



BMT/12/8 Add.

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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



Canadian Grain Commission

The CGC:

- Is the regulator of Canada's grain handling industry
- Is the official certifier of Canadian grain
- 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

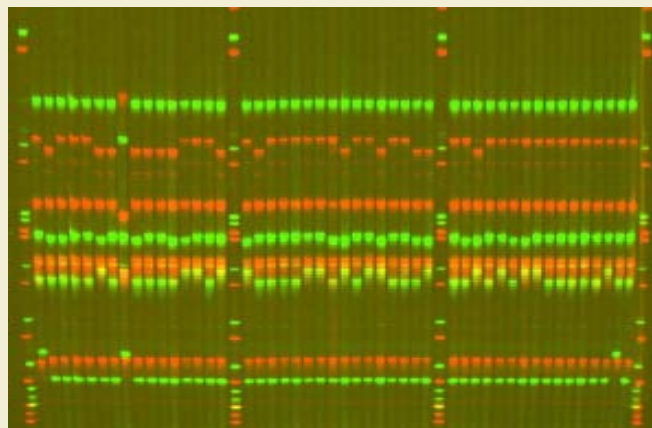
- Certify varietal purity of malting barley cargos

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

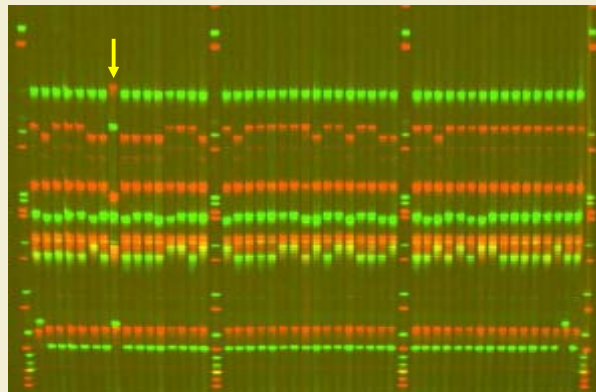


700 Channel ■ 800 Channel ■

Microsatellite methods



Malting barley varietal purity

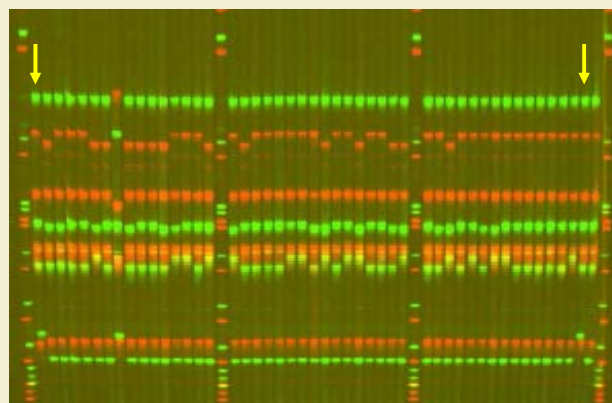


700 Channel ■ 800 Channel ■

Microsatellite methods



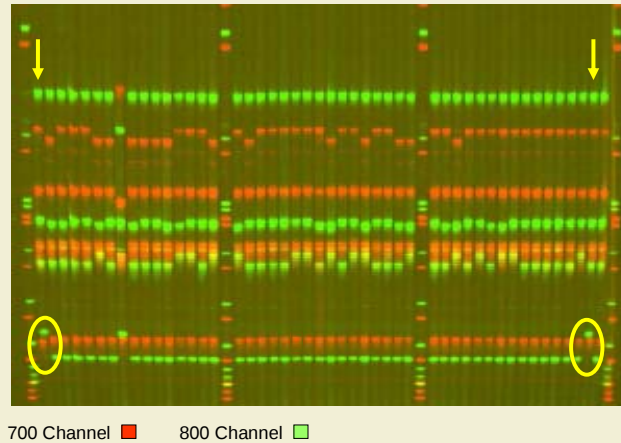
Malting barley varietal purity



700 Channel ■ 800 Channel ■

Microsatellite methods

Malting barley varietal purity



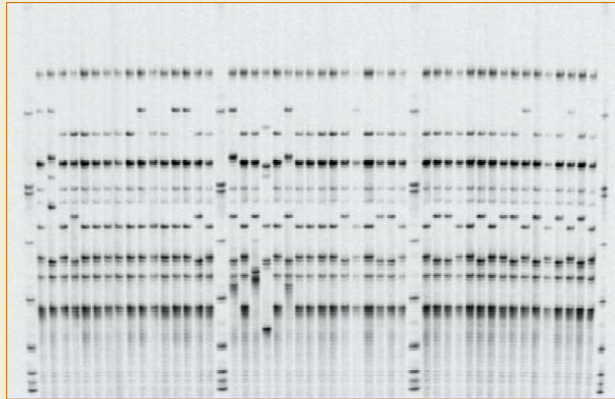
Microsatellite methods

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

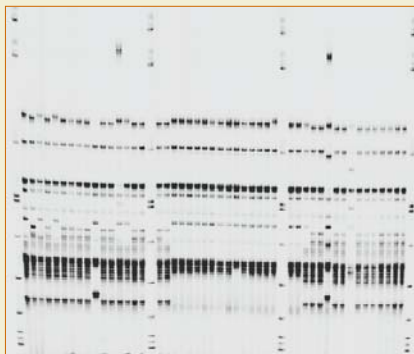


700 or 800 Channel

Theor Appl Genet (2004) 109:55-61

Microsatellite methods

Hexaploid wheat analysis

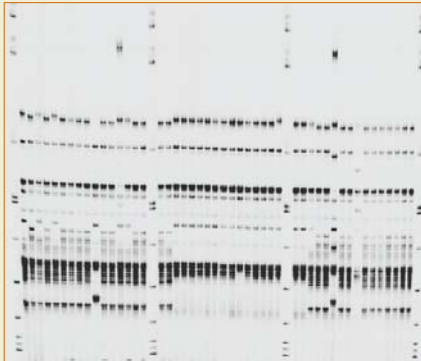


Durum set – 700 Channel

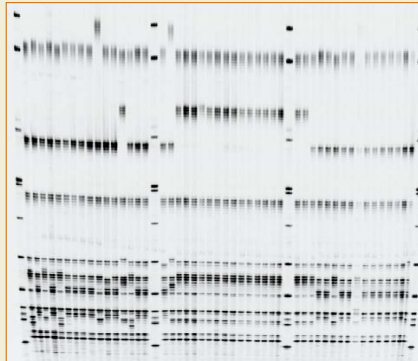
Microsatellite methods



Hexaploid wheat analysis



Durum set – 700 Channel



WOOC set – 800 Channel

SNP Research



SNP-based markers

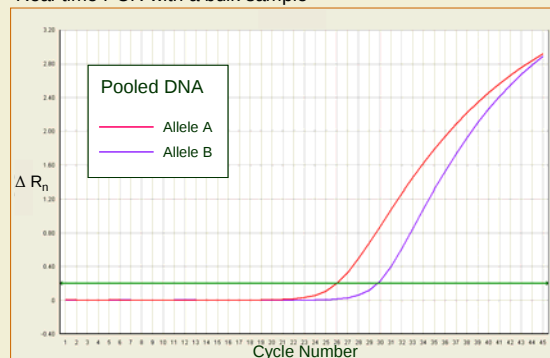
- Less user intervention
- Higher throughput
- Quantitative analyses

SNP Research



Ground sample analysis – barley

Real-time PCR with a bulk sample

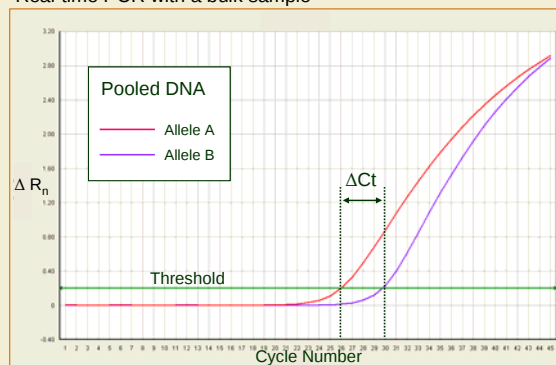


SNP Research



Ground sample analysis – barley

Real-time PCR with a bulk sample

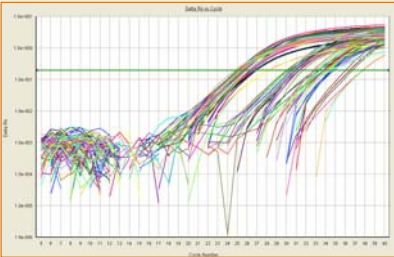


SNP Research



Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells

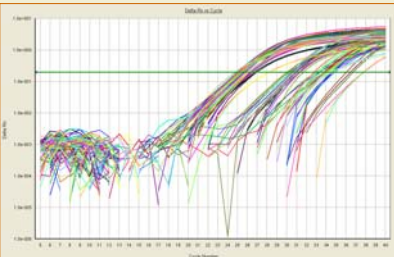


SNP Research



Ground sample analysis – barley

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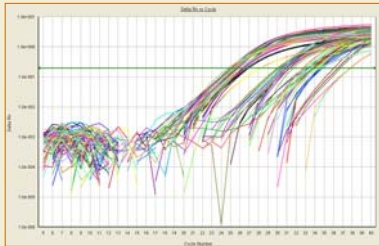


MARKER	Frequency
A*****5	0.7
R**2	1.6
M***1	100.0
A***4	2.6
M****2	0.4
A**8	99.7
A***6	0.4
M*****9	100.0
A***2	97.8
A***1	99.3
B****8	1.3
A*3	98.5
N*1	0.5
A**5	0.0
M*****1	0.3

SNP Research

Ground sample analysis – barley

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A*****5	0.7
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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

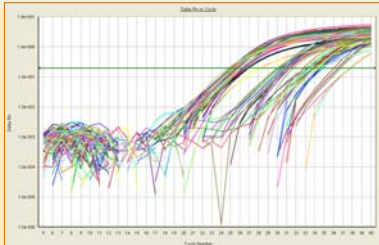
ESTIMATED SAMPLE COMPOSITION

AC Metcalfe	1.48 (0.05)
CDC Copeland	98.06 (0.05)
Harrington	0.46 (0.02)

SNP Research

Ground sample analysis – barley

16 markers x 2 alleles x 3 reps = 96 wells



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A*****5	0.7
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A****1	99.3
B*****8	1.3
A**3	98.5
N**1	0.5
A***5	0.0
M*****1	0.3

ESTIMATED SAMPLE COMPOSITION

AC Metcalfe	1.48 (0.05)
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Harrington	0.46 (0.02)

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 92.6	4 4.1	2 1.9	2 1.4			
B Actual Estimated	2 2.5		94 94.9		2 1.2	2 0.9	– 0.5
C Actual Estimated	2 2.3	96 96.4		2 1.3			
D Actual Estimated	94 94.7	1 0.9	2 2.0	1 1.2	2 1.3		
E Actual Estimated	2 2.3	94 94.8	2 1.8	2 1.2			
F Actual Estimated	2 2.4	2 1.6	2 2.3	94 93.8			

SNP Research

Ground sample analysis – blind samples

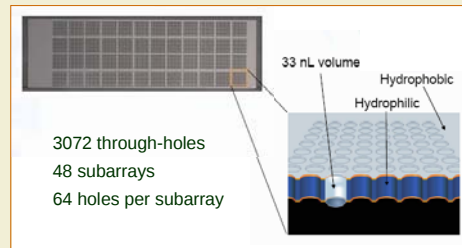
Sample	AC Metcalfe	CDC Copeland	CDC Kendall	Harrington	Stein	Legacy	CDC Dolly
A Actual	92	4	2	2			
A Estimated	92.6	4.1	1.9	1.4			
B Actual	2		94		2	2	–
B Estimated	2.5		94.9		1.2	0.9	0.5
C Actual	2	96		2			
C Estimated	2.3	96.4		1.3			
D Actual	94	1	2	1	2		
D Estimated	94.7	0.9	2.0	1.2	1.3		
E Actual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

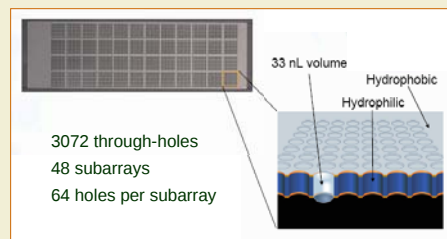
SNP Research

OpenArray for wheat variety ID

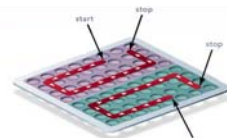


SNP Research

OpenArray for wheat variety ID

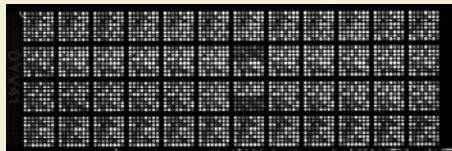


2 samples (kernels) per subarray
96 kernels per plate
32 SNPs per kernel

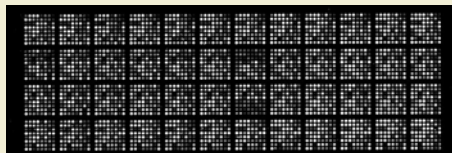


SNP Research

OpenArray – imaging



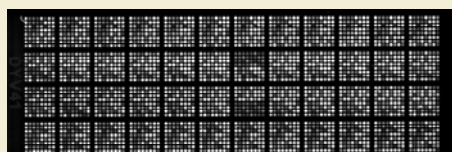
Fam



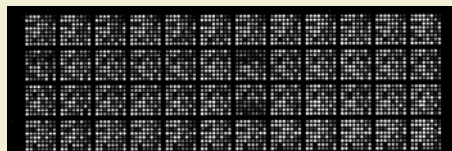
Vic

SNP Research

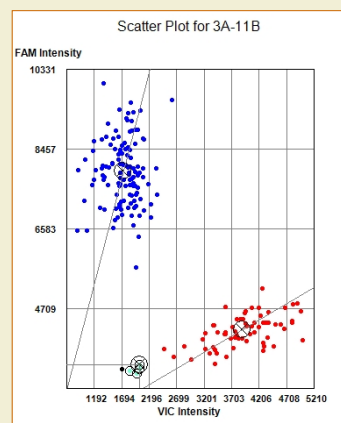
OpenArray – automated genotyping



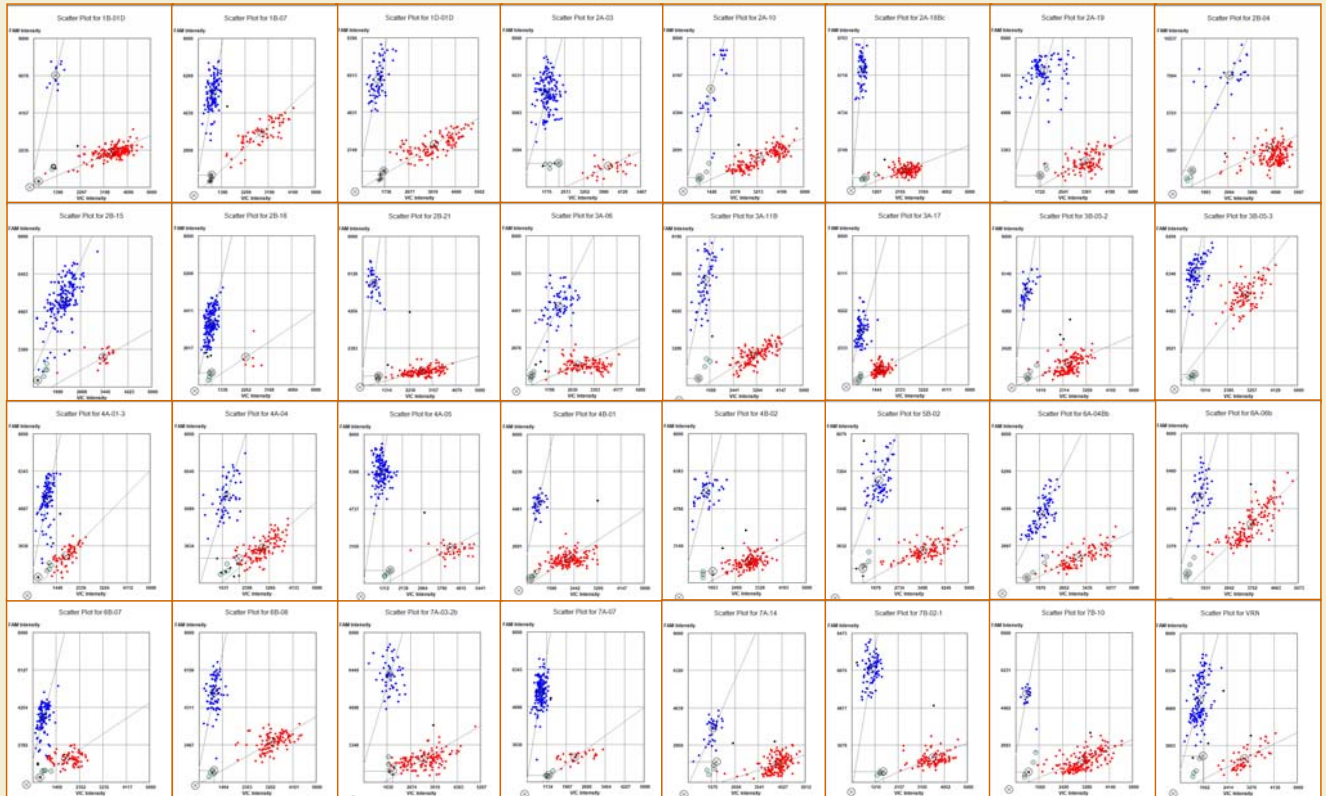
Fam



Vic



SNP Research



SNP Research



Wheat variety identification by SNP profile

88	CM495-86	221211222211121112212121121212	Lillian
89	CM495-87	2111112122112211111111211121112	Harvest
90	CM495-88	1111111111111111111111111111112	CDC Abound Superb
91	CM495-89	1222112122112211222121112112112	CDC Imagine
92	CM495-90	21111121221122111111112101121102	Harvest
93	CM495-91	122222112211121122221210122121102	AC Intrepid
94	CM495-92	12122211222111121111222122121112	Infinity
95	CM495-93	1011111111111111111111111111112	CDC Abound Superb
96	CM495-94	2111112122112211111111211121112	Harvest
97	OCM77-1	22222122222111220122121212221112	5700PR
98	OCM77-2	22222122222111222122121212221112	5700PR
99	OCM77-3	12122121221111222221121212221102	AC Foremost
100	OCM77-4	12122121221111222221121212221112	AC Foremost
101	OCM77-5	12221112222112122121111122221122	5702PR
102	OCM77-6	12122121221111222221121212221102	AC Foremost
103	OCM77-7	22222122222111222122121212221102	5700PR
104	OCM77-8	2222112222221101121121122222112	5701PR
105	OCM77-9	12122121221111222221121212221112	AC Foremost
106	OCM77-10	12122121221111222121112212221112	AC Crystal AC Taber
107	OCM77-11	22222122222111222121121112201102	5700PR

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