

**Genotyping protocol for B6.129 P2-Tacr1<tm1Sph>/H (also known as NK1) –**

(Q545)

FESA code DDG.

Taken from the EMMA submission form.

PCR primers –

NK1 forward - CTGTGGACTCTGATCTCTTCC  
NK1 Reverse - ACAGCTGTCATGGAGTAGATAC  
NeoF cassette - GCAGCGATCGCCTTCTATC

PCR conditions –

1. 94 degrees 5 min.
2. 58 degrees 1 min.
3. 72 degrees 1 min.
4. 94 degrees 1 min.
5. Cycle 35x to 2.
6. 58 degrees 1 min.
7. 72 degrees 7 min.
8. 4 degrees hold.

PCR product sizes.

350bp (NK1+/+) and 260bp (NK1-/-)

Targeting construct for NK1 receptor (NK1 or TACR1) is derived from 129/Sv DNA. A reporter/selection cassette (IRES-Gal-MC1-Neo) was inserted into exon 1 of the targeted allele, rendering the mRNA out-of-frame and thus no functional NK1 receptor can be produced.

Ref:

Genetic background influences the behavioural and molecular consequences of neurokinin-1 receptor knockout.

J. E. McCutcheon, A. S. Fisher, E. Guzdar, S. A. Wood, S. L. Lightman and S. P. Hunt  
European Journal of Neuroscience, Vol. 27, pp. 683–690, 2008.

Ref:

Altered nociception, analgesia and aggression in mice lacking the receptor for substance P.

Carmen De Felipe, Juan F. Herrero, John A. O'Brien, James A. Palmer, Christopher A. Doyle, Andrew J. H. Smith, Jennifer M. A. Laird, Carlos Belmonte<sup>1</sup>, Fernando Cervero & Stephen P. Hunt.  
Nature 392, 394-397 (26 March 1998).

AR May 2012

## Exon 1

GCCACCTTGTGCGCCCCCTCGCGGAGTGCTGGATTTCGTCCGGCCTGCAGAACGCCACAGC  
AGGTCACGAGCAGCAGAAAGGCGTGGTGGCTGCGGTGGTGGAGCGACACAGCTGCCAACAA  
CTGGCGCAGCTGCTGCGCTGTGCTTCGGGACCAGCGAGTAGACAGTCCAGGTACAGCCCC  
TTCCCCGTGACGTCTCTGCAGCGGGTTATAAAAGCCTAGTAGTGCTCACCCCTGCCAAGGC  
TCTGCTAACTCCTGAGCACAGAAGGACCAAGACCTGGAAGCTGCGGGTGGCAGTAGAGGC  
TCTAAGTGAAGCAACGGAAAAAAGGAGCTCACCCAGTTGGATGCGGACCTCAGAAGCCGGC  
CAGAGGACCAGAACTTTTACTAGCTGCGCTGCAAGCAGCCCTTCTTCCCCATCAAGCTCC  
CACCCAGCACCCAGCTTTGCCTTTCTGCTGCACGCATGTTAACGGGAGTTGCTAGTAGC  
AGCAGGTGCCAAAAGGCGGGTTGTCTTGCCTTTTGGAACCGTGTGGAGTGCAGCTGCTTC  
AATGCAAACAAGAGCCTAGCATGAAAGTGGAGGAATTTGGAGAGACCAGGCAAAGAGCTA  
TACAGAAGCATTGCTCTTGTGGACAAGTCTCCTGCTAACCAGGACTAAGTTCTGCCCAGG  
AAACTTCCCACCTCCTGAGTAGCAGGACTCAAGCCTCAGGCATCTGCGACAAGGTCTTAC  
AGCAAGGAGGTGTGTGCGTGTGTGTGTACTTCTGTGTGTACTTGTGAGTACCTGTAGA  
CGCCAGGGCTTTGCTGCCTTGCCGCAAAAATGGATAACGTCCTTCCTGTGGACTCTGATCT  
CTTCCCAACACCTCCACCAACACTTCTGAGTCTAACCAGTTTGTGCAACCTACCTGGCA  
AATTGTCTTTGGGCAGCCGCCTATACGGTCATCGTGGTGAAGTCCCGTGGTGGGCAACGT  
AGTGGTGATATGGATCATTTTGGCCCACAAGAGAATGAGGACAGTGACCAATTATTTCTT  
GGTGAACCTGGCCTTCGCTGAGGCCTGCATGGCTGCATTCAATACAGTGGTGAACCTCAC  
CTACGCAGTCCACAACGTGTGGTACTACGGCCTCTTTTACTGCAAGTTTCACAACCTTCTT  
CCCCATTGCTGCTCTCTTCGCCAGTATCTACTCCATGACAGCTGTGGCCTTTGACAG

xxxx is StuI site

Wt 360bp

Mut 260bp (not shown).