Cloning of gyrB housekeeping gene from *Serratia plymuthica* UBCF_13

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Abstract. *Serratia plymuthica* strain UBCF_13 is a biocontrol agent that has the potential to inhibit pathogenic fungi. *S. plymuthica* has housekeeping genes, one of which is the gyrB gene. The housekeeping gene was cloned to be used as a template for standard curve creation regarding the correlation of gene copy number and Ct value in absolute quantification data. This study was conducted to provide the plasmid housekeeping gene gyrB from *S. plymuthica* which will be used as a template for standard curve creation in absolute quantification. The method used in this study were DNA genome isolation, PCR, ligation, and transformation. The result showed that the housekeeping gene gyrB has been successfully cloning based on the size of the estimated amplicon, which is 114 bp.

Keywords: absolute quantification, a housekeeping gene, Serratia plymuthica, standard curve.

1. Introduction

*Serratia plymuthica* is enabled to suppress the growth of plant pathogenic fungi such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Aspergillus fumigatus*, *Aspergillus euteiches*, *Heterobasidion annosum*, *Microsporum canis*, *Fusarium oxysporum*, *Fusarium culmorum*, *Sclerotium rolfsii*, and *Colletotrichum gloeosporioides* [1] [2] [3].

*Serratia plymuthica* has housekeeping genes, one of which is the gyrB gene. In the qRT-PCR analysis, the housekeeping genes are used as a template for standard curve creation regarding the correlation of gene copy number and Ct value in absolute quantification data and as a reference gene in relative quantification. In absolute quantification using the standard curve method, it calculates the unknown based on a known quantity. The first step is to create a standard curve; then compare the unknown with a standard curve and extrapolate a value [4].

qRT-PCR is quantitative PCR by detecting the fluorescence reporter generated during the PCR reaction. There are three main components used as a tool in the qRT-PCR process, such as a light source, a detection system, and a thermocycling mechanism [5]. So, the housekeeping gene gyrB from *Serratia plymuthica* strain UBCF_13 was cloned to be used as a template for the standard curve in absolute quantification that will be used in the next study related to qRT-PCR.
2. Materials and Methods

2.1. *Serratia plymuthica* UBCF_13 genome isolation
*Serratia plymuthica* strain UBCF_13 isolates were obtained from the internal collection of the Biotechnology Laboratory, Faculty of Agriculture, Andalas University. UBCF_13 bacteria were cultured for 16 hours on a 10 mL liquid LB medium then the genome was isolated using Chen and Kuo's method [6]. The isolated genome was stored at -20°C and the quality and quantity of DNA were checked using electrophoresis with 1% agarose.

2.2. gyrB gene amplification
The isolated gyrB gene contained in strain UBCF_13 is amplified using PCR to obtain the gyrB gene. Amplification of the gene was performed with a pre-designed gyrB-specific primer namely gyrB-F [5′-GAGCTGTCTTTCCTTAACTC-3′] and gyrB-R [5′-GTTCAGATACTCAACAAACG-3′]. PCR master mix using the My Taq [Bioline-USA] PCR kit. Amplification was for 35 cycles on a Biometra thermal cycler as follows: 1 min. 94°C, 1 min. 55°C, and 1 min. 30 sec. 72°C. The quality and quantity of the amplification results were checked by electrophoresis with 1% agarose.

2.3. gyrB gene cloning
To generate the recombinant plasmid of pGEM-T Easy vector with gyrB gene amplicon, the ligation reaction was conducted through the Promega working protocol [Promega-USA]. The ligation reaction was incubated for 16 hours at 4°C. The heat shock method was used to transform recombinant plasmids into *E. coli* DH5α. Bacteria growing and suspected of being transformants were amplified by colony PCR using T7/SP6 primer. The colony PCR was for 29 cycles on a Biometra thermal cycler as follows: 1 min. 94°C, 1 min. 55°C, 1 min. 57°C and 1 min. 30 sec. 72°C. The quality and quantity of the amplification results were checked by electrophoresis with 1% agarose gel.

2.4. Isolation of recombinant plasmid gyrB
Furthermore, the recombinant plasmids were isolated using the *Wizard® Plus SV Minipreps DNA Purification System* kit [Promega, USA]. The quality and quantity of the amplification results were checked by electrophoresis with 1% agarose gel.

3. Results and Discussion

3.1. The isolated genome of *S. plymuthica* UBCF_13

![Figure 1. Visualization of UBCF_13 genome isolation results. Electrophoresis used 1% agarose at 100 Volt. M = Marker [λDNA 50 ng/µL].](image)
The housekeeping gene was cloned to obtain the recombinant plasmid gyrB that was used as a template for standard curve creation regarding the correlation of gene copy number and Ct value in absolute quantification of data. The first step that has to be done to clone the housekeeping gyrB gene was bacterial genome isolation. Figure 1 shown the DNA genome isolated from *S. plymuthica* UBCF_13. λDNA with a concentration of 50 ng/µL was used as a control which showed the migration distance was not far from the gel well. Figure 1 shows there are bands formed in both treatments. In number 1 well, there is a DNA band near the gel that has migrated with a band thickness of ± 50 ng/µL. This indicates that the total DNA intact has been isolated. Whereas well number 2 shows the occurrence of thick and not sharp luminescence at the bottom. This indicates that DNA is fragmented and still contaminated with RNA. Total DNA in an intact state generally shows a wide, large, tailed band shape and has a migration distance not far from the gel well, while total DNA that is fragmented and contaminated with RNA generally gives a thick and not sharp glow to the part of agarose gel [7].

3.2. gyrB gene amplification

Furthermore, the gyrB gene in the bacterial genome of UBCF_13 was amplified with an estimated PCR product of 114 bp. In Figure 2, there is a thick band with a product size of 114 bp compared to the size marker, where the DNA band obtained is far below the 250 bp band. Purnami et al. [8] state that the success of DNA amplification by PCR is determined by several factors, including the purity and concentration of components in the PCR premix solution, gene oligonucleotide primers, the concentration of [α-32P] dCTP, the amount and purity of DNA sputum samples, technical and non-technical factors, for example, contamination.

![Visualization of gyrB gene amplification results with gyrB gene-specific primer [10 pmol/µL]. Electrophoresis used 1% agarose gel at 100 Volt. M = Marker [1 Kb Ladder].]

3.3. Ligation and Transformation

The gyrB gene that was obtained then ligated into the pGEM-T easy vector. Furthermore, the ligation results were transformed into *E. coli* DH5α bacteria which were cells have been competent using the heat shock method, then grown on LB medium added with IPTG, X-gal, and ampicillin. The results of ligation and transformation can be seen in Figure 3.
Figure 3. Visualization of colony PCR results with T7/SP6 primer [10 pmol/µL]. Electrophoresis used 1% agarose gel at 100 Volt. M = Marker [1 Kb Ladder]; 1 = Colony 1; 2 = Colony 2, 3 = Colony 3; 4 = Ligation.

The transformed cultures were then planted on selective LB media for verification using blue-white selection. Colonies containing recombinant pGEM-T plasmids produce white color, while those that do not contain recombinant pGEM-T plasmids produce blue colonies [9]. Three colonies grew on selective LB media. In Figure 3, 3 colonies and ligation results analyzed by colonies PCR showed positive results. This is indicated by the presence of the DNA bands measuring about 255 bp. The size of the DNA band is a combination of the inserted DNA measuring 114 bp plus DNA from the vector plasmid measuring 141 bp.

3.4. Recombinant plasmid isolation

Figure 4. Visualization of gyrB recombinant plasmid result. Electrophoresis used 1% agarose gel at 100 Volt. M1 = Marker [1 Kb Ladder]; M2 = Marker [λDNA 50 ng/µL]; 1 = Plasmid gyrB gene recombinant.
Isolation of recombinant plasmids was carried out using 1 transformed white colony, namely colony 2. Housekeeping gene cloning [partial gyrB] was performed to obtain gyrB recombinant plasmids. Figure 4 shown the visualization of successfully carried out the cloning gyrB gene.

**Figure 5.** Visualization of PCR recombinant plasmid isolation results of gyrB recombinant plasmid isolation. Electrophoresis used agarose gel concentration 1 % at 100 Volt. M1 = Marker [λDNA 50 ng/µL]; M2 = Marker [1 Kb Ladder]; 1 = pGEM-T easy + gyrB gene [255 bp]; 2 = gyrB gene [114 bp].

The empty pGEM-T easy plasmid has a size of 3015 bp and the recombinant plasmid of the gyrB gene has an estimated size of 3129 bp. If the isolated recombinant plasmid is compared with the marker 1 Kb Ladder, the recombinant plasmid has a size of about 2500 bp. These results possibly because the 1 Kb Ladder marker used is linear while the pGEM-T easy plasmid is circular.

The isolated gyrB recombinant plasmid amplified [PCR] using forward and reverse gyrB primers and T7/SP6 primers to confirm the presence of the gyrB gene in the isolated recombinant plasmid. The plasmid recombinant gyrB showed an estimated DNA band of 255 bp, of which 141 bp is the size of the T7/SP6 promoter on pGEM-T easy and 114 bp is the size of the gyrB gene that can be seen in Figure 5.

**4. Conclusion**

Housekeeping gene gyrB has been successfully cloned with pGEM-T Easy. The size of the inserted gene is 114 bp.

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