Name of Mouse model or mutation:
FLJ26957-DEL2061-EM2-B6

Description:
Deletion mutant made by CRISPR/Cas9 gene editing. Made as by-product whilst attempting to make a floxed allele.

Type of mutation:
Deletion of 2061 nt encompassing ENSMUSE00001291889.

Delivery method:
Pronuclear injection into 1-cell stage embryo

Genetic Background:
C57BL/B6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
AATTAAGTGCTATGGCAGAT	GGG
TAATTAAGTGCTATGGCAGA	TGG
TTACGTATTATCTAGGTGAG	TGG
TACGTATTATCTAGGTGAGT	GGG

lssDNA donor sequence (5'-3'):

```
LOCUS       FLJ26957              2878 bp    DNA     linear      03-APR-2020
FEATURES             Location/Qualifiers
     misc_feature    1..350
                     /note="350bp HA"
     PCR_primer      43..62
                     /note="Geno_FLJ26957_F3"
     misc_feature    380..413
                     /note="loxP"
     misc_feature    372..379
                     /note="AsiSI (SfaAI)"
     PCR_primer      351..371
                     /note="LoxPF"
```

misc_feature 414..2466
/note="Critical region"
exon 694..1976
/note="Exon 2: ENSMUSE00001291889 (from transcript
Trappc13-202 as mis annotated) (on reverse strand)"
misc_feature 2467..2500
/note="loxP"
misc_feature 2501..2508
/note="MreI"
PCR_primer complement(2509..2528)
/note="LoxPR"
misc_feature 2529..2878
/note="350bp HA"
source 1..2878
/dnas_title="FLJ26957 donor"
ORIGIN
1 TGAGGCCAGA AGAGACTGTC TGATGGAGCT GGTGTAATAG ACGGTCATGA ACCATCTTGT
61 GGGTAAGGGA ATTGAACTAG AGTCCTCTGG AAGTGTTCTT AACCACTGAG CCATCTCTCC
121 AGCCCCCACC CCCCAAAAAT TTTTTTTGAA ATATTACGTG ATGTGATACG AATTACCAAA
181 TTTTTGTTTC TTTTATGGTT TTAGGACTAT GAATACCAAA CATCTTCATG ATAATATTTC
241 AAAATCCTAT ATATCCACTT AGTTAAACAT GCTGGCTATC AGCATTTTCA GGCTAGATGT
301 AATCTTCATT CCAACCTCAT TCAGATACTT TATTGTGTGA GAAAGTTTTC atccgggggt
361 accgcgtcga gGCGATCGCA TAACTTCGTA TAGCATACAT TATACGAAGT TATATAGCAC
421 TTAATTAAAG AACCATATGC AAGCCGGGCG TGGTGGCGCA CGCCTTTAAT CCCAGCACTC
481 AGGAGGCAGA GGCAGGCGGA TTTCTGAGTT CAAGGCCAGC CTGGTCTACA AAGTGAGTTT
541 CAGGACAGCC AGGGCTACAG AGAAACCCTG TCTCGAAAAG ACCAAAAACA AACCCAAAAC
601 CCATATGCAG AATGGATTCT GATTCTCCTC CCTGCCCATC CGTGTGCAGG CTGTCCCTAC
661 CAAATCTTAC TGTTTGCAAT GCTTCTACTT TAGGTCCTGT TTCTTCTGAT TTGACAAGAA
721 AGACAATTTT GTAATAATAA ATTACATTCT AGCTCTGAAG AATTCAGCTA AGAAATTTTA
781 TCTTCATTAA GAGTTAATGG ATAGCTGCAG AATGACCACT GAAGTAATAT TACATTATCG
841 ACCATATGAA AACGATCCCA AACAGCTGGC AAAAATTGCA GAAAATGTAA TTCAAGACTT
901 TCCTACTCAC CCACTACCAA GATTTATTCC TTGGTTTCCA TACGATGAGT CCAAACTTCC
961 ACTCAAGCCT GAAAGATTAC CACCAGTAAT TTCTGAAGAG GCTGCTGAGA GCGTGAAGCA
1021 GTACTTAGCC ATCTCAGAAC CTGGTGTTAA ATCCCAGAGC TATGACTGCA CAGTAGATCT
1081 CTTGGAGTTT CAGCCTAGCT CAAAACTGCA GCACTTTATC CAATCACACA CAGTGAAGGA
1141 GCAGACCAAT GCGGCACATT TGGATAAAAA TTCAGGAAAA GAAAAACAGC ACAAGCAGAG
1201 ATCCTGGAGT GTTTCGCTTG CCAGCAGCCA CTGTCCAGAA AAGATCTTTC CTTTGTCTAG
1261 AAAATTGCAA GCTAGTTTAA GGACACTACA TTTGCACTCC TTTCATAGAG CAAGATGGAC
1321 TTTAGAATAC AGTGTTTGCA ACAACCAGAC TCTGGAAGAC ATTTGGACAA AACTCAATCG
1381 CCTTATCAGG CGCGATGAAC TCCCTTCTTG TAACGCTACC ATCCAGAGAC AGTTAGGCCA
1441 AATATGGGTG TTCTGTGATA TTAAGTGCTG TGAATATGTA GGAAATCTCC TTAAAGAAAG

1501 ATTATCTCTT ATTGGGAAAA TTGATTTATT TGTACACAAA TATGGTGTTA TTTTTAGTAT
1561 GTAATAAATT TTGATCATCA GGAAAAGAAT TCTGAATGAA ATGAATATGG ACTGAAATTG
1621 TGAAATGAAT AAATTTTATT GATCATTACA TTTTTAATGA CTTAAATTTT GATGCTTCTT
1681 TATAGTATGA GGAAATAATT GTGTAATATG GATACAGTTT GTGTCCAGTA TATAAATATC
1741 ACATAATTCC AAGGCTTGTC AGTCAATTCA GGAACATTCC AGATTATGTT TATTAGCCTT
1801 CTGTTAAGTG AGCTTTGATG AATTACTGTT TGTATTAAAA TTGAACGTAA GTGTTCTAAC
1861 CTTTATCCCA ATGATTTGAG TTTAAATGAA TGATTTTATT TTAGTGAATC CTTAAAAATC
1921 CTTATTCATT TAAATATAAT ATGAGCTGTG TTGTAAAATT AAAAGTCTTA TCAAAGTATG
1981 TCTGATTTTG TATTTTTCAT GATTGGTTGC CAATCAAGAG TTAAAAGTTT GGAAGTCATA
2041 TCACTTTTGT GATAGTTTTG TTATTTTAGA AGGATTATAA CAACTAAAAA GTGTAAGTTG
2101 CAATGAGATT ATAAACAGTT TTTAAATCCA AGAATTAAAA GGAGCGTCTA TGTTTTCATA
2161 TGTGGAAAAT TTGAAGTTTT CTGATGTATT CCACATGAAG TGCTACATCT CAGAATTCTC
2221 TGAGAAACAA TGAAAGTAAA AGCATAGGAA TTGGCAGCGG TGGTAGTGTT CTCCTTTAAT
2281 CCCAGCACTT GAGAAGCAGA GGCAGGTGGA TCTTTGTGAG TTCGAGGCCA GCCCGGTGTA
2341 CAGAGTGTGT ACCAAGACAG CCAGGGCTGC ACAGAGAACC CCTGTTTCAA AAAAACAGAA
2401 AAAGTTAAAA GTTTTTTGCT TCAAATAGTA TAGTGATTCT AAAGACAGAG TTCATTTCCC
2461 ACTCACATAA CTTCGTATAG CATACATTAT ACGAAGTTAT CGCCGGCGgg tctgagctcg
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2641 CACATGCCAC CGTGCATATG TGGAGGTCAG AGAAGGGCCT GAAGGGATTG GTTGGTTATT
2701 TCTTCCCACC TTGTAAGTCC TGGGAATTGA ACTAAGGTCA CCGTGTGCTT TTATCCACTG
2761 AACTCCTTAT CTAGCCCTCT AGAAGCCTTT TAAGATGTCA GATCTTTGAA CGTAGTGCTA
2821 CATGTGGATC CCTGGGGCTG GGTGGCTCGC TATTGGCTGC TACCTTCAGC CTCCCTCC
//

Microinjection mixes:

Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 nm filter and autoclaved. Cas9 mRNA, sgRNAs and lssDNA donor were diluted and mixed in MIB to the working concentrations of 100 ng/µl, 50 ng/µl and 50 ng/µl respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

WT

TGCTGGAACTTCTGGTCACTGCTTTAATTTGGGAAATCACGAGAACTTCTGTGTTGTGCTCTTATTCT
ACATTGGCGTCCAGTAAACTAACATCTTTTTTTTTTTTTTTTTTTTTTTGGTTTTCGAGACAGGGTTTC
TCTGTATAGCCCTGGCTGACCAGGAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCG
CCTGCCTCTGCCTCCCGAGTACTGGGATTTTTTTTAAAACTGTTTTTTAGAAGACAACTTCTATTTACA
TGGATGTCTTACCTACATGTTTGTCTGTACAATGTATTATGTATGTATACCGCGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTATGTGTTTCCCATTGAGGCCAGAAGAGACTGTCTGATGGAGCTGGTGTAA
TAGACGGTCATGAACCATCTTGTGGGTAAGGGAATTGAACTAGAGTCCTCTGGAAGTGTTCTTAACC

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AATCCTATATATCCACTTAGTTAAACATGCTGGCTATCAGCATTTTCAGGCTAGATGTAATCTTCATTC
CAACCTCATTCAGATACTTTATTGTGTGAGAAAGTTTTCAAGTGTGGGATTATAGTCCCATCTGCCAT
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TGCTGGAACTTCTGGTCACTGCTTTAATTTGGGAAATCACGAGAACTTCTGTGTTGTGCTCTTATTCT
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el]**TAGATAATACGTAAGGCATTATCAAGATCTGACAACTTAAAACCCACTGTTAGTTCCTAGAAGTCT
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CCTAGGCACCTCAGGCATCAGCTACCTTGGTGTGTTTTTGTTTTGTTTGCTTTGCTTTTATGGAGACA
GAGTCTCTTACCTGAAGCTTGTCGTGTAGGCTGGGTAA

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_FLJ26957_F1 (5' to 3')	TGCTGGAACTTCTGGTCACTG
Geno_FLJ26957_R1 (5' to 3')	TTACCCAGCCTACACGACAAG
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	4
WT product size (bp)	3650
Mutant product size (bp)	1596
Notes	Sequenced using Geno_FLJ26957_F1 (5' to 3': GGTCATGAACCATCTTGTGG)

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 deleted sequence was carried out by ddPCR at the F1 stage to confirm the fragment had not inserted randomly into the genome. The following Taqman assay was used to copy count the deleted sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	UPL66_1
Forward Primer (5’-3’)	AAGCCTGAAAGATTACCACCAG
Reverse Primer (5’-3’)	GATGGCTAAGTACTGCTTCACG
Probe (5’-3’)	UPL66 (GGCTGCTG)
Label	FAM

The ddPCR assay sits within the region to be deleted. WT controls are expected to call at 2 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

We also checked that the donor used had not been inserted randomly into the genome using the following two mutant assays. No evidence of the floxed donor was found in the genome of these animals.

Assay name	FLJ26957-FLOX-5'MUT1
Forward Primer (5’-3’)	GCTATCAGCATTTTCAGGCTAGATG
Reverse Primer (5’-3’)	GCTTGCATATGGTTCTTTAATTAAGTGC
Probe (5’-3’)	TCGAGGCGATCGCATAACTTCG
Label	FAM

The ddPCR assay is unique to the Floxed allele of the gene as it sits over the 5' LoxP site. WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	Flj26957-FLOX-3’MUT1
Forward Primer (5’-3’)	CCCACTCACATAACTTCGTATAGCA
Reverse Primer (5’-3’)	CAGTGGGTTTTAAGTTGTCAGATCTTG
Probe (5’-3’)	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

The ddPCR assay is unique to the Floxed allele of the gene as it sits over the 3' LoxP site. 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.

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Allele Description

This is a CRISPR/Cas9 induced mutation deleting 2061 nucleotides of the *Flj26957* gene. 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 probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant specific assay with the forward primer binding at the break point

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

FLJ26957-DEL2061-WT1 assay (FAM labelled)

AAAATCCTATATATCCACTTAGTTAAACATGCTGGCTATCAGCATTTTCAGGCTAGATGTAATCTTCAT
TCCAACCTCATTCAGATACTTTATTGTGTGAGAAAGTTTTCAA**GTGTGGGATTATAGTCCCATCTG**CC
ATAGCACTTAATT**AAAGAACCATATGCAAGCCGGGC**GTGGTGGCGCACGCC**TTTAATCCCAGCACT**
CAGGAGGCAGAGGCAGGCGGATTTCTGAGTTCAAGGCCAGCCTGGTCTACAAAGTGAGTTTCAGG
ACAGCCAGGGCTACAGAGAAACCCTGTCTCGA

Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo FLJ26957-DEL2061	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
FLJ26957-DEL2061-WT_F	n/a	**GTGTGGGATTATAGTCCCATCTG**	n/a	Wild type Forward
FLJ26957-DEL2061-WT_PROBE	FAM	**AAAGAACCATATGCAAGCCGGGC**	ZEN/IBFQ	Wild type Probe
FLJ26957-DEL2061-WT_R	n/a	**CTCCTGAGTGCTGGGATTAAAG**	n/a	Wild type Reverse

FLJ26957-DEL2061-MUT1 assay (FAM labelled)

AAAATCCTATATATCCACTTAGTTAAACATGCTGGCTATCAGCATTTTCAGGCTAGATGTAATCTTCAT
TCCAACCTCATTCAGATACTTTATTGTGTGAGAAAGTTTT**CAAGTGTGGGATTATAGTCCCTAG**ATAA
TACGTAAGGCATTATCAAGATCTGACAACTTAAA**ACCCACTGTTAGTTCCTAGAAGTCTTTGGT**TTTT
AGTTACATGTAT**GTATTGTGTGTTTGCACGTGA**ACACACATGCCACCGTGCATATGTGGAGGTCAGA
GAAGGGCCTGAAGGGATTGGTTGGTTAT

Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo FLJ26957-DEL2061	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
FLJ26957-DEL2061-MUT_F	n/a	**CAAGTGTGGGATTATAGTCCCTAG**	n/a	Mutant Forward
FLJ26957-DEL2061-MUT_PROBE	FAM	**ACCCACTGTTAGTTCCTAGAAGTCTTTGGT**	ZEN/IBFQ	Mutant Probe
FLJ26957-DEL2061-MUT_R	n/a	**TCACGTGCAAACACACAATAC**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCCTATGA

Oligo Rabep2-S187AS191A	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

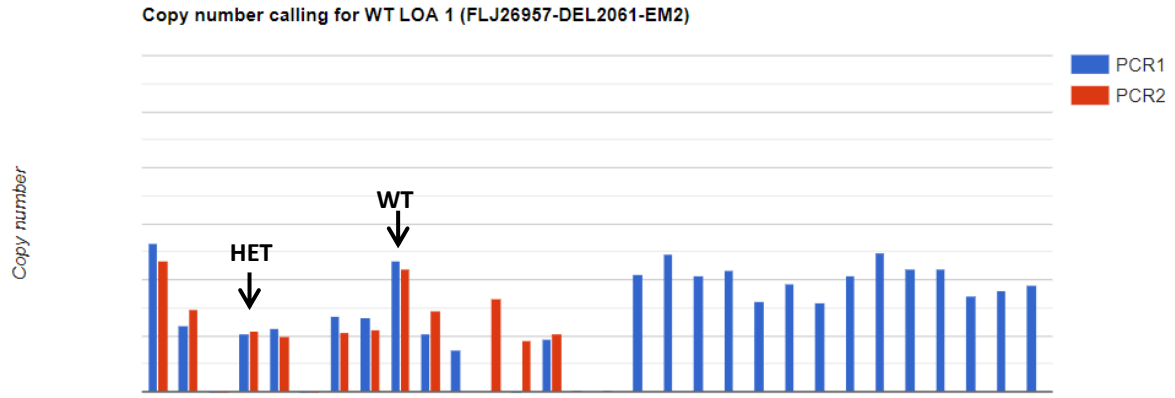
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

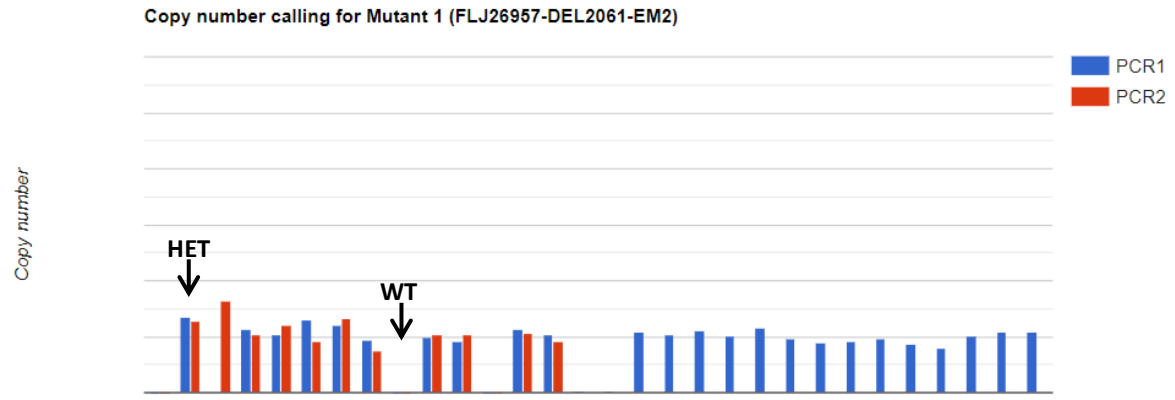
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Example of FLJ26957-DEL2061-WT1 and FLJ26957-DEL2061-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 312914 results).





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