

Genotyping Protocol: **MMRRC 30340**

Assay Type: PCR followed by restriction digest- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain has a T to A transversion at the second position in codon 967 in Exon 23 (nucleotide 72 of Exon 23) of the vacuolar protein sorting 54 gene (*Vps54*). This results in a leucine to glutamine amino acid substitution. Details can be found in Schmitt-John et al (2005) Nat Genet 37(11):1213-5.

Vps54 Exon 23:

CCTACTGTTTCAG**GTTGGTCACAGCAGATGTAGCTTTTTTACTGGAATCTTCAAGCCTTAAAAGGCCTTAAA/GATCTG/GATCTAAA**
TATGGCCGA/GATCTGGGAACAGAAGAGGTGATGACAGACTGGAGACCCAGGTCGTTTCATCTGACCACGGGATGTGTTTCTGAAGAAAA
TCTGG

KEY:

TGA: Stop Codon

R/GATCY: *Bst*YI site (where R is A or G, and Y is T or C)

T: Nucleotide that is changed; T in WT allele, A in Mutant allele

M30340 p F primer

M30340 p R primer

Primer Information:

- 1) Name: M30340 p F
Sequence: 5'-TTTTTACTGGAATCTTCAAGCCTTAAAAGGCCTTAAAATCTGGATC-3'
- 2) Name: M30340 p R
Sequence: 5'-GATGAACGACCTGGGTCTCCAGTCTGTCATCACCTCTTCTGTTCCAGATTTCCGCCATA-3'

Primer location: M30340 p F and R are located on either side of the single nucleotide transversion in Exon 23 of *Vps54*.

Assay name: M30340 wr PCR

PCR Master Mix Components:

| component | manufacturer | concentration | µl/rxn |
|---|--------------------------|---------------|--------|
| Buffer with MgCl ₂ (green cap) | Roche | 10X | 2 |
| dNTPs | Promega (Cat# U1515) | 1.25mM | 3.2 |
| M30340 p F | Sigma | 25µM | 0.3 |
| M30340 p R | Sigma | 25µM | 0.3 |
| FastStart <i>Taq</i> | Roche (Cat# 12032953001) | 5 U/µl | 0.2 |
| sterile water | | | 13 |

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 70°C 30 seconds
- 4) 72°C 30 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

At this step, products may be analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004). *However, this step may be skipped as failure to amplify is rare.*

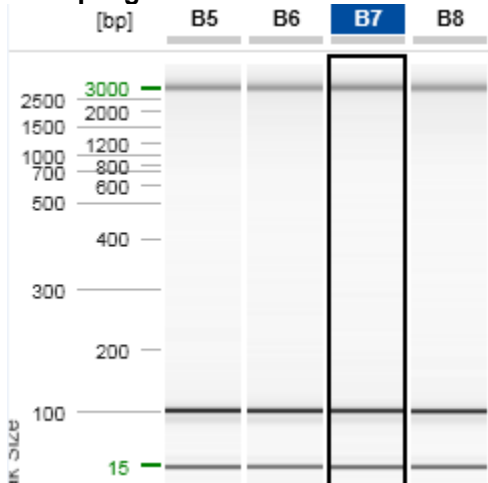
Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100bp-2.5kbbp (Cat# 929559)

Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected product: 114bp

Example gel:



Lanes B5, B6, B7, and B8 display the pre-digest 114bp products that result from the PCR.

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.

BstYI Restriction Digest: (NEB Catalog #R0523S)

BstYI Recognition Site:

5'... R/GATCY ...3'

3'... YCTAG/R ...5'

where R is A or G, and Y is T or C

Wild-Type Sequence, Vps54 gene, Exon 23:

CCTACTGTTTCAG**GTTGGT**CA**CAGCAGATGTAGCTTTTT**TA**CACTGGAAATCTTCAAGCCTTAAAAGGCCTTAAA**/GATCTG/GATCTAAA
TATGGCCGA/GATCTGGGAACAGAAGAGGT**GATGACAGACTGGAGACCCAGGTCGTT**CATCT**GACCACGGGATGTGTTTCTGAAGAAAA**
TCTGG

KEY:

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M30340 p F primer

M30340 p R primer

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Restriction Digest:

10 µl PCR product from PCR above
0.4 µl *Bst*YI (NEB – 10U/µl)
2 µl 10X Buffer 3.1 (NEB)
7.6µl water
20 µl reaction
Incubate at 60°C for 15-30 minutes.

Product Analysis:

All products were analyzed at 80 volts for 40 minutes on a 3% agarose gel with ethidium bromide staining.
Please note: Product sizes may not display correctly if analyzed on the QIAxcel.

Predicted Products:

Wild type (WT): 2 bands (40bp, 74bp)
Heterozygous (Het): 3 bands (40bp, 74bp, 114bp)
Homozygous mutant (Hom): 1 band (114bp)

Example Gel:



Lane B1 displays a 1Kb+ Ladder (Invitrogen Cat# 10787-018).
Lane B2 displays a heterozygous sample (40bp, 74bp and 114bp products)
Lane B3 displays a wild-type sample (40bp and 74bp products)