Application Note

# Direct PCR on Hair: A New Animal-Friendly Genotyping Method

Birute Gagiliene, Daina Nekrasiene and Donata Ulcinaite
Thermo Fisher Scientific, Vilnius, Lithuania

## Abstract

Conventional animal genotyping methods are based on PCR amplification of a transgene usually from tail biopsies or ear punches. Here we present a new, animal-friendly genotyping approach using animal hair and Thermo Scientific™ Phire™ Tissue Direct PCR Master Mix. Phire Tissue Direct PCR Master Mix allows sensitive and specific target DNA amplification from a variety of animal hair samples without prior DNA purification and can be used for simplified, painless and humane animal genotyping applications.

## Introduction

Gene transfer into animals is extensively used to study roles of genes in development, physiology and disease. This approach requires screening for presence of a transgene in animal tissues, often using PCR. Traditionally, it also involves time consuming DNA extraction steps from the biopsies, ear punches or toe tissues. Obtaining tissue from an animal for DNA analysis can cause pain and distress to the animal, it often requires anesthesia and is strictly regulated.

Phire Tissue Direct PCR Master Mix can be used for gene amplification from the animal and human tissue samples without prior DNA purification. It is based on the Thermo Scientific™ Phire™ Hot Start II DNA Polymerase, which was created by fusion protein technology and is extremely robust, sensitive and resistant to tissue derived inhibitors. The Phire DNA Polymerase is a fast enzyme that enables the use of short cycling times and quick PCR turnaround.

Here we present a novel animal-friendly and time-saving genotyping approach based on the use of animal hair samples and Phire Tissue Direct PCR Master Mix. Collecting animal hair is a simple and straightforward procedure that causes minimal discomfort to the animal and does not require anesthesia. A hair sample is added directly into Phire Tissue Direct PCR Master Mix without any DNA extraction step allowing significant savings in both time and cost. The data presented below shows that mouse genotyping using hair and Phire Tissue Direct PCR Master Mix is as effective as genotyping using mouse tail or ear samples.

## Materials and Methods

Phire Tissue Direct PCR Master Mix (F-170S)
Thermo Scientific™ Arktik™ Thermal Cycler, 96-well block (TCA0002 and TCA0096)
0.2 mL Flat Cap Tubes (AB-0620)
Animal hair samples (with folicles)
Mouse tail and ear tissues

## Collection of hair

Tweezers were used to collect the animal hair with follicles. Fresh hair was used in all the experiments.

## Direct Protocol

Animal hair or 0.5 mm punch of the ear or tail tissues was added directly to 50 µL of Phire Tissue Direct PCR Master Mix containing the gene-specific primers (Table 1). PCR was preformed as described in Table 2 and PCR products were loaded directly onto gels.

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## Dilution & Storage Protocol

Hair was placed in 20 µL of Dilution Buffer containing 0.5 µL of DNA Release Additive. The samples were incubated at room temperature for 2 minutes and then at 98 °C for 2 minutes. The samples were spun down and 1 µL of the supernatant was used as a template in a 20 µL reaction with Phire Tissue Direct PCR Master Mix and gene specific primers (Table 1). PCR was preformed as described in Table 2 and PCR products were loaded directly onto gels.

**Table 1.** PCR Reaction Setup.

| Component | 20 µL rxn | 50 µL rxn | Final conc. |
|---|---|---|---|
| $H_2O$ | add to 20 µL | add to 50 µL | |
| 2X Phire Tissue Direct PCR Master Mix | 10 µL | 25 µL | 1X |
| Primer A | X µL | X µL | 0.5 µM |
| Primer B | X µL | X µL | 0.5 µM |
| **Sample** | | | |
| Direct Protocol | – | 0.5 mm punch or hair | |
| Dilution & Storage Protocol | 1 µL | – | |

**Table 2.** PCR 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 | 35 |
| Annealing | – | – | X°C | 5 s | |
| Extension | 72°C | 20 s ≤1 kb<br>20 s/kb >1 kb | 72°C | 20 s ≤1 kb<br>20 s/kb >1 kb | |
| Final Extension | 72°C<br>+4°C | 1 min<br>hold | 72°C<br>+4°C | 1 min<br>hold | 1 |

## Results and Discussion

The ability of Phire Tissue Direct PCR Master Mix to amplify target DNA from the hair samples was tested using mouse hair. Mouse hair samples were collected with tweezers from the animal's back, and only hair with follicles attached were used for PCR. Robust amplification of different length amplicons was achieved even from a single hair sample (Figure 1). Phire Tissue Direct PCR Master Mix can therefore be used for PCR based genotyping applications by using hair as a starting material.

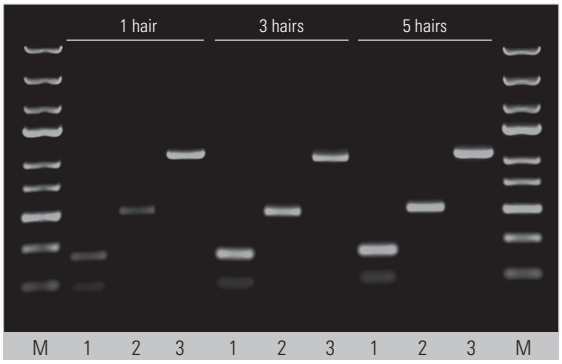


**Figure 1.** Different length amplicons were amplified using different number of mice hair and Phire Tissue Direct PCR Master Mix (direct protocol). Fragments sizes 1 – 237 bp, 2 – 515 bp, 3 – 1.1 kb. M – Thermo Scientific™ O'GeneRuler™ Express DNA Ladder.

Multiplex PCR amplification of five different length fragments was achieved using mouse hair and Phire Tissue Direct PCR Master Mix (Figure 2.). This approach can be used in genotyping applications that require simultaneous determination of several markers in one reaction.

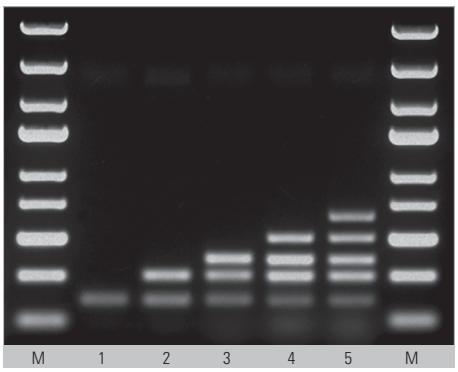


**Figure 2.** Five different length DNA amplicons, ranging from 185 to 650 bp, were amplified from mouse hair in 1-plex to 5-plex reaction with Phire Tissue Direct PCR Master Mix. Dilution & storage protocol and 5 hairs were used for the amplification. M – O'GeneRuler Express DNA Ladder.

The Phire DNA polymerase from Direct PCR Master Mix is optimized to achieve robust and sensitive amplification even from tiny amounts of tissue samples. The performance of Phire Direct PCR Master Mix was compared to that of corresponding kits from other vendors. Figure 3 shows that while other vendors' kits work

with abundant tissue sample types (ear and tail) they fail with hair, which is a more challenging and scarce source of DNA template (Figure 3). Phire Direct PCR Master Mix achieved robust amplification from all the tissue samples tested.

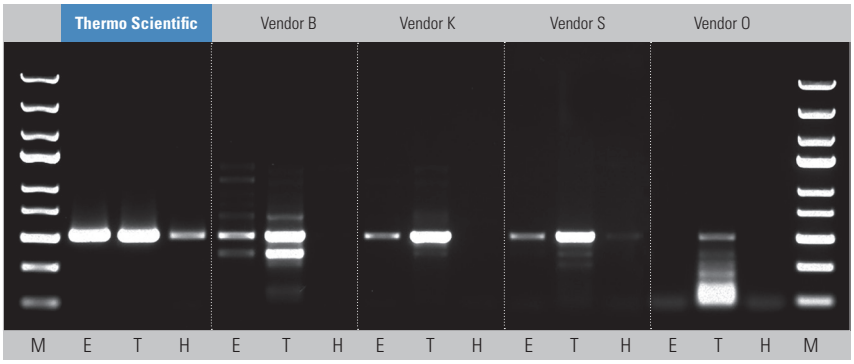


**Figure 3.** A 515 bp DNA fragment was amplified from mouse ear (E), tail (T) and hair (H) tissue samples using Phire Tissue Direct PCR Master Mix (direct protocol) and corresponding kits from different vendors. All reactions were performed according to manufacturers' recommendations. M – O'GeneRuler Express DNA Ladder.

Phire Direct PCR Master Mix can also be used for genotyping of animals other than mice. Figure 4 shows efficient target DNA amplification from the hair of six different species. Direct PCR amplification can also be achieved from human hair samples (data not shown).

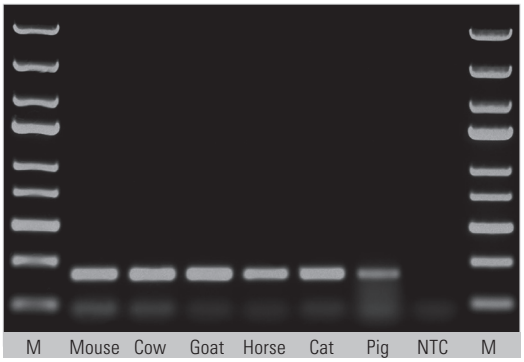


**Figure 4.** A 237 bp DNA fragment was amplified from various animal species hair with Phire Tissue Direct PCR Master Mix using both direct and dilution & storage protocol (data with dilution & storage protocol is shown). Identical PCR cycling conditions were used with 3-5 hairs from each animal. NTC – no template control. M – O'GeneRuler Express DNA Ladder.

## Conclusion

The Phire Tissue Direct PCR Master Mix allows animal genotyping using hair samples. This method is simple, has an abundant sample source and allows repetition of the reactions many times. It is also one of the fastest genotyping methods available allowing results in as short as 30 minutes due to superb efficiency of Phire Hot Start II DNA polymerase and direct PCR approach. Genotyping using hair is painless, has minimum impact on the animal and represents simple, fast and animal-friendly genotyping method.

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**Europe**

Customer Service
cs.molbio.eu@thermofisher.com

Technical Support
ts.molbio.eu@thermofisher.com

Tel 00800 222 00 888
Fax 00800 222 00 889

**United States**

Customer Service
cs.molbio@thermofisher.com

Technical Support
ts.molbio@thermofisher.com

Tel 877 661 8841
Fax 800 854 5395

**Canada**

Customer Service
cs.molbio@thermofisher.com

Technical Support
ts.molbio@thermofisher.com

Tel 800 340 9026
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