Comparison of DNA polymerases for real-time PCR with SYBR® Green I

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Nine commercial DNA polymerases were evaluated for real-time PCR performance. The polymerase with the fastest elongation rate was SpeedSTAR™ from Takara. For non-hotstart enzymes, Finnzymes' Phusion and Biotools' DNA polymerases had the best balance of speed and minimal primer-dimer formation. For hotstart enzymes, the best performers were SpeedSTAR HS from Takara and KAPA2G Robust from Kapa Biosystems.

Introduction

DNA polymerase from *Thermus aquaticus* (Taq) was the first to be used for PCR (Ref 1). Since then, many other heat-resistant DNA polymerase enzymes have been discovered or genetically engineered for improved PCR performance.

Most polymerases have optimal enzymatic activity around 72-76°C, but display some activity at room temperature (Ref 2). This residual activity is believed to be responsible for the formation of non-specific products during PCR. To prevent this problem, hotstart polymerases have been developed where the polymerase is only activated once a permissive temperature has been reached.

On one hand, hotstart polymerases claim to prevent the formation of non-specific products and primer-dimers. On the other hand, non-hotstart polymerases are less expensive and save time by avoiding activation steps.

The purpose of this study was to determine which commercial hotstart and non-hotstart DNA polymerases have the fastest extension rates and lowest formation of non-specific PCR products.

Company	DNA Polymerase	Cat. No.	Hot-Start
Biotools	DNA Polymerase	BT1004B	No
Invitrogen	Platinum Taq DNA Polymerase	19066-018	No
Qiagen	TopTaq DNA Polymerase	200203	No
Lucigen	EconoTaq DNA Polymerase	30031-0	No
Finnzymes	Phusion High-Fidelity DNA Polymeras	F-530	No
KAPA Biosystems	KAPA2G Robust Hotstart DNA Polymerase	KK 5514	Yes
Takara	SpeedSTAR HS DNA Polymerase	RR070A/B	Yes
Bio-Rad	iTaq DNA Polymerase	170-8870	Yes
Finnzymes	Phire Hotstart DNA Polymerase	F-1205	Yes

Table 1. Commercial DNA polymerases.

Materials and Methods

DNA isolation

DNA was isolated from clinical isolates of *Staphylococcus aureus*. For each isolate, 4-5 medium-sized bacterial colonies were re-suspended in 100 µl of lysis buffer (50 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, pH 8) with 2 µl of 1 mg/ml lysostaphin (Sigma-Aldrich). The samples were incubated at 37°C for 30 min. Following this incubation, 5 µl of 20 mg/µl Proteinase K (Sigma-Aldrich) was added to the mixture and the tubes were shaken at 50°C for 1 h. The tubes were then incubated at 100°C for 10 min to inactivate the Proteinase K. Samples were adjusted to a concentration of 750 ng/µl and stored at -20°C.

Template DNA

To ensure consistent results, a 279-bp PCR amplicon was used as the DNA template for all experiments. The amplicon was generated by amplifying a region of the nuclease gene from a clinical isolate of *S. aureus* . The PCR product was diluted 1:16 before being used as the DNA template.

Real-time PCR

Oligonucleotide primers were designed against a region of the *S. aureus* nuclease gene (Ref 3). The forward primer was 5'-gcg att gat

Component	Final Concentration
PCR Reaction Buffer*	1 X
$MgCl_2$ (Invitrogen)**	2.5 mM
dNTP mix (Invitrogen)	0.125 mM
DNA polymerase (9 different brands)	1 U
SYBR Green I (Invitrogen)	0.5 X
PCR primers (Sigma-Aldrich)	0.5 µM
Template DNA	1.5 µg
Sterile water	
Total reaction volume	**20 µl**

**Buffers supplied with the DNA polymerases were used. If no PCR buffer was supplied, then 10X PCR reaction buffer (no $MgCl_2$) from Invitrogen was used. Buffers for low GC templates were used where available.*
*** For Takara's SpeedSTARPolymerase, the supplied buffer had a final concentration of 3mM $MgCl_2$.*

Table 2. Components of the amplification mixtures.

ggt gat acg gtt-3' and the reverse primer was 5'-agc caa gcc ttg acg aac taa agc-3'. The expected amplicon size was 279 bp. SYBR Green I was used as the fluorescent dye for real-time PCR.

Nine different DNA polymerase enzyme were tested (Table 1). Components of the real-time PCR amplification mixtures are listed in Table 2. Cycling parameters for both regular and fast programs are listed in Tables 3 and 4, respectively. In the regular cycling program, the annealing/extension time was 25 s. In the fast program, the time for the denaturation step was shortened to 25 s, and the annealing/extension step was shortened to 14 s. It was anticipated that faster DNA polymerases would not be affected by the decrease in time, and threshold cycle (Ct) values would be equivalent between the regular and fast programs.

To assess the formation of non-specific amplification products, reactions were set up without template DNA. Since SYBR Green binds to any double-stranded DNA, it was anticipated that reactions generating more non-specific products would result in earlier Ct values compared to reactions with higher specificity.

Samples were loaded into 0.2 ml thin-wall, flat-cap PCR tubes (VWR Cat. No. 53550-106) and overlaid with 15 µl of PCR-grade mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation. Real-time PCR was performed using the Spartan DX™. All reactions were performed in triplicate.

Step	Temperature	Time	Cycles
Initial denaturation	97.6°C	30 s	1
Denaturation	97.6°C	30 s	50
Annealing/extension	56.9°C	25 s	50

Table 3. Cycling parameters for regular program.

Step	Temperature	Time	Cycles
Initial denaturation	97.6°C	30 s	1
Denaturation	97.6°C	25 s	50
Annealing/extension	56.9°C	14 s	50

Table 4. Cycling parameters for fast program.

DNA analysis

Fluorescent results from the Spartan DX were graphed using Spartan Analyzer software. Results were also confirmed by agarose gel electrophoresis using 5-7 µl of the amplification products.

Results

With the regular cycling program, Ct values for the 9 polymerases ranged from 7.7 to 14.5 (Table 5). With the fast program, SpeedSTAR polymerase (Takara) had the fastest extension rate as evidenced by the lowest Ct value of 7.7, followed by DNA polymerases from KAPA Biosystems (KAPA2G Robust), and iTaq from Bio-Rad. Although Qiagen's TopTaq DNA polymerase had a delayed Ct when compared with the other polymerases, it has the advantages of stability at 4°C and room-temperature setup. In terms of minimizing non-specific amplification products, the polymerases from Takara and KAPA Biosystems performed the best, as evidenced by Ct values of 40 or greater when reactions were performed without template DNA.

Discussion and Conclusions

DNA Polymerase	Regular Program (Ct)	Fast Program (Ct)	No Template* (Ct)
Polymerase (Biotools)	9.3 (n=18)	13 (n=19)	32.9 (n=19)
TopTaq (Qiagen)	14.5†	NA	NA
iTaq (Bio-Rad)	9	11.9‡	34‡
EconoTaq (Lucigen)	10†	NA	NA
Phusion (Finnzymes)	9.7	9.7	31.7
KAPA2G Robust (KAPA)	8.7	13.7	41.5†
Platinum Taq (Invitrogen)	9.2	NA	NA
SpeedSTAR HS (Takara)	7.7	9.3	42.8
Phire (Finnzymes)	9.33	10.7	31.7

NA = No Amplification

* *Formation of non-specific amplification products was evaluated without template DNA.*

† *Average of 2/3 reactions, 1/3 reactions resulted in no amplification.*

‡ *Data is for n=1, only 1/3 reactions amplified*

Table 5. Threshold cycle (Ct) values for 5 polymerases with regular and fast cycling programs.

With a regular cycling program, most of the DNA polymerases generated similar real-time PCR results with SYBR Green I. For fast cycling, the SpeedStar polymerase from Takara had the fastest elongation rate, followed closely by Phusion Taq by Finnzymes. For a balance between fast elongation and minimal non-specific amplification products, the best performers were the non-hotstart polymerase from Biotools, and the hotstart polymerases from Takara and KAPA Biosystems.

References

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