TBC1D24-P135L

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; P135L in exon ENSMUSE00000475486 of *TBC1D24*. 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:

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-P135L

TBC1D24-P135L-UNI1 assay (FAM labelled)

ACAGTGACATTGTGGGGAAGATTGTGGGCAAGCACAGCAGCAGTAGTCTGCCCTTGCCTGAGTTTG
TAGACAACACTCAGGTGCCCACCTACTGCCTGAACACACGGGGTGAAGGGGCTGT**GCGCAAGATCC**
TCCTGTGTAT**TGCCAACCAGTTCCCTGACATC**TCCTTCTGCCCTGCCCTGCcTGCtGTgGTGGCCTTGC
TACTGCACTACAGCA**TCGATGAAGCTGAGTGTTTCG**AAAAAGCCTGCCGCATCTTATCCTGCAATGA
CCCCACCAAGAAGCTCATTGACCAGAGCTTCCTGGCCTTTGAGTCTTCCTGTATGACATTTGGGGACC

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 TBC1D24-P135L	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
TBC1D24-P135L-WT_F	n/a	**GCGCAAGATCCTCCTGTGTA**	n/a	Wild type Forward
TBC1D24-P135L-WT_PROBE	FAM	**TGCCAACCAGTTCCCTGACATC**	BHQ1	Wild type Probe
TBC1D24-P135L-WT_R	n/a	**CGAAACACTCAGCTTCATCGA**	n/a	Wild type Reverse

TBC1D24-P135L-MUT1 assay (FAM labelled)

ACAGTGACATTGTGGGGAAGATTGTGGGCAAGCACAGCAGCAGTAGTCTGCCCTTGCCTGAGTTTG
TAGACAACACTCAGGTGCCCACCTACTGCCTGAACACACGGGGTGAAGGGGC**TGTGCGCAAGATCC**
TCCTGTGTATTGCCAACCAGTTCCCTGACATCTCCTTCTGCCCTGCCCTGC**tTGCaGTcGTGGCCTTG**C
TACTGCACTACAGCATCGATGAAGCTGAGTGTTTCGAAAAAGCCTGCCGCATCTTATCCTGCAATGA
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 TBC1D24-P135L	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
TBC1D24-P135L-MUT_F	n/a	**TGTGCGCAAGATCCTCCTG**	n/a	Mutant Forward
TBC1D24-P135L-MUT_PROBE	FAM	**TGTATTGCCAACCAGTTCCCTGACA**	BHQ1	Mutant Probe
TBC1D24-P135L-MUT_R	n/a	**CAAGGCCACGACTGCAA**	n/a	Mutant Reverse

TBC1D24-P135L

Dot1l internal control (VIC labelled)

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

Oligo TBC1D24-P135L	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

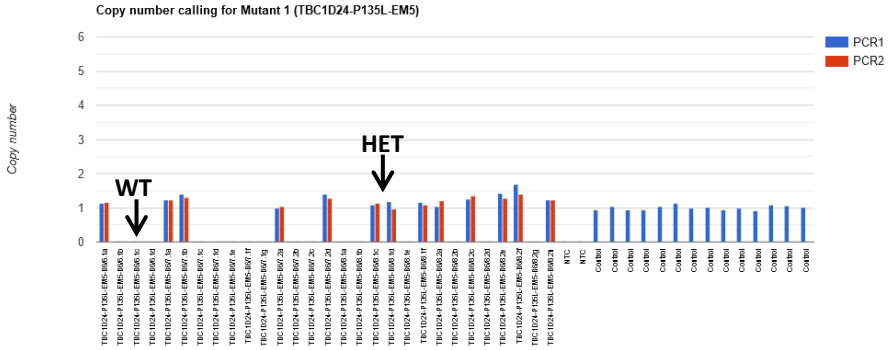
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

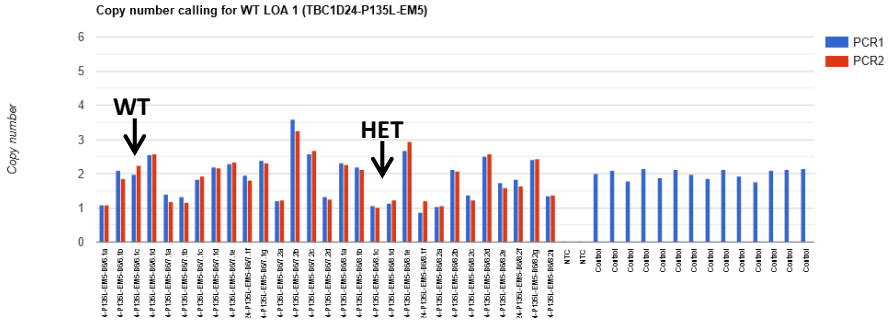
TBC1D24-P135L

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-P135L-WT1 and TBC1D24-P135L -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 289067 results)





Version No.	1
Date:	03/07/2023
Created/Updated by:	DF
Approved by:	DJW