Construction of recombinant plasmid pMTL-*Pthl-F/Xpk(QS)*

1. **The fragment *F/Xpk(QS)* was obtained by PCR amplification on *Bifidobacterium adolescentis* genome**
2. **Linearized vector pMTL-*Pthl* was obtained by PCR amplification using plasmid pMTL82151-*Pthl-adhE2* as template;**
3. **pMTL-*Pthl-F/Xpk(QS)* was obtained by Gibson assembly**

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Recorder：Huaize Zhu

17/07/2023 Monday

Lab Goal: Transplanting, Storing the Final Product

- Cultivate Escherichia coli that successfully accepts the target gene
- Cryopreservation (-23°C) strain
- Plasmid extraction

Steps-*Transplanted strains*:

1. In the biosafety cabinet, transfer 4ml of liquid LB nutrient medium to 12ml shaking tube, and then add one thousandth (4ul) of Chloramphenicol
2. Use the pipette gun and scrape as much bacteria mud (the verified strains) on the Petri dish with the gun head.
3. Throw the tip of the pipette gun into the shaker tube, then keep it in shake bed for 12 hour.

Steps-*Cryopreservations*:

1. Add 900 ul of glycerol
2. Add 900 ul of bacterial fluid
3. Store them in the refrigerator (-23°C)

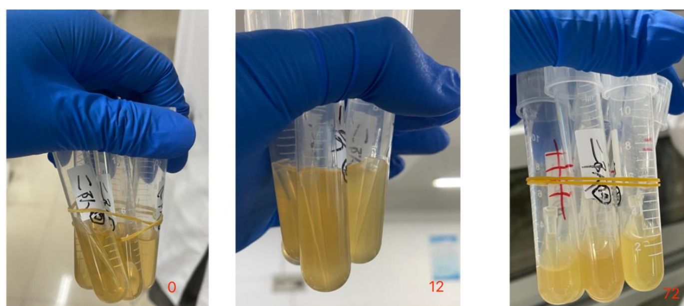
Steps-*Plasmid extraction*:

1. Take 5 ml of bacterial solution and add it to a 1.5ml centrifuge tube. Centrifuge at 10000 rpm for 1 minute. Then abandon the supernatant.
2. Add 250 ul of Buffer P1 to the tube, then shake. (The pH value of the solution can be adjusted to maintain it within an appropriate range by Buffer P1)
3. Add 250 ul of Buffer P2 to the centrifuge tube, then Invert and mix 8-10 times. Shaking would break the DNA (Buffer P2 is a strong alkaline solution which fully lyse the bacterial body)

4. Add 350 ul of Buffer P3 to the centrifuge tube, then invert and mix 8-10 time immediately. (Buffer P3 neutralizes Buffer P2)
5. Centrifuge the solution, then transfer the supernatant into the absorbing column. Then place the absorbing column into the collection tube.
6. Centrifuge the solution, and discard the liquid in the collection tube. (12000rpm for 1minute)
7. Add 500 ul of Buffer Pw1 to the absorbing column, then centrifuge. (12000rpm for 1minute)
8. Discard the liquid in the collection tube, then add 600 ul of Buffer Pw2 with ethanol, and centrifuge. (12000rpm for 1minute)
9. Repeat Step 8
10. Discard the liquid in the collection tube, then centrifuge. (12000rpm for 1minute)
11. Discard the collection tube, and place the absorbing column into a new centrifuge tube. Then add 30 ul of double distilled water (on the membrane of the absorbing column)
12. Centrifuge (12000rpm for 1minute).

Results-*Transplantation*:

The following figures shows bacteria fluids after 0, 12, 72 hours of cultivation in the shake bed.



Results-*Cryopreservations*:

The following figures demonstrates the final status of the bacterias.

Results-*Plasmid extraction*:

Following figure shows the concentration of the plasmic in (ng/ul)

18/07/2023 Tuesday

Lab goal: Preparation/ Plasmid building and transcription

- LB nutrient medium
- PCR DNA fragment&Verify&Gel Recovery
- Plasmid construction (Connecting Vector and Fragment)
- Competent state

Steps-*Blending LB nutrient medium*:

LB nutrient medium specifically cultivates Escherichia coli. Solid nutrient medium cultivates bacteria in Petri Dish which clearly displays bacterias' status; on the other hand, Liquid nutrient medium cultivates a greater amount of bacteria compared to Solid Nutrient Medium.

Steps- *Preparation of LB nutrient medium*:

LB nutrient medium specifically cultivates Escherichia coli. Solid nutrient medium cultivates bacteria in Petri Dish which clearly displays bacterias' status; on the other hand, Liquid nutrient medium cultivates a greater amount of bacteria compared to Solid Nutrient Medium.

Solid LB Nutrient Medium

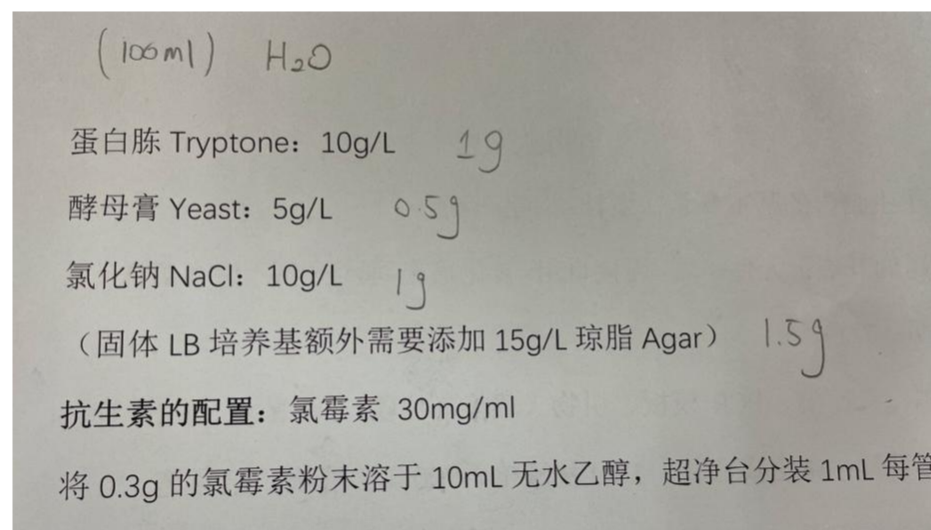
1. Add 1g of Tryptone, 0.5g of Yeast, 1g of NaCl, and 1.5g of Agar into the conical flask
2. Add 100ml of water, then gently shake the solution.
3. Seal the flask with news paper
4. Autoclave the solutions

5. Mix 0.3 grams of chloramphenicol and 10ml of anhydrous ethanol in another conical flask.
6. Separate the solution using filter needles (1ml per centrifuge bottle)
7. Remove the sealed newspaper.
8. Dissolve 100ul of antibiotics (Chloramphenicol) into the Nutrient Medium. (Antibiotics:Solution=1:1000)
9. Evenly distribute the nutrient medium into three petri dishes.
10. Wait for 20 mins for nutrient Medium to cool, then invert the petri dish to prevent condensate water from dropping.
11. Wrap Petri dish with plastic wrap.

(Step 7-10 needs to be done in the Biosafety Cabinet)

Liquid LB Nutrient Medium

Repeat steps 1 to 8, **BUT DO NOT ADD ANY AGAR**.


(100ml) H_2O

蛋白胨 Tryptone：10g/L 1g

酵母膏 Yeast：5g/L 0.5g

氯化钠 NaCl：10g/L 1g

（固体 LB 培养基额外需要添加 15g/L 琼脂 Agar） 1.5g

抗生素的配置：氯霉素 30mg/ml

将 0.3g 的氯霉素粉末溶于 10mL 无水乙醇，超净台分装 1mL 每管

Escherichia coli grows better in aerobic situations, so the bacterial liquid are usually placed in the shaker.

Steps-*Polymerase Chain Reaction (PCR) Fragment*:

1. Deploy the system according to the procedure (50ul)

2. 22 ul of Taq enzyme, 1ul of templated DNA, 1 ul of forward primer, 1 ul of reverse primer, and 22 ul of double distilled water.
3. Program and run PCR (30 cycles)

Steps-*Gel Electrophorus*:

4. Mix 0.6g of Agarose and 60ml of TAE solution. (Agarose:TAE=1:100)
5. Heat the Solution using microwave oven (Medium High/1min)
6. Connect the Gel-tank with comb (8 keys, each key creates 50ul trough) Then pour the solution to the tank.
7. Wait until the Gel is dry (Feels no heat when touching the bottom of the Gel-tank) Then gently remove the comb.
8. Place the Gel in the electrophoresis tank. Trough close to cathode (Black). Ensure that the gel is soaked in running buffer (use your hand)
9. Load Marker (2000bp), and sample.
10. Program and run Gel Electrophorus (110V/30mins)
11. Leave the machine running after visualizing little bubbles coming out of Cathode.

Steps-*Gel Recovery*:

1. Under E-Gel Imager cut off the part of the gel that contained the target DNA. Remove as many gel as possible to seek for higher concentration.
2. Transfer the gel that contained the target DNA into centrifuge tube.

3. Measure the weight of the gel. Then add x ul of Buffer GDP. (xng=xul)
4. Heat the centrifuge tube using water bath to melt the gel. (55 °C; 7mins)
5. Place DNA Mini Absorbing Columns-G inside the Collection Tube.
6. Transfer the melt Gel and GDP solution into the Absorbing Column (700 ul max)
7. Centrifuge the solution (12000 rpm; 1min)
8. Discard the filtrate in the collection tube, then add 300 ul of Buffer.
9. Let the centrifuge tube stand for one minute, then centrifuge the solution (12000 rpm; 1min)

Steps-*Connecting Vector and Fragment*:

1. Calculate the system, then add all the components into a PCR tube.

	上游 1ul
	下游 1ul
	22ul

体系）
片段：bpx0.04=X1/浓度=Y1 ul
载体：bpx0.02=X2/浓度=Y2 ul
*X1、X2 要在 10~100ng 范围内

片段	Xul 1.6ul
载体	Yul 1.9ul
连接酶 buffer	2ul
连接酶	1ul
无菌水	Zul（补齐到 10ul）

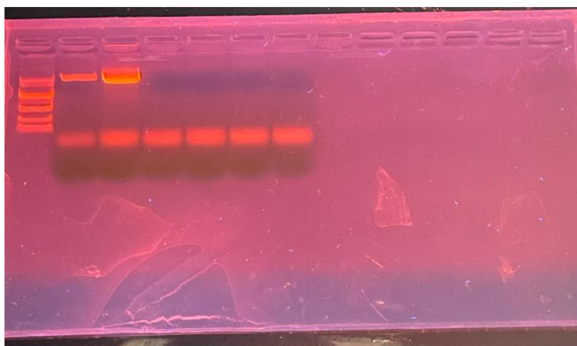
2. Place the tubes into the water bath (37°C /30mins) Product would be referred as “Target plasmid”
3. Steps-Competent State
4. Add 250 ul of cold Calcium Florine into the centrifuge tube.

5. Pick 5 bacteria colonies from the petri dish and add them to a centrifuge tube, stirring
6. Add 10 ul of Target plasmid into the centrifuge tube.
7. Place it on the ice for 15mins or more. At this stage the bacteria has already entered Competent state. Shaking, vibrating, or any severe action will kill the cell.
8. Using water bath, 42°C, 90second. Allow plasmid enter the cell.
9. Put it back onto ice for 2 mins or more. The bacteria are now back to normal stage.
10. Add 250 ul of liquid LB nutrient medium. Then place it into the table concentrator (37°C; 1hour)
11. Finally, we coated the bacteria onto the solid LB nutrient medium. Then, place it the incubator. (37°C; 21 hours)

Results-*LB nutrient medium*：

Results-*Gel electrophoresis*：

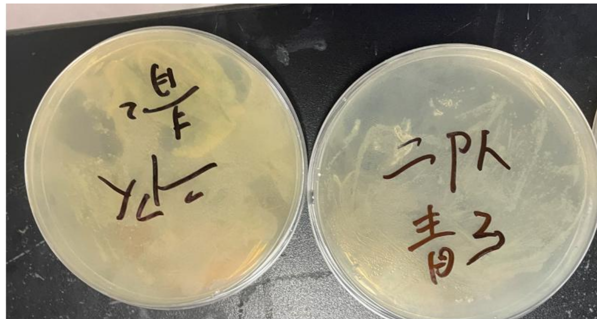
Successful (2/6)

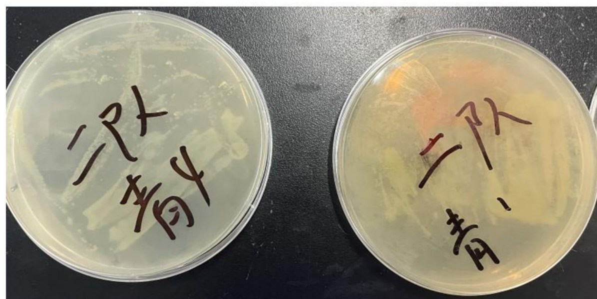


Results-*Competent state*

The pictures show that the bacterias are growing on the solid LB nutrient medium. This means that the stage is very close to successful because we didn't accidentally kill the cell during its competent state.

We will further verify using bacteria PCR and gel electrophoresis.





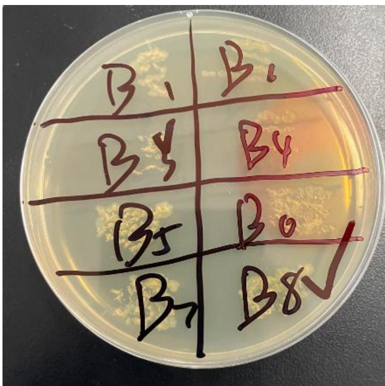
19/07/2023 Wednesday

Lab goal: Verify the final product

- Bacteria PCR
- Gel Electrophoresis

Steps-*Bacteria PCR & Gel Electrophoruses*:

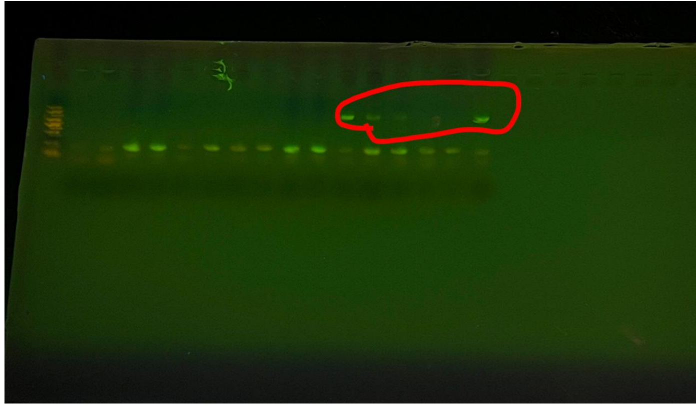
1. Coordinate the system according to the instruction in a PCR tube.
2. Using pipette gun pick 1 bacteria colony from the petri dish and transplant them onto another (Marked)



3. Move pipette gun, solid LB nutrient medium, PCR tube into biosafety cabinet. Then, prob the pipette gun into the PCR tube, mix well.
4. Program and run PCR.
5. After PCR program, carry out gel electrophoruses to verify the success of Plasmid transcription.

Results-*Bacteria PCR & Gel Electrophoruses*

Successful (4/16) A decent amount of bacteria accepted the target DNA we intended to import.



20/07/2023 Thursday

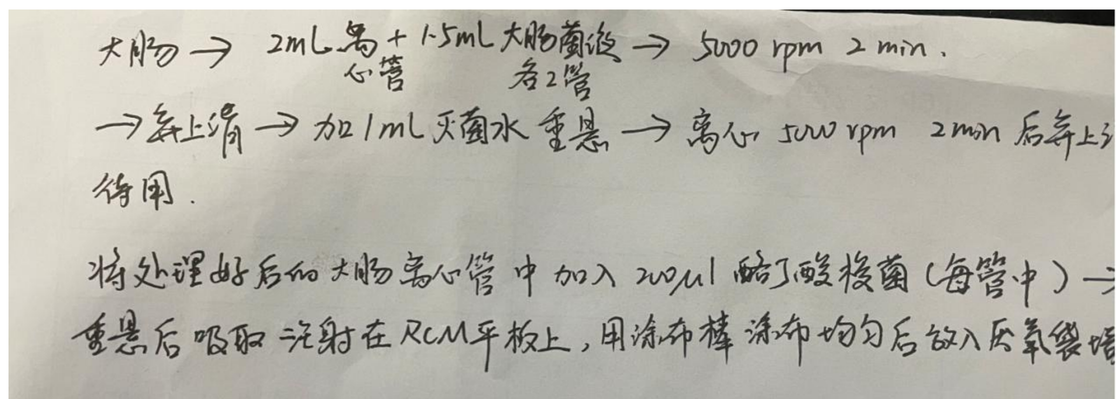
Lab goal: Transplant the final product

- Transplant the final product

Steps-*Transplanted strains*:

1. In the biosafety cabinet, transfer 4ml of liquid LB nutrient medium to 12ml shaking tube, and then add one thousandth (4ul) of Chloramphenicol
2. Use the pipette gun and scrape as much bacteria mud (the verified strains) on the Petri dish with the gun head.
3. Throw the tip of the pipette gun into the shaker tube, then keep it in shake bed for 12 hour.

Steps-*Conjugation transformation*：



大肠 → 2mL离心管 + 1.5mL大肠菌液 各2管 → 5000 rpm 2 min.

→ 弃上清 → 加1mL灭菌水重悬 → 离心 5000 rpm 2min 后弃上

待用.

将处理好后的大肠离心管中加入200ul 酪丁酸梭菌（每管中）→

重悬后吸取 注射在RCM平板上，用涂布棒涂布均匀后放入厌氧袋

Results-*Final product* ：

Even though Electrophoresis results show that there are four strains accepted the target plasmid, we choose to transplant the two with the brightest marks on the Gel.