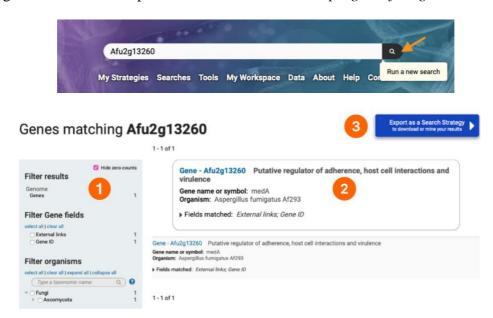
Exploring the gene record page

Learning objectives

- Become familiar with gene page structure and content
- Navigate to and from the gene pages
 - Use the site search to navigate to the gene record page of <u>Afu2g13260</u>, which is a gene known to be important for the virulence of *Aspergillus fumigatus*.

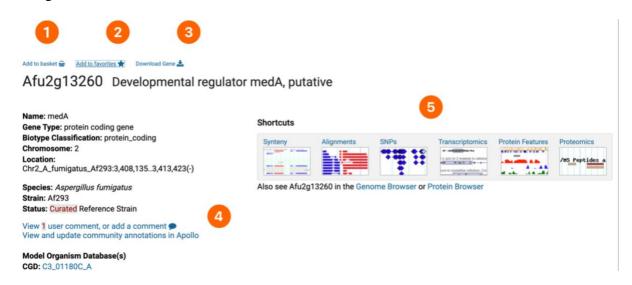


- The panel on the left provides a summary of all record types that match Afu2g13260.
- Click on the gene link to navigate to the gene record page for Afu2g13260.
- Clicking on this button will transform your search into a search strategy.

 Note: If the button is shaded/inactive, limit your search to a single data type using the Filter results panel on the left.

Gene page components

The top section of the gene record page provides a snapshot of the information available for this gene and offers several shortcuts:



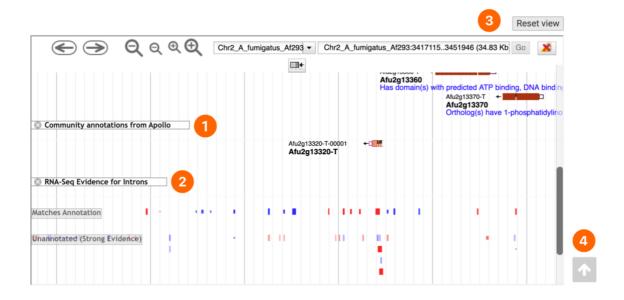
- Add to basket: Save to basket if you want to download gene-specific information for selected genes.
- Add to favorites: Saves genes in the private My favorites section, where you can add notes or keep track of your project.
- Download Gene: Redirects to a download options page where gene records can be exported at text, FASTA, and GFF3 formats.
- Submit a comment or annotate gene in Apollo, a web-based structural and functional gene annotation platform.
- Shortcuts panel provides quick access to the selected section within the gene record page.
 - **Explore the content of this gene record page.** Below are the navigational highlights.

The **Contents** section contains a list of links to various sections in the gene record page and is searchable. Sub-sections of the Contents menu can be hidden by checking the box to the right.



• Explore the gene model section.

The **Gene Models** section is the first section of the gene record page, and it contains information about the structure of the gene (e.g. exon count, transcript number, annotated UTRs, community gene annotation in Apollo, introns, etc.) displayed within the genome browse JBrowse wrapper.



- Community annotation from Apollo provides the latest annotation updates (within 24-48hrs after the changes were submitted to the curation team in Apollo)
- Introns that are matching transcript annotation for which there is an abundance of supporting data from aligned RNA-Seq reads are displayed in bold colours (Blue for a forward gene and red for a gene located on the reverse strand).
- If you navigated away from the gene on interest while scrolling, click on the "Reset view" button to return to the default position within the JBrowse wrapper.
- Click this button to navigate to the top of the gene record page.

The "View in JBrowse genome browser" and "Annotate in Apollo" buttons open in separate tabs. In JBrowse, you can activate additional tracks and build custom evidence views. In Apollo, you can modify and create new genes to improve the genome annotation.

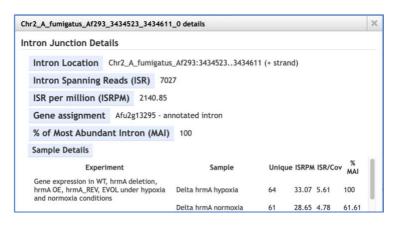


• Explore the RNA-Seq Evidence for Introns track.

Click on the intron. The pop-up window contains a table showing all experiments and samples that provide evidence for this intron junction (generated by in-house automated pipelines):

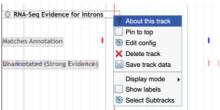
Intron Spanning Reads (ISR): The total number of uniquely mapped reads (all samples) which map across the junction and are on the appropriate strand. GSNAP uses splice site consensus sequences to determine the strand of the mapped read.

ISR per million (ISRPM): Intron Spanning Reads Per Million intron spanning reads and thus represents a normalized count of unique reads.



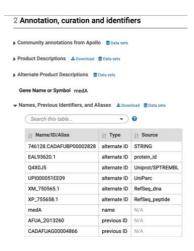
% of Most Abundant Intron (MAI): The percentage (ISRPM of this junction / ISRPM of maximum junction for this gene) of this junction over the maximum for this gene.

Note that the tracks within the JBrowse wrapper have a drop-down menu for further track customization:



• Explore other contents within the page.

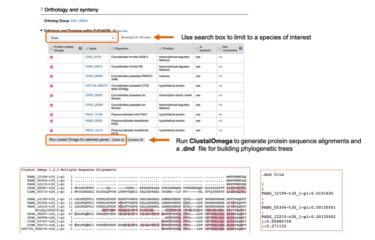
The **Annotation, curation and identifiers** section offers alternate product descriptions, previous identifiers, and aliases, and is populated using data from internal curation, other fungal resources (*e.g.* AspGD, Ensembl, *etc.*), or user-submitted data (user comments).



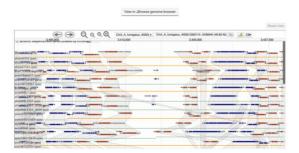
The **Link outs** section offers redirection to other resources (*e.g.*, CGD, Ensembl, MycoCosm, *etc.*).

The **Orthology and synteny** section provides a table of Orthologs and Paralogs within FungiDB produced by OrthoMCL (<u>www.orthomcl.org</u>).

The table has a search box for creating a custom display of orthologs and also deploy the ClustalOmega analysis. The output of this tool can be used to build phylogenetic trees (e.g. iTOL).

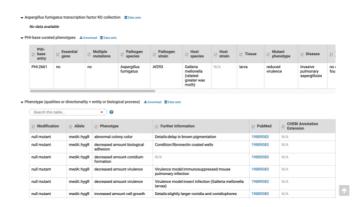


The **Orthology and Synteny** section also contains synteny graphs in JBrowse:



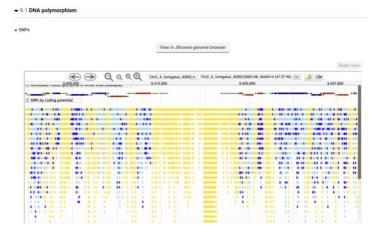
In the screenshot above, the syntenic genes are highlighted in grey.

The **Phenotype** section offers curated information, including annotations from the Pathogen-Host Interactions database, COFUN project (selected transcription factors knockouts) and other sources.



The **Genetic variation** section summarizes integrated SNP data for a given region and classifies SNPs based on the effect on gene function:

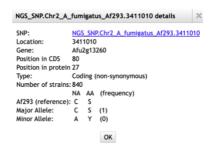
- noncoding (yellow diamonds)
- non-synonymous (dark blue)
- synonymous (light blue)
- nonsense (red)



Note that you can interact with the SNP records by using left and right clicking options on your mouse/touch pad.

Left click brings up a pop-up window containing more information about a particular SNP:

The SNP record linked in blue is linked to the SNP record page, which contains summary of the SNP across different isolates and samples.



Add to basket 🖨 Add to favorites 🛊 Download SNP 🕹

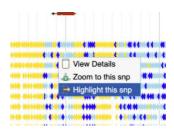
SNP: NGS_SNP.Chr2_A_fumigatus_Af293.3411010

Major allele is the most common allele in the studied population/isolates.

Minor allele frequency is the frequency of the second most common allele. Minor allele

frequency is useful when looking for rare variants or disease-causing SNPs. However, there can be exceptions when minor allele frequency increases under selective pressure (e.g., development of drug resistance).

Right click provides more options for JBrowse view:



The **Transcriptomics** section (RNA-Seq and microarray data).

The Transcript Expression Summary section provides a big picture of gene expression across different samples and experiments, and helps identify experiments in which the current gene is highly regulated.

▼ RNA-Seq Transcription Summary ■ Data sets

▼ Summary of expression values. Each row represents a distinct RNA-Seq experiment. Click to read more...

• Each black dot represents expression in a sample. A boxplot is shown, with the box representing the median and upper/lower quartiles, and the whiskers representing the max/min values (or 1.5 times the interquartile range, in which case values beyond the whiskers are considered outliers).

• Hover over the experiment name to show a table of sample names and values.

• Click on a boxplot to show labels and values for each sample in an experiment. Click again on the boxplot to hide labels and values. A button at the bottom-right removes labels and values for all experiments.

• Use the toggle button at the bottom-left to switch from Log Scale to Linear Scale.

• Log Scale values are log2(TPM+1) for these reasons:

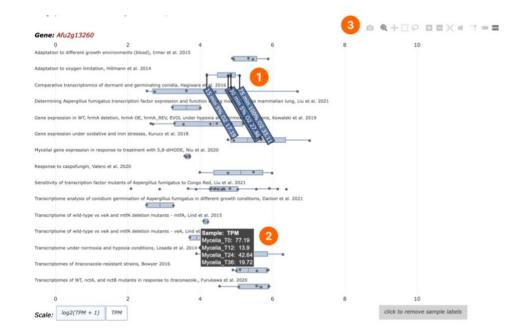
1. TPM+1: to de-emphasize low noisy TPM values (i.e., <1).

2. log2: so that each unit on the x-axis represents a 2-fold difference.

• Navigation buttons appear at the above-right when hovering over the graph. If the buttons do not appear, reload the page.

Zoom in with the navigation button or click and drag within the graph. Zoom out with the navigation button or double-click within the graph.

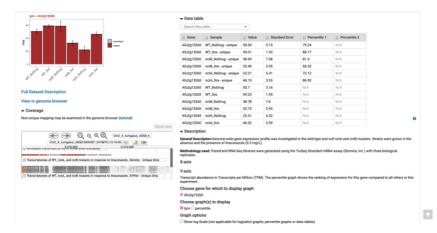
• This graph was created with Plotty. Get more help at their website.



- Clicking on the box plot will bring up sample labels.
- Hovering over the experiments will display sample names.
- The buttons above the summary graph provide additional options (e.g., download data in PNG, zoom, pan, etc.).

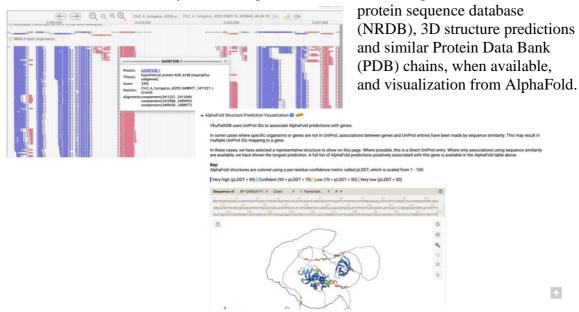
The Transcript Expression section, which is located under the RNA-Seq summary section,

can be expanded to view the expression graph (TPM), data table, full dataset description, coverage plots, a link to the dataset in JBrowse.

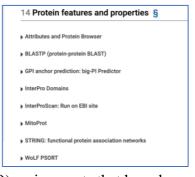


The Sequence analysis,

Sequences, and Structure analysis sections offer sequence information (DNA, RNA, and protein), an interactive summary of EST alignments and BLAT hits against the nonredundant



The **Protein features and properties** section provides access to information about protein domains identifications (InterPro), signal and transmembrane predictions graphics, BLASTP hits, and other tools that can be deployed directly from the gene record page using the amino acid sequence of interest:



The **Function Prediction** section features Gene Ontology (GO) assignments that have been either downloaded from Gene Ontology databases and manually curated by FungiDB. GO Slim terms, Enzyme Commission (EC) numbers with links to EC number and GO terms description and relevant publications are available as well.

Gene ontology provides statements for describing the functions of genes along with three aspects: Molecular function, Biological process, and Cellular component, and it is organized in hierarchies. The GO terms table above provides GO IDs and terms associated with a particular gene and additional metadata that are available for these associations such as source, evidence code (e.g. IDA, IMP, etc.) and reference (PubMed ID).

There are three classes of GO terms in FungiDB:

- 1. Automatically assigned by InterPro2GO
- 2. Assigned by FungiDB curators
- 3. Obtained from external resources such as AspGD, MIPS, and others

For some genes, the **Pathways and interactions** section provides information about metabolic pathways loaded from the KEGG and MetaCyc repositories. Genes are linked to individual pathways by EC numbers when this data is available and clicking on any of the links redirects to an interactive metabolic pathway viewer where the user can explore individual reactions or export all known data for a given pathway. EC numbers are inferred by the OrthoMCL pipeline automatically. As with any automated analysis, use caution when interpreting this data.

For example, Afu2g13260 is not associated with metabolic pathways but its neighbor (Afu2g13250) does:

