Name of Mouse model or mutation:
NEFH-E461K-EM1-B6N-Cdh23
NEFH-E461K-EM2-B6N-Cdh23
NEFH-E461K-EM3-B6N-Cdh23

Description:
Point mutation introduced using CRISPR/Cas9 reagents

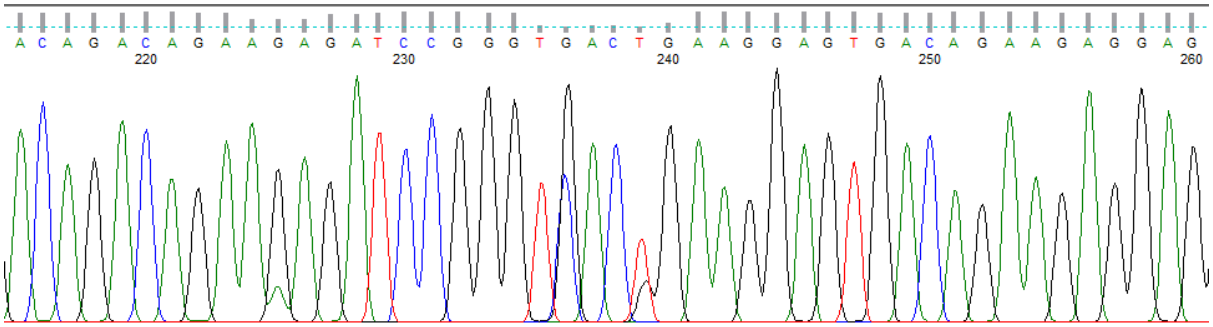
Type of mutation:
SNP: E461K

Sequence details
WT
GGAGGACCACTCTTTAGGGCGAAAGTTCCTCCCTTAAGCAGCAGTGCAGCCCCCATGAGGGTCAGG
CTTTGTAGGCGGCCACCGGAACAGTTCCCGAAGTAACTCTCAAAGTCTTCCTGCCAAGGCTGTGACT
CAGGGCAGGGTATTGTTAGCATAACTCCATCCCCAGCACCAGAAAAAATAAGTGAGTTCAAAAGTCT
ATTAGGAATGAAAATGAAAAATCTGAAAAAAAGAGCAGTAGAGAAGAGAAGTGGAGGGAGCAAG
GGAGAGAGAGAGATTCCTGCCATTGAGTCATACCCTTCATTCACCAAACCCTGTCCCTGCACATACTA
GGGCTTCAGAAAGCCAACCAGGAGTCCAGCTCTGAGGTAACAGTGTATCCCATTATCCACTCCACAG
AAAGCTCCTGGAAGGCGAAGAGTGTCGGATTGGCTTTGGTCCGAGTCCCTTCTCTCTTACTGAAGGA
CTCCCAAAAATTCCCTCCATATCCACGCACATAAAAGTCAAAAGCGAAGAGATGATAAAGGTAGTAG
AGAAATCCGAGAAGGAAACTGTGATTGTAGAAGGACAGACAGAAGAGATCCGGGTGACGGAAGG
AGTGACAGAAGAGGAGGACAAAGAGGCCCAAGGTCAGGAAGGAGAAGAAGCAGAAGAGGGAGA
AGAAAAAGAAGAAGAGGAAGGAGCAGCAGCTACATCTCCCCCTGCAGAAGAGGCTGCATCTCCAG
AAAAAGAAACCAAGTCTCGTGTGAAAGAAGAGGCCAAGTCCCCAGGTGAGGCCAAGTCCCCAGGT
GAGGCCAAGTCCCCAGCTGAGGCCAAGTCCCCAGGTGAGGCCAAGTCCCCAGGTGAGGCCAAGTCC
CCAGGTGAGGCCAAGTCTCCAGCTGAGCCCAAGTCTCCAGCTGAGCCCAAGTCTCCAGCTGAGGCC
AAGTCACCAGCTGAGCCCAAGTCTCCAGCTACAGTGAAGTCTCCAGGT

Mutant
GGAGGACCACTCTTTAGGGCGAAAGTTCCTCCCTTAAGCAGCAGTGCAGCCCCCATGAGGGTCAGG
CTTTGTAGGCGGCCACCGGAACAGTTCCCGAAGTAACTCTCAAAGTCTTCCTGCCAAGGCTGTGACT
CAGGGCAGGGTATTGTTAGCATAACTCCATCCCCAGCACCAGAAAAAATAAGTGAGTTCAAAAGTCT
ATTAGGAATGAAAATGAAAAATCTGAAAAAAAGAGCAGTAGAGAAGAGAAGTGGAGGGAGCAAG
GGAGAGAGAGAGATTCCTGCCATTGAGTCATACCCTTCATTCACCAAACCCTGTCCCTGCACATACTA
GGGCTTCAGAAAGCCAACCAGGAGTCCAGCTCTGAGGTAACAGTGTATCCCATTATCCACTCCACAG
AAAGCTCCTGGAAGGCGAAGAGTGTCGGATTGGCTTTGGTCCGAGTCCCTTCTCTCTTACTGAAGGA
CTCCCAAAAATTCCCTCCATATCCACGCACATAAAAGTCAAAAGCGAAGAGATGATAAAGGTAGTAG

AGAAATCCGAGAAGGAAACTGTGATTGTAGAAGGACAGACAGAAaAGATCCGGGTcACtGAAGGA
GTGACAGAAGAGGAGGACAAAGAGGCCCAAGGTCAGGAAGGAGAAGAAGCAGAAGAGGGAGAA
GAAAAAGAAGAAGAGGAAGGAGCAGCAGCTACATCTCCCCCTGCAGAAGAGGCTGCATCTCCAGA
AAAAGAAACCAAGTCTCGTGTGAAAGAAGAGGCCAAGTCCCCAGGTGAGGCCAAGTCCCCAGGTG
AGGCCAAGTCCCCAGCTGAGGCCAAGTCCCCAGGTGAGGCCAAGTCCCCAGGTGAGGCCAAGTCCC
CAGGTGAGGCCAAGTCTCCAGCTGAGCCCAAGTCTCCAGCTGAGCCCAAGTCTCCAGCTGAGGCCA
AGTCACCAGCTGAGCCCAAGTCTCCAGCTACAGTGAAGTCTCCAGGT

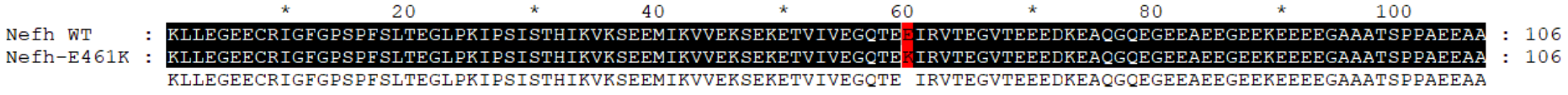
NEFH-E461K-EM1-B6N-Cdh23 Heterozygous F1 animal sequence trace:



Please note the sequences of NEFH-E461K-EM1-B6N-Cdh23, NEFH-E461K-EM2-B6N-Cdh23 and NEFH-E461K-EM3-B6N-Cdh23 are the same, just transmitted from different founder animals.

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_Nefh_E461K_F3 primer (5'-3')	GGAGGACCACTCTTTAGGGC
Geno_Nefh_E461K_R3 primer (5'-3')	ACCTGGAGACTTCACTGTAGC
Taq Polymerase used	ThermoFisher SuperFi Kit
Annealing Temperature (°C)	65
Elongation time (min)	1
WT product size (bp)	970
Mutant product size (bp)	970
Notes	

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 by ddPCR

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	NEFH-E461K-UNI1
Forward Primer (5'-3')	TCCCTCTTCTGCTTCTTCTCC
Reverse Primer (5'-3')	GAAGGAGTGACAGAAGAGGAGGA
Probe (5'-3')	TTCCTGACCTTGGGCCTCTTTG
Label	FAM-BHQ1

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.

NEFH-E461K

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation; E461K in NEFH. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wild type and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the E461K point mutations.

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

NEFH-E461K-WT1 assay (FAM labelled)

AAAGCTCCTG**GAAGGCGAAGAGTGTCGGATTG**GCT**TTGGTCCGAGTCCCTTCTCTCTTAC**TGAAGG
ACTCCCAAAAATTCCCTCCATATCCACGCACATAAAAGTCAAAAGCGAAGAGATGATAAAGGTAGTA
GAGAAATCCGAGAAGGAAACTGTGATTGTAGAAGGACAGACAGA**AgAGATCCGGGTgACgGAAG**G

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo NEFH-E461K	5' label	Sequence 5' → 3'	3' label	Oligo Type
NEFH-E461K-UNI_F	n/a	**GAAGGCGAAGAGTGTCGGATTG**	n/a	Universal Forward
NEFH-E461K-UNI_PROBE	FAM	**TTGGTCCGAGTCCCTTCTCTCTTAC**	ZEN/IBFQ	Universal Probe
NEFH-E461K-WT_R	n/a	**CTTCCGTCACCCGGATCTCT**	n/a	Wild type Reverse

NEFH-E461K-MUT1 assay (FAM labelled)

AAAGCTCCTG**GAAGGCGAAGAGTGTCGGATTG**GCT**TTGGTCCGAGTCCCTTCTCTCTTAC**TGAAGG
ACTCCCAAAAATTCCCTCCATATCCACGCACATAAAAGTCAAAAGCGAAGAGATGATAAAGGTAGTA
GAGAAATCCGAGAAGGAAACTGTGATTGTAGAAGGACAGACA**GAAaAGATCCGGGTcACtGAAG**G

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo NEFH-E461K	5' label	Sequence 5' → 3'	3' label	Oligo Type
NEFH-E461K-UNI_F	n/a	**GAAGGCGAAGAGTGTCGGATTG**	n/a	Universal Forward
NEFH-E461K-UNI_PROBE	FAM	**TTGGTCCGAGTCCCTTCTCTCTTAC**	BHQ	Universal Probe
NEFH-E461K-MUT_R	n/a	**CTTCAGTGACCCGGATCTTTTC**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo NEFH-E461K	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5 µM & primers 15 µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

qPCR cycling conditions

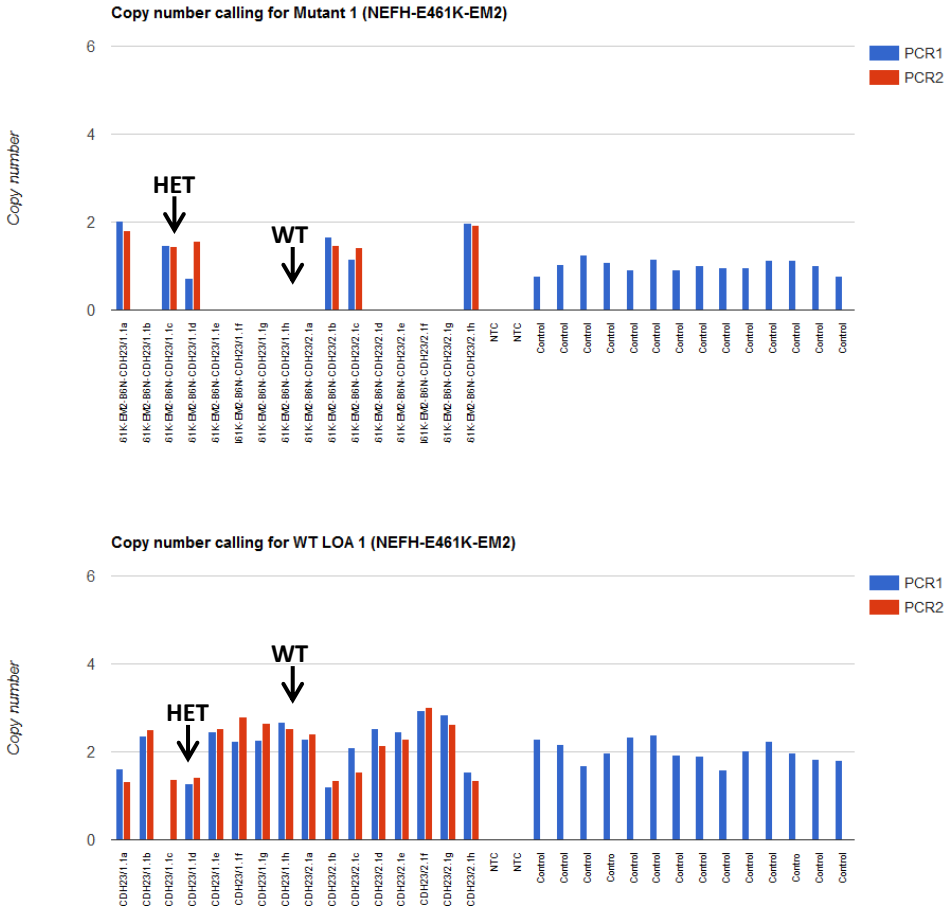
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

NEFH-E461K-WT1 and NEFH-E461K -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 284172 results)



Cdh23 repair Genotyping Strategy

Use B6N or B6J as un repaired (WT) controls and C3H as repaired (HOM) controls

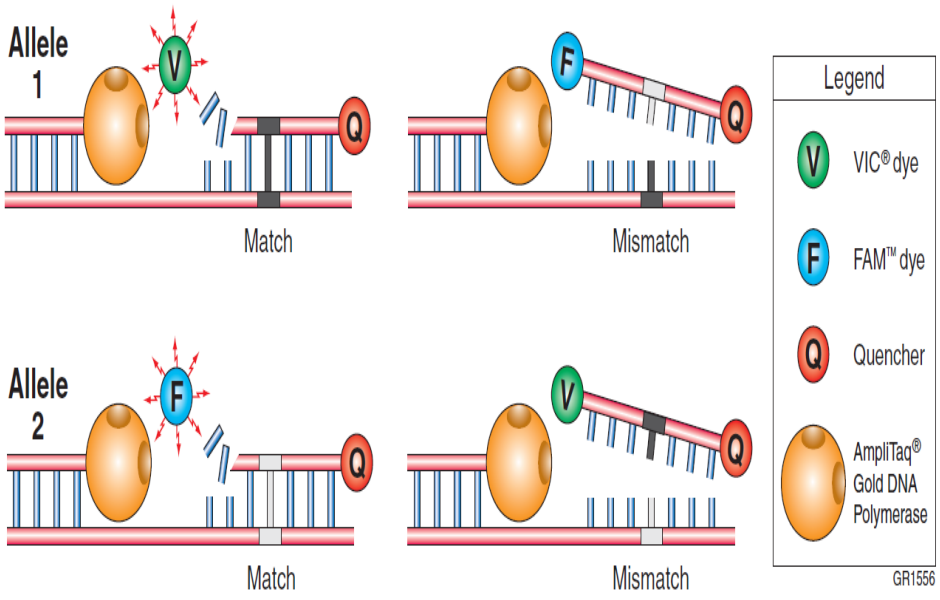
Introduction

An Allelic Discrimination assay can be used to detect two possible variants of a Single Nucleotide Polymorphism (SNP). It is a multiplexed assay (with two primer/probe pairs) with data being collected at the completion of the PCR process.

Two Taqman probes are used in the assay, one detector matching the WT (allele 1) and the other matching the Mutant (allele 2)

The Analysis software produces 2 genotypes:

- Homozygotes (samples having only allele 1 or allele 2)
- Heterozygotes (samples having both allele 1 and allele 2)



Information about running an Allelic Discrimination assay can be found here:

http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042114.pdf

Assay set up

Mouse ear clips arrive for genotyping in task plates.
To retrieve sample IDs and well locations - log into AnonyMus.
https://anonymus.har.mrc.ac.uk/anonymus/core/Login

ALLELIC DISCRIMINATION GENOTYPING

MRC Harwell

Group: Deafness (Sara Wells)
Mutation type: SNP
Mutant allele: G
WT allele: A
Assay Type: Allelic discrimination

Point mutation for the reversion of age related hearing loss in B6 mice. Repaired base is now G where as normal B6 mice will have A
B6=WT (unrepaired)
C3H=MUT (repaired)

Fragment sequence

WT sequence:
CATTGGCCAACCTGGCCATCATCATCACGGACATGCAAGACATGGATCCTATCTTCATCAACCTGCCMTACWSTA
CTAACATCTACGAGCACTCTCCTCCAGTGAGCCCYGCCCCCAGCCCCAGAGCAGGAAGACAAATGCCTGTCCTGC
GTGGGTTCTCTAGCCCGTGCTGGGGATGGCTGTGGACTTAAGCTCGGCCAA

Mutated/repaired sequence (**in red** = single nucleotide polymorphism):
CATTGGCCAACCTGGCCATCATCATCACGGACATGCAAGACATGGATCCTATCTTCATCAACCTGCCMTACWSTA
CTAACATCTACGAGCACTCTCCTCCGGTGAGCCCYGCCCCCAGCCCCAGAGCAGGAAGACAAATGCCTGTCCTGC
GTGGGTTCTCTAGCCCGTGCTGGGGATGGCTGTGGACTTAAGCTCGGCCAA

Primers and Probes

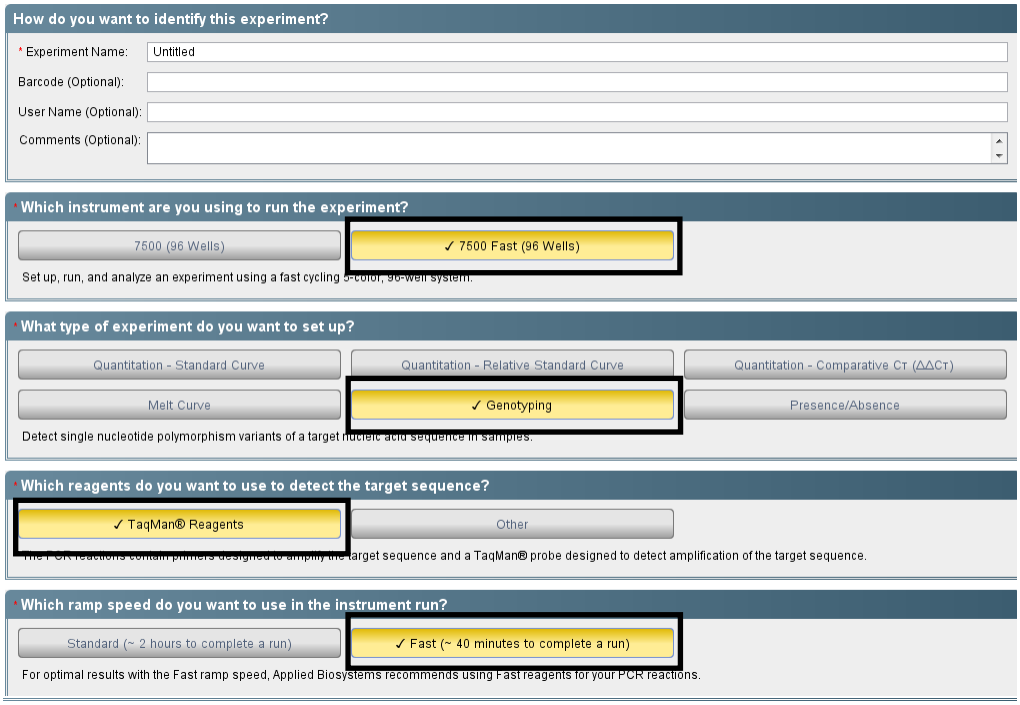
Primer 1	GACATGGATCCTATCTTCATCAACC
Primer 2	CCAGCACGGGCTAGAGAAC
Allele 1 probe - WT (FAM-Labelled)	CACTCTCCTCCAGTGAG
Allele 2 probe - Mutant (TET-Labelled)	CTCCTCCGGTGAGC

qPCR master mix

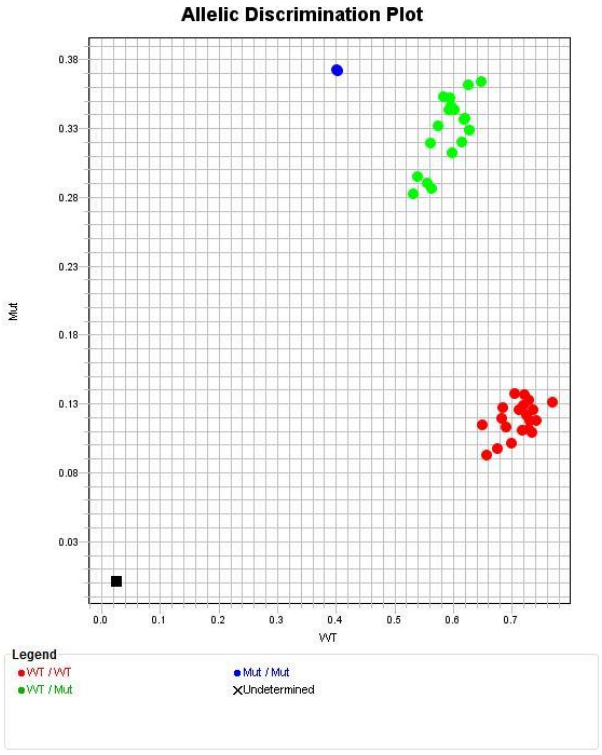
ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

No need to run the samples in duplicates.
Allele 1 = WT on 7500 FAM-labelled. Allele 2 = MUT on 7500 TET-labelled.

7500 Settings for running Allele Discrimination Assay are as shown below



Example of an Allelic Discrimination Plot and Results



from T148050

Please note, use your controls to group and name your samples accordingly.