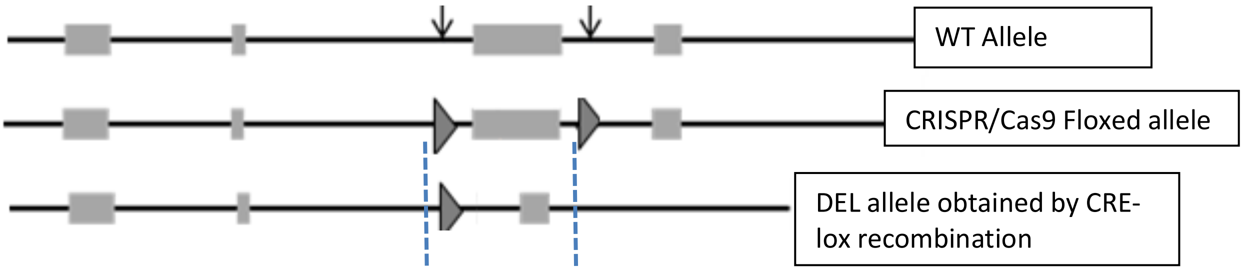
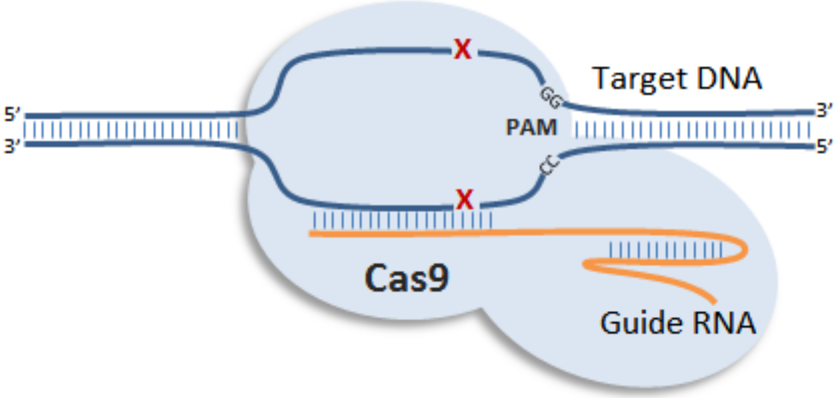
Hnf1a-DEL Genotyping Strategy

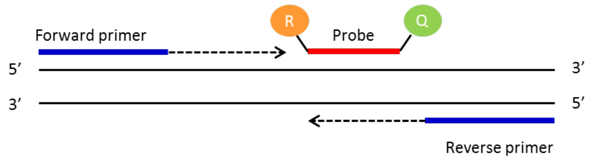
Animals have been engineered using the CRISPR/Cas9 technology. Conditional knockouts are created through this technology by introducing loxp sites targeting around a targeted critical region. This floxed exon is later treated with CRE expressing animals to delete the critical exon.



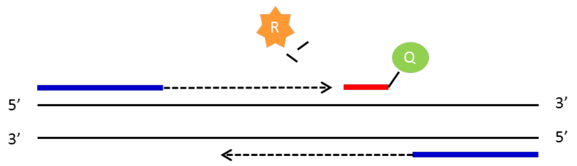


qPCR genotyping strategy

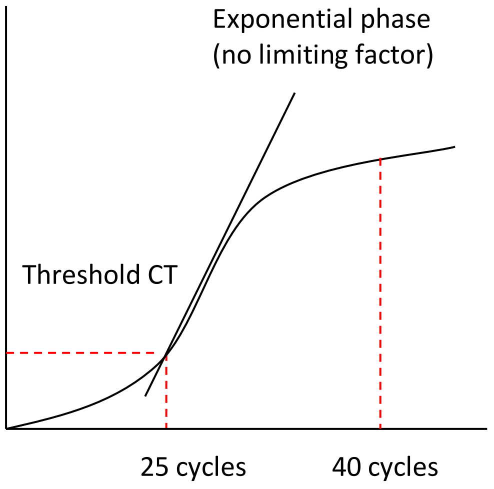
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Hnf1a-FLOX-EM1 & EM2 Genotyping Strategy

Samples are genotyped with a WT loss of allele (WT-LOA) assay. This is a FAM labelled assay that is designed to detect the critical exon that has been floxed. If the animal contains the modified allele the copy number of this assay should drop by 1. For autosomal genes that have been targeted this means the following

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

Hnf1a-FLOX-WT1 assay (FAM labelled probe)

Fragment Sequence

The following sequence is a part of genomic sequence from Hnf1a gene in which sequence in BLACK letters is the breakpoint where 3' loxp sequence is inserted.

GCTCATAGGGTGGCAGTGTCACCCCCTCTTCCATCTCACAGACACATGAGACTGAGAGGGGCCAGAAAGGCGTCC
TCAGACTTTCTGTGCCTTTTGCACAAGGTGCCAAGGGAAGGAAGCAGAATGGGTACACACACTGGGGTCCTCCCT
CCAGGAACTCACTCTCTGCTGAATGGGAGAGAGATCATTATATAATGAG

Hnf1a-FLOX-WT1 primers and probe

Primer 1 = AAAGGCGTCCTCAGACTTTCTG
Primer 2 = GTGTACCCATTCTGCTTCCTTC
Probe = TGCCTTTTGCACAAGGTGCCAAG

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

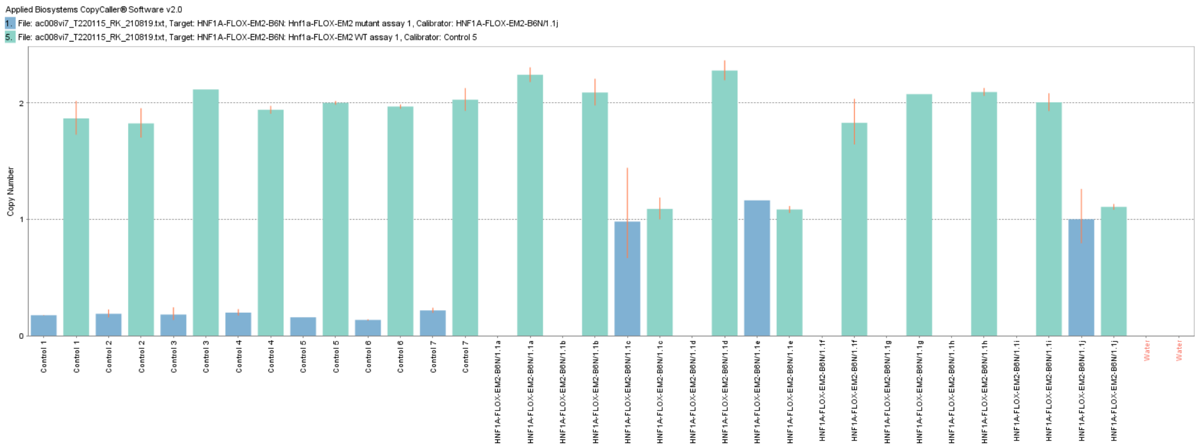
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

Hnf1a-FLOX-EM1 & EM2 LOA copy called result:

Task 220115 Results



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