Name of Mouse model or mutation:
Mc2r-F278C-EM1-B6J
Mc2r-F278C-EM2-B6J

Description:
Point mutation made by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: F278C

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
70:30 ratio Deactivated Cas9 protein: Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
GCTCCGAAAGGCATATATAA	AGG

ssODN donor sequence (5'-3'):
TGCCCAAATAACCCTTACTGTGTTTGCTACATGTCTCTCTTCCAGGTCAATGGCATGTTGATCATG
TGCAATGCAGTTATTGACCCaTTTATATATGCCTgTCGGAGCCCAGAGCTCAGAGATGCATTCAA
AAGGATGCTCTTCTGCAACCGGTATTAGTAGAATTTTTGATCCCTGCTTTGAGTGTTGTAAAGGG
ACCA

Electroporation mixes:
70:30 ratio Deactivated Cas9: Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

Mc2r WT

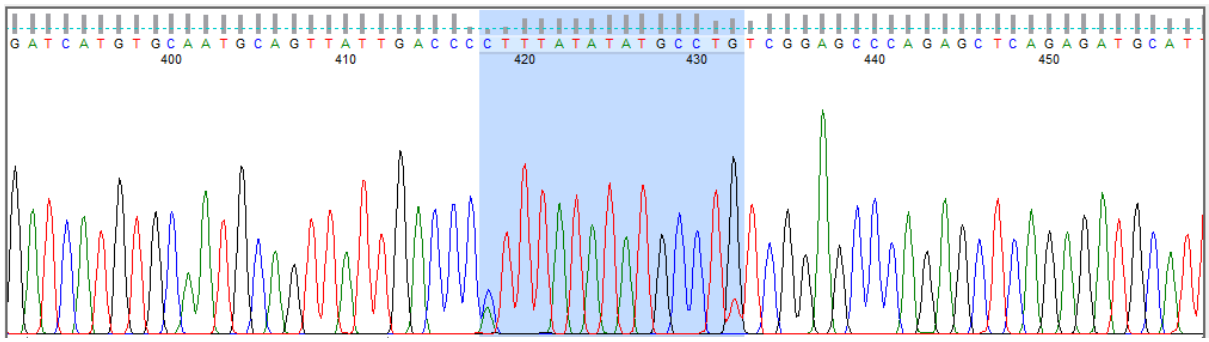
TGCAGCTGACCGTTACATCACCATCTTCCATGCCCTGCAATACCATAGCATTGTGACCATGCGCCGCA
CCATCATCACCCTAACAATTATCTGGATGTTCTGCACAGGGAGCGGCATCACCATGGTGATCTTCTCC
CACCACATCCCCACAGTGCTCACCTTCACATCGCTGTTCCCTTTGATGCTGGTTTTTATCCTGTGTCTCT
ACATCCACATGTTCTTACTTGCCCGCTCCCATGCTAGGAAGATCTCTACCCTTCCTAGAACCAACATG
AAGGGTGCCATGACACTAACCATCCTTCTTGGAGTCTTCATCTTCTGTTGGGCCCCCTTTGTGCTCCAT
GTTCTCTTAATGACCTTCTGCCCAAATAACCCTTACTGTGTTTGCTACATGTCTCTCTTCCAGGTCAAT
GGCATGTTGATCATGTGCAATGCAGTTATTGACCCCTTTATATATGCCTTTCGGAGCCCAGAGCTCAG
AGATGCATTCAAAAGGATGCTCTTCTGCAACCGGTATTAGTAGAATTTTTGATCCCTGCTTTGAGTGT
TGTAAAGGGACCAAATAACACATCAGTCTGACATGTACCAGTGATTCTTCTGACCAGTAGTTCAGTC
CCACTGACTAGTGTTTACCATAACTGCCTTTCTTATTCACAGTCTTACTGTACCTGCATTTTTATATTGT
ATCTAGAGAGATGAAATGTTGTGCTAAACAGGAAAGTTTACAAAGTACACATAGCATACAAATGTG
AGGACTTTGGGATAAACAAATATTTGTTCTTCTGTGGATACCTGTGAAAACTGTCATTGGGAGGCCT
TTCCAAGCAGAAAGCCATGGTAACCAATACTCTAGAAGTTAAAGTACTTTTTGGGCATCTAACTAACT
ATATGTGTTAGTCATATTTCTATGTATGTAGAAAATAAATGCAAACAATCAACTTTGAAAGAGGAAA
TGTTTATTTTGGTGCATGGGTTATTGAGGATTTCGTTTATGACACCTGGCTGT

Mc2r-F278C-EM1-B6J & Mc2r-F278C-EM2-B6J

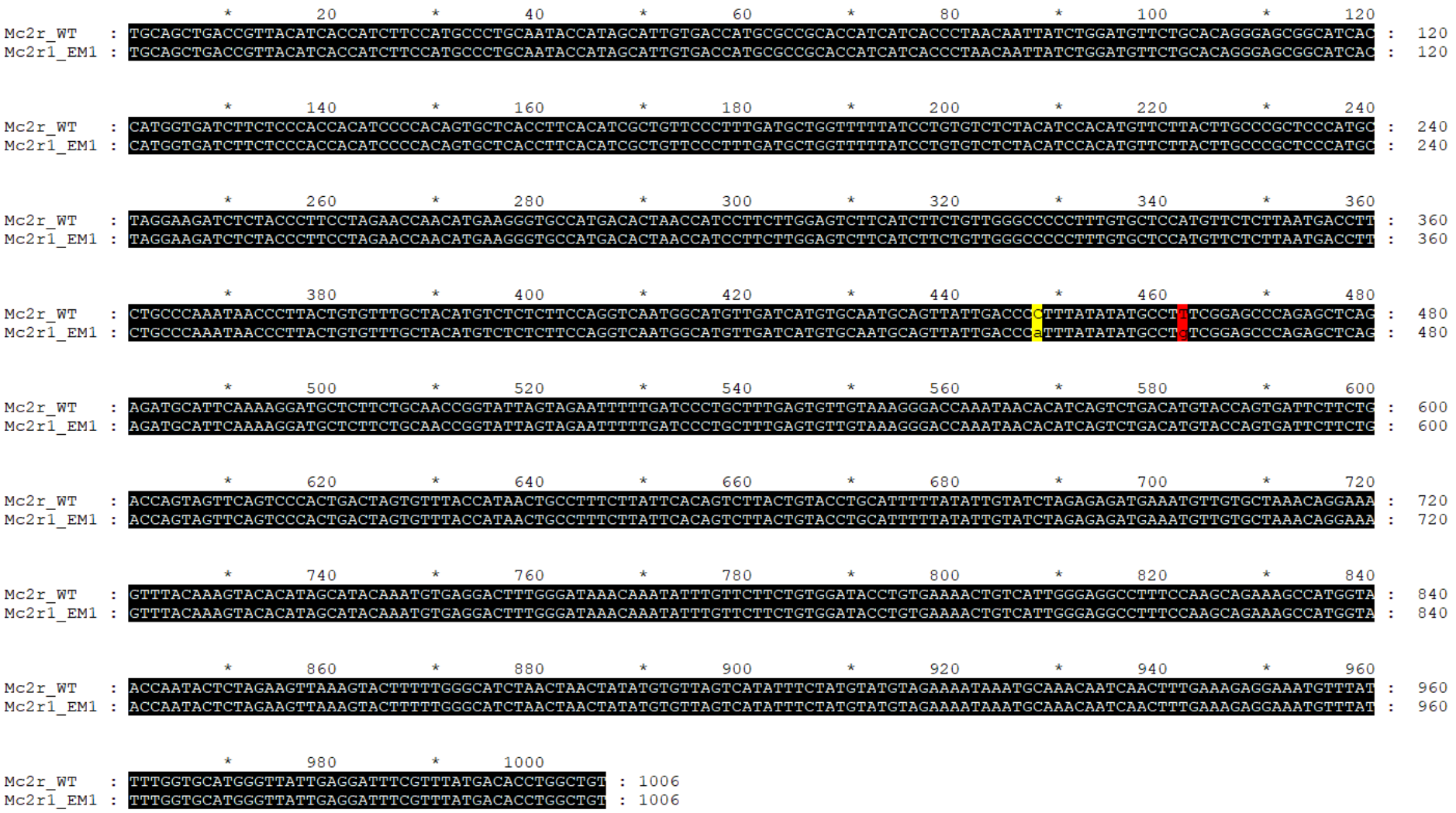
TGCAGCTGACCGTTACATCACCATCTTCCATGCCCTGCAATACCATAGCATTGTGACCATGCGCCGCA
CCATCATCACCCTAACAATTATCTGGATGTTCTGCACAGGGAGCGGCATCACCATGGTGATCTTCTCC
CACCACATCCCCACAGTGCTCACCTTCACATCGCTGTTCCCTTTGATGCTGGTTTTTATCCTGTGTCTCT
ACATCCACATGTTCTTACTTGCCCGCTCCCATGCTAGGAAGATCTCTACCCTTCCTAGAACCAACATG
AAGGGTGCCATGACACTAACCATCCTTCTTGGAGTCTTCATCTTCTGTTGGGCCCCCTTTGTGCTCCAT
GTTCTCTTAATGACCTTCTGCCCAAATAACCCTTACTGTGTTTGCTACATGTCTCTCTTCCAGGTCAAT
GGCATGTTGATCATGTGCAATGCAGTTATTGACCC**a**TTTATATATGCCT**g**TCGGAGCCCAGAGCTCAG
AGATGCATTCAAAAGGATGCTCTTCTGCAACCGGTATTAGTAGAATTTTTGATCCCTGCTTTGAGTGT
TGTAAAGGGACCAAATAACACATCAGTCTGACATGTACCAGTGATTCTTCTGACCAGTAGTTCAGTC
CCACTGACTAGTGTTTACCATAACTGCCTTTCTTATTCACAGTCTTACTGTACCTGCATTTTTATATTGT
ATCTAGAGAGATGAAATGTTGTGCTAAACAGGAAAGTTTACAAAGTACACATAGCATACAAATGTG
AGGACTTTGGGATAAACAAATATTTGTTCTTCTGTGGATACCTGTGAAAACTGTCATTGGGAGGCCT
TTCCAAGCAGAAAGCCATGGTAACCAATACTCTAGAAGTTAAAGTACTTTTTGGGCATCTAACTAACT
ATATGTGTTAGTCATATTTCTATGTATGTAGAAAATAAATGCAAACAATCAACTTTGAAAGAGGAAA
TGTTTATTTTGGTGCATGGGTTATTGAGGATTTCGTTTATGACACCTGGCTGT

Red highlight and in bold = nominated change. **Yellow highlight and in bold = silent mutation** to prevent reprocessing of the engineered allele. Please note that the EM1 and EM2 allele are the same and were generated from separate founders that were derived from the same microinjection session using the same reagents and conditions.

Heterozygous F1 animal sequence trace:



Nucleotide Alignment:



Predicted Protein Alignment:

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                 *       140         *       160         *       180         *       200         *       220         *       240
Mc2r_WT   : AADRYITIFHALQYHSIVTMRRTIITLTIIWMFCTGSGITMVIFSHHIPTVLTFTSLFPLMLVFILCLYIHMFLLARSHARKISTLPRTNMKGAMTLTILLGVFIFCWAPFVLHVLLMTF : 120
Mc2r_EM1  : AADRYITIFHALQYHSIVTMRRTIITLTIIWMFCTGSGITMVIFSHHIPTVLTFTSLFPLMLVFILCLYIHMFLLARSHARKISTLPRTNMKGAMTLTILLGVFIFCWAPFVLHVLLMTF : 120

                 *       260         *       280         *
Mc2r_WT   : CPNNPYCVCYMSLFQVNGMLIMCNAVIDPFIYAFRSPELRDAFKRMLFCNRY* : 172
Mc2r_EM1  : CPNNPYCVCYMSLFQVNGMLIMCNAVIDPFIYAGRSPELRDAFKRMLFCNRY* : 172
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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_Mc2r_F278C_F1 (5' to 3')	TGCAGCTGACCGTTACATCA
Geno_Mc2r_F278C_R1 (5' to 3')	ACAGCCAGGTGTCATAAACGA
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	1006
Mutant product size (bp)	1006
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.

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	Mc2r_F278C_MUT1
Forward Primer (5'-3')	CAGTTATTGACCCATTTATATATGCCTG
Reverse Primer (5'-3')	TGGTCCCTTTACAACACTCAAA
Probe (5'-3')	AATGCATCTCTGAGCTCTGGGCTC
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.

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.