MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11632
MMRRC Strain Name	B6.129P2-*Mtmr2*^{tm1Dgen}/Mmnc
Gene Name(s)	myotubularin related protein 2
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	2020-03-06

PCR Reaction

	1X
ddH_2O	13
5X Buffer	5
25mM MgCl2	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2

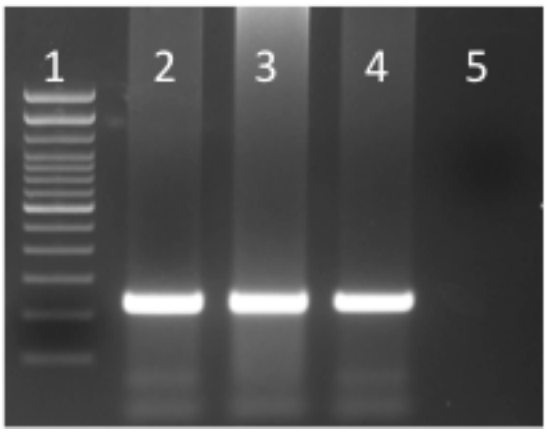
2,2 Primer Reactions
Thermal Cycler:
Step 1: 94C, 5min
Step 2: 94C, 30sec
Step 3: 60C (WT), 55C (Mutant), 30sec
Step 4: 72C, 30sec
Step 2 to 4 Cycles: 30
Step 5: 72C, 7min

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

Bands: MUT: ~840bp WT: 230bp

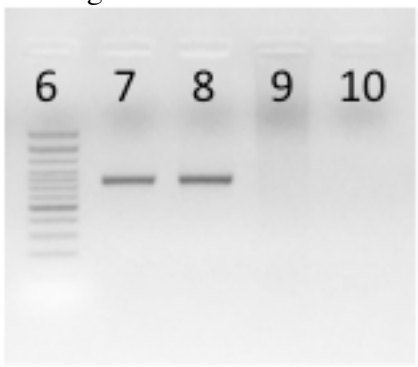
Primer sequences 5' to 3': Primers are 10uM with respect to each primer
NIH0916(11632)WT1: CTCAAGTCAGGAGAAAAGTTGTCTG
NIH0916(11632)WT2: AAGAGGCAACCCAGAATCTGCTGAC
NIH0916(11632)Mut1 CCGCTATCAGGACATAGCGTT
NIH0916(11632)Mut2 CCAGACAGGAACACTCCCAA

Run on 2.0% agarose gel in TAE.



Wild-type Reaction

1. 100bp Ladder (Invitrogen)
2. Heterozygous Sample
3. Heterozygous Sample
4. Wild-type Control
5. Negative Control



Mutant Reaction

6. 100bp Ladder (Invitrogen)
7. Heterozygous Sample
8. Heterozygous Sample
9. Wild-type Control
10. Negative Control

MMRRC at UNC
Mutant Mouse Resource and Research Center supported by NIH

NIH-0916 (11632) Sequencing

CNNNCTAAACTATCACTAGTGCGTGATATCTATCTTGTGACCGTTCCCGCGTGGTTTACGGTAT
CGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGAT
CGATCCGCTTTAAATCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATG
GAAGTTTTTCCTGTCATACTTTGTTAAGAAGGGTGAGAACAGAGTACCTACATTTTGAATGGA
AGGATTGGAGCTACGGGGGTGGGGGTGGGGTGGGATTAGATAAATGCCTGCTCTTTACTGAA
GGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAATTTAAACAAGCAAAA
CCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGATCTCTCGTGGG
ATCATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGT
TTCATAGCCTGAAGAACGAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTT
TTGCCAAGTTCTAATTCCATCAGAAGCTGACTCTAGATCTGGATCTCGAGTGATCAGGTACCA
AGGTCCTCGCTCTGTGTCCGTTGA**ACTGAGGGAGGCAAACAAGTTAGCAGAAATGGAGGA**
ACCAGCCCTGCTTCCAGGAGAGAACATTAAAGATATGGGTCTGTAATGTTTTTTGTCTGC
ACAGCTGTTCATTCTCCTGTAGTGTCCAGCCTGTGTTCTGCAAGCCGAGACCCAGAGCT
GTTGGGGAGTGTCCCTGTCTGGA

Template

ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGA
GAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATG
CCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAG
ACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCT
ATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTG
TCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAG
GATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGC
TGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCC
GGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCC
AGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATC
TCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAAT
GGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCG
CTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCG
GCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGAT
TCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA
CTGAGGGAGGCAAACAAGTTAGCAGAAATGGAGGAACCAGCC
CTGCTTCCAGGAGAGAACATTAAAGATATGGGTCTGTAATGTTTTTTGTC
TGCACAGCTGTTCATTCTCCTGTAGTGTCCAGCCTGTGTTCTGCAAGCCG
AGACCCAGAGCTGTTGGGAGTGTTCCTGTCTGGATGTTGGGAAATGTACA
AATTCAATGTTATTCTCTTTAAATTGTTGCTGAAATCCGTGAAGAGCAGT
TTTATTATTTGAATTACATCATTTAGCCACTGTGTTGGCTAGCTAAAAAC
ATTAAACAAGTGATTTTTATTAAAGTACAGCACTTGGGAGGCAGCGTCTT
ATTTTCTACTTTTTGTAACTTTCTTCCTTGAATAAGTTCAGGTTATAT

Template: The template is pieced together by adding Neo sequence and part of the Mtmr2 gene. Highlighted in yellow are the mutant primers used in the PCR protocol. The Neo gene stops at the blue highlighted region and Mtmr2 beings at the green highlighted region.

Sequencing: The blue highlighted region is the Neo sequence that aligns with the template Neo sequence. The green highlighted region is the Mtmr2 sequence that aligns with the template. The yellow highlighted region is the reverse primer. As with most sequencing assays, the beginning part of the sequence is not very accurate and this is why the forward primer is not matched. The intervening sequence between the Neo and Mtmr2 sequences contains the polyA sequence.