# BioNano Genome Map of Bread Wheat 7DS Arm Supports Sequence Assembly and Analysis

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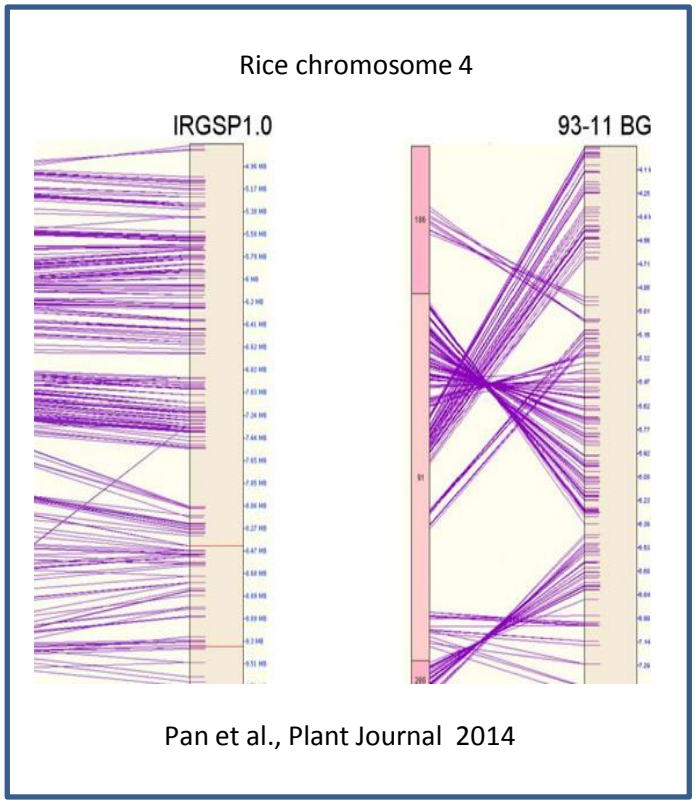
# OUTLINE

- Why BioNano mapping
- Why chromosomal approach

# QUALITY OF GENOME SEQUENCES

- The accepted genome quality has been decreasing
  - The consequence of a massive use of next generation shotgun sequencing
- The bottleneck:
  - NOT the sequencing capacity
  - BUT the ability to assemble many short reads with prevalence of repeated DNA (… and polyploidy)
- Promising:
  - long read technologies (PacBio, Oxford Nanopore, …)



Pan et al., Plant Journal 2014

- BUT read lengths exceeding 200 kb will be needed to unpick long arrays of repeats and segmental duplications

Marx, Nature 501:263, 2013
Shangguan et al., PLOS One 8:e69890, 2013
Pan et al., Plant J. 77 (5): 795-805, 2014
Chaisson et al., Nature Rev. Genet. 16: 627-640, 2015

# How To Make Next Generation Sequences Better?

## Use next generation mapping!

- **Single-molecule mapping** of genomic DNA hundreds to thousands kilobases in size
- Creates **sequence-motif maps**, which provide long-range template for ordering genomic sequences
- **Visualisation of reality**

# GENOME MAPPING ON NANOCHANEL ARRAYS

BIONANO GENOMICS

## Nick labelling

5´-ATGC**GCTCTTC**CATGAATGCG-3´
3´-TACG**CGAGAAG**GTACTTACGC-5´

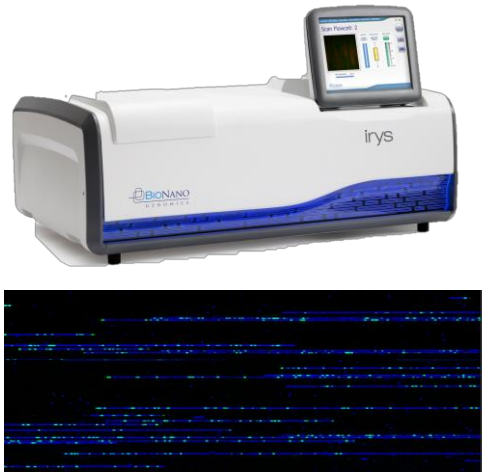
Nt.BspQ1 nicking site

ATGAATGC

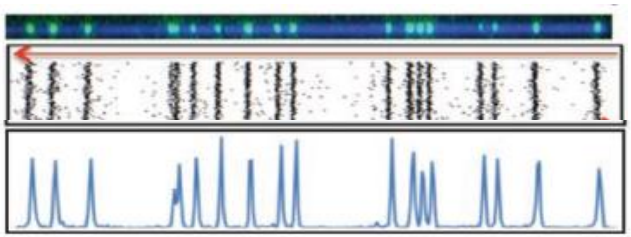
5´-ATGC**GCTCTTC**CAUGAAUGCG-3´
3´-TACG**CGAGAAG**GTACTTACGC-5´

## DNA linearization

## Fluorescence imaging

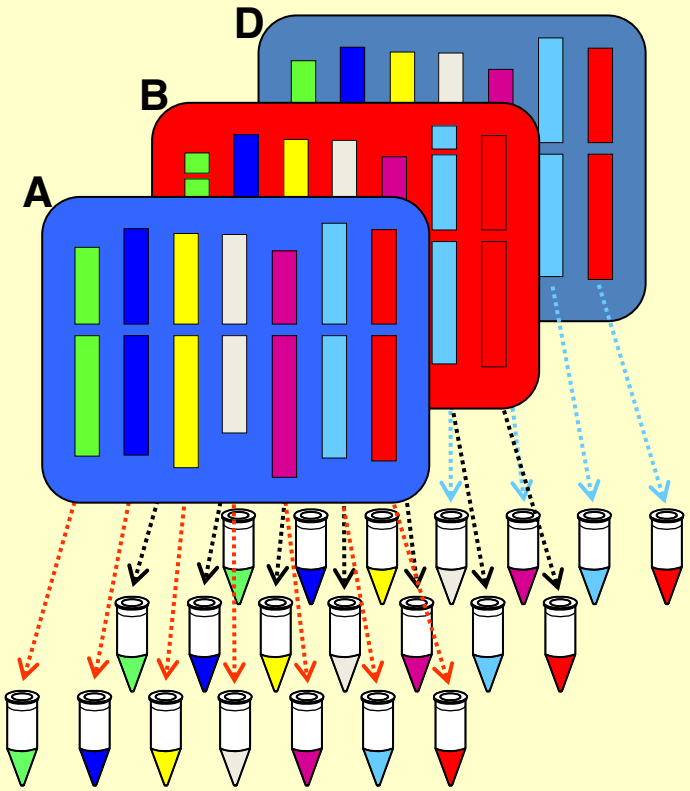



## Single molecule maps



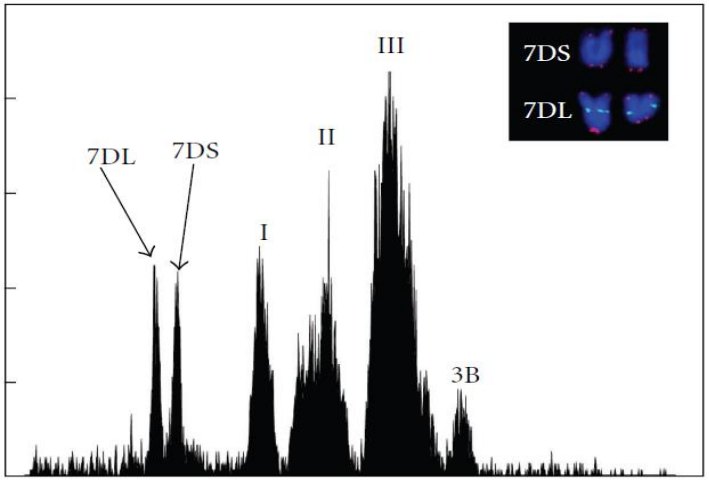
## Consensus genome maps

# Chromosome Mapping on Nanochannel Arrays




Dissecting the bread wheat genome by flow sorting

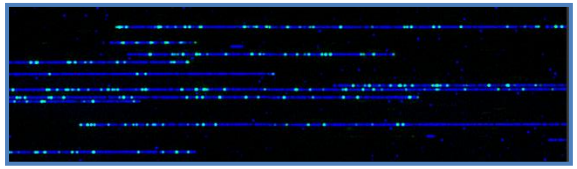
- Pilot study on wheat **7DS** chromosome arm (381 Mb, 2.25% wheat genome)
  - Purified as telocentric chromosome by flow cytometric sorting




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

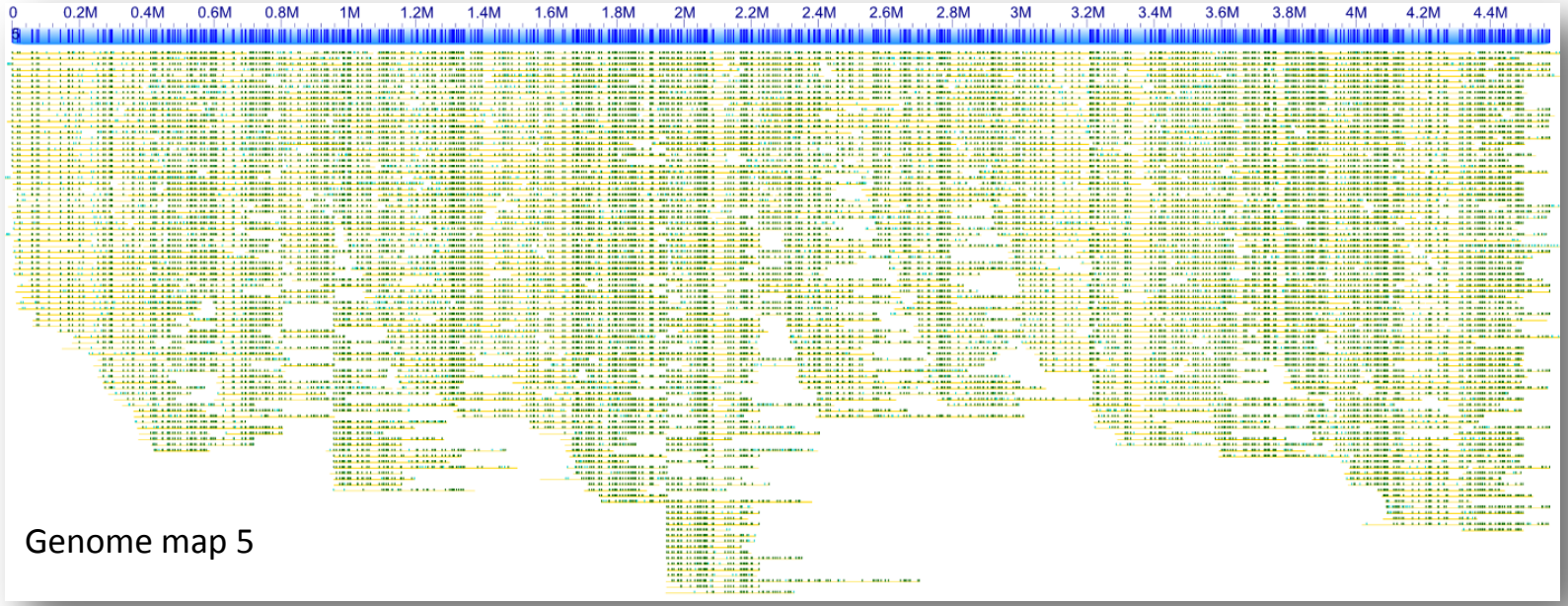
# *De Novo* Assembly of 7DS BioNano Map

- 1.6 million flow-sorted 7DS arms of 'Chinese Spring'



| Length threshold | Total coverage | Molecule N50 |
|---|---|---|
| 150kb | 180x | 344kb |

- A total of **371 genome maps** were *de novo* assembled
- Total assembly length 350 Mb (**92% of estimated 7DS size**)
- **Average map size 0.9 Mb**
- **Map N50 is 1.3 Mb**



# Co-assembly of 7DS and 7AS Data

| | 7AS | 7DS | Theoretical 7AS + 7DS | Co-assembly (% vs. theoretical) |
|---|---|---|---|---|
| **Molecule Coverage** | 194x | 206x | | |
| **Molecule N50 (kb)** | 206 | 219 | | |
| **No. Genome Maps** | 783 | 468 | 1251 | 2043 (163%) |
| **Avg. Genome Map Length (kb)** | 571 | 765 | 0,643 | 0,488 (76%) |
| **Genome Map N50 (kb)** | 1,553 | 1,355 | 1,454 | 1,053 (70%) |

- More fragmented genome maps
- Several percentage of chimeric maps – merged through shared repeats

# 7DS Physical Map and Sequence

## Physical map

- Automatic assembly using FPC
- Manual end-merging of contigs based on integration of *Aegilops tauschii* whole-genome map with the map of the bread wheat **7DS** chromosome arm
- Verification of the assembly using LTC

| | |
|---|---|
| No. contigs > 2 BAC clones | 904 |
| No. contigs > 5 clones | 652 |
| Assembly length | 360 Mb |
| 7DS arm coverage | 95% |
| Contig N50 | 548 kb |
| No. MTP BAC clones | 4,608 |

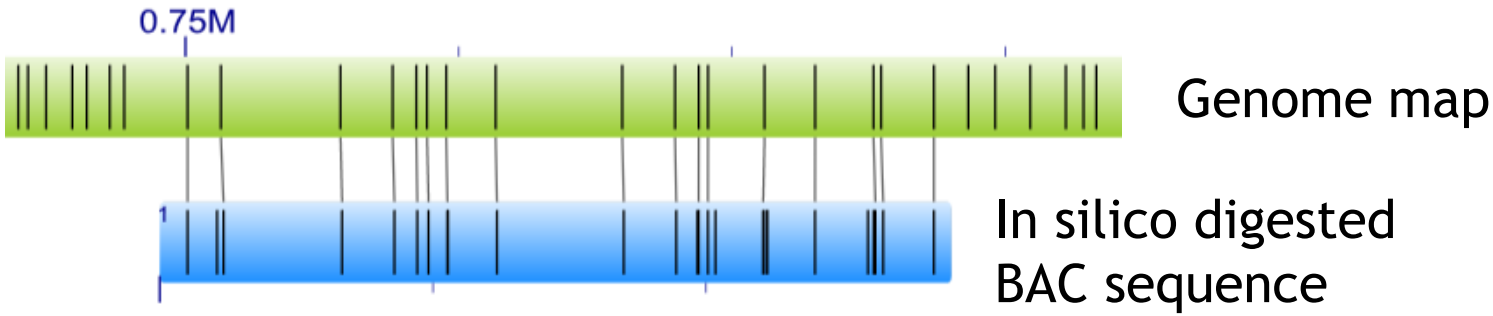
## Sequence

- Sequencing pools of four non-overlapping MTP BAC clones
- Illumina pair-end sequencing - 550bp fragment size, 100 bp read length, coverage >500x → Sassy
- Deconvolution through BAC end sequences
- Scaffolding through mate-pair data obtained from MTP-plate pools (384 clones/pool) → SSPACE

| | |
|---|---|
| Contigs per BAC | 1 - 17 |
| Average No. contigs/BAC | 1.9 |
| Median No. contigs/BAC | 1.5 |
| Average scaffold size | 56 kb |
| Scaffold N50 | 116 kb |
| % N in assembly | 2.9 % |

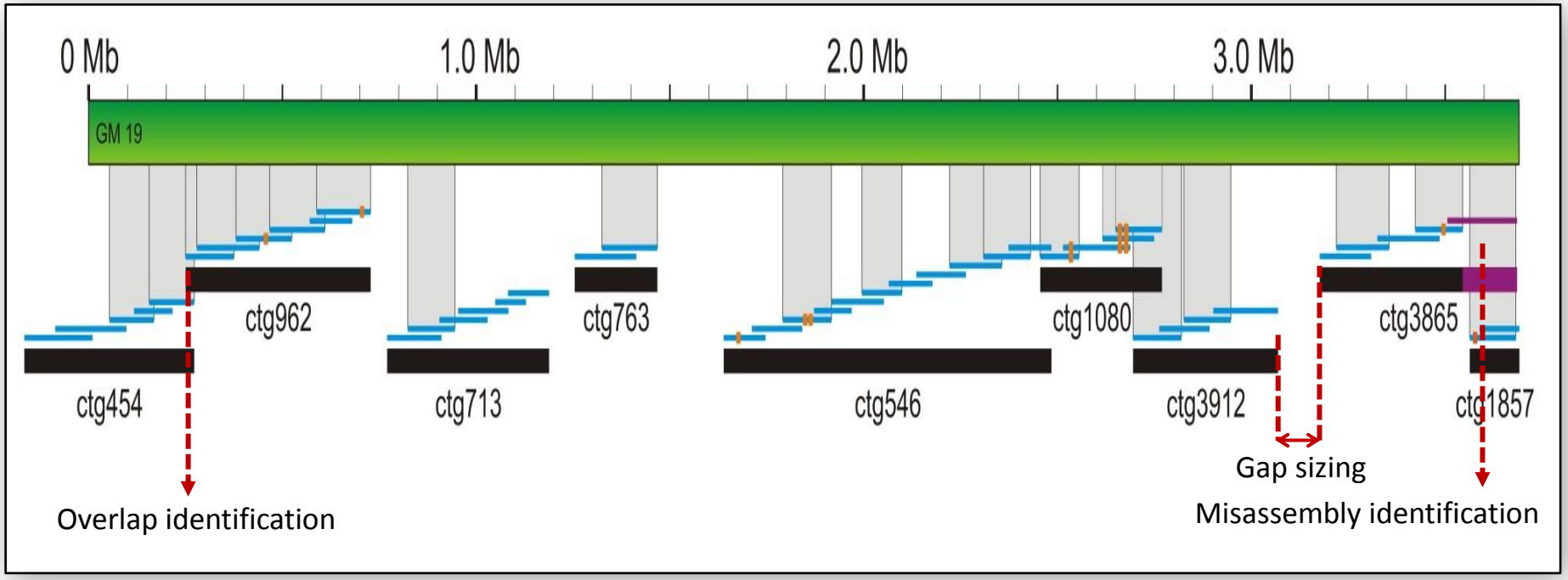
# COMBINING BIONANO MAP WITH THE 7DS SEQUENCE

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



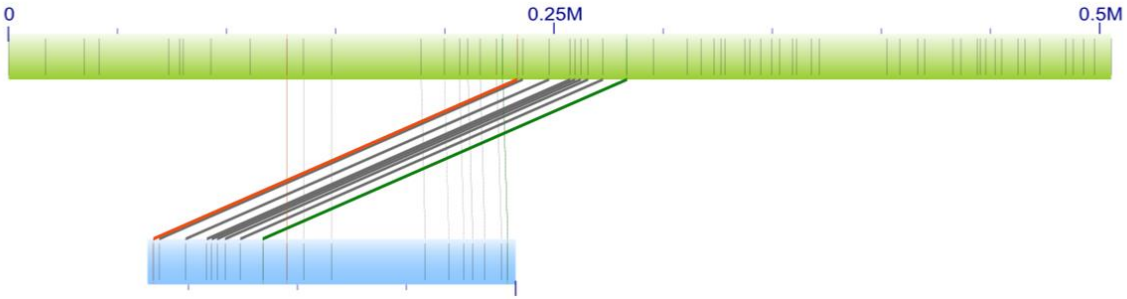
# BioNano Map for Physical Map Improvement

- Enables scaffolding of BAC contigs
  - Reduces demand on number of markers
  - Integrates multiple genetic maps
  - Useful in non-recombing regions
- Sizes gaps
- Identifies and corrects mis-assemblies

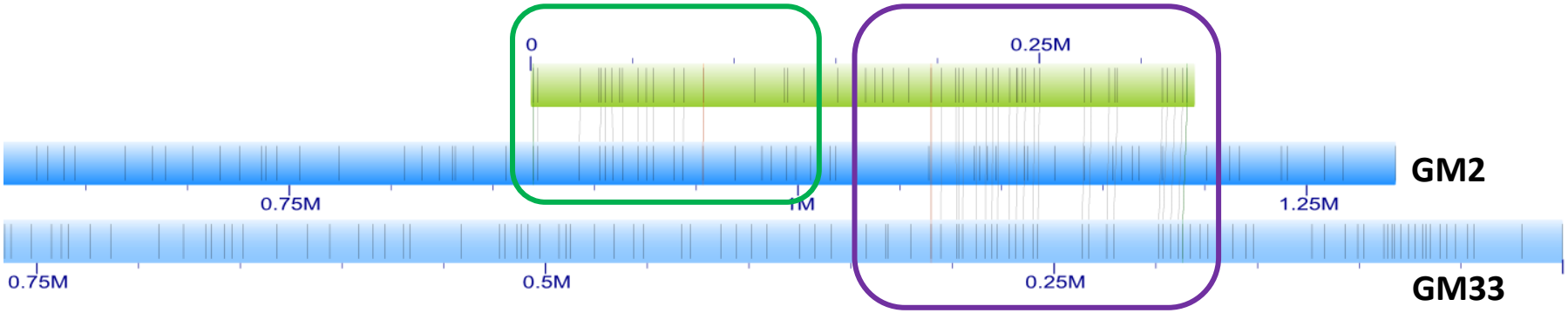


# BioNano Map for Sequence Improvement

- Identification of scaffold mis-assemblies
  - Due to mis-orientation contigs in a scaffold

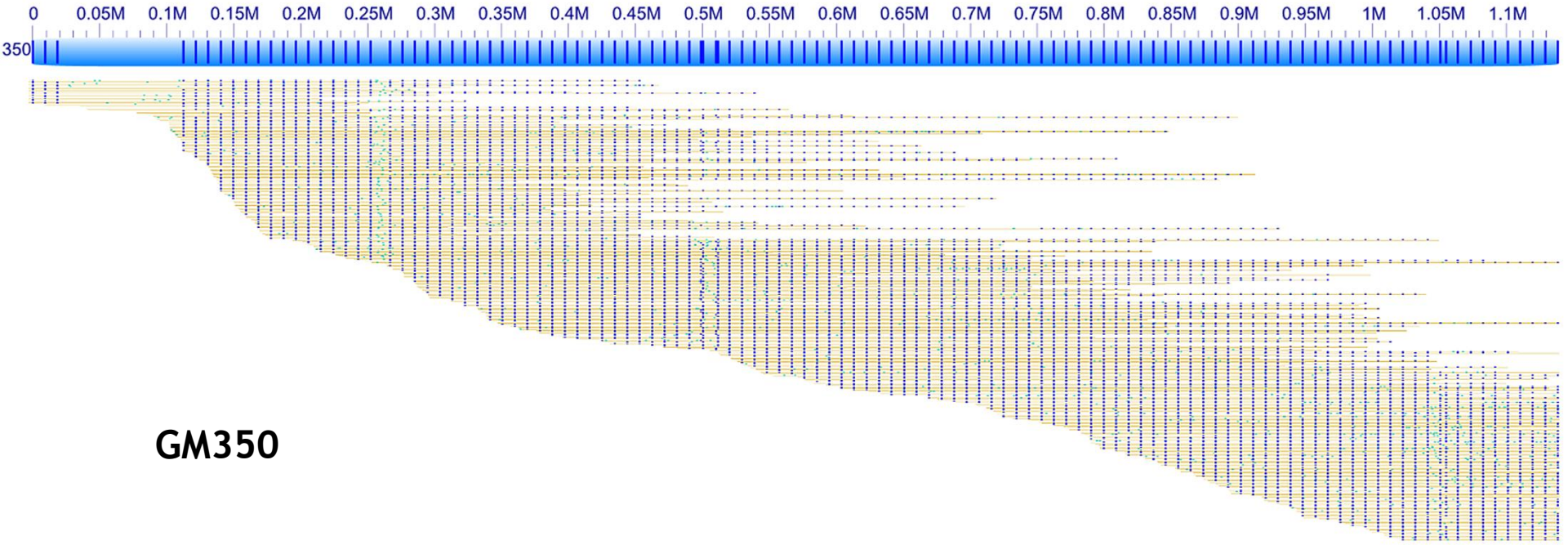



  - Due to mis-joining of contigs within a sequencing pool or cross-contamination



# Large Array of Tandem Repeats in the 7DS

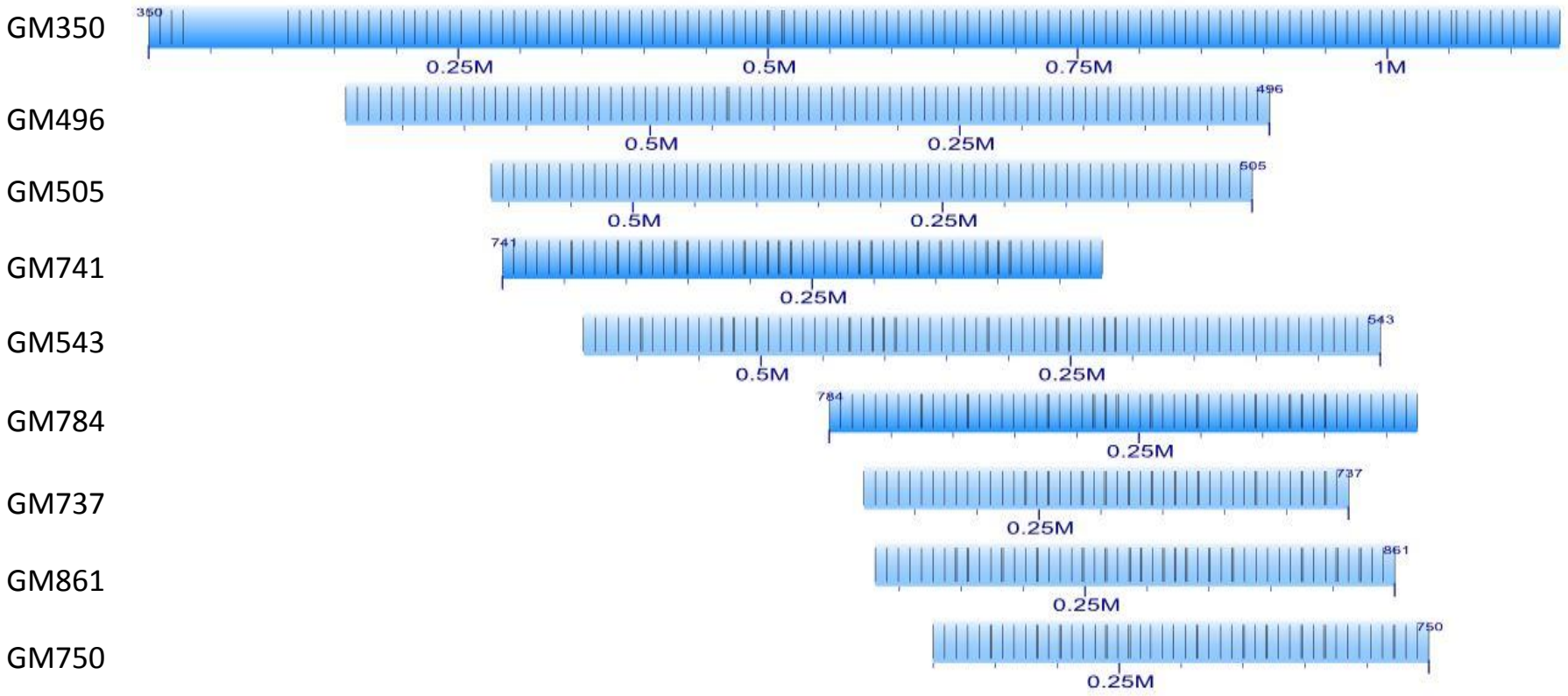
- ~1 Mb-long array of tandem repeats was identified in the genome map No. 350 (longest single molecule ~ 800 kb)
- Unit size ~9.3 kb




**GM350**

# Large Arrays of Tandem Repeats

+ several other maps with identical/similar motif




No high-confidence match in 7DS BAC sequence assemblies

# LARGE ARRAYS OF TANDEM REPEATS

## rDNA locus?

- Corresponds to the size of wheat 25S-18S rDNA unit: ~9 kb (Gerlach and Bedbrook, 1979)
- Major loci on 1B, 6B and 5D

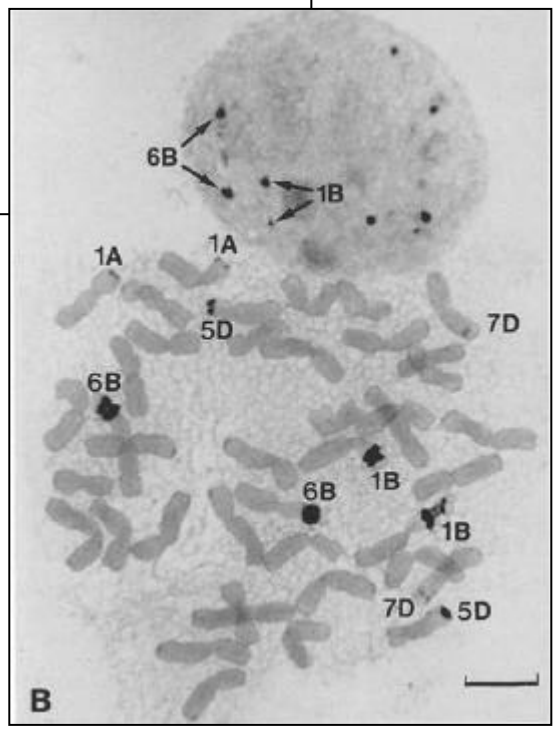
Chromosoma (1991) 100:71–78

CHROMOSOMA
© Springer-Verlag 1991

**Physical mapping of the 18S.26S rRNA multigene family in common wheat: Identification of a new locus**

Y. Mukai*, T.R. Endo**, and B.S. Gill

- Minor locus identified on the 7DS arm



# Studying Local Structural Variation

- Project on cloning a Russian wheat aphid resistance gene *Dn2401*
- Region of 0.81 cM spanned by five BAC clones (~350 kb)

Theor Appl Genet (2015) 128:1373–1383
DOI 10.1007/s00122-015-2512-2

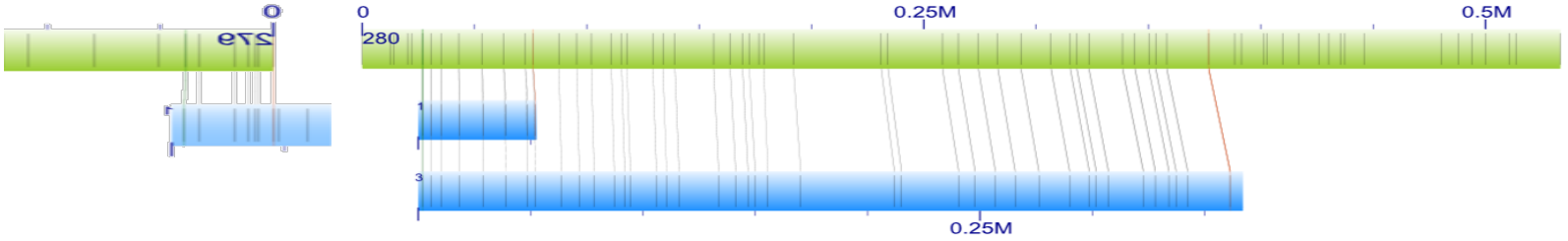
ORIGINAL PAPER

**Chromosomal genomics facilitates fine mapping of a Russian wheat aphid resistance gene**

Helena Staňková[1] · Miroslav Valárik[1] · Nora L. V. Lapitan[2,3] · Paul J. Berkman[4] · Jacqueline Batley[5] · David Edwards[5,6] · Ming-Cheng Luo[7] · Zuzana Tulpová[1] · Marie Kubaláková[1] · Nils Stein[8] · Jaroslav Doležel[1] · Hana Šimková[1]

- BAC clones sequenced, candidate genes identified

CS



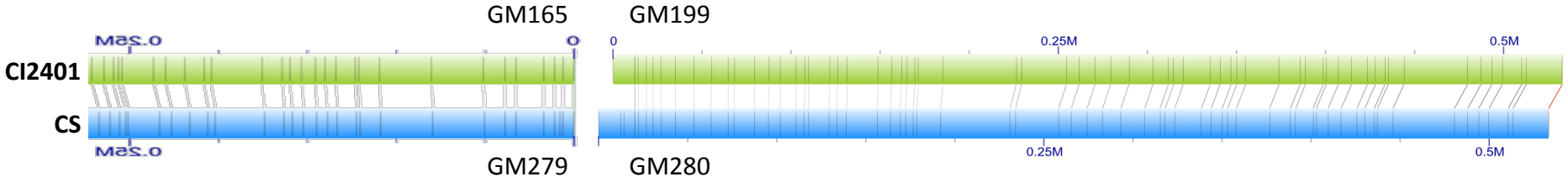

- Sequence available for cv. Chinese Spring (susceptible), while the gene has been mapped in a resistant line CI2401

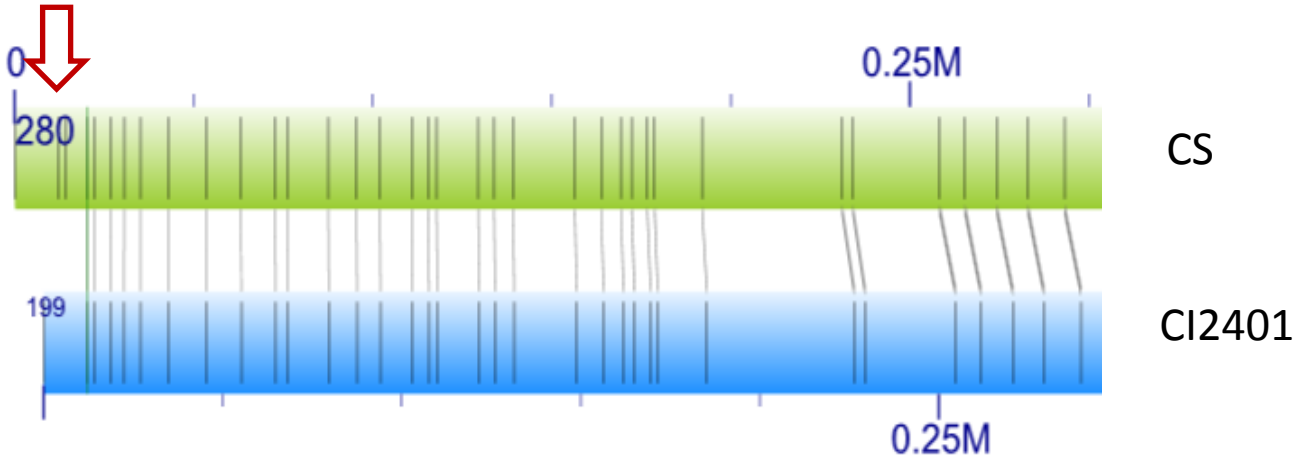
**Do we miss anything when analyzing the CS sequence?**

# Studying Local Structural Variation

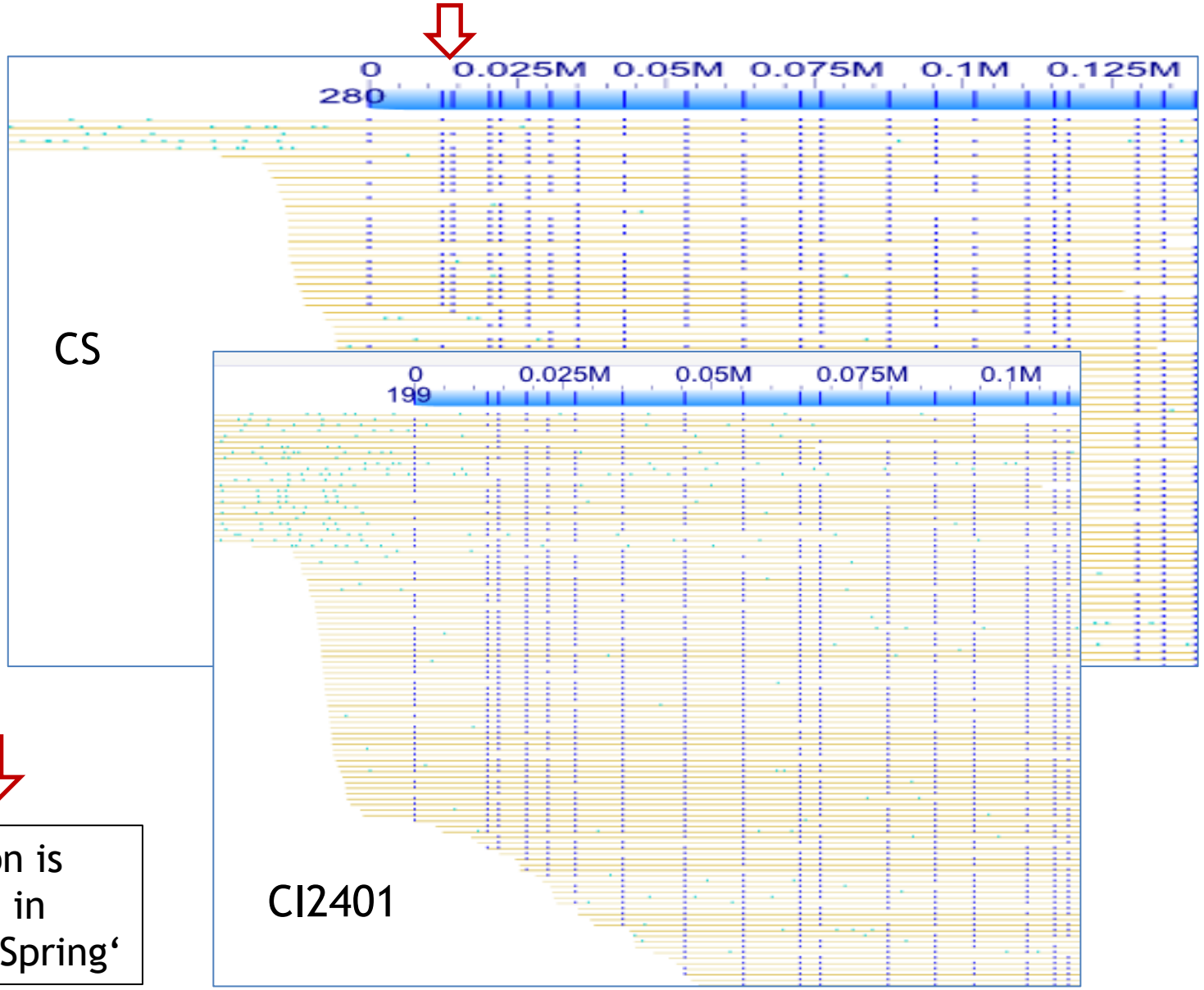
- Construction of BioNano map from 7DS arm of CI2401 (7DS ditelosomic line newly created from CI2401)

| 7DS | CS | CI2401 |
|---|---|---|
| **Molecule Coverage** | 180x | 206x |
| **Molecule N50 (kb)** | 344 | 219 |
| **Genome Map N50 (Mb)** | 1.3 | 1.35 |

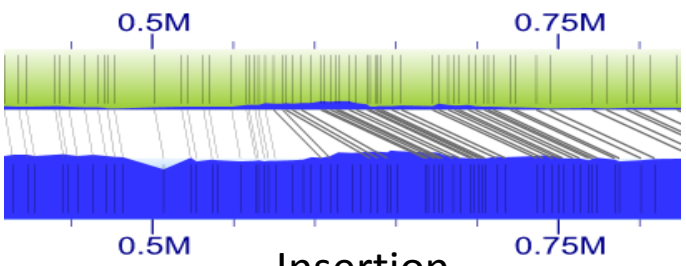


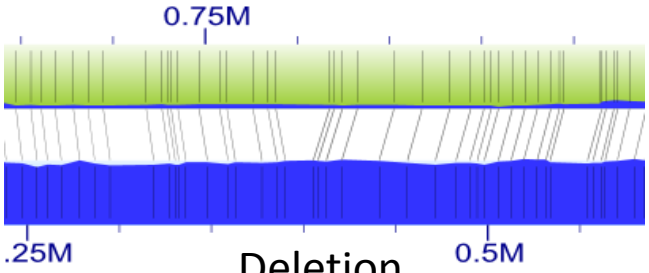
# Studying Local Structural Variation



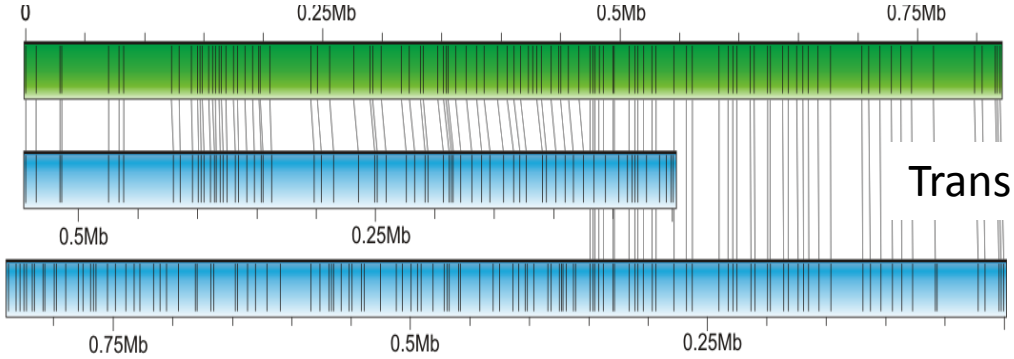
# Studying Structural Variation on Chromosome arm Level




Insertion




Deletion




Translocation

| | No. cases | Total length (% CS arm length) |
|---|---|---|
| Deletions in CI2401 vs. CS | 28 | 1.2 Mbp (0.31%) |
| Insertions in CI2401 vs. CS | 109 | 1.7 Mbp (0.44%) |
| Variable map ends | 73 | 22.8 Mbp (6%) |

- Significant reduction of the long tandem repeat array

# CONCLUSIONS

**The BioNano genome map is useful for**

- Validating and improving physical map and sequence (scaffolding contigs, sizing gaps, identification and correction of misassemblies)
- Identification of large tandem repeats intractable to current sequencing technologies
- Structural variation detection (insertions, deletions, translocations, copy number variations)

**Chromosomal approach**

- Enables higher-quality genome map assembly
- Resolves repeats on chromosomal level
- Focused approach to studying structural variation

# ACKNOWLEDGEMENTS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UC DAVIS UNIVERSITY OF CALIFORNIA

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Bikram Gill
Bernd Friebe

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