Development of CAPS markers for linkage mapping and studies of genetic diversity in barley

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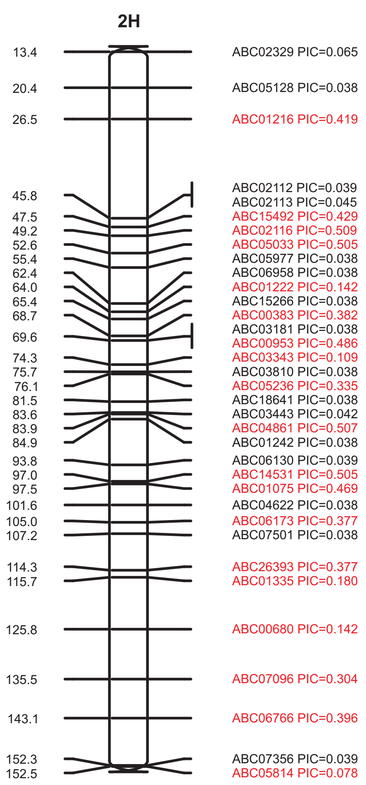
SUMMARY
Molecular markers are applied extensively in basic research to facilitate linkage and QTL mapping and map-based cloning. From an applied perspective, the application of molecular markers in plant breeding can accelerate the development of novel and better adapted varieties, but it is frequently hampered by both high costs and the need to invest in complex technologies. We recently constructed a linkage map for barley based on single nucleotide polymorphism markers (Rostoks et al., 2005). Here we report the conversion of ca. 200 SNP markers into cleaved amplified polymorphic sequences (CAPS), which are both robust and easy to use. To demonstrate the usefulness of this CAPS marker set, we studied polymorphisms detected at 35 mapped loci from barley chromosome 2H in a set of 48 European barley cultivars. The average polymorphism information content (PIC) was 0.178. Approximately 40% of markers were polymorphic among these 48 lines confirming their usefulness for breeding purposes. Recently, a high-throughput SNP genotyping platform was developed for barley (Close, Waugh and Graner, unpublished) which was utilized for analysis of 1524 SNP in 102 diverse barley accessions using Illumina GoldenGate technology. Approximately one third of these SNPs can be converted into CAPS markers, thus, downscaling this genotyping platform for lower throughput analysis and making it more accessible for plant breeding. Since linkage map positions and PICs are known for the majority of these CAPS markers, they are ready to use for trait mapping in wider collections of European barley.

Experimentally validated CAPS markers for linkage mapping and diversity studies

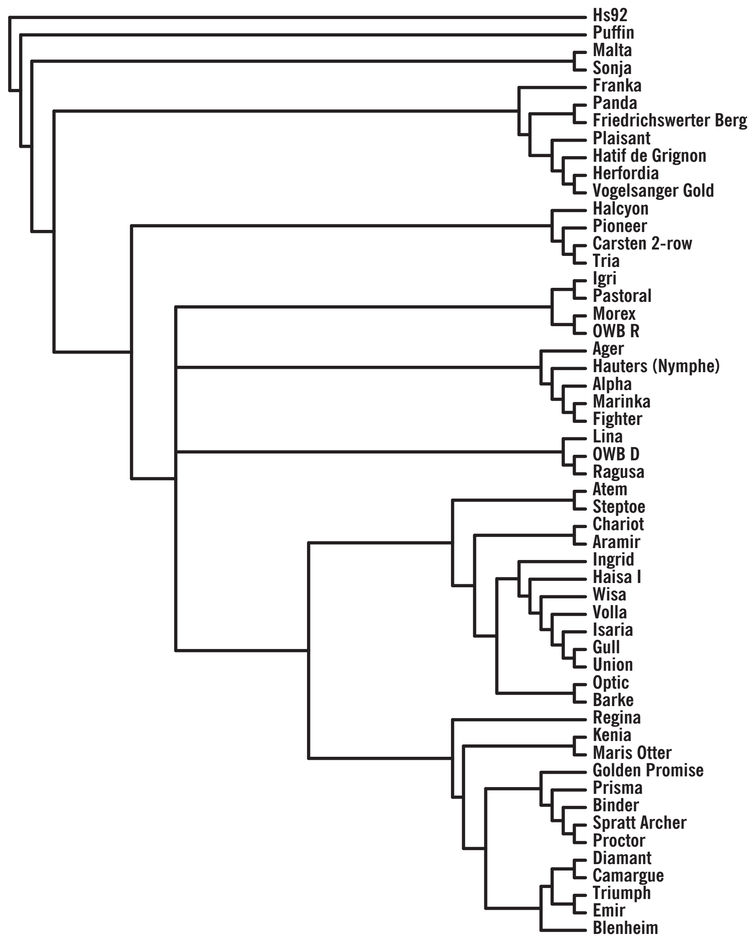
Background
Single nucleotide polymorphisms are abundant type of genetic variation in any organism studied and they also represent an efficient marker system. We recently carried out resequencing-based SNP discovery in barley genes and identified several thousand SNPs of which more than 300 were mapped in three mapping populations (Rostoks et al. 2005). These SNP loci served as a backbone to construct a consensus linkage map integrating RFLP, SSR and AFLP loci. Majority of the SNPs were assayed using Cleaved Amplified Polymorphic Sequences (CAPS) (Konieczny and Ausubel, 1993), which proved to be a simple, robust and cost-effective alternative to other SNP genotyping assays.

Methods
Alignments of sequences from eight genotypes were obtained using either Mutation Surveyor or phred/phrap (Rostoks et al., 2005). SNPs were examined either by eye or using SNP2CAPS software (Thiel et al., 2004) and polymorphisms that affected restriction enzyme sites were identified. PCR amplification was carried out with the same primers that were used for sequencing. Primers, sequences and SNP information is available from SCRI SNP database at http://bioinf.scri.ac.uk/barley_snpdb/. PCR products were digested with appropriate restriction enzymes according to manufacturer's recommendations. Digests were resolved in 1 - 2% 1 x TBE w/v agarose gels.

Results
In total, ca. 200 CAPS markers were developed. Those included mapped loci (Rostoks et al., 2005) and ca. 50 CAPS markers which were not mapped, but their polymorphism was experimentally assessed by assaying the parental lines.
SNP discovery was based on eight barley genotypes (OWB Dominant, OWB Recessive, Steptoe, Morex, Lina, *Hordeum vulgare* ssp. *spontaneum* accession 92, Golden Promise and Optic). In order to test whether these CAPS markers were informative in European barley germplasm, 35 CAPS markers mapping to chromosome 2H were analyzed from 48 European barley accessions (see genetic map of 2H below).

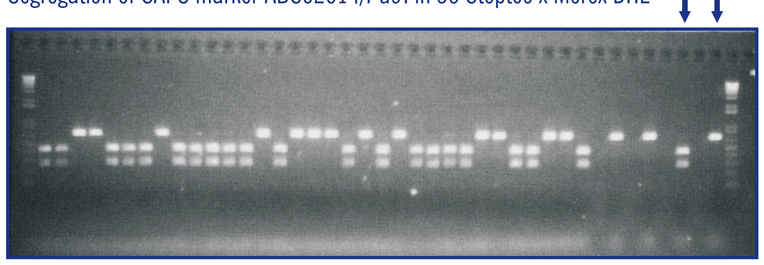


Consensus linkage map of barley chromosome 2H showing only the CAPS loci (Rostoks et al., 2005). Centimorgan distances are on the left. Locus names are on the right followed by the PIC values in the set of 48 European barley varieties and additional five genotypes (OWB D, OWB R, Steptoe, Morex and Hs92). Loci in red were used to construct the dendrogram.



Extended majority rule consensus tree (100 bootstraps) based on 19 segregating sites (mean PIC =0.35, range 0.08 - 0.5) on barley chromosome 2H. The set of germplasm consists of 48 European spring and winter barley varieties with additional four North American lines and a *Hordeum vulgare* ssp. *spontaneum* line used as an outgroup.

Segregation of CAPS marker ABC02614/*Pae*I in 35 Steptoe x Morex DHL



Development of CAPS markers from the high-throughput SNP genotyping platform

Background
Recent advances in marker development and high-throughput SNP genotyping allow whole-genome association mapping and diversity studies not only in model organisms, but also in crop plants, such as barley. We developed a platform based on Illumina GoldenGate technology for high-throughput genotyping of 1524 barley SNP markers (one per gene) and used it to develop a new consensus linkage map (Close et al., in preparation), as well as to study genetic diversity and extent of linkage disequilibrium (LD) in European barley (Rostoks et al., submitted).
Illumina GoldenGate genotyping platform will be applied within two association genetics projects in UK (AGOUEB) and in USA (USDA barley CAP; http://www.barleycap.org/) to map agriculturally important traits and to develop markers useful for marker assisted selection. Illumina GoldenGate SNP genotyping is an efficient and cost-effective way to analyze a large number of SNP loci in a highly multiplex manner, however, it may be prohibitively expensive for small research and plant breeding laboratories. Once diagnostic SNP markers for certain traits are developed, it may be beneficial to convert them to robust, lower throughput CAPS markers that can be applied to a larger number of breeding lines and hybrid progeny. These markers will be with known map locations and polymorphism information content (PIC) facilitating their immediate utility for marker assisted selection.

Methods
Illumina GoldenGate assay was designed from a set of experimentally confirmed and EST-derived electronic SNPs (Close et al., in preparation). Sequences containing SNPs were analyzed by SNP2CAPS software (Thiel et al., 2004) and polymorphisms that affected restriction enzyme sites were identified. SNP PIC was calculated from their allele frequencies in the set of 91 European barley varieties (Rostoks et al., submitted) and their position on the barley linkage map was determined from segregation data in OWB D x OWB R, Steptoe x Morex and Barke x Morex mapping populations (Close et al., in preparation).

Results
In total, 548 CAPS markers (86 enzymes) could be developed for the 1524 SNPs present on OPA1 after manual examination of the SNP2CAPS analysis. The availability of a restriction enzyme and the size difference of the digestion products were the major criteria for assay design.
Not all the SNPs in OPA1 were positioned on the barley consensus linkage map, because they failed to segregate in the three mapping populations. Thus, of the 548 potential CAPS markers, 355 SNPs were with known map locations (see table for their chromosomal distribution).

Chromosome	Number of CAPS	Mean PIC CAPS	Mean PIC all OPA1 SNPs
1H(5)	36	0.249	0.250
2H(2)	64	0.229	0.232
3H(3)	64	0.261	0.257
4H(4)	44	0.325	0.271
5H(7)	53	0.290	0.275
6H(6)	39	0.332	0.331
7H(1)	55	0.213	0.216

Conclusions
Approximately, one third of all the SNP loci in the Illumina barley OPA1 could be detected by a simple and robust CAPS assay making these markers accessible for laboratories with standard molecular biology equipment. Availability of the map locations and PIC values for the majority of the OPA1 SNPs will allow to select CAPS markers for a particular genome region that will be polymorphic in an appropriate set of barley germplasm. As the CAPS marker technology depends on the ability to amplify the polymorphic locus by PCR, the availability of Illumina oligonucleotide sequences will facilitate CAPS assay design.

References
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