

## One-step PCR Genotyping Kit (Cat. #: GT-305/GT-305p)

Cat: GT-305      Size:      1000 Extractions (Reagent-A: 250 mL)      Storage: RT°C

Cat: GT-305p      Size:      1000 Extractions (Reagent-A: 250 mL)      Storage: RT°C  
( 2x Green PCR mixture: 12.5mL )      Storage: -20°C

Benefits and Features:

**Not heating step (DNA extractions at room temperature).**

**No rush (incubation time from 20 minutes to 2 months).**

**No neutralization and no DNA isolation steps. (All-in-one buffer)**

### \* **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, 1-30uL of cells, yeast, bacterial, fungi or plant samples in tubes or plates)
2. Add **250ul reagent-A** into the sample tube or micro-plate well.
3. Incubate at room temperature for 20-30 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

<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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