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PCR-based Gene Synthesis, Cloning, Expression, Purification and Characterization of *Bst* DNA Polymerase in *E. coli* Cells

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Abstract

Gene synthesis is a technique for modifying genes for studying gene function, structure and expression. There are several PCR based strategies and non-PCR based strategies for gene synthesis. Here we perform the 2 step gene synthesis combining assembly PCR and sequential overlap extension PCR strategies to synthesize the full length of *Bst* DNA polymerase (2,648bp) and express the protein in *E. coli* cells. The full length *Bst* DNA polymerase was divided into 5 short DNA Fragments and the oligonucloetides were designed for the entire sequence each with 40-45 mers. In step 1, the oligonuleotides of each fragment were assembled and then the gene fragment was amplified separately through assembly PCR method. In step 2, sequentially the two adjacent fragments were assembled through overlap extension PCR at a time until the full length gene completely synthesized and finally cloned into pCR®2.1-TOPO vector. Then the full length *Bst* DNA pol gene was subcloned into pET28a(+) expression vector and finally expressed in BL21(DE3) *E. coli* cells. The purified protein was identified by MALDITOF analysis. The polymerization activity of the recombinant *Bst* DNA polymerase was compared with commercial enzyme. The 98kDA recombinant *Bst* DNA polymerase succeed in amplification of 100bp DNA fragment via helicase dependent amplification (HDA).

Keywords: Gene synthesis; PCR-based two step DNA synthesis; Assembly PCR; Sequential overlap extension PCR; *Bst* DNA polymerase

Introduction

Synthetic genes are purely man-made genes and being used in multiple applications dependent on the design of the construct. The codon optimized synthetic genes are well preferred for high level expression in heterologous systems hence improves the protein production [1]. Likewise, the site-specific mutations such as defined point mutations, insertions or deletions introduced to design genes allow the progress of exploration and understanding gene structure, function and structure-function relationships [2]. Additionally for gene isolation, gene synthesis may be the only choice if template DNAs are not readily obtainable [3].

Up to date, a number of PCR based gene synthesis methods have been previously reported [4]. Shevchuk, describe improved combination of long PCR and overlap extension PCR method which allows long multiple fusions [5]. Whereas, combined dual asymmetrical PCR and overlap extension PCR enable error free DNA synthesis [6]. On the other hand, a simple PCR based two steps DNA synthesis (PTDS) was presented by Xiong in synthesizing of *via3aI* gene encoding the *Bacillus thuringinesis* vegetative insecticidal protein. The first step involves amplification of five DNA fragments of *via3aI* gene and then followed by the synthesis of full length 2,382bp *via3aI* gene [7].

In the year of 2005, an improved version of PTDS named as modified overlap extension PCR, (M)OE-PCR was demonstrated. Compared to PTDS method, here every two adjacent DNA fragments of *sam I* gene were mixed and pre-extended without amplification primers before synthesis of full length gene [8]. Recently, two-step gene synthesis strategy that combines assembly PCR and overlap extension PCR (AOE) was used in synthesis of codon optimized *Rhizopus oryzae* lipase gene ROL and *Aspergillus niger* phytase gene, *phyA* [9]. However gene synthesis protocol is not consistent for all genes and the increase of cost and error rate still limits the gene synthesis approach.

In this study, we used a two step gene synthesis named as assembly and sequential overlap extension PCR strategy (A-SeqOE PCR) for synthesis of 2,648bp *Bst* DNA polymerase gene. The constructed synthetic gene has been cloned and successfully expressed in *E. coli*. We purified the enzyme and examined its polymerization activity.

Methods and Materials

Chemicals, enzyme and strains

Pfu DNA polymerase, T4 DNA ligase, GeneRuler 1kb DNA Ladder, GeneRuler 100bp DNA Ladder, IPTG and X-Gal were purchased from Fermentas. Restriction enzymes *EcoRI*, *BamHI* and *NotI* were purchased from New England Biolabs. Maxime PCR PreMix Kit (i-StarMAXII), MEGAquick-spin™ PCR & Agarose Gel Extraction System and DNA–Spin™ Plasmid DNA Purification Kit were purchased from Intron, Korea. TOPO Cloning vector was from Invitrogen (USA) and pET28a(+) vector was from Novagen (USA). Two different strains of *Escherichia coli* have been used for transformation purpose in this work. There are TOP10 (Invitrogen, USA) and BL21(DE3) (Novagen, USA). Other chemicals and reagents were purchased from Sigma Chemical.

Gene and oligonucleotides

The sequence of the *Bst* DNA polymerase gene was 2648bp in length. This sequence was divided into five fragments (F1 to F5) with size range from 500bp to 650bp, and overlapped (20 nucleotides) at the end of each fragment. Oligonucleotides of 40-45mer for assembly PCR of each fragment were designed manually. The outermost forward oligonucleotide contain *BamHI* endonuclease restriction site whereas

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the outermost reverse oligonucleotide was designed to have *NotI* endonuclease restriction site. All the olignucleotides were chemically synthesized and PAGE purified by First BASE Laboratories, Malaysia.

Experimental design

A two step PCR based gene synthesis technique was used for synthesis of 2648bp *Bst* DNA polymerase gene. It is called as Assembly and Sequential Overlap Extension PCR (A-SeqOE PCR), which involve an amplification of several DNA fragments and then followed by a sequential overlap extension PCR. The flow work of A-SeqOE PCR was summarized in Figure 1. In step 1, the oligonuleotides of each fragment were assembled and then the gene fragment was amplified separately through traditional assembly PCR method. In step 2, sequentially the two adjacent fragments were assembled through overlap extension PCR at a time until the full length gene completely synthesized and finally cloned into pCR®2.1-TOPO vector for DNA verification.

Gene assembly and amplification of individual fragments

For assembly PCR the mixture of oligonucleotides were act as template and added to 50 µL of PCR mixture, which consisting 1 X *Pfu* Buffer with $MgSO_4$, 0.2 mM each dNTPs, 2.5 U/µL of *Pfu* DNA polymerase and sterile distilled water. The conventional PCR which consists of one cycle at 95°C for 1 minute, 35 cycles at 95°C, 63°C for 30 seconds, 72°C for 1 minute each and one cycle at 72°C for 10 minutes was preformed.

Then the products from assembly PCR were used as template for gene amplification. About 1 µL of the assembly PCR products were mixed with 50 µL PCR reaction mixture which is contain 1 X *Pfu* Buffer with $MgSO_4$, 0.2 mM each dNTP, 2.5 U/µL *Pfu* DNA polymerase, 0.25 µM each of the first forward and last reverse oligonucleotides and sterile distilled water. This mixture was subjected to 37 cycles of amplification consist of one cycle at 95°C for 2 minutes, 35 cycles at 95°C, 63°C for 30 seconds, 72°C for 1 minutes each and one cycle at 72°C for 10 minutes. The amplified DNA fragments observed on 1.2% agarose gel.

Synthesis of full length *Bst* DNA polymerase gene through Sequential OE-PCR

For full length gene synthesis, the adjacent fragments (two at a time) were joined sequentially through overlap extension PCR. These fragments were added into the Maxime PCR PreMix Kit (i-StarMAX II) together with the outermost primers and top up to 20 µL with sterile distilled water. Then this mixture was subjected to 37 cycles of amplification consist of one cycle at 95°C for 4 minutes, 35 cycles at 95°C, 63°C for 30 seconds, 72°C for 3 minutes each and one cycle at 72°C for 10 minutes. Amplified DNA Fragment was purified from agarose gel using MEGAquick-spin™ PCR & Agarose Gel Extraction System and was cloned into the pCR®2.1-TOPO vector for sequence analysis. After sequence verification, the insert was sub-cloned into pET28a(+) expression vector. The *BamHI* and *NotI* digested *Bst* DNA polymerase gene, were inserted into the same restriction sites of linearized pET28a(+) vector. Finally, the ligated mixtures were transformed into *E. coli* BL21 competent cells to generate recombinant proteins.

Protein expression and purification

A fresh bacterial colony, harboring the plasmid pET- *Bst* DNA pol, was inoculated into 10 mL of LB medium containing 50 µg/mL of Kanamycin and was grown at 37°C overnight. Then 5 mL of overnight cultures were inoculated into 500 mL LB and were grown at 37°C while shaking vigorously. Once the cultures OD_{600} reached 0.5, IPTG with final concentration of 0.2 mM was added to the medium and incubated at 37°C for 6 hours with shaking at 180 rpm. After induction for 6 hours, the cells were harvested by centrifugation at 3,000 xg for 15minutes at 4°C. The pellet was resuspended in 10 mL of Lysis Buffer (50 mM sodium phosphate, pH 8, 300 mM NaCl, 10 mM imidazole) containing 5 mM b-mercaptoethanol. The cells were lysed by sonication and the lysate was centrifuged at 3,000 xg for 40 min. Then the collected supernatant was analyzed with SDS-PAGE. The IMAC purification was carried out using nickel charged iminodiacetic acid (IDA)-Sepharose resin column. Initially the supernatant was loaded onto the column and flow through was collected. Then column was washed with Washing Buffer I (50 mM sodium phosphate, pH 8, 300 mM NaCl, 20 mM imidazole) and then followed by washing with Washing Buffer II (50 mM sodium phosphate, pH 8, 300 mM NaCl, 50 mM imidazole) to remove the unbound proteins. Finally the histidine-tagged proteins were eluted with Elution Buffer (50 mM sodium phosphate, pH 8, 300 mM NaCl, 250 mM imidazole) and each fraction were analyzed on SDS-PAGE. The purified proteins were detected by Anti-His antibody in western blot. The recombinant proteins were identified and confirmed by the MALDI-TOF/TOF analysis.

Polymerization activity

Briefly, a real time qPCR reaction mixture was prepared and it contained about 10 µL of 2 X EvaEZ Polymerase Activity Mix, 1 µL of

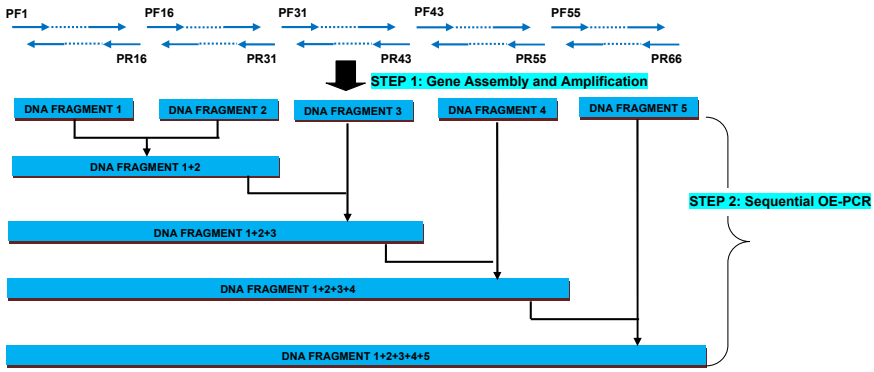

Figure 1: The work flow of gene synthesis via Assembly and Sequential Overlap Extension PCR (A-SeqOE PCR) method. The 2.6kb *Bst* DNA polymerase gene was splitted into five short DNA fragments (each fragment in the molecular weight 500-650bp). The gapless oligonucleotides in the length of 40-45 mers with 20 mers overlapping region were designed. Therefore, each fragment has about 13-16 pairs of oligonucleotides. During step 1 of gene synthesis, the oligonucleotides of each fragment were assembled and then followed by next PCR for amplification of gene fragment. Amplified two adjacent fragments has 60bp overlapping region. Thus, in step 2, sequentially the two adjacent fragments were assembled through overlap extension PCR at a point until the full length gene completely synthesised.

recombinant *Bst* DNA polymerase and 9 µL of sterile deionized water. This mixture was mixed well and placed in real time qPCR 7500 Fast Real Time PCR System (Applied Biosystems). Then an isothermal program at 65°C was run for 90 minutes. The fluorescence reading was collected by Channel 1 (FAM). A reaction profile of EvaEZ fluorometric polymerase activity assay was plotted with X-axis as time in minutes and Y-axis as fluorescence reading. Then the slope of the curve was determined and it is representing the initial rate of fluorescence change (Fluorescence Unit/ minutes). The specific activity of sample was estimated by comparing the initial rate of fluorescence change of recombinant protein with the reaction curve of standard *Bst* DNA polymerase Large Fragment.

Helicase dependent amplification assay (HDA)

The tHDA assay was performed using IsoAmp® II Universal tHDA kit (Biohelix) as described by manufacturer. This reaction mixture contained of 1 X Anneling buffer II, IsoAmp® dNTPs solution, 10 mM $MgSO_4$, 40 mM NaCl, DNA template, 0.25 µM each primers, 0.5 U of termostable Helicase and 1 U of *Bst* DNA polymerase, Larger Fragment or with recombinant *Bst* DNA polymerase. Then an isothermal program at 65°C was run for 90 minutes. The amplified product was analyzed on agarose gel.

Results

In the assembly PCR of each short DNA fragment the 1.0 µM single stranded oligonucleotides (maximum of 16 pairs) were mixed in a single tube, hybridized and extended to form the long dsDNA construct. Then the gene amplification was performed using the assembled products. Five short DNA fragments of *Bst* DNA polymerase gene [named Fragment 1 (662bp), Fragment 2 (659bp), Fragment 3 and 4 (539bp each) and Fragment 5 (494bp)] were assembled and amplified (Figure 2A); Lanes 2 to 6]. Next, synthesis of full length *Bst* DNA polymerase gene was performed with sequential OE-PCR. One pair of adjacent short DNA fragments joined at a time. The Fragment 1 and Fragment 2 were assembled and during overlap extension PCR the ~1.2kb DNA fragment (Fragment1+2) successful amplified. Then these 1.2kb DNA fragment was joined with the short DNA Fragment 3 (539bp) through OE-PCR and results the ~1.7kb DNA fragment (Fragment 1+2+3). This followed by the joining of 1.7kb DNA fragment with the short DNA Fragment 4 and ~2.2kb DNA fragment amplified. Finally the 2.2kb DNA fragment was successfully joined with the short DNA Fragment (494bp) and the full length 2, 650bp *Bst* DNA polymerase gene was amplified (Figure 2B). The full length *Bst* DNA polymerase was successfully cloned into pCR®2.1-TOPO vector and presence of insert confirmed by *EcoRI* digestion as shown in Figure 2C. Subsequent cloning of *Bst* DNA polymerase into pET28a(+) vector was resulted the pET-*Bst* DNA polymerase recombinant plasmid as represented in Figure 2D.

Then, the vector was subjected to expression procedures. Firstly, the optimal induction temperature, IPTG concentration, and induction time for the expression of recombinant *Bst* DNA polymerase in BL21(DE3) *E. coli* were determined. The maximum yield of soluble protein (98kDa) was achieved at an induction temperature of 30°C, an IPTG concentration of 0.2 mM, and with induction for 6 hours. Therefore, the preparation of recombinant *Bst* DNA polymerase was performed under these conditions. As shown in Figure 3A the soluble fraction mainly contained protein products observed with SDS-PAGE. The His-tagged recombinant protein was purified using IMAC purification and the SDS-PAGE analysis (Figure 3B), shows the fractions collected during washing steps. The binding of the histidine proteins with resin were optimum as very little amount of proteins remained after the binding process. Moreover during the washing steps, the contaminant proteins as well as the unbound histidine tagged proteins were removed from the resin. Most of the histidine tagged proteins were eluted at 250 mM imidazole concentration (Figure 3C) and able verified with detection via western blot as shown in Figure 3D. In addition, the MALDI-TOF/TOF analysis (Table 1) was confirms that purified recombinant *Bst* DNA polymerase was matched with DNA polymerase I of *Geobacillus stearothermophilus.*

Thereafter, the polymerase activity of recombinant DNA polymerase was determined. The polymerase activity of recombinant *Bst* DNA polymerase was represented by fluorescence changes in a period of time. Figure 4A represents polymerase activity of recombinant *Bst* DNA polymerase and this curve shows that fluorescence changes were increased with time. Thus the initial rate of fluorescence changes was determined as 2,975 for recombinant *Bst* DNA polymerase. Then the specific activity of recombinant *Bst* DNA polymerase was predicted using comparing with the polymerase activity curve of standard protein

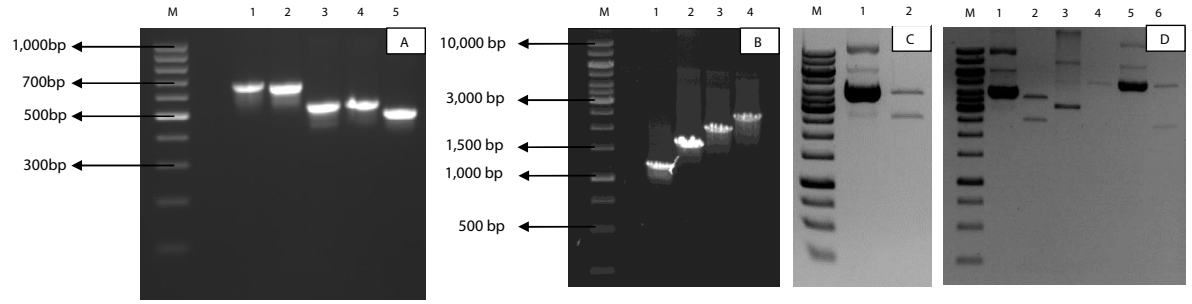

Figure 2. shows the synthesis of full length *Bst* DNA polymerase gene. **A)** Represents the amplified five short DNA Fragments of *Bst* DNA polymerase gene; M: 100bp DNA ladder, Lane 1: Amplified DNA Fragment 1, Lane 2: Amplified DNA Fragment 2, Lane 3: Amplified DNA Fragment 3, Lane 4: Amplified DNA Fragment 4 and Lane 5: Amplified DNA Fragment 5 through assembly PCR technique. **B)** Represents the sequential assembly of adjacent fragments at a time through overlap extension PCR; M: 1kb DNA ladder, Lane 1: Assembled 1.2kb DNA Fragment 1+2, Lane 3: Assembled 1.7kb DNA Fragment 1+2+3, Lane 4: Assembled 2.2kb DNA Fragment 1+2+3+4 and Lane 5: Assembled 2.6kb full length *Bst* DNA polymerase gene**. C)** Represents the analysis of pCR-TOPO-*Bst* DNA pol plasmid; Lane M: 1kb DNA ladder, Lane 1 and Lane 2 shows analysis of isolated recombinant plasmid pCR-TOPO- *Bst* DNA pol and represents two bands after *EcoRI* digestion, the upper band is vector backbone 3.9kb and the lower band is the insert *Bst* DNA polymerase gene, 2.6kb. **D)** Represents *BamHI/ NotI* digestion analysis in construction of pET-*Bst* DNA polymerase recombinant plasmid; Lane M: 1kb DNA ladder, Lane 1: plasmid pCR-TOPO- *Bst* DNA pol plasmid; Lane 2: represents two bands after *BamHI/ NotI* digestion, the upper band is vector backbone 3.9kb and the lower band is the insert *Bst* DNA polymerase gene, 2.6kb; Lane 3 and Lane 4 represent analysis of pET vector backbone preparation, which is uncut and linearized pET28a(+) vector respectively; Lane 5: pET recombinant plasmid pET-*Bst* DNA pol and Lane 6: represents two bands after *BamHI/ NotI* digestion, the upper band is vector backbone 5.3kb and the lower band is the insert *Bst* DNA polymerase gene, 2.6kb.

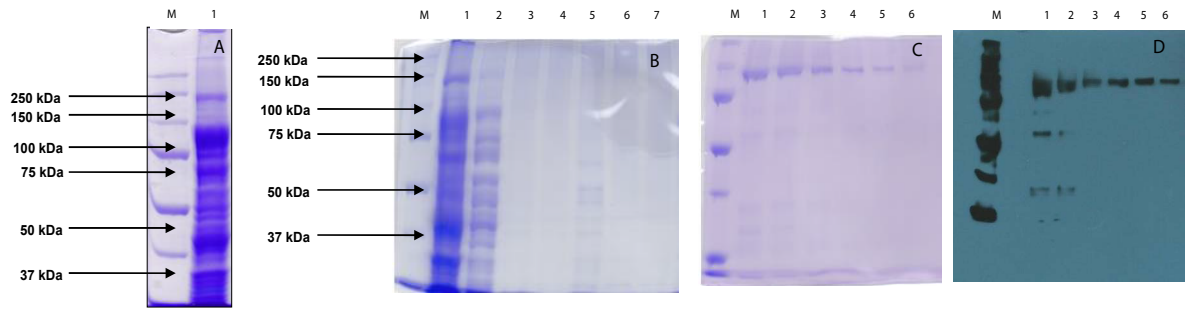

Figure 3. shows the expression and purification of recombinant protein *Bst* DNA polymerase. **A)** Represents the SDS-PAGE analysis of expressed recombinant protein; M: Protein standard marker and Lane 1: Expressed total soluble proteins, **B)** Represents the SDS-PAGE analysis of binding and washing step fractions collected in IMAC purification; M: Protein standard marker, Lane 1: Flow through of the lysate after binding to resin; Lane 2-4: washing with 20mM imidazole and Lane 5-7: washing with 30mM imidazole. **C)** Represents the SDS-PAGE analysis of eluted His-tagged recombinant protein; M: Protein standard marker and Lane 1-6: Elution fraction. D) Represents the Western blotting analysis of eluted His-tagged recombinant proteins; M: Protein standard marker and Lane 1-6: Elution fraction which were detected in Western blot by Anti-histidine antibody.

Sample	Accession number	Protein description	Scores	Molecular weight	pI value
recombinant *Bst* DNA polymerase	sp|P52026|DPO1_GEOSE	DNA polymerase I Taxonomy: *Geobacillus stearothermophilus*	441	98,609	5.65

Table 1: Summary of results of mass spectrometry and database search analyses of the purified recombinant protein.

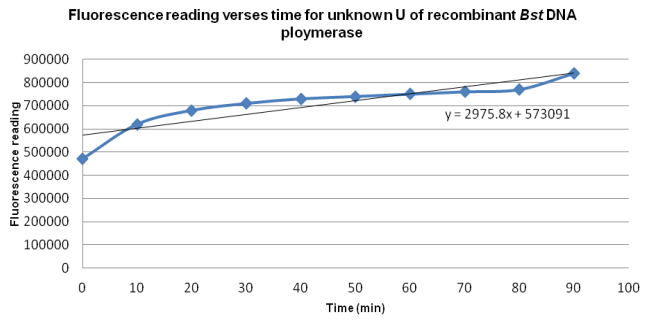

Figure 4(A): Fluorescence reading verses time for unknown U of *Bst* DNA polymerase. The slope of the curve is m or initial rate of fluorescence changes = 2,975 U/min

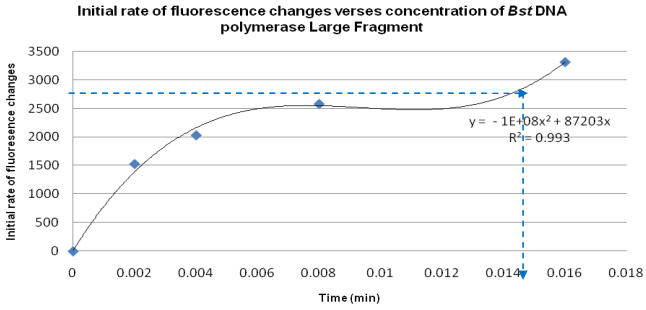

Figure 4(B): Represents prediction of specific activity of *Bst* DNA polymerase using the polymerase activity curve of standard protein, *Bst* DNA polymerase Large Fragment. The Blue line in the graph shows estimation of specific activity of recombinant protein *Bst* DNA polymerase.

(Figure 4B). As shown on figure the specific activity of recombinant *Bst* DNA pol is estimated approximately 0.015 U (blue line).

Finally, in this study the ability of recombinant *Bst* DNA polymerase to perform in HDA were demonstrated. Figure 5 shows the successful amplification of 100bp DNA fragment by *Bst* DNA polymerases via HDA technique. Here the thick band of 100bp amplified product was observed in performing HDA with commercially available *Bst* DNA polymerase Large Fragment as a positive control. The similar result obtained by performing HDA with recombinant *Bst* DNA polymerase. Although, the amplification yield was higher in positive control but the recombinant protein of synthetic *Bst* DNA polymerase was showed that it is biologically active and perform polymerization.

Discussion

Here we used a two step PCR-based gene synthesis strategy for synthesis of *Bst* DNA polymerase gene. The two step gene synthesis involves splitting of the designed oligonucleotides (12-16 pairs in one tube) and short individual fragments amplified separately, and then finally joined together to synthesis the full length gene. In this study, we suggest an improved approach of PCR-based gene synthesis which allows reduction of error rate. Besides, it is a simple, reproducible, and economic method that guarantees the successful synthesis of the error free desired gene. This was done by amplifying and sequentially assembling two adjacent short DNA fragments at a time until synthesis of full length gene.

Xiong and his team have been reported a simple, high-fidelity and

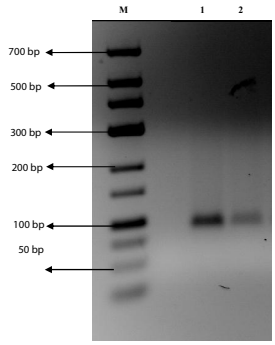

Figure 5: Shows the agarose gel analysis of helicase dependent amplification assay; M: Low range DNA ladder, Lane 1: HDA with *Bst* DNA polymerase Large Fragment (positive control) and Lane 2: HDA with recombinant *Bst* DNA polymerase

cost-effective PCR-based two-step DNA synthesis (PTDS) method for synthesis of long segments of DNA. This team succeeds in amplifying and assembly of 2,382bp *vip3aI* which is encoding for a *Bacillus thuringiensis* vegetative insecticidal protein. Here the PTDS procedures involves, first amplification step of amplification of ~500bp DNA blocks and then followed by mixing of entire blocks in second amplification step to produce the full-length gene [7]. The similar method has been demonstrated during synthesis of modified phytase gene (*phyA-mod*) [1].

However, An et al., were disagree to the earlier method. They suggested that mixing multiple fragments together has great interruptions for correct overlap and thus results in amplification errors. Therefore this team implemented a double-mixing of fragment to avoid amplification errors [8]. Similarly to reduce the amplification interruptions, in this present study, a sequential assembly of two adjacent fragments was performed. However here, the sequential assembly was highlighted as an efficient way to assemble, clone and sequence-validated. The sequential assembly allows monitoring of amplified DNA fragment and improvement via site-directed mutagenesis prior to synthesis of full length gene [10].

Success of gene synthesis highly depends on the quality and purity of the oligonucleotides used. In this present study, the two- step gene synthesis was performed with 40- 45bp oligonucleotides. Although previous researchers reported that the length of oligonucleotides vary from 40bp [11-13], 42 bp, 60 bp [7,14], 90 bp [15], and even over 100 bp [16,17], utilizing longer oligonucloetides it will contribute to low stability and specificity, tendency to form secondary structure [13]. Thus to avoid formation of secondary structure and reduce errors, the shorter oligonucleotides are preferred for gene synthesis [4,18]. Thus generally, the 60bp oligonucleotides were used for two-step gene synthesis methods [6,7,14]. Besides, the gapless short oligonucleotide design would potentially grant an extra point in minimizing errors of gene synthesis [19]. Young, has been reported that absence of gaps between oligonucleotides, make oligos themselves serve as a checking mechanism, in which the mismatched oligos will anneal less favorably than fully match oligos. Thus taking this in consideration, gapless 40bp to 45bp oligonucloetides were designed for gene synthesis of *Bst* DNA polymerase gene.

The designed *Bst* DNA polymerase gene with gapless oligonucleotides was successfully expressed and purified using IMAC purification. The previous reports stated that the *Bst* DNA polymerase I Large Fragment has been cloned and 67.7kDa protein was expressed in *E. coli* cells for functional [20] and structural studies [21]. On the other hand, recently in year 2009 the full length gene of *Bst* DNA polymerase was successfully cloned into pET28a(+) vector and the recombinant proteins were produced [22]. The recombinant proteins with similar molecular weight as stated by Rastgoo were successful produced in this current study. However the culture condition for recombinant protein production was varies compared to condition reported by Rastgoo and his team. They have been reported that gene encoding for DNA polymerase in thermophilic *Geobacillus* sp. MKK successfully expressed with 0.1 mM IPTG [22]. Initially, the similar condition was used for expression in this study. Unfortunately, the expressed proteins were unable to observe on SDS-PAGE and failed to detect via western blotting technique. Thus, in this study the expression of target proteins were conducted with a range of IPTG concentration. The target proteins were expressed with lower IPTG concentration which is 0.2 mM IPTG and amount of target proteins expressed was comparatively similar although the cells were induced with higher concentration of IPTG. The high concentrations of IPTG will results metabolic burden and not lead to maximal expression of a target protein [23]. Furthermore, Rastgoo and his team were failed to eliminate the co-eluted host cells proteins during IMAC purification of his-tagged recombinant proteins. Whereas, in this current study, we improve the purity of the recombinant his –tagged *Bst* DNA polymerase protein by increasing the imidazole and salt concentration in the washing buffer (50 mM and 300 mM respectively).

Then polymerase activity of the recombinant protein was successfully observed. The dependence of the recombinant *Bst* DNA polymerase activity on pH was observed at pH 7.0, with buffer condition 20 mM Tris–HCl and 100 mM NaCl. As reported by Rastgoo and his team, the polymerase activity of *Bst* DNA polymerase observed at temperature 65°C. The polymerase activity assay of recombinant *Bst* DNA polymerase showed that it has considerable polymerase activity. However small reduction was observed in the polymerase activity when comparing with recombinant of DNA polymerase in thermophilic *Geobacillus* sp. MKK as reported by Rastgoo.

Thermostable DNA polymerases are mainly used for the *in vitro* amplification of DNA fragment and for the determination of DNA sequences [24]. The commercially available 67.7kDa *Bst* DNA polymerase Large Fragment has been widely used in isothermal DNA amplification assay; helicase dependent amplification assay (HDA) [25]. So far there is no report on ability of 100kDa *Bst* DNA polymerase in performing HDA assay. Moreover, in this present study, the full length gene was synthetically constructed and the proved work as commercial enzyme in HDA assay.

Conclusion

In summary, result presented in this present study shows that, 2,648bp the full length *Bst* DNA polymerase gene was successfully synthesized via assembly and sequential overlap extension PCR technique. This method allows a rapid and error free gene synthesis that guarantees the successful synthesis of the desired gene. Moreover, according to our findings this synthetic gene was successfully expressed and biologically active. The polymerase activity and helicase dependent activity show as a suitable candidate for biotechnological purposes.

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