Date: Dec. 12, 2017 **CCNB Project #:** Yok-17

Project Title: Improved sensitive detection of the HLB pathogen by new gene primers for real time and droplet digital PCR

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2017 Progress Report

The RNR primer/probe (Zheng et al. 2016) was tested with "*Candidatus* Liberibacter asiaticus" (*C*Las) DNA from California and Florida and found to be ~1.5x more sensitive than 16S rRNA Primer/probe in detecting *C*Las in both real time PCR (qPCR) and droplet digital PCR (ddPCR) from infected citrus tissue (Fig. 1) and bacteriferous Asian citrus psyllid (ACP) (Fig. 2) DNA samples. ddPCR results showed exact low copy number of *C*Las targets in plant and ACP samples were better than qPCR Cq values in making positive *C*Las calls (Fig. 3) (Maheshwari et al. 2018). Since our method includes both 16s and RNR targets in a simultaneous and duplex reaction, the results are double the confidence of a singleplex tests.

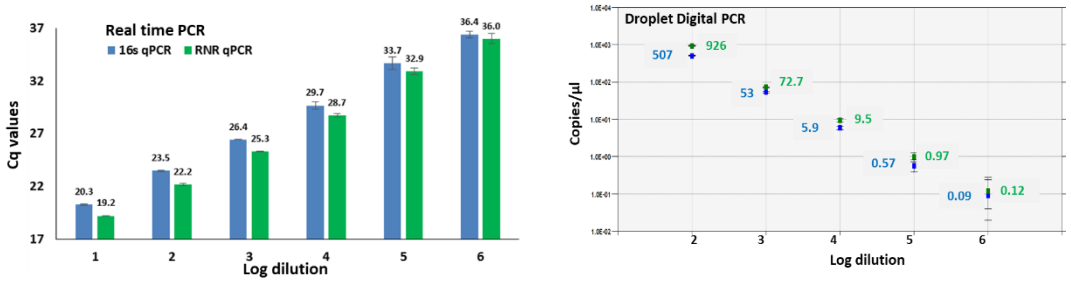

Fig. 1 *C*Las detection by RNR primer/probe compared to 16srRNA primer/probe in citrus tissue in real-time PCR (qPCR) and droplet digital PCR (ddPCR).

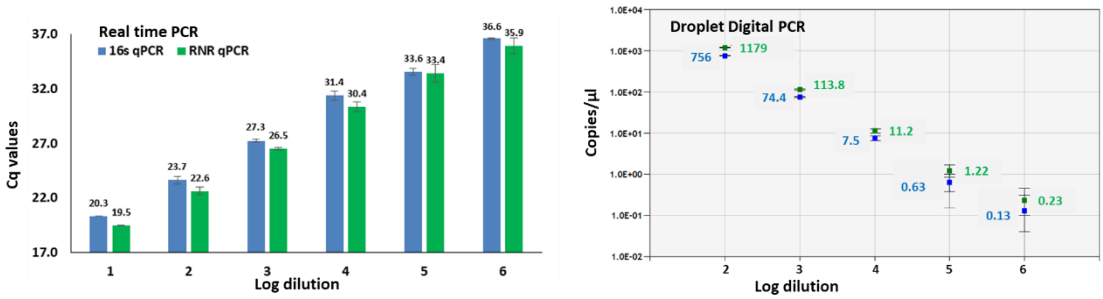

Fig. 2. *C*Las detection by RNR primer/probe compared to 16srRNA primer/probe in Asian citrus psyllid in real-time PCR (qPCR) and droplet digital PCR (ddPCR).

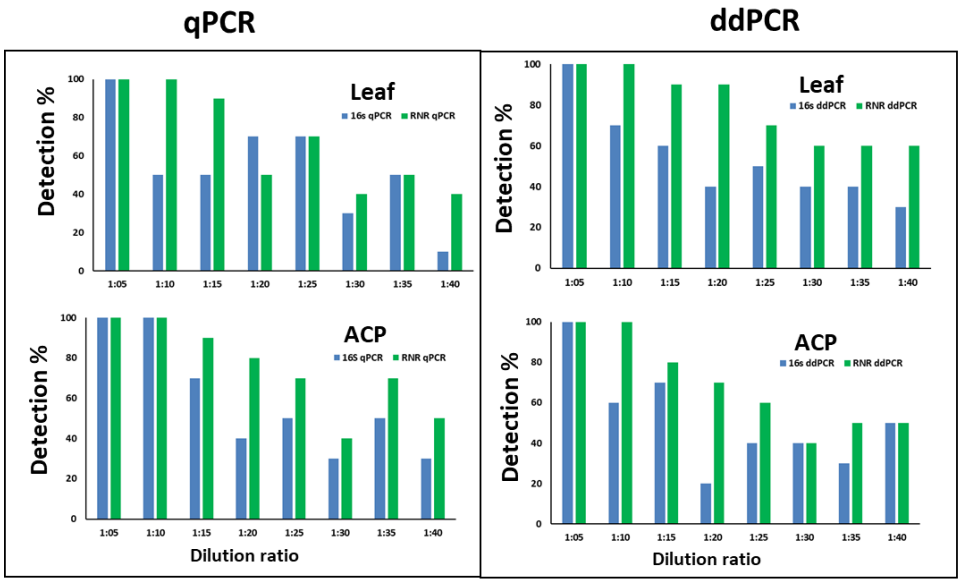

Fig. 3. Detection of *C*Las from serial dilutions of low titer infected DNA samples (Cq 33.5-37.0) from leaf tissue and Asian citrus psyllid by real-time PCR (qPCR) and droplet digital PCR (ddPCR). Percent detection per dilution is based on the number of positives out of ten replications.

Nine *C*Las strains from six southern California cities were analyzed by NGS. No variations detected in 16S rRNA or RNR gene sequences were found. All California strains were of Asiatic, not Florida, origin, based on prophage sequences. California *C*Las populations fell into three prophage groups: Type 1 (SC1) - Anaheim, Cerritos, San Gabriel, and Riverside; Type 2 (SC2) - Hacienda Heights: Type 1 & Type 3 (P-JXGC-3) – Cerritos (Zheng et al. 2018, Dai et al. in press). The RNR sequences of the California *C*Las populations were also compared to those available in the worldwide sequence database for *C*Las and found to be conserved. This information is critical to certify that the RNR sequences of *C*Las can be used to detect all populations of *C*Las regardless of origin. Population differences, however, can be distinguishable by it prophage sequences and, in the future, it may be important for certification programs.

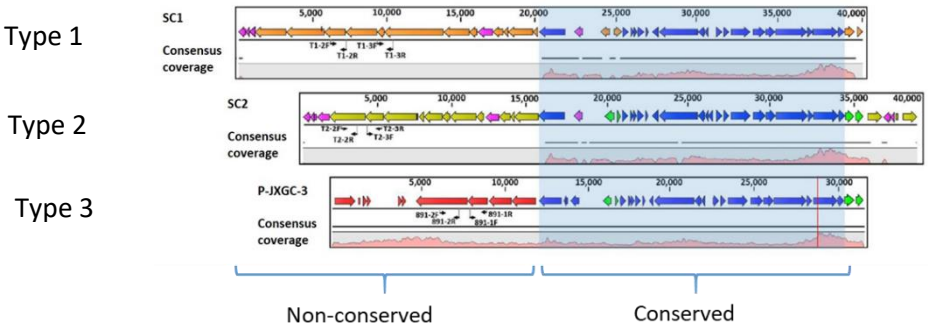

Fig. 4. HiSeq read map of "*Candidatus* Liberibacter asiaticus" (CLas) prophages of SC1 (Type 1), SC2 (Type 2), and P-JXGC-3 (Type 3). Prophage Type 3 carries a restriction-modification system). Based on this map, California *C*Las populations were of Asian, not Florida, origin.

A good question raised during the CCNB Research Committee meeting in Exeter on Dec. 8, 2017 pertained to potential duplication of effort by the USDA, APHIS, PPQ, CPHST and CDFA laboratories with Yok-17 to certify the RNR primer/probe protocol for *C*Las detection. Our efforts are coordinated and we are working together. See Dai et al. (In press) which involves personnel from the CPHST and CDFA labs.

2018 Plans. Obtain CLas DNA from Texas and abroad as well as other liberibacters (e.g. *Ca.* L. africanus, *Ca.* L. solanacearum) and phytoplasmas to test for inclusivity/exclusivity with the RNR primer/probe for *C*Las. We will submit a manuscript based on Yok-17 data to a refereed journal and will work with CPHST and CDFA to certify the methods developed.

References cited.

Dai, Z., Wu, F., Zheng, Z., Yokomi, R., Kumagai, L., Cai, W., Rasco, J., Polek, M., Deng, X. and Chen J. 2018. Diversity of "*Candidatus* Liberibacter asiaticus" strains in California. Abstract for the Intnl. Congress of Plant Pathology. Phytopathology (in press), with support from Yok-17.

Maheshwari, Y., Selvaraj, V., Hajeri, S., Yokomi, R. 2018. Application of duplex droplet digital PCR for detection of "*Candidatus* Liberibacter asiaticus" using 16S rRNA and ribonucleotide reductase genes. Abstract for the Intnl. Congress of Plant Pathology. Phytopathology (in press), with support from Yok-17.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., and Deng, X. 2016. Unusual Five Copies and Dual Forms of *nrdB* in "*Candidatus* Liberibacter asiaticus": Biological Implications and PCR Detection Application. Scientific Reports 6:39020 DOI: 10.1038/srep39020.

Zheng, Z., Bao, M., Wu, F., Van Horn, C., Chen, J., and Deng, X. (2018). A Type 3 prophage of "*Candidatus* Liberibacter asiaticus" carrying 1 a restriction-modification system. Phytopathology (In press).