Application Note

Thermo Scientific Phire Animal Tissue Direct PCR Kit: Genotyping Transgenic Mice

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Abstract

Thermo Scientific™ Phire™ Animal Tissue Direct PCR Kit is designed for DNA amplification directly from animal tissues. Here we present protocols for transgenic mice genotyping achieved directly from mouse ear tissues using the kit. The results obtained with this kit are extremely robust, with greater yields as compared to a commercial DNA purification system used together with a conventional hot start *Taq* DNA polymerase. When combined with Thermo Scientific™ Piko™ Thermal Cycler and UTW PCR Plates, PCR protocols can be completed in as little as 40 minutes.

Introduction

Gene transfer into mice is extensively used to study the roles of genes in development, physiology and human disease. The use of these animals requires screening for the presence of the transgene, usually with PCR. Traditionally, this involves a time consuming DNA isolation step, during which DNA for PCR analysis is purified from ear, tail or toe tissues.[1, 2]

The Phire Animal Tissue Direct PCR Kit can be used for genotyping of transgenic mice without prior DNA purification. Here we present two typical approaches for genotyping. In the first method, two different primer pairs are multiplexed in one PCR reaction. We also tested a more challenging genotyping approach where only one primer set is used for amplification of two different DNA fragments with a large size difference. Finally, we show that the dilution protocol samples are stable in long-term storage.

Materials and Methods

- Phire Animal Direct PCR Kit (Cat #F-140WH)
- SspI (Cat #ER0771)
- Piko 24-well Thermal Cycler (Cat #TCP0024)
- Piko UTW PCR Plates (Cat #SPL0241)
- Mouse ear tissue

Direct protocol:

A 0.50 mm tissue punch was cut using the Harris Uni-Core™ puncher and placed directly into a 50 µL PCR reaction. Thermo Scientific™ DNARelease™ Additive was included in the gel loading dye when analyzing PCR products on an agarose gel.

Dilution protocol:

A 2 mm punch of mouse ear was placed in 20 µL of Dilution Buffer containing 0.5 µL of DNARelease Additive. The samples were incubated at RT for 2 minutes and then at 98 °C for 2 minutes. The samples were spun down and 1 µL of supernatant was used as a template in a 20 µL PCR reaction. The supernatant was transferred to a new tube and stored at -20 °C if not used right away.

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Stability assays:

Mouse ear tissues were prepared as described for dilution protocol. The reactions were either freezed and thawed for 20 times or stored at -20 °C for 1 year before PCR.

Table 1: Reaction conditions for PCR

Components	20 µL reaction	50 µL reaction	Final Conc.
H_2O	add to 20 µL	add to 50 µL	
2x Phire Animal Tissue PCR Buffer	10 µL	25 µL	1x
primer A	x µL	x µL	0.5 µL
primer B	x µL	x µL	0.5 µL
Phire Hot-Start II DNA Polymerase	0.4 µL	1 µL	
Sample: Direct Protocol	-	0.5 mm punch	
Dilution Protocol	1 µL	-	

Table 2: Cycling conditions

	2-step protocol		3-step protocol		Final Conc.
Cycle step	Temp.	Time	Temp.	Time	Cycles
Lysis of cells	98 °C	5 minute	98 °C	5 minute	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 minute hold	72 °C 4 °C	1 minute hold	1

**Recommended annealing temperature is equal to the Tm for primers ≤ 20 nt, and Tm +3 °C for primers > 20 nt.*

Results and Discussion

The performance of the Phire Animal Tissue Direct PCR Kit was first tested with two genotyping protocols. Simply placing a 0.5 mm punch of mouse tissue directly into a 50 µL PCR reaction gave robust results (Figure 1). A more challenging protocol (where only one primer set was used for amplification of two fragments with a large size difference) also produced abundant yields of products of correct sizes (Figure 2). The weaker upper band with heterozygote mice is due to the competition of both templates (alleles) for the same primer pair; however, the genotyping results are still unambiguous.

Next, the Phire Animal Tissue Direct PCR Kit was compared to a combination of a commercial DNA extraction kit designed for animal tissues and a *Taq*-based hot start DNA polymerase in amplification of four DNA fragments. Phire Animal Tissue Direct PCR Kit produced all the four fragments (0.5-1.5 kb) with great yields. Performance of the commercial DNA purification kit combined with *Taq* DNA polymerase varied depending on the amplicon, and amplification of the longest fragment failed completely (Figure 3).

Finally, we tested the stability of the samples prepared according to dilution protocol. Our results show that the dilution samples can be stored at -20 °C for at least one year. Furthermore, repeated freezing and thawing did not significantly affect the reaction effectiveness (Figure 4).

In conclusion, the Phire Animal Tissue Direct PCR Kit can be used for PCR genotyping of transgenic mice. Due to the inhibitor tolerance of Phire Hot Start II DNA Polymerase, the protocols can be completed in minimal time without prior DNA purification. Note that despite the results presented here, it is expected that sometimes the product yields may be lower due to differences in tissue material and/or primers used. Therefore, it is recommended to always start with the dilution protocol as it allows for easier reaction optimization.

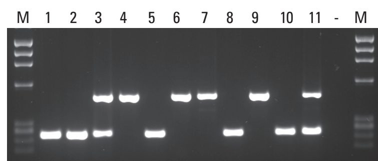

Figure 1. Genotyping of transgenic mice with two primer pairs using the direct protocol. 0.50 mm tissue punches of 11 individual mice were placed directly into 50 µL PCR reactions. Fragment sizes: 490 bp (transgenic) and 250 bp (wildtype).

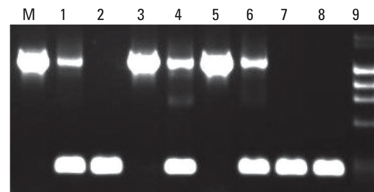

Figure 2. Genotyping of transgenic mice with one primer pair using the dilution protocol. Mouse ear tissues were placed into dilution reactions with 20 µL of Dilution Buffer including DNARelease Additive. After incubations at RT and 98 °C, 1 µL of supernatant was used as a template in PCR reaction. Fragment sizes: 1500 bp (transgenic) and 200 bp (wildtype).

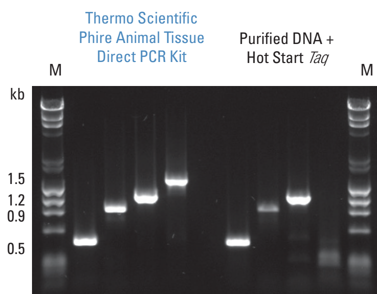

Figure 3. The Thermo Scientific Phire Animal Tissue Direct PCR Kit outperforms a commercial DNA purification system combined with a hot start *Taq* DNA polymerase. Four amplicons (0.5-1.5 kb) were amplified from mouse ear tissues using the dilution protocol of Phire Animal Tissue Direct PCR Kit. For comparison, DNA was first purified using a commercial DNA extraction kit and the same fragments were amplified using a hot start *Taq* DNA polymerase according to manufacturers' recommendations. Only with the Phire Animal Tissue Direct PCR Kit were all four amplicons successfully amplified.

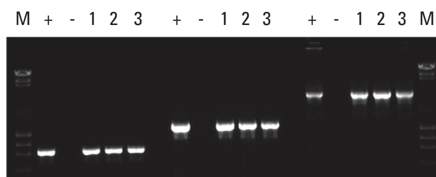

Figure 4. Dilution protocol samples are stable for long term storage. Samples of mouse ear tissues were incubated in 20 µL of Dilution Buffer including DNARelease Additive. Dilution protocol samples were subjected to repeated freezing/thawing (lane 1), stored at -20 °C for one year (lane 2) as described in Materials and Methods, or used immediately for PCR (lane 3). The fragment sizes were 900 bp, 1500 bp and 3200 bp.

Acknowledgements

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