Multiplex PCR with Platinum SuperFi II DNA Polymerase

Introduction

Multiplex PCR is a widespread molecular biology technique that enables simultaneous amplification of many targets in a single tube, using multiple pairs of primers. Multiplexing that uses *Taq* enzyme–based DNA polymerases usually requires optimized reaction conditions, so specialized master mixes are often used. This application note describes multiplex PCR using Invitrogen™ Platinum™ SuperFi™ II DNA Polymerase, a hot-start proofreading enzyme that combines >300x the fidelity of *Taq* polymerase with an innovative reaction buffer that enables universal annealing at 60°C regardless of primer sequences.

Due to its high specificity and processivity, as well as the specially formulated reaction buffer, Platinum SuperFi II DNA Polymerase can multiplex over a broad range of template concentrations without the need for significant optimization of the annealing temperature for each primer pair.

Materials and methods

Materials

- Invitrogen™ Platinum™ SuperFi™ II PCR Master Mix (Cat. No. **12368010**) or Platinum SuperFi II DNA Polymerase (Cat. No. **12361010**)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. **18427013**)
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% and 2% (Cat. No. **G402001** and **G402002**, respectively)
- Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. **10488091**)
- Invitrogen™ TrackIt™ 100 bp DNA Ladder (Cat. No. **10488058**)

Table 1. PCR reaction conditions.

Component	50 µL reaction	Final concentration
PCR with master mix		
2X Platinum SuperFi II PCR Master Mix*	25 µL	1X
10 µM forward primer	1 µL	0.2 µM each
10 µM reverse primer	1 µL	0.2 µM each
Template DNA	0.1–250 ng**	Varies
Water, nuclease-free	to 50 µL	–
PCR with stand-alone enzyme		
5X SuperFi II Buffer*	10 µL	1X
10 mM dNTP mix	1 µL	0.2 mM each
10 µM forward primer	1 µL	0.2 µM each
10 µM reverse primer	1 µL	0.2 µM each
Template DNA	0.1–250 ng**	Varies
Platinum SuperFi II DNA Polymerase	1 µL	–
Water, nuclease-free	to 50 µL	–

* Provides 1.5 mM $MgCl_2$ in final reaction concentration.

** Higher concentrations recommended for DNA of higher complexity.

Primers

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 primer. Avoid significant homology between the primers and self-complementary primer regions, as well as three 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.

The recommended concentration of each primer in the final reaction mix for multiplexing is 0.2 µM (Table 1). If required, the primer concentration may be optimized in the range of 0.1–0.4 µM. We recommend verifying each primer pair in singleplex PCR before starting multiplex reactions.

For effective separation in agarose gel electrophoresis, ensure that the amplicon sizes differ sufficiently. Samples after PCR should be diluted 2- to 20-fold for optimal separation in Invitrogen™ E-Gel™ precast agarose gels.

Size of amplicons	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

Results

We amplified a range of targets, from ~100 bp to ~1,600 bp, from human genomic DNA (gDNA) using Platinum SuperFi II DNA Polymerase in a series of reactions with increasing numbers of targets. Each 50 µL reaction contained 0.2 µM of each primer and 50 ng of the template DNA. The cycling protocol was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 60°C for 30 sec, 72°C for 60 sec; 1 cycle at 72°C for 5 min (Table 2). The products were separated using a 2% E-Gel EX Agarose Gel in TBE buffer. All the expected fragments were clearly visible in the gel, indicating that up to 15 targets can be successfully amplified in the same reaction using Platinum SuperFi II DNA Polymerase (Figure 1).

To demonstrate the efficiency of multiplex PCR using Platinum SuperFi II DNA Polymerase, the reactions were performed with increasing amounts of template DNA. The reactions contained 0.2 µM of each primer and 0 (no-template control), 0.4, 2, 10, 50, and 250 ng of the template DNA per 50 µL reaction. The cycling protocol and analysis were the same as before. All 15 targets from ~100 bp to ~1,600 bp were successfully amplified over a broad range of template concentrations, from 2 to 250 ng (Figure 2).

Table 2. Cycling protocol.

Number of cycles	Steps	Temperature	Time
1	Initial denaturation	98°C	30 sec
25–30	Denaturation	98°C	5–10 sec
	Annealing	60°C	30 sec
	Extension*	72°C	30–60 sec/kb
1	Final extension	72°C	5 min
	Hold	4°C	Indefinitely

* Calculate extension time based on the size of the largest amplicon. The extension time can be extended to up to 60 sec/kb for higher numbers of multiplex targets.

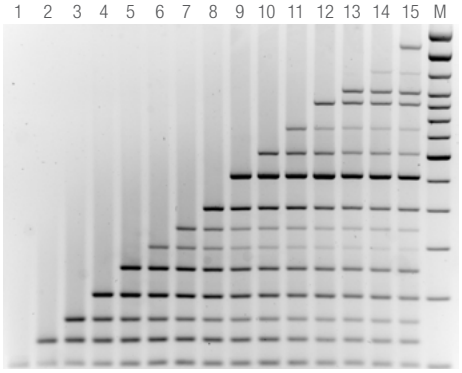

Figure 1. Simultaneous amplification of up to 15 targets using Platinum SuperFi II DNA Polymerase. One or more targets in a series of increasing sizes (lane 1–15: 99, 131, 160, 199, 251, 300, 345, 400, 516, 613, 735, 908, 1,005, 1,190, and 1,606 bp, respectively) was amplified in 50 µL reactions from 50 ng of human gDNA. The Invitrogen™ TrackIt™ 100 bp DNA Ladder was used as a size standard (M).

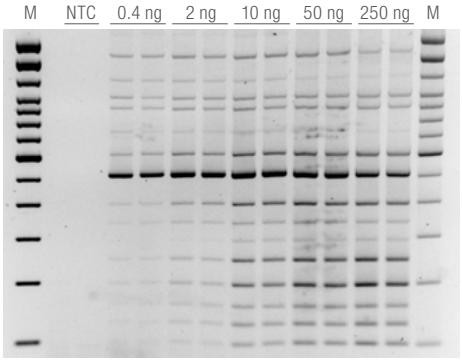

Figure 2. Multiplex PCR over a broad range of template concentrations using Platinum SuperFi II DNA Polymerase. The same 15 targets amplified in Figure 1 were amplified in 50 µL reactions from 0 (NTC), 0.4, 2, 10, 50, and 250 ng of human gDNA. The TrackIt 100 bp DNA Ladder was used as a size standard (M). NTC: no-template control.

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To demonstrate the flexibility of multiplex PCR using Platinum SuperFi II DNA Polymerase, 5 targets from ~0.6 kb to ~5 kb with a broad range of primer melting temperatures were amplified in the same reaction. The cycling protocol was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 60°C for 30 sec, 72°C for 150 sec; 1 cycle at 72°C for 5 min. The reactions contained 0.2 µM of each primer and 0 (no-template control), 2.5, 25, and 250 ng of the template DNA per 50 µL of PCR reaction. Samples were diluted 20-fold and analyzed in a 1% E-Gel EX Agarose Gel. All the expected fragments were clearly visible in the gel (Figure 3).

In parallel, 5 targets from ~0.7 kb to ~5 kb with GC content from 36% to 77% were amplified from 25 ng and 250 ng of human gDNA. The cycling protocol and analysis were the same as described above. All the targets were amplified, indicating that Platinum SuperFi II DNA Polymerase can amplify both AT-rich and GC-rich targets in the same reaction (Figure 4).

Summary

This application note describes a fast and easy method for amplifying up to 15 targets in the same multiplex PCR reaction using Platinum SuperFi II DNA Polymerase. With its high robustness and specificity, Platinum SuperFi II DNA Polymerase can multiplex over a broad range of template concentrations in the buffer provided without the need for significant optimization of template amount, amplification length, and GC content of the sequence. Its innovative reaction buffer also minimizes the need for optimization of annealing temperature for each primer set.

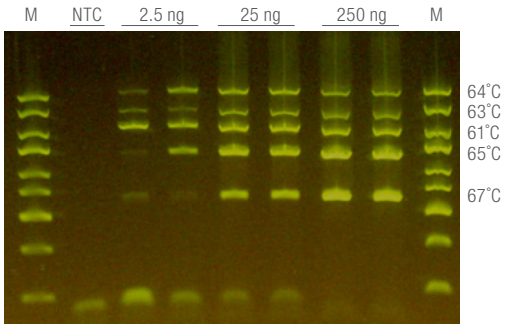

Figure 3. Multiplex PCR over a broad range of primer melting temperatures using Platinum SuperFi II DNA polymerase. 2, 2.7, 4.8, 1.3, and 0.6 kb targets, with their calculated optimal annealing temperatures of 61°C, 63°C, 64°C, 65°C, and 67°C, respectively, were amplified from 0, 2.5, 25, and 250 ng of human gDNA. The E-Gel 1 Kb Plus Express DNA Ladder was used as a size standard (M). NTC: no-template control.

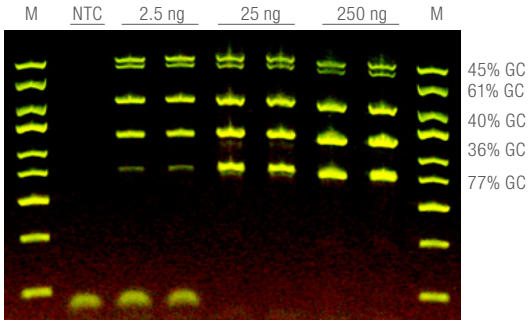

Figure 4. Multiplex PCR over a broad range of amplicon GC content using Platinum SuperFi II DNA Polymerase. 36% GC (1.2 kb), 40% GC (2 kb), 45% GC (4.8 kb), 61% GC (3.5 kb), and 77% GC (0.7 kb) targets were amplified from 0, 2.5, 25, and 250 ng of human gDNA. The E-Gel 1 Kb Plus Express DNA Ladder was used as a size standard (M). NTC: no-template control.

Find out more at **thermofisher.com/platinumsuperfi**

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