PKHD1-T37M-EM1-C3H
PKHD1-T37M-EM2-C3H

Allele Description

This is a CRISPR/Cas9 induced mutation creating point mutation T37M in exon ENSMUSE00001279293 of *Pkhd1*. 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:

- Wild type (WT) assay with a probe binding to the WT bases mutated in the mutant allele.
- Mutant assay with a forward primer binding to the T37M point mutation.

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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Pkhd1-T37M-WT1 assay (FAM labelled)

TGATTGATAGAGCCTTACTCGAGTTTCCAGATTGAACCCG**CAGAAGGTAGCCTTGCAGGGGGAAcaTGGATC ACAG**TTGTATTTGACGGTAG**GTTTGGGCTTTTCTGAATTTGGGTGT**TGAATTTATTGATGTAAAATCCAAAGA ATTATAAATTC

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
Pkhd1-T37M-WT2_F	n/a	**CAGAAGGTAGCCTTGCAGGG**	n/a	Wild type Forward
Pkhd1-T37M-WT2_PROBE	FAM	**CTGTGATCCAtgTTCCCC**	BHQ	Wild type Probe
Pkhd1-T37M-WT2_R	n/a	**ACACCCAAATTCAGAAAAGCCCAAAC**	n/a	Wild type Reverse

Pkhd1-T37M-MUT1 assay (FAM labelled)

TGATTGATAGAGCCTTACTCGAGTTTCCAGATTGAACCCGCAGAAG**GTAGCCTTGCAGGcGGAAtgTGGATCA CAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGT**TGAATTTAT

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
Pkhd1-T37M-DONOR-MUT1_F	n/a	**GTAGCCTTGCAGGCGGAAtg**	n/a	Mutant Forward
Pkhd1-T37M-DONOR-MUT1_PROBE	FAM	**TGGATCACAGTTGTATTTGACGGTAGGT**	BHQ	Mutant Probe
Pkhd1-T37M-DONOR-MUT1_R	n/a	**ACACCCAAATTCAGAAAAGCCCAA**	n/a	Mutant Reverse

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

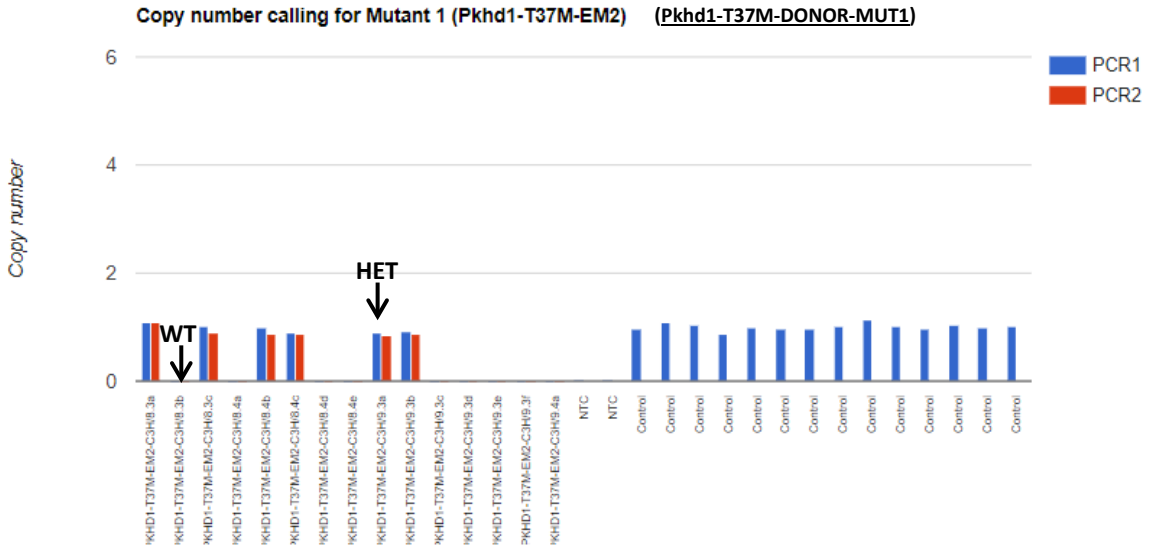
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

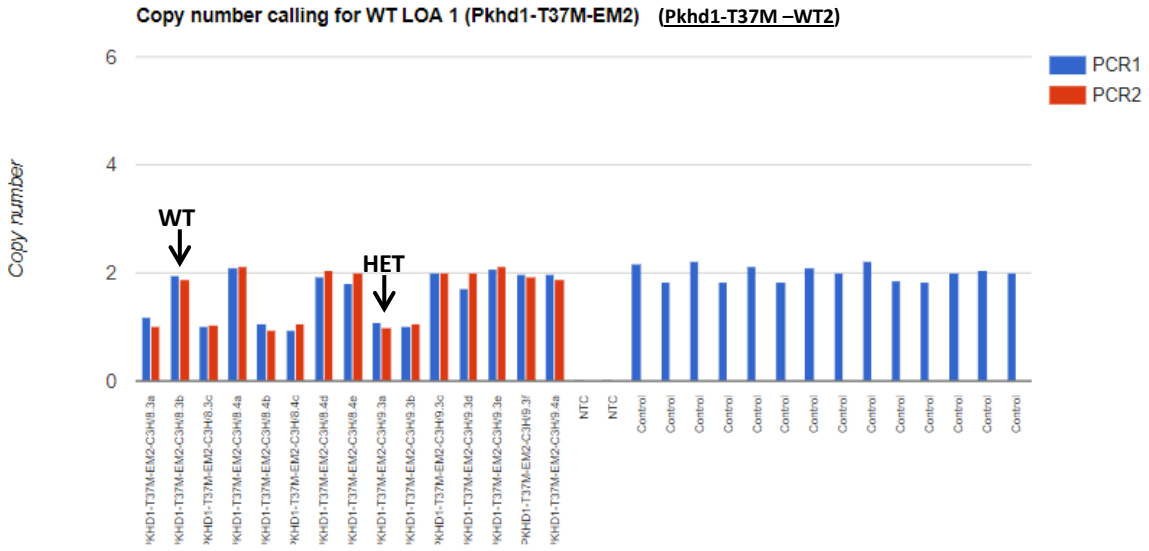
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Pkhd1-T37M-WT2 and Pkhd1-T37M-DONOR-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 273085 results)





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Version No.	1
Date:	18.02.2020
Created/Updated by:	AC

Name of Mouse model or mutation:
Pkhd1-T37M-EM1-C3H

Description:
Point mutant made by CRISPR/Cas9 gene editing.

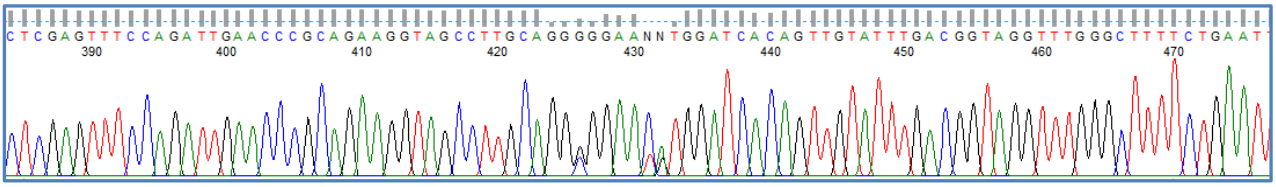
Type of mutation:
SNP: T37M

Sequence details
WT
AGAAGAGAGGCATTGTGGGTTCAAGCATATCTTTGAGATTATTTCTTTTTTTTCCCATTTTTTGTTAGG
TATTTAGCTCATTTACATTTCCAATGCTATACCAAAAGTCCCCCATACCTCCCTACTCCCCTACCCACCC
ACTCCCCCTTTTTGGCCCTGGCATTCCCCTGTACTGGGGCATATTATTTCTAAGGGAAGCAGAAATTC
AGGACTATCCTGATGAACATTGGGATCCTGGATTGGTGAAACGAGAGCCATCCATTTTTCTTAAAGT
AGGACTCTGAAGGTATATTCTCTGGACCAATAATGCCTGAATAGAAGTGGACACGTTGAACCAGAA
ACTCTGGTAATAGGGCCCACTGATCCGTGCTTCATCTGGCCAGTGGATTGTATGTACACCCTAAAGT
CTGCTTTAAAGTATTGGATGTGCTACCGTATTGTACATCACCTTCACAACCGACATGTTCTCTGATTG
ATAGAGCCTTACTCGAGTTTCCAGATTGAACCCGCAGAAGGTAGCCTTGCAGGGGGAACATGGATC
ACAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGTTGAATTTATTGATGTAAAATCC
AAAGAATTATAAATTCTCGCTTCTTTGTTTAAGAACAGATGAACAGAGACCCATCTCTGTCTTTGCAG
ATGCTAAGTAGTCAGTGTCACAGCAGAGGCCACTGTAGCATCAACATCCTCACCGTGGGATGCTTAG
GAAGACGAAATAACATGGAAGTGGACAGCATAGGAATACTACTCCCTCTTGGCAGTTGCCCTTACAA
GTGATTCTGTTAGGAGCCCCTAAACCATGCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGT
GGAGGAATGAAATGCTCTTTCCTAGGGAGTAGGCAGACATGAAGAGAGAGGGGGGGGGGGAGAA
AACATTTATAGGCTCATAGAACTTTTGTAAAACAGTGTAATTGCCTTTGGAAATGAGGATTAATTTTA
TCCTCAGGCAAACGCAATATCT

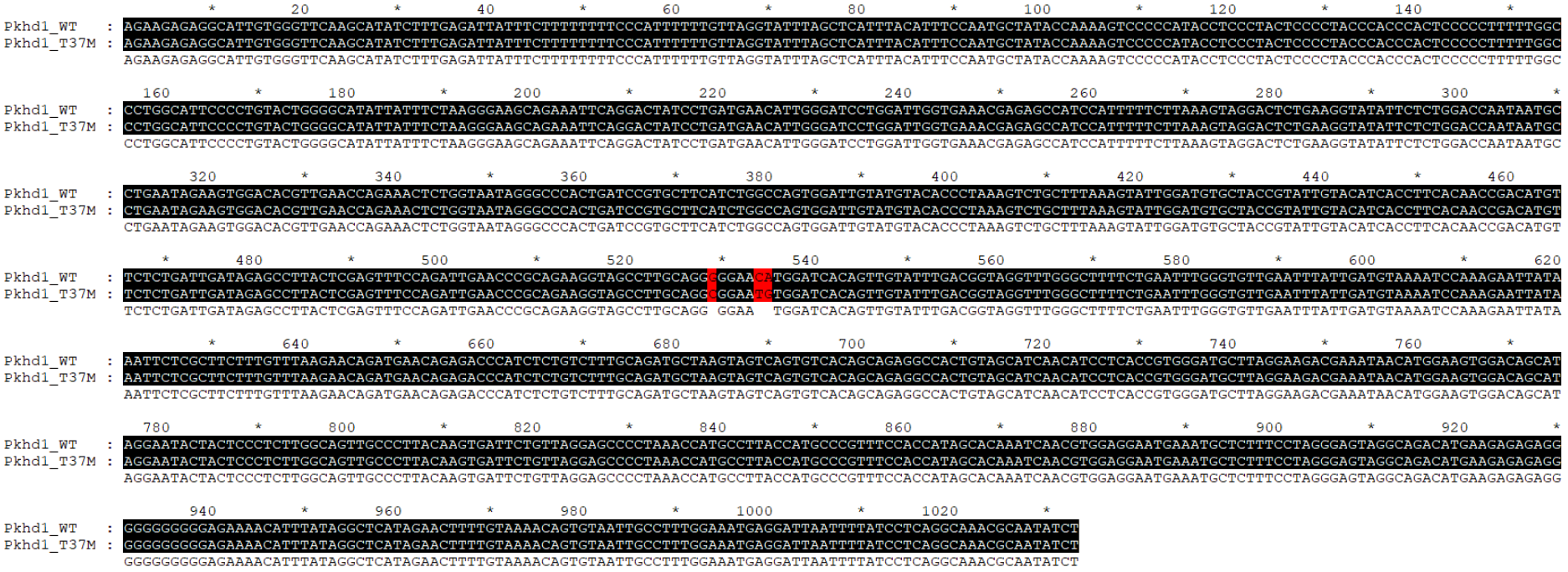
Mutant
AGAAGAGAGGCATTGTGGGTTCAAGCATATCTTTGAGATTATTTCTTTTTTTTCCCATTTTTTGTTAGG
TATTTAGCTCATTTACATTTCCAATGCTATACCAAAAGTCCCCCATACCTCCCTACTCCCCTACCCACCC
ACTCCCCCTTTTTGGCCCTGGCATTCCCCTGTACTGGGGCATATTATTTCTAAGGGAAGCAGAAATTC
AGGACTATCCTGATGAACATTGGGATCCTGGATTGGTGAAACGAGAGCCATCCATTTTTCTTAAAGT
AGGACTCTGAAGGTATATTCTCTGGACCAATAATGCCTGAATAGAAGTGGACACGTTGAACCAGAA
ACTCTGGTAATAGGGCCCACTGATCCGTGCTTCATCTGGCCAGTGGATTGTATGTACACCCTAAAGT
CTGCTTTAAAGTATTGGATGTGCTACCGTATTGTACATCACCTTCACAACCGACATGTTCTCTGATTG
ATAGAGCCTTACTCGAGTTTCCAGATTGAACCCGCAGAAGGTAGCCTTGCAGG**C**GGAA**TG**TGGATC
ACAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGTTGAATTTATTGATGTAAAATCC
AAAGAATTATAAATTCTCGCTTCTTTGTTTAAGAACAGATGAACAGAGACCCATCTCTGTCTTTGCAG

ATGCTAAGTAGTCAGTGTCACAGCAGAGGCCACTGTAGCATCAACATCCTCACCGTGGGATGCTTAG
GAAGACGAAATAACATGGAAGTGGACAGCATAGGAATACTACTCCCTCTTGGCAGTTGCCCTTACAA
GTGATTCTGTTAGGAGCCCCTAAACCATGCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGT
GGAGGAATGAAATGCTCTTTCCTAGGGAGTAGGCAGACATGAAGAGAGAGGGGGGGGGGGAGAA
AACATTTATAGGCTCATAGAACTTTTGTAAAACAGTGTAATTGCCTTTGGAAATGAGGATTAATTTTA
TCCTCAGGCAAACGCAATATCT

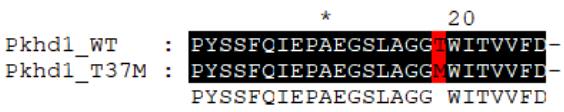
Pkhd1-T37M-EM1-C3H Heterozygous F1 animal sequence trace:



Nucleotide Alignment:



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_ Pkhd1_C3H_F4 (5'-3')	AGAAGAGAGGCATTGTGGGTT
Geno_ Pkhd1_C3H_R4 (5'-3')	AGATATTGCGTTTGCCTGAGGA
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	1.5
Elongation time (min)	3
WT product size (bp)	1033
Mutant product size (bp)	1033
Notes	Sequence PCR products with Geno_Pkhd1_C3H_F4 (AGAAGAGAGGCATTGTGGGTT) and Geno_Pkhd1_C3H_R1 (TTCCTAAGCATCCCACGGTGA)

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.

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	Pkhd1-T37M-DONOR-MUT1
Forward Primer (5'-3')	GTAGCCTTGCAGGCGGAATG
Reverse Primer (5'-3')	ACACCCAAATTCAGAAAAGCCCAA
Probe (5'-3')	TGGATCACAGTTGTATTTGACGGTAGGT
Label	FAM-BHQ1

The Pkhd1-T37M-DONOR-MUT1 ddPCR assay is specific to the T37M mutation in the Pkhd1 gene and only MUT alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	Pkhd1-T37M-DONOR-UNIV1
Forward Primer (5’-3’)	AACCCGCAGAAGGTAGCCTTG
Reverse Primer (5’-3’)	CACCCAAATTCAGAAAAGCCCAA
Probe (5’-3’)	TGGATCACAGTTGTATTTGACGGTAGGT
Label	FAM-BHQ1

The Pkhd1-T37M-DONOR-UNIV1 ddPCR assay is universal to the Pkhd1 gene and all alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies for F1 (HET) animals.

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.