Bacterial DNA detection using Platinum II *Taq* Hot-Start DNA Polymerase

Introduction

In metagenomics, the diversity and composition of a microbial community are often determined by its 16S ribosomal RNA (rRNA) gene content, which is obtained by PCR using primers specific to a broad taxonomic range [1]. At the same time, primers targeting 16S rRNA genes are a convenient tool for detecting bacterial genomic DNA contamination [2]. Due to degeneracy, these primers pair with multiple 16S rRNA gene variants. Degenerate primers in some positions contain a number of possible bases. Hence, instead of a precise single sequence of primers, there is a population of primers with related sequences that cover all possible nucleotide combinations for a given target sequence. However, the problem with degenerate primers is that they do not bind to 16S rRNA targets from different microbes with equal efficiency—contributing to amplification bias, and thus altering representation of bacterial taxa.

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase is provided with the innovative Platinum™ II PCR buffer, which allows one universal temperature for primer annealing. This is due to the fact that isostabilizing molecules in the reaction buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity without the need to optimize annealing temperature for each primer pair. Therefore, different primer variants from a degenerate primer population will bind with similar efficiency to their specific target in the same PCR run.

Moreover, Platinum II *Taq* Hot-Start DNA Polymerase is an enzyme engineered for inhibitor tolerance, enabling successful PCR amplification with samples of suboptimal purity, a common challenge with DNA samples isolated from environmental or clinical research samples. Since Platinum II *Taq* Hot-Start DNA Polymerase can detect even a few copies of the target, it is very important to minimize residual bacterial DNA, which is often found in commercially available enzymes, to prevent unintended amplification of unwanted sequences. During manufacturing of Platinum II *Taq* polymerase, strict measures are taken to control and verify that no more than one copy of residual bacterial genomic DNA is present per unit of the polymerase.

Here we present the use of Platinum II *Taq* Hot-Start DNA Polymerase and its buffer for fast and convenient bacterial DNA detection in samples with low amounts of microbial genomic DNA, using degenerate primers that target conserved regions of bacterial 16S rRNA genes.

Materials and methods

- Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2X) (Cat. No. 14000012) or Platinum II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ dNTP Mix, 10 mM each (Cat. No. 18427013; only required for stand-alone enzyme)
- Forward and reverse primers
- 25 mM $MgCl_2$ (optional)
- Invitrogen™ E-Gel™ General Purpose Agarose Gels or equivalent

When working with new PCR conditions, we recommend starting with the guidelines in Tables 1–3 for reaction setup and thermal cycling. Platinum II *Taq* polymerase allows for flexibility in reaction setup, while Platinum II Hot-Start PCR Master Mix (2X) provides more convenience for researchers since it contains all the necessary reaction components except primers and template DNA, thereby reducing setup time and pipetting steps. Additional recommendations for reaction optimization are provided.

Table 1. Reaction conditions for PCR using master mix.

Component	20 µL reaction	50 µL reaction	Final concentration
Platinum II Hot-Start PCR Master Mix (2X)*	10 µL	25 µL	1X
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Bacterial genomic DNA	Varies	Varies	Up to 500 ng/rxn
Water, nuclease-free	To 20 µL	To 50 µL	—
Optional components for reaction optimization			
Platinum GC Enhancer**	4 µL	10 µL	1X

*Provides 1.5 mM $MgCl_2$ in final reaction. **Recommended for targets with >65% GC content.

Table 2. Reaction conditions for PCR using stand-alone enzyme.

Component	20 µL reaction	50 µL reaction	Final concentration
5X Platinum II PCR Buffer*	4 µL	10 µL	1X
10 mM dNTP mix	0.4 µL	1 µL	0.2 µM each
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Bacterial genomic DNA	Varies	Varies	Up to 500 ng/rxn
Platinum II *Taq* Hot-Start DNA Polymerase	0.16 µL	0.4 µL	0.04 U/µL
Water, nuclease-free	To 20 µL	To 50 µL	—
Optional components for reaction optimization			
Platinum GC Enhancer**	4 µL	10 µL	1X

*Provides 1.5 mM $MgCl_2$ in final reaction. **Recommended for targets with >65% GC content.

Table 3. Cycling protocol.

PCR cycles	Step	Temperature	Time
1	Initial denaturation	94°C	2 min
30*	Denaturation	94°C	15 sec
	Annealing**	60°C	15 sec
	Extension	68°C	15 sec/kb
	Hold	4°C	Indefinitely

*30 PCR cycles are sufficient to amplify targets even from a low load of bacterial genomic DNA. **Due to the unique composition of the Platinum II PCR buffer, the annealing temperature is 60°C for most of the primer pairs designed following general primer design rules. In cases when annealing temperature needs additional optimization, we recommend testing different annealing temperatures or redesigning the primers.

PCR samples should be diluted 5- to 20-fold for optimal separation using E-Gel agarose gels.

Results

Amplification of conserved fragments in bacterial 16S rRNA genes

To evaluate the sensitivity and efficiency of Platinum II *Taq* Hot-Start DNA Polymerase in detecting low amounts of bacterial DNA, we used a degenerate PCR primer system targeting conserved regions of bacterial 16S rRNA genes. Primers (forward 5´-ATT GAC GGG GRC CCG CAC and reverse 5´-CGA GCT GAC GAC ARC CAT GCA, where R can be A or G) were designed for high coverage across almost all bacterial phyla and are therefore suitable for detecting a broad range of microbial genomic DNA [2]. For PCR using the stand-alone enzyme, Platinum II *Taq* polymerase was mixed with its buffer, dNTPs, degenerate primers, and 0.01–1 ng of purified genomic DNA from various bacteria. The cycling protocol was: 1 cycle of 94°C for 2 min; 30 cycles of 94°C for 15 sec, 60°C for 15 sec, and 68°C for 15 sec. For analysis, PCR products were separated by electrophoresis using 1% agarose gels in TAE buffer. As shown in Figure 1, Platinum II *Taq* polymerase successfully amplified 16S rRNA target DNA with high specificity from every tested bacterial species, using DNA input as low as 0.01 ng. At the same time, reactions without added DNA (no-template control) did not show any amplification, indicating the absence of residual bacterial DNA.

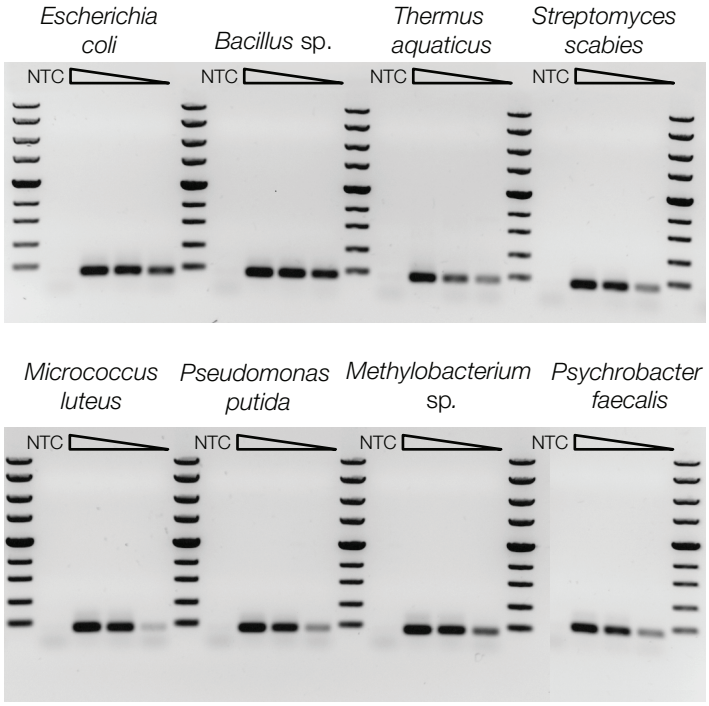

Figure 1. Detection of bacterial DNA with primers targeting 16S rRNA genes. A 16S rRNA gene fragment (153 bp in *E. coli*) was amplified from 0.01–1 ng of purified genomic DNA from different bacteria, using Platinum II *Taq* Hot-Start DNA Polymerase with degenerate primers over 30 PCR cycles. The Thermo Scientific™ ZipRuler™ Express DNA Ladder 2 was used as a size standard. NTC: no-template control.

Detection of bacterial genomic DNA in whole blood samples

Platinum II *Taq* Hot-Start DNA Polymerase was developed to tolerate increased levels of inhibitors that usually impede PCR. We tested whether the efficiency of amplifying bacterial DNA is affected when reactions contain inhibiting substances originating from whole blood. For this experiment, we repeated PCR with Platinum II *Taq* polymerase and degenerate primers using 0.1 ng of bacterial genomic DNA spiked into a 50 µL reaction mix with 1% (v/v) of whole blood. The concentration of $MgCl_2$ was increased to 4.5 mM in the reaction, as recommended for PCR reactions with whole blood. The cycling protocol was the same as described earlier. After PCR was completed, the products were visualized in 2% agarose gels with TAE buffer. Strong amplification signals in all of the reactions demonstrated successful detection of conserved 16S rRNA–coding fragments from all tested bacterial genomic DNA, implying that Platinum II *Taq* polymerase enables efficient DNA detection with degenerate primers, even in samples with suboptimal purity (Figure 2).

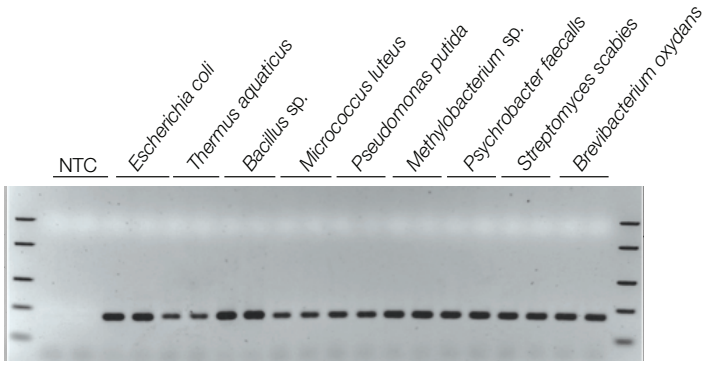

Figure 2. Detection of bacterial DNA in whole blood. Amplification of a conserved bacterial 16S rRNA gene region (153 bp in *E. coli*) in the presence of 1% (v/v) of human blood was performed for 30 PCR cycles using Platinum II *Taq* Hot-Start DNA Polymerase. $MgCl_2$ was added to a final concentration of 4.5 mM. The Thermo Scientific™ FastRuler™ Low Range DNA Ladder was used as a size standard. NTC: no-template control.

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Summary

With its high tolerance to inhibitors and unique Platinum II PCR buffer that allows efficient and specific annealing at 60°C for primers with different sequences, Platinum II *Taq* Hot-Start DNA Polymerase can streamline PCR workflows for sensitive and reliable bacterial DNA detection in environmental or clinical research samples.

References

1. Acinas SG, Marcelino LA, Klepac-Ceraj V, Polz MF (2004) Divergence and redundancy of 16S rRNA sequences in genomes with multiple *rrn* operons. *J Bacteriol* 186:2629–2635.
2. Wang Y, Qian P-Y (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One* 4(10):e7401.

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