

**Name of Mouse model or mutation:****Prdm8-FLOX-EM1-B6N****Description:**

Floxed model made using CRISPR/Cas9.

**Type of mutation:**

Floxed exon: ENSMUSE00000330082

**Sequence details****Prdm8 WT**

AACGCCATATTCGGTCCCTGTGTACTGAGCCATACTCCCTGTATGACAGCATAGCCTTCGTAGCCCTCAAGTC  
CACGGACAAGAGAACAGTACCTTACATCTTCCGGGTGAGTCTCAGCAAGTGTGATGGAGGTGTCTGAGGGTA  
ATTTCTGTTGGGAGTGAGGAAGGAGACATCCTAAGGACAATGTAATCATCTGAGCAGTCAGAGCAGAGCGA  
ACACAACAAAAATTAGAAAAGAAAACTAGATTCAAATCATCAAGTGATTTTACTGAGAAAAAGAAAAAGAC  
AATGCCAAGGTTGCCTGAATCGGTACTCTAGTTCTGGGTTCCCTAGATTAGGGCTTCTAAATTGCTAAGGAG  
ACCTTGTTAGTACTGGGGCTATTTACATATTAAGTAGTCCTTGAGAGAACTTTTACAAGTATTGAGTATATTTAT  
TTTGTAACCTGGGAGGTCTCCCATCGCCTGATTATTGCTTTCTTTAGTTAACATTAGCTAGTGTTCCAGAGTTTG  
TATTTAAATTAACGTTAATTGTTTTGGCAGCAAAGCATCTATAATCCACGTTTGTGTTTGAACAGGCCTCTT  
TCTTCTAGTAGTTCTTTTTTTATTAATTATTTATTTACATGCTAAATGTTACTCCCAGTTTCCCCACCCCA  
GAGTTCCTAGTCTTTCATCATGAAGTAAAGCTGTTAGTCCTTCATGTCAGTTAGGAAGATGGGCGTGAGTGCC  
ATAGAATAATTAATAAGACCCGAACCTTGCTTTTACTTCTGAAACACGGGAAATTTCTCTCCTTTTGTGATGTG  
AGGTAAGTAAAGGGAGATTCTAAAGAGGCTACTCTGTAAGACTGACCCAATATTGCAAAGGATGGCCACTG  
TGCCATTGACCACAAATGCAGGGATGCAGTTCGACTTTGGTCAACTTTTGCAAAGTGGTTTCAGATAAATGT  
TTGGAAGAACAAGCCAGACAGATGAAATATCTCTGGTTTCTCTGGTTGCCGTTTTCACGTGGATCCCTCT  
CTTCTGTCTTTCTAAATACTGTAGGTAGACTTCGGCGGCAAATGGTTCCTCAGAAGGTCTCATGTGGCTG  
CGGCTGGTCCAATCAGCCCAGATAAGGAAGAACAAGTCTCGAAGCTTATATAAAAAATGGACAGCTGTTT  
TACCGCTCTCTCCGCAGGATTGCCAAAGATGAAGAGTTACTAGTTTGGTACGGGAAAGAACTGACTGAATTGC  
TCTTGCTCTGCCGCTAGAGCTCACAAAATGAACGGTAGGTTGGCTCGGGTGCCTGGGCTCACCTTGACAT  
CATAGGGTGGACGAAGCTTCTGAGGTGTTGTCCGAATGGAAGTGGACAGAAGGGGAAGGAAAAGTTGGG  
GAGCGTTTGACTTAGTAAATGCACAAATCAAGAAAGTAAAGTGCACCAATAGGATAGTGATTCATTTTTTTG  
GGCACTCAGCCCAGAAAGATAAAGTGGATTTGTTATGGGGAGGATGGCTAAGTGGCCTAGGGGCGATGCTA  
ACTAACCATGTGGGGT  
CTAAGCAGGGTCTCTCCTTACACATGCCTGGAATGCAGCCAACGTTTCCAGTTTGAGTTCCCTATGTGGC  
ATCTGCGATTCCGCTGCCCCAAGAGACTTCACAGCACTGATGCGAATCCCCAAGACGAGCAAGGGGGCGGCT  
TGGGCACTAAGGACCACGGCGGCGGCGGCTGGTAAAGAGCAACAGCAGCAGCAACAGCAGCAGCAACA  
GGAGGCGCGGTTGATCCCGGGCCCCAAGTCTGCAAAGCCGGCCCCATACCCACTACCCCGGTCATCCCCG  
GAGGCGAGCAACCCGCCGGGCTCCGCGGGTCCGGCAGCGCCAAGCCGTCCACGGACTTCCACAAGTGGCT  
CGGGAAGT

**PRDM8-FLOX-EM1-B6N**

AACGCCATATTCGGTCCCTGTGACTGAGCCATACTCCCTGTATGACAGCATAGCCTTCGTAGCCCTCAAGTC  
CACGGACAAGAGAACAGTACCTTACATCTTCCGGGTGAGTCTCAGCAAGTGTGATGGAGGTGTCTGAGGGTA  
ATTCCTGTTGGGAGTGAGGAAGGAGACATCCTAAGGACAATGTAATCATCTGAGCAGTCAGAGCAGAGCGA  
ACACAACAAAATTAGAAAAGAAAACTAGATTCAAATCATCAAGTGATTTTACTGAGAAAAGAAAAAGAC  
AATGCCAAGGTTGCCTGAATCGGTACTatccgggggtaccgcgtagGCGATCGCATAACTTCGTATAGCATAACATT  
ATACGAAGTTATAAATTGCTAAGGAGACCTTGTAGTACTGGGGCTATTTACATATTAAGTAGTCCTTGAGAG  
AACTTTTACAAGTATTGAGTATATTTATTTTGTAACCTGGGAGGTCTCCCATCGCCTGATTATTGCTTTCTTTA  
GTTAACATTAGCTAGTGTTCCAGAGTTTGTATTTAAATTAACGTTAATTGTTTTGGCAGCAAAGCATCTATAA  
TCCACGTTTGTGTTGTAACAGGCCTCTTTCTCCTAGTAGTTCTTTTTTATTAATTATTTTATTTTACATGC  
TAAATGTTACTCCCAGTTTCCCCACCCAGAGTTCCTAGTCTTTCATCATGAAGTAAAGCTGTTAGTCCTTCATG  
TCAGTTAGGAAGATGGGCGTGAGTGCCATAGAATAACTAAGACCCGAACCTTGCCTTTTACTTCTGAAACA  
CGGGAAATTCCTCTCCTTTTGTCTGTGAGGTAAGTAAAGGGAGATTCTAAAGAGGCTACTCTGTAAGACT  
GACCCAATATTGCAAAGGATGGCCACTGTGCCATTGACCACAAATGCAGGGATGCAGTTGACTTTGGTCAA  
CTTTTGCAAAGTGGTTTCCAGATAATGTTTGAAAGAACAAGCCAGACAGATGAAATATCTCTGGTTTCTC  
TGGTTGCCGTCTTTCACGTGGATCCCTCTCTTCTTGTCTTCTTAATACTGTAGGTAGACACTTCGGCGGCAA  
TGGTTCCTCAGAAGGTCTCATGTGGCTGCGGCTGGTCCAATCAGCCCGAGATAAGGAAGAACAGAATCTCGA  
AGCTTATATAAAAAATGGACAGCTGTTCTACCGCTCTCTCCGAGGATTGCCAAAGATGAAGAGTACTAGTTT  
GGTACGGGAAGAACTGACTGAATTGCTCTTGTCTGCCCCTAGAGCTCACAAAATGAACGGTAGGTTGG  
CTCGGGTGCCTGGGCTCACCTGCACATCATAGGGTGGACGAAGCTTCTGAGATAACTTCGTATAGCATAACAT  
TATACGAAGTTATCGCCGGCGgggctgagctcgccatcaggACAGAAGGGGAAGGAAAAGTTGGGGAGCGTTTGAC  
TTAGTAAATGCACAAATCAAGAAAGTAAAGTGCACCAATAGGATAGTGTATTCTTTTTTTGGGCACTCAGCC  
CAGAAAGATAAAGTGGATTTGTTATGGGAGGATGGCTAAGTGGCCTAGGGGGCGATGCTAACTAACCATGTG  
GGGTTTCTACTAAGCAGGGTC  
GTCTCCTTACACATGCCTGGAATGCAGCCAACGTTTCCAGTTTGTAGTTCCCTATGTGGCACATCTGCGATTCC  
GCTGCCCCAAGAGACTTCACAGCACTGATGCGAATCCCCAAGACGAGCAAGGGGGGCGCTTGGGCACTAAG  
GACCACGGCGGCGGCGGCGGTTAAAGAGCAACAGCAGCAGCAACAGCAGCAGCAACAGGAGGCGCCGT  
TGATCCCGGGCCCAAGTTCTGCAAAGCCGGCCCCATACACCACTACCCCGCGTCATCCCGGAGGGCGAGCAA  
CCC GCCGGGCTCCGCGGGTCCGGCAGCGCCAAGCCGTCCACGGACTTCCACA ACTTGGCTCGGGA ACTG

**Nucleotide Alignment:** Orange is targeted exon, Red is LoxP sites, Yellow are universal primer and RE sites

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160          *          180          *          200          *          220          *          240          *          260          *          280          *          300
Prdm8_WT : CCTGTTGGGAGTGAGGAAGGAGACATCCTAAGGACAATGTAATCATCTGAGCAGTCAGAGCAGAGCGAACCAACAACAAAATAGAAAAGAAAACTAGATTCAAATCATCAAGTGATTTTACTGAGAAAAAGAAAAAGACAATGCCAAGG
Prdm8_Flox : CCTGTTGGGAGTGAGGAAGGAGACATCCTAAGGACAATGTAATCATCTGAGCAGTCAGAGCAGAGCGAACCAACAACAAAATAGAAAAGAAAACTAGATTCAAATCATCAAGTGATTTTACTGAGAAAAAGAAAAAGACAATGCCAAGG

          *          320          *          340          *          360          *          380          *          400          *          420          *          440          *
Prdm8_WT : TTGCCTGAATCGGTACTCTAGTTCTGGTTCCCTAGATTAGGCTTCCT-----AAATTGCTAAGGAGACCTTGTAGTACTGGGGCTATTACATATTAAGTAGTCCCTTGAGAGAACTTTAC
Prdm8_Flox : TTGCCTGAATCGGTACTatccgggggtaccgcgctcgagCGGATCGCAATAACTTCGTATAGCATACATTATACGAAGTTAAATTGCTAAGGAGACCTTGTAGTACTGGGGCTATTACATATTAAGTAGTCCCTTGAGAGAACTTTAC

          460          *          480          *          500          *          520          *          540          *          560          *          580          *          600
Prdm8_WT : AAGTATTGAGTATATTTATTTTGTAACTGGGAGGTCCTCCCATCGCCTGATATTGCTTCTTTAGTTAACATTAGCTAGTGTTCAGAGTTTGTATTTAAATTAACCGTTAATTGTTTTGGCAGCAAAGCATCTATAATCCCGTTG
Prdm8_Flox : AAGTATTGAGTATATTTATTTTGTAACTGGGAGGTCCTCCCATCGCCTGATATTGCTTCTTTAGTTAACATTAGCTAGTGTTCAGAGTTTGTATTTAAATTAACCGTTAATTGTTTTGGCAGCAAAGCATCTATAATCCCGTTG

          *          620          *          640          *          660          *          680          *          700          *          720          *          740          *
Prdm8_WT : TGTTTGTAACAGGCCTCTTCTTCCCTAGTAGTTCTTTTTTTTATTAATTTATTTATTTATTTTACATGCTAAATGTTACTCCAGTTTCCCAACCCAGAGTTCCTAGTCTTTCATCATGAAGTAAAGCTGTTAGTCCCTCATGTCAGTTAG
Prdm8_Flox : TGTTTGTAACAGGCCTCTTCTTCCCTAGTAGTTCTTTTTTTTATTAATTTATTTATTTATTTTACATGCTAAATGTTACTCCAGTTTCCCAACCCAGAGTTCCTAGTCTTTCATCATGAAGTAAAGCTGTTAGTCCCTCATGTCAGTTAG

          760          *          780          *          800          *          820          *          840          *          860          *          880          *          900
Prdm8_WT : GAAGATGGGCGTGAGTGCCATAGAATAAATACTAAGACCCGAACCTTGCCCTTTACTTCTGAAACACGGGAATTTCCCTCTCCTTTTGTCTGAGTAACTGAAAGGGAGATTCTAAAGAGGCTACTCTGTAAGACTGACCCAATATTG
Prdm8_Flox : GAAGATGGGCGTGAGTGCCATAGAATAAATACTAAGACCCGAACCTTGCCCTTTACTTCTGAAACACGGGAATTTCCCTCTCCTTTTGTCTGAGTAACTGAAAGGGAGATTCTAAAGAGGCTACTCTGTAAGACTGACCCAATATTG

          *          920          *          940          *          960          *          980          *          1000          *          1020          *          1040          *
Prdm8_WT : CAAAGGATGGCCACTGTGCCCATTGACCACAAATGCAGGGATGCAGTTCGACTTTGGTCAACTTTTGCAAACCTTGGTTTCAGATAATGTTTGGAAAGAACAAAGCCAGACAGATGAAATATCTCTGGTTCTCTGGTTGCCGTCTTTC
Prdm8_Flox : CAAAGGATGGCCACTGTGCCCATTGACCACAAATGCAGGGATGCAGTTCGACTTTGGTCAACTTTTGCAAACCTTGGTTTCAGATAATGTTTGGAAAGAACAAAGCCAGACAGATGAAATATCTCTGGTTCTCTGGTTGCCGTCTTTC

          1060          *          1080          *          1100          *          1120          *          1140          *          1160          *          1180          *          1200
Prdm8_WT : ACGTGGATCCCTCTCTTCTTGTCTTTCTTAATACTGTAGGTAGACACTTCGGCGGCAAAATGGTTCCCTCAGAAGGCTCATGTGGCTGCGGCTGGTCCAATCAGCCCGAGATAAGGAAGAACAGAATCTCGAAGCTTATATAAAAAATGG
Prdm8_Flox : ACGTGGATCCCTCTCTTCTTGTCTTTCTTAATACTGTAGGTAGACACTTCGGCGGCAAAATGGTTCCCTCAGAAGGCTCATGTGGCTGCGGCTGGTCCAATCAGCCCGAGATAAGGAAGAACAGAATCTCGAAGCTTATATAAAAAATGG

          *          1220          *          1240          *          1260          *          1280          *          1300          *          1320          *          1340          *
Prdm8_WT : ACAGCTGTTCTACCGCTCTCTCCGCAGGATTGCCAAAGATGAAGAGTTACTAGTTTGGTACGGGAAAGAACTGACTGAATGCTCTGCTCTGCCGCTTAGAGCTCACAAAATGAACGGTAGGTTGGCTCGGGTGCCTGGGCTCACCTT
Prdm8_Flox : ACAGCTGTTCTACCGCTCTCTCCGCAGGATTGCCAAAGATGAAGAGTTACTAGTTTGGTACGGGAAAGAACTGACTGAATGCTCTGCTCTGCCGCTTAGAGCTCACAAAATGAACGGTAGGTTGGCTCGGGTGCCTGGGCTCACCTT

          1360          *          1380          *          1400          *          1420          *          1440          *          1460          *          1480          *          1500
Prdm8_WT : CCACATCATAGGGTGGACGAAGCTTCTGAGCTTGTCTGCCGAATGGACTAGG-----ACAGAAGGGGAAGGAAAAGTTGGGAGCGTTTGACTTAGTAAATGCACAAATCAAGAA
Prdm8_Flox : CCACATCATAGGGTGGACGAAGCTTCTGAGATAACTTCGTATAGCATACATTATACGAAGTTAACCGCCGGCGggtctgagctcgccatcaggACAGAAGGGGAAGGAAAAGTTGGGAGCGTTTGACTTAGTAAATGCACAAATCAAGAA

          *          1520          *          1540          *          1560          *          1580          *          1600          *          1620          *          1640          *
Prdm8_WT : AGTAAGTTGCACCAATAGGATAGTGTATTCTATTTTTTGGGCACTCAGCCAGAAAGATAAAGTGGATTTGTTATGGGGAGGATGGCTAAGTGGCCTAGGGCGATGCTAACTAACCATGTGGGGTGTGTGTGTGTGTGTGTGTGTG
Prdm8_Flox : AGTAAGTTGCACCAATAGGATAGTGTATTCTATTTTTTGGGCACTCAGCCAGAAAGATAAAGTGGATTTGTTATGGGGAGGATGGCTAAGTGGCCTAGGGCGATGCTAACTAACCATGTGGGGTGTGTGTGTGTGTGTGTGTGTG

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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_Prmd8_F1 (5'-3')	AACGCCATATTCGGTCCCTG
Geno_Prmd8_R1 (5'-3')	CAGTCCCCGAGCCAAGTTGT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	63
Elongation time (min)	2
WT product size (bp)	1984
Mutant product size (bp)	2054
Notes	Sequence also with Geno_Prmd8_F2 (5'-3'): GCAGGGATGCAGTTCGACTT

LoxPF (5'-3')	atccgggggtaccgctcgag
LoxPR (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	3
WT product size (bp)	-
Mutant product size (bp)	1125
Notes	Sequence also with Geno_Prmd8_F2 (5'-3'): GCAGGGATGCAGTTCGACTT

Geno_Prmd8_F1 (5'-3')	AACGCCATATTCGGTCCCTG
LoxPR (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1.5
WT product size (bp)	-
Mutant product size (bp)	1442
Notes	

LoxPF (5'-3')	atccgggggtaccgctcgag
Geno_Prmd8_R1 (5'-3')	CAGTTCCCAGCCAAGTTGT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1.5
WT product size (bp)	-
Mutant product size (bp)	1737
Notes	Sequence also with Geno_Prmd8_F2 (5'-3'): GCAGGGATGCAGTTCGACTT

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 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	Prdm8-FLOX-DONOR-MUT1
Forward Primer (5'-3')	CGATCGCATAACTTCGTATAGCATACA
Reverse Primer (5'-3')	GGGAGACCTCCCAGGTTACA
Probe (5'-3')	TGCTAAGGAGACCTTGTTAGTACTGGG
Label	FAM-BHQ1

The ddPCR assay recognises the 5' LoxP site specific to the Prdm8 floxed allele. Therefore, WT controls are expected to call at 0 copies and F1 animals with evidence of the floxed exon will call at 1 copy.

Assay name	Prdm8-FLOX-3'-MUT1
Forward Primer (5'-3')	GGCTCACCTTGCACATCATAGG
Reverse Primer (5'-3')	CAAACGCTCCCCAACTTTTCCTT
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM-BHQ1

The ddPCR assay recognises the 3' LoxP site specific to the Prdm8 floxed allele. Therefore, WT controls are expected to call at 0 copies and F1 animals with evidence of the floxed exon will call at 1 copy.

Assay name	PRDM8-CR-LOA-WT1
Forward Primer (5'-3')	TTCGGCGGCAAATGGTTC
Reverse Primer (5'-3')	GCTTCGAGATTCTGTTCTTCCTTA
Probe (5'-3')	TCAGAAGGTCTCATGTGGCTGCG
Label	FAM-BHQ1

The ddPCR assay recognises the floxed region of the Prdm8 gene. Therefore, WT controls are expected to call at 2 copies and F1 animals with evidence of the floxed exon will call at 2 copies.

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.



## Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00000509035 of *Prdm8*. The stock was generated at MRC Harwell via pronuclear injection 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 wildtype loss of allele (WT-LOA) 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:

- Wildtype assay where the probe is situated within the deleted region.
- Mutant assay where the probe binds to the inserted LoxP sequence

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



## PRDM8-FLOX3'-WT1 assay (FAM labelled)

ACGGTAGGTTGGCTCGGGTGCCT**TGGGCTCACCTTGCACATCATAGGGTGGACGAAGCTTCTGAGggtgtgtcccgaatggaactag**ACAGAAGGGGAAGGAAAAGTTGGGGAGCGTTTACTTAGTAAATGCACAAATCAAGAAAGT  
**AAGTTGCACCA**AATAGGATAGTGATTTCATTTTTTTGGGCACTCA

Lower case letters denote the deleted sequence 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
PRDM8-FLOX3'-WT_F	n/a	<b><u>TGGGCTCACCTTGCACATC</u></b>	n/a	WT Forward
PRDM8-FLOX3'-WT_PROBE	FAM	<b>TGAGggtgtgtcccgaatggaac</b>	BHQ	WT Probe
PRDM8-FLOX3'-WT_R	n/a	<b><u>TGGTGCACCTTACTTCTTGATTG</u></b>	n/a	WT Reverse

## PRDM8-FLOX3'-MUT1 assay (FAM labelled)

ACGGTAGGTTGGCTCGGGTGCCT**GGCTCACCTTGCACATCATAGG**GTGGACGAAGCTTCTGAGataacttcgtagcatacattatacga**aagttatcgccggcgggtctga**gctcgccatcagtACAGAAGGGG**AAGGAAAAGTTGGGGAGCGTTT**  
**GACTTAGTAAATGCACAAATCAAGAAAGTAAGTTGCACCAATAGGATAGTGATTTCATTTTTTTGGGCACTC**  
 AG

Lower case letters denote the inserted LoxP sequence

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
PRDM8-FLOX3'-MUT_F	n/a	<b><u>GGCTCACCTTGCACATCATAGG</u></b>	n/a	Mutant Forward
PRDM8-FLOX3'-MUT_PROBE	FAM	<b>aagttatcgccggcgggtctga</b>	BHQ	Mutant Probe
PRDM8-FLOX3'-MUT_R	n/a	<b><u>CAAACGCTCCCCAACTTTTCCTT</u></b>	n/a	Mutant Reverse





## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**  
**CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAAG**GATGAAGCATAAGGATGCCAACTA**CTAACA  
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
CACCTATGA

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

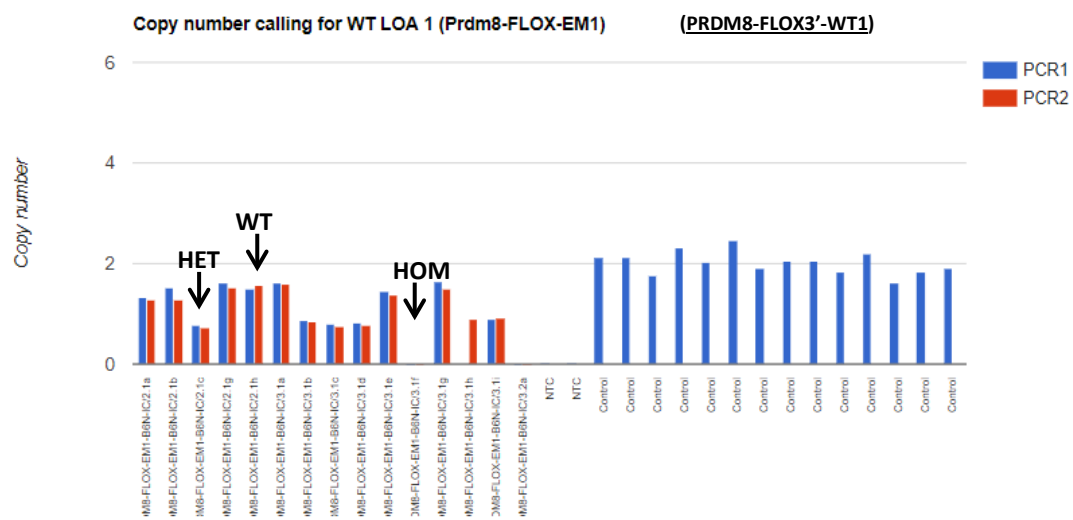
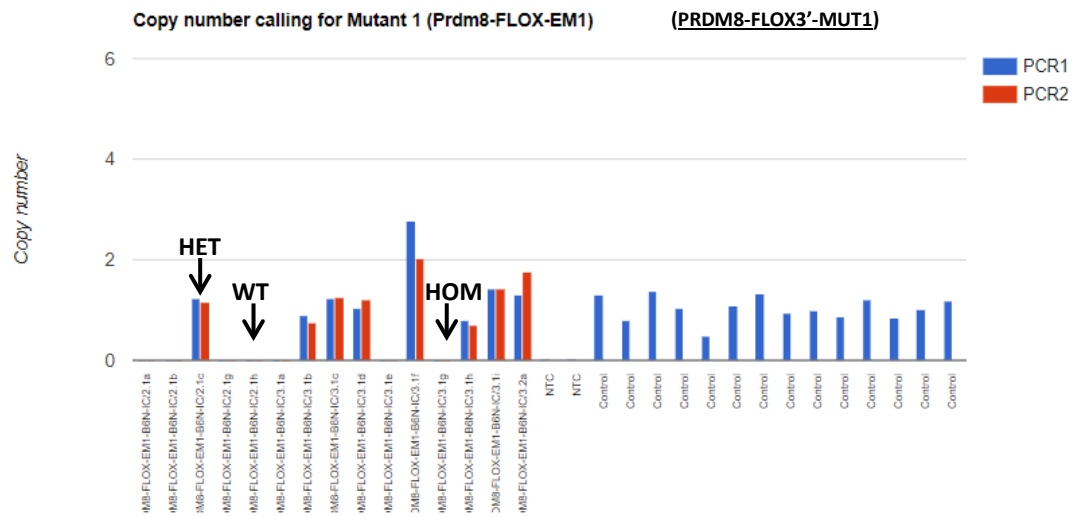
95°C for 20 sec  
Then 40 cycles of;  
95°C for 3 sec  
60°C for 30 sec



## Analysis

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

PRDM8-FLOX3'-WT1 and PRDM8-FLOX3'-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 252874 results)





# PRDM8-FLOX-EM1-B6N

Version No. 1

Date: 25.01.2020

Created/Updated by: AC