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Genomic interference observed in Triplet repeat primed PCR using Homemade Taq Polymerases

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ABSTRACT

Numerous versions and types of Polymerase chain reactions (PCR) are available worldwide, designed and developed to detect different types of mutations and polymorphisms. The triplet repeat primed PCR (TP-PCR) is one such improvised PCR, designed to detect repeat expansions associated with neurodegenerative triplet repeat disorders. High investments in using high quality Taq polymerases for PCR in secondary research labs led them to use homemade Taq polymerases from E.coli strains. This study reports genomic interference observed when homemade Taq polymerases were employed in triplet repeat primed PCR performed for detecting Friedreich Ataxia (GAA repeat expansion disorder). The PCR products obtained from amplification of desired genes with homemade Taq polymerases showed improper and unreliable results when screened through agarose gel electrophoresis comparing to that run with commercial Taq polymerases. The agarose gel electrophoresis results suggest possibility for interference of the bacterial genome present in the Taq enzyme with any of the primers used for TP-PCR reaction. This study highly recommend researchers performing triplet repeat primed PCR to compare the commercial Taq polymerase and homemade Taq Polymerase activity in initial stages of research both experimentally and using database tools to rule out the interference caused by bacterial genome on primer action thus avoiding unreliable PCR results.

Keywords: Triplet repeat primed PCR, Friedreich Ataxia, Pathogenic expansions, Homemade Taq polymerases, Commercial Taq polymerases, Agarose gel electrophoresis.

INTRODUCTION

Triplet repeat primed PCR (TP PCR) is different from conventional PCR in the number and action of primers used. TP PCR was first used in works of Warner *et al.* in the year 1996 [1]. TP PCR for Friedreich Ataxia carried out by Ciotti *et.al.* in 2004 actually is a modified version of the PCR technique developed by Warner *et.al*. in the year 1996 [1,2]. Molecular diagnosis of FRDA is usually done by PCR amplification of the region containing the GAA repeat, followed by agarose gel electrophoresis and capillary electrophoresis of the PCR products to determine their product size and repeat length respectively [3-5]. The high investment cost in purchasing commercial *Taq* polymerases in secondary research labs led them to produce homemade *Taq* polymerases by conventional techniques from *E.coli* strains. In most of the labs the homemade *Taq* polymerases is isolated and purified from cell cultures of *E. coli* strain Fp25 [6]. Nearly every secondary research centres in India and other developing countries use this type of homemade *Taq* polymerases for most of the PCR related study and diagnosis procedures. It doesn't cause much problem when used

in normal PCR process as it involves the use of only two primers with no chance of homology between them in almost all cases. As more than two primer sets are used in TP PCR there is a high chance of genomic interference either from the other primer sequences used or from external reagents present in the master mix [7]. In the present study, TP PCR done using homemade *Taq* polymerases was observed to give unreliable PCR results with spurious bands appearing even on negative controls in post-PCR agarose gel pictures. The implementation of commercial *Taq* polymerases to the TP PCR process completely solved the problem with accurate results as calculated with genomic database tools. As three primers are used in the reaction there is a great possibility for genomic interference between nucleotide sequences present in bacterial genome (Homemade *Taq* Polymerase) with any one of the primer sequences used for amplification.

MATERIALS AND METHODS

DNA samples

A total of 25 samples were analyzed on 1.8 % agarose gel to check the genomic interference of homemade *Taq* polymerases with the TP PCR results.

Technical information

DNA samples required for the study was isolated from white blood cells by phenol chloroform method. Informed consent was obtained from human volunteers. DNA isolated from white blood cells of healthy subjects were checked for quantity and quality by NanoDrop1000™ Spectrophotometer. The DNA concentration ranged from 150-200 ng /µl. 25 samples were isolated and further analyzed for quality by running 1µl of it on a 1% agarose gel at 100 V for 10 minutes. Commercial primers obtained from EUROPHIN MWG OPERON™ (sequences of P1, P3 and P4 are shown in Table 1) were used for amplification of the *frataxin* gene (FRDA) by TP PCR reaction. DNA with high concentration was taken for TP PCR along with three sets of primers. The TP PCR was performed as per the conditions showed in Table 2. About 2 µl PCR products obtained were run on 1.8 % Agarose gel at 100 V for 25 minutes. The gel pictures were recorded and visualized to rule out contamination checking with the negative controls. The TP PCR reaction was repeated five to ten times to confirm that the spurious bands found in negative controls were not due to any other contaminations.

Table 1. Primer sequences TP PCR -FRDA

TP-PCR PRIMER SEQUENCES
P1 : 5'-GCTGGGATTACAGGCGCGCGA-3'
P3 : 5'- TACGCATCCCAGTTTGAGACG-3'
P4 : 5' 6-FAM TACGCATCCCAGTTTGAGACGGAAGAAGAAGAAGAAGAAGAA-3'

Table 2. PCR condition TP PCR -FRDA

TP PCR CONDITION		
• INITIAL DENATURATION	- 95°C- 5MINS	
• DENATURATION	-94°C-30 SEC	35 CYCLES
• ANNEALING	-60°C 30 SEC	
• EXTENSION	-72°C 30 SEC	
• FINAL EXTENSION	-72°C 10 MINS	
• STORAGE	- 4 °C	

RESULTS AND DISCUSSION

Electrophoresis picture of TP PCR products run on 1.8% Agarose gel (shown in figure 1 and figure 2) represents TP PCR performed for two test samples and their equivalent controls. The gel picture showed unexpected equivalent product bands in both test and control samples. We suspected the *Taq* Polymerase prepared in house could be responsible for the unexpected product bands in negative controls. As a result the TP PCR was repeated five times with the same samples set to rule out the chances of other contamination from the master mix. The TP PCR was run using the commercial Taq Polymerase obtained from (AMPLITAQ ®) and the electrophoresis pattern (shown in fig -2). PCR products were compared by running the previous trials on the adjacent lanes (Lane 3 and 4). We were able to see the spurious bands disappearing in negative controls when commercial *Taq* Polymerase was employed for TP PCR instead of homemade *Taq* polymerase enzyme. Thus it can be clearly concluded that there is a typical genomic interference observed when homemade *Taq* Polymerase enzyme was employed in TP PCR.

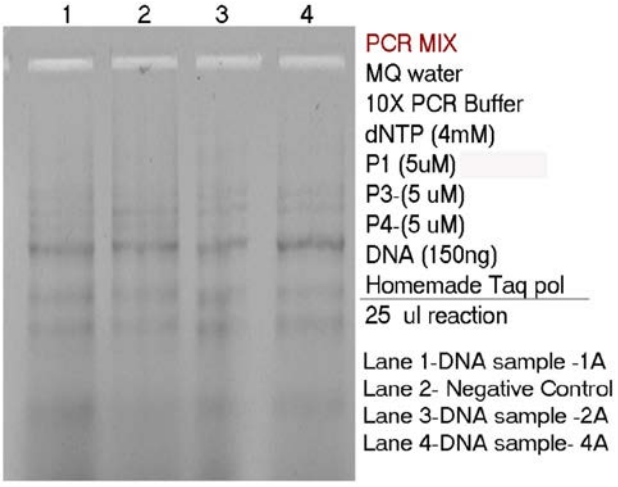

Figure 1. Agarose gel picture of TP PCR with homemade *Taq* polymerase (1.8 % gel).

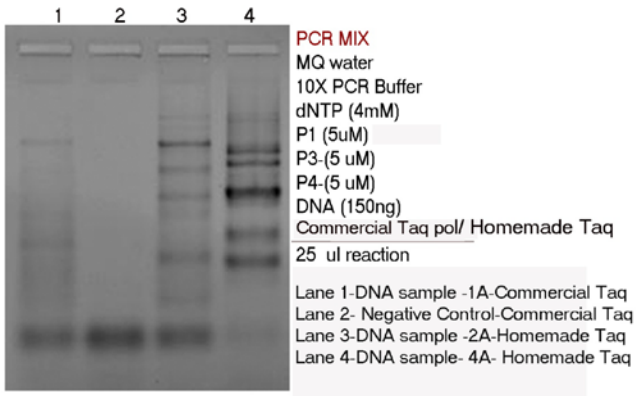

Figure 2. Agarose gel picture of TP PCR with commercial *Taq* polymerase (1.8 % gel).

CONCLUSION

In the present study we were able to observe that use of commercial *Taq* polymerase for the TP PCR clearly abolished the spurious bands, which appeared in the negative controls suggesting a strong chance for bacterial genomic interference between *Taq* polymerase enzyme and the primer sequences used in the master mix during amplification of FRDA gene. Hence forth we highly recommend the use of commercial *Taq* polymerase instead of homemade *Taq* polymerases in TP PCR.

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REFERENCES

1. Warner, Jon P, Barron D, Goudie et al. (1996). A general method for the detection of large CAG repeat expansions by fluorescent PCR. Journal of medical genetics. 33: 1022-1026
2. Ciotti P, Di Maria E, Bellone E et al. (2004). Triplet repeat primed PCR (TP PCR) in molecular diagnostic testing for Friedreich ataxia. The Journal of Molecular Diagnostics. 6: 285-289.
3. Holloway, Timothy P, Simone M et al. (2011). Detection of interruptions in the GAA trinucleotide repeat expansion in the FXN gene of Friedreich ataxia. BioTechniques. 50: 182-186.
4. Klockgether T. (2011). Update on degenerative ataxias. Current Opinion in Neurology. 4:339-345.
5. Marmolin D. (2011). Friedreich's ataxia: Past, present and future. Brain. 67: 311-330.
6. Schmucker S and Puccio H. (2010). Understanding the molecular mechanisms of Friedreich's ataxia to develop therapeutic Approaches. Human Molecular Genetics. 9:103-110.
7. Singh I, Faruq M, Mukherjee O et al. (2010). North and South Indian populations share a common ancestral origin of Friedreich's ataxia but vary in age of GAA repeat expansion. Annals of human genetics. 12: 202-210.
8. Ferralli P, Egan JD and Erickson FL. (2007). Making Taq DNA polymerase in the undergraduate biology laboratory. BIOS. 78: 69-74.

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