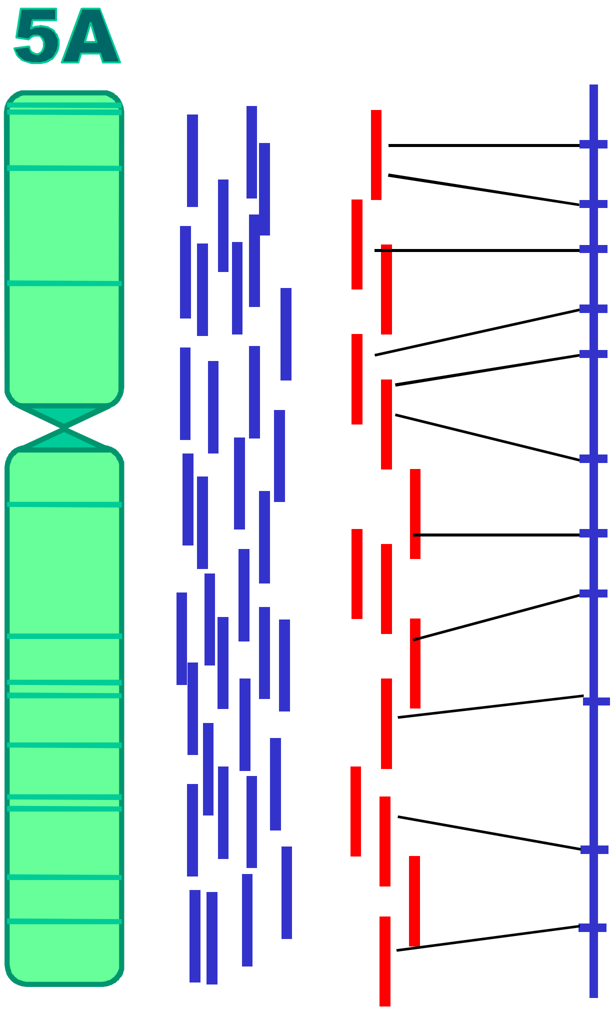
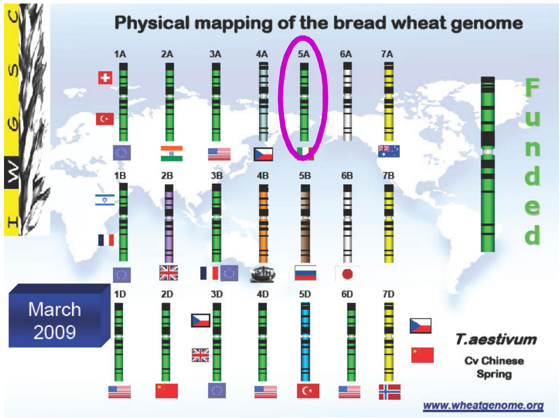
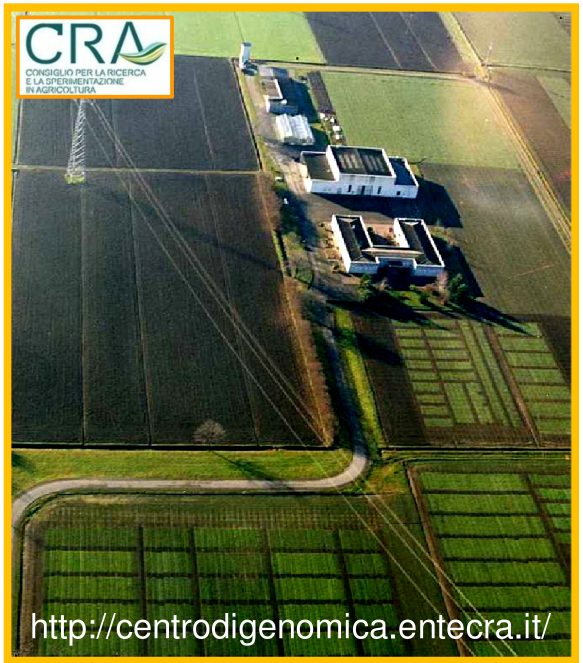
PHYSICAL MAP OF WHEAT CHROMOSOME 5A







22nd International Triticeae Mapping Initiative & 4th National Wheat Genomics Committee Joint Workshop

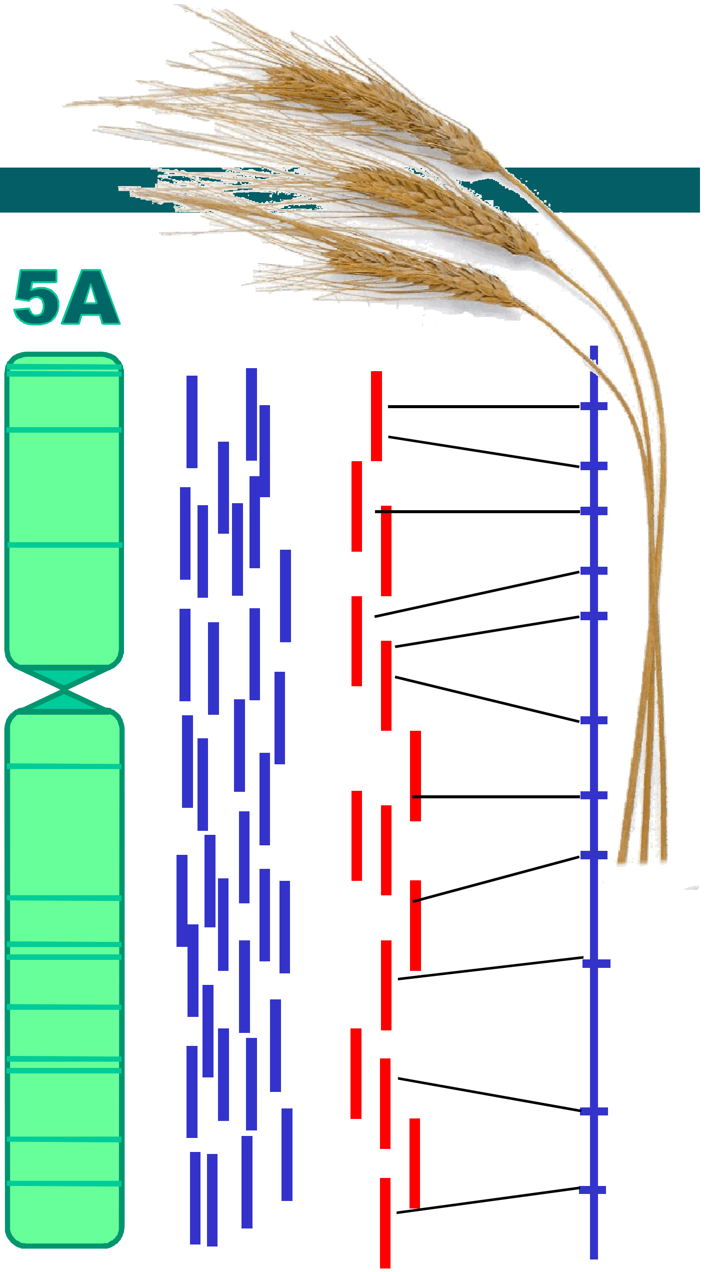
I.T.M.I.

U.S. Wheat Genomics

IWGSC Workshop, Fargo, 24 June 2012

Delfina Barabaschi – Genomics Research Centre, Fiorenzuola d’Arda, Italy

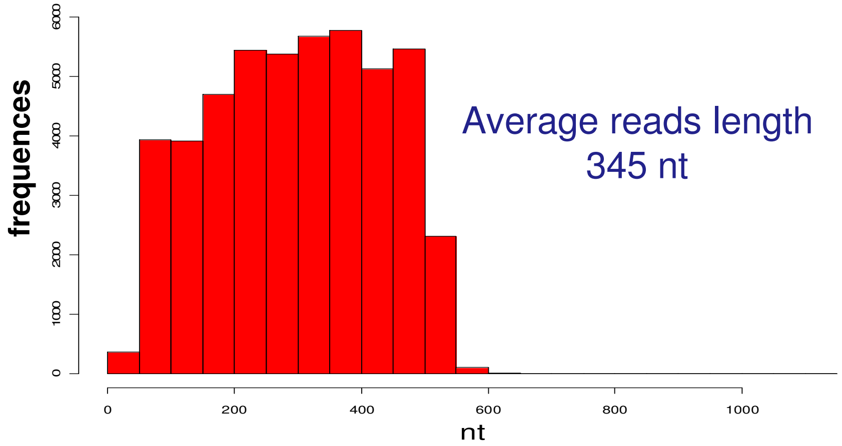
OUTLINE



- Survey sequencing of the 5A composition
- Development of a high-density genetic map
- Construction of a physical map
- Anchoring between the genetic and physical map

Survey sequencing of the chr 5A composition

454 Shot gun sequence of chr 5A,
flow sorted and genomi-Phi amplified 2X



Average reads length
345 nt

5AS (295Mb) 2,407,891 reads → 2,44x

5AL (532Mb) 3,324,512 reads → 2,35x

Vitulo et al, PloSONE, 6 (10), 2011

Survey sequencing of the chr 5A composition

Search for potential markers

Characterization of repetitive elements

	Known TE families (TREP)	Novel TE families (PTREP)	Unknown repeats	Total repeats
5AS	72.67%	2.48%	0.97%	76,13%
5AL	71.14%	2.60%	8.49%	82,23%

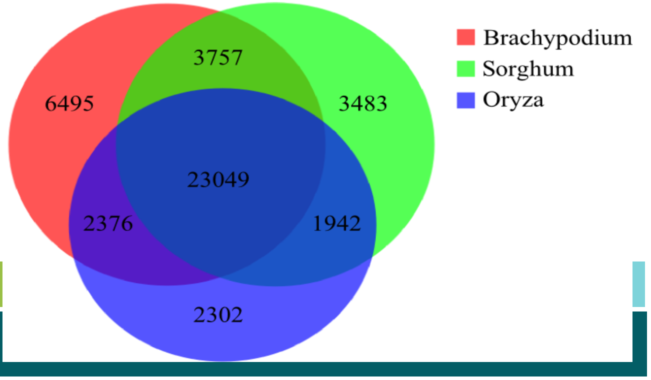
Search for expressed sequences (genes)

	BLAST against UniGene and UniProt	estimated 5A gene content
5AS	1,1%	1593
5AL	1,3%	3496

Search for miRNA

195 candidate miRNA precursors (16 families)

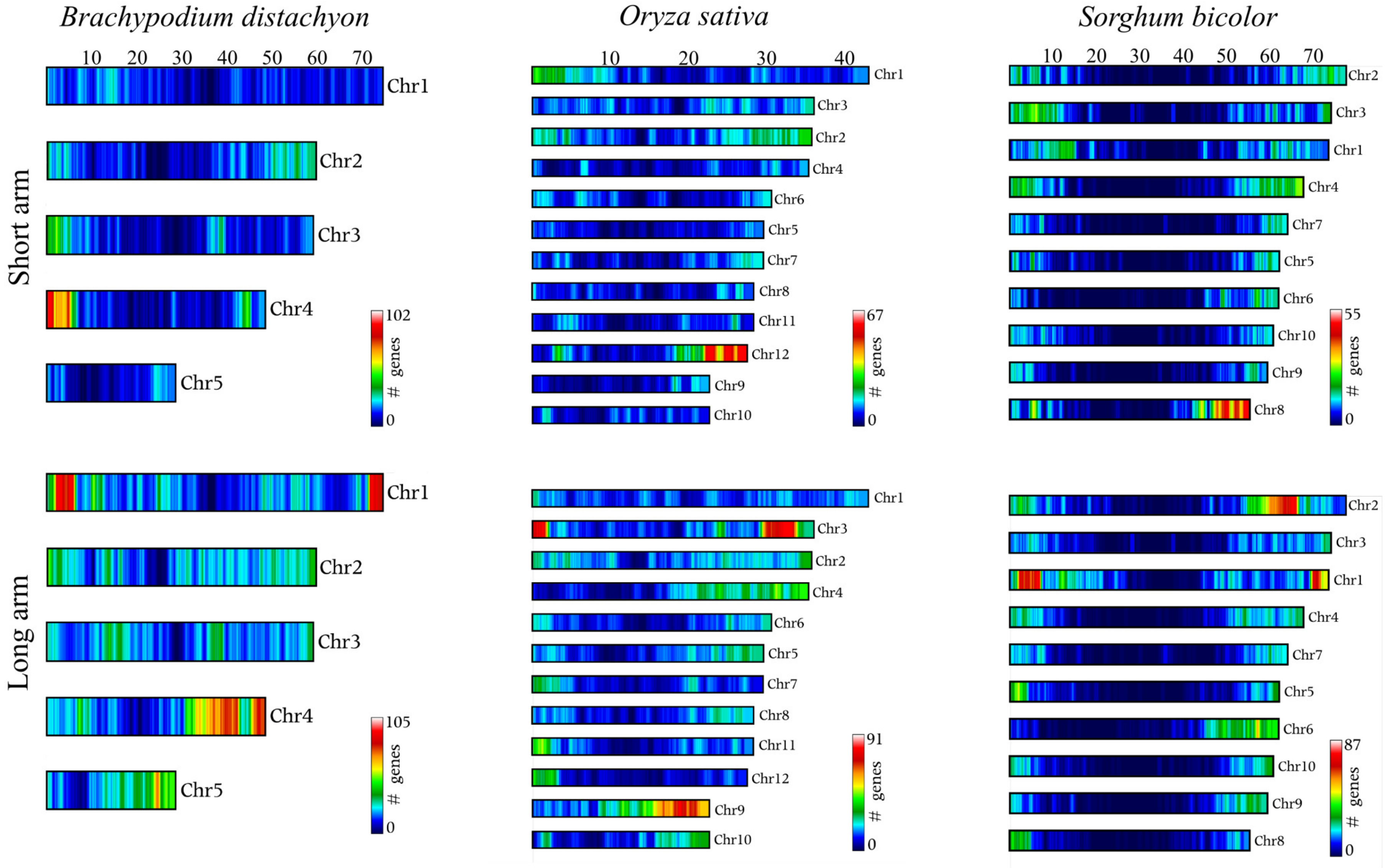
Syntenic relations between wheat 5A and other genomes



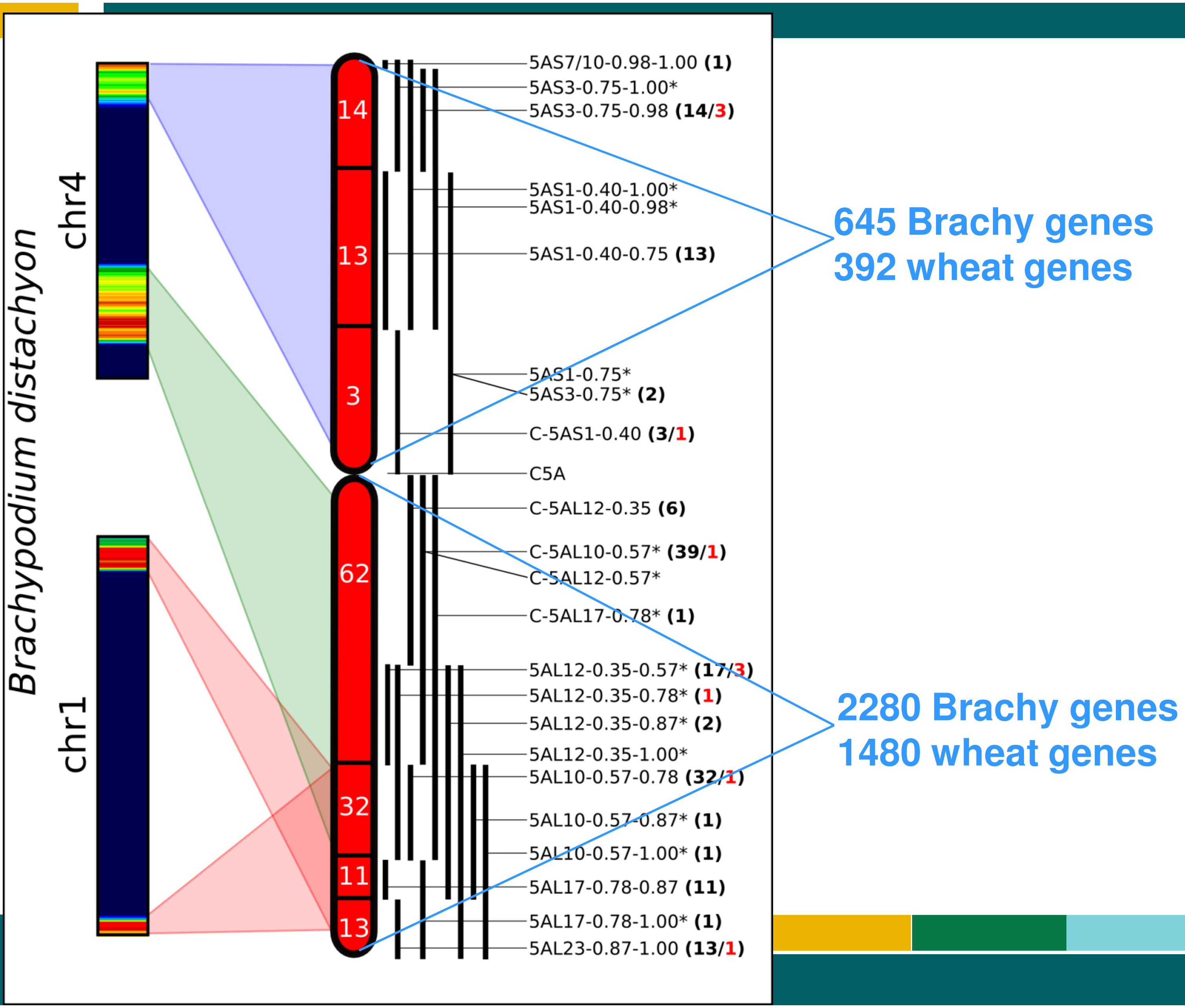
Vitulo et al, PloSONE, 6 (10), 2011

Syntenic relations between wheat 5A and other genomes

Wheat matching proteins in a window of 1Mb with steps of 20Kb



A reference zipper for short and long arm of wheat chromosome 5A



645 Brachy genes
392 wheat genes

2280 Brachy genes
1480 wheat genes

A high resolution genetic map for chr 5A

- ✓Several mapping strategies
- ✓Several kind of molecular markers
- ✓Results: specific and polymorphic markers
  - ✓Genetic Map
  - ✓Cytogenetic Deletion Map

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CRA-GPG &
CRA-CER

Parco
Tecnologico
Padano

Mapping Strategies

DITELOSOMIC LINES
to identify the chr arm

DELETION LINES
to assign each marker in chr BIN

N5AT5B & N5AT5D nulli-tetrasomic lines to assess specific assignment to chr 5A

a set of aneuploid lines derived from the exaploid wheat cv Chinese spring

SEGREGATING POPULATIONS
for meiotic mapping

NEIGHBOR MAP
to increase map density

Under development

Genetic Map: segregating populations

383 F2 Chinese Spring (CS) x Renan
(*T. aestivum* x *T. aestivum*)

from INRA

188 RILs from Chinese Spring x CS-*T. dicoccoides*
Disomic Substitution 5A TDIC (5A CS)
(*T. aestivum* x *T. turgidum dicoccoides*)

from UniBari

132 RILs from DV92 x G3116
(*T. monococcum* x *T. monococcum*)

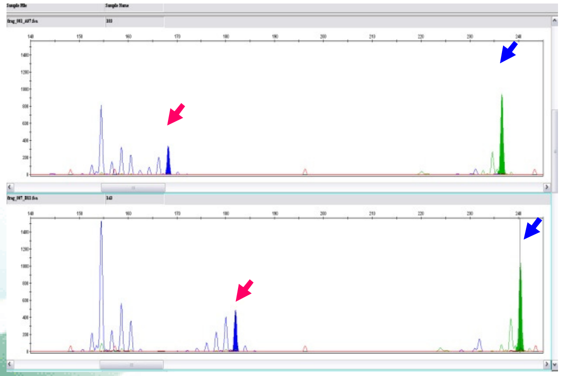
from Czech Republic

124 RILs from Latino x MG5323
(*T. turgidum* ssp *durum* x *T. turgidum* ssp *dicoccum*)

from UniBari

Molecular Markers

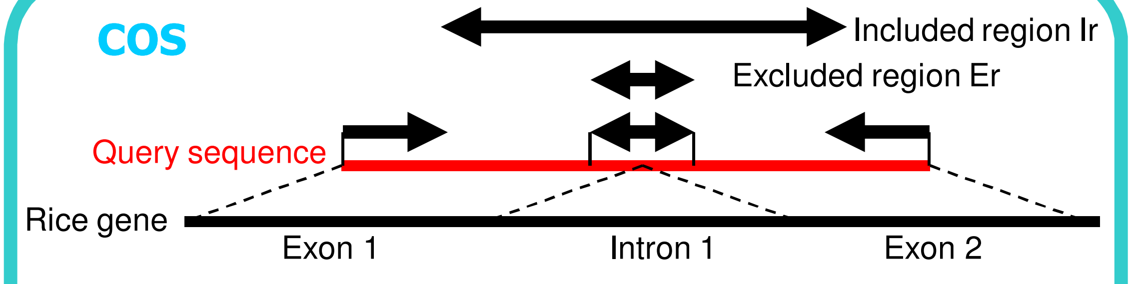
SSR and SSR-EST



High-throughput M13 protocol

Abi3130xl

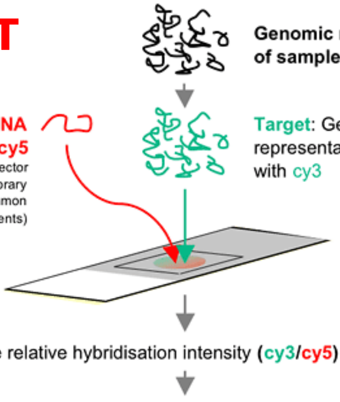
COS



Target region = Ir-Er

Modified from: Salse J. et al (2009). Funct Integr Genomics

DArT



Genomic representation of sample X

Reference DNA labelled with **cy5**
(polylinker sequence of vector used to prepare library spotted on array → common to all spotted fragments)

Target: Genomic representation labelled with cy3

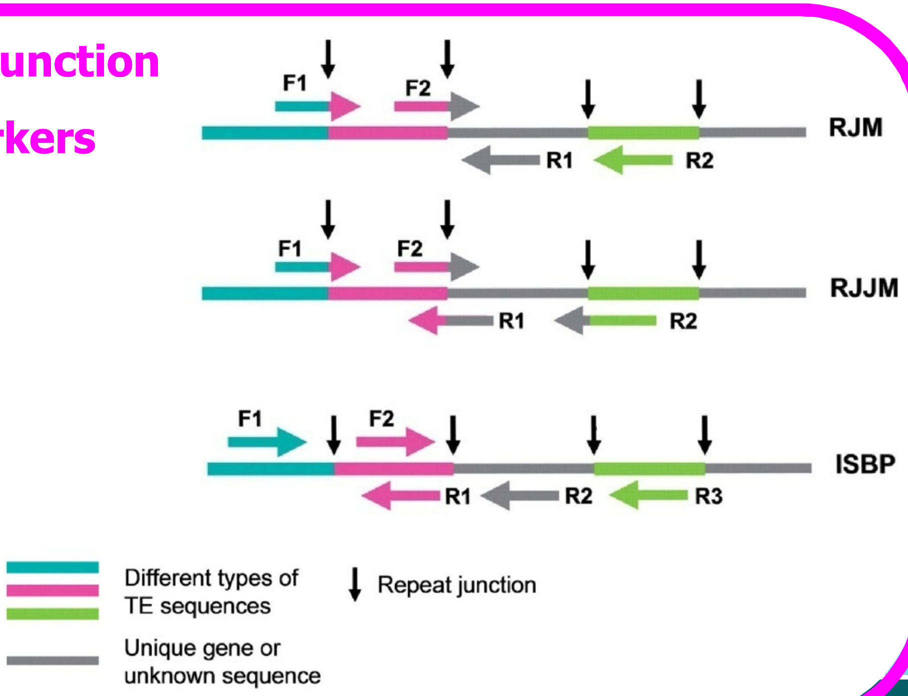
Array of clones from a representation prepared from a mixture of genotypes

Determine relative hybridisation intensity (**cy3**/**cy5**) of each clone

Relative abundance of fragments within genomic representation

Triticarte

TE junction markers



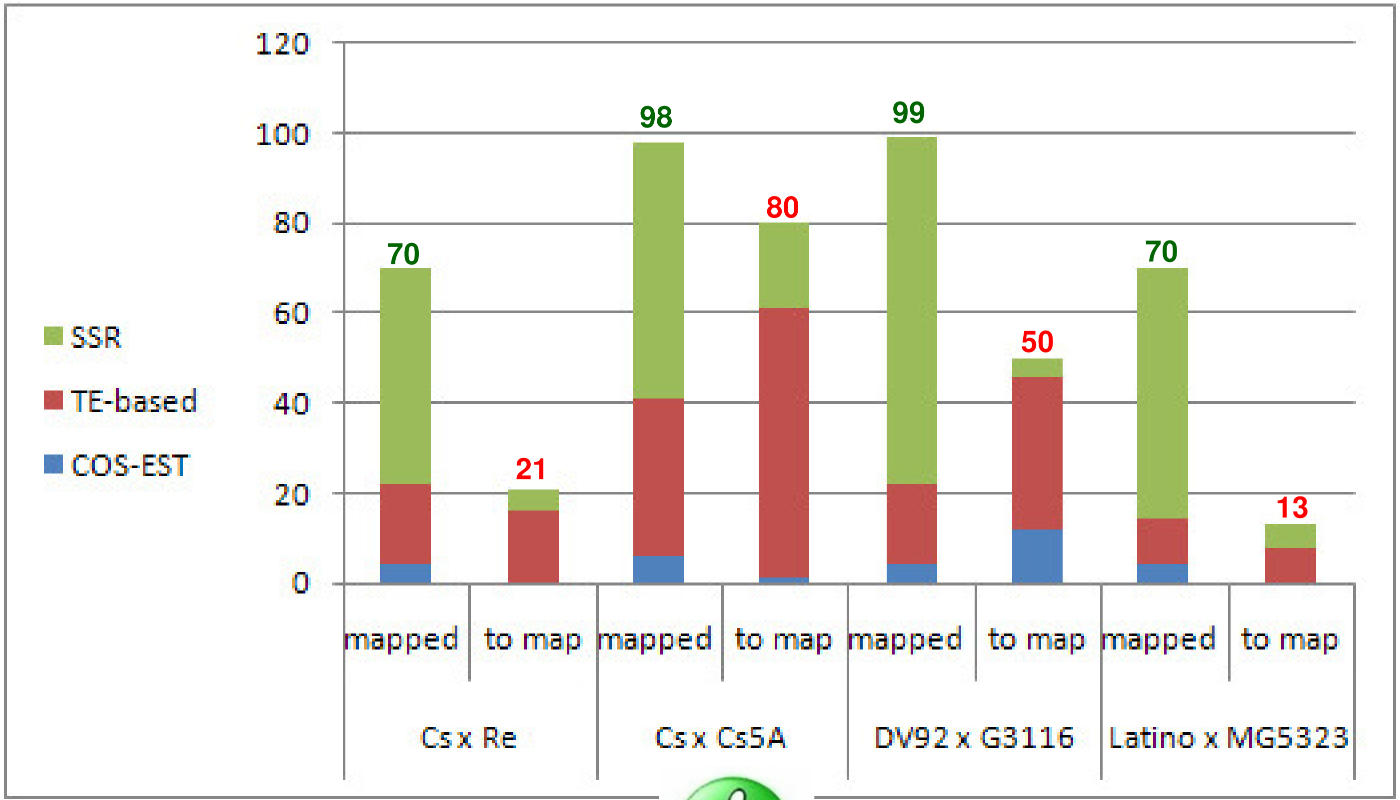
Specific and polymorphic markers

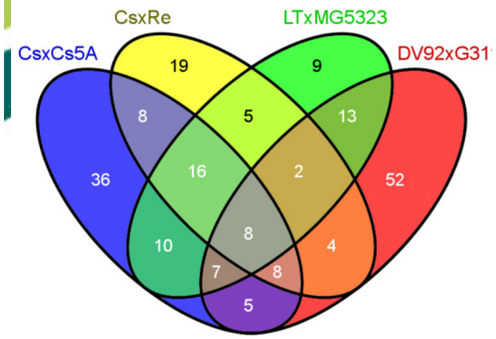
Total marker tested

~400

~700

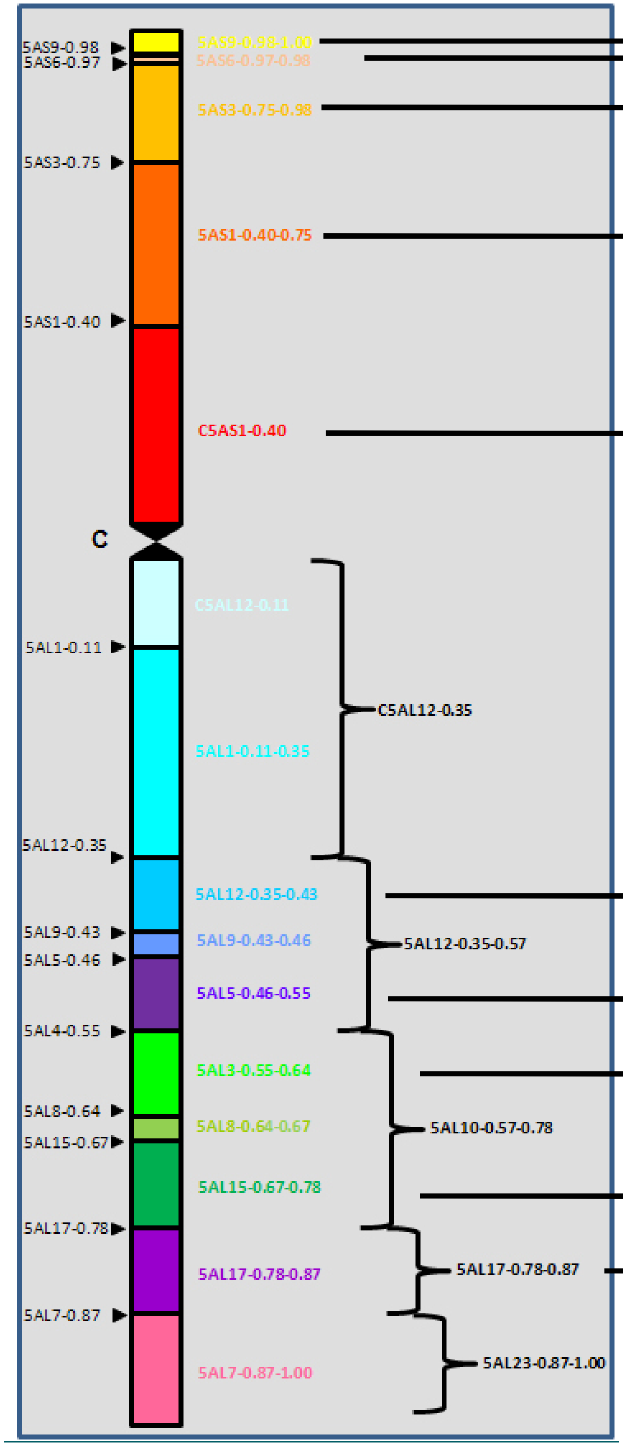
~100





~200 unique markers mapped
~130 unique markers under mapping

Cytogenetic Deletion Map



3 TE-based, 1 SSR

1 TE-based

32 TE-based, 27 SSR, 6 COS-EST

40 TE-based, 16SSR, 5 COS-EST

53 TE-based, 14 SSR, 1 COS-EST

1 SSR

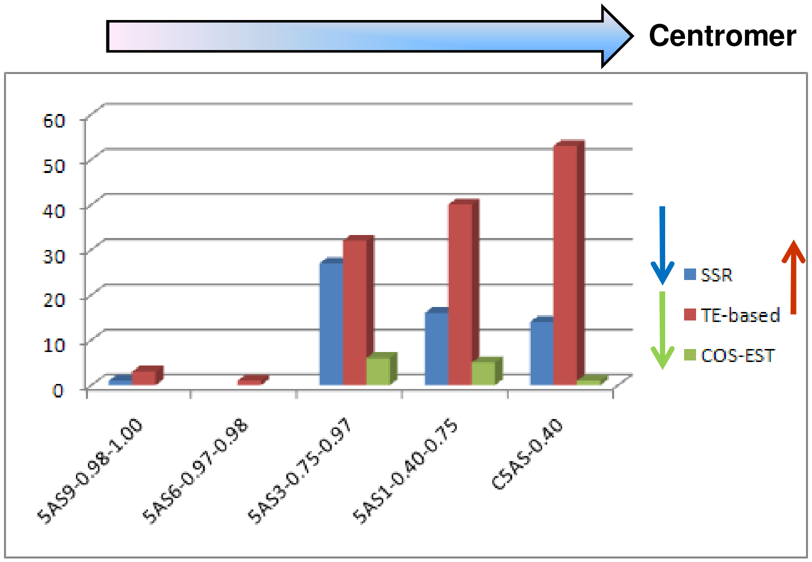
1 TE-based, 15 SSR

12 SSR

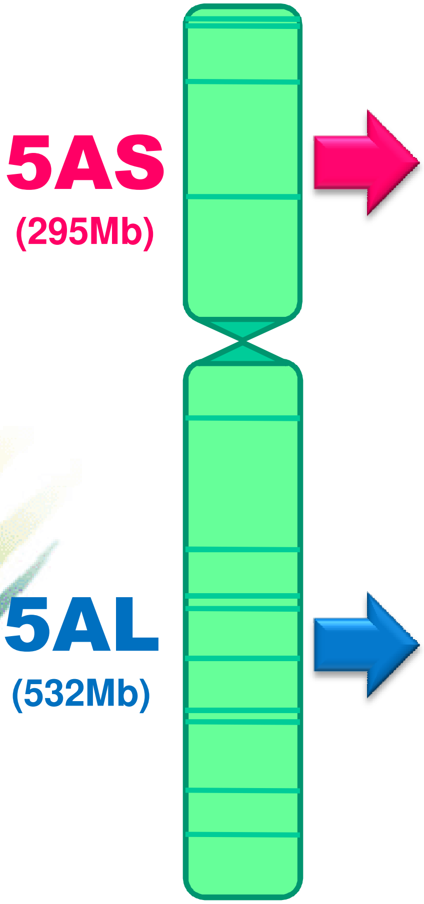
5 SSR

7 SSR, 1 COS-EST

7 SSR, 1 COS-EST



Chromosome-arm specific BAC libraries

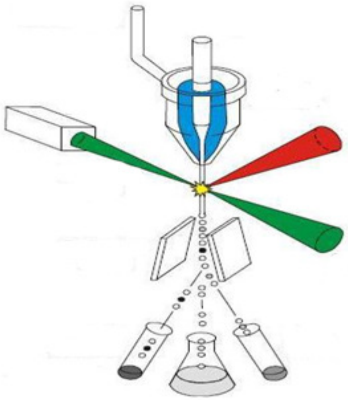


5AS BAC library ”TaaCsp5AShA”

46.080 clones (120 plates)

mean insert size = 120kb

5AS coverage= 16.5X



5AL BAC library ”TaaCsp5ALhA”

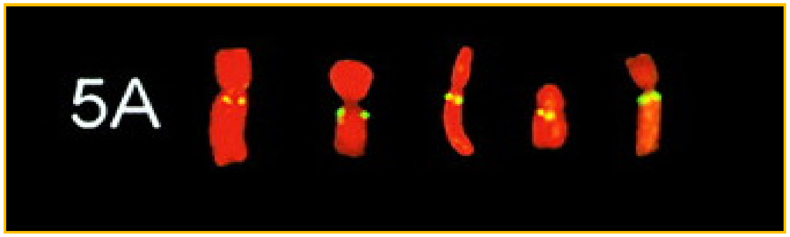
90.240 clones (235 plates)

mean insert size = 123kb

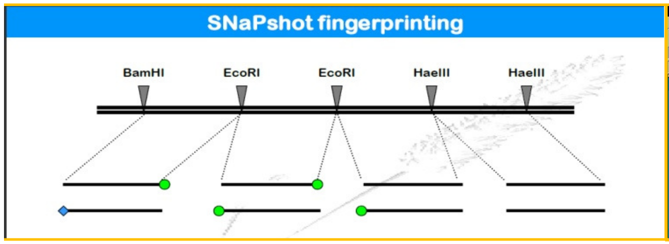
5AS coverage= 18.3X

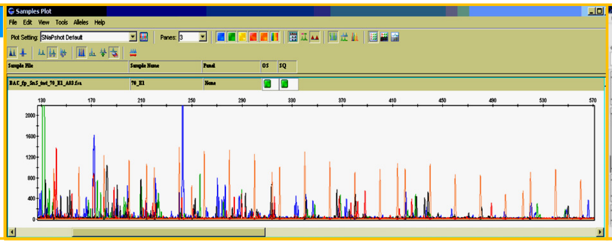
✓The two library were replicated three times

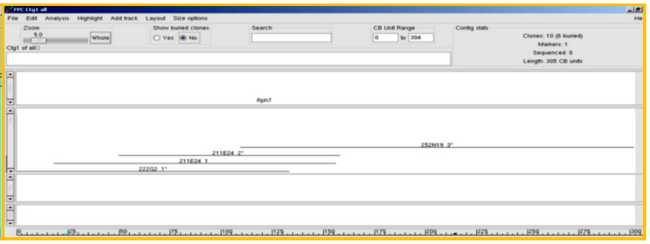
✓A copy in storage at INRA CNRGV



BAC Library Fingerprinting and Contig Assembly







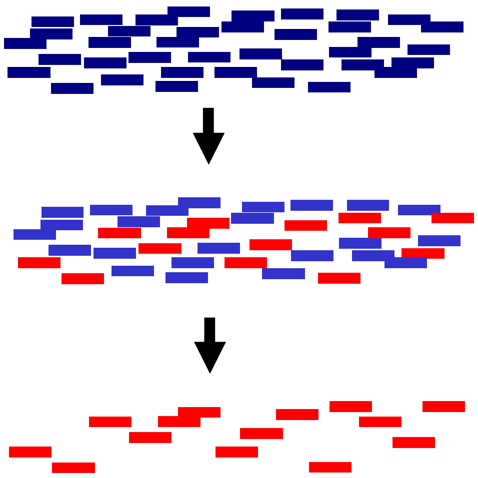
Library	arm size (Mbp)	clone # fingerprinted	insert size (Kbp)	coverage	high quality fingerprints	# MTP clones	total MTP span (Kb)	Percent of map covered (%)
5AS	295	44744	120	16,5X	36165 (80,8%)	4201	309553	99
5AL	532	51072	123	10,4X	39830 (76%)	6560	532577	99

Useful fingerprints were assembled using FPC according to IWGSC rules

- ✓ Several successive cutoffs were used from 1e-75 to 1e-45
- ✓ 25,084 inMap clones and 11,081 singletons for 5AS
- ✓ 27,664 inMap clones and 12,066 singletons for 5AL

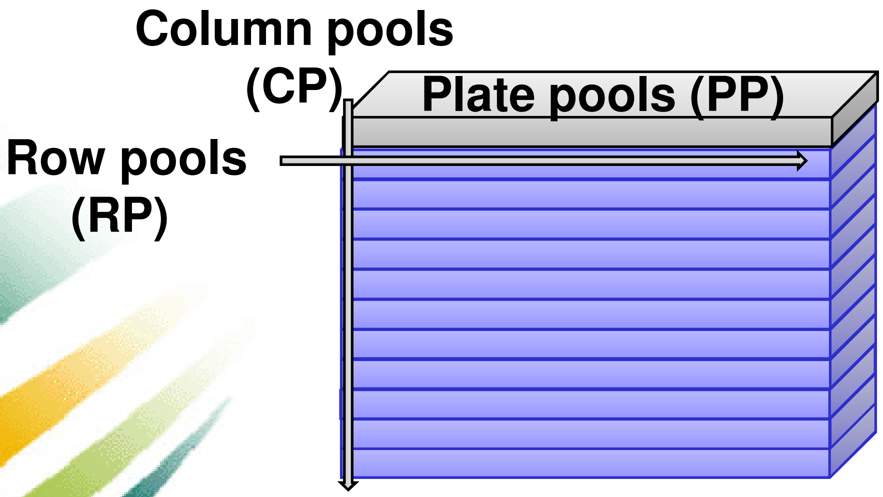
MTP was established after automated assembly

- ✓ 1,308 contigs and N50: 271kb for 5AS
- ✓ 2,556 contigs and N50: 229kb for 5AL



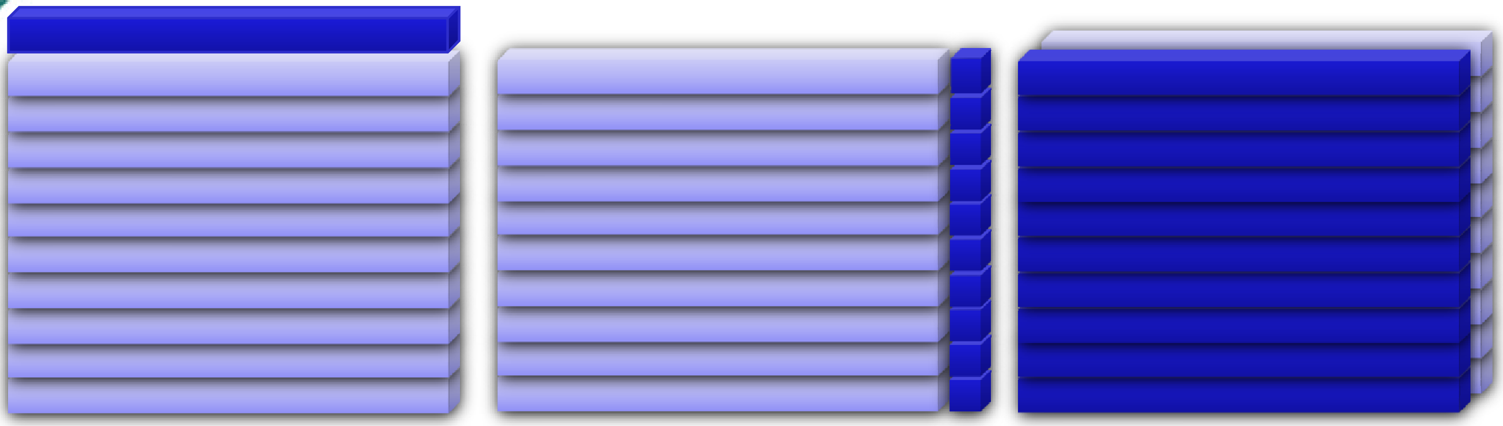
3D Pools of 5A MTP

The 10761 (4201 + 6560) MTP clones were isolated and stored in 11 plates for 5AS and 18 plates for 5AL

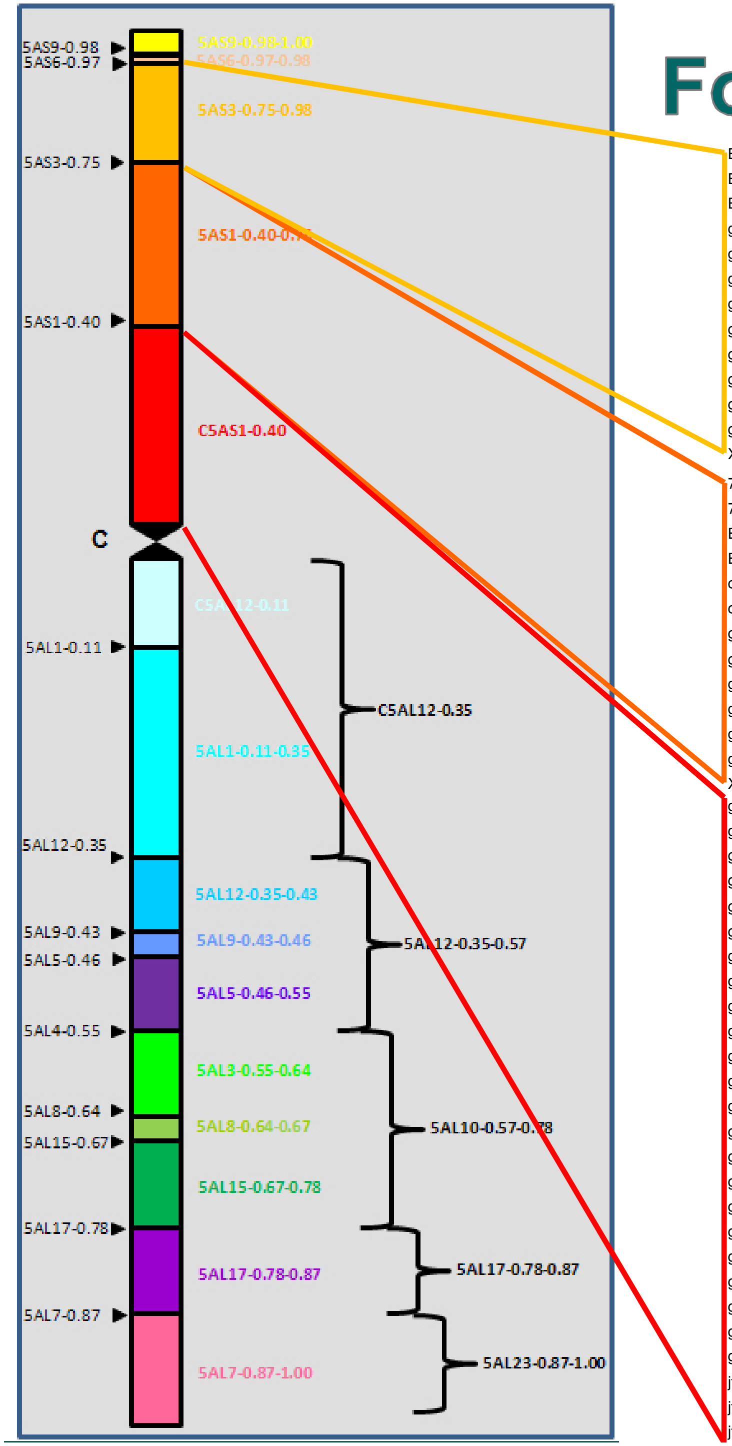


5AS only 51 three-dimensional pools
(11 plates, 24 rows, 16 columns)

5AL only 58 three-dimensional pools
(18 plates, 24 rows, 16 columns)



Forward physical map anchoring



BE444644 ---- [CTG0506]
BE444720 ---- [CTG0928]
BE498768 ---- [CTG0314]
gpg0277 ---- [CTG0185]
gpg0574 ---- [CTG1075]
gpg1139 ---- [CTG0335]
gpg1440 ---- [CTG3169]
gpg2049 ---- [CTG0184]
gpg2072 ---- [CTG0375]
gpg2092 ---- [CTG0364]
gpg2233 ---- [CTG0859]
gpg2250 ---- [CTG0225]
Xgwm293 ---- [CTG2226]

728956 ---- [CTG0563]
730781 ---- [CTG0491]
BE499835 ---- [CTG0500]
BF201102 ---- [CTG0500]
cer121 ---- [CTG0258]
cer140 ---- [CTG0877]
gpg0196 ---- [CTG0332]
gpg0284 ---- [CTG1034]
gpg1040 ---- [CTG0343+0320]
gpg2162 ---- [CTG0006]
gpg2163 ---- [CTG0006]
gpg2298 ---- [CTG0893]
Xmag694 ---- [CTG0081]
gpg0121 ---- [CTG0268]
gpg0158 ---- [CTG0390]
gpg0537 ---- [CTG1222+3019]
gpg1293 ---- [CTG0636]
gpg1321 ---- [CTG0205]
gpg1395 ---- [CTG0678]
gpg1421 ---- [CTG0153]
gpg1438 ---- [CTG0697]
gpg1789 ---- [CTG0541]
gpg2011 ---- [CTG0022]
gpg2019 ---- [CTG0153+0219]
gpg2034 ---- [CTG0468]
gpg2075 ---- [CTG1145]
gpg2083 ---- [CTG0476]
gpg2087 ---- [CTG0712]
gpg2097 ---- [CTG0476]
gpg2117 ---- [CTG0284]
gpg2121 ---- [CTG1728]
gpg2231 ---- [CTG0694]
gpg2309 ---- [CTG1083]
gpg2317 ---- [CTG0184]
gpg2326 ---- [CTG0579]
gpg2328 ---- [CTG0021]
jfio0002 ---- [CTG0329]
jfio0006 ---- [CTG1285]
jfio0021 ---- [CTG0240]

screening of MTP-3D pools using PCR

⇩

anchoring of contigs with all available markers (SSRs, EST- and TE-derived)

⇩

118 markers positive for at least one BAC

⇩

104 contigs anchored by at least one marker

⇩

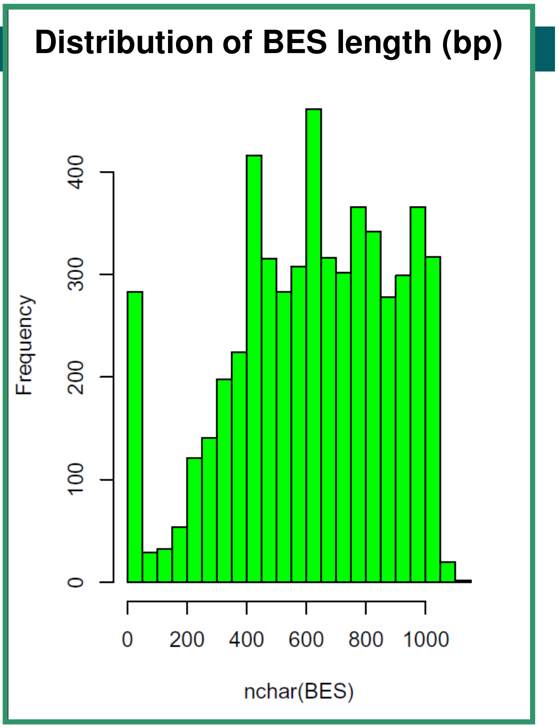
total length anchored 31,5 Mb (10% of assembly)

Anchoring between the genetic and physical map

5AS MTP: BES sequencing

Picking of 2593 clones for BES

3970 sequences already available (6.8Mb, representing 2.3% of 5AS)



Distribution of BES length (bp)

Search for markers directly anchored for integration of the 5AS PM

- 91% BES containing TE (1523 ISBP, 907 other classes)
- 20% BES DNA has been aligned to genes (approximately 480, including transposon associated transcripts)
- 0.14% BES DNA has been aligned to SSRs (240 markers under analysis)

Outlook

- MTP screening with all available mapped markers to anchor contigs
- Location of further markers on genetic map thanks to:
  - neighbor map creation
  - BAC-end sequence-based markers

    …BES for 5AL in progress
  - hybridization of the LTxMG5323 population to 90K Illumina array
- End-merging of contigs
  - Manual assembly with LTC and anchoring information
- Collaboration on 5A
  - <u>FHB resistance </u>with Hermann Buerstmayr,

    Institute for Biotechnology in Plant Production, Tulln, AUSTRIA
  - <u>Stripe rust resistance </u>with Jorge Dubcovsky,

    Department of Plant Sciences, University of California, Davis, CA, USA
  - <u>WYMV resistance </u>with Xiue Wang,

    Cytogenetics Institute, Nanjing Agricultural University, Nanjing, CHINA

All people involved

Coordinators (CRA-GPG)

Antonio Michele Stanca, Luigi Cattivelli, Giampiero Valè

Flow sorting and BAC library preparation

Institute Experimental Botany, Olomouc, Czech Republic
Jaroslav Dolezel
Hana Simkova
Miroslav Valarik

Library Replication

Alice Martini, CRA-GPG

Fingerprinting and BES

IGA Udine
Federica Cattonaro
Federica Magni
Simone Scalabrin

Genetic Mapping

Katia Lacrima, CRA-GPG
Vania Michelotti, CRA-GPG
Luigi Orrù, CRA-GPG
Francesca Desiderio, CRA-GPG
Enrico Francia, UniMore
Agostino Fricano, PTP
Annamaria Mastrangelo, CRA-CER

Citogentic Mapping

UniBari, Italy
Agata Gadaleta
Antonio Blanco

Anchoring Genetic to Physical

CRA-GPG
Andrea Volante
Lucia Prazzoli
Giulio Fulgoni

Survey Sequencing

CRIBI Padova
Giorgio Valle
Nicola Vitulo

Bioinformatic Support

CRA-GPG
Primetta Faccioli
Paolo Bagnaresi
Moreno Colaiacovo