Genotyping protocol

EM:08496

PRDM5 LacZ

Primers

5' forward primer (wt_fwd) - 5´ **tat ggc cct ttg atg tct tca cta** 3´
5' forward primer (ko_fwd) - 5´ **cgc ctt ctt gac gag ttc ttc tg** 3´
3' reverse primer (rev) - 5´ **agt ttg aac agg gta cca tcc at** 3´

PCR profile

94 ˚C, 5 min

94 ˚C, 30 s — 30 cycles
59 ˚C, 30s
72 ˚C, 2 min 30s

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 wt (100 µM)	0.04 ul
forward primer wt (100 µM)	0.2 µl
reverse primer (100 µM)	0.2 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.86 µl
	30 µl

Post-PCR analysis

1% agarose gel.
Expected pattern is **mutant: 2300 bp**
Wt: 1831 bp