

Genotyping protocol

EM:09256

STOCK Tg(Camk2a-Grin2c/itTA)12Rsp Tg(tetO-cre)LC1Bjd/Kctt

Primers

CN12

N2Cinas 5' **GCC GCC TGC TAG GTG CCT TCG TC** 3'
 N2Csaal 5' **GGC TCT GAG GGC TCC AAA AGC AGA G** 3'
 itTArev1b 5' **GCC AGT ACA GGG TAG GCT GCT CCA C** 3'

LC1

LC sense 5' **GCT GGA GCC CGT GCA GTT CCT CAG** 3'
 LC antisense 5' **CCG GGG GCT GCC ATC TTC CAG C** 3'

PCR profile

95 °C, 5 min
 94 °C, 30 s 35 cycles
 55 °C, 45s
 72 °C, 1 min
 72 °C, 10 min
 4 °C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	CN12
MgCl ₂ (25 mM)	3.0 µl
dNTP mix (25 mM of each nucleotide)	1.8 µl
N2Cinas (100 µM)	0.24 µl
N2Csaal (100 µM)	0.15 µl
itTArev1b (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	0.2 µl
H ₂ O	2.0 µl
	<u>22.31 µl</u>
	30 µl

LC1

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
LC sense (100 µM)	0.15 µl
LC antisense (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 NC12: **mutant: 235 bp**

Wt: ~210 bp

LC1: **transgene: 395bp**

Wt: 339bp