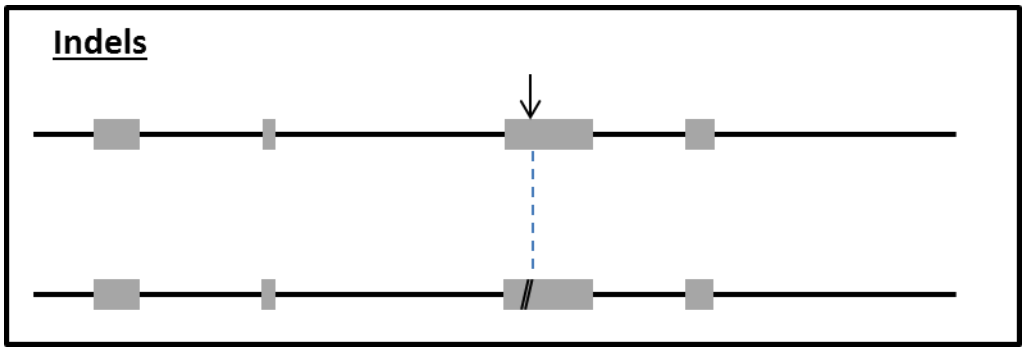
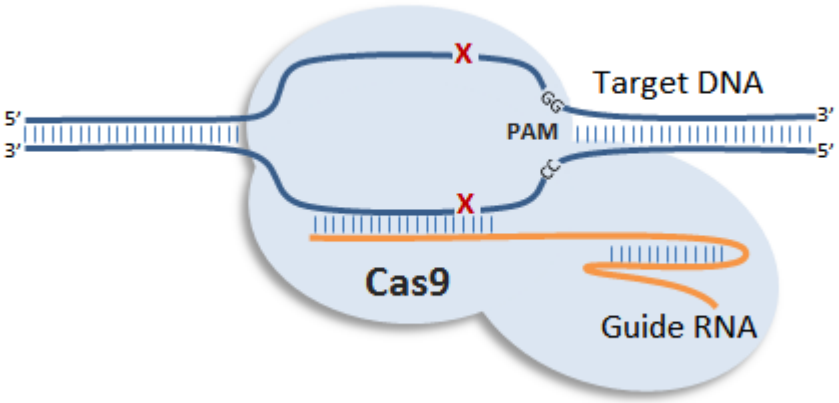
HU-FUS-Q519IFS Genotyping Strategy

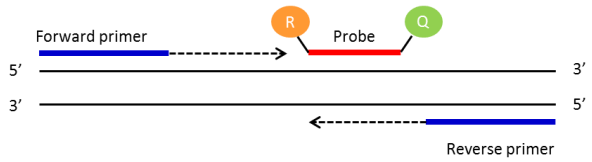
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below). The current mouse model was a CRISPR/Cas9 edit of an existing humanised fus allele to delete four nucleotides and introduce the Q519ifs change. Q519ifs is a mutation associated with human Amyotrophic lateral sclerosis (ALS) motor neuron disease.



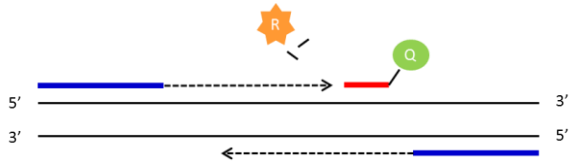


qPCR genotyping strategy

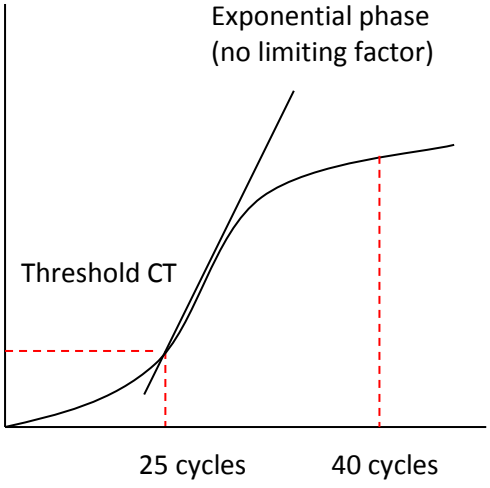
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

HU-FUS-Q519IFS Genotyping Strategy

Group: MmoN
Mutation type: HU-FUS-Q519ifs
Mutant allele: Humanised Fus allele with Q519ifs change
WT allele: Mouse Fus gene
Assay Type: Taqman copy count assays for Mouse WT Fus gene and HU-Fus-P525L.

HU-FUS-Q519ifs

Mouse TCTAACATAACTTTTTCTTTCAGGGGCGAGCACAGACAGGATCGCAGGGAGAGGCC**A
HU-FUS AATTTTTTTTTTTTTTTTTGCAGGGGTGAGCACAGACAGGATCGCAGGGAGAGGCCGTA
HU-FUS-Q519ifs AATTTTTTTTTTTTTTTTTGCAGGGGTGAGCACAG[4del]GATCGCAGGGAGAGGCCGTA

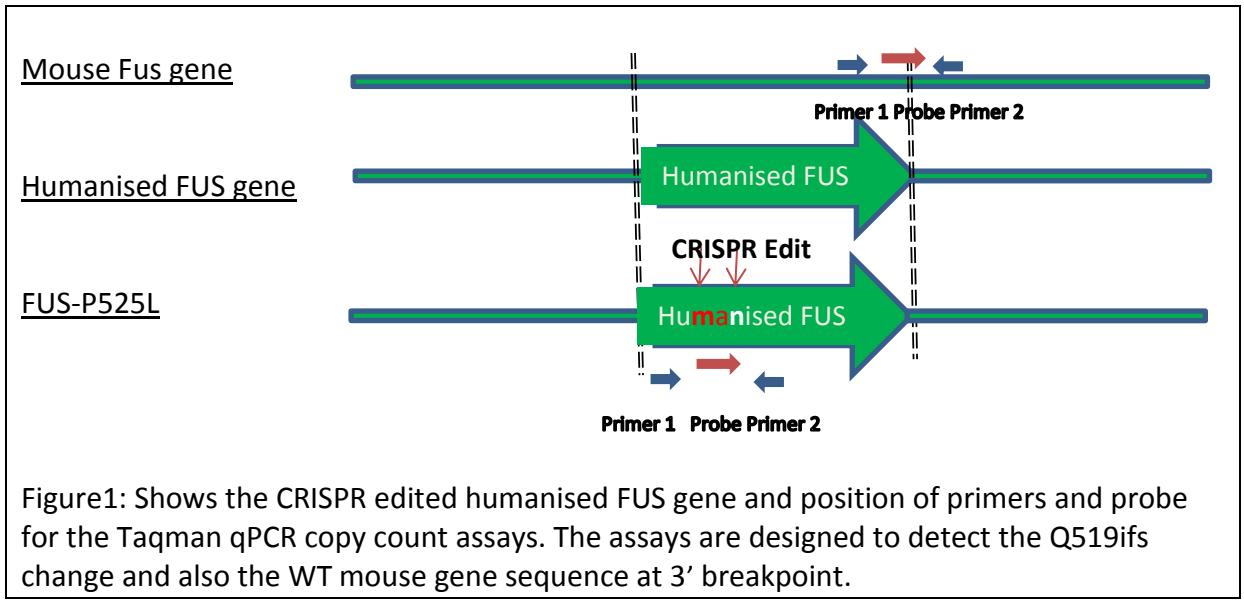


Figure1: Shows the CRISPR edited humanised FUS gene and position of primers and probe for the Taqman qPCR copy count assays. The assays are designed to detect the Q519ifs change and also the WT mouse gene sequence at 3' breakpoint.

FUS_HU-MUS_BP-LOA-WT1 assay: This is on the mouse gene

Assay based on 3' normal mouse sequence that gets disrupted by introduction of humanise FUS cassette.

tatttttttttgggggggggtggggcggttgtgtgtgtatgtgtgtgtgtgtgtgtcagactaccctaa
ttgtaaccatatctctggttcccattaaaaaacatcattttagttaaattctgtttccccccagtttac
tttctgaagaatgggtccatgttattaatgtggggcagtattattccccccagaaattgtcttgcacag
caagaattggaataatttt

Primer 1 = GGCGGTTGTGTGTGTATGTG
Primer 2 = AACATGGACCCATTCTTCAGAAAG
Probe = CATCATTTTAGTTAAATTCTGTTTCC

FUS-Q519Ifs-donor1MUT1 assay (FAM labelled probe)

Assay based on the humanised Fus cassette sequence that has been CRISPR deleted. The probe bridges the new sequence obtained after CRISPR modification.

ATATCTAGGCTTGGAGAGGCTGGTAACTCAAATATAATGGATACTTAATTTTTTTTTTTTTTTTGCAGGGGTGA
GCACAG[4ntDEL]GATCGCAGGGAGAGGCCGTATTAATTAGCCTGGCTCCCCAGGTTCTGGAACAGCTTTTTGT
CCTGTACCCAGTGTTACCCTCGTTATTTTGTAACCTTCCAATTCCTGATCACCCAAGGGTTTT

Primer 1 = GGAGCCAGGCTAATTAATACG
Primer 2 = GGTAACTCAAATATAATGGATACTTAATTTTT
Probe = CCTCTCCCTGCGATCCTGTGCTC

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

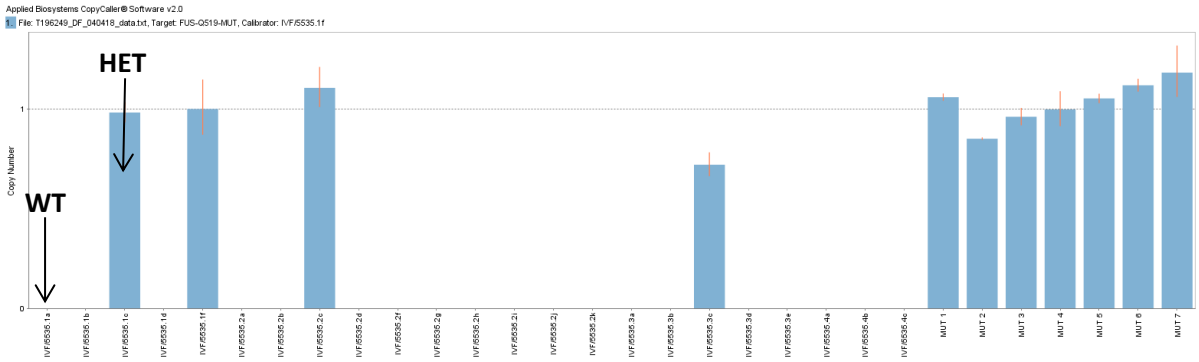
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

HU-FUS-Q519IFS copy called result, image showing samples and controls of the mutant assay only

Task 196249 Results



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