ACAN-MPC227-A1946V-AD Genotyping Strategy

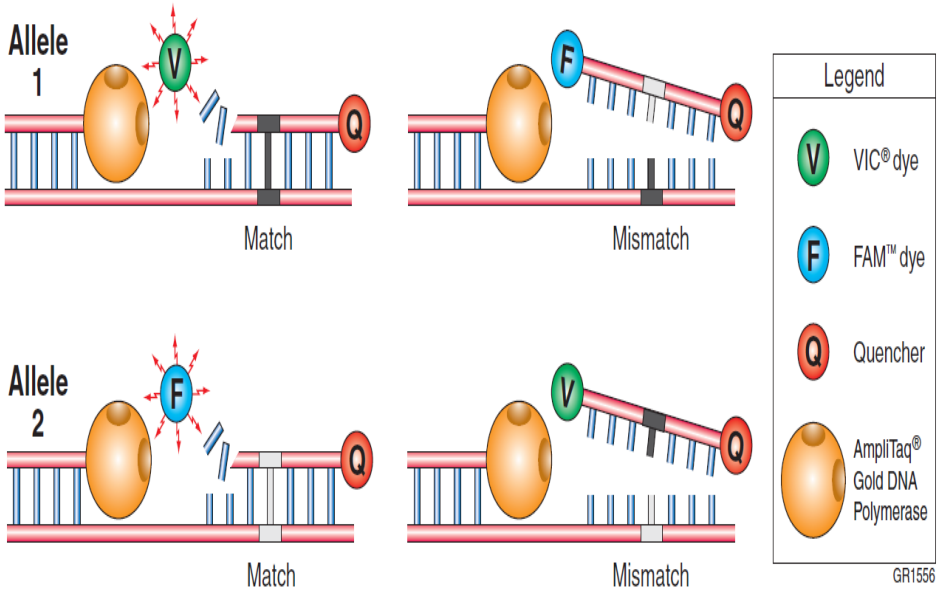
Introduction

An Allelic Discrimination assay can be used to detect two possible variants of a Single Nucleotide Polymorphism (SNP) or small Indels. 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 allele)
- Heterozygotes (samples having both WT and Mutant alleles)



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

Group:	Diabetes
Mutation type:	SNP
Mutant allele:	T
WT allele:	C
Assay Direction:	Forward

Fragment sequences

WT Sequence:

CACTCCCAACCGGCTTTGCCCCCTGTTCAACTTCCTGACCTGTGTTACAGACCAGGAGCAATGTGAGGAGGGGTG
GACTAAGTTCCAGGGTCACTGTTACCGCCACTTTCATGACCGAGAGACCTGGGTGGATGCGGAGAGACGGTGTCG
GGAGCAGCAGTCACATCTGAGCAGCATTGTCACTCCTGAGGAACAGGAGTTCGTCAACAGTGAGTGTGGGCAGGG
GCTGAGGGACAGGACACAGCAGTGAGAGAAAAGA

ACAN-MPC227-A1946V Sequence:

CACTCCCAACCGGCTTTGCCCCCTGTTCAACTTCCTGACCTGTGTTACAGACCAGGAGCAATGTGAGGAGGGGTG
GACTAAGTTCCAGGGTCACTGTTACCGCCACTTTCATGACCGAGAGACCTGGGTGGATGTGGAGAGACGGTGTCG
GGAGCAGCAGTCACATCTGAGCAGCATTGTCACTCCTGAGGAACAGGAGTTCGTCAACAGTGAGTGTGGGCAGGG
GCTGAGGGACAGGACACAGCAGTGAGAGAAAAGA

Primers and Probes

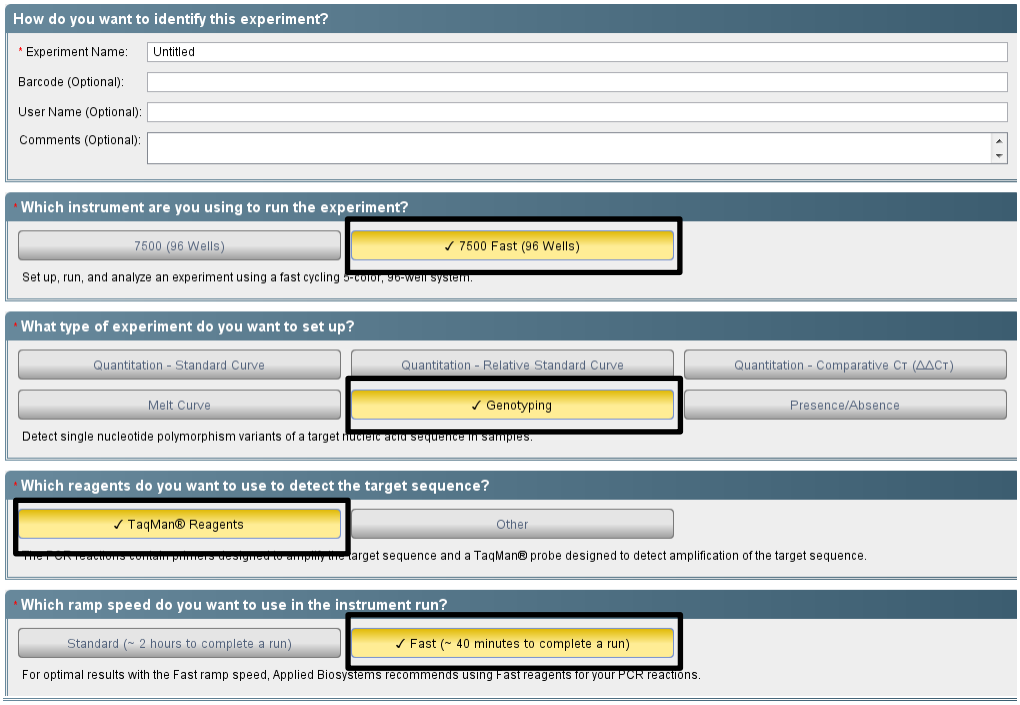
Forward Primer	GCCACTTTCATGACCGAGAGA
Reverse Primer	GGAGTGACAATGCTGCTCAGAT
Allele 1 (WT) probe (FAM-Labelled)	TGGGTGGATGCGGAGA
Allele 2 (Mut) probe (TET-Labelled)	CTGGGTGGATGTGGAGAGA

qPCR master mix

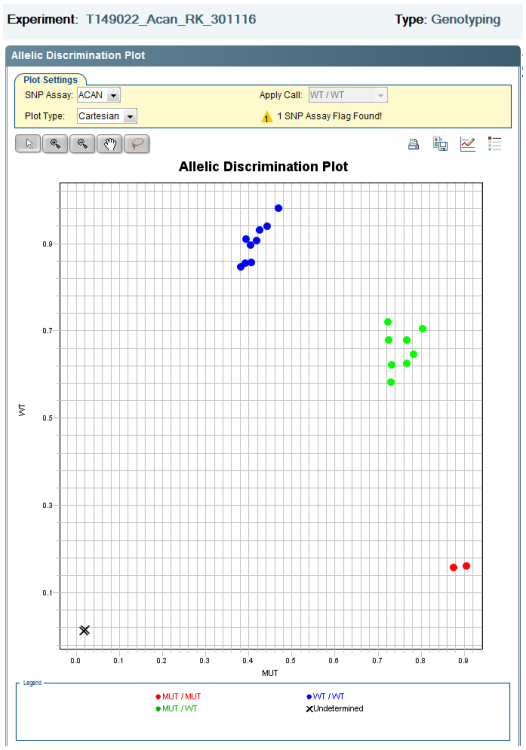
ABI GTX Taqman master mix	5µl
Assay (Probes 5µM each & Primers 15µM each) 20uM	2µl (of 1 in 5 dilution of stock)
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

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



From T149022

Please note, use your controls to group and name your samples accordingly.