

Name of Mouse model or mutation:

FLJ26957-DEL2054-EM1-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 2054 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

IssDNA 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="Mrel"
 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 ATTGAAGTAG AGTCCTCTGG AAGTGTCTT AACCACTGAG CCATCTCTCC
 121 AGCCCCCACC CCCCAAAAAT TTTTTTGGAA ATATTACGTG ATGTGATACG AATTACCAAA
 181 TTTTTGTTTC TTTTATGGTT TTAGGACTAT GAATACCAAA CATCTTCATG ATAATATTTT
 241 AAAATCCTAT ATATCCAATT AGTTAAACAT GCTGGCTATC AGCATTTCAT GGCTAGATGT
 301 AATCTTCATT CCAACCTCAT TCAGATACTT TATTGTGTGA GAAAGTTTTT atccgggggt
 361 accgcgtcga gGCGATCGCA TAACTTCGTA TAGCATAACAT 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 GATTCTCTC CCTGCCATC CGTGTGCAGG CTGTCCCTAC
 661 CAAATCTTAC TGTTTGCAAT GCTTCTACTT TAGGTCCTGT TTCTTCTGAT TTGACAAGAA
 721 AGACAATTTT GTAATAATAA ATTACATTCT AGCTCTGAAG AATTCAGCTA AGAAATTTTA
 781 TCTTCATTA GAGTTAATGG ATAGCTGCAG AATGACCACT GAAGTAATAT TACATTATCG
 841 ACCATATGAA AACGATCCCA AACAGCTGGC AAAAATTGCA GAAAATGTAA TTCAAGACTT
 901 TCCTACTCAC CCACTACCAA GATTTATTCC TTGGTTTCCA TACGATGAGT CCAAACCTCC
 961 ACTCAAGCCT GAAAGATTAC CACCAGTAAT TTCTGAAGAG GCTGCTGAGA GCGTGAAGCA
 1021 GTAAGTAGCC ATCTCAGAAC CTGGTGTTAA ATCCCAGAGC TATGACTGCA CAGTAGATCT
 1081 CTTGGAGTTT CAGCCTAGCT CAAAACCTGCA GCACTTTATC CAATCACACA CAGTGAAGGA
 1141 GCAGACCAAT GCGGCACATT TGGATAAAAA TTCAGGAAAA GAAAACAGC 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 TTTTATAGTAT
1561 GTAATAAATT TTGATCATCA GGAAAAGAAT TCTGAATGAA ATGAATATGG ACTGAAATTG
1621 TGAAATGAAT AAATTTTATT GATCATTACA TTTTAAATGA CTTAAATTTT GATGCTTCTT
1681 TATAGTATGA GGAAATAATT GTGTAATATG GATACAGTTT GTGTCCAGTA TATAAATATC
1741 ACATAATTCC AAGGCTTGTC AGTCAATTCA GGAACATTCC AGATTATGTT TATTAGCCTT
1801 CTGTAAAGTG AGCTTTGATG AATTACTGTT TGTATTAATAA TTGAACGTAA GTGTTCTAAC
1861 CTTTATCCCA ATGATTTGAG TTAAATGAA TGATTTTATT TTAGTGAATC CTAAAAATC
1921 CTTATTCATT TAAATATAAT ATGAGCTGTG TTGTAAAATT AAAAGTCTTA TCAAAGTATG
1981 TCTGATTTTG TATTTTTCAT GATTGGTTGC CAATCAAGAG TAAAAGTTT GGAAGTCATA
2041 TCACTTTTGT GATAGTTTGT TTATTTTAGA AGGATTATAA CAACTAAAAA GTGTAAGTTG
2101 CAATGAGATT ATAAACAGTT TTAAATCCA AGAATTAATAA GGAGCGTCTA TGTTTTTATA
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 CATAATTAT ACGAAGTTAT CGCCGGCGgg tctgagctcg
2521 ccatcagtCT AGATAATACG TAAGGCATTA TCAAGATCTG ACAACTTAAA ACCCACTGTT
2581 AGTTCCTAGA AGTCTTTGGT TTTTAGTTAC ATGTATGTAT TGTGTGTTTG CACGTGAACA
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 CTCCTCC

//

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/ μ l, 50 ng/ μ l and 50 ng/ μ l respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F₀ progeny.

Sequence details

WT

TGCTGGAACCTCTGGTCACTGCTTTAATTTGGGAAATCACGAGAACTTCTGTGTTGTGCTCTTATTCT
ACATTGGCGTCCAGTAAACTAACATCTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTTTTCGAGACAGGGTTTC
TCTGTATAGCCCTGGCTGACCAGGAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCG
CCTGCCTCTGCCTCCCGAGTACTGGGATTTTTTTTAAACTGTTTTTTAGAAGACAACCTTCTATTTACA
TGGATGTCTTACCTACATGTTTGTCTGTACAATGTATTATGTATGTATACCGCGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTATGTGTTTCCATTGAGGCCAGAAGAGACTGTCTGATGGAGCTGGTGTA
TAGACGGTCATGAACCATCTTGTGGGTAAGGGAATTGAACTAGAGTCCTCTGGAAGTGTCTTAACC

ACTGAGCCATCTCTCCAGCCCCACCCCCAAAAATTTTTTTTGAATATTACGTGATGTGATACGAA
TTACCAAATTTTTGTTTCTTTTATGGTTTTAGGACTATGAATACCAAACATCTTCATGATAATATTTCAA
AATCCTATATATCCACTTAGTTAAACATGCTGGCTATCAGCATTTTCAGGCTAGATGTAATCTTCATTC
CAACCTCATTAGATACTTTATTGTGTGAGAAAGTTTTCAAGTGTGGGATTATAGTCCCATCTGCCAT
AGCACTTAATTAAGAACCATATGCAAGCCGGCGTGGTGGCGCACGCCTTTAATCCAGCACTCAG
GAGGCAGAGGCAGGCGGATTTCTGAGTTCAAGGCCAGCCTGGTCTACAAAGTGAGTTTCAGGACA
GCCAGGGCTACAGAGAAACCCTGTCTCGAAAAGACAAAAACAAACCCAAAACCCATATGCAGAAT
GGATTCTGATTCTCCTCCCTGCCATCCGTGTGCAGGCTGTCCCTACCAAATCTTACTGTTTGAATGC
TTCTACTTTAGGTCTGTTTCTTCTGATTTGACAAGAAAGACAATTTTGTAAATAATAATTACATTCTA
GCTCTGAAGAATTCAGCTAAGAAATTTTATCTTCATTAAGAGTTAATGGATAGCTGCAGAATGACCA
CTGAAGTAATATTACATTATCGACCATATGAAAACGATCCCAAACAGCTGGCAAAAATTGCAGAAAA
TGTAATCAAGACTTTCCTACTCACCCACTACCAAGATTTATTCCTTGGTTTCCATACGATGAGTCCAA
ACTTCCACTCAAGCCTGAAAGATTACCACCAGTAATTTCTGAAGAGGCTGCTGAGAGCGTGAAGCAG
TACTTAGCCATCTCAGAACCTGGTGTAAATCCAGAGCTATGACTGCACAGTAGATCTCTTGGAGTT
TCAGCCTAGCTCAAACTGCAGCACTTTATCCAATCACACACAGTGAAGGAGCAGACCAATGCGGCA
CATTGGATAAAAATTCAGGAAAAGAAAACAGCACAAGCAGAGATCCTGGAGTGTTTCGCTTGCC
AGCAGCCACTGTCCAGAAAAGATCTTTCCTTTGTCTAGAAAATTGCAAGCTAGTTTAAGGACACTAC
ATTTGCACTCCTTTCATAGAGCAAGATGGACTTTAGAATACAGTGTTTGCAACAACCAGACTCTGGA
AGACATTTGGACAAAACCTCAATCGCCTTATCAGGCGCGATGAACTCCCTTCTTGTAAACGCTACCATCC
AGAGACAGTTAGGCCAAATATGGGTGTTCTGTGATATTAAGTGCTGTGAATATGTAGGAAATCTCCT
TAAAGAAAGATTATCTTATTGGGAAAATTGATTTATTTGTACACAAATATGGTGTATTTTTAGTAT
GTAATAAATTTGATCATCAGGAAAAGAATTCTGAATGAAATGAATATGGACTGAAATTGTGAAATG
AATAAATTTTATTGATCATTACATTTTAAATGACTTAAATTTTGTGCTTCTTTATAGTATGAGGAAAT
AATTGTGTAATATGGATACAGTTTGTGTCCAGTATATAAATATCACATAATCCAAGGCTTGTGAGTC
AATTCAGGAACATTCCAGATTATGTTTATTAGCCTTCTGTTAAGTGAGCTTTGATGAATTAATGTTTGT
ATTTAAATTTGAACGTAAGTGTCTAACCTTTATCCCAATGATTTGAGTTTAAATGAATGATTTTATTTT
AGTGAATCCTTAAAAATCCTTATTCATTTAAATATAATATGAGCTGTGTTGTAAAATTTAAAGTCTTAT
CAAAGTATGTCTGATTTTGTATTTTTCATGATTGGTTGCCAATCAAGAGTTAAAAGTTTGGAAAGTCAT
ATCACTTTTGTGATAGTTTTGTTATTTTAGAAGGATTATAACAACCTAAAAAGTGTAAGTTGCAATGAG
ATTATAAACAGTTTTTAAATCCAAGAATTAAGGAGGAGCGTCTATGTTTTCATATGTGGAAAATTTGAA
GTTTTCTGATGTATTCCACATGAAGTGCTACATCTCAGAATTCTCTGAGAAACAATGAAAGTAAAAGC
ATAGGAATTGGCAGCGGTGGTAGTGTTCTCCTTAAATCCCAGCACTTGAGAAGCAGAGGCAGGTGG
ATCTTTGTGAGTTGAGGCCAGCCCGGTGTACAGAGTGTGTACCAAGACAGCCAGGGCTGCACAGA
GAACCCCTGTTTCAAAAAACAGAAAAAGTTAAAAGTTTTTTGCTTCAAATAGTATAGTGATTCTAAA
GACAGAGTTCAATTCCTACTCACCTAGATAATACGTAAGGCATTATCAAGATCTGACAACCTAAAACC
CACTGTTAGTTCCTAGAAGTCTTTGGTTTTTGTGTTACATGTATGTATTGTGTGTTTGCACGTGAACACA
CATGCCACCGTGCATATGTGGAGGTCAGAGAAGGGCCTGAAGGGATTGGTTGGTTATTTCTTCCCAC
CTTGTAAGTCTGGGAATTGAACTAAGGTCACCGTGTGCTTTTATCCACTGAACTCCTTATCTAGCCC
TCTAGAAGCCTTTAAGATGTCAGATCTTTGAACGTAGTGCTACATGTGGATCCCTGGGGCTGGGTG
GCTCGCTATTGGCTGCTACCTTCAGCCTCCCTCCCCTTCAAGATCCCCTGTTTAAATCACACATAGAA
CATGCTAGGAAAACAAAATAAGTGTGGAAAGATAAATCAATGAGAGCCTTTGAAAAAATGCAGCC

AGCACTTACTCTCTAGGAAAGAGGCAGACAGGAGATACACAGAGAAAGCTAAGATGCAGAGGCAT
CCAAGTGATAAGCTGTGGTTTGTGTTGAACTTTGACCTGGTCTTAAACAAATCCAACCTTTATTGCCCAA
CTTAACAAATAATAGGTATGGGTTTTGTGGTTTTGGATTGTTGAACCAATAACTTACTTTACCATT
AAGCATAGATATAAGATTACTACCTAATACCTAGTACTTAGTTTTAGTTTCTCTGTCAATGTTTCTGT
GTATGCACATACAGAGGCCAGCCTAGGCACCTCAGGCATCAGCTACCTTGGTGTGTTTTGTTTTGT
TGCTTTGCTTTTATGGAGACAGAGTCTTTACCTGAAGCTTGTCGTGTAGGCTGGGTAA

FLJ26957-FLOX-EM1-B6

TGCTGGAACCTTCTGGTCACTGCTTTAATTTGGGAAATCACGAGAACTTCTGTGTTGTGCTCTTATTCT
ACATTGGCGTCCAGTAACTAACATCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTTTTTCGAGACAGGGTTTC
TCTGTATAGCCCTGGCTGACCAGGAACTCACTCTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCG
CCTGCCTCTGCCTCCCGAGTACTGGGATTTTTTTTTAAAAGTTTTTTTAGAAAGACAACCTTCTATTTACA
TGGATGTCTTACCTACATGTTTGTCTGTACAATGTATTATGTATGTATAACCGCGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTATGTGTTTCCATTGAGGCCAGAAGAGACTGTCTGATGGAGCTGGTGTA
TAGACGGTCATGAACCATCTTGTGGGTAAGGGAATTGAACTAGAGTCCTCTGGAAGTGTCTTAAAC
ACTGAGCCATCTCTCCAGCCCCACCCCCAAAAATTTTTTTTGAATATTACGTGATGTGATACGAA
TTACCAAATTTTTGTTTCTTTTATGGTTTTAGGACTATGAATACCAAACATCTTCATGATAATATTTCAA
AATCCTATATATCCACTTAGTTAAACATGCTGGCTATCAGCATTTTCAGGCTAGATGTAATCTTCATTC
CAACCTCATTGATACTTTATTGTGTGAGAAAGTTTTCAAGTGTGGGATTATAGTCCCATC[2054_n
t_del]CACCTAGATAATACGTAAGGCATTATCAAGATCTGACAACTTAAACCCACTGTTAGTTCCTA
GAAGTCTTTGGTTTTTAGTTACATGTATGTATTGTGTGTTTGCACGTGAACACACATGCCACCGTGCA
TATGTGGAGGTCAGAGAAGGGCCTGAAGGGATTGGTTGGTTATTTCTTCCCACCTTGTAAGTCCTGG
GAATTGAACTAAGGTCACCGTGTGCTTTTATCCACTGAACTCCTTATCTAGCCCTCTAGAAGCCTTTTA
AGATGTCAGATCTTTGAACGTAGTGCTACATGTGGATCCCTGGGGCTGGGTGGCTCGCTATTGGCTG
CTACCTCAGCCTCCCTCCCCTTCAAGATCCCACTGTTAATCACACATAGAACATGCTAGGAAAAC
AAAATAAGTGTGGAAAGATAAATCAATGAGAGCCTTTGAAAAAATGCAGCCAGCACTTACTCTCTA
GGAAAGAGGCAGACAGGAGATACACAGAGAAAGCTAAGATGCAGAGGCATCCAAGTGATAAGCT
GTGGTTTTGTTTGAACCTTTGACCTGGTCTTAAACAAATCCAACCTTTATTGCCCACTTAAACAAATAA
GGTATGGGTTTTGTGGTTTTGGATTGTTGAACCAATAACTTACTTTACCATTCAAGCATAGATATAA
GATTACTACCTAATACCTAGTACTTAGTTTTAGTTTCTCTGTCAATGTTTCTTGTGTATGCACATACAG
AGGCCAGCCTAGGCACCTCAGGCATCAGCTACCTTGGTGTGTTTTGTTTTGTTTGTGCTTTGCTTTTATG
GAGACAGAGTCTTTACCTGAAGCTTGTCGTGTAGGCTGGGTAA

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')	TGCTGGAAGTCTGGTCACTG
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.



Allele Description

This is a CRISPR/Cas9 induced mutation deleting 2054 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 wildtype loss of allele (WT-LOA) 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:

- Wildtype specific assay situated within the deleted region.
- 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-DEL2054-WT1 assay (FAM labelled)

TGCACTCCTTTCATAGAGCAAGATGGACTTTAGAATACAGTGTGGCAACAACCAGACTCTGGAAGA
 CATTGGACAAAACCTCAATCG**CCTTATCAGGCGCGATGAACTCCCTTCTTGTAACGCTACCATCCAG**
 AGACAGTTAGGCCAAATATGGGTGTTCTGTGATATTAAGTG

Lower case letters denote the deleted sequence in the mutant allele.

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Flj26957- DEL2054	5' label	Sequence 5' → 3'	3' label	Oligo Type
Flj26957- DEL2054- WT_F	n/a	<u>GCACTCCTTTCATAGAGCAAGA</u>	n/a	Wildtype Forward
Flj26957- DEL2054- WT_PROBE	FAM	CCTTATCAGGCGCGATGAACTCCC	ZEN- IBFQ	Wildtype Probe
Flj26957- DEL2054- WT_R	n/a	<u>CTGGATGGTAGCGTTACAAGAA</u>	n/a	Wildtype Reverse

Flj26957-DEL2054-MUT1 assay (FAM labelled)

CAACCTCATTAGATACTTTATTGTGTGAGAAAGTTTTCAAGT**GTGGGATTATAGTCCCATCCACCTA**
 GATAATACGTAAGGCATTATCAAGATCTGACAACTTAAA**ACCCACTGTTAGTTCCTAGAAGTCTTTG**
GTTTTTAGTTACATGTAT**GTATTGTGTGTTTGCACGTGA**ACACACATGCCACCGTGCATATGTGGAG

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Flj26957- DEL2054	5' label	Sequence 5' → 3'	3' label	Oligo Type
Flj26957- DEL2054- MUT_F	n/a	<u>GTGGGATTATAGTCCCATCCAC</u>	n/a	Mutant Forward
Flj26957- DEL2054- MUT_PROBE	FAM	ACCCACTGTTAGTTCCTAGAAGTCTTTGGT	ZEN- IBFQ	Mutant Probe
Flj26957- DEL2054- MUT_R	n/a	<u>TCACGTGCAAACACACAATAC</u>	n/a	Mutant Reverse



Dot1l internal control (VIC labelled)

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

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

Oligo Flj26957-DEL2054	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	<u>GCCCCAGCACGACCATT</u>	n/a	WT Forward
Dot1l_Probe	VIC	CCAGCTCTCAAGTCG	BHQ	WT Probe
Dot1l_Reverse	n/a	<u>TAGTTGGCATCCTTATGCTTCATC</u>	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

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 5µM & primers 15µM each)	0.3 µl
ddH2O	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. Non-template controls are also run.

qPCR cycling conditions

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

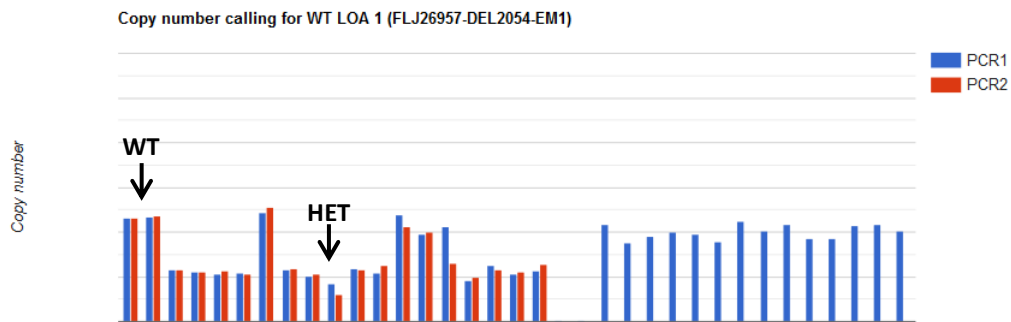
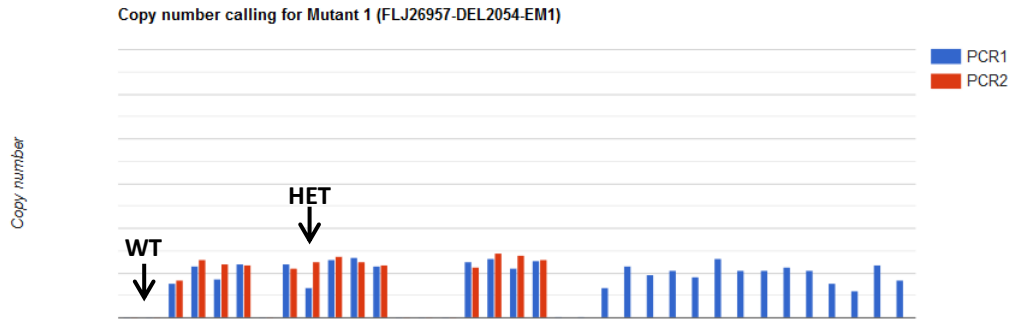
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec



Analysis

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

Flj26957-DEL2054-WT1 and Flj26957-DEL2054-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 296542 results)



Version No. 1

Date: 16/06/2020

Created/Updated by: Daniel Ford

Approved by: AC 16/10/2020