



## Detection of Pathogens in Palm Tissue and Vectors

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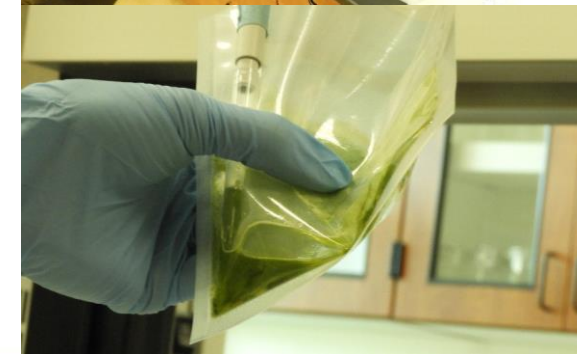
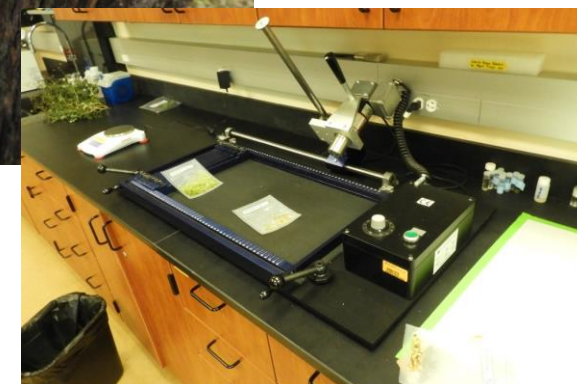
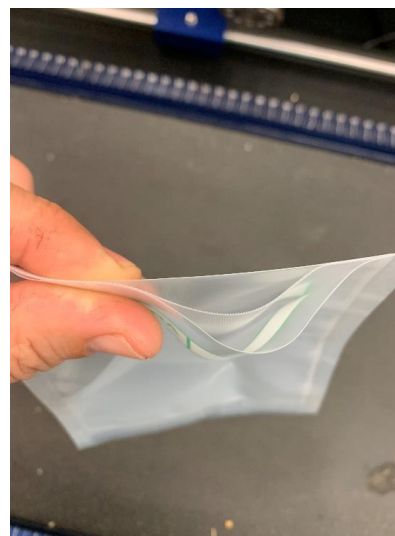


## Protocols

- Sampling and DNA Extraction.
- qPCR and high resolution melt curve análisis.
- Digital PCR.

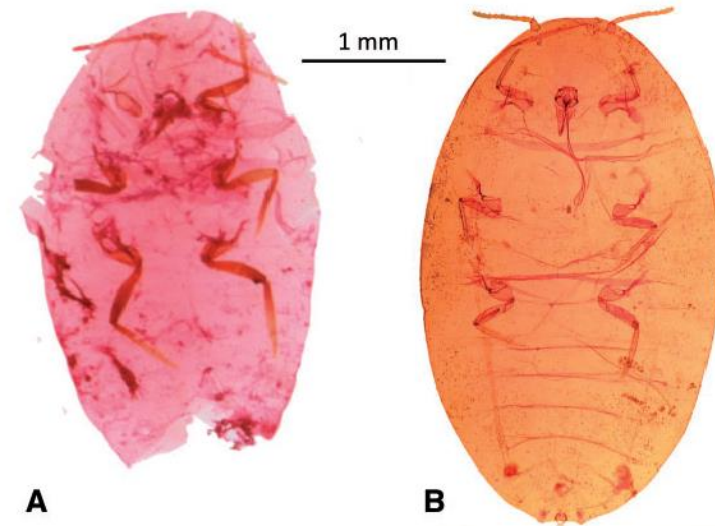
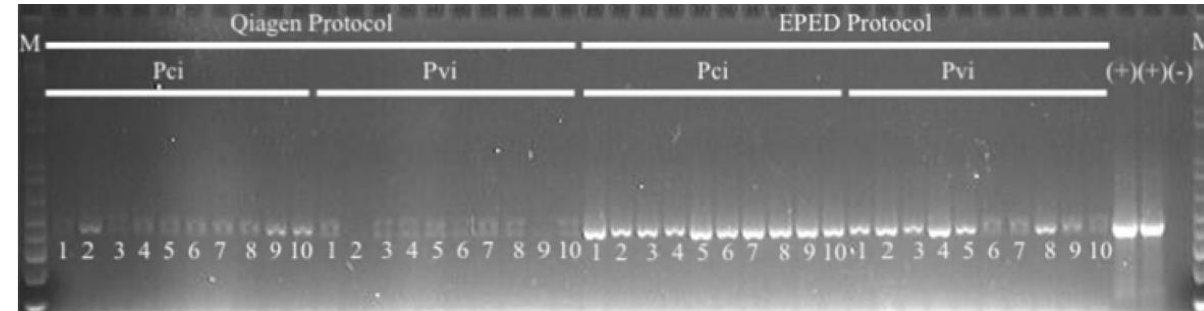
## Total DNA extraction from plant tissue

- Drill into trunk of palm, remove pseudobark, collect 3 g living tissue.
  - 1 g adequate for extraction process.
- Tissue added to BioReba extraction bag, macerated in guanidine buffer, 400  $\mu$ l extracted using Qiagen Plant Mini Kit.
- Originally used for ssDNA virus isolation in grape tissue, versatile and easily translatable to systems dealing with DNA-based pathogens.



## Total DNA extraction from insect vector tissue

- Leave body of insect intact.
  - 180µl buffer ATL + 20µl proteinase K at 56° C for 24 hr. (Qiagen Dneasy Blood and Tissue).
- Complete extraction, evaporate final eluate, conduct second extraction on same insect, elute in tube from previous extraction.
  - Hyperconcentrates DNA yield (~20 ng/µl to >100 ng/µl).



### HRMA

#### 16S rRNA

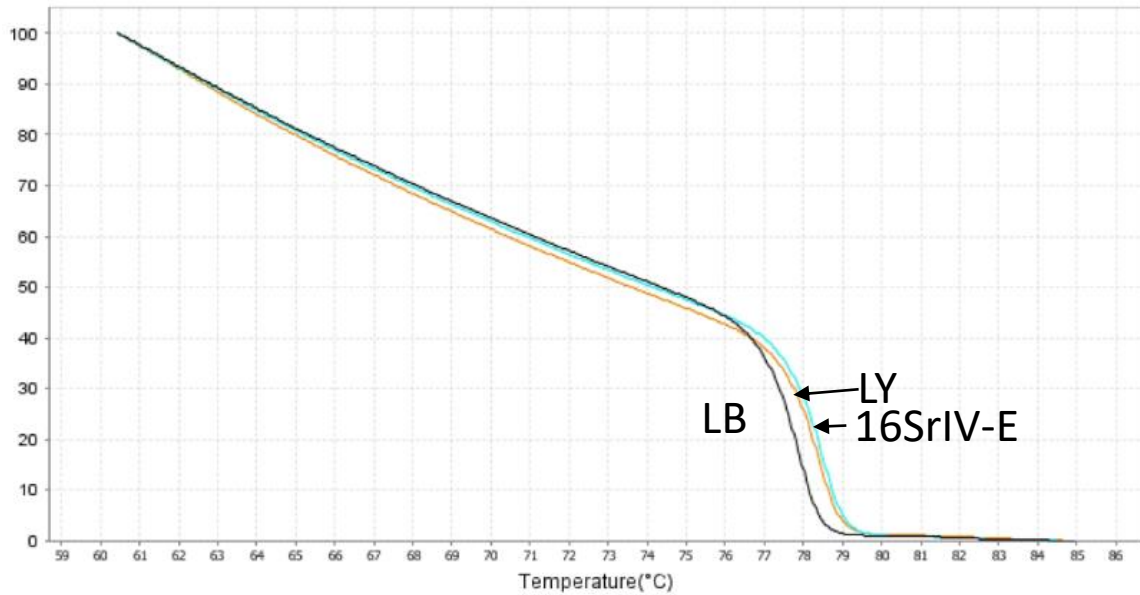
|                     |  |       |
|---------------------|--|-------|
| TPPD (AF434989)     | AGTTTGATCCTGGCTCAGGATTAACGCTGGCGGCGTGCTTAATACATGCAAGTCGAACGGAAATCTTTTAGAGAAATCTTTTAGATTTTAGTGGCGAACGGGTGAGTAACACGTAAGCAACCTGCCTTTAAGACGAGAATAACAATTGGA | [150] |
| Saba11 (HQ613895.1) | .....  | [150] |
| LYJAM (HQ613873.1)  | .....G.....  | [150] |
| LYFL (HQ613874.1)   | .....G.....  | [150] |
| TPPD (AF434989)     | AACAGTTGCTAAGGCTGGATAGGAAATAAAAAGGCATCTTTTATTTTAAAAGACCTTCTTCGGAGGGTATGCTTAAAGAGGGGCTTGCCTCACATTAGTTAGTTGGTAGGGTAATGGCCTACCAAGACGATGATGTGTAGCTGGACTG   | [300] |
| Saba11 (HQ613895.1) | .....  | [300] |
| LYJAM (HQ613873.1)  | .....T.....A.....A.....  | [300] |
| LYFL (HQ613874.1)   | .....T.....A.....A.....  | [300] |

LB = 80.4°C

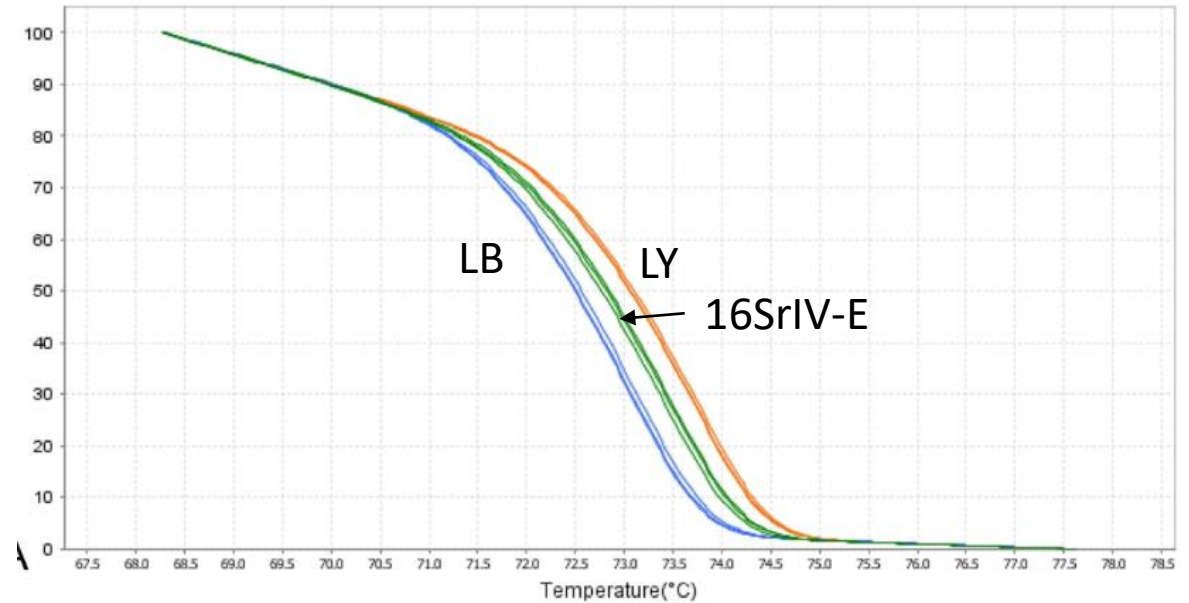
LY = 80.7°C

# HRMA for three phytoplasmas

16S rRNA

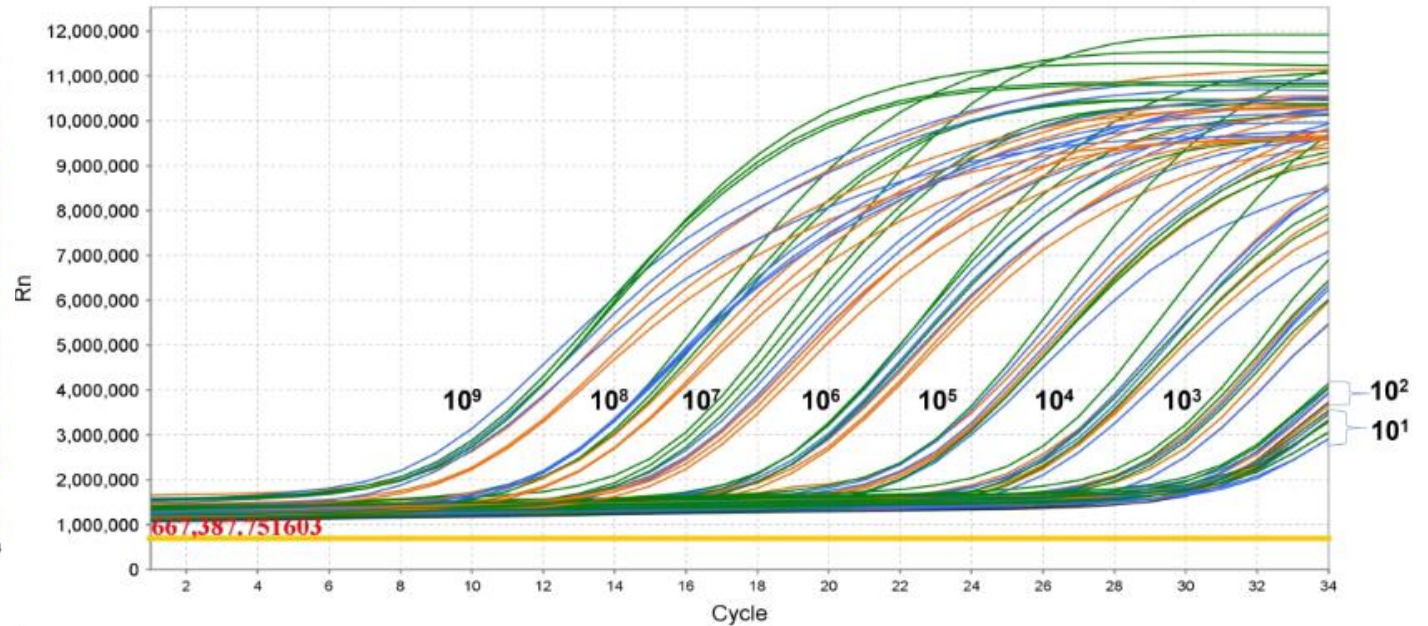
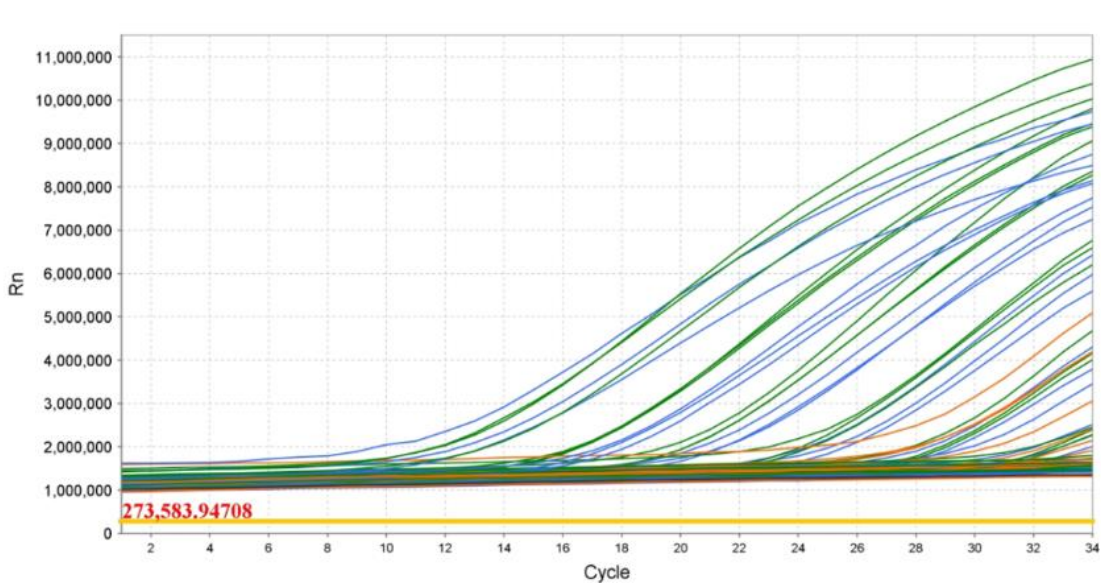


*secA*



# Quantitative PCR (qPCR) assay design

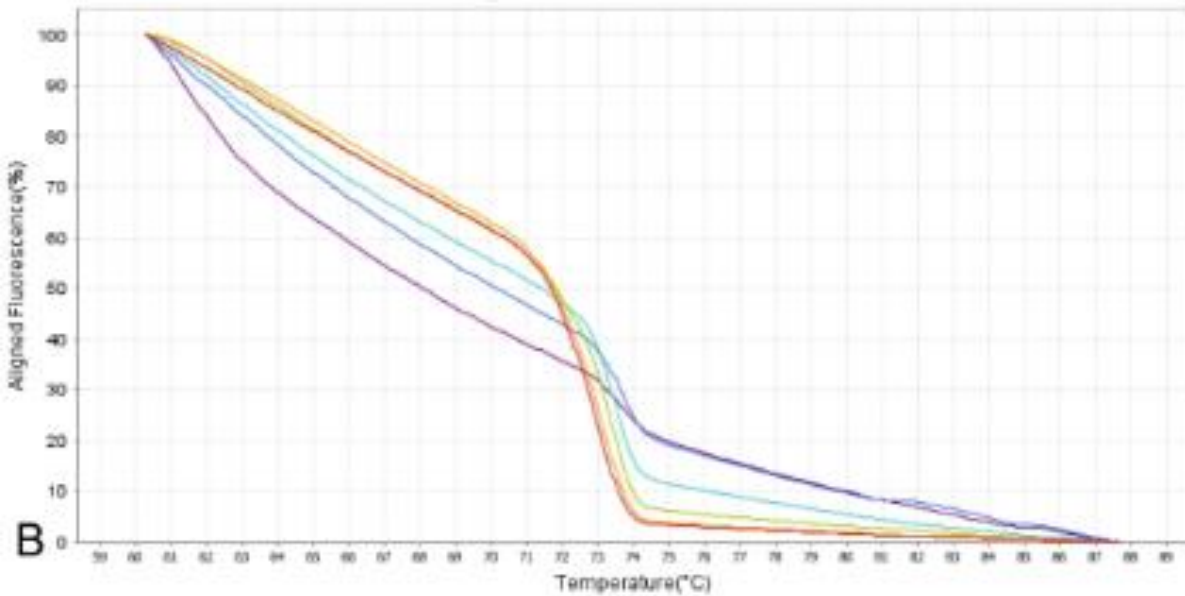
*secA*



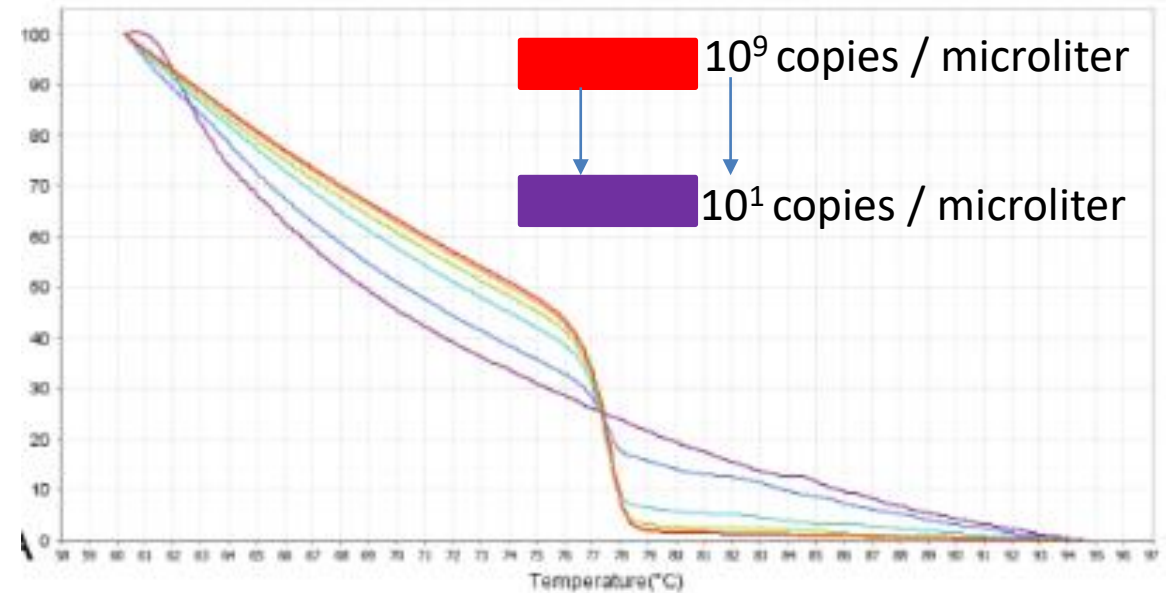


# HRMA for different concentrations

16S rRNA

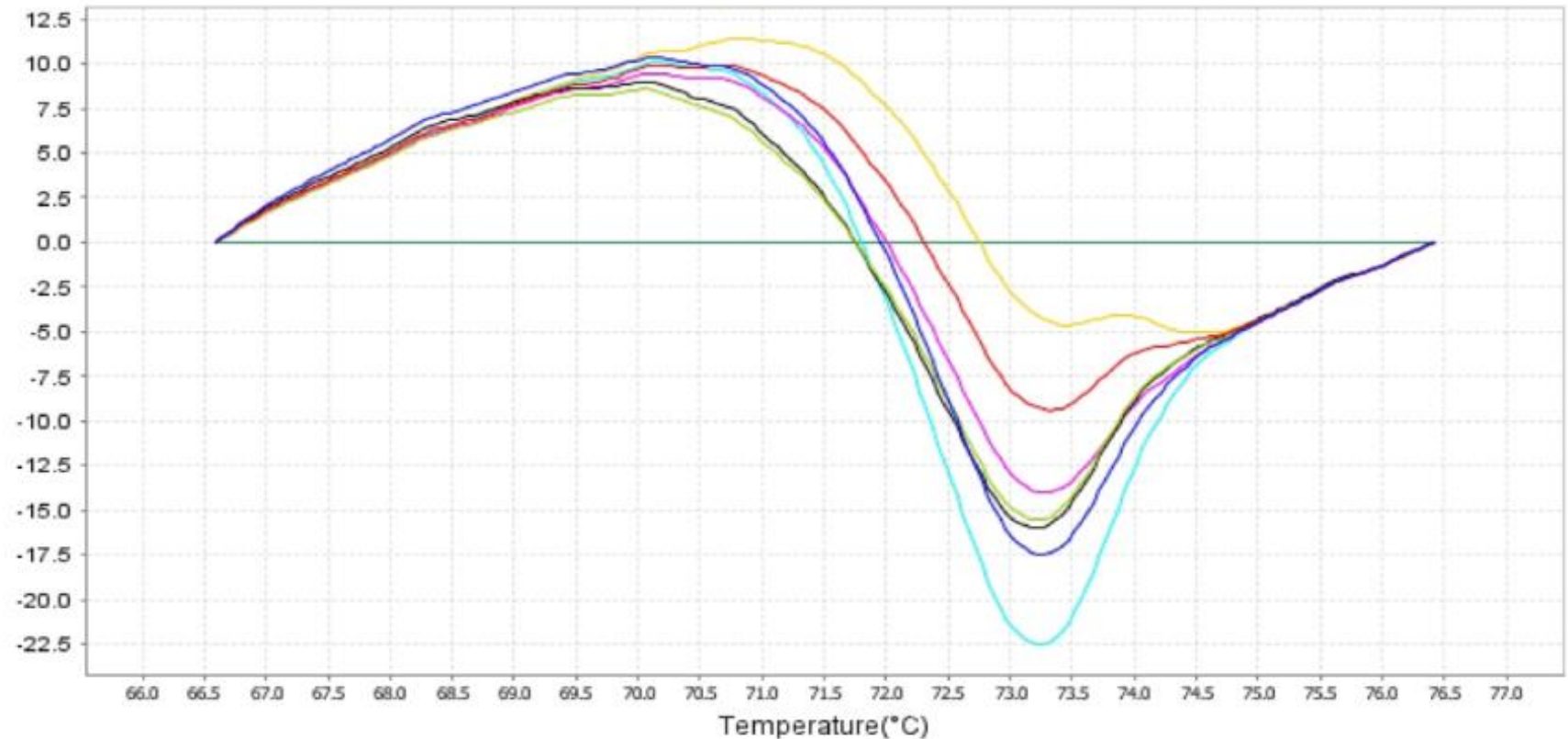


*secA*

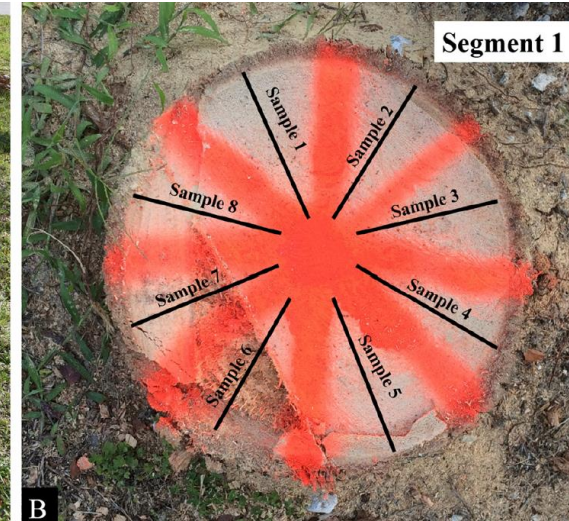


## HRMA for mixed infections

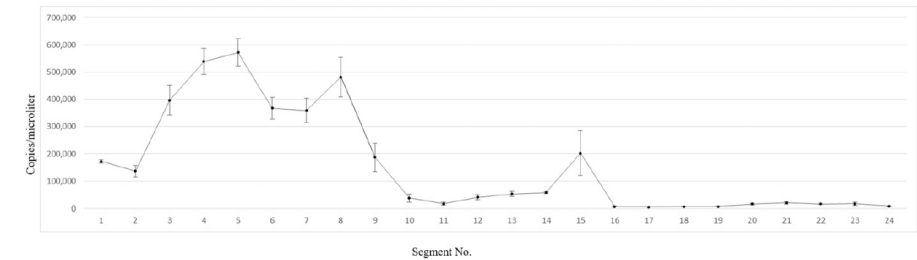
- LY
- E
- LB
- LY+LB
- LY+E
- LB+E
- LY+LB+E
- Healthy control



### Real world application/benefit for stakeholders

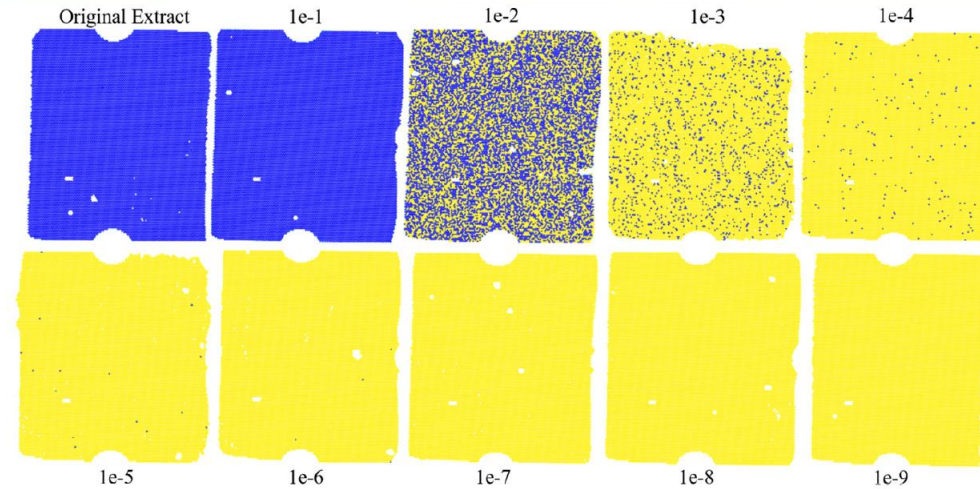


| <i>Syagrus romanzoffiana</i> |            |                       |
|------------------------------|------------|-----------------------|
| Sample                       | Ct         | Quantity <sup>a</sup> |
| Trunk                        | 27 ± 1.3   | 24,948 ± 12,582       |
| Leaf-1                       | No Ct      | 0                     |
| Leaf-2                       | No Ct      | 0                     |
| Leaf-3                       | No Ct      | 0                     |
| Leaf-4                       | No Ct      | 0                     |
| Leaf-5                       | No Ct      | 0                     |
| Leaf-6                       | No Ct      | 0                     |
| Leaf-7                       | 23.7 ± 0.5 | 118,160 ± 9,177       |



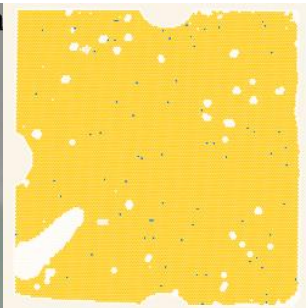
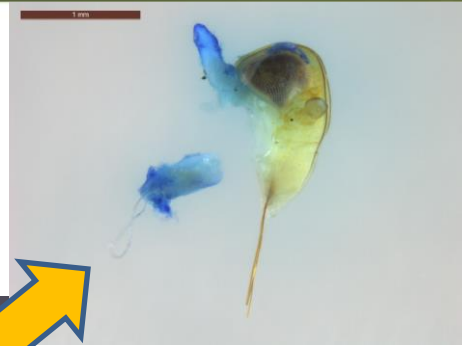
### Digital PCR

- Highly sensitive (100X more than qPCR)
- Versatile
  - Rapid vector Discovery
  - Early pathogen detection
  - More cost effective sampling and testing of large sample sizes.



| Dilution         | Cycle threshold (Ct) value <sup>a</sup> |                         |                 |               |
|------------------|---|-------------------------|-----------------|---------------|
|                  | 16SrIV-A                                | 16SrIV-D                | Healthy control | Water control |
| 1                | 18.9 ± 0.3                              | 16.5 ± 0.1              | No Ct           | No Ct         |
| 1e <sup>-1</sup> | 22.1 ± 0.2                              | 23.5 ± 0.1              | No Ct           | N/A           |
| 1e <sup>-2</sup> | 26.9 ± 0.3                              | 27.7 ± 0.02             | No Ct           | N/A           |
| 1e <sup>-3</sup> | 30.4 ± 0.2                              | 32.0 ± 0.1 <sup>b</sup> | No Ct           | N/A           |
| 1e <sup>-4</sup> | 34.8 ± 0.2 <sup>c</sup>                 | No Ct                   | No Ct           | N/A           |
| 1e <sup>-5</sup> | No Ct                                   | No Ct                   | No Ct           | N/A           |
| 1e <sup>-6</sup> | No Ct                                   | No Ct                   | No Ct           | N/A           |
| 1e <sup>-7</sup> | No Ct                                   | No Ct                   | No Ct           | N/A           |
| 1e <sup>-8</sup> | No Ct                                   | No Ct                   | No Ct           | N/A           |
| 1e <sup>-9</sup> | No Ct                                   | No Ct                   | No Ct           | N/A           |

## Vector discovery with dPCR



# Vector discovery



*Oecleus mackaspringi*

## *Haplaxius*

*crudus*

*jamaciae*

*meadi*

*skarphion*

*pocococo*

*douqwalshi*



## Summary

- Development of strong molecular diagnostic assay is essential for developing management programs.
- Tools are easy to modify for dealing with different pathogen types.
  - Work on viruses, bacteria, fungus.
- qPCR and dPCR significantly expedite pathogen detection and identification, allowing faster dissemination of data to growers.

## Contact Information

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# Thanks