Application Note

Direct PCR from Yeast Cells

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Introduction

Yeast is widely used in scientific research for genetic studies, studies of basic cellular processes as well as for protein expression and bioproduction.

PCR is commonly used in studies based on yeast cells. Yeast mutants are often analyzed by PCR-based assays for the presence of gene insertion or deletion. PCR is also used to screen yeast transformants for the presence of the plasmid of interest. These approaches usually require extraction and purification of DNA prior PCR. DNA purification from yeast is time-consuming and labor-intensive because yeast has a proteinaceous cell wall that is very difficult to lyse, requires harsh treatments and use of reagents that can damage the DNA. These reagents can inhibit subsequent PCR steps by affecting DNA polymerase activity.

Alternatively, colony PCR is often employed to avoid DNA purification steps. In colony PCR a yeast colony is placed into *Taq* DNA polymerase-based PCR master mix to amplify the DNA fragment of interest. This approach allows analysis of transformants directly from liquid cultures or agar plates, thereby allowing significant time savings compared to traditional approaches. This technique however, is limited due to the inhibition of *Taq* DNA polymerase by debris from yeast cells and components of culture media. As a result, inconsistent results are often obtained and only short fragments of cloned inserts can be interrogated. In addition, *Taq* DNA polymerase is very slow enzyme (requires 1 min/kb) which results in very long PCR cycling times.

Here we present an easy, quick and reliable method to amplify DNA from yeast without prior DNA purification by using Thermo Scientific™ Phire™ Plant Direct PCR Master Mix. Yeast grown in liquid medium or agar plates are both suitable for direct amplification. A small aliquot of medium with yeast cells or a punch of a yeast colony is added directly into the Phire Plant Direct PCR Master Mix allowing significant savings in both time and cost. The master mix is based on the Thermo Scientific™ Phire™ Hot Start II DNA Polymerase, which was created by fusion protein technology and is extremely robust, as well as resistant to many inhibitors. The Phire Hot Start II DNA Polymerase is also a very fast enzyme (extension time - 10 s/kb), that enables the use of short cycling times and quick PCR turnaround. The master mix includes two tracking dyes and a density reagent, which enables direct loading of PCR products on gels. Due to all these features, direct amplification from yeast, plants or fungi using Phire Plant Direct PCR Master Mix allows achieving the same robust DNA amplification when compared to conventional PCR.

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Materials and Methods

Two yeast strains were used in the experiments:

- *Saccharomyces cerevisiae*
- *Kluyveromyces lactis*

Both strains were grown in YPD (yeast extract peptone dextrose) liquid medium and on YPD agar plates overnight.

Genomic DNA (gDNA) for positive controls was extracted using Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (K0721), following Yeast Genomic DNA Purification Protocol. 25 ng of gDNA was used for the PCR.

Direct PCR from yeast was done using Phire Plant Direct PCR Master Mix (F-160S). For direct PCR on yeast grown in liquid culture, 1 µL of overnight culture was added to 50 µL of 1X Phire Plant Direct PCR Master Mix. For direct PCR on yeast grown agar plates, yeast cells were grown on agar plates overnight to allow formation of colonies. A well-isolated colony was swiped with a sterile pipette tip and resuspended in 50 µL of 1X Phire Plant Direct PCR Master Mix prior PCR. All amplifications were performed according to standard Direct PCR protocols (Table 1 and 2) by using Thermo Scientific™ Arktik™ Thermal Cycler.

Table 1. PCR reaction set up.

Component	Volume
2X Phire Tissue Direct PCR Master Mix	25 µL
Primer A	X µL (0.5 µM final conc.)
Primer B	X µL (0.5 µM final conc.)
Sample	1 µL of yeast liquid culture, or punch of yeast colony
H_2O	add to 50 µL

Table 2. Cycling conditions.

Cycle step	2-step		3-step		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	5 min	98°C	5 min.	1
Denaturation	98°C	5 s	98°C	5 s	40
Annealing	-	-	X°C	5 s	
Extension	72°C	20 s ≤ 1 kb 20 s/kb > 1 kb	72°C	20 s ≤ 1 kb 20 s/kb > 1 kb	
Final Extension	72°C +4°C	1 min hold	72°C +4°C	1 min hold	1

Results and discussion

In order to examine whether the Direct PCR approach is suitable to detect plasmid DNA from yeast cells, *S. cerevisiae* was first transfected with a plasmid DNA of interest. Cells were then grown overnight in liquid culture and on agar plates. A plasmid DNA fragment was amplified from the samples using the Phire Plant Direct PCR Master Mix. The Plasmid DNA fragment was also amplified from purified DNA. All reactions were completed in less than 30 minutes due to very short cycling times with Phire Hot Start II DNA Polymerase. Robust amplification was achieved with both yeast liquid culture as well as yeast colony by using Phire Plant Direct PCR Master Mix. Efficiency of amplification was comparable to that achieved with purified DNA (Figure 1). Our results show that direct PCR approach can be used for fast and simple detection of plasmid DNA from yeast cells.

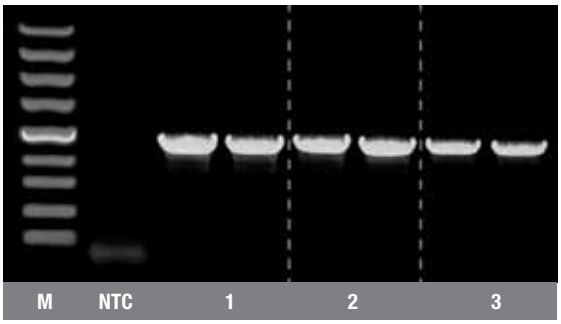

Figure 1. Amplification of 1.5 kb fragment from transfected plasmid DNA.
NTC (No-template control)
1 – PCR with purified *S. cerevisiae* DNA
2 – PCR from colonies grown on agar plates)
3 – PCR from cells grown in liquid culture

M - Thermo Scientific™ ZipRuler™ Express DNA Ladder 2

The second experiment was done to investigate whether Direct PCR can be used to specifically amplify endogenous yeast gDNA. In order to investigate this, a species-identification experiment was done using the two yeast stains: *K. lactis* and *S. cerevisiae*. Phire Plant Direct PCR Master Mix and *K. lactis*-specific primers were used to amplify a 2.5 kb gDNA fragment. In this experiment fresh overnight colonies from agar plates and from liquid culture were used for direct amplification. The amplification was *K. lactis*- specific, allowing easy species confirmation (Figure 2). The results show that indeed Direct PCR can be used for yeast genomic DNA amplification using both liquid medium and colony samples.

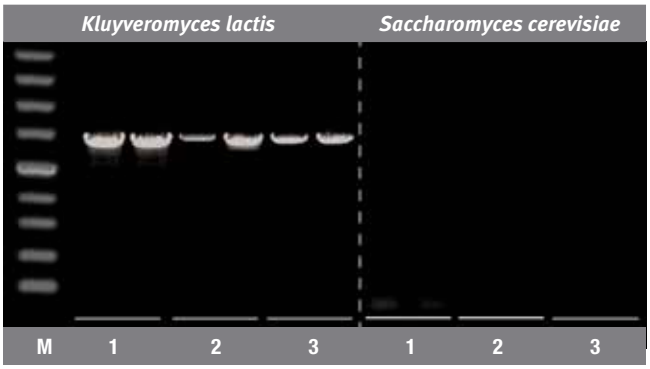

Figure 2. Direct amplification of yeast genomic DNA to identify the species of interest.
1 - PCR with purified yeast gDNA
2 - PCR from yeast colony
3 - PCR from yeast liquid culture

M - ZipRuler Express DNA Ladder 2

Our results show that the Phire Plant Direct PCR Master Mix allows direct DNA amplification from yeast cells. Both ectopic plasmid DNA as well as endogenous gDNA can be amplified with Direct PCR with high efficiency. In comparison to current *Taq*-based methods for DNA amplification from yeast cells, this approach is more simple, reliable and reproducible due to superb features of Phire Hot Start II DNA polymerase. It is also one of the fastest PCR methods available allowing achieving results in as short as 30 minutes. Direct PCR enables DNA amplification from crude samples, because it is optimized to work in an inhibitor-rich environment with the same efficiency as with purified DNA. Not only does this apply to yeast cultures but also to bacterial cultures or cell lines.

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