6430573F11RIK-FLOX-EM2-B6N

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00001075283 of *6430573F11Rik*. The stock was generated at MRC Harwell via pronuclear injection 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:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that binds to the inserted LoxP sequence

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

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6430573F11RIK-FLOX3'-WT1 assay (FAM labelled)

TAGAGGTCTATGCAATGCATTTTGTCTAAATTTAAAATATACACACTCTGTCGCATGACTTATTT**GACTCCTGCC**
TAATACAGTTATGATATGT**AAGCGGTTTTAACTTTCAACTTTCAGGC**TGTTATTGTGTTATTTttgatgatttgttcc**c**
atgtgtttcgctacatgcaagaagggtgtcggaGCGCCTACTCATTGAAAATGTACTTTGCTATATTGAGATCAGGTCAGA
TCCTCCTGACACAAAGGGGATTATTCAGGTTTCATGTTAACAAAAACAGACTACAGCAGTGTCCTTGAATTTCA
ACAGAAAATTC

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
6430573F11RIK-FLOX3'-UNI_F	n/a	**GACTCCTGCCTAATACAGTTATGATATG**	n/a	Universal Forward
6430573F11RIK-FLOX3'-UNI_PROBE	FAM	**AAGCGGTTTTAACTTTCAACTTTCAGGC**	BHQ	Universal Probe
6430573F11RIK-FLOX3'-WT_R	n/a	**tcttgcatgtagcgaaacacatg**	n/a	WT Reverse

6430573F11RIK-FLOX3'-MUT1 assay (FAM labelled)

TAGAGGTCTATGCAATGCATTTTGTCTAAATTTAAAATATACACACTCTGTCGCATGACTTATTT**GACTCCTGCC**
TAATACAGTTATGATATGT**AAGCGGTTTTAACTTTCAACTTTCAGGC**TGTTATTGTGTTATTTataactt**cgtatagc**
atacattatacgaagttatcgccggcgggtctgagctcgccatcagtGCGCCTACTCATTGAAAATGTACTTTGCTATATTGAG
ATCAGGTCAGATCCTCCTGACACAAAGGGGATTATTCAGGTTTCATGTTAACAAAAACAGACTACAGCAGTGT
CCTTGAATTTCAACAGAAAATTC

Lower case letters denote the inserted LoxP sequence
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
6430573F11RIK-FLOX3'-UNI_F	n/a	**GACTCCTGCCTAATACAGTTATGATATG**	n/a	Universal Forward
6430573F11RIK-FLOX3'-UNI_PROBE	FAM	**AAGCGGTTTTAACTTTCAACTTTCAGGC**	BHQ	Universal Probe
6430573F11RIK-FLOX3'-MUT_R	n/a	**gcgataacttcgtataatgtatgctatacg**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo Name	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

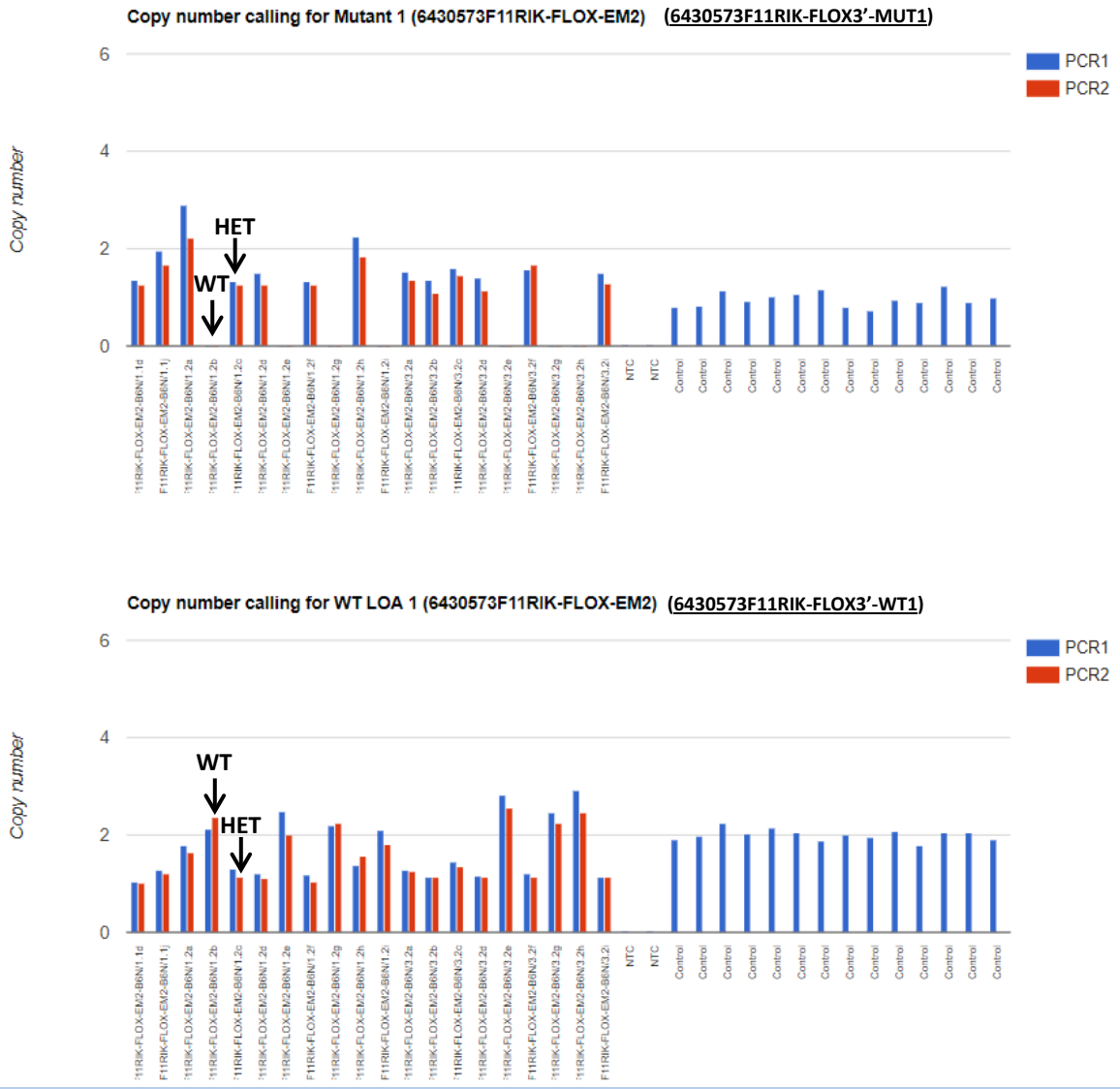
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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.

6430573F11RIK-FLOX3'-WT1 and 6430573F11RIK-FLOX3'-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 259492 results)



Version No.	1
Date:	25.01.2020
Created/Updated by:	AC

Name of Mouse model or mutation:
6430573F11RIK-FLOX-EM2-B6N

Description:
Floxed allele generated using CRISPR/Cas9 editing

Type of mutation:
Floxed exon ENSMUSE00001075283 of 6430573F11Rik.

Sequence details
6430573F11RIK WT
ACCCACCTCTGGGACCTAATTTACATATTTGAGACTGAAGATCATGCTAGGAGCCAGATGCAGATGC
ATGTCCCTAGGGTGCCAATTTCTTGGAAGACTGAAATGGCAAAACTCCCTTGAGCCCAGGAGTTCAG
CCTGGGTAATACAGAGAGACCCAGTCTCAAAAGAGAAGTTGATGGTAGTGCTAAAAAATAGAAGGT
GGGTGTTCCGTGGCAATTTGTCATTTGTGTTACTCTCTATCATACTATAATGTTTGATCTCAGTCTTTG
GATTGGATACTTGGGCTTCTGCCACTAGGAAATGAATGTCAAAAATCATCTGGACTTTTTCCCAGACT
CTCTGTTAGAGCTACAGACTGGCCCAACAGAGAAGCCTTGTACCTGCTATTAACCTAAGAATGTGTG
GGAAGATGTTTCTTCTTCAGTCACAGTCGTGTTTCTTAAAAGCCACATTTTGTTGCCTAGATTTTTCTC
TTCATAGACATTCAAAAGTTGTTAGCAGTAATTTGTTCAACAAGGTCAGAAATTGATAAGACCCATTG
AAACAGACATTTGAGACAGCACACACACACAAAGTAAAATGCATACGTCACTATGAAGACAACGAC
CCCTCATGGTTAACCCCAGTTTCCGGATGTCATAGCTTTTGTTTCTCAGGGATGAGCCAGCTCATGAG
ATTAGGTTGTGCTACTGTGCTGAGCTCCATGGGCAATGCTTCAATAAATATTCCATACATTTCCTCTCT
TGTGTATGAAACAATACGTAAGTGTTCTCTGAAGTGTTGCATCTTGGTCATTTACTATAAAGTTCATC
CATTACAGCACACATTAACTTGCATCGTTCCAGGAAGGACAGCATGTGACGCTGGTTTTTCTCTTACA
GGTTGTGGAACTGGAAAGTATCTTAAAGTGAATAGCCAAGTCCATACTCTGGGCTGTGACTACTGTG
GGCCTCTGGTAGAGATTGCCCGGAATAGAGGGTGTGAAGTCATGGTATGTGACAACCTTAATCTTCC
CTTTAGGGACCAGGGCTTCGATGCTATCATCTCCATAGGAGGTAAGGCAGCCAGGCTTTCATACTTT
GCCATGAAAAAAAAAAAACCCACAATCAGACAACTCACATGACTCAATGTCCATTCTGTATAGAGGT
CTATGCAATGCATTTTGTCTAAATTTAAAATATACACACTCTGTCGCATGACTTATTTGACTCCTGCCT
AATACAGTTATGATATGTAAGCGGTTTTAACTTTCAACTTTCAGGCTGTTATTGTGTTATTTTTGATGA
TTTGTTCCCATGTGTTTCGCTACATGCAAGAAGGGTGTCGGAGCGCCTACTCATTGAAAATGTACTTT
GCTATATTGAGATCAGGTCAGATCCTCCTGACACAAAGGGGATTATTCAGGTTTCATGTTAACAAAA
ACAGACTACAGCAGTGTCCTTGAATTTCAACAGAAAATTCTAGAACTAATGTATTAAACATCCCCCCG
AACAAGTATGCCATCCACTGCTACTCAAGTTCCCGGAAAAGGCAGACCCTACCTGGTCCCAGATTTC
ATTGTCTTAGCATGTCTTTCTGTTCCTAATTCTAGAATTTGTTGATGAATCTAGGGAATTTTTTTTCTCA
CATTTCACAAGCCTCCCCAGGAAACTCCATGCCAAAGTGAACAAAACAACAGGAATATCAGCAAAGT
CCATGCCTGGAACACGCACAGATGCATGAGA

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ACCCACCTCTGGGACCTAATTTACATATTTGAGACTGAAGATCATGCTAGGAGCCAGATGCAGATGC
ATGTCCCTAGGGTGCCAATTTCTTGGAAGACTGAAATGGCAAAACTCCCTTGAGCCCAGGAGTTCAG
CCTGGGTAATACAGAGAGACCCAGTCTCAAAAGAGAAGTTGATGGTAGTGCTAAAAAATAGAAGGT
GGGTGTTCCGTGGCAATTTGTCATTTGTGTTACTCTCTATCATACTATAATGTTTGATCTCAGTCTTTG
GATTGGATACTTGGGCTTCTGCCACTAGGAAATGAATGTCAAAAATCATCTGGACTT*atccgggggtacc*
*gcgtcgagGCGATCGC*ATAACTTCGTATAGCATACATTATACGAAGTTATGAAGATGTTTCTTCTTCAG
TCACAGTCGTGTTTCTTAAAAGCCACATTTTGTTGCCTAGATTTTTCTCTTCATAGACATTCAAAAGTT
GTTAGCAGTAATTTGTTCAACAAGGTCAGAAATTGATAAGACCCATTGAAACAGACATTTGAGACAG
CACACACACACAAAGTAAAATGCATACGTCACTATGAAGACAACGACCCCTCATGGTTAACCCCAGT
TTCCGGATGTCATAGCTTTTGTTTCTCAGGGATGAGCCAGCTCATGAGATTAGGTTGTGCTACTGTGC
TGAGCTCCATGGGCAATGCTTCAATAAATATTCCATACATTTCCTCTCTTGTGTATGAAACAATACGT
AAGTGTTCTCTGAAGTGTTGCATCTTGGTCATTTACTATAAAGTTCATCCATTACAGCACACATTAACT
TGCATCGTTCCAGGAAGGACAGCATGTGACGCTGGTTTTTCTCTTACAGGTTGTGGAACTGGAAAGT
ATCTTAAAGTGAATAGCCAAGTCCATACTCTGGGCTGTGACTACTGTGGGCCTCTGGTAGAGATTGC
CCGGAATAGAGGGTGTGAAGTCATGGTATGTGACAACCTTAATCTTCCCTTTAGGGACCAGGGCTTC
GATGCTATCATCTCCATAGGAGGTAAGGCAGCCAGGCTTTCATACTTTGCCATGAAAAAAAAAAAAC
CCACAATCAGACAACTCACATGACTCAATGTCCATTCTGTATAGAGGTCTATGCAATGCATTTTGTCT
AAATTTAAAATATACACACTCTGTCGCATGACTTATTTGACTCCTGCCTAATACAGTTATGATATGTAA
GCGGTTTTAACTTTCAACTTTCAGGCTGTTATTGTGTTATTTATAACTTCGTATAGCATACATTATACG
AAGTTAT*CGCCGGCGgtctgagctcgccatcagt*GCGCCTACTCATTGAAAATGTACTTTGCTATATTGAG
ATCAGGTCAGATCCTCCTGACACAAAGGGGATTATTCAGGTTTCATGTTAACAAAAACAGACTACAG
CAGTGTCCTTGAATTTCAACAGAAAATTCTAGAACTAATGTATTAAACATCCCCCCGAACAAGTATGC
CATCCACTGCTACTCAAGTTCCCGGAAAAGGCAGACCCTACCTGGTCCCAGATTTCATTGTCTTAGCA
TGTCTTTCTGTTCCTAATTCTAGAATTTGTTGATGAATCTAGGGAATTTTTTTTCTCACATTTCACAAGC
CTCCCCAGGAAACTCCATGCCAAAGTGAACAAAACAACAGGAATATCAGCAAAGTCCATGCCTGGA
ACACGCACAGATGCATGAGA

LoxP sites are underlined. Diagnostic restriction enzyme site and primer sequences are italicised.

Nucleotide Alignment: Orange is targeted exon, Red is LoxP sites, Yellow are universal primer and RE sites

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_6430573F11RIK_F3 (5'-3')	ACCCACCTCTGGGACCTAAT
Geno_6430573F11RIK_R2 (5'-3')	TCTCATGCATCTGTGCGTGT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	2
WT product size (bp)	1724
Mutant product size (bp)	1721
Notes	Amplicon also sequenced with LoxPF and LoxPR (see below).

LoxPF (5'-3')	atccgggggtaccgcgtcgag
LoxPR (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	3
WT product size (bp)	-
Mutant product size (bp)	999
Notes	

Geno_6430573F11RIK_F3 (5'-3')	ACCCACCTCTGGGACCTAAT
LoxPR (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1.5
WT product size (bp)	-
Mutant product size (bp)	1325
Notes	

LoxPF (5’-3’)	atccgggggtaccgcgtcgag
Geno_6430573F11RIK_R2 (5’-3’)	TCTCATGCATCTGTGCGTGT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1.5
WT product size (bp)	-
Mutant product size (bp)	1395
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	643RikFLOX-5’-MUT1
Forward Primer (5’-3’)	GCTTCTGCCACTAGGAAATGAATG
Reverse Primer (5’-3’)	CGACTGTGACTGAAGAAGAAACATC
Probe (5’-3’)	TCGAGGCGATCGCATAACTTCG
Label	FAM-BHQ1

The ddPCR assay recognises the 5' LoxP site of the 6430573F11Rik floxed allele. WT controls are expected to call at 0 copies and correct mutants are expected to call at 1 copy for F1 (HET) animals.

Assay name	6430573F11Rik-CR-LOA-WT1
Forward Primer (5’-3’)	GGTAGAGATTGCCCGGAATAGAG
Reverse Primer (5’-3’)	CGAAGCCCTGGTCCCTAAAG
Probe (5’-3’)	TGTGAAGTCATGGTATGTGACAACCTTA
Label	FAM-BHQ1

The ddPCR assay recognises the critical region of the 6430573F11Rik floxed allele. Correct mutants or WT samples are expected to call at 2. Null mutants are expected to call at 1. Random integration mutants are expected to call >2.

Assay name	6430573F11Rik-DONOR-MUT1
Forward Primer (5’-3’)	GGTCTGAGCTCGCCATCAG
Reverse Primer (5’-3’)	CCCTTTGTGTCAGGAGGATCTG
Probe (5’-3’)	TGCGCCTACTCATTGAAAATGTACTTTGC
Label	FAM-BHQ1

The ddPCR assay recognises the 3' LoxP site of the 6430573F11Rik floxed allele. WT controls are expected to call at 0 copies and correct mutants are 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.