Genotyping protocol

EM:07451
Gt(ROSA)26Sor-mCherry-Rpl10a x CD1

Primers

5' forward primer (R26-mCherry-Rpl10a) - 5´ **tac acc atc gtg gaa cag tac** 3´
3' reverse primer (R26-mCherry-Rpl10a) - 5´ **gta gtt ctt cag gct gat ctg** 3´

5' forward primer (R26-wt)- 5´**gcg gat cac aag caa taa ta** 3´
3´reverse primer (R26-wt)- 5´**ttt ctg gga gtt ctc tgc tg** 3´

PCR profile

95 ˚C, 6 min

94 ˚C, 45 s — 40 cycles
55 ˚C, 45 s
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: 216 bp**
Wt: 330 bp