CTNNB1-H720X-EM1-B6

Allele Description

This is a CRISPR/Cas9 induced mutation creating point mutation H720X in exon ENSMUSE00000786527 of *Ctnnb1*. 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:

- Universal probe and Universal primer designed 5' of the point mutation.
- Wild type (WT) specific primer binding to the WT base mutated in the mutant allele.
- Mutant specific primer binding to the H720X point mutation.

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

Ctnnb1-H720X-WT1 assay (FAM labelled)

TGGCAAGATGTCCTTA**CCACAGTTAATCAGTCTCTCCTCTTTC**C**TTTGCCCATCTTGTGGACACCC**TGACTCCTA
CAGATCCCAGCTACCGTTCTTTTcA**cTctGGTGGATACGGCCAG**GATGCCTTGGGGATGGACCCTATGATGGA

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Ctnnb1-H720X-UNI_F	n/a	**CCACAGTTAATCAGTCTCTCCTCTTTC**	n/a	Universal Forward
Ctnnb1-H720X-UNI_PROBE	FAM	**TTTGCCCATCTTGTGGACACCC**	BHQ	Universal Probe
Ctnnb1-H720X-WT_R	n/a	**CTGGCCGTATCCACCAGAG**	n/a	Wild type Reverse

Ctnnb1-H720X-MUT1 assay (FAM labelled)

TGGCAAGATGTCCTTA**CCACAGTTAATCAGTCTCTCCTCTTTC**C**TTTGCCCATCTTGTGGACACCC**TGACTCCTA
CAGATCCCAGCTACCGTTCTTTTta**gTaaGGTGGATACGGCCAGG**ATGCCTTGGGGATGGACCCTATGATGGA

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Ctnnb1-H720X-UNI_F	n/a	**CCACAGTTAATCAGTCTCTCCTCTTTC**	n/a	Universal Forward
Ctnnb1-H720X-UNI_PROBE	FAM	**TTTGCCCATCTTGTGGACACCC**	BHQ	Universal Probe
Ctnnb1-H720X-MUT_R	n/a	**CCTGGCCGTATCCACCTTAC**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo Name	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

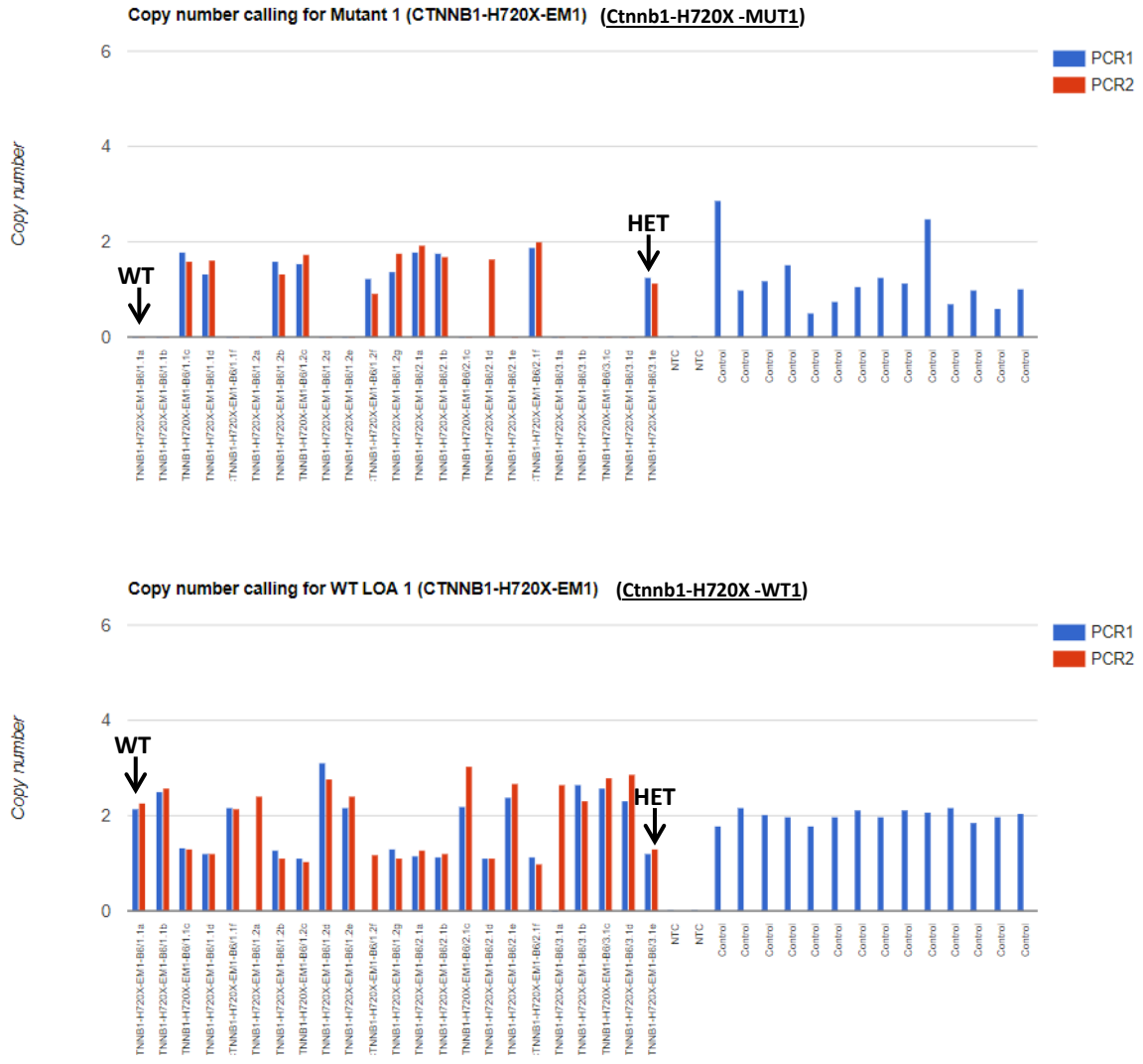
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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

Analysis

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

Ctnnb1-H720X-WT1 and Ctnnb1-H720X -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 266250 results)



Version No.	1
Date:	25.01.2020
Created/Updated by:	AC

Name of Mouse model or mutation:
CTNNB1-H720X-EM1-B6

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

Type of mutation:
SNP: H720X

Delivery method:
Cytoplasmic injection into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
AGCTACCGTTCTTTTCACTC	TGG

ssODN sequence (5'-3'):
ctggcaagatgtccttaccacagttaatcagtctctcctctttcctttgcccatcttgtggacaccctgactcctacagATCCCAGCT
ACCGTTCTTTTTAGTAAGGTGGATACGGCCAGGATGCCTTGGGGATGGACCCTATGATGGAGCATG
AGATGGGTGGCCACCACCCTGGTGCTGACTATCCAGTTGATGGGCT

Microinjection mixes:
Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 µm filter and autoclaved. Cas9 mRNA, sgRNAs and ssODNs were diluted and mixed in MIB to the working concentrations of 50 ng/µl, 6.25 ng/µl each and 50 ng/µl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.