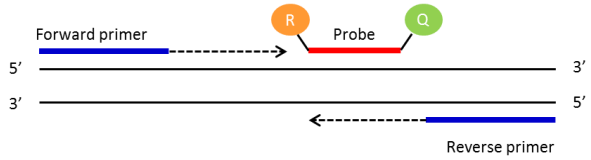
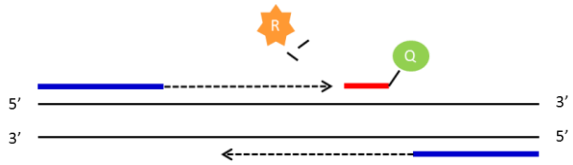
FTO-KO-FLOX Genotyping Strategy

Introduction

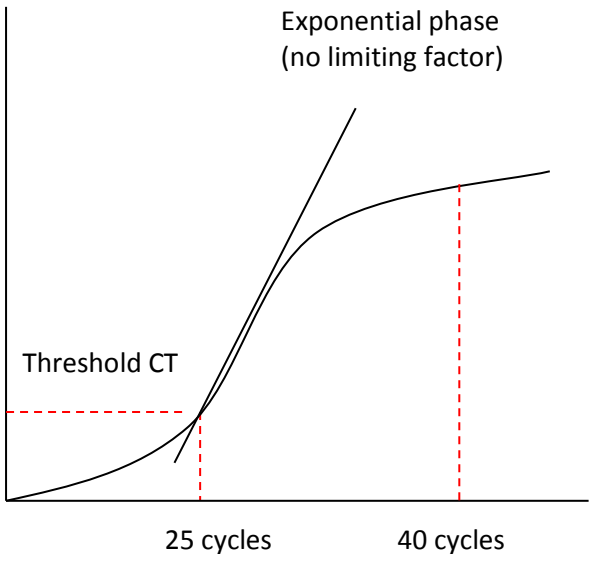
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Group: Diabetes
Mutation type:
Mutant allele: FTO-KO-FLOX-MUT1
WT allele: FTO-KO-FLOX- WT1
Assay Type: 2qPCR copy count Taqman assays

FTO-KO-FLOX- MUT1 (FAM labelled probe)

AACATCGATAAGCTTGATATCGAATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCATCAGTCAGGTA
CATAATATAACTTCGTATAATGTATGCTATACGAAGTTATTAGGTGGATCCACTAGTTCTAGAGCGGCCGCCTA
GG

Primer 1 = TCCGAAGTTCCTATTCTCTAGAAAGTA
Primer 2 = GCCGCTCTAGAACTAGTGGATC
Probe = TCATCAGTCAGGTACATAATATAAC

FTO-KO-FLOX- WT1 (FAM labelled probe)

GGTGGACAGGTCAGCCGTGGCAGTGTACAGCTATAGCTGCGAAGGTACAGTCTGCTCTGGGGGAAAGCATG
CTCGATGGCAGTGTGGCCAGAGCCTCTGAGGCTCTGAGGATGAGTAAGCACACT<CGTGTCTGTGTGTTGGG
T>GTCCTTTCCTTTCCTGGCCGGTCTCACCAGTATATCTGCAGAGCACCCCTCTCCCCCATCTAAATCCTCTCTGG
CTCATTGTTCCATCGTGCGCAGAAT

Primer 1 = GGCTCTGAGGATGAGTAAGCA
Primer 2 = GACCGGCCAGGAAAGGAAAG
Probe = CACTCGTGTCTGTGTGTTGGGTGTC

Dot1l internal control (VIC labelled)

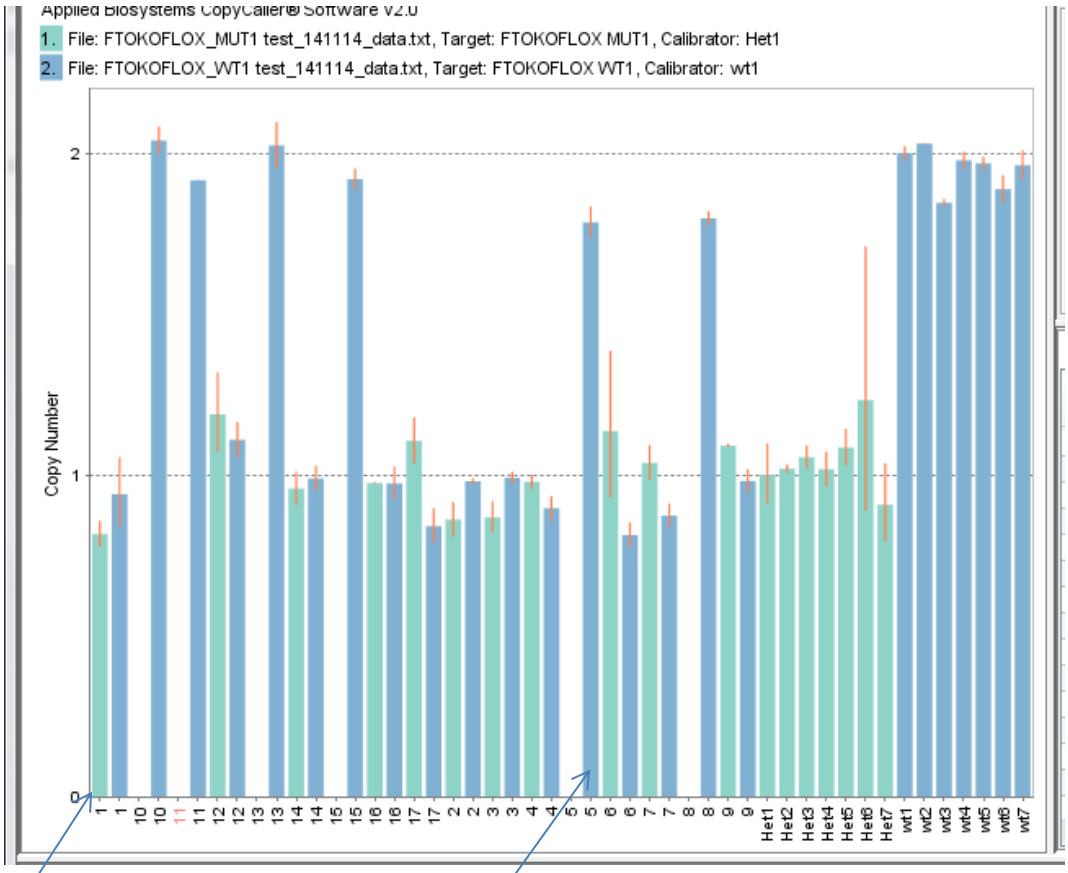
CCTAGCCATGGTGTTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAGTTGCCTTCAT
TACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCAAACCAGTGGGCAGTGGATGTGAAGGGCAGGAAGC
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
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
Water	1.55µl
ALIQUOT 7.5µL	
DNA	2.5µl
(1/10 dilution of ABI Sample-to-SNP prep)	

Example



FTOKOFLOX Het FTOKOFLOX WT