Sequencing the genome of the French wheat variety Renan

Frédéric Choulet

To cite this version:

Frédéric Choulet. Sequencing the genome of the French wheat variety Renan. IWGSC Webinar, IWGSC - International Wheat Genome Sequencing Consortium, Sep 2022, Clermont-Ferrand, France. hal-04090088

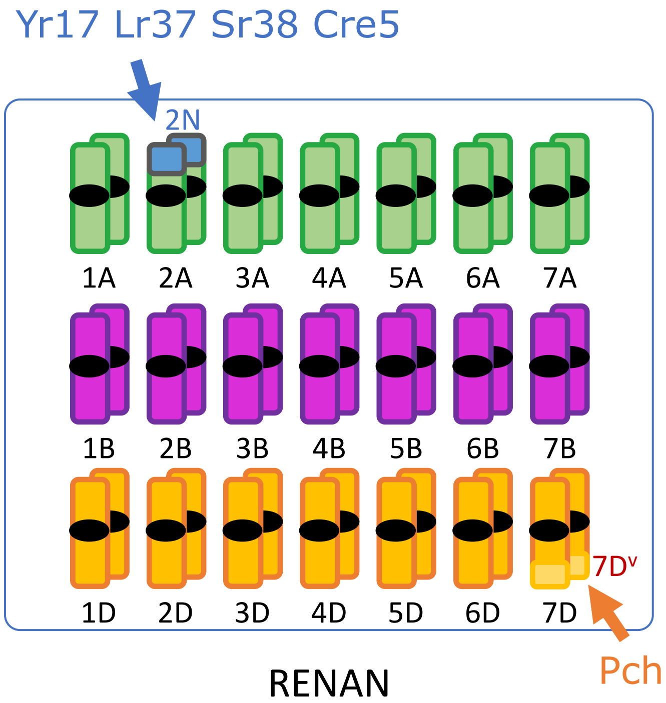
HAL Id: hal-04090088
https://hal.inrae.fr/hal-04090088

Submitted on 5 May 2023

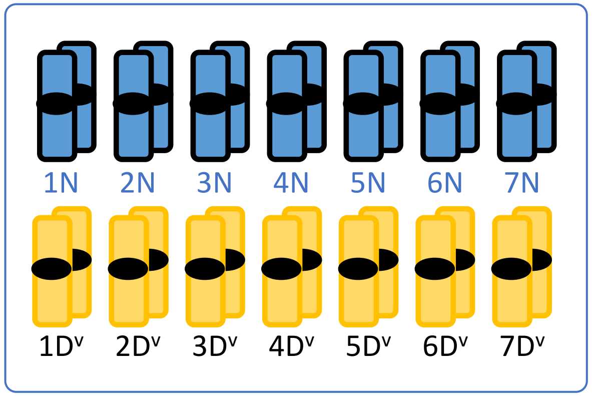
Sequencing the genome of the French wheat variety Renan

- *Frédéric CHOULET*

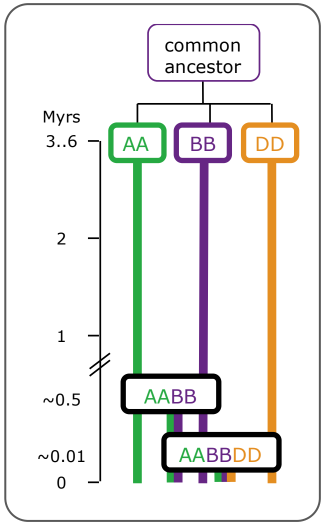
GDEC, INRAE-Univ Clermont-Ferrand



RENAN

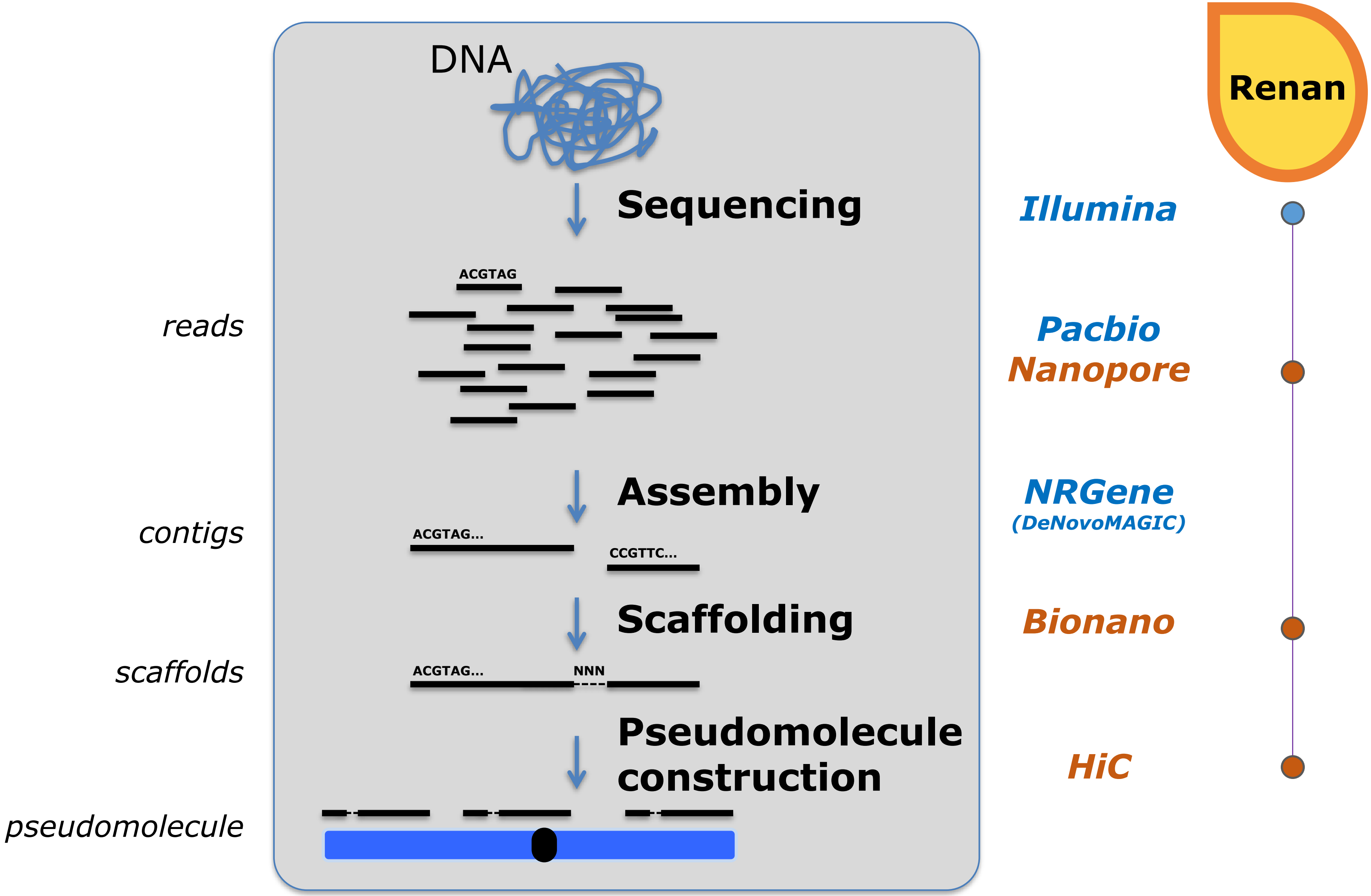


Ae. ventricosa



G. Doussinault, J. Jahier, M. Bernard et al. (1980's 90's 00's)

❑ Genome assembly: steps and techno



Sequencing – *reads* – **Illumina**

Pacbio
Nanopore

Assembly – *contigs* – **NRGene**
(DeNovoMAGIC)

Scaffolding – *scaffolds* – **Bionano**

Pseudomolecule construction – *pseudomolecule* – **HiC**

Renan

❑ *Triticeae* refseq current status (09/2022)

Reference-quality genome assemblies

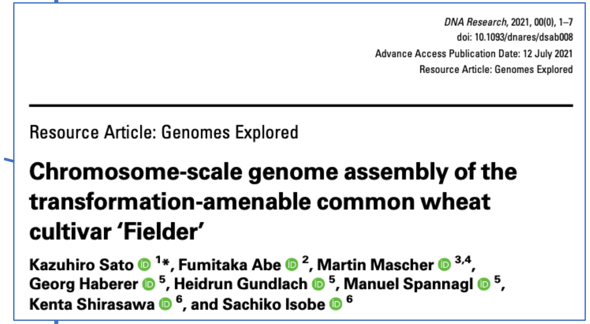
- *T. aestivum*
  - Chinese Spring (NRgene)
  - Arina*LrFor* Jagger Julius CDC_Lancer CDC_Landmark Mace Norin61 Stanley SY-Mattis Spelta (NRGene)
  - Zang1817 (NRGene)
  - Fielder (Pacbio HiFi)
  - Kariega (Pacbio HiFi)
  - Renan (ONT)
  - Attraktion (Pacbio HiFi)
- *T. urartu* AA
- *T. durum* AABB
- *T. dicoccoides* AABB
- *Ae. tauschii* DD
- *Ae. searsii* DD
- *Ae. bicornis* DD
- *Ae. longissima* DD
- *Ae. sharonensis* DD
- *Ae. speltoides* SS

Article | Open Access | Published: 08 October 2020

Origin and adaptation to high altitude of Tibetan semi-wild wheat

Weilong Guo, Mingming Xin, Zihao Wang, Yingyin Yao, Zhaorong Hu, Wanjun Song, Kuohai Yu, Yongming Chen, Xiaobo Wang, Panfeng Guan, Rudi Appels, Huiru Peng ✉, Zhongfu Ni ✉ & Qixin Sun ✉

Nature Communications **11**, Article number: 5085 (2020) | Cite this article

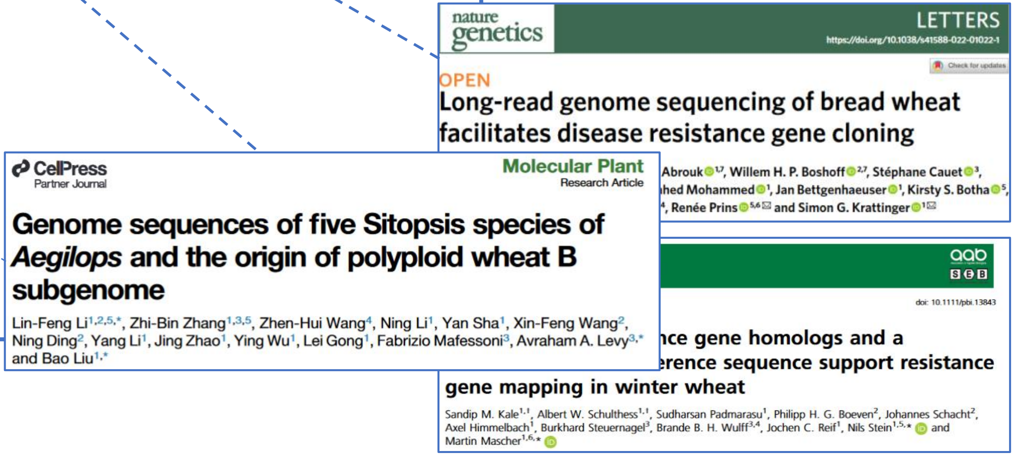


DNA Research, 2021, 00(0), 1–7
doi: 10.1093/dnares/dsab008
Advance Access Publication Date: 12 July 2021
Resource Article: Genomes Explored

Resource Article: Genomes Explored

Chromosome-scale genome assembly of the transformation-amenable common wheat cultivar ‘Fielder’

Kazuhiro Sato[1*], Fumitaka Abe[2], Martin Mascher[3,4], Georg Haberer[5], Heidrun Gundlach[5], Manuel Spannagl[5], Kenta Shirasawa[6], and Sachiko Isobe[6]



https://doi.org/10.1038/s41588-022-01022-1

OPEN

Long-read genome sequencing of bread wheat facilitates disease resistance gene cloning

Abrouk[1,7], Willem H. P. Boshoff[2,7], Stéphane Cauet[3],
hed Mohammed[1], Jan Bettgenhaeuser[1], Kirsty S. Botha[5],
[4], Renée Prins[5,6] ✉ and Simon G. Krattinger[1] ✉

CellPress Partner Journal

Molecular Plant
Research Article

Genome sequences of five Sitopsis species of *Aegilops* and the origin of polyploid wheat B subgenome

Lin-Feng Li[1,2,5,*], Zhi-Bin Zhang[1,3,5], Zhen-Hui Wang[4], Ning Li[1], Yan Sha[1], Xin-Feng Wang[2], Ning Ding[2], Yang Li[1], Jing Zhao[1], Ying Wu[1], Lei Gong[1], Fabrizio Mafessoni[3], Avraham A. Levy[3,*] and Bao Liu[1,*]

doi: 10.1111/pbi.13843

nce gene homologs and a
erence sequence support resistance
gene mapping in winter wheat

Sandip M. Kale[1,†], Albert W. Schulthess[1,†], Sudharsan Padmarasu[1], Philipp H. G. Boeven[2], Johannes Schacht[2], Axel Himmelbach[1], Burkhard Steuernagel[3], Brande B. H. Wulff[3,4], Jochen C. Reif[1], Nils Stein[1,5,*] and Martin Mascher[1,6,*]

GigaScience, 2022, **11**, 1–18
DOI: 10.1093/gigascience/giac034
RESEARCH

Long-read and chromosome-scale assembly of the hexaploid wheat genome achieves high resolution for research and breeding

Jean-Marc Aury[1,*], Stefan Engelen[1], Benjamin Istace[1], Cécile Monat[2], Pauline Lasserre-Zuber[2], Caroline Belser[1], Corinne Cruaud[3], Hélène Rimbert[2], Philippe Leroy[2], Sandrine Arribat[4], Isabelle Dufau[4], Arnaud Bellec[4], David Grimbichler[5], Nathan Papon[2], Etienne Paux[2], Marion Ranoux[2], Adriana Alberti[1,6], Patrick Wincker[1] and Frédéric Choulet[2,*]

[1]Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France
[2]GDEC, Université Clermont Auvergne, INRAE, UMR1095, 63000 Clermont-Ferrand, France
[3]Commissariat à l'Energie Atomique (CEA), Institut François Jacob, Genoscope, F-91057 Evry, France
[4]INRAE, CNRGV French Plant Genomic Resource Center, F-31320, Castanet Tolosan, France
[5]Mésocentre Clermont Auvergne, DOSI / Bâtiment Turing, 7 avenue Blaise Pascal, 63178 Aubière, France
[6]Present address: Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France
[*]Correspondence address. Jean-Marc Aury, Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France. E-mail: jmaury@genoscope.cns.fr; Frédéric Choulet, GDEC, Université Clermont Auvergne, INRAE, UMR1095, 63000 Clermont-Ferrand, France. E-mail: frederic.choulet@inrae.fr

Abstract

Background: The sequencing of the wheat (*Triticum aestivum*) genome has been a methodological challenge for many years owing to its large size (15.5 Gb), repeat content, and hexaploidy. Many initiatives aiming at obtaining a reference genome of cultivar Chinese Spring have been launched in the past years and it was achieved in 2018 as the result of a huge effort to combine short-read sequencing with many other resources. Reference-quality genome assemblies were then produced for other accessions, but the rapid evolution of sequencing technologies offers opportunities to reach high-quality standards at lower cost.

Results: Here, we report on an optimized procedure based on long reads produced on the Oxford Nanopore Technology PromethION device to assemble the genome of the French bread wheat cultivar Renan.

Conclusions: We provide the most contiguous chromosome-scale assembly of a bread wheat genome to date. Coupled with an annotation based on RNA-sequencing data, this resource will be valuable for the crop community and will facilitate the rapid selection of agronomically important traits. We also provide a framework to generate high-quality assemblies of complex genomes using ONT.

Keywords: wheat, hexaploid genome, long-reads, nanopore sequencing, genome assembly, haplotype characterization, introgressions

❑ *Step#1: sequencing*

NovaSeq
30x

Illumina

MinION 2 runs

PromethION 18 runs

- Total 63x
- Select **30x** with longer reads
  (reads>50kb ➔ 15x)

Nanopore

Renan

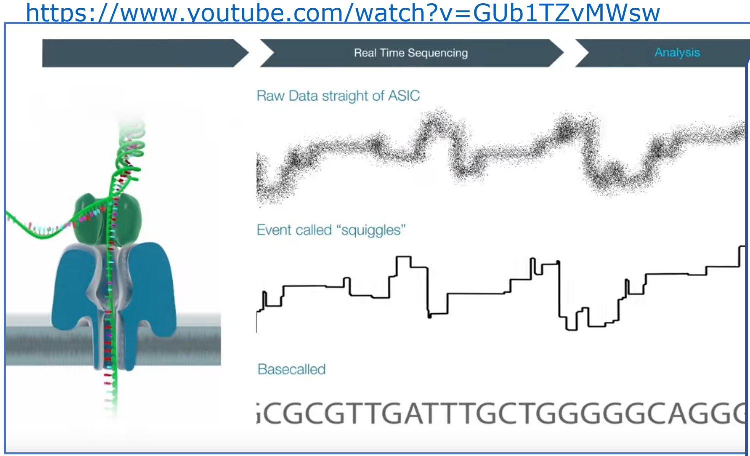
Bionano

HiC

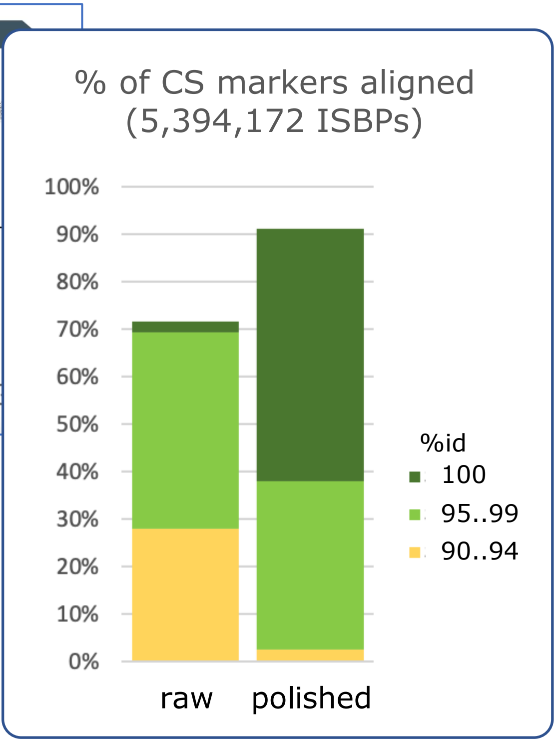
❑ *Step#2: read assembly*

Renan

https://www.youtube.com/watch?v=GUb1TZvMWsw



Early stages
-> error rate in reads: 12%
-> error rate in assembly: 4%



Nanopore

- ○ basecalling
- ○ polishing

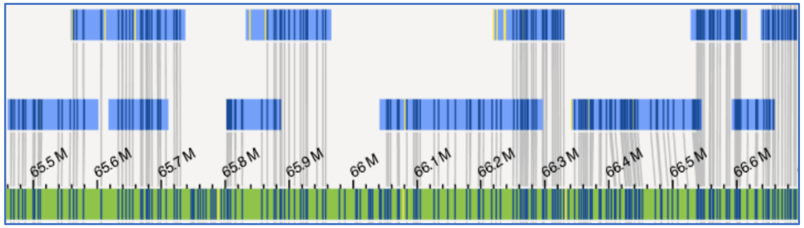
Illumina
30x

ACGTAGACGTACGGCTGCATCGCATTCATCGGCGGGGGGGGCTGCTCGTCGCTCCTCGT assembly

❑ *Step#3: scaffolding*

Renan

11 sequence contigs



1 optical contig

↓

1 scaffold

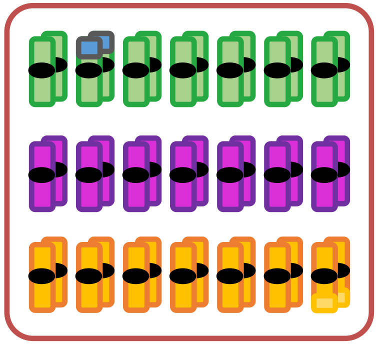
Illumina

Nanopore

Bionano

HiC

❑ *Assembly metrics*



- ❖ **Metrics**
  - ➢ Genome size=14.3 Gb
  - ➢ 2904 scaffolds (=138/chr)

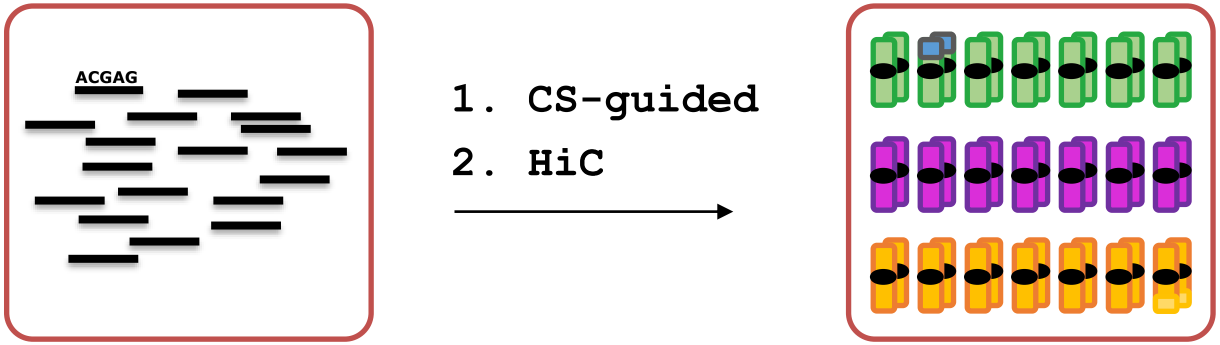
- ❖ **Quality Assessment**

  Genes: 98% CS genes found

  TEs: 92% CS ISBPs found

  QV: lower (99.95% accuracy) than NRGene-based assemblies (99.99%)

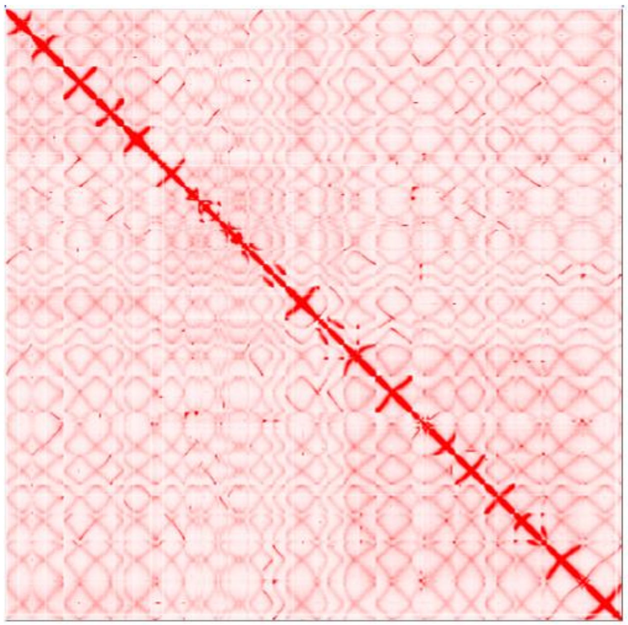
❑ ***Step#4: pseudomolecule construction***



```
1. CS-guided
2. HiC
```

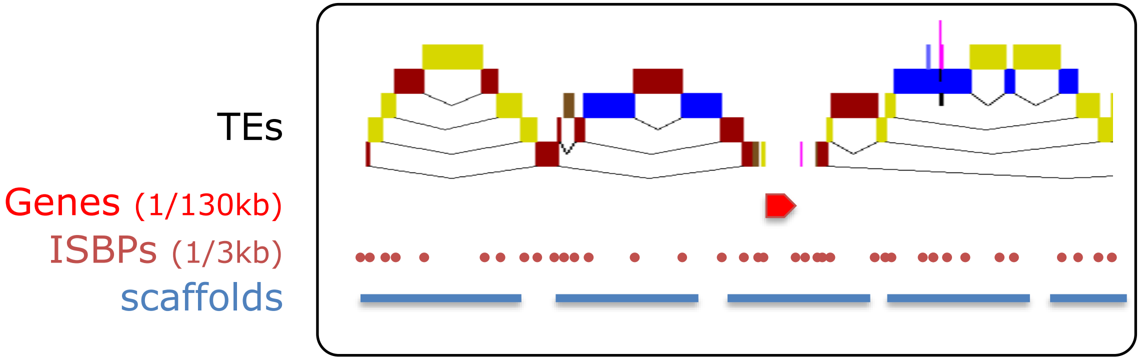
2904 scaff

21 chromosomes



X not efficient enough to build a chromosome-scale assembly

❑ *Step#4-1: CS-guided pseudomol construction*

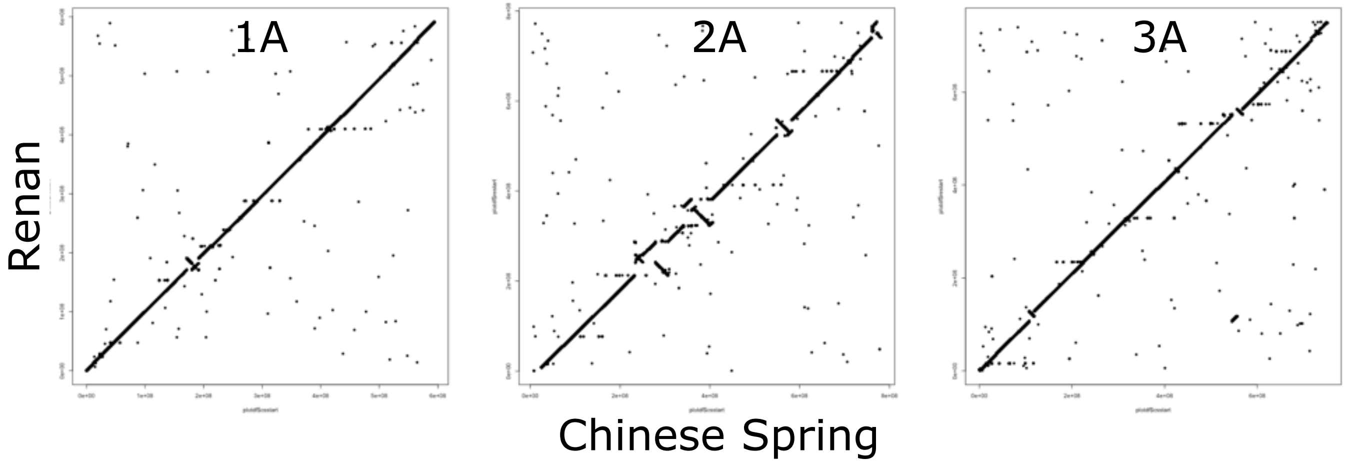


TEs

Genes (1/130kb)

ISBPs (1/3kb)

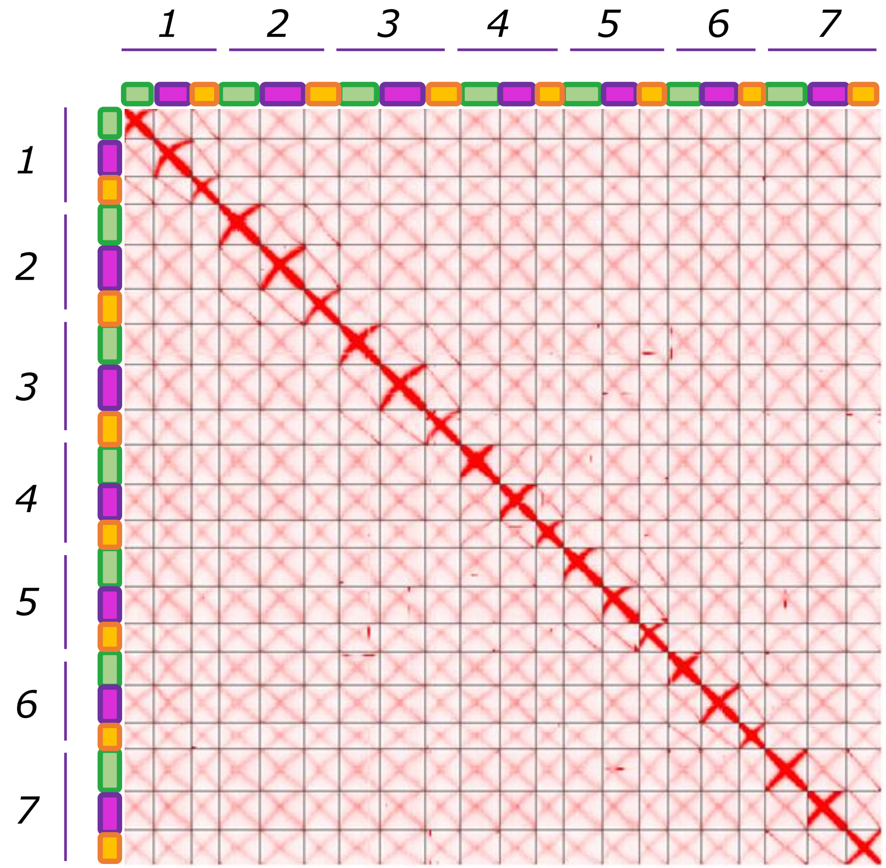
scaffolds



⇒ 14.2 Gb (0.1 Gb unanchored)

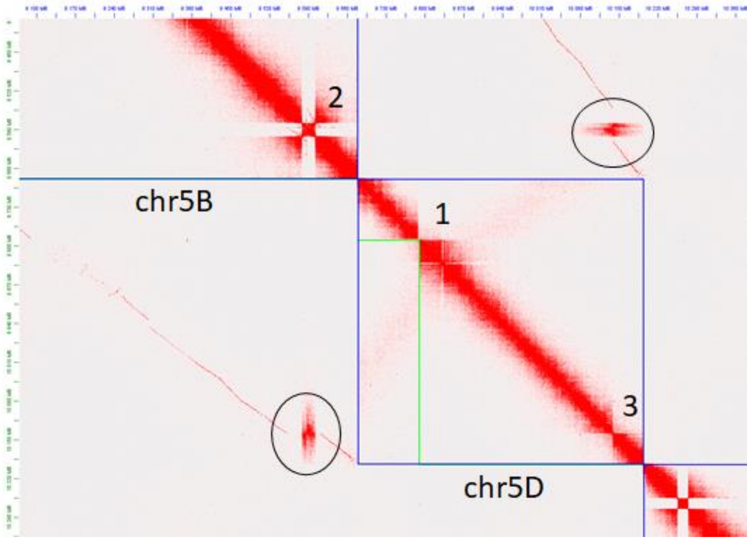
- ☐ Step#4-1: CS-guided pseudomol construction
- ☐ **Step#4-2: HiC corrections**

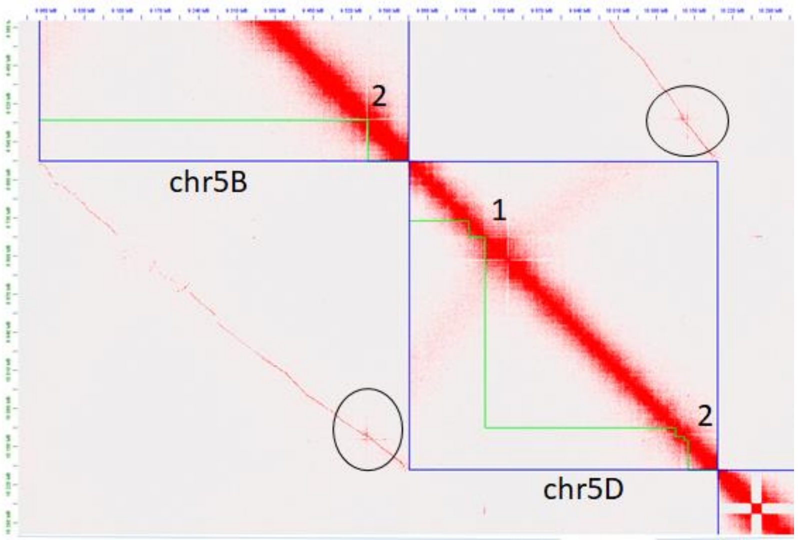
Renan



HiC

Step#4-2: HiC corrections

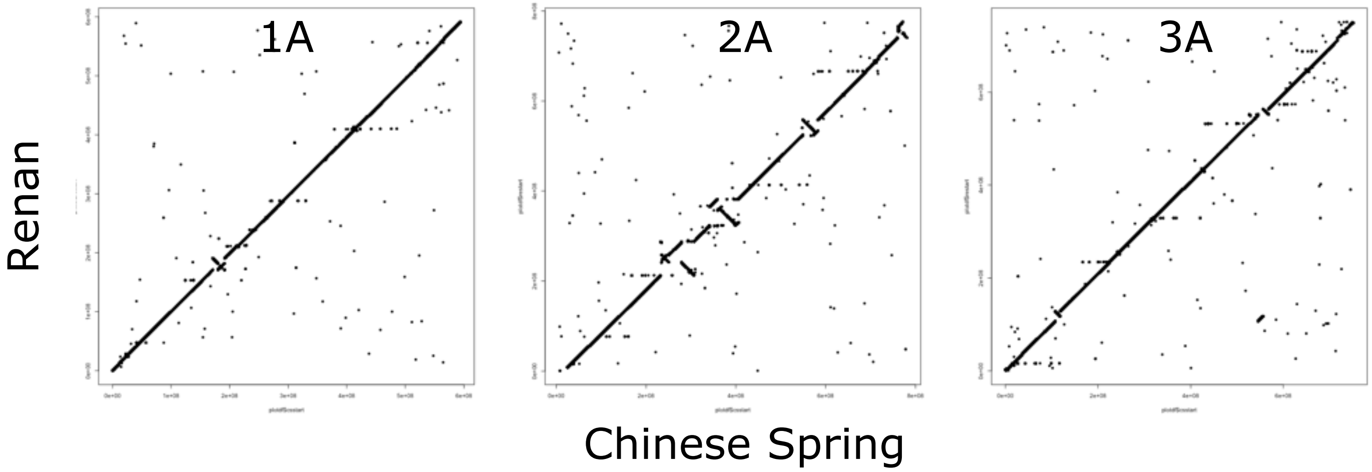




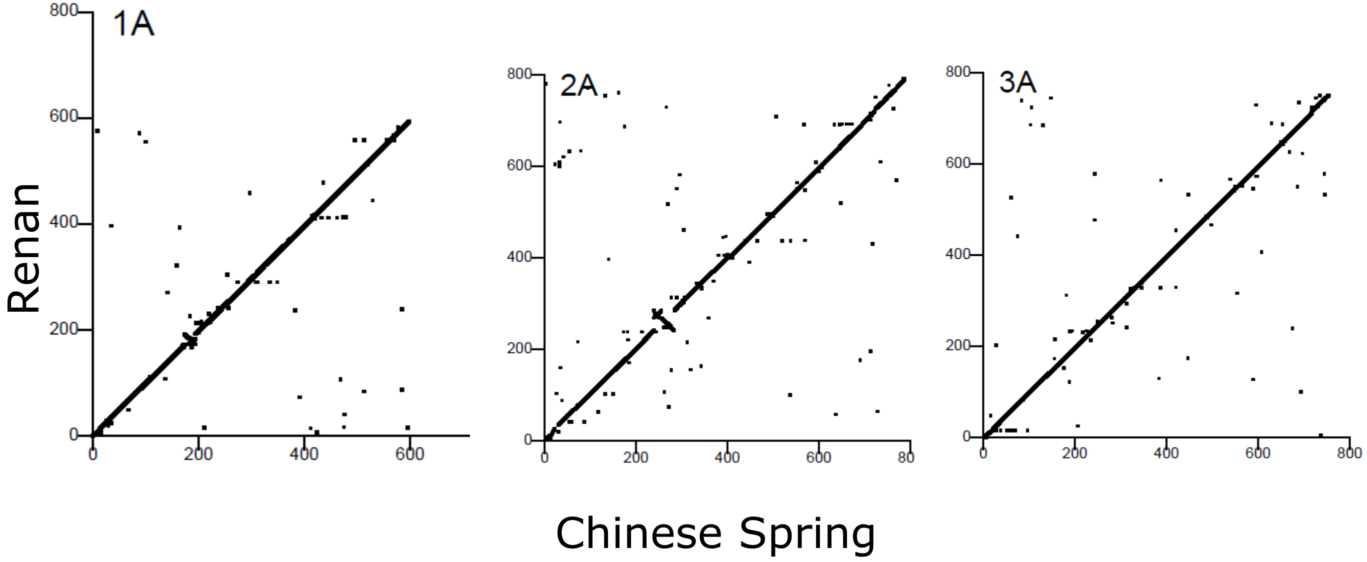
- **18** chimeric scaff
- **198** scaff with correction of location and/or orientation

↓

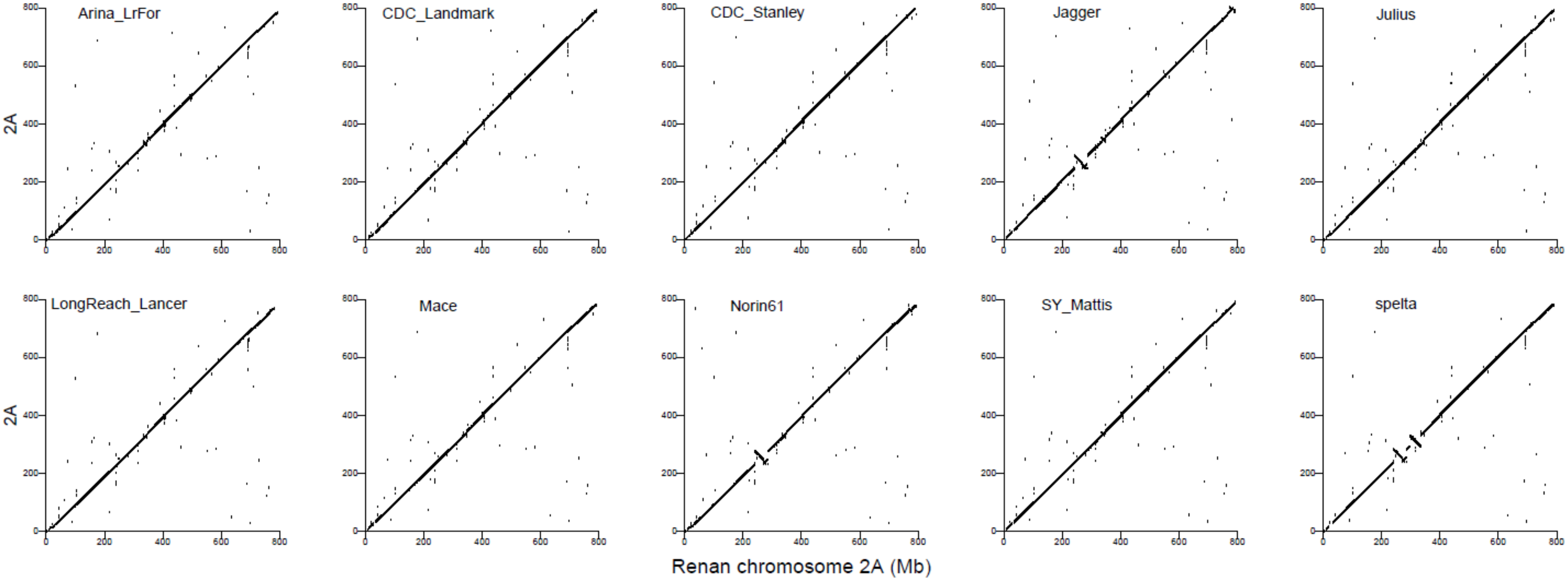
21 chr + chrUn 61 Mb (<1%)

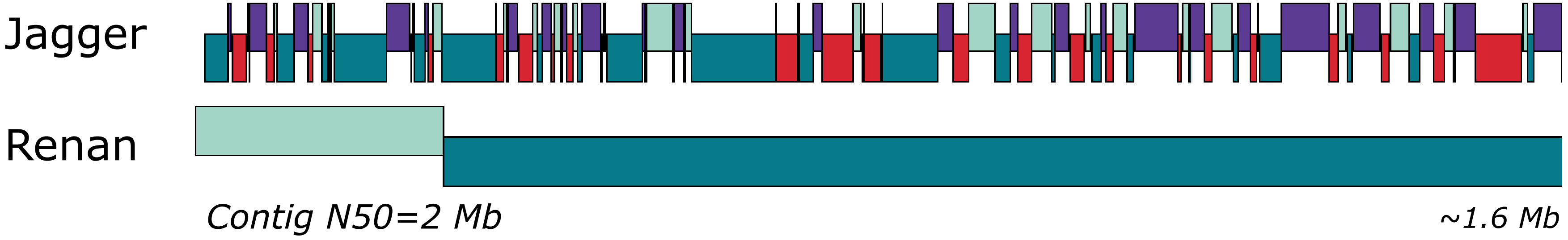


After HiC-based corrections

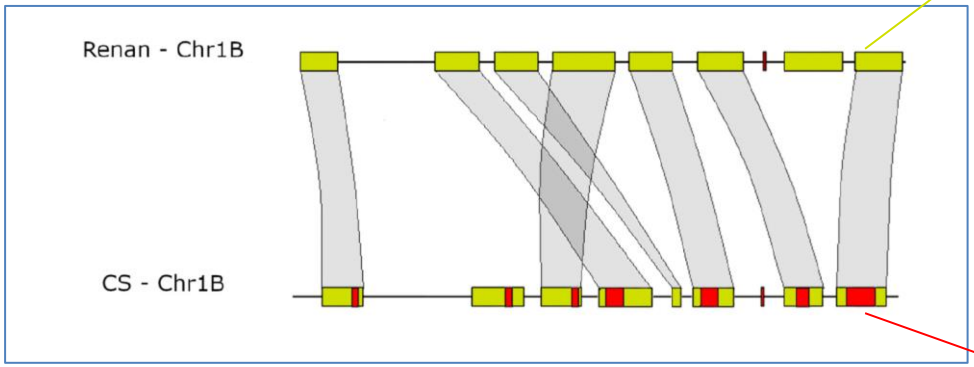


Renan Refseq_v2 *versus* 10 wheat genomes





ω-gliadin gene cluster



ATGAAGACCTTCATCATATTTGTCCTCCTTGCTATGGTGATGAGCATCGCCAGTGCCACTAGGCAACTAA
GCCCTAGAAGCAAGGAGTTGCAAACACCACAAGAACAATTCCCCCAACAGCAATTTCCTCAACCACAACA
AATCCTC**CAACAACAATTCCCCCAACAACAACAAATCCCCCAGCAACCGCAACAATTCCCCCAACAACAA**
ATCCCGCAACAGCAAATCCCGCAACAACAAATCCCCCAACAACAACAAATCCCCCAGCAACCACAACAAT
TCCCCCAACAACAGCAATTCCCCCAACAACAACAAATCCCCCAGCAACCACAACAATTCCCCCAACAACA
GCAATTCCCCCAACAACAACAAATCCTCCAGCAACCACAACAATTCCCCCAACAACAGCAATTCCCCCAA
ACAATTACCCCAACAACAATACCCCGAACA // AACAATTCCCCCAACAGCAATTCCCCCAACAACAAC
AATTTCCCCAACAACACCAGTTACCGCAACAACAATTCCCCCAACAACAACAAATCTCCGAGCAACCACA
ACAATTCCCCCAACAACAACAATTCCCCCAACAACAACAATTCCCCCAACAGCAATTCCCGCAACAACAA
TTTCCCCAACAACAACAATTCCCACAACAACAACAGTTCCCTCAACAACAACAATTCCCGCAACAACAAA
TCCCTGAACAACAATAATTCCCCCAACAACAATTCCCCCAGCAACCATCACAACAATTTCCCCAACAACA
ATTTCCAATACCATACCCACCCCAACAATCACAAGAACCTTCCCCATACCAACAATATCCACAACAACAA
CCATATGGGAGCAACGTTATAAGTATCAGTGGCCGATGA

ATGAAGACCTTCATCATATTTGTCCTCCTTGCTATGGTGATGAGCATCGCCAGTGCCACTAGGCAACTAA
GCCCTAGAAGCAAGGAGTTGCAAACACCACAAGAACAATTCCCCCAACAGCAATTTCCTCAACCACAACA
AATCCTCCAACAACAATTCCCCCAACAACAACAAATCCCCCAGCAACCGCAACAATTCCCCCAACAACAA
ATCCCGCAACAGCAAATCCCGCAACAACAAATCCCCCAACAACAACNNNNNNNNNNNNNNNNNNNNNNNN
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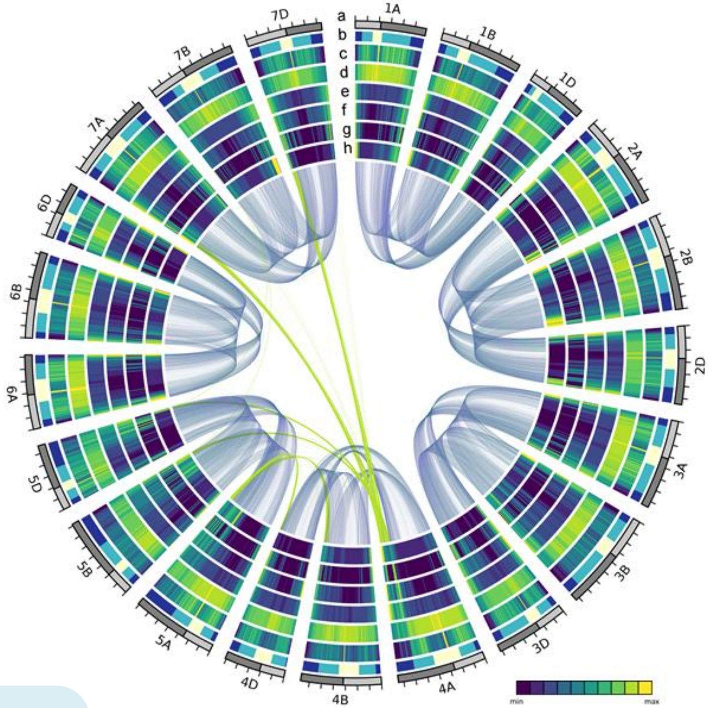
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNACAACAATTCCCCCAACAACCACAACAACAATTCCCCCAACAACAATTTCCCATACCATACC
CACCCCAACAACAATCACAAGAAGCTTCCCCATACCAACAATACCACAACAACAACTATCTGGGAGCGAC
GTTATAA

○ Predicting genes is still NOT routine

GDEC Clermont, France – *Rimbert Leroy Choulet et al.*
PGSB Munich, Germany – *Spannagl Twardziok et al.*
EI Norwich, UK – *Swarbreck Venturini et al.*

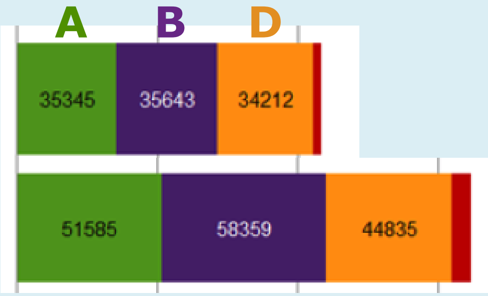


IWGSC RefSeq (Chinese Spring)

- 107,891 genes

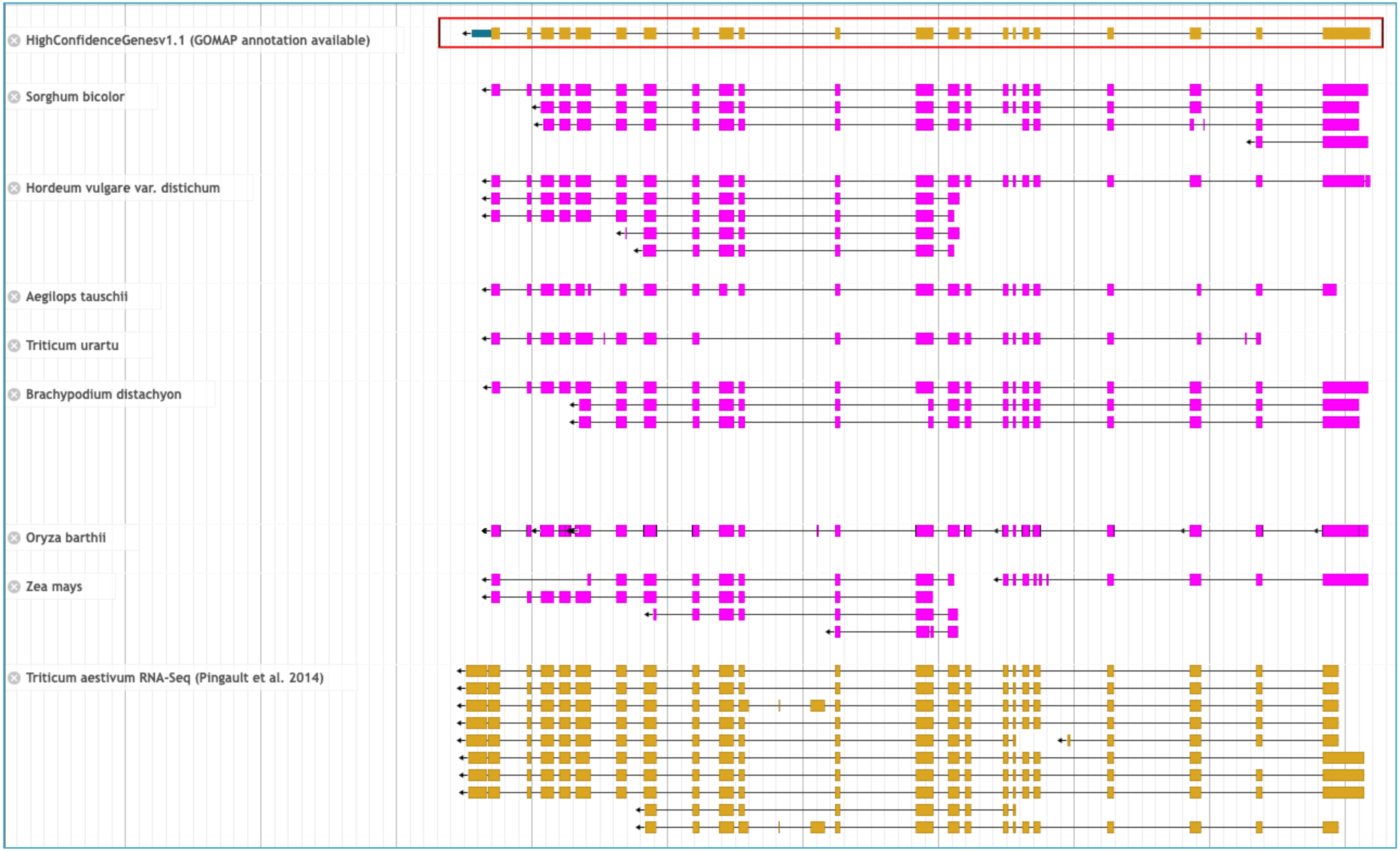
HC genes

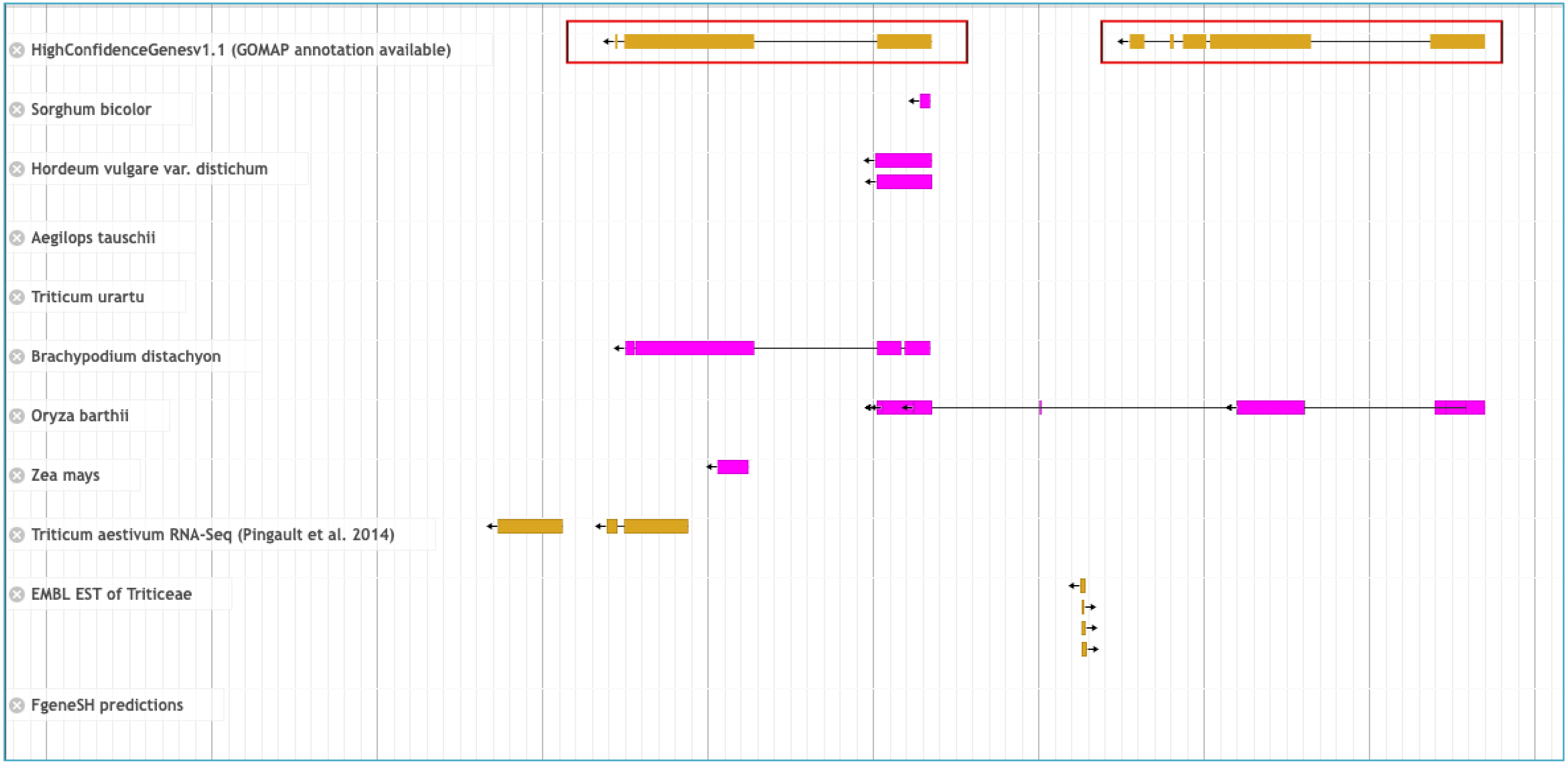
LC genes



107k

161k


HighConfidenceGenesv1.1 (GOMAP annotation available)
Sorghum bicolor
Hordeum vulgare var. distichum
Aegilops tauschii
Triticum urartu
Brachypodium distachyon
Oryza barthii
Zea mays
Triticum aestivum RNA-Seq (Pingault et al. 2014)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Sorghum bicolor
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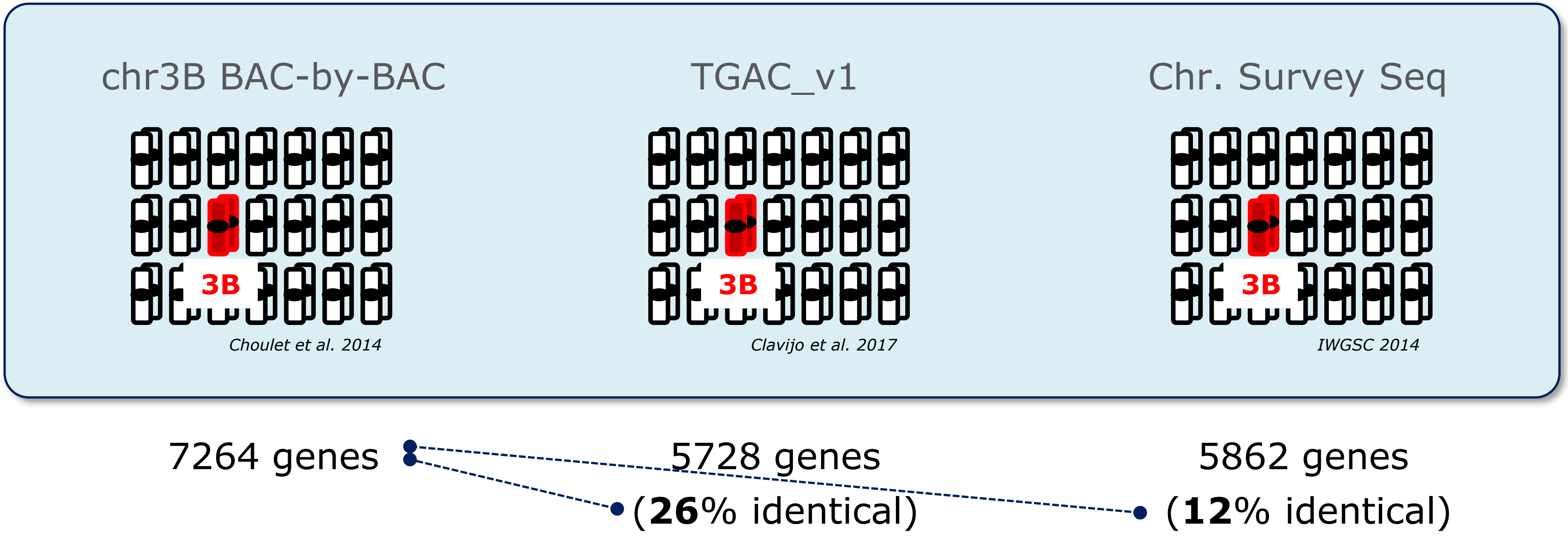
Triticum urartu
Brachypodium distachyon
Oryza barthii
Zea mays
Triticum aestivum RNA-Seq (Pingault et al. 2014)
EMBL EST of Triticeae
FgeneSH predictions



chr3B BAC-by-BAC

Choulet et al. 2014

7264 genes

TGAC_v1

Clavijo et al. 2017

5728 genes

(**26**% identical)

Chr. Survey Seq

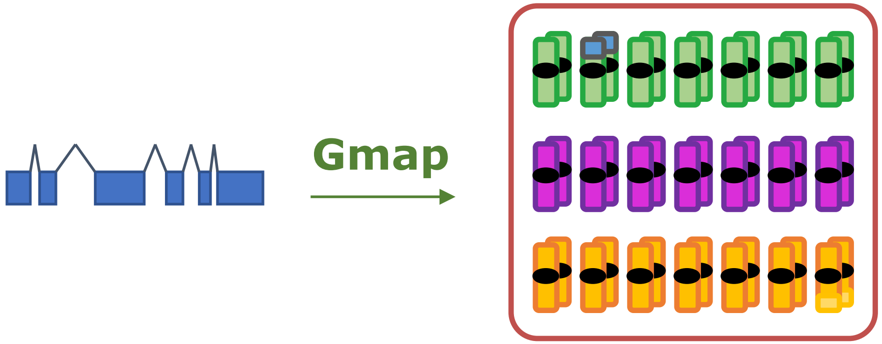
IWGSC 2014

5862 genes

(**12**% identical)

- *denovo* gene annotation not adapted to pangenomics

○ Gene projections / Annotation transfer



Gmap

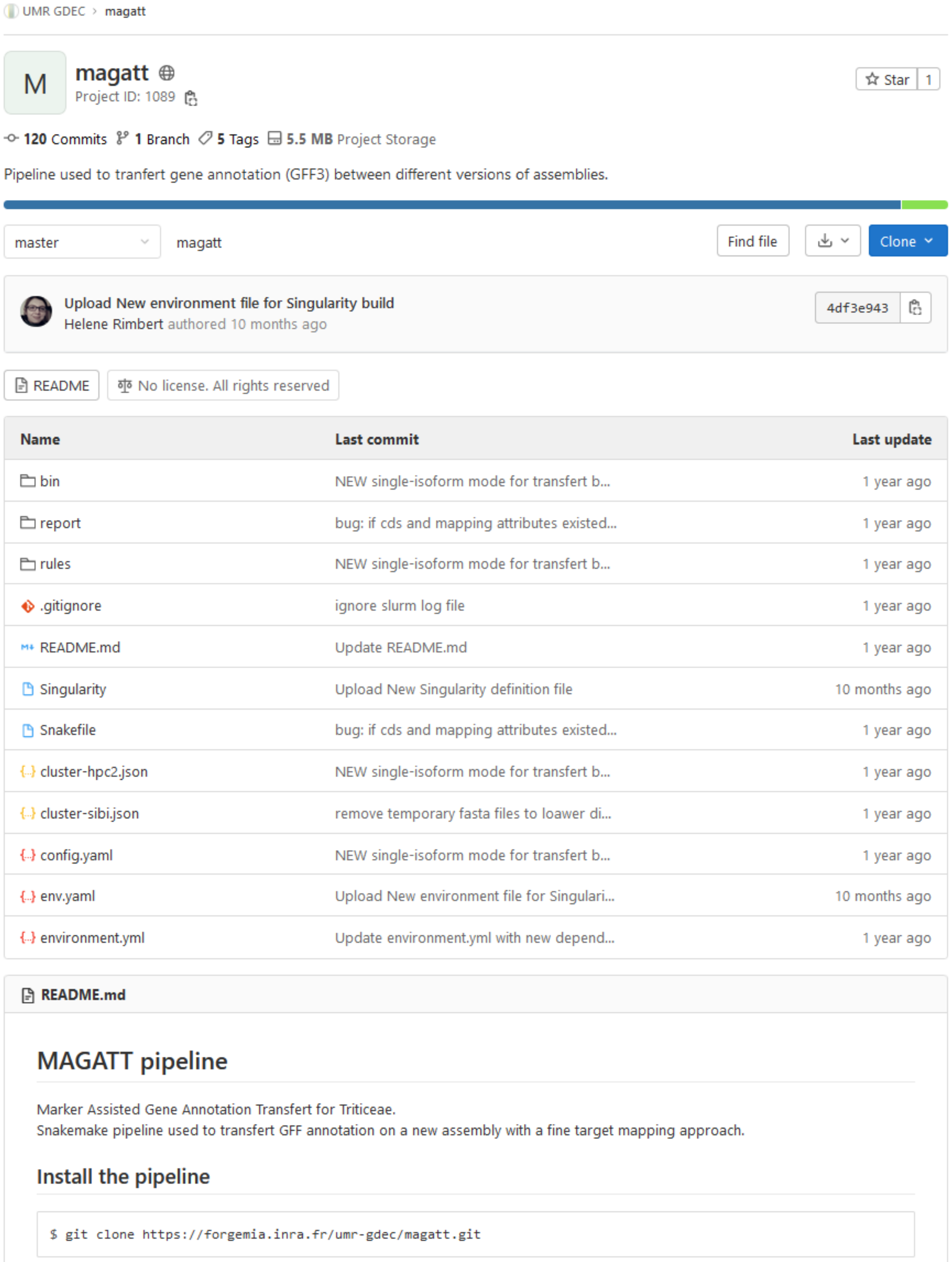
Chinese Spring

Reference annotation

Renan

~12% ambiguous mapping

- ➢ *denovo* gene annotation not adapted to pangenomics
- ➢ Gmap *vs* whole genome introduces mis-alignment errors
  - ➢ Develop **MAGATT** pipeline



magatt

Project ID: 1089

120 Commits 1 Branch 5 Tags 5.5 MB Project Storage

Pipeline used to tranfert gene annotation (GFF3) between different versions of assemblies.

Upload New environment file for Singularity build
Helene Rimbert authored 10 months ago

`4df3e943`

Name	Last commit	Last update
bin	NEW single-isoform mode for transfert b...	1 year ago
report	bug: if cds and mapping attributes existed...	1 year ago
rules	NEW single-isoform mode for transfert b...	1 year ago
.gitignore	ignore slurm log file	1 year ago
README.md	Update README.md	1 year ago
Singularity	Upload New Singularity definition file	10 months ago
Snakefile	bug: if cds and mapping attributes existed...	1 year ago
cluster-hpc2.json	NEW single-isoform mode for transfert b...	1 year ago
cluster-sibi.json	remove temporary fasta files to loawer di...	1 year ago
config.yaml	NEW single-isoform mode for transfert b...	1 year ago
env.yaml	Upload New environment file for Singulari...	10 months ago
environment.yml	Update environment.yml with new depend...	1 year ago

README.md

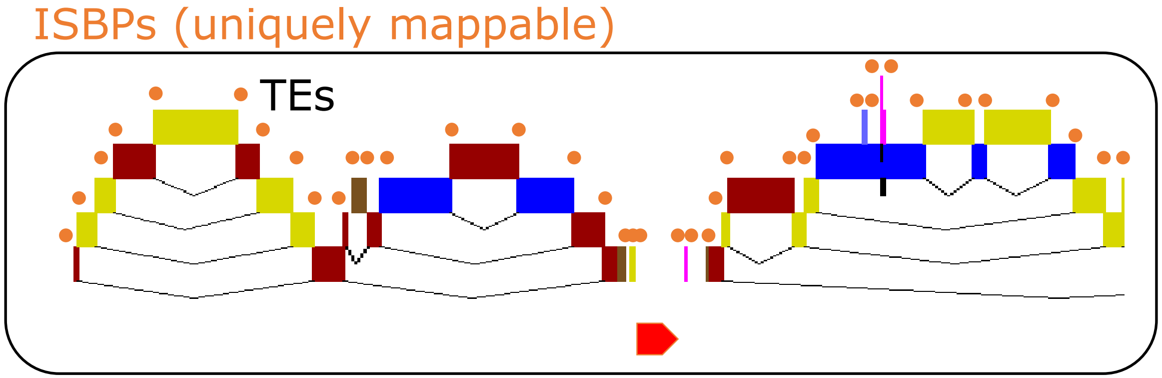
MAGATT pipeline

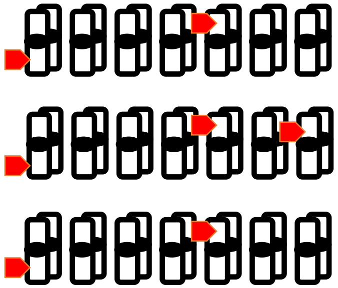
Marker Assisted Gene Annotation Transfert for Triticeae.
Snakemake pipeline used to transfert GFF annotation on a new assembly with a fine target mapping approach.

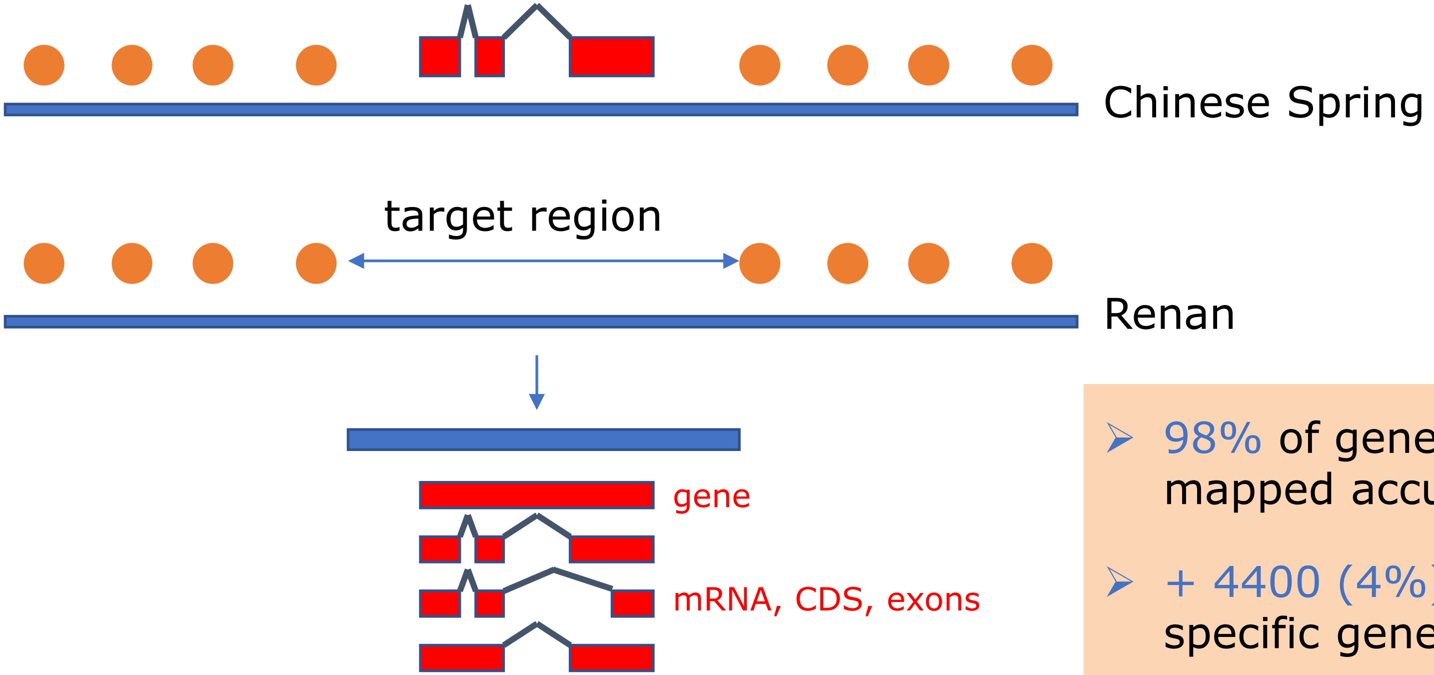
Install the pipeline

```
$ git clone https://forgemia.inra.fr/umr-gdec/magatt.git
```

❑ **MAGATT** (**M**arker-**A**ssisted **G**ene **A**nnotation **T**ransfer for ***T**riticeae*)

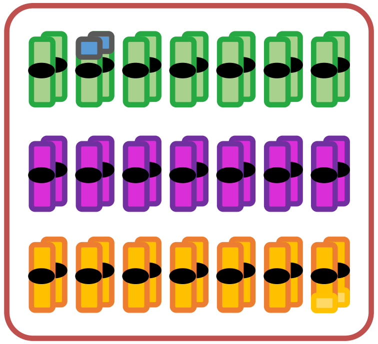


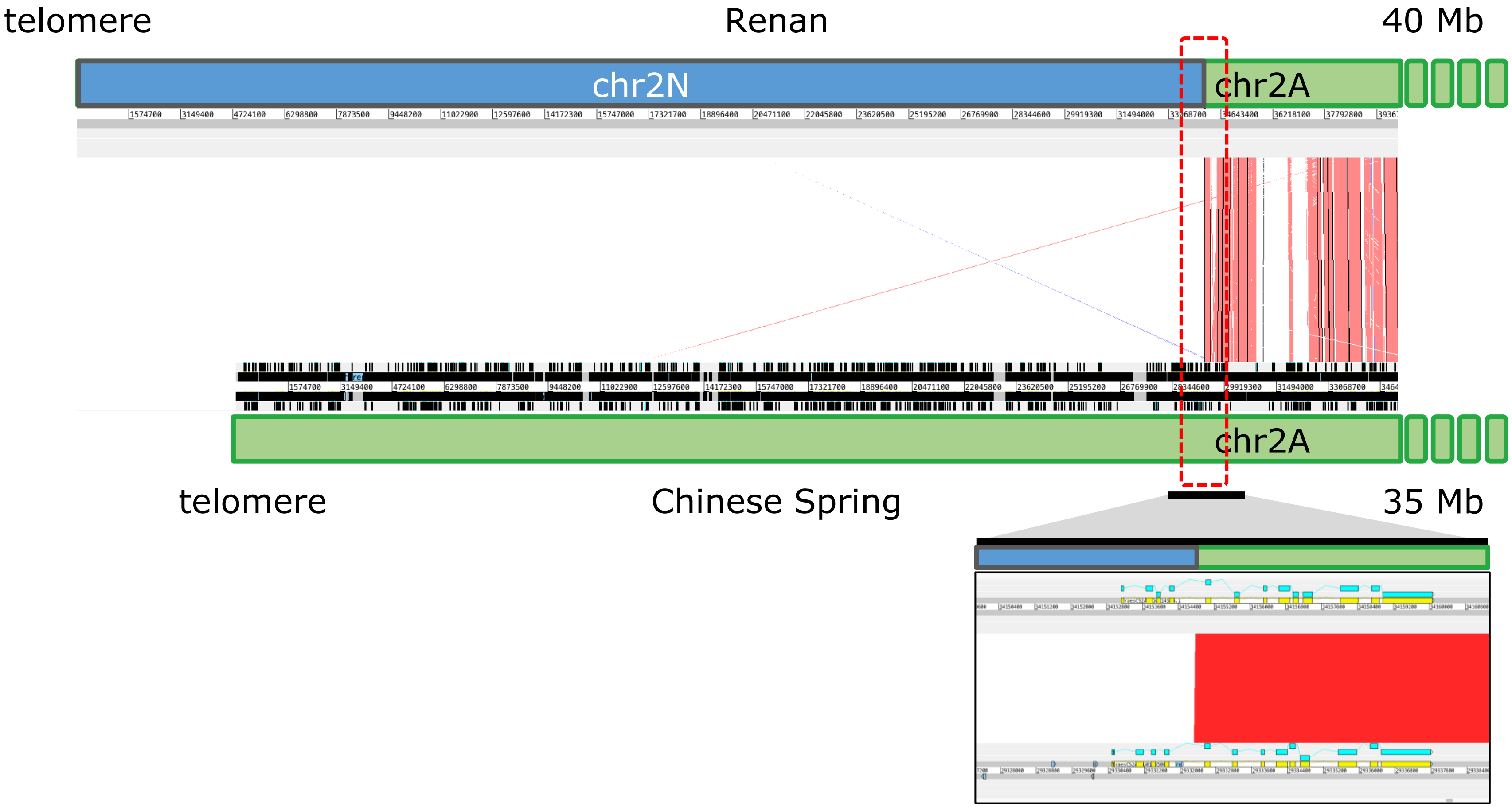


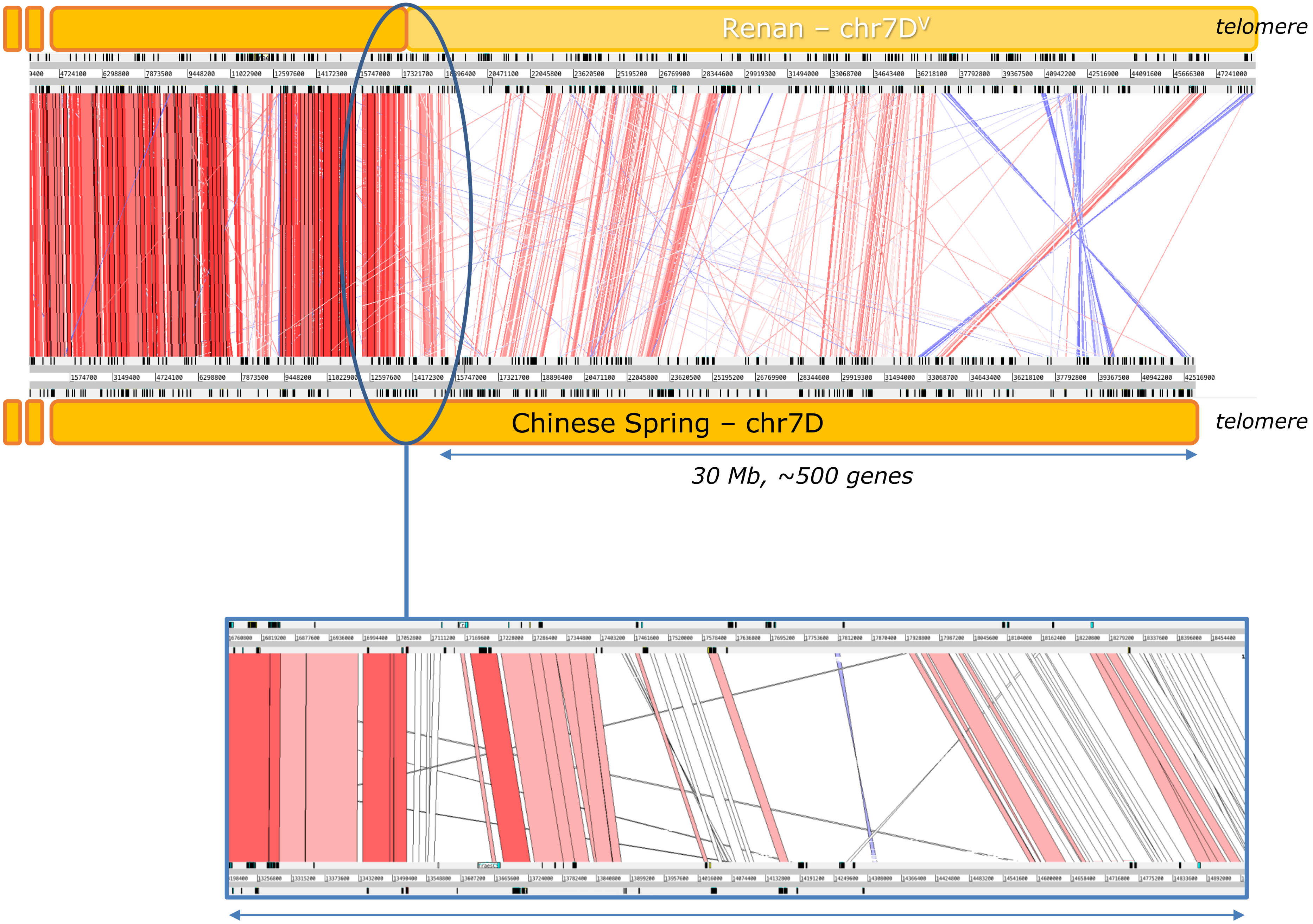


- ➢ 98% of genes mapped accurately
- ➢ + 4400 (4%) Renan specific genes

❑ *Introgressions*







Renan – chr7D^V

telomere

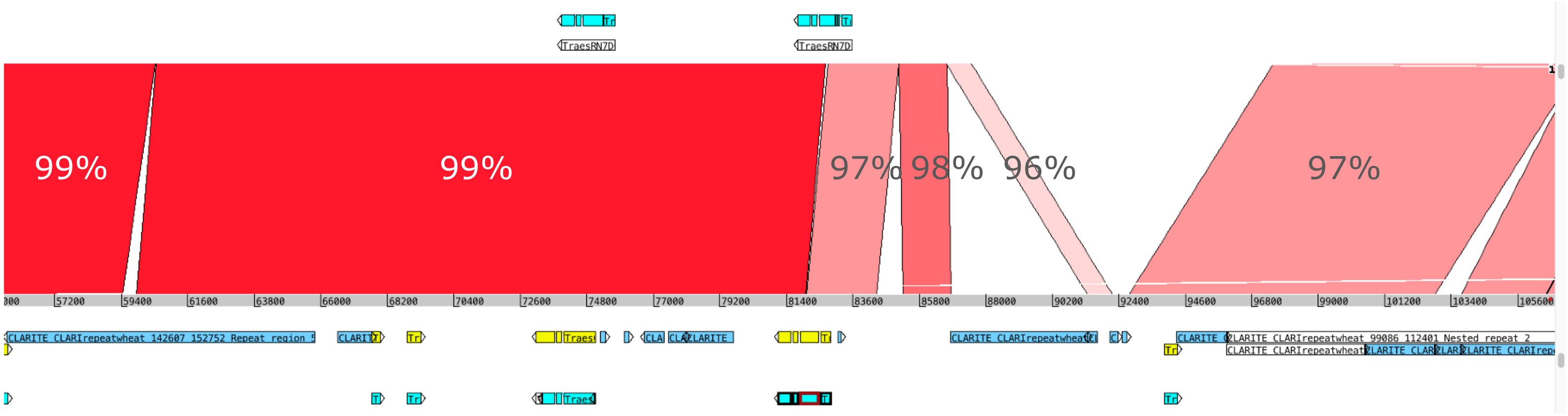
Chinese Spring – chr7D

telomere

30 Mb, ~500 genes

Renan – chr7D

Renan – chr7D^V

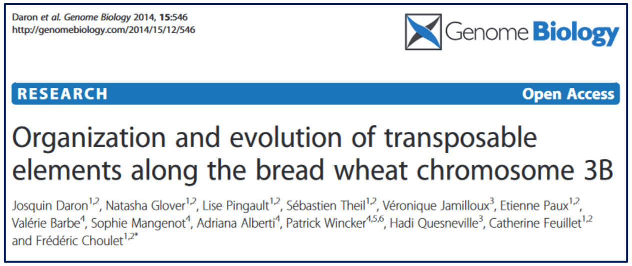


Chinese Spring – chr7D

❑ TE dynamics

- *PhD Nathan Papon (2019-2022)*

Daron et al. 2015



Daron *et al. Genome Biology* 2014, **15**:546
http://genomebiology.com/2014/15/12/546

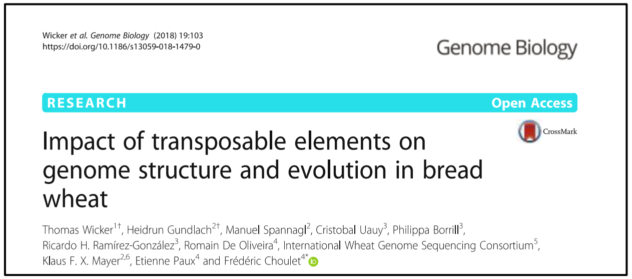
Genome **Biology**

RESEARCH **Open Access**

Organization and evolution of transposable elements along the bread wheat chromosome 3B

Josquin Daron[1,2], Natasha Glover[1,2], Lise Pingault[1,2], Sébastien Theil[1,2], Véronique Jamilloux[3], Etienne Paux[1,2], Valérie Barbe[4], Sophie Mangenot[4], Adriana Alberti[4], Patrick Wincker[4,5,6], Hadi Quesneville[3], Catherine Feuillet[1,2] and Frédéric Choulet[1,2*]

Wicker et al. 2018



Wicker *et al. Genome Biology* (2018) 19:103
https://doi.org/10.1186/s13059-018-1479-0

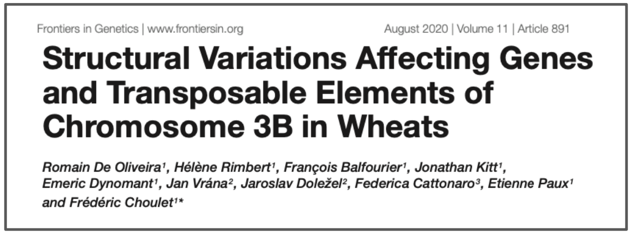
Genome Biology

RESEARCH **Open Access**

Impact of transposable elements on genome structure and evolution in bread wheat

Thomas Wicker[1†], Heidrun Gundlach[2†], Manuel Spannagl[2], Cristobal Uauy[3], Philippa Borrill[3], Ricardo H. Ramírez-González[3], Romain De Oliveira[4], International Wheat Genome Sequencing Consortium[5], Klaus F. X. Mayer[2,6], Etienne Paux[4] and Frédéric Choulet[4*]

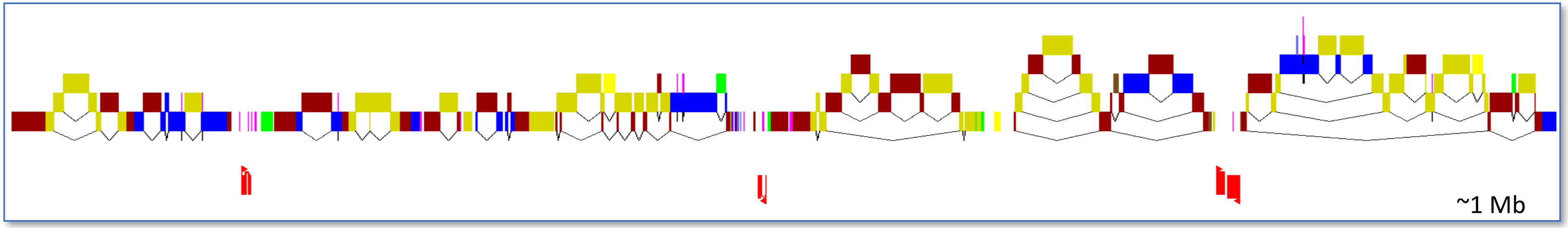
De Oliveira et al. 2020



Frontiers in Genetics | www.frontiersin.org August 2020 | Volume 11 | Article 891

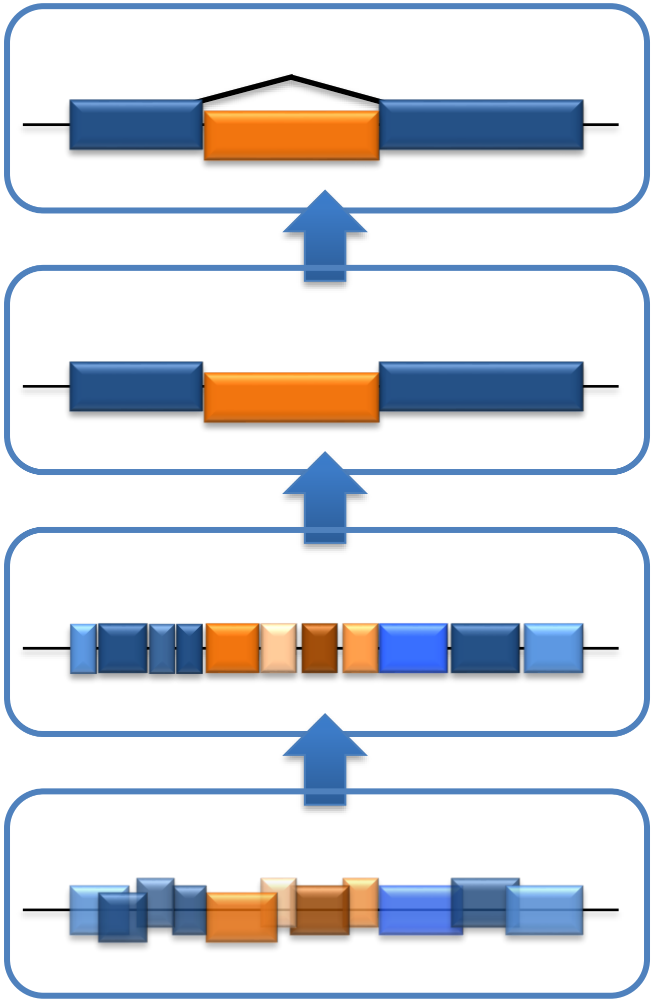
Structural Variations Affecting Genes and Transposable Elements of Chromosome 3B in Wheats

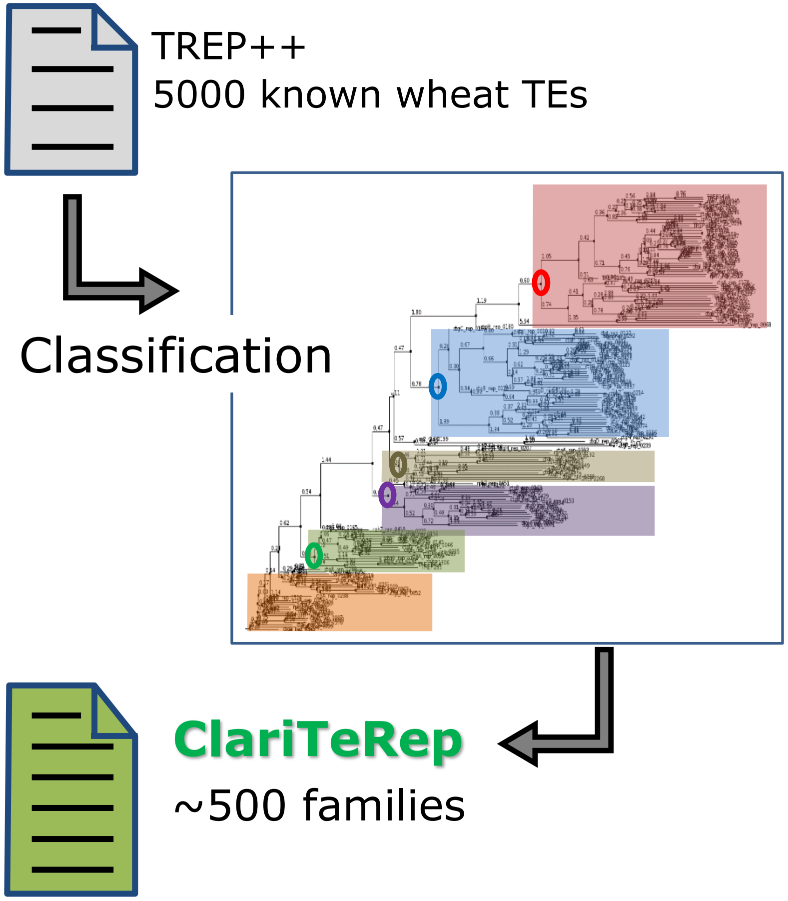
Romain De Oliveira[1], Hélène Rimbert[1], François Balfourier[1], Jonathan Kitt[1], Emeric Dynomant[1], Jan Vrána[2], Jaroslav Doležel[2], Federica Cattonaro[3], Etienne Paux[1] and Frédéric Choulet[1*]


~1 Mb

TE modeling with CLARITE and ClariTeRep

https://github.com/jdaron/CLARI-TE



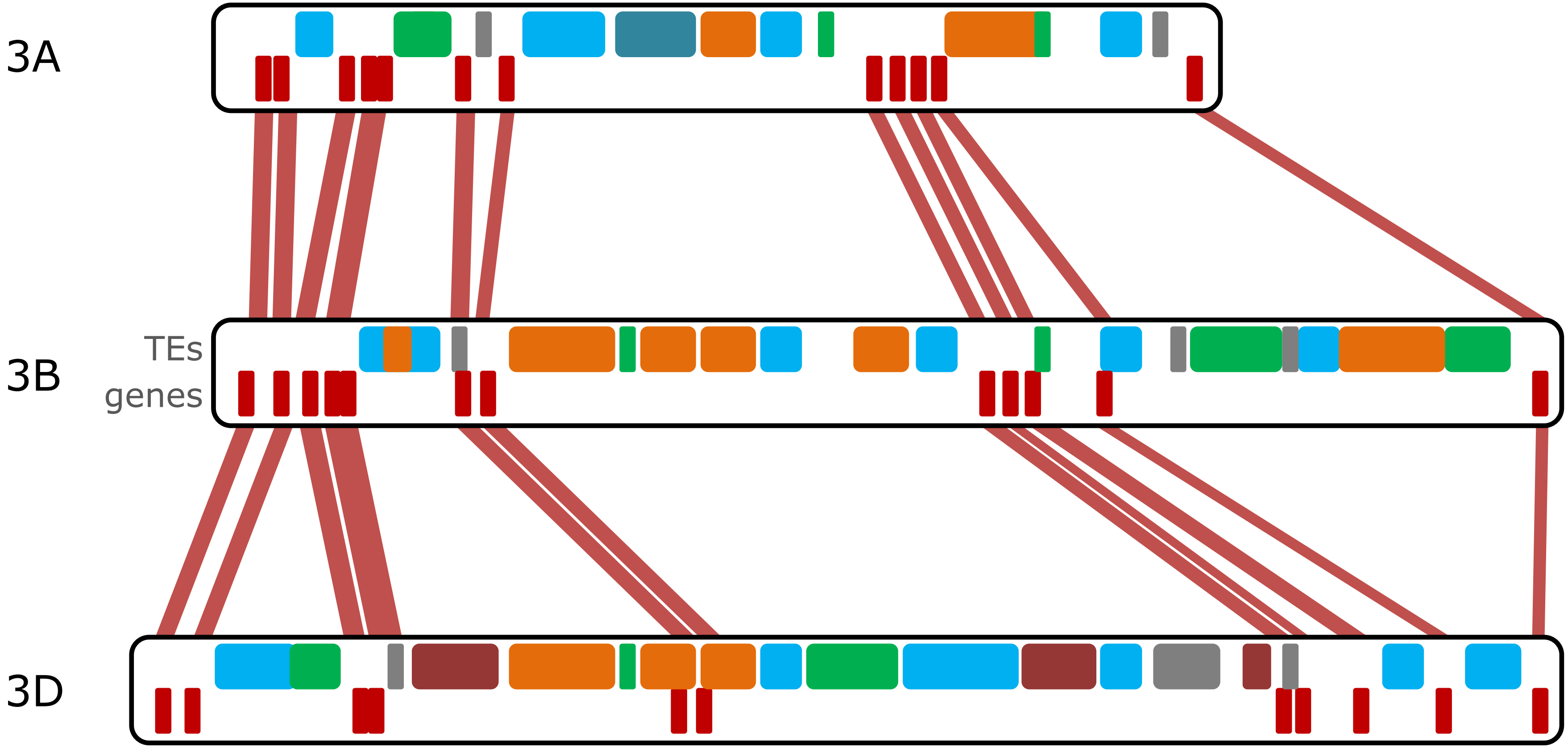


TREP++
5000 known wheat TEs

Classification

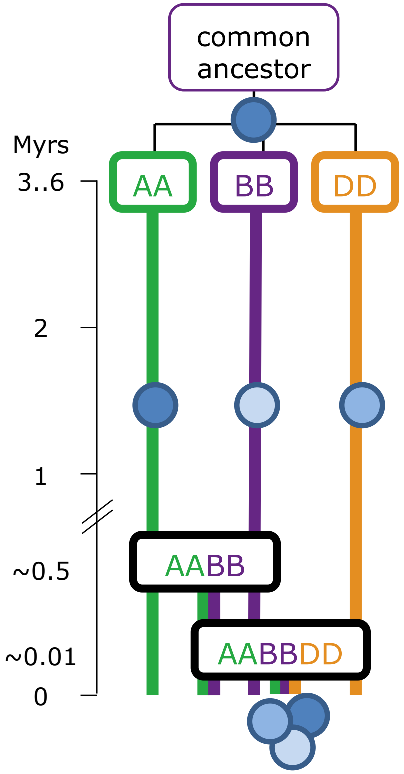
ClariTeRep
~500 families

Daron et al. Genom Biol 2015

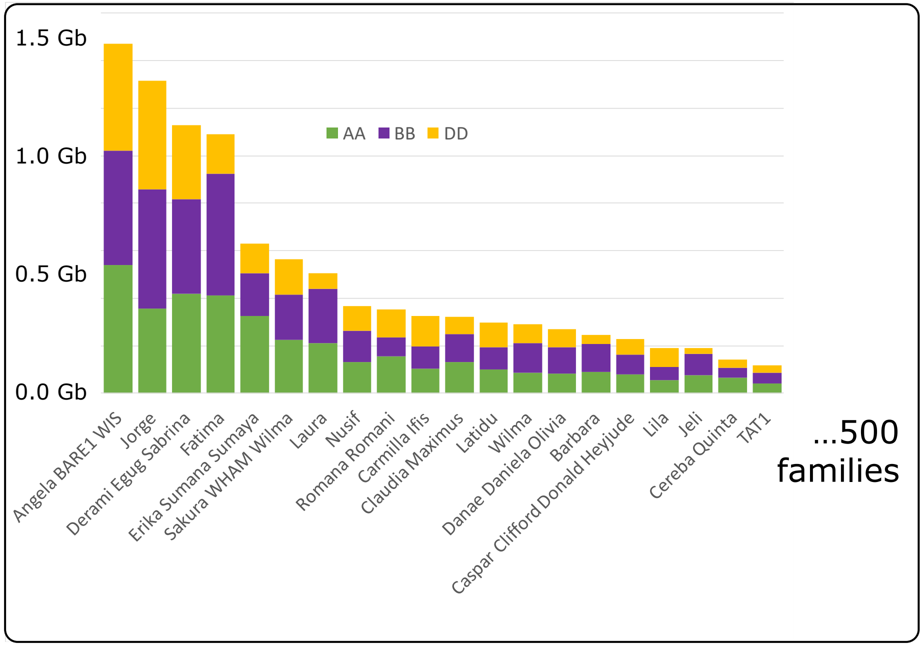


- Near-complete **TE turnover** since A-B-D divergence

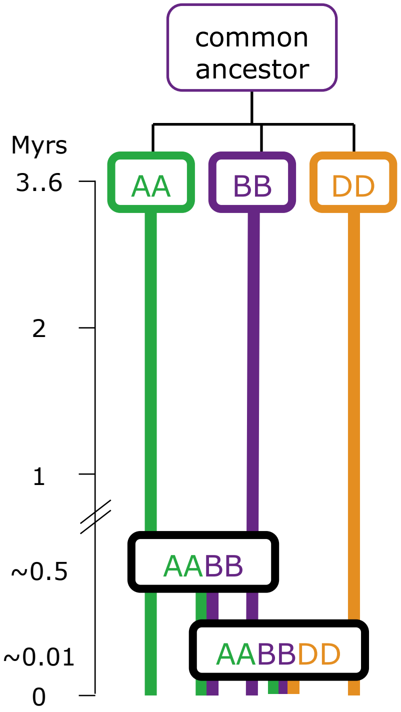
Wicker et al. Genom Biol 2018

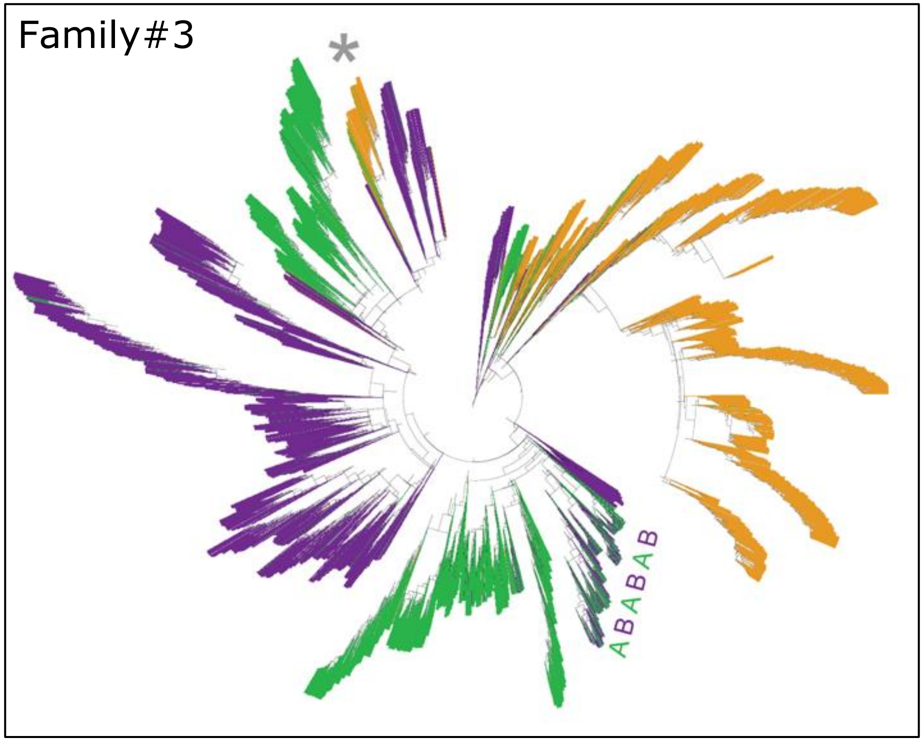


Families

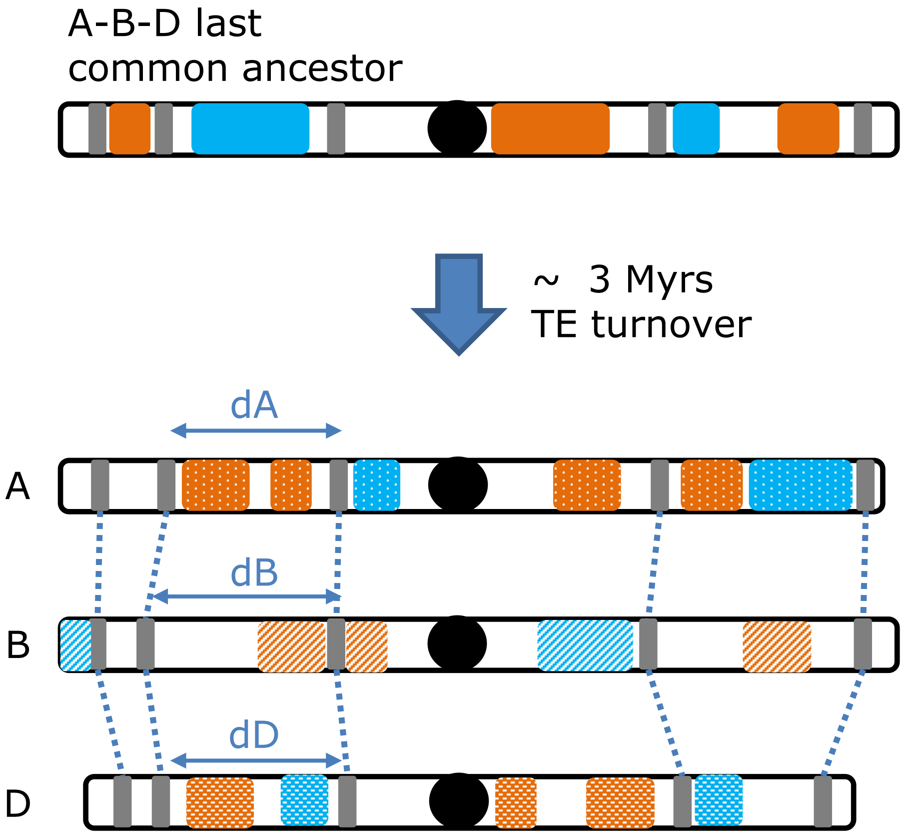


- ➢ A-B-D balanced proportions
- ➢ **0** family specific to 1 subgenome





- ➢ Independent TE evolution in the diploids AA, BB, DD
- ➢ Some rare cases of TE amplif in the tetraploid AABB



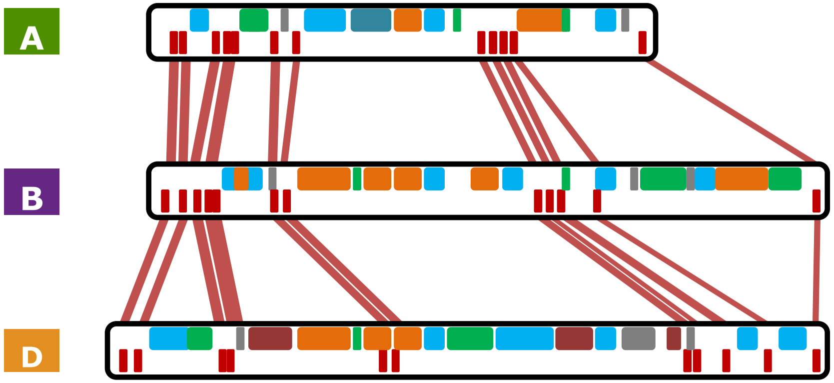
Most families were active since A-B-D divergence

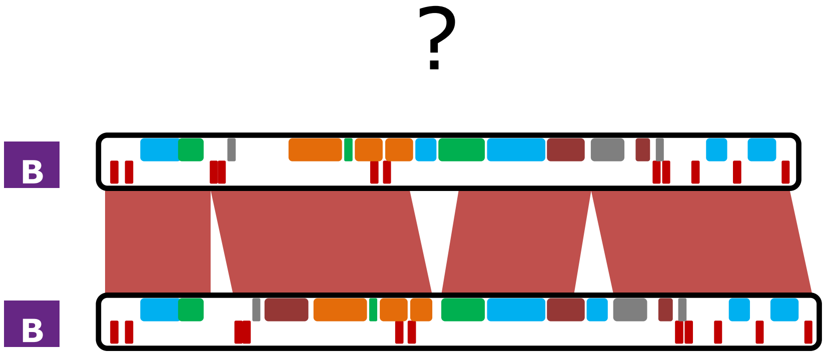
➢ **Equilibrium**

Hypothesis of a structural role of TEs likely under selection pressure?

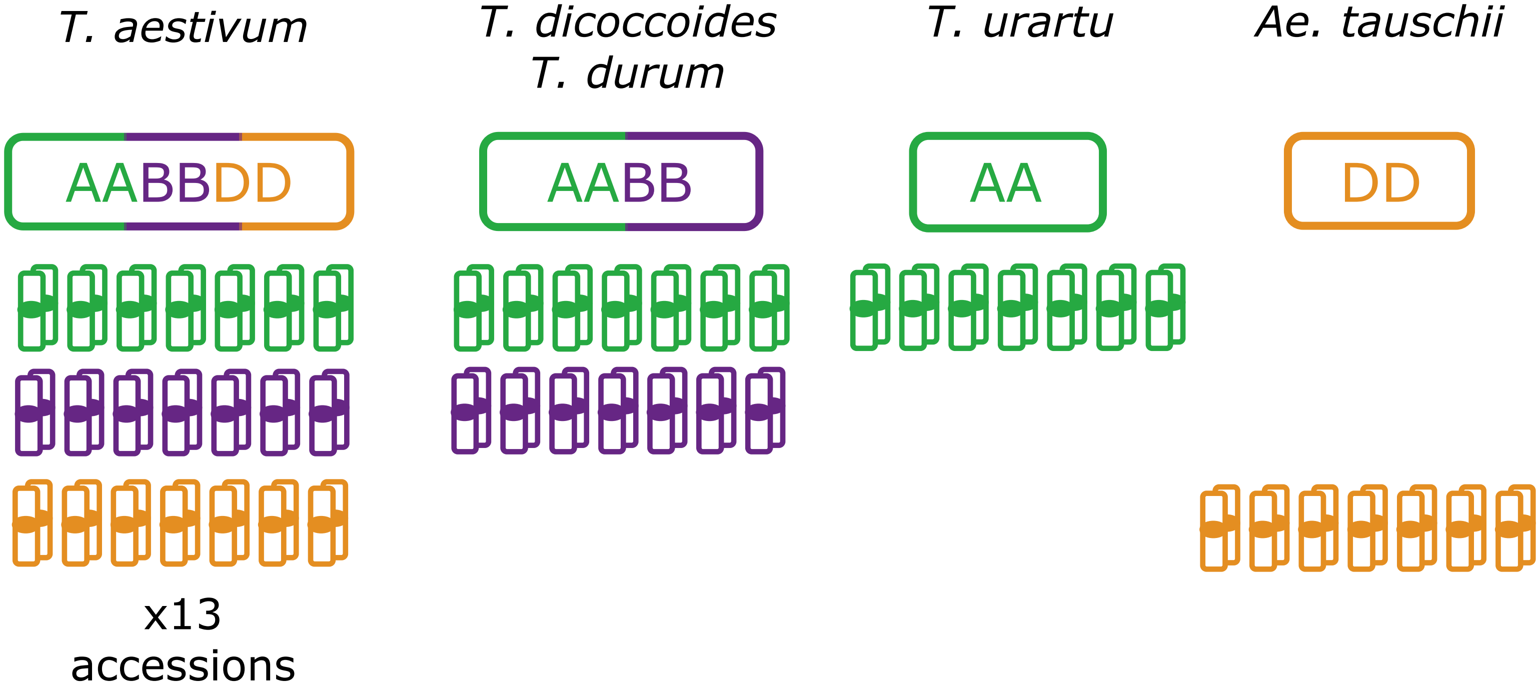
❑ Genomic variability affecting **TEs** in wheat **using assembled genomes**

PhD Nathan Papon (2019-2022)





○ Material = multiple RefSeq now available



T. aestivum

AABBDD

x13 accessions

T. dicoccoides
T. durum

AABB

T. urartu

AA

Ae. tauschii

DD

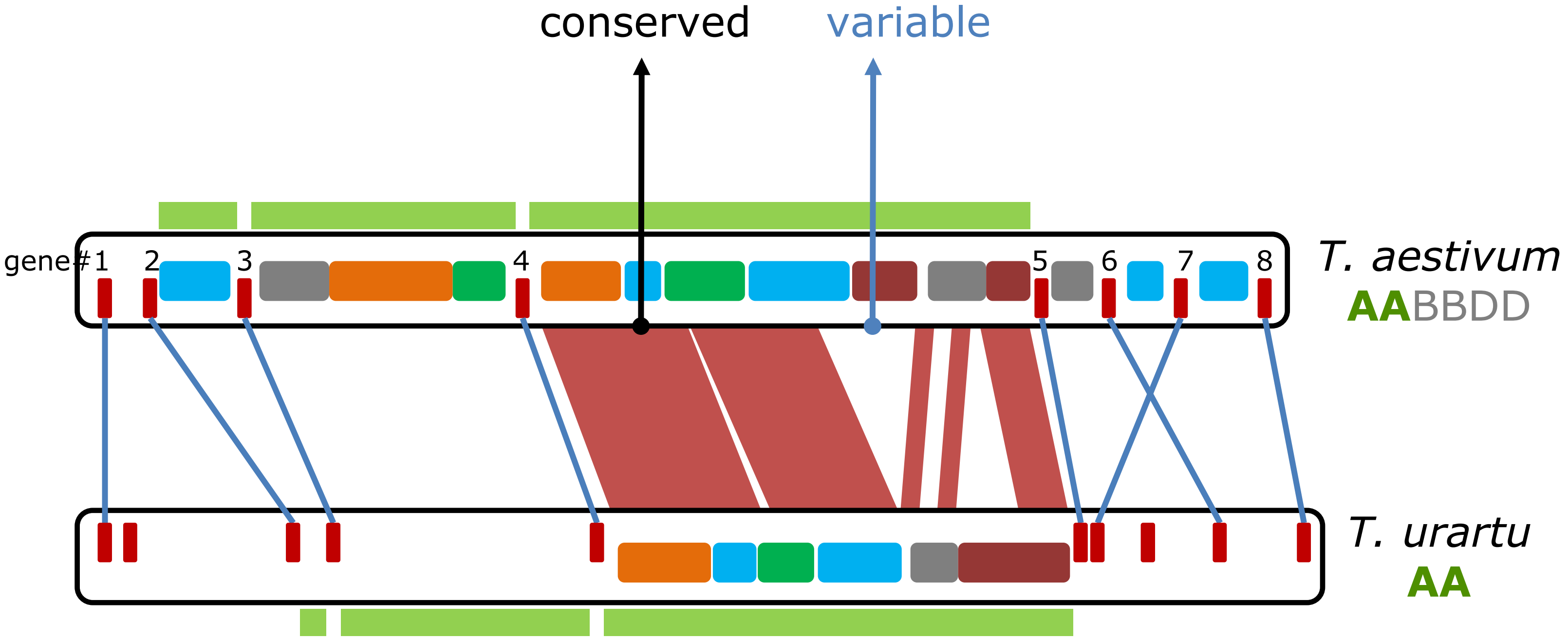
IWGSC 2018
Walkowiak et al. 2020
Guo et al. 2020
Aury et al. 2022

Avni et al. 2017
Maccaferri et al., 2019

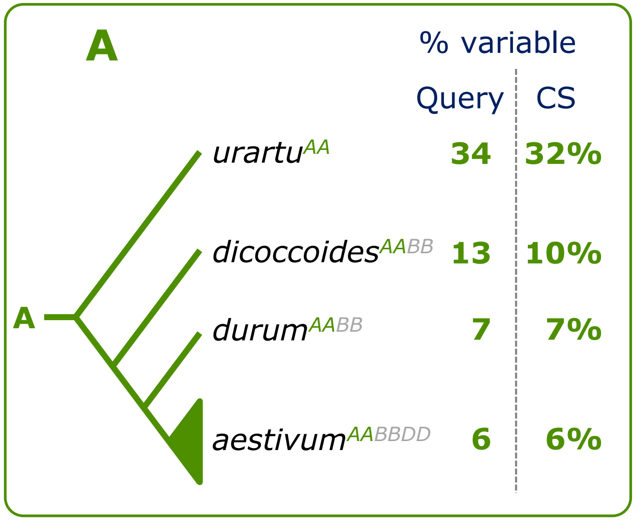
Ling et al. 2018

Luo et al. 2017

○ Method

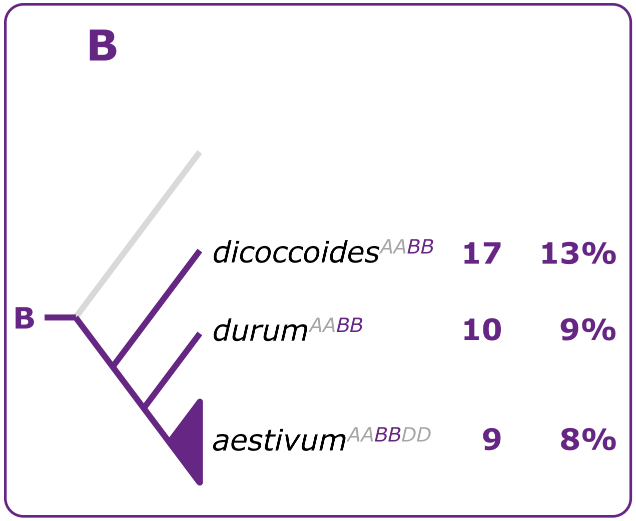

conserved
variable
gene#1
2
3
4
5
6
7
8
T. aestivum
AABBDD
T. urartu
AA

○ Extent of variability affecting TEs

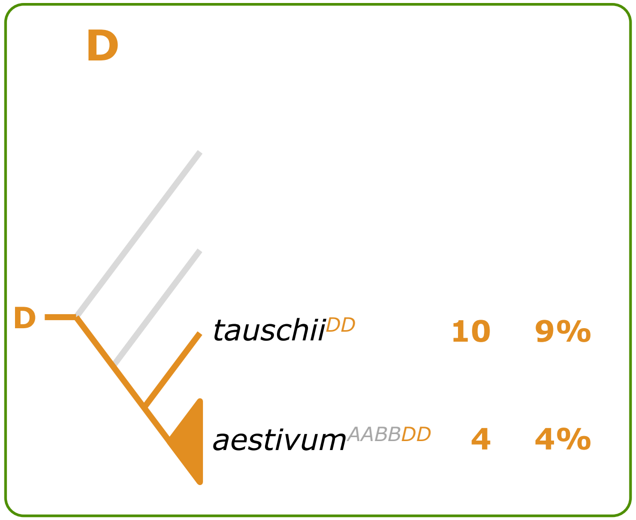


% variable

	Query	CS
urartu^{AA}	**34**	**32%**
dicoccoides^{AABB}	**13**	**10%**
durum^{AABB}	**7**	**7%**
aestivum^{AABBDD}	**6**	**6%**

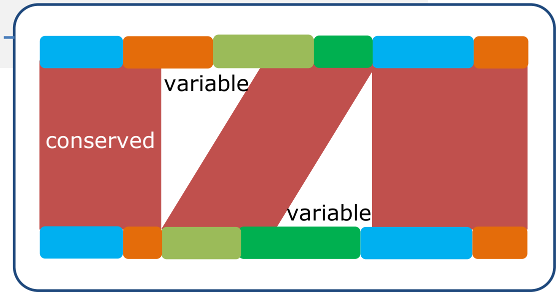


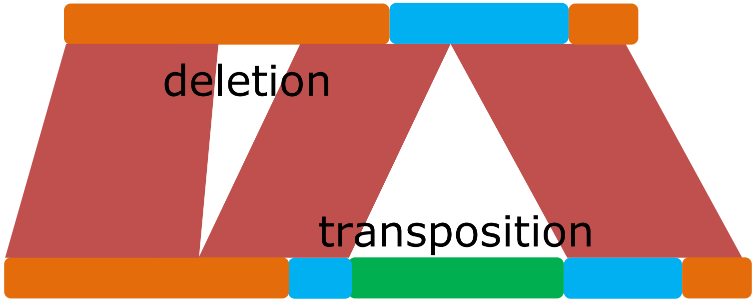
dicoccoides^{AABB}	**17**	**13%**
durum^{AABB}	**10**	**9%**
aestivum^{AABBDD}	**9**	**8%**



tauschii^{DD}	**10**	**9%**
aestivum^{AABBDD}	**4**	**4%**

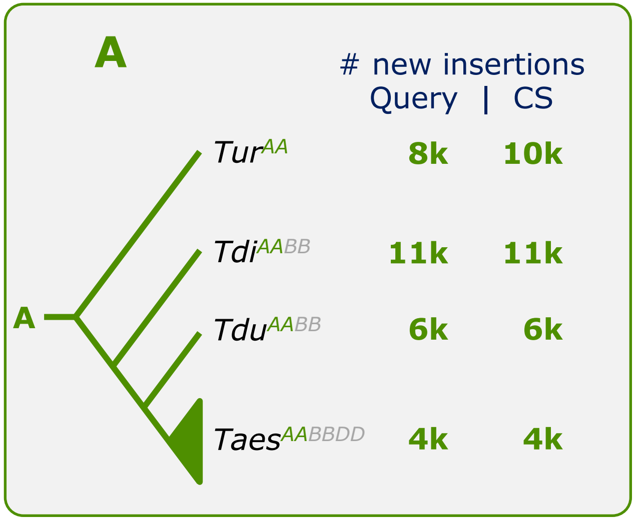
- 5% .. 15%
- B > A > D
- No genome expansion/contraction



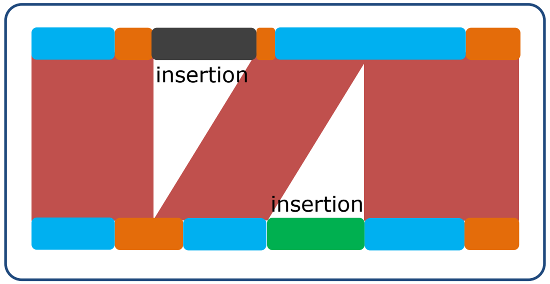


Recent TE insertions

- ~4,000–13,000 transposition events / subgenome (<1% of the genome)



	# new insertions Query	CS
Tur^{AA}	**8k**	**10k**
Tdi^{AABB}	**11k**	**11k**
Tdu^{AABB}	**6k**	**6k**
Taes^{AABBDD}	**4k**	**4k**



Impact of polyploidy?

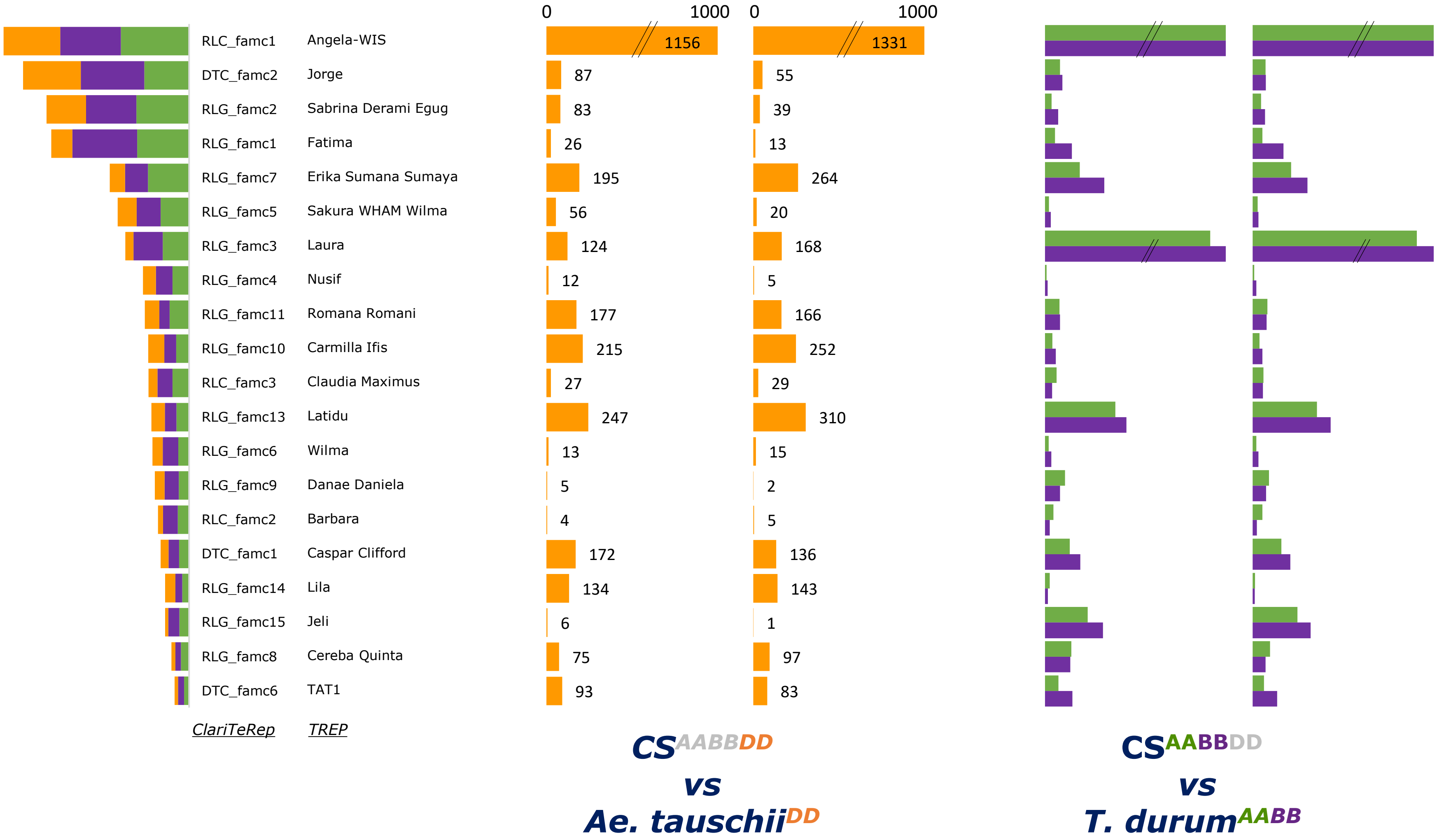
- none

How many families were active recently?

- 346 active families (=99.7% of all TEs)

20 most abundant families

#specific TE insertions



Conclusions

- No genome expansion/contraction due to TE transposition
- No impact of polyploidy
- No TE burst
- All families were active recently
- Transposition rate is stable

- ➢ Equilibrium
- ➢ Role of TEs in genome architecture under evolutionary constraints

❑ Acknowledgments

○ *Génoscope, CEA-CNRS-U Evry*
Jean-Marc Aury
Stefan Engelen
Benjamin Istace
Caroline Belser
Corinne Cruaud
Adriana Alberti
Patrick Wincker

○ *CNRGV, INRAE Toulouse*
Sandrine Arribat
Isabelle Dufau
Arnaud Bellec

○ *URGI, INRAE Versailles*
Michael Alaux
Nicolas Francillonne

○ *Mésocentre, UCA*
David Grimbichler

○ *GDEC, INRAE-UCA*
Cécile Monat
Pauline Lasserre-Zuber
Hélène Rimbert
Philippe Leroy
Etienne Paux
Marion Ranoux
Jérôme Salse
Carline Pont
Cécile Huneau
Nathan Papon
Romain De Oliveira
Josquin Daron

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○ ***Funding***

FRANCE GENOMIQUE

SRESRI/Région ARA