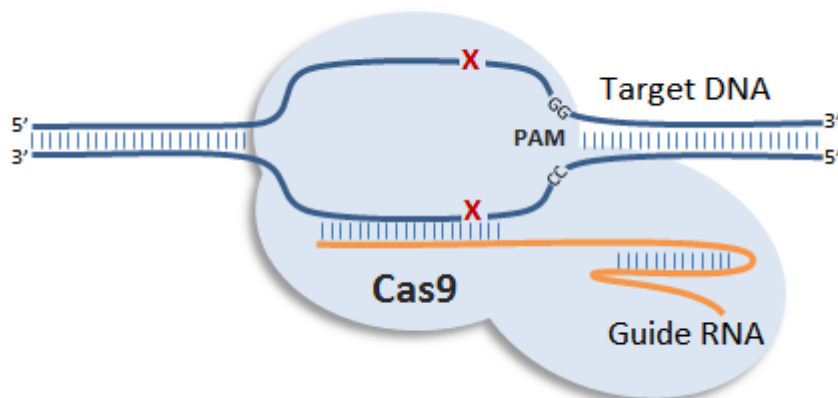
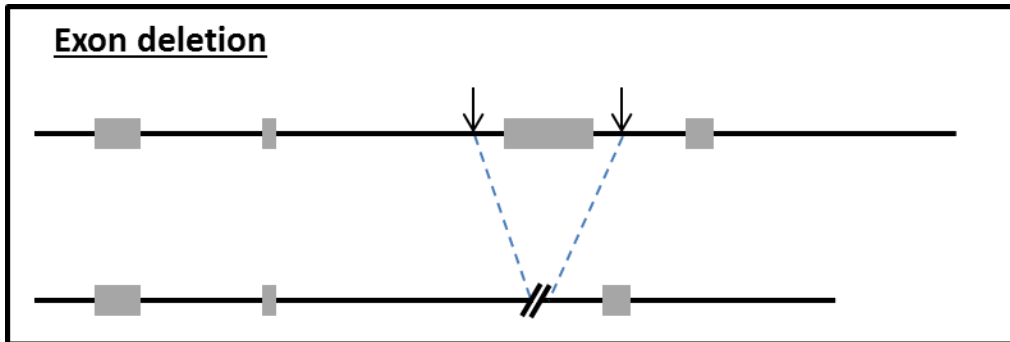


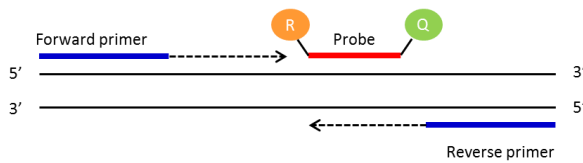
Fto-DEL82 Genotyping Strategy

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).

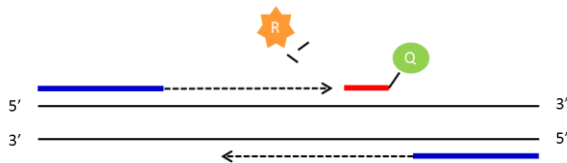


qPCR genotyping strategy

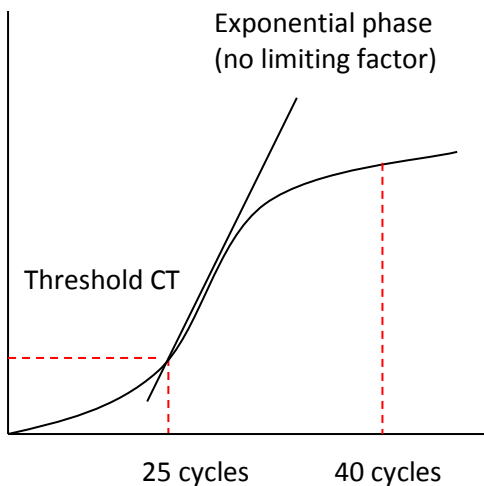
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Fto-DEL82 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.

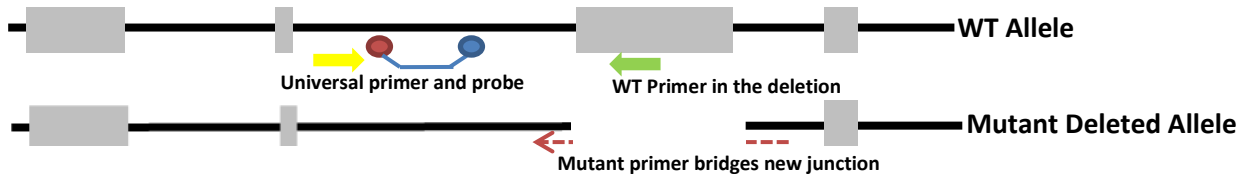


Figure1: Showing position of primers and probes

Fto-DEL82 WT1 assay (FAM labelled)

```
CCCTGCTGCCAGGCTGCCTCCGAGCCCAGCAAACCTCATTCCCTCC CAGGCAAAAGCAAAAGGTGACATAC ACC CAGG
AGCCAGATTGTCCACAGCA GACTTGTAAGCAACAAGATAATCTCATTGTTCCCTCCTGCTACTTAAAATAAAGGTA
ATATTGATTTTACGGTAGCAGTTTCGAGTCCTAAGGCAT CGTATTGATTAAGCGT CTAGTGAGAAATTTGTAGGGCA
GGCTTCTGAGCTGCAGCCACAGGGCATCTCCCCACTGGGCCAGGC CTCTGTGCTGACCCTACTATTAGAAGTGTT
TAGGCTCTGGTCAGAACATTTATTTTACCTGATGGATGGCTTAATCCAGCCCCTCACCTTAATCTGACCTTAAAG
ATGGCAGTCCAACCTCTTTTGTCTGAGCGAATGTGGAGACTGATT
```

Fto-DEL82-Univ-Probe (5nmol)

CAGGAGCCAGATTGTCCACAGCA

Fto-DEL82-WT-R (15nmol)

GGCTGCCCTACAAATTCTCACTAG

Fto-DEL82-Univ-F (15nmol)

CAGGCAAAAGCAAAAGGTGACATAC

Fto-DEL82 MUT1 assay (FAM labelled)

```
CCCTGCTGCCAGGCTGCCTCCGAGCCCAGCAAACCTCATTCCCTCC CAGGCAAAAGCAAAAGGTGACATAC ACC CAGG
AGCCAGATTGTCCACAGCA GACTTGTAAGCAACAAGATAATCTCATTGTTCCCTCCTGCTACTTAAAATAAAGGTA
ATATTGATTTTACGGTAGCAGTTTCGAGTCCTAAG GCAT [82nt DEL]
CTCTGTGCTGACCCT ACTATTAGAAGTGTTTAGGCTCTGGTCAGAACATTTATTTTACCTGATGGATGGCTTAAT
CCAGCCCCTCACCTTAATCTGACCTTAAAGATGGCAGTCCAACCTCTTTTGTCTGAGCGAATGTGGAGACTGATT
```

Fto-DEL82-Univ-Probe (5nmol)

CAGGAGCCAGATTGTCCACAGCA

Fto-DEL82-MUT-R (15nmol)

AGGGTCAGCACAGAGATGC

Fto-DEL82-Univ-F (15nmol)

CAGGCAAAAGCAAAAGGTGACATAC

Dot1l internal control (VIC labelled)

```
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCCTTTTCCCCTCTAGTC
GTTTTCTGTTAG TAGTTGGCATCCTTATGCTTCATC TTACAGT CGACTTGAGAGCTGC CCCTG AATGGTCGTGCT
GGGGC AAGGCTTTATTTTACGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCACACCCATCAG
```

Primer 1 = GCCCCAGCAGACCATT

Primer 2 = TAGTTGGCATCCTTATGCTTCATC

Probe = CCAGCTCTCAAGTCG

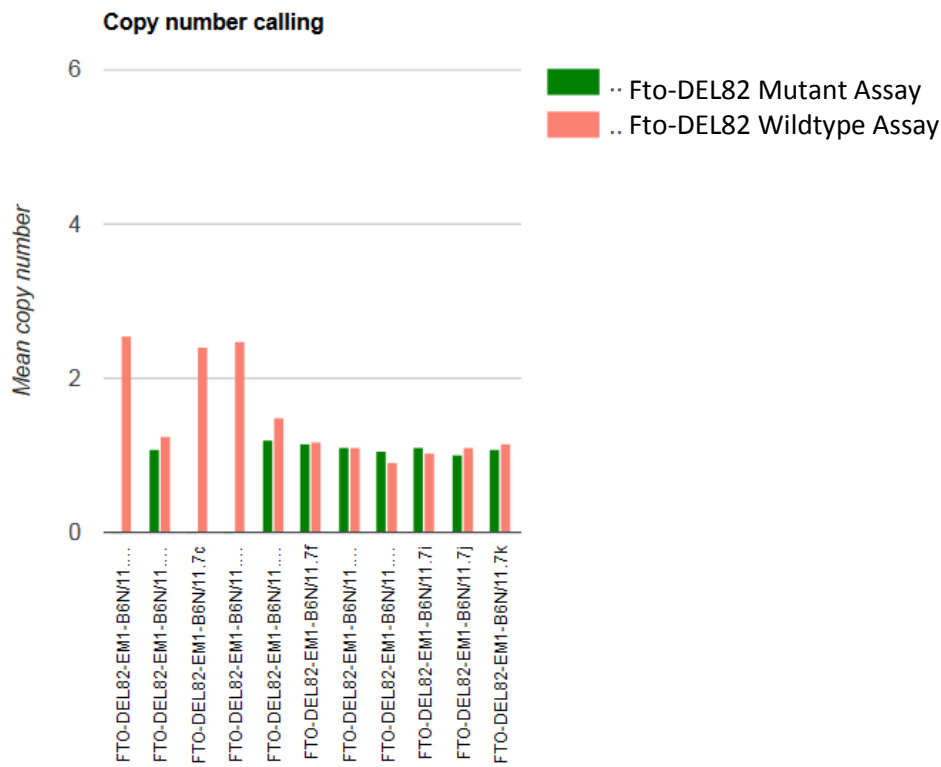


qPCR master mix

| | |
|---|---------|
| 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 |

Fto-DEL82 Assay copy called result, image showing both WT and Mutant assays

Task 185139 Results



Version No. 1

Date: 29.11.2017

Created/Updated by: Ramakrishna Kurapati

Approved by: Debbie Williams