

Date: 20261001

Title: Miniprep(i2, tt, Nc4, i7)

Purpose: to extract plasmid from liquid culture

Procedure:

1. Pellet 1-5 mL of bacterial overnight culture by centrifuging at 6800 xg 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 xg
6. Transfer the supernatant to QIA 2.0 spin column. Centrifuge for 1 min at 17900 xg
7. Discard flow through
8. Wash column by adding 0.5 mL Buffer PB. Centrifuge for 30 sec at 17900 xg. Discard the flow through
9. Wash column by adding 0.75 mL Buffer PE. Centrifuge for 30 sec at 17900 xg. Discard flow through
10. Spin empty column at 17900 xg 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 xg for 1 min
14. Biodrop

Done by Sophie C

Date: 20261001

Title: Colony PCR (A,E,N)

Purpose: Amplify engineered DNA plasmid

Procedure:

Date: 20261001

Title: gel electrophoresis(X,V)

Purpose: Checking Colony PCR result

Procedure:

Date: 20261001

Title: golden gate assembly (B,C)

Purpose: assemble parts

Procedure:

B: (STK) Module 2-PliaG-RBS0-CcaSm3-L3S3P21

Vector (1B) • Concentration (ng/μL): 151	0.1343046358 μ L	1.343046358 μ l (dilute 1/10)
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bp: 3120		
Insert promoter (PliaG) - Concentration (ng/μL): 130 - bp: 2205	0.2205 ul	0.88 ul (1/4)
Insert RBS(RBS0) - Concentration (ng/μL): 188 - bp: 2113	0.1461117021	0.88 ul (1/6)
Insert CDS(CcaSm3) - Concentration (ng/μL): 128 - bp: 1465	0.1487890625 μL	0.9 ul (1/6)
Insert Terminator (L3S3P21) - concentration(ng/μL): 136 ng/ul - Bp: 2137	0.2042720588	0.82 ul (1/4)
T4 DNA ligase buffer	2 μL	1 μL
Sterile water		3.18 μL
T4 DNA ligase	1 μL	0.5 μL
BsaI-HFv2	1 μL	0.5 μL
Total	20 μL	10 μL

C: (STK) Module 2-PlepA-RBS0-CcaSm3-L3S3P21

Vector (1B)	0.1343046358 μL	1.343046358 μl (dilute 1/10)
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- concentration(ng/μL): 151 - bp: 3120		
Insert promoter(PlepA) - concentration(ng/μL): 155 - bp: 2241	0.1879548387 μ l	1.13 μ l (dilute 1/6)
Insert RBS(RBS0) - concentration(ng/μL): 188 - bp: 2113	0.1461117021	0.88 μ l (1/6)
Insert CDS(CcaSm3) - concentration(ng/μL): 128 - bp: 1465	0.1487890625 μ L	0.9 μ l (1/6)
Insert Terminator (L3S3P21) - concentration(ng/μL): 136 ng/μl - Bp: 2137	0.2042720588	0.82 μ l (1/4)
T4 DNA ligase buffer	2 μ L	1 μ L
Sterile water		2.93 μ L
T4 DNA ligase	1 μ L	0.5 μ L
BsaI-HFv2	1 μ L	0.5 μ L
Total	20 μ L	10 μ L

D: Pveg-RBS0-CcaR-bkdB-cold

Vector (1C) - concentration(ng/μL): 142 - bp: 3120	0.1428 μ L	1 ul (1/7)
Insert promoter(Pveg) - concentration(ng/μL): 168 - bp: 2361	0.1826964286 μ L	0.91 (1/5)
Insert RBS(RBS0) - concentration(ng/μL): 188 - bp: 2113	0.1461117 μ L	0.88 ul (1/6)
Insert CDS(CcaR) - concentration(ng/μL): 77 - bp: 3777	0.6376753247	0.6376753247 ul
Insert Terminator (bkdb cold double) - 110 bp - 2151 bp	0.2542090909	0.76 ul (1/3)
T4 DNA ligase buffer	1 μ L	
Sterile water	3.81 μ L	
T4 DNA ligase	0.5 μ L	
Bsal-HFv2	0.5 μ L	
Total	10 μ L	

I:PcpcG2-veg-EEBD-RBS0-Csn:AccD-bkdB-cold
: M3-Pveg-RBS0-CcaR-B0015

Vector (1D)	1.5 uL (dil 1/5)
Pcpcg2-veg	1.2 uL (dilute 1/50)
EEBD-RBS0	1 uL (dilute 1/5)
csn:ACCD	0.6 uL (dil 1/10)
Insert Terminator (bkdb cold double) - 110 bp - 2151 bp	0.2542090909
T4 DNA ligase buffer	1 µL
Sterile water	3.45
T4 DNA ligase	0.5 µL
Bsal-HFv2	0.5 µL
Total	10 µL

1.

NA:Pveg-Bopep4-9-23

- Vector (shuttle vector): pSTK-EXP-1-sfGFP - concentration: 127 ng/ µl 6929 bp	0.3546	1.06 ul (1/3)
Insert promoter(Pveg)	0.182 uL	1.82 uL (dilute 1/10)

• concentration(ng/μL): 168 • bp: 2361		
Insert RBS(EEBD-RBS0) • concentration(ng/μL): 151 • bp: 2392	0.2059337748 μL	1.03 μL (Dilute 1/5)
Insert CDS (Bopep4-9-23) ng/uL - concentration: 193 - 2240 bp	0.150880829	1.06 (1/7)
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	(+0.37)	2.06 uL
T4 DNA ligase	0.5 μL	0.5 μL
Bsal-HFv2	0.5 μL	0.5 μL

Done by Sophie C

Date: 20261003

Title: Colony PCR

Purpose: Amplify engineered DNA plasmid

Procedure:

pSTK-Exp2-CcaSRv1-SamyQ:Acids-2.1 / LBC WB800 2.5kV 25µF 250Ω

pSTK-Exp2-CcaSRv1-SamyQ:Acids-2.1 / LBE WB800 2.5kV 25µF 250Ω 20261001

1. Prepare the following reaction

	1 rxn	10 rxn
Q-amp	10 µL	100 µL
ddH ₂ O	8 µL	80 µL
CcaR fw	1 µL	10 µL
pSTK-Exp-seq rv	1 µL	10 µL

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

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

Done by Alex, Phoebe

Date: 20261007

Title: Transformation (pcyA-CcaS-CcaR)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

1. Set up water bath at 42°C
2. Thaw competent cell and add 2 µL plasmid to 50 µL competent cell
3. Incubate on ice for 20 min
4. Heat shock at 42°C for 25-60sec
5. Spread on LBC plate
6. Incubate at 37°C overnight

Done by Sophie C

Date: 20261007

Title: Golden gate assembly (wi, wv, wt)

Purpose: assemble parts

Procedure:

WÍ : SamyQ-ACCD v3t1

• 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 (SamyQ-ACCD v3t1) ng/uL - concentration: 79 - 3230 bp	0.150880829	1.06 (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	(+0.37)	2.06 uL
T4 DNA ligase	0.5 μL	0.5 μL
BsaI-HFv2	0.5 μL	0.5 μL

W_{IT} : **SamyQ-ACCD**

- 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 (SamyQ-ACCD) ng/uL - concentration: 98 - 3230 bp	0.150880829	0.86 (1/2)
Insert Terminator (B0015) concentration(ng/μL): 148	0.194	0.97 μL (dilute 1/5)

• bp: 2213		
T4 DNA ligase buffer	1 μ L	1 μ L
Sterile water	(+0.37)	2.26 uL
T4 DNA ligase	0.5 μ L	0.5 μ L
BsaI-HFv2	0.5 μ L	0.5 μ L

WÚ : 2.2 LEA

• 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 μ L	1.03 μ L (Dilute 1/5)
Insert CDS (2.2 LEA) ng/uL - concentration: 94 - 2647 bp	0.150880829	0.73 (1/2)
Insert Terminator (B0015) • concentration(ng/μL): 148	0.194	0.97 μ L (dilute 1/5)

• bp: 2213		
T4 DNA ligase buffer	1 μ L	1 μ L
Sterile water	(+0.37)	2.39 μ L
T4 DNA ligase	0.5 μ L	0.5 μ L
BsaI-HFv2	0.5 μ L	0.5 μ L

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Component	Amount	Dilution
Vector STK001_pSTK-0-sfGFP • concentration(ng/ μ L): 346 • bp: 3090	0.058 μ L	1.16 (dilute 1/20)
Insert CDS SamyQ-LEA-STK 1180 bp 10 ng/ μ L	1.53 μ L	
T4 DNA ligase buffer		1 μ L
Sterile water		5.31
T4 DNA ligase		0.5 μ L
Bbs1		0.5 μ L
Total	10 μ L	10 μ L

Done by Sophie C

Date: 20261007

Title: Colony PCR (A,E,N)

Purpose: Amplify engineered DNA plasmid

Procedure:

Date: 20261008

Title: Colony PCR (pcyA-CcaS-CcaR)

Purpose: Amplify engineered DNA plasmid

Procedure:

1. Prepare the following rxn
2. Add 20 μ L to each PCR tube
3. Dispense tips & streak grid (LBC)
4. Spindown
5. Place into thermocycle

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

Done by Sophie & Chloe

Date: 20261008

Title: Transformation (i,wu,wt)

Purpose: Transform and cultivate engineered bacteria on agar plate

Procedure:

1. Set water bath to 42°C
2. Thaw competent cells until 50% thawed and add 10 μ L plasmid
3. Incubate on ice for 3 min
4. Heat shock 35-60sec at 42°C
5. Incubate ice for 3 min
6. Add 1 mL SOC medium and incubate at 37°C
7. Centrifuge 5000xg for 5 min
8. Take out 1 mL supernatant & resuspend pellet
9. Spread on LBA
10. Incubate at 37°C overnight

Done by Chloe & Sophie

Date: 20261008

Title: Gel electrophoresis(A,E,N) (1% gel)

Purpose: Checking Colony PCR result

Procedure:

Date: 20261008

Title: Electroporation

Purpose: Insert cloning product into *Bacillus Subtilis*

Procedure:

1. Mix 1µg DNA with 60 mL WB800 electrocompetent cell
 - a. pSTK-Exp1-Pveg:BoPep4: 3.5 µL
 - b. pSTK-Exp1-Pveg:BoPep-15-23: 3.3 µL
 - c. pSTK-Exp2-CcaSRv1-Acds: 2.6 µL (ABDI)
 - d. pSTK-Exp2-CcaSRv1-Acds-v2t1: 3.0 µL (ABDQ) x3
2. Use 1 mm electron cuvette to do electroporation. Transfer bacteria and DNA mixture to electro cuvette
3. Place electro cuvette on ice for 5 min
4. Place electro cuvette to shockpad , conduct electroporation by the following condition :

pSTK-Exp1-Pveg:BoPep4 Capacitance Resistance TC (ms)

	Voltage (kV)	Capacitance(µF)	Resistance(Ω)	TC (ms)
pSTK-Exp1-Pveg:BoPep4	2.486/2.5	25	250	5.8
pSTK-Exp1-Pveg:BoPep-15-23	2.488/2.5	25	250	5.6
pSTK-Exp2-CcaSRv1-Acds	2.487/2.5	25	250	5.8
pSTK-Exp2-CcaSRv1-Acds-v2t1	2.280/2.3	25	250	5.4
pSTK-Exp2-CcaSRv1-Acds-v2t1	2.082/2.1	25	250	5.7
pSTK-Exp2-CcaSRv1-Acds-v2t1	2.487/2.5	25	250	5.8
pSTK-Exp2-CcaSRv1-Acds-v2t1	Arc/2.7	25	250	Arc

5. Add 1 mL recovery medium immediately, transfer bacteria to tubes
6. Cover the tube with aluminum foil to avoid the light and incubate in 37 °C for 3 hr
7. Transfer to 1.5 mL microtube, centrifuge at 5000 g for 15 min
8. Remove 1 mL supernatant, then resuspend the pellet
9. Spread on the LB plate with antibiotics

10. Incubate in 37 °C for 16 hr

Done by Chloe

Date: 20261009

Title: Gel electrophoresis(pcyA-Ccas-CcaR)

Purpose: Checking Colony PCR result

Procedure:

Date: 20261009

Title: Colony PCR (WI,WU,Wπ)

Purpose: Amplify engineered DNA plasmid

Procedure:

Date: 20261009

Title: Gel electrophoresis(WI,WU,Wπ)

Purpose: Checking Colony PCR result

Procedure:

Date: 20261009

Title: Miniprep(E,N)

Purpose: to extract plasmid from liquid culture

Procedure:

Date: 20261009

Title: Golden Gate Assembly (NA,A,E,N,WO,Wπ)

Purpose: assemble parts

Procedure:

Date: 20261010
Title: Colony PCR (T,Y->WB800)
Purpose: Amplify engineered DNA plasmid
Procedure:

Date: 20261010
Title: PCR (Y->WB800)
Purpose: Amplify plasmid number
Procedure:

Date: 20261010
Title: Gel Electrophoresis(T,Y->WB800)
Purpose: Checking Colony PCR result
Procedure:

Date: 20261010
Title: ELISA
Purpose:
Procedure:

Date: 20261010
Title: Transformation (NA,A,E,N,WO,Wπ)
Purpose: Transform and cultivate engineered bacteria on agar plate
Procedure:

Date: 20261010
Title: Golden Gate Assembly (N,Wπ,WO)
Purpose: assemble parts
Procedure:

Date: 20261010
Title: Transformation (N, Wπ, WO)
Purpose: Transform and cultivate engineered bacteria on agar plate
Procedure:

Date: 20261010
Title: Electroporation(NC, N,V,Ux3)
Purpose: allowing foreign molecules like DNA to enter the cells
Procedure:

Date: 20261010

Title: Liquid Culture(WU,new Y,old Y)

Purpose: bacteria culture from successful colony PCR product

Procedure:

Date: 20261011

Title: Colony PCR (NA,A,E,N,WO,Wπ,N,Wπ,WO,NC,{N,V,U x3} ->WB800

Purpose: Amplify engineered DNA plasmid

Procedure:

Date: 20261011

Title: Miniprep(WU)

Purpose: to extract plasmid from liquid culture

Procedure:

Date: 20261011

Title: Gel electrophoresis (NA,A,E,N,WO,Wπ,N,Wπ,WO,NC,{N,V,U x3}->WB800)

Purpose: Checking Colony PCR result

Procedure: