

of marker systems will yield a better contiguous map. The mapping resolution of these RH panels estimated on the basis of markers spanning known distances was <140 kb. Two sets of informative lines carrying breaks in multiple D-genome chromosomes were selected from *Ae. tauschii* DGRH1s (399 lines) and Chinese Spring DGRH1s (300 lines). First generation RH maps based on 178 lines and 676 markers (641 DaRT and 35 SSR) showed a 17:1 map ratio cR/cM when compared with the genetic maps. A NimbleGen array has been designed and tested for high-throughput mapping, and a total of ~30,000 retro-junction markers and ~6,000 gene-based markers, specific to the D genome were identified. The selected DGRH1 lines currently are undergoing genotyping with this array and, once analyzed, will provide a very dense scaffold for the assembly of the D genome of wheat. This research also provides valuable resources for fine mapping and map based cloning studies of genes present on the D genome along with an unprecedented view into the evolution of grass genomes (<http://avena.pw.usda.gov/RHmapping/>).

Poster 8. Construction of a radiation hybrid map for chromosome 6B of common wheat.

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It is well known that recombination events in wheat are not evenly distributed along the length of chromosomes. This is the case with chromosome 6B, which is the target chromosome assigned to Japan by the International Wheat Genome Sequencing Consortium. The genetic maps of chromosome 6B so far constructed are heavily populated by markers in the pericentromeric region and scarcely in the telomeric regions. This represents that recombinations mostly take place in the telomeric ends of the chromosome. Therefore, we would face problems in determining orientation of contigs in the pericentromeric region if we solely depend on the genetic maps. Our objective of the current study is to establish a radiation hybrid (RH) mapping panel that can be useful in determining marker orders in the recombination-poor, pericentromeric region of chromosome 6B. We crossed nullisomic 6B-tetrasomic 6A plants of Chinese Spring (CS) wheat with the pollen freshly irradiated by γ -ray (15 Gy). We sowed 2,171 M0 seeds and extracted genomic DNA from 461 (21.2%) surviving plants. Additionally, we used 12 6B deletion lines (five deletions in 6BS and seven deletions in 6BL; obtained from NBRP-Wheat, Japan). We scored the presence or absence of 21 previously reported 6B-specific SSR markers and four newly developed EST-based markers. We analyzed the data by CarthaGene software to construct a RH map with the default setting. The resulting RH map consisted of two linkage groups, corresponding to the short and long arms. The gap between the linkage groups may be due to the absence of markers in a pericentromeric bin (6BS-CEN-0.25). The marker order is consistent with that of bin mapping and largely with a previously reported genetic map. All the RH-map markers occupied individual loci. Two pairs of markers genetically mapped to the same loci in the pericentromeric region of 6B were completely separated from each other in the RH map. This result indicates that RH mapping with our panel has better resolution in proximal region of 6B.

Poster 9. A NimbleGen comparative genomic hybridization array for high-throughput physical mapping of genome specific repeat junction and gene-based markers in the D genome of hexaploid wheat.

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Mapping and map-based cloning of agriculturally and economically important traits remain a great challenge in complex highly repetitive genomes such as the grass tribe, Triticeae. This limitation is primarily based upon the availability of polymorphic markers and frequency of genetic recombination. Most markers are gene-based, derived from polymorphisms within coding regions. Non-gene-based markers, such as repeat junction markers, are derived from the noncoding intergenic space. Repeat junction markers take advantage of the repetitive nature of the wheat genome, providing random and equal distribution of these markers throughout the genome and can facilitate mapping efforts. Repeat junction markers are designed upon the junction of nested repetitive elements, and approximately 90% of these markers have been determined to behave as a single copy locus and are genome specific. Repeat junction markers were designed from 454 genome sequences of the wheat D-genome progenitor, *Aegilops tauschii*. Mapping of *Ae. tauschii* repeat junction

markers to deletion bins within the D genome of reference polyploid wheat (Chinese Spring) will allow for the construction of a physical marker scaffold that will aid in genome sequence completion and future mapping and cloning studies. To design an optimal marker array, we tested hybridization temperature, oligo length, and different statistical analysis methods. After determining appropriate marker design and experimental conditions we screened a pool of 206,486 repeat junction markers as well as 26,800 gene markers representing 6,700 genes. Screening results provided 46,224 markers in total that were selected for design of a final mapping array. These 46,224 markers are comprised of 30,900 repeat junction markers and 15,324 gene markers representing 6,330 genes. This final mapping array is being used to construct a high-resolution, physical map using D-genome deletion lines and radiation hybrid panels. Here we present our methods for design and analysis of the NimbleGen comparative genomic hybridization arrays constructed from *Ae. tauschii* repeat junction markers, the construction of a NimbleGen repeat junction array using selected markers, and its use in the development of a physical map for the D genome of hexaploid wheat.

Poster 10. Genome-wide characterization of transposable element repeat junctions in barley and their application in marker development for chromosome 3H.

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Transposable elements (TEs) account for about 70% of the barley (*Hordeum vulgare* L.) genome. TEs move in the genome by inserting to new regions through copy-and-paste or cut-and-paste mechanisms. Insertion of TEs in DNA regions generates unique junctions between the TEs and the sequences in which they land. We investigated the uniqueness of these junctions throughout the barley genome and their potential application for marker development. The 10-Gb survey sequencing data of the seven barley chromosomes was searched with the ‘RJPrimers’ pipeline to estimate the frequency of repeat junctions (RJs). We found 988,750 RJs distributed evenly among the chromosomes with an average of 1 RJ per 10 Kb. Repeat junction markers (RJMs) for each chromosome were designed based on detected repeat junctions. Each RJM consists of one primer that spans unique insertion site of TE, whereas the second primer is designed from any region within 1 Kb of the junction. We randomly chose 36 RJMs from chromosome 3H to amplify five barley cultivars (Betzes, Golden Promise, Bowman, and Haruna Mugi) and two wild barley (*H. bulbosum* L.) accessions. Out of 36 RJMs, 28 (68%) amplified a single band and 21 (58%) were polymorphic. Based on scoring data of 21 RJMs, we were able to separate all tested barley accessions and cluster them into two main groups. The abundance of RJMs makes them an ideal marker for genome saturation. Further, the high level of polymorphism makes them ideal for molecular breeding applications.

Poster 11. Best practices for RNA-Seq differential expression analysis in barley.

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Barley (*Hordeum vulgare*) being a member of the grass family is one of the most important large-genome cereals and a close relative of wheat. It has a complex diploid genome of 5.1 Gb and is being extensively used for genetic studies. As a part of the Barley Genome Sequencing Project, RNA-Seq experiments were performed using eight different tissues representing different developmental stages, including 4-day embryos, roots, and shoots from seedlings, young developing inflorescences (5-mm and 10–15 mm stages), at the six-leaf stage, and from the developing grain (5 and 15 days post anthesis). These datasets have been used to improve the barley gene model predictions and to detect differentially expressed transcripts. Here we discuss the results of the expression analysis that we implemented using different available