7.6 Experiment Report（A）

Experiment 1: 1% Agarose Gel (50ml) Electrophoresis Detection

I. Experimental purpose: Check the success of PCR

II. Experimental principle: There will be bands in the running glue in molecular biology. The speed of the charged biological macromolecules in the electric pool is determined by the molecular weight of the molecule. Therefore, the biological macromolecules of the same molecular weight gather together to form a band, and then realize The purpose of separation and purification of biological macromolecules.

III. Experimental procedure:

1. Glue preparation: Take 0.5g of agarose in a 500-mL Erlenmeyer flask, add 50ml of 1× TAE solution, and heat it in a microwave oven to make all the agarose dissolve;
2. Shake evenly, add 5 μl 10000× GelRed, pour the solution into the gel box inserted into the comb, and wait until the gel solidifies;
3. Adding samples: Take PCR solution (50μl), add 10μl 6× loading buffer, mix well and add to the gel well. At the same time, load the marker for comparison.
4. Electrophoresis: 120V, 30min;
5. Observe and record the results with the Light Transilluminator.

IV. Result: No obvious bands were detected.

V. Analysis: The experiment failure may be caused by the template dilution problem during the PCR solution configuration; it may also be caused by errors in the volume calculation of the 50μl PCR solution reagent configuration.

Experiment 2: Protein Expression

I. Experimental purpose: Cultivate DH5α competent cells containing recombinant plasmids and pick colonies to confirm successful recombination

II. Experiment procedure:

1. Remove DH5α receptor cells from -80°C and place on ice to melt.
2. Add 20 μl of recombinant plasmid product to the strain, flick the wall of the tube, place on ice for 30 min, heat excite in a water bath at 42°C for 45 s, and then place on ice for 2 min to cool.
3. Add 1000μl LB medium (without antibiotics), shake at 37°C, 220 rpm, and incubate for 60 min.
4. Centrifuge at 5000 rpm for 5 min at room temperature, discard 900 μl supernatant, and mix the remaining medium and cells by blowing.
5. Take 100μl of bacterial solution evenly coated on pre-warmed ampicillin-resistant (Amp+) plates and incubated overnight at 37℃ in an inverted incubator;
6. The next day pick monoclonal colonies in 20μl LB liquid medium and take 2μl for PCR sequencing of the bacterial broth to confirm successful recombination.
7. The remaining 18μl was added to 1ml Amp+ LB and incubated for 6-8 hours at 37℃ in shaker, 220rpm.

III. Result: Obvious bands appeared in the colloid, and the cells containing the recombinant plasmid were successfully cultivated.