

ZNRF3-R749G-EM5-B6 Genotyping Strategy

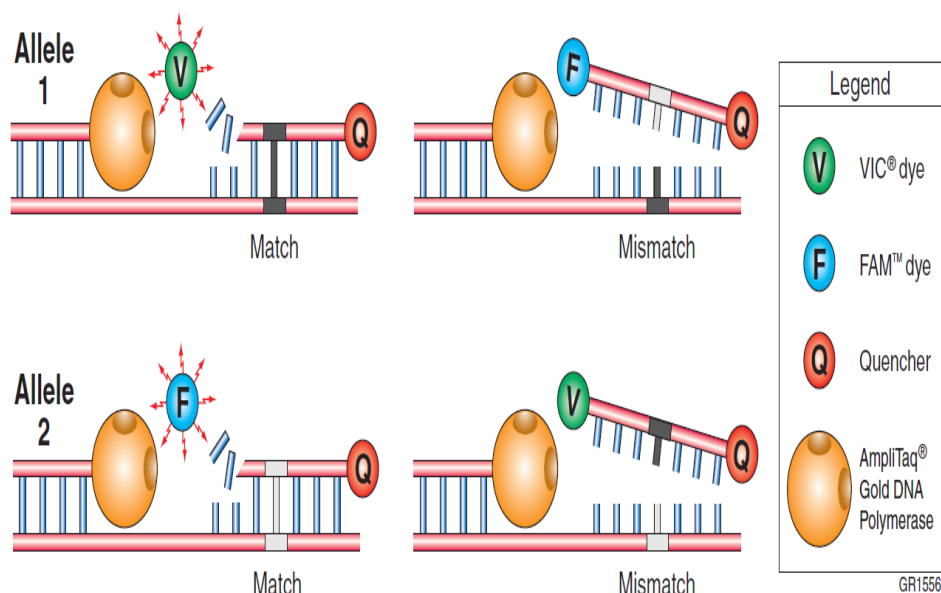
Introduction

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 allele 1 or 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.

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

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



Group: Sex determination - Andy Greenfield/Abigail Harris

Mutation type: SNP
Mutant allele: G
WT allele: A
Assay Direction: Forward

Fragment sequence

WT sequence:

ACCTCAGGAGGACCTGGAAAGGGGGTCCAGAGGGACCATCATGTGCTTGCTGCTTCGAGCCCCAGC
CCTTCCCGCCGGGCTCTGGGATAGAAACAAGTGCAGGTGGCAGCAGTTTATTCTGGGTCCTCGGCT
CCTCGAGGACTGCAACCTCCAAGTGGGGAGCCACAGCTGGGGAGCTCC **CAGGGCCTGTATGGTCT**
TCACTCAGACCATTATCCTAGGACAGATGGGGTGAAATATGAGGGCTTGCCCTGCTGCTTCTATGAA
GAGAAGCAGGTGGCCCACAGTGCTGGCAGGGGCAATGGCTGCTACACTGAGGACTATTCACTGAG
TGTACAGTACACACTCACCGAGGAACCCCCACCCAGCTGTTATGCAGGGCCCCGGGACCTGAGCCA
GCGCATCCCCATTA

Allele 1 = Legitimate repair

ACCTCAGGAGGACCTGGAAAGGGGGTCCAGAGGGACCATCATGTGCTTGCTGCTTCGAGCCCCAGC
CCTTCCCGCCGGGCTCTGGGATAGAAACAAGTGCAGGTGGCAGCAGTTTATTCTGGGTCCTCGGCT
CCTCGAGGACTGCAACCTCCAAGTGGGGAGCCACAGCTGGGGAGCTCC **CAGGGCCTGTATGGTCT**
TCACTCAGACCATTATCCTAGGACAGATGGGGTGAAATATGAGGGCTTGCCCTGCTGCTTCTATGAA
GAGAAGCAGGTGGCCCACAGTGCTGGCAGGGGCAATGGCTGCTACACTGAGGACTATTCACTGAG
TGTACAGTACACACTCACCGAGGAACCCCCACCCAGCTGTTATGCAGGGCCCCGGGACCTGAGCCA
GCGCATCCCCATTA

Primers and Probes

Forward Primer CAGGGCCTGTATGGTCTTCACT
Reverse Primer GGCCACCTGCTTCTTCA
Allele 1 (WT) probe (FAM-Labelled) AGACCATTATCCTAGGACAGA
Allele 2 (Mut) probe (TET-Labelled) CCATTATCCTGGGACAGA

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 5-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?

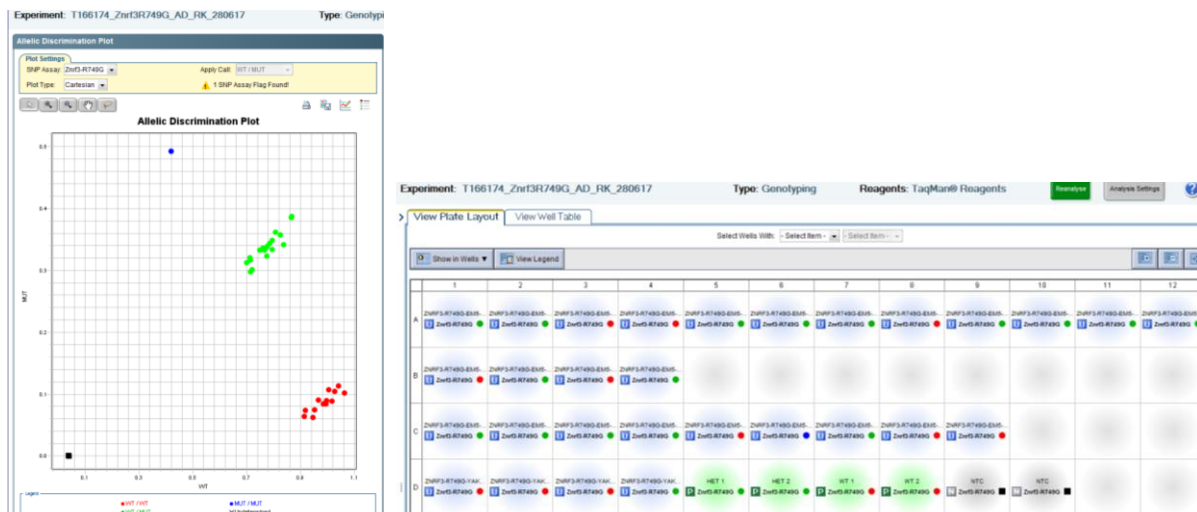
Inter-ON reagents contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.

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

For optimal results with the Fast ramp speed, Applied Biosystems recommends using FastReagents for your PCR reactions.

Example of an Allelic Discrimination Plot and Results

Task 166174 Results



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