Non-gel based SNP-detection using Varioskan and the Invader® Technology

This application note describes the use of Thermo Electron's Varioskan spectrofluoro- and spectrofotometer as a detection platform for the fluorescence based INVADER SNP detection technique (Third Wave Technologies) .

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Abstract

Single-nucleotide polymorphism (SNPs) are the most frequently found polymorphisms in genomes and have been promoted as the genetic markers of choice for the research of complex genetic features (1).

There are several systems for detecting SNP's. Most are PCR-based and usually need gel-electrophoreses. The Invader technology is a good alternative as it is a non-PCR and non-gel based detection system.

The Invader technology uses a Flap Endonuclease for allele discrimination and a universal fluorescence resonance energy transfer (FRET) reporter system. This means that Invader is based on signal rather than product amplification (2, 3). FRET reporter system in the Invader technology uses individual FRET pair for each allele. Each FRET pair is formed by a fluorescent label and label specific fluorescence quencher. Each Invader probe is allele-specific and the combination of results gives the genotype of the sample. For more information about Invader technology, see http://www.twt.com/.

In this application note the Invader assay products (unknown and control samples) were measured by Thermo Varioskan. By using monochromators in stead of filters, Varioskan makes it possible to use a variety of different FRET labels and even multiplex them. In this experiment, a biplex format with two FRET pairs was used, FAM and RED both connected to their specific quenchers.

Experimental

Reagents and materials

- INVADER® Assay FRET Detection Plate 96 Well format Cleavase XI, Third Wave Technologies, Cat No.: 91217 – 96 well PCR plate coated with Cleavase XI and FAM and RED labeled FRET cassettes
- 52mM MgCl2, Third Wave Technologies
- SNP-specific Probe mix (allele 1 and allele 2), specially designed by Third Wave Technologies.
- Tomato DNA >5 ng/µl
- No Target Control (NTC), Third Wave Technologies
- Synthetic controls for Allele1 and Allele 2, Third Wave Technologies
- Mineral Oil, Sigma M-5904

Preparation of reaction-mix

Component	µl/reaction
$MgCL_2$	3.5
ProbeMix (allele1 and allele 2)	3
H2O	0.5
Total	8

Assay procedure

Denaturate the Tomato DNA samples by heating them for 10 minutes at 96°C. Pipet 7 µl of the denaturated samples in the Invader plate. 4 wells are left open for controls (2x 7µl NTC, 1x 7 µl Allele1 and 1x 7µl Allele2 controls). Add 8 µl of the reaction mix to each sample. Overlay each well with 15 µl of mineral oil and incubate at 63°C for 4 hours.

SkanIt protocol

Make plate layout with 96 well plate containing 96 unknowns.
The SkanIt protocol has 1 step:
Fluorescence reading: Multilabelling:
1. For FAM label: Excitation at 485 nm. Emission at 535 nm.
2. For RED label: Excitation at 560 nm. Emission at 620 nm.

After measurement, the raw data is exported for analysis to Invader Analyser software (Third Wave Technologies).

Results and Discussion

The measurement unit in Invader Analyser software is Net FOZ, which is calculated with the following formula:

$$\text{NetFOZ} = \frac{\text{Sample (Rawsignal)} - \text{NTC*(Rawsignal)}}{\text{NTC*(Rawsignal)}}$$

* NTC; No Target Control

These values are put in a graph, where the FAM (Allele 1) values has been put on the Y-axis and RED (Allele 2) has been put on the X-axis. Typically you get 3 different clusters, representing the three possible genotypes (FF, FR, RR). In Invader analyzer, dots can be selected after which an allele call can be chosen.

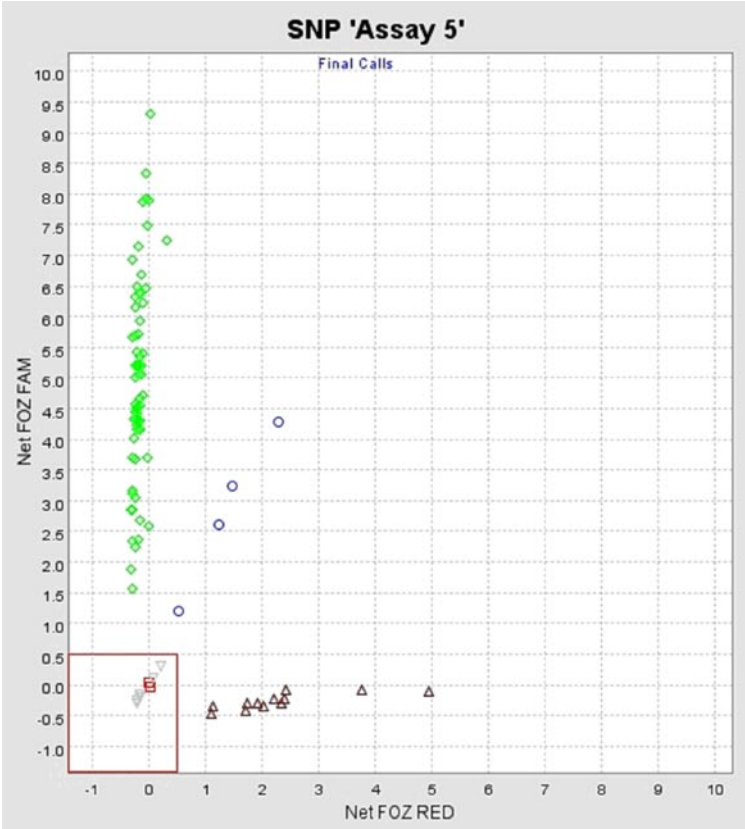

Figure 1: Graph in Invader analyser. Each dot in the graph represents 1 well in the plate. The small red boxes, left below are the NTC's. The gray dots don't have a significant signal over the background. The green dots are scored homozygous Allele 1, the red ones are homozygous allele 2 and the blue dots are hetrozygous.

References

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