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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

Fifteenth Session

Moscow, Russian Federation, May 24 to 27, 2016

VARIETY IDENTIFICATION OF BARLEY USING KASP GENOTYPING

Document prepared by an expert from the United Kingdom

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The Annex to this document contains a copy of a presentation "Variety identification of barley using KASP genotyping" to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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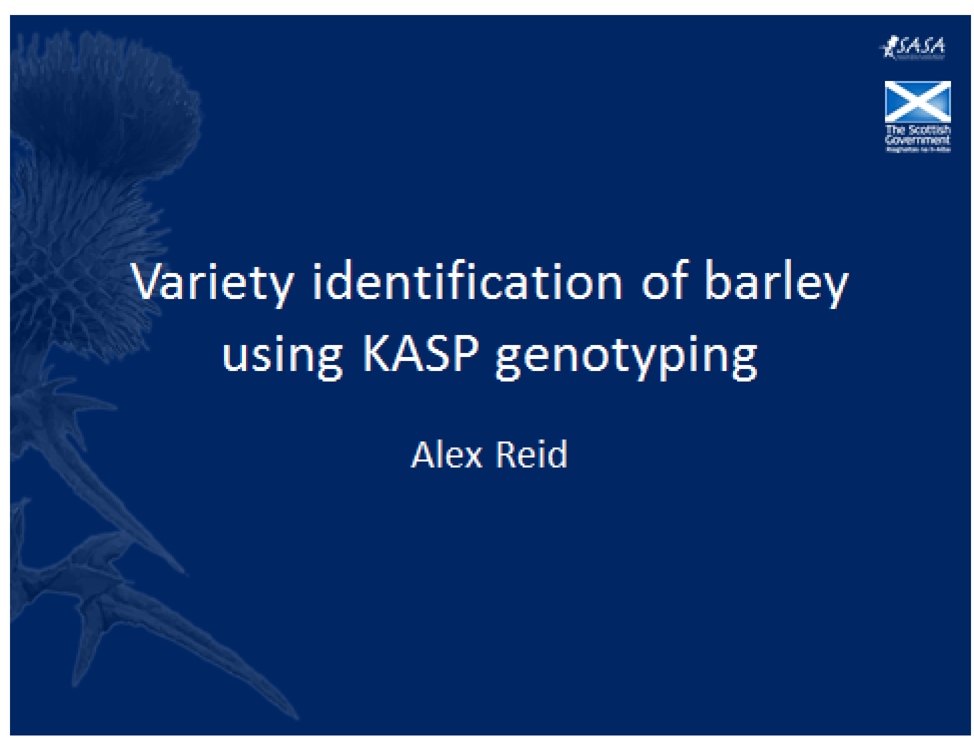
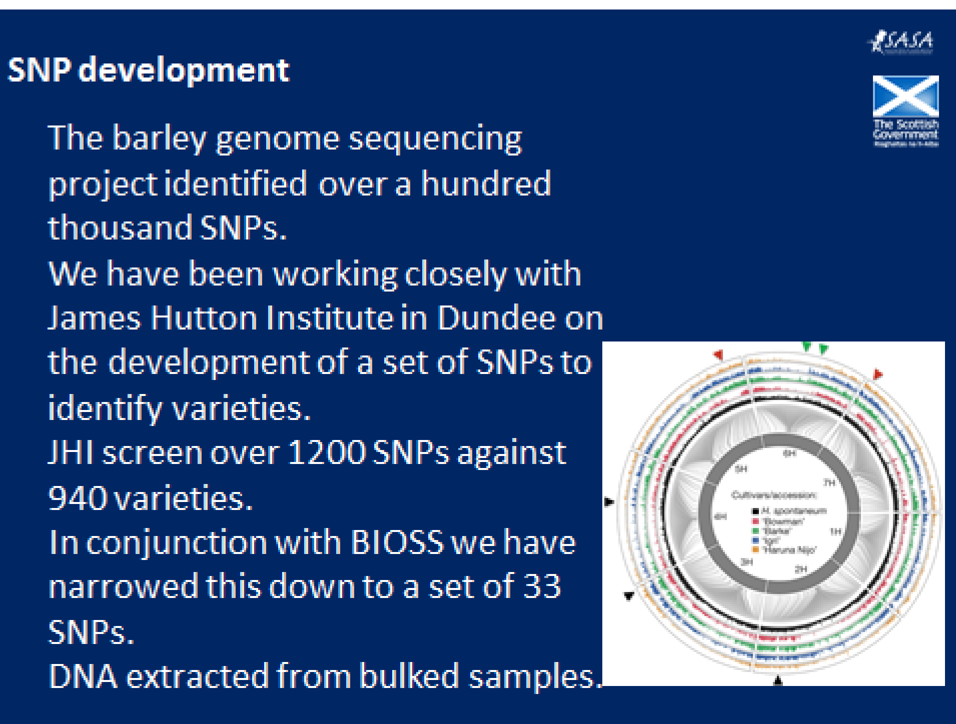
[Annex follows]

ANNEX



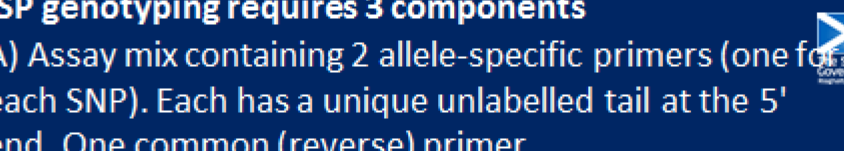


KASP genotyping assay

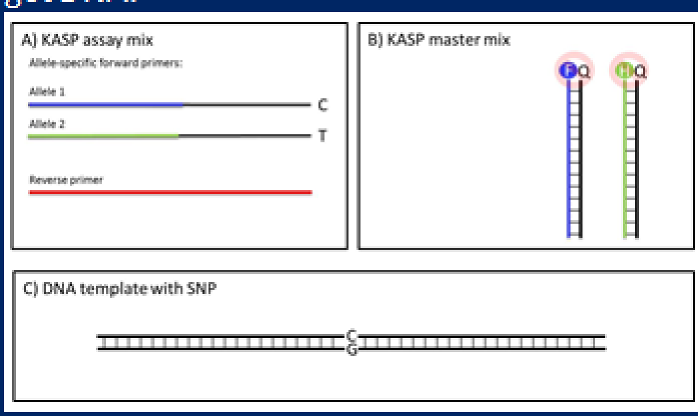
Developed by LGC.

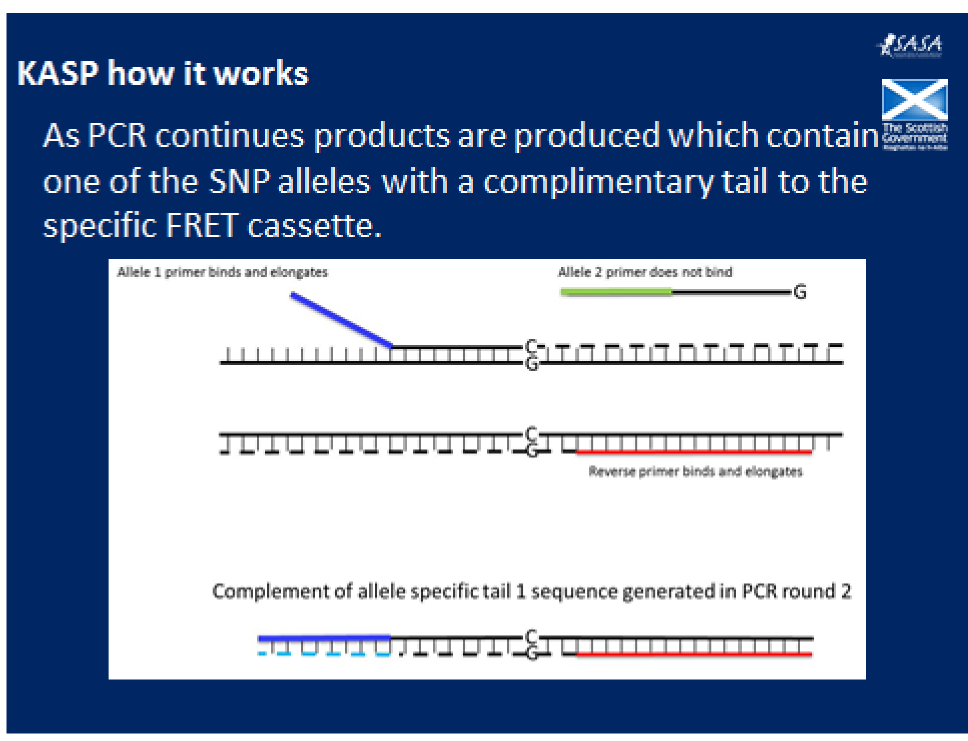
Uses a unique type of Kompetitive Allele-Specific PCR which can yield highly specific bi-allelic discrimination of SNPs and InDels.
There are acouple of very nice YouTube videos that explains how it works
https://www.youtube.com/watch?v=Uq9HhmzOqUQ
https://www.youtube.com/watch?v=GJbM7UbE7ZI

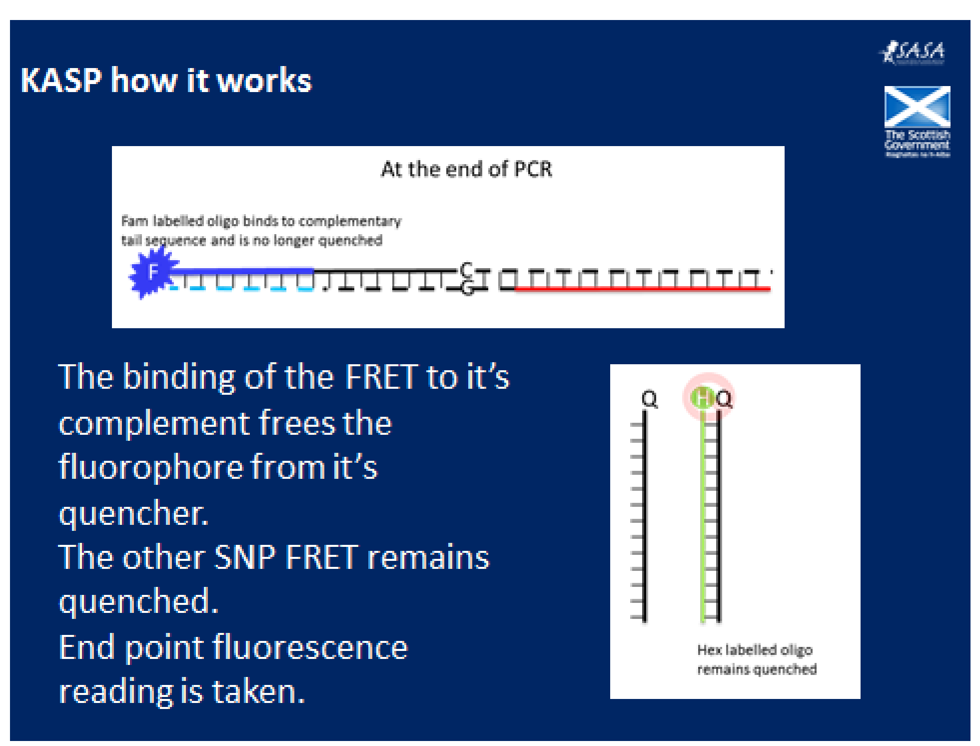
KASP genotyping requires 3 components

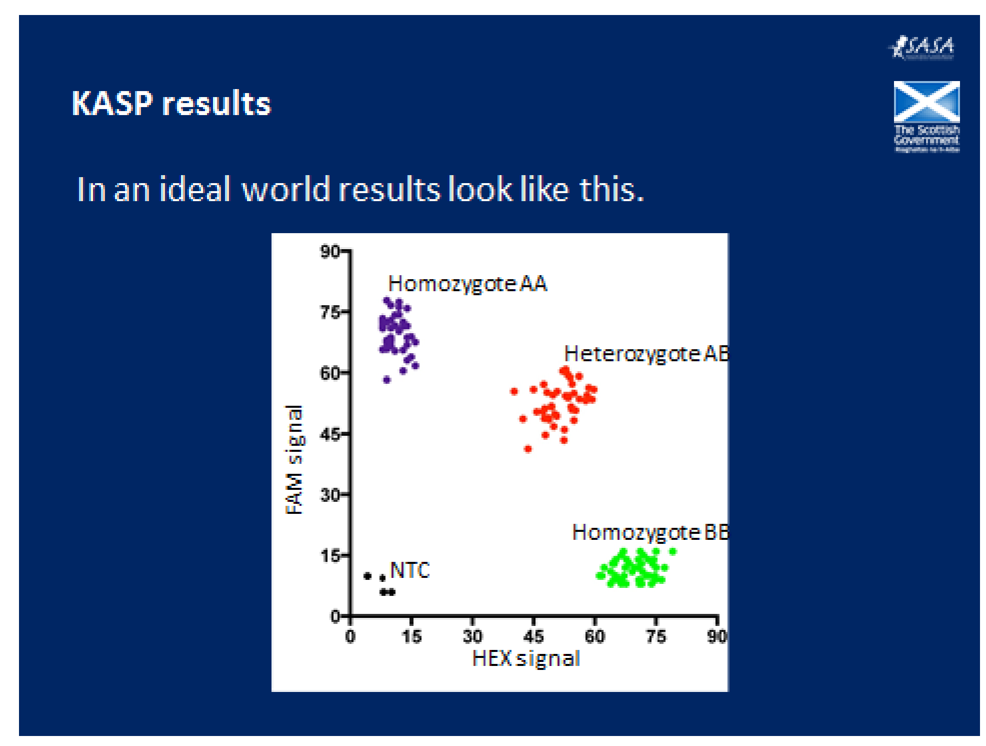


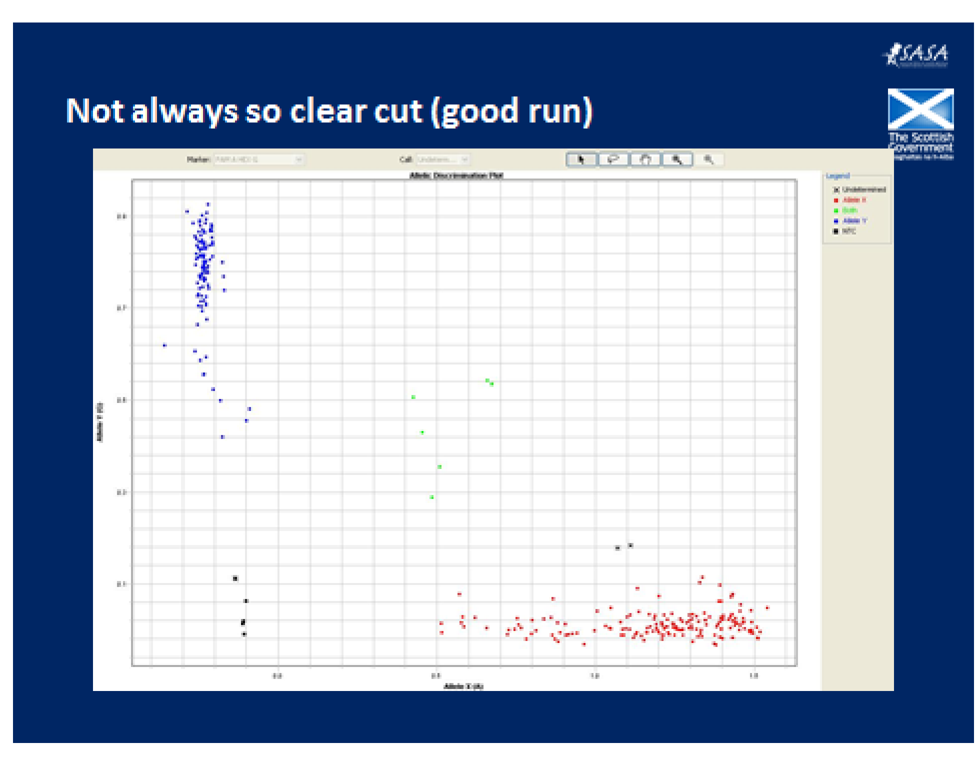
A) Assay mix containing 2 allele-specific primers (one for each SNP). Each has a unique unlabelled tail at the 5' end. One common (reverse) primer.
B) FAM and HEX FRET cassettes.
C) Target DNA.

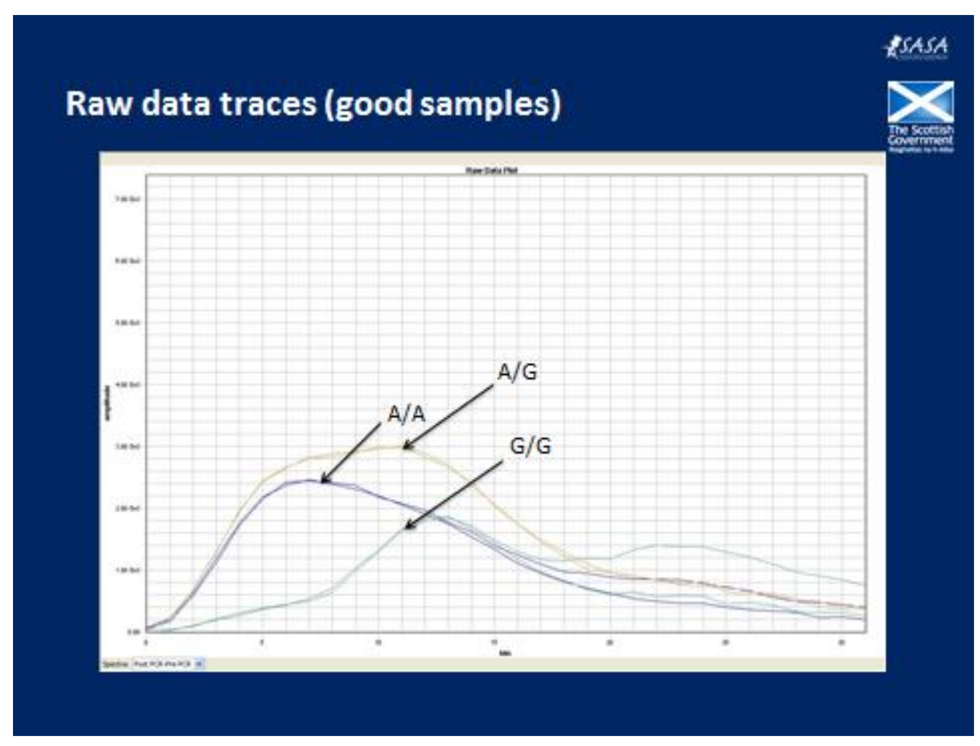


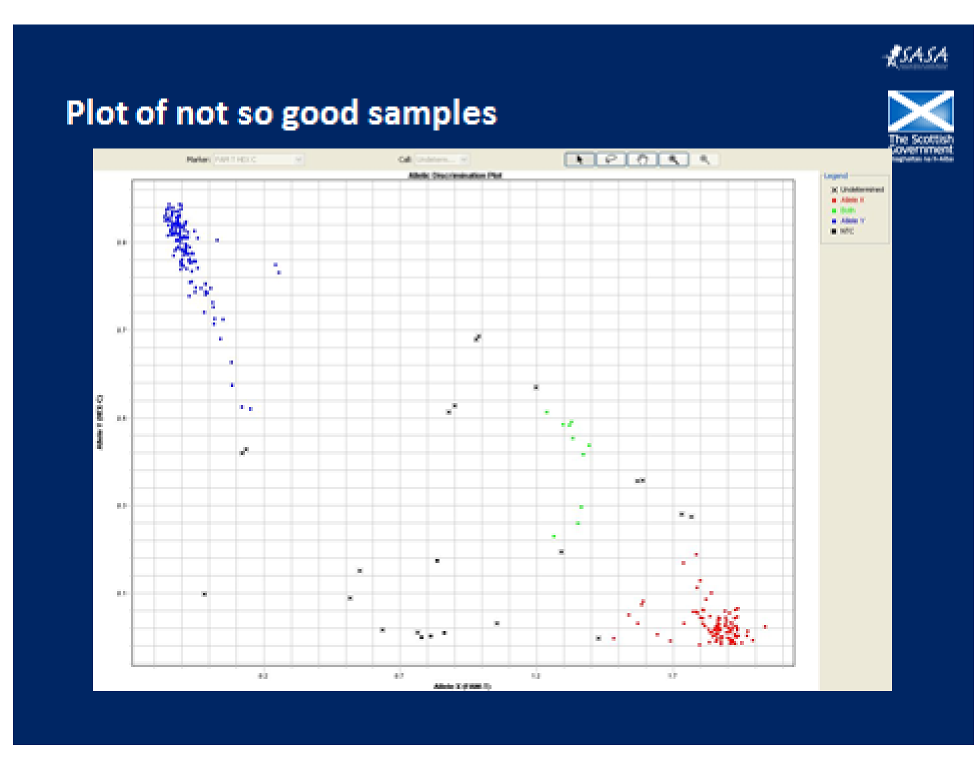

KASP how it works
As PCR continues products are produced which contain one of the SNP alleles with a complimentary tail to the specific FRET cassette.
Allele 1 primer binds and elongates
Allele 2 primer does not bind
G
C
G
C
G
Reverse primer binds and elongates
Complement of allele specific tail 1 sequence generated in PCR round 2
C
G

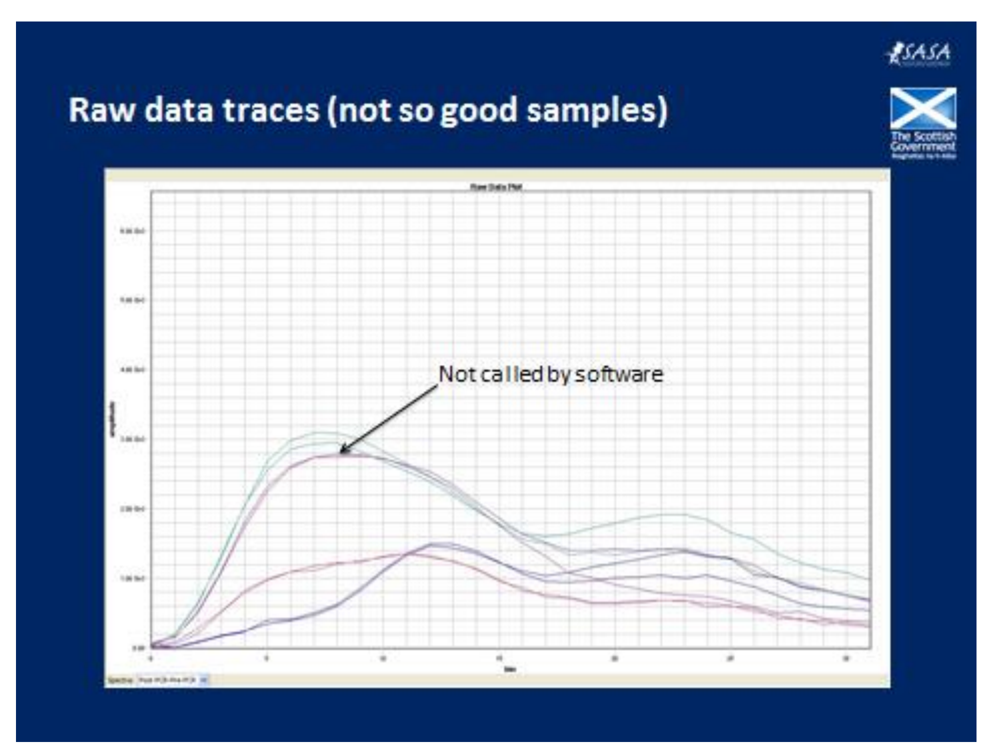

KASP how it works
At the end of PCR
Fam labelled oligo binds to complementary tail sequence and is no longer quenched
F
C
G
The binding of the FRET to it's complement frees the fluorophore from it's quencher.
The other SNP FRET remains quenched.
End point fluorescence reading is taken.
Q
H
Q
Hex labelled oligo remains quenched

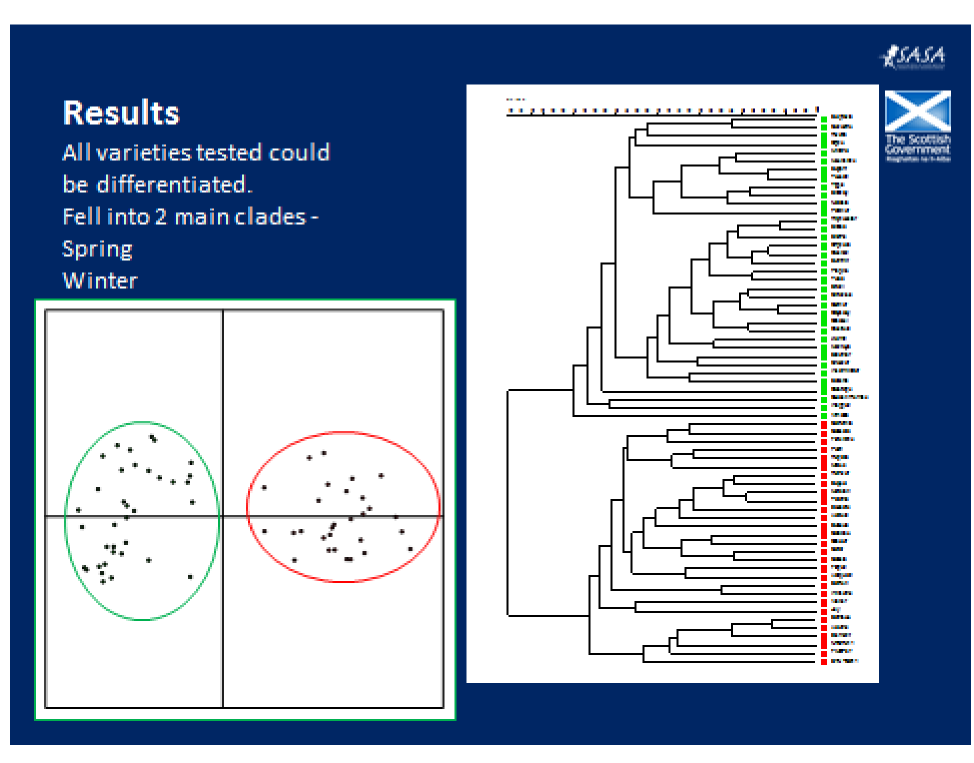

SASA
The Scottish Government
KASP results
In an ideal world results look like this.
Homozygote AA
Heterozygote AB
Homozygote BB
NTC
FAM signal
HEX signal
0
15
30
45
60
75
90


SASA
The Scottish Government
Not always so clear cut (good run)
Allelic Discrimination Plot
Allele X (A)


SASA
Raw data traces (good samples)
The Scottish Government
A/G
A/A
G/G


SASA
Plot of not so good samples
The Scottish Government


SASA
Raw data traces (not so good samples)
The Scottish Government
Not called by software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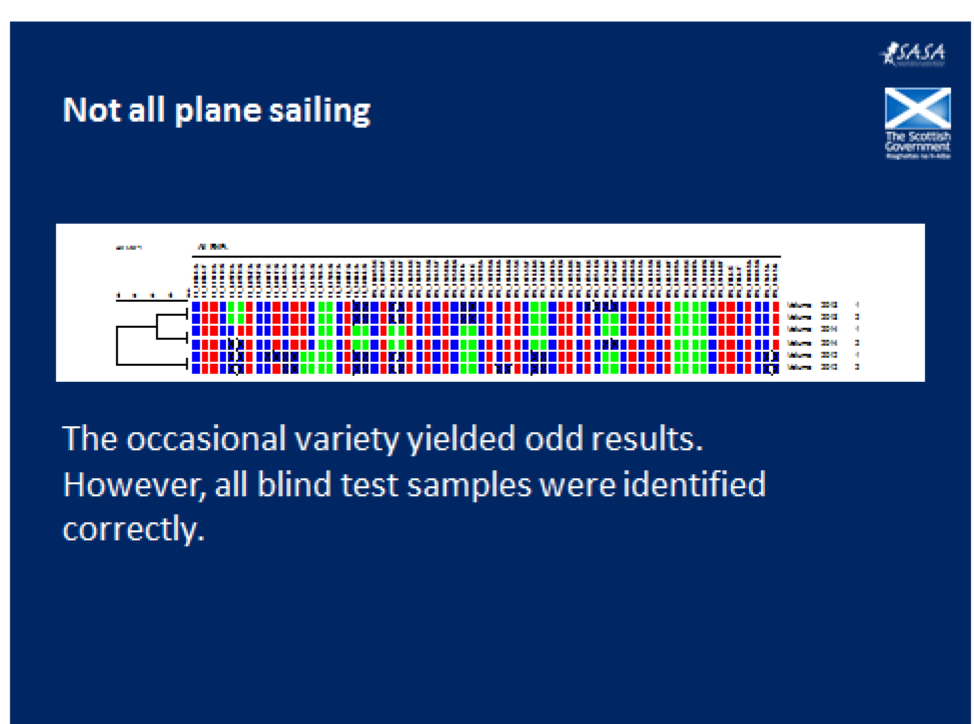
Results
All varieties tested could be differentiated.
Fell into 2 main clades -
Spring
Winter
The Scottish Government



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Pros and Cons of KASP

Pros	**Cons**
Very cheap (ca. £9/sample for all 33 SNPs). This is for 188 samples in duplicate using 384 well plates.	If you only want to run one sample with all 33 SNPs this rises to £51.
Can be automated easily (both DNA extraction and PCR set-up).	Each SNP requires several controls so not really feasible to run one sample with 33 SNPS
Automated scoring.	Not always easy to score (esp. heterozygotes).
Easy to scaled up to 384 and 1536 well plates.	There are limits to rogue detection.

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Summary

We have a working KASP genotyping method for barley variety identification.
Using 33 SNP assays.
Initial test of 66 varieties.
Blind test successful.
Two main clades spring and winter varieties.
Largely automated and therefore fairly high throughput.
Future work to expand the number of varieties tested.

SASA

Acknowledgements

SASA
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Joanne Russell

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