Reference Assembly of Chromosome 7A as a Platform to Study Regions of Agronomic Importance

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GRDC
Grains Research & Development Corporation

Bioplatforms Australia

Murdoch
UNIVERSITY
DISCOVERERS WELCOME

IWGSC
International Wheat Genome Sequencing Consortium

Acknowledgments

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Grains Research Development Corporation
Bioplatforms Australia

ACCWI group
Rudi Appels, Hollie Webster, Shahidul Islam, Xueyan Chen, Yingjun Zhang, Johan Nystrom-Persson

Flow-sorting DNA/BAC library construction
Jaroslav Dolezel, Hana Simkova
Institute of Experimental Botany
Czech Republic

Fingerprinting BAC library
Mingcheng Luo group
UC Davis

Physical map assembly
Zeev Frenkel, Ambraham Korol
Haifa University

Genetic maps
MAGIC: Colin Cavanagh, Emma Huang, Jen Taylor (CSIRO)
MAGIC GBS: Matt Hayden (DEPI)
CSxRenan: Pierre Sourdille, Benoît Darrier (INRA)

***T. monoccocum* genetic map**
Population: Jorge Dubcovsky
90k chip: Matt Hayden, Kerrie Forrest

DNA sequencing
Matt Tinning
AGRF

Annotation
TriAnnot: Philippe Leroy, Aurelien Bernard (INRA)
geneID (CRG): Francisco Camara, Anna Vlasova (CRG, Spain), Juan Carlos Sanchez (ACPFG)
Storage proteins: Angela Juhasz (Hungary)
QTL mapping/Significant genome regions: Delphine Fleury (ACPFG)
Specific genes: Hui-xian Zhao (NW A&F Uni, China)

Pseudomolecule
Fred Choulet, Etienne Paux
INRA

7A mate-pair sequencing of amplified DNA
Matt Hayden, Josquin Tibbits, Sami Hakim
DEPI

Whole-genome mate-pair data
Andy Sharpe, David Konkin, Curtis Pozniak
NRC, Canada

Bionano map
Jaroslav Dolezel, Hana Simkova, Mingcheng Luo

Supercomputing resources
iVEC/Pawsey Supercomputing Centre

Summary of achievements

1. We have produced a high quality, genetically anchored, assembly of chromosome 7A

2. The assembly has been validated using independent genome-level information for specific regions of the chromosome

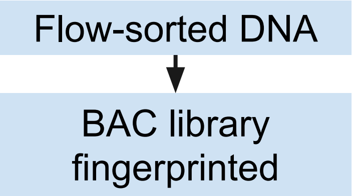
3. The assembly now forms the basis for the analysis of agronomically significant chromosome regions

Reference-level assembly of 7A

Flow-sorted DNA

Dolezel lab, Czech Republic

Reference-level assembly of 7A



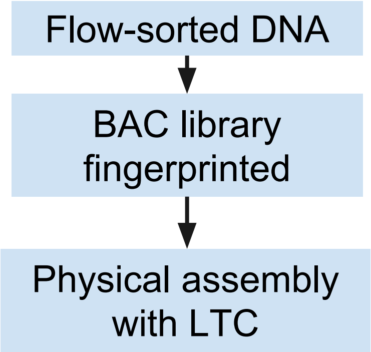
Flow-sorted DNA

BAC library fingerprinted

Dolezel lab, Czech Republic

Mingcheng Luo, UC Davis

Reference-level assembly of 7A



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BAC library fingerprinted — Mingcheng Luo, UC Davis

Physical assembly with LTC — Zeev Frenkel, Korol lab, Haifa University

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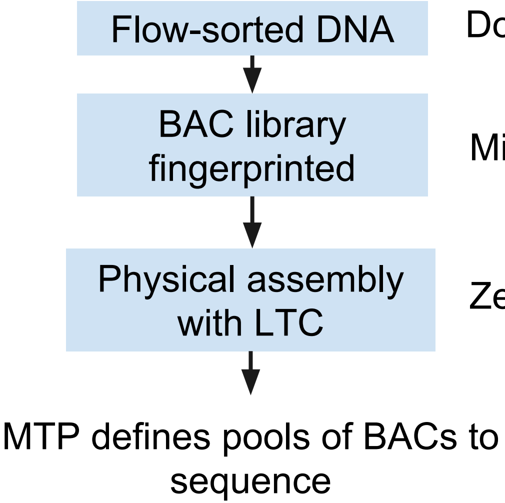

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MTP defines pools of BACs to sequence

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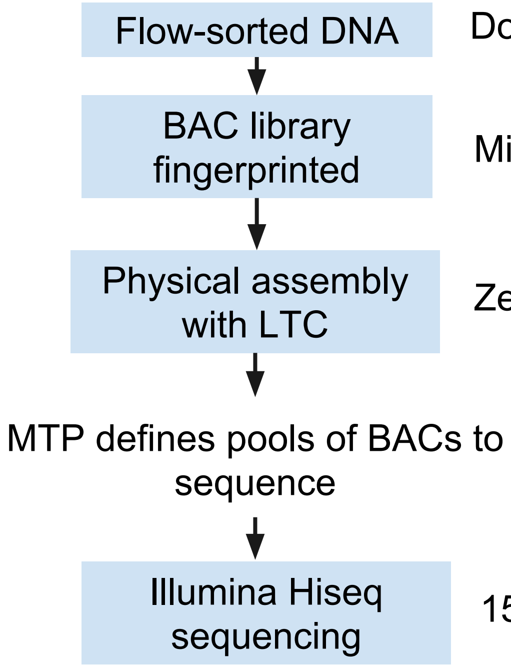

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Physical assembly with LTC

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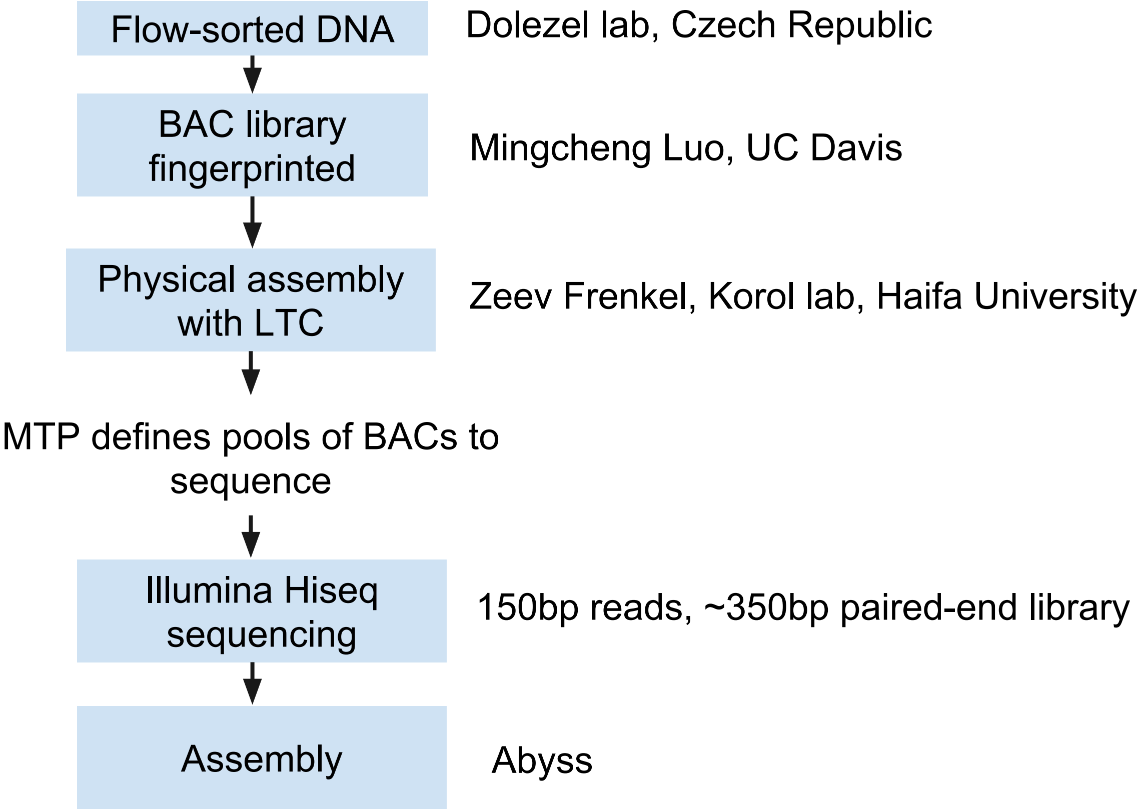
MTP defines pools of BACs to sequence

Illumina Hiseq sequencing

150bp reads, ~350bp paired-end library

Reference-level assembly of 7A



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Assembly — Abyss

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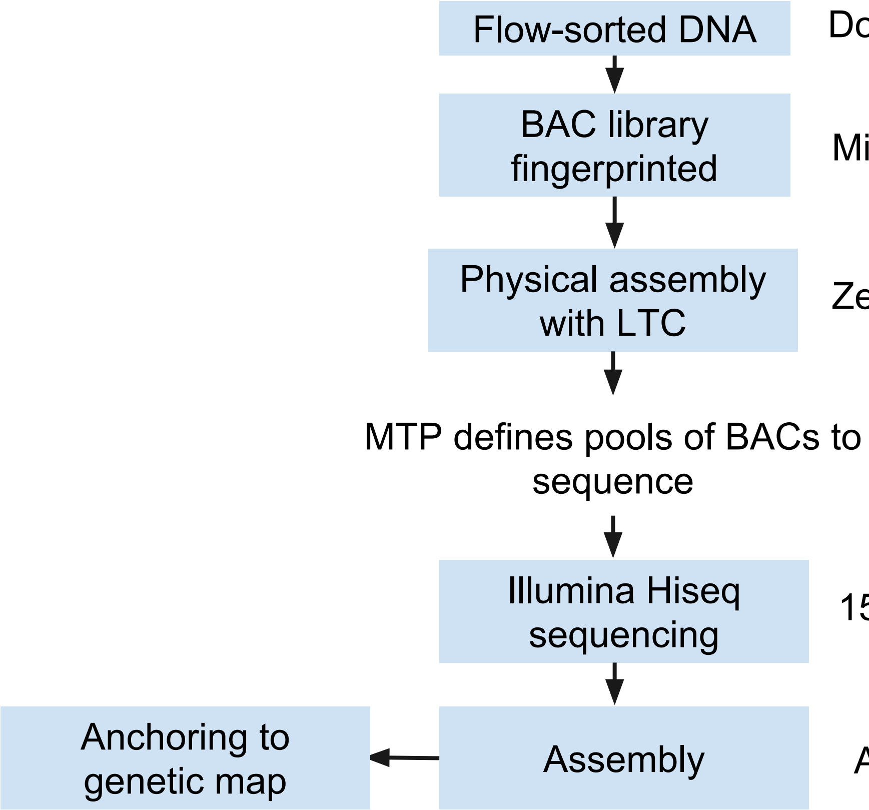

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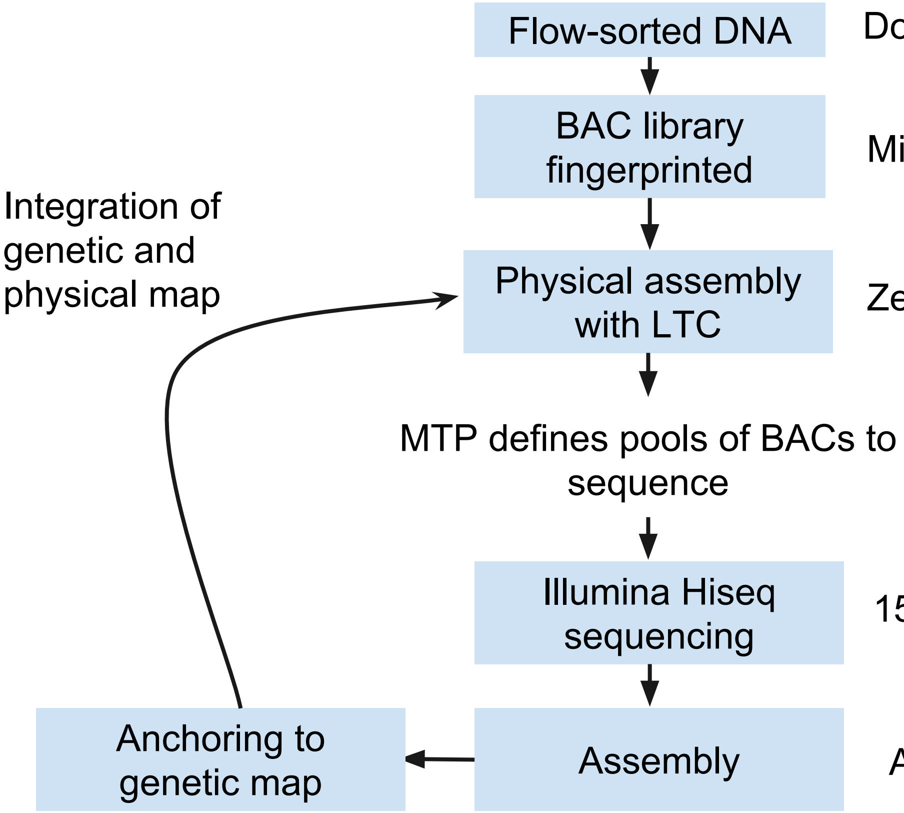
Assembly — Abyss

Anchoring to genetic map

Reference-level assembly of 7A



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Assembly — Abyss

Anchoring to genetic map

Integration of genetic and physical map

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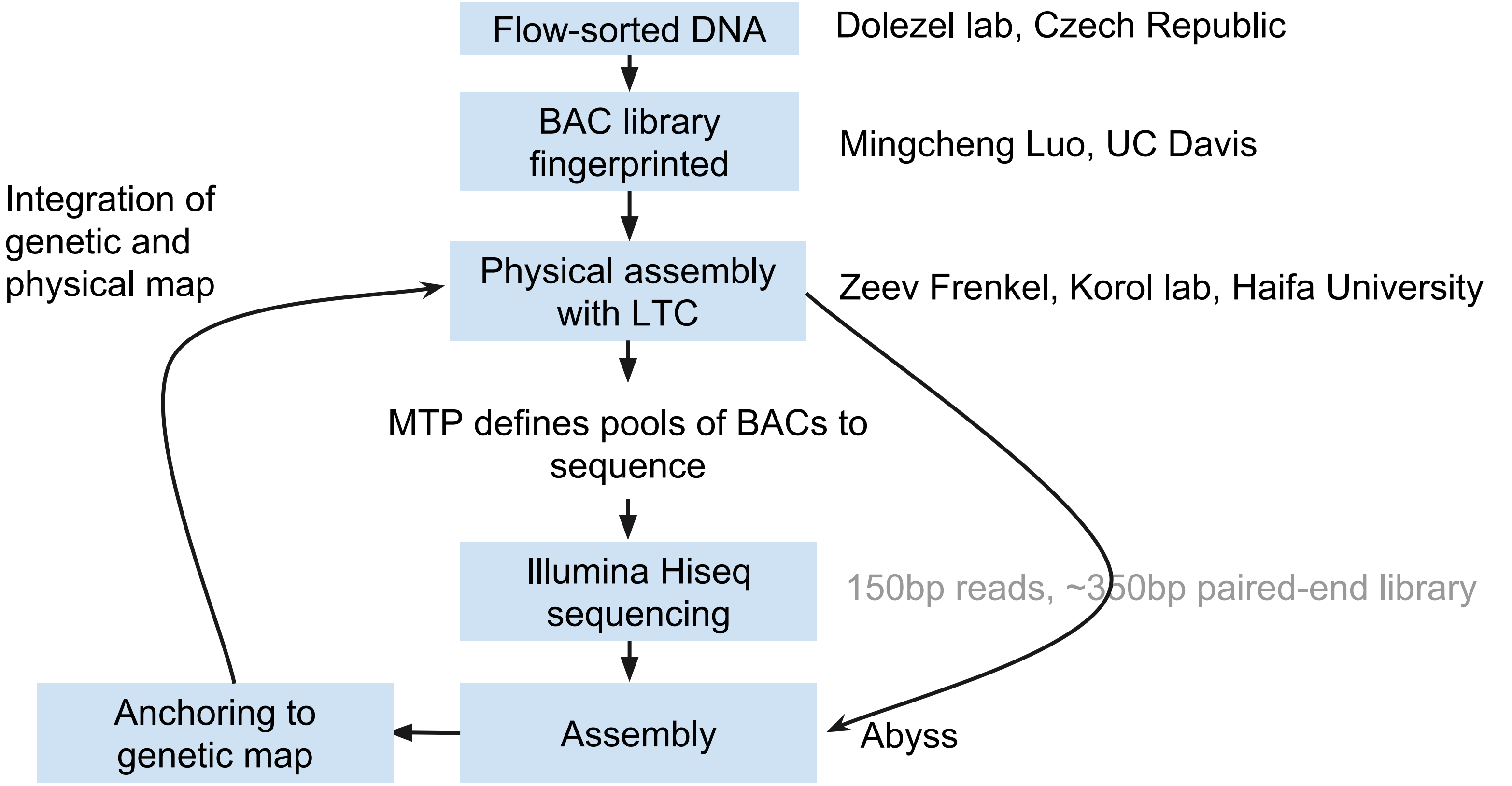

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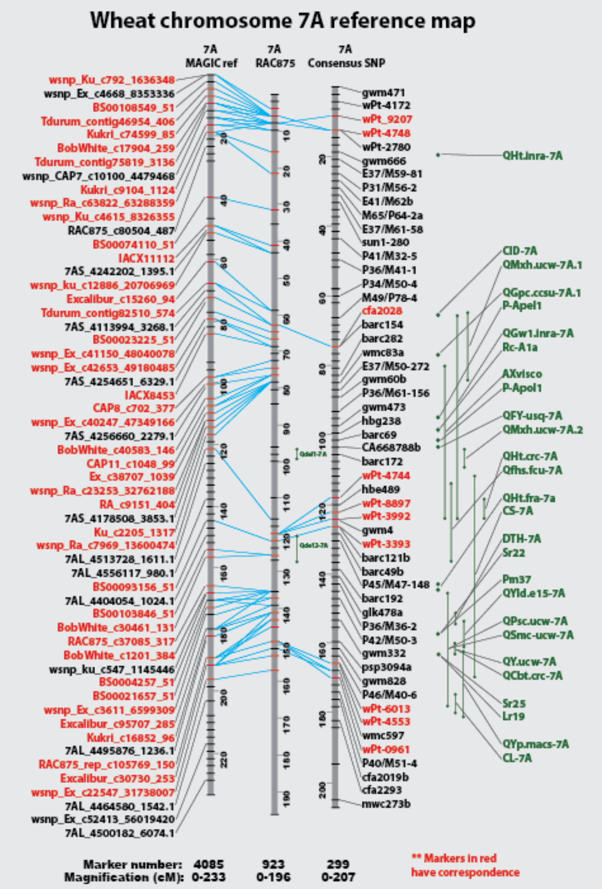
Assembly

Abyss

Anchoring to genetic map

Integration of genetic and physical map

Assembly summary

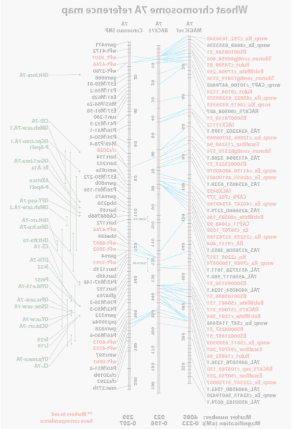


Wheat chromosome 7A reference map

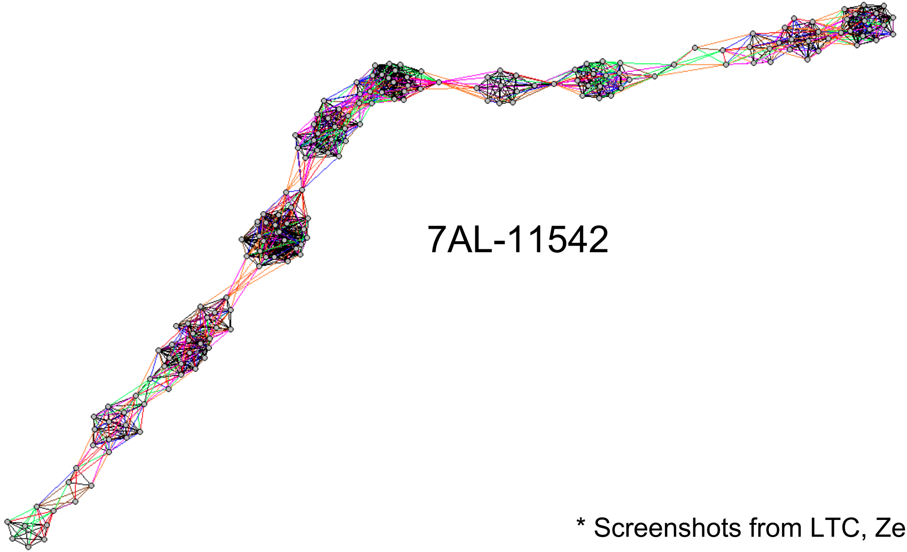
- High-density composite genetic map based on MAGIC (CSIRO) using Chinese Spring x Renan (INRA) map as anchor
    - Over 4,000 markers on 7A

Assembly summary

- High-density composite genetic map based on MAGIC using CSxRenan map as anchor
  - Over 4000 markers



- 732 physical contigs reduced to 316 scaffolds
- 676 physical contigs (92%) anchored via scaffolded physical map



* Screenshots from LTC, Zeev Frenkel

Assembly summary

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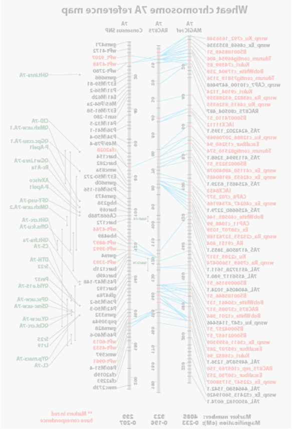
  - Over 4000 markers



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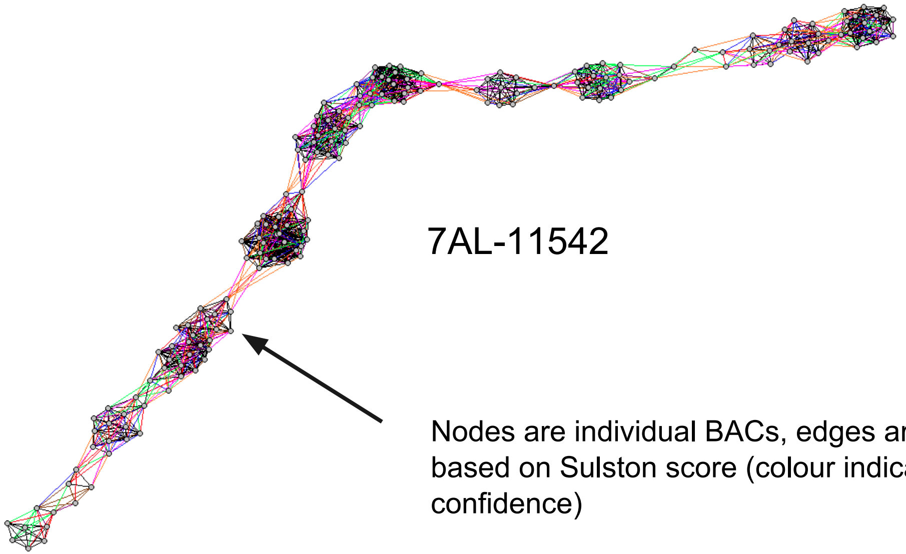


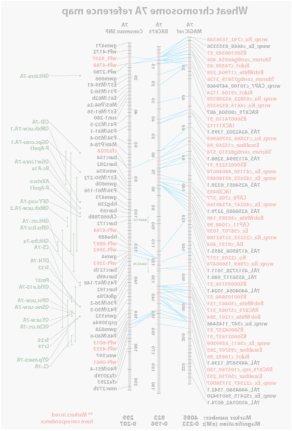
7AL-11542

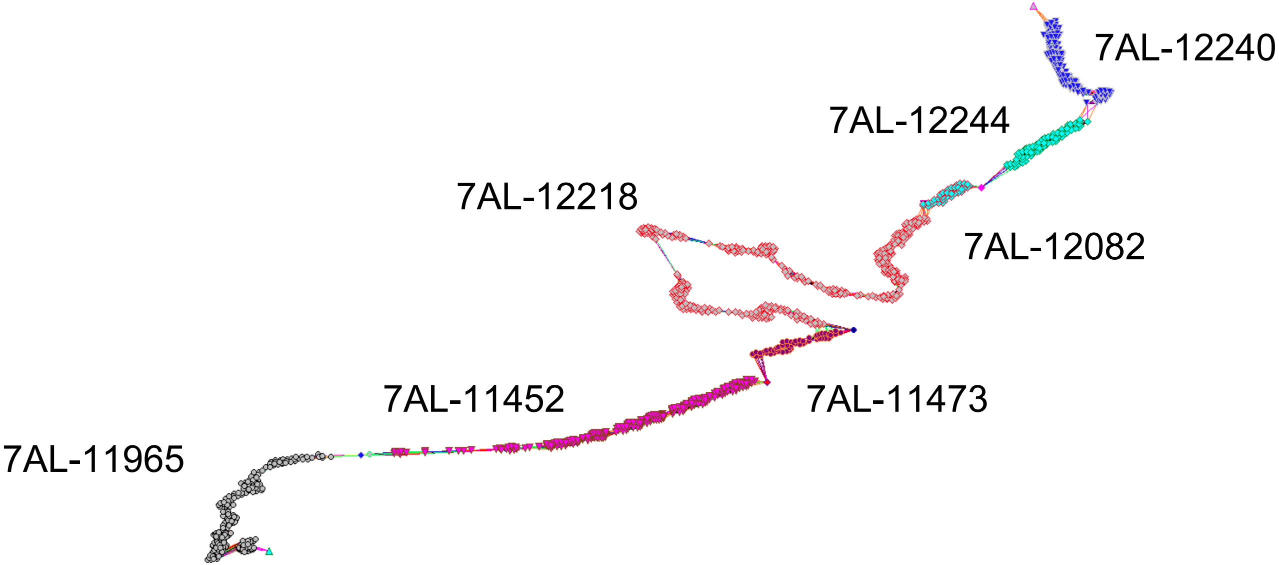
Nodes are individual BACs, edges are overlaps based on Sulston score (colour indicates confidence)

Assembly summary

- 732 physical contigs reduced to 316 *scaffolds*
- 676 physical contigs (92%) anchored via scaffolded physical map

- High-density composite genetic map based on MAGIC using CSxRenan map as anchor
  - Over 4000 markers





Super-scaffolding

Final stats for paired-end-only (pre-mate-pair) assembly:

- 42,441 sequence scaffolds
  - Total length 940Mb
  - N50 137kb
  - Mean 22kb

A large mate-pair dataset was generated by National Research Council, Canada (Andy Sharpe) from a Chinese Spring+7EL line, including 12 insert library sizes from 1.4kb to 20kb.

The read pairs aligning perfectly (no mismatches) to our paired-end-only draft assembly were provided by David Konkin and used for super-scaffolding with SSPACE.

The minimum number of mate-pair joins required to connect two contigs (k) was explored, using k = 2 to 5.

For example, for k = 2, two scaffolds can be joined based on only two connections.

Super-scaffolding stats

Two scaffolding approaches were explored:

1) Chromosome-arm level scaffolding

k	# Scaffolds	Median (bp)	Mean (bp)	N50 (bp)	Max scaffold (bp)	Total length (bp)	% cross-pool joins
2	23,342	4,732	38,839	350,507	2,814,297	906.5e6	3.9
3	27,659	3,941	32,704	289,304	2,148,657	904.5e6	1.6
4	30,690	3,631	29,463	249,246	2,127,911	904.2e6	1
5	33,426	3,449	27,032	214,649	2,117,720	903.5e6	0.7

2) BAC pool-level scaffolding

k	# Scaffolds	Median (bp)	Mean (bp)	N50 (bp)	Max scaffold (bp)	Total length (bp)
2	12,043	5,024	75,216	421,553	2,415,588	905.8e6
3	15,546	3,619	58,172	370,629	2,334,598	904.3e6
4	18,131	3,094	49,848	339,791	2,852,455	903.8e6
5	20,416	2,789	44,242	315,060	1,979,523	903.2e6

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Very few scaffolds from different pools are joined

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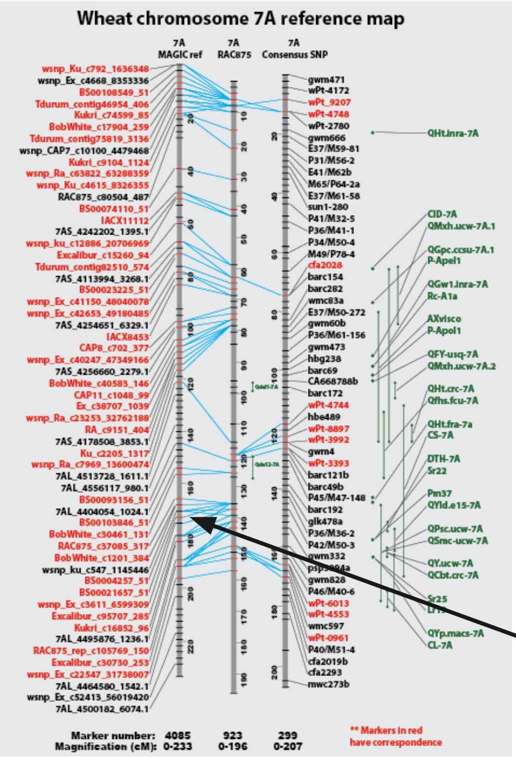
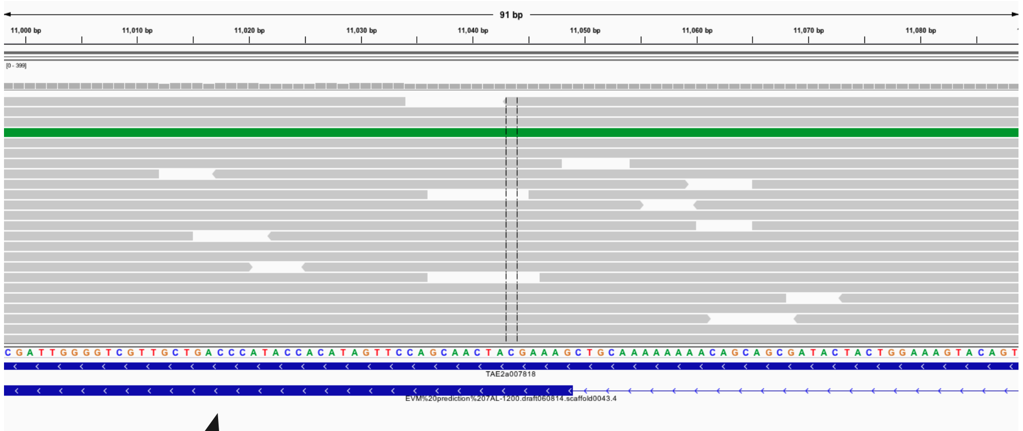
Needs validation, eg: with Bionano maps

From long- to short-range information



Genetic map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11,010 bp
11,020 bp
11,030 bp
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C G A T T G G G G T C G T T G C T G A C C C A T A C C A C A T A G T T C C A G C A A C T A C G A A A G C T G C A A A A A A A A C A G C A G C G A T A C T A C T G G A A A G T A C A G T
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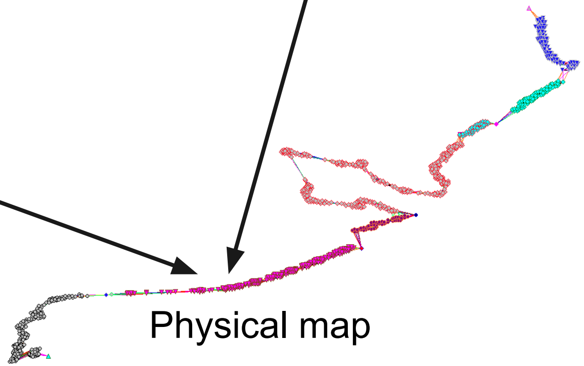
Assembled sequence + annotation



Physical map

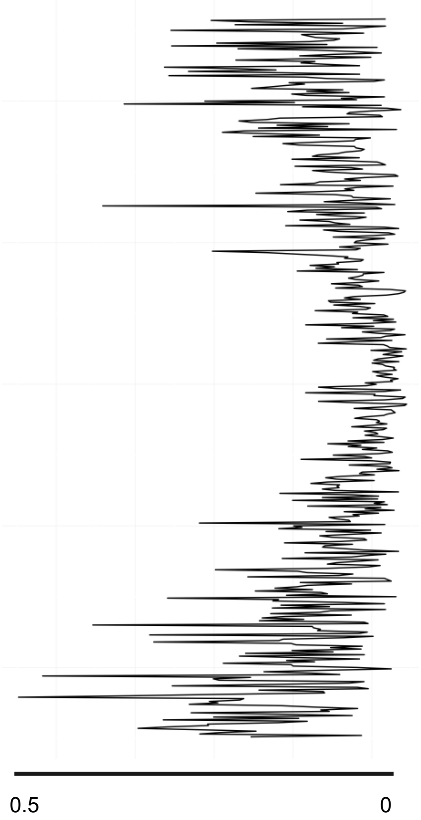
Pseudomolecule

Annotation

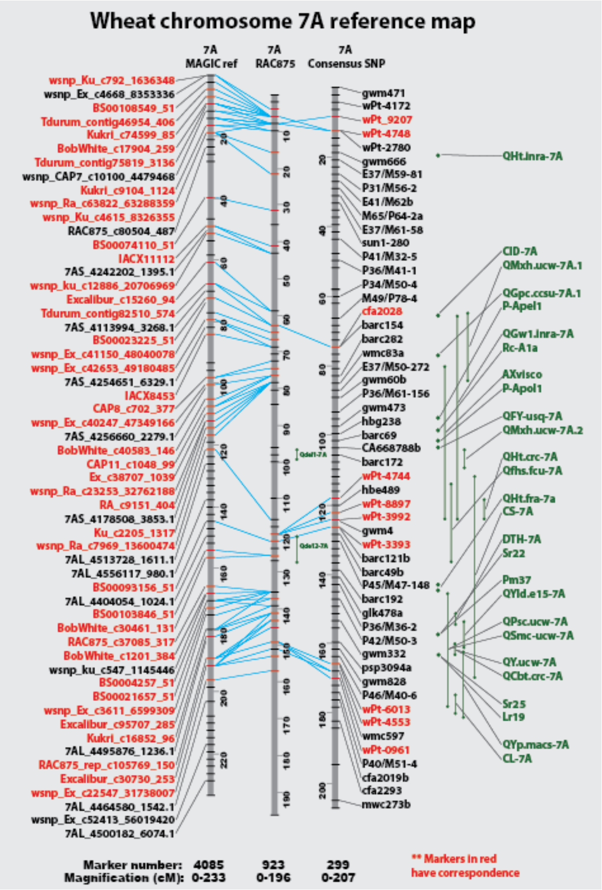
TriAnnot
(Philippe Leroy, INRA)
3897 genes predicted
(1623 “high confidence”, 2274 “low confidence)

CRG annotation
(Francisco Camara group)
24,030 predictions on an earlier draft

Many genes are unique to a particular annotation



Gene density per 10kb
(TriAnnot annotation)



Pseudomolecule

Ann

TriA
(Phi
7256
(3295 “high confidence”, 3961 “low confidence)

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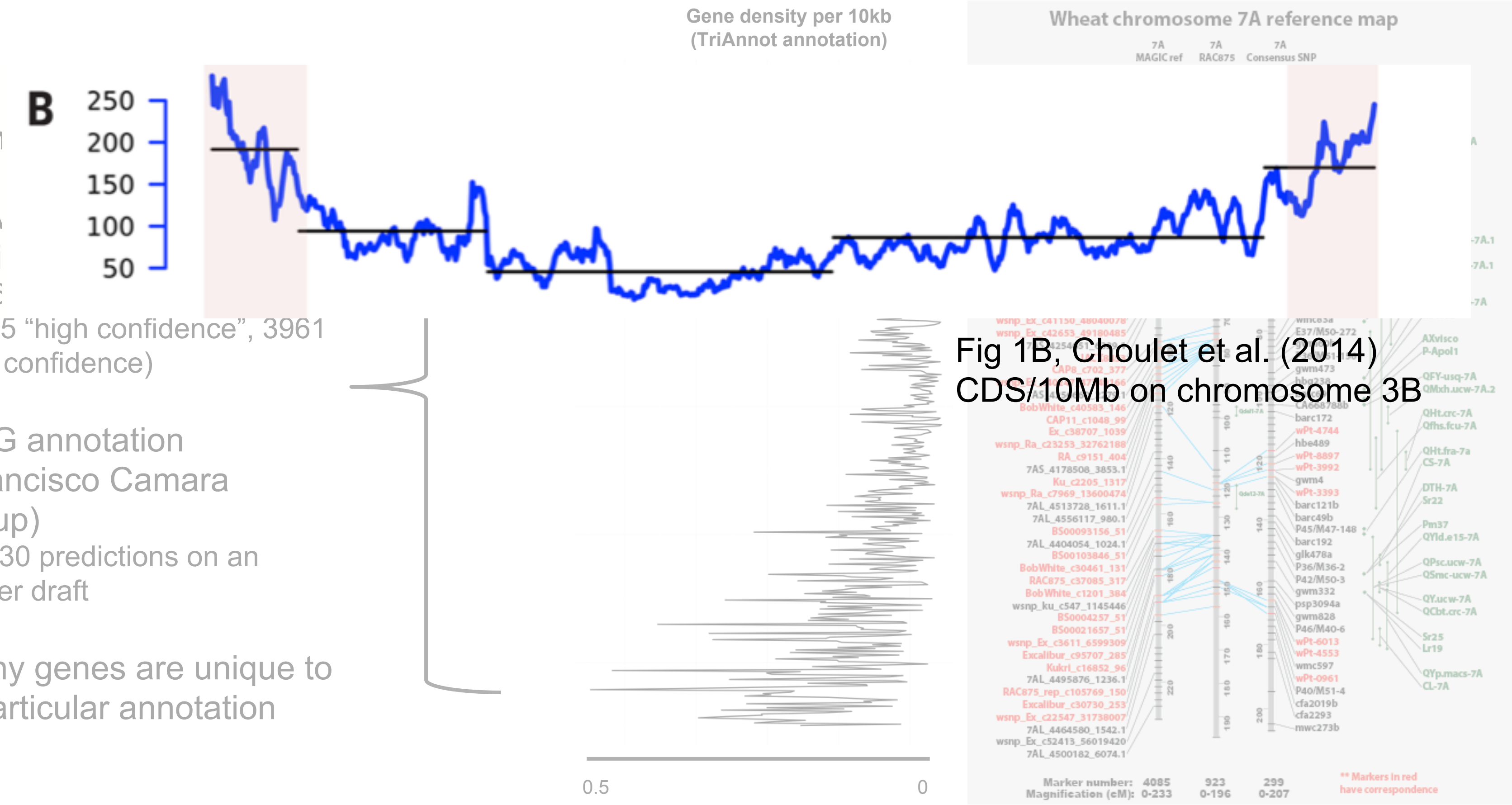

Gene density per 10kb (TriAnnot annotation)

Wheat chromosome 7A reference map

Fig 1B, Choulet et al. (2014)
CDS/10Mb on chromosome 3B

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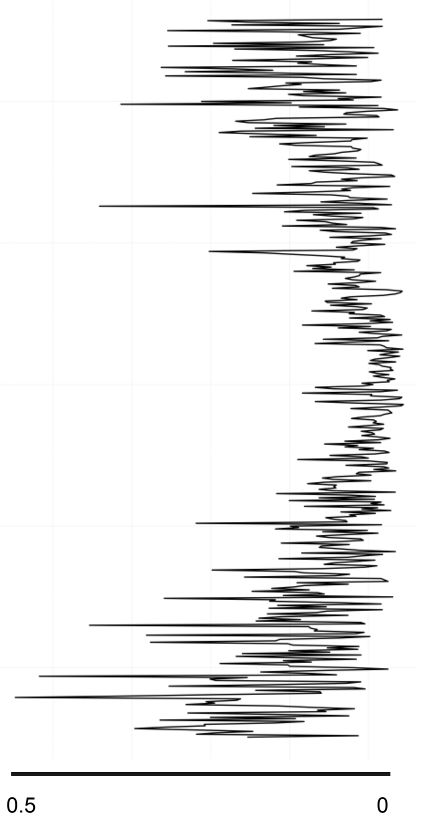

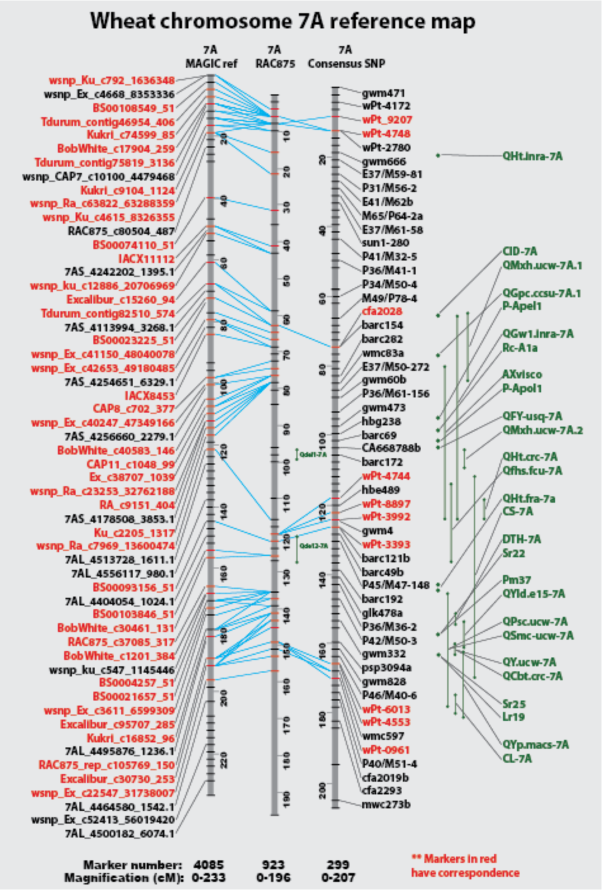

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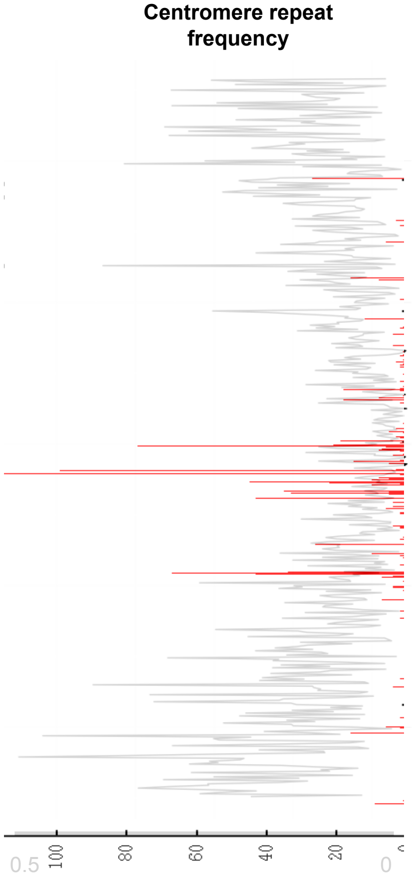
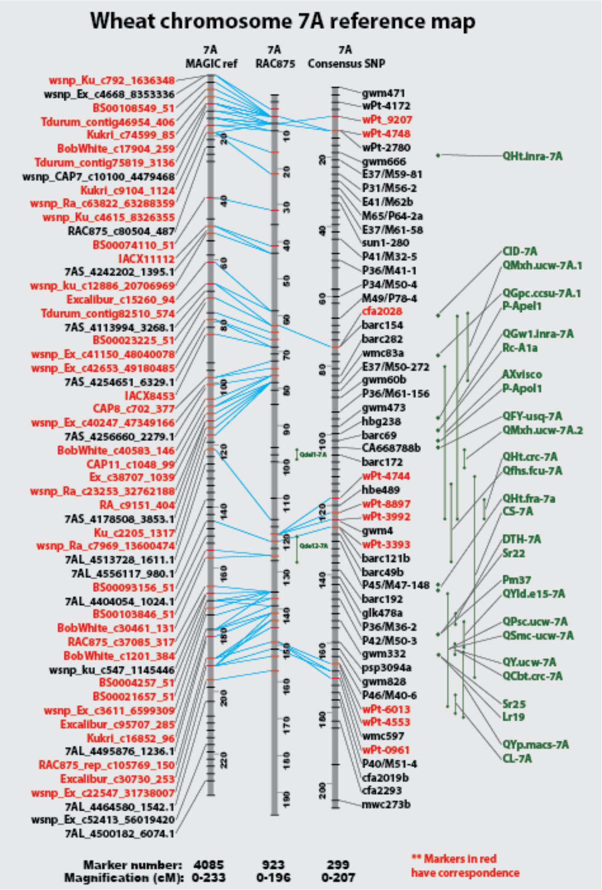

Centromere repeat frequency


Wheat chromosome 7A reference map

Pseudomolecule genes of interest

avenin

granule bound starch synthase 1

cluster of transcription factors

starch synthase 1

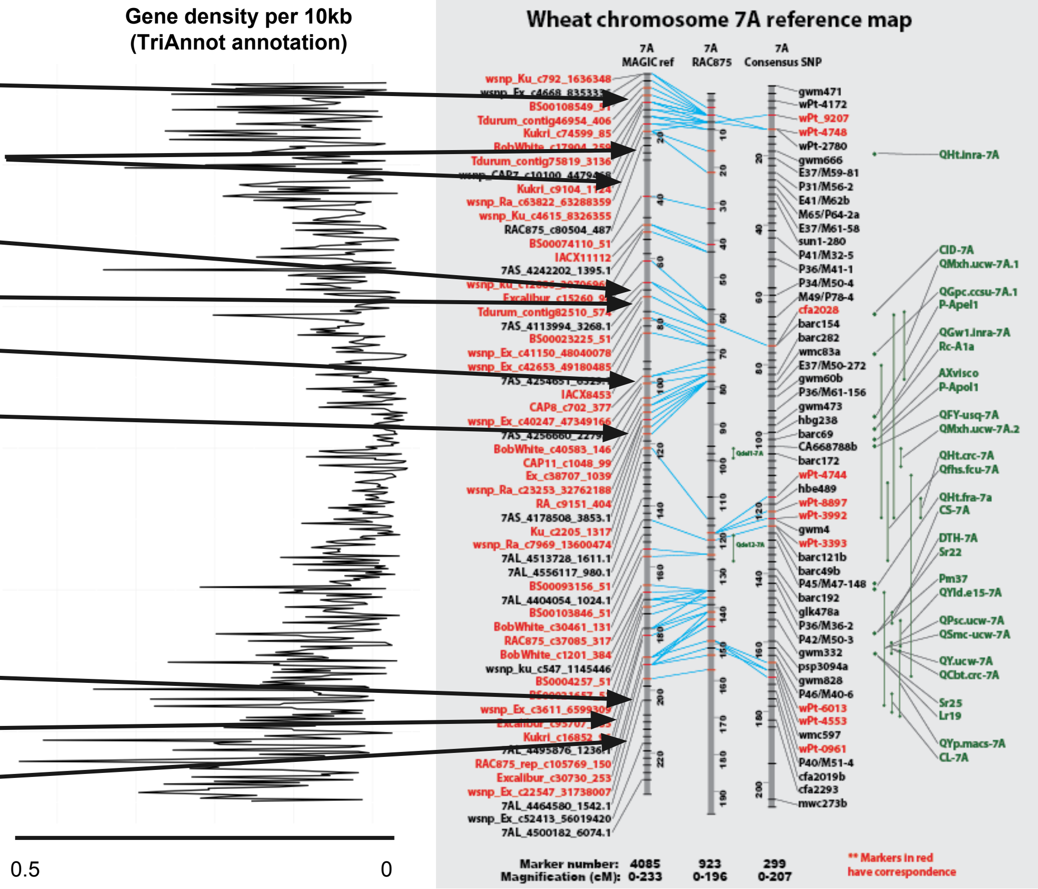
yield QTL 1

yield QTL 2

puroindolin

powdery mildew resistance

starch branching enzyme 1 x 3



Genetic map

- A composite map using the MAGIC 8-way cross population (Emma Huang, Colin Cavanagh, CSIRO and GBS by Matt Hayden, DEPI) with the Chinese Spring/Renan map (INRA) as an “anchor”. Generated with the following procedure:

1. We choose to “trust” the physical map - hence (ideally) we want all markers in a given physical contig to co-locate in the map

* Based on work done at CSIRO with Jen Taylor, Emma Huang, Penghao Wang, Stuart Stephen

Genetic map

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2. For each physical contig three situations to deal with

A) all markers are already tightly linked (which is what we want)

B) one marker is an outlier -> remove to end up in case A

C) multiple groups of tightly linked markers -> separate into “A” and “B” contigs to end up in case A

Genetic map

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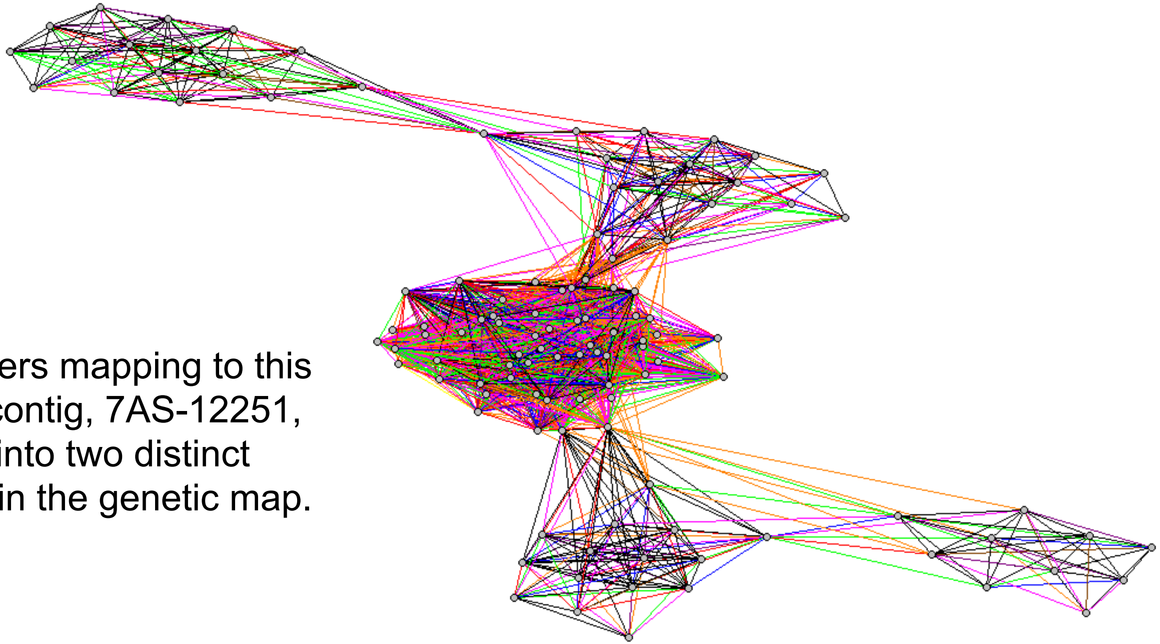
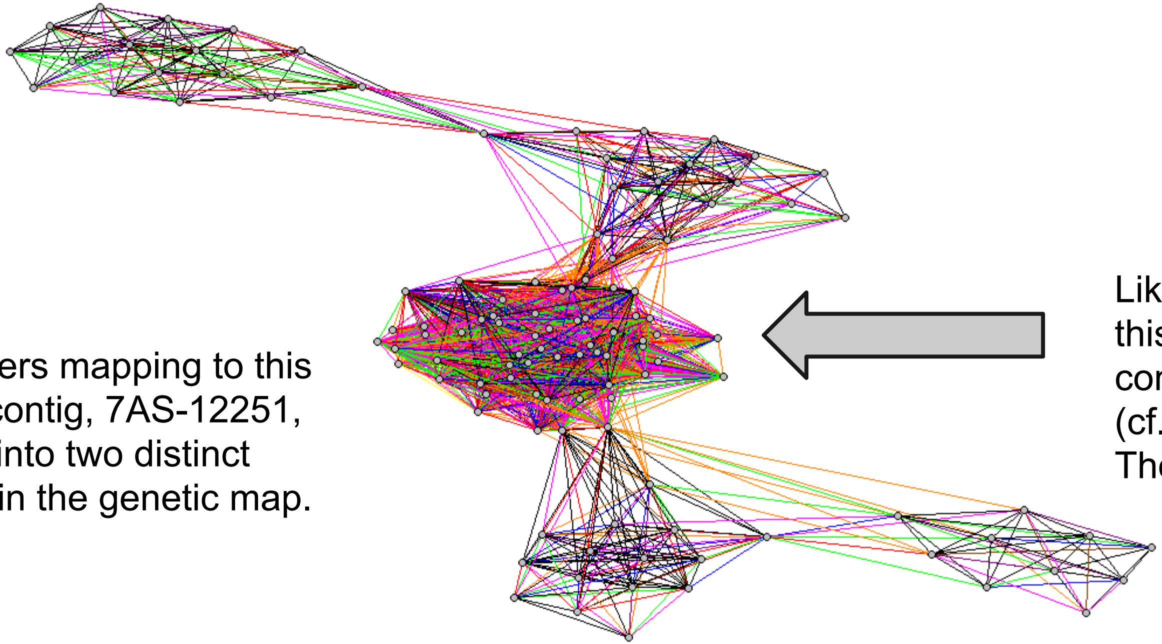
3. Take representative from each group, essentially collapsing contigs
4. Using this data, build clusters around framework markers in CS x Renan
5. Order markers within clusters
6. Estimate positions from full marker order
7. Expand out contigs - forces all markers within a contig to be at same position

Example of a split contig



The markers mapping to this physical contig, 7AS-12251, separate into two distinct locations in the genetic map.

Example of a split contig

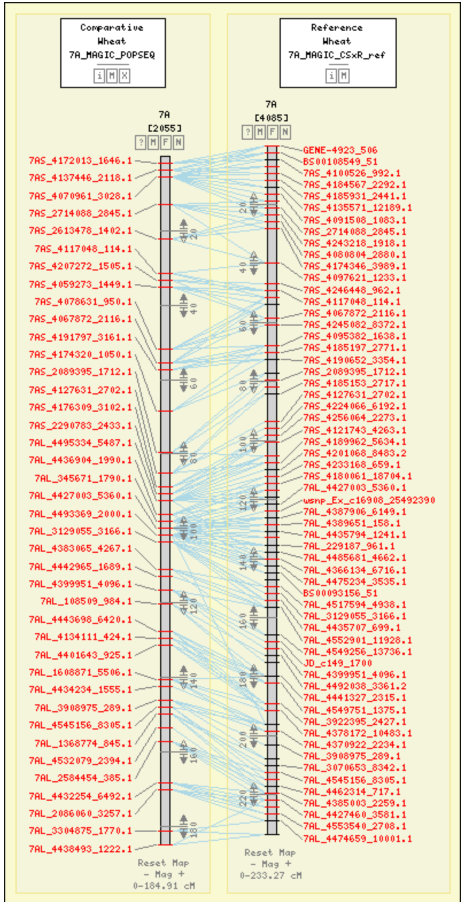


The markers mapping to this physical contig, 7AS-12251, separate into two distinct locations in the genetic map.

Likely caused by this repeat complex ("blob") (cf. talk by by Thomas Wicker)

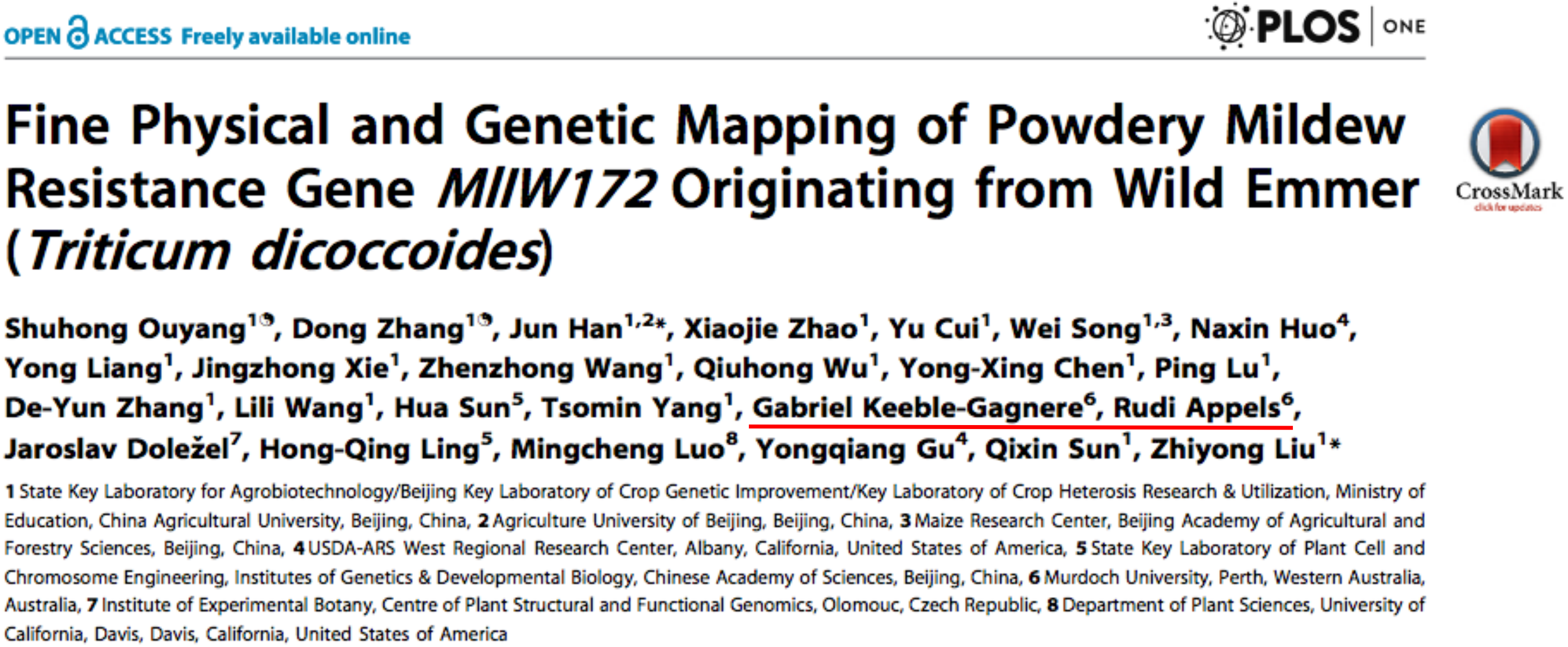
Validating genetic map

7A POPSEQ v1 map (Mascher et al. 2013) shows good alignment



MAGIC/CSxR reference map shows high resolution, with increased detail around centromere

Powdery mildew locus on 7AL



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Fine Physical and Genetic Mapping of Powdery Mildew Resistance Gene *MlIW172* Originating from Wild Emmer (*Triticum dicoccoides*)

Shuhong Ouyang[1☯], Dong Zhang[1☯], Jun Han[1,2*], Xiaojie Zhao[1], Yu Cui[1], Wei Song[1,3], Naxin Huo[4], Yong Liang[1], Jingzhong Xie[1], Zhenzhong Wang[1], Qiuhong Wu[1], Yong-Xing Chen[1], Ping Lu[1], De-Yun Zhang[1], Lili Wang[1], Hua Sun[5], Tsomin Yang[1], Gabriel Keeble-Gagnere[6], Rudi Appels[6], Jaroslav Doležel[7], Hong-Qing Ling[5], Mingcheng Luo[8], Yongqiang Gu[4], Qixin Sun[1], Zhiyong Liu[1*]

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Powdery mildew locus on 7AL

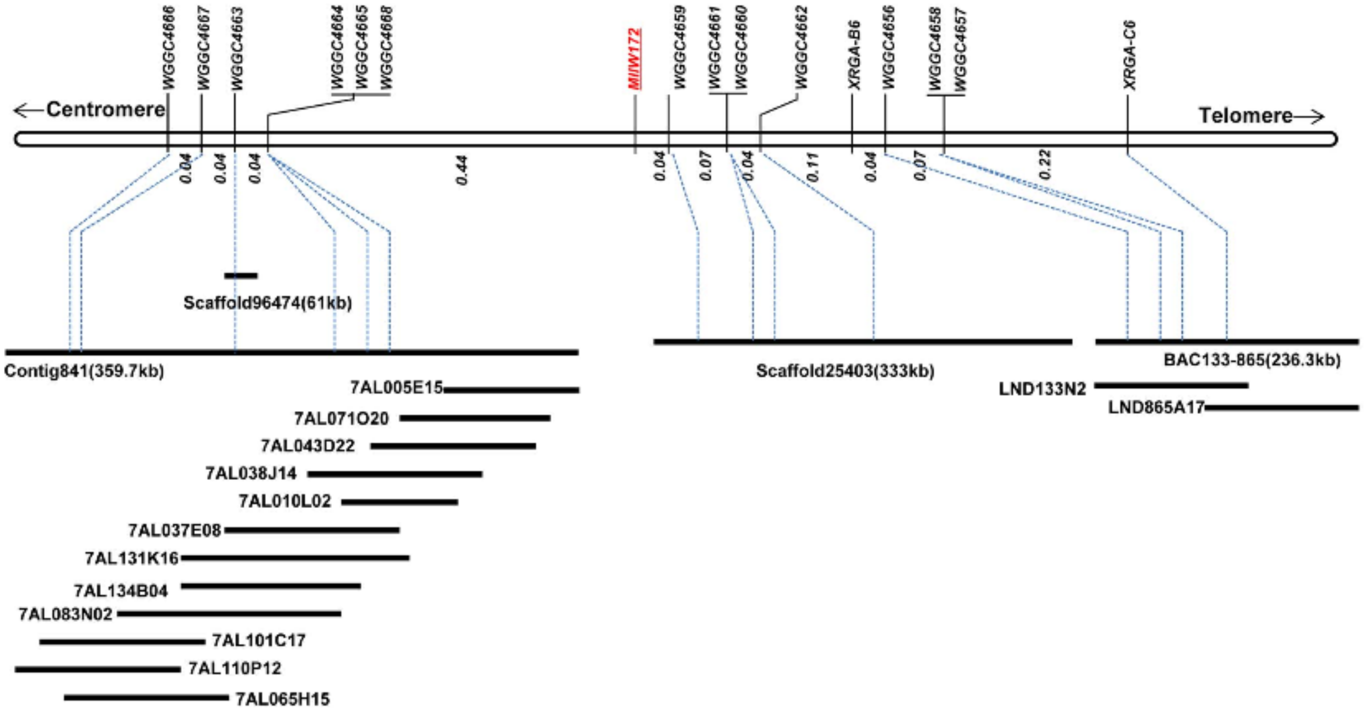

Figure 2. Physical map of the BAC contigs and scaffolds flanking the *MlIW172* locus anchored to the high-resolution genetic map.
The approximate physical locations of all the newly designed markers are given on the BAC contigs or scaffolds.
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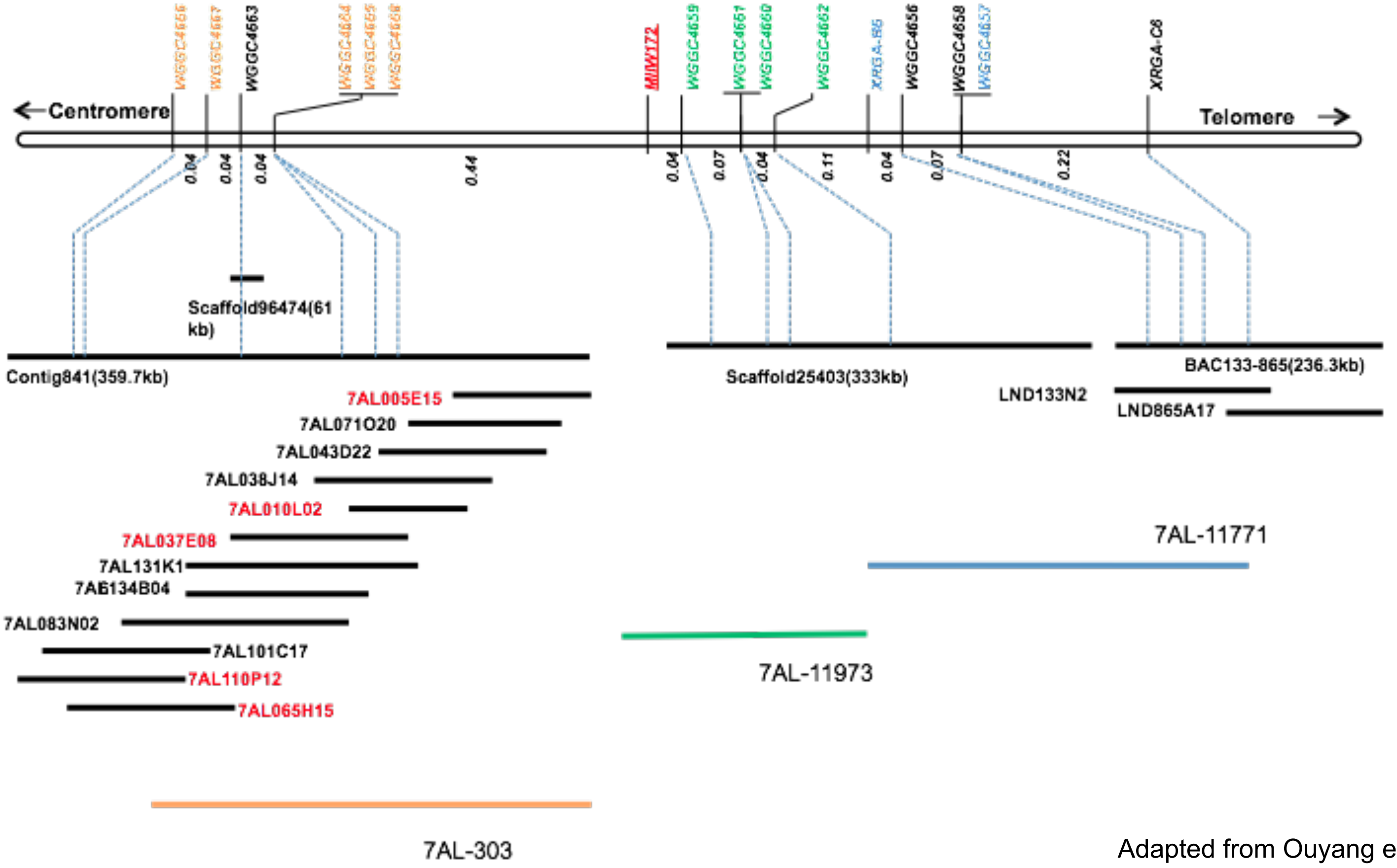
Ouyang et al. 2014

Powdery mildew locus on 7AL



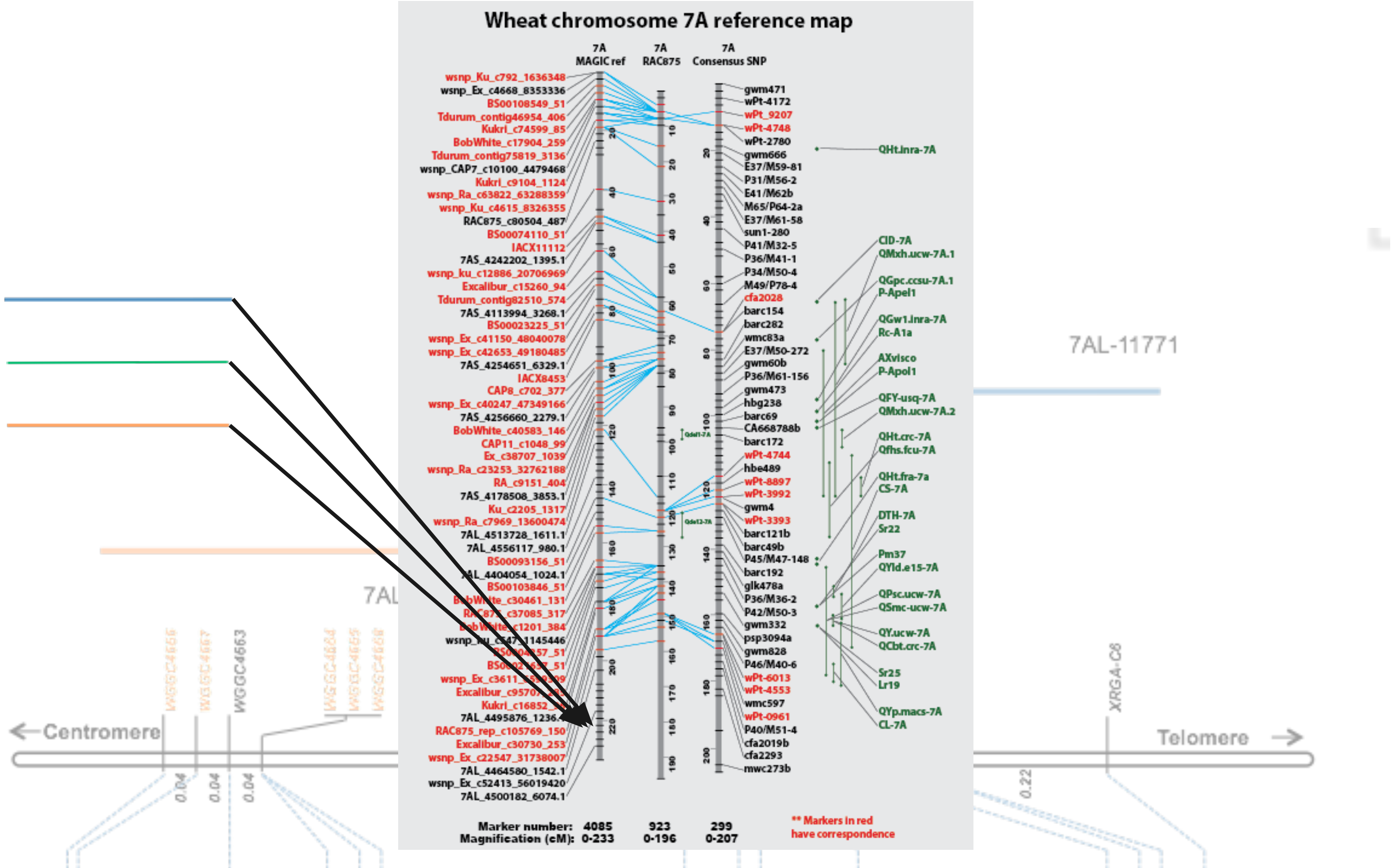
Adapted from Ouyang et al. 2014

Powdery mildew locus on 7AL

7AL-11771
227.4 cM

7AL-11973
227.5 cM

7AL-303
228.1 cM



Powdery mildew locus on 7AL

This provides important validation of our map by a completely independent source

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227.4 cM

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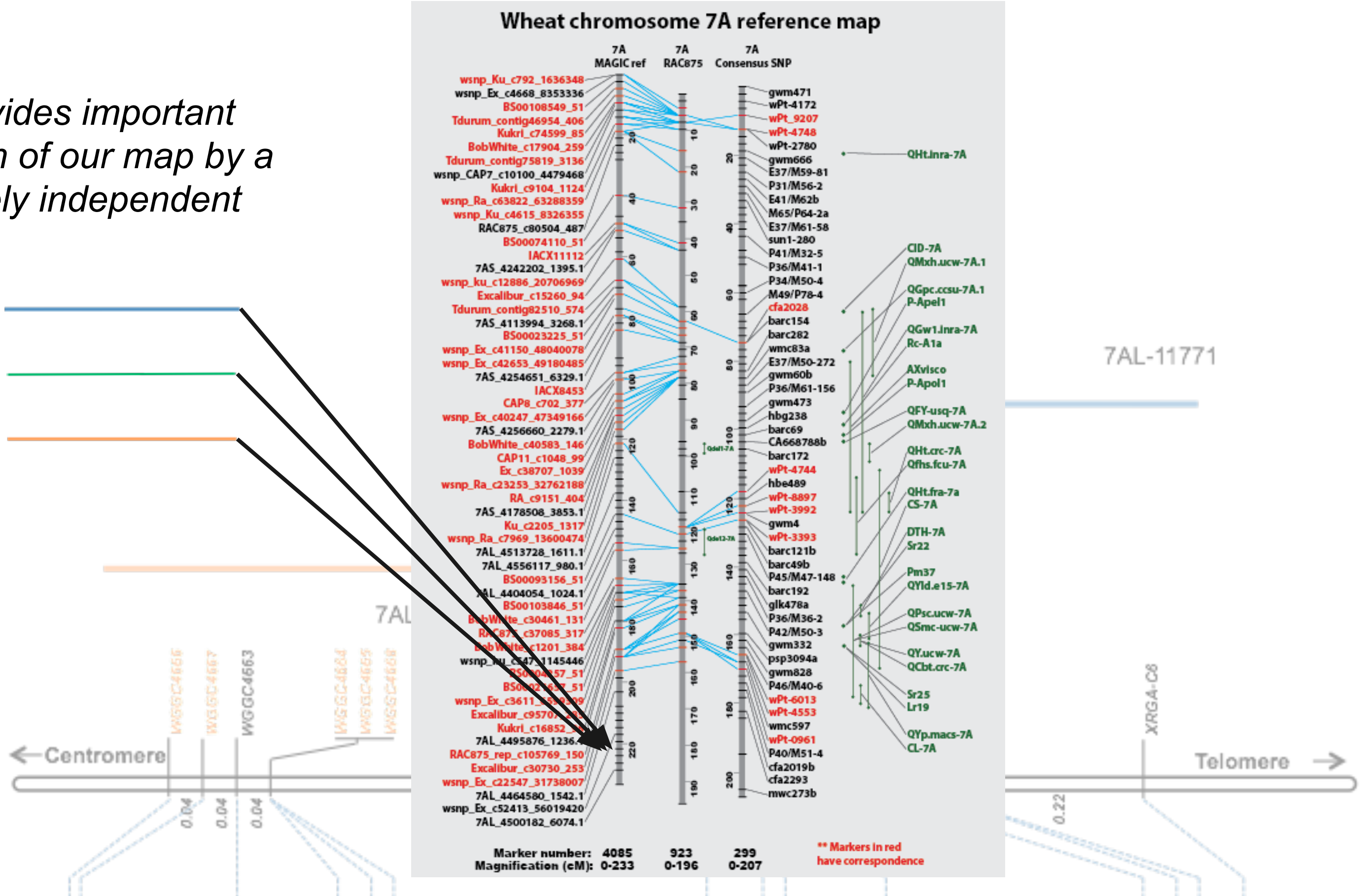
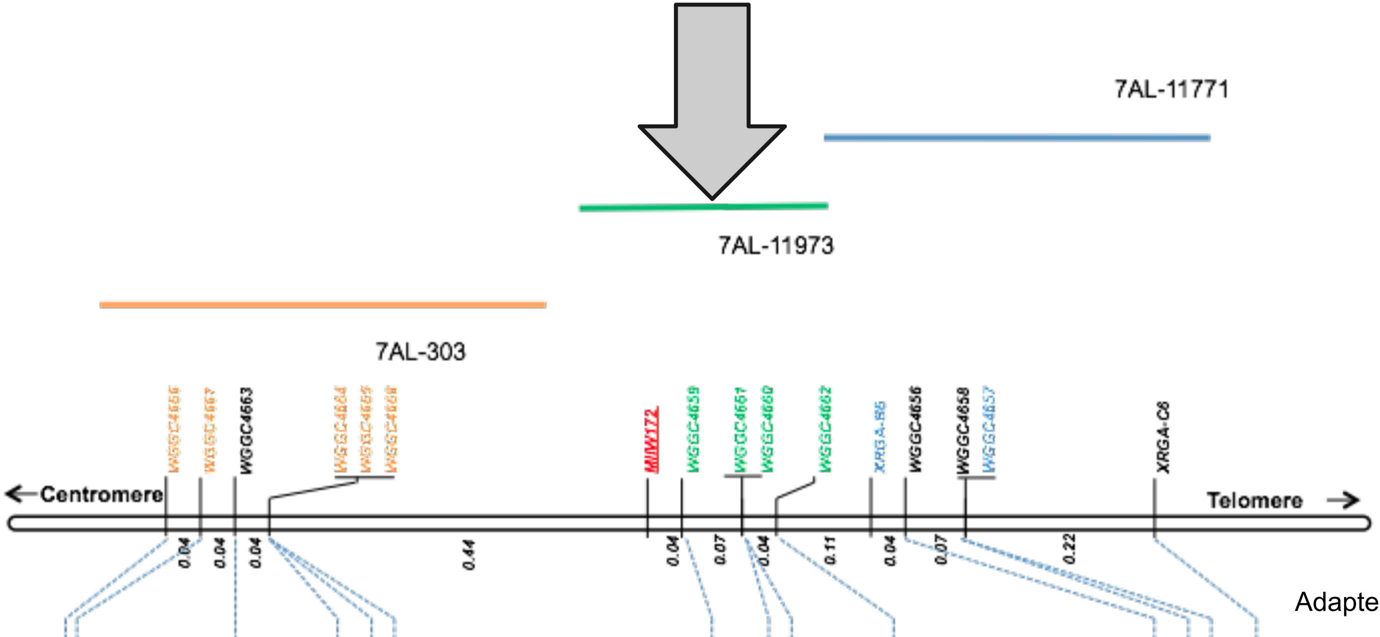


Powdery mildew locus on 7AL

Two genes stand out as candidate genes for powdery mildew resistance:

Disease resistance protein RPP8
Putative disease resistance protein RGA4



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Powdery mildew locus on 7AL

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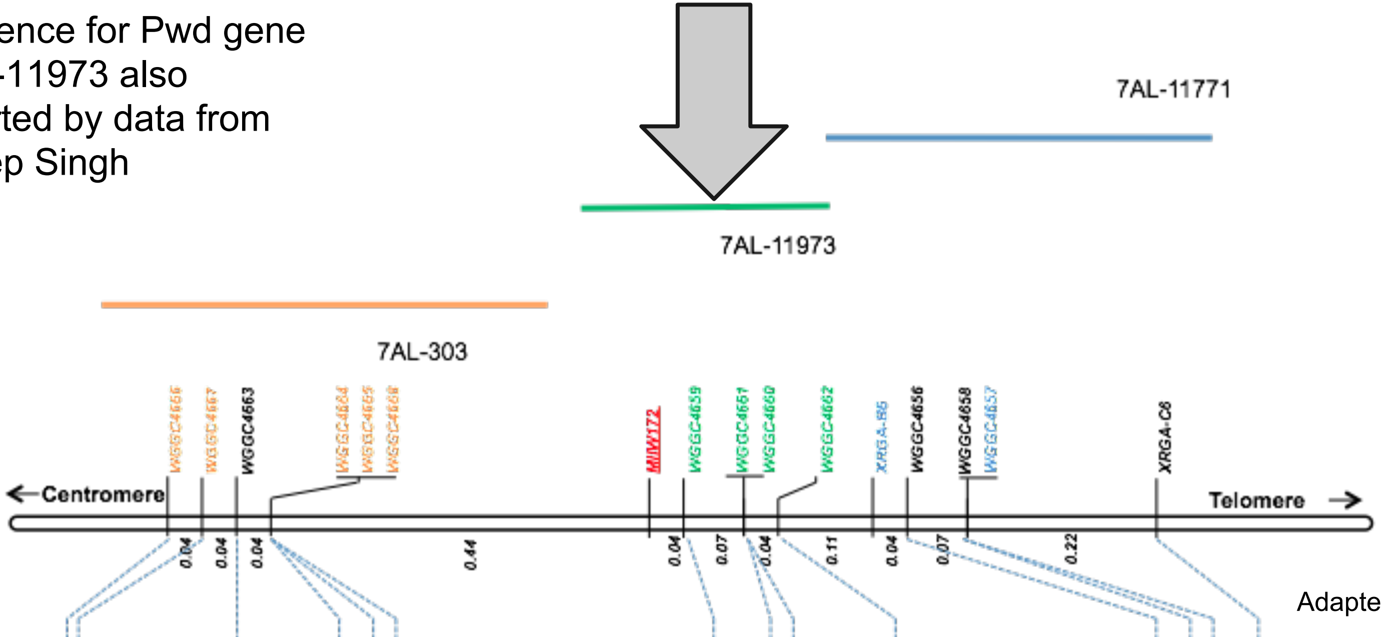
* Evidence for Pwd gene in 7AL-11973 also supported by data from Kuldeep Singh

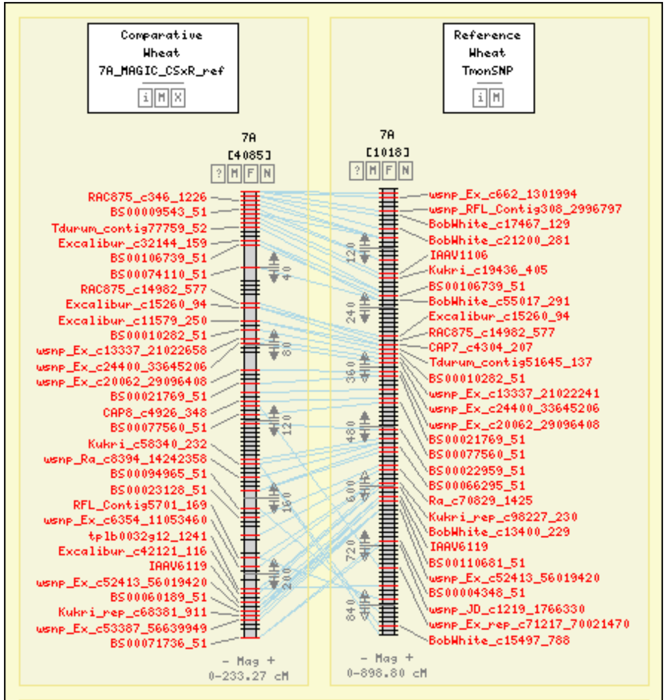


Adapted from Ouyang et al. 2014

Next steps

- Bionano optical mapping data is being generated (Hana Simkova/Jaroslav Dolezel, Mingcheng Luo) from flow-sorted DNA (Dolezel lab)
- Annotation - manual effort
- Diversity analysis and comparison to *T. urartu/T. monococcum* assembly

7A map vs. T. monococcum 90k SNP map (DNA from Jorge Dubcovsky, SNP map by Kerrie Forrest and Matt Hayden)



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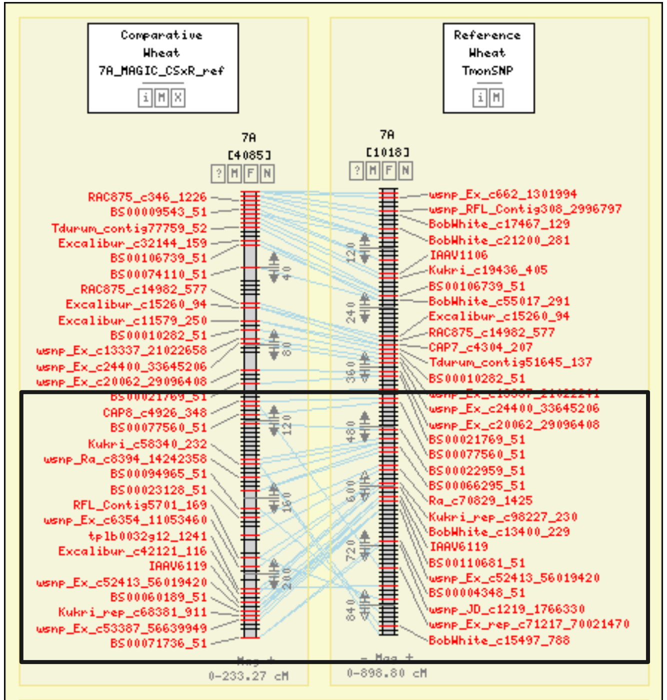

7A map vs. T. monococcum 90k SNP map (DNA from Jorge Dubcovsky, SNP map by Kerrie Forrest and Matt Hayden)

Large inversion?

Summary of achievements

1. We have produced a high quality, genetically anchored, assembly of chromosome 7A

2. The assembly has been validated using independent genome-level information for specific regions of the chromosome

3. The assembly now forms the basis for the analysis of agronomically significant chromosome regions

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Flow-sorting DNA/BAC library construction
Jaroslav Dolezel, Hana Simkova
Institute of Experimental Botany
Czech Republic

Fingerprinting BAC library
Mingcheng Luo group
UC Davis

Physical map assembly
Zeev Frenkel, Ambraham Korol
Haifa University

Genetic maps
MAGIC: Colin Cavanagh, Emma Huang, Jen Taylor (CSIRO)
MAGIC GBS: Matt Hayden (DEPI)
CSxRenan: Pierre Sourdille, Benoît Darrier (INRA)

***T. monoccocum* genetic map**
Population: Jorge Dubcovsky
90k chip: Matt Hayden, Kerrie Forrest

DNA sequencing
Matt Tinning
AGRF

Annotation
TriAnnot: Philippe Leroy, Aurelien Bernard (INRA)
geneID (CRG): Francisco Camara, Anna Vlasova (CRG, Spain), Juan Carlos Sanchez (ACPFG)
Storage proteins: Angela Juhasz (Hungary)
QTL mapping/Significant genome regions: Delphine Fleury (ACPFG)
Specific genes: Hui-xian Zhao (NW A&F Uni, China)

Pseudomolecule
Fred Choulet, Etienne Paux
INRA

7A mate-pair sequencing of amplified DNA
Matt Hayden, Josquin Tibbits, Sami Hakim
DEPI

Whole-genome mate-pair data
Andy Sharpe, David Konkin, Curtis Pozniak
NRC, Canada

Bionano map
Jaroslav Dolezel, Hana Simkova, Mingcheng Luo

Supercomputing resources
iVEC/Pawsey Supercomputing Centre