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**Project:** iGEM uppsala 2019
**Authors:** Irina Ehnström

MONDAY, 17/6/2019

## Lab #14 - BioBrick 3A Assembly - (19-6-24) (Erik Palm)

Aim: Plasmid DNA prep of

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``

Promoter Concentration: 65.6 ng/ul

**Table1**

| | A | B |
|---|---|---|
| 1 | Promoter (BBD - K108ionB) Concentration | 65.6 ng/ul |
| 2 | Glucose Oxidase (PSBIC3 K500003) Concentration | 94.0 ng/ul |
| 3 | Backbone (pSBIK3) Concentration | 25 ng/ul |

Calculation
Amount of ml needed for digestion
psBIC3 - BBD-k108ionb 500ng x 1ul/ 65.6 ng= 7,6 ul
psBIC3- k500003 500ng x 1ul/94.0ng = 5.3 ul
pSBIK3 backbone 500 ng x 1ul/25 ng =20 ul

## Lab #15 - Preparation of 1x TBE solution through Dilution of 1x TBE solution - (19-6-24) (Erik Palm)

Dilution of 10xTBE to make 1 liter of 1x TBE

900 ml of ddH20 added to 100ml of 10x TBE

## Lab #16 - Agarose Gel Electrophoresis - (19-6-24) (Erik Palm)

### Aim:

To separate and analyze nucleic acid samples under non-denaturing conditions

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

See table 18 for components and amounts mixed for agarose gel

Instead of composing 150 mL of gel, 50 mL of gel was used instead.

## Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was weighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel solution was poured into the gel tray and air bubbles were removed with a pipette tip.
7. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

**Table2**

| | A | B |
|---|---|---|
| 1 | Amount | Component |
| 2 | 0,5 g | agarose |
| 3 | 50ml | 1x TBE |
| 4 | | |

## Running the gel

1. The gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. The DNA samples (~0.2 µg)were mix with loading dye (table 18) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply. and the gel was run at **120 V** for **80 min.**
8. After the run, the gel bands were visualized under UV and a picture was taken (figure ...).

Table 21 below shows the components of the loading solutions placed in the wells, well number, and calculations for Erik Palm's gel analysis.

**Table3**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Number | In well | Amount | Calculations if needed |
| 2 | 1 | Ladder (2-log) | 5 ul | -- |
| 3 | 2 | Promotor (PSBIC3 - BBD K108IONB) | 20 ul<br>4ul loading dye | |
| 4 | 3 | Uncut promotor (PSBIC3 - BBD K108IONB) | 5.7 ul of sample 14,3 ul of H20 4 ul loading dye | 200 ngx 1ul/ 35.1 ng= 5.7 ul of sample |
| 5 | 4 | Glucose oxidase (PSB1C3 K500003) | 20 ul sample<br>4 ul loading dye | |
| 6 | | Uncut Glucose oxidase (PSB1C3 K500003) | ------- | |
| 7 | 5 | Backbone pSB1K3 | 20 ul sample<br>4ul loading dye | |
| 8 | 6 | Uncut backbone pSB1KR | 8 ul sample<br>12 ul H20<br>4 ul loading dye | 200ng x 1ul /25ng =8 ul sample |

# Week 1: Jun 17 - Jun 23

**Project:** iGEM uppsala 2019
**Authors:** Irina Ehnström

MONDAY, 17/6/2019

## Lab #1 - Cleaning Pipettes (19-6-17) (Qian + Irina)

### Aim:

To avoid contaminations;

### Methods:

All team pipettes were cleaned with detergent using a toothbrush. The tip-ejecting part of the pipette was taken off and cleaned separately together with the outside of the pipette. The pipettes were then dried with paper towels (Irina & Qian).

## Lab #2 - Prepare solutions and agar plates (19-6-17) (Qian + Irina)

### Aim:

To prepare the solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 90- 101;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Solutions

**A) 0.9% NaCl (Irina)**
Aim: Soduim chloride is used for suspending cells to avoid early lysis by osmosis.
Method: The following protocol was used for preparing NaCl 0.9%:

0.9% NaCl (10ml)

Calculations: m/v = 0.9% = 0.9g/100mL = $m_{NaCl}$/10 mL → $m_{NaCl}$ = 0.09 g
Components and amounts used can be seen in Table 1.

**Table 1 - 0.9% NaCl, 10mL**

| | A | B |
|---|---|---|
| 1 | NaCl | 0.09g |
| 2 | ddH2O | 10ml |

Storage: The solution is stored on the bench.

**B) 1M calcium chloride + autoclave (Irina)**

Aim: Calcium chloride is used for making competent *Esherichia coli* cells. Exposure to $CaCl_2$ loosens the cell wall, making the *E.coli* cells susceptible for taking up foreign plasmids.
Method: The following protcol was used to prepare 1 M of CaCl2:

1 M CaCl2 (10ml)

Calculations:
$Mw(CaCl_2)$ = 110.99 g/mol
$m(CaCl_2) = Mw(CaCl_2) \times n(CaCl_2) = Mw(CaCl_2) \times c(CaCl_2) \times V(CaCl_2)$ = 110.99 g/mol x 1 mol/L x 10 mL x 1 L/1000 mL ≈ 1.11g
See table 2 for components and amounts used.

**Table 2 - 1M calcium chloride, 10mL**

| | A | B |
|---|---|---|
| 1 | CaCl2 | 1.11 g |
| 2 | ddH2O | 10ml |

Storage: The solution is stored in the cold room.

**C) LB medium + autoclave (Irina, Qian)**
Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 3 for components and amounts used to prepare 600 mL of LB.

**Table 3 - LB medium 600mL**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

Storage: The LB medium is stored in the cold room with notation *"G3 - LB medium - 2019.6.17"*

▷ Agar plates and Addition of Antibiotics (Ampicillin 50 ug/mL) + autoclave (Irina + Qian)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for E. coli. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (50 ug/uL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 4 for components and amounts used to prepare 600 mL of LB.

**Table 4 - Agar plates + Ampicillin**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (50mg/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Storage: The agar plates are stored in the cold room in a package with notation *"iGEM 2019.06.17 - G3 - Agar + Amp"*

# Lab #3 - Transformation (19-6-17) (Qian + Irina)

## Aims:

To transform selected plasmids (pSB1C3_BBa_K500003, BBa_K500001) from iG*EM 2019 Distribution Kit* into DH5α competent cells in order to amplify them for future 3A assembly and expression purposes.

## Methods:

Transformation was done according to protocol:

Transformation of Competent E.coli cells

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113 - 115;
- For revisions, refer to iGEM registry's transformation protocol and DNA kit plate instructions;

* All SOB medium in the original protocal was replaced by **LB medium**;
* Competent cells were thawed on ice for more than 20 mins.
* For all parts, **1uL** plasmid was added to 50uL of competent cells in accordance to iGEM registry's transformation protocol;

## Design of control and sample plates:

* Competent cells are E.coli DH5α prepared by iGEM uppsala 2018 team.
* LB medium (Lab #2, 2019.06.17).
* dd$H_2O$ should have been 1uL but was wrongly added as 5uL, the mistake is negligible becuase:
    a. It does not majorly affect the final concentration as the final volume is quite big (1000uL);
    b. It is a negative control, we only need to show that there are no contamination;

* pSB1C3_BBa_K500003 (glyoxal oxidase - GLOX) was prepared from iGEM 2019 Distribution Kit plate 4-19F, and pSB1C3_BBa_K500001 (manganese peroxidase - MnP) was prepared from iGEM 2019 Distribution Kit plate 4-19D in accordance to iGEM registry's DNA kit plate instructions by Jinwen and Katherin.

See table 5 for components and amounts used to prepare transformation mixtures.
See table 6 for details of plates streaked with transformed cells.

**Table 5 - Transformation Mixtures (19-6-17) (Irina)**

| | Amount | Negative Control | pSB1C3_BBa_K500003 Sample | pSB1C3_BBa_K500001 Sample |
|---|---|---|---|---|
| 1 | 1uL | - | pSB1C3_BBa_K500003 (stock conc. 200-300pg/uL) | pSB1C3_BBa_K500001 (stock conc. 200-300pg/uL) |
| 2 | 5uL | ddH2O | - | - |
| 3 | 50uL | Competent cells | Competent cells | Competent cells |
| 4 | 950uL | LB medium | LB medium | LB medium |

**Table 6 - Transformation Plates (Qian)**

| | Plate Name | G3-NC-2019.6.17 | G3-BBa_K500003-1x-2019.6.17 | G3-BBa_K500003-0.1x-2019.6.17 | G3-BBa_K500001-1x-2019.6.17 | G3-BBa_K500001-0.1x-2019.6.17 |
|---|---|---|---|---|---|---|
| 1 | Purpose | Negative Control | BBa_K500003 Sample 1x | BBa_K500003 Sample 0.1x | BBa_K500001 Sample 1x | BBa_K500001 Sample 0.1x |
| 2 | Plasmid quantity /100uL | - | 200-300pg | 20-30pg | 200-300pg | 20-30pg |

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## Lab #4 - Prepare agar plates with addition of antibiotics (19-6-18) (Qian + Irina)

### Aim:

To prepare solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

▷ Agar plates and Addition of Antibiotics (Chloramphenicol 25 ug/mL) + autoclave (Irina + Qian)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (50 ug/uL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 7 for components and amounts used to prepare 600 mL of LB.

**Table 7 - Agar plates + Cm**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Chloramphenicol (25 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

## Lab #5 - Preparation of competent *E.coli* cells using $CaCl_2$ (19-6-18) (Irina + Gustaf)

### Aim:

To increase the competence of *E. coli* DH5α cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;

Calculations for $CaCl_2$ solutions can be seen in table 8.

**Table 8**

| | A | 1M CaCl2 | 50% Glycerol stock | ddH2O | TOTAL volume (ml) |
|---|---|---|---|---|---|
| 1 | 0.1 M CaCl2 (15ml) | 2 ml | / | 18ml | 20ml |
| 2 | 0.1 M CaCl2 + 20% glycerol (3ml) | 0.3ml | 1.2ml | 1.5ml | 3ml |

# Lab #6 - Preparation of LB media (19-6-18) (Qian)

Aim + Method + Calculations: Refer to Lab #2 (19-6-17)
Storage: The LB medium is stored in the cold room with notation *"G3 - LB medium - 2019.6.18"*

# Lab #7 - Preparation of 10x TBE Buffer (19-6-18) (Irina)

## Aim:

To prepare a TBE buffer that inhibits enzymatic degradation of nucleic acids when running agarose gels.

## Methods:

Preparation of 10x TBE Buffer cells for agarose gel electrophoresis was done according to protocol:

10xTBE buffer (500ml stock)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 94;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
m = Mw x n x c
m(Tris) = 121.14 g/mol x 0.89 mol/l x 0.5 L = 53.91 g
m(Boric Acid) = 61.83 g/mol x 0.89 mol/L x 0.5 L = 27.52 g
m(EDTA) = 292.24 g/mol x 0.025 mol/L x 0.5 L = 3.65 g

See table 10 for components and amounts used.

**Table 10 - 10x TBE Buffer (pH = 8.2)**

| | Component | amount |
|---|---|---|
| 1 | Tris | 53.91 g |
| 2 | Boric acid | 27.52 g |
| 3 | EDTA | 3.65 g |
| 4 | ddH2O | 500 mL |

# Lab #8 - O/N cultures (19-6-18) (Irina)

Aim: To start O/N cultures of psB1C3_BBa_K500003 (glyoxal oxidase - GLOX), BBa_K008006 (medium promoter J23110 + medium RBS B0032), BBa_K592009 (amilCP), Bbk_K500001 (manganese peroxidase - MnP) and BBa_K500000 (lignin peroxidase - LiP) containing *E. coli* DH5α cells.
Method: The following protcol was used to prepare the O/N cultures:

E.coli O/N in LB+Antibiotic (5/6mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 2, page 102;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

5 mL of LB media containing chloramphenicol was transferred to 15 mL falcon tubes. One colony was chosen from each O/N agar plate respectively using a pipette tip. The tips were left inside the tubes. The falcon tubes were incubated at 37°C on a shaking table O/N.

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# Lab #9 - Preparation of SOB medium (19-6-19) (Irina)

## Aim:

To prepare SOB Medium, or Super Optimal Broth, which is used for preparing chemically competent cells.

## Methods:

Preparation of SOB was done according to protocol:

SOB Medium

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 96;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations
m(Yeast) = $W_1$/ $V_1$ x $V_2$ = $W_2$ = 0.5g/100 mL x 800 mL = 4g
m(Bacto) = 2g/100mL x 800 mL = 16g
m(NaCl) = 10 mmol/1 L x 800 mL x 58.44 g/mol = 0.47g
m(KCl) = 2.5 mmol/1 L x 800 mL x 74.55 g/mol = 0.15g

See table 11 for components and amounts

**Table 11 - SOB medium (800mL)**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 0.46g |
| 2 | BactoTMtryptone 1% (w/v) | 16g |
| 3 | Yeast extract 0.5% (w/v) | 4g |
| 4 | ddH2O | 800mL |
| 5 | 5 M NaOH | 45uL |
| 6 | KCl | 0.15g |

# Lab #10 - Plasmid MiniPrep (19-6-19) (Irina + Gustav)

## Aim:

To isolatie plasmid DNA from recombinant *E. coli* O/N cultures containing pSB1CE_BBa_K500003 and BBa_K008006.

## Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.

Purified DNA plasmid concentrations can be seen in table 12.

**Table 12 - Purified Plasmid Conc. …**

| | Sample | Concentration (ng/uL) |
|---|---|---|
| 1 | BBa_K500003 | 55.4 |
| 2 | BBa_K608006 | 35.8 |

# Lab #11 - Restriction digestion to get BioBrick parts for 3A Assembly (19-6-19) (Irina + Gustav)

## Aim:

To create three different DNA restriction fragments (BioBrick™ parts) for cloning using "3A Assembly" method:

1. Promoter + RBS (upstream part): pSB1C3_J04500 from iGEM 2019 distribution kit plate 3, well 20_I;
2. CDS (downstream part): pSB1C3_BBa_K500003/K608006;
3. Plasmid vector (backbone part): pSB1A3 vector from 2019 iGEM distribution of linearized plasmid backbones.

## Methods:

Digestion was done according to protocol:

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;

Calculations for digestion mixture total volume 20 uL for BBa_K608006 and BBa_K500003:
V = required mass/intitial concetration
$V_{BBa\_K608006}$ = 200ng/35.8ng/uL = 5.6uL.
$V_{Ba\_K500003}$ = 200ng/55.4ng/uL = 3.6 uL.

For components and amounts for Digestion mixure, see tabel 13.

**Table 13 - Restriction digestion**

| | Component | BBa_K008006 amount | BBa_K500003 amount |
|---|---|---|---|
| 1 | 200 ng DNA | 5.6uL | 3.6uL |
| 2 | 10 x Reaction buffer | 2uL | 2uL |
| 3 | EcoRI | 0.4uL | 0.4uL |
| 4 | PstI | 0.4uL | 0.4uL |
| 5 | ddH2O | 11.6uL | 13.6uL |

# Lab #12 - Agarose gel electrophoresis (19-6-19) (Irina + Gustav)

## Aim:

Gel analysis of digests, to ascertain that the digestion worked properly.

## Method:

Agarose gel casting and analysis was done according to protocol:

Analytical Digestion and Agarose Gel Electrophoresis

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

See table 14 for components and amounts mixed for agarose gel

**Table 14 - 1% Agarose gel for nucleic acid analysis**

| | Component | amount | C |
|---|---|---|---|
| 1 | Agarose | 0.5g | |
| 2 | ddH2O | 50mL | |
| 3 | sybrSafe | 5uL | |

## Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was weighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel solution was poured into the gel tray and air bubbles were removed with a pipette tip.
7. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

## Running the gel

1. the gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. Ther DNA samples (~0.2 µg)were mix with loading dye (table 15) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply. and the gel was run at 100 V for 30–60 min.
8. After the run, the gel bands were visualized under UV and a picture was taken (figure 16).

See table 15 for components and amounts used for running mixture:

**Table 15**

| | Components | amounts |
|---|---|---|
| 1 | Loading dye mix | 4uL |
| 2 | DNA sample | 16uL |

5 uL of 2-Log DNA Ladder (0.1–10.0 kb) (BioLabs) were used as a ladder.

Note:

Due to a misstake that was done by Gustav, the uncut plasmid sample Bba_K500003 (uncut), was accidently placed in the 80 Celcius water bath for 20mins while the cut sample remianed in the 37.5 Celcius bath for a total of 50mins. This will un doubtedly have an effect on how the gel results will be.

From the left in the following order: Ladder ✦ Bba_K608006 (uncut) ✦ Bbk_K608006 (cut) ✦ Bba_K500003 (uncut) ✦ Bba_K500003 (cut) ✦ Ladder ✦ Bba_K592009 (?) ✦ Bba_K500001 (uncut) ✦ Bba_K500001 (cut) ✦ Bba_K500003 (uncut) ✦ Bba_K500003 (cut) ✦ Bba_K608006 (uncut) ✦ Bba_K608006 (cut), see figure 16.

20190619_analytical digestion 1 final.jpg

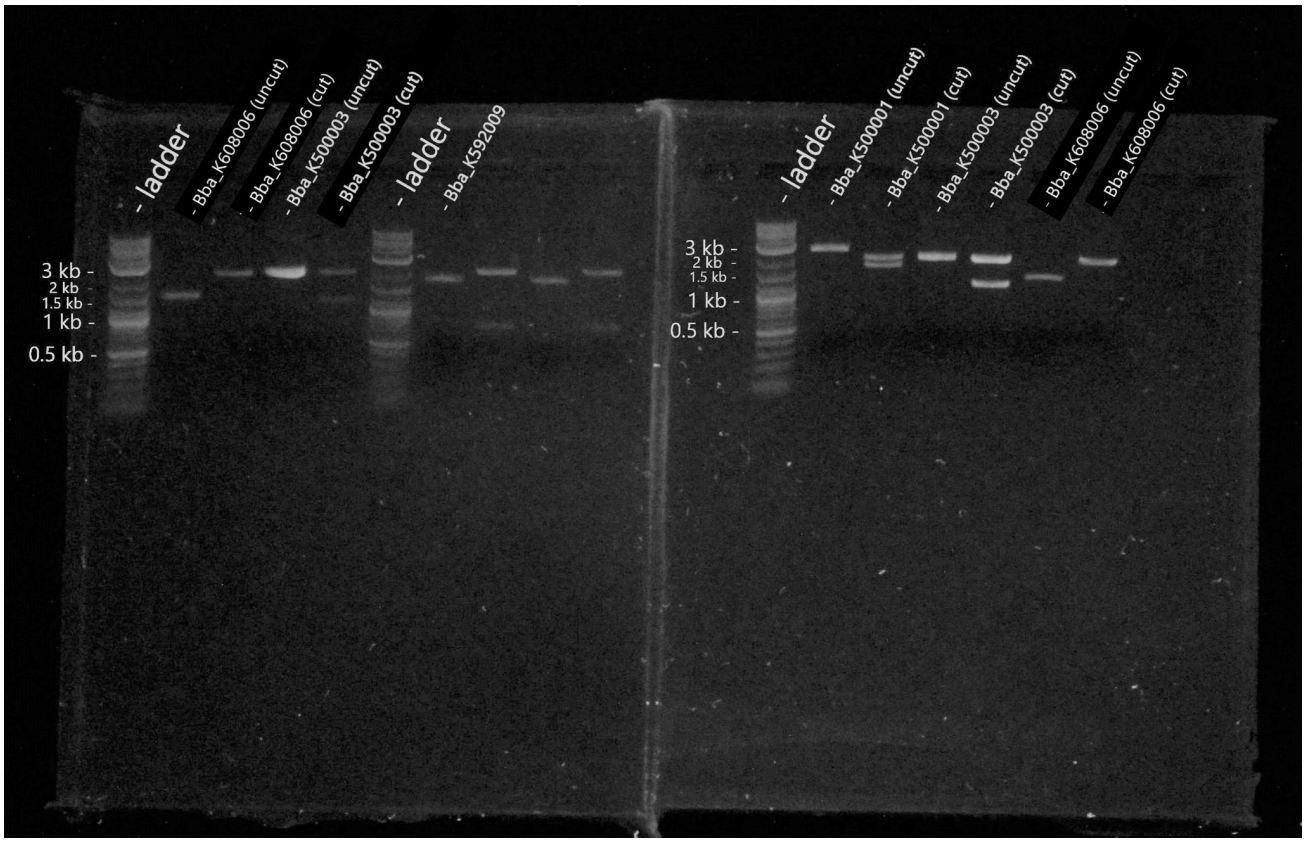



**Figure 16**. Gel elctrophoresis on 1% agarose gel together with ladder 2-Log DNA Ladder (0.1–10.0 kb) BioLabs.

THURSDAY, 20/6/2019

# Lab #13 - BioBrick 3A Assembly (19-6-20)

## Aim:

Plasmid DNA preps of BBa_K608006 BioBricks™

To assemble protein-expressing plasmids from three different DNA restriction fragments:

1. Promoter + RBS (upstream part);
2. BBa_K500003/K608006 CDS (downstream part);
3. Plasmid vector pSB1A3 (backbone part).

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;

Calculations for digestion mixture total volume 50 uL for BBa_K608006:
V = required mass/intitial concetration
V = 500ng/35.8ng/uL = 14uL.

For components and amounts for Digestion mixure, see tabel 17.

**Table 17**

| | Component | BBa_K608006 amount | Bba_K592009 (CDS) | Vector: pSBIK3 |
|---|---|---|---|---|
| 1 | 500 ng DNA | 14uL | 6.9uL | 20uL |
| 2 | 10 x Reaction buffer | 5uL | 5uL | 5ul |
| 3 | EcoRI | 1uL | | 1uL |
| 4 | SpeI | 1uL | | |
| 5 | XbaI | | 1uL | |
| 6 | PstI | | 1uL | 1uL |
| 7 | ddH2O | 29uL | 36.1uL | 23uL |

## Procedure

Digestion

1. Three mixes was made containing 500 ng of Bba_K608006 plasmid, Bba_K592009(CDS) and Vector: pSBIK3 each followed by the addition of ddH2O.
2. To each mix, 5 µL of 10x reaction buffer for restriction enzymes were added.
3. 1 µL each of the appropriate restriction enzymes were added to give a final volume of 50 µL.
4. The tubes were taped to mix.
5. The tubes were incubated at 37°C for 30 min.
6. Heat-inactivatation of the enzymes was done by incubating at 80°C for 20 min.

# Lab #12 - Agarose gel electrophoresis (19-6-19) (Irina + Gustav)

## Aim:

To separate and analyse nucleic acid samples under non- denaturing conditions

## *Method:*

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 18 for components and amounts mixed for agarose gel

**Table 18**

| | Component | amount | C |
|---|---|---|---|
| 1 | Agarose | 0.5g | |
| 2 | 1 x TBE | 50mL | |
| 3 | sybrSafe | 5uL | |

## Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was weighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel solution was poured into the gel tray and air bubbles were removed with a pipette tip.
7. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

## Running the gel

1. the gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. Ther DNA samples (~0.2 µg)were mix with loading dye (table 18) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply. and the gel was run at 100 V for 30–60 min.
8. After the run, the gel bands were visualized under UV and a picture was taken (figure ...).

See table 19 for components and amounts used for running mixture:

**Table 19**

| | Components | amounts |
|---|---|---|
| 1 | Loading dye mix | 4uL |
| 2 | DNA sample | 20uL |

5 uL of 2-Log DNA Ladder (0.1–10.0 kb) (BioLabs) were used as a ladder.

Irina's gel can be seen in figure 20.

Image from iOS_ gel12.jpg

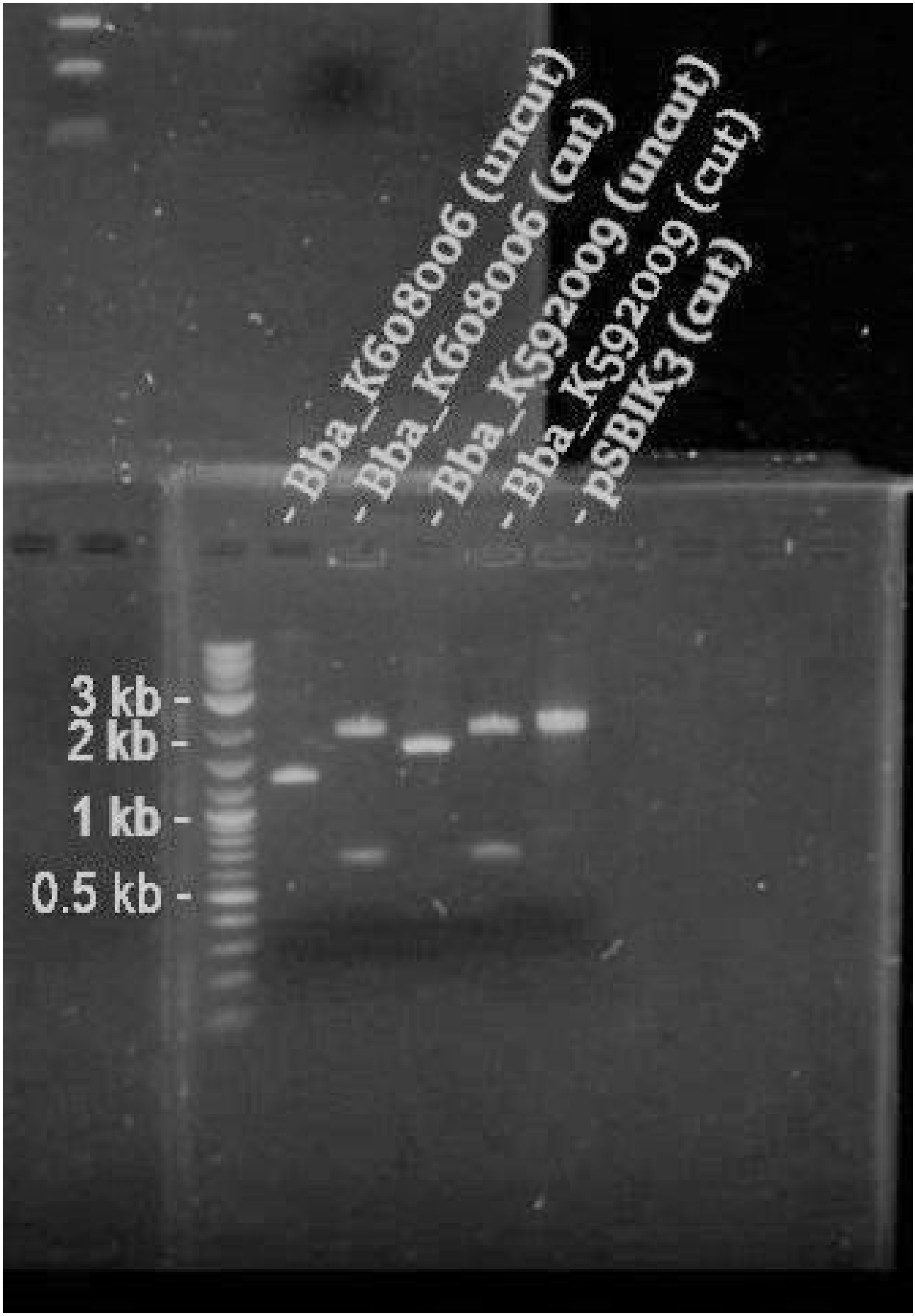


Figure 20. Agarose gel for 3A assembly components (Irina)

Due to poor results, Gustav will repeat the procedure described in lab 12
Results are shown below in figure 21 with a 1 kb ladder:

image.png

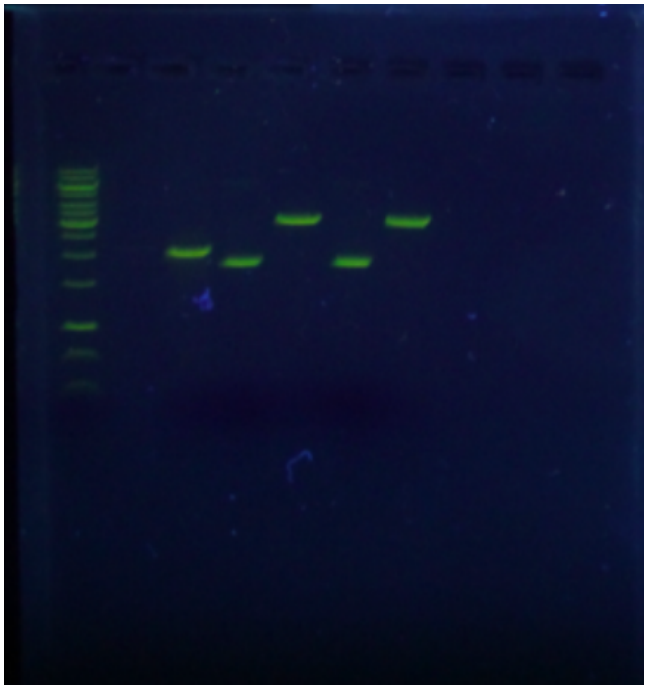


Figure 21. Ladder shown to the very left, from left to right: Back bone, control for PST+XbaI, PST+XbaI cut, control EcoRI+SpeI, EcoRI+SpeI cut

MONDAY, 24/6/2019

## Lab #13 - BioBrick 3A Assembly continued - Ligation and transformation (19-6-24) (Irina)

### Aim:

Ligation of BBa_K608006, Bba_K592009 and pSBIK3 BioBricks™ followed by transformation on Kanamycin LB agar plates.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Note: gel analysis was done on 20/6.

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Components and amounts for ligation can be seen in table 22.

**Table 22**

| | Components | Bba_K608006 (amount) | Bba_K592009 (amount) | pSBIK3 (amount) |
|---|---|---|---|---|
| 1 | Digested sample | 2uL | 2uL | 2uL |
| 2 | T4 10x Reaction buffer | 2uL | 2uL | 2uL |
| 3 | T4 DNA ligase | 1uL | 1uL | 1uL |
| 4 | ddH2O | 11uL | 11uL | 11uL |

### Transformation

Components and amounts can be seen in table 23 for transformation reaction.

**Table 23**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - |
| 2 | DH5α competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

## Lab #15 - BioBrick 3A Assembly - Digestion (19-6-24) (Erik Palm)

### Aim:

Plasmid DNA prep of promotor pSB1C3 BBa_K608006 and gene BBa_K500003 (glucose oxidase) into vector (pSBIK3)

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``
Table 24 shows the initial concentrations of the DNA plasmids:

**Table 24**

| | A | B |
|---|---|---|
| 1 | Promoter (BBa_K608006) Concentration | 65.6 ng/ul |
| 2 | Glucose Oxidase (PSBIC3 BBa_ K500003) Concentration | 94.0 ng/ul |
| 3 | Destination Vector / Backbone (pSBIK3) Concentration | 25 ng/ul |

Table 25 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion.

**Table 25**

| | Component | BBU-KIOSIonb | BBU-K500003 | Vector: pSBIK3 |
|---|---|---|---|---|
| 1 | 500 ng DNA | 7.6uL | 5,3uL | 20uL |
| 2 | 10 x Reaction buffer | 5uL | 5uL | 5ul |
| 3 | EcoRI | 1uL | ------ | 1uL |
| 4 | SpeI | 1uL | ----- | ----- |
| 5 | XbaI | ---- | 1uL | ------ |
| 6 | PstI | ---- | 1uL | 1uL |
| 7 | ddH2O | 35,4 | 37,7 uL | 23uL |

Calculations
Amount of ml needed for digestion
psBIC3 -BBU- KIOSIonb 500ng x 1ul/ 65.6 ng= 7,6 ul
psBIC3- K500003 500ng x 1ul/94.0ng = 5.3 ul
pSBIK3 backbone 500 ng x 1ul/25 ng =20 ul

## Lab #16 - Preparation of 1x TBE solution through Dilution of 1x TBE solution - (19-6-24) (Erik Palm)

Dilution of 10xTBE to make 1 liter of 1x TBE

900 ml of ddH20 added to 100ml of 10x TBE

## Lab #17 - Agarose Gel Electrophoresis - (19-6-24) (Erik Palm)

### Aim:

To separate and analyze nucleic acid samples under non-denaturing conditions

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 18 for components and amounts mixed for agarose gel

Instead of composing 150 mL of gel, 50 mL of gel was used instead. Table 26 shows components in the agarose gel

## Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was wSeighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

**Table 26**

| | A | B |
|---|---|---|
| 1 | Amount | Component |
| 2 | 0,5 g | agarose |
| 3 | 50ml | 1x TBE |
| 4 | 5ul | Sybr Safe |

## Running the gel

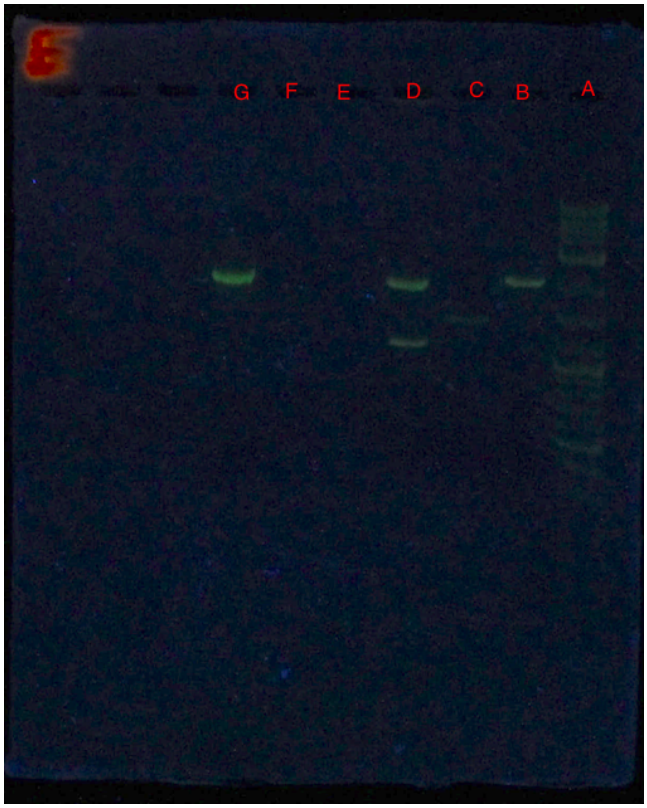
1. The gel tray  was placed into the buffer chamber and the comb was removed carefully.
2.  1x TBE buffer was added until the gel was completely covered.
3. The DNA samples (~0.2 µg)were  mix with loading dye (table 18) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply. and  the gel was run at **120 V** for **80 min.**
8. After the run, the gel bands were visualized under UV and a picture was taken (figure ...).

Table 27 below shows the components of the loading solutions placed in the wells, well number, and calculations for Erik Palm's gel analysis.

**Table 27**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed | Size (kBp) according to iGEM |
| 2 | A | Ladder (2-log) | 5 ul | -- | |
| 3 | B | Promotor (BBa_K608006) | 20 ul<br>4ul loading dye | | 56 |
| 4 | C | Uncut promotor (BBa_K608006) | 5.7 ul of sample 14,3 ul of H20 4 ul loading dye | 200 ngx 1ul/ 35.1 ng= 5.7 ul of sample | |
| 5 | D | Glucose oxidase (BBa_K500003) | 20 ul sample<br>4 ul loading dye | | 1677 |
| 6 | E | Uncut Glucose oxidase (BBa_K500003) | ------- | | |
| 7 | F | Backbone pSB1K3 | 20 ul sample<br>4ul loading dye | | 2204 |
| 8 | G | Uncut backbone pSB1KR | 8 ul sample<br>12 ul H20<br>4 ul loading dye | 200ng x 1ul /25ng =8 ul sample | |

Erik Palm Digestion Analysis 25.06.2019.png




Analysis of gel:
-Lane C had a lower molecular weight because of supercoiling. Not linearized plasmid.
-Lane B Can't see promotor because it was so small and ran off the gel.
-Lane E was left open because glucose oxidase gene ran out. This means that there is no control for the successful digestion of this plasmid.
-Lane F: Possible reason for it being empty is that their no actual sample in the tube. Therefore digestion was recommended again in order to proceed to the next step so that the promotor and gene have a backbone that they can be placed in.
-Erik Palm left the digestion samples at room temperature for more than an hour after gel analysis was done. When the mistake was discovered, sampled were placed in freezer. Since it is dDNA, samples should function normally, but possible source of future error.

TUESDAY, 25/6/2019

## Lab #18 - Preparation of LB media and addition of Chloramphenicol (19-6-25) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g

m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 28 for components and amounts used to prepare 600 mL of LB.

**Table 28**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Chloramphenicol was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"LB + Cm Irina 25/6"*

## Lab #19 - Prepare agar plates with addition of antibiotics (19-6-25) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Chloramphenicol 25 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Chloramphenicol (25 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 29 for components and amounts used to prepare 600 mL of LB.

**Table 29**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Chloramphenicol (25 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"June LB + Cm"*

## Lab #20 - Transformation (19-6-25) (Irina)

### Aim:

Transformation of Bba_K500001 , BBa_K00000 annd Bba_K500003, J04500 and Irinas Competent cells + J04500 on Chlormaphenicol LB agar plates.

### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-114;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Transformation Protocols;

Components and amounts can be seen in table 30 for transformation reactions.

**Table 30**

| | Components | MnP - K500001 | LiP - K500000 | GLOX - K500003 | Lac -J04500 | Irinas competent cells + J04500 | (-) control |
|---|---|---|---|---|---|---|---|
| 1 | Ligation reaction mixture | 1uL | 1uL | 1uL | 1uL | 1uL * | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50uL | 50uL | 50uL (Irinas) | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950uL | 950uL | 950uL | 950uL |
| 4 | ddH2O | - | - | - | - | - | 1uL |

* Might have been 10 uL added

## Lab #21 - Overnight cultures on Transformations from 19-06-24 (19-6-25) (Irina)

### Aim:

O/N cultures to have ready for miniprep the following day.

### Method:

### Lab #21 - Transformation+ligation (25-06-19) (Gustav)

Aim: The aim was to transform and ligate the promoter (k608006), CDS (k500003) and the plasmid backbone (psb1k3.m1) into competent E.coli DH5α and plate onto agar plates.

Method:
The methods described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm concerning protocols 6 and 3 were followed (OBS only the ligation part of protocol 3 was followed). The only deviation from the decribed methods was that when performing the transformation mentioned in protocol 6, 10 microliters of the respective additive was used instead of 5 microliters.

The E. Coli strain used was DH5α.
The ligation was done by adding the following: (all units are in microliters):

2 Promoter
2 backbone
2 CDC
11 ddH2O
2 ligation buffer
1 ligase

In total 4 plates were produced: 1 negative control, 1 ligation transformation, 2 positive controls (1 10% and 1 "regular").
Both of the positive controls were grown on Chloramphenicol media
The ligation and negative controls were grown on kanamycin media

26/6/19: Due to poor quality of competent cells, a new transformation has to be done using another batch of competent E. Coli cells.

Pictures are shown below just above entry #27



## Lab #22 - BioBrick 3A Assembly - Digestion (19-6-25) (Erik Palm)

Aim:

Plasmid DNA prep of only thebackbone (pSB1A3) to make up for the mistake in digestion shown in the gel analysis.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``
Table 31 shows the compents placed in eppendorf tube.

Changes in protocol: Erik Palm ran out of PSB1K3 as was done in the Lab #25. Therefore, the backbone was switched to pSB1A3 incstead (ampicillin resistance).

**Table31**

| | Component | Vector: pSB1A3 |
|---|---|---|
| 1 | 500 ng DNA | 20uL |
| 2 | 10 x Reaction buffer (2.1 New England Buffer) | 5ul |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ----- |
| 5 | XbaI | ------ |
| 6 | PstI | 1uL |
| 7 | ddH2O | 23uL |

Calculations:
500ng x 1ul/25ng= 20 ul pSB1A3

# Lab #23 - BioBrick 3A Assembly continued - Ligation and transformation (19-6-25) (Erik)

## Aim:

Ligation of BBa_K500003, BBa_K608006 and pSB1A3 BioBricks™ followed by transformation on Ampicillin LB agar plates.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Ampicillin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Components and amounts for ligation can be seen in table 32.

**Table32**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500003 | 2uL |
| 2 | Digested BBa_K608006 | 2uL |
| 3 | Digested pSB1AC | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

Changed in Protocol: We did not heat-inactivate DNA ligase.

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

**Table33**

| | Components | Transformation sample | (-) control | (+) control |
|---|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50 uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950 uL |
| 4 | ddH2O | - | 5uL | - |
| 5 | BBa_J04450 | - | - | 5ul |

Positive Control: We used BBa_J04450 pSB1A3
Negative Control: Water

Results:
All of the plates (negative control, positive control and ligation reaction mixture) contained bacterial colonies. Since the negative control had bacterial growth, contamination is expected. Transformation will be done again using the left over ligation solution.

WEDNESDAY, 26/6/2019

# Lab #24 - Overnight cultures on Transformations from 19-06-25 (19-6-26) (Irina)

### Aim:

Prepare O/N cultures of MnP (Bba_K500001), LiP (BBa_K00000), GLOX (Bba_K500003), Lac (J04500) and Irinas' competent cells + Lac J04500 for in 6 mL of LB + Cm miniPrep the 27/6 and Re-streak of same colonies on LB - Cm plates.

### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 114; Step 14.
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Transformation Protocols;

### Procedure:

One colony per plate was transferred to 15 mL Falcon tubes containing 6 mL of LB + Cm medium. The same colony was chosen for re-streaking on LB + Cm plates. Samples were stored in the 37 °C room O/N. The original plates were stored in the refrigerator.

O/N Cultures made 25/6 where discarded (Bba_K608006, Bba_K592009, pSBIK3), as no colors had been seen on the plates or in the cultures.

## Lab #25 - Competent cell count (19-6-26) (Irina)

Transformation efficiency:
Nr of colonies on plate: 308
J04500 - 10pg/uL
Amount used 1 or 10 uL, see table 30.
If 1 ul used: 308/ (10 x $10^{-6}$) = 3.08 x $10^{7}$, but it is more likely 10 uL were used which would give 3,08 x $10^{6}$.

## Lab #26 - Gel Analysis of Restriction Enzyme (19-6-26) (Erik, Vanja and Johanna)

### Aim: To check if the digested backbone (pSB1A3) was actually digested or not.

### Method:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

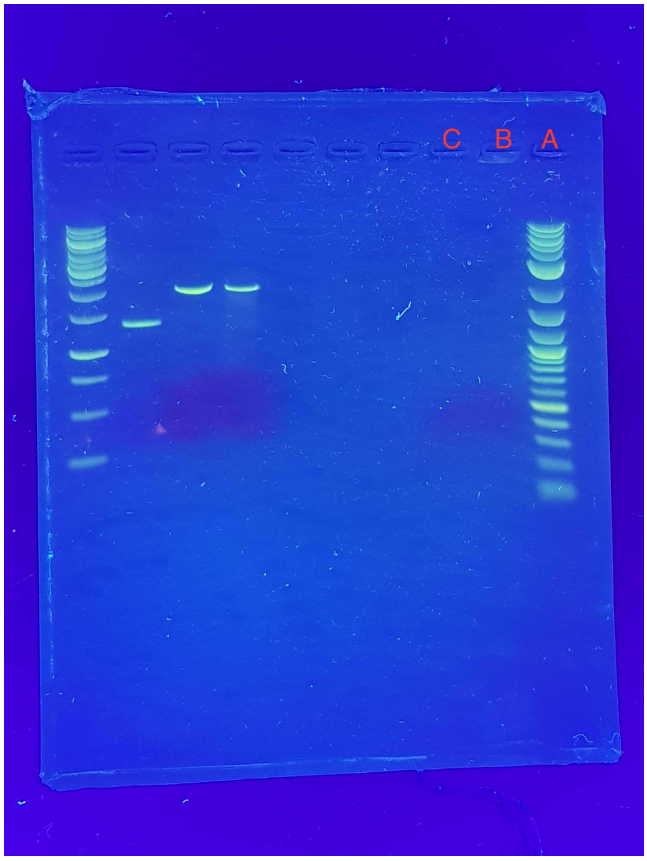
-Johanna and Vanja prepared the gel. Erik simply put in his cut and uncut backbone (pSB1A3)
-When putting in the uncut backbone, there was not enough pSB1A3. Instead of 8 ul, there was 4 ul. The total loaded amount of solution that was loaded into the well was 16 ul.

Table 34 below shows the components of the loading solutions placed in the wells, well number, and calculations for Erik Palm's gel analysis. The other well was part of another experiment

**Table34**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | Ladder (2-log) | 5 ul | -- |
| 3 | B | digested backbone (pSB1A3) | 20 ul<br>4ul loading dye | |
| 4 | C | uncut backbone (pSB1A3) | 4 ul of sample<br>12 ul of H20<br>4 ul loading dye | 200 ngx 1ul/ 25ng ng= 8 ul of sample |

26-06-2019 Gel Analysis backbone.jpg




-Both wells B and C with the cut and uncut backbone were not present in the gel.
-Given the modification to well B, it is possible that that could explain why no bands are present. However, since lane C also has not bands, it could be that pSB1A3 sample is not functioning. It is quite old, from 2014. This will be confirmed or denied if the negative control and the experimental plate is successful.

## Lab #27 - BioBrick 3A Assembly continued - Transformation (27-6-25) (Erik)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Transformation of Competent E.coli cells

Components and amounts can be seen in table 33 for transformation reaction.

**Table35**

| | Components | Transformation sample | (-) control | (+) control |
|---|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50 uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950 uL |
| 4 | ddH2O | - | 5uL | - |
| 5 | BBa_J04450 pSB1C3 | - | - | 5ul |

Components and amounts can be seen in table 33 for transformation reaction.
Positive Control: We used BBa_J04450 psB1C3
Negative Control: Water

There was no pSB1A3 left, which is what is used in the transformation sample, which is why pSB1C3 is used instead. As a result, Erik plated two LB + Cm plates for the positive control, while the negative control and transformation sample was plated on LB + Amp

During Step 6 of the procedure, there was some confusion as to what backbone Erik was supposed to be using. The samples were thus on ice for 15 min instead of 5min.

Prediction for results: if the the gel analysis and experimental results match, the negative control and the sample must be negative, and the positive control should be positive. The transformation sample should be negative because there is no function backbone, since none appeared in the gel.

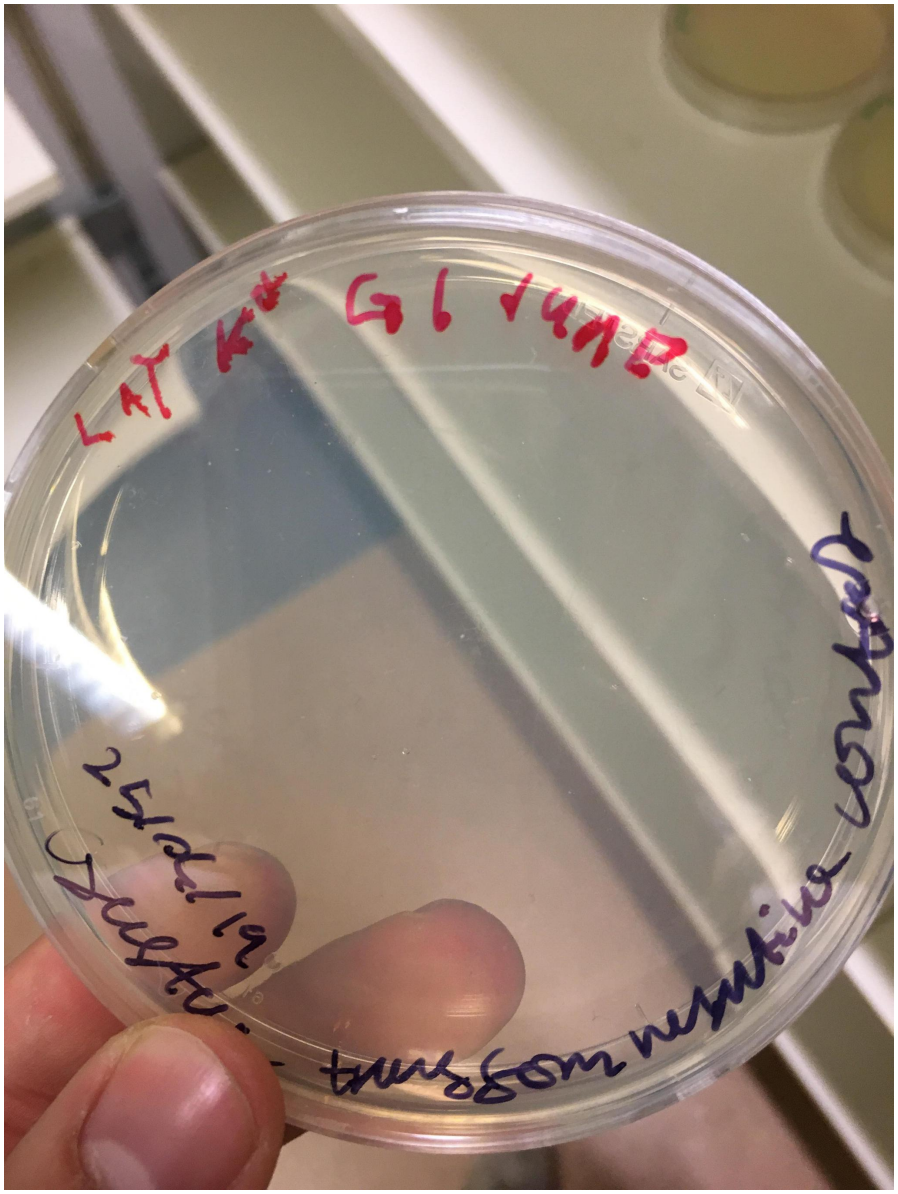
Results: Analysis of plating

All of the plates are postive. The destination was present, making it unclear what happened in the gel analysis. Previous conjectures about how old the sample was must not have been true.

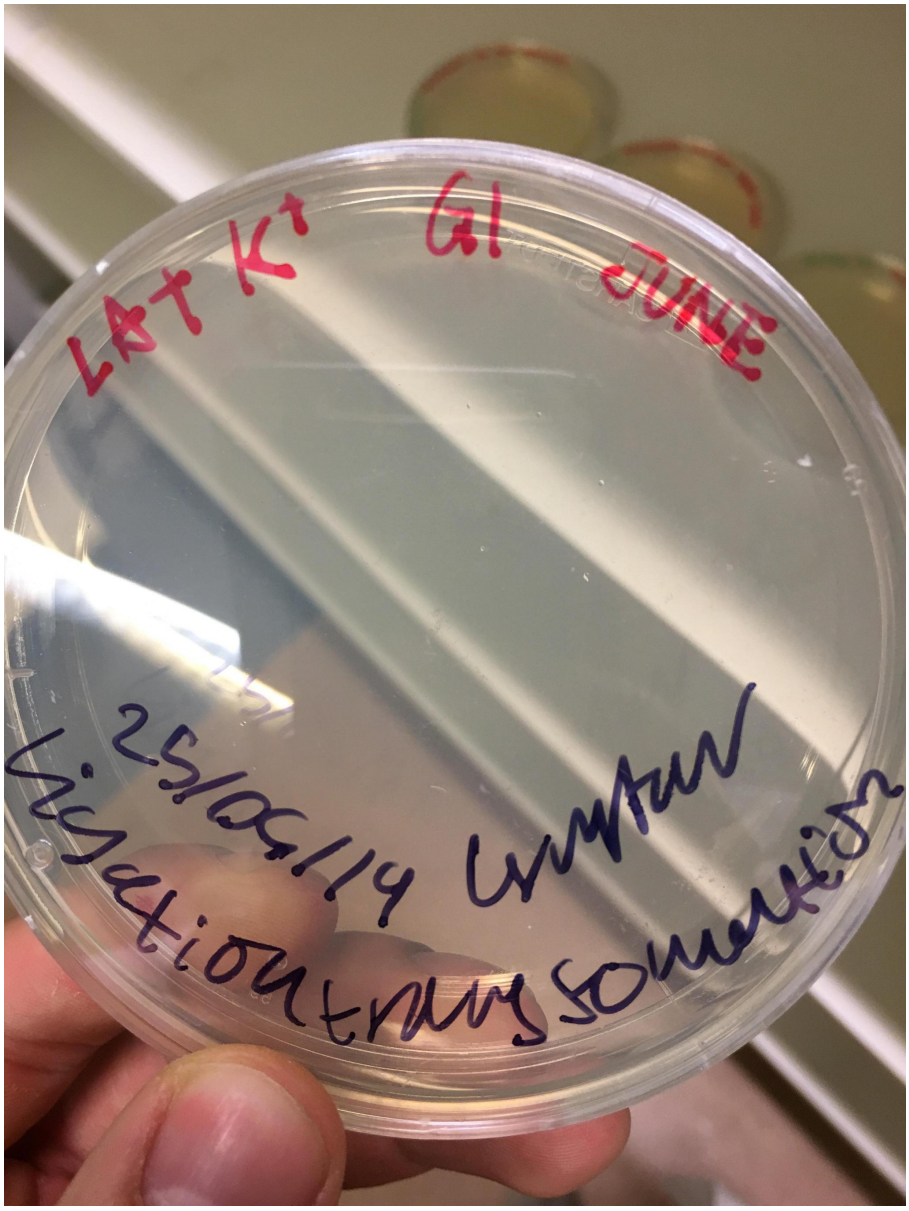
65969539_630288457467569_6380744955584839680_n.pdf

These below are Gustavs, not sure why they are on my Lab transformaiton

65204585_2440422172858938_5685583912948989952_n.jpg



65079508_911057869253882_7529580692162740224_n1.jpg



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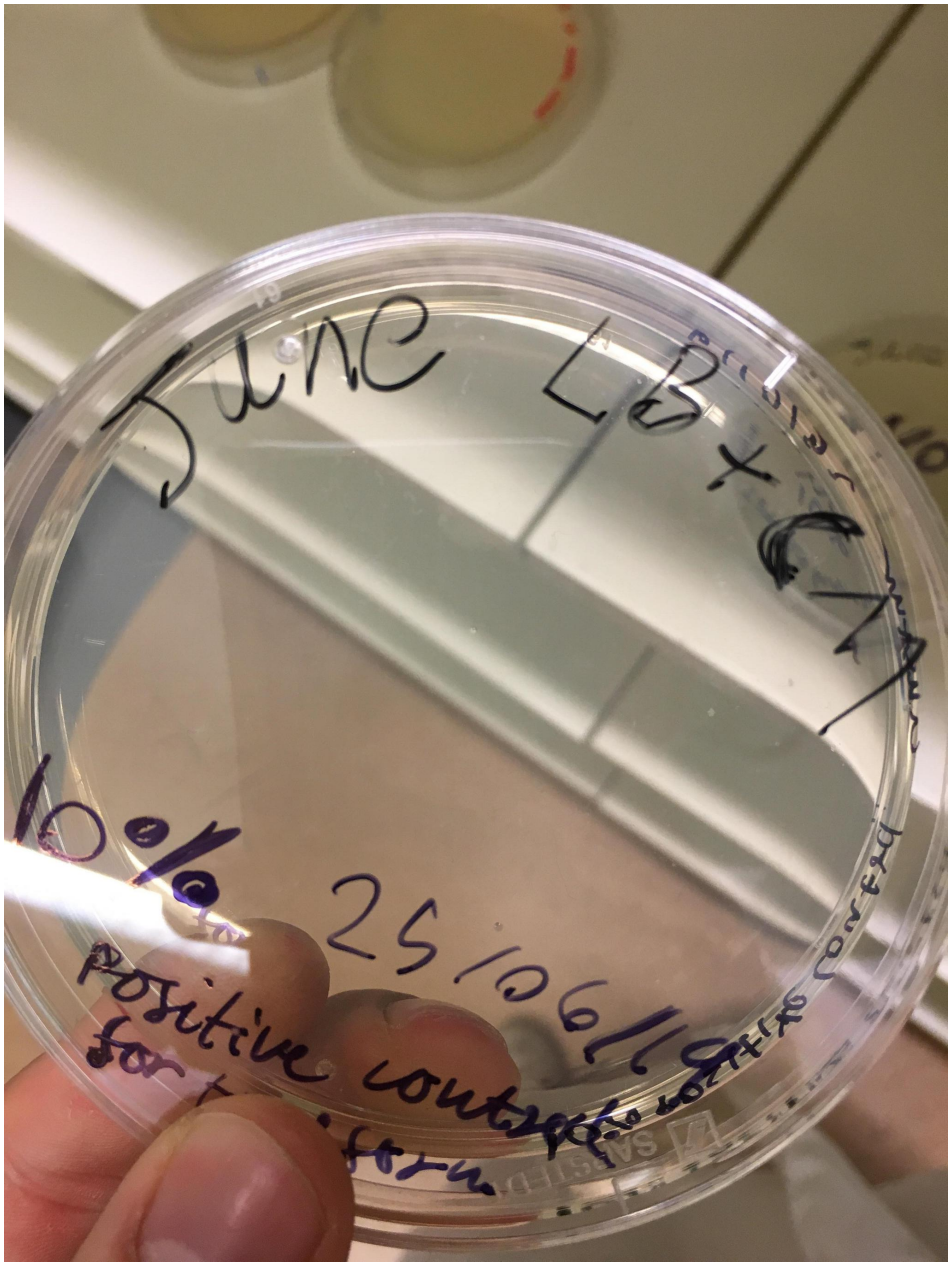
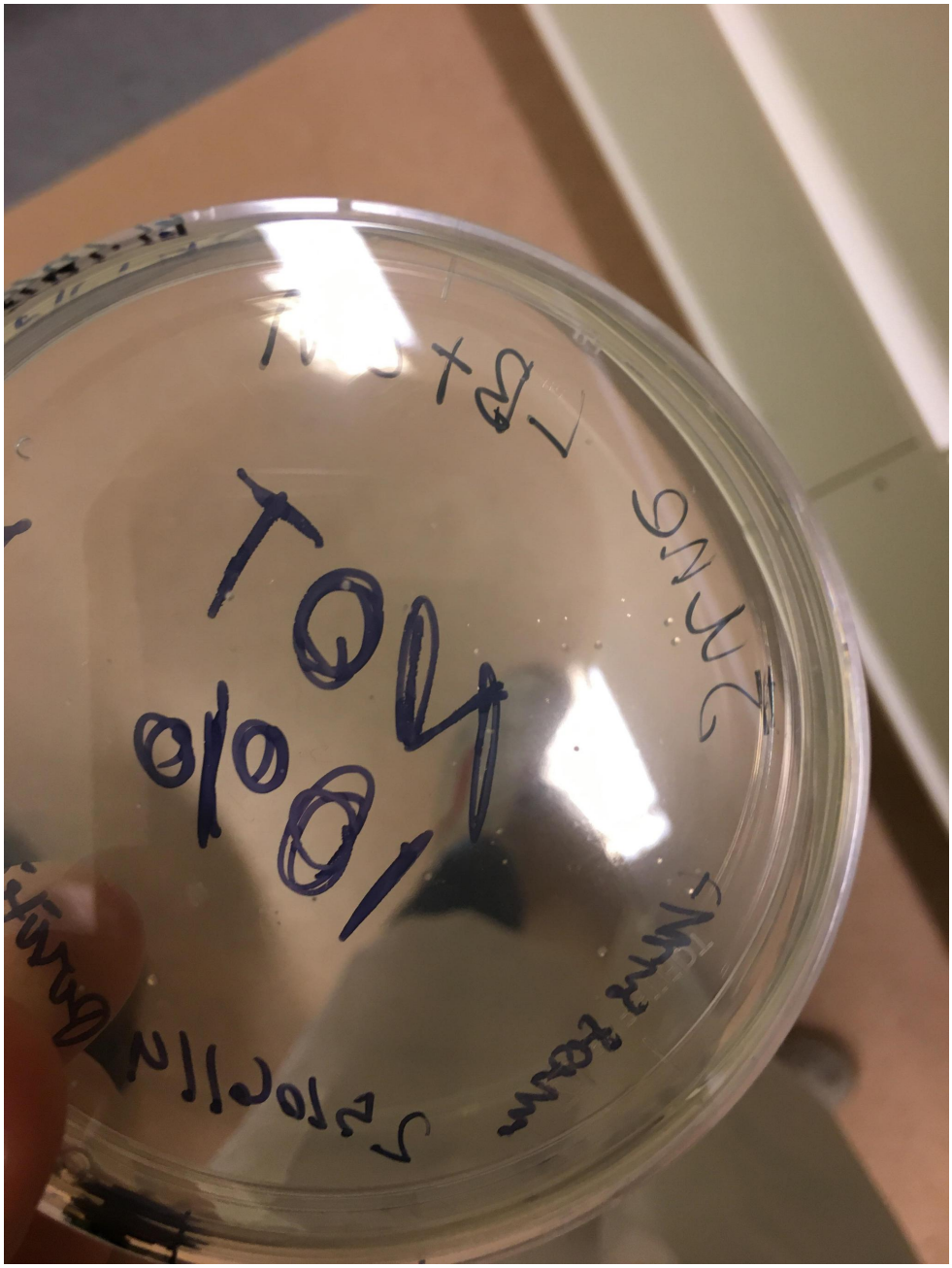


65744568_694188691015425_7994566676400046080_n3.jpg



Lab

## Lab #28 26-6-19 (Gustav)

Aim: To repeat #22 procedure in order to tranform cells

Method: The method, as with entry #21, is specified in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm, Protocol 6. The following deveations were made from said protocol:

- Step 3 10 microliters of the ligation mixture, ddH2O and of the positive cotrol was used due to reccomendation of lab leader.
- Step 6 waiting time was extended from 5 mins to 20 mins due to SOB buffer not being pre-heated to 37°C.
- Step 7 the SOB buffer was not at 37°C when applied to the cell mixture.

- Instead of plating a total of 4 agar plates, 5 were prepard. 3 K+ plates and 2 C+ plates. The extra K+ plate was used to cross reference the competence of Gus cells to another lab partners to see if a) the ligation mixture was working b) see if Gus cells were valid for future usage. The two C+ plates were used for positive control, 1 10% and one regular.

The plasmid used for the positive control was PSB1C3 BBa_J04450.

There was growth on the positive control of the transformation done 25-6-19. Above this entry there is a picture showing the plate. It is noted as "NOT 10%". The compentent efficiency of ths plate was 31*10^4. The calculation went as follows:

- Stock solution used was 10 pg/ul
- 10 ul from stock was used
- This results in 100pg of DNA being present
- 31 colonies were counted
- 31/100pg = 31*10^4.

THURSDAY, 27/6/2019

## Lab #29 - Preparation of competent *E.coli* BL 21 (DE3) cells using $CaCl_2$ (19-6-27) (Irina + Erik)

### Aim:

To increase the competence of *E. coli* BL 21 (DE3) cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Calculations for $CaCl_2$ solutions can be seen in table 35.

**Table 35**

| | A | 1M CaCl2 | 50% Glycerol stock | ddH2O | TOTAL volume (ml) |
|---|---|---|---|---|---|
| 1 | 0.1. M CaCl2 (15ml) | 2 ml | / | 18ml | 20ml |
| 2 | 0.1 M CaCl2 + 20% glycerol (3ml) | 0.3ml | 1.2ml | 1.5ml | 3ml |

## Lab #30 - Plasmid miniprep for pSB1C3_MnP, pSB1C3_LiP, pSB1C3_GLOX, pSB1C3_Lac, continued from lab#24 (Jinwen)

### Aim:

to get enough plasmid containing the genes of peroxidases and oxidases and inducible promoter as above for 3A assembly to build constructs: pSB1K/A3_J04500_K500001(Lac+RBS+MnP), pSB1K/A3_J04500_K500000(Lac+RBS+LiP), pSB1K/A3_J04500_K500003(Lac+RBS+GLOX)

**Also to get enough gene materials for PCR, in order to add overhang on these genes for Gibson assembly to assemble these genes with shuttle vector.**

### Methods:

- Plasmid miniprep kit instruction of **GeneJET Plasmid Miniprep Kit (K0502)**

### Results:

- *Input conc. report*

## Lab #31 - Digestion and gel analysis for pSB1C3_MnP (Bba_K500001), LiP (BBa_K00000), GLOX (Bba_K500003), Lac (J04500) (Jinwen)

### Aim:

To prepare for the 3A assembly for building constructs: pSB1K/A3_J04500_K500001, pSB1K/A3_J04500_K500000, pSB1K/A3_J04500_K500003

### Methods:

3A assembly-Digestion and ligation

### Results:

*Insert gel picuture*

### Discussion:

Digestion for backbone has not been done, due to the lack of backbone. Find a pSB1K/A3_RFP would help. After getting done with digesting the backbone, then the Ligation part can be continued.

After ligation, we can transform ligation mixture into DH5a competent cells. If we are lucky, we can get some colony on our ligation plate, and then re-streak the colony, and grow O/N culture, plasmid miniprep and digestion to examine whether we get the right construct as we designed. If we are lucky, we get the right construct, then we can transform constructs (plasmid miniprep result) into BL21, incubated with **IPTG** to get overexpression, and do ***SDS-PAGE, Coomassie Blue Staining*** to compare the difference of Induced expression and uninduced expression.

MONDAY, 1/7/2019

## Lab #32 3A Digestion with Jinwens samples 1/7/19 (Gustav and Lilli)

Due to uncertanty in labeling of DNA samples, 3A assembly will be postoned until confirmation of samples identification.

The procedure detailed in lab #28 concluded with cell growth on the agar plate with Irenas compentetne cells and Gustavs ligated plasmids. These colonies are not blue so restreaking will be preformed as well as O/N growth to confirm that these cells contain the correct construct. Colony growth was also witnessed on a previous transformed plate, this colony will also be restreaked. (Gustav)

Method: The method followed for restreaking is described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm Protocol 7, Alternativ/Method 1.

After futher discussion, 3A assembly is to be carried out.

Due to lack of backbone, the backbone digestion will not be carried out.
The 3A assembly digestion is described in lab #15. The promoter used was: BBa_K50003, the gene used was that for glyoxal oxidase.
The following calculations were made to recieve a 500ng DNA solution:

| Promoter: | Gene (glyoxal oxidase): |
|---|---|
| 500/83.1 = 6 microL | 500/94.5 = 5.3 microL |
| 5 miroL buffer | 5 microL buffer |
| 1 microL EcoRI | 1 microL XbaI |
| 1 microL SpeI | 1 microL PstI |
| 37 microL ddH2O | 37.7 microL ddH2O |

After gel analysis we concluded that the correct digestion had occured. The gel is displayed below:

image.png


From left to right: Ladder (1kb generuler), digested promoter, undigested promoter, digested GLOX, undigested GLOX

## Lab #33 - Preparation of LB media and addition of Kanamycin (19-7-01) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 36 for components and amounts used to prepare 600 mL of LB.

**Table 36**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Kanamycin was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July LB + Kan"*

## Lab #34 - Prepare agar plates with addition of antibiotics (19-7-01) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Kanamycin 50 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Kanamycin (50 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 37 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 37**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Kanamycin (50 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Kan"*

## Lab #35 - Casting Agarose Gels - (19-7-01) (Irina)

### Aim:

To separate and analyze nucleic acid samples under non-denaturing conditions

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 38 for components and amounts mixed for agarose gel

Instead of composing 150 mL of gel, 50 mL of gel was used instead. Table 26 shows components in the agarose gel

### Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was wSeighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

**Table 38**

| | A | B |
|---|---|---|
| 1 | Amount | Component |
| 2 | 0,5 g | agarose |
| 3 | 50ml | 1x TBE |
| 4 | 5ul | Sybr Safe |

Four gels were cast, one of them where stored in the cold room at 4 °C labelled "1% Agarose gel 1/7"

## Lab #36 - BioBrick 3A Assembly - Digestion (19-7-01) (Erik Palm + Jenny Eriksson)

### Aim:

Digestion of the backbone (pSB1K3), lac operator and BBa_K500001 and gel analysis of the results.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``

Changes in protocol: We ran out of destination vector pSB1K3. Therefore used digested pSB1K3 made by Irina 19-06-20 instead for eventual gel analysis and ligation. Thus only two digestion mixtuers were prepared, the promotor and the MnP gene.

Table 39 shows amounts used in digestion mixture.

**Table 39**

| | Component | Amount of component | ddH20 | Reaction Buffer 10x | EcoRI | Spe1 | Xba1 | Pst1 |
|---|---|---|---|---|---|---|---|---|
| 1 | Lac promotor | 6.02 ul | 37 ul | 5 ul | 1 ul | 1 ul | - | - |
| 2 | BBa_K500001 | 4.07 ul | 39 ul | 5 ul | - | - | 1 ul | 1 ul |

Calculations to calculate amount of solution needed for digestion:
500ng x 1ul/83.1ng= 6.02 ul Lac promotor
500ng x 1ul/122.8ng = 4.07 ul BBa_K500001

## Lab #37 - Agarose gel electrophoresis (1-7-19) (Irina + Erik+Jenny)

### Aim:

To ascertain whether cuts of the of the miniprep sample of the lac promotor and the gene (MnP) were successful or not.

### Method:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

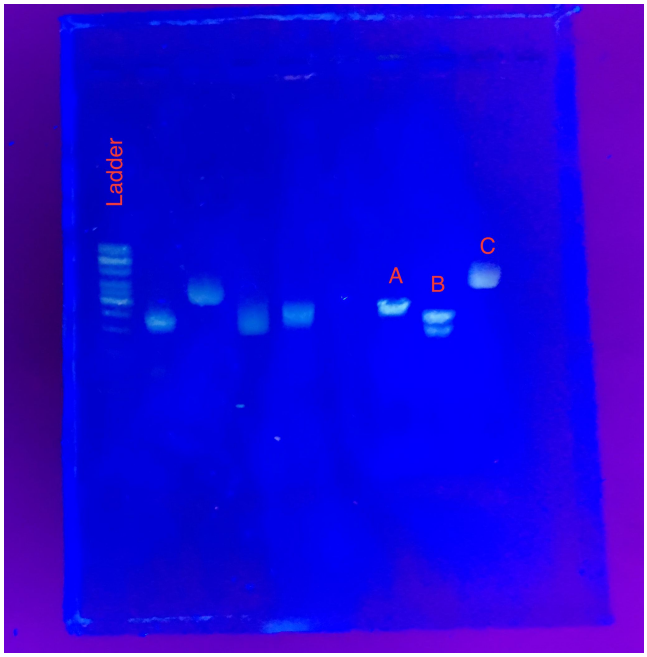
Analytical Digestion and Agarose Gel Electrophoresis

-Agarose gel prepared by Irina.
-See agarose gel preparation for Irina (Lab 35), but again, it was 50 mL plates, not 150mL
-We used a new gel electrophorator at 120 V for 30 min. 120 V was used since the other gel electrophorator took such a long time to run. However, this may not be necessary for the new ge electrophorator, which may have caused the strangely compressed ladder??

**Table40**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | digested lac promotor | 20 ul<br>4ul loading dye | -- |
| 3 | B | the digested gene MnP (BBa_K500001 ) | 20 ul<br>4ul loading dye | |
| 4 | C | undigested gene MnP (BBa_K500001 ) | 1,63 ul of sample<br>18.7 ul of H20<br>4 ul loading dye | 200 ngx 1ul/ 122,8ng = 1,63ul of sample |

01:07:2019EPJAgarosGelDigestionMnP.jpg




From left to right:
Ladder: Generuler 1 kb (ladder) (prepared by Gustav)
Four samples from Gustav Ahlstroms group
One empty well
A: the cut Lac promotor
B: the cut BBa_K500001
C: uncut BBa_K500001.

Observations:
-In terms of the number of bands that we see, it is consistant to what we expect. A, which contains the promotor (only 31 bp) has one bond and the promotor itself is so small it probably has run off the gel or is difficult to see.

-Well B has two cuts, which makes sense since the MnP is 1134 bp and the backbone is 2070 bp. Two bands should be seen.
-Well C has one cut, which reflects the fact that it is a whole plasmid. However, it is a little strange that whole plasmid did not super coil and travel farthern than the linearized DNA fragments of B and C.
-Also the ladder is a weird looking, it looks compressed and the sizes do not match up with the expected sizes.

Conclusions:
-We decided to continue with ligation and transformation despite the weird looking ladder. Adjust voltage for next time.

# Lab #38 -BioBrick 3A Assembly continued - Ligation and transformation (19-7-1) (Irina + Erik)

## Aim:

Ligation of BBa_K500003, lac promotor and pSB1K3 BioBricks™ followed by transformation on Kanamycin LB agar plates.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Ampicillin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

4 ligations were done at the same time.

1. Ligations of the digestions done by Jenny and Erik in lab 36
2. Ligation of Jin Wen's digestion MnP (sample 1) in Lab 31 (marked as a 1 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 2) in Lab 31 (marked as a 2 on the eppendorf tube)
4. Ligation of Jin Wen's digestion MnP (sample 3) in Lab 31 (marked as a 3 on the eppendorf tube)

Components and amounts for ligation can be seen in table 41 (1 above), table 43, (2 above), table 44 (3 above), table 45 (4 above).

**Table41**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (E och J) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table42**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW1) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table43**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW2) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table44**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW3) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Four Transformation were done at the same time, with 1 negative control

1. Erik 0: Erik's ligation mixture
2. JW 1: JIn Wens ligation mixture from eppendorf tube 1
3. JW 2: Jin wens ligation mixture from tube 2
4. JW 3: Jin Wen's ligation mixture from tube 3

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones
2. Certains cells had closer to 150 ul left for resuspension instead of 100ul. Might make calculation of transformation more difficult
3. Mixture of prepared compentent cells done by Jin Wen and Erik, have no idea which are which.

**Table45**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (Erik,I) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

**Table46**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW1 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table47**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW2 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table48**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW3 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

Negative Control: Water
Results:

-All agar plates, including the negative control, have zero colonies. Thus transformation failed.
-Other team memers speculate it is because of a faulty backbone. Digestion of a new backbone has already begun.

2019_02_2019_MnP_Transformation_EP.pdf

2019_02_07_MnP_EP_Transformation_diluted .pdf

TUESDAY, 2/7/2019

***Lab #39 2/7/19 3A Digest of Backbone (pSB1C3 backbone) (Gustav + Lilli)***

Aim: Due to lack of Kanamycin resistant backbone, a new backbone resistant towards Chloramphenicol will be digested and used for ligation.
Method: The method is descirbed in lab #15.

Notes: The restreaking done yesterday (1/7/19) resulted in some cell growth. These cells will be grown overnight to later be digested to double check that the correct construct has been transformed into the cells. A picture of these results is displayed below:

image.png

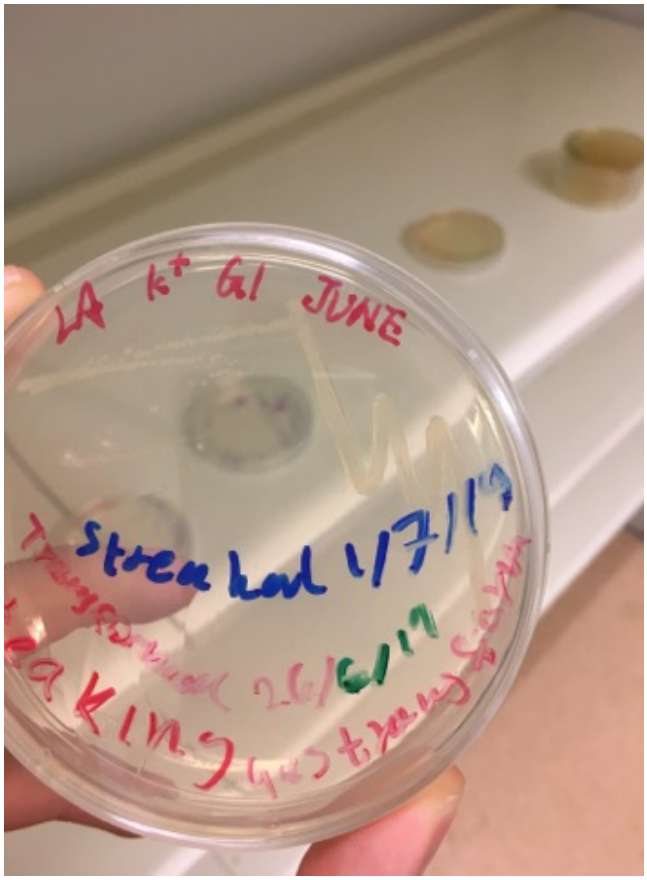
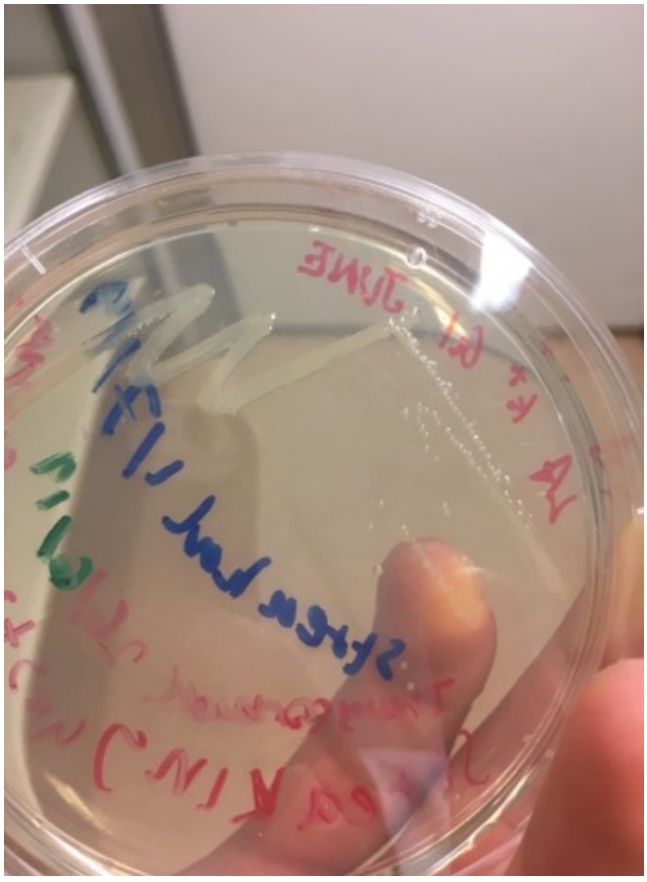


image.png



The digestion of the Chloramphenicol was unsucessful. No bands whatsoever were present. This means that a new backbone will be used, one that codes for ampicilin resistance. The backbone is designated as: pSB1A3.

Transformation of these new ligated plasmids and ligation was done. These protocols have been referenced before in previous labs. More specifically lab #21.  No deveations were made when following the ligation protocol.

## Lab #39 - BioBrick 3A Assembly and gel analysis (19-7-02) (Irina + Jenny)

### Aim:

Plasmid DNA prep of pSB1A3 (2016 iGEM team) will first be made, as pSB1K3 does not seem to work. We will try to do 3A Assembly with pSB1A3 instead together with Jinwens sampels from Lab #31.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;

Calculations for digestion mixture total volume 20 uL for pSB1A3:
V = required mass/intitial concetration
V =  500ng/25 ng/uL = 20 uL.

For components and amounts for Digestion mixure, see tabel 49.

**Table 49**

| | Component | pSB1A3 |
|---|---|---|
| 1 | 500 ng DNA | 20uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | PstI | 1uL |
| 5 | ddH2O | 23uL |

### Procedure

Digestion

1. One mix was made containing 500 ng of one of pSB1A3 backbone and ddH2O.
2. To each mix, 5 µL of 10x reaction buffer (2.1) for restriction enzymes were added.
3. 1 µL each of the appropriate endonucleases (two per tube) was added to give a final volume of 50 µL.
4. The tube were taped to mix.
5. The tube were incubated at 37°C for 30 min.
6. Heat-inactivatation of the enzymes was done by incubating at 80°C for 20 min.

## Lab #40- Agarose gel electrophoresis (19-7-02) (Jenny + Irina)

### Aim:

To separate and analyse nucleic acid samples under non- denaturing conditions

### *Method:*

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 14 for components and amounts mixed for agarose gel

A gel cast from the previous day was used.

### Running the gel

1. the gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. Ther DNA samples (~0.2 µg)were mix with loading dye (table 15) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply and the gel was run at 110 V for 30–60 min.
8. After the run, the gel bands were visualized under UV and a picture was taken (figure x).

See table 50 for components and amounts used for running mixture:

**Table 50**

| | Components | amounts |
|---|---|---|
| 1 | Loading dye mix | 4uL |
| 2 | pSB1A3 sample | 20uL |

Gel analysis on 1% Agarose can be seen in figure 51. Our pSB1A3 sample can be seen in well number 4 from the left. We only had enough backbone to run the cut version, no negative control was loaded.

## Ligation

4 ligations were done at the same time.

1. Ligations of the digestions done by Jenny and Irina using pSB1A3 digested backbone
2. Ligation of Jin Wen's digestion MnP (sample 1) from Lab 31 (marked as a 1 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 2) from Lab 31 (marked as a 2 on the eppendorf tube)
4. Ligation of Jin Wen's digestion  MnP (sample 3) from Lab 31 (marked as a 3 on the eppendorf tube)

Components and amounts for ligation can be seen in table 52,  table 53 and table 54.

**Table 52**

| | Components | Amounts |
|---|---|---|
| 1 | BBa_K500001 (MnP 1) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 53**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP2) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 54**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP3) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Three Transformations were done at the same time, with 1 negative control

1. Mnp1
2. Mnp2
3. MnP3
4. Negative control

In table 55, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones

**Table 55**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (MnP1, MnP2 or MnP3) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

WEDNESDAY, 3/7/2019

## Results from Transformation done 19-7-02:

No growth on any plate. We will try Ligation and Tansformation for Jinwens samples on MnP 4-6 and make suer we take T4 ligase and T4 10x Reactionbuffer from iGGEM 2019 as something might be wrong with the ligation and we did not pay attention to the expiration dates of the ligase/buffer that we used.

# Lab #41 - BioBrick 3A Assembly (19-7-03)

## Ligation (Irina)

4 ligations were done at the same time.

1. Ligations of the digestions done by Irina using pSB1A3 digested backbone
2. Ligation of Jin Wen's digestion MnP (sample 4) from Lab 31 (marked as a Ligation 4 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 5) from Lab 31 (marked as a Ligation 5 on the eppendorf tube)
4. Ligation of Jin Wen's digestion  MnP (sample 6) from Lab 31 (marked as Ligaton 6 on the eppendorf tube)

Components and amounts for ligation can be seen in table 56,  table 57 and table 58.

**Table 56**

| | Components | Amounts |
|---|---|---|
| 1 | BBa_K500001 (MnP 4) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 57**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP 5) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 58**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP 6) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Three Transformations were done at the same time, with 1 negative control

1. Mnp 4
2. Mnp 5
3. MnP 6
4. Negative control

In table 59, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones

**Table 59**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (MnP 4, MnP 5 or MnP 6) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 uL water was added instead of Ligation mixture.

Results from Transformation done 19-7-03:

No growth on any plate.

Image from iOS (4).jpg

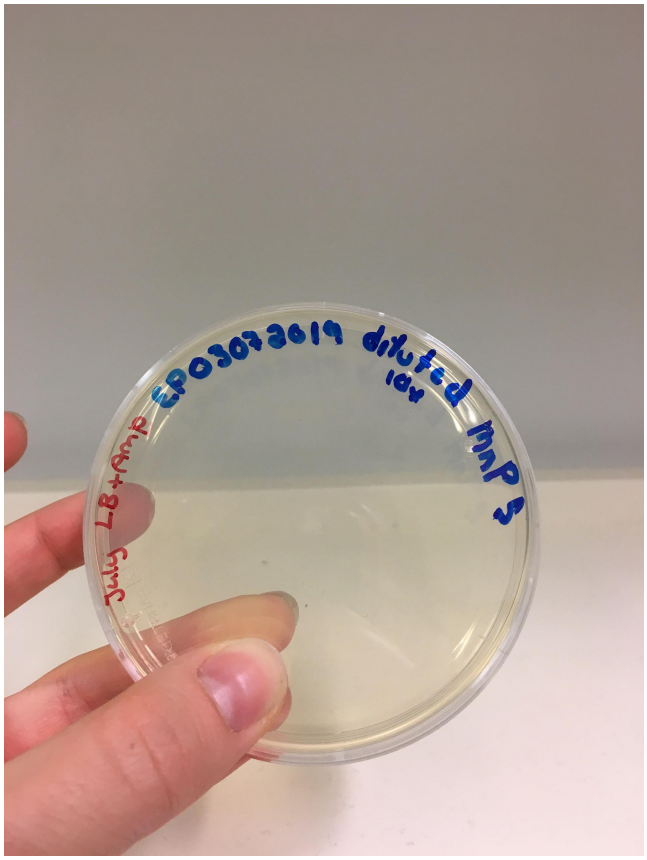


Image from iOS (5).jpg

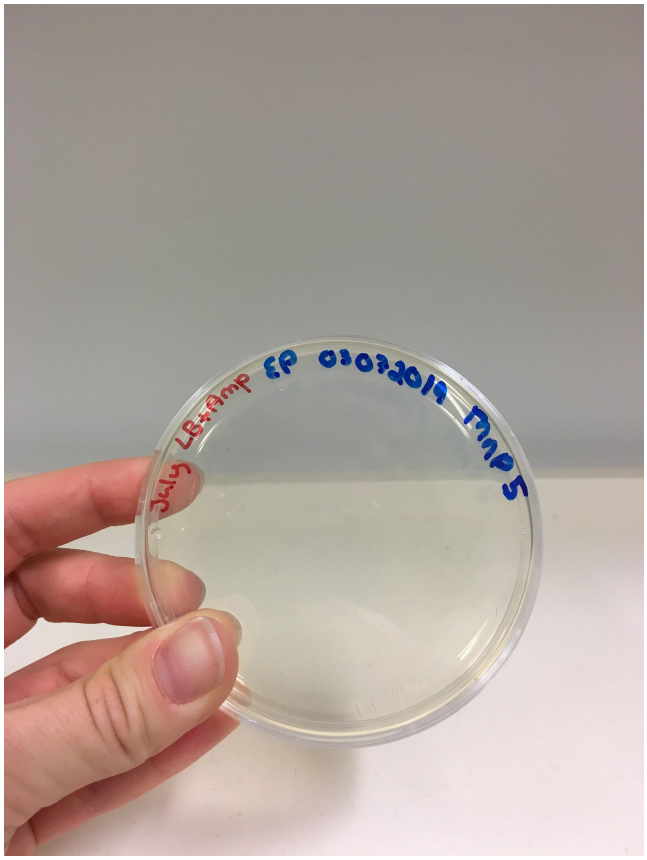


Image from iOS (3).jpg

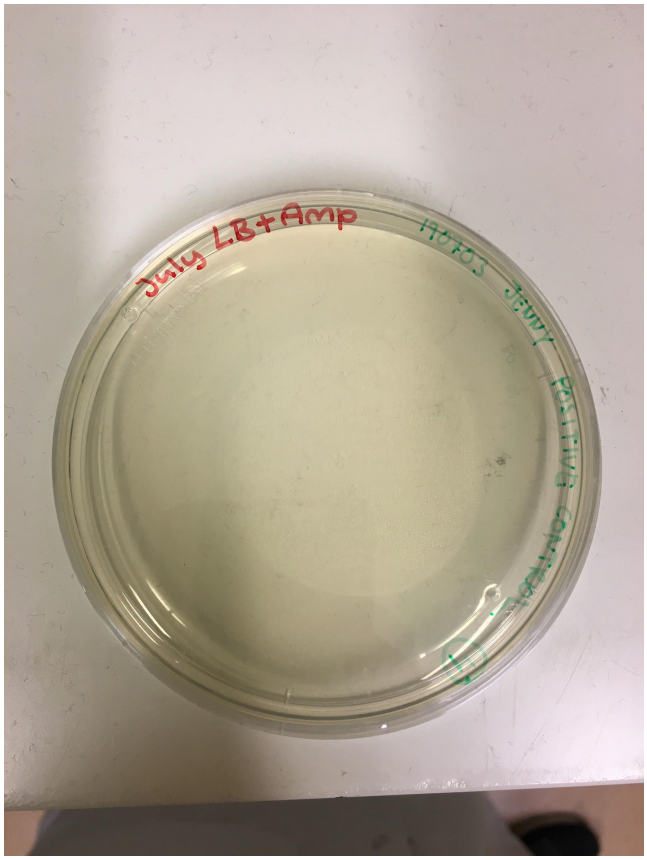
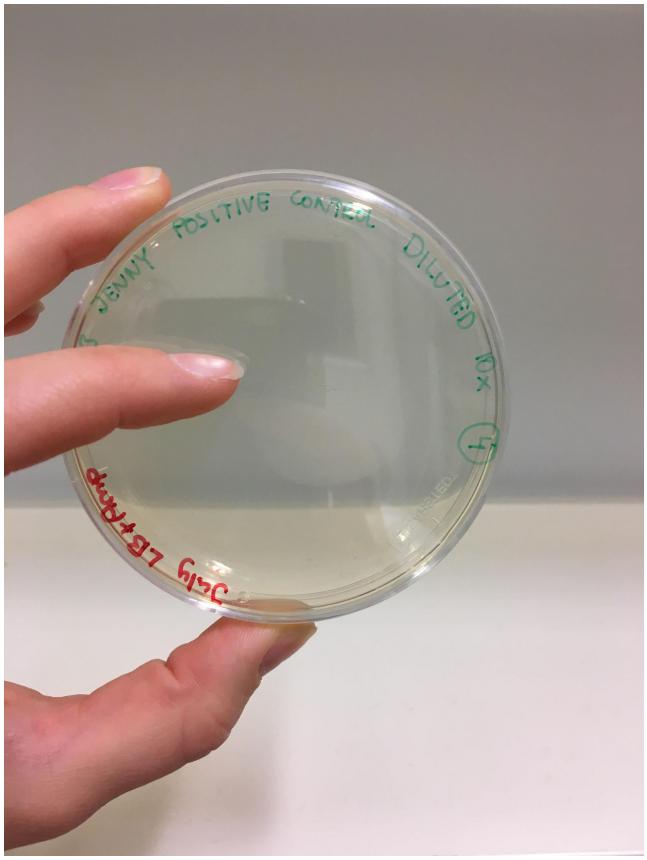


Image from iOS (2).jpg




## Lab #42 - Prepare agar plates with addition of antibiotics (19-7-03) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 60 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 60**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Amp"*

Calculations to calculate amount of solution needed for digestion:
500ng x 1ul/83.1ng= 6.02 ul Lac promotor

## Lab #43 Digest, agarose analysis and 3ADigest (Manish + Gustav) (3/7/19)

Aim: To confirm the presence of the correct plasmid construct in the cells that wee transformed the 26/6/19. This will be done by first digesting the transformed cells and then performing a 3A assembly. The plasmid is cut so that the promoter + gene of interest is displaced from the plasmid backbone. The plasmidbackbone is 2204 bp long and the promoter + gene of interest is 1733 bp long. This means that when a gel is run two bands should be seen at each respective length.
Method: The 3A method has been described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm Protocol 3. The digestion procedure was described in the GenElute$^{TM}$ Plasmid Miniprep Kit manual, pages 4-5, by Sigma-Aldritch while the column procedure was in GeneJET Plasmid Miniprep Kit by ThermoScientific.
Notes: No deveations form each respective protocol was made. However after a missunderstanding of the instructions a ependorf tube was accidentely centrifuged at 5000g for 5mins, in accordance to the Sigma Aldritch protocol not the ThermoScientific protocol. After the mistake was identified the correct protocol was followed meaning that the tube was centrifuged again at 12,000g for 1 min. This tube was marked as tube 1.
Results:

image.png

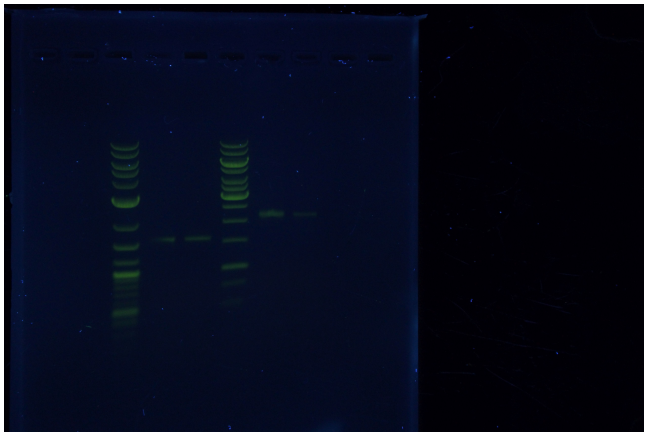
From left to right: Undigested sample, Digested sample, ladder (gene ruler 1kb), control from previous ligation. These samples were all from tube 1.

Calculations: After making two tubes, one following strict protocol marked as "tube 2" and one marked as "tube 1" which had followed the wrong protocol, the DNA concentration was meassured. Tube 1 had a concentration of 12ng/microliters while the other had a concentration of 8 ng/microliters.
It was decided that tube 1 will undergo 3A assembly.
500/12= 42microliters of DNA solution
1 microliters of ddH2O
1 microliters of EcoRI
1 microliters of PstI
5 microliters of buffer
Discussion: As seen from the resulting gel displayed above, the results recieved do not matched the theory. After some dicussion it was decided that due to the similar sizes of each band a 0.7% agarose gel should be made/run instead of the standard of 1%. The experiment was repeated with a 0.7% agarose gel.

## Lab #44 Digest, agarose analysis and 3ADigest (Manish + Gustav) (3/7/19)

Aim: To repeat lab #43 protocol with a 0.7% agarose gel.
Method: Described in lab #43.
Results:

image.png



From left to right: log-2 DNA ladder, Undigested sample "tube 1" described in lab #43, undigested sample, Gene ruler 1 kb ladder , digested sample "tube 1", digested sample

Calculations:
Agarose gel 0.7%: 50*0.007=0.35g agarose.
Digested sample: 500/33 = 15 microliters DNA solutions.
28 microliters ddH2O
5 microliters buffer
1 microliters PstI
1 microliters EcoRI
Discussion: As seen from the gel above the results were easier to see but they did not agree with the theoretical results. Theis implies that the correct construct is not present in the cells.

A further transformation was made using the ampicillin bavkbone but the resuts were negative.

## Lab #41 - Test of Competent Cells Through Transformation of Plasmids (19-7-04) (Erik)

### Aim:

As are all previous transformations have failed with BL21 bacterial strain, we are trying to troubleshoot what is wrong with our method or materials that are causing these transformations. We are therefore testing to see if the competent cells take up plasmids by using the iGEM competent cell test kit 2018 pSB1C3 BBa_J04450.

### Method

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Three transformations were done with the competent cells done by either Irina, Erik, or Irina/Erik (we are not sure who it was). The same RFP construct BBa_J04450 was used for all three transformations.

### Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Calculations:
0,5 ngx 1000pg/1ngx 1ul/1000pg =5ul
Deviations from Protocol:

1. No negative control were done, BBa_J04450 have a RFP reporter that make the colonies red. Any contamination will not have the characteristic red colonies.
2. Competent cells were left on ice for 25 min instead of 15 min.
3. Irina's resuspension liquid was less than 100 ul
4. Erik's resuspension solution was more than 100ul

**Table 61**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Irina's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |
| 4 | ddH2O | - |

**Table 62**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Erik's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table63**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Erik's eller Irina's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

Irina is strating overnight culutre of BL21 cells.

FRIDAY, 5/7/2019

### Discussion (Irina):

Today we are making new Ampicillin stocks as well as LB + Amp plates and LB + Amp medium. We do not trust the stocks used that were made 2018. We will continue to try transformation, but also make sure to use the new plates as well as taking the enzymes from 2019. No more dumpsterdiving for old stuff! Additional SOB medium is also made.

## Lab #42 - Preparation of competent *E.coli* BL 21 (DE3) cells using $CaCl_2$ (19-7-05) (Irina)

### Aim:

To increase the competence of *E. coli* BL 21 (DE3) cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Competent cells are marked with red BL21 and stored in the box with competent cells in the -80 freezer.

## Lab #43 - Prepare agar plates with addition of antibiotics (19-7-05) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

#### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 64 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Tble 64**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

## Lab #44 - Preparation of LB media and addition of Ampicillin (19-7-05) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 64 for components and amounts used to prepare 600 mL of LB.

**Table 64**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Ampicillin was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July LB + Amp"*

## Lab #45 - Preparation of SOB medium (19-7-05) (Irina)

### Aim:

To prepare a SOB Medium, or Super Optimal Broth, is used for preparing chemically competent cells.

### Methods:

Preparation of SOB was done according to protocol:

SOB Medium

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 96;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations
m(Yeast) = $W_1$/ $V_1$ x $V_2$ = $W_2$ = 0.5g/100 mL x 800 mL = 4g
m(Bacto) = 2g/100mL x 800 mL = 16g
m(NaCl) = 10 mmol/1 L x 800 mL x 58.44 g/mol = 0.47g
m(KCl) = 2.5 mmol/1 L x 800 mL x 74.55 g/mol = 0.15g

See table 65 for components and amounts

**Table 65**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 0.46g |
| 2 | BactoTMtryptone 1% (w/v) | 16g |
| 3 | Yeast extract 0.5% (w/v) | 4g |
| 4 | ddH2O | 800mL |
| 5 | 5 M NaOH | 45uL |
| 6 | KCl | 0.15g |

MONDAY, 8/7/2019

# Lab #46 - Digestion of PSB1A3 (19-7-08) (Irina + Jenny)

## Aim:

To digest the plasmid PSB1A3 with two different methods.

## Methods:

- The Protocol 3 form Synthetic Biology
- The iGEM Registry Protocol (http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit)

Notes: EcoRI-HF and Dpn1 was from 2019 but the others were older.

We loaded a gel to measure Irinas sample, figure 66, (from protocol 3) but since the other (mixed w

gel.GIF

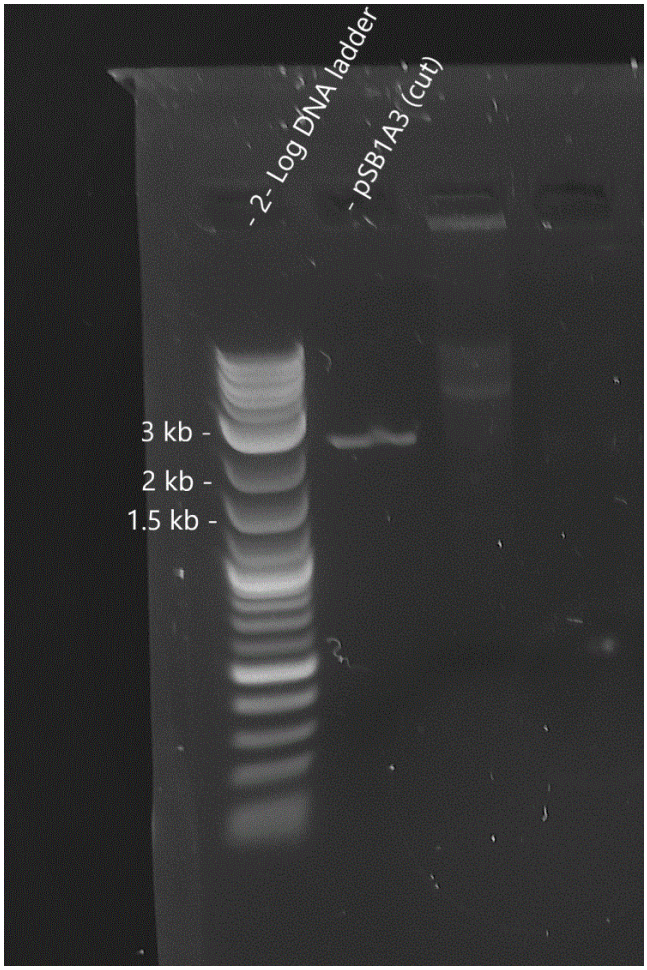



**Figure 66.** Agarose gel analysis of digested pSB1A3.

Lab #39 and #41 was repeated but with new competent cells from 5/7 using BL21 marked competent cells, new LB + Amp plates from 5/7 and with the newly digested plasmid from todays lab (Lab #46).

## Lab #48- Preparation of SOC Media (19-7-8) ( Erik)

Aim: To prepare 10 ml SOC Media

Method: Follow the protocol from Cold Harbor Springs Protocol on preparing SOB media:
http://cshprotocols.cshlp.org/content/2018/3/pdb.rec098863

Since chilled SOB media and 1M glycerol solution was already prepared, 10 ml was simply prepared by putting in 0,2 ml of glucose solution in 10 ml of SOB media.

Calculations:
1000ml of SOB/20ml of glycerol solution= 10ml of SOB/x amount of broth
x= 0,2 ml of glucose solution

## Lab #49- Compentent Cell Test Kit (19-7-8) (Gustav + Erik)

**Aim:** To determine the competency of the cells made 5/7/19. The strain of the E. Coli was BL21 by transforming in the RFP plasmid BBA_J04450.

**Methods:** The protocol followed is detailed in the following link on iGEMS official website:
http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit

1. Clean your working area by wiping down with 70% ethanol.
2. Thaw competent cells on ice. Label one 1.5 mL microcentrifuge tubes for each transformation and then pre-chill by placing the tubes on ice.
    - Do triplicates (3 each) of each concentration if possible, so you can calculate an average colony yield.
3. Spin down the DNA tubes from the Competent Cell Test Kit/Transformation Efficiency Kit to collect all of the DNA into the bottom of each tube prior to use. A quick spin of 20-30 seconds at 8,000-10,000 rpm will be sufficient. *Note:* You should resuspend the DNA in each tube with 50 µL dH2O.
4. Pipet 1 µL of DNA into each microcentrifuge tube.
5. Pipet 50 µL of competent cells into each tube. Flick the tube gently with your finger to mix.
6. Incubate on ice for 30 minutes.
    - Pre-heat waterbath now to 42°C. Otherwise, hot water and an accurate thermometer works, too!
7. Heat-shock the cells by placing into the waterbath for 45 seconds (no longer than 1 min). Be careful to keep the lids of the tubes above the water level, and keep the ice close by.
8. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes.
9. Add 950 µL of **SOC media** per tube, and incubate at 37°C for 1 hour shaking at 200-300rpm.
    - Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.
10. Pipet 100 µL from each tube onto the appropriate plate, and spread the mixture evenly across the plate. Incubate at 37°C overnight or approximately 16 hours. Position the plates with the agar side at the top, and the lid at the bottom.
11. Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.
    - Efficiency (in cfu/µg) = [colonies on plate (cfu) / Amount of DNA plated (ng)] x 1000 (ng/µg)
    - *Note: The measurement "Amount of DNA plated" refers to how much DNA was plated onto each agar plate, not the total amount of DNA used per transformation. You can calculate this number using the following equation:*
    - Amount of DNA plated (ng) = Volume DNA added (1 µL) x concentration of DNA (refer to vial, convert to ng/µL) x [volume plated (100 µL) / total reaction volume (1000 µL)]

Notes: No deviations from the protocol were made.

# Lab #49- Preparation of 10x TBE  (19-7-8) (Erik)

## Aim:

To prepare a TBE buffer that inhibits enzymatic degradation of nucleic acids when running agarose gels.

## Methods:

Preparation of 10x TBE Buffer cells for agarose gel electrophoresis was done according to protocol:

10xTBE buffer (500ml stock)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 94;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
m = Mw x n x c
m(Tris) = 121.14 g/mol x 0.89 mol/l x 0.5 L = 53.91 g

m(Boric Acid) = 61.83 g/mol x 0.89 mol/L x 0.5 L = 27.52 g
m(EDTA) = 292.24 g/mol x 0.025 mol/L x 0.5 L = 3.65 g

See table 10 for components and amounts used. pH = 8.2

**Table1**

| | Component | amount |
|---|---|---|
| 1 | Tris | 53.91 g |
| 2 | Boric acid | 27.52 g |
| 3 | EDTA | 3.65 g |
| 4 | ddH2O | 500 mL |

Notes: I put the ddH20 first before putting in the solid Tris, Boric Acid instead of pouring the water after.

TUESDAY, 9/7/2019

# Lab #50 - Plasmid MiniPrep (19-7-9) (Erik)

## Aim:

To isolate plasmid DNA from recombinant *E. coli BL21* O/N cultures BBa_J04450 (gene RFP), pSB1C3 (backbone), B0034 (ribsome binding site)

## Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.

DNA concentrations can be seen in table 12.

**Table66**

| | Sample | concentration (ng/uL) | 280/260 | 260/230 |
|---|---|---|---|---|
| 1 | BBa_J04450 | 101.4 | 1,22 | 1,77 |

Conclusions:
280nm: Wavelength of that proteins absorb
260nm: Wavelength that DNA absorb
230nm: Wavelength that carbohydrates are absorbed

The DNA concentration is relatively good at 101.4 ng/ul. Values around 2 are good, which means that that our solutions are somewhat contaminated, especially with proteins..

# Lab #51 - Prepare agar plates with addition of antibiotics (19-7-09) (Irina)

Additional LB plates with Ampicillin will be prepared, as the stack is runing low.

## Aim:

To prepare  solutions necessary for future experiments

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 60 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table67**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Amp"*

# Lab #52 - O/N cultures and re- streaking of DH5α started (19-7-09) (Irina)

Discussion: We are going to transform in DH5α as we have no luck with BL21 (DE3).

Colonies were picked from two different plats of DH5α and re-streaked on LB plates. Two additional colonies where chosen fro said plates and placed in 15 mL Falcon tubes containig 6 mL of LB. Plates and Falcon tubes where stored in the 37 degree room.

# Lab #53 - Digestion and gel analysis for pSB1C3 (Bba_J04450) (19-7-9) (Erik)

## Aim:

To doublecheck that the plasmid that we have purified from the miniprep from lab 50 (backbone pSB1C3, gene (RFP) Bba_J04450, B0034, ribosome binding site) is the one that we have gotten out.

## Methods:

3A assembly-Digestion and ligation

**Calculations:**

500 ng x 1ul/101.4 ng= 4,93 ul of plasmid mixture

Table 25 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion.

**Table70**

| | Component | pSB1C3, Bba_J04450 |
|---|---|---|
| 1 | 500 ng DNA | 4,9uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ---- |
| 5 | XbaI | --- |
| 6 | PstI | 1uL |
| 7 | ddH2O | 35,4ul |

Analytical Digestion and Agarose Gel Electrophoresis

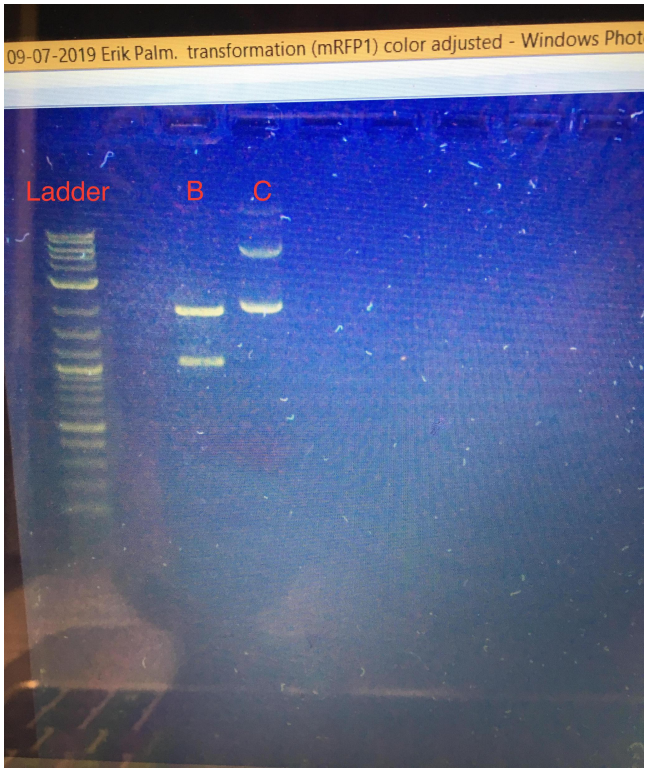
-We used a the standard gel electrophorator at 115 V for 30 min

**Table72**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | latter | 20 ul<br>4ul loading dye | -- |
| 3 | B | digested pSB1C3, Bba_J04450 | 20 ul<br>4ul loading dye | -- |
| 4 | C | undigested pSB1C3, Bba_J04450 | 2,0 ul of sample 18,0 ul H20 4 ul loading sample | 200ng x 1ul/101,4ng |

## Results:

Analytical Digest pSB1C3 mRFP1.jpg




## Discussion:

-Well B was expected, where the length is about 2000 bp and the mRFP is 1000kb, which is what we expect from a digestion from B.
-Well C is a little bit strange. Two bands are expected, as the DNA takes on different conformations in plasmid form. However, the upper bands is higher than 3000 bp, which is what the plasmid would go if it was linear. We think that it is possible that the the plasmid have tangled around one another into a larger mass, which has made it even more difficult to go through.

WEDNESDAY, 10/7/2019

# Lab #54 - Preparation of competent DH5α *E.coli* cells using $CaCl_2$ (19-7-10) (Irina)

Discussion: Today we are continuing the preparation of DH5a cells for future transformations. Two separte cultures where started, but only one shows good results when measuing OD600.

## Aim:

To increase the competence of *E. coli* DH5α cells

## Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Calculations for $CaCl_2$ solutions can be seen in table 73.

**Table 73**

| | A | 1M CaCl2 | 50% Glycerol stock | ddH2O | TOTAL volume (ml) |
|---|---|---|---|---|---|
| 1 | 0.1. M CaCl2 (15ml) | 2 ml | / | 18ml | 20ml |
| 2 | 0.1 M CaCl2 + 20% glycerol (3ml) | 0.3ml | 1.2ml | 1.5ml | 3ml |

Cells are stored in the -80 freezer, marked with the letter "I". Each tube contains 100 uL of competent cells.

## Lab #55 - Transformation of lac promotor (pSB1C3, J04500) and ligation mixture (July 8th, 2019: Mnp, pSB1A3 plus lac promotor) (19-7-10) (Erik)

### Aim:

To see if the plasmid with the lac promotor (pSB1C3, J04500) can get transformed into the competent dH5A

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

**Table 74**

| | A | Erik | Jenny |
|---|---|---|---|
| 1 | competent cell origin | Gustav | Gustav |
| 2 | Lac promotor (J04500) | Lac (5) | Lac (2) |
| 3 | Ligation Mixture (July 8th, 2019: Mnp, pSB1A3 plus lac promotor) | MnP (5) | MnP (2) |
| 4 | Number and Types of Plates | 1x chloroampheni col for unidigested lac promotor<br>1x ampicillin for ligation mixture<br>1x negative control ampicillin | 1x chloroampheni col for unidgested lac promotor plasmid<br>1x ampicillin for ligation mixture<br>1 x negative control ampicillin |

# Lab #56 - Analytical Digestion in Preparation for Gel Extraction (19-7-10) (Erik, Jenny, Qian, Gustav, Irina)

## Aim:

To prepare for gel extraction, we use the same method as analytical digestion to separate out the desired backbone with the Lac promotor (J04500) and the (pSB1C3_MnP (Bba_K500001))

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

Deviations from protocol.

1. Two group were made; one was Jenny and Erik, the other Gustav and Irina. Each group was tasked with preparing a digestion of promotor and Mn gene each per the usual protocol. The goal of doing this analytical digest was to do a gel extraction, hence the many wells of the same thing. No solution remained after gel electrophoresis.
2. Made one large mixture which combined loading dye (10 ul of loading dye, 50 ul total of solution) which we mixed together and and then put into the plates

**Table75**

| | Components | Digestion of Lac promotor (J04500) | Calculations | Digestion of Mn (pSB1C3_MnP (Bba_K500001)) | Calculation |
|---|---|---|---|---|---|
| 1 | 500 ng DNA | 6,9ul | 500ngx 1ul/72,2ng= | 7,6ul | 500ng x 1ul/65,4= |
| 2 | 10 x Reaction buffer (2.1 New England Buffer) | 5ul | | 5ul | |
| 3 | EcoRI | ----- | | ----- | |
| 4 | SpeI | 1ul | | ----- | |
| 5 | XbaI | ----- | | 1ul | |
| 6 | PstI | 1ul | | 1ul | |
| 7 | ddH2O | 36,1 ul | | 35,4ul | |

First Gel: Lac Promotor with 2- log ladder

**Table76**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | 2 log | 6ul | -- |
| 3 | B | uncut lac (pSB1C3, J04500) Irinia + Gustav | 3ul of uncut plasmid + 17 ddH2O + 4ul dye = 24uL (loaded 20ul) | |
| 4 | C | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 5 | D | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 6 | E | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 7 | F | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 8 | G | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 9 | H | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |

Second Gel: Cut ofMn (pSB1C3_MnP (Bba_K500001))

**Table77**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | 2 log | 6ul | |
| 3 | B | uncut Mn gene (pSB1C3, J04500) Irinia + Gustav | | |
| 4 | C | cut Mn gene (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 5 | D | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Irina and gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 6 | E | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Irina and gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 7 | F | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Erik+ Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 8 | G | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Erik+ Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 9 | H | cut  Mn gene (pSB1C3_MnP (Bba_K500001 ))Erik+ Jenny | 16 ul<br>4ul loading dye | |

**Results:**

1. For the lac promotor gel plate, we could not see any lines with the UV at all. For the Mn plate, we could see very thin bands of Mn gene that seemed to be in the right place, but because they were so thin and indistinct, it was deemed not worth it to attempt an extraction.
2. At first we were worried that the gel had run through, however, the ladder was still present. For next time, it would be good to be more careful about the time, as the DNA had progressed quite far.

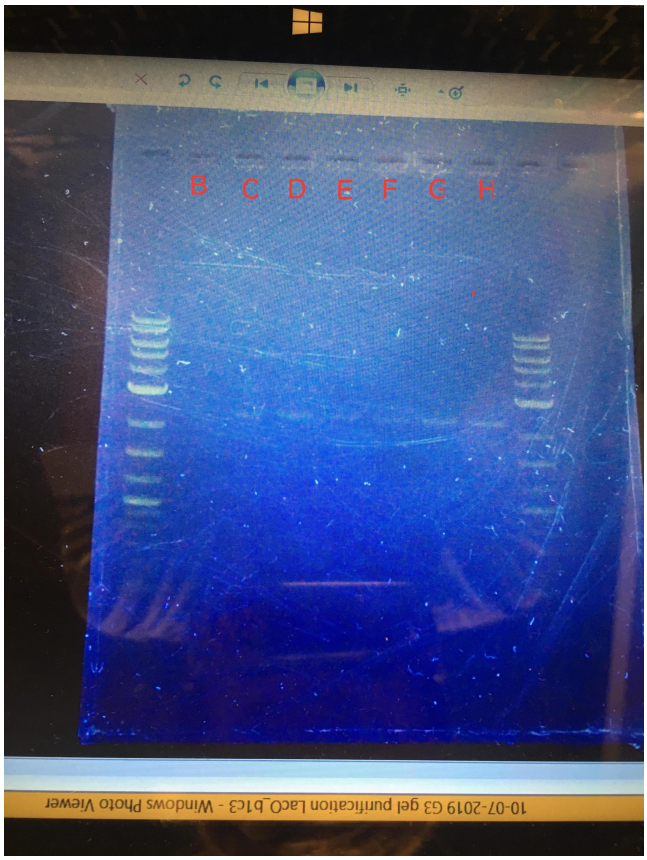
**Conclusions:**

We think that the DNA that Jin Wen prepared a couple of weeks ago now has degraded significantly, meaning that the concentrations that she nanodropped on the side are not accurate. As a result, there is not the necesary amount of DNA for clear bands to be seen and for extraction to be obvious.

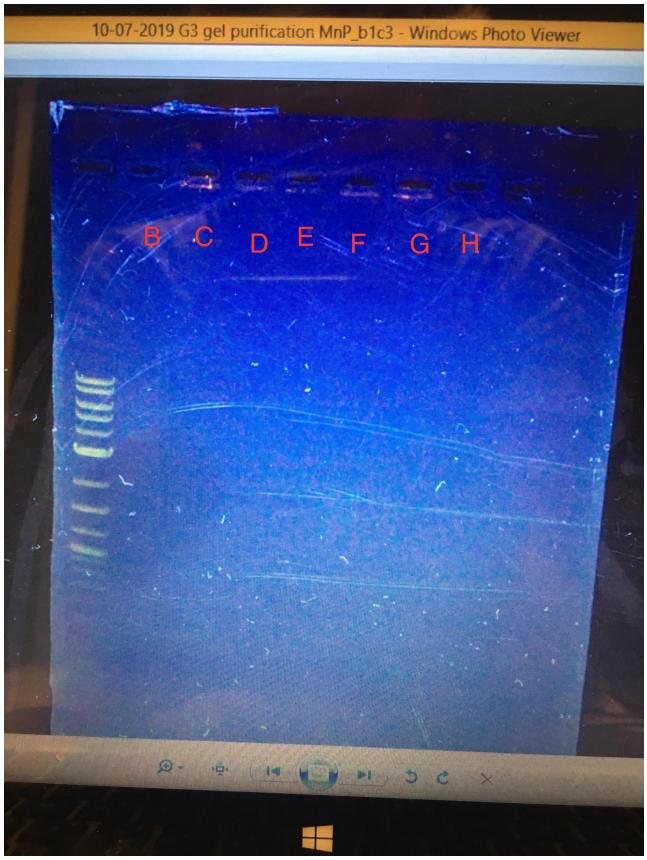
To fix this for next time, a couple of suggestions.

1. More DNA should be loaded into the one well by using the larger well combs while preparing the gel
2. Be careful about what is underneath the gel, as the plastic plate blocked the UV light, alllowing us to not see the bands on the UV light.

Lac Analytical Digest July 10 2019 2.0.jpg



Mn Analytical Digest July 10 2019 2.0.jpg




THURSDAY, 11/7/2019

# Lab #56 - Ligation and transformation into DH5a competent cells (19-7-11) (Irina)

## Aim:

Ligation of pSB1A3, LacI (Jinwen's digestion nr. 6) and MnP (Jinwen's digestion nr.6) on Ampicillin LB agar plates.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

## Ligation

Components and amounts for ligation can be seen in table 78, some modificstions of amounts where done. We took into acount 20% degradation.

**Table 78**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 5uL |
| 4 | Bba_K500001 | 8uL |
| 5 | J04500 | 2uL |
| 6 | pSB1A3 | 2uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

One Transformation was done, with 1 negative control

1. Transformation
2. Negative control

Deviation from protocol: No positive control made. Gustav is making the efficiency count

In table 78, reaction mixtures for transformation can be seen.

**Table 79**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - |
| 2 | DH5a competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

# Lab #57 -Determining the competency of competent cells made 10/7/19 (Gustav, 11/7/19 + 15/7/19)

Aim:
To determine the competency of the competent cells made the 10/7/19

Methods:
The method used can be found on the official iGEM website: http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit.

Notes:
Three deveations from the protocol were made. The first was that no 10ng sample was prepared due to the high concentration of DNA in our DNA source. The second deveation was that the instructions were misread and step 6 was conducted without having any DAN in the samples. When this was realised DAN was added and the wait time in step 6 was extended by 15mins.
The third was that we used the Psb1A3 plasmid miniprepd from Jonas lab group.

Calculations:
100ng of psB1A3 backbone was used with RFP.
Conc. of psb1A3: 92.2 ng/ul. --> 100/92.2 = 1.1 ul.

Competency of plate 1: 1.689*10^(10)
Competency of plate "2": 1.337*10^(10)
Competency of plate "3": 1.22*10^(10)
Average competency: (1.22+1.337+1.689)/3 = 1.415*10^(10) colonies/ng
1.415*10^(7) colonies/ug

The average competency of the cells was to the tenth exponent.

## Lab #58 - Transformation of MnP Gene (BBa_K500001) (19-7-11) (Erik and Jenny)

### Aim:

To see if the plasmid with the MnP gene (pSB1C3, BBa_K500001) can get transformed into the competent dH5A.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Table 80 shows who prepared which sample.

**Table80**

| | A | Erik | Jenny |
|---|---|---|---|
| 1 | competent cell origin | Gustav | Gustav |
| 2 | MnP Gene (BBa_K500001) from Jinwens' miniprep | Mnp (3) | Mnp (2) |
| 3 | Number and Types of Plates | 1x chloroampheni col for pSB1C3_BBa_ K500001 | 1x chloroampheni col for pSB1C3_BBa_ K500001 1 x chloramphenic ol for negative control |

Calculations:

Dilution of the MnP Miniprep Samples. Wanted concentration: 2 ng/ul

Starting concentration sample 2: 109.7 ng/ul

109.7 ng/ul x 1 ul/2 ng = 54,9 ul (total volume) ~55 ul. Add 54 ul dd$H_20$ to 1 ul of the Miniprepped Sample nr 2.

Starting concentraion sample 3: 133,5 ng/ul

133.5 ng/ul x 1 ul/2 ng = 66.75 ul (total volume) ~66.8 ul. Add 65,8 dd$H_20$ to 1 ul of the Miniprepped Sample nr 3.

Differences from the Protocol 6:

*No positive control were done. (skipped point 9)

*At point 11 we saved 60 ul and poured the rest of the liquid as much as possible. Changed to the largest tip and resuspended the pellet before the streaking on the plates.

MONDAY, 15/7/2019

# Lab #56 - Ligation and O/N cultures (19-7-15) (Irina + Gustav)

## Aim:

Ligation of pSB1A3, LacI (Jinwen's digestion nr. 6) and MnP (Jinwen's digestion nr.6). This time, Ligation was done in 4 degrees and 16 degrees for 8 hrs using PCR tubes and PCR machine.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Amounts used can be seen in table 81. Some modifications where done regarding the protocol.

**Table 81**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 3uL |
| 4 | Bba_K500001 | 2uL |
| 5 | J04500 | 10uL |
| 6 | pSB1A3 | 2uL |

Discussion: Mini-prep on O/N cultures from friday the 12th failed on Saturday the 13th. Therefore, new O/N cultures where prepared on Moday the 15th.

TUESDAY, 16/7/2019

## Lab #57 -Transformation of ligation mixture prepared 15-7-19 (Gustav, 16/7/19)

Aim: To tranform the cells with an efficiency factor of 1.415*10^(10) with the ligated samples from 15/7/19.

Methods: The methods followed were those described by protocol 6 in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al.

Notes: Due to missing step 7 was missed. Instead the ependorf tubes went straight to their water baths. The misstake was later realised ca 45mins after. The SOB media was added aaccordingly and the water bath was extended with 45mins.

## Lab #58 - Plasmid MiniPrep (2019-07-16) (Jenny & Irina)

### Aim:

Sample names:

1. LB+Amp pSB1A3_(RFP)
2. LB+Amp pSB1A3_LacI_Boo34_Mnp (3)
3. LB+Cm Lac Prom (2) (J04500)(pSB1C3)
4. LB+Cm Lac (5) in pSB1C3
5. LB+Cm DH5a_BBa_K500001_pSB1C3 (3) MnP

6. LB+Cm DH5a_BBa_K500001_pSB1C3 (2) MnP

The number in brackes is the sample from which the Lac and Mnp derives from (Jinwens samples).

## Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.
with some adjustments:

- When washing the column for the first time we added 750 uL of Washing Solution and centrifuged 1 min/12000g. Thsi was done twice before the dry centrifugation step.
- After dry centrifugation step the column was moved to a new eppendorf tube and 50 uL ddH2O was added. Waited for approximately 5 minutes befor adding another 50 uL and then spinned 1 min/12000g.
- We measuerd the concentrations by Nanodrop2000. See table 1 for results.

Irina made tubes 1, 2 and 3 while Jenny made 4, 5 and 6. Named after the plates with colonies from 10th of July. Two minipreps where done as we had quite low concentrations the first time (we only used 1.5 mL of culture). We modified the protocol according to Manish's protocol for the second miniPrep. No second miniprep was done for sample nr.6 as the cap had flewn off during incubation on shaking table.

DNA concentrations can be seen in table 82.

**Table 82**

| | Sample | concentration (ng/uL) | Concentration (ng/uL) |
|---|---|---|---|
| 1 | 1 | 37.4 | 111.7 |
| 2 | 2 | 10 (discarded) | 43.6 |
| 3 | 3 | 47.2 | 113.9 |
| 4 | 4 | 42.8 | 122.4 |
| 5 | 5 | 70.4 | 185.6 |
| 6 | 6 | 69.7 | - |

Samples where stored in the freezer after NanoDrop reading.

Conclusions:
Since the absorption values was to low for Sampel 1 and 2 Irina and Manish remade them.

WEDNESDAY, 17/7/2019

# Lab #59 - Digestion and gel analysis for pSB1C3 (Bba_J04450) (19-7-17) (Jenny + Irina)

## Aim:

To doublecheck that the plasmid that we have purified from the miniprep from lab 50 (backbone pSB1C3, gene (RFP) Bba_J04450, B0034, ribosome binding site) is the one that we have gotten out.

## Methods:

3A assembly-Digestion and ligation

Deviation from protocol: an 0.8% agarose gel was made and run at 90V for about 1.5 hrs.

In figure 83, Loading and what type of restriction enzymes that where used can be seen.

IMG_20190717_143148751.jpg

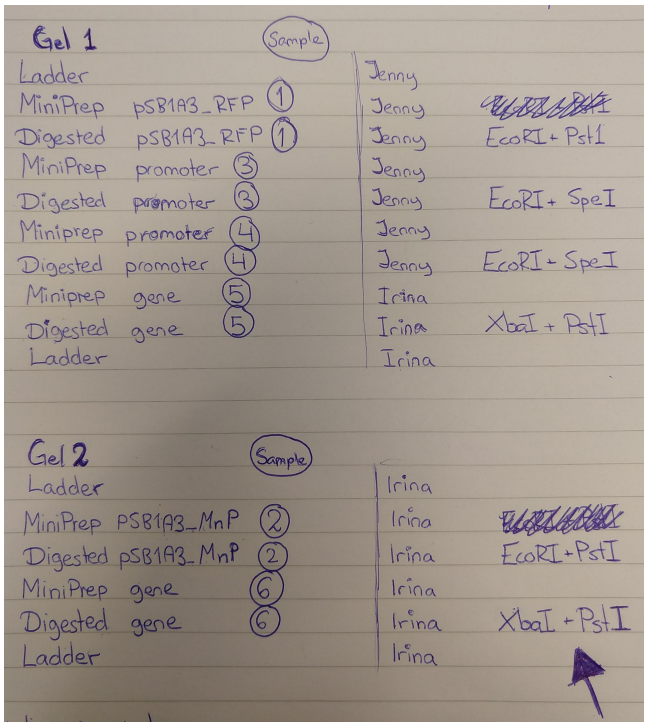



Figure 83. Loading order of 0.8% Agarose gels

GEL1.jpg

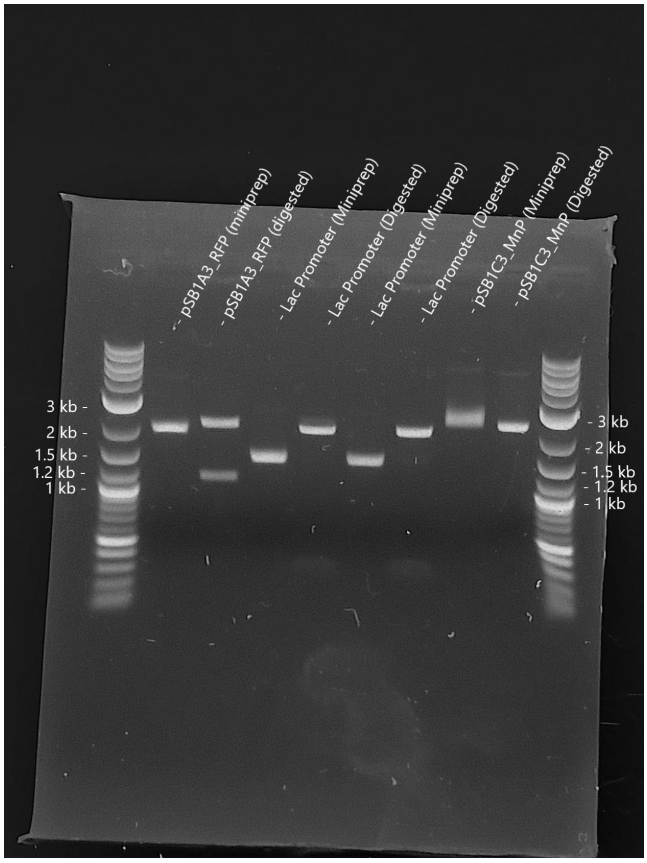



Figure 84. 0.8 % agarose gel loaded with 20 uL sample and 10 uL 2-Log DNA ladder.

GEL2.jpg

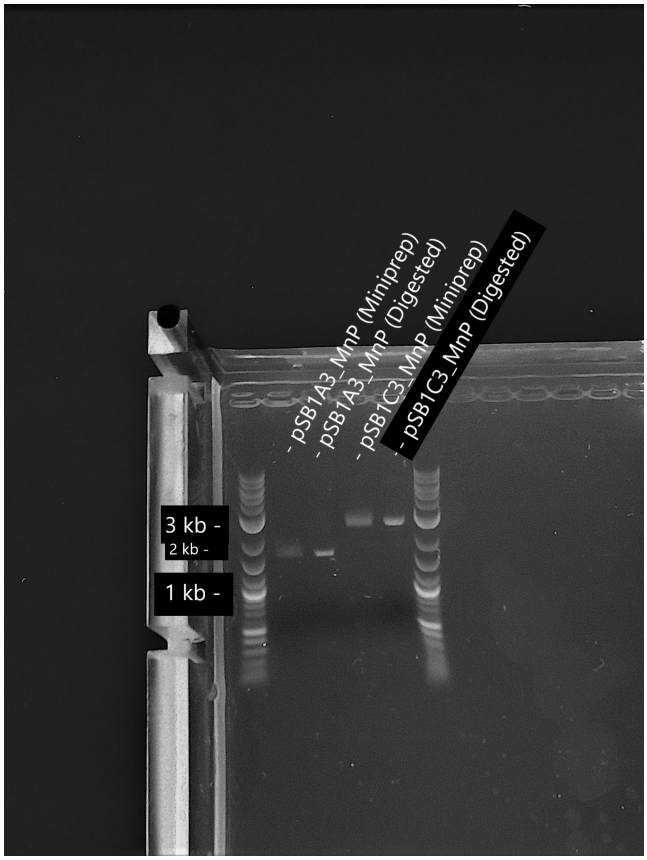



Figure 85. 0.8 % agarose gel loaded with 15 uL sample and 10 uL 2-Log DNA ladder.

THURSDAY, 18/7/2019

# Lab #60 - Digestion and gel analysis for pSB1C3 (Bba_J04450) and pSB1C3_MnP from lab #59 (19-7-18) (Jenny + Irina)

## Discussion:

It appears that there might be a problem with the XbaI enzyme from the gel images from yesterday. Therefore. we will perform digestion again. However, we will take sample from the digestions from yesterday (25uL) and add an additional 0.5uL of XbaI to both sample 5 and 6 (pSB1C3_MnP). We will use the new XbaI for sample 5 again, but an old XbaI for sample 6. Protocol will be followed from there on. We will also perform a digestion on pSB1C3 following protocol.

## Aim:

DIgestion of sample 5 and 6 (pSB1C3_Mnp) again. As well as the digestion of pSB1C3.

## Methods:

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

Deviation from protocol: an 0.8% agarose gel was made and run at 90V for about 1.5 hrs.

Calculations:

500 ng x 1ul/101.4 ng= 4,93 ul of plasmid mixture

Table 86 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion of pSB1C3.

**Table 86**

| | Component | pSB1C3, Bba_J04450 |
|---|---|---|
| 1 | 500 ng DNA | 4,9uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ---- |
| 5 | XbaI | --- |
| 6 | PstI | 1uL |
| 7 | ddH2O | 35,4ul |

MONDAY, 22/7/2019

Discussion:
Looking at the gel in figure 86, it appears that we are woring with GLOX (1700bp) rather than MnP (1134 bp). A mix up of samples could have occured. We will now on continue with ligation and transformation but assuming we are woring with GLOX.

gel.jpg

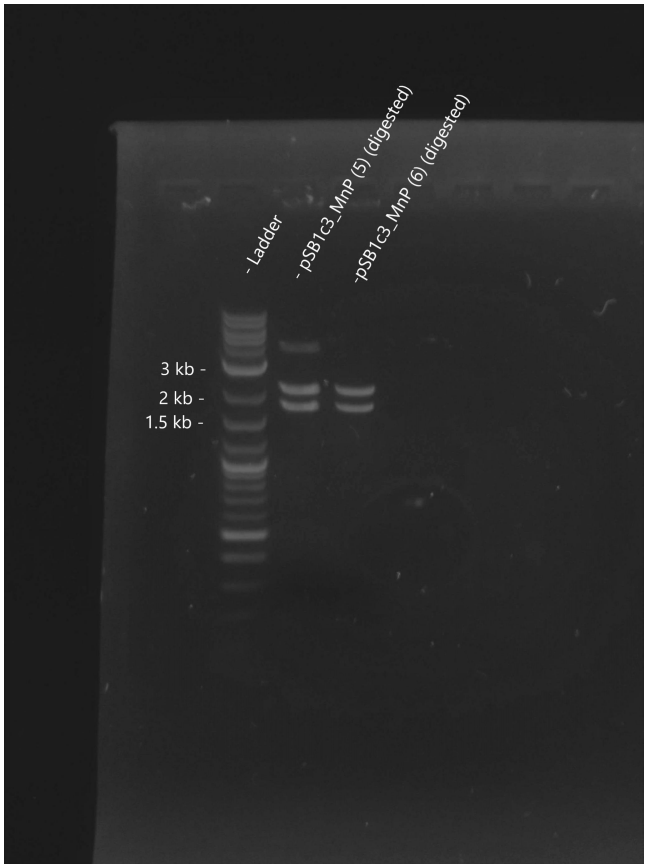



Figure 87. 0.8 % Agarose gel, showing ladder, Mnp (?) sample 5, MnP (?) sample 6.

## Lab #61 - Ligation in PCR tubes (19-7-22) (Irina + Erik)

### Aim:

Ligation of sample 5 in pSB1C3 and ligation of sample 6 in pSB1A3. we assume it is GLOX.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Deviations from protocol: Incubation was done in a PCR macine. 22 degrees for 30 minutes followed by heat inactivation at 65 degrees for 10 minutes.

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Amounts used can be seen in table 88.

**Table 88**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 11uL |
| 4 | GLOX | 2uL |
| 5 | Lac (3) | 2uL |
| 6 | pSB1A3 or pSB1C3 | 2uL |

Samples were stored at -20 degrees for further use, two PCR tubes marjed with encirceld 5 and one marked with an encirceled 6 written with blue pen.

TUESDAY, 23/7/2019

# Lab #62 - Transformation (19-7-23) (Erik + Jenny + Gustav + Irina)

Transformation
Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Four Transformations were done togethre with 2 negative controls

1. GLOX sample 6
2. GLOX sample 6
3. GLOX sample 5
4. GLOX sample 5
5. Negative control on Cm plate
6. Negative control on Amp plate

In table 55, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive oontrols where made

**Table 89**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | GLOX sample 5 or sample 6 | 5uL | - |
| 2 | DH5a competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

## Lab #63 - PCR (2019-07-23) (Gustav + Irina + Erik + Jenny)

Aim: Amplify and create 3' overhangs on AAO, HRP, MnP, GLOX, LiP and pPICZαB for Gibson Assembly.

Method: Following the Protocol 10 - Inverse PCR with PhusioHF DNA Polymerase

- Instead of having 5 min of initial denaturation we put 30 sek.
- Instead of having 30 sek denaturation we put 10 sek each cycle.

PCR for synthesizing DNA fragments

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 126-128;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, PCR;

Irina's PCR reaction mixture for pPICZaB (total volume 50 uL):
23.5 uL of ddH2O
5 uL of 2mM dNTPs
5 uL of Formward primer (5 uM) (pPICZaB_Myc_fwd)
5ul of Reverse primer (5uM) (pPICZaB_afac_end_rev)
10 uL of Phusion HF buffer
1 uL Plasmid DNA (1 ng/ul)
0.5 uL of HF DNA Polymerase

Irinas Assembly Protocol (total volume 20 uL)
NEB calculator was used: http://nebiocalculator.neb.com/#!/ligation
HRP lenght: 1250 bp
Vector lenght: 3600 bp
HRP stock: 64.1 ng/uL
Vector stock: 13.6 ng/uL
Need (3:1) ratio of HRP: 104.2 ng/64.1 ng/uL = 1.7 uL
Need 100 ng vector: 100 ng/13.6 ng/uL = 7.4

9.1 uL of fragments
10 uL of Master mix

0.9 uL of ddH2O

## Transformation

### Aim:

To transform competent DH5a cells with Shuttle-vector containing each gene respectively. First, a 4x dilution was made of the above Assembly protocol by mixing 5 uL of Assembly and 15 uL of ddH2O.

### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Samples where incubated in a thermocycler at 50°C for 15 minutes prior to transformation, following instructions from:
https://international.neb.com/protocols/2012/09/25/gibson-assembly-master-mix-assembly

WEDNESDAY, 24/7/2019

## Discussion:

It appears we have colonies on the transformation plates from GLOX sample 5. Re-streak was done from both of the sample 5 plates, picking four colonies from each plate. The new plates are called "Re-streak Transformation pBS1C3_LacI_GLOX(5):1 in DH5a Irina 24/7" and "Re-streak Transformation pSB1C3_LacI_GLOX(5):2 in DH5a Irina 24/7". The original plates where stored in the refrigerator.

# Lab #64 Gibson assembly (19-07-24) (Erik + Jenny + Gustav + Qian + Irina)

### Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating MnP, HRP, Lip, GLOX and AAO into shadow-vector pPICZαB respectively.

### Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Insert: MnP
Vector: pP1CZab
3 to 1 Insert mass of DNA insert

**GA Description and Values to Use in GA Mix (MnP…**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass (pP1Czab) desired | 100ng | |
| 4 | Number of microliters needed for vector | 7,4 ul | 100ngx 1ul/13,6 ng= |
| 5 | Linearized vector concentration | 13,6 ng/ul | |
| 6 | Insert (MnP) length | 1,2kb | |
| 7 | MnP concentration | 60,9 ng/ul | |
| 8 | Number of microliters needed for insert | 1,6 ul | 95,38ngx 1ul/60,9ng= |
| 9 | Number of ul needed for insert | 3,67 | |

**Gibson Assembly Mixture Compo…**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 1,6 | Insert MnP |
| 3 | 7,4 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 1,00 | water |

# Lab #64 Gibson assembly (19-07-25)

O/N culture (Jinwen's group)

# Lab #65 miniprep and gel digestion (19-07-26). (Irina + Qian + Jinwen + Erik)

## Aim:

To purify plasmid DNA from HRP, AAO, MnP, LiP, GLOX and pPICZaB

## Methods:

MiniPrep was done according to Thermo Fischer Scientific protocol: Thermo Scientific GeneJET Plasmid Miniprep Kit: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0013117_GeneJET_Plasmid_Miniprep_UG.pdf&title=VXNlciBHdWlkZTogR2VuZUpFVCBQbGFzbWlkIE1pbmlwcmVwIEtpdA==

with some adjustments (Irina):

- 300 uL of Resuspension solution where added
- 300 uL of Lysis buffer was adeed
- 350 uL of Neutralization buffer was added
- When washing the column for the first time we added 500 uL of Washing Solution and centrifuged 2 min/12000g. Thsi was done twice before the dry centrifugation step.
- After dry centrifugation step the column was moved to a new eppendorf tube and 50 uL ddH2O was added. Waited for approximately 5 minutes befor adding another 50 uL and then spinned 1 min/12000g.
- We measuerd the concentrations by Nanodrop2000. See table 1 for results.

# Lab #66. Analytical Digestion and Gel Analysis (19-07-26) (Jinwen + Erik)

## Aim:

We used analytical digestions and agarose gel electrophoresis in order see if our Gibson Assembly of 5 different enzymes: Lip, GLOX, MnP, HRP, AAO; were successful.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

Deviations from protocol.

**Analytical Digestion of MnP and HRP (Erik)**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | | ng/ul | DNA Quantity | buffer+enzyme | water |
| 2 | MnP 1 | 61,6 | 3,27 | 2,4 | 14,3 |
| 3 | MnP2 | 148,4 | 1,34 | 2,4 | 16,3 |
| 4 | MnP3 | 114,9 | 1,7 | 2,4 | 15,9 |
| 5 | HRP 1 | 57,9 | 3,5 | 2,4 | 14,1 |
| 6 | HRP 2 | 108,4 | 1,9 | 2,4 | 15,7 |
| 7 | HRP 3 | 91,3 | 2,2 | 2,4 | 15,4 |

Results: We got no bands for any of the samples. We got some results from the control. We think that is has something .to do with the gel itself

# Lab #67. Gibson assembly (19-07-26) (Jinwen + Erik+Tereza)

## Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating MnP, HRP, Lip, GLOX and AAO into shadow-vector pPICZαB respectively.

## Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Construct: HRP-2A-GFP-3
Vector: pP1CZab
2 to 1 Insert mass of DNA insert

**GA Description and Values to Use in GA Mix (HRP…**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 3,67 ul | 50ngx 1ul/13,6 ng= |
| 5 | Linearized vector concentration | 13,6 ng/ul | |
| 6 | Insert length | 1,9kb | |
| 7 | HRP-2A-eGFP concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 5,3 ul | 52,78ngx 1ul/10ng= |
| 9 | Number of ul needed for insert | 3,67 | |

**Gibson Assembly Mixture Compo…**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 5,3 | Insert HRP 2A GFP |
| 3 | 3,67 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 1,00 | water |

Deviations from Protocol:

1. Made a mistake on the PCR machine. Instead of putting 15 min, we put 15 seconds.As soon as it was discovered, we adjusted the the PCR machine, howerver, 10 minutes had elapsed.

Results:

1. We eventually got colonies on the plates. However, we had two different looking microorganisms on the plates. The E. coli looking colonies were restreaked and an overnight culture was done.

MONDAY, 29/7/2019

# Lab #68 - Preparation of Low salt LB media and addition of Zeocine (19-7-29) (Jenny + Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g but since we make low salt, we only add 3 g.
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 90 for components and amounts used to prepare 600 mL of LB.

**Table 90**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 3g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 300ul |

150 uL of Zeocine was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July Low salt LB + Zeo"*

# Lab #69 - Prepare agar plates with addition of antibiotics (19-7-29) (Jenny + Irina)

Additional LB plates with Zeocine will be prepared, as the stack is runing low.

## Aim:

To prepare  solutions necessary for future experiments. High ionic strength and acidity or basicity inhibit the activity of Zeocin. Therefore, it is recommended that the salt is reduced in bacterial medium.  The pH will be adjusted to 7.5 to keep the drug active.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

▷ Agar plates and Addition of Zeocine (Zeocine 100mg/mL stock) + autoclave

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Zeoine (25 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations for Zeocin stock (100 mg/mL) to 25 ug/mL
volume needed of 100 mg/ml stock: (25 uL/mL x 600 mL)/100 000 ug/mL) = 0.15 mL = 150 uL

Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 91 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 91**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Zeocine (100 mg/mL stock) | 150 uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LS LB + Zeo"*

TUESDAY, 30/7/2019

# Lab #67. Gibson assembly (19-07-30) (Jenny + Gustav + Erik + Irina)

## Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating HRP-2A-eGFP, HRP-2A_OOA and AAO-2A-eGFP into shuttle -vector pPICZαB respectively.

## Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Deviation from protocol:
Irina added 1 uL sample into 50 uL Competent cells, Gustav who also worked with HRP-2A-AAo added 2 uL of sample to competent cells.

Irina:
Construct: HRP-2A-AAO
Vector: pP1CZab
2 to 1 Insert mass of DNA insert
Calculations for total fragment amounts in GIbson Assemby mix for Irinas samples can be seen in table 92.

**Table 92**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 1.2 uL | 50ng/43 ng/uL |
| 5 | Linearized vector concentration | 43 ng/uL | |
| 6 | Insert length | 3.0 kb | |
| 7 | HRP-2A-AAO concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 8.3 uL | (2:1) 83.33 ng/uL/10ng |

Total amounts of Irinas fragments, ddH2O and mastermix for gibson assembly can be seen in table 93.

**Table 93**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 8.3 | Insert HRP-2A-AAO |
| 3 | 1.2 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 0.5 | water |

Calculations for total fragment amounts in GIbson Assemby mix for Eriks samples can be seen in table 94.

**Table94**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 1.2 uL | 50ng/43 ng/uL |
| 5 | Linearized vector concentration | 43 ng/uL | |
| 6 | Insert length | 1,9 kb | |
| 7 | HRP-2A-GFP concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 5,3 uL | (2:1) 52,78ng/uL/10ng |

Total amounts of Eriks fragments, ddH2O and mastermix for gibson assembly can be seen in table 93.

**Table95**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 5.3 | Insert HRP-2A-GFP |
| 3 | 1.2 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 3.5 | water |

## Lab #70 Overnight cultures from Lab #67 2019-07-30 (Gustav and Johan)

Aim: To create overnight cultures for midiprep for yeast expression.

Method: 50ml of low salt lb medium was placed into 7 E-flasks (erlenmyer-flasks). Each flask has been designated as to which colony from each respective agar plate th colony was picked from. E.g. Glox 3 was the third colony from the glox plate. After all the E-flasks have been filled with both a colony and lb medium the flasks were placed in a heat room over night.

# Lab #71 Colony PCR 2019-08-02 (Erik and Qian)

## Aim:

To screen two E.coli colonies for the HRP-2A-AAO  in construct from our previous transformations.

## Method:

Colony PCR Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

primers: ppic-fwd
primers: ppic-rev
Deviation from PCR Program

**Table2**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Temp. (*C) | 95 | 95 | 58 | 72 | 4 |
| 2 | Time (min) | 5 | 1 | 1 | 3 | infinity |
| 3 | | | {30cycles | | }}}}}}} | |

**Calculations**
length of amplicon 3,0 kb
taq extension 1min/kb
extension time= 3,0 kbx 1min/kb

**Reaction Mixture: Colony PCR**

| | A | B |
|---|---|---|
| 1 | What | Amount (ul) |
| 2 | ddH20 | 33.7 |
| 3 | 10x Taq PCR buffer | 5 |
| 4 | 2 mM dNTPS | 5 |
| 5 | primer FWD | 2.5 |
| 6 | primer Revers | 2.5 |
| 7 | Taq DNA polymeras | 0.3 |
| 8 | Colony in 30ul of water | 1 |

MONDAY, 5/8/2019

# ~~Lab #72 Electroporation (Irina + Jenny) 2019-08-05~~

~~**Aim:** Electroporate the DH5a to make them competent for further transformation with DNA from Viktors group; AAO-2A-eGFP and HRP-2A-eGFP.~~

**Method:** We follow the protocol **Rapid Protocol for Preparation of Electrocompetent *Escherichia coli* and *Vibrio cholerae*** (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3939052/) and the steps in their methods. Adjustments from the protocol is that instead of taking 1 ng of DNA we took 2 ul. We spun only for 3000g instead of 5000g.

Tubes were spun for 4000g x 5 minutes, 900 mL of supernatant was removed, and the pellet was resuspended in the remaining 100 uL (approx) and plated on low salt LB + Zeocin plates.

## Lab #73 Send for sequencing - AAO and Lip (19-08-05) (Erik + Irina)

Two sequences, AAO and Lip where sent for sequencing.

Calculations for AAO:
C1 = 70.1 ng/uL
C2 = 10 ng/uL
V2 = 15 uL
V1 = 10 ng/uL x 15 uL / 70.1 ng/uL = 3 uL

Stock Forward primer = 100 uM
Diluted 10 x = 2 uL forward primer + 18 ul ddH2O.

Mixture:
3 uL AAO
12 uL ddH2=
2 uL Forward primer (10 uM)

Mixed into a tube named EF30500584

Calculations for Lip:
C1 = 53.2 ng/uL
C2 = 10 ng/uL
V2 = 15 uL
V1 = 10 ng/uL x 15 uL / 53.2 ng/uL = 3 uL

Stock Forward primer = 100 uM
Diluted 10 x = 2 uL forward primer + 18 ul ddH2O.

Mixture:
3 uL Lip
12 uL ddH2O
2 uL Forward primer (10 uM)

Mixed into a tube named EF30500585

WEDNESDAY, 7/8/2019

## Lab 74 Overnight Culture

Aim: To take 6 separate E.coli colonies from the plates with the following constructs: pPICZaphaB_AAO, pPICZaphaB_AAO-2A_GFP, pPICZaphaB, pPICZaphaBHRP-2A-AAO to prepare for colony PCR.
Method:

1. 6ml of Lb low salt media was placedin a erlemeyer flask

2. A pipett tip was used to scoop up a single colony and then dropped into the erlemeyer flask
3. The erlemeyer flask were placed in the 37 degree room

THURSDAY, 8/8/2019

# Lab 75 Colony PCR with Gel Analysis (Erik + Qian+ Jin Wen+ Johan+ Jenny)

## MISSING: WHICH WAS WHICH: Jenny and Johan

### Aim:

To screen 6 E.coli colonies from each of the following constructs that come fromc our previous transformations: pPICZaphaB_AAO, pPICZaphaB_AAO-2A_GFP, pPICZaphaB, pPICZaphaBHRP-2A-AAO- in construct from our previous transformations.

### Method:

Colony PCR Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

primers: ppic-fwd
primers: ppic-rev
Deviations from protocol:

1. 1ml of overnight culture taken from each of the overnight cultures.
2. 400 ul spun down in centrifuge
3. 30 ul of water added to another eppendorf tube
4. Pipett tip used to dab the pellet, then stirred in 30ul of water

## Deviation from PCR Program some differences here that I think Qian wrote down

**Table3**

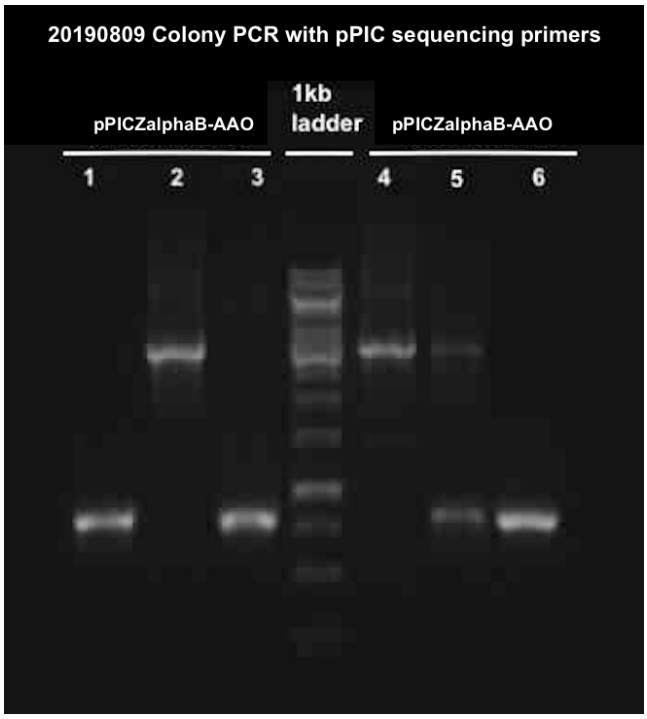
| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Temp. (*C) | 95 | 95 | 58 | 72 | 4 |
| 2 | Time (min) | 5 | 1 | 1 | 3 | infinity |
| 3 | | | {30cycles | | }}}}}}} | |

**Calculations**
length of amplicon 3,0 kb
taq extension 1min/kb
extension time= 3,0 kbx 1min/kb

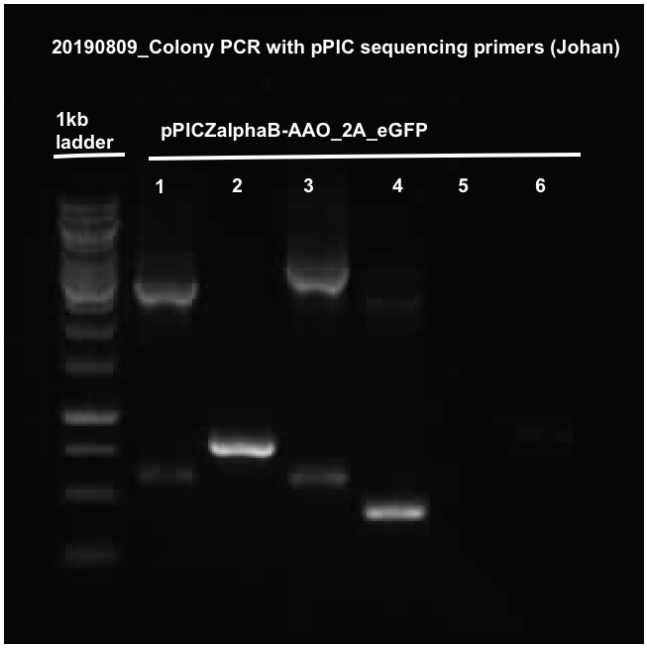
**Table4**

| | A | B |
|---|---|---|
| 1 | What | Amount (ul) |
| 2 | ddH20 | 33.7 |
| 3 | 10x Taq PCR buffer | 5 |
| 4 | 2 mM dNTPS | 5 |
| 5 | primer FWD | 2.5 |
| 6 | primer Revers | 2.5 |
| 7 | Taq DNA polymeras | 0.3 |
| 8 | Colony in 30ul of water | 1 |

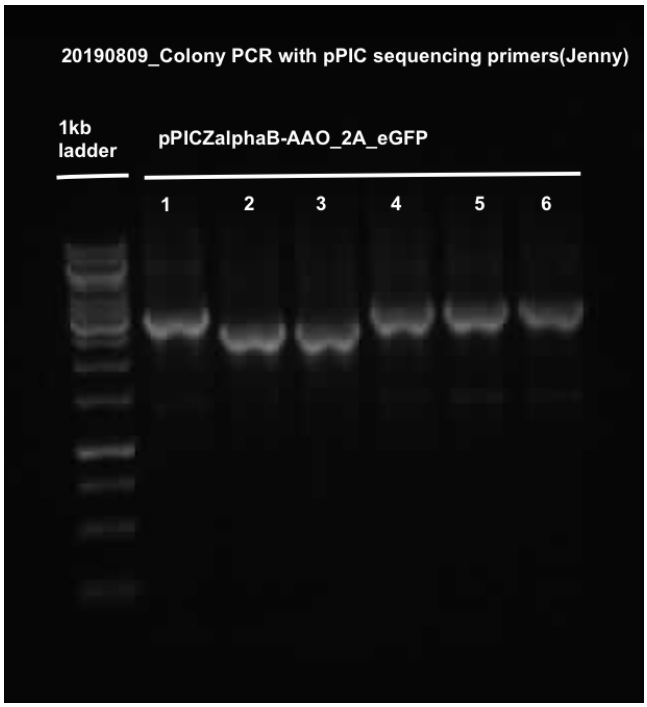
Gel of Colony PCR (AAO)



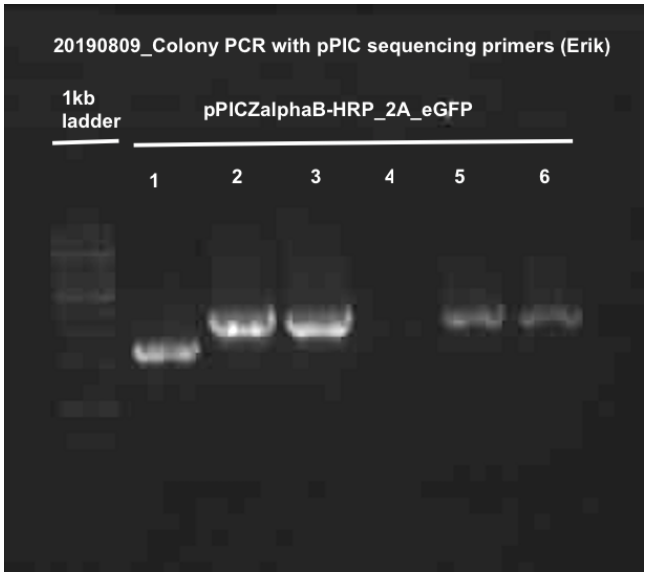
Gel of AAO_2A_eGFP




Gel of AAO_2A_eGFP

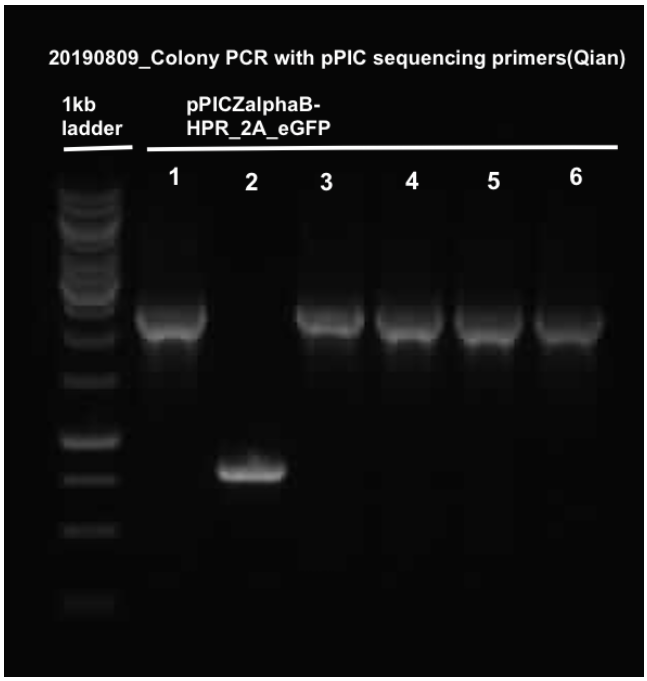


Gel of HRP_2A_eGFP from supercompetent cells




~~Analysis: Wells 2, 3, 5, 6 appear to have approximately the right length for the construct. Eventual glycerol stocks from 1 an4 were discarded.~~

Gel of HRP_2A_eGFP (From non-supercompetent cell s)




## ~~Lab 76 Glycerol Stock of Colony PCR~~

~~Aim:~~

~~To save the E.coli cells with the correct construct from lab 75 to eventually an overnight culture and midi prep..~~

~~Method:~~

1. ~~600 ul of the overnight culture was added to 400 ul of 50% glycerol stock~~
2. ~~Placed in the -80 degree freezer.~~

SATURDAY, 10/8/2019

## ~~Lab 77 4 SDS Page Gels~~

MONDAY, 12/8/2019

## ~~Lab 79: Overnight Culture of AAO and GlOX from Colony PCR (Lab 78) (Qian)~~

## ~~Lab 80: Expression in Recombinant Pichia Strains X33 MnP Pic1~~

# 3A assembly-Digestion and ligation

## Introduction

Cut out the destinated fragment  and ligate them together through 3A assembly method or gel purification.

## Materials

- DNA Sample
- ddH2O
- 10x reaction buffer for restriction enzymes provided by manufacturer
- restriction endonucleases
- 10x reaction buffer for T4 DNA ligase provided by manufacturer

## Procedure

### Digestion

- [ ] 1. Make three mixes: each contains 500 ng of one of the three plasmids and ddH2O to 43 µL.
- [ ] 2. To each mix, add 5 µL of 10x reaction buffer for restriction enzymes.
- [ ] 3. Add 1 µL each of the appropriate endonucleases (two per tube) according to Fig. 25 to give a final volume of 50 µL.
- [ ] 4. Tap on the tubes to mix. If necessary, centrifuge for a few seconds to spin down the liquid.
- [ ] 5. Incubate at 37°C for 30 min.
- [ ] 6. Heat-inactivate the enzymes by incubating at 80°C for 20 min.
- [ ] 7. At this point, samples may be stored at −20°C.

### *Gel analysis of digests (recommended for first time)*

- [ ] 8. Run 20 µl of each digestion mixture (200 ng) on a 1% agarose gel to measure the extent of digestion. Also run the three uncut plasmids (negative controls) directly beside their cut versions, and a DNA ladder marker should be loaded in a middle lane.

### *Ligation*

- [ ] 9. Add 2 µL (20 ng) of each of the three digestion mix- tures to 11 µL of water.

- 10. Add 2 µL 10x reaction buffer for T4 DNA ligase.
- 11. Add 1 µL of T4 DNA ligase to give a final volume of 20 µL.
- 12. Incubate at room temperature (~22°C) for 30 min.
- 13. Heat-inactivate the enzymes by heating at 80°C for 20 min.
- 14. At this point, samples may be stored at −20°C.

# Preparation of solutions

## Introduction

0.9% NaCl in total volume of 10 mL

## Materials

- NaCl
- ddH2O
  -

## Procedure

- [x] 1. 0.09 g of NaCL was weighted and transferred to a flask
- [x] 2. ddH2O was added to a total volume of 10 mL

# Preparation of solutions

## Introduction

0.9% NaCl in total volume of 10 mL

## Materials

- NaCl
- ddH2O
  -

## Procedure

- [x] 1. 0.09 g of NaCL was weighted and transferred to a flask
- [x] 2. ddH2O was added to a total volume of 10 mL

# Week 2: Jun 24 - Jun 30

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

MONDAY, 24/6/2019

## Lab #13 - BioBrick 3A Assembly continued - Ligation and transformation (19-6-24) (Irina)

### Aim:

Ligation of BBa_K608006, Bba_K592009 and pSBIK3 BioBricks™ followed by transformation on Kanamycin LB agar plates.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Note: gel analysis was done on 20/6.

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Components and amounts for ligation can be seen in table 22.

**Table 22**

| | Components | Bba_K608006 (amount) | Bba_K592009 (amount) | pSBIK3 (amount) |
|---|---|---|---|---|
| 1 | Digested sample | 2uL | 2uL | 2uL |
| 2 | T4 10x Reaction buffer | 2uL | 2uL | 2uL |
| 3 | T4 DNA ligase | 1uL | 1uL | 1uL |
| 4 | ddH2O | 11uL | 11uL | 11uL |

### Transformation

Components and amounts can be seen in table 23 for transformation reaction.

**Table 23**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - |
| 2 | DH5α competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

## Lab #15 - BioBrick 3A Assembly - Digestion (19-6-24) (Erik Palm)

### Aim:

Plasmid DNA prep of promotor pSB1C3 BBa_K608006 and gene BBa_K500003 (glucose oxidase) into vector (pSBIK3)

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``
Table 24 shows the initial concentrations of the DNA plasmids:

**Table 24**

| | A | B |
|---|---|---|
| 1 | Promoter (BBa_K608006) Concentration | 65.6 ng/ul |
| 2 | Glucose Oxidase (PSBIC3 BBa_ K500003) Concentration | 94.0 ng/ul |
| 3 | Destination Vector / Backbone (pSBIK3) Concentration | 25 ng/ul |

Table 25 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion.

**Table 25**

| | Component | BBU-KIOSIonb | BBU-K500003 | Vector: pSBIK3 |
|---|---|---|---|---|
| 1 | 500 ng DNA | 7.6uL | 5,3uL | 20uL |
| 2 | 10 x Reaction buffer | 5uL | 5uL | 5ul |
| 3 | EcoRI | 1uL | ------ | 1uL |
| 4 | SpeI | 1uL | ----- | ----- |
| 5 | XbaI | ---- | 1uL | ------ |
| 6 | PstI | ---- | 1uL | 1uL |
| 7 | ddH2O | 35,4 | 37,7 uL | 23uL |

Calculations
Amount of ml needed for digestion
psBIC3 -BBU- KIOSIonb 500ng x 1ul/ 65.6 ng= 7,6 ul
psBIC3- K500003 500ng x 1ul/94.0ng = 5.3 ul
pSBIK3 backbone 500 ng x 1ul/25 ng =20 ul

## Lab #16 - Preparation of 1x TBE solution through Dilution of 1x TBE solution - (19-6-24) (Erik Palm)

Dilution of 10xTBE to make 1 liter of 1x TBE

900 ml of ddH20 added to 100ml of 10x TBE

## Lab #17 - Agarose Gel Electrophoresis - (19-6-24) (Erik Palm)

### Aim:

To separate and analyze nucleic acid samples under non-denaturing conditions

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 18 for components and amounts mixed for agarose gel

Instead of composing 150 mL of gel, 50 mL of gel was used instead. Table 26 shows components in the agarose gel

### Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was wSeighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

**Table 26**

| | A | B |
|---|---|---|
| 1 | Amount | Component |
| 2 | 0,5 g | agarose |
| 3 | 50ml | 1x TBE |
| 4 | 5ul | Sybr Safe |

## Running the gel

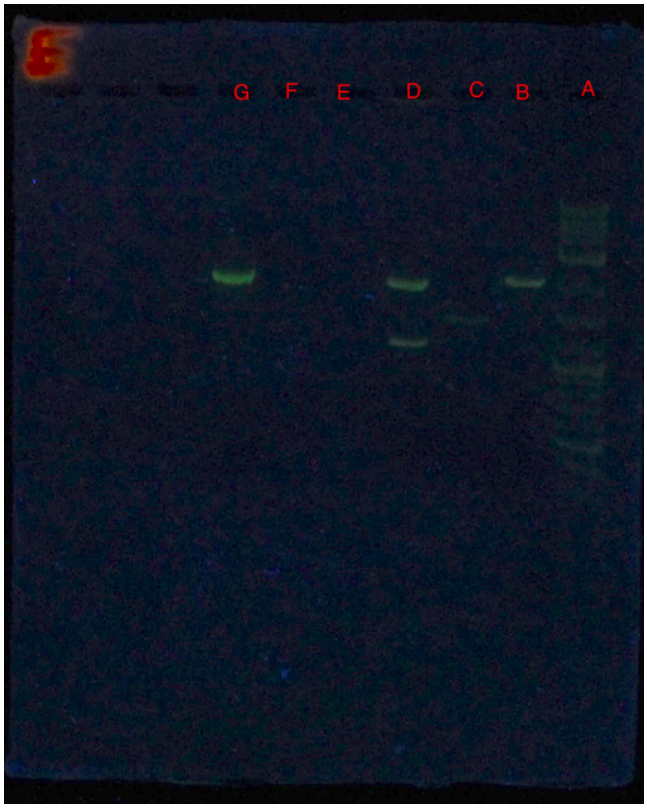
1. The gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. The DNA samples (~0.2 µg)were mix with loading dye (table 18) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply. and the gel was run at **120 V** for **80 min.**
8. After the run, the gel bands were visualized under UV and a picture was taken (figure ...).

Table 27 below shows the components of the loading solutions placed in the wells, well number, and calculations for Erik Palm's gel analysis.

**Table 27**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed | Size (kBp) according to iGEM |
| 2 | A | Ladder (2-log) | 5 ul | -- | |
| 3 | B | Promotor (BBa_K608006) | 20 ul<br>4ul loading dye | | 56 |
| 4 | C | Uncut promotor (BBa_K608006) | 5.7 ul of sample 14,3 ul of H20 4 ul loading dye | 200 ngx 1ul/ 35.1 ng= 5.7 ul of sample | |
| 5 | D | Glucose oxidase (BBa_K500003) | 20 ul sample<br>4 ul loading dye | | 1677 |
| 6 | E | Uncut Glucose oxidase (BBa_K500003) | ------- | | |
| 7 | F | Backbone pSB1K3 | 20 ul sample<br>4ul loading dye | | 2204 |
| 8 | G | Uncut backbone pSB1KR | 8 ul sample<br>12 ul H20<br>4 ul loading dye | 200ng x 1ul /25ng =8 ul sample | |

Erik Palm Digestion Analysis 25.06.2019.png




Analysis of gel:
-Lane C had a lower molecular weight because of supercoiling. Not linearized plasmid.
-Lane B Can't see promotor because it was so small and ran off the gel.
-Lane E was left open because glucose oxidase gene ran out. This means that there is no control for the successful digestion of this plasmid.
-Lane F: Possible reason for it being empty is that their no actual sample in the tube. Therefore digestion was recommended again in order to proceed to the next step so that the promotor and gene have a backbone that they can be placed in.
-Erik Palm left the digestion samples at room temperature for more than an hour after gel analysis was done. When the mistake was discovered, sampled were placed in freezer. Since it is dDNA, samples should function normally, but possible source of future error.

## Lab #18 - Preparation of LB media and addition of Chloramphenicol (19-6-25) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g

m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 28 for components and amounts used to prepare 600 mL of LB.

**Table 28**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Chloramphenicol was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"LB + Cm Irina 25/6"*

## Lab #19 - Prepare agar plates with addition of antibiotics (19-6-25) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Chloramphenicol 25 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Chloramphenicol (25 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 29 for components and amounts used to prepare 600 mL of LB.

**Table 29**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Chloramphenicol (25 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"June LB + Cm"*

## Lab #20 - Transformation (19-6-25) (Irina)

### Aim:

Transformation of Bba_K500001 , BBa_K00000 annd Bba_K500003, J04500 and Irinas Competent cells + J04500 on Chlormaphenicol LB agar plates.

### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-114;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Transformation Protocols;

Components and amounts can be seen in table 30 for transformation reactions.

**Table 30**

| | Components | MnP - K500001 | LiP - K500000 | GLOX - K500003 | Lac -J04500 | Irinas competent cells + J04500 | (-) control |
|---|---|---|---|---|---|---|---|
| 1 | Ligation reaction mixture | 1uL | 1uL | 1uL | 1uL | 1uL * | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50uL | 50uL | 50uL (Irinas) | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950uL | 950uL | 950uL | 950uL |
| 4 | ddH2O | - | - | - | - | - | 1uL |

* Might have been 10 uL added

## Lab #21 - Overnight cultures on Transformations from 19-06-24 (19-6-25) (Irina)

### Aim:

O/N cultures to have ready for miniprep the following day.

### Method:

### Lab #21 - Transformation+ligation (25-06-19) (Gustav)

Aim: The aim was to transform and ligate the promoter (k608006), CDS (k500003) and the plasmid backbone (psb1k3.m1) into competent E.coli DH5α and plate onto agar plates.

Method:
The methods described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm concerning protocols 6 and 3 were followed (OBS only the ligation part of protocol 3 was followed). The only deviation from the decribed methods was that when performing the transformation mentioned in protocol 6, 10 microliters of the respective additive was used instead of 5 microliters.

The E. Coli strain used was DH5α.
The ligation was done by adding the following: (all units are in microliters):

2 Promoter
2 backbone
2 CDC
11 ddH2O
2 ligation buffer
1 ligase

In total 4 plates were produced: 1 negative control, 1 ligation transformation, 2 positive controls (1 10% and 1 "regular").
Both of the positive controls were grown on Chloramphenicol media
The ligation and negative controls were grown on kanamycin media

26/6/19: Due to poor quality of competent cells, a new transformation has to be done using another batch of competent E. Coli cells.

Pictures are shown below just above entry #27



## Lab #22 - BioBrick 3A Assembly - Digestion (19-6-25) (Erik Palm)

Aim:

Plasmid DNA prep of only thebackbone (pSB1A3) to make up for the mistake in digestion shown in the gel analysis.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``
Table 31 shows the compents placed in eppendorf tube.

Changes in protocol: Erik Palm ran out of PSB1K3 as was done in the Lab #25. Therefore, the backbone was switched to pSB1A3 incstead (ampicillin resistance).

**Table31**

| | Component | Vector: pSB1A3 |
|---|---|---|
| 1 | 500 ng DNA | 20uL |
| 2 | 10 x Reaction buffer (2.1 New England Buffer) | 5ul |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ----- |
| 5 | XbaI | ------ |
| 6 | PstI | 1uL |
| 7 | ddH2O | 23uL |

Calculations:
500ng x 1ul/25ng= 20 ul pSB1A3

# Lab #23 - BioBrick 3A Assembly continued - Ligation and transformation (19-6-25) (Erik)

## Aim:

Ligation of BBa_K500003, BBa_K608006 and pSB1A3 BioBricks™ followed by transformation on Ampicillin LB agar plates.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Ampicillin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Components and amounts for ligation can be seen in table 32.

**Table32**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500003 | 2uL |
| 2 | Digested BBa_K608006 | 2uL |
| 3 | Digested pSB1AC | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

Changed in Protocol: We did not heat-inactivate DNA ligase.

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

**Table33**

| | Components | Transformation sample | (-) control | (+) control |
|---|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50 uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950 uL |
| 4 | ddH2O | - | 5uL | - |
| 5 | BBa_J04450 | - | - | 5ul |

Positive Control: We used BBa_J04450 pSB1A3
Negative Control: Water

Results:
All of the plates (negative control, positive control and ligation reaction mixture) contained bacterial colonies. Since the negative control had bacterial growth, contamination is expected. Transformation will be done again using the left over ligation solution.

# Lab #24 - Overnight cultures on Transformations from 19-06-25 (19-6-26) (Irina)

## Aim:

Prepare O/N cultures of MnP (Bba_K500001), LiP (BBa_K00000), GLOX (Bba_K500003), Lac (J04500) and Irinas' competent cells + Lac J04500 for in 6 mL of LB + Cm miniPrep the 27/6 and Re-streak of same colonies on LB - Cm plates.

### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 114; Step 14.
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Transformation Protocols;

### Procedure:

One colony per plate was transferred to 15 mL Falcon tubes containing 6 mL of LB + Cm medium. The same colony was chosen for re-streaking on LB + Cm plates. Samples were stored in the 37 °C room O/N. The original plates were stored in the refrigerator.

O/N Cultures made 25/6 where discarded (Bba_K608006, Bba_K592009, pSBIK3), as no colors had been seen on the plates or in the cultures.

## Lab #25 - Competent cell count (19-6-26) (Irina)

Transformation efficiency:
Nr of colonies on plate: 308
J04500 - 10pg/uL
Amount used 1 or 10 uL, see table 30.
If 1 ul used: 308/ (10 x $10^{-6}$) = 3.08 x $10^{7}$, but it is more likely 10 uL were used which would give 3,08 x $10^{6}$.

## Lab #26 - Gel Analysis of Restriction Enzyme (19-6-26) (Erik, Vanja and Johanna)

### Aim: To check if the digested backbone (pSB1A3) was actually digested or not.

### Method:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

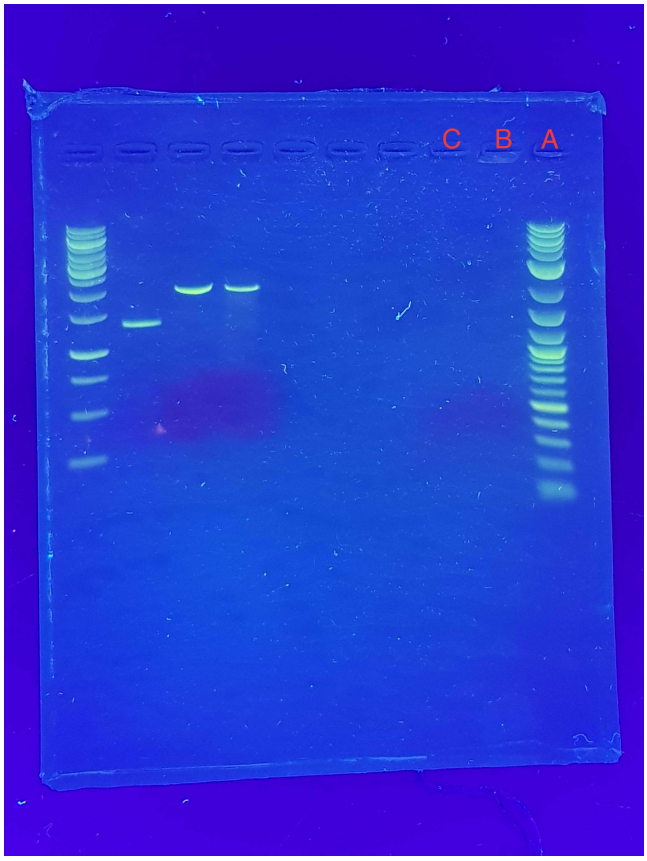
-Johanna and Vanja prepared the gel. Erik simply put in his cut and uncut backbone (pSB1A3)
-When putting in the uncut backbone, there was not enough pSB1A3. Instead of 8 ul, there was 4 ul. The total loaded amount of solution that was loaded into the well was 16 ul.

Table 34 below shows the components of the loading solutions placed in the wells, well number, and calculations for Erik Palm's gel analysis. The other well was part of another experiment

**Table34**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | Ladder (2-log) | 5 ul | -- |
| 3 | B | digested backbone (pSB1A3) | 20 ul<br>4ul loading dye | |
| 4 | C | uncut backbone (pSB1A3) | 4 ul of sample<br>12 ul of H20<br>4 ul loading dye | 200 ngx 1ul/ 25ng ng= 8 ul of sample |

26-06-2019 Gel Analysis backbone.jpg




-Both wells B and C with the cut and uncut backbone were not present in the gel.
-Given the modification to well B, it is possible that that could explain why no bands are present. However, since lane C also has not bands, it could be that pSB1A3 sample is not functioning. It is quite old, from 2014. This will be confirmed or denied if the negative control and the experimental plate is successful.

## Lab #27 - BioBrick 3A Assembly continued - Transformation (27-6-25) (Erik)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Transformation of Competent E.coli cells

Components and amounts can be seen in table 33 for transformation reaction.

**Table35**

| | Components | Transformation sample | (-) control | (+) control |
|---|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - | - |
| 2 | DH5α competent cells | 50uL | 50uL | 50 uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL | 950 uL |
| 4 | ddH2O | - | 5uL | - |
| 5 | BBa_J04450 pSB1C3 | - | - | 5ul |

Components and amounts can be seen in table 33 for transformation reaction.
Positive Control: We used BBa_J04450 psB1C3
Negative Control: Water

There was no pSB1A3 left, which is what is used in the transformation sample, which is why pSB1C3 is used instead. As a result, Erik plated two LB + Cm plates for the positive control, while the negative control and transformation sample was plated on LB + Amp

During Step 6 of the procedure, there was some confusion as to what backbone Erik was supposed to be using. The samples were thus on ice for 15 min instead of 5min.

Prediction for results: if the the gel analysis and experimental results match, the negative control and the sample must be negative, and the positive control should be positive. The transformation sample should be negative because there is no function backbone, since none appeared in the gel.

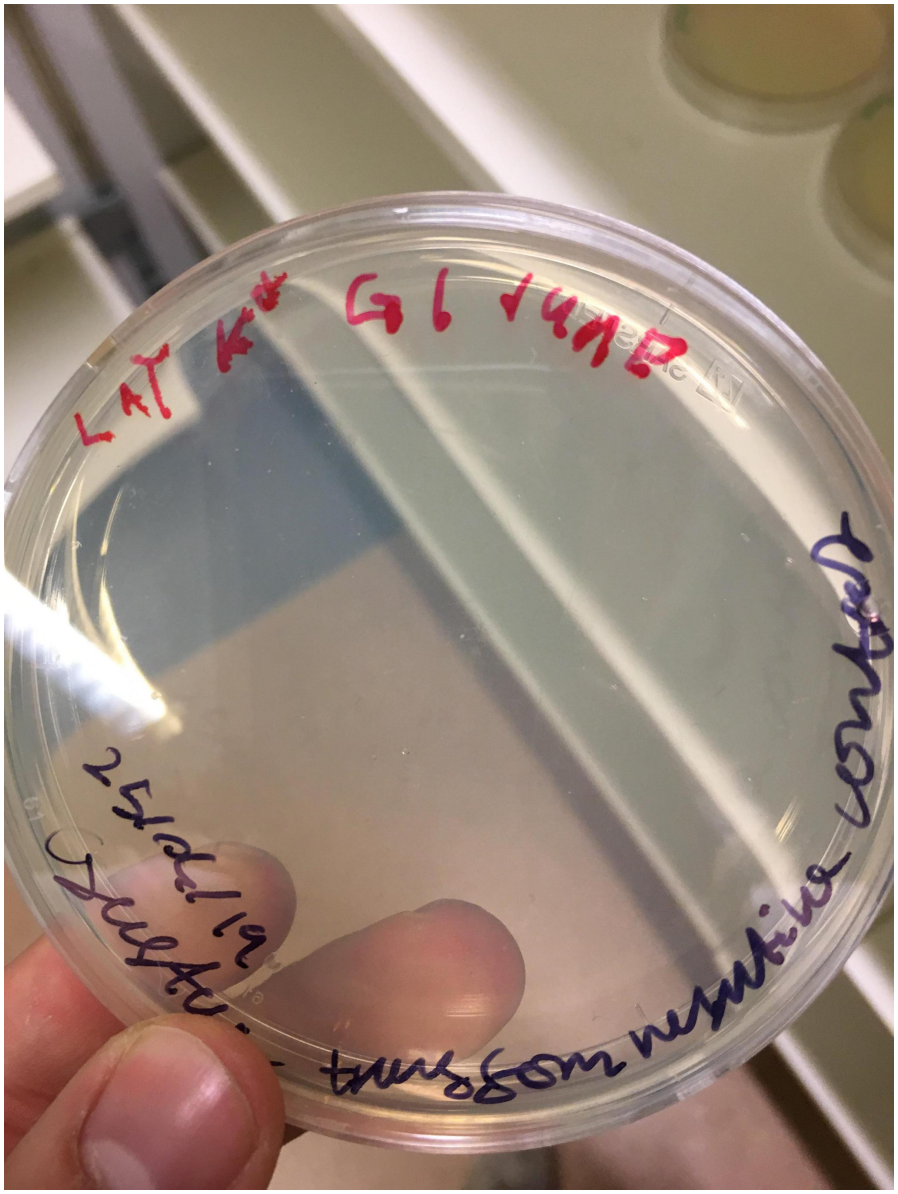
Results: Analysis of plating

All of the plates are postive. The destination was present, making it unclear what happened in the gel analysis. Previous conjectures about how old the sample was must not have been true.

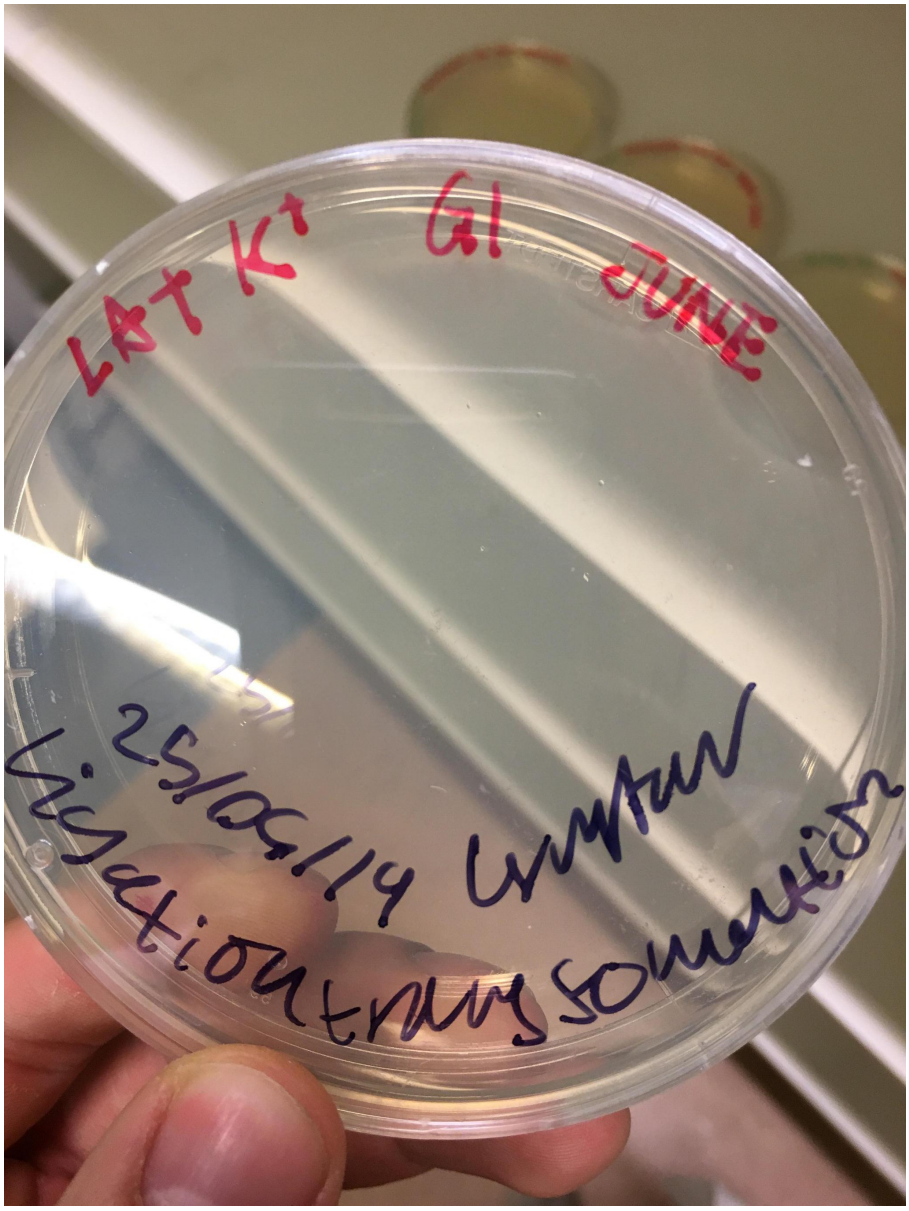
65969539_630288457467569_6380744955584839680_n.pdf

These below are Gustavs, not sure why they are on my Lab transformaiton

65204585_2440422172858938_5685583912948989952_n.jpg



65079508_911057869253882_7529580692162740224_n1.jpg



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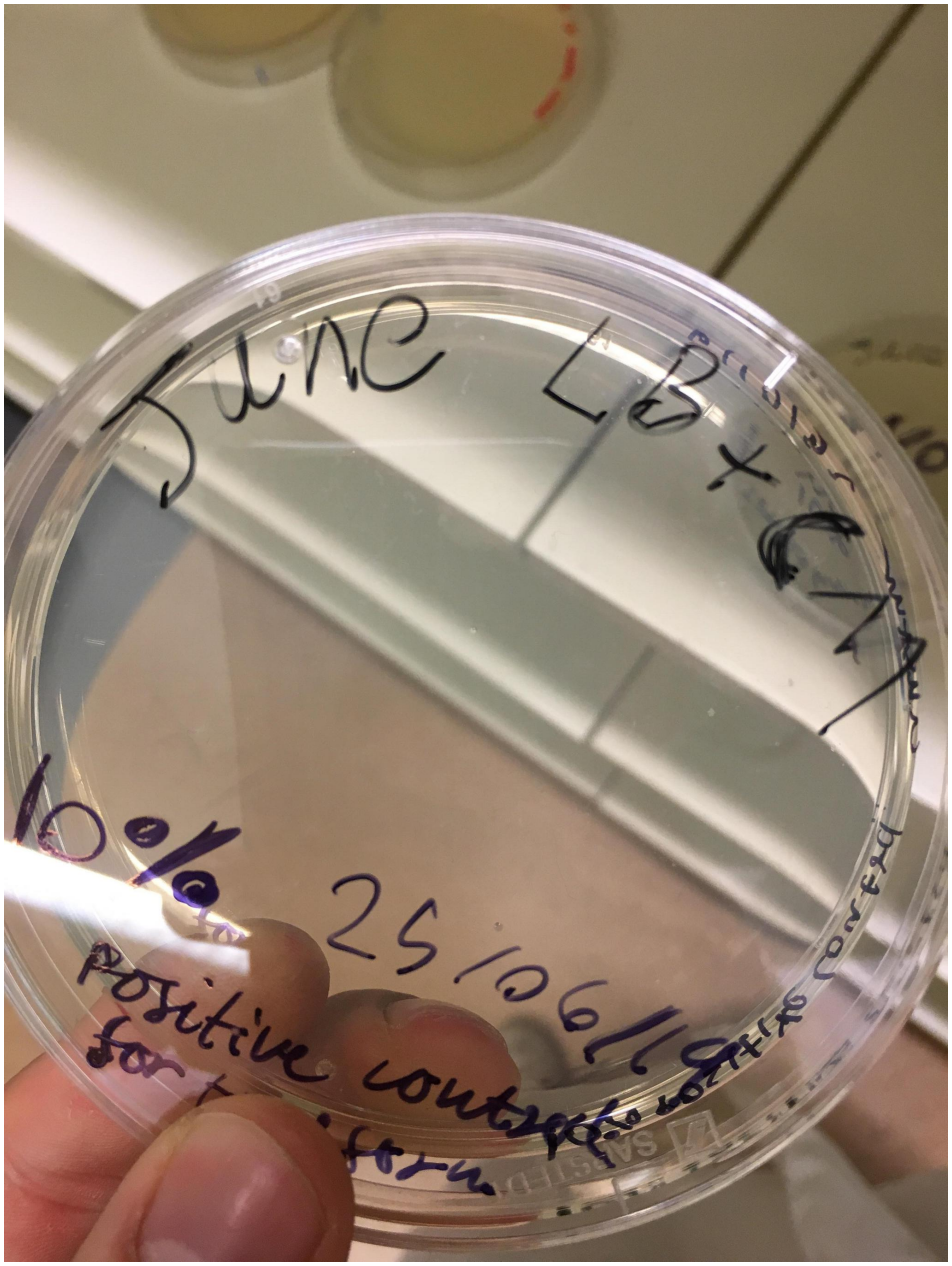
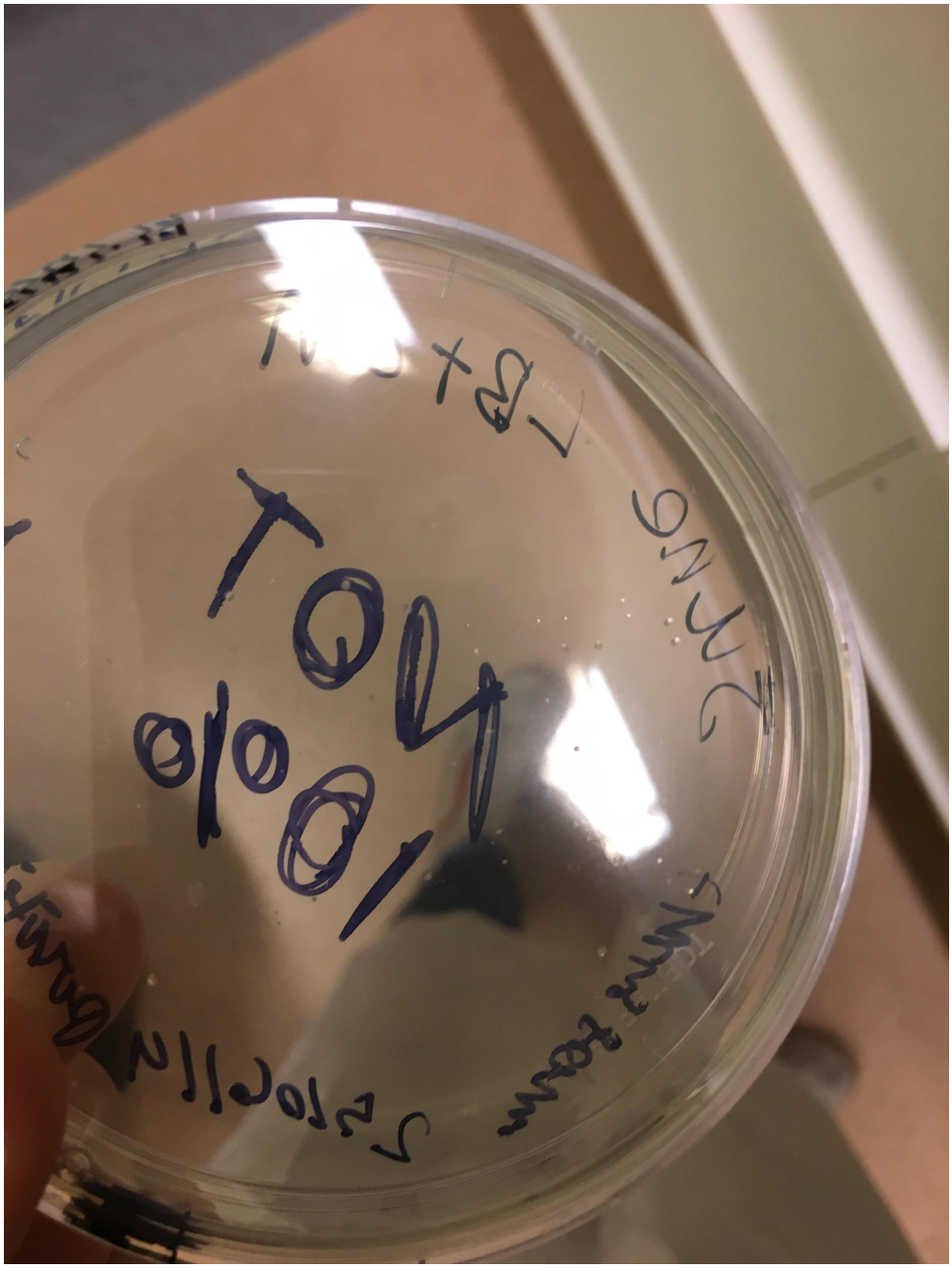


65744568_694188691015425_7994566676400046080_n3.jpg



Lab

## Lab #28 26-6-19 (Gustav)

Aim: To repeat #22 procedure in order to tranform cells
Method: The method, as with entry #21, is specified in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm, Protocol 6. The following deveations were made from said protocol:

- Step 3 10 microliters of the ligation mixture, ddH2O and of the positive cotrol was used due to reccomendation of lab leader.
- Step 6 waiting time was extended from 5 mins to 20 mins due to SOB buffer not being pre-heated to 37°C.
- Step 7 the SOB buffer was not at 37°C when applied to the cell mixture.

- Instead of plating a total of 4 agar plates, 5 were prepard. 3 K+ plates and 2 C+ plates. The extra K+ plate was used to cross reference the competence of Gus cells to another lab partners to see if a) the ligation mixture was working b) see if Gus cells were valid for future usage. The two C+ plates were used for positive control, 1 10% and one regular.

The plasmid used for the positive control was PSB1C3 BBa_J04450.

There was growth on the positive control of the transformation done 25-6-19. Above this entry there is a picture showing the plate. It is noted as "NOT 10%". The compentent efficiency of ths plate was 31*10^4. The calculation went as follows:

- Stock solution used was 10 pg/ul
- 10 ul from stock was used
- This results in 100pg of DNA being present
- 31 colonies were counted
- 31/100pg = 31*10^4.

THURSDAY, 27/6/2019

## Lab #29 - Preparation of competent *E.coli* BL 21 (DE3) cells using $CaCl_2$ (19-6-27) (Irina + Erik)

### Aim:

To increase the competence of *E. coli* BL 21 (DE3) cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Calculations for $CaCl_2$ solutions can be seen in table 35.

**Table 35**

| | A | 1M CaCl2 | 50% Glycerol stock | ddH2O | TOTAL volume (ml) |
|---|---|---|---|---|---|
| 1 | 0.1. M CaCl2 (15ml) | 2 ml | / | 18ml | 20ml |
| 2 | 0.1 M CaCl2 + 20% glycerol (3ml) | 0.3ml | 1.2ml | 1.5ml | 3ml |

## Lab #30 - Plasmid miniprep for pSB1C3_MnP, pSB1C3_LiP, pSB1C3_GLOX, pSB1C3_Lac, continued from lab#24 (Jinwen)

### Aim:

to get enough plasmid containing the genes of peroxidases and oxidases and inducible promoter as above for 3A assembly to build constructs: pSB1K/A3_J04500_K500001(Lac+RBS+MnP), pSB1K/A3_J04500_K500000(Lac+RBS+LiP), pSB1K/A3_J04500_K500003(Lac+RBS+GLOX)

**Also to get enough gene materials for PCR, in order to add overhang on these genes for Gibson assembly to assemble these genes with shuttle vector.**

### Methods:

- Plasmid miniprep kit instruction of **GeneJET Plasmid Miniprep Kit (K0502)**

### Results:

- *Input conc. report*

## Lab #31 - Digestion and gel analysis for pSB1C3_MnP (Bba_K500001), LiP (BBa_K00000), GLOX (Bba_K500003), Lac (J04500) (Jinwen)

### Aim:

To prepare for the 3A assembly for building constructs: pSB1K/A3_J04500_K500001, pSB1K/A3_J04500_K500000, pSB1K/A3_J04500_K500003

### Methods:

3A assembly-Digestion and ligation

### Results:

*Insert gel picuture*

### Discussion:

Digestion for backbone has not been done, due to the lack of backbone. Find a pSB1K/A3_RFP would help. After getting done with digesting the backbone, then the Ligation part can be continued.

After ligation, we can transform ligation mixture into DH5a competent cells. If we are lucky, we can get some colony on our ligation plate, and then re-streak the colony, and grow O/N culture, plasmid miniprep and digestion to examine whether we get the right construct as we designed. If we are lucky, we get the right construct, then we can transform constructs (plasmid miniprep result) into BL21, incubated with **IPTG** to get overexpression, and do ***SDS-PAGE, Coomassie Blue Staining*** to compare the difference of Induced expression and uninduced expression.

# Cloning custom sgRNAs into Zhang lab plasmids

## Introduction

This protocol is for cloning **PX330-based plasmids** including **PX458-462** - SpCas9 (or SpCas9n D10A nickase) + single guide RNA. It also applies to PX260 and PX334 - SpCas9 (or SpCas9n D10A nickase) + CRISPR array + tracrRNA.

It is copied here from information from the Zhang Lab made available on Addgene. Also see the CRISPR forum for any help needed.

To edit this protocol, sign in with Benchling, click the clock icon on the top right, and click the *Clone From Version* button.

**Bolded values in spreadsheets below indicate ones that you should change. Mixture calculations will automatically update.**

## Materials

- SpCas9 Plasmid (Addgene)
- FastDigest BbsI (Fermentas)
- FastAP (Fermentas)
- 10X FastDigest Buffer (Fermentas)
- QIAquick Gel Extraction Kit (Qiagen)
- 10X T4 Ligation Buffer (NEB)
- T4 PNK (NEB)
- sgRNAs in oligo pairs
- 2X Quick Ligase Buffer (NEB)
- Quick Ligase (NEB)

## Procedure

- [x] 1. Digest and dephosphorylate 5 µg of plasmid with BbsI for 30 min at 37°C.

**Table1**

| | A | B |
|---|---|---|
| 1 | | **Amount** |
| 2 | **Plasmid concentration (µg/µL)** | **1** |
| 3 | Plasmid (µg) | 1 |
| 4 | Plasmid volume (µL) | 1 |
| 5 | FastDigest BbsI | 1 |
| 6 | FastAP | 1 |
| 7 | 10X FastDigest Buffer | 2 |
| 8 | ddH2O | 15 |
| 9 | Total (µL) | 20 |

00:30:00

2. Gel purify digested plasmid using QIAquick Gel Extraction Kit and elute in EB. (See protocol.)

3. Phosphorylate and anneal each pair of oligos:

**Table2**

| | A | B |
|---|---|---|
| 1 | **Number of pairs** | **5** |

**Table3**

| | A | B | C |
|---|---|---|---|
| 1 | | **Amount per rxn (µL)** | **Master Mix (µL)** |
| 2 | 10X T4 Ligation Buffer | 1 | 5 |
| 3 | ddH2O | 6.5 | 32.5 |
| 4 | T4 PNK | 0.5 | 2.5 |
| 5 | Master Mix Total | 8 | 40 |
| 6 | Oligo 1 (100 µM) | 1 | |
| 7 | Oligo 2 (100 µM) | 1 | |
| 8 | Reaction Total | 10 | |

Please use the T4 **Ligation** Buffer since the buffer supplied with the T4 PNK enzyme does not include ATP (or supplement to 1mM ATP).
Put the phosphorylation/annealing reaction in a thermocycler using the following parameters:

**Table4**

| | A | B |
|---|---|---|
| 1 | 37°C | 30 min |
| 2 | 95°C | 5 min and then ramp down to 25°C at 5°C/min |

Dilute annealed oligos from Step 3 at a 1:200 dilution into sterile water or EB.

4. Set up ligation reaction and incubate at room temperature for 10 min:

**Table5**

| | A | B | C |
|---|---|---|---|
| 1 | | **Amount** | **Master Mix (µL)** |
| 2 | **BbsI digested plasmid concentration (ng/µL)** | **50** | |
| 3 | BbsI digested plasmid (from step 2, ng) | 50 | |
| 4 | BbsI digested plasmid (volume) | 1 | 5 |
| 5 | 2X Quick Ligase Buffer | 5 | 25 |
| 6 | ddH2O | 3 | 15 |
| 7 | Master Mix Total (µL) | 9 | 0 |
| 8 | Oligo duplex (1:200 dilution) (from step 3) | 1 | |
| 9 | Quick Ligase | 1 | |
| 10 | Reaction Total (µL) | 11 | |

00:10:00

5. *(optional)* Treat ligation reaction with PlasmidSafe exonuclease to prevent unwanted recombination products.

**Table6**

| | A | B | C |
|---|---|---|---|
| 1 | | **Amount (µL)** | **Master Mix (µL)** |
| 2 | 10X PlasmidSafe Buffer | 1.5 | 7.5 |
| 3 | 10mM ATP | 1.5 | 7.5 |
| 4 | ddH2O | 1 | 5 |
| 5 | Master Mix Total | 4 | 20 |
| 6 | Ligation reaction (step 4) | 11 | |
| 7 | Reaction Total | 15 | |

6. Transformation

# Week 3: Jul 1 - Jul 7

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

MONDAY, 1/7/2019

## Lab #32 3A Digestion with Jinwens samples 1/7/19 (Gustav and Lilli)

Due to uncertanty in labeling of DNA samples, 3A assembly will be postoned until confirmation of samples identification.
The procedure detailed in lab #28 concluded with cell growth on the agar plate with Irenas compentetne cells and Gustavs ligated plasmids. These colonies are not blue so restreaking will be preformed as well as O/N growth to confirm that these cells contain the correct construct. Colony growth was also witnessed on a previous transformed plate, this colony will also be restreaked. (Gustav)

Method: The method followed for restreaking is described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm Protocol 7, Alternativ/Method 1.

After futher discussion, 3A assembly is to be carried out.
Due to lack of backbone, the backbone digestion will not be carried out.
The 3A assembly digestion is described in lab #15. The promoter used was: BBa_K50003, the gene used was that for glyoxal oxidase.
The following calculations were made to recieve a 500ng DNA solution:

| Promoter: | Gene (glyoxal oxidase): |
|---|---|
| 500/83.1 = 6 microL | 500/94.5 = 5.3 microL |
| 5 miroL buffer | 5 microL buffer |
| 1 microL EcoRI | 1 microL XbaI |
| 1 microL SpeI | 1 microL PstI |
| 37 microL ddH2O | 37.7 microL ddH2O |

After gel analysis we concluded that the correct digestion had occured. The gel is displayed below:

image.png


From left to right: Ladder (1kb generuler), digested promoter, undigested promoter, digested GLOX, undigested GLOX

## Lab #33 - Preparation of LB media and addition of Kanamycin (19-7-01) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 36 for components and amounts used to prepare 600 mL of LB.

**Table 36**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Kanamycin was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July LB + Kan"*

## Lab #34 - Prepare agar plates with addition of antibiotics (19-7-01) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Kanamycin 50 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Kanamycin (50 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 37 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 37**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Kanamycin (50 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Kan"*

# Lab #35 - Casting Agarose Gels - (19-7-01)  (Irina)

## Aim:

To separate and analyze nucleic acid samples under non-denaturing conditions

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 38 for components and amounts mixed for agarose gel

Instead of composing 150 mL of gel, 50 mL of gel was used instead. Table 26 shows components in the agarose gel

## Procedure Casting a 50 mL gel

1. The ends where closed by a casting stand .
2. the comb was inserted into the gel tray at the ~1 cm from the edge.
3. For a 1% 50 mL agarose gel, 0.5 g agarose was wSeighted into a 150 mL conical flask. 50 mL 1x TBE buffer was added.
4. The agarose was dissolved in the buffer by swirling and microwaved for a few minutes.
5. The agarose solution was left to cool down and 5 uL of Sybr®Safe DNA stain was added.
6. The gel was left to solidify while cooling down to room temperature for approx. 30 min.

**Table 38**

| | A | B |
|---|---|---|
| 1 | Amount | Component |
| 2 | 0,5 g | agarose |
| 3 | 50ml | 1x TBE |
| 4 | 5ul | Sybr Safe |

Four gels were cast, one of them where stored in the cold room at 4 °C labelled "1% Agarose gel 1/7"

# Lab #36 - BioBrick 3A Assembly -  Digestion (19-7-01)  (Erik Palm + Jenny Eriksson)

## Aim:

Digestion of the backbone (pSB1K3), lac operator and BBa_K500001 and gel analysis of the results.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;``

Changes in protocol: We ran out of destination vector pSB1K3. Therefore used digested pSB1K3 made by Irina 19-06-20 instead for eventual gel analysis and ligation. Thus only two digestion mixtuers were prepared, the promotor  and the MnP gene.

Table 39 shows amounts used in digestion mixture.

**Table 39**

| | Component | Amount of component | ddH20 | Reaction Buffer 10x | EcoRI | Spe1 | Xba1 | Pst1 |
|---|---|---|---|---|---|---|---|---|
| 1 | Lac promotor | 6.02 ul | 37 ul | 5 ul | 1 ul | 1 ul | - | - |
| 2 | BBa_K500001 | 4.07 ul | 39 ul | 5 ul | - | - | 1 ul | 1 ul |

Calculations to calculate amount of solution needed for digestion:
500ng x 1ul/83.1ng= 6.02 ul Lac promotor
500ng x 1ul/122.8ng = 4.07 ul BBa_K500001

## Lab #37 - Agarose gel electrophoresis (1-7-19) (Irina + Erik+Jenny)

### Aim:

To ascertain whether cuts of the of the miniprep sample of the lac promotor and the gene (MnP) were successful or not.

### Method:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

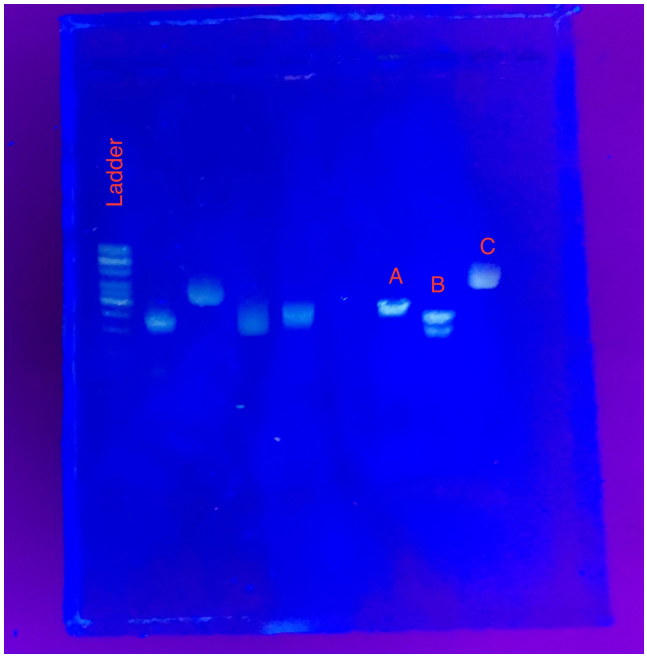
Analytical Digestion and Agarose Gel Electrophoresis

-Agarose gel prepared by Irina.
-See agarose gel preparation for Irina (Lab 35), but again, it was 50 mL plates, not 150mL
-We used a new gel electrophorator at 120 V for 30 min. 120 V was used since the other gel electrophorator took such a long time to run. However, this may not be necessary for the new ge electrophorator, which may have caused the strangely compressed ladder??

**Table40**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | digested lac promotor | 20 ul<br>4ul loading dye | -- |
| 3 | B | the digested gene MnP (BBa_K500001 ) | 20 ul<br>4ul loading dye | |
| 4 | C | undigested gene MnP (BBa_K500001 ) | 1,63 ul of sample<br>18.7 ul of H20<br>4 ul loading dye | 200 ngx 1ul/ 122,8ng = 1,63ul of sample |

01:07:2019EPJAgarosGelDigestionMnP.jpg




From left to right:
Ladder: Generuler 1 kb (ladder) (prepared by Gustav)
Four samples from Gustav Ahlstroms group
One empty well
A: the cut Lac promotor
B: the cut BBa_K500001
C: uncut BBa_K500001.

Observations:
-In terms of the number of bands that we see, it is consistant to what we expect. A, which contains the promotor (only 31 bp) has one bond and the promotor itself is so small it probably has run off the gel or is difficult to see.
-Well B has two cuts, which makes sense since the MnP is 1134 bp and the backbone is 2070 bp. Two bands should be seen.
-Well C has one cut, which reflects the fact that it is a whole plasmid. However, it is a little strange that whole plasmid did not super coil and travel farthern than the linearized DNA fragments of B and C.
-Also the ladder is a weird looking, it looks compressed and the sizes do not match up with the expected sizes.

Conclusions:
-We decided to continue with ligation and transformation despite the weird looking ladder. Adjust voltage for next time.

## Lab #38 -BioBrick 3A Assembly continued - Ligation and transformation (19-7-1) (Irina + Erik)

### Aim:

Ligation of BBa_K500003, lac promotor and pSB1K3 BioBricks™ followed by transformation on Kanamycin LB agar plates.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Ampicillin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

## Ligation

4 ligations were done at the same time.

1. Ligations of the digestions done by Jenny and Erik in lab 36
2. Ligation of Jin Wen's digestion MnP (sample 1) in Lab 31 (marked as a 1 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 2) in Lab 31 (marked as a 2 on the eppendorf tube)
4. Ligation of Jin Wen's digestion  MnP (sample 3) in Lab 31 (marked as a 3 on the eppendorf tube)

Components and amounts for ligation can be seen in table 41 (1 above), table 43, (2 above), table 44 (3 above), table 45 (4 above).

**Table41**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (E och J) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table42**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW1) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table43**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW2) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table44**

| | Components | Bba_K608006 (amount) |
|---|---|---|
| 1 | Digested BBa_K500001 (JW3) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1K3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Four Transformation were done at the same time, with 1 negative control

1. Erik 0: Erik's ligation mixture
2. JW 1: JIn Wens ligation mixture from eppendorf tube 1
3. JW 2: Jin wens ligation mixture from tube 2
4. JW 3: Jin Wen's ligation mixture from tube 3

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones
2. Certains cells had closer to 150 ul left for resuspension instead of 100ul. Might make calculation of transformation more difficult

3. Mixture of prepared compentent cells done by Jin Wen and Erik, have no idea which are which.

**Table45**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (Erik,I) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

**Table46**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW1 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table47**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW2 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table48**

| | Components | Transformation sample |
|---|---|---|
| 1 | Ligation reaction mixture JW3 | 5uL |
| 2 | DH5α competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

Negative Control: Water

Results:

-All agar plates, including the negative control, have zero colonies. Thus transformation failed.
-Other team memers speculate it is because of a faulty backbone. Digestion of a new backbone has already begun.

2019_02_2019_MnP_Transformation_EP.pdf

2019_02_07_MnP_EP_Transformation_diluted .pdf

***Lab #39 2/7/19 3A Digest of Backbone (pSB1C3 backbone) (Gustav + Lilli)***

Aim: Due to lack of Kanamycin resistant backbone, a new backbone resistant towards Chloramphenicol will be digested and used for ligation.
Method: The method is descirbed in lab #15.

Notes: The restreaking done yesterday (1/7/19) resulted in some cell growth. These cells will be grown overnight to later be digested to double check that the correct construct has been transformed into the cells. A picture of these results is displayed below:

image.png

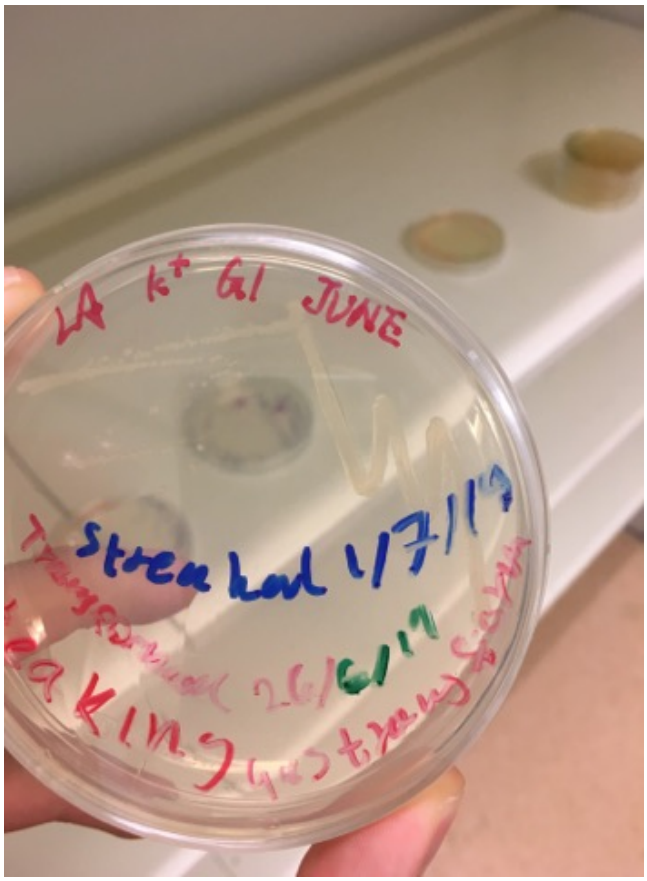
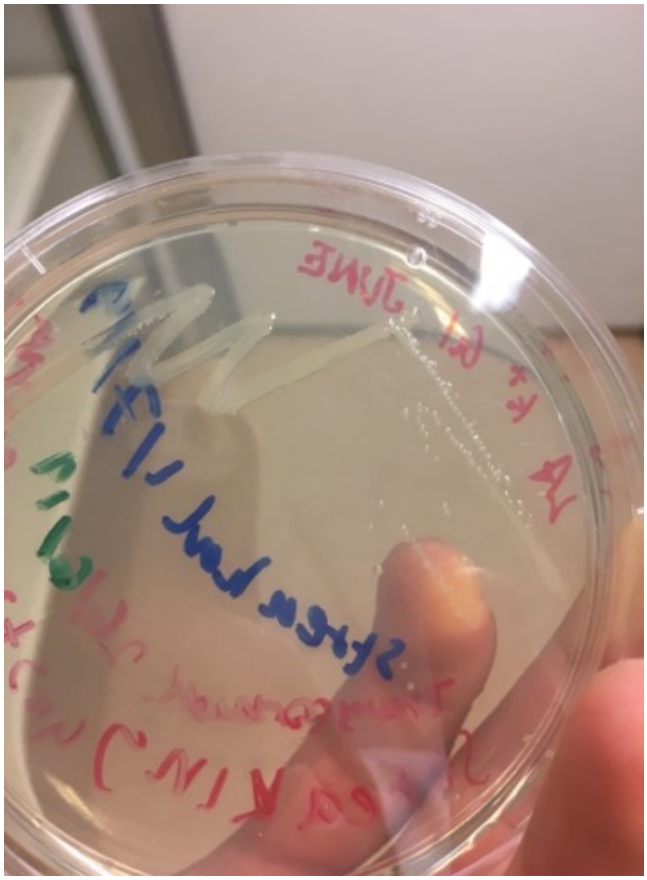


image.png



The digestion of the Chloramphenicol was unsucessful. No bands whatsoever were present. This means that a new backbone will be used, one that codes for ampicilin resistance. The backbone is designated as: pSB1A3.

Transformation of these new ligated plasmids and ligation was done. These protocols have been referenced before in previous labs. More specifically lab #21.  No deveations were made when following the ligation protocol.

## Lab #39 - BioBrick 3A Assembly and gel analysis (19-7-02) (Irina + Jenny)

### Aim:

Plasmid DNA prep of pSB1A3 (2016 iGEM team) will first be made, as pSB1K3 does not seem to work. We will try to do 3A Assembly with pSB1A3 instead together with Jinwens sampels from Lab #31.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 3, page 105-106;

Calculations for digestion mixture total volume 20 uL for pSB1A3:
V = required mass/intitial concetration
V =  500ng/25 ng/uL = 20 uL.

For components and amounts for Digestion mixure, see tabel 49.

**Table 49**

| | Component | pSB1A3 |
|---|---|---|
| 1 | 500 ng DNA | 20uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | PstI | 1uL |
| 5 | ddH2O | 23uL |

### Procedure

Digestion

1. One mix was made containing 500 ng of one of pSB1A3 backbone and ddH2O.
2. To each mix, 5 µL of 10x reaction buffer (2.1) for restriction enzymes were added.
3. 1 µL each of the appropriate endonucleases (two per tube) was added to give a final volume of 50 µL.
4. The tube were taped to mix.
5. The tube were incubated at 37°C for 30 min.
6. Heat-inactivatation of the enzymes was done by incubating at 80°C for 20 min.

## Lab #40- Agarose gel electrophoresis (19-7-02) (Jenny + Irina)

### Aim:

To separate and analyse nucleic acid samples under non- denaturing conditions

### *Method:*

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;
See table 14 for components and amounts mixed for agarose gel

A gel cast from the previous day was used.

### Running the gel

1. the gel tray was placed into the buffer chamber and the comb was removed carefully.
2. 1x TBE buffer was added until the gel was completely covered.
3. Ther DNA samples (~0.2 µg)were mix with loading dye (table 15) in 1.5 eppendorf tubes.
4. The size marker was mixed in 1x loading dye (~6 µL final volume) and loaded onto the gel.
5. Samples were loaded into the other wells while writing down which lanes have which samples.
6. The lod was put onto the buffer chamber and connected to the power supply and the gel was run at 110 V for 30–60 min.
8. After the run, the gel bands were visualized under UV and a picture was taken (figure x).

See table 50 for components and amounts used for running mixture:

**Table 50**

| | Components | amounts |
|---|---|---|
| 1 | Loading dye mix | 4uL |
| 2 | pSB1A3 sample | 20uL |

Gel analysis on 1% Agarose can be seen in figure 51. Our pSB1A3 sample can be seen in well number 4 from the left. We only had enough backbone to run the cut version, no negative control was loaded.

## Ligation

4 ligations were done at the same time.

1. Ligations of the digestions done by Jenny and Irina using pSB1A3 digested backbone
2. Ligation of Jin Wen's digestion MnP (sample 1) from Lab 31 (marked as a 1 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 2) from Lab 31 (marked as a 2 on the eppendorf tube)
4. Ligation of Jin Wen's digestion MnP (sample 3) from Lab 31 (marked as a 3 on the eppendorf tube)

Components and amounts for ligation can be seen in table 52, table 53 and table 54.

**Table 52**

| | Components | Amounts |
|---|---|---|
| 1 | BBa_K500001 (MnP 1) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 53**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP2) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 54**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP3) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Three Transformations were done at the same time, with 1 negative control

1. Mnp1
2. Mnp2
3. MnP3
4. Negative control

In table 55, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones

**Table 55**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (MnP1, MnP2 or MnP3) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

### Results from Transformation done 19-7-02:

No growth on any plate. We will try Ligation and Tansformation for Jinwens samples on MnP 4-6 and make suer we take T4 ligase and T4 10x Reactionbuffer from iGGEM 2019 as something might be wrong with the ligation and we did not pay attention to the expiration dates of the ligase/buffer that we used.

## Lab #41 - BioBrick 3A Assembly (19-7-03)

### Ligation (Irina)

4 ligations were done at the same time.

1. Ligations of the digestions done by Irina using pSB1A3 digested backbone
2. Ligation of Jin Wen's digestion MnP (sample 4) from Lab 31 (marked as a Ligation 4 on the eppendorf tube)
3. Ligation of Jin Wen's digestion MnP (sample 5) from Lab 31 (marked as a Ligation 5 on the eppendorf tube)
4. Ligation of Jin Wen's digestion  MnP (sample 6) from Lab 31 (marked as Ligaton 6 on the eppendorf tube)

Components and amounts for ligation can be seen in table 56,  table 57 and table 58.

**Table 56**

| | Components | Amounts |
|---|---|---|
| 1 | BBa_K500001 (MnP 4) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 57**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP 5) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

**Table 58**

| | Components | Amounts |
|---|---|---|
| 1 | Digested BBa_K500001 (MnP 6) | 2uL |
| 2 | Digested lac promotor | 2uL |
| 3 | Digested pSB1A3 | 2uL |
| 4 | T4 10x Reaction buffer | 2uL |
| 5 | T4 DNA ligase | 1uL |
| 6 | ddH2O | 11uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Three Transformations were done at the same time, with 1 negative control

1. Mnp 4
2. Mnp 5
3. MnP 6
4. Negative control

In table 59, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive control with intact plasmid other than experimental ones

**Table 59**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture (MnP 4, MnP 5 or MnP 6) | 5uL | - |
| 2 | BL21 competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 uL water was added instead of Ligation mixture.

Results from Transformation done 19-7-03:

No growth on any plate.

Image from iOS (4).jpg

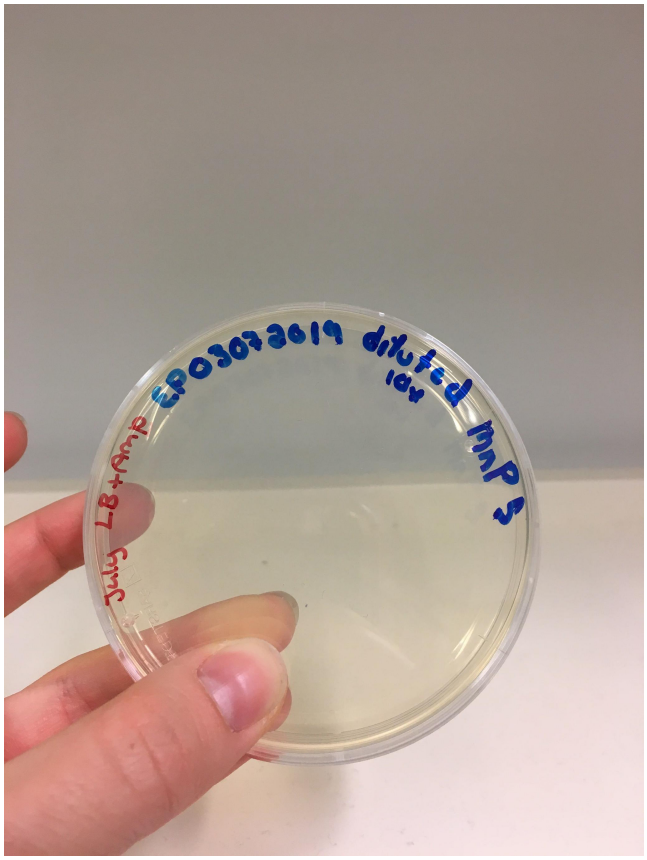


Image from iOS (5).jpg

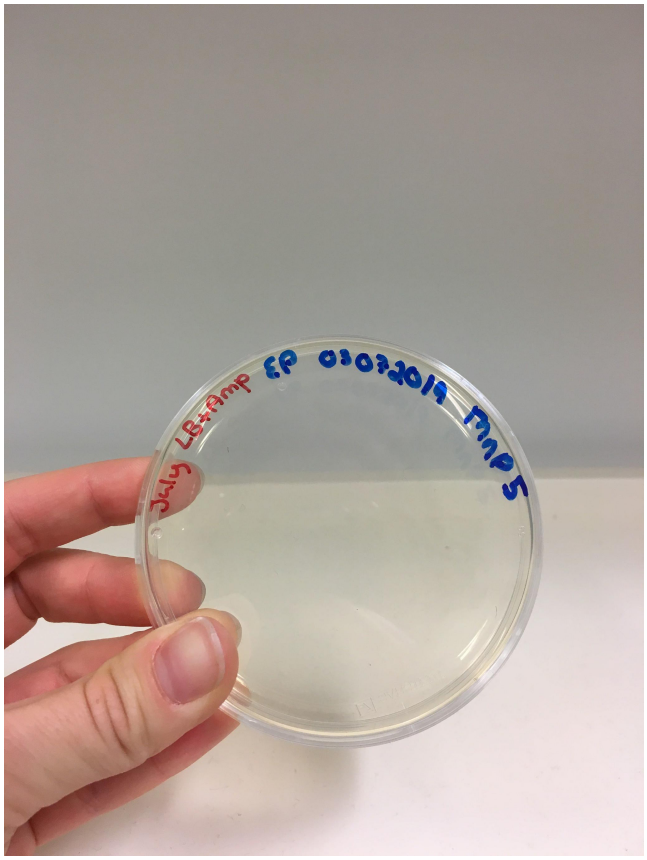


Image from iOS (3).jpg

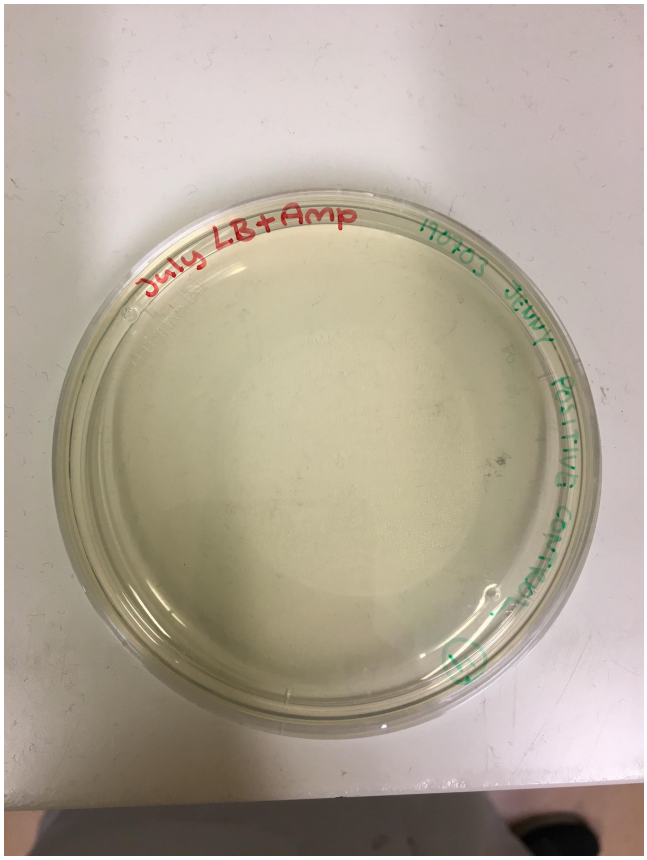
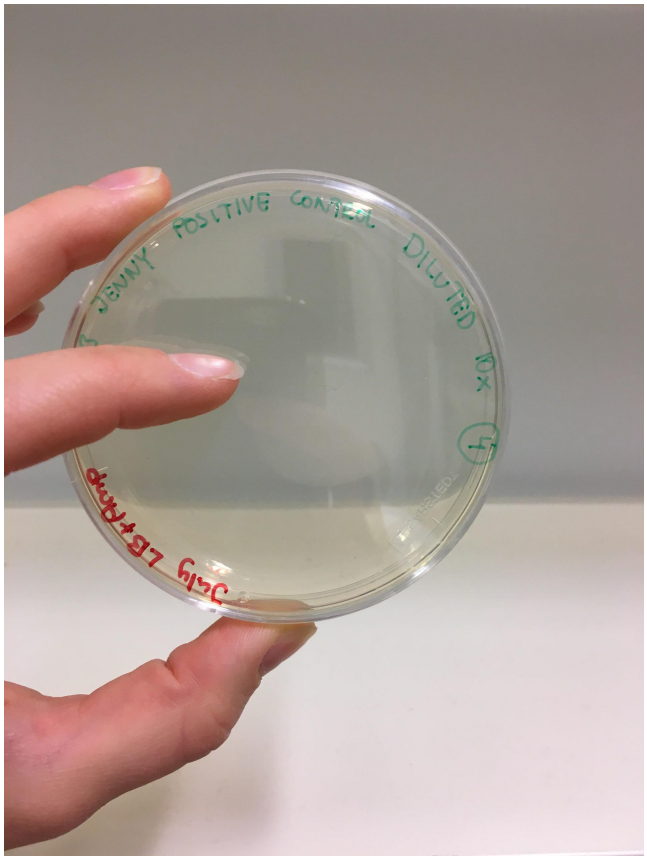


Image from iOS (2).jpg




## Lab #42 - Prepare agar plates with addition of antibiotics (19-7-03) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 60 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 60**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Amp"*

Calculations to calculate amount of solution needed for digestion:
500ng x 1ul/83.1ng= 6.02 ul Lac promotor

## Lab #43 Digest, agarose analysis and 3ADigest (Manish + Gustav) (3/7/19)

Aim: To confirm the presence of the correct plasmid construct in the cells that wee transformed the 26/6/19. This will be done by first digesting the transformed cells and then performing a 3A assembly. The plasmid is cut so that the promoter + gene of interest is displaced from the plasmid backbone. The plasmidbackbone is 2204 bp long and the promoter + gene of interest is 1733 bp long. This means that when a gel is run two bands should be seen at each respective length.
Method: The 3A method has been described in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm Protocol 3. The digestion procedure was described in the GenElute$^{TM}$ Plasmid Miniprep Kit manual, pages 4-5, by Sigma-Aldritch while the column procedure was in GeneJET Plasmid Miniprep Kit by ThermoScientific.
Notes: No deveations form each respective protocol was made. However after a missunderstanding of the instructions a ependorf tube was accidentely centrifuged at 5000g for 5mins, in accordance to the Sigma Aldritch protocol not the ThermoScientific protocol. After the mistake was identified the correct protocol was followed meaning that the tube was centrifuged again at 12,000g for 1 min. This tube was marked as tube 1.
Results:

image.png

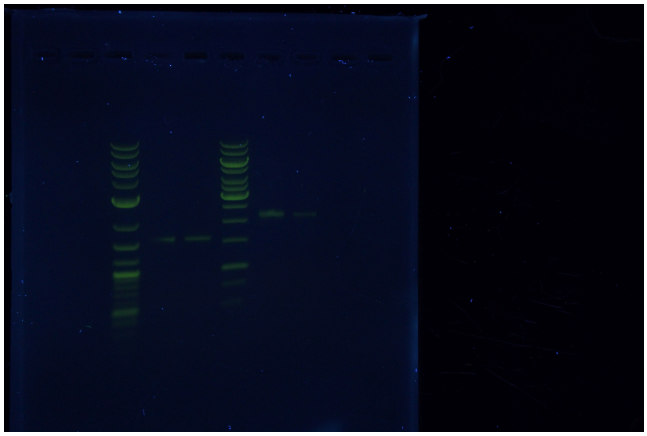
From left to right: Undigested sample, Digested sample, ladder (gene ruler 1kb), control from previous ligation. These samples were all from tube 1.

Calculations: After making two tubes, one following strict protocol marked as "tube 2" and one marked as "tube 1" which had followed the wrong protocol, the DNA concentration was meassured. Tube 1 had a concentration of 12ng/microliters while the other had a concentration of 8 ng/microliters.
It was decided that tube 1 will undergo 3A assembly.
500/12= 42microliters of DNA solution
1 microliters of ddH2O
1 microliters of EcoRI
1 microliters of PstI
5 microliters of buffer
Discussion: As seen from the resulting gel displayed above, the results recieved do not matched the theory. After some dicussion it was decided that due to the similar sizes of each band a 0.7% agarose gel should be made/run instead of the standard of 1%. The experiment was repeated with a 0.7% agarose gel.

## Lab #44 Digest, agarose analysis and 3ADigest (Manish + Gustav) (3/7/19)

Aim: To repeat lab #43 protocol with a 0.7% agarose gel.
Method: Described in lab #43.
Results:

image.png



From left to right: log-2 DNA ladder, Undigested sample "tube 1" described in lab #43, undigested sample, Gene ruler 1 kb ladder , digested sample "tube 1", digested sample

Calculations:
Agarose gel 0.7%: 50*0.007=0.35g agarose.
Digested sample: 500/33 = 15 microliters DNA solutions.
28 microliters ddH2O
5 microliters buffer
1 microliters PstI
1 microliters EcoRI
Discussion: As seen from the gel above the results were easier to see but they did not agree with the theoretical results. Theis implies that the correct construct is not present in the cells.

A further transformation was made using the ampicillin bavkbone but the resuts were negative.

## Lab #41 - Test of Competent Cells Through Transformation of Plasmids (19-7-04) (Erik)

### Aim:

As are all previous transformations have failed with BL21 bacterial strain, we are trying to troubleshoot what is wrong with our method or materials that are causing these transformations. We are therefore testing to see if the competent cells take up plasmids by using the iGEM competent cell test kit 2018 pSB1C3 BBa_J04450.

### Method

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Three transformations were done with the competent cells done by either Irina, Erik, or Irina/Erik (we are not sure who it was). The same RFP construct BBa_J04450 was used for all three transformations.

### Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Calculations:
0,5 ngx 1000pg/1ngx 1ul/1000pg =5ul
Deviations from Protocol:

1. No negative control were done, BBa_J04450 have a RFP reporter that make the colonies red. Any contamination will not have the characteristic red colonies.
2. Competent cells were left on ice for 25 min instead of 15 min.
3. Irina's resuspension liquid was less than 100 ul
4. Erik's resuspension solution was more than 100ul

**Table 61**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Irina's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |
| 4 | ddH2O | - |

**Table 62**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Erik's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

**Table63**

| | Components | Transformation sample |
|---|---|---|
| 1 | BBa_J04450 Erik's eller Irina's | 5uL |
| 2 | BL21 competent cells | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL |

Irina is strating overnight culutre of BL21 cells.

### Discussion (Irina):

Today we are making new Ampicillin stocks as well as LB + Amp plates and LB + Amp medium. We do not trust the stocks used that were made 2018. We will continue to try transformation, but also make sure to use the new plates as well as taking the enzymes from 2019. No more dumpsterdiving for old stuff! Additional SOB medium is also made.

## Lab #42 - Preparation of competent *E.coli* BL 21 (DE3) cells using $CaCl_2$ (19-7-05) (Irina)

### Aim:

To increase the competence of *E. coli* BL 21 (DE3) cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Competent cells are marked with red BL21 and stored in the box with competent cells in the -80 freezer.

## Lab #43 - Prepare agar plates with addition of antibiotics (19-7-05) (Irina)

### Aim:

To prepare  solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 64 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Tble 64**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

## Lab #44 - Preparation of LB media and addition of Ampicillin (19-7-05) (Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 64 for components and amounts used to prepare 600 mL of LB.

**Table 64**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 6g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 100ul |

600 uL of Ampicillin was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July LB + Amp"*

## Lab #45 - Preparation of SOB medium (19-7-05) (Irina)

### Aim:

To prepare a SOB Medium, or Super Optimal Broth, is used for preparing chemically competent cells.

### Methods:

Preparation of SOB was done according to protocol:

SOB Medium

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 96;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations
m(Yeast) = $W_1$/ $V_1$ x $V_2$ = $W_2$ = 0.5g/100 mL x 800 mL = 4g
m(Bacto) = 2g/100mL x 800 mL = 16g
m(NaCl) = 10 mmol/1 L x 800 mL x 58.44 g/mol = 0.47g
m(KCl) = 2.5 mmol/1 L x 800 mL x 74.55 g/mol = 0.15g

See table 65 for components and amounts

**Table 65**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 0.46g |
| 2 | BactoTMtryptone 1% (w/v) | 16g |
| 3 | Yeast extract 0.5% (w/v) | 4g |
| 4 | ddH2O | 800mL |
| 5 | 5 M NaOH | 45uL |
| 6 | KCl | 0.15g |

# 50% (v/v) Glycerol, 50ml

## Introduction

This solution is to be used for making cell glycerol stocks of important bacterial strains.

## Materials

- Glycerol stock
- ddH2O

## Procedure

- 1. Check which percentage glycerol is in the stock.
- 2. Calculate how much volume you need of glycerol and how much water you need to add to reach a final volume of 50 mL.

**Calculation of 50ml 50% glycerol**

| | Components | volume / weight |
|---|---|---|
| 1 | Glycerol stock (85% in stock) | 29.4 ml |
| 2 | ddH2O | 20.6 ml |

- 3. Measure the glycerol in a measuring cylinder.
- 4. Add to a glass bottle and add water to make a 50% glycerol solution.
- 5. Autoclave for 20 min.

# Week 5: Jul 15 - Jul 21

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

MONDAY, 15/7/2019

## Lab #56 - Ligation and O/N cultures (19-7-15) (Irina + Gustav)

### Aim:

Ligation of pSB1A3, LacI (Jinwen's digestion nr. 6) and MnP (Jinwen's digestion nr.6). This time, Ligation was done in 4 degrees and 16 degrees for 8 hrs using PCR tubes and PCR machine.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Amounts used can be seen in table 81. Some modifications where done regarding the protocol.

**Table 81**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 3uL |
| 4 | Bba_K500001 | 2uL |
| 5 | J04500 | 10uL |
| 6 | pSB1A3 | 2uL |

Discussion: Mini-prep on O/N cultures from friday the 12th failed on Saturday the 13th. Therefore, new O/N cultures where prepared on Moday the 15th.

## Lab #57 -Transformation of ligation mixture prepared 15-7-19 (Gustav, 16/7/19)

Aim: To tranform the cells with an efficiency factor of 1.415*10^(10) with the ligated samples from 15/7/19.

Methods: The methods followed were those described by protocol 6 in *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al.

Notes: Due to missing step 7 was missed. Instead the ependorf tubes went straight to their water baths. The misstake was later realised ca 45mins after. The SOB media was added aaccordingly and the water bath was extended with 45mins.

# Lab #58 - Plasmid MiniPrep (2019-07-16) (Jenny & Irina)

## Aim:

Sample names:

1. LB+Amp pSB1A3_(RFP)
2. LB+Amp pSB1A3_LacI_Boo34_Mnp (3)
3. LB+Cm Lac Prom (2) (J04500)(pSB1C3)
4. LB+Cm Lac (5) in pSB1C3
5. LB+Cm DH5a_BBa_K500001_pSB1C3 (3) MnP
6. LB+Cm DH5a_BBa_K500001_pSB1C3 (2) MnP

The number in brackes is the sample from which the Lac and Mnp derives from (Jinwens samples).

## Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.
with some adjustments:

- When washing the column for the first time we added 750 uL of Washing Solution and centrifuged 1 min/12000g. Thsi was done twice before the dry centrifugation step.
- After dry centrifugation step the column was moved to a new eppendorf tube and 50 uL ddH2O was added. Waited for approximately 5 minutes befor adding another 50 uL and then spinned 1 min/12000g.
- We measuerd the concentrations by Nanodrop2000. See table 1 for results.

Irina made tubes 1, 2 and 3 while Jenny made 4, 5 and 6. Named after the plates with colonies from 10th of July. Two minipreps where done as we had quite low concentrations the first time (we only used 1.5 mL of culture). We modified the protocol according to Manish's protocol for the second miniPrep. No second miniprep was done for sample nr.6 as the cap had flewn off during incubation on shaking table.

DNA concentrations can be seen in table 82.

**Table 82**

| | Sample | concentration (ng/uL) | Concentration (ng/uL) |
|---|---|---|---|
| 1 | 1 | 37.4 | 111.7 |
| 2 | 2 | 10 (discarded) | 43.6 |
| 3 | 3 | 47.2 | 113.9 |
| 4 | 4 | 42.8 | 122.4 |
| 5 | 5 | 70.4 | 185.6 |
| 6 | 6 | 69.7 | - |

Samples where stored in the freezer after NanoDrop reading.

Conclusions:

Since the absorption values was to low for Sampel 1 and 2 Irina and Manish remade them.

# Lab #59 - Digestion and gel analysis for pSB1C3 (Bba_J04450) (19-7-17) (Jenny + Irina)

## Aim:

To doublecheck that the plasmid that we have purified from the miniprep from lab 50 (backbone pSB1C3, gene (RFP) Bba_J04450, B0034, ribosome binding site) is the one that we have gotten out.

## Methods:

3A assembly-Digestion and ligation

Deviation from protocol: an 0.8% agarose gel was made and run at 90V for about 1.5 hrs.

In figure 83, Loading and what type of restriction enzymes that where used can be seen.

IMG_20190717_143148751.jpg

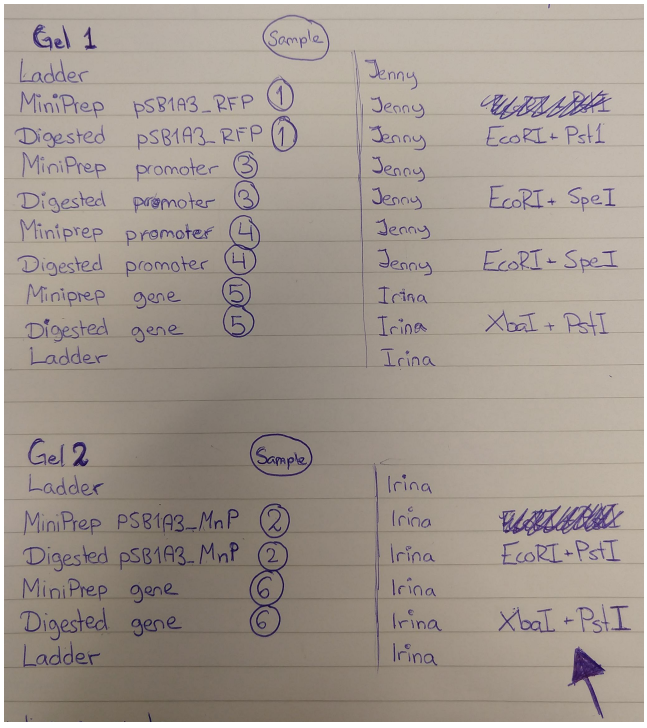



Figure 83. Loading order of 0.8% Agarose gels

GEL1.jpg

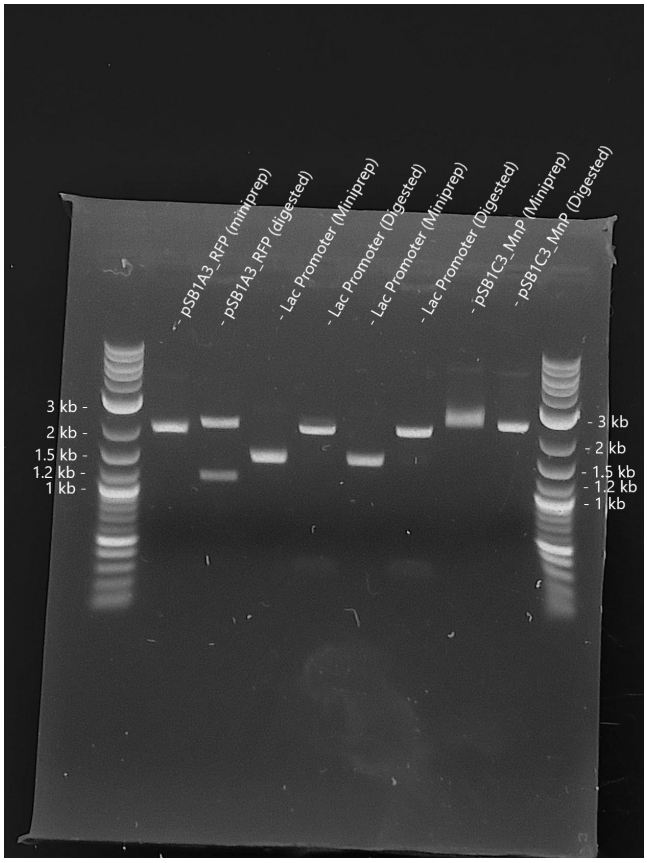



Figure 84. 0.8 % agarose gel loaded with 20 uL sample and 10 uL 2-Log DNA ladder.

GEL2.jpg

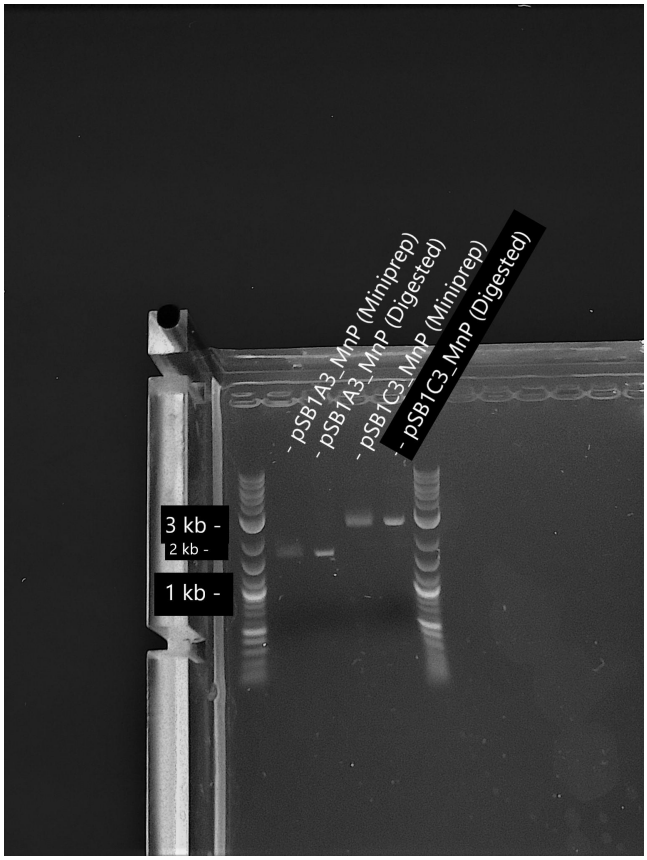



Figure 85. 0.8 % agarose gel loaded with 15 uL sample and 10 uL 2-Log DNA ladder.

## Lab #60 - Digestion and gel analysis for pSB1C3 (Bba_J04450) and pSB1C3_MnP from lab #59 (19-7-18) (Jenny + Irina)

### Discussion:

It appears that there might be a problem with the XbaI enzyme from the gel images from yesterday. Therefore. we will perform digestion again. However, we will take sample from the digestions from yesterday (25uL) and add an additional 0.5uL of XbaI to both sample 5 and 6 (pSB1C3_MnP). We will use the new XbaI for sample 5 again, but an old XbaI for sample 6. Protocol will be followed from there on. We will also perform a digestion on pSB1C3 following protocol.

### Aim:

DIgestion of sample 5 and 6 (pSB1C3_Mnp) again. As well as the digestion of pSB1C3.

### Methods:

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

Deviation from protocol: an 0.8% agarose gel was made and run at 90V for about 1.5 hrs.

Calculations:

500 ng x 1ul/101.4 ng= 4,93 ul of plasmid mixture

Table 86 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion of pSB1C3.

**Table 86**

| | Component | pSB1C3, Bba_J04450 |
|---|---|---|
| 1 | 500 ng DNA | 4,9uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ---- |
| 5 | XbaI | --- |
| 6 | PstI | 1uL |
| 7 | ddH2O | 35,4ul |

# Week 7: Jul 29 - Aug 4

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

MONDAY, 29/7/2019

## Lab #68 - Preparation of Low salt LB media and addition of Zeocine (19-7-29) (Jenny + Irina)

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.6L x 58.44 g/mol ≈ 6g but since we make low salt, we only add 3 g.
m(BactoTMtryptone) = 1g/100 mL x 600 mL = 6g
m(Yeast extract) = 0.5g/100 x 600mL = 3g
See table 90 for components and amounts used to prepare 600 mL of LB.

**Table 90**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 3g |
| 2 | BactoTMtryptone 1% (w/v) | 6g |
| 3 | Yeast extract 0.5% (w/v) | 3g |
| 4 | ddH2O | 600ml |
| 5 | 5 M NaOH | 300ul |

150 uL of Zeocine was added to the LB media.

Storage: The LB medium is stored in the cold room with notation *"July Low salt LB + Zeo"*

## Lab #69 - Prepare agar plates with addition of antibiotics (19-7-29) (Jenny + Irina)

Additional LB plates with Zeocine will be prepared, as the stack is runing low.

### Aim:

To prepare  solutions necessary for future experiments. High ionic strength and acidity or basicity inhibit the activity of Zeocin. Therefore, it is recommended that the salt is reduced in bacterial medium.  The pH will be adjusted to 7.5 to keep the drug active.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Zeocine (Zeocine 100mg/mL stock) + autoclave

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Zeoine (25 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations for Zeocin stock (100 mg/mL) to 25 ug/mL
volume needed of 100 mg/ml stock: (25 uL/mL x 600 mL)/100 000 ug/mL) = 0.15 mL = 150 uL

Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 91 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table 91**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Zeocine (100 mg/mL stock) | 150 uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LS LB + Zeo"*

## Lab #67. Gibson assembly (19-07-30) (Jenny + Gustav + Erik + Irina)

### Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating HRP-2A-eGFP, HRP-2A_OOA and AAO-2A-eGFP into shuttle -vector pPICZαB respectively.

### Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Deviation from protocol:
Irina added 1 uL sample into 50 uL Competent cells, Gustav who also worked with HRP-2A-AAo added 2 uL of sample to competent cells.

Irina:
Construct: HRP-2A-AAO
Vector: pP1CZab
2 to 1 Insert mass of DNA insert
Calculations for total fragment amounts in GIbson Assemby mix for Irinas samples can be seen in table 92.

**Table 92**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 1.2 uL | 50ng/43 ng/uL |
| 5 | Linearized vector concentration | 43 ng/uL | |
| 6 | Insert length | 3.0 kb | |
| 7 | HRP-2A-AAO concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 8.3 uL | (2:1) 83.33 ng/uL/10ng |

Total amounts of Irinas fragments, ddH2O and mastermix for gibson assembly can be seen in table 93.

**Table 93**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 8.3 | Insert HRP-2A-AAO |
| 3 | 1.2 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 0.5 | water |

**Table94**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 1.2 uL | 50ng/43 ng/uL |
| 5 | Linearized vector concentration | 43 ng/uL | |
| 6 | Insert length | 1,9 kb | |
| 7 | HRP-2A-AAO concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 5,3 uL | (2:1) 52,78ng/uL/10ng |

Total amounts of Irinas fragments, ddH2O and mastermix for gibson assembly can be seen in table 93.

**Table95**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 5.3 | Insert HRP-2A-GFP |
| 3 | 1.2 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 3.5 | water |

# Week 6: Jul 22 - Jul 28

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

TUESDAY, 30/7/2019

Discussion:
Looking at the gel in figure 86, it appears that we are woring with GLOX (1700bp) rather than MnP (1134 bp). A mix up of samples could have occured. We will now on continue with ligation and transformation but assuming we are woring with GLOX.

gel.jpg

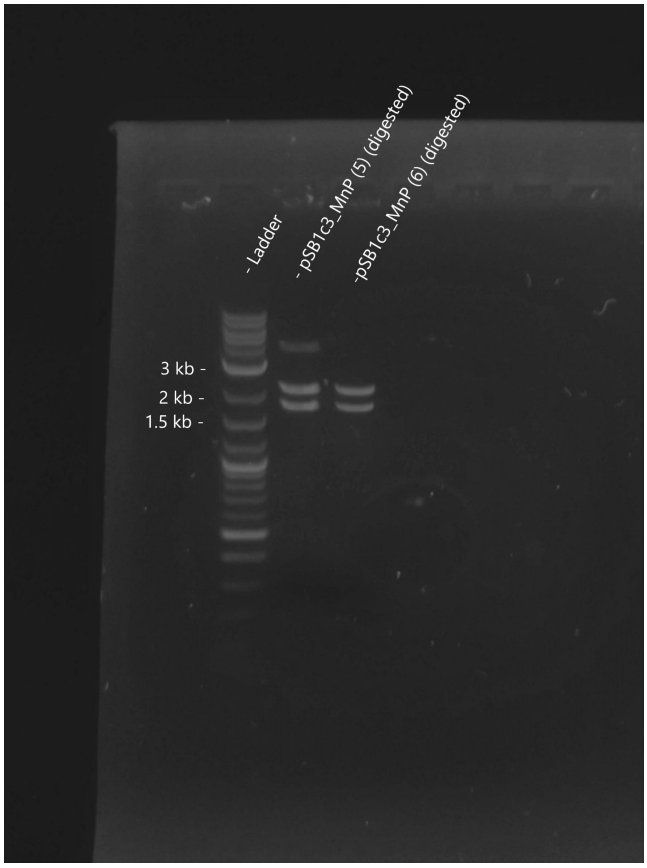



Figure 87. 0.8 % Agarose gel, showing ladder, Mnp (?) sample 5, MnP (?) sample 6.

## Lab #61 - Ligation in PCR tubes (19-7-22) (Irina + Erik)

### Aim:

Ligation of sample 5 in pSB1C3 and ligation of sample 6 in pSB1A3. we assume it is GLOX.

### Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Deviations from protocol: Incubation was done in a PCR macine. 22 degrees for 30 minutes followed by heat inactivation at 65 degrees for 10 minutes.

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Amounts used can be seen in table 88.

**Table 88**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 11uL |
| 4 | GLOX | 2uL |
| 5 | Lac (3) | 2uL |
| 6 | pSB1A3 or pSB1C3 | 2uL |

Samples were stored at -20 degrees for further use, two PCR tubes marjed with encirceld 5 and one marked with an encirceled 6 written with blue pen.

## Lab #62 - Transformation (19-7-23) (Erik + Jenny + Gustav + Irina)

Transformation
Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Four Transformations were done togethre with 2 negative controls

1. GLOX sample 6
2. GLOX sample 6
3. GLOX sample 5
4. GLOX sample 5
5. Negative control on Cm plate
6. Negative control on Amp plate

In table 55, reaction mixtures for transformation can be seen.

Deviations from Protocol:

1. No positive oontrols where made

**Table 89**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | GLOX sample 5 or sample 6 | 5uL | - |
| 2 | DH5a competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

## Lab #63 - PCR (2019-07-23) (Gustav + Irina + Erik + Jenny)

Aim: Amplify and create 3' overhangs on AAO, HRP, MnP, GLOX, LiP and pPICZαB for Gibson Assembly.

Method: Following the Protocol 10 - Inverse PCR with PhusioHF DNA Polymerase

- Instead of having 5 min of initial denaturation we put 30 sek.
- Instead of having 30 sek denaturation we put 10 sek each cycle.

PCR for synthesizing DNA fragments

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 126-128;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, PCR;

Irina's PCR reaction mixture for pPICZaB (total volume 50 uL):
23.5 uL of ddH2O
5 uL of 2mM dNTPs
5 uL of Formward primer (5 uM) (pPICZaB_Myc_fwd)
5ul of Reverse primer (5uM) (pPICZaB_afac_end_rev)
10 uL of Phusion HF buffer
1 uL Plasmid DNA (1 ng/ul)
0.5 uL of HF DNA Polymerase

Irinas Assembly Protocol (total volume 20 uL)
NEB calculator was used: http://nebiocalculator.neb.com/#!/ligation
HRP lenght: 1250 bp
Vector lenght: 3600 bp
HRP stock: 64.1 ng/uL
Vector stock: 13.6 ng/uL
Need (3:1) ratio of HRP: 104.2 ng/64.1 ng/uL = 1.7 uL
Need 100 ng vector: 100 ng/13.6 ng/uL = 7.4

9.1 uL of fragments

10 uL of Master mix
0.9 uL of ddH2O

### Transformation

#### Aim:

To transform competent DH5a cells with Shuttle-vector containing each gene respectively. First, a 4x dilution was made of the above Assembly protocol by mixing 5 uL of Assembly and 15 uL of ddH2O.

#### Method:

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Samples where incubated in a thermocycler at 50°C for 15 minutes prior to transformation, following instructions from:
https://international.neb.com/protocols/2012/09/25/gibson-assembly-master-mix-assembly

### Discussion:

It appears we have colonies on the transformation plates from GLOX sample 5. Re-streak was done from both of the sample 5 plates, picking four colonies from each plate. The new plates are called "Re-streak Transformation pBS1C3_LacI_GLOX(5):1 in DH5a Irina 24/7" and "Re-streak Transformation pSB1C3_LacI_GLOX(5):2 in DH5a Irina 24/7". The original plates where stored in the refrigerator.

## Lab #64 Gibson assembly (19-07-24) (Erik + Jenny + Gustav + Qian + Irina)

#### Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating MnP, HRP, Lip, GLOX and AAO into shadow-vector pPICZαB respectively.

#### Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Insert: MnP

Vector: pP1CZab

3 to 1 Insert mass of DNA insert

**GA Description and Values to Use in GA Mix (MnP…**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass (pP1Czab) desired | 100ng | |
| 4 | Number of microliters needed for vector | 7,4 ul | 100ngx 1ul/13,6 ng= |
| 5 | Linearized vector concentration | 13,6 ng/ul | |
| 6 | Insert (MnP) length | 1,2kb | |
| 7 | MnP concentration | 60,9 ng/ul | |
| 8 | Number of microliters needed for insert | 1,6 ul | 95,38ngx 1ul/60,9ng= |
| 9 | Number of ul needed for insert | 3,67 | |

**Gibson Assembly Mixture Compo…**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 1,6 | Insert MnP |
| 3 | 7,4 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 1,00 | water |

# Lab #64 Gibson assembly (19-07-25)

O/N culture (Jinwen's group)

# Lab #65 miniprep and gel digestion (19-07-26). (Irina + Qian + Jinwen + Erik)

## Aim:

To purify plasmid DNA from HRP, AAO, MnP, LiP, GLOX and pPICZaB

## Methods:

MiniPrep was done according to Thermo Fischer Scientific protocol: Thermo Scientific GeneJET Plasmid Miniprep Kit: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0013117_GeneJET_Plasmid_Miniprep_UG.pdf&title=VXNlciBHdWlkZTogR2VuZUpFVCBQbGFzbWlkIE1pbmlwcmVwIEtpdA==

with some adjustments (Irina):

- 300 uL of Resuspension solution where added
- 300 uL of Lysis buffer was adeed
- 350 uL of Neutralization buffer was added
- When washing the column for the first time we added 500 uL of Washing Solution and centrifuged 2 min/12000g. Thsi was done twice before the dry centrifugation step.
- After dry centrifugation step the column was moved to a new eppendorf tube and 50 uL ddH2O was added. Waited for approximately 5 minutes befor adding another 50 uL and then spinned 1 min/12000g.
- We measuerd the concentrations by Nanodrop2000. See table 1 for results.

# Lab #66. Analytical Digestion and Gel Analysis (19-07-26) (Jinwen + Erik)

## Aim:

We used analytical digestions and agarose gel electrophoresis in order see if our Gibson Assembly of 5 different enzymes: Lip, GLOX, MnP, HRP, AAO; were successful.

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

Deviations from protocol.

**Analytical Digestion of MnP and HRP (Erik)**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | | ng/ul | DNA Quantity | buffer+enzyme | water |
| 2 | MnP 1 | 61,6 | 3,27 | 2,4 | 14,3 |
| 3 | MnP2 | 148,4 | 1,34 | 2,4 | 16,3 |
| 4 | MnP3 | 114,9 | 1,7 | 2,4 | 15,9 |
| 5 | HRP 1 | 57,9 | 3,5 | 2,4 | 14,1 |
| 6 | HRP 2 | 108,4 | 1,9 | 2,4 | 15,7 |
| 7 | HRP 3 | 91,3 | 2,2 | 2,4 | 15,4 |

Results: We got no bands for any of the samples. We got some results from the control. We think that is has something .to do with the gel itself

## Lab #67. Gibson assembly (19-07-26) (Jinwen + Erik+Tereza)

### Aim:

This PCR-based method (Gibson et al., 2009) joins multiple DNA parts in a manner independent of DNA sequence. The 5′ ends of the primers are designed to include 20–150 base overlaps with the fragment that they will be ligated to.
We are ligating MnP, HRP, Lip, GLOX and AAO into shadow-vector pPICZαB respectively.

### Method:

Gibson assembly master kit

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 10, page 135-137;

Transformation of Competent E.coli cells

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Construct: HRP-2A-GFP-3
Vector: pP1CZab
2 to 1 Insert mass of DNA insert

**GA Description and Values to Use in GA Mix (HRP…**

| | A | B | C |
|---|---|---|---|
| 1 | Description | Value | Calculation |
| 2 | Vector length | 3,6 kb | |
| 3 | Vector DNA mass | 50ng | |
| 4 | Number of microliters needed for vector | 3,67 ul | 50ngx 1ul/13,6 ng= |
| 5 | Linearized vector concentration | 13,6 ng/ul | |
| 6 | Insert length | 1,9kb | |
| 7 | HRP-2A-eGFP concentration | 10 ng/ul | |
| 8 | Number of microliters needed for insert | 5,3 ul | 52,78ngx 1ul/10ng= |
| 9 | Number of ul needed for insert | 3,67 | |

**Gibson Assembly Mixture Compo…**

| | A | B |
|---|---|---|
| 1 | Quantity (ul) | What |
| 2 | 5,3 | Insert HRP 2A GFP |
| 3 | 3,67 | Vector pP1CZab |
| 4 | 10 | GA mastermix |
| 5 | 1,00 | water |

Deviations from Protocol:

1. Made a mistake on the PCR machine. Instead of putting 15 min, we put 15 seconds.As soon as it was discovered, we adjusted the the PCR machine, howerver, 10 minutes had elapsed.

Results:

1. We eventually got colonies on the plates. However, we had two different looking microorganisms on the plates. The E. coli looking colonies were restreaked and an overnight culture was done.

# Single-temperature Double Digest

## Introduction

This is the Double Digest Protocol with Standard Restriction Enzymes, using a common reaction and same incubation temperature for both enzymes.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

## Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

## Procedure

### Single Temperature DD Reaction

1. Set up the following reaction (total reaction volume 50 µl).

**Table2**

| | A | B |
|---|---|---|
| 1 | | **Reagent Volumes (µl)** |
| 2 | **Buffer (10x)** | 5 |
| 3 | **DNA *** | Input Volume for ng |
| 4 | **Restriction Enzyme #1 **** | 1 |
| 5 | **Restriction Enzyme #2 **** | 1 |
| 6 | **Deionized Water (µl)** | 48 |
| 7 | **Total Volume (µl)** | 50 |

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.
** Restriction Enzymes should be added to the mixture last.

- [x] 2. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.
- [x] 3. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- [x] 4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

01:00:00

▶

Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

# Week 4: Jul 8 - Jul 14

**Project:** iGEM uppsala 2019
**Authors:** Qian Shi

TUESDAY, 30/7/2019

## Lab #46 - Digestion of PSB1A3 (19-7-08) (Irina + Jenny)

### Aim:

To digest the plasmid PSB1A3 with two different methods.

### Methods:

- The Protocol 3 form Synthetic Biology
- The iGEM Registry Protocol (http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit)

Notes: EcoRI-HF and Dpn1 was from 2019 but the others were older.

We loaded a gel to measure Irinas sample, figure 66, (from protocol 3) but since the other (mixed w

gel.GIF

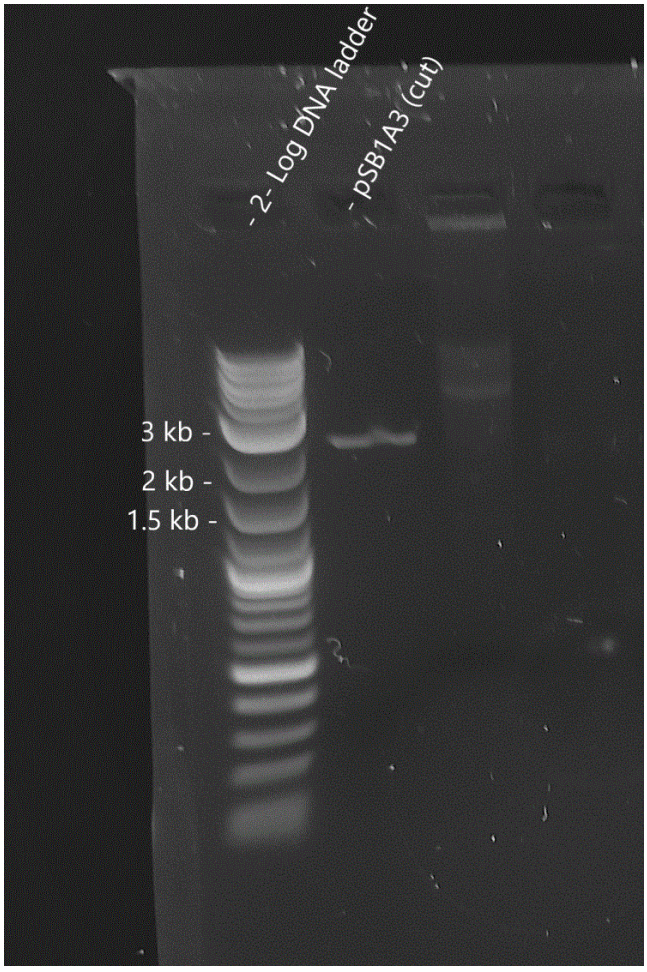



**Figure 66.** Agarose gel analysis of digested pSB1A3.

Lab #39 and #41 was repeated but with new competent cells from 5/7 using BL21 marked competent cells, new LB + Amp plates from 5/7 and with the newly digested plasmid from todays lab (Lab #46).

## Lab #48- Preparation of SOC Media (19-7-8) ( Erik)

Aim: To prepare 10 ml SOC Media

Method: Follow the protocol from Cold Harbor Springs Protocol on preparing SOB media:
http://cshprotocols.cshlp.org/content/2018/3/pdb.rec098863

Since chilled SOB media and 1M glycerol solution was already prepared, 10 ml was simply prepared by putting in 0,2 ml of glucose solution in 10 ml of SOB media.

Calculations:
1000ml of SOB/20ml of glycerol solution= 10ml of SOB/x amount of broth
x= 0,2 ml of glucose solution

## Lab #49- Compentent Cell Test Kit (19-7-8) (Gustav + Erik)

**Aim:** To determine the competency of the cells made 5/7/19. The strain of the E. Coli was BL21 by transforming in the RFP plasmid BBA_J04450.

**Methods:** The protocol followed is detailed in the following link on iGEMS official website:
http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit

1. Clean your working area by wiping down with 70% ethanol.
2. Thaw competent cells on ice. Label one 1.5 mL microcentrifuge tubes for each transformation and then pre-chill by placing the tubes on ice.
    - Do triplicates (3 each) of each concentration if possible, so you can calculate an average colony yield.
3. Spin down the DNA tubes from the Competent Cell Test Kit/Transformation Efficiency Kit to collect all of the DNA into the bottom of each tube prior to use. A quick spin of 20-30 seconds at 8,000-10,000 rpm will be sufficient. *Note:* You should resuspend the DNA in each tube with 50 µL dH2O.
4. Pipet 1 µL of DNA into each microcentrifuge tube.
5. Pipet 50 µL of competent cells into each tube. Flick the tube gently with your finger to mix.
6. Incubate on ice for 30 minutes.
    - Pre-heat waterbath now to 42°C. Otherwise, hot water and an accurate thermometer works, too!
7. Heat-shock the cells by placing into the waterbath for 45 seconds (no longer than 1 min). Be careful to keep the lids of the tubes above the water level, and keep the ice close by.
8. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes.
9. Add 950 µL of **SOC media** per tube, and incubate at 37°C for 1 hour shaking at 200-300rpm.
    - Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.
10. Pipet 100 µL from each tube onto the appropriate plate, and spread the mixture evenly across the plate. Incubate at 37°C overnight or approximately 16 hours. Position the plates with the agar side at the top, and the lid at the bottom.
11. Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.
    - Efficiency (in cfu/µg) = [colonies on plate (cfu) / Amount of DNA plated (ng)] x 1000 (ng/µg)
    - *Note: The measurement "Amount of DNA plated" refers to how much DNA was plated onto each agar plate, not the total amount of DNA used per transformation. You can calculate this number using the following equation:*
    - Amount of DNA plated (ng) = Volume DNA added (1 µL) x concentration of DNA (refer to vial, convert to ng/µL) x [volume plated (100 µL) / total reaction volume (1000 µL)]

Notes: No deviations from the protocol were made.

# Lab #49- Preparation of 10x TBE (19-7-8) (Erik)

## Aim:

To prepare a TBE buffer that inhibits enzymatic degradation of nucleic acids when running agarose gels.

## Methods:

Preparation of 10x TBE Buffer cells for agarose gel electrophoresis was done according to protocol:

10xTBE buffer (500ml stock)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 94;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
m = Mw x n x c
m(Tris) = 121.14 g/mol x 0.89 mol/l x 0.5 L = 53.91 g
m(Boric Acid) = 61.83 g/mol x 0.89 mol/L x 0.5 L = 27.52 g
m(EDTA) = 292.24 g/mol x 0.025 mol/L x 0.5 L = 3.65 g

See table 10 for components and amounts used. pH = 8.2

**Table1**

| | Component | amount |
|---|---|---|
| 1 | Tris | 53.91 g |
| 2 | Boric acid | 27.52 g |
| 3 | EDTA | 3.65 g |
| 4 | ddH2O | 500 mL |

Notes: I put the ddH20 first before putting in the solid Tris, Boric Acid instead of pouring the water after.

# Lab #50 - Plasmid MiniPrep (19-7-9) (Erik)

## Aim:

To isolate plasmid DNA from recombinant *E. coli BL21* O/N cultures BBa_J04450 (gene RFP), pSB1C3 (backbone), B0034 (ribsome binding site)

## Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.

DNA concentrations can be seen in table 12.

**Table66**

| | Sample | concentration (ng/uL) | 280/260 | 260/230 |
|---|---|---|---|---|
| 1 | BBa_J04450 | 101.4 | 1,22 | 1,77 |

Conclusions:
280nm: Wavelength of that proteins absorb
260nm: Wavelength that DNA absorb
230nm: Wavelength that carbohydrates are absorbed

The DNA concentration is relatively good at 101.4 ng/ul. Values around 2 are good, which means that that our solutions are somewhat contaminated, especially with proteins..

## Lab #51 - Prepare agar plates with addition of antibiotics (19-7-09) (Irina)

Additional LB plates with Ampicillin will be prepared, as the stack is runing low.

### Aim:

To prepare solutions necessary for future experiments

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 100;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

### ▷ Agar plates and Addition of Antibiotics (Ampicillin 100 ug/mL) + autoclave (Irina)

Aim: These plates contain solidified lysogeny broth (LB), a rich growth medium for *E. coli*. In contrast to growth in liquid LB where the bacteria are mobile, growth on plates gives colonies originating from one single bacterial cell, an antibiotic is added for resistance selection.
Method: Agar plates containing Ampicillin (100 ug/mL) were prepared according to protocol:

LB Agar Plates (600 ml LA) and Addition of Antibiotics

Calculations:
Refer to calculations for LB medium in lab notebook, *"2019-06-17, Lab #2, LB medium"*
See table 60 for components and amounts used to prepare 600 mL of LB + antibiotic.

**Table67**

| | Component | Amount |
|---|---|---|
| 1 | LB | 600mL |
| 2 | Ampicillin (100 ug/mL stock) | 600uL |
| 3 | Bacto Agar | 9g |

Plates are stored in the cold room with the label *"July LB + Amp"*

# Lab #52 - O/N cultures and re- streaking of DH5α started (19-7-09) (Irina)

Discussion: We are going to transform in DH5α as we have no luck with BL21 (DE3).

Colonies were picked from two different plats of DH5α and re-streaked on LB plates. Two additional colonies where chosen fro said plates and placed in 15 mL Falcon tubes containig 6 mL of LB. Plates and Falcon tubes where stored in the 37 degree room.

# Lab #53 - Digestion and gel analysis for pSB1C3 (Bba_J04450) (19-7-9) (Erik)

## Aim:

To doublecheck that the plasmid that we have purified from the miniprep from lab 50 (backbone pSB1C3, gene (RFP) Bba_J04450, B0034, ribosome binding site) is the one that we have gotten out.

## Methods:

3A assembly-Digestion and ligation

**Calculations:**

500 ng x 1ul/101.4 ng= 4,93 ul of plasmid mixture

Table 25 shows the various solutions and restriction enzymes placed in the eppendorf tubes for digestion.

**Table70**

| | Component | pSB1C3, Bba_J04450 |
|---|---|---|
| 1 | 500 ng DNA | 4,9uL |
| 2 | 10 x Reaction buffer | 5uL |
| 3 | EcoRI | 1uL |
| 4 | SpeI | ---- |
| 5 | XbaI | --- |
| 6 | PstI | 1uL |
| 7 | ddH2O | 35,4ul |

Analytical Digestion and Agarose Gel Electrophoresis

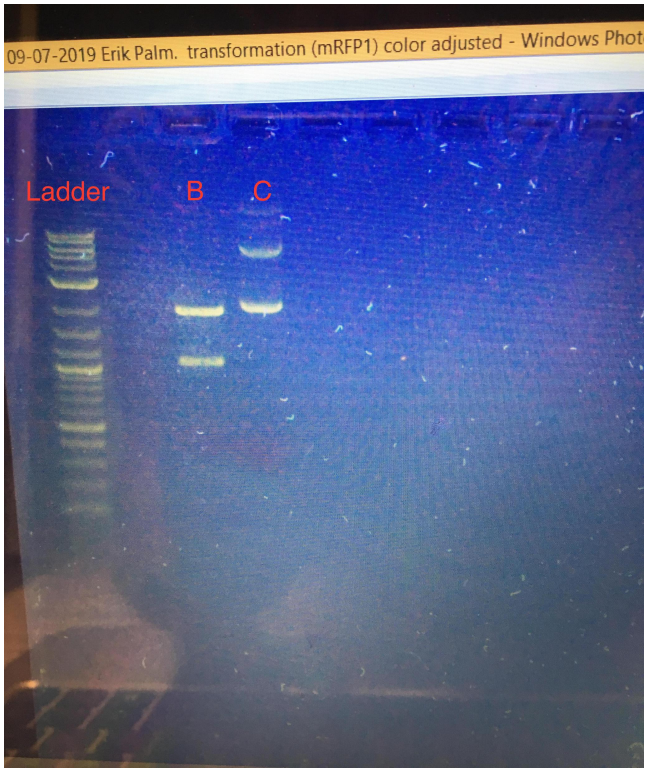
-We used a the standard  gel electrophorator at 115 V for 30 min

**Table72**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | latter | 20 ul<br>4ul loading dye | -- |
| 3 | B | digested pSB1C3, Bba_J04450 | 20 ul<br>4ul loading dye | -- |
| 4 | C | undigested pSB1C3, Bba_J04450 | 2,0 ul of sample 18,0 ul H20 4 ul loading sample | 200ng x 1ul/101,4ng |

## Results:

Analytical Digest pSB1C3 mRFP1.jpg




## Discussion:

-Well B was expected, where the length is about 2000 bp and the mRFP is 1000kb, which is what we expect from a digestion from B.

-Well C is a little bit strange. Two bands are expected, as the DNA takes on different conformations in plasmid form. However, the upper bands is higher than 3000 bp, which is what the plasmid would go if it was linear. We think that it is possible that the the plasmid have tangled around one another into a larger mass, which has made it even more difficult to go through.

## Lab #54 - Preparation of competent DH5α *E.coli* cells using $CaCl_2$ (19-7-10) (Irina)

Discussion: Today we are continuing the preparation of DH5a cells for future transformations. Two separte cultures where started, but only one shows good results when measuing OD600.

### Aim:

To increase the competence of *E. coli* DH5α cells

### Methods:

Preparation of *E.coli* cells for transformation was done according to protocol:

Preperation of E.coli cells for transformation

- For original protocol, refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 5, page 111 - 112;
- Refer to iGEM uppsala 2019's benchling folder, General Protocols, Preparation of E.coli cells for transformation;

Calculations for $CaCl_2$ solutions can be seen in table 73.

**Table 73**

| | A | 1M CaCl2 | 50% Glycerol stock | ddH2O | TOTAL volume (ml) |
|---|---|---|---|---|---|
| 1 | 0.1. M CaCl2 (15ml) | 2 ml | / | 18ml | 20ml |
| 2 | 0.1 M CaCl2 + 20% glycerol (3ml) | 0.3ml | 1.2ml | 1.5ml | 3ml |

Cells are stored in the -80 freezer, marked with the letter "I". Each tube contains 100 uL of competent cells.

## Lab #55 - Transformation of lac promotor (pSB1C3, J04500) and ligation mixture (July 8th, 2019: Mnp, pSB1A3 plus lac promotor) (19-7-10) (Erik)

### Aim:

To see if the plasmid with the lac promotor (pSB1C3, J04500) can get transformed into the competent dH5A

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

**Table 74**

| | A | Erik | Jenny |
|---|---|---|---|
| 1 | competent cell origin | Gustav | Gustav |
| 2 | Lac promotor (J04500) | Lac (5) | Lac (2) |
| 3 | Ligation Mixture (July 8th, 2019: Mnp, pSB1A3 plus lac promotor) | MnP (5) | MnP (2) |
| 4 | Number and Types of Plates | 1x chloroampheni col for unidigested lac promotor<br>1x ampicillin for ligation mixture<br>1x negative control ampicillin | 1x chloroampheni col for unidgested lac promotor plasmid<br>1x ampicillin for ligation mixture<br>1 x negative control ampicillin |

# Lab #56 - Analytical Digestion in Preparation for Gel Extraction (19-7-10) (Erik, Jenny, Qian, Gustav, Irina)

## Aim:

To prepare for gel extraction, we use the same method as analytical digestion to separate out the desired backbone with the Lac promotor (J04500) and the (pSB1C3_MnP (Bba_K500001))

## Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 4, page 108-109;

Analytical Digestion and Agarose Gel Electrophoresis

Deviations from protocol.

1. Two group were made; one was Jenny and Erik, the other Gustav and Irina. Each group was tasked with preparing a digestion of promotor and Mn gene each per the usual protocol. The goal of doing this analytical digest was to do a gel extraction, hence the many wells of the same thing. No solution remained after gel electrophoresis.
2. Made one large mixture which combined loading dye (10 ul of loading dye, 50 ul total of solution) which we mixed together and and then put into the plates

**Table75**

| | Components | Digestion of Lac promotor (J04500) | Calculations | Digestion of Mn (pSB1C3_MnP (Bba_K500001)) | Calculation |
|---|---|---|---|---|---|
| 1 | 500 ng DNA | 6,9ul | 500ngx 1ul/72,2ng= | 7,6ul | 500ng x 1ul/65,4= |
| 2 | 10 x Reaction buffer (2.1 New England Buffer) | 5ul | | 5ul | |
| 3 | EcoRI | ----- | | ----- | |
| 4 | SpeI | 1ul | | ----- | |
| 5 | XbaI | ----- | | 1ul | |
| 6 | PstI | 1ul | | 1ul | |
| 7 | ddH2O | 36,1 ul | | 35,4ul | |

First Gel: Lac Promotor with 2- log ladder

**Table76**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | 2 log | 6ul | -- |
| 3 | B | uncut lac (pSB1C3, J04500) Irinia + Gustav | 3ul of uncut plasmid + 17 ddH2O + 4ul dye = 24uL (loaded 20ul) | |
| 4 | C | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 5 | D | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 6 | E | cut lac (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 7 | F | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 8 | G | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |
| 9 | H | cut lac (pSB1C3, J04500) Erik + Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | -- |

Second Gel: Cut ofMn (pSB1C3_MnP (Bba_K500001))

**Table77**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Well Letter | In well | Amount | Calculations if needed |
| 2 | A | 2 log | 6ul | |
| 3 | B | uncut Mn gene (pSB1C3, J04500) Irinia + Gustav | | |
| 4 | C | cut Mn gene (pSB1C3, J04500) Irinia + Gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 5 | D | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Irina and gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 6 | E | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Irina and gustav | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 7 | F | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Erik+ Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 8 | G | cut Mn gene (pSB1C3_MnP (Bba_K500001 )) Erik+ Jenny | 20ul of 60ul with 50ul of digestion solution and 10 ul dye | |
| 9 | H | cut  Mn gene (pSB1C3_MnP (Bba_K500001 ))Erik+ Jenny | 16 ul<br>4ul loading dye | |

**Results:**

1. For the lac promotor gel plate, we could not see any lines with the UV at all. For the Mn plate, we could see very thin bands of Mn gene that seemed to be in the right place, but because they were so thin and indistinct, it was deemed not worth it to attempt an extraction.
2. At first we were worried that the gel had run through, however, the ladder was still present. For next time, it would be good to be more careful about the time, as the DNA had progressed quite far.

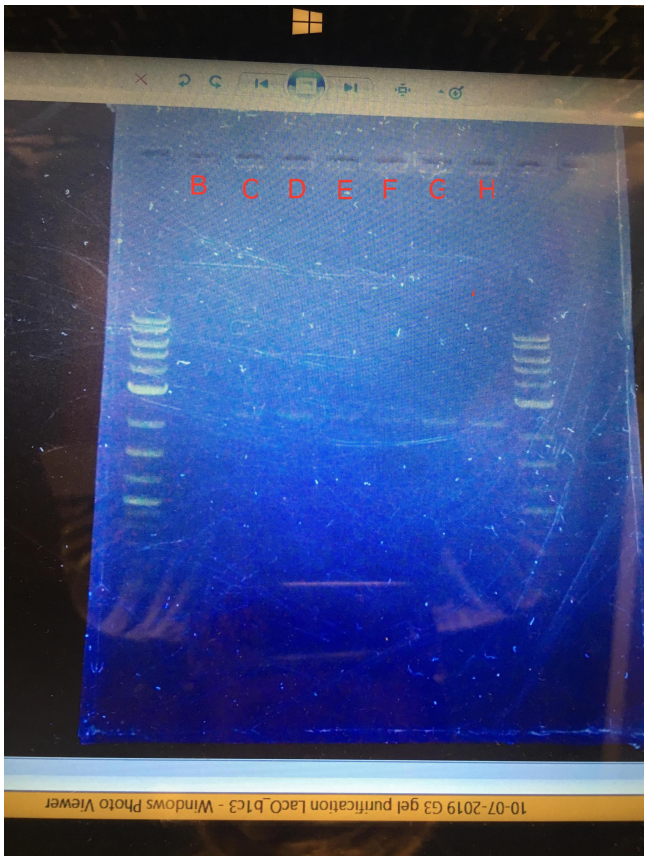
**Conclusions:**

We think that the DNA that Jin Wen prepared a couple of weeks ago now has degraded significantly, meaning that the concentrations that she nanodropped on the side are not accurate. As a result, there is not the necesary amount of DNA for clear bands to be seen and for extraction to be obvious.

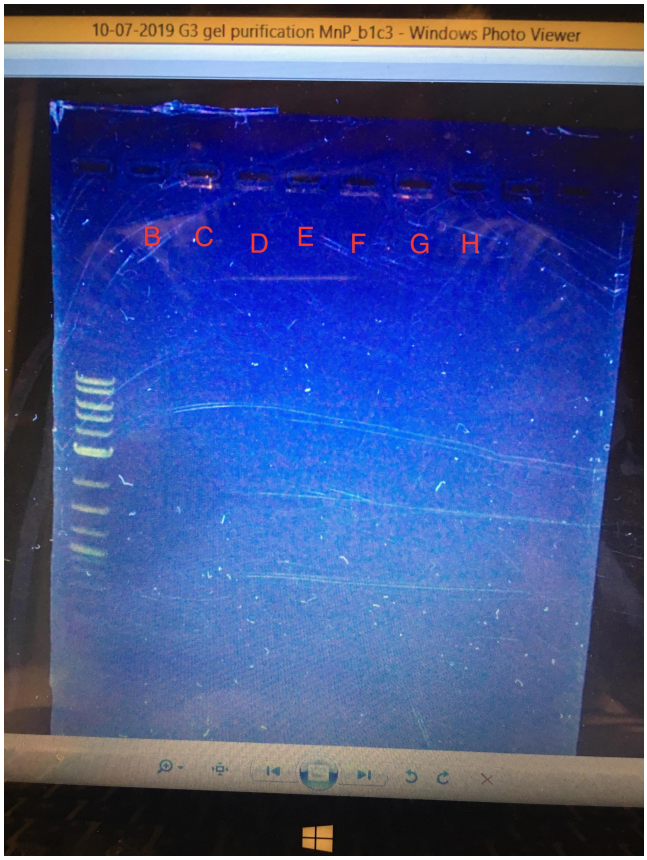
To fix this for next time, a couple of suggestions.

1. More DNA should be loaded into the one well by using the larger well combs while preparing the gel
2. Be careful about what is underneath the gel, as the plastic plate blocked the UV light, alllowing us to not see the bands on the UV light.

Lac Analytical Digest July 10 2019 2.0.jpg



Mn Analytical Digest July 10 2019 2.0.jpg




# Lab #56 - Ligation and transformation into DH5a competent cells (19-7-11) (Irina)

## Aim:

Ligation of pSB1A3, LacI (Jinwen's digestion nr. 6) and MnP (Jinwen's digestion nr.6) on Ampicillin LB agar plates.

## Methods:

E.coli is inefficient at taking up foreign plasmids. Therefore the competent cells (DH5α) have been pre-treated with $CaCl_2$. Ligation of the BioBricks are done prior to Transformation on Kanamycin LB agar plates.

3A assembly-Digestion and ligation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-Digestion and ligation;

### Ligation

Components and amounts for ligation can be seen in table 78, some modificstions of amounts where done. We took into acount 20% degradation.

**Table 78**

| | Components | Amount |
|---|---|---|
| 1 | T4 10x Reaction buffer | 2uL |
| 2 | T4 DNA ligase | 1uL |
| 3 | ddH2O | 5uL |
| 4 | Bba_K500001 | 8uL |
| 5 | J04500 | 2uL |
| 6 | pSB1A3 | 2uL |

## Transformation

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Components and amounts can be seen in table 33 for transformation reaction.

Transformation of Competent E.coli cells

One Transformation was done, with 1 negative control

1. Transformation
2. Negative control

Deviation from protocol: No positive control made. Gustav is making the efficiency count

In table 78, reaction mixtures for transformation can be seen.

**Table 79**

| | Components | Transformation sample | (-) control |
|---|---|---|---|
| 1 | Ligation reaction mixture | 5uL | - |
| 2 | DH5a competent cells | 50uL | 50uL |
| 3 | SOB pre-heated to 37°C | 950uL | 950uL |
| 4 | ddH2O | - | 5uL |

Negative Control: 5 ul water was added instead of Ligation mixture.

# Lab #57 -Determining the competency of competent cells made 10/7/19 (Gustav, 11/7/19 + 15/7/19)

Aim:
To determine the competency of the competent cells made the 10/7/19

Methods:
The method used can be found on the official iGEM website: http://parts.igem.org/Help:2018_Competent_Cell_Test_Kit.

Notes:
Three deveations from the protocol were made. The first was that no 10ng sample was prepared due to the high concentration of DNA in our DNA source. The second deveation was that the instructions were misread and step 6 was conducted without having any DAN in the samples. When this was realised DAN was added and the wait time in step 6 was extended by 15mins.
The third was that we used the Psb1A3 plasmid miniprepd from Jonas lab group.

Calculations:
100ng of psB1A3 backbone was used with RFP.
Conc. of psb1A3: 92.2 ng/ul. --> 100/92.2 = 1.1 ul.

Competency of plate 1: 1.689*10^(10)
Competency of plate "2": 1.337*10^(10)
Competency of plate "3": 1.22*10^(10)
Average competency: (1.22+1.337+1.689)/3 = 1.415*10^(10) colonies/ng
1.415*10^(7) colonies/ug

The average competency of the cells was to the tenth exponent.

## Lab #58 - Transformation of MnP Gene (BBa_K500001) (19-7-11) (Erik and Jenny)

### Aim:

To see if the plasmid with the MnP gene (pSB1C3, BBa_K500001) can get transformed into the competent dH5A.

### Methods:

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;
Refer to iGEM uppsala 2019's benchling folder, General Protocols, 3A assembly-transformation;

Table 80 shows who prepared which sample.

**Table80**

| | A | Erik | Jenny |
|---|---|---|---|
| 1 | competent cell origin | Gustav | Gustav |
| 2 | MnP Gene (BBa_K500001) from Jinwens' miniprep | Mnp (3) | Mnp (2) |
| 3 | Number and Types of Plates | 1x chloroamphenicol for pSB1C3_BBa_K500001 | 1x chloroamphenicol for pSB1C3_BBa_K500001<br>1 x chloramphenicol for negative control |

Calculations:

Dilution of the MnP Miniprep Samples. Wanted concentration: 2 ng/ul

Starting concentration sample 2: 109.7 ng/ul

109.7 ng/ul x 1 ul/2 ng = 54,9 ul (total volume) ~55 ul. Add 54 ul dd$H_20$ to 1 ul of the Miniprepped Sample nr 2.

Starting concentraion sample 3: 133,5 ng/ul

133.5 ng/ul x 1 ul/2 ng = 66.75 ul (total volume) ~66.8 ul. Add 65,8 dd$H_20$ to 1 ul of the Miniprepped Sample nr 3.

Differences from the Protocol 6:

*No positive control were done. (skipped point 9)

*At point 11 we saved 60 ul and poured the rest of the liquid as much as possible. Changed to the largest tip and resuspended the pellet before the streaking on the plates.

# Week 8: Aug 5- Aug 11

**Project:** iGEM uppsala 2019
**Authors:** Erik Palm

MONDAY, 5/8/2019

## Lab #72 Electroporation (Irina + Jenny) 2019-08-05

**Aim:** Electroporate the DH5a to make them competent for further transformation with DNA from Viktors group; AAO-2A-eGFP and HRP-2A-eGFP.

**Method:** We follow the protocol **Rapid Protocol for Preparation of Electrocompetent *Escherichia coli* and *Vibrio cholerae*** (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3939052/) and the steps in their methods. Adjustments from the protocol is that instead of taking 1 ng of DNA we took 2 ul. We spun only for 3000g instead of 5000g.

Tubes were spun for 4000g x 5 minutes, 900 mL of supernatant was removed, and the pellet was resuspended in the remaining 100 uL (approx) and plated on low salt LB + Zeocin plates.

## Lab #73 Send for sequencing - AAO and Lip (19-08-05) (Erik + Irina)

Two sequences, AAO and Lip where sent for sequencing.

Calculations for AAO:
C1 = 70.1 ng/uL
C2 = 10 ng/uL
V2 = 15 uL
V1 = 10 ng/uL x 15 uL / 70.1 ng/uL = 3 uL

Stock Forward primer = 100 uM
Diluted 10 x  = 2 uL forward primer + 18 ul ddH2O.

Mixture:
3 uL AAO
12 uL ddH2=
2 uL Forward primer (10 uM)

Mixed into a tube named EF30500584

Calculations for Lip:
C1 = 53.2 ng/uL
C2 = 10 ng/uL
V2 = 15 uL
V1 = 10 ng/uL x 15 uL / 53.2 ng/uL = 3 uL

Stock Forward primer = 100 uM
Diluted 10 x  = 2 uL forward primer + 18 ul ddH2O.

Mixture:
3 uL Lip
12 uL ddH2O
2 uL Forward primer (10 uM)

Mixed into a tube named EF30500585

THURSDAY, 8/8/2019

## Lab 74 Overnight Culture

Aim: To take 6 separate E.coli colonies from the plates with the following constructs: pPICZaphaB_AAO, pPICZaphaB_AAO-2A_GFP, pPICZaphaB, pPICZaphaBHRP-2A-AAO to prepare for colony PCR.

Method:

1. 6ml of Lb low salt media was placedin a erlemeyer flask
2. A pipett tip was used to scoop up a single colony and then dropped into the erlemeyer flask
3. The erlemeyer flask were placed in the 37 degree room

FRIDAY, 9/8/2019

## Lab 75 Colony PCR with Gel Analysis (Erik + QIan+ Jin Wen+ Johan+ Jenny)

### MISSING: WHICH WAS WHICH: Jenny and Johan

### Aim:

To screen 6 E.coli colonies from each of the following constructs that come frome our previous transformations: pPICZaphaB_AAO, pPICZaphaB_AAO-2A_GFP, pPICZaphaB, pPICZaphaBHRP-2A-AAO in construct from our previous transformations.

### Method:

Colony PCR Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

primers: ppic-fwd

primers: ppic-rev

Deviations from protocol:

1. 1ml of overnight culture taken from each of the overnight cultures.
2. 400 ul spun down in centrifuge
3. 30 ul of water added to another eppendorf tube
4. Pipett tip used to dab the pellet, then stirred in 30ul of water

## Deviation from PCR Program some differences here that I think Qian wrote down

**Table3**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Temp. (*C) | 95 | 95 | 58 | 72 | 4 |
| 2 | Time (min) | 5 | 1 | 1 | 3 | infinity |
| 3 | | | {30cycles | | }}}}}}} | |

**Calculations**

length of amplicon 3,0 kb

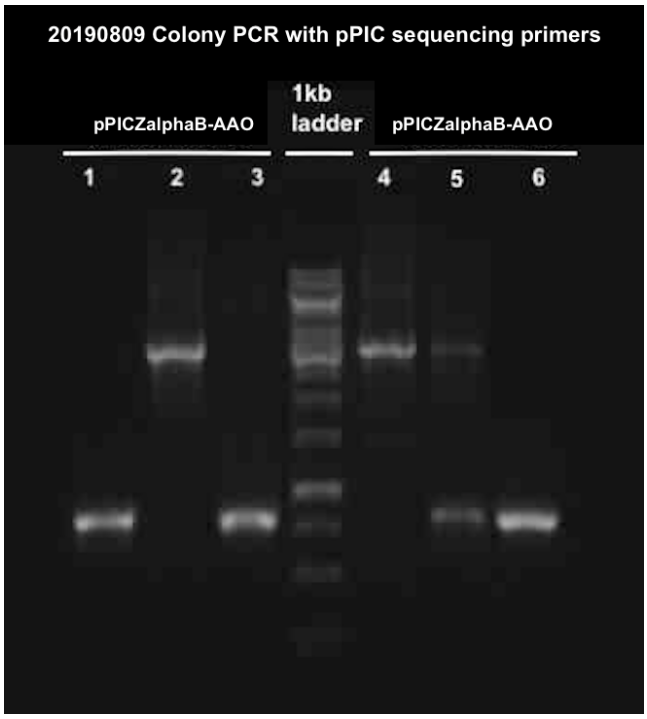
taq extension 1min/kb

extension time= 3,0 kbx 1min/kb

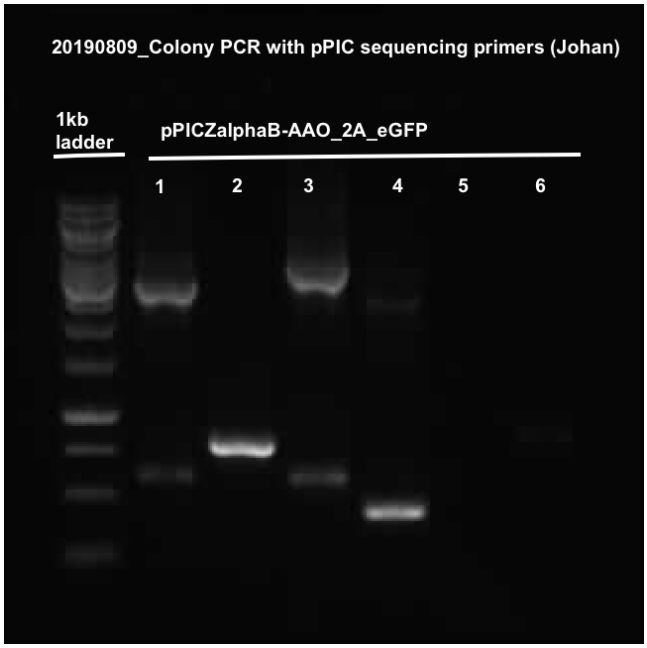
**Table4**

| | A | B |
|---|---|---|
| 1 | What | Amount (ul) |
| 2 | ddH20 | 33.7 |
| 3 | 10x Taq PCR buffer | 5 |
| 4 | 2 mM dNTPS | 5 |
| 5 | primer FWD | 2.5 |
| 6 | primer Revers | 2.5 |
| 7 | Taq DNA polymeras | 0.3 |
| 8 | Colony in 30ul of water | 1 |

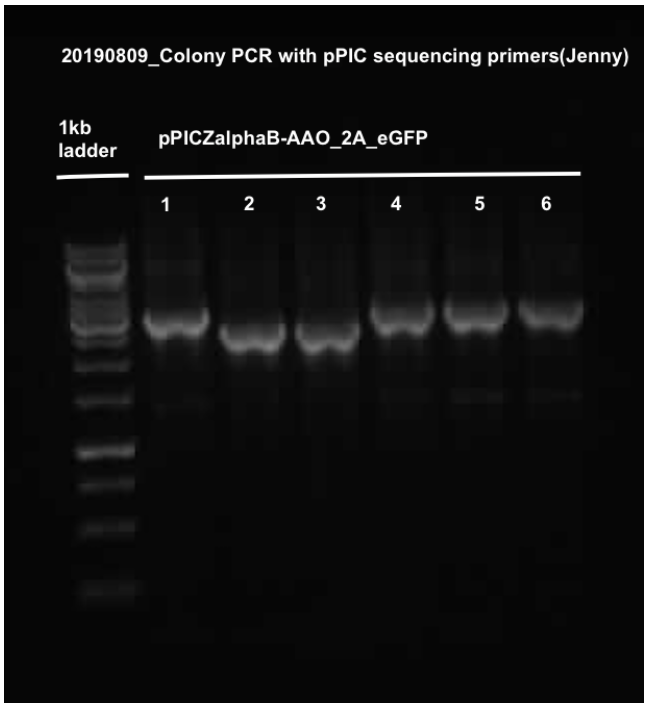
Gel of Colony PCR (AAO)



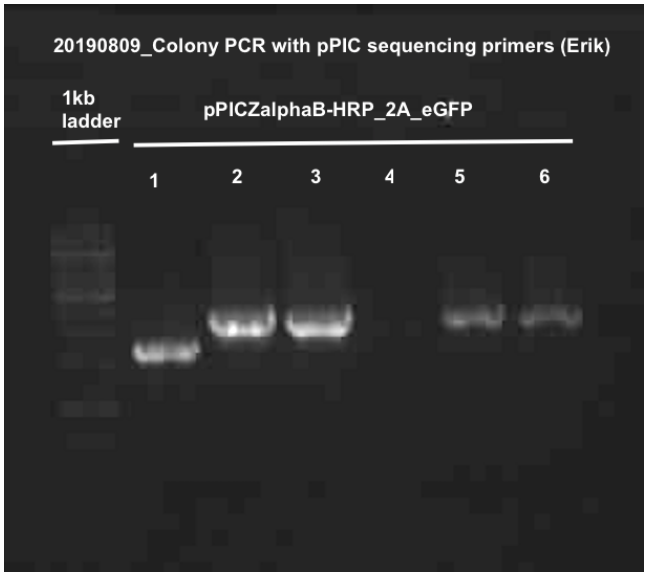
Gel of AAO_2A_eGFP




Gel of AAO_2A_eGFP

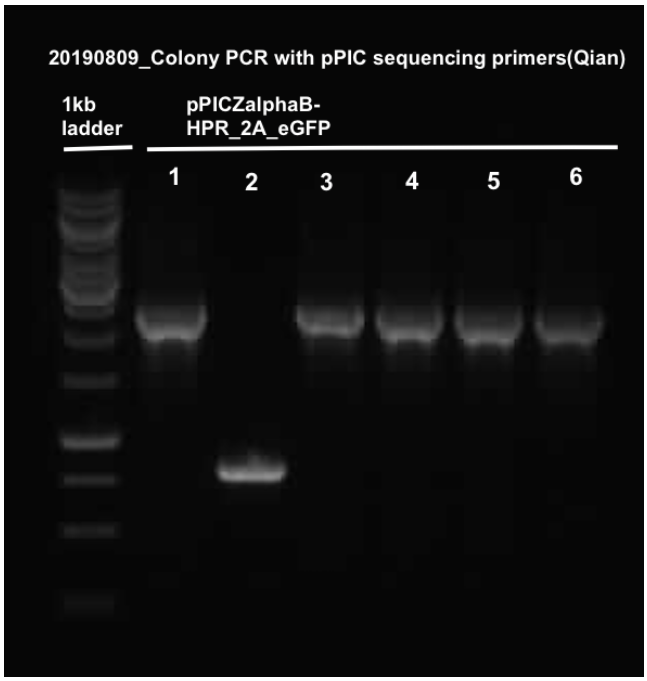


Gel of HRP_2A_eGFP from supercompetent cells




Analysis: Wells 2, 3, 5, 6 appear to have approximately the right length for the construct. Eventual glycerol stocks from 1 an4 were discarded.

Gel of HRP_2A_eGFP (From non-supercompetent cell s)




## Lab 76 Glycerol Stock of Colony PCR

### Aim:

To save the E.coli cells with the correct construct from lab 75 to eventually an overnight culture and midi prep..

### Method:

1. 600 ul of the overnight culture was added to 400 ul of 50% glycerol stock
2. Placed in the -80 degree freezer.

SATURDAY, 10/8/2019

# Lab 78: Four SDS Page Gels

Aim:

Method:

**Separation Gel 15% SDS Page**

| | A | B | C |
|---|---|---|---|
| 1 | What | Amount for 1 Gel | Amount for 4 Gels |
| 2 | water | 1,2 ml | 4,8 ml |
| 3 | Akrylamide | 2,5 ml | 10 ml |
| 4 | 1,5 M Trish pH 8.8 | 1,25 ml | 5 ml |
| 5 | SDS 10% | 50 ul | 200 ul |
| 6 | APS 10% | 50 ul | 200 ul |
| 7 | TEMED | 5 ul | 20 ul |

**10 ml 4% Stacking Gel**

| | A | B | C |
|---|---|---|---|
| 1 | What | Amount | |
| 2 | Water | 6,1 ml | |
| 3 | Akrylamide/Bis 30% | 1.3 ml | |
| 4 | Tris HCl 0,5M pH 6.8 | 2. 5 ml | |
| 5 | SDS 10% | 100 ul | |
| 6 | APS 10% | 100 ul | |
| 7 | Temed | 10 ul | |

# Ethanol precipitation Easyselect-man

## Introduction

Ethanol precipitation after phenol extraction of DNA according to Easyman-select protocol p.26.

## Materials

›

- 3M Sodium acetate
- 100% ethanol
- 80% ethanol
- Sterile deionized water

## Procedure

1. Add 1/10 volume of 3M sodium acetate to the upper aquatious phase and 2.5 volume of 100% ethanol.
2. Centrifuge the solution to pellet DNA, wash the pellet with 80% ethanol, air-dry, and resuspend in 10 µL sterile, deionized water.
3. Use immidiately or store at -20°C.

# Week 9: Aug 12- Aug 18

**Project:** iGEM uppsala 2019
**Authors:** Erik Palm

MONDAY, 12/8/2019

## Lab 78 Colony PCR August 12th with Gel Analysis (Erik + Johan)

### Aim:

To screen 6 E.coli colonies for the p1CZAB_AAO and pP1CZAB_Lip construct from our previous transformations.

### Method:

Colony PCR Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 6, page 113-115;

primers: ppic-fwd
primers: ppic-rev
Deviation from PCR Program

**Table1: PCR Protocol**

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Temp. (*C) | 95 | 95 | 58 | 72 | 72 | 4 |
| 2 | Time (min) | 5 | 1 | 1 | ------ | 7.00 | infinity |
| 3 | | | {30cycles | | }}}}}}} | | |

**Calculations**
AAO length of amplicon 2,0 kb
taq extension 1min/kb
extension time= 2,0 kbx 1min/kb= 2 min

Lip Extension Time Determined by Jin Wen: 1:30

**Table6**

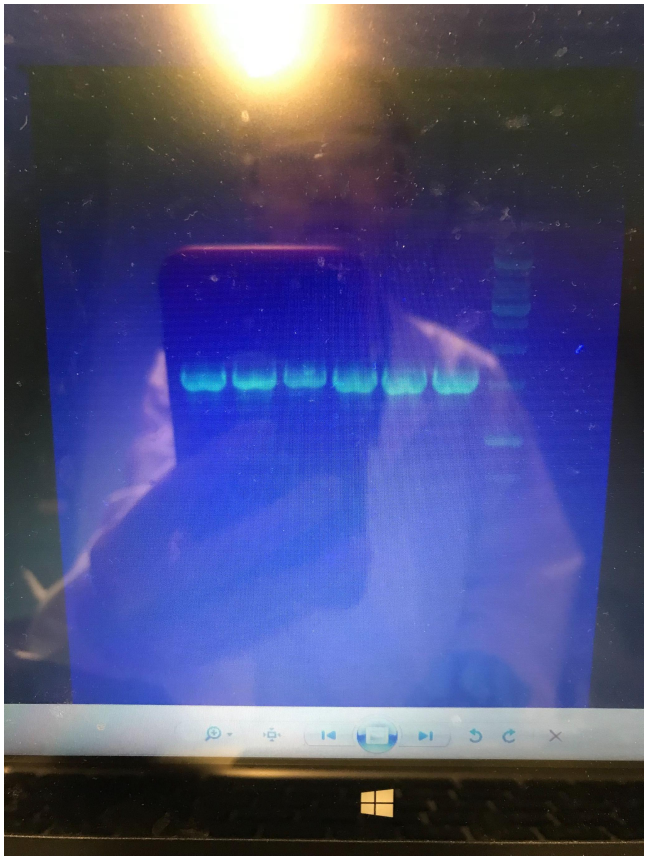
| | A | B |
|---|---|---|
| 1 | What | Amount (ul) |
| 2 | ddH20 | 28.7 |
| 3 | 10x Taq PCR buffer | 5 |
| 4 | 2 mM dNTPS | 5 |
| 5 | primer FWD | 5 |
| 6 | primer Revers | 5 |
| 7 | Taq DNA polymeras | 0.3 |
| 8 | Colony in 30ul of water | 1 |

Deviations from Protocol:

The following gels is the third one done by Johan and Erik. The first gels where difficult to ascertain the length, as not enough of the ladder was added.

Gel Analysis after PCR Screening of AAO

Gel Analysis after PCR Screening of LiP



## Lab 79: Overnight Culture of pP1CZAB_AAO and pP1CZAB_GlOX from Colony PCR (Lab 78) (Qian)

Aim: To get many E. coli cells with the desired pP1CZAB_AAO and pP1CZAB_GlOX constructs.

### Procedure:

~~One colony per plate was transferred to 15 mL Falcon tubes containing 6 mL of LB + Cm medium. The same colony was chosen for re-streaking on LB + Cm plates. Samples were stored in the 37 °C room O/N. The original plates were stored in the refrigerator.~~

## Lab 80: Inoculation of Recombinant Pichia Strains X33 MnP Pic1 (Erik)

### Aim:

To add pP1CZAB_MnP Pichia Strain X33 mut S to a media of BMGY.

### Method:

1. 2ml of PBS was added to an eppendorf tube from large container using pipettboy
2. 50 ul of PBS was taken from this eppendorf tube to a second eppendorf tube
3. A pipett tip was used to touch a single colony on the X33 MnP Pic1 YPD+zeocin plate and then stirred in the 50ml of PBS solution.
4. 25ul of PBS+colony of solution was placed in a 1L baffled flask containing 100ml of BMGY and another 25 ul of PBS+colony solution was placed in the other 1L baffled flask.
5. Placed in water bath/shaker at 28 degrees with RPM at 200 shakes per minute.

## Lab 81: Plating of KM71H pP1CZab (Erik Palm)

### Aim:

To plate AA0-2A-eGFP, pP1CZaB, AAO, HRP-2A-eGFP, GLOX, Control H20 + colony with 10 ul, 25ul, 50 ul, 100ul, 200ul of each to get colonies for eventual inoculation.

### Method:

Spread the remaining suspension on an agar plate containing zeocine. A total of 21 plates were plated, 5 different ul amounts of (10 ul, 25ul, 50 ul, 100ul, 200u )AA0-2A-eGFP, pP1CZaB, AAO, HRP-2A-eGFP, GLOX and 1

i) Dip the spreader into 95% ethanol.

ii) Put it into the flame for a second.

iii) Let the ethanol burn off outside the flame.

iv) Spread the bacterial suspension evenly out on an agar plate. Continue untill all the inoculum has gone into the agar.

v) Put the plates at  28°C overnight in heater.

TUESDAY, 13/8/2019

## Lab 82: Making 15% SDS Page Gel (Erik Palm)

### Aim:

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel asa support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

### Method:

Refer to the SDS Page Gel Protocol below. Four 15% SDS gels were made.

SDS Page Gel (10 and 15%)

## Lab 83: 10% SDS Page Gel (Erik Palm)

### Aim:

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel asa support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

### Method:

Refer to the SDS Page Gel Protocol below. Four 15% SDS gels were made.

Deviations from protocol:

1. SDS Page gel preparation largely failed because of leakage. Water had been used to test leakage, however, Erik maybe have messed with the chambers, resulting in three of the four chambers not retaining enough of the separation gel.
2. Therefore, one chamber could be used to add stacking gel, but the rest could not.
3. The stacking had already been prepared without APS and TEMED. Therefore, 2 aliquots (1,25 ml) were taken, each with the amount of stacking gel needed for 1 gel.
4. The stacking gel was placed in the first gel and allowed to congeal
5. One additional separation gel was made (only one was made because APS ran out). The TEMED and APS was added.
6. The stacking gel was added to the second gel.
7. Two gels were placed in the 4 degree fridge.

SDS Page Gel (10 and 15%)

## Lab 84: OD and Inoculation of Cultures and Taking Samples (Erik Palm+ Yannick)

WEDNESDAY, 14/8/2019

# Lab 85: 10% SDS Page Gel (Erik Palm)

## Aim:

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel asa support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

## Method:

Refer to the SDS Page Gel Protocol below. Four 15% SDS gels were made.

SDS Page Gel (10 and 15%)

THURSDAY, 15/8/2019

# Lab 86: Preparation of BMMY agar plates and Lowsalt LB (19-8-29) (Irina)

## Aim:

For expressing recombinant Pichia strains, BMMY (buffered methanol medium) can be used. BMMY is usually used for expressing secreted proteins, particularly if pH is important for the activity of your protein. This media is buffered with phosphate buffer, and may be used in a wider range of pH values to optimize protein production. BMMY contain yeast extract and peptone to stabilize secreted proteins and to prevent or decrease proteolysis of secreted proteins. Inclusion of yeast extract and peptone allow better growth and biomass accumulation.

Method:
Protcol followed: EasySelect ™ Pichia Expression Kit For Expression of Recombinant Proteins Using pPICZ and pPICZα in Pichia pastoris, Rev. Date 18 June 2010 Manual part no. 25-0172: http://tools.thermofisher.com/content/sfs/manuals/easyselect_man.pdf. Page 58.

Buffered Glycerol-complex Medium Buffered Methanol-complex Medium (100 mL)
1% yeast extract 2% peptone 100 mM potassium phosphate, pH 6.0 1.34% YNB 4 × 10-5% biotin 1% glycerol or 0.5% methanol 1.

1. 1 g of yeast extract,
2. 2 g peptone in 70 mL water.
3. 1.5 g Agar
4. Autoclave 20 minutes on liquid cycle.
5. 10 ml 1 M potassium phosphate buffer, pH 6.0
6. 10 ml 10X YNB
7. 0.2 ml 500X B
8. 10 ml 10X Methanol 4
9. The plates where stored at 4°C

## Preparation of Low salt LB media

Aim: Lysogeny broth (LB) is a rich media for bacterial growth and is the standard choice for *E.coli.*
Method: The following protcol was used to prepare LB medium:

LB media (600 mL)

Refer to the lab manual *"Synthetic Biology - A Lab Manual"* by J. Liljeruhm et al., protocol 1, page 98;

Refer to iGEM uppsala 2019's benchling folder, General Protocols, Solutions Protocols;

Calculations:
Mw(NaCl) = 58.44 g/mol
m(NaCl) = 0.17 mol/L x 0.8L x 58.44 g/mol ≈ 8g but since we make low salt, we only add 4 g.
m(BactoTMtryptone) = 1g/100 mL x 800 mL = 8g
m(Yeast extract) = 0.5g/100 x 800mL = 4g
See table 7 for components and amounts used to prepare 800 mL of LB.

**Table 7**

| | Component | amount |
|---|---|---|
| 1 | NaCl | 4g |
| 2 | BactoTMtryptone 1% (w/v) | 8g |
| 3 | Yeast extract 0.5% (w/v) | 4g |
| 4 | ddH2O | 800ml |
| 5 | 1 M NaOH | 300ul |

# In vitro transcription of guide RNAs

## Introduction

sgRNA template assembly, in vitro T7 transcription, and SPRI bead cleanup

## Materials

- Primers to Generate
  - T7 FwdVar (1 µM)
  - T7 RevLong (1 µM)
  - T7 FwdAmp (100 µM)
  - T7 Rev Amp (100 µM)
- Phusion HF DNA Polymerase (2 units/µl)
- Phusion HF Buffer
- Deoxynucleotide Solution Set
- Ribonucleotide Solution Set
- Magnetic Separation Rack
- SPRI Beads
- 80% Ethanol
- TE Buffer
- Thermocycler

## Procedure

- [x] 1. Set up the following 50 µl reaction on ice.

**Table1**

| | A | B |
|---|---|---|
| 1 | **Reagents (concentration)** | **Reagent Volumes (µl)** |
| 2 | Deionized Water | 35.5 |
| 3 | 5X Phusion HF Buffer | 10 |
| 4 | 10 mM dNTPS | 1 |
| 5 | T7FwdVar (1 µM) | 1 |
| 6 | T7RevLong (1 µM) | 1 |
| 7 | T7 Fwd Amp (100 µM) | 0.5 |
| 8 | T7 Rev Amp (100 µM) | 0.5 |
| 9 | Phusion HF DNA Polymerase (2u/µl) | 0.5 |
| 10 | Total Volume (µl) | **50** |

2. Run PCR (to create template DNA):

1.) 95˚ - 30 sec
2.) 95˚ 10 sec
3.) 57˚ 10 sec
4.) 72˚ 10 sec
**Run 30 cycles of steps 2-4**
5.) 72˚ 2 min
6.) 4˚ hold
No PCR cleanup necessary at this point

3. T7 transcription mix for guide RNAs protocol

**Table2**

| | A | B | C |
|---|---|---|---|
| 1 | | **Volume (µl)** | **Final Concentration (mM)** |
| 2 | **10x Buffer** | 2 | 1X |
| 3 | **ATP (100 mM)** | 2 | **10 mM** |
| 4 | **GTP (100 mM)** | 2 | **10 mM** |
| 5 | **CTP (100 mM)** | 2 | **10 mM** |
| 6 | **UTP (100 mM)** | 2 | **10 mM** |
| 7 | **DNA template (85 ng/µl)** | 8 | **(25 ng/µl)** |
| 8 | **T7 RNA polymerase mix** | 2 | |

4. Incubate transcription mix for ~18 hours at 37˚ in a thermalcycler

18:00:00

- [x] 5. Add 1 µl of RNase-free DNase; incubated 20 min, room Temp

00:20:00

- [x] 6. Bring volume to 150 uL with 100% EtOH (this helps binding of small fragments)
- [x] 7. Add 5X SPRI

    5*10 (IVT sgRNA)= 50 uL of SPRI Beads
    5*20 (IVT sgRNA)= 100 uL SPRI Beads

- [x] 8. Pipette to mix 10 times
- [x] 9. Incubate 5 minutes at room temperature

00:05:00

- [x] 10. Place on magnetic stand, 5 min

00:05:00

- [x] 11. Discard supernatant
- [x] 12. Wash#1 Add 200 uL of 80% EtOH. Wait 2 min. Remove EtOH.

00:02:00

- [x] 13. Wash #2: Add 200 uL of 80% EtOH. Wait 2 min. Remove EtOH.

00:02:00

- [x] 14. Air dry 5-10 min (pellet will change from a glossy/wet to matte/dry. )

00:10:00

- [x] 15. Elute 20 uL of water or TE. Pipette mix 10 times.

- [x] 16. Incubate 2 minutes at room temperature

00:02:00

- [x] 17. Place on magnetic stand, 5 min

00:05:00

- [x] 18. Keep Supernatant. Transfer to a new plate / tubes.

# Week 11: Aug 26- Sept 2

**Project:** iGEM uppsala 2019
**Authors:** Erik Palm

TUESDAY, 27/8/2019

## Plasmid Mini-Prep and Glycerol Stock (Erik Palm)

### Aim:

To isolatie plasmid DNA from recombinant *E. coli* O/N cultures containing pSB1C3_AAO 8/25 and pSB1C3_HRP1 8/25, pSB1C3_HRP2 8/25, pSB1C3_HRP3 8/25 in order to get constructs to send to DTU for expression with their promotors. Glycerol Stocks were also taken.

### Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.

Purified DNA plasmid concentrations can be seen in table 12.

**Deviations from Protocol:** Jin wen and Victor took over from Erik after addition of resuspension solution.
**Results:** The concentrations were extremely low, around 2 ng/ul for all solutions. Might be possible that the new mini-prep kit is not working properly, perhaps due to the fact that somebody has not put in the right ethanol percentage.

Glycerol Stock

1. 600 ul of overnight culture with 400 ul of 50% glycerol
2. Placed in -80 degree Celsius freezer

THURSDAY, 29/8/2019

## Plasmid Mini-Prep (Erik Palm)

### Aim:

To isolatie plasmid DNA from recombinant *E. coli* O/N cultures containing pSB1C3_AAO 8/25 and pSB1C3_HRP1 8/25, pSB1C3_HRP2 8/25, pSB1C3_HRP3 8/25 in order to get constructs to send to DTU for expression with their promotors.

### Methods:

MiniPrep was done according to Sigma- Aldrich protocol:
GenElute™ Plasmid Miniprep Kit - https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/pln70bul.pdf.

Purified DNA plasmid concentrations can be seen in table 12.

**Deviations from protocol:**
Since the last plasmid mini-prep failed, old solutions were tried to see if the kit itself was the problem and not the procedure
Solutions used:
Resuspension: old
Lysis: new
Neutralization: old

**Resultats from Plasmid Mini-Prep 8-29**

| | Sample | Concentration (ng/uL) | 260/280 | 260/230 |
|---|---|---|---|---|
| 1 | pSB1C3_HRP1 8/25 | 237.3 | 1.9 | 2.22 |
| 2 | pSB1C3_HRP2 8/25 | 219 | 1.9 | 2.22 |
| 3 | pSB1C3_HRP3 8/25 | 374 | 1.9 | 2.26 |
| 4 | pSB1C3_AAO 8/25 | 418.4 | 1.9 | 2.28 |

FRIDAY, 30/8/2019

# Week 10. Aug 19- Aug 25

**Project:** iGEM uppsala 2019

**Authors:** Erik Palm

SUNDAY, 8/9/2019