Odyssey of the IWGSC Reference Genome Sequence: 12 years 1 month 28 days 11 hours 10 minutes and 14 seconds.

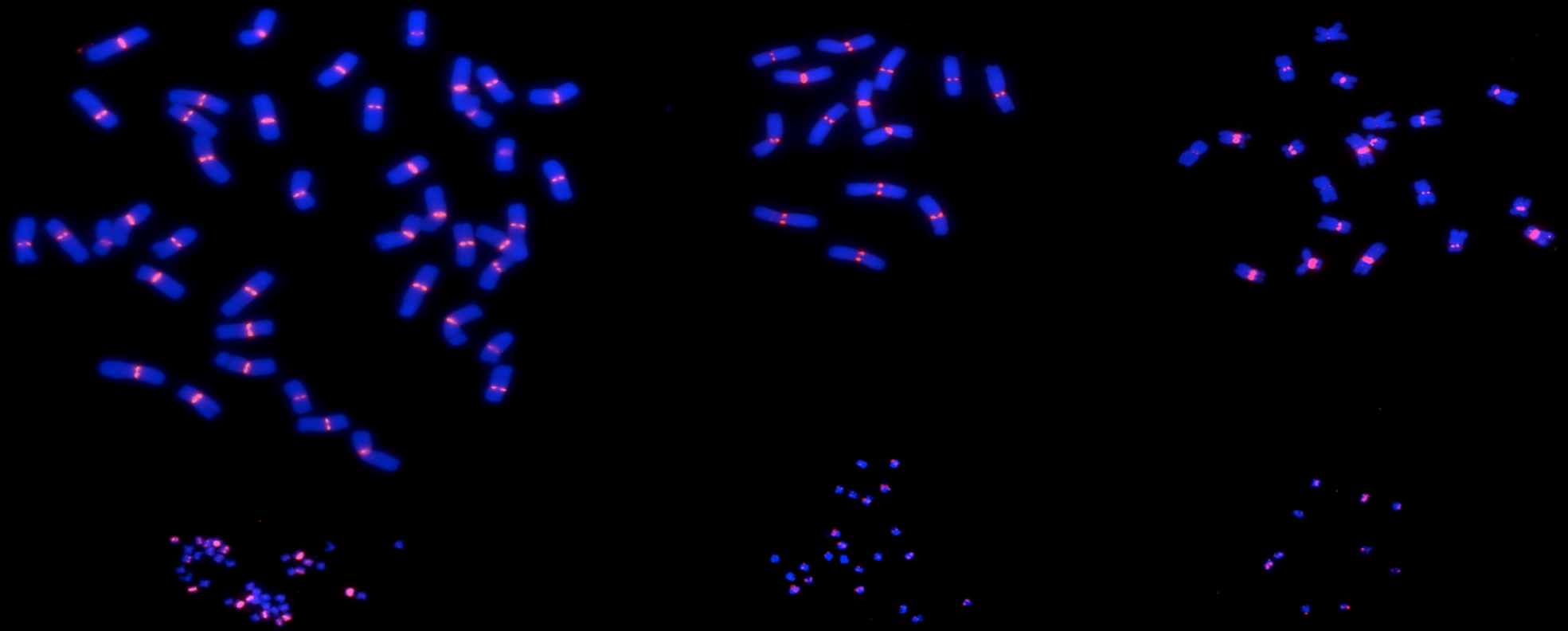
Kellye Eversole
IWGSC Executive Director

Plant Genomics and Gene Editing Congress

Amsterdam, The Netherlands
16 March 2017

The odyssey begins… 2005

2005 - Genome sequencing – the ‘wheat’ challenge



Wheat
Triticum aestivum
(16 Gb)

Barley
Hordeum vulgare
(5 Gb)

Corn
Zea mays
(2.5 Gb)

Soybean
Glycine max
(1.1 Gb)

Rice
Oryza sativa
(0.45 Gb)

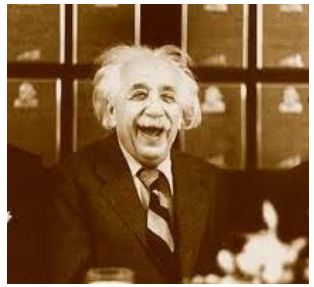
Arabidopsis
Arabidopsis thaliana
(0.15 Gb)

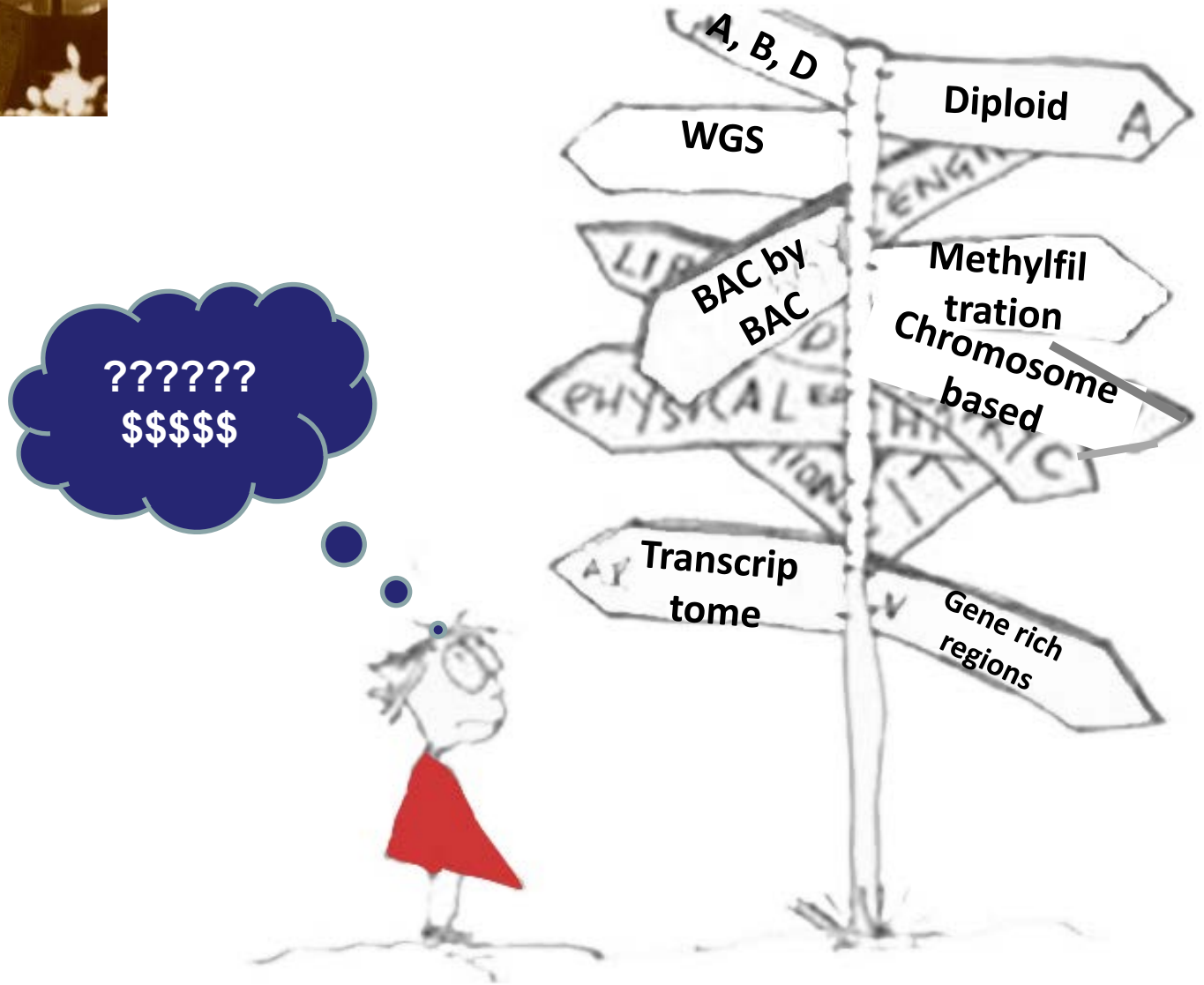
Dal-Hoe Koo, Wheat Genetics Resource Center, Kansas State University

Gb = 1,000,000,000 DNA base pairs

10 µm

How to produce a useful sequence?





Technology Neutral

For what do we want it?

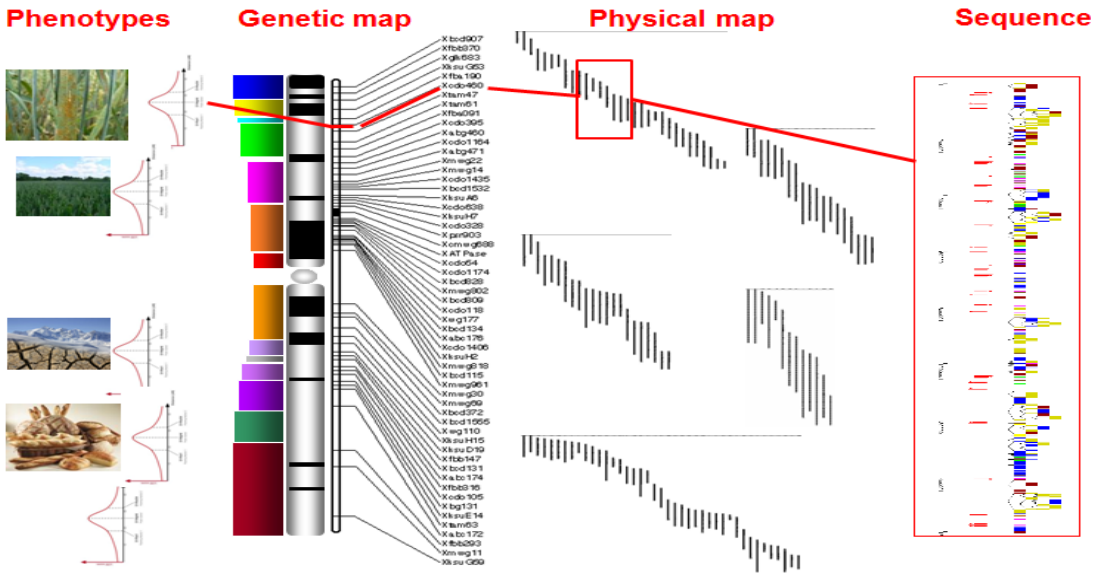
Vision

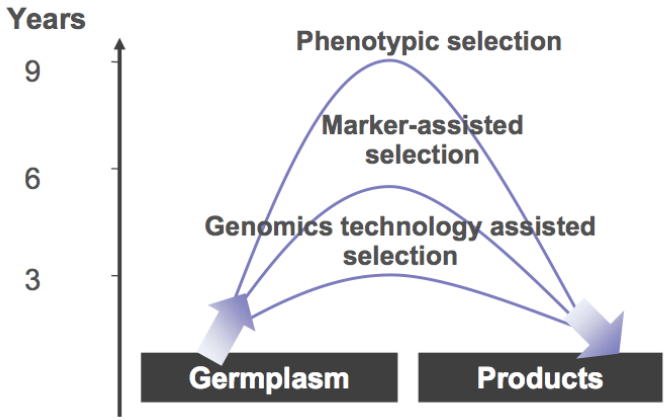
Goal

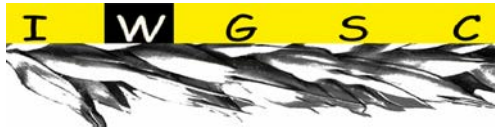
- Lay a foundation to accelerate wheat improvement
- Increase profitability throughout the industry

Vision

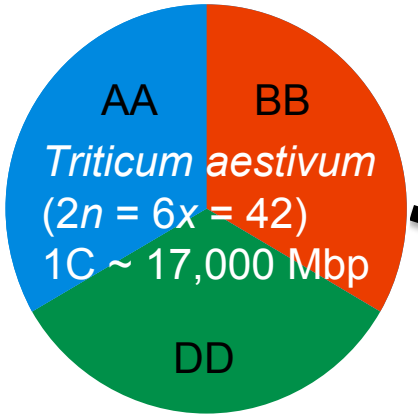
- High quality annotated genome sequence, comparable to rice
- Physical map-based, integrated and ordered sequence

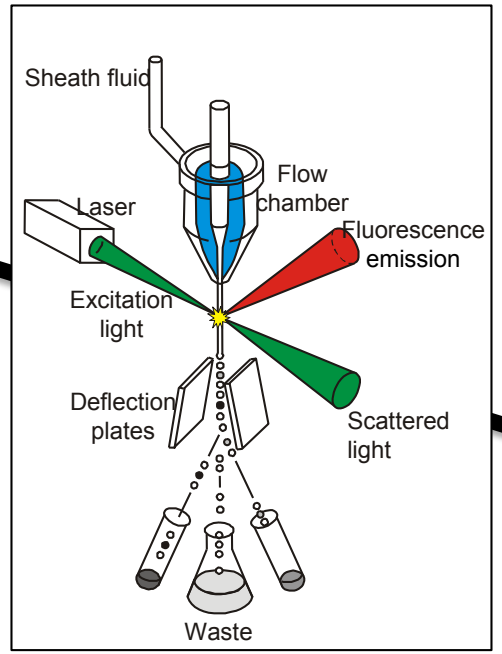




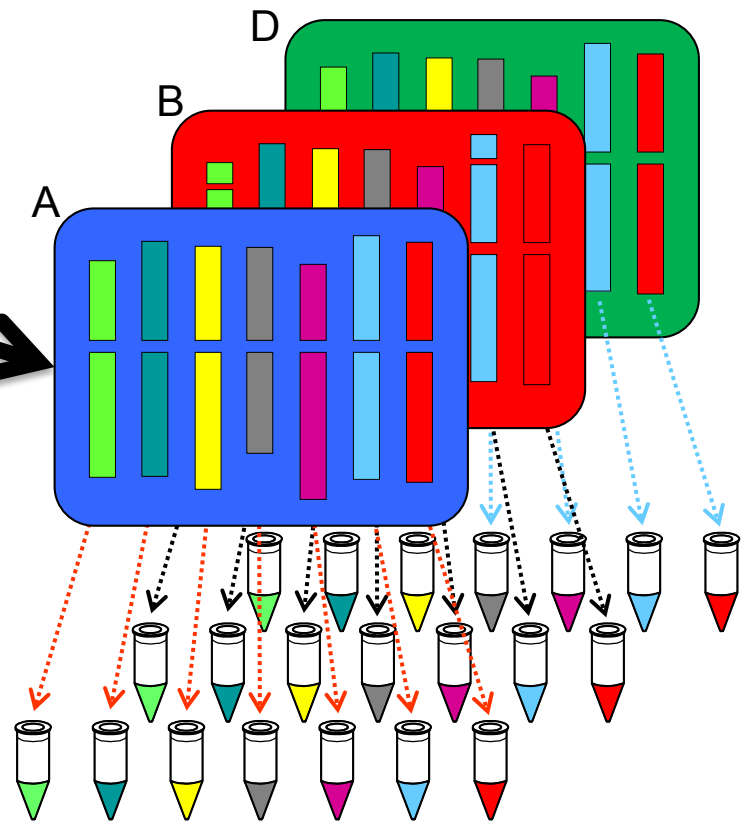


A chromosome-based approach





Dissection of the genome to single chromosomes (arms) representing individual (sub)genomes



Doležel et al., Chromosome Res. 15: 51, 2007

- Chromosomes: 605 - 995 Mbp (3.6 – 5.9% of the genome)
- Chromosome arms: 225 - 585 Mbp (1.3 – 3.4% of the genome)

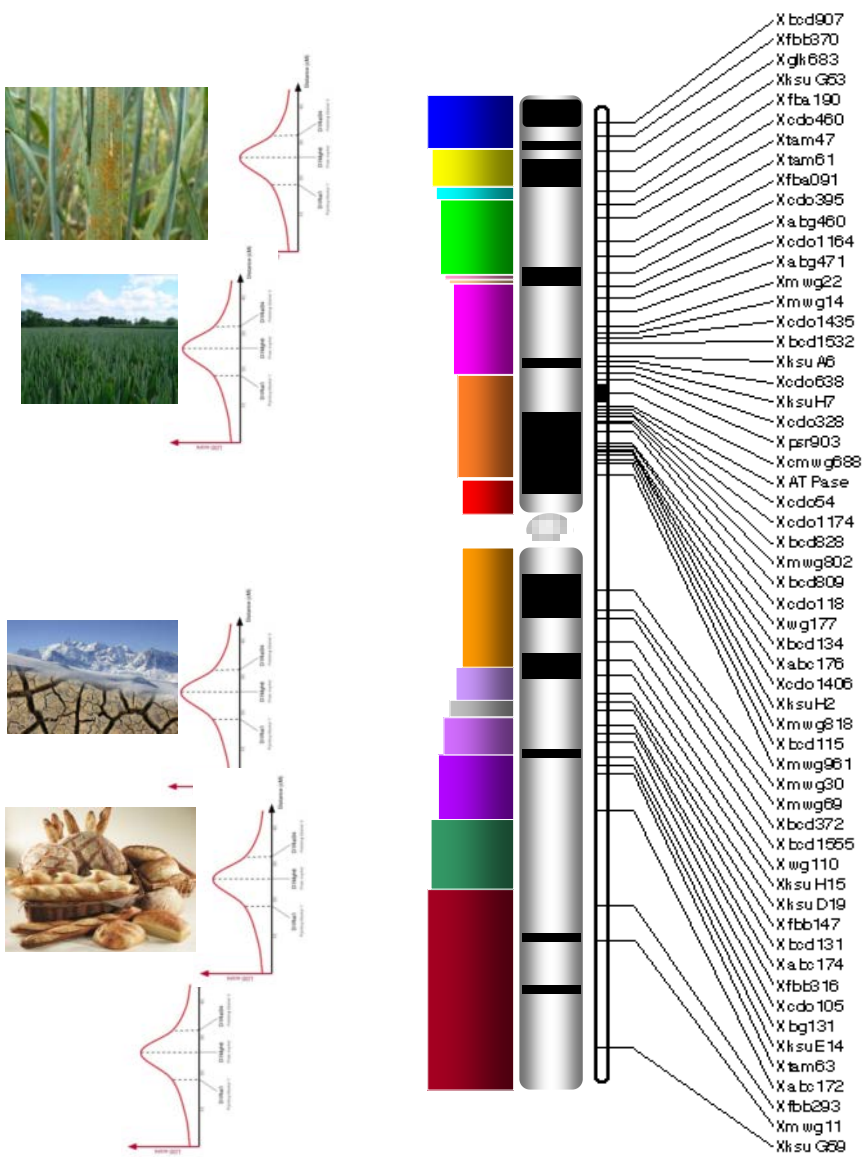
- **Chromosome specific BAC libraries (2006 - 2012)**
- **Amplified DNA for chromosome survey (2010 - 2011)**

IEB


AM /LOBO.WOLF

An integrated and ordered 3B reference sequence

MetaQTL analysis | 3B consensus map (5000 markers) | 3B Physical map | 3B pseudomolecule



Feuillet, Paux, and Choulet, Science 2008 and Science 2014

UMR 1095 GDEC INRA

Roadmap to the Wheat Genome Sequence

Illumina sequencing of individual chromosomes

IWGSC CSS v2 (2014)

+

Whole genome mate pairs

IWGSC CSS v3 (2016)

Physical maps of individual chromosomes

100%

MTP sequencing

62%

Pseudomolecule assembly

100%

Chromosome 3B (2014)
20 chromosomes (2016)

NRGene-Illumina WGS

IWGSC Whole Genome Assembly v0.4 (2016)

+

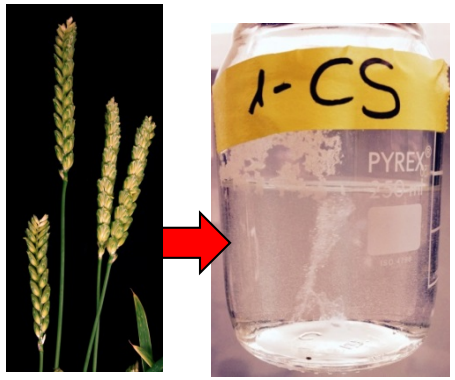
Radiation Hybrid, Hi-C, genetic, LD maps
BioNanoGenomics optical maps
MTP sequence tags…..

Reference Genome Sequence (2017)

I W G S C

IWGSC RefSeq

The IWGSC CS WGA Project – timeline 2015



Agreement Go!

DNA Extraction

Libraries Complete

Sequencing Complete

Assembly V0.1

Assembly V0.2

Complete QA

August | Sept | October | November | December | January | February | March

illumina

DeNovoMAGIC™

~2 months from data accumulation to completion of first assembly

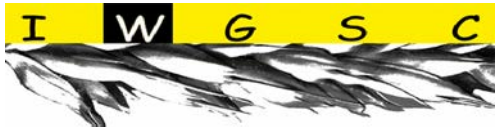
IPK GATERSLEBEN | University of Saskatchewan | GIFS Global Institute for Food Security | K-State | Tel Aviv University | INRA Science & Impact | NRGene | IWGSC

IWGSC Whole Genome Assembly Project

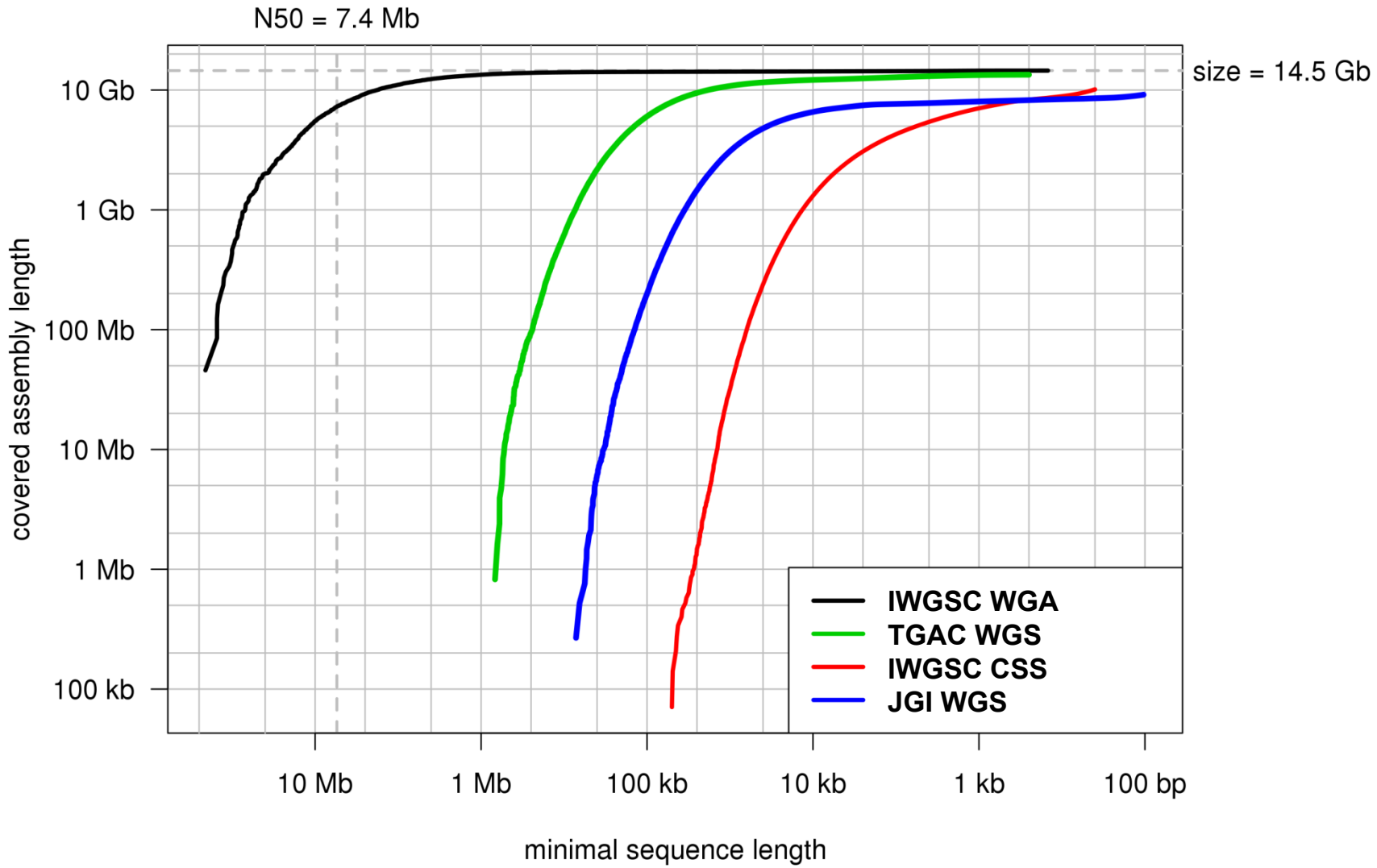
De novo assembly:

- NRGene's DeNovoMagic-2 platform, total run time < 3 weeks, 1Tb RAM computer
- Illumina short-read sequencing data only (200 x coverage)

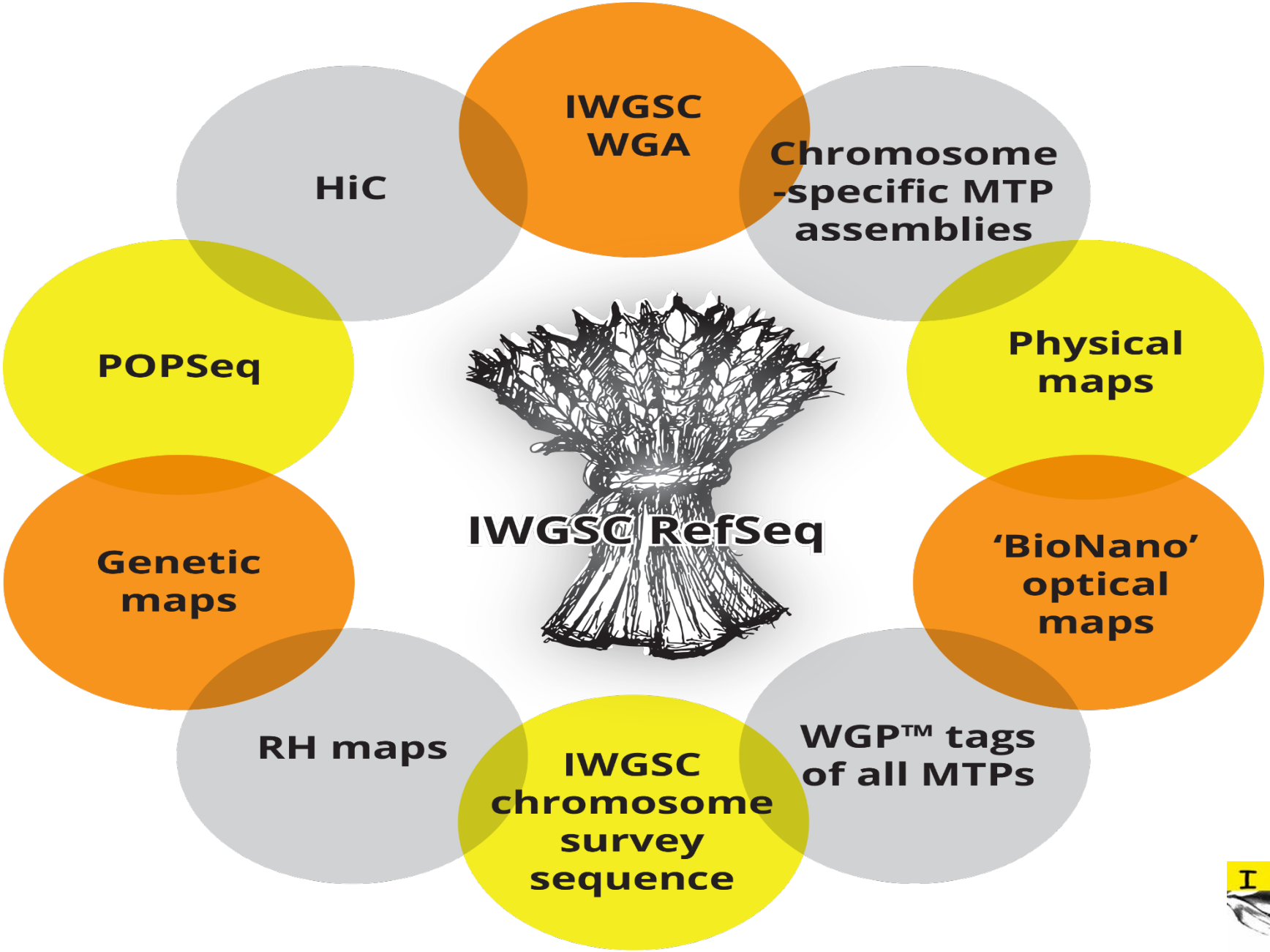
Assembly size:	14.5 Gbp
Est. gaps size:	262 Mbp
Gaps %:	1.80
Total # scaffolds:	138,484
N50:	7.1Mbp
L50 (#sequences):	566
N90:	1.3 Mbp
L90 (#sequences):	2,363
MAX Scaffold:	45.8 Mbp



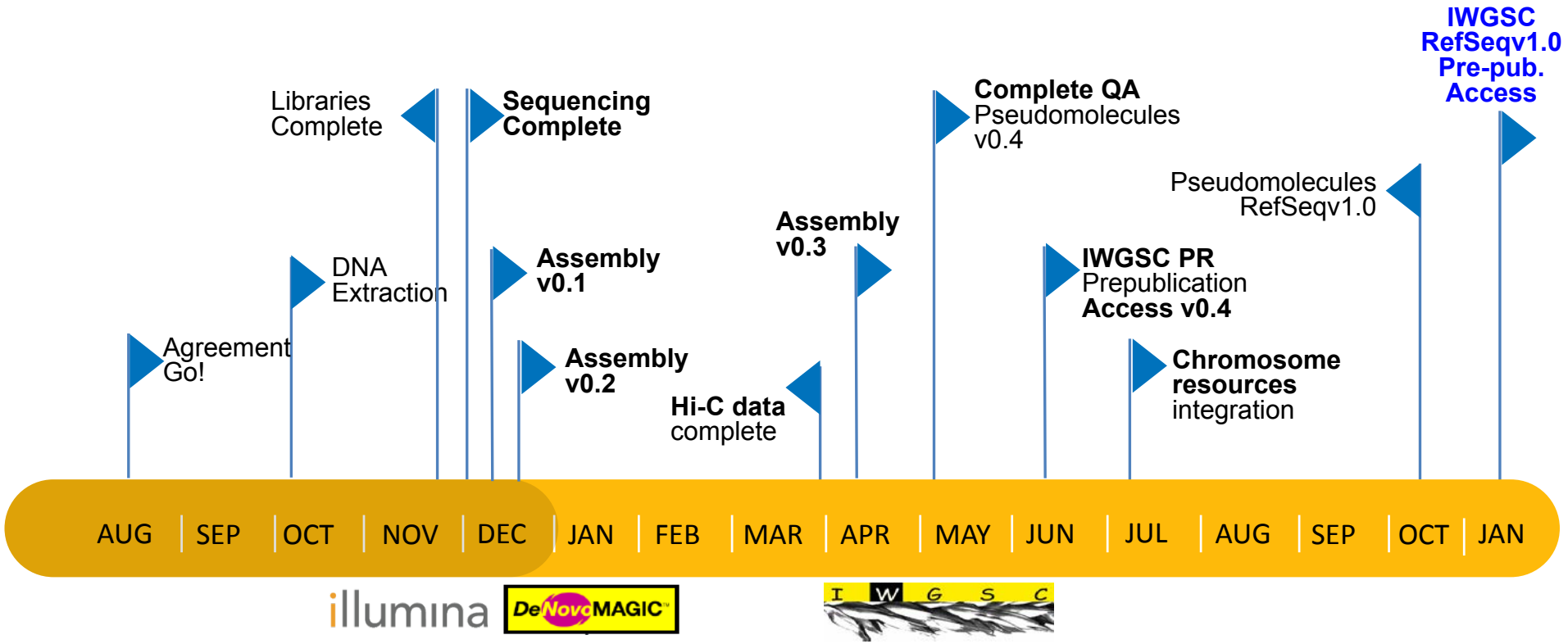
WGA Assembly Statistics



Concerted integration of resources: RefSeq v1.0



IWGSC RefSeq v1.0 Project Timeline



IWGSC RefSeq

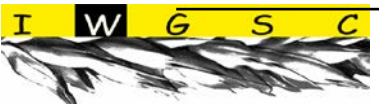
IWGSC RefSeq Project

- Physical maps for all chromosomes
  - 1,839,128 BACs, 47,810 contigs, 380,675 singletons
- WGP tags (mostly from MTP BACs) for all chromosomes except 3B
  - 4,305,249 unique tags, 693,697 BACs
- BAC sequence assemblies for 8 chromosomes (1A, 1B, 3B, 3D, 6B, 7A, 7B, 7D) and partial MTP data for two arms (4AL, 5BS)
  - 52,890 BACs (9.7 Gb), N50 - 68 kb
- Optical maps for 7A, 7B and 7DS
  - 1,335 BioNanoGenomics contigs aligned to the WGA assembly
- GBS map of the SynOp RIL population
  - 179 RILs, 4074 markers

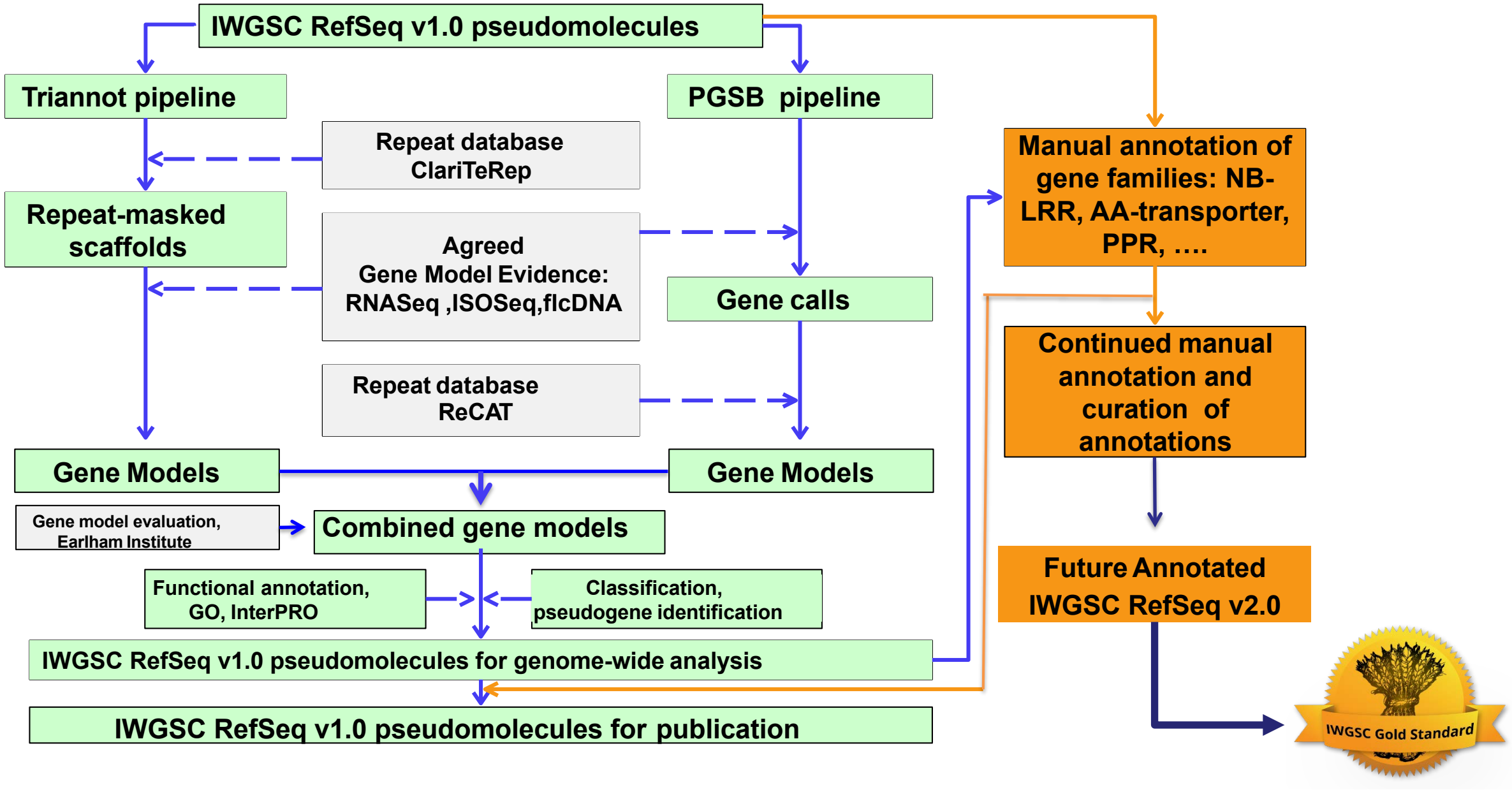
Comparison of IWGSC Assembly Releases

	IWGSCv0.4	RefSeqv1.0
Number / coverage of scaffolds/contigs	138,607 /14.5 Gb	138,665 /14.5 Gb
Number / coverage of scaffolds/contigs >=100kb	4,442 / 14.2 Gb	4,443 / 14.2Gb
N50 scaffolds / superscaffolds	7.0 Mb	22.8 Mb
L50 (no. sequences ➔N50)	566	166
N90 scaffolds / superscaffolds	1.3 Mb	4.1 Mb
L90 (no. sequences ➔N50)	2363	718
Gaps filled with BAC sequences		183 (1.7 Mb)
Average size of inserted BAC sequences		9.5 kb
Sequence assigned to chromosomes	14.1 Gb (96.8%)	14.1 Gb (96.8%)
Sequence assigned to chromosomes (>=100kb)	14.1 Gb (99.1%)	14.1 Gb (99.1%)
No. scaffolds / superscaffolds on chromosomes	3,975	1,601
No. oriented scaffolds / superscaffolds	2,464	1,243
Oriented sequence	13.1 Gb (90.2%)	13.8 Gb (95%)
Oriented sequence >=100kb	13.1 Gb (92.4%)	13.8 Gb (97.3%)

RefSeq v1.0 contains ~ 75 scaffolds per chrom.



IWGSC RefSeq v1.0 Annotation



IWGSC RefSeq Data Access & Availability

Pre-publication data access:

IWGSC WGA v0.4: June 13, 2016
IWGSC RefSeq v1.0: January 14, 2017

Gene models completed: March 2017

Final analyses completed: April/May 2017

Manuscript submission: Summer 2017

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

I W G S C

IWGSC RefSeq

The IWGSC Today

2017

Board of Directors

Role: Overall strategy and organization

Up to 10 directors including at least 1 Outside Director

Leadership Team

Role: Daily management

Members: Appels, Eversole, Feuillet, Keller, & Rogers

Coordinating Committee

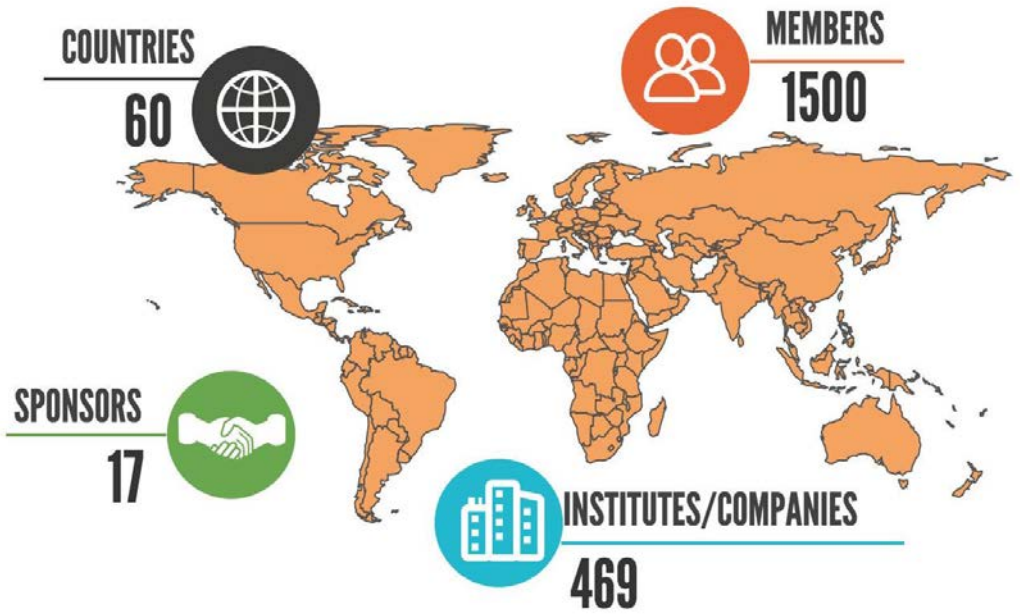
Role: Scientific strategy

Members: Sponsors & Leaders of IWGSC Projects & Initiatives

Members

Role: Participation & Input

Members: Open to Anyone



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IWGSC

www.wheatgenome.org

IWGSC RefSeq

IWGSC 2.0

- Manual and functional annotation of the sequence to empower gene discovery and gene cloning to understand the molecular basis of traits
- Coordination of re-sequencing activities for diversity panels that represent the breadth of geographic distribution of germplasm for breeders
- Support the development of user-friendly, integrated databases

Lessons learned

- At least one high quality, manually annotated reference sequence
- Physical map-based for adaptability to any technology
- Maintain flexibility for new technologies without losing sight of quality
- Stay on the course towards your vision

Acknowledgments

IWGSC Leadership: Rudi Appels, Kellye Eversole, Catherine Feuillet, Beat Keller, Jane Rogers

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THE FEDERAL RESEARCH CENTER Institute of Cytology and Genetics OF SIBERIAN BRANCH OF THE RUSSIAN ACADEMY OF SCIENCES
Elena Salina

NMBU Norwegian University of Life Sciences
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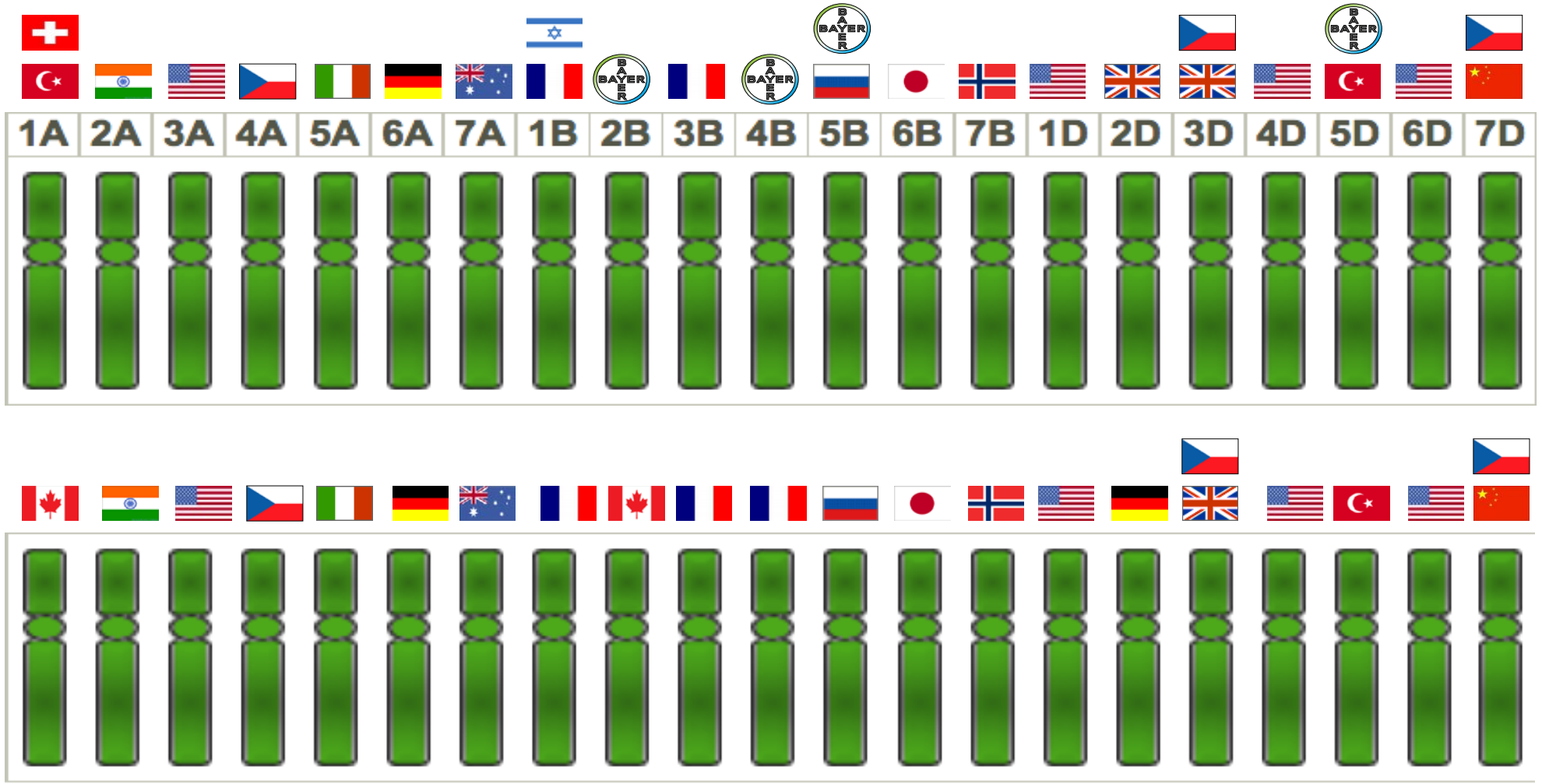
NORTHWEST A&F UNIVERSITY
Song Weining

Nikolai Ravin

Earlham Institute
Matt Clark

Acknowledgments



All physical maps and pseudo-molecule sequences available at IWGSC repository:
https://wheat-urgi.versailles.inra.fr

I W G S C

IWGSC RefSeq v1.0 Team Leaders

IWGSC Sequence Repository

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BAC WGP Tags

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IWGSC Sponsors

Thank you for your attention!