Long PCR products and PCR kits

Hanxiao Zhao[1], Yukun Jia[1], Xianyu Yang[1,2,3*]

[1]College of Animal Science and Technology, Zhejiang A&F University, 666 Wusu Street, Hangzhou 311300, China

[2]Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, 666 Wusu Street, Hangzhou 311300, China

[3]Zhejiang Provincial Engineering Laboratory for Animal Health Inspection and Internet Technology, 666 Wusu Street, Hangzhou 311300, China

**Corresponding author: Xianyu Yang, E-mail: yangxy78@zafu.edu.cn*

Abstract: *To amplify a DNA segment of 5 000 base pairs (5 kb) from genomic DNA of Chinese toad (Bufo gargarizans), several polymerase chain reactions (PCR) were carried out using 4 different PCR kits (Taq kit, PrimeSTAR MAX kit, LA Taq kit and LA Taq with GC Buffer kit), 3 different concentrations of genomic DNA from two individuals as templates (1 μg/μl, 0.1 μg/μl, 0.01 μg/μl), and a pair of specific primers. The results showed that PrimeSTAR MAX kit made the successful amplification from the second individual with the template concentrations of 0.1 μg/μl and 0.01 μg/μl, but not 1 μg/μl. In case of LA Taq kit, PCR products were detected from both individuals with templates of 1 μg/μl and 0.1 μg/μl, but not 0.01 μg/μl. However, neither Taq kit nor LA Taq with GC Buffer kit made PCR product no matter template concentrations and individuals. These results indicated the effects of PCR kit and template concentration on long PCR products, which give us suggestions to choose suitable PCR kits and adjustment of template concentrations when there is no amplification of long DNA segment.*

Keywords: *Bufo gargarizans; Long PCR product; PCR kit; Template concentration*

Introduction

As one of the most important biological molecular techniques, PCR is used to amplify specific DNA segments[1], which has been used in many fields[2,3]. However, the cases of no PCR products are often encountered, therefore optimization of PCR conditions has long been an important topic[4-6]. Addition of DMSO solved the problem of GC-rich template amplification[7], and lowering extension temperature made AT-rich tandem repeats amplified well[8]. In our previous study, qPCR kit gave excellent amplification of short DNA segments relative to routine *Taq* PCR kit[9]. Recently, to amplify 5 kb genomic DNA segment of *B. gargarizans*, we tried several PCR kits and several concentrations of DNA template. The results indicated the effects of PCR kits and template concentrations on the long PCR products, which will be reported here.

Materials and Methods

Materials

Two individuals of *B. gargarizans* were captured on the East Lake Campus of Zhejiang A&F University, and ice anaesthetized before dissection. Their livers were removed and chopped into small pieces for DNA extraction. Four PCR kits [*Taq* (Cat#R001), PrimeSTAR MAX (Cat#R045), LA *Taq* (Cat#RR02MA) and LA *Taq* with GC Buffer (I/II) (Cat#RR02AG)] were from Takara (Dalian, China), agarose (Cat#ST004L) and DNA ladder (Cat#D0107) from Beyotime Biotechnology Institute (Nantong, China). Forward (Fw) primer (5'-CCGTATTAAGATATGGC-3') and reverse (Re) primer (5'-CAATGAATCAGGCTTTTCAT-3') were synthesized by GENEWIZ (Suzhou, China).

Methods

Genomic DNA was extracted by phenol/chloroform method [10], and its concentration was adjusted to about 1 μg/μl in H_2O, which was further diluted to make DNA solutions of 0.1 μg/μl and 0.01 μg/μl. All three solutions were used as PCR templates. Primers were dissolved in sterilized H_2O and diluted to 2 μmol/L. Four sets of PCR were prepared according to the Manufactures' Instructions (Table 1). For each concentration of template, three duplications were carried out. The following are the parameters of PCR cycles. For *Taq*, LA *Taq* and LA *Taq* with GC Buffer (I/II) kits: 94 ℃/2 min; (94 ℃/20 S, 58 ℃/20 S, 72 ℃/6 min) for 35 cycles; for PrimeSTAR MAX kit: 98 ℃/2 min; (98 ℃/10 S, 58 ℃/5 S, 72 ℃/2 min) for 35 cycles. Two μl PCR products were loaded on each lane for detection with 0.8% agarose gel electrophoresis.

Table 1 PCR constitutions for each kit

Taq kit		PrimeSTAR MAX kit		LA *Taq* kit		LA *Taq* with GC Buffer kit	
Components	Vol	Components	Vol	Components	Vol	Components	Vol
10×Buffer	2.0 μl	2×Premix	10.0 μl	10×Buffer	2.0 μl	2×Buffer I/II	10.0 μl
dNTPs	0.8 μl	Fw Primer	1.0 μl	dNTPs	0.8 μl	dNTPs	0.8 μl
Taq	0.2 μl	Re Primer	1.0 μl	LA *Taq*	0.2 μl	LA *Taq*	0.2 μl
Fw Primer	1.0 μl	Template	1.0 μl	Fw Primer	1.0 μl	Fw Primer	1.0 μl
Re Primer	1.0 μl	H_2O	7.0 μl	Re Primer	1.0 μl	Re Primer	1.0 μl
Template	1.0 μl			Template	1.0 μl	Template	1.0 μl
H_2O	14.0 μl			H_2O	14.0 μl	H_2O	6.0 μl

Results and Discussions

DNA extraction

Liver genomic DNA was successfully extracted from two individuals (Figure 1).

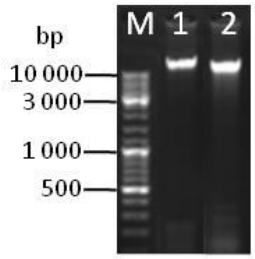

Figure 1 Genomic DNA extracted from livers of two individuals of *Bufo gargarizans*

M: DNA ladder; 1: The first individual (about 0.5 μg); 2: The second individual (about 0.5 μg)

PCR amplification

In current study, 24 combinations of PCR were performed with 4 different PCR kits and 3 concentrations of template from two individuals as summarized in Table 2. PCR products were detected from both experiments with PrimeSTAR MAX kit and LA *Taq* kit, but there was no PCR product with either *Taq* or LA *Taq* with GC Buffer I/II (Table 2).

Table 2 Effects of template concentrations and PCR kits on PCR results (Rs)

Template	*Taq*		PrimeSTAR MAX		LA *Taq*		LA *Taq* (GC)	
(μg/μl)	Rs1	Rs2	Rs1	Rs2	Rs1	Rs2	Rs1	Rs2
1	-	-	-	-	+	+	-	-
1	-	-	-	-	+	+	-	-
1	-	-	-	-	+	+	-	-
10^{-1}	-	-	-	+	+	+	-	-
10^{-1}	-	-	-	+	-	+	-	-
10^{-1}	-	-	-	+	+	-	-	-
10^{2}	-	-	-	+	-	-	-	-
10^{-2}	-	-	-	+	-	-	-	-
10^{-2}	-	-	-	+	-	-	-	-

1: The 1st Individual; 2: The 2nd Individual; ＋: PCR product detected; —: No PCR product

Although PCR products were detected from both experiments with PrimeSTAR MAX kit and LA *Taq* kit, the effective template concentrations (genomic DNA of Chinese toad) are different. In case of PrimeSTAR MAX kit, the effective concentrations were 0.1 μg/μl and 0.01 μg/μl (Figure 2A, lane 13-18), but 1 μg/μl did not work (Figure 2A, lane 10-12). In case of LA *Taq* kit, the effective concentrations are 1 μg/μl and 0.1 μg/μl (Figure 2B, lane 1-4, 6, 10-14), while template of 0.01 μg/μl did not work (Figure 2B, lane 7-9, 16-18). One more difference is that PCR products were only detected from the second individual with PrimeSTAR MAX kit. However, LA *Taq* kit made successful PCR amplification from both individuals.

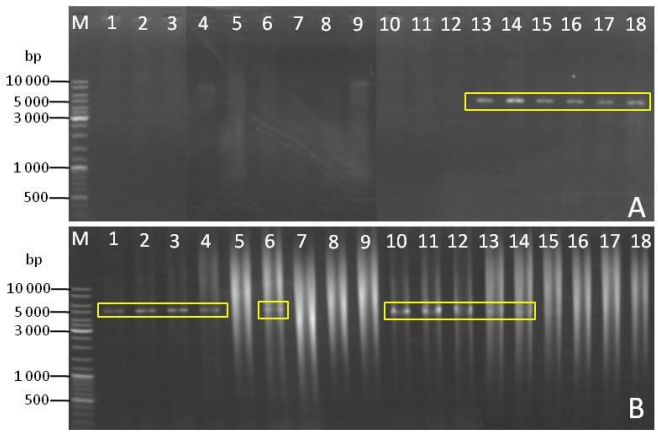

Figure 2 PCR products with different PCR kits and different template concentrations

M: DNA ladder. A: PrimeSTAR Max kit. B: LA *Taq* kit. Lane 1-9: The 1st individual. 10-18: The 2nd individual. Lane 1-3, 10-12: 3 duplicates of 1 μg/μl template; 4-6, 13-15: 3 duplicates of 0.1 μg/μl template; 7-9, 16-18: 3 duplicates of 0.01 μg/μl template.

In fact, these experiments were performed several times and each experiment gave the same results. Concerning the mechanism making such differences, it is not clear currently, which should be clarified in future. Anyway, the results obtained in this study indicated the importance of PCR kits as well as template concentrations.

Concerning the optimization of long DNA segment PCR, there have been some reports [11,12], either suggestion of PCR microtube selection[11] or long-range PCR technique [12]. Here, we tried several different PCR kits and different template concentrations, and the PCR condition for amplification of 5 kb DNA segment was found. Therefore, we emphasize to choose suitable PCR kits and the adjustment of template DNA concentrations before optimization of other parameters when no PCR products are detected. Based on our results, we prefer to use PrimeSTAR MAX kit in future studies, which provides a high fidelity DNA polymerase (PrimeSTAR MAX) with high amplification efficiency due to the shorter denaturing, annealing and elongation time (the reaction time can be greatly shortened) relative to *LA Taq*. In our previous experiments, real-time quantitative fluorescent PCR kit gave excellent amplification for short PCR segment (several hundred base pair DNA segment), which did not made the amplification of 5 kb DNA segment[9]. However, neither the effect of Thermal Cyclers nor the effect of PCR tubes have been confirmed[9].

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