

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

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 30-40 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)
- 96-well Piko™ Thermal Cycler (Finnzymes Oy)
- Piko™ PCR Plates (Finnzymes Oy)

- DNARElease™ Additive for loading buffer (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: ATTCAACAACAGGGGTCAGC 20 nt Tm = 64.3°C

R: AGAAACCTCTCGGACAGCAA 20 nt Tm = 64.3°C

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

F: CCTCCGAGAGCAGCGATTAAAAGTGTGAG 30 nt Tm = 76.6°C

R: TAGAGCTTTGCCACATCACAGGTCATTGAG 30 nt Tm = 74.4°C

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

F: TTGTCTAGAGAGCATGGAGGGCCATGTCAA 30 nt Tm = 77.0°C

R: CCACTCCTGTGACACTTTAGCCCTCCGA 30 nt Tm = 77.4°C

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

F: TGGACAGGACTGGACCTCTGCTTTCCTAGA 30 nt Tm = 75.8°C

R: TAGAGCTTTGCCACATCACAGGTCATTGAG 30 nt Tm = 74.4°C

- Tissue samples

Direct PCR: Previously frozen mouse tissues from ears (1mm punch or ¼ of 2 mm punch) or tails (segments of approximately 1 mm) were used without further preparation. Dilution PCR: 2 mm punch of mouse ear or 1 mm tail were incubated in 50 µl TE buffer for 5 min at 80°C. After centrifugation the supernatant was removed and stored at -20°C if not used immediately.

**Table 1. Pipetting instructions**

Component	20 µl reaction	Final conc.
H <sub>2</sub> O	Add to 20 µl	
5x Phire™ Reaction Buffer	4.0 µl	1x
10 mM dNTP Mix	0.4 µl	200 µM
Primer A	x µl	0.5 µM
Primer B	x µl	0.5 µM
Mouse tissue:	Ear: 1 mm ear punch / Tail: less than 1 mm tail	
Direct PCR		
Dilution PCR	1.0 µl	
Phire™ Hot Start DNA Polymerase	0.4 µl	

**Table 2. Cycling instructions**

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Cell Lysis*	98°C	5 min	98°C	5 min	1
Initial denaturation**	98°C	30 s	98°C	30 s	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	72°C	20 s /1 kb	
Final extension	72°C 4°C	1 min hold	72°C 4°C	1 min hold	1

\* Can be excluded in dilution PCR.

\*\* Can be excluded for protocols with 5 min. cell lysis step.

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

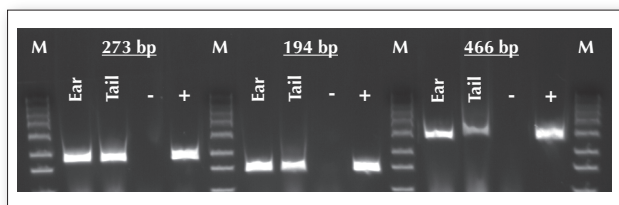
## Results

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). We have found that larger amplicons are more easily amplified from ear than tail tissue, as can be seen in the case of the 466 bp *fabpi* gene fragment.

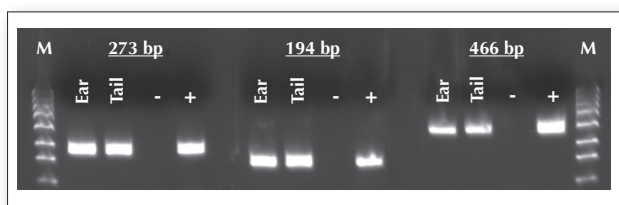
If multiple PCR reactions are to be performed from the same sample, incubating a small amount of tissue in 50  $\mu$ l of TE for 5 minutes at 80°C provides enough template for up to 100 reactions (Figure 2). As with the direct PCR protocol, the larger (466 bp) PCR fragment was amplified slightly less efficiently than the shorter ones. This may indicate some limitation in the release of larger DNA molecules from the tissue.

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).

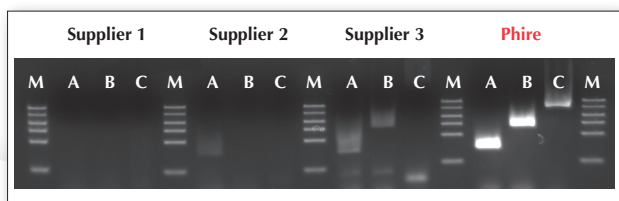
PCR products in the presence of large amounts of unpurified tissue may become trapped in the wells during agarose gel electrophoresis (Figure 4, lanes 1-3). Finnzymes' DNARElease™ Additive for loading buffer eliminates this problem (Figure 4, lanes 4-6; Figures 1 and 3).



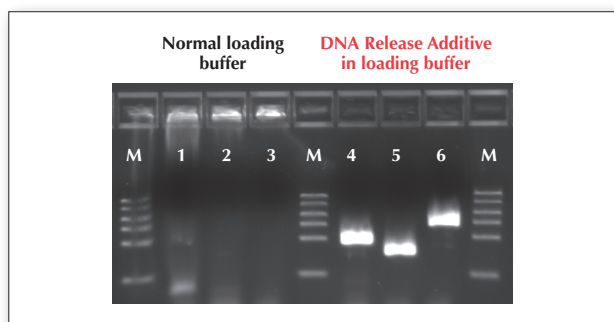
**Figure 1. Direct PCR from mouse ear and tail tissue samples.** Three amplicons (194-466 bp) were amplified as described in Materials and Methods (reaction volume 15  $\mu$ l, 2-step protocol, 40 cycles). 7  $\mu$ l of PCR products were run on FlashGel® System with DNARElease Additive in the FlashGel® Loading Dye. Overall protocol time from tissue to gel image was under 40 minutes. M, Size Marker.



**Figure 2. Dilution PCR from mouse ear and tail samples.** 0.5  $\mu$ l of the pre-incubated tissue sample was added to the reaction (total volume 5  $\mu$ l). Experiments were performed as described in Materials and Methods (no preincubation step, 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  $\mu$ 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  $\mu$ 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. 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.

For more information, please visit our website [www.finnzymes.com](http://www.finnzymes.com) or contact your local distributor.

## References

1. Wang, Z. and Storm, D.R. 2006. Extraction of DNA from mouse tails. *BioTechniques* 41:410-412.
2. Malumbres, M. et al. 1997. Isolation of High Molecular Weight DNA for Reliable Genotyping of Mice. *BioTechniques* 22:1114-1119.
3. Burkhart, C.A. et al. 2002. A simple method for the isolation of genomic DNA from mouse tail free of real-time PCR inhibitors. *Journal of Biophysical and Biochemical Methods* 52:145-149.
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.

Phire, Piko, UTW and DNARElease are trademarks of Finnzymes Oy or its affiliates. FlashGel is a registered trademark of Lonza.