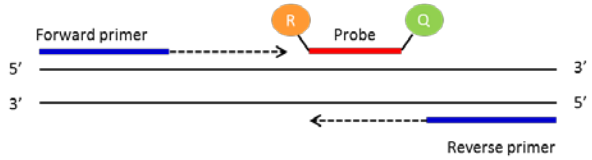
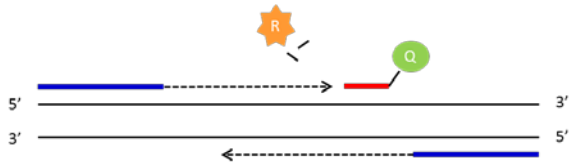
GRIN2D Genotyping Strategy

Introduction

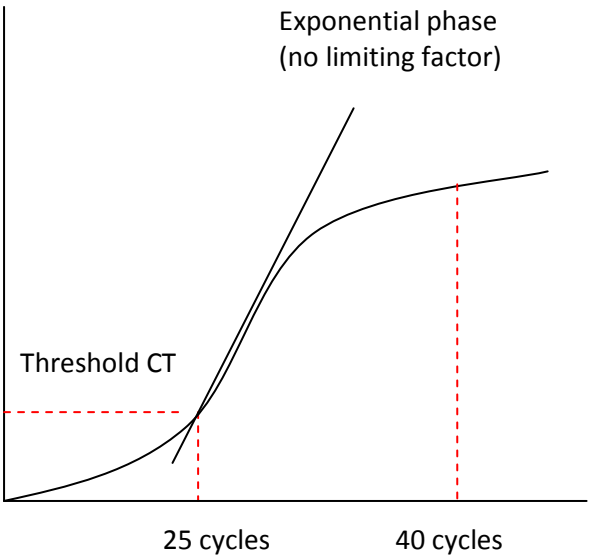
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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



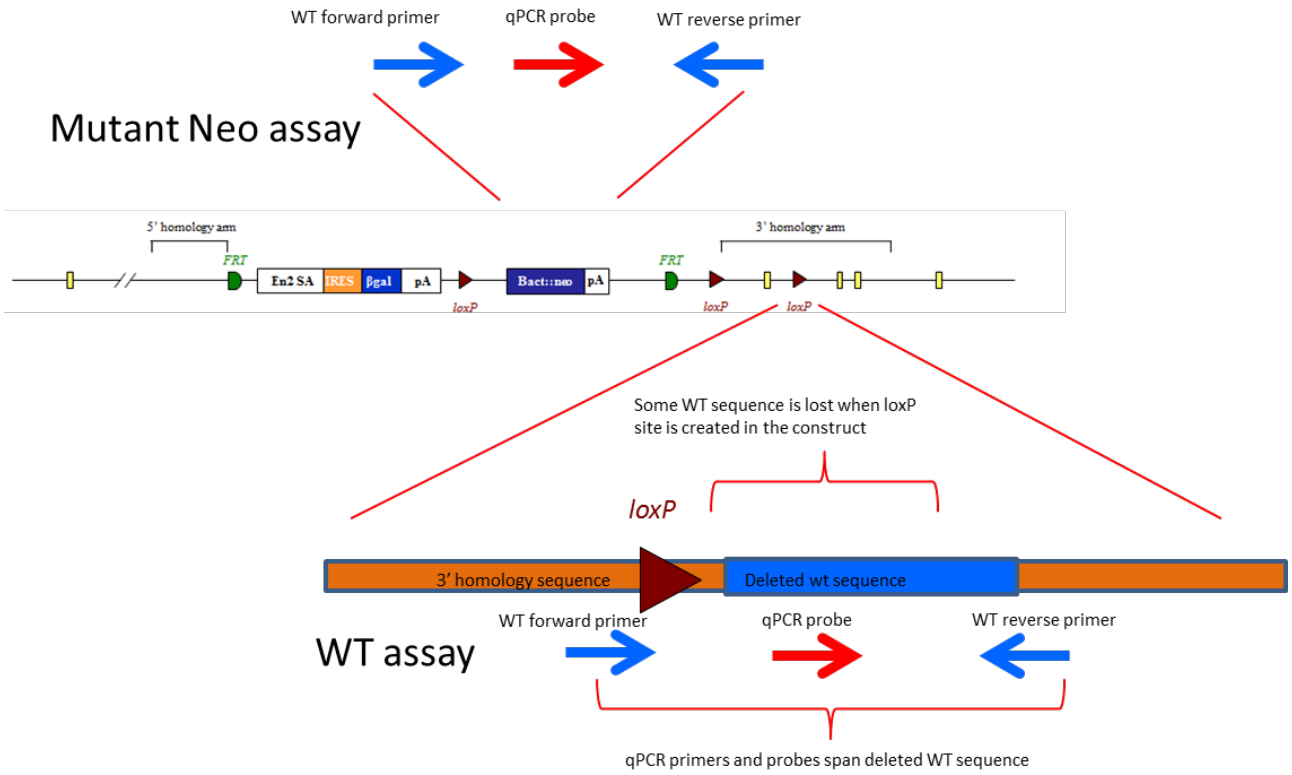
The number of cycles the PCR takes to reach a set threshold is known as the CT value. This is inversely correlated to the amount of template DNA in the sample.

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:	FESA
Mutation type:	Insertion of a EUCOMM cassette
Mutant allele:	Presence of full cassette containing Neomycin resistance sequence
WT allele:	Gene specific assay based on loxP breakpoints
Assay Type:	qPCR copy count Taqman assay

Real time PCR assay for the Tm1a allele is typically designed on the deleted WT sequence around the 3' loxP breakpoint (WT break point loss of allele or BP- LOA assay). Primers or probes are designed to span the deleted WT sequence so will not be present in the EUCOMM allele. Assays may also be designed to the loxP breakpoint that is 5' to the critical region. A mutant Neo qPCR assay is also run to confirm the genotype. Both WT and Neo qPCR assays are FAM labelled and run in duplex with a VIC labelled internal control dot1l. Both sets of results are analysed together and copy counted using the ABI software CopyCaller.



GRIN2D BP-LOA assay (FAM labelled probe)

Blue = Sequence in homology arm Black = breakpoint

GGTGCATTGGGCAGAAACATCAACTCCTGATCTGGGGACAACAGAAGTGGGCAAGAGGACAAGGCCAGCAGGTCA
TATCTGTTGCCCCTTGCTCTTGGCAGTCTGAACAAGGTTCCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
ATCCTTTCCCCTGGGGCCATGAGATTGGGGAGATGGTCCCAGAGAGAAGACTATGCTTTTCTTTGCTGGCCATG
GAGATGGGGAAGACAGGCCCAAGGAGCTTGAGGGACGGGATCAAGGAGCAGGGGGCTGTGACCTGGGGCTGGGTG
GTCACGGC

Primer 1 =GCTCTTGGCAGTCTGAACAAG Primer 2 = CTCCATGGCCAGCAAAGAAA
Probe = AGATGGTCCCAGAGAGAAGACTATGCTT

NEO assay (FAM labelled probe)

ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAA
CAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACC
GACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCT
TGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCT

Primer 1 = GGTGGAGAGGCTATTCGGC Primer 2 = GAACACGGCGGCATCAG
Probe = TGGGCACAACAGACAATCGGCTG

Dot1l internal control (VIC labelled)

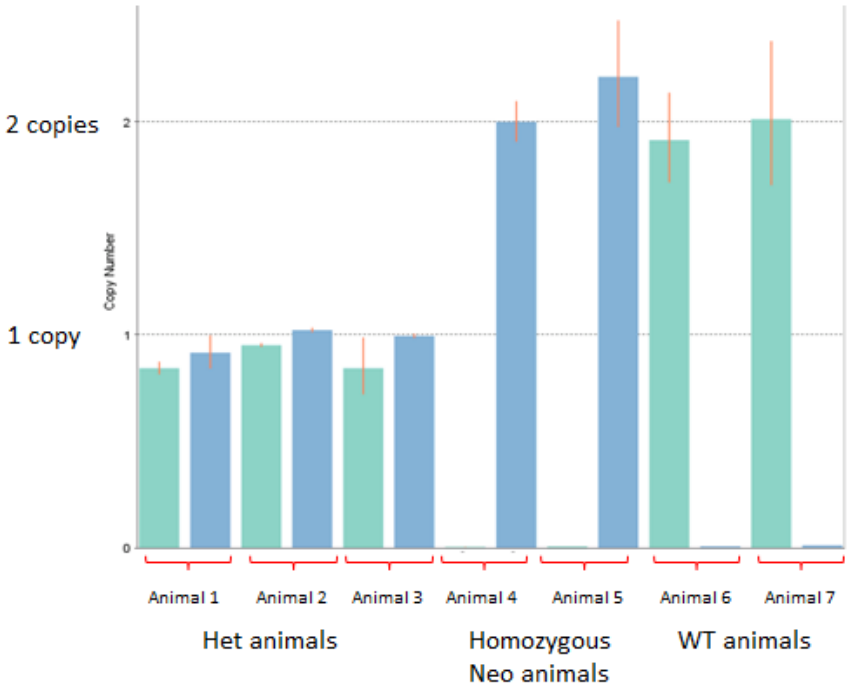
CCTAGCCATGGTGTTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAGTTGCCTTCAT
TACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCAAACCAGTGGGCAGTGGATGTGAAGGGCAGGAAGC
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG
GTGTGCAGGGAGACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTT
GTGCATCATAGAACAAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACTGTGCATCTTTTCTCTTTCAG
GACAAGCACCATGATGCTG

Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20uM)	0.225µl
Primers Dot1L_R (20uM)	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)	

Generic example of a NEO + LOA copy called result



Blue = NEO

Green = WT LOA

WT = NEO copy count of 0
LOA copy count of 2

Het = NEO copy count of 1
LOA copy count of 1

Hom = NEO copy count of 2
LOA copy count of 0