

U.S. WHEAT & BARLEY SCAB INITIATIVE VDUN GENOTYPING WORKSHOP
LABORATORY FOR PLANT GENOME TECHNOLOGY, INSTITUTE FOR PLANT GENOMICS &
BIOTECH, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS
MAY 18, 2004

Host

Monica Menz Texas A&M University, College Station

USWBSI Team Members

James Anderson University of Minnesota, Workshop Coordinator
Guihua Bai USDA-ARS Genotyping Center, Manhattan, KS
Gina Brown-Guedira USDA-ARS, Manhattan, KS
Shiaoman Chao USDA-ARS Genotyping Center, Fargo, ND
Stephen Szalma USDA-ARS, Raleigh, NC (representing David Marshall)

Agenda (May 18, 2004)

8:00 Transportation to Campus & Introductions, welcome by J. Mullet, Director of the
 Institute for Plant Genomics & Biotech
8:30 Overview of LPGT activities/protocols (Menz, No)
10:15 Lab tour and demonstration of techniques/troubleshooting (Menz)
12:00 Lunch
1:30 Final Discussion with breeding programs (J. Betran, B. Rooney)
3:00 Adjourn

Purpose of Workshop:

Four USDA-ARS Genotyping Centers are being established at Raleigh NC, Fargo ND, Manhattan KS and Pullman WA. Research conducted at the Genotyping Centers can speed the short-term objective of bringing FHB resistant varieties into commercial production, and enhance the longer-term objective of utilizing diverse sources of resistance to combat FHB.

This was the second of three workshops designed to produce protocols for 1) the utilization of the Genotyping Centers in marker assisted selection in cultivar development populations, 2) for coordinated multi-state research programs in QTL discovery and validation, and 3) for coordinated multi-state programs in backcrossing of new sources of FHB resistance into adapted genetic backgrounds. Dr. Monica Menz' lab was chosen for this site visit because of their expertise in using DNA markers in a wide variety of species including small grains, diversity of genotyping equipment and marker types utilized, collaboration with breeding programs, and structure of the lab that includes a service component.

Summary of Workshop:

The Laboratory for Plant Genome Technology (LPGT) was established in 1995. Texas A&M University pays salaries of Dr. Menz, a portion of the lab manager – Dr. No, a technician, service contracts, and part of the operational costs. Fees are charged for service activities. In 2003, the service lab processed 35,000 samples, 80% were sequencing reactions and 20% were fragment analysis. Dr. Menz provided a thorough overview of the organizational structure of the LPGT (Fig. 1). The LPGT conducts and facilitates research. Collaborative research in molecular breeding is established with several breeding programs and technology development

includes SNP work and development of marker systems on less-studied orphan crops. Formal training workshops are held periodically and the LPGT also provides informal training. A faculty advisory committee provides input to the LPGT and conducts periodic surveys of its users.

The Lab Manager, Dr. No is responsible for technology development and service activities. Service is his priority and is 60% of his activity. His job evaluation depends on the service component and “making the customers happy.”

Main equipment in the lab are two LiCor 4200’s (fragment analysis, cheaper to run than ABI), Beckman Multimek 96 robot (not used much due to cost of tips), ABI 377 and ABI 3100 sequencers, ABI 7900HT RT-PCR machine, Gene AMP 9700 and MJ tetrad PCR machines. DNA extraction is from coleoptile tissue from seed growing on captan-treated filter paper. Tissue is macerated in 1.5 ml eppendorf tubes (deep well plates too expensive) with a metal rod and shaken in a Genogrider 2000 (~\$10,000 paint shaker). AFLP is used extensively as well as SSRs. PCR products are pooled for fragment analysis, no multiplex PCR is run. Gel images are saved as pdf files and are accessible by ftp.

Texas A&M has more than 20 plant breeders, most of whom are very applied and don’t have molecular labs of their own. Breeders establish the priorities for projects with input from Dr. Menz. Graduate students from the breeding projects work in the lab. There is a general problem in funding marker-assisted selection (MAS) versus conventional breeding or gene/marker discovery research.

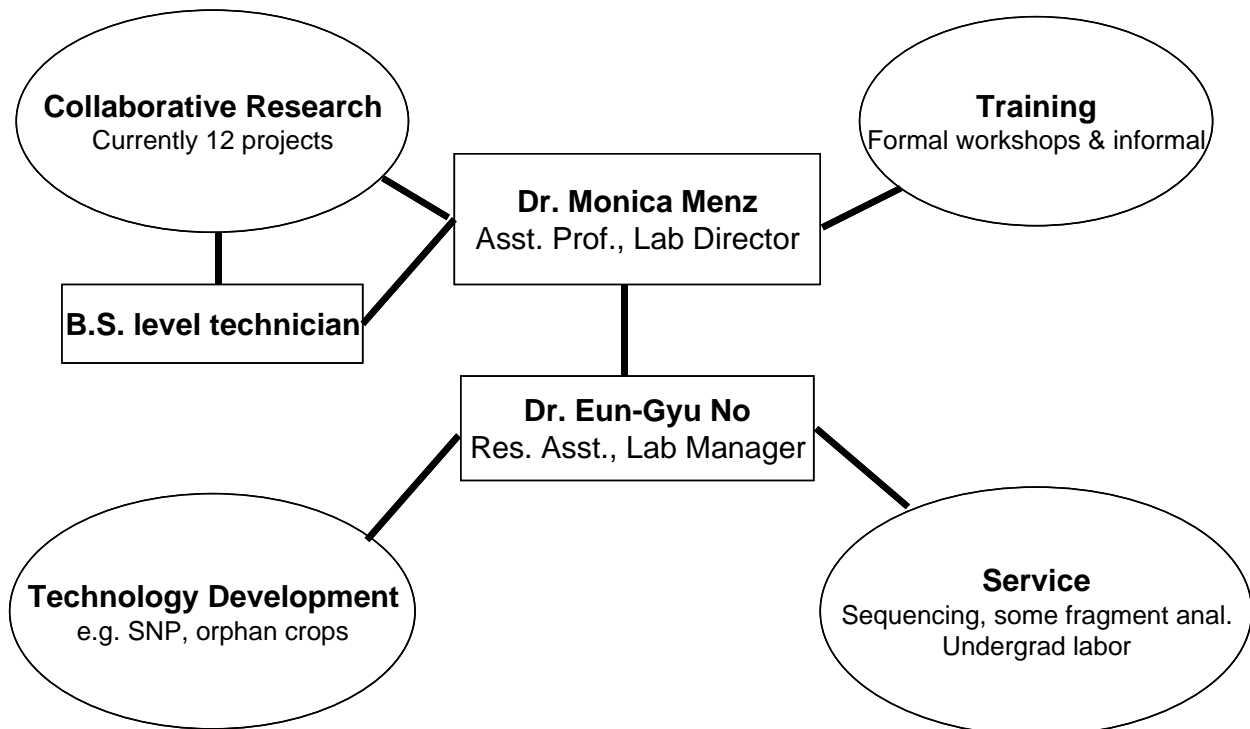


Fig. 1. Organization of the Laboratory for Plant Genome Technology at Texas A&M University.

Relevance to USDA-ARS Genotyping Centers and USWBSI

The Menz lab was a good contrast to the Somers lab we visited the previous week. The Menz lab has a greater defined service role and a Ph.D. level lab manager, both of which were lacking in the Somers lab, but are included in the description of the genotyping centers.

Although the organization of the genotyping centers as indicated in the job descriptions of the Cat. 1 and Cat. 4 scientists does not match the structure of the Menz lab, there should be some similarities:

- The Cat. 4 scientist will likely spend a majority of his/her time at the bench. Dr. No spends at least 6 hours per day at the bench. Can the genotyping centers attract good personnel into such a position? We think that each genotyping center should have discretion to hire a Cat. 4 or technicians. If technicians are hired instead, then the Cat. 1 scientist will bear most, if not all, of the burden of communication with breeders on both collaborative research and MAS.
- The Cat. 1 scientist will need to engage in publishable research, which may include collaborative research with USWBSI investigators. This individual should also interact directly with the breeders – clear protocols for sample submission, expected turn-around time, and data retrieval will minimize the amount of communication necessary.
- The breeders indicated that the collaborative research was an important component and that LTPG personnel would be recognized with co-authorship on cultivar and germplasm release if they contributed to its development. These expectations also apply to the genotyping centers.
- An advisory group needs to be established for each of the genotyping centers, consisting of users of the lab. This group will be relied upon to help set priorities and guide lab activities.

The emphasis of the Menz lab is geared more toward efficiency and fiscal conservation versus throughput as seen in the Somers lab. If funding is adequate, the genotyping centers will need to emphasize throughput to satisfy the demand. This will require at least two or three technicians to process breeder samples, at least during the peak periods. Unless collaborative research funding is acquired for at least one of these technicians, the need for “hands in the lab” may also favor the hiring of technicians versus Cat. 4 scientists.