# Contaminant Detection in Soybean Using Allele-specific Digital PCR on the TaqMan® OpenArray® Platform

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## Abstract

Contamination of seed lots by unexpected varieties is a problem in quality assurance of certified seeds. Identification and elimination of contaminated seed lots are essential to maintain the quality of seed stocks. Cost efficient and high-throughput methods for identifying low contamination level are needed.

We are presenting a feasibility study for contaminant detection in soybean using digital PCR on the TaqMan® OpenArray® platform. Digital PCR is a sensitive approach to detect rare alleles and allows absolute quantification without using endogenous controls. Combining digital PCR with the TaqMan® OpenArray® system enables high-throughput contaminant screening.

Six SNPs were selected to distinguish soybean variety A from the contaminant variety B. Allele specific Custom TaqMan® Assays were designed for the OpenArray® digital PCR. A spike-in experiment was set up mixing soybean variety A DNA with contaminant variety B DNA at a 10,000:1 ratio to mimic 0.01% contamination in the seed lot. Digital PCR was performed on the OpenArray® PCR instrument using real-time signal acquisition followed by data analysis for quantification of detected copies/µl of samples.

Specificity of the assays in discriminating contaminant in a background of high concentration of variety A DNA was tested. All contaminant-specific assays produced no false-positive results in variety A DNA background and achieved the targeted sensitivity of detecting 0.01% contamination.

This methodology combining digital PCR with the high-throughput OpenArray® platform is applicable to any type of rare allele detection like contaminant detection or identifying genetically modified plant seeds in a pool of wild-type seeds.

## The Problem: Contamination

This study aims at detecting contaminant variety B in a population of variety A in the soybean DNA. Targeted sensitivity level of detection is 1:10,000. We mimicked this ratio by spiking contaminant variety B gDNA into variety A gDNA background.

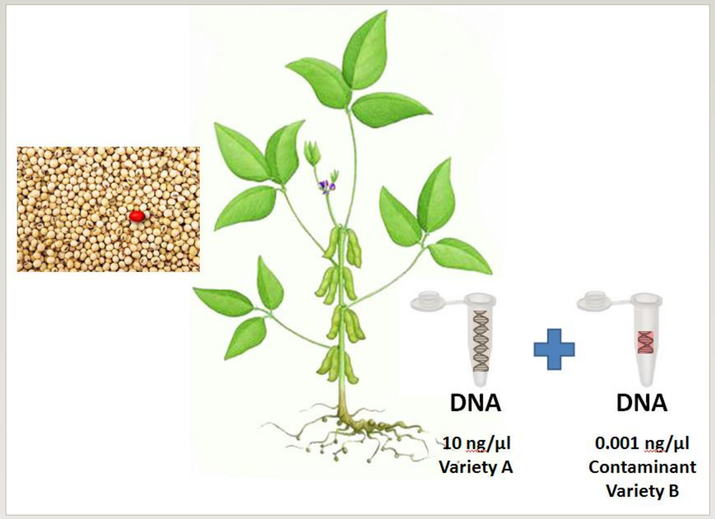


**Figure 1. Simulating seed contamination with a spike–in experiment, adding small amounts of contaminant DNA**

## The Experiment: Digital PCR

Custom TaqMan® assays for detection of variety A and contaminant variety B were designed and validated using gDNA. Contaminant variety B was spiked into variety A at a 1:5,000 and 1:10,000 ratio. We used TaqMan® OpenArray® Digital PCR Plates for detection of contaminant variety B.

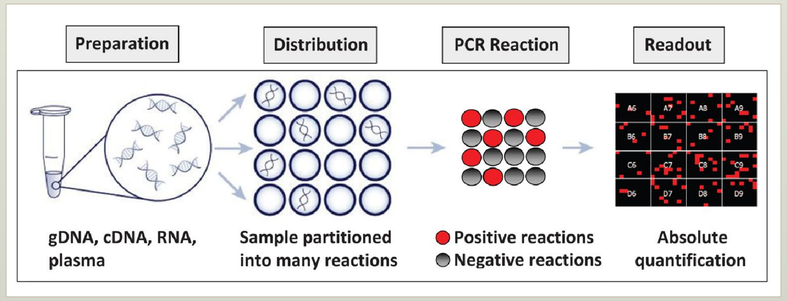


**Figure 2. Digital PCR workflow. Samples are partitioned into individual through-holes at a concentration of 1 copy/µl or lower.**

## The Platform: The OpenArray®

The OpenArray® platform offers high-throughput flexibility for different genomics application including digital PCR.

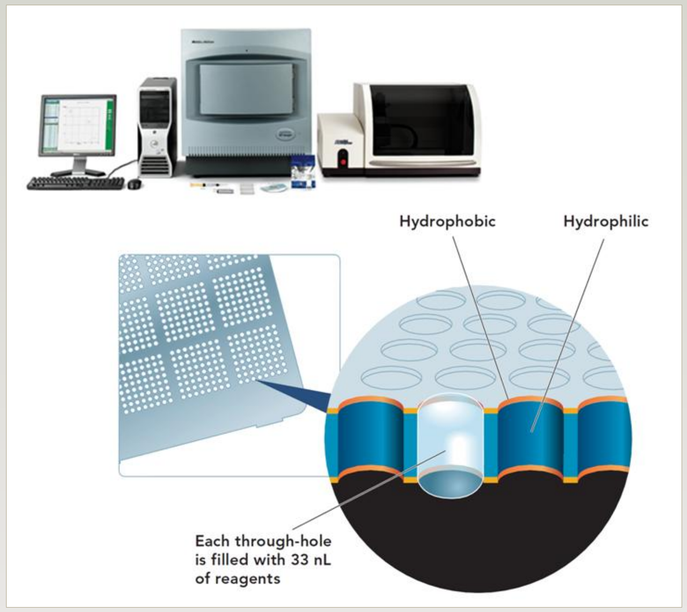


**Figure 3. OpenArray® instrument and detail of the OpenArray® plate showing one of 3,072 through-holes**

## The Result: Sensitive Detection

The experiment was run using TaqMan® OpenArray® Digital PCR Plates. The OpenArray Digital PCR software was used for data analysis. The amplification heat map (below) shows through-holes with detected (red) or undetected (black) target amplification. The DNA copy number was calculated using Poisson analysis by counting the number of wells with detected amplification.

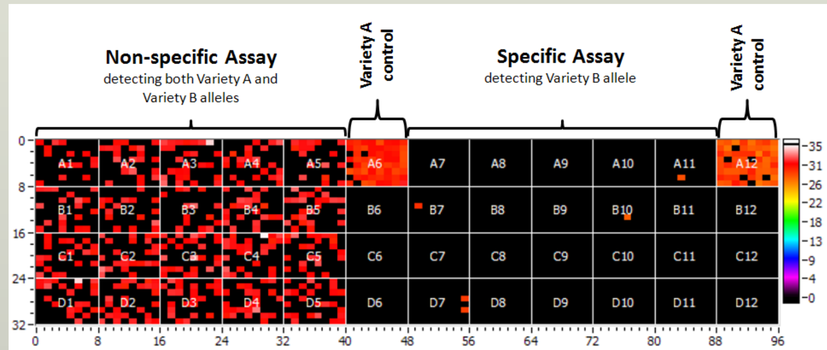


**Figure 4. Heat Map showing a non-specific assay (left) amplifying both variety A and variety B alleles and a specific assay (right) amplifying its target variety B only. Sensitivity level: 1:10,000.**

## Summary

- We designed and validated 6 allele specific TaqMan® assays for SNPs that distinguish our strains of interest.
- Using digital PCR, we achieved the desired detection sensitivity of 1:10,000 contaminant variety B in variety A soybean seed DNA background.
- The digital PCR can be used for variety of AgBio applications including GMO testing.

## References:

*Vogelstein B., Kinzler, KW. Digital PCR, PNAS, 1999, v.96, 9236-9241*

*MorrisonT , et al. Nanoliter high-throughout quantitative PCR. Nucleic Acids Res., 2006, v.34, e123*