Genotyping protocol

EM:08495

mi-R10a KO

Primers

5' forward primer (miR10a K50 Fw_10a.3) - 5´ **cat tca gat caca cc caa aag a** 3´
3' reverse primer (miR10a K50 Rev_10a) - 5´ **ctt ctt gaa ggc agt cac tgg** 3´

PCR profile

94 ˚C, 5 min

94 ˚C, 30 s — 30 cycles
56 ˚C, 1min 30s
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
$MgCl_2$ (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
forward primer (200 µM)	0.15 µl
reverse primer (200 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.46 µl
	30 µl

Post-PCR analysis

1%- 1.5% agarose gel.
Expected pattern is **mutant: 335 bp**
Wt: 246 bp