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

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

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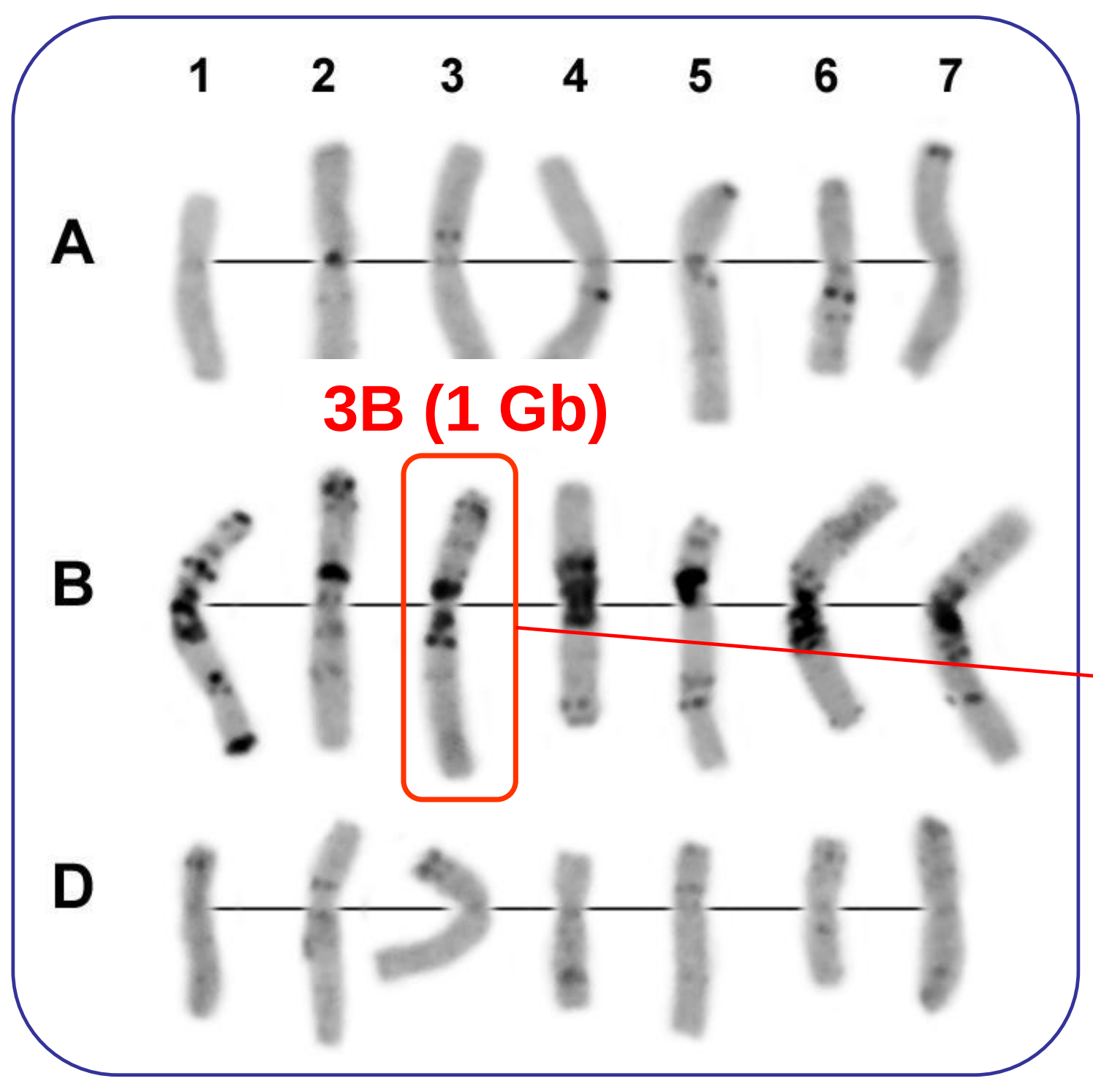
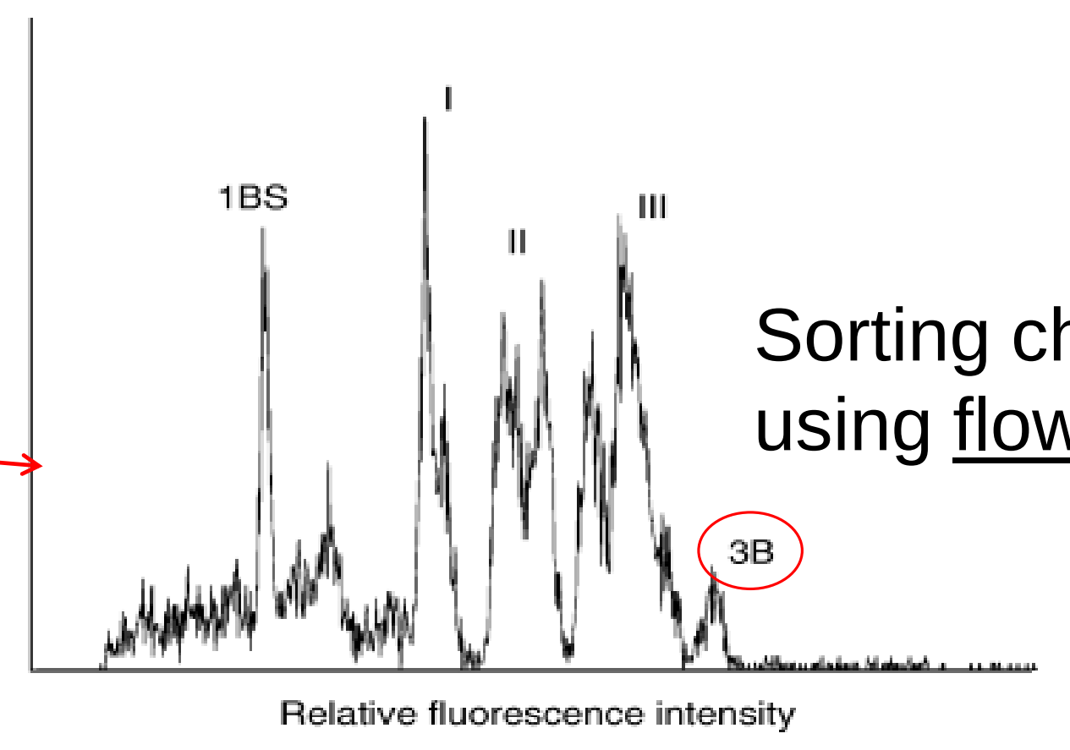
• A chromosome-by-chromosome approach to face the highly complex genome of bread wheat



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

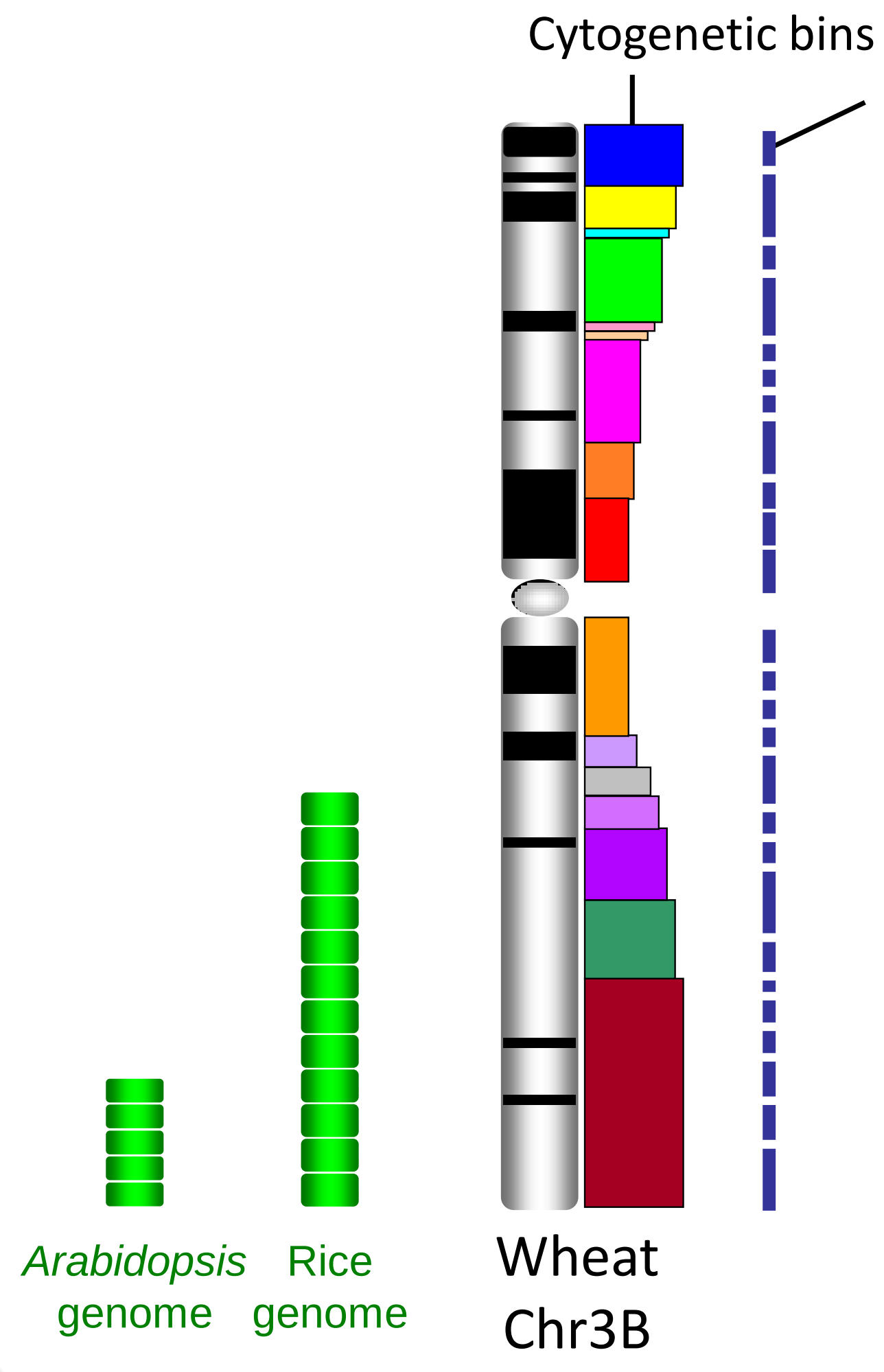


Sorting chromosomes using flow cytometry

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



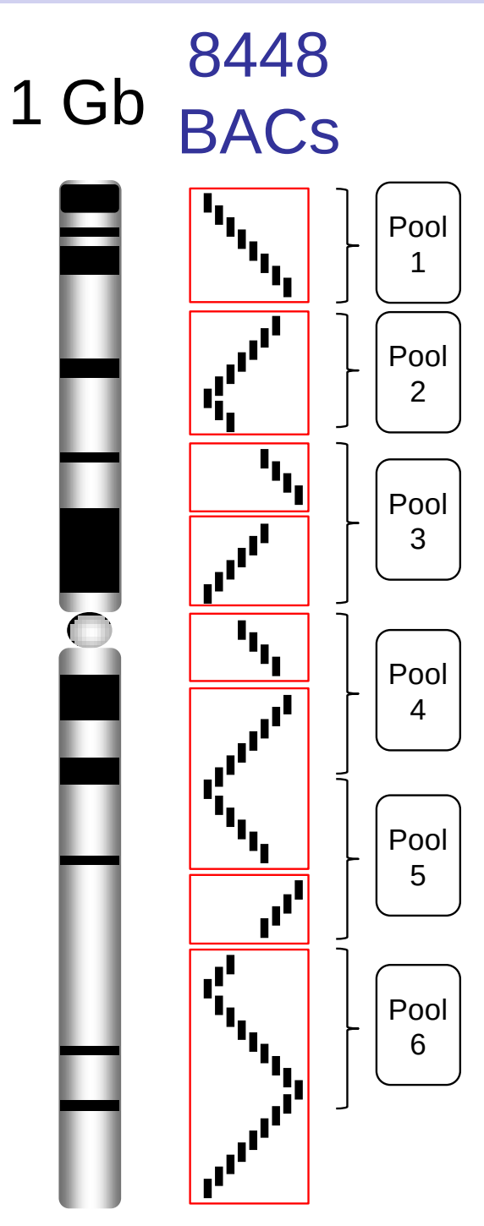
- ❑ 132,000 fingerprinted BACs (19x coverage)
- ❑ 1,282 BAC-contigs (99% of the chromosome)
- ❑ 8448 BAC clones in the Minimal Tilling Path
- ❑ ~4,300 markers assigned to BAC-contigs

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*



928 pools of 10 BACs

928 libraries LPE 8kb

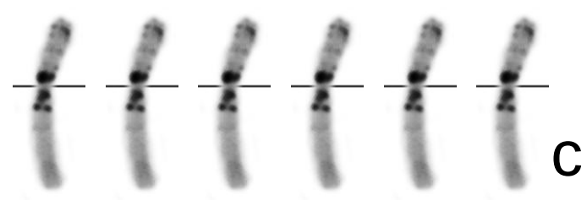
Pool 1 | Pool 2 | Pool 3 | Pool 4 | Pool 5 | Pool 6

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

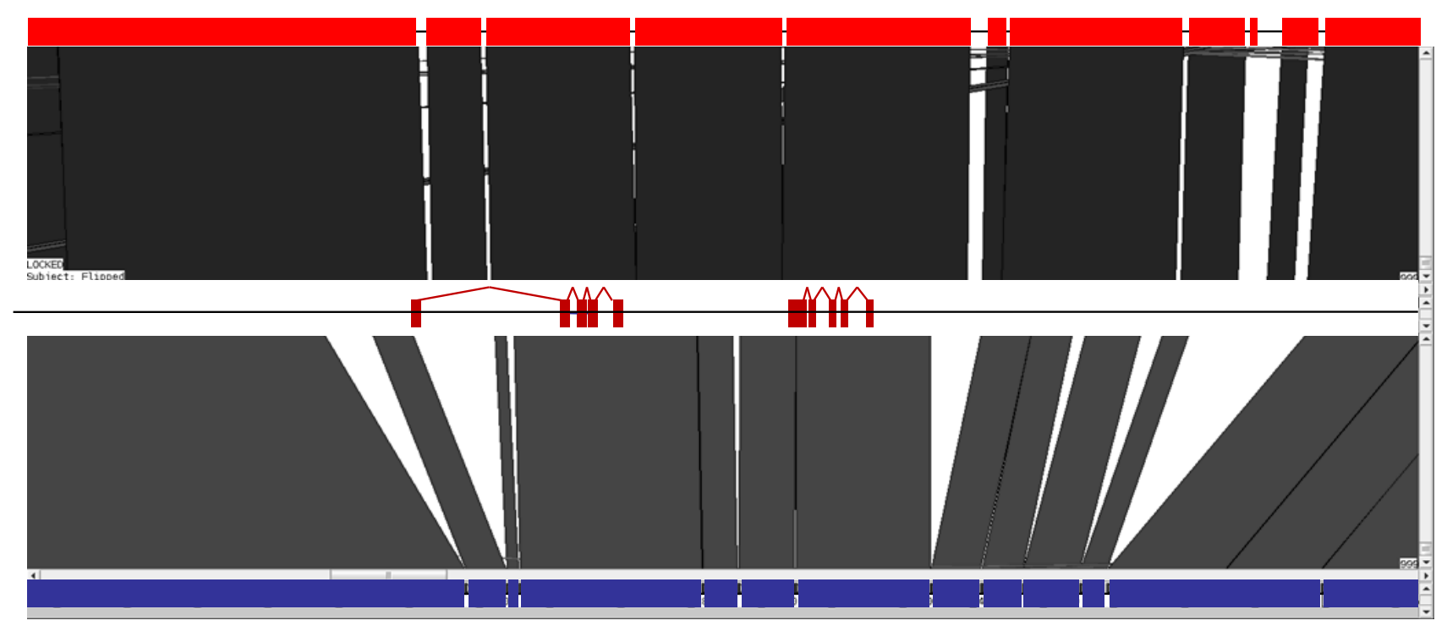
❑ *Roche/454 Sequencing of the Minimal Tilling Path*

	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:



1 scaffold (Roche/454)

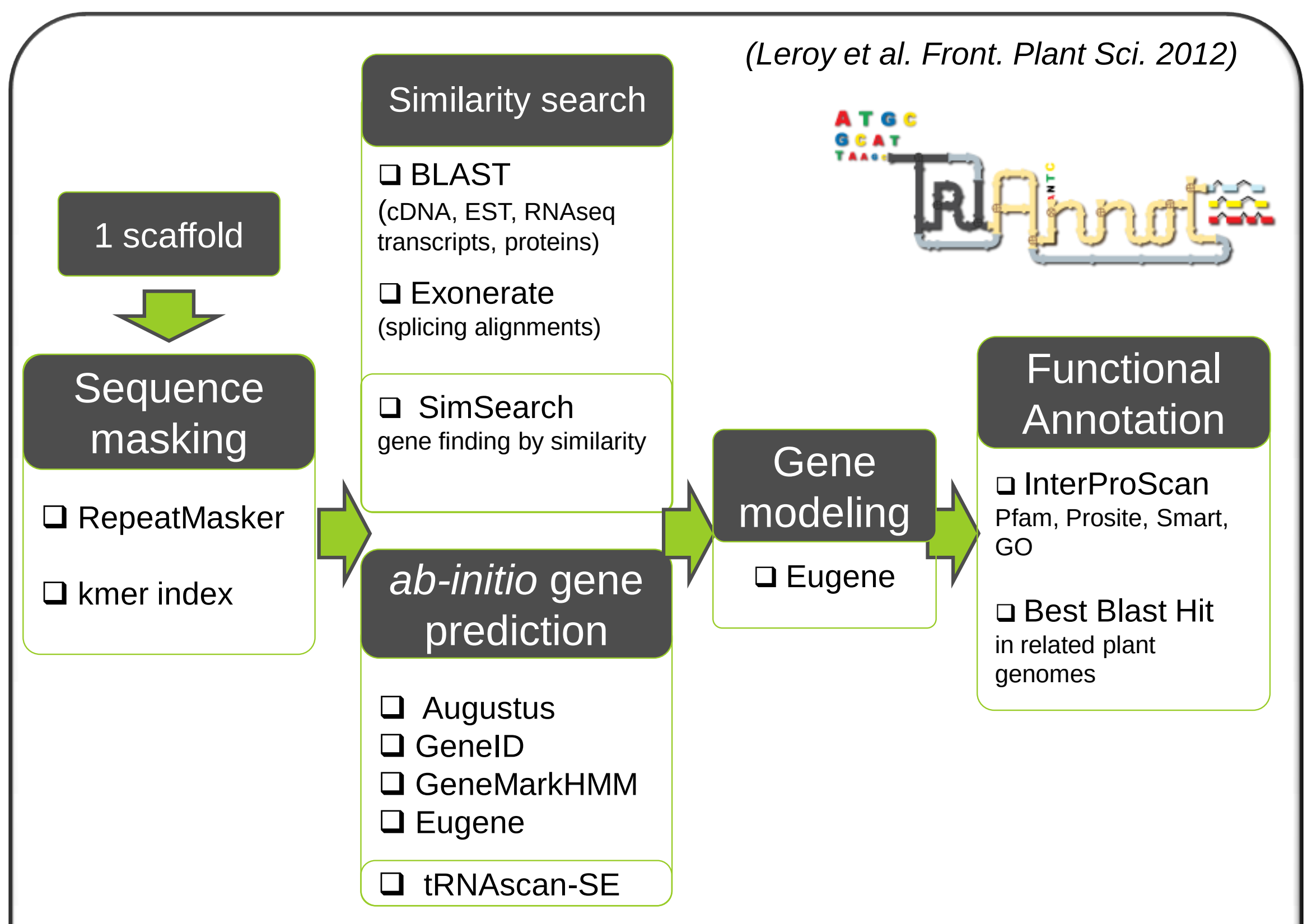
Reference seq. (with 2 genes)

13 unordered contigs (Illumina)

• The TriAnnot pipeline

(http://www.clermont.inra.fr/triannot)

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



1 scaffold

Sequence masking
- ❑ RepeatMasker
- ❑ kmer index

Similarity search
- ❑ BLAST (cDNA, EST, RNAseq transcripts, proteins)
- ❑ Exonerate (splicing alignments)
- ❑ SimSearch gene finding by similarity

***ab-initio* gene prediction**
- ❑ Augustus
- ❑ GeneID
- ❑ GeneMarkHMM
- ❑ Eugene
- ❑ tRNAscan-SE

Gene modeling
- ❑ Eugene

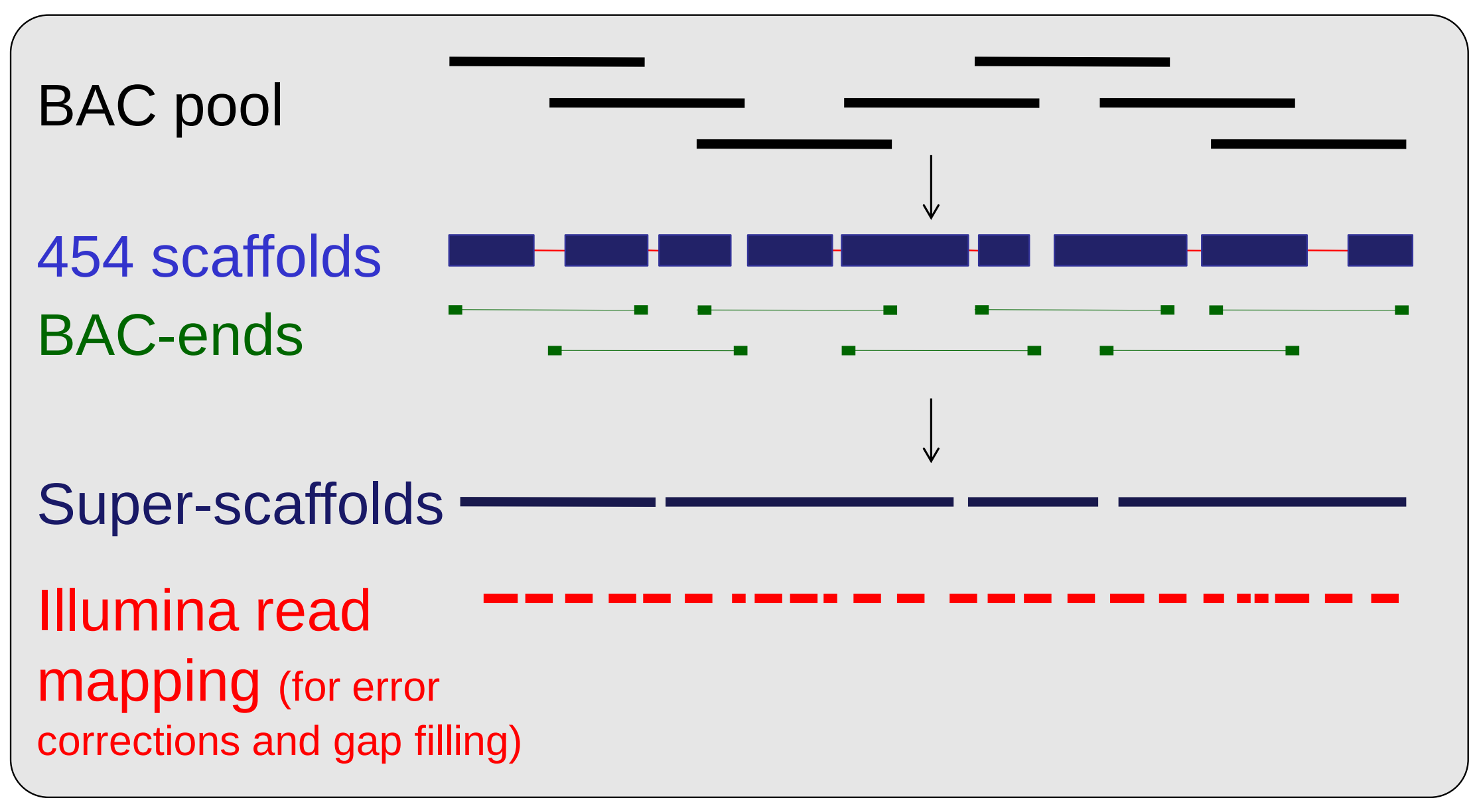
Functional Annotation
- ❑ InterProScan Pfam, Prosite, Smart, GO
- ❑ Best Blast Hit in related plant genomes

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**



BAC pool

454 scaffolds
BAC-ends

Super-scaffolds

Illumina read mapping (for error corrections and gap filling)

• 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

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