Genome Assembly - Galaxy

Clyde Dapat

WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL

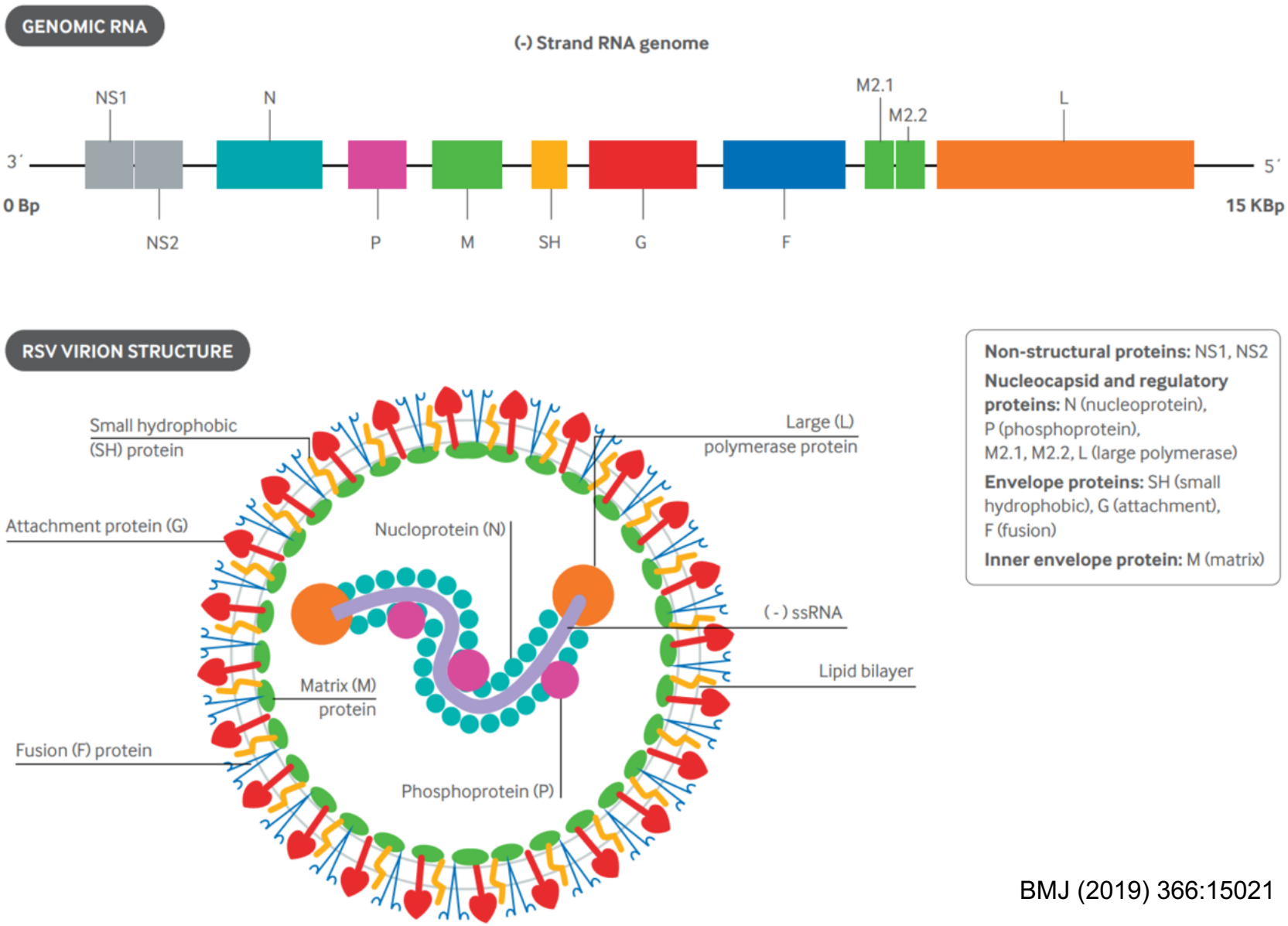
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Objective

- To assemble the genome of RSV using Galaxy

Reads (fastq)

Consensus (fasta)



BMJ (2019) 366:15021

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Workflow Visualize Shared Data Help Log in or Register

Using 0%

Tools

search tools

Upload Data

FILE AND META TOOLS

Get Data

Send Data

Collection Operations

GENERAL TEXT TOOLS

Text Manipulation

Filter and Sort

Join, Subtract and Group

GENOMIC FILE MANIPULATION

FASTA/FASTQ

FASTQ Quality Control

SAM/BAM

BED

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History

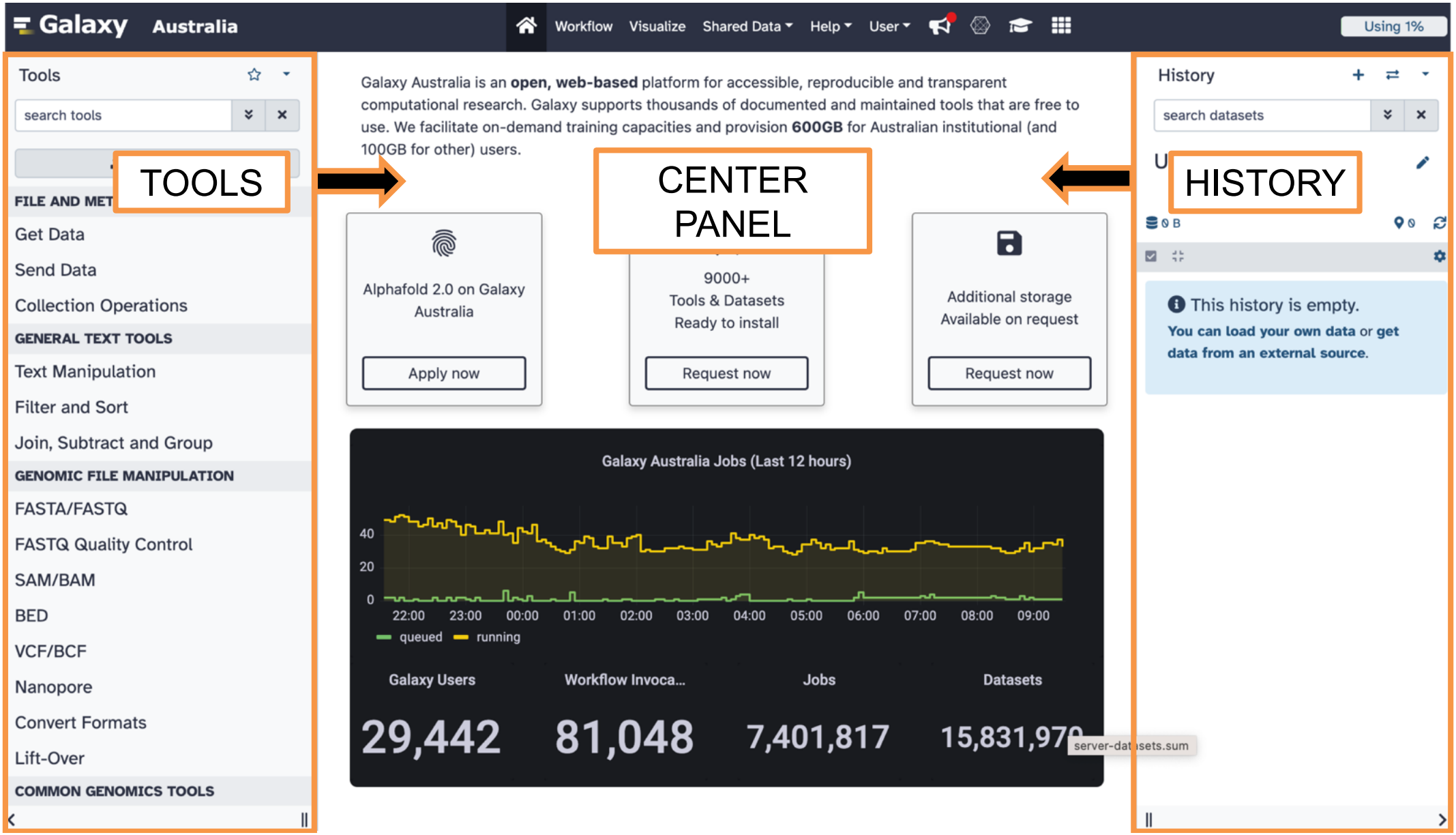
search datasets

Unnamed history

0 B

This history is empty.

You can load your own data or **get data from an external source.**


Galaxy Australia
Workflow
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Help
User
Using 1%
Tools
search tools
TOOLS
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GENERAL TEXT TOOLS
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Apply now
9000+ Tools & Datasets Ready to install
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Additional storage Available on request
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Galaxy Australia Jobs (Last 12 hours)
40
20
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08:00
09:00
queued
running
Galaxy Users
29,442
Workflow Invoca...
81,048
Jobs
7,401,817
Datasets
15,831,970
History
search datasets
HISTORY
This history is empty.
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Workflow Visualize Shared Data Help User

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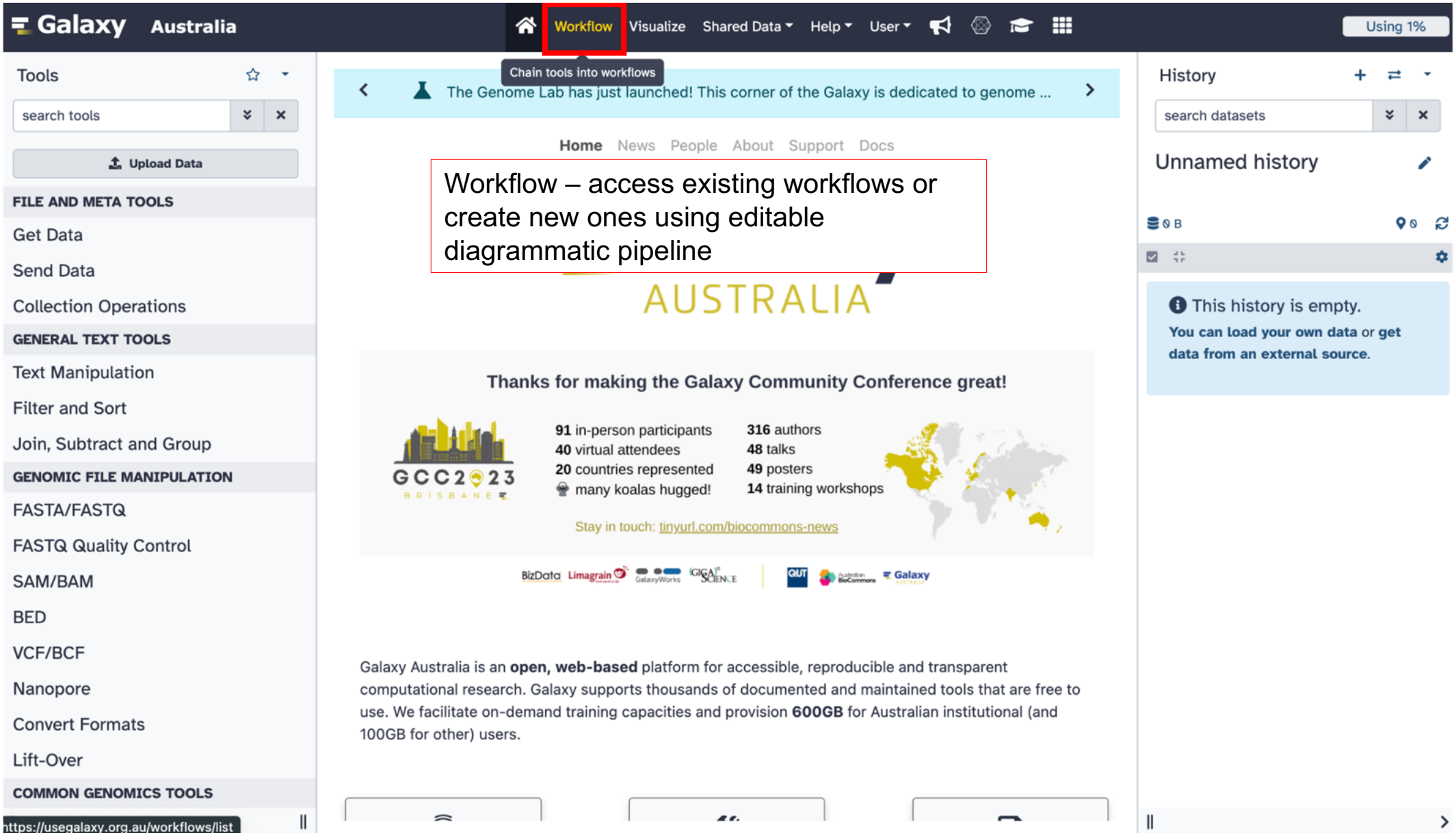
Galaxy Australia
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Chain tools into workflows
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Workflow – access existing workflows or create new ones using editable diagrammatic pipeline
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History
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Workflow | Visualize | Shared Data | Help | User

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Visualize datasets

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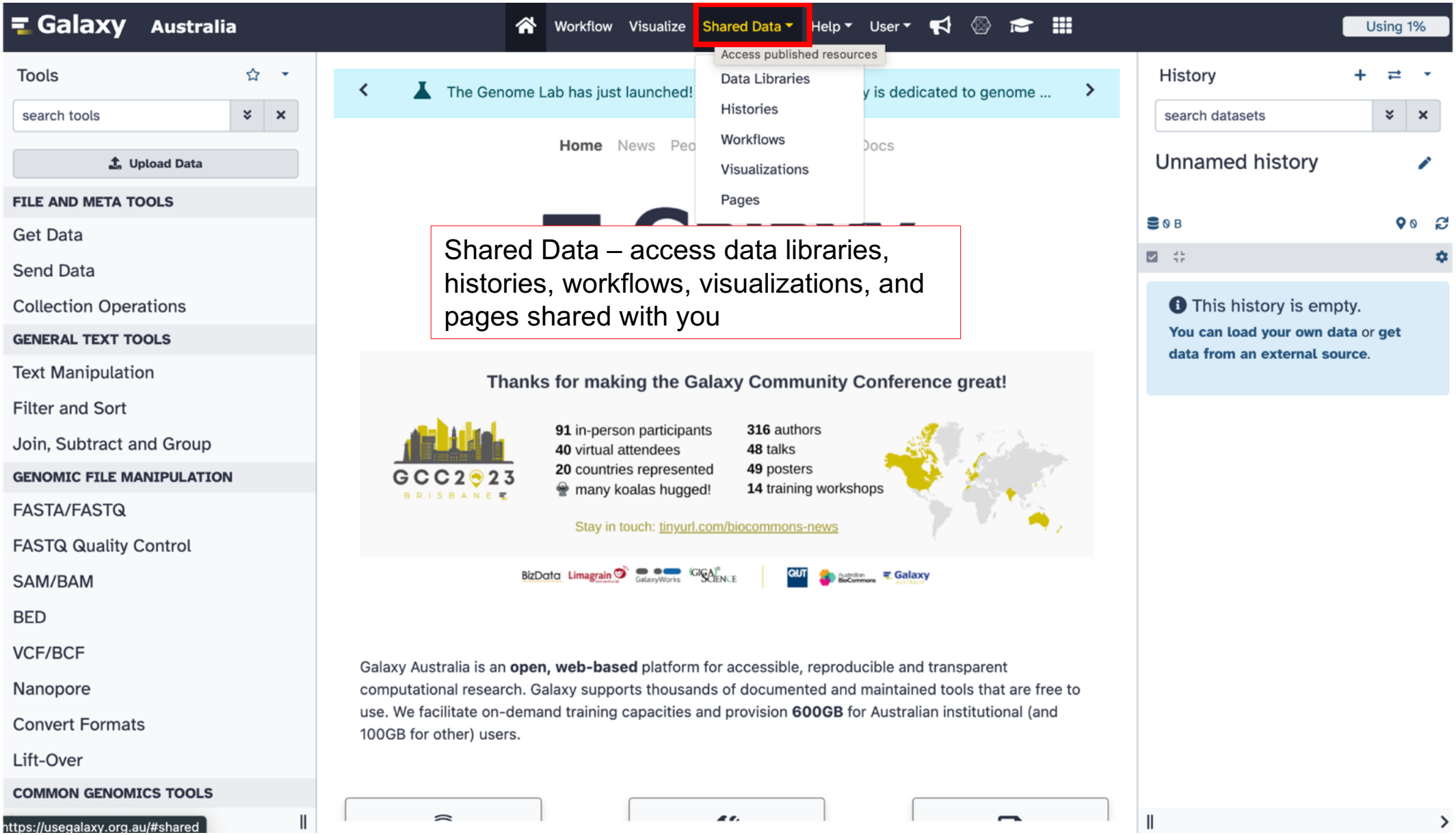
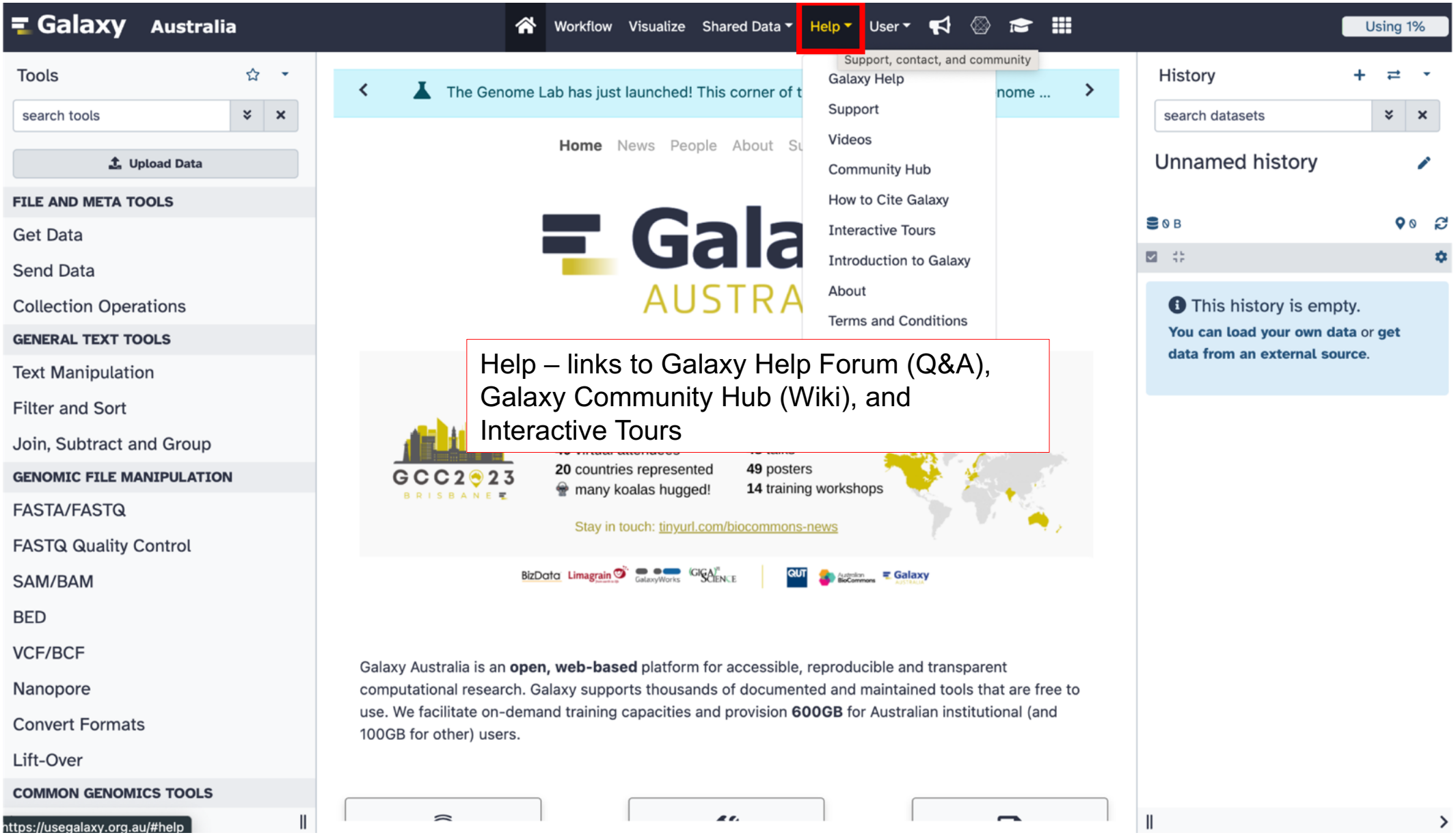


Shared Data – access data libraries, histories, workflows, visualizations, and pages shared with you



Help – links to Galaxy Help Forum (Q&A), Galaxy Community Hub (Wiki), and Interactive Tours

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Workflow Visualize Shared Data Help User

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Account and saved data

Signed in as clyde_dapat

Preferences

Datasets

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Histories shared with me

Archived Histories

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Visualizations

Active InteractiveTools

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User – links to preferences, saved histories, datasets, pages and visualizations

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Create history

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History

search datasets

Unnamed history

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History

search datasets

Unnamed history

RSV_assembly

Annotation (optional)

Add Tags

Save Cancel

0 B

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RSV_assembly

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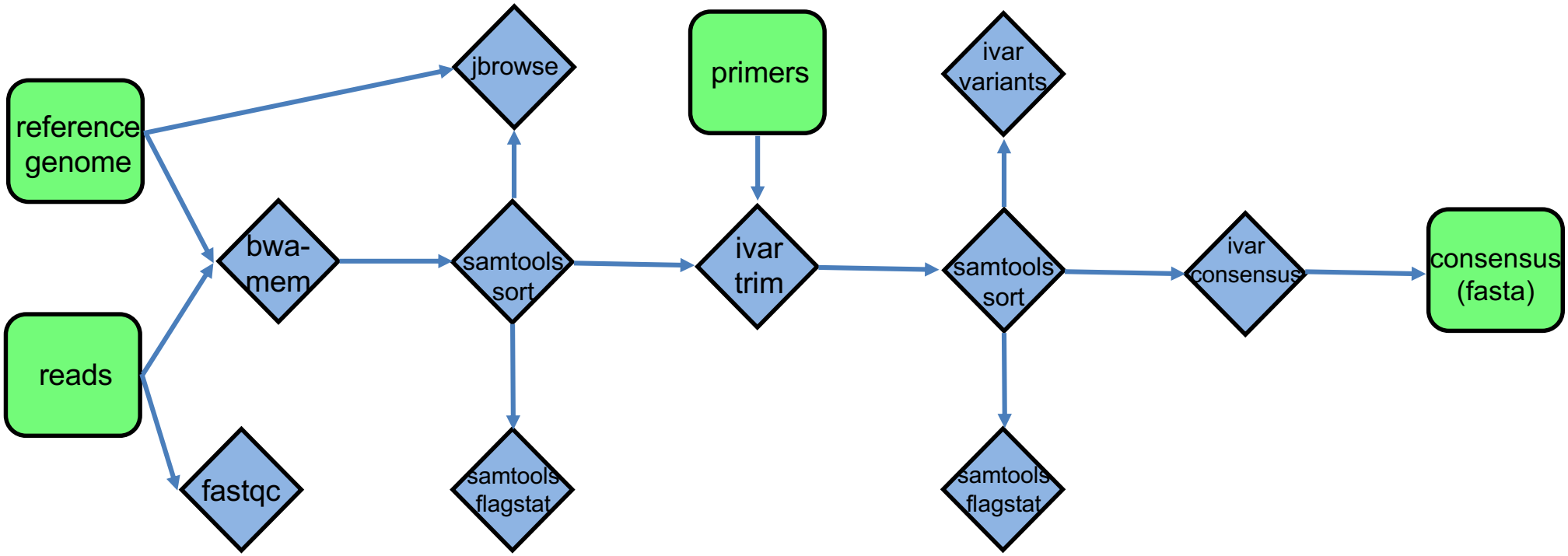
Workflow

RSV genome assembly



Upload data

1. NGS data (fastq file)
2. Reference genome (fasta file)
3. Reference genome annotation (gff file)
4. Primers (bed file)

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Workflow Visualize Shared Data Help User

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Download from URL or upload files from disk

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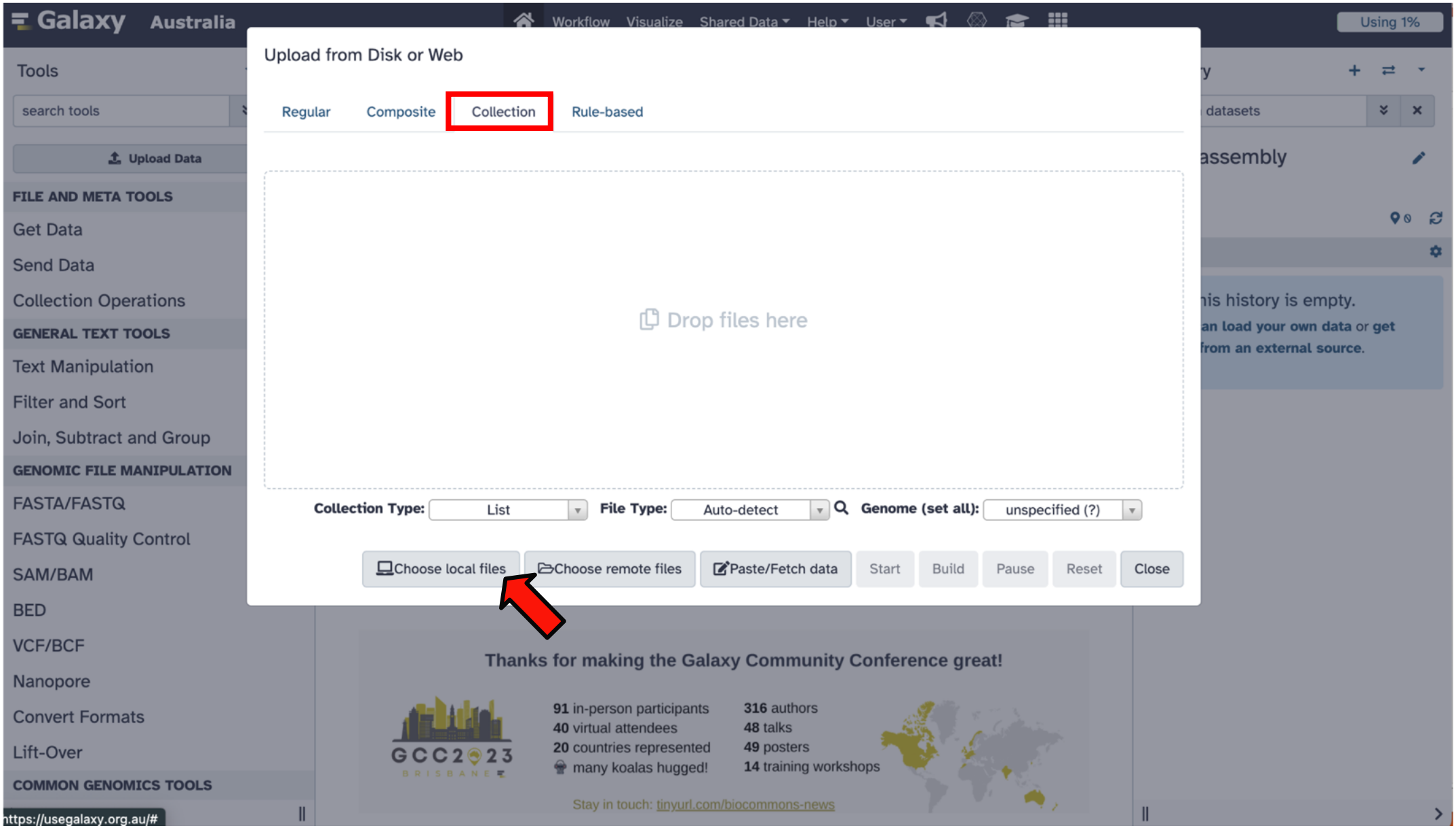
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Drop files here
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Auto-detect
Genome (set all):
unspecified (?)
Choose local files
Choose remote files
Paste/Fetch data
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datasets
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from an external source.
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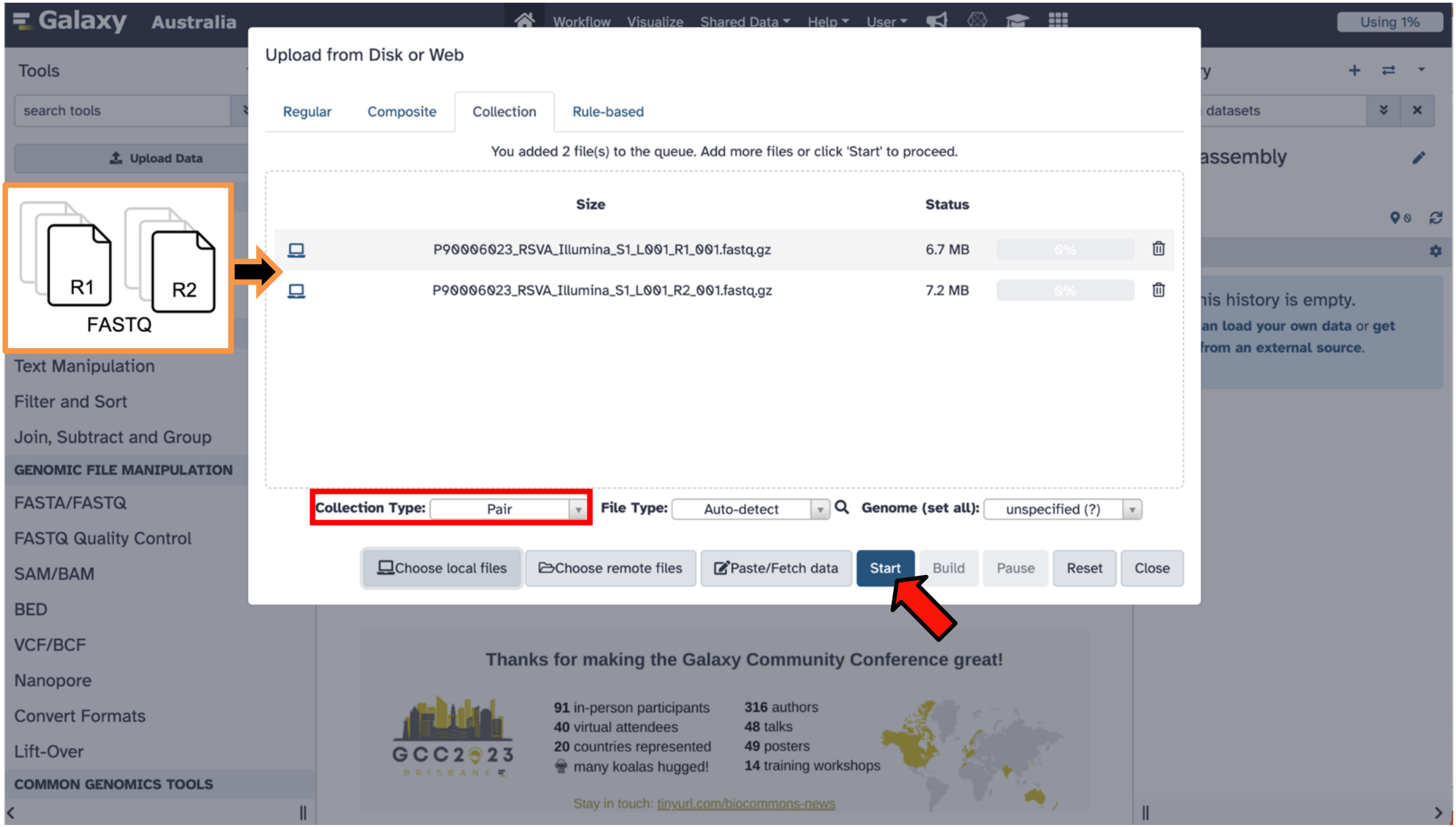
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Rule-based
You added 2 file(s) to the queue. Add more files or click 'Start' to proceed.
Size
Status
P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz
6.7 MB
0%
P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz
7.2 MB
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Collection Type:
Pair
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Auto-detect
Genome (set all):
unspecified (?)
Choose local files
Choose remote files
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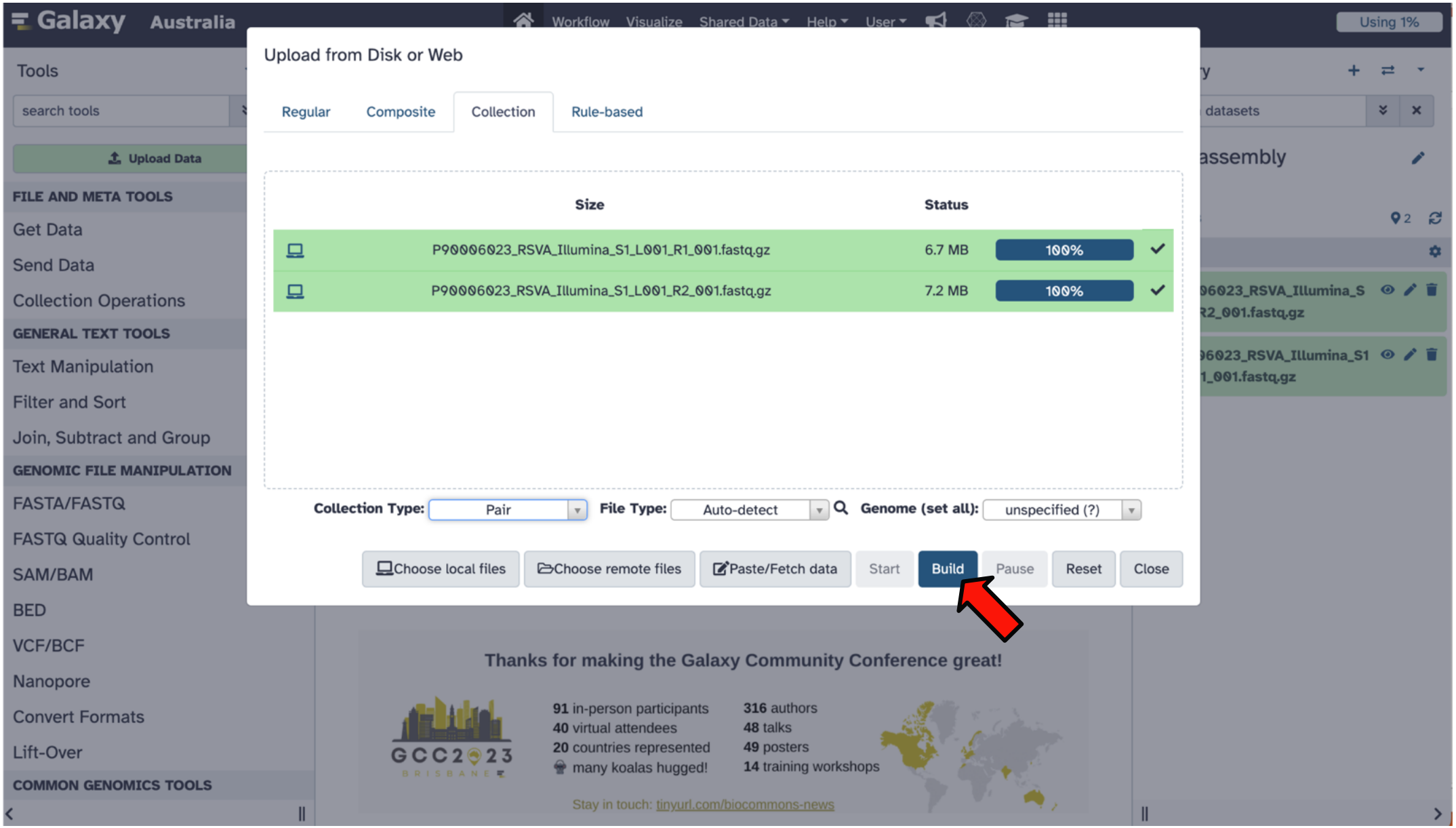
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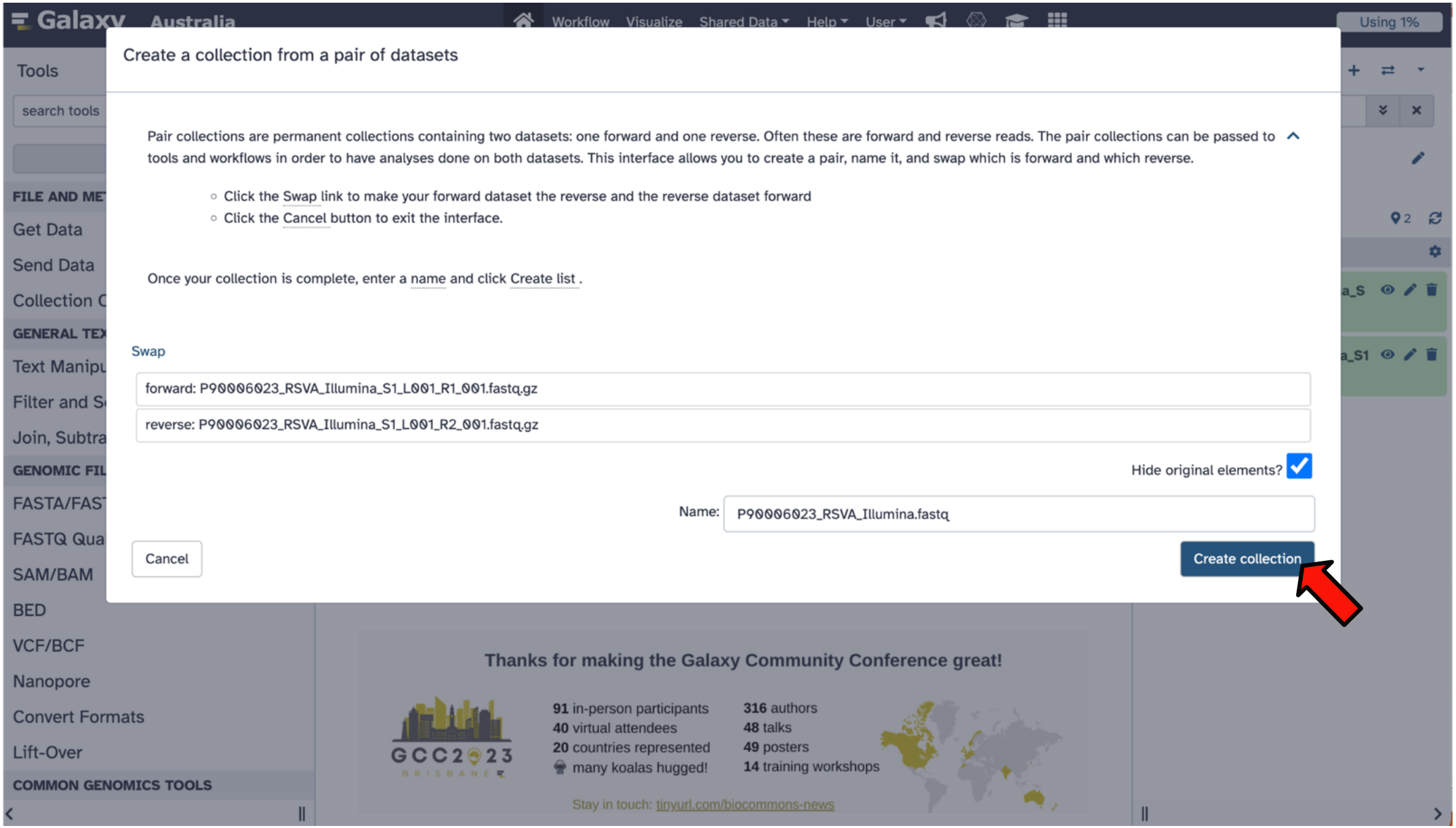
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Create a collection from a pair of datasets
Pair collections are permanent collections containing two datasets: one forward and one reverse. Often these are forward and reverse reads. The pair collections can be passed to tools and workflows in order to have analyses done on both datasets. This interface allows you to create a pair, name it, and swap which is forward and which reverse.
Click the Swap link to make your forward dataset the reverse and the reverse dataset forward
Click the Cancel button to exit the interface.
Once your collection is complete, enter a name and click Create list .
Swap
forward: P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz
reverse: P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz
Hide original elements?
Name:
P90006023_RSVA_Illumina.fastq
Cancel
Create collection
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Outage Notification

Galaxy Australia is planning a full day outage on **Wednesday 25th October** for maintenance. Please plan accordingly and check the usegalaxy.org.au website for any updates.

Job submission will be switched off at midnight and any running jobs will be stopped at 06:00.

We estimate that Galaxy will be unavailable from 06:00 to 18:00 on Wednesday 25th October AEST.

If you have any questions or concerns regarding this outage, please get in contact with us at help@genome.edu.au.

We thank you for your patience and apologise for any inconvenience this may cause.

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History

deleted:false visible:any

RSV_assembly

14.6 MB 1 2

3: P90006023_RSVA_Illumina.fastq

a pair with 2 datasets

2: P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz

1: P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz

Reference genome

rsv.reference.fasta

```
>rsv_reference
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ATTACAAGTAGTGATATTTGCCCTAATAATAATATTGTAGTGAAATCCAATTTCACAACAATGCCAGTATTACAAAATGGAGGTTATATATGGGAAATGATGGAATTAACACACTGCTCTCAACCTAATGGCCTAATAG
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ATTATGATGTGCAGAAACACATTAATAAGTTATGTGGCATGTTATTAATCACAGAAGATGCTAATCATAAATTCACTGGGTTAATAGGTATGTTATATGCTATGTCTAGATTAGGAAGAGAAGACACCATAAAAATACT
CAAAGATGCGGGATATCATGTTAAAGCAAATGGAGTGGATGTAACAACACATCGTCAAGACATTAATGGGAAAGAAATGAAATTTGAAGTGTTAACATTAGCAAGCTTAACAACTGAAATTCAAATCAACATTGAGATA
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GATCAGGTCTTACAGCTGTGATTAGGAGAGCTAACAATGTCCTAAAAAATGAAATGAAACGTTATAAAGGCTTATTACCCAAGGATATAGCCAACAGCTTCTATGAAGTGTTTGAAAAATATCCTCACTTTATAGATGT
TTTTGTTCATTTTGGTATAGCACAATCTTCTACCAGAGGTGGCAGTAGAGTTGAAGGGATTTTTGCAGGATTGTTTATGAATGCCTATGGTGCAGGGCAAGTGATGTTACGGTGGGGGGTCTTAGCAAAATCAGTTAAA
AATATTATGTTAGGACACGCTAGTGTACAAGCAGAAATGGAACAAGTTGTGGAGGTGTATGAGTATGCTCAGAAATTGGGTGGAGAAGCAGGATTCTACCATATATTGAACAACCCAAAAGCATCATTATTATCTTTGA
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TGTGATTAACTACAGTGTATTAGACTTGACAGCAGAAGAACTAGAGGCTATCAAACATCAGCTTAATCCAAAAGATAATGATGTAGAGCTTTGAGTTAATAAAAAGGTGGGGCAAATAAATCATCATGGAAAAGTTTGC
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TTTCAAAACTATACAAAGAAACCATAGAAACATTTGATAACAATGAAGAAGAATCTAGCTATTCATATGAAGAAATAAATGATCAGACAAACGATAATATAACAGCAAGATTAGATAGGATTGATGAGAAATTAAGTGA
AATACTAGGAATGCTTCACACATTAGTAGTAGCAAGTGCAGGACCTACATCTGCTCGGGATGGTATAAGAGATGCCATGGTTGGTTTAAGAGAAGAAATGATAGAAAAAATCAGAACTGAAGCATTAATGACCAATGAC
AGACTAGAAGCTATGGCAAGACTCAGGAATGAGGAAAGTGAAAAGATGGCAAAAGACACATCAGATGAAGTGTCTCTCAATCCAACATCAGAGAAATTGAACAACCTGTTGGAAGGGAATGATAGTGACAATGATCTAT
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TCCAAAACTAGCCACCCGGAAAAAATCGATACTATAGTTACAAAAAAAGATGGGGCAAATATGGAAACATACGTGAACAAACTTCACGAGGGCTCCACATACACAGCTGCTGTTCAATACAATGTCCTAGAAAAAGACG
ATGATCCTGCATCACTTACAATATGGGTGCCCATGTTCCAATCATCCATGCCAGCAGATTTACTCATAAAAGAACTAGCCAATGTCAATATACTAGTGAAACAAATATCCACACCCAAGGGACCYTCATTAAGAGTCAT
GATAAACTCAAGAAGTGCAGTGCTAGCACAAATGCCCAGCAAATTTACCATATGTGCCAATGTGTCCTTGGATGAAAGAAGCAAGCTGGCATATGATGTAACCACACCCTGTGAAATTAAGGCATGCAGTCTAACATGC
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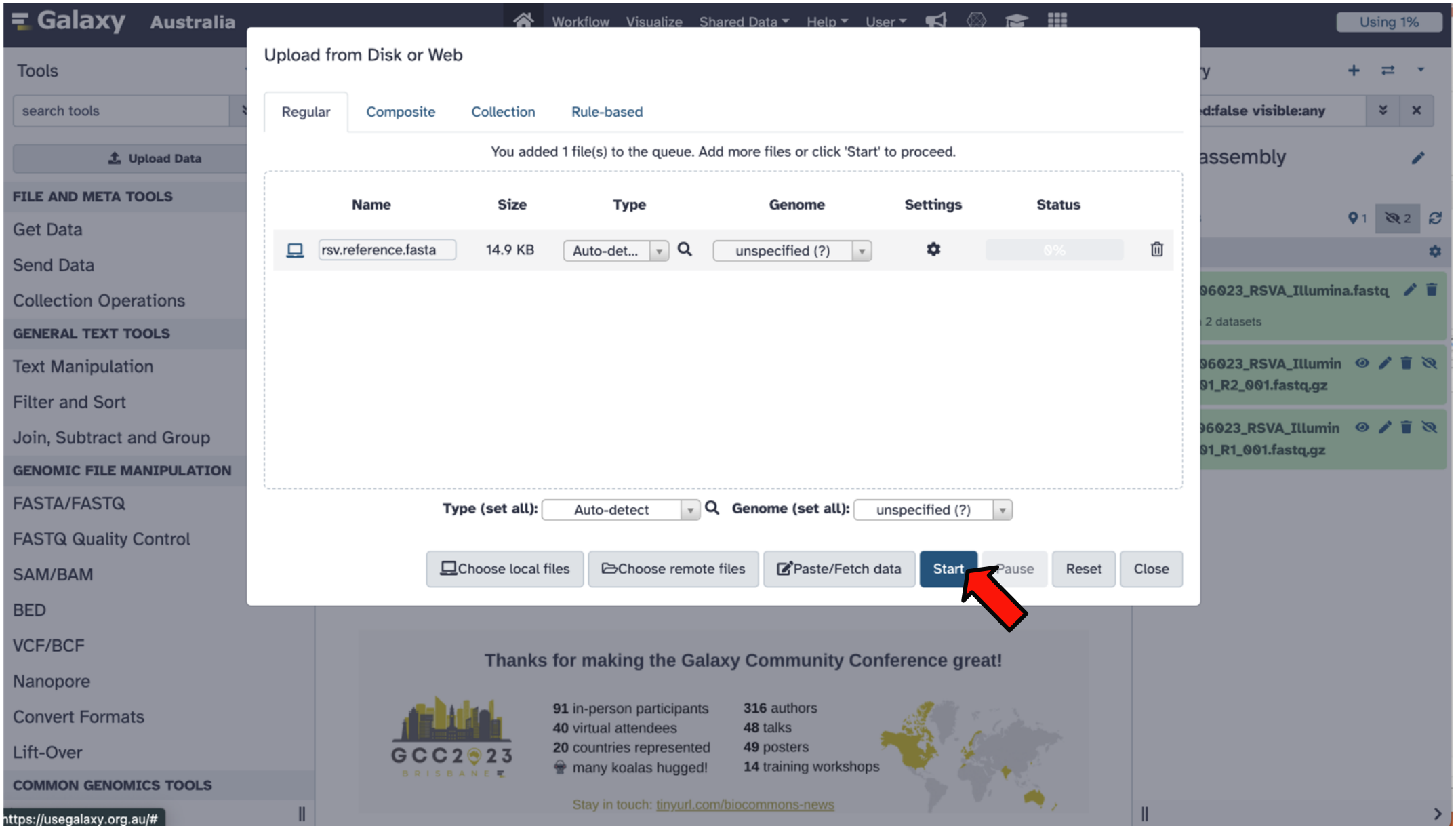
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You added 1 file(s) to the queue. Add more files or click 'Start' to proceed.
Name
Size
Type
Genome
Settings
Status
rsv.reference.fasta
14.9 KB
Auto-det...
unspecified (?)
0%
Type (set all):
Auto-detect
Genome (set all):
unspecified (?)
Choose local files
Choose remote files
Paste/Fetch data
Start
Pause
Reset
Close
assembly
96023_RSVA_Illumina.fastq
2 datasets
96023_RSVA_Illumin
01_R2_001.fastq.gz
96023_RSVA_Illumin
01_R1_001.fastq.gz
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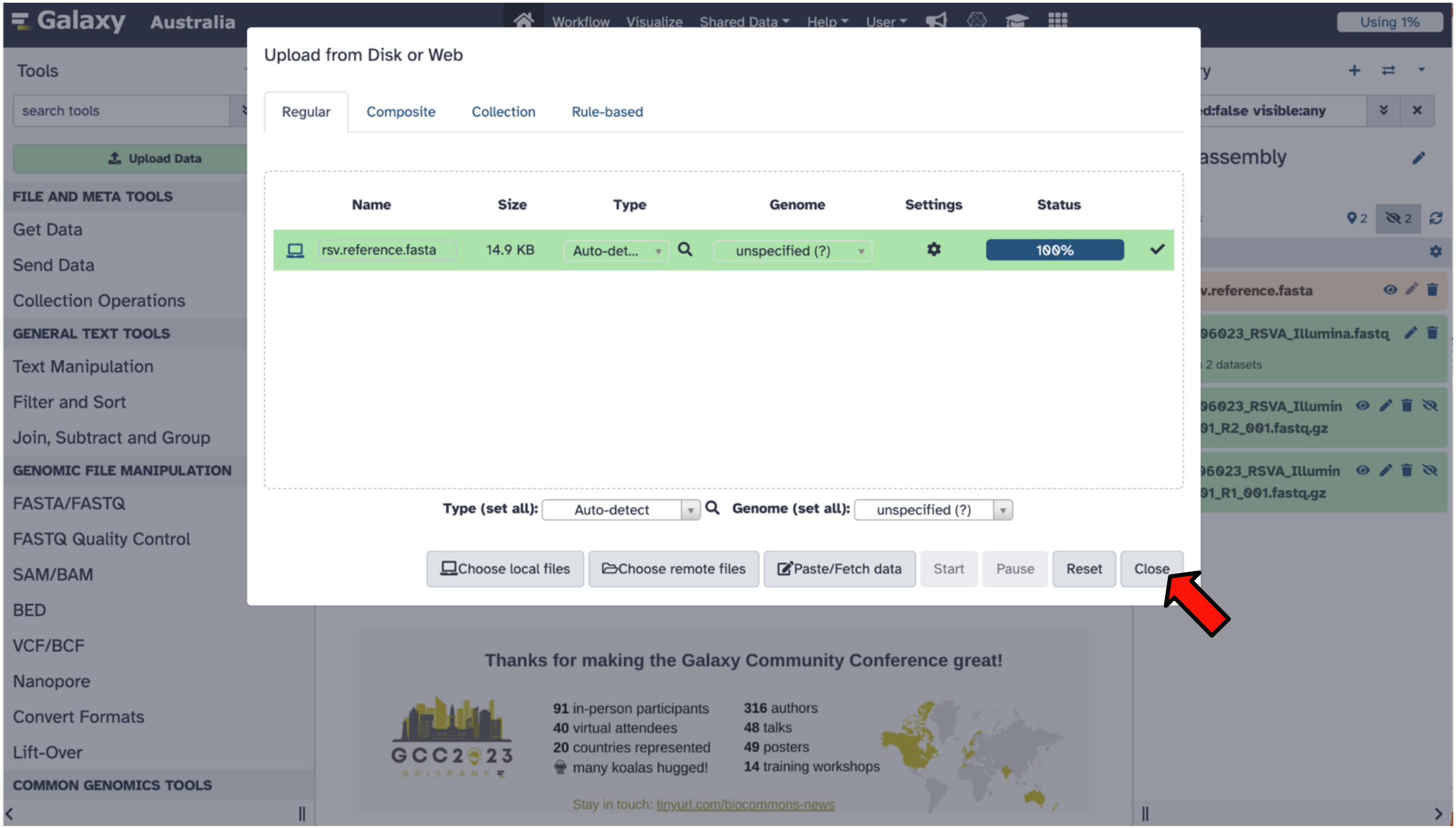
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Status
rsv.reference.fasta
14.9 KB
Auto-det...
unspecified (?)
100%
Type (set all):
Auto-detect
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History

deleted:false visible:any

RSV_assembly

14.6 MB 2 2

4: rsv.reference.fasta

3: P90006023_RSVA_Illumina.fastq

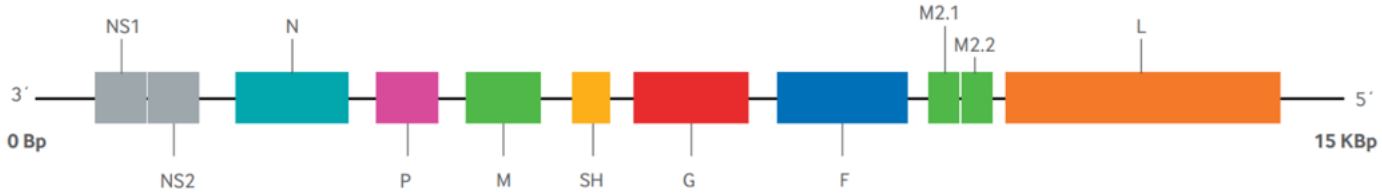
a pair with 2 datasets

2: P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz

1: P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz

Reference genome annotation

RSV genome

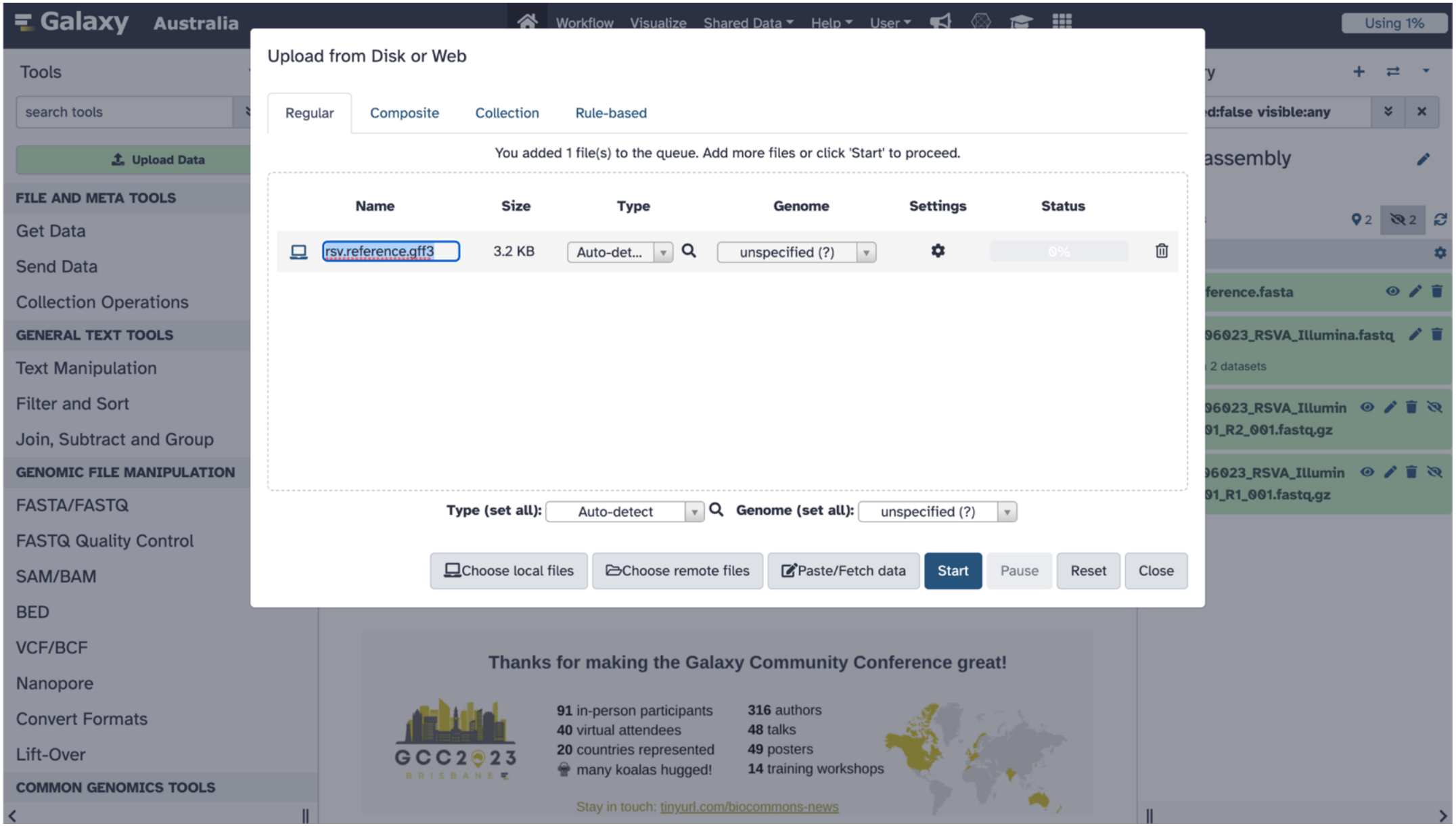


rsv.reference.gff3 — Edited

```
##gff-version 3
rsv_reference	Genbank	region	1	15281	.	+	.	ID=rsv_reference
rsv_reference	Genbank	gene	102	521	.	+	.	ID=gene-NS1;Name=NS1;gbkey=Gene;gene=NS1;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	102	521	.	+	0	ID=cds-WCO07309.1;Parent=gene-
NS1;Dbxref=NCBI_GP:WCO07309.1;Name=WCO07309.1;gbkey=CDS;gene=NS1;product=nonstructural protein 1;protein_id=WCO07309.1
rsv_reference	Genbank	gene	631	1005	.	+	.	ID=gene-NS2;Name=NS2;gbkey=Gene;gene=NS2;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	631	1005	.	+	0	ID=cds-WCO07310.1;Parent=gene-
NS2;Dbxref=NCBI_GP:WCO07310.1;Name=WCO07310.1;gbkey=CDS;gene=NS2;product=nonstructural protein 2;protein_id=WCO07310.1
rsv_reference	Genbank	gene	1143	2318	.	+	.	ID=gene-N;Name=N;gbkey=Gene;gene=N;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	1143	2318	.	+	0	ID=cds-WCO07311.1;Parent=gene-
N;Dbxref=NCBI_GP:WCO07311.1;Name=WCO07311.1;gbkey=CDS;gene=N;product=nucleoprotein;protein_id=WCO07311.1
rsv_reference	Genbank	gene	2350	3075	.	+	.	ID=gene-P;Name=P;gbkey=Gene;gene=P;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	2350	3075	.	+	0	ID=cds-WCO07312.1;Parent=gene-
P;Dbxref=NCBI_GP:WCO07312.1;Name=WCO07312.1;gbkey=CDS;gene=P;product=phosphoprotein;protein_id=WCO07312.1
rsv_reference	Genbank	gene	3258	4028	.	+	.	ID=gene-M;Name=M;gbkey=Gene;gene=M;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	3258	4028	.	+	0	ID=cds-WCO07313.1;Parent=gene-
M;Dbxref=NCBI_GP:WCO07313.1;Name=WCO07313.1;gbkey=CDS;gene=M;product=matrix protein;protein_id=WCO07313.1
rsv_reference	Genbank	gene	4298	4492	.	+	.	ID=gene-SH;Name=SH;gbkey=Gene;gene=SH;gene_biotype=protein_coding
rsv_reference	Genbank	CDS	4298	4492	.	+	0	ID=cds-WCO07314.1;Parent=gene-
SH;Dbxref=NCBI_GP:WCO07314.1;Name=WCO07314.1;gbkey=CDS;gene=SH;product=small hydrophobic protein;protein_id=WCO07314.1
```

General GFF3 structure

Position index	Position name	Description
1	seqid	The name of the sequence where the feature is located.
2	source	The algorithm or procedure that generated the feature. This is typically the name of a software or database.
3	type	The feature type name, like "gene" or "exon". In a well structured GFF file, all the children features always follow their parents in a single block (so all exons of a transcript are put after their parent "transcript" feature line and before any other parent transcript line). In GFF3, all features and their relationships should be compatible with the standards released by the Sequence Ontology Project.
4	start	Genomic start of the feature, with a **1-base offset**. This is in contrast with other 0-offset half-open sequence formats, like BED.
5	end	Genomic end of the feature, with a **1-base offset**. This is the same end coordinate as it is in 0-offset half-open sequence formats, like BED.[*citation needed*]
6	score	Numeric value that generally indicates the confidence of the source in the annotated feature. A value of "." (a dot) is used to define a null value.
7	strand	Single character that indicates the strand of the feature. This can be "+" (positive, or 5'->3'), "-", (negative, or 3'->5'), "." (undetermined), or "?" for features with relevant but unknown strands.
8	phase	phase of CDS features; it can be either one of 0, 1, 2 (for CDS features) or "." (for everything else). See the section below for a detailed explanation.
9	attributes	A list of tag-value pairs separated by a semicolon with additional information about the feature.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Name
Size
Type
Genome
Settings
Status
rsv.reference.gff3
3.2 KB
Auto-det...
unspecified (?)
0%
Type (set all):
Auto-detect
Genome (set all):
unspecified (?)
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RSV_assembly

14.6 MB 3 2

5: rsv.reference.gff3

4: rsv.reference.fasta

3: P90006023_RSVA_Illumina.fastq

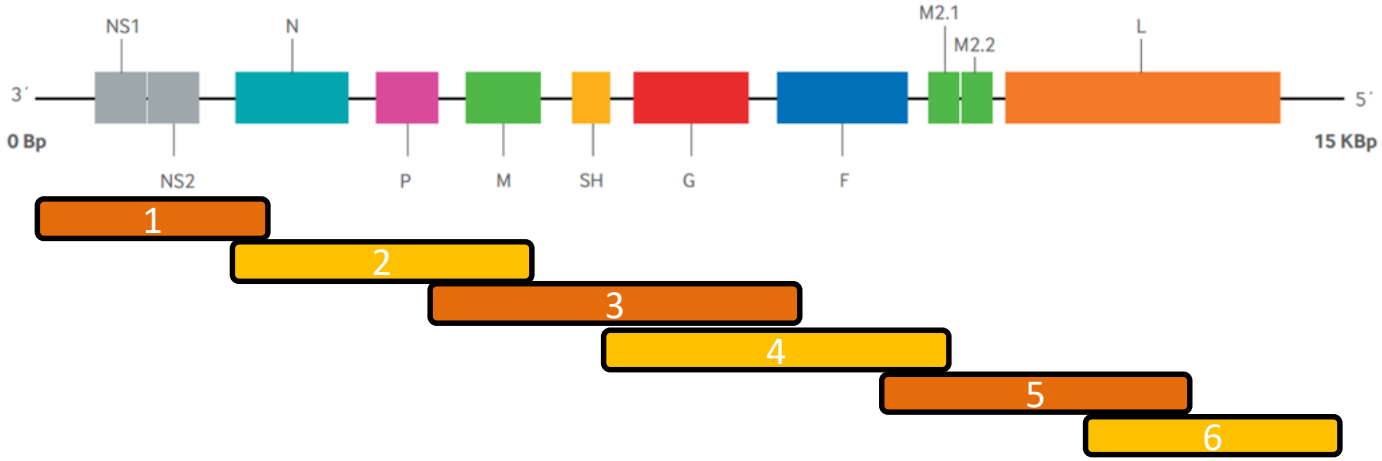
a pair with 2 **fastqsanger.gz** datasets

2: P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz

1: P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz

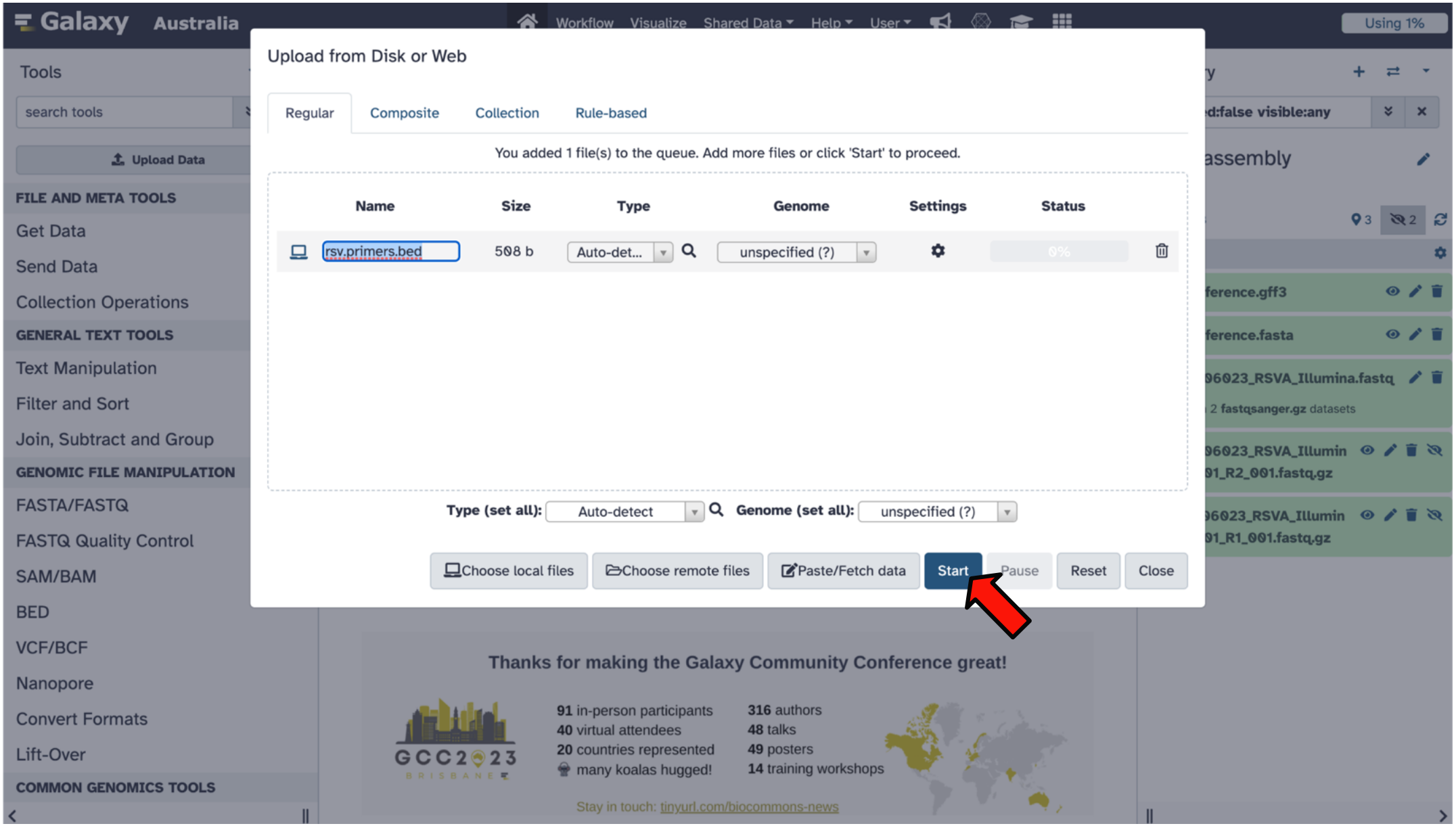
Primers

RSV genome



rsv.primers.bed — Edite

rsv_reference	3	28	1-1F_LEFT	1	+
rsv_reference	1877	1902	1-1R_RIGHT	1	-
rsv_reference	3344	3367	1-3F_3_LEFT	1	+
rsv_reference	6138	6163	1-3R_6_RIGHT	1	-
rsv_reference	7118	7141	1-5F_LEFT	1	+
rsv_reference	11029	11047	1-5R_RIGHT	1	-
rsv_reference	1565	1588	2-2F_LEFT	2	+
rsv_reference	3978	4000	2-2R_RIGHT	2	-
rsv_reference	4330	4353	2-4F_LEFT	2	+
rsv_reference	7874	7897	2-4R1_RIGHT	2	-
rsv_reference	7882	7904	2-4R2_RIGHT	2	-
rsv_reference	10842	10863	2-6F_LEFT	2	+
rsv_reference	15173	15196	2-6R_RIGHT	2	-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Chrom	Start	End	Name	Score	Strand	ThickStart	ThickEnd	ItemRGB	BlockCount	BlockSize
rsv_reference	3	28	1-1F_LEFT	1	+					
rsv_reference	1877	1902	1-1R_RIGHT	1	-					
rsv_reference	3344	3367	1-3F_3_LEFT	1	+					
rsv_reference	6138	6163	1-3R_6_RIGHT	1	-					
rsv_reference	7118	7141	1-5F_LEFT	1	+					
rsv_reference	11029	11047	1-5R_RIGHT	1	-					
rsv_reference	1565	1588	2-2F_LEFT	2	+					
rsv_reference	3978	4000	2-2R_RIGHT	2	-					
rsv_reference	4330	4353	2-4F_LEFT	2	+					
rsv_reference	7874	7897	2-4R1_RIGHT	2	-					
rsv_reference	7882	7904	2-4R2_RIGHT	2	-					
rsv_reference	10842	10863	2-6F_LEFT	2	+					
rsv_reference	15173	15196	2-6R_RIGHT	2	-					

History

deleted:false visible:any

RSV_assembly

14.6 MB | 4 | 2

6: rsv.primers.bed

5: rsv.reference.gff3

4: rsv.reference.fasta

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a pair with 2 fastqsanger.gz datasets

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https://usegalaxy.org.au/datasets/a6e389a98c2d1678be783d07fc638b21/preview

Assess quality

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FastQC Read Quality reports

Tabular to FASTQ converter

Make.fastq Convert fasta and quality to fastq

WORKFLOWS

All workflows

FastQC Read Quality reports (Galaxy Version 0.74+galaxy0) | Run Tool

1

Tool Parameters

! Please provide a value for this option.

Raw read data from your current history * required

2

No fastq, fastq.gz, fastq.bz2, bam or sam dataset available.

Contaminant list - optional

Nothing selected

tab delimited file with 2 columns: name and sequence. For example: Illumina Small RNA RT Primer CAAGCAGAAGACGGCATACGA

Adapter list - optional

Nothing selected

List of adapters adapter sequences which will be explicity searched against the library. It should be a tab-delimited file with 2 columns: name and sequence. (--adapters)

Submodule and Limit specifing file - optional

Nothing selected

a file that specifies which submodules are to be executed (default=all) and also specifies the thresholds for the each submodules warning parameter

Disable grouping of bases for reads >50bp

No

Using this option will cause fastqc to crash and burn if you use it on really long reads, and your plots may end up a ridiculous size. You have been warned! (--nogroup)

Lower limit on the length of the sequence to be shown in the report - optional

History

deleted:false visible:any

RSV_assembly

14.6 MB | 4 | 2

6: rsv.primers.bed

5: rsv.reference.gff3

4: rsv.reference.fasta

3: P90006023_RSVA_Illumina.fastq

a pair with 2 **fastqsanger.gz** datasets

2: P90006023_RSVA_Illumina_S1_L001_R2_001.fastq.gz

1: P90006023_RSVA_Illumina_S1_L001_R1_001.fastq.gz

Galaxy Australia | Workflow | Visualize | Shared Data | Help | User | Using 1%

Tools

fastqc

Upload Data

Show Sections

FastQC Read Quality reports

Tabular to FASTQ converter

Make.fastq Convert fasta and quality to fastq

WORKFLOWS

All workflows

FastQC Read Quality reports (Galaxy Version 0.74+galaxy0) | Run Tool

Tool Parameters

Raw read data from your current history *

3: P90006023_RSVA_Illumina.fastq

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.

Contaminant list - optional

Nothing selected

tab delimited file with 2 columns: name and sequence. For example: Illumina Small RNA RT Primer CAAGCAGAAGACGGCATACGA

Adapter list - optional

Nothing selected

List of adapters adapter sequences which will be explicity searched against the library. It should be a tab-delimited file with 2 columns: name and sequence. (--adapters)

Submodule and Limit specifing file - optional

Nothing selected

a file that specifies which submodules are to be executed (default=all) and also specifies the thresholds for the each submodules warning parameter

Disable grouping of bases for reads >50bp

No

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Additional Options

Email notification

No

Send an email notification when the job completes.

Run Tool

Help

Purpose

FastQC aims to provide a simple way to do some quality control checks on raw sequence data coming from high throughput sequencing pipelines. It provides a set of analyses which you can use to get a quick impression of whether your data has any problems of which you should be aware before doing any further analysis.

The main functions of FastQC are:

- Import of data from BAM, SAM or FastQ/FastQ.gz files (any variant),
- Providing a quick overview to tell you in which areas there may be problems
- Summary graphs and tables to quickly assess your data
- Export of results to an HTML based permanent report
- Offline operation to allow automated generation of reports without running the interactive application

FastQC

This is a Galaxy wrapper. It merely exposes the external package FastQC which is documented at FastQC Kindly acknowledge it as well as this tool if you use it. FastQC incorporates the Picard-tools libraries for SAM/BAM processing.

The contaminants file parameter was borrowed from the independently developed fastqcwrapper contributed to the Galaxy Community Tool Shed by J. Johnson. Adaption to version 0.11.2 by T. McGowan.

History

deleted:false visible:any

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Started tool **FastQC** and successfully added 2 jobs to the queue.

It produces 4 outputs:

- **9: FastQC on data 1: Webpage**
- **10: FastQC on data 1: RawData**
- **11: FastQC on data 2: Webpage**
- **12: FastQC on data 2: RawData**

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

deleted:false visible:any

RSV_assembly

15.9 MB | 6 | 6

12: FastQC on data 2: RawData

11: FastQC on data 2: Webpage

10: FastQC on data 1: RawData

9: FastQC on data 1: Webpage

8: FastQC on collection 3: RawData

a pair with 2 datasets

7: FastQC on collection 3: Webpage

a pair with 2 datasets

6: rsv.primers.bed

5: rsv.reference.gff3

4: rsv.reference.fasta

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FastQC Report

Thu 19 Oct 2023
reverse.gz

Summary

- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

Basic Statistics

Measure	Value
Filename	reverse.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	94076
Total Bases	13.4 Mbp
Sequences flagged as poor quality	0
Sequence length	35-151
%GC	36

Per base sequence quality

Produced by FastQC (version 0.12.1)

History

deleted:false visible:any

RSV_assembly

15.9 MB | 6 | 6

- 12: FastQC on data 2: RawData
- 11: FastQC on data 2: Webpage
- 10: FastQC on data 1: RawData
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- 8: FastQC on collection 3: RawData — a pair with 2 datasets
- 7: FastQC on collection 3: Webpage — a pair with 2 datasets
- 6: rsv.primers.bed
- 5: rsv.reference.gff3
- 4: rsv.reference.fasta

https://usegalaxy.org.au/datasets/a6e389a98c2d1678b4813bfa9333b923/p