Protocol

1. Digestion

1.1. Generate parts of insects, tissues, tails of fishes(~2mm), and digest them 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).
Protease K: 10 mg/ml solution in stock, 1:100 diluted in Lysis buffer freshly prepared before digestion.

1.2. For adherent cells cultured in dishes, wash the cells with PBS and collect them after trypsin digestion. For cells in suspension or blood cells, just take out some cells for genotyping. Digest the cells in 75 µL lysis buffe at 95°C for 10 min to 1h.

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 for each PCR components.)

PCR Reaction Components:

PCR Reaction Components	**20 µL Reaction Volume**	**50 µL Reaction Volume**
ddH2O	Up to 20 µL	Up to 50 µL
Forward Primer (10 µM)	0.8 µL	2 µL
Reverse Primer (10 µM)	0.8 µL	2 µL
Template	Appropriate volume	Appropriate volume
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(Adjustable)
Annealing	50-60	30sec	
Extension	72	60sec/kb	
Final elongation	72	10min	1
Final hold	12	Appropriate time	1

1. Subject your PCR product to agarose gel electrophoresis and generate the result (Our product **SYBR Safe DNA Gel Stain** 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 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 should be applied to the PCR amplification step immediately. If not used immediately, spin down the remaining tissue and store the supernatant at −20 °C.