



## ATP1A3-N879T Genotyping Strategy

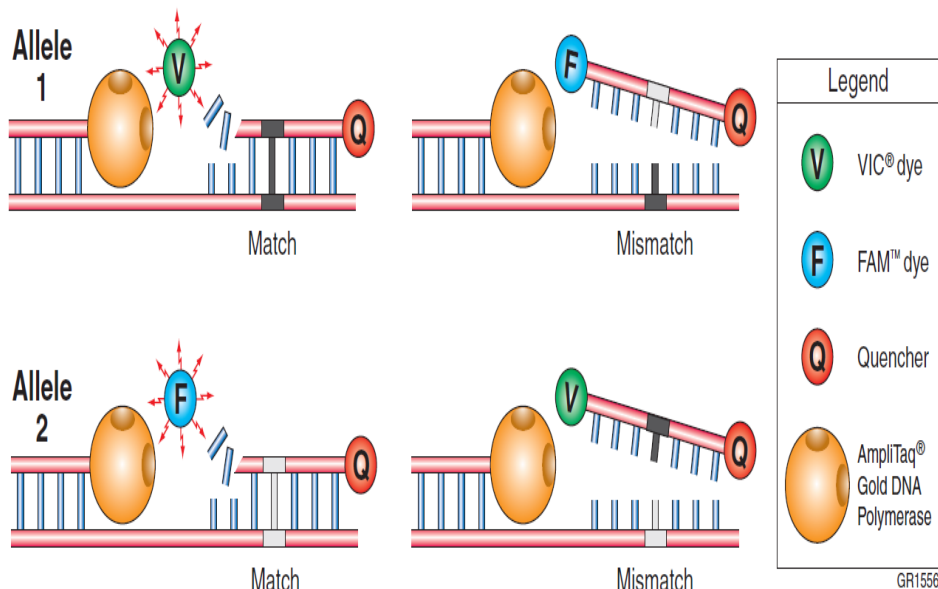
### Introduction

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

**TGCTGCCATAACTGTCTTC**

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):

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Which instrument are you using to run the experiment?

Set up, run, and analyze an experiment using a fast cycling 96-color, 96-well system.

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What type of experiment do you want to set up?

Detect single nucleotide polymorphism variants of a target nucleic acid sequence in samples.

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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.

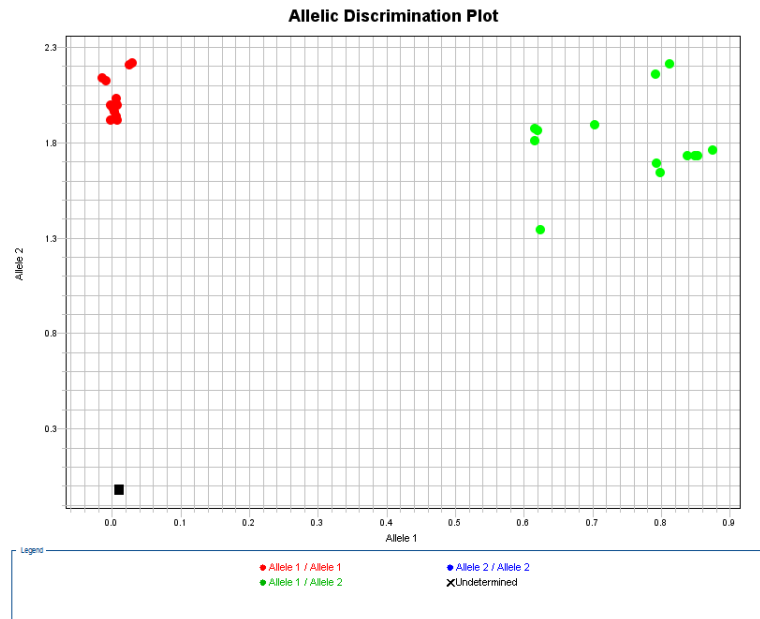
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Which ramp speed do you want to use in the instrument run?

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

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Created/Updated by: Daniel Ford

Approved by: Debbie Williams