

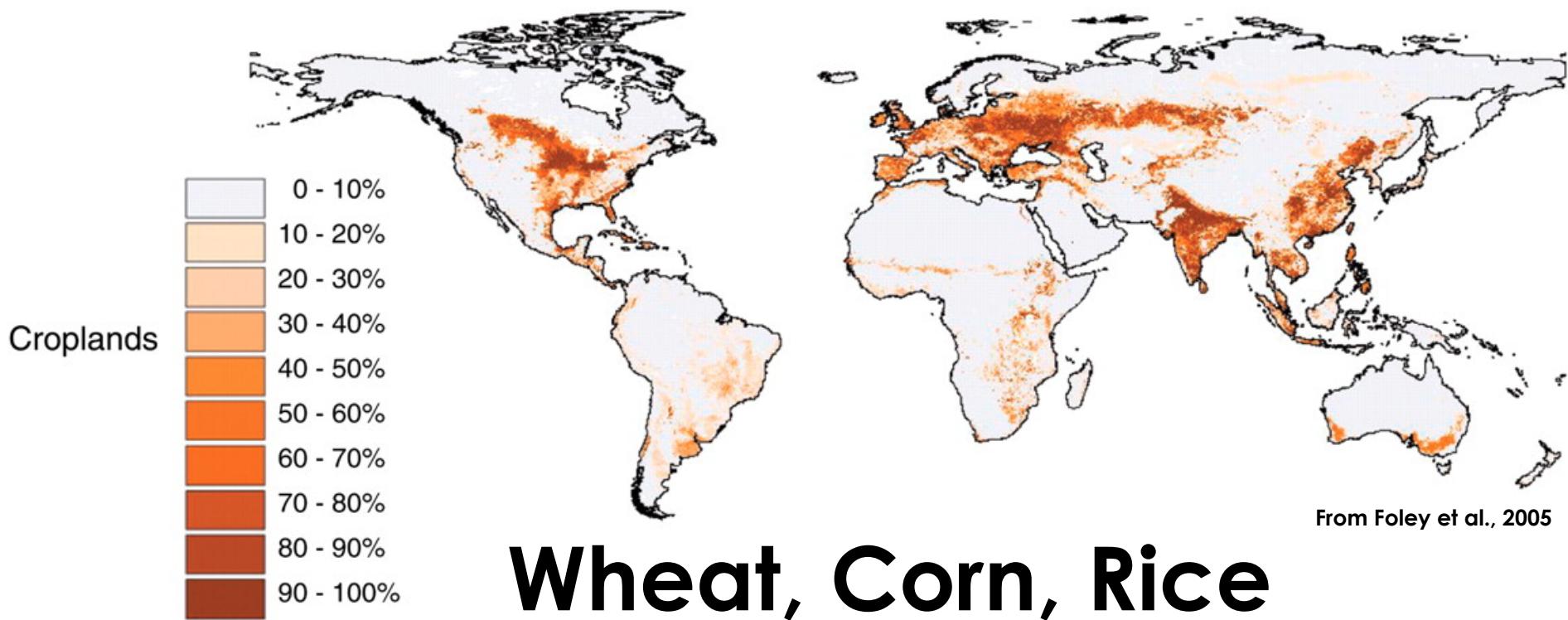
# Genomics-driven development of next generation grain crops

**Kevin Dorn, PhD**  
**USDA-NIFA Postdoctoral Fellow**  
**Poland Lab - Kansas State University**  
[www.kdorn.com](http://www.kdorn.com)



This talk is Twitter friendly!  
 @Dornomics

# 44% of global cropland is occupied by 3 crops

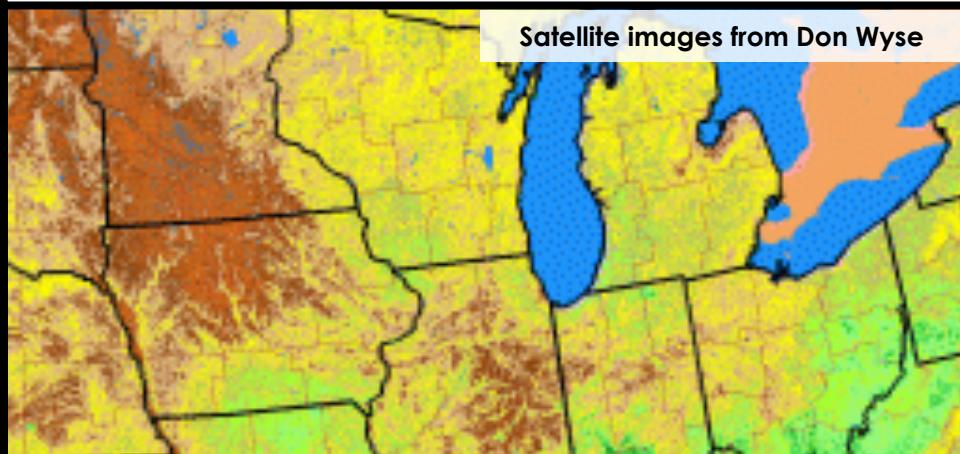


**Challenge: Require annual replanting – often tillage**  
**Opportunity: Decreasing operating costs**

# One common agroecosystem

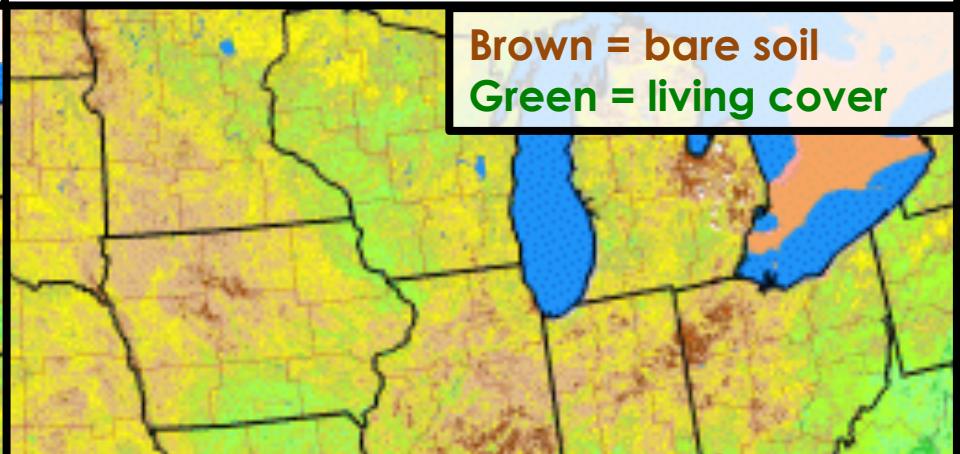


April 20 – May 3



**Challenge:** weed growth  
**Opportunity:** reduce herbicide costs

October 5 - 18



**Challenge:** bare ground, topsoil loss  
**Opportunity:** hold soil in place

# **Genomics-driven development of next generation grain crops**

**Vision: Genomics hub focused on helping growers meet production challenges through delivering new traits/crops/systems**



# One new tools for growers: perennial grain crops

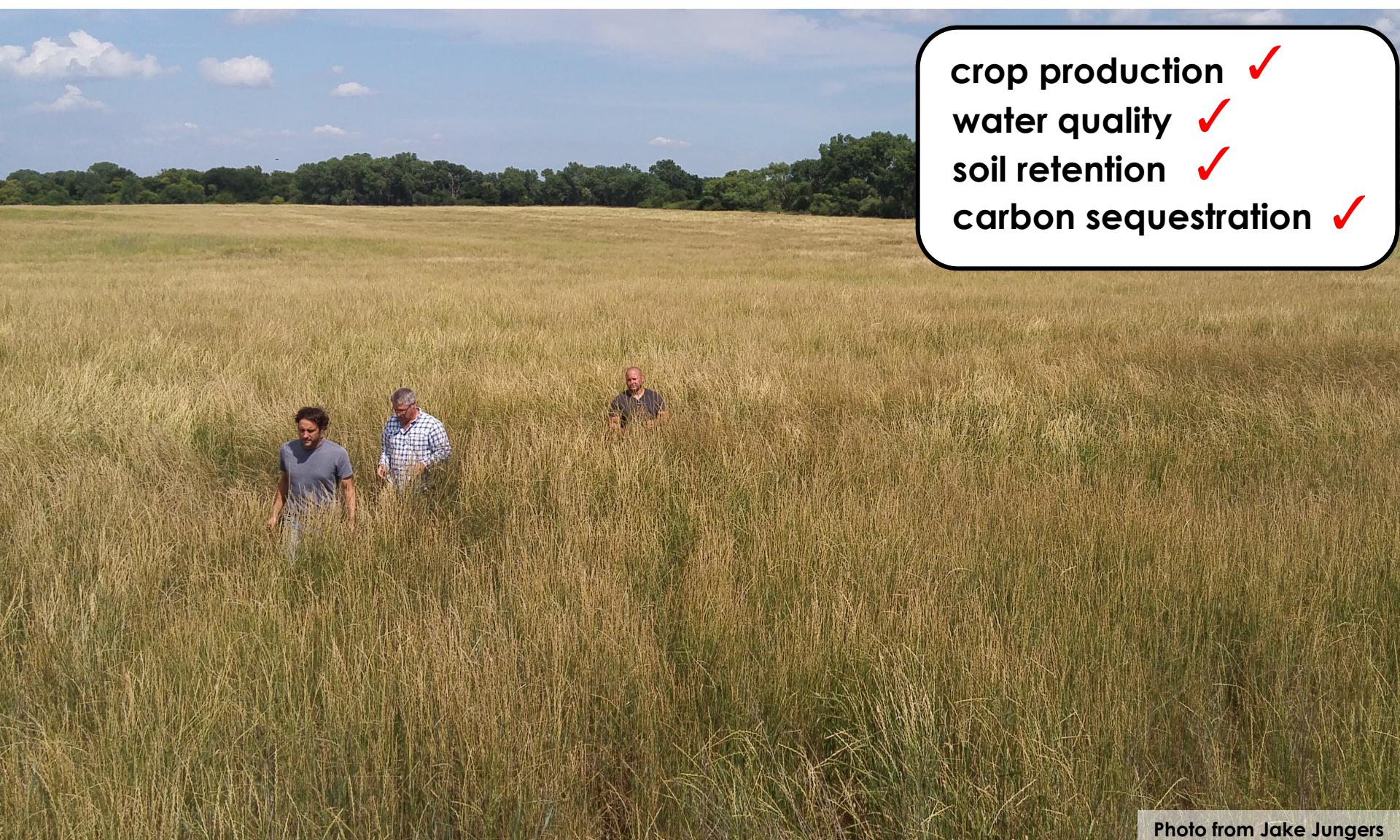


Photo from Jake Jungers

Perennial Kernza field near Salina, Kansas

# Genomics-driven development of next generation grain crops

- 1.) Establishing genomic resources for a new grain crop
- 2.) Genome biology of *Thinopyrum*
- 3.) Genomics of Kernza neo-domestication
- 4.) Disease resistance from wheat relatives



# Domesticating a new perennial grain crop

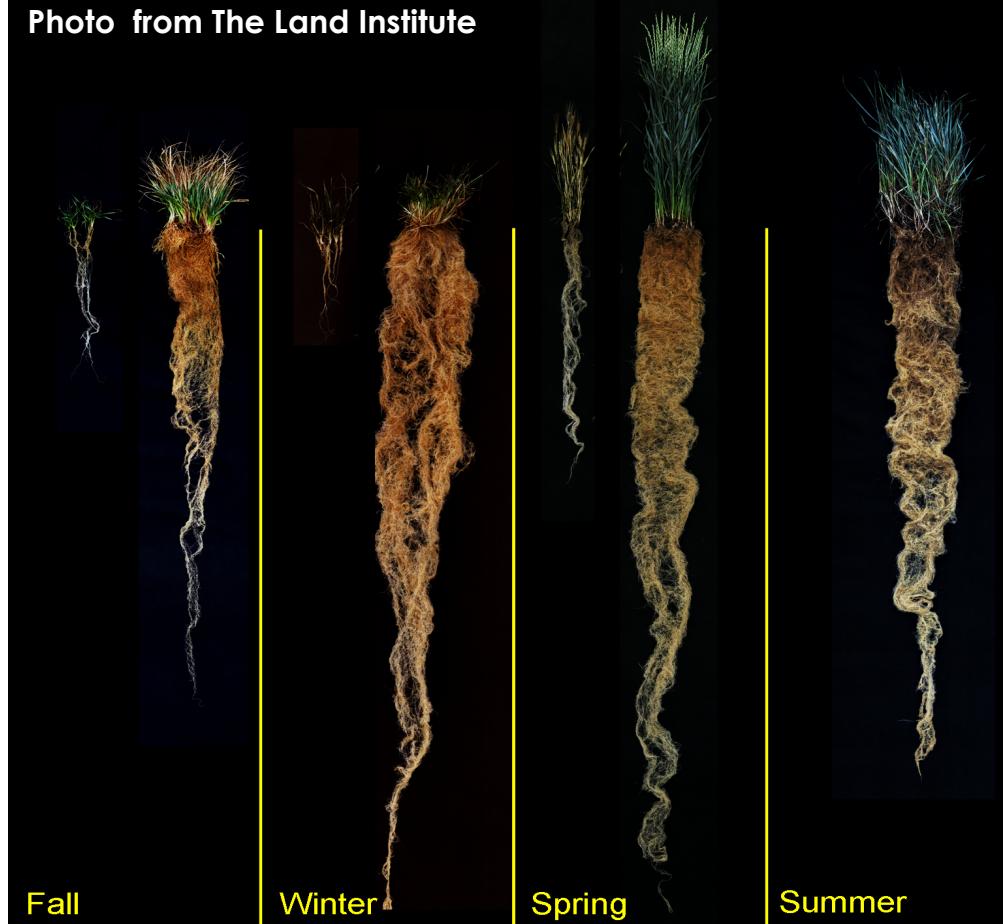


Jerry Glover of The Land Institute:  
Photo by Jim Richardson

*Thinopyrum intermedium*

Common name: intermediate wheatgrass (IWG)

Perennial grass species targeted for direct  
domestication into grain/biomass crop



# Intermediate Wheatgrass (Kernza™) breeding

## Kernza

Founder Current Wheat



Significant progress has been made breeding for key traits

Commercialization is rapidly advancing



Lee DeHaan  
The Land Institute

Cascadian Farms  
(a General Mills brand)  
product coming in 2018

# Challenges still exist = target traits



- **yearly yields**
- **grain size**

**Genomics assisted approach should accelerate improvement**

# The (genomic) challenge with intermediate wheatgrass...



- $2n=6x=42$
- $1C=12.6\text{ Gb}$
- **Obligate outcrosser**

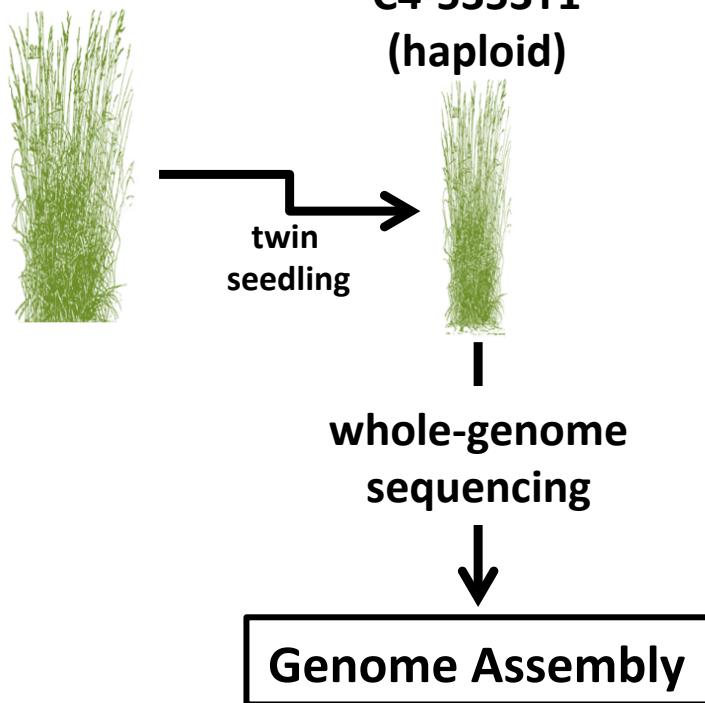
## A reference genome

- Enables functional studies of key traits
- Increases marker quality/quantity for breeding

# Genome sequencing C4-5353 (Parent 1)



Kernza  
genome



**NRGene**

Number	237,622 scaffolds
Assembly Length	11,605,214,228 (92%)
N50 (bp)	2,794,550 bp
L50	1,072 scaffolds

Haploid plant derived  
from twin seedling



31 Billion  
Illumina reads

N50 = half the assembly is composed of fragments this size or larger

L50 = the largest half of the assembly is composed of this many fragments

# Population Sequencing (anchoring the genome)



Parent 2



C4-5353  
(Parent 1)



C4-5353T1  
(haploid)



twin  
seedling

350,885 PopSeq markers

21 chromosomes assembled  
(59,064 anchored scaffolds, 11.3Gb)

F<sub>1</sub> population



whole-genome  
sequencing

Genome Assembly

anchoring

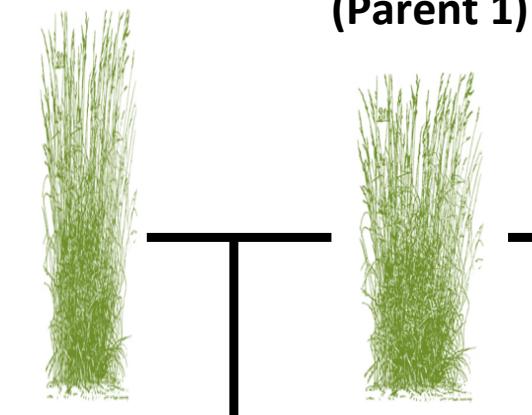
Population Sequence



Jeremy Schmutz & Jerry Jenkins  
(HudsonAlpha / JGI)

# Genome annotation

Parent 2

C4-5353  
(Parent 1)C4-5353T1  
(haploid) $F_1$  populationtwin  
seedlingwhole-genome  
sequencing

RNAseq

Genome Assembly

assembly

Reference  
transcriptome

annotation

anchoring

Population Sequence

RNA from 16 tissue types  
2 Billion Illumina readsPrimary transcripts with  
RNAseq support = 122,028

# Hybridization Events Forming *Th. intermedium*

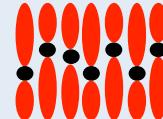
Kernza  
genome



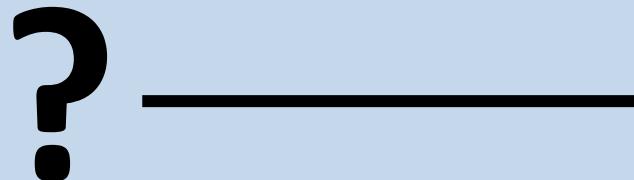
( $2n=2x=14$ , JJ)

( $2n=2x=14$ , J<sup>b</sup>J<sup>b</sup> or StSt)

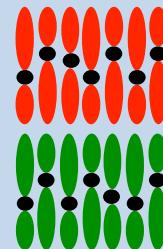
( $2n=2x=14$ , J<sup>b</sup>J<sup>b</sup> or StSt)



Diploid (2X)



( $2n=4x=28$ , JJJ<sup>b</sup>J<sup>b</sup> or JJStSt or J<sup>b</sup>J<sup>b</sup>StSt)

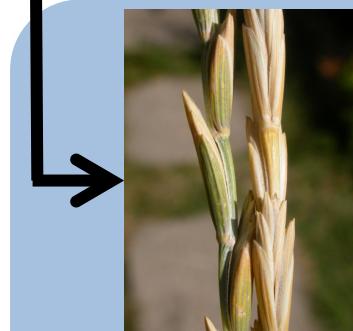


Tetraploid (4X)

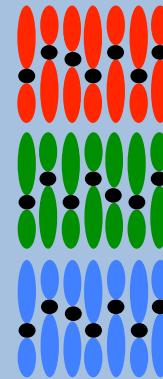
Many conflicting studies on progenitors

Most based on minimal markers / GISH

Best current thought: J J<sup>b</sup> St



*Thinopyrum intermedium*  
( $2n=6x=42$ )



Hexaploid (6X)

# Unraveling the evolutionary history of *Thinopyrum*

Kernza  
genome

Step 1: Parse into 7 homeologous groups (Kantarski et al. 2016) ✓

Step 2: Parse 21 chromosomes into 3 subgenomes

Step 3: Identify the most closely related diploid species

Genotype-By-Sequencing of 46 potential progenitor species\*



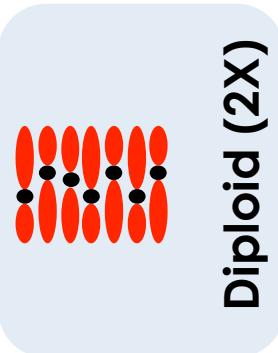
Extract sequences shared within each potential progenitor  
(TASSEL & custom scripts)



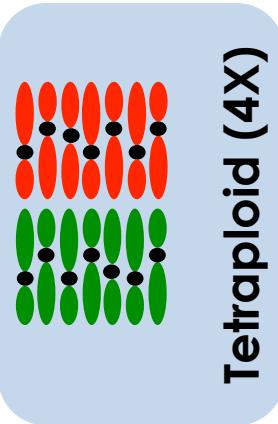
Align non-polymorphic sequences to IWG chromosomes

\*Including...

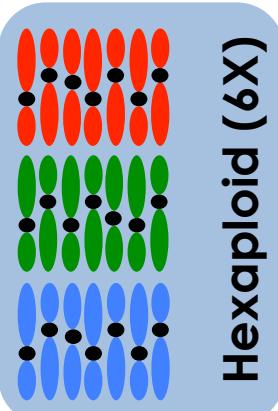
<i>P. gracillima</i>	<i>Th. bessarabicum</i>	<i>A. bicornis</i>
<i>P. libanotica</i>	<i>Th. curvifolium</i>	<i>A. tauschii</i>
<i>P. spicata</i>	<i>Th. elongatum</i>	<i>A. umbellulata</i>
<i>P. strigosa</i>	<i>Th. caepitosum</i>	<i>A. geniculata</i>
<i>P. tauri</i>	<i>Th. curvifolium</i>	<i>A. speltooides</i>
	<i>Th. ponticum</i>	<i>A. ventricosa</i>
	<i>Th. junceiforme</i>	<i>T. urartu</i>
	<i>D. breviaristatum</i>	<i>T. monococcum</i>
	<i>D. villosum</i>	<i>Ag. cristatum</i>
	<i>Taeniatherum</i>	<i>Ag. glaucum</i>



Diploid (2X)



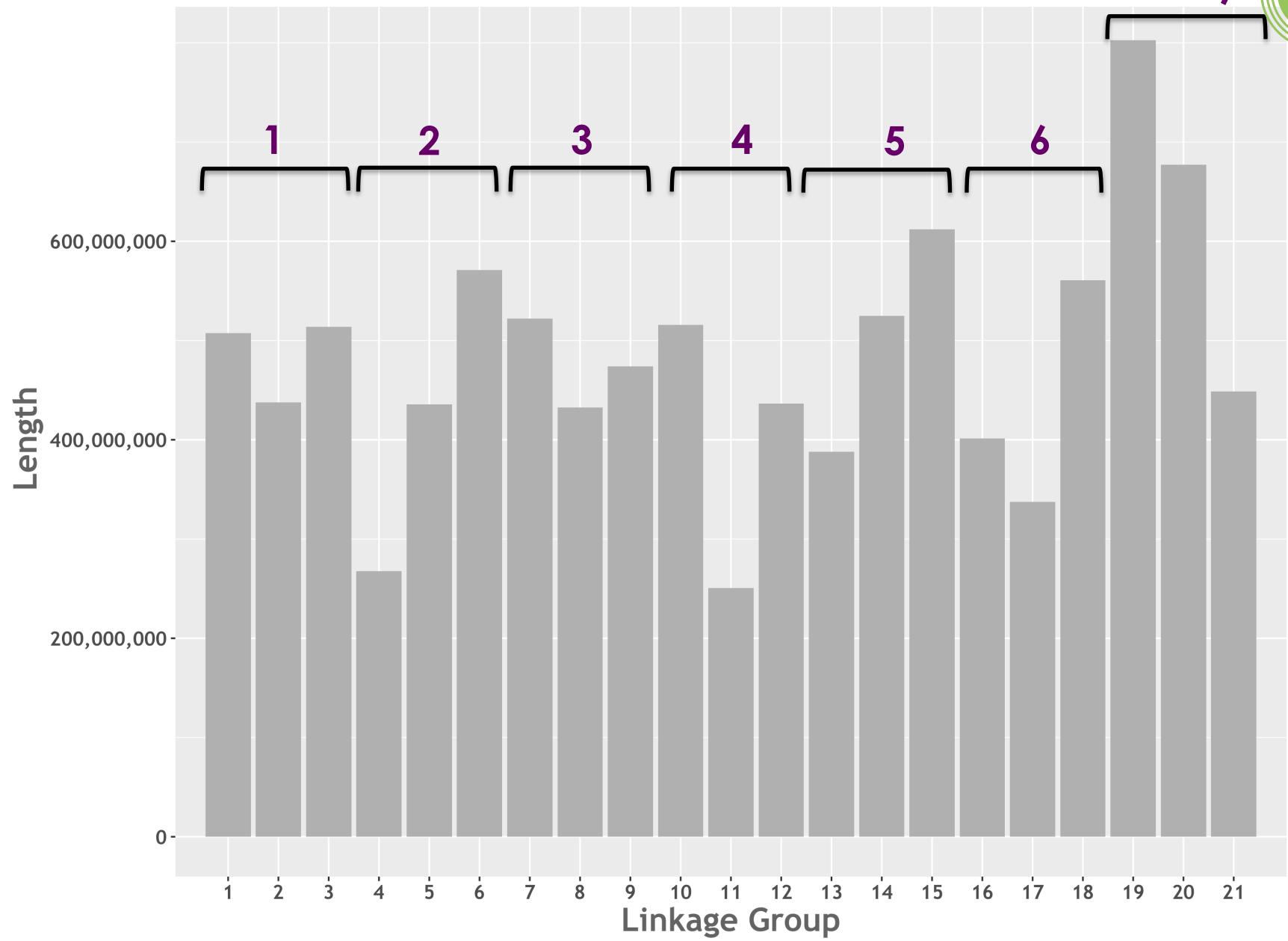
Tetraploid (4X)



Hexaploid (6X)

## Step 2: Parse 21 chromosomes into 3 subgenomes

7

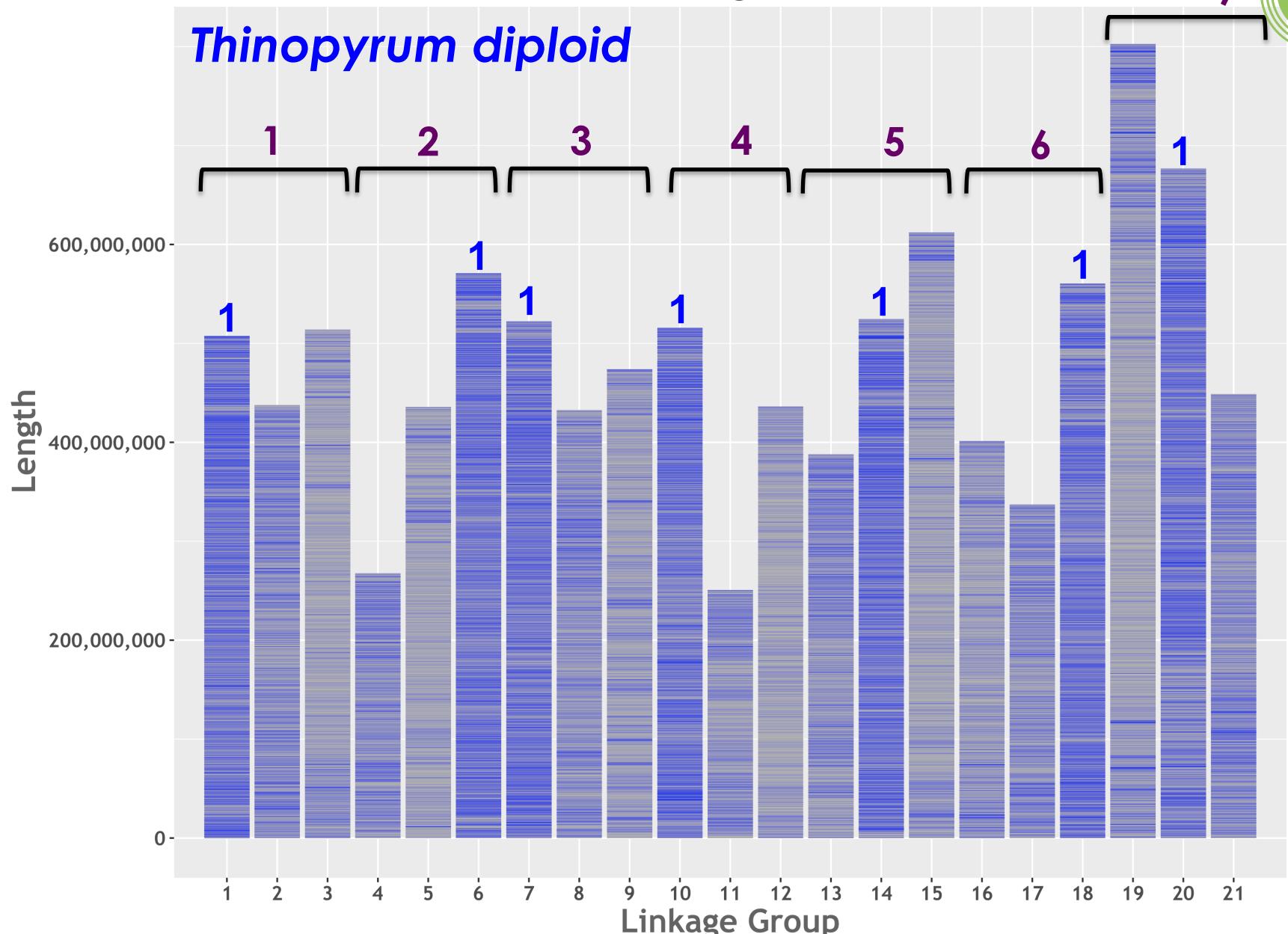


## Step 2: Parse 21 chromosomes into 3 subgenomes

7



### *Thinopyrum diploid*



64mer GBS tag mapping positions including multiple mapping locations

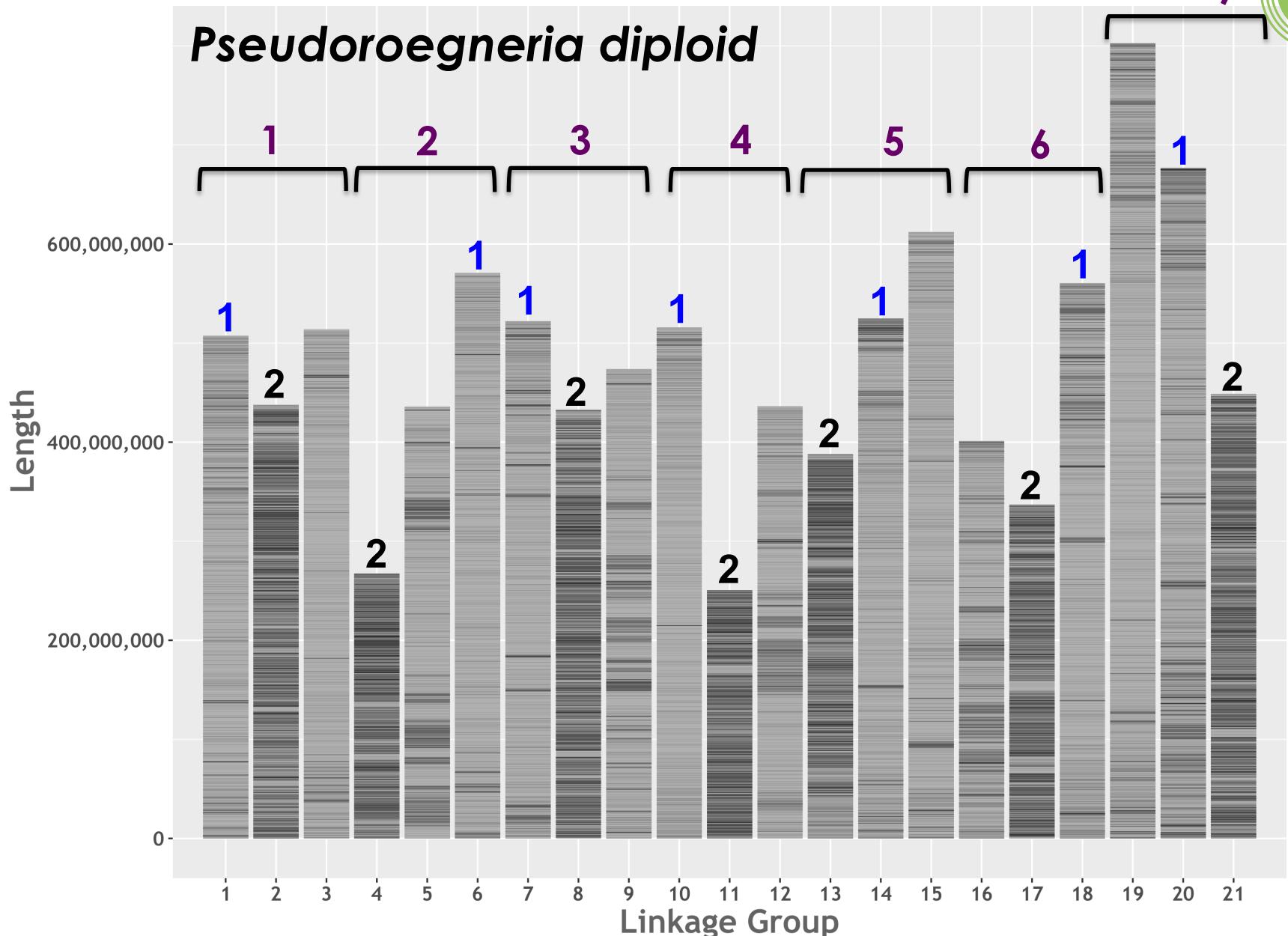
chromosome numbering from Kantarski et al., 2016

## Step 2: Parse 21 chromosomes into 3 subgenomes

7

Kernza genome

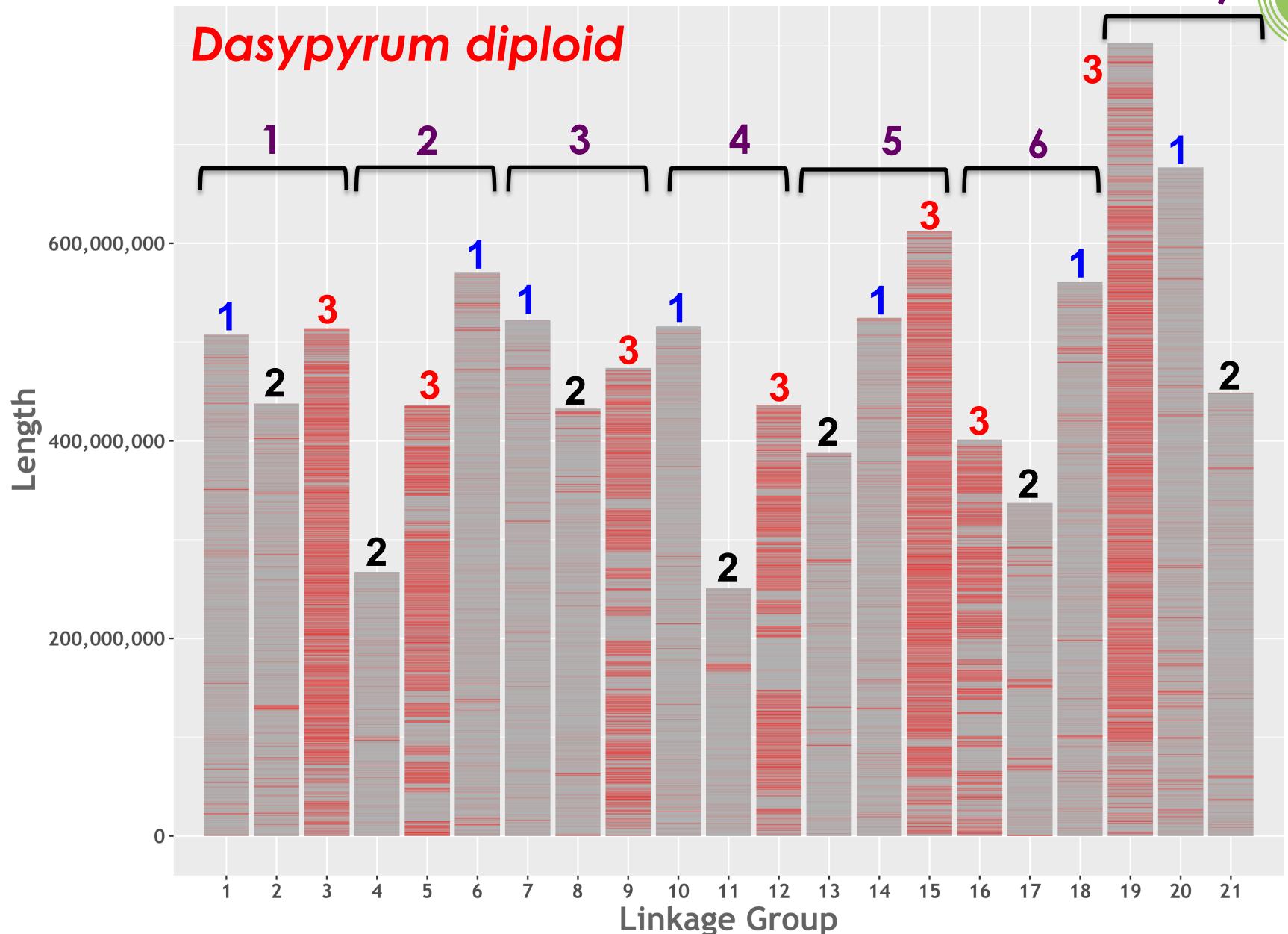
### *Pseudoroegneria diploid*



64mer GBS tag mapping positions including multiple mapping locations  
chromosome numbering from Kantarski et al., 2016

## Step 2: Parse 21 chromosomes into 3 subgenomes

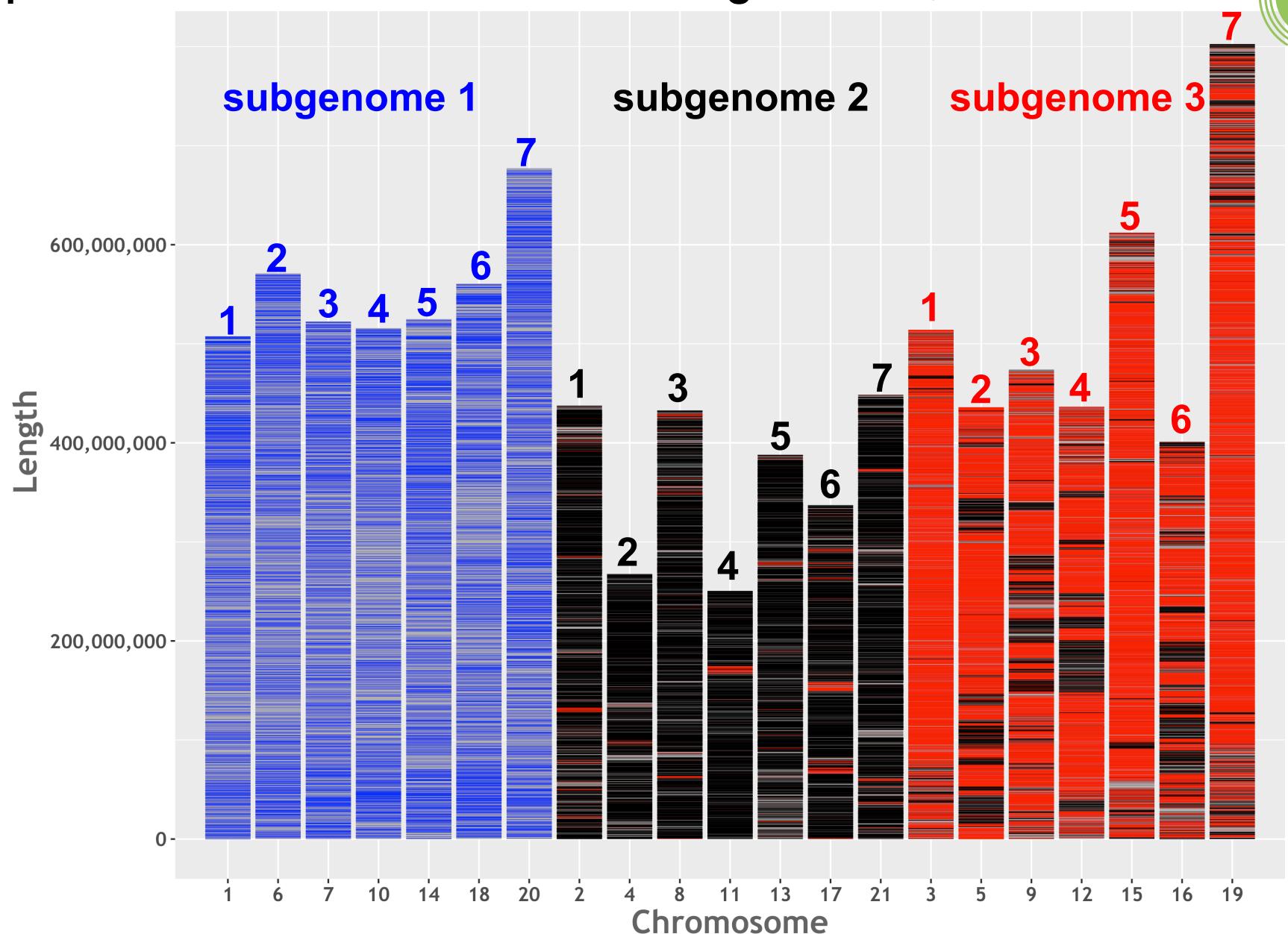
7  
Kernza genome



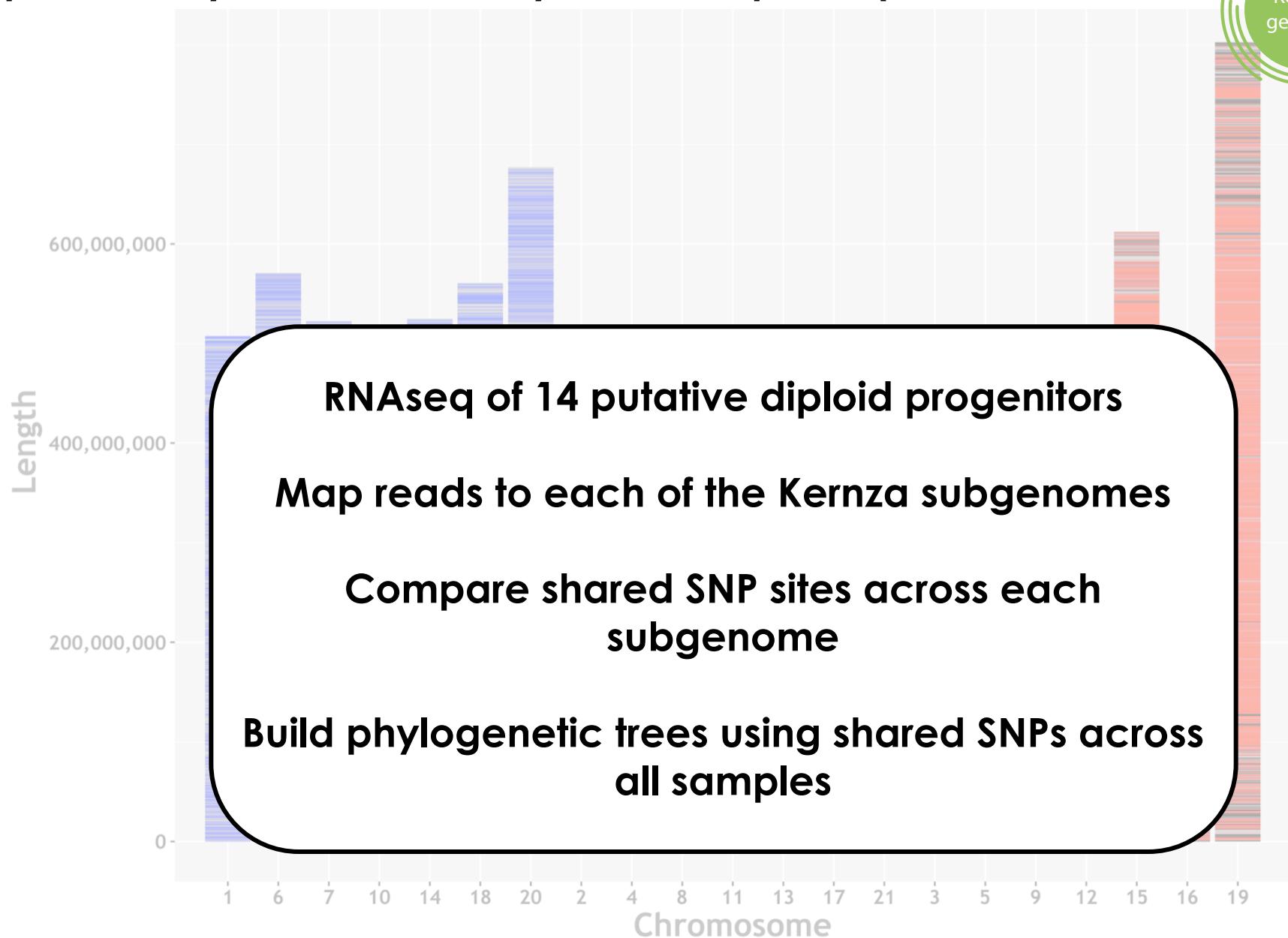
64mer GBS tag mapping positions including multiple mapping locations

chromosome numbering from Kantarski et al., 2016

## Step 2: Parse 21 chromosomes into 3 subgenomes ✓



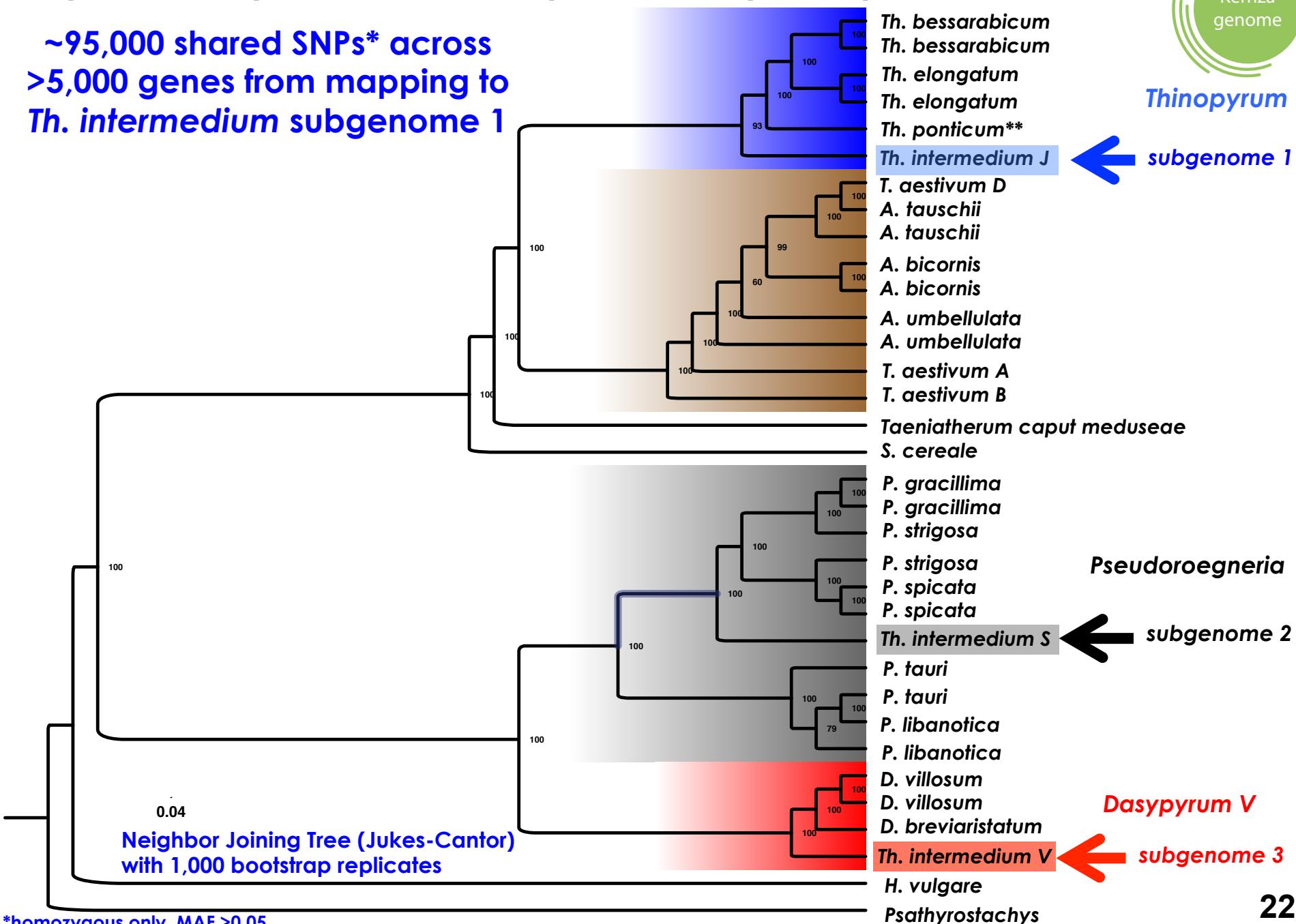
## Step 3: Identify the most closely related diploid species



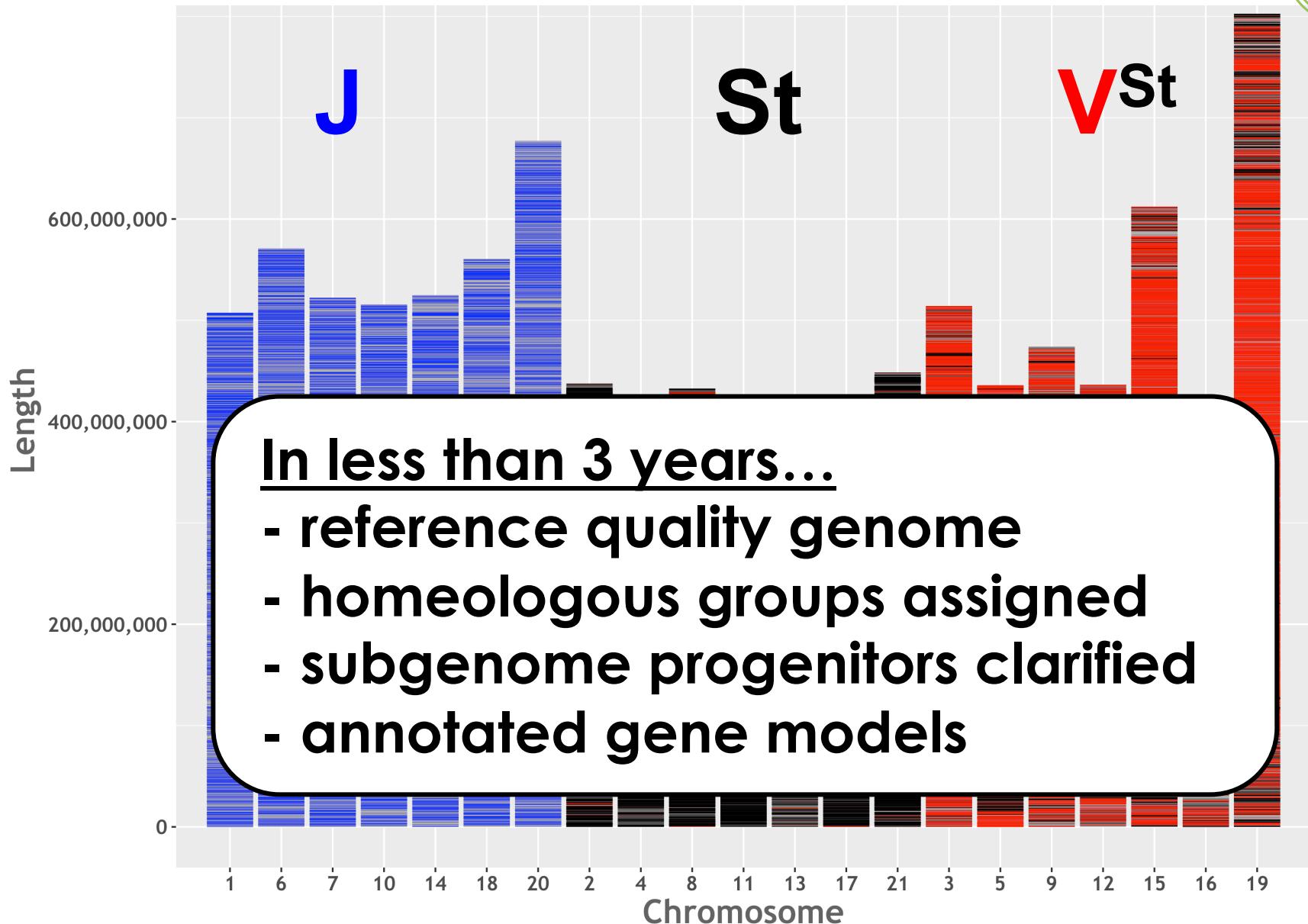
# Step 3: Identify the most closely related diploid species ✓



~95,000 shared SNPs\* across  
>5,000 genes from mapping to  
*Th. intermedium* subgenome 1



# The *Thinopyrum intermedium* genome ✓



# Genomics-driven development of next generation grain crops

- 1.) Establishing genomic resources for a new grain crop
- 2.) Genome biology of *Thinopyrum*
- 3.) Genomics of Kernza neo-domestication
- 4.) Disease resistance from wheat relatives



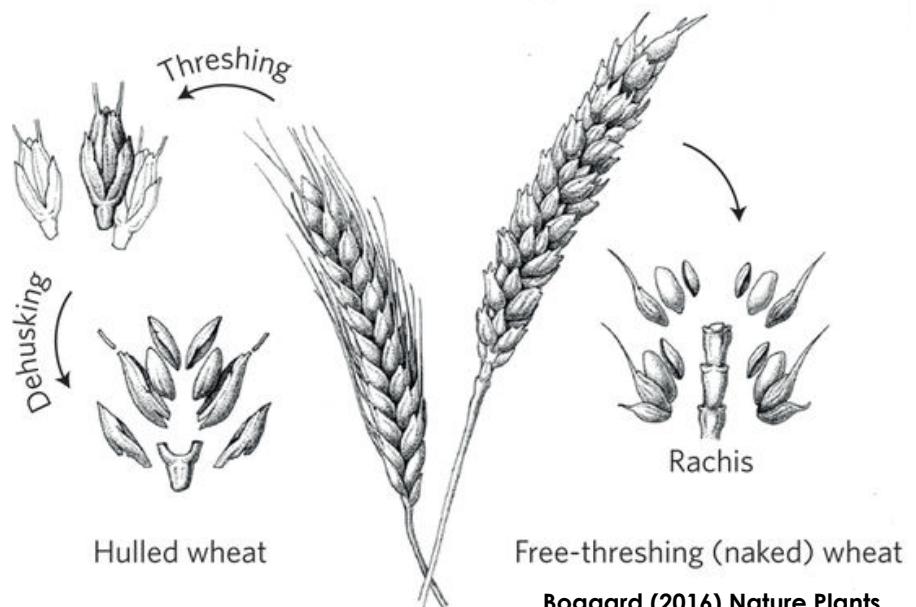
# Advancing molecular tools for Kernza breeding



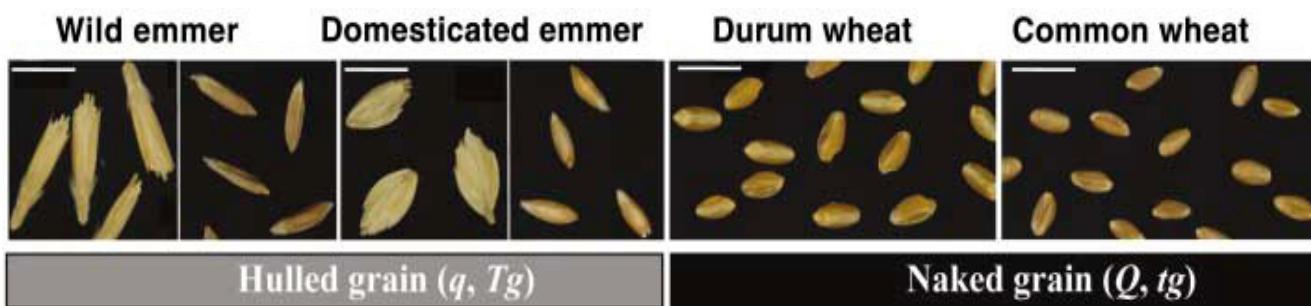
## Shattering



## Free Threshing



Bogaard (2016) Nature Plants



Peng et al. (2011) AJCS

**Do homologs of these wheat genes control these traits in Kernza?**

# Evaluating genetic bottlenecks



Rodale Research Center  
(Pennsylvania)

300 wild collections /  
forage varieties

Best 20

2 breeding cycles

Best 17

The Land Institute (Kansas)

Starting material for first  
breeding cycle

7 breeding cycles

## Central Questions

- 1.) Have the bottlenecks in early Kernza domestication resulted in selection sweeps in homologous regions to other cereal grains?
- 2.) Is there valuable, untapped core and pan-genomic variation across the species?

# Evaluating genetic bottlenecks



Rodale Research Center  
(Pennsylvania)

300 wild collections /  
forage varieties

Best 20

2 breeding cycles

Best 17

The Land Institute (Kansas)

Starting material for first  
breeding cycle

7 breeding cycles

Rodale Selections

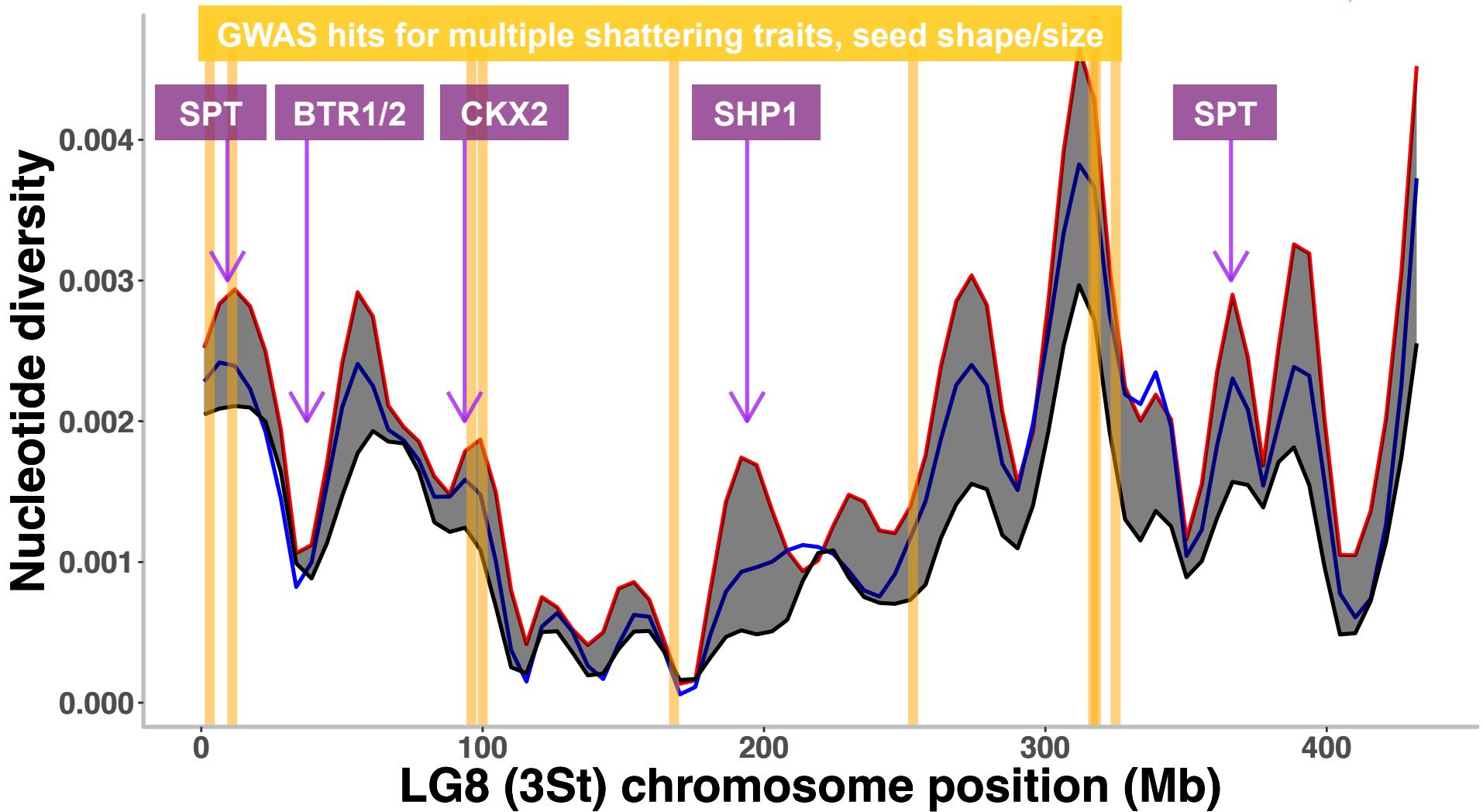
USDA PI accessions

The Land Institute Breeding Cycles

Genotyping (via GBS) of USDA PI accessions  
+ TLI Cycles 5-7 + Rodale Selections

# Evaluating genetic bottlenecks

Genotyping of TLI Cycle 7 selections vs. 'Rodale' selections vs. USDA PI accessions



Ongoing / Planned

- Whole genome sequencing of Rodale selections to refine swept regions

# Genomics-driven development of next generation grain crops

- 1.) Establishing genomic resources for a new grain crop
- 2.) Genome biology of *Thinopyrum*
- 3.) Genomics of Kernza neo-domestication
- 4.) Disease resistance from wheat relatives



# Genomics of disease resistance from wheat relatives

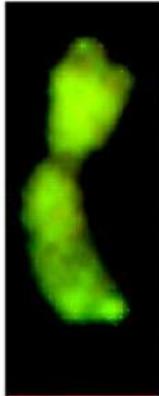
*Aegilops geniculata* – Lr57, Yr40, Sr53

*Aegilops ventricosa* – Lr37, Yr17, Sr38, Pch1, Rkn3

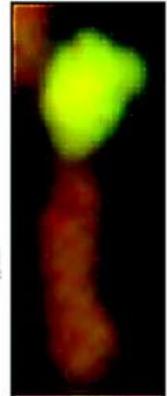
*Thinopyrum elongatum* – Sr24, Sr25, Sr26, Sr43, Lr19, Lr29,

*Thinopyrum intermedium* – WSM1, WSM3, BdV2, BdV3, Sr44, Yr50, Lr38, Pm40

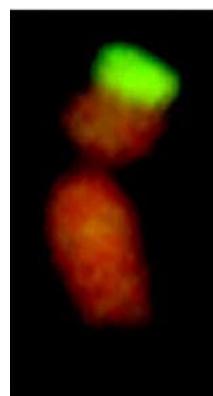
## Wheat Streak Mosaic Virus Resistance (WSM1)



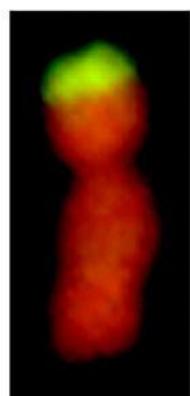
4V<sup>St</sup>



4DL·4V<sup>St</sup>S



rec64



rec213

Friebe et al. (2009) Crop Science



Photo by Jeanne Falk Jones - <http://kswheat.com/>

## Central Questions

- 1.) Will reference genomes of wild relatives increase the speed of mapping resistance genes?
- 2.) Can we rapidly genotype breeding lines harboring alien translocations?

# Tracking WSM1 from *Th. intermedium*



'added' *Th. intermedium* segments (4V<sup>st</sup>)

released introgression →

Shared Tags:

TA5614 / rec213

TA5613 / rec87

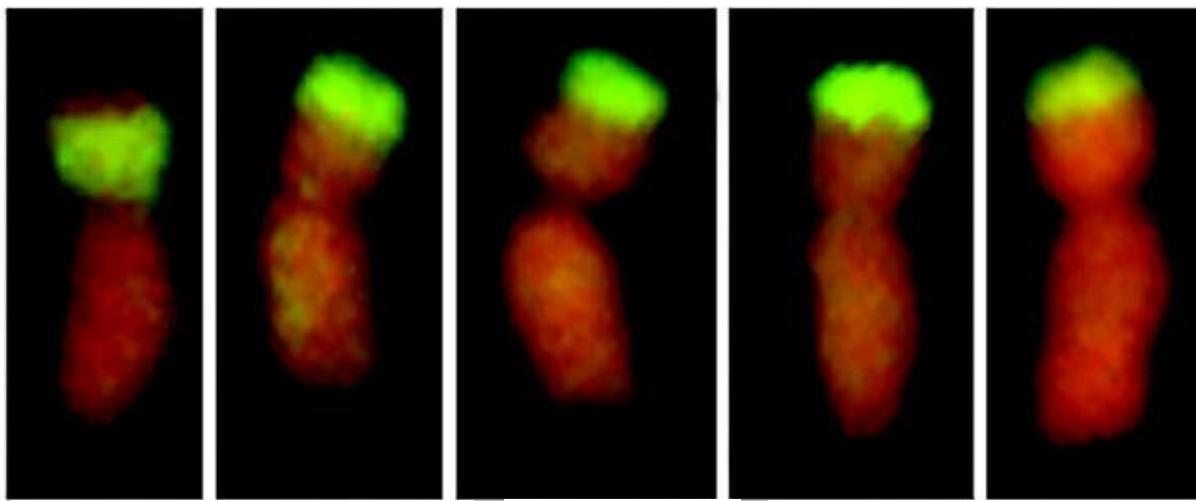
TA5612 / rec 64

TA5611 / rec45

TA5610 / rec36

45 tags (64mers) that map uniquely on 4Vs

smaller introgression →



location lines

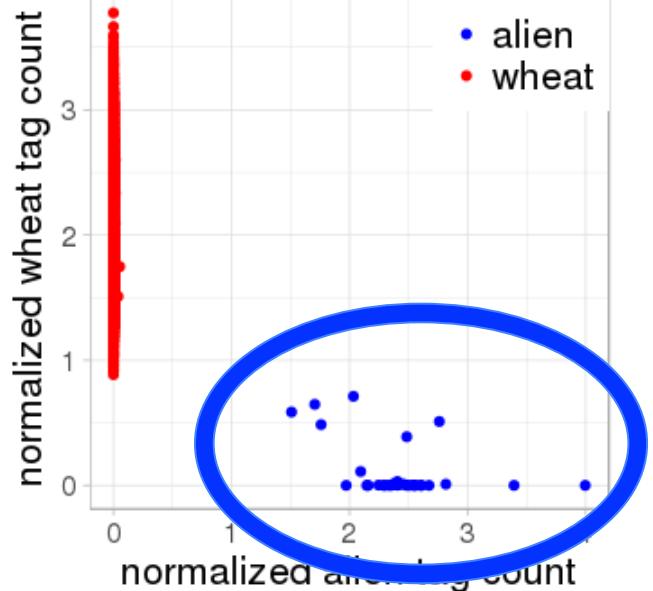
166 tags (64mers) that map uniquely on 4D

rec213 released into breeding program



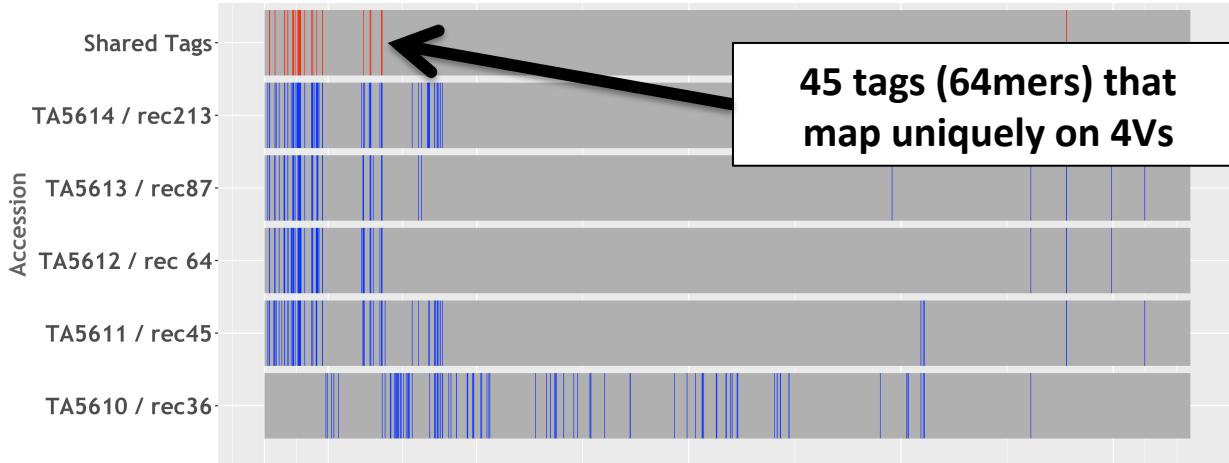
# Tracking WSM1 from *Th. intermedium*

Kansas - WSM1



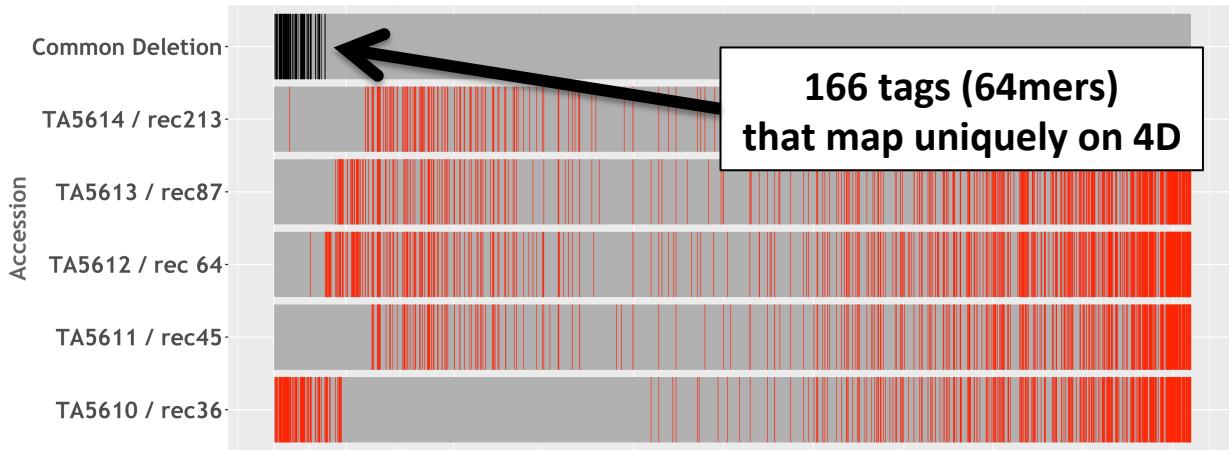
Collaboration with  
Liang Gao, KSU

**'added' *Th. intermedium* segments (4V<sup>st</sup>)**



45 tags (64mers) that  
map uniquely on 4Vs

**'deleted' wheat segments in translocation lines**

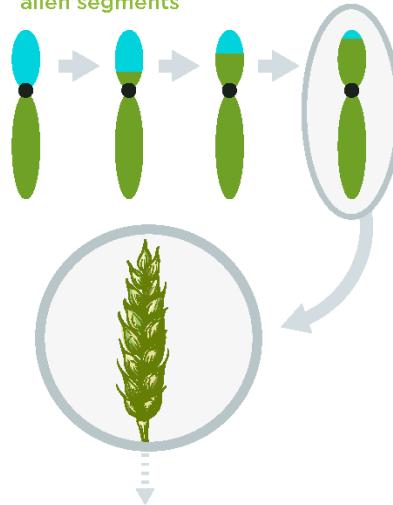


166 tags (64mers)  
that map uniquely on 4D

## UNDERSTANDING THE MOLECULAR BASIS OF VIRUS RESISTANCE TRANSFERRED FROM *TH. INTERMEDIUM* TO WHEAT

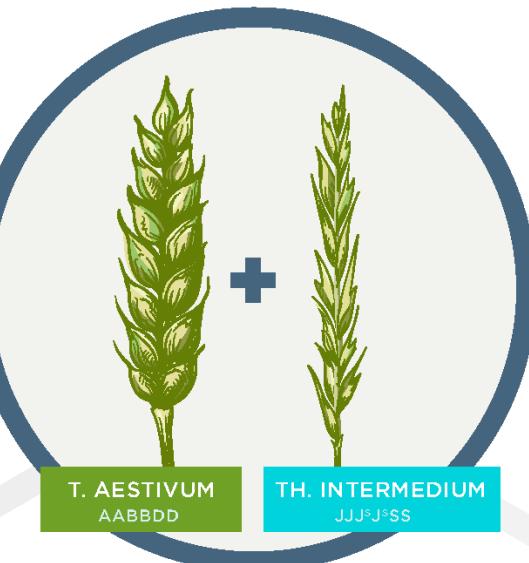
### OBJECTIVE 1

Directed Chromosome Engineering to shorten alien segments

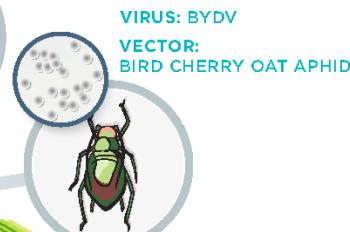
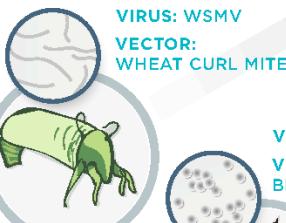
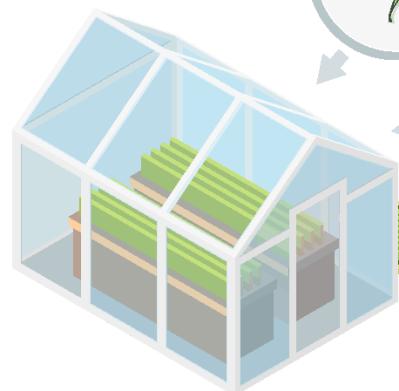


### OBJECTIVE 2

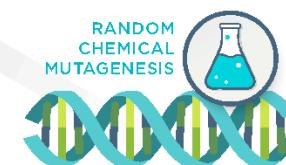
Sorting and sequencing of 3 alien translocations from *Th. intermedium*



Testing viral resistance and physiology of translocation isolines



### OBJECTIVE 3



Identify resistance genes and functional genomics confirmation

### OBJECTIVE 4



# Genomics-driven development of next generation grain crops

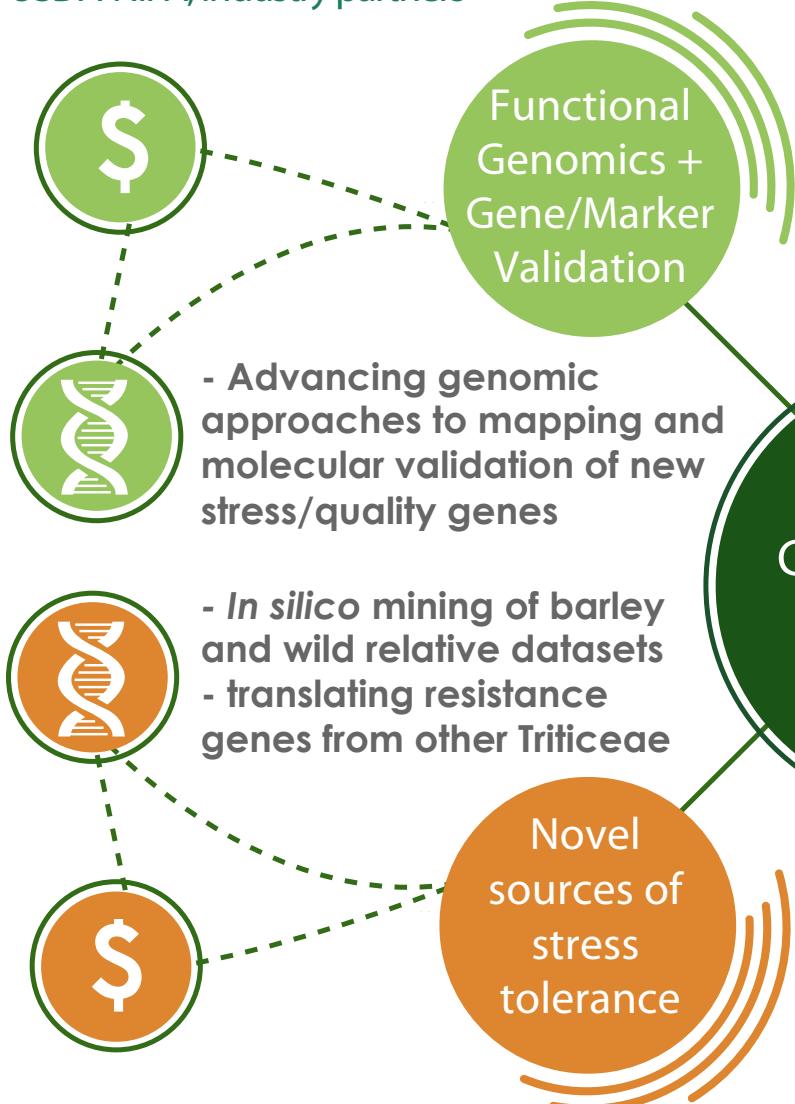
- 1.) Establishing genomic resources for a new grain crop
- 2.) Genome biology of *Thinopyrum*
- 3.) Genomics of Kernza neo-domestication
- 4.) Disease resistance from wheat relatives



helping farmers meet production challenges through delivering new traits/crops/systems to make agriculture more economically and environmentally resilient

# Vision for the Barley Genetics position

USDA-NIFA, Industry partners

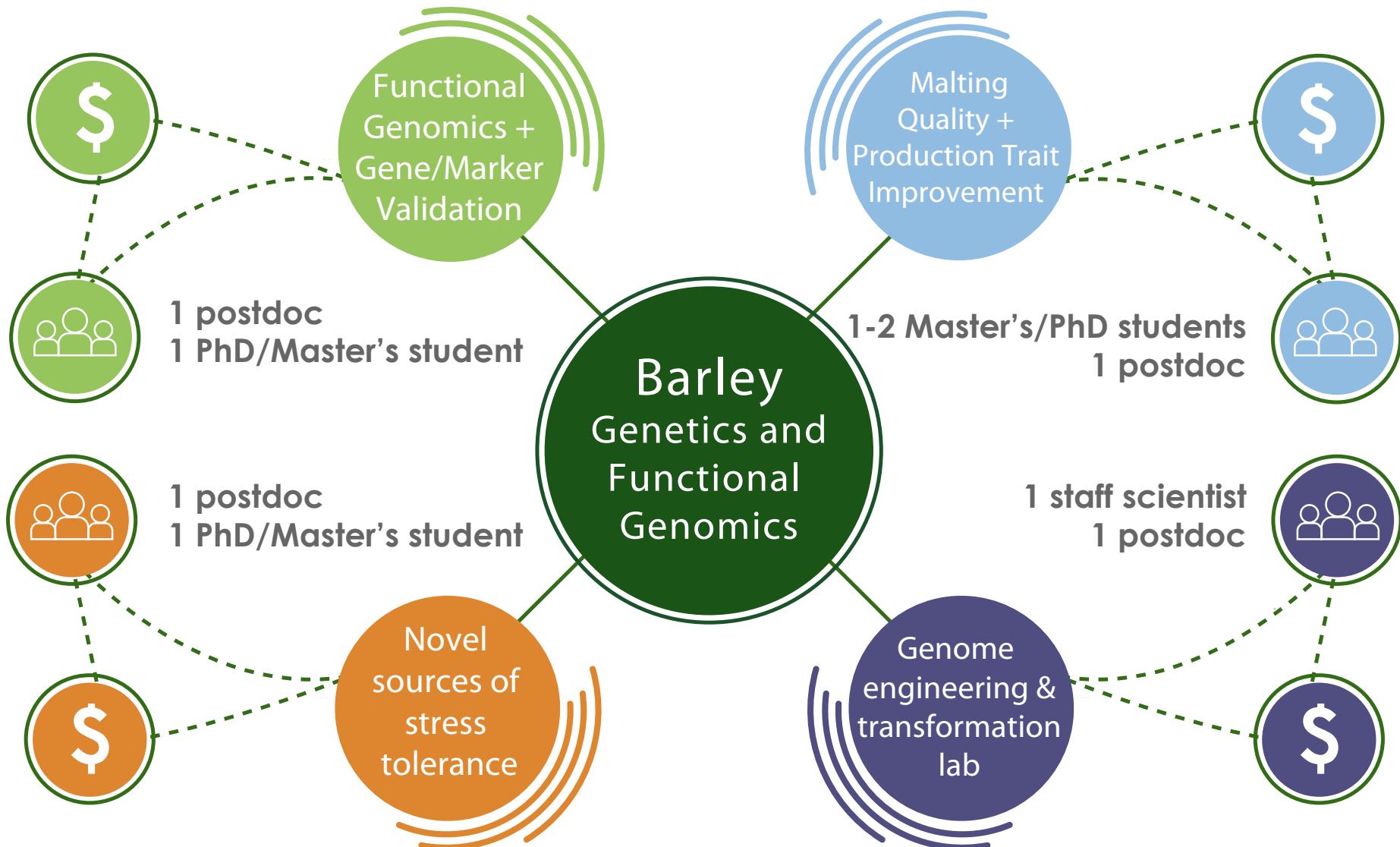


Industry partners, USDA-NIFA

National Science Foundation,  
Industry partners, USDA-NIFA

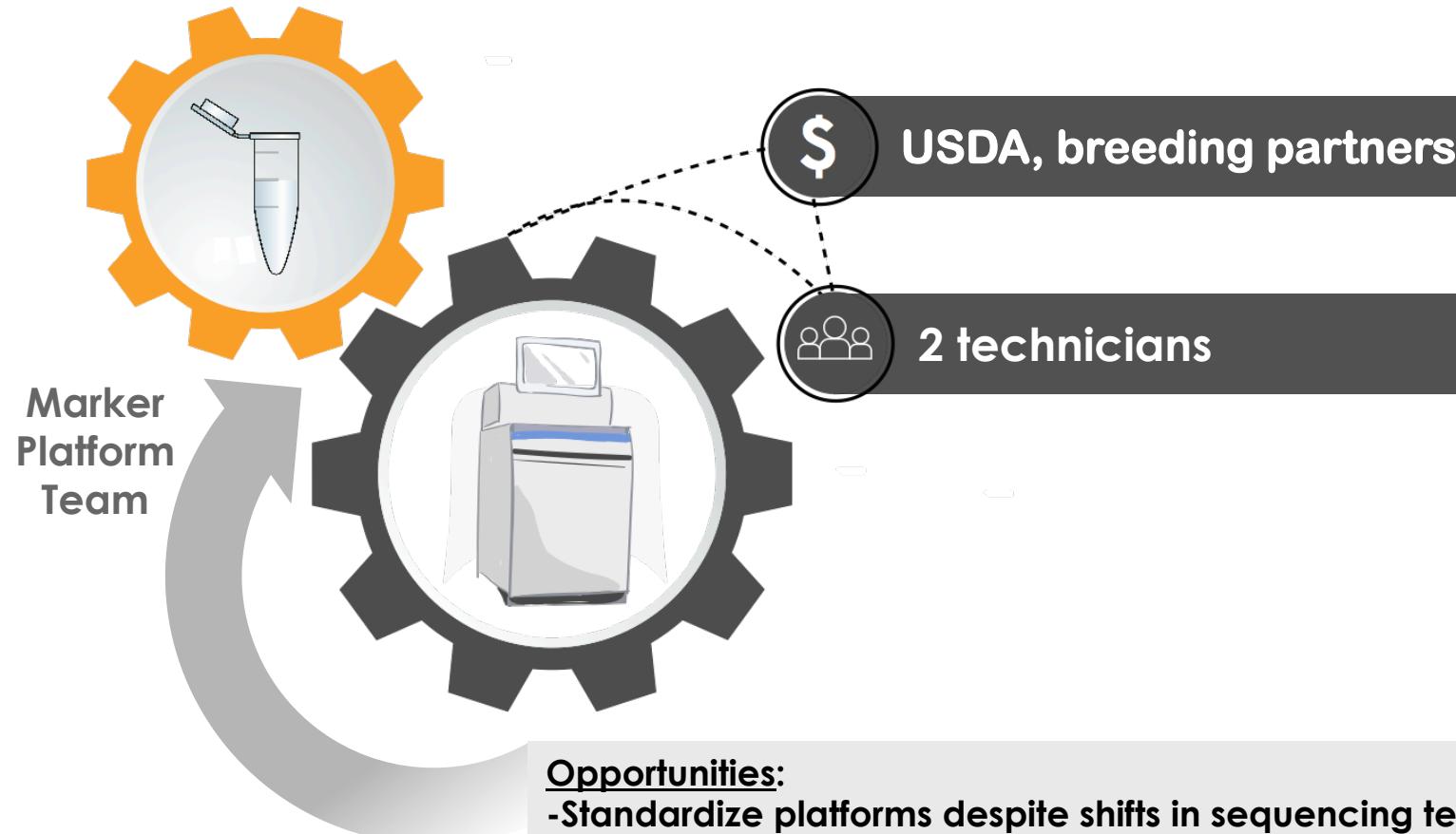
National Science Foundation,  
USDA-NIFA

# Vision for my Barley Genetics group



# Vision for the Genotyping Lab

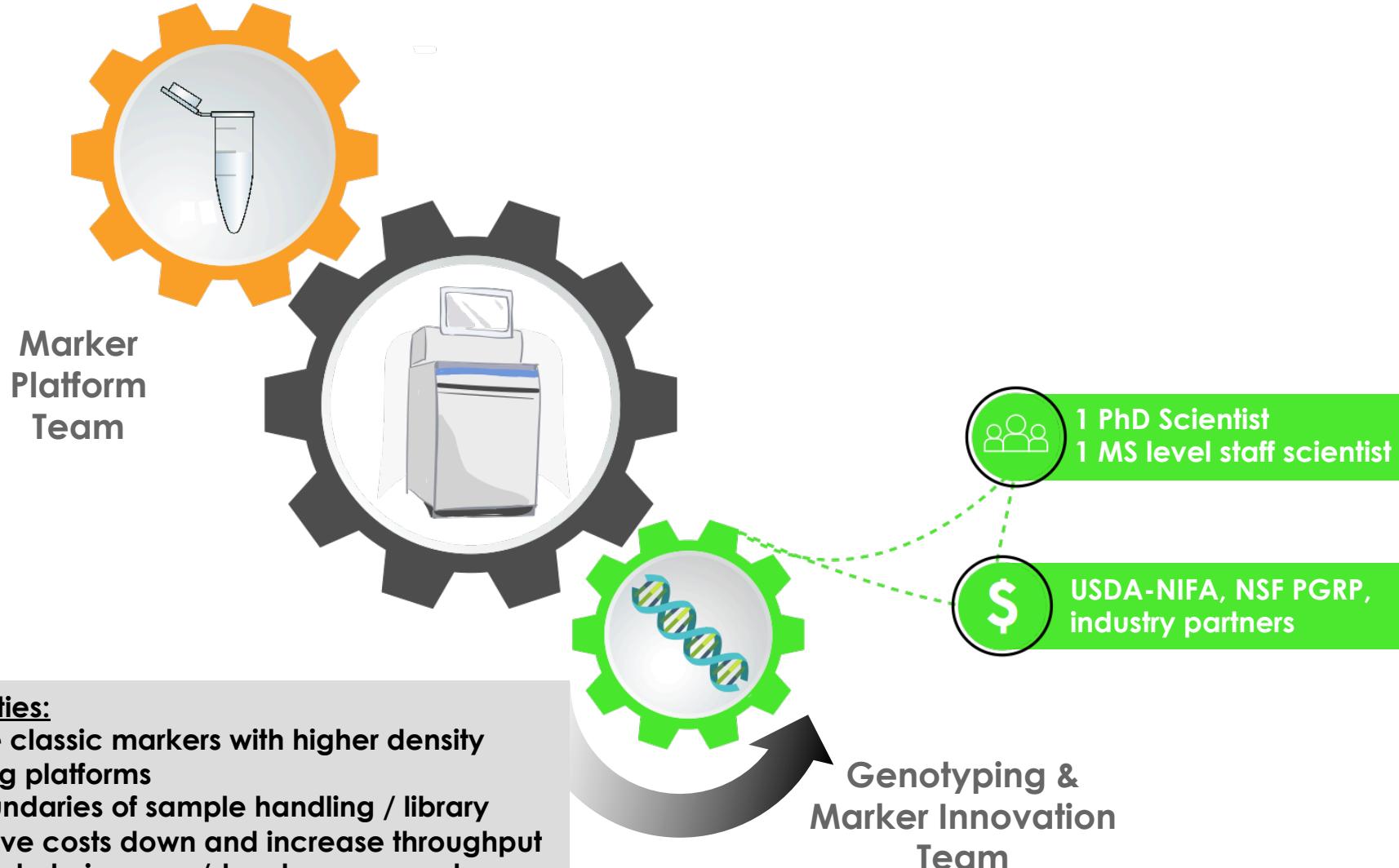
Genomics hub supporting breeders aiming to help growers meet production challenges and make small grain systems sustainable, stress tolerant, and economically resilient



## Opportunities:

- Standardize platforms despite shifts in sequencing tech
- Eliminate timing challenges for breeding programs
- Balancing cost / useful data point
- Increase mechanization / automation

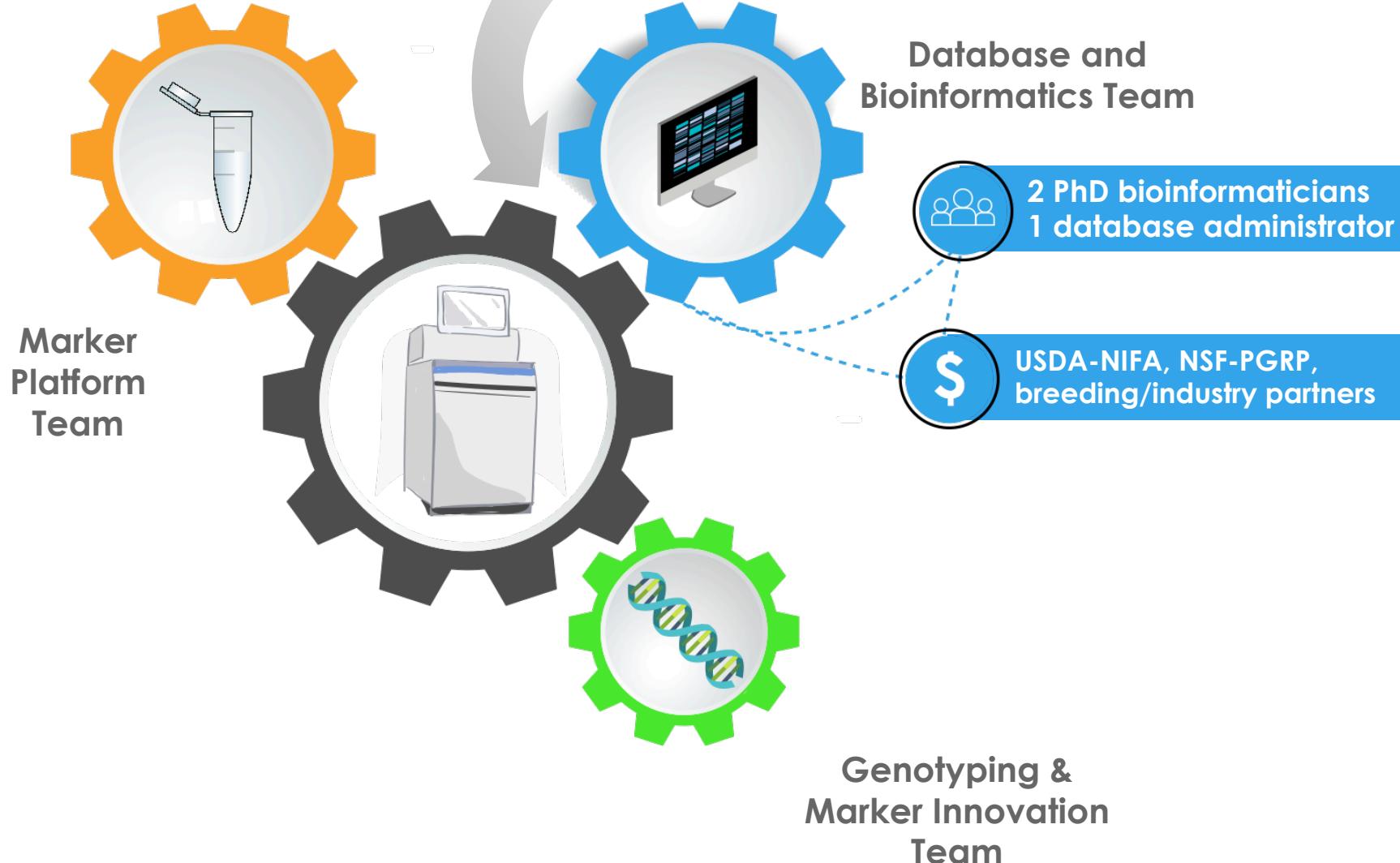
# Vision for the Genotyping Lab



# Vision for the Genotyping Lab

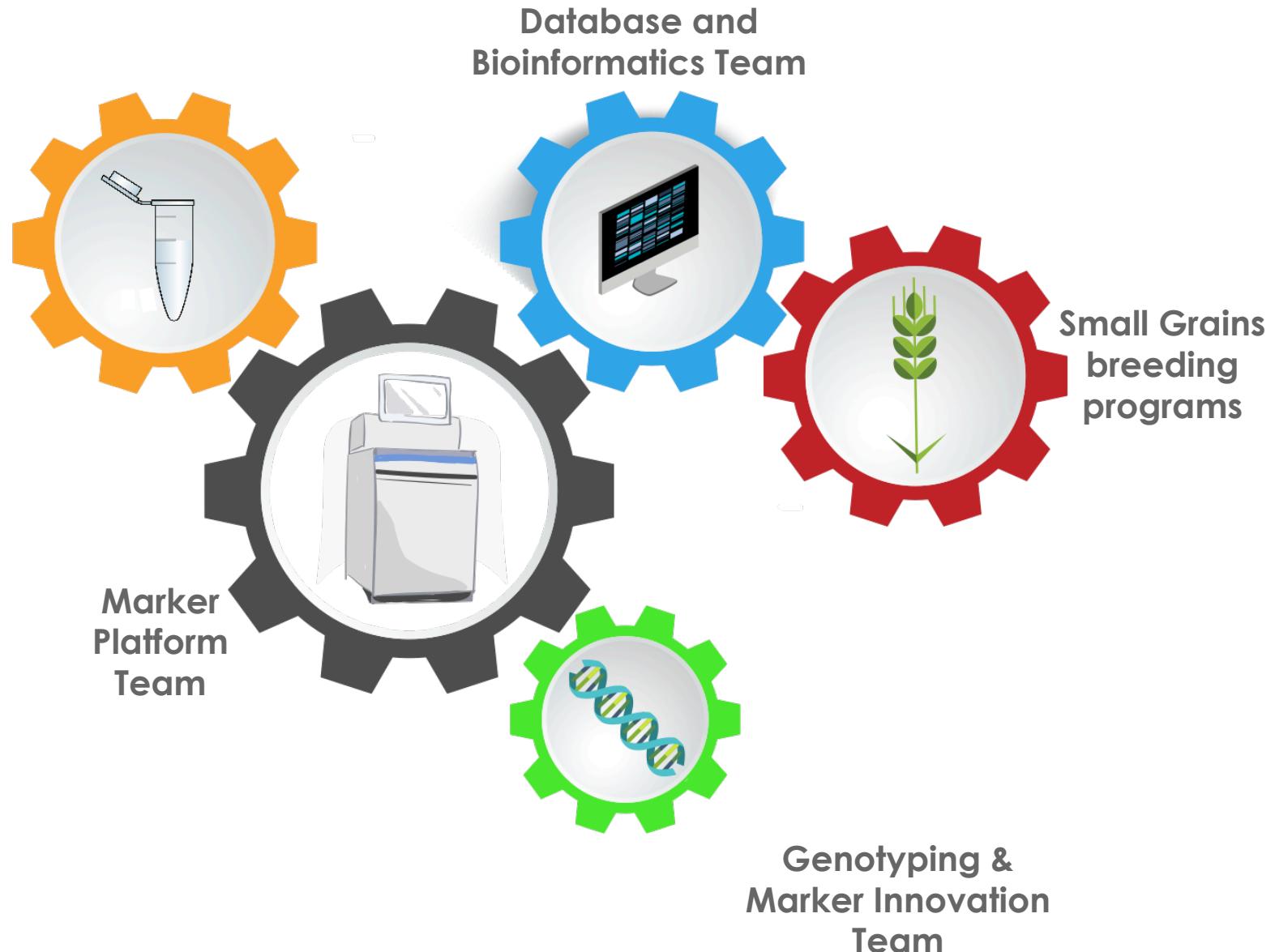
## Opportunities:

- Solve back-end informatics bottlenecks for breeding partners
- Develop methods/database haplotype tracking
- Standardize marker platforms, deliver only desired/useful data



# Vision for the Genotyping Lab

Genomics hub supporting breeders aiming to help growers meet production challenges and make small grain systems sustainable, stress tolerant, and economically resilient



Joint Genome Institute,  
Perennial Agriculture Project

The Land Institute,  
Perennial Agriculture Project



- Establishing gold-standard genomic resources
- Deciphering subgenome progenitors

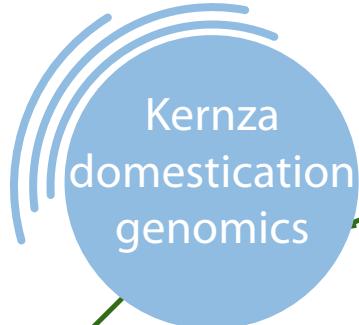


Disease  
resistance  
from wild  
relatives

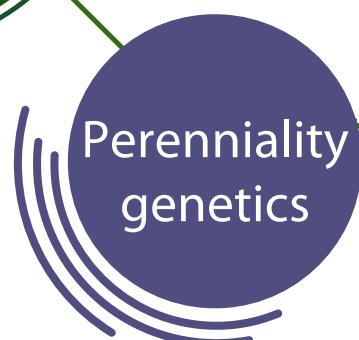
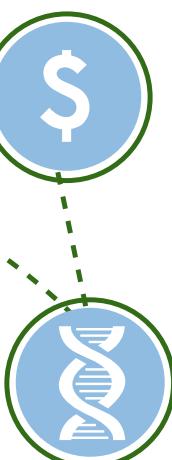
- Establishing high quality reference assemblies and mapping resources for R gene identification
- Directed chromosome engineering to shorten alien segments
- Semi-automated tracking of alien translocations



**Kevin Dorn  
plant molecular  
genetics  
and genomics**



- Population genomics examining selection sweeps
- Dissecting the genetic basis of major domestication traits
- Functional genomics of domestication genes



USDA-NIFA, Joint Genome Institute,  
Perennial Agriculture Project

National Science Foundation,  
Wheat Genetics Resource Center



**Postdoc Projects**

# Acknowledgements

## Kansas State University

Jesse Poland      Bernd Friebe  
Liang Gao           Dal-Hoe Koo  
Traci Kantarski  
Jared Crain  
Mark Lucas

## The Land Institute

Lee DeHaan  
Shuwen Wang  
Kathryn Turner

## HudsonAlpha

Jeremy Schmutz  
Jane Grimwood  
Chris Plott  
Jerry Jenkins

## USDA – Logan, Utah

Steve Larson

## University of Minnesota

Jim Anderson  
Xiaofei Zhang  
Kayla Altendorf  
Don Wyse  
David Marks

## JGI

Kerrie Barry  
John Vogel  
Lifeng Liu

## IEB

Jaroslav Dolezel

## Ag-Canada

Jamie Larson  
Andre Laroche

## Funding Sources

Department of Energy –  
Joint Genomes Institute Community  
Science Program

The Land Institute  
Perennial Agriculture Project &  
Malone Foundation

Minnesota Dept. of Agriculture

UMN Forever Green Initiative

USDA-NIFA Postdoctoral Fellowship



United States  
Department of  
Agriculture

National Institute  
of Food and  
Agriculture



Forever  
Green



JOINT GENOME INSTITUTE  
DEPARTMENT OF ENERGY



HUDSONALPHA  
INSTITUTE FOR BIOTECHNOLOGY

