

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36727
<b>MMRRC Strain Name</b>	C.129X1(B6)- <i>Nkx1-2<sup>tm1Lmgd</sup></i> /Mmnc
<b>Gene Name(s)</b>	NK1 transcription factor related, locus 2 (Drosophila) ( <i>Nkx1-2</i> )
<b>Breeding Protocol(s)</b>	Backcross to Balb/cJ
<b>Protocol Date</b>	8/5/13

### MMRRC #36727 PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25 mM MgCl <sub>2</sub>	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 60°C for 30 sec  
 Step 4: 72°C for 1 min  
 Step 5: Step 2 to 4; Cycles: 30  
 Step 6: 72°C for 7 min

**Taq: Apex and Chromataq 5X Buffer**

**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

WT-F (36727): AGTCCAGCTGCGCCAAGCCAAGGCGC

WT-R (36727): GCGCACCGCCTCCCGCCTGCACGGCG

Neo-F (36727): CTTGGGTGGAGAGGCTATTCGGC

Neo-R (36727): AGGTGAGATGACAGGAGATCCTGC

**Bands expected:** WT [primers NKX1.2-F (36727) + NKX1.2-R (36727)] 237 bp  
 MUTANT[primers Neo-F (36727) + Neo-R (36727)] ~300bp

Run on 2% agarose gel in TAE

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