Cloning Reference Gene rpoB in Serratia plymuthica UBCF_13

R Trivano¹, R Fatiah² and J Jamsari¹
¹Department of Agrotechnology, Agricultural Faculty, Andalas University, Padang, 25163, Indonesia.
²Biotechnology Department, Postgraduate Program, Andalas University, Padang, 25163, Indonesia.
E-mail: jamsari@agr.unand.ac.id

Abstract. The rpoB gene is one of the housekeeping genes in the Serratia plymuthica UBCF_13. This gene is usually used as a reference gene in qPCR analysis to quantify the expression level of the target gene. In the absolute analysis, the reference gene qPCR was cloned to create a standard curve for calculating the copy number of the target gene expression level. The method used in this study was to clone a partial rpoB gene through the ligation to pGEM®-T Easy plasmid cloning vector and transform it to E. coli Dh5α to proliferation recombinant plasmid. The results of this study indicated the reference rpoB gene was successfully cloned using pGEM®-T Easy with the size of insert gene 118 bp length.

Keywords: Cloning, Housekeeping genes, PCR, rpoB, Serratia plymuthica.

1. Introduction

Serratia plymuthica has the ability and potential as a biocontrol agent against various types of pathogenic fungi[4] [5] and [9]. The suppression mechanism against pathogenic fungi from these bacteria can be based on the ability of antibiosis [9] [11].

To measure the level of gene expression in qPCR we need control genes or reference genes as a comparison because these reference genes are always expressed in cells and their expression is relatively stable. In this study we used the rpoB reference gene, this gene is one of the housekeeping genes owned by S. plymuthica UBCF_13[7]. In absolute quantification, the results of cloning reference genes will be made a standard curve, we can find out the exact number of genes expressed so that the results are expected to be more comprehensive. Some of the uses of cloning are to provide sufficient DNA for gene sequencing, directional mutagenesis, gene probe, and cloned gene expression[2].

Cloning of the reference rpoB gene is the initial stage in our research, then we will look at the expression level of the gene derived from the anti-fungal compound produced by S. plymuthica, namely
pyrrolnitrin. This \textit{rpoB} gene will be used as a reference gene for the next research stage. The amplicon size of \textit{rpoB} in this study was 118 bp. Apart from \textit{Serratia plymuthica} UBCF\_13, the \textit{rpoB} gene was also found in other bacteria including \textit{Clavibacter michiganensis}, \textit{Xenorhabdus nematophila}, and \textit{Photorhabdus luminescens} \cite{3} \cite{12}. The length housekeeping gene that is ideally used as a reference gene is 60-150 bp\cite{8}. So, in this study, we cloned the \textit{rpoB} reference gene to be used as a control gene or comparison gene in absolute data analysis at qPCR to determine the copy number of the target gene expression level\cite{7}.

2. Materials and Methods

2.1 Preparation Isolate

The materials used in this study were bacteria \textit{S. plymuthica} strain UBCF\_13 obtained from the internal collection of the Biotechnology Laboratory, Faculty of Agriculture, Andalas University.

2.2 DNA Isolation and Molecular Assay

Extraction DNA of bacterial using the method of Chen and Kuo (1993). The procedure performed was \textit{S. plymuthica} UBCF\_13 from cultured glycerol stock as much as 10 µL in 10 mL of LB medium.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Primer ID & Sequence 5' to 3' & Amplicon Size (bp) \\
\hline
\textit{rpoB-F} & CGTTAAAGACTCTTTCTCTGC & 118 \\
\textit{rpoB-R} & CATTCTCCATTTCCAACG & \\
\textit{T7} & TAATACGACTCACTATAGGG & 141 \\
\textit{SP6} & ATTTAGGTGACACTATAG & \\
\hline
\end{tabular}
\caption{Primer combinations were used in the study}
\end{table}

DNA amplification was performed using the My Taq (Bioline-USA) PCR kit. Amplification was performed with a pre-designed \textit{rpoB}-specific primer. The estimated product size for the \textit{rpoB} gene is 118 bp.

2.3. Ligation of the \textit{rpoB} gene with pGEM®-T Easy

This stage of ligation is carried out to make the recombinant plasmid of the \textit{rpoB} gene by ligating the \textit{rpoB} gene into the pGEM®-T Easy plasmid through the Promega (Promega-USA) working protocol. To see whether the amplified DNA was ligated or not, PCR ligation was performed.

2.4. Transformation of \textit{rpoB}-pGEM to E. coli DH5α

The transformation uses the heat-shock method\cite{10}. The tube containing the suspension mixture was put in a water bath at 42 °C for 45 seconds and cooled in ice for 5 minutes and added 250µL of liquid LB. The liquid culture of the bacteria suspected of being transformant was incubated and shaken in a shaker incubator for 60 minutes at 37 °C with a shaking speed of 150 rpm.
2.5. Transformant Selection
This transformant selection was carried out by blue/white screening on selective media containing ampicillin, IPTG, and X-gal. Bacterial cells that already have the \textit{pGEM\textregistered\,-T Easy} plasmid will be resistant to ampicillin antibiotics. To verify the growth of bacterial clones, it was applied by PCR using the Mytaq (USA) kit with primer T7/SP6.

2.6. Isolation of recombinant plasmid \textit{rpoB}
Isolation of recombinant plasmid \textit{rpoB} using kit isolation by Promega (USA). To see the quality and quantity is checked with electrophoresis and documentary gel.

3. Result and Discussion

3.1. DNA Isolation
DNA isolation is the first step that needs to be done in the process of gene cloning. Isolation of genomic DNA from \textit{S. plymuthica} UBCF\textsubscript{13} was carried out to obtain intact genomic DNA from these bacteria and will be used as a template in the amplification process. DNA isolation from \textit{S. plymuthica} UBCF\textsubscript{13} has been successfully carried out by visualizing the DNA results as shown in Figure 1.

![Figure 1](image.png)

\textbf{Figure 1.} Results of \textit{S.plymuthica} bacterial DNA isolation UBCF\textsubscript{13}. M: Marker Lambda (\(\lambda\)) 50 ng/\(\mu\)l. A: Genomic DNA UBCF\textsubscript{13}.

In Figure 1, the results showed that the genomic DNA of bacteria \textit{S.plymuthica} UBCF\textsubscript{13} has been successfully isolated. Visualization of the resulting DNA bands insulation thickness and brightness are the same compared to DNA lambda (\(\lambda\)), it is stated that the concentration of the isolated DNA was 50 ng/\(\mu\)L. DNA is then used as a DNA template for amplification Activities [1].

3.2. PCR Colony
The ligation steps which is used to connect the amplified DNA fragment to the \textit{pGEM\textregistered\,-T Easy} vector and then transformed into \textit{E. coli} strain DH5\(\alpha\) bacteria. The results of the PCR colony can be seen in Figure 2.
In Figure 2, in the transformation process, two bacterial colonies grow. To ensure whether the transformation process is successful, amplification is carried out by PCR using the T7 / SP6 primer. Primer T7 / SP6 has an amplicon size of 141 bp while the amplicon size of the rpoB gene is 118 bp, based on Figure 2. It can be seen that the colony bands resulting from transformation and ligation show the same results, namely 259 bp, meaning that the ligation and transformation processes were successfully carried out. Henceforth, because colony A had better results, obtained a single band and the ribbon did not smear, isolates were selected for the isolation of rpoB recombinant plasmids[7].

3.3. Isolation of recombinant plasmid rpoB

Reference gene cloning (partial rpoB) was performed to obtain rpoB recombinant plasmids. This rpoB gene cloning has been successfully carried out with visualization as shown in Figure 3.

Figure 2. Results PCR Colony. M: Marker 1 Kb Ladder. A: Bacteria colony 1. B: Bacteria colony 2.

Figure 3. Results of rpoB Recombinant Plasmid Isolation. M: Marker 1 kb Ladder. A: Lamda marker (λ). B: rpoB Recombinant Plasmid.
Cloning of the rpoB reference gene has been successfully carried out. Figure 3. is the result of the isolation of rpoB recombinant plasmid. These results show that a band with the same concentration as the DNA λ marker was obtained, namely 50 ng / µL. Based on the 1 kb ladder marker, the plasmid size obtained is around 2500 bp, while the estimate should be 3133 bp, while the pGEM®-T Easy empty plasmid size is 3015 bp. These results are likely because the 1 kb ladder marker used is linear while the pGem Teasy plasmid is circular, also the isolated plasmid results are stored before electrophoresis so that the possibility of the plasmid in the electrophoresis process runs faster than the marker so that the results are lower than estimated which should.

To ascertain whether the recombinant rpoB gene was embedded in the plasmid, amplification was carried out with the T7/SP6 primer and the rpoB primer. Based on the PCR results in Figure 4, it can be seen that the results of the visualization using the T7/SP6 primer obtained a 259 bp size product and with the rpoB primer a 118 bp size product was obtained. Based on these results, it can be ascertained that the recombinant rpoB has been inserted into the pGEM®-T Easy plasmid because the size of T7/SP6 is 141 bp. If a 118 bp gene is inserted, the T7/SP6 length is 259 bp and it can be said that the rpoB gene cloning is successful [6].

4. Conclusion
The cloning of the rpoB reference gene has been implemented, based on PCR results with primer T7/SP6 and primer rpoB that cloning has successfully performed according to the estimate with an amplicon size of 118 bp.

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