DE LA RECHERCHE À L'INDUSTRIE

PLANT & ANIMAL GENOME XXIII
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IWGSC

Standards and Protocols

CHROMOSOME 1B: A STEP FURTHER IN THE SEQUENCING OF THE HEXAPLOID WHEAT GENOME

Valérie Barbe
CEA/Genoscope
Evry, France

First steps of the wheat complete genome

Introduction to Special Issue

✓ Slicing the wheat genome

Kellye Eversole, Catherine Feuillet, Klaus F. X. Mayer, and Jane Rogers

✓ A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome

IWGSC consortium

✓ Structural and functional partitioning of bread wheat chromosome 3B

Choulet F. *et al.*

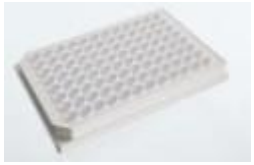
Wheat chromosome sequencing project: library constructions and sequencing process

2 

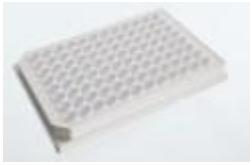
DNA Extraction

Alkaline lysis

Culture plate 96 wells
1 BAC/well

2 

BAC DNA

Pool → 1 

Pool

Pool

Pool equimolar amounts of **96** BAC DNA

Nextera protocol

Mate-paired library
5 kb insert size

HiSeq 2500
2x150 pb, (50x)

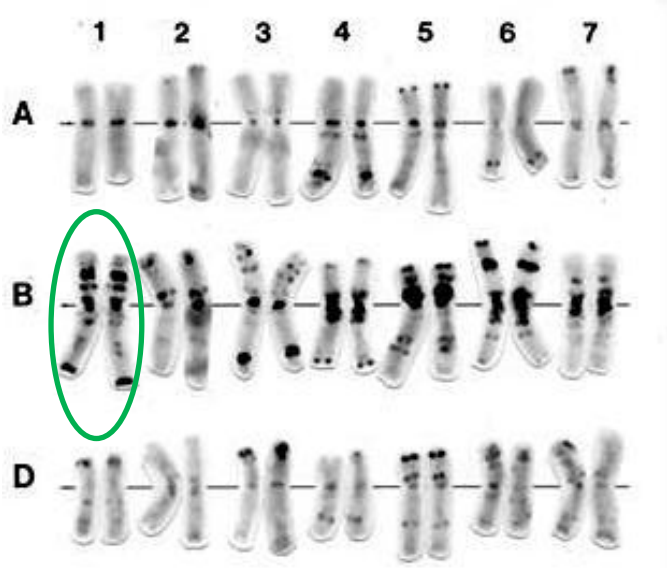
Pool equimolar amounts of **10(or 6)** BAC DNA

Illumina protocol

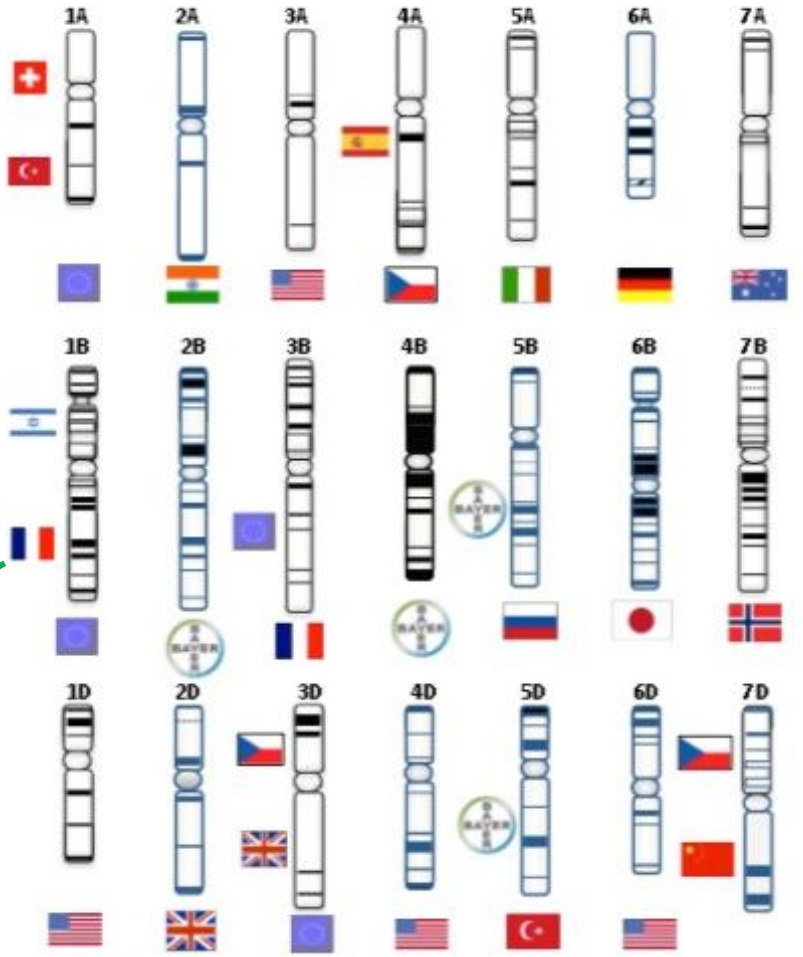
Paired-Ends overlapping libraries
~ 450-550 insert size

MiSeq
2X300 nt, (200x)

France still invests in Wheat genome sequencing



T. aestivum
cv Chinese Spring



1BS: 4372 BACs

Dina Raats *et al.*, *Genome Biology* 2013, 14:R138

1BL: 6023 BACs

Philippe, R. *et al.*, Genome Biol 2013, 14(6):R64

Chromosome 1B sequencing

6023 BACs rearranged in 16 plates 384

630 pools corresponding to 630 PE and 63 MP libraries

40 pools/run => 16 runs MiSeq 2X300

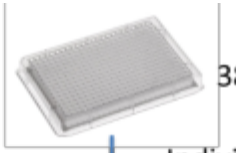
48 pools/lane => 1 run HiSeq2500, 2X150

4372 BACs rearranged in 12 plates 384

456 pools corresponding to 456 PE and 46 MP libraries

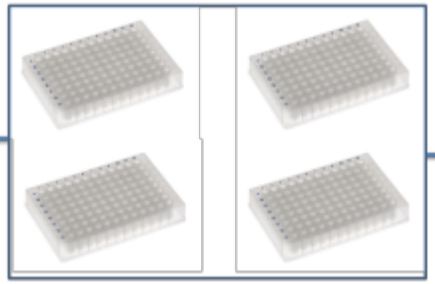
Sequencing in progress (almost finished)

Wheat chromosome sequencing project: Assembly process



384-wells plate

Individual BAC cultures and extraction in 96-wells plates



Pools of 10 BACs

10 overlapping paired end libraries/ plate

Quality filter* and reads trimming**

Paired reads merging

Pools of 96 BACs

One 5kb mate pair library/ plate

Quality filter* and reads trimming**

Newbler

Contigs

Sspace

Scaffolds_V1

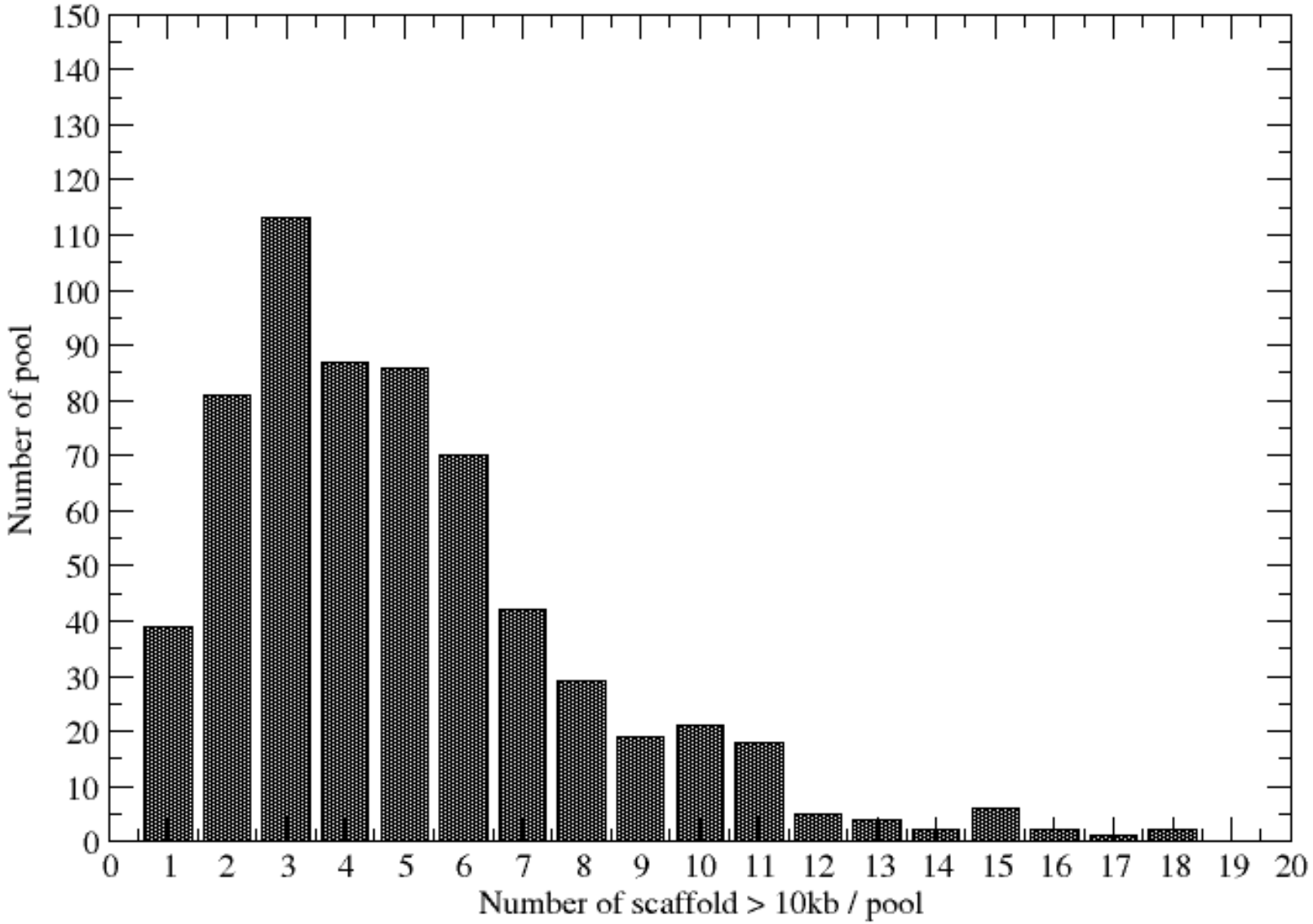
GapCloser

Scaffolds_V2

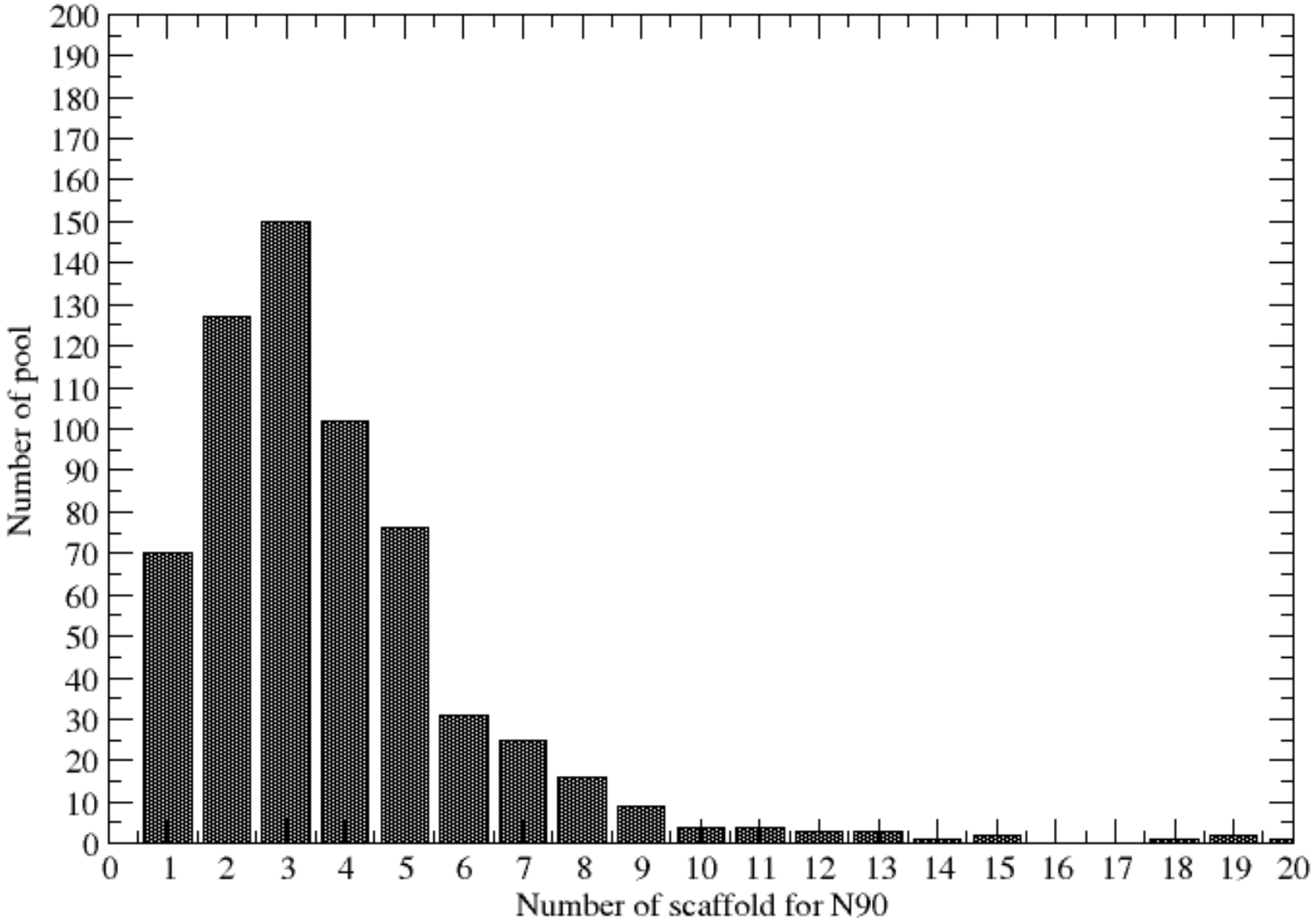
* **Filtering**: Treatment with fastx_clean to remove adaptors and low quality nucleotides (q<20).

** **Trimming** of *E. coli* and vector sequences (+ circularization adaptor for MP library)

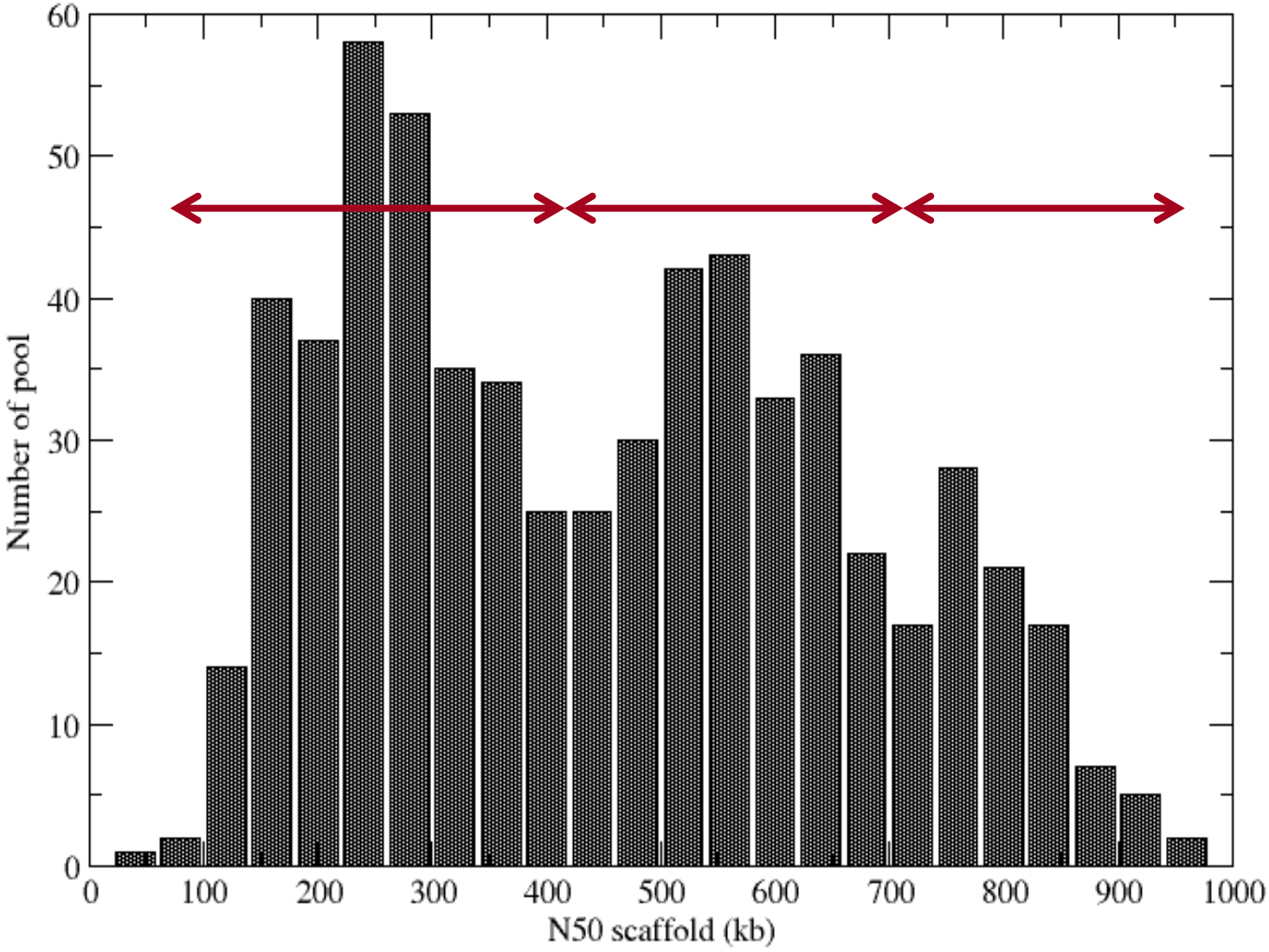
1BL sequencing: first results



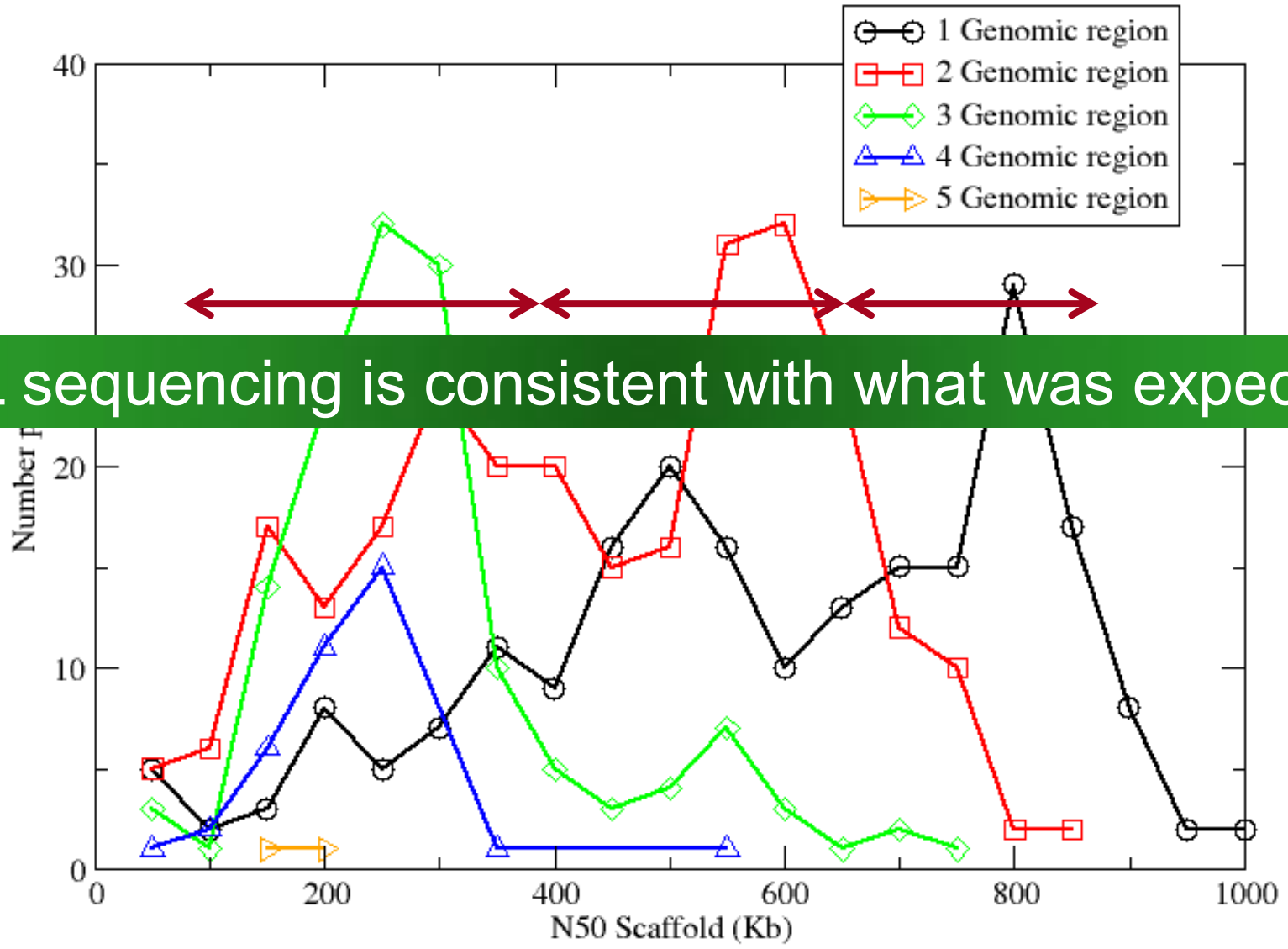
1BL sequencing: first results



1BL sequencing: first results

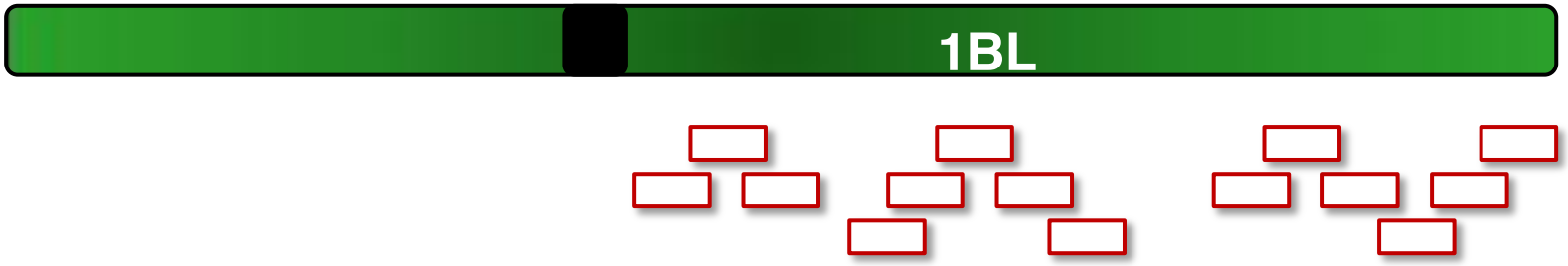


1BL sequencing: first results



1BL sequencing is consistent with what was expected

Assembly validation



Size:	537 Mb (1BL estimated size: 530Mb)
Scaffolds:	3646
N50:	340 kb / 501 scaff
Gap:	1%

Which proportion of 1BL covered?

--> using 1BL IWGSC Survey Sequence gene models:

2713 CDSs

- 2526 found in 1BL MTP-seq **93%**

1BL sequence is similar to that obtained for the 3B

- 139 originate from probable contamination by other chr. (while sorting chromosomes) **5%**

Proportion of 1BL covered ➔ **98%**

Strategies for assembly improvement: Bacs Ends

Fragmentation

A new BAC ends sequencing approach to improve wheat 1B chromosome assembly

Adriana Alberti[1]*, Laura Briñas[1], Caroline Belser[1], Céline Orvain[1], Karine Labadie[1], Laurie Bertrand[1], Arnaud Couloux[1], Stefan Engelen[1], Jean-Marc Aury[1], Valérie Barbe[1], Fréderic Choulet[2], Etienne Paux[2], Patrick Wincker[1,3,4]

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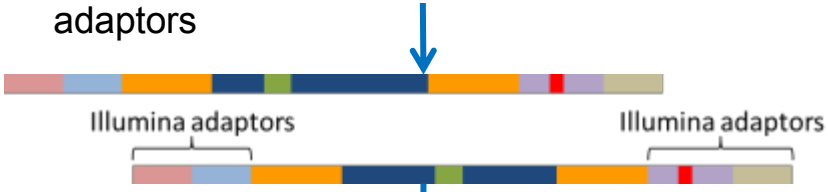
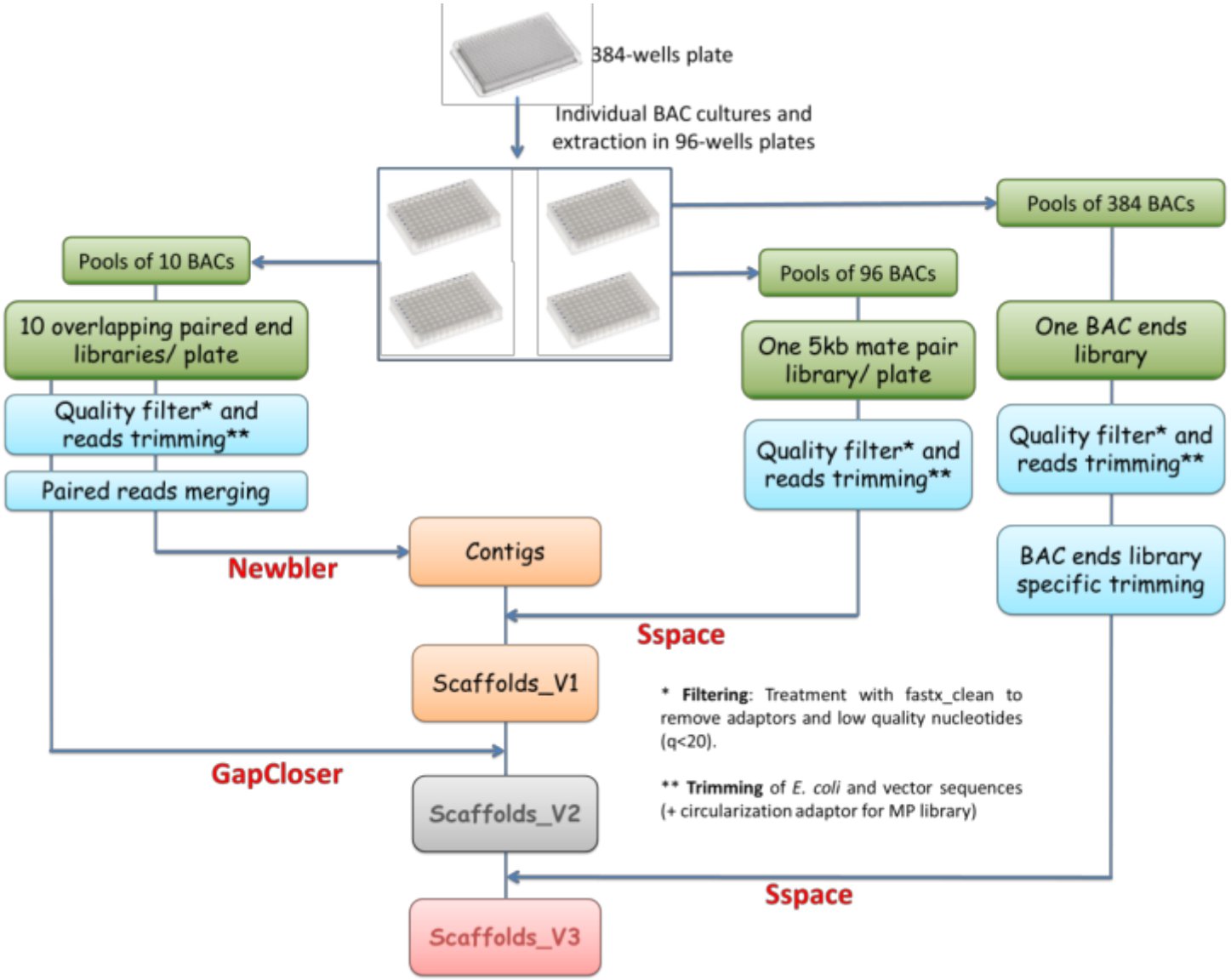
 # P0597

adaptors



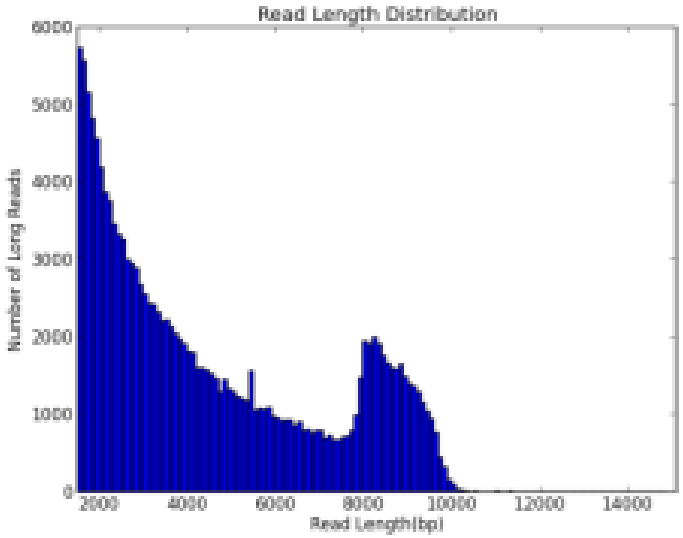
Sequencing

Pipeline using BES



Strategies for assembly improvement: Moleculo

1BL pools



Analysis in progress

Conclusions and perspectives

- ✓ The sequencing strategy we proposed last year provides assembly with the expected quality
- ✓ Shot-gun of the 1BL arm is done
- ✓ Shot gun of the 1BS arm soon complete

To do

- ✓ Bac ends on the whole 1B chromosome
- ✓ Moleculo on 1BL and 1BS arms DNA
- ✓ Whole genome maps for the 1BL and 1BS arms, using Bionano instrument

Acknowledgements

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Thank you for your attention