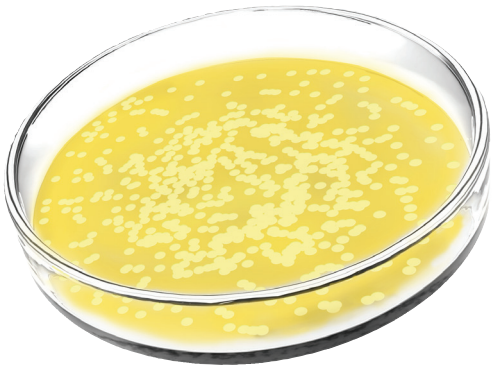
Direct PCR from bacterial and yeast cells using Platinum SuperFi DNA Polymerase



Introduction

Invitrogen™ Platinum™ SuperFi™ DNA Polymerase is engineered with a DNA-binding domain, resulting in high processivity and increased resistance to PCR inhibitors. This application note describes how PCR can successfully be performed from intact *E. coli* and gram-positive bacterial 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 DNA Polymerase allows direct amplification from yeast cells. As accurate DNA sequence is maintained with the exceptionally high fidelity of Platinum SuperFi DNA Polymerase (>100x higher fidelity than *Taq* DNA polymerase), the direct PCR product can be used for downstream cloning or sequencing applications. This protocol minimizes sample loss and significantly reduces protocol time with no DNA purification or cell lysis steps.

Materials and methods

- Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix (Cat. No. 12359-010) or Platinum™ SuperFi™ PCR Master Mix (Cat. No. 12358-010)
- Primers (forward and reverse)
- Well-isolated colonies (ideally fresh) or overnight cultures
- Sterile toothpicks, pipette tips, or microstreakers
- Invitrogen™ E-Gel™ General Purpose Agarose Gels

Important notes

- Annealing temperature rules for Platinum SuperFi DNA Polymerase are different from those used for many common DNA polymerases (such as *Taq* DNA polymerases). For optimal results, use the T_m calculator on our website. Go to **thermofisher.com/tmcalculator**
- 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.

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Reaction composition for PCR using master mix

Component	20 µL reaction	50 µL reaction	Final concentration
2X Platinum SuperFi 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

Number of cycles	Step	2-step protocol		3-step protocol	
		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**	–	–	Varies	10 sec
	Extension	72°C	30 sec/kb	72°C	30 sec/kb
1	Final extension	72°C	5 min	72°C	5 min
		4°C	Indefinitely		

* The initial denaturation step can be extended to up to 5 min to ensure complete cell lysis; however, 30 sec is sufficient in most cases.
** Always use the T_m calculator on our website at **thermofisher.com/tmcalculator** to calculate T_m of your primers and the recommended annealing temperature.

Results

A 1,582 bp target was amplified directly from two commonly used *E. coli* strains using Platinum SuperFi Green PCR Master Mix. BL21 and Invitrogen™ OneShot™ TOP10 Chemically Competent Cells were transferred into the PCR master mix by three methods: (A) a colony was suspended in 20 µL of PCR-grade water, and 1 µL of the suspension was added; (B) a small amount of a colony from an agar plate was transferred directly; (C) 1 µL of overnight culture was added. The cycling protocol was: 1 cycle at 98°C for 30 sec; 35 cycles at 98°C for 10 sec, 61°C for 10 sec, 72°C for 33 sec; 1 cycle at 72°C for 5 min. The products were separated using a 1% agarose gel in TAE buffer. Successful amplification of the target fragment was achieved from BL21 and TOP10 colonies on agar plates and in liquid cultures (Figure 1).

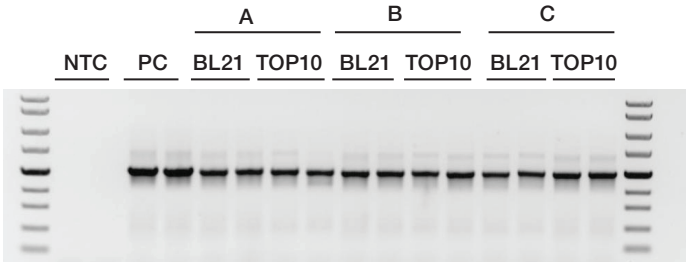

Figure 1. Direct amplification of DNA from *E. coli* colonies on agar plates and in liquid cultures. The Platinum SuperFi Green PCR Master Mix was used to amplify a 1,582 bp target from two commonly used *E. coli* strains (BL21 and TOP10) in the following formats: **(A)** 1 µL of a colony suspended in 20 µL PCR-grade water; **(B)** a small amount of a colony from an agar plate; **(C)** 1 µL of overnight culture. NTC: no-template control; PC: positive control from 5 ng of purified plasmid DNA. Thermo Scientific™ ZipRule™ Express DNA Ladder 2 was used as a size standard.

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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 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 1,500 bp target from *B. subtilis* cells was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 5 sec, 61.5°C for 5 sec, 72°C for 25 sec; 1 cycle at 72°C for 5 min. The cycling protocol to amplify a 576 bp target from *P. pastoris* cells was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 65°C for 10 sec, 72°C for 18 sec; 1 cycle at 72°C for 5 min. The products were separated using a 1% agarose gel in TAE buffer. Successful amplification was achieved from both *B. subtilis* and *P. pastoris* colonies without any lysis steps (boiling, NaOH or Zymolyase treatment) prior to PCR (Figure 2).

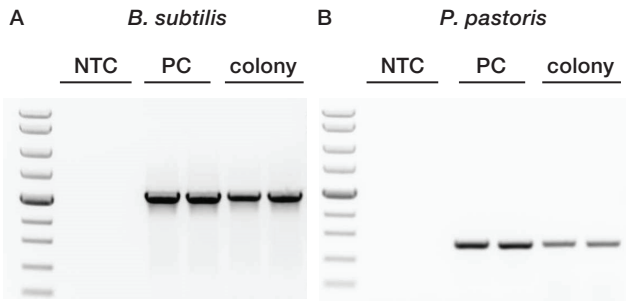

Figure 2. Direct amplification of DNA from *B. subtilis* and *P. pastoris* colonies. (A) A 1,500 bp fragment was amplified from intact *B. subtilis* using Platinum SuperFi Green PCR Master Mix. **(B)** A 576 bp fragment was amplified from intact *P. pastoris* using Platinum SuperFi Green PCR Master Mix. NTC: no template control; PC: positive control from purified plasmid DNA. ZipRuler Express DNA Ladder 2 was used as a size standard.

Summary

With its high processivity and resistance to inhibitors, Platinum SuperFi DNA Polymerase enables direct amplification of DNA from intact *E. coli*, yeast, and gram-positive bacterial cells. High-fidelity PCR with no DNA purification or cell lysis steps can help significantly reduce protocol time in various cloning applications.

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

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