CNTNAP2-FLOX-EM3-B6

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00000509035 of *CNTNAP2*. 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

CNTNAP2-FLOX3'-WT1 assay (FAM labelled)

TCTTTTAACAATCACACACACAC**ACACACACACACACACACACGTA**TGCATGCATGTATATAGCACTGTGTTG
TTTTCTCTGTTTTTAGgatgtaagtataaagactacatta**actgagggagtgaataccacct**agcatacactaagtTTGTTCTTAAGT
GGTTAATGCCTATGATCTAAGCAATAGGCATGTAGAGGCCTGAAAATCAGAAGTGCAACCTCATCCTCATTTA
CATAGTGACTTTGTGACAAGCATGAGCAGCTGAGGTGTATGAGACCTTGTGTCAAA

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
CNTNAP2-FLOX3'-UNI_F	n/a	**ACACACACACACACACACACGTA**	n/a	Universal Forward
CNTNAP2-FLOX3'-UNI_PROBE	FAM	**TGCATGCATGTATATAGCACTGTGTTGTTT**	BHQ	Universal Probe
CNTNAP2-FLOX3'-WT_R	n/a	**aggtggtattcactccctcagt**	n/a	WT Reverse

CNTNAP2-FLOX3'-MUT1 assay (FAM labelled)

TCTTTTAACAATCACACACACAC**ACACACACACACACACACACGTA**TGCATGCATGTATATAGCACTGTGTTG
TTTTCTCTGTTTTTAGataacttcgtatagcatacatta**tacgaagttatcgccggcg**ggtctgagctcgccatcagtTTGTTCTTAAG
TGGTTAATGCCTATGATCTAAGCAATAGGCATGTAGAGGCCTGAAAATCAGAAGTGCAACCTCATCCTCATTT
ACATAGTGACTTTGTGACAAGCATGAGCAGCTGAGGTGTATGAGACCTTGTGTCAAA

Lower case letters denote the inserted LoxP sequence
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
CNTNAP2-FLOX3'-UNI_F	n/a	**ACACACACACACACACACACGTA**	n/a	Universal Forward
CNTNAP2-FLOX3'-UNI_PROBE	FAM	**TGCATGCATGTATATAGCACTGTGTTGTTT**	BHQ	Universal Probe
CNTNAP2-FLOX3'-MUT_R	n/a	**cgccggcgataacttcgta**	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

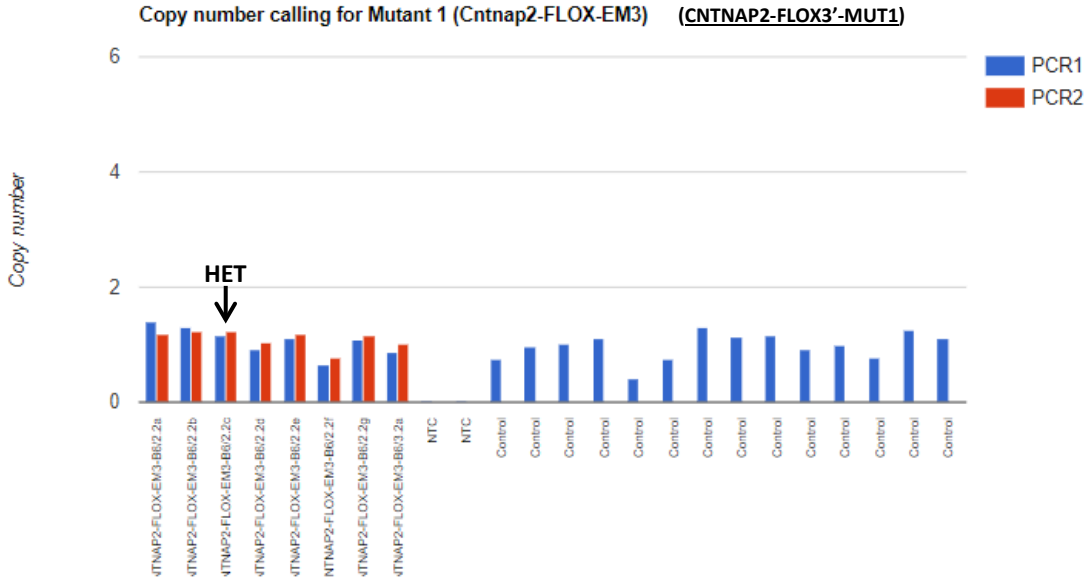
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

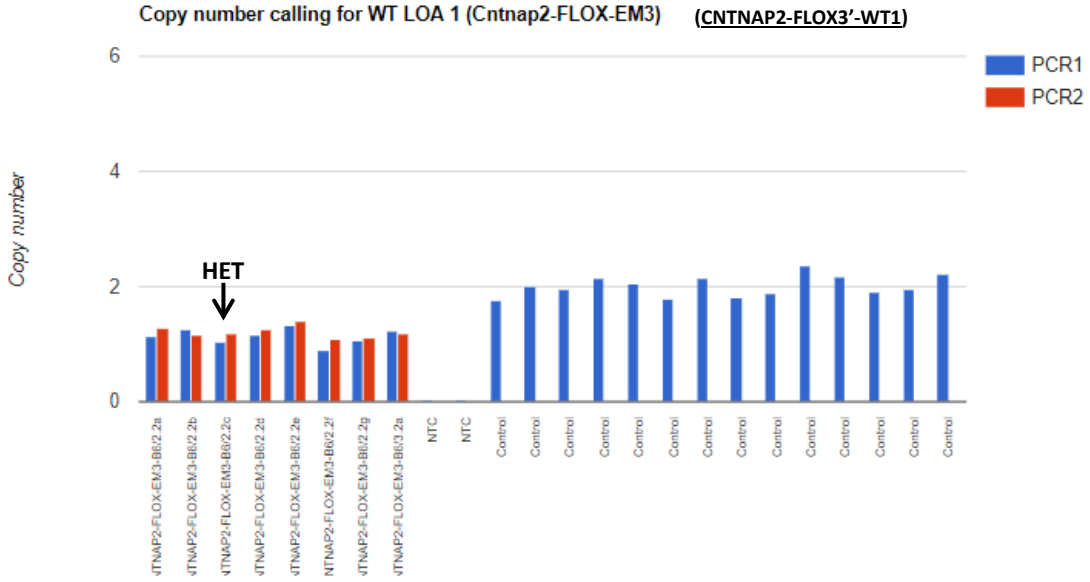
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

CNTNAP2-FLOX3'-WT1 and CNTNAP2-FLOX3'-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 264796 results)





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

Name of Mouse model or mutation:
CNTNAP2-FLOX-EM3-B6

Description:
Floxed model made using CRISPR/Cas9: Exon 4 (ENSMUSE00000509035) flanked by LoxP sites.

Type of mutation:
Flox

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

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
AGCTCTCTAGATTAGCAGAA	GGG
GAGAACCCCATCTTTGACTC	TGG
CAAACTTAGTGTATGCTAGG	TGG
GAACAAACTTAGTGTATGCT	AGG

lssDNA donor sequence (5'-3'):

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LOCUS       CNTNAP2_flox_lss        928 bp    DNA     linear      30-APR-2019
FEATURES             Location/Qualifiers
     misc_feature    130..163
                     /note="LoxP"
     misc_feature    122..129
                     /note="AsiSI (SfaAI)"
     PCR_primer      101..121
                     /note="LoxPF"
     misc_feature    1..100
                     /label=lssDNA donor 5' HA
     misc_feature    464..611
                     /note="Exon 4 (ENSMUSE00000509035)"
     misc_feature    767..800
                     /note="LoxP"
     misc_feature    801..808
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                /note="MreI"
     PCR_primer     complement(809..828)
                /note="LoxPR"
     misc_feature   829..928
                /label=lssDNA donor 3' HA
     PCR_primer     complement(907..928)
                /note="Cntnp2_RNA-R"
     misc_feature   164..766
                /note="Critical region"
     source         1..928
                /dnas_title="CNTNAP2_flox_lssDNA"
ORIGIN
        1 CAAACAAAAG ACTTGTTGGG TCATGTGTGT GTAAGTCCAA TGTTGATTCA CTGTCTCTGT
       61 CTCTTTCCAT TTTATTTTGC AAGACAAGAT CTCACAGGCC atccgggggt accgcgtcga
      121 gGCGATCGCA TAACTTCGTA TAGCATACAT TATACGAAGT TATACTCTGG AGTGCTGTGA
      181 TTTCTGGAGC CCTCACCTGC TAACTTTTTT CTGAGTTCTG GGGATCCAAT GATCTTCAGG
      241 CTTCATGAAA AGTGTTTTAG CTCCTTAGAT ACCCCCTGCA AAGCACCAAA ATAATACATT
      301 TTTAAATAAA AGAAACGAAA AGTTTTATTT TCAGATGTTT CTGATGTAAG AAGATAGAAC
      361 TTGATCACCC AAGAAGAAAA TAAGAGTTTA TTCTTACACA AAGTATACGT TTGTCACTGA
      421 GATGTTCTTC AAAGGTTTTT AATTTTCTTT TCTTTCCTTG AAGGCTTTTC CAGGAAACGT
      481 CAATTCTGAC AGCGTGGTCC GACACGACCT GCAGCATGCA GTAGTTGCCC GTTATGTACG
      541 CGTTGTGCCC TTGGACTGGA ATGGAGAAGG TCACATCGGG CTGCGTGCGG AAGTCTATGG
      601 CTGCGCCTAC TGTGAGTGTC AAGTTTATCG TGGGTCATCT TCATTGAAAT AGCAGTTTTT
      661 TTTAATGTTG CTTGTACTCT TTTAACAATC ACACACACAC ACACACACAC ACACACACAC
      721 GTATGCATGC ATGTATATAG CACTGTGTTG TTTTCTCTGT TTTTAGATAA CTTCGTATAG
      781 CATACATTAT ACGAAGTTAT CGCCGGCGgg tctgagctcg ccatcagtTT GTTCTTAAGT
      841 GGTTAATGCC TATGATCTAA GCAATAGGCA TGTAGAGGCC TGAAAATCAG AAGTGCAACC
      901 TCATCCTCAT TTACATAGTG ACTTTGTG
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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 nm filter and autoclaved. Cas9 mRNA, sgRNAs and lssDNA donor were diluted and mixed in MIB to the working concentrations of 100 ng/µl, 50 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.