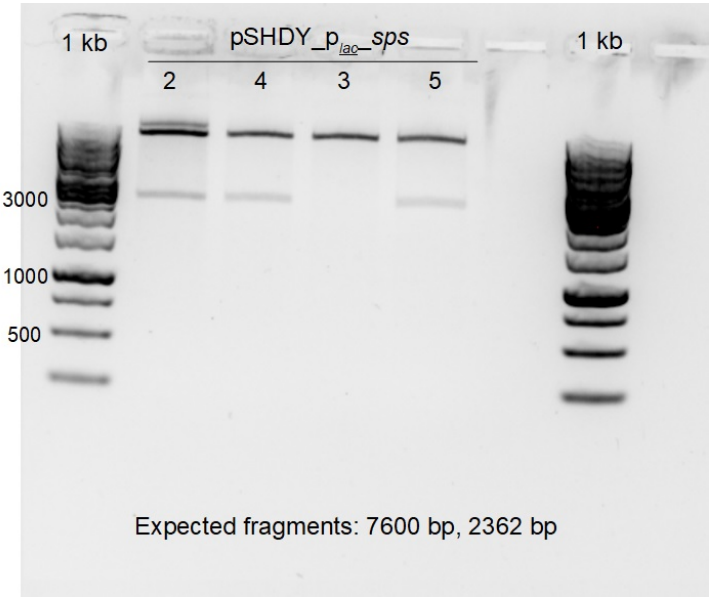
Thereafter reaction was inactivated at 75 °C.

Gelelectrophoresis showed fragment sizes.

Samples 2 & 4 were sent to sequencing before. Sequencing showed an insertion of the expected fragment.

Fragments in gelelectrophoresis showed not the exact ecxpected fragments.

Test_restriction_Gibson_Assembled_pSHDY.jpg



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

Author: Jennifer Denter
created: 19.08.2018 13:47
updated: 19.08.2018 14:11

Entry 202/259: Transformation of S. elongatus (1st & 2nd mating step)
In Project: Level_1
No tags associated

As preparation *S. elongatus* strains (Dany´s strain & Pam´s strain) were inoculated at OD_{750} = 0,4 and incubated o/n.

The color on the next day was fresh green.

For the transformation pre - inoculated *E. coli* RP4 and *E.coli* T10 containing the plasmid for transformation (pSHDY_p_{lac}_*sps*) were diluted in fresh LB medium without antibiotic treatment.

- 4,875 ml fresh LB

- 125 µl of overnight culture

Samples were incubated 2,5 h at 37 °C 220 rpm.

Cells were centrifuged at 2500 rpm for 8 min and the supernatant was discarded.

Cells were resuspended in 1 ml fresh LB media.

(Cultures looked thick at this time)

1 ml of *E.coli* RP4 & 1 ml of *E. coli* T10 containing plasmid of interest were mixed together (not vortexed)!

At this point 2 samples were prepared for the transformation into 2 different *S. elongatus* strains.

Cell mixtures were spinned down at 2500 rpm for 5 min.

Supernatant was discarded and cells resuspended in the backflow (30 µl) and incubated at 30 °C for 1 h (not shaking)!

--> At this point cultures shaked for 30 min, cause I forgot to place them non shaking. I corrected this step after 30 min and incubated cultures for another hour at 30 °C without shaking.

After incubation 0,9 ml of Cyano culture was added to the samples.

Tha samples were spinned down at 2500 rpm for 5 min.

Supernatant was carefully discarded (to not throw away vell pellet).

30 µl of BG11 media was added and cells resuspendet (not vortexed)!

30 µl of mixture was droped onto prepared BG11 plates containing 5% LB and dryed unter sterile conditions.

Plates were closed with Micropore tape and set in the incubator. Plates were also darkened with a papertowel.

Full Protocol is available here:

https://www.protocols.io/view/triparental-mating-of-synechocystis-ftpbnmn?step=7

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

Author: Susanne Vollmer
created: 20.08.2018 11:06
updated: 20.08.2018 17:07
Entry 203/259: PCR of Dur 1,2 p1 and p2
In Project: Level_1
With tags: pcr

PCR to amplify Dur 1,2 p1 and Dur 1,2 p2 with the following protocol:

Mastermix:

- 4 µl Q5 Reaktion Buffer
- 0,5 µl dNTP mix
- 0,5 µl Q5 polymerase
- 0,6 µl DMSO
- 14,4 µl milli Q

add for Dur 1,2 p1 to 20 µl of the mastermix:

- 1 µl p2iGEM0257
- 1 µl O_iGEM18_0036
- 1 µl O_iGEM18_0059

add for Dur 1,2 p2 to 20 µl of the mastermix:

- 1 µl p2iGEM0258
- 1 µl O_iGEM18_0060
- 1 µl O_iGEM18_0061

PCR Programm:

- 98°C 2 min
  - 98°C 30 sec
  - 72°C 30 sec
  - 72°C 90 sec -> repeat 34 times
- 72°C 10 min
- 4°C HOLD

1% Agarose gel

90V 1h

1kb ladder

into the gel pockets:

For Dur 1,2 p1 and Dur 1,2 p2: 3 µl PCR product, 2 µl milli Q, 1 µl loading Dye

For trzE gel Elution: 22 µl PCR product, 3 µl milli Q, 5 µl loading Dye

expected fracment size:

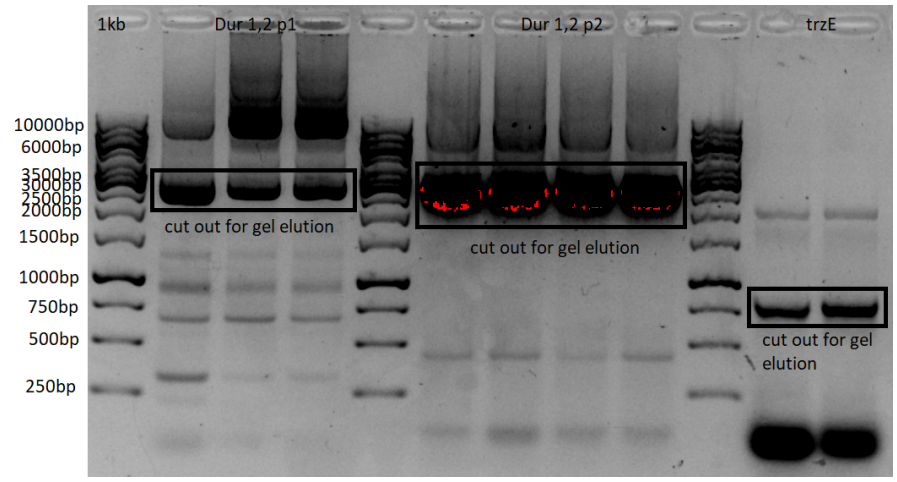
Dur 1,2 p1: 2818 bp

Dur 1,2 p2: 2955 bp

TrzE: 726 bp

gel Picture below:

Gel_2018-08-20_16hr_25min_Test_restriction_DVA_und_Dur1,2+trz_PCRs_bearbeitet.png



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

Author: Thomas Bick

created: 20.08.2018 14:05
updated: 20.08.2018 14:21

Entry 204/259: sequencing of p2iGEM0332, p2iGEM0294 & p2iGEM0295

In Project: Level_1

With tags: sequencing

sequencing of p2iGEM0332, p2iGEM0294 & p2iGEM0295 for possible positiv clones:

Number	Template	Primer
23GI89	p2iGEM0332_TB	pJET1.2 fwd
23GI90	p2iGEM0332_TB	pJET1.2 rev
23GI91	p2iGEM0294_3	O_iGEM_68
23GI92	p2iGEM0294_3	O_iGEM_69
23GI93	p2iGEM0294_4	O_iGEM_68
23GI94	p2iGEM0294_4	O_iGEM_69
23GI95	p2iGEM0294_5	O_iGEM_68
23GI96	p2iGEM0294_5	O_iGEM_69
23GI97	p2iGEM0295_1	O_iGEM_68
23GI98	p2iGEM0295_1	O_iGEM_69
23GI99	p2iGEM0295_2	O_iGEM_68
23GJ00	p2iGEM0295_2	O_iGEM_69
23GJ01	p2iGEM0295_3	O_iGEM_68
23GJ02	p2iGEM0295_3	O_iGEM_69
23GJ03	p2iGEM0295_4	O_iGEM_68
23GJ04	p2iGEM0295_4	O_iGEM_69
23GJ05	p2iGEM0295_5	O_iGEM_68
23GJ06	p2iGEM0295_5	O_iGEM_69

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

Author: Sarah Seyffert
Entry 205/259: PCR of glf
In Project: Level_1
With tags: touchup, pcr

created: 21.08.2018 11:26
updated: 21.08.2018 11:54

Amplification of glf from *Z. mobilis* gDNA as well as from Pamela Silvers *S. elongatus* strain

2xMastermix (done 2 times by two persons)

- 10 µl Q5-Buffer
- 10 µl GCenhancer
- 1 µl dNTP
- 0.5µl Q5-polymerase(at last on ice!)
- 4µl DMSO
- 23 µl miliQ

Add in a tube

- 22µl Mastermix
- 0.5µl fw primer(1039 or 22)
- 0.5µl rv primer(1040 or 25)
- 0.5 gDNA(Pam's strain or *Z. mobilis*)

Touch up PCR programm

- 95°C 3 min
  - 95°C 30 sec
  - 55°C 30 sec
  - Tuch-up 51°C (increase of 0.4°C every cycle)
  - 72 °C 1 min
  - repeat this 30 times
- 72°C 5 min
- hold 12°C

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

Author: Susanne Vollmer
created: 21.08.2018 13:13
updated: 28.08.2018 09:32
Entry 206/259: Testrestriction and testPCR of DVA_guaD, retrafo of p2iGEM0255, 5
In Project: Level_1
With tags: taq pcr, testrestriction, gel electrophoresis, Retransformation

TestPCR to test if guaD was successfully in DVA (p2iGEM0309) with the following protocol:

Mastermix:

- 20,375 µl milli Q
- 2,5 µl 10x Termopol Buffer
- 0,5 µl dNTP
- 0,5 µl O_iGEM18_0068
- 0,5 µl O_iGEM18_0069
- 0,125 µl Taq polymerase

add to 24,5 µl mastermix 0,5 µl Template

PCR Programm:

98°C 30 sec

- 98°C 15 sec
- 47°C 20 sec
- 68°C 100 sec

68°C 2 min

4 °C HOLD

Testrestriction to test if the DVA_guaD cloning was successfully with the following protocol:

mastermix:

- 1 µl Cutsmart
- 0,25 µl PvuI-HF
- 1,5 µl Template
- 7,25 µl milli Q

incubation for 1,5 h at 37°C

1 % Agarose gel

90 V 45 min

1kb ladder

gel pocket:

PCR: 25 µl PCR and 5 µl loading Dye-> 1, 2 : 30 µl on gel and 3-9 : 10 µl on gel

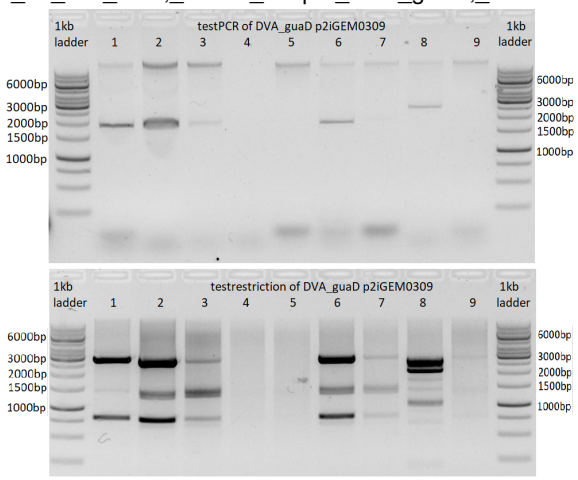
restriction: 10 µl Digest and 2 µl loading Dye

expected bands: 1548 bp for the PCR

2666 bp and 765 bp for the restriction

gel Picture below:

Gel_2018-08-21_15hr_03min_90V_45_min_invA,_ladder_testpcr_DVA_guaD,_testrestriktion_DVA_guaD_bearbeitet.png



retransformation of p2iGEM0255 5 into *E.coli* with the following protocol:

- thraw competent cells (*T10*) 5-10 min on ice
- add 1 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
- add it to 3ml of LB media with the fitting antibiotica (Amp)
- incubate at 37°C over night

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

Author: Carina Gude

created: 21.08.2018 14:32
updated: 21.08.2018 14:37

Entry 207/259: Gel elution of trzE, Dur1,2 p1 and Dur1,2 p2
In Project: Level_1
With tags: trz, Dur12, gel elution

Gel Elution with the Wizard® SV Gel and PCR Clean-Up System and the following protocol:

- add 10 µl Membran binding solution per 10 mg gel
- incubate at 52 °C and vortex until the gel is completely deluted
- Fill gel mixture in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g für 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000g -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000g -> 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 1h + at room temperature
- Centrifuge 1 min 16000g
- Add 20 µl nuclease free water with 10 min at RT, centrifuge again
- Store DNA at -20°C.

measured concentration:

trzE: 3,5 ng/µl

Dur1,2 p1: 6,1 ng/µl

Dur 1,2 p2: 36 ng/µl

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

Author: Carina Gude
Entry 208/259: 3 step PCR for trzE
In Project: Level_1
With tags: Q5 PCR, pcr, trz

created: 21.08.2018 15:42
updated: 23.08.2018 10:05

Mastermix for 4 samples:

- 38,4 µl Milli Q
- 20 µl Q5 buffer
- 20 µl GC enhancer
- 6 µl DMSO
- 2 µl dNTP
- 6 µl rev Primer
- 6 µl fwd Primer
- 0,8 µl Template
- 0,8 µl Q5 Polymerase

25 µl for each sample

program for the cycler:

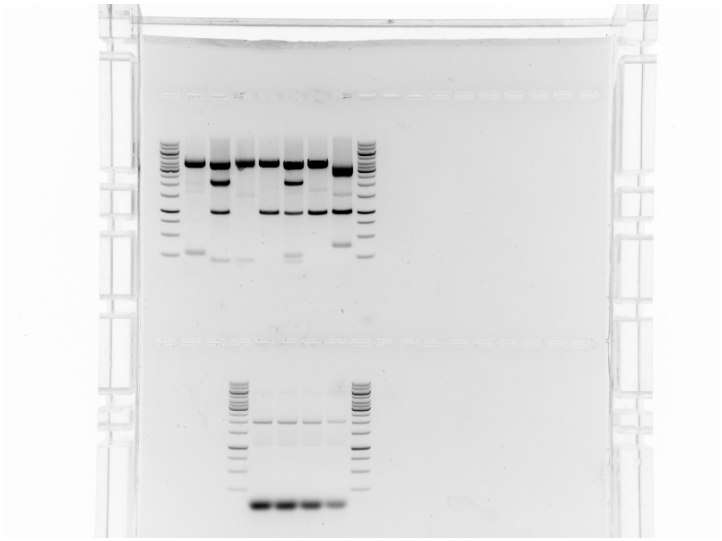
- 95°C 5min
- cycle 40 times:
  - 95°C 20 sec
  - 67°C for 20 sec
  - 72°C 15 sec elongation time
- 72°C 2min
- hold on 4°C

four trzE samples at the bottom

Expected fragment size: 700 bp

Visible bands at ~2000 bp and slightly visible: ~1000 bp

Gel_2018-08-21_18hr_45min_Ylenia_Carina_700.tif



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

Author: Thomas Bick

created: 21.08.2018 16:00
updated: 21.08.2018 16:09

Entry 209/259: Sequencing of p2iGEM0332_TB and p2iGEM0309:
In Project: Level_1
With tags: sequencing

Sequencing of p2iGEM0332_TB and p2iGEM0309:

Nummer	Temp	Primer
3717642	p2iGEM0332_TB	pJET1.2 fwd
3717643	p2iGEM0332_TB	pJET1.2 fwd
23GI74	p2iGEM0309_1	O_iGEM_68
23GI75	p2iGEM0309_1	O_iGEM_69
23GI76	p2iGEM0309_2	O_iGEM_68
23GI77	p2iGEM0309_2	O_iGEM_69
23GI78	p2iGEM0309_3	O_iGEM_68
23GI79	p2iGEM0309_3	O_iGEM_69
23GI80	p2iGEM0309_6	O_iGEM_68
23GI81	p2iGEM0309_6	O_iGEM_69

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

Author: Carina Gude
created: 23.08.2018 09:53
updated: 23.08.2018 10:07
Entry 210/259: trzE PCR and gel electrophoresis
In Project: Level_1
With tags: trz, Q5 PCR, pcr, gel electrophoresis

22.08.2018

for trzE Mastermix:

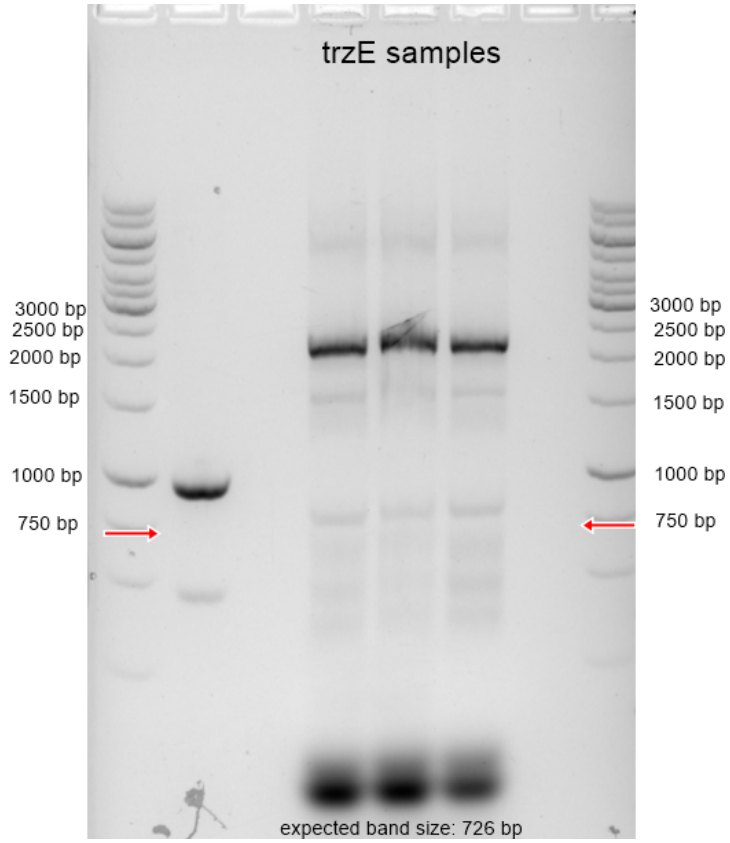
- 38 µl H2O
- 20 µl Buffer
- 20 µl GC enhander
- 6 µl DMSO
- 2µl dNTP
- 6 µl Primer O_iGEM18_0034
- 6 µl Primer O_iGEM18_0035
- 2 µl Template (p2iGEM0256)

distribute in four tubes (25 µl each)

program for the cycler:

- 95°C 5min
- cycle 30 times:
  - 95°C 20 sec
  - 67°C for 20 sec annealing time
  - 72°C 15 sec elongation time
- 72°C 2min
- hold on 4°C

trzE_22.08.2018.png



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

Author: Susanne Vollmer
Entry 211/259: PCR of trzE
In Project: Level_1
With tags: Q5 PCR

created: 23.08.2018 10:18
updated: 23.08.2018 10:23

PCR to amplify trzE from p2iGEM0256 with the folloeing protocol:

mastermix:

- 4 µl Q5 Reaktion buffer
- 1 µl Template p2iGEM0256
- 0,5 µl dNTP mix
- 1 µl Primer O_iGEM18_0034
- 1 µl Primer O_iGEM18_0035
- 0,5 µl Q5 Polymerase
- 0,6 µl DMSO
- 14,4 µl milli Q

PCR program (run on a Pauly Cycler, bio-rad)

- 98°C 2min
  - 98°C 30 sec
  - 67,5 °C 30 sec
  - 72°C 30 sec ->repeat cycle 34 times
- 72°C 10 min
- 4°C HOLD

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

Author: Carina Gude
created: 23.08.2018 10:43
updated: 23.08.2018 15:10
Entry 212/259: glf Touchdown PCR
In Project: Level_1
With tags: touchdown pcr, pcr, Q5 PCR, glf

After many unsuccessful glf PCRs and the occurrence of primer dimers a touchdown PCR was conducted.

Mastermix for 4 samples:

- 51 µl Milli Q
- 20 µl Q5 Buffer
- 20 µl GC enhancer
- 2 µl dNTP MIx
- 2 µl fwd Primer (1039)
- 2 µl rev Primer (1040)
- 2 µl Template (Pamela strain)
- 1 µl Q5 Polymerase

Mastermix was evenly distributed into four tubes (25 µl each)

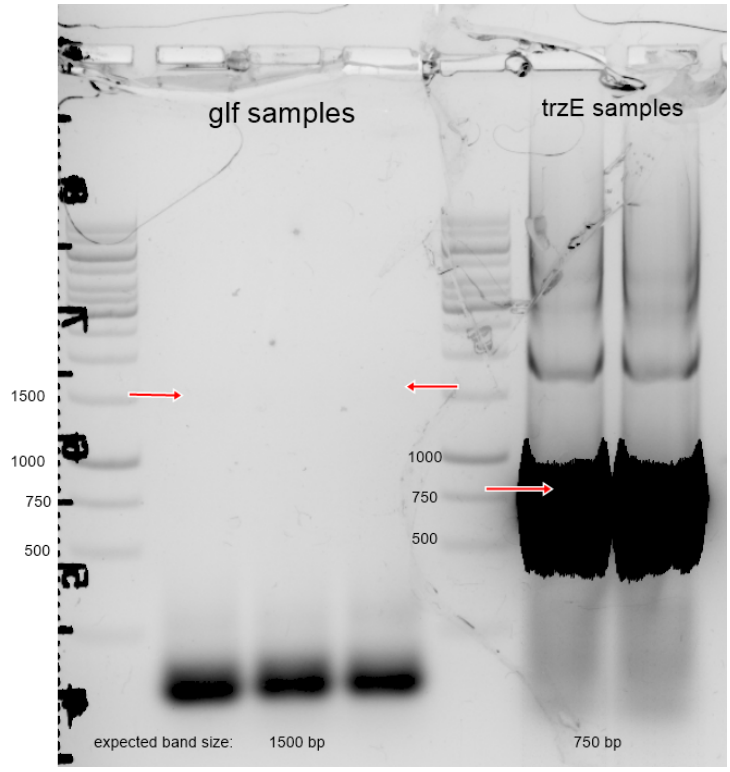
Touchdown PCR program:

- 98°C 5min
- cycle 30x
  - 98°C 30 sec
  - starting at 72°C and decreasing by 0.4°C each cycle - 20sec
  - 72°C 60 sec
- 72°C 5min
- hold on 12°C

Gel was running on 90V for 1h

Results are negative: Only very, very, very slight bands have been able to

Gel_2018-08-23_14hr_40min_glf_trzE_export.tif



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

Author: Susanne Vollmer
created: 23.08.2018 11:02
updated: 23.08.2018 18:46

Entry 213/259: Cryo, Miniprep, testdigest and gel electrophoresis of 2iGEM0373, 2iGEM0353 (delta 5 AA), 2iGEM0347, 2iGEM0348
In Project: Level_1
With tags: testrestriction, Kryo, miniprep, gel electrophoresis

cryo culture of 2iGEM0373, 2iGEM0353 (delta 5 AA), 2iGEM0347, 2iGEM0348 with the following protocol:

700 µl culture and 300 µl 99,5 % Glycerin

store at -80 °C

Miniprep of 2iGEM0373, 2iGEM0353 (delta 5 AA), 2iGEM0347, 2iGEM0348 with the following protocol:

Kit: PureYield™ Plasmid Miniprep System from Promega

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 Sek. max rpm --> discard supernatant
- add 200µ endotoxin removel wash
- add 400µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 2:30 h at RT. Centrifuge for 30 Sek. max

measured concentration:

p2iGEM0294 (2iGEM0348): 242,25 ng/µl

p2iGEM0309 (2iGEM0347): 231,50 ng/µl

p2iGEM0295 (2iGEM0353): 232,95 ng/µl

p2iGEM0332 (2iGEM0373): 226,50 ng/µl

testdigest of p2iGEM0294, p2iGEM0295(delta 5 AA), p2iGEM0309, p2iGEM0332 and p2iGEM0174 with the following protocol:

Mastermix for p2iGEM0294, p2iGEM0295(delta 5 AA), p2iGEM0309, and p2iGEM0174:

- 1 µl Cutsmart
- 0,2µl PvuI-HF
- 1 µl Plasmid
- 7,8 µl milli Q

Mastermix for p2iGEM0332:

- 1 µl Cutsmart
- 0,2µl PstI-HF
- 1 µl Plasmid
- 7,8 µl milli Q

incubate at 37°C for 2 h with 300rpm

50 ml 1 % agarose gel

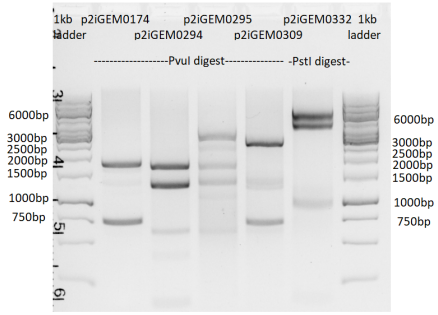
90V 45 min

1kb ladder

in each pocket 10 µl of the restriction (all) and 2 µl loading Dye

gel Picture below:

Gel_2018-08-23_18hr_36min_90_V_45_min_1kb_testdigest_DVA_guaD,_trzC,_atzD_cut_with_pvuI,_pJET_triA_2xsdm_bearbeitet.png



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

Author: Carina Gude
created: 23.08.2018 15:10
updated: 23.08.2018 21:04
Entry 214/259: Gel electrophoresis and gel elution of trzE
In Project: Level_1
With tags: gel electrophoresis, gel elution

The samples from todays trzE PCRs (check Susanne's entry) were put on a gel for 1h at 90V.

Expected band size: ~800 bp

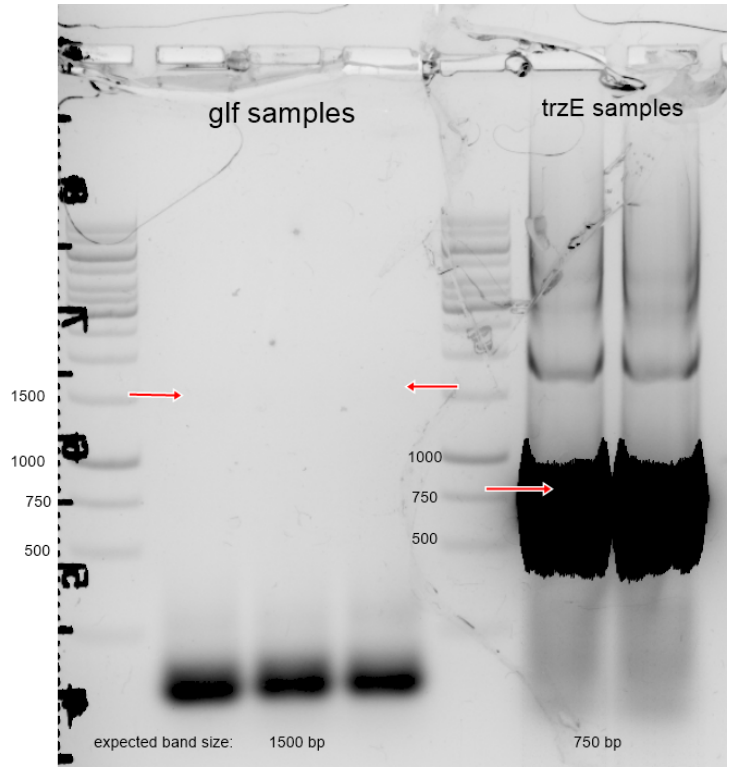
Due two heavy bands on the gel, the bands were cut out and gel elution was conducted.

Gel elution:

- 10 µl Membran binding solution per 10 mg gel was added (510 µl)
- gel mixture was incubate at 52 °C and vortexed until the gel was completely deluted
- gel mixture was filled in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g für 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000g -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000g -> 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 1h + at room temperature
- Centrifuge 1 min 16000g
- Put column into a new tube and add 20 µl nuclease free water, let sit for 10min, centrifuge again
- Store DNA at -20°C.

The gel elution yielded 56,65 ng/µl and ~59 ng/µl

Gel_2018-08-23_14hr_40min_glf_trzE_export.tif



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

Author: Thomas Bick

created: 23.08.2018 15:58
updated: 23.08.2018 16:32

Entry 215/259: PCR atzD and BbsI restriction of DVA_CD
In Project: Level_1
With tags: DVA, gel electrophoresis, pcr

PCR of atzD with the follwing protocol:

sample:

- 5 µl Q5 Buffer
- 5 µl GC enhancer
- 0,2 µl Template
- 0,5 µl dNTP
- 1,5 µl Primer fwd
- 1,5 µl Primer rev
- 0,2 µl Q5 Polymerase
- 1,5 µl DMSO
- 9,6 µl Milli Q
-

Primer:

atzD: O_iGEM18_0078

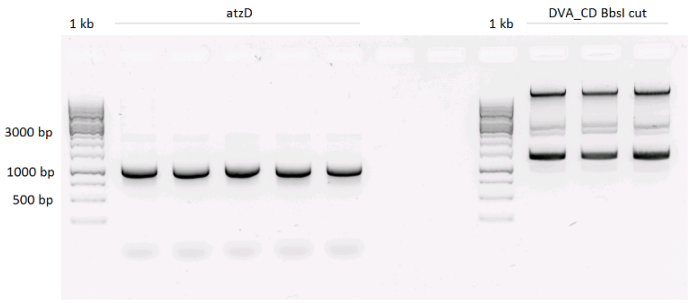
O_iGEM18_0079

PCR programm:

- 95°C 5 min
  - 95°C 20 sec
  - 61°C 20 sec
  - 72 °C 30 sec
  - repeat this 30 times
- 72°C 2 min
- hold 12°C
-

1% Agarose Gel of PCR atzD & trzC:

Gel_2018-08-23_15hr_53min_atzD_Pcr_&_DVA_BBs1_cut.tif



PCR clean up with the Wizard® SV Gel and PCR Clean-Up System and the following protocol:

- add equal Membran binding solution per µl PCR
- Fill gel mixture in column assembly (incubate at room temperature for 5 minute)
- centrifuge at 16000 g für 1 min -> discard flow through
- add 700 µl washing buffer and centrifuge for 1 min at 16000g -> discard flow through
- add 500 µl washing buffer and centrifuge 5 min at 16000g -> 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 1h + at room temperature
- Centrifuge 1 min 16000g

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

Author: Ylenia Longo
created: 24.08.2018 09:46
updated: 24.08.2018 09:57
Entry 216/259: Biochemical analysis of glucose with YSI2590 analyzer
In Project: Level_1
With tags: Glucose, YSI, analysis

To detect the amount of glucose present in the medium after cultivation of the cyanobacteria from Pamela Silver and Danny Ducat, a biochemical assay using the YSI 2590 has been performed.

For this purpose a standard curve with known concentrations of glucose from 1mg/mL to 0.0025 mg/mL was prepared.

The different samples were then meausred, hence samples from Danny and Pamela before induction, at Day1, Day3 and Day 5 after induction.

As a blank the medium used, M2 medium has been used.

To exclude that the addition of IPTG and NaCl (inducer) have an impact on the glucose measurement or on the biochemical measurement overall, also standard curves with the exact concentration of those inducer that was present in the original medium, were prepared.

Results can be seen: https://drive.google.com/drive/folders/1oDx_y_6iSHf7fSecsb1m0_yfRCiD1xMU

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

Author: Carina Gude
created: 24.08.2018 14:52
updated: 24.08.2018 15:00
Entry 217/259: overlap and extension PCR
In Project: Level_1
No tags associated

To fuse TrzE_gene and Dur1,2_gene_p1 by overlap PCR for further isolation, I calculated the following volumes:

Ratio vector:insert 1:3:

3.9 ng TrzE_gene (5,4 ng / µL) --> 0.3 µL

4.15 ng Dur1,2_gene_p1 (4.15 ng / µL) --> 1 µL

to fill a volume of 14 µL: 3,4 µL TrzE_gene & 10 µL Dur1,2_gene_p1

- 5 µL Q5 reaction buffer
- 5 µL High GC buffer
- 0,5 µL dNTPs
- 3,4 µL TrzE_gene
- 10 µL Dur1,2_gene
- 0,5 µL Q5 polymerase

As the annealing temperature of the homologous regions is >65 °C, a Touchdown PCR with the following protocol on the n**exus gradient (previous right) Eppendorf cycler**:

Overhang PCR:

1. Initial denaturation: 98 °C: 2 min
2. Cycling 15x
    1. Denaturation: 98 °C: 30 sec
    2. Annealing: 72°C for 30 sec, each cycle the temperature was lowered by -0,5°C until 67,5°C
    3. Extension: 72 °C: 90 sec
3. initial Extension: 72 °C: 10 min
4. Hold: 4 °C

Extension PCR:
1,5 µl of fwd trzE Primer and 1,5 µl of rev Dur 1,2 p1 Primer was added

Cylcer program:

- Initial denaturation: 98 °C: 2 min
- Cycling 15x
    1. Denaturation: 98 °C: 30 sec
    2. Annealing: 72°C for 30 sec, each cycle the temperature was lowered by -0,5°C until 67,5°C
    3. Extension: 72 °C: 90 sec
- initial Extension: 72 °C: 10 min
- Hold: 4 °C

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

Author: Susanne Vollmer

created: 25.08.2018 14:07
updated: 25.08.2018 16:29

Entry 218/259: gel electrophoresis of overlap extention PCR of trzE and Dur 1,2 p1
In Project: Level_1
No tags associated

1% Agarose gel

90V 1h

1kb ladder

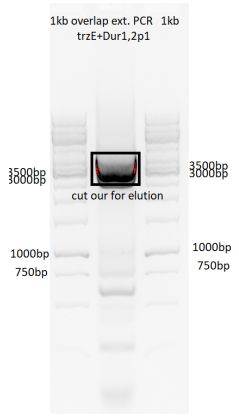
20 µl Overlap extention trzE+Dur1,2 p1 PCR product and 4 µl loading Dye

expected band: ~3650bp

cut out only the upper band

gel Picture below:

Gel_2018-08-25_15hr_55min_90_V_1h_1kb_ladder_Overhang_extention_PCR_trzE+Dur1,2_p1_hell_bearbeitet.png



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

Author: Carina Gude

created: 26.08.2018 18:46
updated: 26.08.2018 20:15

Entry 219/259: Gel elution trzE_Dur1,2p1 overlap

In Project: Level_1

With tags: Dur12, trz, gel elution, overlap

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 (0,18 g = 180µl Membrane binding solution)
- incubate at 52 °C and vortex until the gel is completely deluted
- Fill gel mixture in column assembly (incubate at room temperature for 1 minute)
- centrifuge at 16000 g für 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
- let sit over night
- DNA of tube nr 1 was stored at -20°C.

measured concentration:

trzE_Dur 1,p1 tube 1: 13,58ng/µl

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

Author: Sarah Seyffert
Entry 220/259: Test PCR of glf
In Project: Level_1
With tags: Colony PCR, pcr, cyano

created: 27.08.2018 11:14
updated: 27.08.2018 13:45

To test if in the *glf* PCR the primer form dimers four different tubes were prepared

4xMastermix

- 20µl Q5-buffer
- 20µl High GC
- 41µl MiliQ
- 2µl dNTP
- 2.5µl DMSO

23.5µl ware added in each tube

- tube 1 was filled with 0.5µl template, 0.5µl primer 1039, 0.5µl primer 1040
- tube 2 was filled with 0.5µl primer 1039
- tube 3 was filled with 0.5µl primer 1040
- tube 4 was filled with 0.5µl primer 1039 and 0.5µl primer 1040

PCR programm

1. 95°C 6 min
   - 95°C 20 sec
   - 62°C 20 sec
   - 72 °C 60 sec
   - repeat this 30 times
2. 72°C 5 min
3. hold 12°C

Those samples were loaded on a gel for geleloctrophoresis

2µl 1kb ladder were used

5µl purple loading dye were added to the samples

The gel ran on 90V for 1h

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

Author: Carina Gude
created: 27.08.2018 13:21
updated: 27.08.2018 13:23
Entry 221/259: Gibson assembly of Dur1,2 p1 and trzE overlap
In Project: Level_1
With tags: Gibson Asembly, Gibson, Gibson assembly mix, trz, Dur12

Gibson assembly of Dur1,2 p1 and trzE overlap

15µl of Gibson assembly Mastermix + 5µl of DNA eluted on 26.08.2018

Cycler program:

- 50min 50°C
- afterwards 9°C

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

Author: Susanne Vollmer
created: 28.08.2018 11:03
updated: 28.08.2018 18:29

Entry 222/259: digest of p2iGEM0295 and p2iGEM0199, p2iGEM0198,p2iGEM0196, p2iGEM0200
In Project: Level_1
With tags: digest, gel electrophoresis, golden gate

testrestriction to test if the CIDAR Level 0 cloning of atzD (p2iGEM0295) was succsessfully with following protocol:

Mastermix:

- 1 µl Cutsmart
- 0,25 µl MmeI
- 7,25 µl millli Q

for each sample 8,5 µl mastermix and 1,5 µl template

incubate at 37°C for 2:40 h

inactivation at 65 for 20 min

predigest of the CIDAR Level 1 Backbones (p2iGEM0199, p2iGEM0198, p2iGEM0196, p2iGEM0200) with the following protocol:

Mastermix:

- 5 µl Cutsmart
- 0,5 µl BsaI

for each Sample take 5,5 µl mastermix add 500ng DNA (for p2iGEM0199: 13,2 µl, p2iGEM0198: 5,4 µl , p2iGEM0196: 5,2 µ, p2iGEM0200: 3,2 µl) and fill up to 50 µl with milli Q

incubate at 37°C for 2:25 h

inactivation at 65°C for 20 min

1 % Agarose gel

90 Volt 1 h

1kb ladder

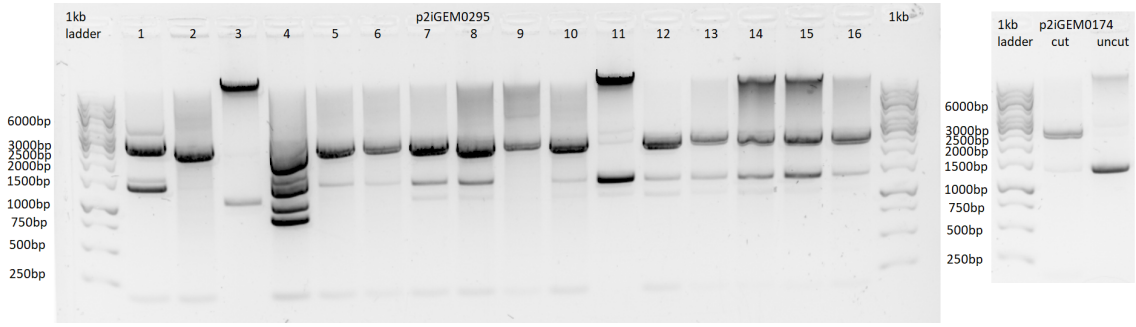
in each pocket:g

for the testrestriction: 10 µl (all) of the restriction and 2 µl of loading Dye

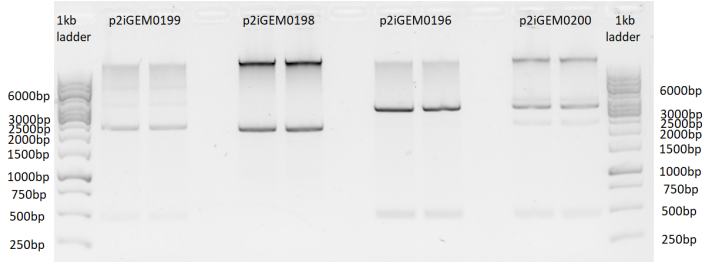
for the Digest: 25 µl of the restriction and 5 µl loading Dye

gel Picture below

Gel_2018-08-28_16hr_05min_testrestriction_DVA_atzD,_bearbeitet.png



Gel_2018-08-28_16hr_05min_restriction_DVK_for_eluation_bearbeitet.png



Golden Gate of trzC and guaD in the CIDAR Level 1 Backbone with the following protocol:

trzC:

- 1,5 µl T4 ligase Buffer
- 0,75 µl T7 Ligase
- 0,75 µl BsaI-HF
- 1,5 µl Cutsmart
- 0,43 µl p2iGEM0295
- 1,9 µl p2iGEM0166
- 1,3 µl p2IGEM0151
- 1,0 µl p2iGEM0111
- 0,26 µl p2iGEM0199
- 5,61 µl milli Q

guaD:

- 1,5 µl T4 ligase Buffer
- 0,75 µl T7 Ligase
- 0,75 µl BsaI-HF
- 1,5 µl Cutsmart
- 0,43 µl p2iGEM0309
- 1,5 µl p2iGEM0165
- 1,3 µl p2IGEM0151
- 2,9 µl p2iGEM0109
- 0,1 µl p2iGEM0196
- 4,27 µl milli Q

in General a Ration of 10 ng:100 ng:100 ng:100 ng:100 ng betwen Backbone and the inserts

Cycler Program:

- 37°C 25 min
  - 37°C 1:30
  - 16°C 3 min ->repeat 60x
- 37°C 60 min
- 50°C 5 min
- 80°C 10 min
- 4° 1 min
- 4°C HOLD

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

Author: Thomas Bick
Entry 223/259: Sequencing of p2iGEM0295
In Project: Level_1
With tags: sequencing, DVK

created: 29.08.2018 10:23
updated: 29.08.2018 12:05

Sequencing of p2iGEM0295 for possible positive clones

ID	template	primer
68IG19	p2iGEM0295_7	O_iGEM_68
68IG20	p2iGEM0295_7	O_iGEM_69
68IG21	p2iGEM0295_8	O_iGEM_68
68IG22	p2iGEM0295_8	O_iGEM_69

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

Author: Jennifer Denter
created: 29.08.2018 10:30
updated: 29.08.2018 10:43

Entry 224/259: Preperation of conjugation plates for Cyano conjugation
In Project: Level_1
With tags: conjugation plates

For the conjugation 2 plates were prepared.

BG11 with 5% LB media without antibiotic treatment.

25 ml per plate.

for both plates 50 ml were prepared in a sterile falcon.

2,5 ml LB media

2X BG11 filled up to 25 ml

Up to 50 ml were filled with preheatet (liquid) Wateragar (2%).

Plates were stored at 4 °C and later on a filter will be placed.

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

Author: Jennifer Denter
Entry 225/259: BG 11 plates with 15 µg/ml Cam
In Project: Level_1
With tags: cyano BG11 plates

created: 29.08.2018 10:43
updated: 29.08.2018 10:54

For plating Dany´s S. elongatus strain which is resistant to chloramphenicol 2 plats with 15 µg/ml Cam were prepared

25 ml 2X BG11 with 15 µl of Chloramphenicol

filled up to 50 ml warm (liquid) Water agar (2%)

2 plates with 25 ml each were prepared.

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

Author: Jennifer Denter
Entry 226/259: Inoculation of Dany´s Cyano strain
In Project: Level_1
With tags: cyano, inoculation

created: 29.08.2018 10:54
updated: 29.08.2018 11:14

2 flasks were prepared either with BG11 medium or with M2 medium.

2x 300 ml sterile flasks were filled with 40 ml of media (here 1x with BG11 and 1x with M2).

From a plate cells were picked and inoculated in prepared flasks.

Incubation 30 °C, 200 rpm for 2-3 days till cultures become fresh green.

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

Author: Susanne Vollmer

created: 29.08.2018 17:22
updated: 29.08.2018 17:31

Entry 227/259: Gel eluttion of the BsaI predigest of p2iGEM0199 and p2iGEM0198
In Project: Level_1
With tags: gel elution

Gel Elution of the BsaI predigested p2iGEM0199 and p2iGEM0198 with the Wizard® SV Gel and PCR Clean-Up System and the follwoing protocol:

- ad 10 µl Membran binding solution per each 10 mg gel
- incubate at 65 °C and Vortex until the gel is completely deluted
- follow the rest of the protocol for both:
- Fill it on the colum (incubate at room temperature for 5 minute)
- centrifuge at 16000 g für 1 min -> discart the flow through
- add 700 µl washing buffer and centrifuge 1min 16000g --> discart the flow through
- add 500 µl washing buffer and centrifuge 5 min 16000g --> discart the flow through
- centrifuge empty column with out lid for 1 min 16000g
- Put column into a new tube
- incubate at roomtemperature until all the Ethanol is evaporated
- Add 30 µl of nuclease free water and elute for 45 min at room temperature
- Centrifuge 1 min 16000g.
- Store DNA at -20°C.

measured concentration:

p2iGEM0199: 3,85 ng/µl

p2iGEM0198: 6,15 ng/µl

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

Author: Carina Gude
created: 29.08.2018 21:08
updated: 29.08.2018 21:12
Entry 228/259: Transformation of 2iGEM0400 (pKiko)
In Project: Level_1
With tags: transformation, trafo

Trasformation of 2iGEM00400 (pKiko)

1. Thaw competent cells 15 minutes on ice
2. Add 1,2 µl plasmid
3. Incubate 30 min on ice
4. 42°C, 45 sec heat shock
5. Incubate 5 minutes on ice
6. add 300 µl LB without antibiotic
7. 1 h at 37°C and 300 rpm
8. cells were plated out (LB+Amp; LB+Kan, LB+Amp+Kan) and stored in the 37°C incubator over night

Next day, only a few small colonies on the LB+Kan plate were to be found

Four colonies were separately inoculated in 3ml LB+Kan

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

Author: Carina Gude
Entry 229/259: Kryo of 2igem0400
In Project: Level_1
With tags: Kryo, kryos

created: 30.08.2018 13:34
updated: 30.08.2018 13:34

700µl of the over night culture and 300 µl Glycerin were mixed and stored at -80°C in the Level 1 Kryo Box

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

Author: Carina Gude
created: 30.08.2018 13:35
updated: 30.08.2018 13:44
Entry 230/259: Miniprep of pKiko (2igem0400/p2iGEM0292)
In Project: Level_1
With tags: miniprep, miniprep kit

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

The four culture were centrifuged for 2min at max rpm, most of the supernatant was discarded and the pellet in the remaining resuspended.

- remaining culture was put into an 1,5 ml Eppi
- 100 µl Lysisbuffer was added and mixed well
- 350 µl neutralisation buffer (cold) was added and mixed
- centrifuge at 3 min max rpm
- Addition of supernatant to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 400µl column wash
- 30 sec max rpm
- centrifuge again for 2min to let any residue of column wash evaporate

For elution 30 µl of Elution Buffer was used at first. After incubation for ~1h and centrifuging for 30sex at max a very low yield (4-6ng /µl) was the result.

Due to the low yiald a new tube was used + 30 µl 37°C warm milli Q water instead of the elution buffer

Incubation for 15min at 37°C, then at RT. Centrifuge for 30 Sek. max

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

Author: Carina Gude
Entry 231/259: Inoculating Cyano preculture and replating strains
In Project: Level_1
With tags: bg11 plates, Cyanobacteria, S.elongatus, preculture

created: 30.08.2018 13:45
updated: 31.08.2018 14:05

Cyano cultures (S. elongatus sp PCC 7942 cscB:::NS3, S.elongatus sp PCC 7492 WT and Pamela Silver's strain) were replated onto new BG11 agar plates. At the same time a pre-culture for fluorescence measurements of S. elongatus sp PCC 7942 cscB:::NS3 was inoculated in150ml BG11.

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

Author: Sarah Seyffert
Entry 232/259: Bg11 plates and cyano culture
In Project: Level_1
With tags: cyano, cscB, bg11 plates

created: 30.08.2018 16:32
updated: 30.08.2018 16:35

For plating S. elongatus cscB:::n53 which is resistant to chloramphenicol 2 plats with 15 µg/ml Cam were prepared

25 ml 2X BG11 with 15 µl of Chloramphenicol

filled up to 50 ml warm (liquid) Water agar (2%)

2 plates with 25 ml each were prepared.

Additionaly a culture S. elongatus cscB:::n53 in 40ml M2 with 26µl chloramphenicol was made.

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

Author: Susanne Vollmer
created: 31.08.2018 10:31
updated: 31.08.2018 16:42

Entry 233/259: Miniprep and digest of p2iGEM0356 and p2iGEM0357
In Project: Level_1
With tags: miniprep, test restriction

Miniprep of p2iGEM0356 and p2iGEM0357 with the following protocol:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 Sek. max rpm --> discard supernatant
- add 200µ endotoxin removel wash
- add 400µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 1 h at RT. Centrifuge for 1 min max rpm

measured concentration:

p2iGEM0356: under 4 ng/µl->not usable be discarded

p2iGEM0357: 282,60 ng/µl

testrestriction of p2iGEM0357 if the goldengate CIDAR Level 1 cloning was successfully and p2iGEM0199 (negative Control) with the followwing protocol:

Mastermix:

- 1 µl cutsmart
- 0,2 µl PvuI-HF
- 100ng template
- fill up to 10 µl with milli Q

incubate at 37°C for 1:10 h

inactivation at 65°C for 20 min

1% Agarose gel

90V 50 min

1 kB ladder

in each pocket 10 µl restriction and 2 µl loading dye

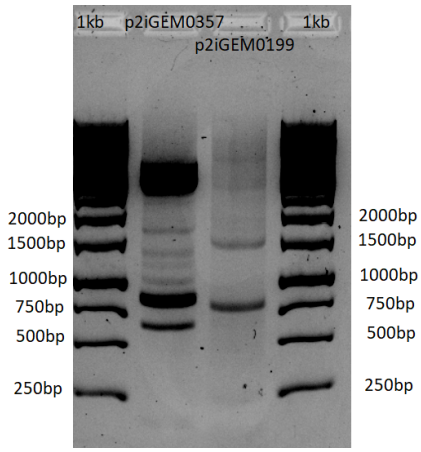
expected bands:

p2iGEM0357: 2033 bp, 1454 bp, 184 bp

p2iGEM0199: 1879 bp, 854 bp

gelpicture below:

Gel_2018-08-31_16hr_10min_testrestriction_goldengate_trzC_level_1_90V_50_min_bright_beaarbeitet.png



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

Author: Thomas Bick
created: 31.08.2018 11:31
updated: 31.08.2018 14:18
Entry 234/259: pJET cloning of ptxD gBlock
In Project: Level_1
With tags: blunt-end cloning, pJET

Blunt-end ligation of *triA* in pJET blunt-end cloning Vector (Thermo Fisher):

2x reaction buffer : 10 µL

ptxD gBlock : 0.5 µL

pJET1.2/blunt Cloning Vector : 1 µL

Water, nuclease-free : 7.5 µL

T4 DNA Ligase: 1 µL

Total volume: 20 µL

Vortex briefly and centrifuge for 3-5 s.

Incubate the ligation mixture at room temperature (22°C) for 30 min.

Stored at -20°C until transformation

Transformation of p2iGEM0373 into *E. coli* T10

1. Thaw competent cells 15 minutes on ice
2. Add 5 µl plasmid
3. Incubate 30 min on ice
4. 42°C, 45 sec heat shock
5. Incubate 5 minutes on ice
6. add 300 µl LB without antibiotic
7. 1 h at 37°C and 300 rpm
8. cells were plated out (LB+Amp) and stored in the 37°C incubator over night

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

Author: Carina Gude
Entry 235/259: Miniprep of pKIKO
In Project: Level_1
With tags: miniprep, miniprep kit

created: 31.08.2018 13:52
updated: 31.08.2018 14:04

30.08.2018: Four over night cultures were inoculated of the four Kryos (2igem0400 1-4)

31.08.2018:

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

The four cultures were centrifuged for 2min at max rpm, most of the supernatant was discarded and the pellet in the remaining resuspended.

- remaining culture was put into an 1,5 ml Eppi
- 100 µl Lysisbuffer was added and mixed well
- 350 µl neutralisation buffer (cold) was added and mixed
- centrifuge at 3 min max rpm
- Addition of supernatant to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 200µl Endotoxin removal wash
- 30 sex max rpm
- add 400µl column wash
- 30 sec max rpm
- centrifuge again for 2min to let any residue of column wash evaporate
- insert collumn into new tube and add 30µl of Elution buffer
- let sit for 1h

measured concentrations were super low

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

Author: Carina Gude

created: 31.08.2018 14:05
updated: 31.08.2018 14:06

Entry 236/259: Inoculation of cscB Cyanobacteria culture in M2
In Project: Level_1
With tags: inoculation, M2-Medium, Cyanobacteria

A pre-culture for fluorescence measurements of S. elongatus sp PCC 7942 cscB:::NS3 was inoculated in 100ml M2.

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

Author: Carina Gude
created: 02.09.2018 16:00
updated: 02.09.2018 16:01
Entry 237/259: Transformation of 2iGEM0400 (pkiko)
In Project: Level_1
With tags: transformation, trafo, pkiko

Due to bad miniprep results and weird colonies following the last transformation of pKiko, it had to be redone.

Trasformation of 2iGEM00400 (pKiko)

1. Thaw competent cells 15 minutes on ice
2. Add 1,2 µl plasmid
3. Incubate 30 min on ice
4. 42°C, 45 sec heat shock
5. Incubate 5 minutes on ice
6. add 300 µl LB without antibiotic
7. 1 h at 37°C and 300 rpm
8. cells were plated out (LB+Amp; LB+Kan, LB+Amp+Kan) and stored in the 37°C incubator over night

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

Author: Susanne Vollmer
Entry 238/259: inoculation of p2iGEM0373 (2iGEM0418)
In Project: Level_1
With tags: inoculation

created: 03.09.2018 17:00
updated: 03.09.2018 17:05

inoculation of 10x 3 ml LB media with each one Colony of 2iGEM0418

incubation over night at 37°C

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

Author: Susanne Vollmer
Entry 239/259: sequencing of p2iGEM0357
In Project: Level_1
With tags: sequencing

created: 03.09.2018 17:06
updated: 03.09.2018 17:10

sequencing of p2iGEM0357 with the following protocol:

Barcode	template	primer	Content plasmid	Content water
68IH08	p2iGEM0357	O_iGEM18_0070	1,77 µl	5,73 µl
68IH07	p2iGEM0357	O_iGEM18_0071	1,77 µl	5,73 µl

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

Author: Carina Gude
created: 05.09.2018 09:01
updated: 05.09.2018 09:06
Entry 240/259: Miniprep of p2iGEM0373 and p2iGEM0292
In Project: Level_1
With tags: miniprep, miniprep kit

03.09.2018 : Four over night cultures were inoculated of the four Kryos (2igem0400 1-4)

04.09.2018:

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

The four cultures and 10 cultures of p2iGEM0373 were centrifuged for 3min at max rpm, most of the supernatant was discarded and the pellet in the remaining resuspended.

- remaining culture was put into an 1,5 ml Eppi
- 100 µl Lysisbuffer was added and mixed well
- 350 µl neutralisation buffer (cold) was added and mixed
- centrifuge at 3 min max rpm
- Addition of supernatant to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 200µl Endotoxin removal wash
- 30 sex max rpm
- add 400µl column wash
- 30 sec max rpm
- centrifuge again for 2min to let any residue of column wash evaporate
- insert collumn into new tube and add 30µl of Elution buffer
- let sit for several hours

Three random sample concentratino measurements of p2iGEM0373: 150 ng/µl, 300 ng/µl and 400ng/µl

The measured concentrations of p2iGEM0292 were very low, with 12ng/µl being the highest.

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

Author: Susanne Vollmer
created: 05.09.2018 10:24
updated: 05.09.2018 18:05
Entry 241/259: Testrestriction, gel electrophoresis and sequencing of p2iGEM0373
In Project: Level_1
With tags: sequencing, testrestriction, gel electrophoresis

testriction if the pJET cloning of ptxDopt worked, with the following protocol:

Mastermix:

- 1 µl Cutsmart
- 0,2 µl PstI-HF
- 1 µ template
- 7,8 µl MilliQ

incubate at 37°C 1 h 30 min

gel electrophoresis with 1% Agarose gel

100 min 105 V

1kb ladder

in each pocket 10 µl restriction product and 2 µl loading Dye

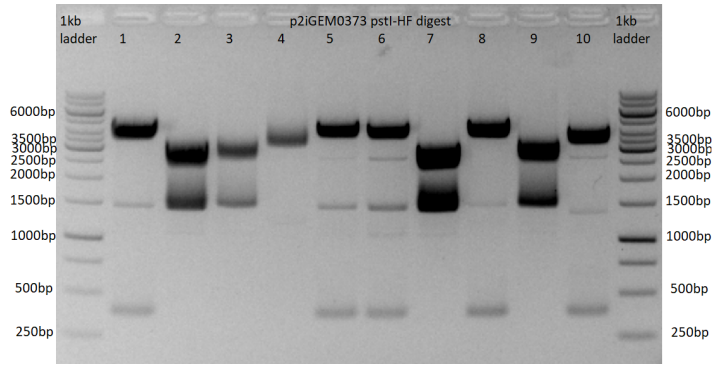
gel Picture below:

expected band:

pJET_ptxDopt: 2613 bp, 1469 bp

pJET: 2974 bp

Gel_2018-09-05_13hr_53min_105_V_100_min_Sarah,_testverdau_pJET_ptxDopt,_Leu2_Salima_bearbeitet.png



Sequencing of p2iGEM0373, 2 to test if it worked, with the following protocoll:

around 500ng Plasmid, 2,5 µl primer and milli Q up to 10 µl, than short spin down, put in a bag and throw in the GATC box, with Barcode on each tube:

Barcode	Template	Primer	Content plasmid	Content water
68IG58	p2iGEM0373, 2	pJET 1,2 rev	1,15 µl	6,35 µl
68iG59	p2iGEM0373, 2	pJET 1,2 few	1,15 µl	6,35 µl

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

Author: Susanne Vollmer
Entry 242/259: Transformation of p2iGEM0292
In Project: Level_1
With tags: Top10, DHSα, Transformation, E.coli

created: 05.09.2018 18:04
updated: 06.09.2018 17:28

transformation of E.coli Top 10 and DH5a with p2iGEM0292 (pKIKOlacZKm) with the following protocoll:

- thraw competent cells (*Top 10 and DH5a*) 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 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
- plate 150 µl of each on LB amp plate and the other 150 µl on LB kan (Amp)-> (150 µl of DH5a on amp, 150 µl top 10 on amp, 150 µl Dh5a on kan, 150 µl top 10 on kan)
- incubate at 37°C over night

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

Author: Susanne Vollmer
created: 06.09.2018 16:06
updated: 06.09.2018 17:38

Entry 243/259: miniprep and sequencing of p2iGEM0332
In Project: Level_1
With tags: miniprep, sequencing

Miniprep of p2iGEM0332 with the follwing protocoll:

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

culture was concentrated in 600µl (centrifuges down by 6000rcf for 2:30 min in the eppi, deacnt the supernatant, repeat until you have only 600µl left, than resuspend the pellet with it) and used for plasmid isolation

- 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 Sek. max rpm --> discard supernatant
- add 200µ endotoxin removel wash
- add 400µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 2:30 h at RT. Centrifuge for 30 Sek. max

measured concentration: 213,85 ng/µl

Sequencing of p2iGEM0332 to test if the mutagenesis worked with the following protocoll:

in each tube 400-500 ng of DNA, 2,5 µl primer and add milli Q up to 10 µl, put a Barcode on the tubes, spin short, collect them in a bag and then bring to the GATC box

barcode	template	primer	Content plasmid
68IH65	p2iGEM0332 from the miniprep of 6.9.2018	pJET 1.2 rev	2,34 µl
68IH66	p2iGEM0332 from the miniprep of 6.9.2018	pJET 1.2 few	2,34 µl

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

Author: Susanne Vollmer

created: 10.09.2018 11:13
updated: 10.09.2018 18:54

Entry 244/259: restriction and gel electrophoresis of p2iGEM0001, 373,2 , 200, 198, 196

In Project: Level_1

With tags: restriction, gel electrophoresis

restriction of p2iGEM0001, p2iGEM0373.2, p2iGEM0200, p2iGEM0198 ,p2iGEM0196 with the following protocol:

p2iGEM0001:

- 6,4 µl milli Q
- 1 µl Neb 3.1 Buffer
- 2 µl Plasmid
- 0,6 µl BsmBI enzyme

p2iGEM0373.2:

- 6,1 µl milli Q
- 1 µl Neb 3.1 Buffer
- 2,3 µl Plasmid
- 0,6 µl BsmBI enzyme

p2iGEM0200:

- 5,6 µl milli Q
- 1 µl CutSmart
- 2,8 µl Plasmid
- 0,6 µl BsaI enzyme

p2iGEM0198:

- 3,1 µl milli Q
- 1 µl CutSmart
- 5,3 µl Plasmid
- 0,6 µl BsaI enzyme

p2iGEM0196:

- 3,2 µl milli Q
- 1 µl CutSmart
- 5,2 µl Plasmid
- 0,6 µl BsaI enzyme

incuabtion at 37°C (p2iGEM0200, p2iGEM0198 ,p2iGEM0196) or 55°C (p2iGEM0001, p2iGEM0373.2) for 1h 15 min

no inaktivation, just add 2 µl loeading dye to each sample

take a 1 % Agarose gel, stored at 4°C

90V 1h

1kb ladder

expected bands:

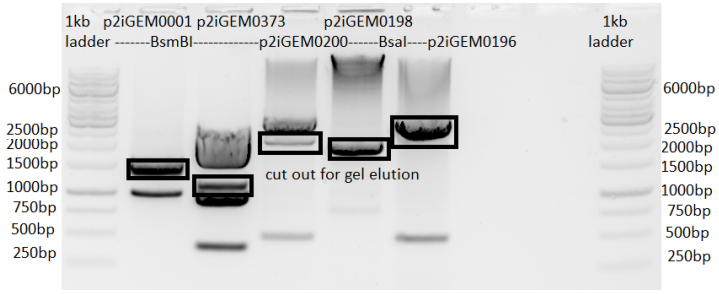
p2iGEM0001: 1600bp, 1000bp

p2iGEM0373: 2600bp, 1000bp, 400bp

p2iGEM0200, p2iGEM0198, p2iGEM0196: environ 2200bp, 500bp

gel picture below:

Gel_2018-09-10_12hr_23min_Donor_knockout_+_ylenja+susanne_bearbeitet.png



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

Author: Susanne Vollmer
Entry 245/259: Cryo of p2iGEM0373, inoculation of p2iGEM0292
In Project: Level_1
With tags: Kryo, inoculation

created: 12.09.2018 20:52
updated: 13.09.2018 13:38

11.09.18: Cryo of p2iGREM0373 (2iGEM0418) with 700 µl culture and 300 µl gylcerin, stored at -80°C, Level 1 box

inoculation of 3ml LB amp with each one Colony of p2iGEM0292 (pKIKOlacZKm) in E.coli DH5a (2iGEM0420). In total two samples from two cultures (from Annas trafo) incubation at 37°C over night.

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

Author: Susanne Vollmer
Entry 246/259: cryo and sequencing of p2iGEM0292, 2iGEM0420
In Project: Level_1
With tags: Kryo, sequencing

created: 13.09.2018 18:49
updated: 13.09.2018 18:59

made kryos from each of the two inoculated colonies of 2iGEM0420 (p2iGEM0292) with 700 µl culture and 300 µl glycerin. stored at -80°C in the level 1 box

sequencing of the miniprep of the two cultures (done by Ylenia) with the following protocol:

in each tube 400-500 ng of DNA, 2,5 µl primer and add milli Q up to 10 µl, put a Barcode on the tubes, spin short, collect them in a bag and then bring to the GATC box

Barcode	Template	primer	content of plasmid
69BC26	p2iGEM0292,1	O_iGEM18_0061	5,26 µl
69BC27	p2iGEM0292, 2	O_iGEM18_0061	4,36 µl

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

Author: Susanne Vollmer
Entry 247/259: ligation of ptxDopt into pYTK0001
In Project: Level_1
With tags: ligation

created: 13.09.2018 18:59
updated: 13.09.2018 19:20

ligation of ptxDopt into the DUBER entryvector pYTK001 to get plasmid (p2iGEM0392) with the following protocol:

Mastermix:

- 2 µl Buffer
- 1 µl T4 ligase
- 7,92 µl insert (precutted and gel eluted) ptxDopt from the p2iGEM0373
- 1-2 µl Backbone (predigested and gel eluted) p2iGEM0001
- 8,09 µl milli Q

incubate for 20 min at Roomtemperature

inactivation at 65°C for 20 min

store at -20°C

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

Author: Susanne Vollmer
created: 14.09.2018 10:38
updated: 15.09.2018 14:25

Entry 248/259: restriction ligation cloning of CIDAR level 1 p2iGEM0356, p2iGEM0357, p2iGEM0393 and transformation of them and of p2iGEM0392
In Project: Level_1
With tags: transformation, restriction, ligation, cloning, Cidar

restriction ligtion cloning of atzD, guaD, trzC in CIDAR Level 1 (p2iGEM0356, p2iGEM0357, p2iGEM0393) with the following protocoll:

Restriction:

Mastermix for (p2iGEM0166, p2iGEM0151, p2iGEM0111, p2iGEM0199, p2iGEM0165, p2iGEM0110, p2iGEM0198, p2iGEM0167, p2iGEM0112, p2iGEM0200, PCR-clean-up of atzD, PCR of trzC):

- 10 µl DNA
- 1,5 µl cutsmart
- 0,2 µl BsaI Enzyme
- 3,3 µl milli Q

Mastermix for (p2iGEM0295, p2iGEM0294, p2iGEM0309):

- 5 µl DNA
- 1 µl cutsmart
- 0,2 µl BsaI Enzyme
- 3,8 µl milli Q

incubation for 1h and 15 min at 37°C

inactivation for 20 min at 65 °C

Ligation:

take the listed volumes from the restriction and add each column to a tube, incubate for 1 h at roomtemperature and inactivate at 65° C for 10 min.

p2iGEM0357 (DVK_trzC)	p2iGEM0357 (DVK_trzC)	p2iGEM0393 (DVK_atzD)	p2iGEM0393 (DVK_atzD)	p2iGEM0393 (DVK_atzD)	p2iGEM0393 (DVK_atzD)	p2iGEM0356 (DVK_guaD)	p2iGEM0356 (DVK_guaD)
p2iGEM0166: 2,88 µl	p2iGEM0166: 2,88 µ	p2iGEM0167: 0,9 µl	p2iGEM0167: 0,9 µl	p2iGEM0167: 0,9 µl	p2iGEM0167: 0,9 µl	p2iGEM0165: 2,27 µl	p2iGEM0165: 2,27 µl
p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl	p2iGEM0151: 1,9 µl
p2iGEM0111: 1,6 µl	p2iGEM0111: 1,6 µl	p2iGEM0112: 3,8 µl	p2iGEM0112: 3,8 µl	p2iGEM0112: 3,8 µl	p2iGEM0112: 3,8 µl	p2iGEM0110: 2,3 µl	p2iGEM0110: 2,3 µl
p2iGEM0199: 0,37 µl from the 1:4 dilution	p2iGEM0199: 0,4 µl from the 1:4 dilution	p2iGEM0200: 0,36 µl from the 1:4 dilution	p2iGEM0200: 2 µl of the predigested gel elution	p2iGEM0200: 0,36 µl from the 1:4 dilution	p2iGEM0200: 2 µl of the predigested gel elution	p2iGEM0198: 0,22 µl	p2iGEM0198: 2,5 µl of the predigested gelelution
p2iGEM0294: 1,25 µl	trzC PCR: 2,5 µl	p2iGEM0295: 1,3 µl	p2iGEM0295: 1,3 µl	atzD PCR Clean-Up: 2,3 µl	atzD PCR Clean-Up: 2,3 µl	p2iGEM0309: 1,3 µl	p2iGEM0309: 1,3 µl
T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl	T4 Ligase Buffer: 2µl
T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl	T4 Ligase: 0,2 µl
milli Q: 9,73 µl	milli Q: 6,52 µl	milli Q: 9,46 µl	milli Q: 8 µl	milli Q: 8,46 µl	milli Q: 6,8 µl	milli Q: 9,73 µl	milli Q: 7,43 µl

Transformation of E.coli Top 10 with p2iGEM0392, p2iGEM0357, p2iGEM0393, p2iGEM0356 with the following protocol:

- thraw competent cells (*Top 10*) 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 ->spread out each 60 µl X-GAL and on some plates IPTG expect for p2iGEM0392
- plate 120µl on LB kan, but p2iGEM0392 on LB cam
- incubate at 37°C over night

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

Author: Susanne Vollmer

created: 15.09.2018 14:15
updated: 15.09.2018 14:31

Entry 249/259: inoculation of the colonies of the transformation of p2iGEM0392, 393, 356, 357

In Project: Level_1

With tags: inoculation, incubation

inoculation of 3ml LB kanamicin with each one colony of the Transformation:

15 colonies of p2iGEM0357 (2IGEM0402) (trzC)

15 colonies of p2iGEM0357 (2iGEM0402) (trzC from PCR)

3 colonies of p2iGEM0393 (2iGEM0440) (atzD from PCR)

3 colonies of p2iGEM0393 (2iGEM0440) (atzD)

2 colonies of p2iGEM0393 (2iGEM0440) (Backbone geleluted)

4 colonies of p2iGEM0356 (2iGEM0401) (guaD)

inoculation of 3ml LB Chloramphenicol with each one colony of the Transformation:

5 colonies of p2iGEM0392 (2iGEM0441) (ptxDopt, both gel eluted)

incubation at 37°C over night

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

Author: Susanne Vollmer

created: 18.09.2018 16:54
updated: 18.09.2018 17:16

Entry 250/259: Miniprep of inoculated colonies of p2iGEM0356, p2iGEM0357, p2iGEM0393 and p2iGEM0392

In Project: Level_1

With tags: miniprep

Miniprep of in total 44 samples of the inoculated Plasmids of p2iGEM0356 (2iGEM0401), p2iGEM0357 (2iGEM0402), p2iGEM0393 (2iGEM0440) and p2iGEM0392 (2iGEM0441) with the following protocol:

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

culture was concentrated in 600µl (centrifuges down by 4000rpm for 4 min in the culture tubes, deacnt the supernatant,until only 600µl is left, than resuspend the pellet with it and stored them at 4°C) than used for plasmid Isolation:

- 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 Sek. max rpm --> discard supernatant
- add 200µ endotoxin removel wash
- 30 sec max rpm
- add 400µl column wash
- 30 sec max rpm
- for elution use new tube+ 30 µl 37°C warm milli Q water
- incubate for 2:10 h at RT. Centrifuge for 30 Sek. max
- randomly checkt concentrations are over 150 ng/ µl
- stored at -20 °C

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

Author: Susanne Vollmer

created: 19.09.2018 14:22
updated: 19.09.2018 18:30

Entry 251/259: testrestriction and gel electrophoresis to test level 1 cloning CIDAR p2iGEM0393, p2iGEM0357, p2iGEM0356 and to test if DUEBER level 1 cloning of p2iGEM0392 worked

In Project: Level_1

With tags: gel electrophoresis, testrestriction

Testretriction if CIDAR Level 1 cloning worked (p2iGEM0393 (DVK_atzD), p2iGEM0357 (DVK_trzC), p2iGEM0356 (DVK_guaD) and to test if DUEBER level 1 cloning of p2iGEM0392 (pYTK001_ptxDopt) worked, using following protocol:

Mastermix for p2iGEM0393, p2iGEM0357, p2iGEM0356:

- 1 µl CutSmart
- 0,2 µl NotI-HF
- 7,8 µl milli Q

add 9 µl mastermix to 1 µl template

incubate for 3h at 37 °C

Mastermix for p2iGEM0392:

- 1 µl CutSmart
- 0,3 µl NcoI-HF
- 0,3 µl PvuII-HF
- 7,4 µl milli Q

add 9 µl mastermix to 1 µl template

incubate for 3h at 37 °C

no inactivation just add 2 µl loading dye and put on gel

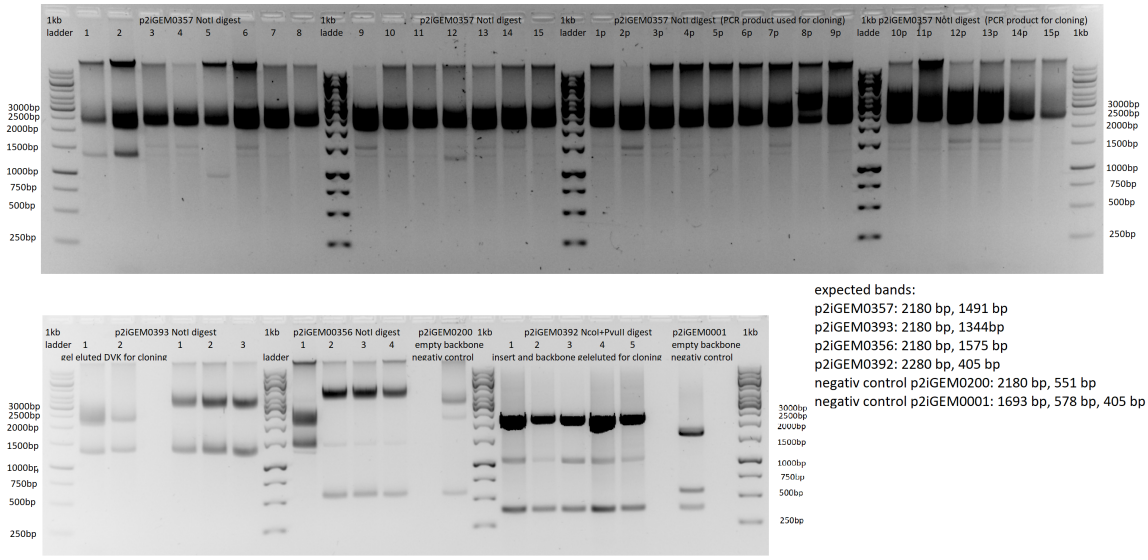
1% Agarose gel

100 V 90 min

1kb ladder

gel Picture below, expecetet bands on Picture

Gel_2018-09-19_17hr_30min_testrestriction_DVK_notI,_pYTK0001_ncoI+pvuII_100V_90min_bearbeitet.png



expected bands:
p2iGEM0357: 2180 bp, 1491 bp
p2iGEM0393: 2180 bp, 1344bp
p2iGEM0356: 2180 bp, 1575 bp
p2iGEM0392: 2280 bp, 405 bp
negativ control p2iGEM0200: 2180 bp, 551 bp
negativ control p2iGEM0001: 1693 bp, 578 bp, 405 bp

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

Author: Susanne Vollmer
created: 20.09.2018 17:23
updated: 20.09.2018 17:34

Entry 252/259: Sequencing of p2iGEM0357, p2iGEM0393, p2iGEM0356, p2iGEM0392
In Project: Level_1
With tags: sequencing

sequencing of p2iGEM0357, p2iGEM0393, p2iGEM0356, p2iGEM0392 with the follwing protocol:

in each tube 400-500 ng of DNA, 2,5 µl primer and add milli Q up to 10 µl, put a Barcode on the tubes, spin short, collect them in a bag and then bring to the GATC box

Barcode	Template	primer	Content of plasmid
ID67EB93	p2iGEM0357 2	O_iGEM0071	1,3 µl
ID67EB94	p2iGEM0357 2	O_iGEM0070	1,3 µl
ID67EB95	p2iGEM0393 2G	O_iGEM0071	3,8 µl
ID67EB96	p2iGEM0393 2G	O_iGEM0070	3,8 µl
ID67EB97	p2iGEM0356 1	O_iGEM0071	2,75 µl
ID67EB98	p2iGEM0356 1	O_iGEM0070	2,75 µl
ID67EB99	p2iGEM0392 2	O_iGEM0082	2,5 µl
ID67EC00	p2iGEM0392 2	O_iGEM0083	2,5 µl

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

Author: Carina Gude

created: 24.09.2018 15:36
updated: 24.09.2018 17:23

Entry 253/259: Second growth curve for E.coli and S.cerevisiae in used Cyano medium

In Project: Level_1

With tags: Growth Curve, Cyanobacteria, co-culture, E.coli, yeast

4 days after the Cyano induction:

Sterile filtration of the Cyano medium:

- 5ml of medium of each culture
- centrifuge at 4000 rpm for 10min
- sterile filtration of each medium

Washing E.coli and yeast from pre-cultures

- taking 1ml of each culture and diluting them with 9ml of their original medium
- centrifuging at 4000rpm
- pouring supernatant off
- refilling with 10ml of M2
- repetition of previous steps

E.coli samples:

- 109µl E.coli in M2 medium
- 892 µl by Cyano sugar enriched medium

Yeast samples:

- 0,029 µl Yeast in M2 medium
- 892 µl by Cyano sugar enriched medium
- 80 µl of AA (20µl of Uracil, Leucin, Lysin and Histidin)

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

Author: Carina Gude

created: 24.09.2018 17:21
updated: 24.09.2018 17:24

Entry 254/259: First growth curve for E.coli and S.cerevisiae in used Cyano medium
In Project: Level_1
With tags: Growth Curve

22.09.2018

2 days after the Cyano induction:

Sterile filtration of the Cyano medium:

- 5ml of medium of each culture
- centrifuge at 4000 rpm for 10min
- sterile filtration of each medium

Washing E.coli and yeast from pre-cultures

- taking 1ml of each culture and diluting them with 9ml of their original medium
- centrifuging at 4000rpm
- pouring supernatant off
- refilling with 10ml of M2
- repetition of previous steps

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

Author: Susanne Vollmer
created: 27.09.2018 10:36
updated: 28.09.2018 10:38

Entry 255/259: entries from the 21.09-2018 - 27.09.2018 : cloning, transformation, inoculation, miniprep, testrestriction, gel electrophoresis, sequencing of p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410 and p2iGEM04011
In Project: Level_1
With tags: testrestriction, gel electrophoresis, inoculation, miniprep, golden gate, cloning, transformation, E.coli, Top10, sequencing

Because Problems with the accout now the entries from the 21.09.2018 until the 27.09.2018

21.09.2018: Goldengate cloning of the Level 1 Plasmids of ptxDopt with DUEBER (p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411) with the following protocol:

p2iGEM0407:

Mastermix:

- 0,05 µl p2iGEM0097
- 0,5 µl p2iGEM0009
- 0,5 µl p2iGEM0392,2
- 0,3 µl p2iGEM0054
- 1 µl T4 Ligase Buffer
- 0,5 µl T7 liagse
- 7,15 µl milli Q

p2iGEM0408:

Mastermix:

- 0,05 µl p2iGEM0097
- 0,3 µl p2iGEM0010
- 0,5 µl p2iGEM0392,2
- 0,3 µl p2iGEM0054
- 1 µl T4 Ligase Buffer
- 0,5 µl T7 liagse
- 7,35 µl milli Q

p2iGEM0409:

Mastermix:

- 0,05 µl p2iGEM0097
- 0,35 µl p2iGEM0011
- 0,5 µl p2iGEM0392,2
- 0,3 µl p2iGEM0054
- 1 µl T4 Ligase Buffer
- 0,5 µl T7 liagse
- 7,3 µl milli Q

p2iGEM0410:

Mastermix:

- 0,05 µl p2iGEM0097
- 0,28 µl p2iGEM0012
- 0,5 µl p2iGEM0392,2
- 0,3 µl p2iGEM0054
- 1 µl T4 Ligase Buffer
- 0,5 µl T7 liagse
- 7,38 µl milli Q

p2iGEM0411:

Mastermix:

- 0,05 µl p2iGEM0097
- 0,30 µl p2iGEM0013
- 0,5 µl p2iGEM0392,2
- 0,3 µl p2iGEM0054
- 1 µl T4 Ligase Buffer
- 0,5 µl T7 liagse
- 7,36 µl milli Q

cycler program:

- 37°C 25 min
  - 37°C 1:30 min
  - 16°C 3 min-> 52 times
- 16°C 10 min
- 37°C 60 min
- 50 °C 5 min
- 80 °C 10 min
- 4°C HOLD

stored at 4°C until transformation

23.09.2018: Transformation into E.coli Top 10 of p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411 and of p2iGEM0392,2 done by Katharina with the following protocol:

- thraw competent cells (*Top 10*) 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 ->spread out each 60 µl X-GAL and on some plates IPTG expect for p2iGEM0392
- plate 120µl on LB kan, but p2iGEM0392 on LB cam
- incubate at 37°C over night

24.09.2018: inoculation of 3ml LB amp with each one Colony of the Transformation p2iGEM0407 (2iGEM0462), p2iGEM0408 (2iGEM0463), p2iGEM0409 (2iGEM0464), p2iGEM0410 (2iGEM0465), p2iGEM0411 (2iGEM0466)

inoculation of 20 colonies of each Plasmid/organsim, exept only one Colony in 3 ml LB cam p2iGEM0392,2 (2iGEM0441) that means 101 samples in total

incubated at 37 °C over night

25.09.2018: Cryo of p2iGEM0932,2 (2iGEM0441) with 700 µl culture and 300 µl Glycerin

stored at -80 °C

25.09.2018: Miniprep of p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411 and of p2iGEM0392,2 with the following protocol:

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

The cultures were centrifuged for 4min at 4000rpm, most of the supernatant was discarded and the pellet in the remaining resuspended (~600µl).

- remaining culture was put into an 1,5 ml Eppi
- 100 µl Lysisbuffer was added and mixed well
- 350 µl neutralisation buffer (cold) was added and mixed
- centrifuge at 3 min max rpm
- Addition of supernatant to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 200µl Endotoxin removal wash
- 30 sex max rpm
- add 400µl column wash
- 30 sec max rpm
- insert collumn into new tube and add 30µl of Elution buffer
- incubation at RT for 45 min
- 30 sec max rpm
- radomly checked conconcentration and Quality:-> most of the tested tubes have a concentration over 180 ng/µl and a good curve
- stored at RT over night

26.09.2018: Testrestriction of the miniprepplasmids (p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411, and p2iGEM0392,2) to test if the Level 1 cloning worked, with the following protocol:

Mastermix for p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411 (100 samples and the negativ Control p2iGEM0097):

- 1 µl CutSmart
- 0,15 µl EcoRI-HF
- 0,15 µl PstI-HF
- 7,5 µl milli Q

add to 8,8 µl mastermix 1,2 µl template

Mastermix for p2iGEM0392,2 (to test the cryo, and negativ kontrol p2iGEM0001):

- 1 µl CutSmart
- 0,25 µl PvuII-HF
- 0,25 µl NcoI-HF
- 7,5 µl milli Q

add to 9 µl mastermix 1 µl template

incubation for 2 h 50 min at 37°C

no heat inactivation, only add 2 µl loading dye and put on gel

26.09.2018: 1 % Agarose gel

90V 60-90 min)

1kb ladder

gel Picture below

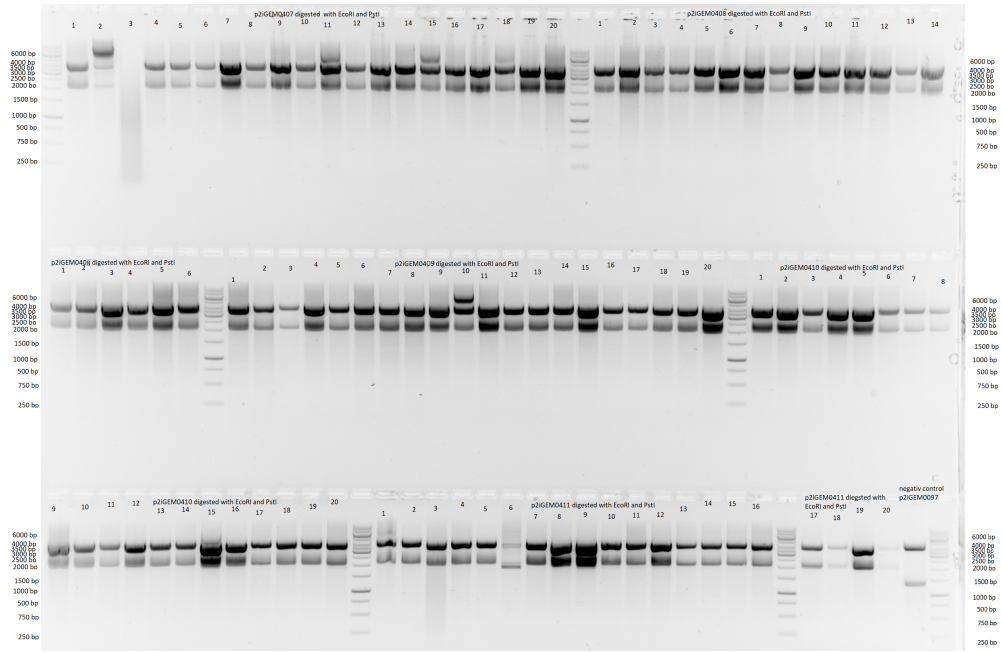
expeceted bands for p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411 are 4000 bp and 2300 bp

for the negativ Control: 4000 bp and 1400 bp

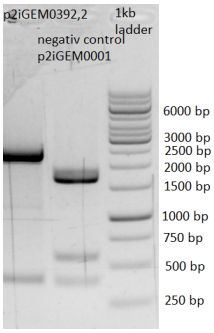
for p2iGEM0392,2: 2300 bp and 400 bp

for the negativ Control: 1700 bp, 600 bp and 400 bp

Gel_2018-09-26_18hr_38min_90_V_90_min_1kb_ladder_testrestriction_EcoRI+PstI_duber_ptxdopt_level_1_bearbeitet.png



Gel_2018-09-26_17hr_58min_iGEM_backbone_cloning_ddh_level_1;_test_kryo_p2iGEM0392_and_p2iGEM0001;_1kb_ladder_90V_60_min_bearbeitet.png



27.09.2018: sequencing of 5 possible possitive samples with the following protocol:

in each tube 400-500 ng of DNA, 2,5 µl primer and add milli Q up to 10 µl, put a Barcode on the tubes, spin short, collect them in a bag and then bring to the GATC box

Barcode	Template	Primer	Content plasmid
67EC01	p2iGEM407, 1	O_iGEM18_0094	2,5 µl
67EC02	p2iGEM407, 1	O_iGEM18_0095	2,5 µl
67EC03	p2iGEM408, 20	O_iGEM18_0094	2,2 µl
67EC04	p2iGEM408, 20	O_iGEM18_0095	2,2 µl
67EC05	p2iGEM409, 13	O_iGEM18_0094	2,65 µl
67EC06	p2iGEM409, 13	O_iGEM18_0095	2,65 µl
67EC07	p2iGEM410, 20	O_iGEM18_0094	2,56 µl
67EC08	p2iGEM410, 20	O_iGEM18_0095	2,56 µl
67EC09	p2iGEM411, 16	O_iGEM18_0094	2,65 µl
67EC10	p2iGEM411, 16	O_iGEM18_0095	2,65 µl

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

Author: Susanne Vollmer

created: 28.09.2018 19:34
updated: 28.09.2018 19:42

Entry 256/259: yeast transformation with p2iGEM0407, p2iGEM0408, p2iGEM0409, p2iGEM0410, p2iGEM0411

In Project: Level_1

With tags: FROZEN EZ Yeast Transformation kit, transformation, S.cerevisiae

yeast BY4742 Transformation with p2iGEM0407 (2iGEM0472), p2iGEM0408 (2iGEM0473), p2iGEM0409 (2iGEM0474), p2iGEM0410 (2iGEM0475), p2iGEM0411 (2iGEM0476) and the following protocol:

using the FROZEN EZ Yeast Transformation kit and following protocol:

- incubate on until the cells are defrosted
- put 4 µl Plasmid DNA on 50µl competent cells
- add 500 µl EZ3 solution
- incubate 45 min at 30°C
- screat 100 µl on a plate
- incubate at 30°C overnight 2-4 days

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

Author: Susanne Vollmer

created: 30.09.2018 15:30
updated: 03.10.2018 17:30

Entry 257/259: inoculation of p2iGEM0407 (2iGEM0472), p2iGEM0408 (2iGEM0473), p2iGEM0409 (2iGEM0474), p2iGEM0410 (2iGEM0475), p2iGEM0411 (2iGEM0476)
In Project: Level_1
With tags: SD medium, inoculation, incubation, S.cerevisiae

inoculation of 3 ml SD -ura media with each one Colony of the transformants p2iGEM0407 (2iGEM0472), p2iGEM0408 (2iGEM0473), p2iGEM0409 (2iGEM0474), p2iGEM0410 (2iGEM0475), p2iGEM0411 (2iGEM0476)

SD-ura for 3 ml:

- 60 µl Histidine
- 60 µl lysine
- 60 µl Leucine
- 300 µl SD 10x media
- 2520 µl milli Q

incubation at 30 °C

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

Author: Susanne Vollmer

created: 03.10.2018 17:27
updated: 03.10.2018 17:30

Entry 258/259: inoculation of p2iGEM0407 (2iGEM0472), p2iGEM0408 (2iGEM0473), p2iGEM0409 (2iGEM0474), p2iGEM0410 (2iGEM0475), p2iGEM0411 (2iGEM0476)
In Project: Level_1
With tags: SD medium, inoculation, incubation, S.cerevisiae

inoculation of SD -ura media with each one Colony of the transformants p2iGEM0407 (2iGEM0472), p2iGEM0408 (2iGEM0473), p2iGEM0409 (2iGEM0474), p2iGEM0410 (2iGEM0475), p2iGEM0411 (2iGEM0476)

SD-ura for 3 ml:

- 60 µl Histidine
- 60 µl lysine
- 60 µl Leucine
- 300 µl SD 10x media
- 1520 µl milli Q
- 1000 µl of the culter from the 30.10.18

incubation at 30 °C

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

Author: Susanne Vollmer

created: 04.10.2018 20:25
updated: 04.10.2018 20:32

Entry 259/259: inoculation of M2 media with phosphite with s.cerevisiae BY4742 and 2iGEM0472

In Project: Level_1

With tags: inoculation, incubation, S.cerevisiae, M2-Medium, H3PO3

inoculation of M2 media with phosphite with *S. cerevisiae* BY4742 and 2iGEM0472 with the following protocol:

first wash the cultures:

- take 1,5 ml of the culture in a falcon tube
- centrifuge with 4000 rpm 5 min
- decant the subernatant
- resuspend in 10 ml sterile milli Q
- repeat
- than measure the OD (5,66 from *S. cerevisiae* BY4742; 1,49 from 2iGEM0472)

prepare culture of *S. cerevisiae* BY4742:

- 300 µl of the 10x stock of Ammoniumsulfat
- 300 µl of the 10x stock Glucose (15%)
- 60 µl Histidine
- 60 µl Lysine
- 60 µl Leucin
- 60 µl Uracil
- 60 µl culture
- 2100 µl of the M2 media with phosphite

prepare culture of 2iGEM0472

- 300 µl of the 10x stock of Ammoniumsulfat
- 300 µl of the 10x stock Glucose (15%)
- 60 µl Histidine
- 60 µl Lysine
- 60 µl Leucin
- 300 µl culture
- 1920 µl of the M2 media with phosphite

incubation at 30°C

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