Discovery of Stress-responsive Cynodon *Genes by cDNA Sequences & Expression Profiles*

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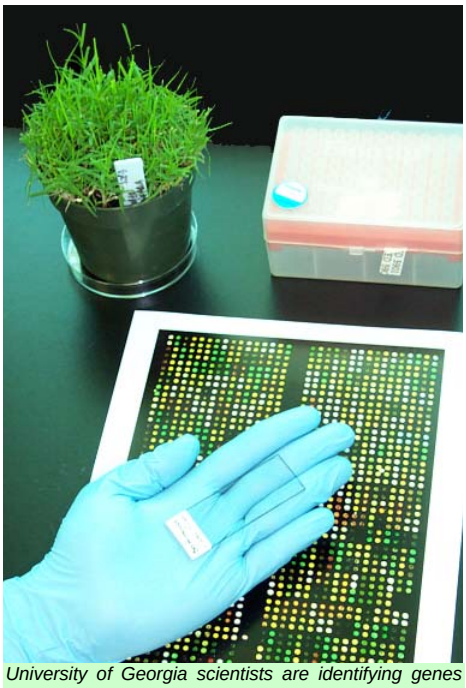
Objectives:

1. Build the first Internet-accessible "gene encyclopedia" for *Cynodon* (bermudagrass).
2. Identify genes responsible for natural adaptation of different *Cynodon* taxa to diverse environments, especially genes associated with drought resistance.

Start Date: 2003
Project Duration: three years
Total Funding: $90,000

Building on our development of the first "genetic map" of the *Cynodon* chromosomes, we will conduct the first large-scale exploration of the "sequences" (spelling) of large numbers of genes in any turfgrass, and use these resources to identify genes that are turned on or off in response to environmental stresses, emphasizing drought.

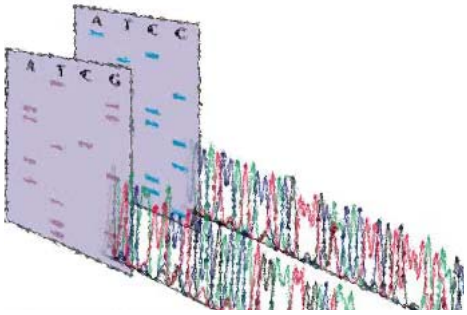
Our analytical approach will leverage a large body of existing data from other plants to determine the functions of many *Cynodon* genes. We will also leverage recent NSF grants to Dr. Paterson of $4 million for grass genomics, and $600,000 for informatics, in applying bioinformatic methods to the *Cynodon* ESTs, and making the resulting information available to the research community.



University of Georgia scientists are identifying genes responsible for natural adapataion of different Cynodon *taxa to diverse environments, especially genes associated with drought resistance.*

At present, very few gene sequences are known in any turfgrass. The world's leading DNA sequence repository, GenBank, presently contains only 269 *Cynodon* sequences, as compared to 247,980 for maize and 109,878 for sorghum. Our lab provided about half of the *Cynodon* gene sequences that are in GenBank. This project will increase the number of *Cynodon* gene sequences by about 15-fold to about 4,000.

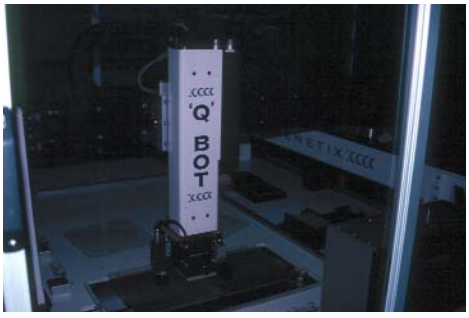
We are engaged in the first crucial step - the production of a high-quality cDNA library for the *C. dactylon* genotype T89. A new graduate student, Mr. Changsoo Kim, is working with a senior graduate student (Neil Skinner) and an Assistant Research Scientist (Terry Kamps) toward this goal.



Univeristy of Georgia researchers are building the first Internet-accessible "gene encyclopedia" for Cynodon *(bermudagrass).*

The primary cDNA library will be constructed using Poly (A)+ RNA cloned directionally in the 3' to 5' orientation at the EcoRI-XhoI restriction sites of the Lambda Zap II vector (Stratagene). Recombinant plasmid clones (in pBlueScript) will be obtained from the primary phage library by *in vivo* excision and blue/white screening.

The use of primary libraries will be employed to obtain a better representation of the genes for a particular tissue and reduce the redundancy of clones that will be chosen for sequencing. The libraries will then be amplified once, amplified



At University of Georgia, Dr. Andrew Patterson uses new robot technology to perform some of the mundane tasks required to create a genetic map of bermudagrass chromosomes.

samples frozen in small aliquots at -80 C, and stored for future distribution to other interested researchers.

Once we have a library of satisfactory quality, 18,432 clones (48 384-well plates) will be picked using a Genetix 'QBOT' (in the Paterson lab). These will be replicated and maintained as a long-term resource that will also be available to other researchers. A minimum of 4,608 clones will be sequenced and subjected to comparison to one another and to known sequences from other organisms. We expect these steps to be complete by the end of next year.

Summary Points

- At present, very few gene sequences are known in any turfgrass. The world's leading DNA sequence repository, GenBank, presently contains only 269 *Cynodon* sequences, as compared to 247,980 for maize and 109,878 for sorghum. This lab provided about half of the *Cynodon* gene sequences that are in GenBank. This project will increase the number of *Cynodon* gene sequences by about 15-fold to about 4,000.
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