

Sep 29, 2025

Version 1

bettercallsal - *Salmonella* serotyping tool based on NCBI Pathogen Detection Project for *Salmonella*. V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzqwd5vx1/v1>

Christopher Duda¹, Padmini Ramachandran¹

¹FDA

GenomeTrakr

Tech. support email: genomeTrakr@fda.hhs.gov



Christopher Duda

FDA

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.rm7vzqwd5vx1/v1>

External link: <https://github.com/CFSAN-Biostatistics/bettercallsal>

Protocol Citation: Christopher Duda, Padmini Ramachandran 2025. bettercallsal - *Salmonella* serotyping tool based on NCBI Pathogen Detection Project for *Salmonella*.. protocols.io <https://dx.doi.org/10.17504/protocols.io.rm7vzqwd5vx1/v1>

**Manuscript citation:**

<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1200983/full>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development

We are still developing and optimizing this protocol

Created: June 25, 2025

Last Modified: September 29, 2025

Protocol Integer ID: 221074

Keywords: Salmonella serotyping, whole genome sequences, shotgun metagenomic sequences, ncbi pathogen detection project for salmonella, salmonella serotyping tool, salmonella from whole genome isolate sequence, multiple salmonella serovar, compatibility with historical salmonella nomenclature scheme, historical salmonella nomenclature scheme, identified salmonella, ncbi pathogen detection project, genome indexing approach, metagenomic sequences since this tool, salmonella, whole genome isolate sequence, sequencing data, profiling integration with ncbi snp, metagenomic sequence, serotyping tool, several confidence metrics sequence quality control assessments sequence, detailed multiqc report, results antimicrobial resistance, profiling integration, more information about this tool, antimicrobial resistance

Disclaimer

This protocol describes a scientific tool developed for research and surveillance purposes. Use of bettercallsal does not constitute FDA endorsement and confers no regulatory benefits or preferential treatment in FDA oversight activities.

Abstract

This document outlines the steps necessary to run the tool - bettercallsal. bettercallsal tool is a *Salmonella* serotyping tool, that accepts both short-read and long-read sequencing data. This tool can serotype *Salmonella* from whole genome isolate sequences or from metagenomic sequences since this tool can identify multiple *Salmonella* serovars. The tool's ability to detect multiple *Salmonella* serovars simultaneously makes it particularly valuable for analyzing complex samples where traditional methods may fail. The tool employs a genome indexing approach while maintaining compatibility with historical *Salmonella* nomenclature schemes, ensuring continuity with existing surveillance systems and databases. More information about this tool can be found at this link :

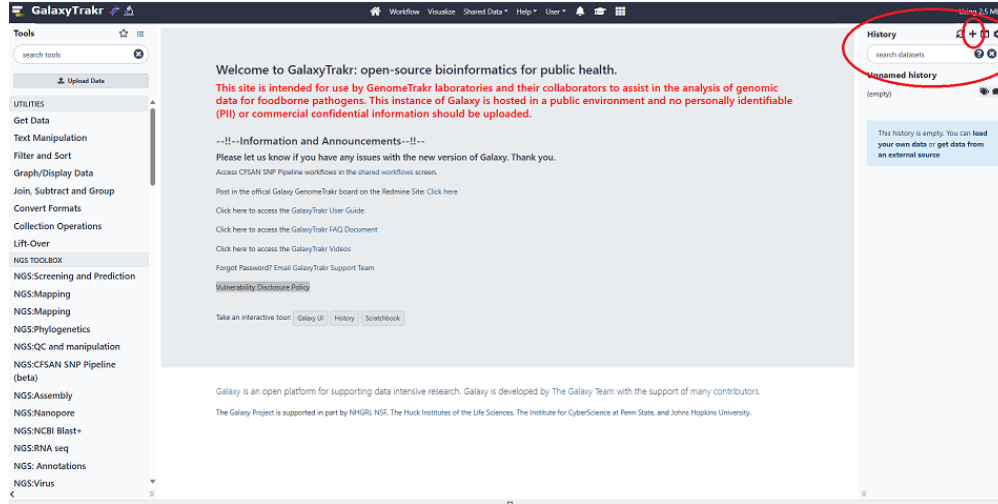
<https://github.com/CFSAN-Biostatistics/bettercallsal> and

<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1200983/full> and

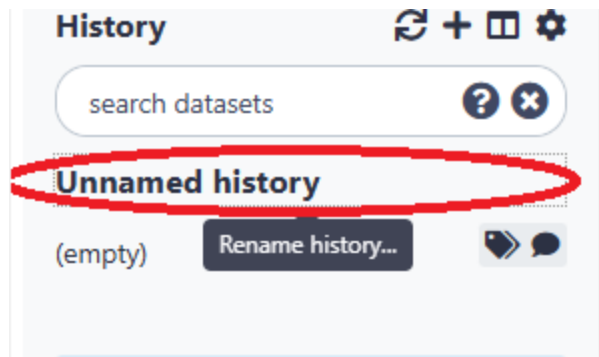
<https://www.fda.gov/media/171312/download>. bettercallsal generates a detailed MultiQC report that includes:

- Identified *Salmonella* serovars with several confidence metrics
- Sequence quality control assessments
- Sequence typing results
- Antimicrobial resistance (AMR) profiling
- Integration with NCBI SNP clustering analysis.

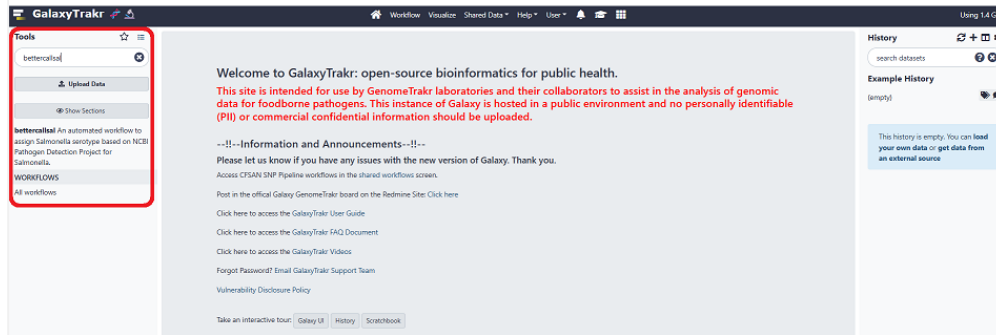
- 1 Go to and log into your GalaxyTrackr account at [Galaxy | GalaxyTrakr](#) .
- 2 On the right-hand side of the screen, click the plus next to the History tab to create a new history.



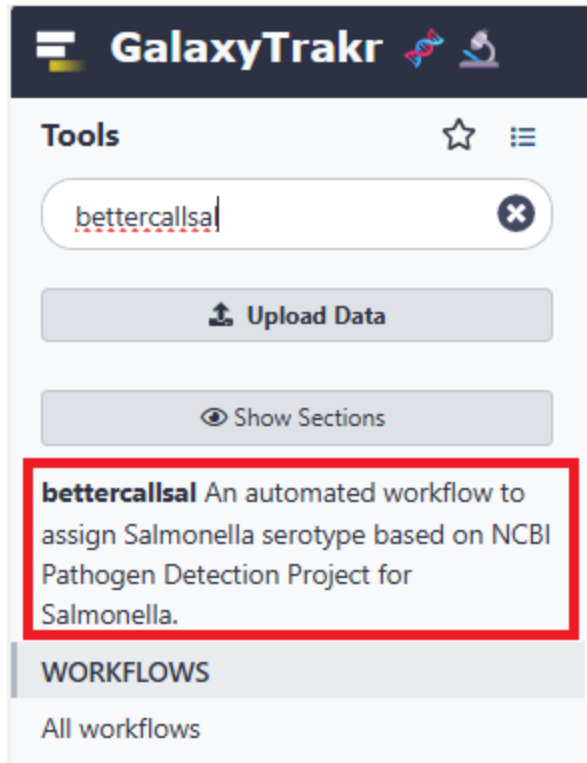
Click on **"Unnamed History"** and rename it according to your system.



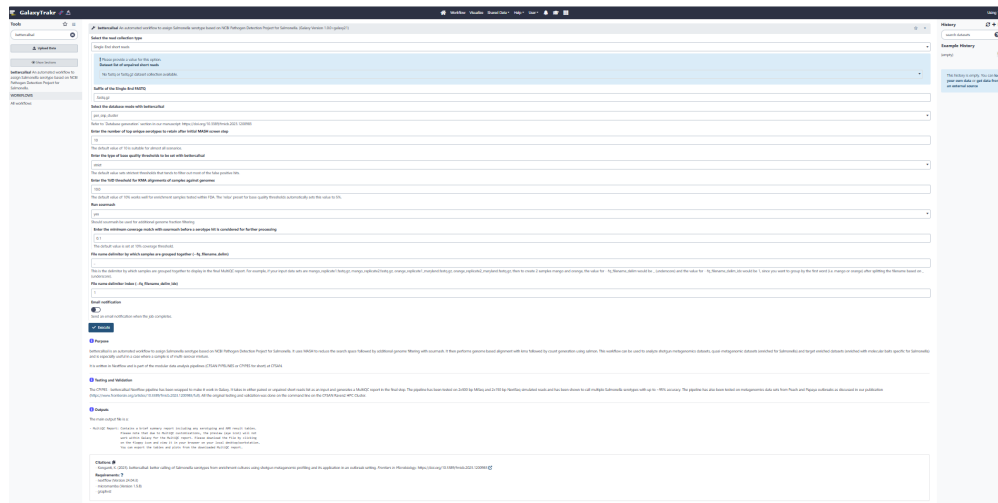
- 3 On the left-hand side of the screen, click the search bar in the "tools" section and search for **"bettercallsal"**.



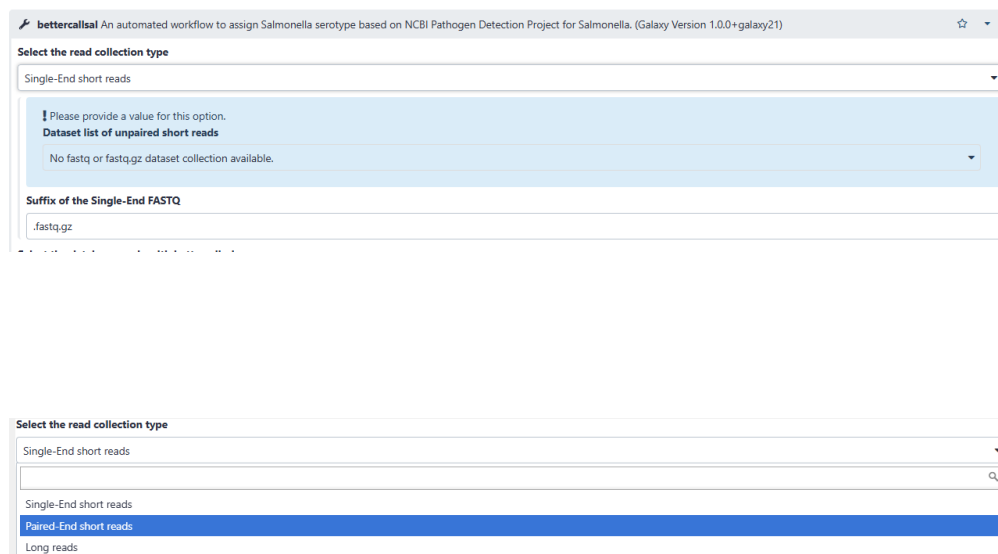
Click on the search result **"bettercallsal An automated workflow to....."**.



- 4 An interface will appear looking like this.

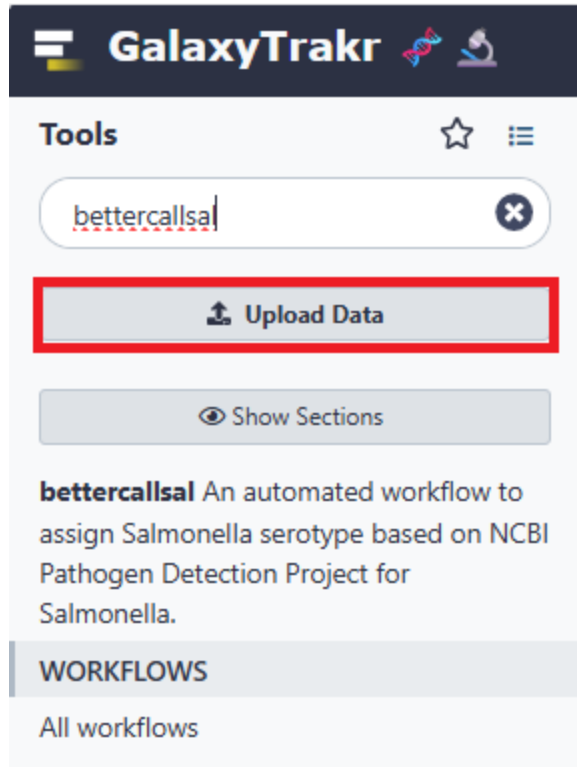


Change the "Select to read collection type" to "paired end short reads". It is the first option from the top.

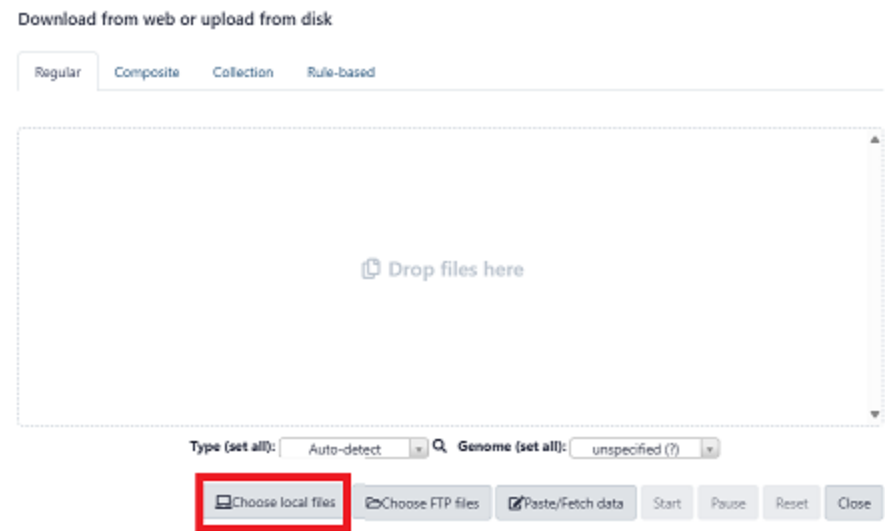


Once selected, the interface will change from "Single-End short pair reads" to the "Paired-End short reads. Everything else can be left alone.

- 5 On the left side of the screen, where we searched for the "bettercallsal" tool, click the "Upload data" button.



An interface where we can find and select files to upload will appear, for most users, the files will be on local files so choose that option unless otherwise noted.



Select all files to be processed, It should resemble this:
Click "Start" to begin uploading.

Download from web or upload from disk

Regular Composite Collection Rule-based

You added 8 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Type	Genome	Settings	Status
4RH_S57_R1_001.fast	186.5 MB	Auto-de...	unspecified (?)		0%
4RH_S57_R2_001.fast	211 MB	Auto-de...	unspecified (?)		0%
4RX_S56_R1_001.fast	186 MB	Auto-de...	unspecified (?)		0%
4RX_S56_R2_001.fast	210.4 MB	Auto-de...	unspecified (?)		0%
4TH_S59_R1_001.fast	148.6 MB	Auto-de...	unspecified (?)		0%
4TH_S59_R2_001.fast	169.8 MB	Auto-de...	unspecified (?)		0%

Type (set all): Auto-detect Genome (set all): unspecified (?)

Choose local files Choose FTP files Paste/Fetch data **Start** Pause Reset Close

Note* Depending on the number of files, it can take a while to upload.
This is what it looks like when uploading has started.

Download from web or upload from disk

Regular Composite Collection Rule-based

Please wait...8 out of 8 remaining.

Name	Size	Type	Genome	Settings	Status
4RH_S57_R1_001.fast	186.5 MB	Auto-de...	unspecified (?)		38%
4RH_S57_R2_001.fast	211 MB	Auto-de...	unspecified (?)		0%
4RX_S56_R1_001.fast	186 MB	Auto-de...	unspecified (?)		0%
4RX_S56_R2_001.fast	210.4 MB	Auto-de...	unspecified (?)		0%
4TH_S59_R1_001.fast	148.6 MB	Auto-de...	unspecified (?)		0%
4TH_S59_R2_001.fast	169.8 MB	Auto-de...	unspecified (?)		0%

Type (set all): Auto-detect Genome (set all): unspecified (?)

Choose local files Choose FTP files Paste/Fetch data Start Pause Reset Close

Once all files have been uploaded, click close.



Download from web or upload from disk

Regular Composite Collection Rule-based

Name	Size	Type	Genome	Settings	Status
 4RH_S57_R1_001.fast	186.5 MB	Auto-de... 	 unspecified (?) 		100% 
 4RH_S57_R2_001.fast	211 MB	Auto-de... 	 unspecified (?) 		100% 
 4RX_S56_R1_001.fast	186 MB	Auto-de... 	 unspecified (?) 		100% 
 4RX_S56_R2_001.fast	210.4 MB	Auto-de... 	 unspecified (?) 		100% 
 4TH_S59_R1_001.fast	148.6 MB	Auto-de... 	 unspecified (?) 		100% 
 4TH_S59_R2_001.fast	169.8 MB	Auto-de... 	 unspecified (?) 		100% 

Type (set all):  Genome (set all): 

 Choose local files  Choose FTP files  Paste/Fetch data  Start  Pause  Reset  Close

- 6 Before running "bettercallsal", the dataset needs to be paired. Click on the square icon, or the "Operations on multiple datasets" on the right side of the tab. select boxes will appear on the green file list. This will allow you to select all files in your dataset.

History

search datasets

?

×

Practice Example Data

8 shown, 10 deleted, 8 hidden

2.72 GB

☒
☐
☐
☐
☐
☐
☐
☐

All

None

For all selected... ▾

☐ 18: 4TX_S58_R2_001.fastq.gz

☐ 17: 4TX_S58_R1_001.fastq.gz

☐ 16: 4TH_S59_R2_001.fastq.gz

☐ 15: 4TH_S59_R1_001.fastq.gz

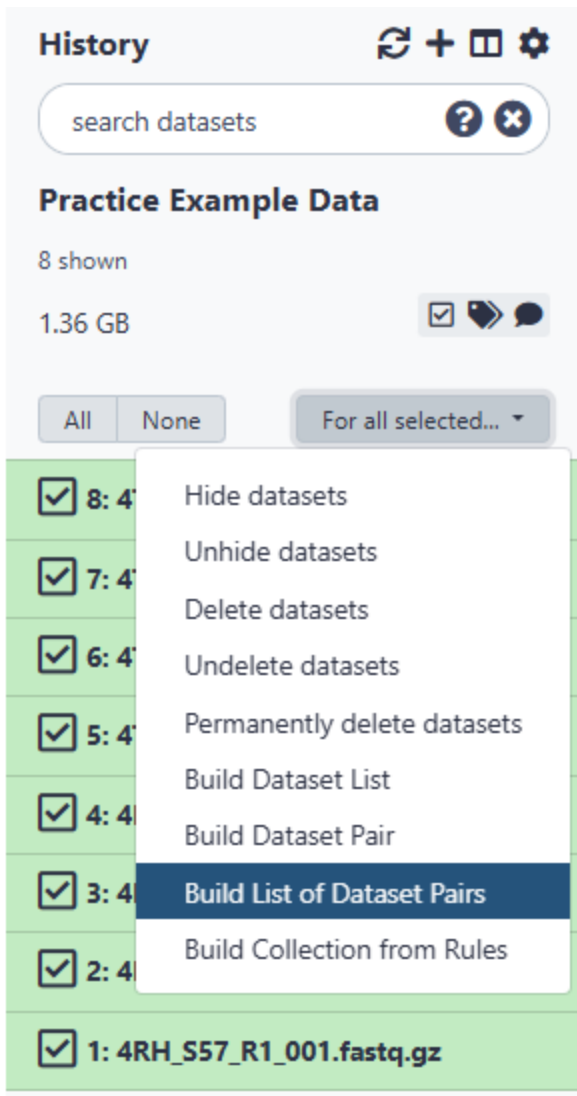
☐ 14: 4RX_S56_R2_001.fastq.gz

☐ 13: 4RX_S56_R1_001.fastq.gz

☐ 12: 4RH_S57_R2_001.fastq.gz

☐ 11: 4RH_S57_R1_001.fastq.gz

Select all files in the workspace, then click the dropdown that says for all selected. A menu will appear, select "Build List of Dataset Pairs".



7 A new interface will appear.

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. **cancel** and reselect new elements.

Collections of paired datasets are ordered lists of dataset pairs (often forward and reverse reads). These collections can be passed to tools and workflows in order to have analyses done on each member of the entire group. This interface allows you to crea...

_1 **0 unpaired forward** - 8 filtered out **Clear Filters** **Auto-pair** **_2** **0 unpaired reverse** - 8 filtered out

No datasets were found matching the current filters.

0 pairs **Unpair all**

Hide original elements? ☒ Remove file extensions? ☒

Name:

Cancel **Create collection**

In the dropdown on the left, select **_R1**. This will auto select **_R2** for the other dropdown.

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. **cancel** and reselect new elements.

Collections of paired datasets are ordered lists of dataset pairs (often forward and reverse reads). These collections can be passed to tools and workflows in order to have analyses done on each member of the entire group. This interface allows you to crea...

_1 **0 unpaired forward** - 8 filtered out **Clear Filters** **Auto-pair** **_2** **0 unpaired reverse** - 8 filtered out

No datasets were found matching the current filters.

0 pairs **Unpair all**

Hide original elements? ☒ Remove file extensions? ☒

Name:

Cancel **Create collection**

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. **cancel** and reselect new elements.

Collections of paired datasets are ordered lists of dataset pairs (often forward and reverse reads). These collections can be passed to tools and workflows in order to have analyses done on each member of the entire group. This interface allows you to crea...

_R1 **4 unpaired forward** - 4 filtered out **Clear Filters** **Auto-pair** **_R2** **4 unpaired reverse** - 4 filtered out

4RH_S57_R1_001.fastq.gz	Pair these datasets	4RH_S57_R2_001.fastq.gz
4RX_S56_R1_001.fastq.gz	Pair these datasets	4RX_S56_R2_001.fastq.gz
4TH_S59_R1_001.fastq.gz	Pair these datasets	4TH_S59_R2_001.fastq.gz
4TX_S58_R1_001.fastq.gz	Pair these datasets	4TX_S58_R2_001.fastq.gz

0 pairs **Unpair all**



You will see both columns populate with filenames. Double check that the files have the same name other than "R1" or "R2". Once you know the program has properly paired the files, Click the "Pair these datasets" for all entries. There two columns will be empty if you do it correctly and they appear in green below the black bar.

8

0 unpaired forward - 8 filtered out

0 unpaired reverse - 8 filtered out

No datasets were found matching the current filters.

4 unpaired forward - 4 filtered out

4 unpaired reverse - 4 filtered out

4RH_S57_R1_001.fastq.gz

4RX_S56_R1_001.fastq.gz

4TH_S59_R1_001.fastq.gz

4TX_S58_R1_001.fastq.gz

4RH_S57_R2_001.fastq.gz

4RX_S56_R2_001.fastq.gz

4TH_S59_R2_001.fastq.gz

4TX_S58_R2_001.fastq.gz

0 pairs Unpair all

9

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, **cancel** and reselect new elements.

0 unpaired forward - 0 filtered out

0 unpaired reverse - 0 filtered out

No datasets were found matching the current filters.

4 pairs Unpair all

4RH_S57_R1_001.fastq.gz → 4RH_S57_001.fastq ← 4RH_S57_R2_001.fastq.gz

4RX_S56_R1_001.fastq.gz → 4RX_S56_001.fastq ← 4RX_S56_R2_001.fastq.gz

4TH_S59_R1_001.fastq.gz → 4TH_S59_001.fastq ← 4TH_S59_R2_001.fastq.gz

4TX_S58_R1_001.fastq.gz → 4TX_S58_001.fastq ← 4TX_S58_R2_001.fastq.gz

Hide original elements? ☒ Remove file extensions? ☒

Name: Enter a name for your new collection

Cancel Create collection

Create a name for your collection and click the "Create collection" button.

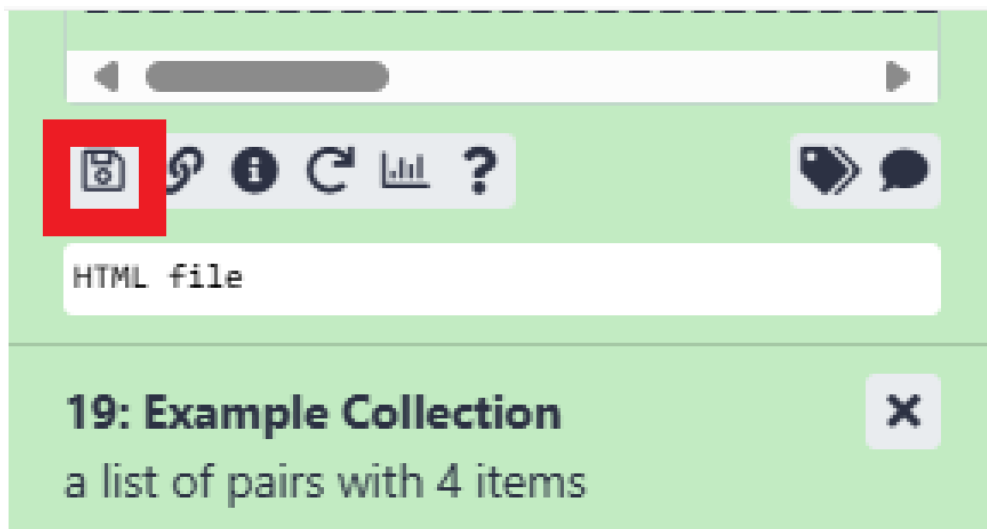
- 10 Double check that you have paired end short reads selected from step 4 and your paired collection is selected under "**List of Dataset pairs**" and match with the example below. If everything matches, click the "Execute" button.

- 11 The program will begin; you can tell it is started by an orange tab appearing above your green paired collection beginning with "bettercallsal: MultiQC Report.....".

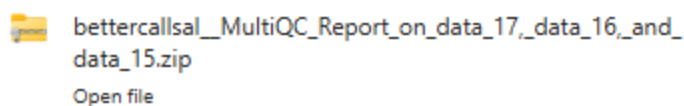
Wait until the job completes. You can tell when the processing is finished by the orange tab turning green. ****This process can take upwards of 45 minutes to an hour**, depending on queue times and number of files uploaded.**

- 12 Once the dataset is done and turns green, click on it. A dropdown will appear.

The screenshot shows the 'History' tab in the protocols.io interface. At the top, there's a search bar labeled 'search datasets' with a question mark and a close button. Below this, the section 'Practice Example Data' is visible, indicating '2 shown, 10 deleted, 16 hidden' and a size of '2.72 GB'. The main content area shows a dataset entry '20: bettercallsal: MultiQC Report on data 14, data 1 3, and others' with a size of '1.3 MB' and format 'html'. The entry is highlighted in green, indicating it is complete. Below the entry, there is a preview of the report content, including the text 'NEXTFLOW ~ version 24.04.3' and 'Launching' followed by a command path. At the bottom, there's a dropdown menu showing 'HTML file' and another entry '19: Example Collection'.



In the bottom left, there will be a button to download the file. This is a zipped HTML file that needs to be downloaded and extracted before it can be opened. It will open in google.



The report will look like this. If you see this, then the program was run successfully!

