

# Rapid point of use DNABLE® assay for *Mycoplasma gallisepticum* 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 instrument was used to perform the *Mycoplasma gallisepticum* assay in the experiment described below. *M. gallisepticum* causes chronic respiratory disease in chickens and can be easily transmitted within a flock. Because of the economic implications of an infected flock, *M. gallisepticum* has become an important target for monitoring flock health.

#### • 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.

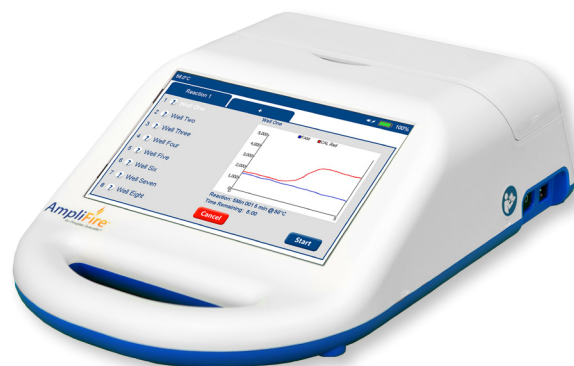


Figure 1. AmpliFire Point of Use Instrument

- **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 microcentrifuge tubes.

A sample containing 500 CFU of heat euthanized bacteria per microliter was used as a positive control in this experiment and buffer was used as a non-contaminant control.

Four 50 µL aliquots of reaction buffer were spiked with 5 µL of bacteria solution. Then 50 µL of the reaction buffer/bacteria solution was used to reconstitute each of four lyophilized reaction tubes. The remaining four tubes received 50 µL of reaction buffer to serve as no template controls. All tubes were sealed and placed into the AmpliFire for incubation and analysis.

The run protocol was programmed using the AmpliFire software and 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 eight samples and calls were assigned by the software as positive or negative based on fluorescent signal amplification, or lack thereof.

## 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. For ease of readability, the results were exported and shown in Figure 2.

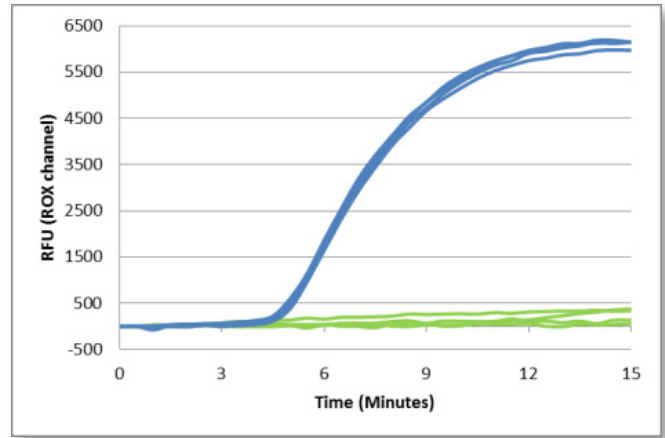


Figure 2. Exported results (Time vs. RFU) for positive samples and no-template controls.

## CONCLUSION

The AmpliFire system for isothermal DNable amplification chemistry has the potential to become a very powerful tool for point of use applications such as detecting or monitoring *M. gallisepticum*. 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.