Name of Mouse model or mutation:
BCAP31-A147P-EM1-B6N
BCAP31-A147P-EM2-B6N

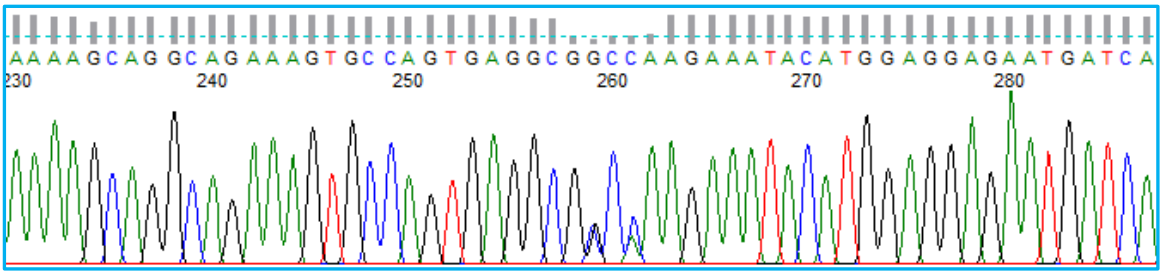
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: A147P

Sequence details
WT
CGCAAGAAGGAAAACAGGGCTGAGGCTTGGTTAGCACATCTAGGCCCTCACCTGGAAAATGCTGGT
GTTCCAAGACCTATTGCTATATATCTAGGTCGTGCCTGGGTGTGCTCTAGCTTCTGATCACTGCCTGA
GATACTTCCCAGAGCCCAATGCAGCTGGTTTTTTTTGGTGAGGGGGGGGATGGCAGAGGCCAGTTCC
TGGGCCCTACTTTGTGTTGCCAGGTCCAGGCTGGAGGCAGGGTAGGCAGGTAGCAGCCAAGAAGA
GTTCTTGCCAGGCCCTTTGCTCTCATGCTTCTCACTTTCCCCTTCTTTTTGTAAACAATGGCAGCCTGCT
TAGACGCCTGGTGACTCTCATCTCCCAGCAGGCCACACTGCTGGCCTCCAATGAAGCCTTTAAAAAG
CAGGCAGAAAGTGCCAGTGAGGCGGCCAAGAAATACATGGAGGAGAATGATCAGCTAAAGAAGGT
AAGTCTGCCCCTACAACCTGAGTGGGATGTCTAGAACCACAGTGCTAGTGGCTCATCTCTAAGAATC
CACAGTAGTTACTATTAGGCTAGGGCCTGTGTGATAATACTGGTCCCTGAAGGAGTTTTTAGTGGGT
AGGGTGTGCCTATTGGAACGATCCAGGATGACTCTGATGCCTTCTTTTCATTCTTGAGGACACAGCA
GAGGAAGAGGGTATTACCATTGATGTCTCCATATGCTGGTTCCAGTGGCAGTTGTTTTGTTTTGTTTT
GTTTTTATGAGAATGACTCTCTAATAACAGAGACCTGTGTTGAAGAAGGCCC

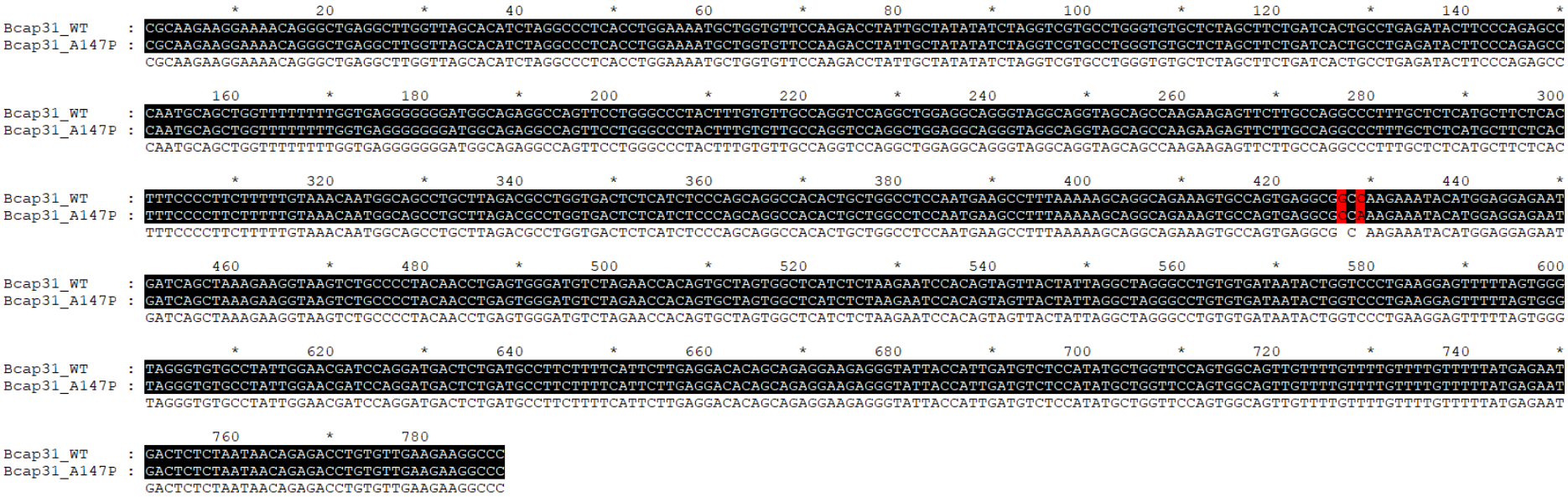
Mutant
CGCAAGAAGGAAAACAGGGCTGAGGCTTGGTTAGCACATCTAGGCCCTCACCTGGAAAATGCTGGT
GTTCCAAGACCTATTGCTATATATCTAGGTCGTGCCTGGGTGTGCTCTAGCTTCTGATCACTGCCTGA
GATACTTCCCAGAGCCCAATGCAGCTGGTTTTTTTTGGTGAGGGGGGGGATGGCAGAGGCCAGTTCC
TGGGCCCTACTTTGTGTTGCCAGGTCCAGGCTGGAGGCAGGGTAGGCAGGTAGCAGCCAAGAAGA
GTTCTTGCCAGGCCCTTTGCTCTCATGCTTCTCACTTTCCCCTTCTTTTTGTAAACAATGGCAGCCTGCT
TAGACGCCTGGTGACTCTCATCTCCCAGCAGGCCACACTGCTGGCCTCCAATGAAGCCTTTAAAAAG
CAGGCAGAAAGTGCCAGTGAGGCG**C**C**A**AAGAAATACATGGAGGAGAATGATCAGCTAAAGAAGGT
AAGTCTGCCCCTACAACCTGAGTGGGATGTCTAGAACCACAGTGCTAGTGGCTCATCTCTAAGAATC
CACAGTAGTTACTATTAGGCTAGGGCCTGTGTGATAATACTGGTCCCTGAAGGAGTTTTTAGTGGGT
AGGGTGTGCCTATTGGAACGATCCAGGATGACTCTGATGCCTTCTTTTCATTCTTGAGGACACAGCA
GAGGAAGAGGGTATTACCATTGATGTCTCCATATGCTGGTTCCAGTGGCAGTTGTTTTGTTTTGTTTT
GTTTTTATGAGAATGACTCTCTAATAACAGAGACCTGTGTTGAAGAAGGCCC

Heterozygous F1 animal sequence trace:

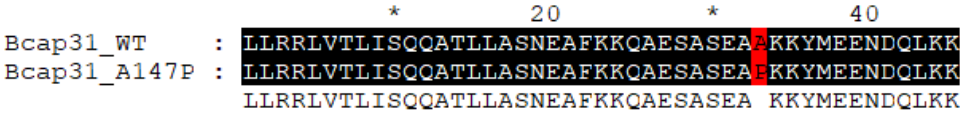


Please note that the BCAP31-A147P-EM1-B6N and BCAP31-A147P-EM1-B6N are the same sequence but derived from two different founders.

Nucleotide Alignment:



Predicted Protein Alignment:



QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_Bcap31-A147P_F1 primer (5'-3')	CGCAAGAAGGAAAACAGGGC
Geno_Bcap31-A147P_R1 primer (5'-3')	GGGCCTTCTTCAACACAGGT
Taq Polymerase used	ThermoFisher SuperFi Taq
Annealing Temperature (°C)	63
Elongation time (min)	0.5
WT product size (bp)	788
Mutant product size (bp)	788
Notes	Sequence with the following primers: Geno_Bcap31-A147P_F2 primer (5'-3': TACTTCCCAGAGCCCAATGC) and Geno_Bcap31-A147P_R2 primer (5'-3': CCTCTGCTGTGTCCTCAAGA)

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

Off-target site with ≤2 mismatches for guide used were checked with the following primers:

Off-target site	Sequence	Type	Primers used
12:31624130-31624152	ATTCTCTTCCATGAATTTCT TGG	Exonic	Amplified using: Bcap31_OT1_F1 (5'-3': CTTCAAATTTGTAAACCAAGAATGGCT) Bcap31_OT1_R1 (5'-3': TTACCTTCCAACAGAGGATGCAA) Sequenced using: Bcap31_OT1_SeqF2 (5'-3': CCTTAGATCAAGGCTATTGTCTC) Bcap31_OT1_SeqR1 (5'-3': CATTTCAATGCCGACTCCACC)

All amplicons were sent for Sanger sequencing. All animals taken forward for breeding did not show evidence of off-target activity at this site.

Copy counting by ddPCR

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Bcap31-A147P-UNI1
Forward Primer (5'-3')	GAGCCACTAGCACTGTGGTT
Reverse Primer (5'-3')	GGAGGAGAATGATCAGCTAAAGAAG
Probe (5'-3')	CCCTACAACCTGAGTGGGATGTCTA
Label	FAM-BHQ1

The ddPCR assay recognises both the WT allele and the mutant allele. As the target is on the X chromosome, WT controls are expected to call at 2 copies for females and 1 copy for males. A correct mutation is expected to call at 2 copy for F1 (HET) female animals and 1 copy for F1 (HET) male animals.

Assay name	Bcap31-A147P-MUT1
Forward Primer (5'-3')	GTGGTTCTAGACATCCCACTCAG
Reverse Primer (5'-3')	GTGCCAGTGAGGCGC
Probe (5'-3')	ACATGGAGGAGAATGATCAGCTAAAGAAGG
Label	FAM-BHQ1

The ddPCR assay is potentially unique to the mutant allele of the gene as it sits across the targeted region. WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

BCAP31-A147P

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; A147P, in exon ENSMUSE00000208823 of *BCAP31*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.
BCAP31 is X-Linked.

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:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

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

BCAP31-A147P-WT1 assay (FAM labelled)

GTTCTTGCCAGGCCCTTTGCTCTCATGCTTCTCACTTTCCCCTTCTTTTTGTAAACAATGGCAGCCTGCT
TAG**ACGCCTGGTGACTCTCATCTC**CCAGCAGG**CCACACTGCTGGCCTCCAATGA**AGCCTTTAAAAAG
CAGGCAGAAAGTGCCAGTGAGGCGg**CcAAGAAATACATGGAGGAGAATGATC**AGCTAAAGAAGG
TAAGTCTGCCCCTACAACCTGAGTGGGATGTCTAGAACCACAGTGCTAGTGGCTCATCTCTAAGAAT

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 BCAP31-A147P	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
BCAP31-A147P-WT_F	n/a	**ACGCCTGGTGACTCTCATCTC**	n/a	Wild type Forward
BCAP31-A147P-WT_PROBE	FAM	**CCACACTGCTGGCCTCCAATGA**	ZEN/IBFQ	Wild type Probe
BCAP31-A147P-WT_R	n/a	**GATCATTCTCCTCCATGTATTTCTTGG**	n/a	Wild type Reverse

BCAP31-A147P-MUT1 assay (FAM labelled)

GTTCTTGCCAGGCCCTTTGCTCTCATGCTTCTCACTTTCCCCTTCTTTTTGTAAACAATGGCAGCCTGCT
TAG**ACGCCTGGTGACTCTCATCTC**CCAGCAGG**CCACACTGCTGGCCTCCAATGA**AGCCTTTAAAAAG
CAGGCAGAAAGTGCCAGTGAGGCG**cCaAAGAAATACATGGAGGAGAATGATCA**GCTAAAGAAGG
TAAGTCTGCCCCTACAACCTGAGTGGGATGTCTAGAACCACAGTGCTAGTGGCTCATCTCTAAGAAT

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 BCAP31-A147P	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
BCAP31-A147P-MUT_F	n/a	**ACGCCTGGTGACTCTCATCTC**	n/a	Mutant Forward
BCAP31-A147P-MUT_PROBE	FAM	**CCACACTGCTGGCCTCCAATGA**	BHQ	Mutant Probe
BCAP31-A147P-MUT_R	n/a	**TGATCATTCTCCTCCATGTATTTCTTTGG**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo BCAP31-A147P	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

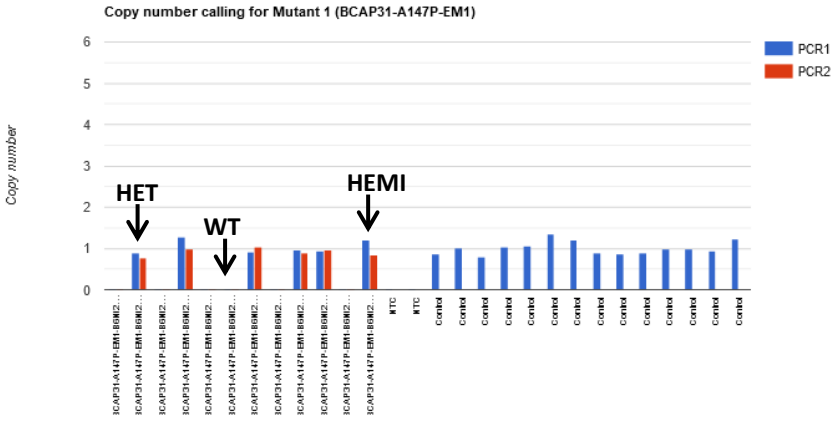
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

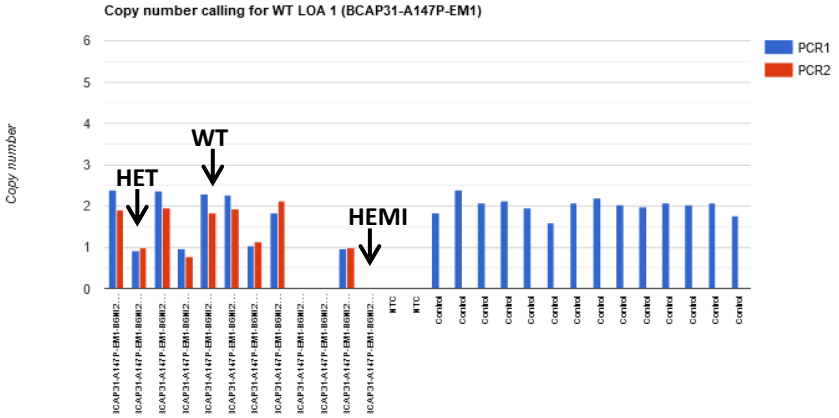
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

BCAP31-A147P-WT1 and BCAP31-A147P -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 350034 results)





Version No.	1
Date:	09/09/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman