Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Genetics	4/15/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

6 EST libraries constructed, 2 libraries sequenced, assembled, and annotated; prelim tests for RNA-Seq

TITLE and CONTACT INFORMATION

Proposal Title The citrus psyllid transcriptome and time course differential gene expression in Ca. Liberibacter-infected/free whole psyllids and organs

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Organization The University of Arizona; University of Florida	*Total Direct Funds (current year)* $164,999 + sub 20,787

REPORT UPDATE (650 words; provide details about your headline)

Obj.1. The construction of six cDNA libraries have been completed from biologically relevant psyllid populations. Ca. Liberibacter was found (project #34) to be overwhelmingly present in guts but not in salivary glands, and liberibacter presence by qPCR in immatures and adults suggested that more information would be gained by focusing on whole adult- and immature instar liberibacter interactions, and on guts instead of salivary glands as originally proposed. The libraries were constructed from adult and immature psyllids, and dissected guts from psyllids, reared on HLB infected-infected and uninfected plants. Plants and psyllids from each treatment were tested by qPCR to confirm infection by, or absence of liberibacter (see report #21).

Obj. 2. All six cDNA libraries have been constructed and two have been sequenced and annotated. The other four are presently being sequenced using Illumina short base read technology. The PG and PI libraries were assembled with PAVE and consensus sequences were annotated and display on a web-based summary and query-system. The unique transcripts (UniTrans) singletons and contigs were blasted against the UniProt Invertebrate subset (db Jan 4, 2010) and blast hits with e-values of >1e-20 were accepted for annotation. The PAVE query system offers the ability to query the UniTrans db by characteristic (e.g. number of ESTs, Invertebrate UniProt hit, EST library composition, R test statistic, etc.) and to query the UniProt Invertebrate proteins that matched the UniTrans. Each UniTrans is allowed multiple protein hits (1e-20 or better) from different organisms. Multiple proteins from the same organism were matched to a UniTrans and only the top match was accepted from that organism ('non-redundant) match. Common proteins can be found in the UniTrans query system by asking for UniTrans with a high NRO count, indicating many organisms had a match to that particular UniTrans. The best annotation is defined as a protein matching a UniTrans with an e-value of 1e-40 and over 60% of the ESTs in the UniTrans matching the same protein with an e-value of 1e-10 or better. To assist with assignment of GO/GOSlim functions or pfam descriptions these annotations are added using the invertebrate matches. To search for protein annotation not yet known in UniProt's invertebrate taxonomic set the assembly is blasted against full UniProt. Because the psyllid assembly contains bacteria, which will not match proteins in the UniProt databases, we blasted contigs against NCBI's nucleotide database (Feb 10). Bast matches with e-values of 1e-20 or better were used from the full UniProt and from the nt databases.

Obj. 3. We proposed a plan that would better and more cost effectively allow us to carry out quantitative analysis of whole immature instars, versus whole adults, guts, and PSG/ASGs from time course exposure (AAPs) to HLB plants within a defined time frame (steady-state qPCR-based titer). This involves extensive direct sequencing of random cDNAs from HLB +/- stages, instars, and organs, and is proposed because the relative cost of sequencing has declined, as the extent of coverage vs. cost has increased. In this way we can more effectively compare expression levels between whole adults and immatures, and adult guts and SGs. To explore the number of guts and salivary glands that would be needed to produce sufficient RNA for RNA-seq quantitative sequencing (4-5 reps each time course AAP) we produced treatments of psyllids and dissected the guts and salivary glands from adult psyllids and isolated the RNA. We determined that 150 salivary glands and 75 guts would each yield about 1 ug of RNA, a sufficient quantity for quantitative analysis, per replicate per treatment. In the remainder of 2010 psyllids will be exposed to Liberibacter-infected citrus over a set of acquisition access periods and subjected to quantitative gene expression using RNA-Seq analysis.