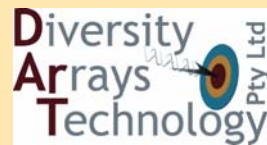




## DArT™ HTP genotyping of barley & wheat



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# What is DArT? - parallel reverse RFLP

## Array development

"Genepool"



Genomic representation  
(Pst1-Taq1 fragments)

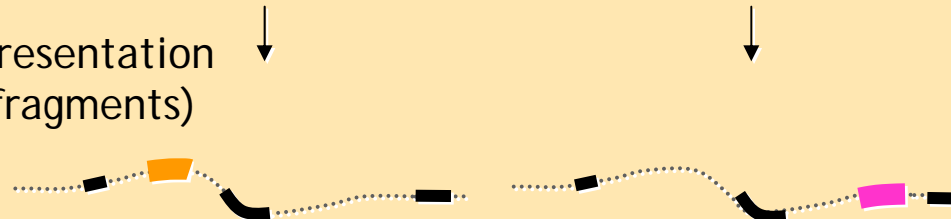


Clone and  
microarray  
fragments

## Routine assays

Sample 1

Genomic DNA Sample 2 ...



Hybridisation

Pattern 1



- 1 - 0 -

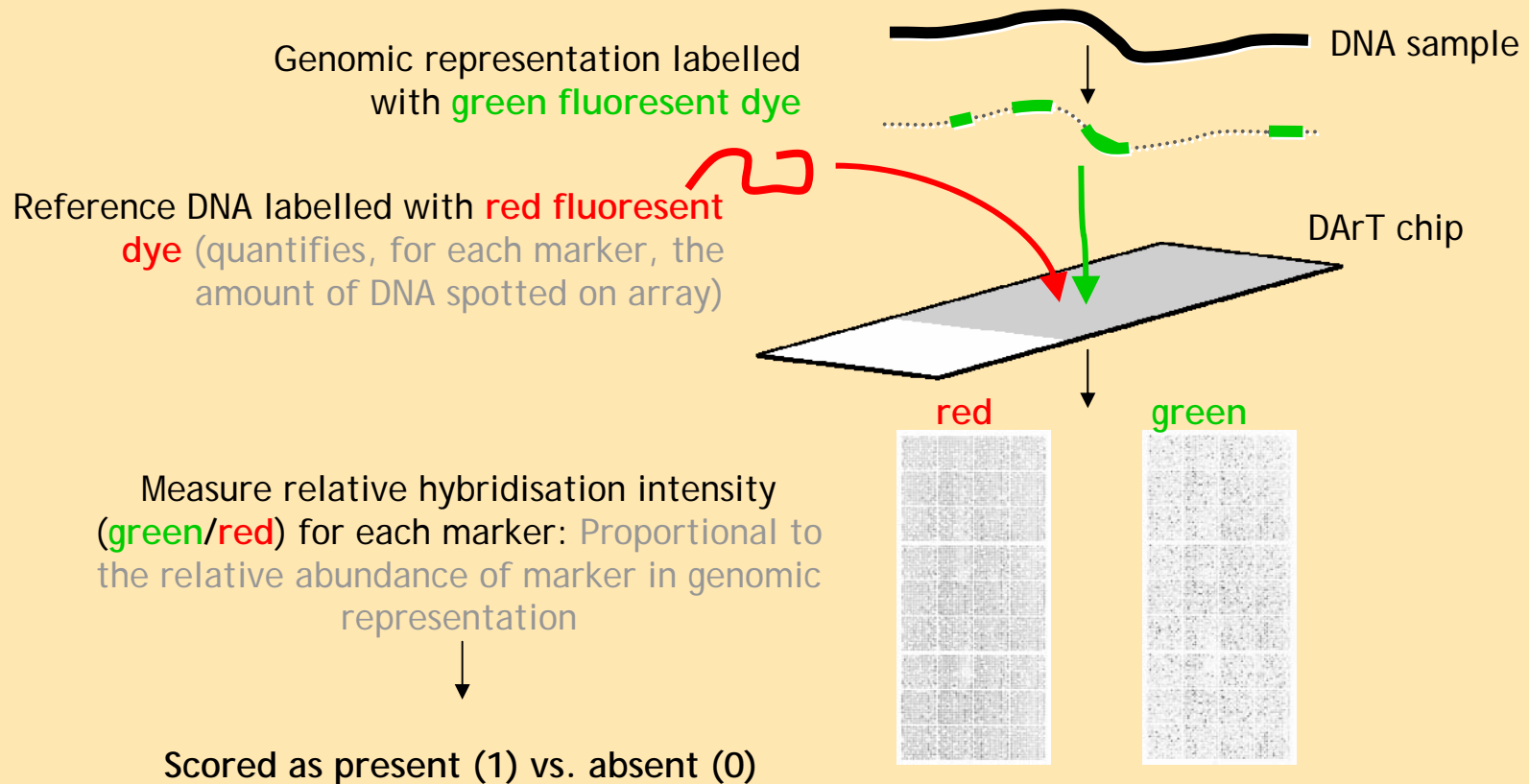
Pattern 2



- 0 - 1 -



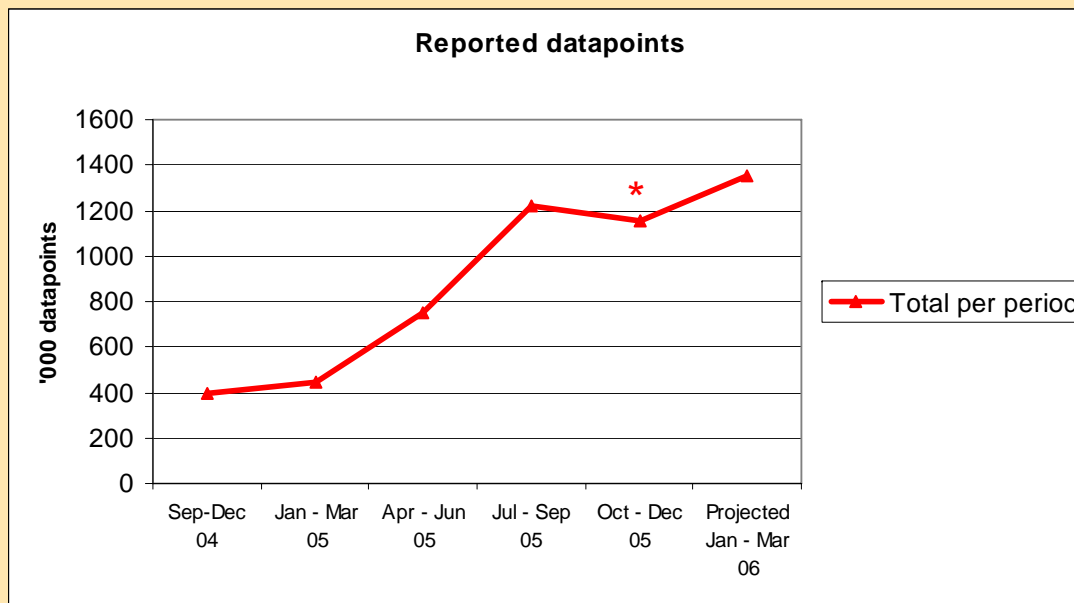
# Routine DArT assay





# The Triticarte Service

Samples last year	Australia	International	Datapoints	AU¢/datapoint
Wheat: 58 analyses	5540	1005	2,696,366	11.5
Barley: 24 analyses	889	1920	1,786,339	7.5



\* Laboratory relocation



## Triticarte service statistics

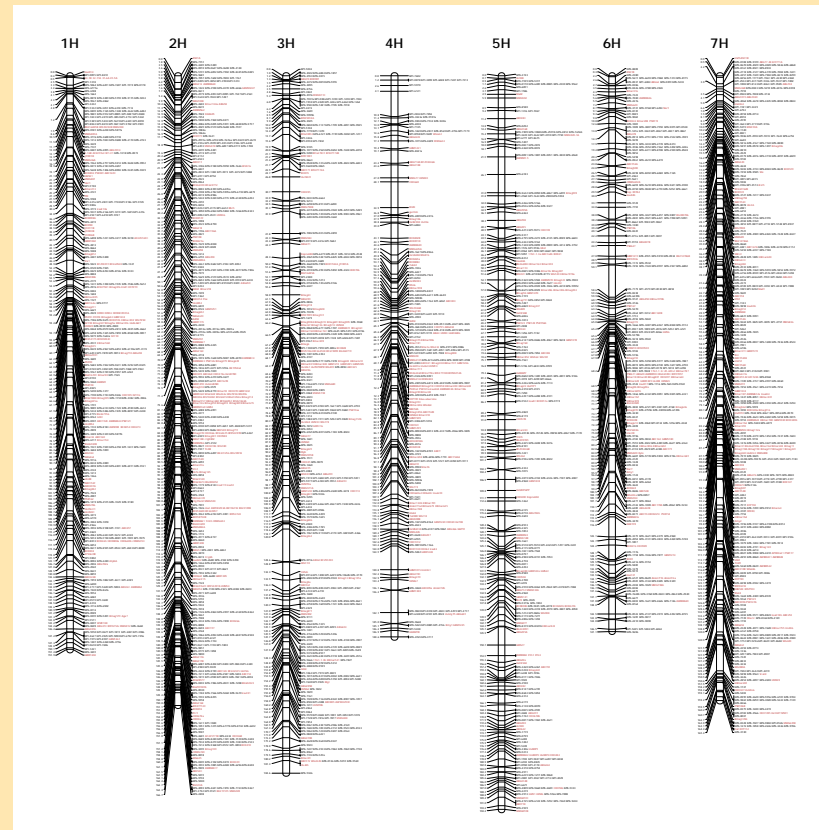
<b>Markers/report</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>StDev</b>
4x wheat	195	256	226	43
6x wheat	130	1,404	496	261
Barley	184	1,130	546	301

<b>Mapping pops</b>	<b>average</b>	<b>markers</b>		
	<b>size</b>	<b>min</b>	<b>max</b>	<b>average</b>
6x wheat (32)	121	121	688	343
barley (16)	105	184	700	418



## Barley consensus map - DArT/SSR/RFLP

- integration of 10 populations
- software limitations
- 2,935 loci - 2,085 DArT
- 1,161cM, median 0.3cM, largest 8.6cM
- very good correlation between consensus and individual maps





## Barley DArT clone sequencing

- ~2,300 polymorphic clones sequenced
- 4438 high quality sequences (both directions)
- 1.6 fold clone redundancy (>1,500 unique markers mapped)
- ~38% with significant DNA sequence homology ( $E < 0.0001$  by BlastN at NCBI)
- >68% with significant protein sequence homology (tBlastX at NCBI)
  - sequences with homology to **barley** sequences:
    - BlastN = 28.7% tBlastX = 21%
  - sequences with homology to **wheat** sequences:
    - BlastN = 22.5% tBlastX = 12%
  - sequences with homology to **rice** sequences:
    - BlastN = 65.4% tBlastX = 50.2%
  - sequences with homology to **mRNA/EST** sequences:
    - BlastN = 58.1% tBlastX = 78.8%



## Wheat DArT assay

- initial wheat discovery arrays with 13 wheats + many others
- 13 wheats for Australian diversity and “alien” segments  
Trident(VPM), Grebe(1B/1R), Janz(Lr24/Sr24), Currawong(Sr26), Sunland(Sr36)  
Westonia, Condor, Cranbrook, More, Frame, Amery, Angus, Halberd
- Version 2.0 array
  - 1,700 selected from 15,000 (polymorphic on 13 wheats)
  - 3,400 unselected, and 300 durum clones
- V2.0 array used to provide services and internal research



## Wheat V2.0 array results

### Results from 15 cross populations, and CS nulli-tetra & deletions

- 2,321 clones mapped in “good” chromosomes
- 236 in unknown groups (3-6 markers), 80 located but not linked in, plus 100 located only in deletion stocks

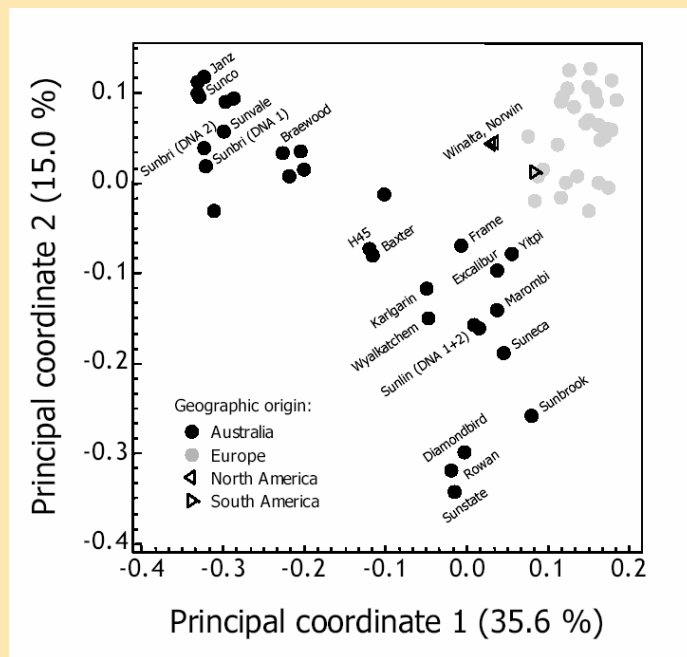
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>
<b>A</b>	99	91	124	180	74	141	137
<b>B</b>	151	176	190	56	128	203	152
<b>D</b>	78	88	77	24	25	30	87

- 1-4 clones diagnostic for the alien segments, and up to 20 “shadow” clones

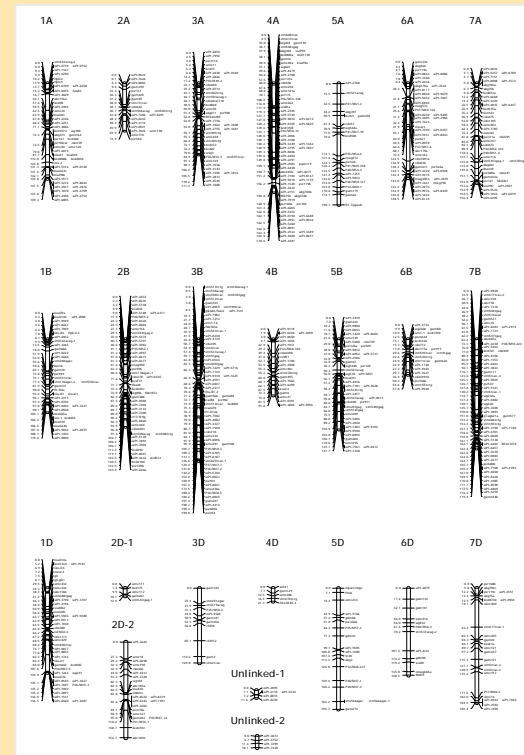


# Wheat V2.0 array results

## Diversity analysis



## Cranbrook/Halberd mapping





# Wheat V2.0 array results

## General Experience

- in new mapping populations, ~400-800 loci typed, 90-95% known
- clusters of clones - most not duplicates (polarity in parents)
  - some cross-specific (re-arrangements)
- some large areas in crosses un-marked                      - identical by descent
- a few clones with “two” map locations in different crosses
  - most are on 5B or 7B, a known translocation difference
  - 7B/7D, 2B/5D



# Triticarte wheat developments

## Development of V2.1 array

- ~ 2,000 clones selected for V2.1 array
- clones re-grown, DNA made, quantified, for spotting
- all clones on V2.1 array sent for sequencing
- V2.1 array reports to include non-polymorphic data
- consensus wheat map in 2006
  - 5-6 wheat populations, including ITMI (Opata/Synthetic)



## Triticarte wheat developments

New platforms - array of arrays, or optically encoded beads

- under development
- decrease in cost
- to allow variation in multiplex level
  - from 10-20 loci in MAS > “full” mapping service

[www.triticarte.com.au](http://www.triticarte.com.au)