Name of Mouse model or mutation:
KCNA1-R417X-EM1-B6
KCNA1-R417X-EM2-B6

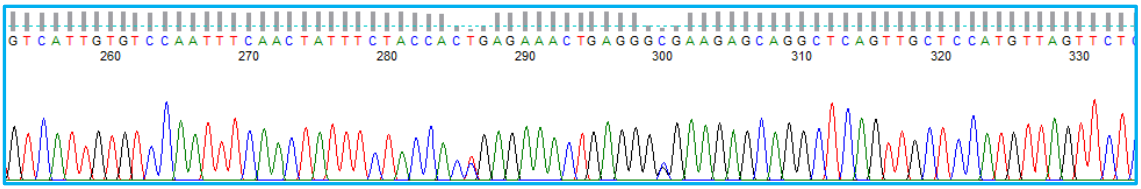
Description:
Point mutation introduced using CRISPR/Cas9 reagents

Type of mutation:
SNP: R417X

Sequence details
WT
CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCA
GGGTCATCCGCTTGGTAAGGGTGTTCAGAATCTTCAAACTCTCCCGCCACTCCAAGGGCCTTCAGAT
CCTGGGCCAGACCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTTCCTCTTCATTGGG
GTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTA
TCCCCGATGCTTTCTGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGT
GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCCC
GTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACCGAGAAACTGAGGGGGAAGAGCAGGCTC
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT
ATCAGCAAGTCTGAGTACATGGAGATCGAAGAGGATATGAACAATAGCATAGCCCATTACAGACAG
GCTAATATCAGAACTGGTAACTGCACCACAGCTGATCAAAACTGCGTTAATAAGAGCAAGCTCCTGA
CCGATGTTTAAAAAGCAACAGGCAAGCCAACAAAAGCCCCAAACA

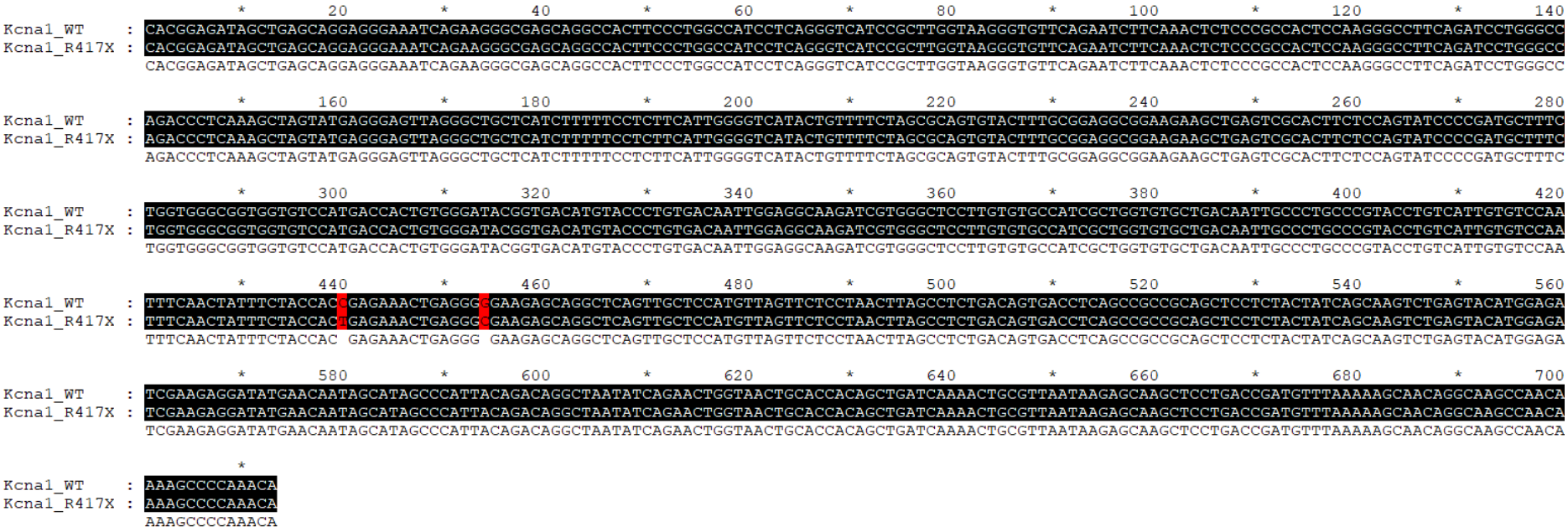
Mutant
CACGGAGATAGCTGAGCAGGAGGGAAATCAGAAGGGCGAGCAGGCCACTTCCCTGGCCATCCTCA
GGGTCATCCGCTTGGTAAGGGTGTTCAGAATCTTCAAACTCTCCCGCCACTCCAAGGGCCTTCAGAT
CCTGGGCCAGACCCTCAAAGCTAGTATGAGGGAGTTAGGGCTGCTCATCTTTTTCCTCTTCATTGGG
GTCATACTGTTTTCTAGCGCAGTGTACTTTGCGGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTA
TCCCCGATGCTTTCTGGTGGGCGGTGGTGTCCATGACCACTGTGGGATACGGTGACATGTACCCTGT
GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCTGCCC
GTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCAC**T**GAGAAACTGAGGG**C**GAAGAGCAGGCTC
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT
ATCAGCAAGTCTGAGTACATGGAGATCGAAGAGGATATGAACAATAGCATAGCCCATTACAGACAG
GCTAATATCAGAACTGGTAACTGCACCACAGCTGATCAAAACTGCGTTAATAAGAGCAAGCTCCTGA
CCGATGTTTAAAAAGCAACAGGCAAGCCAACAAAAGCCCCAAACA

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

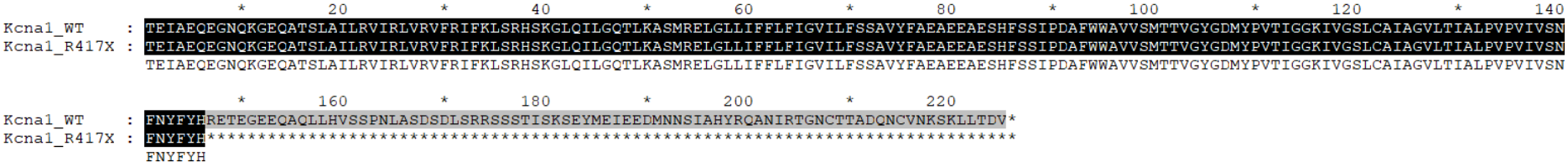


Please note the sequences of KCNA1-R417X-EM1-B6 or KCNA1-R417X-EM2-B6 are the same, just transmitted from different founder animals.

Nucleotide Alignment:



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_Kcna1_F1 primer (5'-3')	CACGGAGATAGCTGAGCAGG
Geno_Kcna1_R1 primer (5'-3')	TGTTTGGGGCTTTTGTTGGC
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.

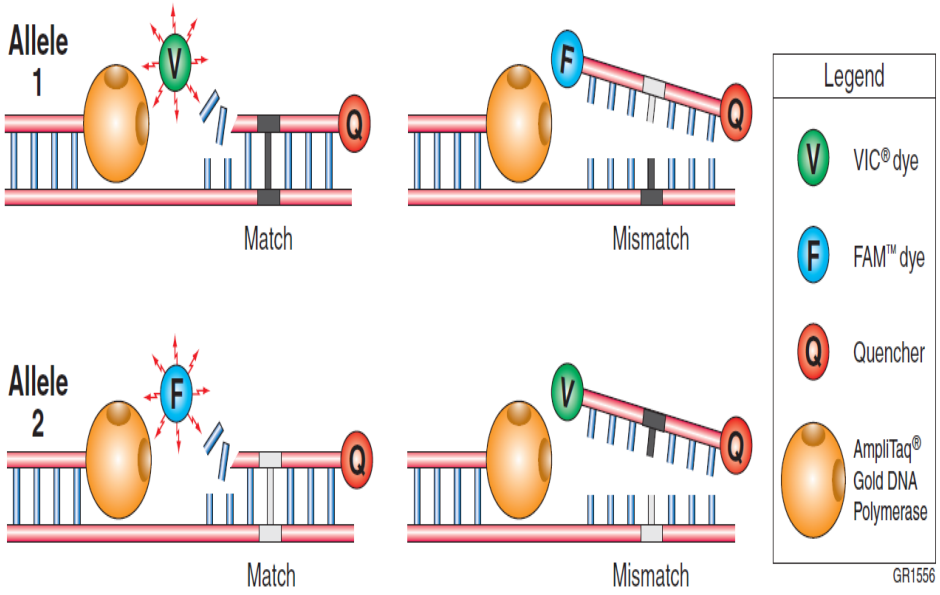
Kcna1-R417X Genotyping Strategy

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: **T**GAGAAACTGAGGG**C**
WT allele: **C**GAGAAACTGAGGG**G**

WT Fragment sequence

GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCT**GCCC**
GTACCTGTCATTGTGTCCAATTTCAACTATT**TCTACCACcGAGAAACTG**AGGGg**GAAGAGCAGGCTC**
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT

MUT Fragment sequence

GACAATTGGAGGCAAGATCGTGGGCTCCTTGTGTGCCATCGCTGGTGTGCTGACAATTGCCCT**GCCC**
GTACCTGTCATTGTGTCCAATTTCAACTATT**TCTACCACtGAGAAACTGA**GGG**c****GAAGAGCAGGCTC**
AGTTGCTCCATGTTAGTTCTCCTAACTTAGCCTCTGACAGTGACCTCAGCCGCCGCAGCTCCTCTACT

Kcna1-R417X-AD
Primers and Probes

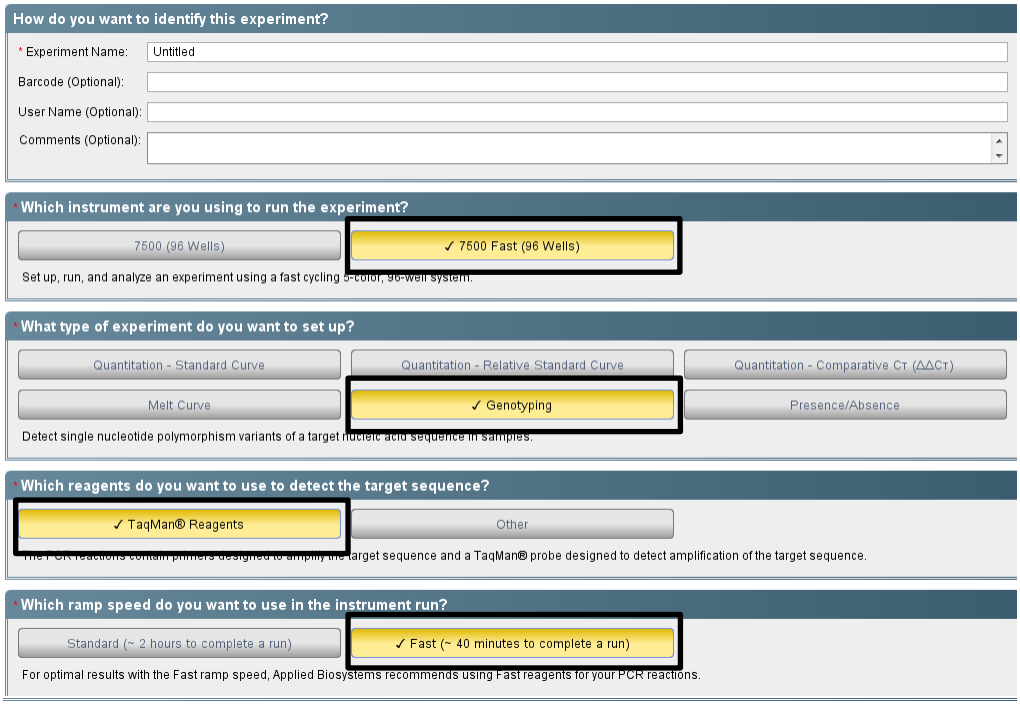
Primer 1	GCCCGTACCTGTCATTGTGT	
Primer 2	GCAACTGAGCCTGCTCTTC	
Allele 1 Mut probe	TCAGTTTCTCAGTGGTAGA	(FAM-Labelled)
Allele 2 WT probe	CAGTTTCTCGGTGGTAGA	(TET-Labelled)

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



Example of an Allelic Discrimination Plot and Results

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