

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**

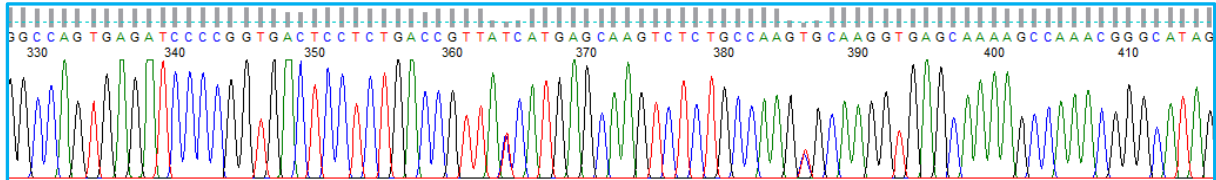
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CAAGGCTGATAAAGTGCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTC
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TGGGAGGCTTGAGGCCCAAAGGCAGAAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGG
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ATACACATATGCACACATATACAGATACACACAAAATACTCATCCATATGTACACACACAGAGACTC
ACATACACACACATAAACACCCATACACGTGTTACACATACCC

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

GTATCCCTTCAAATGTAAGCCAAAATAAATGCTTCCTTCACTAAGCTGCTTCCTGTGACAGGAATAT
TTGCTCACAAAAACAAGAAACGTTATGAACACAGGGAAAAGTCCACATGAAGAATGTAAATTAATC
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CAAGGCTGATAAAGTGCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTC
ACAGGTACCACATCTGTGTGACTGTGCTGATCTACTTCCTGCCTCTGCTGGTGATTGGCTATGCATAC
ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTA**T**CATGAGC
AAGTCTCTGCCAAG**T**GCAAGGTGAGCAAAAGCCAAACGGGCATAGCTTGCCCTGGCACTGACAGTG
TGGGAGGCTTGAGGCCCAAAGGCAGAAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGG
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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:

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*           20           *           40           *           60           *           80           *           100          *           120          *           140          *
Tacrl_WT   : GTATCCCTTCAAAATGTAAGCCAAAATAAATGCTTCCTTCACTAAGCTGCTTCCTGTGACAGGAATATTTGCTCACAAAAACAAGAAACGTTATGAACACAGGGAAAGTCCACATGAAGAATGTAABTTAAATCCGCTTGTTTTCTCCTG
Tacrl_R244C : GTATCCCTTCAAAATGTAAGCCAAAATAAATGCTTCCTTCACTAAGCTGCTTCCTGTGACAGGAATATTTGCTCACAAAAACAAGAAACGTTATGAACACAGGGAAAGTCCACATGAAGAATGTAABTTAAATCCGCTTGTTTTCTCCTG

160          *           180          *           200          *           220          *           240          *           260          *           280          *           300
Tacrl_WT   : CCCATCACCCATTTCATTTGTAATGGTGGGTTGGTATTCCTCCCTGTATGATGACCAAGGCTGATAAAGTGTCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTCACAGGTACCACATCTGTGTGACTGTGCTG
Tacrl_R244C : CCCATCACCCATTTCATTTGTAATGGTGGGTTGGTATTCCTCCCTGTATGATGACCAAGGCTGATAAAGTGTCCACAGACTGTAATTGCTGCTGTTGCTAAGACTGACTCCTCTGTCTACCTCACAGGTACCACATCTGTGTGACTGTGCTG

*           320          *           340          *           360          *           380          *           400          *           420          *           440          *
Tacrl_WT   : ATCTACTTCTGCTGCTGCTGGTGATTTGGCTATGCATACACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTACATGAGCAAGTCTCTGCCAAGGCAAGGTGAGCAAAGCCAAACGGGCATA
Tacrl_R244C : ATCTACTTCTGCTGCTGCTGGTGATTTGGCTATGCATACACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCCCGGTGACTCCTCTGACCGTTACATGAGCAAGTCTCTGCCAAGGCAAGGTGAGCAAAGCCAAACGGGCATA

460          *           480          *           500          *           520          *           540          *           560          *           580          *           600
Tacrl_WT   : GCTTGCCCTGGCACTGACAGTGTGGGAGGCTTGAGGCCCCAAAGGCAGAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGGAAAGCAGGGTCTTCTTCATGTCCACATGTACATGTACACACTCATATACAGTCAAACACATG
Tacrl_R244C : GCTTGCCCTGGCACTGACAGTGTGGGAGGCTTGAGGCCCCAAAGGCAGAGCCATAAAGCATCTCTGGACCTCAGAAACAGAAAAGGAAAGCAGGGTCTTCTTCATGTCCACATGTACATGTACACACTCATATACAGTCAAACACATG

*           620          *           640          *           660          *           680          *           700          *           720          *           740          *
Tacrl_WT   : TACAACTCTCATATATACACATAAATATATACATACAAAAGTAATCATACACAGTCCATATACACACACACATCATATTCATATATATATACCAATACTTATATATGTTTACACATCCATATATAGACACCCATACACACAC
Tacrl_R244C : TACAACTCTCATATATACACATAAATATATACATACAAAAGTAATCATACACAGTCCATATACACACACACATCATATTCATATATATATACCAATACTTATATATGTTTACACATCCATATATAGACACCCATACACACAC

760          *           780          *           800          *           820          *           840          *           860          *           880          *           900
Tacrl_WT   : AAACATTCATACACACGTATATATACACACAAAACCCACTCATACACACACATATACTCATACACATATGCACACATATACAGATACACACAAAATACTCATCCATATGTACACACACAGAGACTCACATACACACACATAAACACCC
Tacrl_R244C : AAACATTCATACACACGTATATATACACACAAAACCCACTCATACACACACATATACTCATACACATATGCACACATATACAGATACACACAAAATACTCATCCATATGTACACACACAGAGACTCACATACACACACATAAACACCC

*           920
Tacrl_WT   : ATACACGTGTTACACATACCC
Tacrl_R244C : ATACACGTGTTACACATACCC

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Predicted Protein Alignment:

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*           20           *           40           *
Tacrl_WT   : YHICVTVLIYFLPLLVIYAYTVVVGITLWASEIPGDSSDRYHEQVSAKRR
Tacrl_R244C : YHICVTVLIYFLPLLVIYAYTVVVGITLWASEIPGDSSDRYHEQVSAKCK

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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')	GTATCCCTTCAAATGTAAGCCA
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	GCTTGGGAGAGACATGCTCA 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	
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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')	GCCCCAGCAGCACCATT
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

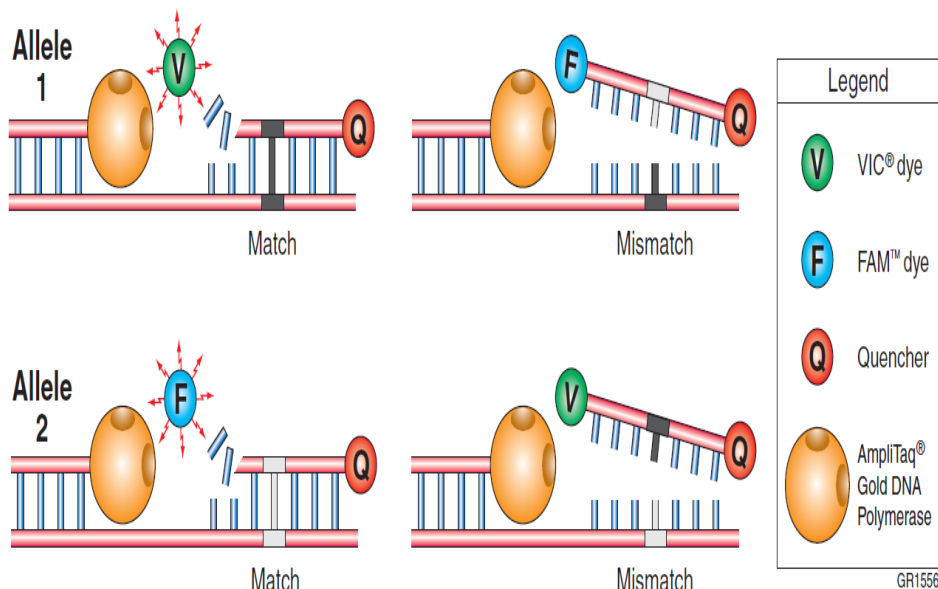
Introduction

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

ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCC**CGGTGACTCCTCTGACCGTT**ACCATGAGC
AAGTCTC**TGCCAAGCGCAAGG**TGAGCAAAGCCAAACGGGCATAGCTTGCCTGGCACTGACAGTG

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

ACTGTGGTAGGGATTACACTGTGGGCCAGTGAGATCCC**CGGTGACTCCTCTGACCGTT**ATCATGAGC
AAGTCT**CTGCCAAGTGCAAGGT**GAGCAAAGCCAAACGGGCATAGCTTGCCTGGCACTGACAGTG

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

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 5-color, 96-well system.

What type of experiment do you want to set up?

Melt Curve

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?

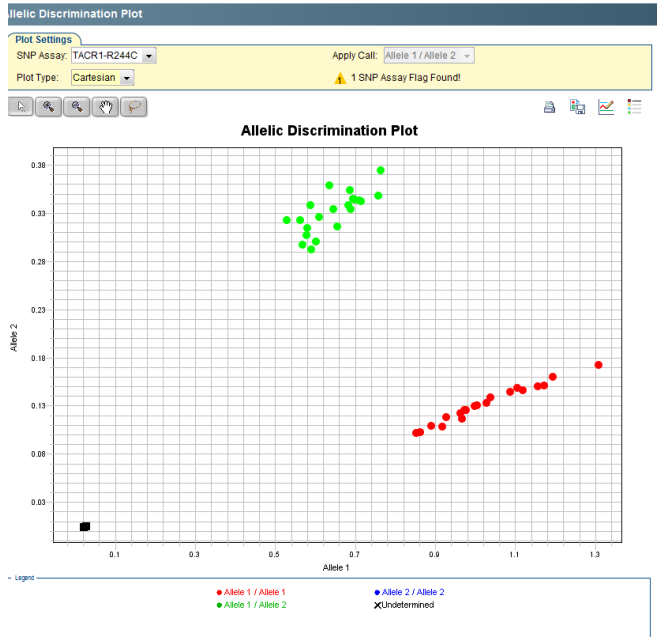
PCR reactions contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.

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 (282399)



	1	2	3	4	5	6	7	8	9	10	11	12
A	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C	TACR1-R244C-EM1-B0V... TACR1-R244C			
B	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C
C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C	TACR1-R244C-EM2-B0V... TACR1-R244C
D	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C	TACR1-R244C-EM3-B0V... TACR1-R244C

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