# Rapid point of use DNAble® assay for citrus greening disease (*Candidatus* Liberibacter spp.) 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 as compared to a real-time PCR 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 the citrus greening disease assay in the experiment described below. Citrus greening, also known as Huanglongbing (HLB), is a bacterial disease transmitted to citrus trees by insects, specifically the Asian or African citrus psyllid. It can cause disfigured and bitter fruit and ultimately kills citrus trees resulting in devastating crop losses. The DNAble assay used in this experiment amplifies the genomic DNA of these bacteria for positive identification.

- **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 the need for 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 a molecular beacon was supplied by EnviroLogix in pre-measured microcentrifuge tubes.

Uninfected psyllids and psyllids known to harbor the bacteria that cause HLB were obtained from collaborators in the citrus industry.

Five psyllids (infected or uninfected) were added to 250 µL of extraction buffer in a 1.5 mL conical tube, pulverized with a plastic pestle for 10 seconds, and particulate matter was allowed to settle for three minutes at room temperature. Samples were diluted 1:4 in extraction buffer and 50 µL of diluted supernatant was added to each tube of lyophilized reaction mix. Two identical 8-tube strips were prepared, each containing four replicates of uninfected psyllid prep and four replicates of HLB-infected psyllid prep. One tube was placed into an AmpliFire instrument and the other into an industry leading real time PCR instrument for incubation and analysis.

The run protocol for each instrument consisted of a 10-minute incubation at 56 °C with fluorescence read every 30 seconds.

Amplification curves for each set were monitored in real time and data from both instruments were exported and analyzed for concordance.

# RESULTS

The samples extracted from infected psyllids resulted in exponential amplification curves for each of the four replicates on the AmpliFire and the real-time PCR instrument. The samples extracted from uninfected psyllids did not produce amplification on either instrument. There was 100% concordance between both instruments. In addition to demonstrating the amplification curves, the AmpliFire software generated positive and negative calls for the infected and uninfected samples, respectively. Figure 2 shows the exported data from the AmpliFire system and figure 3 shows the data from the real-time PCR system.

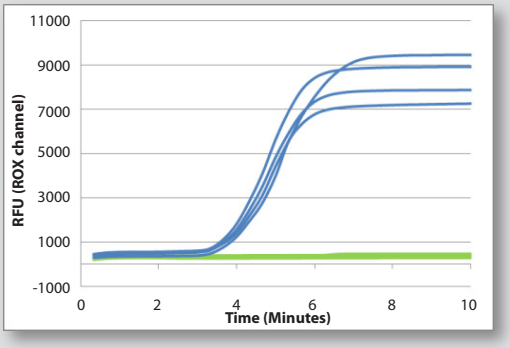


Figure 2. Exported results from AmpliFire (Time vs. RFU) for infected and uninfected psyllids.

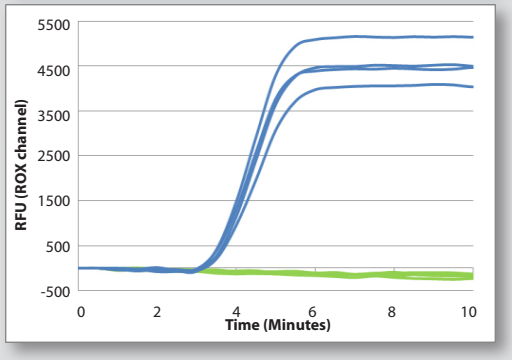


Figure 3. Exported results from a real-time PCR instrument (Time vs. RFU) for infected and uninfected psyllids.

# CONCLUSION

In this experiment, a crude insect preparation and a DNAble assay were used to demonstrate that the AmpliFire instrument accurately detects the presence of HLB-causing bacteria consistent with a real-time PCR system. 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 management of citrus greening disease. 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.