Using the Wheat TILLING Database

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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

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• 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 \sim 12Gb to \sim 112Mbp
 - Screening all targets in the exome capture at once

Most of the wheat genome is non-coding and repetitive



How?

Mutation sequencing and calling



MAPS – (Mutation and Polymorphism Survey)

Noise:

<u>Varietal SNP</u>		
Paralog/Homeolog SNP	Defenence	
Sequencing/PCR error	Reference	CAGTGTGCCCCACCTGTGGCTTTTTGACTAATGTGTACAGCAACGTA
Off target repeats		CAG <mark>G</mark> GTGCC <u>G</u> ACCTGTG <mark>A</mark> CTTTTGAC <u>A</u> AATGTGTACAGCAA <mark>G</mark> GTA
	Mutant 1	CAGTGTGCC <u>G</u> ACCTGTG <mark>A</mark> CTTTTGAC <u>A</u> AATGTGTACAGCAACGTA
C ¹ 1		CAGT <u>C</u> TGCC <mark>G</mark> AC <u>T</u> TGTGGCTTTTGAC <u>A</u> AATGTGTA <u>T</u> AGCAACGTA
Signal:		CAGT <u>C</u> TGCC <mark>G</mark> AC <u>T</u> TGTGGCTTTTGAC <mark>A</mark> AATGTGTACAGCAACGTA
EMS Mutation		
		CAGTGTGCC <u>G</u> ACCTGTG <mark>A</mark> CTTTTGAC <u>A</u> AATGTGTACAGCAACGTA

Mutant 2 CAGTGTGCCGACCTGTGACTTTTGACAAAAGTGTACAGCAACGTA CAGTCTGCCGACCTGTGGCTTTTGACAAATGTATACAGTAACGTA CAGTCTGCCGACCTGTGGCTTTTGACAAATGTGTACAGCAACGTA

 Mutant 3
 CAGTGTGCCGACCTGTGACTTTTGACAAATGTGTACAGCAAGGTA

 CAGTCTGCCGACCTGTGGCTTTTAACAAATGTGTACAGCAACGTA

 CAGTCTGCCGACCTATGGCTTTTAACAAATGTGTACAGCAACGTA

 CAGTCTGCCGACCTGTGGCTTTTAACAAATGTGTACAGCAACGTA

 CAGTCTGCCGACCTGTGGCTTTTAACAAATGTGTACAGCAACGTA

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

The key parameter is coverage cutoff for heterozygous allele



We are discovering ~2350 mutations in each wheat line



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 <u>http://dubcovskylab.ucdvais.edu</u> 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



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 CAATGTTTGCTGAAGAAATGCAAATCAAACAGACTAACATTATTTTGAATACTCACTC
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Please wait here to watch the progress of your job.

This page will update automatically until the search is done.

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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	UWGSC_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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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)
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- 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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SNV Kronos942:C779T

Primary Data

	Туре	SNV
	Position	IWGSC_CSS_5AL_scaff_2756996:779779
	Length	1 bp
A	tributes	
	VEP SNP Effect	SnpEffect_1:
		Transcript = Traes_5AL_21C395CA8
		Mutation Effect = splice_acceptor_variant
	Alternative alle	T
	description	SNV C -> T
	reference_allele	C
	Seed Stock Ava	ailability Available
	seq_id	IWGSC_CSS_5AL_scaff_2756996

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Primary Data

	Туре	SNV
	Position	IWGSC_CSS_5AL_scaff_2756996:413413
	Length	1 bp
A	tributes	
	VEP SNP Effect	SnpEffect_1:
		Transcript = Traes_5AL_21C395CA8
		Mutation Effect = missense_variant
		Codon change/distance = tCt/tTt
		Amino acid change = S89F
	Alternative alle	les A
	description	SNV G -> A
	reference_allel	e G
	Seed Stock Ava	ailability Available
	seq_id	IWGSC_CSS_5AL_scaff_2756996

 \times





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



ave track data X	Ironos16
Region to save	Kronos20
Highlighted region - IWGSC CSS 2BS scalt 5246648:112667 (12.67 KD) Visible region - IWGSC CSS 2BS scalf 5246648:120619 (20.62 Kb)	SINV G ->
Whole reference sequence - IWGSC_CSS_2BS_scaff_5246648:120842 (20.84 Kb)	Kronos3 SNV C -
Format FASTA	Kronos2 SNV G -
Reference sequence-IWGSC_CSS_2BS_scaff_5246648-112667.fasta	Kronos1 SNV C -
X Cancel Uiew Save	Krono SNV C
	Kron
	Kro

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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