Name of Mouse model or mutation:
BACH2-G26K-EM1-B6

Description:
Point mutation model made using CRISPR/Cas9.

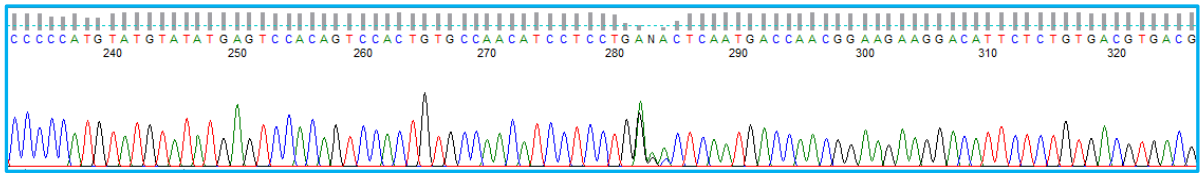
Type of mutation:
Point mutation: G26K

Sequence details
WT
CAGCATATCCTTCAGCCGGTAACCAGGAAAACCTGTAGCTTGCCCTCTGTCCCAGAGGAACAATAAT
ACAGCCACATTACTCAAAATCACACACATTTTAGCCTATTTAGGCCTTTCTAATTTGTCATATCAGGAT
ATTAGCCCAAAATTCCCGTGGATCCCACTGTGTACAGAGTATGAGTCTCCTGCAGACTTTATTATACC
ATCTTTTAAACCATTGATTTAAAACCACACTTCTATTTTCCTTCCTTTTTCCTTTTCCTTTTCCTTTCCTTT
TCTTTCCTCTTCCTTTCTTTTCTTTCCTTTCCTTCCCACTTTTAAGGTGGGAAGCAGGTTTACAGGAAAT
CACCTGAGTGCTGACTCATTCCTTGTTCGCAGCCCCTCACAGGAGAATGTAATTAACATCAATATAAT
TAGCCCCAGGGCTATCCACAACACTACGGATGCTTAGTAGTCCTGCTCCCTCTGCCAGGGCTCATGG
GGACCTGGGAGCTTTGGTGGTCTTTTTTCCTCCTTTGTGACTGAGCCAGCCTCTCTTTATTCCAGGGT
TTGAACGGCATGTCTGTGGATGAGAAGCCTGGCTCCCCCATGTATGTATATGAGTCCACAGTCCACT
GTGCCAACATCCTCCTGGGCCTCAATGACCAACGGAAGAAGGACATTCTCTGTGACGTGACGCTGAT
CGTGGAGAGGAAGGAGTTCCGAGCCCACCGGGCTGTGCTGGCCGCATGCAGTGAATACTTCTGGCA
AGCACTGGTTGGACAGACGAAAGATGACTTGGTGGTCAGCTTGCCTGAGGAGGTACAGTGATTTGG
TGTGGTTGTGGGGTTGTGGTGGAAGTTAGACGTGCACTGTGCCACGTGGATGGCCACGTCACCCAT
GTCCACATCA

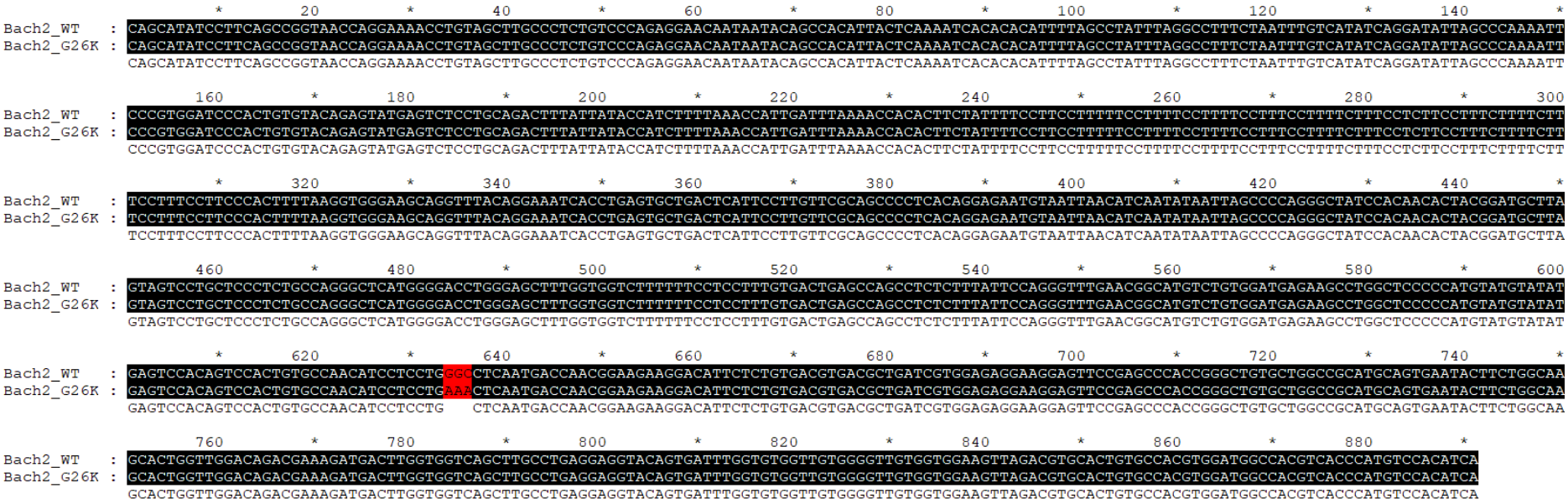
BACH2-G26K-EM1-B6
CAGCATATCCTTCAGCCGGTAACCAGGAAAACCTGTAGCTTGCCCTCTGTCCCAGAGGAACAATAAT
ACAGCCACATTACTCAAAATCACACACATTTTAGCCTATTTAGGCCTTTCTAATTTGTCATATCAGGAT
ATTAGCCCAAAATTCCCGTGGATCCCACTGTGTACAGAGTATGAGTCTCCTGCAGACTTTATTATACC
ATCTTTTAAACCATTGATTTAAAACCACACTTCTATTTTCCTTCCTTTTTCCTTTTCCTTTTCCTTTCCTTT
TCTTTCCTCTTCCTTTCTTTTCTTTCCTTTCCTTCCCACTTTTAAGGTGGGAAGCAGGTTTACAGGAAAT
CACCTGAGTGCTGACTCATTCCTTGTTCGCAGCCCCTCACAGGAGAATGTAATTAACATCAATATAAT
TAGCCCCAGGGCTATCCACAACACTACGGATGCTTAGTAGTCCTGCTCCCTCTGCCAGGGCTCATGG
GGACCTGGGAGCTTTGGTGGTCTTTTTTCCTCCTTTGTGACTGAGCCAGCCTCTCTTTATTCCAGGGT
TTGAACGGCATGTCTGTGGATGAGAAGCCTGGCTCCCCCATGTATGTATATGAGTCCACAGTCCACT
GTGCCAACATCCTCCTG**AAA**CTCAATGACCAACGGAAGAAGGACATTCTCTGTGACGTGACGCTGAT
CGTGGAGAGGAAGGAGTTCCGAGCCCACCGGGCTGTGCTGGCCGCATGCAGTGAATACTTCTGGCA

AGCACTGGTTGGACAGACGAAAGATGACTTGGTGGTCAGCTTGCCTGAGGAGGTACAGTGATTTGG
TGTGGTTGTGGGGTTGTGGTGGAAGTTAGACGTGCACTGTGCCACGTGGATGGCCACGTCACCCAT
GTCCACATCA

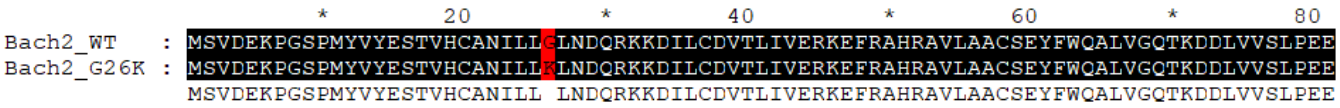
BACH2-G26K-EM1-B6 Heterozygous F1 animal sequence trace:



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_BACH2_G26K_F3 primer (5'-3')	CAGCATATCCTTCAGCCGGT
Geno_BACH2_G26K_R3 primer (5'-3')	TGATGTGGACATGGGTGACG
Taq Polymerase used	Thermofisher Super-Fi
Annealing Temperature (°C)	61
Elongation time (min)	0.5
WT product size (bp)	891
Mutant product size (bp)	891
Notes	Sequence with Geno_BACH2_G26K_F4 primer (5'-3': TAAGGTGGGAAGCAGGTTTAC) and Geno_BACH2_G26K_R3 primer.

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.

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	BACH2-G26K-MUT1
Forward Primer (5'-3')	ACTGTGCCAACATCCTCCTGAAA
Reverse Primer (5'-3')	GGTGGGCTCGGAACTCCTT
Probe (5'-3')	TGACCAACGGAAGAAGGACATTCTCTG
Label	FAM-BHQ1

The ddPCR assay is specific to the G26K mutation in the Bach2 gene and only the donor template sequence is expected to be recognised by this assay. WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	BACH2-G26K-UNI1
Forward Primer (5’-3’)	CACTGTGCCAACATCCTCCT
Reverse Primer (5’-3’)	GAACTCCTTCCTCTCCACGATCAG
Probe (5’-3’)	CAACGGAAGAAGGACATTCTCTGTGACG
Label	FAM-BHQ1

This ddPCR assay is universal to Bach2 WT and G26K alleles are recognised by this assay. WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies 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.

No random insertions of donor sequence were detected in the animals taken forward to establish the colony.

Bach2-G26K

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; G26K in *BACH2*. 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:

- 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

Bach2-G26K

Bach2-G26K-WT1 assay (FAM labelled)

TTGAA**CGGCATGTCTGTGGATGAGAAG**CCTGGCTCCCCC**ATGTATGTATATGAGTCCACAGTCC**AC
TGTGCCAACATCCTCCT**GggcCTCAATGACCAACGG**AAGAAGGACATTCTCTGTGACGTGACGCTGA

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 Bach2-G26K	5' label	Sequence 5' → 3'	3' label	Oligo Type
Bach2-G26K-UNI_F	n/a	**CGGCATGTCTGTGGATGAGAAG**	n/a	Universal Forward
Bach2-G26K-UNI_PROBE	FAM	**ATGTATGTATATGAGTCCACAGTCC**	ZEN/IBFQ	Universal Probe
Bach2-G26K-WT_R	n/a	**CCGTTGGTCATTGAGGCCC**	n/a	Wild type Reverse

Bach2-G26K-MUT1 assay (FAM labelled)

TTGAA**CGGCATGTCTGTGGATGAGAAG**CCTGGCTCCCCC**ATGTATGTATATGAGTCCACAGTCC**AC
TGTGCCAACATCCTCC**TGaaaCTCAATGACCAACGGAAG**AAGGACATTCTCTGTGACGTGACGCTGA

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 Bach2-G26K	5' label	Sequence 5' → 3'	3' label	Oligo Type
Bach2-G26K-UNI_F	n/a	**CGGCATGTCTGTGGATGAGAAG**	n/a	Universal Forward
Bach2-G26K-UNI_PROBE	FAM	**ATGTATGTATATGAGTCCACAGTCC**	BHQ	Universal Probe
Bach2-G26K-MUT_R	n/a	**CTTCCGTTGGTCATTGAGTTTCA**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo Bach2-G26K	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

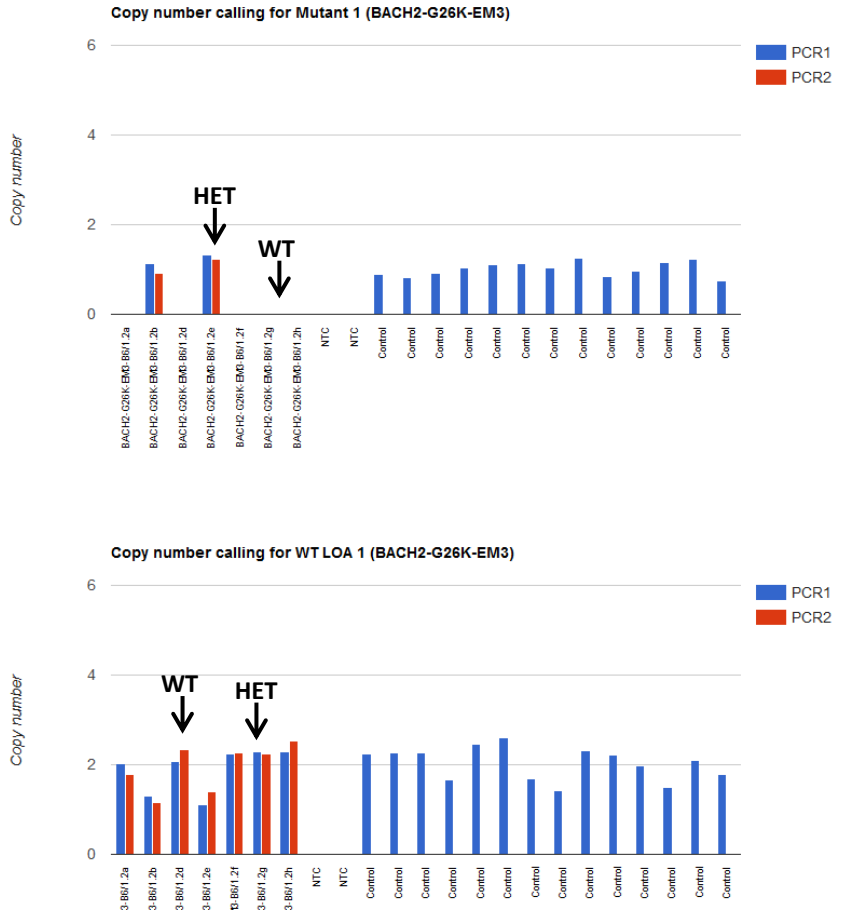
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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.

Bach2-G26K-WT1 and Bach2-G26K -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 280146 results)



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Date:	11/03/2020
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman