405 E Gude Dr, STE 214
Rockville, MD 20850, USA
Web: www.abpbio.com
Email: info@abpbio.com

Direct PCR Genotyping Kit

Catalog Number: D050

Table 1. Kit Components and Storage

Kit Component	D050 (400 rxns)	Storage	Stability
Lysis Buffer	40 mL	4-25°C	The product is stable for one year when stored as directed.
Stop Solution	40 mL	4-25°C	
2×HotStart *Taq* PCR Master Mix	5×1 mL	-20°C	

Product Description

Direct PCR 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 buffer, and incubate at 95℃ for 20 min, then add a stop solution to end the extraction. 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×HotStart Taq PCR Master Mix in this kit contains HotStart Taq 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 Master Mix 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.

Features

- **Direct PCR:** No need for DNA purification steps; crude samples can be added directly to PCR
- **Convenient operation:** Master Mix format with gel loading dye; reduces sample handling and allows direct loading on gel
- **Broad compatibility:** Suitable for ≤ 2kb fragments; up to 4-plex amplification

Applications

- Mouse Genotyping
- Transgenic mice detection
- Knockout mouse analysis

Experiment Process

DNA Extraction

The recommended amount of tissues: 1- 3 mm of mouse tail tip; 2-5 mm^2 of mouse ears, or 1-2 of mouse toes.

1. Place tissue into a PCR micro tube.
2. Add 100 µl of Lysis Buffer.
3. Incubate at 95°C for 20 min on a thermocycler.
4. Add 100 µl of Stop Solution.

   Note: Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.
5. Vortex lysates to mix thoroughly, then centrifuge at 12,000 rpm for 3 min. Take the supernatant for PCR amplification, or transfer the supernatant to another EP tube, and store at -20°C.

PCR amplification

1. Thaw 2×HotStart Taq PCR Master Mix on ice and mix thoroughly. Prepare the reaction mixture in a PCR tube on ice as follows:

Component	Volume	Final Concentration
Lysate (template)	1 µL	-
2×HotStart *Taq* PCR Master Mix	12.5 µL	1×
Forward Primer (10 µM)	1 µL	400 nM
Reverse Primer (10 µM)	1 µL	400 nM
Nuclease-free H_2O	9.5 µL	-

2. Perform PCR using the recommended thermal cycling conditions outlined below:

Steps	Temperature	Duration	Cycle
Initial Denaturation	95°C	1-3 min	1
Denaturation	95°C	10 sec	35
Annealing*	56-64°C	10 sec	
Extension	72°C	15-30 sec/kb	
Final Extension	72°C	10 min	1
Holding	4°C	-	1

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

3. Analyze the amplification products by agarose gel electrophoresis.