

2007

# High-throughput genotyping of barley for variety identification

Julie A. Pattemore  
*Southern Cross University*

Robert J. Henry  
*Southern Cross University*

---

## Publication details

Pattemore, J & Henry, RJ 2007, 'High-throughput genotyping of barley for variety identification', paper presented to 13th Australian Barley Technical Symposium, Fremantle, WA, 26-30 August.

ePublications@SCU is an electronic repository administered by Southern Cross University Library. Its goal is to capture and preserve the intellectual output of Southern Cross University authors and researchers, and to increase visibility and impact through open access to researchers around the world. For further information please contact [epubs@scu.edu.au](mailto:epubs@scu.edu.au).

## HIGH-THROUGHPUT GENOTYPING OF BARLEY FOR VARIETY IDENTIFICATION

J.A. Pattemore<sup>1</sup> and R.J. Henry<sup>1</sup>

<sup>1</sup>Grain Foods CRC, Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, 2480

### INTRODUCTION

Correct identification of cereal variety is vital for quality assurance of goods requiring different grain attributes. For example, the malting and brewing properties of barley (*Hordeum vulgare*) are variety dependent, thus sourcing the correct variety is critical to product consistency. It is becoming increasingly important to be able to identify cultivars rapidly and cheaply to determine purity or levels of admixture in grain samples, in order to make the most appropriate use of agronomic types available and/or quality characteristics. Identification using morphological and physiological characters is still widely used, however these characters can be influenced by environmental conditions and the techniques can be time-consuming. In order to achieve fast and reliable identification, a stable molecular marker and high throughput genotyping platform suitable for screening large numbers of individuals must be found.

Single nucleotide polymorphisms (SNPs) are changes to a single base at discrete loci in the genome and are generally bi-allelic (Gut, 2001). The frequency of SNP markers in the barley genome, approximately 1 per 130 to 200bp (Bundock *et al.*, 2003; Kanazin *et al.*, 2002; Rostoks, Mudie *et al.*, 2005) makes SNPs an attractive genotyping marker. *In silico* SNP discovery is inexpensive and while whole genome data is currently not available for large-genome species such as barley and wheat, large numbers of putative SNPs can be screened from EST-derived unigene databases (Rostoks, Borevitz *et al.*, 2005).

Demand for high throughput, cost-effective genotyping has driven the development of commercial genotyping platforms capable of typing thousands of SNPs from large numbers of individuals. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) provides one of the most powerful and reliable methods for high-throughput SNP genotyping (Buetow *et al.*, 2001). We used Sequenom<sup>®</sup> MALDI-TOF mass spectrometry to develop a rapid, high throughput SNP identification assay capable of distinguishing Australian commercially grown barley varieties with precision and speed.

### MATERIALS AND METHODS

#### *Sample Collection and DNA extraction*

A collection of barley germplasm representing 55 malting and feed cultivars of significance to the Australian barley industry was obtained from the Australian Winter Cereals Collection (AWCC), Tamworth (NSW, Australia). DNA was extracted from leaves of each individual approximately 10-14 days post-germination from a minimum of 10 individual seedlings using a Qiagen MagAttract DNA extraction kit as per manufacturers instructions. The DNA was stored at -20°C.

### *SNP Identification*

SNP sites in 31 barley genes were used for the development of the MALDI-TOF assay. SNPs were identified by aligning barley expressed sequence tags from public databases. The targeted genes form the basis of allele-specific PCRs and correspond to cDNAs used as restriction fragment length polymorphic probes for linkage mapping in barley (Bundock *et al.*, 2006). For each candidate SNP, flanking primers and an extend primer were designed using MassARRAY Assay Design™ software (Sequenom Inc.) and synthesized. Assays were designed for uniplex and multiplex applications. Genomic DNA (2ng) was PCR amplified using 0.5U HotStar Taq DNA polymerase (Qiagen), 0.1µM each flanking primer, 500µM each dNTP, 1.625mM MgCl<sub>2</sub> and 1.25x PCR Buffer (supplied with enzyme including 15mM MgCl<sub>2</sub>) in 5µl. Thermocycling conditions were as follows: 94°C for 15 min, 45 cycles of 94°C for 20 sec, 56°C for 30 sec, 72°C for 1 min, with a final extension step at 72°C for 3min.

### *Genotyping*

Following PCR amplification of the target, reactions were incubated with shrimp alkaline phosphatase (SAP) to neutralize unincorporated dNTPs. The SAP enzyme solution consisted of 0.3µl SAP enzyme (1U/µl), 0.17µl of 10x SAP Buffer (supplied with Sequenom iPLEX™ enzyme) in 2µl. Reactions were incubated at 37°C for 20 min, 85°C for 5 min, then held at 4°C. After SAP incubation, reactions were mixed with 2µl of the iPLEX™ (Sequenom, Inc.) cocktail (1x iPLEX enzyme, 1x iPLEX termination mix and 0.222X iPLEX buffer, 0.625µM extend primer) was added to each reaction bringing the total volume to 9µl. The reaction was thermocycled as follows: 94°C for 30 sec, 45 cycles of 94°C for 5 sec, (5 cycles of 52°C for 5 sec, 80°C for 5 sec); followed by a final extension step at 72°C for 3 min. Next, 25µl water was added to the reactions, followed by addition of Clean Resin™ and mixed by gentle inversion for 15 minutes. Finally, iPLEX reaction products were spotted onto a SpectroCHIP™ for MS analysis, using the MassARRAY Nanodispenser.

The assay was carried out initially in uniplex on a DNA set consisting of one individual per cultivar. The assay was then validated in multiplex on 10 to 25 individuals from each cultivar.

## **RESULTS**

SNPs identified *in silico* were used to design a MALDI-TOF assay. The uniplex assay was successful in identifying homozygous and heterozygous alleles from 31 genes in 55 cultivars of barley. The data was pooled to build a panel of SNPs unique to each cultivar. Further work is continuing in order to identify multiplex capacity and quantification of mixtures of cultivars.

## **DISCUSSION**

Rapid advances in SNP discovery are providing vast amounts of data, ready for detection and diagnostic applications. The priority now is developing cost-effective platforms capable of rapidly and accurately identifying genetic polymorphisms (Shi, 2001), combined with multiplex and automated high-throughput detection capabilities. The challenge for HTP plant genotyping lies in utilizing the available data more effectively, and improving the capacity and cost-effectiveness of screening thousands of polymorphisms from large numbers of individuals. The greatest obstacle is the affordability of testing and validating novel HTP genotyping assays.

The advantage of MALDI-TOF MS genotyping lies in the direct identification of SNPs by determining the mass of the polymorphic nucleotide itself. Genotype calling was precise and coupled with the ability to multiplex up to 25 reactions per well on a 384-well plate in a fully automated process, MALDI-TOF mass spectrometry clearly has the potential to be a high-throughput genotyping method of choice for cereal identification.

#### **ACKNOWLEDGEMENTS**

The authors would like to acknowledge Grains Research and Development Corporation for provision of a Grains Industry Research Scholarship supporting this project.

#### **REFERENCES**

- Buetow, K. H., Edmonson, M., MacDonald, R., Clifford, R., Yip, P., Kelley, J., et al. (2001). High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proceedings of the National Academy of Science, USA*, 98(2), 581-584.
- Bundock, P. C., Christopher, J. T., Egglar, P., Ablett, G., Henry, R. J., & Holton, T. A. (2003). Single nucleotide polymorphisms in cytochrome P450 genes from barley. *Theoretical Applied Genetics*, 106, 676-682.
- Bundock, P. C., Cross, M. J., Shapter, F. M., & Henry, R. J. (2006). Robust allele-specific polymerase chain reaction markers develop for single nucleotide polymorphisms in expressed barley sequences. *Theoretical Applied Genetics*, 112, 358-365.
- Gut, I. G. (2001). Automation in genotyping of single nucleotide polymorphisms. *Human Mutation*, 17, 475-492.
- Kanazin, V., Talbert, H., See, D., DeCamp, P., Nevo, E., & Blake, T. (2002). Discovery and assay of single-nucleotide polymorphisms in barley (*Hordeum vulgare*). *Plant Molecular Biology*, 48, 529-537.
- Rostoks, N., Borevitz, J. O., Hedley, P. E., Russell, J., Mudie, S., Morris, J., et al. (2005). Single-feature polymorphism discovery in the barley transcriptome. *Genome Biology*, 6, R54.
- Rostoks, N., Mudie, S., Cardle, L., Russell, J., Ramsay, L., Booth, A., et al. (2005). Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Molecular and General Genetics*, 274, 515-527.
- Shi, M. M. (2001). Enabling large-scale pharmacogenetic studies by high-throughput mutation detection and genotyping technologies. *Clinical Chemistry*, 47(2), 164-172.