Direct PCR from blood using Platinum SuperFi II DNA Polymerase

Introduction

Invitrogen™ Platinum™ SuperFi™ II DNA Polymerase is engineered with a DNA-binding domain resulting in high processivity and increased tolerance to PCR inhibitors present in blood, as well as to most widely used blood preservatives (e.g., heparin, EDTA, and citrate). This application note describes how researchers can successfully perform PCR from blood samples without DNA purification using Platinum SuperFi II DNA Polymerase. Multiplex PCR can also be used to amplify more than one target sequence directly from blood. This protocol minimizes sample loss and helps significantly reduce protocol time without any DNA purification or isolation steps.

Materials and methods

- Whole blood
- Invitrogen™ Platinum™ SuperFi™ II PCR Green Master Mix (Cat. No. **12369010**) or Platinum SuperFi II DNA Polymerase (Cat. No. **12361010**)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. **18427013**; only required for stand-alone enzyme)
- Forward and reverse primers
- 50 mM $MgCl_2$
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% and 2% (Cat. No. **G401001** and **G401002**)
- Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. **10488091**)

Important notes

- Platinum SuperFi II DNA Polymerase may be used for amplification of DNA from a wide range of blood concentrations in the reaction. The recommended starting amount is 5% blood added directly to the reaction without further modification. Extensive mixing or vortexing after addition of blood to the PCR master mix should be avoided.
- $MgCl_2$ concentration may need to be increased up to 4.5 mM final concentration, especially if a higher blood percentage (≥10%) is used.
- After PCR, centrifuge the reactions at 1,000 × *g* (about 4,000 rpm) for 1–3 min to pellet debris from blood.

Below are recommendations for direct PCR using whole blood.

Reaction conditions for PCR using master mix

Component	20 µL reaction	50 µL reaction	Final concentration
Water, nuclease-free	to 20 µL	to 50 µL	–
2X Platinum SuperFi II PCR Green Master Mix*	10 µL	25 µL	1X
10 µM forward primer	1 µL	2.5 µL	0.5 µM each
10 µM reverse primer	1 µL	2.5 µL	0.5 µM each
Whole blood	0.2–2 µL	0.5–10 µL	1–20%
50 mM $MgCl_2$	0.6–1.2 µL	1.5–3 µL	Up to 4.5 mM

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

or

Reaction conditions for PCR using stand-alone enzyme

Component	20 µL reaction	50 µL reaction	Final concentration
Water, nuclease-free	to 20 µL	to 50 µL	–
5X SuperFi II Buffer*	4 µL	10 µL	1X
10 mM dNTP mix	0.4 µL	1 µL	0.2 mM each
10 µM forward primer	1 µL	2.5 µL	0.5 µM each
10 µM reverse primer	1 µL	2.5 µL	0.5 µM each
Whole blood	0.2–2 µL	0.5–10 µL	1–20%
Platinum SuperFi II DNA Polymerase	0.4 µL	1 µL	–
50 mM $MgCl_2$	0.6–1.2 µL	1.5–3 µL	Up to 4.5 mM

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

Cycling protocol

PCR cycles	Step	2-step protocol (for primers >30 nt in length*)		3-step protocol	
		Temp.	Time	Temp.	Time
1	Initial denaturation**	98°C	30 sec	98°C	30 sec
30–35	Denature	98°C	5–10 sec	98°C	5–10 sec
	Anneal	–	–	60°C	10 sec
	Extend	72°C	30 sec/kb	72°C	30 sec/kb
1	Final extension	72°C	5 min	72°C	5 min
	Hold	4°C	Indefinitely	4°C	Indefinitely

* Counting only bases that are complementary to the template.

** The initial denaturation step can be extended for up to 5 minutes to ensure complete cell lysis; however, 30 sec is usually sufficient.

After amplification, centrifuge the PCR reaction at 1,000 x *g* for 1–3 min to collect the supernatant for analysis. This step separates the various components of blood that might interfere with subsequent assays (e.g., gel electrophoresis). Centrifugation is especially important when high blood concentrations are used, as there can be a substantial amount of cell debris in the tube after the PCR reaction. The sample should be diluted 2- to 20-fold for optimal separation using an E-Gel EX Agarose Gel.

Results

A 585 bp fragment was amplified from whole blood using Platinum SuperFi II PCR Green Master Mix. Varying percentages of human blood (from 1% to 20% (v/v)) preserved with EDTA, citrate, or heparin were added directly to the PCR reactions. $MgCl_2$ concentration was increased to 3.5 mM final concentration by adding 50 mM $MgCl_2$ solution. The cycling protocol was: 1 cycle at 98°C for 30 sec; 35 cycles at 98°C for 10 sec, 60°C for 10 sec, and 72°C for 20 sec; 1 cycle at 72°C for 5 min. The PCR products were diluted 20-fold and analyzed in E-Gel EX 1% Agarose Gels. Successful amplification was achieved with up to 20% EDTA- or citrate-stabilized blood or up to 10% heparin-stabilized blood (Figure 1).

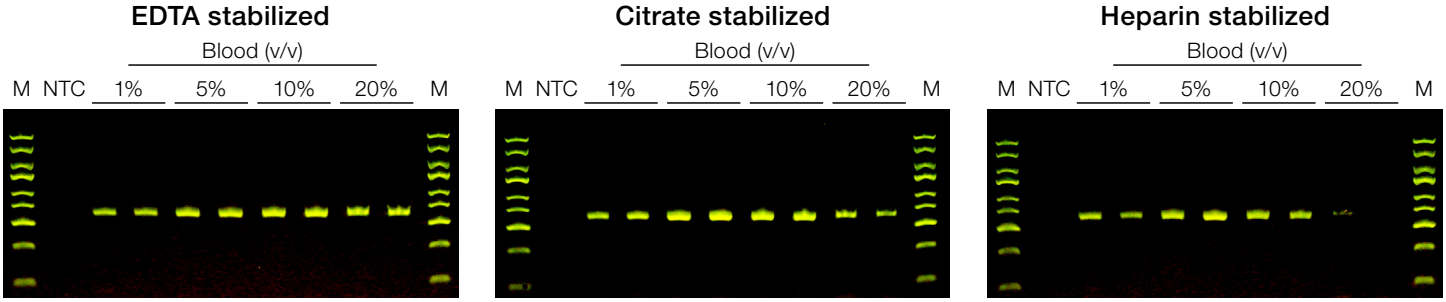

Figure 1. Direct amplification from whole blood. Amplification of a 585 bp fragment from varying percentages of human blood (v/v) was performed using Platinum SuperFi II PCR Green Master Mix. $MgCl_2$ was added to 3.5 mM final concentration. M: E-Gel 1 Kb Plus Express DNA Ladder used as a molecular size standard, NTC: no-template control.

invitrogen

To demonstrate the ability to perform multiplex PCR, 8 targets were simultaneously amplified from whole human blood (Figure 2). PCR reactions contained 0.2 µM of each primer and 1 µL of whole blood per 50 µL of PCR reaction. The cycling protocol was: 1 cycle at 98°C for 30 sec; 35 cycles at 98°C for 10 sec, 60°C for 30 sec, and 72°C for 24 sec (longer annealing and extension times are recommended for multiplex PCR); 1 cycle at 72°C for 5 min. The PCR products were diluted 20-fold and were separated in an E-Gel EX 2% Agarose Gel.

Summary

With its high processivity and tolerance to inhibitors, Platinum SuperFi II DNA Polymerase enables amplification of DNA directly from blood as well as successful multiplex PCR from whole blood. These procedures can help streamline PCR workflows for multiple applications involving blood samples.

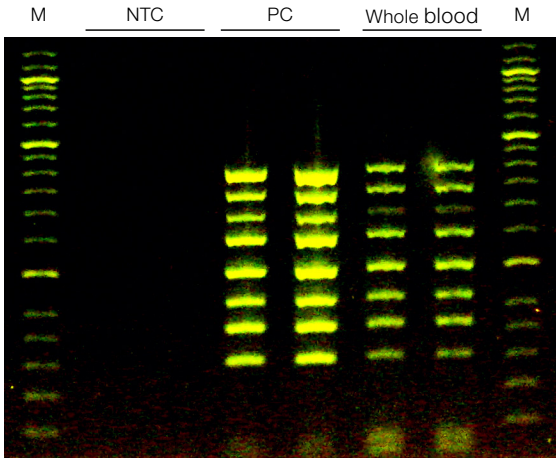

Figure 2. Multiplex PCR from human blood using Platinum SuperFi II DNA Polymerase. Eight targets (99, 131, 160, 199, 251, 300, 345, and 400 bp) were amplified in the same 50 µL PCR reaction from 1 µL of whole blood. M: Invitrogen™ E-Gel™ Sizing DNA Ladder used as a molecular size standard, NTC: no-template control, PC: positive control from 10 ng of purified human genomic DNA.

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

ThermoFisher SCIENTIFIC