

Tgfbr3-Flox

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00001281092 of *Tgfbr3*. 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

Tgfbr3-Flox

Tgfbr3-Flox-WT1 assay (FAM labelled)

TTAAATTATTCCTGGGTTACAGATAGCACCCAATCTAGAATACATACTGTGCAAAGAGTACTTACTGA
TTAGGAAATGACATAGCTAAAACTTTTAAAGTCCATGTT**TGCAAGAGAGGGTGTGTG**TTTCCCTT
AAATGT**CcagttctagacacggCTCTGGT**ATCCATATGTGTTGAAGAGAGGGTACTGTGTTCCCTGGTCAGT
CCACAGACATGGCAACATATAAACAGAGACTATCCAGTACTCAGAAGTTTGATCTTTAATGGAAGTC
TTCTTGGGGTTACCTGGAAGAAGTGGGCTTGGGATTGCTTGATTGATTAGCTCTTAGGGAGCTTGCC

Lower case letters denote the sequence that is replaced in the mutant allele.

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Tgfbr3-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
Tgfbr3-Flox-WT_F	n/a	<u>TGCAAGAGAGGGTGTGTG</u>	n/a	WT Forward
Tgfbr3-Flox-WT_PROBE	FAM	TCCAGTTCTAGACACGGCTCTGGT	ZEN-IBFQ	WT Probe
Tgfbr3-Flox-WT_R	n/a	<u>TGTTGCCATGTCTGTGGA</u>	n/a	WT Reverse

Tgfbr3-Flox -MUT1 assay (FAM labelled)

TTAGGAAATGACATAGCTAAAACTTTTAAAGTCCATGTTTGTCAAGAGAGGGTGTGTGTTTCCCTTA
AATGTCATCCGGGGGTACCGC**GTCGAGGCGATCGC****Cataacttcgt**atagcatacattatacgaagtatCTCTG
GTATCCATATGT**GTTGAAGAGAGGTA****CTGTGTTCC**TGGTCAGTCCACAGACATGGCAACATATAAAC
AGAGACTATCCAGTACTCAGAAGTTTGATCTTTAATGGAAGTCTTCTTGGGGTTACCTGGAAGAAGT

Lower case letters denote the inserted LoxP sequence

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Tgfbr3-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
Tgfbr3-Flox-MUT_F	n/a	<u>ATGTCATCCGGGGGTACC</u>	n/a	MUT Forward
Tgfbr3-Flox-MUT_PROBE	FAM	ACGAAGTTATGCGATCGCCTCGAC	ZEN-IBFQ	MUT Probe
Tgfbr3-Flox-MUT_R	n/a	<u>GGAACACAGTACCTCTCTTCAAC</u>	n/a	MUT Reverse

Tgfb3-Flox

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCTATGA

Oligo Tgfb3-Flox	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

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
ddH ₂ O	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

