

# Genotyping protocol

EM:09255

**NMRI.Cg-Tg(Camk2a-Grin2c/itTA)10Rsp/Kctt**

## Primers

### CN10

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'

## 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) | 3.0 µl          |
| MgCl <sub>2</sub> (25 mM)              | 1.8 µl          |
| dNTP mix (25 mM of each nucleotide)    | 0.24 µl         |
| N2Cinas (100 µM)                       | 0.15 µl         |
| N2Csaal (100 µM)                       | 0.15 µl         |
| itTArev1b (100 µM)                     | 0.15 µl         |
| AmpliTaq Gold (5 U/µl)                 | 0.2 µl          |
| DNA template (~ 0.5 µg tail DNA)       | 2.0 µl          |
| H <sub>2</sub> O                       | <u>22.31 µl</u> |
|  | 30 µl           |

## Post-PCR analysis

1.5% agarose gel.

Expected pattern is **mutant: ~280 bp****Wt: ~210 bp**