COLLECTION OF FIREBLIGHT SYMPTOMATIC TISSUE FROM APPLE TREES

- If available, use bacterial ooze directly for sample preparation (Picture 1).
- From young twigs, collect 1" sample tissue, taking both healthy and diseased tissue from the transition zone (Picture 2), and slice the shoot longitudinally in half.
- For older bark and active cankers, skim off bark using a knife, need only about 1" length exposed, leaving the cambium layer (Picture 3).

COLLECTION OF SAMPLE

- If cambium layer looks dry, add a drop of sterile water onto exposed cambium area.
- Rub the exposed cambium layer with a clean disposable pipet tip 3 times or gently touch pipette tip to the ooze, dip pipette into a 200µl microfuge tube containing 25µl sterile water, repeat the process 3 times.

Note: a discolored sample gives a poor result.

TURNING ON AND CONNECTING THE BIORANGER AND TABLET

- Fully charge tablet and BioRanger.
- Turn on tablet and open BioRanger App.
- Turn on BioRanger device and **wait for green blinking light** on top of device.
- Connect tablet and BioRanger device using Connect menu on the tablet (blue light)
- Tablet top right will display 'connected'.
- Select Analysis or Options/Edit methods.
- Analysis method will be indicated in top center.

BACTERIAL CELL LYSIS

- Collected sample needs to be 'lysed' by heating for 5 minutes at 95°C in the BioRanger.
- Place the 25 µl sample (up to 8 tubes at a time) in the BioRanger.
- Select or Create Lysis Analysis method.
- Options/Edit methods/Add new method/Confirm/Exit.
- Check Lysis step, Set to 95°C for 5 minutes.
- Select Start method, upon start the light on the BioRanger will turn red.
- Status will display in upper left corner of tablet.
- Check lanes you desire to see using the BioRanger App.
- When reaction completed select Options/Quit/Yes.
- When completed cool samples in ice for 5 minutes and let BioRanger rest 5 minutes between runs.

MIXING SAMPLE WITH OTHER REAGENTS

Note: Keep solutions on Ice.

- In new clean tubes, mix the following for each sample needed :
  15µl Optigene mix ISO-001 (Pro-Lab Diagnostics Inc., Round Rock, TX), 2µl DS Primers, 3µl sterile water, 5µl lysed sample.
- Mix four components together by thumping with fingers, let them settle or spin with makeshift centrifuge (Picture 4).
- Run a positive control with pathogen DNA.
- Run a negative control (water, or uninfected plant material).

RUN ANALYSIS PROTOCOL

- Turn on/connect BioRanger and Tablet, and select analysis method or Options/Edit/Methods Settings: (check boxes) in the BioRanger App.
- Setup, isothermal reaction at 65°C for 15-30 minutes, denaturation at 80°C for 5 minutes, and fluorescence read interval every 30 seconds.
- Other options are, reaction mechanism as LAMP, and Classification approach as Auto
- Afterwards, place tubes with prepared sample/reaction mix in BioRanger and select start method. Indicator light will turn red.
- Make sure SAVE Data is checked.
- Label the lanes with sample information and 'Accept Labels'.
- Select positive and negative labels as preinstalled.
- BioRanger will beep when done and status will display in top left corner of tablet.

INTERPRETING THE RESULTS

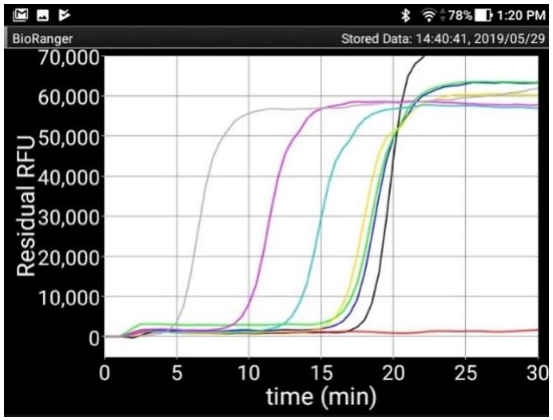
- Watch the results in the BioRanger app on the tablet for real time results.
- A sigmoid curve (S) of positive samples should be observed within 5-10 minutes of the run showing increase in the fluorescence as a peak.

Note:

- Letter colors of the sample refer to same color lines on the graph.
- Irregular peaks or delayed peaks can also be seen in in negative samples. Therefore, look at your positive and negative controls and compare them to your sample results.

Supplies needed: Clean bench space, ethanol 70%, wipes, ice/cooler, 200ul tubes, pipettes, knife, ISO-001 mix (www.pro-lab-direct.com), primer mix, sample, sterile water, BioRanger and an android tablet with BioRanger app installed (diagenetix.com).

Picture 4: Additional supplies needed to run loop mediated isothermal DNA amplification



Picture 5: Sigmoid curves for test samples diluted at different concentrations are in gray, violet, blue, yellow, green and blue. Whereas, red line represents the negative sample.

Note:

- Size of Threshold peak value (default 10000RFU) will be dependent on the run.
- F=fluorescence, T=temperature, t=time.
- 10^2 is the detection limit for this kit.

Picture 1. Bacterial ooze.

Picture 2. Transition zone between healthy and infected tissue.

Picture 3. Scraped bark to expose cambium layer of fire blight canker.

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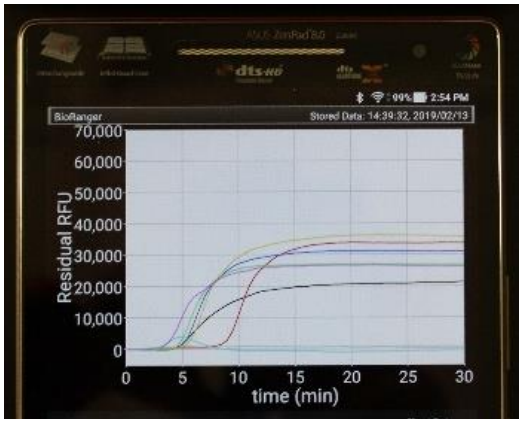
Detection of Fire Blight *Erwinia amylovora* Pathogen with LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION

USING THE BIORANGER© BY DIAGENETIX, INC.



Picture 6: BioRanger device (top) and an android Tablet (bottom)

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