中澳联合研究中心
AUSTRALIA - CHINA JOINT RESEARCH CENTRE

Murdoch
UNIVERSITY
DISCOVERERS WELCOME

Physical map/BAC sequencing of chromosome 7A

Location: Murdoch University, Western Australia

Leadership:
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Research outputs:
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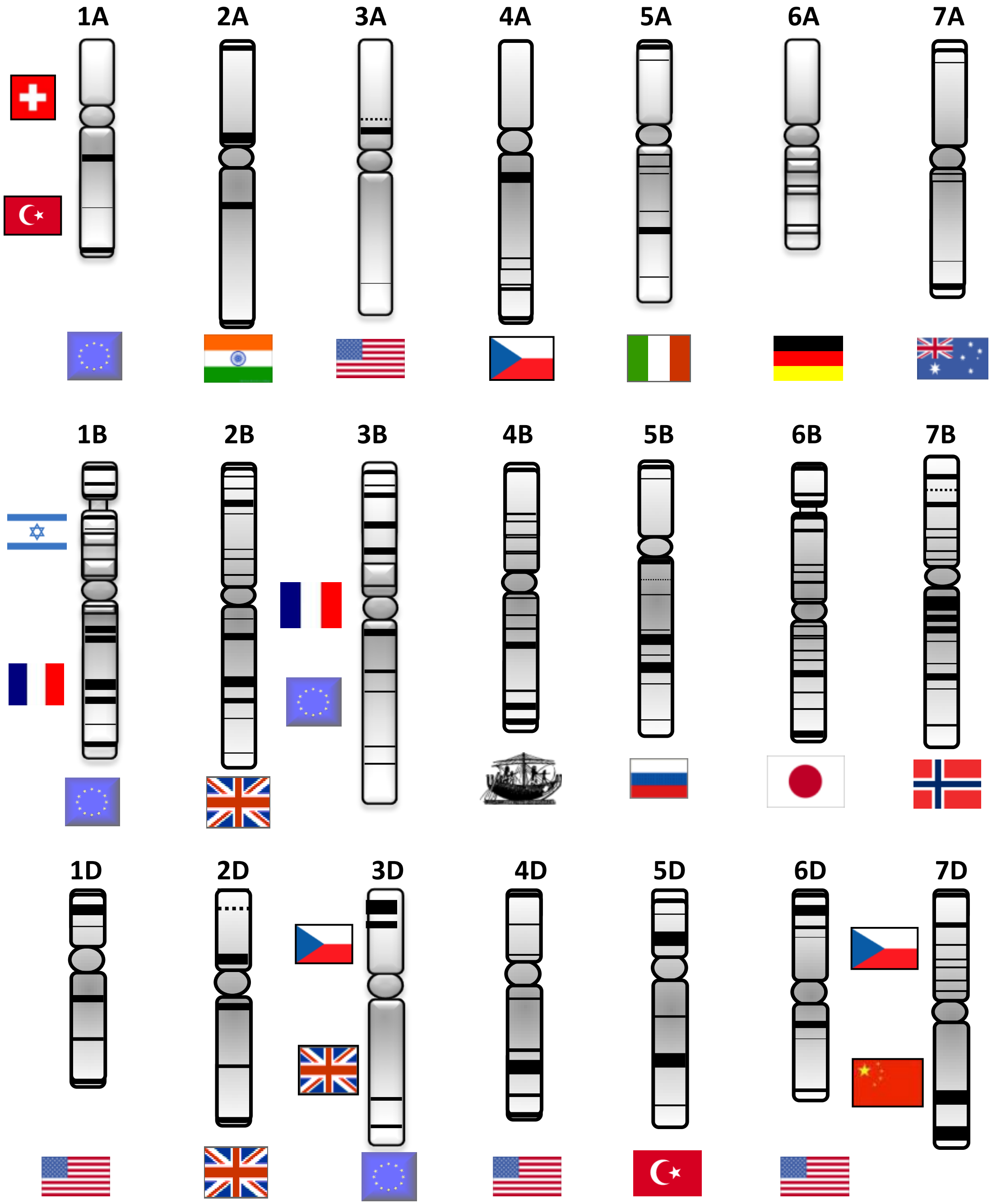
- **Delphine Fleury (ACPFG, Adelaide, South Australia))**
- **Australian Genome Research Facility (AGRF, Melbourne)**

Funding:
- **Grains Research Development Corporation (GRDC)**
- **BioPlatforms Australia**
- **Australian China Centre for Wheat Improvement**

Physical mapping of the bread wheat genome



T. aestivum

cv Chinese Spring

Wheat chromosome 7A

- 7AS and 7AL both around 400Mb in length
  - Both arms longer than rice…
- Has a number of regions of agronomic interest to Australia
  - Yield genes (from genetic studies, one on each of 7AS and 7AL)
  - Quality regions (starch quality-related genes on 7AS)
  - Avenin-like gene
  - Grain size (collaboration with NW-A&F University, Yangling, China)

Australia’s contribution to the International Wheat Genome Sequencing effort is to carry out the analysis of chromosome 7A

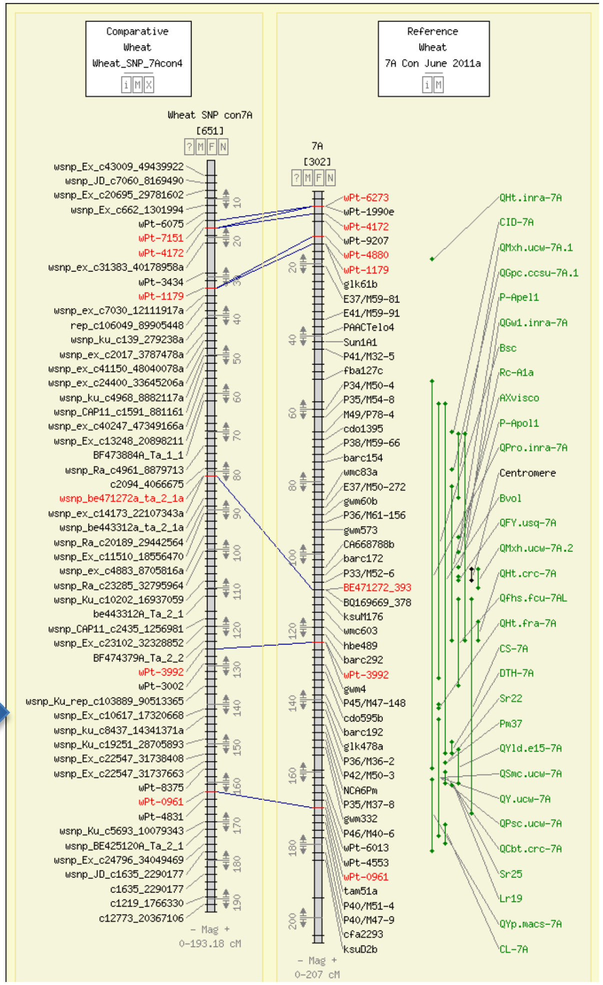
Chromosome sorting of 7AS and 7AL

BAC libraries

BAC compiled into contigs using DNA fingerprinting

814 BAC contigs sequenced in pools of 96

Sequenced contigs assigned to molecular genetic maps



Defining the BACs for sequencing

- **MTP defined the shortest path through the physical contigs and provide the list of BACs for sequencing**

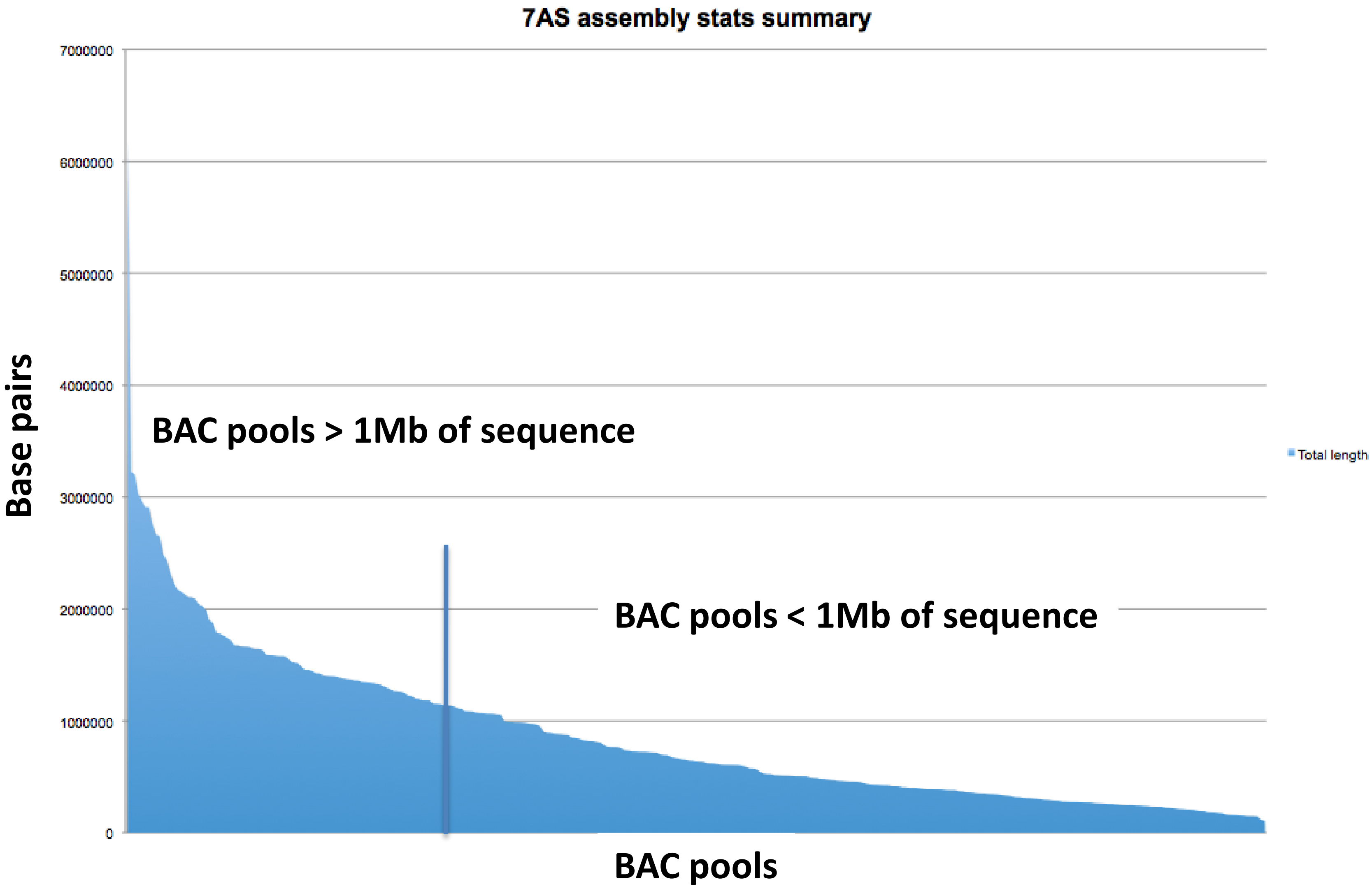
- **7AS**
  - **408 physical contigs in MTP**
  - **=> 5,280 BACs in 408 BAC pools**

- **7AL**
  - **406 physical contigs in MTP**
  - **=> 5,834 BACs in 406 BAC pools**

DNA sequencing strategy

- Sequencing carried out by AGRF
- HiSeq 2000/2500
- Reads were 150bp in length, ~350bp insert library
- BAC pools were bar-coded to allow the multiplexing of 96 to per lane
  - 9 full lanes of data for 800 BAC pools
- To date 7AS complete, now into 7AL sequencing
- Part of the sequencing strategy is to determine all the BAC end sequences using attached vector sequences as a marker

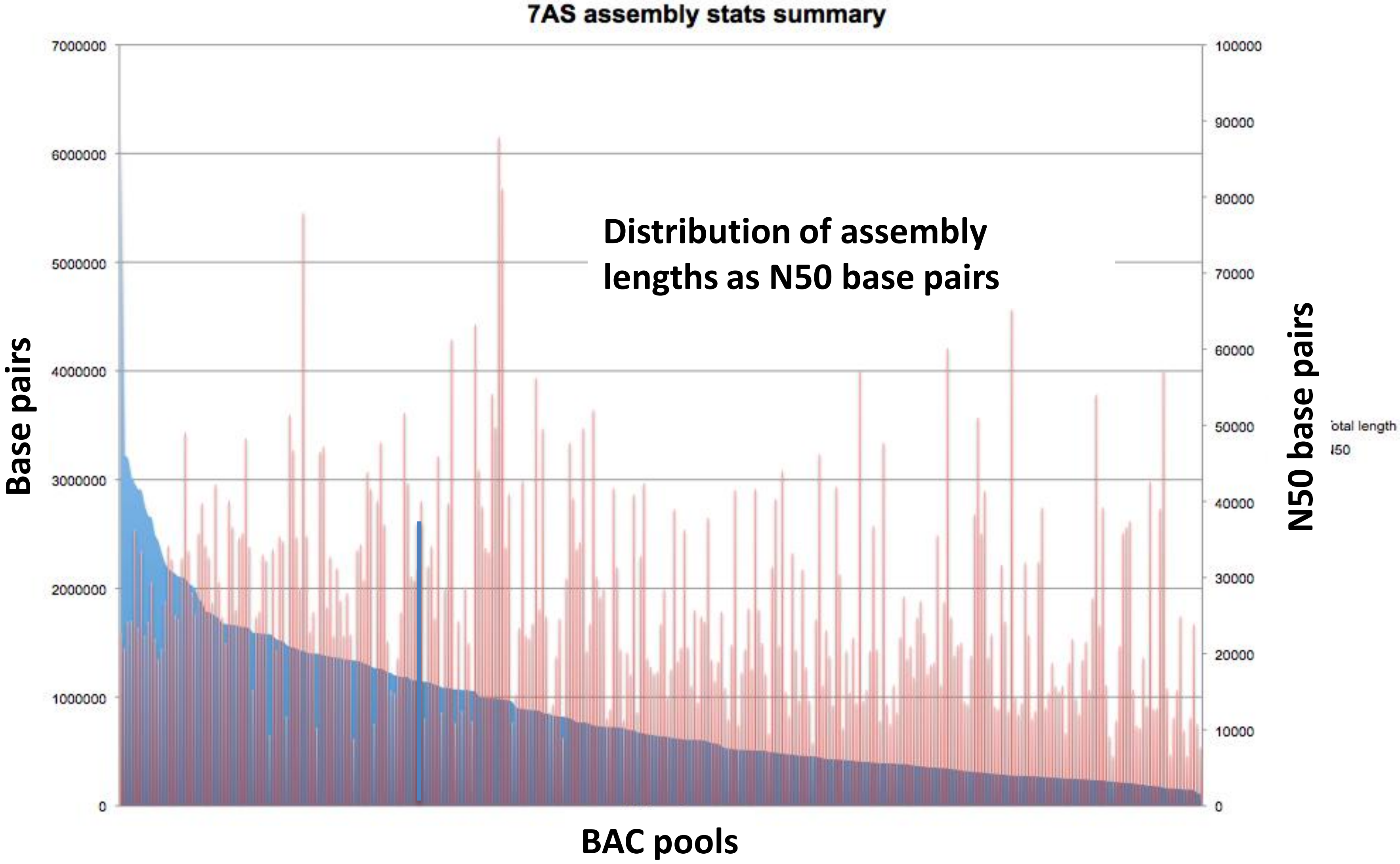
Results summary



BAC pools > 1Mb of sequence

BAC pools < 1Mb of sequence

Results summary

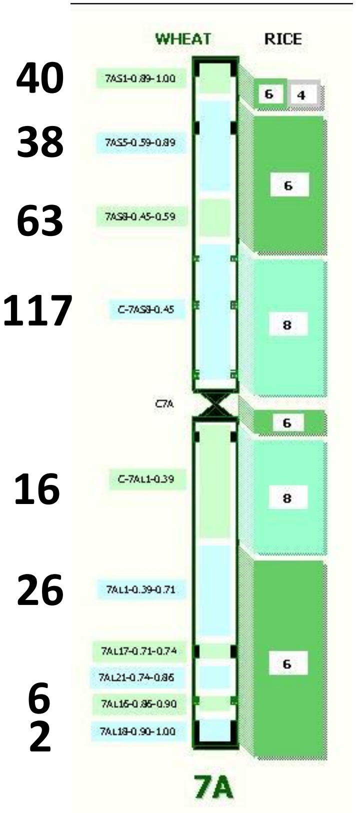


Distribution of assembly lengths as N50 base pairs

Summary for current status of sequencing:

1. 323 contigs/pools sequenced to date using Illumina (150 bp reads)

2. 308 contigs have so far been assigned to the standard deletion bins available for chromosome 7A. This tracks the sequencing work since we started on 7AS contigs/pools

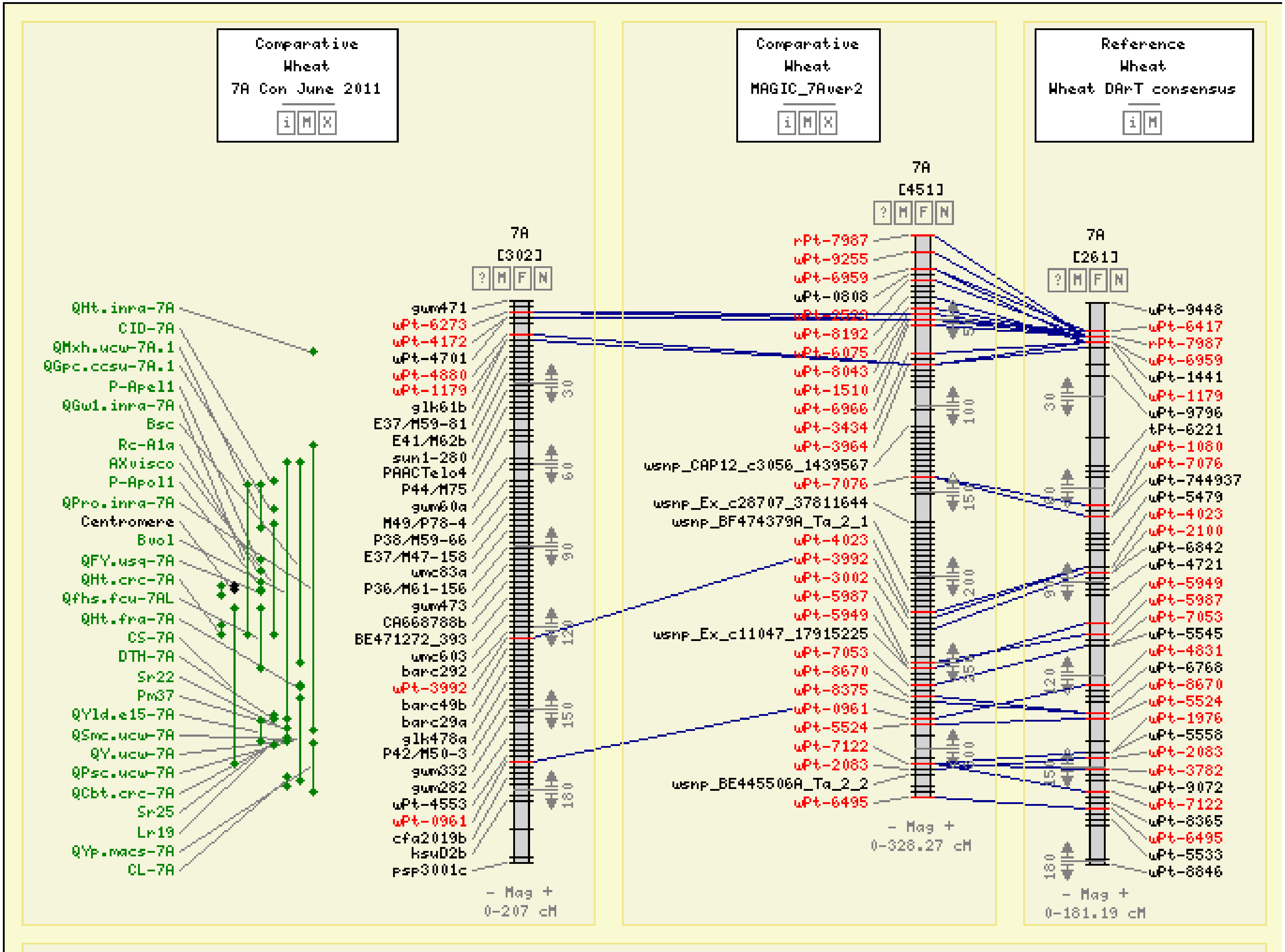


3. One third of contigs/pools are 1 – 2.3 Mb, one third 0.5 – 1 Mb and one third 0.1 – 0.5 Mb

4. One contig/pool of 1.6 Mb, on 7AS, has a major locus for genes coding for the supply of sugars to the developing head and grain (Huynh et al 2012). Another contig/pool has a candidate gene for grain size. Contigs/pools like this are ear-marked for more extensive sequencing using an 8Kb library and pair-end sequencing

5. Extensive RNAseq database from the developing spike is helping to refine assemblies

Molecular genetic maps for anchoring



Genes of interest

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- Sucrose/fructose transferase genes found in a single BAC pool (cl-11582):
  - Fructosyl transferase 7ASpFT
  - sucrose:sucrose 1-fructosytransferase (Ta1-SST)
  - sucrose:fructan 6-fructosyltransferase (Ta6-SFT)
  - fructan:fructan 1 fructosyl transferase (Ta1-FFT)
  - vacuolar invertase (TaWIVRV)

- Locus was identified in B-L Huynh et al (2012) Plant Mol Biol 80: 299-314