Conifer Translational Genomics Network (CTGN – CAP)
Quarterly Progress Report
Year 2 / Quarter 1
October 1 – December 31

General Progress / Administration

Continuous progress was made on scientific objectives 1 and 2: all Cooperative project members are completing selection of study materials, collecting samples for DNA extraction, and actively collecting phenotypic data on their populations. The DNA extraction laboratory at UC Davis is operational and the LIMS System to handle incoming samples and data generated is in place. Patrick McGuire has all but joined the team (official start date of Feb. 1, 2009) as our new project coordinator. He has already made many significant contributions to our workshop preparation and organization. We look forward to his experienced and steady hand in the months to come.

Objective 1: Validate previously discovered SNP by trait associations in Cooperative operational tree improvement populations. (All Partners)

North Carolina State University –

<u>Current Progress</u> - Foliage sampling for DNA extraction and SNP markers continues and is about 35% complete. Permission (via Material Transfer Agreements) has now been received from all cooperators allowing us to collect the remaining 1800 tissue samples. In addition, we have gained access to many selections that we originally deemed "lost" due to some state agencies and companies dropping their tree breeding programs.

NIR spectroscopy work has been completed on the 170 clones collected from replicated trials. A total of 1600 samples were scanned and percent lignin and cellulose content has been estimated for each clone. Of the 170 clones, a subset of 20 clones will soon be selected to be analyzed by the NCSU Wood and Paper Science Department for biofuel conversion efficiency. In addition to lignin and cellulose content of the 170 clones, growth and stem taper assessments have been completed which will add beneficial data to the clone phenotypes. Foliage samples of all of these clones have been collected.

In the Atlantic Coastal Elite Population, a total of 3500 hedges are being managed. The total number of crosses among the 25 elite selections has been reduced from 76 to 51 based on fusiform rust infection levels at the USDA Resistance Screening Center. To date, there has been little additional mortality from the potting/hedging process. Cuttings will be collected in February 2009 for clonal testing.

A template has been developed for submission of NCSU CTIP phenotypic data to the UC Davis database in connection with the CTGN. Phenotypic data for growth parameters (height, diameter, crown form, and stem straightness) will be expressed as percent improvement over wildtype, while values for wood properties will be expressed in absolute terms. All phenotypes will be "best linear unbiased predictions" of the genetic value of each individual tree, based on test data from progeny and relatives.

Jaime Zapata, the PhD student supported by the CTGN at NC State, has successfully completed his first semester of course work, and has also been working in the laboratory to gain experience in molecular marker technologies.

<u>Anticipated Activities Yr2Q2</u> – With all the material transfer agreements in hand, we anticipate completing all tissue collection across loblolly pine tree breeding populations managed by NCSU.

In February, we will begin to take cuttings from the Atlantic Coastal Elite hedges. We plan on taking ~36,000 cuttings in this first round of cuttings. Rooting success of 50+% is achievable and would result in ~18,000 ramets that will be put into field tests in late 2009. These are progeny of parents that are to be genotyped as part of the CTGN and will serve as the verification population to test the utility of molecular marker associations identified in the CTGN for prediction of phenotypic value of progeny in a breeding population.

Stress wave measurements will be taken on the remaining Lower Gulf Elite Population tests to produce wood stiffness values useful for prediction of breeding values of parents in our program.

University of Florida –

<u>Current Progress:</u> MS student Patricio Munoz continues with phenotyping the BC1 population. In collaboration with John Butnor (USDA FS), Patricio has completed taking digital photographs for use in quantifying aboveground biomass area and efforts are being made to allocate leaf area. Further, phenotypes taken on the BC1 in December include height, diameter of the terminal, needle length, branch angle, number of nodes and number of branches. Detailed analysis of phenotypic data will begin in January.

Collection of wood cores and DNA samples from slash pine full-sib block plots has been delayed until January because of the difficulty in obtaining cardboard tubes for storing the cores. A vendor has been located and the tubes ordered for delivery by the end of December.

Work continues on applying Bayesian models to SNP detection. A simulation is now taking place to determine the level of false positives and power to detect given known effects and frequencies for genotypes.
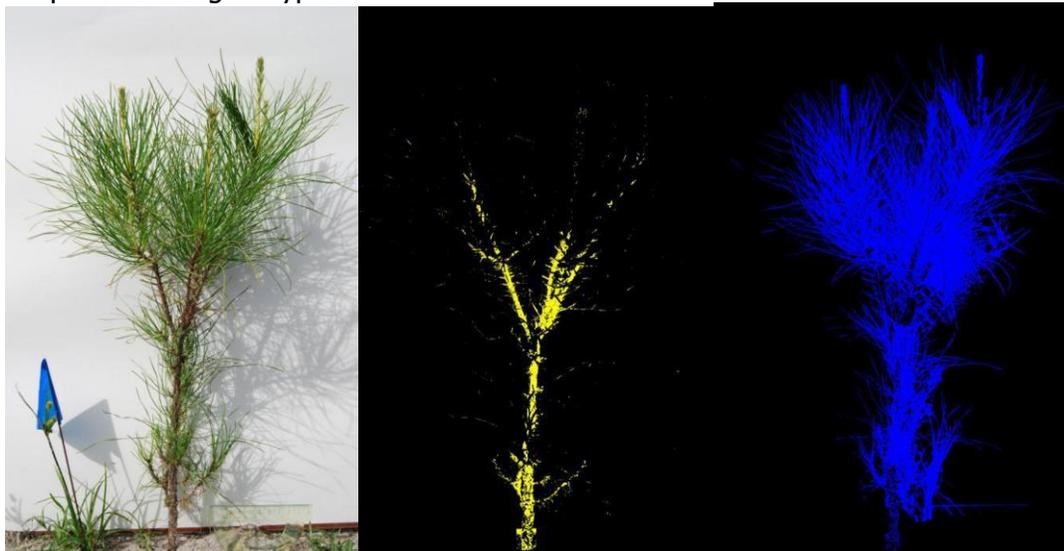

Fig. 1a   Fig. 1b   Fig. 1c

Figure1. Panels (a,b,c) represent alternatively filtered digital photos of test trees that will serve to estimate above ground biomass.

Oregon State University –

Current Progress: Glenn Howe and Jianbin Yu completed the development of the phenotypic module of the *Tree Genome Simulator* (see attached figures). This module is now being proofed and tested and we've begun integrating it with the genotypic module. The genotypic module now simulates simple SNP haplotypes from existing SNP data. Further development of the genotypic module will allow us to simulate more complex haplotypes, including combinations of intergenic and genic regions, and coding and non-coding sequences. The *Tree Genome Simulator* is being designed for both teaching and research. The program functions via a graphic user interface (GUI) (see Figures 1-4).
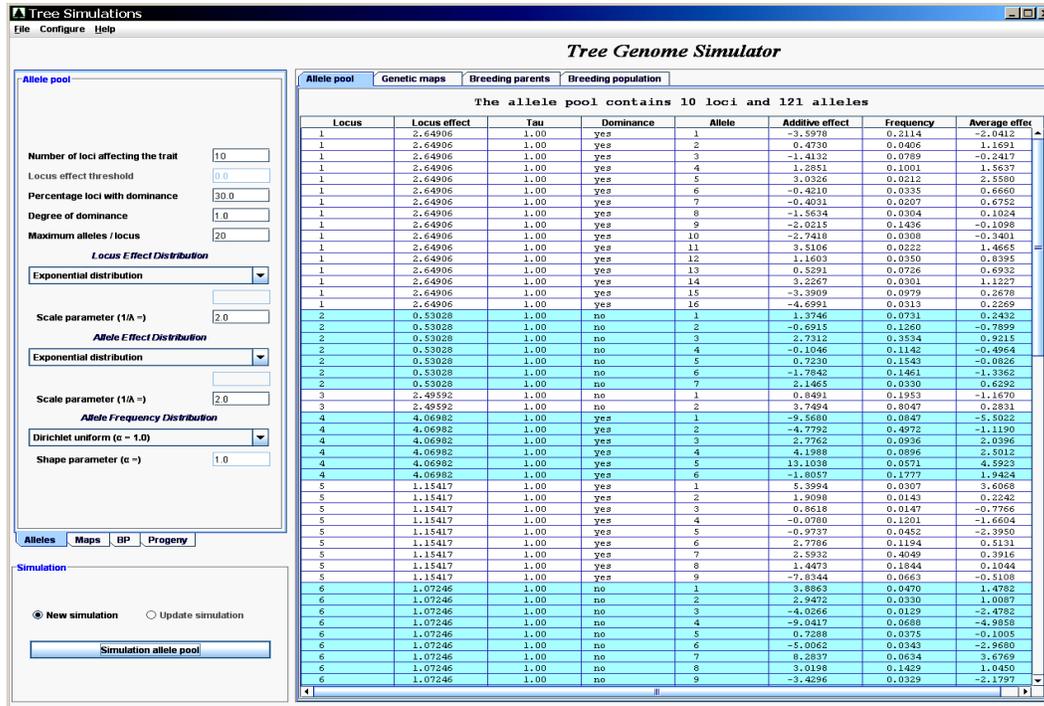

Figure 1. Screenshot of the *Allele* submodule of the *Tree Genome Simulator.* Using this submodule, one can vary the number of loci affecting the trait, percentage of loci with dominance, degree of dominance, maximum number of alleles per locus, distribution of locus effects, distribution of allele effects, and distribution of allele frequencies.
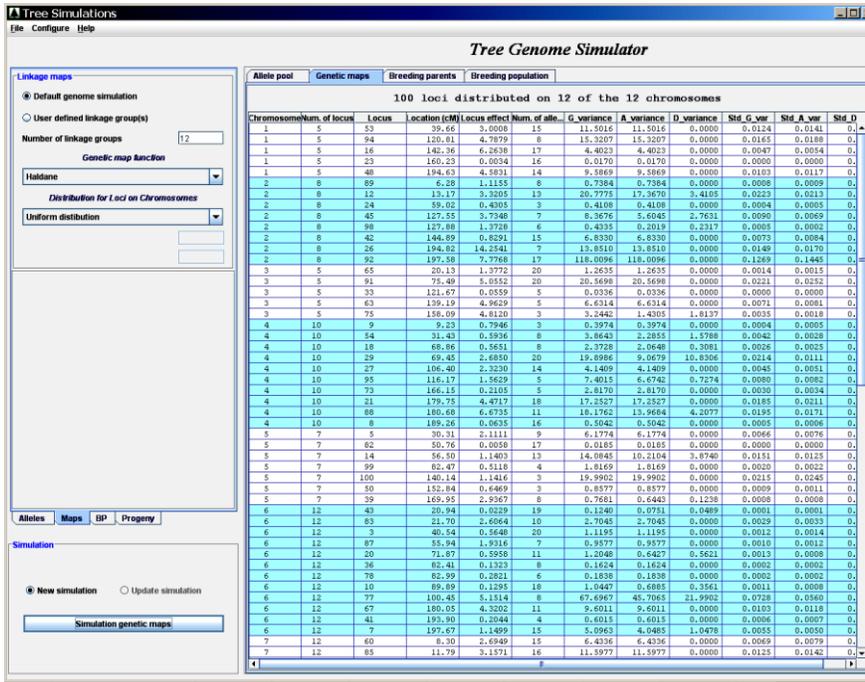
Figure 2. Screenshot of the *Maps* submodule of the *Tree Genome Simulator*. Using this submodule, one can vary the number of linkage groups, genetic map function, and distribution of loci.
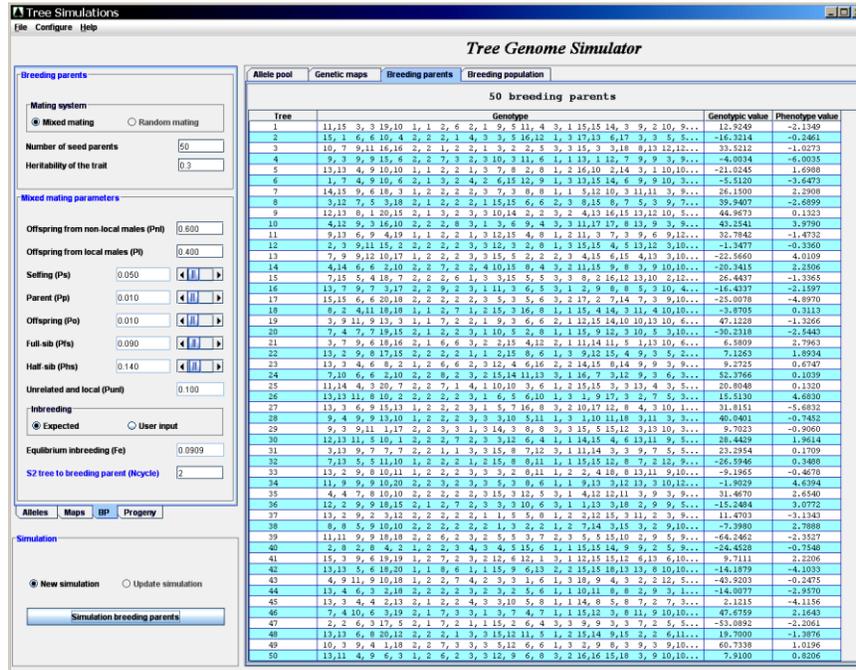

Figure 3. Screenshot of the *Breeding parents* submodule of the *Tree Genome Simulator*. Using this submodule, one can vary the number of breeding parents to simulate, the heritability of the trait, and a wide range of mixed mating parameters to simulate open-pollination in natural populations.
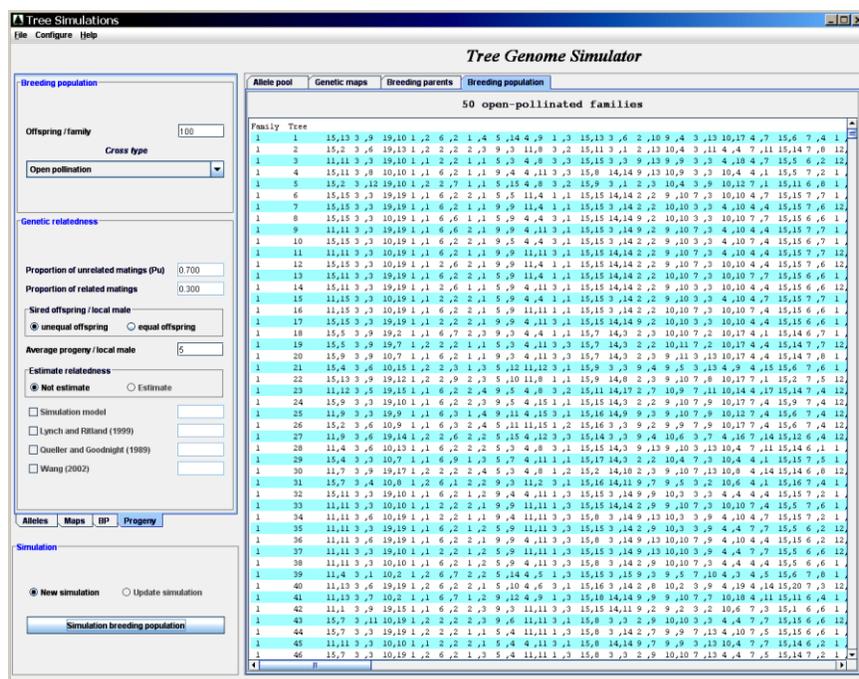
Figure 4. Screenshot of the *Breeding population* submodule of the *Tree Genome Simulator*. Using this submodule, one can simulate the open-pollinated progeny of the breeding parents using a wide range of mixed mating parameters.

We measured cold hardiness of progeny from 141 field-tested parents from the Douglas-fir Puget Sound breeding program using artificial freeze tests (Anekonda et al 2000; W. J. Appl. For. 15:129-136). In collaboration with the Washington State Department of Natural Resources, trees from two sites were tested using six trees per family per site, and two branches per tree (i.e., 2 samples/tree x 6 trees/family x 141 families x 2 sites = 3384 total samples). Site 1 (Matlock) was pre-tested the week of October 6, and the full test was completed the week of October 13. Site 2 (Buckhorn) pre-tested the week of October 27, and the full test was completed the week of November 3.

Texas A&M University –
Current Progress: ASREML software was acquired and TI staff began familiarizing ourselves with the analytical techniques required to generate BLUP values. This included attending the training session sponsored by NC State. Reanalysis of a subset of the East Texas loblolly pine database that will be contributed to the CTGN database is underway.

Foliage samples from 96 loblolly pine clones were sampled from the Texas Forest Service Fastrill Scion Bank and submitted to the UC Davis lab as a test sample to work through our field sampling protocol.

Anticipated Activities Y2Q2: 1) Complete foliage sampling for the E TX parental breeding population. 2) Complete data analysis for the individual tree breeding values and begin uploading phenotypic data. 3) Complete user interface for the Value Simulation Model.

Objective 2: Develop and economically evaluate new methods incorporating marker-assisted selection into conifer tree breeding programs. (TAMU / Wheeler)

Current Progress: The user interface for the Value Simulation of Alternative Breeding Models continues to be developed. The evaluation process for determining how markers would be incorporated in a generic tree improvement program has been incorporated into Module 8 of our upcoming summer workshop.

Future Activities: The value simulation tool will be demonstrated at the summer workshop, and will be refined to allow for individual extension presentations to all cooperative members. A peer-reviewed paper will be written later this year by Wheeler and Byram that will capture all aspects of Objective 2. We anticipate that a contribution to the eXtension effort headed by SolCAP will provide a forum for us to further describe the Value Simulation model and how it is run on Semitar.

Objective 3: Develop databases (TreeGenes) and web-based tools to facilitate all aspects of the CTGN project.

UC Davis –
Current Progress: Substantial progress has been made along several fronts.

CTGN website—has been updated with application information and active forms for students to apply to the June 2009 shortcourse.

The Plone—continues to be updated with relevant files including reports, project status, and presentations.

The Genetic Stock Center—sample tracking interface is complete including database backend. The interface allows cooperatives to login and provide phenotypic data for each sample submitted for genotyping. The genotyping portion of the interface is in final development stage. Developers are finalizing virtual plates that will be used to direct DNA extraction and plate population for delivery to Illumina.

TreeGenes and DiversiTree—development continues to prepare for the display of genotypic and phenotypic data.

Future Activities: As described above, several projects are entering final developmental stages and should be implemented soon.

Objective 4: Develop an international genetic stock center for conifers

Douglas-fir – The Douglas-fir graft-compatible root stock seedlings were moved out of the greenhouse in the fall to shut them down and harden them off. The seedlings will need to be grown for another growing season before they are large enough for grafting. In January we will do an inventory and sow a few more seeds in the winter to have extra seedlings for grafting should some of the first grafts fail.

Objective 5: Develop an education plan for graduate curriculum in genomics-based breeding for forest trees. (Harry, Wheeler, Howe)

Oregon State University -
This was a busy and eventful quarter, highlighted by excellent progress on developing workshop teaching modules (summer shortcourse) and planning, coordinating, and establishing a graduate level course on association genetics at Oregon State University.

Association Genetics and Breeding 599/699 – Harry and Howe, with assistance from Wheeler and Co-PIs from the BarleyCAP, WheatCAP, and SolanaceaeCAP projects developed this graduate level course which is now being delivered (Winter Quarter, 2009) at OSU with an enrollment of 10 students and several observers. Students come from Forestry, Crop Science, and Animal Science backgrounds. See syllabus and website at
http://www.cof.orst.edu/cof/fs/clpg/).

Shortcourse/Workshop "Genomics in Tree Breeding and Forest Ecosystems –
Draft materials have been completed for Modules 1,2,5-8. Howe has worked closely with Harry and Wheeler to facilitate module development, including the hiring of students and science communications specialist (Megan McKenzie) to frame and format teaching materials (PPT). A graphics designer (Greg DeVeer) was used to help create Powerpoint Templates for formatting slides. The designer also created a shortcourse marketing PDF that has been distributed widely to advertise the class. The poster (8" x 11") was posted at the recent PAG meeting. A webpage link on the CTGN site has been completed and is ready to accept student applications for the shortcourse (http://www.pinegenome.org/ctgn/workshop.html). Due to computer classroom space limitations, we can only accept 30 students. Thus, we will have a two-stage registration process where applicants will be screened and project personnel will determine who will be accepted for the course. Those accepted will then complete a registration which will include obtaining baseline evaluation information on the student's current level of knowledge about genomics and applications thereof.

Anticipated Activities Y2Q2 - The graduate course will be completed, and students will be asked to complete both OSU and project evaluation instruments. Workshop modules will be further developed; all modules should be in various stages of review and revision by the end of this quarter.

Objective 6: Develop extension plans for continuing education in genomics-based breeding for practicing tree breeders and forest tree gene resource managers, and develop education and extension evaluation protocols for all activities.

Extension – Project personnel participated in 6 education and extension presentations (see Table 1).

Anticipated Extension Activities - Extension presentations will be given to each of the tree improvement cooperatives this year. Meetings have already been scheduled: Florida Coop CFGRP(1st week in May, Macon, GA), Western Gulf Coop WGRTIP (2nd week in May, Mississippi), NCSU Coop (ICTIP; 3rd week in May). This year's presentations will be 2 to 3 hours in length and will focus on delivering the fundamentals of indirect selection, markers and their various applications in tree improvement, and a brief introduction to the concept of association genetics.

Table 1. Education and Extension presentations, Yr2Q1

Activity	Presenter/Author	Title	Date/Location	Venue/Audience
Workshop	Isik, F. (host and	Advanced ASReml	October 6-10,	Advanced workshop

	organizer) with Drs. Greg Dutkowski and Richard Kerr, quantitative geneticists from PlantPlan Genetics, Australia	Workshop	2008, NC State Univ., Raleigh, NC	on the theory and use of ASReml to predict breeding values, including the use of SNP markers. 25 scientists, breeders, and students from throughout the US
Guest lecture	Harry, D.	Population and Evolutionary Genetics	October 10, 2008. Oregon State University	Non-science undergraduates in Forest Biology (FS 240), ~35 students.
Workshop oral presentation	Wheeler, N.	Education, extension and outreach activities in our genomics projects (CTGN, ADEPT2)	November 17, 2008	Arborea2 Stakeholders Semi-Annual meeting. Guest speaker. 25 attendees.
Workshop	Isik, F. and W.P. Cumbie	ASReml Workshop for beginners	December 8-9, 2008, NC State Univ., Raleigh, NC	Workshop for beginning users of ASReml to predict breeding values. The use of SNP markers was included. 22 scientists, breeders, and students including breeders from 5 company and state members of the Cooperative
Oral Presentation	Whetten, R	Conifer Translational Genomics Network (CTGN)	December 10, 2008. NC State Univ., Raleigh, NC	NC State University Cooperative Tree Improvement Contact Meeting. 54 attendees, mainly members of the Cooperative
Field activity	Howe, Glenn	Field and lab assessments of cold hardiness	December	Assisted employees of the Washington State Department of Natural Resources to measure Douglas-fir cold hardiness

Extension and Education Evaluation –

The CTGN evaluator, Michael Coe, completed a document which summarizes results of surveys and interviews conducted in 2008 with coop staff and membership of the five tree improvement cooperative programs participating in our CTGN project. The report includes baseline information about knowledge, attitudes, skills, and behaviors related to the use of genetic markers in tree selection and breeding and related forestry activities. The complete report, which exceeds 26 pages in length, including forward and summary, will soon be posted to the Plone (see http://dendrome.ucdavis.edu/TGPlone/ctgn/reports/evaluation-reports). The report's summary of findings is excepted below:

"This report contains a summary of survey and interview data collected during 2008 from the staff and membership of five tree improvement cooperative programs that are participating in the Conifer Translational Genomics Network (CTGN). The survey includes baseline information about knowledge, attitudes, skills, and behaviors related to the use of genetic markers in tree selection and breeding and related forestry applications. Survey respondents included all professional staff members of the five participating tree improvement cooperatives, as well as 42 individual representatives of 33 member organizations from around the nation. Nearly 60 percent of coop staff members were also interviewed.

Staff members at the coops reported high levels of knowledge about general forestry, forest regeneration, tree improvement (including selection, breeding, and testing), production seed orchards and clonal forestry. Coop member representatives also reported high levels of knowledge about most of these topics, but were only generally familiar with genetic marker applications, whereas coop staff reported more familiarity with genetic marker applications, more training, and more ability to explain these methods to others.

Both groups had generally positive attitudes toward the development of applied uses of genetic marker research, though many respondents noted that more development is needed before the potential for practical applications can be realized. Interviews revealed a need for additional attention to economic constraints on the application of genetic markers to industry practices. In addition to further research and development, both groups indicated that they would need additional professional development and information resources in order to be confident in applying genetic markers in practice, or explaining their application to others.

As may be expected given the early stage of development in the field, only a minority of respondents indicated having used genetic markers in applied settings to date. Less than a third of respondents expect that they or their organizations will use genetic markers in their operations this year. Nevertheless, the staff and members of the cooperatives view genetic markers as having potential utility over the long term. Respondents were enthusiastic about participating in additional training and gaining access to additional informational resources, and they believe that their organizations will benefit from these developments.

When asked for recommendations about the content or format of future professional development opportunities or information resources, survey respondents offered a number of suggestions. Recommendations were also solicited regarding practical

problems that might be usefully addressed with genetic marker methods. Details of their recommendations, as well as details on all survey topics, are available in the report.

The CTGN 2008 Cooperative Extension Survey was developed with input from the project Principal Investigators and key staff, and was pilot tested during spring 2008. The revised version, administered during summer and fall 2008, was used to gather the baseline data in this report. Data from the items within each survey module are analyzed in detail in the report, and are also combined to produce scale scores for each topic (background, knowledge, attitudes, skills, practices, interest in further learning). Future annual surveys will be used to monitor progress on the project goals for extension audiences, in conjunction with interviews and other data."