

Name of Mouse model or mutation:

KCNA1-R417X-EM1-B6

KCNA1-R417X-EM2-B6

Description:

Point mutation introduced using CRISPR/Cas9 reagents

Type of mutation:

SNP: R417X

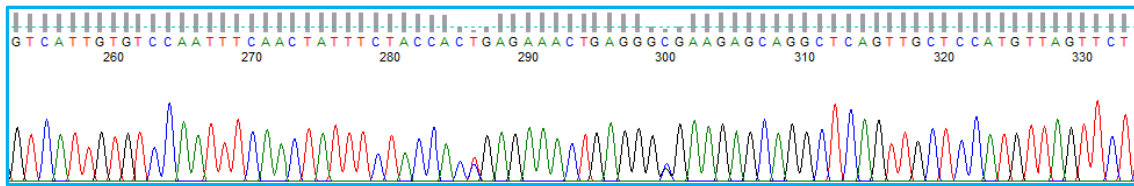
Sequence details**WT**

```
CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCA
GGGTCATCCGCTTGTAAGGGTGTTTTCAGAATCTTCAAACCTCTCCCGCCACTCCAAGGGCCTTCAGAT
CCTGGGCCAGACCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTTCTCTTCATTGGG
GTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTA
TCCCGATGCTTTCTGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGT
GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCC
GTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACCGAGAACTGAGGGGGAAGAGCAGGCTC
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGAGCTCCTCTACT
ATCAGCAAGTCTGAGTACATGGAGATCGAAGAGGATATGAACAATAGCATAGCCATTACAGACAG
GCTAATATCAGAACTGGTAACTGCACCACAGCTGATCAAACCTGCGTTAATAAGAGCAAGCTCCTGA
CCGATGTTTTAAAAGCAACAGGCAAGCCAACAAAAGCCCCAAACA
```

Mutant

```
CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCA
GGGTCATCCGCTTGTAAGGGTGTTTTCAGAATCTTCAAACCTCTCCCGCCACTCCAAGGGCCTTCAGAT
CCTGGGCCAGACCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTTCTCTTCATTGGG
GTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTA
TCCCGATGCTTTCTGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGT
GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCC
GTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACCTGAGAACTGAGGGCGAAGAGCAGGCTC
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGAGCTCCTCTACT
ATCAGCAAGTCTGAGTACATGGAGATCGAAGAGGATATGAACAATAGCATAGCCATTACAGACAG
GCTAATATCAGAACTGGTAACTGCACCACAGCTGATCAAACCTGCGTTAATAAGAGCAAGCTCCTGA
CCGATGTTTTAAAAGCAACAGGCAAGCCAACAAAAGCCCCAAACA
```

KCNA1-R417X-EM1-B6 Heterozygous F1 animal sequence trace:



Please note the sequences of KCNA1-R417X-EM1-B6 or KCNA1-R417X-EM2-B6 are the same, just transmitted from different founder animals.

Nucleotide Alignment:

```

                *      20      *      40      *      60      *      80      *      100     *      120     *      140
Kcna1_WT      : CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCAGGGTCATCCGCTTGGTAAGGGTGTTCAGAATCTTCAAACCTCCTCCCGCCACTCCAAGGGCCTTCAGATCCTGGGCC
Kcna1_R417X   : CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCAGGGTCATCCGCTTGGTAAGGGTGTTCAGAATCTTCAAACCTCCTCCCGCCACTCCAAGGGCCTTCAGATCCTGGGCC
                CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCAGGGTCATCCGCTTGGTAAGGGTGTTCAGAATCTTCAAACCTCCTCCCGCCACTCCAAGGGCCTTCAGATCCTGGGCC

                *      160     *      180     *      200     *      220     *      240     *      260     *      280
Kcna1_WT      : AGACCCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTCCCTTTCATTGGGGTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTATCCCCGATGCTTTC
Kcna1_R417X   : AGACCCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTCCCTTTCATTGGGGTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTATCCCCGATGCTTTC
                AGACCCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTCCCTTTCATTGGGGTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTATCCCCGATGCTTTC

                *      300     *      320     *      340     *      360     *      380     *      400     *      420
Kcna1_WT      : TGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGTGACAAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCCCGTACCTGTGCTATTGTGTCCAA
Kcna1_R417X   : TGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGTGACAAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCCCGTACCTGTGCTATTGTGTCCAA
                TGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGTGACAAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCCCGTACCTGTGCTATTGTGTCCAA

                *      440     *      460     *      480     *      500     *      520     *      540     *      560
Kcna1_WT      : TTTCAACTATTTCTACCACGAGAACTGAGGGGAAGAGCAGGCTCAGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGAGCTCCTCTACTATCAGCAAGTCTGAGTACATGGAGA
Kcna1_R417X   : TTTCAACTATTTCTACCACGAGAACTGAGGGGAAGAGCAGGCTCAGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGAGCTCCTCTACTATCAGCAAGTCTGAGTACATGGAGA
                TTTCAACTATTTCTACCACGAGAACTGAGGGGAAGAGCAGGCTCAGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGAGCTCCTCTACTATCAGCAAGTCTGAGTACATGGAGA

                *      580     *      600     *      620     *      640     *      660     *      680     *      700
Kcna1_WT      : TCGAAGAGGATATGAACAATAGCATAGCCATTACAGACAGGCTAATATCAGAAGTGGTAAGTGCACCACAGCTGATCAAACTGCGTTAATAAGAGCAAGCTCCTGACCGATGTTTAAAAAGCAACAGGCAAGCCAACA
Kcna1_R417X   : TCGAAGAGGATATGAACAATAGCATAGCCATTACAGACAGGCTAATATCAGAAGTGGTAAGTGCACCACAGCTGATCAAACTGCGTTAATAAGAGCAAGCTCCTGACCGATGTTTAAAAAGCAACAGGCAAGCCAACA
                TCGAAGAGGATATGAACAATAGCATAGCCATTACAGACAGGCTAATATCAGAAGTGGTAAGTGCACCACAGCTGATCAAACTGCGTTAATAAGAGCAAGCTCCTGACCGATGTTTAAAAAGCAACAGGCAAGCCAACA

                *
Kcna1_WT      : AAAGCCCCAAACA
Kcna1_R417X   : AAAGCCCCAAACA
                AAAGCCCCAAACA

```

Predicted Protein Alignment:

```

                *      20      *      40      *      60      *      80      *      100     *      120     *      140
Kcna1_WT      : TEIAPEQGNQKGEQATSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAEAEESHFSSIPDAFWWAVVSMTTVGYGDMYPVVTIGGKIVGSLCAIAGVLTIALPVPVIVSN
Kcna1_R417X   : TEIAPEQGNQKGEQATSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAEAEESHFSSIPDAFWWAVVSMTTVGYGDMYPVVTIGGKIVGSLCAIAGVLTIALPVPVIVSN
                TEIAPEQGNQKGEQATSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAEAEESHFSSIPDAFWWAVVSMTTVGYGDMYPVVTIGGKIVGSLCAIAGVLTIALPVPVIVSN

                *      160     *      180     *      200     *      220
Kcna1_WT      : FNYFYHRETEGEEQAQLLHVSSPNLASDLSRRSSSTISKSEYMEIEEDMNNSIAHYRQANIRTNCTADQNCVNSKLLTDV*
Kcna1_R417X   : FNYFYH*****
                FNYFYH

```

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_Kcna1_F1 primer (5'-3')	CACGGAGATAGCTGAGCAGG
Geno_Kcna1_R1 primer (5'-3')	TGTTTGGGGCTTTTGTGGC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	59
Elongation time (min)	1
WT product size (bp)	713
Mutant product size (bp)	713
Notes	6% DMSO required in the PCR mastermix.

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	KCNA1-R417X-UNI2
Forward Primer (5'-3')	CGTACCTGTCATTGTGTCCA
Reverse Primer (5'-3')	GTCAGAGGCTAAGTTAGGAGAAC
Probe (5'-3')	AGCAGGCTCAGTTGCTCCATGTTA
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.



Kcna1-R417X Genotyping Strategy

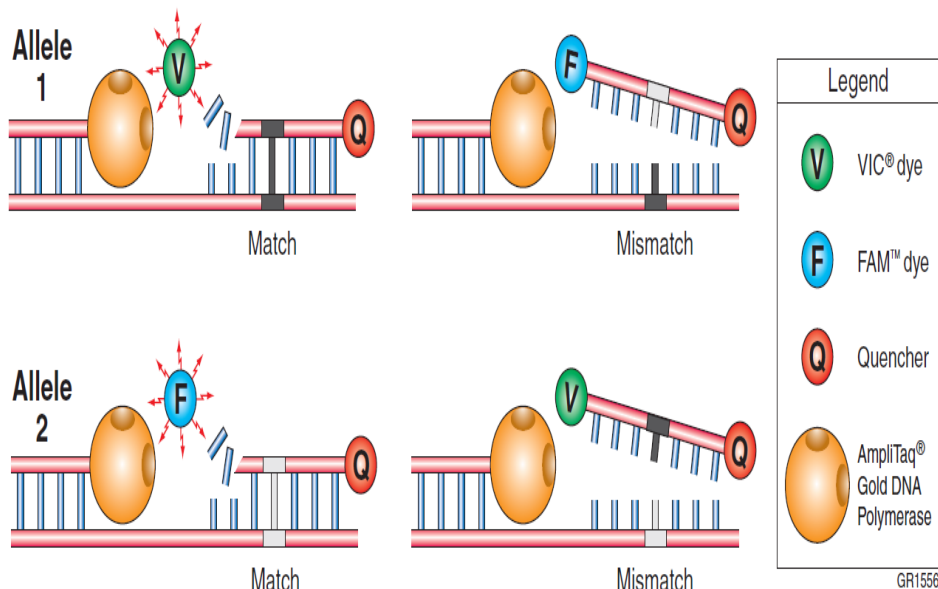
Introduction

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 and the other matching the Mutant

The Analysis software produces 2 genotypes:

- Homozygotes (samples having only WT or Mutant)
- Heterozygotes (samples having both WT and Mutant)



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>

Open and complete sample ID template for Allelic Discrimination assay

[FROM HERE](#)



Mutation type: SNP
Mutant allele: TGAGAACTGAGGGC
WT allele: CGAGAACTGAGGGG

WT Fragment sequence

GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCT**GCCC**
GTACCTGTCATTGTGTCCAATTTCAACTATTT**CTACCACcGAGAACTG**AGGGg**GAAGAGCAGGCTC**
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT

MUT Fragment sequence

GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCT**GCCC**
GTACCTGTCATTGTGTCCAATTTCAACTATTT**CTACCACtGAGAACTGA**GGGc**GAAGAGCAGGCTC**
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT

Kcna1-R417X-AD

Primers and Probes

Primer 1	GCCCGTACCTGTCATTGTGT	
Primer 2	GCAACTGAGCCTGCTCTTC	
Allele 1 Mut probe	TCAGTTTCTCAGTGGTAGA	(FAM-Labelled)
Allele 2 WT probe	CAGTTTCTCGGTGGTAGA	(TET-Labelled)

qPCR master mix

ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl (of 1 in 5 dilution of stock)
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/Mut on 7500 FAM-labelled. Allele 2 = MUT/WT on 7500 TET-labelled.

7500 Settings for running Allele Discrimination Assay are as shown below

How do you want to identify this experiment?

* Experiment Name:

Barcode (Optional):

User Name (Optional):

Comments (Optional):

Which instrument are you using to run the experiment?

Set up, run, and analyze an experiment using a fast cycling 8-color, 96-well system.

What type of experiment do you want to set up?

Detect single nucleotide polymorphism variants of a target nucleic acid sequence in samples.

Which reagents do you want to use to detect the target sequence?

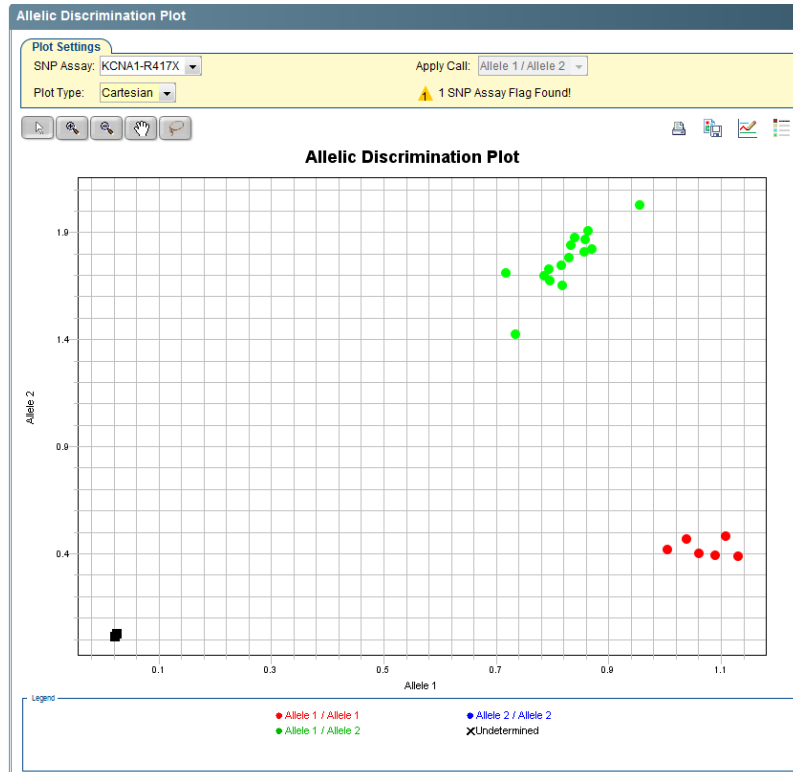
The TaqMan reactions contain primers designed to amplify the target sequence and a TaqMan probe designed to detect amplification of the target sequence.

Which ramp speed do you want to use in the instrument run?

For optimal results with the Fast ramp speed, Applied Biosystems recommends using Fast reagents for your PCR reactions.



Example of an Allelic Discrimination Plot and Results



	1	2	3	4	5	6	7	8	9	10	11	12
A	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01.1f KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X				
B	KCNA1-R417X:EM1-B03... KCNA1-R417X	KCNA1-R417X:EM1-B03... KCNA1-R417X	KCNA1-R417X:EM1-B03... KCNA1-R417X	KCNA1-R417X:EM1-B03... KCNA1-R417X	KCNA1-R417X:EM1-B03... KCNA1-R417X							
C	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X	KCNA1-R417X:EM2-B01... KCNA1-R417X							
D	HET 1 KCNA1-R417X	HET 2 KCNA1-R417X	WT 1 KCNA1-R417X	WT 2 KCNA1-R417X	NTC KCNA1-R417X	NTC KCNA1-R417X						

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

Version No. 1

Date: 03/07/2020

Created/Updated by: Daniel Ford

Approved by: Rumana Zaman