Name of Mouse model or mutation:
ANXA11-D39G-EM1-B6

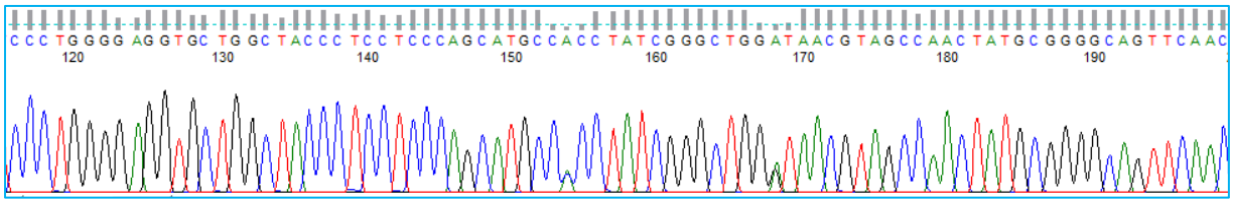
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: D39G

Sequence details
WT
TGGACCTGCCTTCTTCCTCTGCTTTCCCTGGCCCTCTTGTTCCATTCTTTCTGGGTCTTTATAACATTGG
CTGGATGCTGGTCTTCCTTGGTTATCACAGCAGCCCCTCTGCAGCCCTCTTTTCTGTTCTGTCAGTGG
GCCAGGGATACAGAGCTAATAATGGCTTGGTGTTGCTTTCAGGTGGTGGTCCCTGGGGAGGTGCTG
GCTACCCTCCTCCCAGCATGCCCCCTATCGGGCTGGATAACGTAGCCAACTATGCGGGGCAGTTCAA
CCAGGACTACCTCTCAGGCATGGTAAGTCAGGCCCCTGCCAAGGTATCCCTGGGCCAGGGCTATAG
GGATCAAGGCTGCTTACCCCAAGTTCTCAGGGAGACCTCATGCCTGTTTCTGTGAGCATAGAATGCT
GAGCAGCAACTTAGAATATGAGGTCATCTACTTAACACCCACCCTACTCTGGCTCCCCCCTCCCCCCA
TCTATATTAGAGGAAGAGATTCTTGGCACTGAAGGTCTCTCAGGTCCGAGGAGATTGGGTT

Mutant
TGGACCTGCCTTCTTCCTCTGCTTTCCCTGGCCCTCTTGTTCCATTCTTTCTGGGTCTTTATAACATTGG
CTGGATGCTGGTCTTCCTTGGTTATCACAGCAGCCCCTCTGCAGCCCTCTTTTCTGTTCTGTCAGTGG
GCCAGGGATACAGAGCTAATAATGGCTTGGTGTTGCTTTCAGGTGGTGGTCCCTGGGGAGGTGCTG
GCTACCCTCCTCCCAGCATGCC**A**CCTATCGGGCTGG**G**TAACGTAGCCAACTATGCGGGGCAGTTCAA
CCAGGACTACCTCTCAGGCATGGTAAGTCAGGCCCCTGCCAAGGTATCCCTGGGCCAGGGCTATAG
GGATCAAGGCTGCTTACCCCAAGTTCTCAGGGAGACCTCATGCCTGTTTCTGTGAGCATAGAATGCT
GAGCAGCAACTTAGAATATGAGGTCATCTACTTAACACCCACCCTACTCTGGCTCCCCCCTCCCCCCA
TCTATATTAGAGGAAGAGATTCTTGGCACTGAAGGTCTCTCAGGTCCGAGGAGATTGGGTT

ANXA11-D39G-EM1-B6 Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

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                    *        20         *        40         *        60         *        80         *       100         *       120
Anxa11_WT    : TGGACCTGCCTTCTTCCTCTGCTTTCCCTGGCCCTCTTGTTCCATTCTTTCTGGGTCTTTATAACATTGGCTGGATGCTGGTCTTCCTTGGTTATCACAGCAGCCCCTCTGCAGCCCTCTTTTCT
Anxa11_D39G  : TGGACCTGCCTTCTTCCTCTGCTTTCCCTGGCCCTCTTGTTCCATTCTTTCTGGGTCTTTATAACATTGGCTGGATGCTGGTCTTCCTTGGTTATCACAGCAGCCCCTCTGCAGCCCTCTTTTCT

                  *       140         *       160         *       180         *       200         *       220         *       240         *
Anxa11_WT    : GTTCTGTCAGTGGGCCAGGGATACAGAGCTAATAATGGCTTGGTGTTGCTTTCAGGTGGTGGTCCCTGGGGAGGTGCTGGCTACCCTCCTCCCAGCATGCCCCCTATCGGGCTGGATAACGTAGC
Anxa11_D39G  : GTTCTGTCAGTGGGCCAGGGATACAGAGCTAATAATGGCTTGGTGTTGCTTTCAGGTGGTGGTCCCTGGGGAGGTGCTGGCTACCCTCCTCCCAGCATGCCACCTATCGGGCTGGGTAACGTAGC

                260         *       280         *       300         *       320         *       340         *       360         *
Anxa11_WT    : CAACTATGCGGGGCAGTTCAACCAGGACTACCTCTCAGGCATGGTAAGTCAGGCCCCTGCCAAGGTATCCCTGGGCCAGGGCTATAGGGATCAAGGCTGCTTACCCCAAGTTCTCAGGGAGACCT
Anxa11_D39G  : CAACTATGCGGGGCAGTTCAACCAGGACTACCTCTCAGGCATGGTAAGTCAGGCCCCTGCCAAGGTATCCCTGGGCCAGGGCTATAGGGATCAAGGCTGCTTACCCCAAGTTCTCAGGGAGACCT

              380         *       400         *       420         *       440         *       460         *       480         *       500
Anxa11_WT    : CATGCCTGTTTCTGTGAGCATAGAATGCTGAGCAGCAACTTAGAATATGAGGTCATCTACTTAACACCCACCCTACTCTGGCTCCCCCCTCCCCCCATCTATATTAGAGGAAGAGATTCTTGGCA
Anxa11_D39G  : CATGCCTGTTTCTGTGAGCATAGAATGCTGAGCAGCAACTTAGAATATGAGGTCATCTACTTAACACCCACCCTACTCTGGCTCCCCCCTCCCCCCATCTATATTAGAGGAAGAGATTCTTGGCA

                    *       520         *
Anxa11_WT    : CTGAAGGTCTCTCAGGTCCGAGGAGATTGGGTT
Anxa11_D39G  : CTGAAGGTCTCTCAGGTCCGAGGAGATTGGGTT
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Predicted Protein Alignment:

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                    *        20         *
Anxa11_WT    : GGGPWGGAGYPPPSMPPIGLLNVANYAGQFNQDYLSGM
Anxa11_D39G  : GGGPWGGAGYPPPSMPPIGLGNVANYAGQFNQDYLSGM
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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_Anxa11_F1 primer (5'-3')	TGGACCTGCCTTCTTCCTCT
Geno_Anxa11_R1 primer (5'-3')	AACCCAATCTCCTCGGACCT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	64
Elongation time (min)	1
WT product size (bp)	533
Mutant product size (bp)	533
Notes	

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	ANXA11-D39G-UNI1
Forward Primer (5'-3')	GGGCAGTTCAACCAGGACTA
Reverse Primer (5'-3')	TGGCCCAGGGATACCTTG
Probe (5'-3')	CCTCTCAGGCATGGTAAGTCAGGC
Label	FAM-BHQ1

The ANXA11-D39G-UNI1 ddPCR assay is universal with both Anxa11 WT and Anxa11 D39G alleles recognised by this assay. Therefore, both 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.

Assay name	ANXA11-D39G-MUT1
Forward Primer (5’-3’)	CCACCTATCGGGCTGGG
Reverse Primer (5’-3’)	GCCTGACTTACCATGCCTGAG
Probe (5’-3’)	ACGTAGCCAACTATGCGGGGCA
Label	FAM-BHQ1

The ddPCR assay is specific to the oligo donor and so only Anxa11 D39G alleles are expected to be recognised by this assay. 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. Any animals carrying a random integration of the oligo donor will be detected by this assay (copy count >1).

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.