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DNA-free Platinum Taq DNA polymerase for reliable microbiome studies

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ABSTRACT

Residual bacterial DNA in most commercial PCR enzymes poses challenges in analysis of microbial genomes, such as accurate detection of bacterial strains by 16S rRNA gene sequences.
Here, we introduce Invitrogen™ Platinum™ Taq DNA Polymerase, DNA-free — a PCR enzyme with the lowest DNA contamination levels as compared to competing "DNA-free" enzymes. Manufactured using a closed single-use system and stringent quality control, the DNA-free Platinum™ *Taq* DNA Polymerase offers minimized risk of DNA contamination in the enzyme while retaining the performance specifications associated Platinum™ hot-start technology. The purity of this enzyme offers clean backgrounds in reagents-only (or "negative") controls of broad-range PCR and improves confidence in microbiome studies.

INTRODUCTION

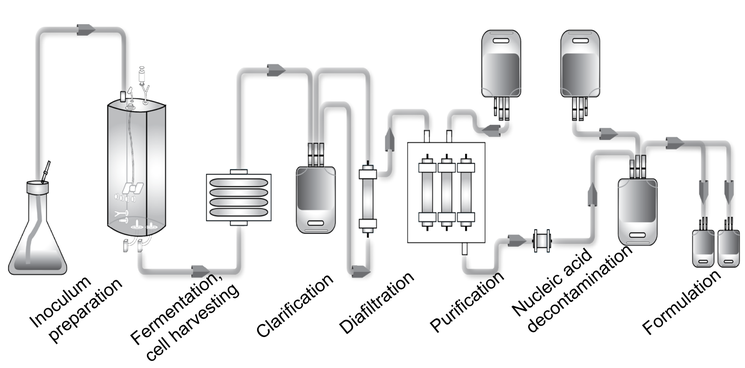
There is a tremendous interest in studies of complex microbial communities, their composition and internal relationships. One of the popular approaches is to sequence 16S rRNA marker genes, which provides both qualitative and quantitative data on the microbiome. Prior to sequencing, DNA requires amplification by PCR. To obtain a reliable representation of the microbial diversity within the sample it is very important to avoid the presence of contaminating bacterial DNA during amplification, especially when the studied sample has low microbial biomass.

Contamination in commercial PCR reagents can arise from manufacturing environment, raw materials, operators, or host cells expressing recombinant proteins. The main source of DNA contamination is the enzyme used for PCR, such as Taq DNA polymerase. Commercially available lots of Taq DNA polymerase have been shown to contain 10–1,000 genome equivalents of bacterial DNA per unit of enzyme. Reported DNA removal methods vary in efficiency and are not universal.

We introduce Invitrogen™ Platinum™ Taq DNA Polymerase, DNA-free – a PCR enzyme with the lowest DNA contamination levels as compared to competing "DNA-free" enzymes. Platinum™ Taq DNA Polymerase, DNA-free is manufactured using a novel single use system. Disposable single-use components (e.g., fermenters, bags, filters, and chromatography columns) are connected by sterile single-use tubes for every stage of production. This closed system helps ensure that the entire process is never exposed to the surrounding environment or operator (Fig. 1). Additional DNA-removal step was added to trap the traces of host-cell DNA after the chromatographic purification. DNA-free enzyme is accompanied by PCR buffer and $MgCl_2$ solution that are manufactured using single-use technology to prevent contamination with DNA.

Here we show how the purity of Platinum™ Taq DNA Polymerase, DNA-free offers clean backgrounds in reagents-only (or "negative") controls of broad-range PCR of rRNA genes and improves confidence in microbiome studies.

Figure 1. Closed single-use system-based manufacturing process



MATERIALS AND METHODS

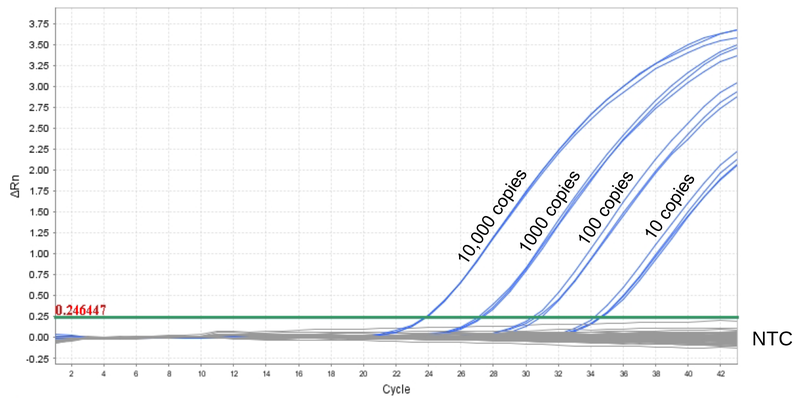
PCR reactions were prepared using Platinum™ Taq DNA Polymerase, DNA-free, 10X PCR buffer (-$MgCl_2$), DNA-free, and $MgCl_2$, DNA-free (Cat. No.15966005), Thermo Scientific™ dNTP Mix (10 mM each) and Thermo Scientific™ Water, nuclease-free (Cat.No. R0581). Reactions were cycled following the recommended protocol. TaqMan assay designed to detect *E.coli* 23S rRNA genes was used for qPCR with a dilution series of purified *E.coli* genomic DNA together with no-template controls. In the end-point PCR reactions broad-range 16S rRNA gene-binding primers were used with varying amount of *E.coli* genomic DNA and no-template controls.

RESULTS

Platinum *Taq* DNA polymerase, DNA-free, has no detectable *E. coli* DNA

During manufacturing Platinum *Taq* DNA polymerase, DNA-free, is expressed in *E.coli* cells. It was very important to adequately remove residual nucleic acids from the bacteria used in production.

Figure 1. Platinum Taq DNA polymerase, DNA-free, testing for the absence of *E. coli* genomic DNA

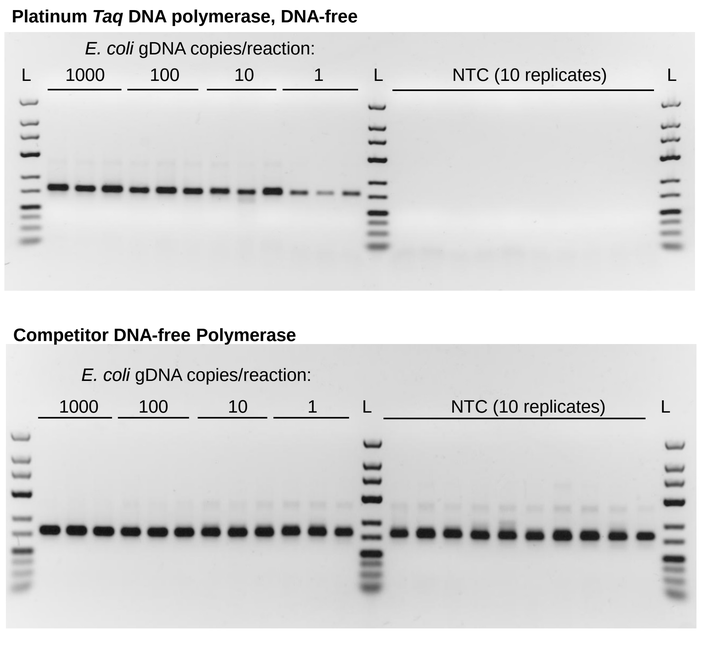


The entire qPCR plate was dedicated to check for the presence of *E.coli* DNA contamination in reactions prepared with Platinum Taq DNA polymerase, DNA-free, and TaqMan assay binding to *E.coli* genomic DNA. 12 wells were used as positive controls with varying amount of *E.coli* genomic DNA per reaction, in remaining wells no DNA was added (no-template control reactions, NTC). In all NTC reactions no amplification was detected, indicating to the purity of the enzyme. When normalized to the enzyme amount used in PCR, this result corresponds to <1 copy of *E.coli* genomic DNA in 100 units of the enzyme.

DNA from any bacteria is undetected in Platinum *Taq* DNA polymerase, DNA-free

Host-cell nucleic acids are not the only contaminating DNA commonly found in PCR enzyme preparations. 16S rRNA gene detection test detects conserved coding sequences present in all bacteria and was used to verify that DNA from any bacteria that may have been transferred into the final product is undetectable.

Figure 2. The purity of Platinum *Taq* DNA polymerase, DNA-free, offers clean background in reagents-only/no-template controls (NTC) while retaining superior sensitivity



16S rRNA gene was amplified by PCR using Platinum *Taq* DNA polymerase, DNA-free, or Promega GoTaq® MDx Hot Start Polymerase with varying amount of *E. coli* gDNA (1–1,000 copies/reaction) or reactions with no added DNA (NTC). In the reactions with template, after amplification for 40 cycles DNA was faithfully detected even when there was only 1 copy of genome added per reaction. No signal was generated in NTC reactions with Platinum Taq DNA Polymerase, DNA-free, indicating to high purity of the enzyme. In contrast, the primers binding to universally-conserved bacterial genomic sequences picked up and amplified contaminating bacterial DNA in competitor polymerase.

Stringent quality control

Proprietary quality control tests, relying on highly sensitive qPCR assays, are used to confirm the absence of nucleic acid contaminants.

Table 1. Purity requirements for Platinum Taq DNA polymerase, DNA-free.

Purity test	Requirement
Taq DNA polymerase purity	Exonucleases and endonucleases: undetected
	RNases: undetected
DNA contamination detection	Bacterial gDNA (16S rRNA gene detection): <0.01 copy/enzyme unit
	Human gDNA (Alu sequence detection): <0.001 copy/enzyme unit
	Plasmid DNA (ori1 sequence detection): <0.01 copy/enzyme unit

CONCLUSIONS

Residual bacterial DNA in most commercial PCR enzymes poses challenges in analysis of microbial genomes, such as accurate detection of bacterial strains by 16S rRNA gene sequences. Manufactured using a closed single-use system and stringent quality control, the DNA-free Invitrogen™ Platinum™ *Taq* DNA Polymerase is the ideal choice for microbiome applications where contaminating DNA can't be tolerated:

- Lower risk of DNA contamination in the enzyme from the novel manufacturing process
- Certified ≤ 0.01 copy of 16S rRNA gene per enzyme unit
- PCR sensitivity down to single copy detection

Find out more at www.thermofisher.com/dna-free.

Product	Quantity	Cat. No.
Platinum™ Taq DNA Polymerase, DNA-free (5 U/µL) Components: • Enzyme • 10X PCR buffer (-$MgCl_2$), DNA-free • 50 mM $MgCl_2$, DNA-free	500 U	15966005
	5 x 500 U	15966025

REFERENCES

1. Goodrich JK, Di Rienzi SC, Poole AC et al. (2014) Conducting a Microbiome Study. Cell 158
2. Salter SJ, Cox MJ, Turek EM et al. (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology 12:87
3. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS (2019) Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. Trends Microbiol 27(2)

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TRADEMARKS/LICENSING

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