



Direct PCR from Mouse Tissue using Phire® Hot Start DNA Polymerase and Piko® Thermal Cycler

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Finnzymes' Phire® Hot Start DNA Polymerase enables robust DNA amplification directly from a variety of mouse tissues without prior DNA extraction or purification. When combined with a fast PCR instrument such as the Piko® Thermal Cycler, Piko® PCR Plates (Finnzymes Oy), and the FlashGel® system (Lonza), protocols can be performed in 30–45 minutes (from sample to gel image) and with reaction volumes as low as 5 µl.

Introduction

Many millions of mice are used in research every year, and PCR is the predominant application used for genotyping these animals. DNA may be obtained from a number of different materials, including ear, tail and toe tissues (1,2,3). Typically, PCR protocols for these tissues call for an initial DNA isolation step which is often time consuming and may require the use of expensive kits or reagents.

Finnzymes' Phire Hot Start DNA Polymerase is a specially engineered enzyme that has been fused to a double stranded DNA binding domain (4) and performs well even in the presence of strong PCR inhibitors. The Piko Thermal Cycler offers superior thermal uniformity and ramping speed allowing the fastest possible protocols with consistent results when used with ultra-thin walled (UTW®) Piko PCR Plates.

By combining these technologies, PCR can be performed directly on small quantities of mouse tissues in minimal time without DNA purification. The recommended PCR protocol for Phire DNA Polymerase is used, with the simple addition of a preliminary 5 minute incubation step to release the DNA template.

PCR products amplified directly from tissues sometimes run poorly in agarose gel, most likely due to the PCR product becoming entangled with cell debris. We describe here an additive for loading buffer that eliminates this problem and allows these PCR products to be electrophoresed without migration artifacts.

Materials and methods

- Phire® Hot Start DNA polymerase (Finnzymes Oy)
- 10 mM dNTP Mix (Finnzymes Oy)
- Piko® Thermal Cycler (Finnzymes Oy)
- UTW® (ultra-thin walled) Piko PCR Plates (Finnzymes Oy)
- DNARElease™ Additive (Finnzymes Oy)
- Harris Uni-Core™ 0.50 mm (available from Finnzymes Oy)
- Harris Cutting Mat™ (available from Finnzymes Oy)
- FlashGel® System with 2.2 % Agarose FlashGel® DNA Cassette (Lonza), run at 275 volts for 4 minutes
- FlashGel® Loading Dye (Lonza)

• Primers

1500 bp fragment of mouse intestinal fatty acid binding protein gene:

F: ATTCACAACAGGGGTCAGC 20 nt T_m = 64.3°C
R: AGAAACCTCTCGGACAGCAA 20 nt T_m = 64.3°C

466 bp fragment of mouse intestinal fatty acid binding protein gene:

F: CCTCCGAGAGCAGCGATTAAGTGTGAG 30 nt T_m = 76.6°C
R: TAGAGCTTGGCCACATCACAGGTCATTGAG 30 nt T_m = 74.4°C

273 bp fragment of mouse male-specific sex-determining Region Y(SRY) gene:

F: TTGTCTAGAGCATGGAGGGCCATGTCAG 30 nt T_m = 77.0°C
R: CCACTCTGTGACACTTTAGCCCTCCGA 30 nt T_m = 77.4°C

194 bp fragment of mouse intestinal fatty acid binding protein gene:

F: TGGACAGGACTGGACCTGTCTTCTAGTA 30 nt T_m = 75.8°C
R: TAGAGCTTGGCCACATCACAGGTCATTGAG 30 nt T_m = 74.4°C

• Tissue samples

Direct protocol: Previously frozen mouse ear or tail tissues were used directly for PCR without further preparation. A 0.50 mm tissue punch was cut using the Harris Uni-Core tool and placed directly into a 50 µl PCR reaction. In direct protocol, DNARElease Additive was added in gel loading dye when analyzing PCR products on gel (see figure 4).

Dilution protocol: A 2 mm punch of mouse ear or 1 mm segment of tail was placed in 20 µl of TE buffer (pH 8.0) containing 0.5 µl of DNARElease Additive. After vortexing for 15 seconds, the samples were incubated at 75°C for 5 min and then at 96°C for 2 min. After centrifugation, the supernatant was removed and stored at -20°C if not used immediately. 0.5–4 µl of supernatant was used as template for 5–20 µl PCR reaction.

Reaction conditions for PCR

Component	20 µl reaction	50 µl reaction	Final conc.
H ₂ O	Add to 20 µl	Add to 50 µl	
5x Phire® Reaction Buffer	4 µl	10 µl	1x
10 mM dNTP Mix	0.4 µl	1 µl	200 µM
primer A	x µl	x µl	0.5 µM
primer B	x µl	x µl	0.5 µM
Phire® Hot Start DNA Polymerase	0.4 µl	1 µl	
Mouse tissue: Direct PCR	-	0.50 mm punch	
Dilution PCR	0.5–4 µl	-	

Cycling conditions

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Lysis of cells*	98°C	5 min	98°C	5 min	1
Denaturation	98°C	5 s	98°C	5 s	35–40
Annealing**	-	-	60–72°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

* Optional in dilution PCR.

** Recommended annealing temperature is equal to the T_m for primers < 21 nt, and T_m +3°C for primers > 21 nt.

Results

We have developed two simple methods for PCR from mouse tissues that render prior DNA purification unnecessary. Direct PCR is a good choice for amplification of a single DNA fragment from mouse ear or tail tissue (Figure 1).

If multiple PCR reactions are to be performed from the same sample, it is convenient to use the dilution protocol. We have found that incubating a small amount of tissue in 20 μ l of TE buffer spiked with DNARelease Additive for 5 minutes at 75°C and then for 2 minutes at 96°C provides enough template for up to 40 reactions (Figure 2). The dilution protocol is also recommended for long and/or difficult amplicons.

For reference, we tested various hot start *Taq*-based DNA polymerases in the Direct PCR assay. All performed poorly producing only weak and/or non-specific bands with the shortest amplicon and no product with amplicons longer than 200 bp, whereas Phire DNA Polymerase successfully amplified all PCR products tested, up to 1.5 kb (Figure 3).

PCR products in the presence of large amounts of unpurified tissue may become trapped in the wells during agarose gel electrophoresis (Figure 4, lanes 1–3). Finnzymes' DNARelease Additive eliminates this problem (Figure 4, lanes 4–6; Figures 1 and 3).

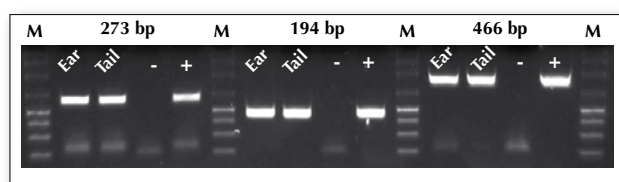


Figure 1. Direct PCR from mouse ear and tail tissue samples. Three amplicons (194–466 bp) were amplified as described in Materials and Methods using 24-well Piko Thermal Cycler (reaction volume 50 μ l, 2-step protocol, 40 cycles). 7 μ l of PCR products were run on FlashGel System with DNARelease Additive in the FlashGel Loading Dye. Overall protocol time from tissue to gel image was under 45 minutes. M, Size Marker.

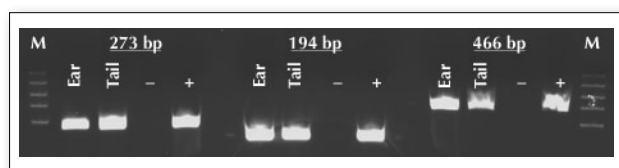


Figure 2. Dilution PCR from mouse ear and tail samples. 0.5 μ l of the sample treated with Dilution buffer was added to 5 μ l PCR reactions. Experiments were performed as described in Materials and Methods using 96-well Piko Thermal Cycler (2-step protocol, 35 cycles) and PCR products were run on FlashGel System. The overall protocol time was under 30 minutes. M, Size Marker.

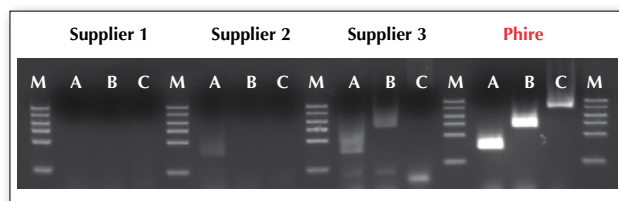


Figure 3. Comparison of Phire Hot Start DNA Polymerase to three hot start Taq DNA Polymerases in direct PCR from tissue. PCR reactions from mouse ear tissue were set up and run according to manufacturers' recommendations. 7 μ l of PCR products were run on FlashGel System with DNARelease Additive in the FlashGel Loading Dye. M, Size Marker. A: 194 bp, B: 466 bp, C: 1500 bp.

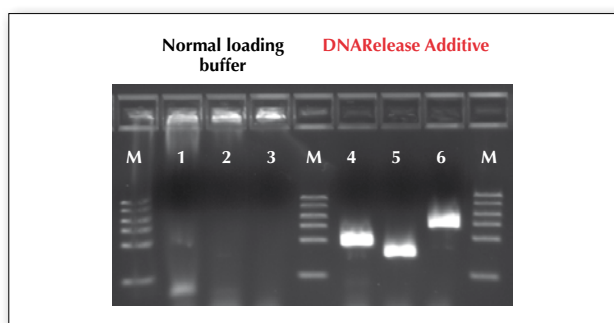


Figure 4. Special loading buffer additive is needed for efficient electrophoresis of PCR products amplified in the presence of tissue. PCR products were amplified directly from mouse ear tissue as described in Figure 1. 7 μ l of PCR products were run on FlashGel System with or without DNARelease Additive in the FlashGel Loading Dye. M, Size Marker. Lanes 1 and 4: 273 bp, 2 and 5: 194 bp, 3 and 6: 466 bp.

Discussion

In this application note we describe two simple protocols for PCR from mouse tissue that require no DNA isolation or purification steps. Mouse ear punches are the preferred sample material for direct PCR applications; results are more consistent than with tail material, especially with larger (> 500 bp) amplicons (data not shown). Ear punching is also used to uniquely identify the animals and the process is simpler and less painful to the mice than removal of toes or tail samples. We have routinely amplified PCR products as large as 1.5 kb from mouse ear punches. However, these protocols are designed to amplify one DNA fragment in each reaction; more complex genotyping experiments amplifying several fragments may require further optimization, especially if the amplicons differ greatly in size.

References

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4. Wang, Y. *et al.* 2004. A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro. *Nucleic Acids Research* 32:1197-1207.

Check the most recent Direct PCR protocols at
www.finnzymes.com/directpcr.

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