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► **To cite this version:**

Frédéric Choulet, Sébastien Theil, Adriana A. Alberti, Valérie Barbe, Sophie Mangenot, et al.. Towards a reference sequence of the 1 Gb wheat chromosome 3B. 16. Annual Advances in Genome Biology and Technology (AGBT), Feb 2012, Marco Island, Floride, United States. hal-02807639

HAL Id: hal-02807639

<https://hal.inrae.fr/hal-02807639v1>

Submitted on 6 Jun 2020

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Towards a reference sequence of the 1 Gb wheat chromosome 3B

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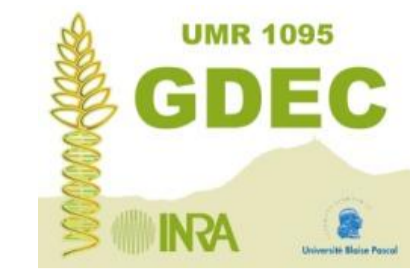
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A chromosome-by-chromosome approach to face the highly complex genome of bread wheat

- Allohexaploid (3 subgenomes: A, B & D)
- 17 Gb (1C)
- 85% of repeated sequences (TEs)

Sorting chromosomes using flow cytometry

The chromosome-based approach is the strategy established by the IWGSC (International Wheat Genome Sequencing Consortium)

➤ 3B is the biggest wheat chromosome
➔ used as a model for physical mapping and sequencing

Establishment of a physical map of chromosome 3B

- 132,000 fingerprinted BACs (19x coverage)
- 1,282 BAC-contigs (99% of the chromosome)
- 8448 BAC clones in the Minimal Tilling Path
- ~4,300 markers assigned to BAC-contigs

Rustenholz et al. Plant Physiol. 2011
Paux et al. Science 2008

3BSEQ project: Getting a reference sequence of chromosome 3B

<http://urgi.versailles.inra.fr/Projects/3BSeq>

Roche/454 Sequencing of the Minimal Tilling Path

1 Gb 8448 BACs

928 pools of 10 BACs

928 libraries LPE 8kb

150 runs Roche/454 GSFLX Titanium

➤ 40 Gb (40x cov.)

Assembly of each pool individually (NEWBLER)

Illumina Whole 3B shotgun

Sorted 3B chromosomes

Amplified DNA

PE library 0.5 kb

Illumina HiSeq2000

➤ 845 M reads (2x108 bp)

➤ 82 Gb (82x cov.)

Assembly (ABYSS)

BAC-ends sequencing

Bac-End sequencing of 3 versions of the MTP

40175 BacEnds ⇔ 25 Mb

3BSEQ assembly results

Roche/454 Sequencing of the Minimal Tilling Path

	Scaffolds
# Scaffolds	16,167 scaff
# bps	1028 Mb
Average	64 kb
L50	1028 scaff
N50	277 kb
Max size	1.3 Mb

Illumina Whole 3B shotgun

	Contigs >200bp
# Contigs	546,922 ctg
# bp	639 Mb
Average size	1.2 kb
N50	2.7 kb
Max size	48 kb

Example of assembly accuracy according to a 50kb reference sequence:

The TriAnnot pipeline

<http://www.clermont.inra.fr/triannot>

(Leroy et al. Front. Plant Sci. 2012)

➔ 8734 genes predicted (1/114 kb)

Work in progress to build a pseudomolecule

- Merging scaffolds of different BAC pools based on sequence comparison
- Integrating 454Scaffolds/Illumina reads/BES

Additional studies

- **Anchoring and orientation of scaffolds**
4000 SNPs to be developed
 - Cytogenetic bin mapping (aneuploid lines)
 - Genetic mapping (381 F2 individuals + 2 parents)
 - Radiation hybrid mapping
- **RNASeq (15 different conditions)**
40x2(PE) M reads x 2 replicates x 15 conditions
- **Structural polymorphism (CNVs, PAVs)**
Illumina shotgun sequencing of chromosomes 3A and 3D
Illumina shotgun sequencing of chromosomes 3B from 18 wheat varieties

