Name of Mouse model or mutation:
TACR1-R244C-EM1-B6N
TACR1-R244C-EM2-B6N

Description:
Point mutation model made using CRISPR/Cas9.

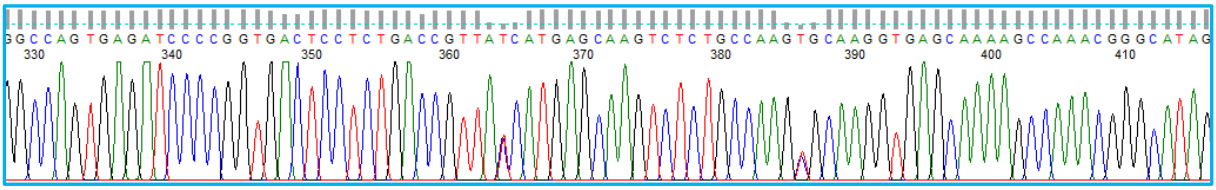
Type of mutation:
SNP: R244C

Sequence details
WT
GTATCCCTTCAAAATGTAAGCCAAAATAAATGCTTCCTTCACTAAGCTGCTTCCTGTGACAGGAATAT
TTGCTCACAAAAACAAGAAACGTTATGAACACAGGGAAAGTCCACATGAAGAATGTAAATTAAATC
CGCTTGTTTTCTCCTGCCCATCACCCATTCATTGTGAATGGTGGGTGGTATTCCTCCCTGTATGATGAC
CAAGGCTGATAAAGTGTCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTC
ACAGGTACCACATCTGTGTGACTGTGCTGATCTACTTCCTGCCTCTGCTGGTGATTGGCTATGCATAC
ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTACCATGAGC
AAGTCTCTGCCAAGCGCAAGGTGAGCAAAAGCCAAACGGGCATAGCTTGCCCTGGCACTGACAGTG
TGGGAGGCTTGAGGCCCCAAAGGCAGAAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGG
AAAGCAGGGTCTTCCTTCATGTCCACATGTACATGTACACACTCATATACAGTCAAACACATGTACAA
ACTCTCATATATACACATAATTATATACATACAAAAGTAATCATACACAGTCATCCATATACACACAC
ACATCATATTCATATACATATATACCAATACTTATATATGTTTACACATCCATATATAGACACCCATAC
ACACACACAAACATTCATACACACGTATATATACACACAAAAACCCACTCATACACACACATATACTC
ATACACATATGCACACATATACAGATACACACACAAATACTCATCCATATGTACACACACAGAGACTC
ACATACACACACATAAACACCCATACACGTGTTACACATACCC

TACR1-R244C-EM1-B6N or TACR1-R244C-EM2-B6N
GTATCCCTTCAAAATGTAAGCCAAAATAAATGCTTCCTTCACTAAGCTGCTTCCTGTGACAGGAATAT
TTGCTCACAAAAACAAGAAACGTTATGAACACAGGGAAAGTCCACATGAAGAATGTAAATTAAATC
CGCTTGTTTTCTCCTGCCCATCACCCATTCATTGTGAATGGTGGGTGGTATTCCTCCCTGTATGATGAC
CAAGGCTGATAAAGTGTCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTC
ACAGGTACCACATCTGTGTGACTGTGCTGATCTACTTCCTGCCTCTGCTGGTGATTGGCTATGCATAC
ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTA**T**CATGAGC
AAGTCTCTGCCAAG**T**GCAAGGTGAGCAAAAGCCAAACGGGCATAGCTTGCCCTGGCACTGACAGTG
TGGGAGGCTTGAGGCCCCAAAGGCAGAAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGG
AAAGCAGGGTCTTCCTTCATGTCCACATGTACATGTACACACTCATATACAGTCAAACACATGTACAA
ACTCTCATATATACACATAATTATATACATACAAAAGTAATCATACACAGTCATCCATATACACACAC

ACATCATATTCATATACATATATACCAATACTTATATATGTTTACACATCCATATATAGACACCCATAC
ACACACACAAACATTCATACACACGTATATATACACACAAAAACCCACTCATACACACACATATACTC
ATACACATATGCACACATATACAGATACACACACAAATACTCATCCATATGTACACACACAGAGACTC
ACATACACACACATAAACACCCATACACGTGTTACACATACCC

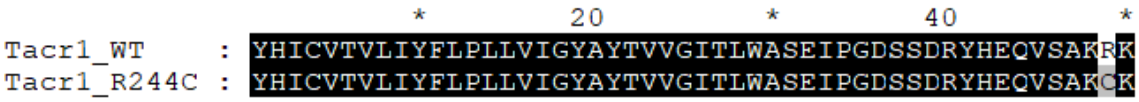
TACR1-R244C-EM1-B6N or TACR1-R244C-EM2-B6N Heterozygous F1 animal sequence trace:



Please note the sequences of TACR1-R244C-EM1-B6N or TACR1-R244C-EM2-B6N 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_Tacr1_R244C_F4 primer (5'-3')	GTATCCCTTCAAAATGTAAGCCA
Geno_Tacr1_R244C_R4 primer (5'-3')	GGGTATGTGTAACACGTGTATGG
Taq Polymerase used	ThermoFisher SuperFi kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	921
Mutant product size (bp)	921
Notes	Sequence in reverse with Geno_Tacr1_seq_R primer (5'-3') TGTGTGTATGAGTGGGTTTTTG

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.

Off-target site with ≤2 mismatches for guide used were checked with the following primers:

Off-target site	Sequence	Type
15:61689434-61689456	GCTTGG**G**AGAGAC**A**TGCTCA AGG	Intergenic

Tacr1_OT1_F1 primer (5'-3')	TAGTTTGCTTCCCTGAGCCTT
Tacr1_OT1_R1 primer (5'-3')	AGGACTGCACTGAGGATAATGC
Taq Polymerase used	ThermoFisher SuperFi kit
Annealing Temperature (°C)	65
Elongation time (min)	0.25
WT product size (bp)	342
Mutant product size (bp)	342

Notes	

All amplicons were sent for Sanger sequencing.

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	TACR1-R244C-UNI1
Forward Primer (5'-3')	GCATACACTGTGGTAGGGATTACA
Reverse Primer (5'-3')	CAGTGCCAGGGCAAGCTATG
Probe (5'-3')	CCCGGTGACTCCTCTGACCGTTA
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.

The ddPCR assay is universal to TACR1 - both WT and MUT alleles are recognised by this assay. Therefore WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies for F1 (HET) animals.

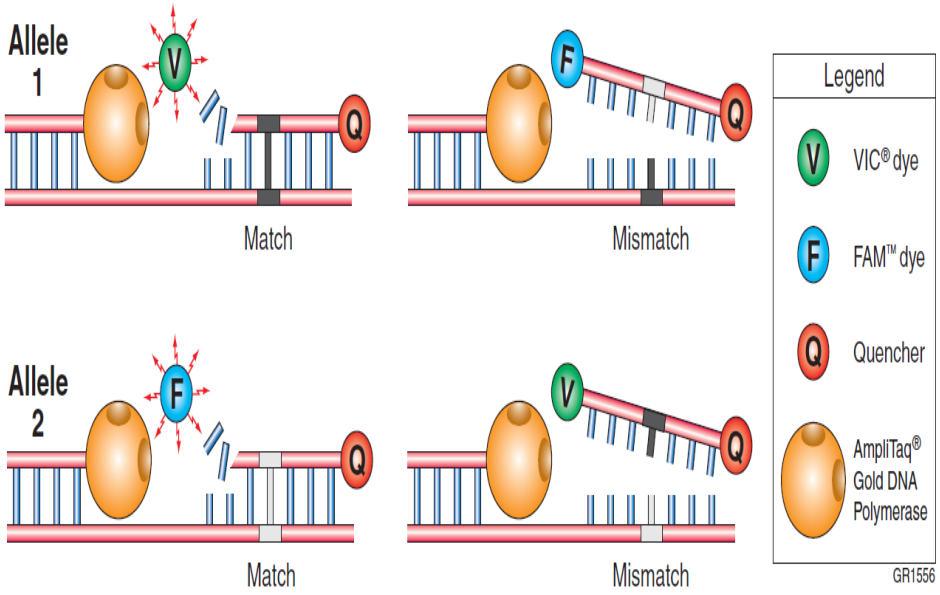
TACR1 R244C Genotyping Strategy

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
WT allele: C

Fragment sequence

WT

ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTACCATGAGC
AAGTCTCTGCCAAGCGCAAGGTGAGCAAAAGCCAAACGGGCATAGCTTGCCCTGGCACTGACAGTG

TACR1-R244C-EM1-B6N or TACR1-R244C-EM2-B6N

ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTA**T**CATGAGC
AAGTCTCTGCCAAG**T**GCAAGGTGAGCAAAAGCCAAACGGGCATAGCTTGCCCTGGCACTGACAGTG

Assay Tacr1 R244C Primers and Probes

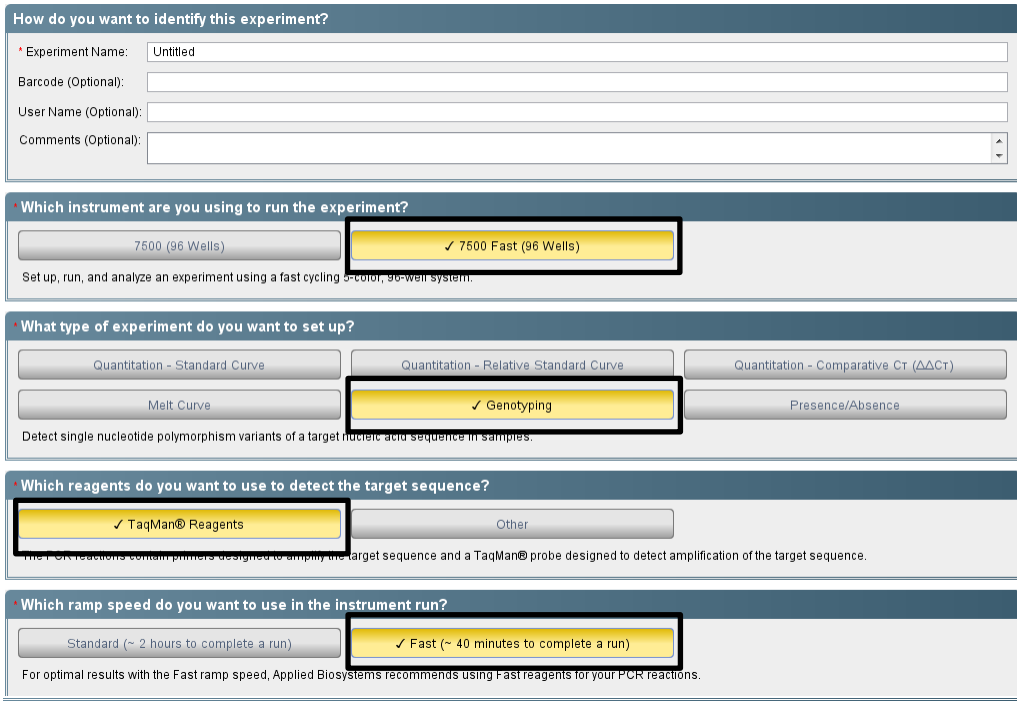
Primer 1	CAGTGCCAGGGCAAGCTATG
Primer 2	CGGTGACTCCTCTGACCGTT
Allele 1 (WT) probe (FAM-Labelled)	TGCCAAGCGCAAGG
Allele 2 (Mut) probe (TET-Labelled)	CTGCCAAGTGCAAGGT

qPCR master mix

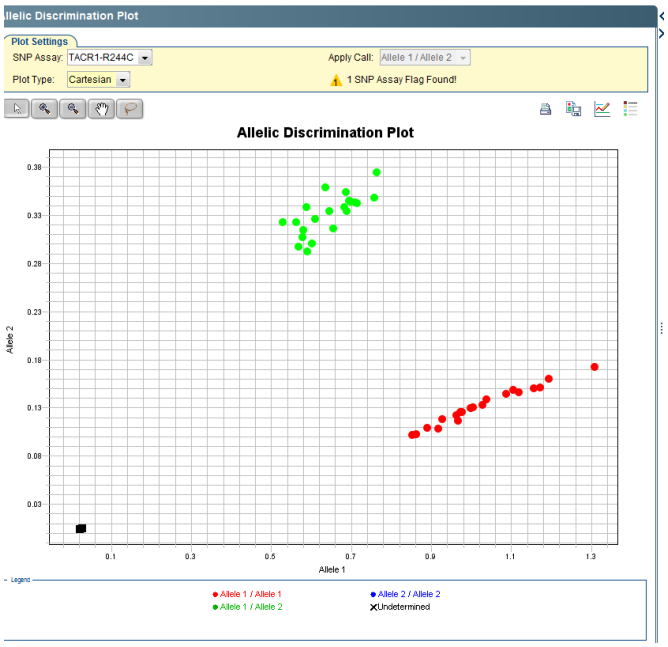
ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl (of 1 in 5 dilution of stock)
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

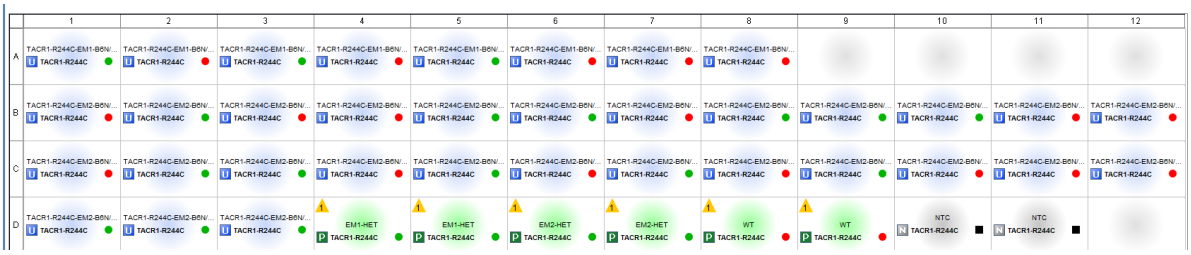
No need to run the samples in duplicates.
Allele 1 = WT/Mut on 7500 FAM-labelled. Allele 2 = MUT/WT on 7500 TET-labelled.

7500 Settings for running Allele Discrimination Assay are as shown below



Example of an Allelic Discrimination Plot and Results (282399)





Please note, use your controls to group and Tacr1 R244C your samples accordingly.

Version No. 1

Date: 10/03/2020

Created/Updated by: Daniel Ford

Approved by: Debbie Williams