

Genotyping protocol

EM:10414

STOCK Noto^{tm2.1(Foxj1)^{Gos}/Kctt}

Primers

F1 5' AGA CCG AGA TAG GGT TGA GTG TTG 3'
 R1 5' GCA ACC CAC ACA CAT AAA AAG GAG 3'
 F2 5' TGA CCA CCT CTC TCT CTC CCA TTG 3'
 R2 5' GAT GAG AGT GGC ATA GGA GTA GGG 3'

PCR profile

95 °C, 5 min

94 °C, 30 s 40 cycles

50 °C, 60s

72 °C, 90s

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
F1 (wt) or F2 (mut) (100 µM)	0.15 µl
R1 (wt) or R2 (mut) (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H ₂ O	<u>22.51 µl</u>
	30 µl

Post-PCR analysis

2 % agarose gel.

Expected pattern is **wt: 250 bp****mut:500bp**