# The effects of PCR kits indicated during gene amplification of *Panthera tigris amoyensis*

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***Abstract:*** *It is not rare that no product could be detected after polymerase chain reaction (PCR). During the cloning of some DNA segments of South China tiger (Panthera tigris amoyensis), it was found that different PCR kits gave different amplification results. To clarify this issue, a series of PCR were carried out by adopting different PCR kits, PCR tubes and Thermal Cyclers from different manufactures. Among 4 DNA segments (A-D), D was amplified well regardless of PCR kits, A was amplified well only with real-time quantitative fluorescent PCR (qPCR) kit, meanwhile the amplification of B and C showed different levels depending on the PCR kits. All these results indicated the importance of PCR kits, while neither Thermal Cyclers nor PCR tubes affect the PCR products.*

***Keywords:*** *Panthera tigris amoyensis; PCR kit; Thermal Cyclers*

## Introduction

PCR is one of the most important biological molecular techniques, which is used to amplify specific DNA segments[1]. Currently, PCR is very popular being used in many fields not limited to the university laboratories or institutes, especially in animal species identification including human[2-4] and livestock[5]. However, the PCR conditions have to be optimized when no PCR products were detected[6-8]. For example, addition of DMSO is helpful for amplification of GC-rich template[9], and lowering extension temperature is useful to amplify AT-rich tandem repeats[10]. Primer screening has been done during the amplification of *Rheum* chloroplast microsatellite[11]. Recently, we found qPCR kit gave excellent amplification, while there were no or few PCR products when *Taq* PCR kit was used for the amplification of some DNA segments from the genomic DNA of *P. tigris amoyensis*. To clarify this issue and to know if other factors, such as Thermal Cyclers and PCR tubes have any effects on PCR products, a series of PCR were carried out and obvious effect of PCR kit was indicated.

## Materials and Methods

### Materials

Genomic DNA was extracted from stillbirth heart of *P. tigris amoyensis* (provided by Hangzhou Safari Park, China) (simplified as tiger). Four sets of primers (F1/R1, F2/R2, F3/R3, F4/R4) were designed and synthesized by GENEWIZ (Suzhou, China) (Table 1) to amplify 4 different DNA segments: A, B, C and D, respectively. The names of manufactures providing PCR kits, tubes and Thermal Cyclers, are not given here due to the sensitivities.

**Table 1 Primers used in current study**

| Primer | Sequence (5'-3') | Primer | Sequence(5'-3') |
|---|---|---|---|
| F1 | CCGGAACACGGTCCTGTC | R1 | GAACGTCCTGAACGTCACG |
| F2 | CTTACCTGGGGGCACTGTA | R2 | GTAGAATTGTTGGAGAAAATGAC |
| F3 | TGTGAGTTTGAGCCCCACAT | R3 | CAGCCAATGGAGAAATGGTG |
| F4 | CTCCCTACGGCTCTTGCTG | R4 | CTGAGATCCTGAAACTATTTGC |

**Table 2 PCR constitutions**

| Components | *Taq* PCR | qPCR |
|---|---|---|
| 10× *Taq* buffer | 1.0 μl | 0.0 μl |
| 10 mM dNTPs | 0.8 μl | 0.0 μl |
| *Taq* (5U/μl) | 0.1 μl | 0.0 μl |
| 2× qPCR Master Mix | 0.0 μl | 5.0 μl |
| Primer F (2 pmol/μl ) | 0.5 μl | 0.5 μl |
| Primer R (2 pmol/μl) | 0.5 μl | 0.5 μl |
| Tiger gDNA(20 ng/μl) | 0.5 μl | 0.5 μl |
| $H_2O$ | 6.6 μl | 3.5 μl |

**Table 3 PCR-related parameters and PCR results (Res)**

| Exp | Res | Exp | Res | Exp | Res | Exp | Res |
|---|---|---|---|---|---|---|---|
| 1-A-qRqTqC* | ++ | 5-A-tRqTqC | - | 9-A-qRqTtC | ++ | 13-A-tRqTtC | - |
| 2-A-qRqTqC | ++ | 6-A-tRqTqC | - | 10-A-qRqTtC | ++ | 14-A-tRqTtC | - |
| 3-A-qRtTqC | ++ | 7-A-tRtTqC | - | 11-A-qRqTtC | ++ | 15-A-tRqTtC | - |
| 4-A-qRtTqC | ++ | 8-A-tRtTqC | - | 12-A-qRqTtC | ++ | 16-A-tRqTtC | - |
| 1-B-qRqTqC | +++ | 5-B-tRqTtC | + | 9-B-qRqTtC | +++ | 13-B-tRqTtC | + |
| 2-B-qRqTqC | +++ | 6-B-tRqTqC | + | 10-B-qRqTtC | +++ | 14-B-tRqTtC | + |
| 3-B-qRtTqC | +++ | 7-B-tRqTqC | + | 11-B-qRqTtC | +++ | 15-B-tRqTtC | + |
| 4-B-qRtTqC | +++ | 8-B-tRqTqC | + | 12-B-qRqTtC | +++ | B-16-tRqTtC | - |
| 1-C-qRqTqC | ++ | C-5-tRqTqC | + | 9-C-qRqTtC | ++ | 13-C-tRqTtC | + |
| 2-C-qRqTqC | ++ | C-6-tRqTqC | + | 10-C-qRqTtC | ++ | 14-C-tRqTtC | + |
| 3-C-qRtTqC | ++ | C-7-tRqTqC | + | 11-C-qRqTtC | ++ | 15-C-tRqTtC | + |
| 4-C-qRtTqC | ++ | C-8-tRqTqC | + | 12-C-qRqTtC | ++ | 16-C-tRqTtC | + |
| 1-D-qRqTqC | ++++ | 5-D-tRqTqC | ++++ | 9-D-qRqTtC | ++++ | 13-D-tRqTtC | ++++ |
| 2-D-qRqTqC | ++++ | 6-D-tRqTqC | ++++ | 10-D-qRqTtC | ++++ | 14-D-tRqTtC | ++++ |
| 3-D-qRtTqC | ++++ | 7-D-tRqTqC | ++++ | 11-D-qRqTtC | ++++ | 15-D-tRqTtC | ++++ |
| 4-D-qRtTqC | ++++ | 8-D-tRqTqC | ++++ | 12-D-qRqTtC | ++++ | 16-D-tRqTtC | ++++ |

Arabic numbers are corresponding to lane numbers of Figure 1. A, B, C and D: DNA segments of A, B, C and D, respectively. qR: qPCR kit; tR: *Taq* PCR kit; qT: qPCR tube; tT: Tubes for regular PCR; qC: qPCR Thermal Cycler; tC: Regular PCR Thermal Cycler. *A-qRqTqC: Segment A was amplified with qPCR regent in specific qPCR tubes in qPCR Thermal Cyclers, and so on. －: No PCR product. ＋: PCR products detected (according to the signal strength, positive results are classified into 4 grades, +, ++, +++, ++++).

## Methods

Genomic DNA (gDNA) was extracted by phenol/chloroform method[12], which was used as PCR template. Four sets of PCR reactions were made as given in Table 2 according to the Manufactures' Instructions. For each experiment, two duplications were carried out under

the following PCR conditions: 94°C /2 min; (94°C /20 s, 60°C /20 s, 72°C /30 s) for 35 cycles.

## Results

All PCR results were summarized in Table 3. Segment D was amplified well in all experiments (Figure 1D). The products of segment A were detected from the experiments with qPCR kit (Figure 1A, lane 1-4, 9-12), but not with *Taq* PCR kit (Figure 1A, lane 5-8, 13-16). While, both segment B and C were amplified in all combinations, but the products with qPCR kit were more than those with *Taq* PCR kit (compare lane 1-4 with 5-8, and 9-12 with 13-16 in Figure 1B and 1C).

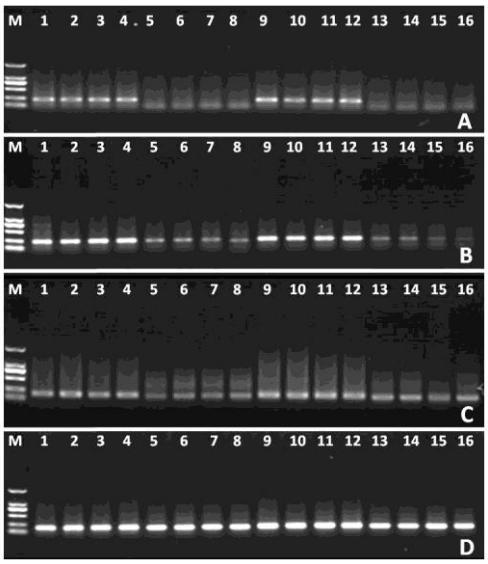


**Figure 1 Detection of PCR products by 1% agarose gel electrophoresis**

M: DNA ladder. Panel A, B, C and D: DNA segment A, B, C and D. 1, 2: qRqTqC; 3, 4: qRtTqC; 5, 6: tRqTqC; 7, 8: tRtTqC; 9, 10: qRqTtC; 11, 12: qRtTtC; 13, 14: tRqTtC; 15, 16: tRtTtC.

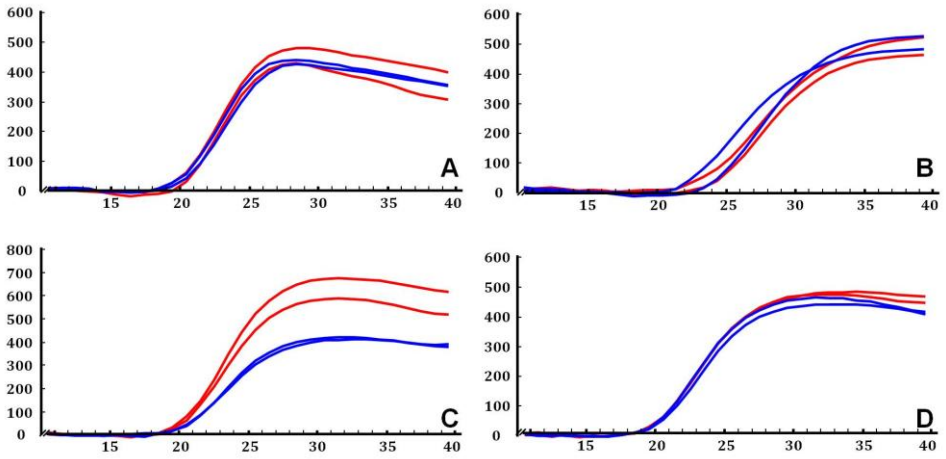


**Figure 2 Dynamics of qPCR amplification of segment A, B, C and D**

Ordinate: Relative fluorescence units. Abscissa: PCR cycles. Panel A, B, C and D: DNA segment A, B, C and D, respectively. Red : qRqTqC. Blue: qRtTqC.

In cases of qRqTqC and qRtTqC, Ct values are 19.80 and 19.41 for segment A, 22.58 and

22.93 for segment B, 19.08 and 19.10 for segment C and 19.01 and 19.13 for segment D, respectively (Figure 2).

## Discussions

In current study, 32 different combinations of PCR were carried out, and only one factor was different between every two combinations in order to clarify the effects of PCR kits, PCR tubes and Thermal Cyclers on the amplification of 4 different DNA segments (Table 3, Figure 1 and 2). Among three factors, Thermal Cyclers had no effect on PCR products (Figure 1, compare lane 1-4 with 9-12; lane 5-8 with 13-16). PCR tubes had no effect on either PCR products (Figure 1, compare lane 1, 2 with 3, 4; lane 5, 6 with 7, 8; lane 9, 10 with 11, 12; lane 13, 14 with 15, 16) or Ct values (Figure 2). However, the effect of PCR kits does exist, which depends on DNA segment (Figure 1). For segment D, both *Taq* PCR kit and qPCR kit gave the same amplification (Figure 1D). While amplification of segment A was heavily dependent on PCR kits, and PCR products were only detected from the experiments with qPCR kit, but not from the experiments with *Taq* PCR kit (Figure 1A). In contrast to segment A and D, segment B and C were amplified from both PCR kits, but the products with qPCR kit were more than those with *Taq* PCR kit (compare lane 1-4 with 5-8, lane 9-12 with 13-16 in Figure 1B and 1C).

Concerning PCR amplification, it is well known that both template and primer are two indispensable factors. In current study, one new important factor, PCR kit, has been indicated. For example, DNA segment A was only amplified by qPCR kit. One more finding here is that qPCR with regular PCR tubes gave the same Ct value as with tubes for specific qPCR (Figure 2). It is suggested to try regular PCR tube for qPCR if funding problem exists or qPCR tubes are out of stock.

Regarding to using the same PCR cycle parameters [94°C/2 min; (94°C/20 s, 60°C/20 s, 72°C/30 s), 35 cycles] for both regular PCR and qPCR in current study, the purpose is to compare the effects of other factors ultimately. In our routine experiments, qPCR cycle parameters are set according to the Manufactures' Guidelines.

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