

Note: Figure1. Gel electrophoresis results of level 2; We tested different vector to insert ratios for Golden Gate assembly and verified the assemblies using primers specific to each module. The vector insert ratio was 1:5 for lanes 1–3 and 1:7 for lanes 4–6.

Done by Sophie C

Date: 20260902

Title: Miniprep L.

Purpose: Isolate and purify small amounts of plasmid DNA from liquid culture of L.

Procedure:

L:3P-EEBD-RBS0-csn:GanaA-Gala-L3S2P21

1. Transfer 1500 μL of overnight liquid culture inoculated from colonies on the streak plates used for the colony (Done in 5/13) to each tube (total 8 tubes)
2. Centrifuge for 3 min at 6800 g, 22°C
3. Aspirate supernatant from 8 tubes with micropipette
4. Add the rest of the liquid culture
5. Centrifuge at 22°C 23944 g for 1 min
6. Collect supernatant transfer to column
7. Centrifuge at 1,7900 g for 30 sec
8. Repeat this step, until collect all the supernatant
9. Add 500 μL PB buffer and centrifuge at 17,900 g for 30 seconds.
10. Discard the flow-through at 750 μL PE buffer
11. Centrifuge at 17,900Xg for 3 minutes
12. Discard the flow through and centrifuge at 17900 g for 3 minutes.
13. Transfer the column to new 1.5 ml microtube
14. Add 50 μL nuclease free water at 65°C
15. Incubate at RT for 3 minutes
16. Centrifuge at 17,900 g for 3 minutes
17. Measure the concentration

	Concentration(ng/ μL)	A260/230	A260/280
L	25.3	2.433	1.888

Done by Sophie C, Abby K.

Date: 20260902

Title: Colony PCR

Purpose: To verify bacterial colonies after a transformation step in molecular cloning

Procedure:

O:pSTK-Exp1-Pveg-EEBD-RBS0-SamyQ:Bopepe4-2.2-B0015

1. Prepare the following reaction X17 in 8-strip PCR tubes

Fw primer (pSTK-Exp)	1 μL
Rv primer (Bopep4)	1 μL
A-amp master mix	10 μL

ddH ₂ O	8μL
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2. Pick up colonies with tip and steak grid
3. Dispense tips in their corresponding tube
4. Take out tips after 5 minutes
5. Close lid and spin down
6. Place in PCR machine for thermal cycle

95°C	5min	x1
95°C	20sec	x35
60°C	20sec	
72°C	1min	
25°C	5min	x1

Done by Chloe, Abby K, Sophie C.

Date: 20260902

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

L:3P-EEBD-RBS0-csn:GanA-Gala-L3S2P21

X6:ACDI

1. Set up water bath at 42°C
2. Add 2μL plasmid to 30μL competent cell
3. Incubate on ice for 20min
4. Heat shock 42°C for 45sec
5. Incubate on ice for 3min
6. Spread on LBA and LBC plates

Done by Sophie C, Abby K

Date: 20260902

Title: Bioreactor Sampling

Purpose: sample and analyze the distribution, stability, and expression of a target protein and host cells within a bioreactor setup over time.

Procedure:

1. Use syringe to sample the liquid from shell side
2. Turn off the pump. Open the lumen side Liner valve
3. Insert the syringe on the sample and aspirate 1mL sample
4. Use filtered air to push liquid back to the lumen side and clean up the sample
5. Check the Lumen valve setting and turn on the pump to PWN 100(~385mL/min)

6. Centrifuge the sample at 5000g in 4°C for 5min
7. Transfer 100mL shell side sample, lumen side sample in new microtubes
8. Add 1mL 100X proteinase inhibitor to sample and storage in -80°C for ELISA and DNPH assay
9. Transfer another 100mL shell side and lumen side sample to new microtubes.
10. Add 20mL 6X sampling buffer and incubate in 95°C for 5 min for SDS-PAGE
11. Remove the supernatant from the lumen side sample. Resuspend pellet in 1X Celbyetic B
12. Add
 - 2.5μL DNase I
 - 1μL Protease inhibitor
 - 2μL Lysozyme
13. Add 20μL 6X sampling buffer and incubate in 95°C for 5min for SDS-PA6B
14. Sample the shell side medium at 16hr and 24hr. Spread on the LB plate overnight antibiotics

Done by Chars

Date: 20260902

Title: SDS-PAGB and Western Blot

Purpose: detect specific protein within a complex biological sample

Procedure:

1. Prepare the 10% polyacrylamide gel
2. Loading sample

(lumen 16hr)		(Lumen 18hr)		(lumen 20hr)		(Lumen 16hr)		(lumen18hr)		(Ilumen 20hr)	
M	Cell lysate	Cell lysate	Cell lysate	Medium	Medium	Medium	Medium	Medium	Medium	Medium	
50	50	50	50	50	50	50	50	50	50	50	
(shell 16hr)		(shell 18hr)		(shell 2hr)							
Medium	Medium	Medium	Medium								
50	50	50	50								
3. Searching in 200V 10min
4. Resolve the gel in 180V 40min
5. Transfer to PVDF (MeOH treated) in 100V for 90min
6. Blocking in 5% BSA in TBST for 2hr in RT
7. Incubate in conti His-tag mouse antibody(CST) in 4°C overnight

Done by Chars

Date: 20260902

Title: Electroporation competent cell preparation

Purpose: To prepare the competent cell for B.sub WB800 electroporation

Procedure:

1. Culture overnight B.sub WB800 in 37°C 200rpm

2. Dilution 16-8old of B.sub WB800(overnight) in GM medium, incube in 37°C 200rpm until O.D. reach 0.926
3. Add final concentration of 1 % threonine, 0.03% glycine and 2% Tween80
4. Incubate for 1hour in 37°C 200rpm
5. Incubate in ice with shake for 5 min
6. Collect the cell with centrifuge at 5000g 10min
7. Resuspend in 1/20 of original volume in EP medium
8. Stored the cell at -80°C

P.S.

1. GM medium: LB+0.5M sorbitol
2. EP medium:0.5M sorbitol, 0.5M mannitol , 10% glycerol in water
 - > The concentration of Glycine and Teen 80 was wrong
 - > It should be 2% Glycine and 0.03% Tween 80

Done by Gabriel

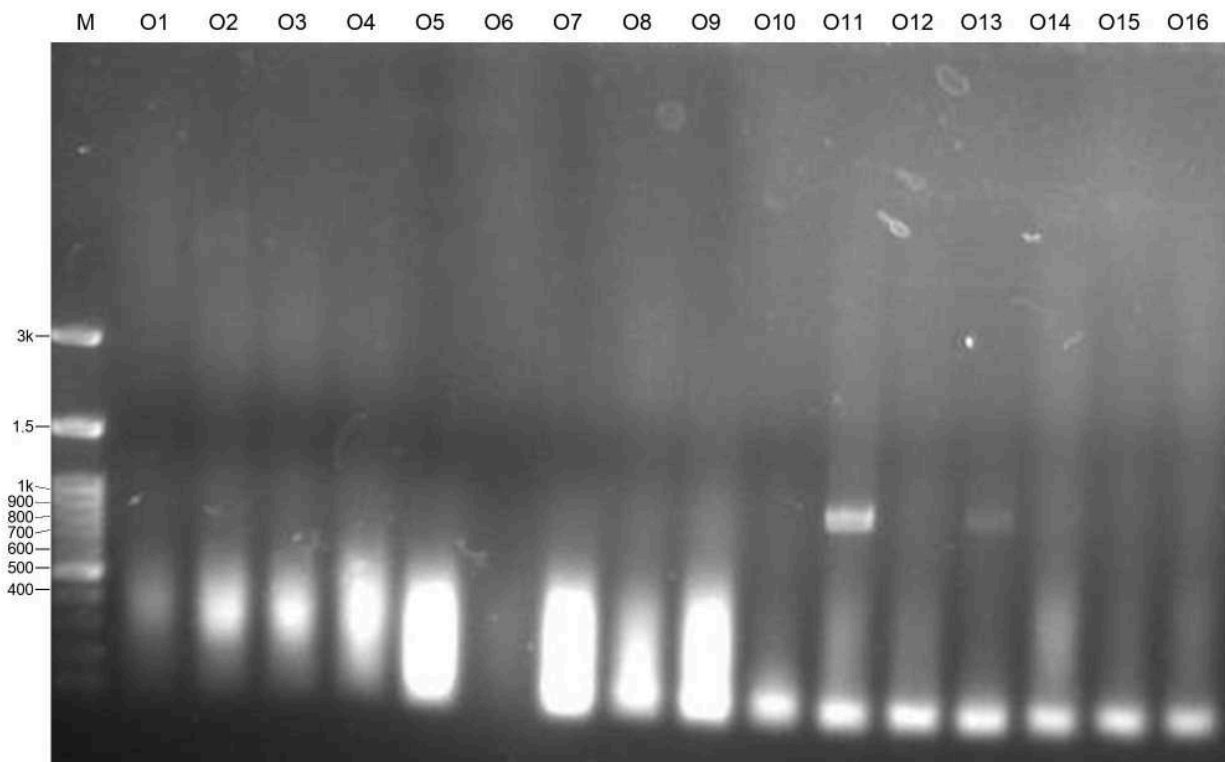
Date: 20260903

Title: gel electrophoresis

Purpose: verify the size of colony PCR product

Procedure:

1. Load the gel in the layout shown below (5 μ L each):
2. Resolve at 100 v for 30 min



M: 3kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
O: Pveg-EEBD-RBS0-SamyQ:Bopep 4 R	EXP fw	Bopep 4 R	678

Done by Sophie C

Date: 20260903

Title: golden gate assembly

Purpose: assemble part

Procedure:

M:PcpcG2-veg-EEBD-RBS0-csn:GanA-Gala-L3S2P21

Part	Amount(μ L)
pSTK-1D-sfGFP 132 ng/ μ L,3120bp	1.5 (1/10)
PcpcG2-veg 168 ng/ μ L,235bp	0.91(1/50)
EEBD-RBS0 151 ng/ μ L,2392bp	1.03(1/5)
GanA-Gala 177 ng/ μ L,4148bp	1.22(1/4)
L3S2P21 170 ng/2145bp	1.64(1/10)
Csn 81 ng/ μ L,2188bp	1.07(1/10)
pSTK-OCZ-Histag6X 135 ng/ μ L,2107bp	1(1/5)
T4 DNA ligase buffer	1
T4 DNA ligase	0.5
Sterile water	0.63
BsaI-HFv2	0.5

O:Pveg-EEBD-RBS0-SamyQ-Bopep4-2.2-B0015

Part	Amount(μ L)
EXP1 162 ng/ μ L,6929bp	1.4 (1/5)
Pveg 168 ng/ μ L,2361bp	1.82(1/50)
EEBD-RBS0 151 ng/ μ L,2392bp	1.03(1/5)
SamyQ:Bopep4 2.2 101 ng/ μ L,2263bp	1.17(1/4)
B0015 107 ng/2213bp	1.34(1/5)

T4 DNA ligase buffer	1
Sterile water	1.24
Bsal-HFv2	0.5
T4 DNA ligase	0.5

N:Pveg-EEBD-RBS0-SamyQ-Bopep4(2.1)-B0015

Part	Amount(μ L)
pSTK-Exp1-sfGFP 162 ng/ μ L,6429bp	1.4 (1/5)
Pveg 168 ng/ μ L,2361bp	1.82(1/50)
EEBD-RBS0 151 ng/ μ L,2392bp	1.03(1/5)
SamyQ:Bopep4 2.2 137 ng/ μ L,2263bp	1.07(1/5)
B0015 107 ng/2213bp	1.34(1/5)
T4 DNA ligase buffer	1
Sterile water	1.34
Bsal-HFv2	0.5
T4 DNA ligase	0.5

Done by Sophie C

Date: 20260904

Title: Transformation(N,O,M)

Purpose: Transform and cultivate engineered bacteria on agar plate

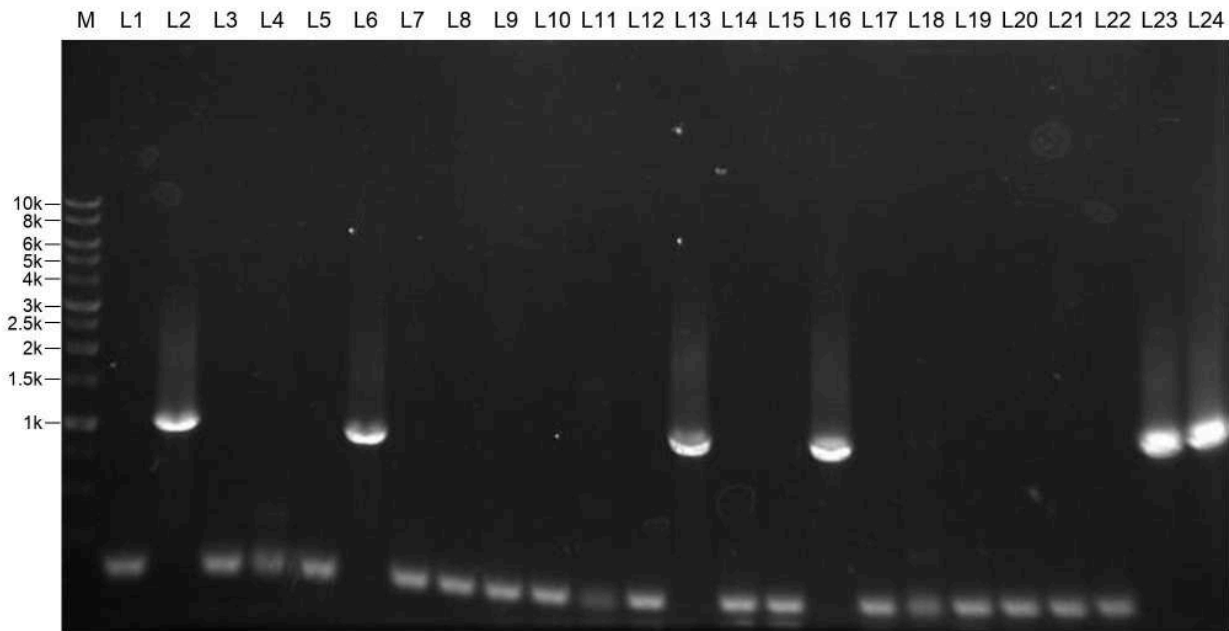
Procedure:

1. Set up water bath at 42 °C
2. Thaw competent cells till 50% thawed, add 5 µL plasmid
3. Incubate on ice for 20min
4. Heat shock for 45 sec
5. Incubate on ice for 3 min
6. Add 1 mL SOC medium
7. Incubate at 37°C for 1 hr
8. Centrifuge for 5 min at 5000 g
9. Take out ~800 µL supernatant
10. Resuspend pellet
11. Spread on LBA
12. Incubate at 37°C overnight

Done by Sophie C

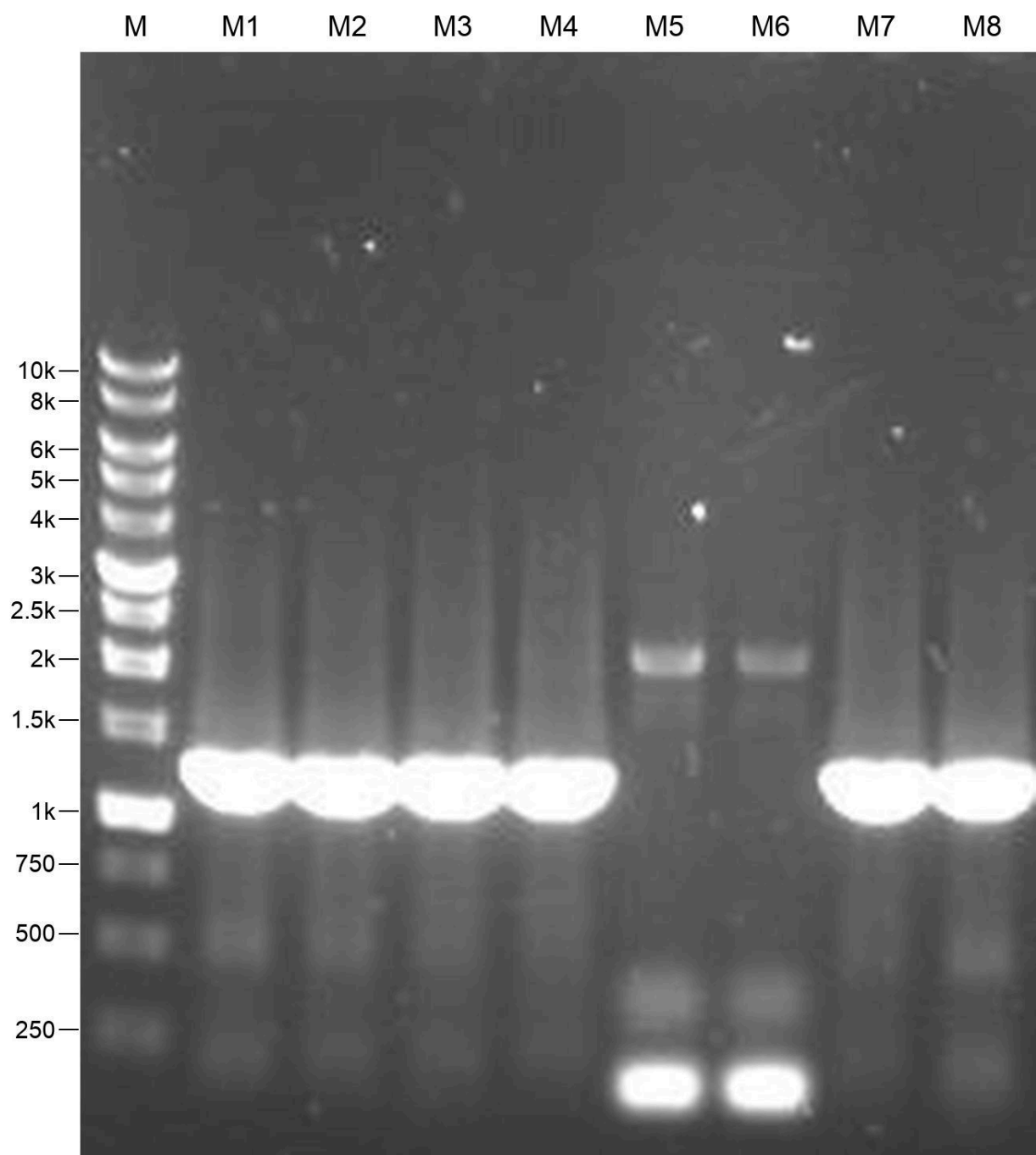
Date: 20260905
Title: Electrophoresis
Purpose: Checking Colony PCR result
Procedure:

- 1. Load the gel in the layout shown below (5 µL each):
- 2. Resolve at 100 v for 30 min



M: 1kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
L1-L24: pSTK-EXP1-3P-EEBD-RBS0-C sn:GanA-Gala-L3S2P21	EXP fw	Csn rv	3535

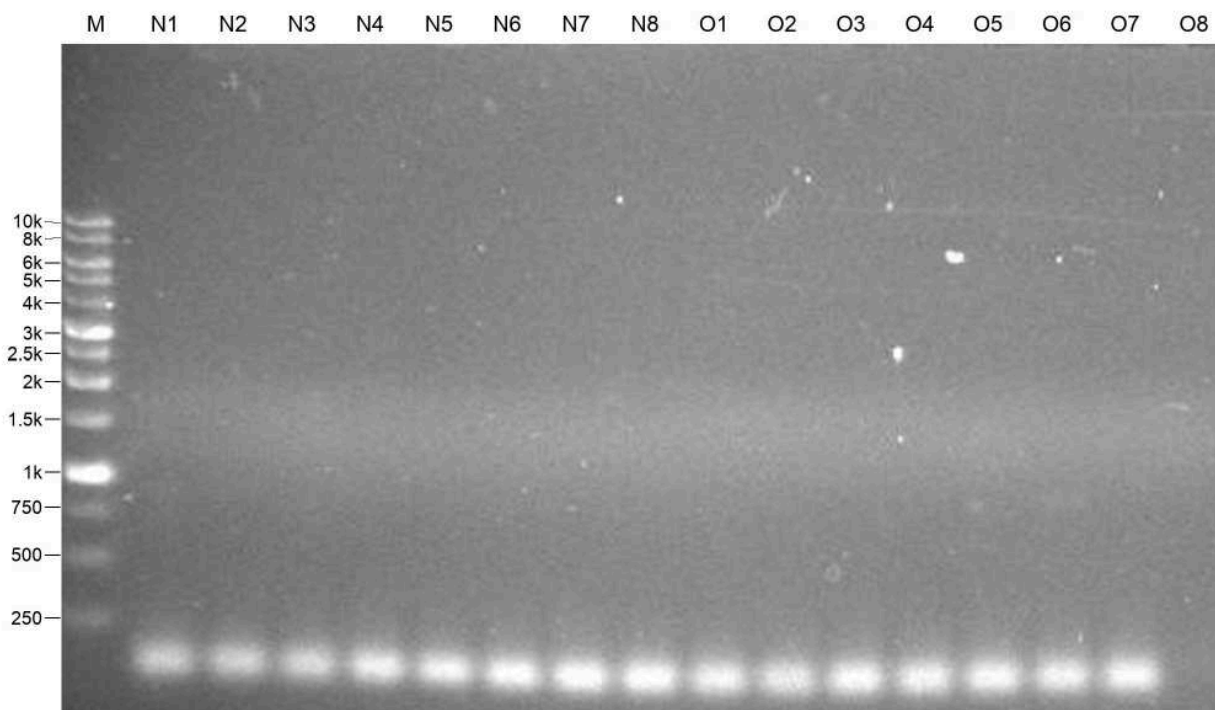
- 3. Load the gel in the layout shown below (5 µL each):
- 4. Resolve at 100 v for 30 min



M: 1kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)

M1-M8: PcpcG2-veg-EEBD-RBS0-Gan A-Gala-L3S2P21	Biobrick fw	pSTK rv	2400-2500
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5. Load the gel in the layout shown below (5 μ L each):
6. Resolve at 100 v for 30 min



M: 1kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
N1~N8: Pveg-EEBD-RBS0-SamyQ:Bopep4-2.1-B0015	EXP fw	Bopep4 R	678
O1~O8: Pveg-EEBD-RBS0-SamyQ:Bopep4-2.2-B0015	EXP fw	Bopep4 R	678

Done by Sophie H, Alex

Date: 20260905

Title: LBA plate with glycerol and LB medium

Purpose: Male plates and medium for further experiments

Procedure:

1. Formula as below:

LBA

LB broth powder	6.25 g
Agar powder	3.75 g
Glycerol	2 mL
ddH ₂ O	250 mL

LB

LB broth powder	6.25 g
Agar powder	0 g
ddH ₂ O	250 mL

1. Add one magnetic stirring bar to the flask
2. Place the flask containing the mixture on the hot plate, and stir until no sediments
3. Remove the stirring bar from the flask
4. Cover the flask with aluminum foil and apply on indicator tape to ensure the mixture is successfully autoclave
5. Place the flask in autoclave
6. Place the new petri dishes in BSC and turn on the UVC
7. Set up water bath at 55 °C
8. Add 250 µL ampicillin
9. Use serological pipette to add 20 mL of melting agar into the petri dish
10. Let the agar plates dry for at least 1 hr in the BSC

Done by Sophie H, Alex

Date: 20260905

Title: Colony PCR of N, O, M, L

Purpose: Amplify engineered DNA plasmid

Procedure:

N: Pveg-EEBD-RBS0-SamyQ-Bopep4-2.1-B0015

O: Pveg-EEBD-RBS0-SamyQ-Bopep4-2.2-B0015

M: PcpcG2-veg

L: 3P-EEBD-RBS0-GanA-Gala-L3S2P21

1. Prepare the reaction as below:

L:

	1 rxn	24 rxn
Exp-Fw-pr	1 µL	24 µL
Csn-Rv-pr	1 µL	24 µL
Qamp master mix	10 µL	240 µL
ddH ₂ O	8 µL	192 µL

O,N

	1 rxn	16 rxn
SamyQ-Fw-pr	1 µL	16 µL
Bopep4-Rv-pr	1 µL	16 µL
Qamp master mix	10 µL	160 µL
ddH ₂ O	8 µL	128 µL

M

	1 rxn	8 rxn
Exp-Fw-pr	1 µL	8 µL
STK-Rv-pr	1 µL	8 µL
Qamp master mix	10 µL	80 µL
ddH ₂ O	8 µL	64 µL

2. Dispense reaction to 8-strip PCR tube
3. Pick up the colonies from the 2.0 kv, 2.1 kv, 2.2 kv condition plate
4. React in the following thermal cycle

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
65°C	20 sec	
72°C	1 min	
72°C	5 min	1 cycle

Done by Alex, Sophie H.

Date: 20260905

Title: Golden gate assembly

Purpose: assemble parts

Procedure:

1. Prepare the following

part	(1:5)	(1:7)
pSTK-EXP2-sfGFP 163ng/μL,5104bp	1 μL(1/5)	1 μL(1/5)
A 107ng/μL,3754bp	1.14 μL	1.6 μL
C 134ng/μL,3828bp	0.93 μL	1.3 μL
D 86ng/μL,3243bp	1.23 μL	1.7 μL
I 108ng/μL,3787bp	1.13 μL	1.58 μL
T4 DNA ligase buffer	1 μL	1 μL
Sterile water	2.57 μL	0.82 μL
T4 DNA ligase	0.5 μL	0.5 μL
BsmBI	0.5 μL	0.5 μL

2. PCR machine for thermal cycle

42°C	5 min	x45
16°C	5 min	
42°C	10 min	x1
80°C	20 min	x1

Done by Sophie C

Date: 20260905

Title: Western blot

Purpose: to identify specific protein within a complex sample mixture using gel separation and antibody binding

Procedure:

1. Make a sandwich with SDS page(refer to Chars' notebook)

2. Transfer to PVDF membrane
3. Block in 5% milk TBST for 1hr
4. Replace blocking solution by anti-Histag-vouse mAb(1:1000) in 5% milk
5. Incubate on orbital shaker at 4 °C overnight

Done by Sophie C

Date: 20260905

Title: Arcylamide gel

Purpose: Create temporary pores in cell membranes, allowing foreign molecules like DNA to enter.

Procedure:

1. Assemble 2 casting frames with 1.5m glass plates
2. Prepare solutions for stacking and resolving gel

Component	Stacker(top)	resolver(bottom)
Resolver A	x	7.5 mL
Resolver B	x	7.5mL
Stacker A	2.5 mL	x
Stacker B	2.5 mL	x
Total volume	5 mL	15 µL
TENED	5 µL	7.5 µL
10% APS	25 µL	7.5 µL

3. Transfer between glass plates(resolver)
4. Add stacking gel to the top of the green part
5. Insert comb for wells
6. Allow it to solidify at RT

Done by Sophie C,Chloe

Date: 20260905

Title: SDS page electrophoresis

Purpose: separate proteins based strictly on their molecular weight by denaturing them and coating them with a uniform negative charge.

Procedure:

1. Mount the gel into electrophoresis tank
2. Fill the inner and outer chambers of gel tank with 1x running buffer
3. Load protein ladder
4. Load prepared protein samples into wells

Gel 1:

Ladder lumen20hr lumen22hr lumen24hr lumensupernatant20hr LS22hr LS24hr

5	5	5	5	60	60	60
<u>shellsupernatant20hr SS22hr SS24hr</u>						
60				60	60	

Gel2:

<u>Ladder</u>	<u>lumen40hr</u>	<u>lumen42hr</u>	<u>lumen44hr</u>	<u>lumensupernatant40hr</u>	<u>LS42hr</u>	<u>LS44hr</u>
5	5	5	5	60	60	60
<u>shellsupernatant40hr SS42hr SS44hr</u>						
60				60	60	

5. Close the lid & connect electrodes
6. Run at 200 V for 10 min until the dye reaches the bottom of stacking gel then continue at 180 V for 40 min until dye reaches the bottom of the gel
7. Carefully disassemble glass plates & collect gel for subsequent analysis

Done by Sophie C

Date: 20260905

Title: electroporation

Purpose: Allowing foreign molecules like DNA to enter the cells

Procedure:

1. Prewarm medium at 37°C and precool a 0.2 cm electroporation cuvette
2. Add 4 µL plasmid AccD in 100 µL electrocompetent cell and transfer mixture of cells & DNA to the 2.1 KV electroporation cuvette & 2.3 KV
3. Set up electroporator & voltage resistance

V	2.1 KV
resistance	300 Ω
capltanu	25 f

V	2.3 KV
resistance	300 Ω
capltanu	25 f

4. Place in shake pod, close lid & wait for beep (1~2s)
5. Immediately add 1 mL SOC medium
6. Transfer to liquid culture tubs and incubate at 37°C for 3 hr shaking at 200 rpm
7. Centrifuge at 5000 g for 5 min
8. Spread LB plate with erythromycin

2.1 KV

TC	4.8
V	2086

2.3 KV

TC	5.6
V	2286

Done by Chloe

Date: 20260906

Title: Miniprep (QIA prep spin Miniprep Kit)

Purpose: To extract plasmid from liquid culture

Procedure:

20260904 V7

20260905 V7-1 ABDI

1. Collect bacterial liquid(1400 μ L) in 1.5 mL microcentrifuge tube
2. Centrifuge for 1 min at 16000 g
3. Repeat step 1~2 until all the liquid were collected
4. Add 250 P1 buffer, then vortex it
5. Add 250 P2 buffer and invert 4~6 times, than add 350 μ L N3 buffer immediately and invert 8~10 times
6. Centrifuge for 10 min at 17900 g
7. Transfer the supernatant in to QIA prep spin column
8. Centrifuge for 60 sec at 17900 g, discard the flow through
9. Add 500 μ L PB buffer and spin for 60s at 17900 g, discard the flow through
10. Add 750 μ L PE buffer and centrifuge for 1 min at 17900 g, discard the flow through
11. Spin the empty column for 1 min at 17900 g
12. Transfer column to 1.5 mL microtube, then add 50 μ L EB buffer
13. Spin for 1 min at 17900 g after let it stand for 1 min

	20260904	20260905
A260/230	2.093	2.329
A260/280	1.890	1.910
Concentration	257.0	384.0

Done by Sophie H

Date: 20260906

Title: Transformation of V

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

V: ABDI

1. Set up water bath at 42 $^{\circ}$ C
2. Thaw competent cells till 50% thawed, add 5 μ L plasmid
3. Incubate on ice for 20min
4. Heat shock for 45 sec
5. Incubate on ice for 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 $^{\circ}$ C for 1 hr
8. Centrifuge for 5 min at 5000 g
9. Take out ~800 μ L supernatant

10. Resuspend pellet

11. Spread on LBA

12. Incubate at 37°C overnight

Done by Sophie C.

Date: 20260907

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

Pveg-Bopep4 15-23 and Pveg-Bopep4 were used

1. Set the water bath to 42 °C and prepare ice
2. Get 1 LBA plate and 4 LBC plates
3. Thaw competent cells until they're 50% thawed and add 5 µL of plasmid
4. Incubate on ice for 20 min
5. Heat shock at 42 °C for 45 sec
6. Incubate on ice for 3 min
7. Add 1 mL SOC medium
8. Incubate for 1 hr at 37 °C
9. Centrifuge at 5000 g for 5 min
10. Remove 1 mL of supernatant then vortex
11. Spread on their respective plates
12. Incubate at 37°C for 16 hr

Done by Gabriela

Date: 20260907

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

X5: ACDI (1:5)

X7: ACDI(1:7)

1. Prepare the following reactions:

X5,X7 X10

Fw primer CcaR F	10µL
Rv primer Csn R	10µL
Q0amp master mix	100µL
ddH ₂ O	80µL

2. Distribute in PCR tubes (20µL) each
3. Dip pipette into colony streak grid
4. Dispense tip into PCR tubes & close lid
5. PCR machine for thermal cycle

95°C	5min	x1
95°C	20sec	x35
60°C	20sec	
72°C	1min	
25°C	5min	x1

Done by Sophie C

Date: 20260907

Title: electroporation

Purpose: Testing and transfer the plasmid into B.sub

Procedure:

Protocol were similar as 9/5

All plasmid were used the condition as below:

2.1KV,300Ω,25μF

-> Pveg-csn::AccD => 6.5ms, 2090V

Pveg-SamyQ::AccD 2.1=> 6.9ms,2091V

Pveg-csn::LEA=>6.9ms,2091V

L2-ABDI=>6.7ms,2091V

Done by Gabirel

Date: 20260907

Title:Golden Gate assembly

Purpose:to clone the fragment DNA into level 0 vector

Procedure:

Bopep4_9-23_NHis

Bopep4_G17A_1.23_NHis

Bopep4_S15A_1.23_NHis

Bopep_WT_1.23_NHis

SamyA-AccD-STK

SamyQ-Lea-STK

Dilute or resuspend the DNA fragment into 10ng/μL

Different vector:insert were used for GGA

Bopep4_9-23_NHis

Bopep4_G17A_1.23_NHis

Bopep4_S15A_1.23_NHis

Bopep_WT_1.23_NHis

=>0.01pmol : 0.03pmol

SamyA-AccD-STK

SamyQ-Lea-STK

=> 0.01 pmol : 0.02 pmol

Done by Gabriel

Date: 20260908

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

L:3P-EEBD-RBS0-csn:GanA-Gala-L3S2P21

1. Set up water bath at 42°C
 2. Thaw competent cells till 50% thawed, add 10 µL plasmid
 3. Incubate on ice for 20 mins
 4. Heat shock 45 sec
 5. Incubate on ice for 3 min
 6. Add 1mL SOC medium
 7. Incubator 1 hr
 8. Centrifuge 5 min 5000 g
 9. Remove ~1mL supernatant
 10. Resuspend pellet
 11. Spread on LBA
- Done by Sophie C

Date: 20260908

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

M:PcpcG2-veg-csn:GanA-Gala-L3S2P21

O:Pveg-SamyQ-Bopep4-2.2

N:Pveg-SamyQ-Bopep4-2.1

1. Prepare the following reactions

Mx1

Fw primer Biobrick prefix	1 µL
Rv primer STK0seq rv	1 µL
A-amp master mix	10 µL
ddH ₂ O	8 µL

N,OX15

Fw primer EXP-seq fw	15 µL
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Rv primer Bopep 4R	15 µL
A-amp master mix	150 µL
ddH ₂ O	120 µL

2. Distribute 20 µL to each PCR tube
3. Dip tip in colony & streak grid
4. Dispense tip in PCR tube & close lid
5. PCR machine for thermal cycle

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
65°C	20 sec	
72°C	3 min	
72°C	5 min	1 cycle

Done by Sophie C

Date: 20260908

Title: LBC+LBE plate s with glycerol

Purpose: prepare LBC+LBE plate for experiment

Procedure:

1. Make the following x2

LB broth powder	6.25 g
Agar powder	3.75 g
ddH ₂ O	250 mL
glycerol	2 mL

2. Add stirring bar & stir till no sediments
3. Cover flasks with aluminum foil, add indicator tape
4. Autoclave for 20 min
5. Place new petri dishes & equipment in BSC (after spray 70% ethanol)
6. Add 250 µL chloramphenicol 50~L erythromycin
7. Use pipette gun to add 20 µL melted agar into each petri dish
8. Let solidify

Done by Sophie C

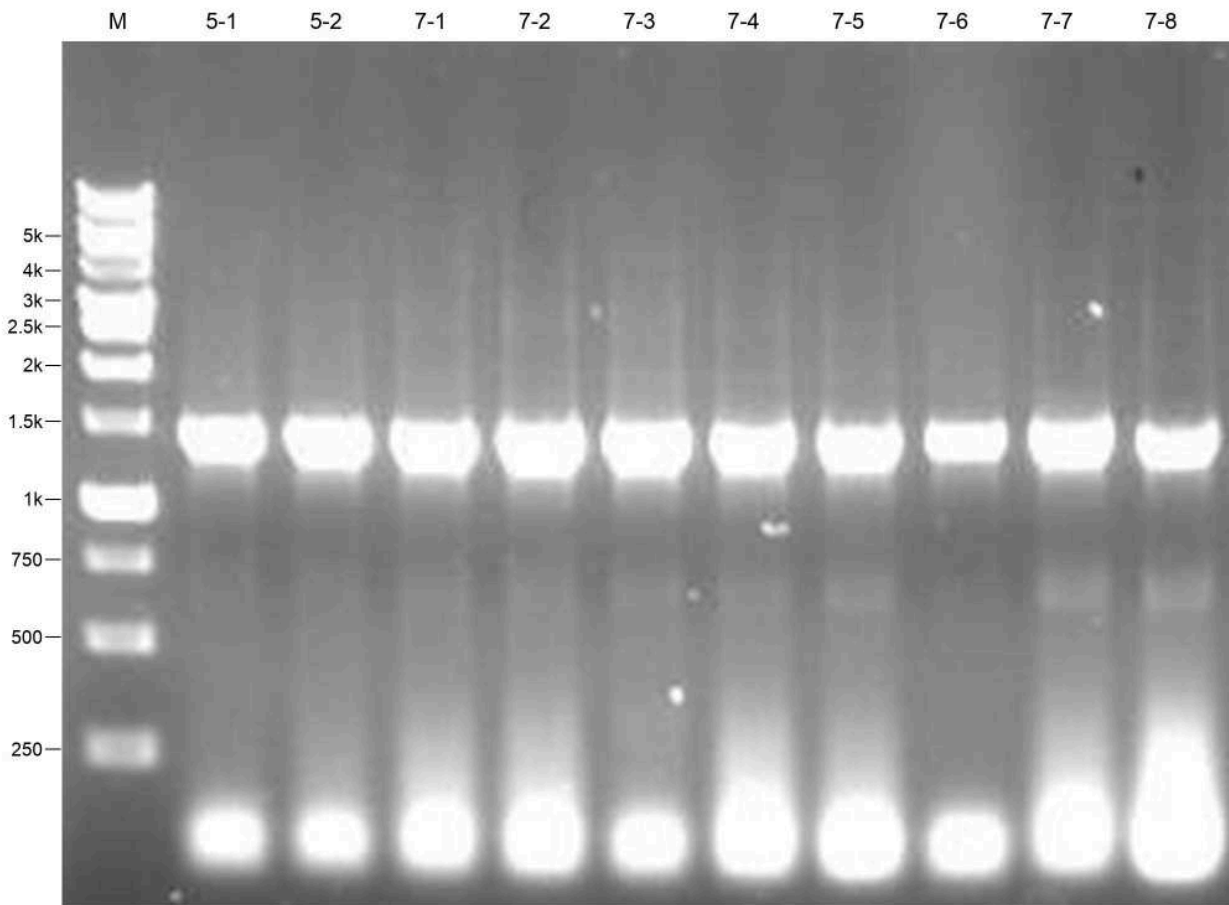
Date: 20260908

Title: Gel electrophoresis

Purpose: Checking Colony PCR result

Procedure:

1. Load the gel in the layout shown below (5 μ L each):
2. Resolve at 100 v for 30 min



M: 1kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
X5, X7 ACDI	CcaR F	Csn R	1424

Done by Sophie C

Date: 20260909

Title: PCR

Purpose: Amplify plasmid number

Procedure:

X:ACDI(X5,X7)

1. Prepare the following

51,71

Fw primer Pcy A F	1 µL
Rv primer CcasM3 R	1 µL
DNA (X5/X7)	1 µL
A-amp master mix	10 µL
ddH ₂ O	7 µL

52,72

Fw primer CcaSm3 F	1 µL
Rv primer CcaR R	1 µL
DNA (X5/X7)	1 µL
A-amp master mix	10 µL
ddH ₂ O	7µL

53,73

Fw primer CcaR F	1 µL
Rv primer Csn rv	1 µL
DNA (X5/X7)	1 µL
A-amp master mix	10 µL
ddH ₂ O	7µL

2. Thermal cycle the PCR machine

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
60°C	20 sec	
72°C	3 min 45 sec	
25°C	5 min	1 cycle

Done by Sophie C

Date: 20260909

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

L:3P-EEBD-RBS0-csn:GanA-Gala-L3S2P21

P1:Pveg-EEBD-RBS0-SamyQ-AccD-2.1 2.1KV

P3:Pveg-EEBD-RBS0-SamyA-AccD-2.1 2.3KV

J:Pveg-EEBD-RBS0-csn:AccD-B0015

1. Prepare the following

Px18

Fw primer Samy QF	18 µL
Rv primer EXP RV	18 µL
Q-amp master mix	180 µL
ddH ₂ O	144 µL

Jx1

Fw primer Exp fw	1 µL
Rv primer Csn R	1 µL
Q-amp master mix	10 µL
ddH ₂ O	8 µL

Lx14

Fw primer Exp fw	14 µL
Rv primer Exp rv	14 µL
Q-amp master mix	140 µL
ddH ₂ O	112 µL

2. Distribute into PCR tubes
3. Dip pipette into colonies & streak grid
4. Dispense tip in PCR tube
5. Take out tip & close lid
6. PCR machine for thermal cycle

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
60°C	20 sec	
72°C	3 min 45 sec	
25°C	5 min	1 cycle

Done by Sophie C, Abby

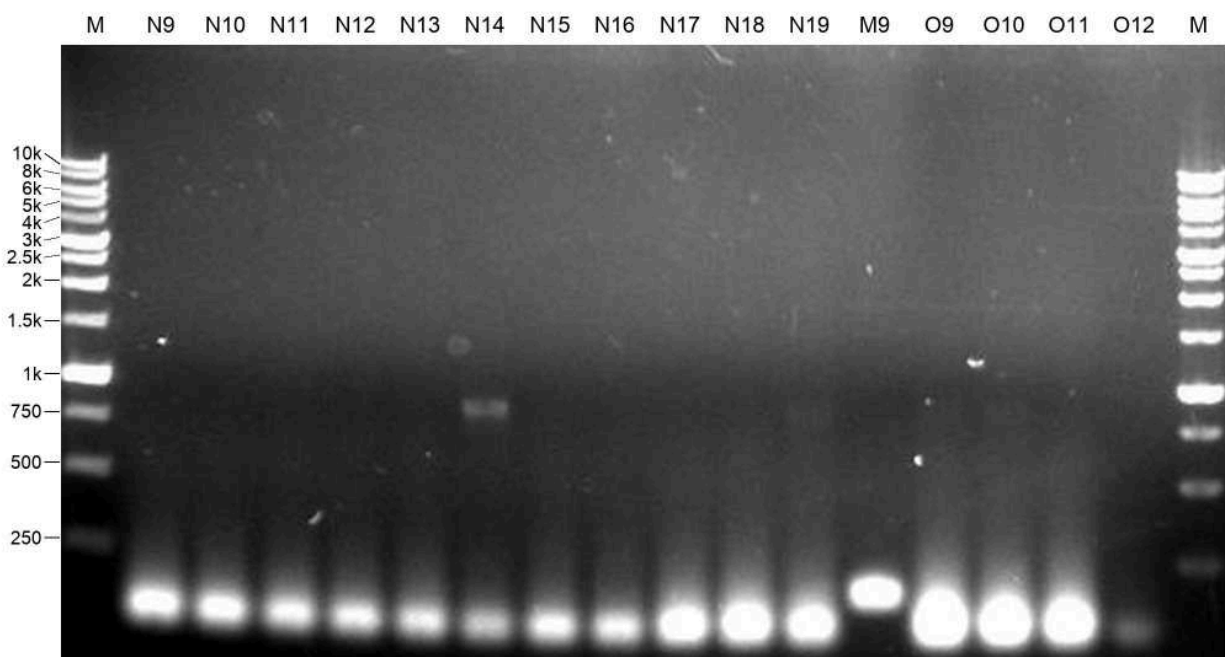
Date: 20260909

Title: Gel electrophoresis of M,O,N

Purpose: Checking Colony PCR result

Procedure:

1. Load the gel in the layout shown below (5 µL each):
2. Resolve at 100 v for 30 min



M: 1kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
N9-N19: Pveg-SamyQ-Bopep4-2.1	EXP-seq fw	Bopep4 R	559
O9-O12: Pveg-SamyQ-Bopep4-2.2	EXP-seq fw	Bopep4 R	559
M9: PcpcG2-veg-Csn:GanA-Gala-L 3S2P21	biobrick prefix	STK-seq rv	2837
Note: N14 positive clone			

Done by Naomi

Date: 20260910

Title: EcoRI Enzyme digestion

Purpose: Prepare the multimer plasmid for *Bacillus subtilis* natural transformation

Procedure:

1. Prepare the reaction by the following receipt:

	J9	R2	7-2
DNA (3.5 µL)	8.5	13.8	9.1
CutSmart buffer	2	2	2
EcoRI	0.5	2.5	0.5
d2W	9	3.7	8.4

2. Incubate in a PCR machine setting at 37 °C for 16 hr.
3. Heat inactivated at 65 °C for 20 min.

Done by Chars

Date: 20260911

Title: Clean up

Purpose: Clean up

Procedure:

1. Mix the reaction from 20260910 EcoRI enzyme digestion 500 μ L
2. Transfer the mixture to the column and centrifuge in 17900 g for 1min
3. Discard the flow-through. Add 500 μ L WN buffer and centrifuge in 17900 g for 1 min
4. Discard the flow-through. Add 500 μ L WS buffer and centrifuge in 17900 g for 1 min
5. Discard the flow-through. Centrifuge the empty column at 23444 g for 5 min
6. Transfer the column to a new microtube. Add 15 μ L D₂W in 65°C
7. Incubate in RT for 5 min. Centrifuge at 17900 g for 3 min
8. Measure the biodrop

	concentration(ng/ μ L)	A260/230	A260/280
J4	113	1.883	1.883
R2	101	1.672	1.865
7-2	84	1.605	1.867

Done by Sophie C.

Date: 20260911

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

J: Pveg-csn:AccD in B.sub 168

1. Prepare the following

Jx9

Fw primer EXP-seq fw	9 μ L
Rv primer Csn R	9 μ L
Q-amp master mix	90 μ L
ddH ₂ O	72 μ L

2. Distribute in PCR tubes
3. Dip pipette tips into colonies & streak grid
4. Dispense tips in PCR tubes

5. Take out tips & close lid
6. Thermal cycle in PCR machine

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
60°C	20 sec	
72°C	1 min	
25°C	5 min	1 cycle

Done by Sophie C

Date: 20260911

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

O:Pveg-EEBD-RBS0-SamyQ-Bopep4-2.2-B0015

1. Set up water bath to 42 °C
2. Thaw competent cell till 50% thawed, add 1 0µL plasmid
3. Incubate on ice for 20 sec
4. Heat shock 45 sec
5. Incubate on ice for 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 °C for 1 hr
8. Centrifuge 5 min at 5000 g
9. Remove 1 mL supernatant
10. Resuspend pellet
11. Spread on LBA plate
12. Place in incubator overnight

Done by Sophie C

Date: 20260911

Title: Miniprep

Purpose: to extract plasmid from liquid culture

Procedure:

A: Qiagon mimiprep kit

1. Collect liquid culture in 2 mL microtubes by centrifuge at 23444 g for 1 min
2. Resuspend pellet in 250 mL P1 buffer
3. ADD 250 µL P2 buffer, gently invert the microtube and incubate at RT for 2 min
4. Add 350 µL N3 buffer, invert the microtube

5. Centrifuge at 17900 g for 10 min
6. Remove the flow-through. Add 500 μ L PB buffer. Centrifuge at 17900 g for 1 min
7. Remove the flow-through. Add 750 μ L PE buffer. Centrifuge at 17900 g for 1 min
8. Remove the flow-through and centrifuge the empty column at 23444 g for 3 min
9. Transfer column to new 1.5 mL microtube
10. Add 50 μ L d2W in 65°C and incubate in RT for 5 min
11. Centrifuge in 17900 g for 3 min
12. Biodrop

B:NEB miniprep kit

1. Collect liquid culture in 2 mL microtubes by centrifuge at 23444 g for 1 min
2. Resuspend pellet in 200 μ L buffer B1
3. Add 200 μ L buffer B2, gently invert the microtube and incubate at RT for 2 min
4. Add 400 μ L buffer B3, invert the microtube
5. Centrifuge at 17900 g for 10 min
6. Remove the flow-through. Add 200 μ L buffer BZ and centrifuge 16000 g for 1 min
7. Remove the flow-through. Add 400 μ L buffer WZ and centrifuge at 16000 g for 2 min
8. Transfer column to new 1.5 mL microtube
9. Add 50 μ L d2W in 65°C and incubate in RT for 5 min
10. Centrifuge in 16000 g for 3 min
11. Biodrop

	Con.(ng/ μ L)	A260/230	A260/280
Pveg-SamyQ:BoPep4-2.1	450	2.454	1.867
Pveg-SamyQ-BoPep4-2.1	305	2.402	1.871

Done by Charsa

Date: 20260912

Title: electrophoresis (redone), 1% gel

Purpose: Checking Colony PCR result

Procedure:

1. Load the samples in the following layout:

M	J ₆	P ₃ 4	P ₃ 5	P ₃ 6	J9	R2	7-2
5	5	5	5	5	2.5	2.5	2.5

M: 1kb DNA ladder

J₆: Pveg-EEBD-RBS0-csn:ACCD-B0015

P₃: Pveg-EEBD-RBS0-samyQ-ACCD-2.1-B0015 2.3 kv

P₁: Pveg-EEBD-RBS0-samyQ-ACCD-2.1-B0015 2.1 kv

J9: ligation results

R2: ligation results

7-2: ligation results

2. Mix 0.5 μ L of loading dye with 2 μ L of J9, R2 and 7-2 before loading all 2.5 μ L
3. Resolve the DNA at 100V for 30 min

Done by Abby K

Date: 20260912

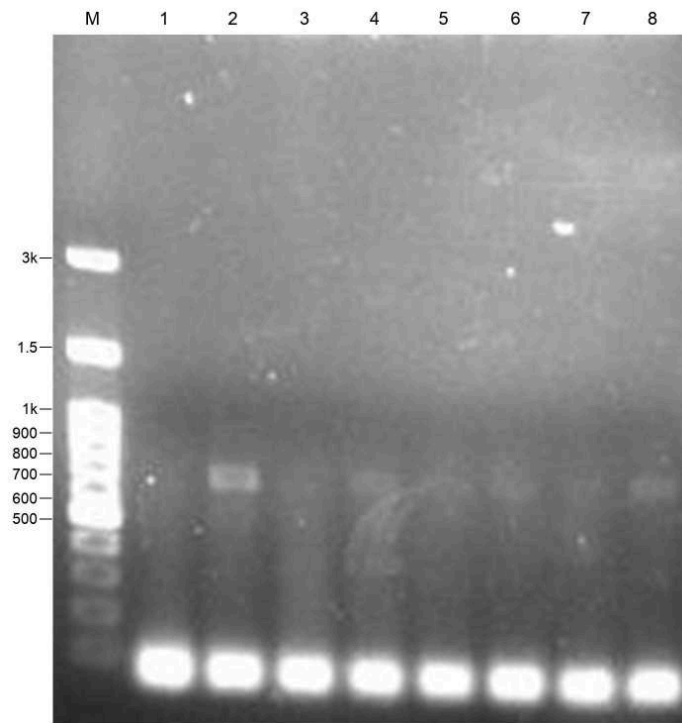
Title: Gel electrophoresis

Purpose: Checking Colony PCR result

Procedure:

Load the gel in the layout shown below (5 μ L each):

1. Resolve at 100 v for 30 min



Done by Sophie C.

Date: 20260912

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

1. Prepare the following:

O ×16

Fw primer (SamyQ fw)	18 µL
Rv primer (EXP rw)	18 µL
Q-amp master mix	180 µL
ddH ₂ O	144 µL

2. Distribute in PCR tubes
3. Dip pipette tips into colonies and streak grid
4. Dispense tips in PCR tubes
5. Take out tip and close lids
6. Thermal cycle in PCR machine:

95°C	5 min	1 cycle
95°C	20 sec	35 cycles
60°C	20 sec	
72°C	1 min	
25°C	5 min	1 cycle

Done by Abby K, Sophie C

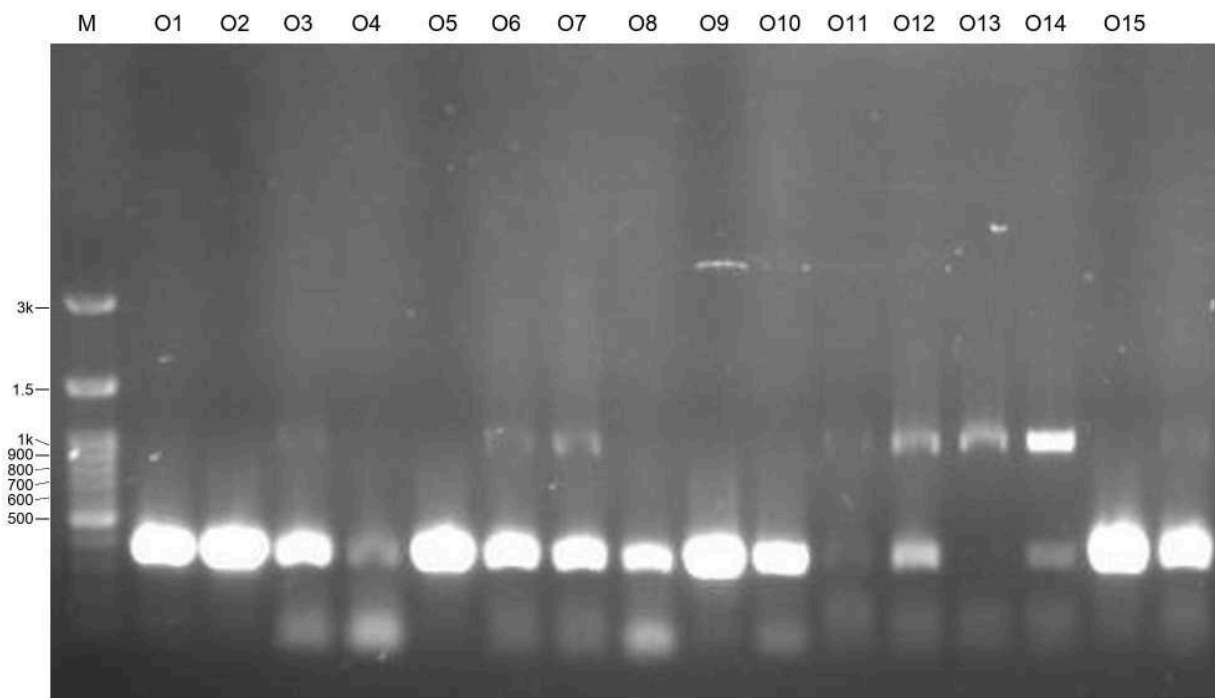
Date: 20260912

Title: electrophoresis

Purpose: Checking Colony PCR result

Procedure:

1. Load the gel in the layout shown below (5 µL each):
2. Resolve at 100 v for 30 min



Sample	Plasmid Construct	Expected Size (bp)	
O5	Pveg-SamyQ-Bop ep4-2.2	559	

Note: O5: positive clone	
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Done by Sophie C

Date: 20260912

Title: Colony PCR (redone from 0909)

Purpose: Amplify engineered DNA plasmid

Procedure:

1. Prepare the following:

$J_6 \times 1$

Fw primer (EXP fw)	1 μ L
Rv primer (Csn rv)	1 μ L
Q-amp master mix	10 μ L
ddH ₂ O	8 μ L

$P_3 \times 3$

Fw primer (SamyQ fw)	3 μ L
Rv primer (EXP rw)	3 μ L
Q-amp master mix	30 μ L
ddH ₂ O	24 μ L

2. Distribute into PCR tubes
3. Pick up colonies and streak the grid
4. Dispense pipette tips into their corresponding PCR tubes
5. Wait for 5 min then remove the tips
6. Put lids on
7. Place in PCR machine for thermal cycles:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	1 min	
25 °C	5 min	1 cycle

Done by Abby K

Date: 20260912

Title: Golden gate assembly

Purpose: assemble parts

Procedure:

T: pTwist-Pveg-Popep4

Part	Amount(μ L)
Vector pSTK-EXP1 162 ng/ μ L, 6929 bp	1.4 (1/5)
Insert (T) 407 ng/ μ L, 2979 bp	0.95 (1/10)
T4 DNA ligase buffer	1
T4 DNA ligase	0.5
Sterile water	6.65
Bsa1-HFv2	0.5

U: pTwist-Pveg-Bopep4 15-23

Part	Amount(μ L)
Vector pSTK-EXP1 162 ng/ μ L, 6929 bp	1.4 (1/5)
Insert (T) 375 ng/ μ L, 2937 bp	1.02 (1/10)
T4 DNA ligase buffer	1
T4 DNA ligase	0.5
Sterile water	5.58
Bsa1-HFv2	0.5

Done by Sophie C

Date: 20260913

Title: Prepare WB800 Electrocompetent cell via the method from Lu et al., 2012

Purpose: prepare electrocompetent *Bacillus subtilis* WB800 cells and preserve them at -80°C for future genetic transformation.

Procedure:

1. Prepare Lu's EP buffer

	Final concentration	Weight
Sorbitol	0.5 M	9.1 g
Mannitol	0.5 M	9.1 g
Trehalose	0.5 M	18.92 g
Glycerol	10 %	10 mL
d ₂ W		Add to 100 mL

2. Autoclave at 127 °C for 30 min. Aliquot in 2 × 50 mL tubes
3. Inoculate WB800 in 5 mL LB medium
4. Inoculate at 36 °C 200 rpm overnight
5. Measure the OD₆₀₀ of the overnight culture: 1.638
6. Dilute 1/16 to GM medium: 625 µL liquid culture to 40 mL GM medium
7. Measure the OD₆₀₀ by Biodrop

Time	OD ₆₀₀	Dilute 3×
10:30	~0.169	
13:50	1.322	
14.20	1.747	$0.694 \times 3 = 2.082$
14:45		$0.782 \times 3 = 2.346$

8. Shake on ice for 10 min. Aliquot 1.8 mL in 2 mL microtubes
9. Centrifuge at 5000g in 4 °C for 5 min
10. Remove supernatant. Resuspend in 1 mL ice cold Lu's EP buffer
11. Repeat step 9 and 10 for 4 times
12. Resuspend all pellet in 1 mL Lu's EP buffer
13. Aliquot 100µl in new 1.5 microtubes
14. Store in -80 °C

Done by Gabriel

Date: 20260914

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

1. Prepare the following:

H

Fw primer (Csn fw)	17 µL
Rv primer (EXP rv seq)	17 µL
Q-amp master mix	170 µL
ddH ₂ O	136 µL

V

Fw primer (ccaR fw)	9 µL
Rv primer (Csn rv)	9 µL
Q-amp master mix	90 µL
ddH ₂ O	72 µL

2. Distribute into PCR tubes
3. Dip tips into colonies and streak grid
4. Dispense tips in PCR tubes
5. Take out tips and centrifuge
6. Apply thermal cycle in PCR machine

Done by Chloe

Date: 20260914

Title: Miniprep

Purpose: To extract plasmid from liquid culture

Procedure:

O: Pveg-EEBD-RBS0-samyQ:Bopep4-2.2-B0015

1. Pellet 1-5 mL liquid culture by centrifuging 13,444 g for 1 min. Discard supernatant
Repeat until done w/ culture
2. Resuspend pellet in 200 µL of Monarch Buffer P1
3. Add 200 µL Monarch Buffer B2, gently invert tube 5-6 times and incubate at room temperature for 1 min
4. Add 400 µL Monarch Buffer B3 and gently invert tube until neutralized incubate at room temperature for 2 min
5. Centrifuge for 5 min at 16,000 g

6. Transfer supernatant to Monarch spin column and centrifuge 1 min at 16,000 g. Discard flow through
7. Re-insert the column in the Monarch spin collection tube and add 200 μ L Monarch Buffer B2 centrifuge 1 min at 16,000 g
8. Wash by adding 400 μ L Monarch Buffer W2 and centrifuge for 1 min at 16,000 g
9. Transfer to a clean 1.5 mL microfuge tube
10. Add ≥ 30 μ L Monarch Buffer EY to the center of the matrix. Incubate at room temperature for 1 min and centrifuge for 1 min at 16,000 g to elute DNA

O:

Concentration (ug/mL)	A260/230	A260/280
212	2.465	1.927

Done by Chloe

Date: 20260914

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

U: pSTK-EXP1-Pveg-Bopep4 15-23

T: pSTK-EXP1-Pveg-Bopep4

1. Set up water bath at 42 $^{\circ}$ C
2. Thaw competent cells until 50 % thawed and add plasmid
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Recover on ice 3 min
6. Add 1 mL SOC medium
7. Incubate 1 hr
8. Centrifuge 5 min 5000 g
9. Remove 900 μ L supernatant
10. Resuspend pellet
11. Spread in LBA

Done by Chloe

Date: 20260914

Title: Gel Electrophoresis (1% gel)

Purpose: Checking Colony PCR result

Procedure:

1. Load the samples in the following layout:

Gel 1: M H₁ H₂ H₃ H₄ H₅ H₆ H₇ H₈ H₉ H₁₀ H₁₁ H₁₂ H₁₃ H₁₄ H₁₅ H₁₆ V₁ V₂ V₃ V₄ V₅ V₆
 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
V₇ V₈

5 5

Gel 2: M J₆ P₃4 P₃5 P₃6
5 5 5 5 5

2. Resolve the gel at 100V for 30 min

Done by Gabriel

Date: 20260915

Title: L0 Golden Gate Assembly

Purpose: assemble parts

Procedure:

Á :Bopep4-S15A_1-23_NHis

Component	Amount	Dilution
Vector STK001_pSTK-0-sfGFP • concentration(ng/μL): 346 • bp: 3090	0.29024566 μL	1.45122832 μL (1/5 dilute)
Insert CDS (Bopep4-S15A_1-23_NHis) • concentration(ng/μL): 10 • bp: 238		1.08 μL
T4 DNA ligase buffer	2 μL	1 μL
Sterile water		5.47μL
T4 DNA ligase	1 μL	0.5 μL
Bbs1	1 μL	0.5 μL
Total	20 μL	10 μL

Ñ: Bopep4-WT_1-23-NHis

Component	Amount	Dilution
Vector STK001_pSTK-0-sfGFP • concentration(ng/μL): 346 • bp: 3090	0.29024566 μL	1.45122832 μL(1/5 dilute)
Insert CDS (Bopep4-WT_1-23-NHis) • concentration(ng/μL): 10		1.08 μL

bp: 238		
T4 DNA ligase buffer	2 μ L	1 μ L
Sterile water		5.47 μ L
T4 DNA ligase	1 μ L	0.5 μ L
Bbs1	1 μ L	0.5 μ L
Total	20 μ L	10 μ L

É: Bopep4_G17A_1-23-NHis

Component	Amount	Dilution
Vector STK001_pSTK-0-sfGFP - concentration(ng/μL): 346 - bp: 3090	0.29024566 μ L	1.45122832 μ L(% dilute)
Insert CDS (Bopep4_G17A_1-23-NHis) - concentration(ng/μL): 10 - bp: 238		1.08 μ L
T4 DNA ligase buffer	2 μ L	1 μ L
Sterile water		5.47 μ L
T4 DNA ligase	1 μ L	0.5 μ L
Bbs1	1 μ L	0.5 μ L
Total	20 μ L	10 μ L

Done by Chloe

Date: 20260915

Title: Electroporation

Purpose: Test the different electroporation competent cell preparation protocol

Procedure:

Protocol: WB800 competent cell using the previous protocol which additionally added the trehalose were used.

1. Pveg-csn:ACCD and ABDI v7-2 plasmid were used
2. 500 ng and 1000 ng plasmid were used for electroporation respectively
3. For ACCD, 2.1 / 2.3 / 2.5 kv, 250 Ω , 25 μ F were tested

2.1 kv $\rightarrow$ TC: 6.3 ms, 2088 V

2.3 kv $\rightarrow$ TC: 6.3 ms, 2280 V

2.5 kv $\rightarrow$ TC: 6.3 ms, 2488 V

4. For ABDI, 2.3 kv, 200 Ω , 25 μ F were tested

TC: 5, 2273 V

Three trials were set: LBC (2.5 μ g/mL), LBC (5 μ g/mL), and LBE(5 μ g/mL)

Done by Gabriel

Date: 20260915

Title: L2 GGA

Purpose: assemble parts

Procedure:

X: ACDI

X: ACDI (1:7)

Vector EXP2 162 ng/ μ L, 5104 bp	1 μ L (1/5)
A 107 ng/ μ L, 3754 bp	1.6 μ L
C 134 ng/ μ L, 3828 bp	1.3 μ L
D 86 ng/ μ L, 3243 bp	1.7 μ L
I 108 ng/ μ L, 3787 bp	1.58 μ L
T4 DNA ligase buffer	1 μ L

ddH ₂ O	0.82 µL
T4 DNA ligase	0.5 µL
BsmB1	0.5 µL

2. PCR machine

42 °C	5 min	45 cycles
16 °C	5 min	
42 °C	10 min	1 cycle
80 °C	20 min	1 cycle

Done by Sophie C

Date: 20260916

Title: LBA & LBE plates

Purpose: prepare plates for experiment

Procedure:

1. Formula as below

LB broth powder	6.25 g
Agar powder	3.95 g
ddH ₂ O	250 mL

2. Add one magnetic stirring bar to the flask
3. Place the flask containing the mixture on the hot plate, and stir until no sediment
4. Cover the flask with double-layer aluminum foil
5. Autoclave for 20 min at 127°C.
6. Add 250 µL ampicillin in the BSC while it's still a bit hot
7. Pour 20 mL into each plate and wait for it to solidify

Done by Olivia, Sophie C

Date: 2026

Title: Miniprep

Purpose: to extract plasmid from liquid culture

Procedure:

1. Pellet 1.5 mL liquid culture by centrifuge 13444 g for 1 min
2. Resuspend pellet in 200 µL of monarch buffer P1
3. Add 200 µL monarch buffer B2, gently invert tube 5-6 times and incubate at RT for 1 min
4. Add 400 µL monarch buffer B3 and gently invert tube until neutralized at RT for 2 min
5. Centrifuge for 5 min at 1000 g
6. Transfer supernatant to monarch spin column and centrifuge 1min at 1000 g. Discard the flow-through
7. Re-invert the column in the monarch spin collection tube and add 200 µL monarch buffer B2 and centrifuge for 1 min at 16000 g
8. Wash by adding 400 µL monarch buffer W2 and centrifuge for 1 min at 16000 g
9. Transfer to clean, 1.5 mL microcentrifuge tube
10. Add 230 µL monarch buffer EY to the center of the matrix. Incubate at RT for 1 min and centrifuge for 1 min at 16000 g to elute DNA5

Done by Chloe, Sophie C

Date: 20260916

Title: Transformation

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

Ñ: pSTK-0C-Bopep4-WT-1-23-NHis

Ã: pSTK-0C-Bopep4-S15A-1-23-NHis

Ê: pSTK-0C-Bopep4-G17A-1-23-NHis

X: ACDI

1. Set up water bath at 42 °C
2. Thaw competent cells until 50 % thawed and add plasmid
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Incubate on ice 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 °C for 1 hr
8. Centrifuge at 5000 xg for 5 min
9. Remove 1 mL supernatant
10. Resuspend pellet
11. Spread in LBC

Done by Chloe, Sophie C

Date: 20260916

Title: Amplification

Purpose: introduce a plasmid into competent bacterial cells via heat-shock transformation and select for transformants on antibiotic agar plates.

Procedure:

1. Set up water bath at 42 °C
2. Thaw competent cells until completely thawed and aliquot 30 µL competent cell with 2 µL plasmid
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Rest on ice for 3 min
6. Spread on LBA and LBC
7. Incubate at 37 °C overnight

Done by Chloe, Sophie C

Date: 20260916

Title: colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

1. Prepare the following reaction:

V:	1 rxn	8 rxn
Fw primer (ccaR fw L2)	1 µL	8 µL
Rv primer (Csn rv)	1 µL	8 µL
Q-amp	10 µL	80 µL

ddH ₂ O	8 µL	64 µL
--------------------	------	-------

P:	1 rxn	10 rxn
Fw primer (SamyQ fw)	1 µL	10 µL
Rv primer (EXP-seq4 rv)	1 µL	10 µL
Q-amp	10 µL	100 µL
ddH ₂ O	8 µL	80 µL

2. Pick up colonies and streak grid
3. Incubate at room temperature for 5 min
4. Run through thermal cycle

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	1 min 30 sec	
72 °C	5 min	1 cycle
25 °C	0 sec	

Done by Sophie

Date: 20260917

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

Ñ: pSTK-0C-Bopep4-WT-1-23-NHis

Ã: pSTK-0C-Bopep4-S15A-1-23-NHis

Ë: pSTK-0C-Bopep4-G17A-1-23-NHis

X: ACDI

1. Prepare the following reaction:

X:	1 rxn	12 rxn
Fw primer (ccasm3 fw)	1 µL	12 µL
Rv primer (ccaR rv)	1 µL	12 µL
Q-amp	10 µL	120 µL
ddH ₂ O	8 µL	96 µL

Ñ, Ã, Ë:	1 rxn	22 rxn
Fw primer (pSTK-seq fw)	1 µL	22 µL
Rv primer (pSTK-seq rv)	1 µL	22 µL
Q-amp	10 µL	220 µL
ddH ₂ O	8 µL	176 µL

2. Pick up colonies and streak on grid
3. Incubate at RT for 5 min
4. Run through thermal cycle as below:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	45 sec	

72 °C	5 min	1 cycle
25 °C	0 sec	

Done by

Date: 20260917

Title: LBC plates

Purpose:

Procedure:

1. Make the following
2. Add a stirring bar and stir till no sediments
3. Cover flasks with aluminum foil, add indicator tape
4. Autoclave for 20 min
5. Place new petri dishes and equipment in BSC
6. Add 250 µL chloramphenicol
7. Use pipette gun to add 15 mL melted agar into each petri dish
8. Let solidify

Done by Sophie C

Date: 20260917

Title: Golden gate assembly: Level 2

Purpose: assemble parts

Procedure:

Y: ABDQ

Z: ACDQ

Y: ABDQ (1:7)

Vector pSTK-EXP2 263 ng/µL, 5014 bp	0.63 µL
A 107 ng/µL, 3754 bp	1.6 µL
B 88 ng/µL, 3792 bp	1.96 µL
D 86 ng/µL, 3243 bp	1.7 µL
Q 71 ng/µL, 3787 bp	2.43 µL
T4 DNA ligase buffer	1 µL
ddH ₂ O	0 µL

T4 DNA ligase	0.5 µL
BsmB1	0.5 µL
Total	10 µL

Y: ACDQ (1:7)

Vector pSTK-EXP2 263 ng/µL, 5014 bp	0.63 µL
A 107 ng/µL, 3754 bp	1.6 µL
B 88 ng/µL, 3792 bp	1.3 µL
D 86 ng/µL, 3243 bp	1.7 µL
Q 71 ng/µL, 3787 bp	2.43 µL
T4 DNA ligase buffer	1 µL
ddH ₂ O	0.34 µL
T4 DNA ligase	0.5 µL
BsmB1	0.5 µL
Total	10 µL

2. PCR machine for thermal cycle

42 °C	5 min	45 cycles
16 °C	5 min	
55 °C	10 min	1 cycle
80 °C	20 min	1 cycle

Done by Sophie C

Date: 20260918

Title: Transformation (Z,Y)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

Y: ABDQ

Z: ACDQ

1. Set up water bath at 42 °C
2. Add 5 µL plasmid to competent cell when 50 % thawed
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Incubate on ice 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 °C 1 hr
8. Centrifuge 5 min 5000 g
9. Resuspend pellet after removing 1 mL supernatant
10. Spread on LBC plate
11. Place in incubator overnight

Done by Sophie C

Date: 20260918

Title: Miniprep (0917 Amplification)

Purpose: To extract plasmid from liquid culture

Procedure:

1. Pellet 1.5 mL of overnight bacterial culture by centrifuging at 6800 g for 3 min
2. Resuspend pelleted bacterial cells in 250 µL Buffer P1
3. Add 250 µL Buffer P2 and mix thoroughly by inverting the tube 4-6 times
4. Add 350 µL Buffer N3, mix immediately by inverting the tube 4-6 times
5. Centrifuge for 10 min at 17900 xg
6. Apply ~800 µL supernatant to QIA 2.0 spin column by pipetting
7. Centrifuge for 30 sec at 17900 xg and discard flow through
8. Wash column by adding 0.5 mL Buffer PB
9. Centrifuge for 30 sec at 17900 xg and discard the flow through
10. Wash column by adding 0.75 mL Buffer PE
11. Centrifuge for 30 sec at 17900 xg and discard flow through
12. Centrifuge for 1 min at 17900 xg to remove residual wash buffer
13. Place column in clean 1.5 mL microcentrifuge tube
14. Add 50 µL Buffer EB to the center of the column
15. Let stand for 1 min
16. Centrifuge for 1 min at 17900 xg
17. Biodrop

	Concentration	A230/260	A260/280
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ABDI	542.0 ng/ μ L	2.336	1.895
J9	408 ng/ μ L	2.254	1.898
P	395 ng/ μ L	2.297	1.890

Done by Sophie C

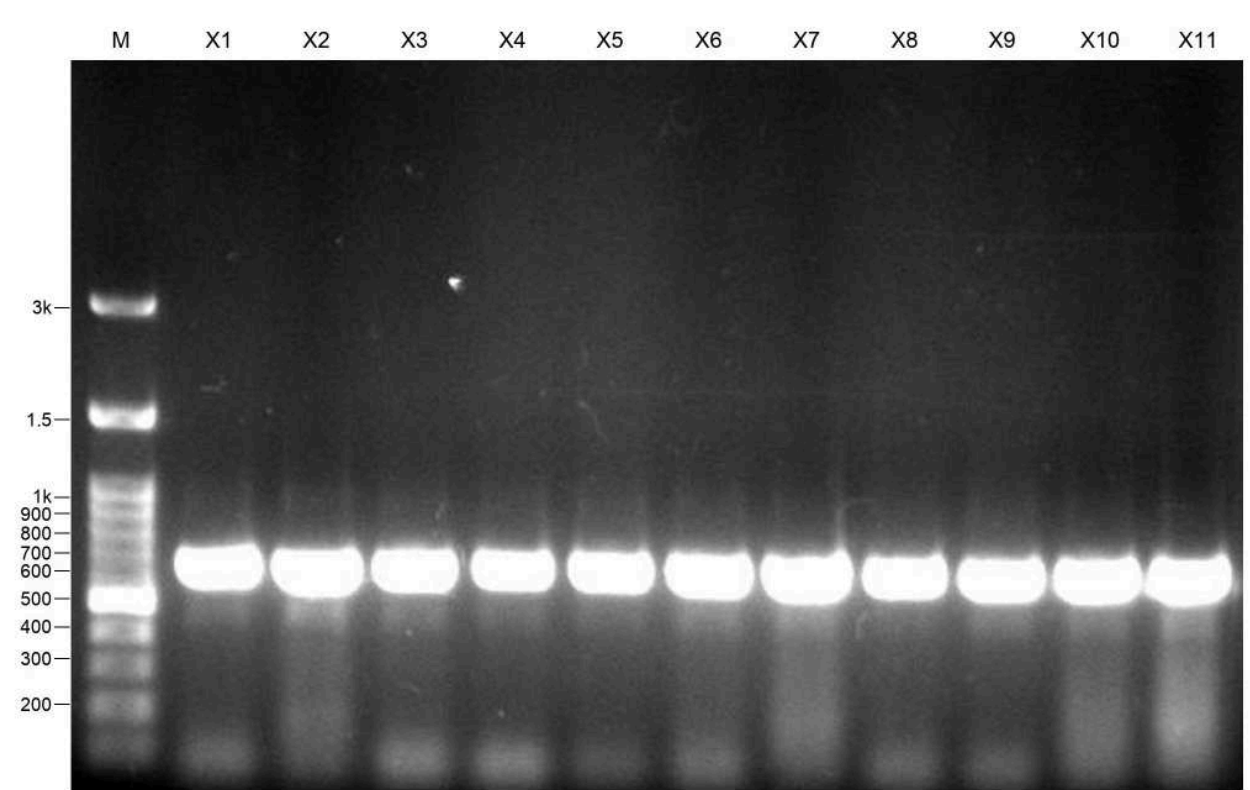
Date: 20260918

Title: Gel electrophoresis

Purpose: Checking Colony PCR result

Procedure:

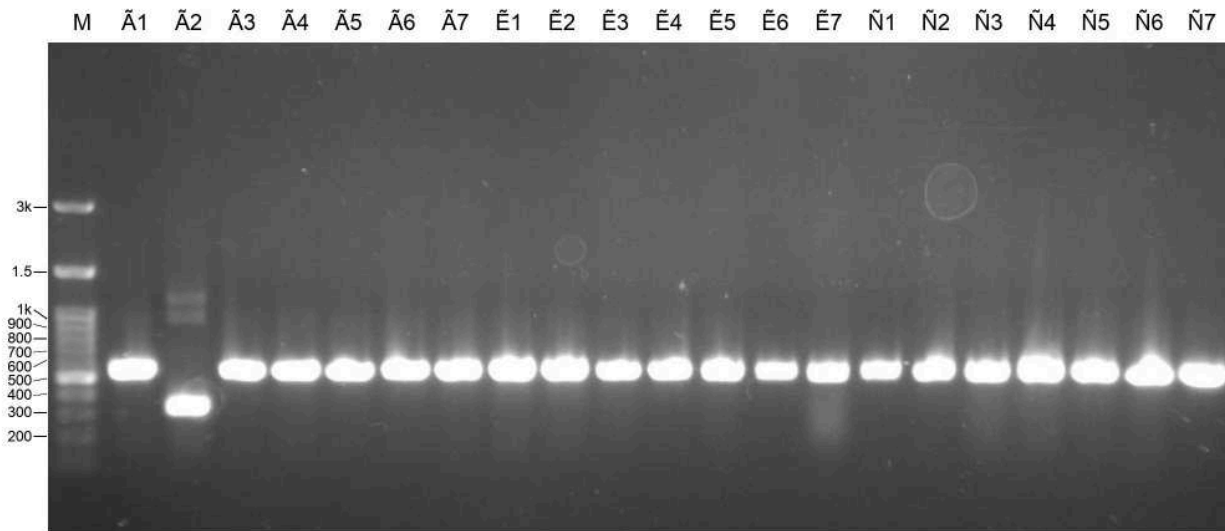
1. Load the gel in the layout shown below (5 μ L each):
2. Resolve at 100 v for 30 min



M: 3kb ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
X: ACDI	L2 CcaSm3 F	L2 CcaR R	632

3. Load the gel in the layout shown below (5 μ L each):

4. Resolve at 100 v for 30 min



M: 3kb ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
Ã: Bopep4_S15A_1-23_NHis	STK-seq-fw	STK-seq-rv	548
Ė: Bopep4_G17A_1-23_NHis	STK-seq-fw	STK-seq-rv	548
Ñ: Bopep4_WT_1-23_NHis	STK-seq-fw	STK-seq-rv	548

Done by Sophie C

Date: 20260919

Title: Miniprep (Ñ, Ã, Ë, X₇)

Purpose: To extract plasmid from liquid culture

Procedure:

Ñ: pSTK-0C-Bopep4-WT-1-23-NHis

Ã: pSTK-0C-Bopep4-S15A-1-23-NHis

Ë: pSTK-0C-Bopep4-G17A-1-23-NHis

X₇: ACDI

1. Pellet 1-8 mL of overnight bacterial culture by centrifuging at 6800 g for 3 min until all culture is used up
2. Discard supernatant and resuspend in 250 µL Buffer P1
3. Add 250 µL Buffer P2 and mix by inverting the tube 4-6 times until the solution becomes clear
4. Add 350 µL Buffer N3, mix immediately by inverting the tube 4-6 times
5. Centrifuge at 17900 g for 10 min
6. Apply 800 µL supernatant to QIA 2.0 spin column
7. Centrifuge for 30 sec at 17900 g and discard flow through
8. Wash column by adding 0.5 mL Buffer PB
9. Centrifuge for 30 sec at 17900 g and discard the flow through
10. Wash column by adding 0.75 mL Buffer PE
11. Centrifuge for 30 sec at 17900 g and discard flow through
12. Centrifuge for 1 min at 17900 g to remove residual wash buffer
13. Place column in clean 1.5 mL microcentrifuge tube
14. Add 50 µL EB Buffer to the center of the column
15. Stand for 1 min
16. Centrifuge for 1 min at 17900 g

	Concentration	A230/260	A260/280
X ₁	94 ng/µL	1.958	1.843
X ₅	72 ng/µL	2.182	1.895
X ₉	87 ng/µL	1.977	1.891
Ñ ₁	69 ng/µL	2.091	1.917
Ñ ₅	117 ng/µL	2.12	1.887
Ñ ₇	78 ng/µL	2.0	1.9
Ã ₁	68 ng/µL	2.0	1.889

$\tilde{A}_5$	86 ng/ μ L	1.755	1.87
$\tilde{A}_7$	80 ng/ μ L	1.9	1.86
$\tilde{E}_1$	73 ng/ μ L	1.78	1.825
$\tilde{E}_5$	61 ng/ μ L	1.811	1.861
$\tilde{E}_7$	71 ng/ μ L	1.29	1.77

Done by Chloe

Date: 20260919

Title: Electroporation

Purpose: Allowing foreign molecules like DNA to enter the cells

Procedure:

N: Pveg-EEBD-RBS0-samyQ-Bopep4-2.1-B0015

O: Pveg-EEBD-RBS0-smayQ-Bopep4-2.2-B0015

J: Pveg-EEBD-RBS0-csn:ACCD-B0015

1. 500 ng plasmids were used for electroporation with 100 μ L competent cell
2. N: Pveg-EEBD-RBS0-samyQ-Bopep4-2.1-B0015
O: Pveg-EEBD-RBS0-smayQ-Bopep4-2.2-B0015
J: Pveg-EEBD-RBS0-csn:ACCD-B0015
2 mL recovery medium was used

Done by Sophie C

Date: 20260919

Title: PCR

Purpose: Amplify plasmid number

Procedure:

1. Prepare the following reactions:

X: ACDI (1;5;9)

X_{1A} , X_{5A} , X_{9A}

Fw primer (L2 pcyA fw)	1 μ L
Rv primer (L2 CcaSm3 rv)	1 μ L
DNA template	1 μ L
Q-amp master mix	10 μ L
ddH ₂ O	7 μ L

X_{1B}, X_{5B}, X_{9B}

Fw primer (L2 CcaSm3 fw)	1 µL
Rv primer (L2 CcaR rv)	1 µL
DNA template	1 µL
Q-amp master mix	10 µL
ddH ₂ O	7 µL

X_{1C}, X_{5C}, X_{9C}

Fw primer (L2 CcaR fw)	1 µL
Rv primer (Csn rv)	1 µL
DNA template	1 µL
Q-amp master mix	10 µL
ddH ₂ O	7 µL

2. PCR machine

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	1 min	
25 °C	5 min	1 cycle

Done by Sophie C

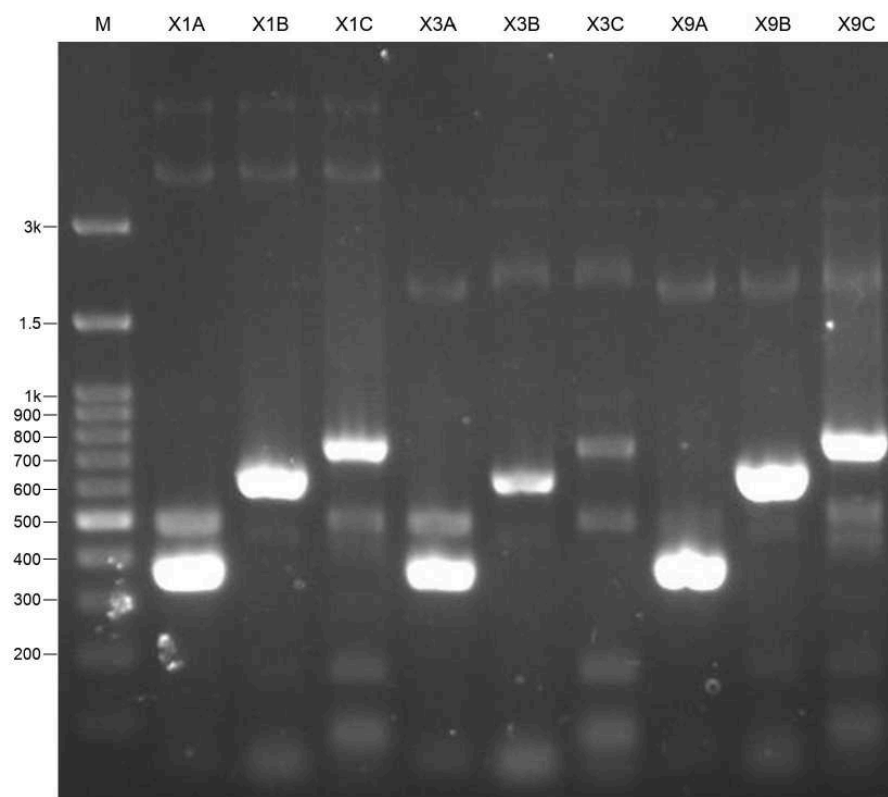
Date: 2026919

Title: Gel electrophoresis

Purpose: Checking Colony PCR result

Procedure:

1. Load the gel in the layout shown below (5 µL each):
2. Resolve at 100 v for 30 min



M: 3kb ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
A (X1A, X3A, X9A) - X: ACDI	L2 pcyA F	L2 CcaSm3 R	375
B (X1B, X3B, X9B) - X: ACDI	L2 CcaSm3 F	CcaR R	632
C (X1C, X3C, X9C) - X: ACDI	CcaR F	Csn R	661

Date: 20260919

Title: Level 2 Golden gat assembly and Level 0 GGA

Purpose:

Procedure:

Y: ABDQ (1:7)

Z: ACDQ (1:7)

Y: ABDQ

Part	Amount(μL)
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Vector pSTK-EXP2 263 ng/μL, 5014 bp	0.63
A 107 ng/μL, 3754 bp	1.6
B 88 ng/μL, 3792 bp	1.96
D 86 ng/μL, 3243 bp	1.7
Q 71 ng/μL, 3787 bp	2.43
T4 DNA ligase buffer	1
ddH ₂ O	0
T4 DNA ligase	0.5
BsmB1	0.5

Z: ACDQ

Vector pSTK-EXP2 263 ng/μL, 5014 bp	0.63
A 107 ng/μL, 3754 bp	1.6
B 88 ng/μL, 3792 bp	1.3
D 86 ng/μL, 3243 bp	1.7
Q 71 ng/μL, 3787 bp	2.43
T4 DNA ligase buffer	1
ddH ₂ O	0.34
T4 DNA ligase	0.5
BsmB1	0.5

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Part	Amount(μL)
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Vector pSTK-0-sfGFP 346 ng/μL, 3090 bp	0.058
samyQ-LEA 10 ng/μL, 1180 bp	1.53
T4 DNA ligase buffer	1
ddH ₂ O	5.31
T4 DNA ligase	0.5
Bbs1	0.5

π

Part	Amount(μL)
Vector pSTK-0-sfGFP 346 ng/μL, 3090 bp	0.058
samyQ-ACCD	1.54
T4 DNA ligase buffer	1
ddH ₂ O	5.3
T4 DNA ligase	0.5
Bbs1	0.5

ú

Part	Amount(μL)
Vector pSTK-0-sfGFP	0.058
smayQ-LEA-2.2	0.8
T4 DNA ligase buffer	1
ddH ₂ O	6.04
T4 DNA ligase	0.5
Bbs1	0.5

í

Part	Amount(μL)
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Vector pSTK-0-sfGFP	0.058
samyQ-ACCD-v3t1	1.53
T4 DNA ligase buffer	1
ddH ₂ O	5.31
T4 DNA ligase	0.5
Bbs1	0.5

Done by Chloe and Sophie

Date: 20260919

Title: Western Blot

Purpose: to identify specific protein within a complex sample mixture using gel separation and antibody binding

Procedure:

Double check LEA, ACCD in supernatant

Prepare SDS-PAGE 10%, 15%

Separating gel	10 %	15 %
30 % liquid gel	2.66 mL	4 mL
4× Tris-Cl (pH 8.8)	2 mL	2 mL
Milli-Q	3.30 mL	1.96 mL
10 % SDS	80 mL	80 mL
10 % APS	80 mL	80 mL
TEMED	12.5 µL	12.5 µL
	8 mL	8 mL

Stacking gel	4 % ×3
30 % liquid gel	1000 µL
4× Tris-Cl (pH 6.8)	1875 µL
Milli-Q	4.625 mL
10 % SDS	69 µL

10 % APS	75 μ L
TEMED	10 μ L
	7.5 mL

1. 95 °C for 10 min (Sample: 50 μ L + 6 SB: 10 μ L)
2. Loading 60 μ L to each well
3. 200V, 0 min $\rightarrow$ 120 V, 1 hr / 0 min
4. 300 mA for 2 hr
5. Wash membrane with Milli-Q
6. Blocking in 5 % non fat milk, 4 °C O/N (begin 20:00)
7. 1 Ab° (histag) T for 1 hr
8. 1 $\times$ TBST wash 3 times (5 min / each)
9. 2 Ab° (g $\propto$ Mouse) T for 1 hr
10. 1 $\times$ TBST wash 5 times (5 min / each)

Δ <u>M₁</u>	<u>ACCD</u>	Δ <u>ACCD</u>		<u>M</u>	<u>ACCD</u>	Δ <u>ACCD</u>	Δ	M ₁ 10 %
	168	WB800			168	WB800		
Δ <u>M₂</u>	<u>LEA</u>	Δ Δ		<u>M</u>	<u>LEA</u>	Δ Δ Δ		M ₂ 15 %
1 Ab° (CST # 2366)				1 Ab° (SC - 53073)				

Done by Gabriel

Date: 20260920

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

N: Pveg-EEBD-RBS0-samyQ-Bopep4-2.1-B0015

O: Pveg-EEBD-RBS0-smayQ-Bopep4-2.2-B0015

J: Pveg-EEBD-RBS0-csn:ACCD-B0015

N, O:	1 rxn	15 rxn
Fw primer (pSTK-EXP-seq-4 fw)	1 µL	15 µL
Rv primer (pSTK-EXP-seq-4 rv)	1 µL	15 µL
Q-amp	10 µL	150 µL
ddH ₂ O	8 µL	120 µL

J:	1 rxn	11 rxn
Fw primer (Csn fw)	1 µL	11 µL
Rv primer (pSTK-EXP-seq-4 rv)	1 µL	11 µL
Q-amp	10 µL	110 µL
ddH ₂ O	8 µL	88 µL

2. Run through thermal cycle

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	1 min 30 sec	
72 °C	5 min	1 cycle
25 °C	0 sec	

Done by Chloe

Date: 20260920

Title: Miniprep of Exp 1

Purpose: To extract plasmid from liquid culture

Procedure:

1. Pellet 1-5 mL liquid culture by centrifuging 1 min at 23,444 g until used up
2. Resuspend pellet in 200 μ L of Monarch Buffer P1
3. Add 200 μ L Monarch Buffer B2 and gently invert the tube 5-6 times. Incubate at room temperature for 2 min
4. Add 400 μ L Monarch Buffer B3 and invert until neutralized incubate at room temperature for 2 min
5. Centrifuge for 5 min at 23,444 g
6. Transfer to column and centrifuge for 1 min at 16,000 g
7. Discard flow through. Re-insert the column in spin collection tube and add 200 μ L Monarch Buffer B2 centrifuge 1 min at 16,000 g
8. Wash by adding 400 μ L Monarch Buffer W2 and centrifuge for 1 min at 16,000 g
9. Transfer to a clean 1.5 mL microfuge tube
10. Add 50 μ L Monarch Buffer EY, wait 1 min and centrifuge for 1 min at 16,000 g

	Concentration	A230/260	A260/280
EXP1	47 ng/ μ L		

Done by Chloe

Date: 20260920

Title: Gel electrophoresis

Purpose: Checking Colony PCR result

Procedure:

1. Load the samples in the following layout:

M	O ₁	O ₂	O ₃	O ₄	O ₅	O ₆	O ₇	O ₈	J ₁	J ₂	J ₃	J ₄	J ₅	J ₆	J ₇	J ₈	J ₉	J ₁₀	N ₁	N ₂	N ₃	N ₄	N ₅	N ₆
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

2. Resolve at 100V for 30 min

Done by Chloe

Date: 20260921

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

Z: ACDQ

ú: Lvl 0 samyQ: LEA 2.2

ó: Lvl 0-samyQ-LEA

π: Lvl 0-samyQ-ACCD

í: Lvl 0-samyQ-ACCD v3t1

Z:	1 rxn	4 rxn
Fw primer (ccaSm3 fw)	1 µL	4 µL
Rv primer (ccaR rv)	1 µL	4 µL
Q-amp	10 µL	40 µL
ddH ₂ O	8 µL	32 µL

:	1 rxn	rxn
Fw primer ()	1 µL	µL
Rv primer ()	1 µL	µL
Q-amp	10 µL	µL
ddH ₂ O	8 µL	µL

Done by

Date: 20260921

Title: Miniprep

Purpose: To extract plasmid from liquid culture

Procedure:

1. Pellet 1-5 mL liquid culture by centrifuging 1 min at 23,444 g until used up
2. Resuspend pellet in 200 µL of Monarch Buffer P1
3. Add 200 µL Monarch Buffer B2 and gently invert the tube 5-6 times. Incubate at room temperature for 2 min
4. Add 400 µL Monarch Buffer B3 and invert until neutralized incubate at room temperature for 2 min
5. Centrifuge for 5 min at 23,444 g

6. Transfer to column and centrifuge for 1 min at 16,000 g
7. Discard flow through. Re-insert the column in spin collection tube and add 200 μ L Monarch Buffer B2 centrifuge 1 min at 16,000 g
8. Wash by adding 400 μ L Monarch Buffer W2 and centrifuge for 1 min at 16,000 g
9. Transfer to a clean 1.5 mL microfuge tube
10. Add 50 μ L Monarch Buffer EY, wait 1 min and centrifuge for 1 min at 16,000 g

Done by Sophie C

Date: 20260922

Title:

Purpose:

Procedure:

1.

Done by

Date: 20260923

Title: Golden gate assembly

Purpose: assemble parts

Procedure:

NA:Pveg-Bopep4-9-23

• Vector (shuttle vector): pSTK-EXP-1-sfGFP • concentration: 127 ng/ μl • 6929 bp	0.3546	1.06 μ l (1/3)
Insert promoter(Pveg) • concentration(ng/μL): 168 • bp: 2361	0.182 μ L	1.82 μ L (dilute 1/10)
Insert RBS(EEBD-RBS0) • concentration(ng/μL): 151 • bp: 2392	0.2059337748 μ L	1.03 μ L (Dilute 1/5)
Insert CDS (Bopep4-9-23) ng/ μ L - concentration: 193 - 2240 bp	0.150880829	1.06 (1/7)
Insert Terminator (B0015) • concentration(ng/μL): 148 • bp: 2213	0.194	0.97 μ L (dilute 1/5)
T4 DNA ligase buffer	1 μ L	1 μ L
Sterile water	(+0.37)	2.06 μ L
T4 DNA ligase	0.5 μ L	0.5 μ L
BsaI-HFv2	0.5 μ L	0.5 μ L

NB :Pveg-Bopep4-S15A_1-23_NHis

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ μl 6929 bp	0.3546	1.06 ul (1/3)
Insert promoter(Pveg) - concentration(ng/μL): 168 - bp: 2361	0.182 uL	1.82 uL (dilute 1/10)
Insert RBS(EEBD-RBS0) - concentration(ng/μL): 151 - bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS (Bopep4-S15A_1-23_NHis) 2200 bp 86 ng/uL	0.33255 uL	1.66 uL (dilute 1/5)
Insert Terminator (B0015) - concentration(ng/μL): 148 - bp: 2213	0.194	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water		1.46 uL
T4 DNA ligase	0.5 μL	0.5 μL
BsaI-HFv2	0.5 μL	0.5 μL

NC: Pveg-Bopep4_G17A_1-23-NHis

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ μl 6929 bp	0.3546	1.06 ul (1/3)
Insert promoter(Pveg) - concentration(ng/μL): 168 - bp: 2361	0.182 uL	1.82 uL (dilute 1/10)
Insert RBS(EEBD-RBS0) - concentration(ng/μL): 151 - bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS Bopep4_G17A_1-23-NHis 2200 bp 73 ng/uL	0.3917	1.18 uL (dilute 1/3)
Insert Terminator (B0015) - concentration(ng/μL): 148 - bp: 2213	0.194	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water		1.94 uL
T4 DNA ligase	0.5 μL	0.5 μL
BsaI-HFv2	0.5 μL	0.5 μL

ND: Pveg-Bopep4-WT_1-23-NHis

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ μl 6929 bp	0.3546	1.06 ul (1/3)
Insert promoter(Pveg) - concentration(ng/μL): 168 - bp: 2361	0.182 uL	1.82 uL (dilute 1/10)
Insert RBS(EEBD-RBS0) - concentration(ng/μL): 151 - bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS Bopep4-WT_1-23-NHis 2200 bp 117 ng/uL	0.2444 uL	1.22 uL (dilute 1/5)
Insert Terminator (B0015) - concentration(ng/μL): 148 - bp: 2213	0.194	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water		1.9 uL
T4 DNA ligase	0.5 μL	0.5 μL
BsaI-HFv2	0.5 μL	0.5 μL

Done by Sophie C

Date: 20260924

Title: Electroporation

Purpose: Introduce Bopep4 expression vector

Procedure:

1. Precool electrocuvette
2. Transfer WB800 electrocompetent cell (260923) to electrocuvette
3. Add following plasmid to electrocuvette

Pveg-Bopep4 290 ng/ μ L Add 3.5 μ L to 100 mL cell (1 mg)
Pveg-Bopep4 15-23 305 ng/ μ L Add 3.3 μ L to 100 mL cell (1 mg)
pSTK-EXP2-ccaSRv1-ACCD 257 ng/ μ L Add 3.9 μ L to 100 mL cell (1 mg) $\times 3$

4. Electroporation

Plasmid	Voltage (kV)	Capacitance (μ F)	Resistance (Ω)	TC (mS)
Pveg-Bopep4	2.280/2.3	25	250	6.3
Pveg-Bopep4 15-23	2.281/2.3	25	250	6.4
pSTK-EXP2-ccaSRv1-ACCD	2.280/2.3	25	250	6.3
pSTK-EXP2-ccaSRv1-ACCD	2.489/2.5	25	250	6.2
pSTK-EXP2-ccaSRv1-ACCD	2.680/2.7	25	250	6.1

5. Add 1 mL Recovery Medium immediately and transfer to culture tubes
6. Cover the tube transform with pSTK-EXP2-ccaSRv1-ACCD with aluminium foil to avoid the light
7. Incubate in 37 °C 200 rpm for 4 hrs

Done by Sophia

Date: 20260924

Title: Transformation (NA, NB, NC, ND)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

1. Set up water bath at 42 °C
2. Add 5 μ L plasmid to competent cell when 50 % thawed
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Incubate on ice for 3 min

6. Add 1 mL SOC medium
7. Incubate at 37 °C for 1 hr
8. Centrifuge for 5 min at 5000 xg
9. Resuspend pellet after removing 1 mL supernatant
10. Spread in LBC plate
11. Place in incubator overnight

Done by Sophie C

Date: 20260924

Title: Miniprep

Purpose: To extract plasmid from liquid culture

Procedure:

Y2, Y7, Y15: ABDQ

Z3: ACDQs

Ú4: pSTK-0C-samyQ-LEA-2.2

1. Pellet 1-5 mL of bacterial overnight culture by centrifuging at 6800 g for 3
2. Resuspend cells in 250 µL Buffer P1 by pipetting
3. Add 250 µL Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear
4. Add 350 µL Buffer N3, mix thoroughly by inverting the tube 4-6 times
5. Centrifuge for 10 min at 17900 g
6. Transfer the supernatant to QIA 2.0 spin column. Centrifuge for 1 min at 17900 g
7. Discard flow through
8. Wash column by adding 0.5 mL Buffer PB. Centrifuge for 30 sec at 17900 g. Discard the flow through
9. Wash column by adding 0.75 mL Buffer PE. Centrifuge for 30 sec at 17900 g. Discard flow through
10. Spin empty column at 17900 g for 1 min to remove residual ethanol
11. Transfer column to new 1.5 mL microcentrifuge tube
12. Add 50 µL EB Buffer to the column membrane
13. Let stand for 1 min then spin at 17900 g for 1 min
14. Biodrop

	Concentration	A230/260	A260/280
Y2	332 ng/µL	2.274	1.897
Y7	402 ng/µL	2.258	1.887
Y15	309 ng/µL	2.356	1.892
Z3	105 ng/µL	1.981	1.875

Ú4	94 ng/μL	1.774	1.880
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Done by Sophie C, Abby K

Date: 20260924

Title: PCR

Purpose: Amplify plasmid number

Procedure:

Y2, Y7, Y15: ABDQ

Z3: ACDQ

1. Prepare master mix as below:

Y2-A, Y7-A, Y15-A, Z3-A :

pcyA F (L2)	1 μL
ccaSm3 R (L2)	1 μL
ddH ₂ O	7 μL
Q-amp master mix	10 μL
DNA	1 μL

Y2-B, Y7-B, Y15-B, Z3-B:

ccaSm3 F (L2)	1 μL
CcaR R (L2)	1 μL
ddH ₂ O	7 μL
Q-amp master mix	10 μL
DNA	1 μL

Y2-C, Y7-C, Y15-C, Z3-C:

CcaR F (L2)	1 μL
Csn R	1 μL
ddH ₂ O	7 μL
Q-amp master mix	10 μL
DNA	1 μL

2. Run through thermal cycle:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	1 min	
25 °C	5 min	1 cycle

Done by Sophie C.

Date: 20260925

Title: Colony PCR

Purpose: Amplify plasmid number

Procedure:

1. Prepare the following reagents:

NA: Pveg-Bopep4-9-23

NB: Pveg-Bopep4-S15A_1-23_NHis

ND: Pveg-Bopep4-WT_1-23-NHis

	1 rxn	26 rxn
Fw primer (SamyQ fw)	1 µL	26 µL
Rv primer (Exp rv)	1 µL	26 µL
Q-amp	10 µL	260 µL
ddH ₂ O	8 µL	208 µL

2. Pick up colonies and streak the grid
3. Incubate in RT for 5 min
4. Run through PCR machine:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	2 min	
25 °C	5 min	1 cycle

Done by Sophie H.

Date: 20260925

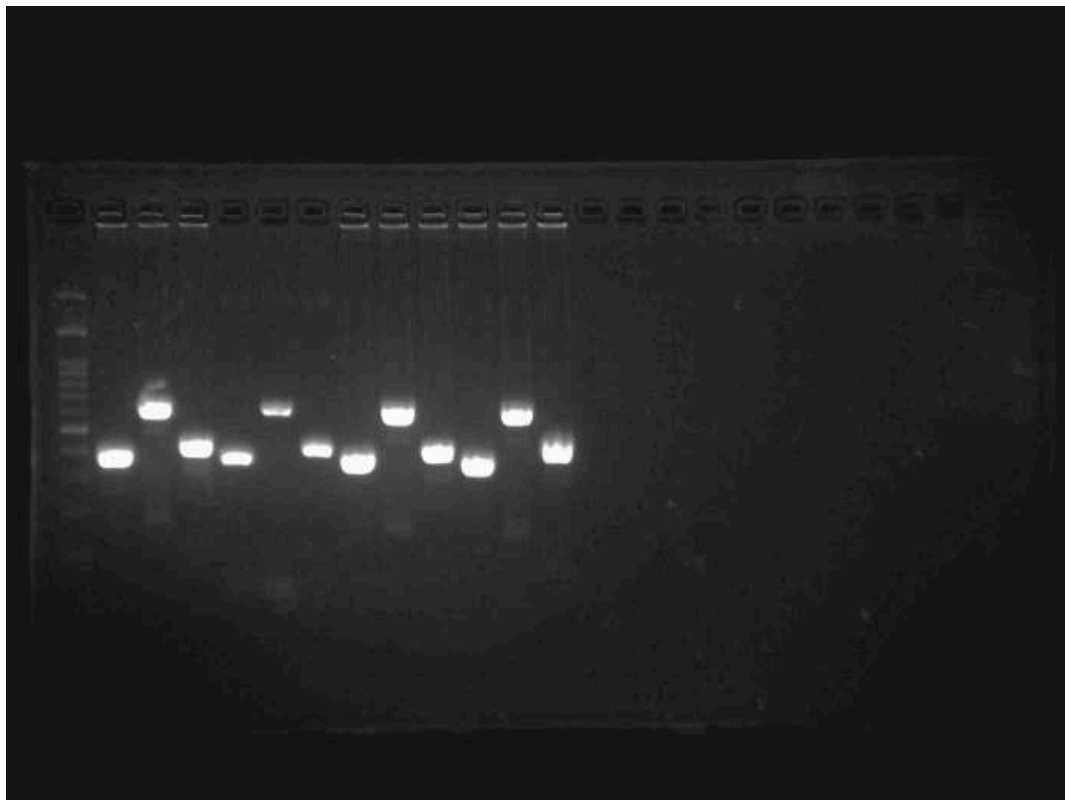
Title: Colony PCR

Purpose: Amplify plasmid number

Procedure:

1. Prepare the following reagents:

E: pSTK-EXP2-CcaSRv1-ACCD (2.3 kV, 2.5 kV, 2.7 kV)



	1 rxn	13 rxn
Fw primer (Ccas fw)	1 μ L	13 μ L
Rv primer (CcaR rv)	1 μ L	13 μ L
Q-amp	10 μ L	130 μ L
ddH ₂ O	8 μ L	104 μ L

2. Pick up colonies and streak the grid
3. Incubate in RT for 5 min
4. Run through PCR machine:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	2 min	

25 °C	5 min	1 cycle
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Done by Sophie C.

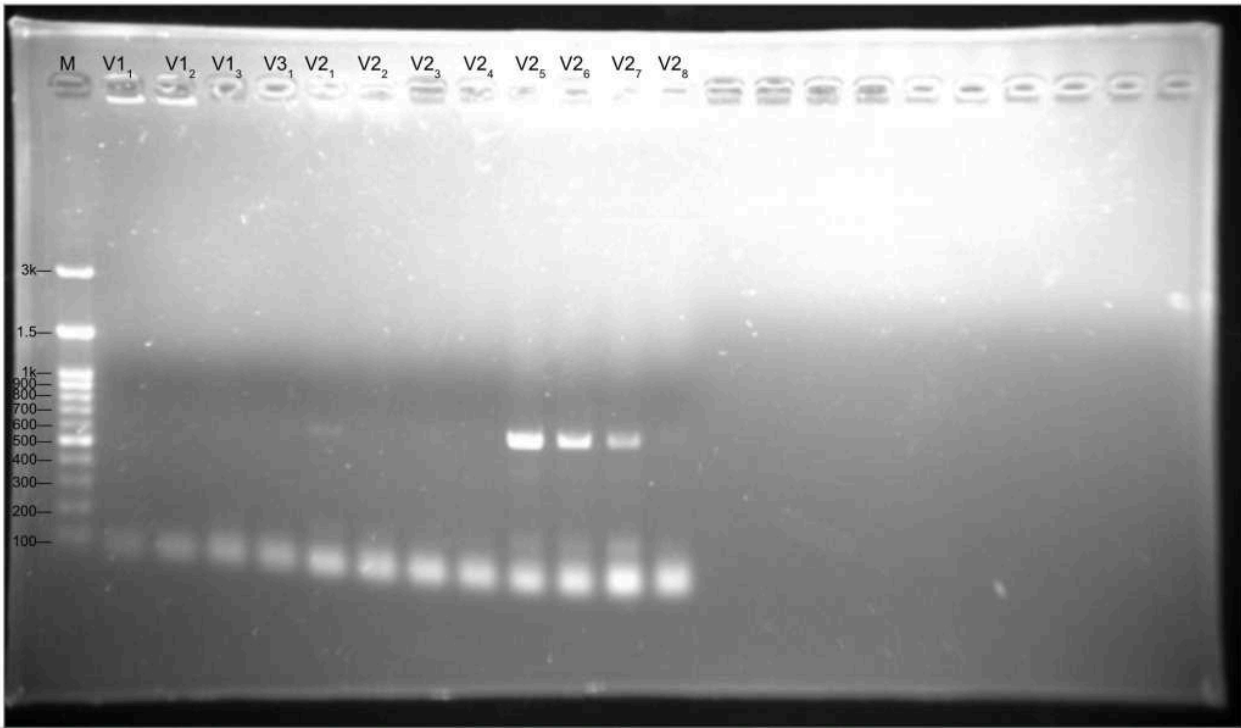
Date: 20260925

Title: Gel Electrophoresis

Purpose: Check Colony PCR product length

Procedure:

1. Load the gel in order
2. Dissolve the gel in 100 V for 30 min



M: 3kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)
V: ACDI electroporation in WB800	CcaR-fw	PcpcG2-veg	640 bp

Date: 20260925

Title: Liquid culture

Purpose: Grow bacteria in liquid medium

Procedure:

N : Pveg-EEBD-RBS0-SamyQ-Bopep4-2.1-B0015

NA: #6, #7, #8

NB: #5, #6, #7

ND: #2, #3, #8

1. Distribute 3 mL of TB medium in each culture tube
1. Add 3 mL of Ampicillin to each culture tube
2. Pick up colonies from streaked grid and dispense the tip in the culture tube
3. Place in 37 °C incubator at a slanted angle for 16 hr

Done by Abby K.

Date: 20260926

Title: Miniprep

Purpose: To extract plasmid from liquid culture

Procedure:

NA(Pveg-Bopep4-9-23): #6, #7, #8

NB(Pveg-Bopep4-S15A_1-23_NHis): #5, #6, #7

ND(Pveg-Bopep4-WT_1-23-NHis): #2, #3, #8

1. Pellet 1-5 mL of bacterial overnight culture by centrifuging at 6800 g for 3 min
2. Resuspend cells in 250 μ L Buffer P1 by pipetting
3. Add 250 μ L Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear
4. Add 350 μ L Buffer N3, mix thoroughly by inverting the tube 4-6 times
5. Centrifuge for 10 min at 17900 g
6. Transfer the supernatant to QIA 2.0 spin column. Centrifuge for 1 min at 17900 g
7. Discard flow through
8. Wash column by adding 0.5 mL Buffer PB. Centrifuge for 30 sec at 17900 g. Discard the flow through
9. Wash column by adding 0.75 mL Buffer PE. Centrifuge for 30 sec at 17900 g. Discard flow through
10. Spin empty column at 17900 g for 1 min to remove residual ethanol
11. Transfer column to new 1.5 mL microcentrifuge tube
12. Add 50 μ L EB Buffer to the column membrane
13. Let stand for 1 min then spin at 17900 g for 1 min
14. Biodrop

Done by Abby K.

Date: 20260927

Title: Transformation (SB, SC, SD, SE, O, I, π , NC)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

1. Set up water bath at 42 °C
2. Add 5 μ L plasmid to competent cell when 50 % thawed
3. Incubate on ice for 20 mins
4. Heat shock 45 sec
5. Incubate on ice for 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 °C for 1 hr
8. Centrifuge for 5 min at 5000 xg
9. Resuspend pellet after removing 1 mL supernatant
10. Spread in LBC plate
11. Place in incubator overnight

Done by Sophie C

Date: 20260927

Title: Golden Gate Assembly (X7)

Purpose: Assemble parts

Procedure:

Ã :Pveg-Bopep4_1-23_S15A

• Vector (shuttle vector): pSTK-EXP-1-sfGFP • concentration: 127 ng/ μl • 6929 bp	0.3546 μ L	0.71 μ L (1/2)
Insert promoter(Pveg) • concentration(ng/μL): 168 • bp: 2361	0.182 μ L	1.82 μ L (dilute 1/10)
Insert RBS(EEBD-RBS0) • concentration(ng/μL): 151 • bp: 2392	0.2059337748 μ L	1.03 μ L (Dilute 1/5)
Insert CDS Ã7	0.3679 μ L	0.74 μ L(1/2)

- concentration: 80 ng/ul - 2264 bp		
Insert Terminator (B0015) • concentration(ng/μL): 148 • bp: 2213	0.194 μL	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water	(+0.37)	2.73 μL
T4 DNA ligase	0.5 μL	0.5 μL
Bsal-HFv2	0.5 μL	0.5 μL

Ě: Pveg-Bopep4_1-23_G17A

• Vector (shuttle vector): pSTK-EXP-1-sfGFP • concentration: 127 ng/ μl • 6929 bp	0.3546 μL	0.71 μL (1/2)
Insert promoter(Pveg) • concentration(ng/μL): 168 • bp: 2361	0.182 μL	1.82 μL (dilute 1/10)
Insert RBS(EEBD-RBS0) • concentration(ng/μL): 151 • bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS Ě7 2264 bp 71 ng/uL	0.4145352113 μL	0.83 (1/2) μL

Insert Terminator (B0015) - concentration(ng/μL): 148 - bp: 2213	0.194 μL	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water		2.64 μL
T4 DNA ligase	0.5 μL	0.5 μL
BsaI-HFv2	0.5 μL	0.5 μL

Ñ: Pveg-Bopep4_1-23_WT:

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ μl 6929 bp	0.3546 μL	0.71 μL (1/2)
Insert promoter(Pveg) - concentration(ng/μL): 168 - bp: 2361	0.182 μL	1.82 μL (dilute 1/10)
Insert RBS(EEBD-RBS0) - concentration(ng/μL): 78 - bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS Ñ7 2264 bp 73 ng/uL	0.4031780822	0.81 (1/2)

Insert Terminator (B0015) - concentration(ng/μL): 148 - bp: 2213	0.194	0.97 μL (dilute 1/5)
T4 DNA ligase buffer	1 μL	1 μL
Sterile water		2.66 μL
T4 DNA ligase	0.5 μL	0.5 μL
Bsal-HFv2	0.5 μL	0.5 μL

Done by Sophie C.

Date: 20260927

Title: Liquid Culture (V21, V25, V27)

Purpose: Grow bacteria in liquid medium

Procedure:

V21, V25, V27: ABDI

1. Distribute 3 mL of TB medium in each culture tube
1. Add 3 mL of Ampicillin to each culture tube
2. Pick up colonies from streaked grid and dispense the tip in the culture tube
3. Place in 37 °C incubator at a slanted angle for 16 hr

Done by Abby K.

Date: 20260928

Title: miniprep (S5, S11)

Purpose: Extract colony PCR product

Procedure:

S5, S11: pSTK-EXP1-Pveg-EEBD-RBS0-SamyQ:LEA-2.1-B0015

1. Pellet 1-5 mL of bacterial overnight culture by centrifuging at 6800 g for 3 min
2. Resuspend pelleted bacterial cells in 250 μ L buffer
3. Add 250 μ L P2 buffer and mix thoroughly by inverting tube 4-6 times
4. Add 350 μ L P3 buffer and mix thoroughly by inverting tube 4-6 times
5. Centrifuge for 10 min at 17900 g

Done by Sophie C

Date: 20260928

Title: Colony PCR(I, π , SB, SC, SD, SE, NC, X)

Purpose: Amplify plasmid number

Procedure:

	1 rxn	14 rxn
Fw primer (CcaSm 3 fw)	1 μ L	14 μ L
Rv primer (CcaR rv)	1 μ L	14 μ L
Q-amp	10 μ L	140 μ L
ddH ₂ O	8 μ L	112 μ L

	1 rxn	5 rxn
Fw primer (SamyQ fw)	1 μ L	5 μ L
Rv primer (Exp rv)	1 μ L	5 μ L
Q-amp	10 μ L	50 μ L
ddH ₂ O	8 μ L	40 μ L

	1 rxn	25 rxn
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Fw primer (STK fw)	1 µL	25 µL
Rv primer (STK rv)	1 µL	26 µL
Q-amp	10 µL	250 µL
ddH ₂ O	8 µL	200 µL

1. Pick up colonies and streak the grid
2. Incubate in RT for 5 min
3. Run through PCR machine:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	2 min	
25 °C	5 min	1 cycle

Done by Sophie C

Date: 20260928

Title:Transformation (X)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

X: ABDI

1. Add 5 µL plasmid to competent cell when 50 % thawed
2. Incubate on ice for 20 mins
3. Heat shock 45 sec
4. Incubate on ice for 3 min
5. Add 1 mL SOC medium
6. Incubate at 37 °C for 1 hr
7. Centrifuge for 5 min at 5000 xg
8. Resuspend pellet after removing 1 mL supernatant
9. Spread in LBC plate
10. Place in incubator overnight

Done by Sophie C

Date: 20260928

Title: Level 1 golden gate assembly (A, E, N)

Purpose: Assemble genetic parts

Procedure:

Ã :Pveg-Bopep4_1-23_S15A

• Vector (shuttle vector): pSTK-EXP-1-sfGFP • concentration: 127 ng/ µl • 6929 bp	0.3546 µL	0.71 µL (1/2)
Insert promoter(Pveg) • concentration(ng/µL): 168 • bp: 2361	0.182 µL	1.82 µL (dilute 1/10)
Insert RBS(EEBD-RBS0) • concentration(ng/µL): 151 • bp: 2392	0.2059337748 µL	1.03 µL (Dilute 1/5)
Insert CDS Ã7 - concentration: 80 ng/ul - 2264 bp	0.3679 µL	0.74 µL (1/2)
Insert Terminator (B0015) • concentration(ng/µL): 148 • bp: 2213	0.194 µL	0.97 µL (dilute 1/5)
T4 DNA ligase buffer	1 µL	1 µL
Sterile water	(+0.37µL)	2.73 µL
T4 DNA ligase	0.5 µL	0.5 µL
BsaI-HFv2	0.5 µL	0.5 µL

Ë: Pveg-Bopep4_1-23_G17A

• Vector (shuttle vector): pSTK-EXP-1-sfGFP • concentration: 127 ng/ µl • 6929 bp	0.3546 µL	0.71 µL (1/2)
Insert promoter(Pveg) • concentration(ng/µL): 168 • bp: 2361	0.182 µL	1.82 µL (dilute 1/10)
Insert RBS(EEBD-RBS0) • concentration(ng/µL): 151 • bp: 2392	0.2059337748 µL	1.03 µL (Dilute 1/5)
Insert CDS Ë7 2264 bp 71 ng/uL	0.4145352113 µL	0.83 µL(1/2)
Insert Terminator (B0015) • concentration(ng/µL): 148 • bp: 2213	0.194 µL	0.97 µL (dilute 1/5)
T4 DNA ligase buffer	1 µL	1 µL
Sterile water		2.64 µL
T4 DNA ligase	0.5 µL	0.5 µL
BsaI-HFv2	0.5 µL	0.5 µL

Ñ: Pveg-Bopep4_1-23_WT:

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ µl 6929 bp	0.3546 µL	0.71 µL (1/2)
Insert promoter(Pveg) - concentration(ng/µL): 168 - bp: 2361	0.182 µL	1.82 µL (dilute 1/10)
Insert RBS(EEBD-RBS0) - concentration(ng/µL): 78 - bp: 2392	0.2059337748 µL	1.03 µL (Dilute 1/5)
Insert CDS Ñ7 2264 bp 73 ng/uL	0.4031780822 µL	0.81 µL (1/2)
Insert Terminator (B0015) - concentration(ng/µL): 148 - bp: 2213	0.194 µL	0.97 µL (dilute 1/5)
T4 DNA ligase buffer	1 µL	1 µL
Sterile water		2.66 µL
T4 DNA ligase	0.5 µL	0.5 µL
Bsal-HFv2	0.5 µL	0.5 µL

Done by Sophie C

Date: 20260928

Title: Liquid Culture

Purpose: Plant colony into liquid medium

Procedure:

N : Pveg-EEBD-RBS0-SamyQ-Bopep4-2.1-B0015

NA: #6, #7, #8

NB: #5, #6, #7

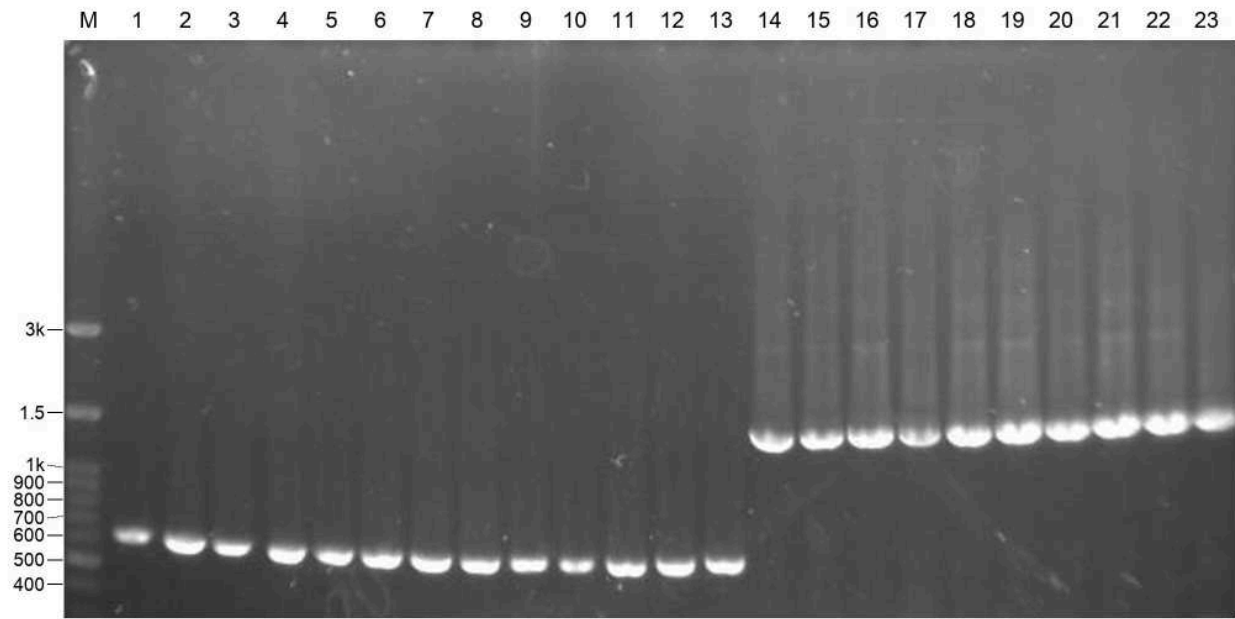
ND: #2, #3, #8

1. Distribute 3 mL of TB medium in each culture tube
1. Add 3 mL of Ampicillin to each culture tube
2. Pick up colonies from streaked grid and dispense the tip in the culture tube
3. Place in 37 °C incubator at a slanted angle for 16 hr

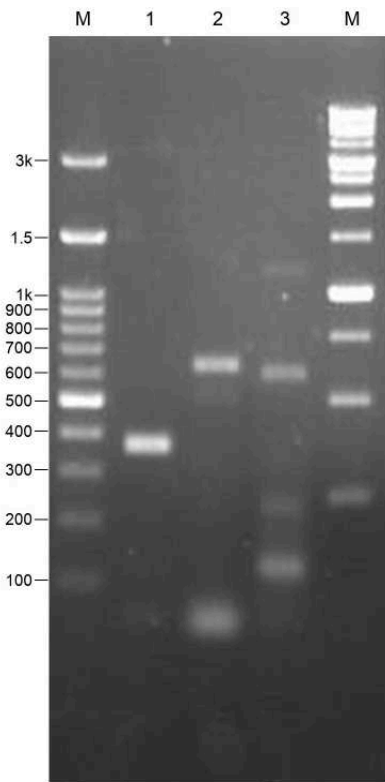
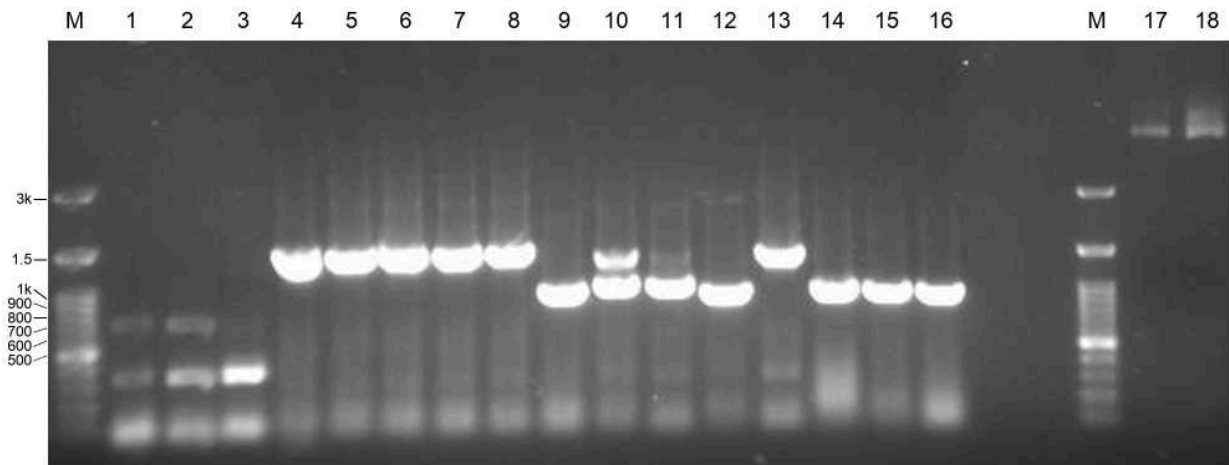
Done by Sophie C

Date: 20260929
Title:Gel electrophoresis
Purpose: Check basepair length
Procedure:

- 1. Load the gel in the layout shown below
- 2. Dissolve the gel at 100 V for 30 min



M: 3kb DNA ladder			
Plasmid Construct	Forward Primer	Reverse Primer	Expected Size (bp)



Done by Chloe, Sophie C

Date: 20260929

Title: LB medium

Purpose: Prepare LB medium for further experiment

Procedure:

1. Prepare the following

LB broth powder	7.5 g
ddH ₂ O	300 mL

2. Add all the ingredients above to a conical flask
3. Add a stirring bar and stir until no powder can be seen
4. Remove the stirring rod
5. Cover the flask with aluminum foil
6. Autoclave for 30 min
7. Store in fridge

Done by Sophie L.

Date: 20260930

Title: Colony PCR (X, V)

Purpose: Amplify plasmid number

Procedure:

X1(A, B, C)

X2(A, B, C)

V7-1(A, B, C)

X1.1 (C)

X1, X2, V7-1 (A)

	1 rxn	3 rxn
Fw primer (SamyQ fw)	1 µL	3 µL
Rv primer (Exp rv)	1 µL	3 µL
Q-amp	10 µL	30 µL
ddH ₂ O	8 µL	24 µL

X1, X2, V7-1(B)

	1 rxn	3 rxn
Fw primer (SamyQ fw)	1 µL	3 µL
Rv primer (Exp rv)	1 µL	3 µL
Q-amp	10 µL	30 µL
ddH ₂ O	8 µL	24 µL

X1, X2, V7-1(C)

	1 rxn	4 rxn
Fw primer (SamyQ fw)	1 µL	4 µL
Rv primer (Exp rv)	1 µL	4 µL
Q-amp	10 µL	40 µL
ddH ₂ O	8 µL	32 µL

1. Pick up colonies and streak the grid

2. Incubate in RT for 5 min
3. Run through PCR machine:

95 °C	5 min	1 cycle
95 °C	20 sec	35 cycles
60 °C	20 sec	
72 °C	2 min	
25 °C	5 min	1 cycle

Done by Chloe, Sophie C

Date: 20260930

Title:Transformation (A,E,N)

Purpose: Grow bacterial colony on plate

Procedure:

1. Set up water bath at 42 °C
2. Add 5 µL plasmid to competent cell when 50% thawed
3. Incubate on ice for 20 min
4. Heat shock for 45 sec
5. Incubate on ice for 3 min
6. Add 1 mL SOC medium
7. Incubate at 37 °C for 1 hr
8. Centrifuge for 5 min at 5000 g
9. Resuspend pellet after removing 1 mL supernatant
10. Spread on LBA plate
11. Place in incubator overnight

Done by Sophie C.

Date: 20260930

Title:Liquid culture (i,NC,π)

Purpose: Plant Liquid culture in medium

Procedure:

1. Distribute 3 mL of TB medium in each culture tube
1. Add 3 mL of Ampicillin to each culture tube
2. Pick up colonies from streaked grid and dispense the tip in the culture tube
3. Place in 37 °C incubator at a slanted angle for 16 hr

Done by Sophie C