Name of Mouse model or mutation:
FGA-4X-EM1-B6

Description:
Series of point mutations made by CRISPR/Cas9 mutagenesis

Type of mutation:
SNP: Q241N, Q243N, Q257N and Q518N

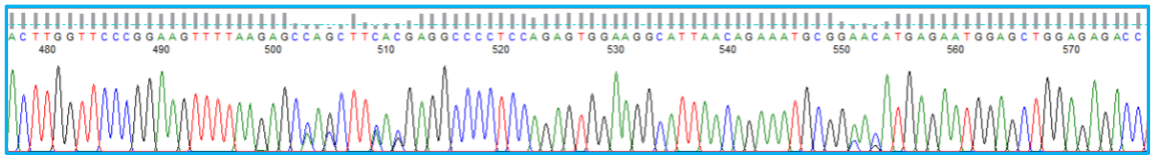
Sequence details
WT
AAGTCCGGAAATGCGAAGGAAATTCTTGAAGGGTGAATAGATATTTGAAAAGAACTCGCTCTGTCC
CGTTTGCCCTCCACTGGCTGAGACCTTGGCTTCAGCTTGGAGGAGGCCCTTCAGCCAGAGTTTACAG
GATTAGAGCCATTCTCCTTCCCCATTCAAATTCTAAAATGGATCCAAATAGTTTGGATCCTCCACACTT
CCTTGTCCCTCCACAGCTTCCTTTGAGGACCAGCCTGAGTAGCTAAAGACAAAGGGTAGGATTGATT
TTTCTGAGAGTCAAAGCAATAAAACCCATTCGCAAAAATGTAAACCAACTACCAGGAACTCAATTAT
TTCAATAGACATAATTCCTTTCTCTGTCTTCCTTTCTTTAGGTGGATATTGATATCAAGATCCGCTCTTG
CAAAGGGTCCTGCAGCAGGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCACCAAAA
ACAGCTTCAACAGGTCATCGCTAAAGAATTGCTTCCTACAAAAGACAGGCAGTACTTGCCAGCACTA
AAAATGTCTCCAGTTCCCGACTTGGTTCCCGGAAGTTTTAAGAGCCAGCTTCAGGAGGCCCCTCCAG
AGTGGAAGGCATTAACAGAAATGCGGCAGATGAGAATGGAGCTGGAGAGACCTGGGAAGGATGG
GGGTTCGCGAGGAGATTCACCAGGAGACTCGCGGGGAGATTCTAGAGGGGACTTTGCAACACGTG
GACCAGGGTCAAAGGCAGAAAACCCCACGAACCCTGGACCTGGTGGATCTGGGTATTGGCGTCCTG
GGAACTCCGGGTCTGGAAGTGATGGAAATCGGAACCCTGGGACCACGGGGTCTGATGGCACTGGA
GACTGGGGTACCGGAAGCCCTAGACCTGGCTCAGACTCTGGGAACTTTAGGCCTGCCAACCCTAACT
GGGGTGTGTTTTCAGAGTTTGGAGACAGTAGCAGCCCAGCCACAAGAAAAGAGTATCACACAGGTA
AAGCGGTCACTTCTAAAGGAGATAAAGAGCTCCTGATTGGAAAGGAGAAAGTCACCTCTTCTGGCA
CAAGCACCACACATCGTTCATGCTCTAAAACCATTACCAAGACTGTCACAGGTCCTGATGGTCGCCG
AGAAGTGGTCAAAGAAGTGATCACCTCGGATGATGGCTCAGACTGTGGCGATGCCACCGAGTTAGA
CATATCCCACAGTTTTAGCGGCAGTCTCGACGAACTCTCTGAAAGGCATCCTGACCTTTCTGGGTTTT
TTGACAACCACTTTGGTTTAATCTCACCTAACTTCAAAGAATTTGGCAGTAAGACCCATTCTGATTCC
GACATCCTCACAAACATTGAGGACCCCAGCTCCCATGTACCTGAGTTTTCTTCCAGTAGTAAAACCTC
AACTGTCAAAAAACAAGTAACCAAGACCTATAAAATGGCAGACGAGGCAGGAAGCGAAGCTCACC
GGGAAGGAGAAACTCGCAACACTAAGAGGGGCCGTGCCAGAGCTCGCCCAACGAGAGGTATCGAC
ACTTAACTTTCGGGAGATTCCCCTGACACCCCCCCCCCCCCTAGATTAAGTTAACCATTACTGCGAAG
TGCTTACCAGGCACGCTGCACCCGTTTCCTAACCTCCTTTAGTGTTTTGTTGGAATCTCATTTTTTTTTT
GGTCAACTTTTCATGCTAGACTGTACGTTCCTTGGGGGCAGGGACTTGACCATGTGTCTATTTCTGTA
ATTCCCAAATGCCTAACAGTGCAGTCATTTCTCAATAAATACATTTTAAATAAATGAACAAATTCTGC

CGAAACTCAGTTCTGAGTCTGTTTAACCGAATTCATTCAAAACGTGTGCGATTGTAATACCCAACCCC
CTAACCTTAAATTTAGTGTATGTTGCAGTTGACATTTAGATCAGGTTAAAAACTGTGTTCCATTAGTA
TGGACTGATGAATGCTTAGCTAGCTTTAAACTATCATTTGAGATTAGCATGGACACGTAAGATTTCA
AATCCATTTGAAGAGAGGTTGCTAAAGGATGAGTATCCTTTACCTGCTAAAAAATTACATCTCATTGT
AGGTGCATCCTTTTCGTGTGGGAGGAGGGAAGGAAGGGAGGAAGGAAGACAGGCAGGCAGGCAG
ACAGACAGGCAGGTAGGCAGTAGCTACTTCAATCTGGGTGATGCCTATCTTTGCA

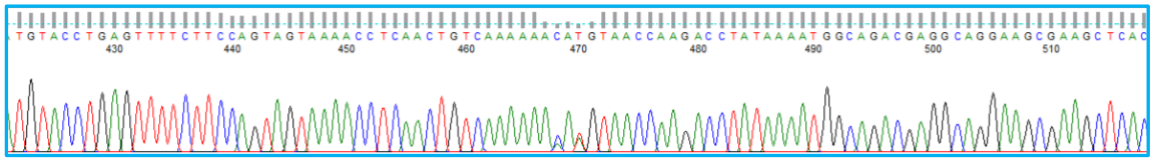
Mutant

AAGTCCGGAAATGCGAAGGAAATTCTTGAAGGGTGAATAGATATTTGAAAAGAACTCGCTCTGTCC
CGTTTGCCCTCCACTGGCTGAGACCTTGGCTTCAGCTTGGAGGAGGCCCTTCAGCCAGAGTTTACAG
GATTAGAGCCATTCTCCTTCCCCATTCAAATTCTAAAATGGATCCAAATAGTTTGGATCCTCCACACTT
CCTTGTCCCTCCACAGCTTCCTTTGAGGACCAGCCTGAGTAGCTAAAGACAAAGGGTAGGATTGATT
TTTCTGAGAGTCAAAGCAATAAAACCCATTCGCAAAAATGTAAACCAACTACCAGGAACTCAATTAT
TTCAATAGACATAATTCCTTTCTCTGTCTTCCTTTCTTTAGGTGGATATTGATATCAAGATCCGCTCTTG
CAAAGGGTCCTGCAGCAGGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCACCAAAA
ACAGCTTCAACAGGTCATCGCTAAAGAATTGCTTCCTACAAAAGACAGGCAGTACTTGCCAGCACTA
AAAATGTCTCCAGTTCCCGACTTGGTTCCCGGAAGTTTTAAGAGC**aac**CTT**aac**GAGGCCCCTCCAGA
GTGGAAGGCATTAACAGAAATGCGG**aac**ATGAGAATGGAGCTGGAGAGACCTGGGAAGGATGGGG
GTTCGCGAGGAGATTCACCAGGAGACTCGCGGGGAGATTCTAGAGGGGACTTTGCAACACGTGGA
CCAGGGTCAAAGGCAGAAAACCCCACGAACCCTGGACCTGGTGGATCTGGGTATTGGCGTCCTGGG
AACTCCGGGTCTGGAAGTGATGGAAATCGGAACCCTGGGACCACGGGGTCTGATGGCACTGGAGA
CTGGGGTACCGGAAGCCCTAGACCTGGCTCAGACTCTGGGAACTTTAGGCCTGCCAACCCTAACTG
GGGTGTGTTTTCAGAGTTTGGAGACAGTAGCAGCCCAGCCACAAGAAAAGAGTATCACACAGGTAA
AGCGGTCACTTCTAAAGGAGATAAAGAGCTCCTGATTGGAAAGGAGAAAGTCACCTCTTCTGGCAC
AAGCACCACACATCGTTCATGCTCTAAAACCATTACCAAGACTGTCACAGGTCCTGATGGTCGCCGA
GAAGTGGTCAAAGAAGTGATCACCTCGGATGATGGCTCAGACTGTGGCGATGCCACCGAGTTAGAC
ATATCCCACAGTTTTAGCGGCAGTCTCGACGAACTCTCTGAAAGGCATCCTGACCTTTCTGGGTTTTT
TGACAACCACTTTGGTTTAATCTCACCTAACTTCAAAGAATTTGGCAGTAAGACCCATTCTGATTCCG
ACATCCTCACAAACATTGAGGACCCCAGCTCCCATGTACCTGAGTTTTCTTCCAGTAGTAAAACCTCA
ACTGTCAAAAAA**aat**GTAACCAAGACCTATAAAATGGCAGACGAGGCAGGAAGCGAAGCTCACCGG
GAAGGAGAAACTCGCAACACTAAGAGGGGCCGTGCCAGAGCTCG**g**CCAACGAGAGGTATCGACAC
TTAACTTTCGGGAGATTCCCCTGACACCCCCCCCCCCCTAGATTAAGTTAACCATTACTGCGAAGTG
CTTACCAGGCACGCTGCACCCGTTTCCTAACCTCCTTTAGTGTTTTGTTGGAATCTCATTTTTTTTTTGG
TCAACTTTTCATGCTAGACTGTACGTTCCTTGGGGGCAGGGACTTGACCATGTGTCTATTTCTGTAAT
TCCCAAATGCCTAACAGTGCAGTCATTTCTCAATAAATACATTTTAAATAAATGAACAAATTCTGCCG
AAACTCAGTTCTGAGTCTGTTTAACCGAATTCATTCAAAACGTGTGCGATTGTAATACCCAACCCCCT
AACCTTAAATTTAGTGTATGTTGCAGTTGACATTTAGATCAGGTTAAAAACTGTGTTCCATTAGTATG
GACTGATGAATGCTTAGCTAGCTTTAAACTATCATTTGAGATTAGCATGGACACGTAAGATTTCAAAT
CCATTTGAAGAGAGGTTGCTAAAGGATGAGTATCCTTTACCTGCTAAAAAATTACATCTCATTGTAG
GTGCATCCTTTTCGTGTGGGAGGAGGGAAGGAAGGGAGGAAGGAAGACAGGCAGGCAGGCAGAC
AGACAGGCAGGTAGGCAGTAGCTACTTCAATCTGGGTGATGCCTATCTTTGCA

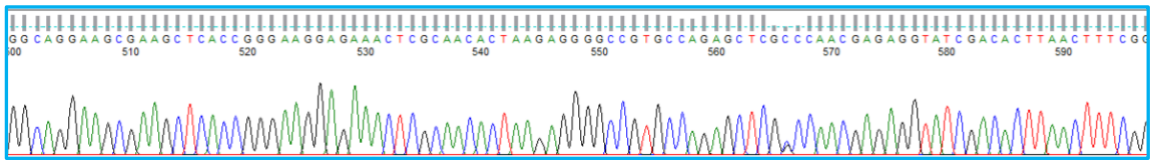
FGA-4X-EM1-B6 Heterozygous F1 animal sequence trace:



Q241N, Q243N, Q257N

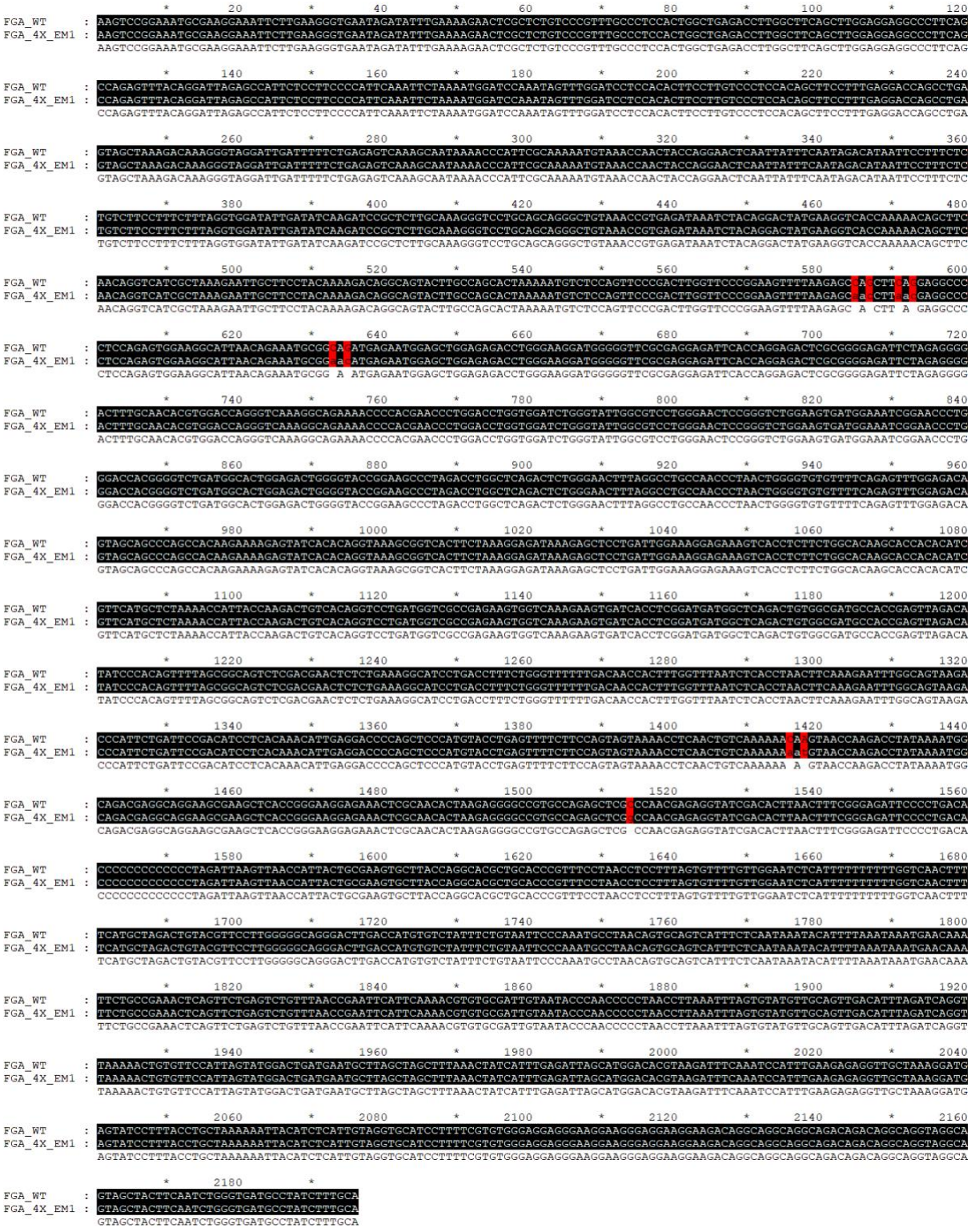


Q518N



Silent change to kill the PAM sequence of sgRNA FGA_3_3_ID: 454794723 (CGC > CGG Arginine)

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_FGA_F3 (5'-3')	AAGTCCGGAAATGCGAAGGA
Geno_FGA_R3 (5'-3')	TGCAAAGATAGGCATCACCC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	57
Elongation time (min)	2.5
WT product size (bp)	2196
Mutant product size (bp)	2196
Notes	To get full coverage of the allele, amplicons were sequenced with the following primers (5'-3'): Geno_Fga_F1: AAAGAACTCGCTCTGTCCCG Geno_Fga_R1: GTGCGATTGTAATACCCAACCC Geno_Fga_R2: AGGATGCACCTACAATGAGATGT Geno_FGA_4x_seqF: GGCCTGCCAACCCTAACTG Geno_FGA_4x_seqR: GACCGCTTTACCTGTGTGATAC

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 Fga_5.3 (ID: 454794574) were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
16:90871803-90871825	TTTTCAGAGTCAGCTTCAGG AGG	Intronic	FGA-4X_OT1_5_3_F1: GCTCTGTACGACAGCATCGG FGA-4X_OT1_5_3_R1: GACTCGGCTCTATTGCCTGTT

All amplicons were sent for Sanger sequencing. No evidence of off-target cutting at tested site(s) was detected.

Copy counting by ddPCR

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor templates were inserted only 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	Fga-4X-518-MUT1
Forward Primer (5'-3')	GATTCCGACATCCTCACAAACATTG
Reverse Primer (5'-3')	TGCCATTTTATAGGTCTTGGTTACatt
Probe (5'-3')	AGGACCCCAGCTCCCATGTACC
Label	FAM-BHQ1

The ddPCR assay is unique to the FGA Q518N point mutation. Therefore, WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	Fga-4X-241_243_257-MUT1
Forward Primer (5'-3')	CCGGAAGTTTTAAGAGCaacCTTaac
Reverse Primer (5'-3')	TCTCTCCAGCTCCATTCTCATgtt
Probe (5'-3')	CCCTCCAGAGTGGAAGGCATTAACA
Label	FAM-BHQ1

The ddPCR assay is unique to the FGA Q241N, Q243N and Q257N point mutations. Therefore, WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	Fga-4X -UNIV1
Forward Primer (5’-3’)	GGCCTGCCAACCCTAACTG
Reverse Primer (5’-3’)	GACCGCTTTACCTGTGTGATAC
Probe (5’-3’)	TTTCAGAGTTTGGAGACAGTAGCAGCC
Label	FAM-BHQ1

The ddPCR assay is universal to the FGA WT and FGA-4X allele. Therefore, WT controls are expected to call at 2 copies and 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 evidence of additional donor integrations was detected in the animals used to establish the colony.

FGA-4X

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; Q241N, Q243N, Q257N and Q518N in ENSMUSG00000059807. 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

FGA-4X-WT1 assay (FAM labelled)

CAAAGGGTCCTGCAGCAGGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCACCAAAA
ACAGCTTCAACAGGT**CATCGCTAAAGAATTGCTTCCTACA**A**AAGACAGGCAGTACTTGCCAGCAC**T
AAAAATGTCTCCAGTTCCCGACTTGGTTCCCGGAAGTTTTAAGAGC**cagCTTcagGAGGCCCCT**CCAG

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 FGA-4X	5' label	Sequence 5' → 3'	3' label	Oligo Type
FGA-4X-UNI_F	n/a	**CATCGCTAAAGAATTGCTTCCTACA**	n/a	Universal Forward
FGA-4X-UNI_PROBE	FAM	**AAGACAGGCAGTACTTGCCAGCAC**	ZEN/IBFQ	Universal Probe
FGA-4X-WT_R	n/a	**AGGGGCCTCCTGAAGCTG**	n/a	Wild type Reverse

FGA-4X-MUT1 assay (FAM labelled)

ACAGCTTCAACAGGT**CATCGCTAAAGAATTGCTTCCTACA**A**AAGACAGGCAGTACTTGCCAGCAC**T
AAAAATGTCTCCAGTTCCCGACTTGGTTCCCGGAAGTTTTAAGAGCaacC**TTaacGAGGCCCCTCCAG**
AGTGGAAGGCATTAACAGAAATGCGGaacATGAGAATGGAGCTGGAGAGACCTGGGAAGGATGGG

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 FGA-4X	5' label	Sequence 5' → 3'	3' label	Oligo Type
FGA-4X-UNI_F	n/a	**CATCGCTAAAGAATTGCTTCCTACA**	n/a	Universal Forward
FGA-4X-UNI_PROBE	FAM	**AAGACAGGCAGTACTTGCCAGCAC**	BHQ	Universal Probe
FGA-4X-MUT_R	n/a	**TCTGGAGGGGCCTCGTTAA**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo FGA-4X	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

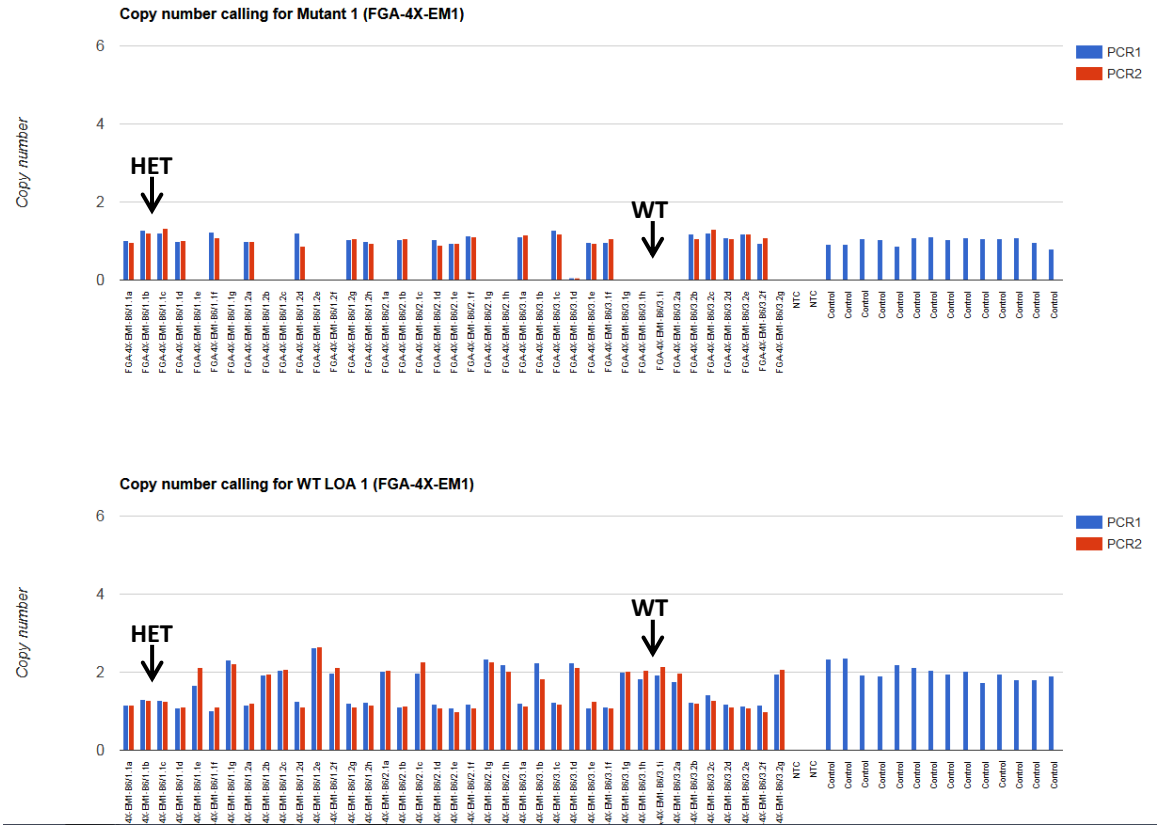
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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.

FGA-4X-WT1 and FGA-4X -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 274565 results)



Version No.	1
Date:	09/03/2020
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman