Sequencing 4.3 Million Mutations in Wheat Promoters to Understand and Modify Gene Expression

Junli Zhang

Dubcovsky Lab

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UCDAVIS

DEPARTMENT OF PLANT SCIENCES

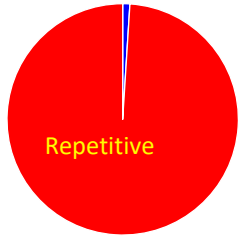
Outline

I. Exome and promoter capture sequencing of wheat TILLING populations

II. A new regulatory capture design for wheat

Exome capture sequencing of wheat TILLING population

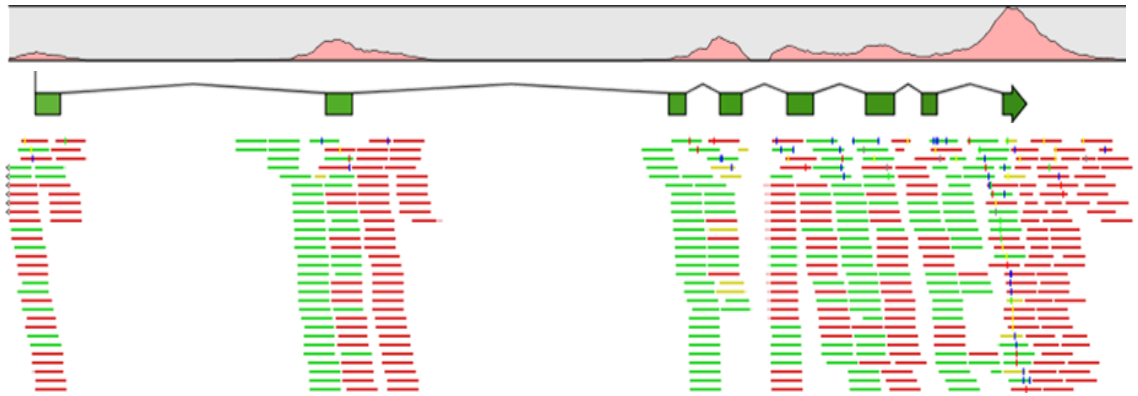
The wheat genome is too big for whole genome sequencing of mutant populations (genes = 2%)



PNAS 2017 114:E913

84 Mb probes

- **119 Mb** captured in 4x
- **162 Mb** captured in 6x



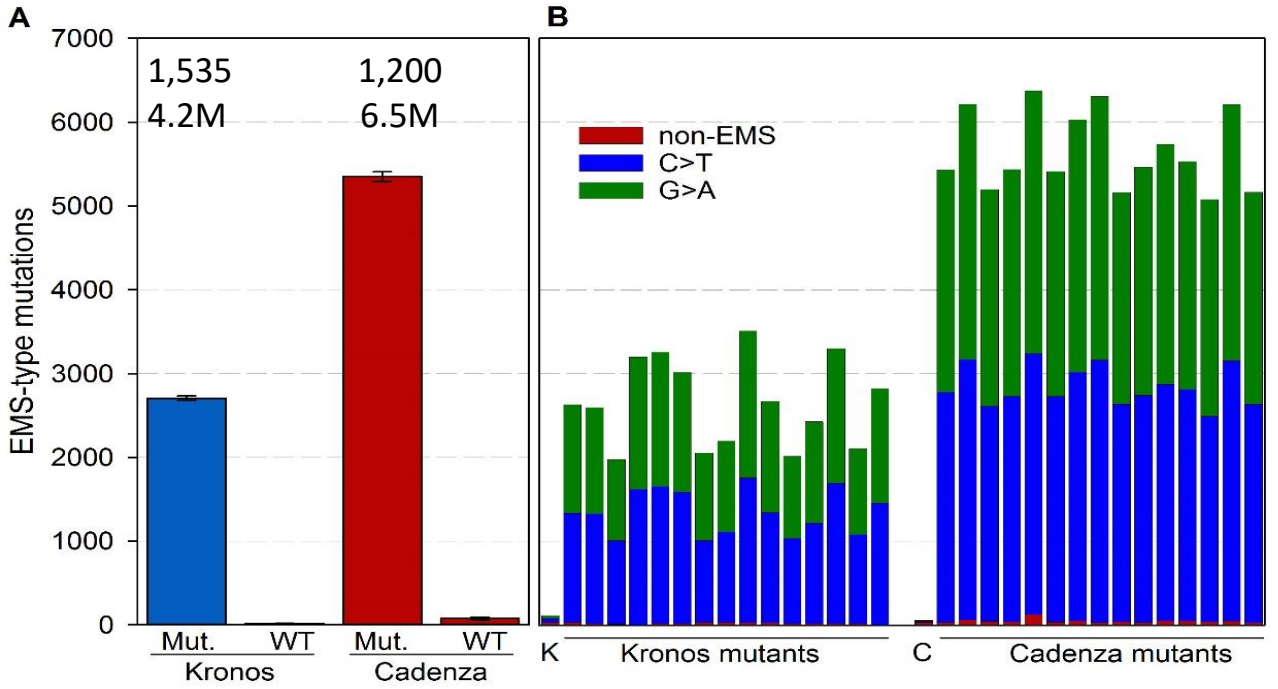
PNAS

Uncovering hidden variation in polyploid wheat

Ksenia V. Krasileva[a,b,c], Hans A. Vasquez-Gross[a], Tyson Howell[a], Paul Bailey[c], Francine Paraiso[a], Leah Clissold[c], James Simmonds[d], Ricardo H. Ramirez-Gonzalez[c,d], Xiaodong Wang[a], Philippa Borrill[d], Christine Fosker[c], Sarah Ayling[c], Andrew L. Phillips[e], Cristobal Uauy[d,1,2], and Jorge Dubcovsky[a,f,1,2]

[a]Department of Plant Sciences, University of California, Davis, CA 95616; [b]The Sainsbury Laboratory, Norwich NR4 7UH, United Kingdom; [c]The Earlham Institute, Norwich NR4 7UG, United Kingdom; [d]John Innes Centre, Norwich NR4 7UH, United Kingdom; [e]Rothamsted Research, Harpenden AL5 2JQ, United Kingdom; and [f]Howard Hughes Medical Institute, Chevy Chase, MD 20815

Contributed by Jorge Dubcovsky, December 20, 2016 (sent for review November 22, 2016; reviewed by Beat Keller and Joachim Messing)



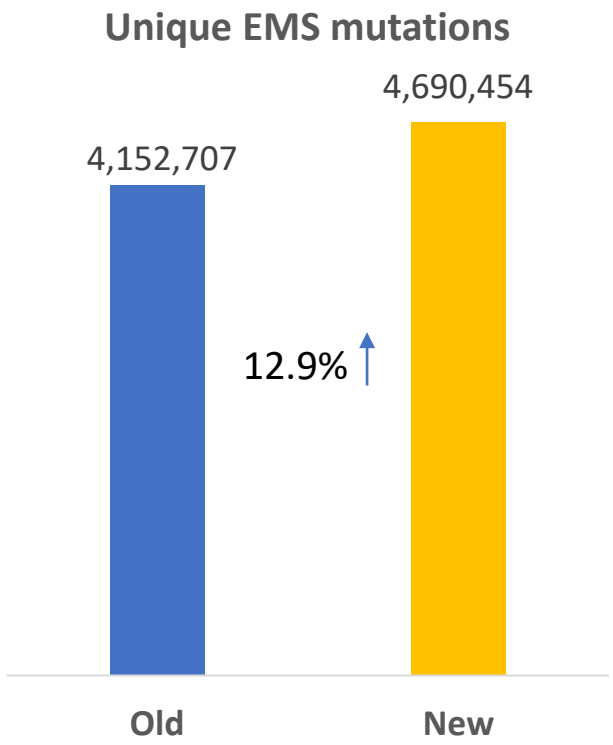
PNAS RESEARCH ARTICLE | AGRICULTURAL SCIENCES OPEN ACCESS

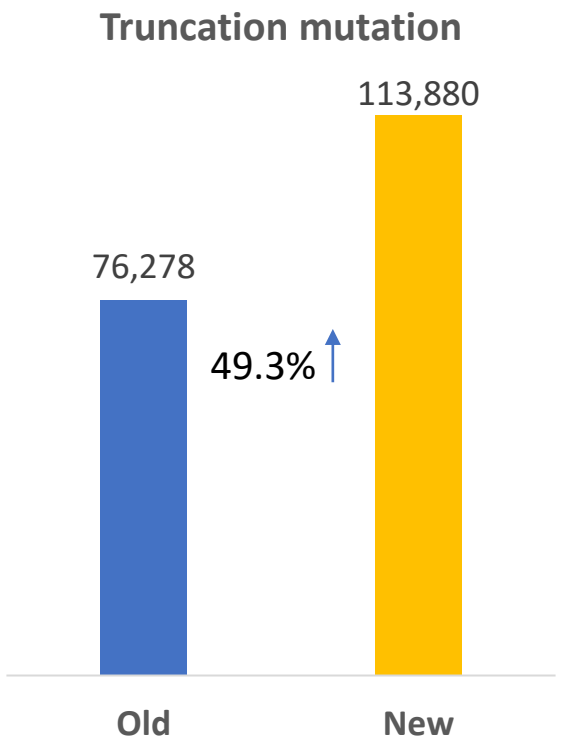
Sequencing 4.3 million mutations in wheat promoters to understand and modify gene expression

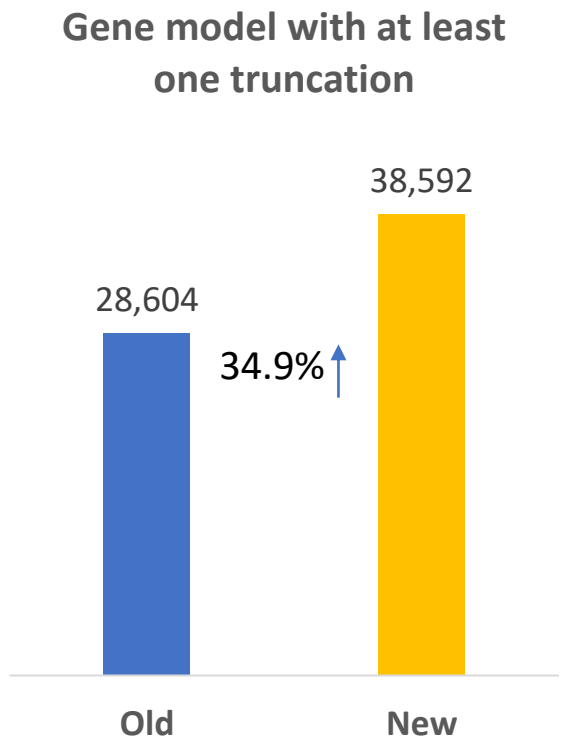
Junli Zhang[a,1], Hongchun Xiong[a,b,1], Germán F. Burguener[a,c], Hans Vasquez-Gross[a,d], Qiujie Liu[a,c], Juan M. Debernardi[a,c], Alina Akhunova[e], Kimberly Garland-Campbell[f], Shahryar F. Kianian[g], Gina Brown-Guedira[h], Curtis Pozniak[i], Justin D. Faris[j], Eduard Akhunov[e], and Jorge Dubcovsky[a,c,2]

Contributed by Jorge Dubcovsky; received April 24, 2023; accepted August 3, 2023; reviewed by Graham Moore, Thorsten Schnurbusch, and Ben Trevaskis

Remapping exome capture to RefSeq v1.1

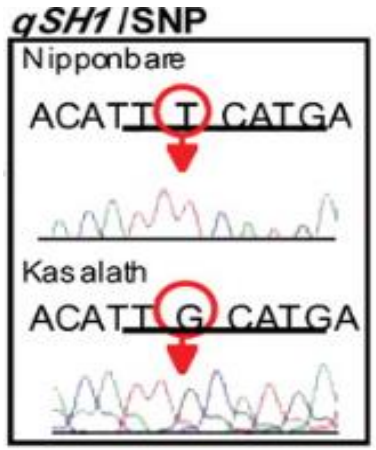






Resequence Kronos TILLING mutants with promoter capture

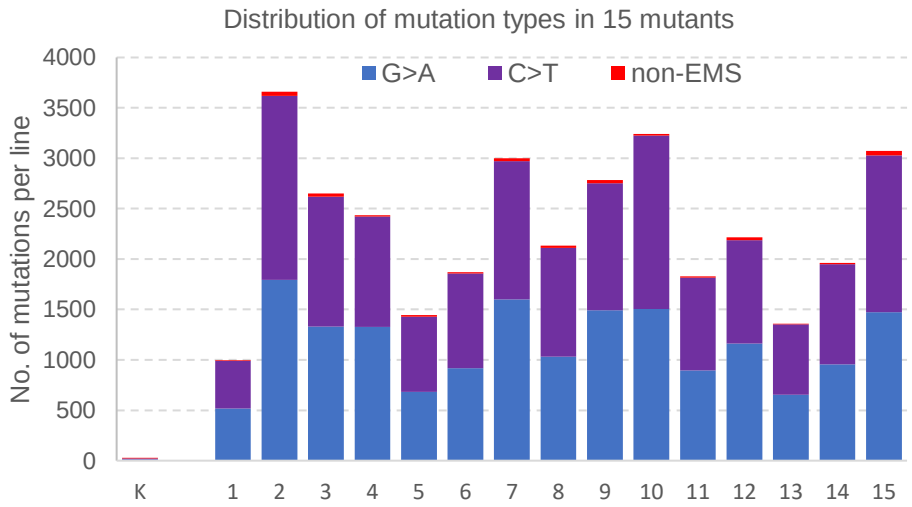
- *Cis*-regulatory variants often cause subtle phenotypic change
  - by modifying the timing, pattern, or level of gene expression (Wittkopp and Kalay, 2011)
- Dr. Anthony Hall's lab developed one global wheat promoter capture assay covering 2 Kb promoter region of all high-confidence annotated genes (Gardiner et al., 2019).



Konishi *et al.*, 2006
A SNP in the 5' regulatory region of the *qSH1* gene caused loss of seed shattering due to loss of expression at at the provisional abscission layer

Promoter capture summary

Summary	Promoter	Exome
Number of lines used (overlap 1,465)	1,513	1,521
Uniquely mapped EMS-type mutations	4.3 M	4.7 M
Avg. EMS-type mutations per kb (population)	**42**	36
Avg. EMS-type mutations/line	**2,834**	3,084
Mapping space	102 Mb	131 Mb



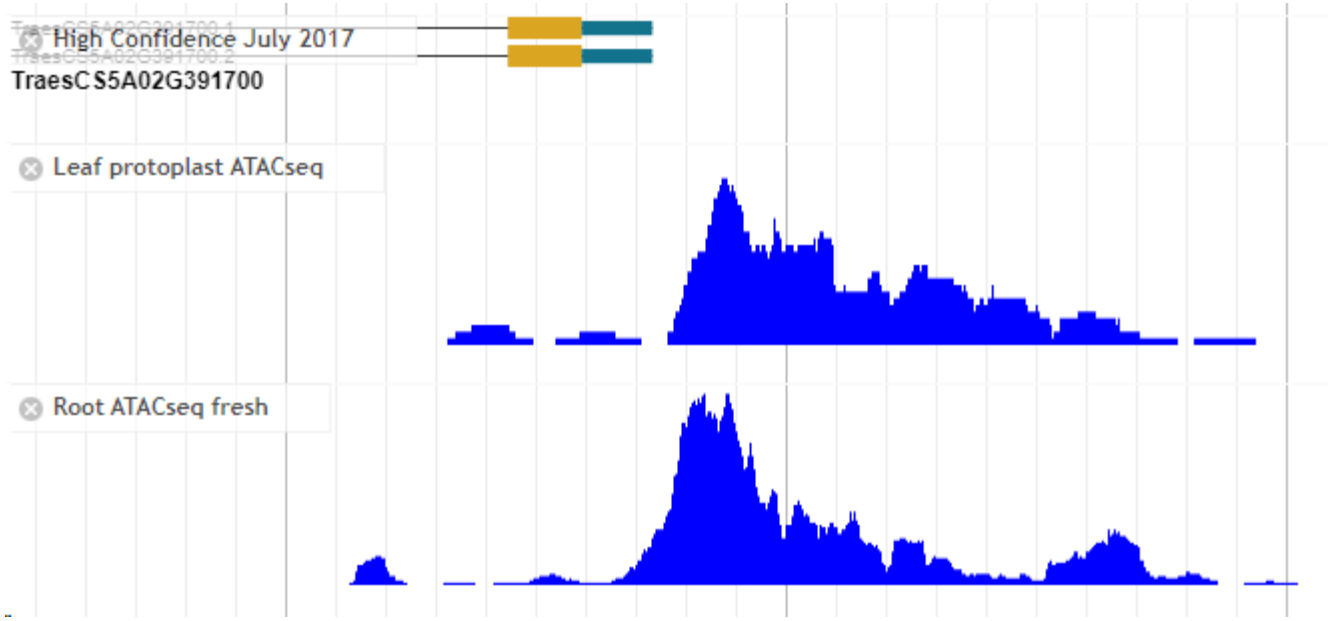
Data availability

- The data is now public available
  - Dubcovsky Lab website: https://dubcovskylab.ucdavis.edu/wheat_blast
  - GrainGenes genome browser: https://wheat.pw.usda.gov/jb?data=/ggds/whe-iwgsc2018
  - Next release of Ensembl Plants
- Kronos mutant seeds can be requested from the Germplasm Resources Unit at the **John Innes Centre** and from **Dr. Dubcovsky Laboratory Tilling Distribution** https://dubcovskylab.ucdavis.edu/wheat-tilling.

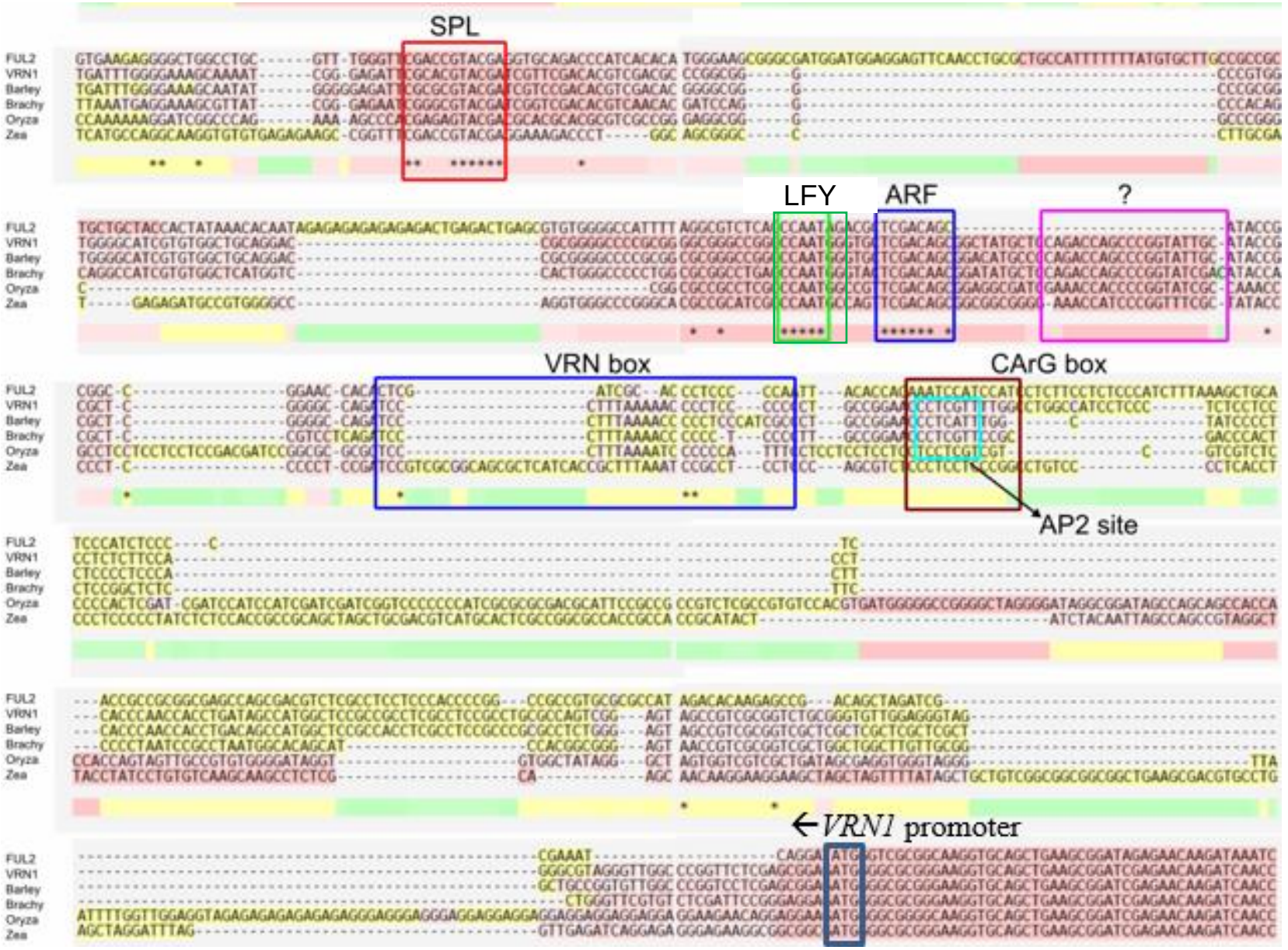
Use the promoter capture data

Promoters are still not well annotated, so users need to find the conserved promoter regions

- Comparisons of promoter region among grass species to identify conserved regions
- Align promoter region (include about 50 bp of the exons)
- Within conserved regions focus on open chromatin regions (ATAC-seq)



VRN1 Promoter



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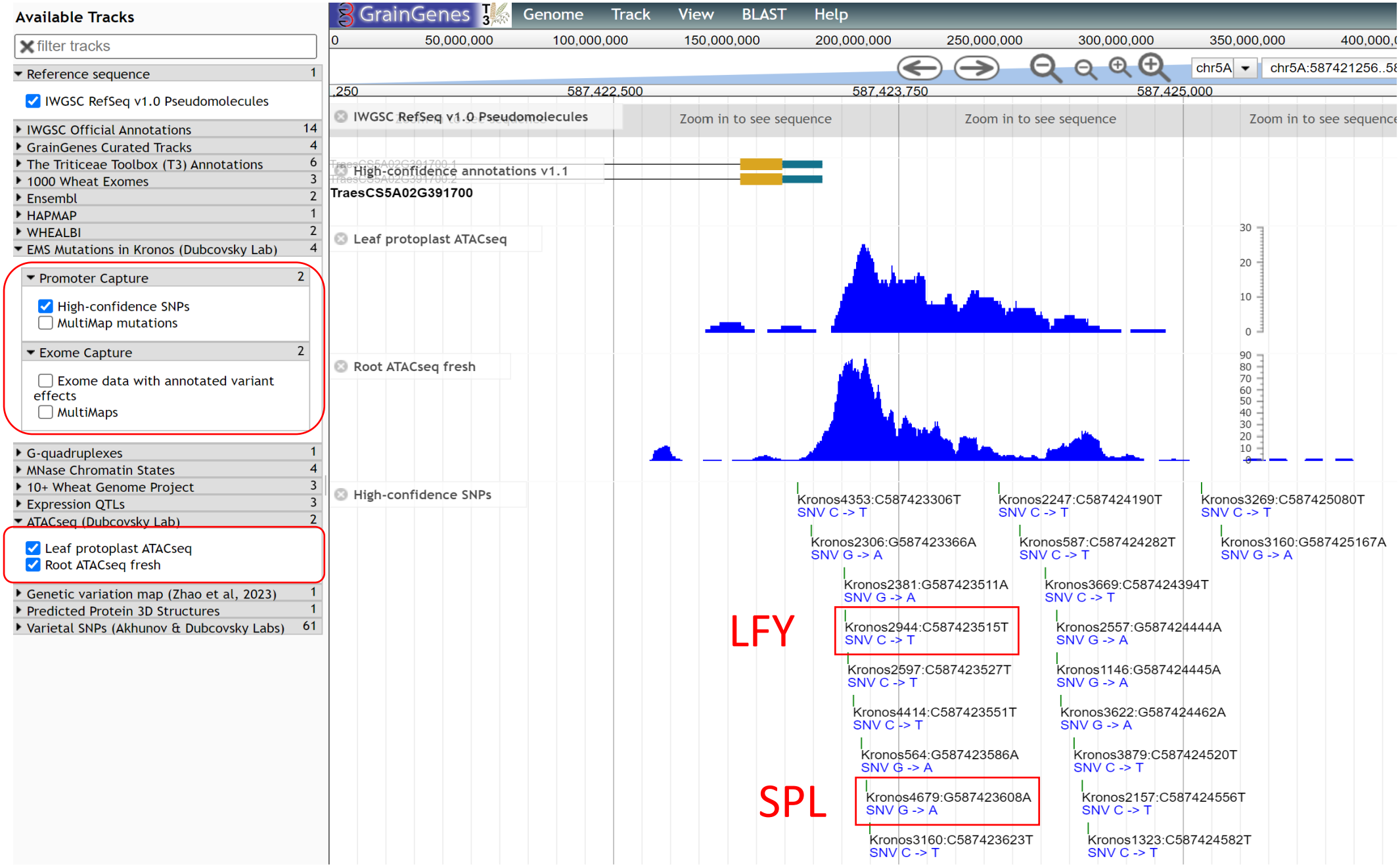

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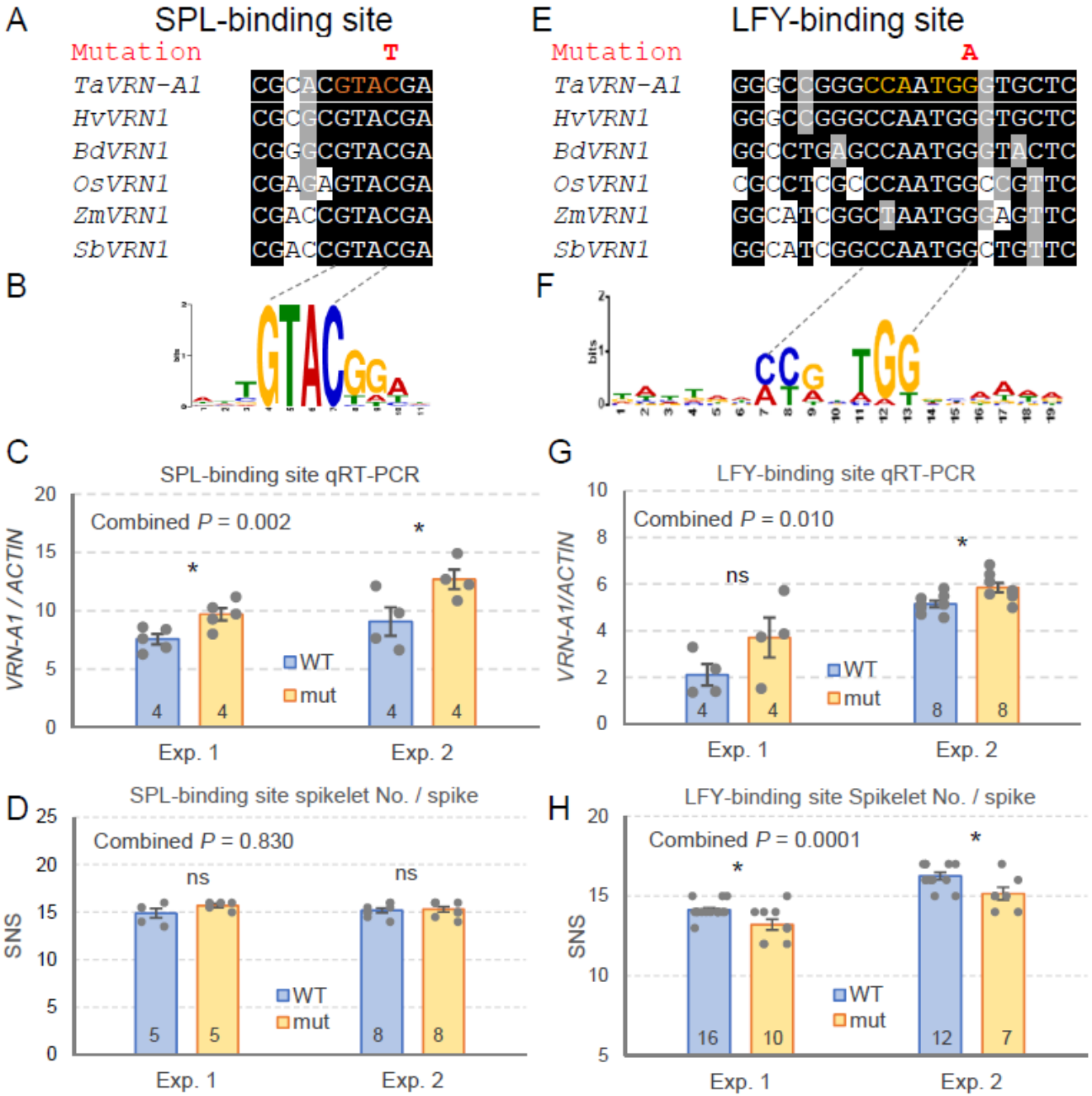

The Plant Genome
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A second-generation capture panel for cost-effective sequencing of genome regulatory regions in wheat and relatives

Junli Zhang, Juan M. Debernardi, Germán F. Burguener, Frédéric Choulet, Etienne Paux, Lauren O'Connor, Jacob Enk, Jorge Dubcovsky

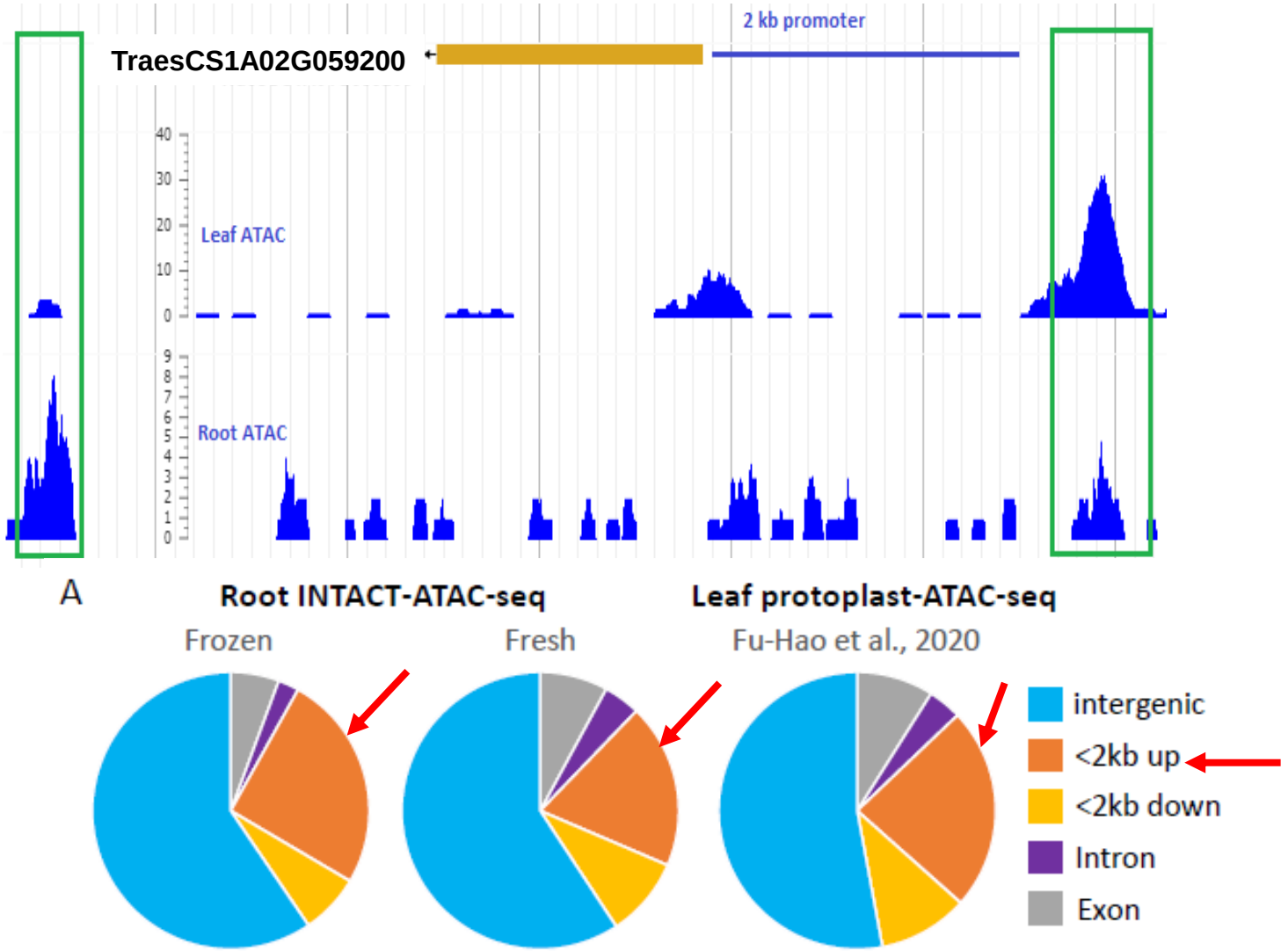
First published: 09 December 2022 | https://doi.org/10.1002/tpg2.20296

A new regulatory capture is needed

- The **Gardiner promoter capture** assay has some limitations
  - Enrichment efficiency is low: 8x to 32x
  - Still have some repetitive regions
  - Only covered 2 Kb of promoter region: ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) revealed more regulatory regions outside the promoter region

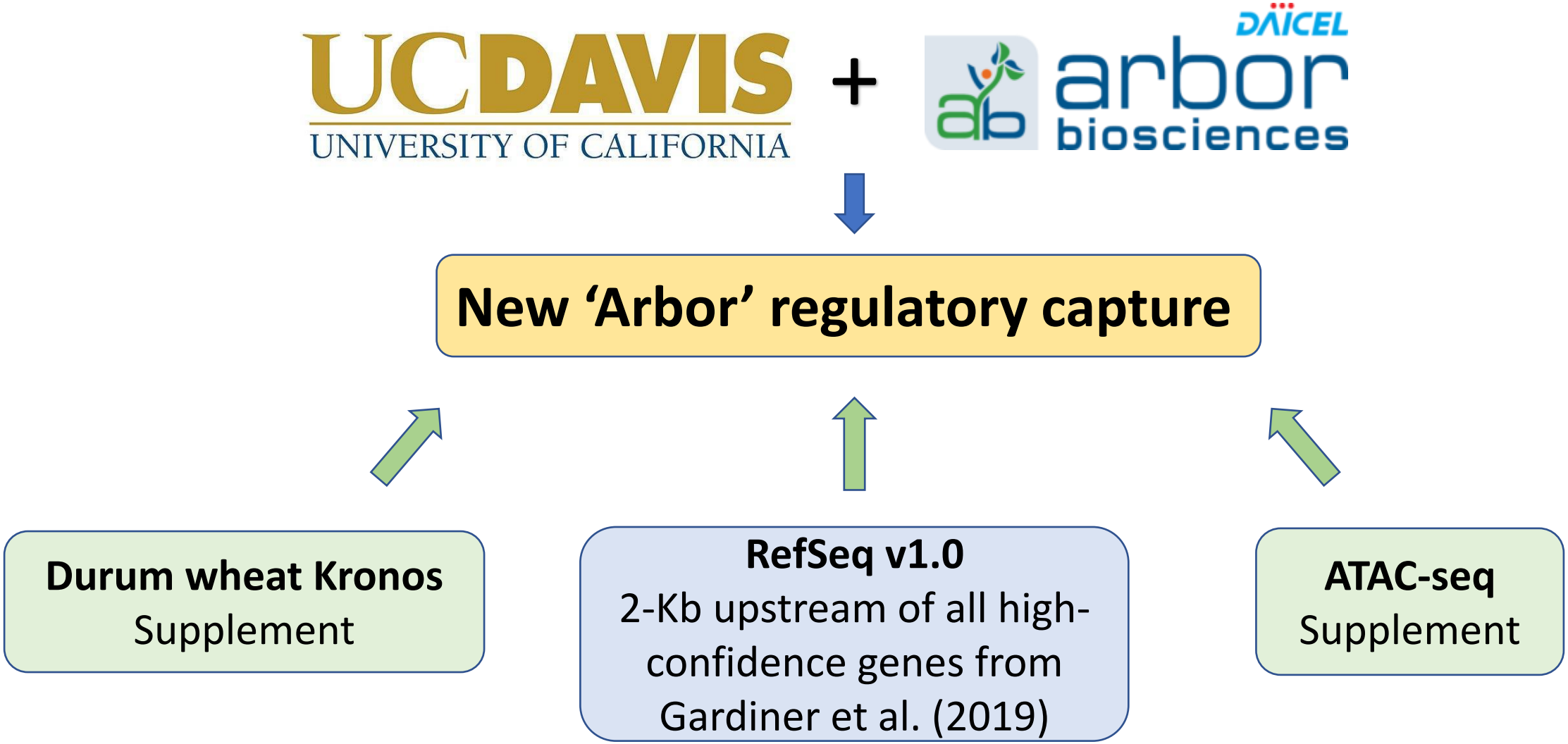
- Gardiner probe set has been discontinued.

ATAC-seq data revealed more regulatory regions

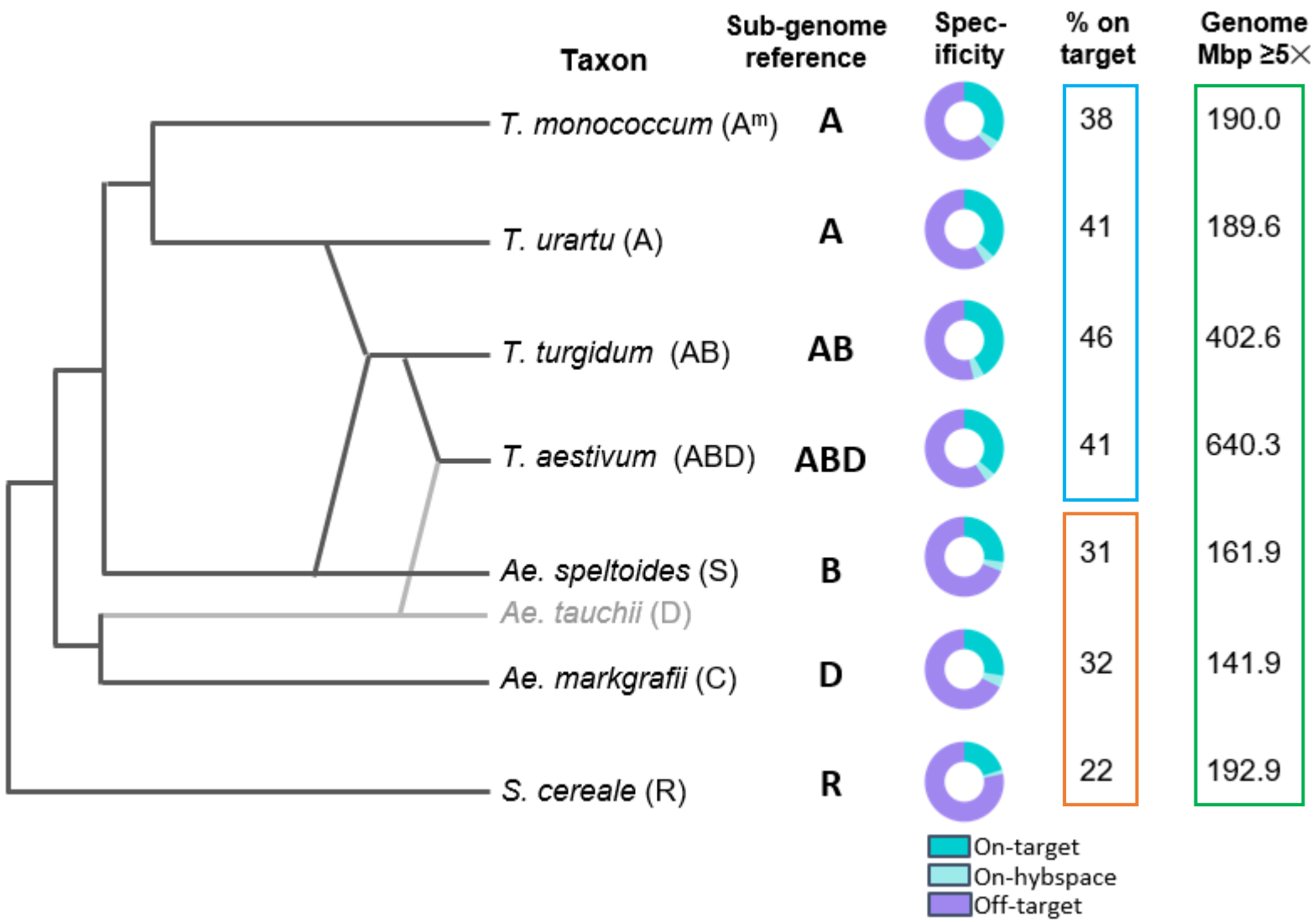


Debernardi et al. 2022

A new improved and expanded regulatory capture design



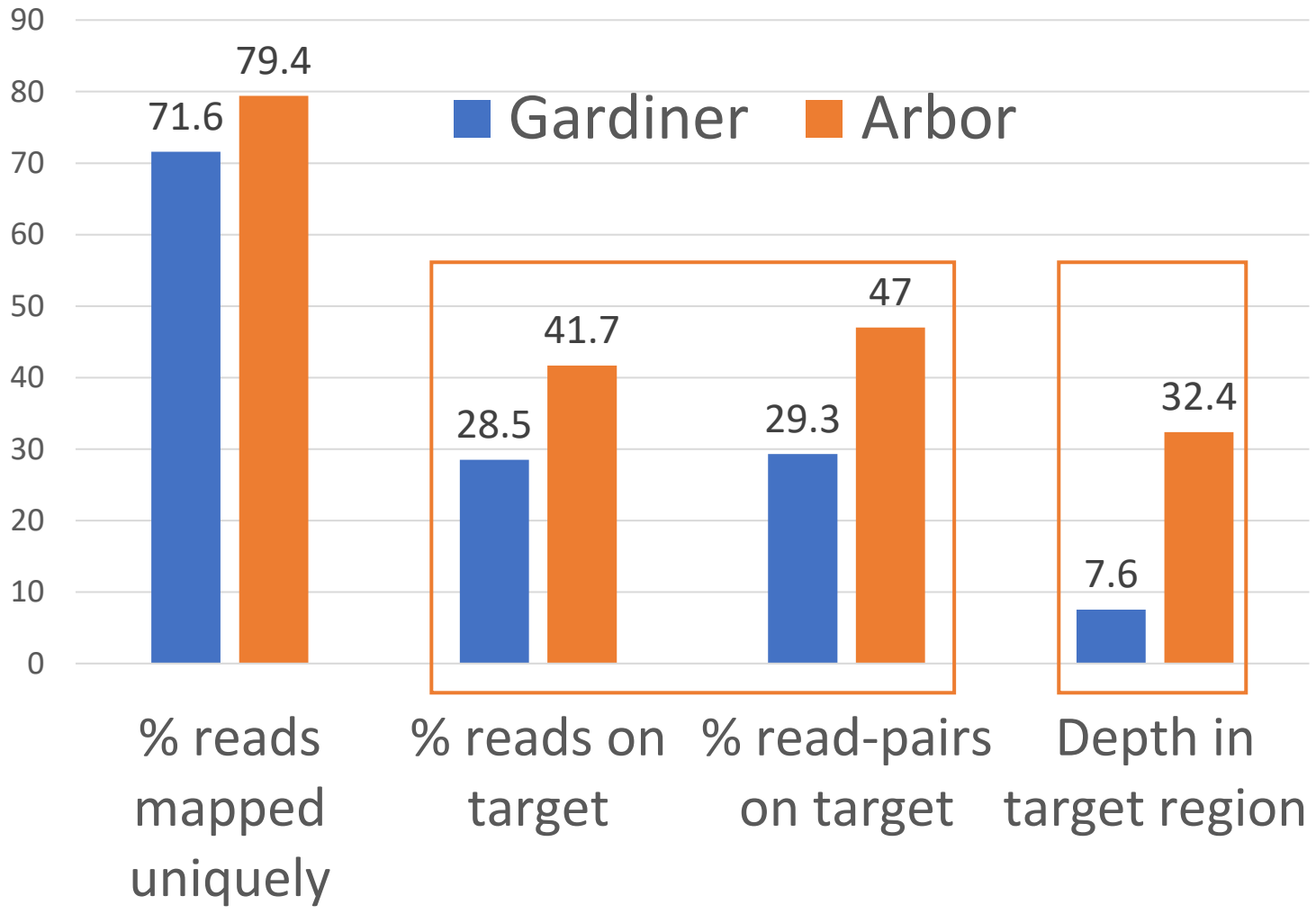
Capture performance evaluation



Comparison between Gardiner and Arbor probes

- 24 Kronos EMS-mutagenized lines
- Captured with either Gardiner or Arbor probes
- Both were down-sampled to 40-M pairs

Arbor probes showed significant increases in **specificity** and **coverage** in the target region.



Summary

- Promoter and exome capture of Kronos EMS mutants
  - 4.3 M SNPs in the promoter and 4.7 M in the coding region
  - Comparisons of promoter region among grass species to identify conserved regions
- New regulatory capture
  - Expanded with open chromatin regions from new ATAC-seq data.
  - Increased specificity and coverage in the target region.
  - Versatile and cost-effective for wheat and related *Triticeae* species.

Acknowledgements

Kronos TILLING promoter capture

University of California, Davis
Howard Hughes Medical Institute
Kansas State University
University of Minnesota
USDA-ARS
Chinese Academy of Agricultural Sciences
University of Saskatchewan

New regulatory capture design

UC Davis
UCA, INRAE, GDEC, France
VetAgro Sup, Lempdes, France
Daicel Arbor Biosciences