

# DATA FORMATS IN NGS INTRODUCTION TO GALAXY

Bioinformàtica per a la Recerca Biomèdica

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**1. Data formats used in NGS**

**2. Introduction to Galaxy**

## 2. Introduction to Galaxy

- An open, web-based platform integrating many popular tools and resources for intensive biomedical research.
- **What can be done?**
  - Obtain data from many data sources like UCSC Table Browser, Biomart, WormBase, or your own data
  - Prepare data for further analysis by rearranging or cutting data columns, filtering data and many other options
  - Analyze data by finding overlapping regions, determining statistics, preprocessing NGS data and much more
  - Share data and workflows

## 2. Introduction to Galaxy

The Galaxy page is divided into three panels:

**Tools** for uploading, processing and analysis

**Viewing panel**  
(menus, data, results)

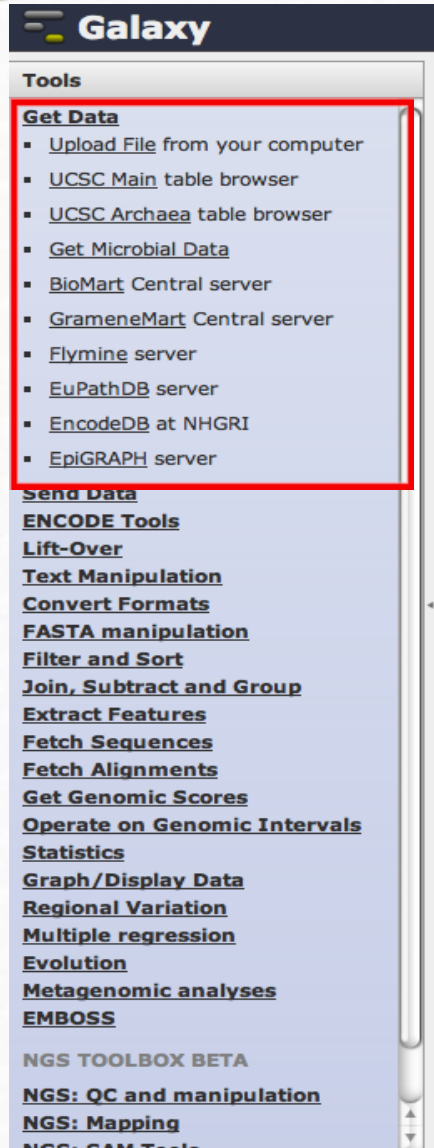
**History** of analysis steps and datasets

The screenshot displays the Galaxy web interface. The top navigation bar includes links for Analyze Data, Workflow, Visualize, Shared Data, Help, User, and a Galaxy logo. The interface is divided into three main panels:

- Tools Panel (Left):** A sidebar with a search bar and a list of tool categories. The categories include: Get Data, Collection Operations, GENERAL TEXT TOOLS, Text Manipulation, Filter and Sort, Join, Subtract and Group, Datamash, GENOMIC FILE MANIPULATION, FASTA/FASTQ, FASTQ Quality Control, SAM/BAM, BED, VCF/BCF, Nanopore, Convert Formats, Lift-Over, COMMON GENOMICS TOOLS, Operate on Genomic Intervals, Fetch Sequences/Alignments, GENOMICS ANALYSIS, Assembly, Annotation, Mapping, and Variant Calling.
- Viewing Panel (Center):** The main content area, outlined in red. It features a header for the JXTX (James P. Taylor Foundation for Open Science) and a large graphic of a sneaker. The text describes the foundation's mission to assist graduate students in computational biology and data science. A "Donate Now" button is present. A blue banner at the bottom of the panel provides information about the Galaxy SARS-CoV-2 portal at [covid19.galaxyproject.org](https://covid19.galaxyproject.org). Logos for PennState, Johns Hopkins University, Oregon Health & Science University, TACC, and CyVerse are displayed at the bottom.
- History Panel (Right):** A sidebar outlined in green, titled "History". It contains a search bar and a message stating "Unnamed history (empty)". A blue banner at the bottom of the panel indicates that the history is empty and provides instructions on how to load data from an external source.



## 2. Introduction to Galaxy



### Tools for data analysis

#### Get Data

- From databases (UCSC Table Browser, ...)
- From uploaded files
- From urls

#### Text manipulation

#### Filter and Sort

#### Operate on Genomic Intervals

#### FASTA manipulation

#### NGS analysis

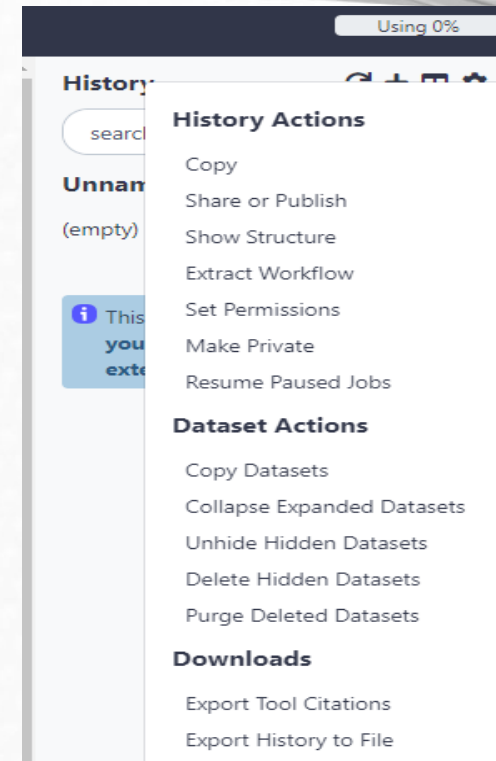
- QC
- Fastq file pre-processing
- Read Alignment / Mapping
- SAM tools

## 2. Introduction to Galaxy

### Histories

List saved histories and shared histories.

Work on Current History, create new, clone, share, create workflow, set permissions, show deleted datasets or delete history.



## 2. Introduction to Galaxy

### Workflows

**Galaxy**

Analyze DataWorkflowVisualizeShared DataHelpUserUsing 2%

Galaxy will be down for six hours beginning at 2:30 PM UTC, Tuesday, November 20 for filesystem maintenance.

Tools

search tools

Inputs

Get Data

Send Data

Lift-Over

Collection Operations

Text Manipulation

Datamash

Convert Formats

Filter and Sort

Join, Subtract and Group

Fetch Alignments/Sequences

NGS: QC and manipulation

NGS: DeepTools

NGS: Mapping

NGS: RNA Analysis

NGS: SAMtools

NGS: BamTools

NGS: Picard

NGS: VCF Manipulation

Workflow Canvas | Coding Exon SNPs

Exons

output

SNPs

output

Join

Join

With

output

Group

Select data

out\_file1 (tabular)

Sort

Sort Dataset

out\_file1

Details

Edit Workflow Attributes

Name:

Coding Exon SNPs

Version:

Version 1, 5 steps (active)

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

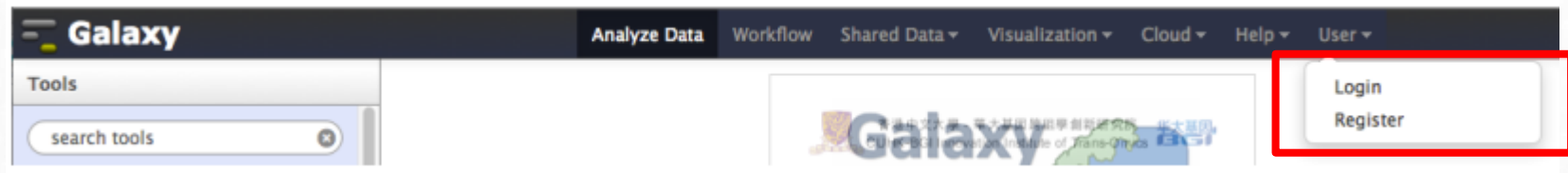
Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

Workflows with all the analysis steps, allows user to repeat analysis using different datasets

## 2. Introduction to Galaxy

### Register for a Galaxy account

This will only take a moment, and will allow all the work that you do to persist between sessions and allow you to name, save, share, and publish Galaxy histories, workflows, datasets and pages. It allows you to store up to 250GB of data on this public server.



<https://usegalaxy.eu/>



## 2. Introduction to Galaxy

### Training Infrastructure as a Service

We want to help you conduct your training seminars. You provide the training, we provide you training infrastructure *at no cost*.

Why use UseGalaxy.eu training infrastructure?

- Free
- Private queue, no wait times
- No Galaxy Maintenance
- No Galaxy Administration
- Official Galaxy Training Materials guaranteed to work



Simply fill out the infrastructure request form and we'll get back to you shortly.

[Find out more](#)

After registration in [European Galaxy server](#)



[https://usegalaxy.eu/join-training/ueb\\_bi2021](https://usegalaxy.eu/join-training/ueb_bi2021)

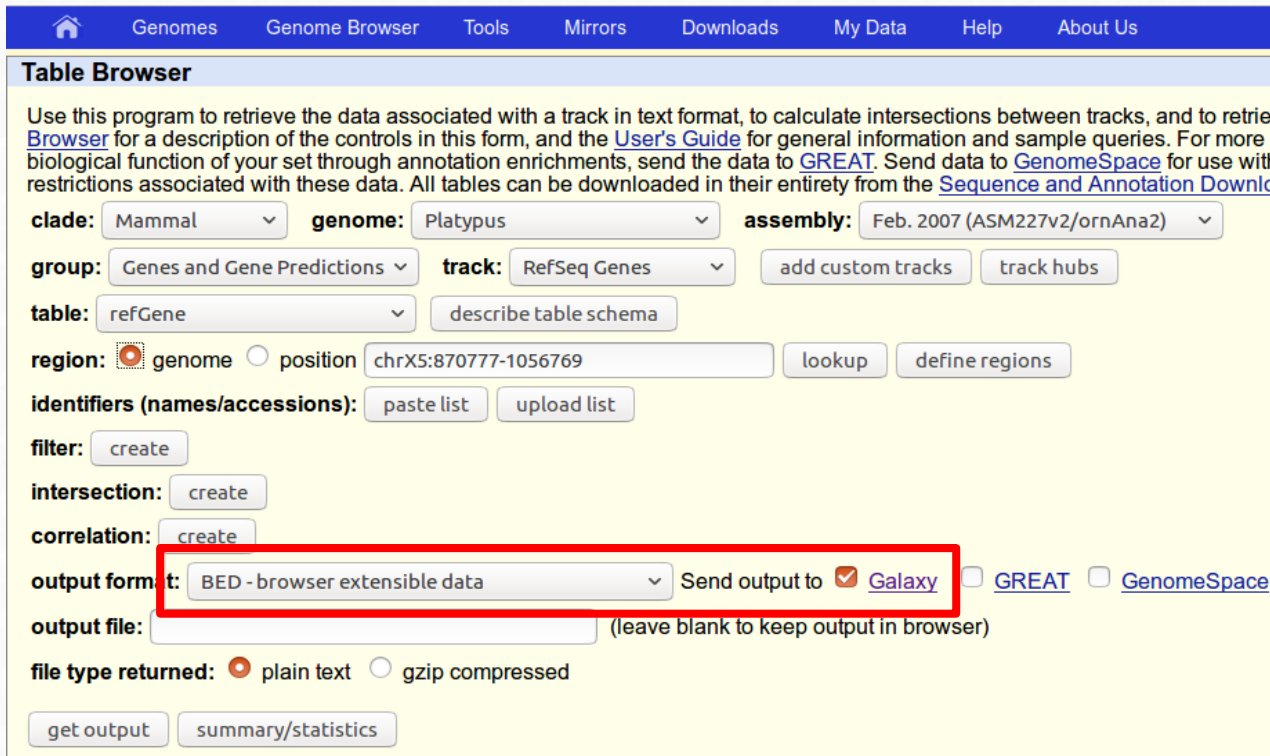
## 2. Introduction to Galaxy

### Importing data into Galaxy

1. From database queries (eg. UCSC): obtain a BED-formatted dataset of all RefSeq genes from platypus.

Get Data > UCSC Main – Table Browser tool

Set genome, RefSeq Genes, and BED output format (send to Galaxy)



**Table Browser**

Use this program to retrieve the data associated with a track in text format, to calculate intersections between tracks, and to retrieve [Browser](#) for a description of the controls in this form, and the [User's Guide](#) for general information and sample queries. For more information on the biological function of your set through annotation enrichments, send the data to [GREAT](#). Send data to [GenomeSpace](#) for use with restrictions associated with these data. All tables can be downloaded in their entirety from the [Sequence and Annotation Download](#)

**clade:** Mammal **genome:** Platypus **assembly:** Feb. 2007 (ASM227v2/ornAna2)

**group:** Genes and Gene Predictions **track:** RefSeq Genes [add custom tracks](#) [track hubs](#)

**table:** refGene [describe table schema](#)

**region:** ☒ genome ☐ position chrX5:870777-1056769 [lookup](#) [define regions](#)

**identifiers (names/accessions):** [paste list](#) [upload list](#)

**filter:** [create](#)

**intersection:** [create](#)

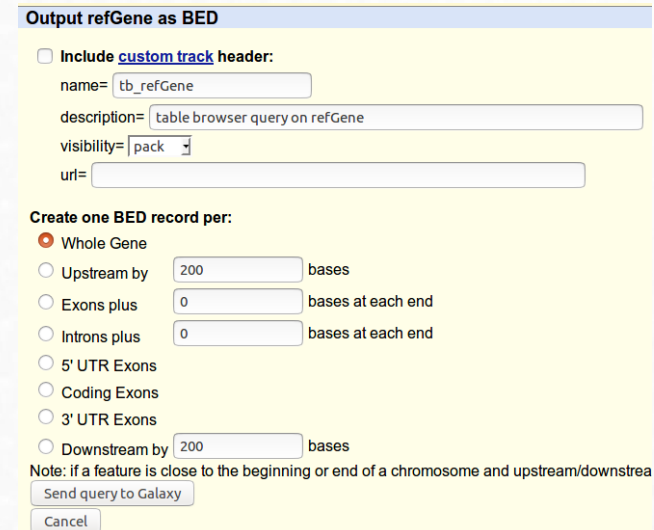
**correlation:** [create](#)

**output format:** BED - browser extensible data **Send output to:** ☒ Galaxy ☐ GREAT ☐ GenomeSpace

**output file:** (leave blank to keep output in browser)

**file type returned:** ☒ plain text ☐ gzip compressed

[get output](#) [summary/statistics](#)



**Output refGene as BED**

☐ Include [custom track](#) header:

name= tb\_refGene

description= table browser query on refGene

visibility= pack

url=

**Create one BED record per:**

☒ Whole Gene

☐ Upstream by 200 bases

☐ Exons plus 0 bases at each end

☐ Introns plus 0 bases at each end

☐ 5' UTR Exons

☐ Coding Exons

☐ 3' UTR Exons

☐ Downstream by 200 bases

Note: If a feature is close to the beginning or end of a chromosome and upstream/downstream

[Send query to Galaxy](#)

[Cancel](#)

## 2. Introduction to Galaxy

### Importing data into Galaxy







2. From a File on your computer / FTP file:

Get Data > Upload File

**Download from web or upload from disk**



Regular Composite Collection Rule-based








You added 1 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Type	Genome	Settings	Status
 New File	72 b	fastqsang... 	 ----- Additional Sp... 		0% 

You can tell Galaxy to download data from web by entering URL in this box (one per line). You can also directly paste the contents of a file.

[http://chagall.med.cornell.edu/galaxy/rnaseq/GM12878\\_rnaseq1.fastqsanger](http://chagall.med.cornell.edu/galaxy/rnaseq/GM12878_rnaseq1.fastqsanger)

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

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

## 2. Introduction to Galaxy

### Importing data into Galaxy

#### 3. From a website:

Get Data > Upload File

Copy this URL into the text-entry box:

url: [https://zenodo.org/record/582600/files/mutant\\_R1.fastq](https://zenodo.org/record/582600/files/mutant_R1.fastq)

Regular Composite Collection

You added 1 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Type	Genome	Settings	Status
New File	-	Auto-det...	unspecified (?)		

You can tell Galaxy to download data from web by entering URL in this box (one per line). You can also directly paste the contents of a file.

← 2. Paste file address in this box

1. click Paste/Fetch data

3. Start 4. Close

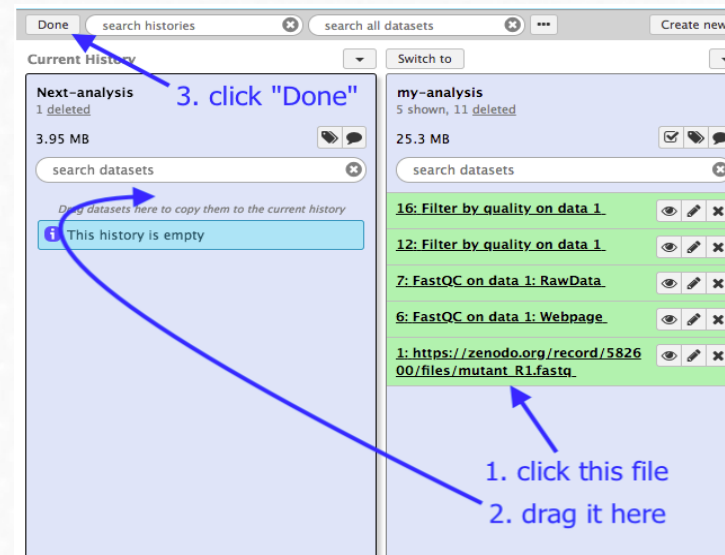
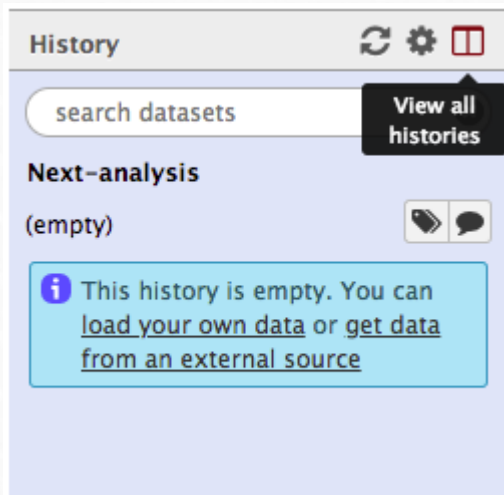
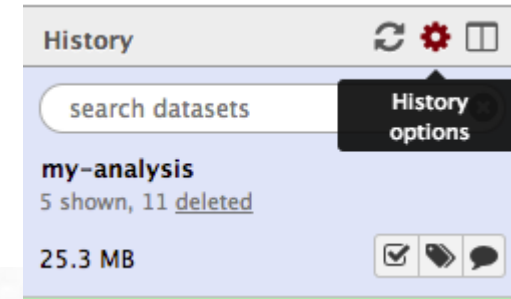
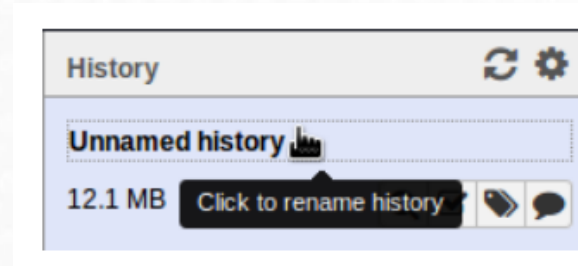
Type (set all): Auto-detect Q Genome (set all): unspecified (?)

Choose local file Paste/Fetch data Pause Reset Start Close

## 2. Introduction to Galaxy

### Managing histories

- Name your current history
- Create new history and rename it
- Manage datasets and histories:
- View all histories
- Drag files between histories (**new history must be set to current**)





## 2. Introduction to Galaxy

### Visualizing

- You can view content by clicking the eye icon on any step in your history.

The mutant\_R1.fastq file contains DNA sequencing reads from a bacteria, in FASTQ format:

```
@mutant-no_snps.gff-24960/1          read 1 sequence
AATGTTGTCACTTGGATTCAAATGACATTTTAAATCTAATTATTCATGAATCGAACTAGTACGAAATGCAATGAG
+
5??A9?BBBDDDBEDDBFF+FGHHIIHHHEIHHIIHIIAHDHIIHIG#IIHIFHHHFGIII*IHHHIIHFIIHGICI
@mutant-no_snps.gff-24958/1
CAAAGTCGTTGGTCATATAAAAAACCGCGTACAGTCAACTATAGATACAATCAAGATAAACTCATGCACAGATTG
+
?A????@?DDDABDE9FGGGFGICFHIIIBGHIIIGICHHIFH=IHAFIHHHHHIFCIIIEIHAIFGIHIDDIHE
@mutant-no_snps.gff-24956/1
TATAAATTCAACTTTGCAACAGAACCATCTAATCTTCAACAACTGGCCCGTTTGTGAACTACTCTTTAATAAA
+
?????BBADD5DDDDGFGCFEECFBBICIII,IIHIICHIIHIFHHHHHIIHIIIIIIAHHHIHHH5FHDHHHH
```

The screenshot shows the Galaxy web interface. At the top, there's a 'History' panel with a search bar labeled 'search datasets'. Below the search bar, a dataset named 'my-analysis' is listed, showing '1 shown' and a size of '3.95 MB'. To the right of the size are icons for a checkmark, a tag, and a speech bubble. Below this, a green bar highlights a specific dataset entry: '1: https://zenodo.org/record/582600/files/mutant\_R1.fastq\_'. To the right of this entry are icons for an eye (selected), a pencil, and a close button. A black tooltip with the text 'View data' is positioned over the eye icon.

## 2. Introduction to Galaxy

### Editing basic attributes

- You can edit several basic attributes by clicking the pencil icon on any step in your history

#### Edit Dataset Attributes

[Attributes](#) [Convert](#) [Datatypes](#) [Permissions](#)

**Name**

**Info**

**Annotation**

Add an annotation or notes to a dataset; annotations are available when a history is viewed.

**Database/Build**

[Save](#) [Auto-detect](#)

#### History

[search datasets](#) [?](#) [x](#)

**my-analysis**  
1 shown  
3.95 MB [✓](#) [▶](#) [💬](#)

**1: mutant\_R1.fastq** [👁](#) [✎](#) [✕](#)  
[Edit attributes](#)

## 2. Introduction to Galaxy

### Galaxy Workflows

- In Galaxy, a Workflow is a defined set of 'tasks' that can be stored and executed on demand in an automated fashion.
- A workflow is composed of :
  - any number of tools and dataset operations available on the 'Tools' panel (*what to do and with what data*).
  - the relationships among them and their specific run parameters (*how to do it*).
- Very useful:
  - Time saving
  - Less error-prone (no need to set any step and parameter again and again manually)
  - Increased repeatability
  - Increased reproducibility

Access your stored workflows:

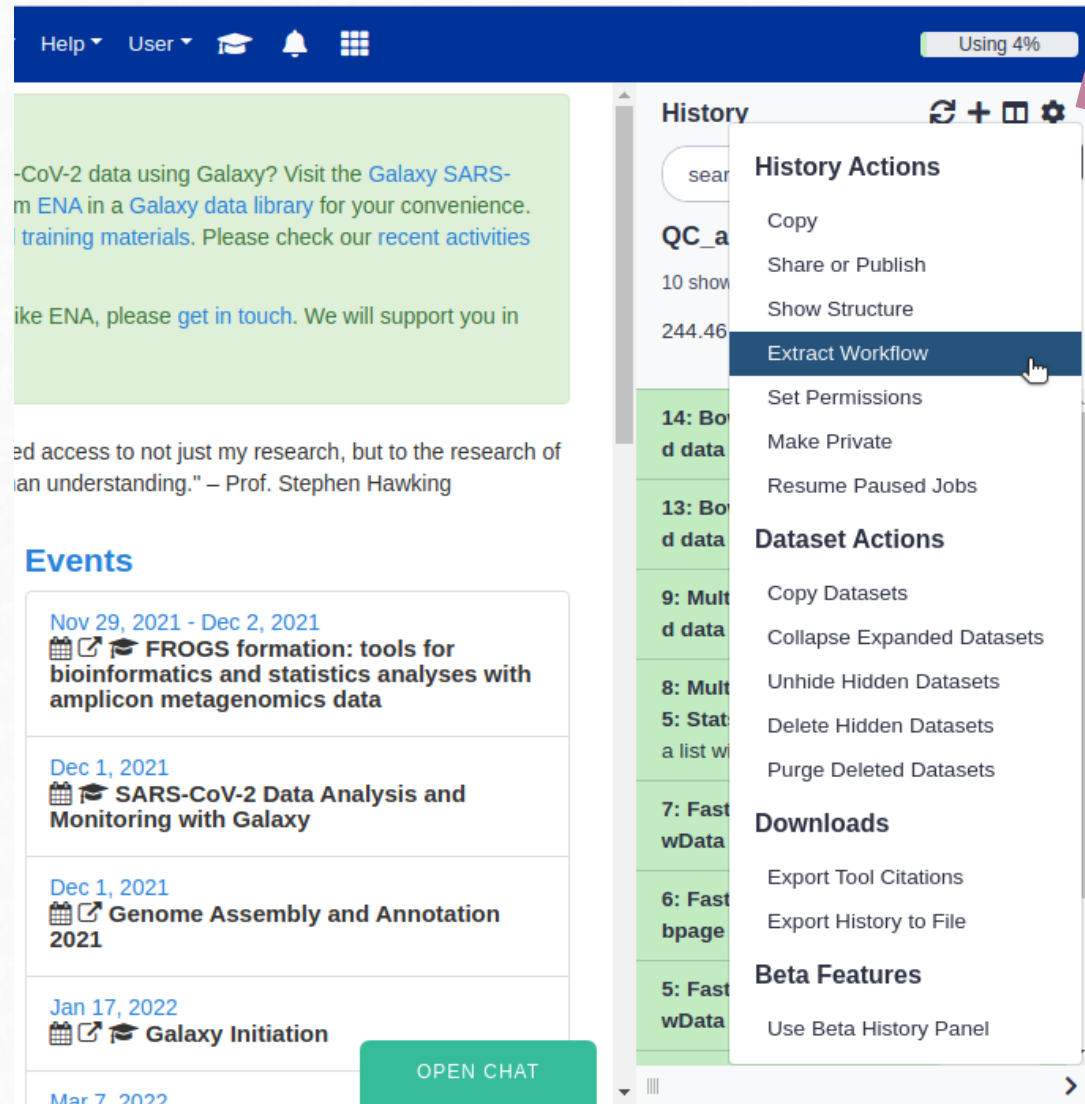


## 2. Introduction to Galaxy

### Galaxy Workflows

Easy to create:

- **From an existing history**
- Using the integrated visual editor



The screenshot displays the Galaxy web interface. At the top, a dark blue header contains navigation links: 'Help', 'User', and icons for a graduation cap, a bell, and a grid. A status bar on the right indicates 'Using 4%'. The main content area is divided into two columns. The left column features a green box with text about SARS-CoV-2 data and a section titled 'Events' listing recent activities with dates and icons. The right column shows a 'History' panel with a search bar and a list of workflow entries. A red arrow points to the 'History Actions' menu, which is open, showing options like 'Copy', 'Share or Publish', 'Show Structure', 'Extract Workflow' (highlighted), 'Set Permissions', 'Make Private', 'Resume Paused Jobs', 'Dataset Actions', 'Downloads', and 'Beta Features'. A green 'OPEN CHAT' button is located at the bottom right.

Help User Using 4%

-CoV-2 data using Galaxy? Visit the [Galaxy SARS-CoV-2 data library](#) for your convenience. Please check our [recent activities](#).

like ENA, please [get in touch](#). We will support you in

ed access to not just my research, but to the research of an understanding." – Prof. Stephen Hawking

### Events

Nov 29, 2021 - Dec 2, 2021  
📅🔗🎓 **FROGS formation: tools for bioinformatics and statistics analyses with amplicon metagenomics data**

Dec 1, 2021  
📅🎓 **SARS-CoV-2 Data Analysis and Monitoring with Galaxy**

Dec 1, 2021  
📅🔗 **Genome Assembly and Annotation 2021**

Jan 17, 2022  
📅🔗🎓 **Galaxy Initiation**

Mar 7, 2022

History

search

QC\_a

10 show

244.46

14: Bo

d data

13: Bo

d data

9: Mult

d data

8: Mult

5: Stat

a list w

7: Fast

wData

6: Fast

bpage

5: Fast

wData

### History Actions

- Copy
- Share or Publish
- Show Structure
- Extract Workflow**
- Set Permissions
- Make Private
- Resume Paused Jobs

### Dataset Actions

- Copy Datasets
- Collapse Expanded Datasets
- Unhide Hidden Datasets
- Delete Hidden Datasets
- Purge Deleted Datasets

### Downloads

- Export Tool Citations
- Export History to File

### Beta Features

- Use Beta History Panel

OPEN CHAT

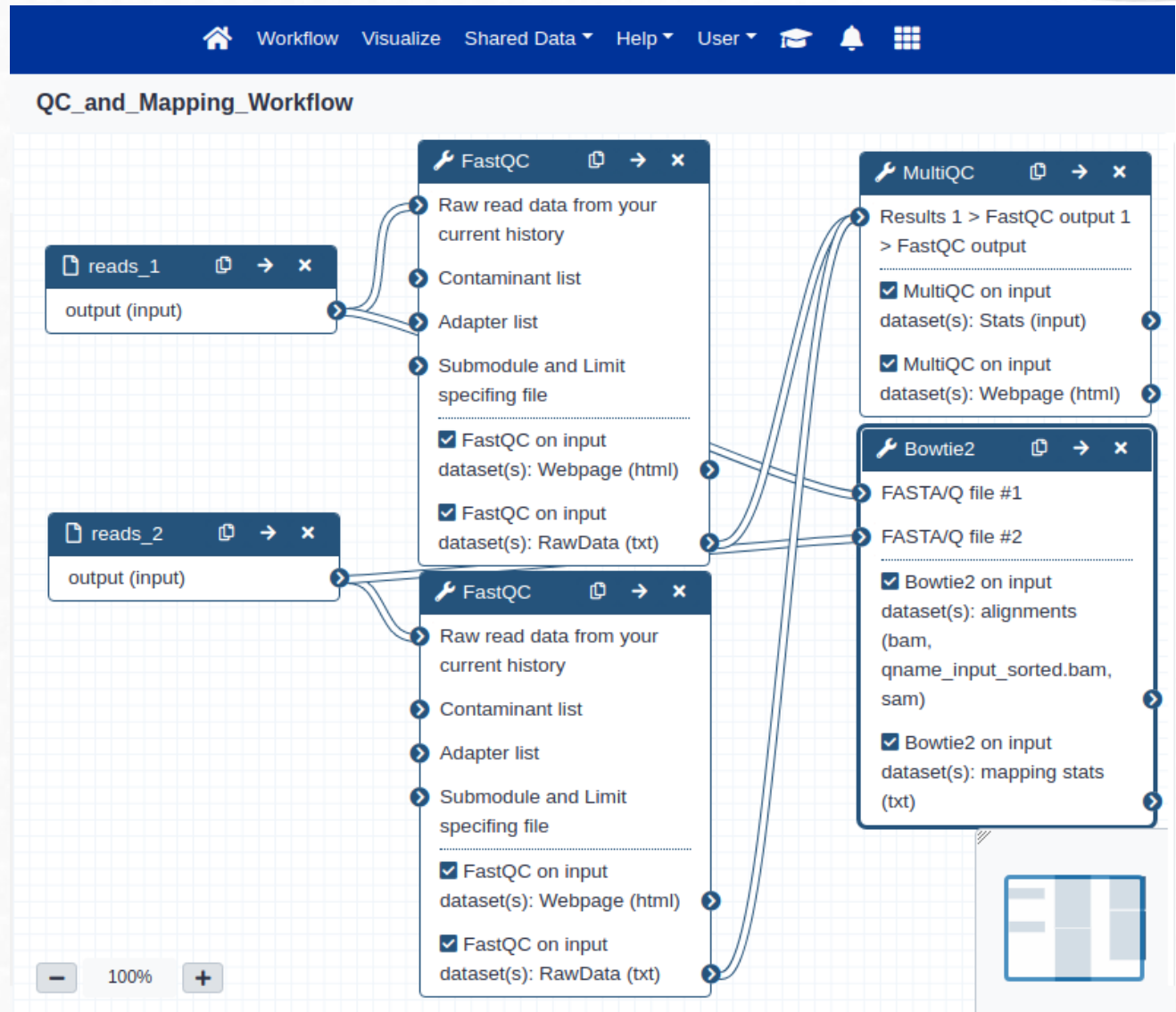


## 2. Introduction to Galaxy

### Galaxy Workflows

Easy to create:

- From an existing history
- **Using the integrated visual editor**





## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads

1. Create a new history and name it 'Paired-End Mapping'
2. Import the following files containing paired-end reads:
  - [https://zenodo.org/record/1324070/files/wt\\_H3K4me3\\_read1.fastq.gz](https://zenodo.org/record/1324070/files/wt_H3K4me3_read1.fastq.gz)
  - [https://zenodo.org/record/1324070/files/wt\\_H3K4me3\\_read2.fastq.gz](https://zenodo.org/record/1324070/files/wt_H3K4me3_read2.fastq.gz)
3. Change their names to 'reads\_1' and 'reads\_2' respectively
4. On the Tools panel, find a tool named '**Bowtie2**' and click on it. This tool will map our reads to a reference genome.
5. Set the following parameters for Bowtie2 on the central panel:
  - *"Is this single or paired library"*: **Paired-end**
  - *"FASTA/Q file #1"*: **reads\_1**
  - *"FASTA/Q file #2"*: **reads\_2**
  - *"Do you want to set paired-end options?"*: **No**
  - *"Will you select a reference genome from your history or use a built-in index?"*: **Use a built-in genome index**
  - *"Select reference genome"*: **Mouse (Mus musculus): mm10**
  - *"Select analysis mode"*: **Default setting only**
  - *"Save the bowtie2 mapping statistics to the history"*: **Yes**
6. Click 'Execute'

## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads

After the mapping process is finished, you should have a history like this:

The screenshot shows the Galaxy History panel. At the top, there's a 'History' header with icons for refresh, add, view, and settings. Below it is a search bar labeled 'search datasets'. The main section is titled 'Paired-End Mapping' and shows '4 shown' and '11.4 MB'. There are icons for checkmark, shield, and chat. Below this, a list of datasets is shown in green boxes:

- 4: Bowtie2 on data 2 and data 1: mapping stats (with view, edit, and delete icons)
- 3: Bowtie2 on data 2 and data 1: alignments (with view, edit, and delete icons)
- 2: reads\_2 (with view, edit, and delete icons)
- 1: reads\_1 (with view, edit, and delete icons)



Now we 'extract' a Workflow from this history:

This screenshot shows the same Galaxy History panel as the previous one, but with a context menu open over the '4: Bowtie2 on data 2 and data 1: mapping stats' dataset. The menu is titled 'History Actions' and includes the following options:

- Copy
- Share or Publish
- Show Structure
- Extract Workflow** (highlighted with a hand icon)
- Set Permissions
- Make Private
- Resume Paused Jobs

Below these are 'Dataset Actions' which include:

- Copy Datasets
- Collapse Expanded Datasets
- Unhide Hidden Datasets
- Delete Hidden Datasets

## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads

Change the name to 'Paired-End Mapping Workflow' and click 'Create Workflow':

Workflow name

Paired-End Mapping Workflow

Create Workflow

Check all

Uncheck all

Tool

Data Fetch

*This tool cannot be used in workflows*

Bowtie2

☒ Include "Bowtie2" in workflow

History items created

1 reads\_1

☒ Treat as input dataset

reads\_1

2 reads\_2

☒ Treat as input dataset

reads\_2

3 Bowtie2 on data 2 and data 1: alignments

4 Bowtie2 on data 2 and data 1: mapping stats

## 2. Introduction to Galaxy

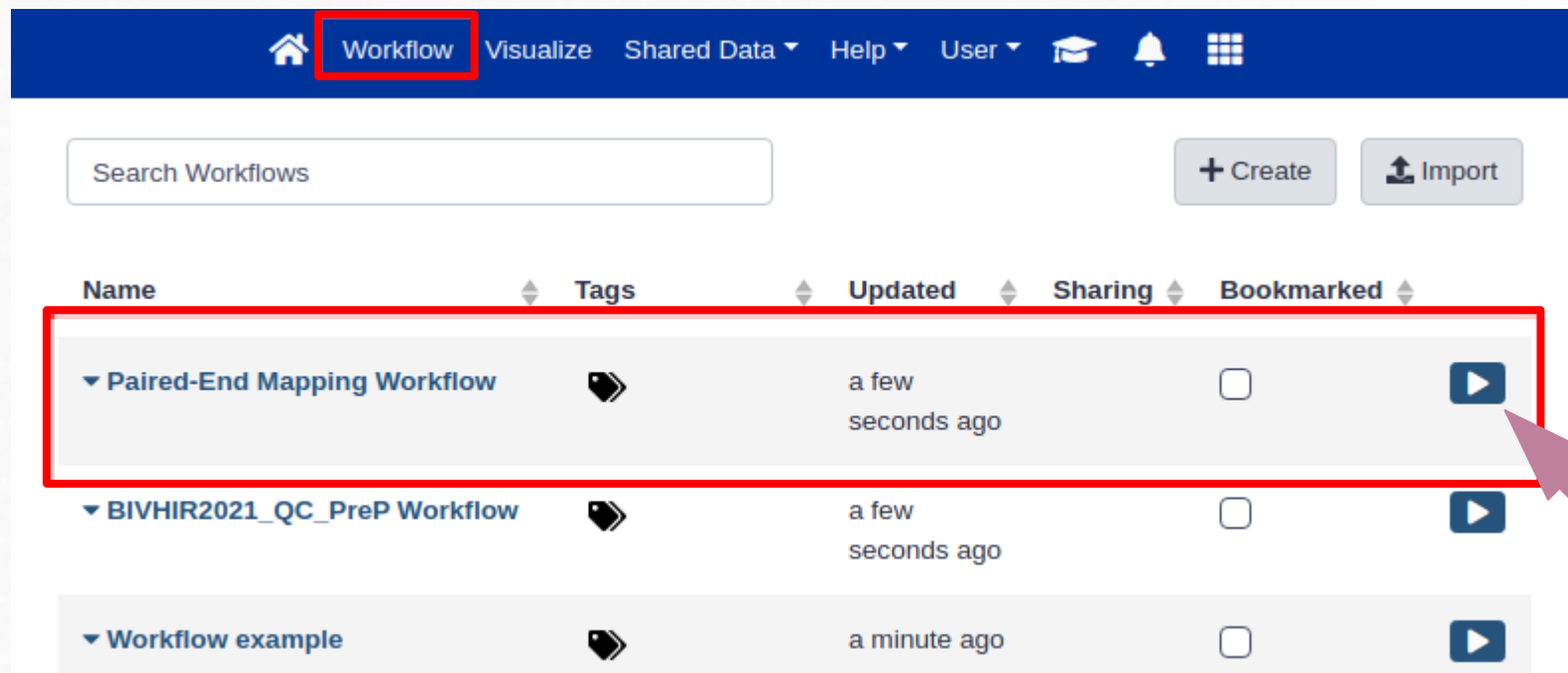
### Micro Hands On: Create a Workflow for mapping paired end reads

Now we are going to run this newly created workflow using a diferent set of paired-end reads:

1. Create a new history and name it with a distinctive name
2. Import the following files containing paired-end reads:
  - [https://zenodo.org/record/3243160/files/father\\_R1.fq.gz](https://zenodo.org/record/3243160/files/father_R1.fq.gz)
  - [https://zenodo.org/record/3243160/files/father\\_R2.fq.gz](https://zenodo.org/record/3243160/files/father_R2.fq.gz)
3. Rename them to 'father\_R1.fq.gz' and 'father\_R2.fq.gz' respectively (if they are not automatically named like that)
4. Go to the 'Workflow' section on the top main menu. You should see your newly created Workflow listed.
5. Click on the arrow icon to run the workflow.







## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads



The screenshot shows the Galaxy web interface. The top navigation bar is dark blue with icons for Home, Workflow (highlighted with a red box), Visualize, Shared Data, Help, User, and a grid icon. Below the navigation bar is a search bar labeled "Search Workflows" and two buttons: "+ Create" and "Import".

Below the search bar is a table of workflows. The table has columns: Name, Tags, Updated, Sharing, and Bookmarked. The first row, "Paired-End Mapping Workflow", is highlighted with a red box. A red arrow points to the play button icon in the first row.

Name	Tags	Updated	Sharing	Bookmarked
▼ Paired-End Mapping Workflow		a few seconds ago	<input type="checkbox"/>	
▼ BIVHIR2021_QC_PreP Workflow		a few seconds ago	<input type="checkbox"/>	
▼ Workflow example		a minute ago	<input type="checkbox"/>	



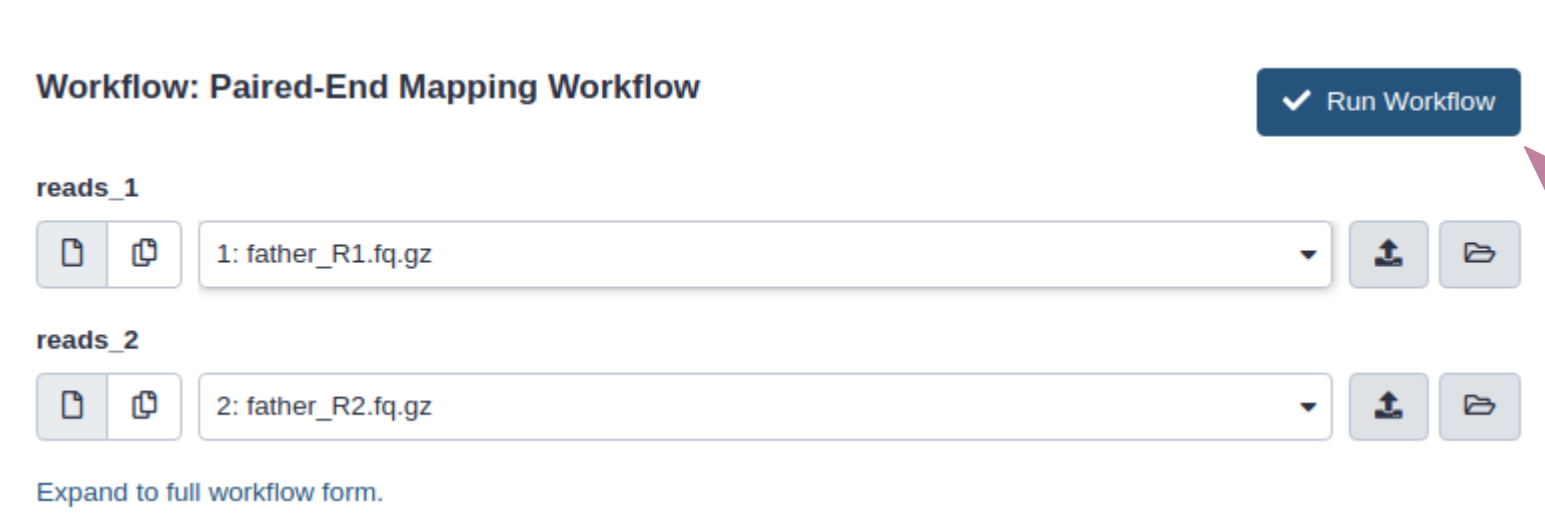
## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads

1. Set the inputs for running your workflow to the new reads:

- “*reads\_1*”: **father\_R1.fq.gz**
- “*reads\_2*”: **father\_R2.fq.gz**

2. Click ‘Run Workflow’



The screenshot shows the Galaxy workflow interface for a workflow titled "Workflow: Paired-End Mapping Workflow". In the top right corner, there is a blue button labeled "✓ Run Workflow". Below the title, there are two input sections. The first section, labeled "reads\_1", contains a dropdown menu with the text "1: father\_R1.fq.gz" and two icons (a document and a folder) to its left. The second section, labeled "reads\_2", contains a dropdown menu with the text "2: father\_R2.fq.gz" and two icons (a document and a folder) to its left. A large purple arrow points from the right side of the image towards the "Run Workflow" button. At the bottom left of the interface, there is a link that says "Expand to full workflow form."

## 2. Introduction to Galaxy

### Micro Hands On: Create a Workflow for mapping paired end reads

Your workflow is running!

The screenshot displays the Galaxy web interface. At the top, a dark blue navigation bar contains icons for home, workflow, visualize, shared data, help, user, and a grid of tools. A status indicator on the right shows 'Using 4%'. Below the navigation bar, a green notification box with a checkmark icon states: 'Successfully invoked workflow **Paired-End Mapping 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.'

Below the notification, a section titled 'Invocation 1...' shows a progress bar. The top bar is green and indicates '3 of 3 steps successfully scheduled.' The bottom bar is orange and indicates '0 of 1 jobs complete....' Below this, there are expandable sections for 'Inputs' and 'Steps'.

At the bottom left, a grey box titled 'We need your support ...' contains a citation: 'If Galaxy helped with the analysis of your data, please do not forget to cite: Afgan E et al. 2016 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44: W2-W10.'

On the right side, the 'History' pane is visible. It has a search bar labeled 'search datasets' and a 'Mapping 'father' reads (from Workflow)' section. Below this, it shows '4 shown' and '285.08 MB'. A list of jobs is displayed, each with a status icon, a description, and action buttons (eye, pencil, and X). The jobs are:

- 4: Bowtie2 on data 2 and data 1: mapping stats (orange bar)
- 3: Bowtie2 on data 2 and data 1: alignments (orange bar)
- 2: father\_R2.fq.gz (green bar)
- 1: father\_R1.fq.gz (green bar)

# 2. Introduction to Galaxy

- <https://galaxyproject.org/learn/>

## Learn Galaxy

There are many approaches to learning how to use Galaxy. The most popular is probably to just dive in and use it. Galaxy is simple enough to use that you can do many analyses just by exploring the interface. However, you may miss much of the power this way.

Have you created or know of a resource that is useful for teaching with Galaxy? Then please share it! This will help others and also help get the word out about your resource. Use [this Google form](#) to describe your resource. **Also:** consider joining Galaxy Training Network and contributing your tutorial as described [here](#)!

## Tutorials by Galaxy Training Network

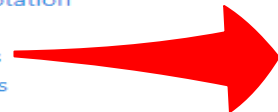
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