Name of Mouse model or mutation:
Prps1-R214W-EM1-B6
Prps1-R214W-EM2-B6

Description:
Point mutation generated using CRISPR/Cas9 reagents.

Type of mutation:
Point mutation: R214W

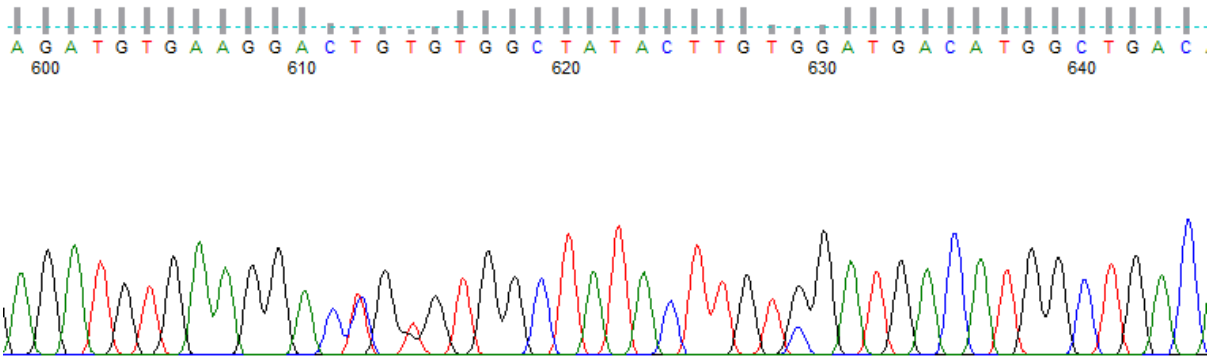
Sequence details
Prps1 WT:
GAGGGTAGGCTTTACTCCACTCCTGCCACCTTCAGGTTCACTTGTCACTAATTCTAGACTCACAAGTC
TTCCATGATGGAAATTGGGATAATCTGTACACTCCCTGCACTAGCTATAAGATCTCTCTCTATCTTCTG
CTTTTAGTTAGCAAGCTAATATACCTTAGTATATGCTGCTTCCCAGGACTGGTGAAGGGTATGCCTAC
CCCATCTATATACTATGGACTGTGCAAGTTTGAATATTATATTCCTATACTGCTGTTGGTATTACAGAA
GCATCAATCCACAAAATCCAGTTTGTATTGTTTGTTTTGTGGGATGTTAATGATAGTTGCTTTCTTCTA
GCTCAGGGATCTGTCTTACTTTCAAGGAGTATTCTCCTACAGTAAAAACATTCCGTTATTTTGTGTAA
GTGGGCACAAGCGATGGAACCTGGGGCCAGCTGTCTACCACTAAATGACACTCCCAGCTTTGGAAG
CCGCTTTGCCTTGGATTTTTGTCTTTCCTAACTTTGAACATCTATTTTACAGAGTGACCTCCATTGCAG
ACCGGCTGAATGTGGATTTTGCTTTGATTCACAAGGAACGGAAGAAGGCCAATGAAGTGGACCGCA
TGGTACTTGTGGGAGATGTGAAGGACCGTGTGGCTATACTTGTGGATGACATGGCTGACACTTGCG
GTACAATCTGTCACGCCGCTGACAAGTGAGTACAGGCTAGTTAGAAATAAAAAGCTAAACTACTCTT
TACTGATTCTTTCTGAAACACAACTGAAGGGTAAGTAGAGTAGTAGCTTTGGGATATTCTGATCATG
TTAACTTTTAATAACATATAGATTAGTAAGTATTCCTCATTTCAGATTTTTTTAAAGATTTATTTTTATA
TCTAAATACACTGTAGCTGTCTTCAGATGCACTAGAAGAGGGCGTCAGATCTCATTATAGCTGGTTG
TGAGCCACCATGTGGTTGCTGGGATTTGTACTCAGGACCTTTGGAAGAGCAGCCAGTGCTCTTAACT
GCTGAGCCATCTCTCCAGCCCTCATTTCAGATTTTTAACTTTTATAAACAGACACTGCTTTTACATCCA
GGATATCCTTAATGAGTTATCAAAAGTTAAATCCATCACATACACTTTACCTTTTAAAAGCCCTTACTT
AGTAACACAATCTACCATAGACTGGGTTGTCTCCCTTCATGATTACATTTCTGCCTGGAAGCTGTGTC
AAGACAGTATATTGATTGCATATTGCTAGACCAGGGAAAGATGAAA

Prps1-R214W-EM1-B6 and Prps1-R214W-EM2-B6:
GAGGGTAGGCTTTACTCCACTCCTGCCACCTTCAGGTTCACTTGTCACTAATTCTAGACTCACAAGTC
TTCCATGATGGAAATTGGGATAATCTGTACACTCCCTGCACTAGCTATAAGATCTCTCTCTATCTTCTG
CTTTTAGTTAGCAAGCTAATATACCTTAGTATATGCTGCTTCCCAGGACTGGTGAAGGGTATGCCTAC
CCCATCTATATACTATGGACTGTGCAAGTTTGAATATTATATTCCTATACTGCTGTTGGTATTACAGAA
GCATCAATCCACAAAATCCAGTTTGTATTGTTTGTTTTGTGGGATGTTAATGATAGTTGCTTTCTTCTA

GCTCAGGGATCTGTCTTACTTTCAAGGAGTATTCTCCTACAGTAAAAACATTCCGTTATTTTGTGTAA
GTGGGCACAAGCGATGGAACCTGGGGCCAGCTGTCTACCACTAAATGACACTCCCAGCTTTGGAAG
CCGCTTTGCCTTGGATTTTTGTCTTTCCTAACTTTGAACATCTATTTTACAGAGTGACCTCCATTGCAG
ACCGGCTGAATGTGGATTTTGCTTTGATTCACAAGGAACGGAAGAAGGCCAATGAAGTGGACCGCA
TGGTACTTGTGGGAGATGTGAAGGAC**T**G**G**GTGGCTATACTTGT**C**GATGACATGGCTGACACTTGCG
GTACAATCTGTCACGCCGCTGACAAGTGAGTACAGGCTAGTTAGAAATAAAAAGCTAAACTACTCTT
TACTGATTCTTTCTGAAACACAACTGAAGGGTAAGTAGAGTAGTAGCTTTGGGATATTCTGATCATG
TTAACTTTTAATAACATATAGATTAGTAAGTATTCCTCATTTCAGATTTTTTTAAAGATTTATTTTTATA
TCTAAATACACTGTAGCTGTCTTCAGATGCACTAGAAGAGGGCGTCAGATCTCATTATAGCTGGTTG
TGAGCCACCATGTGGTTGCTGGGATTTGTACTCAGGACCTTTGGAAGAGCAGCCAGTGCTCTTAACT
GCTGAGCCATCTCTCCAGCCCTCATTTCAGATTTTTAACTTTTATAAACAGACACTGCTTTTACATCCA
GGATATCCTTAATGAGTTATCAAAAGTTAAATCCATCACATACACTTTACCTTTTAAAAGCCCTTACTT
AGTAACACAATCTACCATAGACTGGGTTGTCTCCCTTCATGATTACATTTCTGCCTGGAAGCTGTGTC
AAGACAGTATATTGATTGCATATTGCTAGACCAGGGAAAGATGAAA

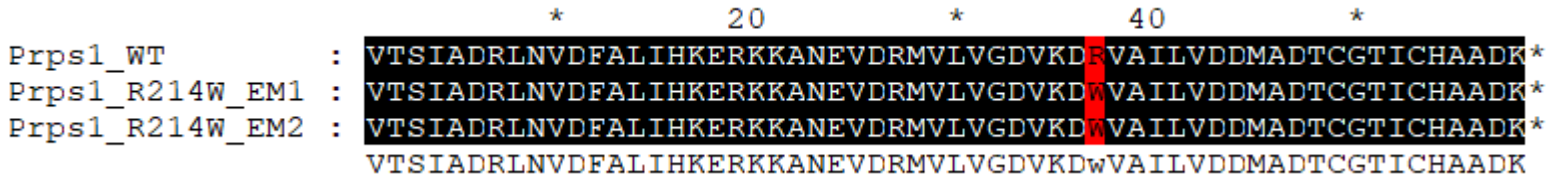
Nucleotide changes highlighted in **red and underlined = nominated change**, silent changes highlighted in red only.

Prps1-R214W-EM1-B6 and Prps1-R214W-EM2-B6 Heterozygous F1 animal sequence trace:



Prps1-R214W-EM1-B6 and Prps1-R214W-EM2-B6 Nucleotide Alignment:

Prps1-R214W-EM1-B6 and Prps1-R214W-EM2-B6 Predicted Protein Alignment:



QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_PRPS1_R214W_F4	GAGGGTAGGCTTTACTCCAC
Geno_PRPS1_R214W_R4	TTTCATCTTTCCCTGGTCTAGCAA
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	64
Elongation time (min)	1
WT product size (bp)	1268
Mutant product size (bp)	1268
Notes	Sequencing primers: Geno_Prps1_R214W_F3 AGGACTGGTGAAGGGTATGC Geno_Prps1_R214W_R3 GGGAGACAACCCAGTCTATGGTA

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

As the sgRNA used had a potential off-target site with two or fewer mismatches, then PCR amplification and Sanger sequencing of this site was performed to confirm its integrity using the following conditions/primer sequences:

Geno_Prps1_OT3_F1	CTGTAGCAGGTGCGGATCAT
Geno_Prps1_OT3_R1	GCAATGGAGCATGAGCTTTGT
Taq Polymerase used	Platinum SuperFi PCR Master Mix
Annealing Temperature (°C)	58
Elongation time (min)	1
WT product size (bp)	927
Mutant product size (bp)	n/a

Notes	

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Prps1-R214W-donor-UNIV1
Forward Primer (5'-3')	ACTTGTGGGAGATGTGAAGGAC
Reverse Primer (5'-3')	GCTTTTTATTTCTAACTAGCCTGTACTCAC
Probe (5'-3')	ATGACATGGCTGACACTTGCGGTA
Label	FAM-BHQ1

The ddPCR assay recognises sequence common to both the WT Prps1 and the R214W mutant. Therefore, WT controls and correctly targeted F1 R214W heterozygote animals will call at 2 copies.

Assay name	Prps1-R214W-donor-MUT1
Forward Primer (5'-3')	GTGGGAGATGTGAAGGACTGG
Reverse Primer (5'-3')	GCTTTTTATTTCTAACTAGCCTGTACTCA
Probe (5'-3')	TGGCTATACTTGTCGATGACATGGC
Label	FAM-BHQ1

The ddPCR assay is specific to the Prps1 R214W mutation. Therefore, WT controls will call at 0 copies and correctly targeted F1 R214W heterozygote animals will call at 1 copy.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

PRPS1-R214W-EM1-B6
PRPS1-R214W-EM2-B6

Allele Description

This is a CRISPR/Cas9 induced mutation creating point mutation R214W in exon ENSMUSE00000384936 of *Prps1*. 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 wild type 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:

- Universal probe and Universal primer designed 5' of the point mutation.
- Wild type (WT) specific primer binding to the WT base mutated in the mutant allele.
- Mutant specific primer binding to the R214W point mutation.

For X-linked genes that have been targeted, the following results would be expected:

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

PRPS1-R214W-EM1-B6
PRPS1-R214W-EM2-B6

Prps1-R214W-WT1 assay (FAM labelled)

ACATCTATTTTACAGAGTGACCTCCATTG**CAGACCGGCTGAATGTGGATTT**TGCTTTGATTCACAAGGAACGG
AAGAAGGCCAATGAAGTGGACCGCATGGTACTTGTGGGAGATGTGAAGGAC**cGtGTGGCTATACTTGTgGAT**
GACATGGCTGACACTTGCGGTACAATCTGTCACGCCGCTGACAAGTGAGTACAGGCTAGTTAGAAATAAA

Lower case letters denote bases changed 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
Prps1-R214W-UNI_F	n/a	**CAGACCGGCTGAATGTGGATTT**	n/a	Universal Forward
Prps1-R214W-UNI_PROBE	FAM	**TGCTTTGATTCACAAGGAACGGAAGA**	BHQ	Universal Probe
Prps1-R214W-WT_R	n/a	**CATCCACAAGTATAGCCACACG**	n/a	Wild type Reverse

Prps1-R214W-MUT1 assay (FAM labelled)

ACATCTATTTTACAGAGTGACCTCCATTG**CAGACCGGCTGAATGTGGATTT**TGCTTTGATTCACAAGGAACGG
AAGAAGGCCAATGAAGTGGACCGCATGGTACTTGTGGGAGATGTGAAGGAC**tGgGTGGCTATACTTGTcGAT**
GACATGGCTGACACTTGCGGTACAATCTGTCACGCCGCTGACAAGTGAGTACAGGCTAGTTAGAAATAAAA

Lower case letters denote bases changed 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
Prps1-R214W-UNI_F	n/a	**CAGACCGGCTGAATGTGGATTT**	n/a	Universal Forward
Prps1-R214W-UNI_PROBE	FAM	**TGCTTTGATTCACAAGGAACGGAAGA**	BHQ	Universal Probe
Prps1-R214W-MUT_R	n/a	**CATCGACAAGTATAGCCACCCA**	n/a	Mutant Reverse

PRPS1-R214W-EM1-B6
PRPS1-R214W-EM2-B6

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

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

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

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 5 µM & primers 15 µM 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 along with non-template controls.

qPCR cycling conditions

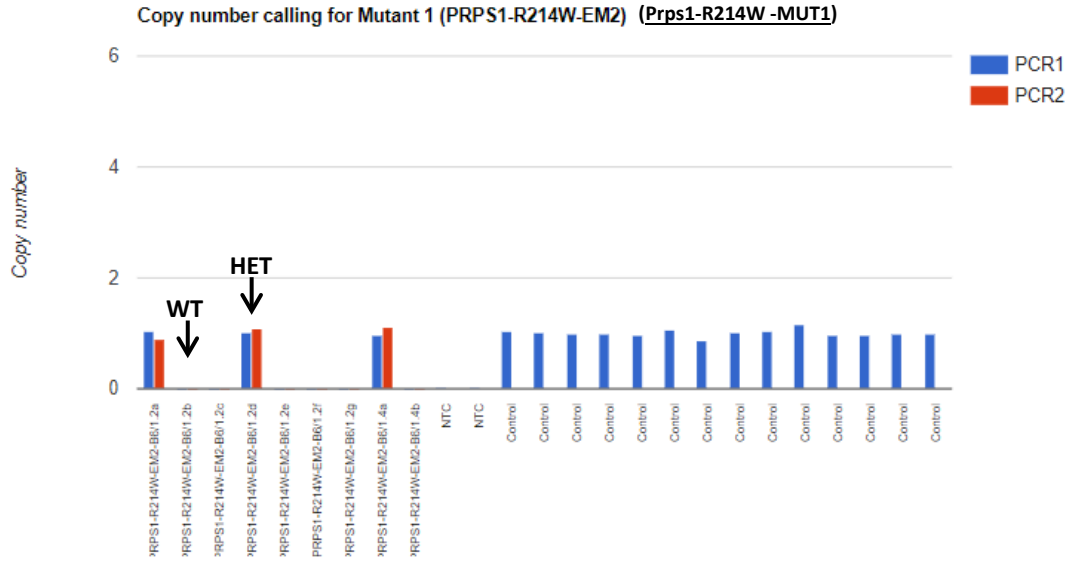
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

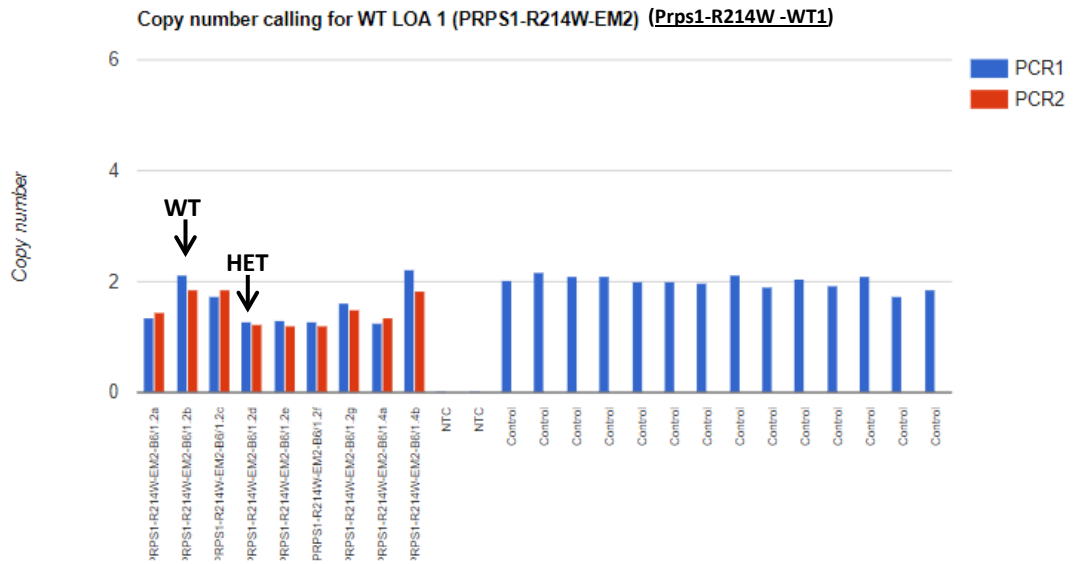
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Prps1-R214W-WT1 and Prps1-R214W -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 263641 results)





PRPS1-R214W-EM1-B6
PRPS1-R214W-EM2-B6

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