Using the Wheat TILLING Database

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PAG 2015 – Computer Demo

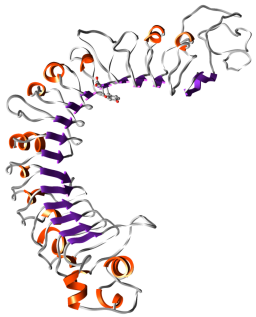
UC Davis – Jorge Dubcovsky Wheat Genetics Lab
Plant Sciences Department

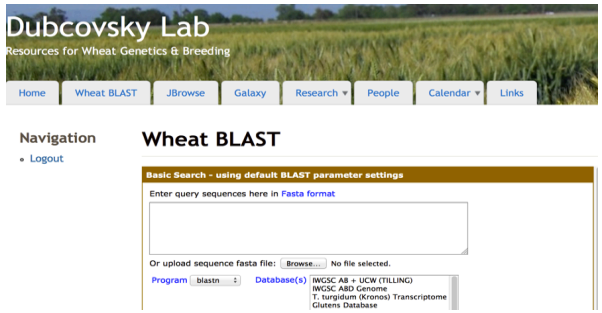
Outline

- Why was the tool made?
- What is the Wheat TILLING Resource?
- How was the tool built?
- Where do I access the data?

Why?

- Functional Genomics
  - Resource for Reverse Genetics
    - isolating alleles of interest in any given gene





ATGGTGTCTCATA
GCA**T**TACTGATGC
TAGCTAGCTGATC
GATTGTGGGCTCG
CTAGCTCGTCCGT

- Accelerates research projects by obtaining samples with knocked-out gene function

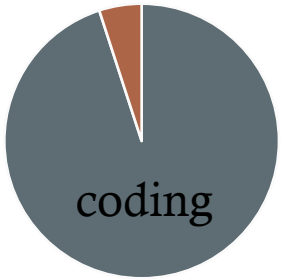
What is the tetraploid Wheat TILLING resource?

- TILLING Population
  - Use of EMS (Ethyl methanesulfanate) to treat lines
    - Mutagenesis on the organism
    - Point mutations C->T and G->A
- Traditional TILLING (Targeting Induced Local Lesions IN Genomes)
  - Targeted region or gene
    - PCR based screening a single target in a population at a time
- High-throughput sequencing of TILLING lines
  - Exome capture
    - Reduces the tetraploid wheat genome from ~12Gb to ~112Mbp
  - Screening all targets in the exome capture at once

Most of the wheat genome is non-coding and repetitive

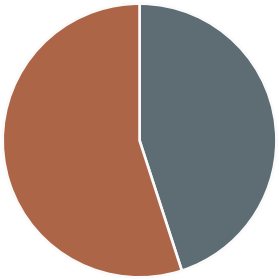
S.cerevisiae

non-coding
repetitive



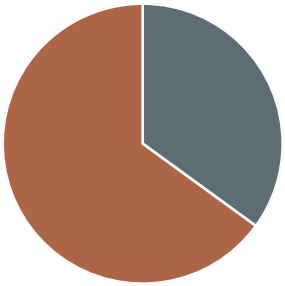
12 Mb

A. thaliana



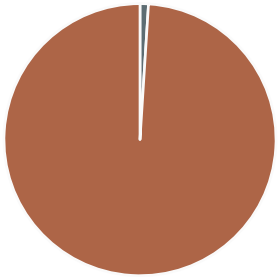
135 Mb
10x

D. melanogaster



180 Mb
15x

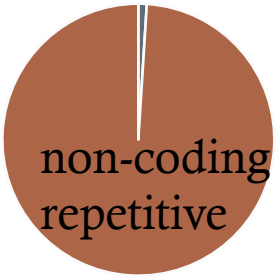
H. sapiens



3.2 Gb
260x

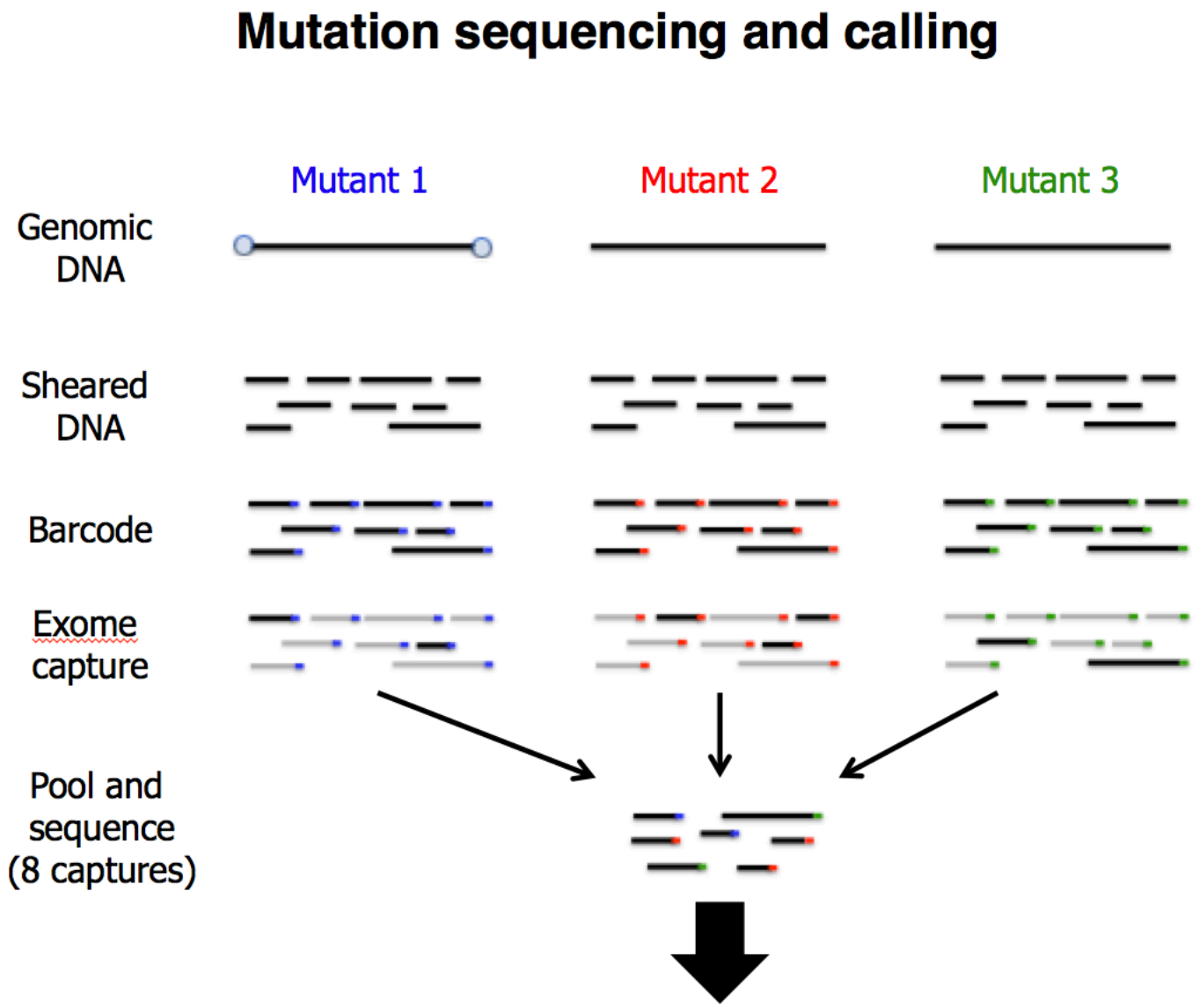
T. turgidum

1-2% coding



12 Gb
1000x

How?


Mutation sequencing and calling
Mutant 1
Mutant 2
Mutant 3
Genomic DNA
Sheared DNA
Barcode
Exome capture
Pool and sequence (8 captures)

MAPS – (Mutation and Polymorphism Survey)

Noise:

Varietal SNP

Paralog/Homeolog SNP

Sequencing/PCR error

Off target repeats

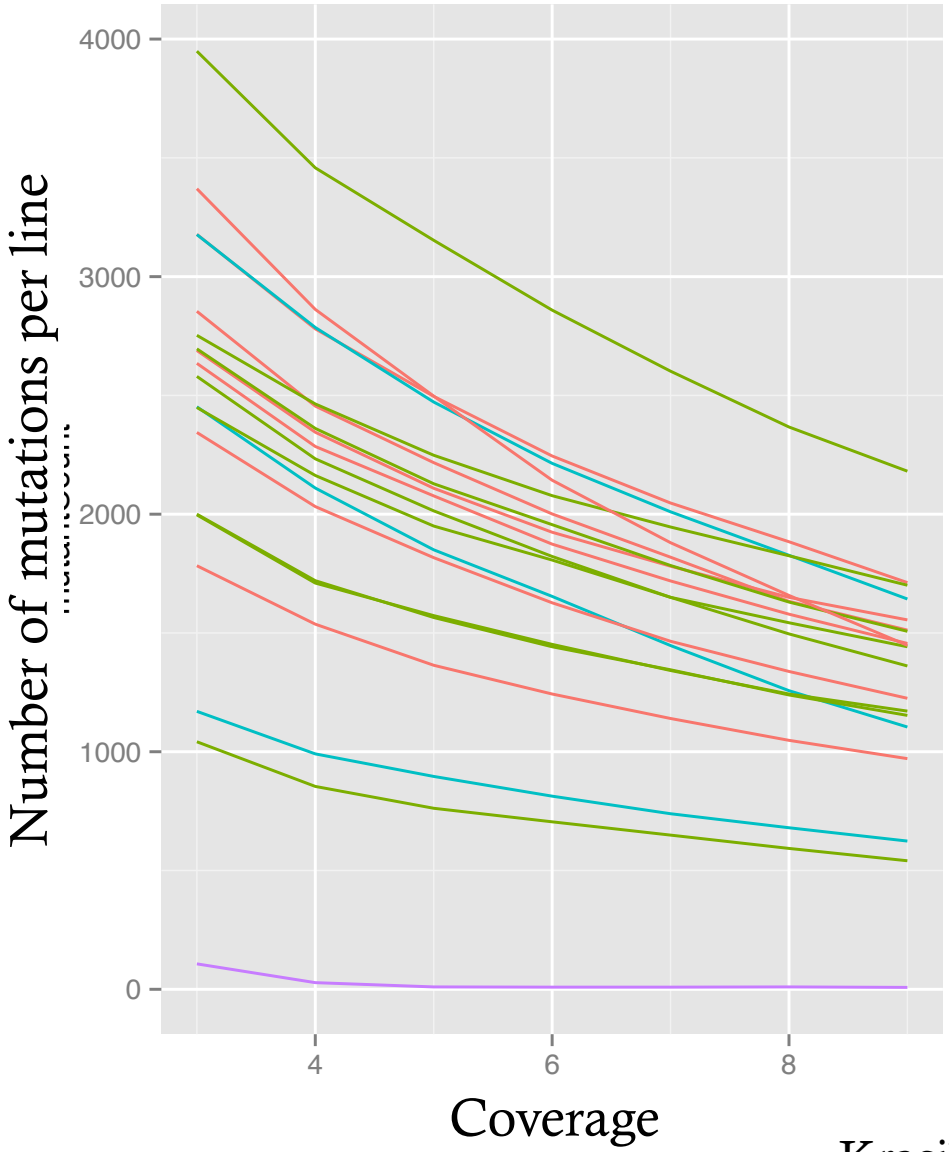
Signal:

EMS Mutation

http://comailab.genomecenter.ucdavis.edu/index.php/MAPS

The key parameter is coverage cutoff for heterozygous allele

2-5% false positive rate

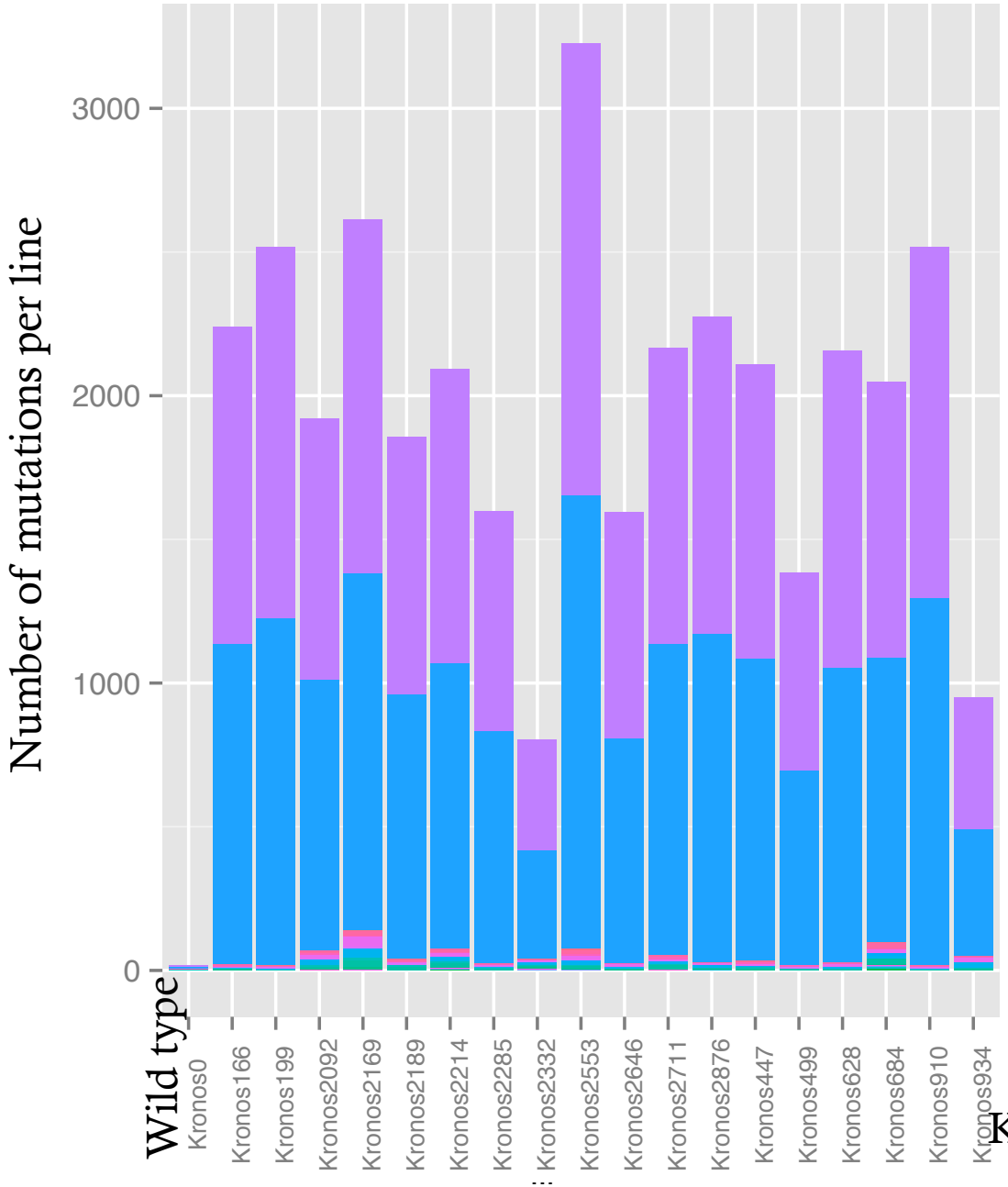


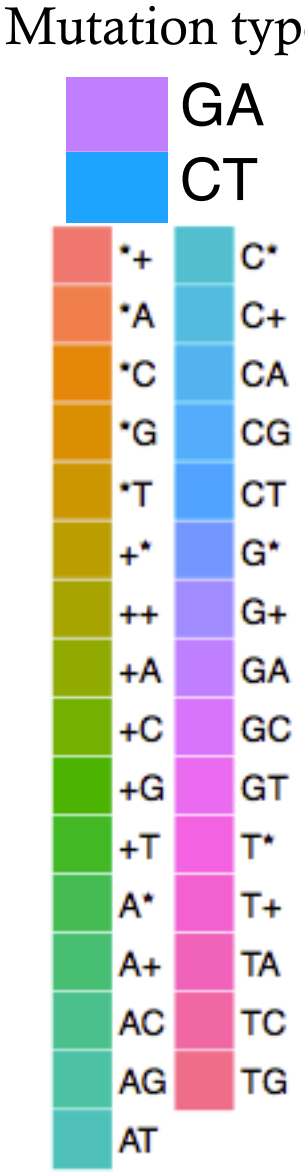
Mutants

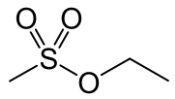
<- Wild type

Krasileva et al., in preparation

We are discovering ~2350 mutations in each wheat line







Krasileva et al., in preparation

How was the tool built?

- Mutation call VCF is run through Ensembl's VEP tool to predict variant effects

- Mutations found with a web blast, ViroBlast
- Visualized in a genome browser, JBrowse
- Data loaded in a relational database, PostGreSQL

Where do I access?

- Visit http://dubcovskylab.ucdvais.edu and sign in to use the tools
- Timeline:
  - Sequencing completed within a couple months
  - Full database available by mid-2015
  - If you need early resource access
    - Email: jdubcovsky@ucdavis.edu and havasquezgross@ucdavis.edu

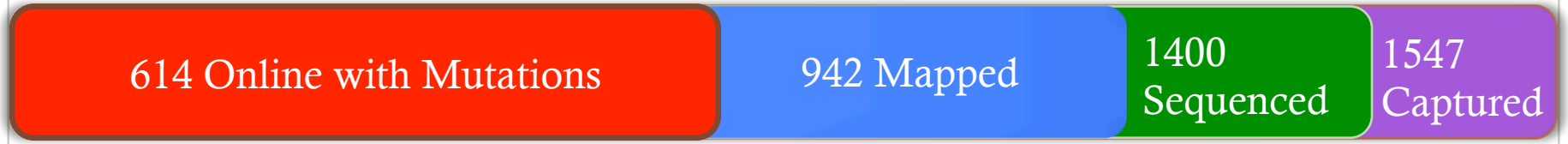
Where do I access?

- Planned Database resources for tetraploid wheat: 1547
- Planned Database resources for hexaploid wheat: 1344

Confidence	Mutation density per KB in a line	Mutation density per KB in the population
4x 95% (lower)	0.0259	39 mut/kb in 1547 lines
4x 99% (higher)	0.0246	37 mut/kb in 1547 lines
6x HetminCOV 4	0.0601	80 mut/kb in 1344 lines
6x HetminCOV 5	0.0588	79 mut/kb in 1344 lines
6x HetminCOV 6	0.0550	73 mut/kb in 1344 lines

TILLING Status

Tetraploid Wheat TILLING



Hexaploid Wheat TILLING

Use Case

- How would I find a mutation in My Favorite Gene (MADS box transcription factor)?

Wheat BLAST

Basic Search - using default BLAST parameter settings

Enter query sequences here in Fasta format

```
>MADS-box transcription factor
CAATTGCACTACCTTTTCTCATTTGTCCAACGACTGGACTGGCTTACCGGCAGTTGGACAGGTACA
CAATGTTTGCTGAAGAATGCAAATCAAACAGACTAACATTATTTTGAATACTCACTCAAAGAGAAG
GTCCACAAATAAGTTTAGTGAATCATAAGCCCATGAGGTTTGAATTAAGATATAACACAGTCGTTG
TGTTTTTGTCAAGACATACACTTAATTCTACACAAGTTCTAGGCTACTAGGTATTATATAGGGGTA
CCAACTTATTGCATATATCCAAAAGATAATCGTAGGTAGATAAATAGTTGATGTTCAACTCTGTTT
TTCATAAGTACTGTATTGTATTTCTATCAAGATCATCACAGCAAGATAGATAAGGTGAAAACCCCT
```

Or upload sequence fasta file: Browse... No file selected.

Program blastn

Database(s)

- IWGSC AB + UCW (TILLING)
- IWGSC ABD Genome
- T. turgidum (Kronos) Transcriptome
- Glutens Database
- Kronos Shotgun Assembly Alpha
- T. urartu Transcriptome
- URGI A chromosome
- URGI B chromosome
- URGI D chromosome
- URGI All Genomes chromosomes

And/or upload sequence fasta file: Browse... No file selected.

Basic search Reset

BLAST Result

Home About BLAST Help

Your job is being processed ■■■

Your job id is 142043299884.

Please wait here to watch the progress of your job.

This page will update automatically until the search is done.

BLAST Result

Home About BLAST Help

Inspect BLAST HTML output

Download BLAST TSV output

Filter current page by score:

Show – All – for each query sequence Filter

Re-parse current blast results (please select cutoff criterion):

Similarity percentage Cutoff %: 95

Blast score Cutoff score: 1000 Parse again

Retrieve and download subject sequences in FASTA format:

Check here to download All sequences... OR select particular sequences of interest below

Submit your selection of sequences to download

Select/Deselect all

Query	Subject	Score	Identities (Query length)	Percentage	Expect	VisualizationLink (if available)	Mutant Line (confidence)
MADS-box	IWGSC_CSS_5AL_scaff_2756996	3243	1798/1798 (1798)	100	0.0	IWGSC_CSS_5AL_scaff_2756996	23 (high)
MADS-box	IWGSC_CSS_4BL_scaff_7032666	2345	1614/1825 (1798)	88	0.0	IWGSC_CSS_4BL_scaff_7032666	1 (low)
MADS-box	IWGSC_CSS_4BL_scaff_6971438	1799	1279/1472 (1798)	87	0.0	IWGSC_CSS_4BL_scaff_6971438	1 (medium) 1 (low) 2 (high)
MADS-box	IWGSC_CSS_4BL_scaff_7041645	1685	1211/1401 (1798)	86	0.0	IWGSC_CSS_4BL_scaff_7041645	32 (high) 5 (low)
MADS-box	UCW_Kronos_U_jcf7180000450852	1068	758/860 (1798)	88	0.0	UCW_Kronos_U_jcf7180000450852	13 (high) 1 (medium) 1 (low)

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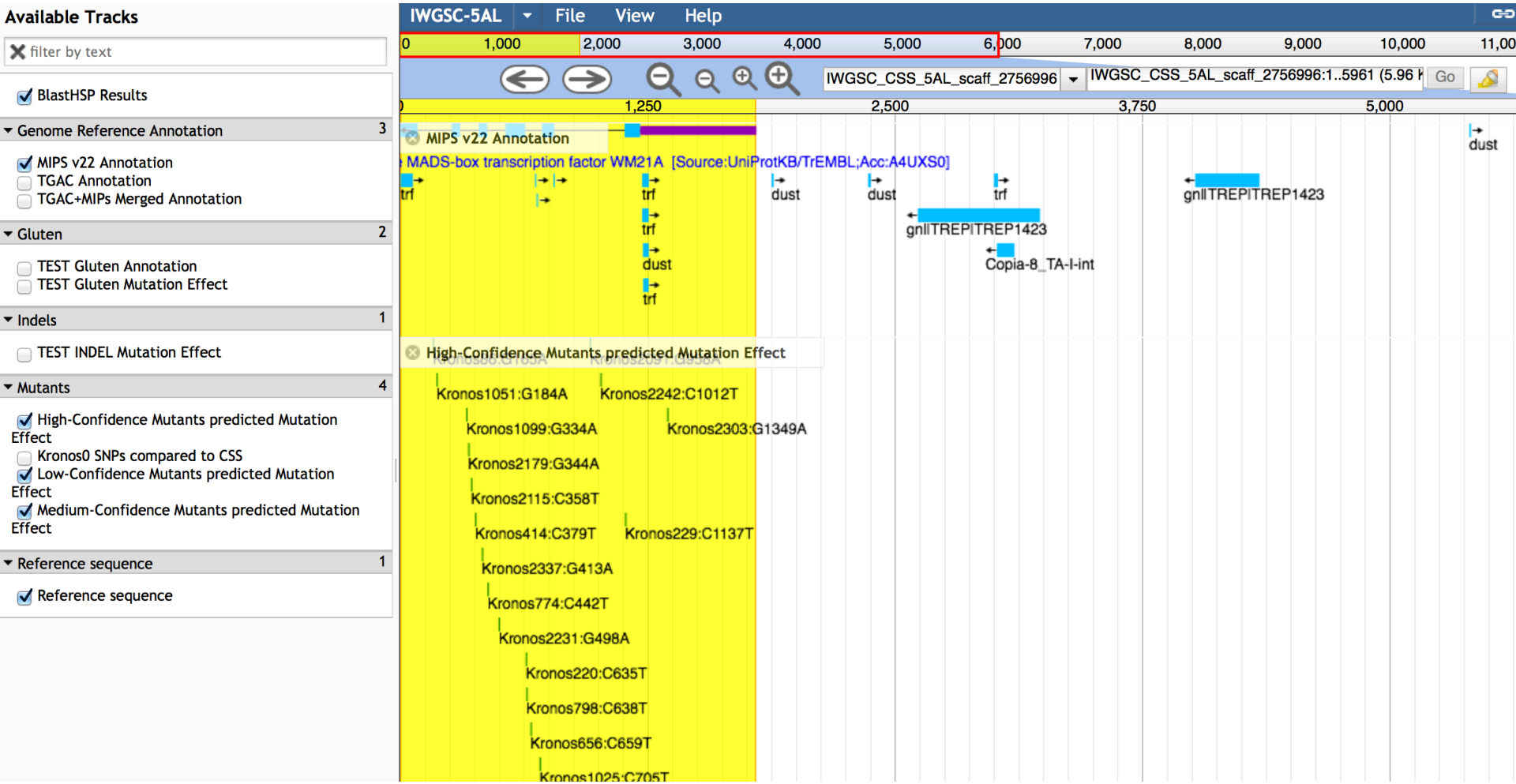
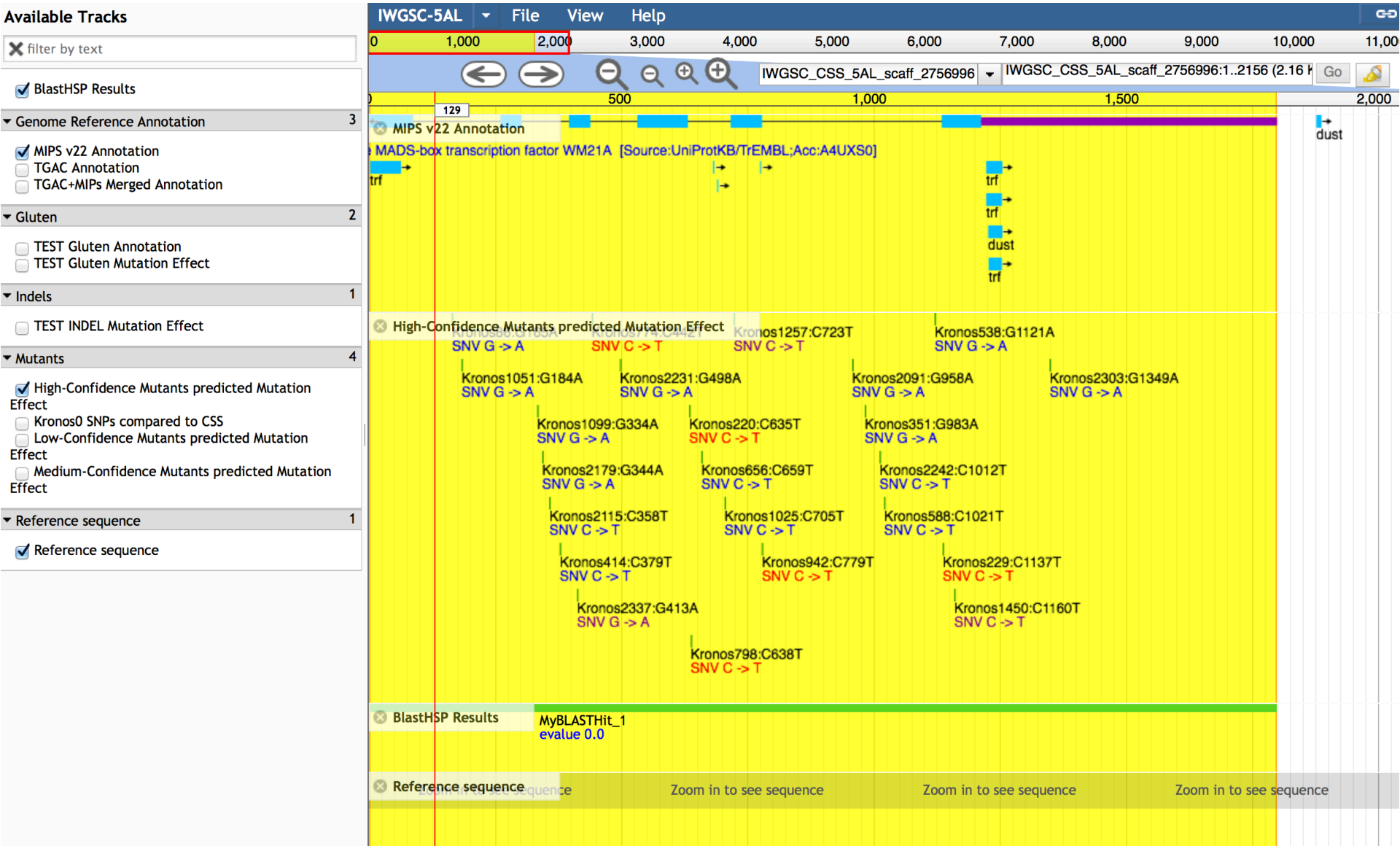

Available Tracks
filter by text
BlastHSP Results
Genome Reference Annotation 3
MIPS v22 Annotation
TGAC Annotation
TGAC+MIPs Merged Annotation
Gluten 2
TEST Gluten Annotation
TEST Gluten Mutation Effect
Indels 1
TEST INDEL Mutation Effect
Mutants 4
High-Confidence Mutants predicted Mutation Effect
Kronos0 SNPs compared to CSS
Low-Confidence Mutants predicted Mutation Effect
Medium-Confidence Mutants predicted Mutation Effect
Reference sequence 1
Reference sequence
IWGSC-5AL
File
View
Help
IWGSC_CSS_5AL_scaff_2756996
IWGSC_CSS_5AL_scaff_2756996:1..5961 (5.96 k
Go
MIPS v22 Annotation
MADS-box transcription factor WM21A [Source:UniProtKB/TrEMBL;Acc:A4UXS0]
gnl|TREP|TREP1423
Copia-8_TA-I-int
High-Confidence Mutants predicted Mutation Effect
Kronos1051:G184A
Kronos2242:C1012T
Kronos1099:G334A
Kronos2303:G1349A
Kronos2179:G344A
Kronos2115:C358T
Kronos414:C379T
Kronos229:C1137T
Kronos2337:G413A
Kronos774:C442T
Kronos2231:G498A
Kronos220:C635T
Kronos798:C638T
Kronos656:C659T

- Mutation Effects are Color Coded
  - Green = Silent mutations
  - Red = Nonsense, stop loss, or splice mutation
  - Purple = Missense mutation
  - Blue = Non-exon mutation
- Data predicted by Ensembl Variant Effect Predictor tool (VEP) using *T. aestivum* reference release 22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Genome Reference Annotation 3
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TGAC Annotation
TGAC+MIPs Merged Annotation
Gluten 2
TEST Gluten Annotation
TEST Gluten Mutation Effect
Indels 1
TEST INDEL Mutation Effect
Mutants 4
High-Confidence Mutants predicted Mutation Effect
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IWGSC-5AL
File
View
Help
IWGSC_CSS_5AL_scaff_2756996
IWGSC_CSS_5AL_scaff_2756996:1..2156 (2.16 k
Go
MIPS v22 Annotation
MADS-box transcription factor WM21A [Source:UniProtKB/TrEMBL;Acc:A4UXS0]
trf
dust
High-Confidence Mutants predicted Mutation Effect
Kronos1257:C723T SNV C -> T
Kronos538:G1121A SNV G -> A
Kronos1051:G184A SNV G -> A
Kronos2231:G498A SNV G -> A
Kronos2091:G958A SNV G -> A
Kronos2303:G1349A SNV G -> A
Kronos1099:G334A SNV G -> A
Kronos220:C635T SNV C -> T
Kronos351:G983A SNV G -> A
Kronos2179:G344A SNV G -> A
Kronos656:C659T SNV C -> T
Kronos2242:C1012T SNV C -> T
Kronos2115:C358T SNV C -> T
Kronos1025:C705T SNV C -> T
Kronos588:C1021T SNV C -> T
Kronos414:C379T SNV C -> T
Kronos942:C779T SNV C -> T
Kronos229:C1137T SNV C -> T
Kronos2337:G413A SNV G -> A
Kronos1450:C1160T SNV C -> T
Kronos798:C638T SNV C -> T
BlastHSP Results
MyBLASTHit_1
evalue 0.0
Reference sequence
Zoom in to see sequence
Zoom in to see sequence
Zoom in to see sequence

SNV Kronos942:C779T

Primary Data

Type	SNV
Position	IWGSC_CSS_5AL_scaff_2756996:779..779
Length	1 bp

Attributes

VEP SNP Effect	**SnpEffect_1:** Transcript = Traes_5AL_21C395CA8 Mutation Effect = splice_acceptor_variant
Alternative alleles	T
description	SNV C -> T
reference_allele	C
Seed Stock Availability	Available
seq_id	IWGSC_CSS_5AL_scaff_2756996

OK

SNV Kronos2337:G413A

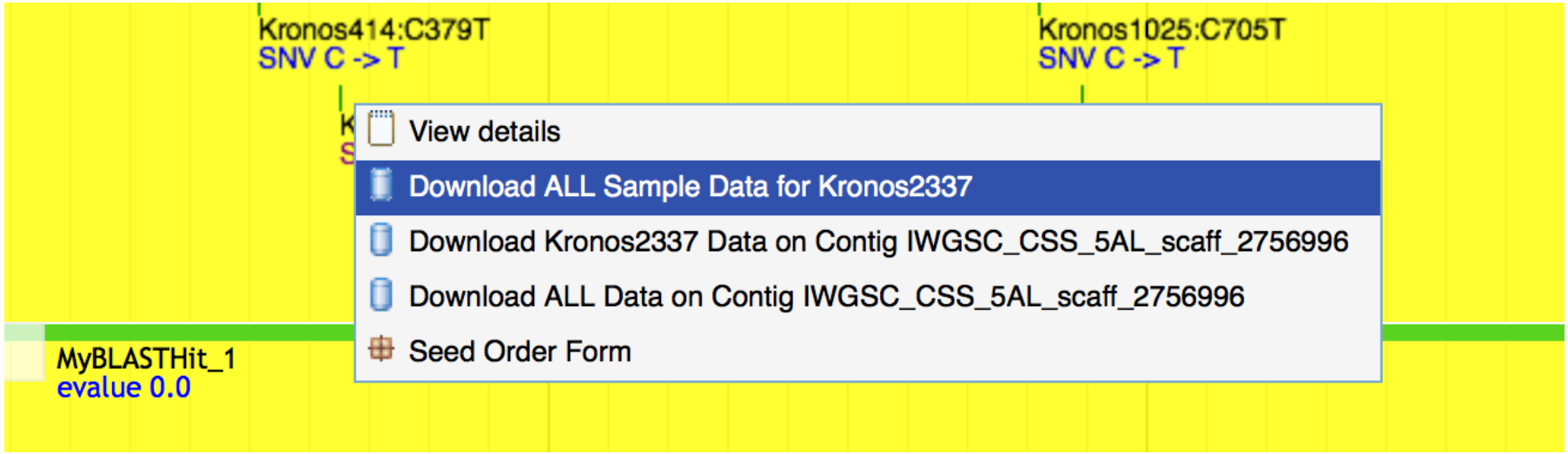
Primary Data

Type	SNV
Position	IWGSC_CSS_5AL_scaff_2756996:413..413
Length	1 bp

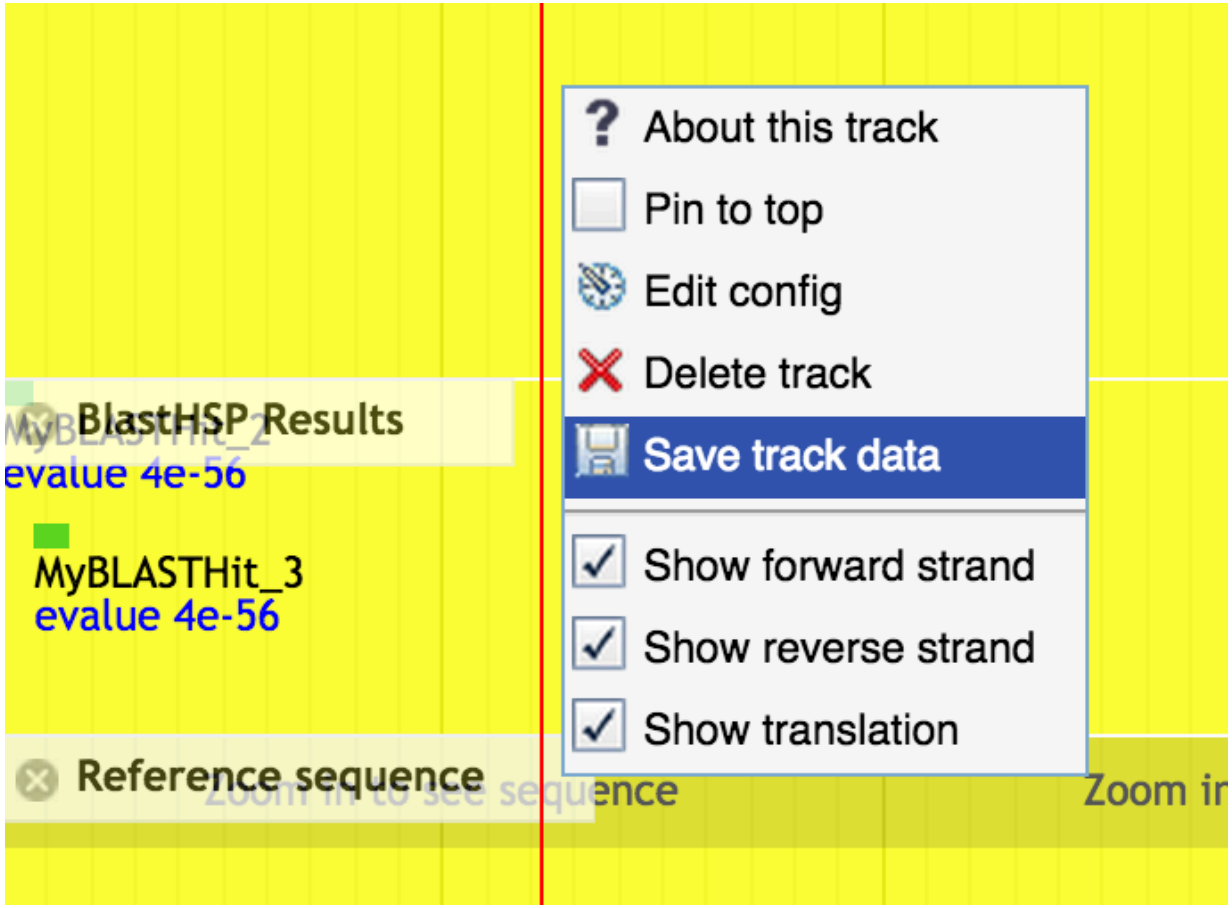
Attributes

VEP SNP Effect	**SnpEffect_1:** Transcript = Traes_5AL_21C395CA8 Mutation Effect = missense_variant Codon change/distance = tCt/tTt Amino acid change = S89F
Alternative alleles	A
description	SNV G -> A
reference_allele	G
Seed Stock Availability	Available
seq_id	IWGSC_CSS_5AL_scaff_2756996

OK


Kronos414:C379T
SNV C -> T
Kronos1025:C705T
SNV C -> T
View details
Download ALL Sample Data for Kronos2337
Download Kronos2337 Data on Contig IWGSC_CSS_5AL_scaff_2756996
Download ALL Data on Contig IWGSC_CSS_5AL_scaff_2756996
Seed Order Form
MyBLASTHit_1
evalue 0.0

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	seqid	library	position	ref_ba	wt_ba	mut_b	hethom	wt_co	mut_co	confiden	Effect_gene_1	Effect_feature_1	Effect_consequence_1
2	IWGSC_CSS_1AL_scaff_139936	Kronos429	5254	G	G	A	hom	0	8	high	Traes_1AL_A2DA8A6AE	Traes_1AL_A2DA8A6AE.1	synonymous_variant
3	IWGSC_CSS_1AL_scaff_271380	Kronos429	3591	C	C	T	het	2	7	high	Traes_1AL_C49C8F4AE	Traes_1AL_C49C8F4AE.1	intron_variant
4	IWGSC_CSS_1AL_scaff_311190	Kronos429	11466	G	G	A	het	7	6	high	Traes_1AL_2D6E49CDD	Traes_1AL_2D6E49CDD.2	synonymous_variant
5	IWGSC_CSS_1AL_scaff_329734	Kronos429	2093	G	G	A	het	20	14	high	Traes_1AL_D7C0EC7C1	Traes_1AL_D7C0EC7C1.1	downstream_gene_variant
6	IWGSC_CSS_1AL_scaff_383476	Kronos429	675	G	G	A	het	9	8	high			intergenic_variant
7	IWGSC_CSS_1AL_scaff_386979	Kronos429	296	G	G	A	het	1	10	high			intergenic_variant
8	IWGSC_CSS_1AL_scaff_387025	Kronos429	520	C	C	T	het	8	4	low	Traes_1AL_0AFB4491D	Traes_1AL_0AFB4491D.2	missense_variant
9	IWGSC_CSS_1AL_scaff_387421	Kronos429	8733	G	G	A	het	2	4	low	Traes_1AL_C6A0E255E	Traes_1AL_C6A0E255E.1	missense_variant
10	IWGSC_CSS_1AL_scaff_387564	Kronos429	11448	C	C	T	het	4	36	high	Traes_1AL_D10D533C3	Traes_1AL_D10D533C3.2	missense_variant
11	IWGSC_CSS_1AL_scaff_387621	Kronos429	6917	G	G	A	het	2	5	medium	Traes_1AL_990E4507E	Traes_1AL_990E4507E.1	missense_variant
12	IWGSC_CSS_1AL_scaff_387933	Kronos429	5298	C	C	T	het	17	11	high	Traes_1AL_B0EAE92EE	Traes_1AL_B0EAE92EE.1	intron_variant
13	IWGSC_CSS_1AL_scaff_387986	Kronos429	6137	G	G	A	het	38	11	high			intergenic_variant
14	IWGSC_CSS_1AL_scaff_388310	Kronos429	18458	C	C	T	het	1	5	medium	Traes_1AL_717D7D16F	Traes_1AL_717D7D16F.2	downstream_gene_variant
15	IWGSC_CSS_1AL_scaff_388347	Kronos429	5649	C	C	T	het	13	32	high	Traes_1AL_A36D5020D	Traes_1AL_A36D5020D.1	missense_variant
16	IWGSC_CSS_1AL_scaff_388576	Kronos429	13132	G	G	A	het	3	8	high	Traes_1AL_E25202CC2	Traes_1AL_E25202CC2.2	intron_variant
17	IWGSC_CSS_1AL_scaff_388718	Kronos429	18261	C	C	T	het	1	15	high	Traes_1AL_4DA7AF7E5	Traes_1AL_4DA7AF7E5.1	splice_region_variant&intron_variant
18	IWGSC_CSS_1AL_scaff_388937	Kronos429	28373	C	C	T	het	18	24	high	Traes_1AL_2AD7BC3B3	Traes_1AL_2AD7BC3B3.2	missense_variant&splice_region_varia
19	IWGSC_CSS_1AL_scaff_389178	Kronos429	9144	G	G	A	het	7	32	high	Traes_1AL_FAA0547B8	Traes_1AL_FAA0547B8.2	intron_variant
20	IWGSC_CSS_1AL_scaff_389539	Kronos429	5772	C	C	T	het	2	21	high	Traes_1AL_E6A6C4E8F	Traes_1AL_E6A6C4E8F.2	intron_variant
21	IWGSC_CSS_1AL_scaff_390127	Kronos429	13548	G	G	A	het	10	13	high	Traes_1AL_39849C8EC	Traes_1AL_39849C8EC.1	splice_donor_variant
22	IWGSC_CSS_1AL_scaff_390390	Kronos429	13317	G	G	A	het	16	4	low	Traes_1AL_FB35B3FDC	Traes_1AL_FB35B3FDC.2	missense_variant
23	IWGSC_CSS_1AL_scaff_390808	Kronos429	7333	G	G	A	het	20	12	high	Traes_1AL_3D6B6B6D1	Traes_1AL_3D6B6B6D1.1	intron_variant
24	IWGSC_CSS_1AL_scaff_391645	Kronos429	3108	C	C	T	het	2	9	high	Traes_1AL_1543CA165	Traes_1AL_1543CA165.2	splice_region_variant&synonymous_v
25	IWGSC_CSS_1AL_scaff_392520	Kronos429	2737	G	G	A	hom	0	14	high	Traes_1AL_49E0DCFBB	Traes_1AL_49E0DCFBB.1	intron_variant
26	IWGSC_CSS_1AL_scaff_392576	Kronos429	6127	C	C	T	het	7	8	high	Traes_1AL_5A569D967	Traes_1AL_5A569D967.1	missense_variant
27	IWGSC_CSS_1AL_scaff_392685	Kronos429	9787	G	G	A	het	16	9	high	Traes_1AL_BA559DE2A	Traes_1AL_BA559DE2A.1	upstream_gene_variant
28	IWGSC_CSS_1AL_scaff_392706	Kronos429	7492	C	C	T	het	2	27	high	Traes_1AL_D74509506	Traes_1AL_D74509506.2	missense_variant
29	IWGSC_CSS_1AL_scaff_392956	Kronos429	1014	C	C	T	het	8	4	low	Traes_1AL_0F8C3E95F	Traes_1AL_0F8C3E95F.1	downstream_gene_variant
30	IWGSC_CSS_1AL_scaff_393112	Kronos429	14370	G	G	A	het	9	8	high	Traes_1AL_FC91B76B1	Traes_1AL_FC91B76B1.2	5_prime_UTR_variant
31	IWGSC_CSS_1AL_scaff_393173	Kronos429	29158	C	C	T	het	1	5	medium	Traes_1AL_FECAB71D5	Traes_1AL_FECAB71D5.1	stop_gained
32	IWGSC_CSS_1AL_scaff_393285	Kronos429	848	G	G	A	het	11	12	high			intergenic_variant
33	IWGSC_CSS_1AL_scaff_393468	Kronos429	673	C	C	T	het	1	4	low			intergenic_variant
34	IWGSC_CSS_1AL_scaff_394294	Kronos429	20299	C	C	T	het	33	24	high	Traes_1AL_699B447C5	Traes_1AL_699B447C5.1	downstream_gene_variant
35	IWGSC_CSS_1AL_scaff_394361	Kronos429	5792	C	C	T	het	3	25	high	Traes_1AL_74A7618B0	Traes_1AL_74A7618B0.2	intron_variant
36	IWGSC_CSS_1AL_scaff_394389	Kronos429	8230	G	G	A	het	2	10	high	Traes_1AL_35182E357	Traes_1AL_35182E357.2	missense_variant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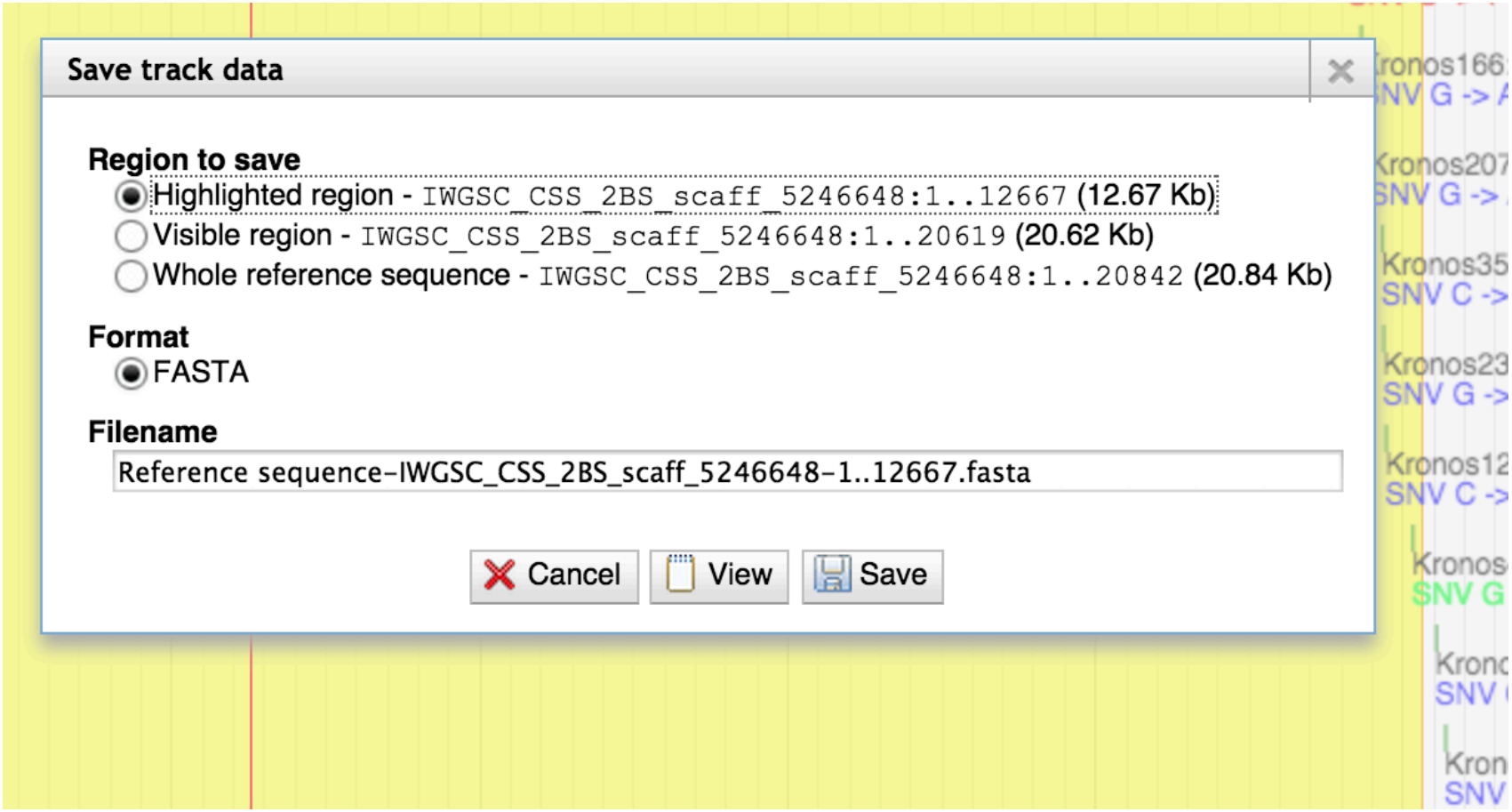
Reference sequence
Zoom in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Cancel
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Conclusion

- Query gene of interest *in silico*
- Find potential candidates with mutations
- Return with an allelic series for your gene of interest!

UC Davis Tetraploid Wheat Genomics Team

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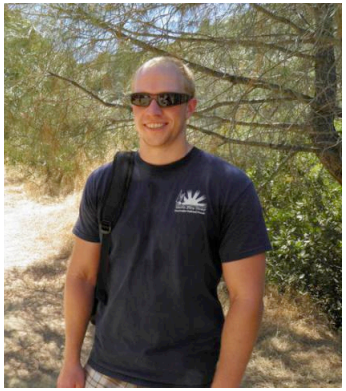
Xiaodong Wang



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