

RNA sequence data analysis via VEuPathDB Galaxy, Part I

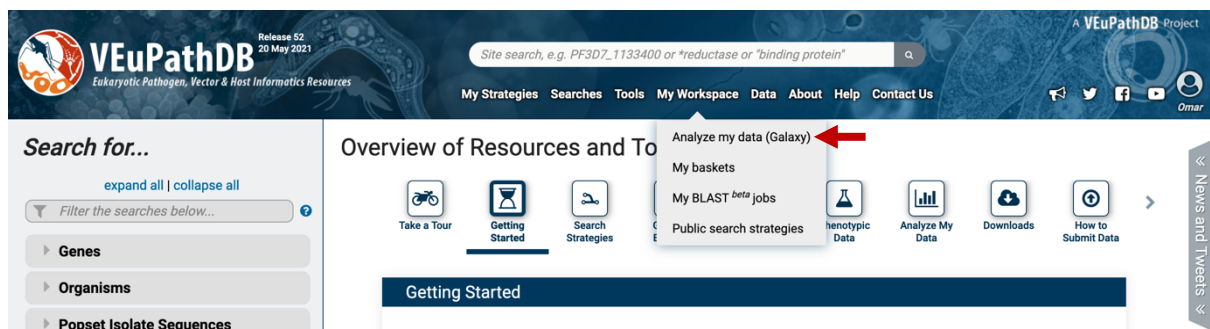
Uploading data and starting the workflow (Group Exercise)

Learning objectives:

- Become familiar with VEuPathDB Galaxy workspace
- Import data from EBI to the VEuPathDB Galaxy
- Create collections of datasets
- Run a pre-configured RNA-Seq workflow

VEuPathDB Galaxy-based workspace offers pre-loaded genomes, private data analysis and display, and the ability to share and export analysis results and also import certain datasets into private workspace within VEuPathDB (My Datasets section).

VEuPathDB Galaxy workspace can be accessed from the *My Workspace* tab on the home page of FungiDB or any other VEuPathDB site. To log in, users must have an account with FungiDB/VEuPathDB, which is free. After an account is created, users



receive access to the VEuPathDB Galaxy services and tools.

The Galaxy instance is not meant for long-term data storage. Datasets are automatically deleted after 60 days or when the total quota for all projects is reached. To save your data, download your analysis results locally and then *delete and purge* files to free up space for your next analysis.

Galaxy is an open, web-based platform for data intensive biomedical research. Galaxy allows you to perform, reproduce, and share complete analyses without the use of command line scripting. VEuPathDB developed its own Galaxy instance in collaboration with Globus Genomics. Many resources are available to learn how to use Galaxy. The following link has information about additional resources to help you learn how to use Galaxy:

https://wiki.galaxyproject.org/Learn#Galaxy_101

For this exercise, we will retrieve raw sequence files from a repository, assess the quality of the data, and then run the data through a workflow (or pipeline) that will align the data to a reference, calculate expression values and determine differential expression. Part 1, uploading data and starting the workflow will be performed today. The workflows will run overnight and we will view / interpret the results tomorrow in Part 2.

We will be working in groups. Each group will have 4-6 members. One person in the group will run the Galaxy controls on one computer. The other members' roles are to ensure that the correct datasets are used and that the correct workflow parameters are selected.

*****IMPORTANT***** During workshop we will NOT be using live sites to access VEuPathDB Galaxy. Use the link below to log in to the workshop VEuPathDB Galaxy with your FungiDB account. If you already have an account with any other VEuPathDB site, this log in will work in FungiDB. If you are creating a new account - remember your password!

Section I: Setting up your VEuPathDB Galaxy account

Step 1: Access the VEuPathDB Galaxy instance at the following URL:

<https://veupathdb1.globusgenomics.org/>

Step 2: On the next page you will be asked to define your organization. Choose VEuPathDB and click Continue.

Log in to use eupathdbworkshop

Use your existing organizational login

e.g., university, national lab, facility, project

VEuPathDB

Didn't find your organization? Then use [Globus ID to sign in](#). ([What's this?](#))

Continue

Step 3: If you are not already logged into VEuPathDB you will be prompted to do so now.



Please log in

Email:

Password:

Login

Cancel

[Forgot Password?](#)

[Register/Subscribe](#)

Step 4: Click on “continue” on the next page (no need to link an existing account).

Welcome – You've Successfully Logged In

This is the first time you are accessing Globus with your **EuPathDB** login.

If you have previously used Globus with another login you can link it to your **EuPathDB** login. When linked, both logins will be able to access the same Globus account permissions and history.

Continue

Link to an existing account

[Why should I link accounts?](#)

Step 5: on the next window select the “non-profit” option and agree to the Terms of Service. Click continue.

Complete Your Sign Up For
[redacted]@eupathdb.org

Name [redacted]

Email [redacted]

Organization test account*

Account will be used for
☒ non-profit research or educational purposes
☐ commercial purposes

☒ I have read and agree to the Globus [Terms of Service](#) and [Privacy Policy](#).

Continue

* This field is specified by the identity provider, and cannot be modified by Globus. If you change it with your identity provider, it will propagate to Globus the next time you log in.

Step 6: The next page will ask for permissions required to use this Galaxy instance. Click on “Allow”

Step 5: Congratulations, you are in!

eupathdbworkshop would like to:

- ☒ Know who you are in Globus. ⓘ
- ☒ Know some details about you. ⓘ
- ☒ Transfer files using Globus Transfer ⓘ
- ☒ Know your email address. ⓘ

To work, the above will need to:

- ☒ View your identities on Globus Auth ⓘ
- ☒ Manage your Globus Groups ⓘ

By clicking "Allow", you allow **eupathdbworkshop** (this client has not provided terms of service or a privacy policy to Globus) to use the above listed information and services. You can rescind this and other [consents](#) at any time.

Allow

Deny

The anatomy of the VEuPathDB Galaxy landing page.

The workspace has four major components:

- a) the top menu controls the main interface
- b) the left panel has a list of available tools
- c) the main welcome page is the interactive interface that houses pre-configured workflows, workflows editor, etc.
- d) the right panel provides access to histories, deleted datasets, and other useful functions

The menu at the top helps to access the landing page, public and private workflows & more.

Main landing page with pre-configured VEuPathDB workflows that also serves as an interactive interface for creating and deploying workflows

Tools to data export into VEuPathDB sites

Sample workflows section

Section featuring available tools. Don't see a tool? – Let us know by sending an email to help@fungidb.org

The history section provides access to workflow history, and much more, including options to delete and purge datasets

Section II: Importing data to Galaxy

There are multiple ways to import data into your Galaxy workspace. For this exercise, we will use the **'Get Data via Globus from the EBI: server using your unique file identifier'**

tool and enter the sequence repository sample IDs based on your group assignments (below). *Remember only one person in your group will be running the workflow.* Although all group members can sign up for an account for later use,

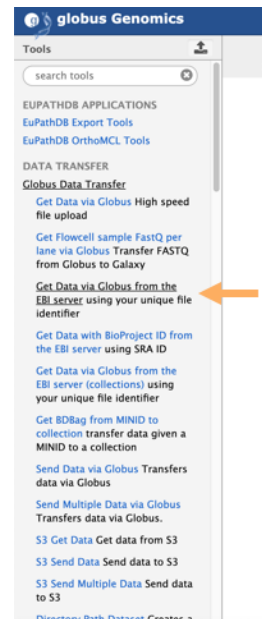
please only one person should start a workflow today because we do not want to overload the servers. The samples below were all generated by paired end sequencing; hence each sample ID will result in transferring two files to your galaxy history. The files are fastq files that are compressed (that is why they end in .gz = gzip).

Group assignments:

See separate group assignment sheet

Step 1: Click on the “Globus Data Transfer” link in the left-hand menu. This will reveal a list of options; click on “Get Data via Globus from the EBI server”. ***important: do not select the option for transferring a collection.

Step 2: In the middle section enter the sample ID and choose whether the run was single or paired end. Click on Execute. Note that the sample ID resulted in importing two files one for each pair. Repeat this process for each sample you want to import. *If you are working with samples from two conditions and the experiment was done in triplicate and paired end sequenced then you should end up with 12 files; six from each condition.*



Get Data via Globus from the EBI server using your unique file identifier (Galaxy Version 1.0.0) Options

Enter your ENA Sample id

SAMN06339669 ←

i.e. SAMN00189025

Data type to be transferred

fastq

Single or Paired-Ended

Paired ←

✓ Execute

WARNING: Be careful not to exceed disk quotas!

✓ 1 job has been successfully added to the queue – resulting in the following datasets:

1: SRR5260546_1.fastq.gz

2: SRR5260546_2.fastq.gz

You can check the status of queued jobs and view the resulting data by refreshing the History pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

Complete

2: SRR5260546_2.fastq.gz

1: SRR5260546_1.fastq.gz

In process

4: SRR5260545_2.fastq.gz

3: SRR5260545_1.fastq.gz

History

search datasets

Unnamed history

2 shown

(empty)

2: SRR5260546_2.fastq.gz

1: SRR5260546_1.fastq.gz

Step 3: If you are working with a dataset with biological replicates it is useful to organize the different conditions of your experiment into “Collections”. For example, if your experiment included RNAseq from *Anopheles stephensi* males (three biological replicates) and females (three biological replicates), it is useful to organize these into two collections, one that includes all male insect files and the other that includes all the female files. Using collections also reduces the complexity of the Galaxy workflows. See below:

The image shows a sequence of five steps to create a dataset collection in Galaxy:

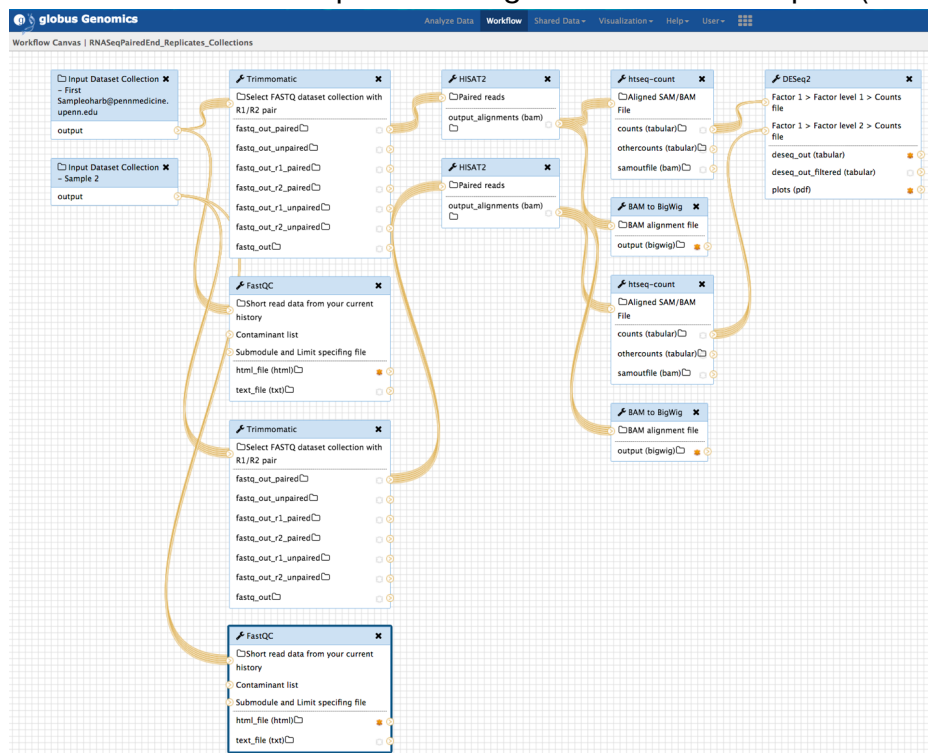
- Step 1:** A context menu is open over a list of datasets. The option "Operations on multiple datasets" is highlighted with a red box and arrow.
- Step 2:** A list of datasets is shown. Several files are selected with checkboxes. A red box and arrow point to the selection.
- Step 3:** A dropdown menu is open for the selected datasets. The option "Build a list of dataset pairs" is highlighted with a red box and arrow.
- Step 4:** A table of dataset pairs is shown. The first pair is highlighted, and a dialog box prompts the user to "Enter a new name for the pair:". The name "male2" is entered in the input field.
- Step 5:** A form to "Create a collection of paired datasets" is shown. The "Name" field is filled with "Miles", and the "Hide original elements" checkbox is checked. A red box and arrow point to the "Create list" button.

Steps to create a collection. **1.** Click on the checkbox function "operation on multiple datasets". **2.** select fastq files that belong to the same condition. **3.** Click on the "For all selected" option and select "Build a list of dataset pairs". **4.** Give each pair a name by clicking on the ID in the middle then renaming it (eg. male1, male2, male3 etc...). **5.** Give the collection a name and select the checkbox "hide original elements", then click on create list.

Section II: Running a workflow in Galaxy

You can create your own workflows in galaxy based on your needs. The tools in the left section can all be added and configured as steps in a workflow that can be run on appropriate datasets. For this exercise we will use a preconfigured workflow that does the following main things:

1. Analyzes the reads in your files and generates FASTQC reports.
2. Trims the reads based on their quality scores and adaptor sequences (Trimmomatic).
3. Aligns the reads to a reference genome using HISAT2 and generates coverage plots.
4. Determines read counts per gene (HTSeq)
5. Determines differential expression of genes between samples (DESeq2).



Additional resources:

Galaxy Project (<https://usegalaxy.org/>)

Trimmomatic manual

FastQC

HISAT2

HTseq

DEseq2

To use one of the VEuPathDB preconfigured workflows, go to the Galaxy home page and select the workflow that you would like to run. For this exercise **“Workflow for paired-end unstranded reads”** – click on this workflow to run it (See A in figure below).

globus Genomics

Analyze Data Workflow Shared Data Visualization Help User

Using 1.1 TB

Tools

search tools

VEUPATHDB APPLICATIONS

VEuPathDB Export Tools
VEuPathDB OrthoMCL Tools
VEuPathDB RNA-Seq Tools

DATA TRANSFER

Globus Data Transfer

Get Data via Globus High speed file upload
Get Flowcell sample FastQ per lane via Globus Transfer FASTQ from Globus to Galaxy
Get Data via Globus from the EBI server using your unique file identifier
Get Data with BioProject ID from the EBI server using SRA ID
Get Data via Globus from the EBI server (collections) using your unique file identifier
Get BDtag from MINID to collection transfer data given a MINID to a collection
Send Data via Globus Transfers data via Globus
Send Multiple Data via Globus Transfers data via Globus
S3 Get Data Get data from S3
S3 Send Data Send data to S3
S3 Send Multiple Data Send data to S3

Get started with VEuPathDB pre-configured workflows:

OrthoMCL

This workflow uses BLASTP and the OrthoMCL algorithm to assign your set of proteins to OrthoMCL groups. Version OG6r1 is the latest set of groups (as of April 2020), but you can also select the previous set (OG5). Explore this [OrthoMCL workflow tutorial](#) to learn more.

- Workflow to map your proteins to OrthoMCL groups

RNA-seq

Use the following workflows to analyze your FASTQ files. The workflows reads, FASTQC for sequencing statistics, and HISAT2 for mapping reads workflow based on your input data and your desired analysis. Explore the workflow results to VEuPathDB.

Examine genome coverage and calculate TPM for each gene

In addition to the tools described above, these workflows use three tools: BigWig and TPM files that can be analyzed on VEuPathDB, in Galaxy, or o and processes them in parallel. To export the results to VEuPathDB, use

- Workflow for paired-end stranded reads
- Workflow for paired-end unstranded reads
- Workflow for single-end stranded reads
- Workflow for single-end unstranded reads

Identify genes with statistically significant expression differences

In addition to the tools described above, these workflows use three tools: each gene exhibits differential expression and to generate BigWig coverage. The workflows compare two samples with any number of replicates. To filter your DESeq2 result file and obtain changes ≥ 2 and $\text{adj-p} < 0.05$; these can be changed, use this workflow on Gene IDs/ question on a VEuPathDB website, as seen here

- Workflow for paired-end stranded reads
- Workflow for paired-end unstranded reads
- Workflow for single-end stranded reads
- Workflow for single-end unstranded reads

Variant calling

Use the following workflows to analyze your FASTQ files. The workflows to a VEuPathDB reference genome, FreeBayes for variant detection, SnpE

Workflow: imported: DESeq2 Workflow for paired-end unstranded reads (v.7) (imported from uploaded file)

History Options

Send results to a new history

Yes No

1: Input Dataset Collection - Sample 1

13: dsGFP_infected

2: Input Dataset Collection - Sample 2

14: dsSARL_infected

Source for the reference genome to align against

Use a built-in genome

Select a reference genome

VectorBase-49_IscaularisWikel_Genome

If your genome of interest is not listed, contact the Galaxy team

Factor level

1: Factor level

Specify a factor level, typical values could be 'tumor', 'normal', 'treated' or 'control'

dsGFP_infected

Only letters, numbers and underscores are allowed in this field

Counts file(s)

2: Factor level

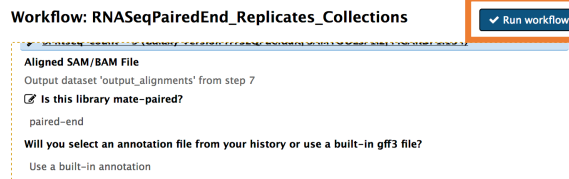
Specify a factor level, typical values could be 'tumor', 'normal', 'treated' or 'control'

Sample2

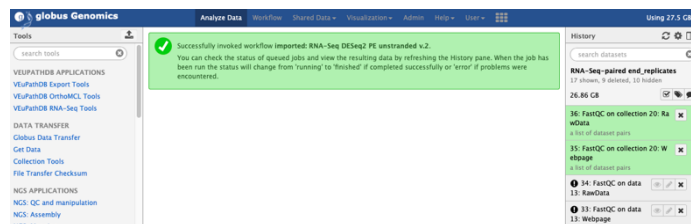
Counts file(s)

- Configure your workflow – there are multiple steps in the workflow, but you do not need to configure all of them. For the purpose of this exercise, you will need to configure the following:
 - Select the input dataset collections. These are the collections of fastq files you just created. Workflow steps 1-2 allow you to select the datasets. (B above figure)
 - Some tools in the workflow require that you select the reference genome to be used. In this workflow, both HISAT2 and HTSeq require this (note that each of these tools is in the workflow twice since you have two collections). It is critical that you select the correct genome that matches the experimental organism. So, for example, if your experiment was performed using *Ixodes scapularis Wikel*, the reference genome you select should be *VectorBase-49_IscaularisWikel_Genome* (C above figure).
 - Name your factor levels. This helps keep everything organized and named properly in your workflow. Each factor level is typically the name of the condition, like Male or Female OR Susceptible or resistant (D & E above figure).

- Once you are sure everything is configured correctly, click on “Run Workflow” at the top.



The steps will start running in the history section on the right. Grey means they are waiting to start. Yellow means they are running. Green means they have completed. Red means there was an error in the step.

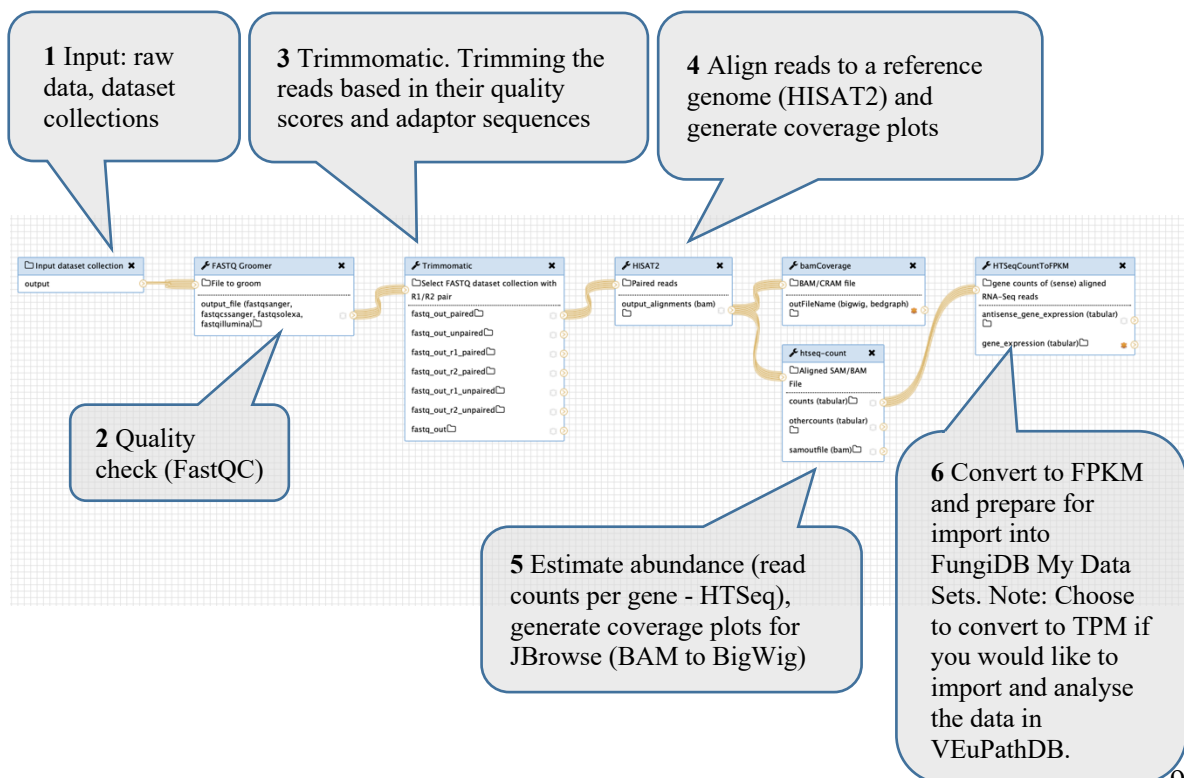


Practice working with Galaxy editor (optional)

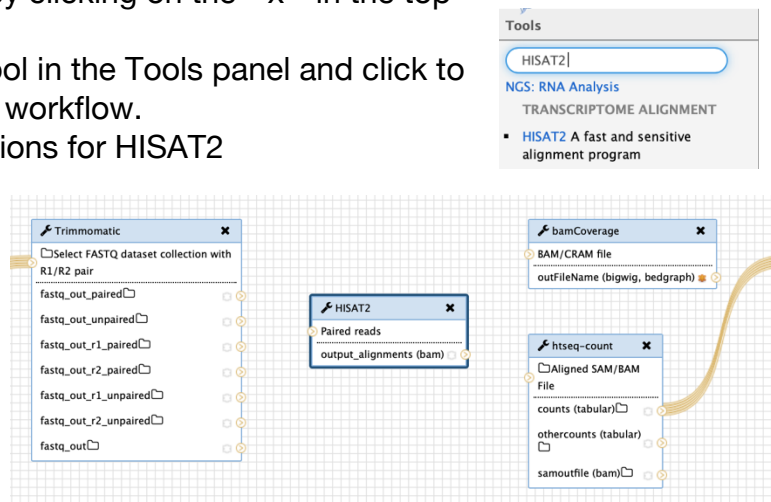
You can create your own workflows. The tools can all be added and configured in a interactive workflow editor.

- Navigate to the Workflow tab from the main menu at the top and select
- Left click on the drop-down icon within the workflow you want to modify and select the “Edit” option.

Sample workflow steps:

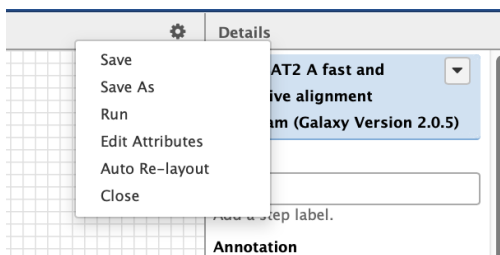


- Delete HISAT2 step by clicking on the “ x “ in the top right corner.
- Locate the HISAT2 tool in the Tools panel and click to insert it back into the workflow.
- Re-establish connections for HISAT2
 - Click on the arrow in the step before HISAT2 and drag to the appropriate input in HISAT2 tool.



- What happens? Can you reconnect it?

Note: Sometimes you may be unable to re-establish connection. When this happens, take a look at the tool documentation notes in the right panel, check your selection for single-read or paired-end setting in particular (paired-end setting must be selected if you are dealing with reverse and forward reads).



Now that you have learned the principals of workflow editing, you can either practice saving the workflow by clicking on the wheel at the far top corner or simply existing the workflow editor without saving.