One Step Mouse Genotyping Kit

PD101

Version 22.1

Product Description

One Step Mouse Genotyping Kit is specially designed for the rapid genotyping of mouse, which contains a complete set of reagents for DNA extraction and PCR amplification. This kit can be used for the 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. During use, immerse the tissue into a lysis solution premixed with Proteinase K, incubate at 55℃ for 20 min, and then heat at 95℃ for 5 min to inactivate Proteinase K. After centrifugation, the lysate can be directly used as the template for PCR amplification. After repeated tests, it is widely applicable to the amplification of target fragments within 2 kb, and is suitable for Multiplex PCR within four fragments.

2 × Taq Plus Master Mix (Dye Plus) in this kit contains Taq Plus DNA Polymerase, dNTP, and an optimized buffer system. It only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results. The amplification system contains protective agents that keep 2 × Taq Plus Master Mix (Dye Plus) stable in activity after repeated freezing and thawing. 2 × Taq Plus Master Mix (Dye Plus) contains loading buffer, PCR products can be directly loaded for electrophoresis after the reaction. The PCR product has an adenine at the 3' end that can be cloned into the T vector. It is also compatible with ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115/C601).

Components

Components	PD101-01 200 rxns (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

* It is applicable for GC-rich PCR systems.

Storage

1 × Mouse tissue Lysis Buffer, store at 2 ~ 8℃;
Other components, store at -30 ~ -15℃;
Transport at ≤0℃.

Applications

- Mouse Genotyping
- Transgenic mice detection
- Knockout mouse analysis

Notes

For research use only. Not for use in diagnostic procedures.

1. Pre-clean all tools used in the tissue separation process with 70% ethanol (self-prepared);
2. Proteinase K inactivation step (95℃, 5 min) must be carried out, otherwise its residual activity will inhibit the subsequent PCR;
3. The preparation process of the PCR system should be carried out in ice water bath to improve the specificity of amplification.

Experiment Process

◇ **DNA Extraction**

The recommended amount of tissues:

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

1. Prepare 1 × lysis buffer according to the amount of samples to be lysed. The recommended preparation method is as follows:

	1 × lysis buffer (single sample)
Proteinase K	4 μl
1 × Mouse tissue Lysis Buffer	200 μl

▲ Use freshly prepared 1 × lysis buffer. After the addition of each component, please vortex to mix thoroughly before use.

2. Add 200 μl of 1 × lysis solution to the tissue to be lysed, mix thoroughly, and then incubate in a 55℃ water bath for 20 min. For target fragments of regular size, 20 min incubation is enough to obtain sufficient amounts of DNA templates. The incubation time can be adjusted according to the actual situation and the following table shows the recommended incubation time for amplified fragments of different lengths at 55℃:

▲ To ensure the efficiency of DNA release, be sure to immerse all tissues in the lysis buffer. After the incubation, the tissue block may not be completely digested, which is normal and does not affect the use.

Size of the amplified fragment	Recommended incubation time at 55℃
~ 500 bp	10 min
~ 1,000 bp	20 min
~ 1,500 bp	30 min

3. After incubation, place the sample at 95℃ or heat it in a boiling water bath for 5 min to inactivate Proteinase K.

4. Vortex lysates to mix thoroughly, then centrifuge at 12,000 rpm (13,400 × g) for 5 min. Take the supernatant for PCR amplification, or transfer the supernatant to another sterilized EP tube, which can be stored at -20°C for at least three months.

◇ **PCR amplification**

1. Thaw 2 × Taq Plus Master Mix (Dye Plus) and mix thoroughly. Prepare the reaction system on ice as follows:

ddH_2O	to 50 μl
2 × Taq Plus Master Mix (Dye Plus)[a]	25 μl
Lysate[b]	2 - 5 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl

a. 2 × Taq Plus Master Mix (Dye Plus) is premixed with Mg^{2+} at a final concentration of 1.5 mM. In actual use, the 25 mM $MgCl_2$ provided in the kit can be used to adjust the concentration of Mg^{2+}, and the adjustment interval is 0.5 mM each time.

b. The amount of lysates added should be ≤1/10 of the total volume of PCR system.

2. Reaction program

94℃	5 min (Initial Denaturation)	
94℃	30 sec	35 cycles
55℃*	30 sec	
72℃	30 sec/kb	
72℃	7 min (Final Extension)	

* The annealing temperature needs to be adjusted according to the Tm value of the primer, generally set to be 1 ~ 2℃ lower than the Tm value of the primer.

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

FAQ & Troubleshooting

◇ No amplification products or low yield

① PCR inhibitors in the tissue are mixed into the lysate: Try to dilute the lysate by 10 times before performing PCR amplification;

② Poor DNA release efficiency: Try to extend the 55℃ incubation time to 3 h;

③ Proteinase K is not fully inactivated: The inactivation step should be carried out in boiling water bath;

④ Insufficient PCR cycles: Generally, 30 - 35 cycles are enough. However, for some fragments, increasing the number of cycles can get better amplification effect;

⑤ The annealing temperature is too high: Decrease the annealing temperature at 3℃ intervals;

⑥ PCR primer error: Set up positive control reaction using the purified mouse genome as a template.

◇ Nonspecific products

① Prepare PCR system at room temperature: The preparation of the reaction system on ice can significantly reduce nonspecific amplification;

② The annealing temperature is too low: Increase the annealing temperature at 2℃ intervals;

③ Serious mismatch of PCR primers: Redesign the primers.

◇ Amplification products appear in the negative control

The PCR system is contaminated: Replace each component in the tissue lysis system and PCR amplification system one by one.