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Original Article

Comparing the efficiency of Taq DNA polymerase and PuRe Taq Ready-To-Go PCR beads in amplifying 12S and 16S ribosomal genes

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Abstract
In the present study, the efficiency of Taq DNA polymerase, an enzyme traditionally used in gene amplification, was compared with the newly developed amplification method, PuRe Taq Ready-To-Go PCR beads. One hundred seventy samples, including both fresh and up to five years old tissue samples, were compared. Taq DNA polymerase was found to be less efficient compared to the PuRe Taq Ready-To-Go PCR beads for amplification of the 12S rDNA gene. However, difference in the efficiency of both procedures was not statistically significant for the 16S rDNA gene. Furthermore, Taq DNA polymerase was found only efficient for fresh samples while PuRe Taq Ready-To-Go PCR beads were equally efficient for both old and new tissue samples.
Key words: Taq method, PCR beads, ribosomal genes, gene amplification

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INTRODUCTION

Currently different molecular techniques are being used for species identification and phylogenetic analyses (Bataille *et al.,* 1999; Wolf *et al.,* 1999). Nucleic acid based phylogenetic analyses rely on identification of various markers within the genome of organisms. Besides nuclear DNA, eukaryotic cells also contain DNA in their mitochondria. Mitochondrial genome is a double stranded DNA molecule encoding genes for various oxidative enzymes (Anderson *et al.,* 1981).

Mitochondrial genome has high mutation rate, making it useful for distinguishing between closely related species (Yang *et al.,* 2014). Usually the mitochondria are inherited maternally (Jenuth *et al.,* 1997). Ribosomal RNA encoded by mitochondria is the product of two ribosomal genes i.e., 12S and 16S and these genes occupy approximately 6.25% and 10% of whole genome respectively (Yang *et al.,* 2014). The mitochondrial 16S rDNA gene has highly conserved regions showing slow evolution (Page & Holmes, 1998) and 12S rDNA gene is used to study recent evolutionary events (Hillis & Dixon, 1991). Combined use of 12S rDNA and 16S rDNA genes as molecular markers provides a competent tool for inter and intra-specific phylogenetic analysis (Li *et al.,* 2010). *Taq* DNA polymerase has widespread usage for the amplification of specific genomic markers. In this method, various reagents i.e., enzymes, primers, nucleotides etc., are mixed manually for preparing reaction mixture. This process requires careful measurements and the chance of cross contamination due to human handling is also high. The recently developed PuRe Taq Ready-To-Go PCR beads method of gene amplification is devoid of all these problems (www.gehealthcare.com/lifesciences). In this method, micro beads of accurate formulation are produced containing all reactants of amplification reaction and only primers, the DNA template and water need to be added. In the present study, the efficiency of the traditional *Taq* DNA polymerase method was compared with the modern and newly developed PuRe Taq Ready-To-Go PCR beads method.

MATERIALS AND METHODS

In the present study, we compared the efficiency of the *Taq* DNA polymerase and PuRe Taq Ready-To-Go PCR beads method for the amplification of 12S rDNA and 16S rDNA genes

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of scorpions in the family Buthidae. In total we used DNA extractions of 170 scorpions belonging to the genus *Androctonus, Hottentota* and *Odontobuthus*. Of these, 135 tissue samples were fresh (less than six months old) while the remaining 35 tissue samples were 5 years old. Scorpions were collected using ultraviolet (UV) lamps from Sargodha, Khushab and Jhang districts of Punjab, Pakistan. Collected scorpions were preserved in 95% ethanol. DNA samples were extracted using Qiagen DNA extraction kit. The primer pairs used for the amplification of 12S and 16S rDNA genes are:

Primer	Sequence of primer
12Sai	5'AAACTAGGATTAGATACCCTATTA3'
12Sbi	5'AAGAGCGACGGGCGATGTGT3'
16SA	5'CTCCGGTTTGAACTCAGATCA3'
16SB	5'CGCCTGTTTATCAAAAACAT3'

For the *Taq* DNA polymerase method, a master mix was prepared by mixing 11 µL molecular grade water, 5 µL Buffer, 2.5 µL dNTPs, 2 µLMg, 1 µL forward primer, 1 µL - reverse primer, 0.25 µL BSA and 0.25 µL Taq polymerase (Thermo Scientific) per sample. Additionally, 2 µL DNA was added at the end to make the total reaction volume 25 µL. However, for the PuRe Taq Ready-To-Go PCR beads method, a master mix was prepared by adding 21 µL molecular grade water, 1 µL forward primer and 1 µL reverse primer. Finally, 2 µL DNA was also added. PCR tubes containing the mixture were spun for few seconds to allow for mixing of reagents and then placed in thermocycler. The PCR product (2 µL) was mixed with 3 µL loading buffer containing SYBR Safe dye. Samples were loaded on the 1% agarose gel which was run for 20 minutes at 200 volts. The gel was studied over UV illuminator and photographed for record. Chi-square test was used to compare the success rate of both methods in amplifying 12S and 16S genes. Results were considered significant if P-value was less than 0.05.

RESULTS AND DISCUSSION

Results of the study showed that efficacy *Taq* DNA polymerase method is comparatively less as compared to the PuRe Taq Ready-To-Go PCR beads method. The success rate of *Taq* DNA polymerase method in amplifying 12S rDNA genes was significantly less compared to PuRe Taq Ready-To-Go PCR beads method ($P < 0.05$; Fig. 1A). Although the PuRe Taq Ready-To-Go PCR beads method had a higher success rate in amplifying the 16S rDNA genes than the *Taq* DNA polymerase method, the difference was statistically non-significant ($P > 0.05$). The *Taq* DNA polymerase method is also efficient for fresh samples but does not work well for old samples (Fig.1B). However, the PuRe Taq Ready-To-Go PCR beads method not only worked well for fresh samples but also had high success rates with old samples. For old samples, statistically significant difference was observed between success rates of two methods for both genes ($P<0.05$ for both genes).

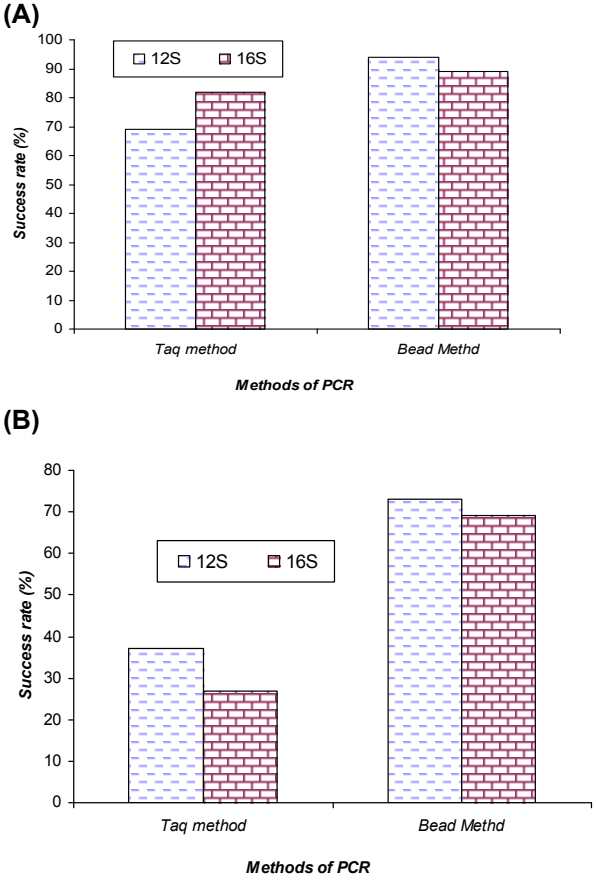

Figure 1: Comparison of Taq and Bead method in amplifying 12S and 16S genes of fresh (A) and old (B) samples.

Many researchers prefer the *Taq* DNA polymerase method over the PuRe Taq Ready-To-Go PCR beads method as there is more flexibility to change the concentration of any reagent while preparing the master mix. On the

other hand, PCR *Beads* are very easy to use and researchers can complete their tasks much more quickly than using the *Taq* DNA polymerase method. Furthermore, this newly developed method does not require prior optimization and is also associated with reduced chance of error and increased reproducibility (Girish *et al.,* 2004). PuRe Taq Ready-To-Go PCR beads are found reproducible even in those reactions where conventional PCR method failed to yield products (Littlewood and Michell, 2008). A researcher only adds water, primers and DNA sample. All other reagents are already present in PCR *Bead.*

Although the PuRe Taq Ready-To-Go PCR beads method is highly efficient for amplification of the 16S rDNA and 12S rDNA genes studied here, it is much more expensive than the *Taq* DNA polymerase method. That is why, in developing countries like Pakistan it is not feasible for the researcher to use PCR *Beads*. Even in well-established laboratories in advanced counties, researchers prefer the *Taq* DNA polymerase procedure instead of PuRe Taq Ready-To-Go PCR beads, as this method is cost effective and PCR *Beads* are only used for the difficult or old samples.

REFERENCES

ANDERSON, S., BANKIER, A. T., BARRELL, B. G., DE BRUIJN, M. H. L., COULSON, A. R., DROUIN, J., EPERON, I. C., NIERLICH, D. P., ROE, B. A., SANGER, F., SCHREIER, P. H., SMITH, A. J., STADEN, R., AND YOUNG, I. G., 1981. Sequence and organizati on of the human mitochondrial genome. *Nature*, **290** (5806), 457-465.

BATAILLE, M., CRAINIC, K., LETERREUX, M., DURIGON, M. AND DE MAZANCOURT, P., 1999. Multiplex amplification of mitochondrial DNA for human and species identification in forensic evaluation. *Forensic Sci. Int.,* **99**: 165–170.

GIRISH, P. S., ANJANEYULU, A.S.R., VISWAS, K.N., ANAND, M., RAJKUMAR, N., SHIVAKUMAR, B.M. AND BHASKAR, S., 2004. Sequence analysis of mitochondrial 12S rRNA gene can identify meat species. *Meat Sci*., **66**: 551–556.

HILLIS, D. M. AND DIXON, M. T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly Rev. Biol.,* **66**: 411–453.

JENUTH, J. P., PERTERSON, A. C., FU, K. AND SHOUBRIDGE, E. A., 1997. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat Genet.,***14**: 146–151.

LI, J., ZHAO, G. H., ZOU, F. C., MO, X. H., YUAN, Z. G., AI, L., LI, H. L., WENG, Y. B., LIN, R. Q. AND ZHU, X. Q., 2010. Combined mitochondrial 16S and 12S rDNA sequences: an effective genetic marker for inter-species phylogenetic analysis of zoonotic trematodes. *Parasitol. Res*., **107**: 561–569

LITTLEWOOD, P. AND MITCHELL, V., 2008. PCR Amplification Performance Comparison. *http://www.americanlaboratory.com/913-Technical-Articles/18716-PCR-Amplification-Performance-Comparison/*

PAGE, R. D. M. AND HOLMES, E. C., 1998. Inferring molecular phylogeny, In: *Molecular evolution: A phylogenetic approach*. Blackwell Science Ltd, Oxford, pp. 172–227.

WOLF, C., RENTSCH, J. AND HUBNER P., 1999. PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification. *J Agric Food Chem*., **47**: 1350–1355.

YANG, L., TAN, Z., WANG, D., XUE, L., GUAN, M., HUANG, T. AND LI, R., 2014. Species identification through mitochondrial rRNA genetic analysis. *Sci. Rep*.**, 4**:4089.