

# Genotyping protocol

EM:10371

STOCK Noto<sup>tm1Gos</sup>/Kctt

## Primers

F1      5' **GAG CAA AGA CCC CAA CGA GAA G** 3'  
 R1      5' **GCA ACC CAC ACA CAT AAA AAG GAG** 3'  
 F2      5' **TGA CCA CCT CTC TCT CTC CCA TTG** 3'  
 R2      5' **TGA TGC CGT TCT TCT GCT TGT C** 3'

## PCR profile

**Wild type allele**

**Mutant allele**

95 °C, 5 min

95 °C, 5 min

94 °C, 30 s    40 cycles

94 °C, 30 s    40 cycles

55 °C, 60s

55 °C, 60s

72 °C, 90s

72 °C, 90s

72 °C, 10 min

72 °C, 10 min

4 °C, ∞

4 °C, ∞

## PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
MgCl <sub>2</sub> (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
F1 (wt) or F2 (mut) (100 µM)	0.15 µl
R1 (wt) or R2 (mut) (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H <sub>2</sub> O	<u>22.51 µl</u>
	30 µl

## Post-PCR analysis

2 % agarose gel.

Expected pattern is **wt: 500bp**  
**mut:550bp**