

Plant Transformation Facility



Marcy Main (left), Rose Schick, Tina Paque (greenhouse manager), Bronwyn Frame, Dan Fredrickson (student assistant), Diane Luth (unavailable for photo). Main, Schick and Frame provide maize and rice services. Luth provides soybean service.

The Plant Transformation Facility at Iowa State is a resource valued and patronized by academic and industry researchers around the world. Research laboratories from Europe, North and South America, and Australia send DNA to Ames to be delivered into plant genomes. It is heavily used by teams working out scientific challenges that range from basic molecular details to crop trait improvements.

Established nearly 12 years ago as one of the first public transformation service

laboratories, the facility is made up of a team of experts who can insert a gene of choice into the chromosomes of maize, soybean or rice. The resulting transgenics are returned as either seed (always for international shipments), plantlets or lines of stable transformed callus—a cluster of undifferentiated plant cells growing in a selective medium.

In addition to service, the team trains individuals who would like to learn the techniques themselves. Thus far, researchers

CONTINUED ON PAGE 2

Proteomics Facility

Many Iowa State scientists have moved up the “-omics” ladder from the genome, through the transcriptome, to the proteome.

The proteome, the entire complement of proteins in an organism, consists of thousands of individual protein molecules. They are the major end products of gene action.

Proteomes confer the character to different cell types, but can change in response to different environmental conditions.

Facility manager, Siquan Luo, helps researchers navigate their way through the complexities of protein sample separation, selection, treatment, mounting and analysis by mass spectrophotometer to identify individual proteins or to build a full-signature protein profile.

“Expertise we have recently added lets us look at protein expression using the iTRAQ technology,” says Guru Rao, professor-in-charge of the facility and interim chair of the Department of Biochemistry, Biophysics and Molecular Biology.

Improving on an earlier isotope-based tagging method (ICAT) that allows researchers to bypass the tricky two-dimensional (2D) gel step for separating protein mixtures, iTRAQ technology from Applied Biosystems employs chemical tags. The technique

CONTINUED ON PAGE 2



Adopt the best of science

The goal of the institute's research initiatives is to enhance the value of Iowa crops. During the past two years, the value of Iowa crops has been greatly enhanced. The price of corn has more than doubled and the price of beans is "in the teens."



But value has not been added in the way we expected. Prices have increased because of the laws of supply and demand that are tempered by many other factors including oil prices, subsidies and biofuels.

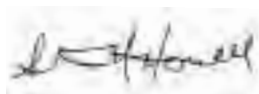
Many years ago we struggled with our agricultural surpluses and price supports. Some of us remember when the U.S. government was agonizing over spending a million dollars a day to store our grain surpluses.

But the world is growing and the picture has changed. Never in recent history has the demand for our agricultural products been so great. To meet these demands, we need an agriculture that can supply all of our needs—with food as the top priority.

To achieve this, we must put an end to many of the restrictions on the use of modern biotechnology in agriculture. Enormous advances have been made in the past two decades in plant biotechnology, but few have been put to practice because of restrictions and limitations placed upon their use.

For example, it has been estimated that it costs \$80-\$100 million to bring a new transgene to market. Much of those costs involve meeting regulatory requirements. Consequently, many biotechnological improvements to crop quality and productivity languish in the pipeline.

If we are serious about enabling an agriculture that can provide us with food, feed and fuel, then we need to allow agriculture to adopt our best science for the benefit of producers and consumers alike.



Stephen Howell
Director

Plant Transformation Facility/CONTINUED

from Argentina, Belgium, China, Israel, Kenya, Morocco, Serbia and South Africa have spent anywhere from a few days to one year working and learning under facility team members' tutelage.

Customer feedback has been overwhelmingly complementary. "We have been called a leading public service for maize transformation," says Kan Wang, facility director and professor in the Departments of Agronomy and Genetics, Development and Cell Biology, "and our staff is always working to improve efficacy and infrastructure."

The facility employs two different methods to transform cells. One uses the natural infection and integration capabilities of a common soil bacterium and plant pathogen, *Agrobacterium tumefaciens*—a valuable tool for plant cell transformation. Researchers outfit the bacterium with their gene of choice, then take advantage of its parasitic ability to introduce its DNA into the host cell genome.

The transformation team also uses a physical delivery system called the biolistic

gun method. Gold particles coated with the desired DNA are physically shot into callus, blasting through the cell wall and into the cytoplasm. Once inside, the DNA finds its way to the cell's chromosomes.

Customer feedback speaks volumes "... service was excellent; amazingly responsive and efficient; always exceeds expectations; best service you can find anywhere; always on time; always able to answer all of my questions; answer with in-depth comments; staff unbelievably skillful, efficient, organized and friendly."

Due to economies of scale, the service is less expensive than it would be for individual labs to take on in-house. But for many it remains a stretch of grant dollars. Nonetheless, users continue to provide requests that require greater greenhouse capacity, growth chamber space and transformation capabilities for additional plant species.

Facility equipment: Bio-Rad Biolistic Apparatus for transformation of immature zygotic embryos and callus of maize and *Agrobacterium tumefaciens*-mediated gene delivery method for maize, rice and soybean.

Proteomics Facility/CONTINUED

reports the relative amounts of the proteins being analyzed along with their identification. The four tagging options allow gene expression level comparisons under four different sample conditions.

Louisa Tabatabai, USDA collaborator professor in the Department of Biochemistry, Biophysics and Molecular Biology, and graduate student Mandy Zimmerli have been using the facility to identify proteins specific to two different bacterial strains, *Haemophilus parasuis* and *Ornithobacterium rhinotracheale*, that cause respiratory diseases in young pigs and poultry, respectively.

Searching the bacterial outer membranes will help them identify candidate proteins that could be used for developing diagnostics and eventually a vaccine. They still use the 2D gel method extensively because it is much less expensive, but "we have collected an amazing amount of data from the few times Mandy used the iTRAQ system," says Tabatabai.



Louisa Tabatabai and Siquan Luo ponder the peaks.

Facility equipment: Typhoon 9410 Variable Imager (1D, 2D gel scans and phosphor imaging), ABI Q-Star quadrupole-TOF mass spectrophotometer equipped with ESI, nanospray and oMALDI sources, LC-Packings UltiMa capillary high pressure liquid chromatography system (plus LC/LC-MS/MS and ICAT analyses).

W. M. Keck Metabolomics Research Laboratory



Ann Perera and Ludmila Rizshsky fractionate fractions.

The W. M. Keck Metabolomics Research Laboratory at Iowa State serves as the base for development of a major initiative to broaden our presence and take leadership in the emerging science of systems biology (a discipline that integrates vast sums of information about the action of genes, proteins and metabolites).

Below the surface of every organism are the metabolites, ever-changing small molecules, such as nutrients, that are central to normal growth, development and reproduction.

Building complete metabolic profiles throughout development or in response to genetic changes or external factors can help pinpoint central metabolic pathway genes. Establishing this framework enables biologically important molecule identification or system manipulation to produce more or less of a particular product.

“Many differences rest in the metabolic phenotype (determined by chemical analysis) and manipulating these systems holds great value,” says Basil Nikolau, professor-in-charge of the facility, director of the Center for Metabolic Biology and pro-

fessor in the Departments of Biochemistry, Biophysics and Molecular Biology and Food Science and Human Nutrition.

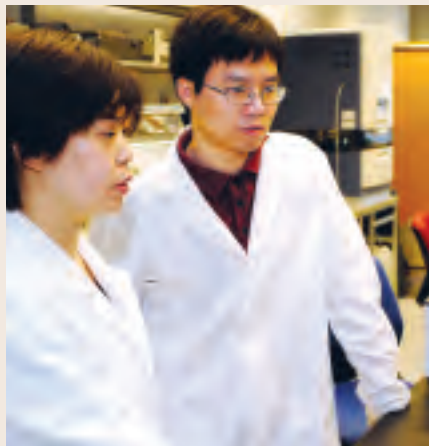
Serving the campus community and those of smaller universities lacking the funds to support such instrumentation and the accompanying technical proficiency onsite keeps Dr. Ann Perera, facility manager, in high demand.

In addition to training an average of three new facility users a week, Perera has been tapped as a collaborator by other major research university groups for facility protocols she develops and her technical expertise.

Dr. Ludmila Rizshsky, assistant scientist in the Department of Biophysics, Biochemistry and Molecular Biology, analyzes plant extracts in the facility. A central component to the efforts of the Center for Research on Dietary Botanical Supplements, her work recently revealed several alkamides from *Echinacea* plants that could be responsible for associated medicinal qualities.

Facility equipment: Beckman Coulter HPLC System, Beckman Coulter CE(P/ACE) system, Agilent Technologies LC/MS-ion Trap, Agilent Technologies GC/MS, Applied Biosystems QSTAR® LC/MS/MS system.

Genomic Technologies Facility



Hailing Jin and Sanzhen Liu zero in on chromosomal coordinants.

Genomic technologies have created a revolution in plant sciences. Iowa State scientists have been leaders in that revolution due, in part, to the availability of cutting-edge instrumentation to carry out their research.

The Genomic Technologies Facility—

part of the Center for Plant Genomics—has provided access to high-throughput instrumentation for studies that range from the mapping of plant genomes to identifying genes that confer specialized traits to crops.

“We are constantly working to identify technologies that will be useful to biologists and to make those technologies available and accessible to Iowa State researchers,” says Patrick Schnable, director of the Center for Plant Genomics and professor in the Departments of Agronomy and Genetics, Development and Cell Biology.

The facility provides researchers with the capability of slicing into specific tissue types and capturing individual cells using a laser-capture microdissection system. Gene activity within these cells can then be studied through microarrays that can also be custom built within the facility.

Researchers involved in maize, pig and now shrimp genome mapping projects

have heavily used the facility where instrumentation allows researchers to scan through genome regions swiftly, searching for specific markers, and to build up functional marker sets or narrow down possible chromosomal positions of specific genes for identification and isolation.

Sanzhen Liu, graduate student in the Department of Genetics, Development and Cell Biology, is working to understand the many steps involved when corn plants synthesize wax in leaves. With a series of low wax-producing mutants called “glossy mutants” in-hand, Liu zeroed in on the chromosomal locations of these genes in just over a week—a process that in the past would have taken months to years.

Facility equipment: Sequenome MassARRAY® system, PerkinElmer ProScanArrayHT microarray scanner, Amersham Biosciences Lucidea SlidePro Hybstation, Leica CM 1850 cryostat, MJ Research, Inc. PTC-225 DNA Engine Tetrad Cyclor, PALM micro laser and Stratagene real-time PCR system Mx4000.

Recent research grants

The following 16 new grants totaling \$4.27 million were awarded recently to plant science researchers at Iowa State.

Development of Bioinformatic Resources to Transfer Biological Information across Species

USDA, CSREES—\$999,961
(J. Reecy, animal science)

Functional Genomics of Cyst Nematode Parasitism of Plants

USDA, CSREES—\$749,624
(T. Baum, plant pathology)

Structural Studies of a T Cell Specific Tyrosine Kinase

National Institutes of Health—\$300,726
(A. Andreotti, biochemistry, biophysics and molecular biology)

Oligomeric Structure of Membrane Peptides from Solid-State NMR

National Science Foundation—\$162,428
(M. Hong, chemistry)

Novel Signaling Components for Plant Steroid Regulated Gene Expression in Arabidopsis

National Science Foundation—\$125,000
(Y. Yin, genetics, development and cell biology)

Seed Systems Development Project

International Crops Research Institute for the Semi-Arid Tropics—\$119,259
(J. Cortes, Seed Science Center)

Statistical Graphics Research in Association with GGobi

National Science Foundation—\$106,233
(D. Cook, statistics)

CAREER: Meshing Synthesis and Biosynthesis in Research and Teaching

National Science Foundation—\$102,000
(N. Pohl, chemistry)

CRI: LAD Acquisition Cluster and High Performance Storage for Data-Intensive Applications in Materials Science, Power Systems, and System Biology

National Science Foundation—\$719,000
(S. Aluru, electrical and computer engineering)

Annotation of Novel Enzymatic Functions in Methanogens Using MS-Based Metabolomics Coupled with Targeted NMR-Based Ligand Screening

U.S. Department of Energy—\$258,712
(B. Nikolau, biochemistry, biophysics and molecular biology)

Biomass Pretreatment and Pyrolysis Experiments

ConocoPhillips Company—\$235,000
(R. C. Brown, mechanical engineering)

Development of High-Throughput DNA-Based Gene Silencing Technology for Soybeans

United Soybean Board—\$125,000
(J. Hill, plant pathology)

Making Improvements to the PCAP Genome Assembly Program

U.S. Department of Health and Human Services—\$94,497
(X. Huang, computer science)

Estimating the Retinol Activity Equivalence of Beta-Carotene-Biofortified Crops

Centro Internacional De Agricultura Tropical—\$85,618
(W. White, food science and human nutrition)

Sequencing the Maize Genome

National Science Foundation—\$48,203
(P. Schnable, agronomy)

Advanced Biorefinery Feedstocks

Metabolix, Inc.—\$36,000
(E. Wurtele, genetics, development and cell biology)

The Plant Sciences Institute Update is published four times each year by the Plant Sciences Institute at Iowa State University, 1060 Roy J. Carver Co-Laboratory, Ames, Iowa 50011-3650; phone 515 294-5255.

The Plant Sciences Institute at Iowa State University is dedicated to becoming one of the world's leading plant science research institutes. More than 200 faculty from the College of Agriculture and Life Sciences, the College of Liberal Arts and Sciences, the College of Human Sciences, and the College of Engineering conduct research in nine centers of the institute. They seek fundamental knowledge about plant systems to help feed the growing world population, strengthen human health and nutrition, improve crop quality and yield, foster environmental sustainability and expand the uses of plants for biobased products and bioenergy. The Plant Sciences Institute supports the training of students for exciting career opportunities and promotes new technologies to aid in the economic development of agriculture and industry throughout the state. The institute is supported through public and private funding.

To be added to our mail list, e-mail psidir@iastate.edu.

On the Web at <http://www.plantsciences.iastate.edu/>



Iowa State University does not discriminate on the basis of race, color, age, religion, national origin, sexual orientation, gender identity, sex, marital status, disability, or status as a U.S. veteran. Inquiries can be directed to the Director of Equal Opportunity and Diversity, 3210 Beardshear Hall, 515 294-7612.