## FungiDB: Navigating gene record pages

#### 1. Components of gene record pages

Navigate to the gene records page for *Aspergillus fumigatus* **Afu2g13260**, which is known to be important in virulence of the filamentous fungus *Aspergillus fumigatus*.

• You can search for the gene using the **Gene ID** shortcut search at the top of the FungiDB main page:



- Notice that **Shortcuts** at the top of the gene page will take you directly to the section containing SNPs, Transcriptomics, Protein Features, *etc.*, while the **Contents** menu on the left brings up additional functionality and data.
- Note: Each thumbnail within the **Shortcuts** shows the number of datasets available within a given category. Clicking on the magnifying glass symbol within the thumbnail will open a preview screen of the evidence.
- The top section of the gene page also provides information about gene name, type (protein coding, etc.), chromosome number and exact location. If a strain is actively under manual EuPathDB curation it will be indicated as shown below as well.
- User comments can be added via the *Add the user comment* link. User comments help improve genome annotations: provide evidence for alternative gene models,

phenotypes, subcellular localization, gene products, *etc*. User comments can be added in bulk (contact <u>help@fungidb.org</u>), and can be edited at any time.

- Gene record page can be saved by clicking on the **Add to basket** link. To bookmark a gene of interest, click on **Add to favorites**.
  - The *Add to basket* function saves the gene record to a basket associated with a user's account. Basket items can be found in the My Strategies section when a user is logged in. The basket serves as a shopping cart where genes in the basket can be downloaded or transferred to a search strategy.
  - Adding a gene to favorites creates a bookmark to that gene in the My Favorites section available within the grey menu bar. In the My Favorites section, users can also add private notes and project descriptions about saved items.
- Practice to export gene records. Click on the **Download Gene** will open up a selection page with available gene records for download, including sequences in FASTA (highlighted):



• Return to the gene record page and look at the **Contents** section on the left. The individual sections comprising the Contents can be quickly identified by using the search box at the top of the Contents menu. In addition, sections in a gene page can be hidden by unchecking the box to the right of any section name in the Contents menu.

• The Gene Models section contains information about the structure of the gene such as exon count, transcript number, annotated UTRs and introns (when available), alternative transcripts, visual GBrowse representation of gene location, *etc.* The color of the gene represents which strand it is encoded on. Red is on the reverse strand (from right to left) and blue is on the forward strand (from left to right). More details are available via the View in genome browser button.

| 1 Gene mode     | ls   |                    |                |             |       |         |
|-----------------|--|--------------------|----------------|-------------|-------|---------|
| # Exons in Gene | 5  |                    |                |             |       |         |
| # Transcripts 1 |  |                    |                |             |       |         |
| ▼ Gene Models   |  |                    |                |             |       |         |
|                 |  |                    | View in genome | browser     |       |         |
|                 | Chr2_A_fumigatus_Af293   |                    |                |             |       |         |
|                 | 3408k 3409k  | 3410k              | 3411k          | 3412k       | 3413k | · · · · |
|                 | Annotated Transcripts ( UTRs in gr<br>Afu2g13250-T(trp8)                     | ay when available) |                |             |       |         |
|                 | Afu2g13260-T(medA)   |                    |                |             |       |         |
|                 | RNASeq evidence for introns (filte<br>Matches Transcript Annotation<br>Novel | red)               | н              | н н         |       | Т       |
|                 | RNRSeq evidence for introns (inclu<br>Matches Transcript Annotation<br>Novel | sive)              |                | н н <u></u> |       | ÷       |
|                 |  |                    | View in genome | browser     |       |         |

• How many introns are reported for this gene?

- 2 • 4
- 9
- Is there any RNA-Seq evidence supporting the intron data? Is there evidence for a possible alternative gene model (introns with RNAseq evidence that are not part of the official gene model)?
- The Annotation, curation and identifiers section offers alternate product descriptions, previous identifiers, and aliases, and is populated using data from internal curation, other fungal specialized resources (*e.g.* AspGD, Ensembl, *etc.*), or user-submitted data (user comments, also see below).

| 2 Anno             | otation, cur                      | ation a         | nd identifiers   |
|--------------------|-----------------------------------|-----------------|--|
| ✓ Alterna<br>No da | ate Product Desc<br>ata available | criptions       | Data sets  |
| Externa            | al DB Version 20                  | 15-09-27        |  |
| Gene N             | Name or Symbol                    | medA            |  |
| ▼ Names,           | , Previous Identi                 | ifiers, and /   | Niases 🕹 Download 🛢 Data sets  |
| Sea                | arch this table                   |                 | Q Showing 4 rows   |
| .↓† N              | Name/ID/Alias                     | <b>↓</b> ↑ Type |  |
| MED                | DA                                | name            |  |
| AFU                | JA_2G13260                        | previous ID     |  |
| AFU                | JB_028890                         | previous ID     |  |
| CAD                | DAFUAG00004866                    | previous ID     |  |
| ▼ Notes f          | from Annotator                    | ≛ Download      | Data sets Q Showing 3 rows   |
| Ut T               | franscript ID(s)                  | <b>↓</b> ↑ Date | ↓↑ Note  |
| Afu2               | 2g13260-T                         | 2010-07-<br>28  | NOTE TYPE=Standard Name NOTE=medA: 19889083 Gravelat FN, et al. (2010) Aspergillus fumigatus MedA governs adherence, host cell interactions and virulence. Cell Microbiol 12(4):473-88 |
| Afu2               | 2g13260-T                         | 2011-01-<br>08  | NOTE TYPE=Sequence Annotation Notes NOTE=Sequence information added to the database.   |
| Afu2               | 2g13260-T                         | 2012-03-<br>06  | NOTE TYPE=Alias Name NOTE=CADAFUAG00004866: AspGD (2012) Update of Aspergillus furnigatus AI293 reference annotation   |

- The Link outs section offers redirection to other resources (*e.g.* CGD, Ensembl, MycoCosm, *etc.*).
  - Find curated information for host-pathogen interactions

Hint: Click on the PHI-Base link out.

|   | Pathogen<br>Gene   | Mutant Phenotype  | Pathog   | jen Species   | Disease                                   |  | Host Species  |
|---|--|---|--|---|---|--|---|
| Ξ | MedA   | reduced virulence   | Aspergil   | rgillus fumigatus Invasive Pulmonary Aspergillosis                                  |   |  | Galleria mellonella (related: greater wax moth)                             |
|   | Pathogen Ger<br>Gene:MedA<br>PHI-base entry<br>Gene ID:EAL93(<br>Protein ID: Q4X<br>Sequence strai<br>Gene function: | PHI:2661<br>620<br>605<br>in:Af293<br>Developmental Regulator |  | Pathogen<br>Pathogen species<br>fumigatus<br>Pathogen ID: 746<br>Pathogen strain II | :Aspergillus<br>128<br>,f293<br>D: 330879 | Host<br>Host spec<br>(related: gr<br>Host class<br>Host ID: 7<br>Tissue:larv | ies:Galleria mellonella<br>eater wax moth)<br>sification:Moths<br>137<br>ra |
|   | Essential gene   | omments   |  | PHI Phenotype   | Pathogen Phenotype                        |  |   |
|   | Pmid:23185496<br>Ref source:Pub<br>Year:2012<br>DOI: 10.1371/jc<br>Author referen                                    | 5<br>med<br>purnal.pone.0049959<br>ccce:Q A Abdallah 2012     | Phenotype:reduce<br>Disease name:lnv<br>Tissue:larva<br>Experimental tec | ed virulence<br>asive Pulmonary Asper<br>hnique:Gene mutatior                       | gillosis<br>n: characteris                | ed; gene complementation   |   |

- What species are mentioned as a host in these manually curated records?
- Is this an essential gene in *A. fumigatus? Hint: Look in the Pathogen Gene section*
- The **Genomic Location** section contains coordinates for a gene or sequence within a chromosome or contig/scaffold and a link out to GBrowse, which is centred on the gene of interest.
- The Literature section offers access to associated publications (internal curation of user comments, direct uploads from other resources The Broad Institute, *etc.*)

• The **Orthology and synteny** section provides a table of Orthologs and Paralogs within FungiDB produced by OrthoMCL (<u>www.orthomcl.org</u>). This section also contains synteny graphs. The table has a search box allowing to create a custom display of orthologs and also deploy the ClustalOmega analysis:





Run ClustalOmega alignment on all sequences and copy .dnd file (without .dnd file line) to build a tree in iTOL (<u>https://itol.embl.de</u>) or other free web application you prefer.

• BLAT is a BLAST-like alignment tool. DNA BLAT database is an index of sequences derived from the assembly of the entire genome. DNA BLAT is designed to quickly find sequences of 95% and greater similarity of length 40 bases or more.



• Click on the **View in genome browser** button to view BLAT and also sequencing reads from *A. fumigatus* isolates.

Note: If you want to zoom in to viewing the B9J08\_000928 only, enter the GeneID in the Landmark or Region search window.



Note: Rates of SNPs can reflect intra-species variation such as more SNPs can occur in less conserved regions while functional regions have fewer SNPs.

• The Genetic Variation section summarizes integrated SNP data for a given region and classifies SNPs into groups based on the resulting effect on gene function: noncoding (white diamonds), non-synonymous (dark blue) or synonymous (light blue), and nonsense (red) nucleotide changes.



*Note: Genetic variation tracks can be explored in GBrowse and SNPs visualized by clicking on the View in genome browser button and activating appropriate tracks from the* **Select Tracks** *tab in GBrowse.* 

- Mouse over the SNP on the gene page to go directly to the SNP record.
- Can you determine the position of the first non-synonymous SNP on the left?



- Determine the minor allele frequency and locate the matching isolate in the strains table
- Are there any geographical data associated with the minor allele isolate/strain?

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| A       a     A1       gdom of the Netherlands     08   | ↓† Strain         | ↓† Sample        | J↑ Allele     | J↑ Allele (gene strand) | J↑ Product | 11 Coverage 🕜 | ↓↑ Read Frequency | J↑ DNA-seq reads for |
| a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           gdom of the Netherlands         B  | Af293 (reference) |                  | G             | c                       | S          |               |                   |                      |
| a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           a         A           gdom of the Netherlands         08  | Afu_1042-09       | EUSMPL0067-1-16  | G             | c                       | S          | 108           | 100               | view DNA-seq reads   |
| a A A A A A A A A A A A A A A A A A A A  | Afu_124-E11       | EUSMPL0067-1-19  | G             | 0                       | S          | 113           | 100               | view DNA-seq reads   |
| a A A A A A A A A A A A A A A A A A A A  | Afu_100-E11       | EUSMPL0067-1-20  | G             | c                       | 3          | 107           | 100               | view DNA-seq reads   |
| a A A A A A A A A A A A A A A A A A A A  | Afu 257-F11       | EUSMPI 0067-1-22 | 6             | c                       | s          | 102           | 100               | view DNA-seq reads   |
| a A A A A A A A A A A A A A A A A A A A  | Afu 343-P-11      | EUSMPL0067-1-17  | G             | c                       | s          | 78            | 100               | view DNA-seg reads   |
| a A A<br>gdom of the Netherlands 08<br>gdom of the Netherlands 08  | Afu_591-12        | EUSMPL0067-1-18  | G             | C                       | s          | 84            | 100               | view DNA-seg reads   |
| gdom of the Netherlands         08           gdom of the Netherlands         10  | Afu_942-09        | EUSMPL0067-1-15  | G             | С                       | S          | 128           | 100               | view DNA-seq reads   |
| gdom of the Netherlands 08<br>gdom of the Netherlands 100  | 08-12-12-13       | EUSMPL0067-1-7   | G             | с                       | s          | 94            | 100               | view DNA-seq reads   |
| gdom of the Netherlands     08   | 08-19-02-10       | EUSMPL0067-1-14  | G             | с                       | s          | 118           | 100               | view DNA-seq reads   |
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| gdom of the Netherlands     08       gdom of the Netherlands     08       gdom of the Netherlands     08       gdom of the Netherlands     10  | 08-19-02-46       | EUSMPL0067-1-13  | G             | С                       | S          | 126           | 100               | view DNA-seq reads   |
| gdom of the Netherlands 08<br>gdom of the Netherlands 08<br>gdom of the Netherlands 10   | 08-19-02-61       | EUSMPL0067-1-10  | G             | С                       | s          | 130           | 100               | view DNA-seq reads   |
| gdom of the Netherlands 08<br>gdom of the Netherlands 10   | 08-31-08-91       | EUSMPL0067-1-9   | G             | с                       | S          | 94            | 100               | view DNA-seq reads   |
| gdom of the Netherlands 10   | 08-36-03-25       | EUSMPL0067-1-8   | G             | с                       | S          | 138           | 100               | view DNA-seq reads   |
|  | 10-01-02-27       | EUSMPL0067-1-12  | G             | с                       | S          | 117           | 100               | view DNA-seq reads   |
| ted Kingdom 09   | 09-7500806        | EUSMPL0067-1-3   | A             | т                       | F          | 90            | 98.8              | view DNA-seq reads   |

• The **Transcriptomics** section (RNA-Seq and microarray data) provides a searchable data table with expandable rows with tabular data, summaries, coverage, expression graphs, and more.

The Expression graph provides an overview of FPKM data, while the **Coverage** section shows uniquely mapped reads. When reads map to several genome locations and therefore could have been derived from multiple transcripts they are labeled as nonunique (shaded in grey).

|  | Transcriptome of wild-type vs veA an<br>mtfA deletion mutants  | type and<br>seconda<br>and Mtf/   | uencing of <i>A. fumigatus</i> wild-<br>I deletion mutants of the<br>ary metabolite regulators, VeA<br>A. | Lind et al.  | RNA-seq               |   |
|--|--|---|---|--------------|-----------------------|---|
| mtfA_fpkm  | - Afu2g13260   |   |   |              |                       |   |
| Dollarshart  | st.  | sounig<br>unique  |   |              |                       |   |
| I Dataset De   | escription   |   |   |              |                       |   |
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| ta table<br>Search this ta<br>Atu2g13260<br>Atu2g13260<br>Atu2g13260<br>Atu2g13260<br>Atu2g13260<br>Atu2g13260           | ablaQ.<br>Li Sample<br>Delta-mtfA TTDS4.1 - mtfA - unique<br>WT CEA10 mtfA matched - mtfA -<br>unique<br>Delta-mtfA TDD54.1 - mtfA<br>WT CEA10 wtA matched - mtfA<br>Delta-veA TSD1.15 - veA - unique<br>Delta-veA TSD1.15 - veA<br>WT CEA10 veA matched - veA | Showing 8 rows<br>12.72<br>13.99<br>12.58<br>13.84<br>13.84<br>13.57<br>7.26<br>12.43 | Li Standard Li Pi<br>Standard Li Pi<br>34.9<br>36.1<br>Detai<br>incluc                                    | rcentile     | ress                  | View in genome browser<br>on values data,<br>and non-unique alignment hit |

- Take a look at the microarray data titled Aspergillus fumigatus hypoxia 0 response transcriptome. Which time point corresponds to the highest expression during stress?
  - i.
  - 2 hr after switch to 0.2% oxygen 6 hr after switch to 0.2% oxygen ii.
  - 12 hr after switch to 0.2% oxygen iii.
  - 24 hr after switch to 0.2% oxygen iv.
- Phenotypes section include manually curated and publicly uploaded data for ٠ mutants (some but now all species).

| nenotype (qualities or directionality + entity or bioligical process) 🛓 Download 🛢 Data sets |                  |                                      |   |                  |                                     |  |  |  |  |  |
|--|------------------|--------------------------------------|---|------------------|-------------------------------------|--|--|--|--|--|
| (Search this table Q) Showing 8 rows   |                  |                                      |   |                  |                                     |  |  |  |  |  |
| ↓↑ Modification  | <b>↓† Allele</b> | <b>↓† Phenotype</b>                  | ↓† Further Information  | <b>↓† PubMed</b> | CHEBI<br>↓↑ Annotation<br>Extension |  |  |  |  |  |
|  | medA::hygR       | abnormal colony color                | Details:delay in brown pigmentation                           | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | decreased amount biological adhesion | Condition:fibronectin coated wells                            | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | decreased amount conidium formation  |   | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | decreased amount virulence           | Virulence model:immunosuppressed mouse pulmonary infection    | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | decreased amount virulence           | Virulence model:insect infection (Galleria mellonella larvae) | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | increased amount cell growth         | Details:slightly larger conidia and<br>conidiophores          | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | increased rate conidial germination  |   | 19889083         |                                     |  |  |  |  |  |
|  | medA::hygR       | viable cell                          |   | 19889083         |                                     |  |  |  |  |  |

• The Sequences, Sequence analysis, Structure analysis sections offer sequence information (DNA, RNA, and protein), an interactive summary of EST alignments and BLAT hits against the nonredundant protein sequence database (NRDB), 3D structure predictions and similar Protein Data Bank (PDB) chains, when available, *etc.* 

| 11 Sequence       | analysis   |   |  |
|-------------------|--|---|--|
| ✓ BLAT Alignments |  | Mauria anama kunuan   |  |
|                   | Chr2_A_fumigatus_Af29                                      | Gl number: 159129715<br>Score: 99.853   |  |
|                   | Afu2g13190-T Afu2g13<br>Afu2g13190-T Afu2g13<br>Afu2g13200 | Location: 3408977 - 3411089<br>Identity %: 100.0<br>Description: transcriptional regulator Meduca (Aspervillus funicatus A1163) | AFU2g13265-T<br>AFU2g13270-T<br>AFU2g13270-T<br>AFU2g13270-T<br>AFU2g13270-T |
|                   | NRDB BLAT Alignments                                       |   |  |
|                   |  |   |  |

- **Protein features and properties** offer protein domains identifications (InterPro), signal and transmembrane predictions graphics, BLASTP hits, and also a selection of tools that can be deployed directly from the gene record page using the amino acid sequence of interest:
  - o BLASTP (protein-protein BLAST) against 8 databases,
  - GPI anchor prediction: big-PI Predictor
  - MitoProt (prediction of mitochondrial proteins)
  - WoLF PSORT (prediction the subcellular localization sites)
- 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.

| Search this table   |                       | Q Sh          | owing 9 rows                |   |             |                     |                                      |
|---------------------|-----------------------|---------------|-----------------------------|---|-------------|---------------------|--------------------------------------|
| ↓↑ Transcript ID(s) | 11 Ontology 😮         | ↓† GO<br>ID Ø | ↓† <mark>Is</mark><br>Not 😮 | J↑ GO Term<br>Name                              | ↓† Source 💡 | ↓↑ Evidence<br>Code | ↓† Reference                         |
| Afu2g13260-T        | Biological<br>Process | GO:0007155    |                             | cell adhesion                                   | AspGD       | IMP                 | AspGD_REF:ASPL0000082448 PMID:198890 |
| Afu2g13260-T        | Biological<br>Process | GO:0007155    |                             | cell adhesion                                   | AspGD       | IMP                 | AspGD_REF:ASPL0000402248 PMID:231854 |
| Afu2g13260-T        | Biological<br>Process | GO:0009405    |                             | pathogenesis                                    | AspGD       | IMP                 | AspGD_REF:ASPL0000082448 PMID:198890 |
| Afu2g13260-T        | Biological<br>Process | GO:0009405    |                             | pathogenesis                                    | AspGD       | IMP                 | AspGD_REF:ASPL0000402248 PMID:231854 |
| Afu2g13260-T        | Biological<br>Process | GO:0044406    |                             | adhesion of<br>symbiont to host                 | AspGD       | IMP                 | AspGD_REF:ASPL0000082448 PMID:198890 |
| Afu2g13260-T        | Biological<br>Process | GO:0048315    |                             | conidium<br>formation                           | AspGD       | IMP                 | AspGD_REF:ASPL0000402248 PMID:231854 |
| Afu2g13260-T        | Biological<br>Process | GO:0075307    |                             | positive regulation<br>of conidium<br>formation | AspGD       | IEA                 | AspGD_REF:ASPL0000000005             |
| Afu2g13260-T        | Cellular<br>Component | GO:0005634    |                             | nucleus   | AspGD       | IDA                 | AspGD_REF:ASPL0000402248 PMID:231854 |
| Afu2g13260-T        | Molecular<br>Function | GO:0003674    |                             | molecular_function                              | AspGD       | ND                  | AspGD_REF:ASPL0000111607             |

• Based on the AspGD manual curation data described in this section how many unique GO terms are used to describe the **Biological process**?

Note: Gene ontology provides statements for describing the functions of genes along 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: GO terms that are automatically assigned by InterPro2GO, GO terms assigned by FungiDB curators, and GO terms obtained from external resources such as AspGD, MIPS, and others.

GO Slim is a subset of high-level terms from Gene Ontology that provides a broader overview of annotations. GO Slim table provides all annotated GO terms/IDs for a gene with the associated top-level GO Slim terms/ IDs. If you click on the individual GO terms you will be redirected to AmiGO 2 site that contains detailed information for GO terms and their hierarchy. Here is an example of Inferred Tree of go terms that are "child" or component terms for cell adhesion (GO:0007155):



• **Pathways and interactions** provide descriptions of 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.

• The **Proteomics** section is populated when Mass Spec. evidence data and phosphoproteomics datasets become available in FungiDB. This data is linked to peptide alignments against the reference genome in GBrowse and also provides a graphic summary of proteomic datasets. Here is an example for Afu2g04620:





• **Immunology** – primarily geared towards host response dataset and contains predicted epitopes from The Immune Epitope Database (IEDB).

# **FungiDB Genome Browser**

### 1. Accessing Genome Browser - GBrowse

To access the Genome Browser features from the gene page click on the **View in genome browser** button:



The Genome Browser default window offers quick access to (see figure on the next page):

1. File sharing and export options

Note: if you modified the default GBrowse window by loading custom tracks or other tracks from FungiDB and would like to share this window with a colleague, you must generate a custom URL address. Copy and paste of the default page url will display the default view only. To create a custom url, navigate to File, Generate URL.

- **2.** Tabs
  - The main **Browser** window, Select Tracks tab where you can activate custom tracks (RNA-Seq or microarray data, SNPs, *etc.*)
  - Snapshots section provides a quick access to snapshots taken while accessing data in various GBrowse sessions
  - **Custom tracks** custom data files (bam or BigWig) uploaded directly from your computer or the EuPathDB Galaxy instance
  - Preferences where you can update GBrowse appearance settings



3. The four main features of the GBrowse window are Landmark or Region that provides information about this gene location and allows you to move to other regions by changing the location or entering a specific Gene ID. The Overview section displays the entire genomic sequence with a marker (red vertical lines) indication your current location. The Region displays the more immediate portion of the genome surrounding the region of interest. The Details section provides a closeup view of the region of interest. Scroll and zoom can be also done using a pull-down menu in the Scroll/Zoom section at the top of the Overview track.





**4.** Each track has 5 track-level controls that can be used to change the display of tracks or remove a track from the GBrowse window.

### 2. Understanding GBrowse display

Navigate to the GBrowse view of the Afu2g13260.

To activate different tracks that are preloaded in FungiDB click on the **Select Tracks** tab and activate 1 RNA-Seq track in addition to default activated **RNASeq evidence for introns** and **Annotated Transcripts tracks** as shown in the figure below:

| Brow       | ser Select Tracl       | ks Snapshots Custom Track                 | ks Preferences            |  |  |
|------------|------------------------|---|---------------------------|--|--|
| << Back    | k to Browser           | Show Active Tracks Only                   | Show Favorites Only 🚖     | Clear All Favorites 🏠                                |  |
| Searc      | th for Specific Tracks |   |                           |  |  |
| Se         | arch:                  | Stick to top when scrolle                 | d                         |  |  |
| Clear      | Search                 |   |                           |  |  |
| Track      | 5                      |   |                           |  |  |
| - Cu       | stom Tracks            |   |                           |  |  |
| <b>1</b> 0 | ene models 🛯           |   |                           |  |  |
| •          | Introns All on All or  | ff  |                           |  |  |
|            | 😭 🗆 RNASeq evidenc     | e for introns (All) [?] [showing 2/2 subt | tracks]                   | e for introns (filtered) [?] [showing 2/2 subtracks] | ☆ SRNASeq evidence for introns (inclusive) [?] [showing 2/2 subtracks] |
| •          | Transcriptional regula | tory sites All on All off                 |                           |  |  |
|            | 😭 🗆 Transcription star | rt sites (A. nidulans) [?] [showing 2/2 s | ubtracks]                 |  |  |
|            | Transcripts All on     | All off                                   |                           |  |  |
|            | Annotated Transc       | cripts ( UTRs in gray when available) [?  | ?] 🛛 😭 🖸 Gene Density (in | Overview) [?]  |  |
|            | 😭 🖬 Gene Density [?]   |   | 😭 🖬 Gene Density (in      | Region) [?]  |  |

As soon as new tracks are selected they begin to load in the Browser tab. Go back to the Browser tab to view the newly activated tracks.

mRNA tracks can provide a comprehensive overview of changing transcriptome environment in response to various stimuli (light, sexual cycle, drug treatment, *etc.*). Visualization of RNA reads aligned against the reference genome may also shed light on transcript diversity, quality of genome annotation and provide evidence for differentially-spliced transcript isoforms and previously-unrecognized coding and non-coding RNAs.



All RNA-Seq datasets integrated in FungiDB are processed through a pipeline generating intron splice site predictions based on available RNA-Seq data. Several parameters are calculated to provide support for the intron tracks:

| RNASeq Unified Splice Site Junctions (All)  |  |  |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|--|--|
| Click here to display in new  | window   |  |  |  |  |  |  |  |  |
| Note that annotated introns are indicated with bold (wider) glyphs.   |  |  |  |  |  |  |  |  |  |
| Intron Spanning Reads (ISR): The total number of uniquely mapped reads (all samples) which map across the junction and are on the appropriate<br>strand. GSNAP uses splice site consensus sequences to determine strand of the mapped read. |  |  |  |  |  |  |  |  |  |
| ISR per million (ISRPM): I  | ntron Spanning Reads Per Million intron s  | panning reads and thus represents a normalized count of unique reads.                  |  |  |  |  |  |  |  |
| % of Most Abundant Intro<br>maximum for this gene.  | n (MAI): The percentage (ISRPM of this j   | unction / ISRPM of maximum junction for this gene) of this junction over the           |  |  |  |  |  |  |  |
| Most abundant in: The exp   | periment and sample that has the highest   | ISRPM for this gene.   |  |  |  |  |  |  |  |
| ISRPM, (ISR / coverage):  | SRPM from sample with highest ISRPM a  | and the ISB/coverage for that same sample.   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.  | nents and samples that provide evidence  | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan   | nents and samples that provide evidence  | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse  | nents and samples that provide evidence<br>ges with the Score as follows:<br>Forward   | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5   | nents and samples that provide evidence<br>ges with the Score as follows:<br>Forward<br>less than 5  | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15   | nents and samples that provide evidence<br>ges with the Score as follows:<br>Forward<br>less than 5<br>5-15  | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15<br>17-64  | nents and samples that provide evidence<br>ges with the Score as follows:<br>Forward<br>less than 5<br>5-15<br>17-64   | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15<br>17-64<br>65-256  | evidence is and samples that provide evidence is ges with the Score as follows:  Forward  less than 5 5-15 17-64 65-256  | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024  | rents and samples that provide evidence is<br>ges with the Score as follows:<br>Forward<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024                            | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024<br>1025-4096   | rents and samples that provide evidence is<br>ges with the Score as follows:<br>Forward<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024<br>1025-4096               | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |
| The table shows all experin<br>sample.<br>The color of the glyph chan<br>Reverse<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024<br>1025-4096<br>4097-16000   | rents and samples that provide evidence is<br>ges with the Score as follows:<br>Forward<br>less than 5<br>5-15<br>17-64<br>65-256<br>257-1024<br>1025-4096<br>4097-16000 | for this intron junction. Note that the values for each row are based on each specific |  |  |  |  |  |  |  |

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 shown here, and red for a gene located on the reverse strand). In the example shown here the large intron has 100% abundance in the integrated RNA datasets. A pop-up window can be initiated by hovering over the intron and will include all supporting documentation generated by in-house automated pipelines:

| E B B 2 B B 2 B RASeq evidence for introns (filtered) (Showing 2 of 2 subtracks)     Matches transcript Annotation     H     H      Foreignment of the state | , F          | Intron Location:<br>Intron Spanning Reads (ISR):<br>ISR per million (ISRPM):<br>Gene assignment:<br>% of Most Abundant Intron<br>(MAI):<br>Most Abundant in:<br>ISRDM //SR / cape | 986556 - 987162 (607<br>8573<br>2744.88<br>NCU02175 - annotate<br>100<br>N. crassa transcripto<br>perithecia 50¢f stc-1<br>255 52 ( 8065) | nt)<br>d intron<br>mes during vegetative | and sexu | ual devel | opment s | ×        |
|---|--------------|---|---|--|----------|-----------|----------|----------|
|   |              | coverage)   | 635.53 (.8985)  |  |          |           |          |          |
|   |              | Experiment  |   | Sample                                   | Unique   | ISRPM     | ISR/Cov  | %<br>MAI |
|   |              | Characterization of light regulate  | ed genes  | WT light 15 min                          | 4        | 2.66      | .4829    | 44.4     |
|   |              |   |   | WT light 60 min                          | 9        | 4.06      | .9948    | 50       |
|   |              |   |   | WT light 120 min                         | 7        | 4.18      | .5455    | 53.8     |
|   |              |   |   | WT light 240 min                         | 7        | 5.74      | .6561    | 53.8     |
|   |              |   |   | WT dark                                  | 12       | 7.74      | .9134    | 92.3     |
|   |              | Conserved and Essential Trans<br>Cellulase Gene Expression  | cription Factors for  | cdr1Del Avicel 1h                        | 50       | 12.95     | .8081    | 100      |
|   |              |   |   | cdr1Del Avicel 4h                        | 48       | 23.81     | .5809    | 90.6     |
| ★ ■ ⊠ I Annotated Transcripts (UTRs in gray when available)<br>NCU02175-t26_1(inl-15)   |              |   |   | cdr1Del Sucrose 1h                       | 18       | 4.56      | .7728    | 100      |
|   |              | 1   |   | cdr1Del Sucrose 4h                       | 56       | 17.56     | .7232    | 90.3     |
|   | Select Track |   |   | cdr2Del Avicel 1h                        | 23       | 7.39      | .4555    | 57.5     |
|   | Select Hack  |   |   | cdr2Del Avicel 4h                        | 56       | 28.83     | .6925    | 74.7     |
| from File menu (above) and cut and paste resulting UBI  |              |   |   | cdr2Del Sucrose 1h                       | 29       | 7.16      | .991     | 82.9     |
|   |              |   |   | cdr2Del Sucrose 4h                       | 44       | 15.32     | .4309    | 61.1     |
| nings:<br>data was mapped   |              |   |   | WT Avicel 1h                             | 24       | 9.14      | .5841    | 60       |
|   |              |   |   | WT Avicel 2h                             | 9        | 12.65     | .9832    | 81.8     |
|   |              |   |   | WT Avicel 30m                            | 10       | 14.11     | .6799    | 100      |
|   |              |   |   | WT Avicel 4h                             | 39       | 21.45     | .8155    | 88.6     |
|   |              |   |   | WT No Carbon 1h                          | 26       | 9.67      | 1.0058   | 100      |
| EuPathDB 3  | · 🔘 🗸 🥐      | l   |   | WT No Carbon 4h                          | 127      | 24.45     | .4336    | 60.8     |

### 3. Accessing Genome Browser- JBrowse (BETA)

In addition to GBrowse, FungiDB has also implemented JBrowse that can be accessed directly from the gene records pages:



Click on *Select tracks* tab to view the list of available datasets. To return to the JBrowse main page click on *Back to browser* button. The pull-down tab next to individual track offer additional customization choices to create custom view of integrated data:



#### Shortcuts