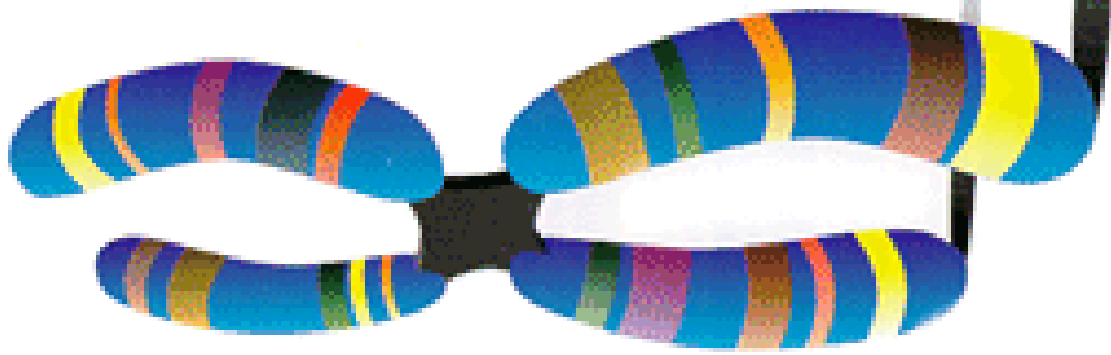


Physical mapping in a Giant Genome: A chromosome landing ready physical map of chromosome 3B of hexaploid wheat

Etienne Paux, Pierre Sourdille, Jerome Salse, Cyril
Saintenac, Frederic Choulet, Philippe Leroy, Abraham
Korol, Wolfgang Spielmeier, Evans Lagudah, Rudi
Appels, Jaroslav Dolezel, Michel Bernard,
Catherine Feuillet



The International Wheat Genome Sequencing Consortium (IWGSC)

Short-mid term goals:

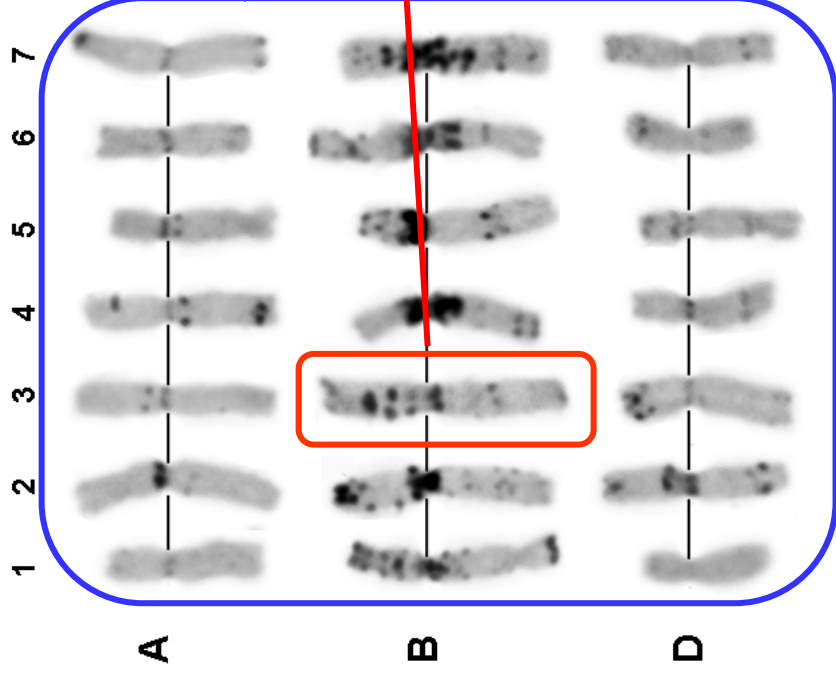
- ✓ **Develop a physical map and link it to the genetic map;**
- ✓ **Sample sequencing** and assessing alternative approaches for sequencing hexaploid wheat;
- ✓ Sequence and annotate the genic regions of hexaploid wheat;

Long term Goals:

- ✓ A complete freely available genome sequence (gene space) of hexaploid wheat;
- ✓ High quality annotation and assembly of the genome.

www.wheatgenome.org

The chromosome-specific approach



Whole genome

- ✓ 21 chromosomes
- ✓ 17 000 Mb
- ✓ homoeologous loci

Chromosome-specific

- ✓ 1 chromosome
- ✓ 1 000 Mb max.
- ✓ no homoeologous loci

Species	BAC library				Coverage
	Chromosome	Cloning site	Number of clones	Insert size (kb)	
<i>T. aestivum</i>	1D, 4D, 6D	HindIII	208 512	97	9.3x
<i>T. aestivum</i>	3B	HindIII	67 986	103	6.2x
<i>T. aestivum</i>	1BS Pavon	HindIII	65 280	82	14.5x
<i>T. aestivum</i>	3AS	HindIII	55 296	80	12x
<i>T. aestivum</i>	3AL	HindIII	55 296	106	12.5x
<i>T. aestivum</i>	3DS	HindIII	36 864	110	12.6x
<i>T. aestivum</i>	3DL	HindIII	55 296	105	12.8x
<i>T. aestivum</i>	3B Hope	HindIII	55 296	85	4.5x
<i>T. aestivum</i>	7DS	HindIII	46 080	115	15.3x

H. Simkova- J. Safar/
P. Suchankova

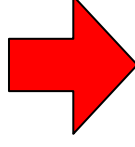
J. Dolezel



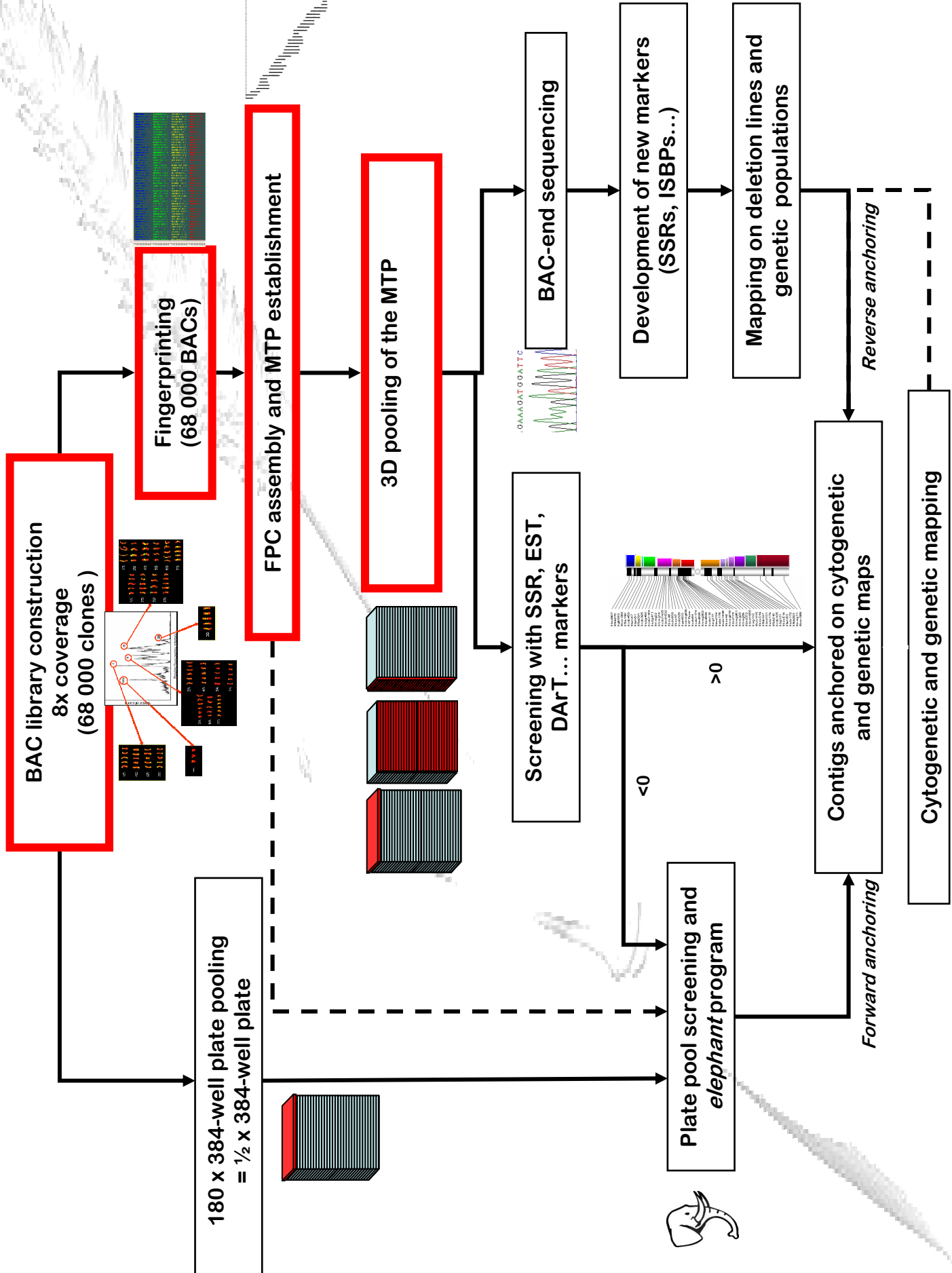
Chromosome based strategies to decipher the hexaploid wheat genome: chromosome 3B, a case study



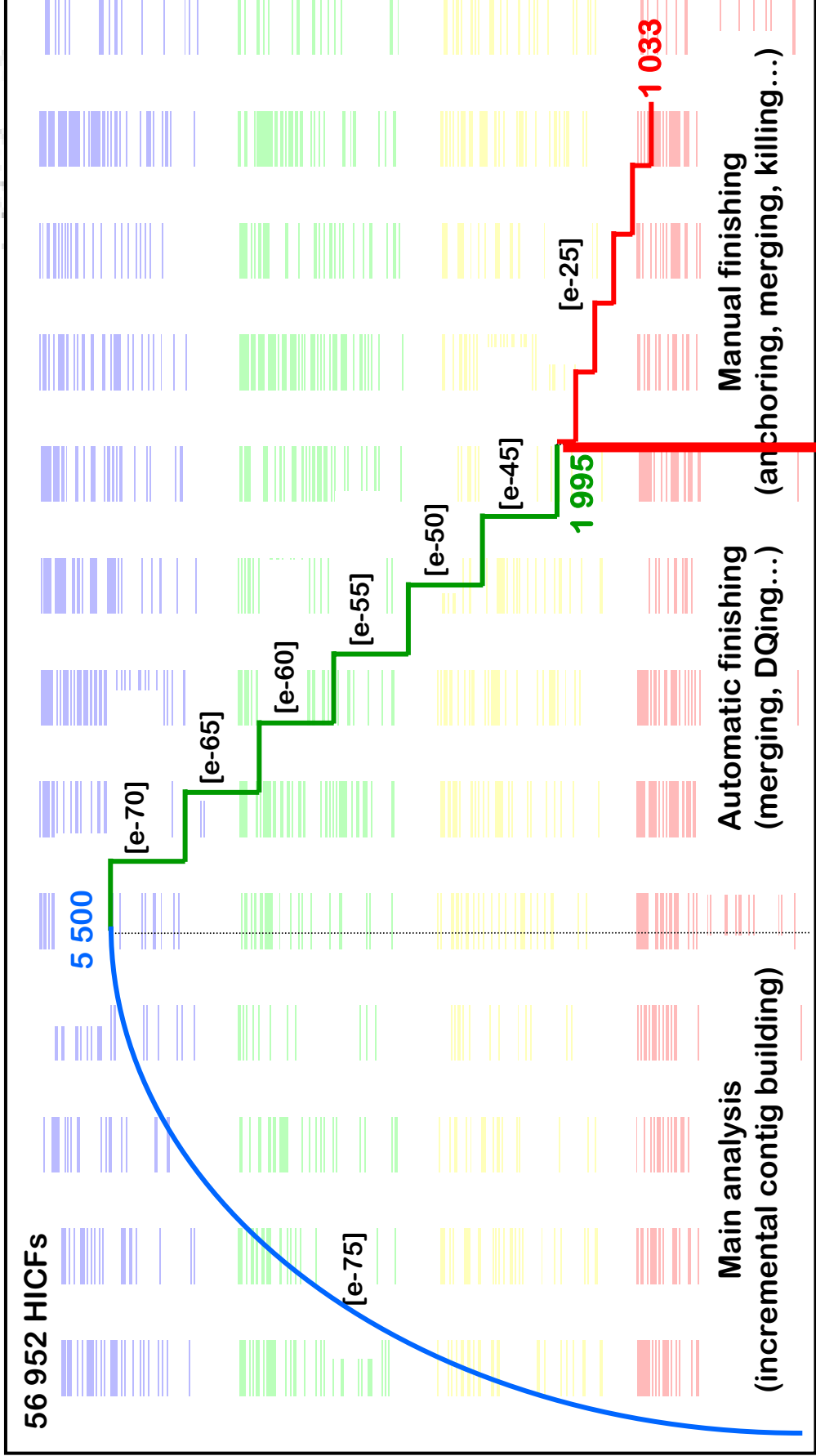
- ✓ Establish an optimized strategy for developing anchored physical map from hexaploid wheat
- ✓ Accelerate map based cloning
- ✓ Study large-scale organization (genic vs repetitive regions)
- ✓ Analyze pattern of recombination and linkage disequilibrium
- ✓ Comparative studies between homoeologous genomes and with other cereal genomes
- ✓ Decipher evolutionary mechanisms (gene movements, genome duplications, TE transposition...)
- ✓ Establish transcriptomic map of 3B



Construction of an integrative map of the chromosome 3B

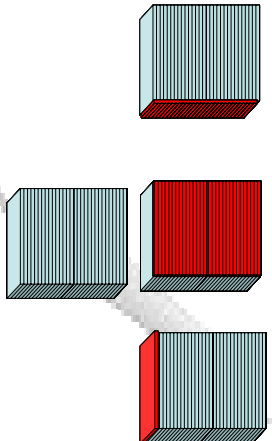


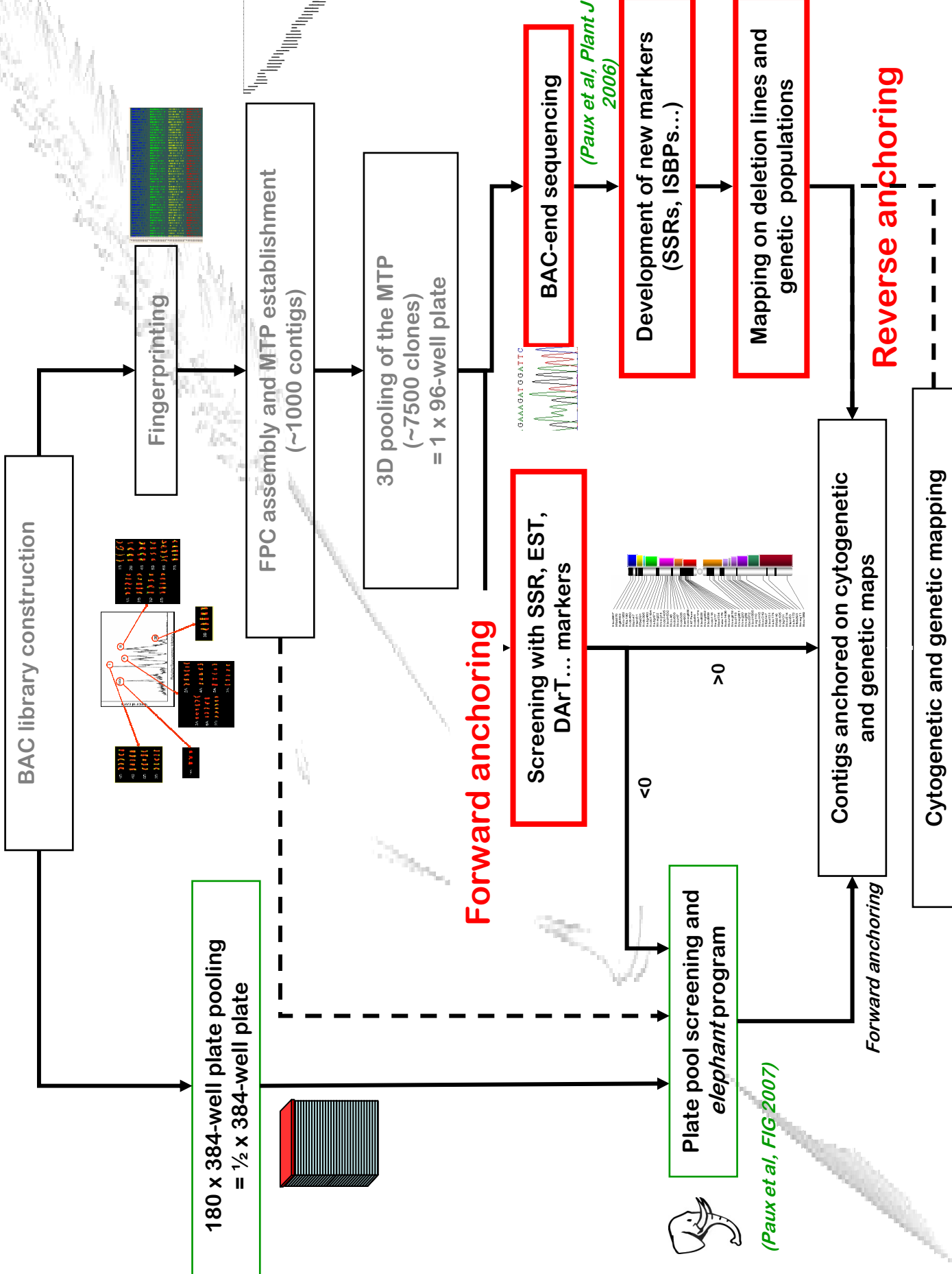
Physical map contig assembly



MTP pooling:

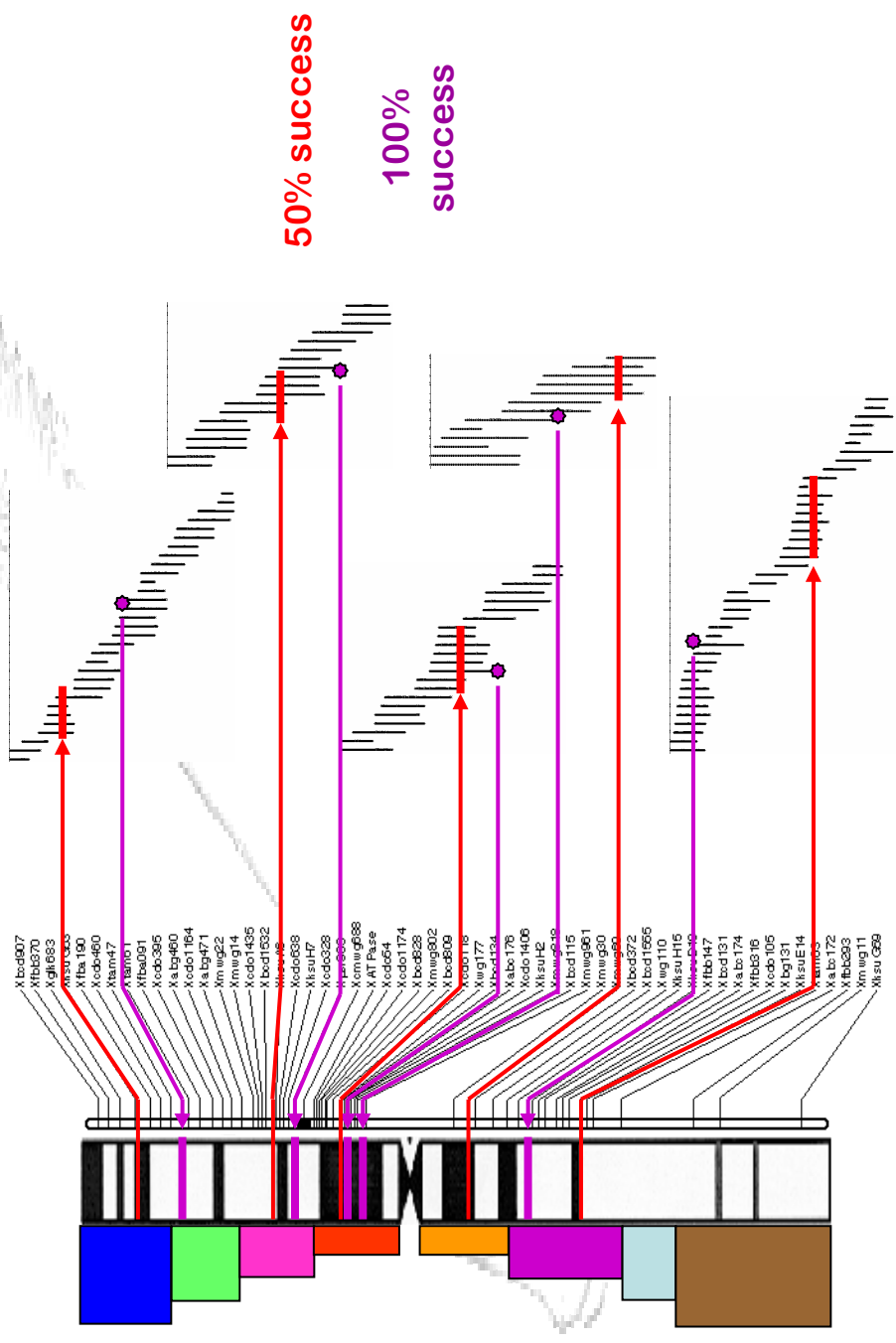
- ✓ 7500 clones for 3B MTP = 20 x 384-well plates pooled in 3 dimensions
- ✓ One 96 well plate (60 PCR) with P/L/C pools for screening





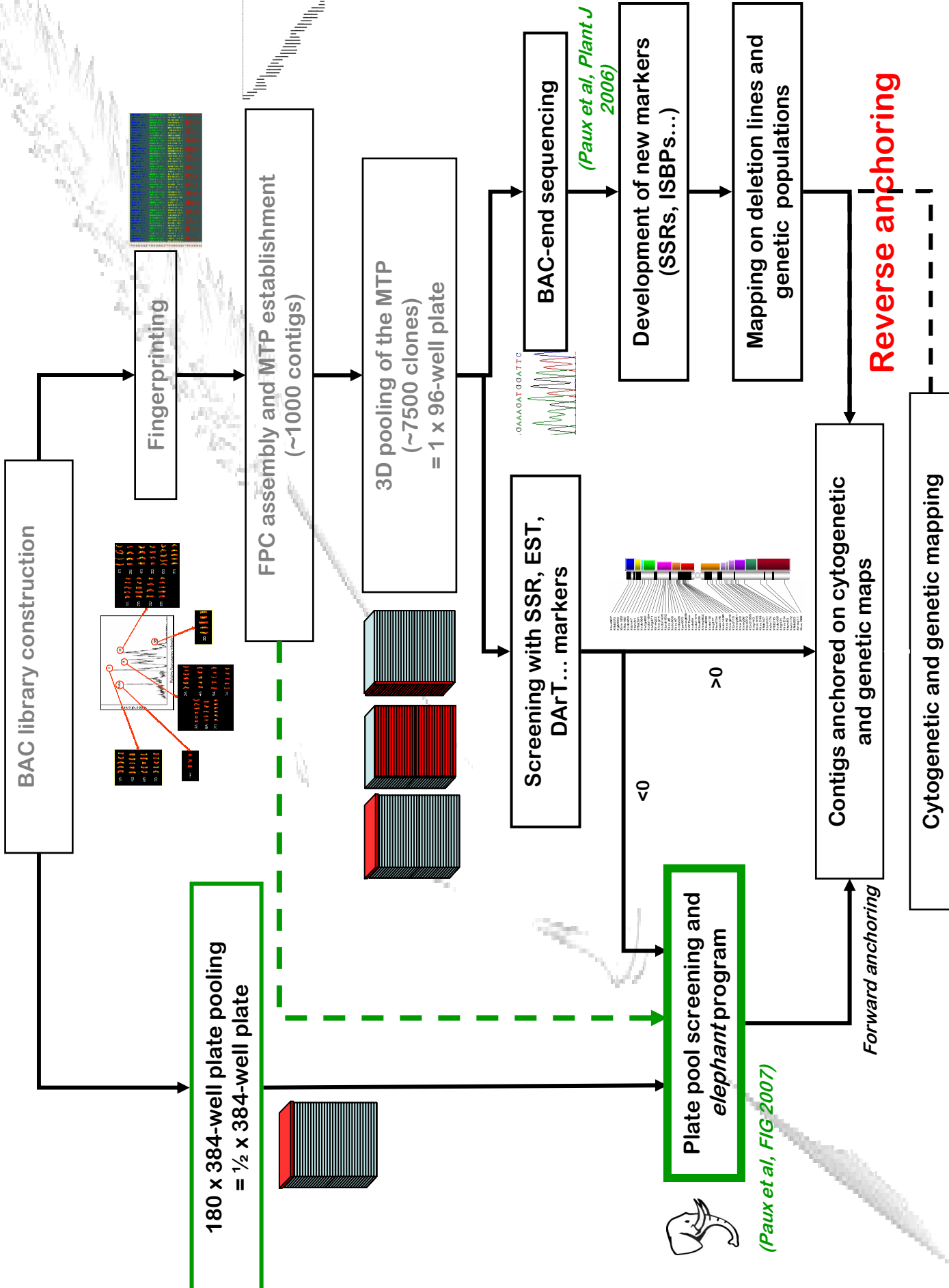
Physical map contig anchoring

« Classical (BAC, BFP, SSTS, RFLP, FISH, ESTs...):
from cytogenetic maps to genetic contigs



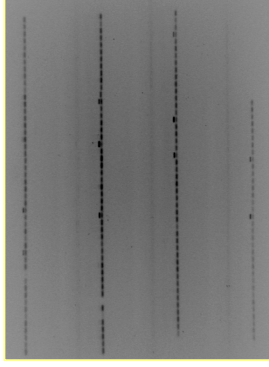
Cytogenetic & genetic maps

Physical map (contigs)



Electronic Physical map Anchoring Tool

Plate pools screening



177
pool
plates

Plate pool addresses
(~8 expected)

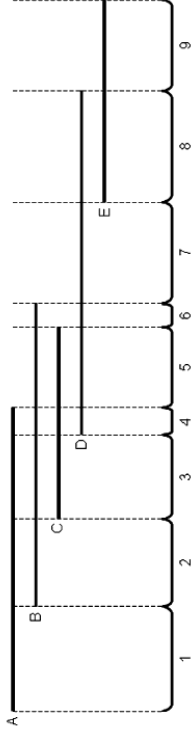
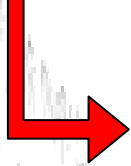
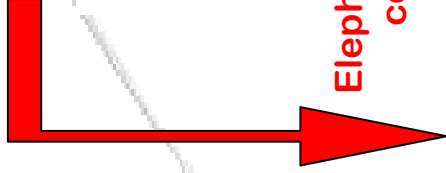


ElePhAnt

FPC contigs

+

(~8 expected)



Results: Using 158 SSR markers,
unambiguous addresses obtained for
52 markers (33%)

= 20'000 PCR reactions spared

Elephant searches in FPC contigs a segment that
contains all BACs from the positive plates

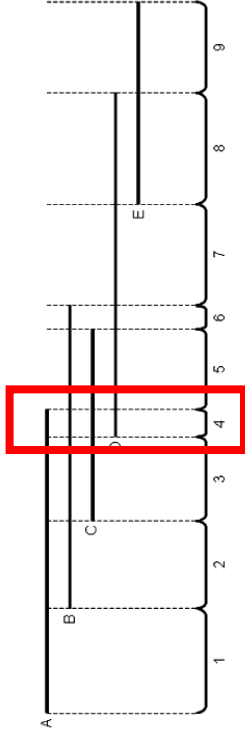


Table 1 Validation of *Elephant* through virtual pool screening

Percentage of screening data used	
100%	50%
98.5%	38.0%
1.5%	2.0%
0.0%	4.0%
0.0%	56.0%

Percentage of markers

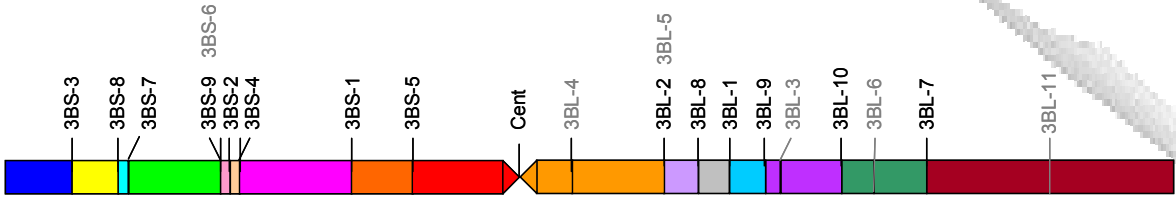
Assigned to the correct contig

Assigned to multiple contigs

Assigned to the wrong contig

Not assigned to a contig

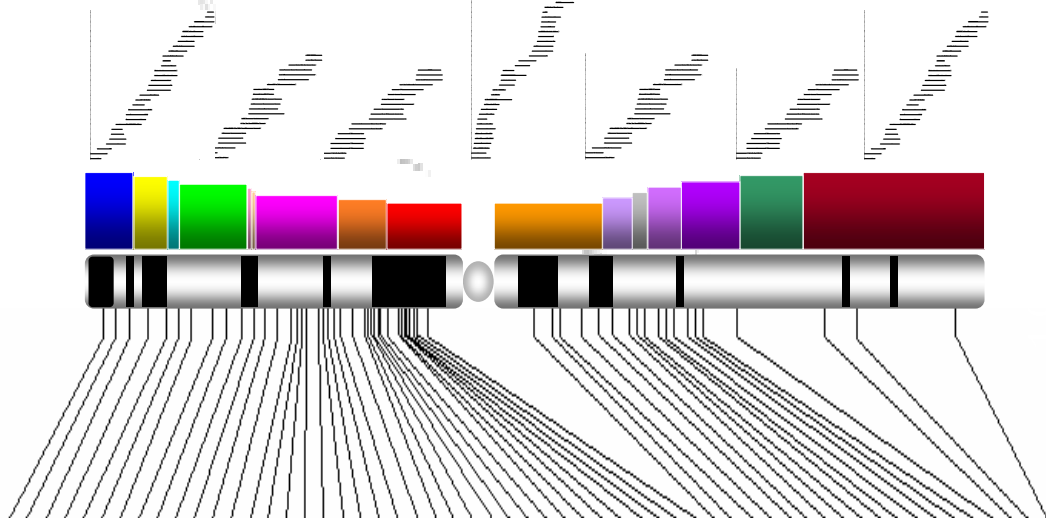
Anchoring using (16) deletion bins



Deletion bin	Markers in contigs			Non redundant contigs anchored		
	ISBP	EST	SSR	ISBP + SSR	EST	ISBP+SSR+EST
Total tested	687	828	322			
3BS	14	3	0	13	2	15
3BS3	11		1	7	5	56
3BS8	53	68	62	44		
3BS7	7		7	7	5	57
3BS9	45	26	15	45		
3BS2	6		4	5		
3BS4	14	52	3	13	5	98
3BS1	100		21	75		
3BS5	58	11	14	44	8	66
C3BS5	11		4	14		
C3BL2	63	16	8	46	7	53
3BL2	21		1	16		
3BL8	6	45	1	6	13	84
3BL1	41		11	25		
3BL9	32		6	24		
3BL10	39	17	21	35	4	39
3BL7	156	123	97	140	31	171
3BL	10	1	4	12	0	12
3B	4	3	11	12	1	13
Total	687	362	280	583	81	664
Success rate (%)	100	43	87			

An integrated physical map of chromosome 3B of hexaploid wheat

Xbcd907
Xfbb870
Xgfh683
Xksu C63
Xfba 190
Xcdo460
Xtam47
Xtam6 1
Xfba091
Xcdo395
Xa.bg460
Xcdo1164
Xa.bg47 1
Xm.wg22
Xm.wg14
Xcdo1436
Xbcd1532
Xksu A6
Xcdo638
Xksu H7
Xcdo328
Xpsr903
Xcm.wg68E
X AT Pase
Xcdo64
Xcdo1174
Xbcd828
Xm.wg802
Xbcd809
Xcdo118
Xwg177
Xbcd134
Xabc 176
Xcdo1406
Xksu H2
Xm.wg8 18
Xbcd115
Xm.wg96 1
Xm.wg30
Xm.wg99
Xbcd372
Xbcd1966
Xwg110
Xksu H15
Xksu D19
Xfbb147
Xbcd13 1
Xabc 174
Xfbb8 16
Xcdo105
Xbg131
Xksu E14
Xtam63
Xabc 172
Xfbb393
Xm.wg11
Xksu C69



✓ 56 952 fingerprinted BACs

✓ 1 033 contigs (average size = 785 kb)

✓ 811 Mb coverage (82% chromosome)

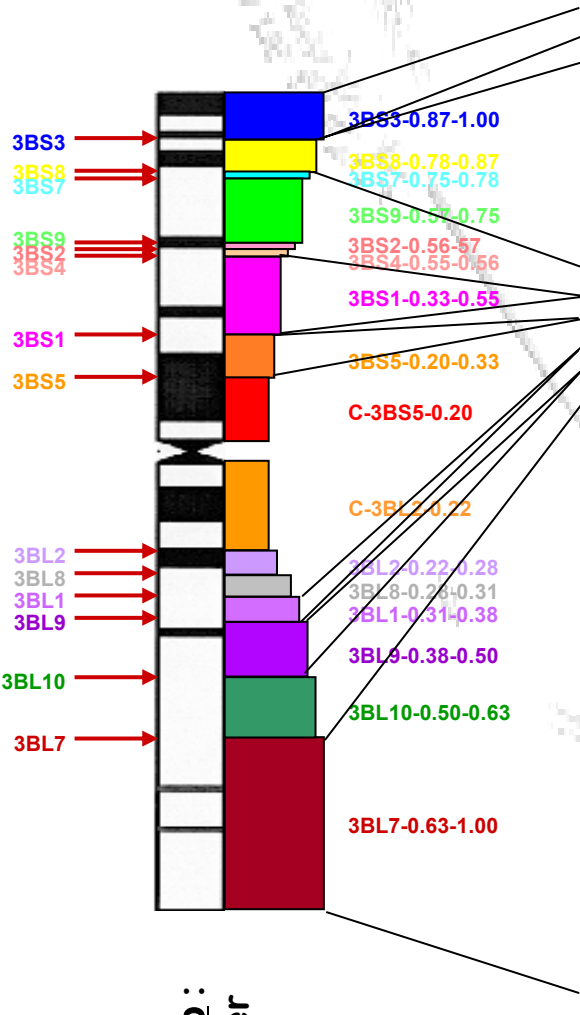
✓ 1 397 molecular markers in contigs

✓ 664 cytogenetically-anchored contigs (612 Mb)

Cytogenetic map :
Nb of contigs per
deletion bin

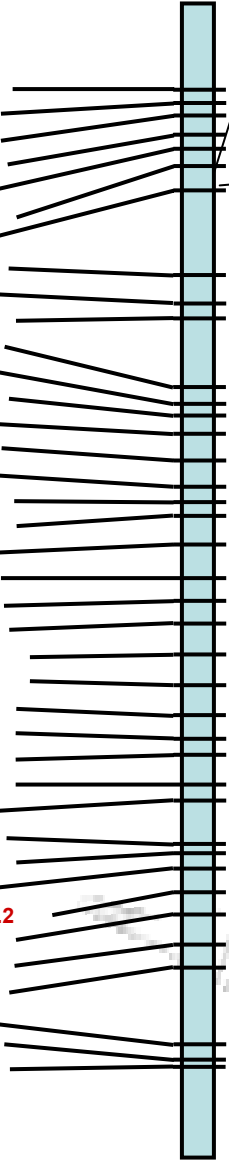


Genetic map :
Contigs order!

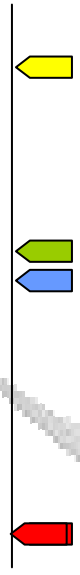


- Xgwm389
- Xnw1600
- Xbarc147
- Xgwm493
- Xbarc87
- Xwmm626
- Xcfd79
- Xwmc500
- Xnw216
- Xgpw3092
- Xwmc231
- Xdw1169
- Xgwm285
- Xdw1221
- Xbarc164
- Xnw2706
- Xgpw8064
- Xgpw4505
- Xnw2860
- Xcfd1871
- Xwmc291
- Xgwm108
- Xwmm1636
- Xwmm1133
- Xwmm702
- Xwmm280
- Xwmm278
- Xwmm638
- Xnw1608
- Xwmc326
- Xgpw3085
- Xbarc77
- Xwmm1966.2
- Xwmm274
- Xgpw3233
- Xgwm299
- Xnw1
- Xgwm247
- Xgwm340

Framework map
(Re xCs 381 F2)
Defines genetic bins



Neighbour map with 12 maps
and relative position of markers
= 552 markers



Traits



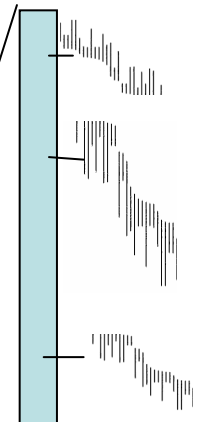
Map based cloning and MAS

HRMP (Re xCs 2000 F2)



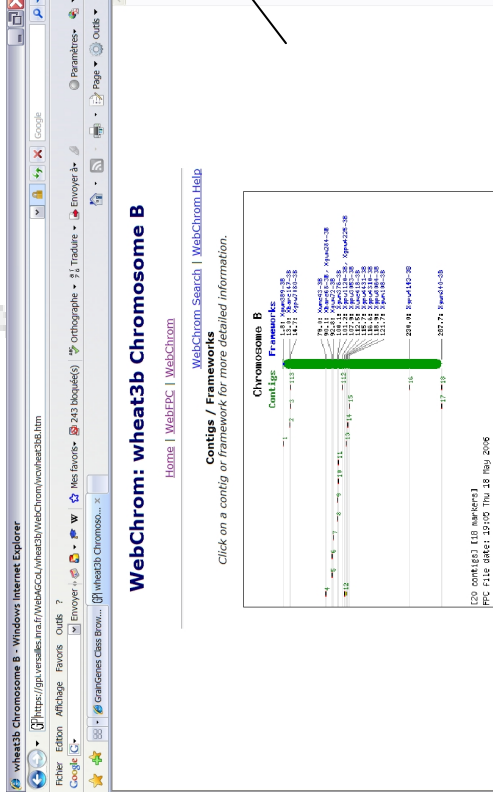
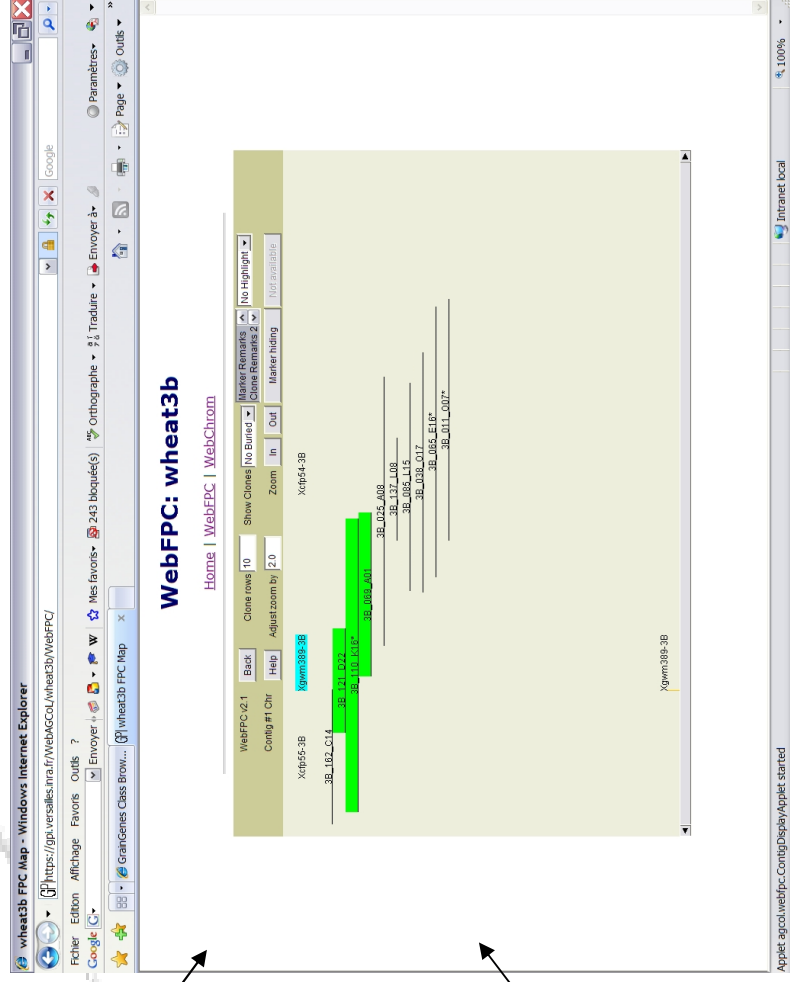
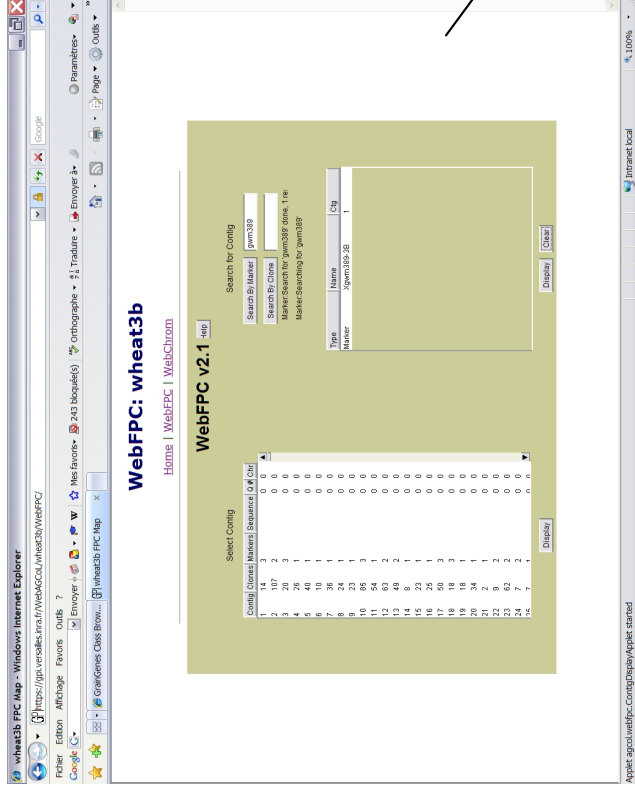
"TriticeaeGenome"

Anchoring



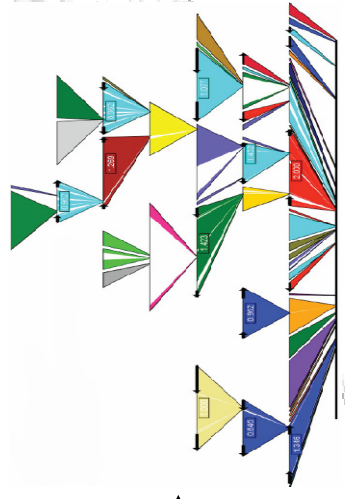
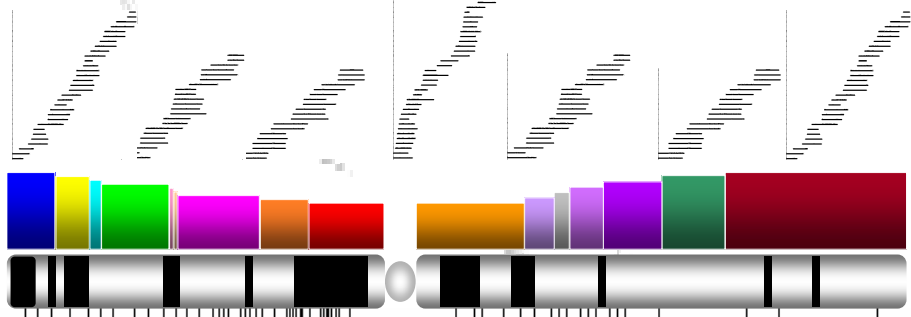
A Web interface to search the 3B physical map for contig, clone or marker

<http://urgi.versailles.inra.fr/projects/xxx>

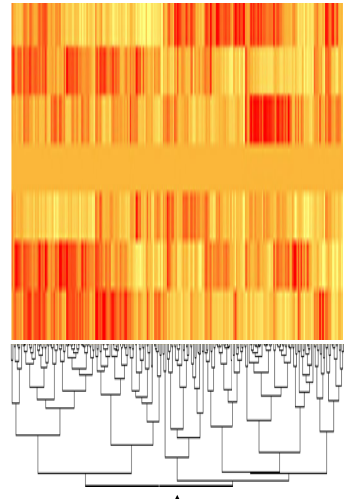


Deciphering the hexaploid wheat genome

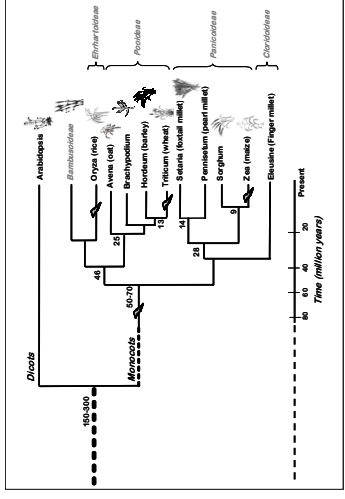
Xbcd907
Xfbb870
Xgfh683
Xksu C63
Xfba 190
Xcd0460
Xtam47
Xtam61
Xfba091
Xcd0395
Xa.bg460
Xcd01164
Xa.bg471
Xm.wg22
Xm.wg14
Xcd01436
Xcd01532
Xksu A6
Xcd0638
Xksu H7
Xcd0328
Xpsr903
Xcm.wg68E
X AT Pase
Xcd064
Xcd01174
Xbcd828
Xm.wg802
Xbcd809
Xcd0118
Xwg177
Xbcd134
Xabc 176
Xcd01406
Xksu H2
Xm.wg818
Xbcd115
Xm.wg961
Xm.wg90
Xm.wg99
Xbcd372
Xbcd1566
Xwg110
Xksu H15
Xksu D19
Xfbb147
Xbcd131
Xa.bc 174
Xfbb816
Xcd0105
Xbg131
Xksu E14
Xtam63
Xa.bc 172
Xfbb393
Xm.wg11
Xksu C59



✓ Structure



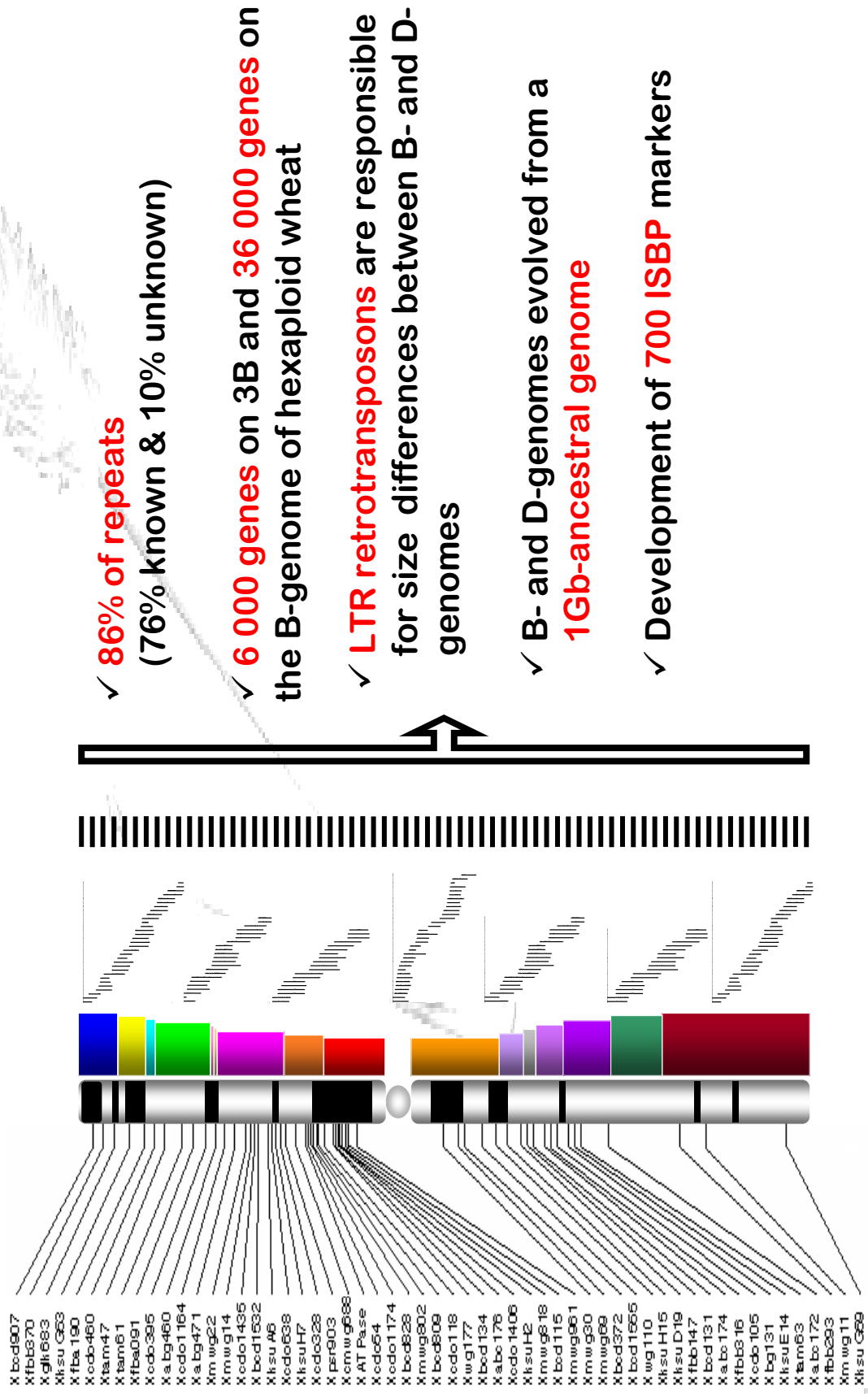
✓ Function



✓ Evolution

Composition and evolution of homeologous genomes in hexaploid wheat

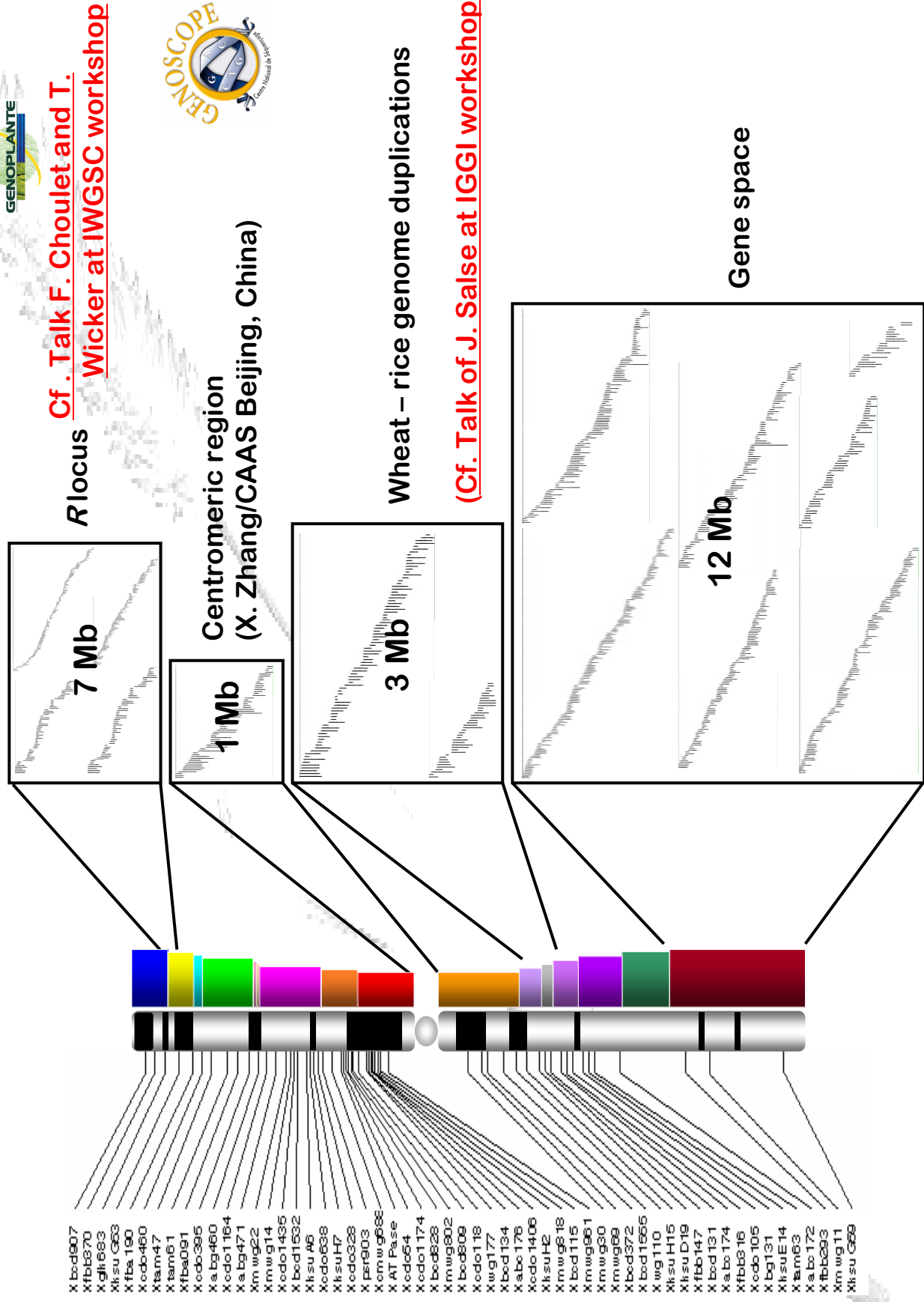
11 Mb of BAC-end sequences evenly distributed along the chromosome 3B



Megabase-sized contig sequencing



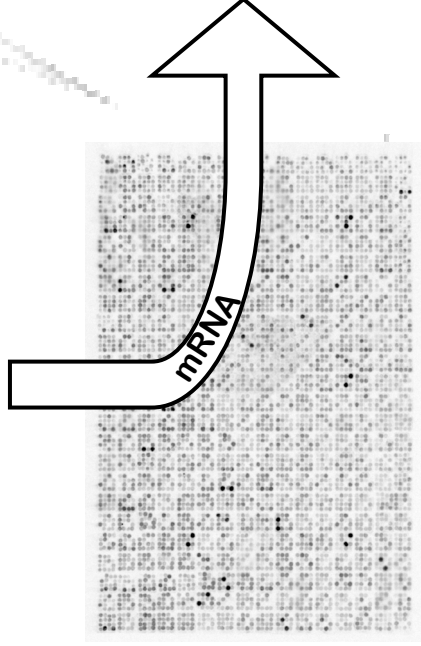
Cf. Talk F. Choulet and T. Wicker at IWGSC workshop



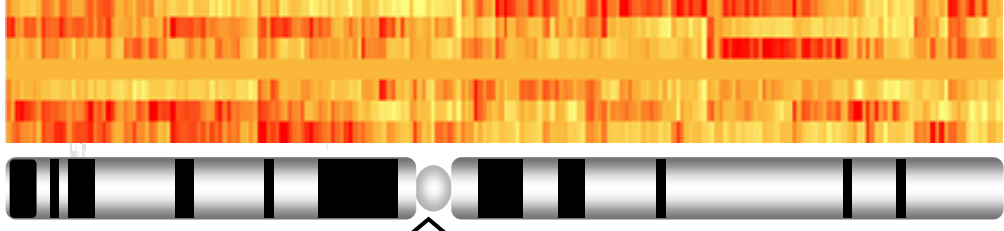
Transcriptional mapping of the chromosome 3B



- ✓ Various organs
- ✓ Various developmental stages
- ✓ Contrasting physiological conditions
- ✓ Aneuploid lines



Genomic tiling array
(7 440 BAC clones
from the MTP)



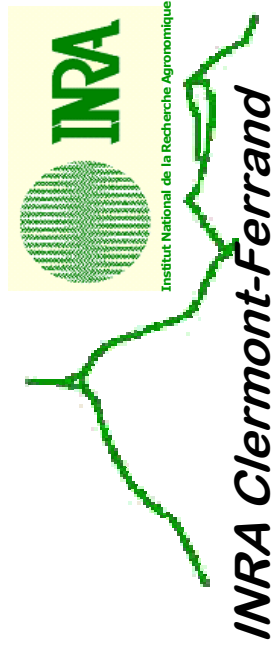
✓ Gene space organization

✓ Co-regulation islands

✓ Relationships between genome structure
and gene function / regulation

✓ Homoeologous gene expression

Acknowledgements



Etienne Paux
Pierre Sourdille
Jérôme Salse
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Georges Gay
Michel Bernard
Catherine Feuillet



CNRGV
Hélène Bergès



Institute of Experimental Botany
Jaroslav Dolezel



Murdoch University
Rudi Appels, Mehmet Kakir



Delphine Roger
Beatrice Gandon



Zurich University
Beat Keller



CSIRO
Evans Lagudah
Wolfgang Spielmeier



Kansas State University
Bikram Gill, Mike Pumphrey



University of Minnesota
Jim Anderson



North Dakota State University
Shrahryar Kianian

