

Barley Coordinated Agricultural Project Work Plan FY07 (4/1/07 – 3/31/08)
Shiaoman Chao, USDA-ARS, Fargo, ND

1) Describe the research, education, and outreach activities you are planning for the next year (4/1/07 – 3/31/08)

Research : We will start genotyping 960 breeding lines collected in 2006 with BarleyOPA1 containing 1,536 SNPs using the Illumina's SNP genotyping system. The DNA quality is the crucial factor affecting the SNP data quality. The results from the pilot studies using the re-synthesized PilotOPA1 have indicated that the quality of the genomic DNA extracted using the SDS-based method is adequate to give satisfactory SNP data. Therefore, this method will be used to extract genomic DNA from all the breeding lines sent to the Fargo genotyping lab. We will continue to genotype 960 breeding lines expected to be collected in 2007.

Education : We will provide the training on the use of the Illumina system to students, postdocs or scientists who intend to use the system and the OPAs generated in the barley CAP for their own research. We are planning to organize a MAS hands-on workshop in 2007 for wheat CAP participants in our region. It is anticipated that the barley researchers in this region will be invited to join this workshop.

Outreach : This will be done through the program set up by the project members at NDSU.

2) List specific outcomes and deliverables that will be accomplished in the first 6 months (4/1 – 9/30). These will be used as benchmarks for your bi-annual progress report.

- Extract genomic DNA for 960 breeding lines collected in 2006.
- Carry out SNP genotyping on 960 breeding lines using BarleyOPA1.
- Store and archive SNP data, both raw and verified, in the database setup in house.
- Send final and verified data set to Iowa State University. The SNP data calls will be verified by Tim Close.

3) List specific outcomes and deliverables that will be accomplished in the second 6 months (10/1 – 3/31). These will be used as benchmarks for the bi-annual progress report.

- Continue DNA extraction for 960 breeding lines collected in 2007
- Carry out SNP genotyping on 960 breeding lines using BarleyOPA1.
- Carry out SNP genotyping using BarleyOPA2.

Barley Coordinated Agricultural Project Biannual Progress Report
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Research.

The preliminary work was carried out to evaluate the tissue preparation format and genomic DNA extracted methods. The Chao lab routinely uses leaf samples prepared in 96 deep-well plate format for high throughput DNA extraction using a robot to conduct wheat and barley MAS projects. However, using the plate format, we could not rule out a low level of cross-contamination between wells, particularly the manual process required to mix well the buffers and solutions added during extraction. To avoid cross contamination for the BarleyCAP materials, Chao collected leaf samples in individual tubes followed by freeze-drying and performing DNA extraction manually. For DNA extraction, the method used in the Chao lab is SDS based. Tim Close previously used Qiagen DNeasy to obtain high quality SNP genotyping data. Thus, we compared the two methods.

The Illumina Bead Station was purchased and shipped in late August and installed October 24. The training was received November 27 through December 1, 2006. There were 10 people attending the training, three from the USDA-ARS Fargo genotyping lab, three students from Rich Horsley's lab, one student from Shahryar Kianian/Elias Elias's lab, one post-doc from Brian Steffenson's lab and two post-docs from Tim Close's lab. The training was done using human samples. The re-synthesized Pilot OPA1 was received shortly after the training. On December 19 the USDA-ARS Fargo genotyping lab first used PilotOPA1 to compare the DNA purification methods and optimize the genotyping techniques. To facilitate the optimization, 96 US barley core germplasm samples provided by Patrick Hayes and Tim Close were used, four of which were previously genotyped with the original batch of Pilot OPA1. Genomic DNAs were individually extracted using the SDS-based method and the Qiagen kit. The results allowed us to cross-check and compare genotyping data generated in different labs, and to determine if both DNA extraction methods are equally adequate to generate high quality of genotyping data. In brief, the results are excellent in all aspects: the data from all samples are of excellent quality so the SDS purification method is fine, and the new batch of PilotOPA1 gave the same genotype calls as the original batch.

Education. We have a temporary intern to assist with the DNA extractions.

Outreach. Chao presented a talk to high school students at Maddock, ND (pop 500) via video conference, and described high throughput genotyping process and the advantage of MAS. A DVD was prepared showing the genotyping lab equipment and facility, and a brief demonstration on how the samples were prepared and the function of the equipment.

Specific outcomes and deliverables accomplished.

- Establish tissue preparation and genomic DNA extraction protocols used for SNP genotyping
- Prepared samples and lab setups ready for SNP genotyping training and optimization
- Installed Illumina Bead Station and BeadStudio software with genotyping module
- Purchased and received re-synthesized PilotOPA1
- Processed 96 project samples using PilotOPA1, shared data with Close
- Analyzed initial dataset