CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Detection probe design complete; testing initiated

Proposal Title

Development, validation, and deployment of rapid, inexpensive, multiplex assays for simultaneous detection and strain characterization of multiple citrus pathogens

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/8/11	California Citrus Research Board	Pathogen/Disease Detection

REPORT UPDATE (500 words; summarize your accomplishments)

We have completed the target selection and design of MOLigo probes for detection of Candidatus Liberibacter, Xylella fastidiosa, Xanthomonas axonopodis pv. citri, and citrus tristeza virus. Characterization probes for Liberibacter have also been designed, and testing of all Liberibacter probes will begin this week using synthetic DNA targets. Because there has been a large influx of new Liberibacter sequence data since probes were designed, we have initiated an in silico evaluation of all probes to determine if they will detect and characterize the new isolates.

We met with Dr. John Hartung of USDA ARS in Beltsville, MD and Dr. Bill Schneider of USDA ARS Ft. Detrick to discuss samples for the validation panels. Both have agreed to send purified nucleic acids from infected plants, insect vectors, and healthy plants. We will assemble these samples into our validation panels for testing once the assays appear to be working properly on synthetic targets.

We recently learned that Luminex plans to discontinue in January 2012 the type of non-magnetic beads that we are using to develop these assays. Because we plan to transfer the technology to service labs, we need to either locate another source of non-magnetic beads, or move our development efforts to the magnetic beads. We have contacted people at Luminex and Radix, a Luminex partner about the availability of tagged, non-magnetic beads past January 2012. We will proceed with development on the type of bead that will be available for the foreseeable future. The advantage of the magnetic beads is that they perform as well or better than the non-magnetic beads, and are the only beads compatible with Luminex's new, less expensive machine. This machine only has a 50-plex capacity, but costs around $35K, making it feasible to deploy our citrus assays to additional labs at lower startup costs. Our concern is that the magnetic beads will cost more, which will increase the cost of each assay. Still, it is anticipated that the total assay cost will still be a fraction of the cost per marker as compared to RT-PCR assays currently in use. We will continue to pursue options for tagged, non-magnetic beads.

Additionally, we have moved the oligo design software to a new server, which is online and accessible to us and the public at http://moligodesigner.lanl.gov . We are in the process of automating the multiplex function of the design tool, which will facilitate probe selection and assay design. This will make the iterative re-design of probes and assays should we encounter some probes that do not perform according to our specifications.

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Organization Los Alamos National Laboratory

Contract Number 5300-10K

Project Duration (years) 2 *Year of Project* 1

Total Funds (current year) $311,000.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.