AQP5-DEL606

Allele Description

This is a CRISPR/Cas9 induced mutation. Deletion of 606 nucleotides from *Aqp5* gene, across two critical exons, (ENSMUSE00000297625 & ENSMUSE00001018343) to introduce a frameshift resulting in a premature stop codon null allele in 3 of 4 transcripts. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wildtype loss of allele (WT-LOA) and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

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AQP5-DEL606-WT1 assay (FAM labelled)

AGTTTCTCCAACAAATCGCCTGTCTGGATCATGCAGGGGACTTGACTTCCAAGAGCTCAGGCTCTAG
GT**GTCCTTGAAGACCAGAACCTTAC**T**TGTCTAGCCTGTCACCGAGCCT**GAAGGCTGCTAGCAGGGT
CCTAACCCAGCTCTTCCTTGCAGCTCAGCAACAACACAACACCAGGCAAGGCCGTGgtggtggagttaatc
ttgactttccagctggccctctg**catcttctcctccacggactcc**cgccgcaccagcccggtgggctccccagccttatccattggcttg
tcggtcacactgggccatcttgtgggggtgagtagtgctggcacaaaccggggtgtcatggagatgaacagtggaaccccagaatg

Lower case letters denote the deleted sequence in the mutant allele.
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
AQP5-DEL606-WT1_F	n/a	**GTCCTTGAAGACCAGAACCTTAC**	n/a	WT Forward
AQP5-DEL606-WT1_PROBE	FAM	**TGTCTAGCCTGTCACCGAGCCT**	BHQ1	WT Probe
AQP5-DEL606-WT1_R	n/a	**GGAGTCCGTGGAGGAGAAGATG**	n/a	WT Reverse

AQP5-DEL606-MUT1 assay (FAM labelled)

GACACCCTACCCTCTGGCTACAGGCCTGAGCTTCAAGCTTGCGCACACTGAAGTGCAGTTTCTCCAACAAATCG
CCTGTCTGGATCATGCAGGGGACTTGACTTCCAAGAGCTCAGGCTCTAGGT**GTCCTTGAAGACCAGAACCTTA**
CT**TGTCTAGCCTGTCACCGAGCCT**GAAGGCTGCTAGCAGGGTCCTAACCCAGCTCTTCCTTGCAGCTCAGCAA
CAACACAACACCAGGCAAGGCC**GTGTCATGAATCGGTTCAGCC**CCTCTCACTGGGTGAGTGCGGGCCTTCCC
CCGGCTCCTGAAAACACGGCAGCGTACTGAACTGAAAATCCAGAATTGACTGGACATTCTGGGTGTTGTCGG

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
AQP5-DEL606-MUT1 _F	n/a	**GTCCTTGAAGACCAGAACCTTAC**	n/a	MUT Forward
AQP5-DEL606-MUT1 _PROBE	FAM	**TGTCTAGCCTGTCACCGAGCCT**	BHQ1	MUT Probe
AQP5-DEL606-MUT1_R	n/a	**GGCTGAACCGATTCATGACAC**	n/a	MUT Reverse

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Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCCTATGA

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5µM & primers 15µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate. Non-template controls are also run.

qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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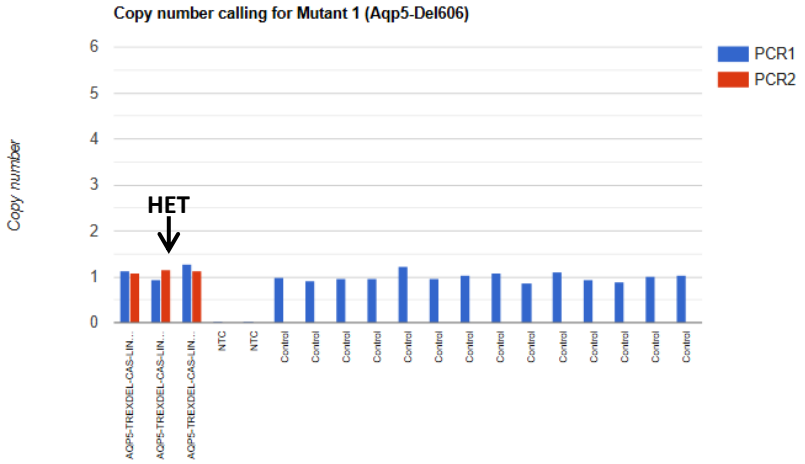
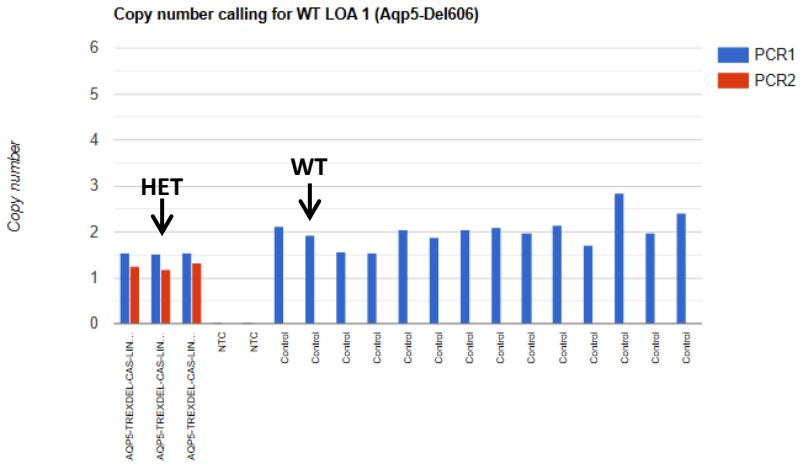
The results are analysed using CopyCaller Software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

AQP5-DEL606-WT1 and AQP5-DEL606-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 232829 results)





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