Multiplex PCR with Platinum SuperFi 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 using *Taq*-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™ DNA Polymerase. Platinum SuperFi DNA Polymerase is a proofreading enzyme that combines >100x the fidelity of *Taq* polymerase with trusted Invitrogen™ Platinum™ hot-start technology. Featuring high specificity and processivity, Platinum SuperFi DNA Polymerase can multiplex over a broad range of template concentrations in the buffer provided, without the need for significant optimization.

Materials and methods

Materials

- Invitrogen™ Platinum™ SuperFi™ PCR Master Mix (Cat. No. 12358010) or Platinum SuperFi DNA Polymerase (Cat. No. 12351010)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. 18427013)

Table 1. PCR reaction conditions.

Component	50 µL reaction	Final concentration
PCR with master mix		
2X Platinum SuperFi PCR Master Mix*	25 µL	1X
10 µM forward primer	1.5 µL	0.3 µM each
10 µM reverse primer	1.5 µL	0.3 µM each
Template DNA	0.1–250 ng**	Varies
Water, nuclease-free	to 50 µL	–
PCR with stand-alone enzyme		
5X SuperFi Buffer*	10 µL	1X
10 mM dNTP mix	1 µL	0.2 mM each
10 µM forward primer	1.5 µL	0.3 µM each
10 µM reverse primer	1.5 µL	0.3 µM each
Template DNA	0.1–250 ng**	Varies
Platinum SuperFi DNA Polymerase (2 U/µL)	0.5 µL	0.02 U/µL
Water, nuclease-free	to 50 µL	–

* Provides 1.5 mM $MgCl_2$ in final reaction concentration.
** Lower concentrations recommended for DNA of lower complexity. 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.

Always use the T_m calculator at **thermofisher.com/tmcalculator** to calculate the T_m of your primers. The recommended T_m of multiplexing primers should be around 60–65°C. We do not recommend using primers that differ in T_m by more than 5°C from other primers in the reaction.

The recommended concentration of each primer in the final reaction mix for multiplexing is 0.3 µM (Table 1). If required, the primer concentration may be optimized in the range of 0.2–0.4 µM. We recommend validating each primer pair in singleplex PCR before starting multiplex reactions.

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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*	Varies	30 sec
	Extension**	72°C	30–60 sec/kb
1	Final extension	72°C	5 min
		4°C	Indefinitely

* Always use the T_m calculator at **thermofisher.com/tmcalculator** to calculate T_m of your primers and the recommended annealing temperature. The recommended T_m of all primers in the reaction should not differ by more than 5°C.
** Calculate extension time based on the size of the largest amplicon. The extension time can be extended up to 60 sec/kb for higher numbers of multiplex targets.

Ensure that the amplicon sizes differ sufficiently for effective separation in electrophoresis:

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 using Platinum SuperFi DNA Polymerase, in a series of reactions with increasing numbers of targets. Each 50 µL reaction contained 0.3 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, 65°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% agarose gel in TAE 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 DNA Polymerase (Figure 1).

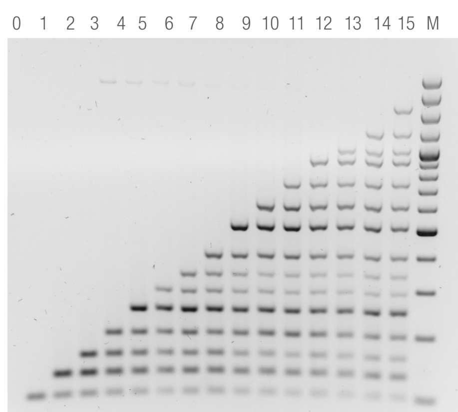

Figure 1. Simultaneous amplification of up to 15 targets using Platinum SuperFi DNA Polymerase. One or more targets in a series of increasing sizes (99, 131, 160, 199, 251, 300, 345, 400, 516, 613, 735, 908, 1,005, 1,190, and 1,606 bp) was amplified in 50 µL reactions from 50 ng of human genomic DNA.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

To demonstrate the efficiency of multiplex PCR using Platinum SuperFi DNA Polymerase, the reactions were performed with increasing amounts of template DNA. The reactions contained 0.3 µM of each primer and 0 (no-template control), 0.08, 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).

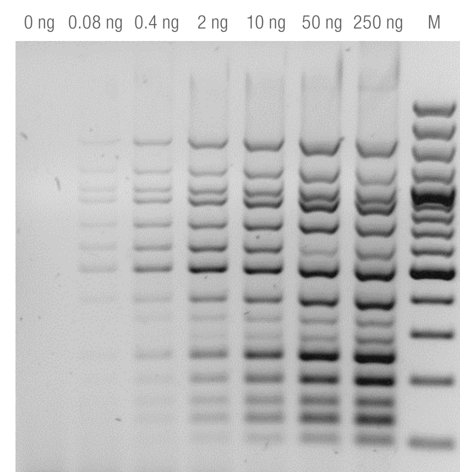

Figure 2. Multiplex PCR over a broad range of template concentrations using Platinum SuperFi DNA Polymerase. The same 15 targets amplified in Figure 1 were amplified in 50 µL reactions from 0, 0.08, 0.4, 2, 10, 50, and 250 ng of human genomic DNA. The GeneRuler 100 bp Plus DNA Ladder was used as a size standard (M).

Summary

This application note describes a fast and easy method for amplifying up to 15 targets in the same multiplex PCR using Platinum SuperFi DNA Polymerase. With its high robustness and specificity, Platinum SuperFi DNA Polymerase can multiplex over a broad range of template concentrations in the buffer provided without the need for significant optimization.

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

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