

7.5 Experiment Report(B)

Experiment : B.1.1.7 Pseudo Virus Packaging--The Transient Transfection of Cell

I. Experimental purpose: packaging pseudo virus to prepare for other experiments

II. Experimental principle: N/A

III. Experimental procedure:

1. Prepare 293T cell with cell confluency of 50-70%, change the medium to DMEM with no serum present.
2. Prepare pcDNA3.1-spikeFL-B.1.17 (870ug/μl) plasmid and pNL4-3.luc.R.E plasmid (1112.9ug/μl);
3. Add 10ug pcDNA3.1-spikeFL-B.1.1.7 (870ug/μl) plasmid and 10ugpNL4-3.luc.R.E plasmid also 1ml DMEM medium in tube A. Add 40ug lipofiter3 reagent and 1mlDMEM medium in tube B;
Leave it for 5min.
4. Mix the solution in tube A and tube B until it is well proportioned then leave it for 20 minutes.
5. Add the mixture formed in step 4 into the cell prepared.

Experiment 2: PCR

I. Experimental purpose: preparation of fragment of gene with purpose for linear amplification

II. Experimental reagents: DNA model, 5X PS buffer, dNTPs, Prime STAR HS DNA polymerase, primers, ddH₂O.

III. Experimental principle:

1. PCR, polymerase chain reaction, is the most commonly used method for rapid amplification of specific genes or DNA sequences in vitro
2. Basic principle: First, the double-stranded DNA molecule is heated and separated into two single-stranded DNA molecules at a temperature close to the boiling point. The DNA polymerase uses the single-stranded DNA as a template and uses the four deoxynucleoside triphosphates in the reaction mixture to synthesize a new complementary DNA strand . During the PCR reaction, as long as the

template DNA, PCR primers, four nucleotides and an appropriate concentration of Mg^{2+} are added to the test tube, the DNA polymerase can amplify the target sequence by more than 1 million times within a few hours.

- (1) The double-stranded template DNA molecule first unwraps the long single strand at high temperature, and the short-stranded primer molecule immediately combines with the specific sequence at both ends of the template DNA to produce a double-stranded region.
- (2) DNA polymerase starts copying its complementary strand from the primer, and quickly produces a copy that is exactly the same as the target sequence.
- (3) In the subsequent reaction, whether it is the starting template DNA or the replicated hybrid DNA double-stranded, it will untie into a single-stranded at high temperature. The primer molecules in the system will combine with their complementary sequence again, and the polymerase will again Copy the template DNA.
- (4) Since the pair of primers selected in the PCR reaction are designed according to the principle of complementing the sequences at both ends of the amplified region, the synthesis of each nascent strand starts from the annealing binding site of the primer and goes in the opposite direction. In the direction of extension, each newly synthesized DNA strand has a new primer binding site.
- (5) The entire PCR reaction process, namely DNA melting (denaturation), primer and template DNA binding (annealing), and DNA synthesis (strand extension) can be repeated continuously. After many cycles, the number of double-stranded DNA molecules contained in the reaction mixture, that is, the number of copies of the DNA segment between the two primer binding sites, should theoretically be 2^n , which can further satisfy heredity The need for analysis.

3. Usage of reagents:

- (1) primers: the start point of DNA replication, aiming at the two edge of copying the parts of the DNA. There are 5'primers and 3'primers
- (2) Taq DNA polymerase: promote dNTPs and the DNA model to combine.
- (3) Buffer: Tris-HCl reaction buffer, Taq DNA polymerase provides a suitable condition for enzyme catalysis.
- (4) dNTPs: substrate, with the leading of primers it will form new DNA chain that is complementary with the model.

IV. Experimental procedure:

- Design 50 μ l system reagent configuration volume:

The configuration of each reagent in the 50 μ l system is as follows:

Reagent	H2O	Buffer	F Primer	R Primer	dNTP	T(model)1ng	E(Enzyme)
Volume/ μ l	32.85	10	1	1	4	1.15	0.5

- Add the reagents from the information provided upward with its specific volume to EP tube then shake it until it is well-proportioned, finally centrifugation.
 - Put PCR tube into PCR, set the time and temperature.
- (1) Warm start: 98°C, 30s

- (2) Denaturation step: 98°C, 10s。
- (3) Annealing step: 60°C, 5s。
- (4) Extension step: 72°C, 2min。
- (5) Step (2) ~ (4) thermal cycling for 30 periods
- (6) Retain the temperature: extend to 72°C, 1min
- Then use 1% agarose gel electrophoresis (60ml) to test

Experiment 3: Plasmid Transformation

I. Experiment purpose: Obtain a Monoclonal Colony

II. Experimental principle: N/A

III. Experimental procedure:

1. Use a pipette to extract 1 μ g of plasmid and add it to 100 μ l of competent cells, and flick to mix evenly;
2. Place the mixture of plasmid and competent cells on ice cubes and let it stand still for 30 minutes;
3. After heat shocking the mixture at 42°C for 45s, immediately place it on ice cubes and let it stand still for 2 minutes;
4. Add the mixture to 900 μ l of LB medium containing ampicillin;
5. Colony expansion: incubate in an incubator at 37°C at 200-250 rpm for 1 hour;