

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	43822
MMRRC Strain Name	B6J;B6N-A ^{tm1Brd} <i>Myo10</i> ^{tm1a(KOMP)Wtsi} /Mmnc
Gene Name(s)	myosin X
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	7/12/2024

PCR Reaction

Myo10 tm1a allele Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
DNA	2

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 387bp MUTANT: 321bp

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

Myo(43822)1 GGGAGCCGACATCAGTGTAG

Myo(43822)2 ACCGCCCATATTACCCCCT

lacZ Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
DNA	2

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 55C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: No Band MUTANT: 248bp

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

Myo(43822)lacZF GGTGAAGTGCCTCTGGATGT

Myo(43822)lacZR CAAAAATCCATTTTCGCTGGT