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Salmonella serotype prediction using the GalaxyTrakr SeqSero2 workflow V.1

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Gangiredla, J., Rand, H., Benisatto, D. et al. GalaxyTrakr: a distributed analysis tool for public health whole genome sequence data accessible to non-bioinformaticians. BMC Genomics 22, 114 (2021).

Protocol status: Working
We use this protocol and it’s working

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Disclaimer

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Abstract

Salmonella serotypes are defined by two surface structures, O antigen and two H antigens. Traditional serotype determination is performed with the *Salmonella* serological somatic (O) and flagellar (H) tests and paired with biochemical confirmation. More than 2,600 *Salmonella* serotypes have been described in the White-Kauffmann-Le Minor scheme. Molecular methods for serotype determination have been developed based on genes responsible for serotype antigens. These genes are encoded in the *rfb* gene cluster, *fliC*, and *fljB*. SeqSero2 is a bioinformatic pipeline that uses whole genome sequence (WGS) data from pure-culture isolates to perform *in silico* analysis to determine the antigenic formula, including somatic (O) antigens and both flagellar (H) antigens. This provides continuity with the well-established scheme for phenotypic *Salmonella* serotypes.

PURPOSE:
This document outlines the steps required to run SeqSero2 v1.1.1 on a collection of isolates in the GalaxyTrakr environment. This is performed by utilizing a custom workflow called “SeqSero2 v1.1.1 collection workflow” and downloading the resulting table.

SCOPE: This protocol covers the following tasks:
1. set up an account in GalaxyTrakr
2. Create a new history/workspace
3. Upload data
4. Execute the SeqSero2 workflow
5. Download the results

Materials

Salmonella WGS fastq files or SRA accessions

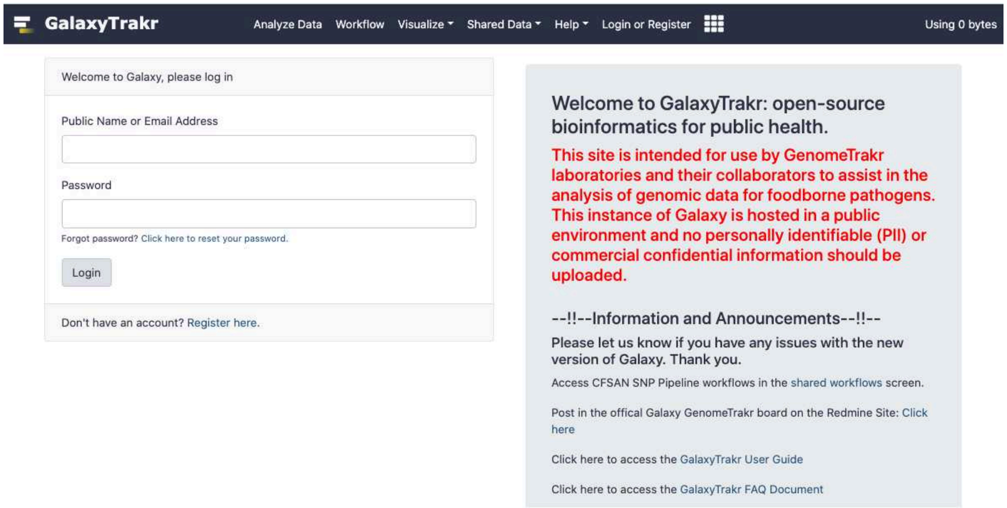
Troubleshooting

Before start

When using GalaxyTrakr, it is recommended to use Google Chrome for optimal browser experience although Microsoft Edge and Safari are also compatible browsers. Internet Explorer and Mozilla FireFox are NOT compatible with GalaxyTrakr.

Login and import workflow

1 Log into GalaxyTrakr 1909 (https://galaxytrakr.org/root/login)



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 offical Galaxy GenomeTrakr board on the Redmine Site: Click here

Click here to access the GalaxyTrakr User Guide

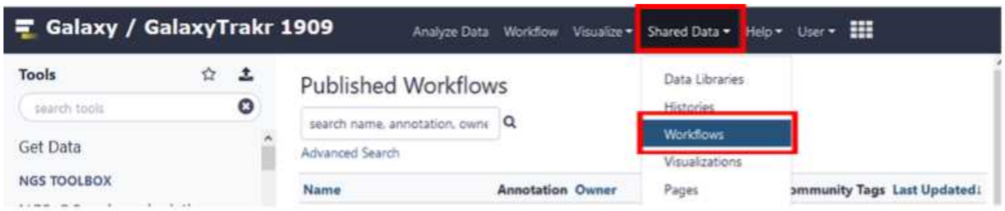
Click here to access the GalaxyTrakr FAQ Document

Link to create a new GalaxyTrakr account: https://account.galaxytrakr.org/Account/Register

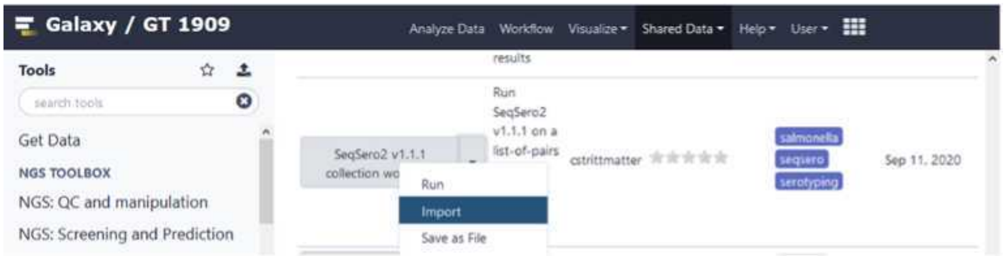
1 **Import** the SeqSero2 workflow into the Tools Panel

> Note
>
> Step 2 only needs to be done once. After this workflow is imported it will be available for use in your Tools Panel.

1.1 Click on **Shared Data** and then **Workflows** from the dropdown menu.



1.2 Select the shared workflow: **SeqSero2 v1.1.1 collection workflow (cstrittmatter 9/11/2020)** and select **import** from the dropdown arrow.

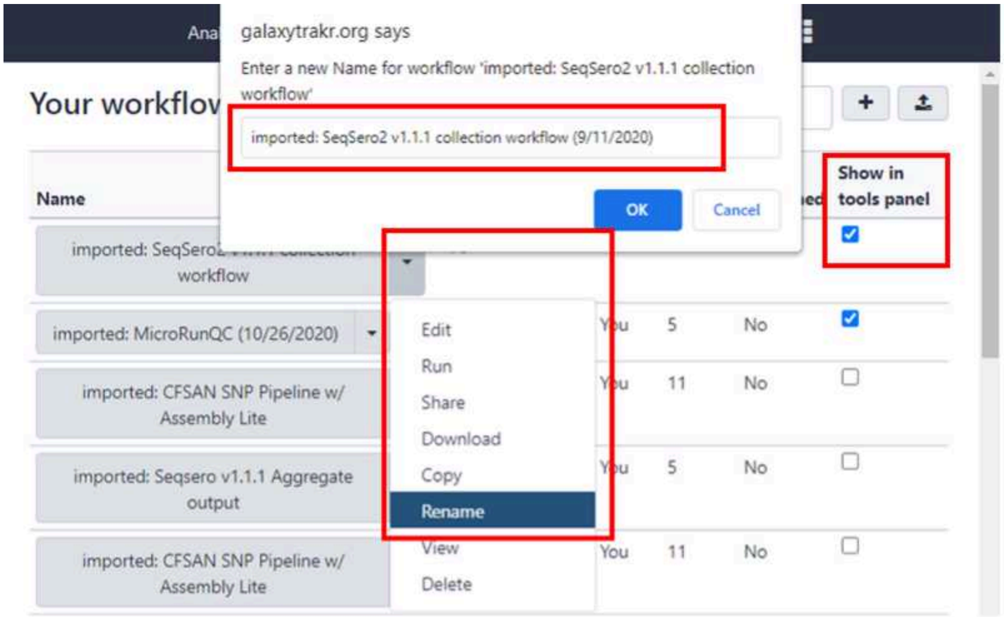


1.3 In **Your Workflows** you can use the dropdown arrow to rename of this workflow.

Adding a date to the name will help you in keeping track of newer versions of this workflow. Workflows do get updated periodically and you want to ensure you are working with the most recent version.

Check the box **"Show in tools panel"**.

This will move the Seqsero2 v1.1.1 collection workflow into your tools panel permanently and you will now have this workflow available to you every time you log into GalaxyTrakr.


galaxytrakr.org says

Enter a new Name for workflow 'imported: SeqSero2 v1.1.1 collection workflow'

imported: SeqSero2 v1.1.1 collection workflow (9/11/2020)

Step 2 only needs to be done once for each workflow that is being imported into your Tools.

Import data for analysis

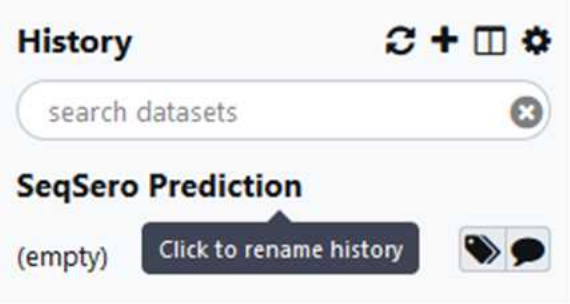
2 If your data is already in GalaxyTrakr, open the history containing that data to be analyzed or move the data to a new history for analysis and proceed to **Step #**. This option may be preferred if the data was already uploaded for other purposes such as MicroRunQC. It's ok if there are non-*Salmonella* isolates in your dataset.They will not return an antigenic formula or serovar name.

For uploading new data proceed to next step to create a new history and upload your data to be analyzed.

2.1 **Create new History:**

Click on the "+" button in the upper right corner.

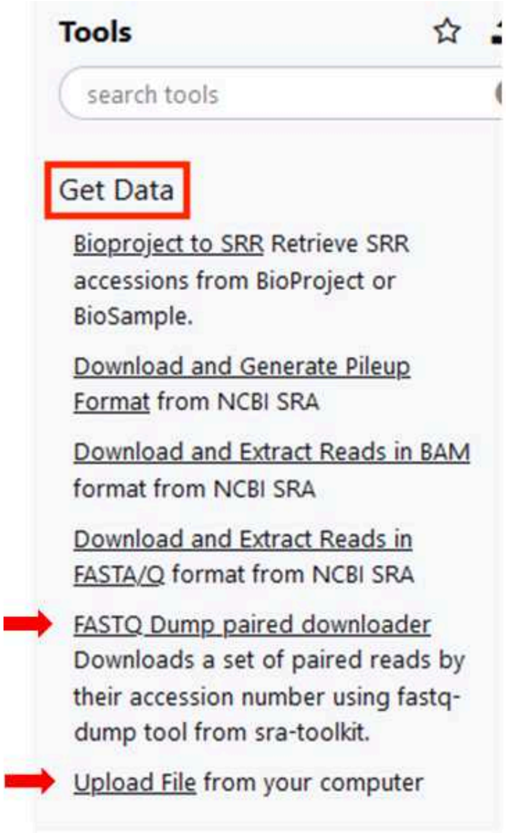
Type in a custom name (i.e., "SeqSero Prediction")



2.2 **Import data:**

Click on the Tool "**Get Data**", top of left panel.

Click on the sub-tool of choice to bring in data for analysis.



Tools

search tools

Get Data

Bioproject to SRR Retrieve SRR accessions from BioProject or BioSample.

Download and Generate Pileup Format from NCBI SRA

Download and Extract Reads in BAM format from NCBI SRA

Download and Extract Reads in FASTA/Q format from NCBI SRA

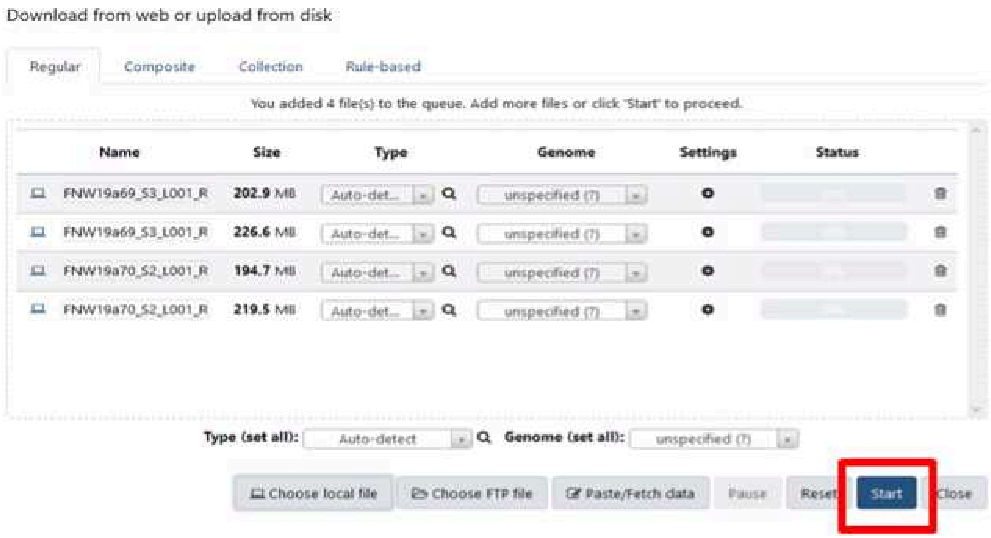
FASTQ Dump paired downloader Downloads a set of paired reads by their accession number using fastq-dump tool from sra-toolkit.

Upload File from your computer

Next steps will show how to import data from NCBI OR upload from your computer.

2.3 “Upload File” for .gz files stored locally.

1. Click on “Choose files”
2. Find your WGS fastq.gz files and select those (2 data files: Read 1 and Read 2 per organism).
3. Click “Start” The amount of time to upload depends on how many files have been selected and the size of those files. The status bar will start to fill as upload progress is made and turn green when completed.



You added 4 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Type	Genome	Settings	Status
FNW19a69_S3_L001_R	202.9 MB	Auto-det...	unspecified (?)		
FNW19a69_S3_L001_R	226.6 MB	Auto-det...	unspecified (?)		
FNW19a70_S2_L001_R	194.7 MB	Auto-det...	unspecified (?)		
FNW19a70_S2_L001_R	219.5 MB	Auto-det...	unspecified (?)		

Type (set all): Auto-detect

Genome (set all): unspecified (?)

2.4 "FASTQ Dump Paired downloader" to import data from NCBI.

1. Enter the NCBI SRR for each sequence to be retrieved.
2. Click "Execute"

FASTQ Dump paired downloader Downloads a set of paired reads by their accession number using fastq-dump tool from sra-toolkit. (Galaxy Version 1.1.4)

2.5 When the data has finished importing, you should see the successfully uploaded files listed in green in the right panel.

Files will be highlighted in RED if they were NOT successfully uploaded.

Example of .gz files uploaded:

History

search datasets

SeqSero Prediction

4 shown

843.76 MB

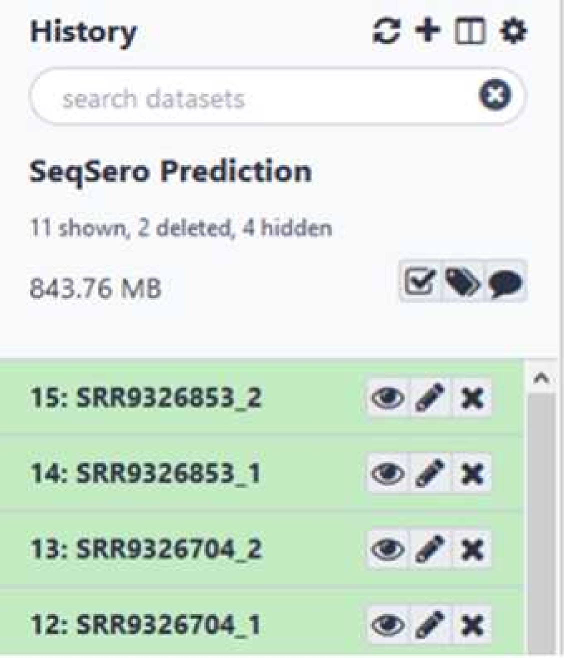
4: FNW19a70_S2_L001_R2_001.fastq.gz

3: FNW19a70_S2_L001_R1_001.fastq.gz

2: FNW19a69_S3_L001_R2_001.fastq.gz

1: FNW19a69_S3_L001_R1_001.fastq.gz

Example of SRR data downloaded from NCBI:



History

search datasets

SeqSero Prediction

11 shown, 2 deleted, 4 hidden

843.76 MB

15: SRR9326853_2

14: SRR9326853_1

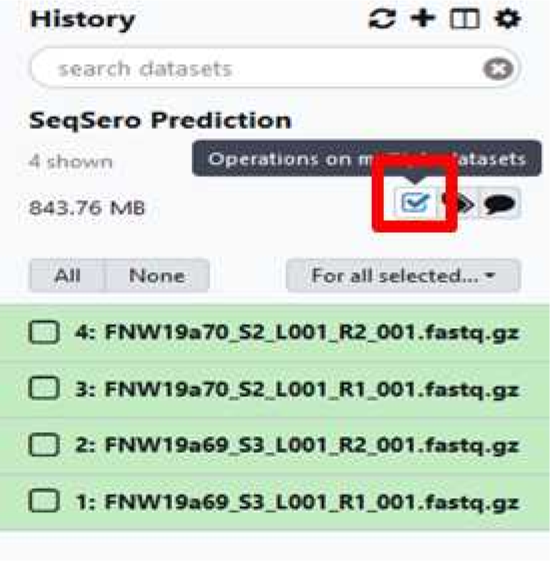
13: SRR9326704_2

12: SRR9326704_1

Build your dataset of paired-reads

3 **Build “list of data set pairs”**

3.1 Click on the check mark in the history panel then select all files you want to include in the data set for SeqSero analysis.



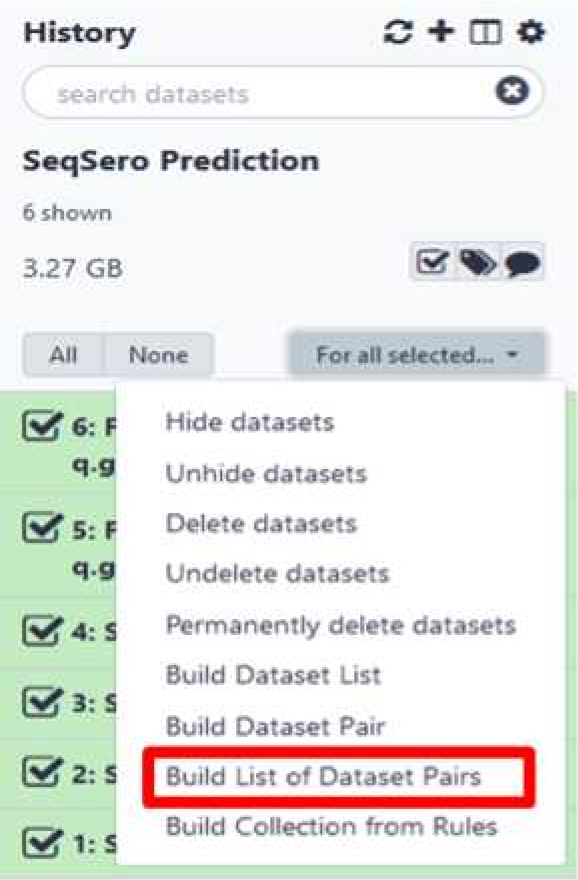
☐ 4: FNW19a70_S2_L001_R2_001.fastq.gz

☐ 3: FNW19a70_S2_L001_R1_001.fastq.gz

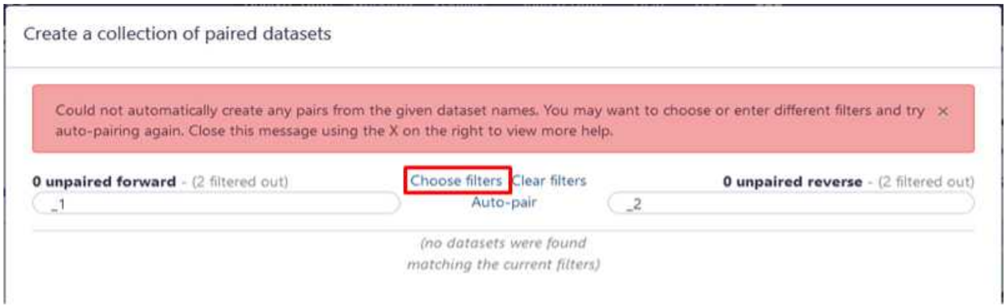
☐ 2: FNW19a69_S3_L001_R2_001.fastq.gz

☐ 1: FNW19a69_S3_L001_R1_001.fastq.gz

3.2 Open options under “For all selected” and then choose “Build List of Dataset Pairs”



3.3 Click “**Choose Filters**”


Create a collection of paired datasets

Could not automatically create any pairs from the given dataset names. You may want to choose or enter different filters and try auto-pairing again. Close this message using the X on the right to view more help.

(no datasets were found matching the current filters)

3.4 Click the correct file extension “**Forward: _R1, Reverse: _R2**”


Create a collection of paired datasets

Could not automatically create any pairs from the given dataset names. You may want to choose or enter different filters and try auto-pairing again. Close this message using the X on the right to view more help.

Choose from the following filters to change which unpaired reads are shown in the display:

3.5 Click “**Auto-pai**r”

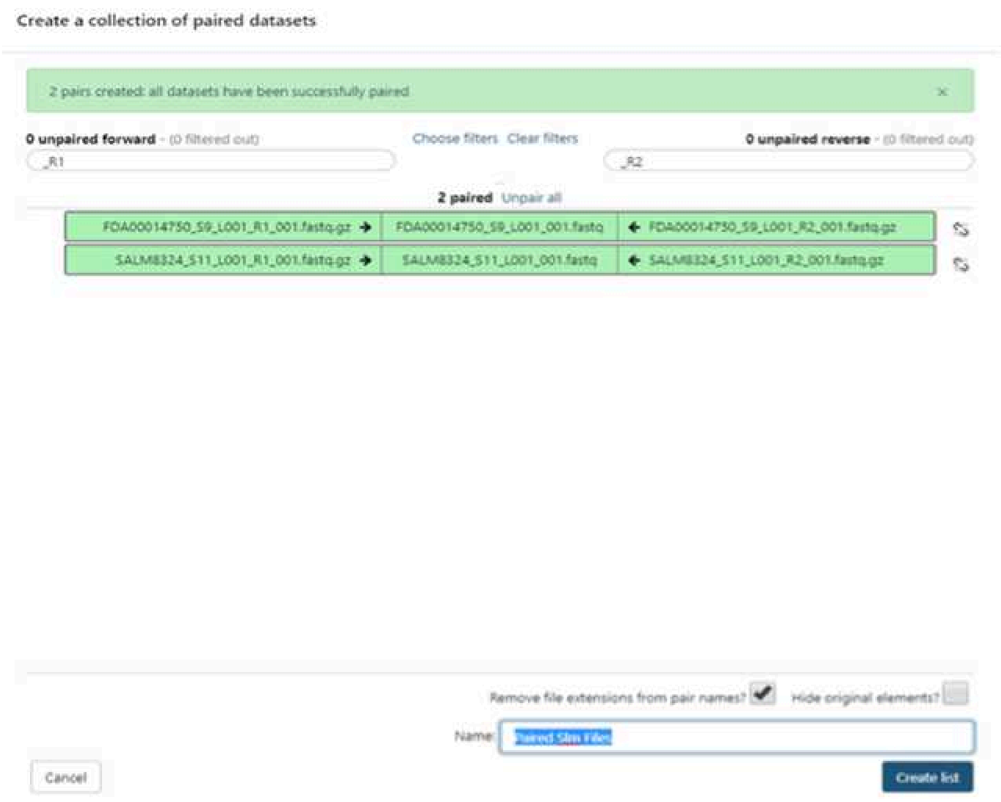
The Read 1 and Read 2 fastq.gz files should automatically pair together. Note: For data downloaded from NCBI the two reads will already be paired and you will not need to select filter and auto-pair.


Create a collection of paired datasets

Could not automatically create any pairs from the given dataset names. You may want to choose or enter different filters and try auto-pairing again. Close this message using the X on the right to view more help.

3.6 Type in a custom name for the dataset (i.e., “Paired Slm Files”)

Click “**Create list”**



Create a collection of paired datasets

2 pairs created: all datasets have been successfully paired

0 unpaired forward - (0 filtered out): _R1

0 unpaired reverse - (0 filtered out): _R2

2 paired

Forward	Pair name	Reverse
FDA00014750_S9_L001_R1_001.fastq.gz →	FDA00014750_S9_L001_001.fastq	← FDA00014750_S9_L001_R2_001.fastq.gz
SALM8324_S11_L001_R1_001.fastq.gz →	SALM8324_S11_L001_001.fastq	← SALM8324_S11_L001_R2_001.fastq.gz

Remove file extensions from pair names? ☑ Hide original elements? ☐

Name: Paired Slm Files

You should see your named list in the history panel

Analyze your data using the SeqSero2 workflow

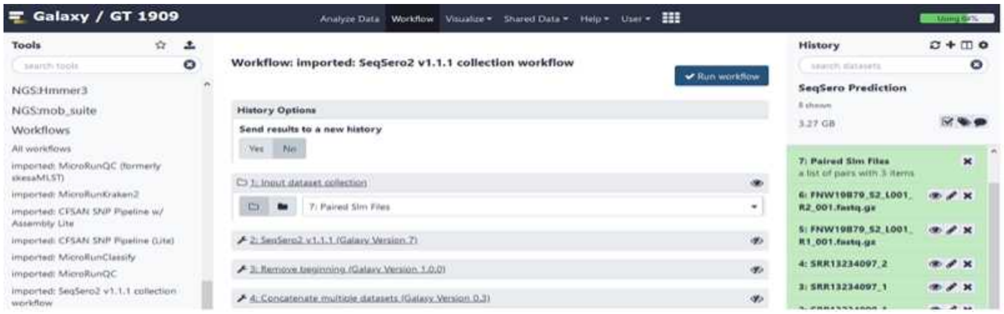
4 In NGS TOOLBOX, left panel:

Click on the imported and saved version of the “**SeqSero2 v1.1.1 collection workflow**”.

4.1 In the Main window, the newly created list of paired files should automatically show up in the “Input dataset collection” window.

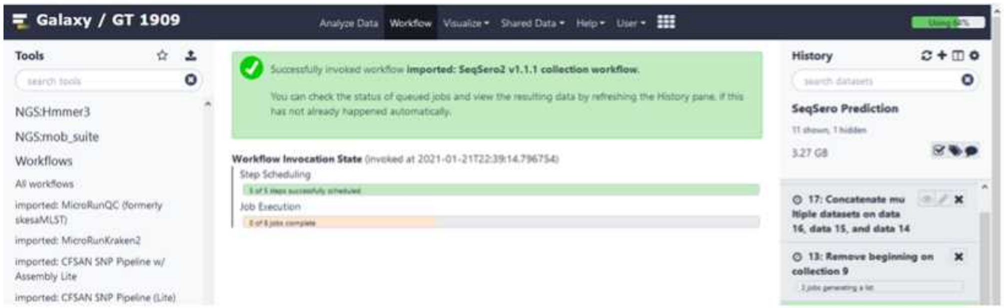
If it doesn’t, click and drag the file from your history panel into the “Input dataset collection” window.

Click “**Run Workflow”**



Workflow: imported: SeqSero2 v1.1.1 collection workflow

4.2 Your working panel should appear green with a white check mark on the upper left-hand corner.



Successfully invoked workflow **imported: SeqSero2 v1.1.1 collection workflow.**

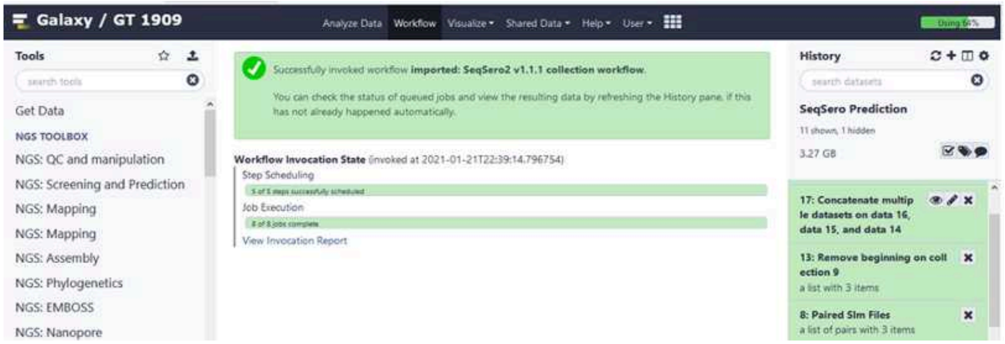
You can check the status of queued jobs and view the resulting data by refreshing the History pane. If this has not already happened automatically.

Workflow Invocation State (invoked at 2021-01-21T22:39:14.796754)

Step Scheduling

Job Execution

4.3 After the SeqSero analysis is complete, the “Concatenate multiple datasets on data” will appear green.



Successfully invoked workflow **imported: SeqSero2 v1.1.1 collection workflow.**

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.

Workflow Invocation State (invoked at 2021-01-21T22:39:14.796754)

Step Scheduling

5 of 5 steps successfully scheduled

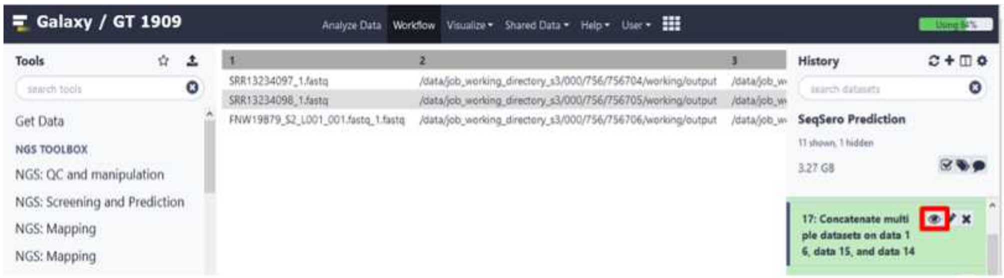
Job Execution

8 of 8 jobs complete

View Invocation Report

View and export results

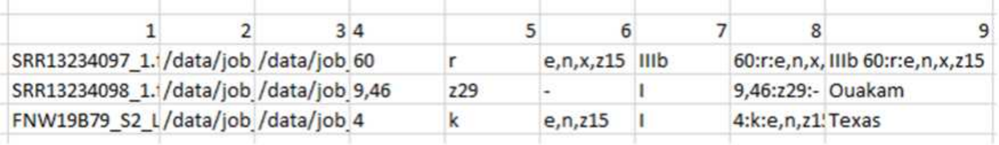
5 Click on the “eyeball” in the “**Concatenate multiple datasets on data”** to view table of predicted serotypes for the collection.



1	2	3
SRR13234097_1.fastq	/data/job_working_directory_s3/000/756/756704/working/output	/data/job_w
SRR13234098_1.fastq	/data/job_working_directory_s3/000/756/756705/working/output	/data/job_w
FNW19879_S2_L001_001.fastq_1.fastq	/data/job_working_directory_s3/000/756/756706/working/output	/data/job_w

Note: Scroll across the table to see additional information.

5.1 **Export SeqSero results:** cut/paste method



1	2	3	4	5	6	7	8	9
SRR13234097_1.	/data/job_	/data/job_	60	r	e,n,x,z15	IIIb	60:r:e,n,x,	IIIb 60:r:e,n,x,z15
SRR13234098_1.	/data/job_	/data/job_	9,46	z29	-	I	9,46:z29:-	Ouakam
FNW19B79_S2_L	/data/job_	/data/job_	4	k	e,n,z15	I	4:k:e,n,z1	Texas

1. Click and drag to highlight text
2. Copy
3. Paste Special as “Text” or “Unicode Text” into Excel

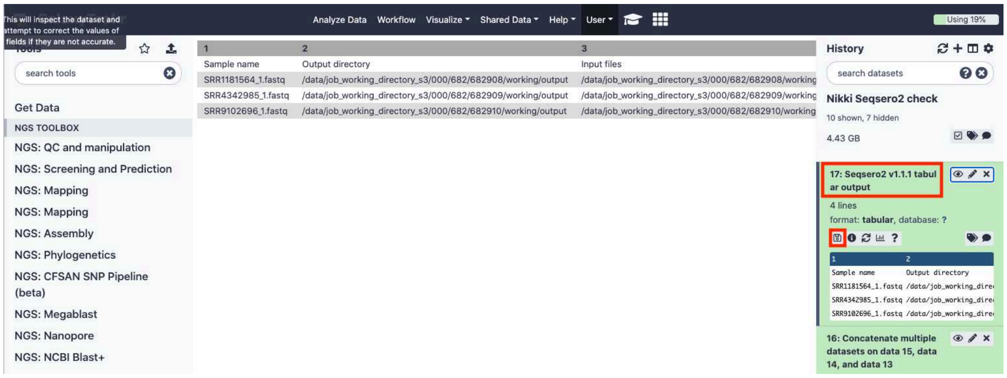
Alternatively, click on the table and “Ctrl-A” to select the entire Table, “Ctrl-C” to copy data and paste the copied data into Excel by “Ctrl-V”

5.2 **Export SeqSero2 results**: download tab-delimited text file

Click the dataset name.

The panel will expand, enabling more options.

Click the "**Save**" icon to download a tab-delimited file of results.

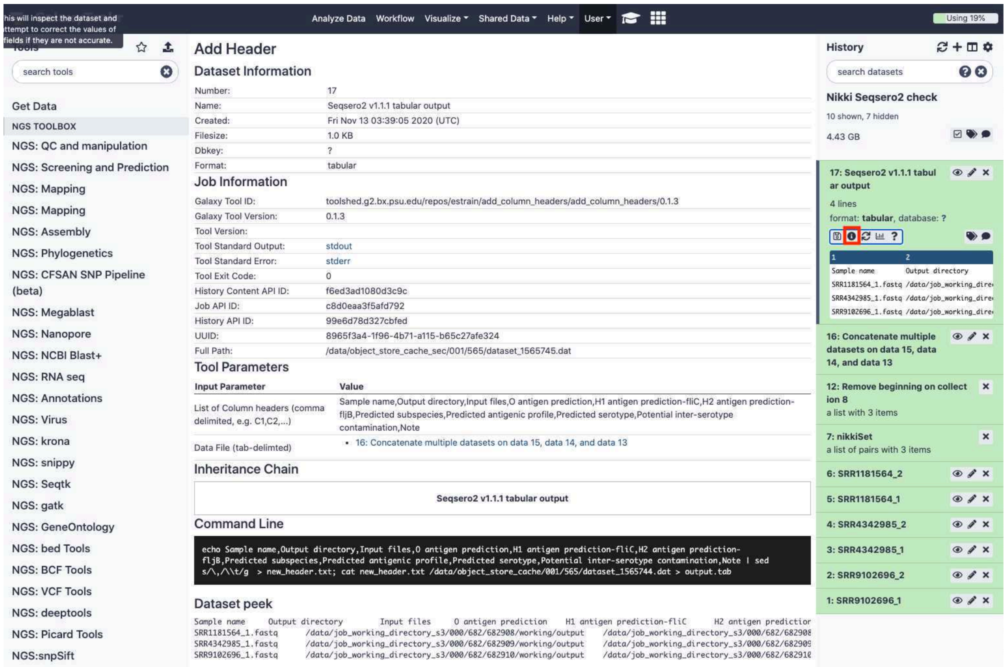


1	2	3
Sample name	Output directory	Input files
SRR1181564_1.fastq	/data/job_working_directory_s3/000/682/682908/working/output	/data/job_working_directory_s3/000/682/682908/working
SRR4342985_1.fastq	/data/job_working_directory_s3/000/682/682909/working/output	/data/job_working_directory_s3/000/682/682909/working
SRR9102696_1.fastq	/data/job_working_directory_s3/000/682/682910/working/output	/data/job_working_directory_s3/000/682/682910/working

Example results file: SeqSeroExampleResults.tabular

5.3 **Optional:**

The small "Info" icon results in a detailed view of the dataset, analysis, parameters used, etc., which can be helpful for troubleshooting.



Add Header

Dataset Information

Number:	17
Name:	Seqsero2 v1.1.1 tabular output
Created:	Fri Nov 13 03:39:05 2020 (UTC)
Filesize:	1.0 KB
Dbkey:	?
Format:	tabular

Job Information

Galaxy Tool ID:	toolshed.g2.bx.psu.edu/repos/estrain/add_column_headers/add_column_headers/0.1.3
Galaxy Tool Version:	0.1.3
Tool Version:	
Tool Standard Output:	stdout
Tool Standard Error:	stderr
Tool Exit Code:	0
History Content API ID:	f6ed3ad1080d3c9c
Job API ID:	c8d0eaa3f5afd792
History API ID:	99e6d78d327cbfed
UUID:	8965f3a4-1f96-4b71-a115-b65c27afe324
Full Path:	/data/object_store_cache_sec/001/565/dataset_1565745.dat

Tool Parameters

Input Parameter	Value
List of Column headers (comma delimited, e.g. C1,C2,...)	Sample name,Output directory,Input files,O antigen prediction,H1 antigen prediction-fliC,H2 antigen prediction-fljB,Predicted subspecies,Predicted antigenic profile,Predicted serotype,Potential inter-serotype contamination,Note
Data File (tab-delimted)	• 16: Concatenate multiple datasets on data 15, data 14, and data 13

Inheritance Chain

Seqsero2 v1.1.1 tabular output

Command Line

```
echo Sample name,Output directory,Input files,O antigen prediction,H1 antigen prediction-fliC,H2 antigen prediction-
fljB,Predicted subspecies,Predicted antigenic profile,Predicted serotype,Potential inter-serotype contamination,Note | sed
s/\,/\\t/g  > new_header.txt; cat new_header.txt /data/object_store_cache/001/565/dataset_1565744.dat > output.tab
```

Dataset peek

```
Sample name     Output directory        Input files     O antigen prediction    H1 antigen prediction-fliC      H2 antigen predictior
SRR1181564_1.fastq      /data/job_working_directory_s3/000/682/682908/working/output    /data/job_working_directory_s3/000/682/682908
SRR4342985_1.fastq      /data/job_working_directory_s3/000/682/682909/working/output    /data/job_working_directory_s3/000/682/682909
SRR9102696_1.fastq      /data/job_working_directory_s3/000/682/682910/working/output    /data/job_working_directory_s3/000/682/682910
```