

## ATP1A3-N879T Genotyping Strategy

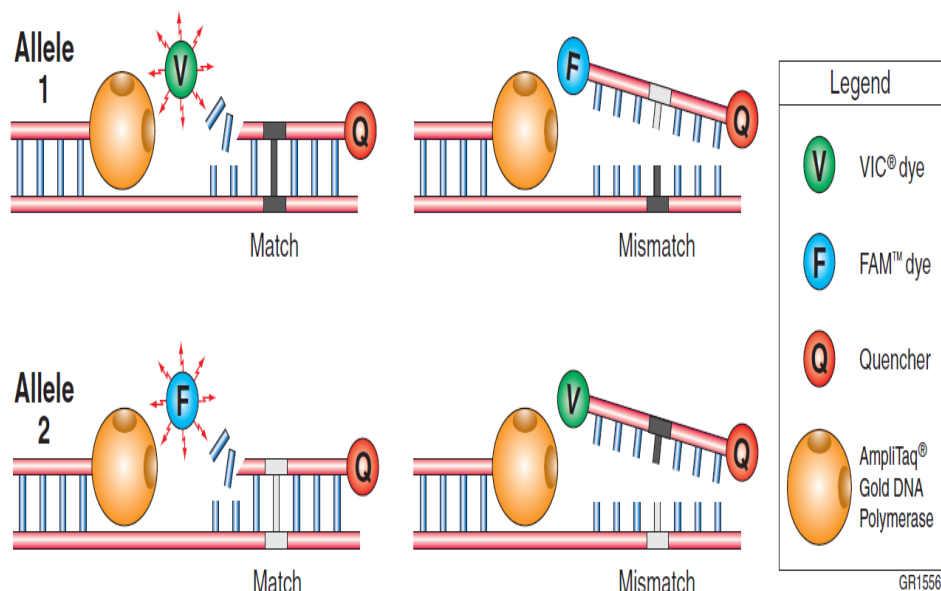
### Introduction

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](http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042114.pdf)

### Assay set up

Mutation type: SNP  
 Mutant allele: ATCCGGCTGACCTGGG  
 WT allele: CATCCGGCTCAACTG



## Fragment sequence

### ATP1A3-N879T WT

TGATCCAGGCCCTCGGTGGTTTCTTCTCCTACTTTGTCATCCTGGCAGAAAATGGCTTCTTGCCCCGGA  
AACCTGGTGGG **CATCCGGCTCAACTG** GGATGATCGCACTGTCAATGACCTA **GAAGACAGTTATGGGCA**  
GCA GTGGGTGAGTTGGCACTGGGCATTGAGCCTCAGGAGCAGACCAACATCTACCTGCAGTCCCATGC

### ATP1A3-N879T MUT

TGATCCAGGCCCTCGGTGGTTTCTTCTCCTACTTTGTCATCCTGGCAGAAAATGGCTTCTTGCCCCGGA  
AACCTGGTGGG **ATCCGGCTGACCTGGG** ATGATCGCACTGTCAATGACCTA **GAAGACAGTTATGGGCA**  
GCA GTGGGTGAGTTGGCACTGGGCATTGAGCCTCAGGAGCAGACCAACATCTACCTGCAGTCCCATGC

## Assay ATP1A3-N879T

### Primers and Probes

Primer 1

GCTTCTTGCCCCGAAACCT

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

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 3-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?

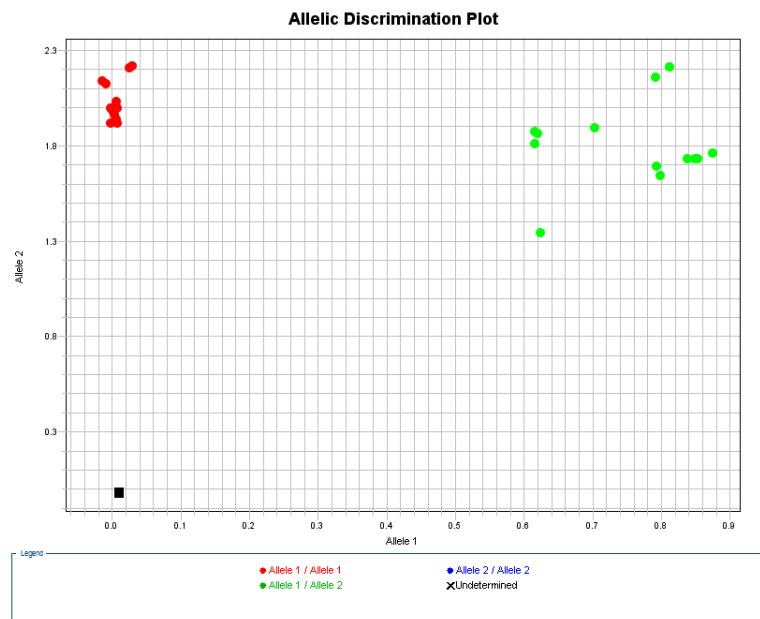
The PCR reactions contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.

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



**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'):**

gtagatgttggtctgctcctgaggctcaatgccagtgccaactcacCCACTGCTGCCATAACTGTCTTCTAGGTCATTGACAG  
TGCGATCATCCCAGgTcAGCCGGATGCCACCAGGTTTCCGGGCAAGAAGCCATTTCTGCCAGGATGACAAA  
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<sub>0</sub> progeny.