



Method For SNP Genotyping And Transgene Detection Using Third Wave Technologies' Invader Plus® Reagents

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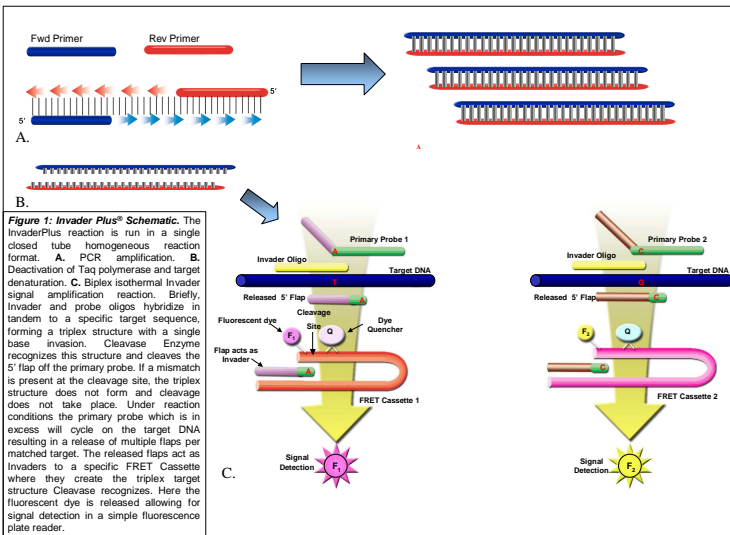
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Introduction

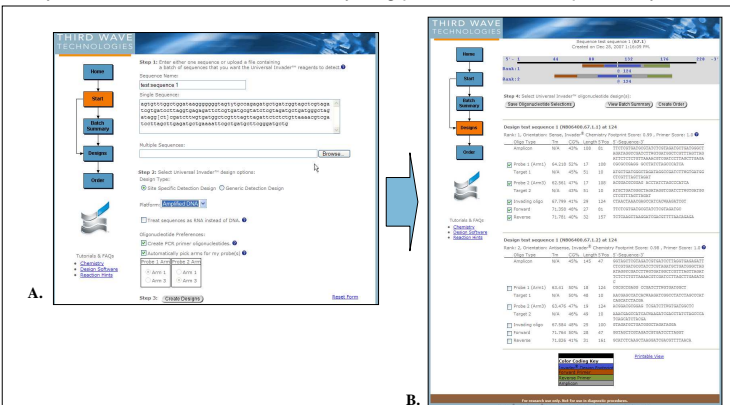
The Invader assay is a homogeneous isothermal signal amplification reaction. It utilizes a biplexed FRET based detection platform, allowing for the simultaneous detection of two target sequences; for instance two SNP alleles, or a transgene and an internal reference sequence. Recent advances in the technology have allowed for combining the Invader Assay with limited cycle PCR in a single closed tube homogeneous format, called Invader Plus (See illustration in figure 1).

In this poster we present data generated using the Invader Plus platform for SNP genotyping as well as transgene detection. Assay oligonucleotides were designed using Third Wave's proprietary design software. This easy to use software generated assay oligonucleotide designs based on input sequence and ranked them according to a "best design" score. The oligonucleotides were then combined with standard assay reagents to assemble the final Invader Plus reactions. In our research, we found that combining the sensitivity of PCR amplification with the specificity of the Invader chemistry, in a single reaction, permitted the use of lower concentration and lower quality input sample DNA than either limited cycle PCR or Invader alone. It also achieved greater sensitivity in transgene detection using pooled samples. The Invader Plus assay is amenable to both 96 and 384 well microtiter plate formats. Only a thermal cycler and a standard fluorescence plate reader were required, thus eliminating the need for expensive real-time PCR equipment.



Method

Assays were designed using the Invader Plus assay design software as outlined in figure 2. Assays were run under standard reaction conditions for oligo, enzyme and buffer concentrations. Cycling parameters varied per assay.



Results

An Invader Plus assay was designed for detection of a single nucleotide polymorphism (SNP) in canola gDNA. To demonstrate the effect of PCR cycling conditions on total Invader signal generation, three samples (homozygous (WT), heterozygous, and homozygous (Mutant)) were amplified for 14, 17, and 20 cycles

in a 2-step protocol followed by a heat denaturation and a 20 minute Invader signal amplification step. All steps were carried out in one homogeneous reaction. All three cycling conditions were suitable for genotype calling, however an increase of signal was observed at higher cycle numbers (see figure 3A). Then a larger sample set was tested at the 20 cycle condition. Clear genotype clusters were observed when the data was graphed in a scatter plot (see figure 3B).

Another Invader Plus assay was used for allele detection in DNA isolated from pooled ground corn seed samples. Seed pools were constructed to contain either 100% WT or Mutant DNA, or 0.67% (1 in 150 kernels) of either WT or Mutant DNA. As can be seen in figure 4 both wild-type and mutant alleles can be detected at a level of 0.67%. No cross-reactivity of wild-type signal on the 100% mutant sample or mutant signal on the 100% wild-type sample was observed.

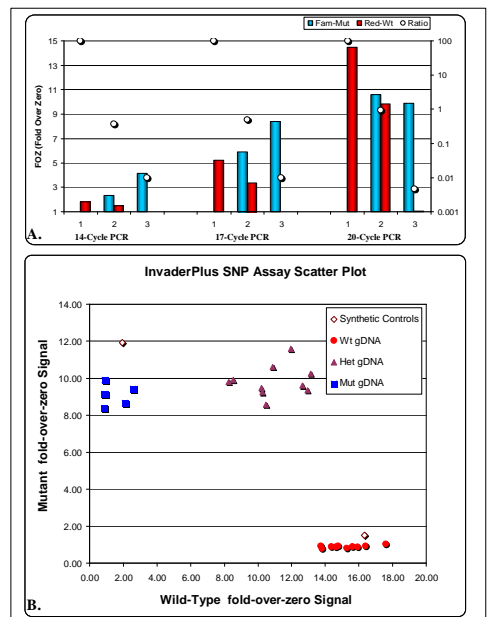


Figure 3: Results of Canola SNP InvaderPlus Assay. A. Bar graph illustrating results of different cycling conditions. Bars represent Wild-type (Red) or Mutant (Blue) signal. Genotype calls are made by ratio of WT signal over Mutant signal (white circles). A ratio between 0.33 and 3.0 indicates a heterozygous result. A ratio ≥ 5 indicates a homozygous wild-type result, and a ratio ≤ 0.2 indicates a homozygous mutant result. B. Scatter plot of Wild-type vs. Mutant signal. Three distinct genotype clusters are observed.

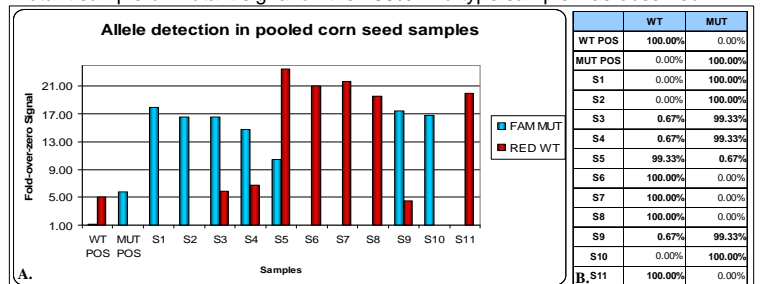


Figure 4: Allele Detection in Pooled Corn Seed Samples. A. Bars represent Wild-type (Red) and Mutant (Blue) signal. Alleles are detected at 0.67% presence in pool, while no cross-reactivity is observed in 0% presence samples for either allele. B. Composition of the pools tested.

Conclusions:

The results presented in this poster demonstrate the versatility of the Invader Plus reaction format. The benefits of the Invader Plus technology include:

- 1) the combined sensitivity of PCR with the specificity of Invader technology;
- 2) the closed tube homogeneous reaction format allowing for a single step setup and reduced risk of amplicon contamination; and
- 3) the elimination of expensive real-time detection equipment.

This new format and available software at www.universalinvader.com gives researchers a convenient way to design a sequence specific test for their own gene of interest. In addition to the DNA target specific assays presented in this poster, the Invader Plus format can also be used for mRNA and microRNA detection.

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