

**PROJECT SUMMARY**  
**Texas Imported Fire Ant Research & Management Plan, FY 1999-00**

**Title of project:** Large Insect Gene Library for Analysis of the Fire Ant Genome

**Principal investigators:**

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**Funding amount/2 years:** \$100,000

**Summary of work to be done:**

Understanding of the biology of the imported fire ant and elucidation of mechanisms by which imported fire ants interact with native ant species can be greatly expedited using molecular genetic

techniques. Furthermore, directed intervention into biological processes for the control and management of imported fire ants necessarily requires precise molecular information about the fire ant genome, its organization and function. Presently, there is very little information available about the fire ant genome, and no genomic DNA libraries are in place to support molecular studies of the fire ant.

The goal of this project is to construct a large insert genomic DNA library of the imported fire ant that will be readily available to support a variety of studies of the molecular biology and genetics of this species. Originally, the library was to be constructed in the pBELO plasmid, a vector of the “bacterial artificial chromosome” or BAC type and consist of at least three genome equivalents of DNA arrayed in replicated sets of 6000 recombinant clones. DNAs will be purified and arrayed so as to facilitate library screening by PCR and by blot hybridization.

To characterize the properties of the library, we proposed to conduct experiments to determine the average insert size, test for unstable DNA clones and isolate 14 unique DNA sequences to verify the representative genomic content of the library. We also proposed to isolate clones of DNA containing R1 and R2 transposable elements as sources of species-specific DNA sequences necessary to develop molecular profiles that identify different species of fire ants and their hybrids. Additionally, we will collaborate with at least four other investigators to isolate specific genes of interest to their respective research programs.

#### **Progress Report:**

##### **Major accomplishments to date (Sept. 1, 1997 through August, 1999):**

In collaboration with the BAC Center of Crop Biotechnology Center (CBC), we have completed construction of a BAC library of genomic DNA from the imported fire ant. The ant DNA is inserted into the *Hind*III cloning site of the pECBAC vector a second generation derivative of pBELO. The library is arrayed in sixty 384 cell plates for a total of 23,040 clones. Preliminary characterization indicates an average insert size of 147 Kbp/clone and a frequency of “empty” vector clones = 3%. Assuming the genome size of the fire ant is  $3 \times 10^8$  bp of DNA, the coverage of this library is about 10 fold. The probability that any given sequence in the fire ant genome is represented at least once in the library is  $>0.95$ . The library has been replicated to provide two working copies (one each at CBC and CVM) and one archived copy at CBC. DNA from the library has been applied to four sets of high density filter arrays in duplicate for gene isolation by blot hybridization. The library is currently available for screening on a collaborative basis.

##### **Relevance/Implication of Project to the Texas Imported Fire Ant Research and Management**

**Plan:** The proposed research addresses the key area of Genetic and Molecular Manipulation as identified by the Fire Ant Research & Management Plan.

The large insert DNA library of the Fire Ant is a research resource available to scientists seeking to characterize specific genes of interest, develop methodologies for gene transfer, and to analyze population structure and evolution of imported fire ants. The ultimate objective is to use this information to devise strategies to control fire ant populations by genetic intervention.