CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

An electrochemical biosensor for rapid and reliable detection of citrus pathogens and pre-symptomatic diagnosis of citrus disease

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/10/13	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

The Main goals of this project are:
(i) to detect citrus pathogens from insect and plant samples
(ii) to perform pre-symptomatic diagnosis of citrus diseases caused by various pathogens.

Currently, the primary focus is on Candidatus Liberibacter that is transmitted by psyllid vector and causes Huanglongbing (HLB). It is extremely important to develop the capability for pre-symptomatic diagnosis of HLB because the disease symptoms appear years after initial Liberibacter exposure.

This project is a collaboration between Los Alamos National Laboratory (LANL) and Sharp Laboratories of America (SLA). LANL is responsible for discovery and validation of pathogen and pre-symptomatic biomarkers whereas SLA is responsible for the design of impedance biosensor for pathogen detection and pre-symptomatic diagnosis. LANL-SLA will jointly design the assays for pathogen detection and pre-symptomatic diagnosis.

During the first two months of this project, we carried out the following tasks.

Task 1. We primarily focused on detection of nucleic acids (DNA and RNA). For this, we measured the change in impedance upon the binding of oligonucleotide probe complementary to target DNA or RNA. In this detection scheme, the specificity is determined by the perfect complementarity of the probe to the target sequence. We further enhanced the specificity of detection by measuring the melting curve (i.e., the melting temperature and the first derivative of melting) for probe-target binding. The notion behind this is that the pattern of impedance change and melting curve would be specific for the perfect complementarity between a given target and its probe.

Task 2. Typically, probes are immobilized on the gold electrodes of the chip by thiol chemistry, which often does not guarantee uniform immobilization of the probes. To remedy that, we developed an alternative method in which we immobilized biotinylated poly-G tract on the gold electrode and constructed probes with poly-C tails. Thus all probes were immobilized with the same G-C pairing and therefore, we eliminated the problem of probe non-uniformity due to non-uniform thiol chemistry.

Task 3. We now have the access to two different Liberibacter asiaticus genome sequences (due to Duan Ping; and Hong Lin and Cliff Han) and two Liberibacter americanus genome sequences (due to Dean Gabriel; and and Hong Lin and Cliff Han). We identified genetic markers that are unique and common to them. We designed appropriate probes for the SLA impedance biosensor. We will soon test the presence of these plus the reference markers used by the CRB Riverside Laboratory in the psyllid samples.

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Project Number 5100-145

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $200.00

Form PR-12 Quarterly Reports **SUBMIT REPORT**

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