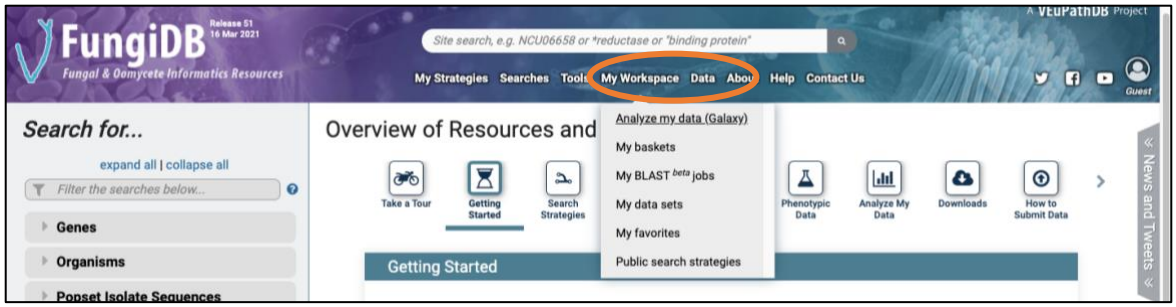
RNA sequence data analysis via VEuPathDB Galaxy, Part I
Uploading data and starting the workflow (Group Exercise)

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 90 days or when the total quota for all projects is . 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.

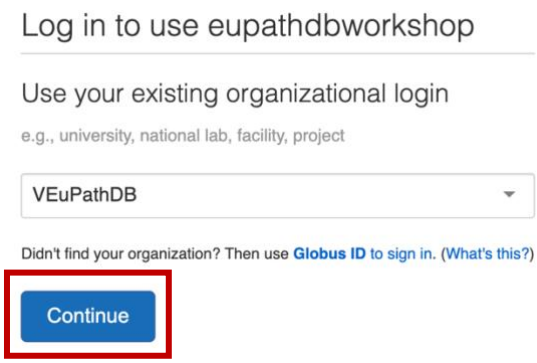
IMPORTANT During workshop we will NOT be using live sites to access VEuPathDB Galaxy. Use the link below to log in to the workshop VEuPathDB Galaxy with your FungiDB account. If you already have an account with any other VEuPathDB site, this log in will work in FungiDB. If you are creating a new account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

Section I: Setting up your VEuPathDB Galaxy account

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

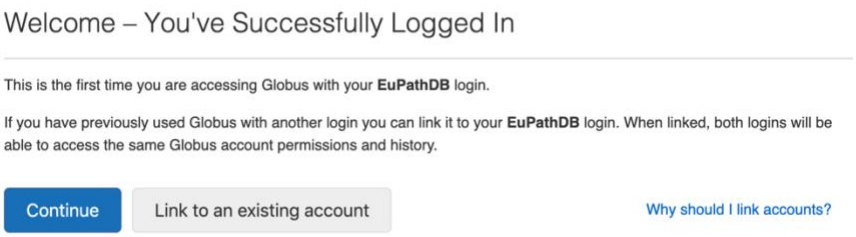
https://veupathdbworkshop.globusgenomics.org/

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

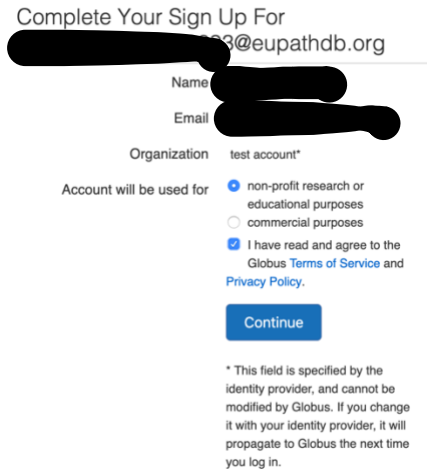


Step 3: If you are not already logged into VEuPathDB you will be prompted to do so now.

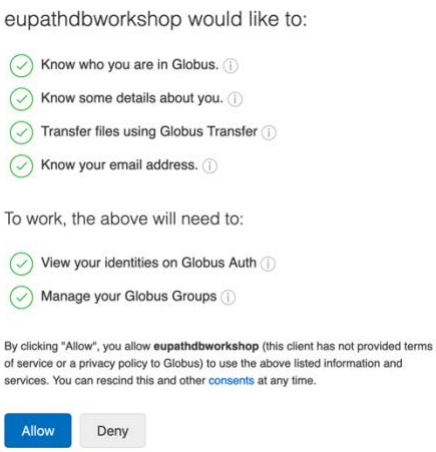
Step 4: Click on "continue" on the next page (no need to link an existing account).



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



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

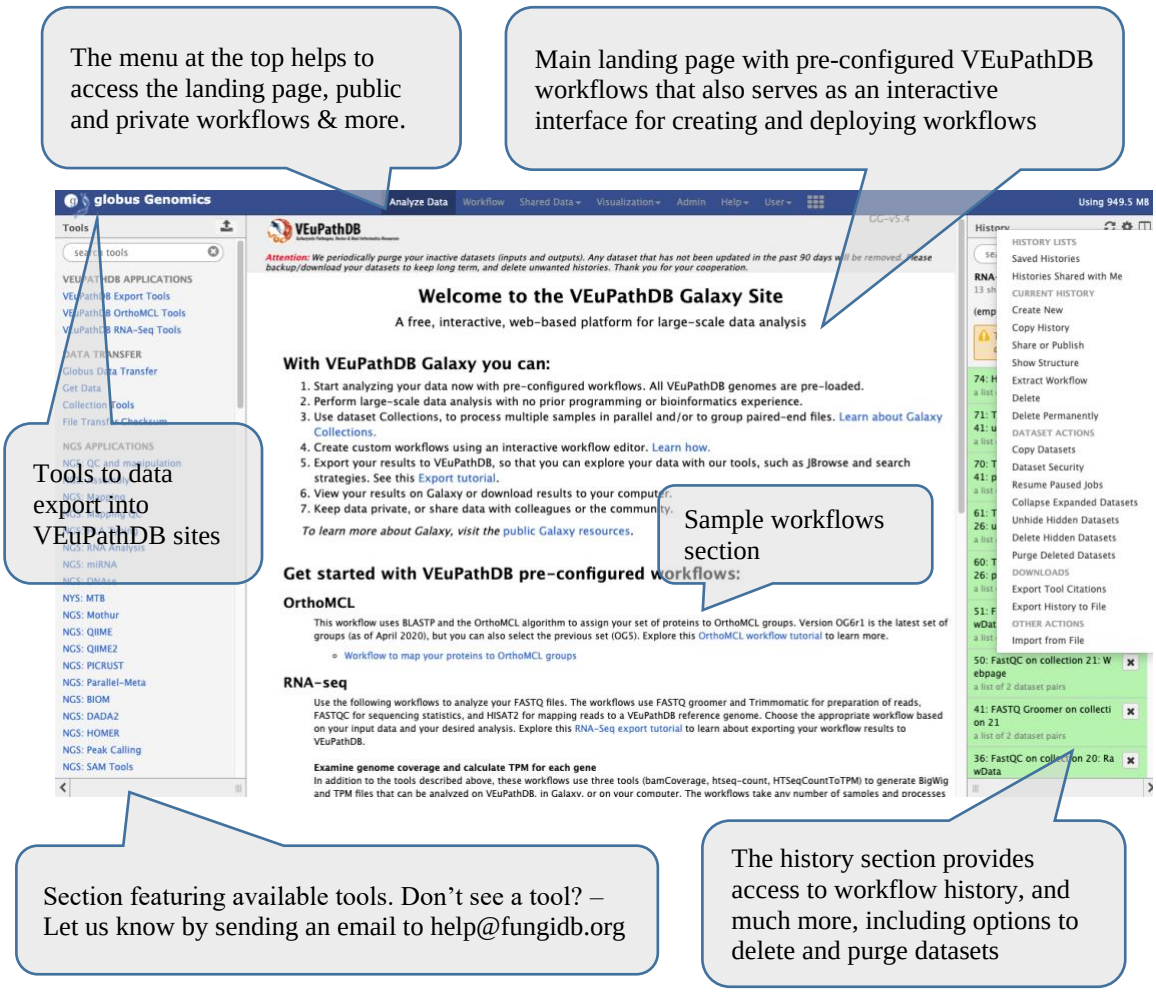


Step 5: Congratulations, you are in!

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



Section II: Importing data to Galaxy

There are multiple ways to important 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:

Groups 1 & 2 will be examining the transcriptome of *Aspergillus fumigatus* incubated in human blood from a study called "*Aspergillus fumigatus in blood reveals a "just wait and see" resting stage behavior*"
https://pubmed.ncbi.nlm.nih.gov/26311470/
The data is available in the sequence repositories:
https://www.ebi.ac.uk/ena/browser/view/PRJNA287921

Sample Name	**Pre-culture media (pre)**	**Blood media 30 min (B30)**	**Blood media 180 min (B180)**
Sample Accession Numbers	SAMN03792073 SAMN03792081	SAMN03792074 SAMN03792077	SAMN03792075 SAMN03792076

Group Number	**1**	**2**
Comparison	**pre vs B30**	**pre vs B180**
Ref genome in Galaxy	FungiDB-29_AfumigatusAf293	

Group 3 will be examining data from a study called "*Transcriptome of Candida parapsilopsis grown under planktonic and biofilm growing conditions*:
https://pubmed.ncbi.nlm.nih.gov/25233198/
https://www.ebi.ac.uk/ena/browser/view/PRJNA246482

Sample Name	**WT planktonic**	**WT biofilm**
Sample Accession Numbers	SAMN02767882 SAMN02767886 SAMN02767883	SAMN02767881 SAMN02767885 SAMN02767890

Group Number	**3**
Comparison	**Mycelia vs Spherules**
Ref genome in Galaxy	FungiDB-29_CposadasiiC735seltSOWgp_Genome

Group 4 & 5 will be examining data from a study called "*DNA damage-induced transcriptome changes in budding yeasts Saccharomyces cerevisiae and Candida glabrata*":
https://pubmed.ncbi.nlm.nih.gov/33323516/
https://www.ebi.ac.uk/ena/browser/view/PRJNA655241

Sample Name	**Sc no MMS**	**Sc + MMS**	**Cg no MMS**	**Cg + MMS**
Sample Accession Numbers	SAMN15731261 SAMN15731262	SAMN15731258 SAMN15731259 SAMN15731260	SAMN15731255 SAMN15731256 SAMN15731257	SAMN15731252 SAMN15731253 SAMN15731254

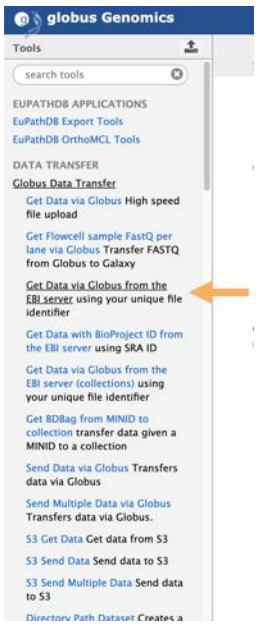
Group Number	4	5
Comparison	**Sc no MMS vs Sc + MMS**	**Cg no MMS vs Cg + MMS**
Ref genome in Galaxy	FungiDB-29_ScerevisiaeS288c	FungiDB-39_CglabrataCBS138

Group 6 will be examining data from a study called "*Genome-wide gene expression analysis of Fusarium graminearum isolate PH-1 in spores and mycelium*":
https://pubmed.ncbi.nlm.nih.gov/24625133/
https://www.ebi.ac.uk/ena/browser/view/PRJNA239711

Sample Name	Spores	Mycelia
Sample Accession Numbers	SAMN02666851 SAMN02666852 SAMN02666849	SAMN02666848 SAMN02666853 SAMN02666850

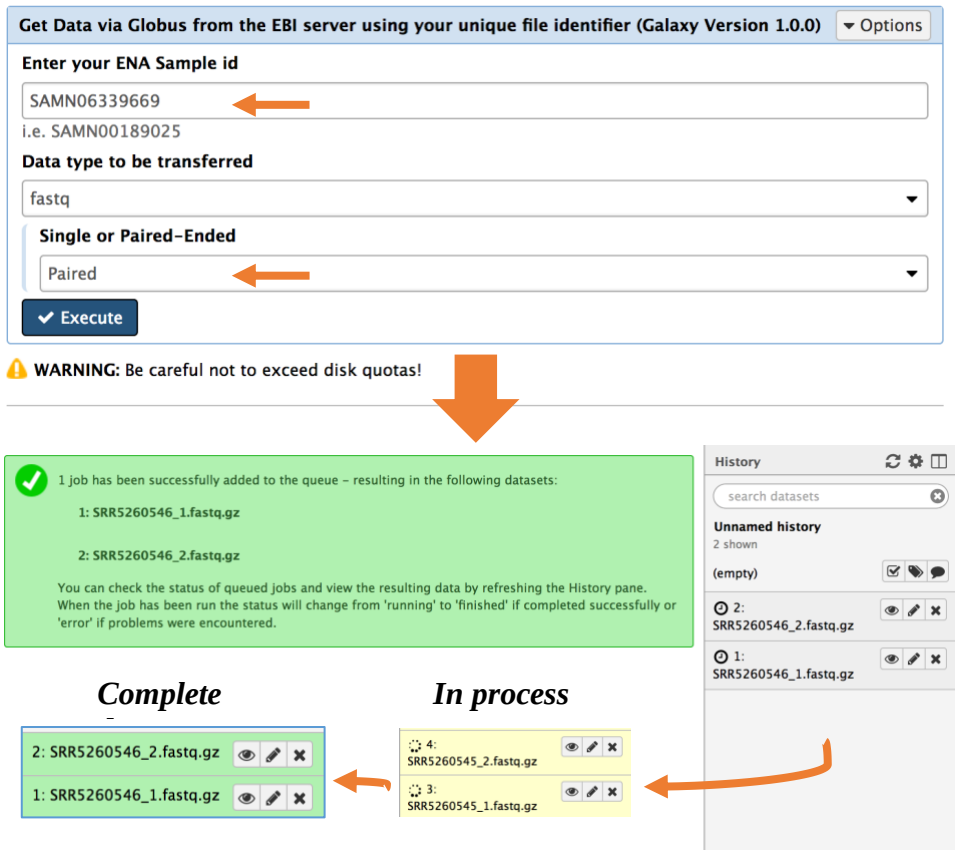
Group Number	6
Comparison	**Spores vs Mycelia**
Ref genome in Galaxy	FungiDB-31_FgraminearumPH-1

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.

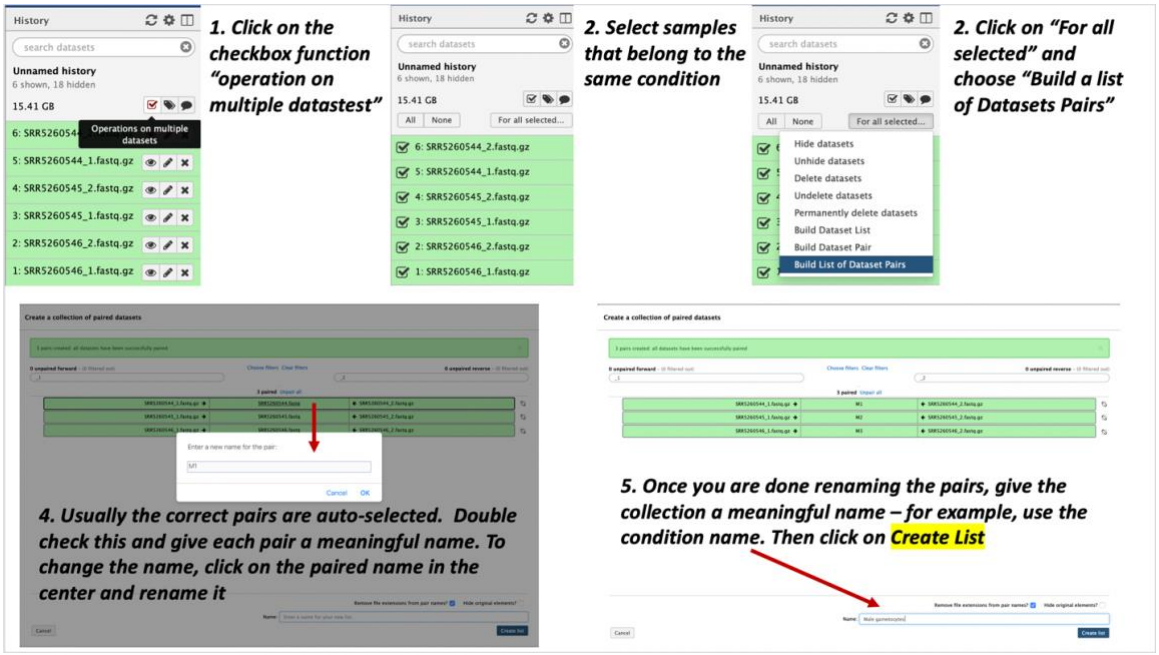


Step 2: In the middle section enter the sample ID and choose whether the run was single or paired end. Click on Execute.

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.*



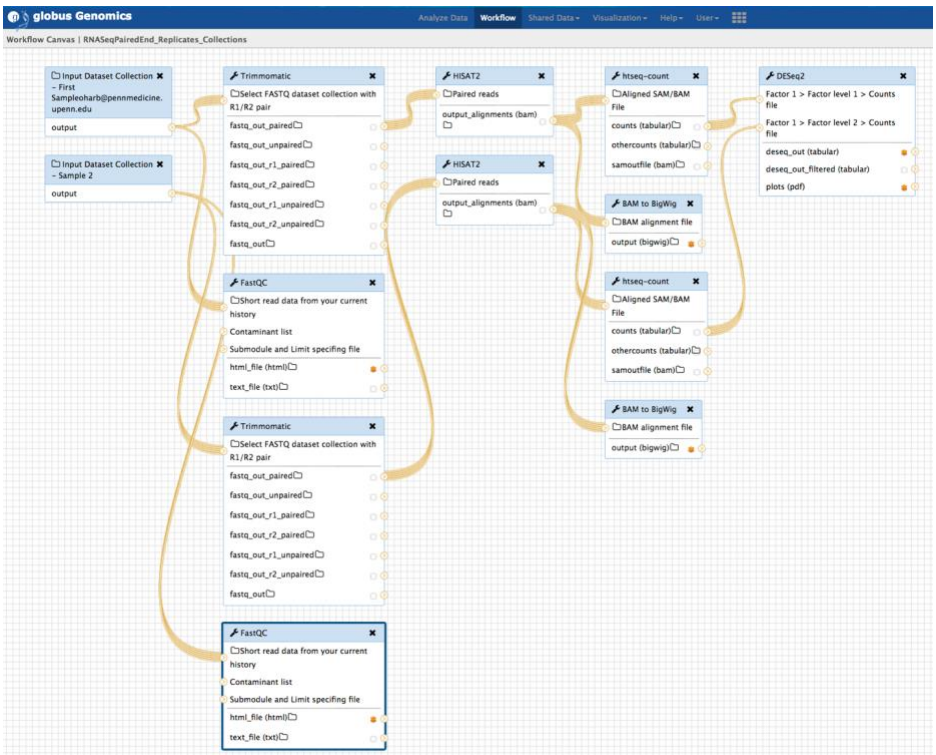
Step 3: If you are working with a dataset with biological replicates it is useful to organize the different conditions of your experiment into "Collections". For example, if your experiment included RNAseq from *Plasmodium falciparum* male gametocyte stages (three biological replicates) and erythrocytic stages (three biological replicates), it is useful to organize these into two collections, one that includes all male gametocyte files and the other that includes all the erythrocytic stage files. Using collections also reduces the complexity of the Galaxy workflows. See below:



Section II: Running a workflow in Galaxy

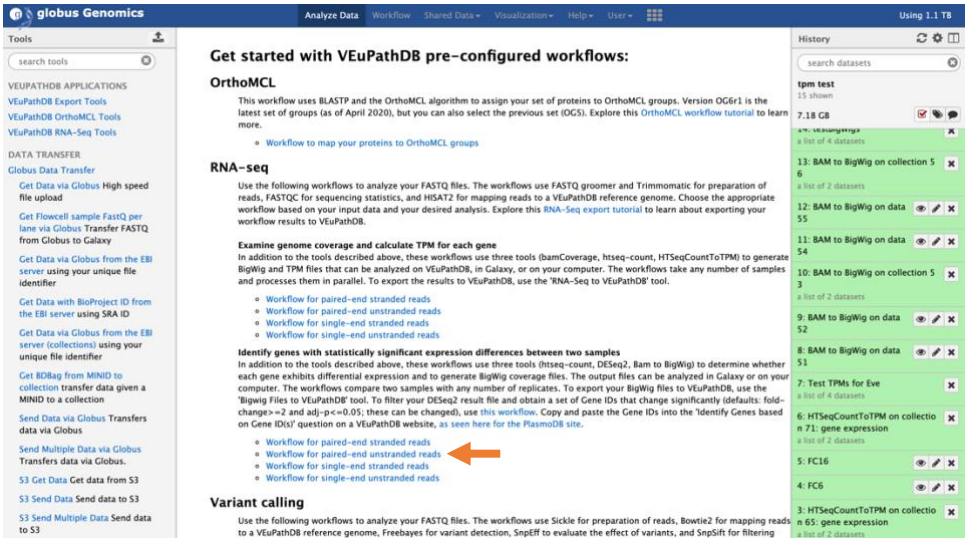
You can create your own workflows in galaxy based on your needs. The tools in the left section can all be added and configured as steps in a workflow that can be run on appropriate datasets. For this exercise we will use a preconfigured workflow that does the following main things:

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).



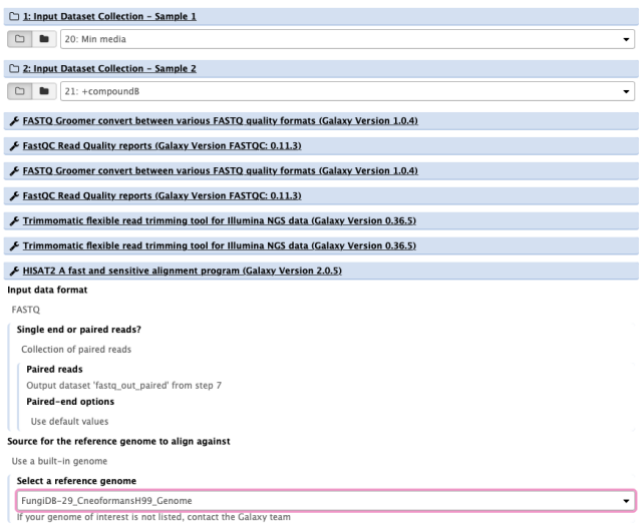
Additional resources:
Galaxy Project (https://usegalaxy.org/)
Trimmomatic manual
FastQC
HISAT2
HTseq
DEseq2

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



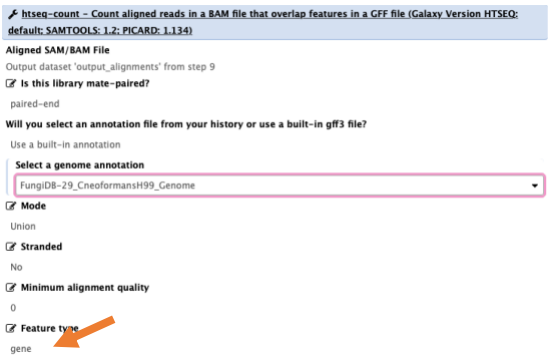
- Configure your workflow – there are multiple steps in the workflow, but you do not need to configure all of them. For the purpose of this exercise you will need to configure the following:

a. 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.

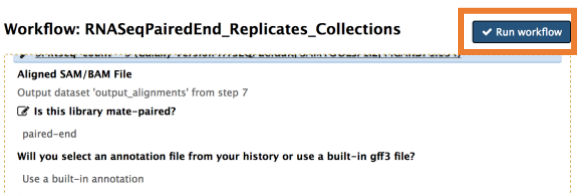


b. Some tools in the workflow require that you select the reference genome to be used. In this workflow both HISAT2 and HTSeq require this (note these tools are in the workflow twice since you have two collections). It is critical that you select the correct genome that matches the experimental organism. So, for example, if your experiment was performed using *Cryptococcus neoformans H99*, the reference genome you select should be *FungiDB-29_CneoformansH99_Genome as shown below*.

c. Another very important parameter to check in the htseq-count step is the Feature type. The default is usually set to exon. Make sure you chance this to gene. To change this to gene, click on the edit icon, the type the word "gene". This is case sensitive so be careful about this.



d. Once you are sure everything is configured correctly, click on "Run Workflow" at the top.



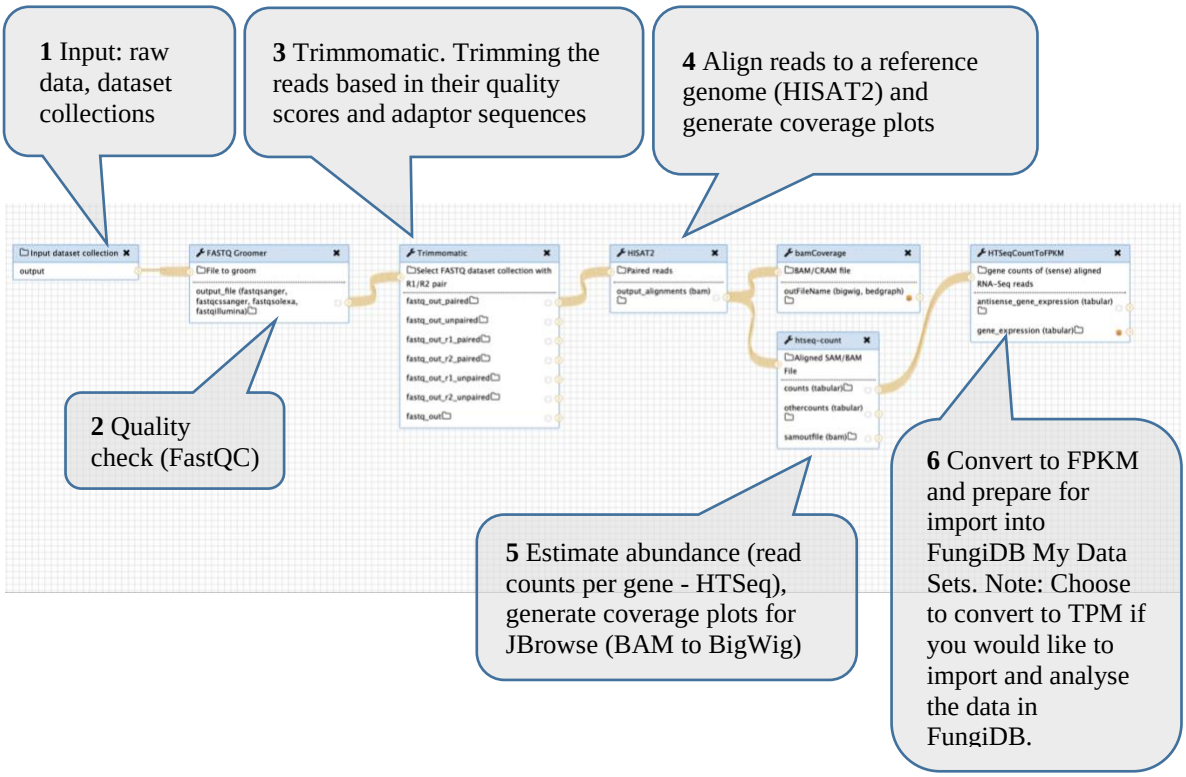
The steps will start running in the history section on the right. Grey means they are waiting to start. Yellow means they are running. Green means they have completed. Red means there was an error in the step.

Practice working with Galaxy editor (optional)

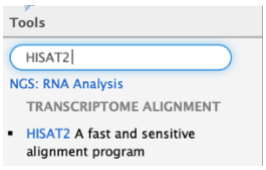
You can create your own workflows. The tools can all be added and configured in a interactive workflow editor.

- 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.

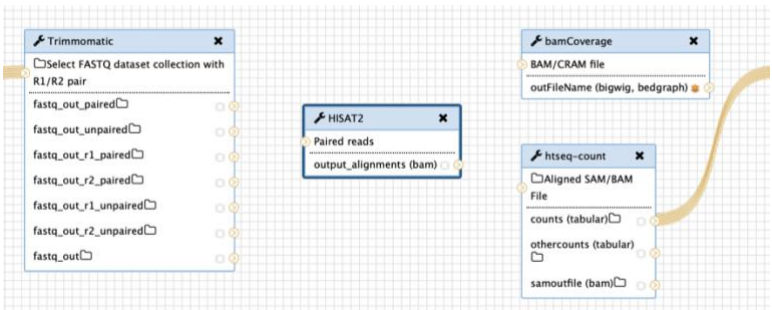
Sample workflow steps:



- 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.

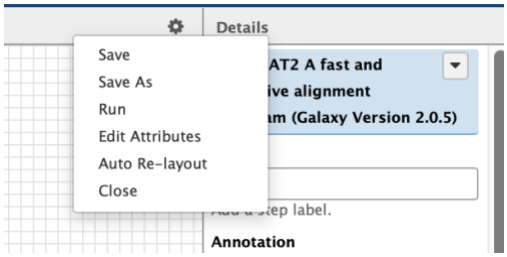


- Re-establish connections for HISAT2
  - Click on the arrow in the step before HISAT2 and drag to the appropriate input in HISAT2 tool.



- 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 you r 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.