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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

**AN OVERVIEW OF DNA-BASED VARIETY IDENTIFICATION AT THE CANADIAN
GRAIN COMMISSION**

Document prepared by an expert from Canada



The slide features a green header bar with the words "QUALITY INNOVATION SERVICE". Below this is a photograph of a vast, rolling landscape of agricultural fields under a blue sky with white clouds. In the foreground, there are some small buildings and vehicles. The Canadian Grain Commission logo, featuring a stylized maple leaf and the text "Canadian Grain Commission / Commission canadienne des grains", is positioned on the left. To the right, the word "Canada" is written in a large, bold font with a small maple leaf icon above the letter "a". The main title "DNA-based Variety Identification at the Canadian Grain Commission" is centered in large, bold, orange letters. Below it, the name "Daniel Perry" and "Grain Research Laboratory" are in smaller orange text. At the bottom left, a small copyright notice reads "© Canadian Grain Commission, 2010".



The slide has a dark green header bar with the text "Canadian Grain Commission" in yellow. To the right of the text is a small image of a wheat stalk. The main body of the slide is a light beige color. The text "The CGC:" is at the top, followed by a bulleted list: "Is the regulator of Canada's grain handling industry", "Is the official certifier of Canadian grain", and "Conducts research into grain quality".

The Grain Research Laboratory



The GRL:

- Conducts research to understand grain quality and grain safety factors
- Develops methods and tests for measuring and evaluating grain quality and grain safety
- Monitors grain in Canada's grain handling system for quality and safety

Variety Identification in the GRL



Wheat

- Marketed by class – designated varieties
- DNA methods to complement protein-based variety monitoring of hexaploid wheat
- DNA methods to monitor variety composition of durum shipments

Barley

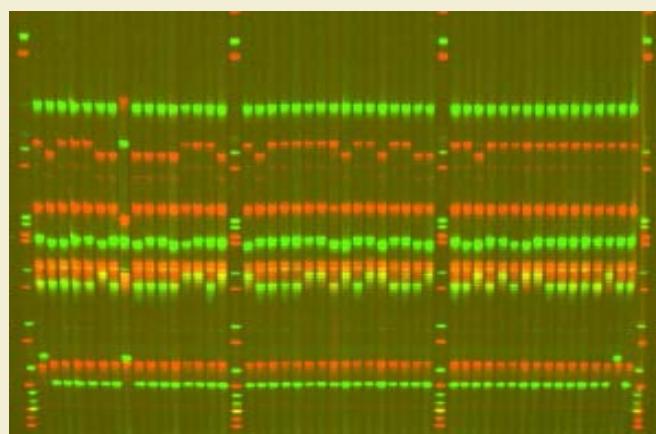
- Certify varietal purity of malting barley cargos

Microsatellite methods

- Multiplexed marker sets
 - published sources
 - tailed primers
 - Li-Cor DNA analyzers
- Single kernel analyses
 - 96-well plate format DNA extraction

Microsatellite methods

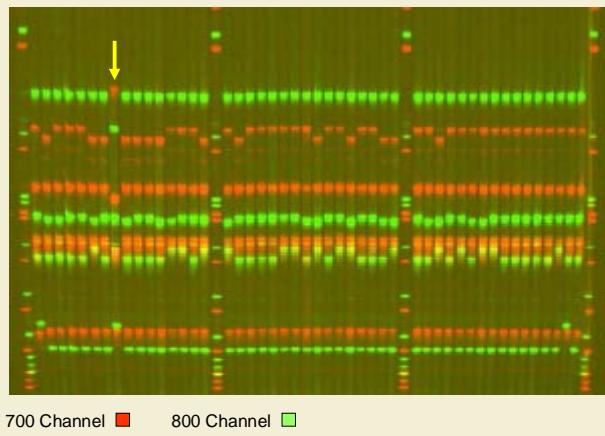
Malting barley varietal purity



700 Channel ■ 800 Channel ■

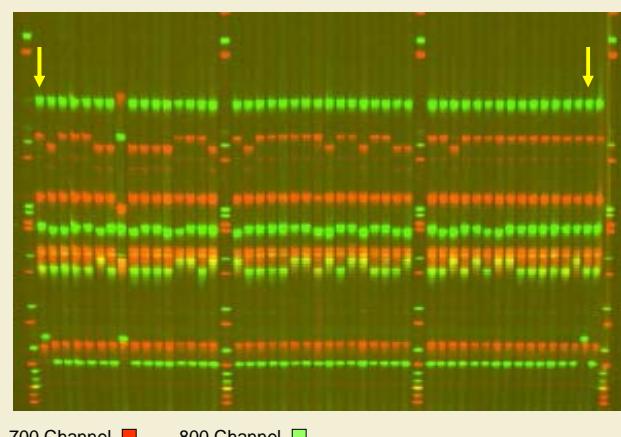
Microsatellite methods

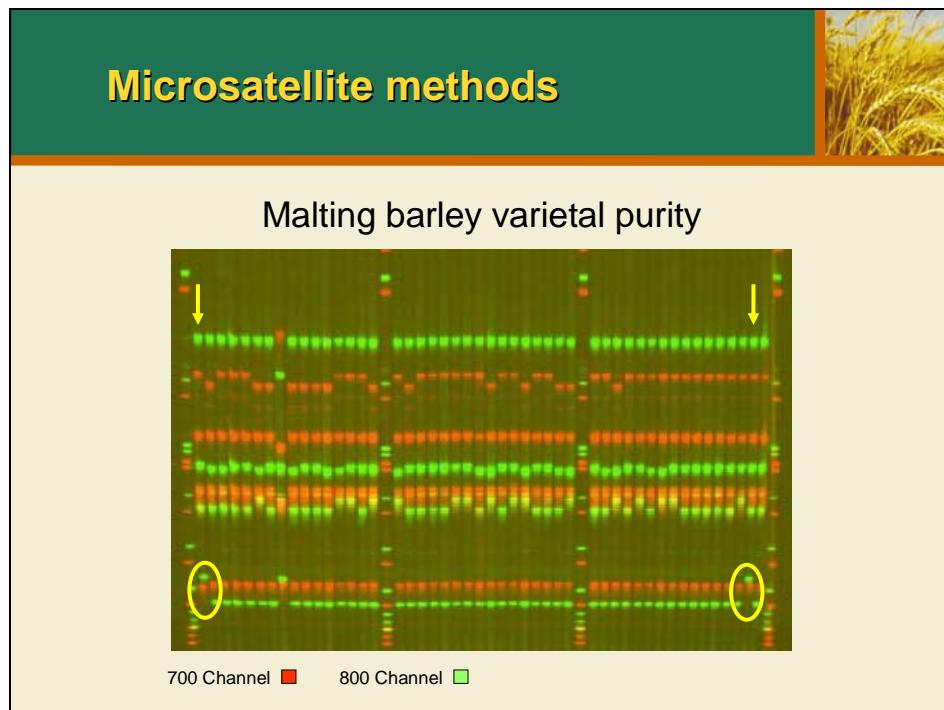
Malting barley varietal purity



Microsatellite methods

Malting barley varietal purity





Microsatellite methods

The figure displays a microsatellite gel electrophoresis (GEL) image used for challenges in barley variety identification. The gel has two main color channels: 700 Channel (red/orange) and 800 Channel (green). The lanes represent different samples, likely different barley varieties. The gel shows various patterns of bands across the lanes, with some bands being more prominent than others.

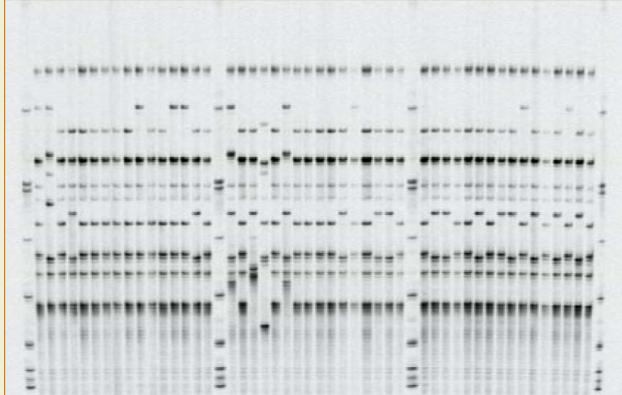
Challenges for barley variety ID

Variety	Pedigree
'CDC PolarStar'	OIU003/CDC Kendall (bx)
'Norman'	Selection from CDC Kendall
'Merit 16'	Merit//Merit/2B95-8129
'Merit 57'	Merit//Merit/2B94-5744

Microsatellite methods



Durum wheat monitoring

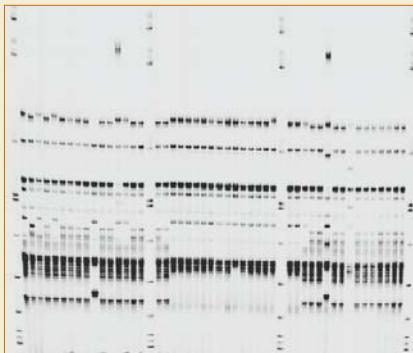


700 or 800 Channel Theor Appl Genet (2004) 109:55-61

Microsatellite methods



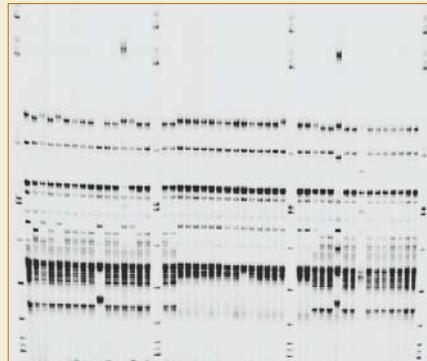
Hexaploid wheat analysis



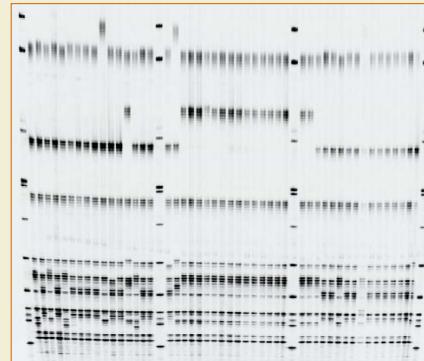
Durum set – 700 Channel

Microsatellite methods

Hexaploid wheat analysis



Durum set – 700 Channel



WOOC set – 800 Channel

SNP Research

SNP-based markers

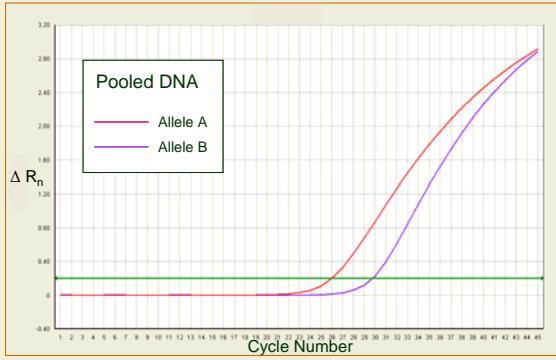
- Less user intervention
- Higher throughput
- Quantitative analyses

SNP Research



Ground sample analysis – barley

Real-time PCR with a bulk sample



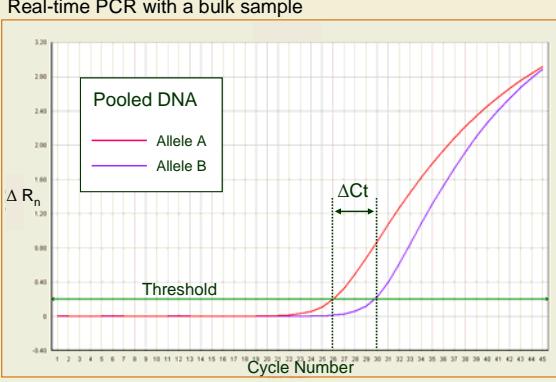
Detailed description: This graph plots the change in fluorescence (ΔR_n) against the cycle number during a real-time PCR experiment. The y-axis ranges from -0.40 to 3.20, and the x-axis ranges from 1 to 45 cycles. Two data series are shown: Allele A (red line) and Allele B (purple line). Both series remain at a baseline level of approximately 0.20 until cycle 18, after which they both increase rapidly. Allele A reaches a plateau around 2.80, while Allele B reaches a plateau around 2.40. A horizontal green line at $\Delta R_n = 0.20$ is labeled 'Threshold'.

SNP Research



Ground sample analysis – barley

Real-time PCR with a bulk sample



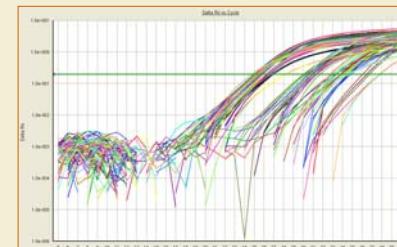
Detailed description: This graph plots the change in fluorescence (ΔR_n) against the cycle number during a real-time PCR experiment. The axes and data series are identical to the previous graph. A vertical double-headed arrow is drawn between the two curves, indicating the difference in cycle number at the threshold, which is labeled ΔC_t .

SNP Research



Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells



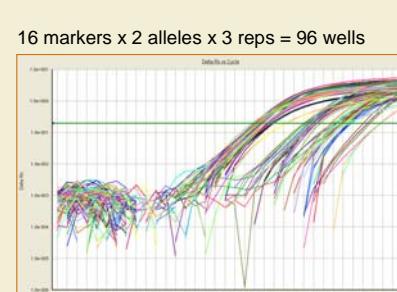
Detailed description: This figure is a line graph titled 'Ground sample analysis – barley'. It shows the fluorescence signal for 16 different SNP markers across 36 cycles. The y-axis represents fluorescence intensity from 0.0 to 1.0, and the x-axis represents the cycle number from 0 to 36. Each marker is represented by a distinct color, and all show a similar trend of increasing fluorescence over time, with most markers reaching a plateau between cycle 30 and 36.

SNP Research



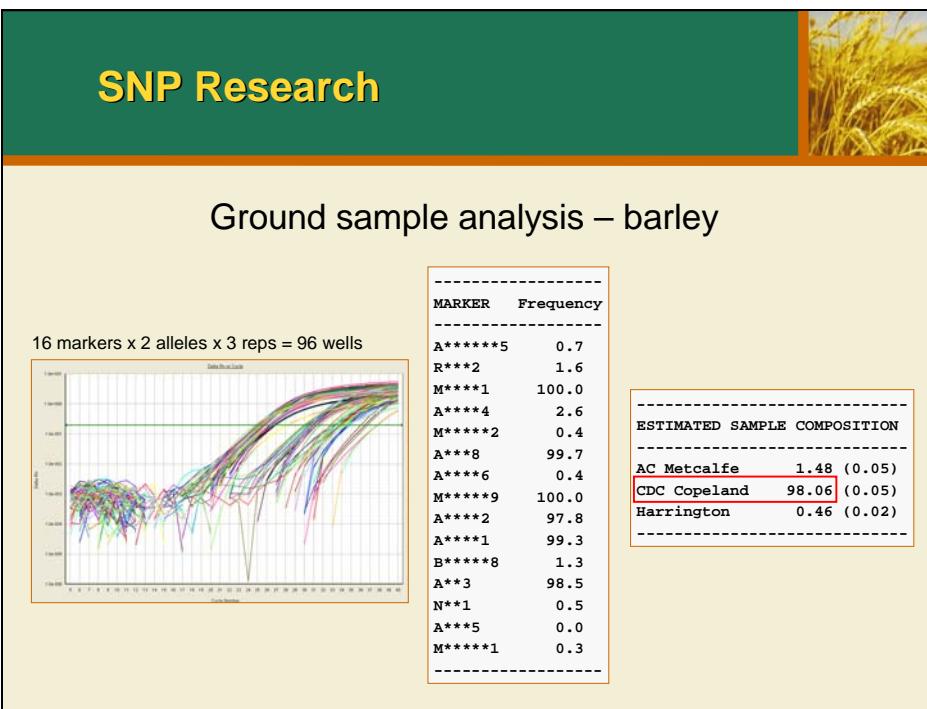
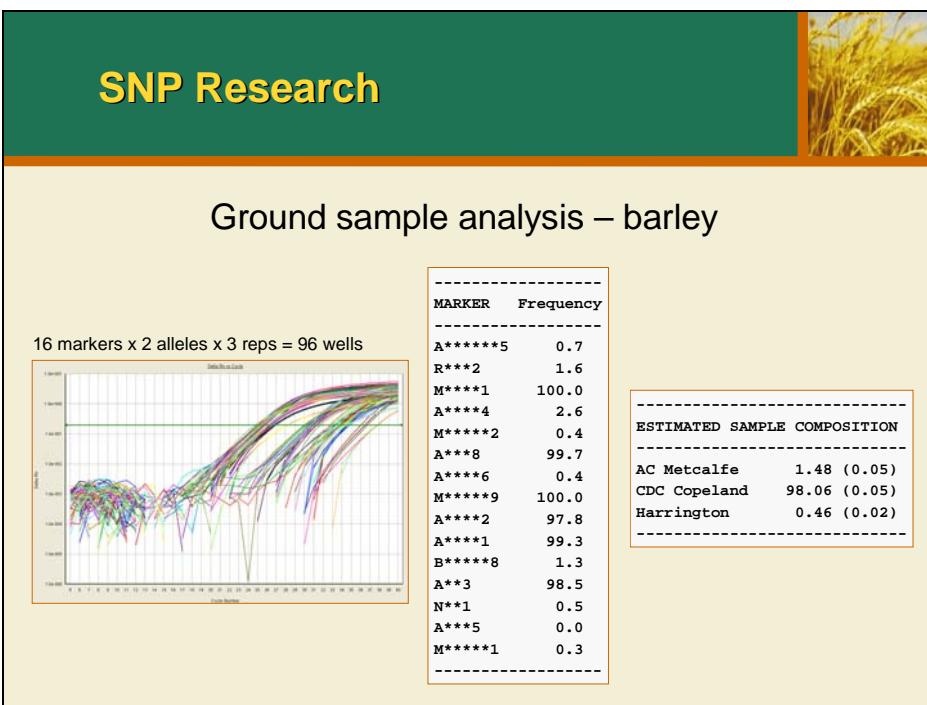
Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells



MARKER	Frequency
A*****5	0.7
R****2	1.6
M*****1	100.0
A****4	2.6
M*****2	0.4
A***8	99.7
A****6	0.4
M*****9	100.0
A****2	97.8
A****1	99.3
B*****8	1.3
A**3	98.5
N**1	0.5
A***5	0.0
M*****1	0.3

Detailed description: This figure is a line graph titled 'Ground sample analysis – barley' showing fluorescence intensity over 36 cycles for 96 SNP markers. To the right of the graph is a table listing the frequency of each marker. The markers are listed in descending order of frequency. Most markers have frequencies of either 0.0, 0.3, 0.4, 0.5, or 100.0, with a few intermediate values.



SNP Research



Ground sample analysis – blind samples

Sample	AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy
A Actual Estimated	92	4	2	2		
B Actual Estimated	2		94		2	2
C Actual Estimated	2	96		2		
D Actual Estimated	94	1	2	1	2	
E Actual Estimated	2	94	2	2		
F Actual Estimated	2	2	2	94		

SNP Research



Ground sample analysis – blind samples

Sample	AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy	CDC Dolly
A Actual Estimated	92	4	2	2			
	92.6	4.1	1.9	1.4			
B Actual Estimated	2		94		2	2	–
	2.5		94.9		1.2	0.9	0.5
C Actual Estimated	2	96		2			
	2.3	96.4		1.3			
D Actual Estimated	94	1	2	1	2		
	94.7	0.9	2.0	1.2	1.3		
E Actual Estimated	2	94	2	2			
	2.3	94.8	1.8	1.2			
F Actual Estimated	2	2	2	94			
	2.4	1.6	2.3	93.8			

SNP Research

Ground sample analysis – blind samples

Sample	AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy	CDC Dolly
A Actual	92	4	2	2			
A Estimated	92.6	4.1	1.9	1.4			
B Actual	2		94		2	2	–
B Estimated	2.5		94.9		1.2	0.9	0.5
C Actual	2	96		2			
C Estimated	2.3	96.4		1.3			
D Actual	94	1	2	1	2		
D Estimated	94.7	0.9	2.0	1.2	1.3		
E Actual	2	94	2	2			
E Estimated	2.3	94.8	1.8	1.2			
F Actual	2	2	2	94			
F Estimated	2.4	1.6	2.3	93.8			

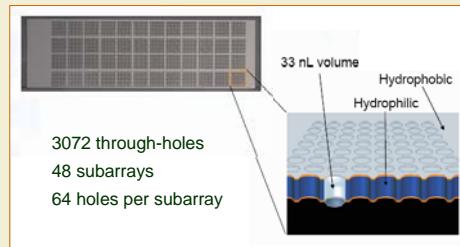
SNP Research

OpenArray for wheat variety ID

SNP Research



OpenArray for wheat variety ID



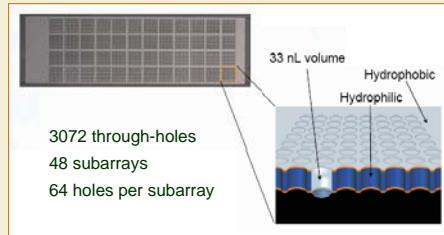
3072 through-holes
48 subarrays
64 holes per subarray

33 nL volume
Hydrophobic
Hydrophilic

SNP Research



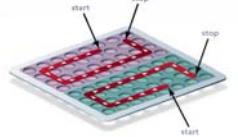
OpenArray for wheat variety ID



3072 through-holes
48 subarrays
64 holes per subarray

33 nL volume
Hydrophobic
Hydrophilic

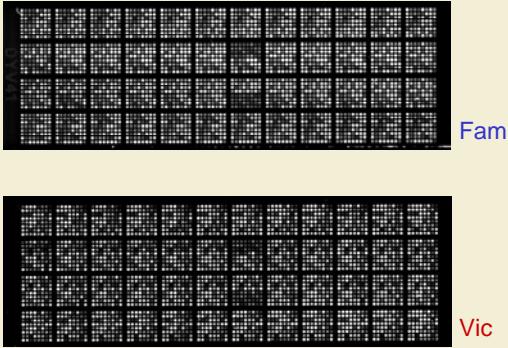
2 samples (kernels) per subarray
96 kernels per plate
32 SNPs per kernel



SNP Research



OpenArray – imaging



Fam

Vic

SNP Research



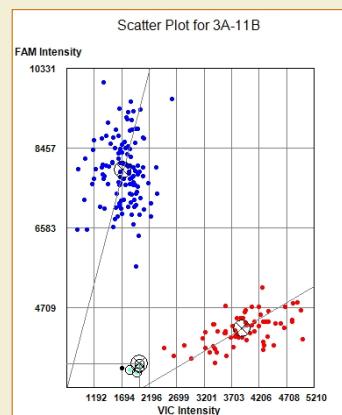
OpenArray – automated genotyping



Fam

Vic

Scatter Plot for 3A-11B



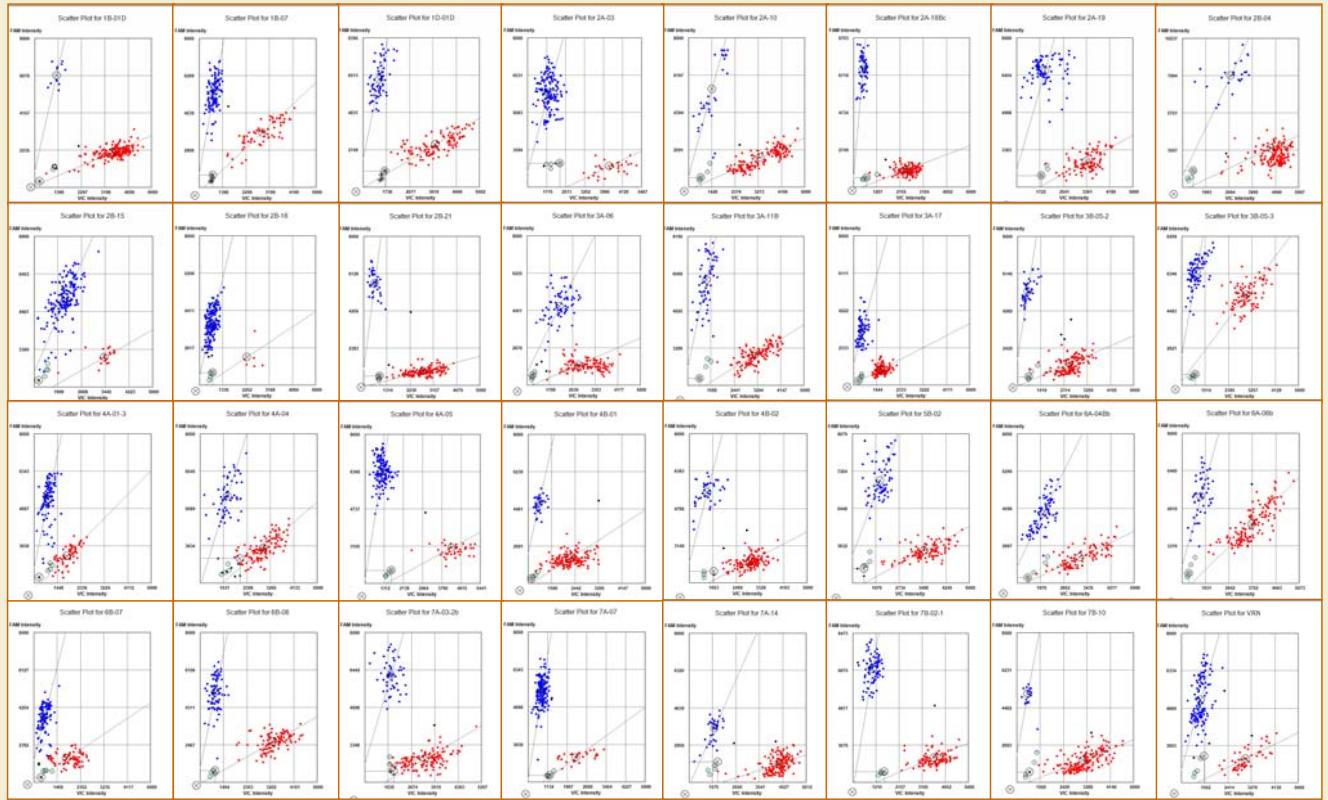
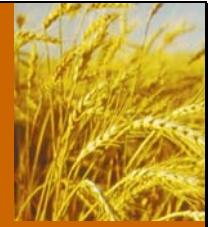
FAM Intensity

VIC Intensity

10331
8457
6583
4709

1192 1694 2196 2699 3201 3703 4206 4708 5210

SNP Research



SNP Research

Wheat variety identification by SNP profile

88 CM495-86	221211222211121122121211212121212	Lillian
89 CM495-87	21111121221122111111112111121112	Harvest
90 CM495-88	111111111111111111111111111111112	CDC Abound Superb
91 CM495-89	12221121221122112221211121121112	CDC Imagine
92 CM495-90	211111212211221111111112101121102	Harvest
93 CM495-91	12222211221112122221210122121102	AC Intrepid
94 CM495-92	1212221122211112111222122121112	Infinity
95 CM495-93	10111111111111111111111111111112	CDC Abound Superb
96 CM495-94	21111121221122111111112111121112	Harvest
97 OCM77-1	2222212222111220122121212221112	5700PR
98 OCM77-2	2222212222111222122121212221112	5700PR
99 OCM77-3	121221212211122221121212221102	AC Foremost
100 OCM77-4	121221212211122221121212221112	AC Foremost
101 OCM77-5	1222111222211212212111122221122	5702PR
102 OCM77-6	121221212211122221121212221102	AC Foremost
103 OCM77-7	2222212222111222122121212221102	5700PR
104 OCM77-8	222211222222110112112112222112	5701PR
105 OCM77-9	121221212211122221121212221112	AC Foremost
106 OCM77-10	1212212122111222121112212221112	AC Crystal AC Taber
107 OCM77-11	2222212222111222121121112201102	5700PR

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