Name of Mouse model or mutation:
DAZL-R115G-EM1-B6

Description:
Point mutant made by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: R115G

Delivery method:
Pronuclear injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 WT protein

sgRNAs:

Protospacer sequence	PAM sequence
AATTTTGTTTCCTGATTGCA	GGG
AAATTTTGTTTCCTGATTGC	AGG

ssODN donor sequence (5'-3'):
TGAAAGATTAAAATTATCGTGTGTTATTTTTAGTCACAGATAAATTTCCATGGTAAAAAGCTGAAACTGGGaCC
TGCAATtgGGAAACAAAATTTATGTGAGTAAAACCAGAAGTTTTCTTGATGAATCTGCCTAGGTTTTCAGAGAA
CGCAATGTAACTTT

Electroporation mixes:
Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl each and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

WT

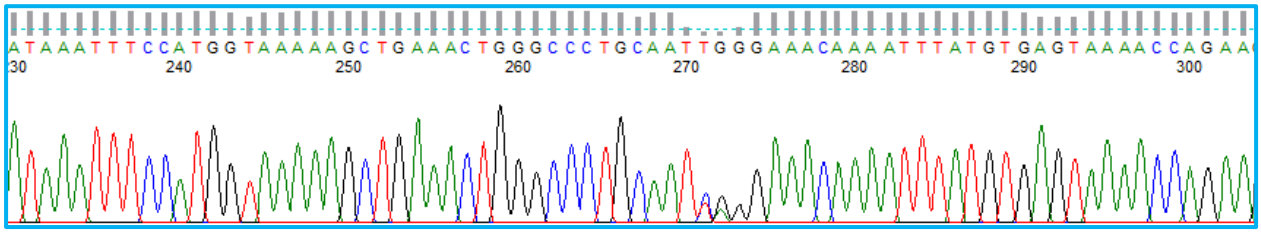
ATGACGTGGATGTGCAGAAGATAGTAGAAGTAAGTAACAGTCTTTGGTAAAACTCTTCTTTAGCAAT
GCTTTCGTGTTGAGTAAGTCCTGGATATTCAATCTTACATAAAATACAGAGAAAATAGGCTTCCTTTC
TGTTTAAGATCAAACTGTAATTTTATGTAACTTGTCAGAACATGCTTGCTTGGAGGCCTCTAGCTTTA
TCAGATTAGAAAAGGGATTTGATGCATTGTGACACTTTGGTTAAAGTAAGGCTGAGCACAATAGTGT
TTGCCAAACTCTTGGAAATGAAAGATTAAAATTATCGTGTGTTATTTTTAGTCACAGATAAATTTCCA
TGGTAAAAAGCTGAAACTGGGCCCTGCAATCAGGAAACAAAATTTATGTGAGTAAAACCAGAAGTT
TTCTTGATGAATCTGCCTAGGTTTTCAGAGAACGCAATGTAACTTTTGTTCTTTGTACTTTTTTAAAGG
TACTTATCATGTGCAGCCACGTCCTTTGATTTTTAATCCTCCTCCTCCACCACAGTTCCAGAGTGTTTG
GAGTAGTCCAAATGCTGAGACTTACATGCAGCCTCCAACCATGATGAATC

DAZL-R115G-EM1-B6

ATGACGTGGATGTGCAGAAGATAGTAGAAGTAAGTAACAGTCTTTGGTAAAACTCTTCTTTAGCAAT
GCTTTCGTGTTGAGTAAGTCCTGGATATTCAATCTTACATAAAATACAGAGAAAATAGGCTTCCTTTC
TGTTTAAGATCAAACTGTAATTTTATGTAACTTGTCAGAACATGCTTGCTTGGAGGCCTCTAGCTTTA
TCAGATTAGAAAAGGGATTTGATGCATTGTGACACTTTGGTTAAAGTAAGGCTGAGCACAATAGTGT
TTGCCAAACTCTTGGAAATGAAAGATTAAAATTATCGTGTGTTATTTTTAGTCACAGATAAATTTCCA
TGGTAAAAAGCTGAAACTGGGCCCTGCAATtgGGAAACAAAATTTATGTGAGTAAAACCAGAAGTTT
TCTTGATGAATCTGCCTAGGTTTTCAGAGAACGCAATGTAACTTTTGTTCTTTGTACTTTTTTAAAGGT
ACTTATCATGTGCAGCCACGTCCTTTGATTTTTAATCCTCCTCCTCCACCACAGTTCCAGAGTGTTTGG
AGTAGTCCAAATGCTGAGACTTACATGCAGCCTCCAACCATGATGAATC

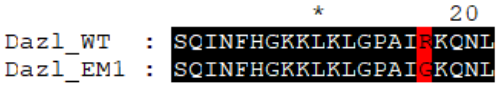
Please note, although a silent mutation was present in the donor template, it does not appear to have been incorporated. As such, only the R115G change, together with the silent change in I114 to create the MfeI site are present in the DAZL-R115G-EM1-B6 allele.

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_Dazl_R115G_F1	ATGACGTGGATGTGCAGAAGATA
Geno_Dazl_R115G_R1	GATTCATCATGGTTGGAGGCTG
Taq Polymerase used	ThermoFisher SuperFi PCR kit
Annealing Temperature (°C)	65
Elongation time (min)	0.5
WT product size (bp)	592
Mutant product size (bp)	592
Notes	

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(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
11:52517671-52517693	AGTTTTGTTTCCTGTTTGCA GGG	Intergenic	Geno_Dazl_OT1F1: GCTTTAGCTCCCTCTGCCAA Geno_Dazl_OT1R1: TGTCACAGACGCAGAAGGAC
7:6397996-6398018	AATTTTGTCTCCTGATTGCT GGG	Intergenic	Geno_Dazl_OT2F1: GTGTGTCTGCGCTTTTAGGG Geno_Dazl_OT2R1: AAGGGCGGACCATAGAACAC
18:9696646-9696668	AAATTTTATTTCCTGAGTGC TGG	Intergenic	Geno_Dazl_OT3F1: GGACAGTCAAGGTTACCCAGG Geno_Dazl_OT3R1: GGCAATGGTTGTGGGGTAGA
19:15162028-15162050	AAATTTAGTTTCATGATTGC AGG	Intergenic	Geno_Dazl_OT4F1: TTCAGCATGATAAGCCCAGGT Geno_Dazl_OT4R1: GGGCGAACAGACTAGGTTAGG
7:6397995-6398017	TAATTTTGTCTCCTGATTGC TGG	Intergenic	Geno_Dazl_OT5F1: GCGCTTTTAGGGGAAAGTCA Geno_Dazl_OT5R1: ATCTTCACCCTATTCGGGGC

All amplicons were sent for Sanger sequencing, no off-target activity was detected.

Additional integrations of the donor sequence

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 allelic discrimination assay was used to copy count the donor sequence compared against a VIC-labelled WT assay:

Assay name	Dazl-R115G-AD1
Forward Primer (5'-3')	GTCACAGATAAATTTCCATGGTAAAAAGCT
Reverse Primer (5'-3')	CTAGGCAGATTCATCAAGAAAACTTCTG
Mutant Probe (5'-3', FAM-labelled)	CTGCAATTGGGAAACA
WT Probe (5'-3', VIC-labelled)	CTGCAATCAGGAAACA
Notes	The ddPCR assay is an allelic discrimination assay and so recognises both the WT (VIC) and MUT (FAM) sequences of Dazl using different probes. A ratio value is provided, as if both alleles are present, a random integration can only be detected if the ratio deviates from the value of 1. No animals showed a ration greater than 1.

Dazl-R115G-EM1-B6

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation, R115G, in exon ENSMUSE00000137060 of *Dazl*. The targeted point mutation R115G is created by a CA > TG conversion, introducing an Arg > Gly conversion as well as an MfeI restriction enzyme cutting site. The stock was generated at MRC Harwell via electroporation 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 the reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with the reverse primer binding to the R115G point mutation.

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

Dazl-R115G-WT1 assay (FAM labelled)

TTAGAAAAGGGATTTGATGCATTGTGACACTTTGGTTAAAGTAAGGCTGAGCACAATAGTGTT**TGCCAAACTC**
TTGGAAATGAAAGATTAAAATTATCGTGTGTTATTTTTAGTCACAGATAAATTTCCATGGTAA**AAAGCTGAAA**
CTGGGCCCTGCAATcaGGAAACAAAATTTATGTGAGTAAAACCAGAAGTTTTCTTGATGAATCTGCCTAGGTT
TTCAGAGAACGCAATGTAACTTTTGTTCTTTGTACTTTTTTAAAGGTACTTATCATGTGCAGCCACGTCCTTTGA
TTTTTAATCCTCCTCCTCCACCACAGTTCCAGAGTGTTTGGAGTAGTCCAAATGCTGAGACTTACATGCAGCCTC

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dazl-R115G-WT1_F	n/a	**TGCCAAACTCTTGGAAATGAAAG**	n/a	Wild type Forward
Dazl-R115G-WT1_PROBE	FAM	**AAAGCTGAAACTGGGCCCTGCAAT**	ZEN(internal quencher)/IBFQ	Wild type Probe
Dazl-R115G-WT1_R	n/a	**GGTTTTACTCACATAAATTTTGTTTCCTG**	n/a	Wild type Reverse

Dazl-R115G-MUT1 assay (FAM labelled)

TTAGAAAAGGGATTTGATGCATTGTGACACTTTGGTTAAAGTAA**GGCTGAGCACAATAGTGTTTG**CCAAACTC
TTGGAAATGAAAGATTAAAATTATCGTGTGTTATTTTTAGTCACAGATAAATTTCCATGGTAA**AAAGCTGAAA**
CTGGGCCCTGCAATtg**GGAAACAAAATTTATGTGAGTAAAA**CCAGAAGTTTTCTTGATGAATCTGCCTAGGTT
TTCAGAGAACGCAATGTAACTTTTGTTCTTTGTACTTTTTTAAAGGTACTTATCATGTGCAGCCACGTCCTTTGA
TTTTTAATCCTCCTCCTCCACCACAGTTCCAGAGTGTTTGGAGTAGTCCAAATGCTGAGACTTACATGCAGCCTC

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 Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dazl-R115G-MUT1_F	n/a	**GGCTGAGCACAATAGTGTTTG**	n/a	Mutant Forward
Dazl-R115G-MUT1_PROBE	FAM	**AAAGCTGAAACTGGGCCCTGCAAT**	ZEN(internal quencher)/IBFQ	Mutant Probe
Dazl-R115G-MUT1_R	n/a	**TTTTACTCACATAAATTTTGTTTCCCA**	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

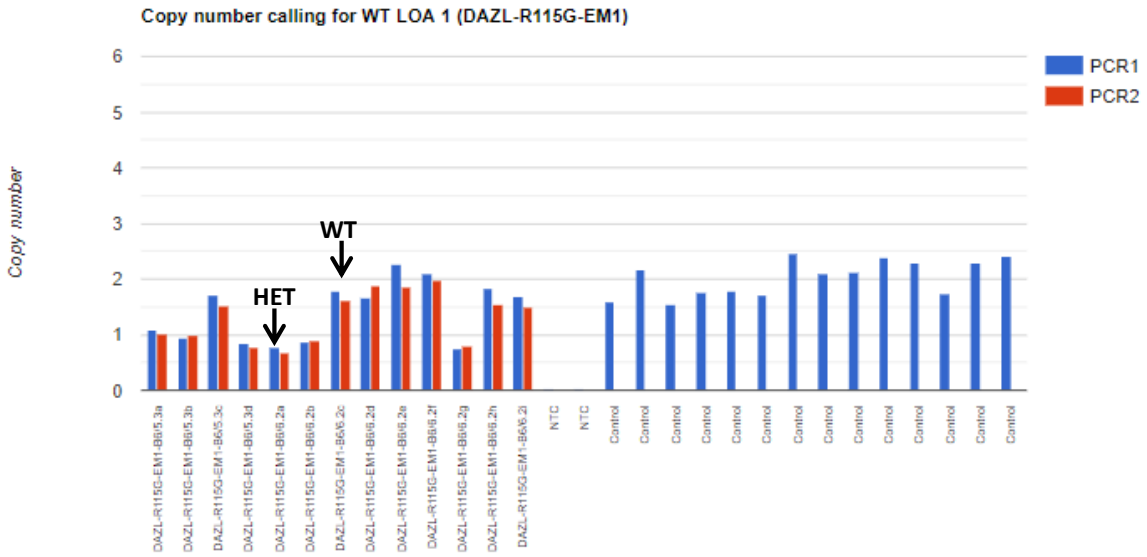
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

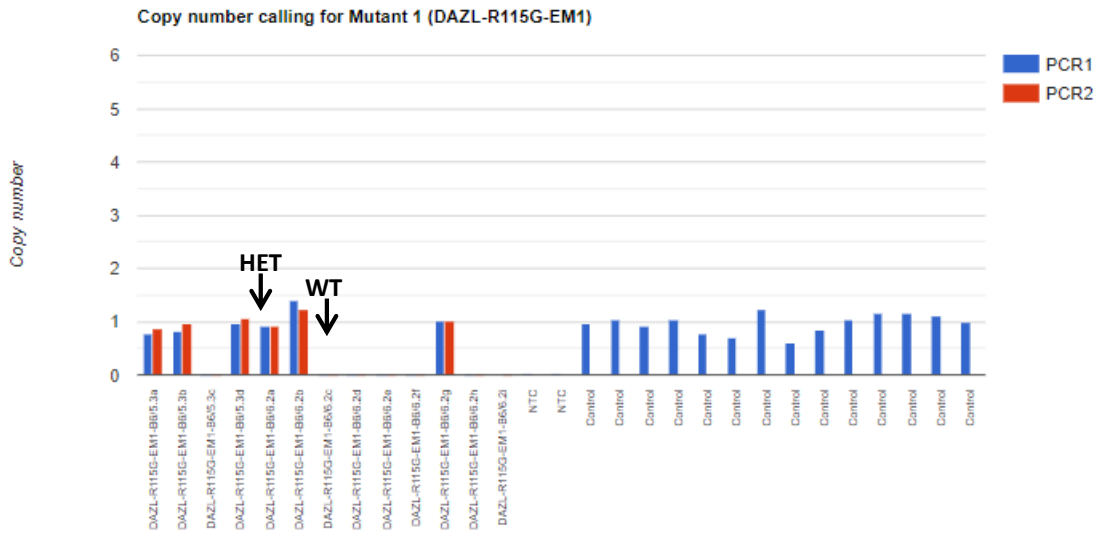
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Dazl-R115G-WT1 and Dazl-R115G -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 329792 results)





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