Name of Mouse model or mutation:
Pax2-S382Lfs-EM1-B6N

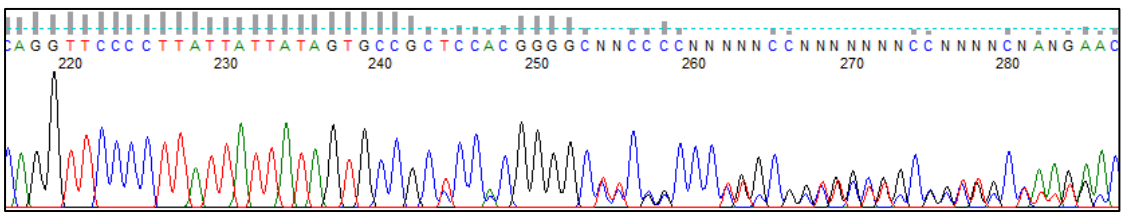
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: S382Lfs

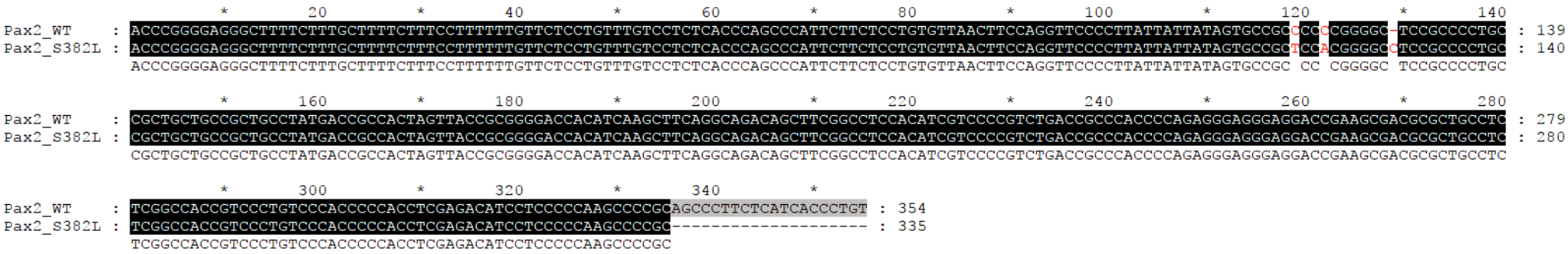
Sequence details
Pax2 WT
CTGGAGGGCTCTTAGCCAACAGTGGCCGTCGGACCCCTTGGGGGATCCGCAGCCGGGTGTCACCTG
TCCTGTCCCTCTCCCGCCCTGTGGATGCAGCACTACTCCTGGGCATCTGGCCTCGGGGCCGAGCCAC
AGTCCTCCAGGCCTGATCCCGCTGGACCCGGGGAGGGCTTTTCTTTGCTTTTCTTTCCTTTTTTGTTCT
CCTGTTTGTCCTCTCACCCAGCCCATTCTTCTCCTGTGTTAACTTCCAGGTTCCCCTTATTATTATAGTG
CCGCCCCCCGGGGCTCCGCCCCTGCCGCTGCTGCCGCTGCCTATGACCGCCACTAGTTACCGCGGGG
ACCACATCAAGCTTCAGGCAGACAGCTTCGGCCTCCACATCGTCCCCGTCTGACCGCCCACCCCAGA
GGGAGGGAGGACCGAAGCGACGCGCTGCCTCTCGGCCACCGTCCCTGTCCCACCCCCACCTCGAGA
CATCCTCCCCCAAGCCCCGCAGCCCTTCTCATCACCCTGT

Pax2 S382Lfs Mutant
CTGGAGGGCTCTTAGCCAACAGTGGCCGTCGGACCCCTTGGGGGATCCGCAGCCGGGTGTCACCTG
TCCTGTCCCTCTCCCGCCCTGTGGATGCAGCACTACTCCTGGGCATCTGGCCTCGGGGCCGAGCCAC
AGTCCTCCAGGCCTGATCCCGCTGGACCCGGGGAGGGCTTTTCTTTGCTTTTCTTTCCTTTTTTGTTCT
CCTGTTTGTCCTCTCACCCAGCCCATTCTTCTCCTGTGTTAACTTCCAGGTTCCCCTTATTATTATAGTG
CCGCTCCACGGGGCCTCCGCCCCTGCCGCTGCTGCCGCTGCCTATGACCGCCACTAGTTACCGCGGG
GACCACATCAAGCTTCAGGCAGACAGCTTCGGCCTCCACATCGTCCCCGTCTGACCGCCCACCCCAG
AGGGAGGGAGGACCGAAGCGACGCGCTGCCTCTCGGCCACCGTCCCTGTCCCACCCCCACCTCGAG
ACATCCTCCCCCAAGCCCCGC

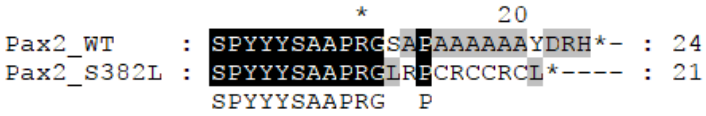
Pax2-S382Lfs-EM1-B6N Heterozygous F1 animal sequence trace:



Nucleotide Alignment:



Predicted Protein Alignment:



QC strategy employed at Harwell to check allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_Pax2 _F1	CTGGAGGGCTCTTAGCCAAC
Geno_ Pax2_R2	ACAGGGTGATGAGAAGGGCT
Taq Polymerase used	Roche Expand DNTPack
Annealing Temperature (°C)	57
Elongation time (min)	1
WT product size (bp)	605
Mutant product size (bp)	605
Notes	Sequence with Pax2_seq_R GGGAGGATGTCTCGAGGTG

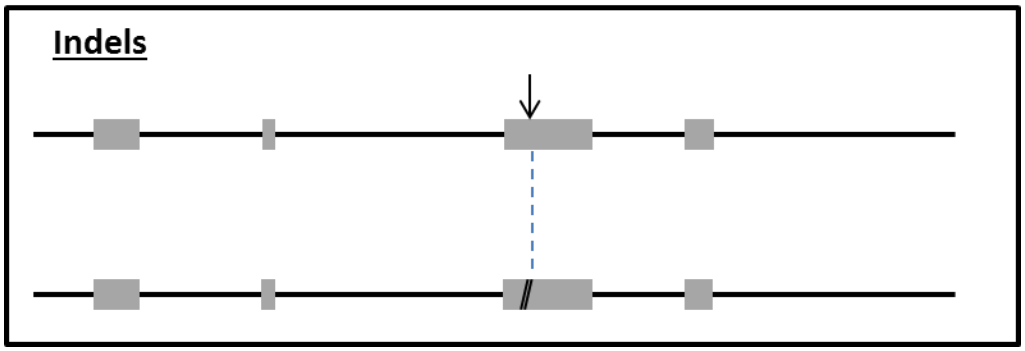
All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on Y chromosome.

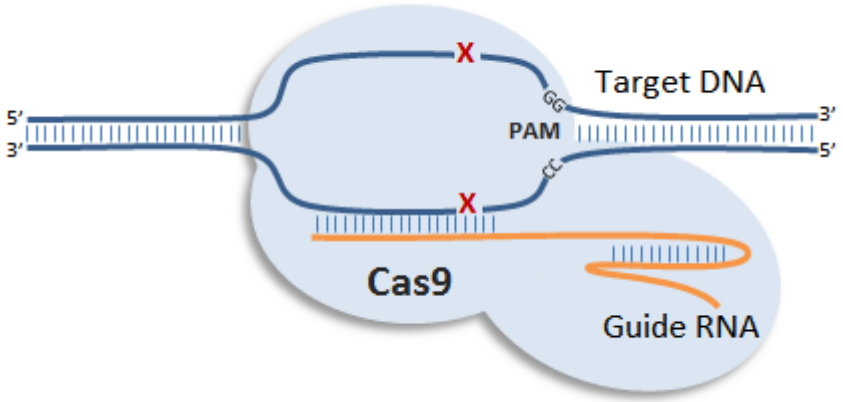
Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to detect the donor sequence:

Assay name	Pax2-S382Lfs-DONOR1-MUT1
Forward Primer	CGGAGGCCCCGTGGAG
Reverse Primer	TCACCCAGCCCATTCTTCTC
Probe	CGGCACTATAATAATAAGGGGAACCTGGA
Label	FAM-BHQ1

Pax2-S382Lfs Genotyping Strategy

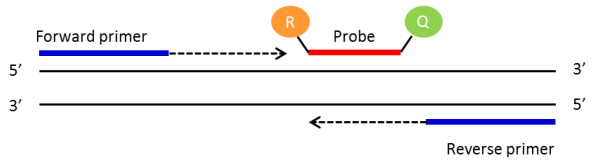
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



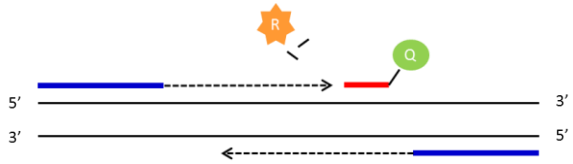


qPCR genotyping strategy

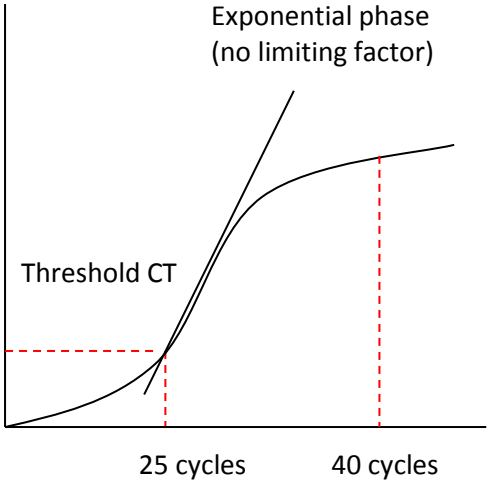
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that is complimentary to part of the DNA sequence between the forward and reverse primers. At the 5' end of the probe there is a fluorescent reporter (R) and at the 3' end a quencher (Q). Whilst they are in close contact with each other there is no fluorescent signal.



As the forward primer is extended the reporter is cleaved from the probe resulting in a fluorescent signal being detected. Once the primer extends enough to release the quencher this signal is blocked. By using probes with different fluorescent signals multiple PCR assays can be multiplexed and run together.



PCR reaction plot



The number of cycles the PCR takes to reach a set threshold is known as the CT value. This is inversely correlated to the amount of template DNA in the sample.

e.g. CT 25 = 2 x template DNA
CT 26 = 1 x template DNA
CT above 30 = no template represented in the sample

CT value can be used to determine how many copies of a particular allele samples have.

All our qPCR are run in duplicate. A FAM labelled genotyping assay is run in multiplex with a VIC labelled internal control Dot1l.

Pax2-S382Lfs Genotyping Strategy

Samples are genotyped with a mutant assay. This is a FAM labelled assay that is designed to detect the critical exon that has been modified. If the animal contains the modified allele the copy number of this assay should increase by 1. For autosomal genes that have been targeted this means the following

WT= 0 copies of the mutant assay
HET = 1 copy of the mutant assay
HOM = 2 copies of the mutant assay

Pax2-S382Lfs-DONOR1-MUT1 assay (FAM labelled probe)

Fragment Sequences
The following sequences are the WT and Mutant sequences for Pax2 gene in which CRISPR modifications are highlighted in **BOLD UNDERLINED** letters

WT

CTGGAGGGCTCTTAGCCAACAGTGGCCGTCGGACCCCTTGGGGGATCCGCAGCCGGGTGTCACCTGTCCTGTCCC
TCTCCCGCCCTGTGGATGCAGCACTACTCCTGGGCATCTGGCCTCGGGGCCGAGCCACAGTCCTCCAGGCCTGAT
CCCGCTGGACCCGGGGAGGGCTTTTCTTTGCTTTTCTTTCCTTTTTTGTTCTCCTGTTTGTCCTCTCACCCAGCC
CATTCTTCTCCTGTGTTAACTTCCAGGTTCCCCTTATTATTATAGTGCCGC**C**CC**C**CGGGG*****CTCCGCCCCTGCCG
CTGCTGCCGCTGCCTATGACCGCCACTAGTTACCGCGGGGACCACATCAAGCTTCAGGCAGACAGCTTCGGCCTC
CACATCGTCCCCGTCTGACCGCCCACCCCAGAGGGAGGGAGGACCGAAGCGACGCGCTGCCTCTCGGCCACCGT

Pax2-S382Lfs-EM1

CTGGAGGGCTCTTAGCCAACAGTGGCCGTCGGACCCCTTGGGGGATCCGCAGCCGGGTGTCACCTGTCCTGTCCC
TCTCCCGCCCTGTGGATGCAGCACTACTCCTGGGCATCTGGCCTCGGGGCCGAGCCACAGTCCTCCAGGCCTGAT
CCCGCTGGACCCGGGGAGGGCTTTTCTTTGCTTTTCTTTCCTTTTTTGTTCTCCTGTTTGTCCTCTCACCCAGCC
CATTCTTCTCCTGTGTTAACTTCCAGGTTCCCCTTATTATTATAGTGCCGC**T**CC**A**CGGGG**C**CTCCGCCCCTGCCG
CTGCTGCCGCTGCCTATGACCGCCACTAGTTACCGCGGGGACCACATCAAGCTTCAGGCAGACAGCTTCGGCCTC
CACATCGTCCCCGTCTGACCGCCCACCCCAGAGGGAGGGAGGACCGAAGCGACGCGCTGCCTCTCGGCCACCGT

Pax2-S382Lfs-DONOR1-MUT1 primers and probe

Primer 1 = CGGAGGCCCCGTGGAG
Primer 2 = TCACCCAGCCCATTCTTCTC
Probe = CGGCACTATAATAATAAGGGGAACCTGGA

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTCGTTTTCT
GTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTT
ATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

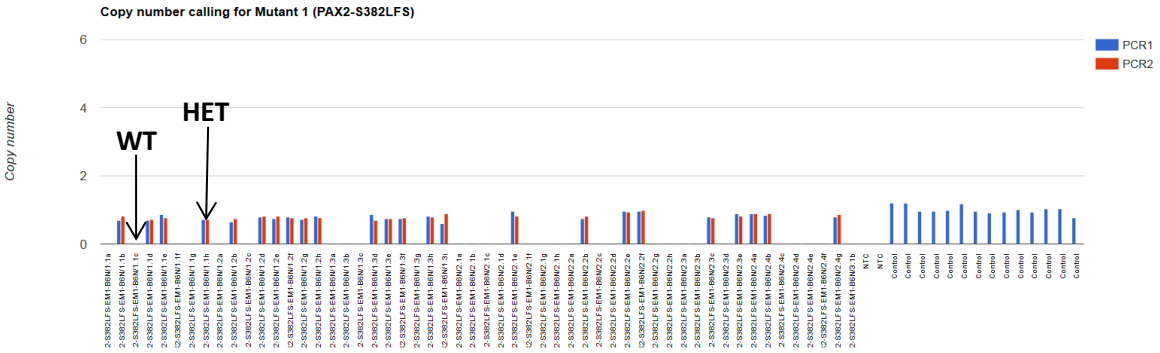
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20µM)	0.225µl
Primers Dot1L_R (20µM)	0.225µl
Probe DotL_2M (5µM)	0.2µl
FAM Assay (probe 5µM & primers 15µM each)	0.3µl
ddH20	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

Pax2-S382Lfs LOA copy called result, image showing both replicates and controls

From T185977



Version No.	1
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Created/Updated by:	Ramakrishna Kurapati
Approved by:	Debbie Williams