Protocol

1. Mouse tail, toe or ear cut (~2mm) are digested in 75 µL lysis buffer with 0.75 µL Proteinase K added at 56 °C for 15 min. After the digestion process, incubate the mixed solution at 95°C for 10 min to 1 h (The undissolved tissue does not interfere with PCR).

2. After heating, samples are cooled to 4°C, and 75 µL balance buffer is added to each sample.

3. 1 µL of the final preparation is used per 20 µL PCR reaction system. (You can adjust the appropriate volume.)

PCR Reaction Components:

PCR Reaction Components	20 µL Reaction Volume	50 µL Reaction Volume
ddH2O	Add to 20 µL	Add to 50 µL
Forward Primer (10 µM)	0.8 µL	2 µL
Reverse Primer (10 µM)	0.8 µL	2 µL
Template	1 µL	2.5 µL
2 x PCR Master Mix plus (With Dye)	10 µL	25 µL

PCR Steps

Procedure	Temperature (°C)	Time	Cycles
Initialization	98	1 min	1
Denaturation	98	15 sec	30-35(Adjustable)
Annealing	55-58	15 sec	
Extension	72	15 – 30sec per kb	
Final elongation	72	2 min	1
Final hold	4	Appropriate time	1

4. Subject your PCR product to agarose gel electrophoresis and generate the result (Our product **SYBR Safe DNA Gel Stain (Cat No A8743)** is available).

Note:

1. For each step, make sure your reagent in the kit is mixed well before use.
2. During the digestion step, shaking the tubes several times will contribute to release the genomic DNA.
3. For major mouse tissue samples, 15 minutes incubation under 56 °C with Proteinase K could suffice for genomic DNA extraction. The tissue may still appear intact, but the lysis has occurred.
4. The acquired genomic DNA can be applied to the PCR amplification step. Spin down the remaining tissue and store the supernatant at −20 °C if not used immediately.