DATE: **19/8**

LUCIFERASE CHARACTERISATION PROJECT

Plates:
Since the backbone have Chloramphenicol marker we will try to make Chloramphenicol plates. ‘
Recipe from iGEM

1. Recommended conc.: Chloramphenicol 25 µg/mL
2. Add agar powder 16g/L -> autoclave
3. Add the appropriate amount of desired antibiotic to autoclaved LB-agar media and swirl to mix.
4. Pour ~20mL per 10cm polystyrene Petri dish.
5. Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified), then invert the plates. Let sit for several more hours or overnight.
6. Label the bottom of plates with antibiotic and date and store in plastic bags or sealed with parafilm at 4°C.

400 ml of LB media ~ 20 plates
25 µg/mL => we need 10 000 µg = 0.01 g of Chloramph.

PLAN
Make plates -> DNA solution from iGEM HQ -> E-coli transformation tomorrow -> Plate on selection media -> incubate overnight -> Colonies containing the plasmid -> liquid culture -> bio lector on Saturday 4 pm

DATE: **26/8**

Arabinose - DH5α experiments

DH5α cells, transformed with biobricks (luciferase) were inoculated in LB (2 ml). Arabinose was added in different amounts. Later, CaCl2 was added, hoping that cells might get porous by it.

Sample	**Arabinose**	**CaCL2**
1		0
2		15 mM
3		100 mM

These cells were later heat shocked as well. 80 C for ~2 min. But no luminescence activity recorded. Conclusion - Should not proceed with this experiment.

DATE: **27/8**

Inoculation of Top 10

We got our plate of Top 10 cells from sysbio. Single colony was inoculated in LB (5 ml)

Date:28/8

Top 10 cells inocluations

10 ml LB was contaminated with Top10 cells from overnight culture

Preprep for competent cells

Autoclaved tips and bottles for competent cells. 1 ml tips in special autoclave bags

SOB medium

SOB medium was prepared by the recipe. pH adjustment: 0.018 gm + 0.445 ml HCl (12 M). Sterile filtered. 250 μl MgSO4 and 250 μl MgCL2 added to 50 ml SOB medium. 100 μl and 200 μl Top 10 cells were added to 2 different flasks

DATE: 9/9

Responsible person: Dharmik

Luciferase expression experiment

Step 1: Grow a colony (Top 10 transformed) in 10 ml (LB+ chloramphenicol) 37°C- 12 hours. The cells are grown in a shake flask that is kept in a beaker with ice/cold water to hold the temperature down.

Step 2: Transfer the cells to a new medium. Contaminate 10 ml (LB+ chloramphenicol) with previously grown cells.
Grown in room temp 20°C til OD (660nm) =0,6.

Step 3: Add arabinose so that the final concentration is 0,25%
No glowing cell could be seen.

DATE: 10/9

Luciferase project

The cells are taken from room temperature shaker (was kept in ice bath overnight)

The OD was checked for the culture and measured to OD=0,9. 350µl of arabinose was added and the celles was kept in the ice bath, room temperature shaker for 30 min.

The cells were checked in the dark room and there were no glowing cells. Kept in shaker for another 30 min and still no glowing cells.

DATE:15/9

Luciferase Experiment

20 ml culture (LB+ chloramphenicol) +
20 μl (25 mg/ml) chloramphenicol @ 20 c
@ 200 rpm

DATE: 17/9

Responsible person: Dharmik

Miniprep of luciferase plasmid.
Luciferase cells were inoculated on the 15th of september at 5pm but they didn't grow anything in 20°C until the 16th of september at 8pm.
The flask was moved to the 37°C incubator and kept there until 17th of September at 1 pm. *Plan: after miniprep, is it still trustworthy? Test 1, try transformation. Test 2, restriction digestion.*
The plasmid miniprep protocol was followed and the concentrations was measured to 232,4 ng/µl.

Inoculation of *E.coli* cells
E.coli cells was inoculated in LB+ chloramphenicol media, both Top10 cells and Top10+ Luciferase.

DATE: 18/9

Responsible person: Dharmik

Luciferase cells experiment

The luciferase cells are growing slower but they are growing. The non-transformed Top10 cells didn't grow in LB+ chloramphenicol.

500 µl of luciferase cells were added to 10 ml of LB + chloramphenicol at 12pm.

DATE: 19/9

Luciferase experiment
100 µl of cells from 18/9 were incubated with 20ml of fresh LB+ chloramphenicol in the shaker incubator 20°C, 180 rpm at 9 am.
No growth was seen after 12,5 h.

Responsible person: Emma

DATE: 20/9

Responsible person: Dharmik

Luciferase incubation

The growth of cells where measured on 8 am and the cells had grown. *Note: OD was not measured.* Added 1-1,5ml of 10% arabinose in 20 ml of culture. *Note: Not completely sterile, should be fine since the cells are only supposed to grow for a couple of hours.*

The working scheme over the last couple of days is summarized below:
17th Sept: Overnight culture of cells in 1 ml media
18th Sept: Overnight culture of cells in 10 ml media
19th Sept: Overnight culture of cells in 20ml media at 20 °C shaker, 180 rpm
20th Sept: added arabinose 1-1,5 ml arabinose
-> result

Restriction digestion of luciferase plasmid.

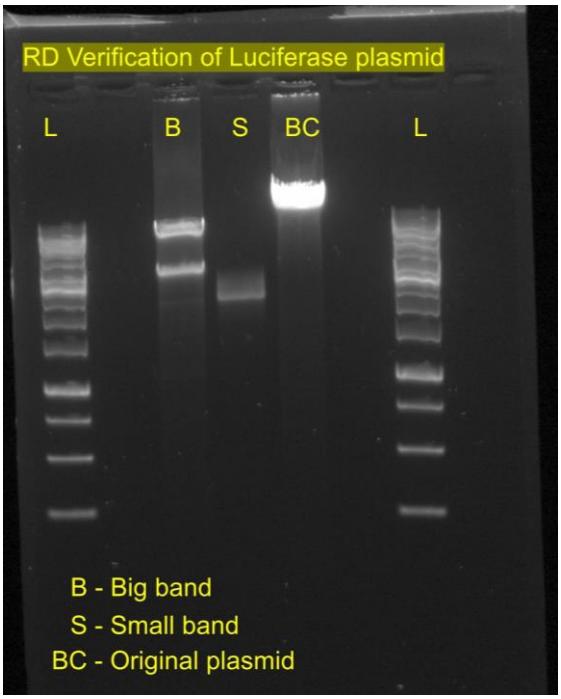
Plan: See if the biobrick is correct

- *EcoR1-PstI combo was chosen*
- *Verified in benchling, cut sites on each side of the biobrick.*

PSB1C3 backbone, the entire plasmid is 9,3kb.

DNA	50µl
water	30µl
Green buffer	10µl
Pst1	5µl
E.coR1	5µl

Kept on 37°C for 30 min and then enzyme inactivation at 87°C for 5 min



DATE: 24/9

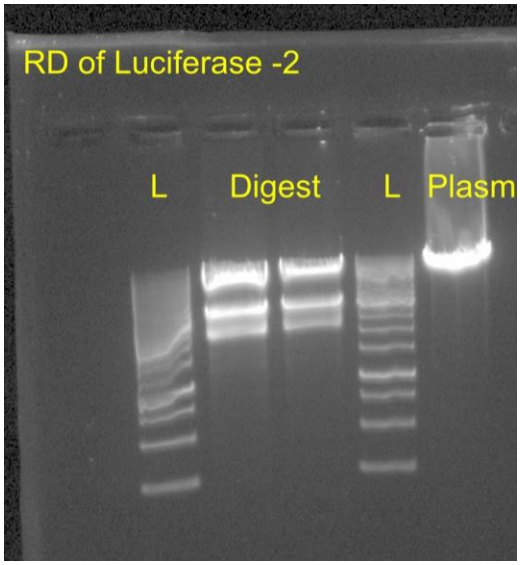
Luciferase digestion project:
Restriction Digestion verification

As suggested by Oliver, All 3 enzymes in one digestion

Luciferase Biobrick - 4.5 μl (234 ng/μl)
Pst1 - 1 μl
EcoR1 - 1 μl
Nde 1 - 1 μl
FD Green Buffer - 3 μl
Water - 19.5 μl

Mixture kept @ 37 C for 15 min

Prestain Gel (1.5 μl Gel Red), small gel, 140 V, 20 min



DATE: 29/9

Characterization experiments

Biobricks used

High Constitutive Expression Cassette (HECE)	1G, Plate 1
Orange Fluorescent Protein (OFP)	9I, Plate 4
Cyan Fluorescent Protein (CFP)	17 J, plate 6
Blue Chromoprotein (Blue)	10B, Plate 5
Terminator	E.Coli plates
GFP with degradation tag (GFP)	E.Coli plates

Biobricks in iGEM plate - Add 10 µl water, take in another tube, use 2 µl for transformation
Biobricks in Chloramphenicol plates - Inoculate cells in 10 ml chloramphenicol LB for miniprep
Transformation - 50 µl cells transformed

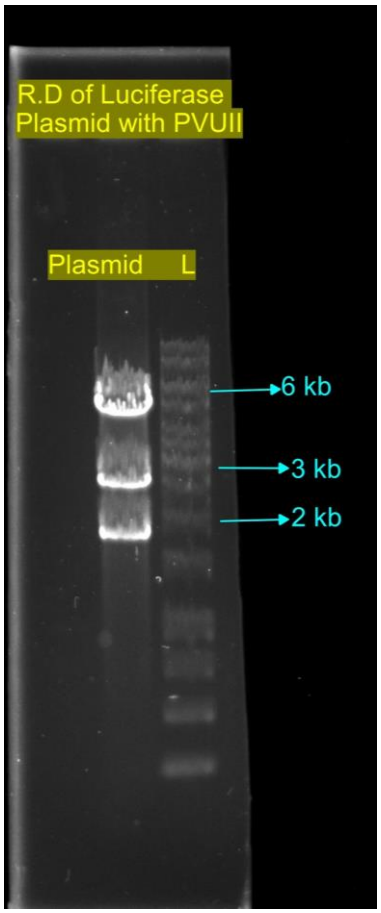
Date: 1/10

Miniprep

OFP	146.1 ng/µl
BFP	148.1 ng/µl
CFP	129.9 ng/µl
GFP	146.1 ng/µl
HECE	133.7 ng/µl

PVU II digestion of luciferase plasmid

PVUII - 2µl
luciferase plasmid - 6 µl
FD green buffer - 2 µl
Water - 10 µl



Gel run - Very good band pattern (worth giving for sequencing)
Biobrick extraction of Terminator from iGEM plate

Biobrick - Double terminator - Plate 1, 3 D
Transformed in DH5 alpha E.Coli, choramphenicol medium

Make Chloramphenicol plates

12.5 mg/ml medium - 24 plates

Date: 2/10

Extraction of Biobricks: Terminator 2

For some reason, double terminator transformation was not successful. So, retransformed it and had a backup plan. Extracted another terminator

Single terminator: 1D, plate 1
(Heads up: 10 µl water used to resuspend DNA in plate. 2 µl used for transformation)

Sequencing: Luciferase plasmid

15 µl DNA + 30 µl water: given for sequencing

Date: 7/10

Restriction Digestion of Biobricks

HCEC - 9.4 µl DNA + 1 µl EcoR1 + 1 µl Spe1
OFP - 5.1 µl DNA + 1 µl Xba1 + 1 µl Spe1
GFP - 6 µl DNA + 1 µl Xba1 + 1 µl Spe1
CFP - 6.7 µl DNA + 1 µl Xba1 + 1 µl Spe1
Blue - 6 µl DNA + 1 µl Xba1 + 1 µl Spe1
Single Terminator - 27.4 µl + 4 µl F.D green buffer + 2 µl Xba1 + 2 µl Pst1

T4 DNA ligase:

Use upto 5 µl of mixture in competent cells in mixture

Buffer: 2 µl
Ligase: 1 unit
Water + DNA; 17 µl

Date: 10/10

Transformation of ligated products

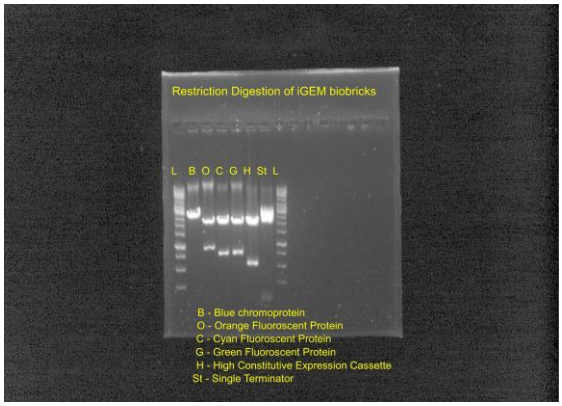
Ligated products -> 5 µl in 50 µl.Rest in - 20 C

DATE: 11/10

GFP - 4 colonies, Blue chromoprotein- 1, CFP - 0, OFP - 0

Gel Run of digests of Biobricks

- 140 V, 25 min, 1 µl ladder, 5 µl sample + 1 µl FD green buffer, pre-stain (1.5 µl Gel Red)



GFP colonies were restreaked on new plate and were inoculated overnight for Fluorescence microscope view with Linnea

DATE: 12/10

Transformation of ligated products

Ligated products were re-transformed (Just to see if they did not work previously due to bad luck)
All of rest of 15 µl ligated products in 50 µl competent cells. Done only for CFP, OFP and Blue chromoprotein