

**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**

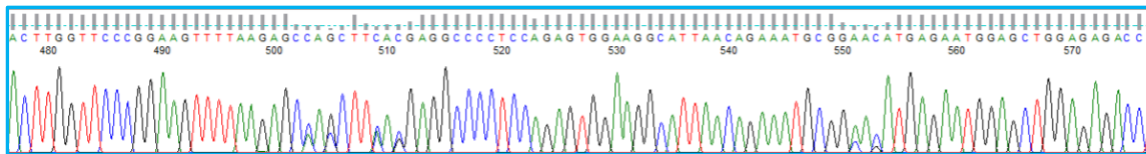
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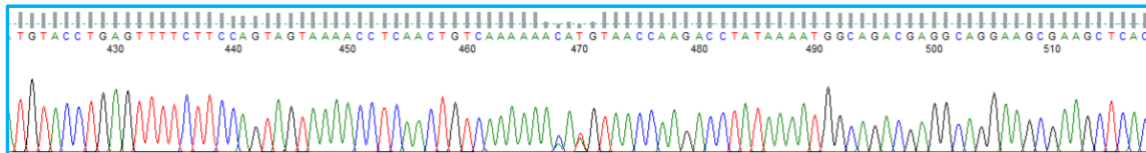
### Mutant

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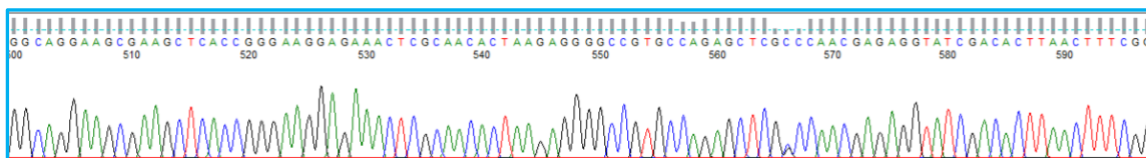
**FGA-4X-EM1-B6 Heterozygous F1 animal sequence trace:**



Q241N, Q243N, Q257N



Q518N



Silent change to kill the PAM sequence of sgRNA FGA\_3\_3\_ID: 454794723 (CGC > CGG Arginine)

## Nucleotide Alignment:

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*      20      *      40      *      60      *      80      *      100      *      120
FGA_WT : AAGTCCGGAATGCGAAGGAATTCCTGAAGGGTGAATAGATATTTGAAAAGAAGTCCGCTCTCCCGTTTGCCCTCCACTGGCTGAGACCTTGGCTCAGCTTGGAGGAGCCCTTCAG
FGA_4X_EM1 : AAGTCCGGAATGCGAAGGAATTCCTGAAGGGTGAATAGATATTTGAAAAGAAGTCCGCTCTCCCGTTTGCCCTCCACTGGCTGAGACCTTGGCTCAGCTTGGAGGAGCCCTTCAG
AAGTCCGGAATGCGAAGGAATTCCTGAAGGGTGAATAGATATTTGAAAAGAAGTCCGCTCTCCCGTTTGCCCTCCACTGGCTGAGACCTTGGCTCAGCTTGGAGGAGCCCTTCAG

*      140      *      160      *      180      *      200      *      220      *      240
FGA_WT : CCAGAGTTTACAGGATTAGGCCATTCTCCTTCCCCATTCAAATTTCAAAATGGATCCAAATAGTTTGGATCCTCCACACTTCTTGTCCCTCCACAGCTTCCTTTGAGGACCAGCCTGA
FGA_4X_EM1 : CCAGAGTTTACAGGATTAGGCCATTCTCCTTCCCCATTCAAATTTCAAAATGGATCCAAATAGTTTGGATCCTCCACACTTCTTGTCCCTCCACAGCTTCCTTTGAGGACCAGCCTGA
CCAGAGTTTACAGGATTAGGCCATTCTCCTTCCCCATTCAAATTTCAAAATGGATCCAAATAGTTTGGATCCTCCACACTTCTTGTCCCTCCACAGCTTCCTTTGAGGACCAGCCTGA

*      260      *      280      *      300      *      320      *      340      *      360
FGA_WT : GTAGCTAAAGACAAGGGTAGGATTGATTTCTGAGAGTCAAAAGCAATAAAACCATTGCAAAAATGTAACCACCTACCAGGAACCAATTTTCAATAGACATAATTCCTTCTC
FGA_4X_EM1 : GTAGCTAAAGACAAGGGTAGGATTGATTTCTGAGAGTCAAAAGCAATAAAACCATTGCAAAAATGTAACCACCTACCAGGAACCAATTTTCAATAGACATAATTCCTTCTC
GTAGCTAAAGACAAGGGTAGGATTGATTTCTGAGAGTCAAAAGCAATAAAACCATTGCAAAAATGTAACCACCTACCAGGAACCAATTTTCAATAGACATAATTCCTTCTC

*      380      *      400      *      420      *      440      *      460      *      480
FGA_WT : TGCCTCCTTCTTAGGTTAGGATTTGATATCAAGATCCGCTCTGCAAGGGTCTCGCAGCAGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCAACAAAACAGCTTC
FGA_4X_EM1 : TGCCTCCTTCTTAGGTTAGGATTTGATATCAAGATCCGCTCTGCAAGGGTCTCGCAGCAGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCAACAAAACAGCTTC
TGCCTCCTTCTTAGGTTAGGATTTGATATCAAGATCCGCTCTGCAAGGGTCTCGCAGCAGGCTGTAAACCGTGAGATAAATCTACAGGACTATGAAGGTCAACAAAACAGCTTC

*      500      *      520      *      540      *      560      *      580      *      600
FGA_WT : AACAGGTCATCGCTAAAGAAATGCTTCTCAAAAGACAGGCAGTACTGCCAGCACTAAAAATGTCCAGTCCCGACTTGGTCCCGGAAGTTTAAAGAGC A CT A GAGGCC
FGA_4X_EM1 : AACAGGTCATCGCTAAAGAAATGCTTCTCAAAAGACAGGCAGTACTGCCAGCACTAAAAATGTCCAGTCCCGACTTGGTCCCGGAAGTTTAAAGAGC a CT a GAGGCC
AACAGGTCATCGCTAAAGAAATGCTTCTCAAAAGACAGGCAGTACTGCCAGCACTAAAAATGTCCAGTCCCGACTTGGTCCCGGAAGTTTAAAGAGC A CT A GAGGCC

*      620      *      640      *      660      *      680      *      700      *      720
FGA_WT : CTCAGAGTGAAGGCATTAACAGAAATGCGG A ATGAGAATGGAGCTGGAGACCTGGGAAGGATGGGGTTCGCGAGGAGATTACACAGGAGACTTCGCGGGGAGATTCTAGAGGGG
FGA_4X_EM1 : CTCAGAGTGAAGGCATTAACAGAAATGCGG a ATGAGAATGGAGCTGGAGACCTGGGAAGGATGGGGTTCGCGAGGAGATTACACAGGAGACTTCGCGGGGAGATTCTAGAGGGG
CTCCAGAGTGAAGGCATTAACAGAAATGCGG A ATGAGAATGGAGCTGGAGACCTGGGAAGGATGGGGTTCGCGAGGAGATTACACAGGAGACTTCGCGGGGAGATTCTAGAGGGG

*      740      *      760      *      780      *      800      *      820      *      840
FGA_WT : ACTTTGCAACACGTGGACCGGTCGCAAGGCAAGAAACCCACGAAACCTGGACCTGGTGGATCTGGGATTGGGCTCTGGGAACTCCGCGTCTGGAAGTGGAAATCGGAAACCTG
FGA_4X_EM1 : ACTTTGCAACACGTGGACCGGTCGCAAGGCAAGAAACCCACGAAACCTGGACCTGGTGGATCTGGGATTGGGCTCTGGGAACTCCGCGTCTGGAAGTGGAAATCGGAAACCTG
ACTTTGCAACACGTGGACCGGTCGCAAGGCAAGAAACCCACGAAACCTGGACCTGGTGGATCTGGGATTGGGCTCTGGGAACTCCGCGTCTGGAAGTGGAAATCGGAAACCTG

*      860      *      880      *      900      *      920      *      940      *      960
FGA_WT : GGACCAGGGGCTGTAGGCACTGGAGCTGGGGTACCGGAAGCCCTAGACCTGGCTCAGACTCTGGGAACTTTAGGCTGCAACCCCTAACTGGGGTGTGTTCAGAGTTTGGAGACA
FGA_4X_EM1 : GGACCAGGGGCTGTAGGCACTGGAGCTGGGGTACCGGAAGCCCTAGACCTGGCTCAGACTCTGGGAACTTTAGGCTGCAACCCCTAACTGGGGTGTGTTCAGAGTTTGGAGACA
GGACCAGGGGCTGTAGGCACTGGAGCTGGGGTACCGGAAGCCCTAGACCTGGCTCAGACTCTGGGAACTTTAGGCTGCAACCCCTAACTGGGGTGTGTTCAGAGTTTGGAGACA

*      980      *      1000      *      1020      *      1040      *      1060      *      1080
FGA_WT : GTAGCAGCCGACCCACAGAAAGAGATATCACACAGGTAAAGCGCTCACTTCTAAAGAGATAAAGAGCTCTGATTTGGAAGGAGAAAGTCACTCTTTCGACAGGACCCACAGC
FGA_4X_EM1 : GTAGCAGCCGACCCACAGAAAGAGATATCACACAGGTAAAGCGCTCACTTCTAAAGAGATAAAGAGCTCTGATTTGGAAGGAGAAAGTCACTCTTTCGACAGGACCCACAGC
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*      1100      *      1120      *      1140      *      1160      *      1200
FGA_WT : GTTCATGCTCTAAAACCAATACCAAGACTGTACAGGCTGATGGTCCGCGAAGTGGTCAAGAAGTGATCACTCCGGATGATGGCTCAGACTTGGCGATGCCACCGATTTAGACA
FGA_4X_EM1 : GTTCATGCTCTAAAACCAATACCAAGACTGTACAGGCTGATGGTCCGCGAAGTGGTCAAGAAGTGATCACTCCGGATGATGGCTCAGACTTGGCGATGCCACCGATTTAGACA
GTTCATGCTCTAAAACCAATACCAAGACTGTACAGGCTGATGGTCCGCGAAGTGGTCAAGAAGTGATCACTCCGGATGATGGCTCAGACTTGGCGATGCCACCGATTTAGACA

*      1220      *      1240      *      1260      *      1280      *      1300      *      1320
FGA_WT : TATCCCAAGTTTTAGCGGAGTCTCGACGAACTCTGAAAGGATCCTGACCTTTCTGGGTTTTTGGACAACCACTTTGGTAAATCTCACTAACTTCAAAGAAATTTGGCAGTAAAG
FGA_4X_EM1 : TATCCCAAGTTTTAGCGGAGTCTCGACGAACTCTGAAAGGATCCTGACCTTTCTGGGTTTTTGGACAACCACTTTGGTAAATCTCACTAACTTCAAAGAAATTTGGCAGTAAAG
TATCCCAAGTTTTAGCGGAGTCTCGACGAACTCTGAAAGGATCCTGACCTTTCTGGGTTTTTGGACAACCACTTTGGTAAATCTCACTAACTTCAAAGAAATTTGGCAGTAAAG

*      1340      *      1360      *      1380      *      1400      *      1420      *      1440
FGA_WT : CCCATTCTGATTCGACATCCTCAAAACATTGAGGACCCAGCTCCATGTACTGCTTCTTCCAGTAGTAAAACCTCACTGTCAA AAA A A GTAACCAAGACCTATAAATGG
FGA_4X_EM1 : CCCATTCTGATTCGACATCCTCAAAACATTGAGGACCCAGCTCCATGTACTGCTTCTTCCAGTAGTAAAACCTCACTGTCAA AAA A a GTAACCAAGACCTATAAATGG
CCCATTCTGATTCGACATCCTCAAAACATTGAGGACCCAGCTCCATGTACTGCTTCTTCCAGTAGTAAAACCTCACTGTCAA AAA A A GTAACCAAGACCTATAAATGG

*      1460      *      1480      *      1500      *      1520      *      1540      *      1560
FGA_WT : CAGACGAGGAGGAGGAGGAGCTCACCGGGAAGGAGAACTCGCAACTAAGAGGGGCGTGCCAGAGCTCG CCAACGAGAGGTATCGCACTTAACTTTGCGGAGATTCCCCTGACA
FGA_4X_EM1 : CAGACGAGGAGGAGGAGGAGGAGCTCACCGGGAAGGAGAACTCGCAACTAAGAGGGGCGTGCCAGAGCTCG CCAACGAGAGGTATCGCACTTAACTTTGCGGAGATTCCCCTGACA
CAGACGAGGAGGAGGAGGAGGAGCTCACCGGGAAGGAGAACTCGCAACTAAGAGGGGCGTGCCAGAGCTCG CCAACGAGAGGTATCGCACTTAACTTTGCGGAGATTCCCCTGACA

*      1580      *      1600      *      1620      *      1640      *      1660      *      1680
FGA_WT : CCCCCCCCCCCTAGATTAAGTTAACCATTACTGCGAAGTCTTACCAGGACGCTGACCCGTTTCTTAACCTCCTTTAGTGTGTTTGGAAATCTCAATTTTTTTTTTGGTCAACTTT
FGA_4X_EM1 : CCCCCCCCCCCTAGATTAAGTTAACCATTACTGCGAAGTCTTACCAGGACGCTGACCCGTTTCTTAACCTCCTTTAGTGTGTTTGGAAATCTCAATTTTTTTTTTGGTCAACTTT
CCCCCCCCCCCCCTAGATTAAGTTAACCATTACTGCGAAGTCTTACCAGGACGCTGACCCGTTTCTTAACCTCCTTTAGTGTGTTTGGAAATCTCAATTTTTTTTTTGGTCAACTTT

*      1700      *      1720      *      1740      *      1760      *      1780      *      1800
FGA_WT : TCATGTAGACTGTACGTTCTTGGGGCAGGGACTTGACCATGTGCTATTTCTGTAATCCCAAATGCCAAGTGCAGTCAATTTCTCAATAAATACATTTAATAAATGAACAAA
FGA_4X_EM1 : TCATGTAGACTGTACGTTCTTGGGGCAGGGACTTGACCATGTGCTATTTCTGTAATCCCAAATGCCAAGTGCAGTCAATTTCTCAATAAATACATTTAATAAATGAACAAA
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*      1820      *      1840      *      1860      *      1880      *      1900      *      1920
FGA_WT : TTCTGCCGAACTCAGTTCTGAGTCTGTTTAAACCGAATTCATTAACAAAGTGTGCGATTGTAATACCAACCCCTAACCTTAAATTTAGTGTATGTTGCAATTAGATCAGGT
FGA_4X_EM1 : TTCTGCCGAACTCAGTTCTGAGTCTGTTTAAACCGAATTCATTAACAAAGTGTGCGATTGTAATACCAACCCCTAACCTTAAATTTAGTGTATGTTGCAATTAGATCAGGT
TTCTGCCGAACTCAGTTCTGAGTCTGTTTAAACCGAATTCATTAACAAAGTGTGCGATTGTAATACCAACCCCTAACCTTAAATTTAGTGTATGTTGCAATTAGATCAGGT

*      1940      *      1960      *      1980      *      2000      *      2020      *      2040
FGA_WT : TAAAACTGTGTTCCATTAGTATGGACTGATGAATGCTTAGCTAGCTTTAAACTATCATTTGAGATTAGCATGGACAGTAAAGATTTCAAATCCATTTGAAGAGAGTTGCTAAAGGATG
FGA_4X_EM1 : TAAAACTGTGTTCCATTAGTATGGACTGATGAATGCTTAGCTAGCTTTAAACTATCATTTGAGATTAGCATGGACAGTAAAGATTTCAAATCCATTTGAAGAGAGTTGCTAAAGGATG
TAAAACTGTGTTCCATTAGTATGGACTGATGAATGCTTAGCTAGCTTTAAACTATCATTTGAGATTAGCATGGACAGTAAAGATTTCAAATCCATTTGAAGAGAGTTGCTAAAGGATG

*      2060      *      2080      *      2100      *      2120      *      2140      *      2160
FGA_WT : AGTATCCTTACCTGTAAAAAATACATCTCATTTGTAGTGCAATCTTTCTGTTGGGAGGAGGAAAGGAGGAAAGGAGACAGGACGACAGACAGGACAGTATGAGCA
FGA_4X_EM1 : AGTATCCTTACCTGTAAAAAATACATCTCATTTGTAGTGCAATCTTTCTGTTGGGAGGAGGAAAGGAGGAAAGGAGACAGGACGACAGACAGGACAGTATGAGCA
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*      2180
FGA_WT : GTAGTACTCAACTGGGTTAGTGCCTATCTTTGCA
FGA_4X_EM1 : GTAGTACTCAACTGGGTTAGTGCCTATCTTTGCA
GTAGTACTCAACTGGGTTAGTGCCTATCTTTGCA
    
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## Predicted Protein Alignment:

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          180      *      200      *      220      *      240      *      260      *
FGA_WT   : V D I D I K I R S C K G S C S R A V N R E I N L Q D Y E G H Q R Q L Q Q V I A K E L L P T K D R Q Y L P A L K M S P V P D L V P G S F K S L E A P P E W K A L T E M R M R M E L E R P G K D G G S
FGA_4X_EM1 : V D I D I K I R S C K G S C S R A V N R E I N L Q D Y E G H Q R Q L Q Q V I A K E L L P T K D R Q Y L P A L K M S P V P D L V P G S F K S L E A P P E W K A L T E M R M R M E L E R P G K D G G S
          V D I D I K I R S C K G S C S R A V N R E I N L Q D Y E G H Q R Q L Q Q V I A K E L L P T K D R Q Y L P A L K M S P V P D L V P G S F K S L E A P P E W K A L T E M R M R M E L E R P G K D G G S

          280      *      300      *      320      *      340      *      360      *
FGA_WT   : R G D S P G D S R G D S R G D F A T R G P G S K A E N P T N P G P G G S G Y W R P G N S G S G S D G N R N P G T T G S D G T G D W G T G S P R P G S D S C N F R P A N P N W G V F S E F G D S S S P A T
FGA_4X_EM1 : R G D S P G D S R G D S R G D F A T R G P G S K A E N P T N P G P G G S G Y W R P G N S G S G S D G N R N P G T T G S D G T G D W G T G S P R P G S D S C N F R P A N P N W G V F S E F G D S S S P A T
          R G D S P G D S R G D S R G D F A T R G P G S K A E N P T N P G P G G S G Y W R P G N S G S G S D G N R N P G T T G S D G T G D W G T G S P R P G S D S C N F R P A N P N W G V F S E F G D S S S P A T

          380      *      400      *      420      *      440      *      460      *
FGA_WT   : R K E Y H T G K A V T S K G D K E L L I G K E K V T S S G T S T T H R S C S K T I T K T V T G P D G R R E V V K E V I T S D D G S D C G D A T E L D I S H S F S G S L D E L S E R H P D L S G F F D N H
FGA_4X_EM1 : R K E Y H T G K A V T S K G D K E L L I G K E K V T S S G T S T T H R S C S K T I T K T V T G P D G R R E V V K E V I T S D D G S D C G D A T E L D I S H S F S G S L D E L S E R H P D L S G F F D N H
          R K E Y H T G K A V T S K G D K E L L I G K E K V T S S G T S T T H R S C S K T I T K T V T G P D G R R E V V K E V I T S D D G S D C G D A T E L D I S H S F S G S L D E L S E R H P D L S G F F D N H

          480      *      500      *      520      *      540      *
FGA_WT   : F G L I S P N F K E F G S K T H S D S D I L T N I E D P S S H V P E F S S S K T S T V K K V T K T Y K M A D E A G S E A H R E G E T R N T K R G R A R A R P T R G I D T *
FGA_4X_EM1 : F G L I S P N F K E F G S K T H S D S D I L T N I E D P S S H V P E F S S S K T S T V K K V T K T Y K M A D E A G S E A H R E G E T R N T K R G R A R A R P T R G I D T *
          F G L I S P N F K E F G S K T H S D S D I L T N I E D P S S H V P E F S S S K T S T V K K V T K T Y K M A D E A G S E A H R E G E T R N T K R G R A R A R P T R G I D T

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## 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')	AAGTCCGGAATGCGAAGGA
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	<p>To get full coverage of the allele, amplicons were sequenced with the following primers (5'-3'):</p> <p>Geno_Fga_F1: AAAGAACTCGCTCTGTCCCC</p> <p>Geno_Fga_R1: GTGCGATTGTAATACCCAACCC</p> <p>Geno_Fga_R2: AGGATGCACCTACAATGAGATGT</p> <p>Geno_FGA_4x_seqF: GGCCTGCCAACCTAACTG</p> <p>Geno_FGA_4x_seqR: GACCGCTTACCTGTGTGATAC</p>

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  $\leq 2$  mismatches for guide Fga\_5.3 (ID: 454794574) were checked with the following primers:**

Off-target site	Sequence	Type	Primers used (5'-3')
<a href="#">16:90871803-90871825</a>	TTTT <b>C</b> AGAG <b>T</b> CAGCTTCAGG 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')	GATCCGACATCCTCACAAACATTG
Reverse Primer (5'-3')	TGCCATTTTATAGGTCTTGTTACatt
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')	TCTCTCCAGCTCCATTCTCATggt
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')	GGCCTGCCAACCTAACTG
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.



## 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**AAAGACAGGCAGTACTTGCCAGCACT  
 AAAAATGTCTCCAGTCCCGACTTGGTTCCCGGAAGTTTTAAGAGC**cagCTTcagGAGGCCCTCCAG**

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	<b><u>CATCGCTAAAGAATTGCTTCCTACA</u></b>	n/a	Universal Forward
FGA-4X-UNI_PROBE	FAM	<b>AAGACAGGCAGTACTTGCCAGCAC</b>	ZEN/IBFQ	Universal Probe
FGA-4X-WT_R	n/a	<b><u>AGGGGCCTCTGAAGCTG</u></b>	n/a	Wild type Reverse

## FGA-4X-MUT1 assay (FAM labelled)

ACAGCTTCAACAGGT**CATCGCTAAAGAATTGCTTCCTACA**AAAGACAGGCAGTACTTGCCAGCACT  
 AAAAATGTCTCCAGTCCCGACTTGGTTCCCGGAAGTTTTAAGAGCaac**CTTaacGAGGCCCTCCAG**  
**AGTGGAAAGGCATTAACAGAAATGCGG**aacATGAGAATGGAGCTGGAGAGACCTGGGAAGGATGGG

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	<b><u>CATCGCTAAAGAATTGCTTCCTACA</u></b>	n/a	Universal Forward
FGA-4X-UNI_PROBE	FAM	<b>AAGACAGGCAGTACTTGCCAGCAC</b>	BHQ	Universal Probe
FGA-4X-MUT_R	n/a	<b><u>TCTGGAGGGGCCTCGTTAA</u></b>	n/a	Mutant Reverse



## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**  
**CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA  
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
CACCTATGA

Oligo FGA-4X	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	<u><b>GCCCCAGCACGACCATT</b></u>	n/a	WT Forward
Dot1l_Probe	VIC	<b>CCAGCTCTCAAGTCG</b>	BHQ	WT Probe
Dot1l_Reverse	n/a	<u><b>TAGTTGGCATCCTTATGCTTCATC</b></u>	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
ddH2O	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

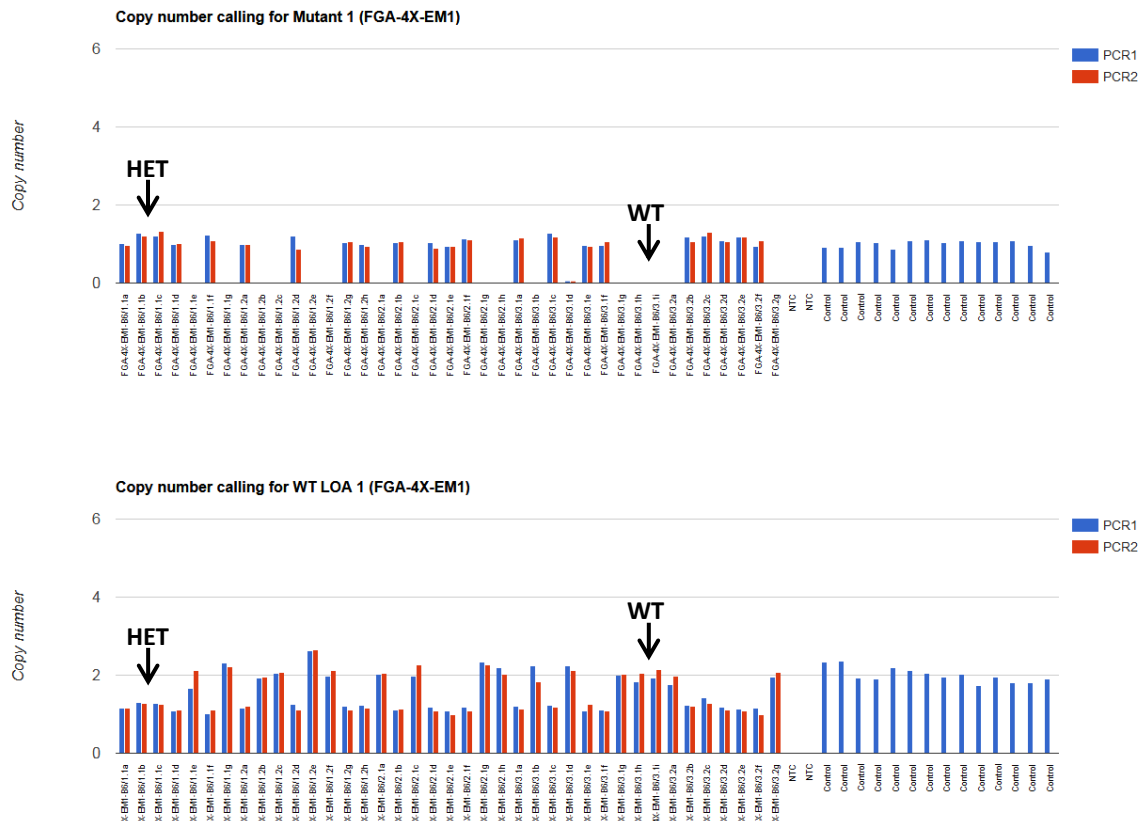
60°C for 30 sec



## 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)



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