Name of Mouse model or mutation:
POLG-A449T-EM1-B6
POLG-A449T-EM2-B6

Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: A449T

Sequence details

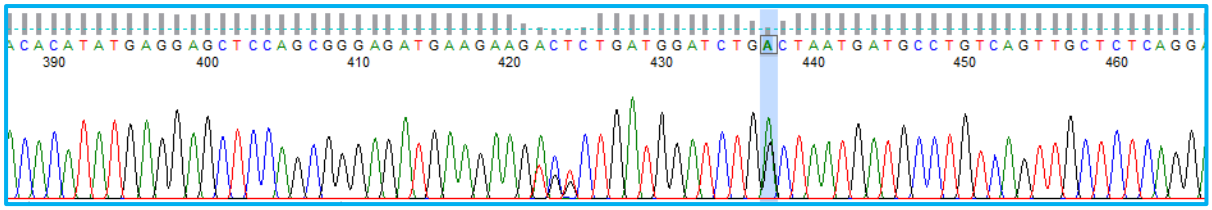
WT

GTCAAAGGCAGCATGAGGGATATCCGAGAGAACTTCCAGGTATGGTGCTGGCACAGCCAATACACA
GGCTGTGAGTAACTGCAACTGAACTTGGGGAGGTAGCTGGGGTTGTCCCTGTCTTCCTCCATTGTCC
CCGCAACAAGTTCCTTGTTACCTTGTGCTAGGATCTGATGCAGTACTGCGCCCGTGATGTGTGGGCC
ACCTTTGAGGTTTTCCAGCAGCAGCTGCCACTCTTCTTGGAGAGGTGAGGGGGAGCCAGGGGAATC
CCTGGGGTGGGTGGGCTCTGGTACTCGGTCCCATTGTAATCAGGTAGGGCTGATTCTTCCTTAGGCT
GGCCACTTAGCTTTTGTAGGCTGAGAGAATGGAGCCCCTATATTCAATGATTGTCTCCTGTGGTCATT
TATGGCAGCCCTTGGTGTTTGTCCCTTAGGTGTCCCCACCCAGTGACTCTGGCTGGCATGCTGGAGA
TGGGTGTGTCCTACCTGCCTGTCAACCAGAACTGGGAGCGTTACCTGACAGAGGCACAGAACACAT
ATGAGGAGCTCCAGCGGGAGATGAAGAAGTCGCTGATGGATCTGGCTAATGATGCCTGTCAGTTGC
TCTCAGGAGAGAGGTAGTCAGGTTCTGGGCAGGCTGGGTCAATGCAGGGTACAGGCAGGTAGGAG
GCCTGAATTTTAGCTCAGCCTTAACCCTAGCCTGGTGGACTGGCTCTGCAGGTACAAAGAAGACCCT
TGGCTCTGGGACCTAGAATGGGATTTGCAGGAGTTTAAGCAGAAAAAGGCAAAGAAGGTGAAGAA
GCCAGCCTCAGCCAGCAAGTTGCCCATCGAGGGAGCTGGGCCCTTTGGGGATCCCATGGATCAGGA
AGGTGGGAAGCTTAGATGGGAATGGGACAGGGTGATCTCTGGGACAGACCTAGAACCCAGGGGAC
CCTTGGAAAGGGTTGCTGTAGTCTTTCAGACCTGTGAGTCCCTTCTGGTTCTAAGCTCCCGAATTGTG
CACAGATCCTGGCCCGCCCAGCGAGGAGGAGGAGCTTCAGCGAAGTGTAACAGCCCACAACCGGTT
ACAGCAGCTGAGGAGCACCACGGACCTCCTGCCTAAGCGACCCCAGCACCTTCCAGGACACCCTGG
GTAAGACTGGCTCTCCA

Mutant

GTCAAAGGCAGCATGAGGGATATCCGAGAGAACTTCCAGGTATGGTGCTGGCACAGCCAATACACA
GGCTGTGAGTAACTGCAACTGAACTTGGGGAGGTAGCTGGGGTTGTCCCTGTCTTCCTCCATTGTCC
CCGCAACAAGTTCCTTGTTACCTTGTGCTAGGATCTGATGCAGTACTGCGCCCGTGATGTGTGGGCC
ACCTTTGAGGTTTTCCAGCAGCAGCTGCCACTCTTCTTGGAGAGGTGAGGGGGAGCCAGGGGAATC
CCTGGGGTGGGTGGGCTCTGGTACTCGGTCCCATTGTAATCAGGTAGGGCTGATTCTTCCTTAGGCT
GGCCACTTAGCTTTTGTAGGCTGAGAGAATGGAGCCCCTATATTCAATGATTGTCTCCTGTGGTCATT
TATGGCAGCCCTTGGTGTTTGTCCCTTAGGTGTCCCCACCCAGTGACTCTGGCTGGCATGCTGGAGA
TGGGTGTGTCCTACCTGCCTGTCAACCAGAACTGGGAGCGTTACCTGACAGAGGCACAGAACACAT
ATGAGGAGCTCCAGCGGGAGATGAAGAAG**AGT**CTGATGGATCTGaCTAATGATGCCTGTCAGTTGC
TCTCAGGAGAGAGGTAGTCAGGTTCTGGGCAGGCTGGGTCAATGCAGGGTACAGGCAGGTAGGAG
GCCTGAATTTTAGCTCAGCCTTAACCCTAGCCTGGTGGACTGGCTCTGCAGGTACAAAGAAGACCCT
TGGCTCTGGGACCTAGAATGGGATTTGCAGGAGTTTAAGCAGAAAAAGGCAAAGAAGGTGAAGAA
GCCAGCCTCAGCCAGCAAGTTGCCCATCGAGGGAGCTGGGCCCTTTGGGGATCCCATGGATCAGGA
AGGTGGGAAGCTTAGATGGGAATGGGACAGGGTGATCTCTGGGACAGACCTAGAACCCAGGGGAC
CCTTGGAAAGGGTTGCTGTAGTCTTTCAGACCTGTGAGTCCCTTCTGGTTCTAAGCTCCCGAATTGTG
CACAGATCCTGGCCCGCCCAGCGAGGAGGAGGAGCTTCAGCGAAGTGTAACAGCCCACAACCGGTT
ACAGCAGCTGAGGAGCACCACGGACCTCCTGCCTAAGCGACCCCAGCACCTTCCAGGACACCCTGG
GTAAGACTGGCTCTCCA

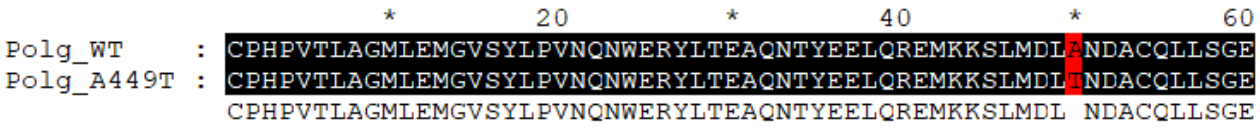
POLG-A449T-EM1-B6 or POLG-A449T-EM2-B6 Heterozygous F1 animal sequence trace:



Please note the sequences of POLG-A449T-EM1-B6 or POLG-A449T-EM2-B6 are the same, just transmitted from different founder animal.

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_Polg_A449T_F1 primer (5'-3')	GTCAAAGGCAGCATGAGGGA
Geno_Polg_A449T_R1 primer (5'-3')	TGGAGAGCCAGTCTTACCCA
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	59
Elongation time (min)	1
WT product size (bp)	1145
Mutant product size (bp)	1145
Notes	Amplicons sequenced with the following primers: Geno_Polg_A449T_F2 primer (5'-3': GTCTTCCTCCATTGTCCCCG) Geno_Polg_A449T_R2 primer (5'-3': TCCCCTGGGTTCTAGGTCTG)

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	POLG-A449T-UNI2
Forward Primer (5'-3')	GACAGAGGCACAGAACACAT
Reverse Primer (5'-3')	CCAGAACCTGACTACCTCTCT
Probe (5'-3')	TGATGCCTGTCAGTTGCTCTCAGG
Label	FAM-BHQ1

This ddPCR assay is universal to POLG - both WT and MUT alleles are recognised by this assay. Therefore, 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.

Polg-A449T

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation; A449T in *Polg*. 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

Polg-A449T

Polg-A449T-WT1 assay (FAM labelled)

ATGAGGAGCTCCAGCGGGAG**ATGAAGAAGtcgCTGATGGAT**C**TGGCTAATGATGCCTGTCAGTTGC**
TCTCAGGAGAGAGGTAGTCAGGTTCTGGGCAGGCTGGG**TCAATGCAGGGTACAGGC**AGGTAGGA

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 Polg-A449T	5' label	Sequence 5' → 3'	3' label	Oligo Type
Polg-A449T-WT_F	n/a	**ATGAAGAAGTCGCTGATGGAT**	n/a	Wild type Forward
Polg-A449T-WT_PROBE	FAM	**TGGCTAATGATGCCTGTCAGTTGC**	ZEN/IBFQ	Wild type Probe
Polg-A449T-WT_R	n/a	**GCCTGTACCCTGCATTGA**	n/a	Wild type Reverse

Polg-A449T-MUT1 assay (FAM labelled)

ATGAGGAGCTCCAGCGGGAG**ATGAAGAAGagtCTGATGGATCTG**aCTAATGATGCCTGTCAGTTGC
TCTCAGGA**GAGAGGTAGTCAGGTTCTGGGCAG**GCTGGG**TCAATGCAGGGTACAGGC**AGGTAGGA

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 Polg-A449T	5' label	Sequence 5' → 3'	3' label	Oligo Type
Polg-A449T-MUT_F	n/a	**ATGAAGAAGAGTCTGATGGATCTG**	n/a	Mutant Forward
Polg-A449T-MUT_PROBE	FAM	**CTGCCCAGAACCTGACTACCTCTC**	BHQ	Mutant Probe
Polg-A449T-MUT_R	n/a	**GCCTGTACCCTGCATTGA**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo Polg-A449T	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

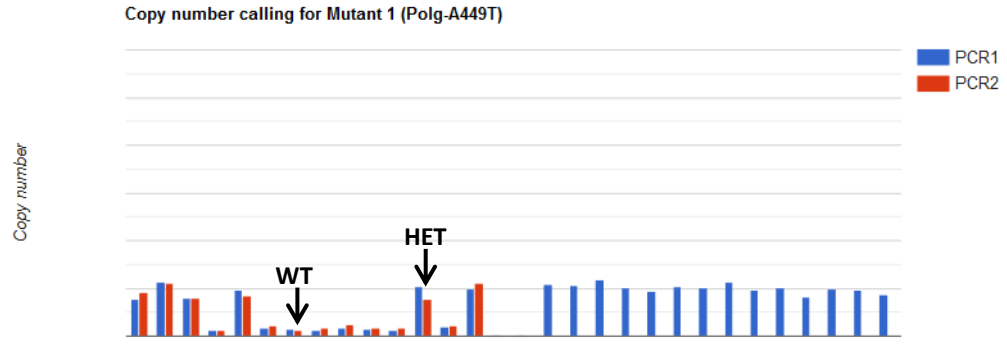
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

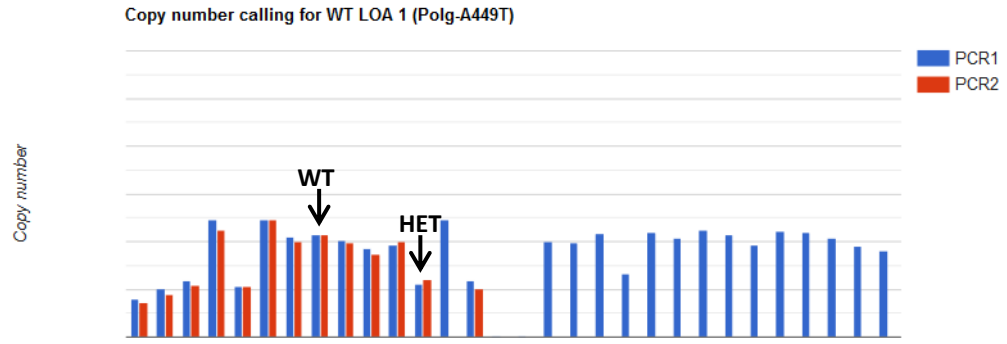
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Polg-A449T-WT1 and Polg-A449T -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 291118 results)





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