Direct PCR from bacterial, yeast, and mammalian cells 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. This application note describes how PCR can successfully be performed from intact *E. coli*, gram-positive bacterial, and HeLa cells. Also, while PCR from yeast colonies using *Taq*-based enzymes requires a cell lysis step (boiling, NaOH, or Zymolyase™ treatment) prior to PCR, Platinum SuperFi II DNA Polymerase allows direct amplification from yeast cells. As accurate DNA sequence is maintained with the exceptionally high fidelity of Platinum SuperFi II DNA Polymerase (>300x higher fidelity than *Taq* DNA polymerase), the direct PCR product can be used for downstream cloning or sequencing applications. This protocol helps minimize sample loss and significantly reduce protocol time without any DNA purification or cell lysis steps.

Materials and methods

- Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix (Cat. No. **12369010**)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. **18427013**)
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% (Cat. No. **G402001**)
- Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. **10488091**)

Important notes

- A sterile toothpick, pipette tip, or microstreaker can be used to transfer a small amount of an individual colony from an agar plate into the PCR master mix. A small amount of the colony is sufficient, and the PCR master mix should not look cloudy. Alternatively, an individual colony can be suspended in 20 µL of PCR-grade water, and 1 µL of this suspension can be added directly to the PCR reaction.
- Residual DNA on agar plates can also serve as a template for PCR. Negative controls from agar plates are recommended in colony-screening experiments after transformation.
- The completed PCR reaction mix should be diluted 2- to 20-fold for optimal separation using E-Gel agarose gels.
- The tracking dyes in the green master mix are compatible with downstream applications such as automated DNA sequencing with fluorescent dyes, ligation, and restriction digestion. For applications that require analysis of PCR products by absorbance or fluorescence excitation, we recommend using the colorless version, or purifying the PCR products prior to analysis.

Reaction composition for PCR using master mix

Component	20 µL reaction	50 µL reaction	Final concentration
2X Platinum SuperFi II Green PCR 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
Water, nuclease-free	To 20 µL	To 50 µL	–

* Provides 1.5 mM $MgCl_2$ final concentration.

Cycling protocol

		2-step protocol (for primers >30 nt in length)		3-step protocol	
Number of cycles	Step	Temperature	Time	Temperature	Time
1	Initial denaturation*	98°C	30 sec	98°C	30 sec
30–35	Denaturation	98°C	5–10 sec	98°C	5–10 sec
	Annealing	–	–	60°C	10 sec
	Extension	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

* The initial denaturation step can be extended up to 5 min to ensure complete cell lysis; however, 30 sec is sufficient in most cases.

Results

A 2.4 kb target from a plasmid was amplified directly from the BL21 *E. coli* strain using Platinum SuperFi II Green PCR Master Mix by three methods: (A) a colony was suspended in 20 µL of PCR-grade water, and 1 µL of this suspension was added; (B) a small amount of a colony from an agar plate was transferred directly; and (C) 1 µL of overnight culture was added. The cycling protocol was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 60°C for 10 sec, 72°C for 75 sec; 1 cycle at 72°C for 5 min. A 4.4 kb genomic target was amplified from Invitrogen™ TOP10 cells by the same methods. The cycling protocol for the 4.4 kb target was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 60°C for 10 sec, 72°C for 175 sec; 1 cycle at 72°C for 5 min.

The completed PCR samples were diluted 20-fold and analyzed on an E-Gel EX 1% Agarose Gel. Amplification was successful with both the plasmid target (Figure 1) and the genomic target (Figure 2) from colonies on agar plates and liquid cultures.

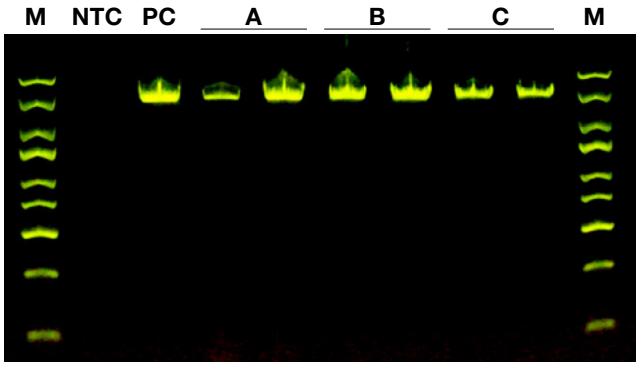

Figure 1. Direct amplification of plasmid DNA from *E. coli* colonies on agar plates and in liquid cultures. Platinum SuperFi II Green PCR Master Mix was used to amplify a 2.4 kb target from BL21 cells in the following formats: **(A)** 1 µL of a colony suspended in 20 µL PCR-grade water; **(B)** direct transfer of a small amount of a colony from an agar plate; and **(C)** 1 µL of overnight culture. M: E-Gel 1 Kb Plus Express DNA Ladder as the size standard, NTC: no template control, and PC: positive control from 5 ng of purified plasmid DNA.

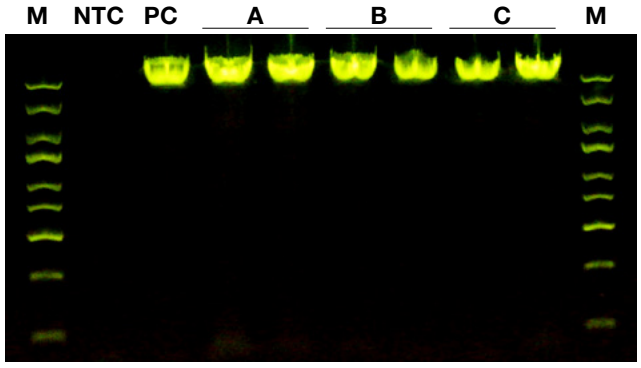

Figure 2. Direct amplification of genomic DNA from *E. coli* colonies on agar plates and liquid cultures. Platinum SuperFi II Green PCR Master Mix was used to amplify a 4.4 kb target from TOP10 cells in the following formats: **(A)** 1 µL of a colony suspended in 20 µL PCR-grade water; **(B)** direct transfer of a small amount of a colony from an agar plate; and **(C)** 1 µL of overnight culture. M: E-Gel 1 Kb Plus Express DNA Ladder as the size standard, NTC: no template control, and PC: positive control from 5 ng of purified genomic DNA.

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To demonstrate the ability to amplify DNA directly from difficult samples, we performed PCR from intact gram-positive bacteria (*Bacillus subtilis*) and yeast (*Pichia pastoris*) cells using Platinum SuperFi II Green PCR Master Mix. A small amount of a colony from an agar plate was placed directly into the PCR mix. The cycling protocol to amplify a 0.7 kb target was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 60°C for 10 sec, 72°C for 20 sec; 1 cycle at 72°C for 5 min. Completed PCR samples were diluted 20-fold and analyzed on an E-Gel EX 1% Agarose Gel. Successful amplification was achieved from both *B. subtilis* and *P. pastoris* colonies without any lysis steps (boiling, NaOH, or Zymolyase treatment) prior to PCR (Figures 3 and 4).

To demonstrate the ability to amplify DNA directly from cultured mammalian cells, we performed PCR from sorted HeLa cells in PBS using Platinum SuperFi II Green PCR Master Mix. The cycling protocol to amplify a 0.6 kb target was: 1 cycle at 98°C for 30 sec; 40 cycles at 98°C for 10 sec, 60°C for 10 sec, 72°C for 16 sec; 1 cycle at 72°C for 5 min. Samples were diluted 20-fold and analyzed on an E-Gel EX 1% Agarose Gel. Successful amplification of the target fragment was achieved from 10 intact HeLa cells (Figure 5).

Summary

With its high processivity and tolerance to inhibitors, Platinum SuperFi II DNA Polymerase enables direct amplification of DNA from intact *E. coli*, yeast, gram-positive bacterial cells, and cultured mammalian cells. High-fidelity PCR without any DNA purification or cell lysis steps can help reduce protocol time significantly in various research applications.

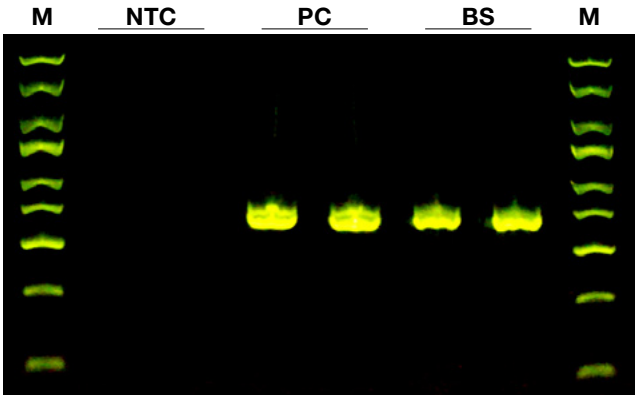

Figure 3. Direct amplification of DNA from gram-positive bacteria. Platinum SuperFi II Green PCR Master Mix was used to amplify a 0.7 kb target from intact *B. subtilis* (BS) cells. M: E-Gel 1 Kb Plus Express DNA Ladder as the size standard, NTC: no template control, and PC: positive control from 5 ng of purified plasmid DNA.

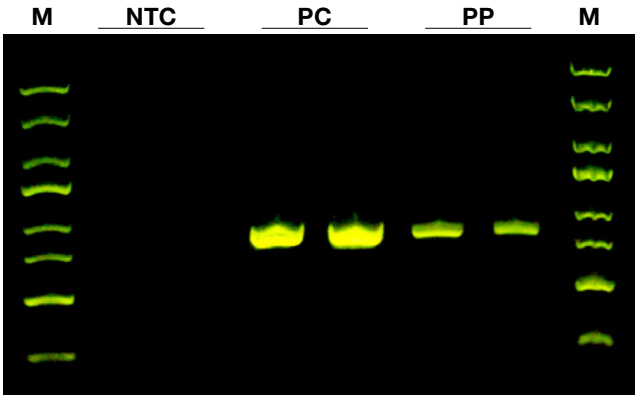

Figure 4. Direct amplification of DNA from yeast colonies. Platinum SuperFi II Green PCR Master Mix was used to amplify a 0.7 kb target from intact *P. pastoris* (PP) cells. M: E-Gel 1 Kb Plus Express DNA Ladder as the size standard, NTC: no template control, and PC: positive control from 5 ng of purified plasmid DNA.

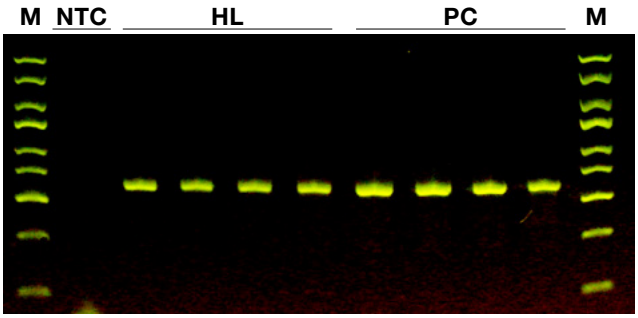

Figure 5. Direct amplification of DNA from cultured mammalian cells. Platinum SuperFi II Green PCR Master Mix was used to amplify a 0.6 kb target from 10 intact HeLa (HL) cells in 2 µL of PBS. M: E-Gel 1 Kb Plus Express DNA Ladder as the size standard, NTC: no template control, and PC: positive control from 5 ng of purified genomic DNA.

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