

Genotyping protocol

EM:09214

Prdm11 Neo

Primers

WT - 5' ccc agg gga tgt tct tac tg 3'
 5' tga agc cat cag aaa caa cc 3'

Mutant- 5' ccc agg gga tgt tct tac tg 3'
 5' acc tgc gtg caa tcc atc tt 3'

PCR profile

94 °C, 5 min

94 °C, 30 s

53 °C, 45 s

72 °C, 2 min

40 cycles

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 (100 µM)	0.15 µl
reverse primer (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H ₂ O	<u>22.46 µl</u>
	30 µl

Post-PCR analysis

1.5% agarose gel.

Expected pattern is **mutant: 700 bp****Wt: 230 bp**