Name of Mouse model or mutation:

PLA2G6-R636Q-EM1-B6N
PLA2G6-R636Q-EM2-B6N
PLA2G6-R636Q-EM3-B6N

Description:

Point mutation model made using CRISPR/Cas9.

Type of mutation:

SNP: R636Q

Sequence details

WT

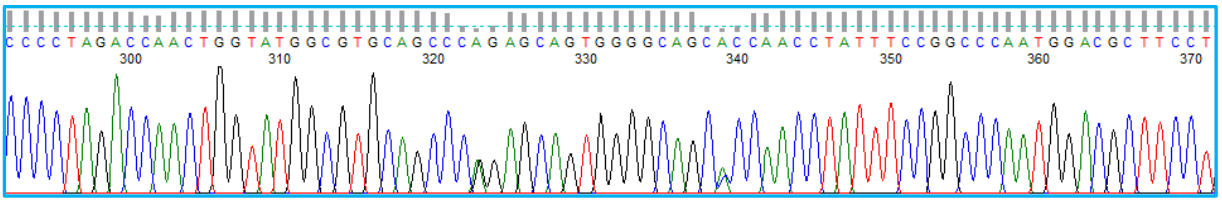
AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTCAGAGCTGAGCGCGTGTGCTGGGGGGGA
GGGCTGGAGGAAGTTGTGGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCCAT
GTCACTGCCCTGGCCTTAGGTGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCAC
CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC
TCCTACCCAGCCTGAGATCTGAAGGGATAGGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCAA
CTGGTATGGCGTGCAGCCCGGAGCAGTGGGGCAGCCCCAACCTATTTCCGGCCCAATGGACGCTTC
CTGGATGGAGGGCTGCTGGCCAACAACCCCACACTGGATGCCATGACTGAAATCCATGAGTACAAT
CAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGA
TGAGAACAGTTTTGGTGTGTGCCTTCACCTATTCAACACTCCTGGAGGGTCTGATGGGCAGAGTCCG
TGCTAGGAGAATTTTGAGGCCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGG
CTGCTGTTAGTACAGGCTGTGTACCTGATGCTACCCAGCCGGGACACACCCTTGCCCTGCAGAGCTT
ACGTTCCTACTTGAAGACTCTATACACACAACAGTCCTGGGCATGTCAGGTCTGAGAGCGCACCCTG
CGGAGGCAGGAGGGGGAGAGTCTGTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTG
GAACTATCCTGAGGCCTCTGGGTGCAAGACAGTGCAGAGGCAGGGAGTCAAGGACAGGCAGTGGC
CCTCACCCAAGTCGTCAGAGCAGGGTGACAAGGCCCACGTGCCAAGGGAGGTGATCTGACCCTGCC
TCAGCTTTCCAATCTTCAGCTAGTGACAGTGCTTGTCTTGATGCCCTGGGGACTCAGGGGGCTGTTG
CCTGTGCCATACTTGGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCCAGTCCAGTCATGGGG
AACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT

Mutant

AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTCAGAGCTGAGCGCGTGTGCTGGGGGGGA
GGGCTGGAGGAAGTTGTGGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCCAT
GTCACTGCCCTGGCCTTAGGTGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCAC
CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC
TCCTACCCAGCCTGAGATCTGAAGGGATAGGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCAA
CTGGTATGGCGTGCAGCCC**A**GAGCAGTGGGGCAGC**A**CCAACCTATTTCCGGCCCAATGGACGCTTC

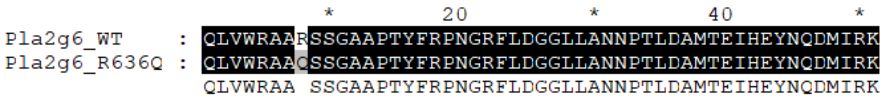
CTGGATGGAGGGCTGCTGGCCAACAACCCCACACTGGATGCCATGACTGAAATCCATGAGTACAAT
CAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGA
TGAGAACAGTTTTGGTGTGTGCCTTCACCTATTCAACACTCCTGGAGGGTCTGATGGGCAGAGTCCG
TGCTAGGAGAATTTTGAGGCCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGG
CTGCTGTTAGTACAGGCTGTGTACCTGATGCTACCCAGCCGGGACACACCCTTGCCCTGCAGAGCTT
ACGTTCCTACTTGAAGACTCTATACACACAACAGTCCTGGGCATGTCAGGTCTGAGAGCGCACCCTG
CGGAGGCAGGAGGGGGAGAGTCTGTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTG
GAACTATCCTGAGGCCTCTGGGTGCAAGACAGTGCAGAGGCAGGGAGTCAAGGACAGGCAGTGGC
CCTCACCCAAGTCGTCAGAGCAGGGTGACAAGGCCCACGTGCCAAGGGAGGTGATCTGACCCTGCC
TCAGCTTTCCAATCTTCAGCTAGTGACAGTGCTTGTCTTGATGCCCTGGGGACTCAGGGGGCTGTTG
CCTGTGCCATACTTGGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCCAGTCCAGTCATGGGG
AACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT

PLA2G6-R636Q-EM1-B6N 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_Pla2g6_F4 primer (5'-3')	AAACATTAACCTGAAGCCACCG
Geno_Pla2g6_R4 primer (5'-3')	ATGTCTGAGGCTGCTAGAACTC
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1191
Mutant product size (bp)	1191
Notes	Sequence with primers: Geno_Pla2g6_F2 primer (5'-3': GGAAGTTGTGGGGGATGGAG) and Geno_Pla2g6_R1 primer (5'-3': GGAGCCCTTCACCAGTTCTC)

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 by ddPCR

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	Pla2g6-R636Q-UNI1
Forward Primer (5'-3')	CACGCCATACCAGTTGGTCTA
Reverse Primer (5'-3')	TCCTACCCAGCCTGAGATC
Probe (5'-3')	TGAAGGGATAGGCCTTATGTCATTGTCC
Label	FAM-BHQ1

This ddPCR assay is universal to both the WT allele and the mutant allele of the gene. WT controls are expected to call at 2 copies and a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	Pla2g6-R636Q-MUT1
Forward Primer (5’-3’)	AGCAGCCCTCCATCCAGGAAG
Reverse Primer (5’-3’)	TGGCGTGCAGCCCA
Probe (5’-3’)	CCAACCTATTTCCGGCCCAATGGA
Label	FAM-BHQ1

This ddPCR assay is unique to the mutant allele of the gene as it sits across the mutated region. WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy 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.

PLA2G6-R636Q

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; R636Q in exon ENSMUSE00000261149 of *PLA2G6*. 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.

An Allelic Discrimination assay is used to detect two possible variants of a Single Nucleotide Polymorphism (SNP). It is a multiplexed assay (with two primer/probe pairs) with data being collected at the completion of the PCR process. The relative level of fluorescence from each probe is used to determine the genotype of an animal.

Samples for this line are genotyped using the following primers and probe:

- Forward and reverse primers common to both Wild Type (WT) and mutant alleles
- WT probe binding to the WT base mutated in the mutant allele.
- Mutant probe binding to the SNP.

PLA2G6-R636Q Allelic Discrimination assay

PLA2G6-R636Q WT sequence

CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC
TCCTACC**CAGCCTGAGATCTGAAGGGATA**GGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCA
ACTGGTATGGCGTGC**AGCCCgGAGCAGTG**GGGCAGCcCCA**ACCTATTTCCGGCCCAATGG**ACGCTT
CCTGGATGGAGGGCTGCTGGCCAACAACCCCACACTGGATGCCATGACTGAAATCCATGAGTACAA

PLA2G6-R636Q mutant sequence

CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC
TCCTACC**CAGCCTGAGATCTGAAGGGATA**GGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCA
ACTGGTATGGCG**TGCAGCCCaGAGCA**GTGGGGCAGCaCCA**ACCTATTTCCGGCCCAATGG**ACGCTT
CCTGGATGGAGGGCTGCTGGCCAACAACCCCACACTGGATGCCATGACTGAAATCCATGAGTACAA

SNP details:
WT=G
MUT=A

Lower case letters denote SNP position.
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**
PLA2G6-R636Q _F	n/a	**CAGCCTGAGATCTGAAGGGATA**	n/a	Common forward primer
PLA2G6-R636Q _WT_PROBE	FAM	**CACTGCTCCGGGCT**	BHQ-plus	Wild type Probe
PLA2G6-R636Q _Mutant_PROBE	TET	**TGCTCTGGGCTGCA**	BHQ-plus	Mutant probe
PLA2G6-R636Q _R	n/a	**CCATTGGGCCGGAAATAGGT**	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

ABI GTX Taqman master mix	5µl
Assay (Probes 5 µM each & Primers 15µM each)	2µl
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

qPCR cycling conditions

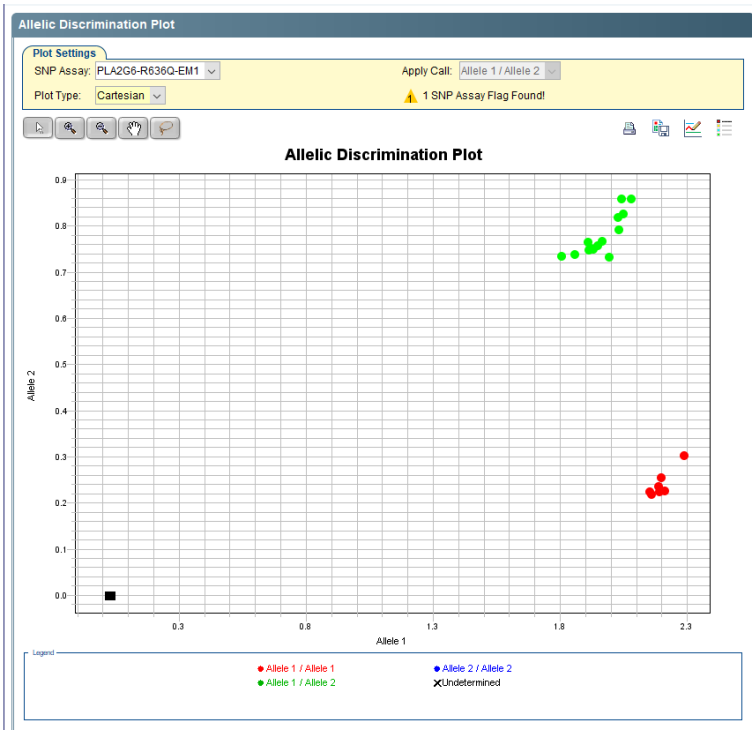
qPCR instrument: Applied Biosystems 7500

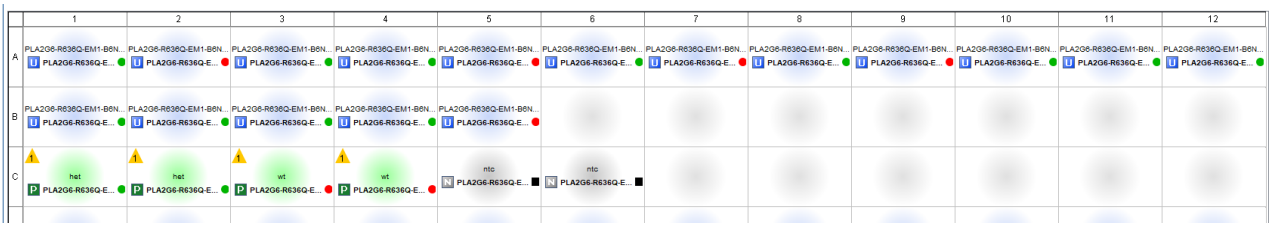
95^{o}C for 20 sec
Then 40 cycles of;
95^{o}C for 3 sec
60^{o}C for 30 sec

Analysis

The results are analysed using 7500 software v2.0.6 from Applied Biosystems

PLA2G6-R636Q Allelic Discrimination assay results (Task 294774 results)





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Created/Updated by: DF

Approved: Rumana Zaman