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Project Title: Construction and Utilization of a Pooled BAC Library of Sumai 3.

PROJECT 2 ABSTRACT

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Funded by USWBSI and NRI, we are using a map-based cloning approach to clone the major FHB QTL on chromosome 3BS (*Fhb1*). Based on FHB phenotypes of recombinants and new DNA markers derived from 'Chinese Spring' BAC sequences, *Fhb1* was narrowed down to a 260 Kb region and seven candidate genes were identified. Transformation appears our last alternative to determine the identity of *Fhb1* and this is in progress for four cosmid clones.

It is possible that Chinese Spring has a null *Fhb1* allele. In this case, it would be very difficult if not impossible to clone *Fhb1* based on the BAC sequence of Chinese Spring. Therefore, the availability of a BAC library of Sumai 3 is an essential resource in cloning *Fhb1* as well as other FHB QTLs, and developing easy to use, diagnostic markers for these QTLs. The specific objectives of this proposal are to:

1) Construct a pooled BAC library of Sumai 3 for the wheat community.
2) Construct a Sumai 3 BAC contig spanning Fhb1.
3) Develop user-friendly markers for Fhb1 and provide the markers to the Regional Genotyping Centers.

Considering the large genome size of common wheat (16,000 Mb) and the amount of funding required to construct a pooled BAC library of Sumai 3, we propose to construct a three genome-equivalent pooled BAC library. Assuming the average insert size of 100 Kb, 480,000 clones would be needed. Due to the technical challenges of constructing a BAC library, we will solicit bids to have a collaborator construct the library.

Our experiences with the construction and screening of a pooled cosmid library of Sumai 3 will be helpful for the proposed BAC library. We have identified suitable pooling densities and screening strategies to facilitate routine PCR-based screening and isolation of desired clones. After construction, the library will replicated and maintained by the Wheat Genetic and Genomic Resource Center, KSU and at the University of Minnesota. We will use the Sumai 3-specific markers developed to screen our pooled cosmid library of Sumai 3 to screen the 3-D pools of BAC clones. After PCR-based identification of positive sub-pools, single positive clones will be identified by DNA hybridization. We will sequence the genic regions of a BAC clone(s) of Sumai 3, and compare the sequences with the corresponding region of CS to identify Sumai 3-specific nucleotides or sequences. We will develop user-friendly markers for *Fhb1* based on the sequences of Sumai 3. The best markers will be provided to the Regional Genotyping Centers, and we will optimize the markers if needed. Our goal is to make sure that the markers work well and are available to all interested parties.