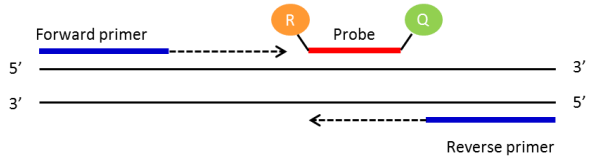
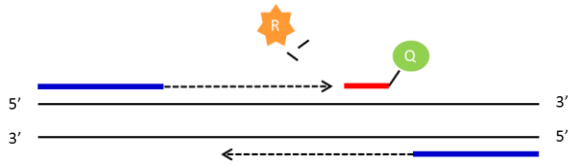
Gt(Rosa26) Genotyping Strategy

Introduction

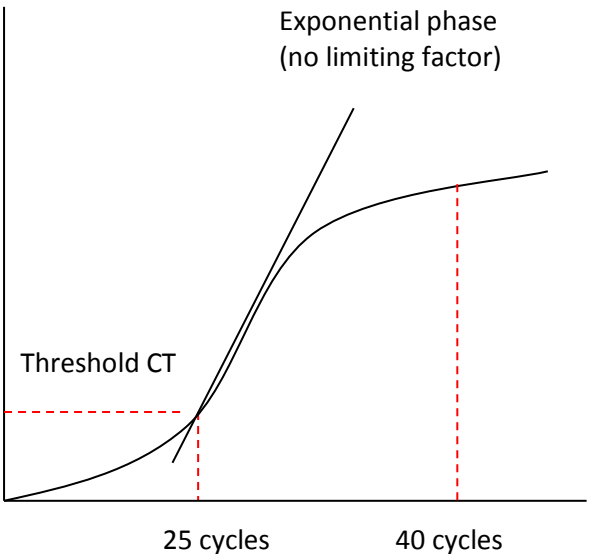
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that 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 details:	Random insertion of retroviral gene trap vector ROSA, Reverse Orientation with Splice Acceptor, which contains the beta-galactosidase gene and accompanying splice acceptor and neo sequences. The construct was inserted in reverse orientation in the retroviral vector pGen-. The transgene is controlled by an unknown endogenous promoter. LacZ is expressed in all tissues of the developing embryo and in most tissues of the adult transgenic mouse. (*J:39814*, *J:79478*, *J:64292)*
Mutation allele:	Presence of Neomycin resistance sequence when not treated with CRE
WT allele:	Gene specific assay based on loxP breakpoints
Assay Type:	qPCR copy count Taqman assay

Gt (Rosa26)

A Cre reporter line called R26R was published in 1999, taking advantage of the ubiquitous expression of the ROSA26 locus (Soriano, 1999). The lacZ reporter gene is not expressed unless the cell also expresses (or has expressed in the past) the Cre recombinase protein. This line has been used in many publications, to determine where Cre is active in Cre expressing transgenic mice. This variant of the R26R reporter mice has been engineered, where, instead of lacZ, ECFP or EYFP (enhanced cyan/yellow fluorescent protein) is expressed as the reporter gene (Srinivas et al., (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 1 (1) 4)

Rosa26 WT2 assay (FAM labelled probe)
Black = Sequence in homology arm

GCCTCCTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGTGATCTGCAACTCCA
GTCTTTCTAGAAGATGGGCGGGAGTCTTCTGGGCAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTTGTCCTGCAG
GGGAATTGAACAGGTGTAAAATTGGAGGGACAAGACTTCCCACAGATTTTCGGTTTTGTCGGGAAGTTTTTTAAT
AGGGGCAAATAAGGAAAATGGGAGGATAGGT

Primer 1 = CCTGGGCCTGGGAGAATC Primer 2 = TTAAGCCTGCCCAGAAGAC
Probe = TGCAACTCCAGTCTTTCTAGAAGATGGG

NEO assay (FAM labelled probe)

ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAA
CAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACC
GACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCT
TGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCT

Primer 1 = GGTGGAGAGGCTATTCGGC Primer 2 = GAACACGGCGGCATCAG
Probe = TGGGCACAACAGACAATCGGCTG

Dot1l internal control (VIC labelled)

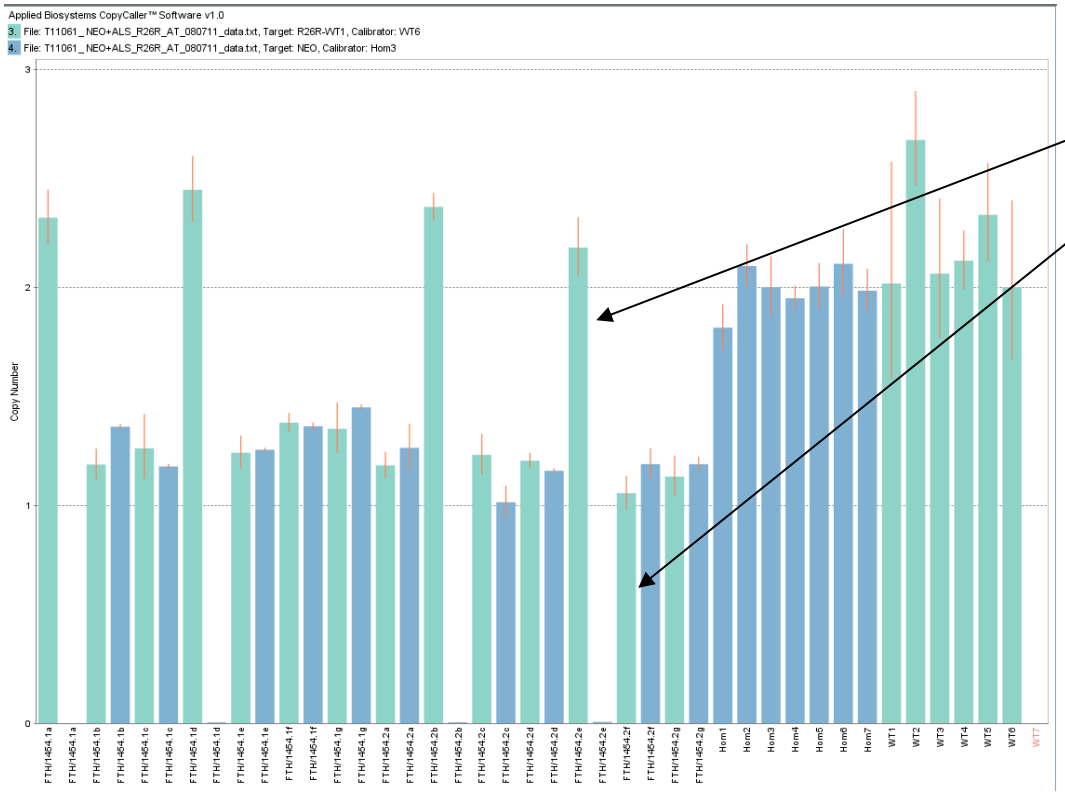
CCTAGCCATGGTGTTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAGTTGCCTTCAT
TACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCAAACCAGTGGGCAGTGGATGTGAAGGGCAGGAAGC
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG
GTGTGCAGGGAGACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTT
GTGCATCATAGAACAAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACTGTGCATCTTTTCTCTTTCAG
GACAAGCACCATGATGCTG

Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

qPCR master mix

ABI GTX Taqman master mix	5.0µl
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 of NEO + LOA copy called result


Applied Biosystems CopyCaller™ Software v1.0
3. File: T11061_NEO+ALS_R26R_AT_080711_data.txt, Target: R26R-WT1, Calibrator: WT6
4. File: T11061_NEO+ALS_R26R_AT_080711_data.txt, Target: NEO, Calibrator: Hom3
Copy Number
0
1
2
3
WT
HET
NEO Assay
Allelic Specific Assay