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Digital PCR on TaqMan® OpenArray® Digital PCR Platform as a Sensitive Tool for Detection of Genetically-Modified Organisms

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Scientific Collaborations

Learn more: www.lifetechnologies.com/agbio

Background on GMO detection

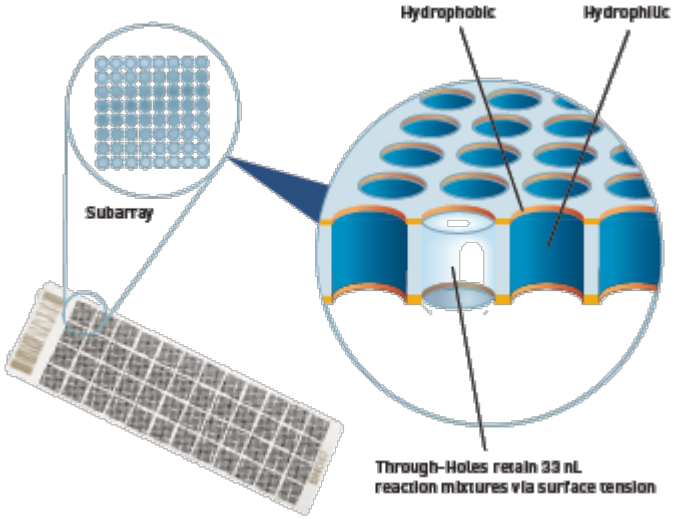
- The presence of genetically-modified seeds in the global seed supply market is regulated in different countries. Europe has the most restrictive rules while a higher percentage of GMO in food is allowed in South America and Asia.

- Soybean continues to be the principal GMO crop followed by maize, cotton and canola. The world-leading producers of GM soybean crops are the USA, Argentina and Brazil.

- The detection and quantification of the GMO present in certified seed lots, and in food products, is essential to fulfill regulatory traceability requirements and downstream food labeling.

Digital PCR for Detection of GMO with TaqMan® OpenArray® Digital PCR

- Used for the absolute quantification of nucleic acids, based on the compartmentalization of single target DNA molecules across many reactions and detection of amplification.
- Allows for detection of single target molecules, even at low concentrations in a heterogeneous sample.
  - Per-well signal-to-noise ratio improves dramatically with dilution.
- TaqMan® Assays can be designed to differentiate between specific DNA molecules present in genetically-modified and wild-type organisms.
- A combination of TaqMan® OpenArray® Digital PCR Plates with TaqMan® chemistry, is a powerful tool for detection of specific, genetically- modified DNA targets present at low levels in certified seed lots.



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Invitrogen™ Applied Biosystems® Gibco® Molecular Probes® Novex® TaqMan® Ambion® Ion Torrent™

Collaboration Details

Subsidiary of BASF located in Quebec, Canada

- Center for Excellence for sequencing and genotyping. In 2003, a full range of marker-assisted breeding services launched to external clients:
  - Sequencing, fingerprinting, genetic mapping, transgene characterization

- Plant-breeding research presented at several national and international conferences.

Wing Cheung
Sequencing Facility Research Manager

- Required technology to detect very low amounts of contaminant GMO DNA in background of wild-type.
  - Desired level of sensitivity to detect 1 molecule of contaminant in the background of 10000 molecules of wild-type DNA.

Experimental Objectives and Workflow

- Project goal: Evaluate digital PCR for detection of GMO DNA in certified soybean seed lots
- 3 main steps: Assay design, assay validation for target specificity, and spike-in experiments where contaminant DNA is spiked-in with wild-type DNA at 0.5% and 0.01%.

1. Assay Design

>ss107912743
CGCCAAGAGAATAAATACCAGTTCTGTCATATTCTTTTCTGGTTTTTCAAGTGCAAGCTCAATGACTTCAATC
ACTGCACCTSCTTCAACTATCTTCATTCTGTTTCTCCCCAAAGGACACGTTTCTATAAGAACATGCAACGCGG
ATTTAACAGCTTGCTGAGAGAGTTCTCTATTTTTCATCACACTCACAACAACTTTGAAGAATTCAAGCTTCAA
GTTCCCTAAAGSGGTTGAGTCTTTGGCTTCAATTACC [A/T] GTTTCAATATTGGCATTGCTTCGTTTGTTAA
TTTGACGTTGTTCTTCGTTTCAACCTCCAAACCCCAAGTCAAACAGTTGAGAAAGTCAAAGTTCTCACCCACC
ATGCGCTTAATNTTGTTGTTGTTGTTGTTGTCGTCSACCATATTGGCACTGCTCCAAAGCAAACGAAGAA
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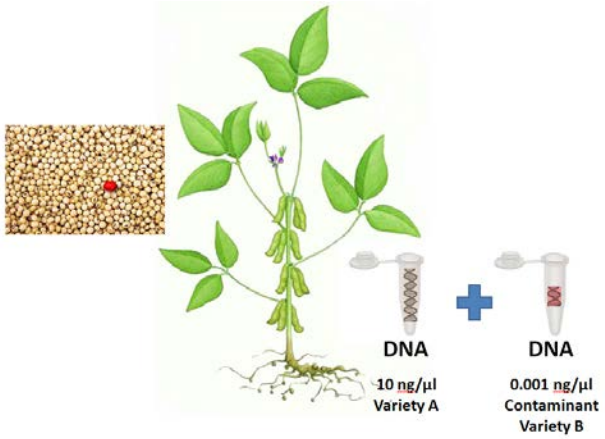
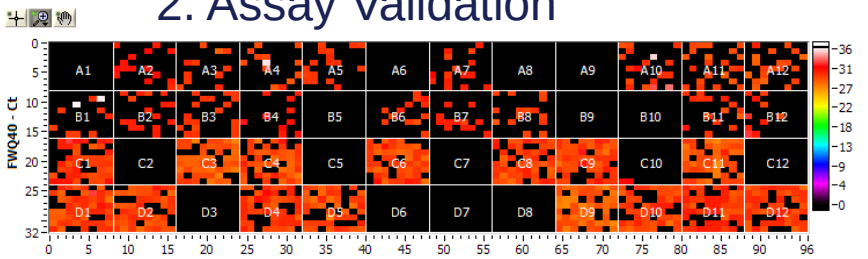
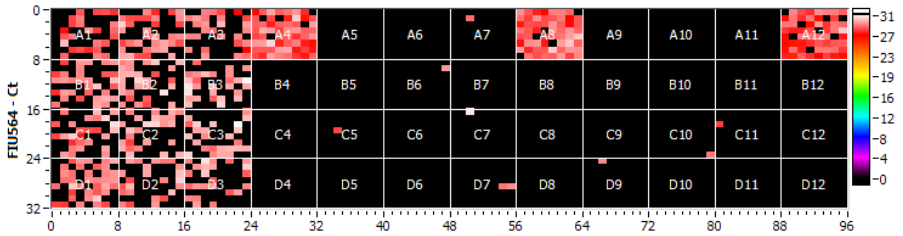


2. Assay Validation



3. Spike-in Experiments



Experimental Objectives and Workflow

- Samples:
  - Genomic DNA isolated from a certified soybean lot (“wild type”).
  - Genomic DNA isolated from GM soybeans.
- Custom TaqMan® Assays designed for detection of 7 different alleles, differentiating GMO and wild-type based on sequence information provided by collaborator (Step I).
- Custom assays validated for specificity prior to experiments for detection of GMO in background of wild-type DNA (Step 2).
- GM soybean DNA diluted to 0.05% and 0.01%, (1:5000 and 1:10000) in a background of wild-type DNA and detected using TaqMan® OpenArray® digital PCR plates (Step 3).

Step 1. Assay Design for Digital PCR Experiments

Input sequence

```
>ss107912743
CGCCAAGAGAATAAATACGAGTTCTGTCATATTCTTTTCTGGTTTTTCAAGTGCAAGCTCAATGAGTTCAATC
ACTGCACCTSCTTCAACTATCTTCATTCTGTTTCTCCCCAAAGGACACGTTTCTATAAGAACATGCAACGCGG
ATTTAACAGCTTGCTGAGAGAGTTCTCTATTTTTCATCACACTCACAACAACTTTGAAGAATTCAAGCTTCAA
GTTCCCTAAAGSGGTTGAGTCTTTGGCTTCAATTACC[A/T]GTTTCAATATTGGCATTGCTTCGTTTGTTAA
TTTGACGTTGTTCTTGGTTTGAAGCTGCAAAGCCCAAGTCAAAGAGTTGAGAAAGTCAAAGTTCTCACCCACC
ATGCGCTTAATNTTGTTGTTGTTGTTGTTGYTGCTGTCSACCATATTGGCACTGCTCCAAAGCAAACGAAGAA
TTCTAAGAGCTTCTTCAACGCACGGCGTCGTTTTGTTACCTTGCTTGAAAATCTTTGTG
```

Standard Assay:
The design pipeline outputs a standard Custom TaqMan® SNP Genotyping Assay with two probes, VIC and FAM

VIC FAM

UID	ProbeSeq1	ProbeSeq2	ForSeq	RevSeq
ss107912743	CCAATATTGAAACtGGTAATT	CAATATTGAAACaGGTAATT	CAGCTTGCTGAGAGAGTTCTCTATT	ACAACGTCAAATTAACAAACGAAGCA

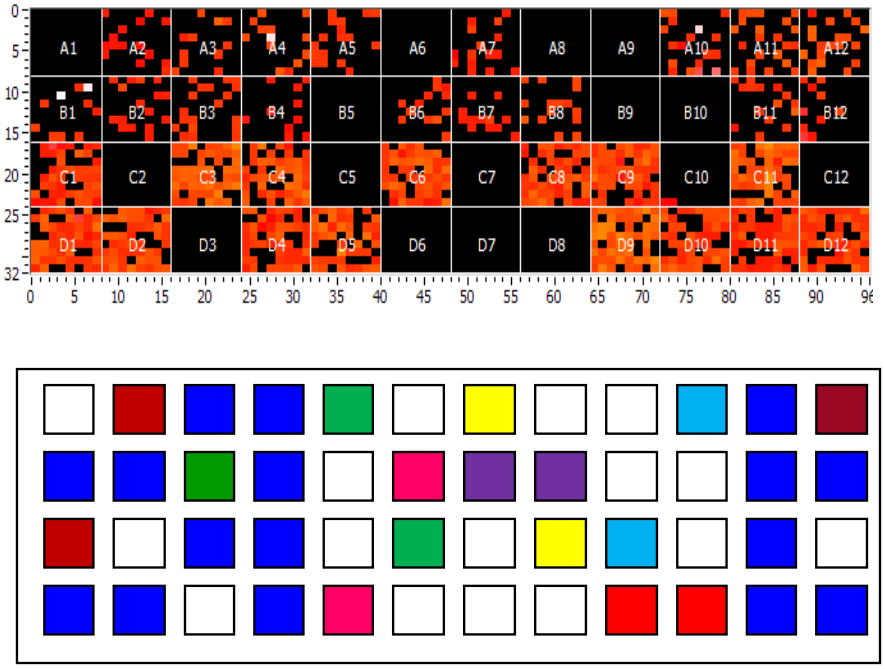
Adaptation to digital PCR:
The standard assay is split into two assays with one probe each. ***No splitting is required for 2-color digital PCR on QuantStudio™ 12K Flex Real-Time PCR System.***

FAM

Assay Name	FAM probe sequence	Forward Primer Sequence	Reverse Primer Sequence
ss107912743a	CCAATATTGAAACtGGTAATT	CAGCTTGCTGAGAGAGTTCTCTATT	ACAACGTCAAATTAACAAACGAAGCA
ss107912743b	CAATATTGAAACaGGTAATT	CAGCTTGCTGAGAGAGTTCTCTATT	ACAACGTCAAATTAACAAACGAAGCA

Step 2. Validation of TaqMan®Assay Specificity for GMO and Wild-Type Targets Using Digital PCR

- Experiment performed 3 times to finalize the list of target-specific TaqMan® assays.
- Wild-type and GMO DNA concentration diluted to digital range for testing assay specificity.
- Each DNA sample tested separately with both assays. Assay specificity confirmed if amplification was detected with a single DNA target.
- If amplification with a single assay was detected with both DNA targets, assay was considered as non-specific and it not used in the spike-in experiments.
- Success rate for assay design - 71%; at least one allele-specific TaqMan® assay for all 7 required alleles to be used in spike-in experiments.



Top - Heatmap shows signal amplification pattern using TaqMan® assays. **Below**- graphical representation of validation results. Each TaqMan® assay pair was tested with wild type and GMO DNA separately. **Blue** squares represent non-specific assays –amplification was detected with both DNA targets, **different colors-** allele-specific TaqMan® assays, **no color-** no amplification detected. **Rows A and B:** wild type DNA; **C and D** – GMO DNA.

Step 3. Setting up a Spike-in Experiment

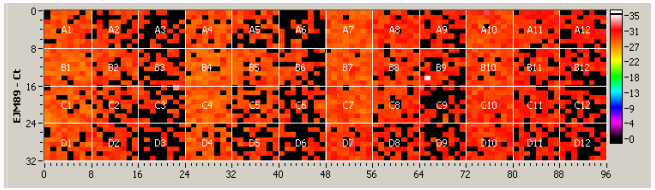
WT

GMO

245.7 ng/ul

27 ng/ul

dPCR



+

20 ng/ul

0.002 ng/ul

Setting up digital PCR:

2.5 uL of 2X digital PCR master mix

-0.25 uL 20X TaqMan® assay

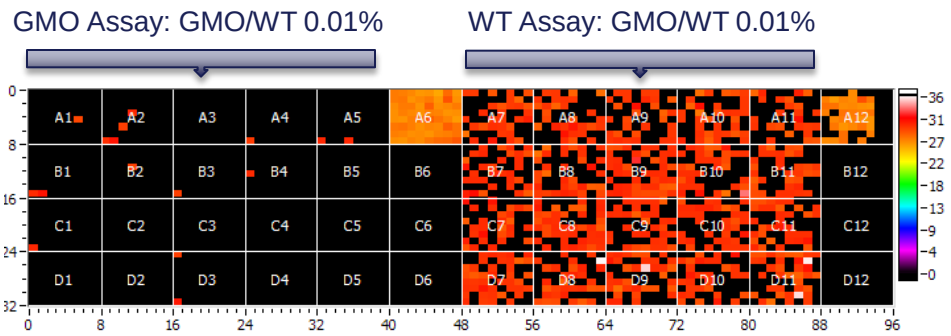
- 0.25 uL of water

- 2 uL of gDNA (WT+GMO)

Estimated Ratio GMO/WT 0.01%

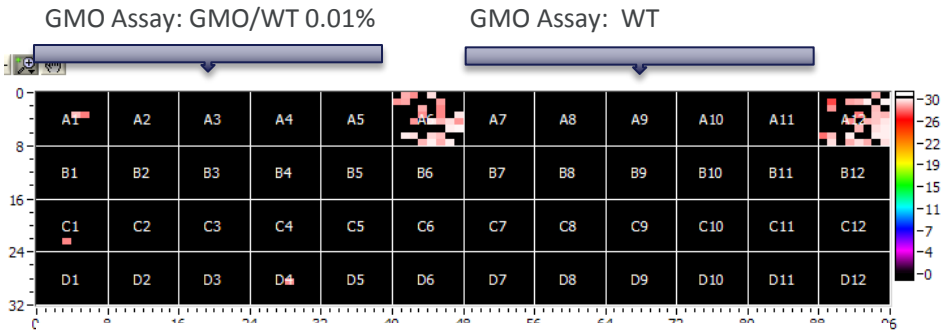
Step 3. Setting up a Spike-in Experiment

- Mixture of genomic DNA for digital PCR prepared to simulate experiments conducted at customer site: 0.01% of GMO added to 20 ng/ul of WT DNA
- Each assay for wild-type (WT) and GMO tested with gDNA mixture. In order to calculate GMO/WT (%) , WT gDNA diluted to digital range for testing with WT TaqMan® assays
- Each GMO assay tested with wild-type DNA to eliminate a possibility of false-positive signal from wild-type background present at high concentration and to confirm assay specificity for GMO target only
- Measured ratio of GMO/WT was in the range from 0.06 to 0.022. 1280 reaction wells is required to achieve 0.01% detection limit of GMO.



Assay Name	Copies/uL	Measured Ratio GMO/WT (%)	Estimated Ratio GMO/WT (%)
CONT	0.4		
WT	1793	0.022	0.01

Target assay specificity is required for discrimination of low percentage of GMO molecules



No amplification signal was detected when GMO-specific assay was used with WT DNA at 20 ng/ul

Summary

- We designed TaqMan® assays for GMO and WT soybean DNA detection and validated assay specificity with target-specific gDNA using dPCR concentration range and saturated gDNA concentration.
- Spike-in experiments were conducted to simulate workflow which could be done by customer, e.g. WT DNA concentration was not increased or decreased.
- Using 1:10,000 GMO/WT spike-in ratio we confirmed that measured ratio for GMO and WT targets produced ratio comparable to estimated GMO/WT.
- Similar experimental workflow can be used for a broad range of "needle-in the haystack" research experiments: for example, fetal samples or gene mutations linked to cancer
- Learn more here or at www.lifetechnologies.com/agbio

Regulatory and Legal Statements

- For Research Use Only. Not for human or animal therapeutic or diagnostic use.