Name of Mouse model or mutation:
USH2A-W3947X-EM1-CDH23-B6

Description:
Point mutation made by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: W3947X

Delivery method:
Electroporation into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
AGGGTGCAGTGGACAGCCCG	TGG

ssODN donor sequence (5'-3'):
GGCTCTGTCCAGTTTAGCATGGCTGAATGAGCACTGGTGGCTTGAACCCGTGGTGCTGGGAGGCCT
CGAGGTGGGGCTTCCAGGGTCTGTATTGTGGACTACGGGCTGTCCACTGCACCCTGGGAGTTCCAG
GCTCTCACCCGGTACTCATACAGTGTAAAAGGCCGCAGAGTGCCTGTATCATCAGTAAACTCCAGAG
C

Electroporation mixes:
Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl each and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

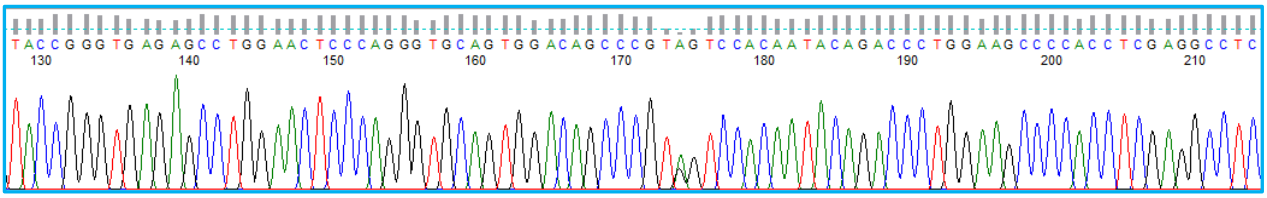
WT

TAGAAATGGCCTTGAGCAGACGTCTAGCACATTCCTCATAAAGAACAATAAGTTGGAAGAAGATAA
CCTTCTCGATAACCAAGAAAGGAGGAAGGAATCCTAAACACAGTGTCTCTCTCTCTCTCTCTCTCT
CGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAAGCTGTGTCTGGGTGTCACGT
GGCTTGCTTGGTTCTTTTGCAGGCGTCCTGCTGACACTGAAGAAGAGTCTCTGCTCTTTGTCTGGTCA
GAAGGAGCTCTGGAGTTTACTGATGATACAGGCACTCTGCGGCCTTTTACACTGTATGAGTACCGGG
TGAGAGCCTGGAACTCCCAGGGTGCAGTGGACAGCCCGTGGTCCACAATACAGACCCTGGAAGCCC
CACCTCGAGGCCTCCCAGCACCACGGGTTCAAGCCACCAGTGCTCATTCAGCCATGCTAAACTGGAC
AGAGCCAGAGGCCCCCAATGGCCTCATCTCCCAGTACCATGTGATCTACCAAGAAAGACCAGATGC
GGCAGCACCCGGCAGTTCTACCGTGCATGCTTTCACGGTGACGGTAAGAACTTAGAATCCATAACAA
GCCCTCAGATTATCTGGTTAACAGGTTATAATTCATTTCCTTATCTGCTTGTCACTTTCCTCACTTAGA
GCATACGTGTATGAATTTTTTTTTTGTATGTGAAAAACAGCAATCTGGTCCTAGGGATAAGATAATCA
GTTGAGTCCGAGCTGTATTTGGGACCGG

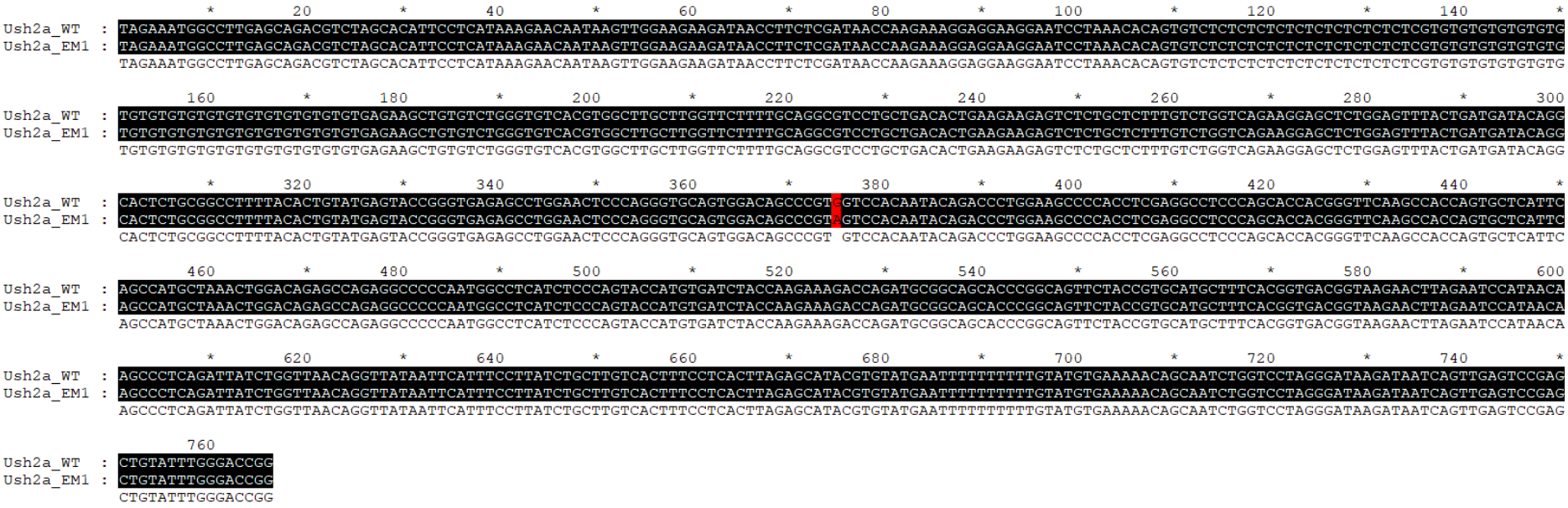
Mutant

TAGAAATGGCCTTGAGCAGACGTCTAGCACATTCCTCATAAAGAACAATAAGTTGGAAGAAGATAA
CCTTCTCGATAACCAAGAAAGGAGGAAGGAATCCTAAACACAGTGTCTCTCTCTCTCTCTCTCTCT
CGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAAGCTGTGTCTGGGTGTCACGT
GGCTTGCTTGGTTCTTTTGCAGGCGTCCTGCTGACACTGAAGAAGAGTCTCTGCTCTTTGTCTGGTCA
GAAGGAGCTCTGGAGTTTACTGATGATACAGGCACTCTGCGGCCTTTTACACTGTATGAGTACCGGG
TGAGAGCCTGGAACTCCCAGGGTGCAGTGGACAGCCCGT**A**GTCCACAATACAGACCCTGGAAGCCC
CACCTCGAGGCCTCCCAGCACCACGGGTTCAAGCCACCAGTGCTCATTCAGCCATGCTAAACTGGAC
AGAGCCAGAGGCCCCCAATGGCCTCATCTCCCAGTACCATGTGATCTACCAAGAAAGACCAGATGC
GGCAGCACCCGGCAGTTCTACCGTGCATGCTTTCACGGTGACGGTAAGAACTTAGAATCCATAACAA
GCCCTCAGATTATCTGGTTAACAGGTTATAATTCATTTCCTTATCTGCTTGTCACTTTCCTCACTTAGA
GCATACGTGTATGAATTTTTTTTTTGTATGTGAAAAACAGCAATCTGGTCCTAGGGATAAGATAATCA
GTTGAGTCCGAGCTGTATTTGGGACCGG

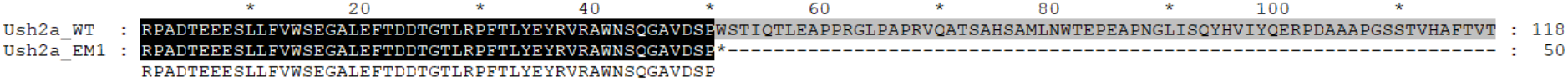
USH2A-W3947X-EM1-CDH23-B6 Heterozygous F1 animal sequence trace:



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_USH2A_F1 primer (5'-3')	TAGAAATGGCCTTGAGCAGACG
Geno_USH2A_R1 primer (5'-3')	CCGGTCCCAAATACAGCTCG
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	57
Elongation time (min)	0.5
WT product size (bp)	766
Mutant product size (bp)	766
Notes	Due to poly-repeats the amplicon was sequenced using the following primers (5'-3'): Geno_USH2A_F2: GTGTGAGAAGCTGTGTCTGG, Geno_USH2A_R2: ATGCTCTAAGTGAGGAAAGTGAC

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.

Additional integrations of the donor sequence

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	USH2A-W3947X_UNI1
Forward Primer (5'-3')	GCTCTGGAGTTTACTGATGATACA
Reverse Primer (5'-3')	CAATACAGACCCTGGAAGCC
Probe (5'-3')	TGTATGAGTACCGGGTGAGAGCCT
Label	FAM

The ddPCR assay is universal to both the WT and mutant allele of the Ush2a gene. WT controls are expected to call at 2 copies and a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	USH2A-W3947X_MUT1
Forward Primer (5’-3’)	GCAGTGGACAGCCCGTa
Reverse Primer (5’-3’)	TGCTCATTCAGCCATGCTAAAC
Probe (5’-3’)	CCACAATACAGACCCTGGAAGCCC
Label	FAM

The ddPCR assay is specific to the W3947X mutation of the Ush2a gene. WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

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.

No evidence of off-target donor insertions was found using these assays.

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation; W3947X in *Ush2a*. 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.

An Allelic Discrimination assay is 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. The relative level of fluorescence from each probe is used to determine the genotype of an animal.

Samples for this line are genotyped using the following primers and probe:

- Forward and reverse primers common to both Wild Type (WT) and mutant alleles
- WT probe binding to the WT base mutated in the mutant allele.
- Mutant probe binding to the SNP.

Ush2a-W3947X Allelic Discrimination assay

Ush2a-W3947X WT sequence

TAGAAATGGCCTTGAGCAGACGTCTAGCACATTCCTCATAAAGAACAATAAGTTGGAAGAAGATAA
CCTTCTCGATAACCAAGAAAGGAGGAAGGAATCCTAAACACAGTGTCTCTCTCTCTCTCTCTCTCT
CGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAAGCTGTGTCTGGGTGTCACGT
GGCTTGCTTGGTTCTTTTGCAGGCGTCCTGCTGACACTGAAGAAGAGTCTCTGCTCTTTGTCTGGTCA
GAAGGAGCTCTGGAGTTTACTGATGATACAGGCACTCTGCGGCCTTTTACACTGTATGAGTACC**GGG**
TGAGAGCCTGGAACTCCCAGGGTGCAGTGGAC**AGCCCGTgGTCCACA**AT**ACAGACCCTGGAAGCC**
CCACCTCGAGGCCTCCCAGCACCACGGGTTCAAGCCACCAGTGCTCATTCAGCCATGCTAAACTGGA
CAGAGCCAGAGGCCCCCAATGGCCTCATCTCCCAGTACCATGTGATCTACCAAGAAAGACCAGATGC
GGCAGCACCCGGCAGTTCTACCGTGCATGCTTTCACGGTGACGGTAAGAACTTAGAATCCATAACAA
GCCCTCAGATTATCTGGTTAACAGGTTATAATTCATTTCCTTATCTGCTTGTCACTTTCCTCACTTAGA
GCATACGTGTATGAATTTTTTTTTTGTATGTGAAAAACAGCAATCTGGTCCTAGGGATAAGATAATCA
GTTGAGTCCGAGCTGTATTTGGGACCGG

Ush2a-W3947X mutant sequence

TAGAAATGGCCTTGAGCAGACGTCTAGCACATTCCTCATAAAGAACAATAAGTTGGAAGAAGATAA
CCTTCTCGATAACCAAGAAAGGAGGAAGGAATCCTAAACACAGTGTCTCTCTCTCTCTCTCTCTCT
CGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAAGCTGTGTCTGGGTGTCACGT
GGCTTGCTTGGTTCTTTTGCAGGCGTCCTGCTGACACTGAAGAAGAGTCTCTGCTCTTTGTCTGGTCA
GAAGGAGCTCTGGAGTTTACTGATGATACAGGCACTCTGCGGCCTTTTACACTGTATGAGTACC**GGG**
TGAGAGCCTGGAACTCCCAGGGTGCAGTGGAC**AGCCCGTaGTCCACA**AT**ACAGACCCTGGAAGCC**
CCACCTCGAGGCCTCCCAGCACCACGGGTTCAAGCCACCAGTGCTCATTCAGCCATGCTAAACTGGA
CAGAGCCAGAGGCCCCCAATGGCCTCATCTCCCAGTACCATGTGATCTACCAAGAAAGACCAGATGC
GGCAGCACCCGGCAGTTCTACCGTGCATGCTTTCACGGTGACGGTAAGAACTTAGAATCCATAACAA
GCCCTCAGATTATCTGGTTAACAGGTTATAATTCATTTCCTTATCTGCTTGTCACTTTCCTCACTTAGA
GCATACGTGTATGAATTTTTTTTTTGTATGTGAAAAACAGCAATCTGGTCCTAGGGATAAGATAATCA
GTTGAGTCCGAGCTGTATTTGGGACCGG

SNP details:
WT= G
MUT= A

Lower case letters denote SNP position.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Ush2a-W3947X_F	n/a	**GGGTGAGAGCCTGGAACTC**	n/a	Common forward primer
Ush2a-W3947X _WT_PROBE	FAM	**AGCCCGTGGTCCACA**	BHQ-plus	Wild type Probe
Ush2a-W3947X _Mutant_PROBE	TET	**AGCCCGTAGTCCACA**	BHQ-plus	Mutant probe
Ush2a-W3947X _R	n/a	**GGGCTTCCAGGGTCTGT**	n/a	WT Reverse

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

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

qPCR cycling conditions

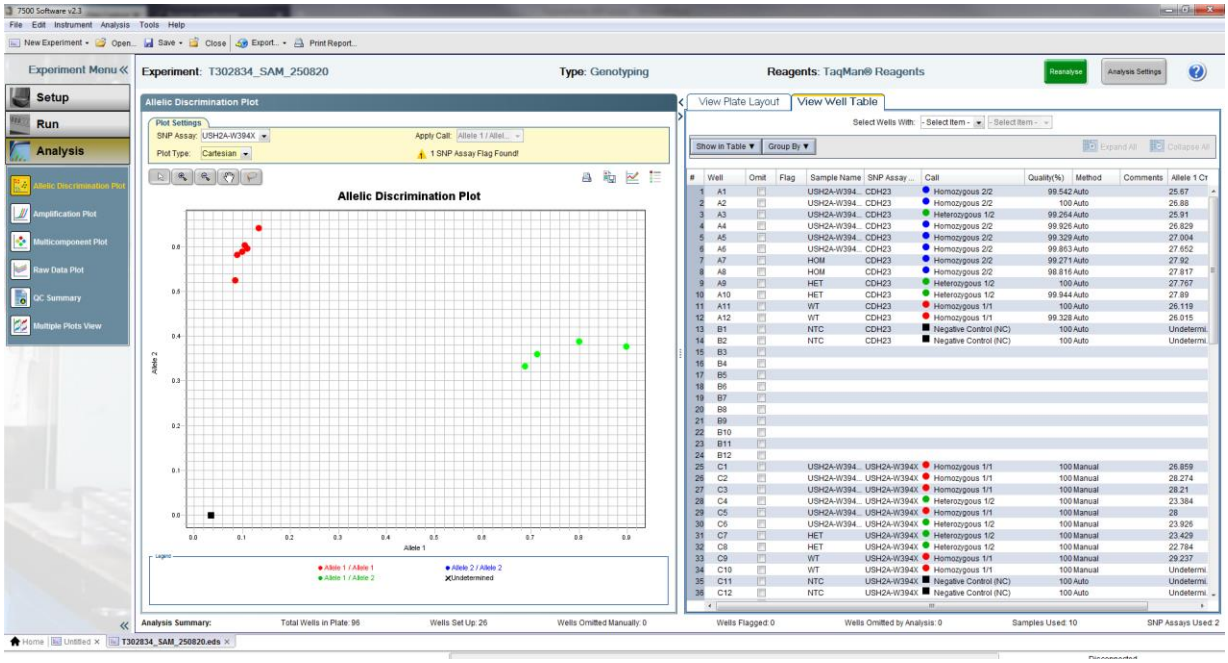
qPCR instrument: Applied Biosystems 7500

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 7500 software v2.0.6 from Applied Biosystems

Ush2a-W3947X Allelic Discrimination assay results (Task 302834 results)



Version No.	1
Date:	06/11/2020
Created/Updated by:	Daniel Ford
Approved:	Rumana Zaman

Cdh23 repair Genotyping Strategy

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

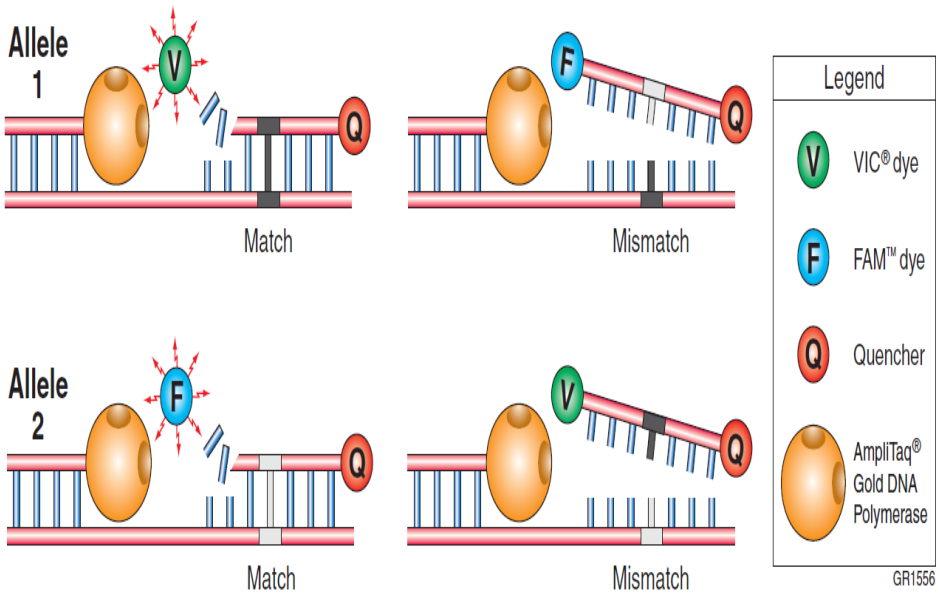
Introduction

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

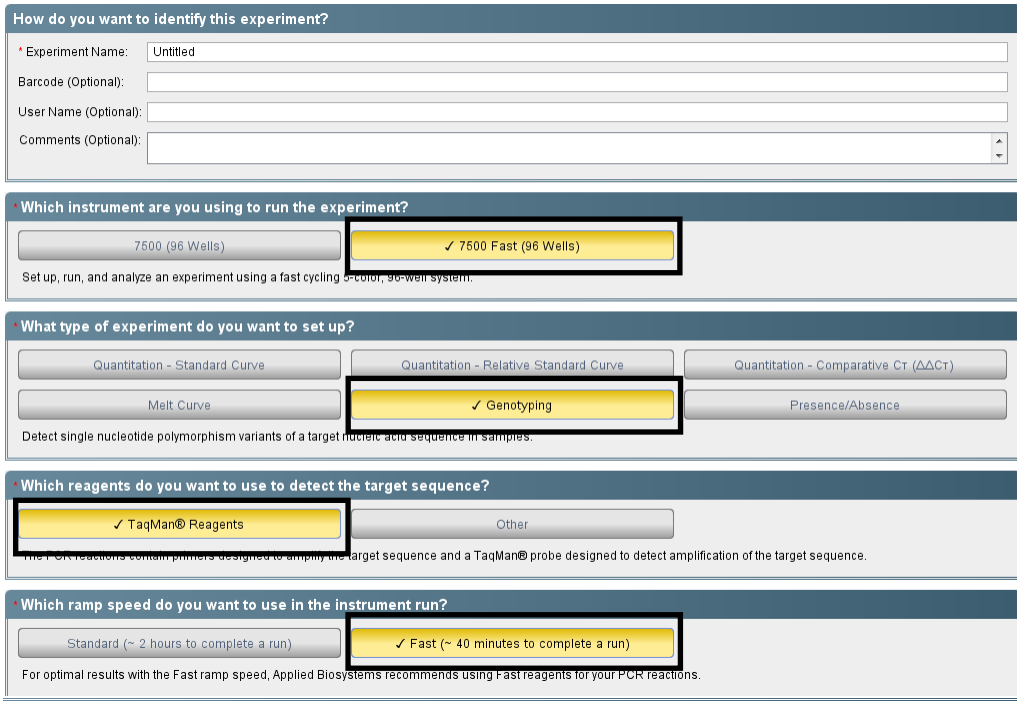
Primer 1	GACATGGATCCTATCTTCATCAACC
Primer 2	CCAGCACGGGCTAGAGAAC
Allele 1 probe - WT (FAM-Labelled)	CACTCTCCTCCAGTGAG
Allele 2 probe - Mutant (TET-Labelled)	CTCCTCCGGTGAGC

qPCR master mix

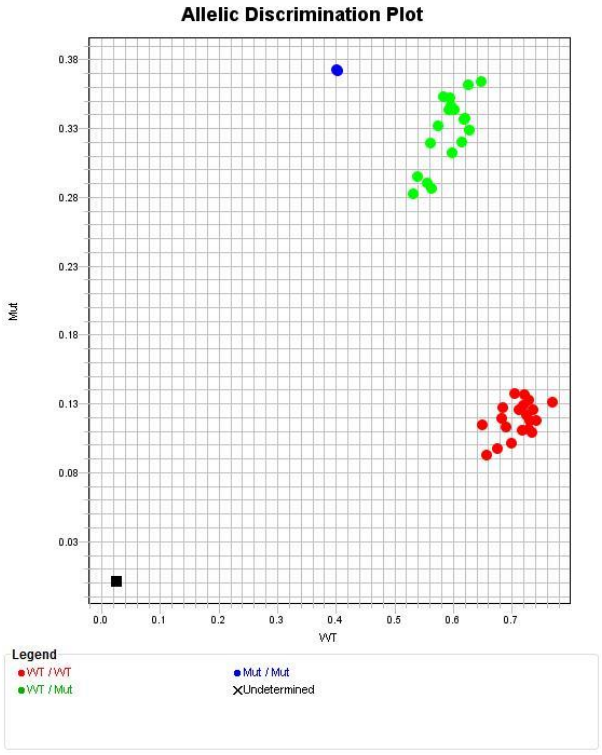
ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

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.