# Rapid point of use DNAble® assay for *Salmonella enterica* 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 using both crude extract and purified DNA samples.

- The AmpliFire is a portable point of use detection instrument optimized for DNAble isothermal nucleic acid amplification.
- 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 system was used to perform the *Salmonella* DNAble assay in the experiment described below. *Salmonella* is a bacterial pathogen commonly implicated in food-borne illness. It is commonly found in poultry and has become an important component of pre-harvest and food safety testing.

- **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 then can 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 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 microcentrifuge tubes.

*Salmonella*-positive and -negative boot swabs from a poultry facility, and purified *Salmonella* genomic DNA were used as samples in this experiment. A rapid crude sample preparation protocol was used to process the boot swabs.

Four 50 µL aliquots of reaction buffer were spiked with 5 µL of *Salmonella* positive boot swab extract. An additional four 50 µL aliquots of reaction buffer were spiked with 5 µL of the *Salmonella* negative boot swab 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 purified *Salmonella* genomic DNA (2,000 genome copies/µL). Then 50 µL of the reaction buffer/extract 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 *Salmonella*-positive samples extracted from boot swabs and the purified *Salmonella* DNA both produced positive calls for each replicate. The *Salmonella*-negative samples from boot swabs and the  template controls did not amplify.

Although the reporter dye intensity was lower with the crude preparation method, there was clear amplification of *Salmonella*-positive samples. Additionally, there was 100% concordance of calls between the samples using the crude preparation protocol from boot swabs and the purified genomic DNA samples. Figures 2 and 3 show the AmpliFire results for the crude preparation method and the purified genomic DNA, respectively.

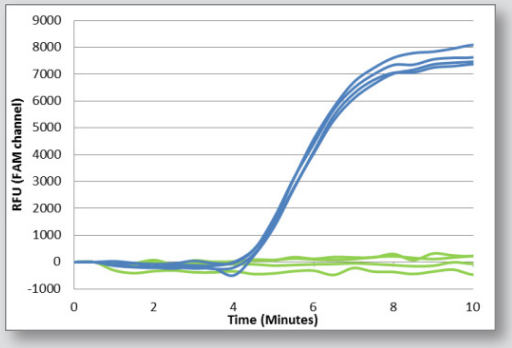


*Figure 2.* Exported results from AmpliFire (Time vs. RFU) using a crude *Salmonella* preparation method from boot swabs.

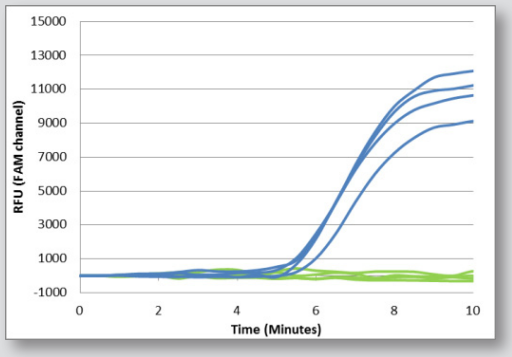


*Figure 3.* Exported results from AmpliFire (Time vs. RFU) using purified *Salmonella* genomic DNA and template controls.

# CONCLUSION

In this experiment, the AmpliFire accurately detected the presence of *Salmonella enterica* using both purified genomic DNA and a crude sample preparation method. 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 detecting *Salmonella* during pre-harvest testing. The 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.

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