Technical Bulletin

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

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

Cat: VGT-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 open ing 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

Enzyme activation step: 95°C 3-5 minutes

25-40 cycles:
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.