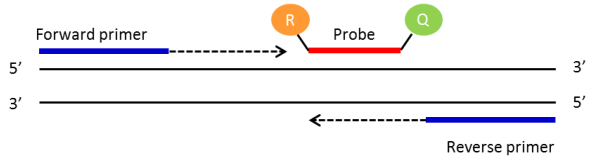
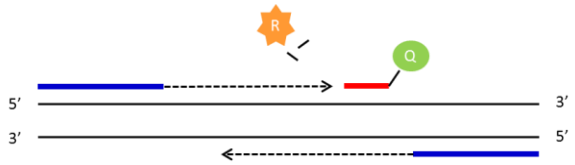
NNT-BACLINE Genotyping Strategy

Introduction

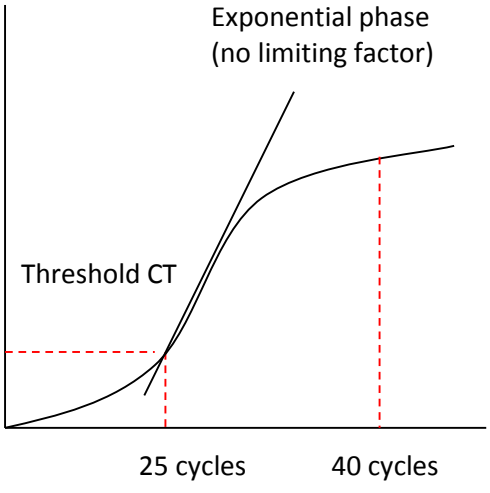
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

C57BL/6J mice have been shown to contain a deletion in NNT. This has been rescued by transgenic expression of the entire Nnt gene sequence contained within a bacterial artificial chromosome (BAC). This BAC transgenic line was prepared using BAC RP22-455H18 derived from a 129S6/SvEvTac mouse BAC library obtained from Children's Hospital Oakland Research Institute. The BAC containing all 21 exons of the Nnt gene, as well as considerable 5 and 3 flanking intergenic DNA, was microinjected into the pronucleus of one-cell C57BL/6J embryos.

The BAC rescue is genotyped by looking at the copy numbers of exon 7 and 11 of NNT which should not be present in the C57BL/6J mice but carried on the BAC or C3H and other strains. Both exons should be present and the copy numbers of homozygous animals should be double the amount of het animals for the BAC. Note that the BAC may be present in multiple copies which may be lost through subsequent rounds of breeding so controls from recent plates are better to use.

NNTex7-LOA-Mut1 (FAM labelled probe)

TAAATATCCGAATACGAGGCAGANGCTTTCTTGCTATACCTTATGAACGTAAAGTTCTCCCGGCTTAGTCGTTTC
AAAGTTTCCTCCAGCCTCAGCAGCTAAATCCACAACAACTGACCCTTCCTTCATGGACTCGATCATTTCTTTACT

Primer 1 = TCTCCCGGCTTAGTCGTTTC Primer 2 = GGAAGGGTCAGTTGTTGTGGATT
Probe = AAAGTTTCCTCCAGCCTCAGCAGC

Dot1l internal control (VIC labelled)

TGTTCTTTTCCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGC
TGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAG

Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

NNTex11-LOA-Mut1 (FAM labelled probe)

TTCCTGCTATTCCTCCTCCTGCCCCTCCTCCTACCTGCAATGTTGACTGATGATATAAATGTAGCAAGAGCAGCA
AGACTCTGAGAAGTTGTGGAGGGATAAAATGCCCTCCCATCAACGCCAGCCCACCAACTGCAGTCAAACCTAGA
AACAAAACATCACCATGGTGAAAAAAATACAGTAAACTCTCCAAATCCAATGTGAAATTTTTCATATCAGGATCT
AATATCCAAAGTCAAGGCAG

Primer 1 = ACTCTGAGAAGTTGTGGAGGGATA Primer 2=GGTGATGTTTTGTTTCTAGGTTTGAC
Probe = AATGCCCTCCCATCAACGCCAGC

Dot1l internal control (VIC labelled)

TGTTCTTTTCCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGC
TGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAG

Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

CmR-Mut1 assay (FAM labelled probe)

GCCCGCCTGATGAATGCTCATCCGGAGTTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC TTGTTACACCG

Primer 1 =GCCCGCCTGATGAATGCT Primer 2 =CGGTGTAACAAGGGTGAACACTA

Probe = TCCGGAGTTCCGTATGGCAATGAA

Dot1l internal control (VIC labelled)

TAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCA

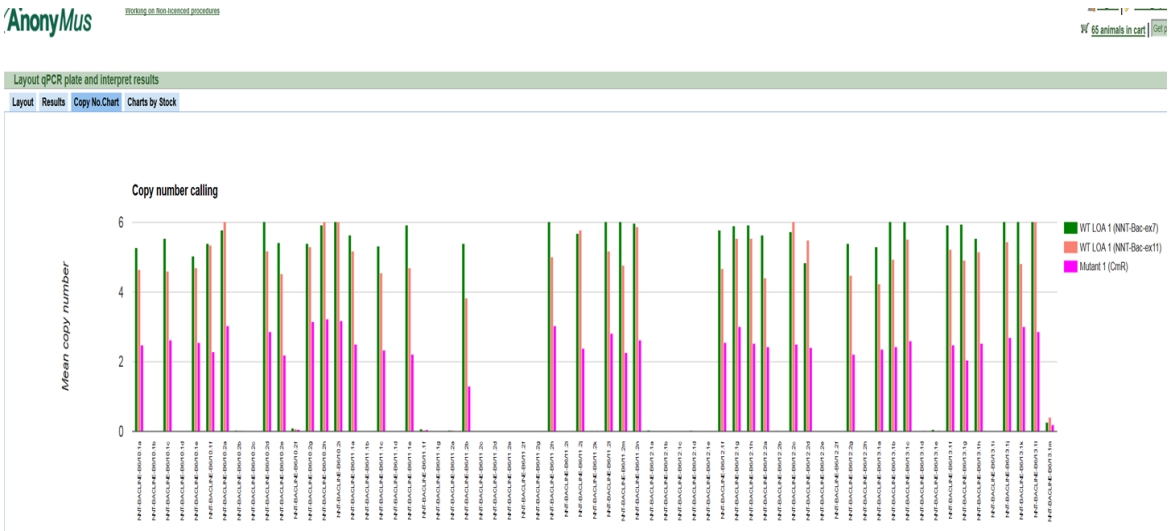
Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC

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



Note in the image above there are multiple copies of the NNT assay as it is contained on the Bac. Homs will be distinguished by having a higher copy number. This assay may also be used for B6N/J genotyping although the CmR will not be needed as a Bac is not present

Version No. 1

Date 17/02/17

Created/Updated by RZ

Approved by