SARS-CoV-2 3CL Protease Gene Cloning

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Abstract

3CL protease (3C-like protease) is the main protease found in coronavirus (SARS-CoV-2) playing an important role in the processing of coronavirus replicase polyprotein [2]. Therefore, it is meaningful to study the 3CL protease and in this experiment we started with cloning the 3CL protease gene.

The major purpose of this experiment was to construct recombinant plasmid vector for future gene expression. So we started from amplifying desired DNA by PCR along with DNA separation and purification by electrophoresis. DNA quantification was needed before assembling the plasmid by Gibson assembly. The final step was to transform the bacteria (*E.coli* DH5α strain) with the plasmid vector to store and reproduce the expression vector.

In this report, I record the experimental methods with the detailed analysis of the results so as to set the cornerstone for future experiments and scientific enquiry.

Keywords: SARS-CoV-2, 3CL protease, PCR, Agarose-gel Electrophoresis, Gel Extraction, Gibson Assembly, Transformation.

1 Interpretation of the Plasmid

The components on the plasmid are important for experimental methods and processes, so I find it deserves a deeper look.

As shown in Figure 1, the 3CL protease gene should be inserted between **T7 promoter and T7 terminator**. However, a **Lac operator** [1] is placed in the upstream of 3CL protease gene, while **LacI gene** expressing **Lac repressor** is also present in the plasmid, which means the expression of 3CL protease gene will be repressed. Indeed, we transform the bacterial strain of **DH5α** which also lacks of **T7 polymerase**. That means in this step we focus on cloning the 3CL protease gene only without expression.

The presence of gene **KanR** brings resistance to **kanamycin** for bacteria, which also enables us to use selective culture medium.

2 Methods

2.1 DNA Amplifying: Polymerase Chain Reaction(PCR)

We used PCR to amplify both **desired DNA fragment** and the **plasmid vector**. Three groups for desired DNA fragment while the other three groups for plasmid vector. The mapping from number to their contents can be seen in Table 1. The forward primer, reverse primer and Phanta Max Master (containing DNA polymerase, dNTP...) were preserved at 0°C in ice. The common procedure for PCR is shown below.

Number	Content
1	Desired DNA
2	Plasmid Vector
3	Desired DNA
4	Desired DNA
5	Plasmid Vector
6	Plasmid Vector

Table 1: Samples and Their Contents

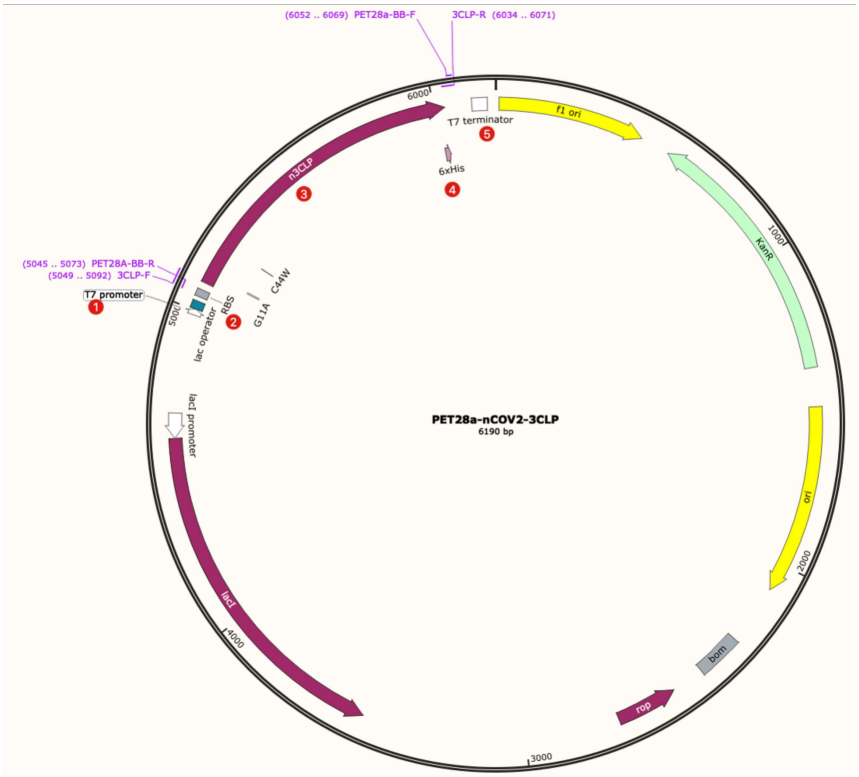


Figure 1: Desired Recombinant Plasmid

- 0.4 μL Forward Primer.
- 0.4 μL Reverse Primer.
- 5 μL Phanta Mas Master Mix.
- Add ddH_2O to 20 μL.

The setting of PCR device is shown below.

1. 98°C, 3 min
2. 98°C, 10 sec
3. 58°C, 15 sec
4. 72°C, 30 sec ($6 \times 10^3 \times 5\text{sec}/10^3 = 30\text{sec}$)
5. Go to step 2 for 33 times
6. 72°C, 5 min
7. 12°C, forever

2.2 DNA Separating: Agagrose-gel Electrophoresis

We prepared the agarose-gel first:

- Weight 0.5 g agarose to 50 mL TAE solution (Tris Base+Acetic Acid+EDTA). (Additional 5 mL TAE compensate for evaporation while heating)
- Boil it in microwave oven.
- Cold the boiled agarose at room temperature until touchable.
- Add in gel-safe dye "GelRed".
- After solidifying add the samples and marker with loading buffer containing blue visible dye(can indicate the frontier roughly).

After that we separated DNA in the gel rank under 150 V for 20 minutes then observed the result under UV light source. The result can be seen in Figure 3.

	Sample 1	Sample 3	Sample 4
Sample 5	(1,5)	(3,5)	(4,5)
Sample 6	(1,6)	(3,6)	(4,6)

Table 2: Combinatorial Arrangement of Samples

2.3 Gel Extraction

- Identify the fragments of interest according to the electrophoresis result under the UV light.
- Isolate the corresponding bands with a blade and weigh them using the scale. The results are shown in Table 3.
- Melt the agarose gel containing DNA at 90°C with equivalent PC solution.
- Add 500 μL BL solution for each spin column CB2 then centrifuge for 1 min at the speed of 12,000 rpm to balance the column. Pour the liquid.
- Add the melted gel to the spin column CB2 then centrifuge for 1 min at 12,000 rpm. Pour the liquid.
- Add 600 μL PW solution with 70% alcohol then centrifuge for 2 min at 12,000 rpm again. Pour the liquid.
- Centrifuge again to eliminate any existing PW solution. Pour the liquid.
- Add elution buffer EB and centrifuge for 2 min at 12,000 rpm. **Collect the solution containing DNA.**

2.4 DNA Quantification: UV Spectrometry

- Sample from the centrifuge tubes.
- Use the UV spectrometer to detect DNA concentration of them. The results are shown in Table 4 and in Figure 4

2.5 Assemble Plasmid: By Gibson Assembly

We calculated the dosage according to the result of DNA quantification as shown in Table 4.

- Desired DNA (3CL Protease Gene) 0.06 pmol.

$$0.04 \times 1650 = 66 \text{ ng}$$

- Plasmid Vector (pet21 bakcone) 0.03 pmol.

$$0.02 \times 5200 = 104 \text{ ng}$$

- Yeasen 2x Gibson mix 5 μL.
- Add ddH_2O to 10 μL.

Since the concentration in Sample 2 was too low to conduct following assays, we decided to abandon it. While we still had 6 groups which was a combinatorial arrangement of 3 groups of Desired DNA and 2 groups of Plasmid Vectors as shown in Table 2 ((1, 3, 4 are desired DNAs; 5, 6 are plasmid vectors)).

Then put them at 50°C for 20 min.

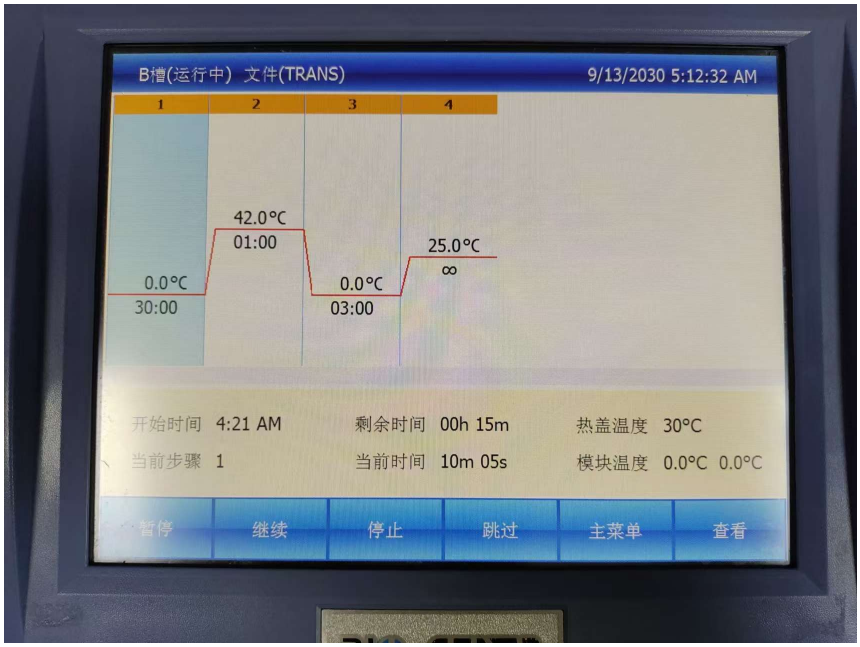


Figure 2: Transformation

Sample Number	Weight (g)
1	0.264
2	0.195
3	0.269
4	0.286
5	0.245
6	0.250

Table 3: Weights of Different Samples

2.6 Introduce Recombinant DNA into Cells: Transformation

- Dilute 5μL Gibson product with 15μL H_2O.
- Add 2$\sim$4 μL dilution to 50 μL DH5α competent cells just above the ice.
- Incubate at 0°C for 30 min.
- Stimulate with heat at 42°C for 1 min.
- Back to 0°C for 3 min.
- 25°C forever.
- Sample 20$\sim$50 μL bacteria to spread on the culture medium.
- Culture at 37°C .

The adjustment of temperature was also completed by PCR device as shown in Figure 2.

3 Experimental Results

The experiment of DNA amplifying was successful both for desired DNA (3CL Protease Gene) and for plasmid vector (pet28) except sample 2, with the result in Figure 4.

The reason why sample 2 failed to be amplified effectively may be the accidental addition of wrong primer which is designed for desired DNA instead of plasmid vector.

The defective amplifying of sample 2 can be deduced from quantification (Table 4 and Table 3).

As for the final result, we saw colonies in group (1,5), (4,5), (3,6) and (4,6). Despite zero colony in (3,5) and (1,6), we can still get enough amplified plasmids from the successful groups given all of the groups were operated in parallel.

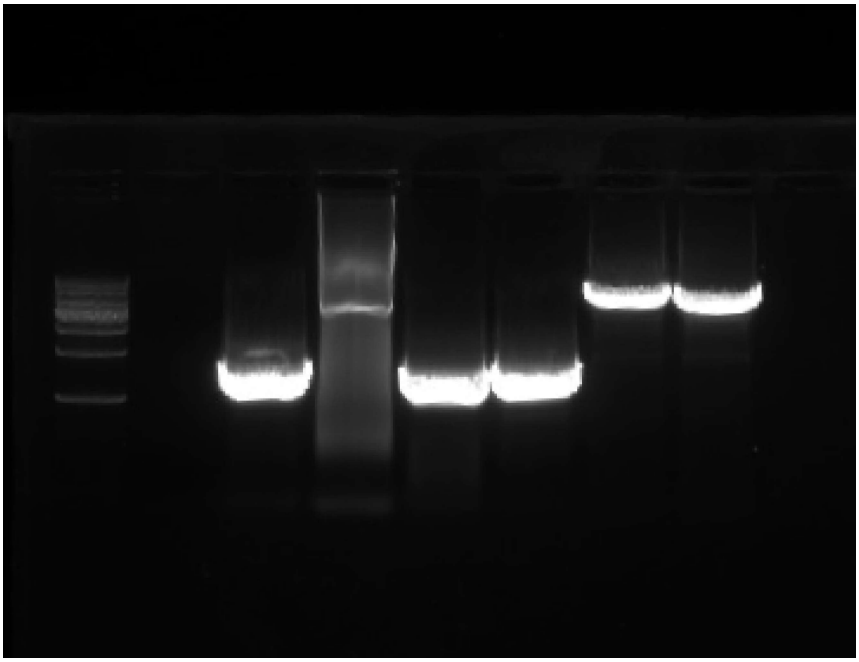


Figure 3: DNA Separation

(a) Sample 1

(b) Sample 2∼6

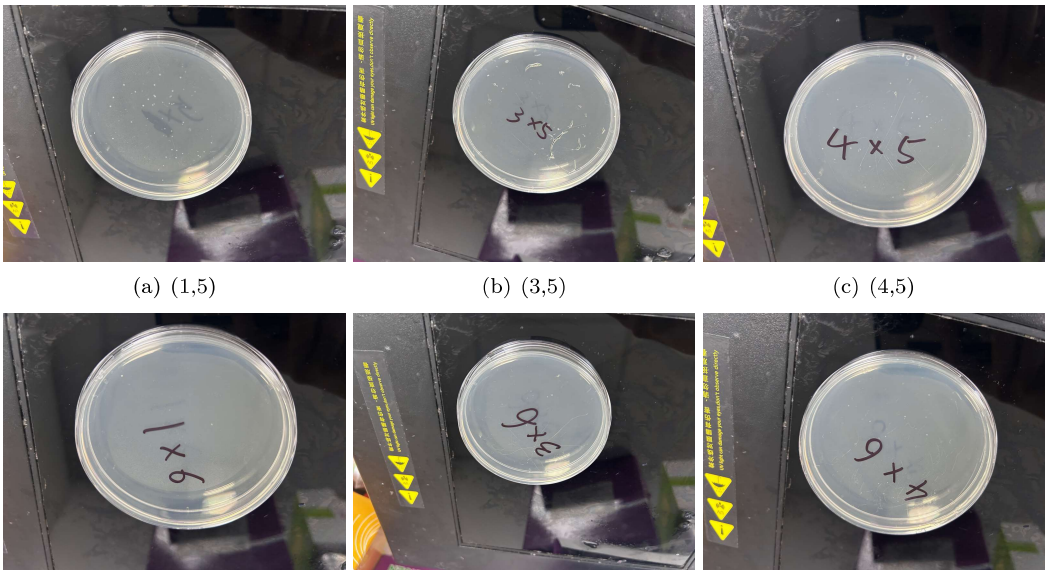
Figure 4: DNA Quantification

Sample Number	DNA Concentration (ng/μL)	Dosage for Gibson Assembly (μL)
1	199.7	0.33
2	10.2	Abandoned for Gibson Assembly
3	161.2	0.41
4	153.7	0.43
5	63.7	1.63
6	83.1	1.25

Table 4: Concentrations of Different Samples

	Sample 1	Sample 3	Sample 4
Sample 5	(1,5) Success	(3,5) Failed	(4,5) Success
Sample 6	(1,6) Failed	(3,6) Success	(4,6) Success

Table 5: Transformation Results



(a) (1,5) (b) (3,5) (c) (4,5)

(d) (1,6) (e) (3,6) (f) (4,6)

Figure 5: Results of Transformation

References

[1] Bruce Alberts, Rebecca Heald, Alexander Johnson, David Morgan, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. W. W. Norton and Company, 7th edition, 2022.

[2] 3C-like protease. `https://en.wikipedia.org/wiki/3C-like_protease`, 2024. Accessed: 2024-09-13.