Name of Mouse model or mutation:
Syne1-R8253Q-EM1-B6

Description:
Point mutation generated using CRISPR/Cas9 reagents.

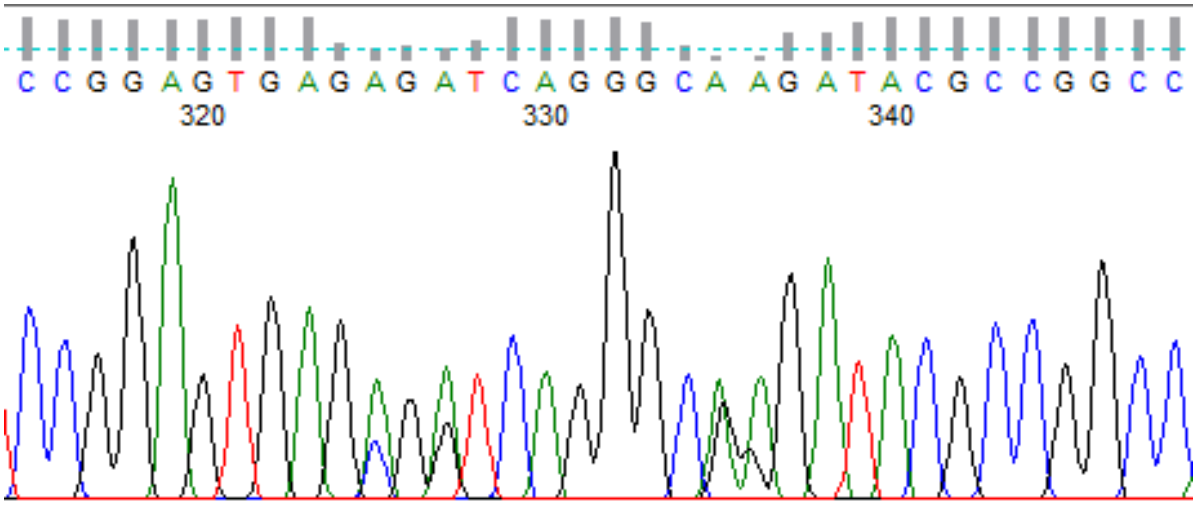
Type of mutation:
Point mutation: R8253Q

Sequence details
Syne1 WT:
GGCCCCTTGGAATAGGTTTTAATATATTCCCACTTTCTCTGTGATAAAACTGACACTGAAACAGATGA
GTATATGAGGTATTTTGAAAGTAATCAGGAAGCTCTCCTGTATCTGTTGATAAAGTTAACTCCATATC
GTCAGCAGGACCTGCTGGCATTCATTTATTGAAGGTCTCACCTGTTTGGAGTAAGAAGTGGATCCTA
TTCTTGCAGTGACAGAGTTGTGACTTCCCTTTTAGCTGCCAGATGACCATGACCTCTCAGACCGAGAA
CTAGATCTCGAAGACTCCACGGCTCTTTCAGACCTCCGCTGGCAGGACCCGTCTGCAGATGGCATGC
CCTCCCCACAGCCTTCTTCCAACCCCTCCCTCTCCCTTCCTCAGCCCCTCCGGAGTGAGCGGTCAGGG
CGAGATACGCCGGCCAGCGTGGACTCCATCCCCCTGGAATGGGACCACGATTACGACCTCAGTCGA
GACCTAGAGTCAGCATCCCGAACTCTGCCCTCAGAAGATGAAGAAGGCGAGGAAGACAAGGAGTT
CTACCTCAGGGGAGCTGTTGGCTTGTCAGGTAGGAGAGAAAACTTCCTGTTGGGAATCCATATGAG
GGACACATCAGCTATCTGGACTCATCTACCCTGAAAGGTATAAGCACTCTCTATATACATGGTCACAC
AGGGACATCATAGAGTGTCAGTCCCAGGGCTGGGTAGAAACCTTTGTGTGAGACTAGTAGGATAAT
GATGAGTCCCTGAGGTGAAAAATAGCAGTGGGCTGAGGAAGGAGGAGAGAGAAGAGCCTTGCCA
GGTGATCAACCTTGGTGCTTTGGGTCATCTGAAGTTTGTCAAGAATAGAATTGTGCTGGATGGCATA
GAGTAGGCTCAATGGAAGATGGGAAAGTGAATAAGGGGCTGGCCCTGAAATGACCAGTTCACCAT
GGAGAGGAGTTTGCCCCTTACCCTG

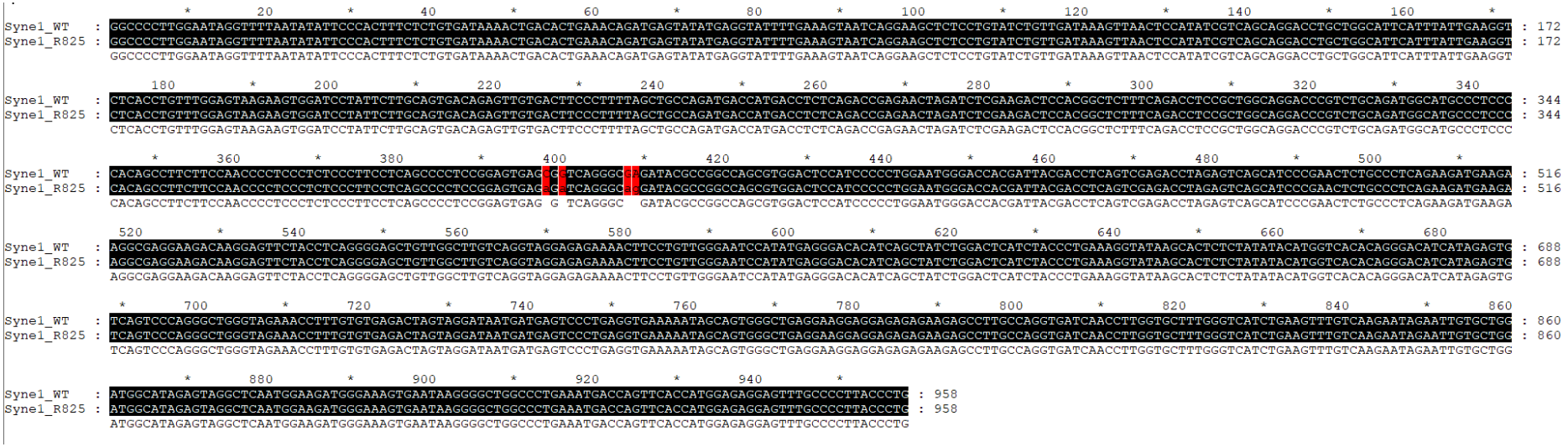
Syne1-R8253Q-EM1-B6:
GGCCCCTTGGAATAGGTTTTAATATATTCCCACTTTCTCTGTGATAAAACTGACACTGAAACAGATGA
GTATATGAGGTATTTTGAAAGTAATCAGGAAGCTCTCCTGTATCTGTTGATAAAGTTAACTCCATATC
GTCAGCAGGACCTGCTGGCATTCATTTATTGAAGGTCTCACCTGTTTGGAGTAAGAAGTGGATCCTA
TTCTTGCAGTGACAGAGTTGTGACTTCCCTTTTAGCTGCCAGATGACCATGACCTCTCAGACCGAGAA
CTAGATCTCGAAGACTCCACGGCTCTTTCAGACCTCCGCTGGCAGGACCCGTCTGCAGATGGCATGC
CCTCCCCACAGCCTTCTTCCAACCCCTCCCTCTCCCTTCCTCAGCCCCTCCGGAGTGAG**A**G**A**TCAGGG
C**AG**GATACGCCGGCCAGCGTGGACTCCATCCCCCTGGAATGGGACCACGATTACGACCTCAGTCGA
GACCTAGAGTCAGCATCCCGAACTCTGCCCTCAGAAGATGAAGAAGGCGAGGAAGACAAGGAGTT
CTACCTCAGGGGAGCTGTTGGCTTGTCAGGTAGGAGAGAAAACTTCCTGTTGGGAATCCATATGAG
GGACACATCAGCTATCTGGACTCATCTACCCTGAAAGGTATAAGCACTCTCTATATACATGGTCACAC

AGGGACATCATAGAGTGTCAGTCCCAGGGCTGGGTAGAAACCTTTGTGTGAGACTAGTAGGATAAT
GATGAGTCCCTGAGGTGAAAAATAGCAGTGGGCTGAGGAAGGAGGAGAGAGAAGAGCCTTGCCA
GGTGATCAACCTTGGTGCTTTGGGTCATCTGAAGTTTGTCAAGAATAGAATTGTGCTGGATGGCATA
GAGTAGGCTCAATGGAAGATGGGAAAGTGAATAAGGGGCTGGCCCTGAAATGACCAGTTCACCAT
GGAGAGGAGTTTGCCCCTTACCCTG

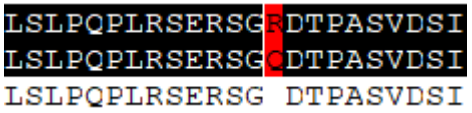
Syne1-R8253Q-EM1-B6 Heterozygous F1 animal sequence trace:



Nucleotide Alignment:



Predicted Protein Alignment:



QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_SYNE1_R8253Q_F2	GGCCCCTTGGAATAGGTTTTAAT
Geno_SYNE1_R8253Q_R2	CAGGGTAAGGGGCAAACTCC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	958
Mutant product size (bp)	958
Notes	Sequenced with the following primers Geno_SYNE1_R8253Q_F1 TGTGATAAAACTGACACTGAAACAG Geno_SYNE1_R8253Q_R1 ATGACCCAAAGCACCAAGGTT

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Syne1-R8253Q-Donor-UNIV1
Forward Primer (5'-3')	GGCCAGCGTGGACTCCAT
Reverse Primer (5'-3')	CTCTAGGTCTCGACTGAGGTCG
Probe (5'-3')	CCCCTGGAATGGGACCACGATTA
Label	FAM-BHQ1

The ddPCR assay recognises sequence common to both the WT Syne1 and the R8253Q mutant. Therefore, WT controls and correctly targeted F1 R8253Q heterozygote animals will call at 2 copies.

Assay name	Syne1-R8253Q-Donor-MUT1
Forward Primer (5'-3')	CGGAGTGAGAGATCAGGGCAG
Reverse Primer (5'-3')	GTTCGGGATGCTGACTCTAGGT
Probe (5'-3')	CCGGCCAGCGTGGACTCCAT
Label	FAM-BHQ1

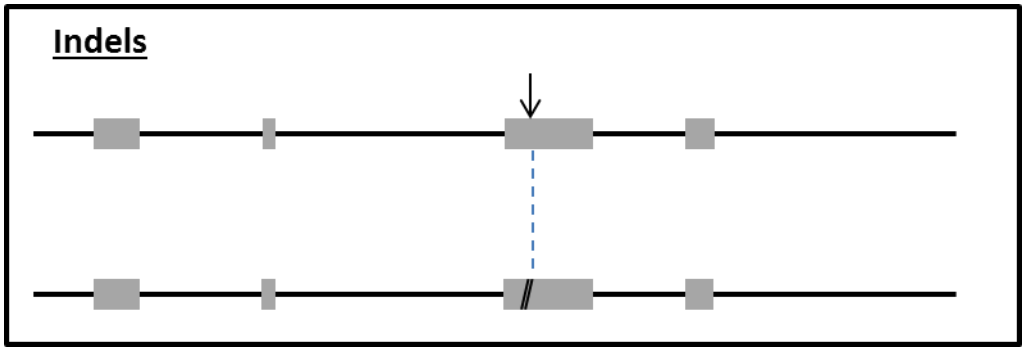
The ddPCR assay is specific to the Syne1 R8253Q mutation. Therefore, WT controls will call at 0 copies and correctly targeted F1 R8253Q heterozygote animals will call at 1 copy.

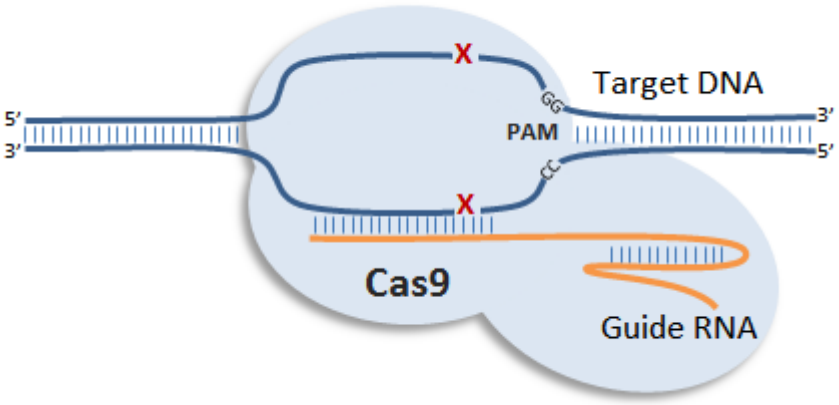
Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

Syne1-T8253Q Genotyping Strategy

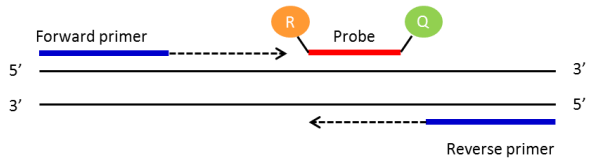
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



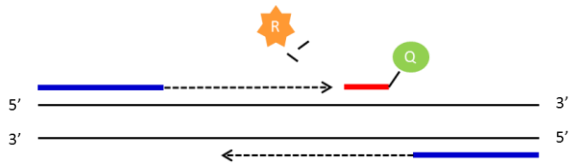


qPCR genotyping strategy

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.



Allele specific primer and probe amplification assay (ASPPAA) PCR

This is a new real-time PCR method (Billard *etal.,* 2012) in which an allele specific primer and an allele specific probe designed specific to the SNPs. The primer is designed such a way that its 3' end ends with a specific SNP. The probe is also designed specific to the SNPs at its 3' end giving a primer probe overlap. A maximum of 3nt overlap between a primer and probe is allowed.

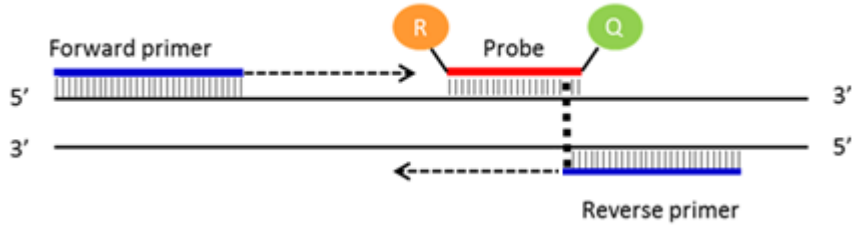
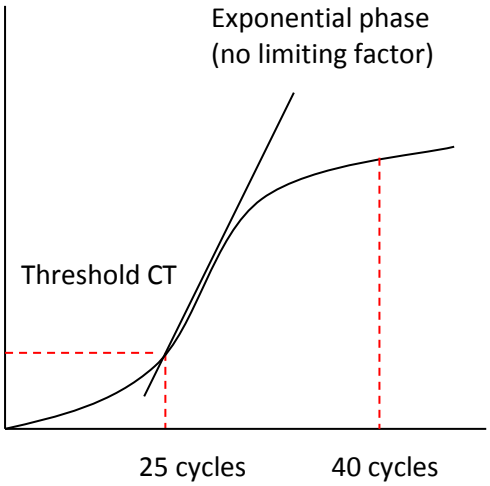


Figure1: Figure showing principle of ASPPAA PCR. The dotted line indicates the position of the SNP.

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.

References:

Billard A., Laval V., Fillinger S., Leroux P., Lachaise H., Beffa R., et al. (2012).
The allele-specific probe and primer amplification assay, a new real-time PCR method for fine quantification of single-nucleotide polymorphisms in pooled DNA.
Appl. Environ. Microbiol. 78 1063–1068. 10.1128/AEM.06957-11

Syne1-T8253Q Genotyping Strategy

Samples are genotyped with both WT and Mutant assays. These are FAM labelled assays that are designed to detect the critical exon that has been modified. If the animal contains the modified allele the copy number of the WT assay should drop by 1 and the mutant assay should raise by 1. For autosomal genes that have been targeted this means the following

WT= 2 copies of the WT assay and 0 copies of the Mutant assay
HET = 1 copy of the WT assay and 1 copy of the Mutant assay
HOM = 0 copies of the WT assay and 2 copies of the Mutant assay

Syne1-T8253Q CRISPR/Cas9 mutant in which SNPs are as highlighted

WT	CGGTCAGGGCGA
Mutant	AGATCAGGGCAG

Syne1-T8253Q-WT1 assay (FAM labelled probe)

TTCTTGCAGTGACAGAGTTGTGACTTCCCTTTTAGCTGCCAGATGACCATGACCTCTCAGACCGAGAA
CTAGATCTCGAAGACTCCACGGCTCTTTCAGACCTCCGCTGGCAGGACCCGTCTGCAGATGGCATGC
CCTCCCCACAGCCTTCTTCCAACCCCTCCCTCTCCCTTCCTCAGCCCCTCCGGAGTGAGCGGTCAGGG
CGAGATACGCCGGCCAGCGTGGACTCCATCCCCCTGGAATGGGACCACGATTACGACCTCAGTCGA
GACCTAGAGTCAGCATCCCGAACTCTGCCCTCAGAAGATGAAGAAGGCGAGGAAGACAAGGAGTT

Syne1-T8253Q-WT1 primers and probe

Primer 1 = CATGACCTCTCAGACCGAGAAC
Primer 2 = GCGTATCTCGCCCTGACCG
Probe = AGATCTCGAAGACTCCACGGCTC

Allele specific primer and probes

SYNE1-T8253Q-MUT1 assay (FAM labelled probe)

TTCTTGCAGTGACAGAGTTGTGACTTCCCTTTTAGCTGCCAGATGACCATGACCTCTCAGACCGAGAA
CTAGATCTCGAAGACTCCACGGCTCTTTCAGACCTCCGCTGGCAGGACCCGTCTGCAGATGGCATGC
CCTCCCCACAGCCTTCTTCCAACCCCTCCCTCTCCCTTCCTCAGCCCCTCCGGAGTGAGAGATCAGGG
CAGGATACGCCGGCCAGCGTGGACTCCATCCCCCTGGAATGGGACCACGATTACGACCTCAGTCGA
GACCTAGAGTCAGCATCCCGAACTCTGCCCTCAGAAGATGAAGAAGGCGAGGAAGACAAGGAGTT

SYNE1-T8253Q-MUT1 primers and probe

Primer 1 = CATGACCTCTCAGACCGAGAAC
Primer 2 = GGCGTATCCTGCCCTGATCT
Probe = AGATCTCGAAGACTCCACGGCTC

Dot1l internal control (VIC labelled)

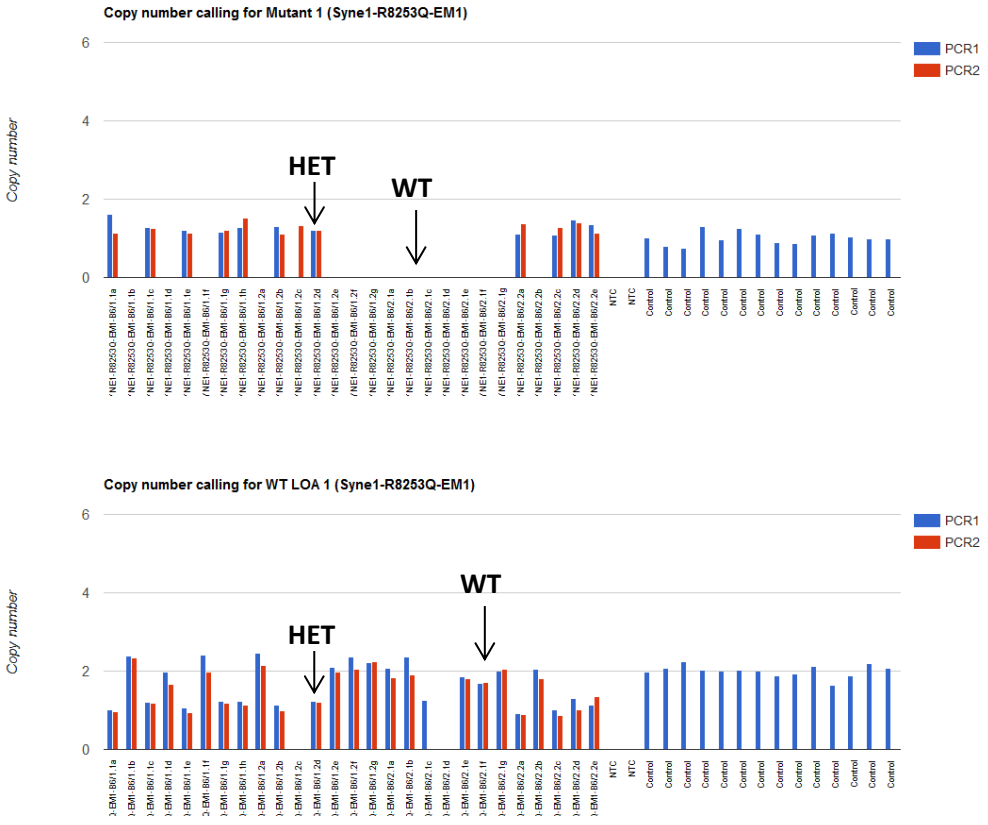
CCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGA
ATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCC

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

Syne1-T8253Q copy called result, image showing both replicates and controls (T249412)



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