Direct Mouse Genotyping Kit

1. Mouse tail, toe or ear cut (~2mm) are digested in 75 µL lysis buffer with 0.75 µL Protease K solution 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 are used per each 20 µL PCR volume. (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 µL
2 x PCR Master Mix (With Dye)	10 µL	25 µL

PCR Steps

Procedure	**Temperature (°C)**	**Time**	**Cycles**
Initialization	94	5min	1
Denaturation	94	30sec	35
Annealing	50-60	30sec	
Extension	72	30sec/kb	
Final elongation	72	10min	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 Protease 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.