Week 1 - gBlock insertion

Project: iGEM2021
Authors: Iris Noordermeer
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TUESDAY, 13/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-13

Goal

- Gblock insertion
- Plasmid isolation

Material

Consumables (media, antibiotics, etc.)

- Plasmid isolation kit
- EcoRI
- NEBuffer
- TAE buffer
- agar
- MiliQ
- 20% glycerol

Bacterial strains

- pJUMP46 a
- pJUMP49 2e GFP
- pJUMP 26 1A - pJUMP 27 1A
- pJUMP 29 1E
- pSB1A3
- pSB1C3

Equipment

- centrifuge
- DNA measure UV
- heat cabinet

Protocol

Plasmid Isolation

For the plasmid isolation, firstly I made glycerol stocks by adding 1 mL of overnight culture to 500 uL glycerol. These were frozen at -20 C.
The plasmids were then isolated using a ... plasmid isolation kit. After isolation, the concentrations were measured using a ...
The concentrations of the isolated plasmids can be found in table 1. The plasmids were stored in -20 C in a box and added in the same order as described in table 1.

Finally, aliquots of the isolated plasmids were digested for electrophoresis. A gel was also made, however due to time constraints, the rest will be done tomorrow. The gel was placed overnight in the fridge.

GBlock insertion

This was not done today due to time constraints.

Figures:

Nanodrop result:

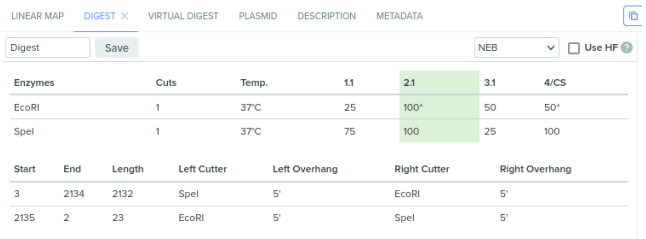
Table1

	Location in box	Plasmid	concentration (ng/uL)
1	1	pJUMP 46 2a GFM	33.3
2	2	pJUMP 46 8f GFP	105.3
3	3	pJUMP 49 2e 54 GFP	45.9
4	4	pJUMP 49 2e 54 GFP	46.8
5	5	pJUMP 26 1A	34.2
6	6	pJUMP 27 1A	44
7	7	pJUMP 28 1A	152.5
8	8	pJUMP 29 1A	49.2
9	9	pSB1A3	96.2
10	10	pSB1C3	348.2

In Silico Cloning

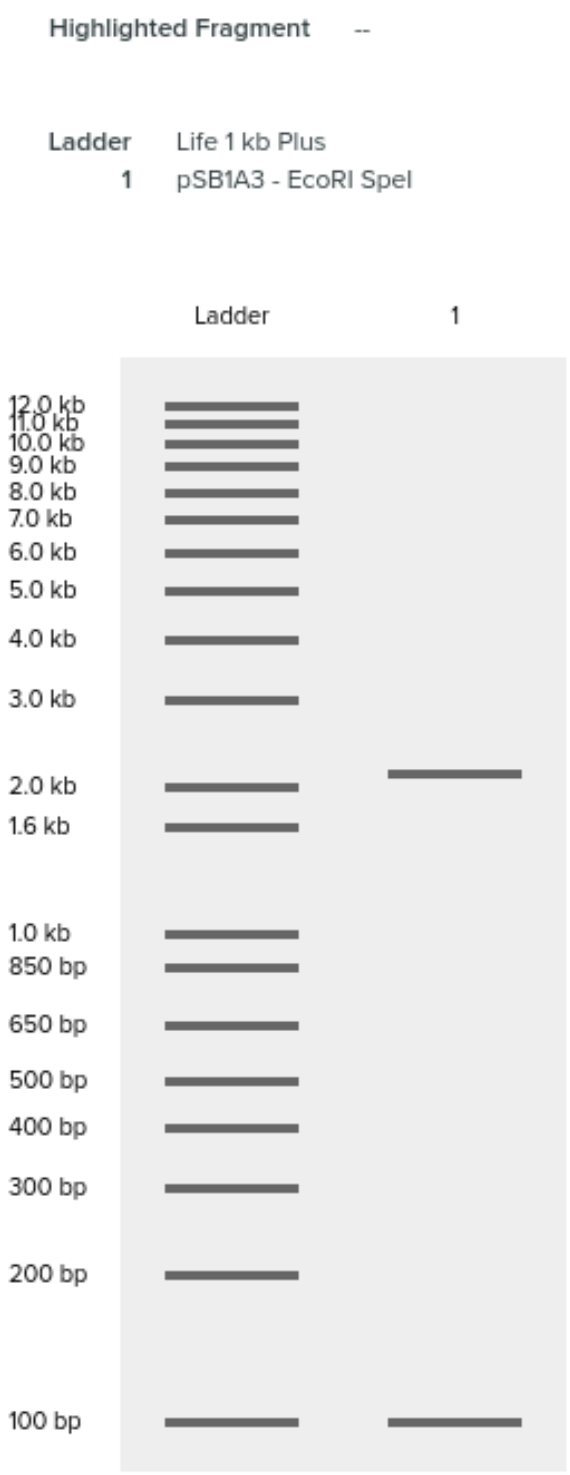
In silico restriction of pSB1A3 with EcoR1 and Spe1

image.png



Pre-digestion of the pSB1A3 plasmid:

image.png



Result

Plasmid pSB1C3 was possibly not digested well, since due to the pipettes being not callibrated well, there was less than 1 uL restriction mix for it left.

Extras

Tomorrow: start with the electrophoresis, continue into the ligation of our plasmids, this will be done by Bas van Woudenberg.
Further, when you have time, make the ethidium bromide baths and get the shaker.

Week 1 - gBlock Labjournal

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TUESDAY, 13/7/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-13

Goal

- Digestion and ligation of gBlocks in pSB1A3 and pSB1C3, which are the iGEM plasmids.

Materials

- AE buffer
- EcoRI
- PstI
- IDT gBlocks DNA
- 10x Buffer
- MiliQ
- pSB1A3
- pSB1C3
- gBlocks ordered from IDT (toxins, antitoxins, promoters)

Protocol

- IDT gBlocks are disolved in 20 AE buffer
- IDT gBlocks digested and the plasmids are with EcoRI and PstI.

Digested from: 12:15 - 15:15

Table1

	A	B	C
1	Components	1 reaction (µL)	Master mix for 30 reactions (µL)
2	gBlock DNA	7	N/A
3	EcoRI-HF	1	30
4	PstI	1	30
5	10 xBuffer 2.1	1	30
6	MiliQ	0	
7	Total vollume	10	

pSB1A3 digestion

Table2

	A	B
1	Components	Reaction (uL)
2	pSB1C3 (85.6 ng/ul)	22
3	EcoRI-HF	2.5
4	PstI	2.5
5	Buffer	3
6	MiliQ	0
7	Total vollume	30

pSB1C3 digestion

Table3

	A	B
1	Components	Reaction (uL)
2	pSB1A3 (96.4 ng/ul)	22
3	EcoRI-HF	2.5
4	PstI	2.5
5	Buffer	3
6	MiliQ	0
7	Total vollume	30

All digestions are heat inactivated at 80 degrees for 20 min

Ligation of gBlocks in apropriate plasmid at RT O/N.
And ligations are stored at -20 °C the next day

Extra

Pipets are not accruate, make more master mix than Xreactions + 1.

THURSDAY, 15/7/2021

Week 1 - gBlock Labjournal : Transformation of ligated plasmids (2)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-15

Goal

- Transformation of the Ligated plasmids of 2021-07-15

Materials

- Ligation mixture
- Competent cells of the different bacterial strains

Method

Table4

	A	B
1	Name	Name
2	pSB1C3	pBAD
3	pSB1C3	pLacI
4	pSB1C3	pTetOn
5	pSB1C3	P1
6	pSB1C3	P21
7	pSB1C3	P162
8	pSB1C3	P387
9	pSB1C3	P623
10	pSB1C3	P908
11	pSB1A3	P1303
12	pSB1C3	P1487
13	pSB1C3	P1831
14	pSB1C3	P2547
15	pSB1C3	lambda t0 terminator
16	pSB1A3	P_pBAD_CcdB_T_S
17	pSB1A3	P_pLacI_CcdA_T_S
18	pSB1A3	CcdB T
19	pSB1A3	CcdA AT
20	pSB1A3	MazF T
21	pSB1A3	MazE AT
22	pSB1A3	RelE T
23	pSB1A3	RelB AT
24	pSB1A3	Hok T
25	pSB1A3	Sok AT
26	pSB1A3	LacI RFP casette
27	pSB1A3	GFP
28	pSB1A3	mCherry

Protocol

- Thaw competent cells on ice
- Chill approximately 5 ng (2ul) of the ligation mixture in a 1.5 ml microcentrifuge tube

- Add 50 ul 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.
- Place the mixture on ice for 30 minutes. Do not mix.
- Heat shock at 42 °C for 30s. Do not mix.
- Place tube at 37 °C for 60 minutes. Shake vigorously (250rpm) or rotate.
- Warm selection plates to 37 °C
- Spread 50-100 ul of the cells and ligation mixture onto plates.
- Incubate overnight at 37 °C

FRIDAY, 16/7/2021

Week 1 - gBlock Labjournal : count and transfere colonies (3)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-16

Goal

- Count the resulted colonies after transformation and inoculte in liquid LB cultures.

Materials

- LB medium
- Bacterial strains on plates

Protocol

- Red and white Colonies are counted, and the white colonies are transfered to 5 mL liquid LB medium and stored in 5degrees.
- On 2020-07-18 The tubes are put in the 37 degrees shaker (200 RPM) at 1800h.

Results

Table5

	A	B	C	D	E
1				colonies(16/07/21	
2		Name	Name	Red	White
3	2	pSB1C3	pBAD	0	1
4	1	pSB1C3	pLacI	6	8
5	3	pSB1C3	pTetOn	2	5
6	4	pSB1C3	P1	1	2
7	5	pSB1C3	P21	0	2
8	6	pSB1C3	P162	2	3
9	7	pSB1C3	P387	0	1
10	8	pSB1C3	P623	0	6
11	9	pSB1C3	P908	3	6
12	10	pSB1A3	P1303	2	10
13	11	pSB1C3	P1487	0	2
14	12	pSB1C3	P1831	2	2
15	13	pSB1C3	P2547	1	0
16	14	pSB1C3	lambda t0 terminator	3	7
17	15	pSB1A3	P_pBAD_CcdB_T_S	0	1
18	16	pSB1A3	P_pLacI_CcdA_T_S	4	2
19	17	pSB1A3	CcdB T	12	0
20	18	pSB1A3	CcdA AT	10	8
21	19	pSB1A3	MazF T	14	16
22	20	pSB1A3	MazE AT	8	15
23	21	pSB1A3	RelE T	6	5
24	22	pSB1A3	RelB AT	3	5
25	23	pSB1A3	Hok T	7	18
26	24	pSB1A3	Sok AT	1	3
27	25	pSB1A3	LacI RFP casette	7	0
28	26	pSB1A3	GFP	24	1
29	27	pSB1A3	mCherry	27	12

Week 1 - OD to CFU preparation

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TUESDAY, 13/7/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-13

Goal

- Prepare the solution and agar plates for OD to CFU calibration

Material

Consumables (media, antibiotics, etc.)

- LB
- LB-agar

Bacterial strains

- DH5α

Equipment

- Autoclave

Protocol

- The protocol OD to CFU calibration was used
- Pour the 45 agar without antibiotics with standard protocol
- Add LB with dilution gradiant volumn

Extras

Learn the FACS cytometry from Noortje

Check all the liquid bottle on the left chamber of the machine is not empty and the waste bottle is not full.

Start-up the machine for around 20 minutes

200uL is needed for each sample

When the sample is tested, pour out the bacteria, put it in the soap (instrction on the wall)

After using the machine, wash it with washing fluid then with ethanol

Week 1 - Start culturing bacterial strains and isolate plasmids

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MONDAY, 12/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2020-07-12

Pouring plates

Materials

- Consumables
  - LB medim 25 g/L
  - Bacterial Agar 15g/L
- Antibiotic stocks (1000X)
  - Ampicillin (100 mg/mL)
  - Kanamycin: (50 mg/mL)
  - Chlorampenicol: (25 mg/mL)
  - Spectinomycin: (50 mg/mL)
- Equipment
  - Autoclace
  - Bunsen burner
  - Incubator

Methods

Microwave solidified LB agar bottles until they are almost boiling or use freshly autoclaved LB agar.
Let the agar cool untill you can hold it in your hands for 10 seconds
Optional: For selection plates add for **1 ml** of LB agar, **1 µL** of antibiotic stock
Pour 25 mL LB agar in a petri-dish and let the agar solidify.
Label the petridishes with the apropriate collor corresoponding to the antibiotics used! Additionally, label with the date.
Store the plates upside down at 5°C

Week 1 - Starting cultures for preparation competent cells and extraction plasmids

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2020-07-12

Goal

- Preparation: start culturing TOP10, DB3.1 and DH5α *E.coli* strains to make competent cells later out of it.

Materials

- pJUMP bacterial cultures
- iGEM standard plasmid
- Liquid LB medium
- Toothpicks

Equipment

Bunsen burner

Vortex

Shaker 37 degrees C

Methods

Liquid LB medium was used with added antibiotics (ampicillin, kanamycin, chloramphenicol and spectinomycin).

Single colonies were picked from the bacteria that carry our plasmids using sterilised wooden toothpicks.

The tootpicks were added to 10 mL tubes and added overnight to a shaker.

TUESDAY, 13/7/2021

Week 1 - Plasmid Isolation

Project: Lab Notebook

Authors: Sebastiaan Ketelaar

Created at: 2020-07-13

For the plasmid isolation, firstly I made glycerol stocks by adding 1 mL of overnight culture to 500 uL glycerol. These were frozen at -20 C.

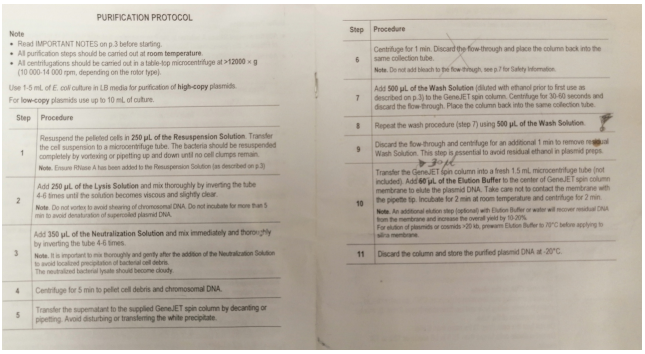
The plasmids were then isolated using a Thermoscientific GeneJET plasmid Miniprep Kit. After isolation, the concentrations were measured using a nanodrop.

The concentrations of the isolated plasmids can be found in table 1. The plasmids were stored in -20 C in a box and added in the same order as described in table 1.

Finally, aliquots of the isolated plasmids were digested for electrophoresis. A gel was also made, however due to time constraints, the rest will be done tomorrow. The gel was placed overnight in the fridge.

Protocols

Protocol Thermoscientific GeneJET plasmid Miniprep Kit



PURIFICATION PROTOCOL

Note
- Read IMPORTANT NOTES on p.3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g (10 000-14 000 rpm, depending on the rotor type).

Use 1-5 mL of *E. coli* culture in LB media for purification of **high-copy** plasmids.
For **low-copy** plasmids use up to 10 mL of culture.

Step	Procedure
1	Resuspend the pelleted cells in **250 µL of the Resuspension Solution**. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. **Note.** Ensure RNase A has been added to the Resuspension Solution (as described on p.3)
2	Add **250 µL of the Lysis Solution** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. **Note.** Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
3	Add **350 µL of the Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times. **Note.** It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudy.
4	Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
5	Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate.

Step	Procedure
6	Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube. **Note.** Do not add bleach to the flow-through; see p.7 for Safety Information.
7	Add **500 µL of the Wash Solution** (diluted with ethanol prior to first use as described on p.3) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
8	Repeat the wash procedure (step 7) using **500 µL of the Wash Solution**.
9	Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
10	Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add **60 µL of the Elution Buffer** to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min. **Note.** An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70°C before applying to silica membrane.
11	Discard the column and store the purified plasmid DNA at -20°C.

Table 3 contains the protocol for making restrictions for analysing plasmids after isolation to screen for the correct lenght.

Table3

	Ingredient	Amount
1	Restriction enzyme 1 EcoRI	5.5 µL
2	10x restriction buffer	1.1 µL
3	MiliQ	4.4 µL
4	Total	11 µL

Results

Table1

	Location in box	Plasmid	concentration (ng/uL)
1	1	pJUMP 46 2a GFM	33.3
2	2	pJUMP 46 8f GFP	105.3
3	3	pJUMP 49 2e 54 GFP	45.9
4	4	pJUMP 49 2e 54 GFP	46.8
5	5	pJUMP 26 1A	34.2
6	6	pJUMP 27 1A	44
7	7	pJUMP 28 1A	152.5
8	8	pJUMP 29 1A	49.2
9	9	pSB1A3	96.2
10	10	pSB1C3	348.2

WEDNESDAY, 14/7/2021

Week 1 - Plasmid isolation : Checking the plasmids through gel-electrophoresis (2)

Project: Lab Notebook

Authors: Sebastiaan Ketelaar

Created at: 2020-07-12

Goal

- The plasmids that were isolated yesterday were checked by performing a gel electrophoresis.

Material

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

Protocol

The plasmids were first digested with EcoRI.

Set up the station for staining the gels in ethidium bromide.
The etbr was diluted to a final concentration of 0.5 ug/mL by adding 50 uL to a final volume 1 L of TAE (1x).

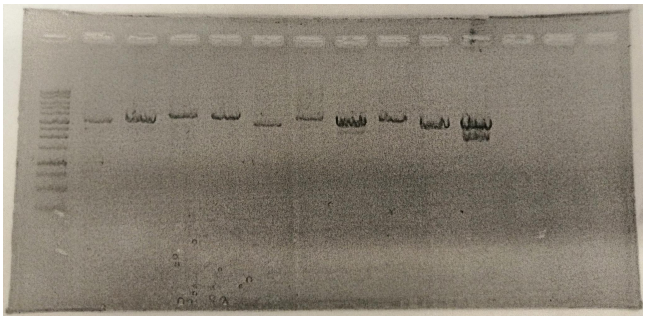
Gelelectrophoresis legenda:

Table4

	Lane nr.	Contents
1	1	DNA ladder
2	2	pJUMP 46 2a GFM
3	3	pJUMP 46 8f GFP
4	4	pJUMP 49 2e 54 GFP
5	5	pJUMP 49 2e 54 GFP
6	6	pJUMP 26 1A
7	7	pJUMP 27 1A
8	8	pJUMP 28 1A
9	9	pJUMP 29 1A
10	10	pSB1A3
11	11	pSB1C3

Result

Gel electrophoresis EcoRI digestion isolated plasmids 1 4-07



The gel electrophoresis ran for 30 minutes at 100 V.
Set up the station for staining the gels in ethidium bromide. The etbr was diluted to a final concentration of 0.5 ug/mL by adding 50 uL to a final volume 1 L of TAE (1x).
See table 2 for contents. The bands are roughly equal to virtual digests. However, it seems there is a little shift due to protein binding of the DNA for pJUMP 28 and pSB1C3.

Week 2 - Colony qPCR

Project: iGEM2021
Authors: Iris Noordermeer
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FRIDAY, 23/7/2021

Project: Lab Notebook
Authors: Iris Noordermeer
Created at: 2020-07-23

Goal

- Performing a Colony qPCR to test the primers.
- See is the colony qPCR is succeeded with a gelelectrophoresis.

Material

- MiliQ
- Primers: Prefix R, Suffix, VF iGEMplasmids, VR iGEMplasmids
- Colonys: DH5 α PSB IA3 a red colony , DH5 α PSB IA3 white colony, DH5 α PSB IC_3, PSB IC_3, PSB IA3.
- DNTP
- TAQ polymerase
- PCR Buffer
- qPCR machine
- 1% Agarose gel
- 1% TAE buffer
- Ethidiumbromide
- 10x Loading dye
- 1 Kb DNA Ladder
- 1000µL Pipet
- 200µL pipet
- 10µL pipet
- eppendorf tubes

Protocol

Diluting Primers

1. FIrst spin-off the new derived primers for 30s 8000rpm.
2. Added the amount of MiliQ mentioned on the Sigma-Aldrich form to the Primers to get 100µM. Including the P-ENTER primer (not used for the colony qPCR).
3. Make a stock of each primer of 100µL of 10µM of primer.

Table1

	A	B	C
1		A	B
2	1	90uL of MiliQ	
3	2	10uL of primer	

Performing Colony qPCR

1. Take one colony of the plate.
2. Dilute the colony in 100µL MiliQ
3. Dilute 50x the two DNA samples PSB IC_3 and PSB IA3

Table2

	A	B	C
1		A	B
2	1	MiliQ	49uL
3	2	DNA sample	1uL

Make a PCR MasterMix (MM)

Table3

	A	B	C
1		A	B
2	1	DNTP	11uL
3	2	Taq	5,5uL
4	3	PCR buffer	55uL
5	4	MiliQ	170,5uL
6	5	**Total**	242uL

25µL per PCR tube

Table4

	A	B	C
1		A	B
2	1	MM	22uL
3	2	Primer F	1uL
4	3	Primer R	1uL
5	4	Colony	1uL
6	5	**Total**	**25**

qPCR protocol

Table5

	A	B
1		A
2	1	95°C for 15 minutes
3	2	94°C for 30 seconds
4	3	63°C for 30 seconds
5	4	72°C for 3 minutes
6	5	Repeat 2-4 39 times
7	6	72°C for 15:00 minutes
8	7	4°C forever

Gel electrophoresis

Table6

	Lane	Sample
1	1	DNA Ladder
2	2	PSBIA3 VF + VR
3	3	DH5a PSBIA3 red colony prefix+ suffix
4	4	DH5a PSBIA3 red colony VF + VR
5	5	DH5a PSBIA3 white colony prefix + suffix
6	6	DH5a PSBIA3 white colony VF + VR
7	7	DH5a PSBIC3 orefix + suffix
8	8	DH5a PSBIC3 VF + VR
9	9	PSBIC3 prefix + suffix
10	10	PSBIC3 VF + VR
11	11	PSBIA3 prefix + suffix
12	Slots 12 untill 14	Empty

Week 2 - Competent cell CFU test

Project: iGEM2021
Authors: Iris Noordermeer
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MONDAY, 19/7/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-19

Goal

- Plasmid isolation with Sebastiaan Ketelaar
- Competent cell CFU test

Material

Consumables (media, antibiotics, etc.)

- media with ampicillin
- agar-LB with ampicillin
- plasmid isolation kit

Bacterial strains

- DH5alpha with lacI-RFP/pSB1A3 plasmid

Equipment

- **Centrifuger**
- Heatshock device

Protocol

- Isolate all the plasmid from strain incubate last week.
- The plamsid lacI-RFP is used for testing the competent cell viability following the protocol.
  The plasmid concentration is 42ng/uL
  We do triplicate of the dishes. In each triplicate, we add 1uL, 2uL and 4uL plasmid respectively.
  The cells used are the first batch of competent cells (Bas's protocol)

TUESDAY, 20/7/2021

Week 2 - Competent cell CFU test (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-20

Goal

- See if red colonies have grown after a 2-day incubation on agar.
- Isolate iGEM plasmid

Materials

- Thermoscientific GeneJET plasmid mini prep kit.
- MiliQ

Protocols

Corresponding protocol that comes along with the Thermoscientific kit is used to isolate the plasmids.

Result

The competent cell tests have more red colony after 2-day inocubation on agar.

WEDNESDAY, 21/7/2021

Week 2 - Competent cell CFU test: Plasmid Isolation (3)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-21

Goal

- Isolate pJUMP49 plasmids to be able to make integrate mCherry with a promoter for the next day.

Materials

- Thermoscientific GeneJET plasmid mini prep kit
- MiliQ

Protocol

The protocol that comes along with the Thermoscientific GeneJET plasmid mini prep kit is used to extract the plasmids out of DH5α.

THURSDAY, 22/7/2021

Week 2 - Competent cell CFU test: Construct Assembly (4)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-21

Goal

- Make a construct with the isolated plasmids pJUMP49 + promoter + mCherry

Materials

- T4 ligase buffer
- T4 ligase
- MiliQ

Target construct:

- pJUMP-49 + P1487 + mCherry
- pJUMP-49 + P1303 + mCherry
- pKIKO + P1487 + mCherry
- pKIKO + P1303 + mCherry

Protocol

Ligation protocol was according to: https://www.promega.com/-/media/files/resources/protocols/product-information-sheets/g/t4-dna-ligase-blue-white-cloning-qualified-protocol.pdf

ligation ration vector:insert was 1:3.
The ligation reaction was performed for 2 hours.

Table1

	A	B
1	Vector DNA	3 ul
2	Insert DNA	5 ul
3	T4 ligase buffer (10X)	1.5 ul
4	T4 ligase	0.7 ul
5	MiliQ	0.8 ul
6	**Total**	**10 ul**

Week 2 - gBlock : Plasmid isolation

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Authors: Iris Noordermeer
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MONDAY, 19/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-19

Goal

- IDT received gBlocks that were transformed last week are now isolated, restricted and checked on gel.

Materials

- Overnight cultures from Bas
- Glycerol
- Plasmid extraction kit
- 1% TAE Buffer
- Gelelectrophoresis kit

Protocol

The overnight cultures were turned into glycerol stocks by adding 400 uL of 20% glycerol to 800 uL of bacterial culture.
These were frozen at -20 C.
For the rest of the plasmid isolation, the instructions of the plasmid extraction kit were used.
Transformations No. 13, 15 and 17 did not grow any colonies and thus were not included in this culture. Neither did nr. 1 and 2.
In the end, 22 cultures had their plasmids extracted.

Growing extra plasmids for future use

Further cultures were made from the existing glycerol stocks with the strains containing all plasmid backbones.
50 mL LB medium and 20 uL of glycerol stock was added to these cultures. They were then added to erlenmeyer flasks that were grown at 37 degrees, 120 rpm.
For pSB1A3 and pSB1C3, 75 mL were used, all with the appropriate antibiotics added (pSB1A3 chloramphenicol, pSB1C3 ampicillin, pJUMP spectinomycin and kanamycin).

Gel electrophoresis

The plasmids that were transformed by Bas and extracted on Friday were restricted by making the following master mix:

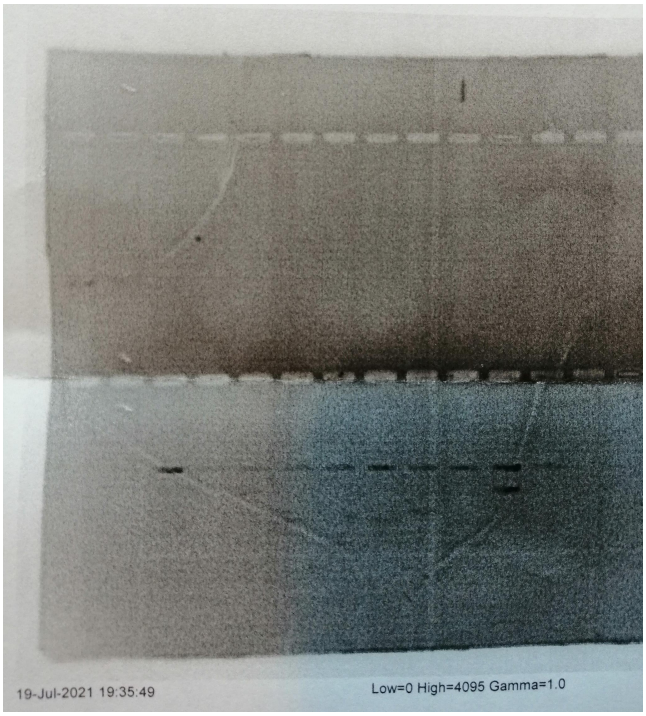
Table1

	Component	Volume
1	NEBuffer 2.1	25 uL
2	EcoRI	12.5 uL
3	PstI	12.5 uL
4	MiliQ	85 uL
5	Total	135 uL

The restriction was performed for 85 minutes at 37 C. After this, immediately loading dye was added and electrophoresis was performed.
For the electrohporesis, some things went wrong. After running for about 3 minutes at 150 V, the machine gave an signal noise, giving the error code that the lid was not fastened. However, after removing the lid, adding it again and restarting the machine, the same error came up. Then, the gel was transferred to a new electrophoresis machine and set again, this time at 120 V since we hypothesised that the voltage might be the issue. It was run for about 5 minutes until this machine too gave the same signal. During this time, I had a meeting, so Hoda and Siheng selected a new program for me, which then ran smoothly for around 20 minutes until the dye was almost around halfway the gel.

Results

2021_07_19 gel.jpg



orientation bands on gel.jpg

Samples of the gel above. See Table 2 for the corresponding plasmids.

As can be seen from the picture, only the lower half of the gel shows any bands. Furthermore, the gene ladder which should be in the leftmost lanes does not show any DNA at all, which is exceedingly strange, since the same gene ladder was used as the previous gel. Currently, my hypothesis is that not enough ethidium bromide was used in staining the gel, although it would then be strange that around half of the samples do show bands.

Table2

	Sample nr.	Plasmid	Insert
1	3	pSB1C3	pTetOn
2	4	pSB1C3	P1
3	5	pSB1C3	P21
4	6	pSB1C3	P162
5	7	pSB1C3	P387
6	8	pSB1C3	P623
7	9	pSB1C3	P908
8	10	pSB1A3	P1303
9	11	pSB1A3	P1487
10	12	pSB1C3	P1831
11	14	pSB1C3	P1487
12	16	pSB1A3	Lambda t0 terminator
13	18	pSB1A3	Promoter pLacI_CcdA
14	19	pSB1A3	MazF
15	20	pSB1A3	KMazE
16	21	pSB1A3	RelE
17	22	pSB1A3	RelB
18	23	pSB1A3	Hok
19	24	pSB1A3	Sok
20	25	pSB1A3	LacI RFP
21	26	pSB1A3	GFP
22	27	pSB1A3	mCherry

TUESDAY, 20/7/2021

Week 2 - gBlock Labjournal - Reperformed restriction (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-20

The cause of yesterday's failed electrophoresis was probably soaking in EtBr for too short. Today, I did the same restriction again, and failed the electrophoresis again, this time due to adding too little DNA. Therefore, in the afternoon, I did another restriction.

Goal

- Reperform the restriction of 2020-07-19

Materials

- EcoRI
- PstI
- 10xBuffer
- MiliQ
- 1% Agarose gel
- 1% TAE Buffer

Protocol

- The master mix for the restriction was made as follows:

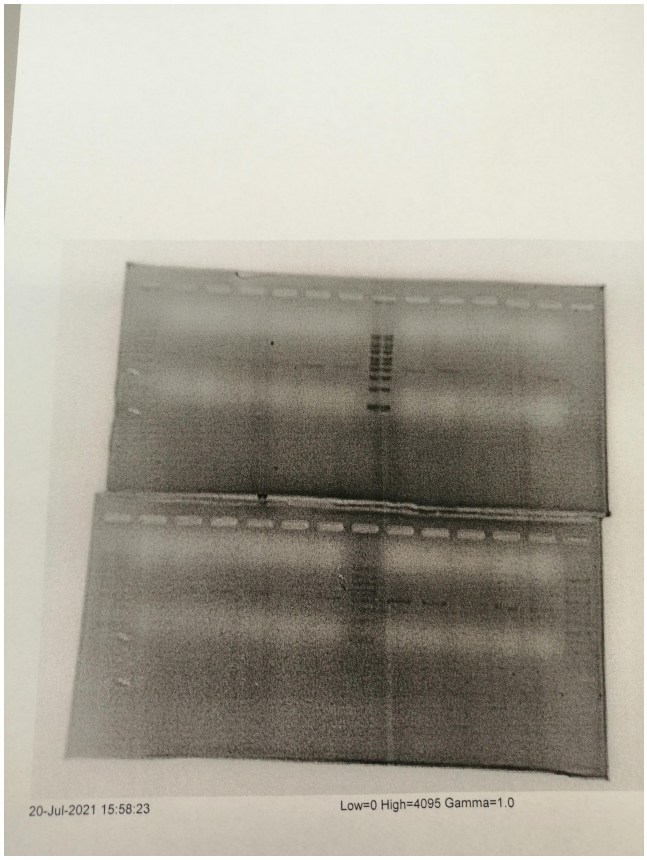
Table3

	A	B
1	EcoRI	12.5
2	PstI	12.5
3	Buffer	57.5
4	MiliQ	375
5	**Total**	**457.5**

For each reaction, 5 uL of DNA was added to 18 uL of Master Mix.

Result

2021-07-20 gel nr 1.jpg



The lanes were as follows:

Table4

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Ladder	3	4	5	6	7	8	Ladder	9	10	11	12	14	-
2	Ladder	16	18	19	20	21	22	Ladder	23	24	25	26	27	Ladder iGEM

Another restriction ran with the same composition for 1 hour, and then heat inactevated at 65 C for 15 minutes. It was then added to the freezer for electrophoresis tomorrow morning.

Isolation Plasmid Backbones

Yesterday, 50 mL cultures as well as 75 mL cultures were grown. Siheng and Iggy isolated the plasmids pSB1A3 and pSB1C3 from the 75 mL cultures. The isolation of the other plasmids was not performed, but the cells were pelleted and stored at -20 C for isolation tomorrow.

WEDNESDAY, 21/7/2021

Week 2 - gBlock Labjournal - Reperformed restriction (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-21

Goal

- Redigestion was again performed as the labels again did not correspond as expected.

Materials

- 10x restriction buffer
- 1% Agarose
- 1% TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

Protocol

In the morning another gel was poured as in the previous days and then ran the digestion again.
Thereafter, a gelelectrophoresis is performed. 9 ul sample with 1 ul 10x loading dye is added to the slot and 6 ul of the 1 kB DNA Ladder and runned at 100V for 30 minutes.The gel was stained in ethidiumbromide for 22 minutes.

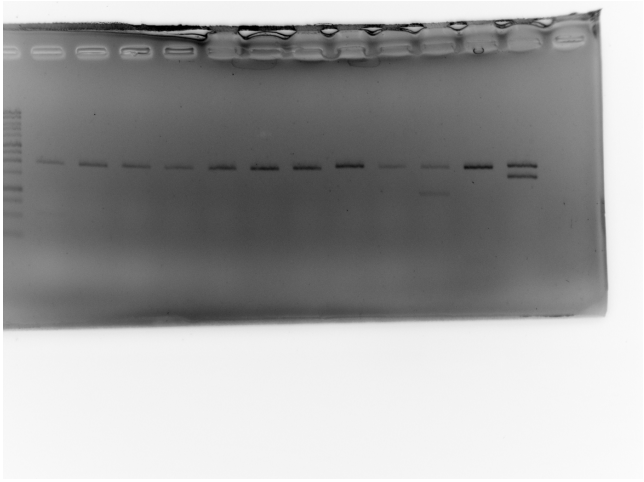
Legenda gel electrophoresis:

Table6

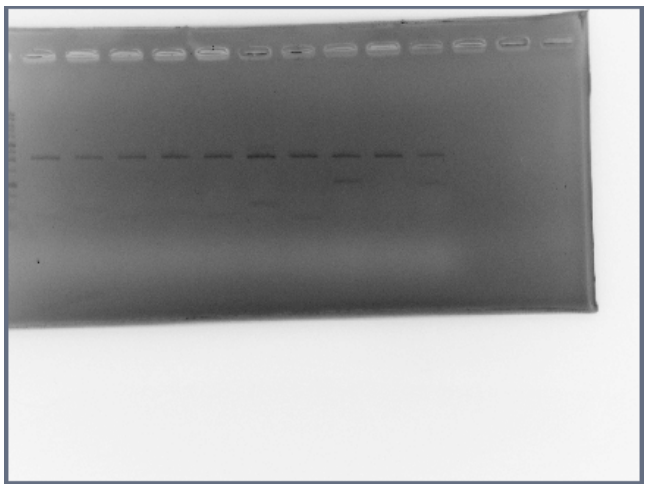
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	1	2	3	4	5	6	7	8	9	10	11	12	14
2	pSB1C3_TetOn	pSB1C3_P1	pSB1C3_P21	pSB1C3_P162	pSB1C3_P387	pSB1C3_P623	pSB1C3_P908	pSB1C3_P1303	pSB1A3_P1487	pSB1C3_P1831	pSB1C3_P1487	pSB1C3_	pSB1C3_

Results

20210721 sk 3-16 restrictions destained.tif



20210721 sk 18-27 restricctions destained.tif



The labels of the DNA tubes seem to have been wrong. Bas and I sorted out the labels. We removed the tubes that had wrong labels and of which the restriction and gel electrophoration showed wrong results.
Lane with nr. 3 and 26 do not show the expected results. 4-16 are according to expectations, as is 27.
The results of lane 18 - 25 are ambiguous. They have a very vague lane. Therefore, they need to be done again.
However, Bas will do a colony PCR tomorrow to verify the constructs. In the meantime, I will digest nr. 27 with XbaI, EcoRI and nr. 11 with SpeI and EcoRI and ligate them together. Then we can see the effect of the GFP with the strong promoter upstream.

It seems that we have found the reason for the high background and low detection of DNA: The gel for running the plasmid isolations was made with agar instead of agarose. This could explain the high levels of staining in the gel.
Bas has made a new gel with agarose on 2020-07-23 and will recheck the restrictions.
They turned out perfectly. All the expected bands can be seen for all constructs, even including the roughly 35 bp promoter sequences in the pSB1C3 constructs.

Week 2 - gBlock Labjournal

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-11 03:27:45 PM +0000
Entry Last Modified: 2021-10-11 03:29:50 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 19/7/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-19

Goal

- Make glycerol stocks of the white colonies

Materials

- 50% glycerol

Protocol

- Put the grown culture in 50% glycerol to make a glycerol stock out of the bacterial strain.
- The colonies that remained white are stored in glycerol stocks at -80 degrees and palleted for plasmid prepartion.

FRIDAY, 23/7/2021

Week 2 - gBlock Journal : Redigestion and Ligation

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-23

Goal

- Redigest the IDT oligos of pLacI, pBAD, P1831, P2547, GFP, pBAD_CcbD, CcbD and pSB1A3:P1303 due to the wrong use of Agar in the gel the days before.

Materials

- EcoRi
- PstI
- 10x Buffer
- MiliQ
- IDT oligos
- DH5α

Protocol

- Redigested with EcoRI and PstI for 1h and heat inactivated for 20 min.

Reaction scemes of the digestions:

Table7

	A	B	C	D	E	F	G	H	I
1	Components	Reaction (uL)		Components	Reaction (uL)		Components	1 reaction (µL)	Master mix for 10 reactions (µL)
2	pSB1C3 (85.6 ng/ul)	9		pSB1A3 (96.4 ng/ul)	7		gBlock DNA	7	N/A
3	EcoRI-HF	1		EcoRI-HF	1		EcoRI-HF	1	11
4	PstI	1		PstI	1		PstI	1	11
5	10x Buffer 2.1	1		10x Buffer 2.1	1		10x Buffer 2.1	1	11
6	MiliQ	0		MiliQ	0		MiliQ	0	
7	Total vollume	12		Total vollume	10		Total vollume	10	

The rest of the constructs are retransformed in DH5a to generate new plasmid and -80 degrees stocks.
Plates are left at RT over the weekend.

Week 2 - Making p1487::mCherry construct in pSB1A3

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 07:50:27 AM +0000
Entry Last Modified: 2021-09-28 09:01:13 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 22/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2020-07-22

Goal

- Digestion of the components p1487-mCherry

Materials

- MiliQ
- NEBuffer 2.1
- EcoRI
- SpeI
- XbaI
- pSB1A3-mCherry (extraction nr. 27 from Plasmid isolation)
- pSB1A3-P1487 (extraction nr. 11 from Plasmid isolation)

Methods

The ligation will be done at a 3:1 ratio of insert:vector.
For calculating the amounts of DNA needed, I used the NEBioCalculator webtool. The lengths of the DNA segments were taken from virtual digestions in benchling.
NEBioCalculator showed I need 2.426 ng of insert of 58 bp to get a 3:1 ratio when the vector length is 2977 bp and a mass of 41.5 ng (1 uL of plasmid 27).
Since the insert is only 58 bp of a plasmid of about 3 kb, I inferred that around 2% of the mass of the plasmid is my insert. The insert DNA was 19.7 ng/uL, meaning it contains 0.394 ng of my insert per uL. In order to get 2.426 ng, I needed 6.1 uL of DNA.
Restriction reactions:

Table1

	Insert restrictioningredients	Volume (uL)	C	Vector restriction ingredients	Volume (uL)
1	Insert DNA	6.1		Vector DNA	1
2	SpeI	0.5		XbaI	0.5
3	EcoRI	0.5		EcoRI	0.5
4	NEBuffer 2.1	1		NEBuffer 2.1	1
5	MiliQ	1.9		MiliQ	7
6	Total	10		Total	10

After the restriction, which ran for 1 hour and 15 minutes at 37 C, the enzymes were heat inactivated. For the insert, the reaction was set at 80 C for 35 minutes and for the vector, the reaction was inactivated at 65 C for 35 minutes.

Ligation of the reaction

Materials

- MiliQ
- T4 ligase
- T4 ligase buffer
- Vector and Insert DNA from previous step

Table2

	Ingredient	Volume (uL)
1	Vector DNA	5
2	Insert DNA	10
3	T4 Ligase Buffer	2
4	T4 Ligase	1
5	MiliQ	2
6	**Total**	**20**

The reaction was done at room temperature for 2.5 hours.

FRIDAY, 23/7/2021

Making p1487-mCherry construct in pSB1A3 (2)

Project: Lab Notebook

Authors: Sebastiaan Ketelaar

Created at: 2020-07-23

Goal

- Transform E coli DH5a with yesterdays ligation.

Materials

- LB medium
- Heat-shock

Protocol

I used 50 uL of competent cells made in week 1, and added 10 uL of the ligation mix from yesterday.
The cells were then put on ice for 30 minutes and heat-shocked for 90 s. at 42 C. 1 mL fresh LB was added and then the cells were stored at 37 °C for roughly 1 hour without shaking.
After this, I helped Bas with doing the transformations of the promotersequences that were made over the past week, see 'plasmid isolation'.
For these transformations, I used 5 uL of the plasmid with 50 uL of competent cells.
Bas plated these transformations for me. They will grow over the weekend on the bench.

TUESDAY, 27/7/2021

Making p1487-mCherry construct in pSB1A3 (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2020-07-24

Goal

The plasmids are still not visible as red colonies, even though we know we have them. However, I found some literature that mCherry sometimes has problems when grown at 37 °C, so I will try to put it in the fridge for 24 hours and hope it turns red. If not, I will leave it on the bench for 24 more hours and some colonies should turn red, at least according to https://www.researchgate.net/post/Do-mCherry-fusion-proteins-mature-poorly-at-37C-in-E-coli

If this doesn't work, we will have to try to make new construct.
Today, I used the rest of the ligation mix that was leftover to do a new heatshock with the same bacterial strain and protocol. Hopefully it will matter and we can get some nice and red colonies.

Week 2 - Primer test PCR (2)

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:04:34 AM +0000
Entry Last Modified: 2021-10-11 11:26:59 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

WEDNESDAY, 21/7/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2021-07-21

Goal

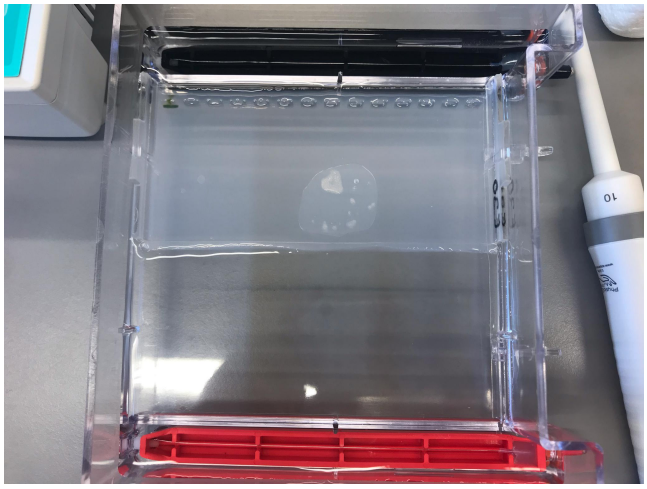
- Perform a gelelectrophoresis for testing primer after PCR.

Materials

- 1% Agarose gel
- 1% TAE Buffer
- Loading dye
- 10kb Ladder
- Ethidium bromide

image0 (1).jpeg

image1 (1).jpeg

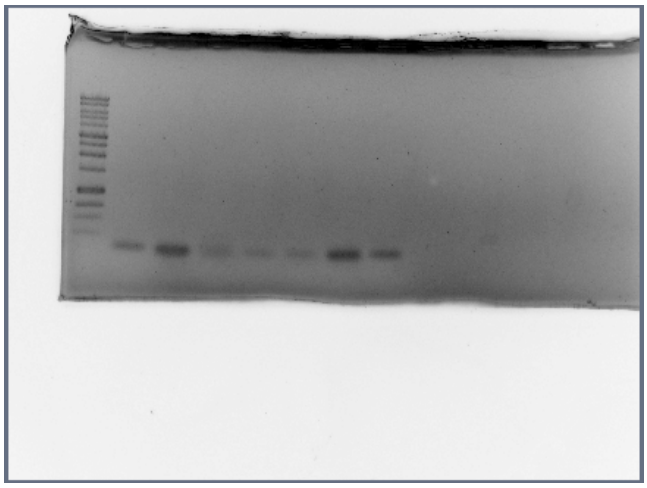


Protocol:

- Added 2 µl of gel loading dye to 10 µl of plasmids
- add 10 µl of each sample on the gel
- run gel electrophoresis for around 40 mins
- put the gel on Ethidium Bromide
- wait for 30 mins
- take pictures of the gel

Results

20210721 HE PCR 1.tif



The gelelectrophoresis of the PCR primer test did not correpsond to the expected bands.

Reperform the primertest with PCR

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2020-07-21

Goal

- Reperform the primertest with PCR to check if the primers are correct and correspond to the expected length.

Materials

- 10xBuffer
- Taq polymerase
- DNTPs
- MiliQ

Protocol

Used master mix:

Table1

	A	B
1	DNTPs	8 µl
2	10x pcr buffer	40 µl
3	MLQ water	292 µl
4	taq polymerase	4 µl

Add primers with the same dilutions as 20-07 to plasmids.

Add 43 µl of master mix to each tube of plasmids and primers.

Put them in PCR over night.

Change in PCR program:

Table2

	A	B
1	95°C	5 min
2	95°C	30 sec
3	60 °C	2 min
4	72°C	3 min
5	72°C	5 min
6	12°C	infinity

unnamed (2).jpg

Week 2 - Primer test PCR (3)

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:04:42 AM +0000
Entry Last Modified: 2021-10-11 11:24:49 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 22/7/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2020-07-22

Goal

- Perform a gelelectrophoresis with PCR products of 2020-07-21.

Materials

- pJUMP 26
- pJUMP46
- Primers
  - K2
  - K3
  - Smr2
  - Smr3
  - House keeping gene

Protocol

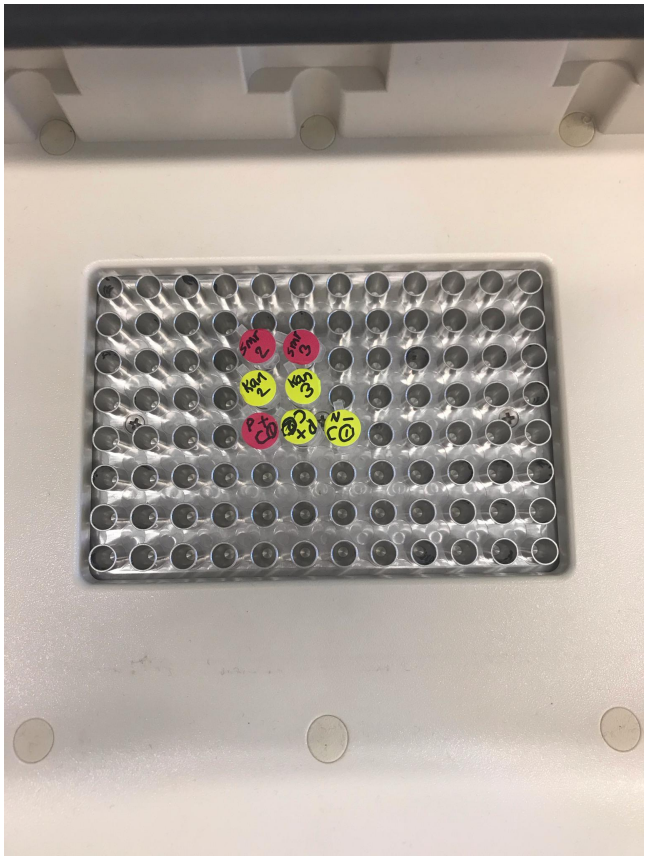
Did PCR with 5 samples, 2 positive controls and 1 negative control
Plasmid pJUMP 26, pJUMP 46
Primer: K2, K3, Smr2, Smr 3, House keeping gene
positive control: 2 primers Bas gave with both plasmids
Negative control: Smr2 primer+yellow plasmid

PCR cycle used:

Table2

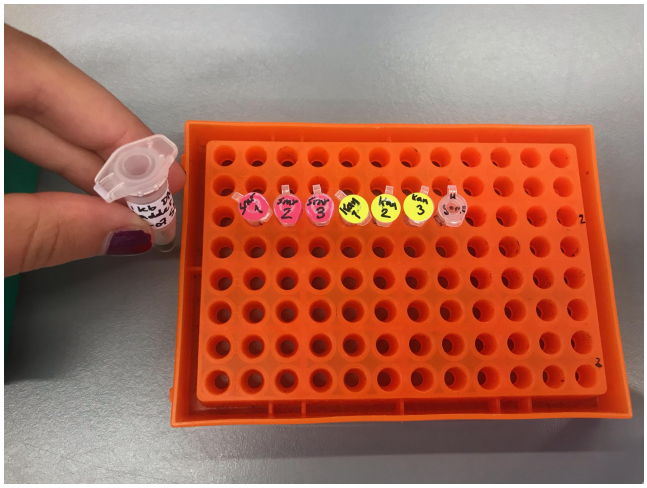
	A	B
1	95°C	5 min
2	95°C	30 sec
3	60 °C	2 min
4	72°C	3 min
5	72°C	5 min
6	12°C	infinity

image0 (2).jpeg



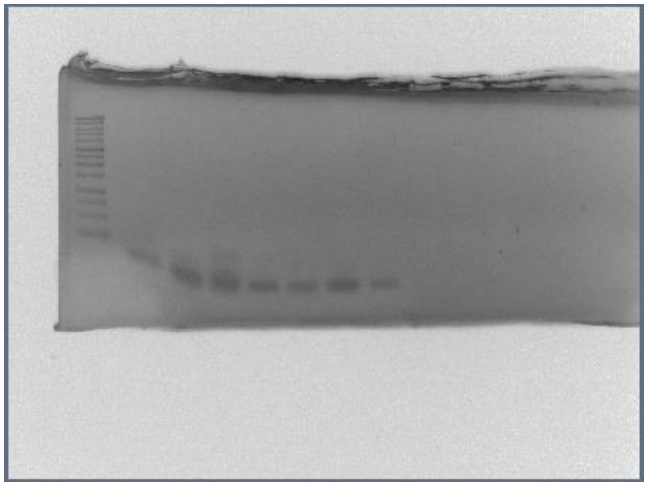
Gelelectrophoresis legenda:

unnamed (1).jpg



Result

22 july pcr 2 H.E.jpg



The gel does not look good. It seems like the gel was not straight in the gelelectrophoresis machine, as the gel bands turned out in a really bad shape.

Week 2 - Primer test PCR

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:04:26 AM +0000
Entry Last Modified: 2021-10-11 11:32:16 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 20/7/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2021-07-20

Goal:

- Test the Sigma-Aldrich primers with PCR.

Materials:

- Plasmids → 1) pJUMP 46
  2) pJUMP 26
- DNTP
- PRIMERS
- Taq polymerase
- 10X PCR buffer
- MiliQ

Primers used:

Table1

	A	B
1	Name	µl of water for 100 µM
2	qPCR_Smr1_forward	616
3	qPCR_Smr1_reverse	621
4	qPCR_Smr2_forward	647
5	qPCR_Smr2_reverse	532
6	qPCR_Smr3_forward	549
7	qPCR_Smr3_reverse	636
8	qPCR_Kan1_forward	560
9	qPCR_Kan1_reverse	664
10	qPCR_Kan2_forward	542
11	qPCR_Kan2_reverse	617
12	qPCR_Kan3_forward	623
13	qPCR_Kan3_reverse	575
14	House keeping gene forward	511
15	House keeping gene reverse	508

PCR programme

Table2

	A	B
1	95°C	5 min
2	95°C	30 sec
3	55°C	2 min
4	72°C	3 min
5	72°C	5 min
6	12°C	Infinity

Protocol:

- Spin the primer tubes, add specific amount of M.L.Q water to each one, use vurtex to dilute DNA in water.
- Dilute plasmids 100 times (1 µl of plasmid + 99 µl M.L.Q wate)
- Add 5 Micro liter of each plasmid (we had 2 so 5 micro of each on 3 tubes)
- In 1 tube we add 5 Micro liter of DH5**α DNA**
- add 1 µl DNTP
- add 1 µl of forward primer to each tube

- add 1 µl of reverse primer to each tube
- add 0.5 µl Taq polymerase
- 5 µl 10x PCR buffer
- Add 36.5 µl M.L.Q water

put them in PCR machine

Extra

Next time make this changes:
3) 60
DNA+PRIMERS
BUFFER +WATER+ DNTP+TAQ polymerase
43 reaction mix
40 buffer+8(36.5) water+8 MICRO DNTP+ 4 MICRO TAQ

Week 3 - Colony qPCR

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:59:36 AM +0000
Entry Last Modified: 2021-10-11 02:45:04 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

WEDNESDAY, 28/7/2021

Project: Lab Notebook
Authors: Iris Noordermeer, Jetse van Os
Created at: 2021-07-28

Goal

- Perform a Colony qPCR to see if the constructs are present in the iGEM plasmids pSB1A3 and pSB1C3.
- This is part of the gBlock journal of Bas Woudenberg

Materials

- MiliQ
- Primers: Prefix R, Suffix, VF iGEMplasmids, VR iGEMplasmids
- Colonys: DH5 α PSB IA3 a red colony , DH5 α PSB IA3 white colony, DH5 α PSB IC3, PSB IC3, PSB IA3.
- DNTP
- TAQ polymerase
- PCR Buffer
- qPCR machine
- 1000 uL Pipet
- 200uL pipet
- 10uL pipet
- eppendorf tubes

Protocol

Constructs to varify:

Table1

	A	B	C
1	Name	Name	Catalogue number
2	pSB1C3	pLacI	1
3	pSB1C3	pBAD	2
4	pSB1C3	P1831	12
5	pSB1C3	P2547	13
6	pSB1A3	P_pBAD_CcdB_T_S	15
7	pSB1A3	GFP	26

Numbers come from the labjournal of Bas from the gBlock Labjournal

Used MasterMix (MM):

Table2

	A	B
1	DNTP	19uL
2	Preffix - F	19uL
3	Suffix - R	19uL
4	Taq	8.5uL
5	PCR buffer	85uL
6	MiliQ	286.50uL
7	**Total**	380uL

PCR reaction mix:

Table3

	A	B
1	Mastermix	20uL
2	Colony	5uL
3	**Total**	**25uL**

Colony were directly from the medium pipetted in the PCR reaction mix.

PCR reaction cycle

Table4

	A
1	95°C for 15 minutes
2	94°C for 30 seconds
3	60°C for 30 seconds
4	72°C for 3 minutes
5	Repeat 2-4 39 times
6	72°C for 15:00 minutes
7	4°C forever

Gelelectrophoresis

- Gel was made by 1% TEA and was dried for 15 minutes.
- The samples were first pipetted in the gel, before laying the gel in the 1% TAE running buffer.

Table5

	A	B
1	Gel 1	Gel 2
2	1 Kb Ladder	1 Kb Ladder
3	1	15
4	1	15
5	1	15
6	2	26
7	2	26
8	2	26
9	1 Kb ladder	Slots 8-14 empty
10	12	
11	13	
12	13	
13	13	
14	Slot 13 & 14 empty	

    - The gel was runned on 120 V for 35 minutes.
    - Stained in Ethidium bromide for 20 minutes.

week 3 - Co-transformation

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:36:00 AM +0000
Entry Last Modified: 2021-10-11 02:23:48 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 29/7/2021

Project: Lab Notebook
Authors: Ehsan Razaghi Siahroudi
Created at: 2021-07-29

Goal

- Perform a co-transformation of tje JUMP plasmid family.

Materials

- LB agar

Protocol

Prepare dilutions of the plasmids with a concentration of 2 ng/µL • Switch on the water bath and set temperature at 42 °C. Also turn on the heat/shaking-block and set up to 37 °C • Load a bucket with ice from the ice machine • Take the bacterial cells and SOC (Super optimal broth with catabolite repression) out of the - 80 °C freezer. Transfer the cells directly to ice. Do not touch the bottom of the tube that contains the cells. • Thaw the cells on ice for ~5 minutes • Add 1 µL of each plasmid into 20 µL bacteria. Mix well. Make sure you work near the Bunsen burner flame • Leave the cells on ice for 5 minutes • Heat-shock the cells for 30 seconds (exactly!) at 42°C • Return the cells directly to ice for 2 minutes • Add 80 µL of SOC solution to the bacteria • Incubate for 60 minutes at 37 °C and 300 rpm • Dry agar plate, supplemented with Spectinomycen(25 µg/mL) and Kanamycen(30 µg/mL) in the 37 °C incubators. Place plate upside down and slightly opened.
Plating the cells on agar plate • Take the dried agar plate out of the 37 °C incubator • Label the bottom of the plates • Open an agar plate in close proximity of the Bunsen burner flame • Pipette the cells (100 µL) on the plate • Sterilize the Trigalski spatula by burning the alcohol on it, shortly let it cool down • Spread the cells on the plate using the sterile spatula • Transfer the agar plate to the 37°C incubator • Place the plate upside down, closed • Let the cells grow on the plate overnight .

Co-transformation reaction mix:

Table1

	Plasmid name	Amount of plasmid	Amount of M.L.Q
1	26	3.65 µl	1.35
2	26	3.65 µl	1.35
3	27	2.82 µl	2.17
4	27	2.82 µl	2.17
5	29	2.52 µl	2.47
6	29	2.52 µl	2.47
7	46	3.75 µl	1.25
8	46	3.75 µl	1.25
9	47	2 µl	3
10	47	2 µl	3
11	49	2.65 µl	2.35
12	49	2.65 µl	2.35

Add 5 µl of plasmids to 50 µl of old competent and new competent cells.

Week 3 - Creating promoter: Toxin/antitoxin gene fusions

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:37:49 AM +0000
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WEDNESDAY, 28/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-28

Goal

- Perform a plasmid restriction of pSB1A3 and restrict the toxin, antitoxins, and the pTetOn promoter.
  Also, a ligation is performed of the promoter with the toxin and antitoxins.

Materials

- EcoRI
- PstI
- XbaI
- SpeI
- NEBuffer 2.1
- MiliQ
- pSB1A3 with
  - ccdA
  - Sok
  - MazE
  - RelB
  - ccdB
  - Hok
  - MazF
  - RelE
  - PTetOn
- pJUMP 49
- pJUMP 27

Protocol

The plasmids containing the gene fragments were restricted by mixing them with the following components:

Plasmid restriction:

Table1

	Plasmid restriction	Volume
1	EcoRI	0.5
2	PstI	0.5
3	NEBuffer 2.1	1
4	DNA	1
5	MiliQ	7
6	**Total**	10

Toxin/Antitoxin restriction:

Table2

	Toxin/Antitoxin components	Volume (uL)
1	XbaI	0.5
2	PstI	0.5
3	NEBuffer 2.1	1
4	DNA	2.5
5	MiliQ	5.5
6	**Total**	10

pTetOn restriction:

Table3

	A	B
1	EcoRI	1
2	SpeI	1
3	NEBuffer 2.1	2
4	DNA	14
5	MiliQ	2
6	**Total**	20

Ligation:

Table4

	Component	Volume/reaction (uL)
1	T4 Ligase	1
2	T4 Ligase buffer	2
3	Vector DNA	1
4	promoter DNA	1.5
5	Insert DNA	10
6	MiliQ	4.5
7	**Total**	**20**

These restrictions were then performed at 37 °C for 2 hours. Following this, there was a heat-shock at 85 °C for 20 minutes.
They will then be ligated for 2 hours at room temperature and then inactivated again to be transformed tomorrow.

THURSDAY, 29/7/2021

Week 3 - Creating promoter: Toxin/antitoxin gene fusions (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-29

Goal

Transormation of the ligated constructs in Top10 competent cells.
Restriction of the pBAD promoter in order to ligate it in the pJUMP27 plasmid infront of the toxins.

Materials

- EcoRI
- SpeI
- XbaI
- PstI
- NEBuffer 2.1
- MiliQ
-

Protocol

The full 20 uL of the ligations were added to one vial of chemically competent cells Top10 made by Mark van Loosdrecht. They were then left for 30 minutes on ice.
A heat-bath was used to heat-shock the cells for 90 s. After this, 400 uL of LB without antibiotics was added.
The transformations were then plated on LB containing the appropriate antibiotics and put in the 37 °C.

Restriction reaction of the pBAD and pJUMP27 plasmid with toxins

Table5

	pBAD restriction	Volume (uL)	C	Toxin restriction	Volume (uL)
1	EcoRI	1		XbaI	0.5
2	SpeI	1		PstI	0.5
3	NEBuffer 2.1	2		NEBuffer 2.1	1
4	MiliQ	2		MiliQ	6
5	DNA	14		DNA	2
6	**Total**	20		**Total**	10

Ligation reaction of in pJUMP27 of the pBAD

Table6

	Ligation	Volume (uL)
1	T4 DNA ligase	1
2	T4 ligase buffer	2
3	Vector DNA	1.5
4	Insert DNA	3
5	Insert DNA	13
6	MiliQ	4.5
7	**Total**	25

Restriction done for 2 hours at 37 °C. Heat inactivation done at 80 °C for 20 minutes. Ligation done for 2 hours at room temperature.

FRIDAY, 30/7/2021

Week 3 - Creating promoter- Toxin/antitoxin gene fusions (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-07-30

Goal

- Transformation of the ligated pBAD in pJUMP27 in Top10.

Materials

- Competent cells Top10
- LB medium

Protocol

Transformation protocol followed according to https://international.neb.com/protocols/2012/05/21/transformation-protocol
Further, I transformed the pBad-toxin fusions in the same batch of competent cells I used yesterday.
They were heat-shocked for 90 s. at 42 °C. Then 400 uL of LB was added to the cells and they were incubated at 37 °C for 45 minutes, after which they were plated on plates containing the appropriate antibiotics.

Results

The transformations were a great success! All of them worked, which is surprising considering pTetOn has a leaky promoter, but still the toxin-expressing plasmids grew (albeit in roughly 100x reduced quantity compared to the antitoxins).
I will isolate plasmids from the plates on Monday (or ask Iggy and Lisa).

Week 3 - gBlock Labjournal: Redigestion and Ligation

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-11 03:30:25 PM +0000
Entry Last Modified: 2021-10-11 03:32:40 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 26/7/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2020-07-26

Goal

- Ligate the redigested inserts in pSB1C3 and pSB1A3

Materials

- 10x ligase buffer
- T4 ligase
- MiliQ
- DH5α TSS competent cells

Protocols

redigested inserts are ligated in the folling plasmids.

Table8

	A	B
1	**pSB1C3:**	**pSB1A3:**
2	pLacI	pBAD_CcdB
3	pBAD	CcdB
4	P1303	GFP
5	P1831	
6	P2547	

According to the following ligation

Table9

	A	B	C	D
1	Component	1 reaction	**pSB1C3:**	**pSB1A3:**
2	Digested plasmid	2	12	10
3	insert	10		
4	10x ligase buffer	2	12	10
5	T4 ligase	1	6	5
6	miliQ	5	30	25

After 2H of ligation 10 ul of the plasmids are transformed in DH5a TSS competent cells.

TUESDAY, 27/7/2021

Week 3 - gBlock Journal : Plasmid stocks (7)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2020-07-27

Goal

- Transfere white colonies to generate plasmid stocks

Materials

- LB medium
- Tooth picks
- Antibiotics

Protocol

- Pick white colonies and inoculate in LB medium.

Results

The red samples did not show any white colonies, the others were transfered to liquid LB to generate plasmid stocks.

Table10

	A	B
1	**pSB1C3:**	**pSB1A3:**
2	pLacI	pBAD_CcdB
3	pBAD	CcdB
4	P1303	GFP
5	P1831	
6	P2547	

WEDNESDAY, 28/7/2021

Week 3 - gBlock Journal : Plasmid Isolation and Digestion (8)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2020-07-28

Goal

- Plasmid isolation and digestion

Materials

- ThermoScientific GeneJET mini prep kit
- MiliQ
- Restriction enzymes
  - EcoRI
  - PstI
- Restriction buffer

Protocols

Plasmid isolation is performed according to the protocol of the Thermoscientific GeneJET plasmid miniprep kit.

Digestion for 1 H:

Table6

	A	B
1	DNA	1 ul
2	10x Buffer	2 ul
3	EcoRI	1 ul
4	PstI	1 ul
5	MiliQ	15 ul

Next, the digestion is loaded on a 1% Agarose gel, 100V for 30 min.

Extra

The CcdB harboring plasmids were not able to propiate in liquid cultre, so plates were covalently streaked to generate plasmid stocks.

Week 3 - Gel electrophoresis of primer check

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:36:13 AM +0000
Entry Last Modified: 2021-10-11 01:04:03 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 30/7/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2021-07-30

Goal

- Perform a gelelectrophoresis to check the functionality of the primers

Material

- Used the stored co-transformed plates in fridge
- 10x restriction buffer
- 1% Agarose
- 1% TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

Protocol

Gel electrophoresis is performed using 9 ul sample with 1 ul 10x loading dye is added to the slot and 6 ul of the 1 kB DNA Ladder. The gel ran at 100V for 30 minutes.
Next, the gel was stained in ethidiumbromide for 25 minutes.

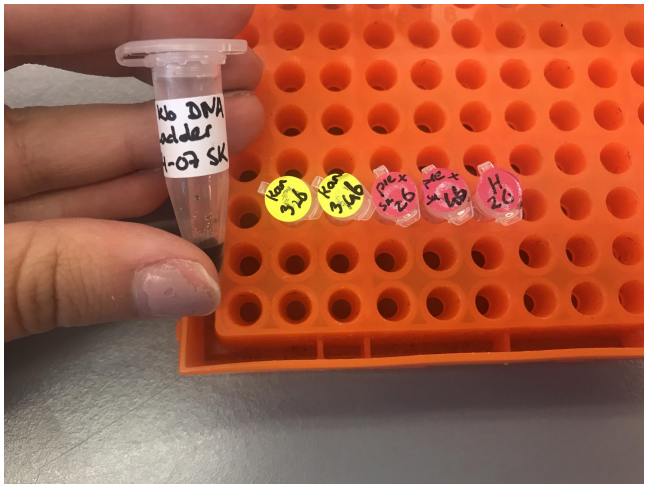
Legenda used for gelelectrophoresis

Legenda gel 1:

IMG_1832.jpg

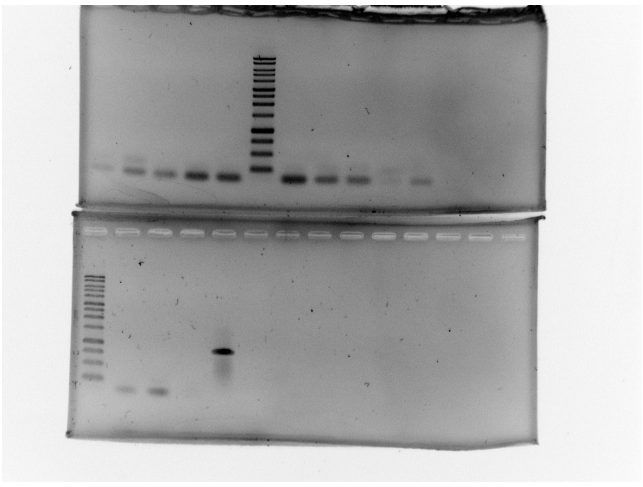
Legenda Gel 2:

image0 (5).jpeg



Result

30 July July pcr 3 H.E.tif



It seems that the gel electrophoresis did not succeed as all the bands are at the bottom of the gel what is not the right size. Only one band on gel 2 lane 4th lane shows a band. This is the household primer with pJUMP26.

Week 3 - Jump plasmids RFP switch

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:35:10 AM +0000
Entry Last Modified: 2021-10-11 12:42:10 PM +0000
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THURSDAY, 29/7/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-29

Goal

To obtian pJUMP plasmids with a RFP casette, we switch the lacI RFP biobrick from the pSB1A3 to the jump40 plasmids.

Materials

- EcoRI
- PstI
- 10x Buffer
- PSB1A3
- pJUMP40, pJUMP46, pJUMP47, pJUMP48, pJUMP49, and pKiKo plasmids
- MiliQ

Protocol

Restriction reaction:

Table1

	A	B	C	D	E	F
1		**pJump 46**	**pJump 47**	**pJump 48**	**pJump 49**	**pKiKo**
2	Acceptor plasmid DNA	1	1	1	1	1
3	PSB1A3	5	5	5	5	5
4	10x Buffer 2.1	1	1	1	1	1
5	EcoRI	0.5	0.5	0.5	0.5	0.5
6	PstI	0.5	0.5	0.5	0.5	0.5
7	MiliQ	2	2	2	2	2
8	Total	10	10	10	10	10

- Plasmids are digeested for 1,5h and heat inactivated for 20 min at 80degrees.
- plasmdis are ligated over night.

FRIDAY, 30/7/2021

Week 3 - Jump plasmids RFP switch (2)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-07-30

Goal

- Transform the ligated pJUMP40 plasmids in TOP10 *E.coli* strain

Materials

- LB medium
- Competent cells TOP10
- Agar plates

Protocol

Plasmids are transformed according to the protocol: https://international.neb.com/protocols/2012/05/21/transformation-protocol and placed in the 37 degrees over the weekend.

Result

Red colonies had grown over the weekend, this means that the RFP was integrated in the plasmid.

MONDAY, 2/8/2021

Week 4 - Jump plasmids RFP switch (3)

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-08-02

Goal

Make liquid cultures of the colonies and isolate the plasmids.

Materials

- Liquid LB medium
- 50 ml tubes

Protocol

Red colonies are picked and isolated at the end of the day and put in liquid LB medium.
The isolation protocol is followed according to the Thermoscientific GeneJET Plasmid mini prep.

THURSDAY, 5/8/2021

Week 4 -Jump plasmids RFP switch (4)

Project: Lab Notebook

Authors: Bas van Woudenberg

Created at: 2021-08-05

Goal

- To verify if the plasmids are right a gelelectrophoresis will be done after a digestion of the isolated plasmids.

Materials

- EcoRI
- PstI
- 10x restriction buffer
- MiliQ
- 1% Agarose gel
- 1% TAE buffer

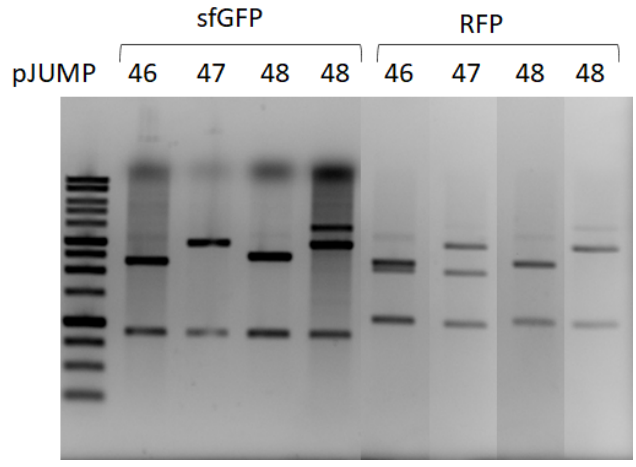
Protocol

The digestion protocol on https://www.sigmaaldrich.com/NL/en/technical-documents/protocol/genomics/cloning-and-expression/restriction-enzyme-digest-protocol was followed. The restriction reaction was performed for 1H at room temperature.

Then, the plasmids are ran on a 1% agarose gel at 100V for 30 minutes.

Results

image.png



The gel result of RFP switches. The first lane was the DNA ladder. The four lanes on the left were original pJUMP-4x plasmids with sfGFP as control (pJUMP46: 2311bp, 902bp; pJUMP47: 3039bp, 902bp; pJUMP48: 2508bp, 902bp; pJUMP49: 3039bp, 902bp). The other four lanes are pJUMP-4x plasmids with RFP cassette after switching (pJUMP46: 2311bp, 1110bp; pJUMP47: 3039bp, 1110bp; pJUMP48: 2508bp, 1110bp; pJUMP49: 3039bp, 1110bp). Some contamination was observed in pJUMP47-RFP.

Week 3 - Ligation and transformation

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:37:27 AM +0000
Entry Last Modified: 2021-10-11 12:32:40 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

WEDNESDAY, 28/7/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-28

Goal

- Ligation and transformation of pJUMP49 with GFP or mCherry in DH5α.

Materials

- T4 ligase
- Ligase buffer
- Competent cells of DH5α
- MiliQ
- LB medium
- agar plates
- Streptomycin

Protocol

Build construct:

Table1

	A	B	C	D	E
1	5003	P1831	GFP	pJUMP-49	
2	5004	PTetOn	GFP	pJUMP-49	
3	5005	P1831	mCherry	pJUMP-49	
4	5006	PTetOn	mCherry	pJUMP-49	

Transformation them in plates with streptinmycin
Arabinose stock solution: >2% (w/v) 1g/50mL ; max: 834g/L 40g/50mL
Tetracyclin stock solution: >100ng/mL
IPTG stock solution: >1mmol/L
https://www.protocols.io/view/IPTG-1-M-100-x-Stock-Solution-j93ugm?step=4

THURSDAY, 29/7/2021

Week 3 - Ligation and transformation (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-07-29

Goal:

- Transformation did not succeed as there was no growht on the plates. Transformation will be reperformed with new competent cells.

Materials

- New competent cells of DH5α
- LB medium
- Agar plates
- Streptomycin

Protocol

Retransform the ligation plasmids using competent cells of DH5α from Floor according to the same protocol as used on 2020-07-28.

Week 3 - Reporter Gene Swap

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:38:04 AM +0000
Entry Last Modified: 2021-10-11 11:22:09 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 26/7/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Hoda Ekhlasi
Created at: 2020-07-26

Goal:

This week, Hoda and I will attempt to swap the reporter genes in the JUMP plasmid to add RFP to the plasmid.
For this, we will restrict the pSB1A3 and pJUMP at the same time (cutting out the GFP/RFP) and then ligate it in the same reaction.

Materials

- pJUMP46-GFP
- pJUMP47-GFP
- pJUMP48-GFP
- pJUMP49-GFP
- pSB1C3
- EcoRI
- PstI
- NEBuffer 2.1
- T4 ligase
- T4 ligase buffer

Protocol

A restriction mastermix was made with the following method:

Table1

	Component	Amount (uL)
1	PstI	3
2	EcoRI	3
3	NEBuffer 2.1	6
4	MiliQ	42
5	DNA (not added to master mix)	6
6	**Total**	60

The restriction was run at 37 °C for 1 hour.

Then, the whole reaction was heat inactivated at 85 °C for 20 minutes. Following this step, cells were taken from the -80 °C and we added 10 uL of DNA to every tube containing 50 uL of competent cells following gentle thawing of the cells on ice. This was then left to incubate on the ice for 30 minutes.

Following this step, the cells were heat-shocked at 42 °C for 90 seconds. Then, they were immediately placed back on ice and 500 uL of LB liquid medium was added and they were grown for 60 minutes at 37 °C without aeration.

Next, the transformed cells were plated on LB plates containing spectinomycin.

They were grown overnight at 37 °C.

TUESDAY, 27/7/2021

Week 3 - Reporter Gene Swap (2)

Project: Lab Notebook

Authors: Sebastiaan Ketelaar, Hoda Ekhlasi

Created at: 2020-07-27

Goal

- See if the plates show bacterial growth and if the cultures appears to be red.

Results

The plates have grown with bacteria on them, but they do not appear to be red.

Perhaps the ligation did not contain enough insert DNA in relation to the amount of vector, or something else was wrong. However, we don't know exactly why the cells are not red.

Week 3 - Result co-transformation and check

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-28 09:36:07 AM +0000
Entry Last Modified: 2021-10-11 11:20:07 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 29/7/2021

Project: Lab Notebook
Authors: Hoda Ekhasi
Created at: 2021-07-29

Goal

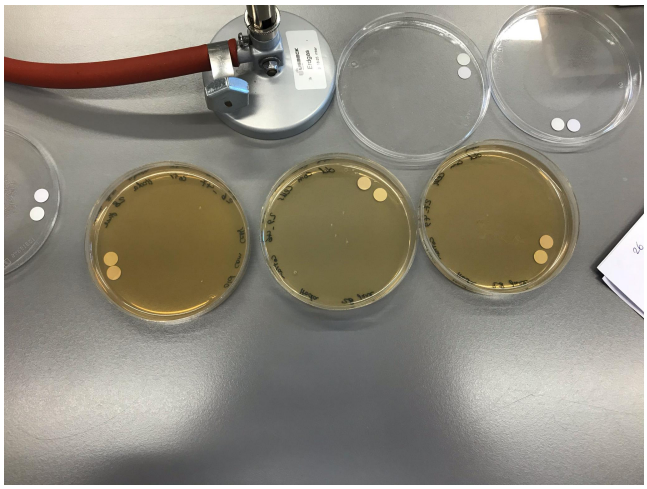
- Check the result of co-transfomation on the agar plates.
- Perform a PCR reaction to see if co-transformants contain the right construction of plasmids.

Result co-transformation

The co-transformed plasmids in old competent cells have these amounts of colonies :

1. pJUMP26-pJUMP47: 3 colonies
2. pJUMP29-pJUMP46: 7 colonies
3. pJUMP27-pJUMP49: 1 colony

image0 (4).jpeg

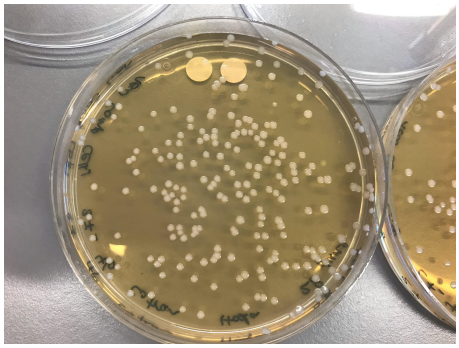


From left to right: 1) 26-47 2) 29-46 3) 27-49

The co-transformed plasmids in new competent cells have these amounts of colonies :

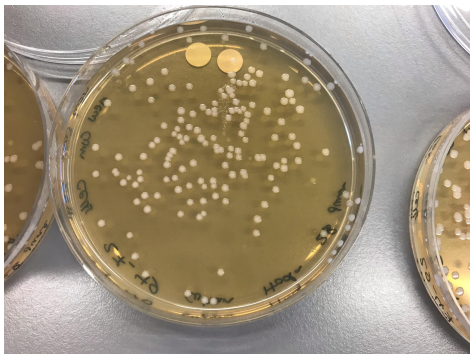
1. pJUMP27-pJUMP46: 1 colony
2. pJUMP27-pJUMP49: 5 colonies
3. pJUMP29-pJUMP47: 7 colonies
4. pJUMP26-pJUMP49: 4 colonies

image1 (2).jpeg



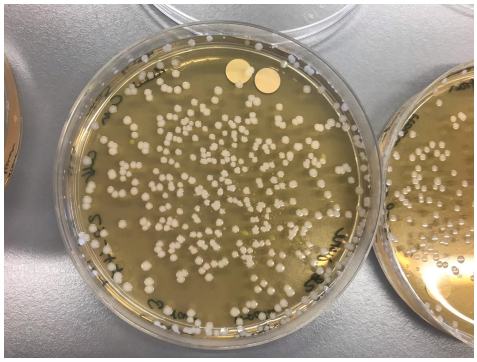
27-46

image6.jpeg



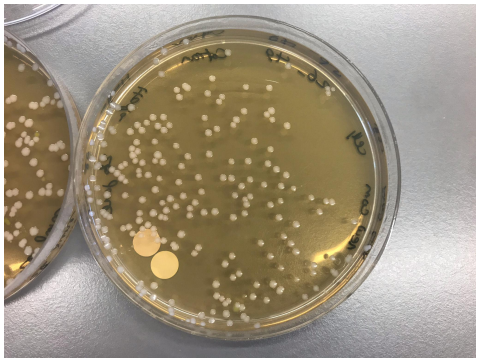
27-49

image3.jpeg



29-47

image7.jpeg



26-49

I took 3 colonies on each and inoculate them in 3 other plates with both antibiotics.

Protocol PCR reaction

Do PCR with primers with negative and positive control as follows:

Table1

	Primer	Plasmid
1	SMR 1	46
2	SMR 1	26
3	SMR 2	46
4	SMR 2	26
5	SMR3	46
6	SMR3	26
7	KAN 1	46
8	KAN 1	26
9	KAN 2	46
10	KAN 2	26
11	KAN 3	46
12	KAN 3	26
13	HOUSEHOLD	DH5 alpha
14	PREFIX-SUFFIX	26
15	PREFIX-SUFFIX	46

PCR cycle used:

Table2

	A	B
1	95°C	5 min
2	95°C	30 sec
3	60 °C	2 min
4	72°C	3 min
5	72°C	5 min
6	12°C	infinity

Week 4 - Assembly Plan

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:57:05 AM +0000
Entry Last Modified: 2021-09-30 01:13:47 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

WEDNESDAY, 4/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-04

Goal

- Make an over view of what already is assembled and what is checked so far with gel electrophoresis and Testing.

Assembly

- [x] P2549-mCherry on pJUMP-49
- [x] Pbad-mCherry
- [ ] Pbad-GFP
- [x] P1831-mCherry
- [x] P1487-mCherry
- [x] P1303-mCherry
- [x] P908-mCherry
- [x] P623-mCherry
- [x] P387-mCherry
- [x] P162-mCherry
- [x] P21-mCherry
- [x] P1-mCherry

Gel check

- [x] P2549-mCherry on pJUMP-49
- [x] Pbad-mCherry
- [ ] Pbad-GFP
- [x] P1831-mCherry
- [ ] P1487-mCherry
- [x] P1303-mCherry
- [x] P908-mCherry
- [ ] P623-mCherry
- [ ] P387-mCherry
- [ ] P162-mCherry
- [ ] P21-mCherry
- [ ] P1-mCherry

Testing

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	26-49						29-46					
B	26-49						29-47					
C	26-49						29-47					
D	27-46						29-47					
E	27-46						29-49					
F	27-46						29-49					
G	29-46						29-49					
H	29-46						Blank					

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	26						29					
B	26						47					
C	26						47					
D	27						47					
E	27											
F	27											
G	29											
H	29											

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	P2549						P908					
B	P2549						49					
C	P2549						49					
D	P1303						49					
E	P1303						Blank					
F	P1303											
G	P908											
H	P908											

Week 4 - Biobricks : Digestion isolated plasmids

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:47:50 AM +0000
Entry Last Modified: 2021-10-11 01:42:40 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-08-06

Goal

- Perform a digestion of the isolated plasmids Biobricks of today

Materials

- EcoRI
- PstI
- 10x buffer 2.1
- MiliQ

Protocol

The following plasmids are digested with EcoRI and PstI

Table1

	A	B
1	Sample	Plasmid
2	1	Jump49_P2459_mCherry
3	2	Jump29_pBad_Hok A
4	3	Jump29_pBad_Hok B
5	4	Jump49_sfGFP
6	5	Jump29_sfGFP
7	6	pSB1A3
8	7	psb11C3
9	8	psb11C3_lacI

The following reaction sceme is used to digest the plasmids:

Table2

	A	B	C
1	Components	1 reaction	10 reactions
2	Plasmid DNA	7	x
3	10x Buffer 2.1	1.5	15
4	EcoRI	0.5	5
5	PstI	0.5	5
6	MiliQ	5.5	55
7	**Total**	**15**	**x**

Week 4 - Co-transformation preparation plate reader

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:50:16 AM +0000
Entry Last Modified: 2021-10-11 02:32:51 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Ehsan Razaghi Siahroudi
Created at: 2021-08-06

Goal

- Co-transform different pJUMP plasmids in TOP10 as preparation for plate reader assay.

Material

- LB agar
- Agar plates
- Ice
- Plasmids
    - pJUMP26
    - pJUMP27
    - pJUMP29
    - pJUMP46
    - pJUMP47
    - pJUMP49

Protocol

Co-transformation of the following plasmid combinations and transformation, according to protocol
26 - 46
26 - 47
26 - 49
27 - 46
27 - 47
27 - 49
29 - 46
29 - 47
29 - 49
26 - control
27 - control
29 - control
46 - control
47 - control
49 - control

Week 4 - Creating constitutive: AT fusions

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:54:19 AM +0000
Entry Last Modified: 2021-10-11 02:23:31 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 3/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-03

Goal

- Perfom a digestion reaction in order to create constitutive antitoxin fusions in pJUMP49 plasmids.

Materials

- Vector: Jump49
  Promoter: pSB1C3:P1/P21/P162
  Antitoxin: pSB1A3:relB/MazE/Sok
- NEBuffer 2.1
- EcoRI
- SpeI
- XbaI
- PstI
- MiliQ

Protocol

According to http://www.protocol-online.org/biology-forums-2/posts/6540.html, 3-way ligations are best performed in a 1:1:1 ratio.
Therefore, that is what I will attempt for my restrictions.
Vector: Jump49
Promoter: pSB1C3:P1/P21/P162
Antitoxin: pSB1A3:relB/MazE/Sok
Using https://nebiocalculator.neb.com/#!/ligation, I calculated the amounts of DNA needed to be for each uL JUMP49:
1 uL RelB
0.99 (1) uL MazE
0.98 (1) uL Sok
1.1 uL P1
1.6 uL P21
2.1 uL P162
I will use 3 promoters * 3 antitoxins -> 9 ligations
Therefore, I will want my backbone cut 9 times, my promoters and at's 3 times.

Table1

	P1	Volume (uL)	C	P21	Volume (uL)	F	P162	Volume (uL)	I	Antitoxins (separate per ligation)	Volume (uL)	L	pJUMP49	Volume (uL)
1	EcoRI	0.8		EcoRI	0.8		EcoRI	1.13		XbaI	0.5		EcoRI	1.13
2	SpeI	0.8		SpeI	0.8		SpeI	1.13		PstI	0.5		SpeI	1.13
3	NEBuffer 2.1	1.8		NEBuffer 2.1	1.8		NEBuffer 2.1	2.25		NEBuffer 2.1	1		NEBuffer 2.1	2.25
4	P1 DNA	8.8		P21 DNA	12.8		P162 DNA	16.8		AT DNA	6		pJUMP49 DNA	18
5	MiliQ	5.8		MiliQ	1.8		MiliQ	1.2		MiliQ	2		MiliQ	0
6														
7	Total	18		Total	18		Total	22.5		Total	10		Total	22.5
8	per ligation	6		per ligation	6		per ligation	7.5		per ligation	-		per ligation	2.5

These were restricted at 37 °C for 1 hour. Then, they were frozen overnight to heatshock them before ligation tomorrow morning.
I made the ligation protocol as follows:

Table2

	Component	Volume (uL)
1	T4 DNA ligase	1
2	T4 buffer	2
3	Vector DNA	2.5
4	Promoter DNA	6 (7.5)
5	Insert DNA	6
6	MiliQ	2.5 (1)
7	**Total**	**20**

WEDNESDAY, 4/8/2021

Week 4 - Creating constitutive - AT fusions (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-04

Goal

- Perform a ligation reaction of the digested constructs of yesterday.

Materials

- T4 Ligase buffer
- T4 ligase
- MiliQ

Protocol

Table3

	Component	Volume (uL)
1	T4 DNA ligase	1
2	T4 buffer	2
3	Vector DNA	2.5
4	Promoter DNA	6 (7.5)
5	Insert DNA	6
6	MiliQ	2.5 (1)
7	**Total**	**20**

I did the ligation. It seems I made a small error and had too little of every restricted plasmid containing AT, so I made the entire ligation less volume, to keep the same ratio (1:1:1).
Then, I put the ligation in the freezer, since it was too late to do the transformation as well.

THURSDAY, 5/8/2021

Week 4 - Creating constitutive - AT fusions (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-05

Goal

- Perform a transformation with the ligated constructs Promoter: pSB1C3:P1/P21/P162 Antitoxin: pSB1A3:relB/MazE/Sok in pJUMP49 into DH5α.

Materials

- LB medium agar
- Agar plates

Protocol

The transformed constructs were as follows;
pJUMP49:[constitutive promoter]:[antitoxin].
I had P1, P21 and P162, in combination with RelB, MazE and Sok.
The ligations were all fully added to a tube of competent cells. Then, they were incubated on ice for 30 minutes.
After this, they were heatshocked for 90 s at 42 °C, and immediately put back on ice.
Then, 400 uL of LB was added and they were plated after recovering for 1 hour at 37 °C.
Transformed bacteria are then plated out on LB medium agar plates.

Week 4 - Creating pBAD-Toxin fusions

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:54:36 AM +0000
Entry Last Modified: 2021-10-11 02:01:25 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 2/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-02

Goal

- Make construct of pBAD with toxins in pJUMP27. To do this a restriction, ligation and transformation are performed today.

Materials

- EcoRI
- SpeI
- XbaI
- PstI
- NEBuffer 2.1
- MiliQ
- T4 ligase buffer
- T4 ligase
- Toxins
    - Hok
    - RelE
    - MazF
- pJUMP27

Protocol

The ligation transformations from Monday were not a big success. However, it does seem like some colonies may have grown in there, so I put the plates in the 37 °C incubator and I will see if I can pick colonies from them tomorrow, and check on Wednesday if they were succesful.
Additionally, I redid the restriciton and ligation today of pSB1C3 containing pBad, as well as Hok, RelE and MazF and ligated them. As vector DNA, leftover restricted pJUMP 27 was used from 26-7.
These were done according to the following schedule:

Table1

	pBAD restriction	Volume (uL)	C	Toxin restriction	Volume (uL)	F	Ligation	Volume (uL)
1	EcoRI	1		XbaI	1		T4 ligase	1.5
2	SpeI	1		PstI	1		T4 ligase buffer	2.5
3	NEBuffer 2.1	2		NEBuffer 2.1	2		MiliQ	1
4	MiliQ	13		MiliQ	11		Vector DNA	4
5	DNA pBAD	3		DNA toxin	5		pBAD DNA	6
6							Toxin DNA	10
7	**Total**	20		**Total**	20		**Total**	25

Then, these were restricted for 90 minutes at 37 °C, after which they were heat-inactivated at 80 °C for 30 minutes, and then ligated for 2 hours at room temperature.
Next, these were transformed according to my usual protocol:
The full ligation mixes were added to one eppendorf of competent cells, and the cells were incubated for 30 minutes on ice. Next, the cells were heat-shocked at 42 °C for 90 s., and then immediately placed back on ice again. Following the shock, 400 uL of LB without antibiotics was added to the cells, and they were left to incubate for 30 minutes at 37 °C. Next, the cells were plated on plates containing the appropriate antibiotics and put in the 37 °C incubator overnight.

TUESDAY, 3/8/2021

Week 4 - Creating pBAD-Toxin fusions (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-03

Goal

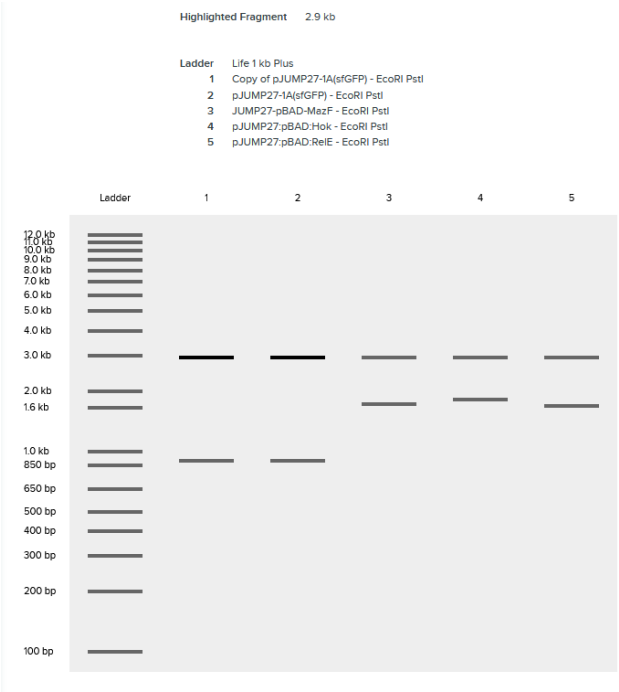
- Small colonies seem to have formed, but this will need to be confirmed. Therefore a gelelectrophoresis will be performed.

Materials

- Thermoscientific GeneJET plasmid mini prep kit
- MiliQ
- 1% Agarose
- 1% TAE buffer
- 10x loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

A virtual digests is made to compare with the gel we have of our isolated promoters.

afbeelding.png



Protocol

The plasmids were isolated, along with many other plasmids from Ehsan and Siheng.
Then, they were restricted and put on a gel to run. However, due to time constraints, I will not be able to analyse the gel myself today, so I will see tomorrow what looks well and what doesn't.

Table2

	Restriction reaction	microliters
1	EcoRI	1 ul
2	PstI	1 ul
3	NEBuffer 2.1	2 ul
4	DNA	1 ul
5	MiliQ	5 ul
6	**Total**	**10 ul**

THURSDAY, 5/8/2021

Weell 4 - Creating pBAD-Toxin fusions (3)

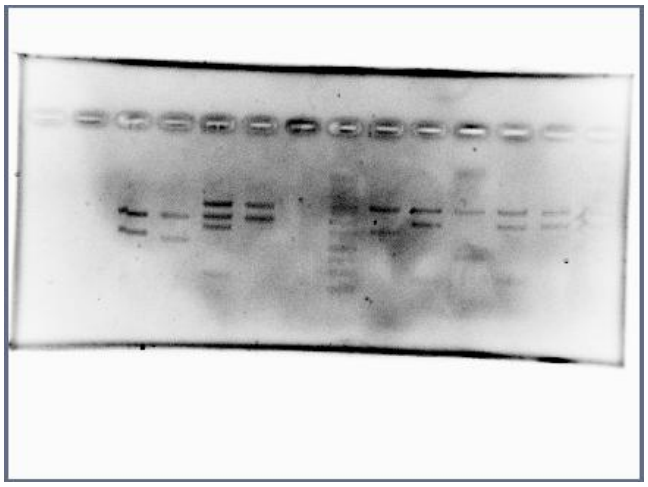
Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-05

Goal

- See if the photo of the gel is as expected (see the virtual digestion of the constructs on 2020-08-03).

Results

20210805 digestion of PBad toxin constructs Sebas.jpg



There is something slightly strange going on with the Hok fusion; in the gel from yesterday, we saw a band in the correct place, but in today's gel, it doesn't show up.
It might be that the people who did the gel today used too little DNA; the concentration was 2 pg/uL.
The other toxins seem to have fused in there correctly.
Tomorrow, I will grow them over the weekend, so that we will have lot of plasmid to isolate on Monday. Then, we can continue by ligating pBAD to (hopefully).

Week 4 - Extraction of Biobricks

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:47:59 AM +0000
Entry Last Modified: 2021-10-11 01:41:31 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-08-06

Goal

- Extract the Biobrick plasmids from the distribution kit

Materials

- Thermoscientific GeneJET plasmid mini prep kit
- MiliQ

Protocol

The protocol of the Thermoscientific GeneJET plasmid mini prep kit was followed to extract the Biobrick plasmids.

Week 4 - Gel electrophoresis check

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:55:13 AM +0000
Entry Last Modified: 2021-10-11 01:08:24 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 2/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-02

Goal

Digest the isolated plasmids as preparation for the gelelectrophoresis tomorrow to see if the construct is correct.

Materials

- Restriction enzyme 1
- Restriction enzyme 2
- Cutsmart buffer
- MiliQ

Protocol

- Get the isolated plasmid from Lisa and Iggy
- The concentration of the plasmids and the digestion solutions are shown below:

Table1

	A	B	C	D	E	F	G	H
1	Enzyme 1	0.5		Plasmid ID	Plasmid concentration	Plasmid	water	ID
2	Enzyme 2	0.5		1	53.3	1.8761726079	6.1238273921	P1831-GFP-red
3	cutsmart buffer	1		2	17.7	5.6497175141	2.3502824859	P1831-mCherry-red
4	MiniQ	8-X		3	30.4	3.2894736842	4.7105263158	P1831-mCherry-yellow
5	Plamsid	X	100ng in total	4	13.3	7.5187969925	0.4812030075	P1831-mCherry-yellow
6				5	29.4	3.4013605442	4.5986394558	P1831-mCherry-black
7				6	29.1	3.4364261168	4.5635738832	P1831-mCherry-black
8				7	16.8	5.9523809524	2.0476190476	P1831-GFP-black
9				8	19.6	5.1020408163	2.8979591837	P1831-GFP-black
10				9	9.3	8	0	P1831-GFP-yellow
11				10	49.8	2.0080321285	5.9919678715	P1831-GFP-yellow
12					71.1	1.4064697609	6.5935302391	pJUMP29
13					47	2.1276595745	5.8723404255	pJUMP46
14					27.3	3.663003663	4.336996337	pJUMP48
15					71.2	1.404494382	6.595505618	pJUMP49
16					63.5	1.5748031496	6.4251968504	Control
17					97.2	1.0288065844	6.9711934156	pJUMP47
18					10.8	8	0	psbc1

- digestion solution were incubate in 37 degree for 1.5h
- Heatshock at 80 degree for 30min to kill the enzymes
- Store the DNA in the fridge

TUESDAY, 3/8/2021

Week 4 - Gel electrophoresis check (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-03

Goal

- Perform a gelelectrophoresis to check if the plasmid construct is correct of week 3 ligation promoter::GFP and promoter::mCherry
- Also te pJUMP plasmid stock was varified if they were correct.

Materials

- 1% Agarose

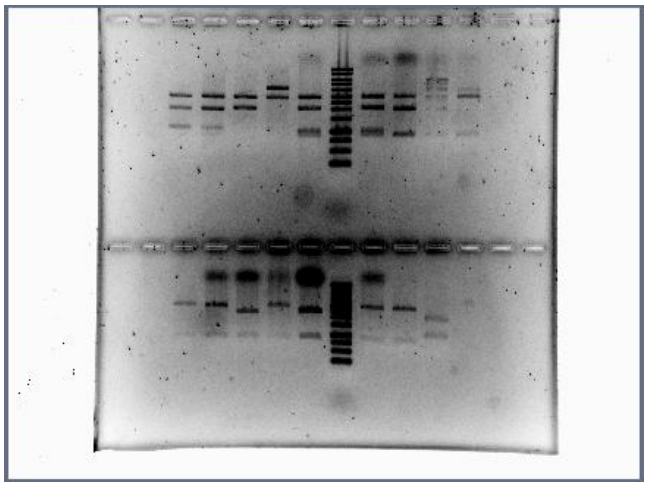
- 1% TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

Protocol

Of the samples 9 ul was loaded with 1 ul of the 10x loading dye in the lanes of the gel and 6 ul of the DNA Ladder. The gel runned at 100V for 30 minutes. Thereafter, the gel was stained in ethidiumbromide for 25 minutes.

Results

20210803 sl.jpg



First time running the gel didn't goes well, as all the band were not correct.

Week 4 - Plasmid assembly

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:57:15 AM +0000
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Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 2/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-02

Goal

- Assemble mCherry and ccdB behind a promoter in pJUMP49 by ligation.

Materials

- T4 ligase buffer
- T4 ligase
- MiliQ
- Genes
  - mCherry
  - ccdB
- Promoters
  - P2549
  - p1303
  - p908
  - pBad
  - pTet
- Plasmids
  - pJUMP49
  - pJUMP46

protocol

Ligate plasmids of:

Table1

	A	B	C	D	E
1	Backbone	promoter	gene		
2	PJUMP49	P2549	mCherry		
3	PJUMP49	P1303	mCherry		
4	PJUMP49	P908	mCherry		
5	PJUMP49	Pbad	mCherry		
6	PJUMP49	Ptet	mCherry		
7	PJUMP46	Pbad	ccdB		
8	PJUMP46	Ptet	ccdB		
9					
10					
11					

Ligation reaction:

Table3

	A	B
1	Insert	5 ul
2	Insert	5 ul
3	Vector	5 ul
4	T4 ligase buffer	2 ul
5	T4 ligase	1 ul
6	MiliQ	2 ul
7	**Total**	**20 ul**

Ligation reaction was performed for 2H at room termperture.

THURSDAY, 5/8/2021

Plasmid assembly (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-06

Goal

- More different promoters will be ligated in front of mCherry to use for the plate reader assay that is planned for this friday.

Materials

- pJUMP49 plasmid
- Promoters
  - p1
  - p21
  - p162
  - p387
  - p623
  - p1487
- Gene mCherry
- T4 ligase
- T4 ligation buffer

Protcol

Ligate plasmids of:

Table2

	A	B	C
1	pJUMP49	P1	mCherry
2	pJUMP49	P21	mCherry
3	pJUMP49	P162	mCherry
4	pJUMP49	P387	mCherry
5	pJUMP49	P623	mCherry
6	pJUMP49	P1487	mCherry

Result

First tranformation on kanamycin failed, as no red cultures had grown the next day.

FRIDAY, 6/8/2021

Plasmid assembly (3)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-06

Goal

- Transform the ligated constructs made on 2020-08-02

Materials

- LB agar medium
- Created constructs on 2020-08-02
- Competent cells
- Spectinomycin
- Agar plates

Protocol

The transformation is performed according to the protocol: https://international.neb.com/protocols/2012/05/21/transformation-protocol

After transformation the transformants are plated on agar plates with spectinomycin and grown over the weekend

Result

The Promoter::mCherry was successful and the mCherry constructs will be used today for the plate reader assay.

Week 4 - Plate reader

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:57:25 AM +0000
Entry Last Modified: 2021-10-11 12:03:29 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-06

Goal

- Performing a plate reader with GFP and mCherry constructs.

Materials

- Plate reader machine
- Plasmids
  - pJUMP26
  - pJUMP29
  - pJUMP49
- Promoters
  - p908
  - p1303
  - p2549
- co-transformed plasmids pJUMP26 and pJUMP49.

Protocol

The OD value read from the spectumphotometer:

Table1

	A	B
1	Construct	OD value
2	26_49	0.685
3	26	1.115
4	49	0.906
5	29	0.727
6	P908	1.392
7	P1303	0.914
8	P2549	1.013
9		
10		

Plate reader layout:

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	49-RFP	/2	/4	/8	/16	/32	P908-mCherry					
B	49-RFP						P908-mCherry					
C	26-GFP						P1303-mCherry					
D	26-GFP						P1303-mCherry					
E	26_49-GFP+RFP						P2549-mCherry					
F	26_49-GFP+RFP						P2549-mCherry					
G	29-GFP						Blank	Blank	Blank	Blank	Blank	Blank
H	29-GFP											

- Put 200mL of bacteria solution in the 1st and 7th columns.
- Add 100mL of LB medium to all the rest wells
- Transfer 100mL of column 1 to column 2.
- Pipet up and down >3 times
- Transfer 100mL of column 2 to column 3 and so on

Result

OD 600

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.4564	0.2872	0.1968	0.1503	0.1256	0.1111	0.7508	0.4357	0.273	0.1878	0.1467	0.1208
B	0.4901	0.2968	0.2041	0.1529	0.1309	0.1142	0.7557	0.4543	0.2903	0.1992	0.1519	0.1316
C	0.4405	0.2664	0.1906	0.1474	0.1257	0.1121	0.487	0.288	0.1965	0.1499	0.1256	0.1113
D	0.4327	0.2713	0.1911	0.1445	0.1232	0.11	0.497	0.3055	0.2038	0.1515	0.1265	0.1125
E	0.4022	0.2365	0.1721	0.1392	0.1198	0.1089	0.5042	0.3078	0.2104	0.1559	0.1281	0.113
F	0.373	0.2407	0.1743	0.1349	0.1195	0.1018	0.5197	0.324	0.2183	0.161	0.1294	0.1145
G	0.3943	0.2598	0.1805	0.1402	0.1194	0.1115	0.0964	0.0979	0.0983	0.0986	0.0988	0.0993
H	0.3769	0.2471	0.1757	0.1384	0.1226	0.1078						

sfGFP

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	3254	3185	3179	3222	3269	3280	3652	3524	3272	3095	3324	3091
B	3348	3091	3195	3174	3239	3261	3581	3351	3310	3306	3239	3185
C	49686	24652	15258	9285	6594	4993	3398	3175	3201	3243	3228	3179
D	51677	25781	14949	9594	6465	4980	3389	3277	3243	3222	3206	3238
E	25713	13623	8803	6168	4726	4001	3143	3133	3173	3199	3224	3180
F	24899	14366	8596	6350	4794	4278	3098	3229	3243	3248	3241	3171
G	51024	27104	15135	9353	6527	4971	2772	3225	3256	3095	3185	3171
H	49106	26055	15331	9556	6412	4943						

RFP

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	48108	23755	13304	7560	4231	2454	360	394	424	428	463	465
B	48735	23102	13177	7412	4194	2469	323	444	449	466	561	456
C	379	364	426	464	507	532	317	388	432	466	454	469
D	284	391	441	460	483	493	313	396	445	444	475	461
E	25595	12901	7934	4652	2574	1510	3113	1797	1273	936	734	615
F	24975	13550	7886	4625	2596	1699	3145	1848	1298	967	746	656
G	269	381	449	436	503	474	423	477	499	477	493	490
H	258	356	433	458	529	517						

Week 4 - Varify plasmid construct and plate co-transformants

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:53:37 AM +0000
Entry Last Modified: 2021-10-11 10:06:27 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 5/8/2021

Project: Lab Notebook
Authors: Hoda Ekhasi
Created at: 2021-08-05

Goal

- Perform a digestion of isolated primer **OF WHAAAAAT??** to varify if the construct with gel electrophoresis.
- co-tranformation colonies where prepared to be plated. **????**

Materials

- EcoRI
- PstI
- 10x buffer 2.1
- MiliQ
- 10x Loading buffer
- 1 Kb DNA Ladder
- 1% Agarose
- 1% TAE buffer
- Ethidiumbromide
- LB medium
- LB agar plates

Protocol

Digestion of the plasmids was performed using the protocol bellow:

Table6

	A	B	C
1	Components	1 reaction	20 reactions
2	Plasmid DNA	7	x
3	10x Buffer 2.1	1.5	30
4	EcoRI	0.5	10
5	PstI	0.5	10
6	MiliQ	5.5	110
7	Total	15	x

After making this mix, put the tubes in 37 °C incubator for 1 hour.

Gelelectrophoresis

After 1 hour, I did gel electrophoresis to check plasmids.
To 9 ul of the samples 1 ul of Loading dye was used to pipet in the lanes and 6 ul of the DNA Ladder was used. Let the gel run for 30 minutes on 100V. Thereafter, stain the gel in ethidiumbromide for 25 minutes.

Gelelectrophoresis legenda:

image1 (3).jpeg

Plating of co-transformants

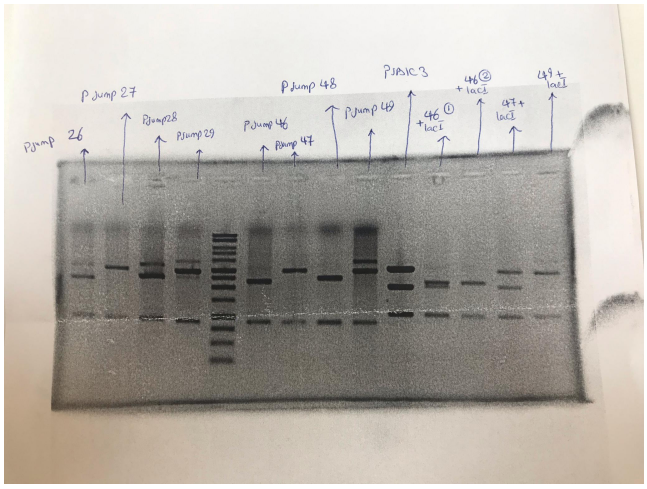
Furthermore, concentrations of different co-tranformation colonies where prepared to be plated, and put in incubater.

Method: added 1 colony to 10 microliter LB. 5 microliter of them were plated.
added 1 mililiter LB to the same tube and put them in incubator.

The co-transformation plates were placed in the fridge.

Result

image0 (6).jpeg



Week 5 - Co-transformation plate reader assay

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:37:16 AM +0000
Entry Last Modified: 2021-10-11 09:22:05 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 13/8/2021

Project: Lab Notebook
Authors: Ehsan Razaghi Siahroudi, Siheng Li
Created at: 2021-08-13

Goal

- Perform a plate reader assay of the co-transformation of the constructs pJUMP::GFP, pJUMP::LacI, and pJUMP::RFP

Material

- plasmids
  - pJUMP26
  - pJUMP27
  - pJUMP28
  - pJUMP29
  - pJUMP46
  - pJUMP47
  - pJUMP48
- Genes
  - sfGFP
  - RFP
- LB liquid medium

Protocol

- To perform the plate reader assay the protol of the OD to CFU calibration was used.

See which Ori's compatibility works best with co-transformation of the following sixteen pJUMP plasmid combinations:

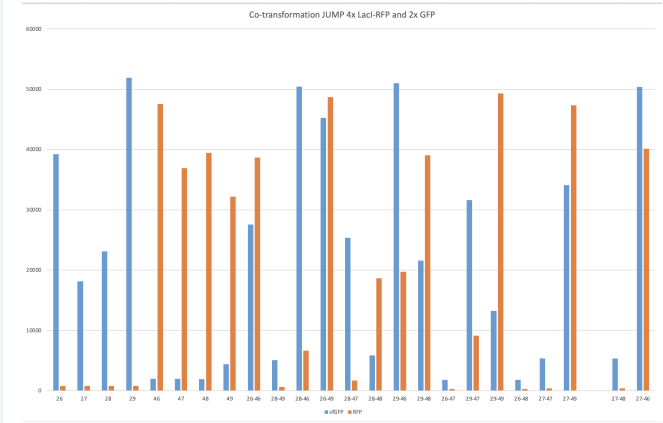
Table1

	A
1	26-46
2	28-49
3	28-46
4	26-49
5	28-47
6	28-48
7	29-46
8	29-48
9	26-47
10	29-47
11	29-49
12	26-48
13	27-47
14	27-49
15	
16	27-46

After co-transformation on Tuesday with JUMP 2x GFP and JUMP4x LacI+RFP, we had platereader measurements today.

Result plate reader

image.png



platereader results co-transformation 13-8.xlsx

It seems that pJUMP26 and pJUMP49 have the best compatible Ori's and therefore the Oris p15A and pBR322/ROB are most optimal to sustain in the cell.

Week 5 - Assembly of LacI infront of toxins

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:39:14 AM +0000
Entry Last Modified: 2021-10-05 12:06:26 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 10/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-10

Goal

- Assemble LacI infront of toxins and GFP in the pJUMP49 backbone.

Materials

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

Protocol

Target:

Table1

	A	B	C	D	E
1	pJUMP49	lacI	sok		
2	pJUMP49	lacI	MazE		
3	pJUMP49	lacI	RelB		
4	pJUMP49	lacI	ccdA		
5	pJUMP49	lacI	mCherry		
6	pJUMP49	lacI	sfGFP	use 49-RFP	
7	pJUMP49	lacI	GFP	use 49-RFP	
8	pJUMP49	pBad	sfGFP	use 49-RFP	
9	pJUMP49	pBad	GFP	use 49-RFP	
10					
11					
12					
13					

Restriction reaction performed as followed:

Table2

	A	B	C	D	E
1	Plasmid ID	Plasmid concentration	Plasmid	water	bp
2	pJUMP49	27.3	3.663003663	4.336996337	2964
3	pJUMP-49-RFP	24.7	4.048582996	3.951417004	2964
4	lacI	323.4	0.3092145949	7.6907854051	1293
5	pBad	575	0.1739130435	7.8260869565	1233
6	MazE	39.36	2.5406504065	5.4593495935	398
7	ccdA	22.9	4.3668122271	3.6331877729	368
8	RelB	36.8	2.7173913043	5.2826086957	389
9	sok	37.5	2.6666666667	5.3333333333	362
10	mCherry	24.5	4.0816326531	3.9183673469	863
11	sfGFP	84.2	1.1876484561	6.8123515439	711
12	GFP	27.9	3.5842293907	4.4157706093	869
13					
14		3.535			

Restriction reaction was performed for 1,5H at 37 °C. Next, the digestion was stopped by heatinactivating the enzymes at 85 °C.

Then after digestion, the LacI can be ligated in front of the toxins and GFP in the pJUMP49 plasmid. 1 ul of T4 ligase and 2 ul of T4 ligase buffer were added to the samples.

Table3

	A	B	C	D	E	F	G	H
1	Plasmid backbone	gene1	gene2	water				
2	2	4.2857142857	1.221	0.9932857143	pJUMP49	lacI	sok	
3	2	4.2857142857	1.343	0.8712857143	pJUMP49	lacI	MazE	
4	2	4.2857142857	1.221	0.9932857143	pJUMP49	lacI	RelB	
5	2	4.2857142857	1.343	0.8712857143	pJUMP49	lacI	ccdA	
6	2	4.2857142857	2.912	-0.6977142857	pJUMP49	lacI	mCherry	
7	*2*	*4.2857142857*	*2.399*	*-0.1847142857*	*pJUMP49-RFP*	*lacI*	*sfGFP*	
8	*2*	*4.2857142857*	*2.932*	*-0.7177142857*	*pJUMP49-RFP*	*lacI*	*GFP*	
9	*2*	*4.16*	*2.399*	*-0.059*	*pJUMP49-RFP*	*pBad*	*sfGFP*	
10	*2*	*4.16*	*2.932*	*-0.592*	*pJUMP49-RFP*	*pBad*	*GFP*	
11	1.8482852883	3.960611332	2.6911033797	-0	pJUMP49	lacI	mCherry	9.1977142857
12	1.9574622078	4.1945618739	2.3479759183	-0	pJUMP49-RFP	lacI	sfGFP	8.6847142857
13	1.8442749985	3.960611332	2.7037071477	-0.0085934782	pJUMP49-RFP	lacI	GFP	9.2177142857
14	1.9862133427	4.1313237528	2.3824629045	0	pJUMP49-RFP	pBad	sfGFP	8.559
15	1.8697756269	3.889133304	2.7410910691	0	pJUMP49-RFP	pBad	GFP	9.092
16								

Week 5 - Construct pJUMP49:promoter:Antitoxin

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:38:16 AM +0000
Entry Last Modified: 2021-10-11 12:12:37 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 10/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-10

Goal for this week

Last week the fusion of the antitoxins infront of a constitutive promoter did not succeed. Therefore, the construction will tried to be assemled this week.

As soon as I have the correct constructs, I will start to double transform the following:

pJUMP27:pBAD:Toxin and pJUMP49:promoter:Antitoxin.
Since the second ones were not correct, I could not do this today. We will see if we can start this tomorrow.

WEDNESDAY, 11/8/2021

Week 5 - Construct pJUMP49:promoter:Antitoxin (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-11

Goal

- Isolate new plasmids, since the other ones were not correct.

Material

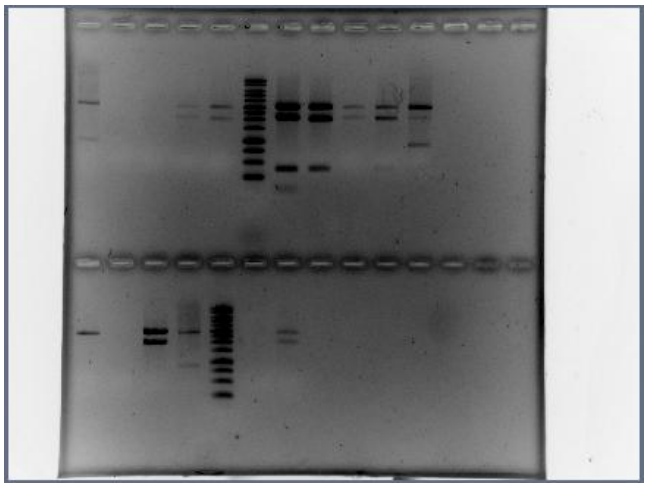
- Restriction enzymes
  - EcoRI
  - PstI
- 10x restriction buffer
- 1% Agarose
- 1% TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

Protocol

I restricted them using EcoRI and PstI for 2 hours, then put them on a gel to run again on 100V for 30 minutes.
If the bands are correct, I will cotransform them into TOP10 with pBAD and their respective toxins.

Result

SK 2021-08-11 17hr 54min.jpg



Most of the bands were not correct or not visible on the gel. Therefore, I will make axenic cultures, since I suspect that it might be due to contamination. Next time, I will try to put single colonies from these plates in liquid medium over the weekend for isolation on Monday.

FRIDAY, 13/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-13

Goal

- Grow liquid cultures of the pJUMP49::constitutive promoter::Antitoxin plates over the weekend.

Material

- LB liquid medium
- 50ml tube

Protocol

Pick the JUMP49:constitutive promoter:Antitoxin from the LB agar plates with a tooth pick to inoculate in LB liquid culture. Grow the culture over the weekend.

Week 5 - Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:54:28 AM +0000
Entry Last Modified: 2021-10-06 02:23:12 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-06

Goal

- Create the DOPL LOCK system by: Ligate the pBAD toxin fusions (Creating pBAD-Toxin fusions entry) to the constitutive antitoxin fusions (Creating constitutive AT fusions entry.

Materials

- T4 ligase buffer
- T4 ligase
- MiliQ
- Constructs pBAD toxin & constitutive AT
- DH5α

Protocol

Next, we will start making the full system. We will do this in several ways. Firstly, we will ligate the pBAD toxin fusions to the constitutive-AT fusions and clone them into DH5α.

Ligation reaction:

Table1

	Ligation reaction	microliter
1	Insert	5 ul
2	Insert	5 ul
3	Vector	5 ul
4	T4 ligase buffer	2 ul
5	T4 ligase	1 ul
6	MiliQ	2 ul
7	**Total**	**20 ul**

Ligation reaction was performed for 2H at room temperature.

Extra

Further, as soon as we have access to the LacI promoter, we will clone this into the system. For that end, I will also try to make AT:pBAD:T plasmids, so all we will have to do is clone the LacI in there.

Today, I started cultures of pBAD-T and const:AT to isolate plasmids from, so that I can immediately start digesting them and then ligate them into each other, perhaps even transform them on Monday.

Week 5 - Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:38:08 AM +0000
Entry Last Modified: 2021-10-11 02:20:51 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

SUNDAY, 8/8/2021

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

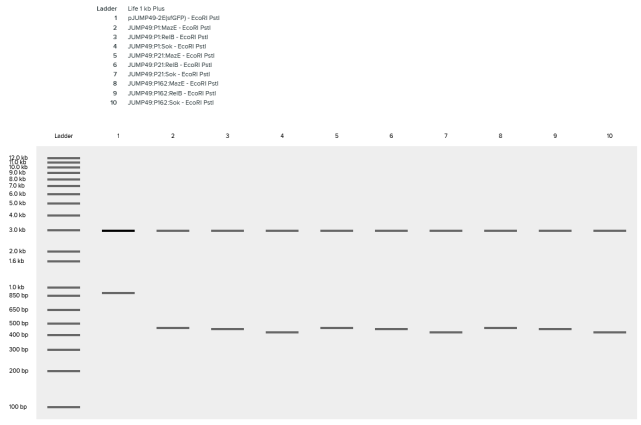
Goal

- Make virtual digestions of all the constitutive:antitoxin plasmids for tomorrow.
- Also a virtual digestion of Con:AT:pBAD:T plasmids is made.

Protocol

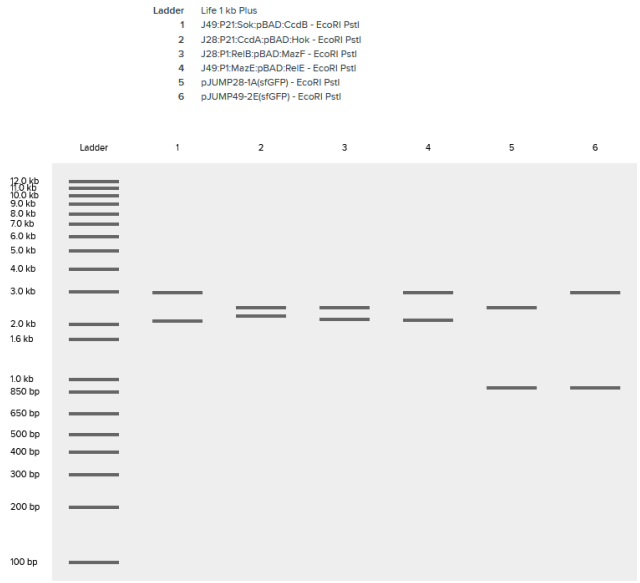
What we should get for the constitutive:antitoxin plasmids:

afbeelding.png



Expected digest of the Con:AT:pBAD:T plasmids;

afbeelding.png



MONDAY, 9/8/2021

Week 5 - Creating DOPL Lock (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-10

Today, I will restrict a lot of plasmids.

First of all, I will do restrictions to check if the plasmids we are isolating today are the correct size.
Next, I will do a lot of restrictions to create the new system.
I will fuse the con:at to pBAD:t. Further, I will add p2549:mcherry into the pJUMP plasmids.
Also, I will ligate LacP in front of the AT's.

However, we do not have the amount of SpeI required to do this, therefore, this will be put on hold until we have more.

TUESDAY, 10/8/2021

Week 5 - Creating DOPL Lock (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-10

Goal:

- Do restrictions to check if the plasmids we are isolating today are the correct size.
- Do a lot of restrictions and ligations to create the new system, if the gel electrophoresis is correct.
    - Fuse the con:at to pBAD:t. Further, I will add p2549:mcherry into the pJUMP plasmids.

Also, I will ligate LacP in front of the AT's.

Materials

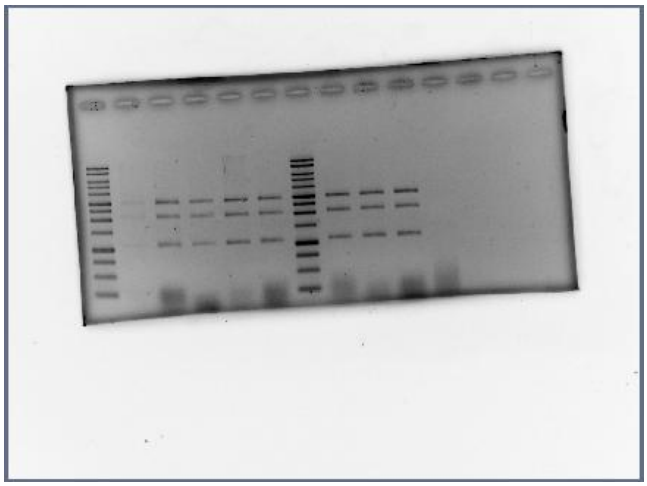
- 10x Loading dye
- 1 Kb DNA Ladder
- 1% TAE buffer
- 1% Agarose gel

Protocol

1 ul Of Loading dye was added to 9 ul sample and loaded in the gel. Also 6 ul of DNA Ladder was added to the gel. The gel was runned at 100V for 30 minutes and stained in ethidiumbromide for 25 minutes.

Result

20210810 IN restrictie const_at.jpg



Additionally, it seems that the con:at constructs were not correct. They showed 3 bands on the gel electrophoresis, one at 3 kb, one at 2 kb and one at 1 kb. They had no band of around 500 bp, which would indicate the con:at.
Therefore, I took new colonies from the plates of the transformation and put them in liquid LB with spectinomycin to grow overnight, so that I can isolate the plasmids tomorrow and run a new digestion.

Extra

There is a small break in this line of experiments due to the SpeI shortage, and uncertainty about the constructs.
However, we have decided to make a small change in our plans. We will start on Monday with isolating the constitutive:AT, as well as the constitutive:T.
Then, we can fuse these together and transform the whole system with constitutive promoters, as well as doing a cotransformation with only one pair.
Next, we can do a horizontal gene transfer experiment with these cells: let them grow in liquid overnight, then autoclave them and add the rest of the cells to *Bacillus subtilis*, and then check if there is any plasmid uptake.
My hypothesis is that it will only grow if the antitoxin plasmid is added.

Week 5 - p2549::mCherry in p40's

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:38:24 AM +0000
Entry Last Modified: 2021-10-11 12:28:14 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 10/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-10

Goal

- Perform a restriction and ligation of the p249:mCherry

Materials

- Restriction enzymes:
  - EcoRI
  - PstI
- NEBuffer 2.1
- MiliQ
- T4 DNA ligase
- T4 ligase buffer

Protocol

I restricted and ligated these constructs today by following Bas' trick:
restrict the new backbone and the p2549:mCherry construct in the same eppendorf tube, and then ligate.
The restriction and ligation were done according to:

Table2

	A	B	C	D	E
1	PstI	0.5		T4 DNA ligase	0.5
2	EcoRI	0.5		T4 buffer	1.2
3	NEB 2.1	1		Restriction mix	10
4	DNA	1		MiliQ	0.3
5	MiliQ	7.5			
6	**Total**	10		**Total**	12

The restriction was done at 37 °C for 2 hours and the ligation was done for 2 hours at room temperature, then they were put in the freezer overnight.

WEDNESDAY, 11/8/2021

Week 5 - p2549::mCherry in p40's (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-11

Goal

- Transformation of TOP10 bacterial strain with constructed plasmids on 2020-08-10.

Materials

- LB medium

Protocol

I transformed TOP10 with these ligations following standard protocol.
I let them incubate on ice with the plasmids for 70 minutes, because I was also doing a plasmid isolation. Then, I heatshocked for 90 s at 42 °C.
They were incubated at 37 °C for (12:47 till 13:52).

Then, they were plated and grown for 1.5 day at 37 °C.

FRIDAY, 13/8/2021

p2549-mCherry in p40's (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-13

The plates of the transformation of 2020-08-11 have grown colonies.

Goal

- Transfer the grown colonies in duplo to liquid to grow a liquid cultures.

Materials

- LB medium liquid.
- Toothpicks
- Agar plates

Protocol

The colonies were picked under sterile circumstances with tooth picks to transfer the colonies to the liquid cultures. The liquid cultures are grown over the weekend at 37 °C.
Monday we will be able to isolate a good amount of plasmids and check if they are correct. If they are correct, we can then start the fluorescence experiments with them.

Week 5 - Plate reader

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:39:41 AM +0000
Entry Last Modified: 2021-10-11 11:55:51 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 9/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-09

Goal

- Perform a plate reader assay to see what plasmids are most optimal to co-transform

Materials

- Plate reader assay
- Plasmids
  - pJUMP26
  - pJUMP27
  - pJUMP29
  - pJUMP46
  - pJUMP47
  - pJUMP48
  - pJUMP49
  - pJUMP50
  - pJUMP51
  - pJUMP53
- Genes
  - sfGFP
  - RFP

Protocol

Plate reader layout:

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	26-49	26-50	26-51	26-52	26-53	26-54	27-49	27-49	27-49	27-49	27-49	27-49
B	26-49	26-50	26-51	26-52	26-53	26-54	29-46	29-46	29-46	29-46	29-46	29-46
C	26-49	26-50	26-51	26-52	26-53	26-54	29-46	29-46	29-46	29-46	29-46	29-46
D	27-46	27-47	27-48	27-49	27-50	27-51	29-46	29-46	29-46	29-46	29-46	29-46
E	27-46	27-47	27-48	27-49	27-50	27-51						
F	27-46	27-47	27-48	27-49	27-50	27-51						
G	27-49	27-50	27-51	27-52	27-53	27-54						
H	27-49	27-50	27-51	27-52	27-53	27-54						

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A	29-47	29-47	29-47	29-47	29-47	29-47	26	26	26	26	26	26
B	29-47	29-47	29-47	29-47	29-47	29-47	27	27	27	27	27	27
C	29-47	29-47	29-47	29-47	29-47	29-47	27	27	27	27	27	27
D	29-49	29-49	29-49	29-49	29-49	29-49	27	27	27	27	27	27
E	29-49	29-49	29-49	29-49	29-49	29-49						
F	29-49	29-49	29-49	29-49	29-49	29-49						
G	26	26	26	26	26	26						
H	26	26	26	26	26	26						

Well3

	1	2	3	4	5	6	7	8	9	10	11	12
A	29	29	29	29	29	29	49	49	49	49	49	49
B	29	29	29	29	29	29						
C	29	29	29	29	29	29						
D	47	47	47	47	47	47						
E	47	47	47	47	47	47						
F	47	47	47	47	47	47						
G	49	49	49	49	49	49						
H	49	49	49	49	49	49						

Results of the plate reader are stored in a CSV file and will be analyzed.

WEDNESDAY, 11/8/2021

Week 5 - Plate reader (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-11

Goal

- Test the inducibility of the pBad infront of the toxins with arabinose in the plate reader assay.

Materials

- Toxins
  - MaxF
  - Hok
  - RelE
- Bacerial strain TOP10
- Arabinose concentrations:
  - 1%
  - 0.001%
  - 0.04%
  - 0.2%

Protocol

pBad toxin arabinose assay target layout:

Well4

	1	2	3	4	5	6	7	8	9	10	11	12
A	5%-MazF	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	5%	1%	0.2%
B	5%-MazF	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.04%	0.008%	0.0016%
C	5%-Hok	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.00032	0.000064%	0
D	5%-Hok	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	5%	1%	0.2%
E	5%-RelE	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.04%	0.008%	0.0016%
F	5%-RelE	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.00032	0.000064%	0
G	5%-Top10	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0			
H	5%-Top10	1%	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0			

Arabinose (XuL Ara + XuL LB medium spectinomycin)

Well5

	1	2	3	4	5	6	7	8	9	10	11	12
A	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	125uL +125uL	0uL+ 200uL	0uL+ 200uL
B	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL
C	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL
D	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	125uL +125uL	0uL+ 200uL	0uL+ 200uL
E	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL
F	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL
G	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL			
H	125uL +125uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL	0uL+ 200uL			

Bacteria TOP10

Well6

	1	2	3	4	5	6	7	8	9	10	11	12
A	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF			
B	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF	5uL-MazF			
C	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok			
D	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok	5uL-Hok			
E	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE			
F	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE	5uL-RelE			
G	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10			
H	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10	5uL-Top10			

Results of the plate reader are stored in a CSV file and will be analyzed.

THURSDAY, 12/8/2021

Plate reader (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-12

Goal

- Perform a plate reader assay of the inducibility of pBad infront of the toxins MazF, Hok, or RelE and mCherry

Materials

- Toxins
  - MaxF
  - Hok
  - RelE
- Bacerial strain TOP10
- Arabinose concentrations:
  - 1%
  - 0.001%
  - 0.04%
  - 0.2%

Protocol

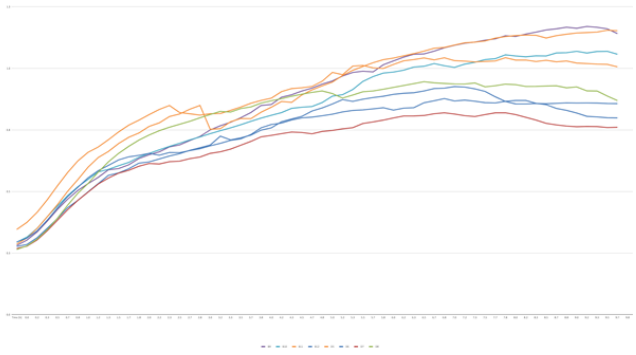
Plate reader layout:

Well7

	1	2	3	4	5	6	7	8	9	10	11	12
A	1%-MazF	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	1%-Top10	0.2%	0.04%	0.008%
B	1%-MazF	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	1%-Top10	0.2%	0.04%	0.008%
C	1%-Hok	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.0016%	0.00032	0.000064%	0
D	1%-Hok	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	0.0016%	0.00032	0.000064%	0
E	1%-RelE	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	Blank	Blank	Blank	Blank
F	1%-RelE	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0	Blank	Blank	Blank	Blank
G	1%-mCherry	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0				
H	1%-mCherry	0.2%	0.04%	0.008%	0.0016%	0.00032	0.000064%	0				

Result

Platereader 08-12.png



Here the growth is shown of the pBAD that is induced by different concentration of Arabinose. The plate reader assay was performed for 10H over night. Here in this graph you can see the bacterial growth of TOP10 containing pBAD::toxin. The bacteria is more prohibited in growth by the toxin CcdB than by the other toxins.

Week 5 - Plate reader

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:39:50 AM +0000
Entry Last Modified: 2021-10-11 12:00:42 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 13/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-13

Goal

- Perform a plate reader assay to test the growth of several pJUMP plasmids.

Materials

- Arabinose
- Plate reader assay
- pJUMP plasmids
  - 26
  - 27
  - 28
  - 29
- LB liquid medium as a blank

Protocol

Plate reader legenda:

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	26						28					
B	26						29					
C	26						29					
D	27						29					
E	27						Blank					
F	27											
G	28											
H	28											

Result is as a CSV file and will be analyzed.

Week 5 - Testing inducibility pBAD

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:38:33 AM +0000
Entry Last Modified: 2021-10-11 10:56:25 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 10/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-10

Goal

- Try induce cell-death by adding arabinose to the medium of cells growing with a pBAD-toxin fusion.

Material

- Different arabinose concentrations
  - 1%
  - 0.1%
  - 0.01%
  - 0.001%
- LB-agar
- Kanamycin
- TOP10 bacterial strain

Protocol

The constructs are the ones I made previously, pJUMP27:pBAD:toxin (RelE, Hok and MazF) during week 4 Creating pBAD-Toxin fusions experiments.

For this experiment, I made plates with dilutions of arabinose, 1%, 0.1%, 0.01% and 0.001%, since it seems that 1% arabinose in the medium should yield the maximum induction.
I did this by adding 2, 20, 200 and 2000 uL of 10% arabinose solution to 20 mL of LB-agar and plated it with kanamycin.
Then, I spotted 20 uL of the TOP10 strain with the toxins in them and let the plates dry and put them overnight in the 37 °C.

Result of pBad induction on 2021-08-10

It looks like the induction worked on all concentrations; the colonies with mCherry were all slightly red. However, there was no concentration which killed off colonies completely.
Therefore, it might be that I accidentally used DH5α instead of TOP10, which should be more vulnerable to the toxins, since it is better at protein expression.
I will make all the plates once more, this time with TOP10 for certain.

WEDNESDAY, 11/8/2021

Week 5 - Testing inducibility pBAD (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-11

Goal

- Reperform the experiment of 2021-08-10 in order to acctually induce the pBad promoter infront of the toxins with arabinose in TOP10 bacterial strain.

Materials

- See materials used on 2021-08-10

Protocol

Same protocol used as used on 2021-08-10

Result

All the plates look precisely the same. It seems that pBAD is not working in this system.

Week 5 - Transfer of P2549 mCherry to pJump29

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-09-30 09:48:09 AM +0000
Entry Last Modified: 2021-10-11 10:24:41 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 6/8/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-08-06

Goal

- To transfer the P2549_mCherry frament to pJump29

Materials

- EcoRI
- PstI
- 10X buffer
- MiliQ
- 10x ligation buffer
- T4 ligase
- DH5 α
- Kanamycin
- ThermoScientific GeneJET mini prep kit

Protocol

The following reactions scheme is used:

Table1

	A	B
1	Components	Raction (uL)
2	Jump29_sfGFP	1
3	Jump49_P2459_mCherry	5
4	10x Buffer 2.1	1
5	EcoRI	0.5
6	PstI	0.5
7	MiliQ	2
8	Total	10

After 1 h of digestion the reaction was heat inactivated at 80 °C for 20 minutes.

Ligation reaction:

Table3

	A	B
1	inactivated Restriction reaction	10 ul
2	T4 ligase buffer	2 ul
3	T4 ligase	1 ul
4	MiliQ	7 ul
5	**Total**	**20 ul**

Transformation protocol:

10 ul of the ligation reaction is transformed in DH5α and plated out on Kanamycin plates.

Result

Over the weekend the colonies had grown. Red colonies are visible on the LB agar medium, which means that the mCherry constructs are integrated in the DH5α strain.

Red colonies are picked and grown in liquid LB suplemented with spectinomicin, followed by a plasmid extraction.

Non green plasmids are picked and plasmids are isolated by SK with the ThermoScientific GeneJET mini prep kit protocol.

Week 6 - Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-04 02:18:04 PM +0000
Entry Last Modified: 2021-10-11 02:09:30 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 16/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-16

Goal

- Isolate the p2549:mCherry in the 40 JUMP plasmids and all the constitutive:toxins and the constitutive antitoxins that we have so far.
- Restriction of these plasmid and check on gel
- Additionally, I started a ligation of p162:ccdA so that we can make the full system.

Material

- Thermoscientific plasmid mini prep kit.
- MiliQ

Protocol

The plasmid isolations were done using the standard protocol of the kit.
For the restrictions of the plasmids, I used the following reactions:

Digstions were performed as follows:

Table1

	Vector DNA	Volume (uL)	C	Toxin DNA	Volume (uL)	F	AT DNA (MazE)	Volume (uL)	I	Restriction to check construct	Volume (uL)	L	pBAD restriction	Volume (uL)	O	ccdB restriction	Volume (uL)	R	S	T
1	EcoRI	1		EcoRI	1.5		XbaI	0.5		EcoRI	0.25		EcoRI	0.5		XbaI	0.5			
2	PstI	1		SpeI	1.5		PstI	0.5		PstI	0.25		SpeI	0.5		PstI	0.5			
3	NEB 2.1	2		NEB 2.1	3		NEB 2.1	1.3		NEB 2.1	0.5		NEB 2.1	0.5		NEB 2.1	0.5			
4	pSB1C3 DNA	16		162:tox	24		162: MazE	11		DNA	1		pBAD DNA	3		ccdB DNA	2.5			
5										MQ	3		MQ	0.5		MQ	1			
6	**Total**	**20**		**Total**	**30**		**Total**	**13.3**		**Total**	**5**		**Total**	**5**		**Total**	**5**			

For the ligations, I used the following schemes:

Table2

	J27:pBAD: ccdB	Volume (uL)	C	1C3:162:MazF:162:ccdB	Volume (uL)	F	1C3:162:MazE	Volume (uL)	I	J27:162:ccdA	Volume (uL)	L	M	N	O	P	Q	R	S	T
1	T4 DNA Ligase	1		T4 DNA Ligase	2.5		T4 DNA Ligase	2.5		T4 DNA Ligase	1									
2	T4 ligase buffer	2		T4 ligase buffer	3		T4 ligase buffer	3		T4 ligase buffer	2									
3	JUMP27	1		pSB1C3 restriction	4.5		pSB1C3 restriction	4.5		JUMP27	1									
4	pBAD	3		p162:MazF restriciton	10		p162:MazE restriciton	10		p162	3									
5	ccdB	5		p162:ccdB restriction	10					ccdA	5									
6	MiliQ	8								MiliQ	8									
7	**Total**	**20**		**Total**	**30**		**Total**	**20**		**Total**	**20**									

The restrictions were done for 1 hour 45 min. at 37 °C, and the ligations were all done overnight at room temperature.
Tomorrow morning, I will transform all the restrictions first thing in the morning.
Next, (probably the day after tomorrow), I will make a liquid culture of the p162:ccdA and isolate plasmids, so that I can immediately fuse the whole system together.

The scheme of the gel is as follows:

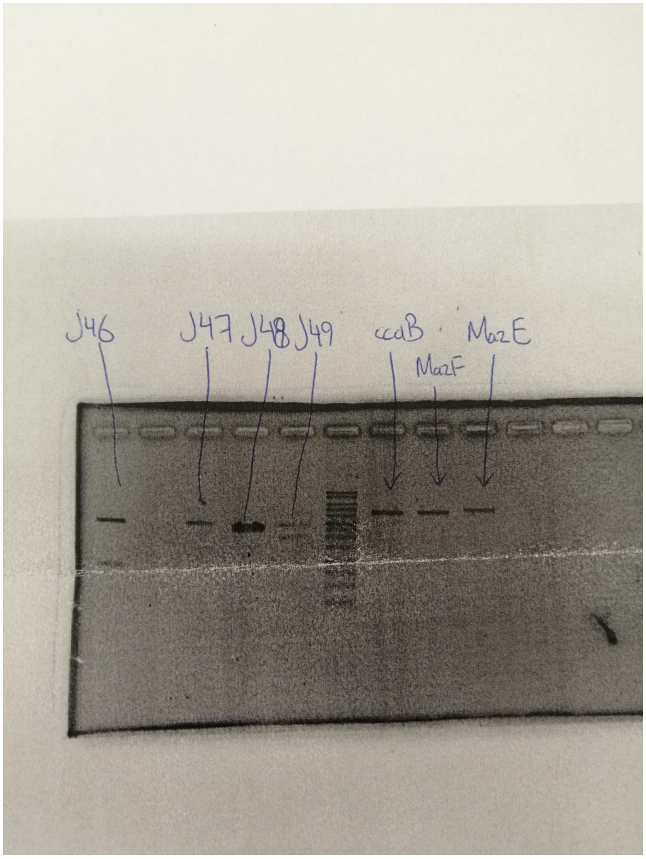
Table3

	A	B	C	D	E	F	G	H
1	1	2	3	4	5	6	7	8
2	J49:p2549:mCherry	J48:p2549:mCherry	J47:p2549:mCherry	J46:p2549:mCherry	DNA ladder	p162:ccdB	p162:MazF	p162:MazE

Result

This gel was not correct. See the gel in the picture below:

IMG_20210817_151142.jpg



TUESDAY, 17/8/2021

Week 6 - Creating DOPL LOCK (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-17

Goal

- Restriction of p2549:mCherry in the 40 JUMP plasmids and all the constitutive:toxins and the constitutive antitoxins with different plasmid isolations.
- Start with making DOPL LOCK with pBAD:AT:162:T.

  For this, I will use pBAD:MazE:p162:ccdB and pBAD:ccdA:p162:MazF
  - pBAD:ccdA and p162:MazF must be grown today to be able to make the construct

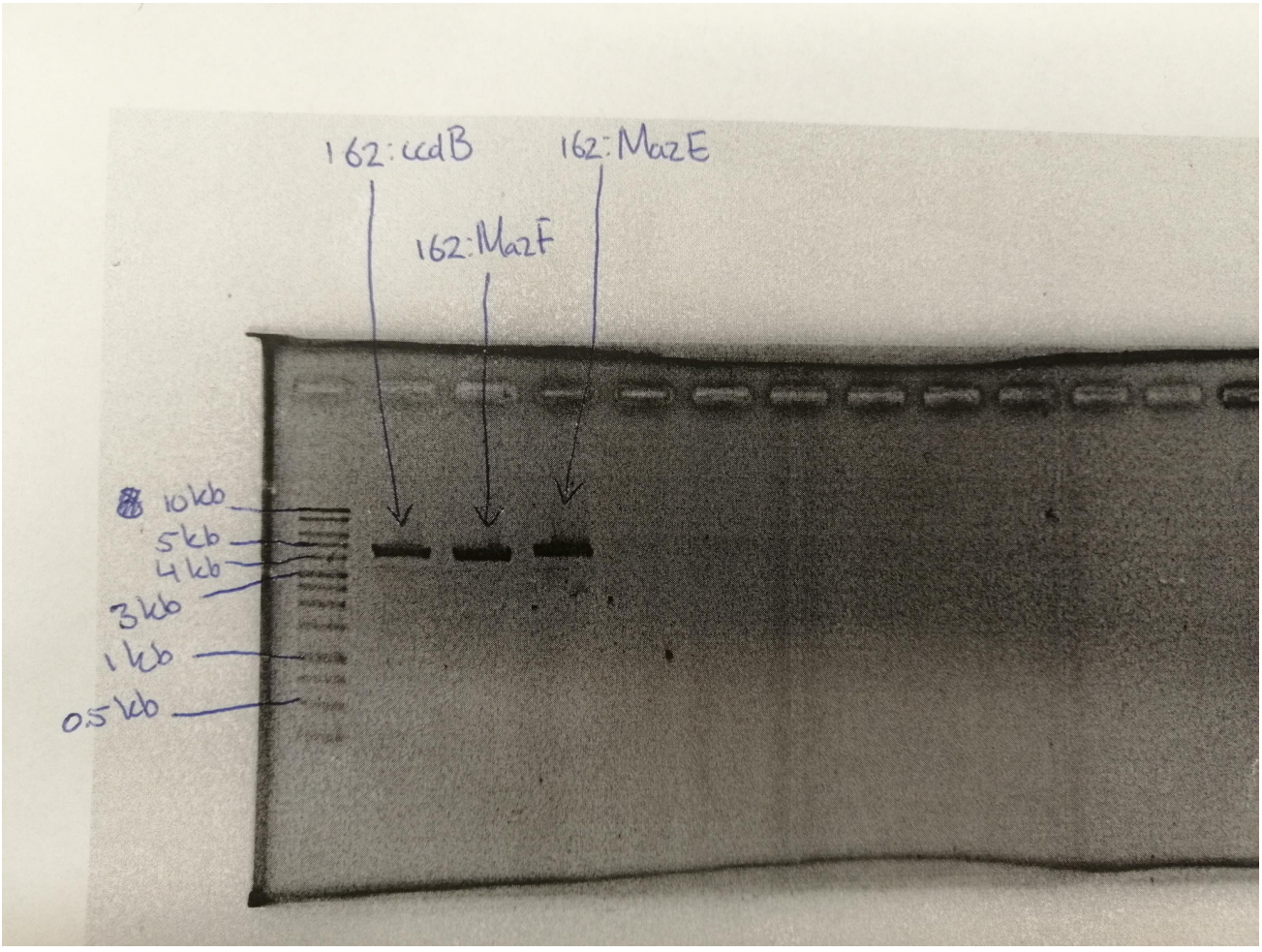
Protocol

Thermoscientific standard plasmid extraction kit protocol was used. The same restriction was performed as on 2021-08-16.

Result

However, again the gel was not correct. See the gel below:

IMG_20210817_145942.jpg



The bands should be at 3 kb and 0.5 kb. Therefore, I will assume that this is a plasmid from a contamination.
I regrew new colonies from the transformation plates, really making sure to pick colonies with a different morphology this time. I will check tomorrow if they are correct.

To start with making DOPL LOCK with pBAD:AT:162:T, the following constructs were used: pBAD:MazE:p162:ccdB and pBAD:ccdA:p162:MazF.

pBAD:ccdA and p162:MazF are currently being made and I will see tomorrow if there are colonies on the plate.
Thursday I will be able to see if the colonies contain the correct plasmids, meaning I can then start restriction and hopefully ligating and transforming, although that might only happen on Friday.

WEDNESDAY, 18/8/2021

Week 6 - Creating DOPL LOCK (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-18

Most of the overnight cultures grew, except for one of the pBAD:CcdA ones. and the ccdB plate did not look to be super correct. Therefore, I will start new restrictions for that transformation and this time I will remember to do it in 3.1.

Goal

- Start new restrictions of the isolated plasmids and check it on gel.

Material

- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide
- Thermoscientific plasmid mini prept kit.
- Restriction enzymes
    - EcoRI
    - PstI
    - SpeI
    - XbaI
- Restriction buffer
    - NEB2.1
    - Cutsmart

Protocol

Most of the overnight cultures grew, except for one of the pBAD:CcdA ones. and the ccdB plate did not look to be super correct. Therefore, I will start new restrictions for that transformation and this time I will remember to do it in 3.1 (hopefully).

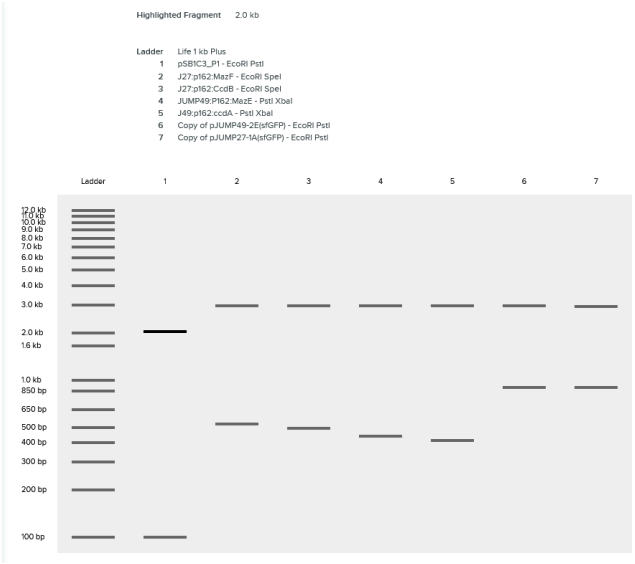
I did some restrictions on the plasmids after isolation according to the next scheme:

Table4

	pBAD:ccdB fusion	Volume (uL)	C	Toxin	Volume (uL)	F	AT 1	Volume (uL)	I	AT 2	Volume (uL)	L	Volume plasmids	Volume (uL)	O	P	Volume (uL)	R	S	T
1	EcoRI	0.5		EcoRI	0.5		XbaI	0.5		XbaI	1		EcoRI	0.5						
2	PstI	0.5		SpeI	0.5		PstI	0.5		PstI	1		PstI	0.5						
3	NEB 2.1	1		Cutsmart	1		NEB 2.1	1		NEB 2.1	2		NEB 2.1	1						
4	pBad:ccdB	8		p162:MazF /p162:ccdB	8		p162:MazE	8		p162:ccdA	16		J27/J49/pSB1C3	8						
5	**Total**	**10**		**Total**	**10**		**Total**	**10**		**Total**	**20**		**Total**	**10**						

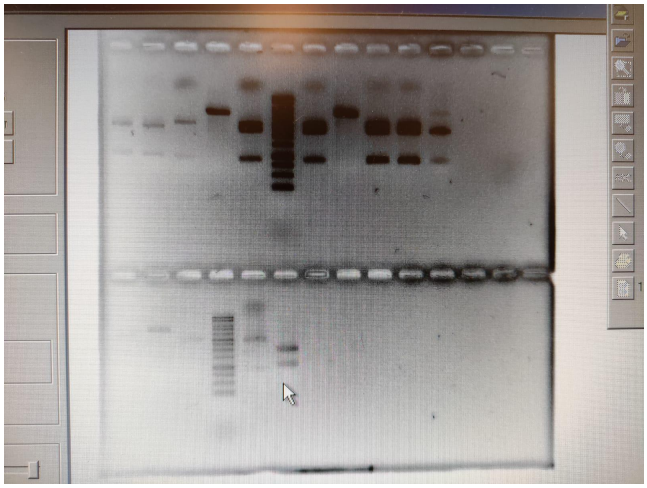
Then, I ran a gel with these restrictions. It should look like this:

afbeelding.png



Result

WhatsApp Image 2021-08-18 at 16.58.36.jpeg



All of the lanes are wrong, except for the controls.

THURSDAY, 19/8/2021

Week 6 - Creating DOPL LOCK (4)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-19

Goal

- Isolate plasmids of the transformation performed on 12-08-2021

Materials

- ThermoScientific GeneJET plasmid mini prep kit
- MiliQ

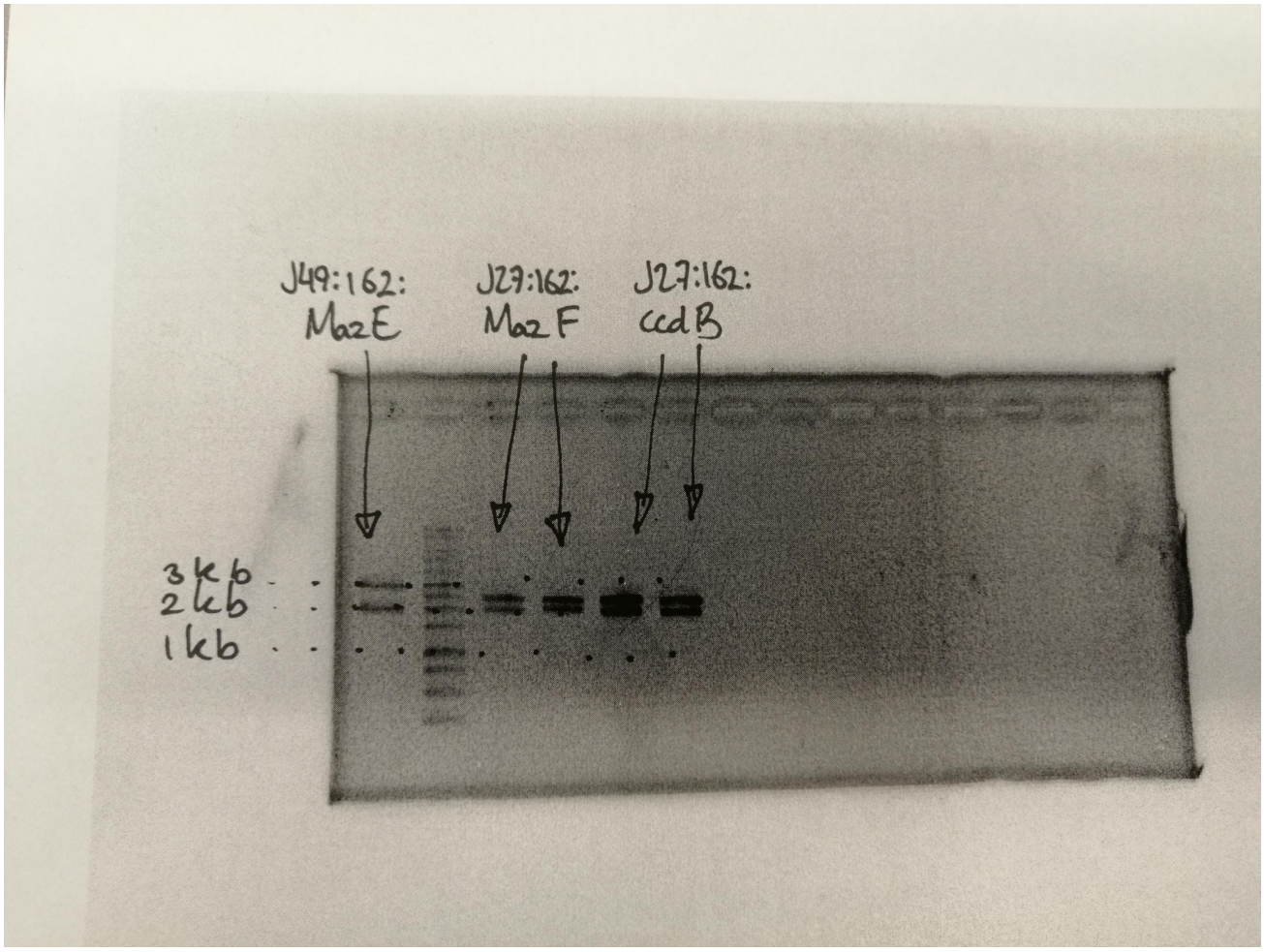
Protocol

Standard isolation protocol of the ThermoScientific GeneJET kit was used.
Today, I isolated more plasmids that were grown with the ligation transformation from 12-8.

Result

It was again not correct, so for now Bas will redo the restriction-ligations of constitutive:antitoxin, constitutive:toxin and pBAD:ccdB.

IMG_20210819_170028.jpg



The inserts should be at roughly 500 bp, since that is the size of the TA's with the promoters.
Tomorrow, I will transform the ligations Bas made.

FRIDAY, 20/8/2021

Week 6 - Creating DOPL LOCK (5)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-20

Goal

- Transform the ligations from Bas and plated them on kanamycin.
- Isolated the pSB1C3:pBAD:ccdB that I grew yesterday, as well as J46, J46, J48, J49:P2594:mCherry from Siheng.
- Check pSB1C3:pBAD:ccdB construct on gel.

Materials

- 1% Agarose gel
- 1& TAE buffer

- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide
- Thermoscientific GeneJET plasmid mini prep kit

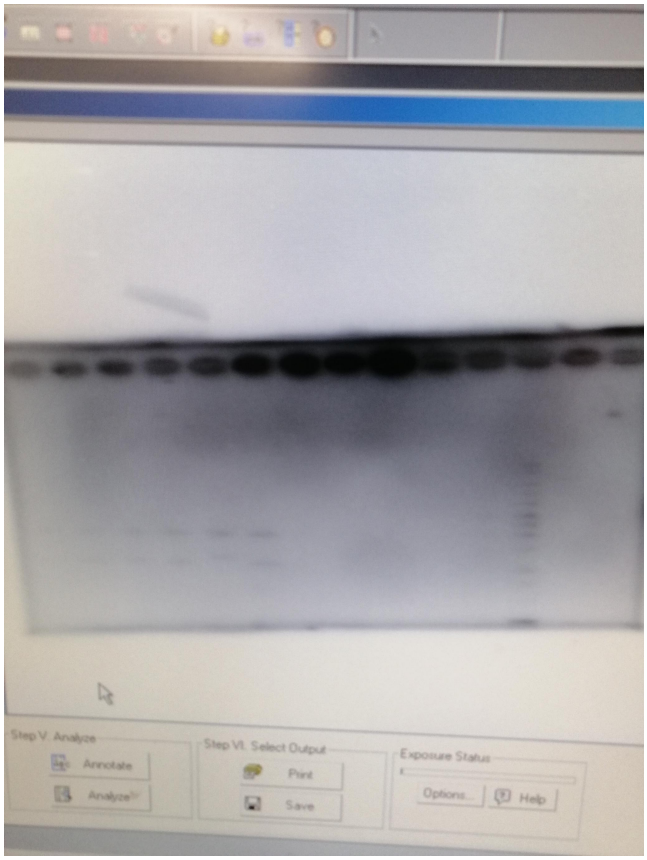
Protocol

Plasmid isolations were done according to the standard protocol of the Thermoscientific isolation kit.
I followed the usual protocol (incubate DNA 30 min, HS 90 s 42 °C, incubate 60 m 37 °C, plate, grow overnight).
The plasmids from Siheng were already checked, but I restricted mine in 5 uL, 0.25 uL restriction enzyme, 0.5 uL buffer, 3 uL MQ, 2 uL DNA.

Result

The gel looks as follows:

IMG_20210820_172744.jpg



Almost no bands were visible.

SATURDAY, 21/8/2021

week 6 - Creating DOPL LOCK (6)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar

Created at: 2021-08-21

Goal

- Check the plates of yesterday
- Make liquid cultures of Chanel's plates.

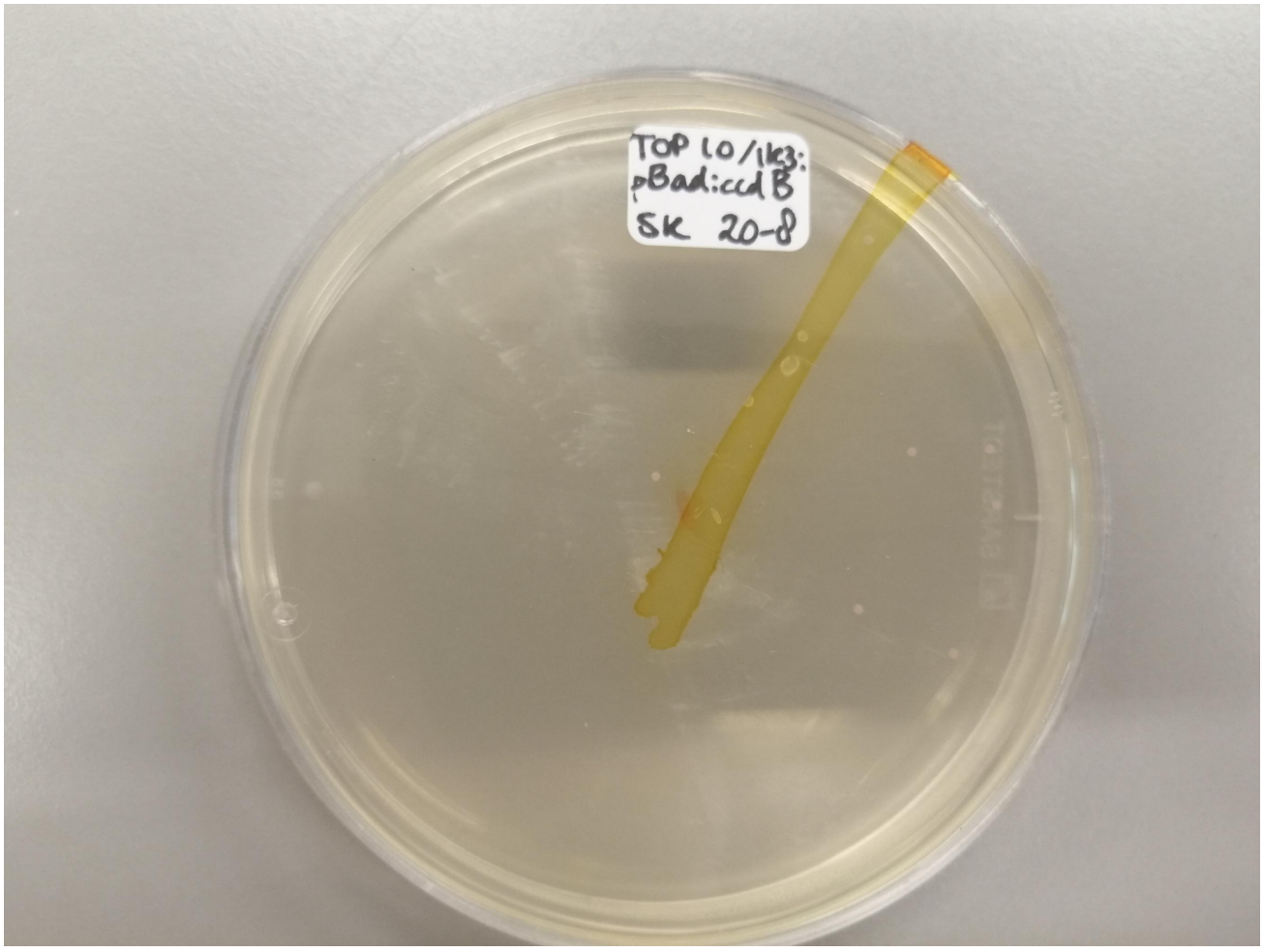
Material

- Liquid LB medium

Result

A lot of the plates contained colonies. Most were red (self-ligations), but I was able to select several other colonies for plasmid isolation tomorrow.
However, the pSB1K3:pBAD:ccdB did not have any colonies except for self-ligators. Therefore, I added 1.666 mL of 10% glucose to the plate and evaporated it, to reach a final volume of 1% glucose (w/v) to inhibit the ccdB production through tightening of the pBAD regulation. However, it might be that all the RFP-containing colonies will spread throughout the plate to become confluent and I might need to streak it again. However, my hope is that I can identify several white colonies, which I will grow in liquid containing glucose overnight for plasmid isolation as well.
This is how the plate looked before:

IMG_20210821_144119.jpg



Extra

Further, I put several of Chanel's plates in liquid culture. However, the transformation looked to be quite low efficiency, so not all of the plates had non-fluorescent colonies on them. I will check again tomorrow to see if new colonies grew, since Chanel told me beforehand that they grow extremely slow.

SUNDAY, 22/8/2021

Week 6 - Creating DOPL LOCK (7)

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

Goal

- Make liquid culture of the TOP10/pSB1K3:pBAD:ccdB
- Check on gel other isolated constructs T/AT constructs on gel.

Material

- 1% Agarose gel
- 1& TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide
- Thermoscientific GeneJET plasmid mini prep kit

Protocol

Layout ot the gel:

Table6

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	J49:pBad:MazE 1	J49:pBad:MazE 2	J49:pBad:MazE 2.1	J49:pBad:RBS:RepA:MazE 2	J49:p2547:RBS:Ntag:MazE 2	J49:pBad:RBS:Ntag:MazE 2	ladder	pSB1K3:p908:MazE1	pSB1K3:p908:MazE2	pSB1K3:p908:Sok 1	pSB1K3:p908:Sok 2			
2	pSB1K3:p908:RelB 1	pSB1K3:p908:RelB 2	pSB1K3:p908:ccdA 1	pSB1K3:p908:ccdA 2	:pSB1K3:p162:MazF 1	pSB1K3:p162:MazF 2	ladder	pSB1K3:p162:Hok 1	pSB1K3:p162:Hok 2	pSB1K3:p162:RelE 1	pSB1K3:p162:RelE 2	pSB1K3:p162:ccdB 1	pSB1K3:pBad:MazE 1	

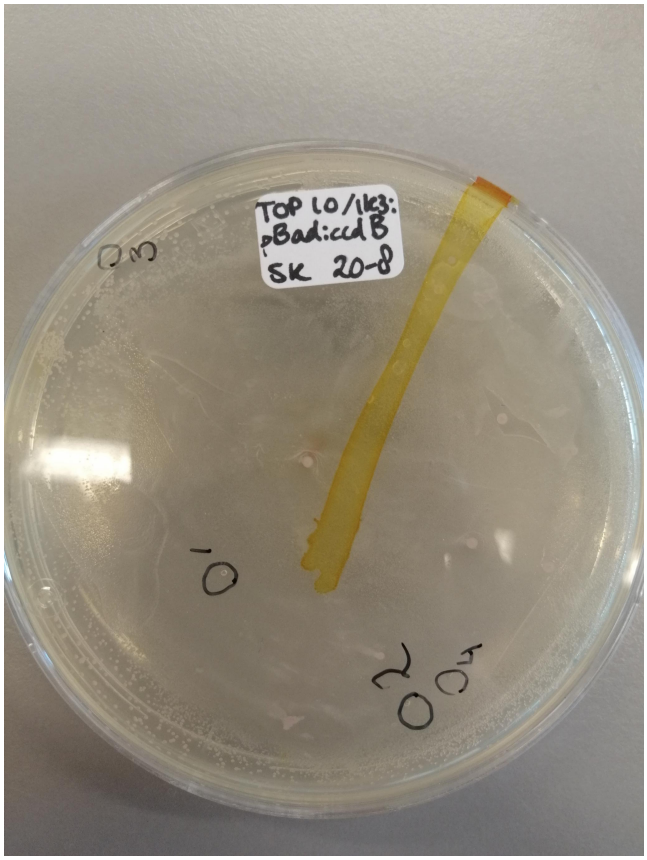
Lane 1-6 of the top row are Chanel's samples.
The other rows are my samples. Here is the layout:

I only restricted for 1 hour at 37 °C, so it might be that there is still some uncut DNA left.

Result

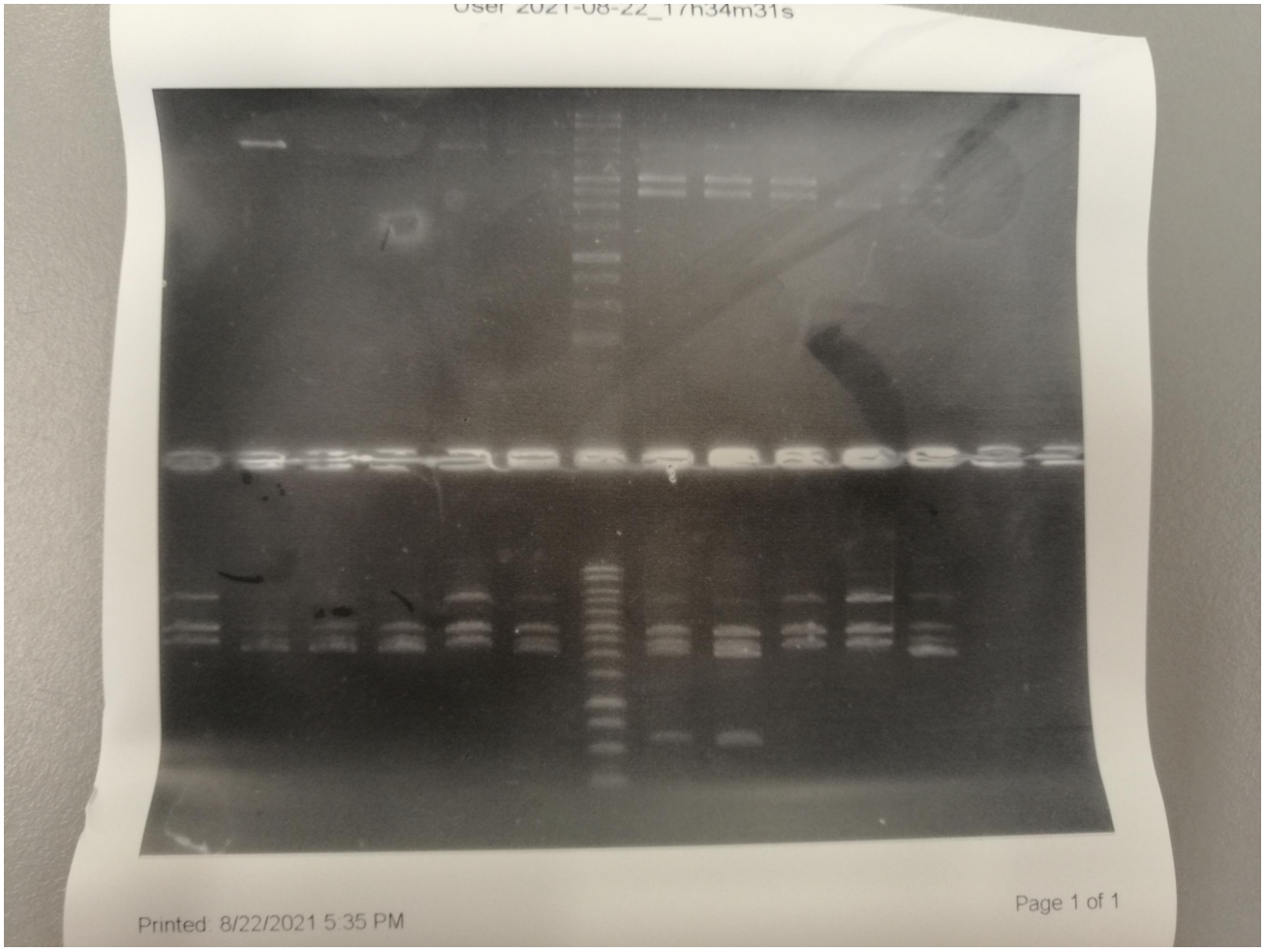
This is what the plate looks like after one day:

IMG_20210822_154619.jpg



I took these 4 colonies and streaked them. Hopefully they will not be red tomorrow, but white. I also put the tips that I put the first dot on the plate with in liquid culture in case all of them are white.
Further, I did a plasmid isolation/gel electrophoresis today.
This is a picture of the gel:

IMG_20210822_173737.jpg



Only really apparent from HOK that it worked, according to the length. However, the other bands are quite similar.

Week 6 - LacI antitoxin fusion

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-01 09:36:33 AM +0000
Entry Last Modified: 2021-10-01 12:34:49 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

FRIDAY, 13/8/2021

Project: Lab Notebook
Authors: Bas van Woudenberg
Created at: 2021-08-13

Goal

- Restriction, ligation, and transformation of the LacI promoter infront of the antitoxins in the Jump29, pSB1C3, and pSB1A3 plasmids. Next, the construct will be transformed in the DH5α bacterial strain.

Materials

- Restriction enzymes
  - EcoRI
  - PstI
  - Spe1
  - XbaI
- Promoters
  - LacI
- Plasmids
  - Jump29
  - pSB1C3
  - pSB1A3
- Antitoxins
  - Sok
  - ccdA
  - RelE
  - MazE
- Fluoresent genes
  - GFP
  - mCherry
- 10x restriction buffer
- MiliQ
- T4 Ligase
- T4 ligase buffer

Protocol

The following constructs are digested:

Table1

	A	B	C
1	**Backbone:**		lane
2	Jump29	sfGfP	1
3	pSB1C3	RFP	2
4			
5	**promotor:**		
6	pSB1C3	LacP1	3
7	pSB1C3	LacP2	4
8	pSB1C3	LacI	5
9	pSB1C3	LacR1	6
10	pSB1C3	LacR2	7
11			
12	**CDS:**		
13	pSB1A3	SOK	8
14	pSB1A3	CcdA	9
15	pSB1A3	MazE	10
16	pSB1A3	RelE	11
17	pSB1A3	GFP	12
18	pSB1A3	mCherry	13

According to the following digestion tables:

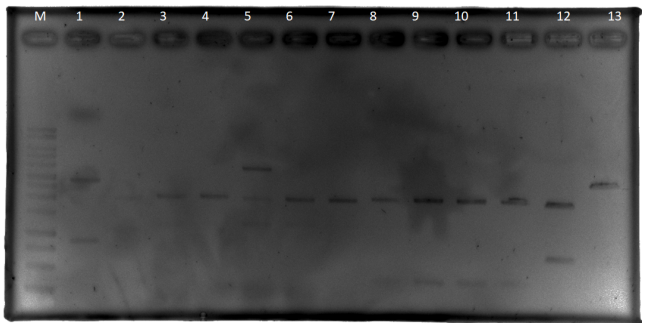
Table2

	A	B
1	**Backbone**	
2	Jump29 sfGfP	16
3	10x Buffer 2.1	2
4	EcoRI	1
5	PstI	1
6	MiliQ	0
7		
8	**promotor**	
9	psb1C3 Promotor	8
10	10x Buffer 2.1	1
11	EcoRI	0.5
12	Spe1 HF	0.5
13	MiliQ	0
14		
15	**CDS**	
16	psb1A3 CDS	8
17	10x Buffer 2.1	1
18	xba	0.5
19	psti	0.5
20	MiliQ	0

After 1 h 3 ul of the digestion is put on gel.

Result

image.png



LacI and GFP are ligated and transformed in DH5α, the white colonies are picked and grown with and without iPTG.

Week 6 - Make GFP and sfGFP constructs

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-04 02:18:51 PM +0000
Entry Last Modified: 2021-10-11 08:49:57 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 16/8/2021

Goal

- Ligation of pSB1K3::Promoter::sfGFP and pSB1K3::Promoter::GFP

Material

- Plasmids
  - pSB1K3
- Promoters
  - p2547
  - p1303
  - p162
  - pBAD
- Gene
  - GFP
  - sfGFP
- T4 ligase
- T4 ligase buffer

Protocol

Ligation of the following constructs:

Table5

	A	B	C	D	E
1	pSB1K3	P2549	GFP		
2	pSB1K3	P1303	GFP		
3	pSB1K3	P162	GFP		
4	pSB1K3	pBad	GFP		
5	pSB1K3	P2549	sfGFP		
6	pSB1K3	P1303	sfGFP		
7	pSB1K3	P162	sfGFP		
8	pSB1K3	pBad	sfGFP		
9					

Calculation amount DNA ng/ul necessary for ligation:

Table6

	A	B	C	D	E	F	G	H
1	Plasmid ID	Plasmid concentration	Plasmid	water	bp			
2	pSB1K3	48.2	2.0746887967	5.9253112033	2163	20	4.1493775934	11.8506224066
3	pBad	575	0.1739130435	7.8260869565	1233	20	0.347826087	15.652173913
4	P2549	69.8	1.4326647564	6.5673352436	58	10	2.8653295129	13.1346704871
5	P1487	44.4	2.2522522523	5.7477477477	58	10	4.5045045045	11.4954954955
6	P908	66.2	1.5105740181	6.4894259819	58	10	3.0211480363	12.9788519637
7	P387	46.5	5	3	58	10	10	6
8	P21	56.9	1.7574692443	6.2425307557	58	10		
9	P1	66.2	1.5105740181	6.4894259819	58	10		
10	sfGFP	84.2	1.1876484561	6.8123515439	711	20	2.3752969121	13.6247030879
11	GFP	27.9	3.5842293907	4.4157706093	869	20	7.1684587814	8.8315412186
12	RelE1	53.8	1.8587360595	6.1412639405			3.717472119	12.282527881
13	RepAMazE2	61.1	1.6366612111	6.3633387889			3.2733224223	12.7266775777
14	NtagMazE1	121	0.826446281	7.173553719			1.652892562	14.347107438
15	NtagMazE2	63.5	1.5748031496	6.4251968504			3.1496062992	12.8503937008
16	RepAmazE1	80.6	1.2406947891	6.7593052109			2.4813895782	13.5186104218
17	RelE2	78.5	1.2738853503	6.7261146497			2.5477707006	13.4522292994
18								

Ligation mixture:

Table7

	A	B	C	D	E	F	G
1	Plasmid backbone	gene1	gene2	water			
2	2	1	4	1.5	pSB1K3	P2549	GFP
3	2	1	4	1.5	pSB1K3	P1303	GFP
4	2	1	4	1.5	pSB1K3	P162	GFP
5	2	5.7	4	-3.2	pSB1K3	pBad	GFP
6	2	1	3.287	2.213	pSB1K3	P2549	sfGFP
7	*2*	*1*	3.287	*2.213*	pSB1K3	P1303	sfGFP
8	*2*	*1*	3.287	*2.213*	pSB1K3	P162	sfGFP
9	*2*	*5.7*	3.287	*-2.487*	pSB1K3	pBad	sfGFP
10	*1.452991453*	*4.141025641*	*2.905982906*	8.5			*11.7*
11	*1.5472831528*	*4.4097569855*	*2.5429598617*	8.5			10.987
12	15.0002746058						
13							

Add to each ligation mixture 1 ul of T4 ligase buffer and 0.25 ul of T4 ligase. Let the ligation reaction perform for 2H at room temperature.

TUESDAY, 17/8/2021

Week 6 - Make GFP and sfGFP constructs (2)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-17

Goal

- Gel check with electrophoresis
- Ligate pBAD::GFP and pBAD::sfGFP

Material

- 10x Loading dye
- 1 Kb Ladder
- 1% TAE buffer
- 1% Agarose gel
- Ethidiumbromide
- T4 Ligase buffer
- T4 ligase

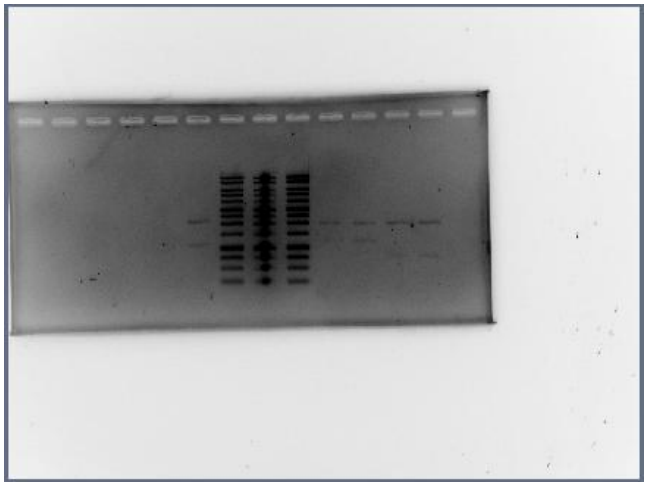
Protocol

9 ul of sample is mixed with 1ul of 10x loading dye and pipetted in the gel slots. 6 ul of 1 Kb of DNA ladder was used. The gel has runned on 100V for 25 minutes. After that, the gel was stained in Ethidiumbromide bath for 25 mintues.

For the ligation the same protocol as on 2021-08-16 was used to measure and perform the ligation reaction for the pBAD::GFP and pBAD::sfGFP constructs.

Result

20210817 SL.jpg



Constitutive promoter on the left show no bands. The other parts on the right of the DNA ladder have correct bands.
FACS test for all co-transformation and single plasmids

WEDNESDAY, 18/8/2021

Week 6 - Make GFP and sfGFP constructs (3)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-18

Goal

- Digest plasmid with constitutive promoter again with higher concentration: 10uL of plasmid, 6.1 uL of water
- Ligate again constitutive promoters in front of GFP and sfGFP
- After Ligation, transform the constructs in TOP10 strain

Material

- Restriction enzymes
- 10x buffer 2.1
- Promoters
    - p2547
    - p908
    - p21
- Genes
    - GFP
    - sfGFP
- T4 ligase
- T4 ligase buffer

- MiliQ
- LB agar

Protocol

Digestion mixture:

Table3

	A	B	C	D	E
1	Components	Mixture (ul)			
2	Plasmid DNA	10			
3	10x Buffer 2.1	1.5			
4	EcoRI	1.2			
5	SpeI	1.2			
6	MiliQ	6.1			
7	**Total**	**20**			

Ligations:

- P2547-GFP
- P2547-sfGFP
- P908-GFP
- P908-sfGFP
- P21-GFP
- P21-sfGFP

Ligation reaction mixture:

Table4

	A	B	C	D
1	Plasmid	Promoter	Gene	
2	2	3	3	GFP
3	2	3	2.5	sfGFP

Add to each ligation mixture 1 ul of T4 ligase buffer and 0.25 ul of T4 ligase. Let the ligation reaction perform for 2H at room temperature.

Transformation protocol used as our written co-transformation protocol, only in this case adding one plasmid instead of two.

Result

Transformation of pBad-GFP/sfGFP failed, no colony in the plate the next day 2021-08-19.

THURSDAY, 19/8/2021

Week 6 - Make GFP and sfGFP constructs (4)

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-19

Goal

- Retransform pBad-GFP/sfGFP as well as constitute promoter + GFP/sfGFP as no colonies have grown on the LB agar plates.

Material

- Ice
- Ice bucket
- LB agar

Protocol

For the retransformation the same protocol as mentioned in the protocol of 2021-08-18 was used. Transformation was performed in the TOP10 *E.coli* strain.

Week 6 - Plate reader : mCherry

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-04 02:19:14 PM +0000
Entry Last Modified: 2021-10-11 11:58:51 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

THURSDAY, 19/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-19

Goal

- Perform a mChery calibration in a plate reader assay with the promoters p2547 and pBad infront of mCherry.

Material

- L-arabinose concentrations
  - 1%
  - 0.0016%
- Promoters
  - p2547
  - pBAD
- Genes
  - mCherry
  - Hok
  - RelE

Protocol

Use same layout but use arabinose-LB as inducer.

Cells layout:

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry			P2547-mCherry-1%			
B	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry						
C	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry	P2547-mCherry			P2547-mCherry-0.0016%			
D	pBad-Hok											
E	RelE-1%											
F									Blank			
G	pBad-mCherry-1%											
H												

Result of the plate reader is a CSV file that will be analyzed.

Week 7 - Co-transformation of toxin / antitoxin (1)

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 02:11:24 AM +0000
Entry Last Modified: 2021-10-11 02:55:56 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 24/8/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2021-08-24

Goal

- Digest, ligate and transform toxins and antitoxins in pJUMP26 and pJUMP46 plasmids.

material

- pJUMP26 and pJUMP49
- EcoRI
- PstI
- T4 ligase
- T4 ligase buffer
- MiliQ
- Ice
- LB agar
- Agar plates

Protocol

Digest protocol used:

Table1

	A	B
1	EcoRI	0.5 ul
2	PstI	0.5 ul
3	10x buffer	1 ul
4	MiliQ	7 ul
5	DNA	1 ul
6	**Total**	**10 ul**

After 1,5 H of restriction, the restriction reaction was stopped by heat-inactivating at 85 °C.
Next, to the digestion mixture, 0.25 ul of T4 ligase and 1 ul of T4 ligase buffer was added to the mixture. The ligation reaction was performed for 2H at room temperature.

Co-transformation of the toxin/antitoxin system was performed with the TOP10 bacterial strain.

Competent TOP10 cells were kept on ice during the experiment. After heat-shocking the constructs into TOP10 at 45°C the cells were plated out on LB agar with the appropriate antibiotics.

Result

No colonies had grown on the plates.

Week 7 - Co-transformation of toxin / antitoxin (2)

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 02:11:36 AM +0000
Entry Last Modified: 2021-10-11 02:58:26 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

WEDNESDAY, 25/8/2021

Project: Lab Notebook
Authors: Hoda Ekhlasi
Created at: 2021-08-25

Goal

- As no cultures had grown. The ligation and transformation was re-performed into DH5α competent cells.

Material

- See materials used at 2021-08-24

Protocol

- Also same protocol was used as on 2021-08-24.

Result

Again, no colonies pJUMP26::T/A construct had grown on the agar plates. Also no colonies of the pJUMP46::T/A were grown on the plates.

Week 7 - Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 02:12:14 AM +0000
Entry Last Modified: 2021-10-11 02:11:49 PM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

MONDAY, 23/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-23

The streaked plates seem to not contain RFP, so that is positive, it seems it worked to rescue the old transformed cells with the glucose.

Goal

- Reperform the digestion of yesterday and check again on gel. It does not seem to be contamination on the plates, so I think it should be fine.
- Restrict the cultures on the plates to put on gel after restriction.
    - If they are correct, I will ligate and transform them today to make pSB1A3:p908:AT:p162:t and pSB1A3:p908:at:p162:T. These will be transformed in pSB1A3 so that I have the right selection markers for the double transformation.

Material

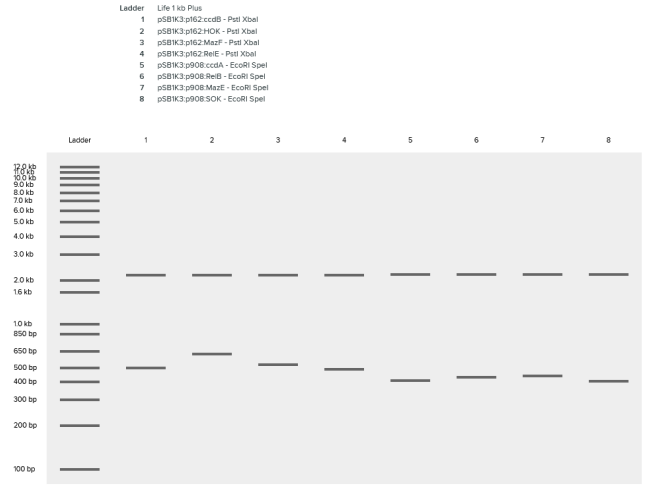
- Restricion enzymes
    - EcoRI
    - PstI
- Restriction buffer
- MiliQ
- 10x restriction buffer
- 1% Agarose
- 1% TAE buffer
- 10x Loading dye
- 1 Kb DNA Ladder
- Ethidiumbromide

Protocol

When they have succeeded, I will do a plasmid backbone switch, so that they are in J27/J49 and I can select with kanamycin and spectinomycin. Since the fragments are currently in pSB1K3, this would not be possible, since it also contains kanamycin and you would not be able to select away kanamycin self-ligations.

This is what the gel should look like:

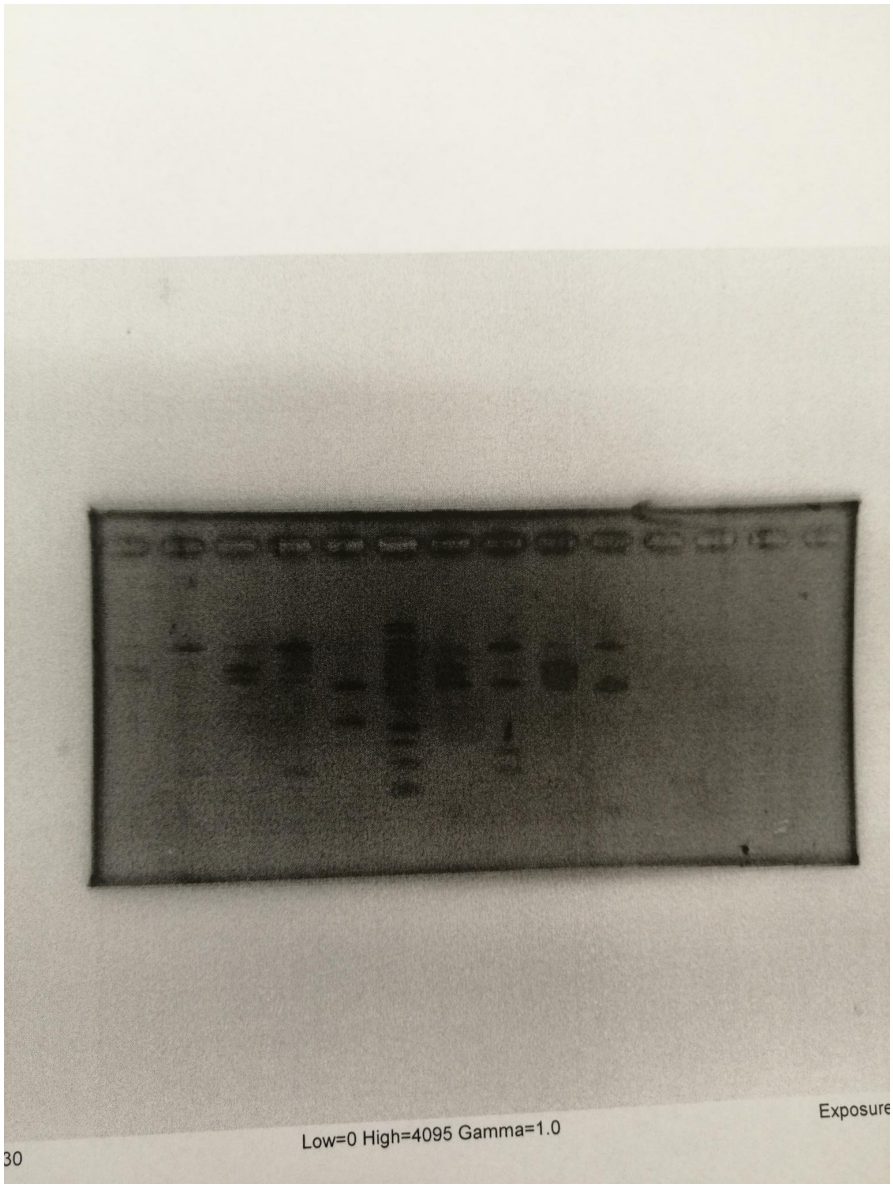
afbeelding.png



Result

This is what we actually see:

IMG_20210823_155148.jpg



Therefore, none of the sequences seem to be the correct one. The cloning of the full system seems to be more difficult than expected. One reason for this might be the fact that the constitutive primer is so short that the efficiency of the reaction decreases drastically.

TUESDAY, 24/8/2021

Week 7 - Creating DOPL LOCK (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-24

Today, I ordered primers for an overhang PCR. Since cloning seems to be so difficult, we will PCR the constitutive promoters in front of the toxin and antitoxin genes.
The primers can be found not in this folder, these were the first draft, but in the map shared by Jo-Anne.
I will put them below as well in a table.

primers.xlsx

All the reverse ones are the same, since it is just the biobrick flank and also the flanking region contains the same sequence in all plasmids.

Goal

- Started new restrictions for doing the transformations in a different way.

Material

- Restriction enzymes
  - PstI
  - SpeI
  - XbaI
  - PstI
- Restriction buffer
- MiliQ

Protocol

I use the plasmid pSB1C3 containing the promoters, cutting it open with PstI and SpeI, so that we keep the promotersequence included.
Then, I cut the other plasmids with XbaI and PstI, so we can add the other biobrick after it.
Then, I will run it on gel tomorrow and cut out the bands for ligation, so that we know all the DNA is cut successfully and pure.

WEDNESDAY, 25/8/2021

Week 7 - Creating DOPL LOCK (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-24

Goal

- Isolate the DNA from gel using the genejet plasmid gel isolation kit.

Material

- Thermoscientific GeneJET plasmid gel isolation kit.
- MiliQ

Protocol

For the isolation, the standard protocol of the Thermoscientific isolation kit was used.
The concentrations I will determine tomorrow. After that, I will ligate and transform them.

THURSDAY, 26/8/2021

Week 7 - Creating DOPL LOCK (4)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar
Created at: 2021-08-25

Goal

- Restrict, ligate, and transformation of the DOPL LOCK system will be performed.

Material

- Restriction enzymes
  - EcoRI
  - SpeI
  - XbaI
  - PstI
- NEB2.1 restriction buffer
- MiliQ
- T4 Ligase buffer
- T4 Ligase
- TOP10

Protocol

Restrictions (per reaction):

Table2

	Component	Volume (uL)	C	Component	Volume (uL)	F	Component	Volume (uL)
1	EcoRI	0.25		XbaI	0.25		EcoRI	0.25
2	SpeI	0.25		PstI	0.25		PstI	0.25
3	NEB2.1	0.5		NEB2.1	0.5		NEB2.1	0.5
4	DNA	4		DNA	4		DNA	4
5	**Total**	**5**		**Total**	**5**		**Total**	**5**

Restricitons were done for 2 hours at 37 °C.

Ligations (per reaction):

Table1

	Component	Volume (uL)
1	T4 DNA ligase	1
2	T4 ligase buffer	2
3	pSB1C3	2
4	con:tox	5
5	con:atox	5
6	Miliq	5
7	**total**	**20**

The combinations of toxin/antitoxin were:
ccdA + RelE, ccdB + RelB
MazE + Hok, MazF + Sok
pBAD:MazE + ccdB
pBAD:ccdA + MazF

The ligations were done for 2 hours at room T.

However, I forgot to heat-inactivate the restriction enzymes.
Therefore, I heat inactivated everything and then proceeded to add new ligase and buffer. I added 3 uL buffer and 1 uL enzyme to each tube.

Then, the standard protocol was followed for cloning with heatshock.

Week 7 - Digestion and Ligation : mCherry

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 02:12:29 AM +0000
Entry Last Modified: 2021-10-06 02:46:18 AM +0000
Export Generated On: 2021-10-11 03:48:55 PM +0000

TUESDAY, 24/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-24

Goal

- Digestion and ligation of target constructs written in the protocol of today.

Materials

- Restriciton enzymes
- Restriction buffer
- MiliQ
- T4 Ligase
- T4 Ligase buffer

Protocol

Target construct
PJUMP49-P1-mCherry
PJUMP49-P21-mCherry
PJUMP49-P382-mCherry
PJUMP49-P908-mCherry
PJUMP49-P1487-mCherry

Digestion:

Table1

	A	B	C	D	E	F	G	H	I
1	Enzyme 1	0.5		Plasmid ID	Plasmid concentration	Plasmid	water		
2	Enzyme 2	0.5		pJUMP49-1	55.4	1.8050541516	6.1949458484		
3	cutsmart buffer	1		pJUMP49-2	0	#DIV/0!	#DIV/0!		
4	MiniQ	8-X		pJUMP49-3	57.1	1.7513134851	6.2486865149	2.6269702277	9.3730297723
5	Plamsid	X	100ng in total	P1		8	0		
6				P21		8	0		
7				P382		8	0		
8				P908		8	0		
9				P1487		8	0		
10									
11									
12									
13									

Ligation:

Table2

	A	B	C	D
1	Plasmid backbone	gene1	gene2	water
2	1.5	1	4	2
3	1.5	1	4	2
4	1.5	1	4	2
5	1.5	1	4	2
6	1.5	1	4	2
7	2			#VALUE!

Week 7 - OD callibration to cfu : mCherry

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 02:12:39 AM +0000
Entry Last Modified: 2021-10-06 02:51:50 AM +0000
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FRIDAY, 27/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2021-08-27

Goal

- OD calibration

Material

- 96-well plate
- Liquid LB medium
- LB agar medium

Protocol

Steps to calibrate the OD:

1. Grow DH5-alpha pSB1K3 in liquid LB overnight.
2. Preload 96-well plate with LB
3. Dilute them to certain OD (0.1, 0.2, 0.4, 0.8)
4. Dilute them immediately in 96-well plate in triplicate.
5. Use the 100uL of the last dilution and plate them in agar.

Dilution layout:

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A	OD=0.1 190uL	190uL	190uL	180uL	180uL	OD=0.4 150uL	190uL	190uL	190uL	180uL	180uL	
B	190uL	190uL	190uL	180uL	180uL	150uL	190uL	190uL	190uL	180uL	180uL	
C	190uL	190uL	190uL	180uL	180uL	150uL	190uL	190uL	190uL	180uL	180uL	
D	OD=0.2 150uL	190uL	190uL	180uL	180uL	180uL	OD=0.8 190uL	190uL	190uL	180uL	180uL	180uL
E	150uL	190uL	190uL	180uL	180uL	180uL	190uL	190uL	190uL	180uL	180uL	180uL
F	150uL	190uL	190uL	180uL	180uL	180uL	190uL	190uL	190uL	180uL	180uL	180uL
G												
H												

Final dilution factor:
OD=0.1 (1600, 16000, 160000)
OD=0.2 (3200, 32000, 320000)
OD=0.4 (6400, 64000, 640000)
OD=0.8 (16000, 160000, 1600000)

Table1

	A	B	C	D	E	F
1	OD	1	2	3	Ave	Dilution factor
2	0.1A					
3	0.1B					
4	0.1C	16	18	104	46	160000
5	0.2A					
6	0.2B					
7	0.2C	47	8	9	21.3333333333	320000
8	0.4A					
9	0.4B					
10	0.4C	133	288	134	185	640000
11	0.8A					
12	0.8B					
13	0.8C	123	340		231.5	1600000
14						

Week 7 - Preparation : mCherry

Project: iGEM2021
Authors: Iris Noordermeer
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MONDAY, 23/8/2021

Project: Lab Notebook
Authors: Siheng Li
Created at: 2020-08-23

Prepare for a plate reader assay this week of the inducible promoter pBAD::mCherry and the constitutive promoter p2547::mCherry.

Goal

- Put pBad-toxin strain for in new LB medium
- Culture mCherry for plasmid ligation
- Culture P2547-mCherry and pBad-mCherry. pJUMP-49 as control

Material

- Liquid LB medium
- LB agar medium

Protocol

Pick pBAD::toxin strain to inoculate in new LB liquid medium.
Also, make a cell culture of mCherry by inoculating the strain in LB liquid medium. After one day of growth, the culture willbe ready to isolate the plasmids.
Ultimately, make cell cultures of p2547::mCherry and pBAD::mCherry. pJUMP49 will also be grown in LB liquid medium to use as a control this week for the plate reader assay.

Week 8 - PCR : Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 03:01:49 AM +0000
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WEDNESDAY, 1/9/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Iris Noordermeer
Created at: 2020-09-01

This afternoon, the primers arrived!

Goal

- Perform a PCR- based cloning reaction, to ligate the promoter in front of toxins.

Material

- Primers
- DNTPs
- Taq polymerase
- Buffer

Protocol

PCR mix:

Table3

	A	B
1	Promoter	1 ul
2	Plasmid	1 ul
3	Forward primer	1 ul
4	Reverse primer	1 ul
5	Taq polymerase buffer	2 ul
6	DNTPs	1 ul
7	Taq polymerase	0.25 ul
8	MiliQ	17,75 ul
9	**Total**	**25 ul**

We made several different PCR programs to spread our chances.
The protocol was made by Le, and we made variations on it.

Here is the schedule:

Table4

	Phase	Repetitions	Temperature (C)	Time (s)	Notes
1	1	1 time	95	30	
2	2	15 times	95	30	
3	2		68	30	-0.6 C / cycle
4	2		72	60	
5	3	20 times	95	30	
6	3		59	30	
7	3		72	60	
8	4	1 time	72	300	
9	4	1 time	4	-	

The variations were that the inital annealing T was decreased to 65 and 62 degrees and the decreases in T per cycle were also decreased to 0.3 and 0.1 C per cycle respectively.
Further, we did the primers once 10 x diluted and once 100 x diluted.
This means that every reaction was done 6 times.

THURSDAY, 2/9/2021

Week 8 - PCR : Creating DOPL LOCK (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Iris Noordermeer
Created at: 2020-09-02

Goal

- Restrict the PCR products and check on gel if the construct is right

Material

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

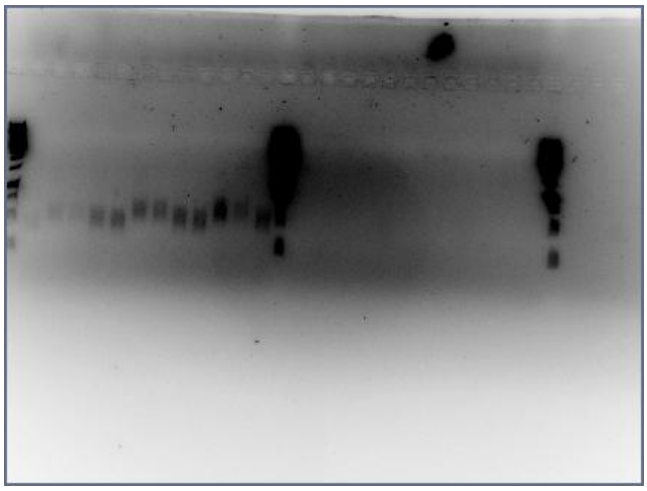
- Ethidiumbromide

Protocol

Restriction reaction was performed for 1,5H at 37 °C. After 1,5H the restriction reaction was stopped by heat-inactivating the enzymes at 85°C.

Result

20210902 SK IN Overhang PCR.jpg



It worked, the bands are the correct size! This means that after PCR-based cloning the promoters are now in front of the toxins.

Week 8 - TA testing : Creating DOPL LOCK

Project: iGEM2021
Authors: Iris Noordermeer
Entry Created On: 2021-10-06 03:05:41 AM +0000
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MONDAY, 30/8/2021

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Iris Noordermeer
Created at: 2021-08-30

Goal

- Transformation for pBAD:ccdB, since this was not succesful over the weekend.

Material

- LB agar
- Agar plates
- Baterial strains
    - DB3.1
    - TOP10

Protocol

Tomorrow, we will see if the transformation was succesful and if it is, we can grow new cultures for doing the co-transformation of pBAD:ccdA and pBAD:ccdB.
I did everything according to the standard protocol for the heatshock.
The restriction was done in a volume of 11 uL. 2 uL backbone, 7 uL insert, and 2 uL buffer and enzyme, 37 °C for 1 hour. Heat inactivation for 20 minutes at 80 °C.
The ligation was then done at room temperature, for 2 hours. I added 2 uL in total extra, enzyme and buffer. Then, half of the ligation was transformed to TOP10 and half to DB3.1, and incubated o/n at 37 °C.

Result

Transformations seemed to be succesful with little self-ligations, therefore the constructs should now be in the correct backbones (J26 and J49).

TUESDAY, 31/8/2021

Week 8 - TA testing : Creating DOPL LOCK (2)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Iris Noordermeer
Created at: 2021-08-31

Goal

- Isolate the plasmids with the constructs to check them on gel tomorrow.
- At the same time, we will co-transform the plasmids into TOP10
    - To see if they behave differently in the platereader from the ones with only toxin or antitoxin.

Material

- Thermoscientific GeneJET plasmid mini prep kit
- TOP10 bacterial strain
- Spectinomycen(25 µg/mL)
- Kanamycen(30 µg/mL)
- LB agar medium
- Agar plates

Protocol

The plasmid isolation protocol followed was the standard protocol of the Thermoscientific kit that came along with it.
Furhtermore, the co-transformation protocol that we used was the standard protocol that we use for performing a co-transformation, which you can find under "Protocols".

The transformants were grown in liquid culture at 37 °C, 200 rpm.

WEDNESDAY, 1/9/2021

Week 8 - TA testing : Creating DOPL LOCK (3)

Project: Lab Notebook
Authors: Sebastiaan Ketelaar, Iris Noordermeer
Created at: 2021-09-01

Goal

- Check the isolated plasmids of the previous days on gel by loading a digestion of the plasmids cut with EcoRI and PstI.

Material

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

Protocol

Digestion mixture:

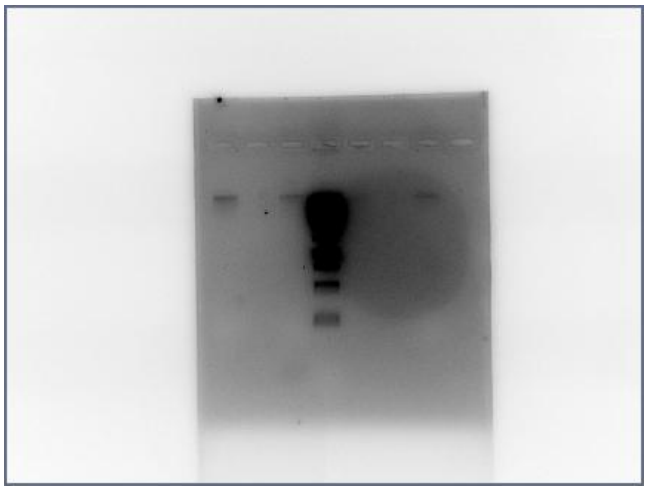
Table1

	A	B
1	EcoRI	0.5 ul
2	PstI	0.5 ul
3	10x buffer	1 ul
4	MiliQ	7 ul
5	DNA	1 ul
6	**Total**	**10 ul**

The gelelectrophoresis was performed to mix 9 ul sample with 1 ul 10x loading dye. This mix was added to the slots in the gel. 6 ul of the 1 kB DNA Ladder and runned at 100V for 30 minutes. The gel was stained for 25 minutes in ethidiumbromide.

Result

20210901 SK plasmid check.jpg



The constructs on the gel are not correct, as we expect two bands due to the restriction reaction you expect more than one band. In addition, in lane 2,5,6, and 8 there aren't any band visible at all. Therefore, we expect that the pBAD:ccdB construct were not present in either TOP10 and the DB3.1.