

Oct 22, 2024

Version 1

Hepatitis A virus genotyping and phylogeny analysis_ViroTrakr workflow 2_v.1 V.1

DOI

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ViroTrakr



Zhihui Yang

FDA

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Protocol status: Working

We use this protocol and it's working

Created: October 21, 2024

Last Modified: October 22, 2024

Protocol Integer ID: 110440

Keywords: virotrakr database, galaxytrakrmicrorunqc workflow, virotrakr, sequencing platform, analysis within the galaxytrakr, sequencing data, quality control assessment for microbial genome, galaxy trakr, phylogenetic result, drafting de novo assembly, sequence genotype, de novo assembly, account in galaxy trakr, galaxytrakr, microbial genome, hepatitis, workflow, virus, assembled sequence, sequences from local folder, foodborne virus, raw data

Disclaimer

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Abstract

This workflow provides step-by-step instructions for hepatitis A virus (HAV) analysis within the GalaxyTrakr platform. It includes the quality assessment for raw sequencing data (from most next-generation sequencing platforms), drafting de novo assemblies, executing the workflow either from the raw sequencing data or assembled sequences, and reporting the sequence genotype and phylogenetic results. This workflow was designed for HAV, which is one of the major targets of our ViroTrakr database.

This protocol covers how to:

- Set up an account in Galaxy Trakr (Item 1);
- Create a new history/workspace for a new submission (Item 2);
- Upload raw data obtained from local folders (Item 3.1) or download from NCBI (Item 3.2);
- Upload assembled sequences from local folders (Item 4);
- Execute the ViroTrakr workflow with either raw data or assembled sequences (Item 3.1.8-3.1.12);
- Interpret the results (Item 3.1.13-3.1.14).

ViroTrakr: [foodborne viruses \(ID 396739\) - BioProject - NCBI \(nih.gov\)](#).

-Reference: Quality control assessment for microbial genomes: GalaxyTrakrMicroRunQC workflow V.5:
[Quality control assessment for microbial genomes: GalaxyTrakrMicroRunQC workflow \(protocols.io\)](#)



- 1 Log into your GalaxyTrakr account
 - 1.1. Create a GalaxyTrakr account if you are the first-time user:
User Registration Form - Galaxy Genome Trakr (galaxytrakr.org)

User Registration Form

Location

FDA MOD1

[Add New Location](#)

First Name

Enter First Name, Do not use characters: \/\;\.:\|'\"'<?>@.

Last Name

Enter First Name, Do not use characters: \/\;\.:\|'\"'<?>@.

Email

Email will be used for automated messages to include registration information!

Primary Phone

Please enter number with country code, without dashes, for example +17035456789

If possible please use a mobile number than can accept text messages, only used for support

Title

Requirements

Please annotate intended use of Galaxy and Analysis tools. List specific tools you would like to see deployed in Galaxy.

Register

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- 1.2. Log into your GalaxyTrakr account if you already have one:
Galaxy (galaxytrakr.org)

Workflow Visualize Shared Data Help Login or Register

Welcome to Galaxy, please log in

Public Name or Email Address

Password

Forgot password? Click here to reset your password.

Login

Don't have an account? Register here.

Welcome to GalaxyTrakr: open-source bioinformatics for public health.

This site is intended for use by GenomeTrakr laboratories and their collaborators to assist in the analysis of genomic data for foodborne pathogens. This instance of Galaxy is hosted in a public environment and no personally identifiable (PII) or commercial confidential information should be uploaded.

--!!--Information and Announcements--!!--

Please let us know if you have any issues with the new version of Galaxy. Thank you.

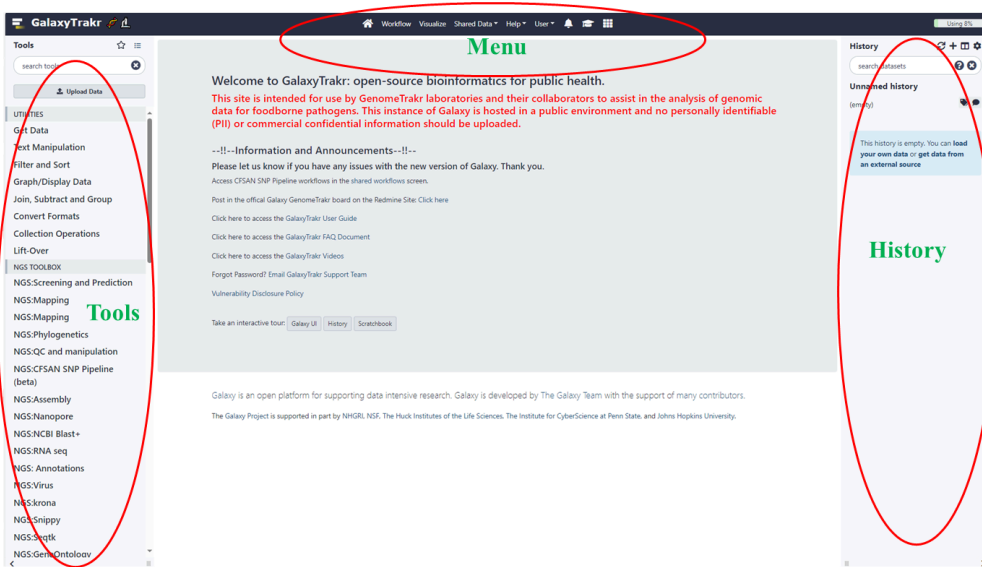
Access CFSAN SNP Pipeline workflows in the shared workflows screen.

Post in the official Galaxy GenomeTrakr board on the Redmine Site: Click here

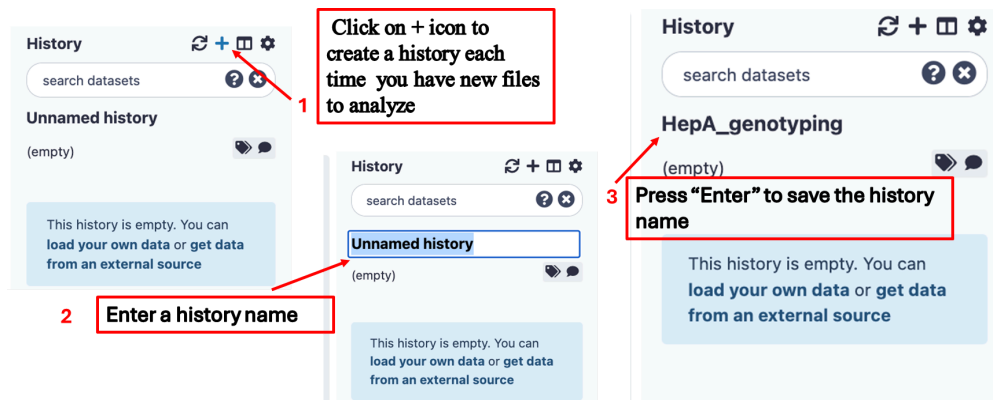
Click here to access the GalaxyTrakr User Guide

Click here to access the GalaxyTrakr FAQ Document

1.3. Get familiar with Galaxy components: Tools, Menu and History



2 Create a new history.



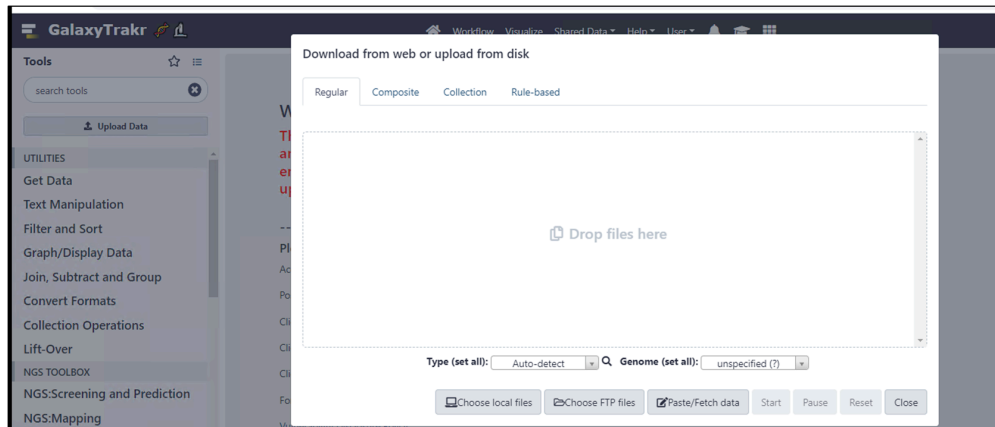
3 Upload raw sequencing data.

The raw sequencing data in fastq files can be imported into GalaxyTrakr directly from your local folder (instructions shown in 3.1); or downloaded from SRA (instructions shown as in 3.2) if the files have been already submitted to ViroTrakr in NCBI (Submission protocol: **NCBI submission protocol for foodborne virus surveillance (protocols.io)**).

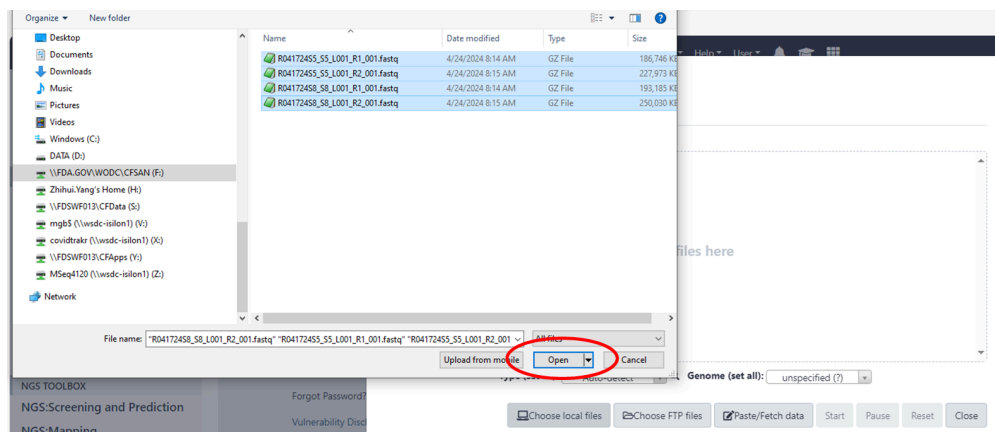
After being uploaded to GalaxyTrakr, the files will remain in your account until they are deleted.

3.1. Upload raw data from local folder.

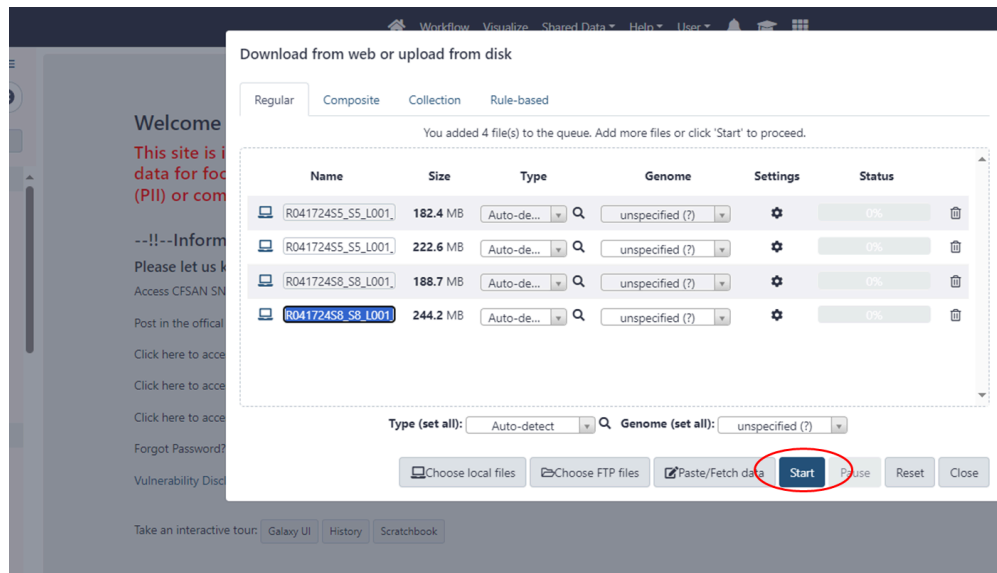
3.1.1. Click on the button "Upload Data", then "Choose local files".



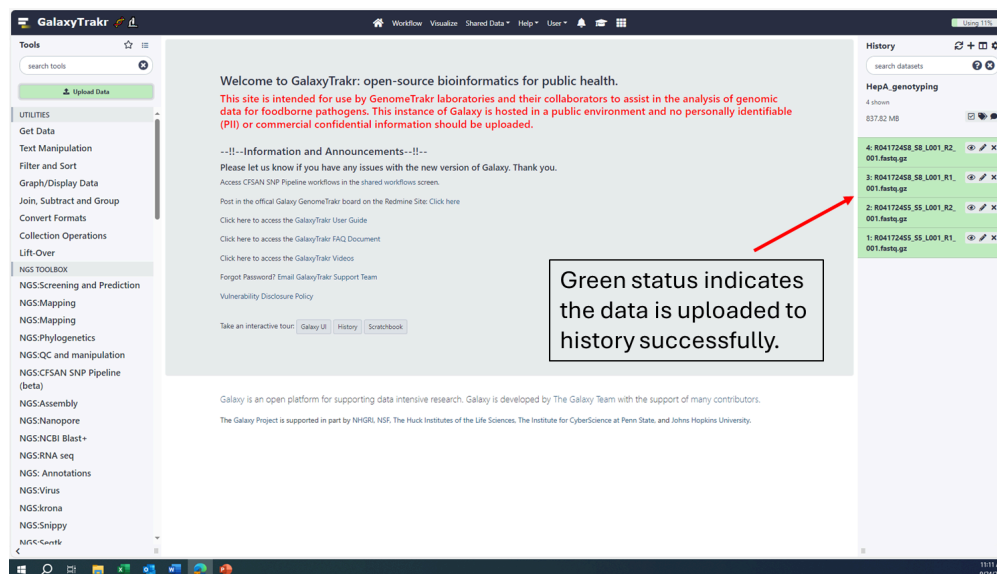
3.1.2. Select fastq files from your local folder.



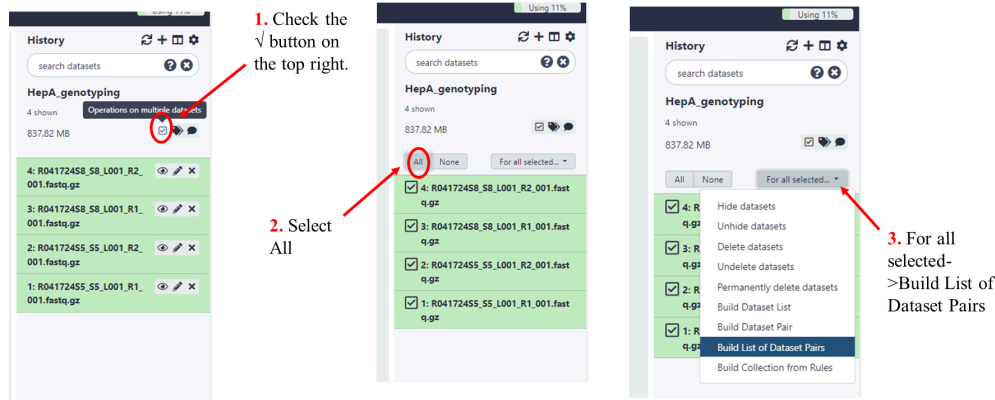
3.1.3. Select the files and click "Start" to upload.



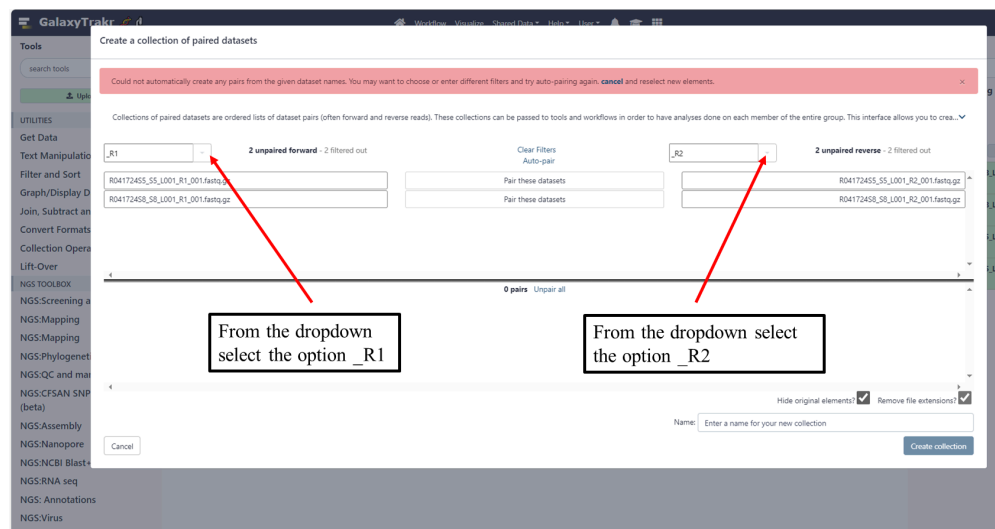
3.1.4. Check the status of data upload.



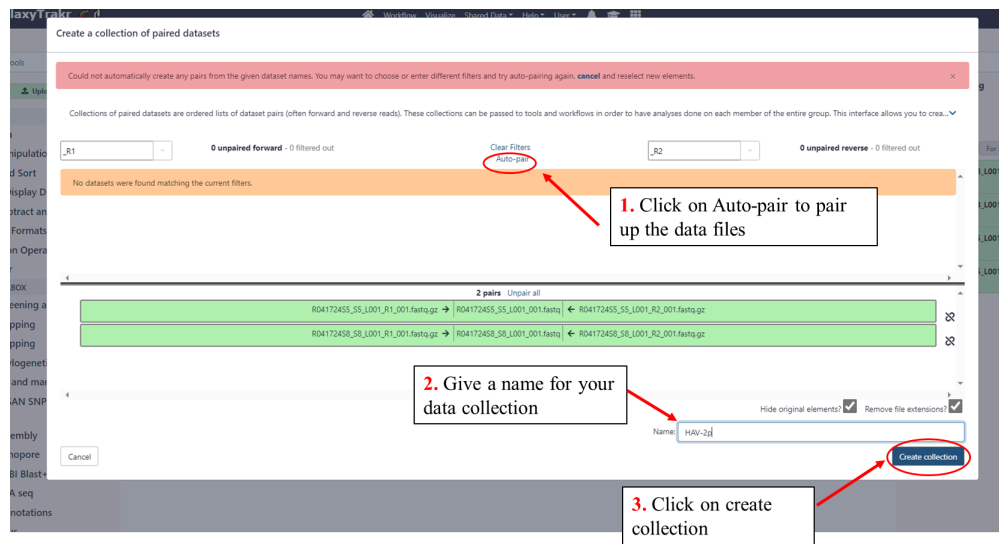
3.1.5. Build a list of Dataset Pairs (pairing the forward and reverse files into their respective samples for batch analysis (Follow steps1, 2 and 3).



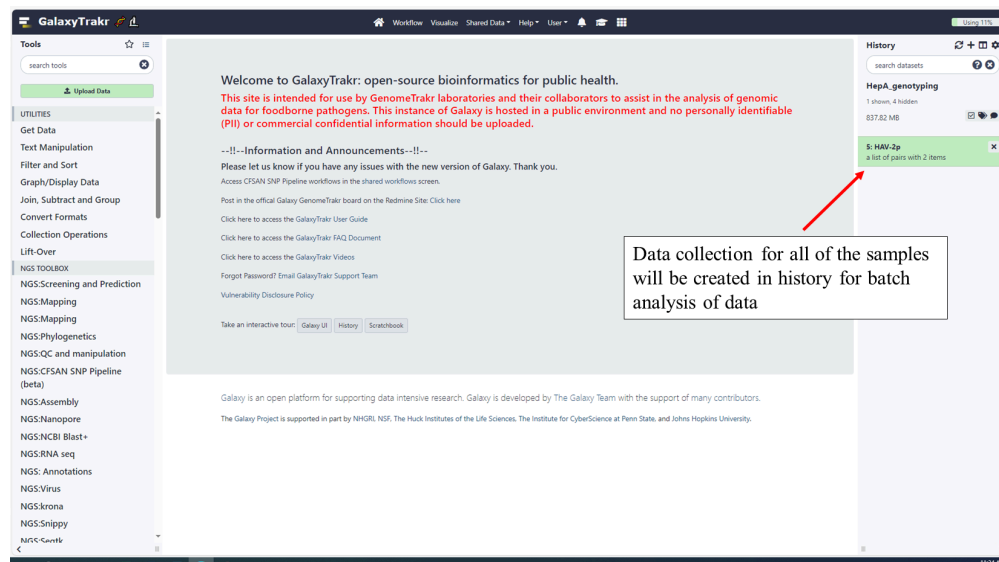
3.1.6. Create a collection of paired datasets.



3.1.6. Create a collection of paired datasets - Cont.



3.1.7. Data collection will be created in history.



3.1.8. Import the reference data files from Shared Data folder following the steps 1-4 as shown below.

1. Click on Shared Data -> Data Libraries.

2. Click on OARSA-MOD1-HepA folder.

3. Select all files from the folder.

4. Click on Export to History -> as Datasets.

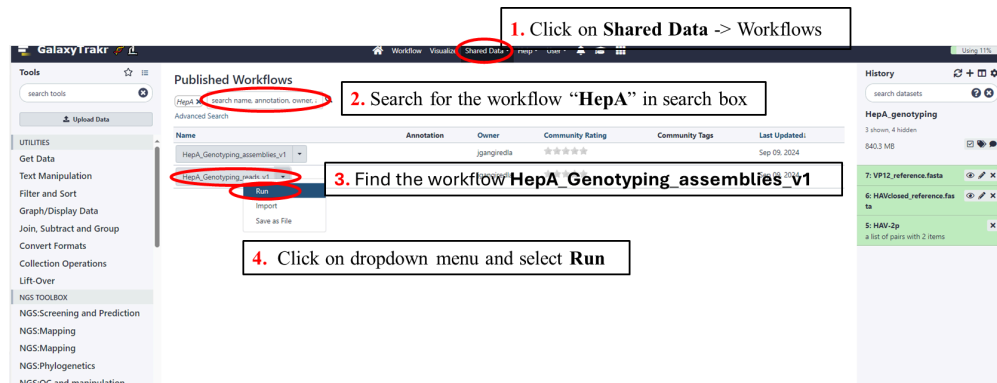
The screenshots show the GalaxyTrakr interface. The first screenshot shows the 'Shared Data' menu and the 'Data Libraries' section. The second screenshot shows the 'OARSA-MOD1-HepA' folder selected in the left sidebar. The third screenshot shows the files in the folder selected. The fourth screenshot shows the 'Export to History' button and the 'as Datasets' option.

3.1.9. Select all files from the folder and Import them as Datasets to your current history following the steps 1-3 as shown below.

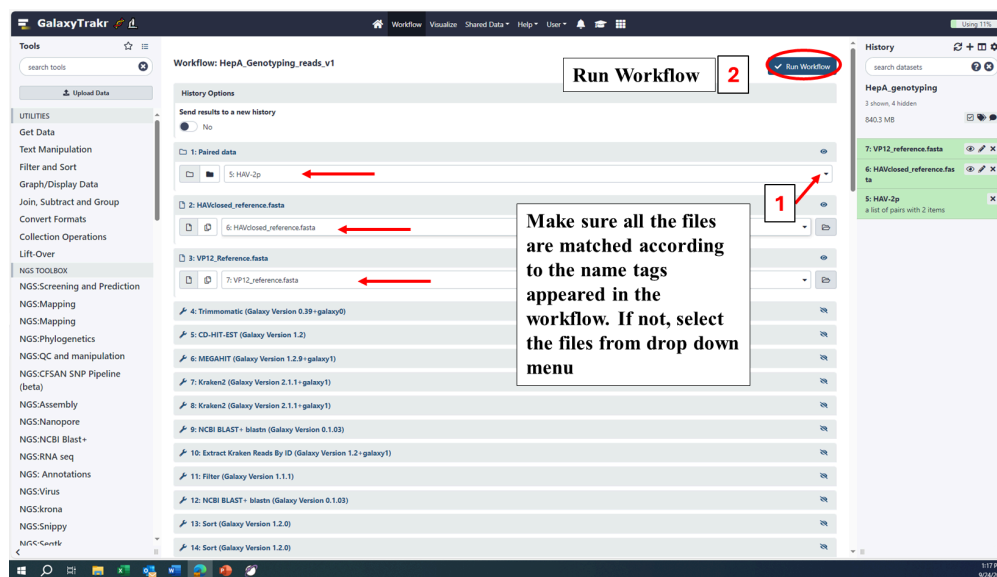
The screenshot shows the 'Import into History' dialog box in GalaxyTrakr. The 'Select history' dropdown is set to 'HepA_genotyping'. The 'Import' button is circled in red.

The screenshot shows the 'History' tab in GalaxyTrakr. The 'HepA_genotyping' workflow is selected, and the 'Import' button is circled in red.

3.1.10. Click on WorkFlow tab from main menu, select and run the HepA_Genotyping_Reads workflow.



3.1.11. Select all the appropriate files from each dropdown menu and run workflow.



3.1.12. Once the workflow run is successful (Green status), results will appear in the history. Note: workflow takes approximately 5 – 10 minutes for the processing to complete.

GalaxyTrakr

Tools

UTILITIES

Get Data

Text Manipulation

Filter and Sort

Graph/Display Data

Join, Subtract and Group

Convert Formats

Collection Operations

Lift-Over

NGS TOOLBOX

NGS:Screening and Prediction

NGS:Mapping

NGS:Mapping

NGS:Phylogenetics

NGS:QC and manipulation

NGS:CFSAN SNP Pipeline (beta)

NGS:Assembly

NGS:Nanopore

NGS:NCBI Blast+

NGS:RNA seq

NGS: Annotations

NGS:Virus

NGS:krona

NGS:Snippy

NGS:Guvvle

Successfully invoked workflow **HepA_genotyping_reads_v1**.

You can check the status of queued jobs and view the resulting data by refreshing the History pane, if this has not already happened automatically.

View Report 1

Download BioCompute Object

Inputs

Outputs

Output Collections

Steps

History

search datasets

HepA_genotyping

49 shown, 77 hidden

1.72 GB

181: Newick Display on data 180: Tree Graph

180: Best-scoring ML Tree

174: MAFFT on data 173

172: Concatenate datasets on data 169, data 166, and others

171: seqtk_seq on data 168

170: Genotype_report

169: Tabular-to-FASTA on data 163

168: Tabular-to-FASTA on data 162

163: Filter on data 155

162: Filter on data 155

155: Collapse Collection on data 148 and data 147

140: Sort on collection 131 a list with 2 items

137: Sort on collection 131 a list with 2 items

134: Sort on collection 128 a list with 2 items

All the results files will appear in your current history

3.1.13. Select and view the result files in the middle panel.

GalaxyTrakr

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NGS:Nanopore

NGS:NCBI Blast+

NGS:RNA seq

NGS: Annotations

NGS:Virus

NGS:krona

Phylogenetic tree visualization showing relationships between various HepA sequences.

History

search datasets

HepA_genotyping

49 shown, 132 hidden

2.62 GB

181: Newick Display on data 180: Tree Graph

180: Best-scoring ML Tree

174: MAFFT on data 173

172: Concatenate datasets on data 169, data 166, and others

171: seqtk_seq on data 168

170: Genotype_report

169: Tabular-to-FASTA on data 163

168: Tabular-to-FASTA on data 162

163: Filter on data 155

162: Filter on data 155

155: Collapse Collection on data 148 and data 147

140: Sort on collection 131 a list with 2 items

137: Sort on collection 131 a list with 2 items

Click on the 'eye' icon to view the results from each file in the middle panel.

3.1.14. Download the result files to your local folder.



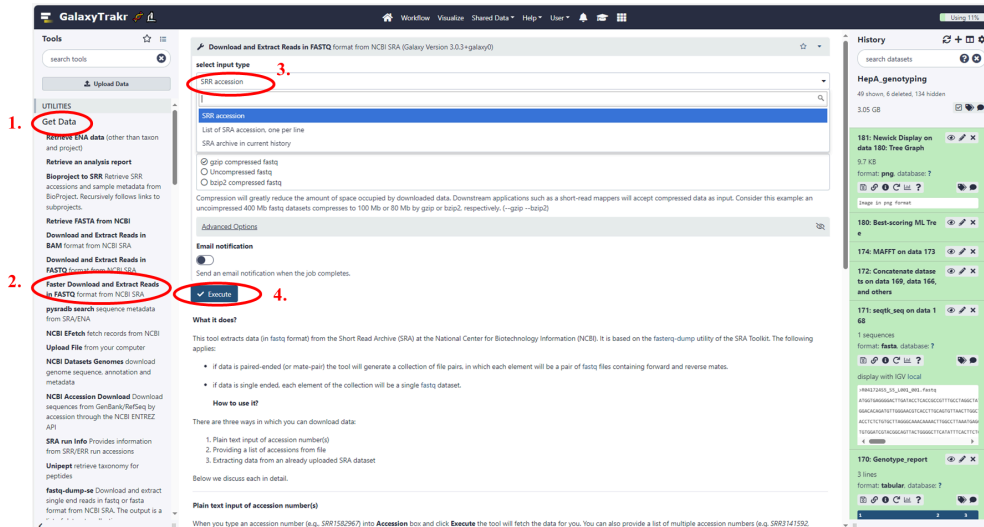
Results files include:

- Assembly with MEGAHIT: Metagenomic assemblies
- Report: Kraken2: Kraken2 reports
- Report_blasthits_Genotype: Reporting Best BLAST Hits against reference sequences
- Noro_genotyping_report: Final report contains QC stats and genotyping results
- HAV_contigs: HAV specific contigs extracted from metagenomics assembly
- Reference_query_phylogenetic_tree: Phylogenetic tree represents the input genomes along with the reference genomes from all groups .png format and .txt format.

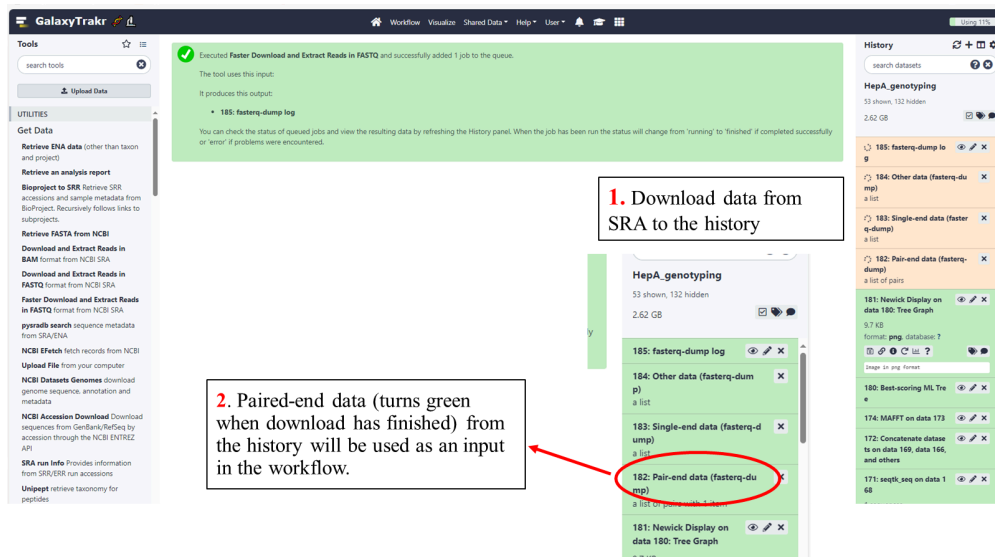
3.2. Download data from SRA database.

You may also download raw data from SRA if you already submit it to the ViroTrakr, or from other Bioprojects in NCB. You need to get the SRR# or SRA accession# ready for the download.

3.2.1. From "Get Data" on the left menu, select "Faster Download and Extract reads in FASTQ from NCBI SRA", and select option by clicking on drop down menu, three options available: (1) "SRR accession" number(s); (2) "List of SRA accessions, one per line"; or (3) SRA archive in current history". Click button "Execute" once the information is put in.



3.2.2. Data files will be downloading from NCBI SRA database. (Download time varies, depending on the number of files downloading and the NCBI server status).



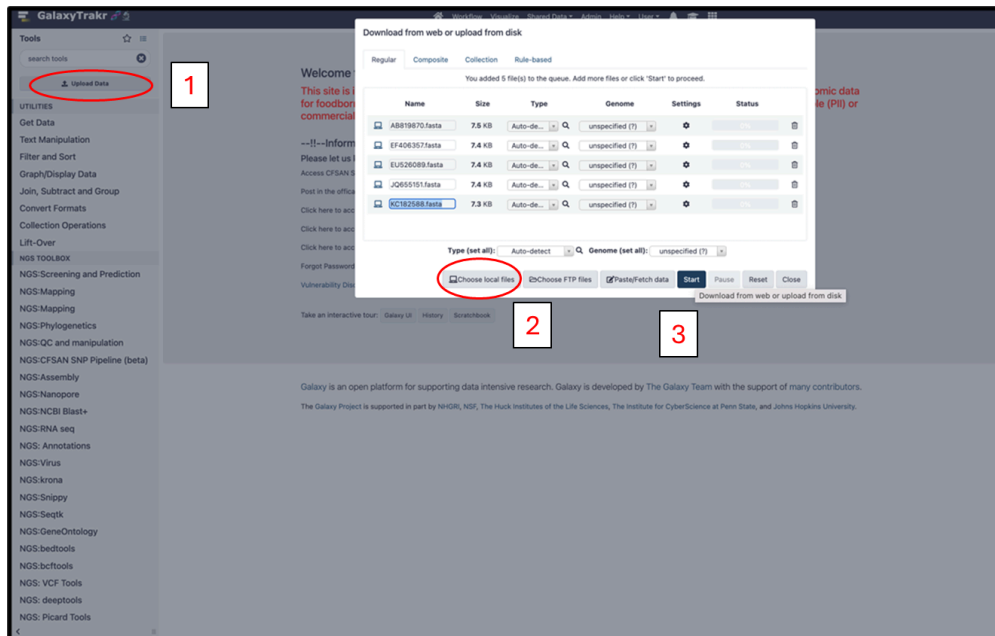
3.2.3. Follow the steps from 3.1.8. to 3.1.14 to run the workflow and collect the results.

4 Upload assembled sequences.

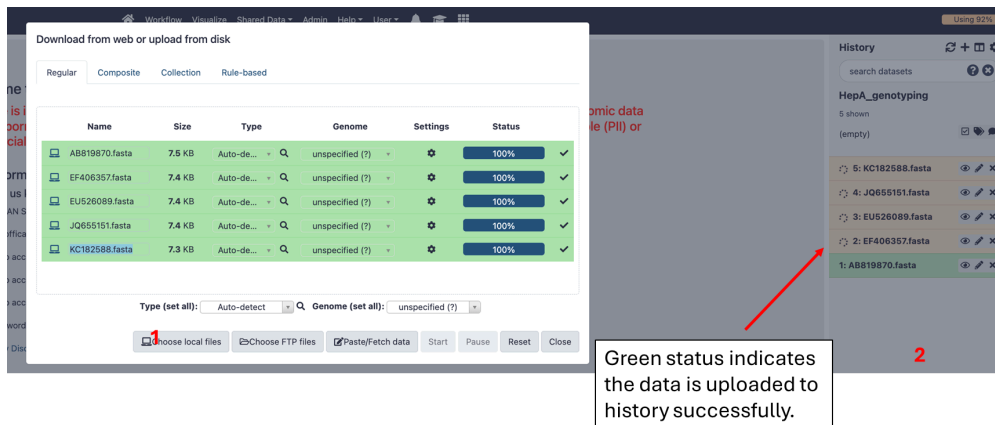
This ViroTrakr workflow can be used with raw sequencing data, as well as assembled sequences that you may already obtained with another platform and stored in your local folder.

4.1. Upload assembled sequences from local folder.

4.1.1. Click on the button "Upload Data", "Choose local files", then click the button "Start".

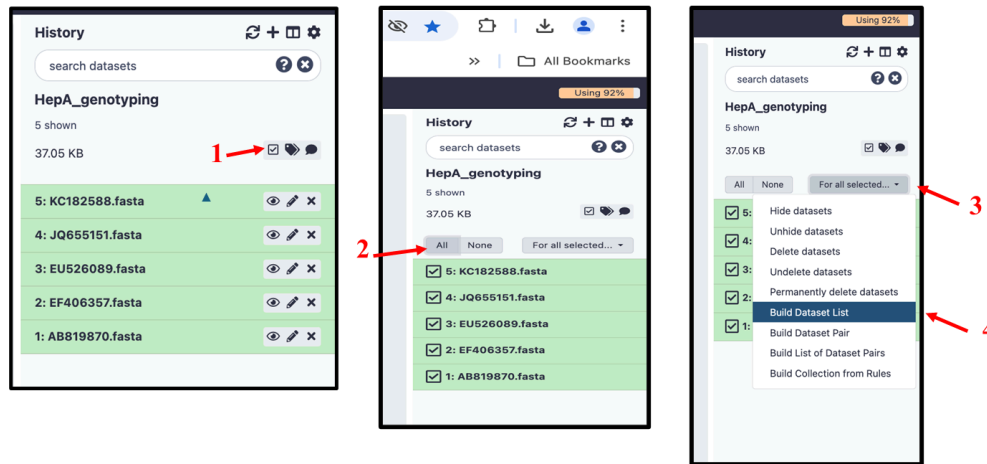


4.1.2. Check the status of data upload.

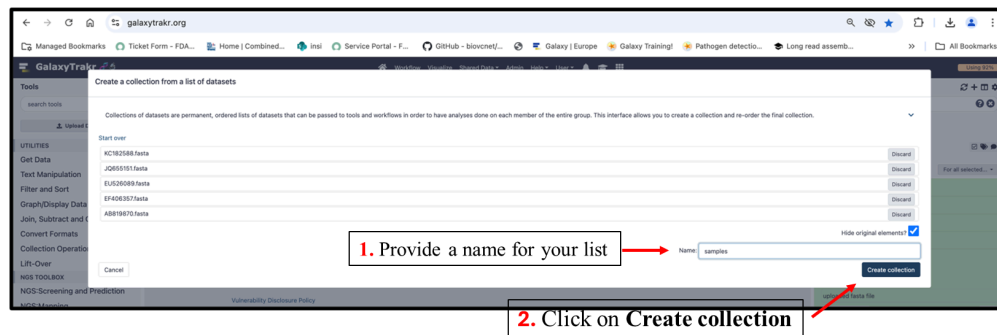


4.1.3. Create a dataset list.

1.click on Checkbox; 2. Click on All to select all the assemblies; 3. click on Drop down on for all selected. 4. Select Build Dataset List.



4.1.4. Build Dataset list on selected samples.



4.1.5. Follow the steps from 3.1.8. to 3.1.14 to run the workflow and collect the results.