1 Molecular Cloning

1.1 *E. coli* transformation

1.1.1 Strains, materials

E. coli DH5α competent cells (TaKaRa, Japan), SOC medium, LB solid medium (with antibiotics), sterile ddH_2O

1.1.2 Procedures

1) Take out DH5*α* competent cells (100 μL) from the ultra-low temperature freezer (-80 ℃), melt it with fingers immediately and insert it into the ice. Bathe in ice for 5~10 min, and adjust the temperature of the constant temperature water bath to 42℃.
2) Add 5 μL plasmid (no more than 100 ng of DNA), shake gently and place on ice for 20 minutes.
3) Gently shake well and incubate the tubes at 42 ℃ water bath for 55 s, then quickly put them back into ice for 2min and incubate them for 5 minutes at room temprature.
4) Add 500 μL SOC to each tube and mix them gently, then fix them on the spring frame of the shaking table and shake them at 37℃ for 1 h at 120 rpm/min.
5) Take 100 ～300 μL of the above conversion mixture, and spread respectively into the solid LB plate containing antibiotics evenly with glass coating rod.
6) Slowly add the samples into each tank with a micropipette with a volume of about 25 μL. Take care to avoid the overflow of samples and contamination of adjacent samples.
7) Mark the coated petri dish and place it in a constant temperature incubator at 37℃ for 30~60 min until all the liquid on the surface permeates into the culture medium. Then turn it upside down and put it in the incubator at 37℃ overnight.

1.2 Plasmid extraction

1.2.1 Materials

LB culture medium、MiniBEST Plasmid Purification Kit Ver.4.0 (TaKaRa, Japan)

1.2.3 Procedures

1) Select a single colony from the selective medium plate and transfer it to LB liquid medium containing corresponding antibiotics, and incubate it at 37℃ overnight (no more than 16 h).
2) Take 1 mL of bacteria solution into a 1.5 mL centrifuge tube and centrifuge at 12000 r/min for 1min, then remove the supernatant. After that, take 1 mL of bacteria solution into a 1.5 mL centrifuge tube and centrifuge at 12000 r/min for 1 min again. Repeat three times to obtain thallus from 3 mL of bacteria solution.
3) Follow the instructions of plasmid extraction kit, for the next steps. (https://www.takarabiomed.com.cn/ProductShow.aspx?m=20141215114017640217&productID=20141226133731220739)。

1.3 DNA agarose gel electrophoresis

1.3.1 Equipments

Horizontal electrophoresis tank, electrophoresis apparatus, rubber mold, sample comb, electric furnace, and triangular bottle (50 mL)

1.3.2 Materials

Agarose, GoldView™ nucleic acid dye (Solarbio, China), TAE buffer.

1.3.3 Procedures

1) Place the mold on the surface of the table and insert the sample comb into the electrophoresis tank. At this time, the sample comb should be about 1 cm away from one end of the mold, with a gap of 0.5-1 mm between the bottom edge of the comb teeth and the surface of the mold.
2) Weigh 0.25 g of agarose in a triangular flask and add 25 mL 1×TAE buffer to configurate 1% gel. Heat the agarose solution on an electric furnace until it is completely melted and shake gently.
3) When agarose solution is cooled to about 65 ℃, add 2.5 μL GoldView™, mix slowly, and then carefully pour the solution into the rubber mold (note to avoid bubbles). The gel is slowly expanded to form a uniform rubber layer about 3 mm thick on the surface of the mold, and left for 0.5-1 h at room temperature.
4) After complete solidification, gently pull out the sample comb with both hands with equal force (be careful not to break the sample groove), then separate sample

grooves were formed on the rubber mold.

5) Place the gel along with the gel mold on the platform of the electrophoresis tank. Pour TAE buffer till it immerses the gel surface for 2-3 mm. Carefully remove the bubbles from the sample tank.
6) Slowly add the DNA samples into each tank with a micropipette with a volume of about 25 μL. Pay attention to avoid the overflow of samples and contamination of adjacent samples.
7) After adding the samples, connect one end near the sample tank to the negative electrode and the other end to the positive electrode, switch on the power and start the electrophoresis. A slightly higher voltage can be used to prevent the sample from spreading before the sample is injected into the gel. After the sample is injected into the gel, the voltage should not be higher than 5 V/cm (voltage value divided by the distance between the two ends of the electrophoresis tank).
8) When the dye bands move to about 1 cm from the front of the gel, stop the electrophoresis.
9) After electrophoresis, push the gel plate to the plastic film which is pre-soaked and spread on the ultraviolet observation table. Then observe under the ultraviolet lamp with a wavelength of 254 nm. Take photos of and record the electrophoresis results. Determine the molecular weight of the tested samples according to the corresponding standard DNA bands.

1.4 Double digestion of plasmid DNA

1.4.1 Materials

Restriction enzyme, sterile ddH_2O

1.4.2 Procedures

Reaction System：

Component	Volume (μL)
*Eco*R I	0.5
Bgl II	0.5
10x H Buffer	2.0
plasmids DNA	10.0 (about 1.0 μg)
ddH_2O	7.0
Total	20.0

37℃ water bath insulation for 4 h.

1.5 DNA extraction

1.5.1 Materials

DNA agarose Gel and SanPrep Column DNA Gel Extraction Kit (Sangon Biotech)

1.5.2 Procedures

See operation instructions of SanPrep Column DNA Gel Extraction Kit （https://www.sangon.com/productDetail?productInfo.code=B518131#）

1.6 DNA ligation reaction

1.6.1 Reaction System

Component	Volume (μL)
T4 DNA ligase	2.0
T4 DNA ligase buffer	2.0
vector	3.0 (about 100 ng)
insert DNA	6.0 (about 200 ng)
ddH_2O	7.0
Total	20.0

16℃ overnight ligation.

1.7 Transgenic experiment of microalgae

1.7.1 Equipments

Gene Pulser II (BioRad, USA)

1.7.2 Materials

TAP medium, 40mM sucrose-tap solution, plasmid, TAP plate containing antibiotics

1.7.3 Procedures

1) Dilute the preculture *C. reinhardtii* solution, and culture in 30 mL of TAP medium for 2 to 3 days, and continuously observe the cell concentration.
2) When the cell concentration reach 1×10^6 -2×10^6 cells/mL, centrifuge the cells at 2500 rpm for 5 min to harvest the cells, carefully remove the supernatant.
3) Use 10 mL TAP-40 mm sucrose solution to re-suspended the cells, centrifuge at 2500 rpm for 5 min, and remove the supernatant was carefully and quickly.
4) Repeat steps 3 again.
5) Use 40 mM sucrose-TAP solution to re-suspended the cells to achieve cell concentration of 1×10^8 cells/mL。

6) Add 250 µL cell suspensions and 1-2 µg plasmid DNA to the electric shock cup with a 4 mm gap in the middle. Transfer the cup to a 16°C tank and let sit for 5 min.

7) Electrocute the cells and, after electroporation, set the cells in room temperature on the worktable for 15 min. The parameters were set as follows:

Gene Pulser II Parameters (BioRad):

Voltage	Capacitance	Resistance
1000 V	50 µF	800 Ω

8) Transfer the cells to a flat bottom glass tube containing 10 mL 40 mM sucrose-tap solution at room temperature. Place the cells in an algal chamber under dark light and culture at 120 rpm for 14-16 h.

9) Centrifuge the cells at a speed of 2500 rpm for 10 min. Discard the supernatant and precipitate and re-suspend cells in a TAP medium of 200 µL. Coat the cells on TAP agar plate containing paromycin and culture in dark light for 3-5 days in an algal chamber. Switch to high-light culture when clones grow on the plate.