

## A559V Genotyping Protocol

### Tail Digest: REDExtract-N-Amp Tissue PCR Kit

1. Combine 50 µl Extraction Solution + 10 µl Tissue Prep Solution per tail, vortex
2. Add 60 µl ES/TP solution to each tail, vortex
3. Incubate at room temp for 10 minutes
4. Incubate at 95°C for 3 minutes
5. Add 50 µl Neutralization solution to each tube
6. Proceed immediately to PCR set up or store tails in 4°C

### PCR: KAPA2G Fast HotStart Genotyping PCR Kit

#### PCR RXN (1)

KAPA	12.5 µl
Primer 1	1.25 µl
Primer 2	1.25 µl
RNase Free H <sub>2</sub> O	9 µl
DNA	1 µl

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Total	25 µl
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Primer stocks kept at 200 µM, diluted in RNase Free H<sub>2</sub>O 1:10 for final working solution (20 µM)

#### Primer Sequences:

RB3826	agctatattcaccatcaaaagg
RB3827	ctctctattcttgagacaata

#### Products:

WT, **490** bp

homozygous Val559, **561** bp product

### Thermal Profile (Gwynne: KAPA)

- 1) 95°C for 3 min
- 2) 95°C for 30 sec
- 3) 57°C for 30 sec
- 4) 72°C for 45 sec  
2-4 X 35
- 5) 72°C for 10 min
- 6) 4°C Forever