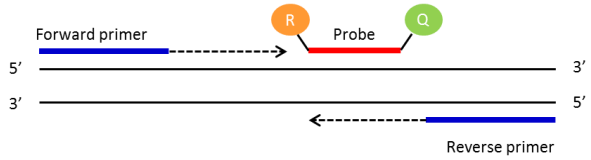
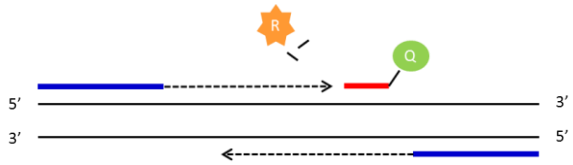
FUS-P525L Genotyping Strategy

Introduction

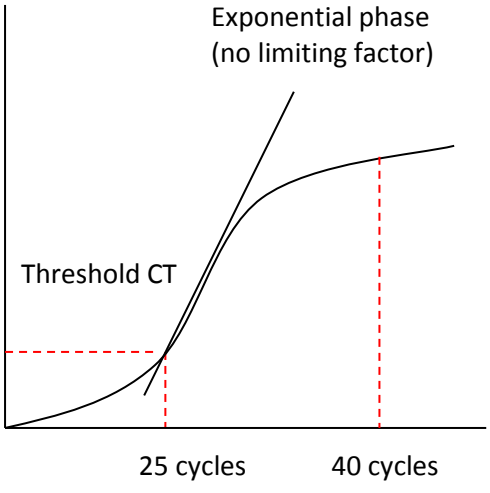
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Group:	MmoN
Mutation type:	HU-FUS-P525L
Mutant allele:	Humanised Fus allele with P525 change
WT allele:	Mouse Fus gene
Assay Type:	Taqman copy count assays for Mouse WT Fus gene and HU-Fus-P525L.

FUS-P525L

This is a CRISPR edit of an existing Humanised mouse model; HU-Fus (Please refer to assay details sheet). Using CRSIPR technology a P525L change was introduced. Please see picture below

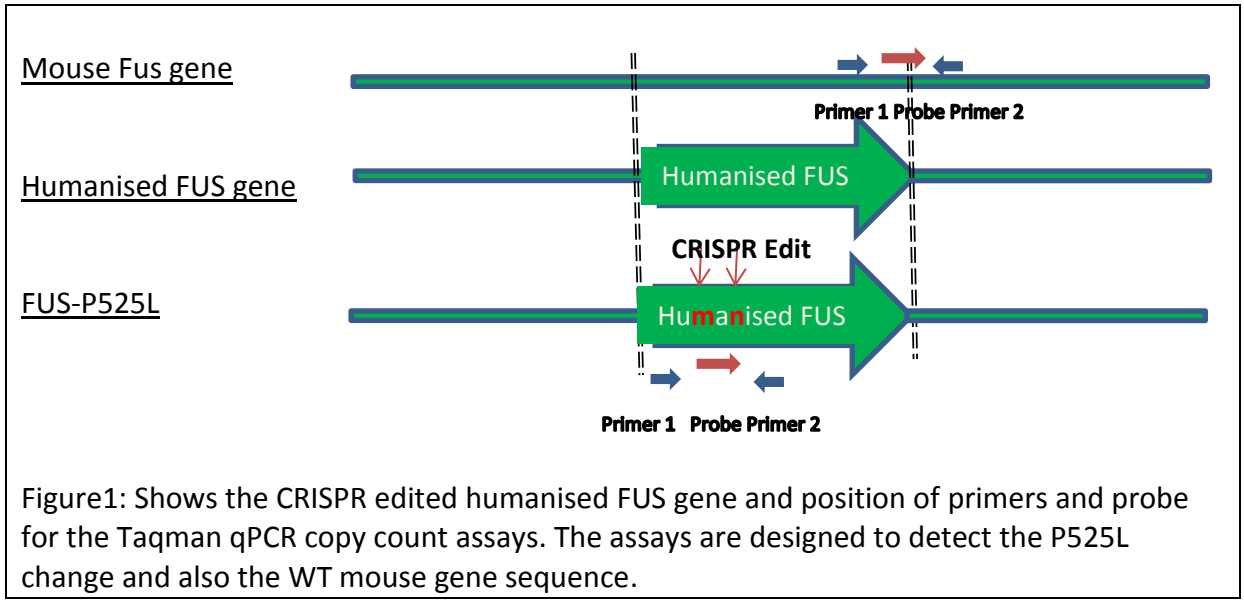


Figure1: Shows the CRISPR edited humanised FUS gene and position of primers and probe for the Taqman qPCR copy count assays. The assays are designed to detect the P525L change and also the WT mouse gene sequence.

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

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

tatttttttttgggggggtggggcggttgtgtgtgtatgtgtgtgtgtgtgtcagactaccctaa
ttgtaaccatatctctggttcccattaaaaaacatcattttagttaaattctgtttcccccagtttac
tttctgaagaatgggtccatgttattaatgtggggcagtattattccccagaaattgtcttgcacag
caagaattggaataatttt

Primer 1 = GGCGGTTGTGTGTGTATGTG
Primer 2 = AACATGGACCCATTCTTCAGAAAG
Probe = CATCATTTTAGTTAAATTCTGTTTCC

Humanised-FUS-P525L-MUT1 assay (FAM labelled probe)
This is on human Fus gene that detects the P525L change

The following sequence is the Humanised Fus gene sequence in which the P525L change is shown in BOLD UNDERLINED letters

TGTAGACCCACTTGAGATAAGATACTCGCTGGGTTAGGTAGGAGGGGCAGATAGGATATCTAGGCTTGGAGAGGC
TGGTAACTCAAATATAATGGATACTTAATTTTTTTTTTTTTTTTGCAGGGGTGAGCACAGACAGGATCGCAGGG
AGAGGC**T**GTA**C**TAATTAGCCTGGCTCCCCAGGTTCTGGAACAGCTTTTTGTCCTGTACCCAGTGTTACCCTCGT
TATTTTGTAACCTTCCAATTCCTGATCACCCAAGGGTTTTTTTGTGTCGGACTATGTAATTGTAACTATACCTCT

Primer 1 = CAGGACAAAAAGCTGTTCCAGAAC
Primer 2 = AGGATCGCAGGGAGAGGCT
Probe = AGCCAGGCTAATTAGTACAGC

Dot1l internal control (VIC labelled)

CCTAGCCATGGTGTTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAGTTGCCTTCAT
TACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCAAACCAGTGGGCAGTGGATGTGAAGGGCAGGAAGC
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG
GTGTGCAGGGAGACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTT
GTGCATCATAGAACAAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACTGTGCATCTTTTCTCTTTCAG
GACAAGCACCATGATGCTG

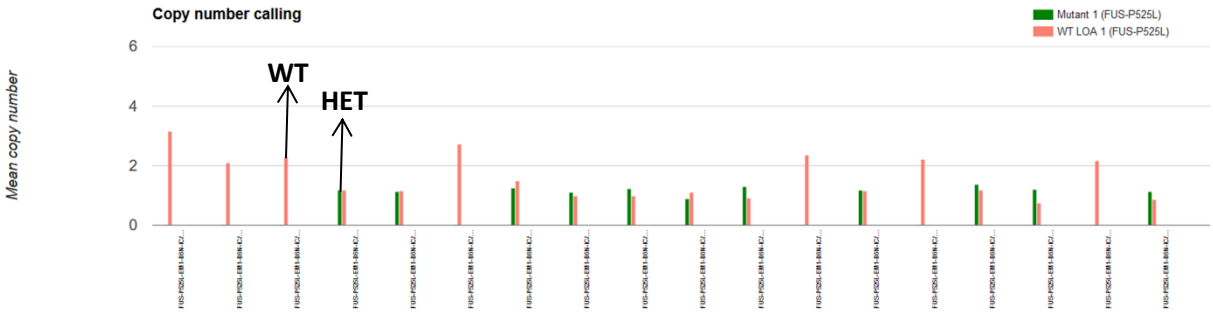
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

Example image

Task 200671 Results



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Date:	25/04/2018
Created/Updated by:	Ramakrishna Kurapati
Approved by:	Debbie Williams

HU-FUS Genotyping Strategy

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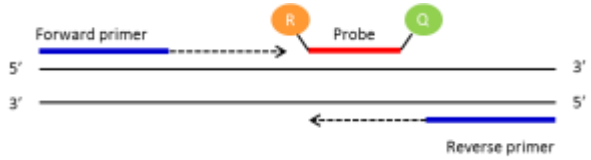

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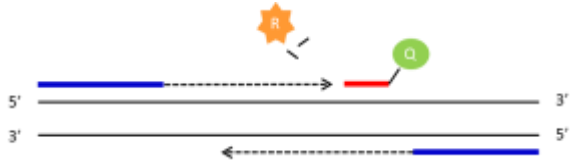

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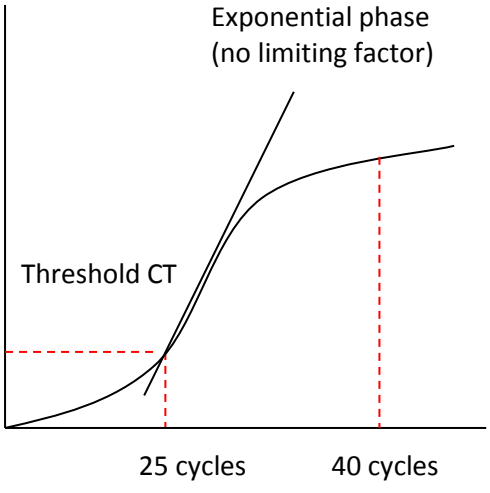

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HU-FUS

This model is a humanised mouse model for understanding pathobiology of amyotrophic lateral sclerosis (ALS) in which part of a mouse Fus gene is replaced by human FUS gene (see image below). This is done by incorporating the human mutant transgene into mouse DNA. The humanised FUS cassette is incorporated using a BAC that contains Neo as well. (Please note mouse sequence and gene nomenclature will be shown in lower case letters while human in uppercase letters.)

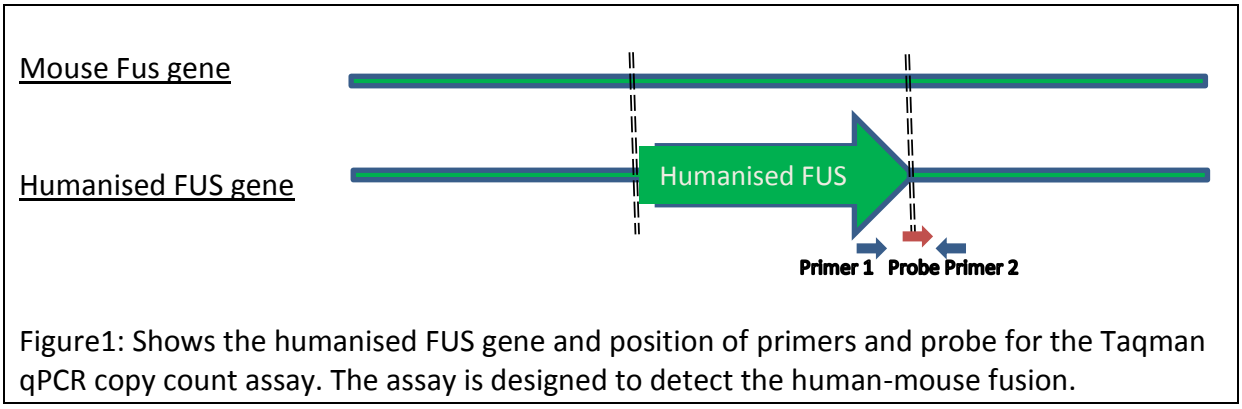

Figure1: Shows the humanised FUS gene and position of primers and probe for the Taqman qPCR copy count assay. The assay is designed to detect the human-mouse fusion.

FUS HU-FUS_BP-LOA-Mut1 assay (FAM labelled probe)

The following sequence is the Humanised Fus gene sequence in which sequence in Upper case letters represents the Human sequence from BAC and lower case letters represent the mouse sequence.

TATACCTCTGGTTCCCATTAAAAGTGACCATTTTAGTTAAATTTTGTTCCTCTTCCCCCTTTTCACTTTCCTGGA
AGATCGATGTCCCGATCAGGAAGGTAGAGAGTTTTCCTGTTCAGATTACCCTGCCCAGCAGGAACTGGAATACAG
TGTTCGGGGAGAAGGCCAAATGATATCCTTGAGAGCAGAGATTAAACTTTTCTGTCATGGGGaaattctgtttcc
cccagtttactttctgaagaatgggtccatgttattaatgtggggcagtattattcccccagaaattgtcttgca
cagcaagaattggaataatttttttcctacctagcaggaagtaagataaatacatttttttttttcctgagaca

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Probe = TTCTGTCATGGGGAAATTCTGTTTCCC

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TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG
GTGTGCAGGGAGACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTT
GTGCATCATAGAACAAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACTGTGCATCTTTTCTCTTTCAG
GACAAGCACCATGATGCTG

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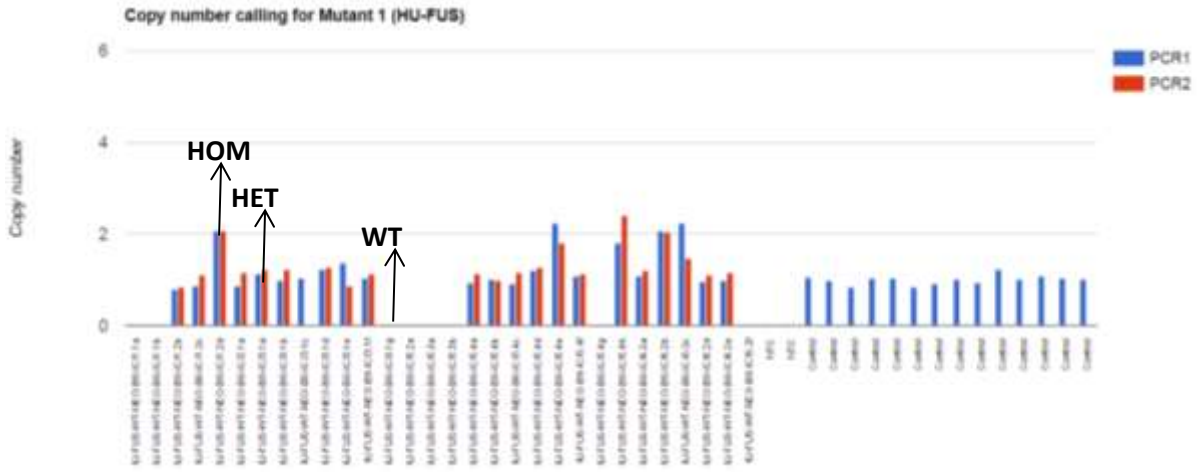
Example image

Task162546 Results



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Date:	03/07/2017
Created/Updated by:	Ramakrishna Kurapati
Approved by:	Deen Quwailid