

Genotyping protocol

EM:11487

STOCK Gt(ROSA)26Sor^{tm2(CAG-GCaMP3)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'

GCaMP specific insert

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

GCaMP3 rev2 5' ACG CCG TAG GTC AGG GTG GTC 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 GCaMP

95 °C, 5 min

95 °C, 20 s

53 °C, 45 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 GCaMP

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
GCaMP3rev2 (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
GCaMP insert: ~850bp