

Genotyping protocol

EM:12387

STOCK Mirc35tm1Arte/Kctt

Primers

Mir-31 5' GCA GAG TCA CAA ACA TGT GTA GC 3'
Mir-32 5' GAG TGT GCT GAC AGT CTA GAC TGC 3'

PCR profile

95 °C, 5 min

95 °C, 30 s

60 °C, 30 s

35 cycles

72 °C, 1 min

72 °C, 10 min

4 °C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl ₂ (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
Mir-31 (100 µM)	0.1 µl
Mir-32 (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H ₂ O	<u>15.31 µl</u>
	20 µl

Post-PCR analysis

1.5% agarose gel.

Expected pattern is wt:188bp, mut:348bp