Improving Physical Map and Sequence of the Wheat 7DS Arm Through a BioNano Map

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CHALLENGES IN PLANT GENOME SEQUENCING

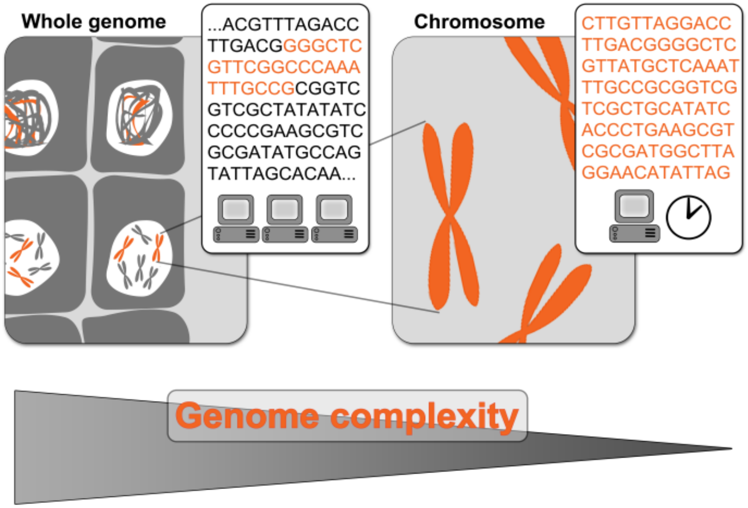
De novo genome assemblies using only short read data of NGS technologies are generally incomplete and highly fragmented due to

- Large duplications - chromosomal approach, BAC-by-BAC sequencing
- High proportion of repetitive DNA - challenge

- Large genome size (~17 Gb)
- Polyploidy (AABBDD genome)

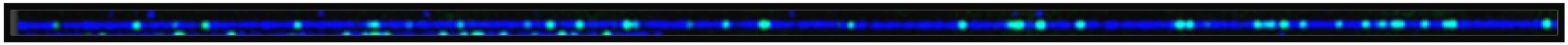
IWGSC

Chromosomal approach



SOLUTIONS FOR THE REPEATS

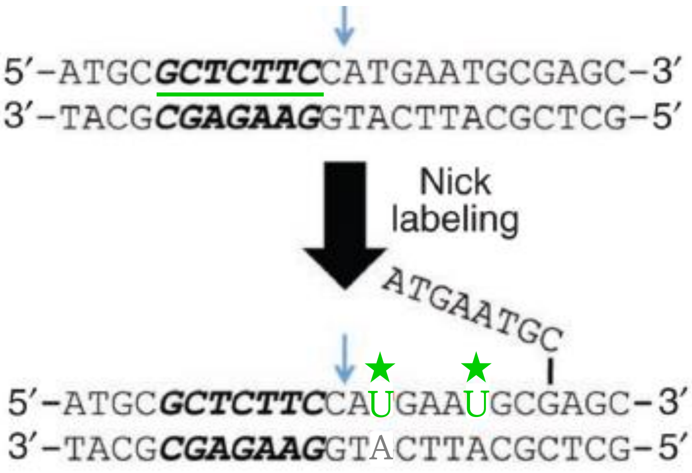
- **Long mate-pair reads**
- **Long read technologies** – PacBio, Oxford Nanopore
- **Optical mapping/genome mapping in nanochannel arrays**
    - Single-molecule mapping of genomic DNA **hundreds of kilobases to several megabases in size**
    - Creates **sequence-motif maps**, which provide long-range template for ordering genomic sequences
    - **Visualisation of reality** “Seeing is Believing”



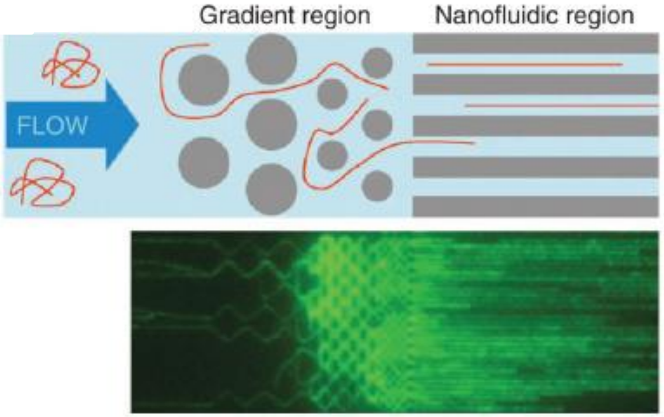
BIONANO GENOME MAPPING ON NANOCHANEL ARRAYS

1 Sequence-specific labeling

Nickase (Nt.BspQI)



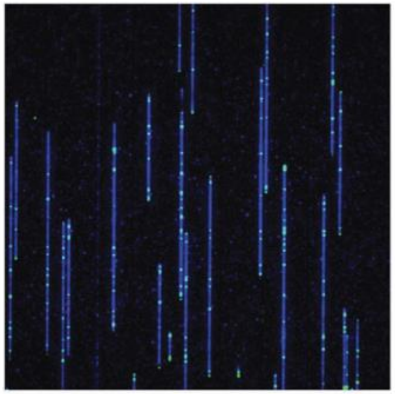
2 DNA linearization



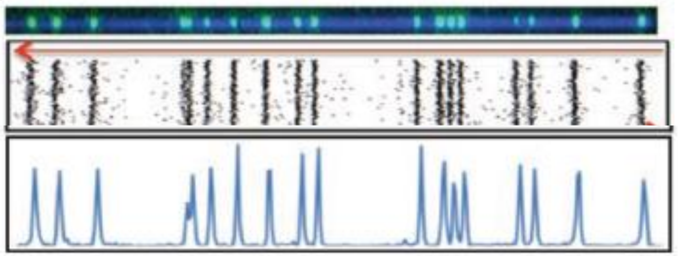
Lam et al., Nat. Biotechnol. 30(8) 2012

Irys

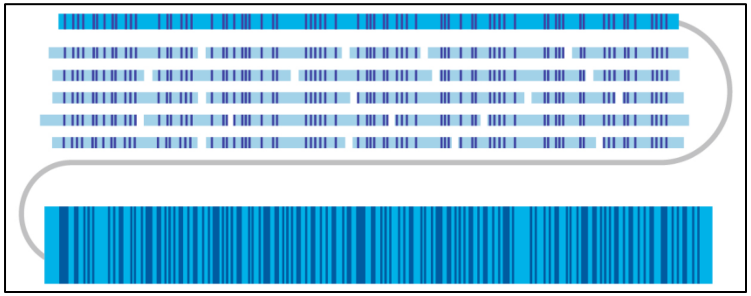
3 Fluorescence imaging



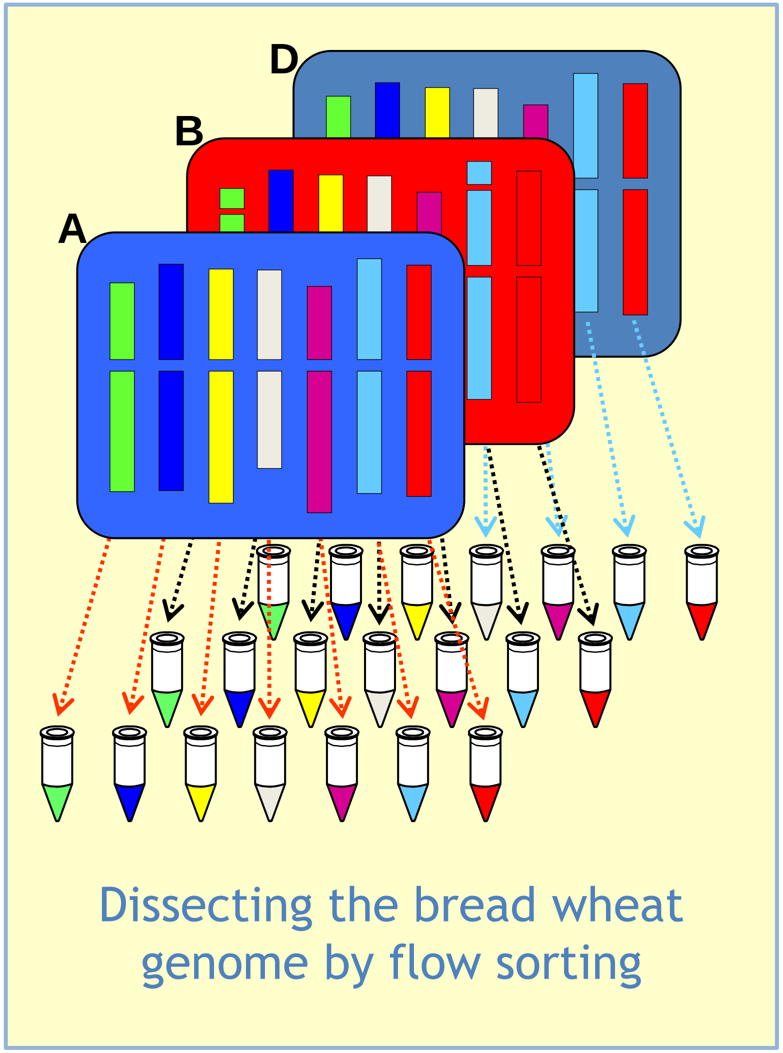
4 Map construction



5 Building consensus map

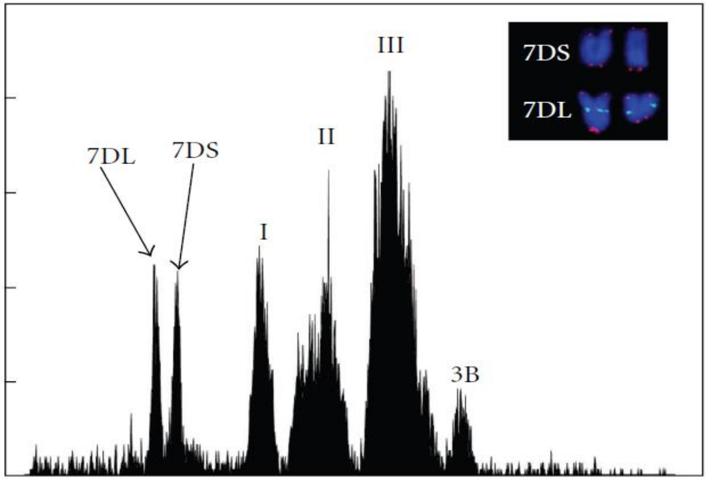


CHROMOSOME MAPPING ON NANOCHANNEL ARRAYS



Dissecting the bread wheat genome by flow sorting

- Pilot study on wheat **7DS** chromosome arm (381 Mb, 2.25% wheat genome)
  - Purified as telocentric chromosome by flow cytometric sorting

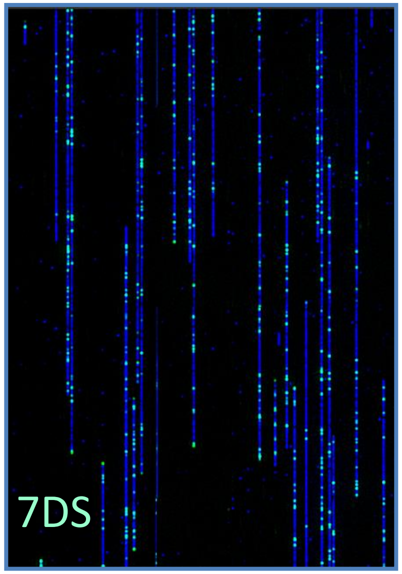


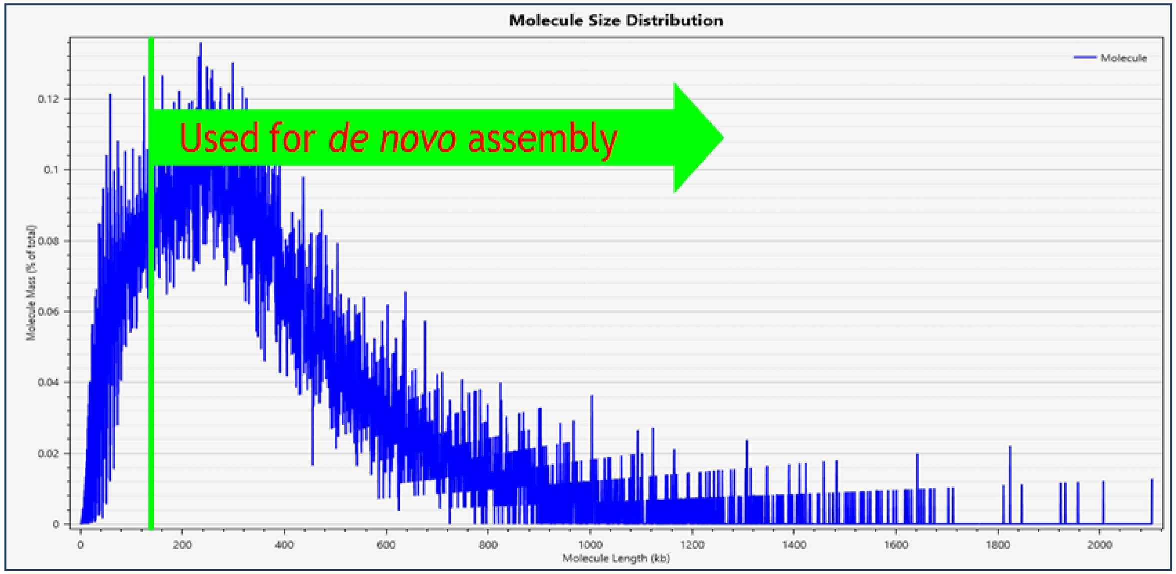
- *In silico* analysis (7DS CSS sequence) for chromosome mapping
  - Nt.BspQI ~13 sites per 100kb
  - Nb.BbvCI ~7 sites per 100kb

BIONANO MAP OF 7DS: DATA ACQUISITION

- Three miniplugs from flow-sorted 7DS chromosome arm:
  - flow sorted equivalent of 950 ng, recovered **575 ng** at 25ng/µl
- Labelling - **Nt.BspQI** (GCTCTTC motif)
- Collecting data from **one version-2 chip**

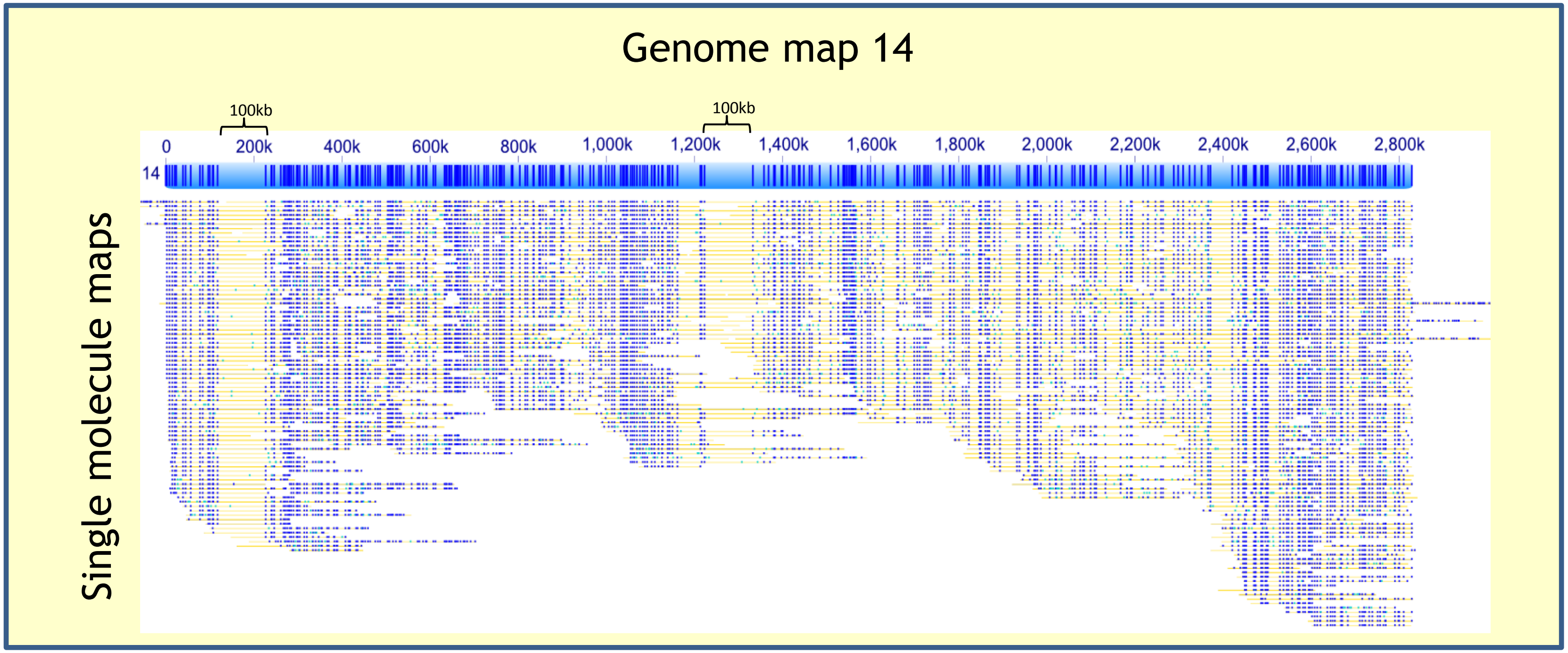
Length treshold	**Total coverage**	**n50**
150kb	200x	344kb





DE NOVO ASSEMBLY OF A 7DS MAP

- A total of **371 genome maps** were *de novo* assembled
- Total assembly length is 350 Mb (**92% of estimated 7DS size**)
- **Average map size is 0.9 Mb**
- **n50 is 1.3 Mb**


Genome map 14

7DS SEQUENCING STRATEGY

- BAC-by-BAC sequencing based on 7DS physical map, sequencing contigs ≥ 3 BAC clones
- 4608 MTP clones → 1152 pools of four non-overlapping BAC clones
- Illumina pair-end seuqencing - 550bp fragment size, 96 pools per lane of HiSeq, 100bp read length, coverage ~500x
- Assembler – Sassy (Kazakoff *et al*. 2012)
- Deconvolution through BAC end sequences, inner contigs unresolved

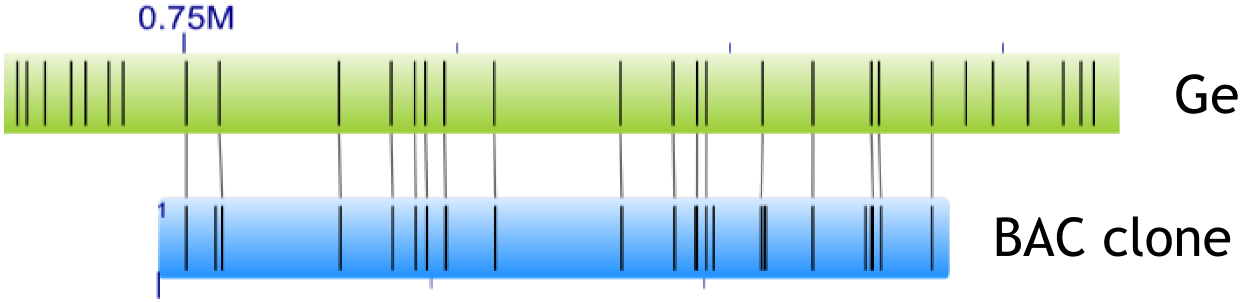
⇩

  - **1-20 contigs per BAC clone, median 3.8**
  - **average contig size 24.3 kb, N50: 65 kb**

- Assignment of inner contigs based on
  - mate-pair data obtained from MTP-plate pools (384 clones)
  - information from overlapping BAC clones (BLAST on BAC pools)
  - BioNano genome map

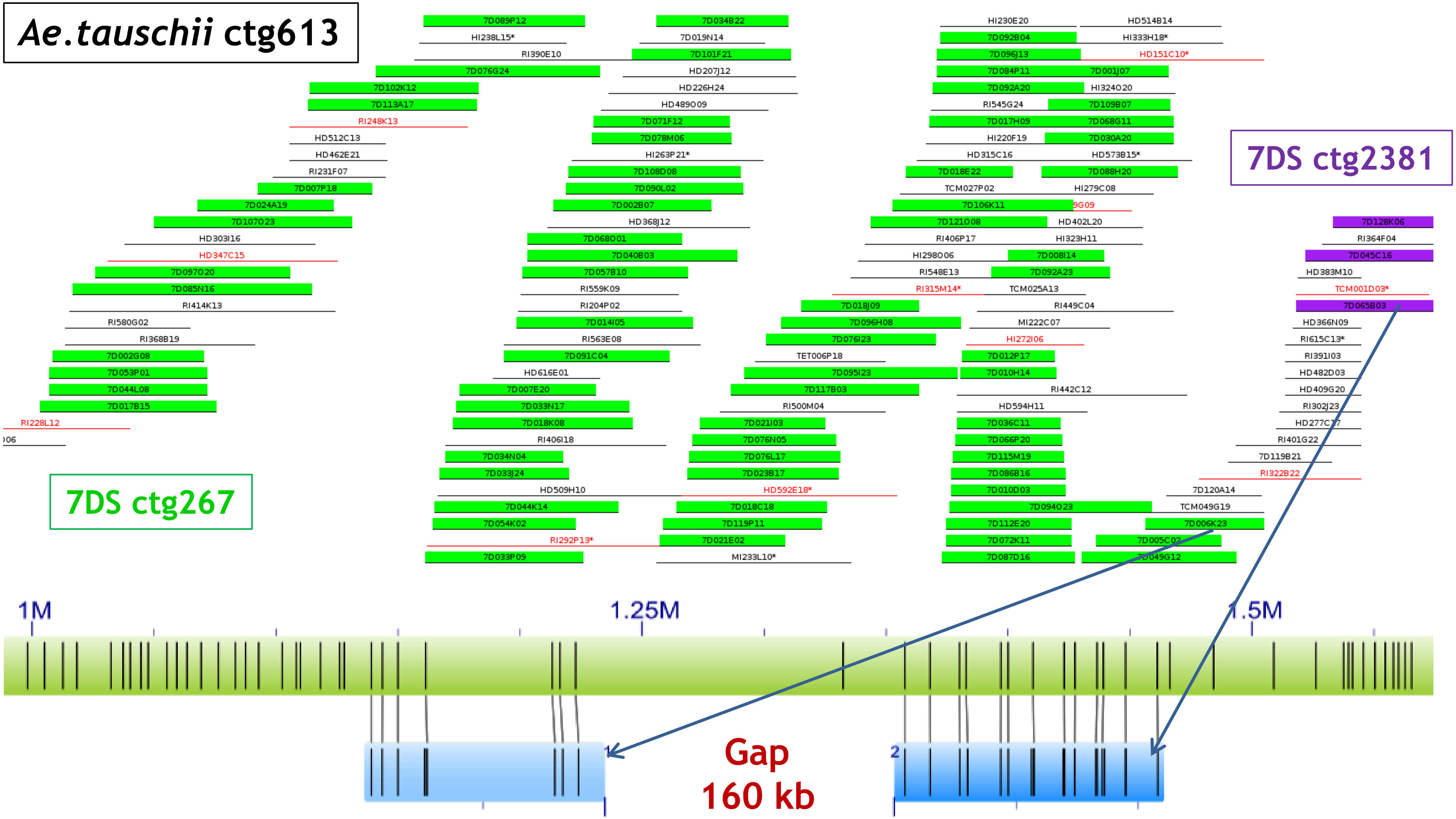
COMBINING BIONANO MAP WITH THE 7DS SEQUENCE

By aligning BAC clone sequences to the BioNano genome map through **IrysView sofware**



BIONANO MAP FOR PHYSICAL MAP IMPROVEMENT

Co-assembly of 7DS with *Ae. tauschii*



***Ae.tauschii* ctg613**

7DS ctg2381

7DS ctg267

Gap
160 kb

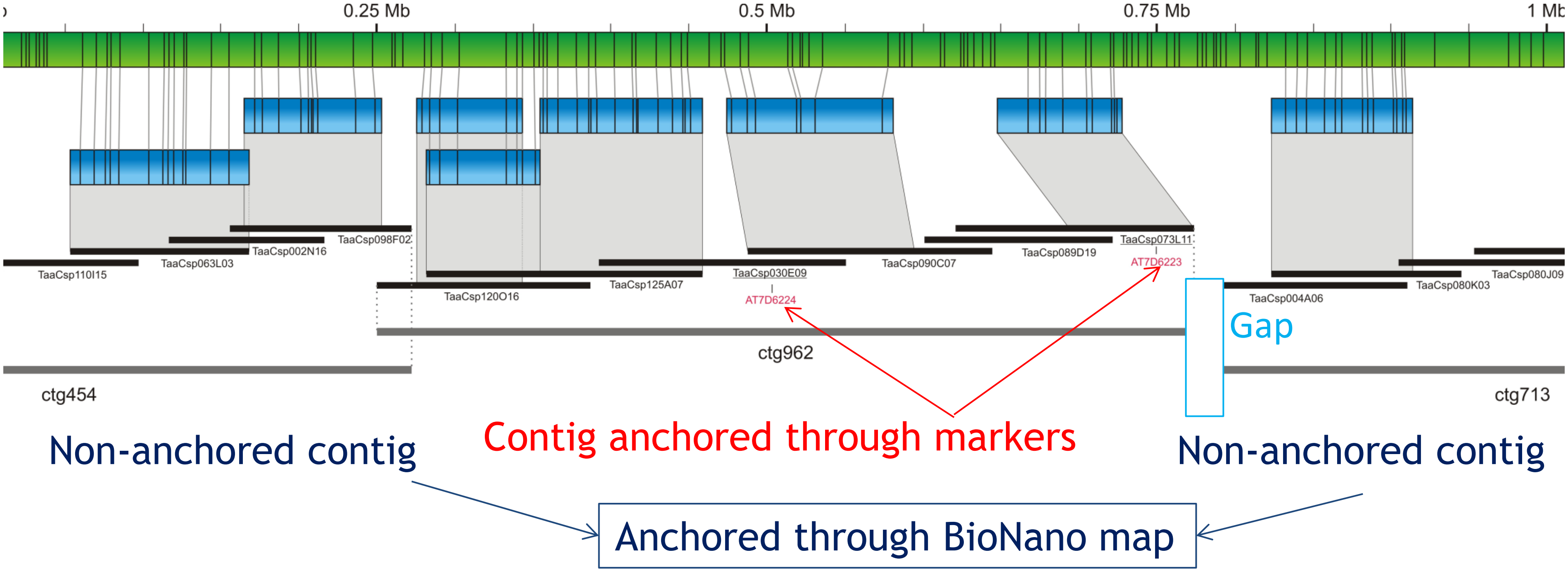
7D006K23

7D065B03

BIONANO MAP FOR PHYSICAL MAP IMPROVEMENT

7DS physical map

- 931 BAC contigs → reduced hitherto to 905 using information from the BioNano map
- 65% anchored through markers
- the rest might be anchored through the BioNano map



Gap

Non-anchored contig

Contig anchored through markers

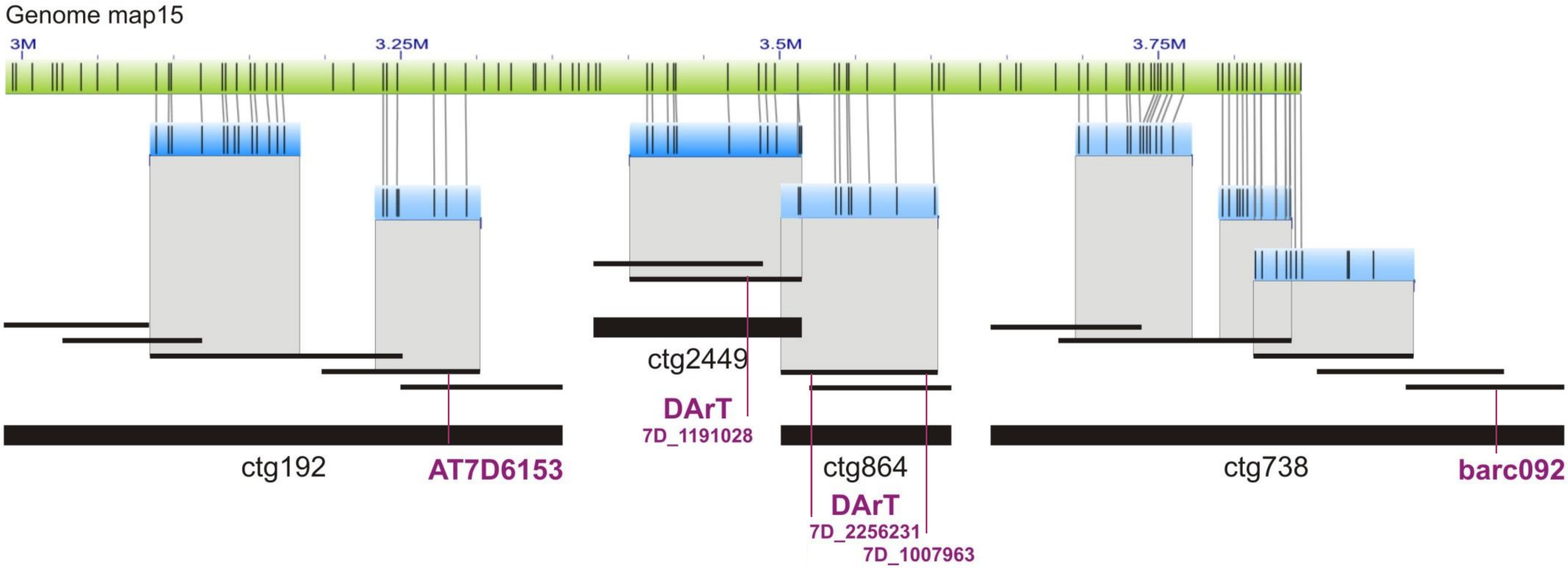
Non-anchored contig

Anchored through BioNano map

BIONANO MAP FOR PHYSICAL MAP IMPROVEMENT

7DS physical map anchored by

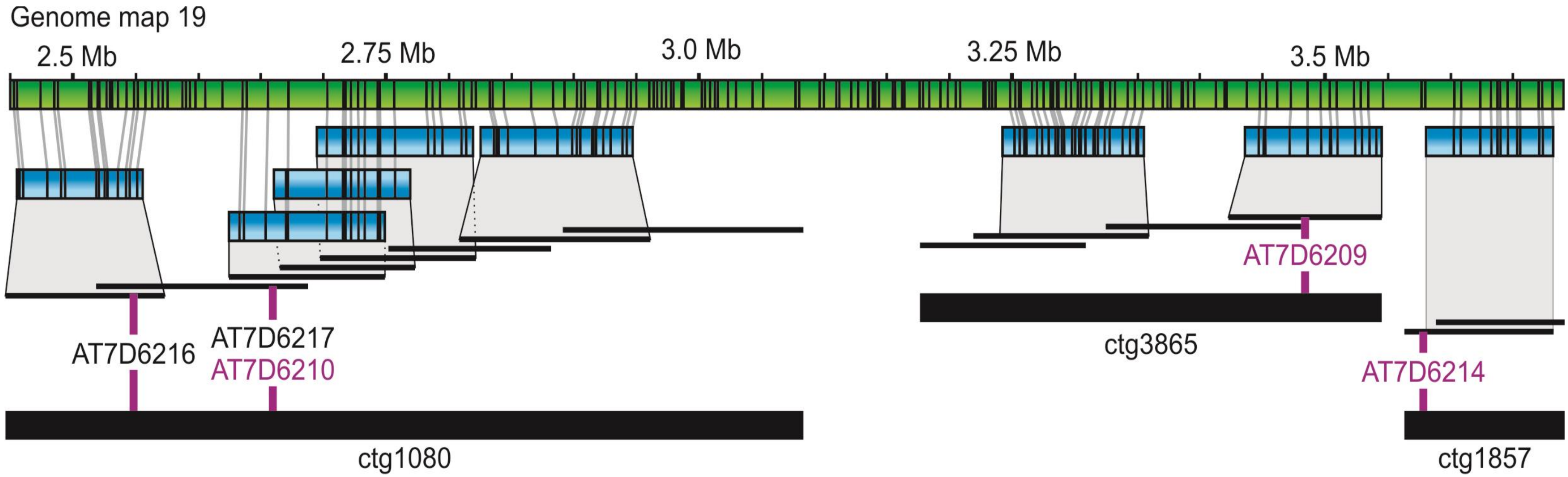
- 583 *Ae. tauschii* SNP markers
- 30 STS markers from *Ae. tauschii* RH map
- 134 SNP markers from CS RH map
- 76 DArT markers
- 23 SSR markers and 11 STS markers



- BioNano map enables integrating various genetic/radiation hybrid maps used for landing BAC contigs on chromosomes

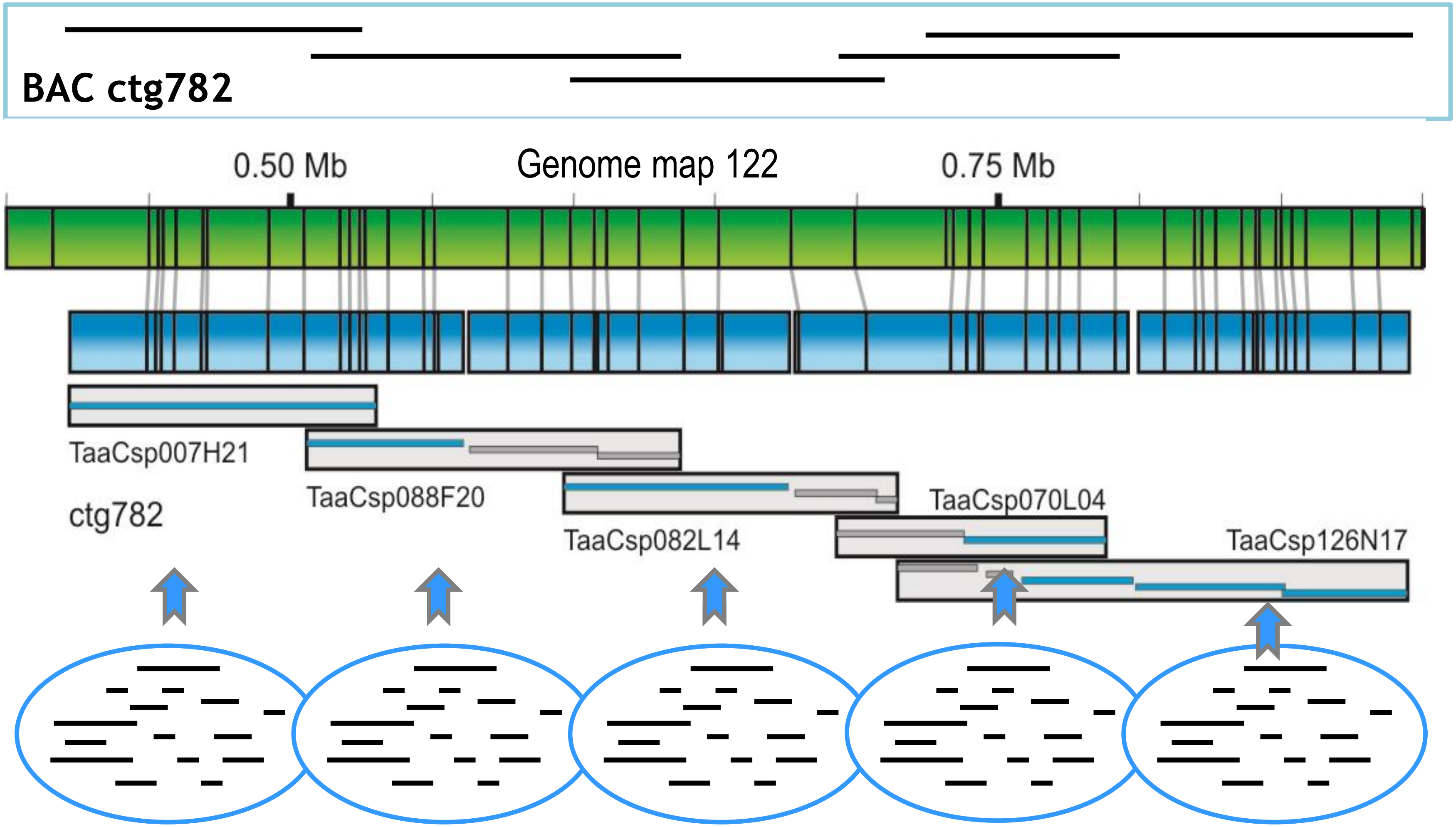
BIONANO MAP FOR PHYSICAL MAP IMPROVEMENT

- Helps ordering contigs in regions with clustering genetic markers



- Genome maps for centromeric region available but are relatively short

BIONANO MAP FOR POOL DECONVOLUTION

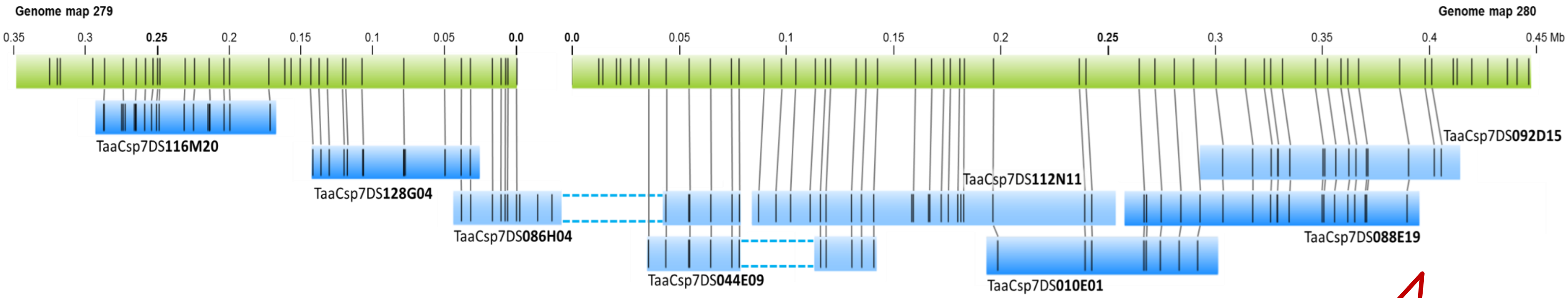


1) Sequences of five BAC pools aligned to the 7DS BioNano map
→ 1-3 contigs per clone anchored to genome map 122

2) The remaining contigs resolved through BLASTing BAC pools against each other

BIONANO MAP FOR IDENTIFYING AND CORRECTING MISASSEMBLIES

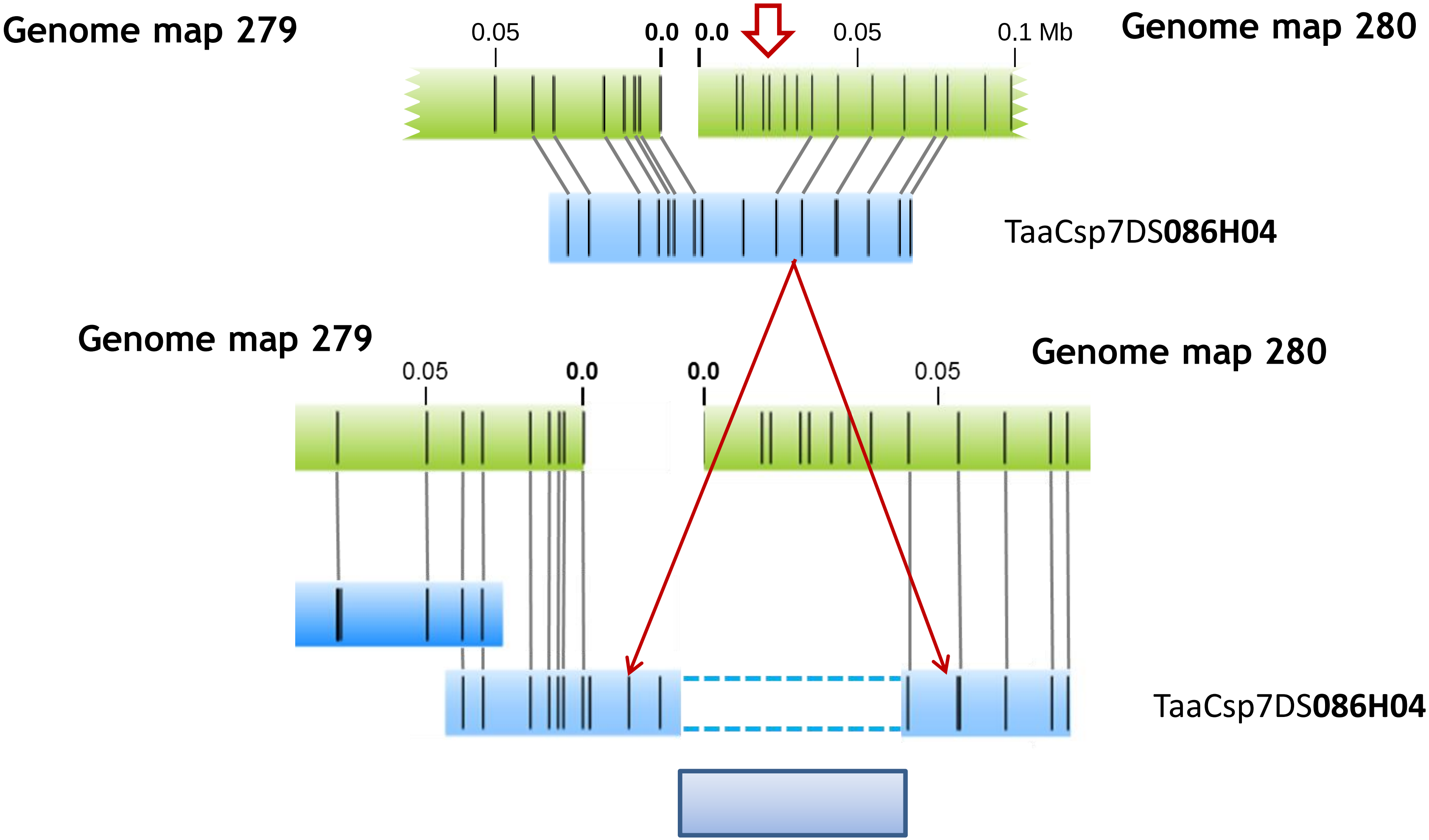
~700 kb



Poster
No. P590

http://olomouc.ueb.cas.cz/

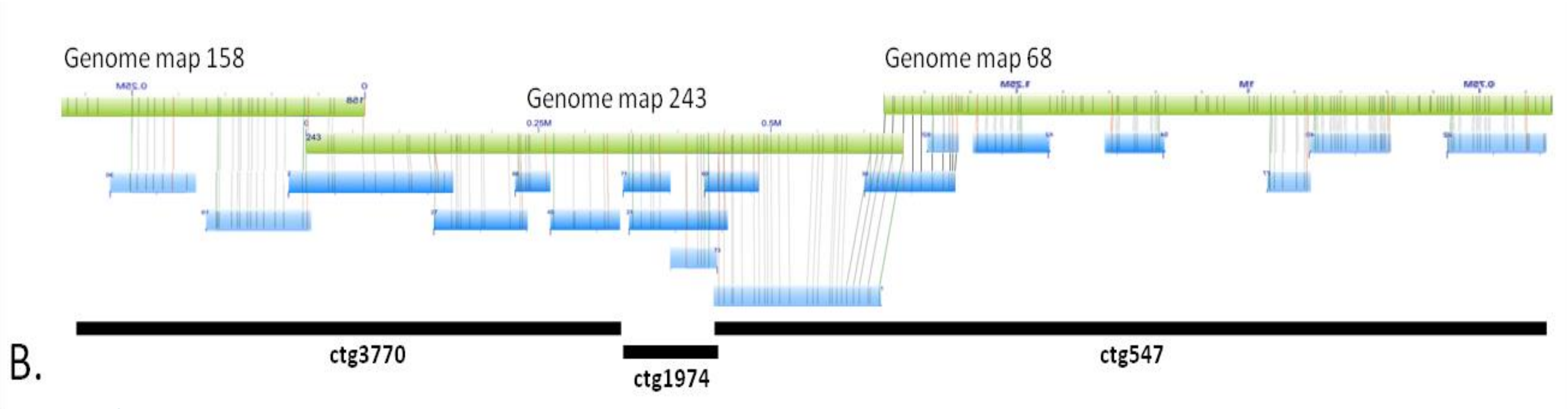
BIONANO MAP FOR IDENTIFYING AND CORRECTING MISASSEMBLIES



7DS086H04-Ctg2 – match in size (19 kb)
mismatch in sequence

WHAT HAS NOT BEEN MENTIONED YET…

- Size estimation is very precise (± 3/1000 bp)
- Parametres of the BioNano map can be further improved through alignment with BAC contig map and sequence



BAC clone sequences

Physical map contigs

CONCLUSIONS

- Coupling chromosome sorting with BioNano technology enables producing quality *de novo* genome maps for particular wheat chromosomes/arms

The genome map shows big potential for

- Improving physical maps - anchoring and orientating BAC contigs, scaffolding, validation of the assembly. The map reduces # markers needed for anchoring, facilates ordering contigs in non-recombining regions
- Genome sequence assembling – identifying misassemblies, deconvolution of sequence contigs, sizing gaps, assembly improvement, building pseudomolucule

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