One Step Mouse Genotyping Kit V2

PD111

Version 24.1

Vazyme

Product Description

One Step Mouse Genotyping Kit V2 is specially designed for the rapid genotyping of mouse. The kit includes a lysis buffer that can quickly release genomic DNA from mouse tissues (mouse tail, mouse ear, mouse toe, liver, etc.), and it is also compatible with plant leaves, blood, and 293 cell samples. The lysate can be directly used as the PCR amplification template after centrifugation. The kit is equipped with 2 × Rapid Taq Plus Master Mix (Dye Plus), which contains a new generation hot-start Taq DNA polymerase and an optimized buffer system. It only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, and improving detection throughput and reproducibility of results. After repeated tests, it is widely applicable to the amplification of fragments within 8 kb. In addition, this product contains tracking dyes, enabling direct electrophoresis analysis after the reaction.

Components

	Components	PD111-01 200 rxns (50 μl/rxn)
	Mouse Direct Lysis Buffer A	10 ml
	Mouse Direct Lysis Buffer B	10 ml
□	2 × Rapid Taq Plus Master Mix (Dye Plus)	5 × 1 ml

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

▲ Mouse Direct Lysis Buffer A, and Mouse Direct Lysis Buffer B should be stored at 2 ~ 8°C for long-term storage.

Applications

◇ Mouse genotyping, transgenic detection and gene knockout analysis, etc.

◇ Cell gene knockout identification

◇ Plant transgenic identification

Notes

1. Please pre-clean all tools used in the tissue separation process with 70% ethanol (self-provided).
2. The reaction system preparation process should be conducted on ice to improve amplification specificity.

Experiment Process

◇ DNA extraction

1. Before use, gently mix Mouse Direct Lysis Buffer A and Mouse Direct Lysis Buffer B by inversion to avoid generating a large number of bubbles.
2. When in use, cut the sample to be lysed into small pieces and place it in a centrifuge tube, add an appropriate amount of Mouse Direct Lysis Buffer A, vortex to mix, briefly centrifuge at low speed to the bottom of the centrifuge tube, and incubate under recommended conditions (for recommended sample amount to be lysed, amount of lysis reagent to be added, and incubation conditions, please refer to Table 1).
   ▲ To ensure DNA release efficiency, please be sure to immerse the tissue in lysis buffer completely.
   ▲ If the sample volume is relatively large, the user can proportionally increase the addition volume of Mouse Direct Lysis Buffer A and Mouse Direct Lysis Buffer B for lysis.
3. After incubation, add an equal volume of Mouse Direct Lysis Buffer B to Mouse Direct Lysis Buffer A, vortex to mix, briefly centrifuge at low speed to the bottom of the centrifuge tube, collect the supernatant into a new centrifuge tube, and complete the preparation of the lysate products.
   ▲ The DNA solution after lysis can be stored at 4°C for 1 month. For long-term storage, please freeze the DNA solution after lysis at -20°C and mix by inversion before use.

Table 1. Lysis conditions for different types of samples

Sample Types	Sample Size	Mouse Direct Lysis Buffer A Volume	Incubation Conditions*	Mouse Direct Lysis Buffer B Volume
Mouse Tail	1 - 2 mm	50 μl	95°C, 3 min	50 μl
Mouse Ear	3 - 3.5 mg	50 μl	95°C, 3 min	50 μl
Mouse Toe	2 mg	50 μl	95°C, 3 min	50 μl
Mouse Liver	3 - 3.5 mg	50 μl	95°C, 3 min	50 μl
Fresh Whole Blood (EDTA/Citrate/Heparin/Anticoagulant)	5 - 7.5 μl	50 μl	Room Temperature, 3 min	50 μl
Cell Suspension	5×10^3 - 5×10^5	50 μl	Room Temperature, 3 min	50 μl
Plant Leaf	3 - 4.5 mm	50 μl	95°C, 3 min	50 μl

* Room Temperature: 20 ~ 25°C. The processing time can be appropriately extended for samples that are difficult to lyse. For incubation at 95°C, it is recommended to use a PCR machine or a metal bath to control the temperature.

◇ Reaction system

Keep all components on ice during the experiment. Mix, and briefly centrifuge each component before use.

Components	Volume	
2 × Rapid Taq Plus Master Mix (Dye Plus)	25 μl	☐
Forward primer (10 μM)	1.5 μl	
Reverse primer (10 μM)	1.5 μl	
lysate products (≤1/10 of the total volume of PCR system)	2 - 5 μl	
ddH_2O	up to 50 μl	

◇ Reaction program

Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	
Denaturation	98°C	10 sec	28 - 35
Annealing[a]	Tm	30 sec	
Extension[b]	68°C	15 sec/kb	
Final Extension	68°C	5 min	

a. Please set the annealing temperature according to the Tm value of the primers. If necessary, the annealing temperature can be further optimized through setting the temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Increasing the annealing temperature can improve the specificity of amplification.

b. To increase the yield of PCR products, please extend the extension time appropriately.

FAQ & Troubleshooting

◆ No amplification products or low yield

① The amount of sample to be lysed is relatively small: it is advisable to increase the input amount of lysis samples and the amount of template in the amplification system. Taking the mouse tail as an example, the recommended amount of lysis buffer in Table 1 can lyse 1 - 2 mm sample, 50 μl of lysis buffer can be compatible with 1.5 - 2 times the sample, and does not affect subsequent PCR amplification.

② The impurities of lysate products are relatively high: try diluting the lysate products 2 - 10 times before performing PCR amplification.

③ Annealing temperature set too high: appropriately decrease annealing temperature at 2°C intervally.

④ Poor primer design: use the purified mouse genome as a template to set a positive control and verify the primer effectiveness.

◆ Nonspecific products or smeared bands

① Reaction system is prepared at room temperature: preparing the reaction system on ice can significantly reduce non-specific amplification.

② Inappropriate annealing temperature setting: appropriately increase annealing temperature at 2°C intervally.

③ Serious PCR primer mismatch: redesign primer.

◆ Negative control showed amplification

① Reaction system contamination: replace each component of the reaction system one by one.

② Existence of aerosol pollution: Conduct thorough cleaning of the experimental environment, consumables, etc.