

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

The goal of this exercise is to use a Galaxy workflow to analyze RNA sequencing data. 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. EuPathDB 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.

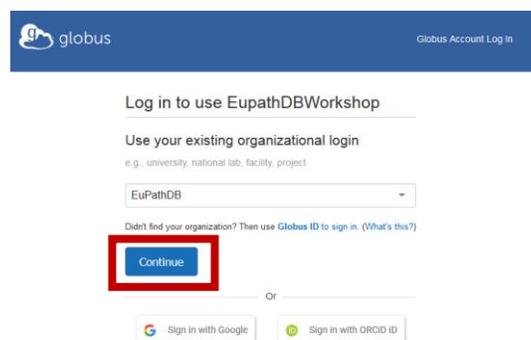
We will be working in groups. Each group will have 4-6 members. One person in the group will run the Galaxy controls on one computer. The other members' roles are to ensure that the correct datasets are used and that the correct workflow parameters are selected.

Section I: Setting up your EuPathDB Galaxy account

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

<http://eupathdbworkshop.globusgenomics.org/>

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



The screenshot shows the login interface for EupathDBWorkshop on the Globus platform. At the top left is the Globus logo, and at the top right is a link for 'Globus Account Log In'. The main heading is 'Log in to use EupathDBWorkshop'. Below this, it prompts the user to 'Use your existing organizational login' with examples like 'university, national lab, facility, project'. A dropdown menu is set to 'EuPathDB'. A note asks if the user didn't find their organization, suggesting they use Globus ID to sign in. A blue 'Continue' button is highlighted with a red rectangular box. Below this, there are two alternative login options: 'Sign in with Google' and 'Sign in with ORCID ID'.

Step 3: Log in to EuPathDB (if you are not logged in already).



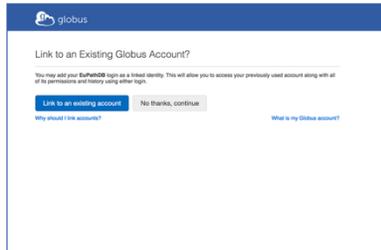
Step 4: Next, sign up for the EuPathDB Galaxy instance.

Analyze My Experiment

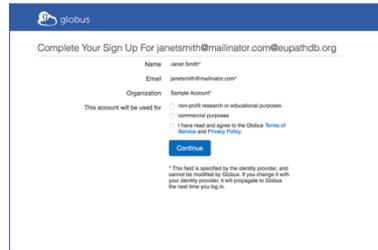
The first time you visit EuPathDB Galaxy you will be asked to sign up with Globus, EuPathDB's Galaxy instance manager. This is a three-step sign-up process (screenshots below).

Click **"Continue to Galaxy"** to sign up for EuPathDB Galaxy services.

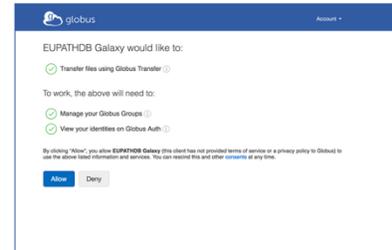
Contact us if you experience any difficulties.



(1) If you already have a Globus account, you can link it to your EuPathDB account. **Your choice.** If you don't have a prior Globus account, choose **No Thanks**.



(2) Complete your account information and agree to Globus's Terms and Conditions. Please read, make your selections, and click **Continue**.

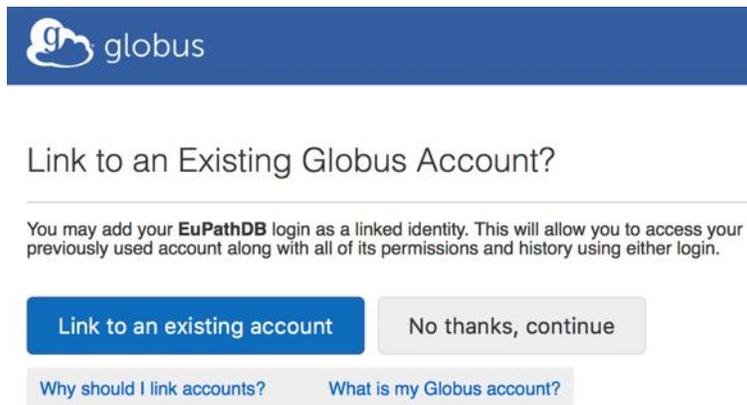


(3) Grant permission to share your Globus identity and files with us. Please click **Allow**. (We will only perform file transfers that you explicitly request, between Galaxy and other resources, including EuPathDB.)

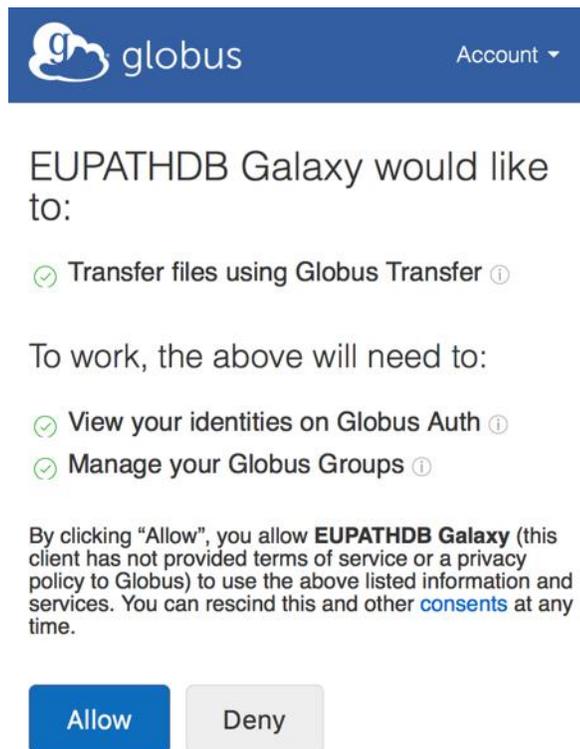
[Continue to Galaxy](#)

Step 5: Click on "Continue to Galaxy" and follow the instructions.

Step 6: Click on "No thanks, continue"



Step 7: Click on “Allow”



Step 8: Congratulations, you are in!

Section II: Importing data to Galaxy

There are multiple ways to important data into your Galaxy workspace. For this exercise, we will use the ‘Download from web or upload from disk’ tool and enter the direct data repository links listed below under ‘Group Assignments’. Remember one person in your group will be starting 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 don’t want to overload the servers. The samples below were all generated by paired end sequencing, hence there are two files for each sample. The files are fastq files that are compressed (that is why they end in .gz = gzip).

Step 1: Click on the “Get data” link in the left-hand menu. This will reveal a list of options; click on “Get Data via Globus from the EBI server”

The screenshot shows the EuPathDB Galaxy Site interface. The left-hand menu is expanded to show 'Get Data' options. A red arrow points to 'Get Data via Globus from the EBI server using your unique file identifier'. The main content area displays a welcome message and a list of pre-configured workflows.

Tools

search tools

Get Data

- Upload File from your computer
- Get Data via Globus High speed file upload
- Get Data via Globus from the EBI server using your unique file identifier
- Send Data via Globus Transfers data via Globus

EUPATHDB APPLICATIONS

- EuPathDB Export Tools
- NGS APPLICATIONS
- NGS: QC and manipulation
- NGS: Assembly
- NGS: Mapping
- NGS: Mapping QC
- NGS: RNA Analysis
- NGS: DNase
- NGS: Mothur
- NGS: QIIME
- NGS: PICRUST
- NGS: Parallel-Meta
- NGS: BIOM
- NGS: HOMER
- NGS: Peak Calling
- NGS: SAM Tools
- NGS: SAM Tools (1.1)
- NGS: BAM Tools
- NGS: SNPIR Tools
- NGS: Picard

EuPathDB
EuPathDB Pathogen Database Resource

system status

Welcome to the EuPathDB Galaxy Site

A free, interactive, web-based platform for large-scale data analysis

With EuPathDB Galaxy you can:

1. Start analyzing your data now. All EuPathDB genomes are pre-loaded. Pre-configured workflows are available.
2. Perform large-scale data analysis with no prior programming or bioinformatics experience.
3. Create custom workflows using an interactive workflow editor. [Learn how](#)
4. Visualize your results (BigWig) in GBrowse.
5. Keep data private, or share it with colleagues or the community.

To learn more about Galaxy check out public Galaxy resources: [Learn Galaxy](#)

Get started with pre-configured workflows:
(additional workflows will be added soon)

- EuPathDB Workflow for Illumina paired-end RNA-seq, without replicates**
Profile a transcriptome and analyze differential gene expression.
Tools: FastQC, Sickle, GSNAP, CuffLinks, CuffDiff.
- EuPathDB Workflow for Illumina paired-end RNA-seq, without replicates**
Profile a transcriptome and analyze differential gene expression.
Tools: FastQC, Trimmomatic, TopHat2, CuffLinks, CuffDiff.
- EuPathDB Workflow for Illumina paired-end RNA-seq, biological replicates**
Profile a transcriptome and analyze differential gene expression.
Tools: FastQC, Trimmomatic, TopHat2, HTseq, DESeq2.
- EuPathDB Workflow for Illumina paired-end RNA-seq, biological replicates**
Profile a transcriptome and analyze differential gene expression.
Tools: FastQC, Trimmomatic, TopHat2, CuffLinks, CuffDiff.
- EuPathDB Workflow for Variant Calling, single-read sequencing**
Profile and analyse SNPs.
Tools: Sickle, Bowtie2, FreeBayes, and SnpEff

History

search datasets

Unnamed history
(empty)

This history is empty. You can load your own data or get data from an external source

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

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.

History refresh settings close

search datasets

Unnamed history
2 shown

(empty) checkbox refresh help

2: SRR5260546_2.fastq.gz eye edit close

1: SRR5260546_1.fastq.gz eye edit close

Completed

2: SRR5260546_2.fastq.gz eye edit close

1: SRR5260546_1.fastq.gz eye edit close

In process

4: SRR5260545_2.fastq.gz eye edit close

3: SRR5260545_1.fastq.gz eye edit close

Queued for import

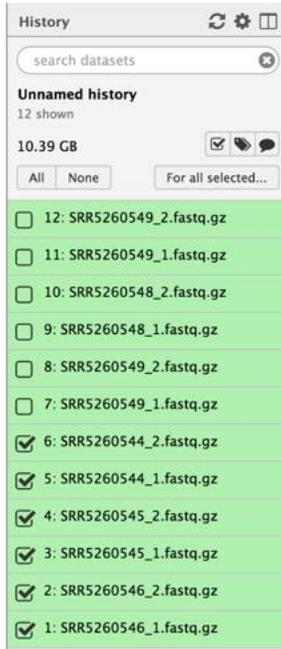
Note that the sample ID resulted in importing two files one for each pair. Repeat this process for each sample you want to import. *If you are working with samples from two conditions and the experiment was done in triplicate and paired end sequenced then you should end up with 12 files; six from each 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:

1. Click on the checkbox function "Operation on multiple datasets"



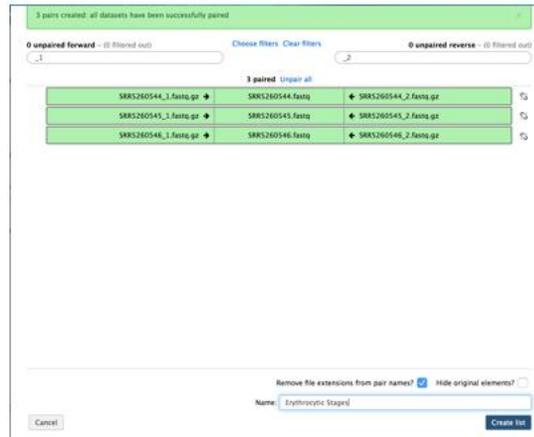
2. Select files that belong to the same condition



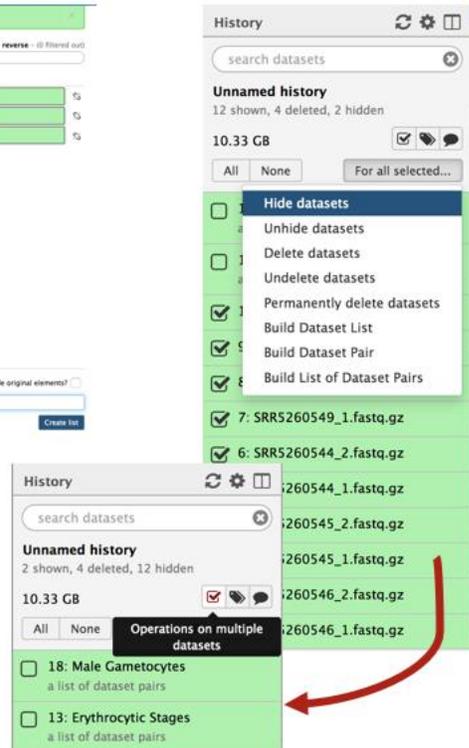
3. Click on for all selected and choose "Build List of Dataset Pairs"



4. Usually the corrected pairs are matched. Double check this and give this collection a meaningful name



5. You can clean up your history by hiding the files that you just put into a collection



Group assignments:

Groups 1, 2 & 3 will be examining data from a study called “*Plasmodium berghei* transcriptome for female gametocytes, male gametocytes, and asexual erythrocytic stages”

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5604118/>

The data is available in the sequence repositories:

<https://www.ebi.ac.uk/ena/data/view/PRJNA374918>

Samples:

Erythrocyte stages (Asexual):

SAMN06339669

SAMN06339670

SAMN06339671

Male gametocytes:

SAMN06339666

SAMN06339667

SAMN06339668

Female gametocytes:

SAMN06339663

SAMN06339664

SAMN06339665

Group 1:

Plasmodium berghei male gametocytes vs. erythrocytic stages

Group 2:

Plasmodium berghei female gametocytes vs. erythrocytic stages

Group 3:

Plasmodium berghei male gametocytes vs. female gametocytes

Groups 4, 5 & 6 will be examining data from a study called “*Plasmodium falciparum* NF54 Transcriptome” which examines RNAseq from 3 stages: erythrocytic, salivary gland and cultured sporozoite stages. This study is unpublished but data is accessible in the sequence repositories: <https://www.ebi.ac.uk/ena/data/view/PRJNA230379>

Samples:

Erythrocytic stages (Asexual):

SAMN02428730

SAMN02428734

Salivary gland sporozoites:

SAMN02428726

SAMN02428729

Cultured sporozoites:

SAMN02428728

SAMN02428727

Group 4:

Plasmodium falciparum salivary sporozoites vs. erythrocytic stages

Group 5:

Plasmodium falciparum cultured sporozoites vs. erythrocytic stages

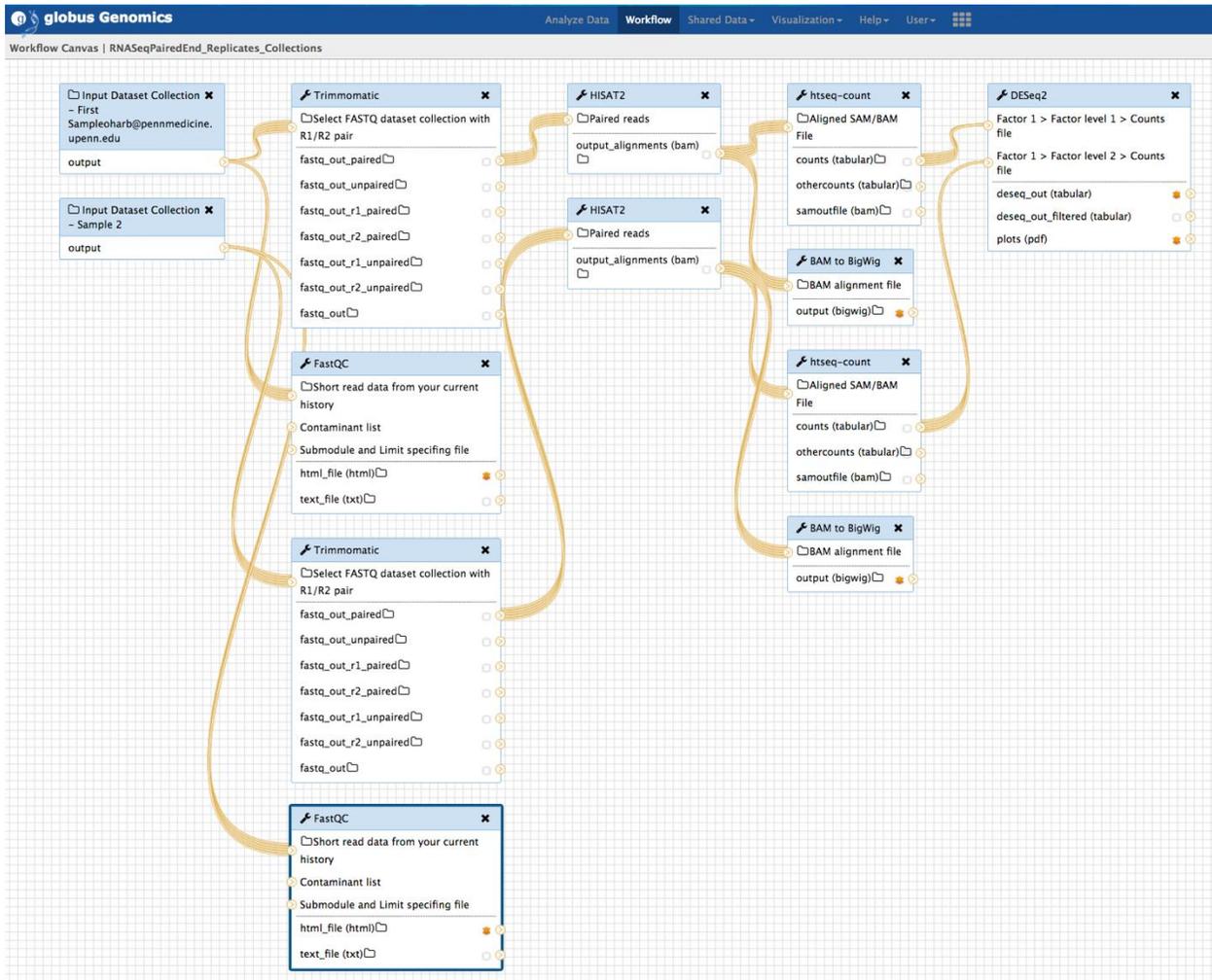
Group 6:

Plasmodium falciparum salivary sporozoites vs. cultured sporozoites

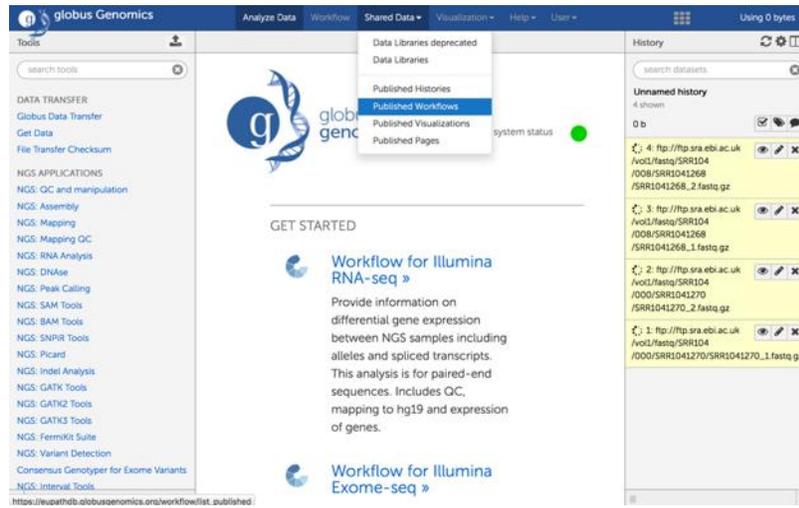
Section II: Running a workflow in Galaxy

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:

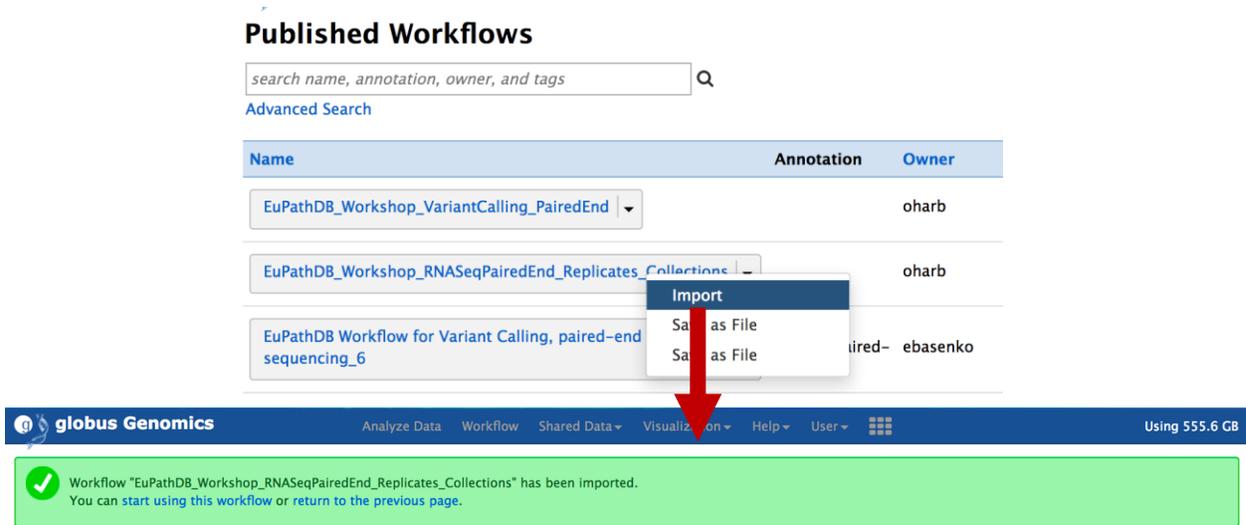
1. Analyzes the reads in your files and generates FASTQC reports.
2. Trims the reads based on their quality scores and adaptor sequences (Trimmomatic).
3. Aligns the reads to a reference genome using HISAT2 and generates coverage plots.
4. Determines read counts per gene (HTSeq)
5. Determines differential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



Step 1: Import the workflow called “EuPathDB_Workshop_RNASeqPairedEnd_Replicates_Collections” – click on the shared data menu item and select “Published Workflows” from the menu.

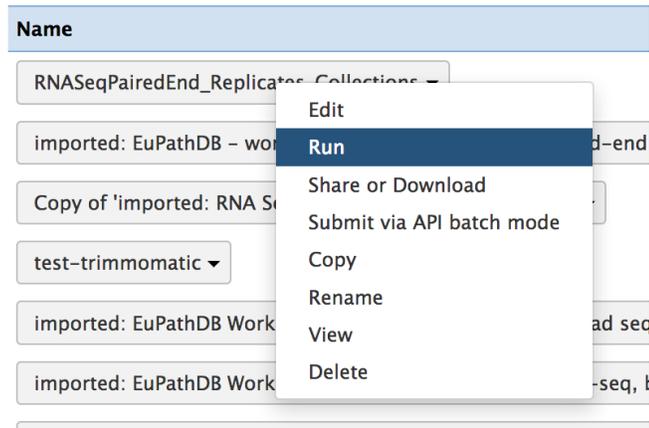


Step 2: Click on the arrow next to the appropriate workflow and select import.



Step 3: Click on “Workflow” in the menu at the top of the page. On the next page click on the arrow next to workflow you just imported and select the “Run” option.

Your workflows



Step 4: 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.

Workflow: RNASeqPairedEnd_Replicates_Collections Run workflow

History Options

Send results to a new history
 Yes No

1: Input dataset collection - 1
13: Erythrocytic Stages

2: Input dataset collection - 13
18: Male Gametocytes

3: Trimmomatic - 3 (Galaxy Version 0.36.5)

4: FastQC - 2 (Galaxy Version FASTQC: 0.11.3)

5: Trimmomatic - 9 (Galaxy Version 0.36.5)

6: FastQC - 8 (Galaxy Version FASTQC: 0.11.3)

7: HISAT2 - 4 (Galaxy Version 2.0.5)

Input data format
FASTQ

Single end or paired reads?
Collection of paired reads

Paired reads
Output dataset 'fastq_out_paired' from step 3

Paired-end options
Use default values

Source for the reference genome to align against
Use a built-in genome

Select a reference genome
AmoebaDB-29_AastronyxisUnknown_Genome

- 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 *Plasmodium berghei*, the reference genome you select should be *Plasmodium berghei*.

Source for the reference genome to align against

Use a built-in genome

Select a reference genome

- PlasmoDB-29_Pchabaudichabaudi_Genome
- PlasmoDB-29_PcynomolgiB_Genome
- PlasmoDB-29_Pfalciparum3D7_Genome
- PlasmoDB-29_PfalciparumIT_Genome
- PlasmoDB-29_PknowlesiH_Genome
- PlasmoDB-29_PreichenowCDC_Genome
- PlasmoDB-29_PvivaxP01_Genome
- PlasmoDB-29_PvivaxSal1_Genome
- PlasmoDB-29_Pyoeliiyoelii17XNL_Genome
- PlasmoDB-29_PyoeliiyoeliiYM_Genome
- PlasmoDB-30_PcoatneyiHackeri_Genome
- PlasmoDB-30_PfragileNilgiri_Genome
- PlasmoDB-30_PinuiSanAntonio1_Genome
- PlasmoDB-30_PmalariaeUG01_Genome
- PlasmoDB-30_PvinckeipetteriCR_Genome
- PlasmoDB-30_Pvinckeivinckeivinckeii_Genome
- PlasmoDB-30_Pyoeliiyoelii17X_Genome
- PlasmoDB-32_PbergheiANKA_Genome**
- PlasmoDB-32_Pgallinaceum8A_Genome
- PlasmoDB-32_PovalecurtisiGH01_Genome

Paired alignment parameters

Use default values

- c. Click on “Run Workflow” at the top.

Workflow: RNASeqPairedEnd_Replicates_Collections

Run workflow

Aligned SAM/BAM File

Output dataset 'output_alignments' from step 7

Is this library mate-paired?

paired-end

Will you select an annotation file from your history or use a built-in gff3 file?

Use a built-in annotation

Select a genome annotation

PlasmoDB-32_PbergheiANKA_Genome

Mode

Union

The screenshot displays the globus Genomics interface. At the top, there is a navigation bar with 'Analyze Data', 'Workflow', 'Shared Data', 'Visualization', 'Help', and 'User' menus. The main area is divided into three sections:

- Tools:** A sidebar on the left containing a search bar and a list of applications categorized under 'EUPATHDB APPLICATIONS' and 'NGS APPLICATIONS'. The NGS applications include QC and manipulation, Assembly, Mapping, RNA Analysis, DNase, Mothur, QIIME, PICRUST, Parallel-Meta, BIOM, HOMER, Peak Calling, SAM Tools, and various GATK tools.
- Message:** A central green notification box with a checkmark icon. It reads: 'Successfully invoked workflow RNASeqPairedEnd_Replicates_Collections. 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.'
- History:** A panel on the right showing a list of datasets and jobs. The top section is titled 'Cultured vs. Salivary' and shows 53 datasets (8 hidden) with a size of 9.7 GB. Below this, a list of jobs is shown, each with a status icon (eye, pencil, or X) and a close button. The jobs are:
 - 28: FastQC on data 4: RawData (Grey)
 - 27: FastQC on data 4: Webpage (Grey)
 - 26: FastQC on data 3: RawData (Grey)
 - 25: FastQC on data 3: Webpage (Grey)
 - 24: FastQC on data 2: RawData (Grey)
 - 23: FastQC on data 2: Webpage (Grey)
 - 22: FastQC on data 1: RawData (Grey)
 - 21: FastQC on data 1: Webpage (Grey)
 - 20: Trimmomatic on collection 5: unpaired (Green)
 - 19: Trimmomatic on collection 5: paired (Green)
 - 10: Cultured sporozoites (Green)
 - 5: Sporozoites (Green)

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.

Appendix:

FASTQ files are text files (similar to FASTA) that include sequence quality information and details in addition to the sequence (ie. name, quality scores, sequencing machine ID, lane number etc.). FASTQ files are large and as a result not all sequencing repositories will store this format. However, tools are available to convert, for example, NCBI's SRA format to FASTQ. Sequence data is housed in three repositories that are synchronized on a regular basis.

- The sequence read archive at GenBank
- The European Nucleotide Archive at EMBL
- The DNA data bank of Japan

