Rapid point of use Grouper Chek™ assay using the Douglas Scientific® AmpliFire® detects seafood fraud

ABSTRACT

The AmpliFire by Douglas Scientific along with GrouperChek isothermal amplification chemistry from PureMolecular, LLC provide a simple and portable tool to perform genetic analysis at the point of use. This paper describes a proof-of-concept experiment demonstrating the ability of the AmpliFire combined with the GrouperChek assay to differentiate the highly prized grouper from tilapia, a common illegal substitute.

INTRODUCTION

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

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

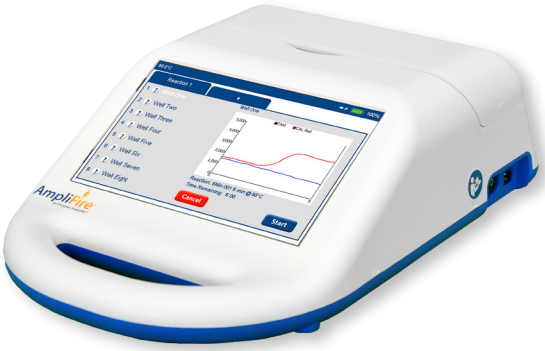

Figure 1. AmpliFire Point of Use Instrument

- **GrouperChek Isothermal Chemistry**
  PureMolecular's GrouperChek assay utilizes nucleic acid sequence-based amplification (NASBA) to amplify a genetic region and molecular beacons for detection. Three enzymes work in concert to achieve exponential RNA amplification without the need for thermal cycling. Reactions are completed in under 45 minutes, allowing users to perform fast qualitative analysis. Additionally, GrouperChek enables the use of crude sample preparation, simplifying the overall testing process.

MATERIALS AND METHODS

One fillet of grouper and one of tilapia were used for this experiment. The fillets were frozen prior to the experiment. Two samples were taken from the grouper fillet and one from the tilapia.

Each 10 mg sample was placed in a tube containing 500 µL of a salt detergent buffer solution and allowed to incubate for 10 minutes at room temperature. The resulting solution (5 µL) was added to a tube containing 15 µL of reagent mix consisting of buffer, dNTPs, primers, enzymes, and molecular beacons for a total reaction volume of 20 µL. A fourth tube was used for a no template control (NTC).

The four tubes were sealed and placed in the AmpliFire for incubation and analysis. A 45-minute protocol was run at 41 °C. The amplification curves were monitored in real time for all of the samples. Data for the reactions were exported and analyzed.

RESULTS

The two grouper samples amplified while the tilapia sample and NTC showed no amplification. The grouper reactions provided strong positive signals easily differentiated from the tilapia and NTC reactions (Figure 2).

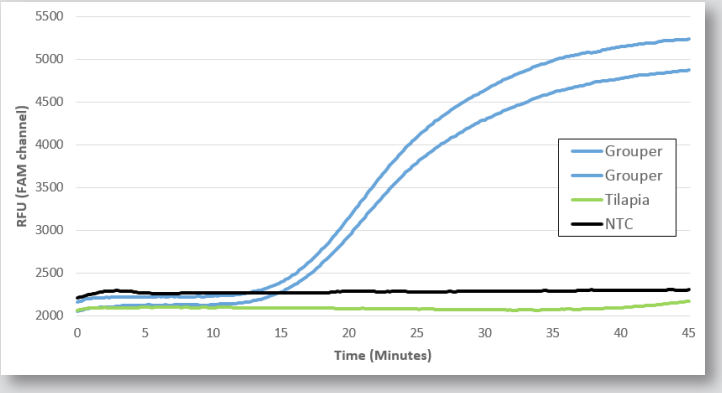

Figure 2. Exported results from the AmpliFire (Time vs. RFU) for grouper and non-grouper samples.

CONCLUSION

The AmpliFire successfully differentiated between grouper and tilapia. This experiment demonstrates the ability of GrouperChek to amplify specific RNA targets in crude samples without interference from inhibitors or compromising sensitivity. With greater portability than real-time PCR instrumentation, the AmpliFire is a very powerful tool for point of use applications such as seafood fraud detection in grouper and other seafood species. 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.