

Tbc1d24-Flox

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exons, ENSMUSE00000309806 and ENSMUSE00000309780 of Tbc1d24. 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

Tbc1d24-Flox

Tbc1d24-Flox-3-WT1 assay (FAM labelled)

CTGGAGAATGCTTTGTTTTAGGGTGAGTTGGACTGAATGTGGGGTCCCTCTGGCCACGCCCTCCTG
CCCCAGCTGTTCCCAT**TTTCTCAGATGTTCTAAGCACT**TCAATCTTTCCTCCAA**AAACTAAATGCAGGC**
CCGGTCTGCAGTGGGCCAGGGTTCCCTGACCCCAGG**CTGTCCCAGAGGCTGGTA**GAAAGTCACCAG
GCCATGGACCTGGGCAGCCCTGCACAGGAGCCCAGCTTTATTTCCACCGTTCACTGTGGCCAAGATC

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	5' label	Sequence 5' → 3'	3' label	Oligo Type
Tbc1d24-Flox-WT_F	n/a	<u>TTTCTCAGATGTTCTAAGCACT</u>	n/a	WT Forward
Tbc1d24-Flox-WT_PROBE	FAM	<u>AAACTAAATGCAGGCCCGGTCTG</u>	ZEN-IBFQ	WT Probe
Tbc1d24-Flox-WT_R	n/a	<u>TACCAGCCTCTGGGACAG</u>	n/a	WT Reverse

Tbc1d24-Flox-5-MUT1 assay (FAM labelled)

CAAGCTAGCTTGCTCAGGTACAGGTAGTATTCAAGTCTCAAATGACAATTCACAAACTCTTGACCACC
CTACCCCAACCTGAAGGCCCTGC**CTCCAACCACACAAGCAAT**CCGGGGGTACCGC**GTCGAGGCGAT**
CGCataacttcgtatagcatacattatacgaag**gttatTTGAGTTCCTTCTTCCAG**CCCTGGGAGTGCCTGAGCG
CCACAGTGGCACTCACCTTCTTACGTCCAGGGCTCAGCTCCCCTGGGTCTCTGCTTTCTGCTTCCAG

Lower case letters denote the inserted LoxP sequence

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo	5' label	Sequence 5' → 3'	3' label	Oligo Type
Tbc1d24-Flox-MUT_F	n/a	<u>CTCCAACCACACAAGCAATC</u>	n/a	Mutant Forward
Tbc1d24-Flox-MUT_PROBE	FAM	<u>ACGAAGTTATGCGATCGCCTCGAC</u>	ZEN-IBFQ	Mutant Probe
Tbc1d24-Flox-MUT_R	n/a	<u>GCTGGAAGAAGGAACTCAAATAAC</u>	n/a	Mutant Reverse

Tbc1d24-Flox

Dot1l internal control (VIC labelled)

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

Oligo Tbc1d24-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

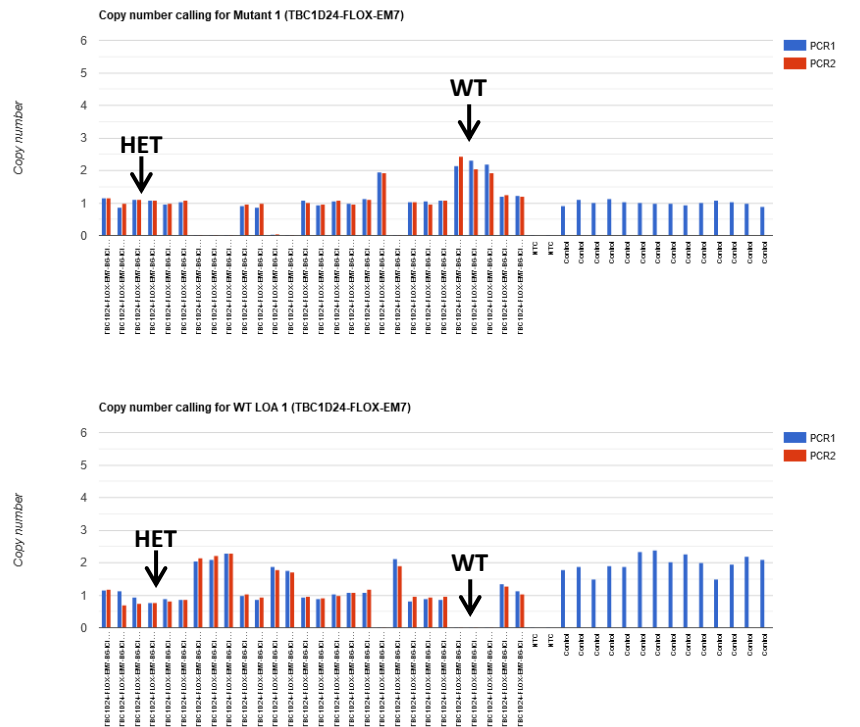
60°C for 30 sec

Tbc1d24-Flox

Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

Tbc1d24-Flox-3-WT1 and Tbc1d24-Flox-5-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 314862 results)



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