Module 5 NCBI's Zebrafish Resources





Workshop Overview

- How to access information for a known gene
- Viewing genes in context of a Genome Browser
- Managing data tracks in the Genome Browser
- BLAST within the genome browser
- Comparative genomics
- NCBI resources for other fish species
- Additional NCBI resources

How do I search NCBI for my gene of interest?

- Start from the NCBI homepage (<u>www.ncbi.nlm.nih.gov</u>)
- Choose the 'Gene' database from the dropdown menu
- Query by organism name combined with gene name or gene symbol
 - Example: enter 'Zebrafish pitx2'

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Search results are displayed as a tabular list of Gene records that correspond to a single gene from a specific organism. The table can be further sorted by defined filters in the left column or by taxon on the right column.

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What resources will I find on the Gene page?

The Gene record provides access to gene-specific information including nomenclature, reference sequences, genomic sequence viewer, publications and functional data.



How do I access the Reference Sequences (RefSeqs) for my gene of interest?

- Expand the 'NCBI Reference Sequences (RefSeq)' section of the Gene record
- Expanded view shows both curated (NM_,NR_ and NP_) and model (XM_, XR_ and XP_) RefSeqs



Nucleotide record provides access to FASTA sequence, feature annotation and sequence analysis tools – BLAST and Genome Data Viewer

Exploring the Genomic context section of the Gene Page

The default view of the 'Genomic regions, transcripts and products ' section of the Gene page which shows the gene annotation on the current reference assembly. This section provides a quick link to NCBI's Genome browser as well as access to download and BLAST the Refseqs.

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How do I search for my gene of interest in NCBI's Genome Data Viewer (GDV)?

Gene searches can also be performed in NCBI's new genome browser which offers easy access to the expansive range and number of organisms and assemblies represented in the GDV browser.

- Starting from the landing page
 - Select an organism by entering an organism name (common or scientific) in the search box
 Example: 'Zebrafish'
 - Or select an organism from the tree. More organism can be exposed by clicking on the nodes within the tree
 - The knowledge panel to the right will update to the selected organism



Using GDV's knowledge panel

The GDV knowledge panel provides multiple modes of access to the genome browser

- Search by query term. The search box accepts a broad range of search terms such as gene names and symbols, protein names, dbSNP ids (human only), phenotypes or sequence accessions.
 - Example query 'pitx2'
- Select Assembly version 'GRCz11' which defaults to the most current reference assembly
- Alternatively, use the 'Browse Genome' button which allows for searching directly in the genome browser view
- The 'BLAST Genome button allows for a nucleotide or translated protein search against the selected genome



What is displayed in the GDV default view?

A text search for 'pitx2' returns the default GDV browser view zoomed into the pitx2 gene on chr 14. The browser display consists of a series of widgets on the left that control the graphical display on the right. You can customize the look and feel of the control panel. Use the toggle icon found at the left side of each widget header to collapse or expand the various panels. Re-order the panels by clicking in the headers and dragging and dropping them into their desired location. The Sequence Viewer displays the chromosomal location and the annotated gene features.



Sequence viewer panel shows the current annotation of the selected gene along with default tracks for Ensembl's annotation and RNA-seq intron and exon aggregate reads

How do I adjust my view within sequence viewer?

- 1. Exon navigator allows you to jump between gene features within a view or between exons
- 2. The 'Show All' icon 📰 expands the green gene bar to show transcripts (purple) and proteins (red)
- 3. 'Tools' allows you to adjust the gene orientation, view sequence text, and create pdfs
- 4. RefSeq annotation default view shows both model and curated RefSeqs
- 5. RefSeq transcripts shaded in grey indicate discrepancies with the reference genome
- 6. Ensembl annotation
- 7. RNA-seq exon and intron aggregate tracks
- 8. Clicking on the orange 'x' removes the data track



How do I display additional data tracks?

In addition to the default display, you can view other data tracks by selecting the 'Tracks' button which opens a dialog box containing additional data tracks. In the example shown, the 'Expression' tab offers the choice of tissue-specific RNA-seq intron and exon reads.

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How do I view protein and mRNA feature annotation?

Gene features can be projected onto the transcript and protein products by selecting the 'Genes' tab within the 'Tracks' menu then highlighting 'Genes, Known RefSeqs, NCBI Danio rerio Annotation release 106 ' and using the 'Track Settings' section to configure the display. Selecting 'Product Features' option displays feature annotation in the GDV Sequence Viewer.







How can I view RefSeq annotation on a previous version of the reference assembly?

RefSeq annotations on previous versions of the reference assembly can be viewed using the 'Comparative genomics' tab and selecting previous assemblies. The annotations can be projected onto the assembly alignment using the 'Project features' setting.

In some cases cross-species alignments can be provided if the assemblies are similar enough.

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How can I use GDV to view BLAST results in a graphical display?

GDV recently added BLAST functionality to the genome browser. Clicking on the 'BLAST genome' button opens a BLAST page that offers blastn, tblastn and tblastx searches against the selected reference assembly. The BLAST results page provides a direct link to the GDV alignment.



How do I visualize my BLAST results in GDV? Using the 'BLAST Genome' button automatically populates the BLAST widget with recent BLAST searches. In this example we selected RID: HXPYZA2P015 which is then displayed in the GDV Sequence Viewer Genome Data Viewer Ideogram View Search The 'Tools' dropdown menu takes you to Your Data the BLAST page to run a new search BLAST HXPYZA2P015 (NM_131311.2) Tools New BLAST \sim Show BLAST Copy RID Details MH Alignments Ð BLAST alignment results are grouped by Query Hit location Score Identity chromosomal location and can be sorted by Chr9 (NC 007120.7) any column or filtered by identity 1 > 4843.400.5K (-) 874 100% $484 \gg 671$ 3.399.6K (-) 340 100% $523 \gg 670$ 3,388.9K (+) 80% 133 The 'accessions menu' allows you to quickly $668 \gg 1,704$ 3.397.9K (-) 1871 100% jump within the table to specific sequence $1.173 \gg 1.211$ 46.249M (-) 82% 39 on which alignments are found. Chr1 (NC 007112.7) 169 > 30430.979M (-) 84 73% $484 \gg 675$ 30.979M (-) 158 78% 666 > 76730.979M (-) 90 79% Sequence Ident Cover $1,173 \gg 1,206$ 21.697M (-) 45 88% ✓ Chr9 (NC_007120.7) 100% 100% $1,173 \gg 1,209$ NW_018394493.1 79% 25% 22.100M (-) 41 83% Chr1 (NC_007112.7) 79% 25% $1,173 \gg 1,206$ 3,409.6K (-) 39 85% Chr12 (NC_007123.7) 87% 12% NW 018394493.1 Chr19 (NC_007130.7) 77% 11% Chr3 (NC_007114.7) 71% 8% $169 \gg 304$ 135.57K (-) 84 73% Chr14 (NC_007125.7) 74% 4% $484 \gg 675$ 135.09K (-) 158 78% Chr13 (NC_007124.7) 79% 3% Chr17 (NC_007128.7) 84% $666 \gg 767$ 2% 134.83K (-) 90 79% Chr19 (NC 007130.7) $484 \gg 672$ 41.471M (-) 147 77% 518 > 671 44 ACCM (-) 700/ 70 Only alignments on the current assembly are shown Add Tracks

Viewing the BLAST result in the Sequence Viewer?

Continuing with this example (RID: HXPYZA2P015) we will use the 'alignment inspector' to select a BLAST hit to view in the Sequence Viewer.



How can I use the BLAST widget to search for paralogs?

The BLAST widget can also be used to search to location of lower quality hits that may represent paralogs or gene family members. In this example the dlx2a transcript, NM_131311.2 (RID# HXPYZA2P015) was searched against the zebrafish reference assembly. Additional genomic alignments can selected from the Ideogram view or from the BLAST widget.



How do I access NCBI's resources for other fish species?

Access to RefSeq annotated genomes for other fish species can be found at

<u>https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/</u>. Genomes are organized by taxonomic groups. Opening the dropdown menu for each group provides a species list and links to FTP, BLAST (**B**), a detailed annotation report (**AR**) and the Genome Data Viewer (**GDV**).

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Searching NCBI's Assembly database?

NCBI's Assembly database is an archival database of submitted genome assemblies across a broad range of organisms. The database also tracks changes to assemblies that are updated by submitting groups over time with a versioned Assembly accession number. The Assembly interface also provides easy access to download genomic datasets.

Example:

- Enter 'fishes' in the Assembly search field
- Use the filter on the left side to select 'Latest RefSeq'
- Select one or multiple assemblies
- Use the 'Download Assemblies' button to Select 'RefSeq' or 'GenBank' datasets
- Use the file type dropdown menu to select from a selection of FTP download choices





Using the Assembly database to find more information on a submitted assembly?

The individual assembly pages provide information on the structure and quality of the assembled genome in addition to assembly names and other meta-data, statistical reports, and links to genomic sequence data.

In this example the Assembly page for coho salmon (Oncorhynchus kisutch) is shown.



How do I use NCBI resources to find orthologs?

When available, ortholog sets can be found as a link on the Gene record. Clicking on 'all' opens a tabular list of Gene records for all orthologs within the set. The entire ortholog dataset can be downloaded as a nucleotide or protein FASTA file by following links to the Nucleotide and Protein databases using the 'related data' dropdown menu to the right of the Gene search page.



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How do I use BLAST in GDV to locate orthologs of my gene?

Potential orthologs can be searched using the BLAST widget which supports translated protein sequence to the genomic sequence (tblastn). In this example the zebrafish MyD88 protein, NP_997979.2 (RID # HYBHRHS9014) was searched against the coho salmon (*Oncorhynchus kisutch*) reference assembly.

Job title: ref NP_997979.2 (284 letters	9							
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Coming Soon to the GDV browser

- Load your own data
 - For example: GFF3, BED, BAM formats
- Track Hubs
 - Search Track Hub Registry from GDV



Additional NCBI resources

- Bulk Data Download
 - FTP
 - <u>ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/</u>
 - <a>ftp://ftp.ncbi.nlm.nih.gov/refseq/
 - NCBI Genome Pages provides direct access to datasets <u>https://www.ncbi.nlm.nih.gov/genome/?term=danio+rerio</u>
 - NCBI Assembly Database allows for bulk download of data across multiple assemblies. https://www.ncbi.nlm.nih.gov/assembly/?term=Neopterygii
- Programmatic access Eutils/Edirect
 - https://www.ncbi.nlm.nih.gov/books/NBK25500/
 - <u>https://www.ncbi.nlm.nih.gov/books/NBK179288/</u>
 - YouTube Videos <u>https://www.youtube.com/user/NCBINLM</u>
 - Tutorials
 <u>ftp://ftp.ncbi.nih.gov/pub/factsheets/Factsheet_bulk_download.pdf</u>

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- <u>https://ncbiinsights.ncbi.nlm.nih.gov/</u>
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Contact Us!

NCBI Help Desk https://support.nlm.nih.gov

RefSeq https://www.ncbi.nlm.nih.gov/projects/RefSeq/update.cgi

Nuala O'Leary – RefSeq Curator, Zebrafish Champion <u>olearyna@ncbi.nlm.nih.gov</u>

Thank you.

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RefSeq/Gene

Terence Murphy Eric Cox Catherine Farrell Tamara Goldfarb Diana Haddad John Jackson Vinita Joardar Kelly McGarvey Michael Murphy Nuala O'Leary

RefSeq Developers

Alex Astashyn Olga Ermolaeva Vamsi Kodali Craig Wallin Shashi Pujar Bhanu Rajput Sanjida Rangwala Lillian Riddick Barbara Robbertse Brian Smith-White Pooja Strope Anjana Vatsan David Webb

Annotation Pipeline

Francoise Thibaud-Nissen Paul Kitts Mike Dicuccio Wratko Hlavina Avi Kimchi

Jinna Choi Boris Kiryutin Patrick Masterson Eyal Mozes Anton Perkov Dan Rausch Robert Smith Alexandre Souvorov

GDV/Remap/GBench

Valerie Schneider Peter Meric Nathan Bouk Hsiu-Chuan Chen Cliff Clausen Anatoliy Kuznetsov Andrei Shkeda

A cast of thousands

Ken Katz Michael Ovetsky Lukas Wagner Andrei Shkeda Donna Maglott **Kim Pruitt** Jim Ostell