

1810073N04Rik / Slc38a10 / NACARAT
Clone EPD0023_1_F07

qPCR

Assay 1 – Neo Assay

Assay run in duplex with an internal control Dot1L

PCR master mix for Assay 1-NEO

AB GTX Taqman master mix	5µL
Primer Dot1L_2F (20µM)	0.45µL
Primer Dot1L_R (20µM)	0.40µL
Primer Neo F (20µM)	0.45µL
Primer Neo R (µM)	0.45µL
Probe DotL_2M (5µM)	0.4µL
Probe Neo _M (5µM)	0.4µL
DNA	2.5µL

Aliquot 7.5µL per well

Internal qPCR control (VIC labelled probe)

Forward Primer: Dot1L_2F	GCCCCAGCAGACCATT
Reverse Primer: Dot1L_2R	TAGTTGGCATCCTTATGCTTCATC
Probe: Dot1L_2M1	VIC-CCAGCTCTCAAGTCCG

CCTAGCCATGGTGTGTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAG
TTGCCTTCATTACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCAAACCAGTGGGCAG
TGGATGTGAAGGGCAGGAAGCTCATAGGGTGAAGTGGCCAACCCAGGGAAGCCGGAGTGCTGCC
TCTTCTGTTTCTTGTGTTTCTTTCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCA
TCATTACAGTCCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTAGGGC
TAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCACACCCATCAGGTGTGCAGGGA
GACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTT
GTGCATCATAGAACAAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACCTGTGCATCTTT
TCTCTTTCAGGACAAGCACCATGATGCTG

Neo Assay (FAM labelled probe)

Looks for the presence of the neomycin Eucomm allele

Forward Primer: NeoF	GGTGGAGAGGCTATTCGGC
Reverse Primer: NeoR	GAACACGGCGGCATCAG
Probe: NeoM1	6FAM-TGGGCACAACAGACAATCGGCTG

ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATG
ACGGGCACAACAGACAACTCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGC
GCCCGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAAGTGCAGGACGAGGCAGC
GCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCAGCAGCTGTGCTCGACGTTGTCACTGA
AGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCACTCACCT
TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCCGGCGGCTGCATACGCTTGATCCG
GCTACCTGCCATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAG
CCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTGT
TCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCTGTGACCCATGGCGATGCC
GCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGG
TGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
GAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCT
TCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCG
ACGCCAACCTGCCATCACGAGATTTGATTCCACCGCCGCTTCTATGAAAGGTTGGGCTTCG

GAATCGTTTTCCGGACGCCGGCTGGATGATCCTCCAGCGCGGGATCTCATGCTGGAGTTCTT
CGCCACCCCCGGATCTAAGCTCTAGA

Assay 2 – Allelic Specific Assay (1810073N04Rik) (FAM labelled probe)

Looks for the presence of the targeted allele

AB GTX Taqman master mix	5µL
Primer Dot1L_2F (20µM)	0.45µL
Primer Dot1L_R (20µM)	0.40µL
Probe DotL_2M (5µM)	0.4µL
Assay (probe 5uM & primers 15uM each)	0.6 µL
Water	0.6µL
DNA	2.5µL

Aliquot 7.5µL per well

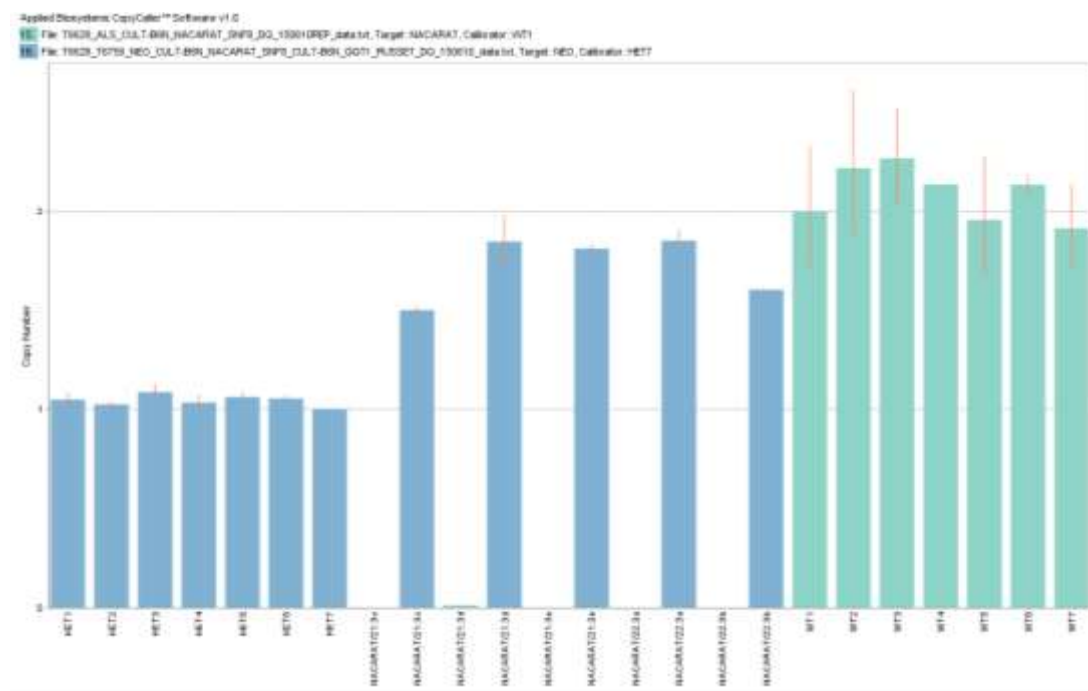
Forward primer CACCTCTGAAGGCCACTGAA
Reverse primer GAGACCCTGCAACCACTCTTAG
Probe CGTGTA AACGTTTGTCCGTAGGCA

Labelled with FAM at 5' end and MGB non-fluorescent quencher at 3' end (bought from Applied Biosystems).

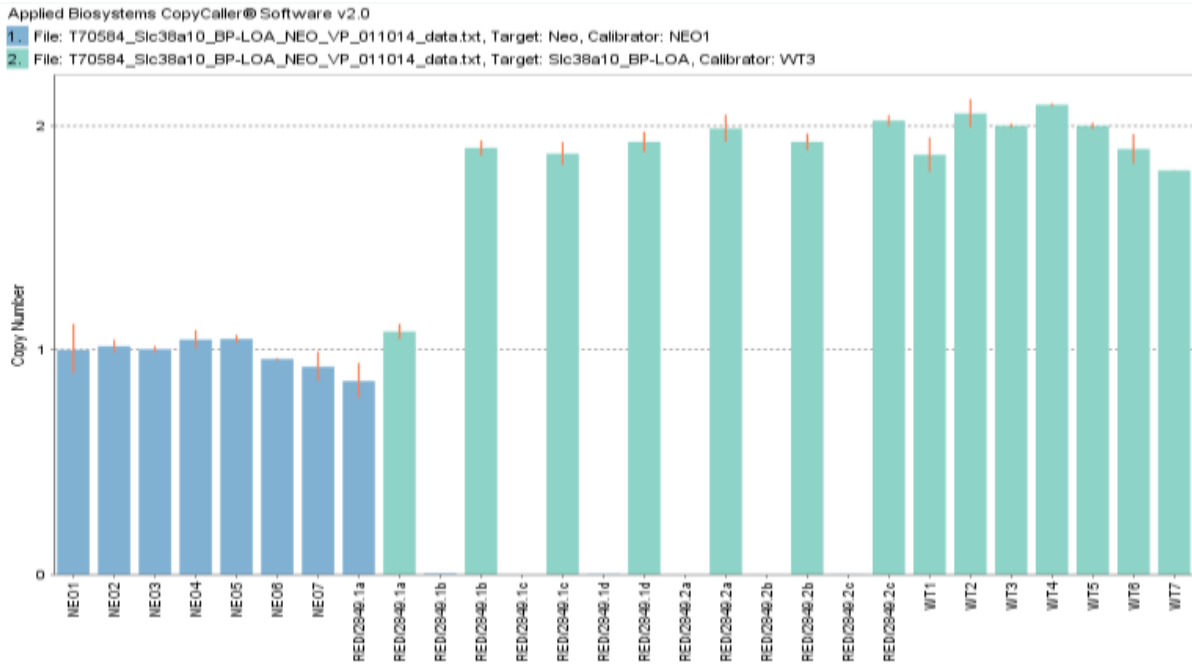
TCTTTCTTAAGCATTCTGTGCCTGGCAGCACGCAGAAAGCTGTCTTCATGGCCCTCCTTCTCTGC
TGCACGGGGCAGTTGTGACGTTGATCATTAAATAAACTTCTTTGCATGAATGGACTAGACTTGATTT
TCTAGT**CACCTCTGAAGGCCACTGAACTTTTTGTTTTGTTGGTTATGGCTAAACCCACGTGTA
AACGGTTTGTCCGTAGGCATTTCAATCCTGAAGGAGAAAGGCC**TAAGAGTGTTGCAGGGTCTC
ATGGCCAACATCTGTTAATTTGTAAGTAAGCGCACACCATCCTTAGTCCTTCAAGGCTATGTTT
ACCACATAGGGCTGTTGAGGAGGTGGTTAAGACGCTGCGTGTGAGGTGCGTGTAGGGAGTGTG
GGATGCTAGTTGATAGCCCAACAGACTTAG

Example of Copycall results including controls: for Homozygous mice:

■ Neo result ■ Allele specific result



And for heterozygous mouse (RED/2849.1a) - rest are WT.



And below – all the mice are heterozygous.

