2010

1st Annual Scientific Advisory Board Review Meeting
on North Carolina State University
NSF Plant Genome Research Program Project
May 6 & 7, 2010
Raleigh, NC

Board Members

Wout Boerjan, Ghent University
Richard Dixon, Samuel Roberts Nobel Foundation
Catherine Lapierre, AgroParisTech
Debra Mohnen, University of Georgia
Ernest Retzel, National Center for Genome Resources

Project DBI-0922391
(Sept. 2009 – Sept. 2013)

Regulation and modeling of lignin biosynthesis

(Progress report: September 15, 2009 to April 30, 2010)

PI
Vincent L. Chiang, NCSU

CoPIs
Ron Sederoff, NCSU
John Ralph, UW, Madison
Joel Ducoste, NCSU
Fikret Isik, NCSU

Room 246, Monteith Research Center, Centennial Campus

AGENDA

May 6

08:00 am Transportation of Board Members from hotel to meeting location

08:30 am Welcome
Introducing the Board Members
Overview of research, management, and project personnel

Vincent Chiang

Project Progress Report Presentations

Transcriptome:

09:15 am Full transcriptome analysis for candidate lignin biosynthesis genes and potential redundancy in *Populus trichocarpa*

Ron Sederoff, Rui Shi, Ying-Hsuan Sun, Quanzi Li, Steffen Heber, and Vincent Chiang

09:40 am Circadian and seasonal transcript and enzyme activity levels of candidate monolignol genes in *P. trichocarpa* stem differentiating xylem

Ying-Hsuan Sun, Rui Shi, Jack Wang, Hsi-Chuan Chen, Ron Sederoff, and Vincent Chiang

10:05 am Break

Lignin proteome:

10:20 am Production and purification of recombinant protein products from candidate lignin pathway genes

Jack Wang, Hsi-Chuan Chen, Quanzi Li, Rui Shi, Ying-Hsuan Sun, Sermsawat Tunlaya-anukit, Ron Sederoff, and Vincent Chiang

10:35 am Absolute quantitation of lignin biosynthesis proteins using protein cleavage coupled with isotope dilution MS (PC-IDMS) based LC-MS/MS

David Muddiman, Chris Shuford, Hsi-Chuan Chen, Jack Wang, Rui Shi, Quanzi Li, Ying-Hsuan Sun, Ron Sederoff and Vincent Chiang

Monolignol metabolome:

11:05 am Absolute quantitation of monolignol pathway metabolites using isotope dilution based GC-MS and LC-MS/MS

Vincent Chiang, Toshiaki Umezawa, Shiro Suzuki, Tin-Feng Yeh, David Muddiman, Jie Liu, Hsi-Chuan Chen, Jack Wang, Hou-min Chang and Ron Sederoff

Wood composition and lignin structural quantitation:

11:20 am Lignin preparations and NMR characterization

Ewellyn Capanema, Jie Liu, Tin-Feng Yeh, Ron Sederoff, Vincent Chiang, Hou-min Chang and John Ralph

11:50 am Lunch Break

Transgenics:

1:20 pm Transgenic *P. trichocarpa* as the systems tool: Strategy and status

Vincent Chiang, Quanzi Li, Rui Shi, Jack Wang, Hsi-Chuan Chen, Chenmin Yang, Yufuko Nishimura, Sermsawat Tunlaya-anukit, Shaobing Peng, and Ron Sederoff

Monolignol metabolic flux controls:

1:35 pm Kinetic models: Enzyme reaction and inhibition of PAL protein members

Rui Shi, Hsi-Chuan Chen, Ron Sederoff and Vincent Chiang

2:05 pm Kinetic models: Enzyme reaction and inhibition of protein members involved in CoA ligation fluxes

Hsi-Chuan Chen, Jina Song, Cranos William, Joel Ducoste, Ron Sederoff and Vincent Chiang

2:35 pm Kinetic models: Enzyme reaction, inhibition and activation of protein members involved in 4- and 3-hydroxylation fluxes

Hsi-Chuan Chen, Ron Sederoff and Vincent Chiang

3:05 pm Break

3:20 pm Kinetic models: Enzyme reaction and inhibition of protein members involved in 5-hydroxylation fluxes

Jack Wang, Hsi-Chuan Chen, Ron Sederoff and Vincent Chiang

3:50 pm Kinetic models: Enzyme reaction and inhibition of protein members involved in dehydrogenation fluxes

Quanzi Li, Hsi-Chuan Chen, Ron Sederoff and Vincent Chiang

4:20 pm Kinetic models: Enzyme reaction of peroxidases

Hsi-Chuan Chen, Ron Sederoff and Vincent Chiang

4:50 pm Meeting Adjourns, transportation of Board Members to hotel

May 7

08:00 am Transportation of Board Members from hotel to meeting location

Project Progress Report Presentations

Systems modeling:

08:30 am Overview

Joel Ducoste, Cranos William, Ron Sederoff and Vincent Chiang

08:35 am Multivariate Statistical Analysis: Development of pathway correlations

Fikret Isik, Ron Sederoff and Vincent Chiang

09:00 am Predicting regulatory control of lignin biosynthesis using signaling graph methodology

Cranos William, Joel Ducoste, Jina Song, Fikret Isik, Ron Sederoff and Vincent Chiang

09:25 am Regulatory constrained flux balance analysis of monolignol biosynthesis

Joel Ducoste, Cranos William, Jina Song, His-Chuan Chen, Fikret Isik, Ron Sederoff and Vincent Chiang

09:50 am Development of mass action relationships for lignin biosynthesis pathway

Jina Song, Cranos William, Joel Ducoste, His-Chuan Chen, Fikret Isik, Ron Sederoff and Vincent Chiang

10:30 am Break

Project database and website:

10:45 am Database (Entity Relationship Diagram) and website structures

Chris Smith, **Ying-Hsuan Sun**, and all other project personnel

Outreach programs:

11:15 am Kenan Fellow

Danielle Seneschal, Joel Ducoste and Ron Sederoff

11:35 am Undergraduate summer research for students from St. Augustine's College and NCSU

Joel Ducoste, Mark Melton, Thomas Easly, Ron Sederoff and Vincent Chiang

12:00 pm Lunch

Project Progress Summary

1:30 pm Progress summary: (1) Publications, (2) Research accomplishments in relation to overall project goal, (3) Plans for the next year

Vincent Chiang

Scientific Advisory Board Session

2:00 pm Preparation of Scientific Advisory Board annual report to NSF

Scientific Advisory Board members only

4:30 pm Comments and suggestions from the Scientific Advisory Board members

Board members and all project personnel

5:00 pm **Meeting Adjourns,** transportation of Board Members to hotel

PROJECT SUMMARY

List of Senior Personnel: This is a collaborative project between scientists at North Carolina State University (NCSU) and University of Wisconsin (UW, Madison). The **PI** is Vincent Chiang (Forest Biotech, NCSU) and the **Co-PI's** are Ron Sederoff (Forest Biotech, NCSU), John Ralph (Organic Chemistry, UW), Joel Ducoste (Systems Biology, NCSU), and Fikret Isik (Statistics, NCSU). **Senior Personnel** are David Muddiman (Proteomics, NCSU), Cranos Williams (Systems Biology, NCSU), Chris Smith (Bioinformatics, NCSU), Reza Ghiladi (Metalloenzyme Chemistry, NCSU), Hou-min Chang (Lignin Chemistry, NCSU), and Ewellyn Capanema (NMR/Lignin Chemistry, NCSU). Others are listed in Management Plan A-2. Collaborators for the outreach/education program are Thomas Easley (NCSU), Valerie Brown-Schild (Kenan Institute) and Mark Melton (St. Augustine's, a Historically Black College).

Objectives and Approaches: Climate change has become the most important factor affecting plants in agriculture and natural ecosystems through increasing biotic and abiotic environmental stress. Fundamental to the adaptation of plants to land, the evolution of vascular transport and the resistance of plants to pests and pathogens are intimately dependent on lignin. Lignin is a phenolic polymer and a structural component of many plant cell walls. Much is known about lignin biosynthesis, but the underlying mechanisms of regulation are largely unknown. Our objective is to build models to quantitatively illustrate how the entire pathway is organized and regulated and to reveal new regulatory and metabolic flux control mechanisms, leading to lignin structures. To do this, we propose a systems approach, using advanced quantitative methods of genomics, proteomics, biochemistry and structural chemistry, to provide the most comprehensive analysis of the regulation of lignin biosynthesis ever undertaken. The model woody plant, *Populus trichocarpa* (Torr. & Gray), is our system. We will use a transgenic perturbation strategy to systematically knock down all known pathway and regulatory genes involved in lignin biosynthesis during wood formation. We will analyze all knock-downs with transcriptomics, mass spectrometry proteomics, enzymology, metabolite profiling, and 2D NMR for lignin structure quantification. We will integrate this information using correlation matrices and path analysis to formulate mechanisms of regulatory and metabolic pathway interactions. Such mechanisms will be iteratively refined and validated by a signaling graph approach, providing specific regulatory constrains to flux distribution analysis and lignin structural predictions for a quantitative model of the biosynthesis of the lignin polymer.

Broader Impacts: Lignin in plant cell walls provides hydrophobicity for water transport, strength to cells and tissues and defense against diseases and pests. The ability of woody plants to establish forest ecosystems depends on lignin. Understanding the fundamental nature of lignin biosynthesis will lead to improved crops and can also aid in resistance to pests and pathogens. Lignin is the main barrier to the utilization of biomass for energy, for papermaking, and for forage digestibility due to the interaction of lignin with cellulosics in the plant cell secondary wall. Quantitative models of lignin biosynthesis would guide strategies for more predictive genetic manipulation for improved plant productivity, production of materials, energy, and food.

All data and materials generated, including a large collection of transgenic trees, will be available to the research community for further research and evaluation. Our database will increase the community genome resources, improve annotation of the *Populus* genome and provide new information for iPlant. Dissemination of project results will be made to major parts of the global forest products and bioenergy industries through our NCSU Forest Biotech Industrial Consortium.

Graduate and postdoctoral education and training in systems biology is a major emphasis of this proposal. NCSU will support two graduate students and a total of six will conduct part of the proposed research for their dissertations, with each focusing on an integral part of the lignin pathway—from genetic transformation to lignin structural characterization to quantitative modeling. They will be trained in a systems approach to develop a rigorous understanding of plant metabolic networks and will be directly supervised by senior project members and other experts in the subject areas of this proposal. The outreach/education efforts focus on under-represented groups at the university and high school levels. Project-specific research training/learning opportunities will be offered annually on the NCSU campus with a total of 8 St. Augustine's students. The Kenan Fellow efforts focus on developing Biosystems-education modules with curricular materials for state- and nation-wide dissemination to bring cutting-edge plant genomics/systems biology to high school classrooms across the nation. We hope to motivate

students, particularly women from under-represented groups, to gain an interest in science, and to consider careers in plant genomics.

INTRODUCTION: Lignin is a unique and complex phenylpropanoid polymer, important in plant development and response to environment. We propose to advance our fundamental knowledge of lignin biosynthesis by developing a systems biology based pathway model of regulatory and metabolic flux control mechanisms. New quantitative technology in genomics, proteomics, and lignin chemistry make such an approach possible. There are few opportunities in higher plants to integrate genomics, proteomics, biochemistry, chemistry and modeling to develop a comprehensive understanding of biosynthesis and structure of a major component of morphology and adaptation. Our primary tool will be systematic gene specific perturbation in transgenic *Populus trichocarpa*. We will perturb all 34 known lignin pathway and regulatory network genes in *P. trichocarpa* using artificial microRNA (amiRNA) and RNAi suppression. From each independent transgenic perturbation, we will obtain quantitative information on transcript and protein abundance, enzyme activities, metabolite concentrations, and lignin structural chemistry. Using statistical correlation and path analysis, we will integrate this information to develop a mechanistic-based signaling graph and metabolic flux model for the pathway and its regulation leading to specific lignin structures. This model will reveal regulatory constraints on flux distributions and show how genes and other process components affect flux activity of lignin precursors, composition, and linkages.

More than a century of investigation has established a basic pathway for lignin biosynthesis.[1-12] The weight of the evidence supports a combinatorial mode of biosynthesis generated by oxidative free radical-based coupling of precursors (monolignols).[7-12] The combinatorial concept explains how monolignol composition and regiochemistry dictate the diverse linkage structures.[12] The lignin structure also explains its resistance to chemical and biochemical degradation. Although our knowledge of lignin chemistry is substantial, many aspects of lignin biosynthesis are not yet quantified or sufficiently understood for model development. Modeling requires the systematic analysis of quantitative relationships of all key processing components[13-16]—genes, transcripts, proteins, enzyme activities, metabolites, and lignin structural units and linkages (Fig. 1). New quantitative tools and the feasibility of identifying and modifying the expression of every gene involved by knock-downs now make such an analysis possible. Our system is the lignin biosynthetic machinery and our output is lignin structure in the wood of *P. trichocarpa*.

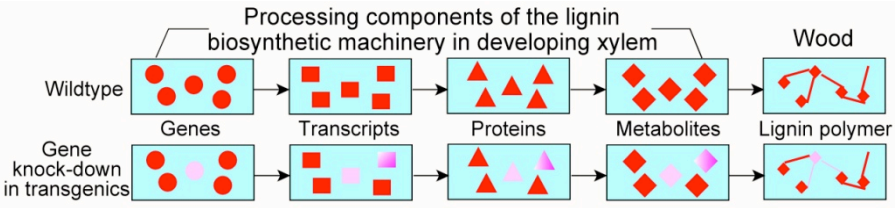


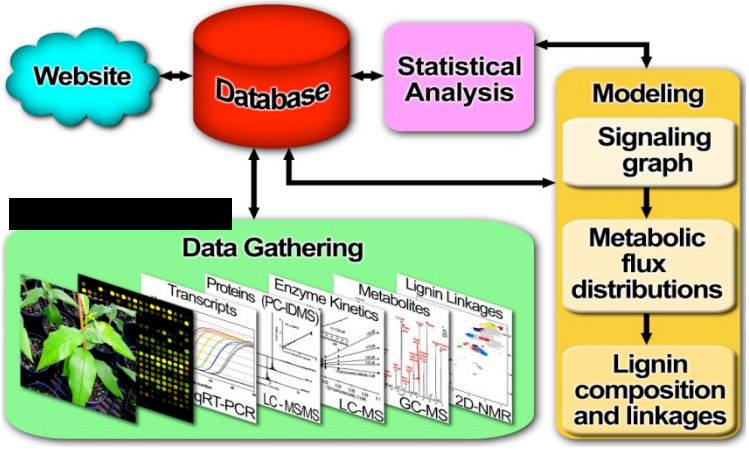
Fig. 1. Pathway gene perturbation is the basis for testing and integrating quantitative contributions of process components to lignin biosynthesis and structure for modeling.

OBJECTIVES AND RESEARCH APPROACHES: Our primary objective is to quantitatively describe how the flux and direction of the entire pathway for the biosynthesis of the lignin polymer is integrated and regulated. We have identified, in the *P. trichocarpa* genome, all known pathway and transcription factor (*TF*) genes[17] and their corresponding proteins (using LC-tandem mass spectrometry (MS/MS)) affecting lignin biosynthesis in differentiating xylem. We will: **(1)** Quantify the responses (transcripts, proteins, metabolites, lignin composition and linkages) for every relevant gene through xylem-specific and gene-specific knock-down. **(2)** Quantify redundancy where more than one member of a gene family is expressed[17] using gene and family specific suppression. **(3)** Generate new hypotheses about mechanisms of regulation and metabolic flux from data summaries and statistical analyses. **(4)** Model and illustrate how changes in process components affect pathway flux and lignin structure. Consequently, we also expect to reveal new regulatory mechanisms and to answer the following questions: To what extent can the relative abundance of transcripts of specific genes predict the protein quantity? To what extent can the abundance and activity of individual enzymes predict the composition of lignin monomers? To what extent can the relative abundance of lignin monomers predict the quantity, composition, and specific linkages of lignin? Our long term goal is a predictive model of lignin biosynthesis and structure.

We will create a public database and website to make readily accessible all of the information generated in this project, as it is collected and analyzed. All biological materials, including transgenic plants, will also be made available.

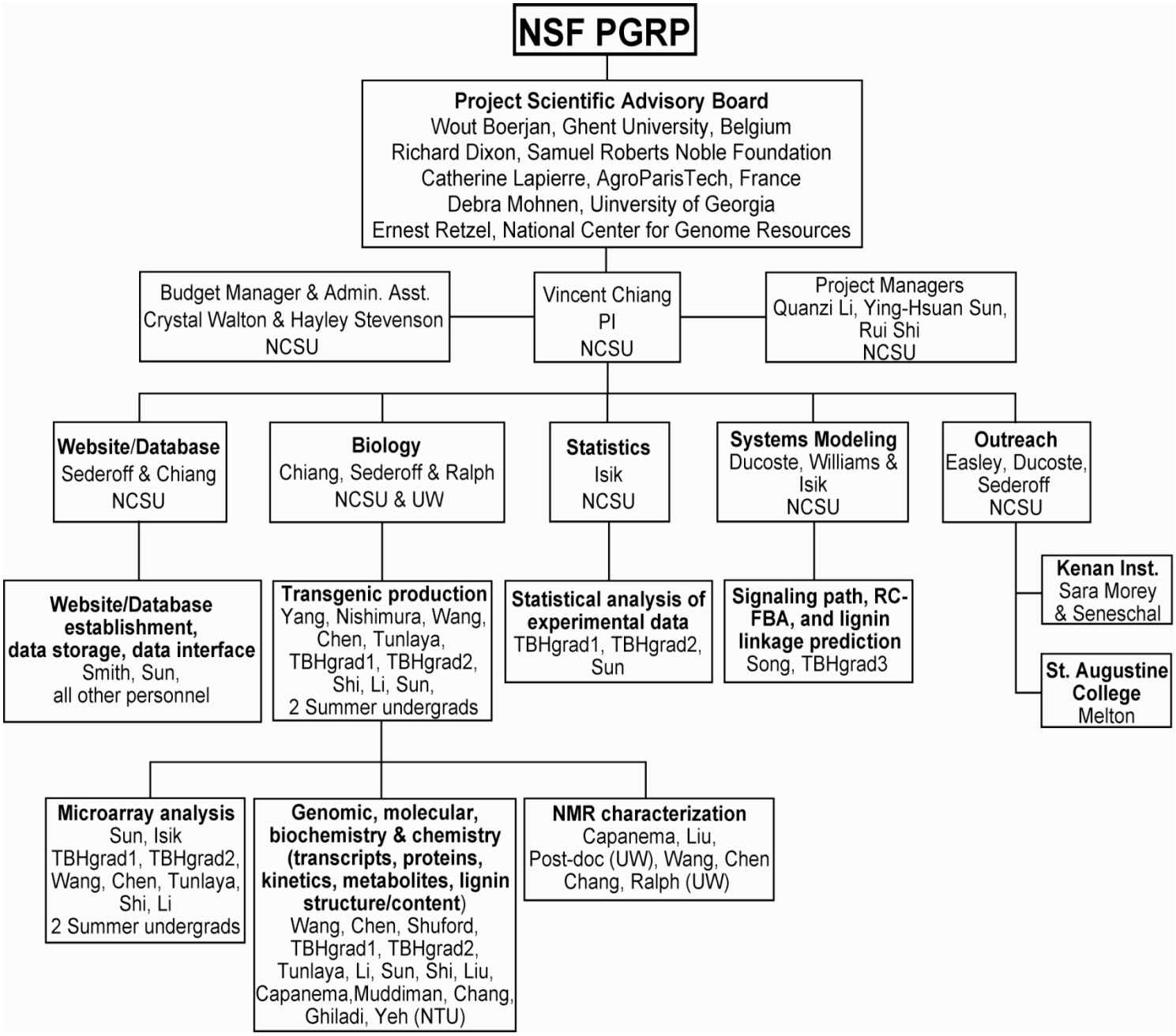
SPECIFIC OBJECTIVES (Fig. 2):

1. **Transgene Perturbation:** We will generate transgenic *P. trichocarpa* with modified expression of all identified lignin pathway and *TF* genes.



2. **Transcriptome Analysis:** Transgenics for each gene will be analyzed to test for specificity and pleiotropic effects on transcripts within and outside of the lignin pathway using microarrays, qRT-PCR, and targeted sequencing.
3. **Proteomic Analysis:** Changes in abundance of protein components will be characterized by protein cleavage coupled with isotope dilution MS (PC-IDMS)-based LC-MS/MS for absolute protein quantity, and by LC-MS for enzyme activity. Kinetic parameters will be determined for recombinant proteins from all lignin biosynthesis genes.
4. **Metabolite Quantitation:** GC-MS and LC-MS will be used to quantify the effect of transgenic perturbations on type and concentration of lignin pathway metabolites to correlate metabolites and lignin structure.
5. **Lignin Quantity and Structure:** NIR will be used to quantify lignin content of the wood for each transgenic line. Lignin monomer composition and inter-unit linkages will be quantified by 1D and 2D NMR to identify correlations with pathway components and for modeling formation of lignin structures.
6. **Database and Website:** A public database/website will be setup at the beginning of the project to integrate all project data and information, including a comprehensive NMR structure library for lignin.
7. **Statistical Analysis:** Univariate and multivariate statistical methods will be applied to describe the degree, direction and significance of relationships among all pathway components and inter-unit linkages. Statistics is essential to establish significance of correlations and to provide quantitative information to mechanistic modeling.
8. **Mechanistic and Systems Modeling:** Modeling techniques will integrate experimental results and statistical inference to develop mathematical representations of lignin biosynthesis and linkage structure. We will construct a signaling graph and integrate the information into steady-state regulatory-constrained flux balance analysis (RC-FBA).

Project Members			
Member	**Institution**	**Role**	**Responsibilities**
Vincent Chiang	Forest Biotech., NCSU	**PI**	Chiang, Professor, oversees the progress of the entire project and supervise project tasks.
Ron Sederoff	Forest Biotech., NCSU	**Co-PI**	Sederoff, Professor, supervises transcriptome related subjects and helps supervise lignin biochemistry and biosynthesis tasks.
John Ralph	Department of Biochem, UW, Madison	**Co-PI**	Ralph, Professor, supervises the NMR characterization and all tasks related to lignin structural analysis.
Joel Ducoste	Civil & Environ. Engineering, NCSU	**Co-PI**	Ducoste, Associate Professor, supervises all modeling efforts outlined in the proposal.
Fikret Isik	Dept. of Forestry, NCSU	**Co-PI**	Isik, Associate Professor of Quantitative Genetics, is responsible for and supervises all statistical analyses of the experimental data.
David Muddiman	Dept. of Chemistry, NCSU	**Senior Personnel**	Muddiman, Professor and Director of NCSU Mass Spectrometry Center, supervises all absolute protein quantification tasks.
Cranos Williams	Dept. of Electrical & Computer Eng., NCSU	**Senior Personnel**	Williams, Assistant Professor, supervises modeling tasks together with Ducoste.
Chris Smith	Bioinformatics Res. Center, NCSU	**Senior Personnel**	Smith, Bioinformatician, establishes and maintains website/databases for the entire project, and computer support for sequence analysis and microarray applications.
Reza Ghiladi	Dept. of Chemistry, NCSU	**Senior Personnel**	Ghiladi, Assistant Professor, help supervises kinetics of peroxidases.
Hou-min Chang	Wood & Paper Science Group, NCSU	**Senior Personnel**	Chang, Professor, helps supervising tasks in metabolomics, cell-wall composition and lignin structure analyses.
Rui Shi	Forest Biotech., NCSU	**Senior Personnel**	Shi, Senior Research Associate, conducts and supervises graduate students on qRT-PCR, transgenic production.
Ying-Hsuan Sun	Forest Biotech., NCSU	**Senior Personnel**	Sun, Senior Research Associate, conducts and supervises tasks in microarray preparations and characterizations, transcriptome (RNA-seq) and proteome data analysis, and statistics.
Quanzi Li	Forest Biotech., NCSU	**Senior Personnel**	Li, Senior Research Associate, conducts and supervises protein expression, enzyme kinetics and transgenic production.
Ewellyn Capanema	Wood & Paper Science Group, NCSU	**Senior Personnel**	Capanema, Assistant Research Professor, conducts lignin NMR analysis.
Jie Liu	Forest Biotech., NCSU	**Senior Personnel**	Liu, Post-doc, conducts organic synthesis, metabolomics, and lignin NMR analysis.
Jack Wang	Forest Biotech., NCSU	**PhD grad student**	Transgenic production and characterization, recombinant protein production, enzyme functional analysis, data analysis.
His-Chuan Chen	Forest Biotech., NCSU	**PhD grad student**	Transgenic production and characterization, recombinant protein production, enzyme functional analysis, data analysis.
Chris Shuford	Dept. of Chemistry, NCSU	**PhD grad student**	Conducts all tasks related to absolute protein quantification.
Jina Song	Dept. of Electrical & Computer Eng., NCSU	**PhD grad student**	Signaling flow graph, flux balance analysis, modeling.
Sermsawat Tunlaya	Forest Biotech., NCSU	**PhD grad student**	Transgenic production and characterization, recombinant protein production, enzyme functional analysis, data analysis.
TBH1	Forest Biotech., NCSU	**PhD grad student**	Transgenic production and characterization, statistics.
TBH2	Forest Biotech., NCSU	**PhD grad student**	Transgenic production and characterization, statistics.
TBH3	Civil & Environ. Engineering, NCSU	**PhD grad student**	Signaling flow graph, flux balance analysis, modeling.
Chenmin Yang	Forest Biotech., NCSU	**Lab Techn**	Transgenic production and greenhouse maintenance.
Yufuko Nishimura	Forest Biotech., NCSU	**Lab Techn**	Transgenic production and greenhouse maintenance.
Courtney Mosley	St. Augustine	**Under grad**	Transgenic production, qRT-PCR and microarray (Summer 2010).
Jaron Hinton	NCSU	**Under grad**	Transgenic production, qRT-PCR and microarray (Summer 2010).
Danielle Seneschal	Kenan Institute	**Outreach**	Coordinates Kenan Fellow activities with Joel Ducoste and Ron Sederoff.
Sara Morey	Kenan Fellow	**Wakefield High Schl**	Develops Biosystems curriculua with Seneschal, Ducoste and Sederoff.
Mark Melton	St. Augustine	**Outreach**	Melton coordinates outreach/education activities at St. Aug.
Thomas Easley	NCSU	**Outreach**	Easley coordinates outreach/education program.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Project Scientific Advisory Board
Wout Boerjan, Ghent University, Belgium
Richard Dixon, Samuel Roberts Noble Foundation
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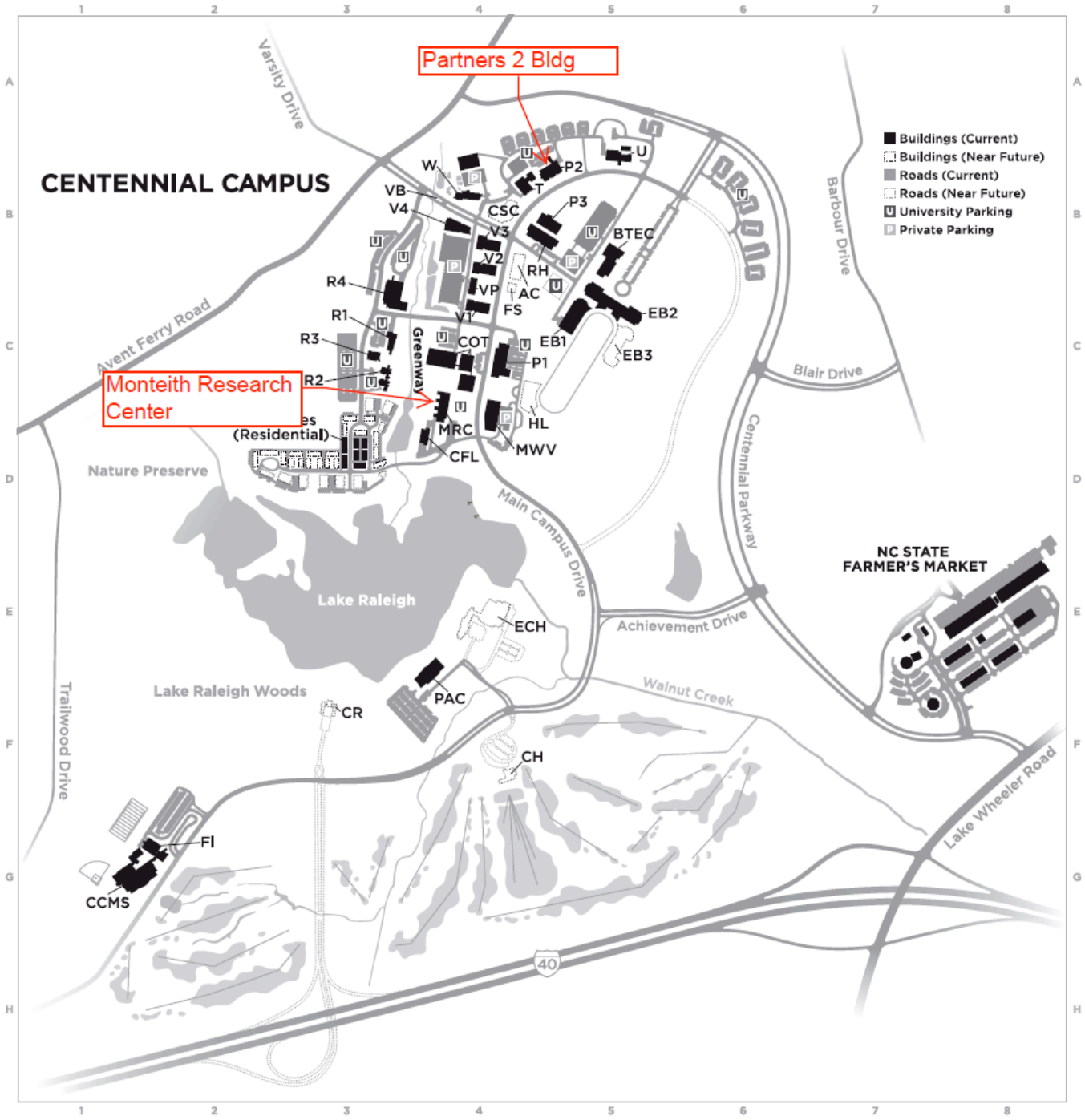
Ernest Retzel, National Center for Genome Resources
Budget Manager & Admin. Asst.
Crystal Walton & Hayley Stevenson
NCSU
Vincent Chiang
PI
NCSU
Project Managers
Quanzi Li, Ying-Hsuan Sun,
Rui Shi
NCSU
Website/Database
Sederoff & Chiang
NCSU
Biology
Chiang, Sederoff & Ralph
NCSU & UW
Statistics
Isik
NCSU
Systems Modeling
Ducoste, Williams &
Isik
NCSU
Outreach
Easley, Ducoste,
Sederoff
NCSU
Kenan Inst.
Sara Morey
& Seneschal
St. Augustine
College
Melton
Website/Database
establishment,
data storage, data interface
Smith, Sun,
all other personnel
Transgenic production
Yang, Nishimura, Wang,
Chen, Tunlaya,
TBHgrad1, TBHgrad2,
Shi, Li, Sun,
2 Summer undergrads
Statistical analysis of
experimental data
TBHgrad1, TBHgrad2,
Sun
Signaling path, RC-
FBA, and lignin
linkage prediction
Song, TBHgrad3
Microarray analysis
Sun, Isik
TBHgrad1, TBHgrad2,
Wang, Chen, Tunlaya,
Shi, Li
2 Summer undergrads
Genomic, molecular,
biochemistry & chemistry
(transcripts, proteins,
kinetics, metabolites, lignin
structure/content)
Wang, Chen, Shuford,
TBHgrad1, TBHgrad2,
Tunlaya, Li, Sun, Shi, Liu,
Capanema,Muddiman, Chang,
Ghiladi, Yeh (NTU)
NMR characterization
Capanema, Liu,
Post-doc (UW), Wang, Chen
Chang, Ralph (UW)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CENTENNIAL CAMPUS
Buildings (Current)
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Blair Drive
Centennial Parkway
Greenway
Main Campus Drive
Achievement Drive
Walnut Creek
Lake Wheeler Road
Trailwood Drive
Nature Preserve
Lake Raleigh
Lake Raleigh Woods
NC STATE FARMER'S MARKET
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