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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

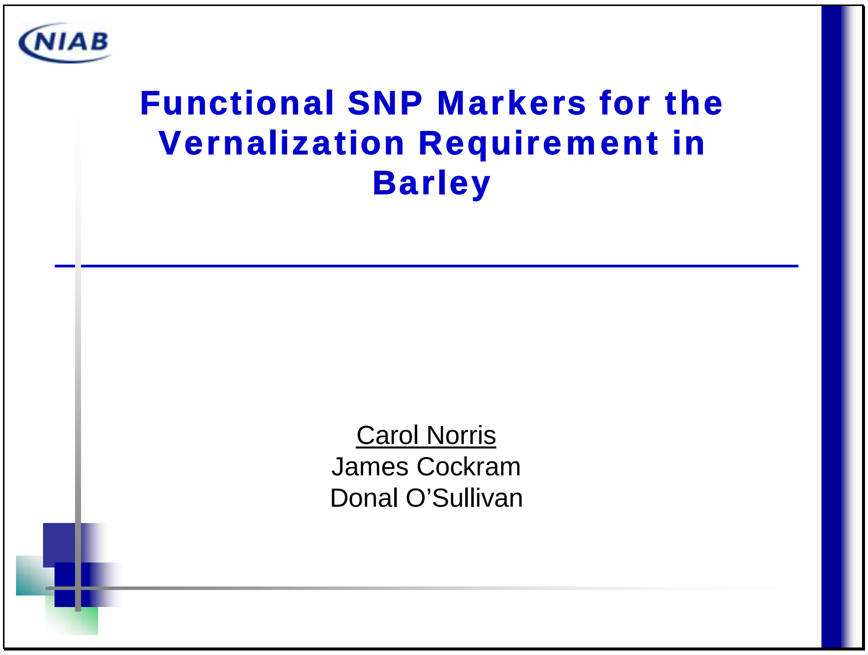
WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

FUNCTIONAL SNP MARKERS FOR THE VERNALIZATION REQUIREMENTS IN BARLEY: AN OPTION 1 APPROACH

Document prepared by experts from the United Kingdom



Functional SNP Markers for the Vernalization Requirement in Barley

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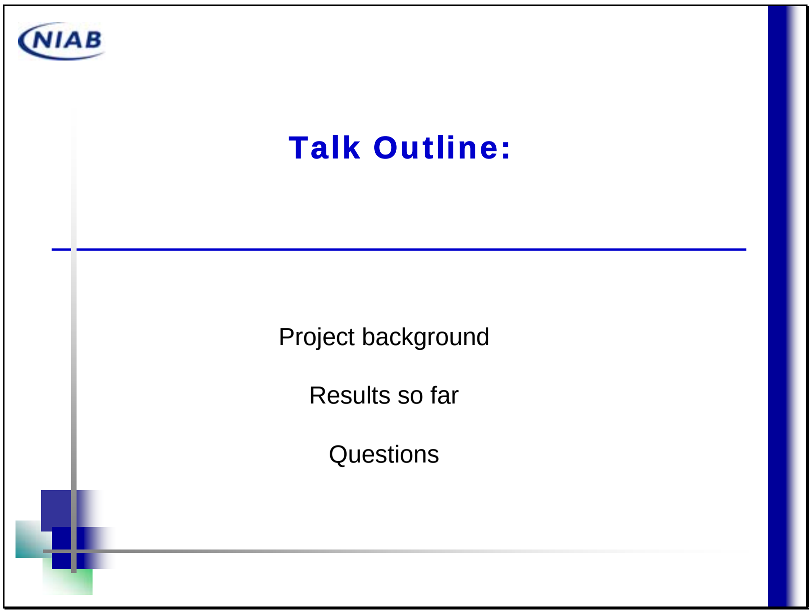
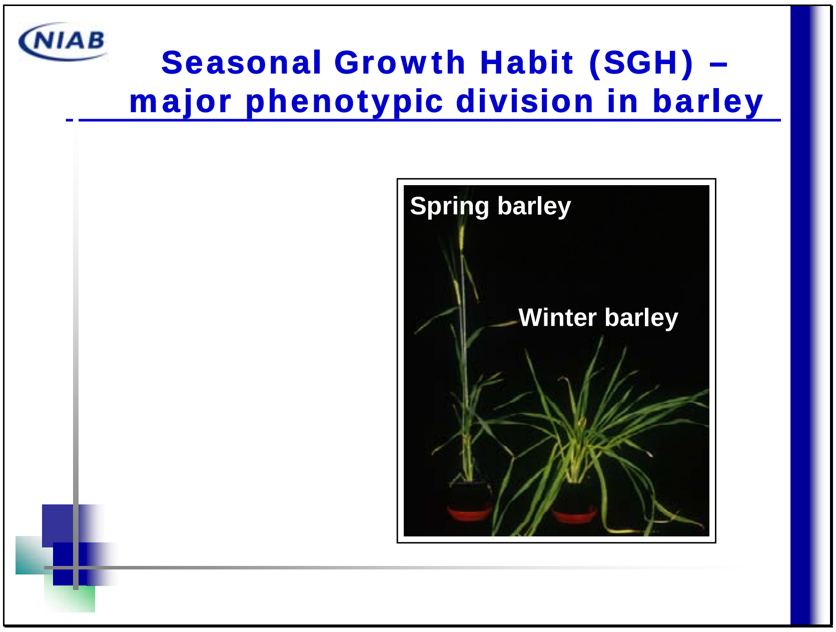


Talk Outline:

Project background

Results so far

Questions



Seasonal Growth Habit (SGH) – major phenotypic division in barley

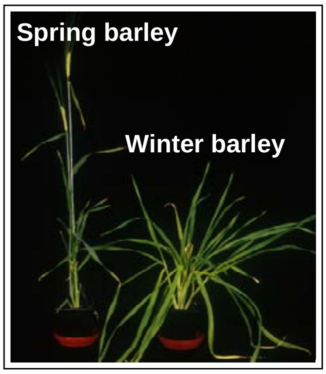
SGH – UPOV TG/19/10 characteristic 29

SGH states:

1 = spring

2 = alternative

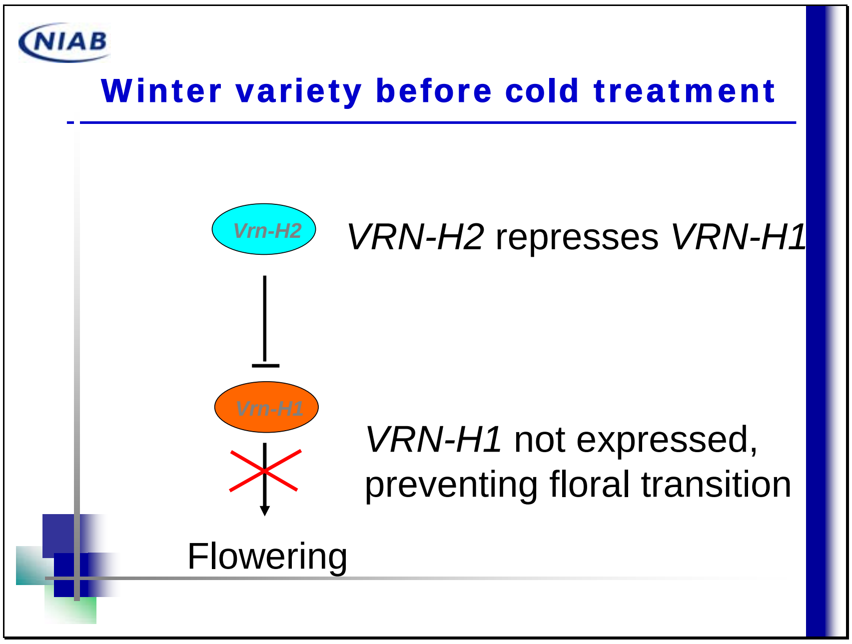
3 = winter



LD -V

Genetic control of SGH

- Two major loci in European barley (*VRN-H1* and *VRN-H2*)
- *VRN-H1* responsible for flowering mechanism
- *VRN-H2* represses *VRN-H1*
- During cold treatment repression is removed
- Mutation in either gene = spring type

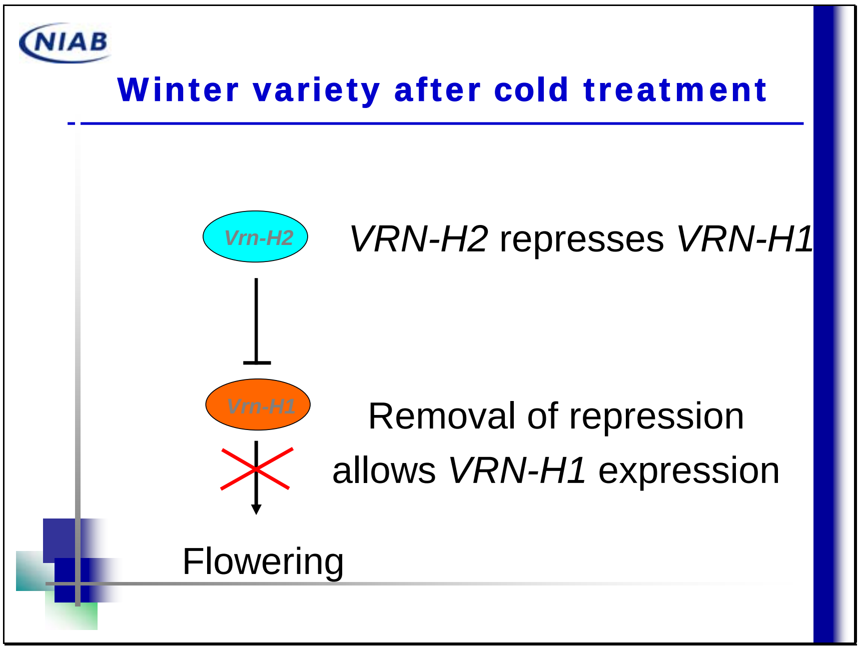


Winter variety before cold treatment

VRN-H2 represses *VRN-H1*

VRN-H1 not expressed,
preventing floral transition

Flowering



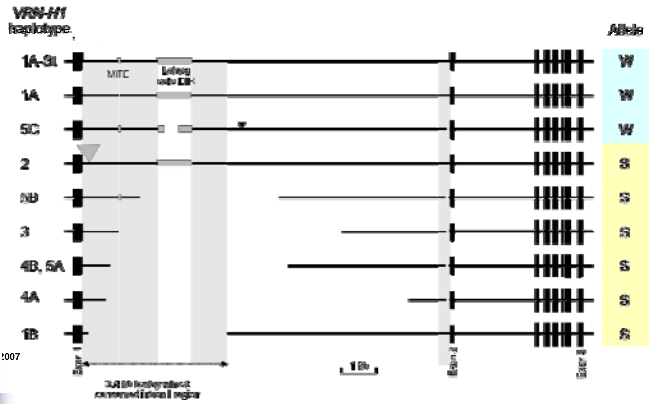
Winter variety after cold treatment

VRN-H2 represses *VRN-H1*

Removal of repression
allows *VRN-H1* expression

Flowering

VRN-H1: 6 spring, 3 winter alleles



Aims

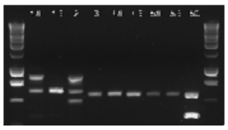
- ➢UPOV option 1 approach – “Molecular characteristics as a predictor of traditional characters
- ➢Investigate ‘Alternative’ lines
- ➢Detect SGH off types

New *VRN-H1* assay + *VRN-H2* assay

100 UK varieties recorded on the UK NL/RL 1991-2007 (50W, 50S)

Combined haplotypes predict SGH in all 100 varieties

VRN-H1 assay + *VRN-H2* assay = predicted SGH



(Cockram *et al* 2008)

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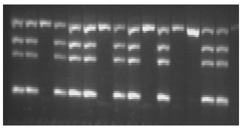

(Karsai *et al* 2005)

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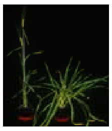

Generated a reference database of *VRN-H1* and *VRN-H2* alleles

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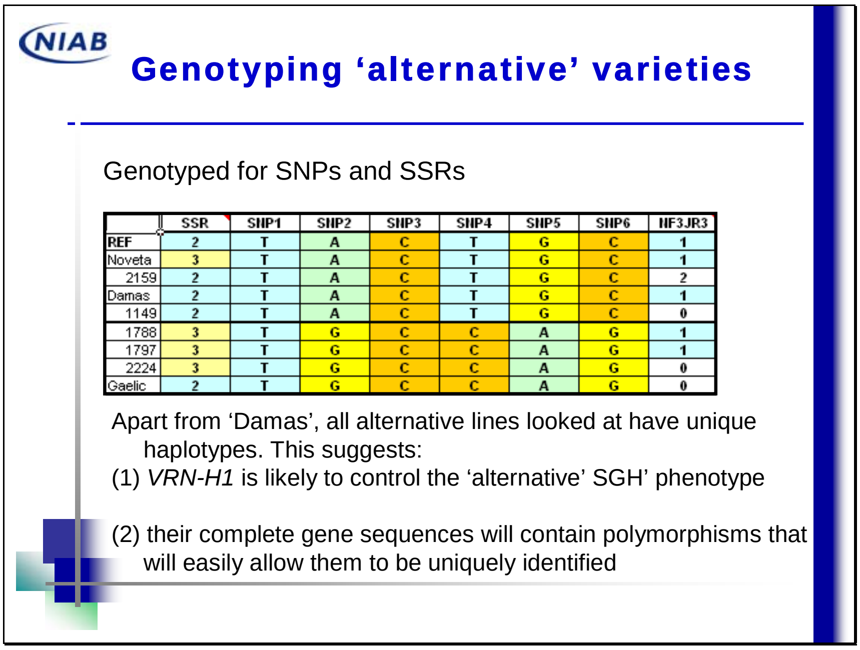
Additional work…

- Now single well markers for *VRN-H1* and *VRN-H2* have been designed and validated:
- Use vernalization field data from 2 years
- Test and sequence ‘Alternative’ lines
- Develop PCR marker to detect and quantify off-types (uniformity)



NIAB

Genotyping ‘alternative’ varieties

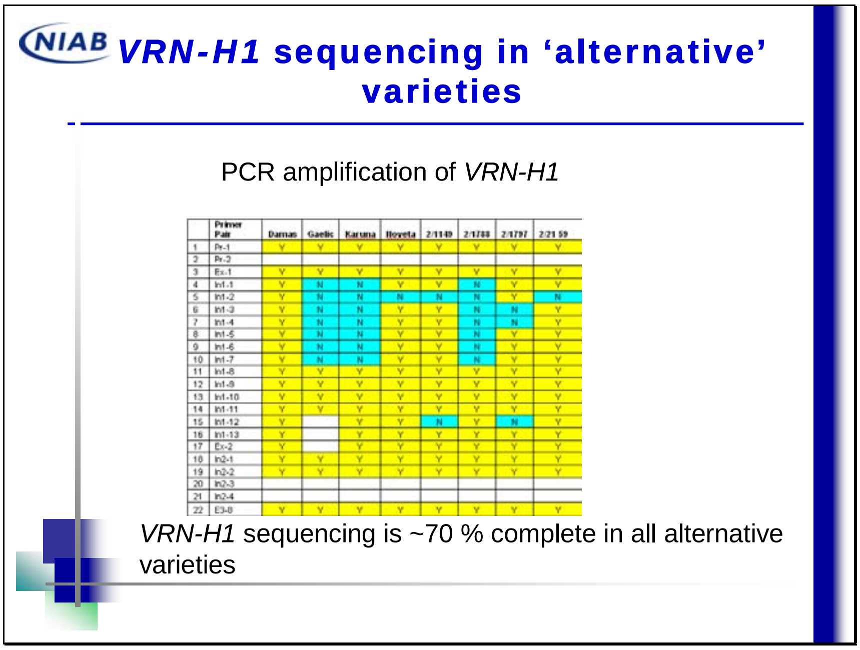
Genotyped for SNPs and SSRs

	SSR	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	NF3JR3
REF	2	T	A	C	T	G	C	1
Noveta	3	T	A	C	T	G	C	1
2159	2	T	A	C	T	G	C	2
Damas	2	T	A	C	T	G	C	1
1149	2	T	A	C	T	G	C	0
1788	3	T	G	C	C	A	G	1
1797	3	T	G	C	C	A	G	1
2224	3	T	G	C	C	A	G	0
Gaelic	2	T	G	C	C	A	G	0

Apart from ‘Damas’, all alternative lines looked at have unique haplotypes. This suggests:

(1) *VRN-H1* is likely to control the ‘alternative’ SGH’ phenotype

(2) their complete gene sequences will contain polymorphisms that will easily allow them to be uniquely identified



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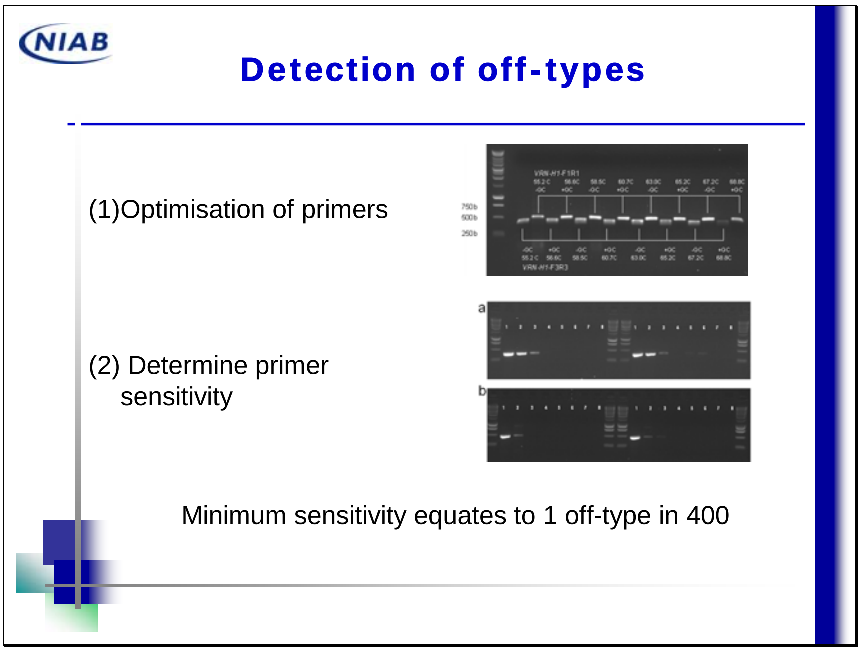
VRN-H1 sequencing in ‘alternative’ varieties

PCR amplification of *VRN-H1*

	Primer Pair	Damas	Gaelic	Karuna	Noveta	2/1149	2/1788	2/1797	2/21 59
1	Pr-1	Y	Y	Y	Y	Y	Y	Y	Y
2	Pr-2								
3	Ex-1	Y	Y	Y	Y	Y	Y	Y	Y
4	Int-1	Y	N	N	Y	Y	N	Y	Y
5	Int-2	Y	N	N	N	N	N	Y	N
6	Int-3	Y	N	N	Y	Y	N	N	Y
7	Int-4	Y	N	N	Y	Y	N	N	Y
8	Int-5	Y	N	N	Y	Y	N	Y	Y
9	Int-6	Y	N	N	Y	Y	N	Y	Y
10	Int-7	Y	N	N	Y	Y	N	Y	Y
11	Int-8	Y	Y	Y	Y	Y	Y	Y	Y
12	Int-9	Y	Y	Y	Y	Y	Y	Y	Y
13	Int-10	Y	Y	Y	Y	Y	Y	Y	Y
14	Int-11	Y	Y	Y	Y	Y	Y	Y	Y
15	Int-12	Y		Y	Y	N	Y	N	Y
16	Int-13	Y		Y	Y	Y	Y	Y	Y
17	Ex-2	Y		Y	Y	Y	Y	Y	Y
18	In2-1	Y	Y	Y	Y	Y	Y	Y	Y
19	In2-2	Y	Y	Y	Y	Y	Y	Y	Y
20	In2-3								
21	In2-4								
22	E3-8	Y	Y	Y	Y	Y	Y	Y	Y

VRN-H1 sequencing is ~70 % complete in all alternative varieties

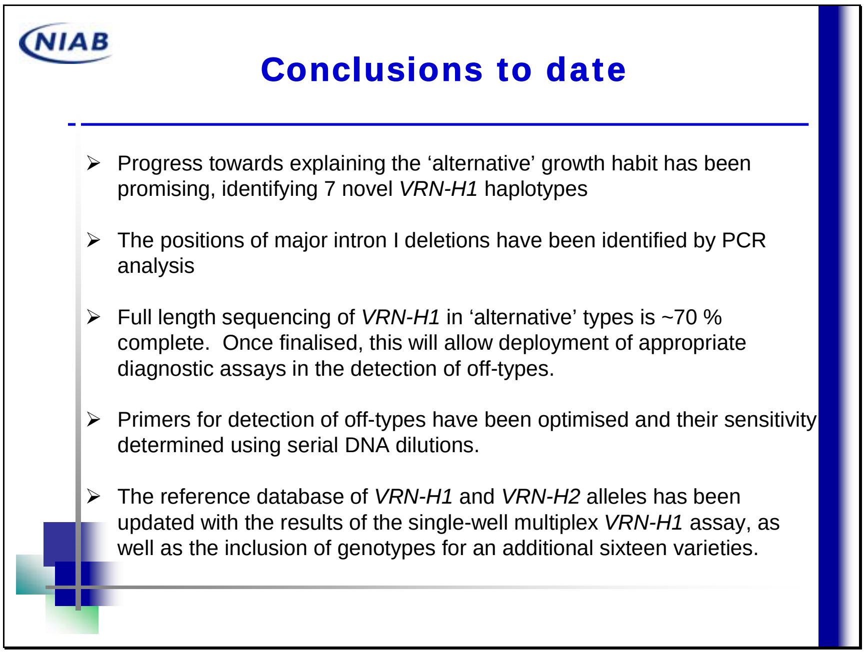


Detection of off-types

(1)Optimisation of primers

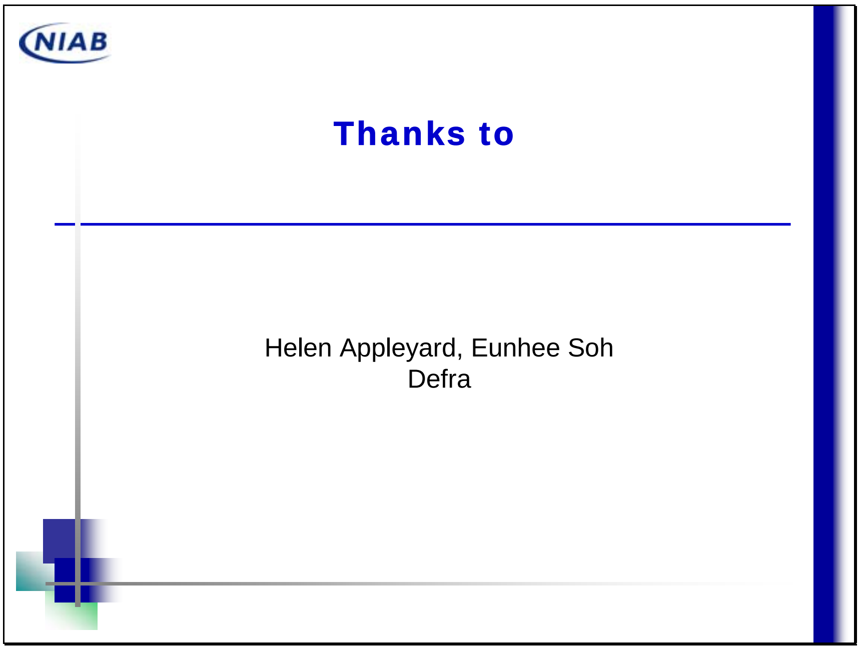
(2) Determine primer sensitivity

Minimum sensitivity equates to 1 off-type in 400



Conclusions to date

- Progress towards explaining the ‘alternative’ growth habit has been promising, identifying 7 novel *VRN-H1* haplotypes
- The positions of major intron I deletions have been identified by PCR analysis
- Full length sequencing of *VRN-H1* in ‘alternative’ types is ~70 % complete. Once finalised, this will allow deployment of appropriate diagnostic assays in the detection of off-types.
- Primers for detection of off-types have been optimised and their sensitivity determined using serial DNA dilutions.
- The reference database of *VRN-H1* and *VRN-H2* alleles has been updated with the results of the single-well multiplex *VRN-H1* assay, as well as the inclusion of genotypes for an additional sixteen varieties.



Thanks to

Helen Appleyard, Eunhee Soh
Defra

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