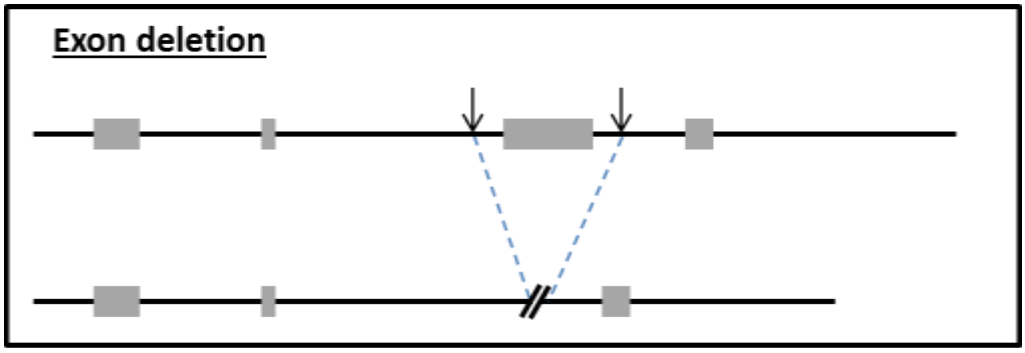
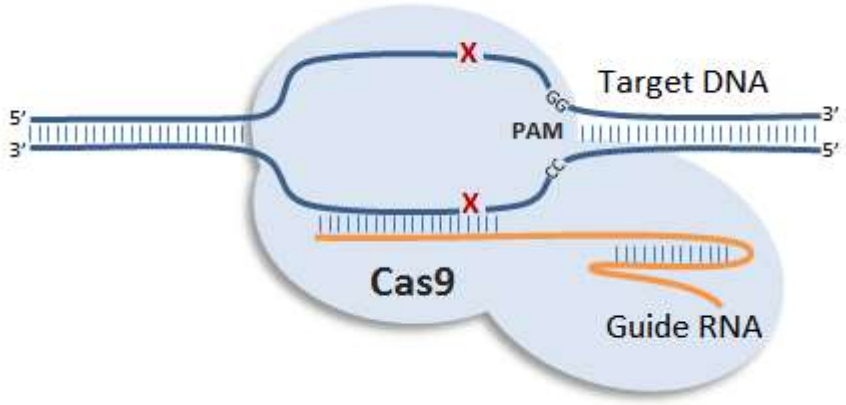
Clrn2-DEL629 Genotyping Strategy

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).

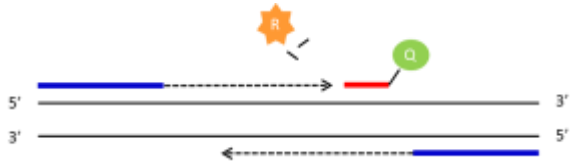




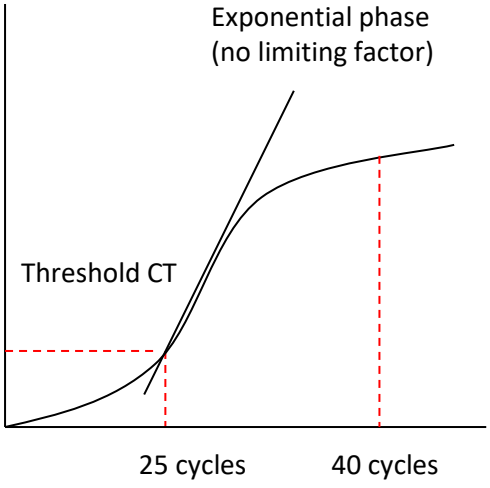
qPCR genotyping strategy

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.

Clrn2-DEL629 Genotyping Strategy

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

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

Clrn2-CR-LOA-WT1 assay (FAM labelled probe)

From the sequence below sequences between <> are the 629nt and 8nt deletions.

GAGTATTTCACTAACCCTTCCTGCCACATTCATATGGGAAAATCAATATTTTCATGAAGTAGTACACAACAAGAG
TATTATGTGTTCTTGATACTTGATGGCAATAACATTTACTCTGCCAAGCCA<ATGCCTTGGGCTTATGGGAAGTG
CAGCAGTTGACATCAGATCCCAGAACTGCAGGATCACTGTAATGTCAACTGTGAGGCACATTCAGATTTTGTGTC
CACTCCAAGACAGAGAAGATTGCTGGCCAGTTAACAAGTGGCAGCTCTAGGGTTTGAGGACCCGTGTCAGGAAGT
CTCTTGGCTTTTGAAAACCGAGCATTCTTGTGTCTTGCTCTCACCCTCTACTTGATTACCTTGGGCTATGCAAGT
TCTGGCATTTGTGAAAGGAGCCACACTCTGACCTTGAAATATCATTTCTCTCTGCAGTCTTCCCGCATCTGGTGA
AGGAGCTCAATGCAGGCCTCCACGTCACAATTCTCTTGCTCCTCTTCCTGGCGTTGGCCCTGGCTCTGGTCAGCA
TGGGTTTTGCTATTCTCAACATCATTCAGGTCCCCTACAGAGCAGTCAATGGCCCTGGTGGCATCTGCCTTTGGA
ATGTCCTCGCAGGTAAGAAGTCCCCAAGAGAGAGAAACTAGTGTCCACACCATCAAGCTGGGCTCCCAAGTGTTA
GCCAGGGAATCTGGGTGTTCCTTTTGGGGGTTCTTGGTCTTGTTAATGAAAGAATTTATAAACAGACTCAAAAGG
AAGCTA>AAGGGCACTTTTA<TTGCAGTC>TGGAAGAGAAAAAATGAGAGAGCAGACAAGCTGTAAACCTTGCCA
GCTGCCAG

Clrn2-CR-LOA-WT1 primers and probe

Primer 1 = GCTCTGGTCAGCATGGGTT
Primer 2 = GGGCCATTGACTGCTCTGTA
Probe = TTGCTATTCTCAACATCATTCAGGTCCC

Clrn2-DEL629-MUT1 assay (FAM labelled probe)

GAGTATTTCACTAACCCTTCCTGCCACATTCATATGGGAAAATCAATATTTTCATGAAGTAGTACACAACAAGAG
TATTATGTGTTCTTGATACTTGATGGCAATAACATTTACTCTGCCAAGCCA[629nt_replaced_by]**tacat**
AAGGGCACTTTTA[8nt_DEL]TGGAAGAGAAAAAATGAGAGAGCAGACAAGCTGTAAACCTTGCCAGCTGCCAG

Primer 1 = AACCCTTCCTGCCACATTCATAT
Primer 2 = GGCAGCTGGCAAGGTTTAC
Probe = TGCCAAGCCATACATAAGGGCAC

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

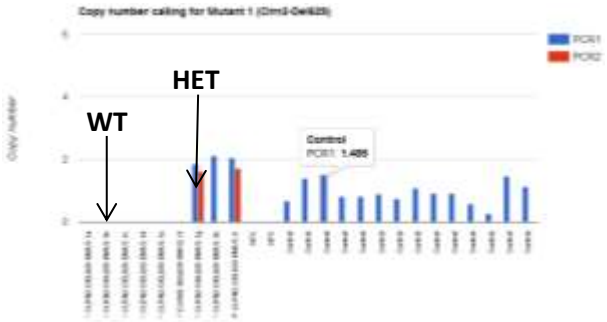
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

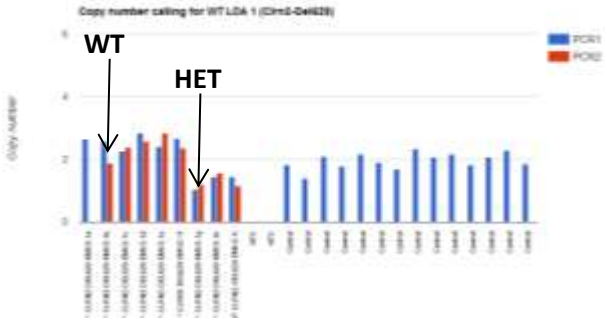
qPCR master mix

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

Clrn2-DEL629 LOA copy called result, image showing both replicates and controls from both WT-LOA and Mutant assays

Task 207638 Results





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