Small Grains
Genotyping

Wheat CAP PI meeting

Sept 30, 2002

2022 Wheat CAP Genotyping

Western Region (2 T3 projects)			
2688	GBS	WSU	Carter, Pumphrey, Campbell (in collaboration with NC lab)
384	GBMAS	UI	Chen
96	GBMAS	UCD	Dubcovsky
192	SPET, Agriplex	UCD	Dubcovsky (NC lab)
Central Region			
500	MRASeq	KSU	Zhang
500	MRASeq	ARS-KS	Guttieri
2000	70 KASP, STS	Regional	Regional
6372	GBS	CSU	Mason (in-house)
Northern Region			
3500	Illumina 3K	Regional	Regional
384	Illumina 3K	Montana	Cook
1623	GBS	UMN	Anderson (in-house)
2000	8 KASP	UMN	Anderson
1100	Illumina 90K	USDA, Fargo	Faris, Justin
192	Illumina 90K	NDSU	Green, Andrew
288	AVR, 25K	Montana	Cook, Jason
18240	KASP	NDSU/USDA	NDSU/USDA-ARS, fargo
Eastern Region			
2784, 2880	GBS	MSU	Olson (in-house)
1344	GBS	U IL	Rutkoski
384	GBS	Cornell	Sorrells
1056	GBS	VA Tech	Santantonio
288	GBS	ARS-NC	DeWitt
256	GBS	Regional	Training populations
1344	11 KASP	ARS-NC	DeWitt
960	Agriplex	Regional	Regional
960	28 KASP	Regional	Regional
2112	SPET	Regional	Mid-density testing

Targeted Genotyping Options

Agriplex – *available to this group*

PlexSeq primer pool 216 targets, ~150 loci; $5 sample
Includes Rht, Ppd, Vrn, quality, disease, insect resistance genes
They are working with CIMMYT for “PlexSeq plus” (50 bp read reported)

Illumina arrays -

Northern Lab developed 3,000 SNP wheat array – $14 sample
Currently testing broad germplasm
Includes many of the trait related targets

Single Primer Extension (Allegro)

Eastern lab has second generation design ~3,000 markers
Includes trait related markers

ThermoFisher AgriSeq (amplicon sequencing) – in design phase

Four labs are investing in development of 5,000 SNP pool
Each region has submitted 1500 selected targets (intersect with other platforms)
Will include trait related targets
Working on unified system for SNP naming
ThermoFisher will do the genotyping with this pool for 3 years at $10/sample

SPET (Allegro) EW-Design2 EXO-CTRL study

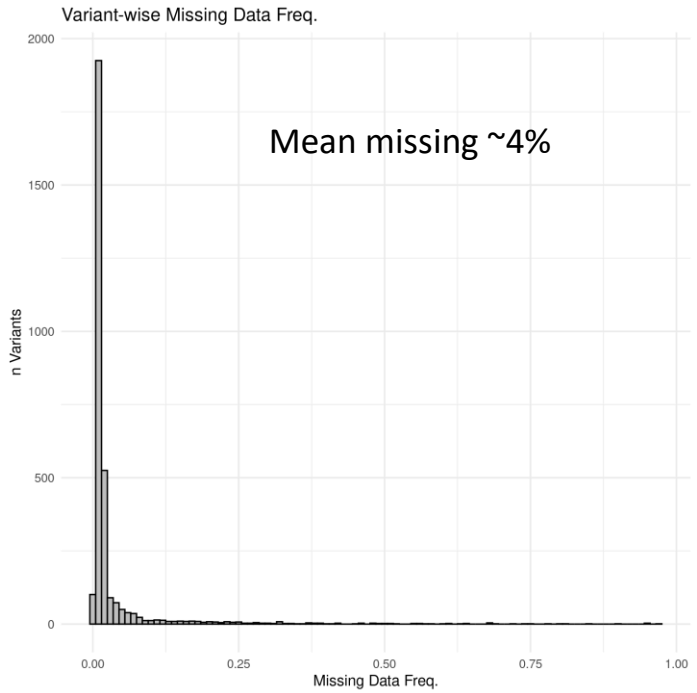
- ~ 180 targets used in routine screening eastern germplasm/uniform nurseries
- ~ 150 targets of interest from collaborators at CIMMYT, HWWGRU, NCSGGL
- currently determining concordance between Allegro data and historical Agriplex/KASP data

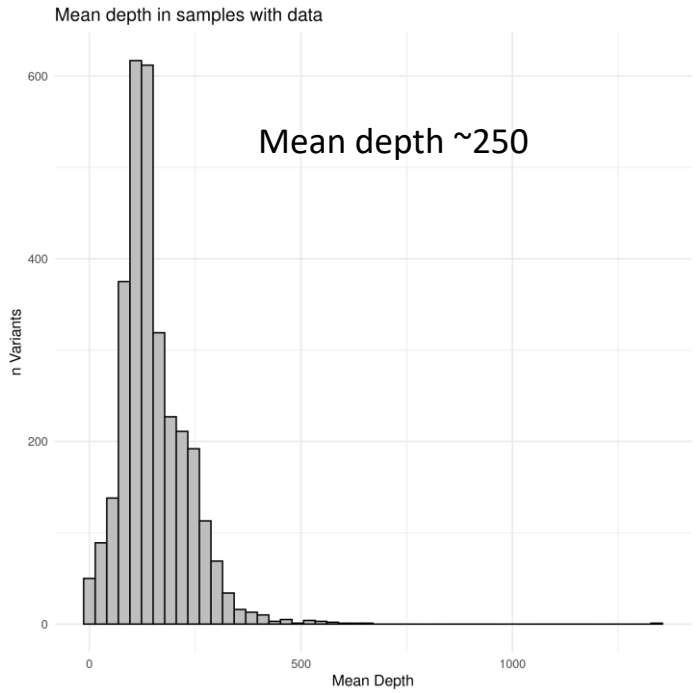
- 190 samples – mix of exome capture samples and known controls for various traits
- NovaSeq 6000 SP single end 100 cycle
- align to composite reference (CS refseq v1 + contig fastas)
- variant calling performed with Bcftools

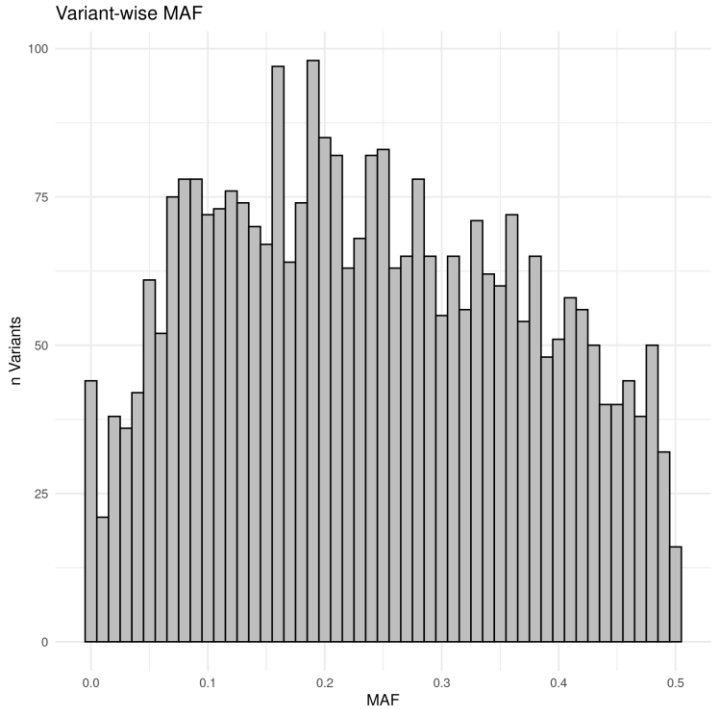
	aligned to refseq v1 (SNPs/indels <10bp)	aligned to contigs (indels >10bp/alien introgressions)
targets in design	**297**	**32**
targets reported in VCF	272 (92%)	24 (75%)
reported targets with mean per sample DP > 50	248 **(91%)**	18 **(56%)**
reported targets polymorphic	220 (80%)	18 (56%)
reported targets multiallelic	5 (2%)	0

SPET (Allegro)
EW-Design2

5000 probes, 3680 targets
2845 biallelic SNP, MAF >0.05
84% success rate







1344 eastern wheat samples + 192 UC Davis

Reagents + supplies - $5.39 (0.4 vol rxn)
NovaSeq S1 (2 lanes) - ***1536 samples, $3.54/sample*** *2304 samples, $2.35/sample* *3072 sample, $1.77/sample*

Mid-Density genotyping TAKE-AWAYS

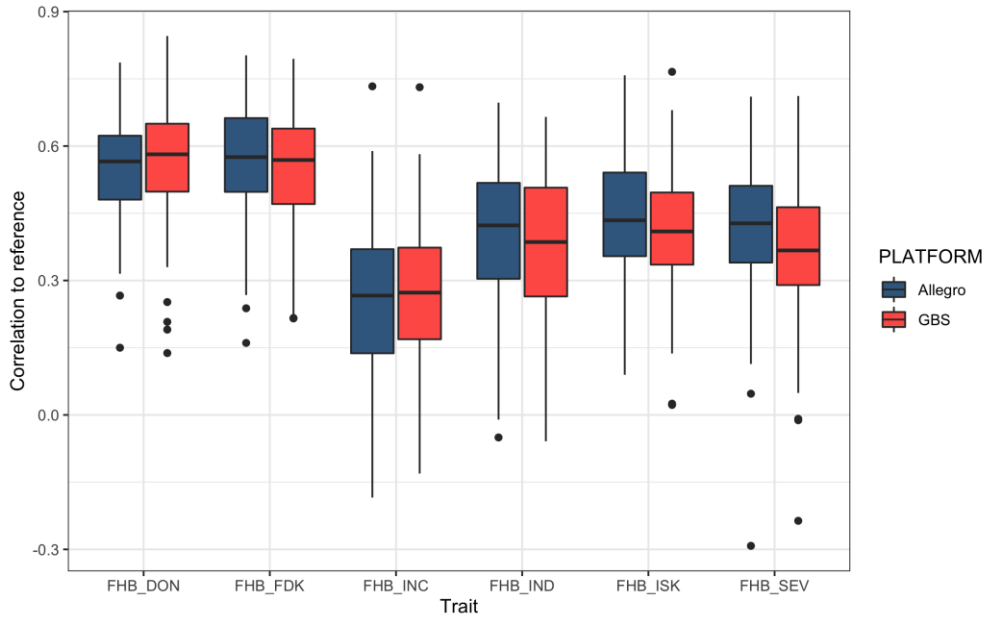
Promising prediction accuracy from relatively small numbers of markers
1000 SNP (EW Design1) versus 6000 GBS markers

Promising imputations accuracy with PHG

Promising for broader US wheat SNP panel
EW-Design2 included 450 SNP selected by Northern lab
and from 450 GBS that passed MAF filters

Success rate with indels >10 bp or alien segments is only about 50%
Target nearby SNP in high LD as back up

Cross validations for FHB resistance



Mid-Density SNP calling pipeline in place –
Genome wide SNP: align to whole genome and extract SNP in target regions (Bowtie2, Bcf tools)
KIMs: targets not in reference genome can be aligned to “accessory” fasta
requires intervention for SNP calling