

Name of Mouse model or mutation:**PDE2A-D479G-EM3-B6N****Description:**

Point mutation model made using CRISPR/Cas9.

Type of mutation:

SNP: D479G

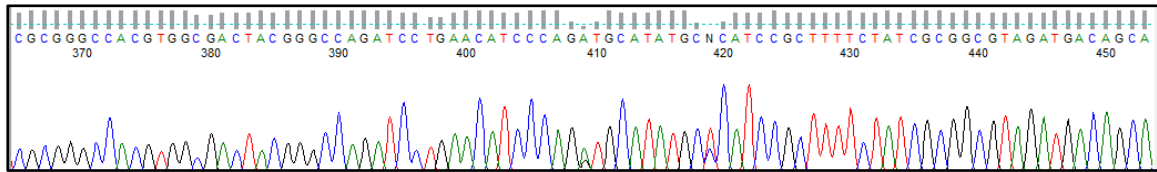
Sequence details**WT**

GTTTGG AATTGTCCAGCTCCGAGGAGAGTGTCTCACTTTAGGACACTTTCCAGGGTCTCAGTAATCG
CTACTAGCCATATGTGGTGGTTTCGAGAGGCGTCAGAACCCACGAACCAACCTCTTCTCCACA
GCTGCTCGGTGTTCTGCTGGATCAGAACGAGCTGGTGGCCAAGGTGTTTCGATGGTGGCGTTGTGG
ACGATGAGGTGAGGGAGTATGGAGGGTGTCCGGGAGTAAGCAAGAGGACCTGGGGGAAAAGCCA
CATTTGAAAAGTGTCTATACGGCAGGCTAACTTCTTCTTTCTATTCCATCCCTGTTTGGGCAGAGTT
ATGAGATCCGCATCCCGGCGGACCAAGGCATCGCGGGCCACGTGGCGACTACGGGCCAGATCCTG
AACATCCCAGATGCATATGCCATCCGCTTTTCTATCGCGGCGTAGATGACAGCACTGGCTTCCGCAC
GCGCAACATTCTGCTTCCCTATCAAGAACGAGAACCAGGGTGCCGACTCGTGGTGGCCAGGGGC
GGGGCCTGAAGGGGCTGTACGGGGGTGGAGCCGGGGCGGGGACTAGGGGAAAGTGGTTCTAGG
GTCGGAACCTAAAGAGCCAGGGATTGTCTAGTCTTGAGCCATTTCTTCCAGAGGAGTTAGGAGAAG
GCAGGTCTCACTGGACCCCTTACCAGCGATTCCCATTTTCTCTCTCTTCCCAGAGGTCATTGGTGTG
GCTGAGCTAG

Mutant

GTTTGG AATTGTCCAGCTCCGAGGAGAGTGTCTCACTTTAGGACACTTTCCAGGGTCTCAGTAATCG
CTACTAGCCATATGTGGTGGTTTCGAGAGGCGTCAGAACCCACGAACCAACCTCTTCTCCACA
GCTGCTCGGTGTTCTGCTGGATCAGAACGAGCTGGTGGCCAAGGTGTTTCGATGGTGGCGTTGTGG
ACGATGAGGTGAGGGAGTATGGAGGGTGTCCGGGAGTAAGCAAGAGGACCTGGGGGAAAAGCCA
CATTTGAAAAGTGTCTATACGGCAGGCTAACTTCTTCTTTCTATTCCATCCCTGTTTGGGCAGAGTT
ATGAGATCCGCATCCCGGCGGACCAAGGCATCGCGGGCCACGTGGCGACTACGGGCCAGATCCTG
AACATCCCAGGTCATATGCTCATCCGCTTTTCTATCGCGGCGTAGATGACAGCACTGGCTTCCGCAC
GCGCAACATTCTGCTTCCCTATCAAGAACGAGAACCAGGGTGCCGACTCGTGGTGGCCAGGGGC
GGGGCCTGAAGGGGCTGTACGGGGGTGGAGCCGGGGCGGGGACTAGGGGAAAGTGGTTCTAGG
GTCGGAACCTAAAGAGCCAGGGATTGTCTAGTCTTGAGCCATTTCTTCCAGAGGAGTTAGGAGAAG
GCAGGTCTCACTGGACCCCTTACCAGCGATTCCCATTTTCTCTCTCTTCCCAGAGGTCATTGGTGTG
GCTGAGCTAG

PDE2A-D479G-EM3-B6N Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

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                *      20      *      40      *      60      *      80      *      100     *
Pde2a_WT       : GTTTGGAATTGTCCAGCTCCGAGGAGAGTGTCTCACTTTAGGACACTTTCCAGGGTCTCAGTAATCGCTACACTAGCCATATGTGGTGGTTCGAGAGGCGTCAGAACCCC
Pde2a_D479G    : GTTTGGAATTGTCCAGCTCCGAGGAGAGTGTCTCACTTTAGGACACTTTCCAGGGTCTCAGTAATCGCTACACTAGCCATATGTGGTGGTTCGAGAGGCGTCAGAACCCC

                120     *      140     *      160     *      180     *      200     *      220
Pde2a_WT       : ACGAACCAACCTCTTCTCTCCACAGCTGCTCGGTGTTCTGCTGGATCAGAACGAGCTGGTGGCCAAGGTGTTTCGATGGTGGCGTTGTGGACGATGAGGTGAGGGAGTAT
Pde2a_D479G    : ACGAACCAACCTCTTCTCTCCACAGCTGCTCGGTGTTCTGCTGGATCAGAACGAGCTGGTGGCCAAGGTGTTTCGATGGTGGCGTTGTGGACGATGAGGTGAGGGAGTAT

                *      240     *      260     *      280     *      300     *      320     *
Pde2a_WT       : GGAGGGTGTCCGGGAGTAAGCAAGAGGACCTGGGGGAAAAGCCACATTTGAAAAGTGCTATACGGCAGGCTAACTTCTTGCTTTCTATTCCATCCCTGTTTGGGCAGAG
Pde2a_D479G    : GGAGGGTGTCCGGGAGTAAGCAAGAGGACCTGGGGGAAAAGCCACATTTGAAAAGTGCTATACGGCAGGCTAACTTCTTGCTTTCTATTCCATCCCTGTTTGGGCAGAG

                340     *      360     *      380     *      400     *      420     *      440
Pde2a_WT       : TTATGAGATCCGCATCCCGGGCGGACCAAGGCATCGCGGGCCACGTGGCGACTACGGGCCAGATCCTGAACATCCAGATGCATATGCCATCCGCTTTTCTATCGCGGCG
Pde2a_D479G    : TTATGAGATCCGCATCCCGGGCGGACCAAGGCATCGCGGGCCACGTGGCGACTACGGGCCAGATCCTGAACATCCAGATGCATATGCCATCCGCTTTTCTATCGCGGCG

                *      460     *      480     *      500     *      520     *      540     *
Pde2a_WT       : TAGATGACAGCACTGGCTTCCGCACGCGCAACATTCTCTGCTTCCCTATCAAGAACGAGAACCAGGGTGCCGACTCGTGGTGGCCAGGGGCGGGGCTGAAGGGCTGTA
Pde2a_D479G    : TAGATGACAGCACTGGCTTCCGCACGCGCAACATTCTCTGCTTCCCTATCAAGAACGAGAACCAGGGTGCCGACTCGTGGTGGCCAGGGGCGGGGCTGAAGGGCTGTA

                560     *      580     *      600     *      620     *      640     *      660
Pde2a_WT       : CGGGGGTGGAGCCGGGGCGGGGACTAGGGGAAAAGTGGTTCAGGGTCCGGAACCTAAAGAGCCAGGGATTGTCTAGTCTTGAGCCATTTCTTCCAGAGGAGTTAGGAGAAG
Pde2a_D479G    : CGGGGGTGGAGCCGGGGCGGGGACTAGGGGAAAAGTGGTTCAGGGTCCGGAACCTAAAGAGCCAGGGATTGTCTAGTCTTGAGCCATTTCTTCCAGAGGAGTTAGGAGAAG

                *      680     *      700     *      720     *
Pde2a_WT       : GCAGGTCTCACTGGACCCCTTACCAGCGATTCCCATTTTCTCTCTTCCCAGAGGTCATTGGTGTGGCTGAGCTAG
Pde2a_D479G    : GCAGGTCTCACTGGACCCCTTACCAGCGATTCCCATTTTCTCTCTTCCCAGAGGTCATTGGTGTGGCTGAGCTAG
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Predicted Protein Alignment:

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                *      20      *      40      *
Pde2a_WT       : SYEIRIPADQGIAGHVATTGQILNIP AYAHPLFYRGVDDSTGFRTRNILCFPIKNENQ
Pde2a_D479    : SYEIRIPADQGIAGHVATTGQILNIP AYAHPLFYRGVDDSTGFRTRNILCFPIKNENQ
SYEIRIPADQGIAGHVATTGQILNIP AYAHPLFYRGVDDSTGFRTRNILCFPIKNENQ
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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_ Pde2a_D479_F1 (5'-3')	GTTTGGAAATTGTCCAGCTCCG
Geno_ Pde2a_D479_R1 (5'-3')	CTAGCTCAGCCACACCAATGA
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	63
Elongation time (min)	1
WT product size (bp)	738
Mutant product size (bp)	738
Notes	Sequence with the following primers Geno_ Pde2a_D479_F2 (5'-3'): TTCCAGGGTCTCAGTAATCGC Geno_ Pde2a_D479_R2 (5'-3'): AATCGCTGGTGAAGGGGTC

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	Pde2a-D479G-UNIV1
Forward Primer	CGGGCCAGATCCTGAACATC
Reverse Primer	CGGAAGCCAGTGCTGTCA
Probe	CATCCGCTTTTCTATCGCGGCGTA
Label	FAM-BHQ1

Reference Assay Name	Dot1l
Forward primer	GCCCCAGCACGACCATT
Reverse primer	TAGTTGGCATCCTTATGCTTCATC
Probe	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

The ddPCR copy counting assay is universal to both the Pde2a WT allele and the engineered D479G allele. Therefore, both WT animals or correct mutants with a single integration for a correct mutation are expected to call at 2 copies for F1 (HET) animals. Animals with more than one donor integration are expected to call at 2+ copies.