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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

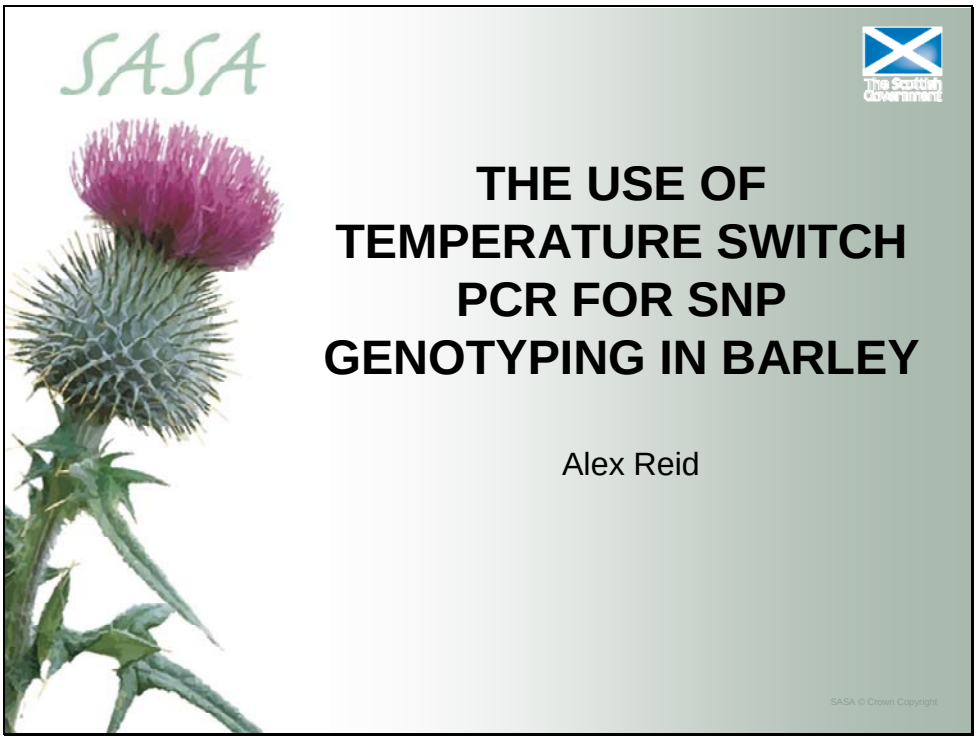
WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

THE USE OF TEMPERATURE SWITCH PCR FOR SNP GENOTYPING IN BARLEY

Document prepared by an expert from the United Kingdom


SASA
THE USE OF TEMPERATURE SWITCH PCR FOR SNP GENOTYPING IN BARLEY
Alex Reid
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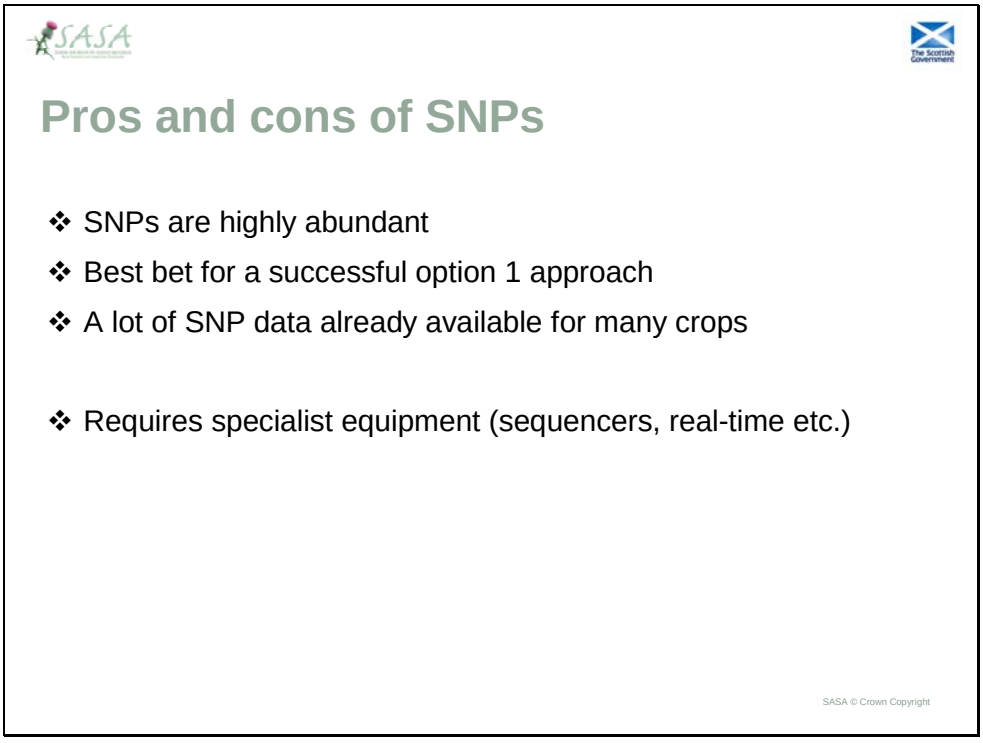
SASA
Pros and cons of SNPs
❖ SNPs are highly abundant
❖ Best bet for a successful option 1 approach
❖ A lot of SNP data already available for many crops
❖ Requires specialist equipment (sequencers, real-time etc.)
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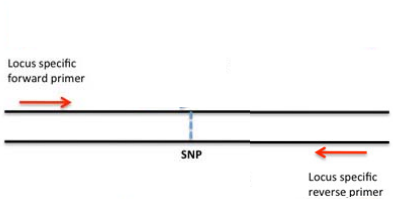
Temperature Switch PCR (TSP)

- Tania Tabone, Diane E Mather and Matthew J Hayden (2009) Temperature Switch PCR (TSP): Robust assay design for reliable amplification and genotyping of SNPs. *BMC Genomics*, **10:**580
- A biphasic PCR system with a universal primer design that permits amplification of the target locus in the first phase of thermal cycling before switching to the detection of the alleles.
- TSP can simplify assay design for a range of commonly used single-marker SNP genotyping methods, and reduce the requirement for individual assay optimization and operator expertise in the deployment of SNP assays.

So how does it work?



Application for barley SNP genotyping

- Matthew J Hayden, Tania Tabone and Diane E Mather (2009) Development and assessment of simple PCR markers for SNP genotyping in barley. *TAG*, **119**:939-951.
- Here they detail a total of 87 TSP markers which give good coverage of the genome.

Chromosome	# TSP markers
1H	12
2H	17
3H	15
4H	8
5H	13
6H	13
7H	9

The SASA adaptation

- We selected 29 of the 87 markers to trial
- The universal primer is labelled with FAM
- Fragments detected on a capillary sequencer
- Scored as present or absent
- DNA extracted from 100 grain bulks
- Initially looking at 12 varieties
- Also tested individual plants

Results

- Not all markers informative for the varieties tested but most were
- Can detect differences between individual plants
- Combined data for all markers differentiates all the varieties
- Encouraging start!

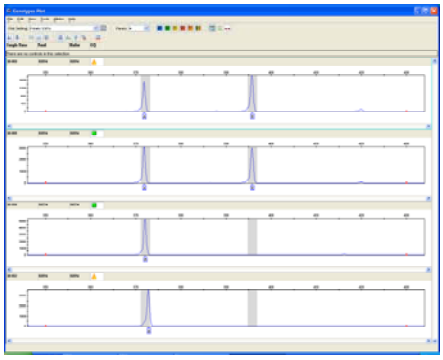
Summary

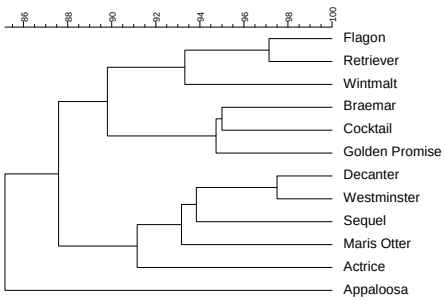
- Temperature switch PCR appears to be a good low cost alternative to more conventional genotyping.
- Equipment set up costs are relatively low (PCR machine, gel tank & power pack and uv transilluminator).
- Next generation sequencing undertaken by SCRI and collaborators has resulted in a high resolution gene map for barley.
- Underway is a project to genotype 2400 barley lines with 3000 SNPs.
- This can only be good news for an option 1 approach.

Future work

- Expand the number of varieties to include all of the UK National List.
- Multiplex markers to get more information out of a single run.
- Look for markers linked to DUS traits.

Thank you





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