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Application Note

MolTaq 16S/18S – DNA-Free *Taq* Polymerase for 40-Cycles PCR Analysis of Low Load Microbial DNA Targets

Keywords: *PCR detection, Real-Time PCR, 16S rRNA gene, 18S rRNA gene, ultra-clean Taq, low bacterial and fungal loads, DNA contamination*

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Abstract: The analysis of microbial pathogens and environmental microbes by PCR needs a *Taq* polymerase that is highly active over extended cycle numbers and, at the same time, free of contaminating microbial DNA. These two qualities are important characteristics for a sensitive analytical system that is able to discriminate signal-to-noise in the broad-range detection of bacteria and fungi at low abundance. Examples of applications where highly sensitive detection is required are molecular diagnostics of infections by pathogens, quality control of blood and pharmaceutical products, quality management of potable water, and analysis of microbial communities by next generation sequencing. Here, the performance of Molzym's MolTaq16S/18S was compared with other Taq products. In particular, quality parameters included measurements of activity at DNA dilutions between 5ng and 2.5pg and determination of false-positive rates at 40-cylces PCR runs.

Introduction

In the past years, demands for ultra-clean, molecular-grade reagents increased. In particular, amplification analysis of microbial pathogens at low concentration in clinical samples and Next Generation Sequencing analysis of microbial communities at low loads depend on reagents that are essentially free of microbial DNA contaminations [1, 2]. Clearly, at the limit of molecular detection methods, DNA contamination can pose serious problems to accurate data collection as regards false positive results and wrong community structure analysis. A prominent source of DNA contamination is the amplification enzyme together with other components of the master mix for PCR or Real-Time PCR reactions [3]. In this short note, a comparison of Molzym's Mol-Taq 16S/18S with other Taq polymerases declared low DNA contaminated was performed as regards the amplification activity and presence of contaminating microbial DNA.

Methods

Three *Taq* DNA polymerases (*Taq* D, E, M) declared low DNA contaminated or free of contaminating microbial DNA over 30 to 35 cycles amplification, were used in the comparative study with MolTaq 16S/18S. The setup of master mixes was performed following the instructions of the manufacturers. Forward and reverse primers (0.25µM final concentration) were used to amplify a 460bp sequence of the V3/V4 region of the 16S rRNA gene. PCR was performed in 25µl reactions using 4U of *Taq* DNA polymerase. The conditions for amplification in an Eppendorf Mastercycler were as follows: initial denaturation at 95°C for 1min, 40 cycles each at 95°C for 5s, 55°C for 5s and 72°C for 25s, and cooling to 10°C at the end of the programme. For analysis, 8µl of the PCR reaction were mixed with 2µl gel loading solution. Fragment size analysis was done by agarose gel electrophoresis.

Results and Discussion

Activity of *Taq* DNA polymerases. Activity of the *Taq* DNA polymerases was determined by gel electrophoretic analysis of amplicons gener-

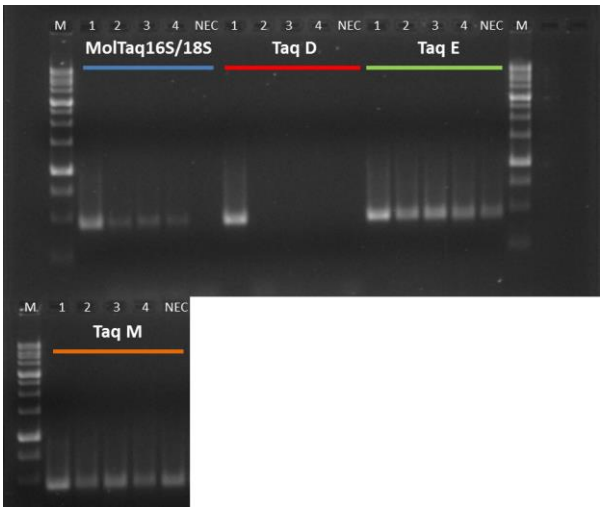

Fig. 1: Activity of *Taq* DNA polymerases at different target amounts per assay (25µl). M: marker; 1: 5ng *Bacillus subtilis* genomic DNA; 2: 10pg; 3: 5pg; 4: 2.5pg; NEC: no template control.

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ated over 40 cycles with *Bacillus subtilis* genomic DNA amounts per reaction, ranging from 5ng to 2.5pg (Fig. 1). All *Taq* products except *Taq* D repeatedly (n = 4-6) showed amplicons at each target amount. In fact, *Taq* D was active only at the highest target amount (5ng) and failed at lower amounts, hence showing the poorest activity.

Table 1: Results of no-template-control PCRs.

Taq	**Positives/ total tests**	**%**
MolTaq 16S/18S	0/14	<7
Taq E	12/13	92
Taq M	14/14	100

DNA contamination. Of the remaining *Taq* DNA polymerases that were active at 2.5pg target DNA, MolTaq 16S/18S did not show an amplicon in the no template control (DNA-free water instead of target DNA), whereas *Taq* E and *Taq* M generated strong signals (Fig. 1, NEC). Repeated no control runs confirmed the results, with 92% and 100% contamination of *Taq* E and *Taq* M, respectively, and no signals (<7%) with MolTaq 16S/18S (Table 1). Sequencing and BLAST analysis of amplicons from *Taq* E and *Taq* M reactions both resulted in *Aquabacterium parvum* contaminations. This result was surprising, because both *Taq*s are declared low contamination products, at least for 30 to 35 cycles. Apparently, the appearance of strong signals at 40 cycles uncovers the presence of considerable loads of bacterial DNA in both *Taq* products. Even another, standard *Taq* showed a lower contamination rate (5 of 18 tests, 28%) than *Taq* E and *Taq* M under the described conditions. With MolTaq 16S/18S, besides absence of bacterial DNA, also absence of fungal DNA was demonstrated (30 no control runs) in an assay containing primers for the amplification of a 310 bp sequence of the V8/V9 region of the 18S rRNA gene. Activity of MolTaq 16S/18S was very high: 16S rRNA gene amplicons were still generated at target DNA amounts ranging from 1.25pg to 300fg (not shown).

Summary and Conclusions

Activity and absence of contaminating DNA in amplification enzymes like *Taq* DNA polymerase are the determinative factors for highly sensitive and reliable analysis of low loads of microorganisms in clinical samples and other specimens. Usually PCR amplification is limited to 30 to 35 cycles to avoid upcoming signals from traces of contaminating microbial DNA. This limits the sensitivity of analysis of microorganisms at low abundance. In this study, amplification was extended to 40 cycles to determine the activity and DNA contamination of four Taq DNA polymerases declared low DNA contaminated. One product (Taq D) showed activity only at 5ng whereas the other enzymes amplified the target over the full range of DNA amounts tested (5ng to 2.5pg). Two products, *Taq* E and *Taq* M, amplified bacterial sequences from the no template control, whereas no signals came up with MolTaq 16S/18S. By this, the reliable detection of spiked target DNA even at 300fg per assay was possible. Thus, only MolTaq 16S/18S fulfilled the criteria of a highly active and pure enzyme regarding absence of contaminating bacterial and fungal DNA.

References

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Further Literature on the Topic

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