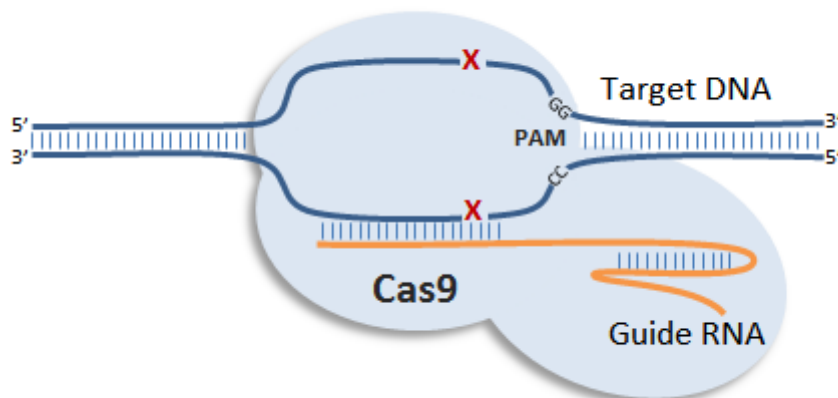
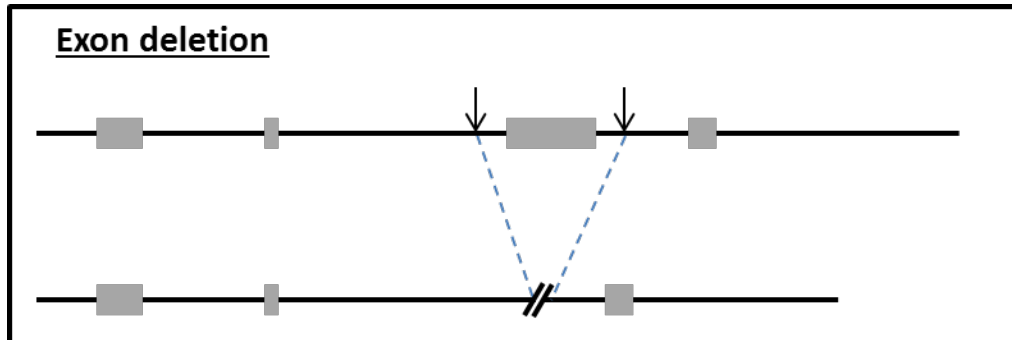


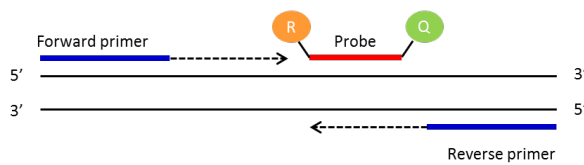
Tbx15-DEL964 Genotyping Strategy

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).

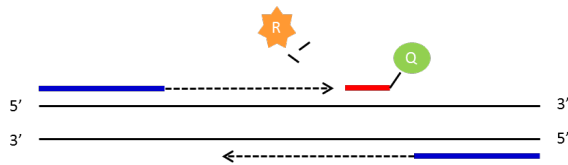


qPCR genotyping strategy

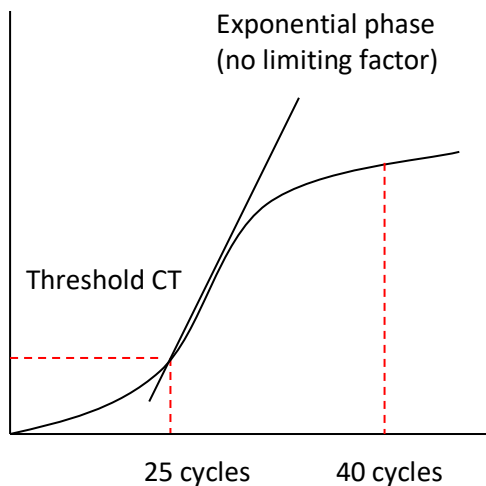
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Tbx15-DEL964 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.

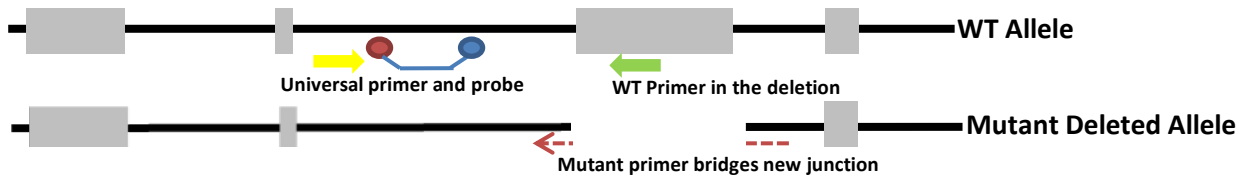


Figure1: Showing position of primers and probes

Tbx15-DEL964 WT1 assay (FAM labelled)

```
TTCATATCAGTGTTGGGCTGCCCCCTCTGTTCTCCATGTAGTTTCTGTATGCTGTTAGGTTAGACTTTCCAACCA
TAGGCTAACTTGGCGCACTTCTCTTATCTCTTTTCTGGAAAGCTTTAGTCTTCAAGGACGAAAGGCCTAATA
AACAAGTCTGCTTCTGAGGTACCTTCTAACTGTGTAATAATAATTGCTGAATTTACACAGATAACATGT
ACATGTTTACGGATGCCTGGAGATTATCCCTGGCCTCGACCATACAAATTTGCTTTGATGTTTCAATGTGTCCA
TGCCCCATACCATGCAGAGCCATAGAGAGAGAAGAGCCTTACAGCTTGGGATTTCTATTTTATGGGACGGTGAAGA
CTGTAGTTTAGCACATGCCACAGTCTTTCTGAGAGTCTTTGGGATGACGCACCTGTACTTCACACCTGATGAA
GAGCCTTAGCCATTCTTCTTTTGCCTGTATGGATGACTCCAGGGCCTTGGGACAAAGAACCCAGCCATTTGAG
TATATGAGAATACTGGTACTTGGCTTCTGGCCACTTTGACTAGACATTCATCAAGGACAGAGAGACAACCTTCCCC
TTCCTTCCACGCTTCTGCCAGAGCTCAGATAAAGACACCACGCCCATGTGCCATGAGGCAGTTACTAGAGAAGC
TAAGCATCTGAGTTGTAATACTGAATGTGACTTTATTTCTGATATCTGGCCTTTCTCCATACACTGAAACAAAGA
TCCTTTGCCCTATAGAAGACAGATATCTTTTAAAAAATAAATTCATTTTTTACACTCCATATCCACTCCC
TGCCCCGTTCACCGAAGACAGACATCTTATCCAGATAATGAGTGCCGTGTAGACAGGGAGACTGATCTGTAC
CACAGTCATGGTCTTACAGTCCAGCAGAGAAAACAAATCACATGTTTCATCTTAGCAGTACTCCTTTGCTCATTC
AACAAGCAGAATTCTAGGTGCTGGAAGGGATAAGGGTGAGTAAGCGTGGACCTTGCTCTTTAGAAGATAACACAT
ATTCAGATAAAATAAAATATAGGATGATGAGTATCCTGTCAGGTTGTGGACAGAAGCTAGGAAAATGACCTGATTT
```

Tbx15-DEL964-Univ-Probe (5nmol)

CCAACCATAGGCTAACTTGGCGCA

Tbx15-DEL964-WT-R (15nmol)

GACCTCAGAAGCAGAAGCTGTTC

Tbx15-DEL964-Univ-F (15nmol)

AGTTTCTGTATGCTGTTAGGTTAGAC

Tbx15-DEL964 MUT1 assay (FAM labelled)

```
TTCATATCAGTGTTGGGCTGCCCCCTCTGTTCTCCATGTAGTTTCTGTATGCTGTTAGGTTAGACTTTCCAACCA
TAGGCTAACTTGGCGCACTTCTCTTATCTCTTTTCTGGAAAGCTTTAGTCTTCA [964nt del]
TGGACAGAAGCTAGGAAAATGACCTGATTT
```

Tbx15-DEL964-Univ-Probe (5nmol)

CCAACCATAGGCTAACTTGGCGCA

Tbx15-DEL964-MUT-R (15nmol)

CATTTTCTAGCTTCTGTCCATGAAG

Tbx15-DEL964-Univ-F (15nmol)

AGTTTCTGTATGCTGTTAGGTTAGAC

Dot1l internal control (VIC labelled)

```
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTTCGACTTGAGAGCTGCCCCGTAATGGTCGTGCT
GGGGCAAGGCTTTATTTTACGGCGTAGCACACATGGTGGCAATGGGACTCTGTAGGATCTGCCACACCCATCAG
```

Primer 1 = GCCCCAGCACGACCATT

Primer 2 = TAGTTGGCATCCTTATGCTTCATC

Probe = CCAGCTCTCAAGTCG

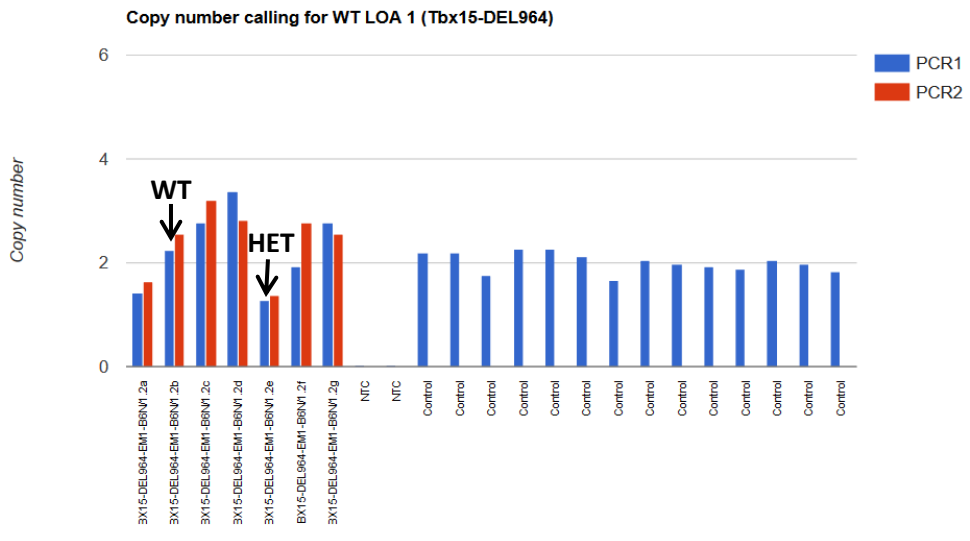
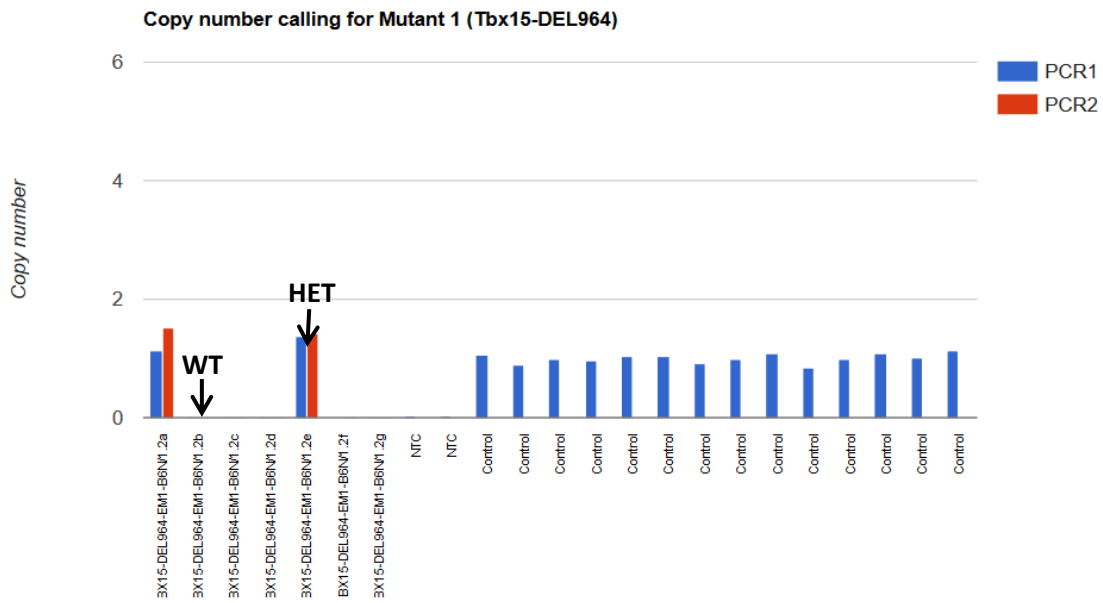


qPCR master mix

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
ddH2O	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

Tbx15-DEL964 Assay copy called result, image showing both replicates and controls for WT and Mutant assays

Task 220119 Results





Version No. 1
Date: 09.11.2018
Created/Updated by: Ramakrishna Kurapati
Approved by: Daniel Ford