

Name of Mouse model or mutation:

NELFE-FLOX-EM1-B6J

Description:

Floxed allele made by CRISPR/Cas9 gene editing.

Type of mutation:

Floxing of exons ENSMUSE00000141419 and ENSMUSE00001249621.

Delivery method:

Pronuclear injection into 2-cell stage embryo.

Genetic Background:

C57BL/6J

Nuclease:

Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
CCTGAACTTTCCAGTCACTC	TGG
TAAGCATGAGCAACATCCCG	GGG
GTAAGCATGAGCAACATCCC	GGG

Plasmid donor sequence (5'-3'):

LOCUS pUC-GW-Amp.dna 2671 bp ds-DNA circular SYN 06-MAY-2020
 SOURCE synthetic DNA construct
 ORGANISM synthetic DNA construct
 REFERENCE 1 (bases 1 to 2671)
 AUTHORS 123
 TITLE Direct Submission
 JOURNAL Exported Jun 8, 2020 from SnapGene Viewer 4.2.6 to Vector NTI(R)
 format
<http://www.snapgene.com>
 COMMENT <http://www.informaxinc.com/>
 ORIGDB|GenBank
 COMMENT VNTDATE|578275200|
 COMMENT VNTDBDATE|912902400|
 COMMENT VNTNAME|pUC-GW-Amp|
 COMMENT VNTAUTHORNAME|123|

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661 AGAGGAAATC TCTGTATGAA AGGTCAGAGA GAGCACCGTT TGGTGTGGGG TCAGAGGCCA
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781 AGTTATTGAC TGAAAGTTC AGGGCTAGAG TGGGAGGAGC CAGCTGCAGA GGCAGTGTG
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//

Plasmid donor 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 protein, sgRNAs and plasmid were diluted and mixed in MIB to the working concentrations of 100 ng/μl, 50 ng/μl each and 50 ng/μl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F₀ progeny.

Sequence details

Nelfe WT

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NELFE-FLOX-CAS-EM1-B6J

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TCTcagtggtggcacacacctcagccacaacacgtgggaggtggagccaggtgagttcaaggccagcctggtctacagagcaga
ggtcagaacagccagggtacacagagaaatctgtcttgaagccaTTTGgagggcagtggtggcacacaccttaatccc
agcacttgggagggcagaggaaggtggttctgagttcaaggccagcttggctacagagtgagttccaggacagccaagactaca
cagagaaacctgtctcagaaaaacaaaaaaagaaagaaagaaaaGCCATTTGAAAAACAGAACAGG

GTCCTCTGTCACAGACAGGAAAGCAGAAGCCTAGTTGTCGCCTCTGAGCTGGAGTGTTACCTGAACT
TGGTATTGTGGGTCTGGACCTCAAGTAGAAGACCAGGCATGGAAACATAGATTAGTGTTCTGTGTAT
CTGGAACCATCAGAGGTGGGGATTTTGGAGATTGAAAAGTGACCTGAGACTGGTTTAGAGAATCAA
GAGCATTAGGAAGCTAGGCTCTACTGTCTGTGCCTAGCATACTCCAGCAG

LoxP sites are red and underlined and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in *yellow and italics*. Exons highlighted in **blue**. Floxed exon highlighted in **blue and bold**.

Nucleotide Alignment:

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      *      20      *      40      *      60      *      80      *      100      *      120
Nelife_WT : ACAGGCCAAACAACCTGGTGAAGTCAGGAGCCATCAGTGCCATCAAGGCTGAAACTAAGAACTCAGGCTTCAAACGTTCTCGGACCCTGGAAGGGAAGTTAAAGGTGAGCACACAGGAAGA : 120
Nelife_EM1 : ACAGGCCAAACAACCTGGTGAAGTCAGGAGCCATCAGTGCCATCAAGGCTGAAACTAAGAACTCAGGCTTCAAACGTTCTCGGACCCTGGAAGGGAAGTTAAAGGTGAGCACACAGGAAGA : 120

      *      140      *      160      *      180      *      200      *      220      *      240
Nelife_WT : TTGTCTGGAACGCTCTGGCCTCAGCTGGAAGGGGGTGGGTGGGGGAAGTCATGCATGCTCACATATGAGCAACCTgtgctgttctgagtttaggggtgtttctttctgttccttgagaca : 240
Nelife_EM1 : TTGTCTGGAACGCTCTGGCCTCAGCTGGAAGGGGGTGGGTGGGGGAAGTCATGCATGCTCACATATGAGCAACCTgtgctgttctgagtttaggggtgtttctttctgttccttgagaca : 240

      *      260      *      280      *      300      *      320      *      340      *      360
Nelife_WT : gggctctgactgtactcatggagtcagtgatgctctggcctcagcctcagaagtagttTTTGCCACTGCAGCCCACTGGAGCGCTAGGTTTCTGTCTATCACAAGAAACCTGagcccag : 360
Nelife_EM1 : gggctctgactgtactcatggagtcagtgatgctctggcctcagcctcagaagtagttTTTGCCACTGCAGCCCACTGGAGCGCTAGGTTTCTGTCTATCACAAGAAACCTGagcccag : 360

      *      380      *      400      *      420      *      440      *      460      *      480
Nelife_WT : tgtggtggcacatgcctttaatcccagcacttgtggggcagaggcagcagatttctgagtttgaggccagcccggctctacagagtgagttctaggacagccagggctatacagagaaac : 480
Nelife_EM1 : tgtggtggcacatgcctttaatcccagcacttgtggggcagaggcagcagatttctgagtttgaggccagcccggctctacagagtgagttctaggacagccagggctatacagagaaac : 480

      *      500      *      520      *      540      *      560      *      580      *      600
Nelife_WT : cctgtctcgaaaaacaaaacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaGCCTGGGTGATGCCACATCTCTTTTCCATATTTGTGCTTCTGGATTGAAGGGAGGTTTGTAGTAATGAAACCA : 600
Nelife_EM1 : cctgtctcgaaaaacaaaacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaGCCTGGGTGATGCCACATCTCTTTTCCATATTTGTGCTTCTGGATTGAAGGGAGGTTTGTAGTAATGAAACCA : 600

      *      620      *      640      *      660      *      680      *      700      *      720
Nelife_WT : GTTACTCACATCAGCTGATgtagtggcacacacacctttaatcccagcacttggagcagacagggccgagagctctaacataaaggacagcctggttctactgcgcaagttcaggatagccag : 720
Nelife_EM1 : GTTACTCACATCAGCTGATgtagtggcacacacacctttaatcccagcacttggagcagacagggccgagagctctaacataaaggacagcctggttctactgcgcaagttcaggatagccag : 720

      *      740      *      760      *      780      *      800      *      820      *      840
Nelife_WT : ggccacacagagaagcactatcttgaaaaaccaGTCAACCGGTCCAGCACATCATATAAGAGTGACAGGAGCAAAGAGAAAGCAGAGAAGAGAGAGGGGAAGAGCTCTCCTGATCCACGC : 840
Nelife_EM1 : ggccacacagagaagcactatcttgaaaaaccaGTCAACCGGTCCAGCACATCATATAAGAGTGACAGGAGCAAAGAGAAAGCAGAGAAGAGAGAGGGGAAGAGCTCTCCTGATCCACGC : 840

      *      860      *      880      *      900      *      920      *      940      *      960
Nelife_WT : TAACATGGGTATCTGCCCTTTTCCAGGACCCAGAGAAGGGGCTGTCCCACTTTCCAGCCGTTCCAGAGGAGCATGTCTGCTGATGAGGACCTACAGGAGGTAATGGTTTCTGTGGGGC : 960
Nelife_EM1 : TAACATGGGTATCTGCCCTTTTCCAGGACCCAGAGAAGGGGCTGTCCCACTTTCCAGCCGTTCCAGAGGAGCATGTCTGCTGATGAGGACCTACAGGAGGTAATGGTTTCTGTGGGGC : 960

      *      980      *      1000      *      1020      *      1040      *      1060      *      1080
Nelife_WT : CAGGGTTGGGGACAAGAAGAGACCCACACACTGTGGACCAACTCCGGCACCTGGGAATCATTTGAATTGTTCTCTGTCTTTTCTTTCCCTTAGCCATCCAGACGTCCCAGAGGAAATC : 1080
Nelife_EM1 : CAGGGTTGGGGACAAGAAGAGACCCACACACTGTGGACCAACTCCGGCACCTGGGAATCATTTGAATTGTTCTCTGTCTTTTCTTTCCCTTAGCCATCCAGACGTCCCAGAGGAAATC : 1080
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      *      1100      *      1120      *      1140      *      1160      *      1180      *      1200
Nelife_WT : TCTGTATGAAAGGTCAGAGAGAGCACCGTTTGGTGTGGGGTCAGAGGCCAGAG-----TGAC : 1137
Nelife_EM1 : TCTGTATGAAAGGTCAGAGAGAGCACCGTTTGGTGTGGGGTCAGAGGCCAGAGatccgggggtaccgcgtcgagCGGATCGATAAAGTTCGATAGCATAACATTATACGAAGTAT : 1200

      *      1220      *      1240      *      1260      *      1280      *      1300      *      1320
Nelife_WT : TGGAAAGTTCAGGGCTAGAGTGGGAGGAGCCAGCTGCAGAGGCAGTGTGACTCCACAAGGTTTGAAGAAGGTCGAACCCCTTTGGTCCCTTACTGTCTGCCTCTCCCAGCTTTGTGT : 1257
Nelife_EM1 : TGGAAAGTTCAGGGCTAGAGTGGGAGGAGCCAGCTGCAGAGGCAGTGTGACTCCACAAGGTTTGAAGAAGGTCGAACCCCTTTGGTCCCTTACTGTCTGCCTCTCCCAGCTTTGTGT : 1320

      *      1340      *      1360      *      1380      *      1400      *      1420      *      1440
Nelife_WT : CTTCTAGTGATCGGCTTCGGGAAGTGGGCAAGATGGAGAAGAAGCAGAGGCCCGGGGCTGGTGTATGGCCACCTCGAGGCTTTGACTGGAGCTATGAAGAGCATGGTAGCGCCCGCT : 1377
Nelife_EM1 : CTTCTAGTGATCGGCTTCGGGAAGTGGGCAAGATGGAGAAGAAGCAGAGGCCCGGGGCTGGTGTATGGCCACCTCGAGGCTTTGACTGGAGCTATGAAGAGCATGGTAGCGCCCGCT : 1440

      *      1460      *      1480      *      1500      *      1520      *      1540      *      1560
Nelife_WT : CCTCAGCCTCCCCTCCCGAAGCCGCAGCAGGGACCGAAGTCATGACCCGGAGCCGAGATAGGGATCGGgacaaagagagagacagagaccgagatcgagatcgagacagagaccgggata : 1497
Nelife_EM1 : CCTCAGCCTCCCCTCCCGAAGCCGCAGCAGGGACCGAAGTCATGACCCGGAGCCGAGATAGGGATCGGgacaaagagagagacagagaccgagatcgagatcgagacagagaccgggata : 1560

      *      1580      *      1600      *      1620      *      1640      *      1660      *      1680
Nelife_WT : aagacaaagacagagaccgggatcgagacagagaccgggaccgagatcgggaacgagaccgagaGGGCCCTTTCCGCAGTGAGTGATCTTAGCCAGGGTTTAGGTGGGTCTCTGCTAAGTA : 1617
Nelife_EM1 : aagacaaagacagagaccgggatcgagacagagaccgggaccgagatcgggaacgagaccgagaGGGCCCTTTCCGCAGTGAGTGATCTTAGCCAGGGTTTAGGTGGGTCTCTGCTAAGTA : 1680

      *      1700      *      1720      *      1740      *      1760      *      1780      *      1800
Nelife_WT : GAAGGTGAGGTGGACCCAGTAATGACTGTGGTTCCTAACCCACAGGGTCAGACTCATTCGCCGACGGAGGGCCCTCGAAAGGGGAACACACTGTATGTGTATGGAGAGGACATGACGC : 1737
Nelife_EM1 : GAAGGTGAGGTGGACCCAGTAATGACTGTGGTTCCTAACCCACAGGGTCAGACTCATTCGCCGACGGAGGGCCCTCGAAAGGGGAACACACTGTATGTGTATGGAGAGGACATGACGC : 1800

      *      1820      *      1840      *      1860      *      1880      *      1900      *      1920
Nelife_WT : CCACCTCCTCCGCGGGGCTTCTCTCCCTTTGGAAATATCATCGACCTCTCCATGGACCCACCCAGAAAGTAAGCATGAGCAACATC----- : 1825
Nelife_EM1 : CCACCTCCTCCGCGGGGCTTCTCTCCCTTTGGAAATATCATCGACCTCTCCATGGACCCACCCAGAAAGTAAGCATGAGCAACATCATAAAGTTCGATAGCATAACATTATACGAAGTT : 1920

      *      1940      *      1960      *      1980      *      2000      *      2020      *      2040
Nelife_WT : -----CCGGGGAGGGAGCTGCCTGGTCTCCATGGAGTTTCTGACTCTGCTTTCTCTCTCACCAGCTGTGCCTTCGTACCTACGAGAAGATGG : 1915
Nelife_EM1 : ATCGCCGGCGggtctgagctcgccatcagtCCGGGGAGGGAGCTGCCTGGTCTCCATGGAGTTTCTGACTCTGCTTTCTCTCTCACCAGCTGTGCCTTCGTACCTACGAGAAGATGG : 2040

      *      2060      *      2080      *      2100      *      2120      *      2140      *      2160
Nelife_WT : AGTCTGCAGATCAGGCTGTTGCTGAGGTTGGGACTTCTCAGACCGTTCCCTCCCATCCCTCCTGTCATCCCTGCTTCTTCTTTCAATATCTGCTTCTAGAGGAAACTTTTCTCCTGAC : 2035
Nelife_EM1 : AGTCTGCAGATCAGGCTGTTGCTGAGGTTGGGACTTCTCAGACCGTTCCCTCCCATCCCTCCTGTCATCCCTGCTTCTTCTTTCAATATCTGCTTCTAGAGGAAACTTTTCTCCTGAC : 2160

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* 2180 * 2200 * 2220 * 2240 * 2260 * 2280
 Nelfe_WT : TGAGAAAACCTTCATCCTCACATTCCTGGTTGGTCTCCCAGGGCAGCAGCACCTGGCTCCATGTAATGCCCGTCTCTGTCCCCTCCCCTCTTGTCCCACAGCTCAATGGGACCCAGGTG : 2155
 Nelfe_EM1 : TGAGAAAACCTTCATCCTCACATTCCTGGTTGGTCTCCCAGGGCAGCAGCACCTGGCTCCATGTAATGCCCGTCTCTGTCCCCTCCCCTCTTGTCCCACAGCTCAATGGGACCCAGGTG : 2280

* 2300 * 2320 * 2340 * 2360 * 2380 * 2400
 Nelfe_WT : GAATCTGTGCAGCTTAAAGTCAACATAGCCCCGAAAACAGCCCATGCTGGACGCTGCTACTGGCAAATCTGTCTGGGGCTCCCTTGGTAAGGATGAGGCTCCTCCCCTCCCCTCTCTGCAG : 2275
 Nelfe_EM1 : GAATCTGTGCAGCTTAAAGTCAACATAGCCCCGAAAACAGCCCATGCTGGACGCTGCTACTGGCAAATCTGTCTGGGGCTCCCTTGGTAAGGATGAGGCTCCTCCCCTCCCCTCTCTGCAG : 2400

* 2420 * 2440 * 2460 * 2480 * 2500 * 2520
 Nelfe_WT : CATAGCACTTCCTTGAGTTCCTTAAAGTGCCCAAGGTCAGGATCTTACGTTAGGTAGTGGATGAAGAATTGCCAGGGCATCTGTCAGGTGAGGGAAGGAGAGCAGCACTGAAATGTGCC : 2395
 Nelfe_EM1 : CATAGCACTTCCTTGAGTTCCTTAAAGTGCCCAAGGTCAGGATCTTACGTTAGGTAGTGGATGAAGAATTGCCAGGGCATCTGTCAGGTGAGGGAAGGAGAGCAGCACTGAAATGTGCC : 2520

* 2540 * 2560 * 2580 * 2600 * 2620 * 2640
 Nelfe_WT : TCTcagtggtggcacacaccttcagccacaacacgtgggaggtggagccaggtgagttcaaggccagcctggtctacagagcagagttcagaacagccagggctacacagagaaatcttg : 2515
 Nelfe_EM1 : TCTcagtggtggcacacaccttcagccacaacacgtgggaggtggagccaggtgagttcaaggccagcctggtctacagagcagagttcagaacagccagggctacacagagaaatcttg : 2640

* 2660 * 2680 * 2700 * 2720 * 2740 * 2760
 Nelfe_WT : tcttgaaaagccaTTTGGGcagggcagtggtggcacacacctttaatcccagcacttgggagggcagaggaaggtggatttctgagttcaaggccagcttggctacagagtgagttccag : 2635
 Nelfe_EM1 : tcttgaaaagccaTTTGGGcagggcagtggtggcacacacctttaatcccagcacttgggagggcagaggaaggtggatttctgagttcaaggccagcttggctacagagtgagttccag : 2760

* 2780 * 2800 * 2820 * 2840 * 2860 * 2880
 Nelfe_WT : gacagccaagactacacagagaaaccctgtctcagaaaaccc : 2755
 Nelfe_EM1 : gacagccaagactacacagagaaaccctgtctcagaaaaccc : 2880

* 2900 * 2920 * 2940 * 2960 * 2980 * 3000
 Nelfe_WT : CCTAGTTGTCGCCTCTGAGCTGGAGTGTACCTGAACTTGGTATTGTGGGTCTGGACCTCAAGTAGAAGACCAGGCATGGAAACATAGATTAGTGTCTGTGTATCTGGAACCATCAGAG : 2875
 Nelfe_EM1 : CCTAGTTGTCGCCTCTGAGCTGGAGTGTACCTGAACTTGGTATTGTGGGTCTGGACCTCAAGTAGAAGACCAGGCATGGAAACATAGATTAGTGTCTGTGTATCTGGAACCATCAGAG : 3000

* 3020 * 3040 * 3060 * 3080 * 3100
 Nelfe_WT : GTGGGGATTTTGGAGATTGAAAAGTGACCTGAGACTGGTTTLAGAGAATCAAGAGCATTAGGAAGCTAGGCTCTACTGTCTGTGCCTAGCATACTCCAGCAG : 2977
 Nelfe_EM1 : GTGGGGATTTTGGAGATTGAAAAGTGACCTGAGACTGGTTTLAGAGAATCAAGAGCATTAGGAAGCTAGGCTCTACTGTCTGTGCCTAGCATACTCCAGCAG : 3102

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_Nelfe_Flox_F3 (5'-3')	ACAGGCCAAACAACACTGGTGA
Geno_Nelfe_Flox_R3 (5'-3')	CTGCTGGAGGTATGCTAGGC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	2977
Mutant product size (bp)	3102
Notes	This PCR sits external to the donor site.

LoxP_F (5'-3')	atccgggggtaccgctcgag
LoxP_R (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	-
Mutant product size (bp)	817
Notes	This PCR is specific to the donor.

Geno_Nelfe_Flox_F3 (5'-3')	ACAGGCCAAACAACACTGGTGA
LoxP_R (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	-

Mutant product size (bp)	1950
Notes	This PCR is used to screen if the donor has inserted on target.

LoxP_F (5'-3')	atccgggggtaccgcgtcgag
Geno_Nelfe_Flox_R3 (5'-3')	CTGCTGGAGGTATGCTAGGC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	-
Mutant product size (bp)	1969
Notes	This PCR is used to screen if the donor has inserted on target.

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.

Off-target site with ≤ 2 mismatches for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
5:120203755-120203777	GCTCAC CTTTCCAGTCACTC TGG	Intergenic	Nelfe_G2OT1_F1: GGGTAGTGCTCAGAAAGGGAC Nelfe_G2OT1_R1: ATTCCAGGAAGGTGCAAGAGG
8:100865760-100865782	TCTGTACTTCC CAGTCACTC TGG	Intergenic	Nelfe_G2OT2_F2: CTCAGGTCTTGCCCATAGCC Nelfe_G2OT2_R2: CATGGGCAGATGGATACTTAGATA
6:72673102-72673124	CC AGAAGTTCC CAGTCACTC GGG	Intronic	Nelfe_G2OT3_F1: CCCCTCCCAGTAAACAGATT Nelfe_G2OT3_R1: TCAGTAAAACGGAAGTGGCAG
2:95586382-95586404	GTAT TC ATGAGCAACATCCC TGG	Intergenic	Geno_Nelfe_OT4F1: CCAGGCGACAAATCAATGCT Geno_Nelfe_OT4R1: ACCCCAATCCATGATATCCC

All amplicons were sent for Sanger sequencing.

No off-target activity was detected in the animals selected to establish the colony.

Additional integrations of the donor sequence

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	Nelfe_Flox_5'_MUT1
Forward Primer (5'-3')	GTCAGAGAGAGCACCGTTTG
Reverse Primer (5'-3')	GCCCTGAACCTTCCAGTCAATA
Probe (5'-3')	tcgagGCGATCGCATAACTTCG
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, 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.

Assay name	Nelfe_Flox_3'_MUT1
Forward Primer (5'-3')	CATGGACCCACCCAGAAAG
Reverse Primer (5'-3')	TCCATGGAGACCAGGCA
Probe (5'-3')	AAGTTATCGCCGGCGggtctga
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, 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.

Assay name	Nelfe_Flox_CR-LOA_WT1
Forward Primer (5'-3')	agacagagaccgggataaaga
Reverse Primer (5'-3')	CCTAAACCCTGGCTAAGATCAC
Probe (5'-3')	aagacagagaccgggatcgagaca
Label	FAM

This ddPCR assay is universal; both the WT and mutant alleles are recognised by this assay. Therefore, 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	pUC57_backbone_assay2
Forward Primer (5'-3')	GGCGAAACCCGACAGGACTATAA
Reverse Primer (5'-3')	GGGAGAAAGGCGGACAGGTATC
Probe (5'-3')	TCTCCTGTTCCGACCCTGCCGCTTA
Label	FAM

The ddPCR assay is designed to highlight the plasmid backbone as it sits within the backbone region. Therefore, WT controls are expected to call at 0 copies and correct mutants are expected to call at 0 copies 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.

No additional donor integrations were detected in the animals taken forward to establish the colony.