Rapid point of use DNAble® assay for soybean GMO detection using the Douglas Scientific® AmpliFire®

ABSTRACT

The AmpliFire by Douglas Scientific along with DNAble isothermal amplification chemistry from EnviroLogix® provide a simple and portable tool to perform genetic analysis at the point of use. The highly specific and accurate DNAble chemistry resolves past challenges for isothermal DNA amplification such as noisy background, interference from inhibitors and false positives. This paper describes a proof-of-concept experiment that demonstrates the performance characteristics of a DNAble assay performed on the AmpliFire instrument.

- The AmpliFire is a portable point of use device optimized for DNAble isothermal nucleic acid amplification.
- EnviroLogix DNAble is a rapid and robust isothermal DNA amplification chemistry using a fluorescent-labeled molecular beacon for detection.

INTRODUCTION

Douglas Scientific has developed a portable, point of use testing solution for rapid genetic analysis using DNAble isothermal nucleic acid amplification chemistry in combination with the AmpliFire instrument.

Douglas Scientific Instrumentation and DNAble Chemistry

The AmpliFire instrument was used to perform a DNAble assay designed for detection of a genetic modification event in soybean genomic DNA. Genetic modification is commonly used to add specific gene sequences or alter gene expression in agriculturally important crops such as corn and soybeans. Testing for the presence of genetic modification is required to classify crops at many stages of use, including in the field, at the grain elevator, in transit, or at the final point of use. Tests of this nature must be fast, convenient, and accurate. The DNAble assay used in this experiment demonstratesthese characteristics.

- **AmpliFire Point of Use Instrument** (Figure 1)
  The AmpliFire point of use instrument supports genetic analysis of up to eight samples in 15 minutes or less. Samples are incubated at a constant temperature using a built in heat block and fluorescence is read in real time by an integrated detection system capable of multichannel fluorescence detection.
  Data can then be displayed and analyzed on the touch screen interface as the reaction progresses, or exported for further analysis.

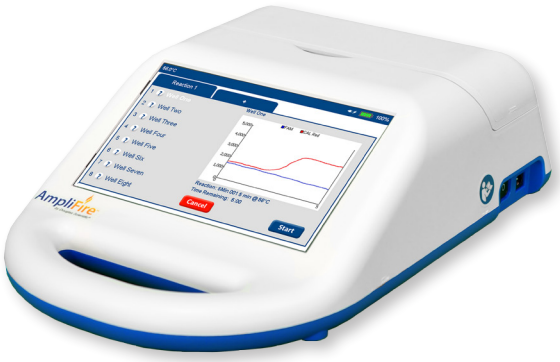

Figure 1. AmpliFire Point of Use Instrument

- **DNAble Isothermal Amplification Chemistry**
  DNAble is an isothermal amplification chemistry that utilizes sequence-specific primers to amplify a genetic region and a molecular beacon for detection. A nicking enzyme and DNA polymerase work together at a single temperature to achieve exponential DNA amplification without the need for thermal cycling. Reactions are completed in as little as 15 minutes, allowing users to perform rapid qualitative analysis. Unlike many other isothermal chemistries, DNAble can tolerate crude sample matrices.

MATERIALS AND METHODS

Lyophilized reaction mix containing buffer, dNTPs, primers, nicking and polymerase enzymes, and a molecular beacon was supplied by EnviroLogix in pre-measured microentrifuge tubes.

Soybeans with and without genetic modification (GMO) were ground into a fine powder and a mixture containing 5% GMO: 95% non-GMO was compared to a 100% non-GMO sample. Synthetic DNA containing the GMO sequence was also analyzed and compared to a no template control.

A very simple and quick crude sample preparation protocol was used for processing the ground GMO-mixed and non-GMO samples.

Four 50 µL aliquots of reaction buffer were spiked with 5 µL of the 5% GMO soybean crude prep extract. An additional four 50 µL aliquots of reaction buffer were spiked with 5 µL of the non-GMO soybean crude prep extract. Then 50 µL of each of the reaction buffer/extract solutions were used to reconstitute eight lyophilized reaction tubes. The tubes were sealed and placed into the AmpliFire for incubation and analysis.

An additional four 50 µL aliquots of reaction buffer were spiked with 5 µL of synthetic GMO soybean DNA. Then 50 µL of the reaction buffer/DNA solutions were used to reconstitute four lyophilized reaction tubes. An additional four tubes were reconstituted with 50 µL of reaction buffer to serve as no template controls. The tubes were sealed and placed into the AmpliFire for incubation and analysis.

The run protocols consisted of a 15-minute incubation at 56 °C with fluorescence read every 30 seconds.

The amplification curve was monitored in real time for each of the samples. Data for the runs were exported and analyzed.

RESULTS

The samples containing bacterial target DNA produced positive calls for each of the four replicates. The four no template controls did not amplify and were correctly assigned negative calls by the AmpliFire software. Figure 2 and Figure 3 show the data output for the experiment.

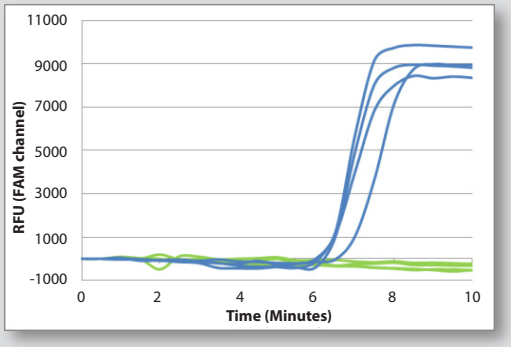

Figure 2. Exported results from AmpliFire (Time vs. RFU) using a crude preparation method of GMO and non-GMO soybeans.

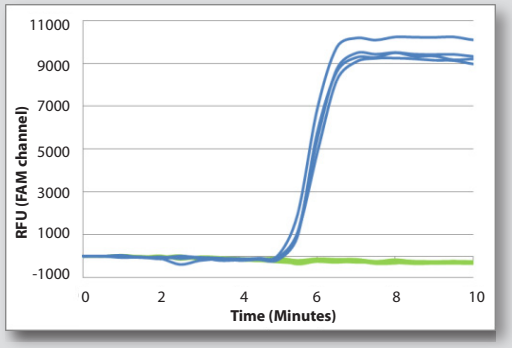

Figure 3. Exported results from AmpliFire (Time vs. RFU) using synthetic genomic DNA and no template controls.

CONCLUSION

The AmpliFire accurately detected the presence of a genetic modification event DNA sequence with both synthetic DNA and crude soybean sample extracts. This experiment demonstrates the ability of DNAble to amplify specific DNA targets in crude samples without interference from inhibitors or compromising sensitivity. With greater portability than real-time PCR instrumentation, the AmpliFire has potential to become a very powerful tool for point of use applications such as GMO detection in soybeans and other agriculturally important crops. AmpliFire produces rapid and accurate results in the field or in the lab without cumbersome equipment or reagents.

*For research use only. The products of Douglas Scientific, LLC are not FDA-approved for use in human diagnostic procedures.