FungiDB: Synteny in GBrowse

Fungal organisms produce a wide range of extracellular enzymes to break down organic materials. Glycoside hydrolases (GH) are a large family of enzymes that facilitate degradation processes.

1. Initiate a gene search for Glycosyl hydrolase family 47 proteins in *Cryptococcus*. *Use the InterPro Domain search and enter* **PF01532** (the PFAM ID for Glycosyl hydrolase family 47).

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2. Navigate to the gene page of CNBG_9313. This gene belongs to the family of CAZy genes

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🗧 🗘 Gene ID	Transcript ID	🤤 Organism 🥝	Genomic Location () (Gene)	Product Description Q	🖨 Sco	re/E Je
CNBG_2351	CNBG_2351-t26_1	C. deuterogattii R265	KQ410561:860,016862,851(+)	mannosyl-oligosaccharide 1,2-alpha-mannosidase		.0E+00
CNBG_5230	CNBG_5230-t26_1	C. deuterogattii R265	KQ410571:160,190162,783(-)	mannosyl-oligosaccharide alpha-1,2-mannosidase		.0E+00
CNBG_9313	CNBG_9313-t26_1	C. deuterogattii R265	KQ410563:85,56189,006(-)	carbohydrate binding protein		1.6E-121
CGB_E6120W	CGB_E6120W-t26_1	C. gattii WM276	CP000290:1,169,9051,172,487(+)	endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase (ER alpha- 1,2-mannosidase)		.0E+00
CGB_G2060C	CGB_G2060C-t26_1	C. gattii WM276	CP000292:392,095394,973(-)	mannosyl-oligosaccharide 1,2-alpha-mannosidase		.0E+00
CGB_K0370C	CGB_K0370C-t26_1	C. gattii WM276	CP000296:94,57397,577(-)	carbohydrate binding protein		3.4E-99
CNAG_02081	CNAG_02081-t26_1	C. neoformans var. grubii H99	CP003825.1:1,248,6061,251,401(+)	mannosyl-oligosaccharide alpha-1,2-mannosidase		.0E+00
CNAG_03240	CNAG_03240-t26_1	C. neoformans var. grubii H99	CP003827.1:419,423422,891(-)	mannosyl-oligosaccharide 1,2-alpha-mannosidase		.0E+00

Note: You can further explore clusters and other data in MycoCosm CAZy Browser as was shown previously.

3. Click on the Synteny shortcut to examine tracks in closely related species.



4. Next, let's explore synteny in Basiodiomycota phylum. Click on the *View in genome browser* button to be re-directed to GBrowse session.



- 5. Activate custom tracks by clicking on the Select Tracks tab.
- 6. Navigate to the *Orthology and synteny* menu and click on the "showing... subtracks" link. Now you have an option to select tracks for Basidiomycetes.

Select th	ie tracks you	wish to display. Sort the tracks by	clicking on the column head	dings, or by clicking an
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	Fungi	Tremellomycetes	Basidiomycota	Filobasidiella
	Fungi	Agaricomycetes	Basidiomycota	Coprinopsis
	Fungi	Agaricomycetes	Basidiomycota	Coprinopsis
	Fungi	Agaricomycetes	Basidiomycota	Phanerochaete
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Once you are done selecting tracks, click on the Change button at the bottom of the pop-up window.

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7. Is the region containing this gene syntenic in all species you selected?

- What can you conclude about the conservation of this gene across various species?
- What is the direction of the CNBG_9313 (highlighted in red) gene relative to the chromosome?
- What genes are upstream or downstream of the CNBG_9313?
- Do you observe changes in the number of exons?
- Notice that some of the genomes have contigs that are not contiguous. Why is that?
- Mouse over the two contigs and look at the information in the popups do these pieces belong to the same chromosome? What does this mean?
- Observe the last track on the bottom of the screen *Ustilago maydis*. Why do you think this gene is not detected here? *Hint: examine contigs*.

- Examine neighboring genes in *Cryptococcus* species. Which genes have undergone expansions or possible truncations (highlighted in blue)?
- 8. Navigate to FOXG_17458, a hypothetical protein in *Fusarium oxysporum* f. sp. *lycopercisi* 4287



a. Generate GBrowse view of syntenic genes in *Fusarium* species only: Notice that there are no syntenic genes shown for FOXG 17458:

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Why do you think this is?

Investigate this further by looking into non-syntenic *Fusarium* orthologs in FungiDB and examine Fusarium resources offered at MycoCosm.

b. Create a search for FOXG_17458. To generate a search query with a single gene, use the text search box at the top of FungiDB page:

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FOXG_17458	FOXG_17458- t26_1	F. oxysporum f. sp. lycopersici 4287	CM000602.2:1,300,2731,303,560(+)	hypothetical protein		Product, InterPro, GOTerms, Geneld	4

c. Next, look for non-syntenic orthologs in *Fusarium* species:

Add Step 2 : Transform by Orthology			
Organism			
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What can you conclude about conservation and function of this gene?

d. Navigate to Mycocosm main page and select a Fusarium oxysporum f. sp. *lycopersici* strain 4287 genome. [genome.jgi.doe.gov/Fusox2]

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Fusarium w lycopersici. Image Cred	it of tomato ca it: David B. La	nused by Fusario ngston, Univers	um oxysporum f.sp. ity of Georgia,	of F. oxysporum f. s	parison led to p. lycopersici se (Ma et al., 2	(race 2 - VCG 0 2010).	030) cont	aining gene	es required for host specific
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- · Alabouvette, C., Olivain, C., Migheli, Q., and Steinberg, C. (2009) Microbiological control of soil-borne
- phytopathogenic fungi with special emphasis on wilt-inducing Fusarium oxysporum. New Phytologist 184: 529-544.
 Ma,L.J., van der Does,H.C., Borkovich,K.A., Coleman,J.J., Daboussi,M.J., Di Pietro,A. et al. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. Nature 464: 367-373.
- O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Magnon, K.C., Cox, P.A., Revankar, S.G. et al. (2004) Genetic diversity of human pathogenic members of the Fusarium oxysporum complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analyses: Evidence for the recent dispersion of a geographically widespread clonal lineage and nosocomial origin. Journal of Clinical Microbiology 42: 5109-5120.
 - e. Use the Fusox2 portal's search page to identify the proteinID of "FOXG_17458T0" (Transcript 0 of FOXG_17458). You will find that the proteinID of FOXG 17458 in Fusox2 is 23236.

FOXG_17458T0 Search By: Across Keywords Defa 	Search ss: Terms: ault V exact - fast V					
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f. Click on MCL clusters tab and then use the pull down menu to select clustering run "Fusarium-orthomcl 1.5.2900".

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https://genome.jgi.doe.gov/clm/run/Fusarium-orthomcl.2900?organism=Fusox2

g. Enter keyword Fusox2.23236 (databaseID.proteinID) to find clusters with that protein in it. Remember, for FOXG_17458 (FOXG_17458T0 protein ID in MycoCosm is 23236 and genome ID is Fusox2)



This will bring up cluster #386. Notice that this family is expanded only in the two known pathogens of the *Fusarium oxysporum* species complex with dispensable chromosomes (Fusox2 and FusoxT415), but not in other Fusarium species including endophytic *Fusarium oxysporum* like Fusoxys1 and Fusoxy1.

Now having this information at hand, you can either return to FungiDB and examine underlying transcriptomics, proteomics, *etc.* data or use other databases to enrich your analysis. For example:

• Navigate to Ensembl Fungi, search for FOXG_17458 and visualize the gene-tree:





Take a look at the between species paralogues. Is your data consistent with observations in MycoCosm? (Hint: look for duplication nodes).

• Click on the link at the bottom of the gene tree image to view all paralogues on the tree:

View options:

- View current gene only (Default)
- View paralogs of current gene
- View all duplication nodes
- View fully expanded tree



• To export this data you can click on the *Download data for this image* button and choose form multiple formats:



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Or you can choose to download the image as shown by clicking on the Export this image button:



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• PDF file - Standard image as PDF file						
Presentation - Saturated image, better suited to projectors						
O Poster - Very high resolution,	Poster - Very high resolution, suitable for posters and other large print uses					
O Journal/report - High resolut	ion, suitable for printing at A4/letter size					
O Web - Standard image, suitab	ble for web pages, blog posts, etc.					
Custom image - Select from	Custom image - Select from a range of formats and sizes					
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