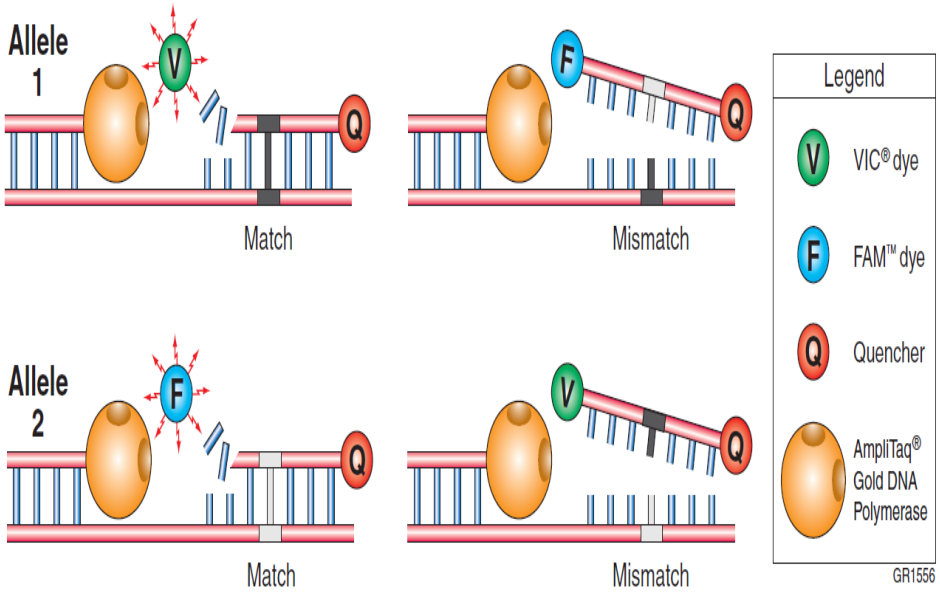
ATP1A3-N879T Genotyping Strategy

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

Mutation type:	SNP
Mutant allele:	ATCCGGCT**G**A**C**CTGGG
WT allele:	CATCCGGCT**C**A**A**CTG

Fragment sequence

ATP1A3-N879T WT

TGATCCAGGCCCTCGGTGGTTTCTTCTCCTACTTTGTCATCCTGGCAGAAAATGGCTTCTTGCCCGGA
AACCTGGTGGGCATCCGGCTCAACTGGGATGATCGCACTGTCAATGACCTAGAAGACAGTTATGGGCA
GCAGTGGGTGAGTTGGCACTGGGCATTGAGCCTCAGGAGCAGACCAACATCTACCTGCAGTCCCATGC

ATP1A3-N879T MUT

TGATCCAGGCCCTCGGTGGTTTCTTCTCCTACTTTGTCATCCTGGCAGAAAATGGCTTCTTGCCCGGA
AACCTGGTGGGCATCCGGCTGACCTGGGATGATCGCACTGTCAATGACCTAGAAGACAGTTATGGGCA
GCAGTGGGTGAGTTGGCACTGGGCATTGAGCCTCAGGAGCAGACCAACATCTACCTGCAGTCCCATGC

Assay ATP1A3-N879T

Primers and Probes

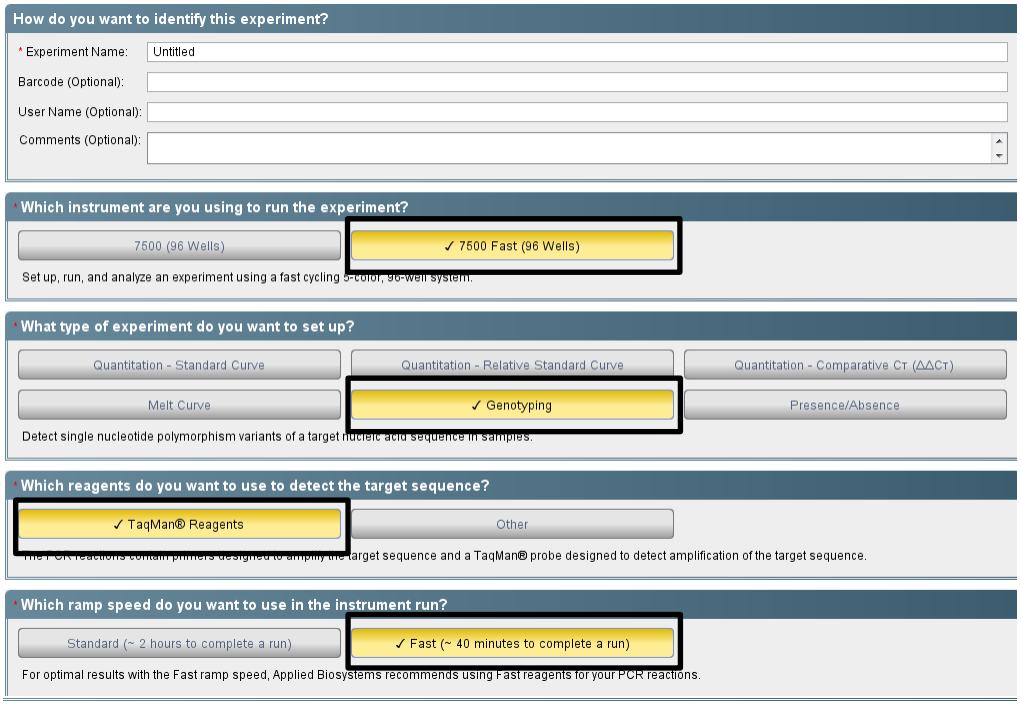
Primer 1	GCTTCTTGCCCGGAAACCT
Primer 2	TGCTGCCCATAACTGTCTTC
Allele 1 (WT) probe (FAM-Labelled)	CATCCGGCTCAACTG
Allele 2 (Mut) probe (TET-Labelled)	ATCCGGCTGACCTGGG

qPCR master mix

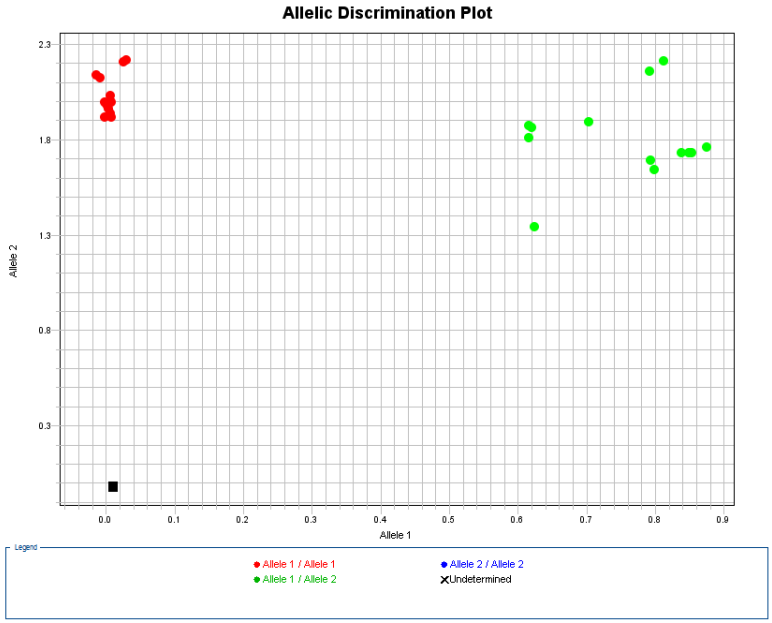
ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl (of 1 in 5 dilution of stock)
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

No need to run the samples in duplicates.
Allele 1 = WT on 7500 FAM-labelled. Allele 2 = MUT 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 ATP1A3-N879T your samples accordingly.

Version No.	1
Date:	03/02/2020
Created/Updated by:	Daniel Ford
Approved by:	Debbie Williams

Name of Mouse model or mutation:
ATP1A3-N879T-EM3-B6N
ATP1A3-N879T-EM4-B6N

Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: N879T

Delivery method:
Cytoplasmic injection into 1-cell stage embryo.

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
TGGTGGGCATCCGGCTCAAC	TGG

ssODN sequence (5'-3'):
gtagatgttggtctgctcctgaggctcaatgcccagtgccaactcacCCACTGCTGCCCATAACTGTCTTCTAGGTCATTGACAG
TGCGATCATCCCAGgTcAGCCGGATGCCCACCAGGTTTCCGGGCAAGAAGCCATTTTCTGCCAGGATGACAAA
GTAGGAGAAGAAACCACCGAGGGCCTGGATCATCCctggggg

Microinjection mixes:
Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 µm filter and autoclaved. Cas9 mRNA, sgRNAs and ssODNs were diluted and mixed in MIB to the working concentrations of 50 ng/µl, 6.25 ng/µl each and 50 ng/µl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.