FORAGE AND RANGE RESEARCH

Logan, UT

Evaluation of ‘Allegro’ targeted DNA sequencing to accelerate domestication and improvement of Kernza...

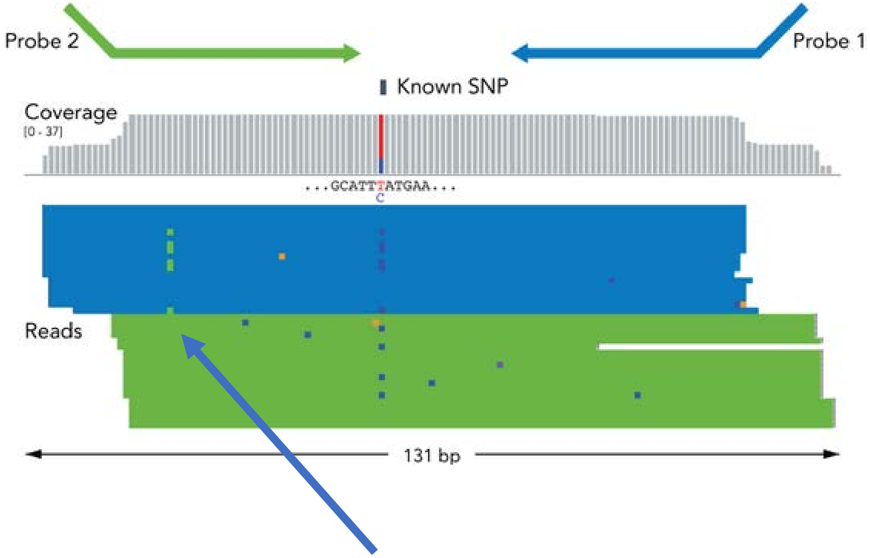
Steve Larson

Pilot experiment

4,200 non-synonymous mutations in 1,500 genes (400 candidate genes)

800 GWAS SNPs (TLI and UMN)

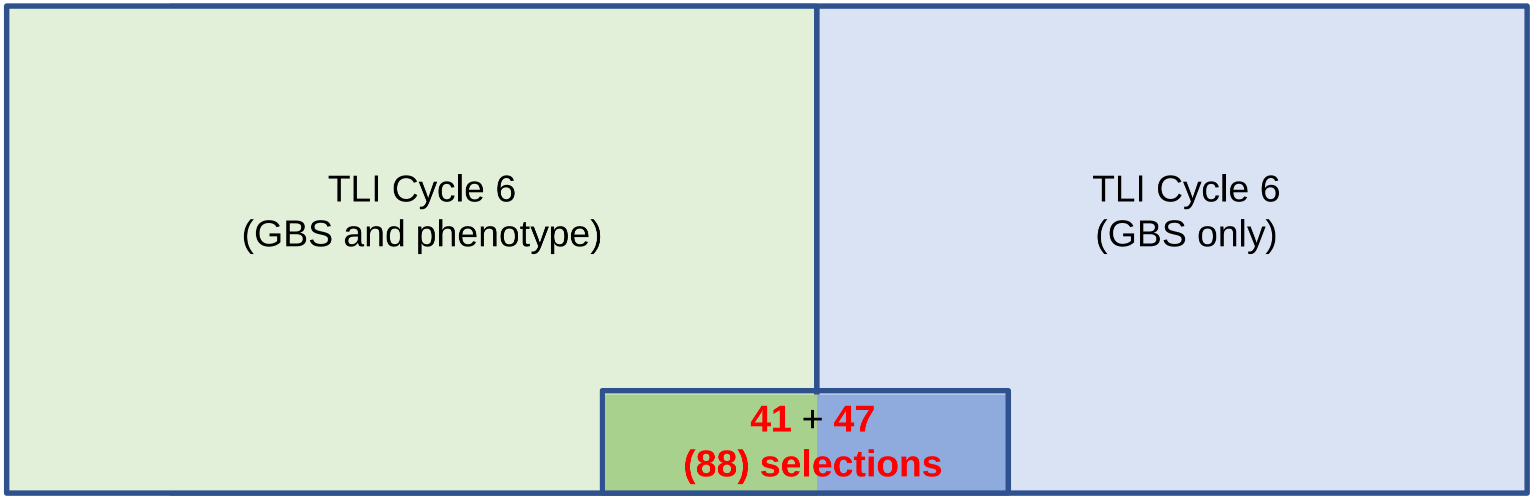
Probes designed from the Kernza genome reference sequence with resequencing data from four plants (C3_3471 and POPSEQ parents)



Potential to call other "non-target" variants

# Samples	# targets	Cost (USD)
96	**5,000**	**1,488**
96	10,000	1,728
96	50,000	2,064
96	100,000	2,400
192	5,000	2,976
192	10,000	3,456
192	50,000	4,128
192	100,000	4,800
384	5,000	5,952
384	10,000	6,912
384	50,000	8,256
384	100,000	9,600

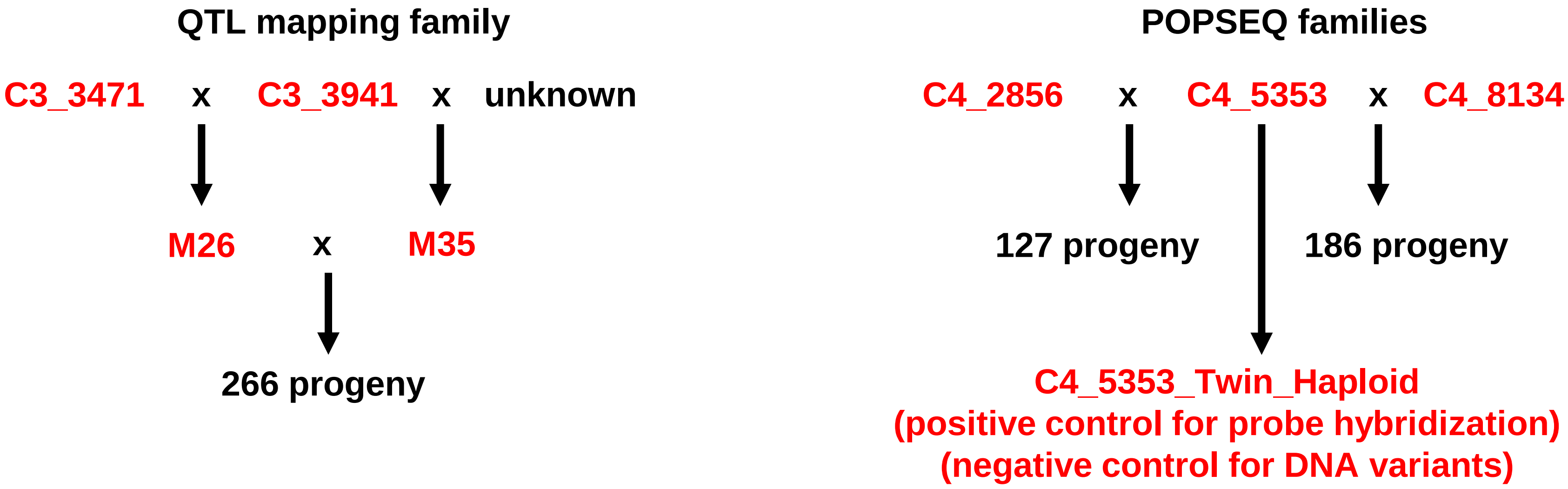
96 test plants



TLI Cycle 6
(GBS and phenotype)

TLI Cycle 6
(GBS only)

41 + 47
(88) selections



QTL mapping family

C3_3471 x C3_3941 x unknown

M26 x M35

266 progeny

POPSEQ families

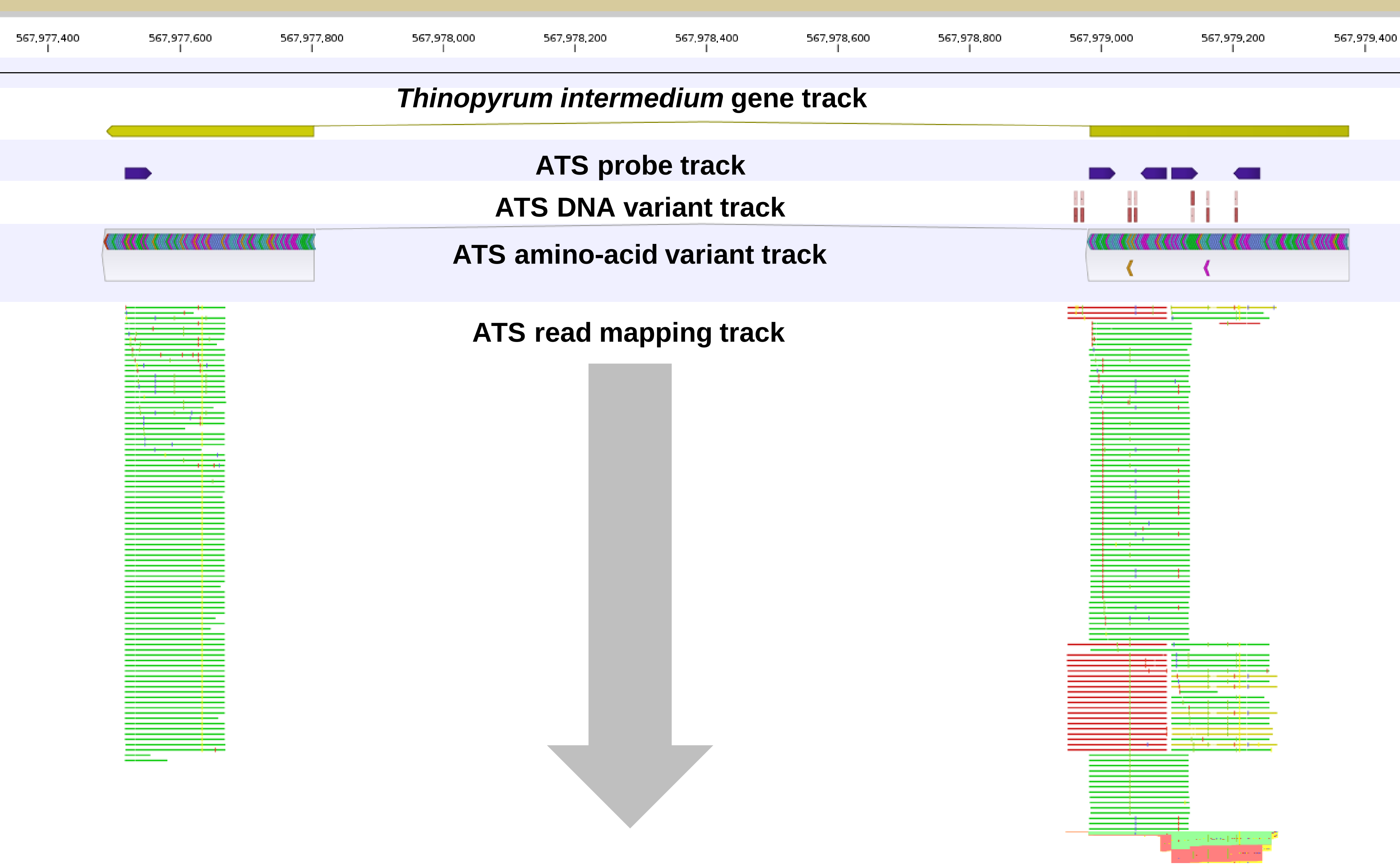
C4_2856 x C4_5353 x C4_8134

127 progeny

186 progeny

C4_5353_Twin_Haploid
(positive control for probe hybridization)
(negative control for DNA variants)

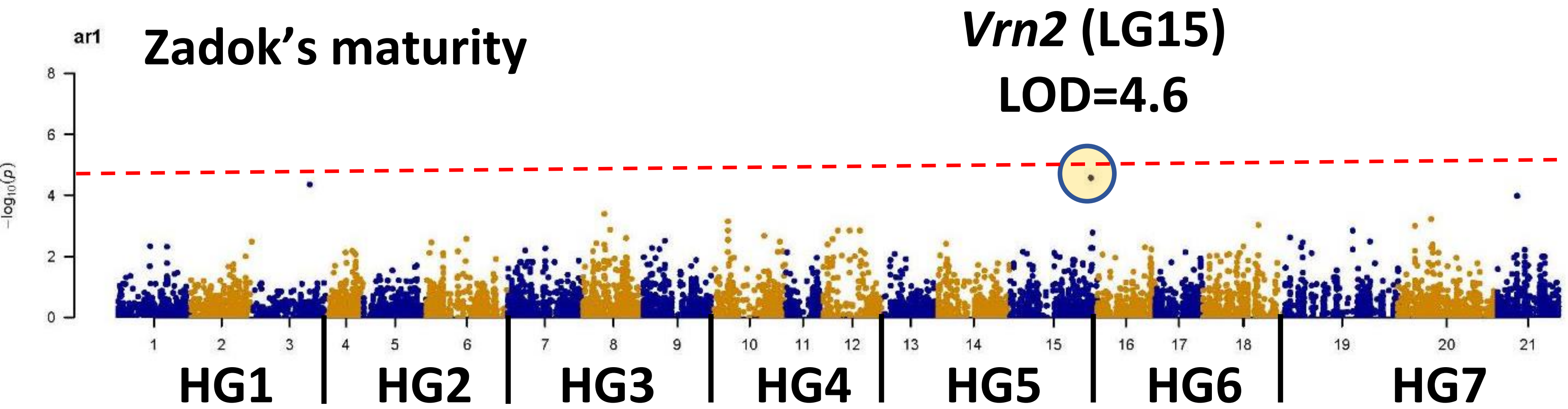
Example of DNA read mappings (1plant) on the Kernza ortholog of *Vernalization 2* (*Vrn2*)



Calling and filtering of variants

- Minimum coverage of 8 reads with at least 2 variants to call SNP genotype (each plant)
- 99,000 variants present >10% or <90% plants
- 80,000 variants not found in the haploid reference control
- 10,845 biallelic variants, NO MISSING DATA

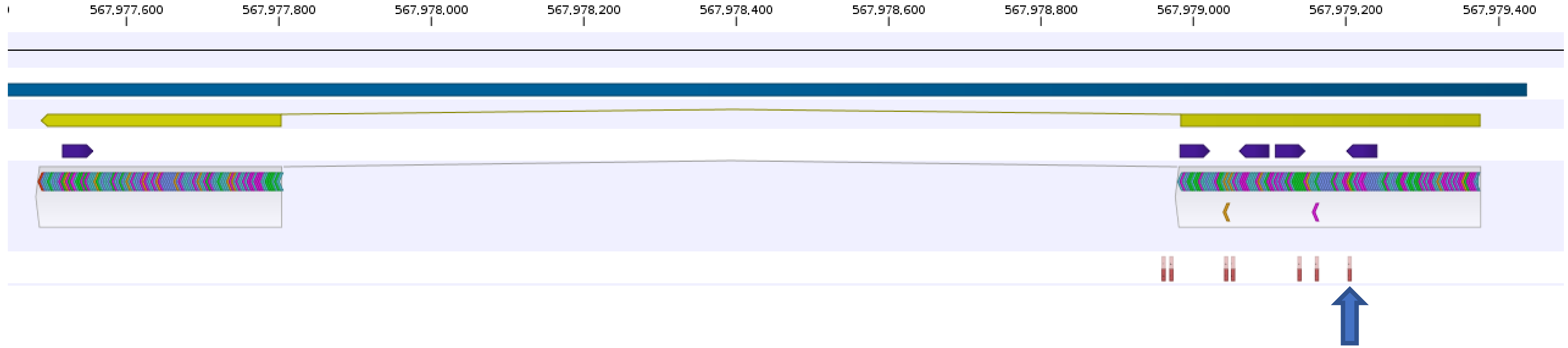
Log-odds from GWAS for Zadok's maturity: 41 plants from TLI cycle 6



Zadok's maturity

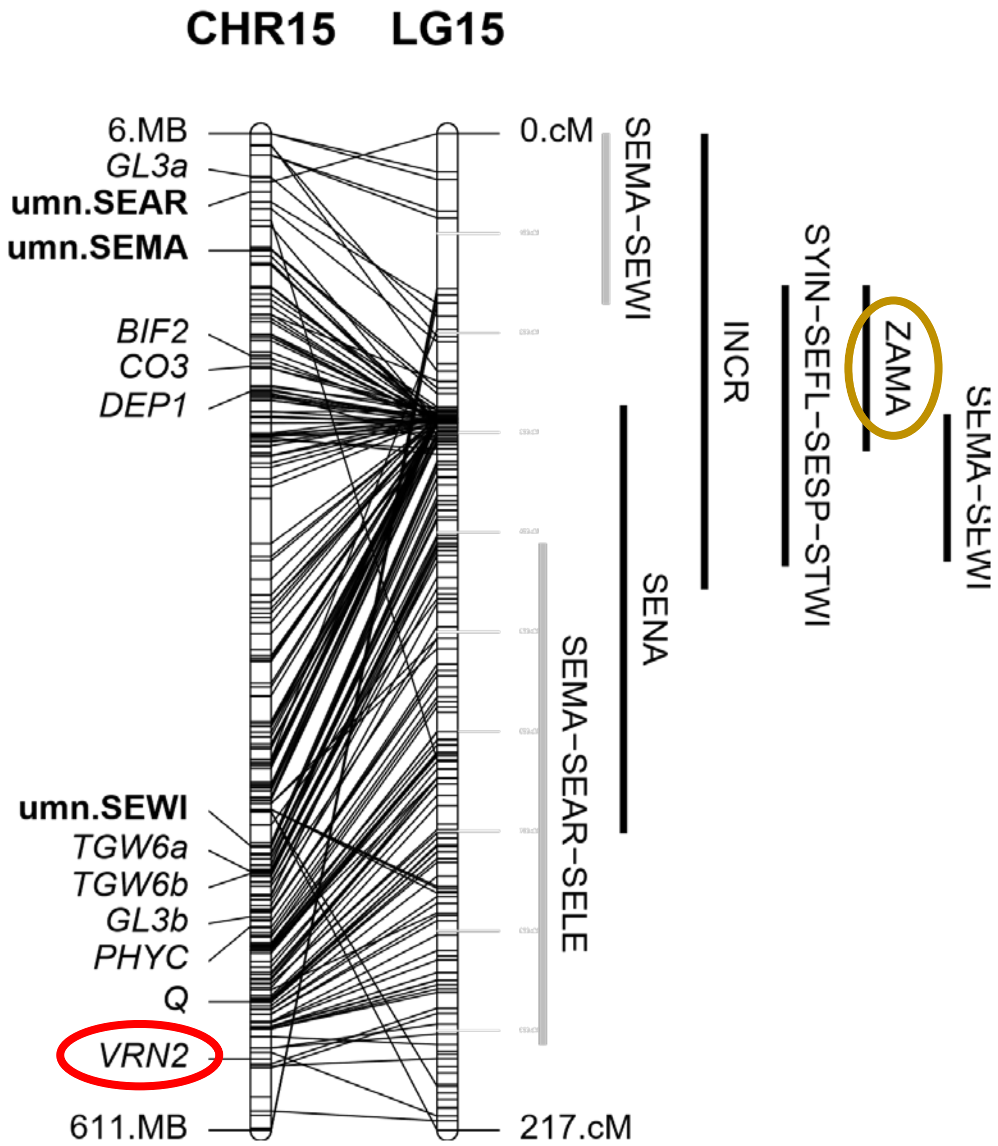
***Vrn2* (LG15)**
LOD=4.6

HG1 **HG2** **HG3** **HG4** **HG5** **HG6** **HG7**



off-target synonymous variant

A Zadok's maturity QTL on LG15 (M26xM35) is syntenous with *Vrn2* on Chr15



No significant GBS markers in cycle 6 GWAS

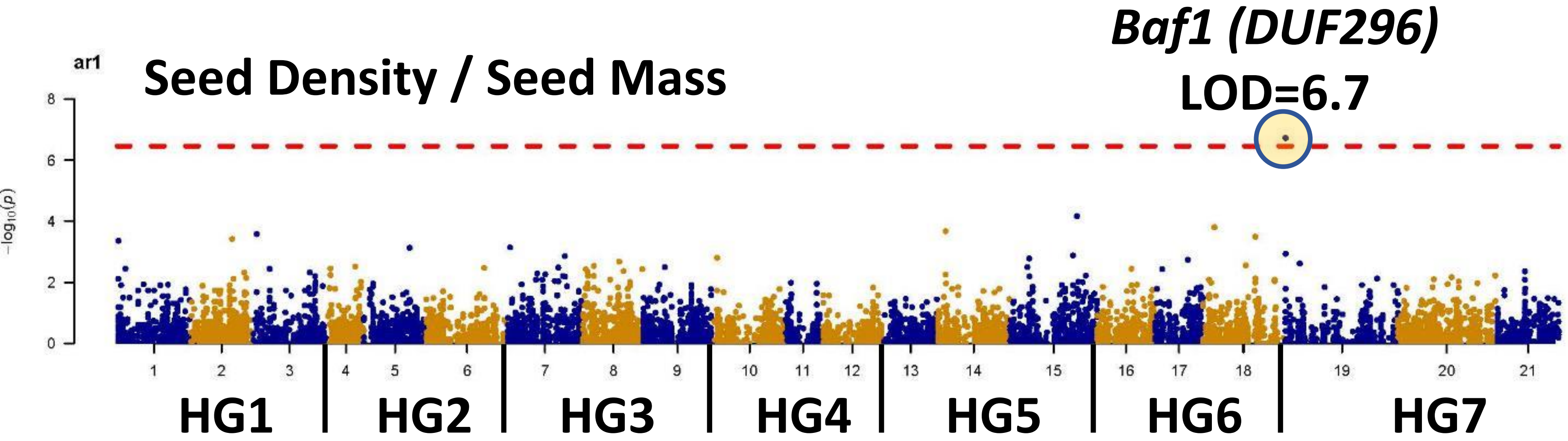
Variant calls from *VRN2* gene show M26 and M36 are monomorphic for the mutation of interest

Variant calls from POPSEQ (C4_5353 x C4_2856)

Variant calls from POPSEQ (C4_5353 x C4_81346)

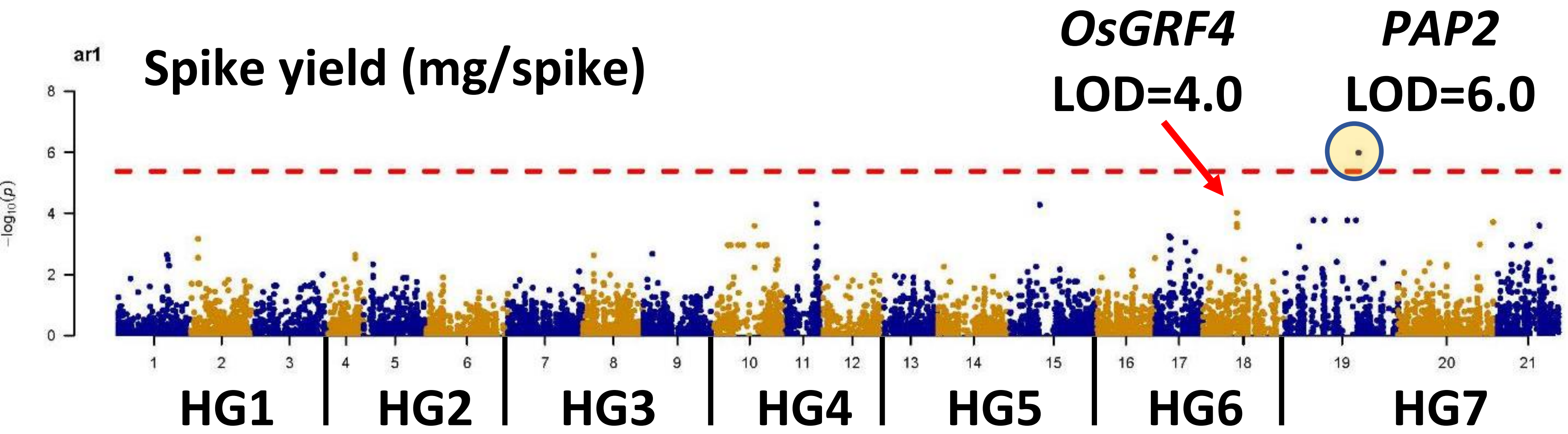
Variant calls from ALLEGRO (parents of mapping & TLI cycle-7)

Most significant GWAS hits



Seed Density / Seed Mass

Baf1 (DUF296)
LOD=6.7



Spike yield (mg/spike)

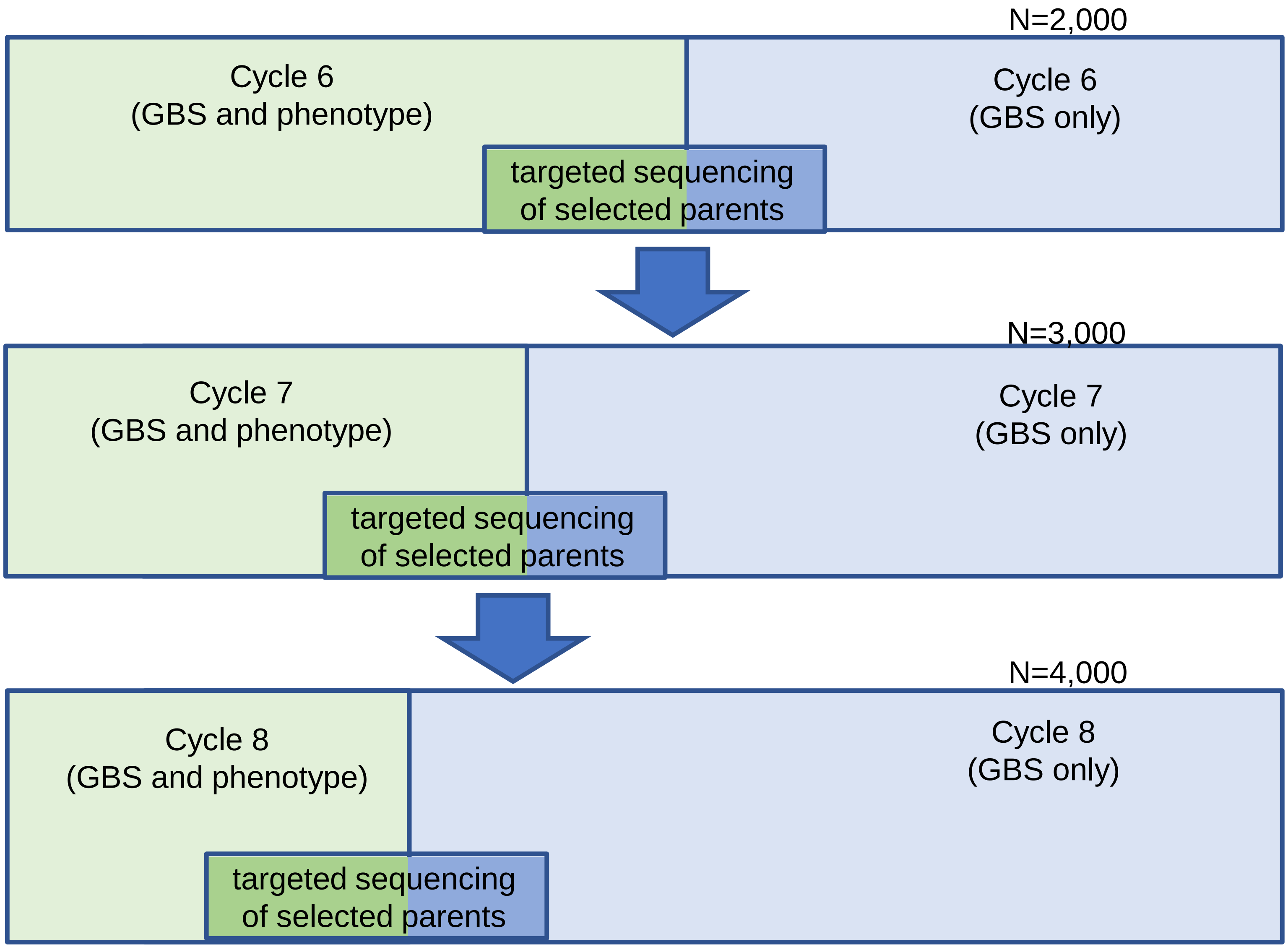
OsGRF4
LOD=4.0

PAP2
LOD=6.0

Some important points

- This was a pilot test of 5,000 probes on 41 plants that could be used for GWAS
- We obtained 80,000 potentially useful SNPs including nearly 11,000 SNPs with no missing data
- Observed potentially meaningful GWAS hits
- Technique can scale up to 100,000 probes that may detect 200K – 1.6M or more SNPs
- The number of probes scales up more efficiently (cheaply) than the number of plants…so may not be feasible for thousands of plants

Application of targeted sequencing for breeding & genetics by imputation from parents to progeny



N=2,000

Cycle 6
(GBS and phenotype)

Cycle 6
(GBS only)

targeted sequencing
of selected parents

N=3,000

Cycle 7
(GBS and phenotype)

Cycle 7
(GBS only)

targeted sequencing
of selected parents

N=4,000

Cycle 8
(GBS and phenotype)

Cycle 8
(GBS only)

targeted sequencing
of selected parents

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