

Amplification of whole human mitochondrial DNA with Phusion™ High-Fidelity DNA Polymerases

In this study, the performance of five different polymerases was compared when amplifying whole human mitochondrial DNA. Finnzymes' Phusion™ High-Fidelity Polymerases were compared to three enzyme mixes recommended for amplification of long templates by the manufacturers. Phusion™ High-Fidelity DNA Polymerases performed the amplification in less time and with fewer units of enzyme than the other polymerases. Reliable amplification of human mitochondrial DNA can be accomplished more quickly and cost-effectively with Phusion™ High-Fidelity DNA Polymerases.

Introduction

Human mitochondrial DNA is a 16.6 kb circular double-stranded molecule. Mutations (deletions, duplications and point mutations) in the mitochondrial genome leading to mitochondrial dysfunction are increasingly recognized as a contributor to a wide range of human diseases. Mitochondrial dysfunction is involved in diseases such as diabetes, cancer, heart diseases and migraine. In addition, neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease are associated with mitochondrial dysfunction.

Amplifying the whole mitochondrial genome using PCR has been found to be an efficient method for detecting mitochondrial DNA deletions involved in human diseases¹. Whereas amplifying the whole mitochondrial genome is generally useful for detecting mitochondrial deletions, that are typically relatively large, amplifying shorter target fragments is also widely used for detecting other types of mutations (e.g. point mutations) in the mitochondrial genome.

Materials and Methods

Finnzymes' Phusion™ High-Fidelity DNA Polymerases consist of a novel *Pyrococcus*-like enzyme with a double-stranded DNA-binding domain, which gives the fusion polymerase high processivity and fidelity. The performance of Phusion™ High-Fidelity DNA Polymerases and three polymerase mixes from two other vendors were compared in amplification of whole human mitochondrial DNA. Total DNA isolated from human blood was used as a template. The amplified product was 16.5 kb. The primers used for the amplification anneal to mtDNA at the following positions: forward 10-40; reverse 16 494-16 463¹. All reactions were conducted using conditions recommended by the manufacturers. The amounts of enzymes in units and total cycling times are shown in Figure 1. The reactions were set up on ice and run on a DNA engine Tetrad 2 thermal cycler (Bio-Rad Laboratories, Inc.).

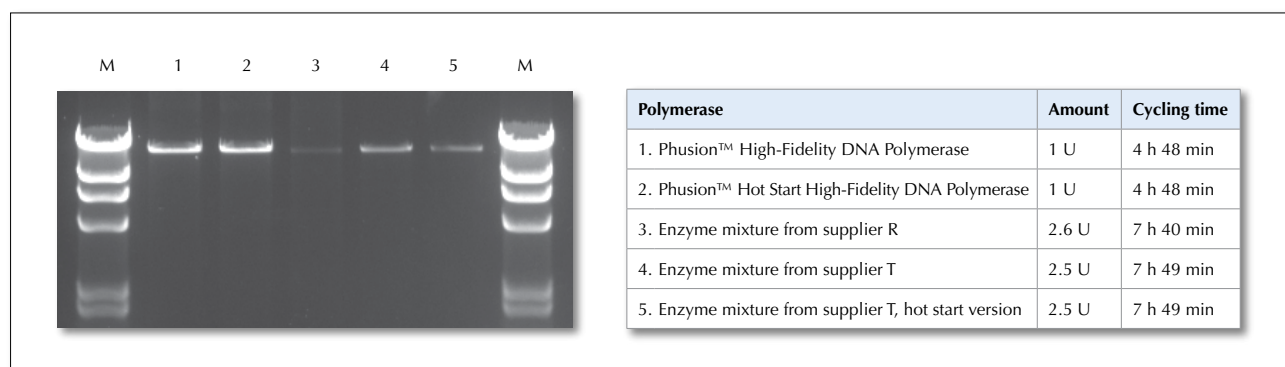


Figure 1. Amplification of whole mitochondrial genome using Phusion™ DNA Polymerases and three polymerases from two other suppliers. Phusion DNA Polymerases provided higher yield with shorter cycling times with less enzyme than the other polymerases.

The conditions for Phusion™ High-Fidelity DNA Polymerase and Phusion™ Hot Start DNA Polymerase were as follows:

<ul style="list-style-type: none">• 50 ng template• 0.5 µM primers• 200 µM dNTPs• 1 x GC buffer• 1 U enzyme
<ul style="list-style-type: none">• Total volume 50 µl

The cycling conditions for Phusion™ High-Fidelity DNA Polymerases were as follows:

Temperature	Time	Number of cycles
98 °C	30 s	1
98 °C	10 s	30 cycles
72 °C	8 min 15 s	
72 °C 4 °C	10 min hold	1

Conclusions

The performance of five different polymerases was compared when amplifying whole human mitochondrial DNA. Phusion DNA Polymerases completed the entire PCR reaction in less than 5 hours, while the other polymerases required almost 8 hours. Compared to the three other polymerases tested, which are recommended by the manufacturers for amplification of long genomic targets, Phusion DNA Polymerases provided high yield with lower enzyme amounts.

An important advantage of Phusion DNA Polymerases is their extremely low error rate. It is 50-fold lower than that of *Taq* polymerase. Two different buffers are provided with Phusion DNA Polymerases: Phusion HF Buffer (error rate 4.4×10^{-7}) and Phusion GC Buffer (error rate 9.5×10^{-7}). HF Buffer should be used as the default buffer for high-fidelity amplification. GC Buffer can, in turn, improve the performance on some difficult or long templates. Due to their high accuracy, Phusion DNA Polymerases can reliably be used for studying mitochondrial DNA point mutations. In conclusion, Phusion DNA Polymerases perform accurate and fast amplification of mitochondrial DNA.

Acknowledgements

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References

1. Tengan C.H. and Moraes C.T. (1996) Detection and analysis of mitochondrial DNA deletions by whole genome PCR. *Biochem Mol Med* 58: 130-134.

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