Rapid point of use DNAble® assay for *Escherichia coli* 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 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 an assay for detection of *E. coli* genomic DNA. Although most common strains of *E. coli* are harmless, the Shigatoxigenic group is pathogenic to humans and is often implicated in food poisoning. Stx1 and Stx2 genes encode for Shiga toxins 1 and 2 and are particularly important for 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 integrated touch screen interface as the reaction progresses, or exported for further analysis.

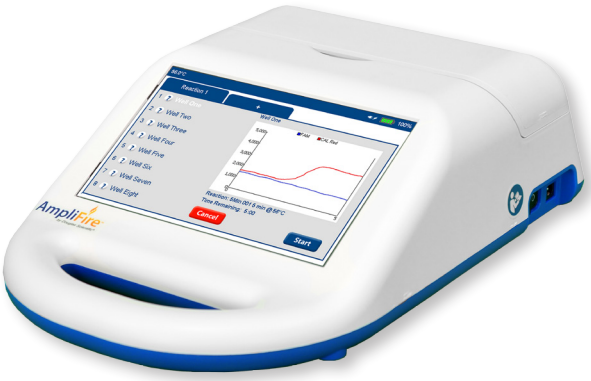

Figure 1. AmpliFire Point of Use Instrument

- **DNAble Isothermal Amplification Chemistry** (Figure 2)
  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. Additionally, 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 multiplexed molecular beacons were supplied by EnviroLogix in pre-measured microcentrifuge tubes.

Stx1 positive and Stx2 positive *E. coli* cultures and corresponding purified DNA samples were used as positive controls in this experiment.

Three cultures of ground beef were used as crude testing sources; Stx1 *E. coli* was introduced to one culture, Stx2 was introduced to another, and the third culture was left in a natural state.

Three 50 µL aliquots of reaction buffer were spiked with 5 µL of the Stx1 positive crude prep extract or purified DNA. An additional three 50 µL aliquots of reaction buffer were spiked with 5 µL of the Stx2 positive crude prep extract or purified DNA. Two 50 µL aliquots were spiked with either 5 µL of ground beef crude prep without *E. coli* or nothing was added to the reaction buffer. 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.

The run protocols consisted of a 10-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 purified samples and *E.coli*-positive beef samples both produced positive calls for each of the three replicates. The no template controls and crude prep samples without *E. coli* did not produce any amplification. The AmpliFire results for the multiplexed Stx1 and Stx2 assays can be seen in figures 2 and 3 respectively.

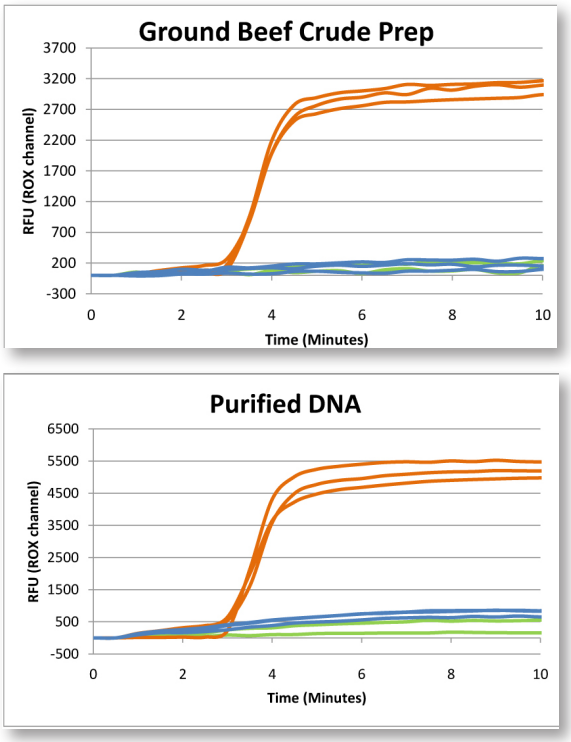

Figure 2. Detection of Stx1

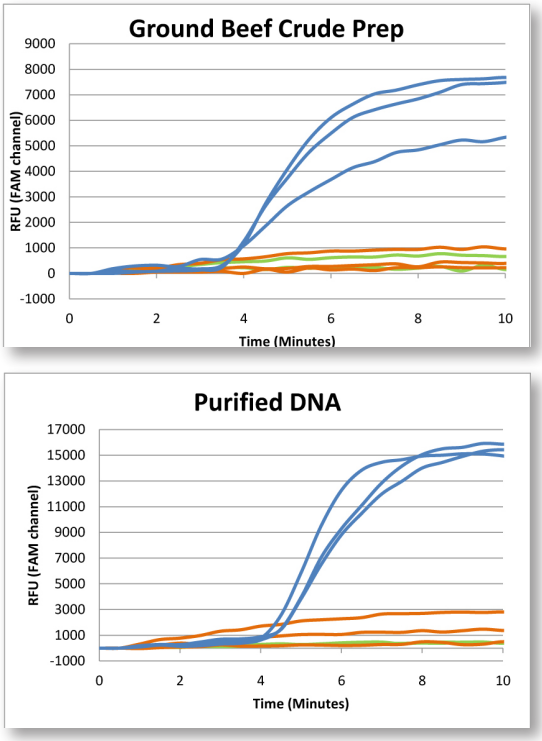

Figure 3. Detection of Stx2

CONCLUSION

The AmpliFire accurately detected the presence of Stx1 and Stx2 *E . coli* strains with a crude sample preparation method and in purified genomic DNA samples. This demonstrates that the assay has the ability to multiplex without 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 detecting the presence of pathogenic *E. coli* strains in food samples. 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.