Coupling Expressed Sequences and Bacterial Artificial Chromosome Resources To Access The Barley Genome

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Abstract: Barley genomic resources now include BAC and cDNA libraries, several widely used mapping populations, about 400,000 ESTs representing about 70% of all barley genes, and a 22K microarray. In work supported by North American Barley Genome Project and NSF Plant Genome Research Program, we aim to couple these resources to facilitate access to the barley genome. The unifying objective is to accelerate a transition to comprehensive physical mapping and sequencing of the "gene-space". We utilize knowledge of unigene sequences to create 36-mer "overgo" probes to identify Morex BAC clones that carry expressed gene; ca 12,000 such overgos will be used to find most of the "gene-space". We then apply a fingerprinting technique to create contigs of these BAC clones, from which a minimal set can be identified. In total ~ 5000 genes (January, 2005) have been screened for in our own work and that of collaborators Andris Kleinhofs, Gary Muehlbauer, Roger Wise, Patrick Hayes, Kulvinder Gill, Nils Stein, MA Saghai Maroof and their co-workers. 21,161 BACs were fingerprinted and 13,067 BACs were assembled into 2262 contigs comprising ca 9.4% (470 Mbp) of the genome. All data is publicly available: (1) the "Barley Genome" website http://phymap.ucdavis.edu:8080/barley/ provides access to BAC contig data, (2) the "HarvEST" website http://harvest.ucr.edu provides access to sequence assemblies, unigene sequences and function annotations and (3) the "OligoSpawn" website http://oligospawn.ucr.edu provides access to elements of our oligo design algorithms. Special emphasis is given to 1000 genes related to abiotic stress, including drought, low temperature and salinity, such that these genes and their corresponding BACs will be anchored to the genetic linkage map. For this purpose we investigated single feature polymorphisms (SFPs) using the Affymetrix Barley1 GeneChip hybridized with labeled cRNA from several genotypes including Morex, Barke, Steptoe, Oregon Wolfe Barley (OWB) dominant and OWB recessive. We developed a detection method using the robustified projection pursuit (RPP) method in order to evaluate the overall differentiations of signal intensities of probe sets comparing two genotypes and to measure the individual contribution of each probe, from which the probes covering polymorphisms (SNPs or INDELs) can be identified. We found a total of 1665 SFPs of which 844 were abiotic stress responsive as defined by expression data (salinity, drought, ABA and low temperature). Using the RPP method, it is clear that single 25-mers on GeneChips designed for expression analysis can be used to query barley genotypes for nucleotide polymorphisms. Design of high throughput SFP and SNP mapping tools with collaborators (Robbie Waugh, Nils Rostoks, Nils Stein and Andreas Graner) are in progress.