iGEM_BIT Genetic Engineering Experimental Procedure

A-B-C-F-D-F-E-B-C-D-F

A. Medium Preparation

1	Preparation of Liquid LB Medium	① calculate the required volume of liquid culture medium and the quality of the added substances; ②take a conical flask; ③ add 1% NaCl, 1% peptone, 0.5% yeast extract, and the required volume of deionized water; ④ remove the sub-instrument and sub-tubes marked with LB; ⑤ dispense liquid medium, small tubes carry 5mL, large tubes carry 7mL.
2	Preparation of Liquid M9 Medium	① calculate the required volume of liquid culture medium and the quality of the added substances; ②0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4CL, 0.05%$MgSO_4$, 0.2% glucose, 0.0015% $CaCl_2$.
3	Preparation of Solid LB Medium	① calculate the volume of solid culture medium required and the quality of the added substance; ②take a conical flask; ③ add 1% NaCl, 1% peptone, 0.5% yeast extract, 1.5% agar and the required volume of deionized water; ④ seal the conical flask with a sealing film.
4	High Temperature Sterilization	① open the high pressure steam pot; ② make sure the water is over the hole; ③ put a iron basket in the pot, then place the medium in the iron basket, and covered with iron cover; ④ close the steam pot, close the exhaust valve; ⑤ adjustment mode for the "sterilization", press the start button 4s; ⑥ sterilization at least 2h.

5	Cool Down	① close the high pressure steam pot; ② check the air pressure is reduced to 0, if not, then continue to wait; ③ if so, then open the exhaust valve, open the steam pot when the gas is released; ④ place the liquid medium in the beaker, stored at room temperature; ⑤ put solid medium on the table to cool; ⑥ Wait for the solid medium to cool to 60 ° C.
6	Add Antibiotics	① calculate the amount of chlorogenic or ampicillin, add chloramphenicol 2 ‰, or ampicillin 1 ‰; ② remove and ignite the alcohol lights; ③use sterile tips to add antibiotics in the medium beside the alcohol lights.
7	Preparation of the Solid Culture Dishes	① next to the alcohol lamp, open the culture dishes; ② gently pour the solid medium liquid to one half of the volume of a solid culture dish; ③ shock culture dishes lightly to make the liquid evenly distributed; ④ repeat steps to complete all solid medium.
8	Refrigeration	① at room temperature, standing for about 20min; ② after the solid medium is solidified, wrap these dishes up ③ inverte in the 617 room 4 ℃ freezer storage.

B. Plasmid Transformation and Cultivating

1	Prepartation	② take the foam box, carrying about 2/3 volume of ice; ② remove 100μL competent cells from the -80 ℃ refrigerator.B
2	Packing	① remove the alcohol lights, tweezers, sterile centrifuge tube and sterile tip; ② ignite the alcohol lights, burning the tweezers 5s on it;

		③ remove the 1.5mL sterile centrifuge tube, cover the tube cover and put it on the tube box; ④ open the centrifuge tube with competent cells, remove 50μL competent cells into the sterile centrifuge tube; do these beside the alcohol lights; ⑤ put the above two centrifuge tubes equipped with competent cells in the ice box in the spare.
3	Adhesion	① use a sterile tip to remove 5μL plasmid on the plasmid tray, add it 50μL competent cells; do these beside the alcohol lights, ② open the water bath, and set the temperature of 42 ℃; ③ put the centrifuge tube in the ice box, standing 30min;
4	Heat Shock	① put the ice-bath-after centrifuge tube in 42 ℃ water bath, heat shock 45s; ② Remove the centrifuge tube, placed in the ice box 2min.
5	Recovery	① remove the non-antibiotic liquid medium; ② use sterile tip to remove 500μL liquid medium and add to the centrifuge tube which has been heat shocked; ③ put the centrifuge tube into the 37 ℃ shaker with the tube pad, incubated 1h.
6	Coat Dishes	① remove centrifuge tube from the shaker, centrifuge 7000rpm for 30s or 5000rpm for 1min; ② use sterile tips to abandon 400uL supernatant beside alcohol lights; use a pipette make remaining bacteria liquid well mixed; ③ according to plasmid's resistance, remove the chloramphenicol resistant solid medium or ampicillin resistant solid medium; ④ use sterile tips to take 100uL bacteria liquid mentioned in step 3 then add to the surface of solid medium beside alcohol lights; ⑤ apply the bacteria on the solid medium with a coated rake and spread it evenly, cover the culture dish; ⑥ inverted the solid medium, placed in 37 ℃ incubator, cultured 12h.
7	Picking Colony	① remove the non-antibiotic liquid medium, absorb antibiotics, added chloramphenicol 5 ‰ or ampicillin 1 ‰; ② tilt the liquid medium to add antibiotics;

		③ open the solid medium, looking for a single colony, then use a tip to row the colony, and put the tip into the liquid medium; ③ place it in the 37 ℃ shaker, culture 12h.

C. Mini Plasmid

1	Preservation of Bacteria	① remove and ignite the alcohol lights; ② folder 1.5mL centrifuge tube with a pair of tweezers and cover the tube cover; ③ take 700μL bacteria from the liquid medium into the 1.5mL centrifuge tube beside alcohol lights; ④ take 700μL glycerol into the 1.5mL centrifuge tube; ⑤ place the centrifuge tube in -20 ℃ refrigerator.
2	Pretreatment	Column equilibration: Place a Spin Column CP3 in a clean collection tube, and add 500 µl Buffer BL to CP3. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Discard the flow-through, and put the Spin Column CP3 back into the collection tube. (Please use freshly treated spin column).
3	Collect Bacteria	Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 × g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15- 25°C), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained (For large volume of bacterial cells, please harvest to one tube by several centrifugation step.)
4	Remove RNA	Re-suspend the bacterial pellet in 250 µl Buffer P1 (Ensure that RNase A has been added). The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.
5	Lysis of Cells	Add 250 µl Buffer P2 and mix gently and thoroughly by inverting the tube 6-8 times.

6	Remove Protein	Add 350 µl Buffer P3 and mix immediately and gently by inverting the tube 6-8 times. The solution should become cloudy. Centrifuge for 10 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Transfer the supernatant from step 5 to the Spin Column CP3 (place CP3 in a collection tube) by decanting or pipetting. Centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through and set the Spin Column CP3 back into the Collection Tube.
7	Wash	Wash the Spin Column CP3 by adding 600 µl Buffer PW (ensure that ethanol (96%-100%) has been added) and centrifuge for 30-60 s at 12,000 rpm (~13,400 × g). Discard the flow-through, and put the Spin Colum CP3 back into the Collection Tube. Repeat the previous step . Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual wash Buffer PW.
8	Collection of Plasmids	Place the Spin Column CP3 in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Buffer EB to the center of the Spin Column CP3, incubate for 2 min, and centrifuge for 2 min at 12,000 rpm (~13,400 × g).

D. Digestion

1	Preparation	① Take the PCR tubes; ② add 20 µL of the digestion system: 10 µL of the plasmid solution, 7 µL of sterile water, 2 µL of Buffer 2.1, 0.5 µL of enzyme A and 0.5 µL of enzyme B.
2	Digestion	① shock the PCR tube lightly to mix the digestion system up; ② use sealing film to seal the PCR tube, place the tube in a tube pad; ④ place in the 37 ℃ incubator, digested at least 2h.

E. Enzyme Linked

1	Preparation	② add 2 μL of 5 × Buffer, 0.4 μL of T4 ligase, 2 μL of the carrier solution and 6μl of the target gene solution;
2	Enzyme Linked	① shock the PCR tube lightly to mix the enzyme linked system up; ② Place the PCR tube in the PCR tube pad; ③ take the foam box, carrying about 2/3 volume of tap water; ④ place the PCR tube pad in water for 0.5h at 16℃.

F. Electrophoresis

1	Preparation	① take a glass bottle; ② measure 30mL 1 × TAE buffer into the bottle; ③ weighing 0.3g agarose added to the bottle; ④ use microwave oven heating about 1min to make the liquid boil; ⑤ add 3μl of Genecolor, shake the bottle to mix up; ⑥ remove the plastic box, put in the plastic sheet, insert the comb, pour the gel solution into the box; ⑦ at room temperature conditions, standing for more than 20min to make the gel solution to curdle;
2	Electrophoresis	① remove the plastic sheet, and put into the TAE buffer in electrophoresis instrument; ②remove the marker from 4 ℃ refrigerator, extract 3μL to inject into the first hole; ③ extract plasmid to inject into the PCR tube, add 1/5 plasmid's volume of 6 × DNA loading Buffer, mix up and inject into the gel hole, make sure the number was recorded; ④ open the electrophoresis device, adjust voltage to 110V, running 35min.
3	Check the Results	①turn off the electrophoresis instrument, remove the plastic sheet;

		② use E-Gel™ Imager System to get the results; ③ analysis and save the image; ⑤ turn off the device; ⑥ throw the gel need not recycle into the trash, clean the plastic box and plastic sheet, put back in the drawer;
4	Calculation	Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the volume. For example, 100 mg of gel is equivalent to a 100 µl volume. Transfer the gel slice into a 1.5 ml microfuge tube.
5	Melting the Gel	Add a 3x sample volume of Buffer DE-A. Resuspend the gel in Buffer DE-A by vortexing. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). Heat at 40°C if low-melt agarose gel is used. Intermittently vortexing (every 2-3 minutes) will accelerate gel solubilization.
6	Combination	Add 0.5x Buffer DE-A volume of Buffer DE-B, mix. Attach the vacuum manifold to a vacuum source. Position a Miniprep column securely into one of the complementary fittings. Transfer the binding mix from Step 4 to the Miniprep column(s). Switch on the vacuum source and adjust the negative pressure to -25-30 inches Hg. Continue to apply vacuum until no liquid remains in the Miniprep column.
7	Wash	Pipette 500 µl of Buffer W1 into the Miniprep column(s). Draw all liquid through the column(s).
8	Remove Salt	Pipette 700 µl of Buffer W2 along the wall of the Miniprep column(s) to wash off all residual Buffer W1. Draw all liquid through the column(s) . Repeat this wash step with a second 700 µl aliquot of Buffer W2. Transfer the Miniprep column into a 2 ml microfuge tube (provided) and centrifuge at 12,000xg for 1 minute to purge residual Buffer W2 from the binding membrane.
9	Collection	Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.