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THE USE OF TEMPERATURE SWITCH PCR FOR SNP GENOTYPING IN BARLEY

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Introduction

1. Single nucleotide polymorphism (SNP) represents the smallest unit of genetic variability. SNPs are widespread in both animal and plant genomes, occurring at high frequencies and many thousands have been, or are in the process of being mapped (see *http://bioinf.scri.ac.uk/barley_snpdb/maps*). SNP markers have been found that are linked to traits such as disease resistance and malting quality and the search for SNPs for specific quantitative trait loci (QTLs) is well underway. It is therefore not inconceivable that before long SNP markers may be used to determine the physical characteristics a barley plant displays.

2. One problem precludes the use of molecular techniques on a wide scale basis, namely the technology required and the costs involved. Standard SNP genotyping requires expensive equipment and consumables and this is, more often than not, a barrier to its use by DUS testing stations. It would therefore be desirable to be able to take the data from the next generation sequencing projects and use it to produce a low cost SNP genotyping alternative. One possible solution is the use of temperature switch PCR (TSP) (Tabone *et al*., 2009) which allows SNP assays to be run on standard agarose gels after conventional PCR. This paper describes preliminary work carried out using this method to examine barley varieties on the United Kingdom national list.

Materials and Methods

3. DNA was extracted from bulked samples of 100 grains using the method described in Reid *et al*. (2009). The only variation being the initial grinding step, which was carried out with a mortar and pestle.

4. The TSP reactions were performed using a selection of the primers listed in Hayden *et al*. (2009) (see Table 1). In each reaction one of the universal primers was labelled with FAM for detection on a 3130xl capillary sequencer but the products can also be separated by agarose gel electrophoresis. Alleles were scored as present or absent and analyzed in BioNumerics (Applied Maths).

Table 1. Details of SNPs analyzed during this study

SNP name	Chromosome	HarvEST contig	SNP alleles	SNP allele assayed
scsnp17647_248[T/C]top	1H	ABC17647	T/C	T
scsnp02329_170[A/G]top	2H	ABC02329	A/G	A
scsnp01327_275[C/T]bot	2H	ABC01327	C/T	T
scsnp05033_332[G/A]bot	2H	ABC05033	G/A	G
scsnp02403_54[T/C]top	2H	ABC02403	T/C	T
scsnp14531_165[G/A]top	2H	ABC14531	G/A	A
scsnp06766_249[G/A]top	2H	ABC06766	G/A	A
scsnp05814_321[A/G]top	2H	ABC05814	A/G	A
scsnp05814_98[G/A]top	2H	ABC05814	G/A	A
scsnp03814_188[A/G]top	3H	ABC03814	A/G	A
scsnp14307_683[T/C]top	3H	ABC14307	T/C	T
scsnp19616_322[G/A]top	3H	ABC19616	G/A	G
scsnp05754_646[G/A]top	3H	ABC05754	G/A	A
scsnp06172_369[G/A]bot	4H	ABC06172	G/A	G
scsnp07010_126[A/C]top	5H	ABC07010	A/C	C
scsnp05926_188[G/C]top	5H	ABC05926	C/G	G
scsnp05926_55[T/C]top	5H	ABC05926	T/C	C
scsnp02265_354[A/G]bot	5H	ABC02265	A/G	G
scsnp03594_515[T/C]top	5H	ABC03594	T/C	T
scsnp02739_543[T/C]bot	5H	ABC02739	T/C	C
scsnp07305_298[T/A]bot	6H	ABC07305	T/A	A
scsnp06204_63[A/G]top	6H	ABC06204	A/G	A
scsnp02895_423[T/C]bot	6H	ABC02895	T/C	C
scsnp03149_168[C/A]top	6H	ABC03149	C/A	C
scsnp04220_436[C/T]bot	6H	ABC04220	C/T	C
scsnp04220_508[G/T]bot	6H	ABC04220	G/T	G
scsnp02493_292[G/C]bot	7H	ABC02493	G/C	G
scsnp02493_317[C/T]bot	7H	ABC02493	C/T	C
scsnp06931_64[A/G]top	7H	ABC06931	A/G	G

Results

5. All of the primer sets tested yielded products, however not all of them were informative as all of the samples were either homozygous for one allele or heterozygous for both. Testing further varieties may alter this outcome. A tree constructed from all of the data (including non-informative markers) yielded a tree which differentiated all of the varieties (Figure 1).

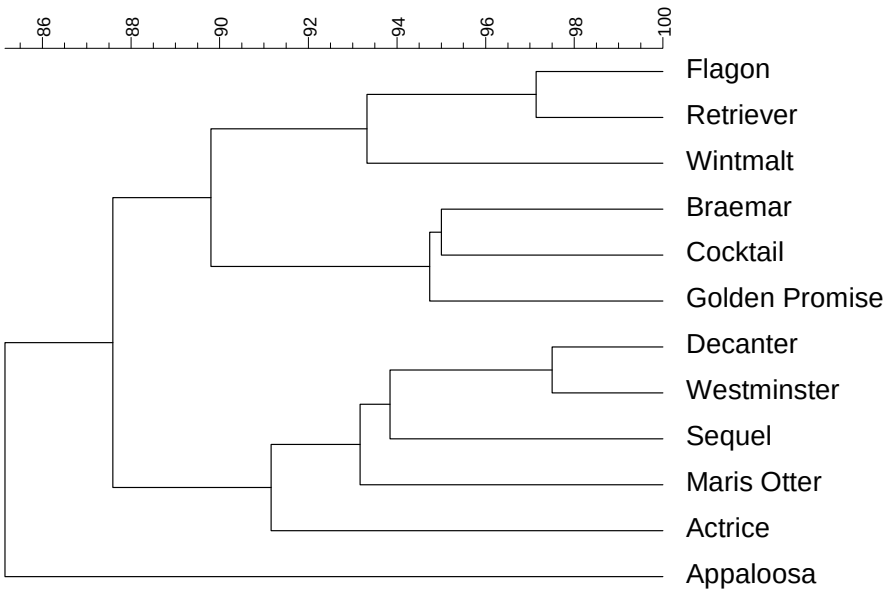

Figure 1. Dendogram constructed using Jaccard and UPGMA for 12 barley varieties.

Summary

6. Temperature switch PCR appears to be a viable low cost alternative for SNP genotyping in barley. The equipment needed to set up the technique and the running costs involved are now within reach of most laboratories. As more SNPs are discovered and the genome sequencing of barley progresses it become more likely that SNPs linked to specific traits with relevance to the DUS test will become available.

References

Tabone, T., Mather, D.E. and Hayden, M.J. (2009) Temperature switch PCR (TSP): Robust assay design for reliable amplification and genotyping of SNPs. *BMC Genomics*, **10**, 580.

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