Application Note

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High Temperature PCR with DyNAzyme™ II DNA Polymerase Provides Fast and Efficient Results

DyNAzyme™ II DNA Polymerase, a recombinant thermostable DNA polymerase, exhibits some advantages over *Taq* polymerase when performing PCR, including better thermal stability at denaturation temperatures and superior tolerance to DMSO. DyNAzyme II DNA Polymerase works well as a replacement for *Taq* polymerase for all standard PCR protocols. This application note describes improved protocols that can be used with DyNAzyme II DNA Polymerase on fast PCR instruments such as the Piko™ Thermal Cycler and ultra-thin walled (UTW™) tubes and plates (Finnzymes Oy). With these improved protocols, PCR can be performed in less than 1 hour for amplicons smaller than 1kb. Moreover, the consistency and yield of the resulting fragments are typically equal to or better than that derived from *Taq* DNA polymerase.

Introduction

Mostly for historical reasons, *Taq* DNA polymerase, isolated from the thermophile *Thermus aquaticus*, is the most popular enzyme for performing routine PCR. Subsequently, newer polymerases with improved properties have been discovered or engineered. Finnzymes' DyNAzyme II DNA Polymerase, a recombinant form of a DNA polymerase derived from *Thermus brockianus* was first introduced in 1991. Although similar to *Taq* polymerase, DyNAzyme II DNA Polymerase exhibits greater thermal stability at temperatures above 94°C, allowing for denaturation at higher temperatures during PCR. Higher denaturation temperatures can improve PCR performance, especially with amplicons that have a high GC-content or particularly strong secondary structures (1). *Taq* polymerase is less well suited for high temperature protocols as the activity of the enzyme above 94°C is relatively short-lived.

In most cases DyNAzyme II DNA Polymerase can be substituted directly for *Taq* polymerase in standard PCR reactions with no change in protocol conditions (detailed instructions available on www.finnzymes.com). However, when combined with a fast instrument such as the Piko Thermal Cycler and ultra-thin walled UTW reaction vessels, protocols can be reduced to as little as 40 minutes for small amplicons. This application note describes how to achieve faster protocol times with DyNAzyme II DNA Polymerase while maintaining or improving product yield.

Protocol for DyNAzyme II Polymerase on fast PCR systems

DyNAzyme II DNA Polymerase is well suited for protocols run on fast thermal cyclers (i.e. those that are able to maintain an average thermal ramp rate of sample of 4°C per second or greater, such as the Piko Thermal Cyclers used for these experiments). For conventional cyclers, it is recommended to use the standard reaction conditions for DyNAzyme II DNA Polymerase.

The Piko Thermal Cycler, when combined with UTW vessels, allows denaturation and annealing hold times during PCR to be reduced by 2-fold to 5-fold, even as compared to other fast thermal cyclers. The exceptional thermal characteristics offered by the combination of these technologies ensure that even at these ultra-short incubation times, very consistent yields are obtained from every well in the sample block. Other thermal cyclers and consumables often do not provide this level of consistency with such rapid protocols.

Materials and methods

- DyNAzyme™ II DNA polymerase (F-501, Finnzymes Oy)
- 10x Optimized DyNAzyme™ Buffer
- 10 mM dNTP Mix
- Purified Human genomic DNA (50 ng/µl)
- Piko™ Thermal Cycler (Finnzymes Oy)
- UTW™ tubes or plates (Finnzymes Oy)
- Primers

588 bp fragment of Glutathione peroxidase 3 gene:

Forward	TGTGGCGGTCTAGGGTGTATT	21nt	Tm 65.7
Reverse	GGTGGGCTGGTCGTGATG	18nt	Tm 68.4

1217 bp fragment of Glutathione peroxidase 3 gene:

Forward	CTGACCCCCACTATCCCTTGACA	23nt	Tm 70.3
Reverse	CTTGGACTGGCCCTTTCTTTTCTT	24nt	Tm 68.3

Table 1. Pipetting instructions

Component	15 µl react.	Final conc.
H_2O	add to 15 µl	
10x Optimized DyNAzyme™ Buffer	1.5 µl	1x
10 mM dNTP Mix	0.3 µl	200 µM
Primer A	x µl	0.5 µM
Primer B	x µl	0.5 µM
Purified DNA (50 ng/µl)	0.3µl	15 ng
DyNAzyme™ II DNA Polymerase (2 U/µl)	0.15 µl	20 U/ml

Table 2. Cycling instructions (Piko Thermal Cycler & UTW vessels only)

	3-step protocol		
Cycle step	**Temp.**	**Time**	**Cycles**
Initial denaturation	96°C	30-60 s	1
Denaturation Annealing* Extension	96°C X°C 72°C	5-15 s 5-15 s 40 s/1 kb	30-35
Final extension	72°C 4°C	1-3 min hold	1

* Recommended annealing temperature is 5°C below the Tm of the primers, as calculated by the nearest neighbor method (www.finnzymes.com/tm_determination.html).

Results

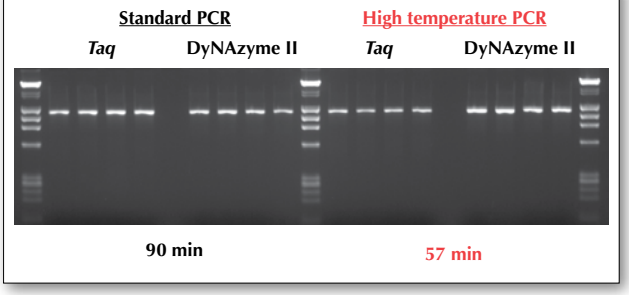

Figure 1. Comparison of standard and modified PCR protocols in amplification of a 1.2 kb fragment of glutathione peroxidase 3 gene showing improved results with DyNAzyme II DNA Polymerase compared to Taq. All reactions were setup as instructed above and run in quadruplicate. Cycling conditions for standard PCR: 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 72 s, followed by a final extension step of 72°C for 5 min. Total protocol time was 90 min. Cycling conditions for a shortened protocol using higher denaturation temperature: 96°C for 30 s, followed by 30 cycles of 96°C for 10 s, 65°C for 15 s and 72°C for 48 s, followed by a final extensions step of 72°C for 1 min. Total protocol time was 57 minutes.

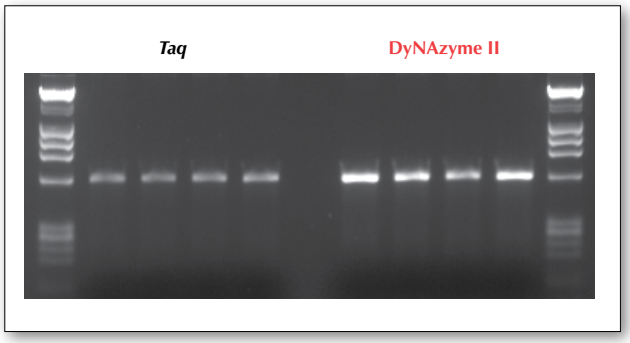

Figure 2. Improved yield using DyNAzyme II DNA Polymerase in a fast PCR protocol. PCR reactions were set up as described above. A 0.6 kb fragment of the human glutathione peroxidase 3 gene was amplified. Cycling conditions were: 96°C for 30 s, followed by 30 cycles of 96°C for 5 s, 60°C for 10 s and 72°C for 24 s, followed by a final extensions step of 72°C for 3 min. Total protocol time was 41 minutes.

Discussion

DyNAzyme II DNA Polymerase works well for standard PCR applications where *Taq* polymerase is typically used. Taking advantage of the improved stability of DyNAzyme II DNA Polymerase at high temperatures, we have here developed a protocol featuring hotter and faster denaturation steps. When combined with the fast Piko Thermal Cycler and ultra-thin wall consumables, protocol times can be reduced while maintaining good product yields.

Finnzymes also offers a line of High Performance PCR products that allow the fastest possible PCR reactions while maintaining maximum fidelity and product yields. For more information please visit the High Performance PCR website (www.highperformancePCR.com).

References

Dutton, C. *et al.* (1993) "General method for amplifying regions of very high G + C content". *Nucleic Acids Research*, Vol. 21, No. 12, pp 2953-2954.