iGEM Strasbourg (Lab Notebook)

Project: iGEM Strasbourg Shared Project
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MONDAY, 27/5/19

LB Medium and petry dishes preparation

Summary:
-Preparation of LB Medium
Petry dishes preparation:
-Preparation of LB Medium + Chloramphenicol
-Preparation of LB Medium + Ampicillin
-Preparation of LB Medium + Kanamicyne
-Preparation of LB Medium + Spectinomycine
-Measurement of the plasmids in the nanodrop

Preparation of LB Medium

Table1

	A	B	C	D	E	F
1		**Solid medium**	**Liquid medium**			
2	**Components**	**Quantite**				
3	Bacto-tryptone	10 g	10g			
4	Yeast extract	5 g	5 g			
5	NaCl	5 g	5 g			
6	NaOH 1N	1 mL	1 mL			
7	Bacto-agar	15 g	-			
8	Adjust with water to 1000mL					
9	Autoclave					
10	**Peparing petry dishes**					
11		Stock Solution		Dilution		
12	Ampicillin	100mg/mL in water stored at -20°C		1000X		
13	Kanamicyne	50mg/mL in water stored at -20°C		1000X		
14	Chloramphenicol	34mg/mL in 100% ethanol stored at -20°C		2000X		
15	Spectinomycine	50mg/mL		1000X		
16	Put in petry dishes and let them solidify					

The medium is sterilized by autoclaving at 120°C with a pressure of 1,2 bars for 20 minutes.

The measurements described before are for 1 L.

Measurement of the plasmids in the nanodrop

1mL of each plasmid was put it in the nanodrop in order to quantify the quantity of DNA.

Table2

	A	B	C
1	Plasmid	DNA	Antibiotic R.
2	pKB1094	140,1 ng/µL	Spectinomycin
3	pKB1081	109,6 ng/µL	Ampicillin
4	pKB989	85,8 ng/µL	Chloramphenicol

TUESDAY, 28/5/19

Summary:

-Protocol for produce CaCl2 competent cells using the FW102 O_L2-62 strain

-Preparation of Tris-HCl 1mM with a pH of 7,5

-Preparation of CaCl2 1M

-Preparation of competent cells buffer: Tris-HCl 10mM pH 7,5 containing CaCl2 50mM

-Filtration of competent cell buffer

-Launch the FW102 O_L2-62 strain in culture for the competent cells

Measurement of the plasmids in the nanodrop

1mL of each plasmid was put it in the nanodrop in order to quantify the quantity of DNA.

Table3

	A	B	C
1	Plasmid	DNA	Antibiotic R.
2	pKB822	81,5 ng/µL	Spectinomycin

Preparation of Tris-HCl 1M with a pH of 7,5 (Stock)

The soulution 1 M Tris-HCl was prepared in a volume of 100 mL

Using 12.11 g of Tris-HCl

Adjusting the pH to 7,5 using HCL 1M

Filling the tube until 100mL using MiliQ water and mix vigorously

Preparation of $CaCl_2$ 1M (Stock)

The soulution 1 M CaCl2 was prepared in a volume of 100 mL

Using 14.70 g of CaCl2 dihydratated

Filling the tube until 100mL using MiliQ water and mix vigorously

Preparation of Tris-HCl 10mM with a pH of 7,5 containing CaCl2 50mM (Buffer for competent cells)

-Add 1mL of Tris-HCl 1M with a pH of 7,5

-Add 5mL of CaCl2 1M

-Filling the bottle until 100mL using MiliQ water and mix vigorously

-Filtrate the buffer and 50% glycerol solution using a Thermo Scientific Nalgagene filtration bottletop

Competent cells preparation using FW102 O_L2-62 strain

- Put a little piece of agarose from the tube which contains the FW102 O_L2-62 strain in 25mL of sterile LB medium, leave it at 37°C overnight under rotation.

WEDNESDAY, 29/5/19

Summary:

-Dilutions of the plasmids: pKB1094, pKB1081, pKB989

-Creating Bacterial Glycerol Stocks for Long-term Storage of FW102 O_L2-62 strain

-Preparing FW102 O_L2-62 competent cells

-Transformation of the plasmids pKB1094, pKB1081, pKB989

Dilutions of the plasmids: pKB1094, pKB1081, pKB989

Table4

	A	B	C	D
1	Plasmid	DNA	Dilution	Final Concentration
2	pKB1094	140,1 ng/µL	9µL H2O + 1 µL plasmid	14,01 ng/µL
3	pKB1081	109,6 ng/µL	9µL H2O + 1 µL plasmid	10,96 ng/µL
4	pKB989	85,8 ng/µL	9µL H2O + 1 µL plasmid	8,58 ng/µL

Creating Bacterial Glycerol Stocks for Long-term Storage of FW102 OL2-62 strain

-Make an inoculation overnight in liquid culture medium LB

-After you have bacterial growth

-Add 500 µL of the overnight culture to 500 µL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.

Note: *The 50% glycerol solution was made by diluting 100% glycerol in dH20.*

-The the glycerol stock tube were freeze at -80°C.

Preparing FW102 O_L2-62 competent cells

-The cells from a culture of 60 mL stoped in exponential phase of growth (A700nm = 0,6)

-The cells are recolected by centrifugacion at 4°C for 15 min at 3000g and gently put in 15 mL of Tris-HCl 10 mM pH 7,5 containing 50 mM of CaCl2, in order to, permeabilize the membrane.

-After 1 hour of incubation on ice

-The suspension is centrifuge at 4°C for 5 min at 1200g.

-The cells are put carefully in suspension in 2mL of the precedent solution and it is incubated 1 hour on ice.

-The suspension of the competent cells is additionated with 10% (v/v) of glycerol

-It is aliquotee in fractions of 0,2 and stocked at -80°C.

Transformation of the plasmids pKB1094, pKB1081, pKB989

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at room temperature for 1 day

FRIDAY, 31/5/19

Summary:
Petry dishes preparation:
-Preparation of LB Medium + Chloramphenicol
-Dilutions for the transformation for the Competent Cell Test kit (iGEM)
-Mini prep protocol
-Transformation of the plasmid from the competent cell test kit (iGEM) using FW102 OL2-62 ourcompetent cells
-Preparation of the tubes of LB with antobiotic for mini prep

Preparation of LB Medium + Chloramphenicol

Table5

	A	B	C	D	E	F
1		**Solid medium**	**Liquid medium**			
2	**Components**	**Quantite**				
3	Bacto-tryptone	10 g	10g			
4	Yeast extract	5 g	5 g			
5	NaCl	5 g	5 g			
6	NaOH 1N	1 mL	1 mL			
7	Bacto-agar	15 g	-			
8	Adjust with water to 1000mL					
9	Autoclave					
10	**Peparing petry dishes**					
11		Stock Solution		Dilution		
12	Chloramphenicol	34mg/mL in 100% ethanol stored at -20°C		2000X		
13	Put in petry dishes and let them solidify					

Dilution for the transformation for the Competent Cell Test kit (iGEM)
Stock solutions: 10 pg/µL and 100 pg/µL.

Table6

	A	B	C
1	0.01 ng/µL	0.1 ng/µL	0.5 ng/µL
2	Stock 10 pg/µL	Stock 100 pg/µL	5 µL of 100 pg/µL in 5 µL of dH2O

Transformation of the plasmids from the cell test kit (iGEM)

-Thaw 100 µL of chemically competent FW102 OL2-62 cells on ice
-Add 1 µL of plasmid (0.01 ng/µL, 0.1 ng/µL, 0.5 ng/µL)
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes (Important for Cam)
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at room temperature for 1 day

Tube with LB + antibiotic (cell culture for miniprep)

2 mL of LB in a sterile tube with 2 µL of antibiotic (Amp, Cam, Spc)

Table7

	A	B	C	D
1	Antiobiotic	Stock Solution		Dilution
2	Ampicillin	100mg/mL in water stored at -20°C		1000X
3	Chloramphenicol	34mg/mL in 100% ethanol stored at -20°C		2000X
4	Spectinomycine	50mg/mL		1000X

MONDAY, 3/6/19

Summary:
-Miniprep of the plasmids: pKB989, pKB1081, pKB1094
-Results of the competent cell test kit (iGEM)
-Check another protocol to make the competent cells using electroporation

Miniprep of the plasmids: pKB989, pKB1081, pKB1094

-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
-Add 100µl of Solution I
-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.

-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
-Repeat wash procedure in step 8.
-Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution.
-Transfer the column to a clean 1.5ml microfuge tube.
-Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes.
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.

Note: It is extremely important to add the Elution Buffer into the center part of the column. Incubating the column with the Elution Buffer at higher temperature (37ºC to 50ºC) may slightly increase the yield especially for large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

Concentration of the purified DNA measured by the nanodrop.

Table8

	A	B
1	Plasmid eltuted in 50 µL of MiliQ water	DNA concentration
2	pKB989 (1)	160,4 ng/µL
3	pKB989 (2)	179,0 ng/µL
4	pKB1081 (1)	62,6 ng/µL
5	pKB1081 (2)	112,3 ng/µL
6	pKB1094 (1)	176,3 ng/µL
7	pKB1094 (2)	17,8 ng/µL

Results of the competent cell test kit (iGEM)

Table9

	A	B	C
1	Concentration	Number of colonies	
2	0.01 ng/µL	1	
3	0.1 ng/µL	1	
4	0.5 ng/µL	3	

Competent cells trying another method "Okayama et al., GENE 96 pp 23-28

-Put 50 mL of LB in a 250 mL flask and add 25 µL of Kanamycine under the hood
-Add a tip with the bacteria strain FW102 OL2-62
-Put at 37°C culture ovenight

TUESDAY, 4/6/19

Summary:
-Prepare the dilution for make the FW102 OL2-62 competent cells and incubation at 18°C
-Transformation of the plasmids pKB1094, pKB1081, pKB989
-Glycerol stock bacteria
-Primer dilutions

Prepare the dilution for make the FW102 OL2-62 competent cells and incubation at 18°C
Inoculate Stratagene strain FW102 OL2-62 (from tube from USA group) + 20 ug/mL kanamycine (stock: 50 mg/mL in water, 2000X)
Incubate at 18°C to in three different OD concentration: 0.05, 0.1 and 0.2 (it will take approx. 24 hrs to reach OD of 0.6 at 600 nm) * The flask, and the LB medium were precooled.

Transformation of the plasmids pKB1094, pKB1081, pKB989

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

Creating Bacterial Glycerol Stocks for Long-term Storage of FW102 OL2-62 strain

-Make an inoculation overnight in liquid culture medium LB
-After you have bacterial growth
-Add 500 µL of the overnight culture to 500 µL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.

Note: *The 50% glycerol solution was made by diluting 100% glycerol in dH20.*

-The the glycerol stock tube were freeze at -80°C.

Primer dilutions

The primers stock were diluted in 100 µM using MiliQ sterile water and aliquoted in a tube at 10µM (it was taken 10 µL from the primer stock and diluted in 90 µL of MiliQ sterile water.

Table10

	A	B	C	D
1	Primer	Code Letter	Stock solution	Aliquot
2	pKB1081-alphaPP7-F1	A	100µM	10µM
3	pKB1081-alphaPP7-R1	B	100µM	10µM
4	pKB989-lambdaMS2-F1	C	100µM	10µM
5	pKB989-lambdaMS2-R1	D	100µM	10µM
6	PKB1094-RNA-F1	E	100µM	10µM
7	PKB1094-RNA-R1	F	100µM	10µM
8	pKB989-lambdaMS2-Seq-F	G +	100µM	10µM
9	pKB989-lambdaMS2-Seq-R	H °	100µM	10µM
10	pKB1081-alphaPP7-Seq-F	I	100µM	10µM
11	pKB1081-alphaPP7-Seq-R	J	100µM	10µM
12	PKB1094-RNA-Seq-	K	100µM	10µM

WEDNESDAY, 5/6/19

Summary:

-Culture of the transformer plasmids: pKB1094, pKB1081, pKB989

-Preparation of competent cells using method "Okayama et al., GENE 96 pp 23-28"

-Transformation using the just made FW102 OL2-62 strain competent cells and the Competent Cell Test kit (iGEM)

Culture of the transformer plasmids: pKB1094, pKB1081, pKB989 (for miniprep)

-Add 3 mL of LB to a sterile tube + 3 µL of antibiotic in each case it was.

Table12

	A	B	C	D
1	Colony	Antibiotic	Concentration of antibiotic	
2	pKB1094	Spectinomicyne	50mg/mL	
3	pKB1081	Ampicillin	100mg/mL in water stored at -20°C	
4	pKB989	Chloramphenicol	34mg/mL in 100% ethanol stored at -20°C	

Preparation of competent cells using method "Okayama et al., GENE 96 pp 23-28"

-Incubate at 18°C to an OD of 0.6 at 600 nm (will take approx. 24 hrs) *GS3 rotor should be precooled
-Place Erlenmeyer on ice for 10 min.
-Pour culture in Sorvall GS3 centrifuge tube (use clear GS3 centrifuge tubes which have not been used for other plasmids before)
-Prepare 100 mL of "TBjap" + 2mL of 100% DMSO on ice (in blue cap glass bottle)
-Spin GS3 rotor (must be precooled) at 2500 g and 4°C, for 10 minutes
-Discard supernatant completely and redissolve peller in 80 mL of "TBjap" (Precoled, from above).
-Keep suspension on ice (for 10 min)
-Spin again for 10 min. at 2500 g and 4°C, (in precooled rotor)
-Walk to the cold room
-Discard supernatant and resuspend pellet in 18.6 mL of precooled "TBjap"
-Add 1.4 mL of DMSO (->final conc, of 7%)
-Let bacteria sit on ice for 10 min and prelabel cryotubes on the lid ("C" = comp. cells)
-Dispens suspension gently into cryotubes (200uL), freeze immediately in liquid nitrogen
-Store immediately in a prelabeled Box at -70°C

Transformation using the just made FW102 OL2-62 strain competent cells and the Competent Cell Test kit (iGEM)

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid (0.1 ng/µL and 0.01 ng/µL)
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

THURSDAY, 6/6/19

Summary
-Miniprep of the plasmids: pKB989, pKB1081, pKB1094 for sequencing
-Results of the competent cell test kit (iGEM) using the new competent cells
-Run the purified plasmids pKB989, pKB1081, pKB1094 in an agarose gel 1%

Miniprep of the plasmids: pKB989, pKB1081, pKB1094

-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
-Add 100µl of Solution I
-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.
-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.

-Repeat wash procedure in step 8.
-Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution.
-Transfer the column to a clean 1.5ml microfuge tube.
-Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes.
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.

Note: It is extremely important to add the Elution Buffer into the center part of the column. Incubating the column with the Elution Buffer at higher temperature (37ºC to 50ºC) may slightly increase the yield especially for large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

Concentration of the purified DNA measured by the nanodrop.

Table11

	A	B
1	Plasmid eltuted in 50 µL of MiliQ water	DNA concentration
2	pKB989 (1)	89,3 ng/µL
3	pKB989 (2)	82,1 ng/µL
4	pKB1081 (1)	78,6 ng/µL
5	pKB1081 (2)	79,3 ng/µL
6	pKB1094 (1)	105,8 ng/µL
7	pKB1094 (2)	67,8 ng/µL

Results of the competent cell test kit (iGEM)

Table13

	A	B	C
1	Concentration	Number of colonies	
2	0.01 ng/µL	2	
3	0.1 ng/µL	16	

Run the purified plasmids pKB989, pKB1081, pKB1094 in an agarose gel 1%
-For the agarose gel we used:
-1g of agarose in 100mL of TAE 1%
-Run the gel at 100v for 30 minutes
-Put the gel on Ethidium bromide for 15 minutes
-Visualize the gel under an UV light machine

igem plasmid picture.png

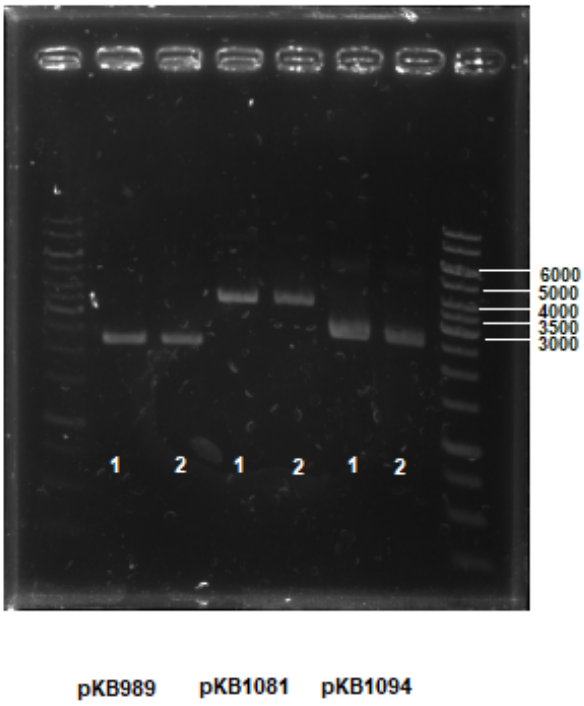


Table14

	A	B
1	Plasmid	Length (bp)
2	pKB989	3,545 bp
3	pKB1081	5,480 bp
4	pKB1094	4,009 bp

Note: The plasmid could look smaller in the scale, due to its a close plasmid and it tends to form coils

For sequencing in eurofins

All the plasmids were diluted at 50 ng /µL in 20 µL which will be used for sequencing

All the primers were diluted in a concentration of 10 mM in 50 µL

FRIDAY, 7/6/19

Summary:

-Transformation of the plasmids:pKB989, pKB1081, pKB1094 and pK822 using the FW102 OL2-62 competent cells

-Adjust the primers for sequencing in sanger conditions

-Transformation of the plasmids:pKB989, pKB1081, pKB1094 and pK822 using the FW102 OL2-62 competent cells

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid (0.1 ng/µL and 0.01 ng/µL)
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

-Adjust the primers for sequencing in sanger conditions

Table15

	A	B	C	D	E
1	**Nom de l'échantillon**	**Quantité (ng)**	**A(260 /280)**	**Amorce**	**Taille bp**
2	989-1	600,2	1,94	Forward: CTCATGTTTGACAGCTTATCATCG	1311
3	989-1	600,2	1,94	Reverse: GCGCTTCGTTAATACAGATGTAG	1311
4	989-2	600,7	1,92	Forward: CTCATGTTTGACAGCTTATCATCG	1311
5	989-2	600,7	1,92	Reverse: GCGCTTCGTTAATACAGATGTAG	1311
6	1081-2	570,8	1,99	Forward: aaacccagcgttcgatgcttc	1755
7	1081-2	570,8	1,99	Reverse: CTCCTGCATTAGGAAGCAGC	1755
8	pkb1094-1	600,3	1,88	Forward: aaagtgtctataatcacggcag	728
9	pkb1094-2	600,7	1,97	Forward: aaagtgtctataatcacggcag	728

TUESDAY, 11/6/19

-Petry dishes preparation:
-Preparation of LB Medium + Chloramphenicol + Ampicillin + Spectinomycine
-Transformation of BH3 (pkb989. pkb1081 and pkb1094 and BH2 as a control (pkb822, pkb1081 and pkb1094)
-Preparation of Na2CO3 1M
-Preparation of SDS 0.1%

Preparation of LB Medium + agar

Table16

	A	B	C	D	E	F
1		**Solid medium**				
2	**Components**	**Quantite**				
3	Bacto-tryptone	10 g				
4	Yeast extract	5 g				
5	NaCl	5 g				
6	NaOH 1N	1 mL				
7	Bacto-agar	15 g				
8	Adjust with water to 1000mL					
9	Autoclave					
10	**Peparing petry dishes with 3 antibiotics combined**					
11		Stock Solution		Dilution		
12	Ampicillin	100mg/mL in water stored at -20°C		1000X		
13	Chloramphenicol	34mg/mL in 100% ethanol stored at -20°C		2000X		
14	Spectinomycine	100mg/mL		2000X		
15	Put in petry dishes and let them solidify					

Transformation using the just made FW102 OL2-62 strain competent cells and the BH3 and BH2 plasmids

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of each plasmid (pkb989, pkb1081 and pkb1094 for the BH3) and (pkb822, pkb1081 and pkb1094 for BH2)
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes

-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

Preparation of Na2CO3 1M

Add 10.6g of Na2CO3 and fill until 100mL with desionized water

Preparation of SDS 0.1%

Add 1mL of SDS 10% to 99mL of desionized water

WEDNESDAY, 12/6/19

- PCR of the plasmids pKB989, pKB1081, pKB1094 obtained from the transformation with FW102 OL2-62 strain competent cells
-Transformation using the just made FW102 OL2-62 strain competent cells and the BH3 and BH2 plasmids using the Nickels protocol
-Culture preparation of pKB989, pKB1081, pKB1094 plasmids.

- PCR of the plasmids pKB989, pKB1081, pKB1094 obtained from the transformation with FW102 OL2-62 strain competent cells

Transformation using the just made FW102 OL2-62 strain competent cells and the BH3 and BH2 plasmids using the Nickels protocol
-Thaw competent cells on ice.
-Add 10 nanograms of both the plasmid carrying the a fusion and the plasmid carrying the kCI fusion to a sterile microcentrifuge tube, place on ice for 5 min. Be sure to include appropriate negative controls (testing the kCI fusion with plasmid pBRaLN and the a fusion with plasmid pACkCI is recommended).
-Add 50 lL competent FW 102 OL262 cells to tubes containing plasmid DNA and incubate on ice for 10 min.
-Heat shock at 42 for 2 min, then place tubes back on ice for 2 min.
-At this point cells can either be plated directly onto LB-agar plates containing the neccesary antibiotics, a recovery step can be done to allow efficient expression of the antibiotic resistance genes (inclusion of the recovery step yields higher numbers of transformants). If a recovery step is desired add 1 mL of LB to each tube and incubate at 37 for 1 h. (We did the recovery step)
-Pellet cells by centrifugation and pour off all but 100 uL of the supernatant.
-Use the 100 lL of remaining supernatant to resuspend the cell pellet and plate on LB-agar plates containing the antibiotics required (Amp, Cam, Spec)
-Incubate plates overnight at 37.

PCR of the plasmids pKB989, pKB1081, pKB1094 obtained from the transformation with FW102 OL2-62 strain competent cells
Mix together
- 1 µl of primer 1
- 1 µl of primer 2
- 1 µl of plasmid (4 ng optimal)
- 9.5 µl of MilliQ
- 12.5 µl of Master Mix

Use the following cycles
- 1:00 at 98°C
- 0:10 at 98°C
- 0:15 at 58°C (change according to primers Tm)
- 0:05 at 72°C (5 seconds for 1 kb below 5 kb fragment, 10 seconds for 1 kb above 5 kb fragment)

- Repeat 30 times (35 cycles for PCR on colonies)
- 1:00 at 72°C
- ∞ at 12 °C

For the gel verification mix together (Charge in an agarose gel of 2%)
• 5 µl of PCR product
• 5 µl of Loading buffer (green one is better)
100 watts for 30 minutes

Culture of plasmids: pKB1094, pKB1081, pKB989 (for miniprep)
-Add 3 mL of LB to a sterile tube + 3 µL of antibiotic in each tube.

THURSDAY, 13/6/19

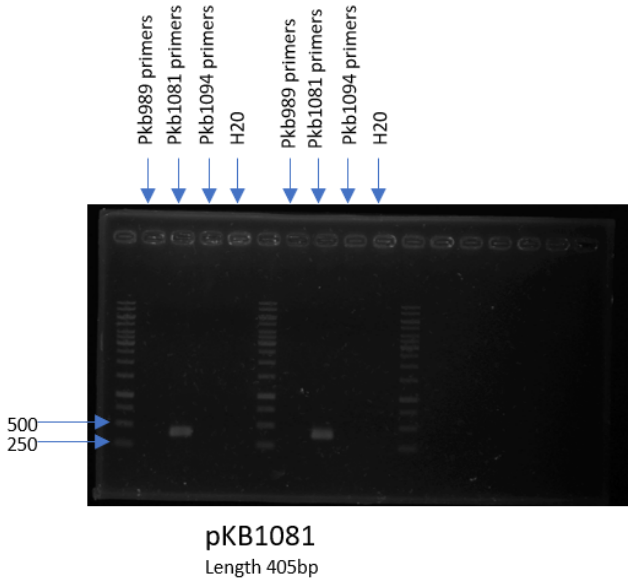
Summary:
-Mini prep preparation of the plasmids pkb989, pkb1081, pkb1094 (Adding the mising steps in the protocol*)
-PCR results to check if the primers are specific
-Plasmid DNA concentration
-Transformation for BH3 and BH2 using more cells and more plasmid (Becker's suggestion)

Mini prep preparation of the plasmids pkb989, pkb1081, pkb1094 (Adding the missing steps in the protocol*)

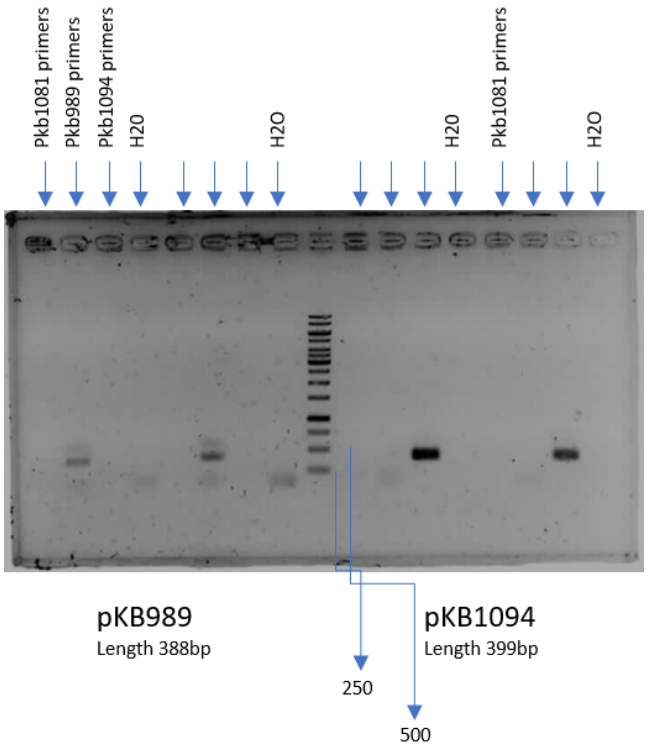
-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
-Add 100µl of Solution I
-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.
-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
-Repeat wash procedure in step 8.
-Centrifuge again at 10,000rpm for 2 minutes without adding anything to remove any residual Wash Solution.*
-Discard the flow-through in the collection tube.
-Transfer the column to a clean 1.5ml microfuge tube.
-Put in the warm box at 50°C during 5 minutes to dry the ethanol in the column
-Put 25uL of warm milliq water into the center part of the column incubate it in the warm box at 50°C during 5 minutes
-Take the tube out from the warm box and incubate at room temperature for 5 minutes
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.
-Measure the DNA concentration using the nanodrop

PCR results to check if the primers are specific

plasmids pkb1081 pcr with primers.png



pkb 1094 and pkb989 pcr with primers.png



Plasmid DNA concentration

Nanodrop results plasmids.png

	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
Pkb989 1	Default	13/06/2019	08:35	103,32	2,066	1,054	1,96	2,07	50,00	230	0,999	0,024
Pkb989 2	Default	13/06/2019	08:36	131,58	2,632	1,353	1,95	2,11	50,00	230	1,246	0,010
Pkb1081 1	Default	13/06/2019	08:39	116,79	2,336	1,171	1,99	1,98	50,00	230	1,178	0,028
Pkb1081 2	Default	13/06/2019	08:40	144,66	2,893	1,527	1,89	1,58	50,00	230	1,828	0,090
Pkb1094 1°	Default	13/06/2019	08:41	101,42	2,028	1,047	1,94	2,02	50,00	230	1,005	0,051
Pkb1094 1	Default	13/06/2019	08:42	186,08	3,722	2,022	1,84	1,78	50,00	230	2,093	0,234
Pkb1094 2°	Default	13/06/2019	08:43	169,71	3,394	1,813	1,87	1,88	50,00	230	1,806	0,123
Pkb1094 2	Default	13/06/2019	08:44	218,83	4,377	2,290	1,91	2,11	50,00	230	2,073	0,009

Transformation using the just made FW102 OL2-62 strain competent cells and the BH3 and BH2 plasmids

-Thaw 200 µL of chemically competent bacteria on ice
-Add 1 µL of each plasmid (pkb989, pkb1081 and pkb1094 for the BH3) and (pkb822, pkb1081 and pkb1094 for BH2)
Concentration used for each plasmid:
pkb989: 103,3 ng/uL, pkb1081: 116,8 ng/uL and pkb1094: 101,4 ng/uL for the BH3) and (pkb822: 80,3ng/uL, pkb1081:116,8 ng/uL and pkb1094: 101,4 ng/uL for BH2)
-Put on ice for 30 minutes
-Heatshock at 42°C for 1 minute
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 1 hour
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

FRIDAY, 14/6/19

Summary
-PCR of the colonies from the BH3 and BH2 transformations
-Making petry dish plates with the three antibiotics
-Making agarose gels 1%
-Run the gels for the PCR

PCR of the colonies from the BH3 and BH2 transformations
-1uL of primer 1
-1uL of primer 2
-10 uL of master mix
-7 uL of milliQ water
-1 tip with a little quantity of each colonie separately

Use the following cycles
- 1:00 at 98°C
- 0:10 at 98°C
- 0:15 at 58°C (change according to primers Tm)
- 0:05 at 72°C (5 seconds for 1 kb below 5 kb fragment, 10 seconds for 1 kb above 5 kb fragment)
- Repeat 30 times (35 cycles for PCR on colonies)
- 1:00 at 72°C

- ∞ at 12 °C

For the gel verification mix together (Charge in an agarose gel of 1%)
• 5 µl of PCR product
• 5 µl of Loading buffer (green one is better)
100 watts for 30 minutes

Making agarose gels 1%
-4g of agarose
-Disolved in 400 mL of TAE 1X
-Melt in the microwave
-Cold it and put in mold for agar gels

Making petry dish plates with the three antibiotics
For 20 plates:
-400 mL of LB + Agar sterilized
-400 uL Ampicillin 100mg/mL - 1000X
-200 uL Spectinomycine 100mg/mL - 2000X
-400 uL Chloramphenicol 34mg/mL - 1000X

Results to the PCR gels
(Put the pictures) Looks that we have BH2 and BH3 :D
A pcr for confirm the pKB989 band was done

MONDAY, 17/6/19

Summary:
-Spread the positive colonies in new plates with the three antibiotics
-Disolve the Gibson primers
-Make plates with MacConkey agar for the agar art
-Prepare cultures for the B Gal assay
-Preparation of the IPTG, ONPG and the Z buffer

Spread the positive colonies in new plates with the three antibiotics
-Take the colony and disolve in 100 uL of LB and plate under the hood

Disolve the Gibson primers
Disolved in a concentration 100uM, by adding 40uL of milliQ water to 4nmol

Make plates with MacConkey agar for the agar art
The agar plates were made using:
-7g of MacConkey agar and disolving it in 140 mL of milliQ water
-It was autoclaved at 121°C for 15 minutes as the protocol indicate
-It was put 25mL in a petry dish and let on the bench for solidify
- Afterwards it was plated the "agar art" using E.coli strain *FW102 OL2-62*

Prepare cultures for the B Gal assay
It was prepared 6 tubes with:

-15uL of ITPG (Diluted 10uL of IPTG stock at 100uM + 90 uL of LB medium)

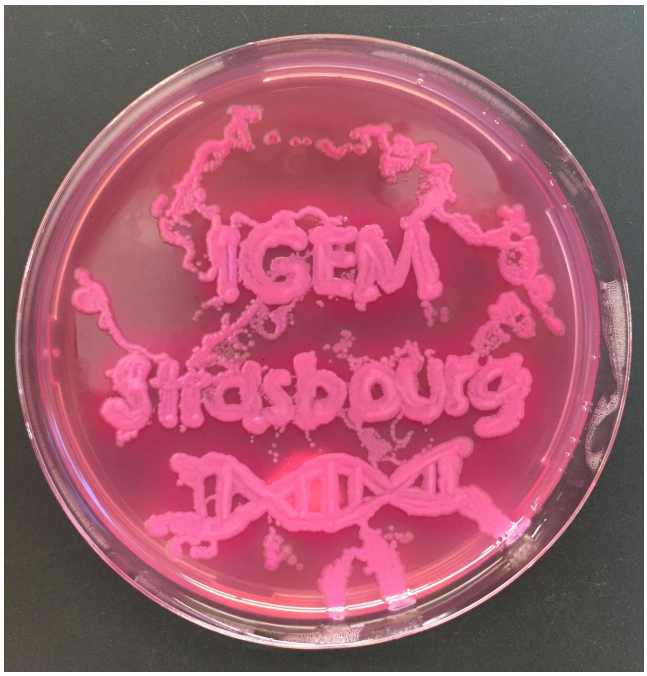
-3 uL of Ampicillin
-3 uL of Chloramphenicol
-1.5 uL of Spectinomycine
-2985 uL LB medium
And it was added a tip which contain the culture positive for BH3 and for BH2
3 colonies for each (BH3 and BH2) were cultured.

TUESDAY, 18/6/19

Summary:
-Results for agar art
-B Galactosidase assay with the BH3 and BH2 plasmids
-Perfom the Gibson assembly for Guanosine active

Results for agar art

agart art igem.png



agar art tree.png

agar art sugar free.png

B Galactosidase assay with the BH3 and BH2 plasmids

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the same concentration that was present in the overnight culture).

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

WEDNESDAY, 19/6/19

Summary:
-Plates with Spectinomycine for the Gibson transformation
-Gibson assembly using 3 different ratios between the vector and the insert for Guanosine +
-Transformation for the Gibson Guanosine + assembly
-Cultures for the B Galactosidase test with arabinose 0.2%

Plates with Spectinomycine for the Gibson transformation
For 10 plates:
-200 mL of LB + Agar sterilized
-100 uL Spectinomycine 100mg/mL - 2000X

Gibson assembly using 3 different ratios between the vector and the insert for Guanosine +
Total amount 20 uL per reaction

Table18

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Guanosine +	0.2 uL (826,3 ng/uL)
4	Plasmid	1uL (101,4 ng/uL)
5	H20	7.8uL

Table19

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Guanosine +	0.4 uL (826,3 ng/uL)
4	Plasmid	1 uL (101,4 ng/uL)
5	H20	7.6 uL

Table20

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Guanosine +	0.6 uL (826,3 ng/uL)
4	Plasmid	1uL (101,4 ng/uL)
5	H20	7.4 uL

Incubate 30 min at 50°C and it is ready

Transformation for the Gibson Guanosine + assembly

-Thaw 100 µL of chemically competent bacteria on ice
-Add 5 µL of each Gibson guanosine + assembly with different ratios (0.2, 0.4 and 0.6)
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

Cultures for the B Galactosidase test with arabinose 0.2% over night
-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

for 6 tubes (3 Colonies of BH3 and 3 colonies of BH2)

THURSDAY, 20/6/19

Summary:
-PCR for gibson guanosine active colonies
-Results analysis
-B Galactosidase test

For PCR in colony
-Put 15 uL of water and disolve a part of the colony on it
-Incubate it at 100°C for 10 minutes

PCR reaction
-1uL Primer 1
-1uL Primer 2
-2uL of the (colony diluted in water)
-12.5uL Master mix saphire
-8.5 uL milliQ water

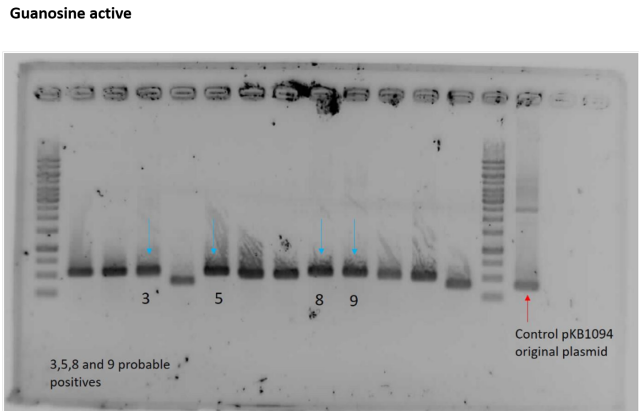
Use the following cycles
- 1:00 at 98°C
- 0:10 at 98°C
- 0:15 at 58°C (change according to primers Tm)
- 1:15 at 72°C
- Repeat 30 times (35 cycles for PCR on colonies)
- 1:00 at 72°C
- ∞ at 12 °C

For the gel verification mix together (Charge in an agarose gel of 1%)
• 5 µl of PCR product (Spahire mix already has the loading buffer)
• 5 µl of Ruler
•100 watts for 30 minutes

Making agarose gels 1%
-4g of agarose
-Disolved in 400 mL of TAE 1X
-Melt in the microwave
-Cold it and put in mold for agar gels

Results:

Guanosine active gibson.png



B Galactosidase assay with the BH3 and BH2 plasmids

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

β galactosidase activity is expressed as Miller Units which are calculated using the equation:
Miller units = $1000 \times [OD_{420} - (1.75 \times OD_{550})/t \times v \times OD_{600}]$
Where *t* is the total time of the reaction expressed in minutes and *v* is the volume of culture used in the assay (which for this protocol is 0.2 mL).

Table21

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH3 Colony 2	0.487	0.011	0.260	77	0.2		
3	BH3 Colony 3	0.286	0.001	0.342	77	0.2		
4	BH2 Colony 3	0.174	0.005	0.464	77	0.2		
5	BH2 Colony 4	0.286	0.018	0.340	78	0.2		

FRIDAY, 21/6/19

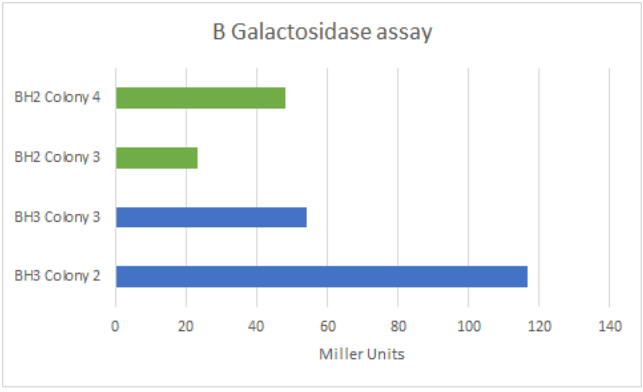
Summary:
Analyze the results of the B Galactosidase
Gibson assembly for Guanosine inactive, Theophylline active, Theophylline inactive
Transformation of the Gibson Asembly

Results of the B Galactosidase

Table17

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH3 Colony 2	0.487	0.011	0.260	77	0.2	116.820	
3	BH3 Colony 3	0.286	0.001	0.342	77	0.2	53.97	
4	BH2 Colony 3	0.174	0.005	0.464	77	0.2	23.126	
5	BH2 Colony 4	0.286	0.018	0.340	78	0.2	47.98	

b galatosidase assay.png



Gibson assembly of Guanosine inactive, Theophylline active, Theophylline inactive using a ratio 1:5
Total amount 20 uL per reaction.

Table22

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Guanosine -	0.5 uL (827,1 ng/uL)
4	Plasmid	1uL (101,4 ng/uL)
5	H20	7.5 uL

Table23

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Theophylline +	0.5 uL (807,9 ng/uL)
4	Plasmid	1uL (101,4 ng/uL)
5	H20	7.5 uL

Table24

	A	B
1	Isothermal assembly reaction mix	equimolar amounts of DNA fragments have to be used
2	Reaction master mix	10uL
3	PCR Theophylline -	0.5 uL (637,3 ng/uL)
4	Plasmid	1uL (101,4 ng/uL)
5	H20	7.5 uL

Incubate 30 min at 50°C and it is ready

Transformation for the Gibson assembly Guanosine -

-Thaw 100 µL of chemically competent bacteria on ice
-Add 5 µL of Gibson assembly guanosine -
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB

Let grow at room temperature for 2 days

Transformation for the Gibson assembly Theophylline +

-Thaw 100 µL of chemically competent bacteria on ice
-Add 5 µL of Gibson assembly theophylline +
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at room temperature for 2 days

Transformation for the Gibson assembly Theophylline -

-Thaw 100 µL of chemically competent bacteria on ice
-Add 5 µL of Gibson assembly theophylline -
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at room temperature for 2 days

MONDAY, 24/6/19

Summary:
-PCR for gibson guanosine inactive, theophylline active, theophylline inactive colonies
-Results analysis
-Cultures for the B Galactosidase test with arabinose 0.2% over night

PCR for gibson guanosine inactive, theophylline active, theophylline inactive colonies
For PCR in colony
-Put 15 uL of water and disolve a part of the colony on it
-Incubate it at 100°C for 10 minutes

PCR reaction
-1uL Primer 1
-1uL Primer 2
-2uL of the (colony diluted in water)
-12.5uL Master mix saphire
-8.5 uL milliQ water

Use the following cycles
- 1:00 at 98°C
- 0:10 at 98°C
- 0:15 at 58°C (change according to primers Tm)

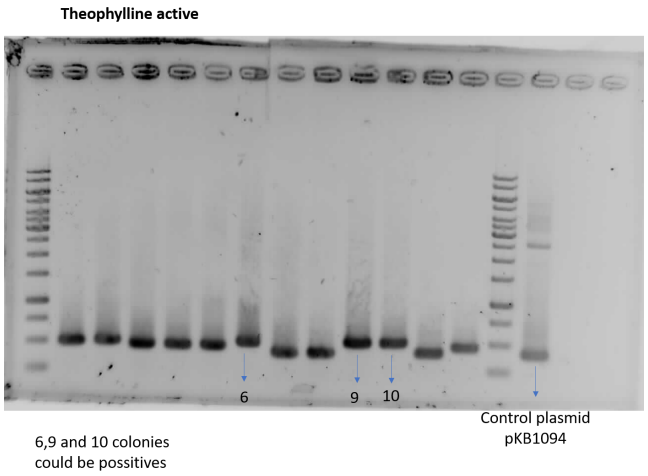
- 1:15 at 72°C
- Repeat 30 times (35 cycles for PCR on colonies)
- 1:00 at 72°C
- ∞ at 12 °C

For the gel verification mix together (Charge in an agarose gel of 1%)
• 5 µl of PCR product (Spahire mix already has the loading buffer)
• 5 µl of Ruler
•100 watts for 30 minutes

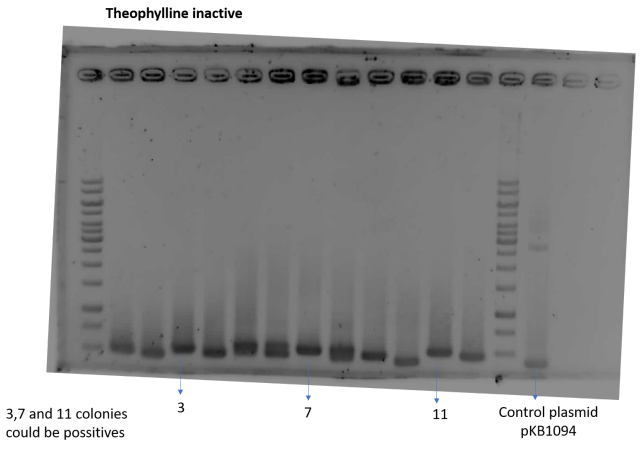
Making agarose gels 1%
-4g of agarose
-Disolved in 400 mL of TAE 1X
-Melt in the microwave
-Cold it and put in mold for agar gels

Results

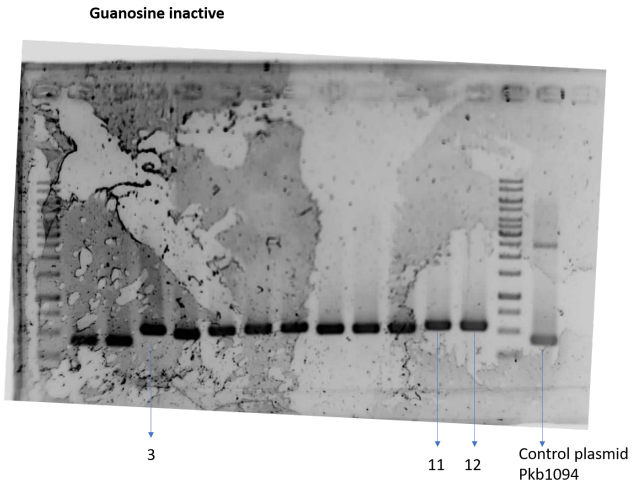
Theophylline active gibson.png



Theophylline inactive gibson.png



Guanosine inactive gibson.png



Cultures for the B Galactosidase test with arabinose 0.2% over night

-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

for 4 tubes (2 Colonies of BH3 and 2 colonies of BH2)

TUESDAY, 25/6/19

Summary:
-B Galactosidase assay with the BH3 and BH2 plasmids
-Cultures for the B Galactosidase test with arabinose 0.2% over night

B Galactosidase assay with the BH3 and BH2 plasmids

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7. Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table25

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH3 Colony 2	0.207	0.015	0.327	10	0.2	276.376147	
3	BH3 Colony 3	0.158	0.005	0.546	9	0.2	151.862027	
4	BH2 Colony 3	0.217	0.033	0.64	10	0.2	124.414063	
5	BH2 Colony 4	0.07	0.002	0.473	10	0.2	70.2959831	

Cultures for the B Galactosidase test with arabinose 0.2% over night

-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

for 4 tubes (2 Colonies of BH3 and 2 colonies of BH2)

WEDNESDAY, 26/6/19

Summary:
-B Galactosidase assay
-Cultures for the B Galactosidase test with arabinose 0.2% over night
-Cultures of the positives colonies (Gibson assembly: Guanosine active, Guanosine inactive, Theophylline active and Theophylline inactive) for miniprep

B Galactosidase assay with the BH3 and BH2 plasmids

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

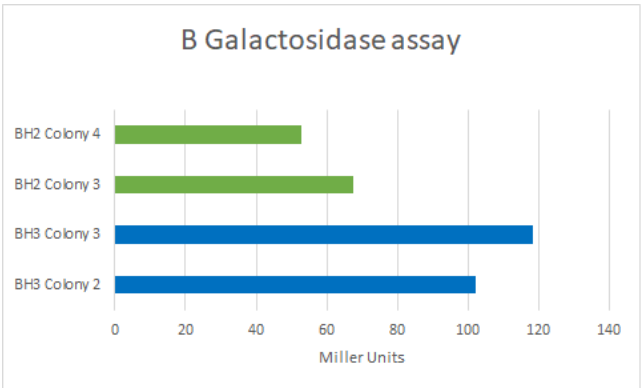
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table26

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH3 Colony 2	0.5	-0.01	0.702	36	0.2	102.38604	
3	BH3 Colony 3	0.679	0.061	0.654	37	0.2	118.243243	
4	BH2 Colony 3	0.316	0.016	0.592	36	0.2	67.5675676	
5	BH2 Colony 4	0.389	0.071	0.697	36	0.2	52.7558584	

B gal assay 3.png



Cultures of the positives colonies (Gibson assembly: Guanosine active, Guanosine inactive, Theophylline active and Theophylline inactive) for miniprep

-Add 3 mL of LB to a sterile tube + 1.5 µL of spectinomycin in each tube.

THURSDAY, 27/6/19

Summary:

-Miniprep of the Gibson colonies (Guanosine active, Guanosine inactive, Theophylline active and Theophylline inactive)

-Growth test using the SAGA method

Miniprep of the positives colonies (Gibson assembly: Guanosine active, Guanosine inactive, Theophylline active and Theophylline inactive)

-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.

-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.

-Add 100µl of Solution I

-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.

-To prevent contamination from genomic DNA, do not vortex.

-A homogenously blue suspension should also be observed.

-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.

-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.

-A fluffy white material forms and lysate should become less viscous.

-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless Centrifuge at 12,000rpm for 5 minutes.

-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.

-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.

-Repeat wash procedure in step 8.

-Centrifuge again at 10,000rpm for 2 minutes without adding anything to remove any residual Wash Solution.*

-Discard the flow-through in the collection tube.

-Transfer the column to a clean 1.5ml microfuge tube.

-Put in the warm box at 50°C during 5 minutes to dry the ethanol in the column

-Put 25uL of warm milliq water into the center part of the column incubate it in the warm box at 50°C during 5 minutes

-Take the tube out from the warm box and incubate at room temperature for 5 minutes

-Centrifuge at 10,000 rpm for 2 minutes.

-Store purified DNA at -20ºC.

-Measure the DNA concentration using the nanodrop

Results

Table27

	A	B	C
1	Sample	Concentration	260/280
2	G+8	243,7 ng/uL	1,92
3	G+8°	68,7 ng/uL	2,01
4	G+9	230,1 ng/uL	1,92
5	G+9°	56,9 ng/uL	1,96
6	G-3	221,5 ng/uL	1,91
7	G-3°	71,0 ng/uL	1,90
8	G-11	229,0 ng/uL	1,90
9	G-11°	81,6 ng/uL	1,85
10	T+10	238,4 ng/uL	1,91
11	T+10°	50,9 ng/uL	1,86
12	T-7	273,8 ng/uL	1,92
13	T-7°	77,3 ng/uL	1,89
14	T+9	157,1 ng/uL	1,91
15	T -11	146,1 ng/uL	1,93

Growth test using the SAGA method
-Add 25mL of LB in a 250mL flask
-Add 25 uL of Ampicillin
-Add 25 uL of Chloramphenicol
-Add 12.5 uL of Spectinomycine
-Add 12.5 uL of Kanamycine
-A tip with the colony
-Growth at 37°C
-Let it 24 hours to measure the samples and the OD precisely

FRIDAY, 28/6/19

Summary:
-Agarose gels 2%
-Miniprep
-Colaboration with Marlbourg
-Repeat growth test using the SAGA method
-Growth test (Manually) BH3 and BH2 strains

Miniprep
Miniprep of the positives colonies (Gibson assembly: Guanosine active, Guanosine inactive, Theophylline active and Theophylline inactive)

-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
-Add 100µl of Solution I
-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.
-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
-Repeat wash procedure in step 8.
-Centrifuge again at 10,000rpm for 2 minutes without adding anything to remove any residual Wash Solution.*
-Discard the flow-through in the collection tube.
-Transfer the column to a clean 1.5ml microfuge tube.
-Put in the warm box at 50°C during 5 minutes to dry the ethanol in the column
-Put 25uL of warm milliq water into the center part of the column incubate it in the warm box at 50°C during 5 minutes
-Take the tube out from the warm box and incubate at room temperature for 5 minutes
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.
-Measure the DNA concentration using the nanodrop

Results

Colaboration with Marlbourg
-Took the pictures from the colonies and count the colonies using ImgLab

Growth test using the SAGA method
-Add 25mL of LB in a 250mL flask
-Add 25 uL of Ampicillin
-Add 25 uL of Chloramphenicol
-Add 12.5 uL of Spectinomycine
-Add 12.5 uL of Kanamycine
-A tip with the colony
-Growth at 37°C
-Let it 24 hours to measure the samples and the OD precisely

MONDAY, 1/7/19

Summary:
-Agarose gels 2%
-Sagas test set up 96 plate
-B galactosidase test of BH3 and BH2 colonies
-Cultures for the B Galactosidase test with arabinose 0.2% over night

Agarose gels 2%
-4g of Agarose
-200mL of TAE X1
-Disolve and melt everything using the microwave
-Let them cold down and put it in molds

-Save at 4°C

Sagas test set up 96 plate

-From the cultures put it on Sunday
-Mesuare the OD of each one
-Adjust 10mL of each culture at 0.01 OD
-Put 100 uL of the 0.01 OD cultures i each file of the plate
-Set up a blanck (LB)
-Let it run for 24 hours at 37°C taking the OD every 10 minutes

B galactosidase test of BH3 and BH2 colonies

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7 (0.5 is ideal). Typically this takes between 90 and 120 min. Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

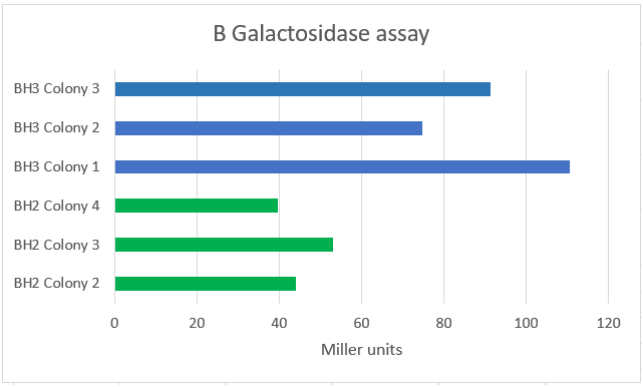
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table28

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH2 Colony 2	0.153	-0.018	0.418	50	0.2	44.138756	
3	BH2 Colony 3	0.183	-0.009	0.374	50	0.2	53.1417112	
4	BH2 Colony 4	0.144	-0.015	0.43	50	0.2	39.5930233	
5	BH3 Colony 1	0.232	-0.019	0.24	50	0.2	110.520833	
6	BH3 Colony 2	0.247	0.03	0.26	50	0.2	74.8076923	
7	BH3 Colony 3	0.325	-0.015	0.385	50	0.2	91.2337662	

b gasl assay 01-07-19.PNG



Cultures for the B Galactosidase test with arabinose 0.2% over night

-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

TUESDAY, 2/7/19

Summary:
-Budget summary of the materials used in Becker's Lab
-Analyzing the data from the Safas test
-Protocols for the characterization part
-Cultures for the B Galactosidase test with arabinose 0.2% over night
-PCR for the 4 aptazymes (Guanosine active, Guanosine inactive, Theophillyn active, Theophillyn inactive)

Budget summary of the materials used in Becker's Lab

https://docs.google.com/spreadsheets/d/11fXH4jt4hBK-DhuOFjI1Em0midnX8zgx/edit?dls=true#gid=1708038100

Analyzing the data from the Safas test

Layout 1.pdf

Cultures for the B Galactosidase test with arabinose 0.2% over night

-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

PCR for the 4 aptazymes (Guanosine active, Guanosine inactive, Theophillyn active, Theophillyn inactive)

For PCR in colony
-Put 15 uL of water and disolve a part of the colony on it
-Incubate it at 100°C for 10 minutes

PCR reaction
-1uL Primer 1
-1uL Primer 2
-2uL of the (colony diluted in water)
-12.5uL Master mix saphire
-8.5 uL milliQ water

Use the following cycles
- 1:00 at 98°C
- 0:10 at 98°C
- 0:15 at 55°C for the Theophillyn and 54°C for the Guanosine
- 1:15 at 72°C
- Repeat 30 times (35 cycles for PCR on colonies)
- 1:00 at 72°C
- ∞ at 12 °C

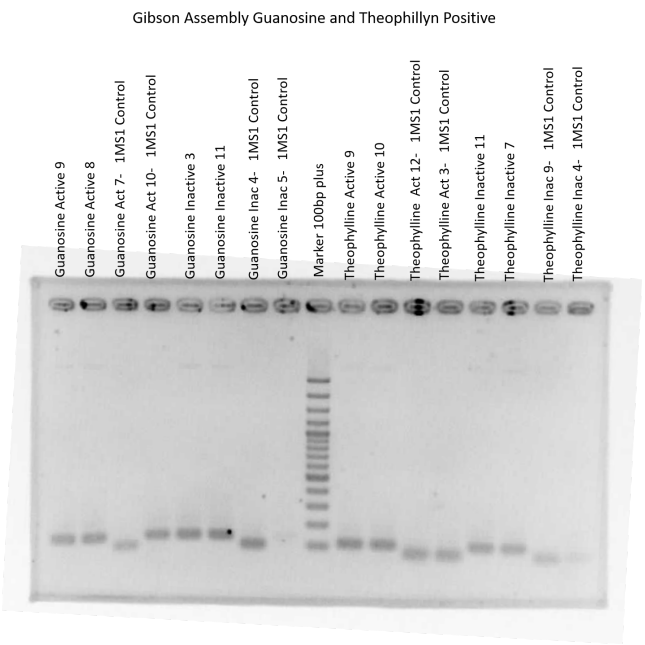
WEDNESDAY, 3/7/19

Summary
-PCR results
-B galactosidase assay
-Cultures for the B Galactosidase test with arabinose 0.2% over night

PCR results

-For the gel verification mix together (Charge in an agarose gel of 2%)
• 12 µl of PCR product (Spahire mix already has the loading buffer)
• 5 µl of Ruler
•100 watts for 30 minutes

Primers to test the Guanosine and theophillyn.png



B galactosidase assay (Modified)

-We measure the OD600 of the of the precultures O.N.

-Add the necessary quentity of the overnight cultures to inoculate 5 mL of LB containing the antibiotics and IPTG (using the 50uM) which should contain a OD600 of 0.1.

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7 (0.5 is ideal). (In our curve test it resulted to take 4 hours 10 minute)

-Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table29

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH2 Colony 2	0.171	0.011	0.255	40	0.2	74.3872549	
3	BH2 Colony 3	-	0.009	0.352	39	0.2		
4	BH2 Colony 4	0.29	0.067	0.354	40	0.2	60.9992938	
5	BH3 Colony 1	0.193	0.008	0.206	40	0.2	108.616505	
6	BH3 Colony 2	-	0.005	0.222	40	0.2		
7	BH3 Colony 3	0.441	0.032	0.325	41	0.2	144.465291	

THURSDAY, 4/7/19

Summary
-Prepare the samples for sequencing
-Prepare plates with spectinomycine
-Transformation for the colonies of Guanosine and Theophillyn positives
-Triple transformation with the aptazymes
-Cultures for the B Galactosidase test with arabinose 0.2% over night

Triple transformation with the aptazymes

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 minute seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

Triple transformation with the aptazymes

-Thaw 200 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid (100ng) of each of the three plasmids

-Put on ice for 30 minutes
-Heatshock at 42°C for 1 minute seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 1 hour
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

Cultures for the B Galactosidase test with arabinose 0.2% over night
-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

FRIDAY, 5/7/19

Summary
-PCR for the colonies of the triple transformation
-B Galactosidase BH3 and BH2

B galactosidase assay (Modified)

-We measure the OD600 of the of the precultures O.N.

-Add the necessary quentity of the overnight cultures to inoculate 5 mL of LB containing the antibiotics and IPTG (using the 50uM) which should contain a OD600 of 0.1.

-Grow cultures at 37° until the cells reach an OD_{600} between 0.3 and 0.7 (0.5 is ideal). (In our curve test it resulted to take 4 hours 10 minute)

-Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped. The ideal range for the OD_{420} is between 0.6 and 0.9. It is important to try and stop each of the assays when they have reached the same OD_{420}.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table30

	A	B	C	D	E	F	G	H
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU	
2	BH2 Colony 2	0.164	0.008	0.269	53	0.2	52.6057375	
3	BH2 Colony 3	0.13	0.007	0.256	52	0.2	44.2270132	
4	BH2 Colony 4	0.252	0.019	0.294	52	0.2	71.5430403	
5	BH3 Colony 1	0.358	0.034	0.295	52	0.2	97.2946545	
6	BH3 Colony 2	0.297	0.007	0.321	52	0.2	85.2953511	
7	BH3 Colony 3	0.439	0.036	0.296	52	0.2	122.141372	

MONDAY, 8/7/19

Summary
-Plates with the three antibiotics
-Spread the positive colonies in plates
-PCR for the colonies of the triple transformation
-Cultures for the B Galactosidase test with arabinose 0.2% over night

Cultures for the B Galactosidase test with arabinose 0.2% over night
-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

TUESDAY, 9/7/19

-Summary
-Transformation of the pBAD and GFP
-B Galastosidase test
-Cultures for the B galactosidase test with arabinose 0.2% over night

Transformation of the pBAD and GFP

-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid to the cells on ice
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

B Galactosidase assay

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table31

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	BH2 Colony 2	0.158	0.032	0.372	53	0.2	25.8673159
3	BH2 Colony 3	0.187	0.02	0.344	53	0.2	41.6849495
4	BH3 Colony 2	0.379	0.014	0.347	52	0.2	98.2320993
5	G+9	0.184	0.008	0.394	45	0.2	47.9413424
6	G-11	0.169	0.009	0.316	46	0.2	52.7139516
7	T+10	0.134	0.005	0.378	46	0.2	36.0162181

Cultures for the B Galactosidase test with arabinose 0.2% over night

-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

WEDNESDAY, 10/7/19

Summary:
-Culture of pBAD and GFP plasmids
-B galactosidase assay of Guanosine active and Guanosine inactive
-Cultures for the B Galactosidase test with arabinose 0.2% over night

Culture of pBAD and GFP plasmids

-3uL Chloramphenicol for pBAD
-3uL Ampicilline for GFP
-3 mL of LB

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table32

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	Guanosine active						40.5380136
3	Guanosine active + 2.5mM						62.3189617
4	Guanosine Inactive						50.7163167
5	Guanosine Inactive + 2.5mM						54.3228988

Cultures for the B Galactosidase test with arabinose 0.2% over night
-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

THURSDAY, 11/7/19

Summary:
-Miniprep of pBAD and GFP
-Digestion of pBad and GFP
-Digestion of the linearized vector
-Run gel to confirm the digestion
-Ligation using the 3A Assembly iGEM approach
-Transformation of the 3A Assembly in a Kanamycine plate
-B Galactosidase assay
-Cultures for the B Galactosidase test with arabinose 0.2% over night

Miniprep of pBAD and GFP

-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
-Add 100µl of Solution I

-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.
-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless
Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
-Repeat wash procedure in step 8.
-Centrifuge again at 10,000rpm for 2 minutes without adding anything to remove any residual Wash Solution.*
-Discard the flow-through in the collection tube.
-Transfer the column to a clean 1.5ml microfuge tube.
-Put in the warm box at 50°C during 5 minutes to dry the ethanol in the column
-Put 25uL of warm milliq water into the center part of the column incubate it in the warm box at 50°C during 5 minutes
-Take the tube out from the warm box and incubate at room temperature for 5 minutes
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.
-Measure the DNA concentration using the nanodrop

Digestion of pBad and GFP

10uL of plasmid
1uL of

Digestion of pBad and GFP
Digestion of the linearized vector
Run gel to confirm the digestion

Transformation of the 3A Assembly in a Kanamycine plate
-Thaw 100 µL of chemically competent bacteria on ice
-Add 1 µL of plasmid to the cells on ice
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C overnight

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped.

-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table33

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	Guanosine active	0.127	0.047	0.334	47	0.2	14.2534081
3	Guanosine active + 2.5mM	0.216	0.053	0.291	47	0.2	45.0573956
4	Guanosine Inactive	0.153	0.042	0.364	47	0.2	23.234744
5	Guanosine Inactive + 2.5mM	0.18	0.022	0.333	47	0.2	45.2047792
6	BH3	0.118	0.049	0.393	47	0.2	8.72990093
7	BH3 + 2.5mM	0.208	0.056	0.285	47	0.2	41.0600971
8	BH2	0.243	0.084	0.385	47	0.2	26.5266648
9	BH2 + 2.5mM	0.207	0.053	0.362	47	0.2	33.5752909

Cultures for the B Galactosidase test with arabinose 0.2% over night
-1.5 uL IPTG (50uM)
-24 uL Arabinose (0.2%)
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB

Digestion of pBad and GFP and the linearized vector ON

Vector
-10 uL plasmid
-1 uL EcoRI
-1 uL PstI
-2 uL fastdigest buffer
-6 uL MilliQ water

GFP
-10 uL plasmid
-1 uL XbaI
-1 uL PstI
-2 uL fastdigest buffer
-6 uL MilliQ water

pBAD
-10 uL plasmid
-1 uL EcoRI
-1 uL SpeI
-2 uL fastdigest buffer
-6 uL MilliQ water

at 37°C Overnight

FRIDAY, 12/7/19

Summary:
-Run gel to confirm the digestion
-Ligation using the 3A Assembly iGEM approach
-Transformation of the 3A Assembly (Characterization construct)
- B galactosidase assay

Run gel to confirm the digestion
-3 uL of each plasmid (individually)
-2 uL of Lx6 Loading buffer
Run it using a 2% Agarose gel at 100 walts for 25 minutes
(The bands presented the correct size: 130 pBAD, 720 GFP and 2209 vector)

Ligation using the 3A Assembly iGEM approach

1:1:3
-10 uL buffer T4 DNA ligase
-1 uL vector
-1 uL GFP
-3 uL pBAD
-1 uL T4 DNA ligase
-4 uL MilliQ water

1:1:5
-10 uL buffer T4 DNA ligase
-1 uL vector
-1 uL GFP

-5 uL pBAD
-1 uL T4 DNA ligase
-2 uL MilliQ water

Leave it at room temperature for 2 hours

-Transformation of the 3A Assembly (Characterization construct)
-Thaw 100 µL of chemically competent bacteria on ice (DH5-Alpha Cells)
-Add 1 µL of 3A Assembly (ligation) to the cells on ice
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow for the weekend on the bench

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).

-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.

-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.

-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.

-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.

-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.

-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.

-Record the time at which each reaction was stopped.

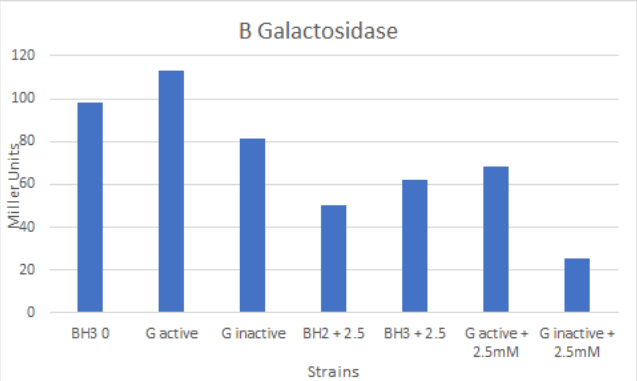
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.

-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table34

	A	B	C	D	E	F	G	H	I
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU		
2	BH2 0	0.126	0.014	0.298	45	0.2	37.8448919		
3	BH3 0	0.343	0.023	0.342	45	0.2	98.3593242		
4	G active	0.328	0.016	0.294	45	0.2	113.378685		
5	G inactive	0.289	0.022	0.342	45	0.2	81.3840156		
6	BH2 + 2.5	0.193	0.022	0.34	45	0.2	50.4901961		
7	BH3 + 2.5	0.248	0.029	0.354	45	0.2	61.9114878		
8	G active + 2.5mM	0.212	0.024	0.277	45	0.2	68.1909346		
9	G inactive + 2.5mM	0.281	0.111	0.378	45	0.2	25.4997061		

beta gal 12-07-2019.png



MONDAY, 15/7/19

Summary:
-Digestion pBad plasmid
-Ligation 1:1:3 (Vector, pBad, GFP)
-Transformation (ligation)
-Preculture with arabinose powder
-Preculture colony obtained in the digestion from Friday
-Colaboration with Marburg (Pictures and counting colonies)

Digestion of pBad plasmid

pBAD

-10 uL plasmid
-1 uL EcoRI
-1 uL SpeI
-2 uL fastdigest buffer
-6 uL MilliQ water

at 37°C 2 hours

Ligation using the 3A Assembly iGEM approach

1:1:3
-10 uL buffer T4 DNA ligase
-1 uL vector
-1 uL GFP
-3 uL pBAD
-1 uL T4 DNA ligase
-4 uL MilliQ water
Leave it at room temperature for 2 hours

-Transformation of the 3A Assembly (Characterization construct)
-Thaw 100 µL of chemically competent bacteria on ice (DH5-Alpha Cells)
-Add 1 µL of 3A Assembly (ligation) to the cells on ice
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C over night

Cultures for the B Galactosidase test with arabinose 0.2% powder over night
-1.5 uL IPTG (50uM)
-6mg of arabinose powder
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB
at 37°C overnight

Precultures for the ligation colony
-A tip qith the culture
-1.5 uL Kananamycine
-3 mL of LB
at 37°C overnight

Pictures of the Marburg collaboration

TUESDAY, 16/7/19

Summary:
-Miniprep for the culture (ligation)
-Restriction enzyme to verify the construct (ligation)
-Run in an agarose gel
-B galactosidase assay (using agarose in powder directly in the precultures)
-Precultures for the B Galactosidase test with arabinose 0.2% (using powder) over night

Miniprep for the preculture (ligation)
-Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes.
-Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.

-Add 100µl of Solution I
-Add 200µl of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.
-To prevent contamination from genomic DNA, do not vortex.
-A homogenously blue suspension should also be observed.
-If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
-Add 350µl of Solution III, and mix gently. Incubate at room temperature for 1 minute.
-A fluffy white material forms and lysate should become less viscous.
-If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless
Centrifuge at 12,000rpm for 5 minutes.
-Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
-Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
-Repeat wash procedure in step 8.
-Centrifuge again at 10,000rpm for 2 minutes without adding anything to remove any residual Wash Solution.*
-Discard the flow-through in the collection tube.
-Transfer the column to a clean 1.5ml microfuge tube.
-Put in the warm box at 50°C during 5 minutes to dry the ethanol in the column
-Put 25uL of warm milliq water into the center part of the column incubate it in the warm box at 50°C during 5 minutes
-Take the tube out from the warm box and incubate at room temperature for 5 minutes
-Centrifuge at 10,000 rpm for 2 minutes.
-Store purified DNA at -20ºC.
-Measure the DNA concentration using the nanodrop

Restriction enzyme to verify the construct (ligation)

GFP
-10 uL plasmid
-1 uL XbaI
-1 uL PstI
-2 uL fastdigest buffer
-6 uL MilliQ water

pBAD
-10 uL plasmid
-1 uL EcoRI
-1 uL SpeI
-2 uL fastdigest buffer
-6 uL MilliQ water

at 37°C for 2 hours
Run in a 2% agarose gel

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use 50 µL of the overnight cultures to inoculate 3 mL of LB containing the antibiotics and IPTG (using the 50uM).
-Grow cultures at 37° until the cells reach an OD_{600} at 0.3 . Typically this takes between 90 and 120 min.
Place the cultures on ice for 30 min.
-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.
-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.
-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.
-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.
-Mix the tubes by gently shaking or vortexing them at a low speed.
-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.
-Record the time at which each reaction was stopped.
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.
-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table35

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	BH2 0	0.252	0.027	0.32	53	0.2	60.3626179
3	BH3 0	0.423	0.021	0.372	53	0.2	97.9534388
4	G active	0.332	0.024	0.312	53	0.2	87.6874698
5	G inactive	0.371	0.03	0.295	53	0.2	101.854813
6	BH2 + 2.5	0.113	0.016	0.292	54	0.2	26.9533232
7	BH3 + 2.5	0.392	0.023	0.304	54	0.2	107.13633
8	G active + 2.5mM	0.222	0.023	0.344	53	0.2	49.8436814
9	G inactive + 2.5mM	0.277	0.023	0.296	53	0.2	75.4557624

Preultures for the B Galactosidase test with arabinose 0.2% powder over night
-1.5 uL IPTG (50uM)
-6mg of arabinose powder
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB
at 37°C overnight

WEDNESDAY, 17/7/19

Summary:
-Transformation of the ligation (10uL)
-B galactosidase assay (Using the precultures directly)
-Precultures for the B Galactosidase test with arabinose 0.2% (using powder) over night
-Precultures for all the bacterias with important plasmids to keep it on glycerol

B Galactosidase assay of Guanosine active and Guanosine inactive

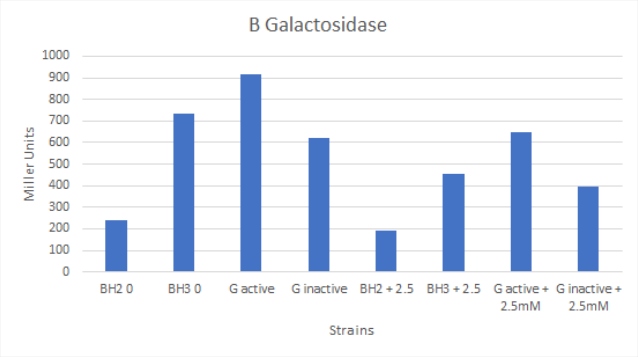
-Use the overnight precultures in order to have an OD of 0.5
-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.
-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.
-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.
-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.

-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.
-Mix the tubes by gently shaking or vortexing them at a low speed.
-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.
-Record the time at which each reaction was stopped.
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.
-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table36

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	BH2 0	1.11	0	0.5	46	0.2	241.304348
3	BH3 0	3.312	0	0.5	45	0.2	736
4	G active	4.208	0	0.5	46	0.2	914.782609
5	G inactive	2.852	0	0.5	46	0.2	620
6	BH2 + 2.5	0.878	0	0.5	46	0.2	190.869565
7	BH3 + 2.5	2.1	0	0.5	46	0.2	456.521739
8	G active + 2.5mM	3.252	0	0.5	50	0.2	650.4
9	G inactive + 2.5mM	1.966	0	0.5	50	0.2	393.2

beta gal precultures directly.png



-Transformation of the 3A Assembly (Characterization construct)
-Thaw 100 µL of chemically competent bacteria on ice (DH5-Alpha Cells)
-Add 10 µL of 3A Assembly (ligation) to the cells on ice
-Put on ice for 30 minutes
-Heatshock at 42°C for 45 seconds
-Return on ice for 5 minutes
-Add liquid LB to 1 mL
-Regenerate at 37°C for 30 minutes
-Centrifuge cells and resuspend in 100 µL of liquid LB
-Plate cells and resuspend in 100 µL of liquid LB
-Let grow at 37°C over night

Preultures for the B Galactosidase test with arabinose 0.2% powder over night

-1.5 uL IPTG (50uM)
-6mg of arabinose powder
- 1.5 uL Spectinomycine
-3uL Chloramphenicol
-3uL Ampicilline
-1.5 uL Kananamycine
-3 mL of LB
at 37°C overnight

THURSDAY, 18/7/19

Summary:
-B Galactosidase assay of Guanosine active and Guanosine inactive
-B Galactosidase assay using the M.R. idea
-Precultures of the miniprep for the ligation
-Precultures for the B Galactosidase test with arabinose 0.2% powder over night
-Glycerol stock of the BH3 and BH2 strains as well as the individual plasmids
-Starter for beta-gal test (Katya)

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use the overnight precultures in order to have an OD of 0.5
-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.
-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.
-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.
-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.
-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.
-Mix the tubes by gently shaking or vortexing them at a low speed.
-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.
-Record the time at which each reaction was stopped.
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.
-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table37

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	BH2 0	3.524	0	0.5	48	0.2	734.166667
3	BH3 0	4.536	0	0.5	48	0.2	945
4	G active	3.756	0	0.5	48	0.2	782.5
5	G inactive	3.312	0	0.5	48	0.2	690
6	BH2 + 2.5	3.596	0	0.5	48	0.2	749.166667
7	BH3 + 2.5	4.692	0	0.5	48	0.2	977.5
8	G active + 2.5mM	2.496	0	0.5	49	0.2	509.387755
9	G inactive + 2.5mM	3.224	0	0.5	49	0.2	657.959184

Katya

Media preparation for 14 cultures :

-LB 1x 150 ml + 150 ml Spectinomycin, Ampecilin, Chloramphenicol + 300 mg Arabinose (Cf = 2 g/L)

- 50 ml of this culture + 14 mg Guanosine (Cf = 1M)
- 50 ml of this culture + 13 mg Theophiline (Cf = 2,5M)

Bacteria was added from petrie dish with tips :

- Theophinine inactive 7
- Theophiline active 11
- Guanosine inactive 9
- Guanosine active 10
- BH3
- BH2

The cultures was incubated overnight at 37 C with shaking in 3 ml of discribed media

FRIDAY, 19/7/19

Summary:
-B Galactosidase assay of Guanosine active and Guanosine inactive
-B Galactosidase assay using the M.R. idea
-Digestions with the minipreps using (XbaI and SpeI) (EcoRI and PstI)
-Pictures collaboration (Marburg)
-Precaltures preparetion and freeze of culture (Yasmine)
-Transformation of DH10beta cells for caracterisation

B Galactosidase assay of Guanosine active and Guanosine inactive

-Use the overnight precultures in order to have an OD of 0.5
-Place 1 mL of the cultures in a cuvette to read and record the OD_{600} value.
-Set up the assay tube (in duplicate) by placing 200 µL of the cultures in a small glass test tubes that contain 800 µL of Z-buffer (with BME). Be sure to set up a tube that will serve as a blank using 200 µL of LB.
-Add 30 µL of 0.1% SDS and 60 µL of chloroform to each tube and vortex for 10 s.
-Allow tubes and the 4 mg/mL ONPG solution to equilibrate at 28° in a water bath for 15–20 min.
-Start the assays by adding 200 µL of the ONPG solution to each assay tube, be sure to record the time at which each of the assays was started. It is recommended to add ONPG to each tube at 5 s intervals.
-Mix the tubes by gently shaking or vortexing them at a low speed.

-When the tubes turn yellow, stop the reactions by adding 500 µL of 1 M Na_2CO_3.
-Record the time at which each reaction was stopped.
-Gently vortex the reaction tubes then allow them to sit at room temperature for 10–15 min to allow the chloroform and cell debris to settle.
-Place 1 mL of the reaction into a cuvette and read and record the OD_{420} and the OD_{550} value.

Table38

	A	B	C	D	E	F	G
1	Sample	OD420	OD550	OD600	t (min)	v (ml)	MU
2	BH2 0	2.108	0	0.5	46	0.2	458.26087
3	BH3 0	3.124	0	0.5	46	0.2	679.130435
4	G active	2.704	0	0.5	46	0.2	587.826087
5	G inactive	3.444	0	0.5	46	0.2	748.695652
6	BH2 + 2.5	2.032	0	0.5	46	0.2	441.73913
7	BH3 + 2.5	2.548	0	0.5	46	0.2	553.913043
8	G active + 2.5mM	2.036	0	0.5	46	0.2	442.608696
9	G inactive + 2.5mM	2.292	0	0.5	46	0.2	498.26087

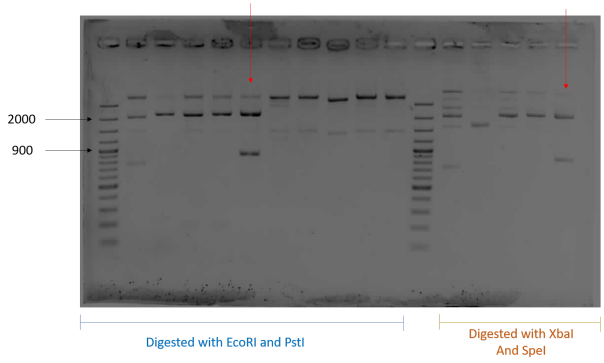
Digestions with the minipreps using (XbaI and SpeI) (EcoRI and PstI)

-4 uL plasmid
-1 uL XbaI
-1 uL SpeI
-2 uL fastdigest buffer
-12 uL MilliQ water

-4 uL plasmid
-1 uL EcoRI
-1 uL PstI
-2 uL fastdigest buffer
-6 uL MilliQ water

at 37°C for 2 hours / 80°C for 20 minutes to kill the raction
Run in a 2% agarose gel

Characterization digestion.png



pBAD + GFP = 130 bp + 720 bp =850 bp
Vector = 2204 bp

Precaltures preparetion and freeze of culture

The OD600 of starter was taken and 50 ul of each culture were incubate in previosly discribed media + 5uM IPTG (100 uM IPTG in prepared before)
The cultures was incubated 1h30 at 37 C with shaking
In 1h30 the cultures are spun and freeze for the week-end

Transformation of DH10beta cells for caracterisation

- 100 ng of plasmide was aded in 50 ul of bacteria without mixing
- DH10beta cells are stock in ice during 20 min
- Heat chock is performed 30 sec in water bath 42 C ans then 2 min on ice
- 500 ul of fresh LB was added
- Bacteria are shaked in 37 C durind 1 hour
- The LB + Kana plates were spreaded with 500 ul and 50 ul of bacteria
- The plates are stocked in aluminium on banch whole week-end

Lundi 21/07 ----------

- Incubate starter of clone for caracterisation
- Beta-gal test on 96 well plate
- Incubate new starter for bgal test
- Incubate preculture for caracterisation test

Incubate starter of clone for caracterisation

3 clone from fransformation was selected and incubated in 3 ml of LB + Kana for 10 hours

Beta-gal test on 96 well plate

The pellet was dilutes in 400 ul of PBS and OD600 was measured
The cultures are concentrated of diluted for arrive to OD600 = 0,5
50 ul of cultures + 50 ul of P-ber + 150 ul of ONPG (0,8 g/ml) are vortexed
50 each culture mix putud on 96-well plate in triplicate and b-gal activity is mesured during 1h.
The graph of evolution is constracted by comparaison of slope of each culture

Incubate new starter for bgal test

-LB 1x 250 ml + 250 ml Spectinomycin, Ampecilin, Chloramphenicol + 500 mg Arabinose (Cf = 2 g/L)

- 80 ml of this culture + 36 mg Guanosine (Cf = 1M)
- 80 ml of this culture + 28 mg Theophiline (Cf = 2,5M)

Bacteria was added from petrie dish with tips :

- Theophinine inactive 7
- Theophiline active 11
- Guanosine inactive 9
- Guanosine active 10
- BH3
- BH2

The cultures was incubated overnight at 37 C with shaking in 3 ml of discribed media

Incubate preculture for caracterisation test

100 of each of 3 different starters was added in

- LB media + Kana + Arabinose 12 uM
- LB media + Kana +Arabinose 6uM
- LB media + Kana +Arabinose 3 uM
- LB media + Kana +Arabinose 1,5 uM
- LB media + Kana

The cultures was incubated 20h at 37 C with shaking

Mardi 22/07 ----------

- **Precaltures preparetion + Beta-gal test on 96 well plate**
- **GFP measure for caracterisation**

Precaltures preparetion + Beta-gal test on 96 well plate

The OD600 of starter was take and 50 ul of each culture incubated in previously described media + 5uM IPTG (100 uM IPTG in prepared before) repeat twice
Theophiline level is 5M for increase aptazyme efficiency
The cultures was incubated 1h30 at 37 C with shaking
The cultures are dilutes in 1ml of PBS and OD600 was measured
The cultures are concentrated of diluted for arrive to OD600 = 0,5
50 ul of cultures + 50 ul of P-ber + 150 ul of ONPG (0,8 g/ml) are vortexed
50 each culture mix putud on 96-well plate in triplicate and b-gal activity is mesured during 1h. Theo in PBS and PBS are used like a blanc.
The graph of evolution is constracted by comparaison of slope of each culture

GFP meseare for caracterisation

GFP is detected but the levels are simular between different concantration. After 20 hours we arrived to saturation of expession = try meseare time evolution.