



UMODC2 Genotyping Strategy

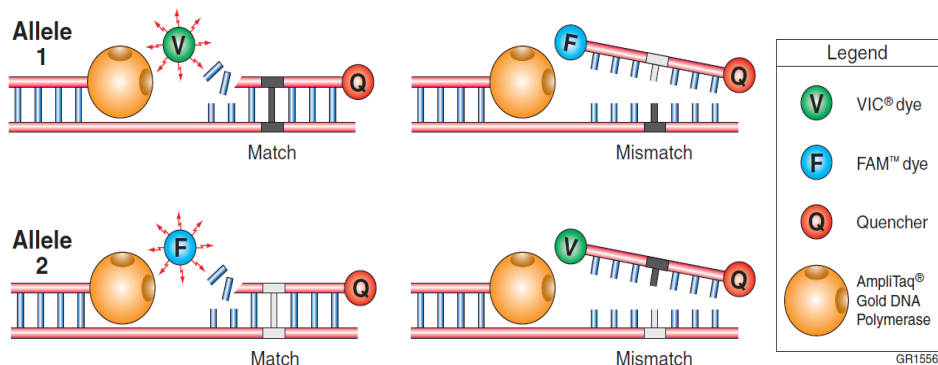
Introduction

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 (allele 1) and the other matching the Mutant (allele 2)

The Analysis software produces 3 genotypes:

- Homozygotes (samples having only allele 1)
- Homozygotes (samples having only allele 2)
- Heterozygotes (samples having both allele 1 and allele 2)



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. Samples are prepared using ABI Sample-to-SNP crude lysis reagents.

To retrieve sample IDs and well locations - log into Anonymus.

<https://anonymus.har.mrc.ac.uk/anonymus/core/Login>



UMODC2 sequence

CTCCTGTCAGGATGGTTTTTCGTCTGACGCCTGAGCTGAGCTGCAC**TGATGTGGATGAGTGCTCAGAG**
 CAGGGGCTCAGTAAGTGCATGCCCTGG**CCACCTGTGTCAACAC**AGAAGGCCG**ACTACTTGTGCGTGT**
GTCCCGAGGGCTTTACAGGGGATGGTTGGTACTGTGAGTGCTCCCAGGCTCCTGTGAGCCAGGAC
 TGG

Primers and Probes

Forward	TGATGTGGATGAGTGCTCAGA
Reverse	GGGACACACGCACAAGTAGT
Mut Probe Rev (TET-Labelled)	CCACCCGAGTCAACA
WT Probe Rev (FAM-Labelled)	CCACCTGTGTCAACAC

qPCR master mix

ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl
Water	0.5µl
ALIQUOT	7.5µL
DNA	2.5µl

(1/10 dilution of ABI Sample-to-SNP prep)

No need to run the samples in duplicates.

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?

7500 (96 Wells) 7500 Fast (96 Wells)

Set up, run, and analyze an experiment using a fast cycling 8-color, 96-well system.

What type of experiment do you want to set up?

Quantitation - Standard Curve Quantitation - Relative Standard Curve Quantitation - Comparative Ct ($\Delta\Delta C_t$)

Melt Curve Genotyping Presence/Absence

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?

TaqMan® Reagents Other

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

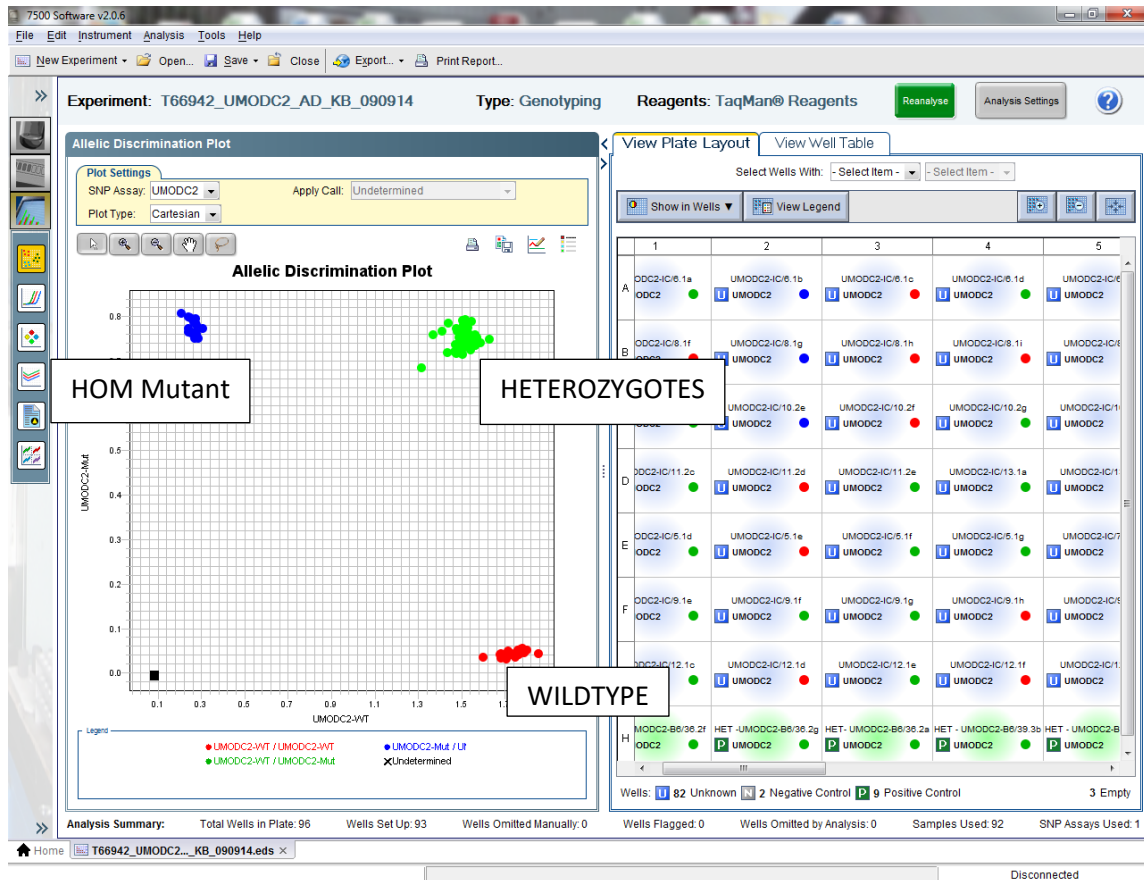
Which ramp speed do you want to use in the instrument run?

Standard (~ 2 hours to complete a run) Fast (~ 40 minutes to complete a 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 that the WT and MUT results may vary in the actual plot and the HETs always in the middle. Samples usually group automatically based upon the location of the controls. If necessary, you can manually use your controls to group and name them accordingly.

Alternative assay

The allelic discrimination assay should be the first choice.

If it is not possible to use this one, an alternative assay (allele-specific PCR) is available – please see other protocol.