

I172M Genotyping Protocol

DNA extraction

1. Gather materials: (from REExtract-N-Amp Tissue PCR Kit)
 - a. 50 µL extraction solution
 - b. 10 µL tissue prep solution
2. mix and add 60 µL per tail.
3. Incubate for 10 min at RT, then for 3 min at 95 °C.
4. Add 50 µL neutralization solution per tail, vortex.

PCR

1. Materials needed
 - a. 10 µL RedTaq Master Mix
 - b. 1 µL fwd primer (20 µM stock conc.)
 - c. 1 µL rev primer (20 µM stock conc.)
 - d. 7 µL RNase free water
 - e. 1 µL DNA extract

Primers I172M: RB2830 (cctggccctcttagcaggtt) - sense ; RB2871 (aggaggagaagccagcaagg) – antisense

Thermal profile:

- 1) 95 °C for 5 min
- 2) 95 °C for 30 sec
- 3) 63 °C for 30 sec
- 4) 72 °C for 30 sec
- 5) 2-4 30x
- 6) 72 °C for 8 min
- 7) 4 °C forever

Run on 3% agarose gel, so – 4.5 g agarose powder + 150 mL 1x TAE buffer

KI = 750 bp, WT = 700 bp