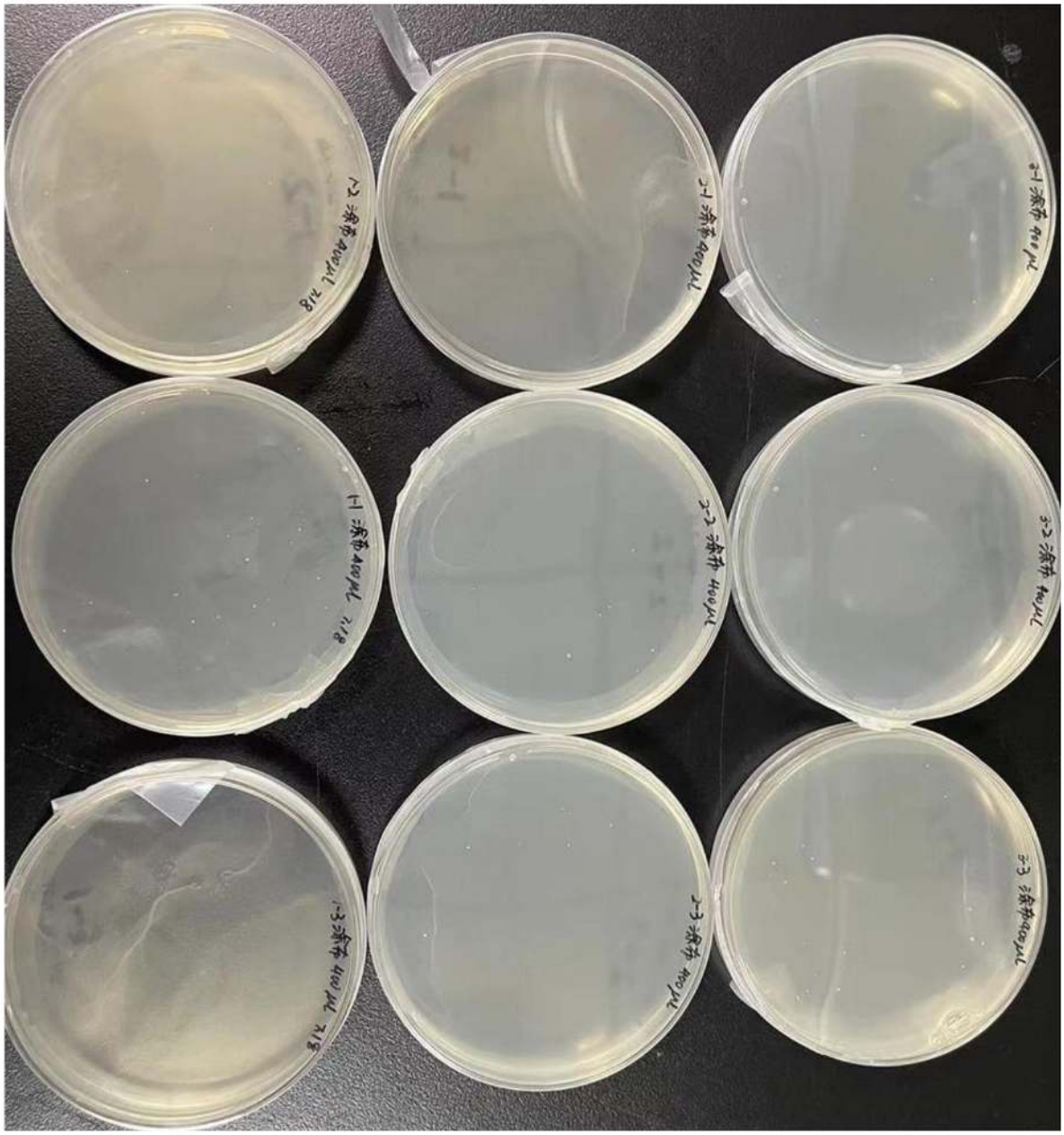
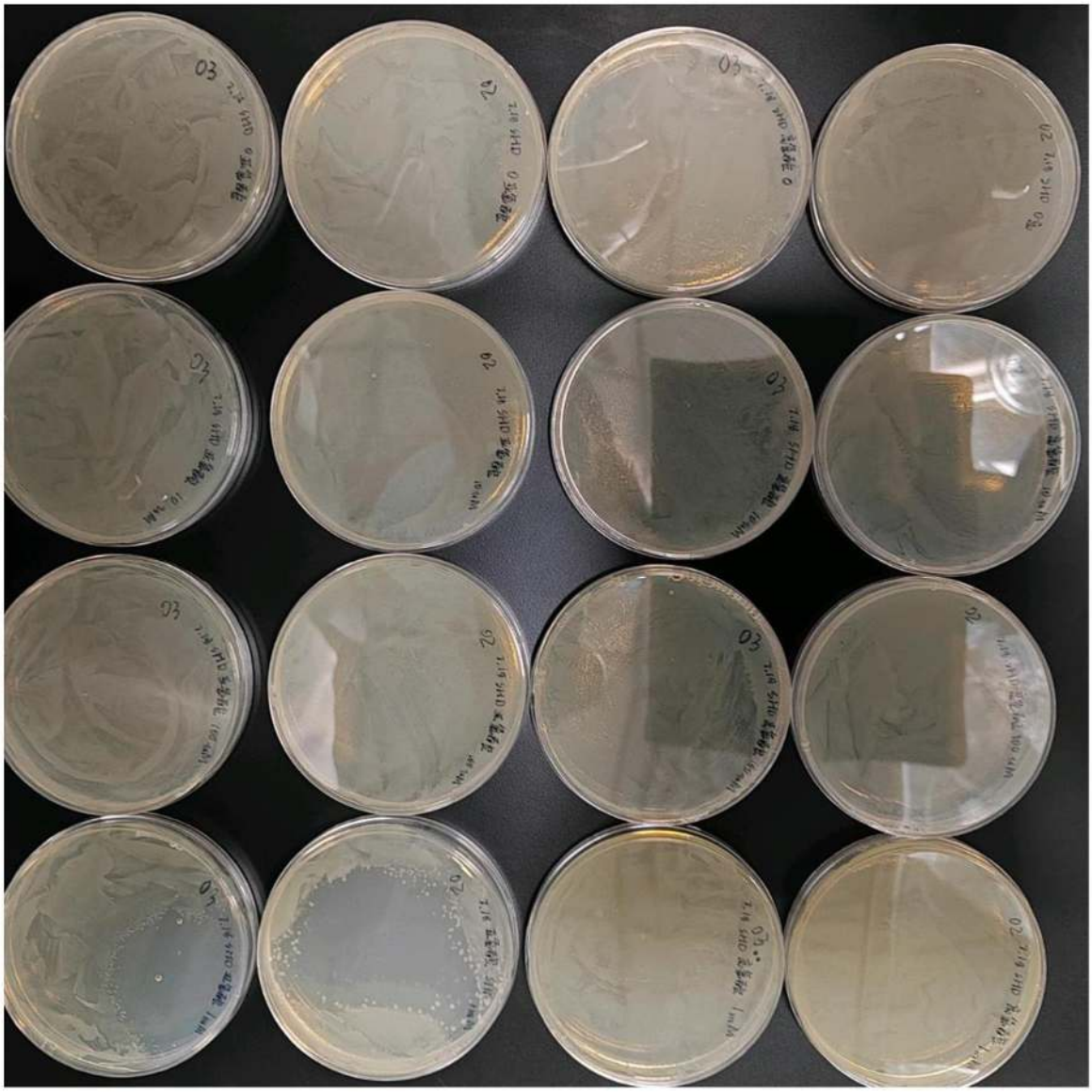


There is no sign of growth after we place these dishes for one night in 30 celcius. Some dots of white matter should not be the yeast since they are irregular and it seems that they cling on the bottem of the dish. There should have some contamination.

We will place them in incubator for another day.

Growth of SHD02 and SHD03



2024/07/19 DAY 21

Experiment content: Charlotte Gou, Jerry Zhu, Frank Li, Jack Bian

1. Do the preliminary experiment for the yeast(CEN-PK2) PCR.
2. PCR for yeast that were received plasmids yesterday.

Experiment processes:

1. Preliminary experiment of yeast PCR:

   1. Pick 8 colonies of yeast from the dish CEN-PK2.

   2. Add them into 10ul of lysate, M5 Hiper 超光速 mix 直接扩增最佳伴侣(from Mei5 biotechnology, MF859-10). Blow and mix them well.

   3. Put them in hot water bath in 95 celcius for about 3-5 mins.

   4. Extract 1-2ul of each sample as the PCR template after centrifuge.

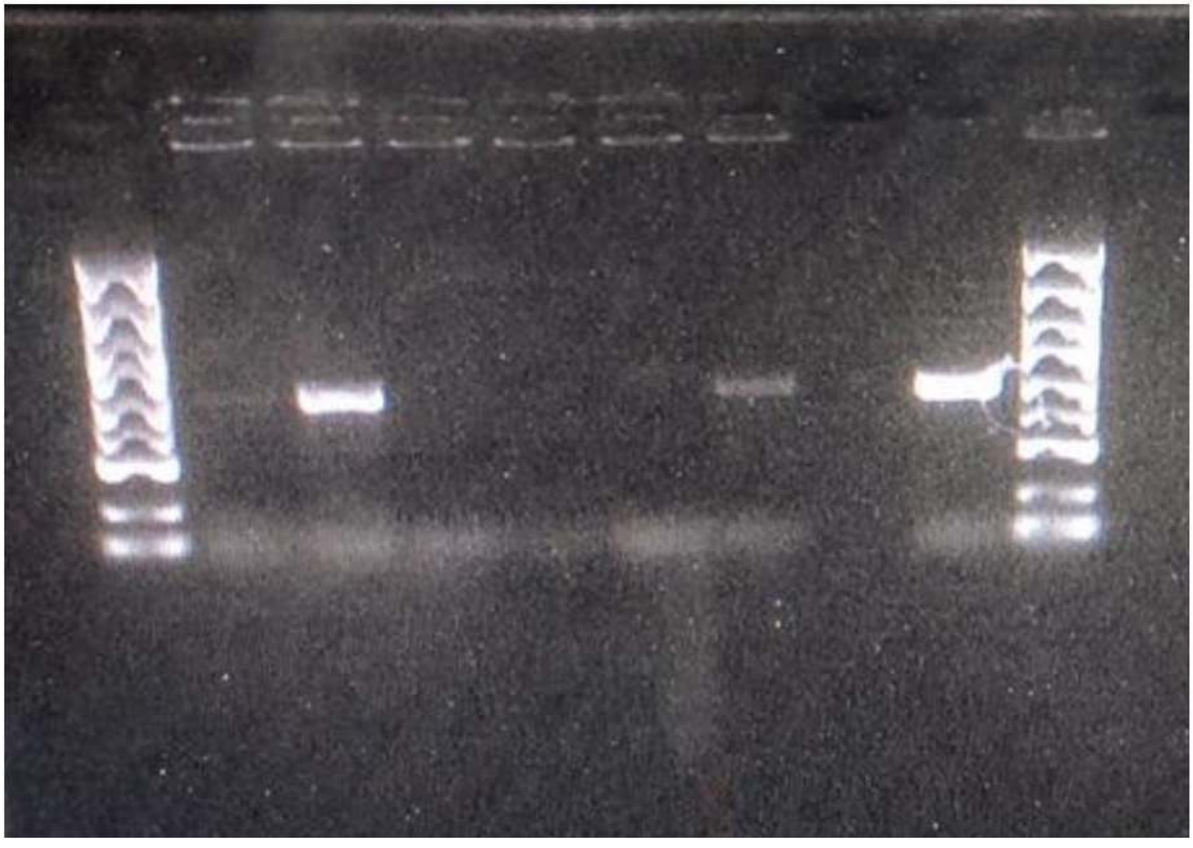
   5. Add primer UP1622-R + UP1622F into the template and do the PCR.

6. Run the gel and see which sample have the greatest result in 1000bp.

2. Verify the result of the plasmid combination of yeast from yesterday. The PCR process repeated the same processes as the preliminary experiment.

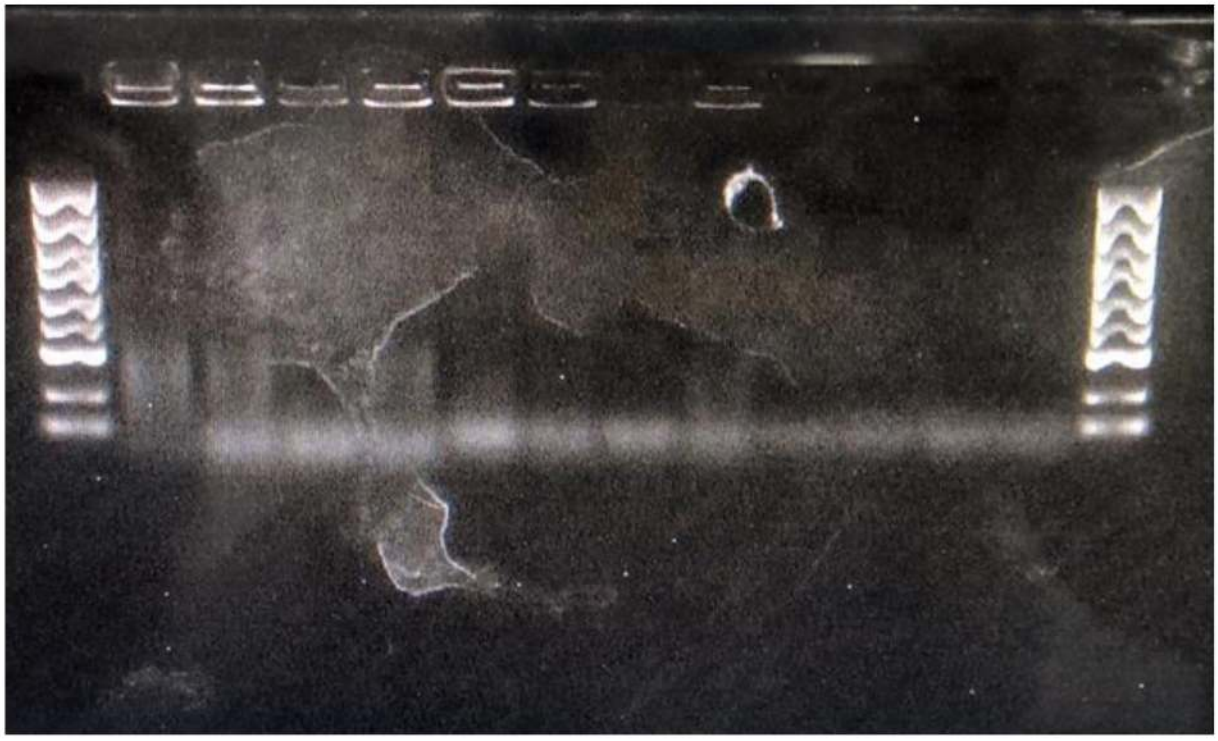
Experiment result:

PCR for CEN-PK2



Sample 2 and 8 are avaliable. They are cutted for the gel recycle in the future.

PCR for plasmids PINEM5, PILAC3 and PIT6EG



None of the samples are useful. It is plausible that it takes more time for the yeast to grow so we still keep them in the incubator. But we still need to repeat the plasmid combination just in case if the plasmid combination really failed.

2024/07/20 DAY 22

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang

Experiment content:

1. Observe yeast dishes which were grown 2 days ago. Y1 should produce substances with red color since it is designed to produce lycopene.
2. Reconstruct 3 plasmids PINEM5, PILAC and PIT6EG. Construct a new one 11-14 KanR, which aim to develop kanamycin and chloramphencol resistance in SHD01.
3. Gel recycle for the sample 2 tracted yesterday. It will be used as the template.
4. Activate the SHD01 bacteria solution since it have been placed in fridge for one month.

Experiment processes:

1. Constructing plasmids

Group	Premier	Template
1(Amp)	KILEU2 7.13 SH05-leu-R/F	one of the addgene plasmid(Dilute 1000 times)
	Skeleton 7.13 SH05-BB-R/F	miaolingbio plasmid pRs3B 100μl water soluble
	TEFI 7.13 SH05-TEF-F/R	miaolingbio plasmid p406TEF 100μl water soluble
2(Amp)	KILEU2 7.13 SH06-Red-F/R	Cloning transformed by killred
	Skeleton SHD03-BB-R/F	SHD03 bacterial fluid
	This plasmid needs to be kept away from light	
3(Amp)	Skeleton 7.15 SHD07-BB-R/F	SHD03 bacterial fluid
	EBI-1 EBI-1-F/R	EBI-1 plasmid 100μl water soluble
	EBI-2 EBI-2-F/R	crtI-1 plasmid 100μl water soluble
	EBI-3 EBI-3-F/R	EBI-3 plasmid 100μl water soluble
	EBI-4 EBI-4-F/R	crtI-2 plasmid 100μl water soluble
4(KanR)	CmR-A 7.18 SHD08-FA-F/R	the chloramphenicol resistant plasmid colony transformed last time
	CmR-B 7.18 SHD08-FB-F/R	the chloramphenicol resistant plasmid colony transformed last time
	PUC19 7.18 SHD08-FC-F/R	miaolingbio plasmid pRs3B
	KanR 7.18 SHD08-FD-F/R	PBb(RSF1010)IK-GFPuv colony transformed from 7.5

System structure:

Substance	Amount/μl
ddH2O	20
Premier-R	2
Premier-F	2
template	1
2xphanta Flash Master Mix	25

PCR instrument:

Temperature	Time
98°C	30s
98°C	10s
57°C	5s
72°C	30s
72°C	1min

Make & Run & Recycling gel

Prepare two bottles of gel. One bottle contains 60ml of TAE and 0.6g of agarose, and 6 μl of dye is added after heating(for the first 10 amp plasmids). The other bottle contains 30ml of TAE and 0.3g of agarose, and 3 μl of dye is added after heating(for the 11-14 KanR).

2. Process of gel recycle:
   Cut the gel and divide it into 14 tubes. Add the sol solution and wait for it to dissolve. Then, add each solution to 14 adsorption columns CA5 and let it stand at room temperature for 2 minutes. Centrifuge at 12000rpm for 1 minute and discard the waste liquid. Add 600 μ l of PW to each of the 14 adsorption columns CA5. Centrifuge at 12000rpm for 1 minute and discard the waste liquid. Add 600 μ l of PW to each of the 14 adsorption columns CA5. Centrifuge at 12000rpm for 1 minute and discard the waste liquid. Centrifuge again at 12000rpm for 2 minutes. Place the adsorption column at room temperature until it dries. Place the adsorption column CA5 in a clean centrifuge tube, suspend an appropriate amount of elution buffer TB in the middle of the adsorption membrane, and let it stand at room temperature for 2 minutes. Centrifuge at 1200rpm for 2 minutes and collect the DNA solution.

3. Implant SHD01 bacterial fluid on petri dishes.

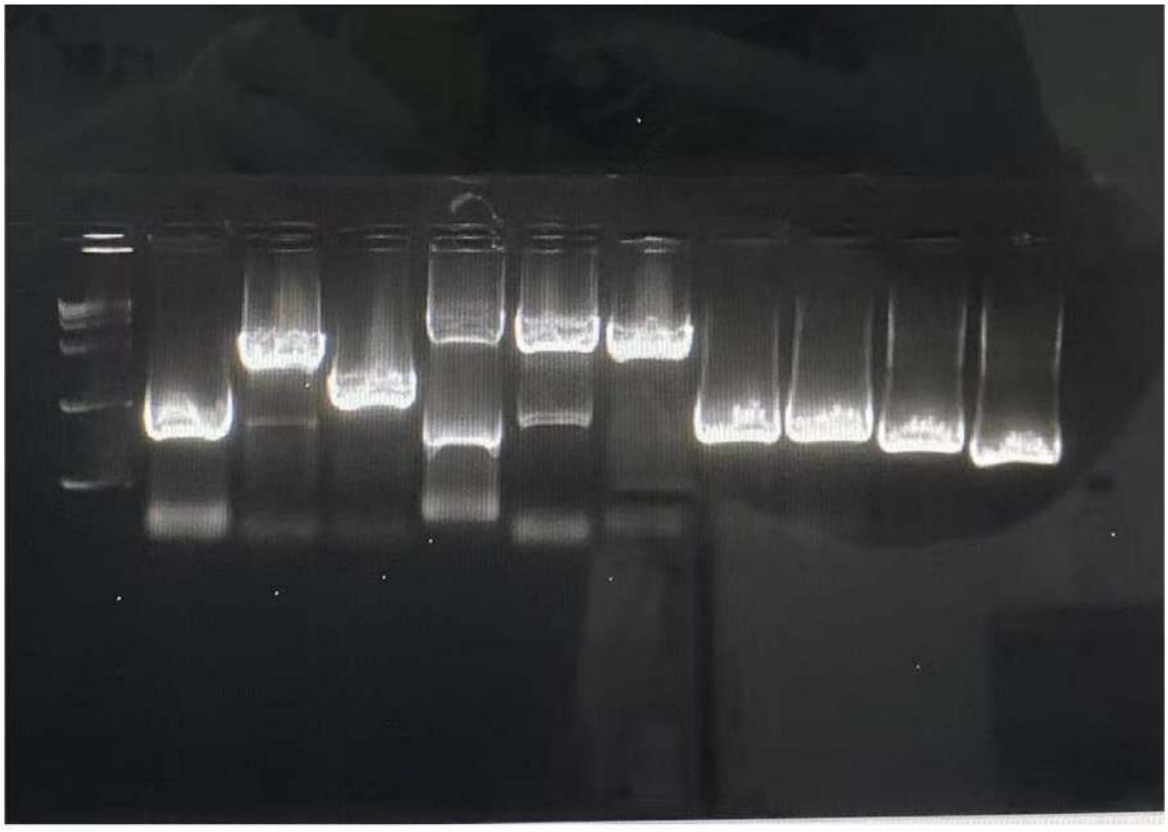
Three tubes, each add 5 μl of antibiotic (Amp) and a little bacterial fluid to 5000 μ l of LB medium.

4. Transformation of 4 connection products into E.coli competent cell

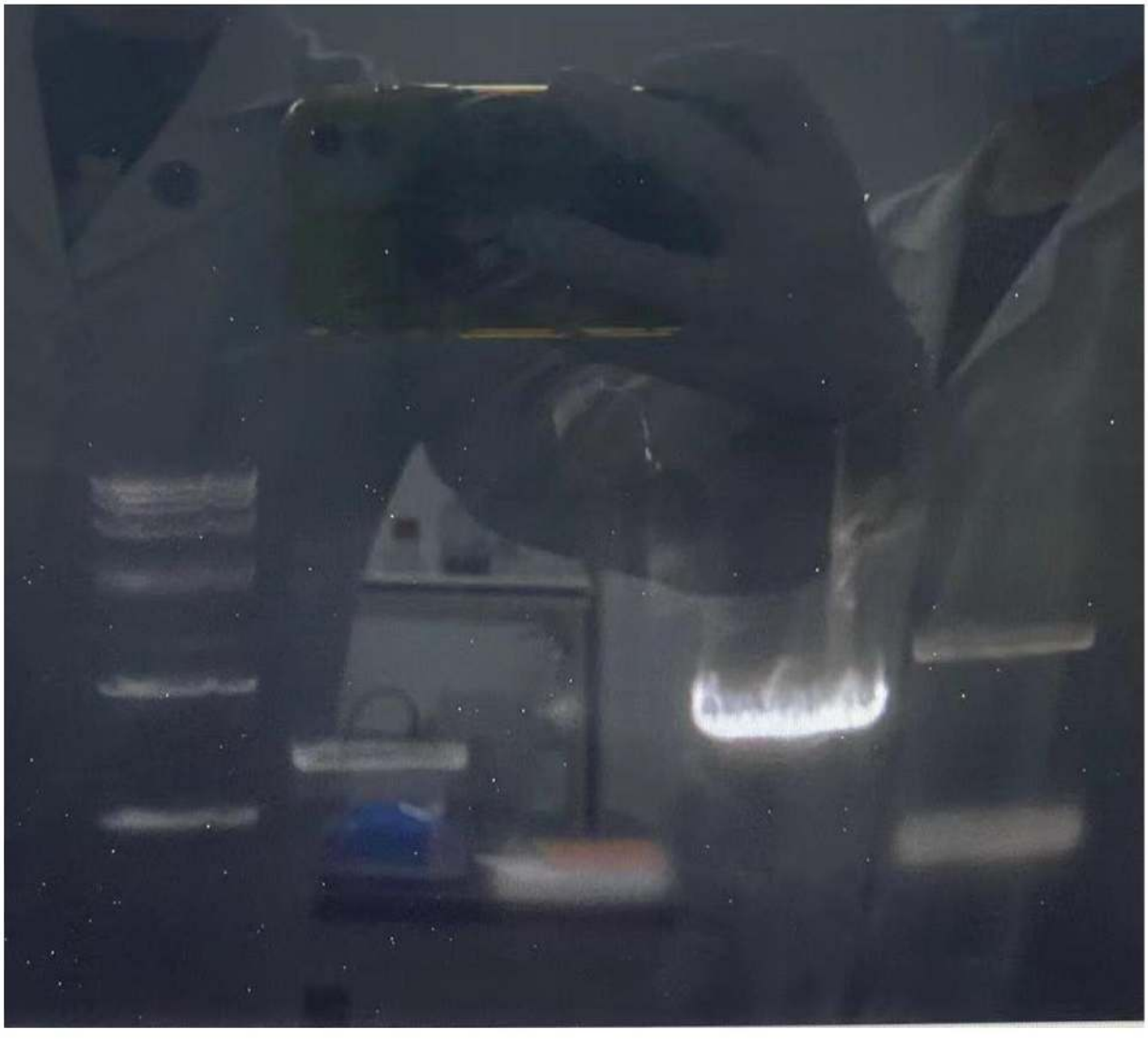
Transfer of 2 μ l of each connecting product into competent cells. Remember that one of them requires avoidance of light. Leave it on ice for 30 minutes, then put it in a water bath at 42 degrees Celsius for 45 seconds. Place on ice for two minutes and add 700 μ l of liquid LB to each tube. Shake the bacteria at 37 degrees Celcius for 45 minutes.

Experiment result:

Gel results for the connection products:



Gel results for the plasmid construction in the competent cell:



Concentration of connection products in the sample:

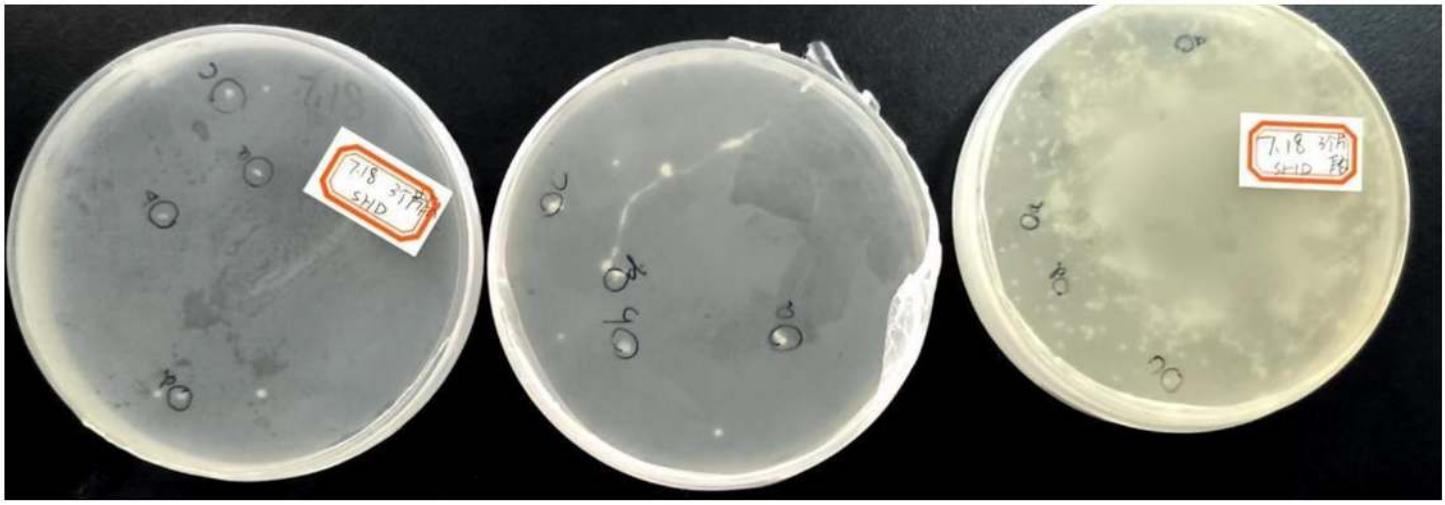
Tube number	Concentration（ng/μl）
1	74.801
2	35.003
3	57.406
4	21.995
5	23.973
6	28.316
7	90.472
8	129.656
9	81.416
10	73.032
11	8.301
12	8.334
13	76.758
14	4.251

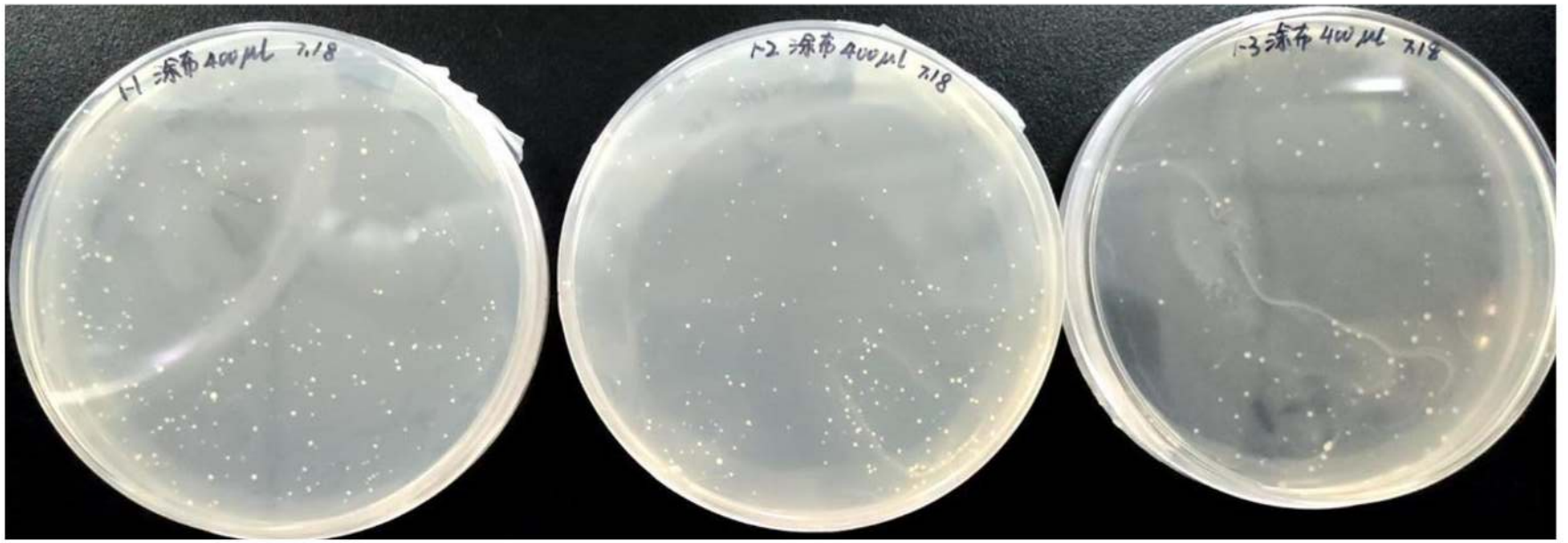
Yeast colonies:

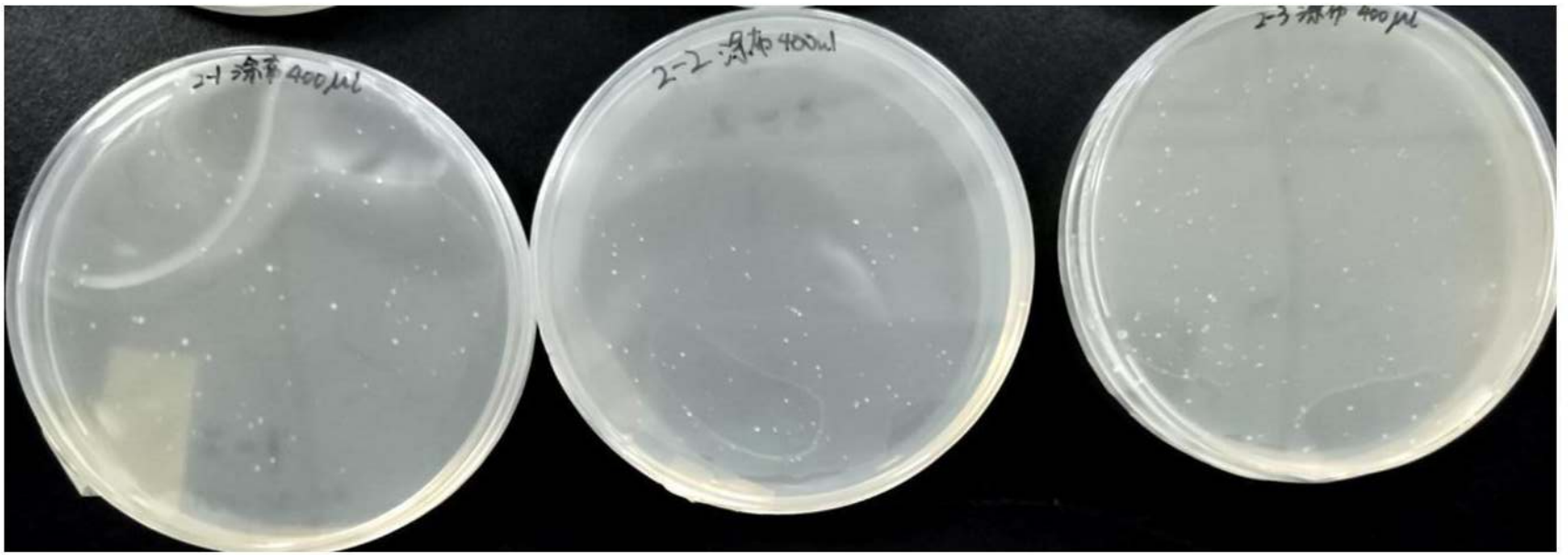
Implanted in 7/20:

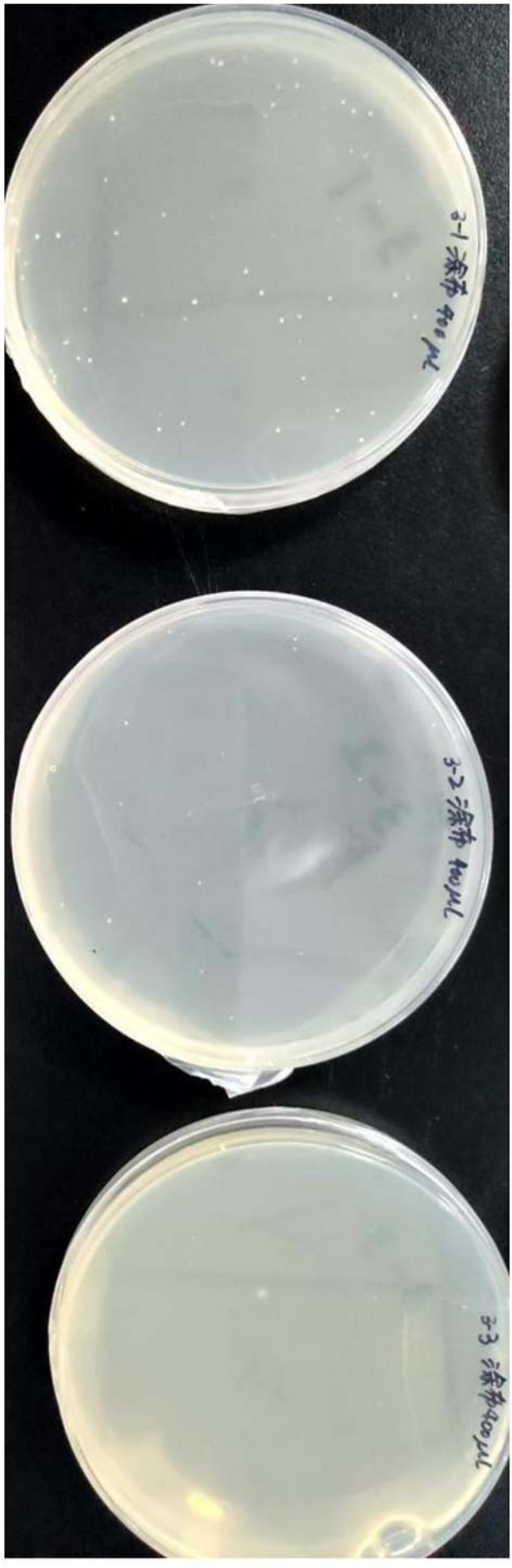
Too much of the bacteria are grown on the dish. Probably contaminated by other bacteria in environment since there don't have a single colony.

Implanted in 7/18:




1-1 涂布 400μL 7.18
1-2 涂布 400μL 7.18
1-3 涂布 400μL 7.18


2-1 涂布 400μl
2-2 涂布 400μl



1:Piner5 2: pILAC3; 3:pIT6EG7ml

2024/07/21 DAY 23

Participants: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

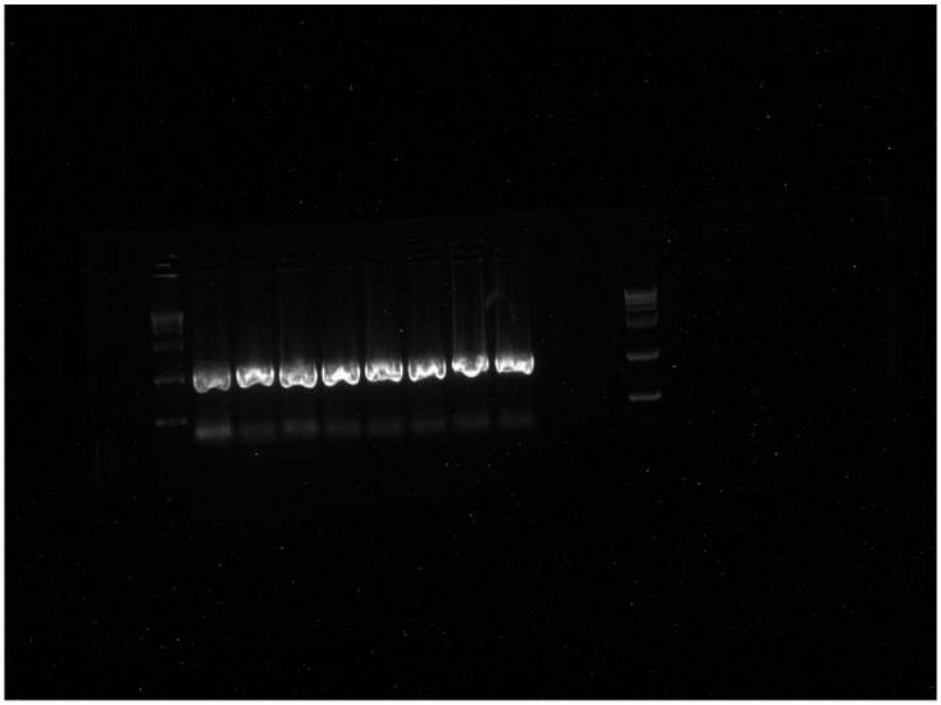
1. Verify the yeast in 1-1 by PCR.
2. Reconstruct plasmid 1:Piner5 2: pILAC3; 3:pIT6EG7ml.

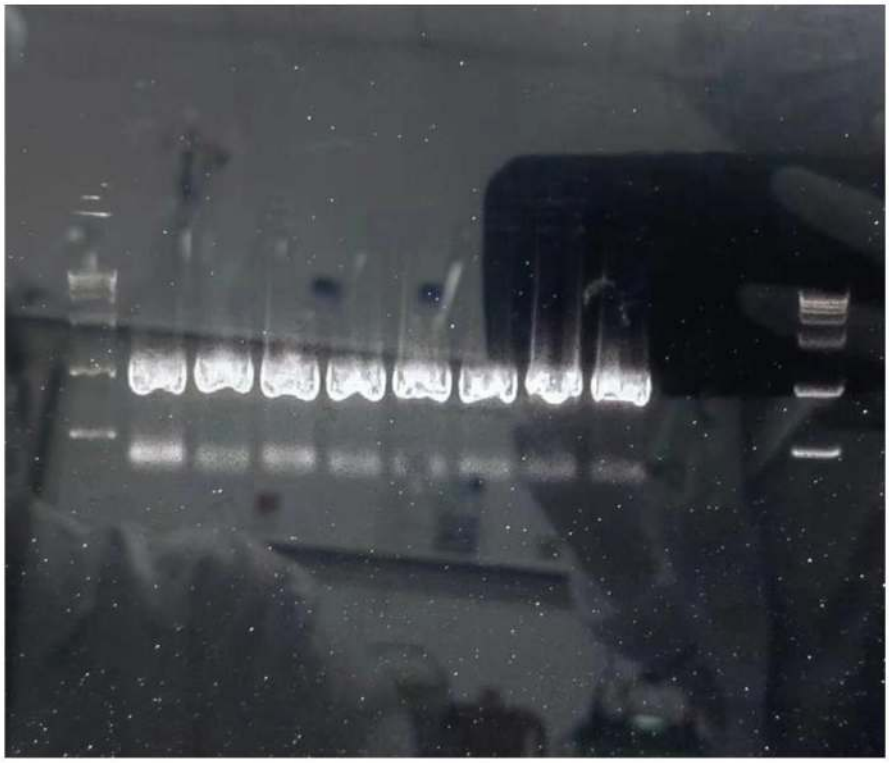
Experiment processes:

1. Repeated processes that were done before.
2. Pick 8 colonies from 1-1. Use the same lysate. The template is 7.13 SHDDo5-Leu-F/R. Transfer the plasmid to the competent cell as usual. Implant the competent cell on the petri dishes.

Experiment result:

Gel result of 1-1:





Perfect PCR result.

2024/07/23 DAY 24

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

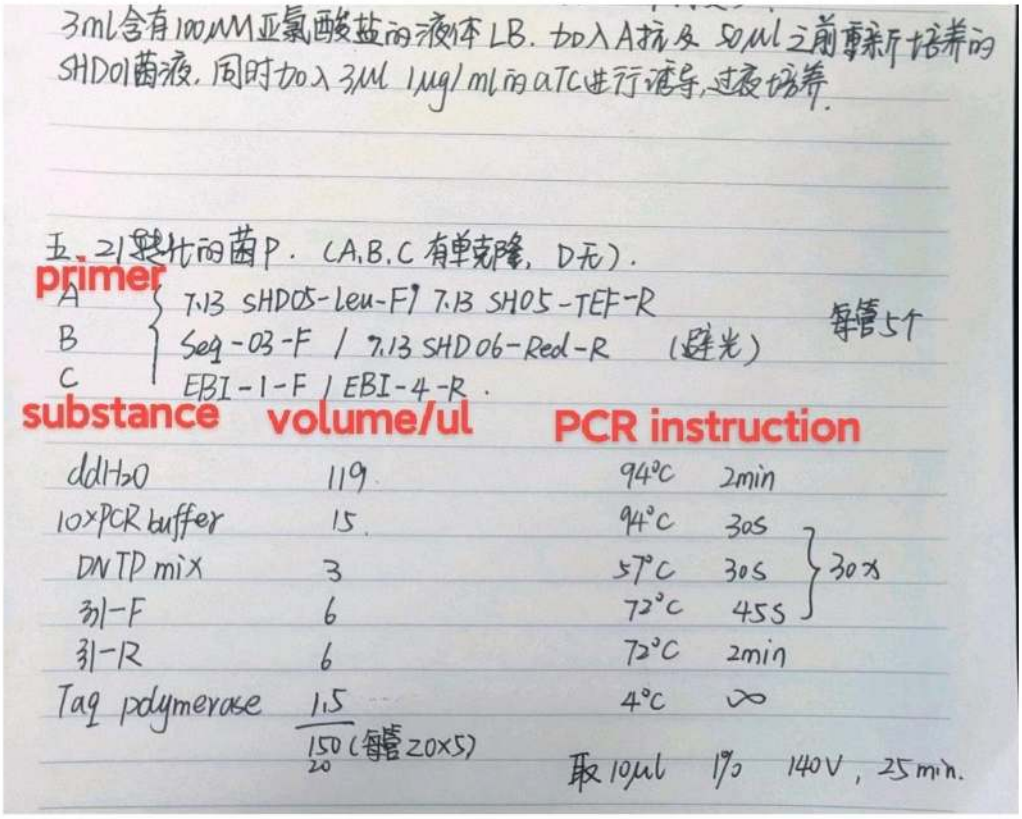
Experiment content:

1. PCR for plasmids which transfered to competent cells in 7/21
2. PCR for the plasmid pLIAC3 (2) PIT6G (3) and do the gel recycle.
3. Prepare 12 petri dishes with SD-Leu medium and 3 YPD dishes. They will further verify the genome intergration in yeast by nutritional screening.
4. Transfer the plasmid BBa_K3317004 into the competent cell for biosafty.

Experiment processes:

1. PCR for competent cell.

- Pick 5 bacteria colonies from each petri dish.
- Set up of PCR:



- Make and run the gel.

2. PCR for plasmid pLIAC3 and PIT6G in yeast.

- Pick 8 colonies of yeast from each petri dish.
- Add 10 ul M5Mix into 8 colonies respectively

Component of M5Mix:

M5Mix

Primer 7.13SHDO5-Leu-F/R

Substance	Amount/ul
ddH2O	4.0
Primer-F	0.4
Primer-R	0.4
Template	0.2
2xFhanta Flash Master Mix	5.0

8 Primers

Primer

7.19pADH1-F/R

7.19pTDH3-F/R

7.19TEF1-F/R

7.19TEF1t-F/R

7.19pPGK1-F/R

7.19CYC1t-F/R

7.19pTEF1-F/R

7.19ENO2t-F/R

7.19PGK1t-F/R

3. Put them in hot water bath in 95 Celsius for about 3-5 mins

PCR instrument:

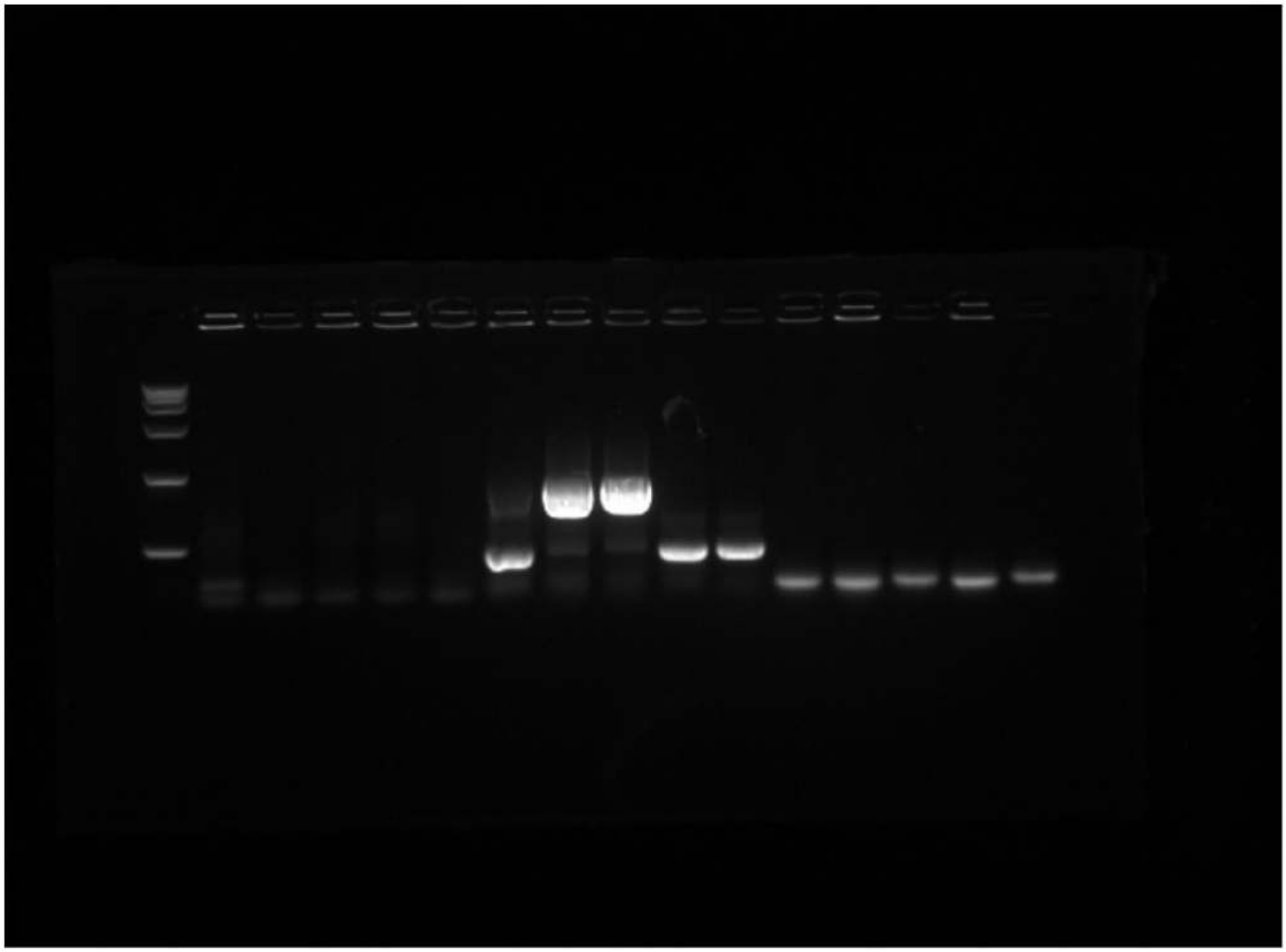
Temperature	Time
98 ° c	30s
98 ° c	10s
57 ° c	5s
72 ° c	30s
72 ° c	1min

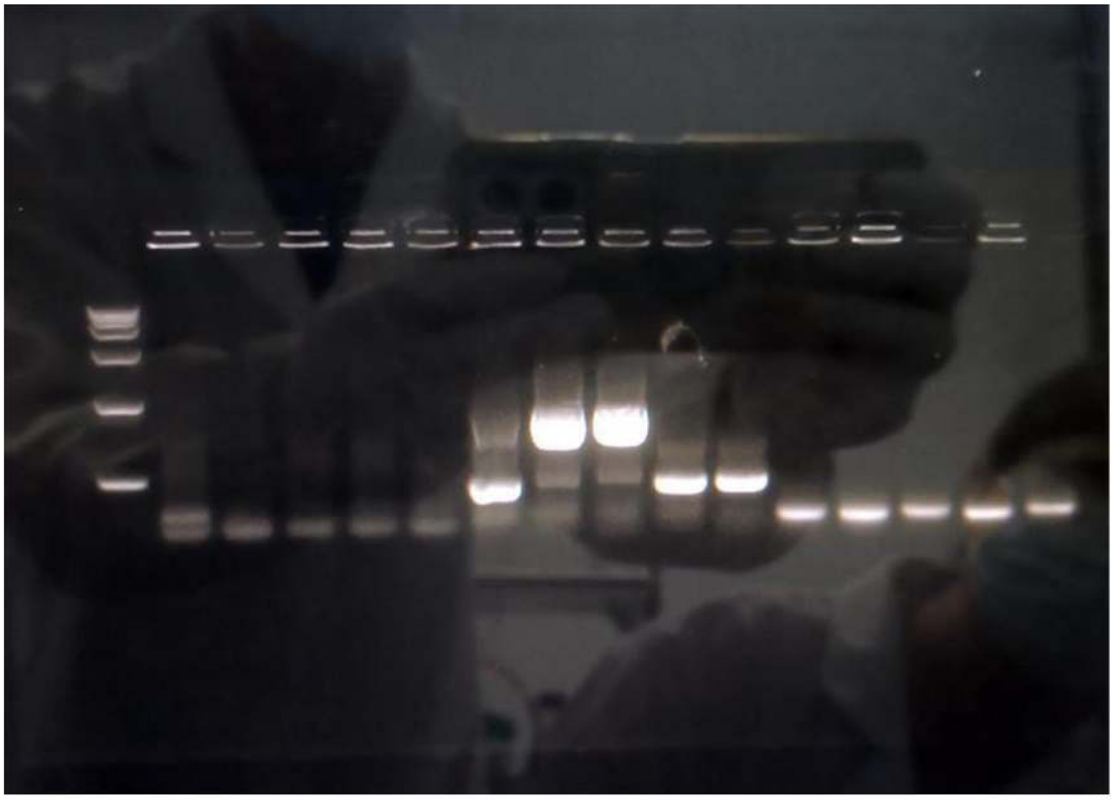
Make & run gel and recycle the gel.

3. Repeat the same processes as before.
4. Repeatthe same processes as before.

Experiment result:

1. Gel result of competent cell:



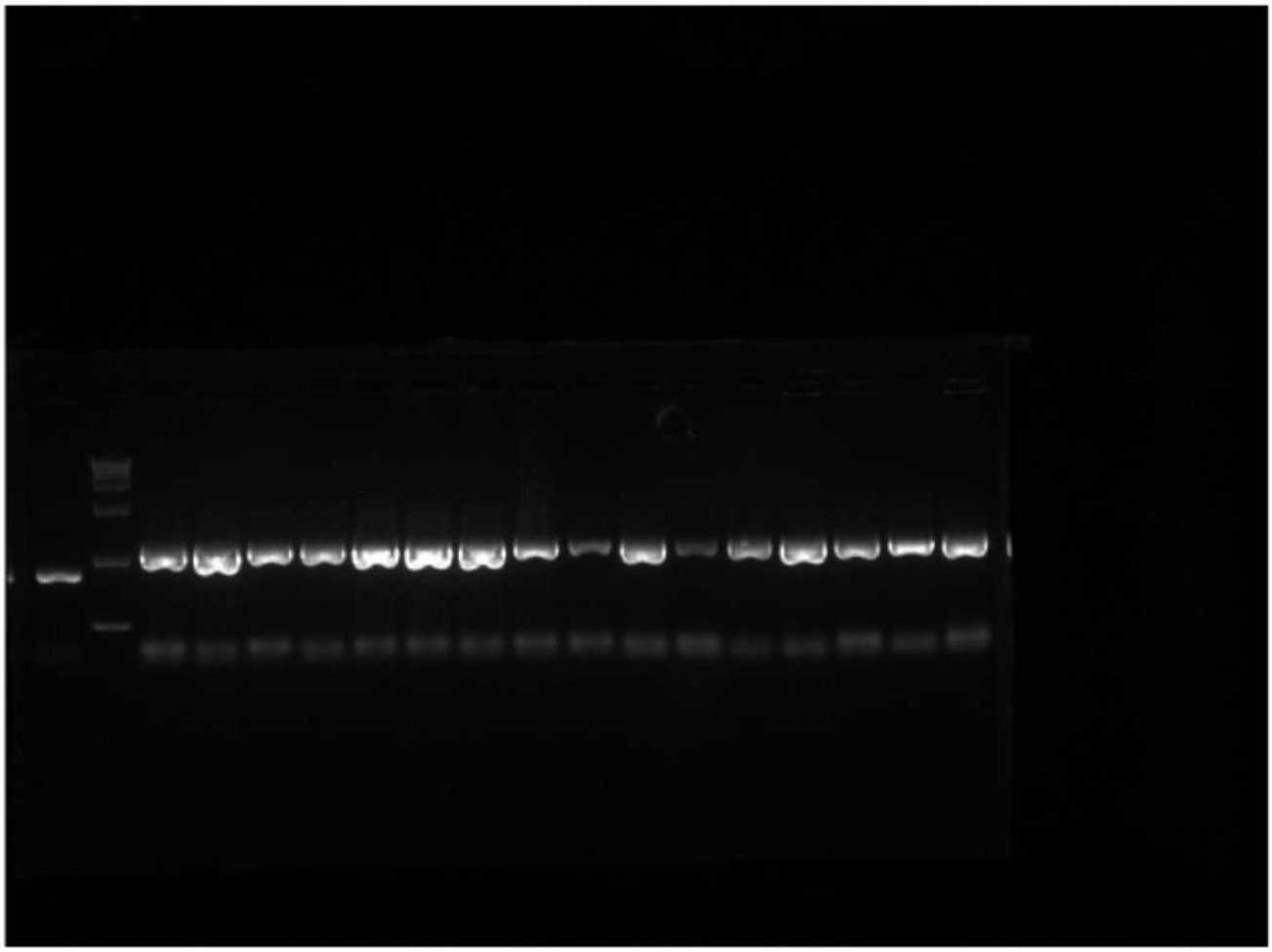


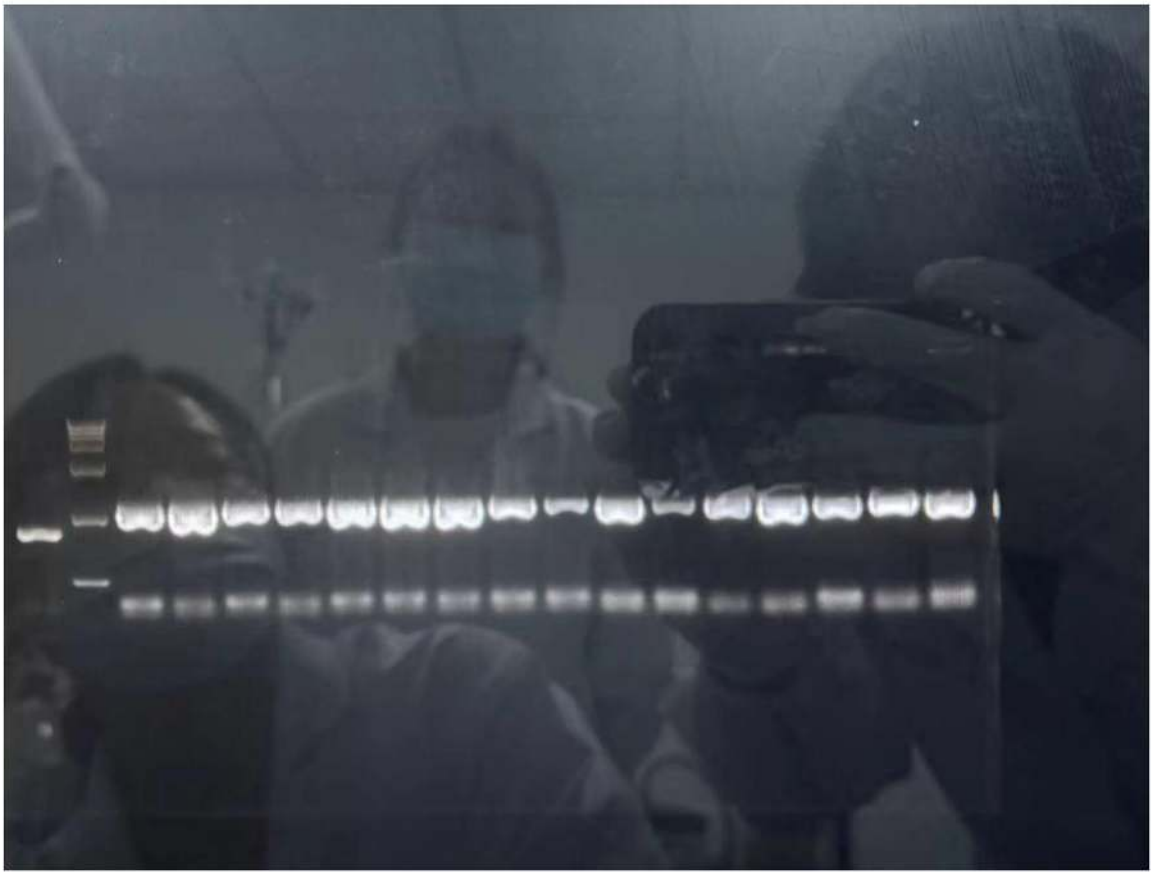
B1, B2 and B3 might be successful. The competent cell colonies are implanted into liquid LB tubes with Amp for each.

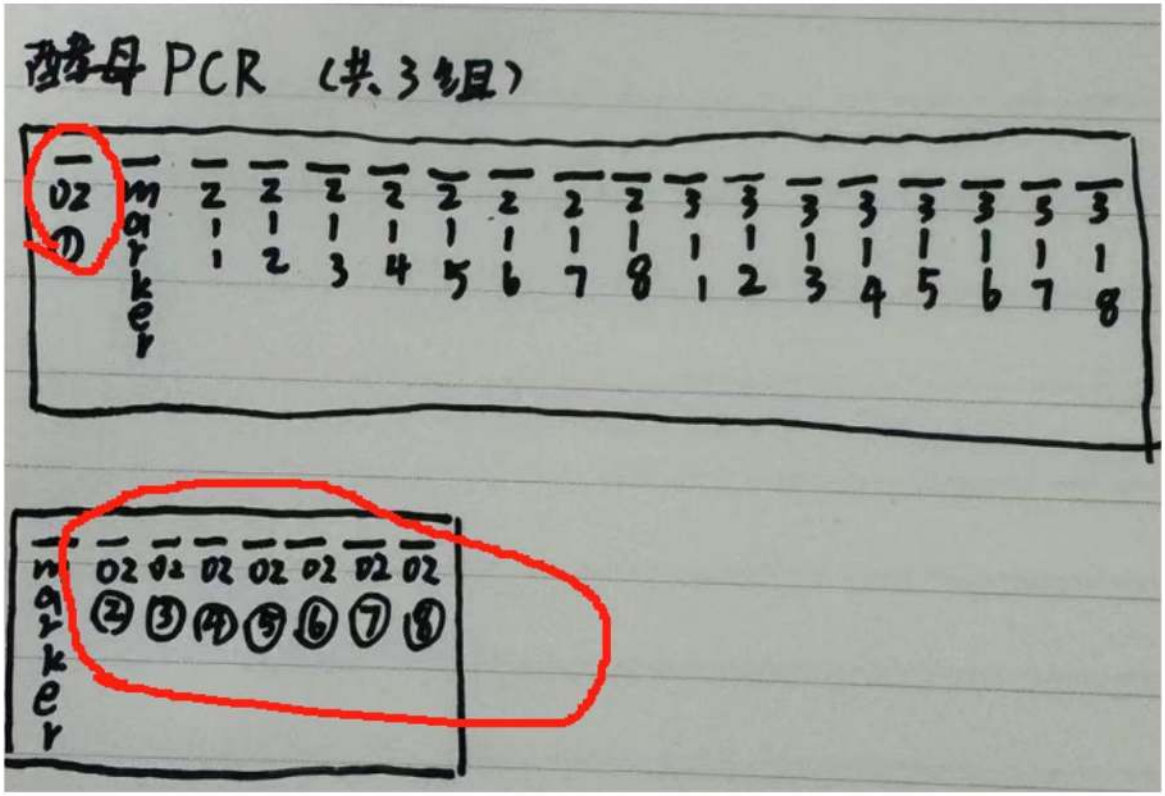
Petri dishes A and C + 3 tubes of B colonies will be sent to external institutes for sequencing.

Preserve 10 tubes of A, B and C colonies for each.

2. Gel result for yeast:







Gels inside the red circle are cutted and do the gel recycle.

2024/07/24 DAY 25

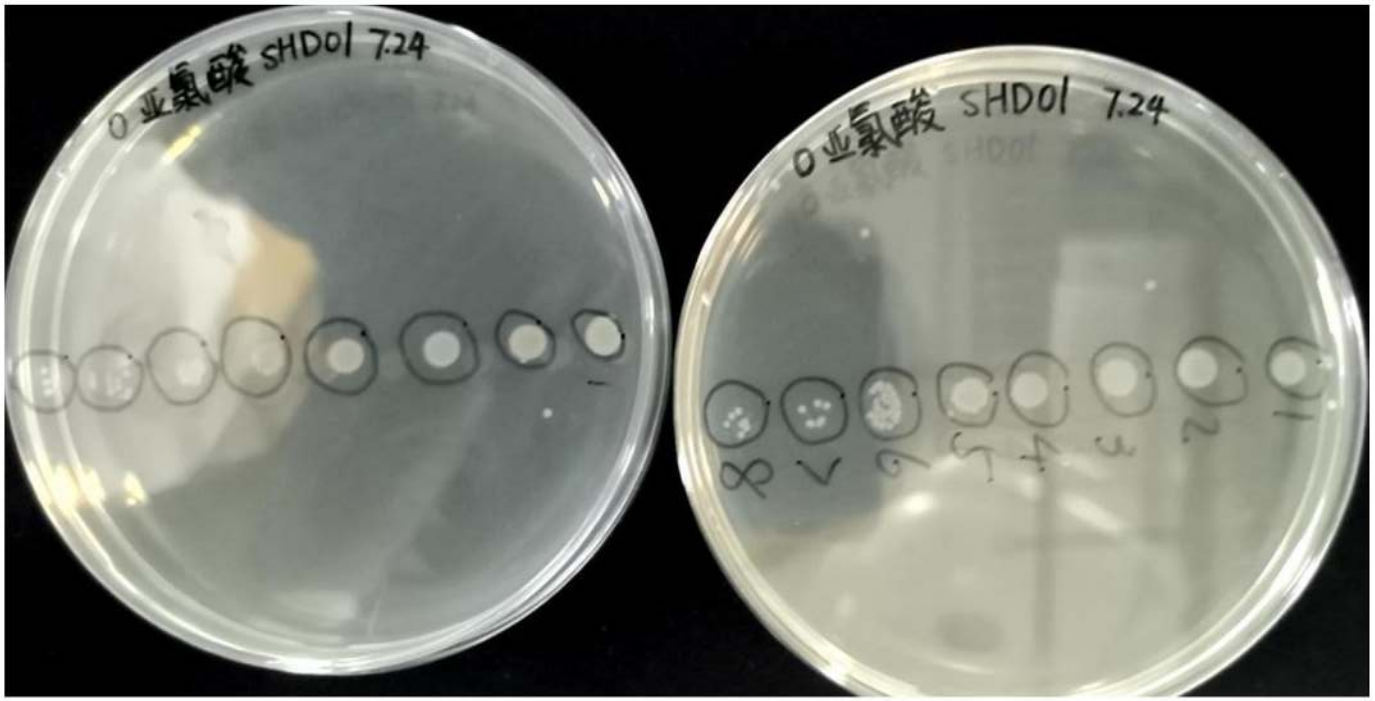
Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

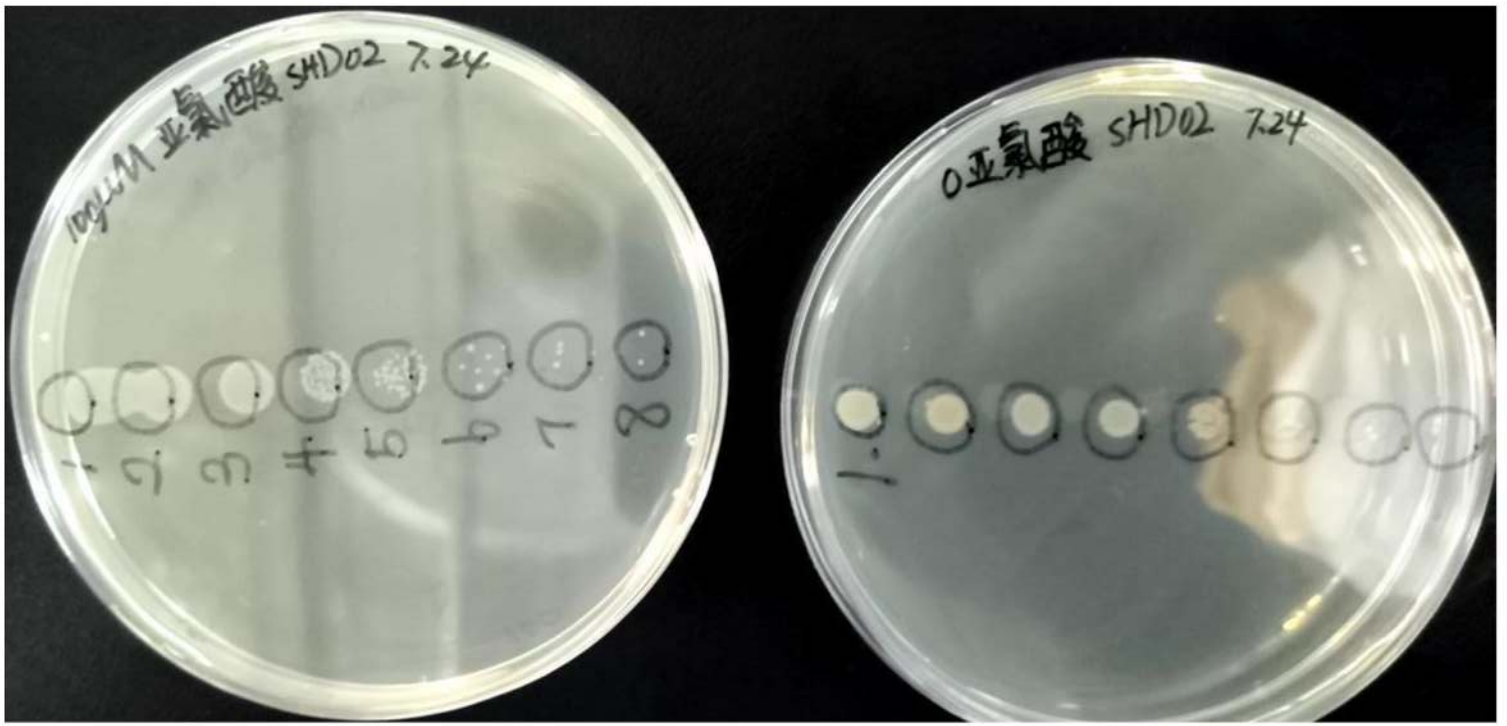
Experiment processes:

1. Implant bacteria solution SHD01 SHD02 and SHD03 on petri dishes with hypochlorite.
2. Implant 4 yeast colonies in the dishes that were verified yesterday into liquid SD-Leu medium.
3. Subpackage and send the competent cell A, B and C to the external institutes for sequencing.

Experiment results:

Petri dishes


0.1% 氯酸 SHD01 7.24
0.1% 氯酸 SHD01 7.24


100μL/ml 亚氯酸 SHD02 7.24
1 2 3 4 5 6 7 8
0亚氯酸 SHD02 7.24
1

Yeast solution:

2024/07/25 DAY26

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

1. aTc solution set up. aTc will be used for the inducible expression.

2. PCR for 2 plasmids pSHD08-RSF101 (do the conditional cracking for biosafety) and pSHD09-RSF101 (controls CSCB translocator protein. Can provide sucrose as carbon source to other microorganisms)for synechococcus. PCR for 2 plasmids pSHD10-TswEF1p(helping express ERG20 mutant which can level up the lycopene synthesis) and pSHD11-TEF1p(Helping expressing NtGGPS mutant which can level up the lycopene synthesis) which will be transfered to yeast. Do the gel recycle for any valid PCR results.
3. Coercive evolution set up for SHD01, which aim for them to develop hypochlorite resistance.

Experiment processes:

1. Using pure ethanol solution to dissolve aTc.
   1. Adding 2ml of ethanol into the amber bottle of aTc.
   2. Transfer them into the 50ml centrifuge tube.
   3. Adding ethanol until the total volume reach 12.5ml.
   4. Subpackage them into 10 tubes. Label as 2mg/ml.
   5. Dilute one of the tube in 1:2. Label as 1mg/ml.
   6. Add 1ul of the solution in 1mg/ml into 999ul of ethanol so the solution is diluted for 100 times. Label as 1ug/ml.
2. Repeating the same processes as before.
3. 1. Adding 50ul of SHD01 bacteria solution into 3ml of liquid LB with 500uM of hypochlorite.
   2. Adding 3ug of 1ug/ml aTc solution into the tube.
   3. Culture the bacteria solution for one night.

Experiment results:

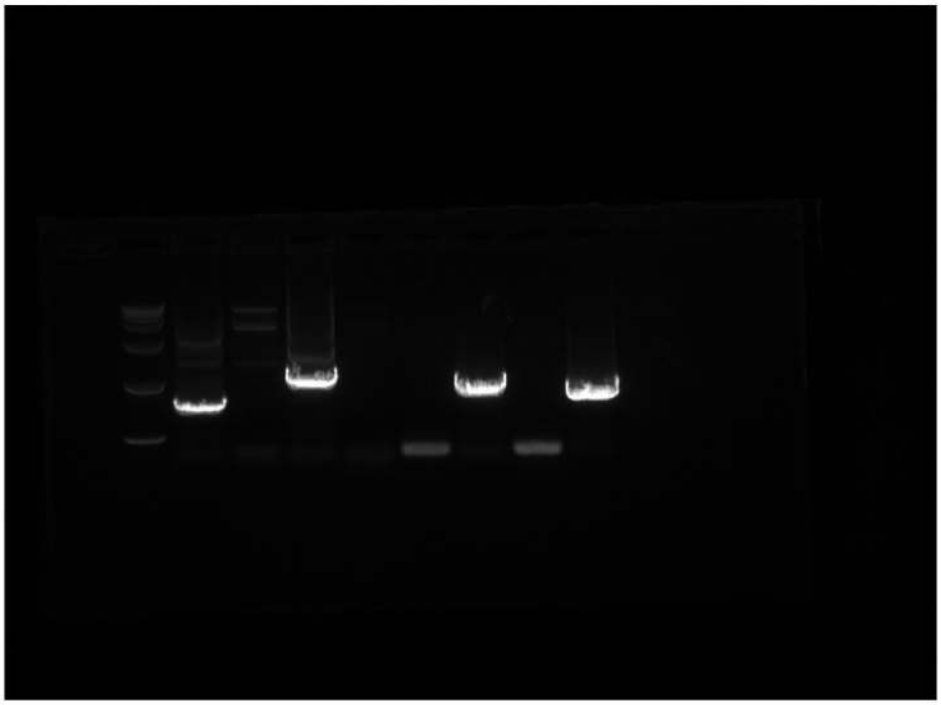
PCR

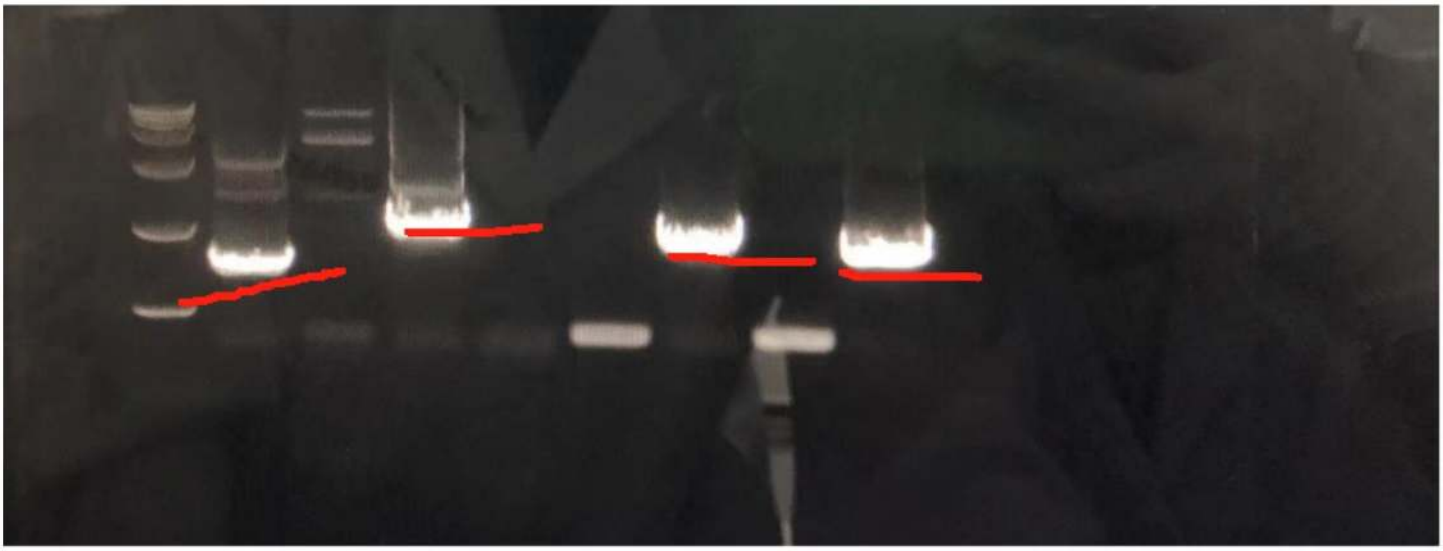
DATE / / /

引物

片段1	S0766	7.24 SHD08-S0766-F/R	1	S0
片段2.	SHD08骨架	7.24 SHD08-BB-R/F	2	✓上次
片段1	S0766	7.24 SHD09-CSCB-F/R	3	CS
片段2	SHD08骨架	7.24 SHD08-BB-F/R	4	✓上次
片段1	SHD10.11骨架	7.24 SHD11-BB-R/F	5	✓SHD
片段2	ERG20	7.24 SHD10-F/R	6	✓PIT 取1
片段1	SHD01.11骨架	7.24 SHD11-BB-F/R	7	✓SHD
片段2	NtGGPPS	7.24 SHD11-NtGGPPS-F/R	8	只需 NtGG

S0766 CSCB ERG20 NtGGPPS are cutted for gel recycle.





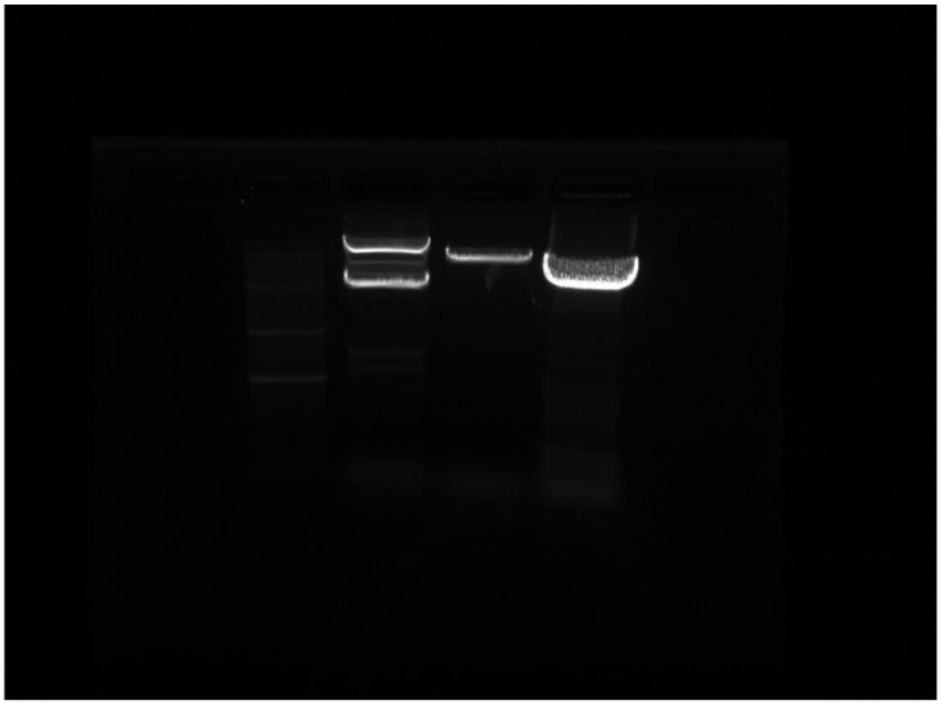
2024/07/26 DAY27

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

1. Continue the coercive evolution for SHD01 bacteria solution.
2. Draw the yeast solution on the SD-Leu medium.
3. Prepare 6 petri dishes wih 500uM of hypochlorite.
4. PCR for failed samples(SHD08, 10 and 11 skeleton) in 7.25

Experiment result:



2024/07/27 DAY28

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

1. Continue the coercive evolution of SHD01 bacteria solution.
2. Run the gel for the PCR result from yesterday. Do the gel extraction.
3. Construct the plasmid SHD12(for lycopene synthesis). Do the gel extraction.
4. Verify the expression of killred plasmid.

Experiment processes:

1. 3ml aTc + 500uM hypochlorite.
2. Repeat the same processes as before.
3. Repeat the same processes as before.
4. Implant bacteria solution B3(with killred plasmid) and cultivate it under light.
    1. Prepare 3 petri dishes with LB medium + Amp and diluted B3 solution.
    2. Add 0 aTc, 1ng/ml aTc and 10ng/ml aTc to each dish respectively.
    3. Cover half of each petri dish with tinfoil.
    4. Cultivate 3 petri dishes in illumination incubator for one night.

Experiment results:

The portion folded by tinfoil have higher density of bacteria colonies. But many bacteria colonies are still growing on the half with light.

2024/07/29 DAY29

Participant: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

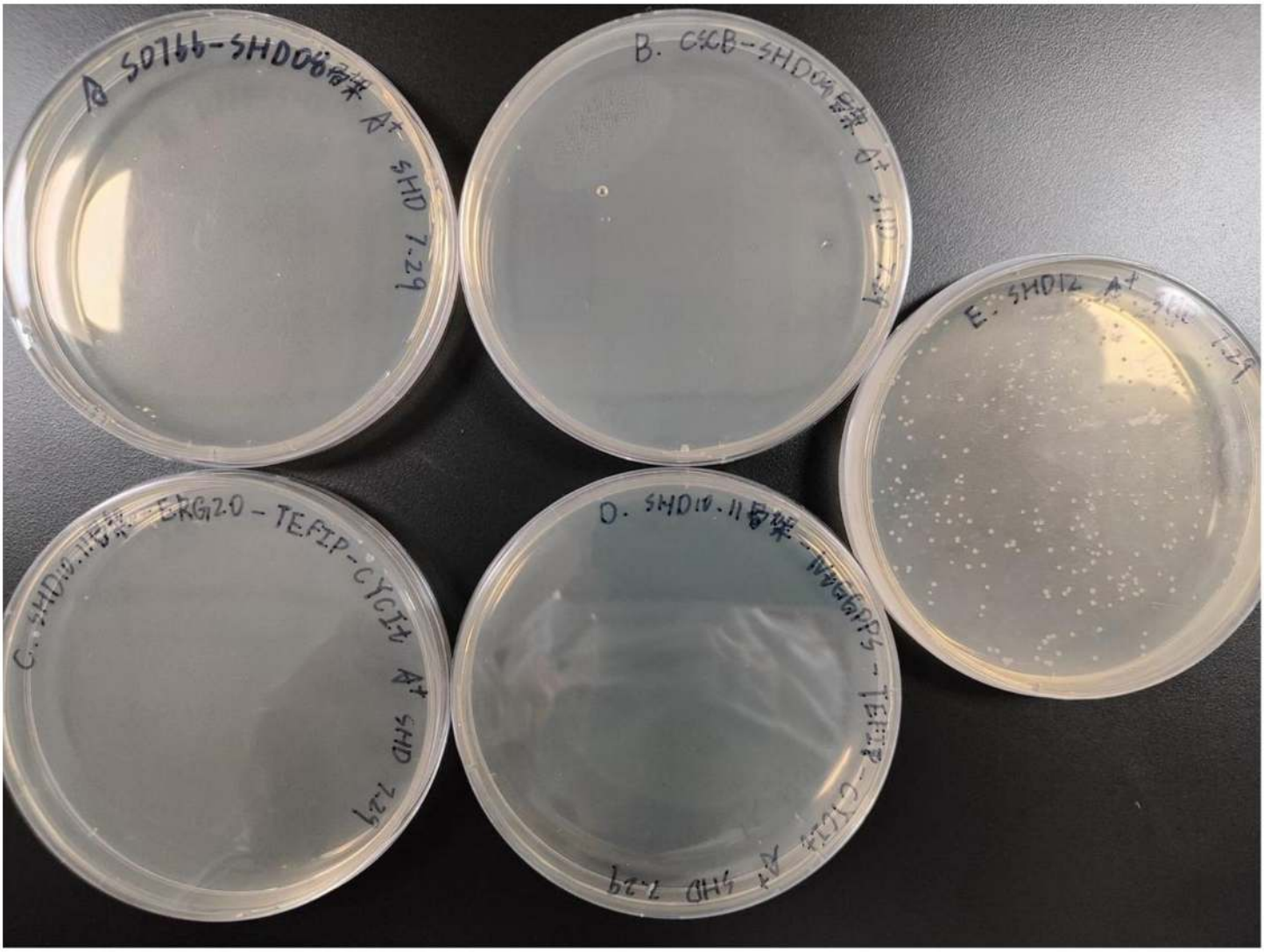
1. Continue the coersive evolution of SHD01 bacteria solution.
2. Spot bacteria solution SHD01 and SHD02 on LB medium with hypochlorite again since the trend is not so obvious last time.
3. Transfer plasmid pSHD08-RSF101, pSHD09-RSF101, pSHD10-TswEF1p, pSHD11-TEF1p and SHD12 to competent cell.

Experiment processes:

1. Prepare 3ml of liquid LB medium with 500uM of hypochlorite + Amp + aTc.
2. Repeat same processes as before.
3. Repeat same processes as before.

Experiment results:

Competent cell



Only SHD12 grow successfully.

2024/07/30 DAY30

Participants: Charlotte Gou, Angelina Liu, Annie Tao, Tony Wang, Jerry Zhu, Jack Bian

Experiment content:

1. Continue the coersive evolution of SHD01. Change the condition into 1ng/ml aTc and 100/200uM of hypochlorite.
2. Reconstruct the plasmid pSHD08-RSF101 and pSHD09-RSF101 in competent cell. We made a mistake yesterday since these 2 plasmids are Kan resistance but we used Amp instead.

Experiment processes:

Repeat the same process as before.

2024/07/31 DAY31

Participants: Angelina Liu, Mingda Wang, Tony Wang, Cynthia Zhao, Jerry Zhu, Annie Tao

Experiment content:

Experiment 1: Transfer 30 μL of SHD01 induced bacterial culture from yesterday into 3 mL of LB medium with antibiotic Amp. (Continue the coersive evolution of SHD01.)

Experiment 2: Divide three YPD solid media plates into four sections. Each plate corresponds to three different plasmids(PILAC3, Pinar5, PITEG)transfered into yeast. (Note: When streaking, just touch the yeast with the pipette tip without aspirating, otherwise it won’ t separate properly). Repeat for two groups of SHD01 and SHD02.

Experiment 3: 500 μM chlorite plating experiment. Follow the wiki diagram. Prepare six solid LB plates with chlorite and antibiotic A (A) and six without chlorite but with antibiotic A (B).

- SHD01: Spot three A plates and three B plates.
- SHD02: Spot three A plates and three B plates.

Experiment 4:

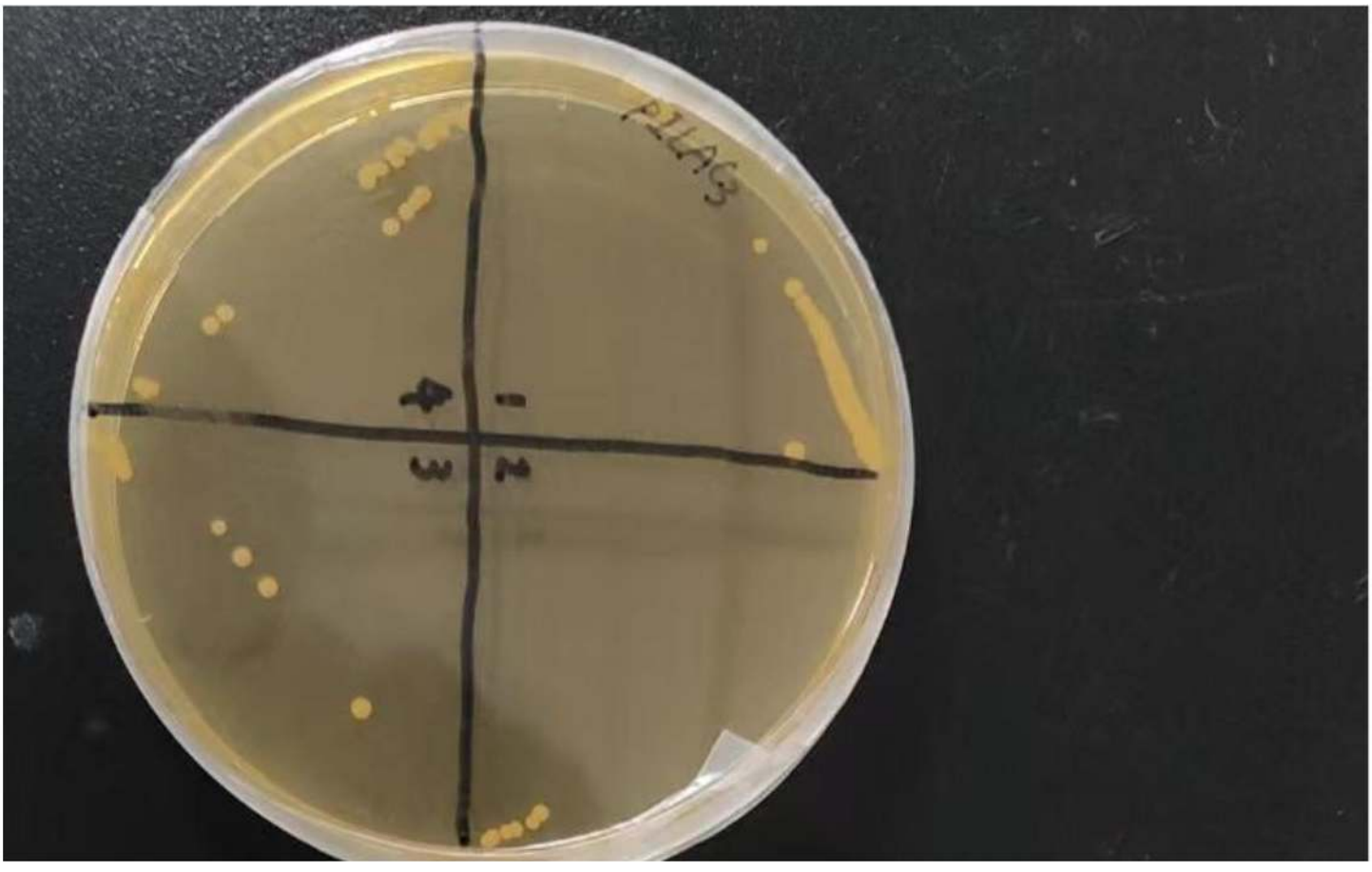
- **Limonene:** Measure at 252 nm.
- **Nerol:** Measure at 195 nm.

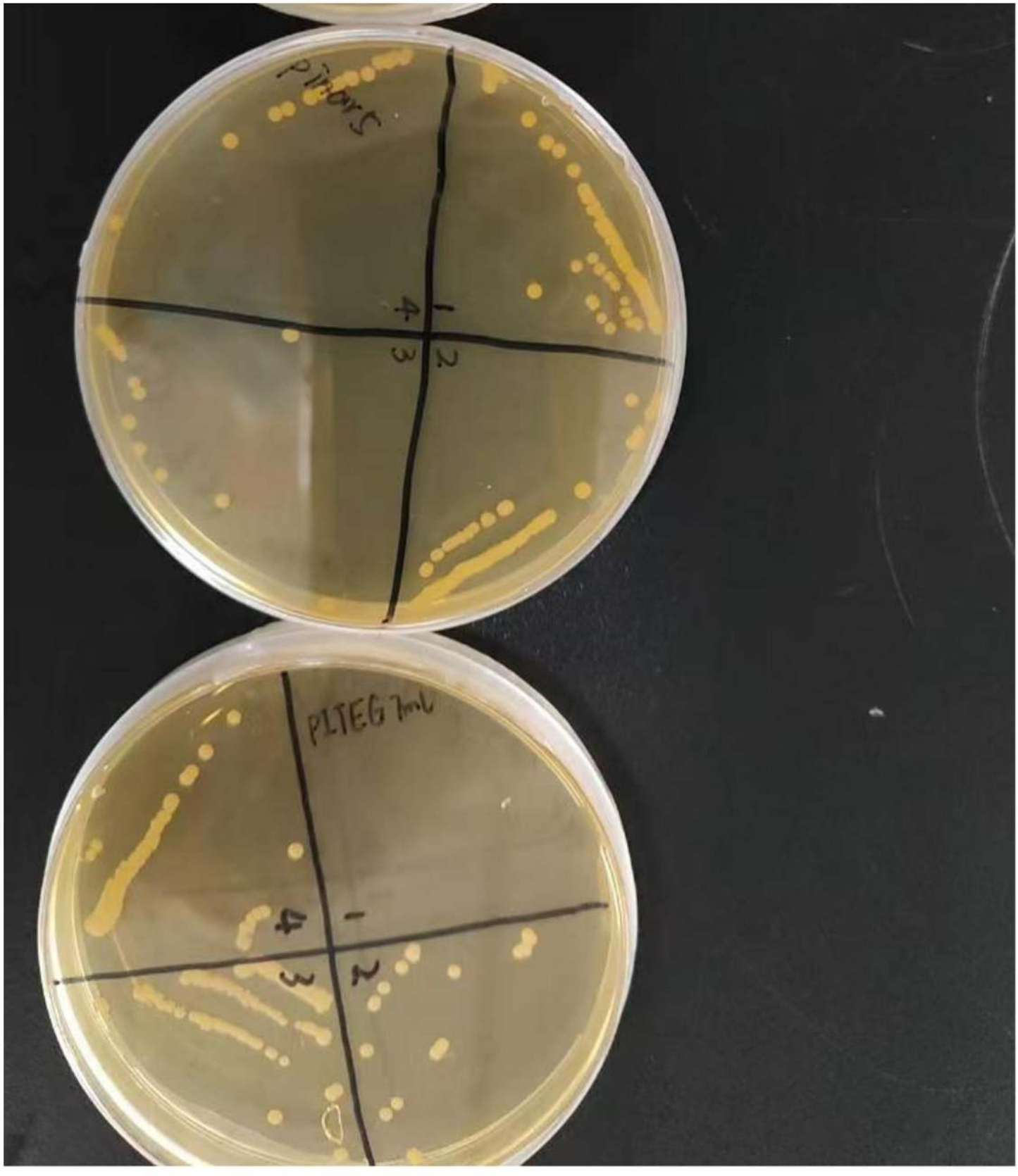
Nerol: 1 mL -> 9 mL anhydrous ethanol (100 mg/mL). Gradually dilute to obtain 10 mg/mL, 1 mg/mL, and 100 μg/mL solutions. Measure at 195 nm with anhydrous ethanol.

Limonene: 1 g -> 10 mL anhydrous ethanol (100 mg/mL). Gradually dilute to obtain 10 mg/mL, 1 mg/mL, and 100 μg/mL solutions. Measure at 252 nm with anhydrous ethanol.

Experiment result:

Yeast implantation after 2 days of incubation





2024/08/01 DAY32

Participants: Cynthia Zhao, Tony Wang, Charlotte Gou

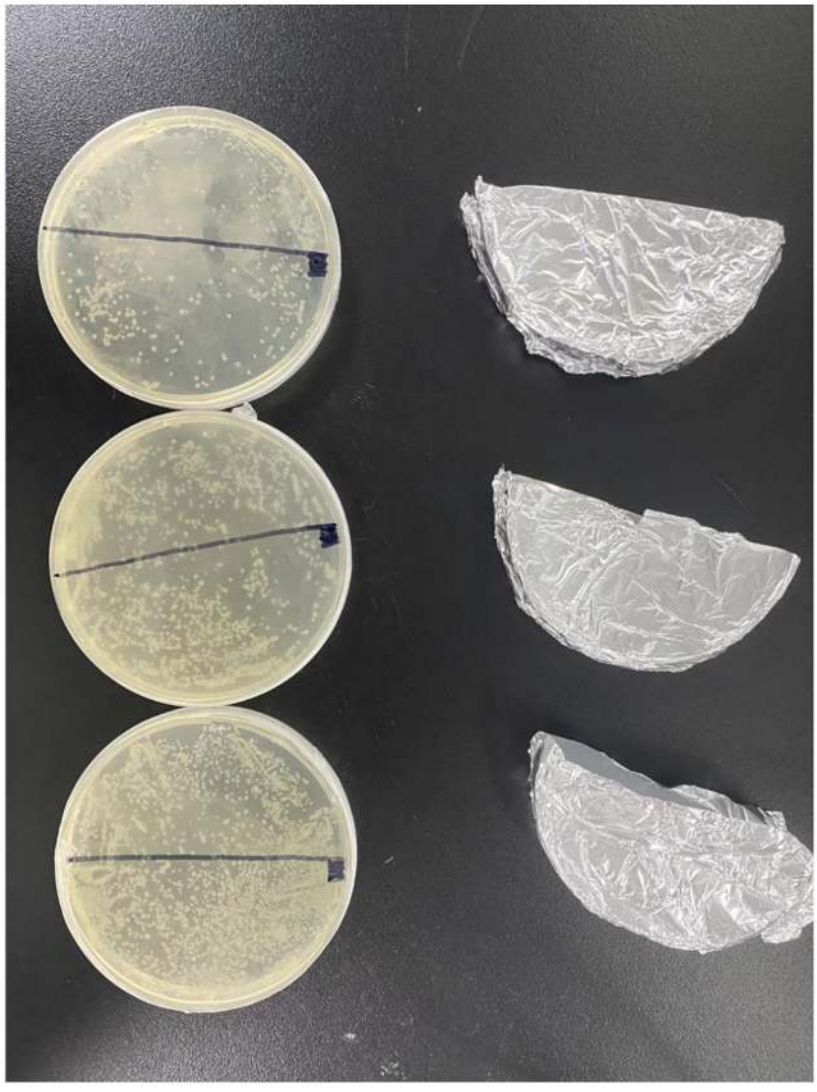
Experiment content:

1. Continue the coersive evolution of E.coli in 100uM of hypochlorite.
2. Prepare 20 petri dishes with Ashby medium with 250uM of hypochlorite, which will be used to culture nitrogen fixing bacteria in the future.
3. Centrifuge the yeast with plasmid plLAC3 to see if it produced lycopene or not.
4. Repeat the light experiment for competent cell with plasmid killred.
5. Check the light absorbance of lemonene and nerol solution.

Experiment result:

Yeast centrifuge:

killred



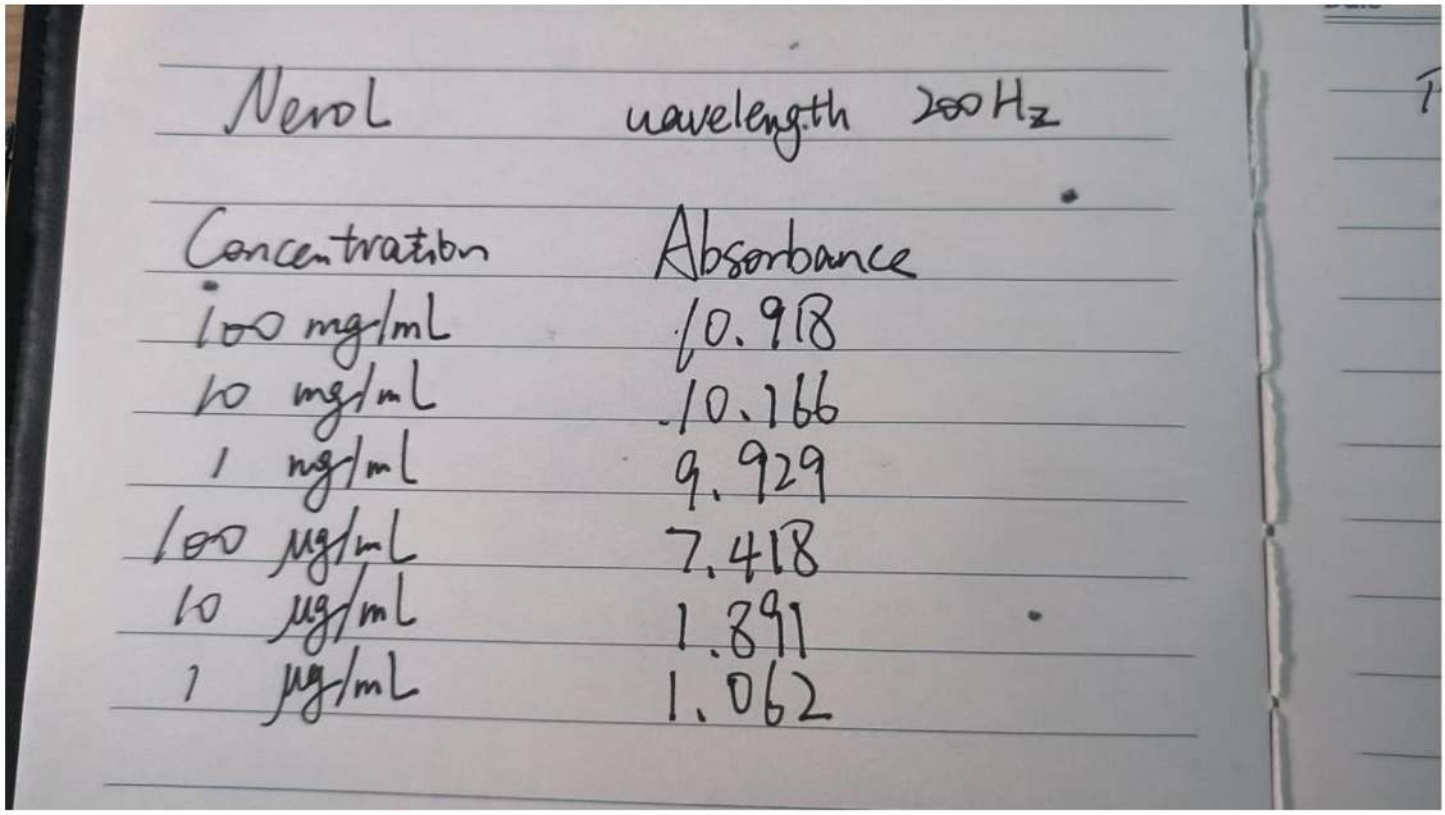
Lemonene:

Date / /

- Limonene Wavelength: 252

Concentration	Absorbancy
100 mg/mL	5.135
10 mg/mL	2.343
1 mg/mL	0.229
~~100~~ 100 µg/mL	0.09

Nerol:



2024/08/02 DAY33

Participant: Cynthia Zhao, Tony Wang, Charlotte Gou, Jack Bian, Angelina Liu, Roslyn Huang, Mingda Wang

Experiment content:

Experiment 1:

Observe the E. coli cultured in two tubes from yesterday, and measure the OD600. If it is relatively clear, transfer to LB medium with A antibiotic only without adding anything else; if it is somewhat dense, transfer 30 μL to 3 mL LB medium containing 100 μM chlorite, A antibiotic, and 1 ng/mL aTc.

Experiment 2:

Isolate microorganisms from soil, and plate them: Take 1 g of root nodule, add it to 20 mL of sterile water (using a 50 mL centrifuge tube), shake, then let it stand for 10 minutes. Afterward, dilute it 1000 times according to the spot plate experiment method, then use two previously poured ash-based solid media (containing chlorite), and spread 40 μL each on the plates.

Experiment 3:

Observe the yeast in YPD medium from yesterday. If it appears very turbid (more turbid than when making competent cells), collect these tubes, place them in sequencing bags, and freeze at -20°C; if not very turbid, observe again tomorrow to decide whether to preserve them.

Experiment 4: Construct SHD00.

SHD00 construction:

Fragment 1:

pBBR1-R

S01-AmpR-F Template is SHD01 Band 3k

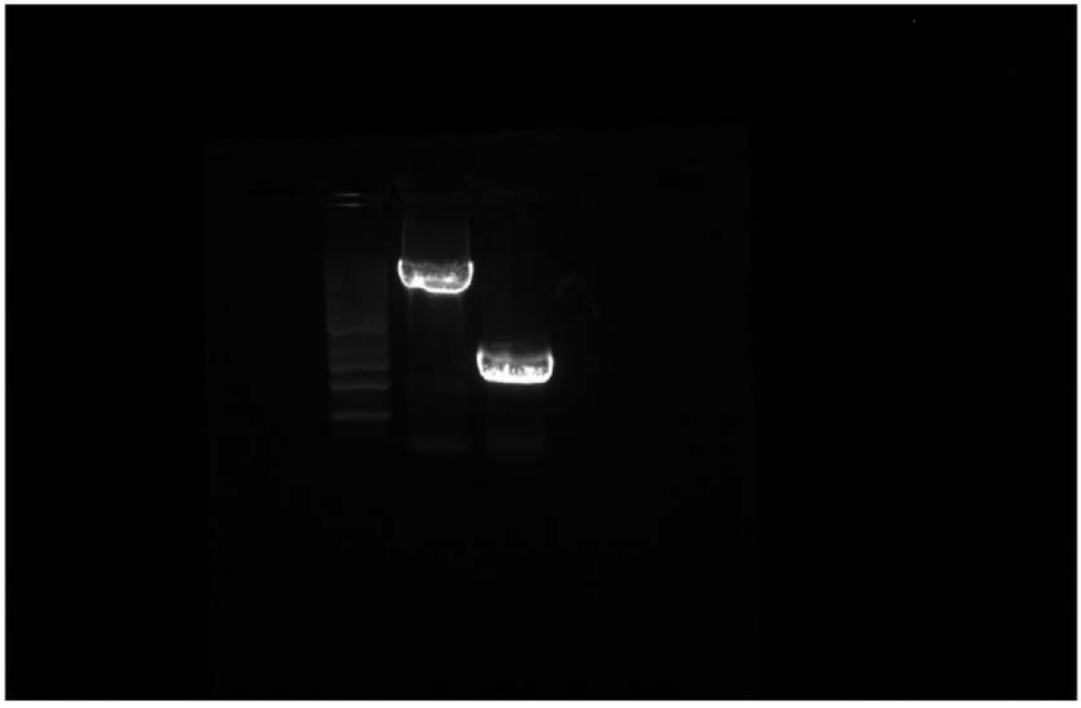
Fragment 2:

S01-TETR2-F 8.1 SHD00-R Template is also SHD01 Band 1k

Experiment 5:

Streak the returned SHD012 bacterial liquid onto solid LB with A antibiotic plates.

Gel result:



2024/08/03 DAY34

Participant: Cynthia Zhao, Tony Wang, Charlotte Gou, Jack Bian, Angelina Liu, Roslyn Huang, Mingda Wang

Experiment content:

Experiment 1:

Observe the two tubes of E. coli transferred yesterday and measure the OD600. If it is relatively clear, transfer to LB with A-antibiotic only; if it is a bit dense, transfer 30 μL to 3 mL LB with 100 μM chlorite, A-antibiotic, and 1 ng/mL aTc.

Experiment 2:

Pick clones of SHD012 and transfer to 5 mL liquid LB with A-antibiotic.

Experiment 3:

Transfer clones from the CEN-PK2 plate to 5 mL liquid YPD medium, and also prepare 50 mL of fresh liquid YPD, as you need to prepare competent cells.

Experiment 4:

Pick P SHD00 colonies and transfer to LB with A-antibiotic.

2024/08/05 DAY35

Participant: Cynthia Zhao, Tony Wang, Charlotte Gou, Jack Bian, Angelina Liu, Roslyn Huang, Mingda Wang

Experimeent content:

Experiment 1:

Observe the two tubes of E. coli transferred yesterday and measure the OD600. If it is relatively clear, transfer to LB with A-antibiotic only; if it is a bit dense, transfer 30 μL to 3 mL LB with 100 μM chlorite, A-antibiotic, and 1 ng/mL aTc.

Experiment 2:

Extract the plasmid of SHD012.

Experiment 3:

Prepare three SD-URA solid plates. We have the SD powder from previous purchases, and for the –URA supplement, ask Dr. Zhang Hui as the Alpha team has bought it.

Experiment 4:

Prepare competent cells of CEN-PK2 yeast following the original procedure [Ask Dr. Sang to inoculate the yeast before lunch].

Experiment 5:

Transform the yeast.

Experiment 6:

Streak SHD00 on A+ LB plates. The bacteria with the Killred (SHD06) plasmid also need to be streaked on A+ LB plates.

Experiment 7:

Streak SHD03 on A+ solid LB plates.

2024/08/06 DAY36

Participant: Cynthia Zhao, Tony Wang, Charlotte Gou, Jack Bian, Angelina Liu, Roslyn Huang, Mingda Wang

Experiment content:

Experiment 1:

Observe the two tubes of E. coli cultured yesterday and measure OD600. If the cultures are relatively clear, transfer them to LB media containing only ampicillin. If the cultures are somewhat dense, transfer 30 μL to 3 mL of LB with 100 μM chlorite, ampicillin, and 1 ng/mL aTc.

Experiment 2:

For SHD00 and killred plasmid cloning, pick 3 colonies for each and inoculate them into 3 mL of LB broth.

Experiment 3:

Prepare 20 mL of BG11 cyanobacteria culture medium (containing 100 mM sodium bicarbonate, calculate this beforehand), and note that it should not be sterilized but only dissolved and then filtered. Divide it into two parts, with each tube containing 10 mL, then add 1 mL of the newly received cyanobacteria culture, mix well, and take 500 µL to measure OD730.

Place one tube in the light incubator under the previous parameters and the other on a laboratory shelf near a light source.

Experiment 4:

Prepare 50 mL of BG11 solid culture medium (containing 100 mM sodium bicarbonate). Since sodium bicarbonate decomposes into carbon dioxide when heated, it should be added last after autoclaving the medium. You can first prepare a 10x sodium bicarbonate stock solution and add it after autoclaving. Then streak cyanobacteria on the medium and place it on a laboratory shelf near a light source.

2024/08/07 DAY37

Participant: Cynthia Zhao, Tony Wang, Charlotte Gou, Jack Bian, Angelina Liu, Roslyn Huang, Mingda Wang

Experiment content:

Experiment 1: Plasmid extraction of SHD012

Experiment 2: Prepare 3 agar SHD-URA plates

Experiment 3: Prepare 50ml BG11 medium.

Add sodium bicarbonate after autoclaving.

Streak the blue algae and culture it close to light source.

Experiment 4:

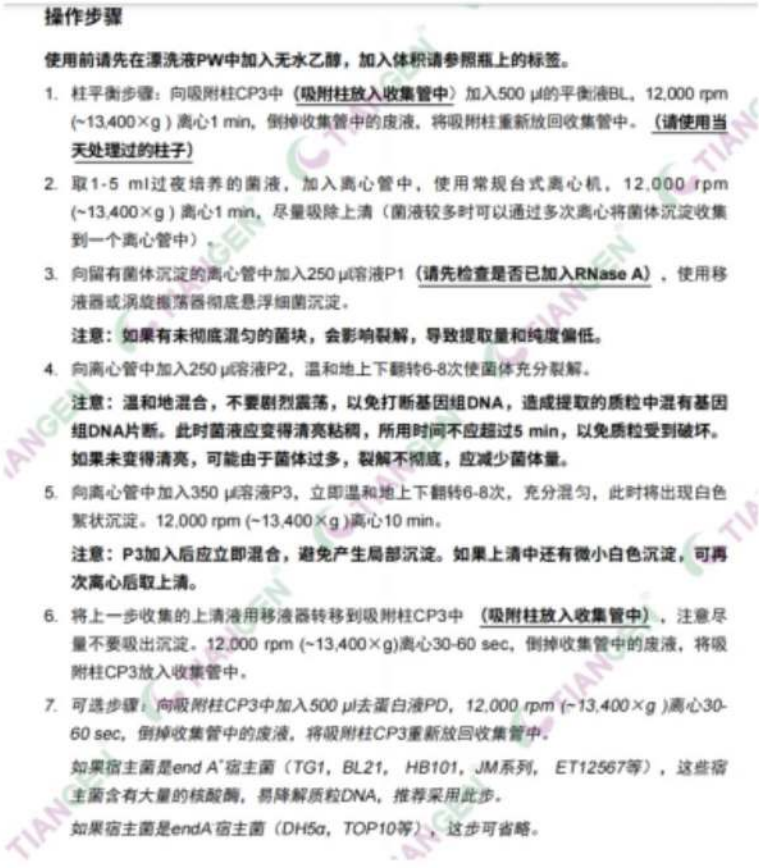
Observe the E.coli conjugated yesterday and examine the OD600

If the cell suspension is diluted, conjugate it on the LB plates with ampicilin antibiotics only.

If the cell suspension is concentrated, conjugate 30ul on 3ml LB plates with 100uM sodium chlorite and 1ng/ml Anhydrotetracycline Hydrochloride.

Experiment 5:

Observe the streaked blue algae.



操作步骤

使用前请先在漂洗液PW中加入无水乙醇，加入体积请参照瓶上的标签。

1. 柱平衡步骤：向吸附柱CP3中（**吸附柱放入收集管中**）加入500 μl的平衡液BL，12,000 rpm (~13,400×g) 离心1 min，倒掉收集管中的废液，将吸附柱重新放回收集管中。（**请使用当天处理过的柱子**）
2. 取1-5 ml过夜培养的菌液，加入离心管中，使用常规台式离心机，12,000 rpm (~13,400×g) 离心1 min，尽量吸除上清（菌液较多时可以通过多次离心将菌体沉淀收集到一个离心管中）。
3. 向留有菌体沉淀的离心管中加入250 μl溶液P1（**请先检查是否已加入RNase A**），使用移液器或涡旋振荡器彻底悬浮细菌沉淀。

   注意：如果有未彻底混匀的菌块，会影响裂解，导致提取量和纯度偏低。
4. 向离心管中加入250 μl溶液P2，温和地上下翻转6-8次使菌体充分裂解。

   注意：温和地混合，不要剧烈震荡，以免打断基因组DNA，造成提取的质粒中混有基因组DNA片断。此时菌液应变得清亮粘稠，所用时间不应超过5 min，以免质粒受到破坏。如果未变得清亮，可能由于菌体过多，裂解不彻底，应减少菌体量。
5. 向离心管中加入350 μl溶液P3，立即温和地上下翻转6-8次，充分混匀，此时将出现白色絮状沉淀。12,000 rpm (~13,400×g)离心10 min。

   注意：P3加入后应立即混合，避免产生局部沉淀。如果上清中还有微小白色沉淀，可再次离心后取上清。
6. 将上一步收集的上清液用移液器转移到吸附柱CP3中（**吸附柱放入收集管中**），注意尽量不要吸出沉淀。12,000 rpm (~13,400×g)离心30-60 sec，倒掉收集管中的废液，将吸附柱CP3放入收集管中。
7. *可选步骤：向吸附柱CP3中加入500 μl去蛋白液PD，12,000 rpm (~13,400×g)离心30-60 sec，倒掉收集管中的废液，将吸附柱CP3重新放回收集管中。*

   如果宿主菌是end A+宿主菌（TG1，BL21，HB101，JM系列，ET12567等），这些宿主菌含有大量的核酸酶，易降解质粒DNA，推荐采用此步。

   如果宿主菌是endA-宿主菌（DH5α，TOP10等），这步可省略。

2024/08/08 DAY38

Participant: Jack Bian, Jialei Wang, Angelina Liu, Tony Wang, Dingxuan He

Experiment content:

Experiment 1: Prepare competent CEN-PK2 yeast cells.

Experiment 2: Observe two tubes of E. coli that were passaged yesterday and test OD600 (1.347). If it's relatively clear, transfer to LB with Ampicillin and nothing else; if it's somewhat dense, inoculate 30 μL into 3 mL LB containing 100 μM chlorite, Ampicillin, and 1 ng/mL aTc.

Experiment 3: Pick three colonies from the SHD03 streaked plates into fresh LB with Ampicillin.

Experiment 4: Streak SHD00 in LB with Ampicillin. The Killed (SHD06) plasmid bacteria also need to be streaked, also in LB with Ampicillin.

Experiment 1: Prepare competent CEN-PK2 yeast cells.

Competent cell preparation:

1. Transfer all of the newly cultured 5 mL yeast culture into 50 mL YPD and incubate on the shaker.
2. 30 minutes in advance, take the Y1, Y2, and Y3 solutions out and place them at 4°C. Also, prepare a 30°C water bath.
3. When the OD reaches 0.4-0.8 (around 1 PM), centrifuge the yeast culture at 4000 rpm for 5 minutes, discard the supernatant, resuspend the pellet in 3 mL of filtered sterile water, centrifuge again, and discard the supernatant. Resuspend in 3 mL of sterile water, centrifuge, and discard the supernatant again.
4. Resuspend the pellet in 3.4 mL Y1 solution, centrifuge at 4000 rpm for 5 minutes, and discard the supernatant.
5. Add 0.34 mL Y2 solution to resuspend the pellet and aliquot 110 µL into 3 sterile EP tubes.

Plasmid yeast transformation:

Component | Plasmid Transformation Premix

Y3 solution :350 µL

SHD12 plasmid : 10 µL

Total volume :360 µL

6. Take 360 µL of the premix and add it to the 110 µL competent cells, mix thoroughly to ensure the yeast cells are completely suspended in the premix.
7. Heat shock at 30°C for 60 minutes, mixing every 10 minutes.
8. Centrifuge at 12000 rpm for 15 seconds and discard the supernatant.
9. Resuspend the cells in 400 µL of sterile water and plate onto SD-URA selection agar, incubate at 30°C.

Experiment 2: Observe two tubes of E. coli that were passaged yesterday and test OD600 (1.347). If it's relatively clear, transfer to LB with Ampicillin and nothing else; if it's somewhat dense, inoculate 30 µL into 3 mL LB containing 100 µM chlorite, Ampicillin, and 1 ng/mL aTc.

Experiment 3: Pick three colonies from the SHD03 streaked plates into fresh LB with Ampicillin.

Experiment 4: Streak SHD00 in LB with Ampicillin. The Killed (SHD06) plasmid bacteria also need to be streaked, also in LB with Ampicillin.

Experiment result:

2024/08/09 DAY39

Participants: Jack Bian, Angelina Liu, Dingxuan He

Experiment content:

Experiment 1:

Cloning of SHD00 and killred plasmids, select 3 colonies for each and culture them in 3 ml of liquid LB.

Experiment 2:

Observe the two E. coli cultures transferred yesterday and measure their OD600. If the cultures are relatively clear, transfer them to LB with only A-antibiotic added; if they are somewhat concentrated, transfer 30 µL to 3 ml of LB containing 100 µM chlorite, A-antibiotic, and 1 ng/ml aTc.

Experiment 3:

Identify whether SHD012 has successfully integrated into yeast:

Use the previously prepared amplification helpers for 8 clones, then use the primers 7.26 SHD12-crtEBI-F / 7.26 SHD12-crtEBI-R for PCR amplification. The desired result should be a 3k band.

Experiment 4:

The second streak plate of cyanobacteria has grown.

2024/08/12 DAY40

Participants: Jack Bian, Dingxuan He

Experiment content:

Experiment 1:

Conjugate 30ul SHD00 suspension with killred plasmid into LB medium with ampicillin antibiotics (6 tubes in total).

After 3 hours, obtain 3ul from each tube and add to two alternative tubes: One only consists LB liquid medium with 3ml ampicillin antibiotics; another one consists Lb medium with ampicillin antibiotics and 3ml 1ng/ml aTc with inducing expression.

Culture overnight and examine the OD600 tomorrow.

Experiment 2:

Send to sequence nitro fixing bacteria and blue algae.

Experiment 3:

Take photos

Implant 1ml blue algae to 4ml BG11 medium with sodium bicarbonate.

Obtain 1ml blue algae and examine OD600(0.130). Then take a photo.

Take another photo next week.

Experiment 4:

Observe the E.coli conjugated yesterday and examine the OD600

If the cell suspension is diluted, conjugate it on the LB plates with ampicilin antibiotics only.

If the cell suspension is concentrated, conjugate 100ul into 4ml LB with 100uM sodium chlorite and Anhydrotetracycline Hydrochloride.

Experiment 5:

PCR: 27F 1492R

Streak blue algae on the plates.

1-1，1-2，2-1，2-2，3-1，3-2

Then send for sequencing

2024/08/26

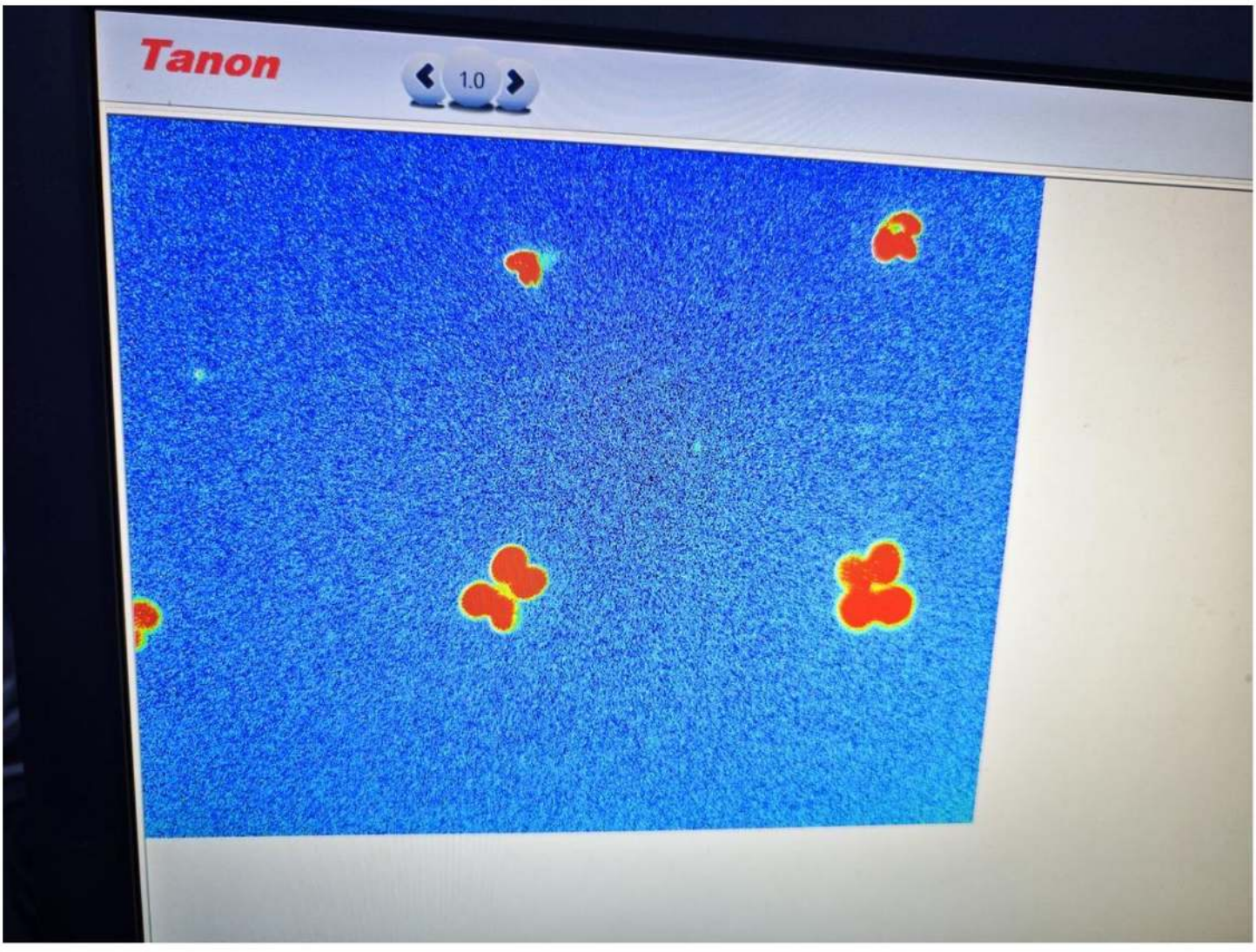
Check the transformants of nerol,limonene, lycopene synthesis cassette by PCR

1. M5 mix treatment
2. PCR

Inoculate correct colonies

Construct plasmid for cscB

Construct plasmid for katG



2024/08/27

Send for sequencing

Affect of Chlorite to plant growth

2024/08/29

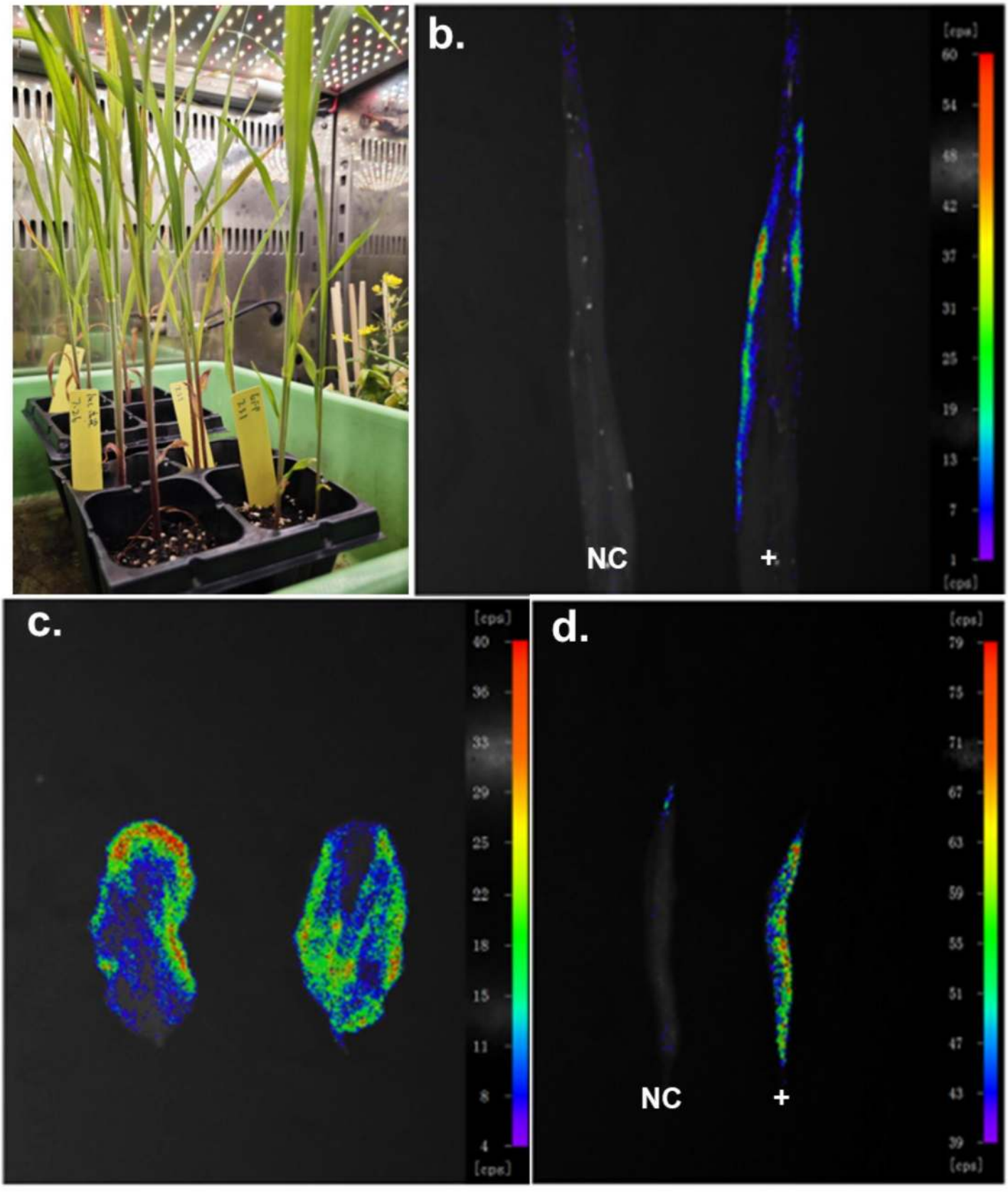
Inoculate correct plasmid of cscB and katG

Transformed sorghum

2024/08/30

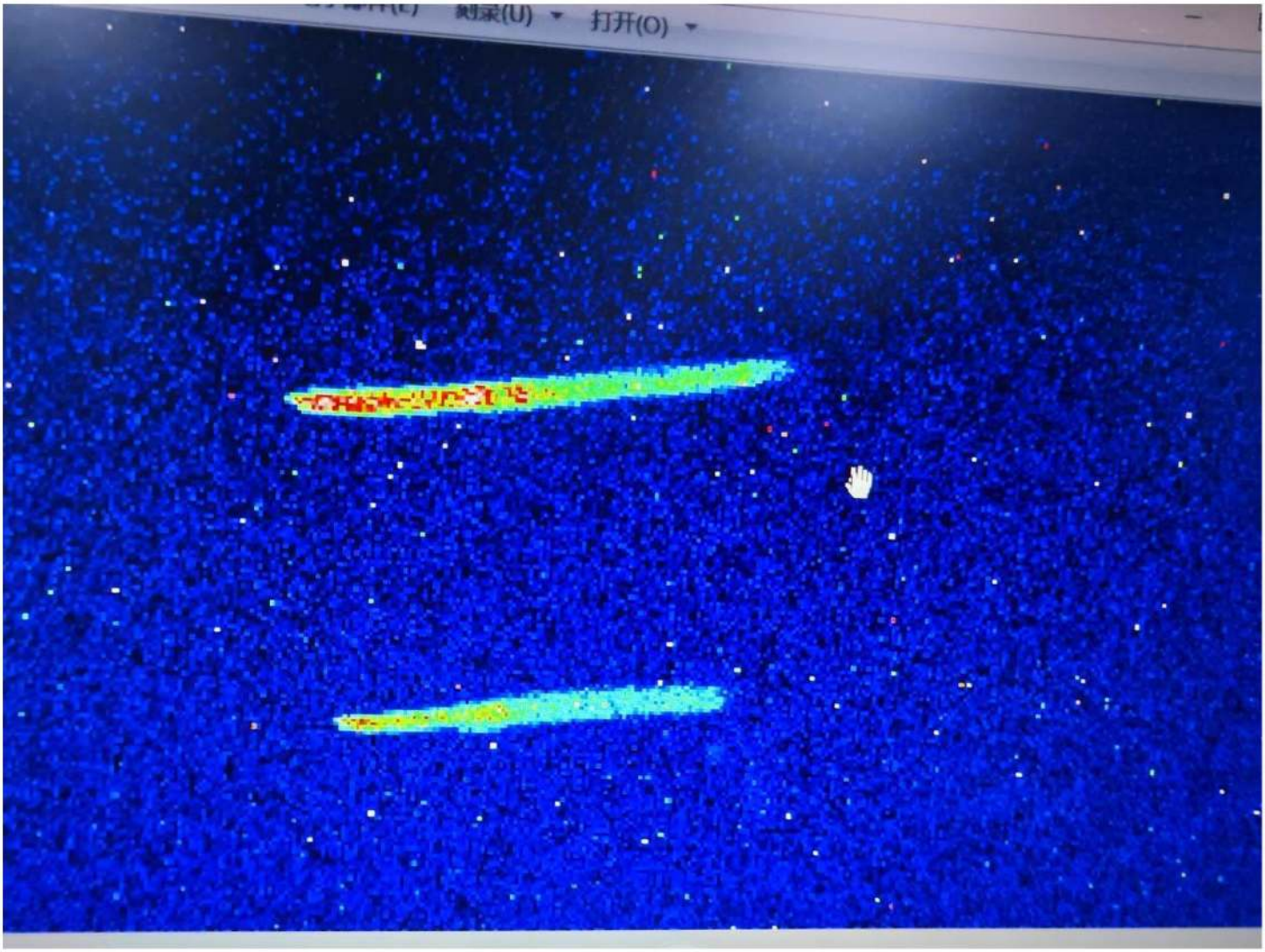
Test lycopene, nerol and limonene by different absorbency

Minipre, transinto cyanobacteria prepared



2024/09/08

CPCR to check engineered cyanobacteria, inoculate



2024/09/11

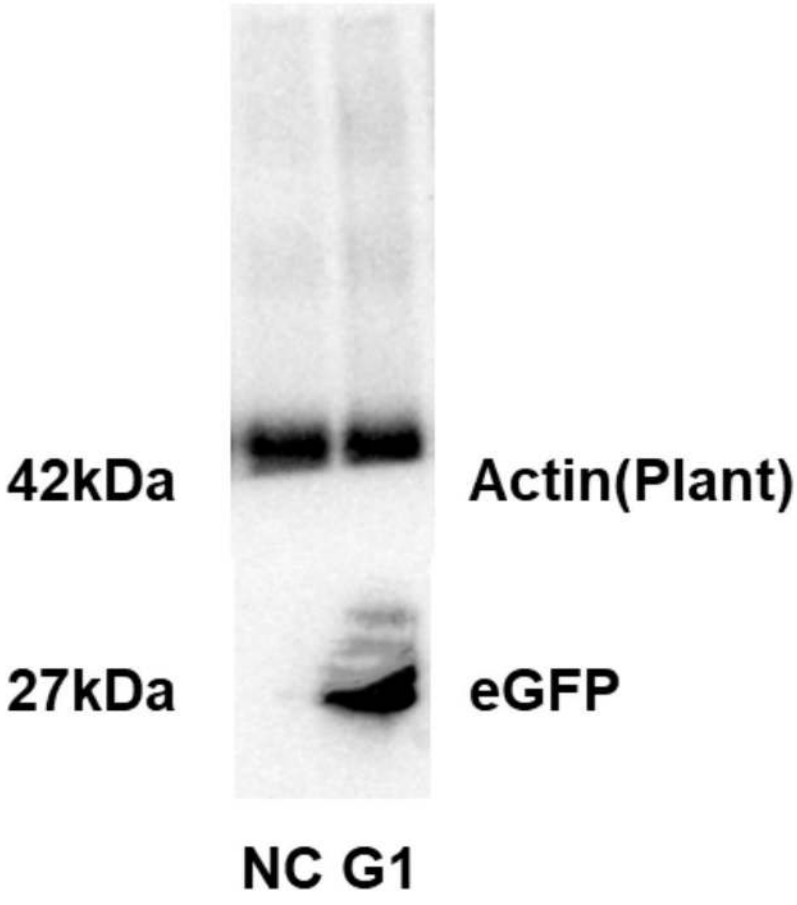
Inoculate successful construction of cscB, glgC, SP

2024/09/12

Minipre, transinto cyanobacteria prepared

2024/09/09-16

Test A600 of cynaobacteria



2024/09/18

CPCR to check engineered cyanobacteria

2024/09/20

Inoculate engineered cyanobacteria

2024/09/27

Test sucrose