



positive



detection and diagnostics

and

SEQID 

February 2017

PositiveID and seqID Pilot Study - Overview

- PositiveID and seqID are collaborating on a phased Pilot Study to demonstrate the utility of Firefly Dx in Point of Need (PON) detection of GMO markers in corn and soybeans intended for export
 - Phase I: Demonstrate ability of Firefly Dx bench prototype to detect 35S promoter gene at different concentrations
 - Phase II: Develop cartridge-based sample preparation of plant/seed materials
 - Phase III: Field demonstration of hand-held Firefly Dx prototype



PositiveID: Detection and Diagnostics

- PositiveID develops innovative, disruptive products positioned to revolutionize biological testing, detection and diagnostics
 - M-BAND – developed for U.S. Department of Homeland Security for automated detection of airborne bio-threats
 - Firefly Dx – a handheld lab for point-of-need detection and diagnostics
- Leading molecular diagnostic technologies using multiplex polymerase chain reaction (PCR) chemistry that are accurate, rapid, easy-to-use, cost-effective, assay agnostic
- Evolving, growing need for infectious disease and bio-threat detection requires point-of-need testing capabilities
- Proven technologies with 22 patents/patents pending



seqID: Genomic Solutions for Agriculture

- Technology scouting
- Development and collaboration
- Consulting and evaluation
- Genomic services
- 25+ years of agricultural experience
- 12 years in ag genomics



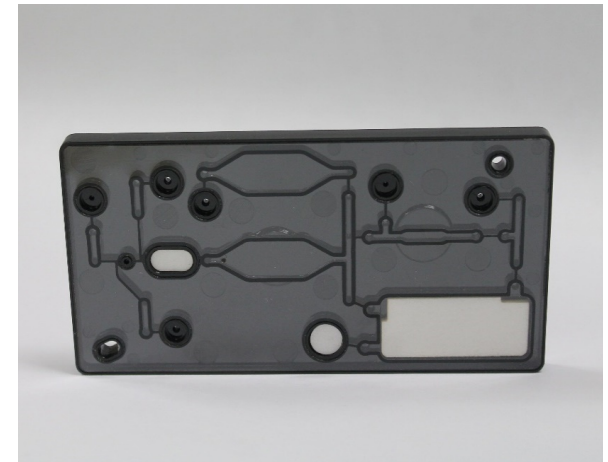
Firefly Dx Features

- A hand-held laboratory designed to provide integrated sample purification, biological analysis, and communication of test results in diverse settings and environments
- Uses real-time TaqMan® PCR (industry gold standard)
- Designed to incorporate single-use, disposable cartridges with pre-programmed RFID chips (no end-user programming or setup required)
- Reagents and waste all within cartridge for containment and archive
- Data to be processed in real time and communicated through Bluetooth® or wireless connectivity
- Battery powered and stand-alone for 10 to 15 cartridges based on sample type
- Designed for resource limited areas and minimally trained personnel



Cartridge Design

- Injection molded
- All reagents will be preloaded and ready to use
- Lyophilized reagents stable at room temperature (approx. 0°C–40°C) for >12 months
- RFID chip contains required protocol – no user programming
- Automatic chain of custody
- Fast results – designed to produce sample-in to results-out in 15-20 minutes with high integrity
- Affordable, disposable and portable (\$10-\$50 per test depending on assay and multiplex)
- Fully automated sample processing
- All waste and sample are contained



Microfluidic Cartridges

- Single-use, disposable cartridge currently running full sample preparation on breadboard prototype
- Integration of ultrasonic sample lysis, nucleic acid purification, and PCR amplification with real-time detection
- Sample purification and PCR amplification demonstrated across multiple organisms :
 - Zika Virus
 - Ebola
 - BWA agents (Ba, Yp, Ft)
 - Influenza A and B
 - Clinical and Environmental samples (E. coli)
 - Clinical (HPV)
 - Antibiotic resistant bacteria (MRSA, MSSA, C. diff)
- Sample Types Processed
 - Whole blood, nasal swabs, buccal swabs, simulated urine, environmental
 - Plant and seed material under development



Selected Product Specifications

- Size: current design of the Firefly Dx platform system is 170 mm long x 115mm wide x 50mm thick
- Weight: total operational weight of the Firefly Dx platform is estimated to weigh less than 2 lbs
- Power Requirement: Firefly Dx platform will operate on lithium ion batteries or from an AC or DC power source
- One sample per cartridge
- Current design is a four-color (four nucleic acid target) multiplex
- Sample size: ~50-250 µl – standard laboratory volumes

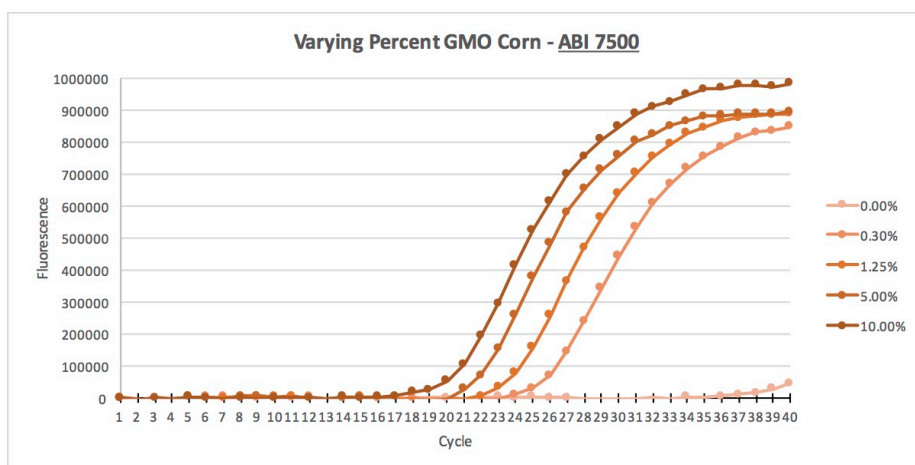


Pilot Study

- Samples:
 - Received: 0%, 0.1%, 1%, 10% and 100% GMO for both corn and soybean
 - Tested: 0%, 0.3%, 1.25%, 5.00% and 10% GMO for both corn and soybean
 - Demonstrated that 10% could easily be detected so used this titration ladder instead
- Sample Prep:
 - Biotecon Diagnostics DNA Purification - foodproof® Sample Preparation Kit III
- Assays:
 - Biotecon Diagnostics PCR kit - foodproof® GMO Screening Kit, 4 Target
 - 35S promoter assay was used in preliminary study
- Platforms:
 - Firefly Dx benchtop prototype
 - ABI 7500 for benchmark comparison

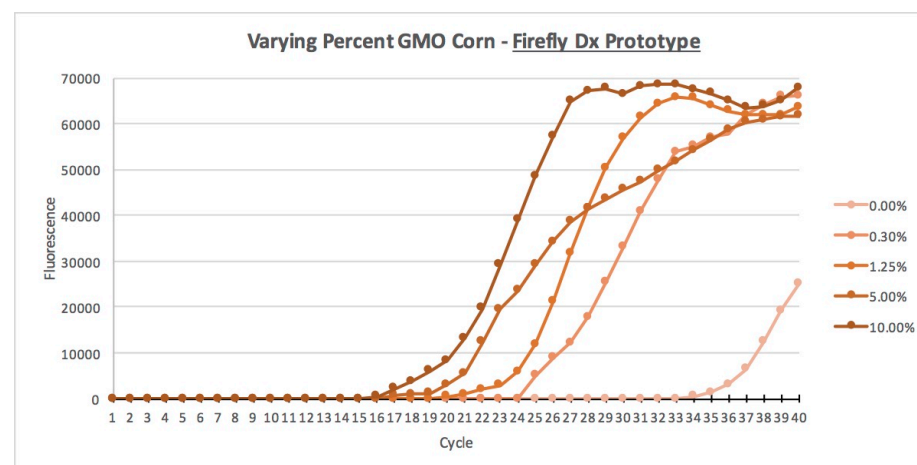


Results from Varying Percentages GMO Corn



% GMO	Ct
10.00%	20
5.00%	21
1.25%	23
0.3%	25

Data from the ABI 7500 is baselined and normalized to an internal ROX dye standard and smoothed.

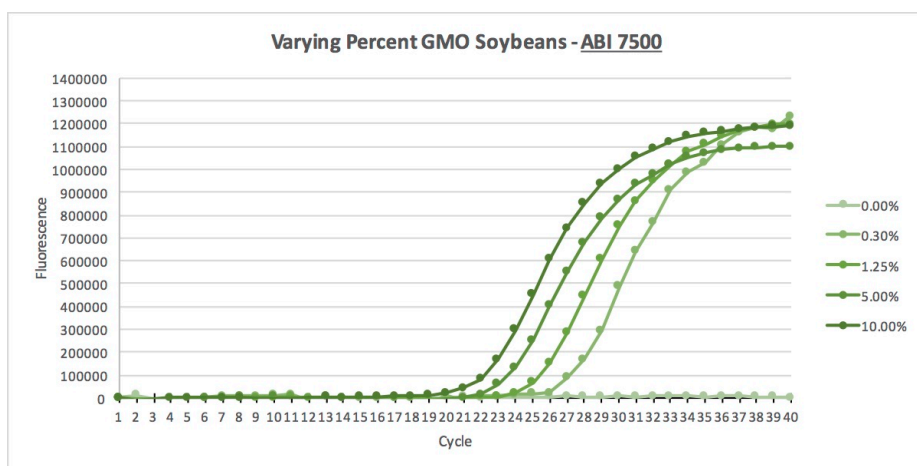


% GMO	Ct
10.00%	21
5.00%	22
1.25%	23
0.3%	27

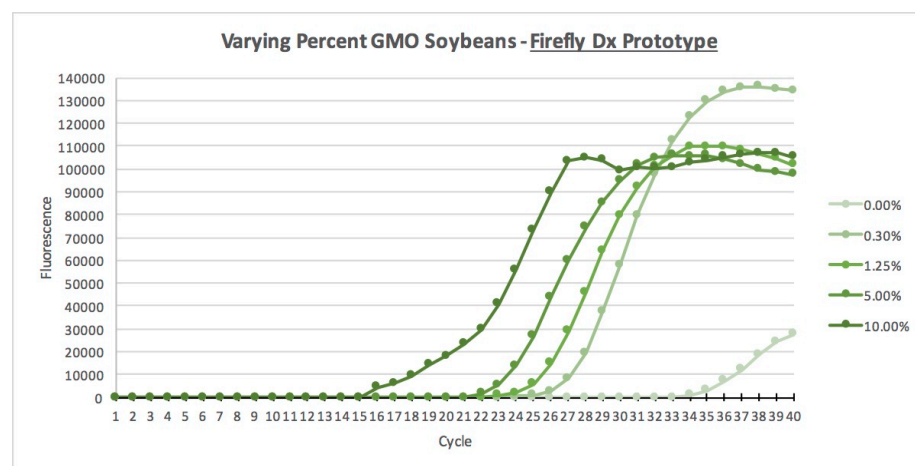
Data from the Firefly Dx Prototype is baselined to minimum values and smoothed with moving average.



Results from Varying Percentages GMO Soybean



% GMO	Ct
10.00%	21
5.00%	23
1.25%	25
0.3%	27



% GMO	Ct
10.00%	20
5.00%	24
1.25%	25
0.3%	29

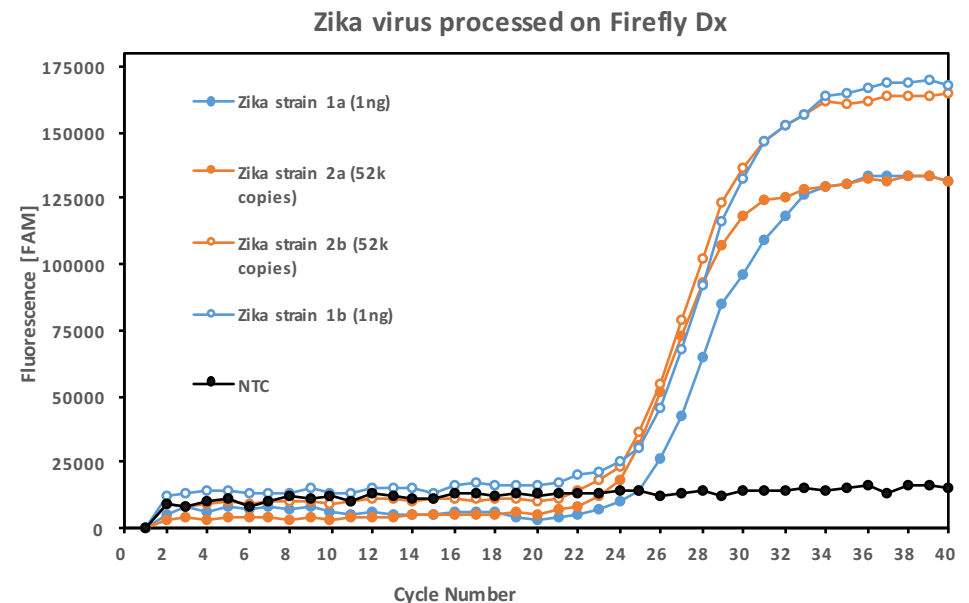
Data from the ABI 7500 is baselined and normalized to an internal ROX dye standard and smoothed.

Data from the Firefly Dx Prototype is baselined to minimum values and smoothed with moving average.



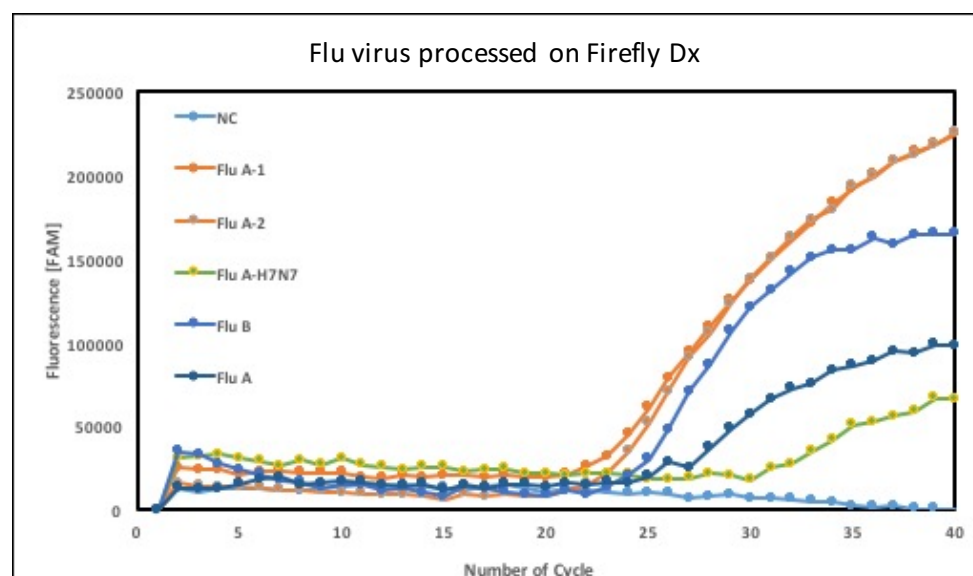
Zika Virus Data Sample

In a collaborative effort with GenArraytion Inc., PositveID's assay testing partner, Zika Virus was successfully detected on the Firefly Dx breadboard prototype pathogen detection system. Two different strains of Zika virus were tested at low number of copies via real time polymerase chain reaction (PCR) and the data curves crossing threshold at 26 Ct. The disposable chip was loaded with the reaction and then inserted onto the Firefly Dx prototype system. The automated runs successfully synthesized cDNA using a reverse transcriptase (RT) step and then completed a 40 cycle PCR to produce the detected target results.



Influenza Virus Data Sample

In a collaborative effort with GenArray Inc., PositveID's assay testing partner, Influenza Virus was successfully detected on the Firefly Dx breadboard prototype pathogen detection system. Five different flu virus assays were tested at low number of copies via real time polymerase chain reaction (PCR) and the data curves crossing threshold at 25 to 27 Ct. The disposable chip was loaded with the reaction and then inserted onto the Firefly Dx prototype system. The automated runs successfully synthesized cDNA using a reverse transcriptase (RT) step and then completed a 40 cycle PCR to produce the detected target results.



E.coli Data Sample

Samples containing 100pg of E.coli were successfully processed and detected on the Firefly Dx breadboard prototype pathogen detection system. E.coli DNA was isolated and purified through the cartridge sample preparation and detected via real time polymerase chain reaction (PCR) on the Firefly Dx PCR chip. 40 PCR cycles were performed and the data curves crossed threshold at 28 - 30 Ct. Throughout development, the Firefly Dx prototype system has consistently shown its effectiveness and repeatability through a wide range of organisms.

