

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	11608
<b>MMRRC Strain Name</b>	B6.129P2- <i>Bdkrb1</i> <sup>tm1Dgen</sup> /Mmnc
<b>Gene Name(s)</b>	bradykinin receptor, beta 1
<b>Breeding Protocol(s)</b>	Backcross to B6/J
<b>Protocol Date</b>	7/9/2019

### PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	12
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
10uM Primer 3	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 94C, 5min  
 Step 2: 94C, 45sec  
 Step 3: 60C, 45sec  
 Step 4: 72C, 90sec  
 Step 2 to 4 Cycles: 35  
 Step 5: 72C, 7min

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

Bands: WT: 241bp MUTANT: 425bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

NIH0173(11608)1 CTGAACATCTCTGCCTGCATCCTGC  
 NIH0173(11608)2 CCCAGCAGACCAGGAAGGAGGCTAC  
 NIH0173(11608)N GGGTGGGATTAGATAAATGCCTGCTCT



1. Heterozygous Sample
2. Wild-type Control
3. 100bp Marker (Invitrogen)