

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**

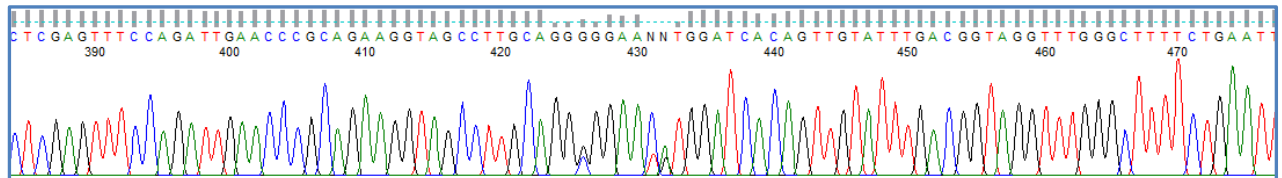
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TATTTAGCTCATTACATTTCCAATGCTATACCAAAGTCCCCATACCTCCCTACTCCCCTACCCACCC
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AGGACTATCCTGATGAACATTGGGATCCTGGATTGGTGAACGAGAGCCATCCATTTTTCTTAAAGT
AGGACTCTGAAGGTATATTCTCTGGACCAATAATGCCTGAATAGAAGTGGACACGTTGAACCAGAA
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GAAGACGAAATAACATGGAAGTGGACAGCATAGGAATACTACTCCCTCTTGGCAGTTGCCCTTACAA
GTGATTCTGTTAGGAGCCCTAAACCATGCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGT
GGAGGAATGAAATGCTCTTTCCTAGGGAGTAGGCAGACATGAAGAGAGAGGGGGGGGGGAGAA
AACATTTATAGGCTCATAGAACTTTTGTA AACAGTGAATTGCCTTTGGAAATGAGGATTAATTTTA
TCCTCAGGCAAACGCAATATCT

Mutant

AGAAGAGAGGCATTGTGGGTTCAAGCATATCTTTGAGATTATTTCTTTTTTTTCCCATTTTTTGTAGG
TATTTAGCTCATTACATTTCCAATGCTATACCAAAGTCCCCATACCTCCCTACTCCCCTACCCACCC
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CTGCTTTAAAGTATTGGATGTGCTACCGTATTGTACATCACCTTCACAACCGACATGTTCTCTGATTG
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ACAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGTTGAATTTATTGATGTAAAATCC
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GTGATTCTGTTAGGAGCCCTAAACCATGCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGT
GGAGGAATGAAATGCTCTTTCCTAGGGAGTAGGCAGACATGAAGAGAGAGGGGGGGGGGAGAA
AACATTTATAGGCTCATAGAACTTTTGTAAACAGTGTAATTGCCTTTGAAATGAGGATTAATTTTA
TCCTCAGGCAAACGCAATATCT

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



Nucleotide Alignment:

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                *      20      *      40      *      60      *      80      *      100     *      120     *      140     *
Pkhd1_WT : AGAAGAGAGGCATTGTGGGTTCAAGCATATCTTTGAGATTATTTCTTTTTTTTCCCATTTTTTGTAGGATATTTAGCTCATTACATTTCCAATGCTATACCAAAGTCCCCATACCTCCCTACTCCCCTACCCACCCTCCCCTTTTTGGC
Pkhd1_T37M : AGAAGAGAGGCATTGTGGGTTCAAGCATATCTTTGAGATTATTTCTTTTTTTTCCCATTTTTTGTAGGATATTTAGCTCATTACATTTCCAATGCTATACCAAAGTCCCCATACCTCCCTACTCCCCTACCCACCCTCCCCTTTTTGGC
                160     *      180     *      200     *      220     *      240     *      260     *      280     *      300     *
Pkhd1_WT : CCTGGCATTCCCCTGTACTGGGGCATATTAATTTCTAAGGGAAGCAGAAATTCAGGACTATCCTGATGAACATTTGGGATCCTGGATTGGTGAAACGAGAGCCATCCATTTTTCTTAAAGTAGGACTCTGAAGGTATATTTCTTGGACCAATAATGC
Pkhd1_T37M : CCTGGCATTCCCCTGTACTGGGGCATATTAATTTCTAAGGGAAGCAGAAATTCAGGACTATCCTGATGAACATTTGGGATCCTGGATTGGTGAAACGAGAGCCATCCATTTTTCTTAAAGTAGGACTCTGAAGGTATATTTCTTGGACCAATAATGC
                320     *      340     *      360     *      380     *      400     *      420     *      440     *      460
Pkhd1_WT : CTGAATAGAAGTGGACACGTTGAACCCAGAAACTCTGGTAATAGGGCCCACTGATCCGTGCTTCATCTGGCCAGTGGATTGTATGTACACCCTAAAGTCTGCTTTAAAGTATTGGATGTGCTACCGTATTGTACATCACCTTCACAACCGACATGT
Pkhd1_T37M : CTGAATAGAAGTGGACACGTTGAACCCAGAAACTCTGGTAATAGGGCCCACTGATCCGTGCTTCATCTGGCCAGTGGATTGTATGTACACCCTAAAGTCTGCTTTAAAGTATTGGATGTGCTACCGTATTGTACATCACCTTCACAACCGACATGT
                *      480     *      500     *      520     *      540     *      560     *      580     *      600     *      620
Pkhd1_WT : TCTCTGATTGATAGAGCCCTTACTCGAGTTTCCAGATTGAACCCGACAGAGGTAGCCCTTGCAGG GGAAC TGGATCACAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGTTGAATTTATTTGATGTAATAATCCAAAGAATTATA
Pkhd1_T37M : TCTCTGATTGATAGAGCCCTTACTCGAGTTTCCAGATTGAACCCGACAGAGGTAGCCCTTGCAGG GGAAC TGGATCACAGTTGTATTTGACGGTAGGTTTGGGCTTTTCTGAATTTGGGTGTTGAATTTATTTGATGTAATAATCCAAAGAATTATA
                *      640     *      660     *      680     *      700     *      720     *      740     *      760     *
Pkhd1_WT : AATTCCTCGCTTCTTTGTTTAAAGAACAGATGAACAGAGACCCATCTCTGTCTTTTGCAGATGCTAAGTAGTCAGTGTACAGCAGAGGCCACTGTAGCATCAACATCCTCACCGTGGGATGCTTAGGAAGACGAAATAACATGGAAGTGGACAGCAT
Pkhd1_T37M : AATTCCTCGCTTCTTTGTTTAAAGAACAGATGAACAGAGACCCATCTCTGTCTTTTGCAGATGCTAAGTAGTCAGTGTACAGCAGAGGCCACTGTAGCATCAACATCCTCACCGTGGGATGCTTAGGAAGACGAAATAACATGGAAGTGGACAGCAT
                780     *      800     *      820     *      840     *      860     *      880     *      900     *      920     *
Pkhd1_WT : AGGAATACTACTCCCTCTTGGCAGTTGCCCTTACAAGTGATTTCTGTTAGGAGCCCTTAAACCATGCCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGTGGAGGAATGAAATGCTCTTTTCCCTAGGGAGTAGGCAGACATGAAGAGAGAGG
Pkhd1_T37M : AGGAATACTACTCCCTCTTGGCAGTTGCCCTTACAAGTGATTTCTGTTAGGAGCCCTTAAACCATGCCCTTACCATGCCCGTTTCCACCATAGCACAAATCAACGTGGAGGAATGAAATGCTCTTTTCCCTAGGGAGTAGGCAGACATGAAGAGAGAGG
                940     *      960     *      980     *      1000    *      1020    *
Pkhd1_WT : GGGGGGGGGAGAAAACATTTATAGGCTCATAGAAGCTTTTGTAAAACAGTGAATTTGCCTTTGGAAATGAGGATTAATTTTATCCTCAGGCAAACGCAATATCT
Pkhd1_T37M : GGGGGGGGGAGAAAACATTTATAGGCTCATAGAAGCTTTTGTAAAACAGTGAATTTGCCTTTGGAAATGAGGATTAATTTTATCCTCAGGCAAACGCAATATCT
                GGGGGGGGGAGAAAACATTTATAGGCTCATAGAAGCTTTTGTAAAACAGTGAATTTGCCTTTGGAAATGAGGATTAATTTTATCCTCAGGCAAACGCAATATCT

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Predicted Protein Alignment:

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                *      20
Pkhd1_WT : PYSSFQIEPAEGLAGG WITVVFD-
Pkhd1_T37M : PYSSFQIEPAEGLAGG WITVVFD-
                PYSSFQIEPAEGLAGG WITVVFD

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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.