Multiplex PCR using Platinum II *Taq* Hot-Start DNA Polymerase

Introduction

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase combines an enzyme engineered for speed and inhibitor tolerance with a proprietary buffer that allows for a universal annealing temperature. Here we describe how Platinum II *Taq* Hot-Start DNA Polymerase can be used for simultaneous amplification of many targets in a single tube using multiple pairs of primers.

Materials and methods

- Platinum II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. 18427013)
- Forward and reverse primers
- Invitrogen™ E-Gel™ General Purpose Agarose Gels, or equivalent

Important notes

Special attention to primer design parameters is critical for the success of multiplex PCR. Optimal primers for multiplexing should be 21–34 nt in length and have 40–60% GC content. Ideally, G and C nucleotides should be distributed uniformly along the primers. Avoid significant homology between the primers, as well as self-complementary primer regions, and also 3 or more G or C nucleotides at the 3′ end. If possible, the primer should terminate with a G or C at the 3′ end. We recommend verifying the performance of each primer pair in singleplex PCR before starting multiplex reactions.

Ensure that the amplicon sizes differ sufficiently for effective separation during electrophoresis.

Recommended amplicon sizes

Amplicon size range	Minimum difference
50–200 bp	25 bp
200–700 bp	50 bp
700–1,000 bp	300 bp
1,000–2,500 bp	500 bp

A PCR sample should be diluted 2- to 20-fold for optimal separation on E-Gel agarose gels.

Reaction components for multiplex PCR

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 mM each
Forward primers	Varies	Varies	100–200 nM each
Reverse primers	Varies	Varies	100–200 nM each
Template DNA	Varies	Varies	<500 ng/rxn
Platinum II *Taq* Hot-Start DNA Polymerase	0.32 µL	0.8 µL	0.08 U/µL
Water, nuclease-free	To 20 µL	To 50 µL	–

* Provides 1.5 mM $MgCl_2$ final concentration.

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Cycling protocol for multiplex PCR

Number of cycles	Step	Temperature	Time
1	Initial denaturation	94°C	2 min
30–35	Denaturation	94°C	15 sec
	Annealing	60°C	30 sec
	Extension	68°C	30–60 sec/kb
1	Hold	4°C	Indefinite

Results

The reaction conditions were optimized to amplify 15 targets from human genomic DNA using Platinum II *Taq* Hot-Start DNA Polymerase. Amplification of 99–1,606 bp fragments was performed from 0 (no-template control), 1.6, 8, 40, 200, and 1,000 ng of template DNA, in 50 µL reactions containing 100 nM of each primer. The cycling protocol was: 1 cycle at 94°C for 2 min; 35 cycles at 94°C for 15 sec, 60°C for 30 sec, and 68°C for 96 sec. The products were separated on a 2% agarose gel in TBE buffer. All of the expected fragments were clearly visible on the gel, indicating that up to 15 targets can be successfully amplified in the same reaction using Platinum II *Taq* Hot-Start DNA Polymerase (Figure 1).

Summary

With the high performance and unique composition of the Platinum II PCR Buffer that allows universal primer annealing, Platinum II *Taq* Hot-Start DNA Polymerase enables amplification of up to 15 targets in the same reaction.

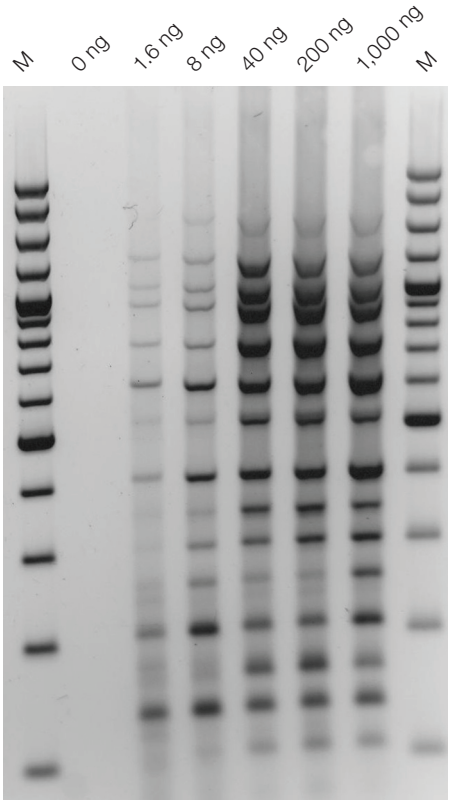

Figure 1. Multiplex PCR with Platinum II *Taq* Hot-Start DNA Polymerase. Fifteen targets (99, 131, 160, 199, 251, 300, 345, 400, 516, 613, 735, 908, 1,005, 1,190, and 1,606 bp) were simultaneously amplified from 0, 1.6, 8, 40, 200, and 1,000 ng of human genomic DNA, in 50 µL reactions. The Thermo Scientific™ GeneRuler™ 100 bp Plus DNA Ladder was used as a size standard/marker (M).

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