Name of Mouse model or mutation:
EGFR-Y1018F-EM1-B6 & EGFR-Y1018F-EM2-B6

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

Type of mutation:
SNP: Y1018F

Delivery method:
Electroporation into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
GAGTATCTTATCCCACAGCA	AGG

ssODN donor sequence (5'-3'):
gtaaatcaatttaaatcaagaggggggcataccagagaactcaagaggggagtcctcgacgtggacgggctgttgaagaagccttgctgGg
ggataagGAaTtcatcagcatcaactacatcctccatgtcctcttcatccatcagggctcggtaaaagttggagtctgtagggcttggcaaatgc
attctttcatccc

Electroporation mixes:
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 each 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

WT

ccaatcggcttagttctaattctggaagaacaaaaatcaagataatttgtctttgtgaatttaaagcttcagtaaaatgatgtatgaa
aagtcattatgcagtggcatagcattgtgtagtgggggttaaatgttcccacggctttcttttctcctctctcttaaactatttttcatatg
gaggctgtcagactcaggagacttctgtgttttattacattccaaagtatccacacactgagatggaaaagggacatatatatgaga
gctaccataggggttatattataactaaactggcaaacccataggcacctattgcttcctatagccaggaagcagagcaagcttcgt
tgccctttctaatagtatcaaaatctctgcaccaggggatgaaagaatgcatttgccaagccctacagactccaacttttaccgagc
cctgatggatgaagaggacatggaggatgtagttgatgctgatgagtatcttatcccacagcaaggcttcttcaacagcccgtccac
gtcgaggactcccctcttgagttctctggtatgccccccctcttgatttaaattgatttactttccattttccatattttaattagaaaaag
agggataaattctaaccaaatagaacctggaatcataatcaacagttaaggattttagttatttaattgcctatgtttttctaaagcag
taatttaagacaaaaattcttctctccaagaaagcattcgacacattaaacgccttaaaatatttttactctatgagacctacctcctg
cattcaacagaaatggatgagaaagtgccagtgtacatgaccaacattgctttctttttttccagAGTGCAACTAGCAACAA
TTCCACTGTGGCTTGCATTAATAGAAATGGGgtatgtatggacatggtataaaccagaatcaaataagctgcgcagg
gaaagtgcccaacttagcccacatcgcactggctttccctcctgtacattcttttccat

EGFR-Y1018F-EM1-B6 & EGFR-Y1018F-EM2-B6

ccaatcggcttagttctaattctggaagaacaaaaatcaagataatttgtctttgtgaatttaaagcttcagtaaaatgatgtatgaa
aagtcattatgcagtggcatagcattgtgtagtgggggttaaatgttcccacggctttcttttctcctctctcttaaactatttttcatatg
gaggctgtcagactcaggagacttctgtgttttattacattccaaagtatccacacactgagatggaaaagggacatatatatgaga
gctaccataggggttatattataactaaactggcaaacccataggcacctattgcttcctatagccaggaagcagagcaagcttcgt
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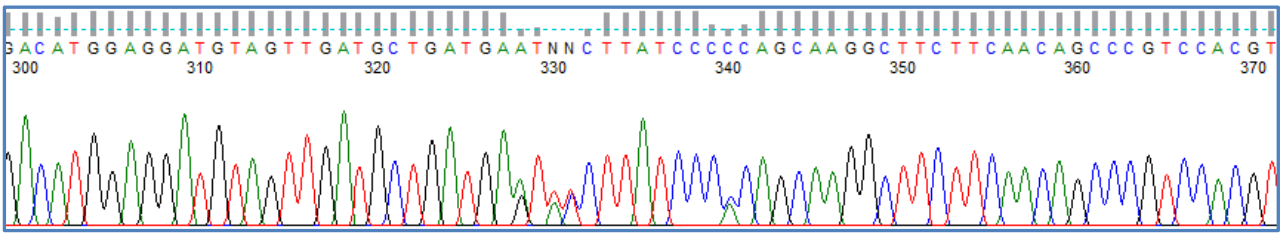
cctgatggatgaagaggacatggaggatgtagttgatgctgatgaAtTCcttatcccCcagcaaggcttcttcaacagcccgtcca
cgtcgaggactcccctcttgagttctctggtatgccccccctcttgatttaaattgatttactttccattttccatattttaattagaaaaa
gagggataaattctaaccaaatagaacctggaatcataatcaacagttaaggattttagttatttaattgcctatgtttttctaaagca
gtaatttaagacaaaaattcttctctccaagaaagcattcgacacattaaacgccttaaaatatttttactctatgagacctacctcct
gcattcaacagaaatggatgagaaagtgccagtgtacatgaccaacattgctttctttttttccagAGTGCAACTAGCAACA
ATTCCACTGTGGCTTGCATTAATAGAAATGGGgtatgtatggacatggtataaaccagaatcaaataagctgcgcag
ggaaagtgcccaacttagcccacatcgcactggctttccctcctgtacattcttttccat

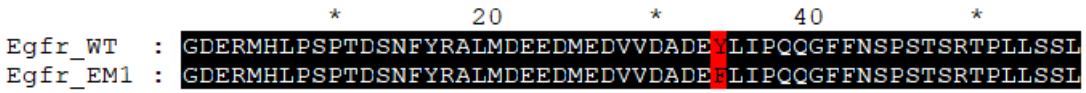
Nucleotide changes highlighted in **red and underlined = nominated change**, silent changes highlighted in red only. Blue highlight is BsaBI restriction enzyme site in WT allele, Yellow highlight is EcoRI site in Y1018F allele.

Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

Predicted Protein Alignment:



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_Egfr_Y1018F_F1	ccaatcggcttagttctaattctgg
Geno_Egfr_Y1018F_R1	atggaaaagaatgtacaggagggaa
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	1018
Mutant product size (bp)	1018
Notes	Sequenced using following primers (5'-3'): Geno_Egfr_Y1018F_F2: taaatgttcccacggctttcttttc Geno_Egfr_Y1018F_R2: ccatgtccatacatacCCCATTTCT

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')
5:44920380-44920402	GAGT**G**TCTTATC**T**CACAGCA AGG	Intergenic	Egfr_Y1018F_OT1F1: GTAGAATGGACTTACAGAAACGCAC Egfr_Y1018F_OT1R1: TATCCCCTTAGAAATTGAGGCAGAG

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	EGFR-Y1018F-UNI1
Forward Primer (5’-3’)	AGAGGACATGGAGGATGTAG
Reverse Primer (5’-3’)	GGCATACCAGAGAACTCAAG
Probe (5’-3’)	CTTCTTCAACAGCCCGTCCACGTC
Label	FAM

This ddPCR assay is universal to Egfr - both WT and Y1018F 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	EGFR-Y1018F-MUT1
Forward Primer (5’-3’)	CATTTGCCAAGCCCTACA
Reverse Primer (5’-3’)	TGCTGGGGGATAAGGAAT
Probe (5’-3’)	CCATGTCCTCTTCATCCATCAGGGC
Label	FAM

This ddPCR assay is specific to the Y1018F mutation in the Egfr gene 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.

EGFR-Y1018F

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; Y1018F, in *EGFR*. 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 assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

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

Mutant assay only available at the moment.

EGFR-Y1018F

EGFR-Y1018F-MUT1 assay (FAM labelled)

CATTTGCCAAGCCCTACAGACTCCAACTTTTACCGA**GCCCTGATGGATGAAGAGGACATGG**AGGATGT
AGTTGATGCTGATGA**aTtcCTTATCCCcCAGCA**AGGCTTCTTCAACAGCCCGTCCACGTCGAGGACTC
CCCTCTTGAGTTCTCTGGTATGCCCCCCCTCTTGATTTAAATTGATTTACTTTCCATTTTCCATATTTT

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo EGFR-Y1018F	5' label	Sequence 5' → 3'	3' label	Oligo Type
EGFR-Y1018F-MUT_F	n/a	**CATTTGCCAAGCCCTACA**	n/a	Mutant Forward
EGFR-Y1018F-MUT_PROBE	FAM	**CCATGTCCTCTTCATCCATCAGGGC**	BHQ	Mutant Probe
EGFR-Y1018F-MUT_R	n/a	**TGCTGGGGGATAAGGAAT**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo EGFR-Y1018F	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

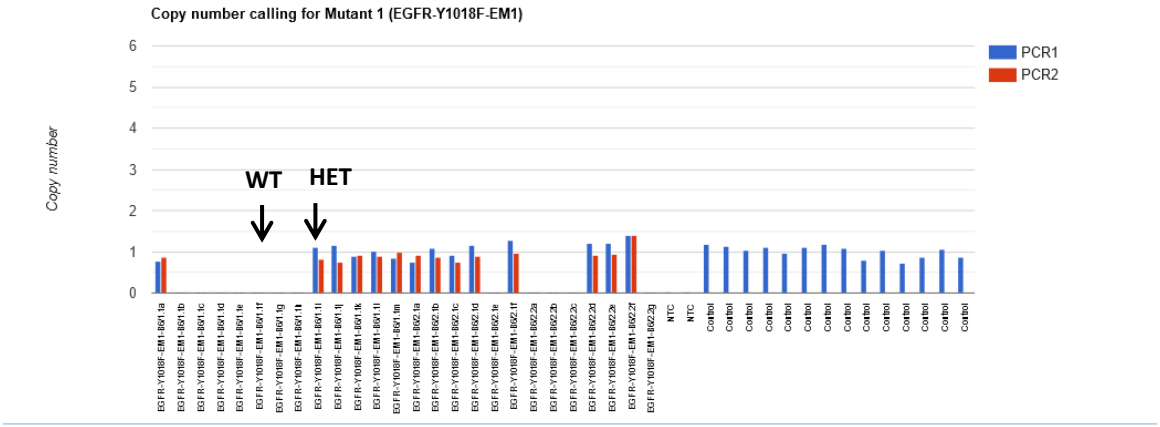
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95^{o}C for 20 sec
Then 40 cycles of;
95^{o}C for 3 sec
60^{o}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.

EGFR- EGFR-Y1018F -MUT1 assays copy called results, image showing copy number chart for Mutant assay (Task 331610 results)



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Date: 07/06/2021

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