

## **M-Fast PCR Genotyping Kit (Cat. #: GT-003/GT-003p)**

**Cat: GT-003**      **Size:**      500 Extractions (Reagent-A: 250mL; Reagent-B: 25mL)  
**Storage:** Room Temperature

**Cat: GT-003p**      **Size:**      100 Extractions  
(Reagent-A: 50mL; Reagent-B: 5mL; 2x Green PCR mixture: 1.25mL )  
**Storage:** -20°C

### **\* Protocol for gDNA Extractions**

1. Prepare 0.1-0.5cm mouse tail biopsy sample in a 1.5ml microcentrifuge tube.
2. Add **500ul reagent-A** into the sample tube.
3. Place the tube in a PCR machine (or dry heat bath or heat block) and incubate at 95°C for 30-50 minutes or until the tail complete digestions (around 1 hour for the old mouse tails). (Cover the sample tubes with a heavy book or others to prevent the cap opening during incubation)
4. Turn off the power and continue incubate for another 20 minutes.
5. Add **50ul reagent-B** into the sample tube and mix well by simple vortexing.
6. Centrifuge at 12,000 xg for 3 minutes at 4°C.
7. Pipette **2ul lysate supernatant** into an 23ul PCR mastermix (total: 25ul reaction)
8. Run PCR reactions at thermal cyclers.

**Note:** These DNA samples are stable at room temperature for 1-3 weeks, or 1-3 months at 2-8°C and more than 3 years at -20°C.

### **Suggested PCR Protocol:**

I. Preparation of PCR Master Mix for a single reaction (total volume: 25uL) in a 0.2mL tube.

Component	Volume (μL)	Final Concentration
2x Green PCR Mastermix	12.5	1x
Forward primer (10μM)	1	250nM
Reverse primer (10μM)	1	250nM
DNA Template	1-5	Determined by user
PCR grade water	up to 25 μL	

II. Setup typical thermal cycling parameters

<b>Enzyme activation step:</b>	95°C	3-5 minutes
<b>25-40 cycles:</b>		
Denaturation	95°C	30 seconds
Annealing	X°C	dependent on Tm of primers
Extension	72°C	30 seconds (1min per kb amplicon)

Hold at 4-8°C

After thermal cycling, the PCR products can be loaded directly onto an agarose gel and run gels as usual.

**Precautions and Disclaimer:** This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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