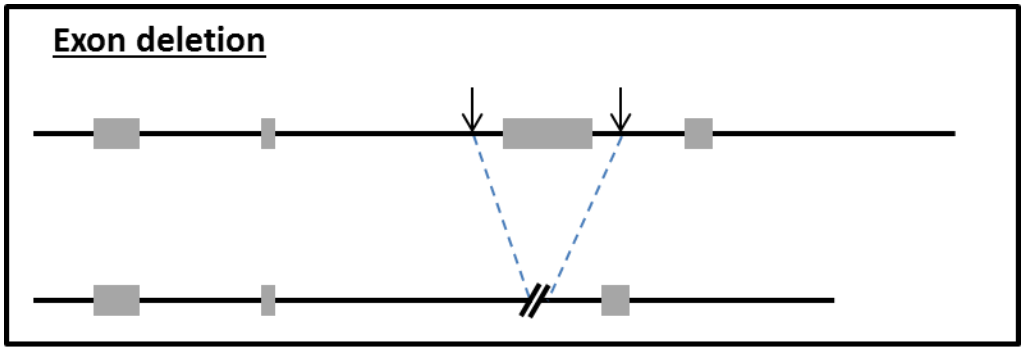
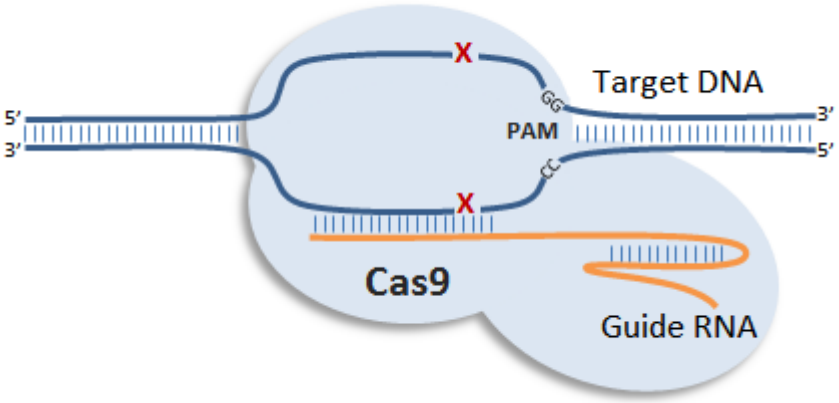
Mpeg1-CRE Genotyping Strategy

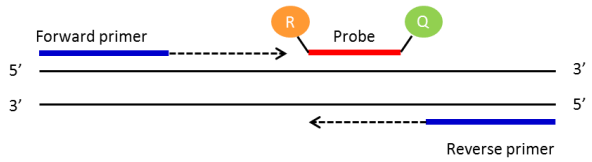
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



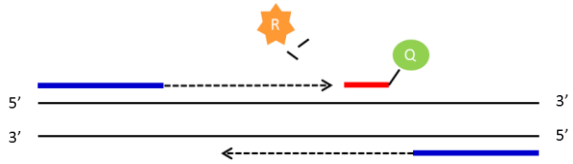


qPCR genotyping strategy

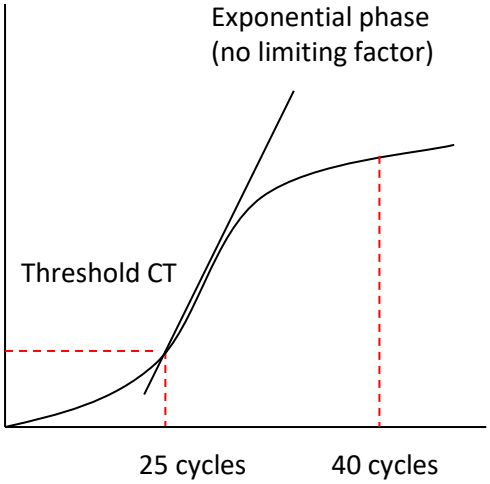
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that 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.

Mpeg1-CRE Genotyping Strategy

Samples are genotyped with both WT loss of allele (WT-LOA) and Mutant assays. Samples for this line are genotyped using the following primers and probe (see Figure1)

- Universal probe and Universal primer designed near the CRISPR deletion for both alleles.
- Wildtype specific primer in the deletion designed for the WT allele.
- Mutant specific primer that bridges the junction designed for the CRISPR mutant allele.

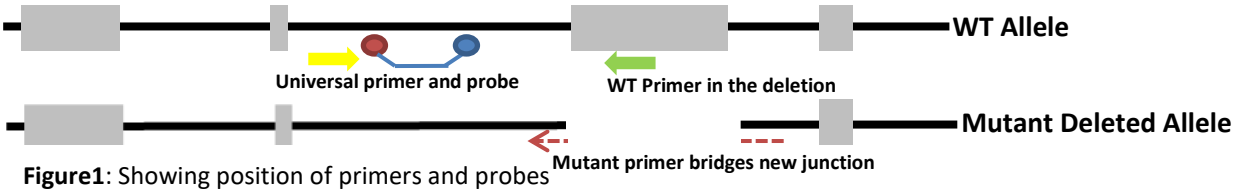


Figure1: Showing position of primers and probes

Mpeg1-CRE WT1 assay (FAM labelled)

TCGGAAGTACAAGAAGAAGGAATACCAGGAAATTGAGGAGCAGGAGAGTTTGGTTGGAAGCTTAGCAACAGATGCAACAGTCC
TTAATGGAGAAGAGGATCCAAGTCCAGCTTAA [iCRE Insertion]TTGTCTCCAAAGGAAACAGTTTCCAGCCACAGCT

Mpeg1-CRE-Univ-Probe (5nmol)	TGGAAGCTTAGCAACAGATGCAACA
Mpeg1-CRE-WT-R (15nmol)	AACTGTTTCCTTTGGAGACAATTAAGC
Mpeg1-CRE-Univ-F (15nmol)	GAGGAGCAGGAGAGTTTGGT

Mpeg1-CRE MUT1 assay (FAM labelled)

TCGGAAGTACAAGAAGAAGGAATACCAGGAAATTGAGGAGCAGGAGAGTTTGGTTGGAAGCTTAGCAACAGATGCAACAGTCC
TTAATGGAGAAGAGGATCCAAGTCCAGCTTAAcctGAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGC
CGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAA
ACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGA
AGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGC
GACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTG
GATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTA
TGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACG
GGGACGTGGTTTTCCTTTGAAAAACACGATGATAAGCTTGCCACAACCATGTCCAATTTACTGACCGTACACCAAAATTTGCC
TGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGC
ATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAA
CCTGAAGATGTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCT
AAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAG
AAAACGTTGATGCCGGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAA
AATAGCGATCGCTGCCAGGATATACGTAATCTGGCATTTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGC
CAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCA
CCGCAGGTGTAGAGAAGGCACTTAGCCTGGGGGTAACTAAACTGGTCGAGCGATGGATTTCCGTCTCTGGTGTAGCTGATGAT
CCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCT
GGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGAC
ACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACC
AATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATTG
ATTGTCTCCAAAGGAAACAGTTTCCAGCCACAGCT

Mpeg1-CRE-Univ-Probe (5nmol)	TGGAAGCTTAGCAACAGATGCAACA
Mpeg1-CRE-MUT-R (15nmol)	AGCGGCTTCGGCCAGTAAC
Mpeg1-CRE-Univ-F (15nmol)	GAGGAGCAGGAGAGTTTGGT

Dot1l internal control (VIC labelled)

TGTTTCCTTGTTCTTTTCCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGC
TGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCC

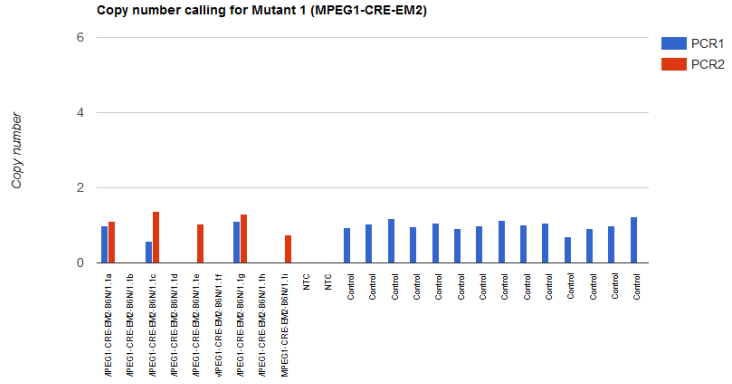
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

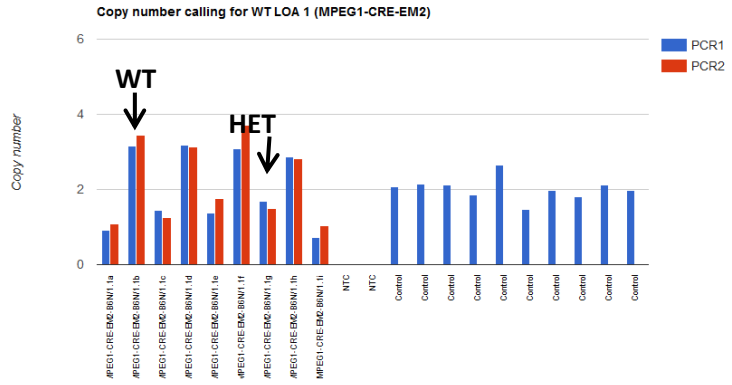
qPCR master mix

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

The EM3 line contains a random integration.

Mpeg1-CRE Assay copy called result, image showing both replicates and controls for WT and Mutant assays (T215656)





Version No. 1

Date: 05/09/18

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Approved by: Debbie Williams