Ppp1r15a-DEL14

Allele Description

This is a CRISPR/Cas9 induced mutation deleting 14 nucleotides from the *Ppp1r15a* gene, encompassing/within a critical exon to introduce a frameshift resulting in a premature stop codon/null allele. 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:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that either bridges the junction designed for the CRISPR mutant allele or is 3' of the junction

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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Ppp1r15a-DEL14-WT1 assay (FAM labelled)

GACCCCTCCAACTCTCCTTCTTCAGGCTCCCACTCCAGAGCCTGGGAGTACTACTC**TAGAGAGAAGC**
CTAAGCAGGAGGGAGAAGC**CAAGGTAGAGGCACACAGGGCAGGG**CAGGGTCACccttgtcg**gaatgc**
TGAGGCTGAGGAAGGAGGACCTGAGACAACTTTTGTCTGTACTGGAAATGCCTTCCTGAAGGCCTG
GGTGTATCGCCCAGGAGAGGACACAGAGGAAGAAGACAACAGCGATTCGGATTCAGCTGAGGAAG

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 Ppp1r15a-DEL14	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
Ppp1r15a-DEL14-UNI_F	n/a	**TAGAGAGAAGCCTAAGCAGG**	n/a	Universal Forward
Ppp1r15a-DEL14-UNI_PROBE	FAM	**CCCTGCCCTGTGTGCCTCTACCTTG**	BHQ1	Universal Probe
Ppp1r15a-DEL14-WT_R	n/a	**TCCTCAGCCTCAGCATTC**	n/a	WT Reverse

Ppp1r15a-DEL14-MUT1 assay (FAM labelled)

CTGTGCCTTTCTTGGGGGAGGCAGAACATCAAGCCACGGAAGAAAAAGGAACAGAAAACAAGGCT
GACCCCTCCAACTCTCCTTCTTCAGGCTCCCACTCCAGAGCCTGGGAGTACTACTC**TAGAGAGAAGC**
CTAAGCAGGAGGGAGAAGC**CAAGGTAGAGGCACACAGGGCAGGG**CAG**GGTCAC[14ntdel]TGA**
GGCTGAGGAAGGAGGACCTGAGACAACTTTTGTCTGTACTGGAAATGCCTTCCTGAAGGCCTGGGT
GTATCGCCCAGGAGAGGACACAGAGGAAGAAGACAACAGCGATTCGGATTCAGCTGAGGAAGACA

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

Oligo Ppp1r15a-DEL14	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
Ppp1r15a-DEL14- UNI_F	n/a	**TAGAGAGAAGCCTAAGCAGG**	n/a	Universal Forward
Ppp1r15a-DEL14-UNI_PROBE	FAM	**CCCTGCCCTGTGTGCCTCTACCTTG**	BHQ1	Universal Probe
Ppp1r15a-DEL14-MUT_R	n/a	**TCCTCAGCCTCAGTGACC**	n/a	MUT Reverse

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Dot1l internal control (VIC labelled)

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

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

Oligo Ppp1r15a-DEL14	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

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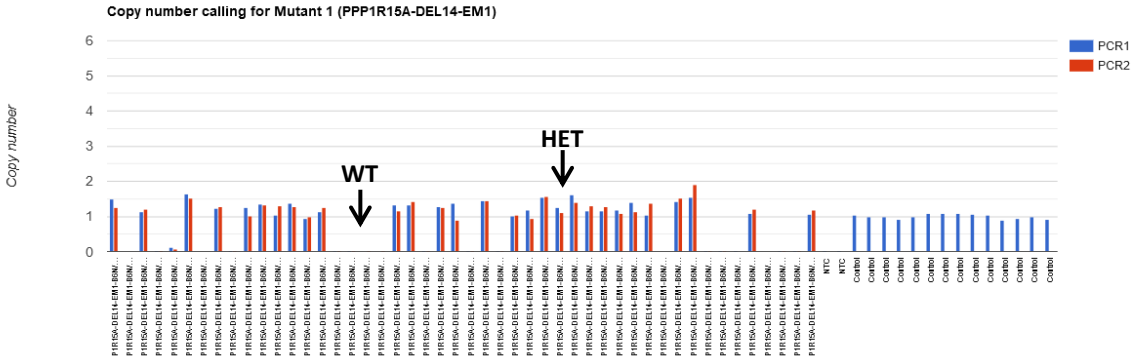
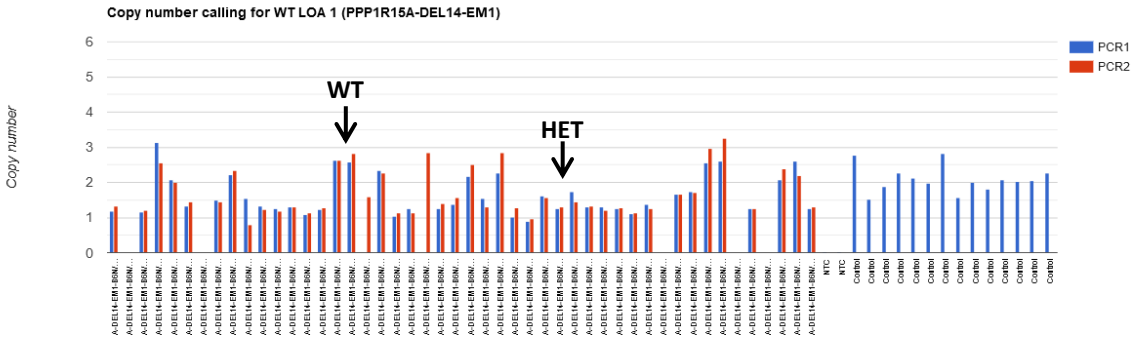
Analysis

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

Ppp1r15a-DEL14-WT1 and Ppp1r15a-DEL14-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 327866 results)





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