Application Note

Mastermix 16S – Ultra Sensitive Detection of Microbial DNA

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Highly Active and DNA-Free *Taq* DNA Polymerase

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 low abundance bacteria. Examples, where sensitive detection is demanded, are molecular diagnostics of systemic infections by pathogens, quality control of blood and pharmaceutical products, quality management of potable water, and analysis of microbial communities by next generation sequencing.

The majority of commercially available *Taq* DNA polymerase products were not designed and are not quality-controlled for the use in applications for the detection of microorganisms at high analytical sensitivity. This is illustrated by a study by Mühl et al. [10] who investigated the amplification activity of 5 commercial Taq products. The authors used a quantitative Real-Time PCR assay to determine the threshold values (C(t)) of the amplification of a 450 bp fragment of the V3/V4 region of the 16S rRNA gene at various input amounts of *Klebsiella pneumoniae* DNA. The results showed that the amplification activities varied greatly among the products at decreasing target DNA amounts. Only one product (Mastermix 16S) gave a signal (C(t) = 30) well above the limit of detection (threshold value with water, C(t) ≥ 35) at the lowest DNA amount (1.7pg/assay).

Contamination has been found in all *Taq* polymerases or master mixes from a variety of suppliers (Table 1). Unexpectedly, most DNA was identified to belong to species other than the production strains. The rate of contamination by exogenous DNA appearing in no template control runs can be very high (Table 1). It is generally recognized that some step(s) in the purification or reagents added to the enzyme are the sources of this bacterial contamination. Philipp et al. [13] state that "it seems unsatisfying to accept detection limits that high for diagnostic bacterial PCR...Reliable methods for DNA decontamination of *Taq* polymerase are needed and would present one important step towards bacterial DNA detection with high sensitivity."

Molzym's PCR products MolTaq and Mastermix 16S are characterized by high amplification activities (Fig. 1) and guaranteed absence of contaminating exogenous DNA (less than 3% false-positives) verified lot-by-lot.

Application: Microsystem for Universal 16S rDNA PCR

The early diagnosis of infectious diseases by molecular means is an application where highly pure PCR reagents are demanded. Peham et al. [11]

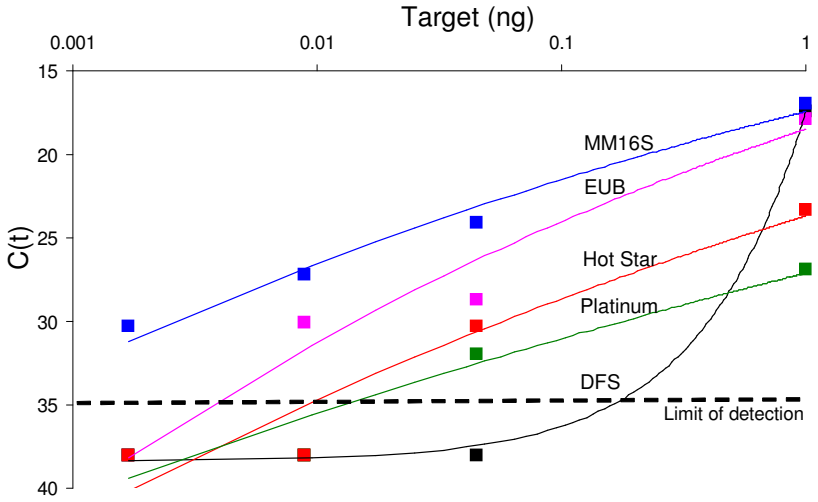

Fig. 1: Comparison of amplification activities of commercial *Taq* DNA polymerases. Target: *Klebsiella pneumoniae* DNA at indicated amounts per assay (25µl); assay: 16S rRNA gene-targeted SYBR Green 1 Real-Time PCR (40 cycles). MM16S, Mastermix 16S Complete kit (Molzym), Onar EUB kit (Minerva Biolabs, Berlin, Germany), Hot Star Plus kit (Qiagen, Hilden, Germany), Platinum kit (Invitrogen, Carlsbad, CA), DFS kit (Bioron, Ludwigshafen, Germany). Data modified from [10].

Table 1: Contamination of commercial PCR products by microbial DNA

PCR reagent	False positives(%) /origin [a]	Ref
HotStart Taq (Protech) Fast Hot Start Taq (KAPA Biosystems) Low-DNA Taq (Takara) ULTRATOOLS Taq (Biotools)	100 bacteria	[1]
Platinum Taq (Invitrogen)	100 *Pseudomonas* spp.	[10]
Taq DNA polymerase (Promega) AmpliTaq (Perkin Elmer) Low-DNA AmpliTaq (Perkin Elmer)	100 Corynebacterium spp., Arthrobacter spp., Mycobacterium spp.	[7]
TaqMan® Universal PCR Master Mix (Applied Biosystems)	100 bacteria	[13]
Taq polymerase (Amersham)	65-85 bacteria	[6]
Unknown commercial Mastermix	10-30 *Coxiella burnetii*	[16]
Taq polymerase (Promega)	11 *Thermus* spp.	[2]
Onar EUB kit (Minerva Biolabs)	8 *Sphingomonas* spp., *Moraxella* spp.	[10]
Mastermix 16S (Molzym)	2 *Acinetobacter junii*	[10]
FastStart (Roche), Platinum HiFi (Invitrogen), Platinum (Invitrogen)	n.d. *Pseudomonas* spp.	[15]
Amplitaq (ABI)	n.d. *Escherichia coli*, *Salmonella* spp., *Shigella* spp.	[15]
Hot Star Taq (Qiagen)	n.d. *Serratia marcescens*	[15]
Taq DNA polymerase (Qiagen)	n.d. mixed bacteria	[15]
10x PCR buffer (Boehringer Mannheim)	n.d. *Acremonium* spp. [b]	[9]
Hot Star PCR kit (Qiagen)	n.d. bacteria	[14]

[a] Signals generated in assays with molecular grade water instead of experimental DNA added; signal strength in the gel analysis reached from strong to faint; sequence analysis of amplicons; n.d., not determined, positive for bacterial DNA in the universal 16S rRNA gene assay used

[b] Lot dependent

used Mastermix 16S Basic (Molzym) to test a long target droplet microdevice for the amplification of the 16S ribosomal RNA gene with different primer sets and following microarray identification of species. They were able to increase the PCR efficiency as compared to conventional thermocyclers. Among a selection of mastermixes from different suppliers Mastermix 16S Basic was the most active allowing the detection of 100cfu *S. aureus*/reaction. Runs with water instead of target DNA did not show any false-positive results. The authors concluded that the system of Mastermix 16S in combination with the microdevice achieved detection limits which are appropriate for pathogen detection/identification in clinical applications.

Table 2 summarizes a selection of referenced applications involving MolTaq 16S and Mastermix 16S products.

Table 2: Selection of applications of highly active and DNA-free Molzym PCR products

PCR assay	Issue	Analysis and target	Ref
Mastermix 16S Basic	Systemic infection	16S rRNA gene PCR, microarray hybridization	[17]
Mastermix 16S Complete	Sensitivity and activity	16S rRNA gene PCR, sequencing	[10]
Mastermix 16S Complete	Reference for MALDI-TOF MS identification	16S rRNA gene PCR, sequencing	[3]
Mastermix 16S Dye	Sensitivity and activity	Hypervariable fungal ITS2 Real-Time PCR, pyrosequencing	own results
MolTaq 16S	Bacterial communities in sponges	16S rRNA gene PCR, denaturing gradient gel electrophoresis, sequencing	[4]
MolTaq 16S	Intrauterine infection	16S rRNA gene PCR, sequencing	[8]
MolTaq 16S	*Candidatus* Neoehrlichia mikurensis infection	16S rRNA gene PCR, sequencing	[12]
MolTaq 16S	Systemic infection	16S rRNA gene PCR, sequencing	[5]

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