Author: Katharina Polzen
created: 06.04.2018 13:25
updated: 06.04.2018 13:54
Entry 1/143: Retransformation in E.coli
In Project: General
With tags: E.coli, Retransformation

We discriminate 3 types of transformations:

1. Retransformation of plasmids (Retrafo)
2. Transformation of TOPO ligation (Topcloning)
3. Transformation of ligations (ligation)

- Thaw competent E.coli cells on ice for about 10 min
- Put the reaction tubes needed for the transformation on ice to cool them
- For each transformation mix carefully:

	Retrafo	**Topocloning**	**Ligation**
DNA amount	1 µl	5 µl	10 µl
Comp. E.coli	15 µl	50 µl	50 µl

! We used for the Retrafo 10 µl Comp. E.coli !

- Incubate on ice for 30 min (or 2 min for Retrafo)
- Heat shock: 45 sec; 42°C
- Incubate 2 min on ice
- Add + 5 Vol dyT medium (leave this step out for Retrafo) *
- Incubate 30 min, 37°C (only IF you added dYT: Careful: If you use kanamycin resistence this time needs to be increased 60 min!)
- Plate the cells on selective plates that contain the antibiotic that fits the resistence mediated by your plasmid (In case you do a Topcloning add 60 mL x-Gal (2%) to each plate in advance and dry the plate before planting the transformation mix)
- Incubate the plates at 37°C, o/n

*Remark: for other antibiotics it may be necessary to include or recovery step also for retrafos: Kanamycin 60 min Gentamycin: 30 min

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

Author: Katharina Polzen

created: 06.04.2018 14:01
updated: 06.04.2018 14:10

Entry 4/143: Preparation of chemically competent cells (E. Coli DHSa)
In Project: General
With tags: E.coli, DHSα, competent cells

E. coli DHSα

- Inoculated in the morning (05.04.18) (≈ ONculture), normally overnight
  --> Form this culture: 1 mL in 100 mL fresh LB-Medium --> OD_{600} = 0,627 (conditions: 37°C, 230 rpm)
- Transfer in two mL Falcons --> incubation on ice for 20 min
- Centrifugation: 5 min, 4000 rpm, 4°C
- Discarded supernatant, added 2 mL cold 85 mM
- ***Carefully*** suspend each pellet in 2 ml icecold 85 mM CaCl2 containing 15% Glycerol.
- Alliquots of 50 µL --> shockfreeze in nitrogen, stored in -80°C freezer

https://www.protocols.io/view/preparation-of-chemically-competent-cells-n8mdhu6?step=5

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

Author: Matthias Willmann
Entry 6/143: Transformation-efficiency-test
In Project: General
No tags associated

created: 06.04.2018 17:19
updated: 04.05.2018 10:20

We used old competent cells (E.coli T10) and new competent cells we made at 5.4.2018 (E.coli DH5 α) to make a transformation-efficiency-test.

The Plasmid has a Kannamycin resistance

Per sample (E.coli T10 / E.coli DH5 α) we did one with our Plasmid #84 (from Düber Toolbox) and one with MilliQ water (control group)The samples were splitted in two containers and exposed to heat 42 °C (heat shock).

LB Medium was added (600 µl) to each sample.

Incubation (37°C, 400rpm) for 1 hour)

we plated 2 agar plates (every plate was splitted in 2 subparts) with 1: DH5α 2: T10. one side of the plate was the controlgroup.

we incubated over night with 37°C

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

Author: Katharina Polzen
created: 07.04.2018 09:02
updated: 08.04.2018 18:47
Entry 7/143: Retransformation of the last Dueber plasmids
In Project: General
With tags: dueber, Retransformation, E.coli

- Incubate competent cells 10 minutes on ice
- Add 10-15 µl cells + 1 µl plasmids in Eppis (sterile)
- Incubate 5 min on ice
- 42°C, 45 sec heatshock (not longer)
- Incubate 2 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
- 100 µl cells + 3 mL LB with antibiotic --> Overnight 37°C 200 rpm

We did it with the last plasmids with another antibiotic than chloramphenicol.

Ampicillin: **#89***, #83, #95

Kanamycin: #84, #90, #96

Spectinomycin: #85, #91

*#89 was missing in the Dueber toolbox, so there is **no** retransformation.

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

Author: Matthias Willmann
Entry 10/143: Retrafo Duber #86
In Project: General
No tags associated

created: 08.04.2018 17:32
updated: 04.05.2018 12:21

We did a retransformation with a chloramphenikolresistance in the plasmid with the number #86 from the Duber toolbox in E.coli

1 Control with : 1 µl plasmid

15 µl competent cells

First we incubated the competent cells 10 minutes on ice

then we put 1 µl plasmid (#86) into 15 µl competent cellsand incubated 5 minutes on ice

Now we heatshocked them for 45 seconds on 42°C

Then we incubated for 2 minutes on ice

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

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

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

Author: Katharina Polzen
created: 08.04.2018 18:47
updated: 08.04.2018 18:49
Entry 11/143: Retransformation of the last Dueber plasmids
In Project: General
With tags: dueber, Retransformation, E.coli

With #91:

- Incubate competent cells 10 minutes on ice
- Add 10-15 µl cells + 1 µl plasmids in Eppis (sterile)
- Incubate 5 min on ice
- 42°C, 45 sec heatshock (not longer)
- Incubate 2 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
- 100 µl cells + 3 mL LB with antibiotic --> Overnight 37°C 200 rpm

For the other cells we used the stocks in the 4°C freezer and added 100 µl cells and 3 mL LB with antibiotic.

- --> Overnight 37°C 200 rpm

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

Author: Matthias Willmann
Entry 13/143: Retrafo NS15 CM resistance
In Project: General
No tags associated

created: 09.04.2018 15:23
updated: 09.04.2018 17:05

We used different competent cells (Pauly(P) / iGEM new(I) / iGEM old(O, 2017) for our retransformation

- Incubate competent cells 10 minutes on ice
- Add 15 µl cells + 1 µl plasmids in Eppis (sterile) (P+, P-, I+, I-, O+, O-) Control = - Retrafos = +
  - in the control group we used 1µl MilliQ Water instead of Plasmid
- Incubate 30 min on ice
- 42°C, 30 sec heatshock
- Incubate 5 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 300rpm (incubator)
- 5 min with max rpm centrifugation. decand the supernatant and resuspend the cells in the remaining suspension
- spread the suspension on the respective agar

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

Author: Matthias Willmann Entry 14/143: No entry title yet In Project: General No tags associated	created: 09.04.2018 15:31 updated: 09.04.2018 15:31

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

Author: Nicholas Schmitt
created: 09.04.2018 16:48
updated: 09.04.2018 18:25

Entry 15/143: Retransformation of the last Dueber plasmids
In Project: General
No tags associated

In a lot of the tubes that have been set up last time transformation did not seem to occur, so the process was done again. However #6 and #59 are empty and it was not possible to replenish them.

- Incubate competent cells 10 minutes on ice
- Add 10-15 µl cells + 1 µl plasmids in Eppis (sterile)
- Incubate 5 min on ice
- 42°C, 45 sec heatshock (not longer)
- Incubate 2 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
- 100 µl cells + 3 mL LB with antibiotic --> Overnight 37°C 200 rpm

We did it with the last plasmids with another antibiotic than chloramphenicol.

Ampicillin: **#89***, #83, #95

Kanamycin: #84, #90, #96

Spectinomycin: #85, #91

*#89 was missing in the Dueber toolbox, so there is **no** retransformation.

The process was performed on:

#11

#12

#13

#18

#22

#23

#51

#52

#54

#55

#58

#60

#61

#62

#63

#64

#65

#66

#68

#67

#69

#70

#76

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

Author: Jennifer Denter
created: 10.04.2018 19:37
updated: 01.08.2018 19:18
Entry 17/143: Preparation of Kryo cultures & plasmidisolation
In Project: General
With tags: Kryo, plasmidisolation, miniprep

Preparation of Kryostocks for -80 storage

We used: 730 µl overnight culture

fill up to 1 ml with Glycerin (Sterile)

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

E. coli strains with DUBER toolbox plasmids

Number of DUBER plasmids for the stocks and isolations:

11, 12, 13, 18, 22, 23, 51, 55, 57, 58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 76

After creating stocks:

Isolation of plasmids wit the Promega MiniPrep Kit

Measure the concentrations of the DNA (at the next day)

Storage of plasmids at -20°C.

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

Author: Susanne Vollmer
Entry 18/143: Testtransformation of competent cells
In Project: General
No tags associated

created: 11.04.2018 18:33
updated: 11.04.2018 18:39

Testtransformation of the competent cells from 10.4.18 with the DUBER Plasmid 0051, by followring the protocol:

- Incubate competent cells 10 minutes on ice
- Add 15 µl cells + 1 µl plasmids in Eppis (sterile) (P+, P-, I+, I-, O+, O-) Control = - Retrafos = +
  - in the control group we used 1µl MilliQ Water instead of Plasmid
- Incubate 30 min on ice
- 42°C, 30 sec heatshock
- Incubate 5 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 300rpm (incubator)
- 5 min with max rpm centrifugation. decand the supernatant and resuspend the cells in the remaining suspension
- spread the suspension on the respective agar

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

Author: Lev Petrov Entry 19/143: Plasmid Concentration In Project: General No tags associated	created: 11.04.2018 21:29 updated: 11.04.2018 21:34

Concentration of plasmids has been measured using an in-house photometer. See attached scans for values.

Plasmid_Concentration.pdf

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

Author: Lev Petrov
Entry 20/143: Retransformation Dueber 52
In Project: General
No tags associated

created: 11.04.2018 21:36
updated: 11.04.2018 21:41

The following process has been performed on **#52**; Vessel containing plasmid p0054 was empty, so it has been left out of the procedure.

- Incubate competent cells 10 minutes on ice
- Add 10-15 µl cells + 1 µl plasmids in Eppis (sterile)
- Incubate 5 min on ice
- 42°C, 45 sec heatshock (not longer)
- Incubate 2 minutes on ice
- + 300 µl LB without antibiotic **STERILE**
- 60 min at 37°C and 400rpm (incubator) (200 rpm also nice)
- 100 µl cells + 3 mL LB with antibiotic --> Overnight 37°C 200 rpm

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

Author: Katharina Polzen

created: 11.04.2018 22:31
updated: 11.04.2018 22:50

Entry 21/143: Inoculation of a liquid culture

In Project: General

With tags: inoculation, Corynebacterium, Corynebacterium, Zymomonas

A smear auf *Corynebactericum glutamicum* and *Zymomonas mobilis* was added in 3 mL LB and incubated at 30°C overnight. The procedure was made two times with each organism.

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

Author: Nicholas Schmitt
Entry 22/143: Kryo-Prep
In Project: General
No tags associated

created: 12.04.2018 13:54
updated: 12.04.2018 14:58

Preparation of Kryostocks for -80 storage

We used: 730 µl overnight culture

fill up to 1 ml with Glycerin (Sterile)

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

E. coli strains with DUBER toolbox plasmids

Number of DUBER plasmids for the stocks and isolations:

52

After creating stocks:

Isolation of plasmids with the Promega MiniPrep Kit

Measure the concentrations of the DNA (same day)

Storage of plasmids at -20°C.

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

Author: Nicholas Schmitt
Entry 23/143: Plasmid Concentration
In Project: General
No tags associated

created: 12.04.2018 14:56
updated: 12.04.2018 14:58

Concentration of Deuber plasmid #52 has been measured using an in-house photometer. #52 was measured at 223,20 ng/µl

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

Author: Nicholas Schmitt
Entry 24/143: MiniPrep for Deuber #52
In Project: General
No tags associated

created: 12.04.2018 14:58
updated: 12.04.2018 15:45

After successful incubation, the Promega MiniPrep Tollkit was used to isolate the plasmids.

To 600 µl Culture 100 µl Lysisbuffer was added and, after a five seconds, 350µl Neutralization was added.

The solution was put into the centrifuge for 3 min at max rpm.

After that we separated the fluid from the pallet and filtrated the fluid through a column by putting into the centrifuge for 30sek at max rpm.

After that we used 400µl Column Wash and filtrated by putting it into the centrifuge for 30sek at max rpm.

After that we washed out with 37°C hot water and filtrated by putting it into the centrifuge for 15sek at max rpm.

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

Author: Carina Gude
Entry 25/143: Preparing Yeast strain BY4742
In Project: General
With tags: yeast, S.cerevisiae

created: 13.04.2018 14:44
updated: 13.04.2018 15:55

11.04.2018: Waking up the Yeast strain BY4247

- inoculate on agar plate
- over night incubation

12.04.2018: Preparation of pre culture

- inoculating in 3ml medium
  - 2x single colonies
  - 2x multi colonies (thin and thick
- over night incubation

13.04.2018: Preparation of main culture

- 12.00 o'clock: OD measurment:
  - thick multi colonies: OD=0,8
  - rest: OD=0
- adding 6ml of medium to the 2ml of culture in order to get and OD of 0,2
- let sit for 5h --> OD=1

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

Author: Nicholas Schmitt
Entry 26/143: Preparing Medium and Agar
In Project: General
No tags associated

created: 13.04.2018 17:47
updated: 13.04.2018 18:13

In order to replenish our stocks, Medium and LB Agar was prepared

The medium was prepared according to the guide-lines, in other words 12.5 grams per 500 ml.

Two 0.5 Liter and one 1 Liter false were prepared and put into the 4°C room.

LB Agar was prepared with Kanamycin and Ampicillin.

The target concentrations are 100µg/ml for Ampicillin and 25µg/ml for Kanamycin. In both case, the concentration of the stock was 10mg/ml.

The Agar was then used to pour Kanamycin and Ampicillin plates.

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

Author: Katharina Polzen Entry 27/143: Preparation of chemically competent cells (E. Coli DHSa) In Project: General With tags: E.coli, competent cells	created: 13.04.2018 18:04 updated: 13.04.2018 18:06

preparation-of-chemically-competent-cells-n8mdhu6.pdf

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

Author: Nicholas Schmitt
Entry 29/143: Sorting of Ordered Enzyms
In Project: General
No tags associated

created: 16.04.2018 16:36
updated: 16.04.2018 16:54

Ordered entymes war sorted into boxes and stored at -20°C.

Box 1: Blue plastic box for restrictionenzymes

Enzymes stored in:

BbsI - HF (NEB) with CutSmart buffer and 6X gel loading dye

BsaI - HF (NEB) with CutSmart buffer and 6X gel loading dye

DpI (NEB) with CutSmart buffer ans 6X gel loading dye

BsmBI (NEB) with 3.1 buffer and 6X gel loading dye

Enzymes standing side by side, the belonging buffer below

Box2: White paper box for Additions for PCR & Gelelectrophorase

Q5 High-fidelity DNA polymerase, 4x High GC Enhancer, 4x Reaction Buffer

Taq DNA polymerase with 2x ThermoPol Buffer

Taq DNA Ligase, 2x Buffer

T4 DNA Ligase, 1x Buffer

T5 Exonuclease, 1x Buffer

In the right corner on the bottom:

4X 1kb Ladder template (not equated till now)

2X Loading dye

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

Author: Carina Gude
Entry 30/143: Testcloning GoGate
In Project: General
With tags: goldenGate, golden gate

created: 17.04.2018 09:59
updated: 17.04.2018 10:14

Mastermix:

2µl piGEM0051

2µl piGEM0013

2µl piGEM0002

2µl piGEM0067

2µl piGEM0074

2µl piGEM0082

2µl piGEM0083

4µl T4 Ligase buffer

2µl bsaI

2µl T7 Ligase

16µl H2O

Fluorescence marker solutions:

piGEM0032 +9,5µl Mastermix

piGEM0033 +9,5µl Mastermix

piGEM0034 + 9,5µl Mastermix

Thermocycler (Programm GoGate BasI)

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

Next day: Trafo

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

Author: Katharina Polzen
Entry 31/143: Preparation of chemically competent cells (Yeast)
In Project: General
With tags: yeast, competent cells, YPD

created: 17.04.2018 10:06
updated: 17.04.2018 10:12

- Plating the yeast on YPD agar and icubate at 30 ° C overnight

- Next day: Seeding a preculture (3-5 mL) at 30 ° C and 200-250 rpm overnight

- Next day: Seeding the preculture: 10 mL with OD 0,8-1

- Creating competent cells via Frozen-EZ Yeast Transformation

- aliquot in 50 µl
  --> 4 °C 13 min

  --> -20 °C 13 min
  --> -80 °C

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

Author: Salima Rüdiger
created: 17.04.2018 12:20
updated: 20.04.2018 17:14
Entry 32/143: Restriction of DUEBER plasmids for testing
In Project: General
With tags: dueber, plasmid, pdna, digest, restriction, enzyme, bsai, ladder

Yesterday 10 different concentrated DUEBER plasmid samples were digested with BsaI-HF (NEB).

p2iGEM0003 150 ng in 10 µl

p2iGEM0014 70 ng in 10 µl

p2iGEM0025 500 ng in 50 µl

p2iGEM0036 300 ng in 30 µl

p2iGEM0037 50 ng in 10 µl

p2iGEM0038 30 ng in 10 µl

p2iGEM0049 25 ng in 10 µl

p2iGEM0058 300 ng in 30 µl

p2iGEM0066 60 ng in 10 µl

We tried to use the absolute minimum to safe plasmid and normal amounts for digestions.

https://www.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions

Later we put the digested plasmid on a 1% agarose-gel + old ladders that have to be tested.

Purple 2-log DNA Ladder (2x)

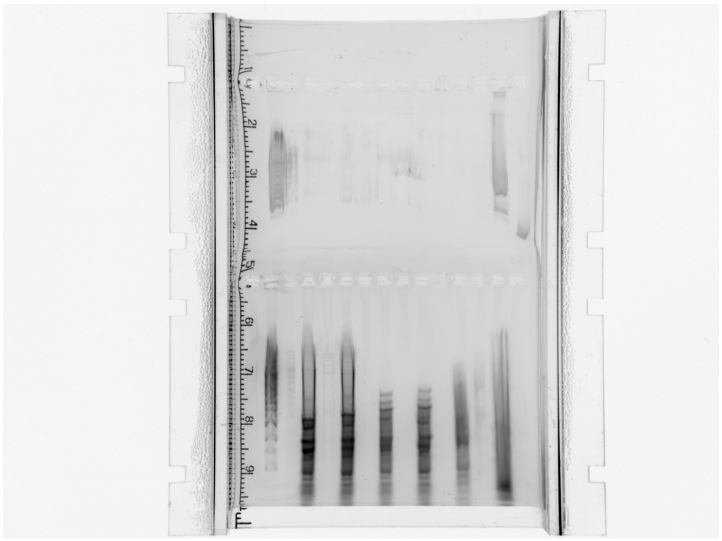
Purple 100 bp DNA Ladder (2x)

Quantitas 2 kb DNA Ladder

Ladder | 3 | 14 | 25 | 36 | 37 | 38 | 49 | 58 | 66 |

Ladder | 2-log (1) | 2-log (2) | 100 bp | 100 bp | 2 kb |

Gel_2018-04-16_21hr_25min_Testgel_DUEBER_+_alte_Ladder.jpg



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

Author: Matthias Willmann
Entry 34/143: minipräp Ura 1,2 cassete, lvl2 casette, 083
In Project: General
No tags associated

created: 18.04.2018 10:52
updated: 08.05.2018 20:20

minipräp for Ura cassette 1 , Ura cassette 2, lvl 2, 083

all from last year from Jan

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

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

100 µl Lysisbuffer were added and inverted.

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

now we centrifugated with max rpm for 3 minutes.

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

the flow was disposed.

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

then we used a new eppi under the column and put 30 µl Eluationbuffer into the column

we waited 5 minutes and centrifugated again for 30 seconds with max rpm

we added the plasmids to our Plasmid list and put them in "-20° iGem_drawer(2)green box - labeld 'Plasmids iGEM'"

p2iGEM0097	is	Ura1 cassette
p2iGEM0098	is	Ura2 cassette
p2iGEM0099	is	Lvl 2 cassette
p2iGEM0100	is	083 cassette

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

Author: Ylenia Longo
Entry 35/143: Dueber fluorescent proteins transfer onto liquid medium
In Project: General
No tags associated

created: 18.04.2018 13:58
updated: 18.04.2018 14:03

Clones from the fluorescent cassettes #32,#33,#34 of the previous day , which were plated out on LB-Amp, are simply inoculated on fresh medium (LB-Amp, 100ug/mL) and incubated over night at 37°C, 225 rpm.

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

Author: Katharina Polzen
created: 19.04.2018 09:37
updated: 14.05.2018 09:42
Entry 36/143: Test restriction with BsaI
In Project: General
With tags: agarose gel, bsai, gel electrophoresis, ladder

Mastermix:

- 3,5µl pro Plasmid (p2iGEM047,p2iGEM082,p2iGEM083)
- 7µl T4 DNA Ligase Buffer
- 3,5µl T7 DNA Ligase
- 35µl H2O

--> kurz vortexen

cassette leu:

- 8µl Mastermix
- 0.5µl p2iGEM003
- 0.5µl p2iGEM072
- 0.5µl p2iGEM075
- 0.5 BsaI

cassette GOI3/4_2:

- 8µl Mastermix
- 0.5µl p2iGEM003
- 0.5µl p2iGEM068
- 0.5µl p2iGEM075
- 0.5 BsaI

cassette GOI3_3:

- 8µl Mastermix
- 0.5µl p2iGEM004
- 0.5µl p2iGEM076
- 0.5µl p2iGEM072
- 0.5 BsaI

cassette GOI4_3:

- 8µl Mastermix
- 0.5µl p2iGEM004
- 0.5µl p2iGEM069
- 0.5µl p2iGEM076
- 0.5 BsaI

cassette GOI4_4:

- 8µl Mastermix
- 0.5µl p2iGEM005
- 0.5µl p2iGEM072
- 0.5µl p2iGEM074
- 0.5 BsaI

Thermocycler (Programm GoGate BasI)

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

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

Author: Katharina Polzen

created: 19.04.2018 11:25
updated: 14.05.2018 09:43

Entry 37/143: Inoculation cassettes
In Project: General
With tags: inoculation

Ura1 cassette, Ura2 cassette, Lvl 2 cassette, 083 cassette were inoculated and stored overnight at 37 ° C 225 rpm.

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

Author: Katharina Polzen
created: 19.04.2018 11:28
updated: 19.04.2018 14:45
Entry 38/143: Test restriction
In Project: General
With tags: agarose gel, gel electrophoresis, gel, bsai

With p2iGEM100

3 approaches

1. 1 µl p2iGEM100, 1 µl NEBuffer, 1 µl BsaI filled with 22 µl H_2O
2. 1 µl p2iGEM100 1:10 dilution, 1 µl NEBuffer, 1µl BsaI filled with 22 µl H_2O
3. 1 µl p2iGEM100 1:10 dilution, 1 µl NEBuffer, 1 µl BsaI filled with 47 µl H_2O

Thermocycler (Programm GoGate BsaI)

- 37 ° C 1 h
- 65 ° C 20 min
- 4 ° C hold

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

Author: Ylenia Longo

created: 19.04.2018 14:54
updated: 19.04.2018 15:07

Entry 39/143: Minirep of fluorescent plasmids
In Project: General
No tags associated

Isolation of plasmids p2iGEM0101/p2iGEM0102/p2iGEM0103 according to the PureYield Miniprep Plasmid Kit from Promega. .

1. 600µl culture+ 100 µl Lysisbuffer --> mix+ 350 µl Neutralisation buffer (cold) --> mix
2. centrifuge at 3 min max rpm
3. --> Repeat step 1 and 2 if necessary
4. Add supernatant (~ 800 µl) to column
5. centrifuge 15 Sek. max rpm --> discard supernatant
6. + 200 µl Endotoxin removal wash
7. 15 Sek. max rpm
8. For elution use new tube+ 30 µl 37°C prewarmed water on column
9. Let incubate for 5 Min at RT. Centrifuge for 15 Sek. max

The concentration of the plasmids were

236,55 ng/ul--> #101

200,4 ng/ul--> #102

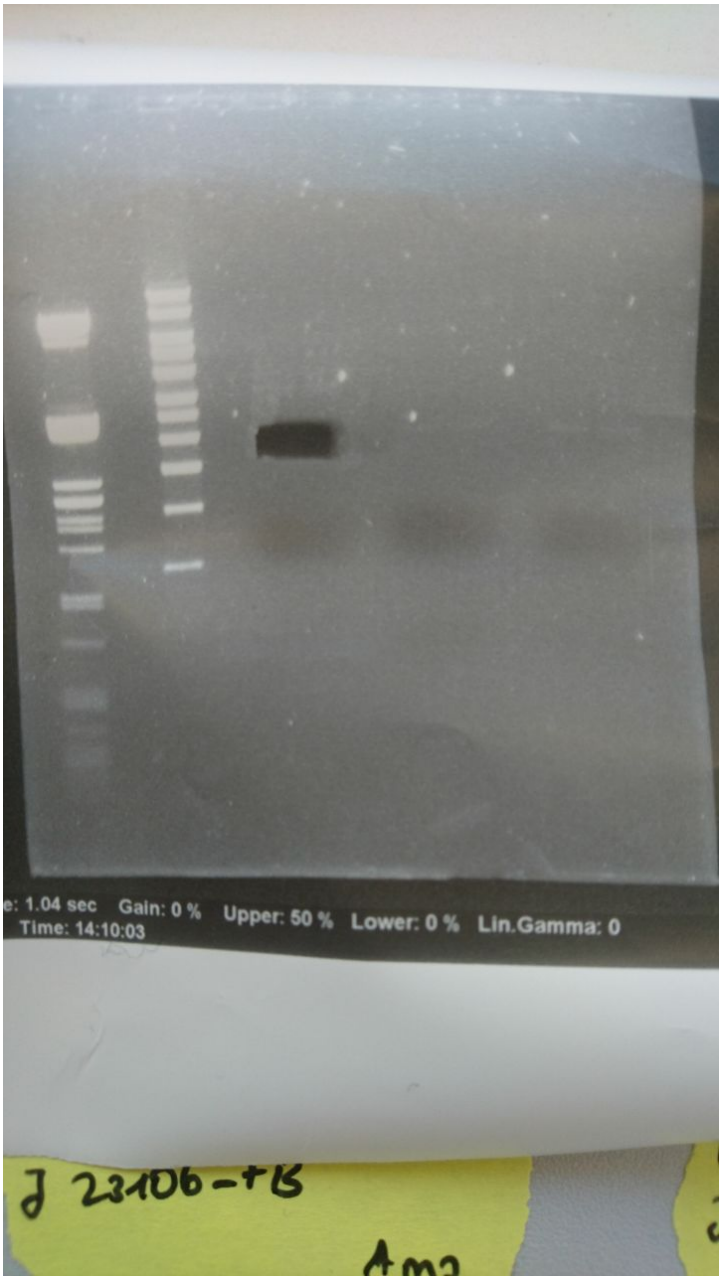
258,55 ng/ul--># 103

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

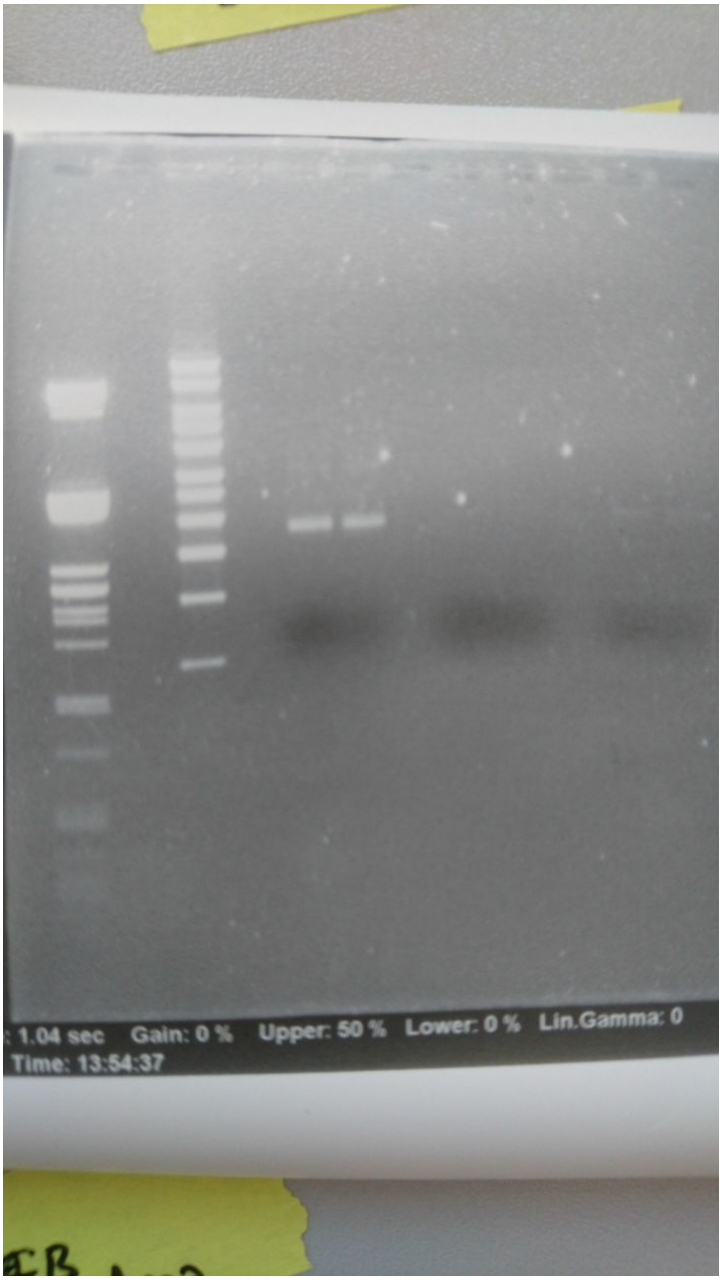
Author: Ylenia Longo
created: 20.04.2018 14:46
updated: 20.04.2018 15:06
Entry 40/143: Isolation of AmpR from p2iGEM0083
In Project: General
No tags associated

p2iGEM0083 was previously cut with BsaI. To the reaction 10ul loading dye was added and then each lane was loaded with 20ul sample (ladder: 1kbp Rothi ladder). The samples were then separated by size as can be seen in the figure below. Also, the band at approx.1800bp is gel extracted.

Gel1.jpeg


e: 1.04 sec Gain: 0 % Upper: 50 % Lower: 0 % Lin.Gamma: 0
Time: 14:10:03
J 23106-FB

Gel2.jpeg



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

Author: Salima Rüdiger
created: 20.04.2018 17:03
updated: 20.04.2018 18:58

Entry 41/143: Gel for plasmid testing
In Project: General
With tags: bsai, digest, dueber, enzyme, ladder, pdna, plasmid, restriction, Corynebacterium, gDNA, glutamicum, mobilis, Zymomonas, gel

Today five different DUEBER plasmids were digested with BsaI for one hour at 37°C.

--> 500 ng plasmid in 20 µl reaction medium.

p2iGEM: #0011

#0012

#0018

#0019

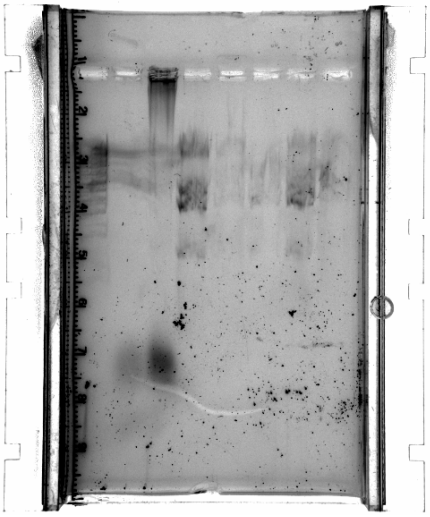
#0020

Then the reaction was inactivated at 65°C for 20 min.

10 µl of each reaction, plus gDNA of *Zymomonas mobilis* and *Corynebacterium glutamicum*, were applied to a 1% agarose gel (stained with 2 µl GelRed) at 90 V (-> 4,5 V/cm) for 45 min.

1 kb Ladder | C. g. | Z. m. | 11 | 12 | 18 | 19 | 20 |

Gel_2018-04-20_18hr_49min_sad_Test_digestion_Dueber_gDNA_11,12,18,19,20_90V_45min.tif



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

Author: Sarah Seyffert
Entry 43/143: Overnightculture
In Project: General
With tags: overnight cultures

created: 21.04.2018 12:05
updated: 31.07.2018 16:19

Today we prepared the main culture of 2iGEM0108,2iGEM0109,2iGEM0110,2iGEM0111,2iGEM0112

- 10 culture tubes were filled with 3ml LB-medium (sterile)
- 30 µl ampicillin were added from a 10mg/ml stock (sterile)
- 1 culture of the strains on the agar plate was transferred in the culture tubes (sterile)
- overnight incubation by 37°C

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

Author: Matthias Willmann
Entry 44/143: Gelelectrophoresis Duber 12/19/20
In Project: General
No tags associated

created: 22.04.2018 15:10
updated: 25.04.2018 12:24

Today we did a gelelectrophoresis with restricted plasmids("R") (with Bsa1) and with unrestricted plasmids ("N") with the Plasmids p2iGEM0012, p2iGEM0019, p2iGEM0020.

We want to test, if the loading dye is the reason for our bad looking gel images

We used a new made agarose gel (1%, 40 ml, 2 µl gelred)

we tested with two different loading dye:

LD = Gel loading dye purple (6x) from NEB

B = 1x Gel loading buffer Rothi load

For the Electrophoresis we used 5 µl plasmid per eppi and 5 µl B or 2 µl LD with 10 µl MilliQ

1	Ladder (10 µl)		
2	p2iGEM0012	R	B
3	p2iGEM0019	R	LD
4	p2iGEM0020	R	B
5	p2iGEM0012	N	LD
6	p2iGEM0019	N	B
7	p2iGEM0020	N	LD

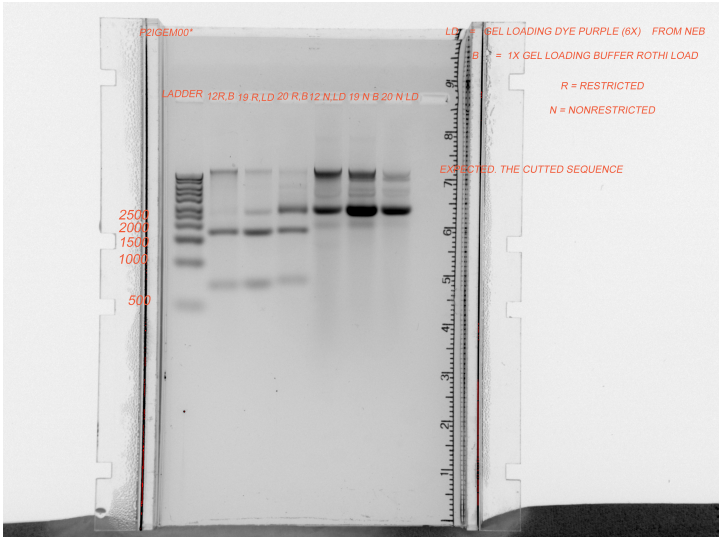
we expected a bond with 1662 bp for the restricted plasmids and also found the bonds

we ran our gel with 135 V for 20 minutes

we ran our gel again with 135 for 20 minutes

This was a testrestriction to find the problems we had with our gelpictures.
the solution: the only difference was the gel, it was the first time we use a complete new made gel and used many different conditions and all restrictions worked.

Gel_2018-04-24_16hr_40min_Test_of_Testgel_SR_135_V_40_min_fresh_gel.png



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

Author: Sarah Seyffert
created: 22.04.2018 21:08
updated: 03.05.2018 23:30
Entry 45/143: Miniprep and cryocultures
In Project: General
With tags: Pure yield miniprep, cryogenic culture

Mini prep of the Plasmids p2iGEM0104-p2IGEM0108 with the following Protocoll of the Pure Yield Miniprep:

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

The concentrations were measured after this

p2iGEM0104 = 453,4ng/µl

p2iGEM0105 = 406,25ng/µl

p2iGEM0106 = 404,9ng/µl

p2iGEM0107 = 622,15ng/µl

p2iGEM0108 = 339,1ng/µl

Cryoculture were made with the strains 2iGEM0108- p2iGEM0112

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

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

Author: Jennifer Denter
Entry 46/143: Preperation and dilution of primers
In Project: General
With tags: dilution

created: 23.04.2018 11:28
updated: 01.08.2018 19:20

Preparation of ordered primers

add fresh strerile Milli Q Water to the primer pellet (amount of water needed for 100µM)

diluted primers to a work concentration of 10µM (prepared 100µl)

Storage: Paper box, -20°C.

Note: Primer 2O_iGEM_0039 we got 2 times.

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

Author: Carina Gude
Entry 47/143: CIDAR Toolbox
In Project: General
With tags: incubation

created: 23.04.2018 15:23
updated: 23.04.2018 15:46

Cidar Toolbox incubation:

per Glycerol stock sample (A1-B12/ 2iGEM0113-2iGEM0136):

- 3ml LB Medium
- 30µl Amp (100µg/ml)

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

Author: Katharina Polzen
Entry 48/143: Golden Gate Assembly
In Project: General
With tags: goldenGate, bsai

created: 23.04.2018 15:46
updated: 14.05.2018 09:45

With p2iGEM100

3 approaches

1. 1 µl p2iGEM100, 1 µl NEBuffer, 1 µl BsaI filled with 22 µl H_2O
2. 1 µl p2iGEM100, 1 µl NEBuffer, 1 µl BsaI filled with 47 µl H_2O

Thermocycler (Programm GoGate BsaI)

- 37 ° C 1 h
- 65 ° C 20 min
- 4 ° C hold

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

Author: Nicholas Schmitt

created: 24.04.2018 11:59
updated: 25.04.2018 21:03

Entry 49/143: Mini prep of the Plasmids p2iGEM0109-p2IGEM0132

In Project: General

No tags associated

Mini prep of the Plasmids p2iGEM0109-p2IGEM0132 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

The concentration of the isolated Plasmids:

Plasmidnumber:	Concentration [ng/µl]:
p2iGEM0109	78,5
p2iGEM0110	115,45
p2iGEM0111	90,8
p2iGEM0112	59,7
p2iGEM0113	49,9
p2iGEM0114	65,5
p2iGEM0115	40,95
p2iGEM0116	16,5
p2iGEM0117	45,35
p2iGEM0118	34,95
p2iGEM0119	37,75
p2iGEM0120	45,8
p2iGEM0121	34
p2iGEM0122	50,4
p2iGEM0123	46
p2iGEM0124	26
p2iGEM0125	59,95
p2iGEM0126	45,85
p2iGEM0127	46,75
p2iGEM0128	51,65
p2iGEM0129	51,1
p2iGEM0130	19,35
p2iGEM0131	59,05
p2iGEM0132	52,95

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

Author: Katharina Polzen
created: 24.04.2018 12:08
updated: 24.04.2018 12:11
Entry 50/143: Making of the first 24 CIDER Kryos
In Project: General
With tags: Kryo, Cidar, E.coli

300 µl glycerol and 300 µl culture added in one kryo tube.

Formblatt Z checked and signed by Pauly!

Made with:

2iGEM0113
2iGEM0114
2iGEM0115
2iGEM0116
2iGEM0117
2iGEM0118
2iGEM0119
2iGEM0120
2iGEM0121
2iGEM0122
2iGEM0123
2iGEM0124
2iGEM0125
2iGEM0126
2iGEM0127
2iGEM0128
2iGEM0129
2iGEM0130
2iGEM0131
2iGEM0132
2iGEM0133
2iGEM0134
2iGEM0135
2iGEM0136

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

Author: Katharina Polzen Entry 51/143: No entry title yet In Project: General No tags associated	created: 24.04.2018 22:44 updated: 24.04.2018 22:44

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

Author: Matthias Willmann
created: 25.04.2018 16:18
updated: 26.04.2018 03:02
Entry 52/143: Duber testrestriction with BsaI
In Project: General
No tags associated

Duber Testrestriction

- 2µl Buffer per Plasmid
- 0,5µl Enzyme (cold!) per plasmide

first we put the Water in the eppis

second we put the buffer in the eppis

then we put the Plasmids in the eppis

at least we put the Enzyme BsaI in the eppis

We used a 1 % agarose gel with gelred

					Plasmid Concentration (ng/µl) for 500ng we used(µl):
DUBER025	254,6	1,96386489	0,5	15,5361351	2
DUBER029	281,2	1,77809388	0,5	15,7219061	2
DUBER036	293,1	1,70590242	0,5	15,7940976	2
DUBER052	223,2	2,24014337	0,5	15,2598566	2
DUBER058	316,8	1,57828283	0,5	15,9217172	2
DUBER074	231,4	2,16076059	0,5	15,3392394	2
DUBER081	242	2,0661157	0,5	15,4338843	2
DUBER083	260,3	1,92086055	0,5	15,5791395	2
DUBER087	348	1,43678161	0,5	16,0632184	2
DUBER088	277,9	1,79920835	0,5	15,7007917	2

BsaI (µl) MilliQ(µl) Cutsmart buffer(µl)

15min 37°C incubation time

20min 65°C inactivation time

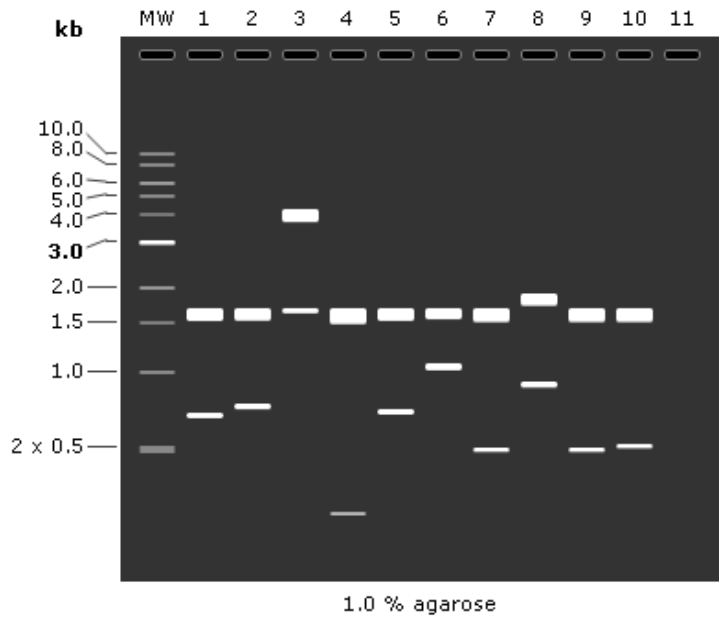
after that: add 5µl loading dye to the restricted plasmids

Gelelectrophoresis: 100V 60min

expected bands:

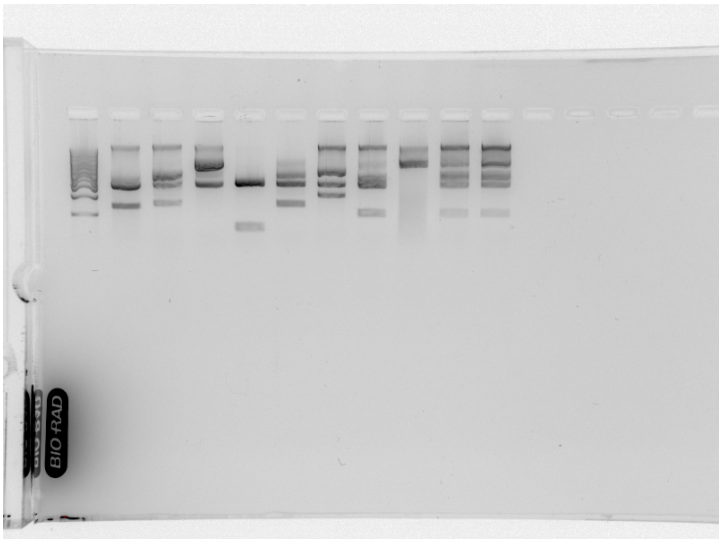
Duber025:1662	703	(1)
Duber029:1662	759	(2)
Duber036:4167	1662	(3)
Duber052:1662	238	(4)
Duber058:1662	724	(5)
Duber074:1662	1063	(6)
Duber081:1662	512	(7)
Duber083:1870	919	(8)
Duber087:1662	514	(9)
Duber088:1662	524	(10)

Gel_Image25.04.png



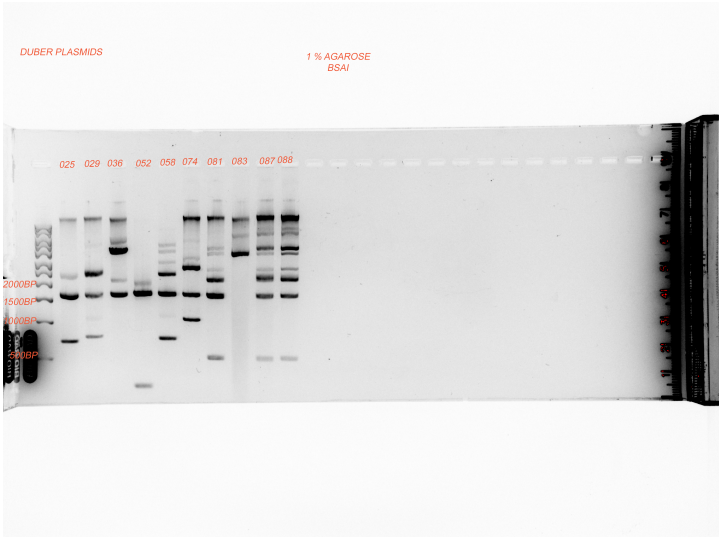
This was our Gel after 30 minutes. It was not clear to see so we ran it again for 30 minutes more

Gel_2018-04-25_18hr_52min_Testgel_DUEBER_1_kb_ladder_30_min_100_v.tif



Here we can see our restricted plasmids after 1 Hour. exept the Plasmid Duber 083 each Plasmid was restricted correct.

Gel_2018-04-25_18hr_52min_Testgel_DUEBER_1_kb_ladder_1_h_100_v,_1_h_50_v.png



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

Author: Katharina Polzen
created: 25.04.2018 21:54
updated: 25.04.2018 22:06
Entry 53/143: CIDAR kryos and preps
In Project: General
With tags: Pure yield miniprep, Kryo, E.coli, Cidar

Cidar Toolbox kryos and mini preps:

- 700µl culture + 300µl Glycerin **sterile**
- stored in -80°C freezer CIDAR holy stocks

We also prepared plasmids according to these steps:

- 600µl Kultur + 100 µl Lysisbuffer --> mixen+ 350 µl Neutralisation (kalt) --> mixen
- 3 min max rpm zentrifugieren
- --> Schritte 1 und 2 wiederholen, bis keine Kultur mehr da ist
- Überstand (~ 800 µl) auf Säule geben
- 15 Sek. max rpm zentrifugieren--> Durchfluss verwerfen
- --> restlichen Überstand auf Säule geben, Schritt 5 wiederholen
- + 200 µl Endotoxin removal wash
- 15 Sek. max rpm
- Neues Eppi drunter+ 30 µl aus 37°c erwämrters Wasser auf Säule
- 5 Min stehen lassen
- 15 Sek. max runter zentrifugieren
- Konzentration messen

Made with:

2iGEM0113
2iGEM0114
2iGEM0115
2iGEM0116
2iGEM0117
2iGEM0118
2iGEM0119
2iGEM0120
2iGEM0121

2iGEM0122
2iGEM0123
2iGEM0124
2iGEM0125
2iGEM0126
2iGEM0127
2iGEM0128
2iGEM0129
2iGEM0130
2iGEM0131
2iGEM0132
2iGEM0133
2iGEM0134
2iGEM0135
2iGEM0136
2iGEM0137
2iGEM0138
2iGEM0139
2iGEM0140
2iGEM0141
2iGEM0142
2iGEM0143
2iGEM0144
2iGEM0145
2iGEM0146
2iGEM0147
2iGEM0148
2iGEM0149
2iGEM0150
2iGEM0151

2iGEM0152
2iGEM0153
2iGEM0154
2iGEM0155
2iGEM0156
2iGEM0157
2iGEM0158
2iGEM0159
2iGEM0160
2iGEM0161
2iGEM0162
2iGEM0163
2iGEM0164
2iGEM0165
2iGEM0166
2iGEM0167
2iGEM0168
2iGEM0169
2iGEM0170
2iGEM0171
2iGEM0172
2iGEM0173
2iGEM0174
2iGEM0175
2iGEM0176
2iGEM0177
2iGEM0178
2iGEM0179
2iGEM0180
2iGEM0181

2iGEM0182
2iGEM0183
2iGEM0184
2iGEM0185
2iGEM0186
2iGEM0187
2iGEM0188
2iGEM0189
2iGEM0190
2iGEM0191
2iGEM0192
2iGEM0193
2iGEM0194
2iGEM0195
2iGEM0196
2iGEM0197
2iGEM0198
2iGEM0199
2iGEM0200
2iGEM0201
2iGEM0202
2iGEM0203
2iGEM0204
2iGEM0205

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

Author: Katharina Polzen
created: 25.04.2018 22:01
updated: 25.04.2018 22:05
Entry 54/143: DUEBRR Toolbox incubation
In Project: General
With tags: dueber, incubation

DUEBER Toolbox incubation:

per Glycerol stock sample:

- 6 ml LB Medium
- 4 µl Cam

Made with:

E. coli T10_DUBER001

E. coli T10_DUBER002

E. coli T10_DUBER004

E. coli T10_DUBER005

E. coli T10_DUBER007

E. coli T10_DUBER008

E. coli T10_DUBER009

E. coli T10_DUBER010

E. coli T10_DUBER013

E. coli T10_DUBER014

E. coli T10_DUBER015

E. coli T10_DUBER016

E. coli T10_DUBER017

E. coli T10_DUBER021

E. coli T10_DUBER024

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

Author: Matthias Willmann
created: 26.04.2018 15:48
updated: 04.05.2018 13:19
Entry 55/143: Duber testrestriction with BsaI
In Project: General
No tags associated

Duber Testrestriction

- 2 µl Buffer per Plasmid
- 0,5µl Enzyme (cold!) per plasmide

first we put the Water in the eppis

second we put the buffer in the eppis

then we put the Plasmids in the eppis

at least we put the Enzyme BsaI in the eppis

We used a 1 % agarose gel with gelred

Content	ng/µl	µl benötigt	BsaI (µl)	cutsmart buffer(µl)	MilliQ (µl)
DUBER001	85,9	2	0,5!!!!BsmBI	2	#WERT!
DUBER002	87,7	2	0,5	2	15,5
DUBER003	150,3	2	0,5	2	15,5
DUBER004	96,1	2	0,5	2	15,5
DUBER005	119,6	2	0,5	2	15,5
DUBER006		2	0,5	2	15,5
DUBER007	73,6	2	0,5	2	15,5
DUBER008	53,5	2	0,5	2	15,5
DUBER009	105,5	2	0,5	2	15,5
DUBER010	107,3	2	0,5	2	15,5
DUBER011	128,1	2	0,5	2	15,5
DUBER012	93,1	2	0,5	2	15,5
DUBER013	46,6	2	0,5	2	15,5
DUBER014	70,6	2	0,5	2	15,5
DUBER015	54	2	0,5	2	15,5
DUBER016	58	2	0,5	2	15,5
DUBER017	50,9	2	0,5	2	15,5
DUBER018	113,5	2	0,5	2	15,5
DUBER019	175,6	2	0,5	2	15,5
DUBER020	168,7	2	0,5	2	15,5
DUBER021	85,8	2	0,5	2	15,5
DUBER022	66,9	2	0,5	2	15,5
DUBER023	66,3	2	0,5	2	15,5
DUBER024		2	0,5	2	15,5

60min 37°C incubation time

20min 65°C inactivation time

after that: add 5µl loading dye to the restricted plasmids

Gelelectrophoresis: 100V 60min

Here are the expected Restrictions:

Dueber001 1646 1030

Dueber002 1662 194

Dueber003 1662 194

Dueber004 1662 194

Dueber005 1662 194

Dueber006 1662 194

Dueber007 1662 194

Dueber008 1662 173

Dueber009 1662 689

Dueber010 1662 709

Dueber011 1662 709

Dueber012 1662 685

Dueber013 1662 709

Dueber014 1662 709

Dueber015 1662 709

Dueber016 1662 708

Dueber017 1662 709

Dueber018 1662 709

Dueber019 1662 709

Dueber020 1662 709

Dueber021 1662 709

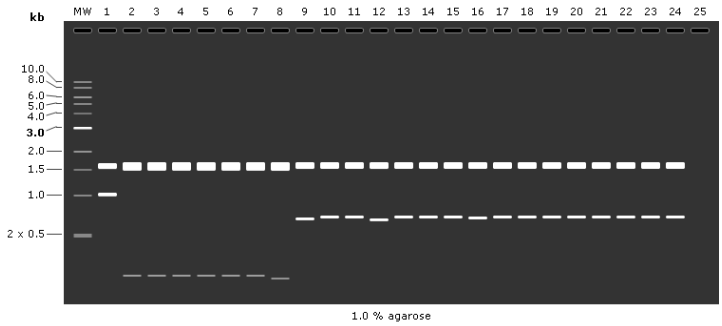
Dueber022 1662 709

Dueber023 1662 709

Dueber024 1662 709

Here is the expected gel image:

Gel_Image_Duber_01-25___26,04,2018.png

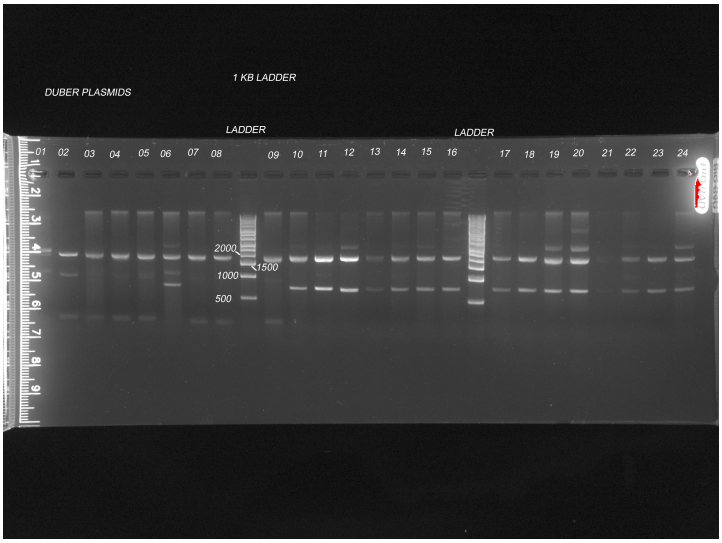


This was our testrestriction after one hour.
You can see every band clearly exept plasmid number 21. there might be a pipettemistake, but its also possible to see the bands..

Every upper band is restricted correct.

exept the Plasmid 06, every lower band is correct too.

Gel_2018-04-26_20hr_34min_Testgel_DUEBER_1kb_Ladder_1h_100V.png



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

Author: Katharina Polzen
Entry 56/143: Pure Yield Minipreps System Promega and cryos
In Project: General
With tags: Promega, Pure yield miniprep, Kryo

created: 27.04.2018 08:44
updated: 27.04.2018 08:56

Mini prep of plasmids with the following protocol:

- 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

Cryos:

300 µl glycerol and 700 µl culture added in one kryo tube.

Made with:

p2iGEM0001, p2iGEM0002, p2iGEM0004, p2iGEM0005, p2iGEM0007, p2iGEM0008, p2iGEM0009, p2iGEM00010, p2iGEM0014, p2iGEM0015, p2iGEM0016, p2iGEM0017, p2iGEM0021, p2iGEM0024, p2iGEM0021, p2iGEM0028, p2iGEM0029, p2iGEM0051, p2iGEM0033, p2iGEM0105, p2iGEM0106, p2iGEM0107, p2iGEM0108, p2iGEM0104

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

Author: Carina Gude
created: 27.04.2018 10:35
updated: 27.04.2018 10:49
Entry 57/143: Test restriction p2iGEM0109-p2iGEM0120
In Project: General
With tags: test restriction

Restriction with MmeI

- since the concentrations of the plasmids were to low to use 1µg for each restriction without using too much volume, 3µl of each plasmid (p2iGEM0109-p2iGEM0120) was used instead
- 41µl H2O

Mastermix:

- 13µl enzyme
- 65µl Buffer

→6µl to each H2O-plasmid mixture

Restriction with BsaI

- p2iGEM0104: 7,09 µl plasmid + 36,91 µl H2O
- p2iGEM0105: 2,09 µl plasmid + 41,91 µl H2O
- p2iGEM0106: 1,92 µl plasmid + 42,08 µl H2O
- p2iGEM0107: 8,12 µl plasmid + 35,88 µl H2O
- p2iGEM0108: 2,70 µl plasmid + 41,3 µl H2O

Mastermix:

- 7 µl BsaI
- 35 µl Buffer

→ 6µl to each H2O-plasmid mixture

Incubation: 37°C for 15min

Inactivation: 10µl loading dye was added to each PCR tube and mixed well

Stored: -20°C Quorum Sensing Plasmid box

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

Author: Nicholas Schmitt
Entry 58/143: Creating new 1x TAE Buffer
In Project: General
No tags associated

created: 27.04.2018 15:23
updated: 27.04.2018 15:26

Due to lacking 1x TAE Buffer, 4 liters were created by using MilliQ and the 50x TAE Buffer.

Of these, 2 were used up for gel electrophoresis.

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

Author: Nicholas Schmitt
created: 27.04.2018 16:12
updated: 02.05.2018 14:06
Entry 59/143: Testrestriction Dueber Plasmids
In Project: General
No tags associated

Testrestriction for the Dueber plasmids #26, #27, #28. #30, #31, #32, #33. #34, #35, #37, #38, #39, #40, #41, #42, #43, #44, #45, #46, #47, #48, #49, #50, #51

- 3 µl Plasmid
- 1 µl Cutsmart Buffer per Plasmid
- 0,25µl Enzyme (BsaI) (cold!) per plasmid
- 5,75 µl MilliQ Destille Water

First, a master solution of the MilliQ Water, the Buffer and the Enzym was created, then the plasmids were put into their respective tubes and the restriction-solution was added.

Then, the plasmids were incubated for;

60min at 37°C

20mina at 65°C

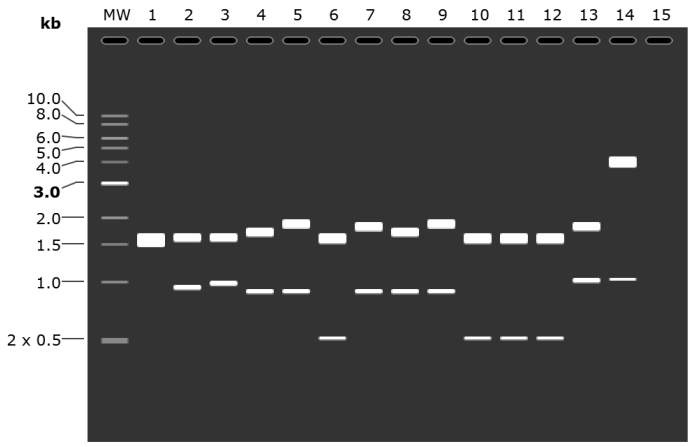
after that 5µl of purple loading dye were added to the incubated plasmids.

After that, we loaded them into a Gel and performed Gel electrophoresis at 90V for 60min

((Here are the expected Restrictions:

Here is the expected gel image:))(Not possible, as I can't open the Geneious files)

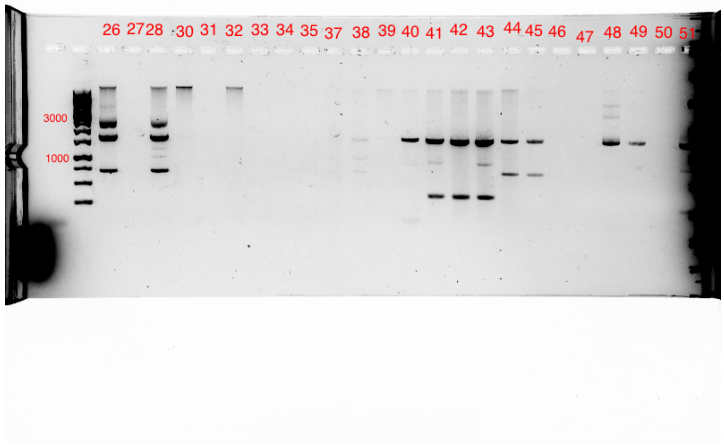
Gel_ImageDuber_Ende.png



1.0 % agarose

This is the result after the electrophoresis:

Smol_Gel_2018-04-27_18hr_08min_SV_PCR_links_NS_DUEBER_Testgel_rechts_90_V_90_Min_1_kb_ladder.tiff



26, 28, 41, 42, 43, 44, 45 turned out well. The others didn't. This is probably due to their low concentration.

Note, that number 46 dispersed upon deposition and as such has to be done again

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

Author: Katharina Polzen
Entry 60/143: Test restriction DUEBER & CIDAR
In Project: General
With tags: gel electrophoresis, test restriction, dueber, Cidar

created: 30.04.2018 12:44
updated: 30.04.2018 14:25

Gel preparation:

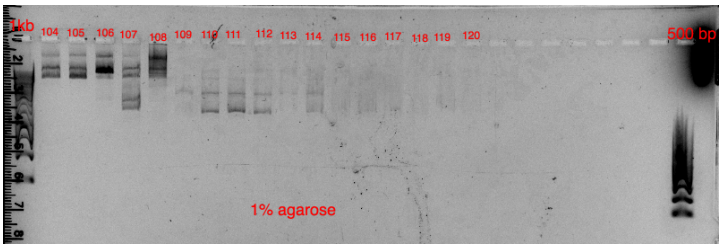
160 mL TAE and 1,6g agarose and 8 µl gelred

1x TAE buffer

Made with:

p2iGEM0104 - p2iGEM0120

iGEM_30-04-2018.tif



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

Author: Nicholas Schmitt
Entry 61/143: Testrestriktion Dueber Plasmids
In Project: General
No tags associated

created: 30.04.2018 13:59
updated: 01.05.2018 12:45

Testrestriction for the Dueber plasmids #53, #55, #56. #57, #60, #61, #62. #63, #64, #65, #66, #67, #68, #69, #70, #71, #72, #73, #74, #75, #76, #77, #78

- 3 µl Plasmid
- 2 µl Cutsmart Buffer per Plasmid
- 0,5µl Enzyme (BsaI) (cold!) per plasmid
- 14,5 µl MilliQ Destille Water

First, a master solution of the MilliQ Water, the Buffer and the Enzym was created, then the plasmids were put into their respective tubes and the restriction-solution was added.

Then, the plasmids were incubated for;

60min at 37°C

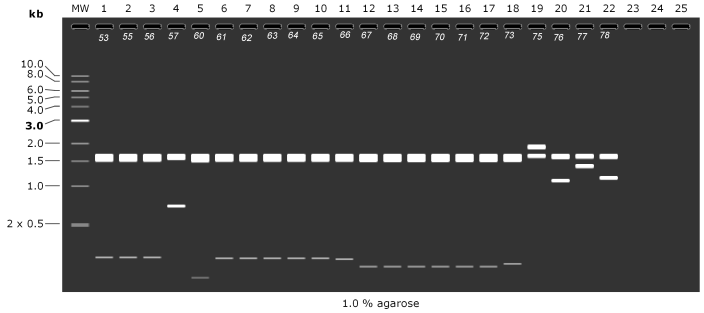
20mina at 65°C

after that 5µl of purple loading dye were added to the incubated plasmids.

After that, we loaded them into a Gel and performed Gel electrophoresis at 90V for 90min

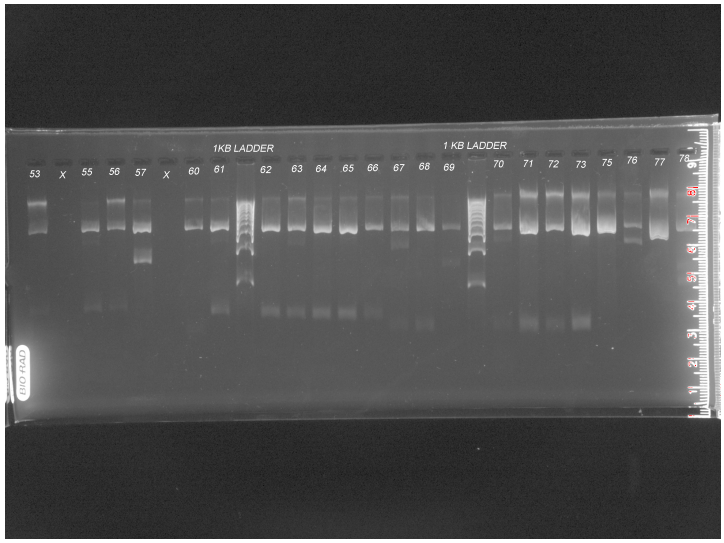
Here is the expected gel image:

Gel_Image_Duber_30.04.2018_.png



This is the result after the electrophoresis:

Gel_2018-04-30_17hr_56min_Testgel_DUEBER_1kb_Ladder_1h_100V.png



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

Author: Nicholas Schmitt
created: 01.05.2018 12:45
updated: 02.05.2018 07:52
Entry 62/143: No entry title yet
In Project: General
No tags associated

Testrestriction for the Dueber plasmids #79, #80, #82, #84, #85, #86 & #89-#96

- 3 µl Plasmid
- 1 µl Cutsmart Buffer per Plasmid
- 0,25µl Enzyme (BsaI) (cold!) per plasmid
- 5,75 µl MilliQ Destille Water

First, a master solution of the MilliQ Water, the Buffer and the Enzym was created, then the plasmids were put into their respective tubes and the restriction-solution was added.

Then, the plasmids were incubated for;

60min at 37°C

20mina at 65°C

after that 5µl of purple loading dye were added to the incubated plasmids.

After that, we loaded them into a Gel and performed Gel electrophoresis at 90V for 60min, then for 30min at the same Voltage

These are our expected bands:

79:1662, 1618

80:1662, 964

82:1662, 1005

84:1775, 919

85:1929, 919

86:1662, 524

89:1870, 919

90:1775, 919

91:1929, 919

92:1662,524

93:1662, 524

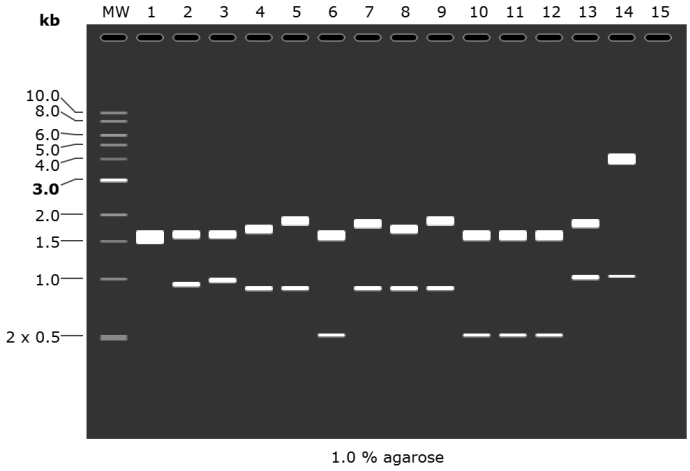
94:1662, 524

95:1870, 1036

96:4253,1030

Here is the expected gel image:

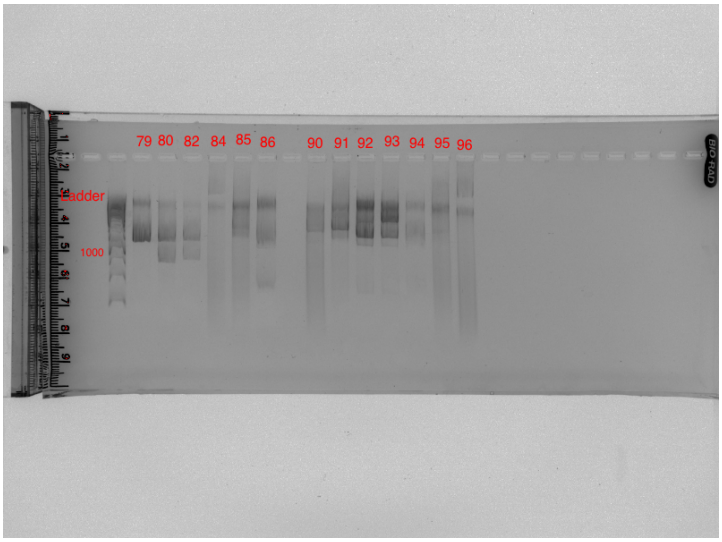
Gel_ImageDuber_Ende.png



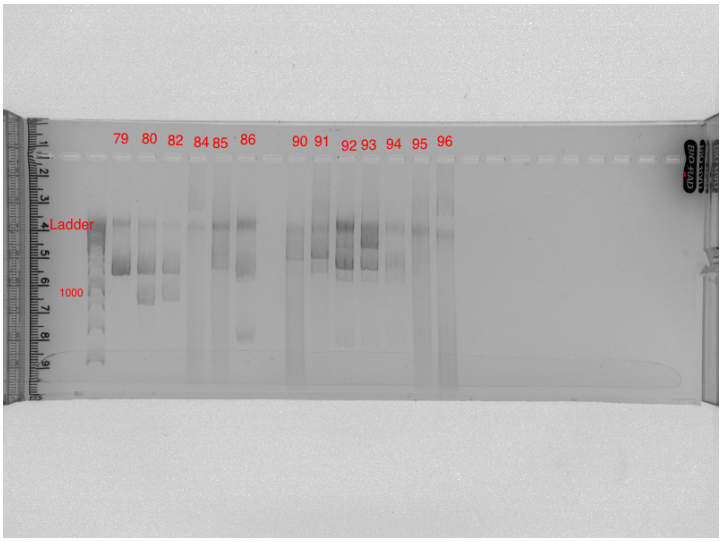
1.0 % agarose

This is the result after the electrophoresis (first after 60 Minutes, second after 90 Minutes):

Smol_Gel_2018-05-01_Test_Gel_DUEBER_1kb_79_-_96_V1_15hr_00min.tiff



Smol_Gel_2018-05-01Test_Gel_DUEBER_1kb_79-96_V2_15hr_44min.tiff



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

Author: Nicholas Schmitt
Entry 63/143: Taking Stock
In Project: General
No tags associated

created: 01.05.2018 16:25
updated: 02.05.2018 07:50

As was asked of her, Jennifer;

Verified our Kryo-Stocks

and checked the plasmid concentrations

For updates and missing kryos please refer to the respective sheets

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

Author: Salima Rüdiger
Entry 64/143: Preparation for measuring yeast growth
In Project: General
No tags associated

created: 02.05.2018 00:03
updated: 02.05.2018 00:06

Today we inoculated 3x *Saccharomyces cerevisiae* WT BY4742 in 10 ml YPD.

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

Author: Katharina Polzen
Entry 65/143: Test restriction CIDAR
In Project: General
With tags: Cidar, test restriction, gBlocks

created: 02.05.2018 14:22
updated: 02.05.2018 15:55

Gel preparation:

160 mL TAE and 1,6g agarose and 8 µl gelred

1x TAE buffer

90 V, 50 min.

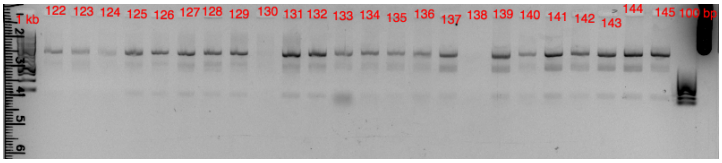
Made with:

p2iGEM0122 - p2iGEM0170.

Mix:

- 1,5 µl plasmid
- 20,5 µl H2O
- 0,5 µl MmeI
- 2,5 µl CutSmart

Gel_2018-05-02_15hr_13min_Testrestriction_CIDAR.tif



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

Author: Matthias Willmann
created: 02.05.2018 14:55
updated: 04.05.2018 13:18
Entry 66/143: Duber testrestriction with BsaI
In Project: General
No tags associated

Duber Testrestriction

- 2 µl Buffer per Plasmid
- 0,5µl Enzyme (cold!) per plasmide
-

first we put the Water in the eppis

second we put the buffer in the eppis

then we put the Plasmids in the eppis

at least we put the Enzyme BsaI in the eppis

We used a 1 % agarose gel with gelred

Content	ng/µl	µl benötigt	Bsal (µl)	cutsmart buffer(µl)	MilliQ (µl)
DUBER027	188,3	2	0,5		#WERT!
DUBER030	14,85	2	0,5	2	15,5
DUBER031	16,35	2	0,5	2	15,5
DUBER032	11,4	2	0,5	2	15,5
DUBER033	14,9	2	0,5	2	15,
DUBER034	8,65	2	0,5	2	15,5
DUBER035	10,85	2	0,5	2	15,5
DUBER037	28,95	2	0,5	2	15,5
DUBER038	25,45	2	0,5	2	15,5
DUBER039		2	0,5	2	15,5
DUBER046	68,7	2	0,5	2	15,5
DUBER047	100,5	2	0,5	2	15,5
DUBER050	92,3	2	0,5	2	15,5
DUBER051	80	2	0,5	2	15,5
DUBER069	83,2	2	0,5	2	15,5
DUBER078	54,7	2	0,5	2	15,5
DUBER083	230,9	2	0,5	2	15,5
DUBER084	103,4	2	0,5	2	15,5
DUBER085	171,5	2	0,5	2	15,5
DUBER086	95,6	2	0,5	2	15,5
DUBER090	104,1	2	0,5	2	15,5
DUBER091	161	2	0,5	2	15,5
DUBER092	178,1	2	0,5	2	15,5
DUBER093	153,5	2	0,5	2	15,5

60min 37°C incubation time

20min 65°C inactivation time

after that: add 5µl loading dye to the restricted plasmids

Gelelectrophoresis: 100V 60min

Here are the expected Restrictions:

Dueber027 1662 709

Dueber030 1662 542

Dueber031 1662 315

Dueber032 1662 720

Dueber033 1662 717

Dueber034 1662 714

Dueber035 1662 711

Dueber037 1662 720

Dueber038 1662 717

Dueber039 1662 714

Dueber046 1662 714

Dueber047 1654 1030

Dueber050 1662 1446

Dueber051 1662 238

Dueber069 1662 173

Dueber078 1662 1162

Dueber083 1870 919

Dueber084 1775 919

Dueber085 1929 919

Dueber086 1662 524

Dueber090 1775 919

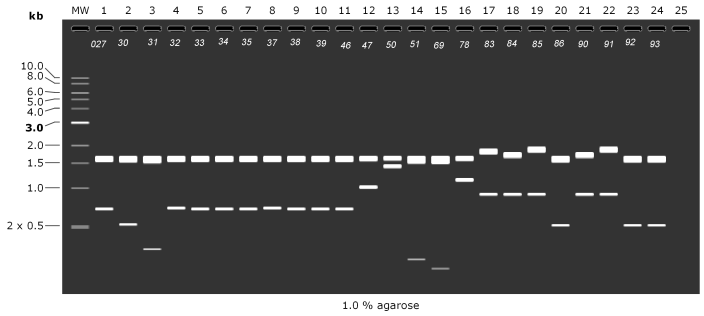
Dueber091 1929 919

Dueber092 1662 524

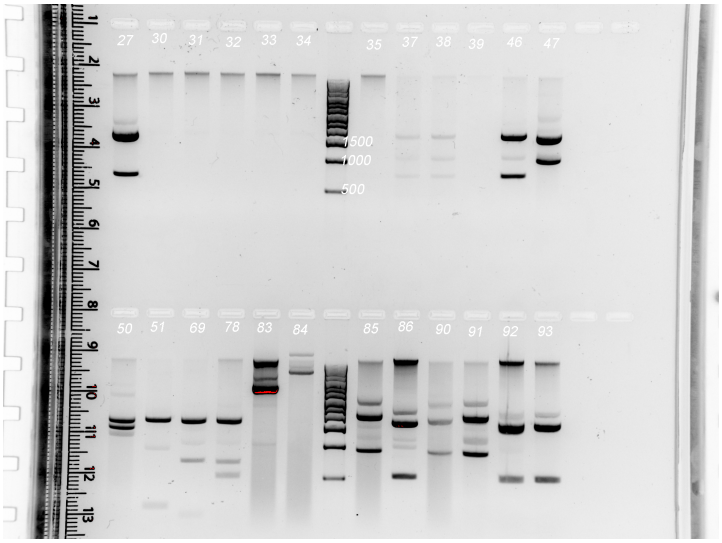
Dueber093 1662 524

Here is the expected gel image:

Gel_Image_Duber_again.png



Gel_2018-05-02_17hr_24min_Testgel_DUEBER_1_kb_MW_1_h_120_V_hell.png



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

Author: Lev Petrov
Entry 68/143: Testrestriction for DUEBER(94-96)
In Project: General
No tags associated

created: 04.05.2018 16:53
updated: 05.05.2018 12:14

Testrestriction for DUEBER(94-96)

Performed by: Lev on 04.05.2018

-2 µl Buffer per Plasmid

-0,5µl Enzyme (cold!) per plasmide

1. Pipetting step: MilliQ Water, Buffer, Plasmids, Enzyme BsaI. All mixed in a reaction tube.
2. 60min 37°C incubation time
3. 20min 65°C inactivation time
4. Add 5µl loading dye to the restricted plasmids
5. 5 µl ladder+5 µl water for reference

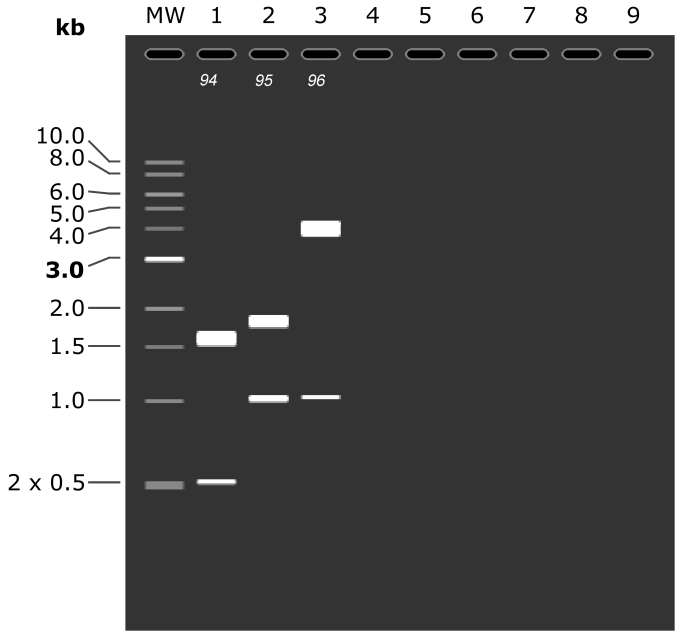
Content	ng/µl	ignored here (µl needed for 500 ng)	BsaI (µl)	cutsmart buffer(µl)	MilliQ (µl)
DUEBER094	126,4	2	0.5	2	15.5
DUEBER095	160,2	2	0,5	2	15,5
DUEBER096	93,5	2	0.5	2	15.5

Gel:	1 % agarose	gelred (2µl for 40ml)	120V	60 min

Resulting gel attached.

Gel_2018-05-04_16hr_41min_Testrestiction_DUEBER_94-96.pdf

Gel_Image5,05,2018.png



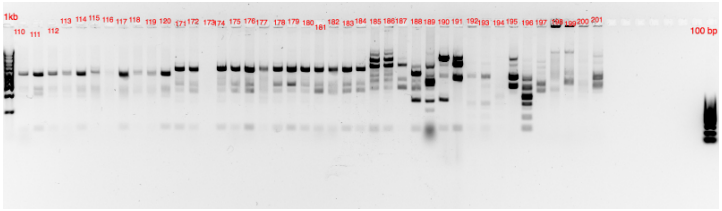
1.0 % agarose

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

Author: Katharina Polzen
Entry 69/143: Test restriction CIDAR
In Project: General
With tags: E.coli, gel electrophoresis, test restriction

created: 06.05.2018 15:42
updated: 14.05.2018 09:49

Gel_2018-05-04_13hr_51minCIDARfinalones.tif



Gel preparation:

160 mL TAE and 1,6g agarose and 8 µl gelred

1x TAE buffer

90 V, 60 min.

Made with:

p2iGEM0110 - p2iGEM0120.

p2iGEM0171 - p2iGEM0201

Mix:

- 1,5 µl plasmid
- 20,5 µl H2O
- 0,5 µl MmeI
- 2,5 µl CutSmart
- 0,08 µl S-adenosylmethionine

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

Author: Matthias Willmann
created: 08.05.2018 20:25
updated: 14.05.2018 16:53
Entry 72/143: growth rates s.cerevisiae
In Project: General
No tags associated

We made growth rates with *s.cerevisiae* in the following steps:

Day 1:

Inoculate your organism into the respective medium (10 ml): *S.cerevisiae*: YPD. Consider for later growth analysis the medium composition.

Day 2

- Measure the OD of the preculture *S.cerevisiae* OD600 Use the SAME photometer throughout the experiments.

- inoculate each medium with OD=0.1 (Formula: wanted OD/actual OD x 25mL)

-> final medium Volume:

25mL Perform your experiments as a triplicate at 30°C.

Also use different media composition

We will use:

LB-

YPD-

LB+YPD (1:1)

LB+YPD (1:2)

Measure OD directly after adding the culture to the medium (this is your t0)

then every hour

BLANK WITH THE RESPECTIVE MEDIUM.

- Measure OD for *S.cerevisiae*:

every hour --> usually only for *S.cerevisiae* after approx.8 hours

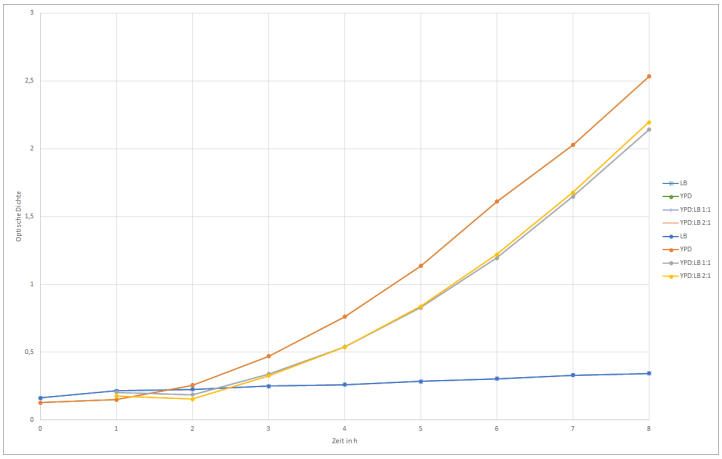
(1:10 dilution should be sufficient)

For the different temperatures try to use the same dilution for the same time point.

- Plot the data: (mean values of)OD600 against time

Measured data:

Wachstumsrate1.test.png



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

Author: Jennifer Denter
created: 12.05.2018 13:09
updated: 01.08.2018 19:22

Entry 74/143: Preparation of LB-Amp media
In Project: General
With tags: LB, Medium

200 ml LB-Agar was heated

Amp was filled in with a final concentration of 100 µg/ml

LB-Amp plated were prepared and stored at 4 °C (cooling room)

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

Author: Miriam Dreesbach
Entry 75/143: Primer preparation & dilution
In Project: General
With tags: stock, primer, dilution

created: 13.05.2018 13:29
updated: 13.05.2018 13:51

Wear gloves!

To prepare ordered primers as **100 µM primer stocks**, I added **1 µL nuclease-free water (milliQ) / 1 µg primer to the primer pellet**.

I **vortexed** the sample briefly for **3 seconds** and **centrifugated it for 3-5 second** (short button). I stored the primer stock at -20 °C.

To dilute primer stocks for the work stock, I diluted **10 µL 100 µM primer** with **90 µL nuclease-free water (milliQ)**.

I **vortexed** the sample briefly for **3 seconds** and **centrifugated it for 3-5 second** (short button). I stored the work stock at -20 °C.

Thus, I prepared the primer and work stock for the primers O_iGEM18_0059, O_iGEM18_0060, O_iGEM18_0061.

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

Author: Miriam Dreesbach
created: 13.05.2018 13:34
updated: 13.05.2018 13:50

Entry 76/143: Synthesises (IDT) preparation & dilution
In Project: General
With tags: stock, dilution, synthesises

Wear gloves!

Centrifuge the tube for **3-5 sec at minimum of 3,000 rpm** to ensure the material is in the bottom of the tube.

To prepare ordered synthesises from IDT, add 1 x TE to reach a **final concentration of 10 ng / µL**.

To reach a concentration of 10 ng / µL out of a 10,000 ng synthesis, I added **100 µL 1xTE to the synthesis pellet**.

Afterwards, I **votexed** the sample briefly for 3 seconds. I incubated the sample at **50 °C for 20 minutes** and **vortexed** it afterwards.

After another centrifugation for **3-5 sec at minimum of 3,000 rpm**, I stored the synthesises at -20 °C (g-block box).

Thus, I prepared the synthesises S_iGEM18_0016 and S_iGEM18_0017.

To dilute synthesises for the work stock, I added **9 µL nuclease-free water (milliQ) to 1 µL of the synthesis stock**.

Thus, I reached a **final concentration of 1 ng / µL**. I **vortexed** the sample briefly for **3 seconds** and **centrifugated it for 3-5 second** (short button).

I stored the work stock in the Level 1 box for upcoming PCRs.

Thus, I prepared the synthesis work stocks of the synthesises S_iGEM18_0016 and S_iGEM18_0017.

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

Author: Ylenia Longo
Entry 77/143: PBS preparation
In Project: General
With tags: PBS, Interlab

created: 14.05.2018 15:53
updated: 14.05.2018 15:59

For the interlab challenge PBS (pH 7,4 to 7,6 is needed)

Recipe:

Reagent	Amount to add (for 1× solution)	Final concentration (1×)
NaCl	8 g	137 mM
KCl	0.2 g	2.7 mM
Na_2HPO_4	1.44 g	10 mM
KH_2PO_4	0.24 g	

The reagents are dissolved in 1L H20 and the pH is adjusted to 7.4.

The solution is autoclaved.

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

Author: Carina Gude
created: 15.05.2018 16:32
updated: 16.05.2018 16:51
Entry 79/143: E.coli Test-Growth curve in LB and YPD
In Project: General
With tags: LB, YPD, Growth Curve, E.coli

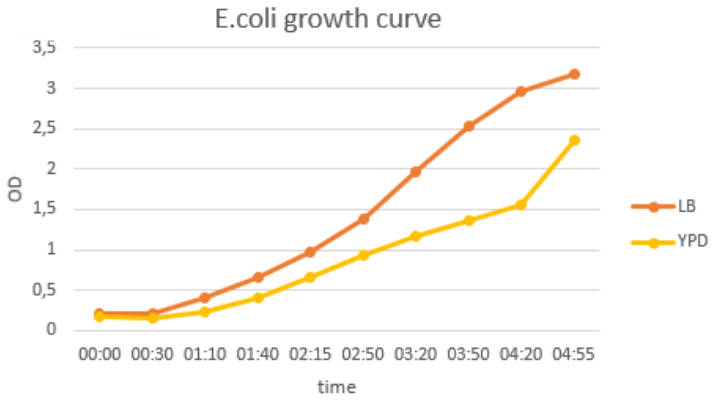
Over night pre culture with E.coli DH5a:

- 30ml LB-medium
- DH5a colonies

Growth:

time	LB 1 OD (diluted)	LB 2 OD (diluted)	LB 3 OD (diluted)	YPD 1 OD (diluted)	YPD 2 OD (diluted)	YPD 3 OD (diluted)	LB - undiluted	YPD - undiluted
00:00	0,023	0,019	0,019	0,018	0,018	0,017	0,20333333	0,17666667
00:30	0,021	0,022	0,022	0,015	0,014	0,017	0,21666667	0,15333333
01:10	0,036	0,044	0,04	0,028	0,014	0,025	0,4	0,22333333
01:40	0,073	0,059	0,066	0,045	0,036	0,038	0,66	0,39666667
02:15	0,091	0,11	0,091	0,059	0,064	0,073	0,97333333	0,65333333
02:50	0,135	0,145	0,134	0,097	0,089	0,094	1,38	0,93333333
03:20	0,225	0,173	0,195	0,1	0,119	0,129	1,97666667	1,16
03:50	0,268	0,254	0,237	0,14	0,139	0,132	2,53	1,37
04:20	0,31	0,309	0,267	0,171	0,159	0,139	2,95333333	1,56333333
04:55	0,29	0,341	0,321	0,193	0,196	0,206	3,17333333	2,36666667

capture-20180516-164908.png



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

Author: Miriam Dreesbach
created: 16.05.2018 12:13
updated: 16.05.2018 12:30
Entry 80/143: Blunt-End Cloning Protocol with pJET (Thermo Scientific)
In Project: General
With tags: pJET, blunt-end cloning, T4 ligase

Blunt-End Cloning Protocol with pJET (Thermo Scientific)

Gel-purify the DNA fragment prior to ligation anduse in a 3:1 molar ratio with pJET1.2/blunt (50ng/µL).

1. **Set up the ligation reaction on ice**:

2X Reaction Buffer: 10 µL

Non-purified **PCR product** or purified PCR product/other **blunt-end DNA fragment**: 1 µL 0.15 pmol ends

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

Water, nuclease-free (Edit Mimi: milliQ): up to 19 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 **5 min**.

Note: For PCR products > 3 kb, ligation can be prolonged to 30 min.

Edit (Mimi): You can also incubate the ligation mixture at room temperature for 30 minutes or at 4 °C overnight.

3. **Use the ligation mixture directly for transformation.**

Note: **Keep the ligation mixture at - 20 °C if transformation is postponed.**

Thaw on ice and mix carefully before transformation.

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

Author: Susanne Vollmer Entry 82/143: No entry title yet In Project: General No tags associated	created: 17.05.2018 11:37 updated: 17.05.2018 11:38

poured 8 LB 2%argar plates with 1970µl ampicillin

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

Author: Miriam Dreesbach
created: 17.05.2018 14:09
updated: 17.05.2018 16:54

Entry 83/143: 1x BG11 out of 100xBG11 stock
In Project: General
With tags: 1xBG11, Media

Today I prepared 2 L of 1x BG11 for Carina's growth mesurement. Therefore, I followed the following protocol:

Everything, except for the 100x BG11 media and the TES buffer is stored at 4 °C. Actually we do not own any 5000x $CuSO_4$ stock. Please ask AG Axmann for it, if you need it.

To prepare a 1xBG11 media out of a 100x BG11 stock, I pipetted the following solutions in a 1 L bottle:

- 10 mL 100x BG11 (sterile autoclaved)
- 1 mL 1000x Na_2CO_3 (sterile filtered)
- 10 mL 100x TES-buffer, pH 8.0 (1M), adjusted with KOH (sterile autoclaved)
- 1 mL 1000x Trace metal **-Cu** mix containing H_3BO_3, $MnCl_2$, $ZnSO_4$, Na_2MoO_4, $Co(NO_3)_2$ (sterile filtered)
  - For BG11 lacking certain metals (e.g. for working with metal inducible promoters P_{petE}, P_{coaT}, P_{ziaA} etc., trace metal mix can be prepared lacking these chemicals and used instead of standard trace metal mix.
- 1 mL 1000x K_2HPO_4 (sterile filtered)
- fill up to 1 L with nuclease-free water (milliQ)

Autoclave it directly or store it for few hours at 4 °C.

If you don't use a Cu-inducible promoter, add the following nutrients after autoclaving it.

Make sure that the nutrients are sterile filtered and **work in a sterile environment**!

- 1 mL 1000x Fe(III) ammonium citrate
- 0.2 mL 5000x $CuSO_4$ (optional)

Store the media at room temperature. Make sure to work with it under sterile conditions.

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

Author: Ylenia Longo
Entry 84/143: LB Ampicillin plates
In Project: General
With tags: ampicillin, plates

created: 17.05.2018 14:41
updated: 18.05.2018 16:37

New LB Ampicillin(100µg/mL) plates have been poured.

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

Author: Ylenia Longo
Entry 85/143: Interlab challenge: Calibration 1
In Project: General
With tags: Interlab, challenge, calibration, ludox

created: 17.05.2018 18:11
updated: 17.05.2018 18:15

For the interlab challenge the first calibration has been performed. For this purpose 100µl of LUDOX CL-X (45% colloidal silica suspension) was pipettet in well A1,B1,C1,D1 of a 96 well plate. In A2,B2, C2 and D2 100µl of H20 were pipetted. Absorbance at 584nm was measured.

Well Row	Well Col	Content	Raw Data (584)
A	1	Sample X1	0,259
A	2	Sample X2	0,258
B	1	Sample X1	0,253
B	2	Sample X2	0,2
C	1	Sample X1	0,223
C	2	Sample X2	0,208
D	1	Sample X1	0,304
D	2	Sample X2	0,235

Since the iGEM Headquarter requires measurement at 600nm, this experiment will be repeated soon.

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

Author: Carina Gude
Entry 86/143: Test growth curve
In Project: General
With tags: E.coli, Growth Curve, LB, YPD

created: 18.05.2018 13:19
updated: 18.05.2018 13:40

Over night culture: 50ml LB with cultures of E.coli DH5a

Test growth curve:

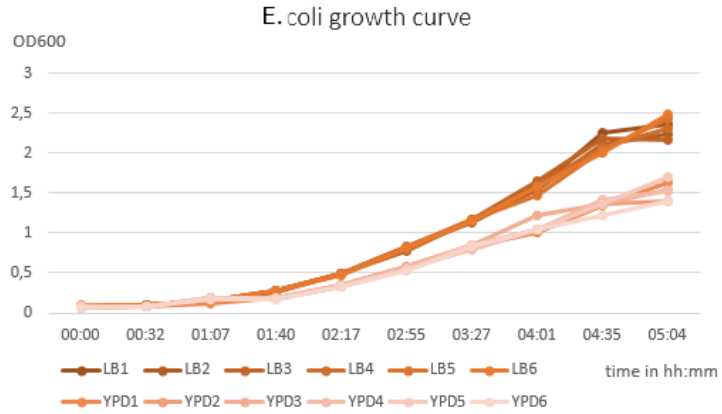
12 cultures, each:

- 24,32 ml medium (6 LB and 6 YPD)
- 0,30ml overnight culture

measured ODs:

Time	dilution	LB1 diluted	LB2 diluted	LB3 diluted	LB4 diluted	LB5 diluted	LB6 diluted	YPD1 diluted	YPD2 diluted	YPD3 diluted	YPD4 diluted	YPD5 diluted	YPD6 diluted	LB mean (undiluted)
00:00	-	0,084	0,082	0,09	0,089	0,085	0,083	0,092	0,071	0,072	0,08	0,073	0,072	0,0855
00:32	-	0,098	0,098	0,099	0,099	0,098	0,098	0,079	0,077	0,079	0,077	0,079	0,078	0,09833333
01:07	-	0,151	0,154	0,154	0,154	0,153	0,153	0,112	0,184	0,181	0,172	0,181	0,176	0,15316667
01:40	-	0,271	0,267	0,271	0,274	0,276	0,279	0,182	0,184	0,181	0,172	0,181	0,176	0,273
02:17	-	0,487	0,485	0,492	0,492	0,49	0,479	0,337	0,351	0,347	0,328	0,338	0,329	0,4875
02:55	1:2	0,415	0,389	0,404	0,4	0,411	0,405	0,283	0,294	0,293	0,28	0,27	0,267	0,808
03:27	1:2	0,565	0,584	0,586	0,578	0,582	0,577	0,411	0,42	0,428	0,398	0,426	0,419	1,15733333
04:01	1:4	0,389	0,386	0,411	0,4	0,37	0,396	0,253	0,263	0,307	0,261	0,262	0,261	1,568
04:35	1:4	0,562	0,529	0,547	0,52	0,511	0,501	0,335	0,343	0,342	0,353	0,339	0,306	2,11333333
05:04	1:8	0,296	0,279	0,27	0,288	0,304	0,311	0,203	0,175	0,193	0,191	0,214	0,175	2,33066667

capture-20180518-134020.png



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

Author: Miriam Dreesbach
Entry 87/143: Trace metal addition to 1x BG11
In Project: General
With tags: 1xBG11, Fe(III)NH4Citrate, CuSO4

created: 20.05.2018 17:57
updated: 20.05.2018 18:01

To prepare a **full media for *Synechococcus elongatus* sp. PCC 7942**, I added the following metals to the prepared and **autoclaved pre-version of 1x BG11 under sterile conditions**:

- **1 mL 1000x Fe(III)NH_4Citrate**
- **200 μL 5000x $CuSO_4$ (for not Cu-induced cultures)**

The media is now ready for cultivation.

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

Author: Ylenia Longo
Entry 88/143: LB Ampicillin plates
In Project: General
With tags: ampicillin, plates

created: 21.05.2018 08:53
updated: 21.05.2018 08:53

New LB Ampicillin(100µg/mL) plates have been poured.

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

Author: Carina Gude
Entry 89/143: Cyano growth curve
In Project: General
With tags: Growth Curve, Cyanobacteria, BG-11, LB, YPD

created: 22.05.2018 15:36
updated: 04.06.2018 15:11

21.05.2018

OD-measurement of pre-pre culture of Synechococcus elongatus sp. PCC 7942 WT

15:15: OD750= 0,393

Preparation of 200ml Preculture of pre-pre culture:

- 50,891ml Pre-preculture
- + 149,109 ml BG11 Medium
- -> 15:30: OD750= 0,100

22.05.2018

8:00: OD750 of preculture: 0,377

Preparation of 9 Samples with the OD 0,1

For each sample:

- 10,610 ml preculture
- 29,390ml medium (3 samples with LB, 3 samples with BG11, 3 samples with YPD)

8:40: OD750

- 0LB1: 0,013
- 0LB2: 0,093
- 0LB3: 0,015
- 0YPD1: 0,016
- 0YPD2: 0,024
- 0YPD3: 0,015
- 0BG11-1: 0,034
- 0BG11-2: 0,334 -> probably not mixed well
- 0BG11-3; 0,330 -> probably not mixed well

--> ODs are way to low, to be correctly measured; Samples aren't replicates

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

Author: Ylenia Longo
Entry 90/143: LB Ampicillin plates
In Project: General
With tags: LB, ampicillin

created: 23.05.2018 12:06
updated: 23.05.2018 12:07

New LB Ampicillin(100µg/mL) plates have been poured

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

Author: Susanne Vollmer Entry 91/143: poured amp plates In Project: General With tags: amp, plates	created: 24.05.2018 09:28 updated: 24.05.2018 10:46

poured 7 ampicilin plates with 200ml LB and 2ml ampicilin

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

Author: Miriam Dreesbach
created: 24.05.2018 16:42
updated: 24.05.2018 16:46
Entry 92/143: Permanent culture S. elongatus cscB
In Project: General
With tags: Cultivation, 1xBG11

To prepare a permanent culture of *Synechococcus elongatus* sp. PCC 7942 cscB 2iGEM301, I inoculated 200 mL 1x BG11 with supplemented $CuSO_4$ and Fe(III)NH_4-Citrate.

Afterwards, I incubated it at 30 °C and 150 rpm in the light chamber together with the colonies on a BG11 agar plate.

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

Author: Carina Gude

created: 26.05.2018 19:21
updated: 04.06.2018 14:25

Entry 93/143: Preparation of pre culture of S.elongatus sp PCC 7942 cscB ::: N53
In Project: General
With tags: Cyanobacteria, preculture, Growth Curve

Preculture of S. elongatus sp. PCC 7942 cscB:::N53 (2iGEM301)

OD750: 0,245

In order to create a preculture with an OD of 0,1 (0,1/0,25*200ml)

- 81,632ml culture and
- 118,368 ml BG11 was used

Incubation at 150rpm and 30°C with constant light.

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

Author: Ylenia Longo Entry 95/143: No entry title yet In Project: General No tags associated	created: 29.05.2018 16:00 updated: 29.05.2018 16:00

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

Author: Ylenia Longo
created: 29.05.2018 16:01
updated: 29.05.2018 16:16
Entry 96/143: Interlab challenge: Calibration 1 and 2
In Project: General
With tags: Interlab, challenge, ludox, silica beads, calibration

1st Calibration: For the first Calibration LUDOX CL-X (45% colloidal silica suspension) is used.

For this purpose 100µl Ludox are pipetted into well A1,B1,C1 and D1 of a 96 well plate.

Also, 100µl ddH20 are pipetted into well A2,B2,C2 and D2.

Absorbance at 600nm was measured:

	1	2
A	0,0659	0,0479
B	0,056	0,0434
C	0,0566	0,0564
D	0,0578	0,054

2nd Calibration:

For the second calibration a dilution series of monodisperse microspheres is performed and absorbance at 600nm is measured.

The tube"Silica beads" from the Interlab Measurement Kit is heavily vortexed for 45seconds. 96µl of microspheres are immediately pipetted into a new 1.5µl tube. Then 904µl ddH20 are added to the microspheres. This is the Microspheres Stock solution.

100µl ddH20 are pipetted in A2-A12/B2-B12/C2-C12 and D2-D12.

The tube containing the stock solution is again vortexted for 10 seconds and then 200µl are pipetted into wells A1/B1/C1 and D1.

100µl from A1 are transferred into A2. A2 is mixed by pipetting up and down 3 times. Then 100µl are transferred into A3. The same procedure is repeated until well A11. The last 100µl are then discarded and NOT pipetted into well A12. The same is also done for row B,C and D.

Immediately before measurement, the wells are re-mixed, so that settled down beads are resuspended.

Absorbance at 600nm is measured:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0,6755	0,4406	0,2767	0,1635	0,0996	0,0775	0,0608	0,0684	0,0557	0,0528	0,0527	0,0489
B	0,7355	0,4835	0,3022	0,1888	0,1212	0,0836	0,0686	0,0727	0,0492	0,0492	0,0449	0,0532
C	0,6268	0,4084	0,2693	0,1461	0,099	0,0826	0,0688	0,0677	0,0603	0,0458	0,0453	0,0573
D	0,7601	0,3842	0,2411	0,145	0,1035	0,0969	0,0734	0,0646	0,0594	0,0617	0,0597	0,0487

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

Author: Carina Gude
Entry 97/143: BG11-plates
In Project: General
With tags: BG-11, Cyanobacteria, LB

created: 30.05.2018 08:20
updated: 04.06.2018 14:24

Finishing the preparation of 2x BG11 from 28.05.2018

Addition of $CuSO_4$ and Fe(III) to 2x BG11

LB-Bg11 plates:

- 20ml 20% LB agar plus 20ml 2xBg11
- stored in 4°C room

Bg11-plates:

- 40ml 2x BG11 and 40ml water agar
- 300 µl S. elongatus sp PCC 7942 was spread onto one plate
- 300µl S. elongatus sp PCC cscB :::N53 was spread onto antoher plate

Inoculation of S.elongtus sp PCC 7942 cscB:::N53 preculture

- 29,412 ml culture
- 170,588 ml BG11

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

Author: Sarah Seyffert
Entry 98/143: Transformation
In Project: General
With tags: Transformation, E.coli

created: 30.05.2018 16:00
updated: 30.05.2018 16:04

E.coli was transformed with p2iGEM0289(pbBba-GFP) for the co-culture measurements

- thaw competent cells 5-10 min on ice
- add 3µl 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 Antibiotics (sterile)
- 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)
- spread the Suspension on an agar plate with chloramphenicol
- incubate at 37°C overnight

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

Author: Ylenia Longo
created: 01.06.2018 13:28
updated: 01.06.2018 13:39
Entry 99/143: Interlab challenge: Calibration 3
In Project: General
With tags: fluorescein, measurement, Interlab, challenge, fluorescence

For the third calibration, a fluorescein standard curve is prepared. For this purpose first the supplied fluorescein solution in the Interlab Measurement Kit is spun down. 1mL PBS (pH7.4) is added to the tube and the solution is pipetted up and down until a homogenous solution is visible.

This is the 10x fluorescein stock solution (100µM). To obtain a 10µM (1x fluorescein) stock solution, the beginning stock solution is diluted with PBS (1:10). Hence, 100µl of 10x fluorescein stock solution are mixed with 900µl of PBS (pH 7.4).

Then serial dilutions are performed across columns 1-12 of a 96 well plate (replicates of 4: row A-D)

100µl of PBS (pH 7.4) are pipetted in A2-12, B2-12, C2-12 and D2-12. 200µl of a 1x fluorescein stock solution are pipetted in A1,B1, C1 and D1.

100µl of fluorescein stock solution are transferred from A1 to A2. A2 is mixed by pipetting up and down three times and then 100µl are transferred to B2. The same procedure is performed until A11. The final 100µl are transferred into the liquid waste and NOT in A12.

The same serial dilutions are performed for row B,C and D.

Afterwards, fluorescence of all samples is measured with the following settings:

Mode				Fluorescence Top Reading		
Excitation Wavelength				485	nm	
Emission Wavelength				525	nm	
Excitation Bandwidth				9	nm	
Emission Bandwidth				20	nm	
Gain				68	Optimal (100%)	
Number of Flashes				10		
Integration Time				20	µs	
Lag Time				0	µs	
Settle Time				0	ms	

The following results were obtained:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	39821	22567	12009	6018	3104	1573	803	416	204	101	49	1
B	38452	22204	10261	6538	3300	1612	809	406	216	104	56	4
C	39259	22161	11574	5939	2988	1528	782	346	230	102	55	3
D	42331	23412	12728	6196	3225	1491	838	368	197	97	51	4

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

Author: Carina Gude
Entry 100/143: Cyano growth curve
In Project: General
With tags: Cyanobacteria, S.elongatus, BG-11, Growth Curve

created: 04.06.2018 14:28
updated: 05.06.2018 17:27

21.05: preparation of pre culture:

- 50,891 ml stock culture of S. elongatus sp PCC 7942
- 149,109 ml BG11 medium

23.05: preparation of samples:

OD of preculture = 0,233

Mix:

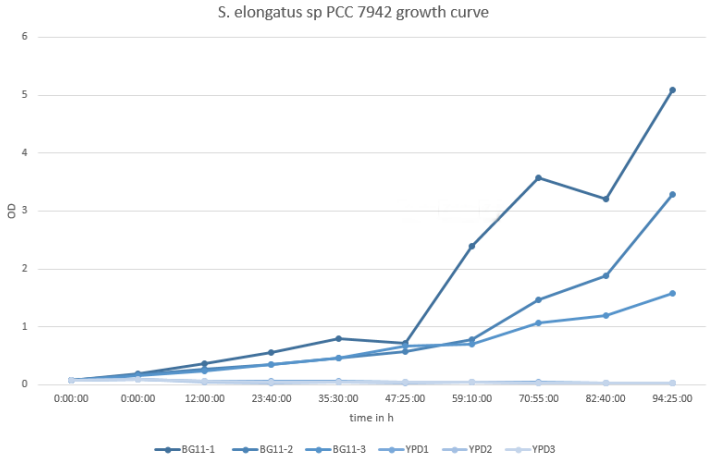
- 32.188 ml preculture
- 42,811 ml desired medium (LB/ BG11)

seperate each sample into three 200ml flasks

date time	time in h	BG11-1	BG11-2	BG11-3	YPD1	YPD2	YPD3
23.05.2018, 20:15	0:00:00	0,078	0,078	0,078	0,078	0,078	0,78
24.05.2018, 08:15	12:00:00	0,184	0,166	0,16	0,092	0,085	0,089
19:55	23:40:00	0,369	0,268	0,24	0,063	0,051	0,055
25.05.2018, 08:05	35:30:00	0,556	0,35	0,35	0,056	0,035	0,043
20:00	47:25:00	0,799	0,452	0,453	0,053	0,042	0,046
26.05.2018, 08:15	59:10:00	0,72	0,576	0,665	0,045	0,035	0,045
20:00	70:55:00	2,39	0,78	0,695	0,049	0,037	0,043
27.05.2018, 08:15	82:40:00	3,57	1,47	1,06	0,039	0,026	0,034
20:00	94:25:00	3,21	1,88	1,19	0,036	0,027	0,025
28.05.2018, 08:15	106:10:00	5,09	3,29	1,58	0,03	0,023	0,028
20:50	118:45:00	2,68	3,7	1,48	dead	dead	dead
29.05.2018, 08:00	131:35:00	not enough volume	3,648	2,22	dead	dead	dead

Since not enough volume was used, the Cyanobacteria culture in BG11 dried out pretty quickly.

Cyano_growth_curve_(zu_wenig_Volumen).png



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

Author: Susanne Vollmer
Entry 101/143: -URA SD platten
In Project: General
With tags: plates, SD, -URA

created: 05.06.2018 17:22
updated: 05.06.2018 17:26

Poured 5 plates -URA SD plates using 1ml SD medium and 1ml each of LEU, HIS, TRP and fill up to100ml 2%water agar

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

Author: Carina Gude
Entry 102/143: E.coli DH5a Gefrierstock
In Project: General
With tags: E.coli, Growth Curve, Kryo, kryos, preculture

created: 05.06.2018 19:23
updated: 07.06.2018 08:45

E.coli DH5a Gefrierstock for growth curves

4.6.18: over night culture: 5ml LB with E.coli DH5a from plate

5.6.18:

OD=0,879

1. 2ml over night culture with 2 ml glycerin was mixed
2. 100µl in Kryo tube
3. frozen with lliquid nitrogen
4. stored in -80°C freezer (E.coli DH5a Gefrierstock box)

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

Author: Carina Gude
Entry 103/143: LG BG11 coculture plates
In Project: General
With tags: LB, BG-11, Synechococcus, E.coli, co-culture, S.elongatus, S.cerevisiae

created: 05.06.2018 19:27
updated: 06.06.2018 11:45

4.6.2018:

1 LB-BG11 plate was streaked with E.coli DH5a, S.elongatus sp PCC 7942 and S.cerevisiae BY4742: seperately

1 LB-BG11 plate was streaked with E.coli DH5a, S.elongatus sp PCC 7942 and S.cerevisiae BY4742: criss cross

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

Author: Carina Gude

created: 06.06.2018 11:45
updated: 07.06.2018 06:47

Entry 104/143: Kryo of S.elongatus sp PCC cscB:::N53
In Project: General
With tags: Kryo, Cyanobacteria, cscB

Preparing cryo culture of S. elongatus sp PCC cscB:::N53 (2igem301)

1. OD of stock culture: 0,358 (1:10 dilution) --> 3,58
2. dilution to OD=1 --> 1,397ml stock culture + 3,603 ml media
3. After mixing, 1 ml was transfered in an 1,5ml Eppi
4. Centrifugation under **4,500 rpm** for **5 minutes**.
5. Supernatant was discarded
6. Pallet was resuspended in **500μl 1 x BG11 with 8% DMSO**
7. Sample was cooled with **liquid nitrogen**
8. Stored under **-80 °C** for a further storage

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

Author: Katharina Polzen
Entry 105/143: -URA S plates
In Project: General
With tags: Ura, plates

created: 06.06.2018 15:33
updated: 06.06.2018 15:34

Poured 5 plates -URA SD plates using 1ml SD medium and 1ml each of LEU, HIS, TRP and fill up to 100ml 2% water agar

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

Author: Sarah Seyffert
Entry 106/143: Overnightculture & Primer aliquots
In Project: General
No tags associated

created: 06.06.2018 16:50
updated: 06.06.2018 21:58

The primer O_iGEM18_0066 +O_iGEM18_0067 and O_iGEM18_0055 + O_iGEM18_0056 were disolved in MiliQ-water up to 100µM

Overnightculture of Rosetta E. coli strain

- 5ml LB
- 3,6 µl Cam

Overnight incubation at 37°C

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

Author: Ylenia Longo
Entry 107/143: Water agar 2%
In Project: General
With tags: water, Agar, 2%, for SD

created: 07.06.2018 13:11
updated: 07.06.2018 13:12

2% water agar is prepared: For this purpose 3g Agar Agar were added to 150mL ddH20. The final solution is autoclaved.

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

Author: Carina Gude
created: 08.06.2018 15:24
updated: 08.06.2018 15:38
Entry 108/143: Yeast growth curve
In Project: General
With tags: preculture, YPD, BG-11, Growth Curve, S.cerevisiae

Preparation of the S. cerevisiae BY 4742 pre culture:

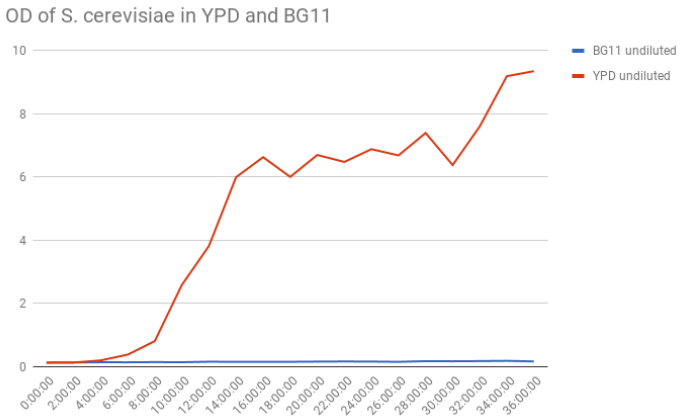
- 12:00 in 200ml YPD
- Incubation at 30°C and 300rpm

Preparation of the samples:

- OD in the morning: 0,592 (1/10) --> 5,92
- 2,027ml of preculture + 117,972ml of medium (YPD/BG11)
- for replicates: transfer 40ml of each 120ml culture into two falsks

time in h	BG11 undiluted	YPD undiluted
0:00:00	0.129	0.125
2:00:00	0.130	0.130
4:00:00	0.138	0.196
6:00:00	0.138	0.380
8:00:00	0.143	0.809
10:00:00	0.139	2.587
12:00:00	0.155	3.821
14:00:00	0.152	5.997
16:00:00	0.150	6.630
18:00:00	0.152	6.007
20:00:00	0.159	6.700
22:00:00	0.163	6.480
24:00:00	0.160	6.883
26:00:00	0.151	6.687
28:00:00	0.173	7.397
30:00:00	0.170	6.380
32:00:00	0.175	7.605
34:00:00	0.183	9.195
36:00:00	0.164	9.353

chart.png



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

Author: Carina Gude
Entry 109/143: BG11+2% sucrose (+NaCl)
In Project: General
With tags: BG-11

created: 09.06.2018 06:36
updated: 09.06.2018 06:38

For 300ml BG11 2%sucrose:

- Mix
  - 20ml 30% sucrose
  - 580ml BG11

For 300ml BG11 2% sucrose 150mM NaCl:

- Mix:
  - 90ml 500mM NaCl
  - 20ml 30% sucrose
  - 190ml BG11

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

Author: Carina Gude
created: 09.06.2018 06:39
updated: 09.06.2018 06:54
Entry 110/143: E.coli Rosetta2 pLysS "UMa" 971 Kryos (2
In Project: General
With tags: E.coli, Rosetta, Kryo

Overnight culture:

- 30ml LB
- colonys from the E.coli Rosetta2 pLysS "UMa" 971 plate
- Chloramphenicol

10:30

- OD600=2.04

1. Mixed 2ml culture in 2ml glycerin
2. 100µl in kryo tube
3. shock freeze with nitrogen

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

Author: Carina Gude
created: 09.06.2018 08:32
updated: 13.06.2018 19:34

Entry 111/143: S. elongatus sp PCC 7942 cscB:::N53 growth curve
In Project: General
With tags: preculture, cscB, Growth Curve, BG-11, LB, Cyanobacteria, S.elongatus

26.05.2018: Preparation of pre culture

- OD of stock culture: 0,245
- Mix:
  - 81,633 ml culture
  - 118,367 ml BG11

03.06.2018: Preparation of samples

- OD of pre culture: 0,202
- Mixed in 2 500ml flasks:
  - 59,406 ml culture
  - 60,594 ml medium (1x BG11/ 1x LB)
- Transfer 2x 40ml of the BG11 and LB-sample into two other flasks -> triplicates

After three LB measurements, LB-1, the preculture and the stock culture were tested for contamination due to the fast growth in the LB samples. LB-plates were streaked with 200µl of each culture --> the next day many colonies were growing on the LB-1 plates. Because of that the LB measurements were stopped.

Preparation of chlorophyll extraction:

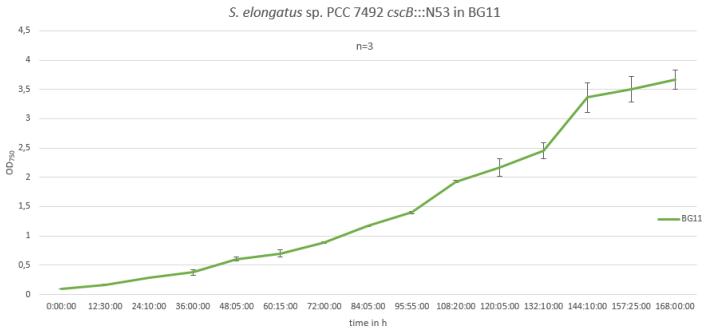
- During the measurement for each sample 500µl of BG11 and 500µl sample was pippetted into a 1,5ml Eppi
- centrifuge at max rpm
- 0,9 ml of supernatant was discarded
- pellet was resuspended in remaining 100µl
- Eppis were frozen with nitrogen and then stored in -80°C freezer for further tests

time	time in h	LB-1	LB-2	LB-3	BG11-1	BG11-1	BG11-3	dilution	BG11-1 undiluted	BG11-2 undiluted	BG11-3 undiluted	Standardabweichung
03.06.2018 20:00	0:00:00	0.101	0.101	0.101	0,098	0,098	0,098	1	0,098	0,098	0,098	1,388E-17

04.06.2018 08:30	12: 30: 00	0.166	0.166	0.166	0,168	0,173	0,167	1	0,168	0,173	0,167	0,00262467
04.06.2018 20:10	24: 10: 00	0.396	0.397	0.481	0,286	0,293	0,291	1	0,286	0,293	0,291	0,00294392
05.06.2018 08:00	36: 00: 00	contamination!			0,217	0,194	0,154	2	0,434	0,388	0,308	0,05205979
05.06.2018 20:05	48: 05: 00				0,303	0,323	0,284	2	0,606	0,646	0,568	0,03184686
06.06.2018 08:15	60: 15: 00				0,378	0,367	0,306	2	0,756	0,734	0,612	0,06333684
06.06.2018 20:00	72: 00: 00				0,455	0,442	0,432	2	0,91	0,884	0,864	0,0188326
07.06.2018 08:05	84: 05: 00				0,591	0,584	0,579	2	1,182	1,168	1,158	0,00984322
07.06.2018 19:55	95: 55: 00				0,713	0,701	0,689	2	1,426	1,402	1,378	0,01959592
08.06.2018 08:20	108: 20: 00				0,473	0,486	0,487	4	1,892	1,944	1,948	0,02550817
08.06.2018 20:05	120: 05: 00				0,493	0,545	0,584	4	1,972	2,18	2,336	0,14910697
09.06.2018 08:10	132: 10: 00				0,655	0,574	0,609	4	2,62	2,296	2,436	0,13267839
09.06.2018 20:10	144: 10: 00				0,796	0,795	0,93	4	3,184	3,18	3,72	0,25362089
10.06.2018 09:25	157: 25: 00				0,355	0,322	0,375	10	3,55	3,22	3,75	0,21853045

10.06.2018 20:00	168: 00: 00		0,36	0,352	0,39	10	3,6	3,52	3,9	0,16357126

Cyano_cscB_BG11_curve.png



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

Author: Carina Gude

created: 11.06.2018 15:26
updated: 11.06.2018 15:46

Entry 112/143: E.coli Rosetta pLys "UMa" 791 growth curve in different BG11 media
In Project: General
With tags: Growth Curve, E.coli, BG-11

8.6.2018 Preparation of samples

- E.coli Rosetta pLys "UMa" 791 pre culture was inoculated the night before
- OD in the morning: 6.90
- Mix in three 300ml flasks:
  - 2,609 ml culture + 177,391 ml medium (BG11; BG11+2% sucrose, BG11+2%sucrose +150mM NaCl)
  - Triplicate: Transfer 2x 60ml of each mixture into 2 other 300ml Flasks

Due to the unexpected slow growth in all media, the measurement interval was decreased from every 30min to every hour

time	time [h]	BG11-1	BG11-2	BG11-3	dilution	Standardabweichung	BG11-mean	NaCl-1	NaCl-2	NaCl-3	dilution	Standardab
09:30	00:00:00	0,087	0,087	0,087	1	1,3878E-17	0,087	0,082	0,082	0,082	1	0
10:00	00:30:00	0,036	0,086	0,087	1	0,02380943	0,07	0,082	0,082	0,082	1	0
10:30	01:00:00	0,086	0,086	0,088	1	0,00094281	0,087	0,085	0,085	0,088	1	0,0014142
11:00	01:30:00	0,091	0,097	0,093	1	0,00249444	0,094	0,09	0,097	0,089	1	0,0035590
11:30	02:00:00	0,091	0,093	0,091	1	0,00094281	0,092	0,08	0,083	0,08	1	0,0014142
12:00	02:30:00	0,091	0,099	0,093	1	0,00339935	0,094	0,09	0,091	0,096	1	0,0026246

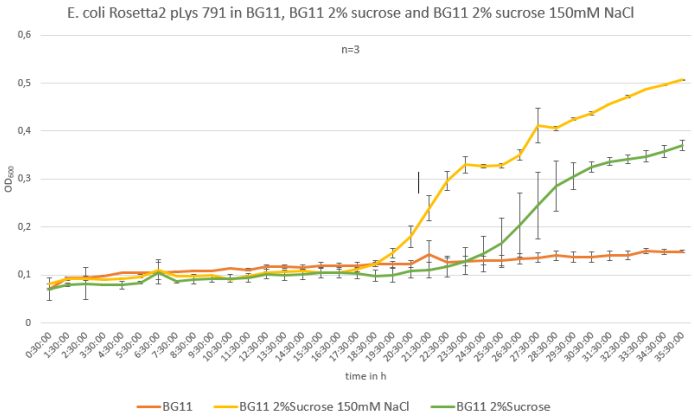
12:30	03:00:00	0,096	0,095	0,097	1	0,0008165	0,096	0,087	0,088	0,087	1	0,0004714
13:00	03:30:00	0,097	0,098	0,099	1	0,0008165	0,098	0,091	0,091	0,088	1	0,0014142
13:30	04:00:00	0,098	0,101	0,1	1	0,00124722	0,1	0,091	0,092	0,093	1	0,0008165
14:00	04:30:00	0,103	0,104	0,106	1	0,00124722	0,104	0,092	0,095	0,093	1	0,0012472
14:30	05:00:00	0,102	0,107	0,104	1	0,0020548	0,104	0,094	0,094	0,093	1	0,0004714
15:00	05:30:00	0,102	0,102	0,108	1	0,00282843	0,104	0,094	0,096	0,096	1	0,0009428
15:30	06:00:00	0,105	0,104	0,106	1	0,0008165	0,105	0,094	0,095	0,094	1	0,0004714
16:00	06:30:00	0,103	0,104	0,107	1	0,00169967	0,105	0,094	0,097	0,141	1	0,0214838
16:30	07:00:00	0,105	0,106	0,107	1	0,0008165	0,106	0,096	0,096	0,095	1	0,0004714
17:00	07:30:00	0,104	0,105	0,108	1	0,00169967	0,106	0,097	0,099	0,097	1	0,0009428
17:30	08:00:00	0,107	0,11	0,109	1	0,00124722	0,109	0,093	0,095	0,095	1	0,0009428
18:00	08:30:00	0,107	0,108	0,111	1	0,00169967	0,109	0,097	0,098	0,098	1	0,0004714
18:30	09:00:00	0,116	0,11	0,111	1	0,00262467	0,112	0,094	0,094	0,093	1	0,0004714

19:00	09:30:00	0,107	0,11	0,11	1	0,00141421	0,109	0,1	0,1	0,098	1	0,0009428
20:00	10:30:00	0,112	0,113	0,114	1	0,0008165	0,113	0,091	0,092	0,091	1	0,0004714
21:00	11:30:00	0,108	0,11	0,116	1	0,00339935	0,111	0,099	0,097	0,098	1	0,0008165
22:00	12:30:00	0,111	0,123	0,116	1	0,00492161	0,117	0,108	0,103	0,103	1	0,0023570
23:00	13:30:00	0,113	0,121	0,12	1	0,00355903	0,118	0,107	0,107	0,105	1	0,0009428
00:00	14:30:00	0,11	0,121	0,116	1	0,00449691	0,116	0,11	0,109	0,106	1	0,0016996
01:00	15:30:00	0,112	0,127	0,12	1	0,00612826	0,12	0,106	0,104	0,105	1	0,0008165
02:00	16:30:00	0,111	0,127	0,12	1	0,00654896	0,119	0,106	0,104	0,105	1	0,0008165
03:00	17:30:00	0,112	0,126	0,122	1	0,00588784	0,12	0,117	0,112	0,106	1	0,0044969
04:00	18:30:00	0,115	0,128	0,122	1	0,00531246	0,122	0,131	0,122	0,116	1	0,0061644
05:00	19:30:00	0,113	0,13	0,124	1	0,00703957	0,122	0,159	0,144	0,135	1	0,0098994
06:00	20:30:00	0,114	0,132	0,123	1	0,00734847	0,123	0,212	0,171	0,158	1	0,0230120
07:00	21:30:00	0,114	0,184	0,128	1	0,03024346	0,142	0,274	0,229	0,213	1	0,0258241

08:00	22:30:00	0,116	0,137	0,128	1	0,00860233	0,127	0,321	0,295	0,271	1	0,02041786
09:00	23:30:00	0,117	0,141	0,129	1	0,00979796	0,129	0,355	0,319	0,317	1	0,01746107
10:00	24:30:00	0,119	0,142	0,129	1	0,0094163	0,13	empty	0,329	0,322	1	0,0035
11:00	25:30:00	0,118	0,143	0,129	1	0,01023067	0,13		0,332	0,324	1	0,004
12:00	26:30:00	0,12	0,145	0,137	1	0,01042433	0,134		0,361	0,338	1	0,0115
13:00	27:30:00	0,123	0,148	0,136	1	0,01020893	0,136		0,448	0,375	1	0,0365
14:00	28:30:00	0,128	0,152	0,141	1	0,00980929	0,14		0,41	0,401	1	0,0045
15:00	29:30:00	0,123	0,149	0,138	1	0,01065624	0,137		0,427	0,422	1	0,0025
16:00	30:30:00	0,125	0,15	0,135	1	0,01027402	0,137		0,44	0,434	1	0,003
17:00	31:30:00	0,127	0,152	0,142	1	0,01027402	0,14		0,456	0,456	1	0
18:00	32:30:00	0,129	0,152	0,142	1	0,0094163	0,141		0,474	0,469	1	0,0025
19:00	33:30:00	empty	0,155	0,145	1	0,005	0,15		0,486	0,487	1	0,0005
20:00	34:30:00		0,154	0,142	1	0,006	0,148		0,495	0,497	1	0,001

21:00	35:30:00		0,152	0,146	1	0,003	0,149		0,508	0,506	1	0,001

E.coli_Rosetta2_BG11+_curve.png



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

Author: Ylenia Longo
created: 12.06.2018 17:51
updated: 12.06.2018 18:08

Entry 113/143: Test-PCR for p2iGEM0174 and Lvl0
In Project: General
With tags: entry vector

To check whether p2iGEM0174 is contaminated, a test PCR is done on p2iGEM0174 of the Quroum sensing group, as well as on Lvl0 plasmids containing p2iGEM0174, such as p2iGEM0263,270 and 265, and the 4 p2iGEM0174 samples belonging to Jennifer (1.5 µl each).

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

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

4 µl	Buffer (Q5)
4 µl	Enhancer (Q5)
1.5 µl	Primer fwd
1.5 µl	Primer rev
0.5 µl	dNTPs
0.5 µl	Q5 Polymerase
8 µl	Milli Q Water
1.5µl	DMSO

Following protocole was used:

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

The same was done using the Tap Polymerase:

Taq reaction buffer: 2.5µl

dNTP: 0.5µl

O_iGEM0013 & O_iGEM0014: 1.5µl

Taq Polymerase: 0.5µl

water: 20µl

According to the following conditions

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 60°C 68°C	20 seconds 20 seconds 100sec
Final Extension	68°C	5 minutes
Hold	4-10°C	hold

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

Author: Carina Gude

created: 13.06.2018 19:32
updated: 14.06.2018 10:59

Entry 114/143: Chlorophyll extraction and measurment for Cyano cscB growth curve
In Project: General
With tags: BG-11, cscB, Cyanobacteria, Growth Curve, chlorophyll, S.elongatus

Chlorophyll Extraction in Cyanobacteria for measurements for the growth curves from 03.06.2018 - 10.06.2018

- Frozen Chlorophyll-samples were defrosted
- Pellet was resuspended in remaining 100µl if it wasn't done before
- 0,9ml of 100% methanol was aded to the sample and mixed thoroughly by vortexing
- Incubation of samples in the dark for 30min at 4°C in the fridge
- Spinning of samples at 14000rpm for 5min
- Supernatant was transfered into a cuvette
- Extinction was measured at 655nm. 90% methanol was used as the reference solution.

time	time in h	BG11-1 Chlorophyll	BG11-2 Chlorophyll	BG11-3 Chlorophyll
03.06.2018 20:00	0:00:00	0,054	0,013	0,011
04.06.2018 08:30	12:30:00			
04.06.2018 20:10	24:10:00	0,029	0,022	0,033
05.06.2018 08:00	36:00:00	0,046	0,046	0,049
05.06.2018 20:05	48:05:00	0,073	0,075	0,074
06.06.2018 08:15	60:15:00	0,094	0,098	0,096
06.06.2018 20:00	72:00:00	0,121	0,139	0,122
07.06.2018 08:05	84:05:00	0,163	0,16	0,161
07.06.2018 19:55	95:55:00	0,191	0,205	0,198
08.06.2018 08:20	108:20:00	0,218	0,21	0,082
08.06.2018 20:05	120:05:00	0,292	0,268	0,251
09.06.2018 08:10	132:10:00	0,269	0,284	0,267
09.06.2018 20:10	144:10:00	0,226	0,288	0,271
10.06.2018 09:25	157:25:00	0,193	0,179	0,197
10.06.2018 20:00	168:00:00	0,31	0,352	0,305

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

Author: Carina Gude
Entry 115/143: Cyanobacteria pre culture
In Project: General
With tags: BG-11, Cyanobacteria, Growth Curve, S.elongatus, preculture

created: 18.06.2018 20:59
updated: 18.06.2018 20:59

Preparation of Cyanobacteria preculture:

Synechococcus elongatus sp PCC 7942 stock culture: OD_{750}= 1,18

Mix:

- 16,949 ml culture
- 183,051 ml BG11
- inoculate at 30°C at 200rpm

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

Author: Carina Gude
created: 23.06.2018 13:11
updated: 02.07.2018 11:41
Entry 116/143: S.cerevisiae in LB, SD and M2
In Project: General
With tags: Growth Curve, LB, S.cerevisiae, M2-Medium, SD medium

19.06.2018

S. cerevisiae BY4742 over night culture:

- 200ml YPD
- a drop of S.cerevisiae BY4742 Kryo

20.06.2018

OD of over night culture: 0,874

Preparation of samples:

- 20,595 ml over night culture
- 159,405 ml medium (LB/SD/M2)

after everything inside the three falcons is mixed well, 2x 60ml each of each falcon is refilled into another one

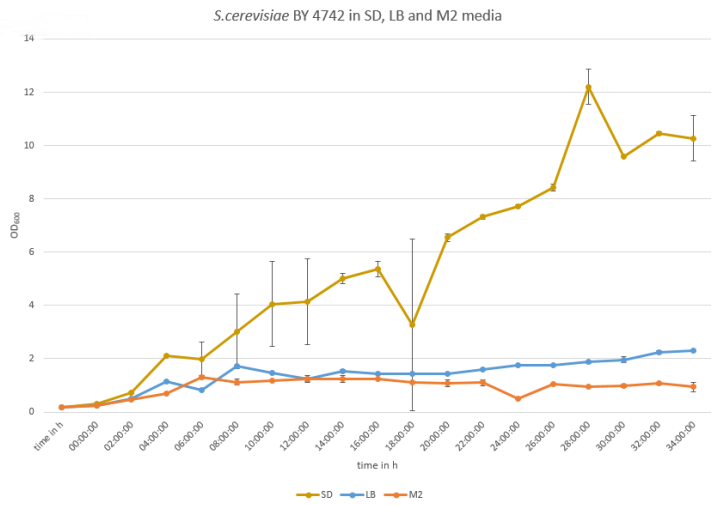
time	time in h	LB tR1 undiluted	LB tR2 undiluted	LB tR3 undiluted	Standardabweichung	LB mean	SD-1 undiluted	SD-2 undiluted	SD-3 undiluted	Standardab
09:00	00:00:00	0,173	0,173	0,173	2,77556E-17	0,173	0,188	0,188	0,188	2,77556E-1
11:00	02:00:00	0,265	0,244		0,0105	0,2545	0,324	0,318	0,312	0,0048989
13:00	04:00:00	0,503	0,472		0,0155	0,4875	0,715	0,761	0,686	0,0308796

15:00	06:00:00	1,168	1,128		0,02	1,148	2,148	2,092	2,1	0,0247296
17:00	08:00:00	0,82			0	0,82	1,196	2,092	2,7	0,6177464
19:00	10:00:00	1,72			0	1,72	4,1	1,03	3,96	1,415368(
21:00	12:00:00	1,46			0	1,46	4,2	2,03	5,95	1,6033922
23:00	14:00:00	1,3	1,07	1,36	0,124988888	1,24333333	4,43	2,03	5,95	1,6137189
01:00	16:00:00	1,43	1,61	1,55	0,074833148	1,53	4,76	5,23	5,06	0,1943078
03:00	18:00:00	1,47	1,43	1,45	0,016329932	1,45	5,016	5,709	5,346	0,2830229
05:00	20:00:00	1,46	1,46	1,38	0,037712362	1,43333333	7,84	1,16	0,84	3,2270523
07:00	22:00:00	1,4	1,4	1,48	0,037712362	1,42666667	6,6	6,69	6,34	0,1483988
09:00	24:00:00	1,61	1,56	1,63	0,029439203	1,6	7,27	7,43	7,27	0,0754247
11:00	26:00:00	1,79	1,76	1,71	0,032998316	1,75333333	7,66	7,78	7,72	0,0489897
13:00	28:00:00	1,75	1,72	1,8	0,032998316	1,75666667	8,29	8,43	8,6	0,1267543
15:00	30:00:00	1,94	1,83	1,85	0,047842334	1,87333333	12,26	13	11,36	0,6705884

17:00	32:00:00	2,02	2,05	1,82	0,102089286	1,96333333	9,64	9,58	9,58	0,0282842
19:00	34:00:00	2,34	2,24	2,17	0,069761498	2,25	10,46	10,54	10,38	0,0653197
21:00	36:00:00	2,34	2,28	2,3	0,024944383	2,30666667	9,34	11,4	10,06	0,8535937

Two LB samples weren't usable after a while which is why at 23:00 technical replicates were made.

Yeast_SD,_LB_and_M2.png



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

Author: Jennifer Denter Entry 117/143: No entry title yet In Project: General No tags associated	created: 24.06.2018 16:05 updated: 24.06.2018 16:05

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

Author: Sarah Seyffert
Entry 118/143: Chlorophyll extraction
In Project: General
With tags: chlorophyll, Growth Curve

created: 26.06.2018 18:37
updated: 26.06.2018 18:39

Chlorophyllextraction of the first 81 samples for the growth curve of the first co-culture

- **Add 0.9 ml** of **100% methanol** to the sample and **mix thoroughly** by vortexing.
- Incubate the samples in the **dark** for **30 min** at **4 °C** in the fridge.
- Spin down samples again at **14,000 rpm** for **5 min**.
- **Transfer supernatant** into a cuvette and measure the **extinction** at **665 nm**. Use **90% methanol** as the **reference** solution.

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

Author: Sarah Seyffert
Entry 119/143: Chorophyll extraction
In Project: General
With tags: chlorophyll

created: 28.06.2018 13:20
updated: 28.06.2018 13:21

(27.06.18)

Chlorophyllextraction of the last 81 samples for the growth curve of the first co-culture

- **Add 0.9 ml** of **100% methanol** to the sample and **mix thoroughly** by vortexing.
- Incubate the samples in the **dark** for **30 min** at **4 °C** in the fridge.
- Spin down samples again at **14,000 rpm** for **5 min**.
- **Transfer supernatant** into a cuvette and measure the **extinction** at **665 nm**. Use **90% methanol** as the **reference** solution.

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

Author: Sarah Seyffert
Entry 120/143: S. elongatus culture
In Project: General
With tags: Cyanobacteria, cscB

created: 02.07.2018 16:09
updated: 02.07.2018 16:15

Because the main culture of *S. elongatus* cscB was contaminated another mainculture is prepared from the plates

- Add 200ml BG11 in a 500ml culture flasks (sterile)
- inocultae with cyano bacteria (sterile)

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

Author: Sarah Seyffert
Entry 121/143: Strain from Pam. Silver
In Project: General
With tags: Cyanobacteria

created: 10.07.2018 14:01
updated: 10.07.2018 14:27

Preparing of a plate with the strain of *S. elongatus* from Pamela Silver

- from the cryo 2iGEM0314 cells were plated out on a BG11-agar plate
- incubation in light panel at 30°C, but the plate is covered with paper

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

Author: Ylenia Longo
Entry 122/143: LB Kan
In Project: General
With tags: Lb kan

created: 11.07.2018 16:23
updated: 11.07.2018 16:24

LB plates were prepared with Kanamycin (50µg/mL)

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

Author: Susanne Vollmer
Entry 123/143: LB amp plates
In Project: General
With tags: amp, plates

created: 18.07.2018 14:18
updated: 18.07.2018 14:20

20 LB amp plates were poured with 500 ml LB and 5 ml ampiciline

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

Author: Susanne Vollmer
Entry 124/143: primer aliquot O_iGEM18_0068
In Project: General
With tags: primer

created: 18.07.2018 14:20
updated: 18.07.2018 14:22

make a new aliquot (10mM) of O_iGEM18_0068 (CIDAR entryvektor DVA_CD primer) with 90 µl milli Q and 10 µl from the stock

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

Author: Ylenia Longo
Entry 125/143: Test competent cells
In Project: General
No tags associated

created: 25.07.2018 15:13
updated: 25.07.2018 15:15

To exclude that the competent cells used have unwanted resistancies, E.coli DH5a, E.coli BL21 (DE3) C43 and C41 were plated on plates containing antibiotics (Kan,Cam, Amp --> one resistance per plate). As expected, on none of those plates the cells grew. As a positive control, the cells were also streaked out on plates with no antibiotics. Indeed growth was observed here.

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

Author: Susanne Vollmer
Entry 126/143: LB amp plates
In Project: General
With tags: amp, plates

created: 26.07.2018 14:33
updated: 26.07.2018 14:34

20 LB amp plates were poured with 500 ml LB and 5 ml ampiciline

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

Author: Kai Huß
created: 01.08.2018 11:25
updated: 07.08.2018 10:44
Entry 127/143: Retransformation of Nootkatone Plasmids
In Project: General
With tags: Retransformation, Transformation, Nootkatone, Plasmids

Retransformation of iGEM 2017 plasmids with *E. coli* Top Ten:

Vals lvl 0 (pD_044)

ADH lvl 0 (pD_042)

AIPLF (BM3 variant) lvl 1 (no numeration, based on pD_049, ask Marvin Hubert)

AIPLF (BM3 variant) Pts1 lvl1 (pK_#4)

Detailed description of constructs: Marvin Hubert/Jan Maika, iGEM HHU/UzK 2017.

Transformed storage plasmids are marked with a blue dot at the bottom of the reaction tube.

Thaw competent *E. coli* cells on ice for about **10 min**

Put the reaction tubes, containing the plasmids needed for the transformation, on ice to cool them, too

For each transformation mix carefully: **10 µl competent cells + 1 µl plasmid**

Incubate on **ice for 2 min**

Heat shock: **45 sec, 42°C**

Incubate **2 min on ice**

Add 150 µl dYT and **incubate** at 37°C, 1000 rpm, 1.5 h (Regeneration Step)

Use **20 µl** to **inoculate** 5 mL dYT-Amp or LB-Cam, respectively

Use **20 µl** to **plate** YT-Amp or LB-Cam-plates, respectively

--> For **Lvl0 Plasmids** (pD_042 and pD_042) use **Cam**, for **Lvl1 Plasmids** (AIPLF plasmids: w/o number and pK_#4) use **Amp**

Incubate over Night at 37°C

Centrifuge remaining culture 13000 rpm, 1 min, remove supernatant, resuspend in dYT-Glycerol and store at -80°C

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

Author: Carina Gude
created: 01.08.2018 15:21
updated: 01.08.2018 15:34
Entry 128/143: Growth curve of corynebacterium glutamicum in M2 and LB
In Project: General
With tags: Growth Curve, LB, M2-Medium, Corynebacterium

24th of July:

Preparation of Corynebacterium glutamicum 200ml preculture

25.07.18

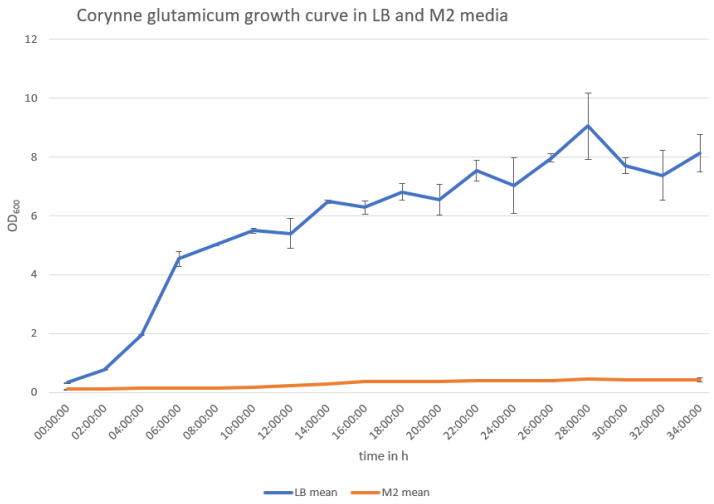
OD600 of preculture: 3,90

For the samples of LB and M2:

- 4,615ml preculture
- 175,385 ml preculture
- pipetting 60ml each into two other flasks

time in h	LB 1	LB 2	LB 3	LB mean	M2-1	M2-2	M2-3	M2 mean
00:00:00	0,117	0,115	0,128	0,12	0,105	0,102	0,102	0,103
02:00:00	0,338	0,336		0,337	0,12	0,116	0,12	0,11866667
04:00:00	0,775	0,771		0,773	0,132	0,128	0,134	0,13133333
06:00:00	1,968	1,956		1,962	0,138	0,132	0,139	0,13633333
08:00:00	4,288	4,792		4,54	0,147	0,144	0,147	0,146
10:00:00	5,055	4,98		5,0175	0,167	0,159	0,164	0,16333333
12:00:00	5,58	5,42		5,5	0,182	0,176	0,191	0,183
14:00:00	6,08	5,28	4,86	5,40666667	0,23	0,219	0,22	0,223
16:00:00	6,46	6,44	6,56	6,48666667	0,301	0,288	0,289	0,29266667
18:00:00	6,48	5,98	6,4	6,28666667	0,374	0,362	0,356	0,364
20:00:00	7,14	6,86	6,44	6,81333333	0,385	0,374	0,374	0,37766667
22:00:00	5,84	7,08	6,76	6,56	0,412	0,345	0,367	0,37466667
24:00:00	8	7,44	7,18	7,54	0,423	0,403	0,392	0,406
26:00:00	5,92	8,22	6,98	7,04	0,443	0,382	0,405	0,41
28:00:00	8,06	7,76	8,1	7,97333333	0,477	0,373	0,391	0,41366667
30:00:00	10,6	8,6	7,96	9,05333333	0,485	0,436	0,422	0,44766667
32:00:00	7,52	7,52	8,1	7,71333333	0,487	0,442	0,402	0,44366667
34:00:00	7,96	8	6,18	7,38	0,483	0,363	0,43	0,42533333
36:00:00	8,76	8,36	7,28	8,13333333	0,513	0,401	0,348	0,42066667

Kurve_Corynbacterium_glutamicum.png



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

Author: Kai Huß

created: 02.08.2018 12:10
updated: 02.08.2018 14:03

Entry 129/143: Plasmid prep of Nootkatone plasmids

In Project: General

With tags: Nootkatone, Plasmids, miniprep kit, plasmid isolation, Plasmid prep

Isolation of Nootkatone Plasmids using Macherey Nagel NucleoSpin® Plasmid (NoLid) Kit:

Cultivate and harvest bacterial cells: Use 5 mL of a saturated *E.coli* LB/dYT culture, pellet cells in a standard benchtop microcentrifuge for 1 min at 13000 rpm. Discard the supernatant and remove as much of the liquid as possible.

Cell lysis: Add 250 µL Buffer A1. Resuspend the cell pellet completely by vortexing

Precipitation: Add 250 µL Buffer A2. Mix gently by inverting the tube 6–8 times. Room temperature 5 minutes

Neutralization: Add 300 µL Buffer A3. Mix thoroughly by inverting the tube 6–8 times until blue samples turn colorless completely!

Clarification of lysate: Centrifuge for 10 min at 13000 rpm at room temperature.

Bind DNA: Place a NucleoSpin® Plasmid/Plasmid (NoLid) Column in a Collection Tube (2 mL) and transfer the supernatant onto the column.

Centrifuge for 1 min at 13000 rpm. Discard flowthrough and place the NucleoSpin® Plasmid/Plasmid (NoLid) Column back into the collection tube.

Wash: Add 600 µL Buffer A4

Centrifuge for 1 min at 13000 rpm. Discard flowthrough and place the NucleoSpin® Plasmid / Plasmid (NoLid) Column back into the empty collection tube.

Dry silica membrane: Centrifuge for 2 min at 13000 rpm and discard the collection tube.

Elute DNA: Place the NucleoSpin® Plasmid / Plasmid (NoLid) Column in a 1.5 mL microcentrifuge tube (not provided) and add 25 µl H_2O (heat to 70°C before). Incubate for 1 min at room temperature. Centrifuge for 1 min at 13000 rpm.

Repeat this step 1 time

Measure concentration using nanodrop

Sample ID	Nucleic Acid	Unit	260/280	260/230
ADH	392,3	ng/µl	1,88	1,84
ValS	268,6	ng/µl	1,83	1,37
AIPLF	678	ng/µl	1,85	2,12
AIPLF-pts1	486,1	ng/µl	1,85	1,89

Label Eppis and freeze at -20°C

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

Author: Sarah Seyffert
Entry 130/143: Cyanos in M2
In Project: General
With tags: cscB, Cyanobacteria

created: 03.08.2018 17:33
updated: 03.08.2018 17:36

Transfer from 2iGEM0314 and S.elongatus cscB:::N53 culture into 150ml M2-medium

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

Author: Jennifer Denter
Entry 131/143: Refill 1X TAE
In Project: General
With tags: TAE

created: 08.08.2018 15:22
updated: 08.08.2018 15:27

Dilute 50X TAE to 1X TAE

20 ml 50X TAE

980 ml Milli Q Water

mix and fill into flask.

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

Author: Jennifer Denter
Entry 132/143: Preparation of X-GAL solution
In Project: General
No tags associated

created: 08.08.2018 15:27
updated: 08.08.2018 15:34

Use aluminium foil to cover all tubes cause X-GAL is light sensitive!

20 mg X-Gal stock powder

1 ml DMSO

Carefully invert for 3 times. & wait for 1,5 min till powder is in solution.

Sterile filtrate solution into a new sterile falcon.

Storage at -20 °C.

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

Author: Carina Gude
created: 09.08.2018 10:54
updated: 09.08.2018 11:04
Entry 133/143: Growth curve of E.coli BL21 (DE3) C43
In Project: General
With tags: Growth Curve, growth rate, E.coli, LB, M2-Medium

06.08.2018

Preculture preparation: 200ml LB with Kryo

08.08.2018

Growth curve measurements:

Preculture OD_{600}: 3,8

For each sample: 1,579 ml preculture + 58,421 ml medium

time [h]	LB-1	LB-2	LB-3	dilution	Standardabweichung	LB-mean	M2-1	M2-2	M2-3	Standardabweichung	M2-mean
0,0	0,107	0,103	0,1	1	0,00286744	0,10333333	0,074	0,089	0,083	0,00616441	0,082
0,5	0,118	0,115	0,112	1	0,00244949	0,115	0,087	0,078	0,076	0,00478423	0,0803333
1,0	0,192	0,184	0,184	1	0,00377124	0,18666667	0,086	0,078	0,078	0,00377124	0,0806666
1,5	0,313	0,313	0,307	1	0,00282843	0,311	0,102	0,095	0,089	0,00531246	0,0953333
2,0	0,557	0,56	0,554	1	0,00244949	0,557	0,109	0,098	0,097	0,0054365	0,1013333
2,5	0,445	0,445	0,42	2	0,01178511	0,87333333	0,118	0,106	0,104	0,00618241	0,1093333
3,0	0,818	0,814	0,809	2	0,00368179	1,62733333	0,114	0,099	0,104	0,0062361	0,1056666
3,5	0,151	0,15	0,154	10	0,00169967	1,51666667	0,125	0,114	0,111	0,00601849	0,1166666
4,0	0,177	0,176	0,179	10	0,00124722	1,77333333	0,112	0,095	0,09	0,0094163	0,099
4,5	0,239	0,239	0,221	10	0,00848528	2,33	0,126	0,109	0,119	0,00697615	0,118

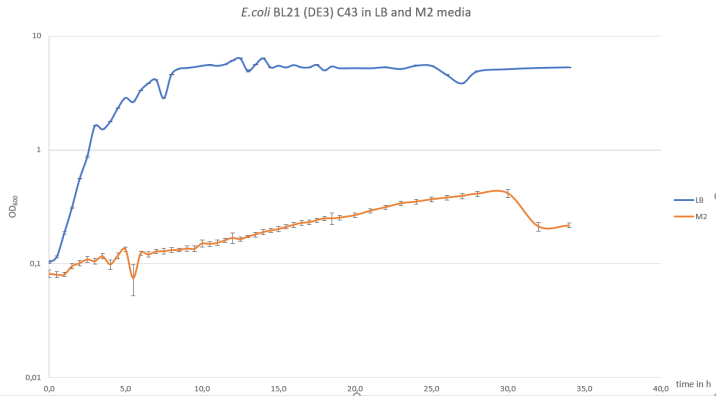
5,0	0,288	0,294	0,28	10	0,00573488	2,87333333	0,126	0,135	0,14	0,00579272	0,1336666
5,5	0,265	0,283	0,244	10	0,01593738	2,64	0,104	0,073	0,048	0,0229056	0,075
6,0	0,344	0,344	0,316	10	0,01319933	3,34666667	0,131	0,121	0,12	0,00496655	0,124
6,5	0,388	0,382	0,388	10	0,00282843	3,86	0,129	0,119	0,114	0,0062361	0,1206666
7,0	0,415	0,409	0,416	10	0,00309121	4,13333333	0,135	0,129	0,121	0,00573488	0,1283333
7,5	0,279	0,294	0,282	10	0,00648074	2,85	0,139	0,122	0,125	0,0074087	0,1286666
8,0	0,47	0,459	0,451	10	0,00778888	4,6	0,14	0,128	0,128	0,00565685	0,132
8,5	0,503	0,532	0,517	10	0,01184155	5,17333333	0,139	0,131	0,126	0,00535413	0,132
9,0	0,527	0,529	0,521	10	0,00339935	5,25666667	0,146	0,133	0,13	0,00694422	0,1363333
9,5	0,567	0,512	0,527	10	0,02321398	5,35333333	0,147	0,13	0,129	0,00825967	0,1353333
10,0	0,538	0,56	0,55	10	0,00899383	5,49333333	0,164	0,148	0,14	0,00997775	0,1506666
10,5	0,546	0,552	0,579	10	0,0143527	5,59	0,159	0,147	0,142	0,00713364	0,1493333
11,0	0,524	0,544	0,578	10	0,022291	5,48666667	0,164	0,149	0,145	0,00817856	0,1526666
11,5	0,568	0,565	0,562	10	0,00244949	5,65	0,17	0,156	0,155	0,00684755	0,1603333
12,0	0,654	0,636	0,54	10	0,05003998	6,1	0,194	0,157	0,155	0,01793197	0,1686666
12,5	0,654	0,602	0,659	10	0,02577251	6,38333333	0,174	0,159	0,159	0,00707107	0,164
13,0	0,588	0,562	0,336	10	0,11316458	4,95333333	0,179	0,172	0,169	0,00418994	0,1733333
13,5	0,6	0,54	0,546	10	0,02698148	5,62	0,192	0,172	0,178	0,00837987	0,1806666
14,0	0,586	0,643	0,684	10	0,04018568	6,37666667	0,204	0,184	0,181	0,01020893	0,1896666
14,5	0,55	0,532	0,511	10	0,01593738	5,31	0,207	0,192	0,188	0,00817856	0,1956666
15,0	0,571	0,543	0,537	10	0,01481741	5,50333333	0,215	0,196	0,193	0,00974109	0,2013333
15,5	0,571	0,502	0,519	10	0,02935227	5,30666667	0,223	0,205	0,203	0,00899383	0,2103333
16,0	0,556	0,559	0,558	10	0,00124722	5,57666667	0,234	0,216	0,208	0,010873	0,2193333
16,5	0,541	0,528	0,53	10	0,00571548	5,33	0,243	0,222	0,22	0,01040299	0,2283333
17,0	0,537	0,506	0,55	10	0,01845716	5,31	0,245	0,225	0,223	0,00993311	0,231
17,5	0,572	0,547	0,559	10	0,01020893	5,59333333	0,256	0,235	0,232	0,01067708	0,241
18,0	0,504	0,505	0,493	10	0,0054365	5,00666667	0,264	0,243	0,242	0,01014342	0,2496666
18,5	0,529	0,535	0,563	10	0,01481741	5,42333333	0,278	0,264	0,208	0,03024346	0,25
19,0	0,536	0,51	0,522	10	0,01062492	5,22666667	0,271	0,247	0,244	0,01208305	0,254
20,0	0,531	0,528	0,513	10	0,00787401	5,24	0,285	0,26	0,254	0,01342469	0,2663333

21,0	0,462	0,59	0,515	10	0,05251243	5,22333333	0,309	0,283	0,282	0,01249889	0,2913333
22,0	0,522	0,557	0,519	10	0,0172498	5,32666667	0,33	0,3	0,309	0,01256981	0,313
23,0	0,473	0,52	0,55	10	0,03168947	5,14333333	0,357	0,333	0,325	0,01359739	0,3383333
24,0	0,491	0,56	0,6	10	0,04502098	5,50333333	0,372	0,345	0,334	0,01596524	0,3503333
25,0	0,566	0,548	0,534	10	0,01309792	5,49333333	0,391	0,358	0,351	0,01744197	0,3666666
26,0	0,5	0,453	0,418	10	0,03359563	4,57	0,405	0,371	0,365	0,01761313	0,3803333
27,0	0,342	0,404	0,407	10	0,02995923	3,84333333	0,421	0,385	0,372	0,02072572	0,3926666
28,0	0,5	0,523	0,446	10	0,03227314	4,89666667	0,439	0,395	0,399	0,01986622	0,411
30,0	0,505	0,54	0,494	10	0,01961292	5,13	0,453	0,419	0,373	0,03278211	0,415
32,0	0,515	0,526	0,538	10	0,00939267	5,26333333	0,233	0,217	0,188	0,01862495	0,2126666
34,0	0,519	0,531	0,548	10	0,01189771	5,32666667	0,203	0,228	0,219	0,01033871	0,2166666

M2 estimated growth rate: 0,0326 h^1

LB estimated growth rate: 0,5274 h^1

Kurve_E.coli_BL21_(DE3)_C43.png



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

Author: Susanne Vollmer
Entry 134/143: renew WT plates of S.cerevisiae
In Project: General
With tags: WT, plates

created: 10.08.2018 18:05
updated: 10.08.2018 18:10

to renew the WT plates:

inoculation of 3ml YPD each with, 50 µl *S. cerevisiae* BY4742 and from the WT plate VWIA from Feldbrügge

incubation at 30 °C

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

Author: Sarah Seyffert
Entry 135/143: Induction of the cyanos
In Project: General
With tags: Cyanobacteria, cscB

created: 14.08.2018 10:08
updated: 14.08.2018 10:16

Indution of the sugar excreting Cyanobacteria strains 2iGEM0314 and S.elongatus cscB:::N53 culture

- 45ml was taken away from the cultures
- 45ml 500mM NaCl was added to the remaining 105 ml culture(150mM in culture media)
- 150µl of 1M IPTG was added to the cultures(1mM in Culture media)

Afterwards the OD was measured at 750nm

1. 2iGEM0314: 0,143
2. S.elongatus cscB:::N53 : 0,109

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

Author: Jennifer Denter
created: 15.08.2018 17:30
updated: 15.08.2018 19:01

Entry 137/143: Transformation of iGEM Part for iGEM Bielefeld.
In Project: General
With tags: Transformation, iGEM part, Kit Plate

Since iGEM Bielefeld 2018 came up with troubles with the iGEM part 4A of the 2018 Kit Plate 4.

We decided to transform and sequence this part to make sure the part is correct.

10 µl Milli Q Water was added to the well 4A on Kit Plate 4.

incubarion for 10 min on RT.

1,2 µl of DNA was used for an heat shock transformation

After 1 h at 37 °C, 300 rpm 100% of the cells were plated at LB-chloramphenicol.

Plates were incubated at 37 °C o/n.

After 19 h incubation 2 colonies appeared.

Both were picked and inoculated in 3 ml LB-chloramphenicol and incubated o/n at 37 °C, 200 rpm.

Plasmids of both cultures were isolated using the Promega Pure Yield Miniprep Kit and stored at -20 °C.

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

Author: Sarah Seyffert
created: 17.08.2018 18:06
updated: 18.08.2018 11:26
Entry 138/143: Growth curve of sugar enriched M2-media
In Project: General
With tags: Cyanobacteria, Growth Curve

The Cyanoculture was induced one day before the measurement started

Today was the first measure day. Therefore one 96 wellplate was prepared.

Preparation of the M2 media

- 4ml from both cyanobacteria strains(2iGEM0314 and S.elongatus cscB:::N53 culture) was taken and added in a falcon tube
- centrifuge it down for 6 min at 4000rpm
- take the supernatant and sterile filtrate it into another falcon tube

Preparation of the *E.coli* and *S.cerevisiae* cultures

- 3.5ml was taken from each culture
- centrifuge it down for 6 min at 4000rpm
- discard supernatant
- add 2ml M2-media and resuspend the pellet
- centrifuge it down for 4 min at 4000rpm
- discard supernatant and add 2ml M2-media
- OD-measurement

Each well was filled with the prepared media and the cultures. The density was adjusted to OD: 0.1 in each well

The plate was filled with the following scheme

M2-blank fromS. elongatus cscB:::N53	E.coli C43	E.coli C43	E.coli C43	S. cerevisiae	S. cerevisiae	S. cerevisiae
M2-blank from2iGEM0314	E.coli C43	E.coli C43	E.coli C43	S. cerevisiae	S. cerevisiae	S. cerevisiae

Note: The media taken for the cultures in the wells in a row is the same as the one for the blank in the same row.

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

Author: Sarah Seyffert
created: 19.08.2018 14:10
updated: 19.08.2018 14:17
Entry 139/143: Growth curve in sugar enriched M2 media
In Project: General
With tags: Cyanobacteria, cscB, Growth Curve, M2-Medium

The Cyanoculture was induced three days before the measurement started

Today was the first measure day. Therefore one 96 wellplate was prepared.

Preparation of the M2 media

- 4ml from both cyanobacteria strains(2iGEM0314 and S.elongatus cscB:::N53 culture) was taken and added in a falcon tube
- centrifuge it down for 9 min at 2500rpm
- take the supernatant and sterile filtrate it into another falcon tube

Preparation of the *E.coli* and *S.cerevisiae* cultures

- 3.5ml was taken from each culture
- centrifuge it down for 9 min at 2500rpm
- discard supernatant
- add 2ml M2-media and resuspend the pellet
- centrifuge it down for 4 min at 4000rpm
- discard supernatant and add 10ml M2-media
- OD-measurement

Each well was filled with the prepared media and the cultures. The density was adjusted to OD: 0.1 in each well

The plate was filled with the following scheme

M2-blank fromS.elongatus cscB:::N53	E.coli C43	E.coli C43	E.coli C43	S.cerevisiae	S.cerevisiae	S.cerevisiae
M2-blank from2iGEM0314	E.coli C43	E.coli C43	E.coli C43	S.cerevisiae	S.cerevisiae	S.cerevisiae

Note: The media taken for the cultures in the wells in a row is the same as the one for the blank in the same row.

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

Author: Sarah Seyffert
created: 21.08.2018 14:07
updated: 21.08.2018 14:08
Entry 140/143: Growth curve in sugar enriched M2 media
In Project: General
With tags: Growth Curve, cscB, Cyanobacteria, M2-Medium, E.coli, S.cerevisiae

The Cyanoculture was induced five days before the measurement started

Today was the first measure day. Therefore one 96 wellplate was prepared.

Preparation of the M2 media

- 4ml from both cyanobacteria strains(2iGEM0314 and S.elongatus cscB:::N53 culture) was taken and added in a falcon tube
- centrifuge it down for 4 min at 4000rpm
- take the supernatant and sterile filtrate it into another falcon tube

Preparation of the *E.coli* and *S.cerevisiae* cultures

- 3.5ml was taken from each culture
- centrifuge it down for 4 min at 40000rpm
- discard supernatant
- add 2ml M2-media and resuspend the pellet
- centrifuge it down for 4 min at 4000rpm
- discard supernatant and add 10ml M2-media
- OD-measurement

Each well was filled with the prepared media and the cultures. The density was adjusted to OD: 0.1 in each well

The plate was filled with the following scheme

M2-blank fromS.elongatus cscB:::N53	E.coli C43	E.coli C43	E.coli C43	S.cerevisiae	S.cerevisiae	S.cerevisiae
M2-blank from2iGEM0314	E.coli C43	E.coli C43	E.coli C43	S.cerevisiae	S.cerevisiae	S.cerevisiae

Note: The media taken for the cultures in the wells in a row is the same as the one for the blank in the same row.

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

Author: Katharina Polzen

created: 09.09.2018 20:59
updated: 09.09.2018 21:01

Entry 141/143: Overnight culture for fluorescence measurements
In Project: General
With tags: yeast, overnight, culture, fluorescence, mTurquoise

Overnight culture with p2iGEM0374 in 3mL SD medium -Ura for the yeast fluorescence measurements.

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

Author: Carina Gude

created: 11.09.2018 17:25
updated: 11.09.2018 17:26

Entry 142/143: S. elongatus cscB preculture
In Project: General
With tags: inoculation, S.elongatus, preculture, M2-Medium

Inoculated new S. elongatus PCC 7942 cscB:::NS3 culture in freshly made M2 for the fluorescent measurements.

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

Author: Sarah Seyffert
Entry 143/143: Growth curve of E.coli and S. cerevisisae in sucrose enriched M2 medium and togehter with S. elonagatus cscB:::NS3
In Project: General
With tags: Growth Curve, E.coli, cscB, S.cerevisiae, M2-Medium, sucrose

created: 08.10.2018 16:54
updated: 08.10.2018 16:56

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