

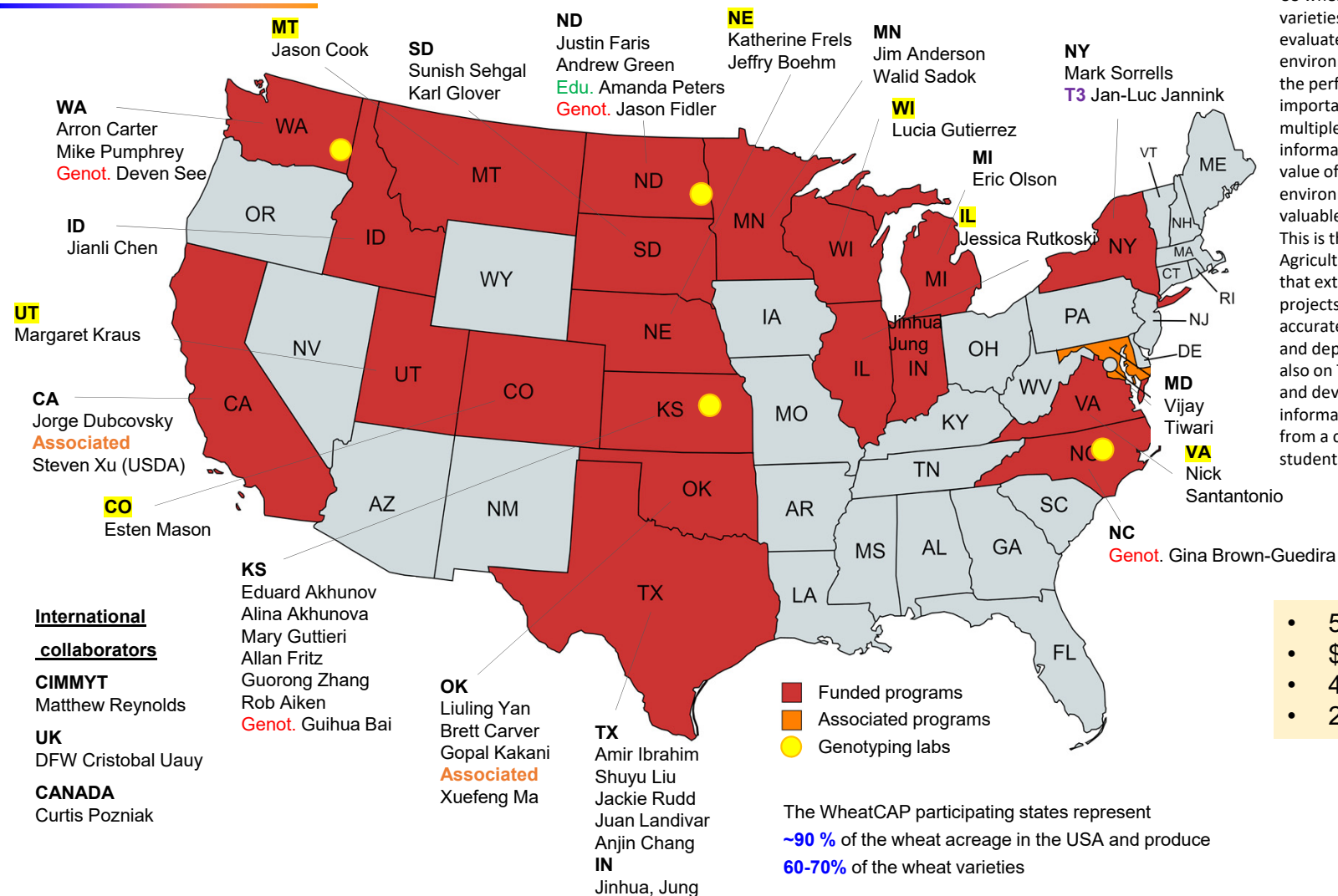
NIFA CAP for Innovation in Genomic Technology to Accelerate Breeding

# **Leveraging high-throughput genotyping and phenotyping technologies to accelerate wheat improvement and mitigate the impacts of climate change**

September 30, 2022



# WheatCAP 22-26



## WheatCAP graduate students

Student profiles can be found at:

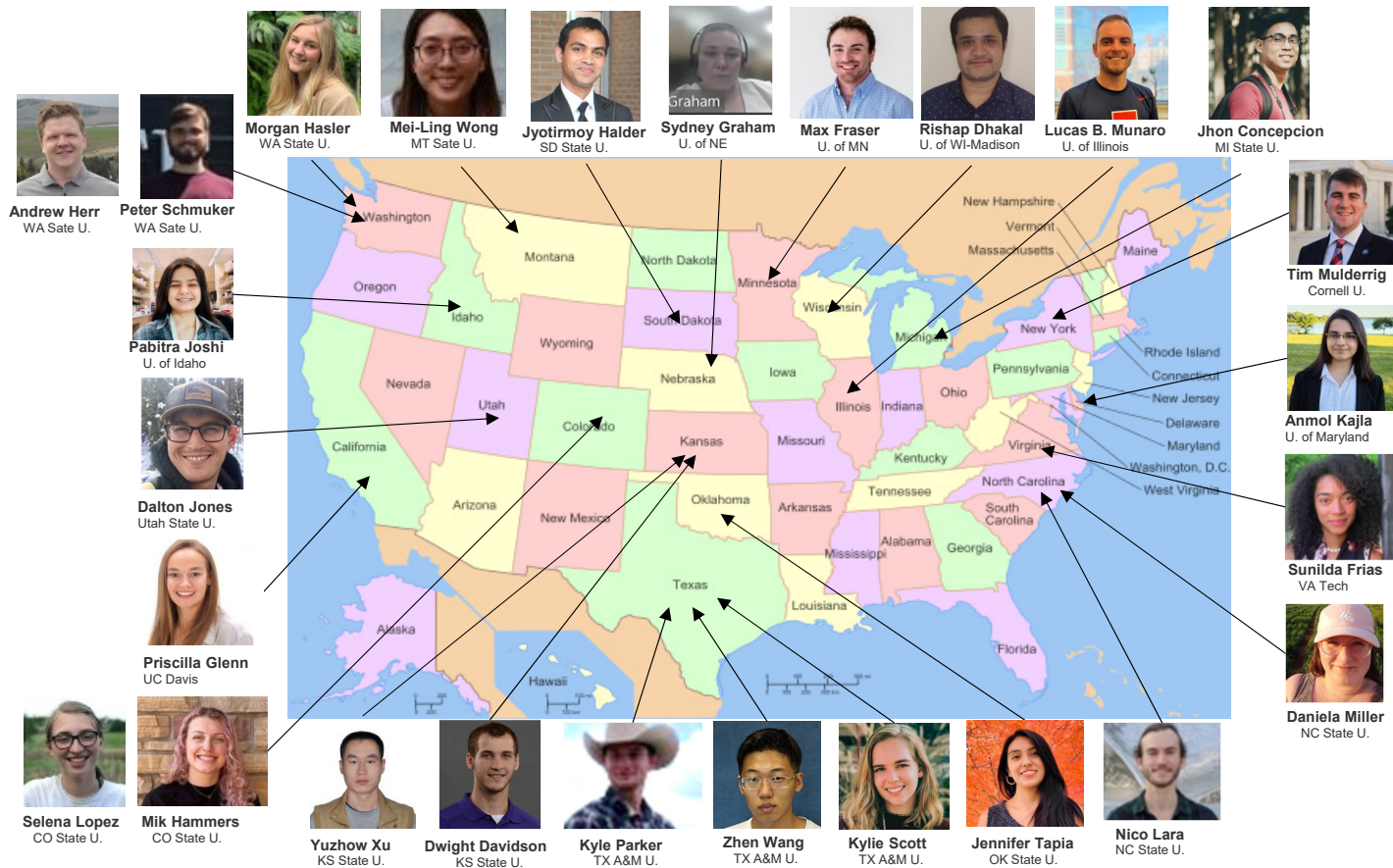
<https://www.triticeacap.org/meet-our-students/>



United States Department of Agriculture  
National Institute of Food and Agriculture



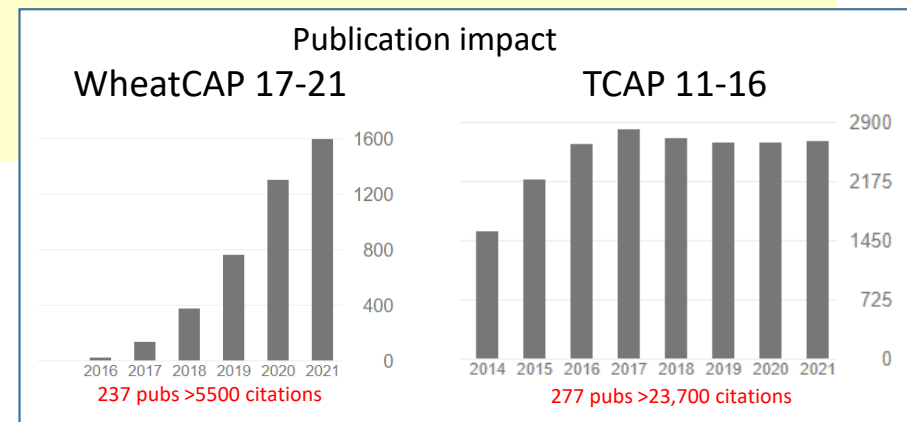
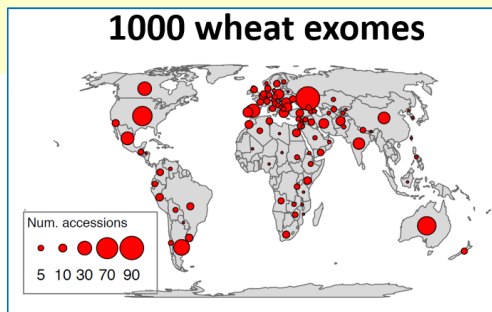
Research to Deliver Wheat for the Future



2022: **34** students + 6 graduated. Since 2001 WheatCAP projects trained **>200 graduate students**

## WheatCAP metrics to evaluate progress. **November 1<sup>st</sup>** deadline for individual reports

1. **Released varieties and germplasm.** This is the central metric that USDA uses to ask for support for our program to congress. **PVP application number and day of application for REEport.**
2. **Publications** explicitly acknowledging this grant: “This project was supported by the Agriculture and Food Research Initiative Competitive Grant **2022-68013-36439** (WheatCAP) from the USDA National Institute of Food and Agriculture”
3. **Trained students.** Number, quality of the training and jobs they get.
4. **Information deposited in T3/BreedBase**
  - Number of lines genotyped and submitted to T3/Breedbase (500/program/y).
  - Number of field trials & lines phenotyped (agronomic and UAS-HTP) deposited in T3/Breedbase.
  - Adoption of BreedBase in the public breeding programs.
5. **Genomic resources generated.**



## WheatCAP 22-26 Objectives

---

1. **Train a new cohort of 20 plant breeders** within an active Community of Practice in plant breeding, by integrating them into active wheat breeding programs. **Amanda will tell us what was done and the results of a recent survey**
2. Develop cost-effective medium-density SNP assays for effective genome-wide imputation using practical haplotype graphs and generate a **centralized automated platform for GS**. **Gina will present current status on the SNP platforms.**
3. Implement a **centralized pipeline for UAS-HTP** in public wheat breeding programs and deposit of UAS data in T3. **Amir will present progress and needs in this area.**
4. Develop **improved tools for T3/BreedBase** to accelerate the automatic deposit of genotypic and agronomic data from public breeding programs into T3, and develop tools for the use of this information. **Jean-Luc will present progress and needs in this area.**
5. Develop public **genomic resources** including natural and induced genetic variants that regulate gene expression and integrate this information into GS and T3. **Eduard and I will present new resources and discuss what is needed**

## Wheat Exome capture re-mapped to RefSeq v1.1

PNAS 2017	Old PNAS 2017	New RefSeq v1.1
Missense variant	1,030,287	2,136,531
Splice acceptor variant	14,624	29,758
Splice donor variant	15,074	30,549
Stop gained	46,580	95,756
Synonymous variant	550,556	1,145,611

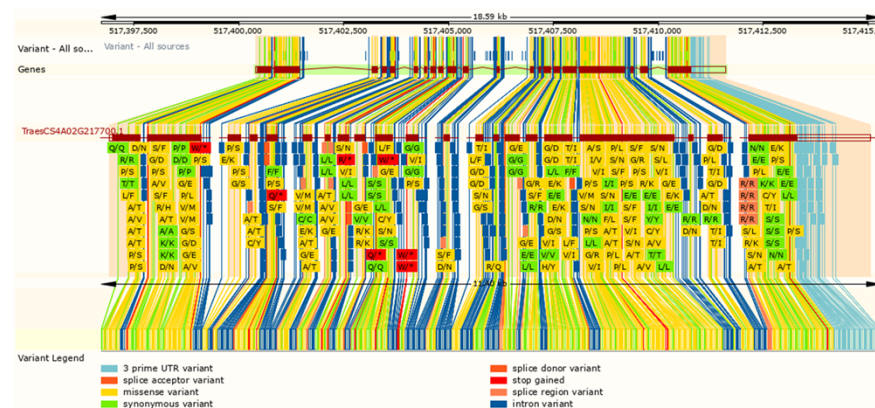
- 10.7 M mutations at <1% error or 14 M at <5% error
- 1.1 million new missense variants identified.
- 80,000 new truncation mutations called.

Data available in Ensembl new release, GrainGenes Genome Browser and Dubcovsky lab:  
[https://dubcovskylab.ucdavis.edu/wheat\\_blast](https://dubcovskylab.ucdavis.edu/wheat_blast)

**e!EnsemblPlants**  
**Triticum aestivum**  
 Location: 4A:517,404,659-517,407,5

**Location-based displays**

- Whole genome
- Chromosome summary
- Region overview
- Region in detail
- Comparative Genomics
  - Synteny
  - Alignments (image)
  - Alignments (text)
  - Region Comparison
  - Polyploid view
- Genetic Variation
  - Variant table**
  - Resequencing
  - Linkage Data



## Wheat promoter capture mapped to RefSeq v1.1

**NimbleGen promoter design.** Anthony Hall. GigaScience, 8, 2019, 1–13.

### Wheat Promoter Capture (RefSeq v1.1)

Kronos EMS populations = 1,513

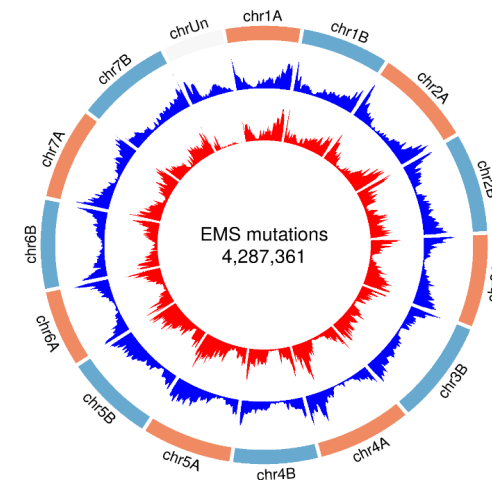
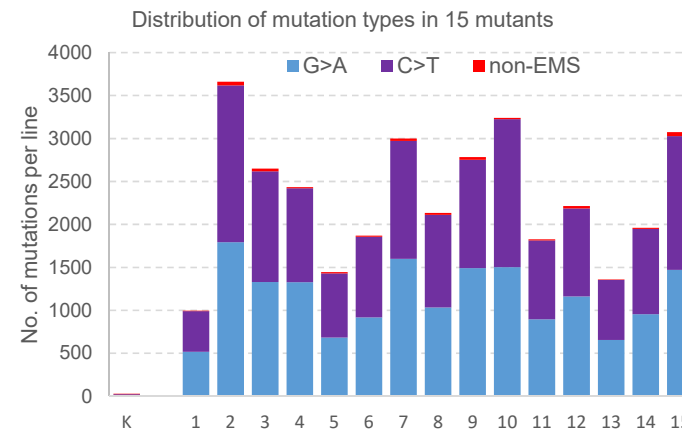
EMS mutations detected = 4,287,361

Error rate = 0.21% (99.8% accuracy)

Mutation density = 36 mutations / kb

Data available in

- Ensembl new release
- GrainGenes Genome Browser
- Dubcovsky lab: [https://dubcovskylab.ucdavis.edu/wheat\\_blast](https://dubcovskylab.ucdavis.edu/wheat_blast)

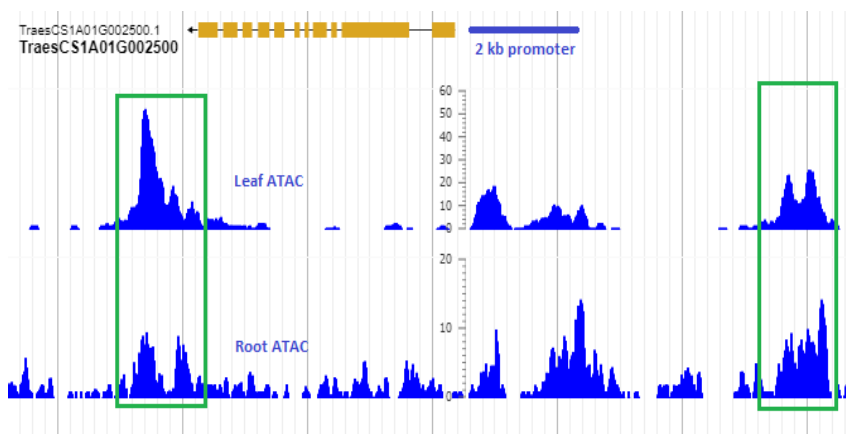


## New: Wheat Regulatory Region Capture with Arbor Biosciences

- NimbleGen promoter design was Discontinued 12/2019 (25% reads on target)
- 2-kb promoter high confidence genes in RefSeq v1.0 minus repetitive elements (>99% identity eliminated).
- Masked regions with high 17 kmer frequencies (>100) and/or high coverage in previous design: increased specificity 50%.

Complemented with open chromatin ATAC peak regions from leaves and roots outside the promoter region (23.5 Mb)

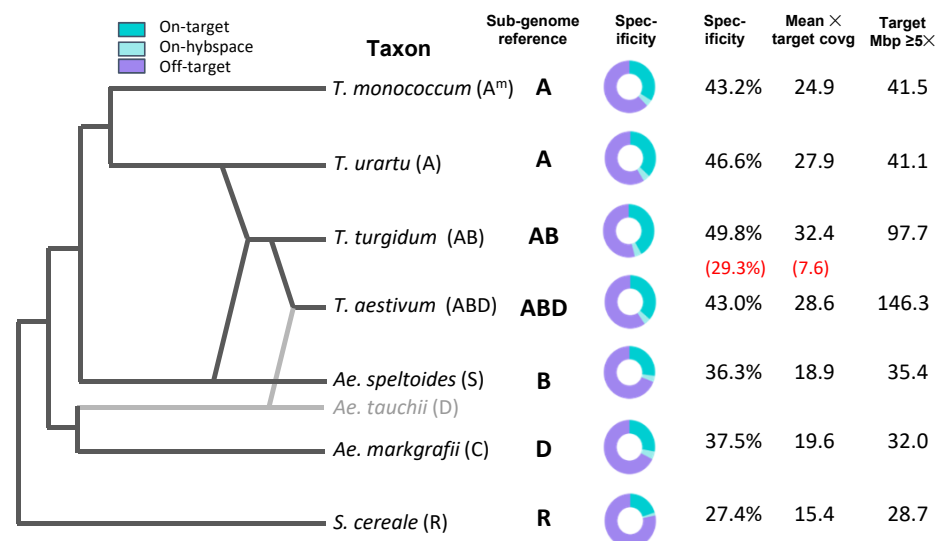
**ATAC-seq:** Assay for Transposase-Accessible Chromatin using sequencing



Green rectangles: open chromatin outside 2kb promoter region

**162.5 Mb promoter + 23.5 Mb open chromatin = 186 Mb**

bioRxiv <https://biorxiv.org/cgi/content/short/2022.09.16.508311v1>

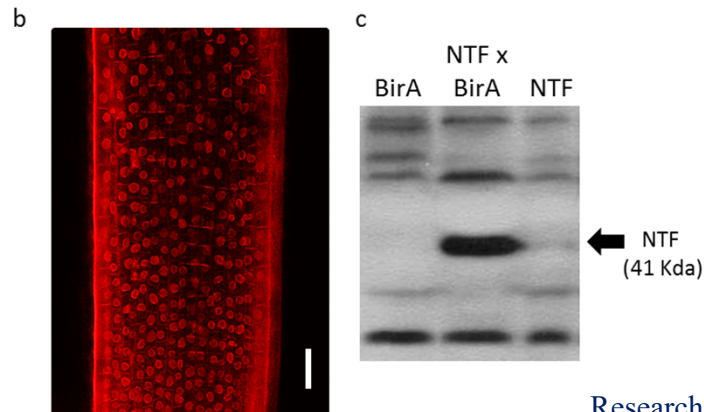
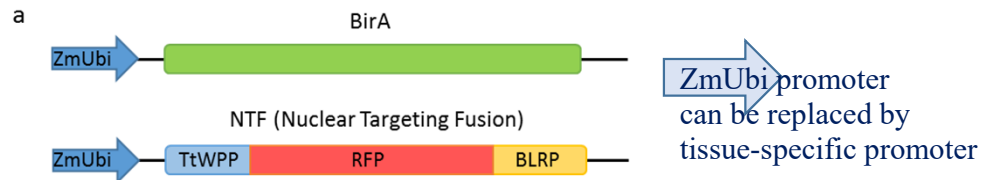


- 43-50% specificity *Triticum* sp.
- Decreases with phylogenetic distance (27.4% in rye)
- Target at 5x coverage: 41 Mb diploid, 98 Mb 4x and 146 Mb 6x.
- 61% ↑ reads on target, >3-fold ↑ on-target coverage than previous design

## INTACT technology for high quality ATAC-seq data

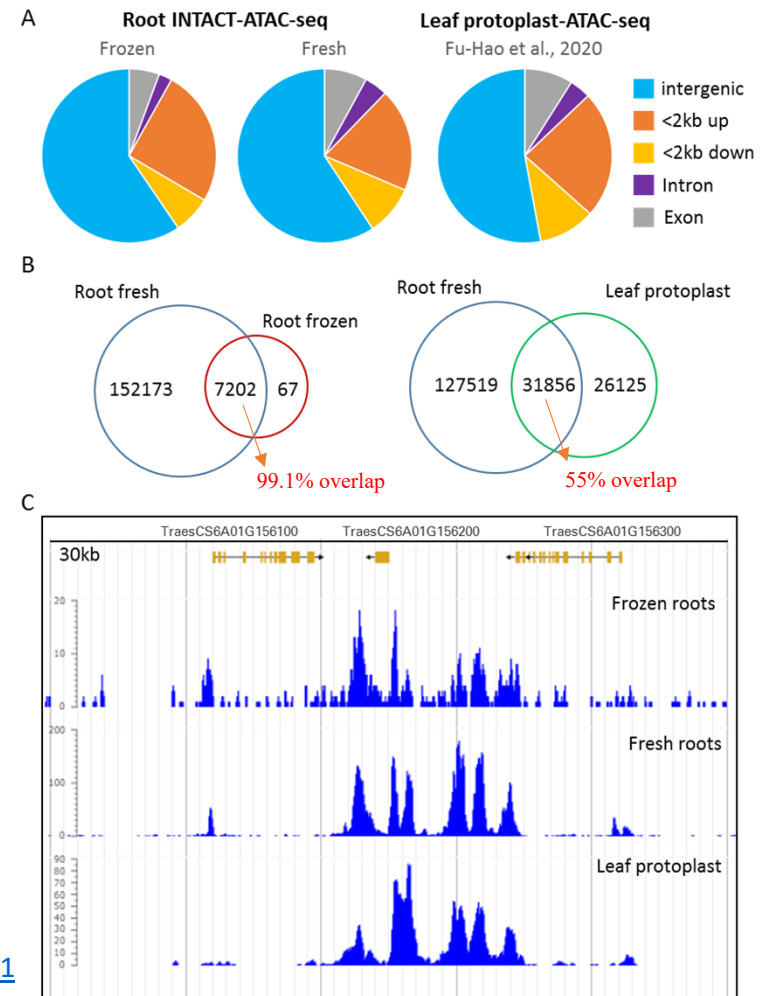
- ATAC-Seq quality and signal to noise ratio depends on the quality of nuclei extraction
- INTACT: isolation of nuclei tagged in specific cell types. Uses 2 transgenic lines expressing:

- A biotinylating enzyme **BirA**
- A chimeric nuclear envelope targeting fusion protein (NTF) that includes a nuclear binding domain (**TtWPP**), a reporter gene (**RFP**) and a biotin ligase recognition peptide (**BLRP**).



Research Square Preprint online

<https://doi.org/10.21203/rs.3.rs-2058892/v1>



## Tetraploid wheat haploid inducer using *MATRILINEAL* mutants

### CRISPR mutations in *MATL* in tetraploid wheat Kronos

- Combined editing in both *MATL* genomes
- Kronos *matl*-null used as male induces haploids
  - Avg. 4 tetraploid tester lines 10.9 % haploids
  - 1 hexaploid tester line 5.7 % haploid
- Seeds are available

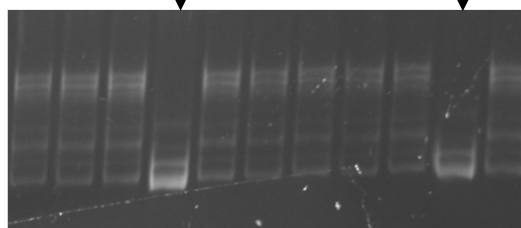
CRISPR *matl* **A** genome (TraesCS1A02G395800)



CRISPR *matl* **B** genome (TraesCS1B02G424300)



SSR polymorphic marker screening



Kronos haploid inducer line (male parent, *matl*-null)

Test line	No. of Flowers	Fert. %	No. of seeds	No. of plants	Germ. rate %	Haploid	HIR %
Rusty (4x)	208	22.1	46	36	78	7	<b>19.4</b>
Tipai (4x)	182	20.9	38	22	58	1	<b>4.5</b>
UC1850 (4x)	212	27.8	59	46	78	3	<b>6.5</b>
UC1771 (4x)	246	24.8	61	43	70	5	<b>11.6</b>
<b>Central Red (6x)</b>	238	19.8	47	35	74	2	<b>5.7</b>



Chromosome count confirmation



Diploid Haploid

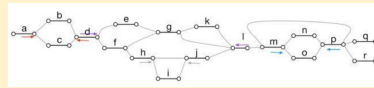
## Agenda Zoom meeting September 30, 2022

8:00 – 8:10. **J Dubcovsky**. Opening remarks.

- Objectives and metrics by which the project will be evaluated
- Coming report
- New resources

8:10 – 8:20. **Eduard Akhunov** Genomic resources.

- Natural variation on wheat expression.
- Additional genomic resources



8:20 – 8:30. **Gina Brown-Guedira**. Genotyping labs.

- Medium throughput genotyping platforms.
- Data submitted to Genotyping labs.
- Status of GS in the Eastern breeding programs and possibilities to expand to US?



8:30 – 8:40. **Amir Ibrahim**. UAV high-throughput phenotyping

- Status of the UAS-HTP pipeline and standardization needs.
- Data submitted to UAS-HTP.
- Bottlenecks and future activities.

8:40 – 8:55. **Jean-Luc Jannink** T3/Breedbase

- Status of the pipelines connecting UAV and Genotyping data to T3
- Summary data submitted to T3 by breeders.
- Plans for the GS pipelines
- Current tools provided by BreedBase to breeders.
- Bottlenecks and future activities.



8:55 – 9:05. **Amanda Peters Haugrud** Education coordinator

- Summary of educational activities.
- Requested support from coPIs.
- New Educational coordinator.

9:05 – 9:30. Open discussion

- What should be the priorities for the 2<sup>nd</sup> year

