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NGS Technique for Palindromic Sequencing of DNA Through Effective PST-PCR

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ABSTRACT

DNA Sequencing technologies have been in use since 1970 and has diversified to much more effective transformations till the date. Initially due to certain drawbacks like cost, time period and requirement of toxic and radioactive elements for the compilation of the process, it remained unintroduced to research setting for complex data in earlier times. One of a technique named as the Sanger technique had more practical approach for sequencing the desired data of the fragments. But the need of DNA sequencing surged after the commencement of the Human Genome Project (HGP) which was a 13 year long collaboration to sequence human genome for understanding its applicable uses.[1]

At the current stage, the progress moved towards Next Generation Sequencing (NGS) to sequence the fragments of DNA for a better acknowledgement but somehow this did not reported for a fair outcome for palindromic DNA while sequencing. In this rugged sequencing platforms, Roche's 454 with emulsion PCR technique amplified the templates through beads which later went through pyrosequencing with DNA Polymerase gave the desired output but had a major drawback of high cost.[2] Therefore, this study deals with effective PST – PCR technique for studying palindromic DNA in NGS and how it can be considered to fit in all advantages for the above task.

Keywords- Palindrome DNA Sequence, Next Generation Sequencing (NGS), PST- PCR.

I. INTRODUCTION

Next Generation Sequencing (NGS) inclines potentially towards a parallel sequencing, resulting in ultrahigh throughput, scalability and speed. This technique is especially used to determine nucleotides in genomes or targeted site of DNA and RNA. Beginning the process with extraction and isolation of nucleic acid, a library is prepared and clonal amplification is performed of the sample. The retrieved sample is then analyzed with the help of bioinformatics tools.

In this study we see major recalling of Genome Walking (GW). As per the studies, Next Generation Sequencing adopts principles of Genome Walking at in-silico methods generally.[3] Here, PCR based Genome Walking comes under consideration that has a role to perform sequencing of palindromic strands either by synthesis or ligation through restriction endonucleases. In the PCR technique, the targeted sequence is allowed for generating multiple NGS libraries and sequencing of multiple targeted regions simultaneously.[4] In this, two different PCR techniques stands for a controversy- Emulsion PCR and Bridge PCR. Emulsion does the amplification of each sequence promising no contamination to the sample whereas Bridge PCR is the other method prior for NGS with two types of oligos fixed to flow cell. The average cost of this entire application was around $600 that led to high cost and dives for a budget friendly option.

Optimizing for a cost-efficient approach, PST-PCR (Palindromic Sequence Targeting- Polymerase Chain Reaction) stands as a better option for limited time equipment and is budget friendly in use. [5] The process sums up with a duration of 2-3 hours with two phase rounds running within. The question arises how this technique can be made less expensive and less time

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taking. Either by changing the concentration of master mix materials, fluctuating the thermocycling phase or any other.

II. METHODOLOGIES

The PST-PCR can be successfully done by the preparation of master mixture with required components. But this still remains a delicate process because there are other factors too that may affect the desired results.[6] The table given below describes the materials and their components required for running PST-PCR for 30µl reaction mixture.

Template DNA	*30ng*
TAQ Buffer	*1 x*
DNA Polymerase	*1U*
Mg $^{2+}$	*2mM*
DNTP	*200µm*
SSP	*0.2µM*
PST Primer	*0.5µM*

There could be no possible fluctuations made in the composition of master mixture to accelerate the PST-PCR process. Doing so will lead to following defaults in running the PCR and getting the desired bands successfully like-

i. Increasing the amount of TAQ buffer may lead to increase in excessive unwanted DNA fragments and poor background during imaging.
ii. Increasing the amount of DNA Polymerase may lead to increase in false priming and poor DNA synthesis.
iii. Increasing the amount of Mg^{2+} may increase the annealing of primers to wrong template sites and reduce specificity if PCR.
iv. Increasing the amount of primer may increase the chance of primers binding non- specifically to unwanted sites.
v. Thus, changing the concentration of reactors in a master mix may not result in a successful band result.[7]

III. TEMPERATURE

Thermal profile holds a major sensitivity for running the PCR setup. The entire process can be completed in 2-3 hours of time. For successful results temperature of each phase can be controlled as given below-

For first round (combining PST primer with SSP for 7-12 cycles)		
Phase	Temperature	Altered Temperature
Linear Amplification phase	68° C	65° C
Exponential Amplification phase	55° C	50° C
**For increasing the cycles to 12-18 annealing temperature is reduced.*		
For second round (same SSP or nested SSP with tail primer)		
By Denaturing and Extension		
Exponential phase | 68°-72° C | 72° C		

Thus, temperature alteration in first round of annealing phases resulted in successful band retrieval. This can be more precisely obtained by-

i. Binding of targets with a particular PST site and template.
ii. Quality of the template used.
iii. Increasing the units of Taq Polymerase to increase primer concentration.
iv. Avoiding selection of rare PST sites.[7]

IV. CONCLUSION

On running the PST-PCR for palindromic sequencing of templates of DNA and RNA efficiently, the study focuses on importance of thermal hindrance, templates chosen for the PCR, reactant's composition and binding of sites of primer as a major role in getting the desired bands of the template within a minimum period of time. On the current times of the surge in genome sequencing, NGS has diversified into more adaptive techniques for fostering a reliable, hence, less time taking advancements in procedures of sequencing. But, the palindromic sequencing was a challenge to the technique for a time of its earlier evolution. Application of PST- PCR promises to sequence palindromic templates with accurate result formation. Avoiding to these precautions listed in the study may lead to poor band formation or no band formation and lastly require more time period to complete the process. Therefore, this review paper suggests measures for redeeming a successful band result of a palindromic template through PST-PCR.

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