Genotyping from mouse tail using Platinum II *Taq* Hot-Start DNA Polymerase

Introduction

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase combines speed and inhibitor tolerance with an innovative buffer that allows one universal temperature for primer annealing. The unique formulation of Platinum II *Taq* Hot-Start DNA Polymerase and its buffer enables DNA amplification from minimally processed tissue samples without a DNA purification step prior to PCR. Here we provide recommendations for fast and convenient PCR with DNA extracted from mouse tail by alkaline lysis.

Materials and methods

- Mouse tail tissue
- 50 mM NaOH
- 1 M Tris-HCl (pH 8.0)
- Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2X) (Cat. No. 14000012), Platinum II Hot-Start Green PCR Master Mix (2X) (Cat. No. 14001012), or Platinum II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ dNTP Mix, 10 mM each (Cat. No. 18427013; only required for stand-alone enzyme)
- Forward and reverse primers
- Invitrogen™ E-Gel™ General Purpose Agarose Gels or equivalent

Important notes

- Due to the unique composition of the Platinum II PCR buffer, the annealing temperature is 60°C for any primer pair designed following general primer design rules. Isostabilizing molecules in the reaction buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity without the need to optimize the annealing temperature for each primer pair.
- DNA can be extracted from mouse tail tissue by heating a 3–6 mm piece in 180 µL of 50 mM NaOH for 10 min at 95°C, followed by addition of 20 µL of 1 M Tris-HCl (pH 8.0).

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When working with new PCR conditions, we recommend starting with the guidelines below for reaction setup and thermal cycling (Tables 1–3). Platinum II *Taq* Hot-Start DNA Polymerase allows for flexibility in reaction setup, while Platinum II Hot-Start PCR Master Mix (2X) provides more convenience for researchers since it contains all the necessary reaction components except primers and template DNA, thereby reducing setup time and pipetting steps. Platinum II Hot-Start Green PCR Master Mix (2X) also contains premixed loading buffer and allows for direct gel loading after PCR. Additional recommendations for reaction optimization are provided.

Table 1. Reaction conditions for PCR using master mix.

Component	20 µL reaction	50 µL reaction	Final concentration
Platinum II Hot-Start PCR Master Mix (2X)*	10 µL	25 µL	1X
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Mouse tail lysate**	0.2–2 µL	0.5–5 µL	1–10%
Water, nuclease-free	To 20 µL	To 50 µL	—
Optional components for reaction optimization			
Platinum GC Enhancer†	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction.
** Prepared by heating a 3–6 mm piece from mouse tail in 180 µL of 50 mM NaOH for 10 min at 95°C, followed by addition of 20 µL of 1 M Tris-HCl (pH 8.0).
† Recommended for targets with >65% GC content.

Table 2. Reaction conditions for PCR using stand-alone enzyme.

Component	20 µL reaction	50 µL reaction	Final concentration
5X Platinum II PCR Buffer*	4 µL	10 µL	1X
10 mM dNTP Mix	0.4 µL	1 µL	0.2 mM each
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Mouse tail lysate**	0.2–2 µL	0.5–5 µL	1–10%
Platinum II *Taq* Hot-Start DNA Polymerase	0.2 µL	0.5 µL	0.02 U/µL
Water, nuclease-free	To 20 µL	To 50 µL	—
Optional components for reaction optimization			
Platinum GC Enhancer***	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction.
** Prepared by heating a 3–6 mm piece from mouse tail in 180 µL of 50 mM NaOH for 10 min at 95°C, followed by addition of 20 µL of 1 M Tris-HCl (pH 8.0).
† Recommended for targets with >65% GC content.

Table 3. Cycling protocol.

PCR cycles	Step	Temperature	Time
1	Initial denaturation	94°C	2 min
30-35	Denaturation	94°C	15 sec
	Annealing*	60°C	15 sec
	Extension	68°C	15 sec/kb
Hold		4°C	Indefinitely

* An annealing temperature of 60°C works for most primers. In cases when annealing temperature needs additional optimization, we recommend performing gradient PCR or redesigning the primers.

The PCR sample should be diluted 2- to 20-fold for optimal separation using E-Gel agarose gels.

Results

Amplification from various amounts of crude mouse tail lysate

To evaluate the possibility of amplifying DNA from crude tissue extracts with Platinum II *Taq* Hot-Start DNA Polymerase, we used tail samples from BALB/c mice. Tail tissue lysates were prepared by an alkaline lysis method:

- **Step 1.** A 3–6 mm piece of mouse tail was placed into a PCR tube containing 180 µL of 50 mM NaOH, vortexed, and incubated for 10 min at 95°C.
- **Step 2.** The lysate was neutralized by adding 20 µL of 1 M Tris-HCl (pH 8.0), and centrifuged at 12,000 rpm for 5 min.

The supernatant obtained from the above steps was used as the template in 50 µL PCR reactions, in various amounts. The reactions included primers (forward 5´-AAA GTC GCT CTG AGT TGT TAT; reverse 5´-GGA GCG GGA GAA ATG GAT ATG) that target the *ROSA26* locus on the mouse genome to amplify a 650 bp fragment. The cycling protocol was: 1 cycle of 94°C for 2 min; 35 cycles of 94°C for 15 sec, 60°C for 15 sec, and 68°C for 15 sec. For analysis, the amplification products were separated by electrophoresis using 1% agarose gels in TAE buffer. The results demonstrate that Platinum II *Taq* Hot-Start DNA Polymerase can amplify a specific target from crude mouse tail lysate. Successful amplification with lysate volumes ranging from 1% to 10% of the final reaction volume predicts robust target detection regardless of the extent of lysis during the extract preparation (Figure 1).

Sex genotyping of mice

Generally, we seek to detect the presence or absence of a particular sequence variant through genotyping. To ensure that absence of a signal is not due to a failed PCR, a positive control is included. We used Platinum II *Taq* Hot-Start DNA Polymerase and primers (sry1, 5´-AAC AAC TGG GCT TTG CAC ATT G; sry2, 5´-GTT TAT CAG GGT TTC TCT CTA GC) to amplify a pair of fragments, 144 bp and 166 bp long, from a sex-determining gene on the Y chromosome, to determine the sex of mice. A non–sex-related fragment (527 bp) of the gene for Rho guanine nucleotide exchange factor 7 was utilized as an internal positive control. The lysates from known adult male and female mouse tails were prepared as described above. For PCR, the two primer pairs were multiplexed at the standard concentration (0.2 µM for each primer) with mouse tail lysate at 5% (v/v) in the reaction. The cycling protocol was: 1 cycle of 94°C for 2 min; 35 cycles of 94°C for 15 sec, 60°C for 15 sec, and 68°C for 15 sec. PCR products were separated by electrophoresis using 1% agarose gels in TAE buffer. Based on our results, the Platinum II *Taq* Hot-Start DNA Polymerase gave robust amplification of the internal control target (527 bp fragment) in both female and male tail samples, while the sex-determining target (144 bp and 166 bp) was specifically amplified only from the male tail lysate, consistent with the known sex of the mouse (Figure 2).

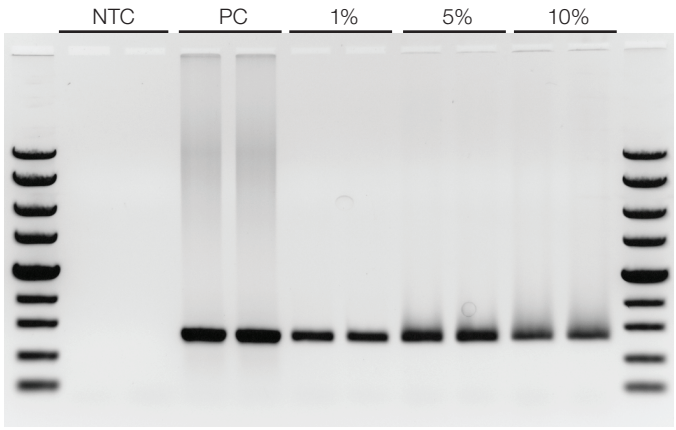

Figure 1. Amplification from mouse tail lysate prepared by alkaline lysis. A 650 bp fragment was amplified in PCR reactions containing varying percentages (v/v) of mouse tail lysate, using Platinum II *Taq* Hot-Start DNA Polymerase. The Thermo Scientific™ ZipRuler™ Express DNA Ladder 2 was used as a size standard. NTC: no-template control; PC: positive control from 1 ng of purified mouse genomic DNA.

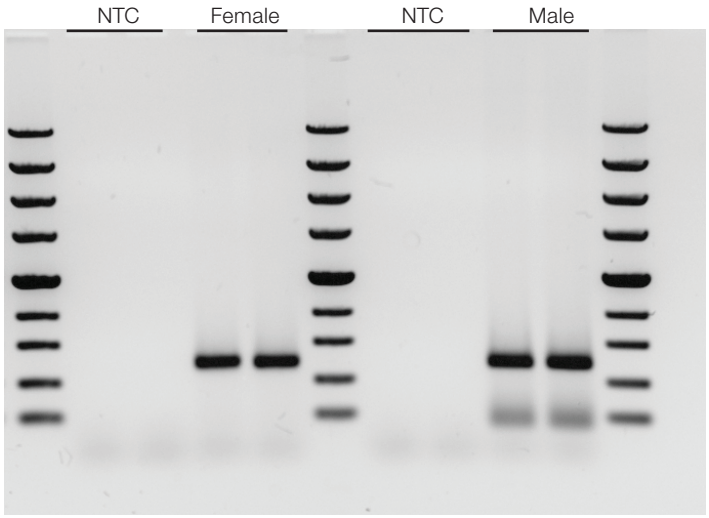

Figure 2. Sex genotyping from mouse tail. Amplification of the sex-determining target (144 bp and 166 bp doublet for males, and no product for females) and internal control (527 bp) fragment was performed using Platinum II *Taq* Hot-Start DNA Polymerase. Tail tissue lysates from known adult males and females were prepared by alkaline lysis. The ZipRuler Express DNA Ladder 2 was used as a size standard. The 144 bp and 166 bp fragments are seen as a single band at ~150 bp in an agarose gel. NTC: no-template control.

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Summary

With its high tolerance to inhibitors and the unique buffer composition, Platinum II *Taq* Hot-Start DNA Polymerase enables fast and convenient amplification of DNA extracted from mouse tail by a simple alkaline lysis method. This procedure can help streamline PCR workflows for multiple applications involving tissue samples.

Find out more at **thermofisher.com/platinumiitaq**

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