UBA1-E557V

Allele Description

This line is X-linked

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; E557V in exon ENSMUSE00000624599 of *UBA1*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

SNP 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.

UBA1-E557V

UBA1-E557V Allelic Discrimination assay

UBA1-E557V WT sequence

GCATCTCCTGATTTCACAGAAGTTAAAGTCTGACACGGCCGCTGCAGCTGTGCGCCAGATGAATCCT
TACATCCAGGTGACAAGCCACCA**GAACCGTGTAGGTCCTGAC**ACt**GaGCGCATCTATGATGA**TGATT
TCTTCCAAAATTTGGATGGTGTGGCCAATGCTCTGGACAACATAGATGCCCGTAAGTTTTGAAGG**CT**
GGTAAAGAAGGCAGGGGCAAAAGAGTCGGCGCCTGGGTTTTCCTGTTCTTTCCCAGTATGCTTTTTC
TTGTCTCAAGGCTATCACATCCTTGTCCCCACCCCATCATACAGGCATGTACATGGATCGCCGATGTG

UBA1-E557V mutant sequence

GCATCTCCTGATTTCACAGAAGTTAAAGTCTGACACGGCCGCTGCAGCTGTGCGCCAGATGAATCCT
TACATCCAGGTGACAAGCCACCA**GAACCGTGTAGGTCCTGAC**ACa**GtGCGCATCTATGATGA**TGATT
TCTTCCAAAATTTGGATGGTGTGGCCAATGCTCTGGACAACATAGATGCCCGTAAGTTTTGAAGG**CT**
GGTAAAGAAGGCAGGGGCAAAAGAGTCGGCGCCTGGGTTTTCCTGTTCTTTCCCAGTATGCTTTTTC
TTGTCTCAAGGCTATCACATCCTTGTCCCCACCCCATCATACAGGCATGTACATGGATCGCCGATGTG

SNP details:
WT= A
MUT= T

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
UBA1-E557V _F	n/a	**GAACCGTGTAGGTCCTGAC**	n/a	Common forward primer
UBA1-E557V _WT_PROBE	FAM	**GAGCGCATCTATGATGA**	BHQ-plus	Wild type Probe
UBA1-E557V _Mutant_PROBE	TET	**GTGCGCATCTATGATGA**	BHQ-plus	Mutant probe
UBA1-E557V _R	n/a	**CCCCTGCCTTCTTTACCAG**	n/a	WT Reverse

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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

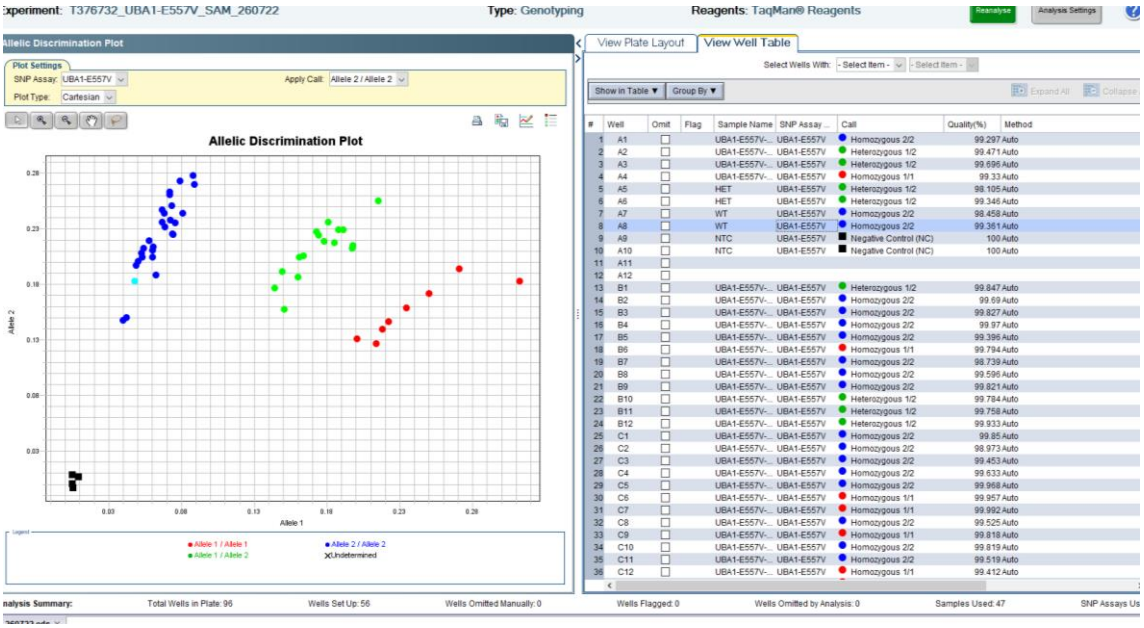
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

UBA1-E557V

Analysis

The results are analysed using 7500 software v2.0.6 from Applied Biosystems

UBA1-E557V Allelic Discrimination assay results (Task 376732 results)



Hemizygous males will appear as Homozygotes.

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Approved:	Debbie Williams