The Reference Sequence of Wheat and IWGSC Phase II:

Ensuring a Full Genomic Toolbox for Wheat

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IWGSC Executive Director
Hélène Bergès
Member of the IWGSC Coordinating Committee

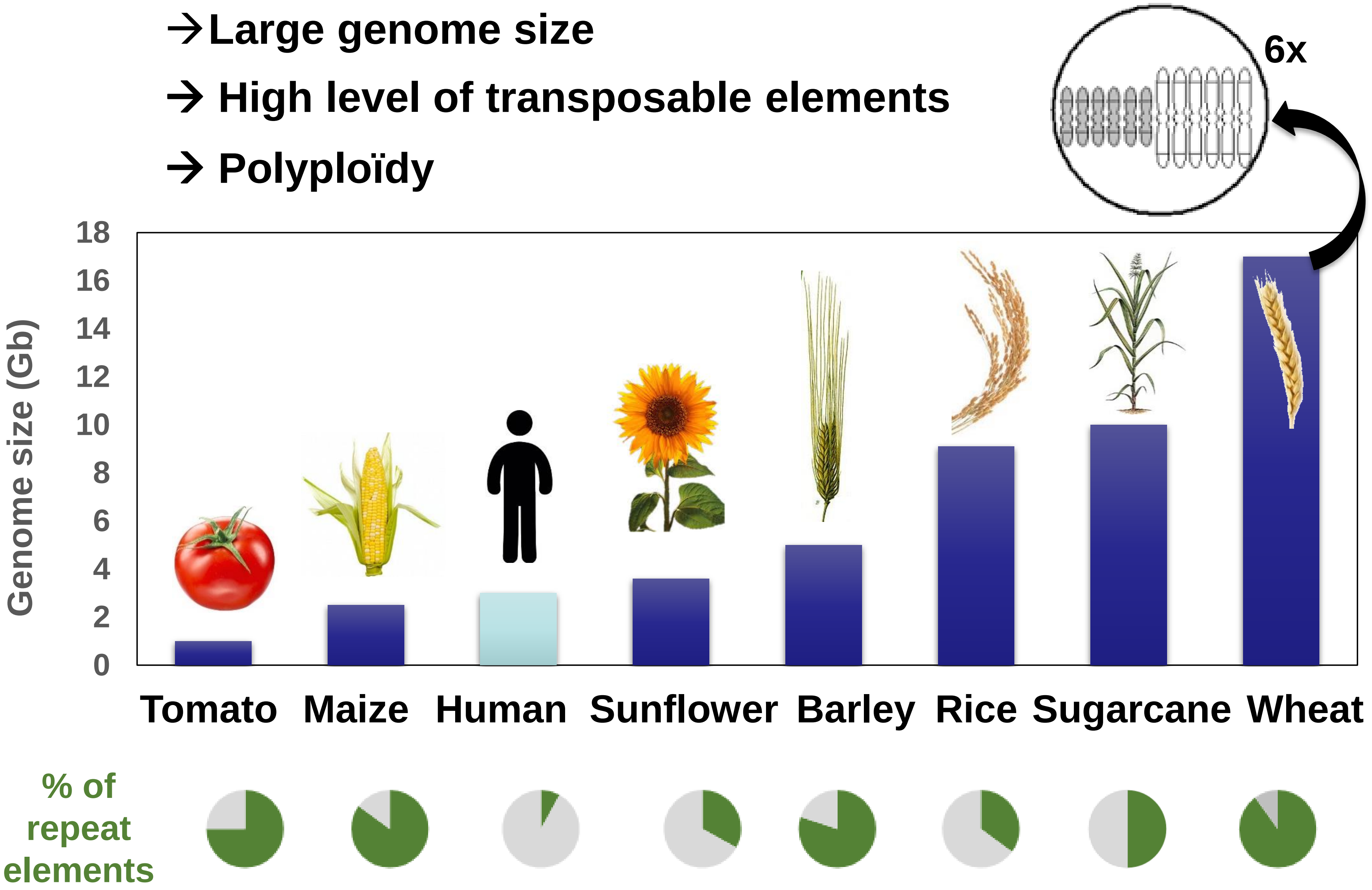
WHEATS&WOMEN INTERNATIONAL CONFERENCE
Roma – June 2018

Plant's genome exhibits high levels of complexity

→Large genome size

→ High level of transposable elements

→ Polyploïdy



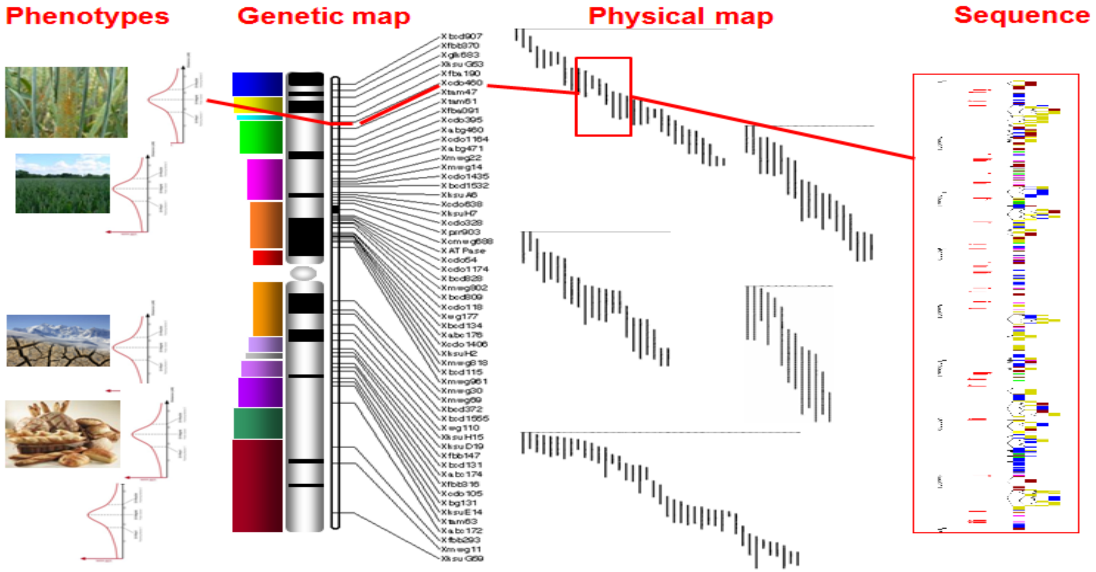
2005 – IWGSC Goal & Vision

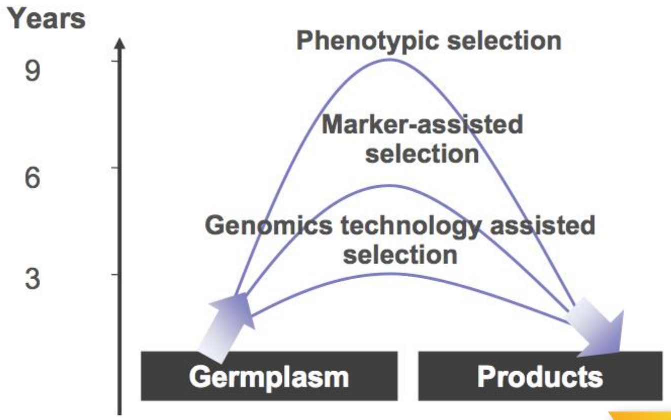
Goal

- Lay a foundation to accelerate wheat improvement
- Increase profitability throughout the industry

Vision

- High quality annotated genome sequence, comparable to rice
- Physical map-based, integrated and ordered sequence



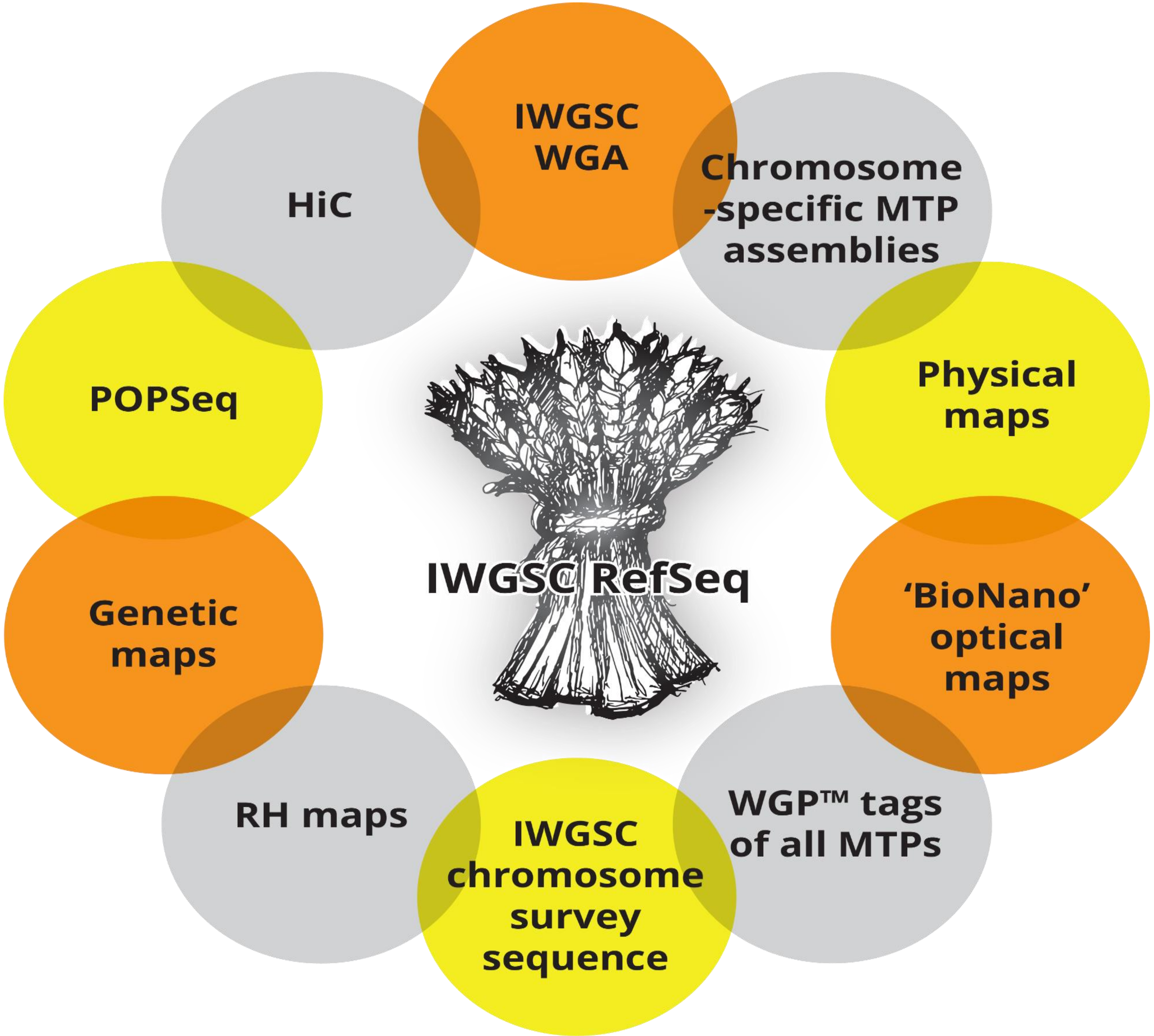


IWGSC RefSeq

The Reference Sequence for the Bread Wheat Genome

Frédéric Choulet
GDEC, INRA, UCA, Clermont-Ferrand, France

Concerted integration of resources: RefSeq v1.0



IWGSC WGA

Chromosome-specific MTP assemblies

Physical maps

'BioNano' optical maps

WGP™ tags of all MTPs

IWGSC chromosome survey sequence

RH maps

Genetic maps

POPSeq

HiC

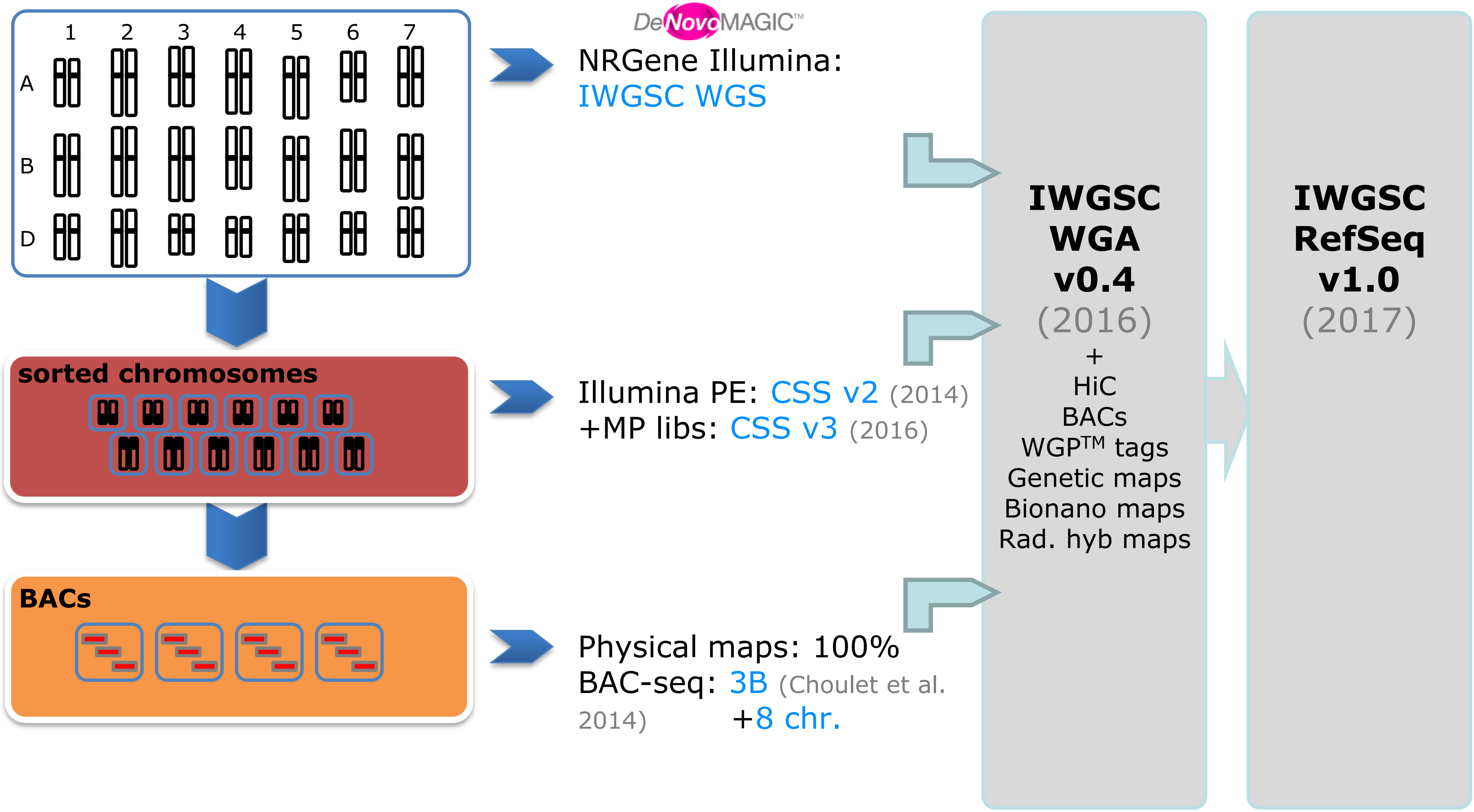
IWGSC RefSeq

IWGSC

IWGSC RefSeq

RefSeq v1.0

A fully annotated and anchored reference sequence assembly of the wheat genome



sorted chromosomes

BACs

DeNovoMAGIC™
NRGene Illumina:
IWGSC WGS

Illumina PE: CSS v2 (2014)
+MP libs: CSS v3 (2016)

Physical maps: 100%
BAC-seq: 3B (Choulet et al. 2014) +8 chr.

IWGSC
WGA
v0.4
(2016)
\+
HiC
BACs
WGP™ tags
Genetic maps
Bionano maps
Rad. hyb maps

IWGSC
RefSeq
v1.0
(2017)

❑ **IWGSC RefSeq v1.0** – *Community resources*

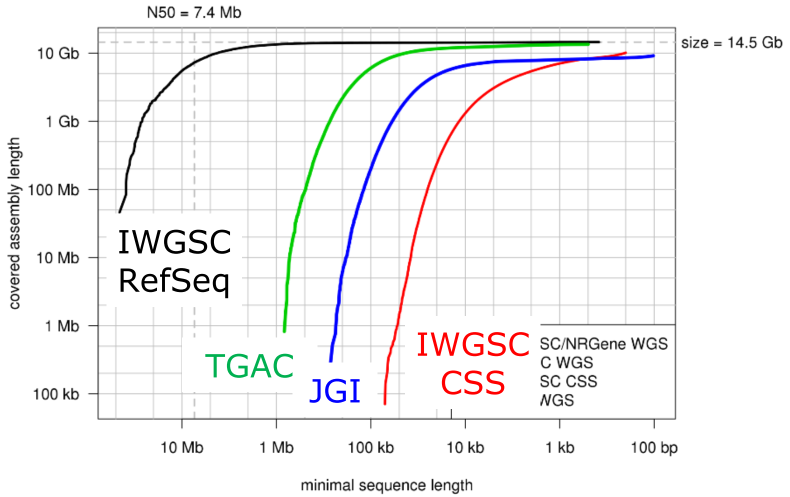
- Physical maps for all chromosomes
  - 1,839,128 BACs
- WGP tags (mostly from MTP BACs) for all chromosomes except 3B
  - 4,305,249 unique tags for 693,697 BACs
- BAC sequence assemblies
  - 8 chr: 1A, 1B, 3B, 3D, 6B, 7A, 7B, 7D + 2 chr arms: 4AL, 5BS
  - 52,890 BACs (9.7 Gb), N50=68 kb
- BioNano maps for 7A, 7B and 7DS
  - 1,335 BioNano contigs
- 4.7 M molecular markers

❑ **IWGSC RefSeq v1.0** – *Metrics*

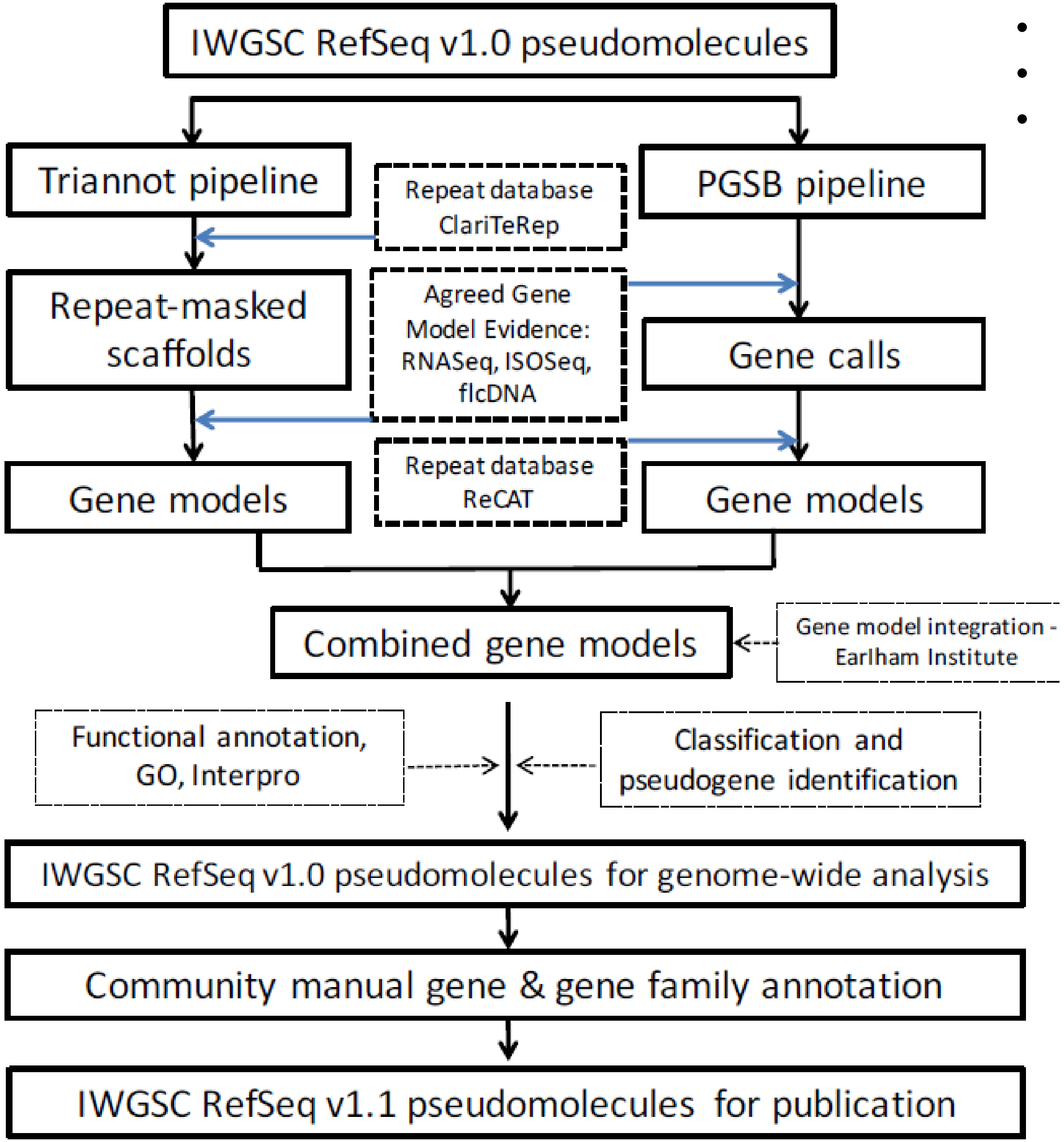
total size	14.5 Gb (genome size ~15.5 -> 94%)
completeness	97-99%
contig N50	52 kb
scaffold N50	7 Mb
superscaffold N50	23 Mb

21 pseudomolecules:

total size	14.1 Gb (97%)
superscaffolds	1601 (avg 76 per chr.)



❑ **IWGSC RefSeq v1.0** – *Genome annotation pipeline*



IWGSC RefSeq v1.0 pseudomolecules

Triannot pipeline

Repeat database ClariTeRep

PGSB pipeline

Repeat-masked scaffolds

Agreed Gene Model Evidence: RNASeq, ISOSeq, flcDNA

Gene calls

Gene models

Repeat database ReCAT

Gene models

Combined gene models

Gene model integration - Earlham Institute

Functional annotation, GO, Interpro

Classification and pseudogene identification

IWGSC RefSeq v1.0 pseudomolecules for genome-wide analysis

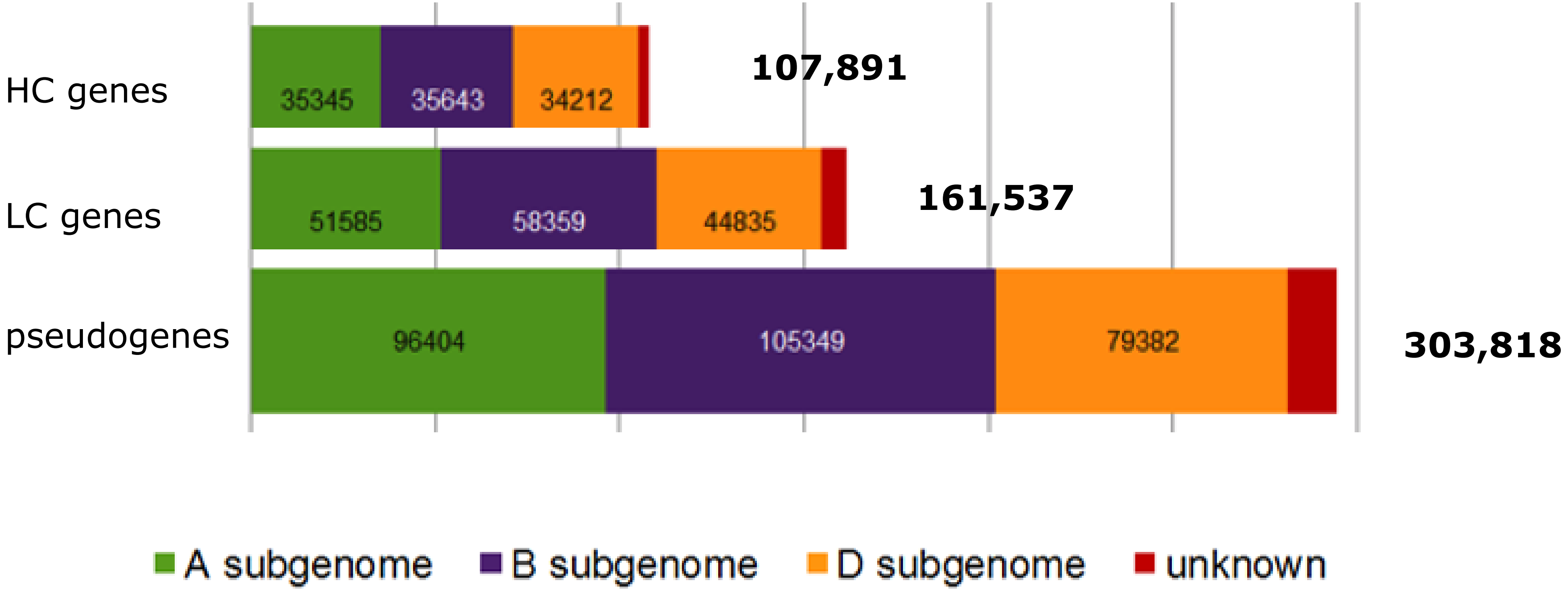
Community manual gene & gene family annotation

IWGSC RefSeq v1.1 pseudomolecules for publication

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

❑ Annotation – gene models

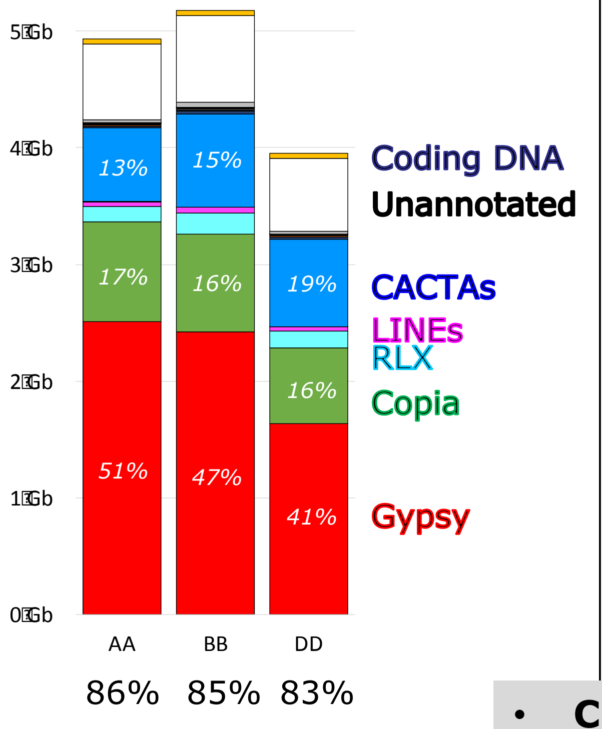
v1.0: automated
v1.1: incorporate 3685 manually annotated genes (available upon publication)

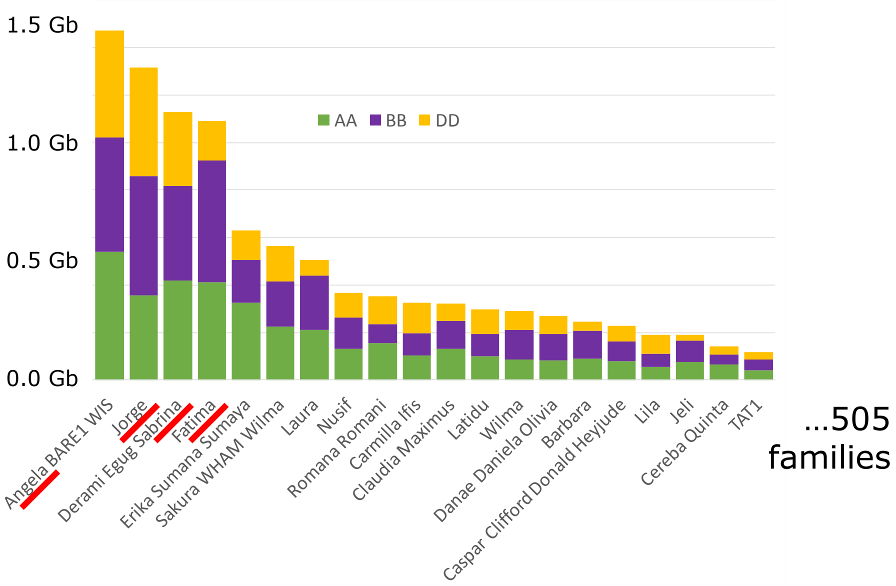


- *T. Wicker (UZH)*
- *H. Gundlach (PGSB)*
- *F. Choulet (GDEC)*

❑ Annotation – transposable elements

- CLARITE, TREP, *denovo* fl-LTR-RTs detection…
- 3,968,974 elements belonging to 505 families (84% <=> 11.9 / 14.1 Gb)





- **Conserved TE composition of A-B-D (no family specific to 1 subgenome)**
- **Complete reshuffling of TEs between homeologous loci**
- **TE activity after polyploidization very limited**

TE companion paper…

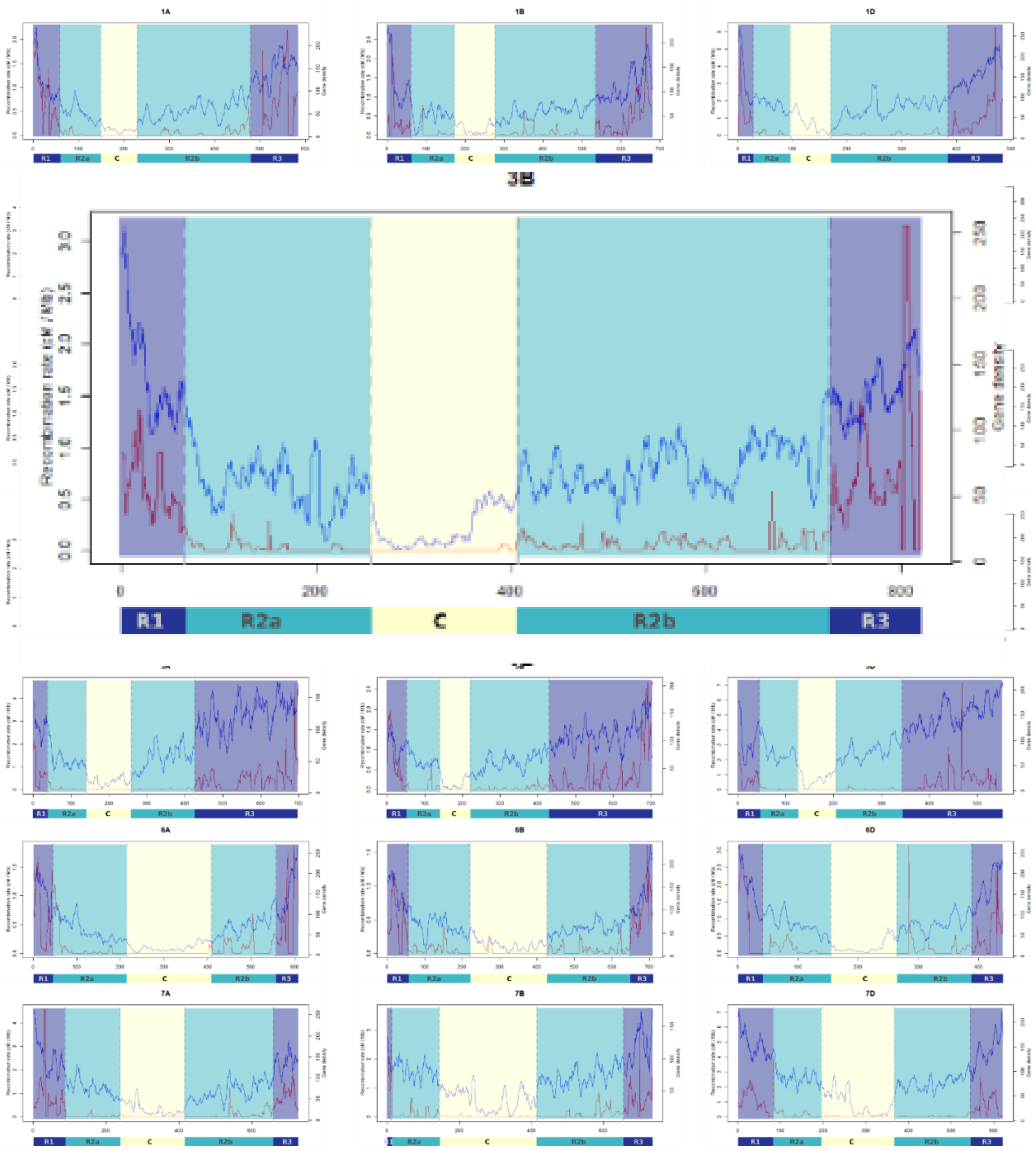
- *Etienne Paux (INRA GDEC)*

❑ Genome partitioning

R1/3=distal

C=proximal

R2a/b=interstitial



R1/3= high gene / low TE

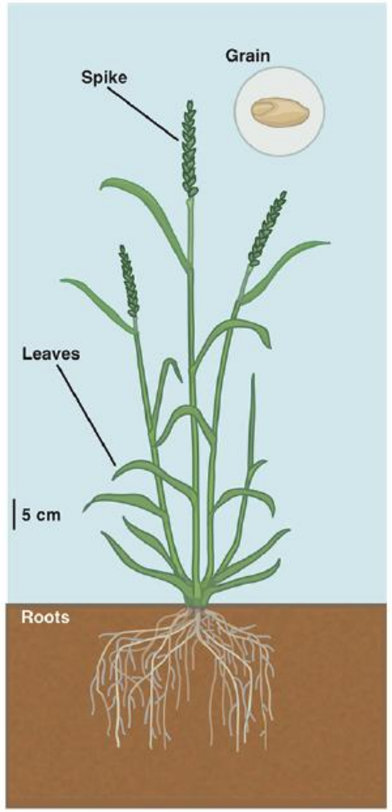
- *Daniel Lang (PGSB)*
- *Manuel Spannagl (PGSB)*

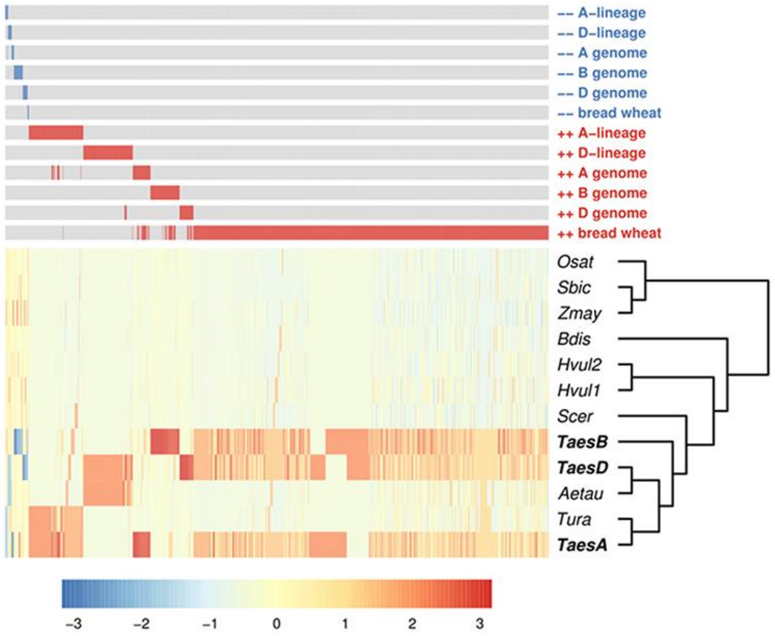
❑ A-B-D comparative analyses

Analysis of gene families of wheat

Gene family expansion/contraction associated with traits targeted by breeder selection

- 26080 gene families
- 33% expanded in the wheat lineage / 0.1% contracted families
  72% expanded in A+B+D





GO/PO/TO term enrichment and expanded gene families:

- **morphological traits** related to leaf, spike and root development, leaf size, tillering, vegetative growth and development, seed, endosperm, and embryo-development and morphology
- **grain yield** and **quality** (seed maturation, dormancy and germination)
- **abiotic stress** tolerance (salt stress, cold stress)

B sub-genome expanded families enriched for genes related to plant and leaf size and development, tillering and vegetative growth time:

predominant impact of the B genome on vegetative plant growth and development

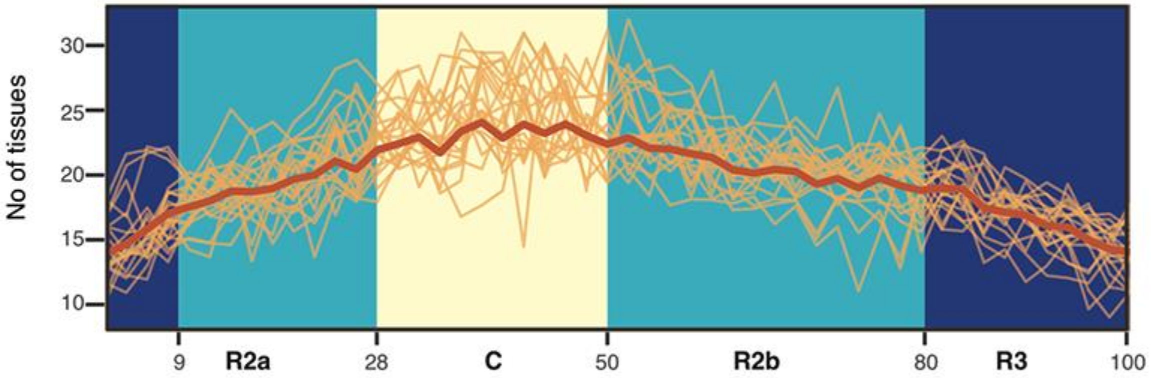
- *C. Uauy (JIC)*
- *P. Borrill*
- *R. Ramirez-Gonzalez*

❑ Transcriptome atlas

Atlas of transcription reveals trait associated gene co-regulation networks

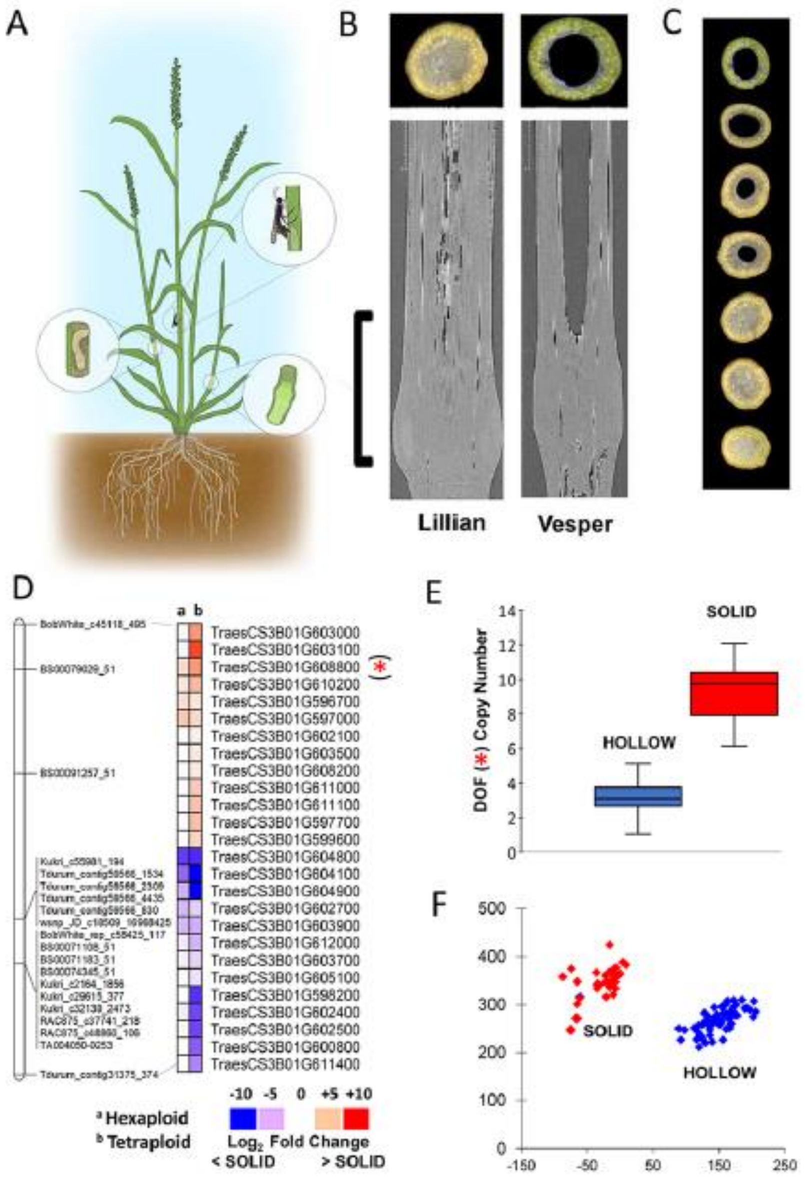
- 850 RNASeq samples – 32 tissues
  - 85% HC genes expressed
  - 49% LC genes expressed

- Chromosome partitioning
  - Distal region genes expressed in fewer tissues and enriched for responses to stress



Genes located in the distal R1 and R3 regions : lower expression breadth than those in the proximal regions

❑ Reference-guided dissection of an insect/abiotic stress resistance QTL for improved marker assisted selection



- traits of agronomic importance are inherited as QTL

- informative DNA markers in strong linkage disequilibrium with QTL are essential
- IWGSC RefSeq v1.0 provides template for assigning molecular markers to coordinates in linear DNA molecules anchored to high density molecular maps
- Example described : dissecting a QTL associated with insect/abiotic stress resistance
  - ➢ **Diagnosis marker**

- ➢ **Combining this knowledge with major loci selected for breeding programs provides a new framework for breeders to tackle the challenges of the new agriculture (balance the selection processes for adaptation to biotic and abiotic stress, end-use quality, and yield improvement)**

- *M. Alaux (INRA URGI)*

❑ IWGSC RefSeq Data Access & Availability

https://wheat-urgi.versailles.inra.fr/Seq-Repository/

Xwmc430

Examples: Xwmc430, QTL, TaeCsp3B

Pre-publication data access under Toronto Agreement:

- IWGSC **WGA v0.4**: June 2016
- IWGSC **RefSeq v1.0**: January 2017
- IWGSC **Annotation v1.0**: May 2017
- IWGSC **Annotation v1.1**: upon publication
- Manuscript under review

http://www.wheatgenome.org/Tools-and-Resources

IWGSC Phase II:

What’s Next for the IWGSC

WHEATS&WOMEN INTERNATIONAL CONFERENCE
Roma – June 2018

Measuring the Impact of IWGSC RefSeq v1.0

476,595 BLAST searches
22,935 downloads

2017

> 100 papers

Genome structure (9)

Genome expression (4)

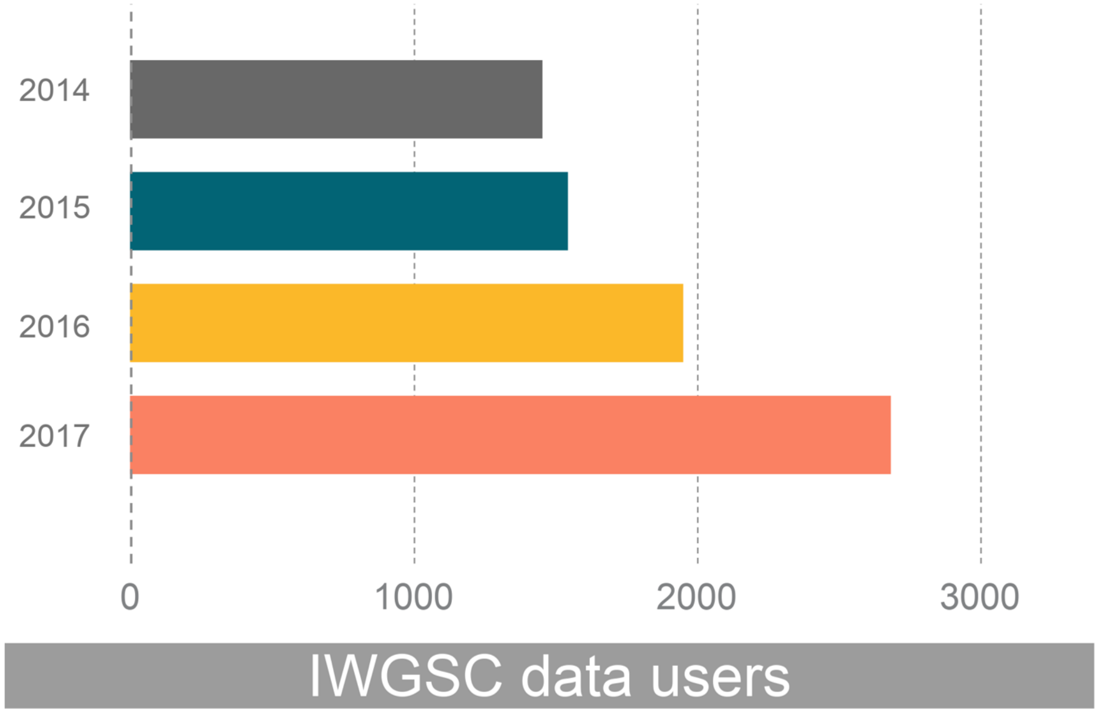
Genetic diversity (18)

Trait genomics (67)

Genome enabled breeding (9)

# of papers referencing use of IWGSC RefSeq related resources since January 2017

Measuring the Impact of IWGSC RefSeq v1.0



Progress

2005

Vision

- High quality annotated genome sequence, comparable to rice
- Physical map-based, integrated and ordered sequence

MISSION ACCOMPLISHED

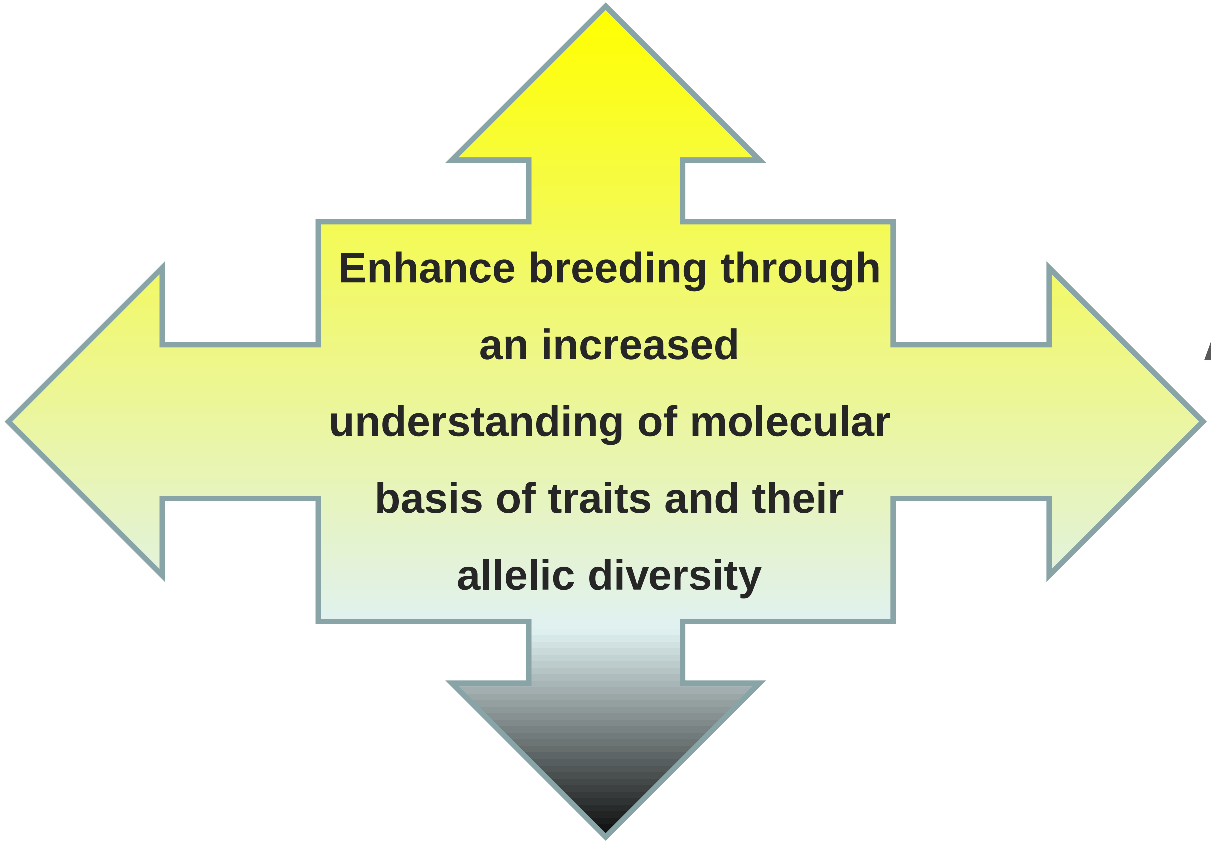
Looking into the Future

- **Achieve Gold Standard Reference**
- **Reach beyond reference to ensure a full genome-sequence based toolbox for wheat**

Goal: Accelerate wheat improvement

Vision and strategy for the IWGSC 2.0

Reference sequences with high quality functional annotations



Public, user-friendly, integrated databases and tools for all users

Enhance breeding through an increased understanding of molecular basis of traits and their allelic diversity

A wheat Pan-genome representing the worldwide diversity

An international, well connected and coordinated community

IWGSC

From RefSeq v1.0 to IWGSC Gold Standard

IWGSC RefSeq + **Manual & Functional Annotation** = IWGSC Gold Standard

- Pipeline for community generated manual annotation with future annotation releases
- Functional annotation
  - Gene family leaders
  - Applying machine learning techniques
- Closing gaps by incorporating new resources to build a IWGSC RefSeq v2.0
- IWGSC leaders: Rudi Appels, Fred Choulet…..

IWGSC 2.0 Activities: Pan-Genome

- Develop a wheat pan-genome that represents the breadth of worldwide wheat diversity
- Reference quality for 8-12 landraces
- Diversity panels at draft quality
- Haplotype database
- Skim-sequencing and high throughput genotyping of many lines
- IWGSC team: Etienne Paux, Sébastien Praud,…..

IWGSC 2.0 Activities: Database

- Develop user-friendly, integrated databases and tools

- IWGSC Pan-genome Database Task Force
  - Fred van Ex, Bayer CropScience
  - Magalie Leveugle, Biogemma
  - Matthieu Conte, Syngenta
  - Michael Alaux, URGI-INRA
  - …….

- What tools do you want?

IWGSC 2.0 Activities: IWGSC Exome Array

- Wheat exome capture array based on IWGSC RefSeq annotation v1.1.
- Arbor Biosciences will offer enrichment reagents and an enrichment service
- Exome Team: Burkhard Steuernagel, Sreya Ghosh, Sébastien Praud, Hikmet Budak, Etienne Paux, Ute Bauhman
- Discussion: What modules would you like to see?

❑ Conclusion

- IWGSC goal for high quality reference achieved
- IWGSC RefSeq v1.0 incorporates highly diverse community resources (WGS assembly + Hi-C links + physical maps + CSS + BAC sequences + millions of markers)
- Draft v0.4 + pseudomolecules v1.0 + annotation v1.0 available for download prior to publication
- IWGSC Phase II
  - Continued improvements from manual and functional annotation
  - IWGSC Exome Array based on the sequence
  - Pan-genome that covers the breadth of wheat diversity (landraces and elite var.)
  - Pan-genome database, visualization, and tools for breeders

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Histone Mark Analyses: **Moussa Benhamed and Etienne Paux**

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1B BAC Sequencing & Assembly: Frédéric Choulet and Etienne Paux

1D, 4D, 6D Physical Mapping: Bikram Gill

2AL Physical Mapping: Kuldeep Singh

2AS Physical Mapping: Nagendra K. Singh and **Jitendra P. Khurana**

2B, 2D, 4B, 5BL & 5DL IWGSC-Bayer Whole Genome Profiling(WGP™) Physical Maps: Jane Rogers and John Jacobs

3AL Physical Mapping: Bikram Gill

3DS Physical Mapping & BAC Sequencing & Assembly: Jan Bartoš

4A Physical Mapping, BAC Sequencing, Assembly, & Annotation: Miroslav Valárik

5BS BAC Sequencing, & Assembly: Elena Salina, Nikolai Ravin and **Konstantin Skryabin**

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GRAINS RESEARCH & DEVELOPMENT CORPORATION

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Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

DEPARTMENT OF BIOTECHNOLOGY
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