One Step Mouse Genotyping Kit

Cat# NB-54-0161 size: 200 rxns (50 μl/rxn)

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 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, incubated at 55˚C for 20 min, then heat at 95˚C for 5 min to inactivate the Proteinase K. After centrifugation, the obtained lysate can be used as PCR template directly. After repeated tests, it is widely applicable to the amplification of target fragments within 2 kb, and is suitable for multiple PCR reactions within 4 pairs of primers.

This Kit is equipped with 2 × Taq Plus Master Mix (Dye Plus), which includes high-performance Taq Plus DNA Polymerase, dNTP, and optimized buffer system. Only need to add primers and template in PCR reaction to perform amplification, which reduces operations such as tube opening/pipetting, significantly reduces sample cross-contamination and improves detection throughput and results reproducibility. The unique protective agent allows Taq Plus Master Mix to maintain stable activity after repeated freezing and thawing. The system is premixed with electrophoresis buffer and dyes, and electrophoresis can be carried out directly after the reaction, which is convenient and quick to use.

The PCR product has A-tailing at the 3' end, which can be cloned into T vector and is suitable for ClonExpress and topological cloning kit (Neo Biotech NB-54-0002/NB-54-0003/).

Components

Components	NB-54-0161 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 used when the GC content of PCR amplification product is high.

Storage

1 × Mouse tissue Lysis Buffer should be stored at 2 ~ 8˚C.

Other components should be stored at -30 ~ -15˚C;

Transport at ≤0˚C.

Applications

- Mouse Genotyping
- Detection of mouse transgenic
- Analysis of mouse knockout

Notes

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

Experiment Process

◇ **DNA Extraction**

The recommended tissue amount:

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

1. Prepare 1 × lysis buffer according to the number of samples to be lysed. The method for preparing lysis buffer for single sample 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. Vortex and shake after the addition of each component, and mix throughly before use.

2. Add 200 μl of 1 × lysis buffer to the tissue, mix by vortexing, and then incubate at 55˚C for 20 min. For target fragments of regular size, 20 min incubation is sufficient to release sufficient DNA template. The incubation time can also be adjusted according to the actual situation and the following table shows the recommended incubation time for amplified fragments of different lengths at 55˚C:

▲ 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˚C
~ 500 bp	10 min
~ 1,000 bp	20 min
~ 1,500 bp	30 min

3. Incubate the samples in boiling water bath or at 95˚C for 5 min to inactivate Proteinase K.

4. Mix the lysates thoroughly by vortexing, and centrifuge at 12,000 rpm (13,400 × g) for 5 min. The supernatant can be directly used as a PCR template or transferred into a new tube and stored at - 20˚C for at least three months.

◇ **PCR Amplification**

1. 2 × Taq Plus Master Mix (Dye Plus) after thawing completely, mix up and down, then prepare the reaction system on ice as follows:

ddH_2O	to 50 μl
2 × Taq Plus Master Mix (Dye Plus)[a]	25 μl
Lysates[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 lysate added should not exceed 1/10 of the total volume of the PCR reaction.

2. Recommended PCR reaction condition setting:

94˚C	5 min (Initial 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, and generally set to 1 ~ 2˚C 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

◇ Low yield of amplificated products or no amplification

① Some 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˚C 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 speaking, 30 - 35 cycles are enough to amplify sufficient amount of product. However, for some fragments, increasing the number of cycles can get better amplification effect;

⑤ The annealing temperature of the amplification reaction is set too high: Reduce the annealing temperature (by 3˚C each time);

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

◇ Many non-specific products

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

② The annealing temperature of the amplification reaction is set too low: Increase the annealing temperature (increased by 2˚C each time);

③ Serious mismatch of PCR primers: Redesign the primers.

◇ The negative control also amplified

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