One Step Mouse Genotyping Kit

Catalog # PD101-01

Version 5.1

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1. Introduction

One Step Mouse Genotyping Kit is specially designed for the rapid genotyping of mouse and rat, which contains a complete set of reagents for DNA extraction and PCR amplification.

This kit can be used for rapid extraction of genomic DNA from mouse tails, ears, toes, and other tissues. The extracted genomic DNA can be used directly as template for PCR amplification with no need of homogenization, crushing, overnight digestion, phenol chloroform extraction, DNA precipitation or column purification operations, which greatly shortens the experimental time. For using, the tissue should be soaked in lysis buffer with Proteinase K, and then incubated at 55℃ for 20 min, followed by 5 min heating at 95℃ to inactivate the Proteinase K. After centrifugation, the obtained lysis buffer can be used as PCR template directly. Confirmed with repeated tests, the template enables amplification of fragments < 2 kb and multiplex PCR with no more than four pairs of primers. This Kit supplies 2 × Taq Plus Master Mix (Dye Plus) (P212), which includes a high-performance Taq Plus DNA Polymerase, dNTP, and optimized buffer system to ensures high amplification efficiency of target DNA. There is dA overhang on the 3' end of the PCR product that can be cloned into T vector and applied to One Step Express rapid cloning kit, the ClonExpress kits (C112/C113/C114).

2. Applications

- Mouse Genotyping
- Transgenic Mouse Identification
- Mouse Knockout Analysis

3. Package Information

Components	PD101-01 200 rxn (50 μl/rxn)
1 × Mouse tissue Lysis Buffer	40 ml
Proteinase K	800 μl
2 × Taq Plus Master Mix (Dye Plus)	5 ml
25 mM $MgCl_2$	500 μl
5 × PCR Enhancer	2 ml

4. Storage

Store 1 × Mouse tissue Lysis Buffer at 4℃; Store other components at -20℃.

5. Application Examples

Notes before operation:

- Wash all the to-be-used tools in the tissue separation process with 70% ethanol.
- Must do the inactivation of Proteinase K step (95℃, 5 min). Otherwise, the residual activity of Proteinase K will inhibit the subsequent PCR reactions.
- Prepare the PCR reaction mixture in ice-water bath, in order to improve the specificity of the PCR amplification.

1. DNA Extraction

The recommended tissue amount:

- 3 ~ 5 mm of mouse tail tip
- 5 ~ 10 mm^2 of mouse ears
- 1 ~ 2 of mouse toes

1.1 Prepare 1 × lysis buffer according to the number of to-be-treated samples. The following table shows the strategy to prepare lysis buffer for one sample.

Proteinase K	4 μl
1 × Mouse tissue Lysis Buffer	200 μl

Note: The 1 × lysis buffer should be prepared freshly. Please mix the reaction throughly by vortexing.

1.2. Place the to-be-lysed mouse tissue, such as tail, ear, or toe, in a 1.5 ml EP tube. Add 200 μl of 1 × lysis buffer to the tissue, mix by vortexing, and then incubate at 55℃ for 20 min. To improve the efficiency of DNA release, please make sure the lysis buffer covers all the tissue in the tube. Incubation time may vary, depending on the size of the target DNA fragment. The recommended incubation time at 55 ℃ is as follows:

Size of the amplified fragment	Recommended incubation time at 55℃
~500 bp	10 min
~1000 bp	20 min
~1500 bp	30 min

Note: It is normal that tissue blocks are not digested completely, which do not affect the follow-up experiment.

1.3. Incubate the samples in boiling water bath or at 95℃ for 5 min to inactivate Proteinase K.

1.4. Mix the lysates thoroughly by vortexing, and centrifuge at 12000 rpm for 5 min. The supernatant can be directly used as a PCR template. The supernatant can be transferred into a new tube and stored at - 20℃ for at least three months.

2. PCR Amplification

2.1. General Reaction Setup for PCR:
Mix 2 × Taq Plus Master Mix (Dye Plus) by inverting after thawed completely. Prepare the reaction system in ice-water bath as follows:

ddH_2O	to 50 μl
2 × Taq Plus Master Mix (Dye Plus)*1	25 μl
Lysates*2	2~5 μl
Primer 1 (10 μM)	2μl
Primer 2 (10 μM)	2 μl

2.2. Thermocycling Program for a Routine PCR:

94°C	5 min (Pre-denaturation)	
94°C	30 sec	35 Cycles
55°C*	30 sec	
72°C	30 sec/kb	
72°C	7 min (Complete extension)	

* The annealing temperature should be adjusted according to the Tm value of primer, at a temperature that is 1-2℃ lower than the Tm of primer.

2.3. The PCR products can be used directly for agarose gel electrophoresis, without addition of DNA Loading Buffer.

6. Trouble shooting

A. No amplification product in test or control samples
1. Amplification reaction was incorrectly set up: Optimize the proper reaction set up;
2. Improper storage has led to loss of activity of PCR reagents: Replace all components with fresh reagents;
3. Primers were not optimal and did not anneal: Redesign primers;

B. Amplification worked in the control samples, but not in test samples
1. Digestion was incomplete: Extend digestion time up to 60 min at 55℃;
2. Lysis solution was mixed with PCR mixture for too long: Collect fresh mouse tail samples for genomic DNA extraction;
3. The quantity of amplified product was not sufficient: Increase the number of PCR cycles to 35-40 to yield more amplification product.

C. Non-specific amplification product(s)
1. Annealing temperature was too low: Increase the annealing temperature;
2. The number of PCR cycles was too high: Decrease the number of cycles to 30-35;
3. Primer concentration was too high: Decrease primer concentration;
4. Template concentration was too high: Dilute template in purified H_2O or TE buffer.