John Innes Centre

JIC WGIN Nov 2005

- Part 3-

COS Markers

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COS Markers: intron-based markers for targeted wheat genotyping and comparative mapping

Develop using wheat genomic information :

- Homoeologous markers
- Single copy
- Polymorphic between and within species

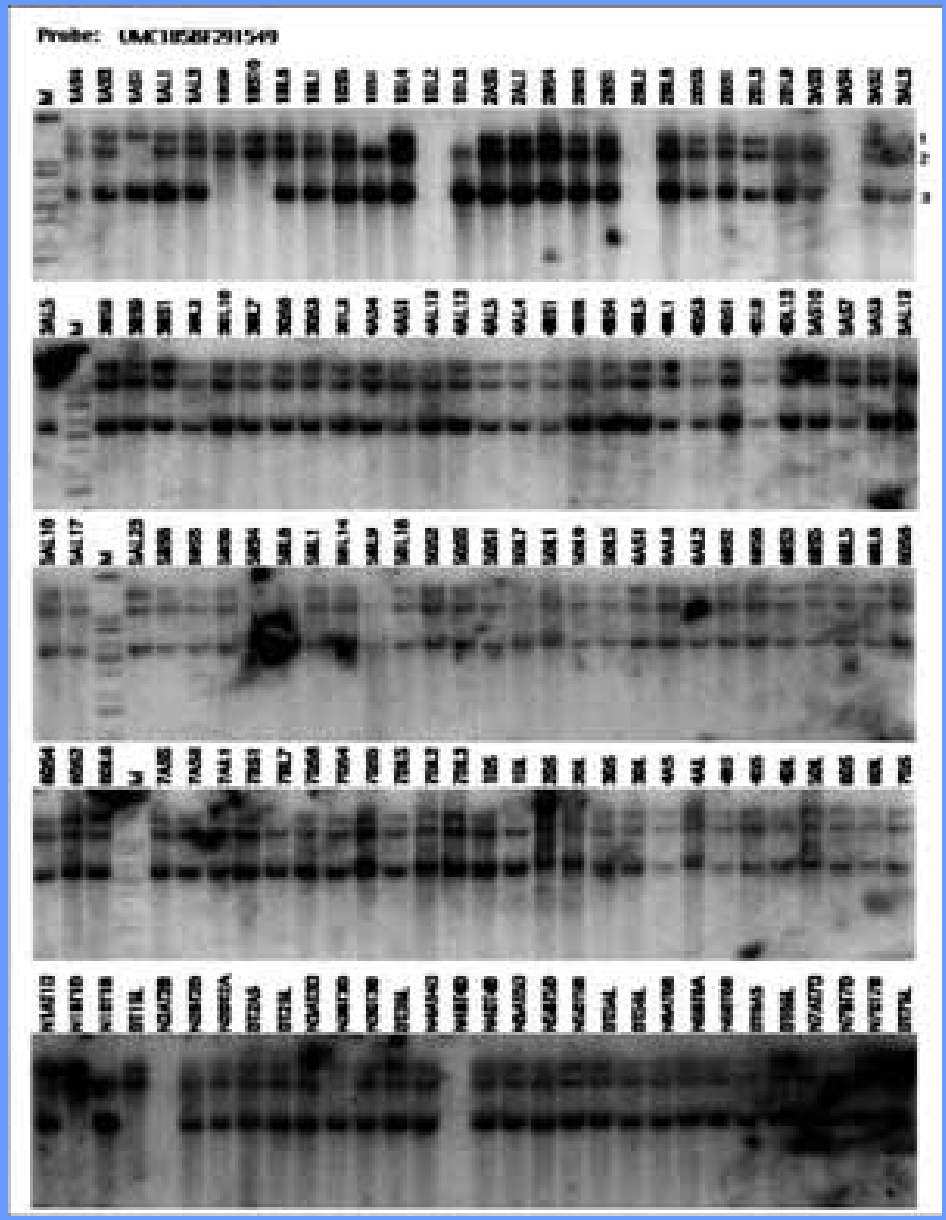
Strategy for COS marker Development

- Bin mapped ESTs
- ITMI EST contigs
- Blast to rice
- Annotated rice sequence
- SNPF analysis and primer design

Bin mapped ESTs

http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi

Mapped Loci for UMC185BF291549.jpg			
Locus	**Chr.**	**Line(s)**	**Bin**
UMC185 BF291549-1	1DS	1DS1, 1DL5, 7BL7/1DS3, N1D, DT1DL	1DS1-0.59-0.70
UMC185 BF291549-2	1AS	1AS1, N1A	1AS1-0.47-0.86
UMC185 BF291549-3	1BS	1BS9, 1BS10, N1B, DT1BL	1BS9-0.84-1.06



Wheat group 1

BE500570

BF291549

BG262247

BE443071

BG606097

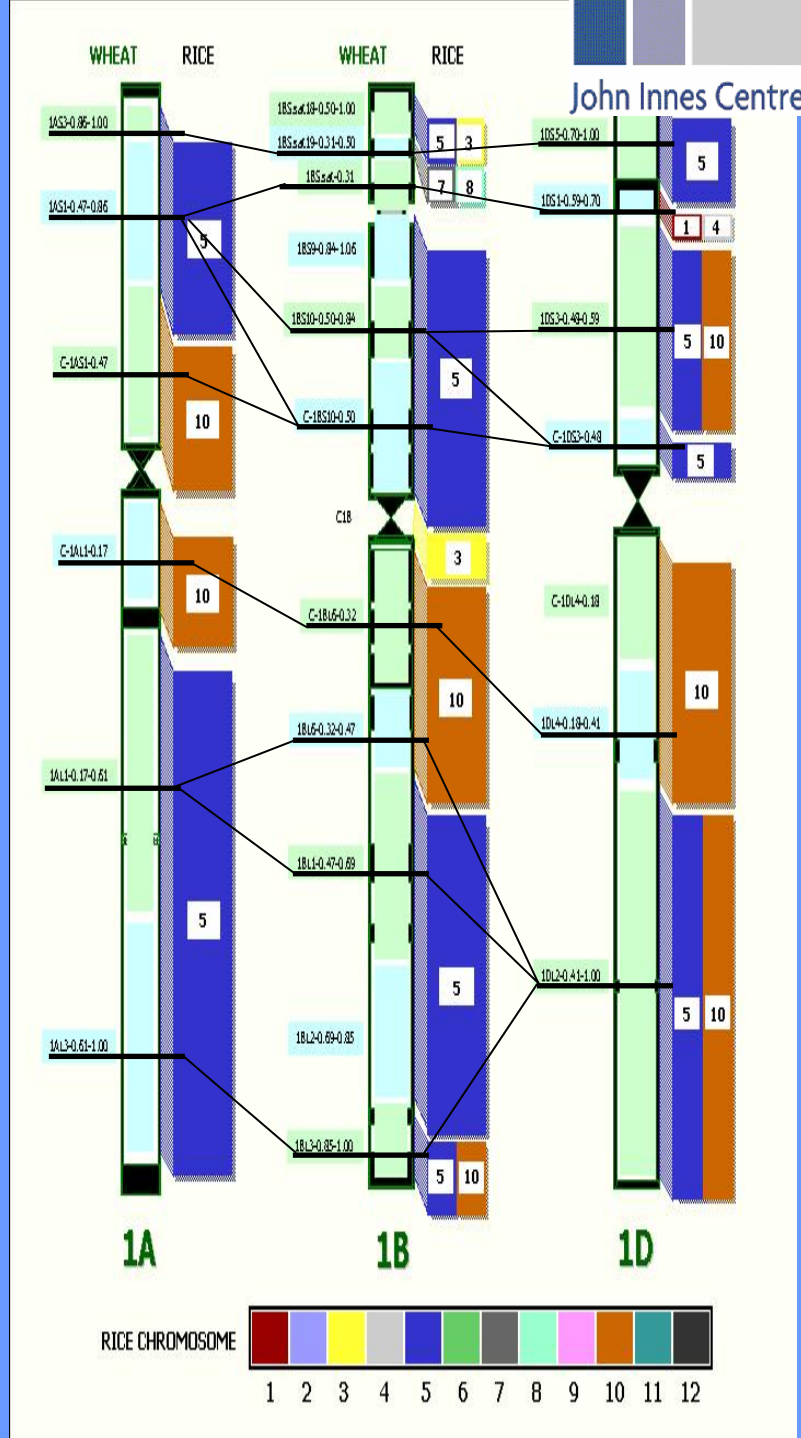
BE500714

BE403420

BE590719

BF200980

BE398263



ITMI Group 1 EST

BF291549

```
  1 gcctgtaact cttattgggt aattctacca cattatggcc tcggttggtg cggtgcgtcc
 61 ttcctcgcgc ttccagaatg acacgagtac tagtggtgat gctgaccgac ttccgaacga
121 gatgggcaat atgagcataa gggatgacag ggacactgaa gatatagtag tcttcggcaa
181 tgggacggaa ccaggccata ttatagtcac aagcattgag ggaagaaatg ggcaagcaaa
241 acagaccatt agctacatgg ctgagcgcgt ggttggtaat gggtcatttg gaactgtttt
301 cc
```

BLAST this to the ITMI EST contigs

ITMI EST contigs

>contig-6336.1
length = 1827

GCACGAGGCTCGCCGCCCCCTCCTCTCCTCCGATCCTCCCCCCACGTCCCGCGCCGGAGCTGGGGAGGGAGGGGGGAAATTCACCC
CTGCGTCAGGCGGATTCCGCCCCGGATCCCCGCGTCGGGCAGGCCGGCCTTCTGATCCGGCAGGTTAACCAAGTACGGTGCATTCT
GAACGGTA**GCCTGTAACTCTTATTGGGTAATTCTACCACATTATGGCCTCGGTTGGTGCGGTGCGTCCTTCCTCGCGCTTCCAGAA**
TGACACGAGTAC*TAGTGGTGATGCCGACCGACTTCCGAACGAGATGGGCAATATGAGCATAAGGGATGACAGGGACACTGAAGAT
ATAGTAGTCAACGGCAATGGGACGGAACCAGGCCATATTATAGTCACAAGCATTGAGGGAAGAAATGGGCAAGCAAAACAGACCAT
TAGCTACATGGCTGAGCGCGTGGTTG*GTAATGGGTCATTTGGAACTGTTTTCCAGGCTAAGTGTCTTGAAACTGGCG*AGACGGT
GGCTATAAAGAAGGTTCTTCAAGACAAGAGATATAAGAA*CCGTGAGCTGCAAACGATGCGAGTTCTTGACCACCCAAATGTTGTG
GCTTTAAAGCATT**GTTTTTTCTCAAAGACTGAGAAGGAGGAGCTTTACCTCAACCTGG*TGCTTGAGTATGTGCCGGAGACTGC
TCATCGTGTCATTAAGCATTACAACAAGATGAACCAACGCATGCCCTTGATATATGCAAAACTGTACATGTATCAGATATGTAGAT
CTTTGGCATACATTCACAACAGCATTGGAGTATGCCACAGAGACATCAAGCCTCAAAATCTTCTGGTGAATCCACATACACACCAA
TTGAAATTATGTGACTTCGGAAGTGCGAAAGTGTTGGTAAAAGGAGAACCAAATATTTCCTATATCTGTTCAAGGTACTATAGAGC
CCCAGAGCTCATATTTGGTGCTACTGAATACACAACGGCAATTGACGTTTGGTCTGCTGGCTGTGTTCTTGCTGAACTCCTTCTAG
GACAGCCTATATTCCCTGGCGACAGTGGTGTTGATCAGCTTGTTGAAATCATCAAGGTTTTAGGTACCCCTACAAGAGAAGAAATT
AAGTGCATGAATCCAAATTATACAGAGTTTAAATTCCCACAAATCAAAGCTCACCCATGGCACAAGATCTTCCATAAAAGAATGCC
TGCTGAAGCAGTAGATCTTGTCTCCA*GACTCTTGCAATATTCACCAA*GCCTGCGTTCAACTGCTTTGGA*AGCATTAATTCATC
CATTCTTCGATGAACTCCGGGACCCAAACACCCGTTTACCGAACGGCCGTTTTCTTCCTCCCCTCTTTAACTTTAAGCCCCATGAG
TTGAAGGGCGTGCCAATGGACATCCTG*GTGAAGCTCATCCCTGAACATGCTCGGA*AGAACTGTGCCTTTGTAGGATGGTGATCC
GTCAGACGGCTGCTTGAAGTTTAGTTCAGAACAAATCCAGTTGTCGTCTACTAGAAACCCCAGGAATTTGAGATTGCCTGCAGCCA
CACGGGATATAGGCGATGACACATGTGATTATTATTCTTTTTCTCGTCCGAGACCTCGATGTCATGTATTCTTTCCCCCTACTGCC
GATGTAACAAA*CCACCCATGATTCTGTAAGTAGATGAGAAGTGTTTCGACCGTTTTCCCCTGAGCTCATGTGCTATGCAATGAAG
GATGCACCCTATGTACCGCCAATATTTGGTCCAGTATTTGTTCATGGATCGAGGCTATAATTCATTTGTACTCATCACATTGTTCT
GTCNNNNNNNNNNNNNNNNN

BLAST this to rice

BLAST to Oryza sativa (japonica cultivar-group) chromosome 5 clone P0519E07, complete sequence Length = 151399

```
Score =  107 bits (54), Expect = 1e-19 Identities = 99/114 (86%) Strand = Plus / Minus
Query: 214  tatggcctcggttggtgcggtgcgtccttcctcgcgcttccagaatgacacgagtactag 273
            |||||||||||| |||| ||||||| |||||| | | || ||||| || || ||||| ||
Sbjct: 9278 tatggcctcggtaggtgtggtgcgttcttccttggggtttcagaacgaaacaagtacaag 9219

Query: 274  tggtgatgccgaccgacttccgaacgagatgggcaatatgagcataagggatga 327
            ||| |||||||||||||||||| |||||| |||||||||| ||||||||||
Sbjct: 9218 tggcgatgccgaccgacttccgaatgagatgagcaatatgagtataagggatga 9165

Score = 67.9 bits (34), Expect = 9e-08 Identities = 79/94 (84%) Strand = Plus / Minus
Query: 331  ggacactgaagatatagtagtcaacggcaatgggacggaaccaggccatattatagtcac 390
            ||||| ||| || ||||| ||||| |||||||||| ||||| || ||| |||| || ||
Sbjct: 9079 ggacattgacgacatagttgtcaatggcaatgggacagaacctggtcatgttattgtgac 9020

Query: 391  aagcattgagggaagaaatgggcaagcaaaacag 424
            |||||||| |||||||||| || || ||||||
Sbjct: 9019 aagcattgatggaagaaatggacaggctaaacag 8986

Score = 71.9 bits (36), Expect = 6e-09 Identities = 57/64 (89%) Strand = Plus / Minus
Query: 422  cagaccattagctacatggctgagcgcgtggttggtaatgggtcatttggaactgttttc 481
            |||||||||||||||||||| ||||| || || ||| |||| || ||||||||||||||
Sbjct: 8874 cagaccattagctacatggccgagcgtgtcgtaggtcatggatcctttggaactgttttc 8815

Query: 482  cagg 485
            ||||
Sbjct: 8814 cagg 8811       etc…etc…..
```

Gene No. : P0519E07.Predgene01

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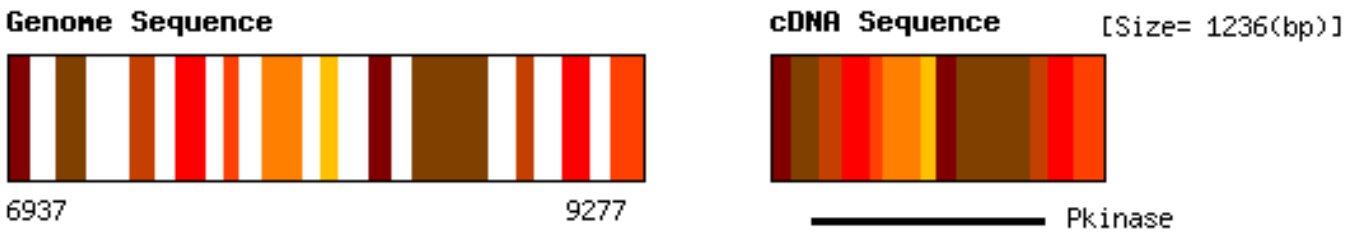
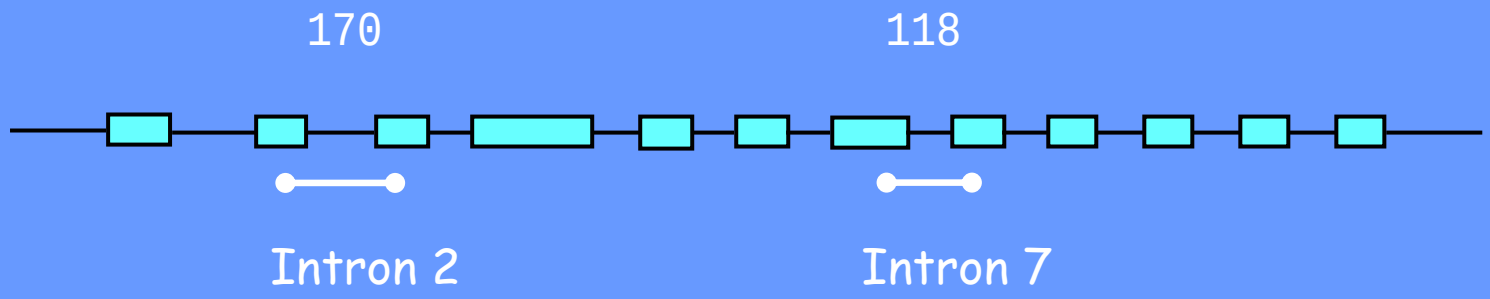


Chr.	05			
Clone	P0519E07			
Position	6937..7019	7120..7222	7393..7476	7564..7659
	7736..7786	7878..8018	8098..8154	8273..8347
	8433..8705	8812..8871	8986..9078	9158..9277
Direction	-			
C+G%	46.16 %			

Predicted Function [GFS]	**by Gene Function Selector (GFSelector)**
	putative Glycogen synthase kinase-3 homolog MsK-3

Design primers to two introns per seq.

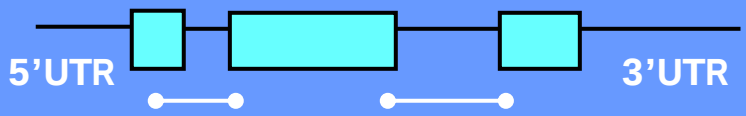


Look for introns that give variation in size/sequence between homoeologues and polymorphisms between varieties

Variation in intron sizes in genes

BE590719

Contig-6122.2

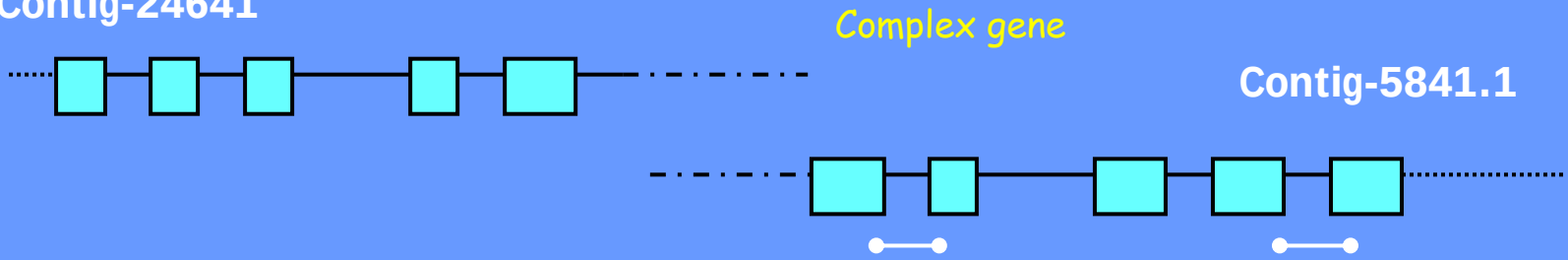


BF200774

Contig-24641

Complex gene

Contig-5841.1

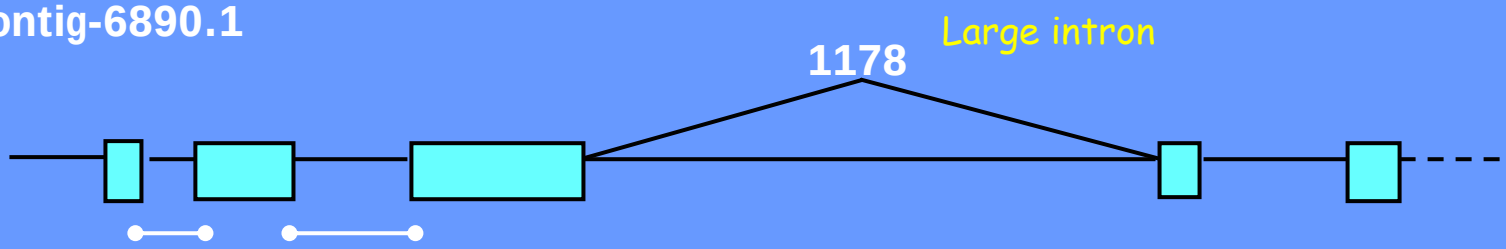


BG262247

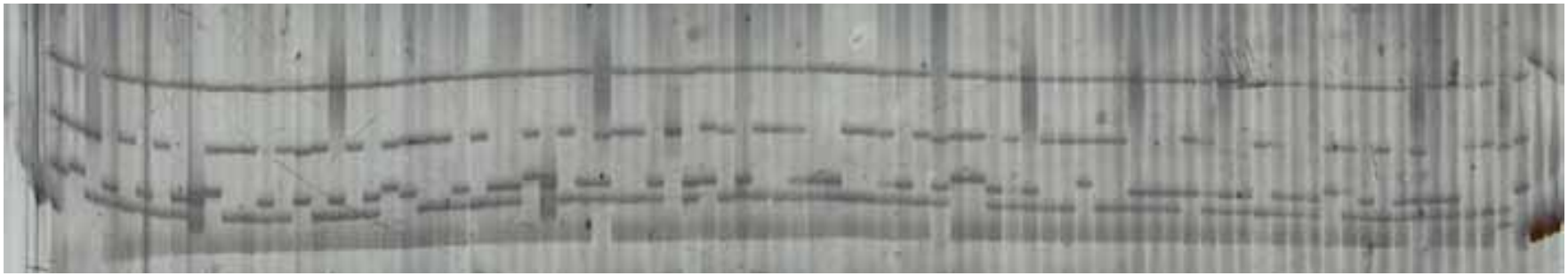
Contig-6890.1

Large intron

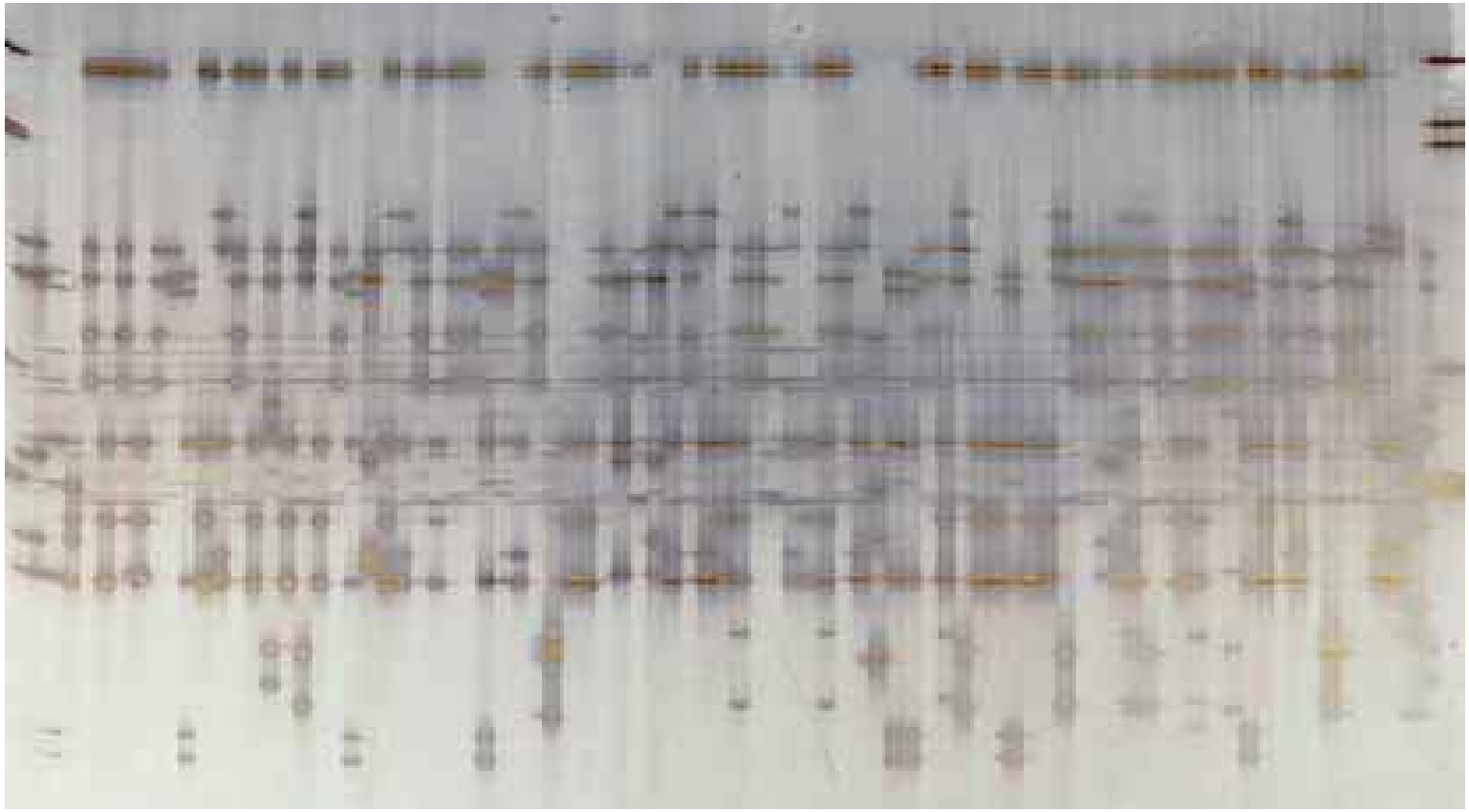
1178



Analysis of products

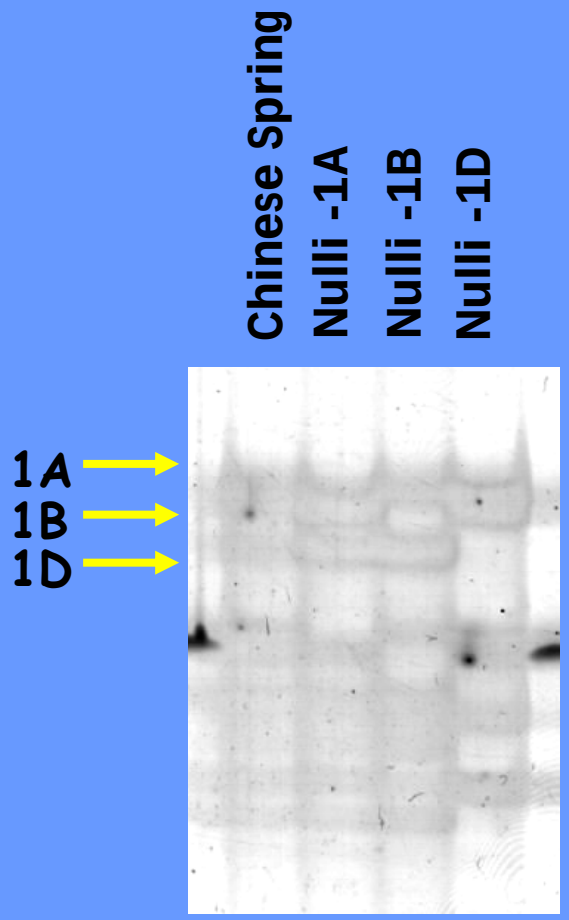


12 patterns are resolved using denaturing acrylamide gels

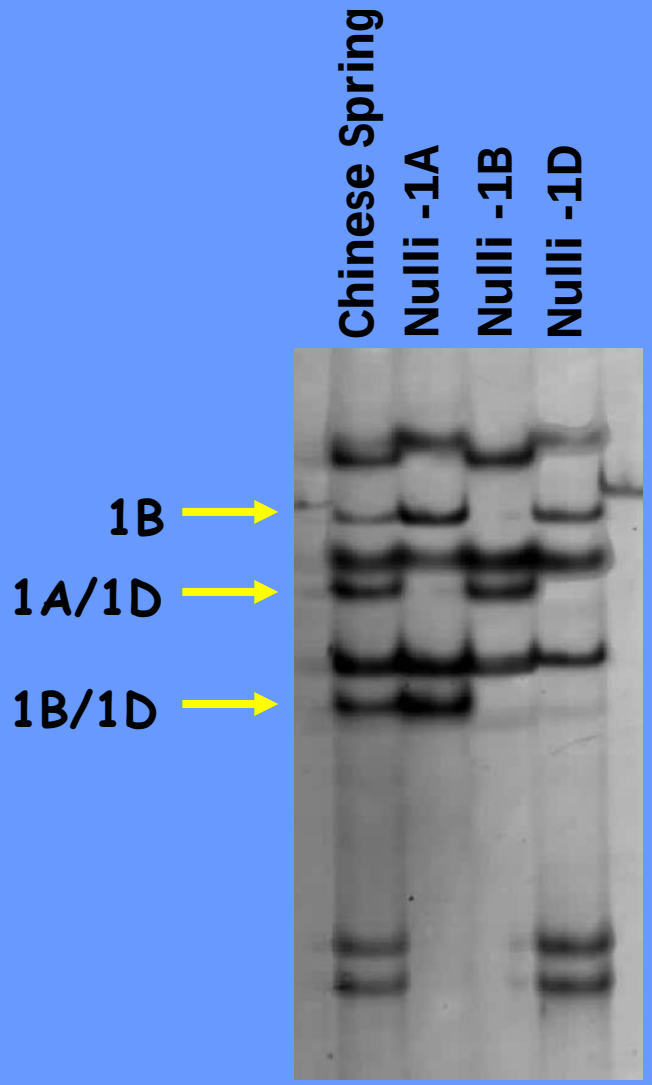


17 patterns are resolved using SSCP

SSCP Gel Analysis



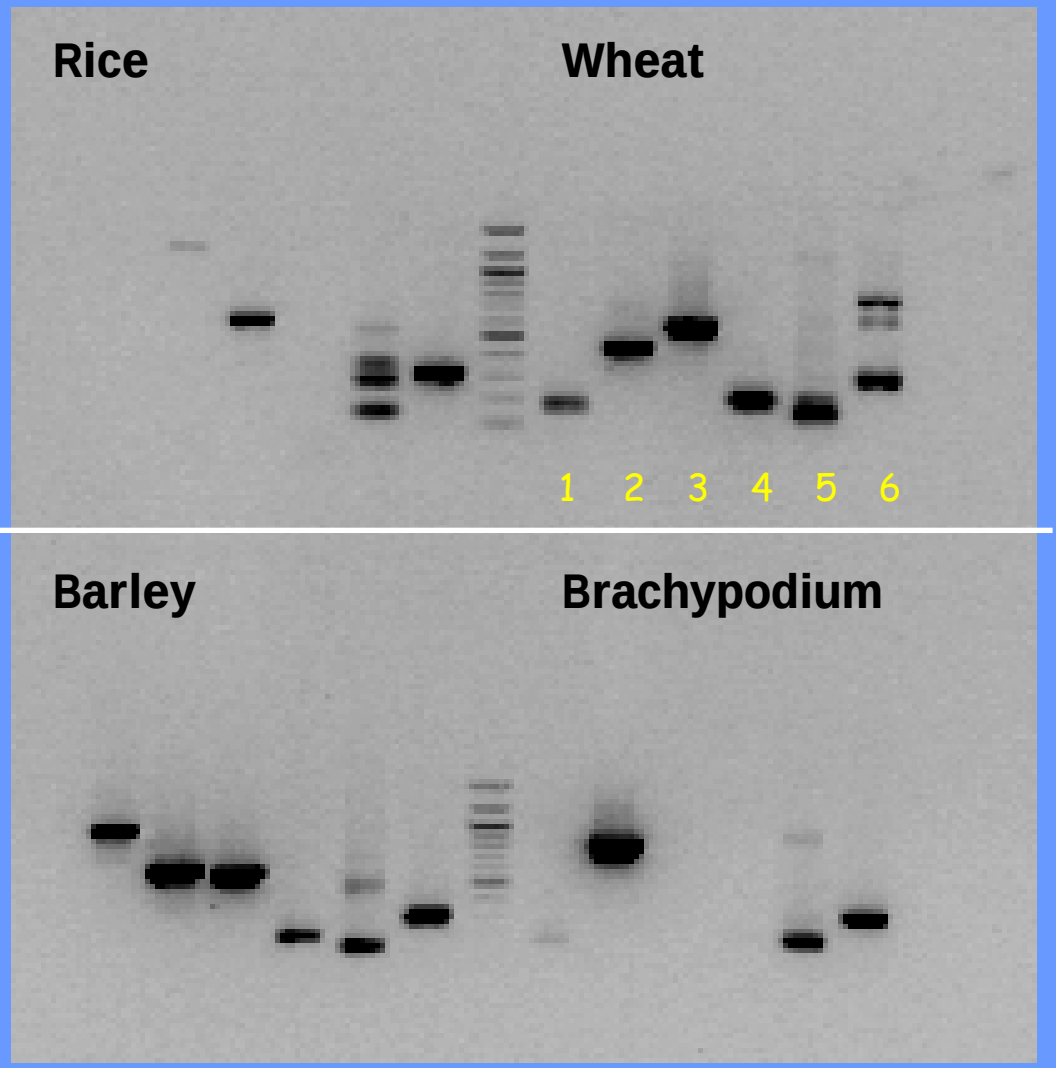
BG262247
intron 2



BE200980
intron 1

Transferability of wheat COS markers

1 BF291549-2
2 BG262247-3
3 BE443071-3
4 BE403420-3
5 BE590719-2
6 pearl millet



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Future Work

- Aim initially for at least 10 COS markers per wheat chromosome
- Use to develop a *B. distachyon* genetic map & comparative map with wheat
- Provide anchors for genetic and physical mapping in wheat, barley and forage grasses
- Provide resource for *Brachypodium* genetic, physical mapping as preliminary to full *Brachypodium* sequence