

Genotyping protocol

EM:11486

STOCK Gt(ROSA)26Sor^{tm1}(CAG-ChR2/tdTomato)Oki /Kctt

Primers

ROSA 26 locus

RR711 5' GCA CTT GCT CTC CCA AAG TC 3'

RR712 5' GGG CGT ACT TGG CAT ATG AT 3'

RR713 5' CTT TAA GCC TGC CCA GAA GA 3'

ChR2 specific insert

pFPH 5' CTG GAT CTG ACA TGG TAA GTA AGC 3'

ChRRev1 5' TCA ATA TGT GCT CGT GGA TCA G 3'

PCR profile ROSA26

95 °C, 5 min

95 °C, 15 s

57 °C, 45 s

35 cycles

72 °C, 90 s

72 °C, 10 min

4 °C, ∞

PCR profile ChR2

95 °C, 5 min

95 °C, 20 s

53 °C, 30 s

35 cycles

72 °C, 90 s

72 °C, 10 min

4 °C, ∞

PCR mix ROSA26

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl ₂ (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
RR711 (100 µM)	0.1 µl
RR712 (100 µM)	0.1 µl
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AmpliTaq Gold (5 U/µl)	0.13 µl

DNA template (~ 0.5 µg tail DNA)	1.0 µl
H ₂ O	<u>15.21 µl</u>
	20 µl

PCR mix ChR2

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl ₂ (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
pFPH (100 µM)	0.1 µl
ChRRev1 (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H ₂ O	<u>15.11 µl</u>
	20 µl

Post-PCR analysis

1.5% agarose gel.

Expected pattern is ROSA26:wt:~250bp, mut:~450bp

ChR2 insert: ~850bp