Readme: Please follow this template when writing notes

2022年8月16日 12:27

Please include:

- Date
- Name(s)/Initial(s) of the operator(s)
- Name(s) of the protein(s)/gene(s) etc. you're working on
- Name(s) of the kit(s)
    - Please specify the brand! e.g., TIANGEN Plasmid Miniprep
- Procedure (no details needed if a protocol is present)
- Any data/picture you've obtained, e.g., the picture of the gel
- Anything you want to add, e.g., 'there seems something wrong with the band, it's too dim/the size is incorrect', etc.

in your notes, so that other people can easily follow what you've done today.

Thanks!

RYY

Initials
RYY = Ru Yunyi
SC = Shen Chang
WYX/Lisa = Wu Yixuan
IJ/JJY/Irene = Jin Jingyun

7.28 SDS-PAGE Practice

Monday, July 25, 2022 2:59 PM

Operators: WYX/SC/JQZ/YY/RYY
Note taken by: RYY

Sample Used: Cell pellets with some His-tagged protein from last year's iGEM project, 1.5mL tubes noted 'H3.1沉淀' 1&2

Procedure:

1. Preparing Sample Lysis
   - Add 100uL 裂解缓冲液 (from GST Purification Kit) into each tube
   - Add 2uL lysosome (50mg/mL from ? kit) into each tube, mix well by pipetting and vortex
   - Let it stand on ice for 30 minutes
   - Centrifuge the samples at 15000g, 4*C
   - Aspirate the supernatant, discard the pellet
   - Add 25uL sample + 25uL loading buffer to make a total of 50uL
   - Put the samples on dry bath (90*C, 5mins)
2. SDS-PAGE Electrophoresis
   - Samples were loaded in this order: ladder ('多彩预染'___), sample1, (N/A), ladder, sample2, ladder
   - Run at 120V for ~1-2 hrs
   - Stain with Coomassie Brilliant Blue (protocol by Yang)
   - Shake for 5 minutes
   - Wash with MiliQ Water for 5 minutes (change water in between)
   - Imaging using the ChemiDoc Imaging system

8.10 LB Plate making + bacterial culture

Tuesday, August 9, 2022 1:43 PM

LB plate making+ LB liquid medium making
LB + Kanamycin plate making (For pET30a transformation from last year to test the competence transformation efficiency)

Total media: 1L LB liquid, 200mL LB agar, 200mL LB+Amp (final conc: 20ug/mL)

Plate made by Lisa, Irene, Yunyi, Chang

8.15 Plasmid extraction

Monday, August 15, 2022 11:07 AM

OD600 of E. coli cultivated since August 14th 8pm

Measured at 10:15am， diluted *10

	20IpaD	IpaD-HA	JPS-G3
LB+amp	0.147	0.149	0.145
LB	0.104	0.162	0.133

Meaured at 1pm, diluted*10

	20IpaD	IpaD-HA	JPS-G3
LB+amp	0.161	0.170	0.162
LB	0.115	0.167	0.135

OD measured by Chang

Plasmid extracted by Lisa, Chang, Yuxin (TIANGEN Plasmid Miniprep)

8.16 Plasmid Enzyme Digestion

Tuesday, August 16, 2022 13:44

Double Digestion

Protocol

Operator: SC, RYY

Nanodrop result of extracted plasmid and the reaction system

Name	μg/μl	Applied amount in the reaction system	Enzyme(1μl)	Enzyme(1μl)	Buffer (2μl)
20IpaD_digested	0.085	11.8μl	XhoI	SacI	rcutsmart
IpaD-HA_digested	0.092	10.8μl	BamHI	XhoI	rcutsmart
JPS-G3_digested	0.119	8.4μl	XhoI	SacI	rcutsmart

Add milliQ to a reaction system of total 20 uL

*Placed in dry bath at 37˚C for 1 h for heat inactivation

Put back in -20*C for storage

8.22 Gel Electrophoresis & Purification

2022年8月22日 14:12

Operator: RYY

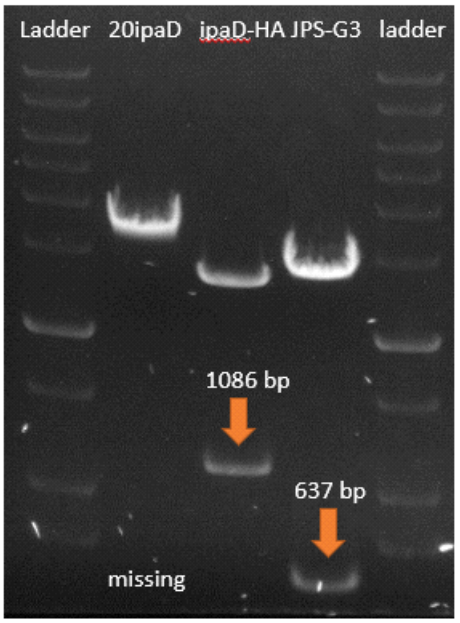
Digested plasmids 20ipaD_digested, ipaD-HA_digested, JPS-G3_digested

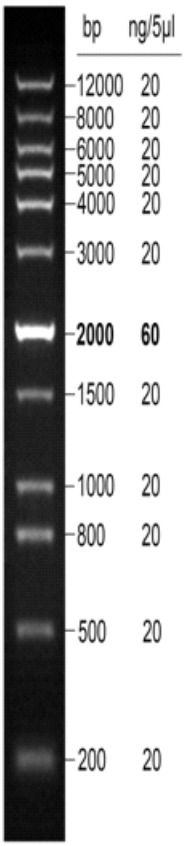
Gel Electrophoresis

1% agarose gel (TAE), run at 120V for 60 min

ladder	20ipaD	IpaD_HA	JPS-G3	ladder

Remaining samples put back to -20*C for storage (<1uL left)





5μl/lane
1% agarose gel
TAE, 7V/min, 45min

*20ipaD missing due to running time too long
Another double digestion session needed

Gel Purification

Protocol

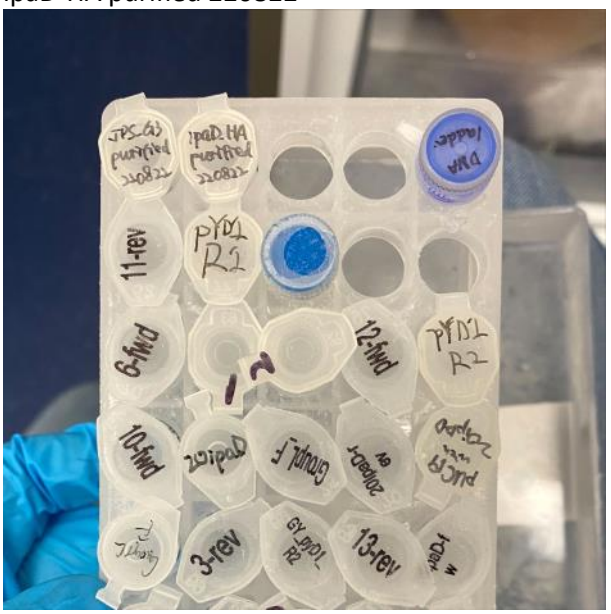
Beyotime kit

IpaD-HA: 124.2 mg
JPS_G3: 113.5 mg

55*C dry bath for 5 mins
16.0xg, 1 min, 23*C

Extracted ~20uL DNA

Put in -20*C
Named as

- JPS-G3 purified 220822
- ipaD-HA purified 220822
- 

8.23 Gel Electrophoresis

Tuesday, August 23, 2022 10:28

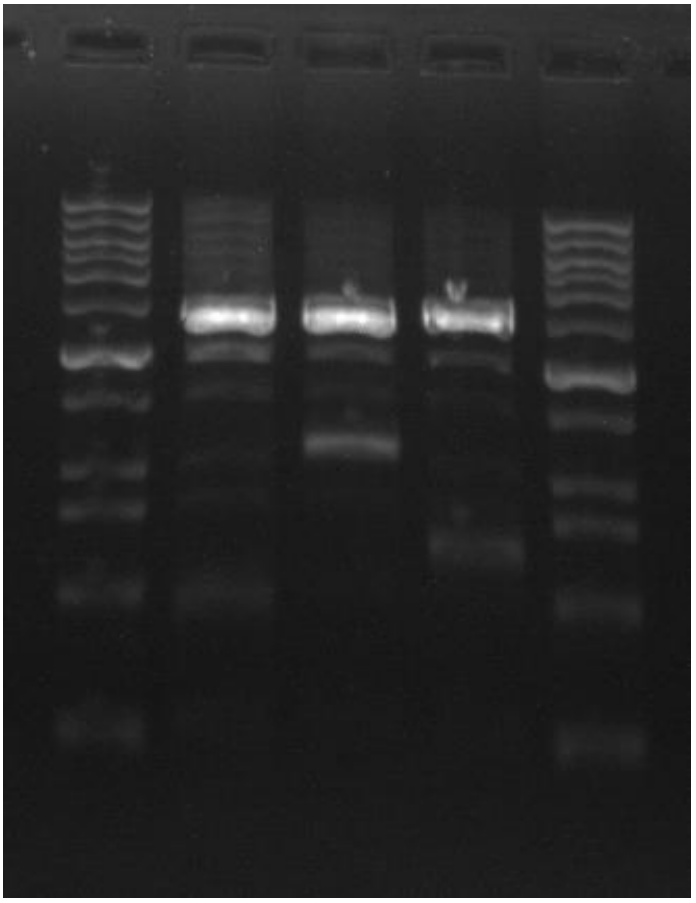
Gel electrophoresis
Operator: Chang (RYY nearby)

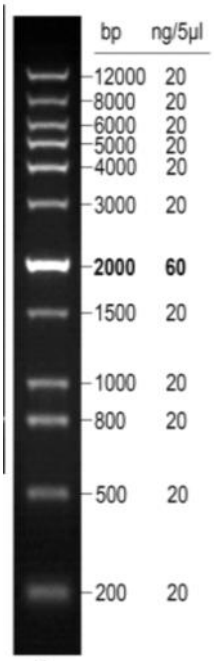
DNA sample: Purified IpaD-HA, Purified JPS-G3
No result

Digestion & Gel electrophoresis
Operator: Chang Shen

#	Name	μg/μl	Applied amount in the reaction system (μl)	Enzyme(1μl)	Enzyme(1μl)	Buffer (2μl)	Milli Q (μl)
1	20IpaD_digested	0.094	10.6	BamHI	SacI	rcutsmart	5.4
2	IpaD-HA digested	0.079	12.6	BamHI	XhoI	rcutsmart	3.4
3	JPS-G3_digested	0.127	7.9	XhoI	SacI	rcutsmart	10.1

Ladder 1 2 3 ladder





Conclusion: The sample lanes were contaminated by the DNA ladder because of improper loading method, so the result was not reliable.

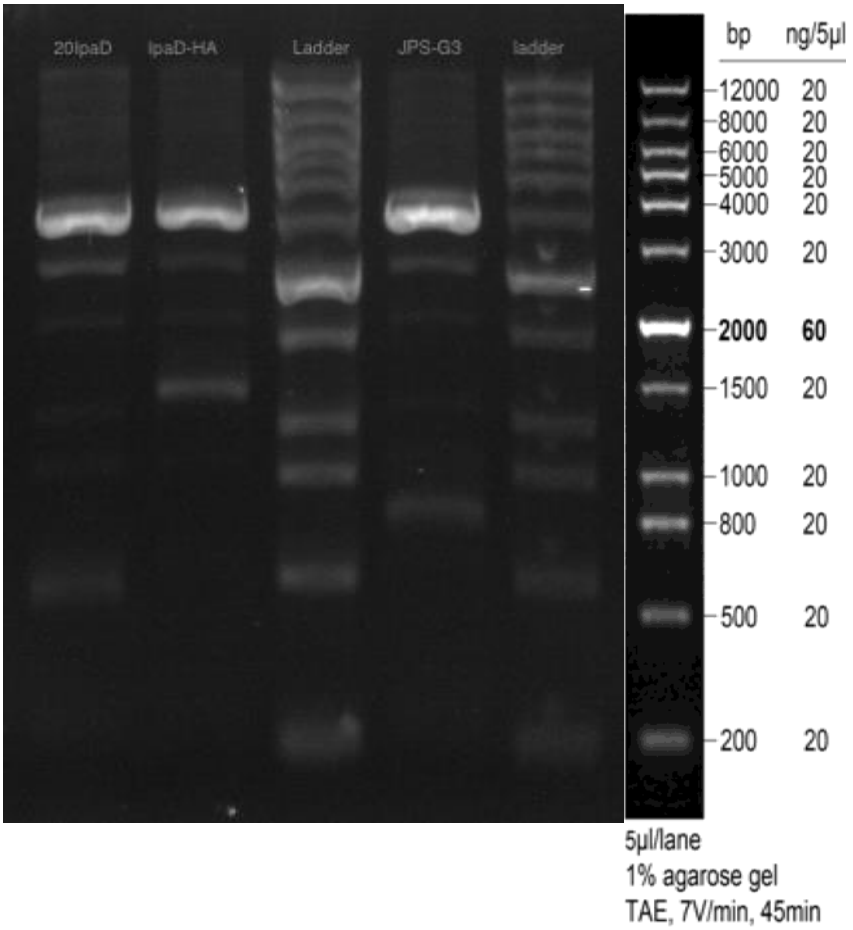
8.24 Gel Electrophoresis, Gel Purification, & Enlarge amplification of E. coli with csgA-smt gene (from XJTLU)

Wednesday, August 24, 2022 12:40

Gel Electrophoresis

Gel made by Qizhou

Operator: Chang
Samples:
Digested Samples (9µl): 20IpaD, IpaD-HA, JPS-G3
Loaded Ladder (1.5 µl * 2 lanes): Beyotime
Loaded mixture: 10µl
Result:



Gel Purification

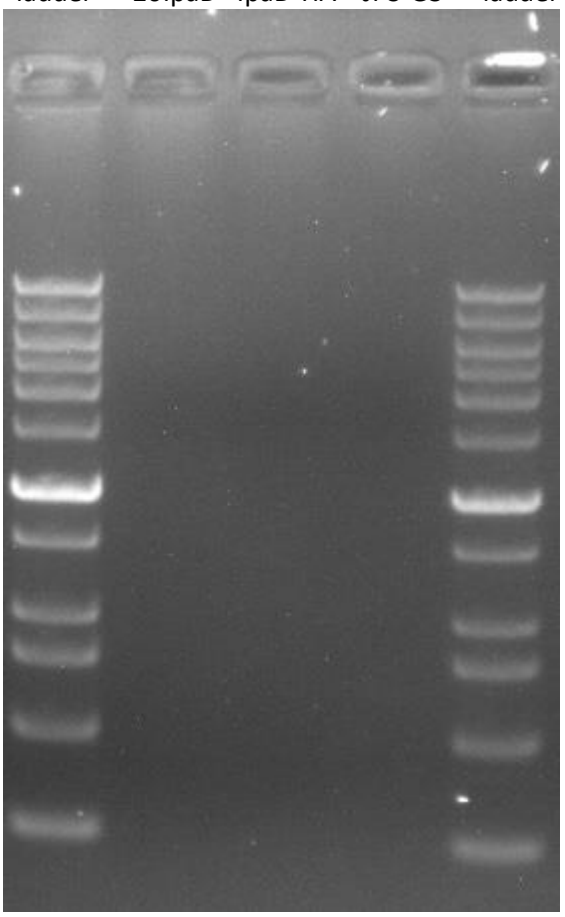
Protocol
Beyotime kit

Operator: RYY

20ipaD: 47.7 mg
ipaD_HA: 31.6 mg
JPG_G3: 41.9 mg

failed, AGAIN. What's the reason?????
Maybe the agarose isn't fully melted?

ladder 20IpaD IpaD-HA JPS-G3 ladder



E. coli (ssgA-smt, from XJTLU, No sign of bacteria on the plate after 15 h

8.30 Double Digestion + Gel Purification

2022年8月30日 10:29

Operator: RYY

Double Digestion

#	Name	Concentration (μg/μl)	Applied amount in the reaction system (μl)	Enzyme-1 (1μl)	Enzyme-2 (1μl)	Buffer (5μl)	MilliQ (μl)
1	20IpaD_digested	0.139	7.2	BamHI	SacI	rcutsmart	35.8
2	IpaD-HA digested	0.113	8.8	BamHI	XhoI	rcutsmart	34.2
3	JPS-G3_digested	0.198	5.1	SacI	XhoI	rcutsmart	37.9

Incubate at 37°C for 1 hr
Incubate at 65°C for 20 mins
35uL run gel electrophoresis

Gel Extraction

By ThermoFisher GeneJET Gel Extraction Kit

Conc:
1(20ipaD) - 0.7ng/uL;
2(ipaD_HA) - 1.9ng/uL;
3(JPS-G3) - 1.7ng/uL

8.31 PCR

2022年8月31日 11:06

Operator: RYY

PCR + Gel Electrophoresis

Primer pairs:

10/11 used for ipaD, 13/15 used for JPS-G3
10: CGCGGATCCATGAATATTACAACTC (ipaD_FW with BamHI cleavage site)
11: GGCCTCGAGTCAAGCGTAG (ipaD_RV with XhoI cleavage site)

13: GGCCTCGAGTTATTTGTCGTCGTCATCCTTG (JPS-G3_RV with XhoI cleavage site)
15: CGCGGATCCGGAAGTACACAAGTACAGCTAG (JPS-G3_FW with BamHI cleavage site)

Protocol:

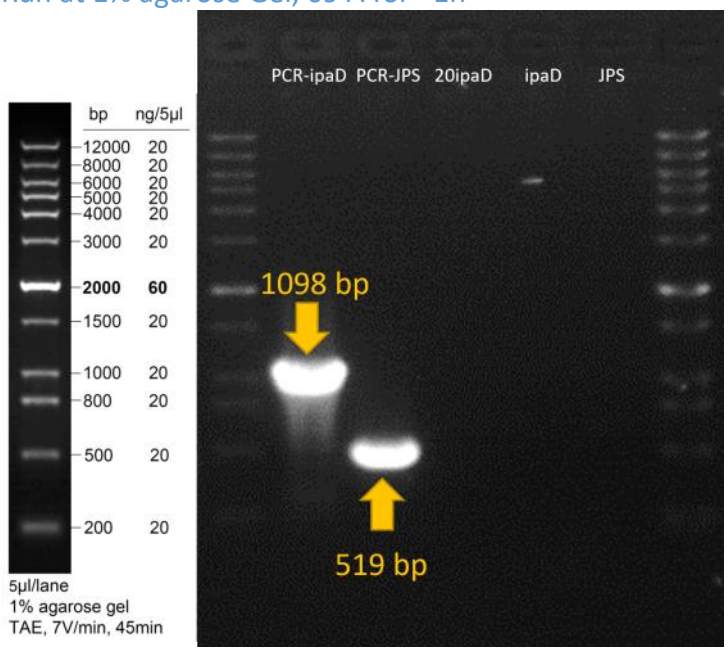
Reaction system:

- 0.2uL DNA template
- 1uL FP
- 1uL RP
- 12.5uL TIANGEN Taq 2xPCR Mix
- Add ddH2O to a reaction system of 25uL in total

Run at Bio-Rad Thermo Cycler

- 95°C for 3 min
- 98°C for 20 sec -
- 60°C for 15 sec | *35
- 72°C for 15 sec -
- 72°C for 15 sec
- 4°C hold

Run at 1% agarose Gel, 69 A for ~1h



9.1 PCR product Purification + Double Digestion

2022年9月1日 18:15

Operator: RYY

PCR Purification

Beyotime PCR clean-up Kit
After purification, dsDNA concentration: ipaD: 117.3ng/uL, JPS-G3: 57.7 ng/uL

Double Digestion

#	Name	Concentration (μg/μl)	Applied amount in the reaction system (μl)	Enzyme-1 (1μl)	Enzyme-2 (1μl)	Buffer (5μl)	MilliQ (μl)
1	IpaD-HA ppcr	0.117	10	BamHI	XhoI	rcutsmart	33
2	JPS-G3 ppcr	0.057	20	BamHI	XhoI	rcutsmart	23

incubate on 37°C dry bath overnight (9.1 20:00-9.2 12:00, 16 hrs in total) + 65°C for 20 min

9.2 Transformation + Plate Preparation

2022年9月2日 17:18

Operator: RYY

Transformation

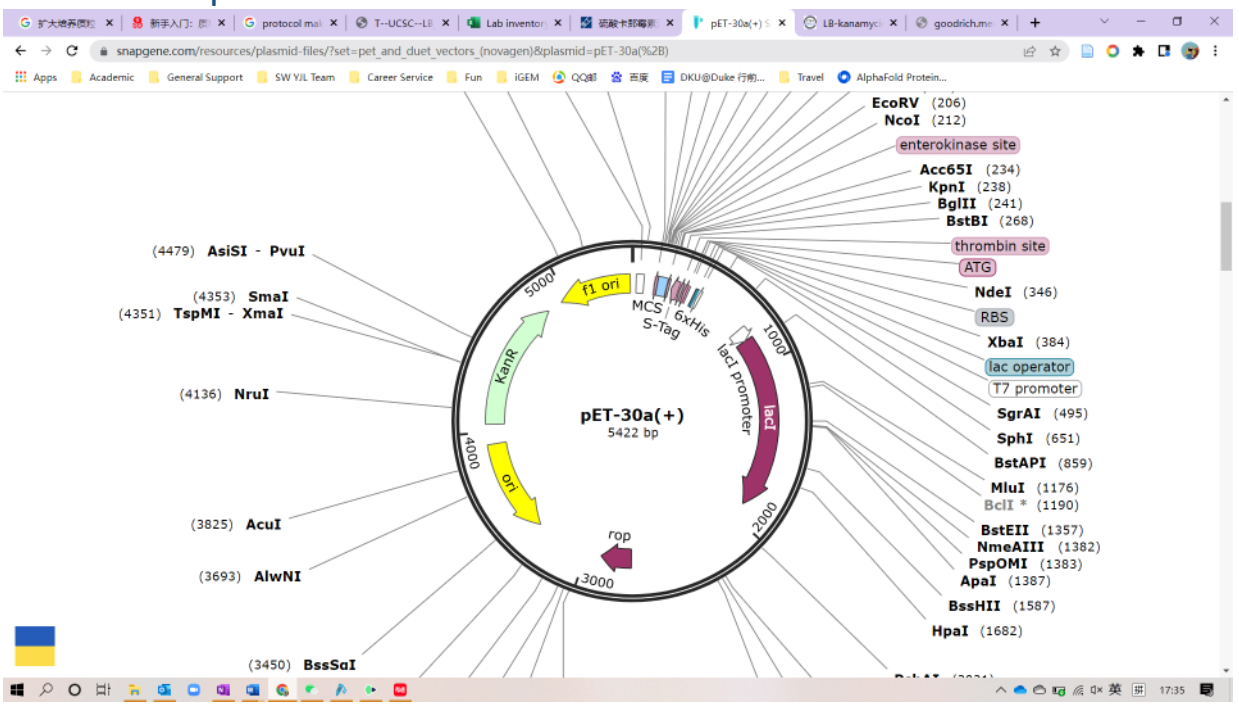
pET30a from Huang Lab (KanR)

BeyoTime DH5a competent cell (stored at -80°C)

Protocol

To step 4, overnight culture (IB 2052)

LB Plate Preparation



Bottle 1: 500mL MilliQ, 12.5g LB broth, 7.5 g agar
Bottle 2: 500mL MilliQ, 12.5g LB broth

Shake well

Sterilize at 120°C for 30 min

For bottle 1, add 0.4mL Sangon Kanamycin Sulfate Solution, mix well, make agar plates

Stored at -4°C fridge

9.3 Strains fetched from XJTLU/Coat Plate & Overnight Culture

Saturday, September 3, 2022 19:54

Operator: RYY/JJY

Coat Plate & Overnight Culture

Use overnight cultured pET30a to coat plates
Protocol: Transformation

1 liquid culture (5mL LB + 4uL Kan)
7 plates (1 streak plate, 1 resuspension plate, 5 serial dilution from 10X to 10^5X)

Remaining DH5a stored in -80°C in IB 2052 (labelled 'E')

Operator: SC

Strains fetched from XJTLU

pGEX-4T-1-Csga-MBP3 E. coli LB liquid culture, stored in 15ml centrifuge tube at 4 ˚C

pGEX-4T-1-Csga-shMT E. coli Glycerol stock, stored at -20˚C at 3080

The CsgA-shMT strain was inoculated in LB medium for recovery at 8pm.

pGEX4T-1-csgA-shMT

pGEX-4T-1-Csga-MBP3

About pGEX-4T-csgA-SmtA strain which was fialed to culture:

The first LB+amp liquid medium inoculated with the csgA-smtA strain (for recovery) was considered to be problematic by yy as the medium didn't show that the strain was growing and the result of the plate was not normal. The medium was then stored at 4˚C before it was taken out again and cultured on I placed the medium in the incubator again on August 30.

On August 31 I checked it and found the liquid to be opacite, and placed it in 4˚C.

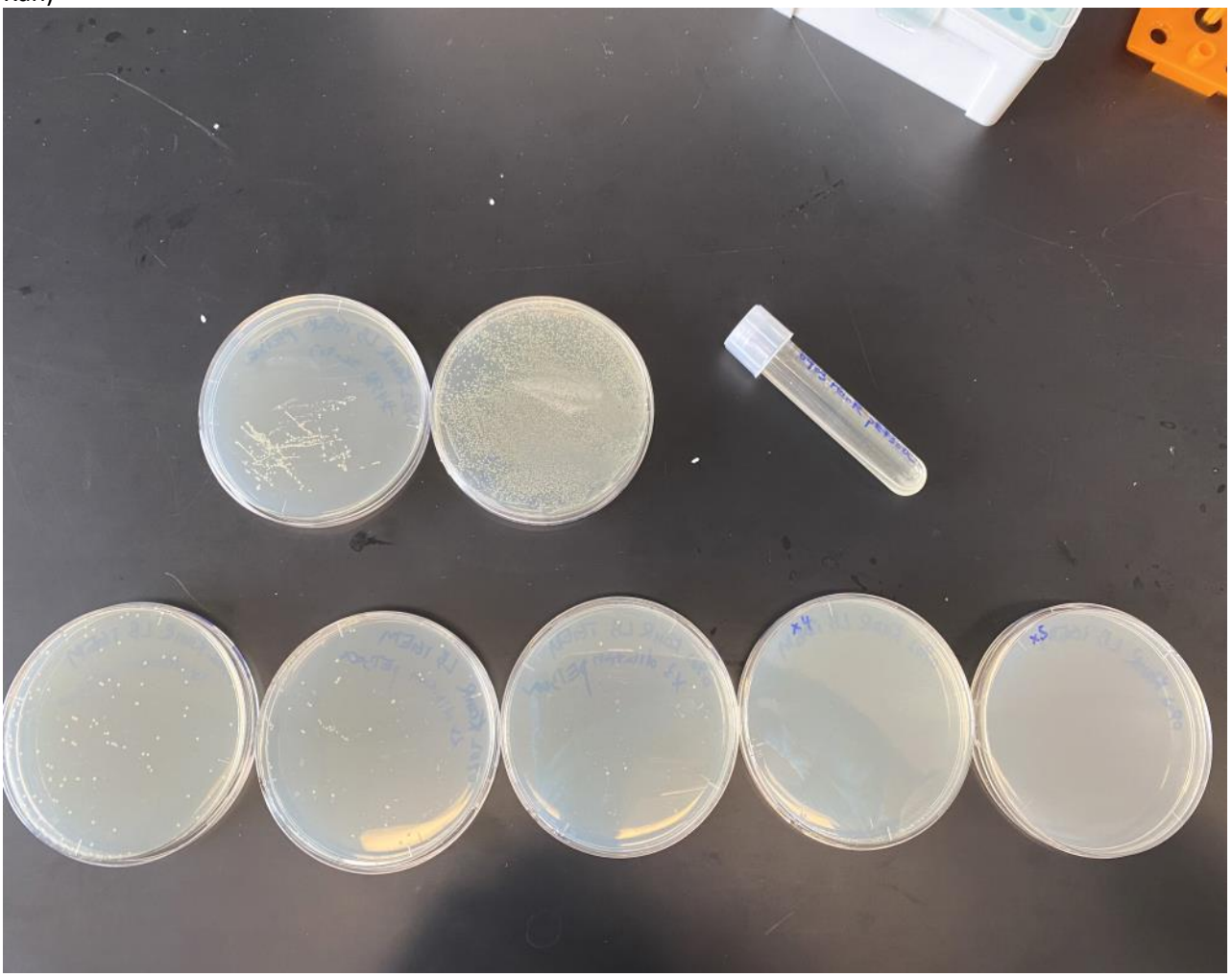
9.4 Monoclonal Culture + Plasmid Extraction

2022年9月4日 22:10

Operator: RYY

Monoclonal Culture

9:59AM single colony from overnight culture plates found, transplanted to 2* 5mL liquid culture (LB + Kan)



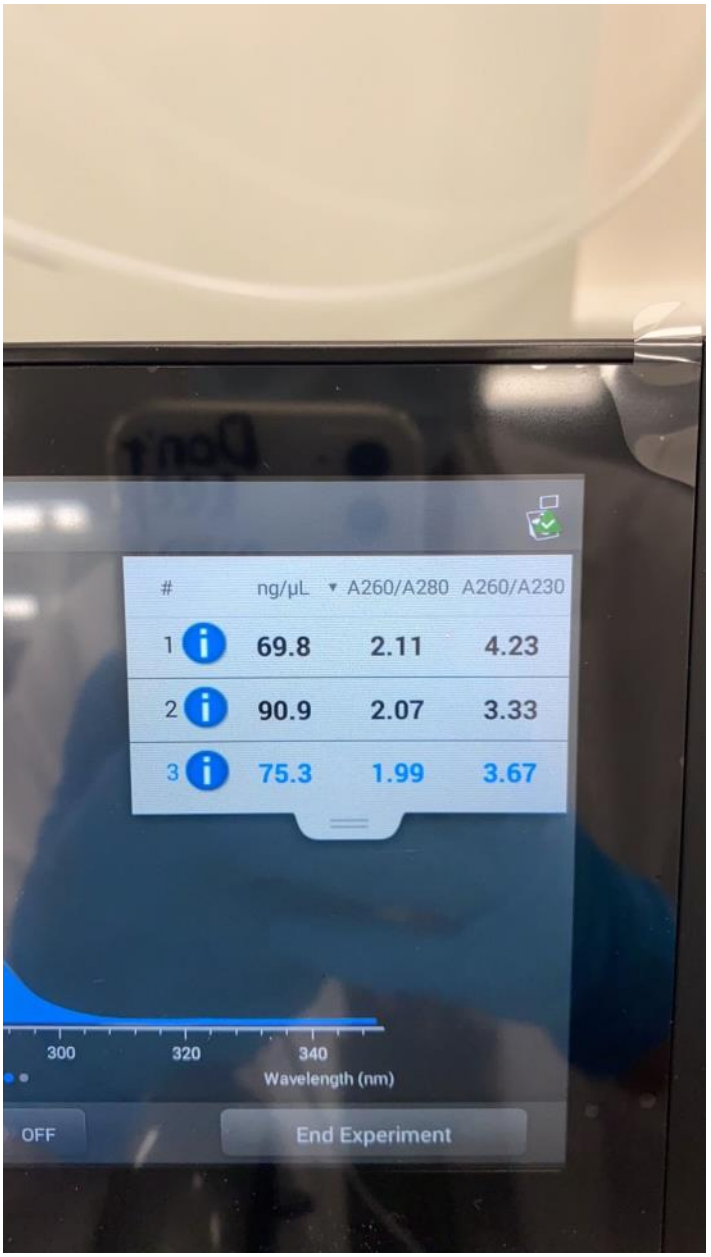
Plasmid Extraction

9:30PM start (culture time: ~12 hrs)

Protocol: TIANGEN Plasmid MiniPrep Kit

Bacterial Solution used:

- 2* 5mL Liquid culture made today (labelled **pET30a 1 & 2**),
- polyclonal culture made yesterday (labelled **pET30a - non-MC**)



75uL/tube, stored at -20

9.5 Streak Plate

2022年9月5日 10:29

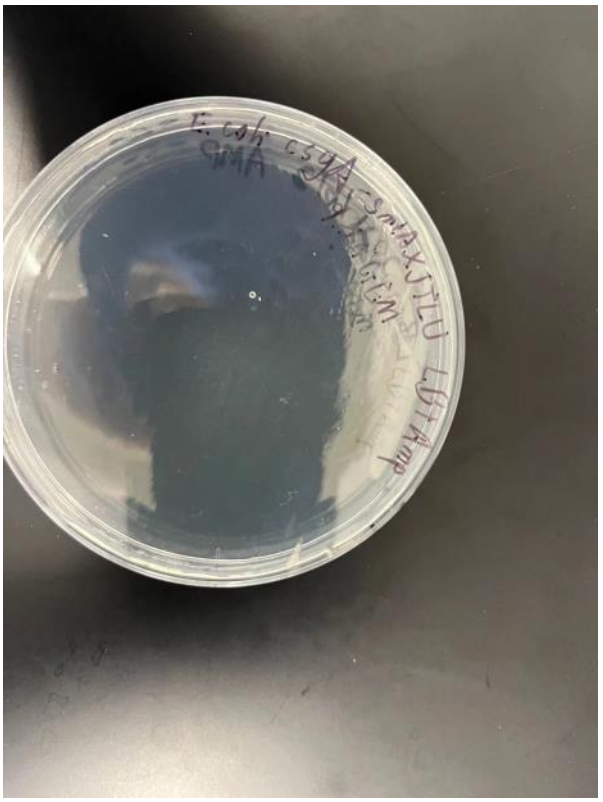
Operator: SC

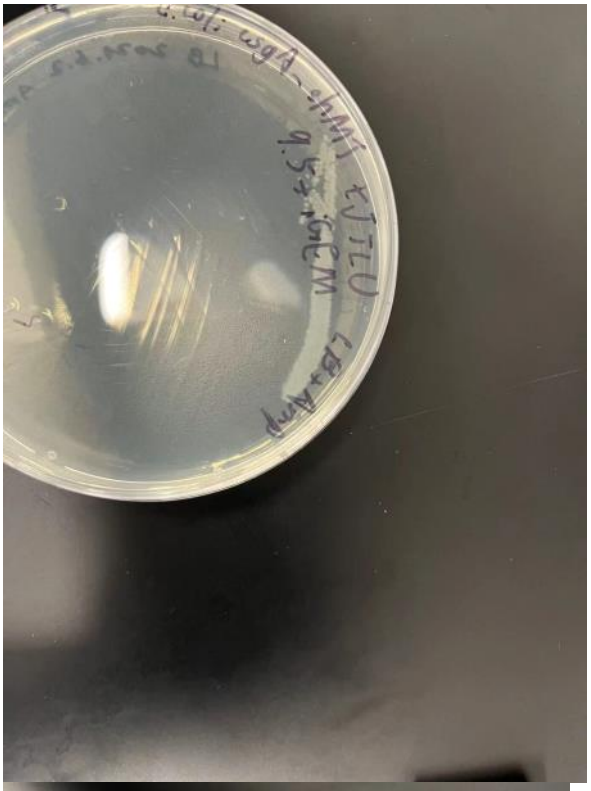
Plate streaking

Plate: LB+amp (made on June 2)

Strains	Source
pGEX-4T-1-Csga-MBP3	LB liquid culture from XJTLU
pGEX-4T-1-Csga-shMT	LB liquid, cultured before for 36 h
pGEX-4T-1-Csga-smtA	LB+amp liquid, from the tube considered to be problematic

Picking colony (at 12pm)





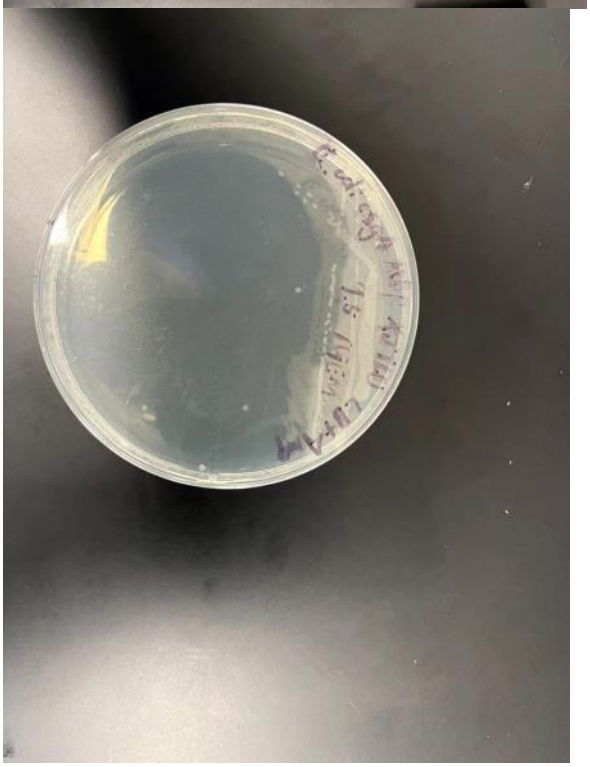


Plate streaking

Plate: LB+amp (made by Qizhou on August 26)(Time: September 6, 1am)

Strains	Source
pGEX-4T-1-Csga-MBP3	LB liquid culture from XJTLU
pGEX-4T-1-Csga-shMT	LB liquid, cultured before for 36 h
pGEX-4T-1-Csga-smtA	LB+amp liquid, from the tube considered to be problematic

*As suggested by yy, I used more reliable plates and streaked them again

9.6 Double Digestion, Gel Extraction (Failed)

2022年9月6日 22:09

Operator: RYY

Double Digestion

#	Name	Concentration (μg/μl)	Applied amount in the reaction system (μl)	Enzyme-1 (2μl)	Enzyme-2 (2μl)	Buffer (10μl)	MilliQ (μl)
1	pET30a-1	0.07	30	BamHI	XhoI	rcutsmart	56

protocol

Gel Extraction

Failed again……. I won't use Beyotime Kit anymore

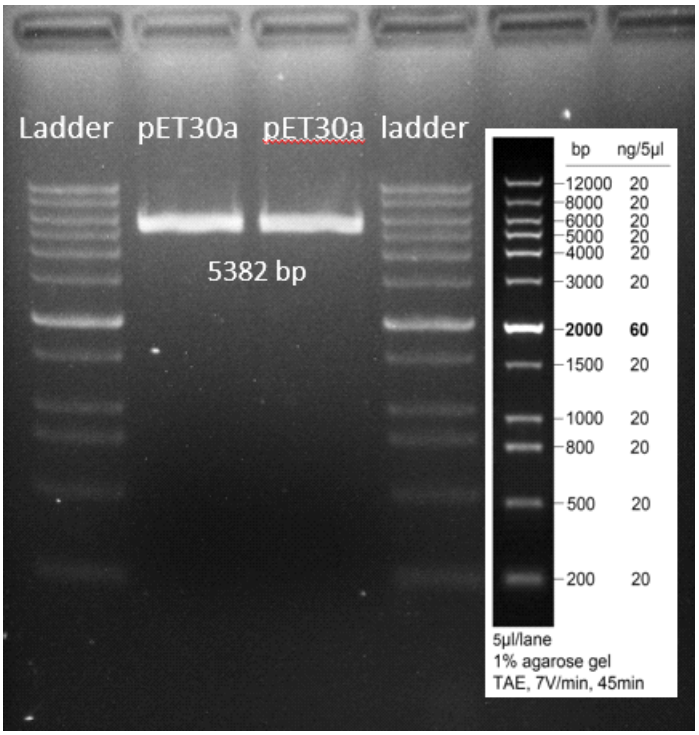
Double Digestion

Same as above, I will try ligation tmr

9.7 Gel Extraction

2022年9月7日 16:40

Operator: RYY



Gel Extraction

pET30a: 10.8ng/uL

9.8 Transformation

2022年9月9日 9:01

Operator: RYY

pUC19 + JPS-G3
pUC19 + ipaD
Transformed into E. coli DH5a, overnight culture (8:40p.m.)

Experimental Design

2022年9月9日 11:50

What we have:

1. **Target Sequences**
   a. ipaD_HA sequence, purified PCR product (F: BamHI/R: XhoI)
   b. JPS-G3_FLAG sequence, purified PCR product (F: BamHI/R: XhoI)
2. **Empty Vectors**
   a. pET30a empty vector (KanR, LacI/Lac Operon)
   b. pET30a empty vector digested, purified (F: BamHI/R: XhoI) (KanR, LacI/Lac Operon)
3. **Sequences in plasmids**
   a. ipaD_HA + pUC19 (insertion site unknown, synthesized by Sangon)
   b. JPS-G3 + pUC19 (insertion site unknown, synthesized by Sangon)
   c. pGEX-4T-1-CsgA-MBP3 (from XJTLU)
   d. pGEX-4T-1-CsgA-shMT (from XJTLU)
4. **E.coli strains**
   a. DH5a competent cell (T7 polymerase-)
   b. BL21(DE3) competent cell (T7 polymerase+)
5. **Primers**

Goal 1: Express & Purify proteins ipaD_HA & JPS-G3_FLAG, Test Affinity

- [x] ***Ligate*** *(1)* (1.a + 2.b) (1.b + 2.b) to make pET30a_ipaD_HA and pET30a_JPS_G3
- [x] Use ***colony PCR*** *(2)* (two plasmids transformed into DH5a competent cells) to test whether the plasmid is intact, then
- [ ] ***Transform*** *(3)* useful plasmids to BL21(DE3) competent cells, and
- [ ] ***Induce*** *(4)* the expression of target proteins via IPTG, then
- [ ] ***Purify*** *(5)* proteins, and
- [ ] protein ***affinity analysis via ELISA(6)***

Goal 2: Bind CsgA to ipaD_HA & JPS-G3 (i.e., Surface Display)

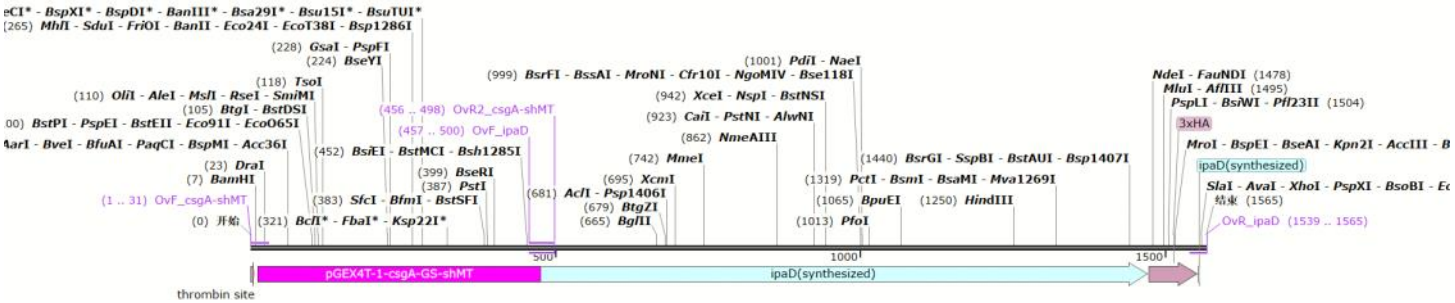
- [ ] Overlap extension PCR (or called gene SOE):

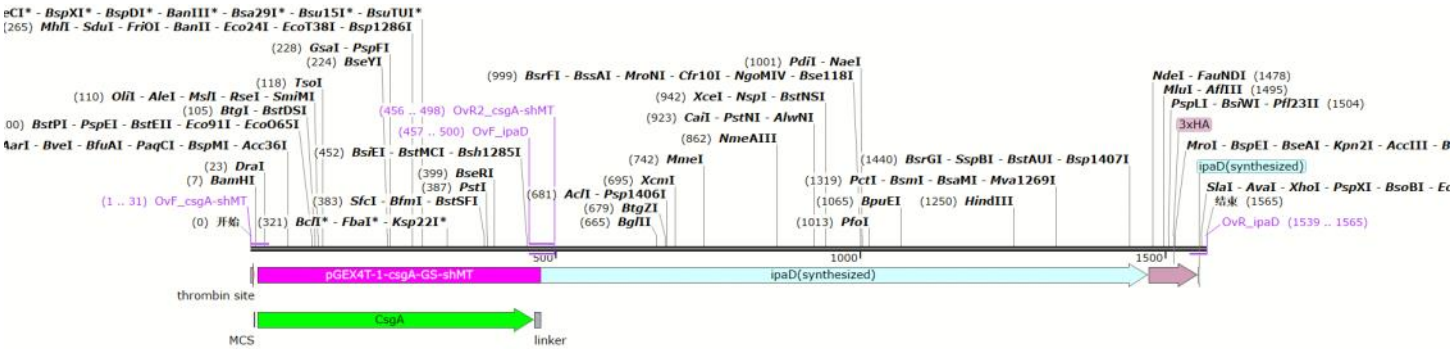
CsgA+Linker with ipaD_HA = *BamHI*_CsgA+Linker+ipaD_HA_*XhoI*

- 1st round PCR:
  - Tube 1: pGEX-4T-1-CsgA-shMT + (OvF_csgA-shMT + OvR1_csgA-shMT), Tm = 63
  - Tube 2: pET30a_ipaD_HA + (OvF_ipaD + 一开始订的Primer11), Tm = 51
- 2nd round PCR:
  - Tube 1 product + Tube 2 product + (OvF_csgA-shMT + OvR_ipaD), Tm = 61

Expected Final Product:

csgA-linker-ipaD-3xHA





- [ ] Next step: Ligate into pET30a, Transform into BL21(DE3), induce via IPTG, then test surface display

Same for JPS-G3

9.9 Ligation + Transformation

2022年9月9日 12:51

Operator: RYY

Ligation

pET30a	6uL (~50ng, 0.014pmol)
JPS-G3	0.3uL (~14ng, 0.042pmol)
10X Ligation Buffer	2µl
双蒸水或Milli-Q水	11.2uL
T4 DNA ligase	0.5uL
总体积	20µl

pET30a	6uL (~50ng, 0.014pmol)
ipaD	0.3uL (~30ng, 0.042pmol)
10X Ligation Buffer	2µl
双蒸水或Milli-Q水	11.2uL
T4 DNA ligase	0.5uL
总体积	20µl

JPS-G3: 57ng/uL, ipaD: 117ng/uL
Incubate at 25 from 15:30 to 17:00 (1.5 hr), then stored at -20
Biomass calculator: https://www.promega.com.cn/resources/tools/biomath/

Transformation

Transformed into DH5a competent cells, overnight culture

No results

9.12 Purification of Plasmids and Ligation

Monday, September 12, 2022 21:56

Operator: RYY

Purification of Plasmids and Insertion Sequences

Double digestion of plasmid again, purified by both GeneJET gel extraction kit (pET30a-2) and Beyotime DNA purification kit (pET30a-1; proved to be better);
used Beyotime DNA purification kit to purify insertion sequences again

dsDNA Concentration

Conc.	**ng/uL**
ipaD (~1000bp)	41.5
JPS-G3 (~600bp)	35.4
pET30a-1	7.9
pET30a-2	16.9

Overnight Ligation

	Empty vector	**Insert sequence**	**T4**	**Ligation Buffer**	**DNAse-free water**	**Result**
i-1	1: 6.5uL	ipaD: 1uL	0.5uL	2uL	10uL	++
J-1	1: 6.5uL	JPS-G3: 1uL	0.5uL	2uL	10uL	++
i-2	2: 3uL	ipaD: 1uL	0.5uL	2uL	13.5uL	-
J-2	2: 3uL	JPS-G3: 1uL	0.5uL	2uL	13.5uL	+

Plasmid: 0.015 pmol
Insert sequence: 0.05 pmol

Overnight culture at 16°C

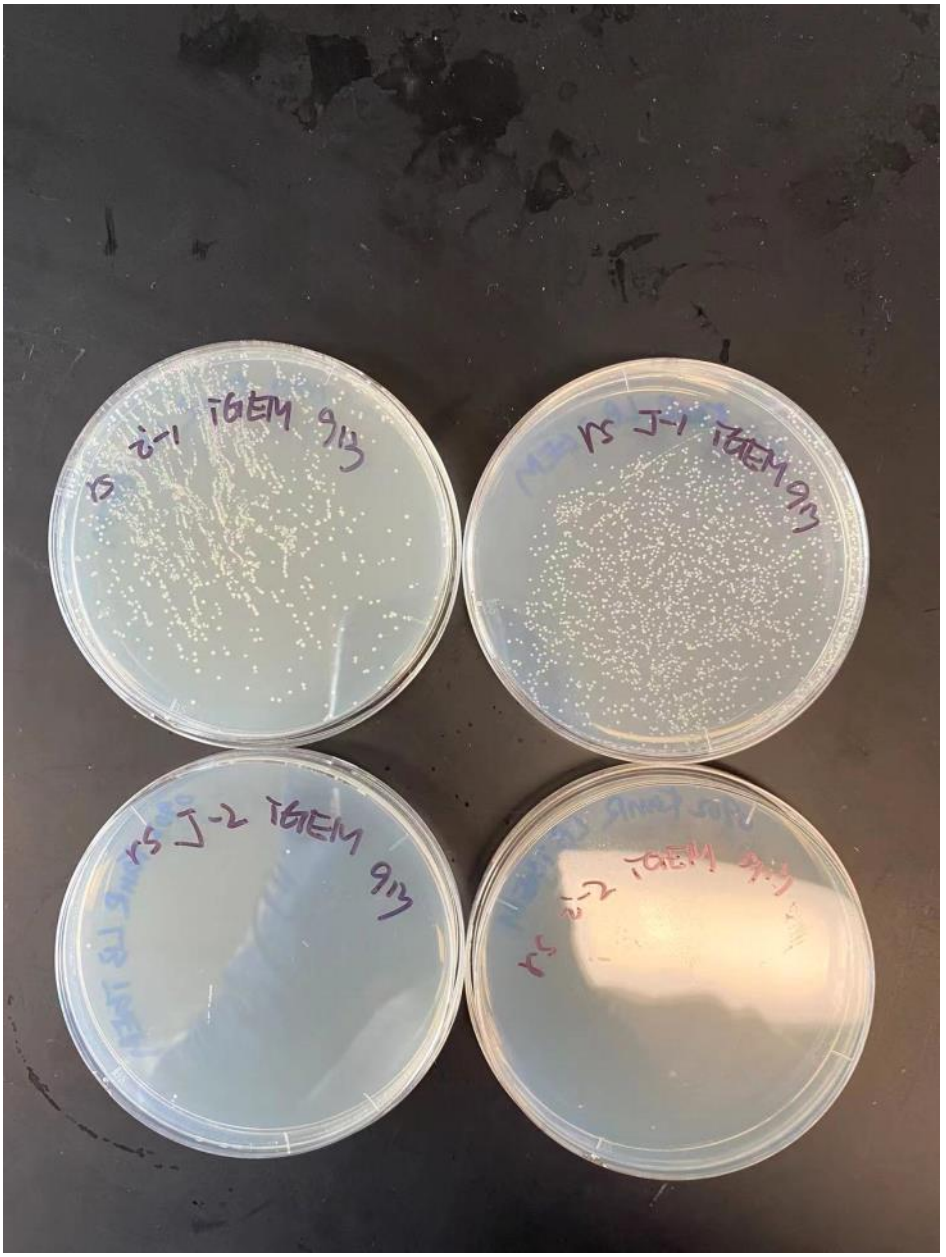
9.13 Transformation

Tuesday, September 13, 2022 20:42

Operator: RYY

J-1, J-2, I-1, I-2 transformed into DH5a, overnight culture

Result:


J-1 iGEM 913
J-1 iGEM 913
J-2 iGEM 913

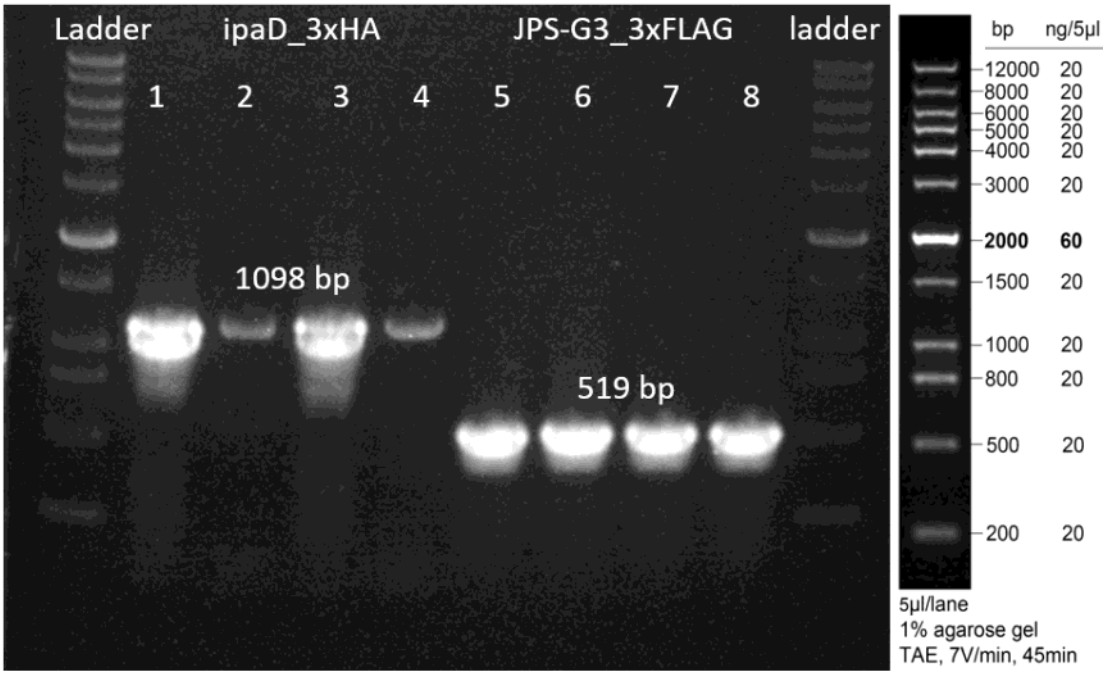
9.14 Colony PCR + Overnight Culture; Picking colonies + overnight culture for strains from XJTLU

2022年9月14日 16:22

Operator: RYY

Colony PCR

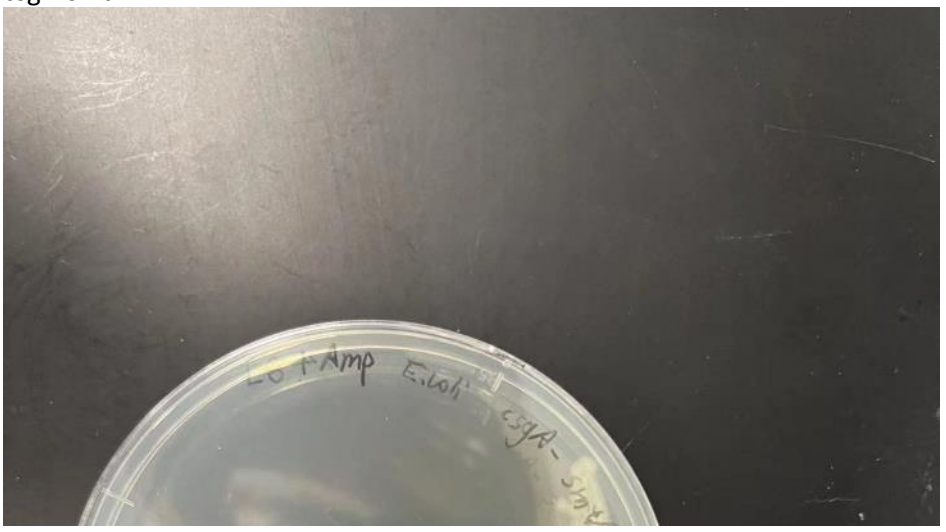
4 colonies were picked for each gene, run PCR + Gel electrophoresis
All positive

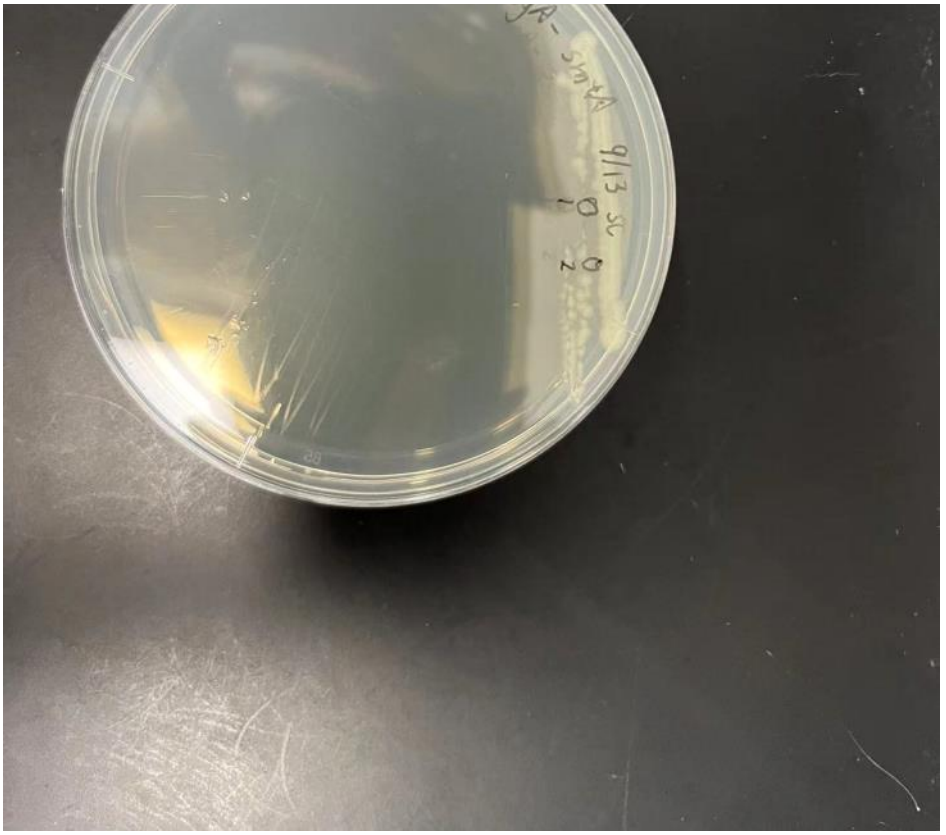


Operator: Chang

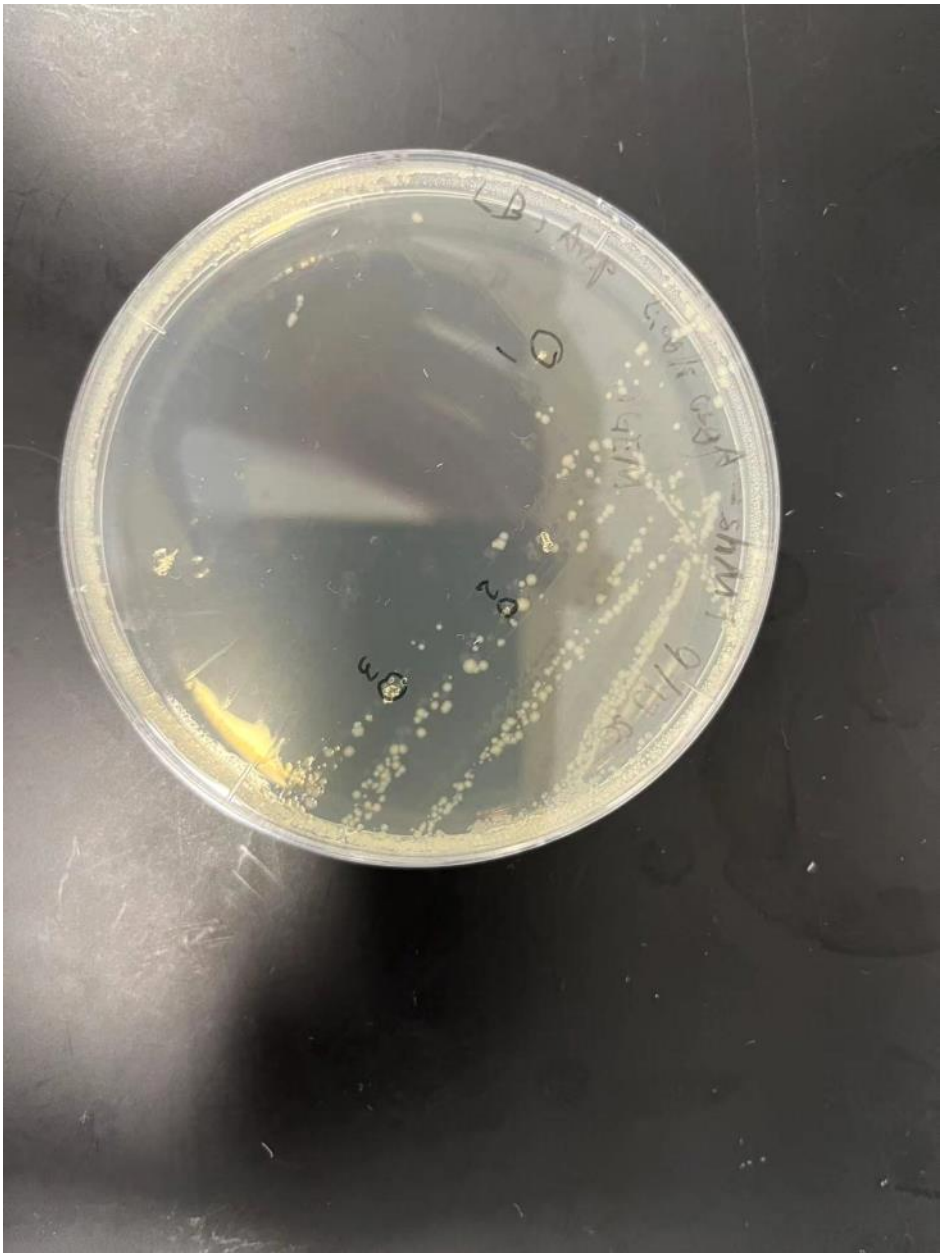
Two colonies were picked for strain csgA-smtA
Four colonies were picked for strain csgA-smht (one by RYY)
Plate:

csgA-smtA





csgA-smht


LB AMP
1
2
3

9.15 Plasmid extraction, enzyme digestion and Gel electrophoresis for csgA; ipaD & JPS-G3 recombinant plasmids sent for sequencing

Thursday, September 15, 2022 13:51

Operator: Chang Shen

Plasmid Extraction

Source	Plasmid concentration (ng/µl)
smht-1	66.7
smht-2	95.4
smht-3	98.2
smht-4(RYY)	69.1
smtA-1	99.0
smtA-2	41.8

Enzyme digestion

Applied Enzyme: BamHI & XhoI

Predicted length:

csgA-smtA	656bp
csgA-smht	656bp

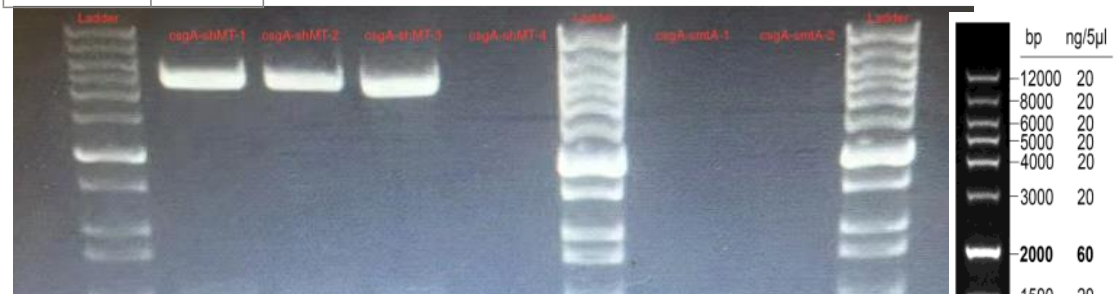
Reaction system:

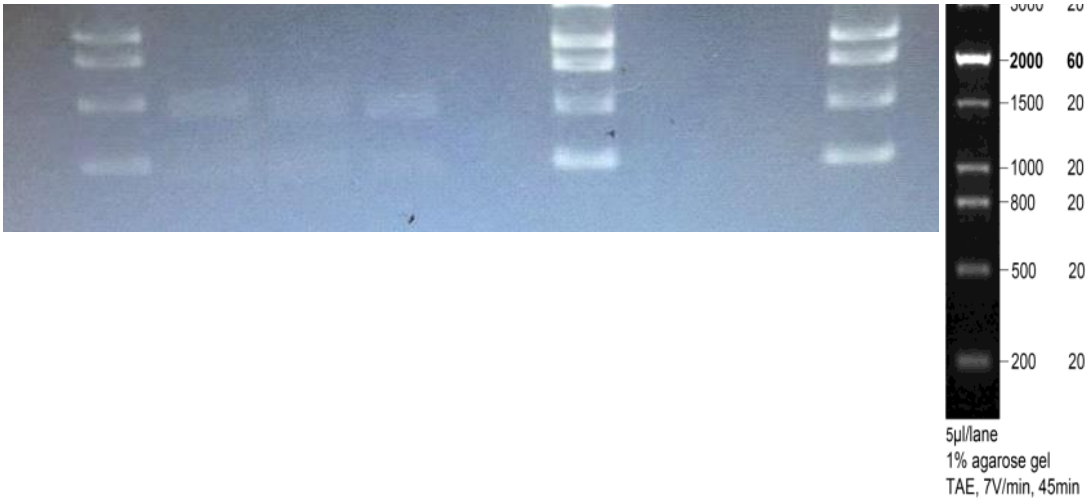
Plasmid	Plasmid volume (µl)	BamHI (µl)	XhoI(µl)	rCutSmart	MilliQ (µl)	Total (µl)
smht-1	15.00	1	1	5	8	30
Smht-2	10.48	1	1	5	12.52	30
Smht-3	10.18	1	1	5	12.82	30
Smht-4(RYY)	14.47	1	1	5	8.53	30
smtA-1	10.10	1	1	5	12.9	30
smtA-2	23.93	1	1	5	4.07	30

Gel electrophoresis result

Supposed length

csgA-shMT	656bp
csgA-smtA	656bp





Operator: RYY

Plasmid Extraction & Sequencing

ipaD-1 (D1) & ipaD-3 (D2) & JPS-G3-5 (J1) & JPS-G3-6 (J2) Plasmids extracted, sent for sequencing

Name	Conc (ng/uL)	Sequencing result	.ab file (T7)	.ab file (T7t)
D1	60.2	Point Mutation A>G	G220901353_D1_T7_E03	D1
D2	60.4	No problem	G220901354_D2_T7_F03	D2
J1	58.3	No problem	G220901355_J1_T7_A07	G220901355_J1_T7t_B07
J2	57.7	Point mutation A>G	G220901356_J2_T7_C07	G220901356_J2_T7t_D07

9.19 Overlap Extension PCR

2022年9月19日 15:55

Operator: RYY

PCR 1

Goal: Amplify csgA for

1. F/R1: ipaD (expected length: 497 bp)
2. F/R2: JPS-G3 (expected length: 498 bp)

Templates used: shmt-2/shmt-3 plasmids

Reaction system:

- 0.5uL DNA template
- 1uL FP
- 1uL RP
- 12.5uL TIANGEN Taq 2xPCR Mix
- Add ddH2O (10uL) to a reaction system of 25uL in total

Protocol:

- 95°C for 3 min
- 98°C for 20 sec -
- 58°C for 15 sec | *35 <- 58 = 63-5
- 72°C for 15 sec -
- 72°C for 15 sec
- 4°C hold

Purified by Beyotime PCR product purification kit

PCR 2

Goal: Amplify JPS-G3 (expected length: 531 bp)

Templates used: pET30a_JPS-G3

Reaction system:

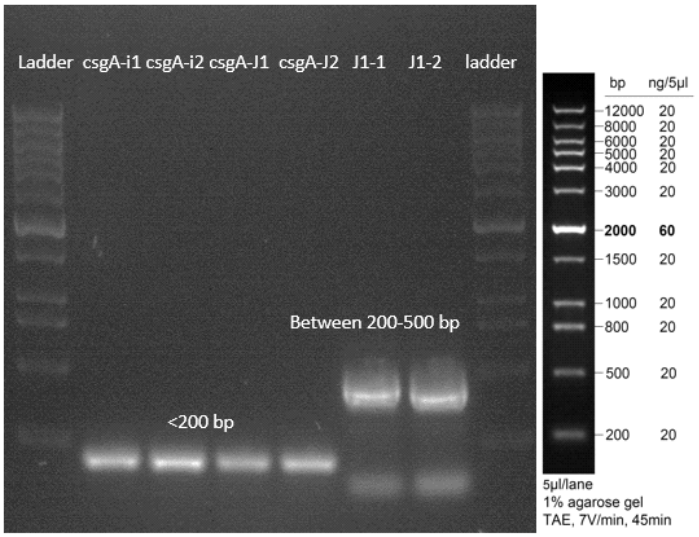
- 0.5uL DNA template
- 1uL FP
- 1uL RP
- 12.5uL TIANGEN Taq 2xPCR Mix
- Add ddH2O (10uL) to a reaction system of 25uL in total

Protocol:

- 95°C for 3 min
- 98°C for 20 sec -
- 54°C for 15 sec | *35 <- 54 = 59-5
- 72°C for 15 sec -
- 72°C for 15 sec
- 4°C hold

Purified by Beyotime PCR product purification kit

csgA (expected: 497bp)/JPS-G3 (expected: 531bp), length error



9.20 Overlap Extension PCR

2022年9月22日 15:23

Operator: RYY

PCR 1

Goal: Amplify ipaD (expected length: 1109bp)
Templates used: pET30a-ipaD

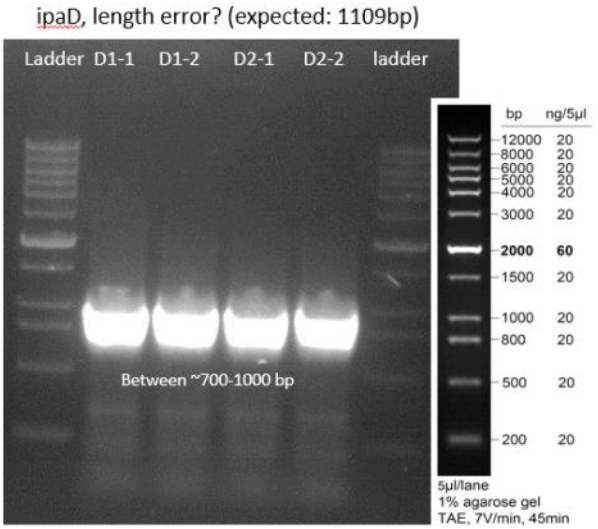
Reaction system:

- 0.4uL DNA template
- 1uL FP
- 1uL RP
- 12.5uL TIANGEN Taq 2xPCR Mix
- Add ddH2O (10uL) to a reaction system of 25uL in total

Protocol:

- 95°C for 3 min
- 98°C for 20 sec -
- 50°C for 15 sec | *35
- 72°C for 15 sec -
- 72°C for 15 sec
- 4°C hold

Purified by Beyotime PCR product purification kit



PCR 2

Goal: Overlap Extension
Templates used: product from 19-PCR1, 19-PCR2, 20-PCR1

Reaction system:

- 0.4uL + 0.4uL DNA templates
- 1uL FP
- 1uL RP
- 12.5uL TIANGEN Taq 2xPCR Mix
- Add ddH2O (10uL) to a reaction system of 25uL in total

Protocol:

- 95°C for 3 min
- 98°C for 20 sec -
- 55°C for 15 sec | *35
- 72°C for 15 sec -
- 72°C for 15 sec
- 4°C hold