

A roadmap for gene functional characterisation in wheat

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 @CristobalUauy


John Innes Centre

Unlocking Nature's Diversity



Genotype

```
>Sequence_1  
GAGCATCGCACGATCGACGACTAGC  
AGCAGCATCAGCAGCATCAGCAGCA  
GCATACGAGGGACACACGCAGCTTA  
GCATCAGCATCGACGATCGACTACG  
CAGTACGACGACTACGCAGCATCAG  
GGCAGCATCGACTACGCATCAGCAT  
CGACTAGACTACGCATCAGCATCGA
```



Phenotype



In the past 4 years

Jordan et al. 2015 *Genome Biology*
 He et al 2019 *Nature Genetics*
 Pont et al 2019 *Nature Genetics*
 Zhou et al 2020 *bioRxiv*

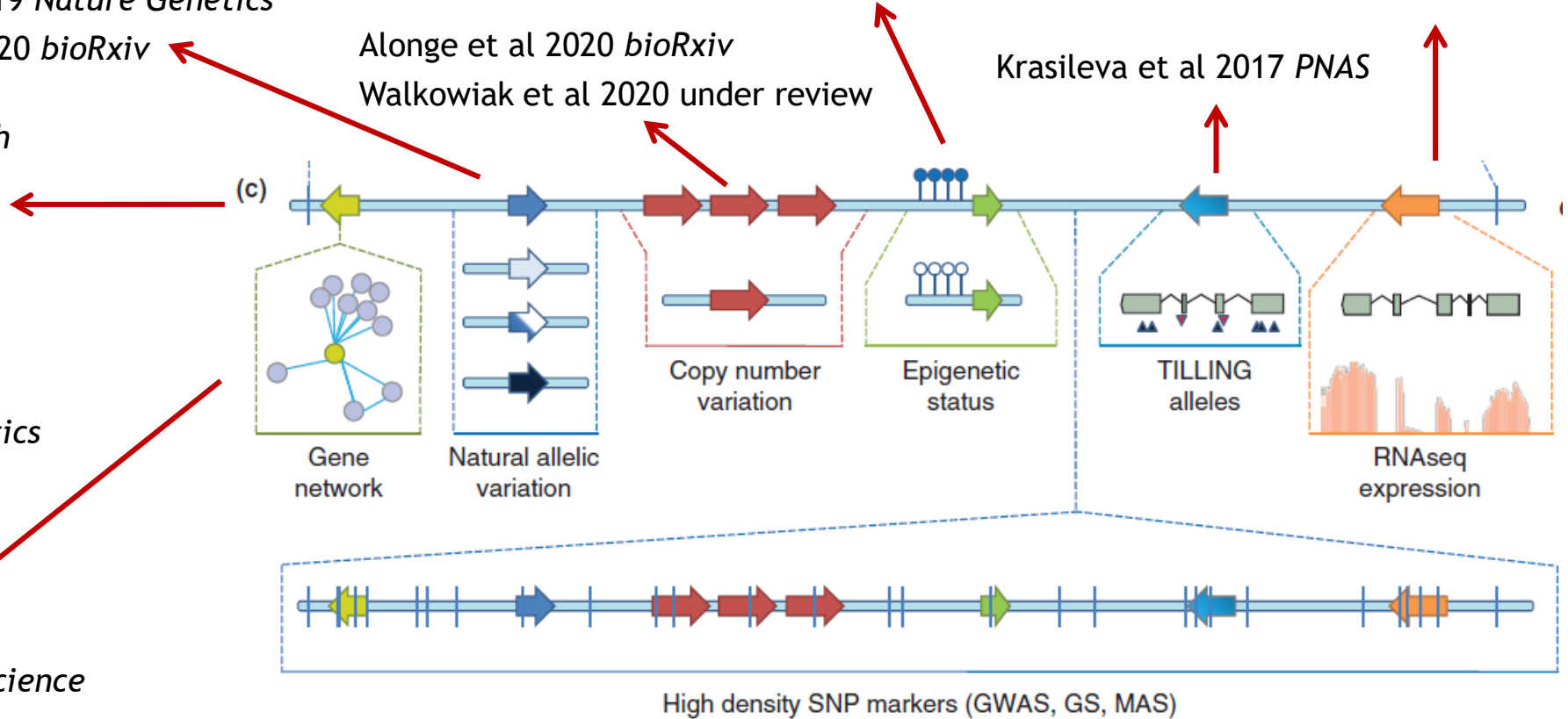
Gardiner et al 2015 *Genome Biology*
 Gardiner et al 2018 *Genome Research*
 Li et al 2019 *Genome Biology*

Pearce et al 2015 *BMC Plant Biol*
 Borrill et al 2016 *Plant Physiol*
 Ramirez-Gonzalez et al 2018 *Science*

IWGSC et al 2014 *Science*
 Clavijo et al 2017 *Genome Research*
 Avni et al 2017 *Science*
 Luo et al 2017 *Nature*
 Zimin et al 2017 *GigaScience*
 Ling et al 2018 *Nature*
 IWGSC et al 2018 *Science*
 Maccaferri et al 2019 *Nature Genetics*
 Alonge et al 2020 *bioRxiv*
 Walkowiak et al 2020 under review

Alonge et al 2020 *bioRxiv*
 Walkowiak et al 2020 under review

Krasileva et al 2017 *PNAS*



Ramirez-Gonzalez et al 2018 *Science*
 Hassani-Pak et al 2020 *bioRxiv*



Wilkinson et al 2012/2016
BMC Bioinformatics



Howe et al 2020
NAR



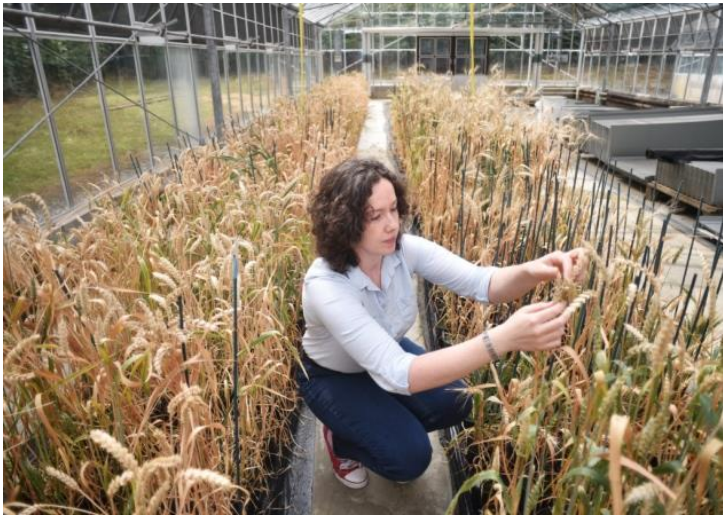
Blake et al 2019
Database



Alaux et al 2018
Genome Biology



Ramirez-Gonzalez et al 2015 *Bioinformatics*
 Bevan and Uauy 2013 *Genome Biology*



Philippa Borrill
(JIC)
University of Birmingham



Clemence Marchal
(JIC)



Nikolai Adamski
(JIC)



Sophie Harrington
(JIC)



Jemima Brinton
(JIC)

Plus countless
additional
contribuors !



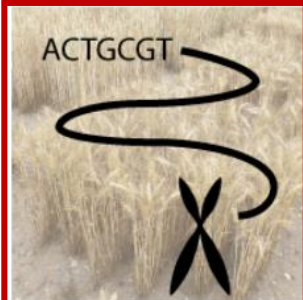
This website provides background information and practical resources to help both budding wheat scientists as well as researchers looking to expand their work into wheat.

There is a need to improve crops to feed the world's growing population with the backdrop of climate change. Translation of fundamental plant biology research (e.g. from *Arabidopsis thaliana*) into crops such as wheat provides a potential route to deal with this challenge. However learning even simple tasks such as growing and crossing wheat plants requires time and effort, while material and methods sections in published articles are often short and cannot substitute teaching aids. This is also true for more complex topics such as the genomics aspect of wheat.

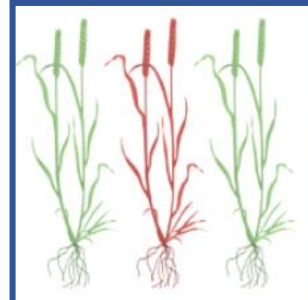
Here we provide information and training about:



Growing wheat




Wheat genomic resources



Functional studies

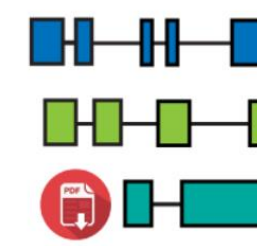


Useful wheat links




ACTGCGT

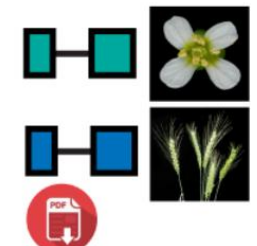
Genome assemblies



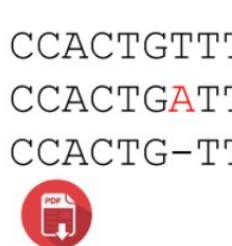
Gene models



Using Ensembl Plants

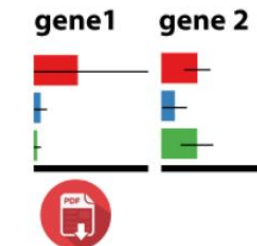


Finding wheat orthologs




CCACTGTTT
CCACTGATT
CCACTG-TT

Variation data




gene1 gene2


Expression data



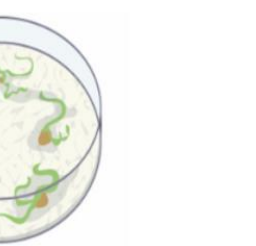
Gene Networks




Selecting TILLING mutants




Wheat Transformation and CRISPR/Cas9



Virus Induced Gene Silencing




Populations



A GTCGTC AACCAG
B GTCGTGAACCAG
D GTCGTC AACCAG

Designing genome specific primers

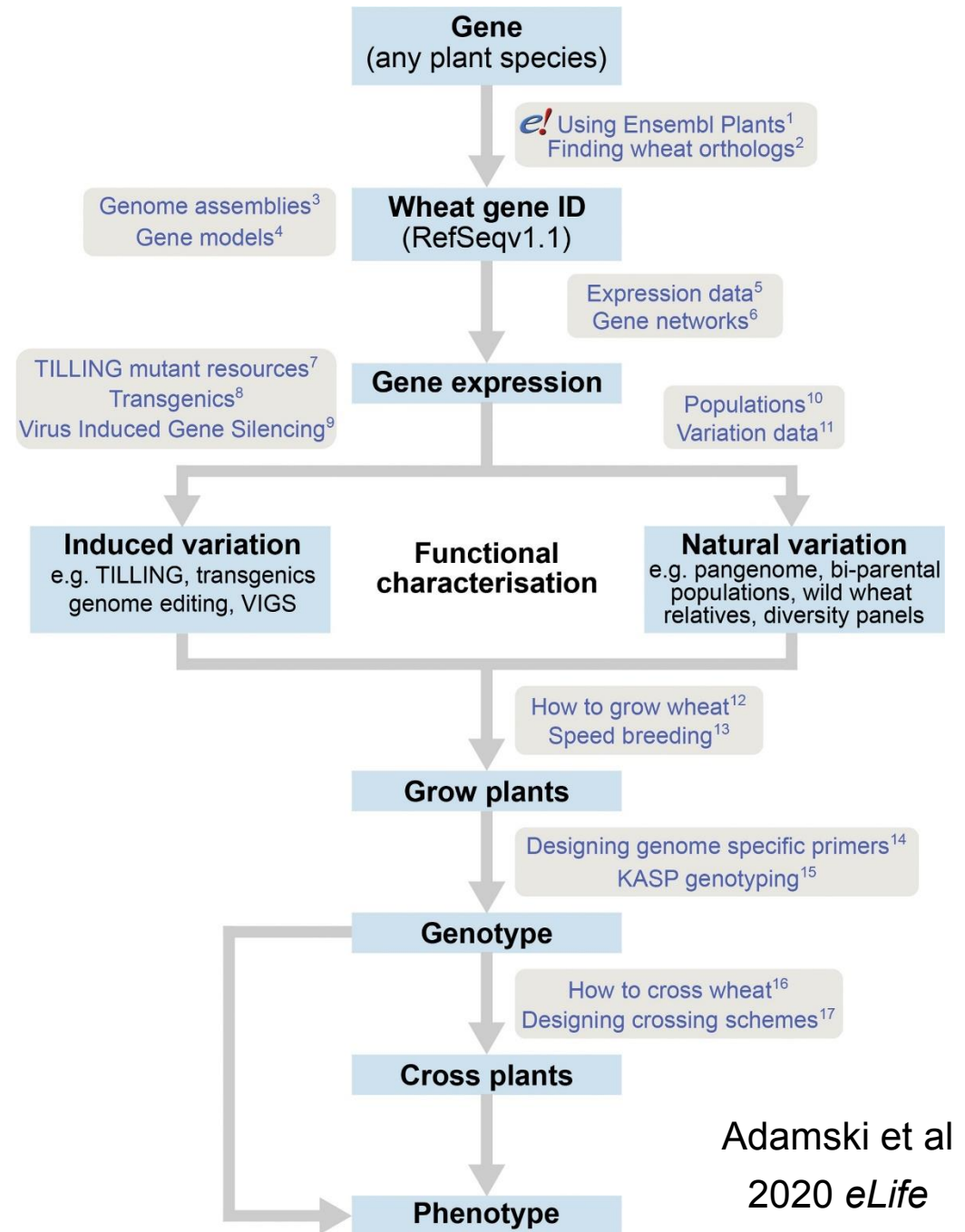


Designing crossing schemes

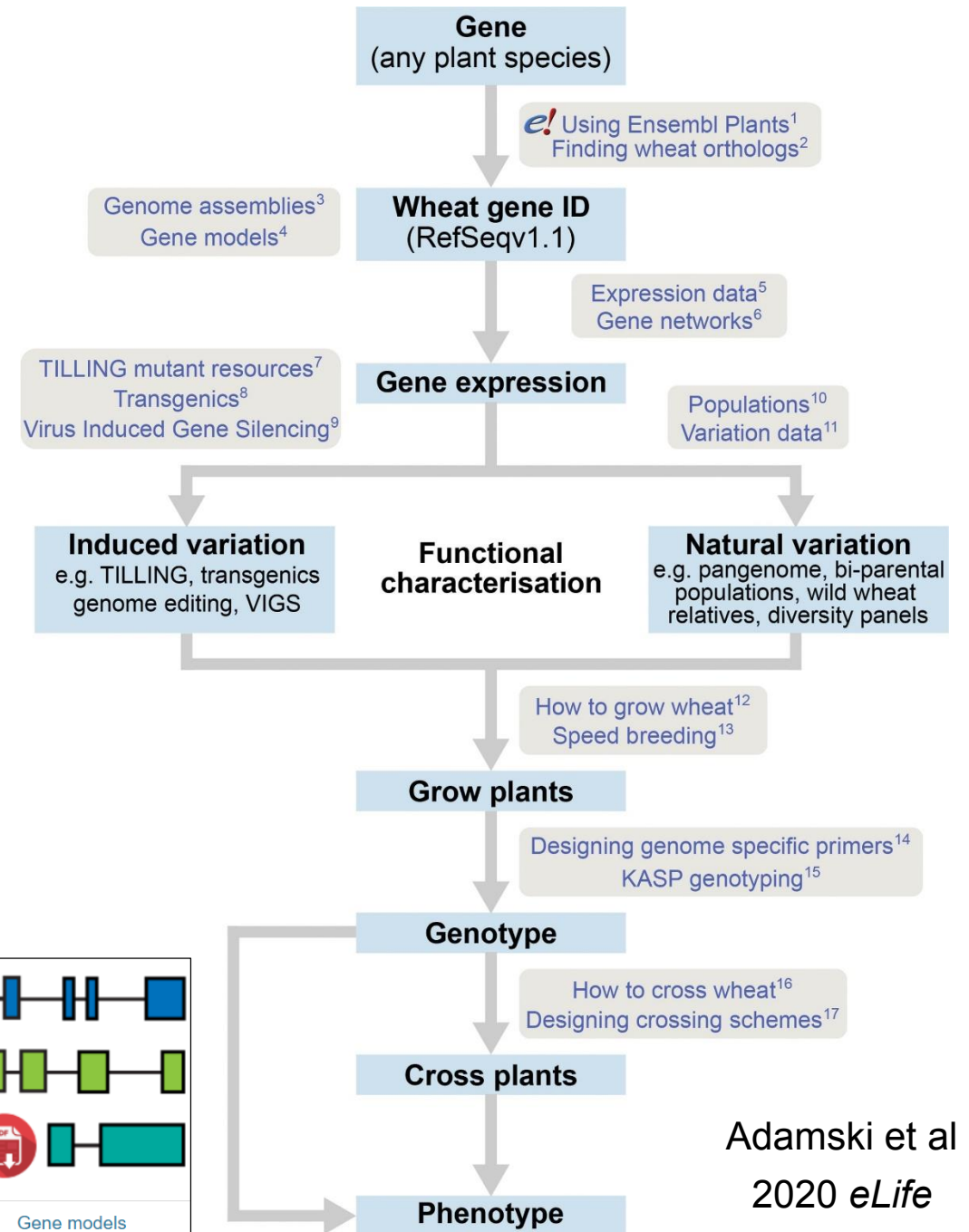
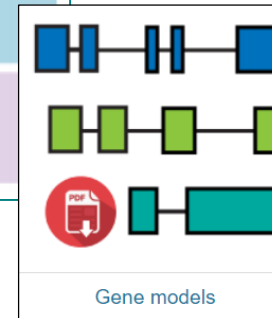
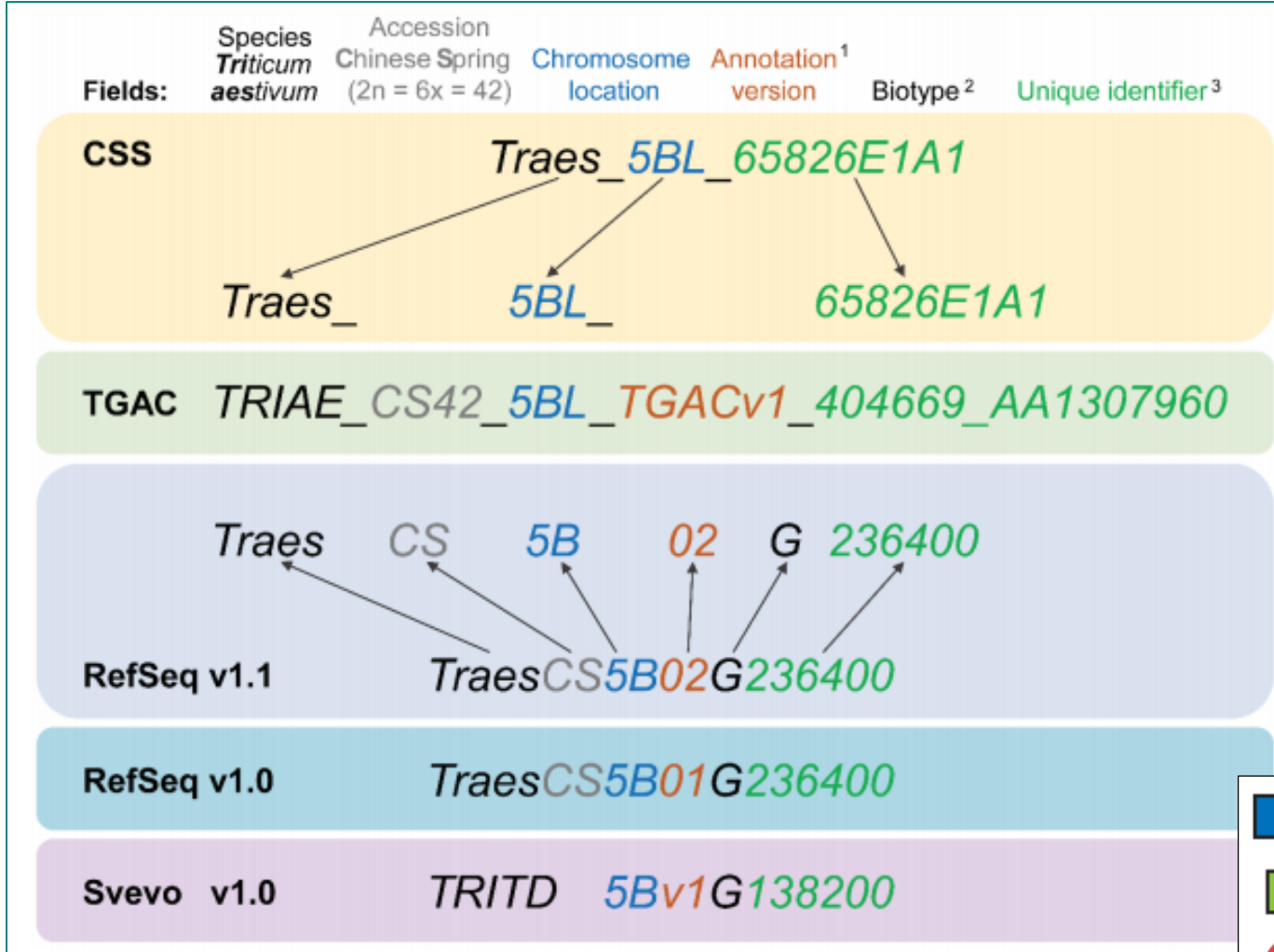


A roadmap for gene functional characterisation in crops with large genomes: Lessons from polyploid wheat

Nikolai M Adamski^{1†}, Philippa Borrill^{2†}, Jemima Brinton^{1†}, Sophie A Harrington^{1†}, Clémence Marchal^{1†}, Alison R Bentley³, William D Bovill⁴, Luigi Cattivelli⁵, James Cockram³, Bruno Contreras-Moreira⁶, Brett Ford⁴, Sreya Ghosh¹, Wendy Harwood¹, Keywan Hassani-Pak⁷, Sadiye Hayta¹, Lee T Hickey⁸, Kostya Kanyuka⁷, Julie King⁹, Marco Maccaferri¹⁰, Guy Naamati⁶, Curtis J Pozniak¹¹, Ricardo H Ramirez-Gonzalez¹, Carolina Sansaloni¹², Ben Trevaskis⁴, Luzie U Wingen¹, Brande BH Wulff¹, Cristobal Uauy^{1*}

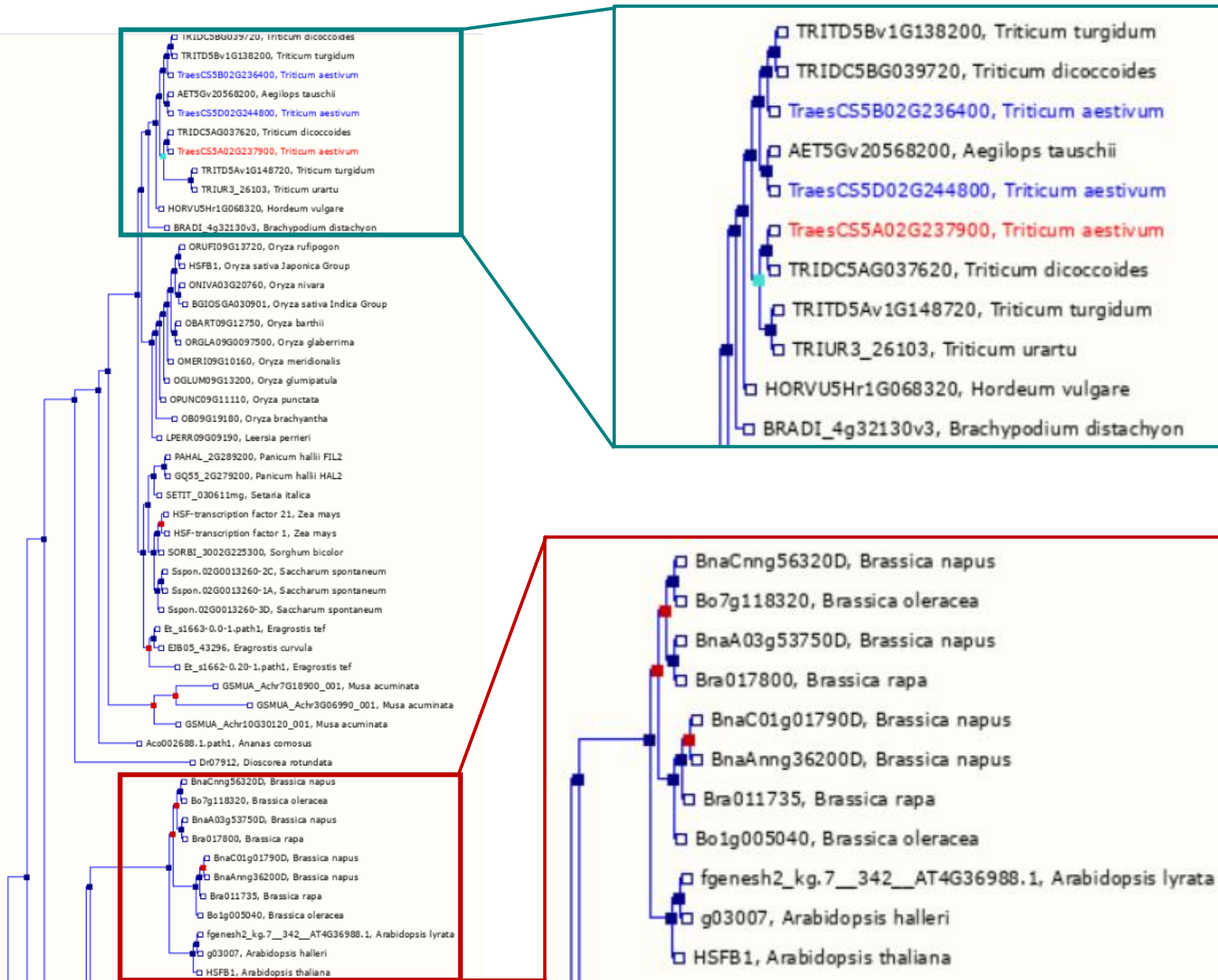


The importance of good gene models (and clear nomenclature)



Adamski et al
2020 *eLife*

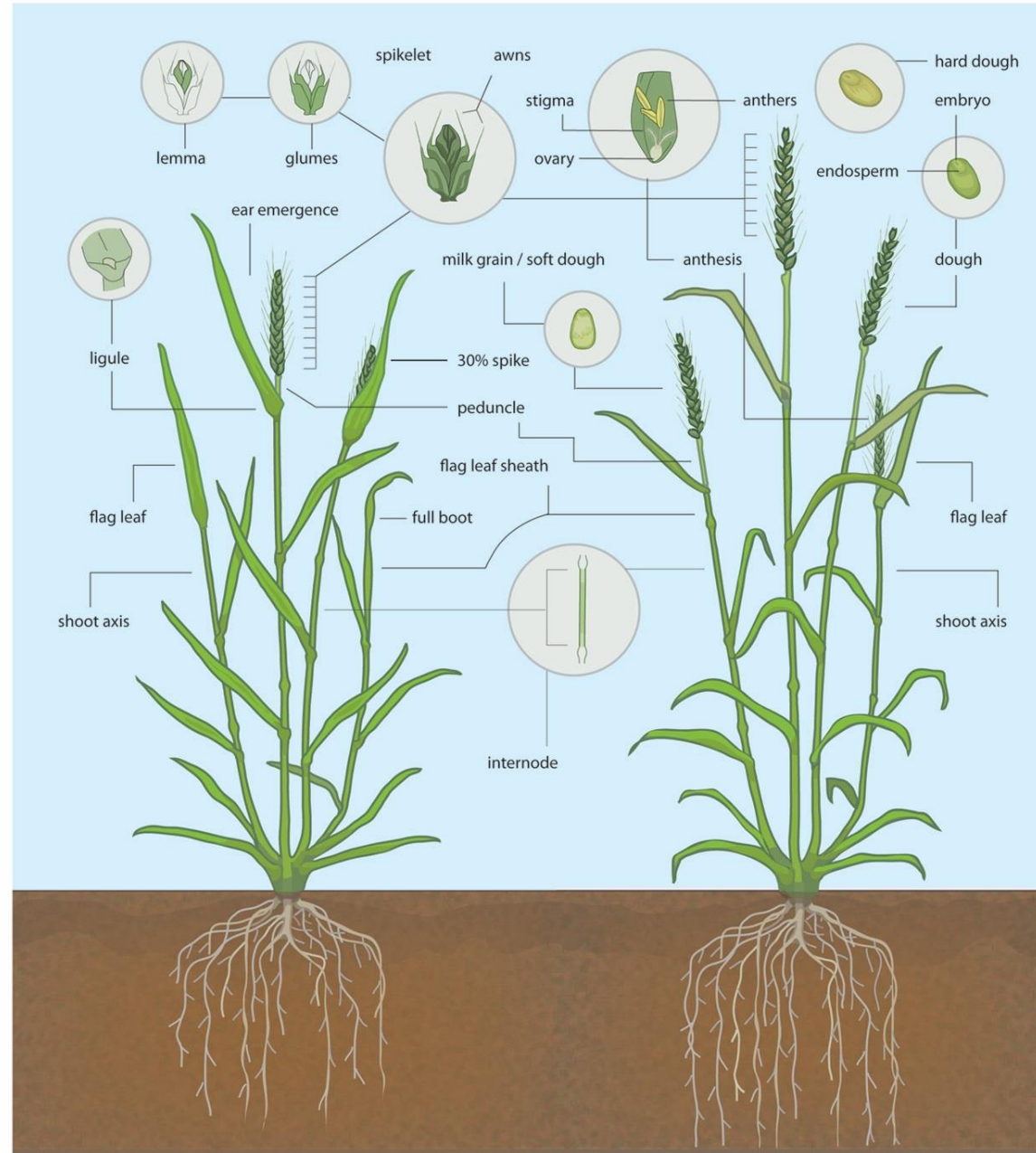
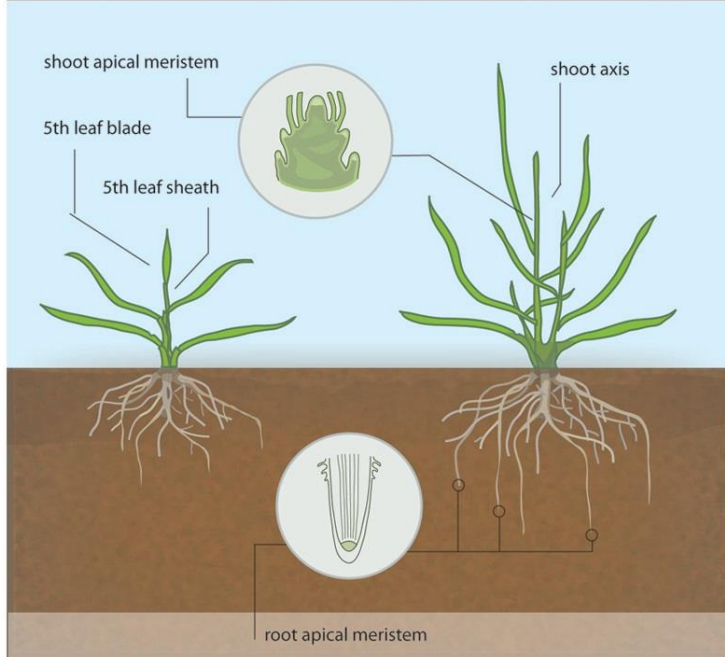
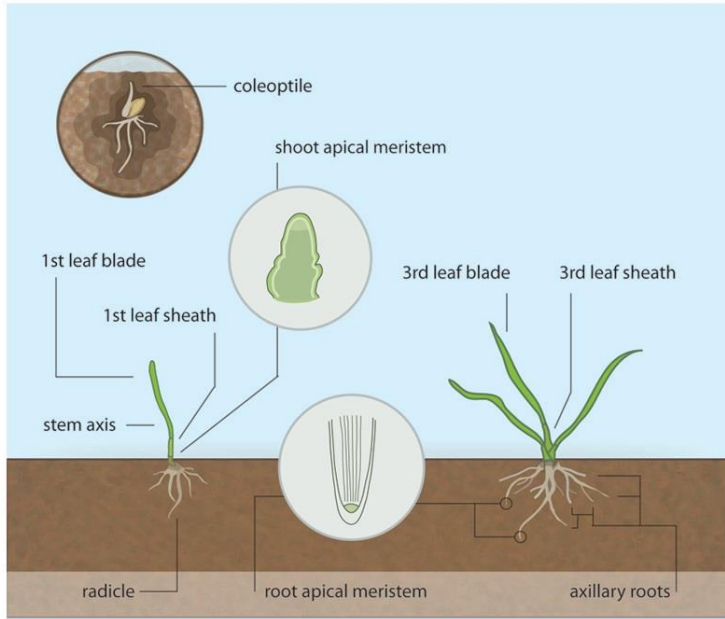
Pre-computed gene trees in *e!* to identify wheat orthologs



Using Ensembl Plants

Finding wheat orthologs

Where is my gene expressed?



Philippa Borrill
(JIC)
University of Birmingham



Ricardo Ramirez-Gonzalez (JIC)

Wheat Expression Browser powered by expVIP

 Gene set **RefSeq1.1**

[Home](#)

[Studies](#)

[Download](#)

[Add your data](#)

[Tutorials](#)

[Videos](#)

[Cite](#)

One or two genes

Gene set **RefSeq1.1** Gene:

Examples: *TraesCS2A02G103900* | *TraesCS4B02G075700* | *TraesCS2A02G103900.1* | *TraesCS4B02G075700.1*

Multiple genes

If you want to compare more than two genes, you can add a list of genes separated by commas or one gene in each line.

Select studies to display ▼

- Grain tissue-specific developmental timecourse
- Developmental time-course of Chinese Spring
- Chinese Spring seedling (leaves and roots) and spikes at anthesis
- Chinese Spring leaves and roots from seven leaf stage
- Six unreplicated tissues from Chinese Spring.
- Chinese Spring flag leaves, 6 timepoints
- Chinese Spring early meiosis, early prophase

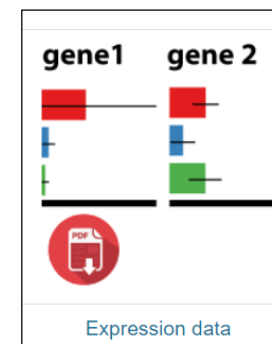
BLAST Scaffold

Paste query sequence(s) or drag file containing query sequence(s) in FASTA format here ...

Nucleotide databases

- IWGSC 2.26
- TGACv1
- RefSeq 1.0
- RefSeq 1.1

Advanced Parameters:



Bray et al 2016 *Nature Biotechnology*
Borrill et al 2016 *Plant Physiology*
Ramirez-Gonzalez et al 2018 *Science*

Visual interface (>1,000 samples)

Wheat Expression Browser powered by expVIP

Traes_4AL_F99FCB25F.1

Search

Compare

Gene set

RefSeq1.0

Home

Studies

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Add your data

Tutorials

Videos

Cite

Share

Expression unit: **tpm**

Log₂

Save as SVG

Save as PNG

Save data

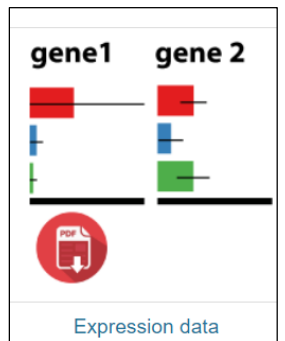
Save raw data

Restore Defaults

Zoom

study
High level tissue
Tissue
High level age
Age
High level stress-disease
Stress-disease
High level variety
Variety
Intermediate
Intermediate stress

● spike, spike (n=77)
● spike, spikelets (n=149)
● spike, awns (n=6)
● spike, glumes (n=12)
● spike, anther (n=5)
● spike, stigma & ovary (n=4)
● spike, microspores (n=9)
● spike, rachis (n=6)
● grain, gr_mi (n=68)
● grain, gr_ha (n=36)
● grain, endosperm (n=25)
● grain, embryo (n=3)
● grain, aleurone (n=12)
● grain, seed coat (n=6)
● grain, e_sc (n=3)
● grain, transfer cells (n=4)
● grain, al_e (n=4)
● leaves/shoots, s_aer (n=168)
● leaves/shoots, sam (n=6)
● leaves/shoots, lea_b (n=27)
● leaves/shoots, lea_s (n=12)
● leaves/shoots, shoot axis (n=12)
● leaves/shoots, flag leaf blade (n=30)
● leaves/shoots, leaf ligule (n=3)
● leaves/shoots, flag leaf sheath (n=12)
● leaves/shoots, intermode (n=15)
● leaves/shoots, peduncle (n=9)
● leaves/shoots, leaf excl flag leaf (n=73)
● leaves/shoots, flag leaf (n=26)
● leaves/shoots, v_aer (n=12)
● roots, roots (n=42)
● roots, root apical meristem (n=6)



Borrill et al 2016 *Plant Physiology*
Ramirez-Gonzalez et al 2018 *Science*

Wheat eFP (70 tissue*development stage)



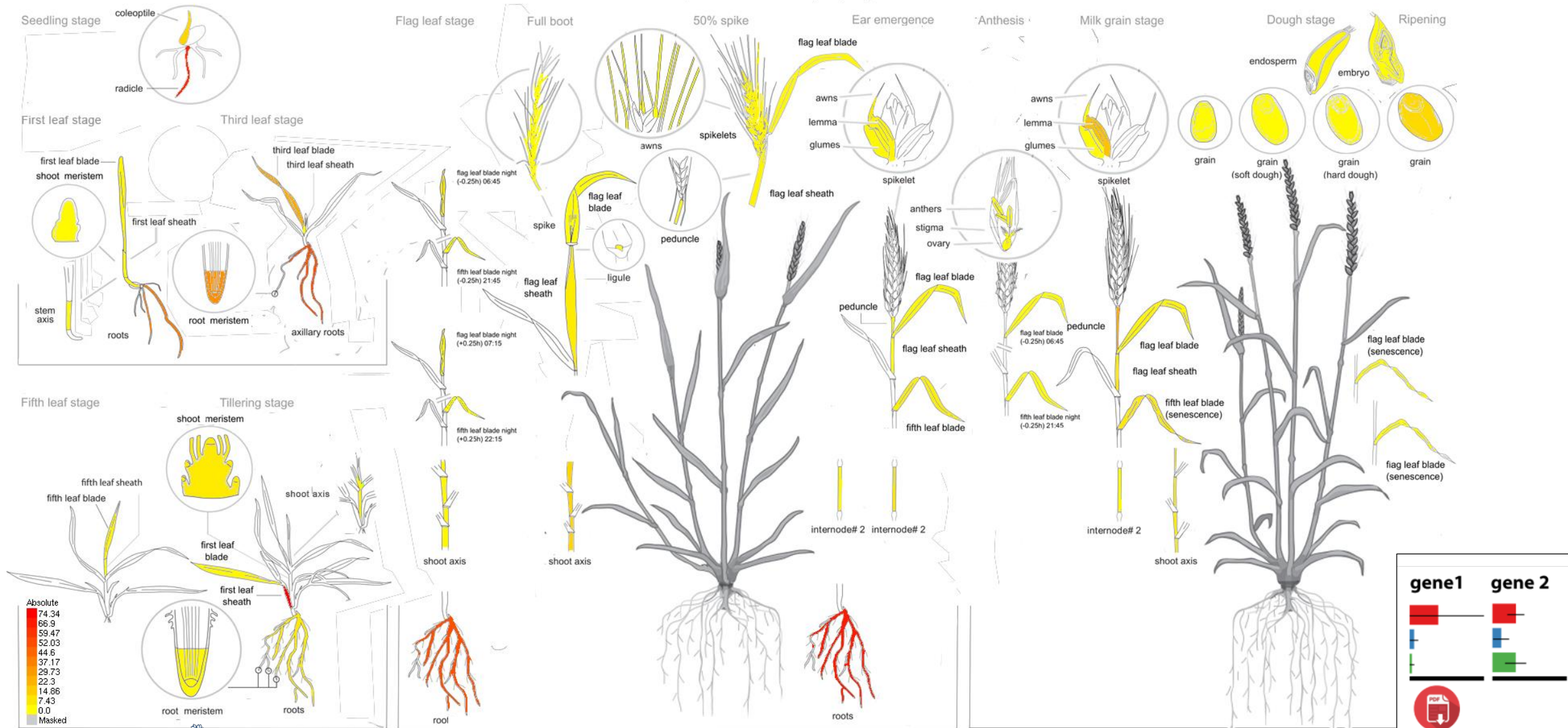
Wheat eFP Browser

TraesCS1A01G000100

TraesCS1A01G000100

Wheat (*Triticum aestivum* L.) eFP Browser at bar.utoronto.ca
RNA-seq data from Azhurnaya spring wheat

P



UNIVERSITY OF
TORONTO



UNIVERSITY OF
SASKATCHEWAN



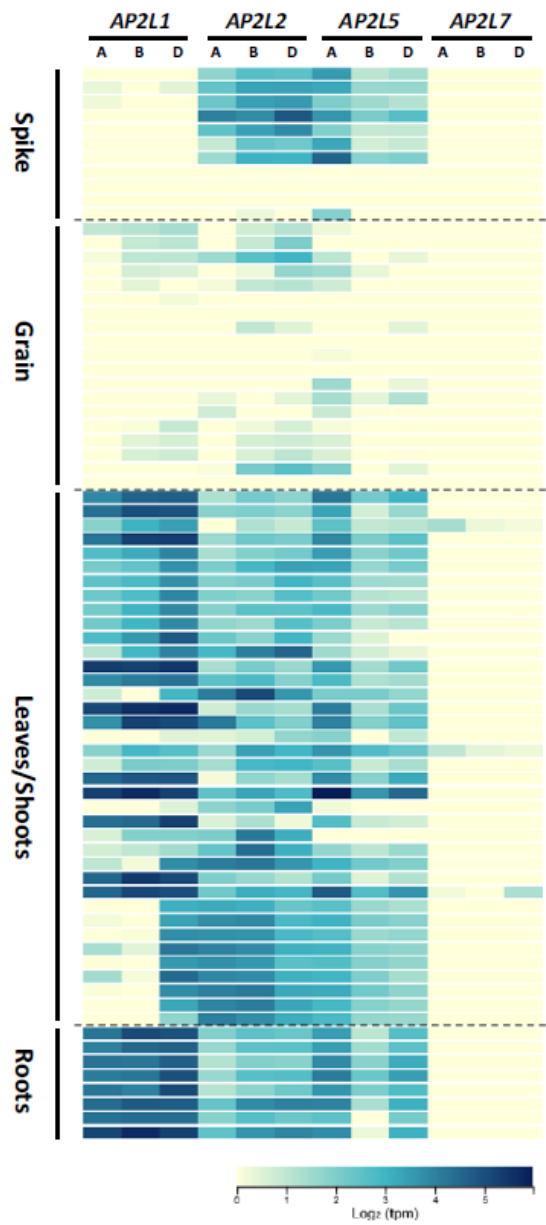
Agriculture and
Agri-Food Canada



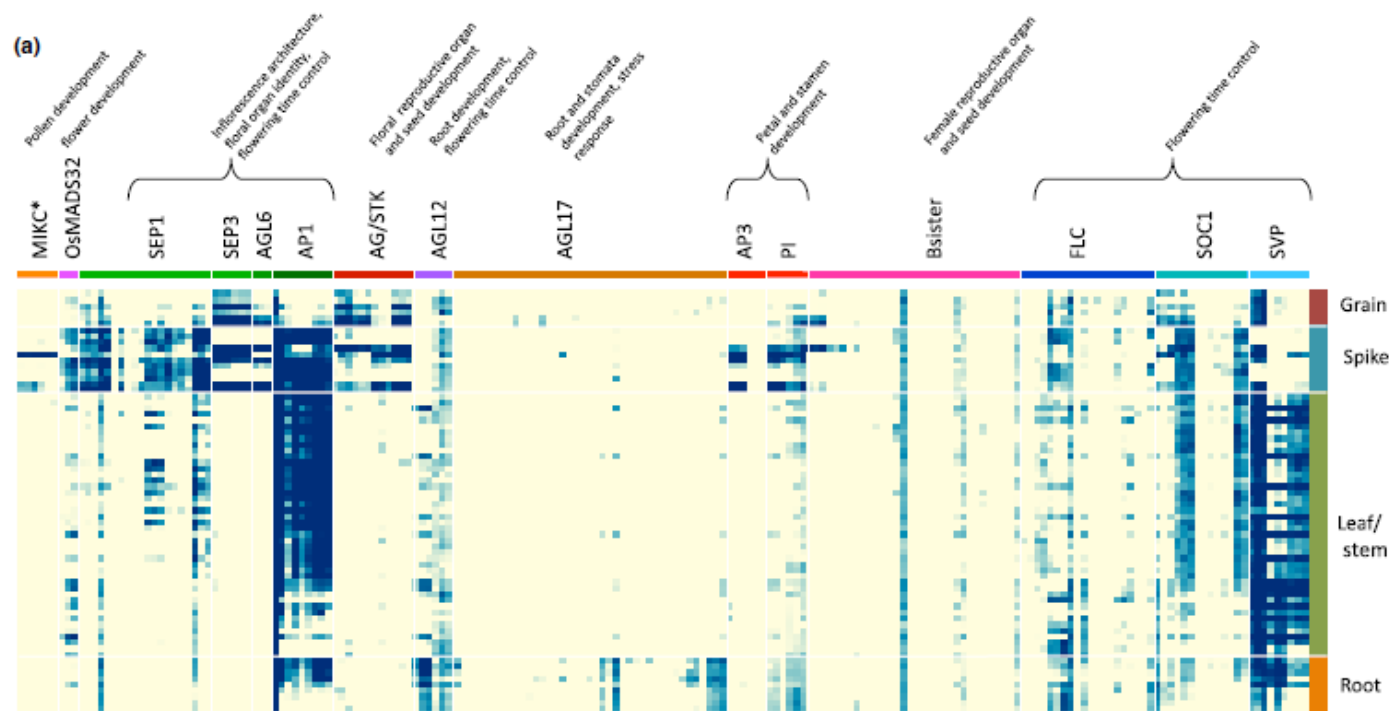
Bayer CropScience



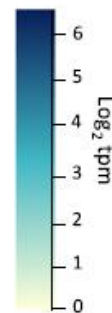
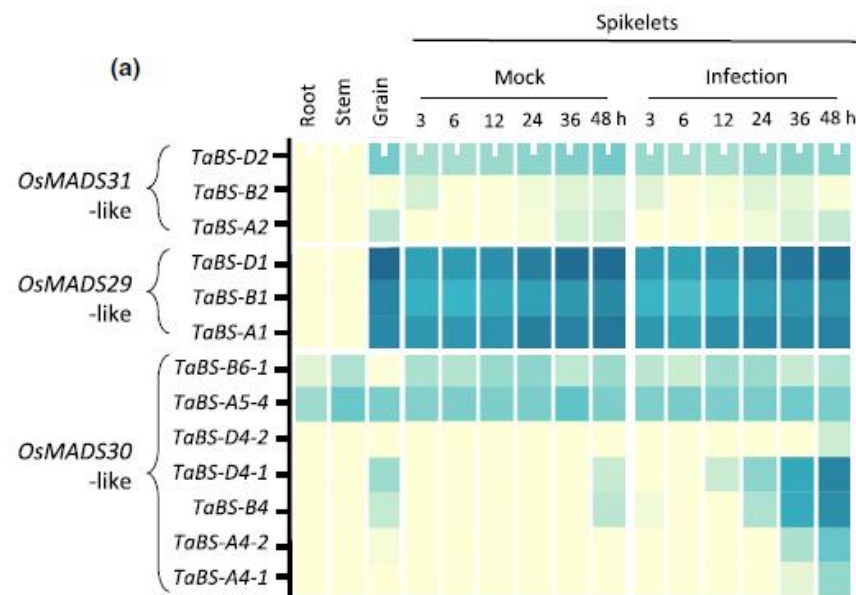
Expression data



Debernardi et al 2020
Plant Journal



Schilling et al 2020 *New Phytologist*



Susanne Schilling Rainer Melzer
Univ. College Dublin

Data Availability

<http://www.wheat-expression.com>



Ricardo Ramirez-Gonzalez (JIC)

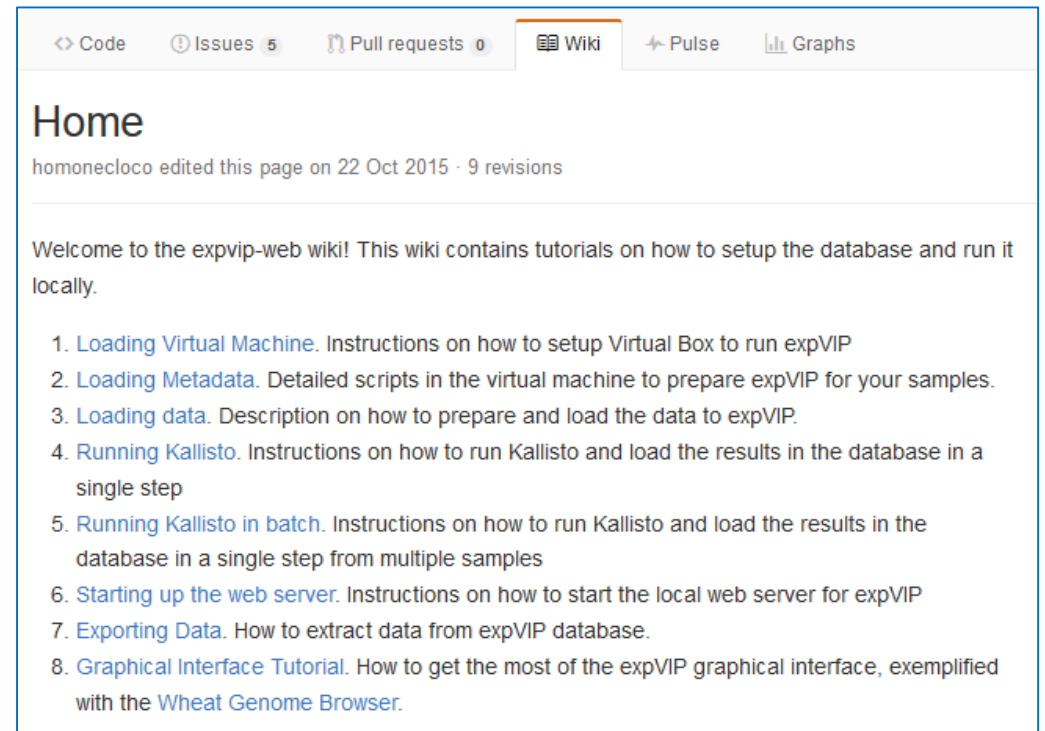
Source code

Database and interface setup:

<https://github.com/Uauy-Lab/expvip-web>

BioJS visualisation component:

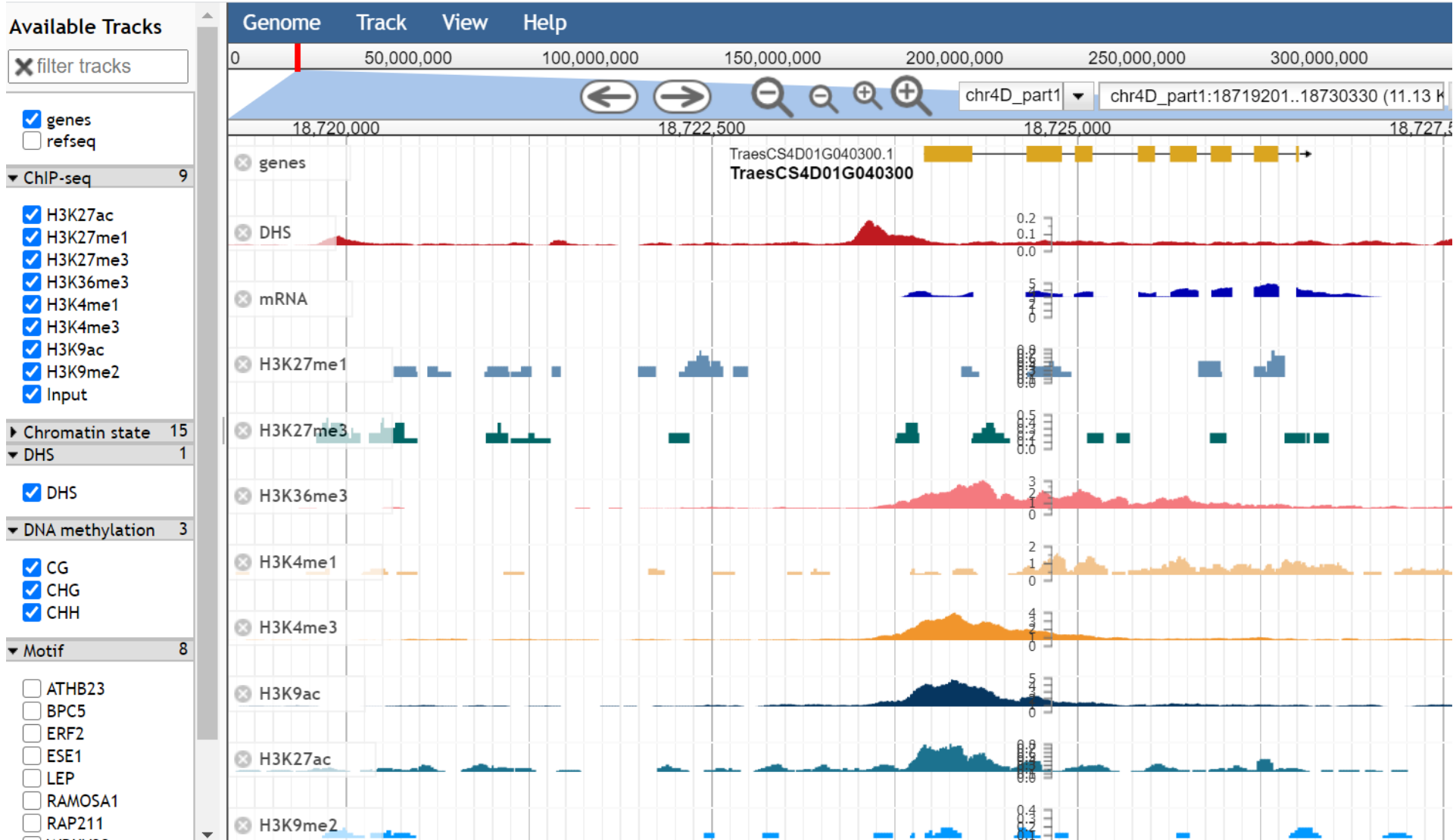
<http://biojs.io/d/bio-vis-expression-bar>



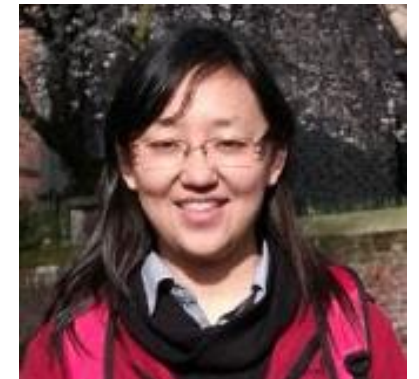
https://opendata.earlham.ac.uk/wheat/under_license/toronto/Ramirez-Gonzalez_etal_2018-06025-Transcriptome-Landscape/

Epigenetic marks

http://bioinfo.sibs.ac.cn/cs_epigenome



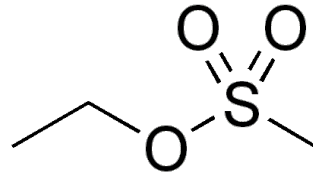
Zijuan Li
(CAS)



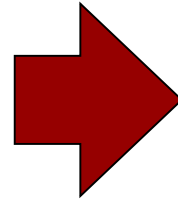
Yijing Zhang
(CAS)



Mutants in every wheat gene: *in silico* TILLING



EMS



Mutant Population



Ksenia Krasileva
(Earlham)-
Berkeley



Jorge Dubcovsky
(UC Davis)



Andy Phillips
(RRes)

Seeds



SeedStor

DNA



Exome
capture



Mutant
Identification

ATG T	ATGC	AT A C	ATGC	ATGC
T A CT	TG T T	TGCT	TGCT	TGCT
CGTG	A GTG	CGTG	T GTG	CGT A

Krasileva et al 2017 *PNAS*

Uauy, Wulff, Dubcovsky 2017 *ANR Genetics*

Access to >10M wheat mutants online



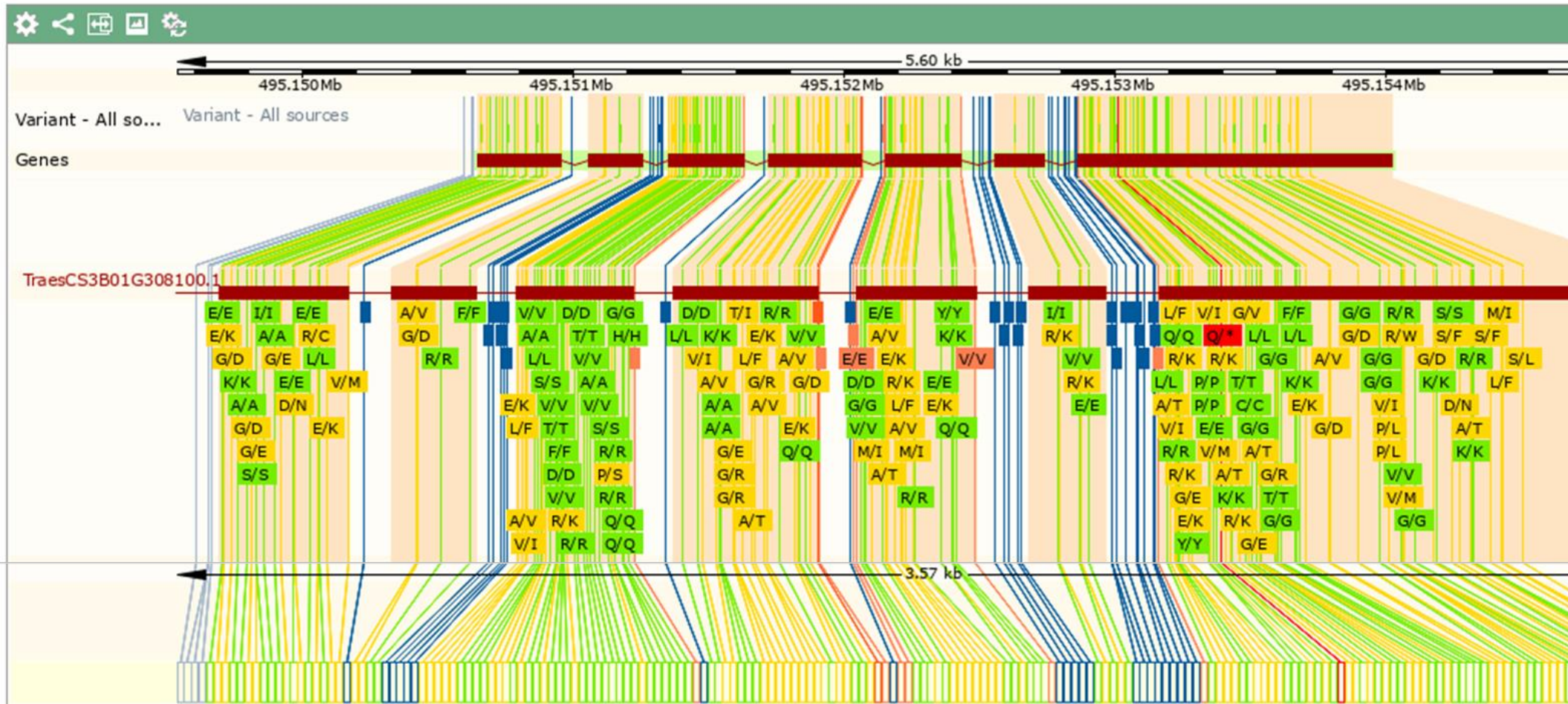
Bruno Contreras
Moreira



Guy Naamati



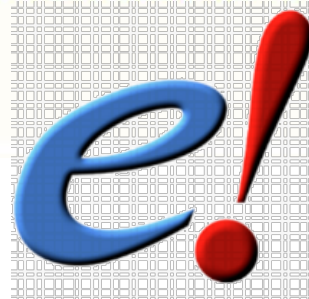
Dan Bolser



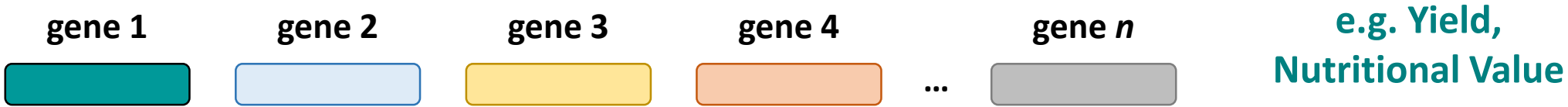
Variant Legend

- splice acceptor variant
- missense variant
- synonymous variant
- downstream gene variant

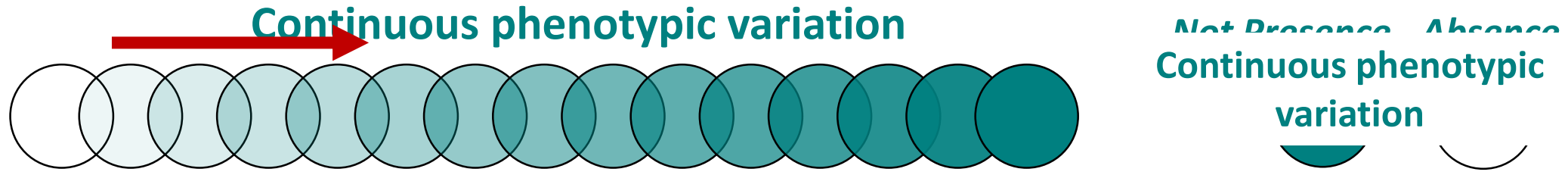
- stop gained
- splice region variant
- intron variant



Phenotypes of agricultural importance are “complex”



Diploids



Polyploids



Sugar cane



Coffee

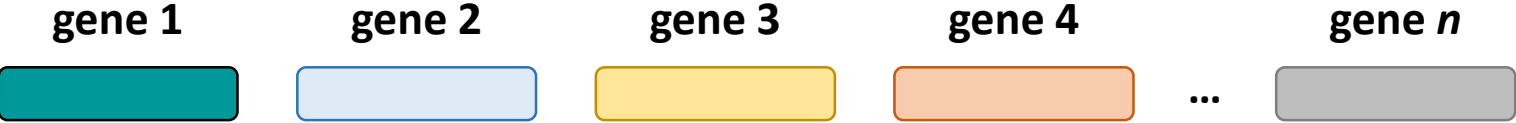


Rapeseed

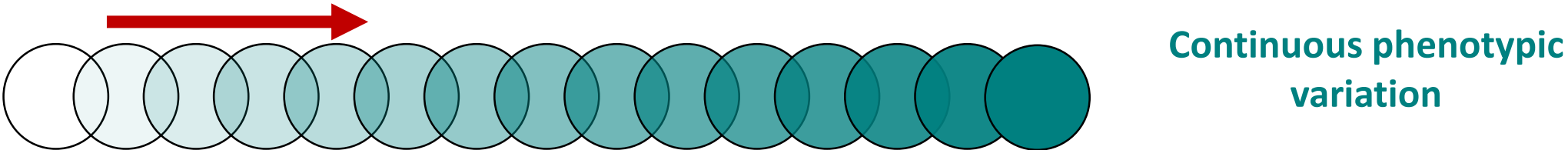


Wheat

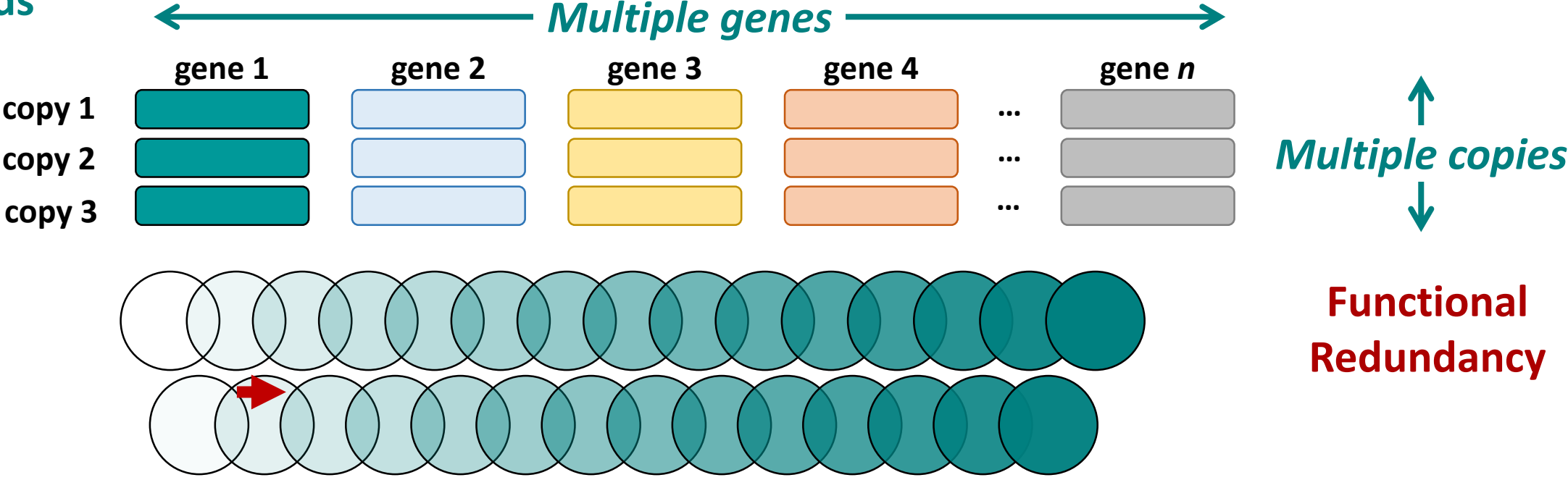
Phenotypes of agricultural importance are “complex”



Diploids

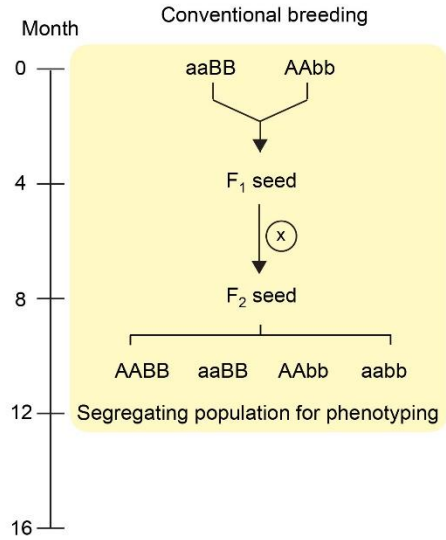


Polyploids

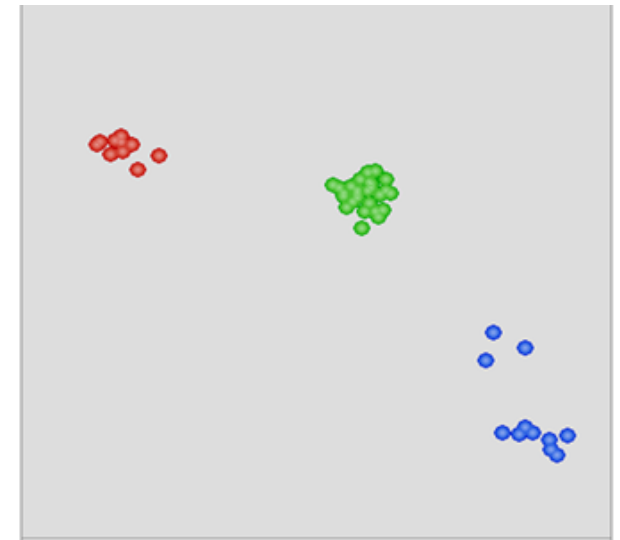
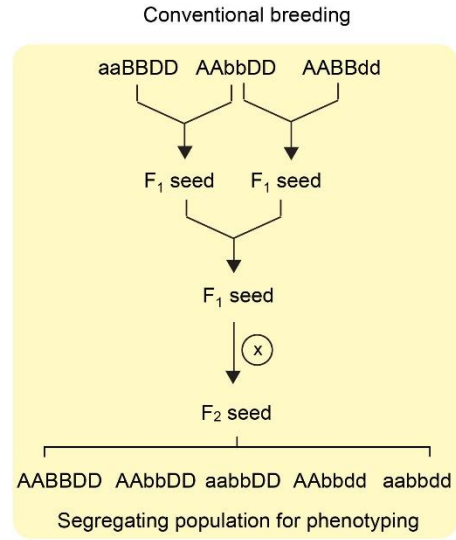


Combining mutations for full gene knock-outs

Tetraploid



Hexaploid



Speed Breeding

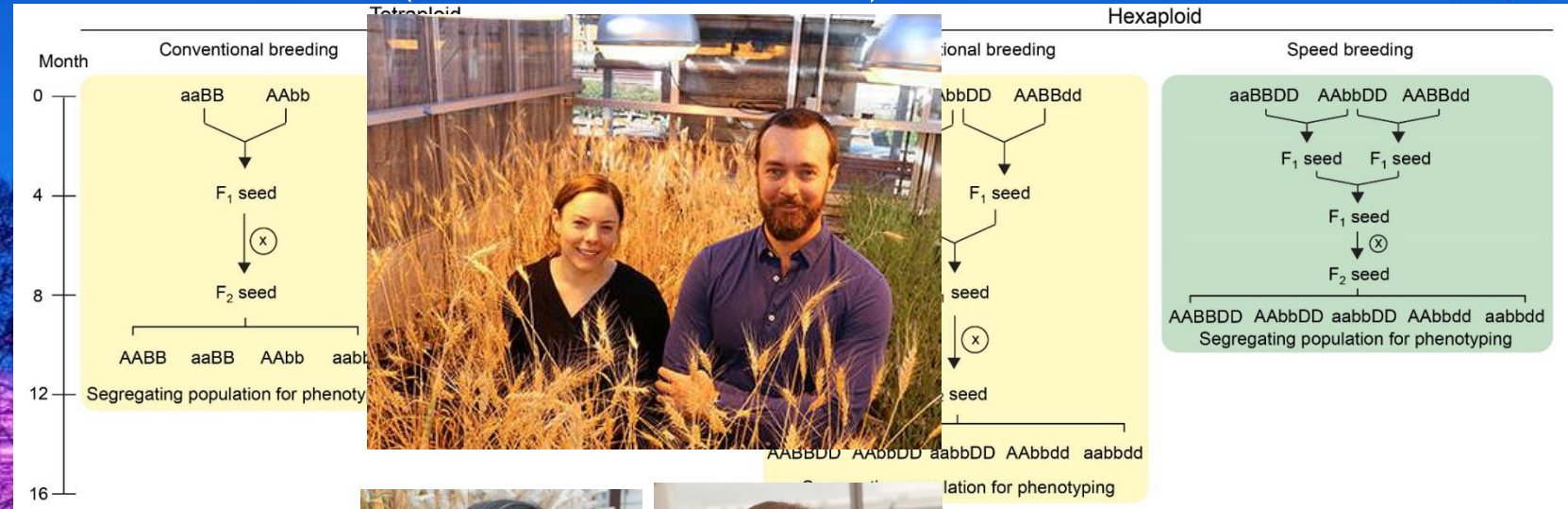
Cadenza 38 days after sowing



Normal growth

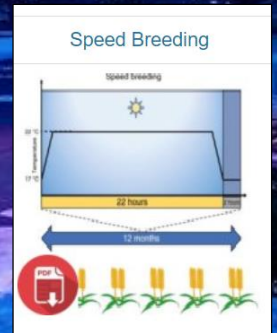
Accelerated growth

Amy Watson & Lee Hickey
(Univ Queensland)

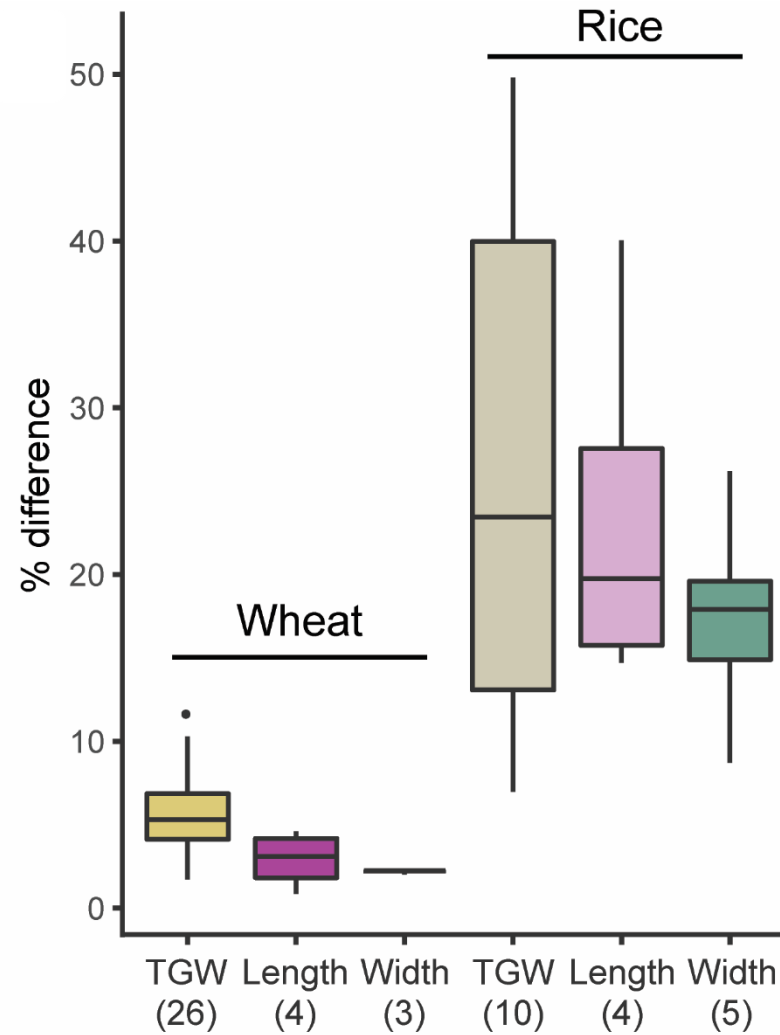


Sreya Ghosh &
Brande Wulff (JIC)

Watson et al 2018 *Nature Plants*
Ghosh et al 2018 *Nature Protocols*



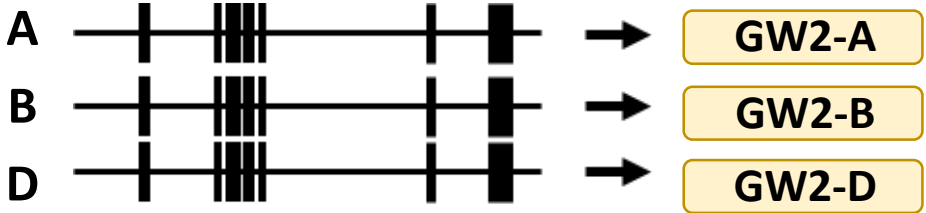
QTL effects in wheat are subtle compared to diploid



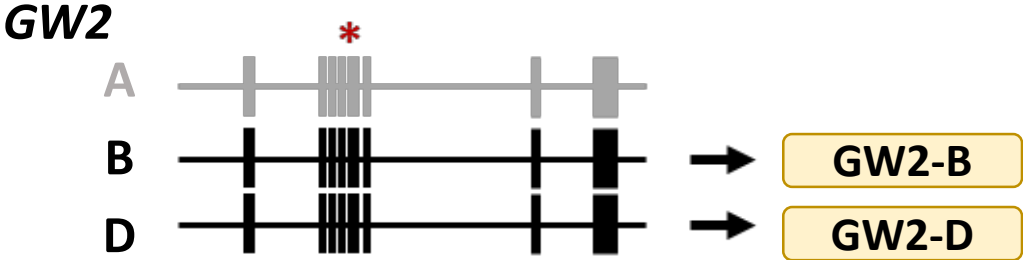
Combining mutations to overcome redundancy

Wildtype

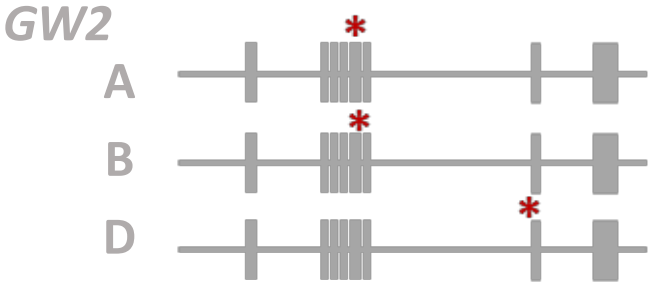
Grain Width 2 (GW2)



Single mutant



Triple mutant



James Simmonds (JIC)

Simmonds et al 2016 TAG
Wang et al 2018 TAG

20 grains
Increase grain weight





Natural diversity populations

Collection	Short description	Number of accessions	Genotyping	Data/seed availability	More information/ Reference
<i>Wheat diversity panels</i>					
Watkins historic collection of landrace wheats	World collection of wheat landraces grown as farmer saved seed before the 1930s. Genetically stable collection developed by two generations of single seed descent	829 accessions (core set of 119 represent majority of assayed genotypic variation). F _{4.5} mapping populations against Paragon, mainly for the core set.	35K Axiom breeders array (<i>Allen et al., 2017</i>); subset exome sequenced (<i>Gardiner et al., 2018</i>)	Genotype: https://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/axiom_download.php Seed: https://www.seedstor.ac.uk/ (store codes WATDE0001-WATDE1063)	http://wisplandrangepillar.jic.ac.uk/results_resources.htm ; <i>Wingen et al., 2014</i> ; <i>Wingen et al., 2017</i>
NIAB, UK	8-way (parents Alchemy, Brompton, Claire, Hereward, Rialto, Robigus, Xi19, Soissions); 16-way (Banco, Bersee, Brigadier, Copain, Cordiale, Flamingo, Gladiator, Holdfast, Kloka, Maris Fundin, Robigus, Slejpner, Soissons, Spark, Steadfast, Stetson)	NIAB 8-way MAGIC:>1,000 RILs; NIAB 16-way MAGIC: ~600 RILs	35K Axiom breeders array. Genome sequence (Claire, Robigus, others underway). Exome capture sequence of 16-way parents. Skim-seq of all RILs underway.	Claire and Robigus genomes: https://opendata.earlham.ac.uk/opendata/data/Triticum_aestivum/EI/v1.1/ Genotyping and Seed: https://www.niab.com/research/research-projects/resources	<i>Mackay et al., 2014</i> ; <i>Gardner et al., 2016</i>



Adamski et al 2020 *eLife*
(Table 2)



Keith Gardner



James Cockram (NIAB)

Publicly available germplasm collections (eg SeedStor)

 <p>Reference panel of 376 European winter wheats used in association mapping research 2008-2009</p> <p>NIAB  </p>	 <p>Wheat TILLING in silico wheat TILLING populations (Exome capture)</p>	 <p>DFW Nested Association Mapping Populations</p>	 <p>Wheat Pan Genome Pangenome Collection</p>	 <p>Open Wild Wheat Consortium: <i>Aegilops tauschii</i> Diversity Panel</p>
 <p>The Paragon Library Paragon Near Isogenic Line Library</p>	 <p>DFW BREEDERS TOOL KIT Designing Future Wheat Breeders Toolkit</p>	 <p>GEDIFLUX Collection 1945-2000 European Commission</p>	 <p>Paragon X Chinese Spring SSD Paragon x Chinese Spring Mapping Population</p>	 <p>Paragon gamma Deletions Paragon Gamma Irradiated deletions</p>



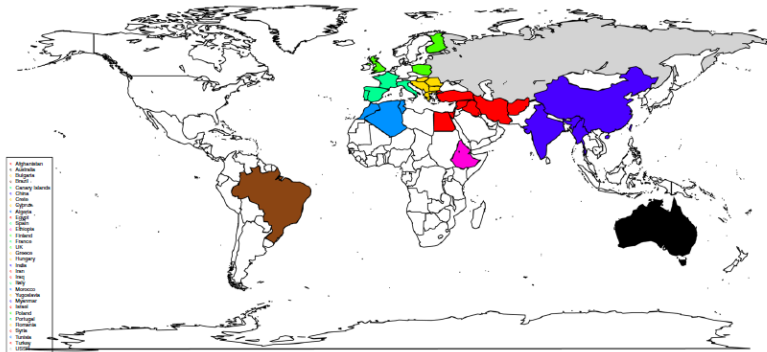
Adamski et al 2020 *eLife*
(Table 2)

<https://www.seedstor.ac.uk/search-browsecollections.php>

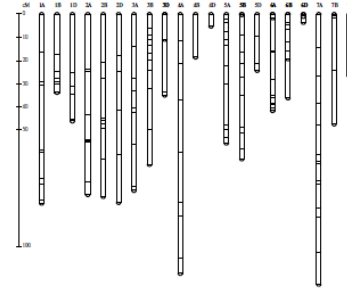
Watkins NAM population

<http://wisplandracepillar.jic.ac.uk/>

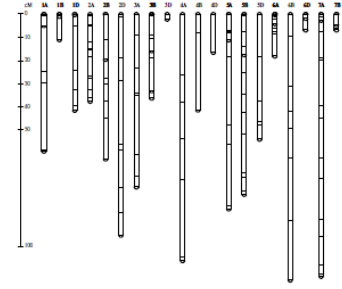
AE Watkins: Countries of Origin – Regions



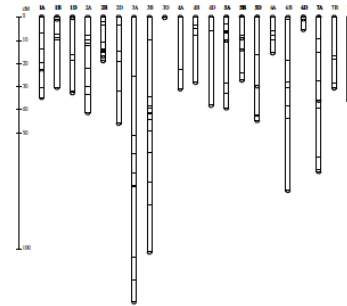
ParW219



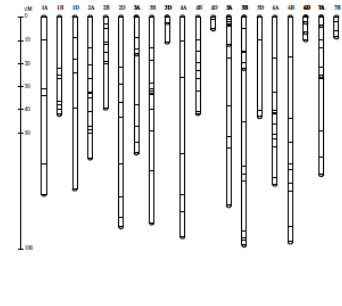
ParW224



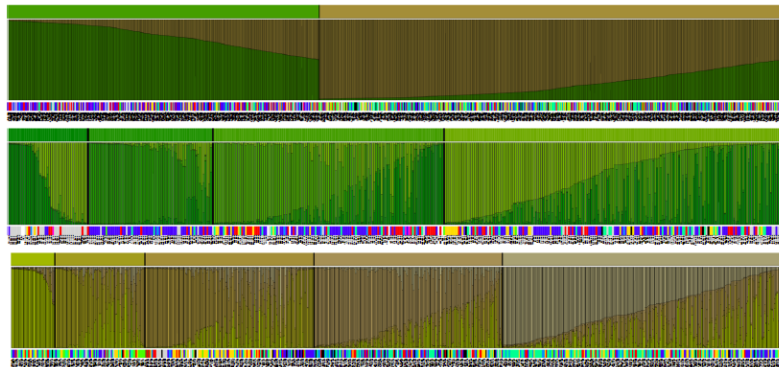
ParW254



ParW273



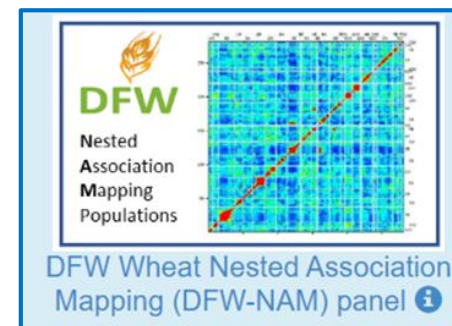
>100 F₆ "off-the shelf"
mapping populations
(many phenotyped)



Simon Griffiths (JIC)

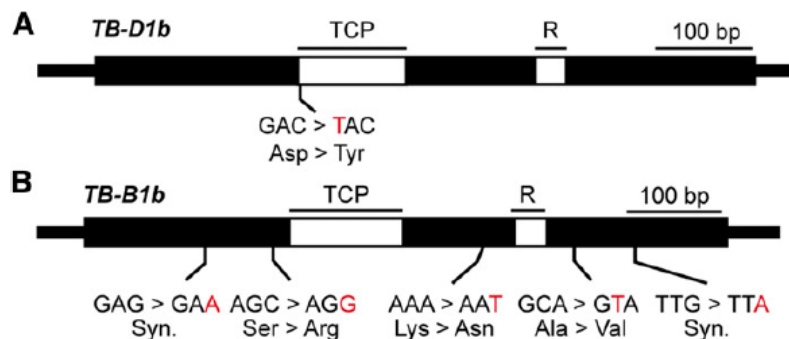
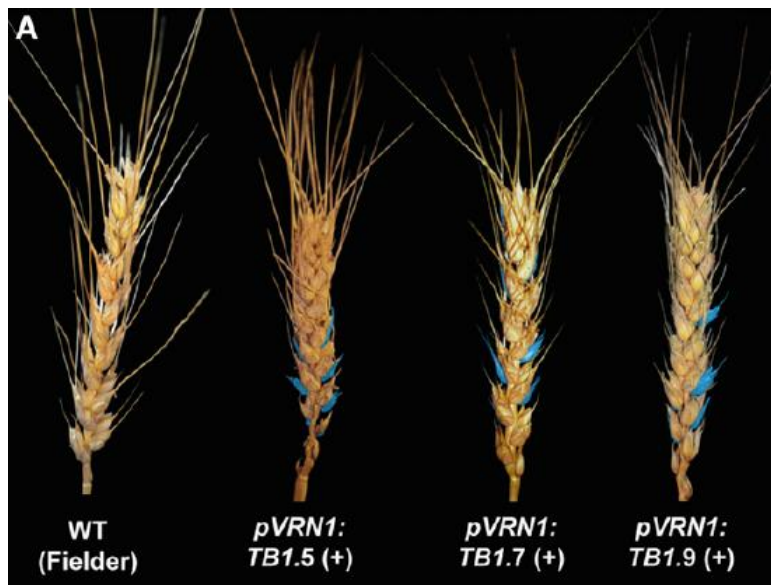
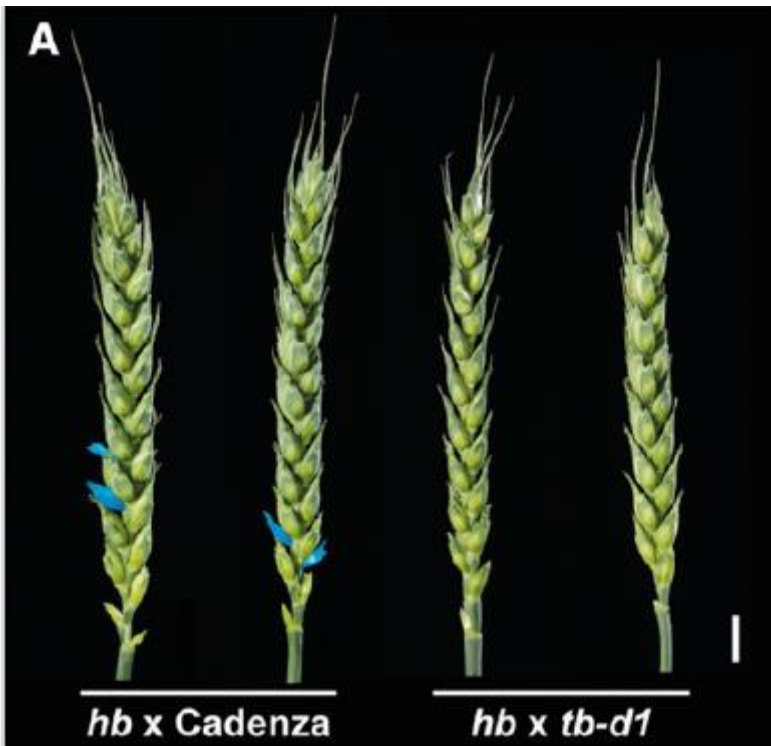


Luzie Wingen (JIC)



Wingen et al 2014 *TAG*
Wingen et al 2017 *Genetics*

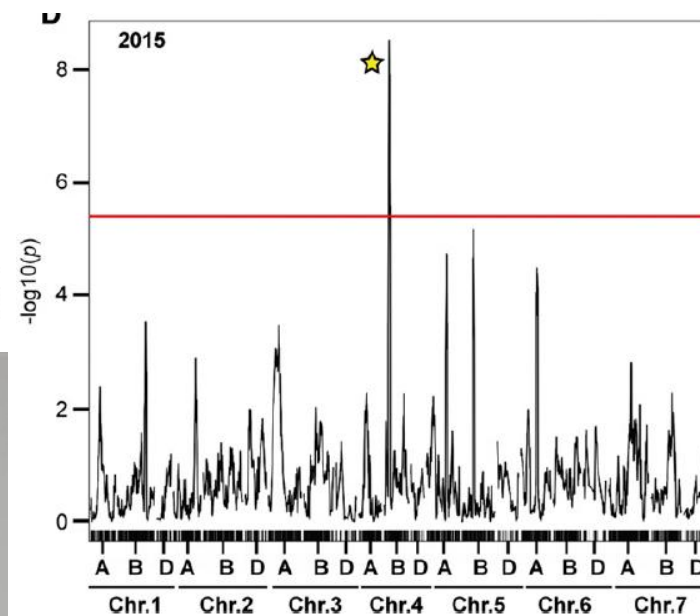
Original allele (*hb*) from CSIRO 4-way MAGIC
 Candidate gene validated with TILLING mutant (*tb-d1*)
 Functional validation with transgenic lines (Fielder)
 Identify natural variation
 NIAB 8-way MAGIC QTL identifies B genome (*TB-B1*)



Laura Dixon (Leeds)



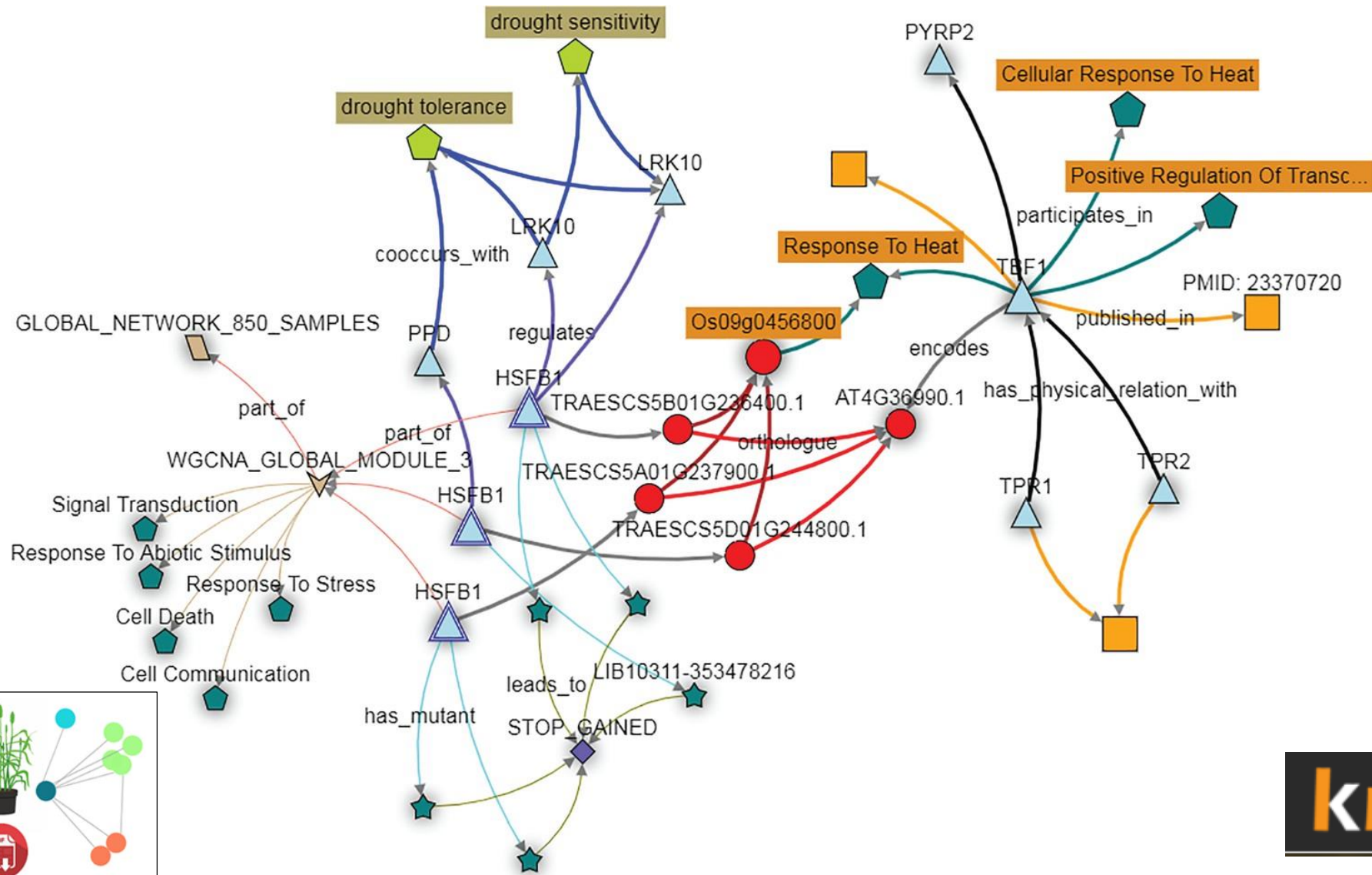
Scott Boden (Adelaide)



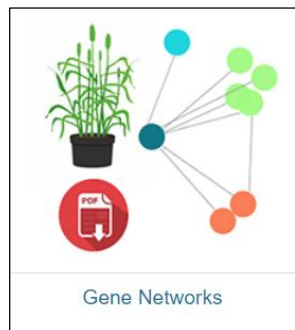
Dixon et al 2018 *Plant Cell*

Knowledge Networks

https://knetminer.rothamsted.ac.uk/Triticum_aestivum/



Keywan Hassani-Pak
(Rothamsted)



Hassani-Pak et al 2020 *bioRxiv*

From one cultivar to over 10 sequenced cultivars



Curtis Pozniak
University of Saskatchewan



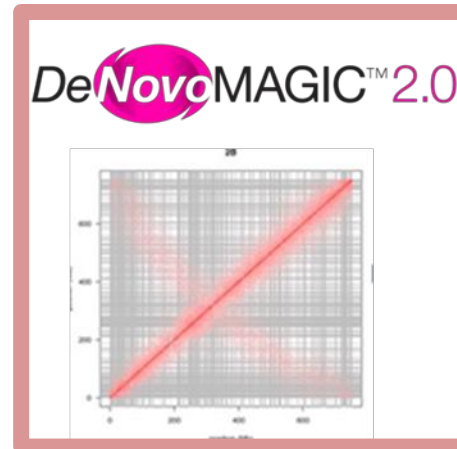
Variety	Habit	Origin
Lancer	spring	Australia
Mace	spring	Australia
CDC Landmark	spring	Canada
CDC Stanley	spring	Canada
Paragon	spring	UK
Cadenza	spring	UK
Weebil	spring	CIMMYT
Robigus	winter	UK
Claire	winter	UK
Arina	winter	Switzerland
Julius	winter	Germany
Norin-61	winter	Japan
Jagger	winter	US
SY Mattis	winter	Syngenta
Kronos	spring	US
Svevo	spring	Italy
Zavitan	-	Israel

Bread wheat (6x)

Durum wheat (4x)

Wild emmer (4x)

Variety	Habit	Origin
Lancer	spring	Australia
Mace	spring	Australia
CDC Landmark	spring	Canada
CDC Stanley	spring	Canada
Paragon	spring	UK
Cadenza	spring	UK
Weebil	spring	CIMMYT
Robigus	winter	UK
Claire	winter	UK
Arina	winter	Switzerland
Julius	winter	Germany
Norin-61	winter	Japan
Jagger	winter	US
SY Mattis	winter	Syngenta
Kronos	spring	US
Svevo	spring	Italy
Zavitan	-	Israel



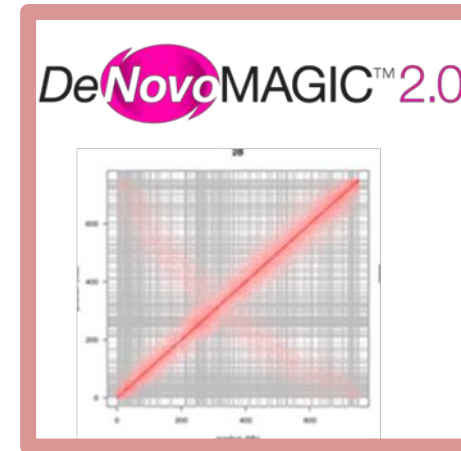
Sean Walkowiak
(Canadian Grain Commission)

Variety	Habit	Origin
Lancer	spring	Australia
Mace	spring	Australia
CDC Landmark	spring	Canada
CDC Stanley	spring	Canada
Paragon	spring	UK
Cadenza	spring	UK
Weebil	spring	CIMMYT

Robigus	winter	UK
Claire	winter	UK
Arina	winter	Switzerland
Julius	winter	Germany
Norin-61	winter	Japan
Jagger	winter	US
SY Mattis	winter	Syngenta

Kronos	spring	US
--------	--------	----

Svevo	spring	Italy
Zavitan	-	Israel



The screenshot shows the bioRxiv preprint server interface. The title is "W2RAP: a pipeline for high quality, robust assemblies of large complex genomes from short read data". The authors listed are Bernardo Clavijo, Gonzalo Garcia Accinelli, Jonathan Wright, Darren Heavens, Katie Barr, Luis Yanes, and Federica Di Palma. The DOI is https://doi.org/10.1101/110999. The Cold Spring Harbor Laboratory logo is visible in the top left corner.

Clavijo et al 2017 *bioRxiv*



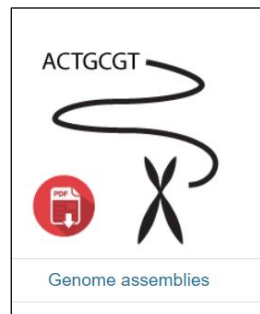
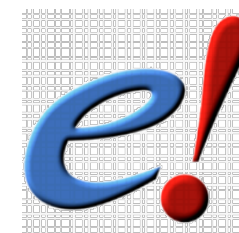
Bernardo Clavijo
(Earlham Institute)

Currently available

Variety	Habit	Origin		Website
CDC Landmark	spring	Canada	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Arina	winter	Switzerland	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Julius	winter	Germany	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Jagger	winter	US	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Zavitan	-	Israel	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Lancer	spring	Australia	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Mace	spring	Australia	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Norin-61	winter	Japan	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
CDC Stanley	spring	Canada	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
SY Mattis	winter	Syngenta	10G	http://webblast.ipk-gatersleben.de/wheat_ten_genomes/
Svevo	spring	Italy	Interomics	https://www.interomics.eu/durum-wheat-genome
Paragon	spring	UK	Earlham	https://wheatis.tgac.ac.uk/grassroots-portal/blast
Cadenza	spring	UK	Earlham	https://wheatis.tgac.ac.uk/grassroots-portal/blast
Robigus	winter	UK	Earlham	https://wheatis.tgac.ac.uk/grassroots-portal/blast
Claire	winter	UK	Earlham	https://wheatis.tgac.ac.uk/grassroots-portal/blast
Kronos	spring	US	Earlham	https://wheatis.tgac.ac.uk/grassroots-portal/blast

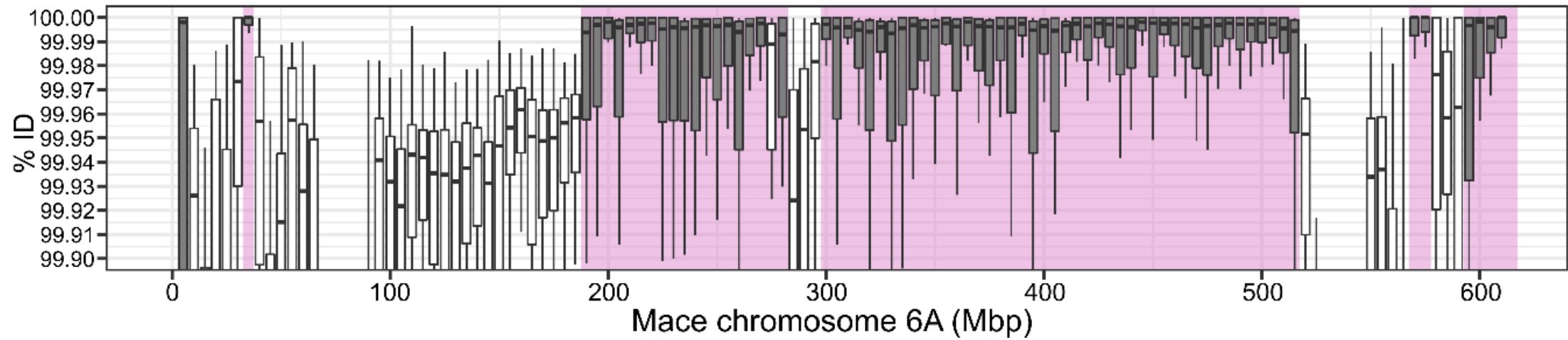


Fei Lu (CAS)
25 *Triticum/Aegilops* ssp.
Zhou et al 2020 *bioRxiv*



Coming to EnsemblPlants Soon!

Using the wheat pangenome to define haplotypes



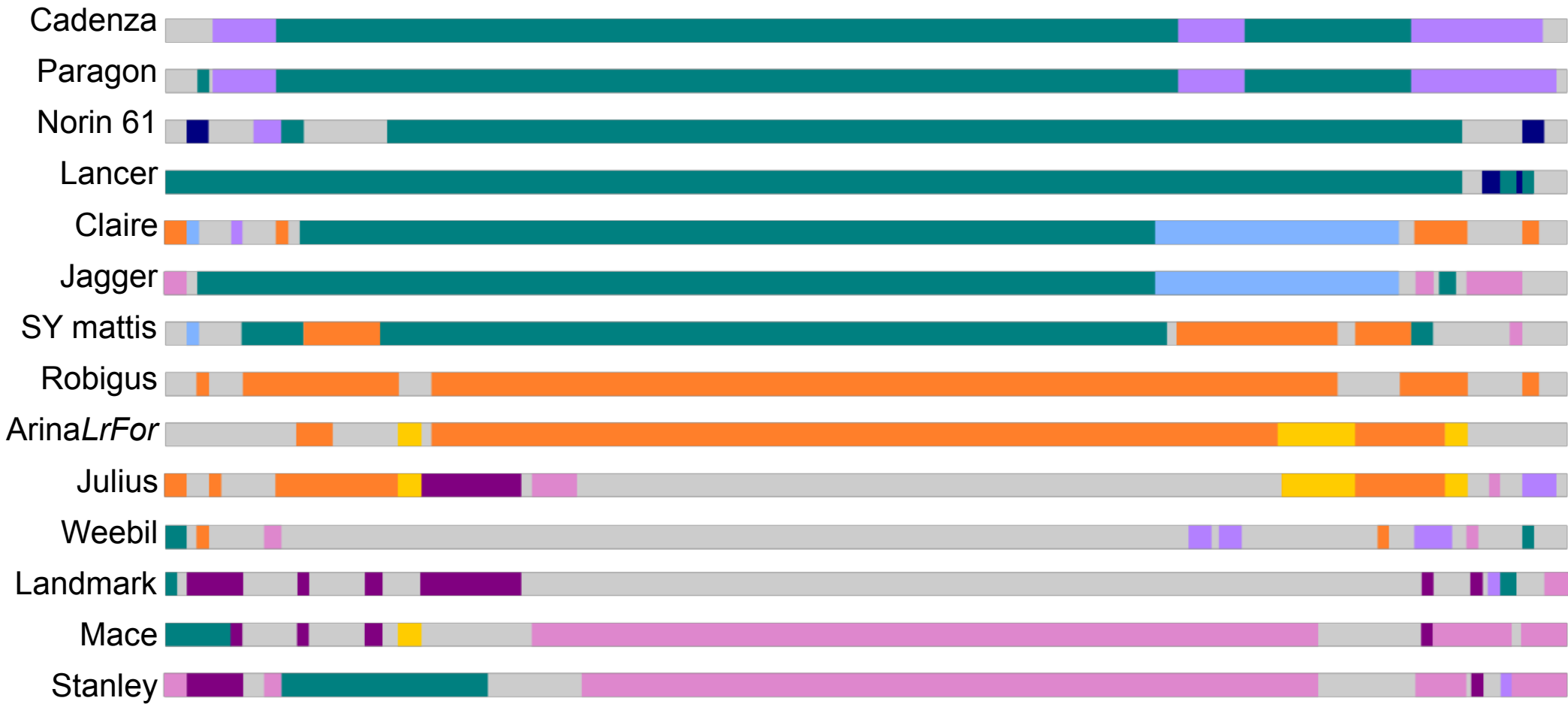
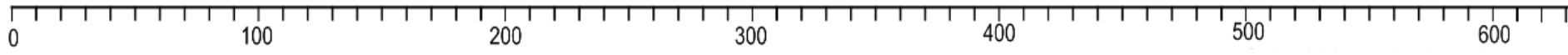
Jemima Brinton
(JIC)



Ricardo Ramirez-Gonzalez
(JIC)

Defining haplotypes across all cultivars (chr 6A)

Chromosome
6A
(Mbp)



Visualisation interface (chr 4B example)

<http://www.crop-haplotypes.com/>



Block no	Assembly	Start	End	Length
3896	Jagger	30,000,000	625,000,000	595,000,000
3896	Weebill	28,632,000	625,400,000	596,770,000
3897	Jagger	50,000,000	600,000,000	550,000,000
3897	Norin61	50,000,000	595,000,000	545,000,000
3898	Jagger	30,000,000	425,000,000	395,000,000
3898	SY Mattis	30,000,000	420,000,000	390,000,000
3899	SY Mattis	15,000,000	280,000,000	265,000,000
3899	Weebill	15,409,000	282,890,000	267,480,000
3900	Norin61	295,000,000	515,000,000	220,000,000
3900	Weebill	296,290,000	517,620,000	221,330,000
3901	Robigus	426,770,000	633,310,000	206,540,000
3901	SY Mattis	420,000,000	625,000,000	205,000,000
3902	CDC Landmark	505,000,000	689,710,000	184,710,000
3902	CDC Stanley	510,000,000	660,000,000	150,000,000
3902	CDC Stanley	675,000,000	695,000,000	20,000,000

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[Michael Purugganan](#) – New York University, USA

Concluding thoughts

- Open-access resources to enable biological understanding
- Polyploidy 'hides' useful phenotypic variation
- Be collaborative and open
- For more info:
 - www.wheat-training.com
 - Adamski et al 2020 *eLife*



Wheat (and human) diversity



DESIGNING
FUTURE WHEAT



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cristobal.uauy@jic.ac.uk