Technical Bulletin

One-step PCR Genotyping Kit (Cat. #: VGT-205/GT-V 205p)

Cat: GT-205 Size: 1250 Extractions (Reagent-A: 125 mL) Storage: RT°C

Cat: GT-205p Size: 1250 Extractions (Reagent-A: 125 mL) Storage: RT°C
(2x Green PCR mixture: 12.5mL) Storage: -20°C

* Protocol for gDNA Extractions and PCR reactions

1. Prepare 0.1-0.5cm mouse tail/ear biopsy sample in a 1.5ml microcentrifuge tube.
   (1-5mg plant leaf, 10-30uL of blood, saliva , buccal swab media, yeast, bacterial or viral samples)
2. Add **100ul reagent-A** into the sample tube.
3. Incubate at room temperature for 15-20 minutes, vortex 2-3 seconds.
4. Pipette **2.5ul lysate supernatant** into an 22.5ul PCR mastermix (total: 25ul)
5. 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	2.5	Determined by user
PCR grade water	8	

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.