# Week 1 - Improve a part

**Project:** iGEM2021
**Authors:** Iris Noordermeer
**Entry Created On:** 2021-10-04 02:17:19 PM +0000
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FRIDAY, 13/8/2021

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-13

Goal

- Make the following constructs:
    - pJump29-RBS_NTag_Maze_Term
    - pJump29-RBS_Ntag_RelE_Term
    - pJump29-RBS_RepA_MazE_Term
    - pSB1C3-RBS_Ntag_Maze_Term
    - pSB1C3-RBS_Ntag_RelE_Term
    - pSB1C3-RBS_RepA_MazE_Term

However only these grew colonies:

- pSB1C3-RBS_Ntag_Maze_Term
- pJump29-RBS_Ntag_RelE_Term
- pJump29-RBS_RepA_MazE_Term

MONDAY, 16/8/2021

## Improve a part (2)

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-16

## Goal

- Single non-fluorescent colonies were ented into liquid cultures.

## Material

- Liquid LB medium
- 50 ml tubes
- Toothpick

TUESDAY, 17/8/2021

## Improve a part (3)

**Project:** Lab Notebook

**Authors:** Iris Noordermeer, Chanel Naar

**Created at:** 2021-08-17

## Goal

- Plasmids isolation.
- Check if transformation succeded on gel

## Material

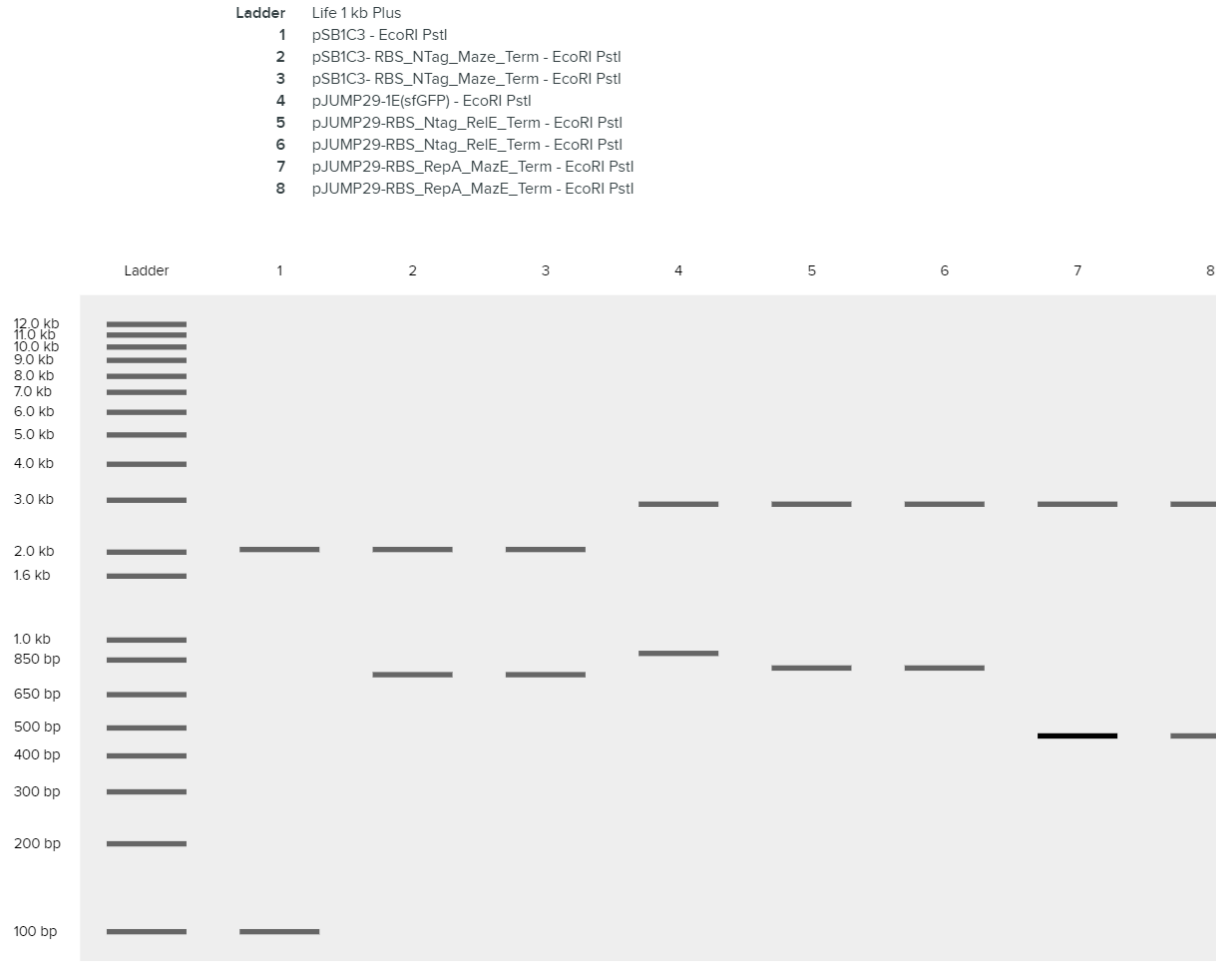
- EcoRI
- PstI
- Restriction buffer
- MiliQ
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide

## Protocol

Standard isolation protocol.

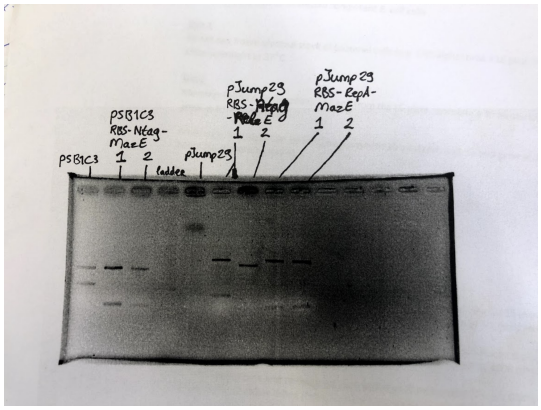
Check on gel, should be:

image.png




## Result

image.png



Unfortunately, the ladder came out quite vague...
However on the computer it was better visible and when compared with the other bands of which we know the size (controls), it could be that the PSB1C3-RBS-Ntag-MazE are right but they are a bit on the lower side. The first colony of the pJUMP29-RBS-Ntag-RelE seems to be right and both the colonies of the pJUMP29-RBS-RepA-MazE seem to be right.

--> a new colony of the PSB1C3-RBS-Ntag-MazE was put in liquid culture

Also the old one with promotors will be checked on gel on friday anyway

## Continue with making constructs

- Make constructs (restriction/ligation protocols) :
  - DH5α strain
  - Jump 29 (EcoRI/PstI) / pSB1C3 (PstI/EcoRI)
  - Put promotors in front of the construct: PBad and p2547
    - Cut backbone with insert plasmid with: XbaI and EcoRI
    - Cut promotors with: EcoRI and SpeI

**Protocollen:**

*Restriction*

**Table2**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | 1 reaction | EcoRI + PstI MM | EcoRI + XbaI MM | EcoRI + SpeI MM |
| 2 | Plasmid DNA | 2\|3\|0.5 | | | |
| 3 | 10x Buffer 2.1 | 1.5 | 13.5 | 21 | 21 |
| 4 | Enzyme 1 | 0.5 | 4.5 | 7 | 7 |
| 5 | Enzyme 2 | 0.5 | 4.5 | 7 | 7 |
| 6 | MiliQ | 10.5\|9.5\|12 | 94.5 | 133 | 168 |
| 7 | Total | 15 | 135 | 210 | 210 |

Digest for 1,5 hour.
Stop the digestion by heatshocking at 65 °C for 15 minutes.

*Ligation*

Measure Nucleac acid concentration and add vector / insert ratio 1:3.
Used: NEBioCalculator to calculate the needed amounts

**Table1**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | | bp | pBad needed | p2547 needed | |
| 2 | RelE.1 | 3716 | 264.8 ng | 169.9 ng | |
| 3 | RelE.2 | 3716 | 264.8 ng | 169.9 ng | |
| 4 | RepAMazE1 | 3380 | 291.1 ng | 186.8 ng | |
| 5 | RepAMazE2 | 3380 | 291.1 ng | 186.8 ng | |
| 6 | NtagMazE1 | 2787 | 353.1 ng | 226.6 ng | |
| 7 | NtagMazE2 | 2787 | 353.1 ng | 226.6 ng | |

So we need 1818 ng pBad and 1166.6 ng p2547.

Restriction of the promotors

**Table3**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | pBad | p2547 | | |
| 2 | Plasmid DNA | 2.7 | 16.7 | | |
| 3 | 10x Buffer 2.1 | 1.5 | 2.5 | | |
| 4 | EcoRI | 1.2 | 1 | | |
| 5 | SpeI | 1.2 | 1 | | |
| 6 | MiliQ | 8.2 | 3.8 | | |
| 7 | Total | 15 | 25 | | |

Ligation:

**Table4**

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Components | pBad RelE.1 | pBad RelE.2 | pBad RepA.1 | pBad RepA.2 | pBad Ntag.1 | pBad Ntag.2 | p2547 RelE.1 | p2547 RelE.2 | p2547 RepA.1 | p2547 RepA.2 | p2547 Ntag.1 | p2547 Ntag.2 |
| 2 | T4 DNA ligase buffer | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 3 | Vector DNA 100 ng | 9.3 | 6.4 | 6.2 | 8.2 | 4.1 | 7.9 | 9.3 | 6.4 | 6.2 | 8.2 | 4.1 | 7.9 |
| 4 | Insert DNA | 13.8 | 2,6 | 2.8 | 2.8 | 3.4 | 3.4 | 3.6 | 3.6 | 4 | 4 | 4.9 | 4.9 |
| 5 | Nuclease free water | 1.9 | 8 | 8 | 6 | 9.5 | 5.7 | 4.1 | 7 | 6.8 | 4.8 | 8 | 4.2 |
| 6 | T4 DNA ligase | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 7 | Total | 30 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

See protocol --> https://international.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202

WEDNESDAY, 18/8/2021

## Improve a part (3)

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-18

### Goal

- Transformation of the constructs in Top10

### Material

- Top10 competent cells
- Liquid LB medium

### Protocol

Protocol from --> https://international.neb.com/protocols/2012/05/21/transformation-protocol

I. Thaw competent cells on ice.
II. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.
III. Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.
IV. Place the mixture on ice for 30 minutes. Do not mix.
V. Heat shock at 42°C for 30 seconds*. Do not mix.
VI. Add 950 µl of room temperature media* to the tube.
VII. Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
VIII. Warm selection plates to 37°C.
IX. Spread 50–100 µl of the cells and ligation mixture onto the plates.
X. Incubate overnight at 37°C.
* Please note: For the duration and temperature of the heat-shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.

THURSDAY, 19/8/2021

## Improve a part (4)

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-19

### Goal

Redo for PSB1C3-RBS-Ntag-MazE:

- Isolate plasmid with the standard protocol of the kit
- Restriction with EcoRI and PstI to check on gel

### Material

- EcoRI
- PstI
- Restriction buffer
- MiliQ
- 10x Loading dye

- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide

## Protocol

Restriction reaction:

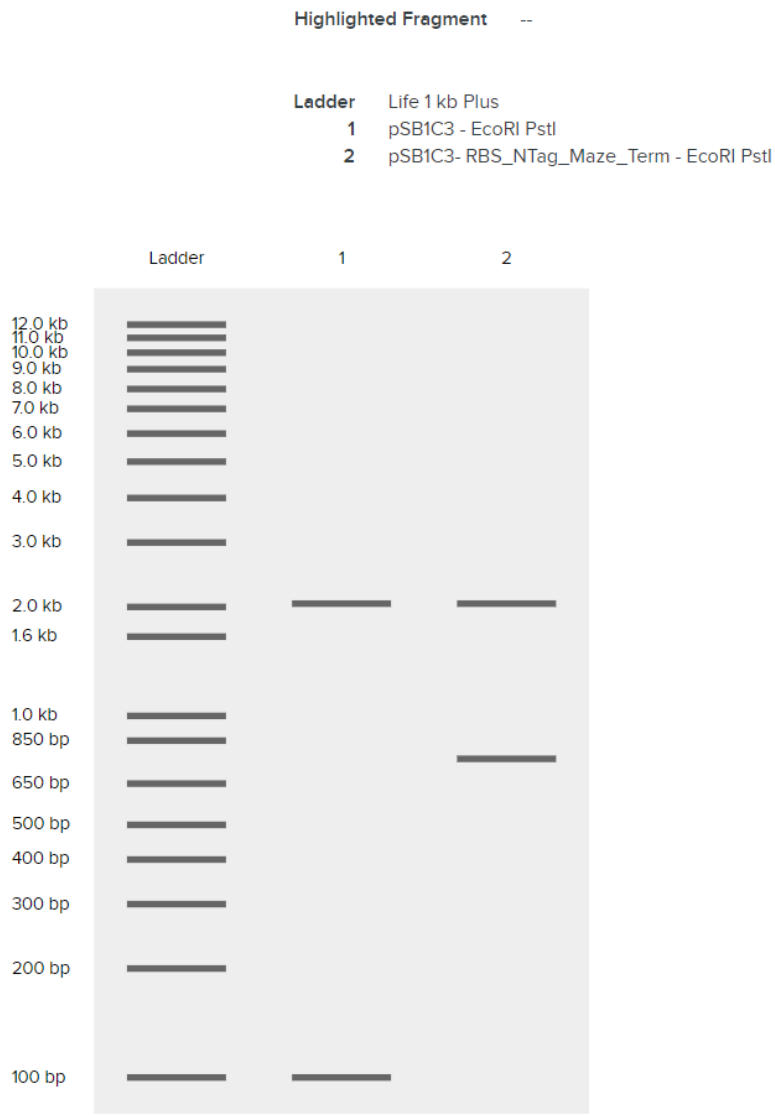
**Table5**

| | A | B |
|---|---|---|
| 1 | Components | 1 reaction |
| 2 | Plasmid DNA | 1 |
| 3 | 10x Buffer 2.1 | 1.5 |
| 4 | Enzyme 1 | 0.5 |
| 5 | Enzyme 2 | 0.5 |
| 6 | MiliQ | 7 |
| 7 | Total | 10 |

Restriction reaction performed for 1,5H at 37 °C. Next, the enzymes are heat inactivated at 85 °C.

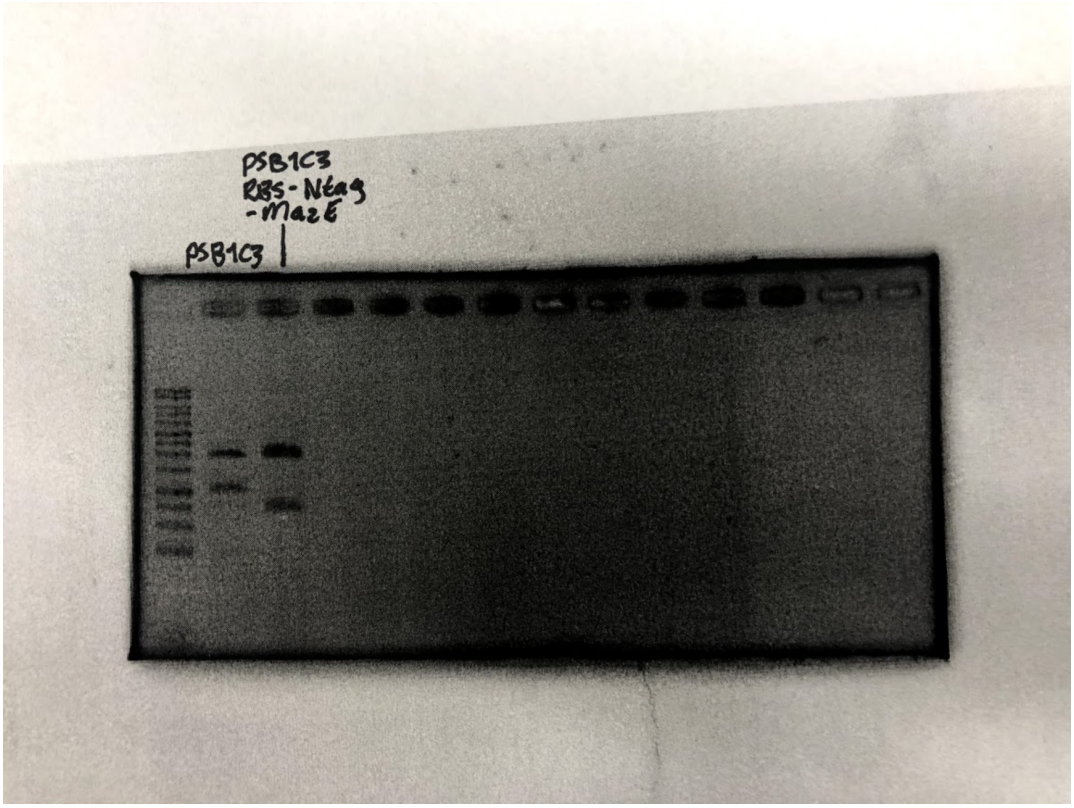
Gel should look like this:

image.png




## Result

image.png




This colony is right, and when comparing this with the last gel, the other two colonies for the PSB1C3-RBS-Ntag-MazE were not right.

FRIDAY, 20/8/2021

## Improve a part (5)

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-20

### Goal

- Check if transformation succeeded.
- Cut and put on gel.

### Material

- EcoRI
- PstI
- Restriction buffer
- MiliQ
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel

- Ethidiumbromide

## Protocol

Restriction reaction:

**Table6**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | 1 reaction | EcoRI + PstI MM | | |
| 2 | Plasmid DNA | 1.0 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | 13.5 | | |
| 4 | Enzyme 1 | 0.5 | 4.5 | | |
| 5 | Enzyme 2 | 0.5 | 4.5 | | |
| 6 | MiliQ | 7 | 63 | | |
| 7 | Total | 10 | 90 | | |

Restriction reaction performed for 1,5H at 37 °C. Next, the enzymes are heat inactivated at 85 °C.

Ligation of promotors in front of the antitoxin or change backbone:

Change the pBad-RBS-RepA-MazE to the Jump49 backbone

- 1:3

**Table7**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | | bp | Jump49 | J29-pBad-RBS-RepA-MazE needed |
| 2 | J29-pBad-RBS-RepA-MazE | 4643 | 100 ng | 360.3 ng |
| 3 | Jump49 (vector) | 3866 | | |

Put in promotor and change backbone:

- 1:1:1

**Table8**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | bp | Jump49 | J29-RBS-RepA-MazE needed | pBad needed | p2547 needed |
| 2 | J29-RBS-RepA-MazE | 3380 | 100 ng | 87.43 ng | 86.21 ng | 56.78 ng |
| 3 | Jump49 (vector) | 3866 | | | | |
| 4 | pBAD | 3333 | | | | |
| 5 | p2547 | 2195 | | | | |

**Table9**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | bp | Jump49 | PSB1C3-RBS-Ntag-MazE | pBad needed | p2547 needed |
| 2 | PSB1C3-RBS-Ntag-MazE | 2787 | 100 ng | 72.09 ng | 86.21 ng | 56.78 ng |
| 3 | Jump49 (vector) | 3866 | | | | |
| 4 | pBAD | 3333 | | | | |
| 5 | p2547 | 2195 | | | | |

So we need 86.21 ng (575 ng/ul) pBad and 113.56 ng p2547 (69.8 ng/ul)
Restriction of the promotors

**Table10**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | pBad | p2547 | | |
| 2 | Plasmid DNA | 0.5 | 2.0 | | |
| 3 | 10x Buffer 2.1 | 1.0 | 1.0 | | |
| 4 | EcoRI | 0.5 | 0.5 | | |
| 5 | SpeI | 0.5 | 0.5 | | |
| 6 | MiliQ | 7.5 | 6 | | |
| 7 | Total | 10 | 10 | | |

So we need 500 ng Jump49 (57.1 ng/ul)
Restriction of the vector:

**Table11**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | Jump49 | | | |
| 2 | Plasmid DNA | 10.0 | | | |
| 3 | 10x Buffer 2.1 | 1.5 | | | |
| 4 | EcoRI | 1 | | | |
| 5 | Pst1 | 1 | | | |
| 6 | MiliQ | 1.5 | | | |
| 7 | Total | 15 | | | |

So we need 144.18 ng PSB1C3-RBS-Ntag-MazE (154.7 ng/ul)

**Table12**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | PSB1C3-RBS-Ntag-MazE | | | |
| 2 | Plasmid DNA | 1.5 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | | | |
| 4 | XbaI | 0.5 | | | |
| 5 | Pst1 | 0.5 | | | |
| 6 | MiliQ | 6.5 | | | |
| 7 | Total | 10 | | | |

So we need 87.43 ng J29-RBS-RepA-MazE (only once if the last one correct, otherwise x2) (80.6 ng/ul)

**Table13**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | J29-RBS-RepA-MazE | | | |
| 2 | Plasmid DNA | 1.5 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | | | |
| 4 | XbaI | 0.5 | | | |
| 5 | Pst1 | 0.5 | | | |
| 6 | MiliQ | 6.5 | | | |
| 7 | Total | 10 | | | |

So we need 360.3 ng J29-pBad-RBS-RepA-MazE if correct. (see concentration of the right colony: 25.3 ng/ul)

**Table14**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | J29-pBad-RBS-RepA-MazE | | | |
| 2 | Plasmid DNA | 14.3 | | | |
| 3 | 10x Buffer 2.1 | 2.0 | | | |
| 4 | EcoRI | 1 | | | |
| 5 | Pst1 | 1 | | | |
| 6 | MiliQ | 1.7 | | | |
| 7 | Total | 20 | | | |

Change the Jump27-pBad-MazE to the Jump49 backbone

- 1:3

**Table16**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | | bp | Jump49 | Jump27-pBad-MazE needed |
| 2 | Jump27-pBad-MazE | 4578 | 100 ng | 355.3 ng |
| 3 | Jump49 (vector) | 3866 | | |

Concentration is 160 ng/ul so

**Table17**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | Jump27-pBad-MazE | | | |
| 2 | Plasmid DNA | 2.5 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | | | |
| 4 | EcoRI | 0.5 | | | |
| 5 | Pst1 | 0.5 | | | |
| 6 | MiliQ | 5.5 | | | |
| 7 | Total | 10 | | | |

Ligation:

**Table15**

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Components | Jump49 - J29-pBad-RBS-RepA-MazE | Jump49 - p2547 - J29-RBS-RepA-MazE | Jump49 - pBad - PSB1C3-RBS-Ntag-MazE | Jump49 - p2547 - PSB1C3-RBS-Ntag-MazE | Controle: Jump49-J27-pBad-MazE | |
| 2 | T4 DNA ligase buffer | 3 | 2 | 2 | 2 | 2 | |
| 3 | Vector DNA 100 ng | 2.63 | 2.63 | 2.63 | 2.63 | 2.63 | |
| 4 | Insert promotor | - | 4.07 | 3.0 | 4.07 | - | |
| 5 | Insert DNA | 20 | 7,23 | 3.1 | 3.1 | 8.89 | |
| 6 | Nuclease free water | 2.37 | 3.5 | 8.7 | 7.63 | 5.48 | |
| 7 | T4 DNA ligase | 2 | 1 | 1 | 1 | 1 | |
| 8 | Total | 20 | 20 | 20 | 20 | 20 | |

See protocol --> https://international.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202

*Transformation*

Protocol from --> https://international.neb.com/protocols/2012/05/21/transformation-protocol

I. Thaw competent cells on ice.
II. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.
III. Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.
IV. Place the mixture on ice for 30 minutes. Do not mix.
V. Heat shock at 42°C for 30 seconds*. Do not mix.
VI. Add 950 µl of room temperature media* to the tube.
VII. Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
VIII. Warm selection plates to 37°C.
IX. Spread 50–100 µl of the cells and ligation mixture onto the plates.
X. Incubate overnight at 37°C.

* Please note: For the duration and temperature of the heat-shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.

# Week 1 - pLacI promotor

**Project:** iGEM2021
**Authors:** Iris Noordermeer
**Entry Created On:** 2021-10-04 02:17:28 PM +0000
**Entry Last Modified:** 2021-10-06 12:26:07 PM +0000
**Export Generated On:** 2021-10-11 03:40:46 PM +0000

MONDAY, 16/8/2021

**Project:** Lab Notebook
**Authors:** Iris Noordermeer, Chanel Naar
**Created at:** 2021-08-16

## Goal

- Culture LacI and LacP2 promoters.

## Material

- Liquid LB medium
- Kanamycin
- LB agar medium
- IPTG

## Protocol

The non-fluorescent colonies of Bas´s LacI-GFP and LacP2-GFP plates were put in 2ml LB (with 2 ul Kanamycin)
These were put in the shaker for 6 hours at 37°C.

Thereafter, a bit of the culture was plated on both a normal Kanamycin plate and a Kanamycin + IPTG plate.

These were incubated overnight at 37°C

# Week 2 - Improve a part

**Project:** iGEM2021
**Authors:** Iris Noordermeer
**Entry Created On:** 2021-10-06 02:11:05 AM +0000
**Entry Last Modified:** 2021-10-06 01:23:26 PM +0000
**Export Generated On:** 2021-10-11 03:40:47 PM +0000

SATURDAY, 21/8/2021

**Project:** Lab Notebook
**Authors:** Sebastiaan Ketelaar
**Created at:** 2021-08-21

## Goal

- Pick non-fluorescent colonies and put them in liquid culture and grow them over the weekend.

MONDAY, 23/8/2021

## Week 2 - Improve a part (2)

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-23

## Goal

- Plasmid isolation and check the constructs on gel that have grown over the weekend.

## Material

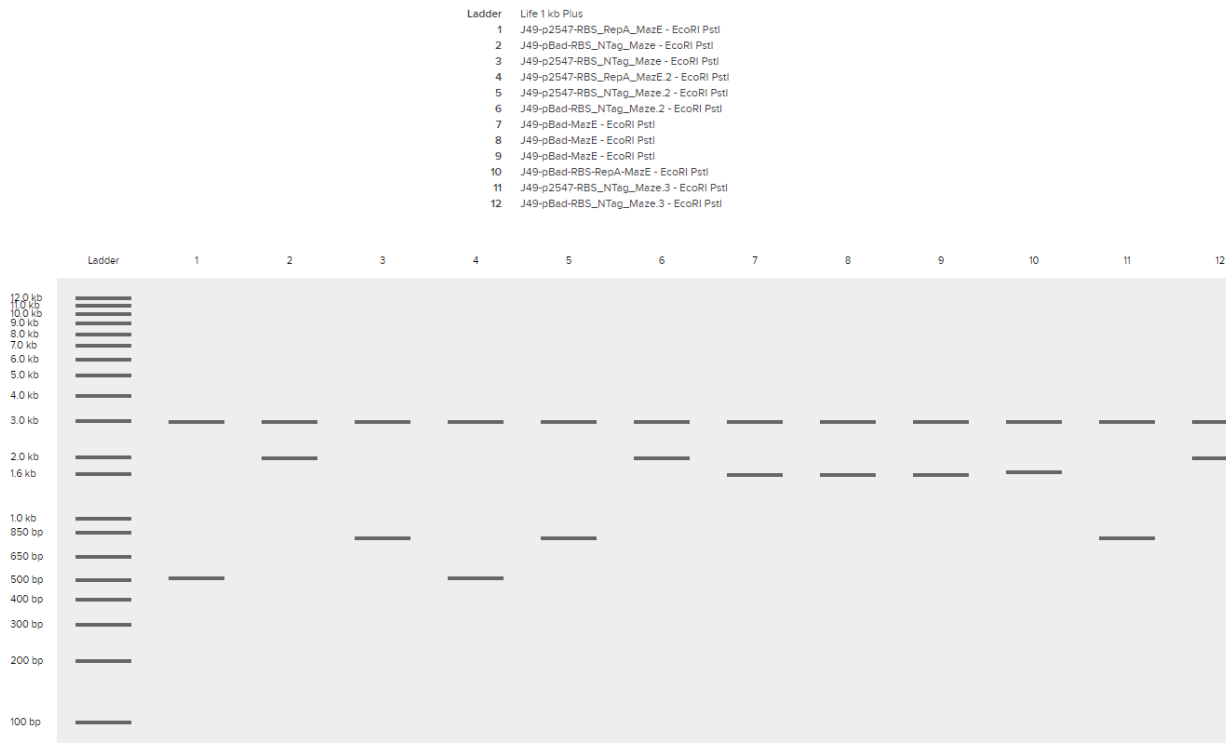
- ThermoScientific GeneJET plasmid mini prep kit
- EcoRI
- PstI
- Restriction buffer
- MiliQ
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide

## Protocol

- The stanadard protocol for plasmid isolation of the ThermoScientific kit was used.
- Check on gel:
    - 1: J49:p2547:RBS:RepA:MazE 2
    - 2: J49:pBad:RBS:Ntag:MazE 1.1
    - 3: J49:p2547:RBS:Ntag:MazE 1.1
    - 4: J49:p2547:RBS:RepA:MazE 1
    - 5: J49:p2547:RBS:Ntag:MazE 1.2
    - 6: J49:pBad:RBS:Ntag:MazE 1.2
    - Alfa: J49:pBad:MazE 1
    - Beta: J49:pBad:MazE 1.2

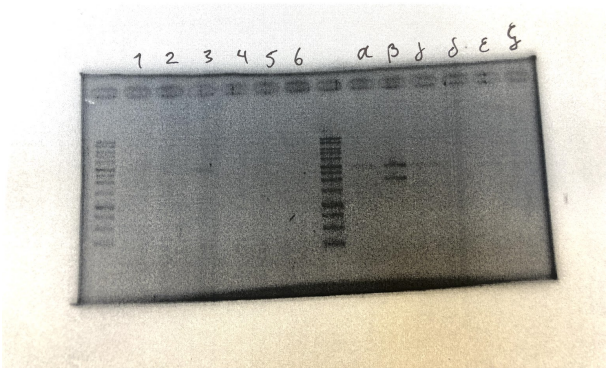
- Gamma: J49:pBad:MazE 2.1
- Delta: J49:pBad:RBS:RepA:MazE 2
- Epsilon: J49:p2547:RBS:Ntag:MazE 2
- Dzeta: J49:pBad:RBS:Ntag:MazE 2.1
  - 

image.png




## Result

image.png



So unfortunately, none of the constructs are right.
Beta seems nice, but the lower band should be 1.6 kb not 2.0 kb.

This means we do not have the J49-pBad-MazE for the control as we thought. Sebas will do a new backbone switch on 30-08-2021.

TUESDAY, 24/8/2021

# Week 2 - Improve a part (3)

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-24

## Goal

- Ligation of promotors in front of the antitoxin or change backbone:
    - Change the pBad-RBS-RepA-MazE to the Jump49 backbone

## Material

- T4 ligase
- T4 ligase buffer
- Restriction enzymes
    - EcoRI
    - PstI
    - XbaI
    - SpeI
- Restriction buffer
- MiliQ

## Protocol

Ligation mix ratio of vector:insert is 1:3

**Table7**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | | bp | Jump49 | J29-pBad-RBS-RepA-MazE needed |
| 2 | J29-pBad-RBS-RepA-MazE | 4643 | 100 ng | 360.3 ng |
| 3 | Jump49 (vector) | 3866 | | |

Put in promotor and change backbone:

- 1:1:1

**Table8**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | bp | Jump49 | J29-RBS-RepA-MazE needed | pBad needed | p2547 needed |
| 2 | J29-RBS-RepA-MazE | 3380 | 100 ng | 87.43 ng | 86.21 ng | 56.78 ng |
| 3 | Jump49 (vector) | 3866 | | | | |
| 4 | pBAD | 3333 | | | | |
| 5 | p2547 | 2195 | | | | |

**Table9**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | bp | Jump49 | PSB1C3-RBS-Ntag-MazE | pBad needed | p2547 needed |
| 2 | PSB1C3-RBS-Ntag-MazE | 2787 | 100 ng | 72.09 ng | 86.21 ng | 56.78 ng |
| 3 | Jump49 (vector) | 3866 | | | | |
| 4 | pBAD | 3333 | | | | |
| 5 | p2547 | 2195 | | | | |

So we need 86.21 ng (575 ng/ul) pBad and 113.56 ng p2547 (69.8 ng/ul)
Restriction of the promotors

**Table10**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | pBad | p2547 | | |
| 2 | Plasmid DNA | 0.5 | 3.0 | | |
| 3 | 10x Buffer 2.1 | 1.0 | 1.0 | | |
| 4 | EcoRI | 0.5 | 0.5 | | |
| 5 | SpeI | 0.5 | 0.5 | | |
| 6 | MiliQ | 7.5 | 5 | | |
| 7 | Total | 10 | 10 | | |

So we need 500 ng Jump49 (71.2 ng/ul)

Restriction of the vector:

**Table11**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | Jump49 | | | |
| 2 | Plasmid DNA | 7.0 | | | |
| 3 | 10x Buffer 2.1 | 1.5 | | | |
| 4 | EcoRI | 1 | | | |
| 5 | Pst1 | 1 | | | |
| 6 | MiliQ | 4.5 | | | |
| 7 | Total | 15 | | | |

So we need 144.18 ng PSB1C3-RBS-Ntag-MazE (154.7 ng/ul)

**Table12**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | PSB1C3-RBS-Ntag-MazE | | | |
| 2 | Plasmid DNA | 1.5 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | | | |
| 4 | XbaI | 0.5 | | | |
| 5 | Pst1 | 0.5 | | | |
| 6 | MiliQ | 6.5 | | | |
| 7 | Total | 10 | | | |

So we need 87.43 ng J29-RBS-RepA-MazE (80.6 ng/ul)

**Table13**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | J29-RBS-RepA-MazE | | | |
| 2 | Plasmid DNA | 2.5 | | | |
| 3 | 10x Buffer 2.1 | 1.0 | | | |
| 4 | XbaI | 0.5 | | | |
| 5 | Pst1 | 0.5 | | | |
| 6 | MiliQ | 5.5 | | | |
| 7 | Total | 10 | | | |

So we need 360.3 ng J29-pBad-RBS-RepA-MazE if correct. (see concentration of the right colony: 25.3 ng/ul)

**Table14**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Components | J29-pBad-RBS-RepA-MazE | | | |
| 2 | Plasmid DNA | 18.3 | | | |
| 3 | 10x Buffer 2.1 | 2.5 | | | |
| 4 | EcoRI | 1 | | | |
| 5 | Pst1 | 1 | | | |
| 6 | MiliQ | 2.2 | | | |
| 7 | Total | 25 | | | |

Ligation:

**Table15**

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Components | Jump49 - J29-pBad-RBS-RepA-MazE | Jump49 - p2547 - J29-RBS-RepA-MazE | Jump49 - pBad - PSB1C3-RBS-Ntag-MazE | Jump49 - p2547 - PSB1C3-RBS-Ntag-MazE | | |
| 2 | T4 DNA ligase buffer | 3 | 2 | 2 | 2 | | |
| 3 | Vector DNA 100 ng | 3.0 | 3 | 3 | 3 | | |
| 4 | Insert promotor | - | 2.71 | 3.0 | 2.71 | | |
| 5 | Insert DNA | 19.46 | 4.34 | 3.1 | 3.1 | | |
| 6 | Nuclease free water | 2.54 | 6.95 | 7.9 | 8.19 | | |
| 7 | T4 DNA ligase | 2 | 1 | 1 | 1 | | |
| 8 | Total | 30 | 20 | 20 | 20 | | |

See protocol --> https://international.neb.com/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202

WEDNESDAY, 25/8/2021

# Week 2 - Improve a part (4)

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-25

## Goal

- Transform the Top10 with the MazE constructs and check on gel.

## Material

- Liquid LB medium
- LB agar medium
- DH5α & Top10
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide

## Protocol

Transformation

Protocol from --> https://international.neb.com/protocols/2012/05/21/transformation-protocol

I. Thaw competent cells on ice.
II. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.
III. Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.
IV. Place the mixture on ice for 30 minutes. Do not mix.
V. Heat shock at 42°C for 30 seconds*. Do not mix.
VI. Add 950 µl of room temperature media* to the tube.
VII. Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
VIII. Warm selection plates to 37°C.
IX. Spread 50–100 µl of the cells and ligation mixture onto the plates.
X. Incubate overnight at 37°C.

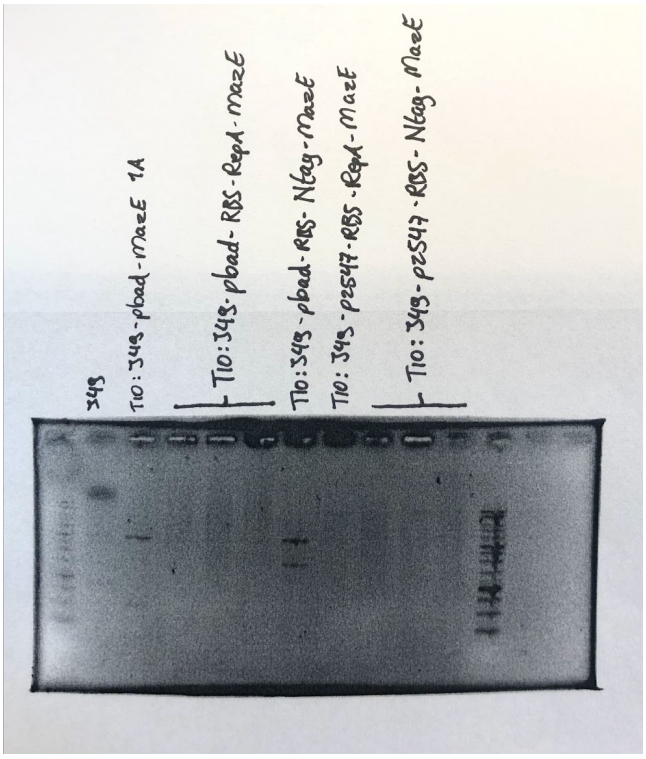
* Please note: For the duration and temperature of the heat-shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.

- Co-transform the new plasmids with Toxin plasmids (MazF, RelB with the promotors: Pbad, P1, P162)
  - Strains: DH5α & Top10
- Grow overnight

## Result

Check new white colonies of old transformed plates on gel:

image.png




However none were right again.

THURSDAY, 26/8/2021

# Week 2 - Improve a part (5)

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-26

## Goal

- Perform another restriction and gelelectrophoresis, as in the previous gel quite a lot band weren't visible.

## Material

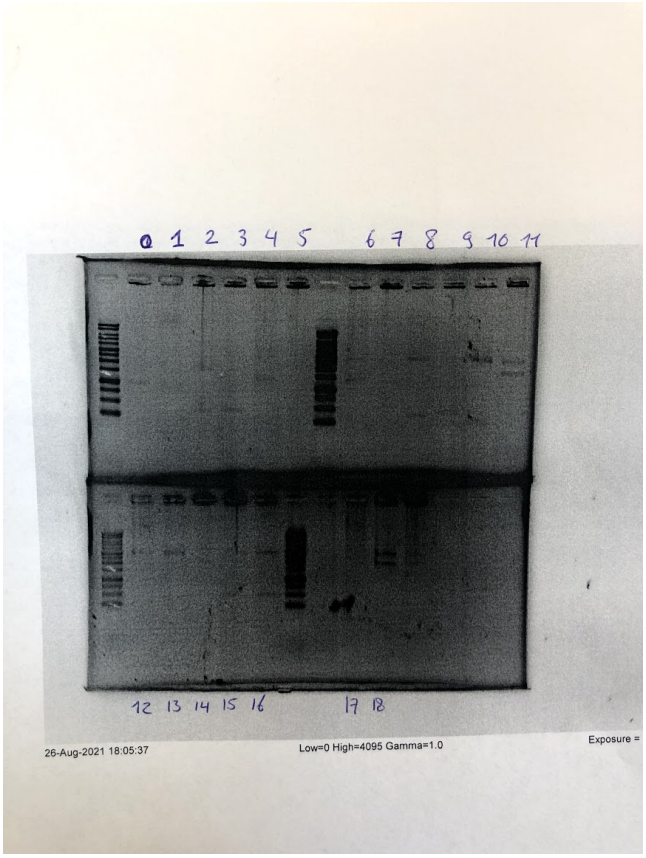
- Restriction enzymes
    - EcoRI
    - PstI
- Restriction buffer
- MiliQ
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide

## Protocol

Same restriction protocol was used as on 2021-08-24.

## Result

image.png




Not right

FRIDAY, 27/8/2021

# Week 2 - Improve a part (6)

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-27

## Goal

- Perform another restriction and gelelectrophoresis, as in the previous gel the bands were not the correct length. only now more insert was added to the samples (5 ul).

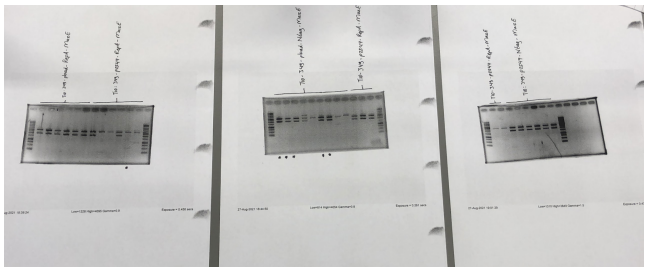
## Material

- Restriction enzymes
  - EcoRI
  - PstI
- Restriction buffer
- MiliQ
- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel

- Ethidiumbromide

## Protocol

image.png



The 6 with a star are correct! Therefore, we now have these constructs:

- Top10/J49::p2547::RepA::MazE
- Top10/J49::pBad::Ntag::MazE

# Week 3 - Improve a part

**Project:** iGEM2021
**Authors:** Iris Noordermeer
**Entry Created On:** 2021-10-06 03:01:57 AM +0000
**Entry Last Modified:** 2021-10-08 06:40:51 AM +0000
**Export Generated On:** 2021-10-11 03:40:47 PM +0000

TUESDAY, 31/8/2021

**Project:** Lab Notebook
**Authors:** Chanel Naar, Iris Noordermeer
**Created at:** 2021-08-31

## Goal

Control is missing of the Improve a Part. Therefore, Pbad:MazE will be newly integrated in a Jump 49 plasmid and transformed in Top10.

## Material

- Restriction enzymes
  - EcoRI
  - PstI
- Restriction buffer
  - NEB 2.1
- MiliQ
- Bacterial strain
  - Top10
  - DH5α
- pJump49 plasmid
- T4 ligase buffer
- T4 ligase

## Protocol

**Restriction** of Jump 49 and Top 10.Ik3:badL:MazE

**Table1**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Jump 49 GFP | 2,5 ul | | Top10/Ik3:pbad:MazE | 1,5 ul |
| 2 | EcoRI | 0,5 ul | | EcoRI | 0,5 ul |
| 3 | PstI | 0,5 ul | | PstI | 0,5ul |
| 4 | Ned2.1 | 1 ul | | Ned2.1 | 1 ul |
| 5 | MiliQ | 5,5 ul | | MiliQ | 6,5 ul |
| 6 | **Total** | **10 ul** | | **Total** | **10 ul** |

After 1 hour of restriction heat inactivate by 80 °C for 15 minutes.

**Ligation reaction** of Jump 49 and Pbad MazE.

**Table2**

| | A | B |
|---|---|---|
| 1 | Vector | 10 ul |
| 2 | Insert | 10 ul |
| 3 | T4 ligase buffer | 2,5 ul |
| 4 | T4 ligase | 1 ul |
| 5 | MiliQ | 1 ul |
| 6 | **Total** | **25 ul** |

Ligate for 2 hrs.

**Transformation**

Transform the Jump 49/Pbad:MazE in Top 10 and let the culture grow overnight.

Protocol from --> https://international.neb.com/protocols/2012/05/21/transformation-protocol

I. Thaw competent cells on ice.
II. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.
III. Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.
IV. Place the mixture on ice for 30 minutes. Do not mix.
V. Heat shock at 42°C for 30 seconds*. Do not mix.
VI. Add 950 µl of room temperature media* to the tube.
VII. Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
VIII. Warm selection plates to 37°C.
IX. Spread 50–100 µl of the cells and ligation mixture onto the plates.
X. Incubate overnight at 37°C.

* Please note: For the duration and temperature of the heat-shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.

- Co-transform the new plasmids with Toxin plasmids (MazF, RelB with the promotors: Pbad, P1, P162)
  - Strains: DH5α & Top10
- Grow overnight

## Extra

The gel isolation kit's lysis buffer is the wrong one, so we have not been able to check the plasmid sizes.

We will try again at the end of the day.