Galaxy Workflow for RNAseq

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Contents

Chapter 1

About

These instructions show how to use pre-made workflows on usegalaxy.org.au to analyse single stranded RNAseq files outputted from an Illumina system.

It is broken into 2 workflows. A workflow is a set of tools on galaxy organised together to do certain tasks. These workflows are shareable and are included here.

Workflow Step 1

The first workflow is designed to rename files, run quality check for each file and join any files together that are from the same sample (see 3). It also calculates some other values required for Step 2, such as the length of bp of the transcripts.

Once the output is checked (FastQC via a MultiQC report), the concatenated files can be used as input to Step 2.

Workflow Step 2

Step 2

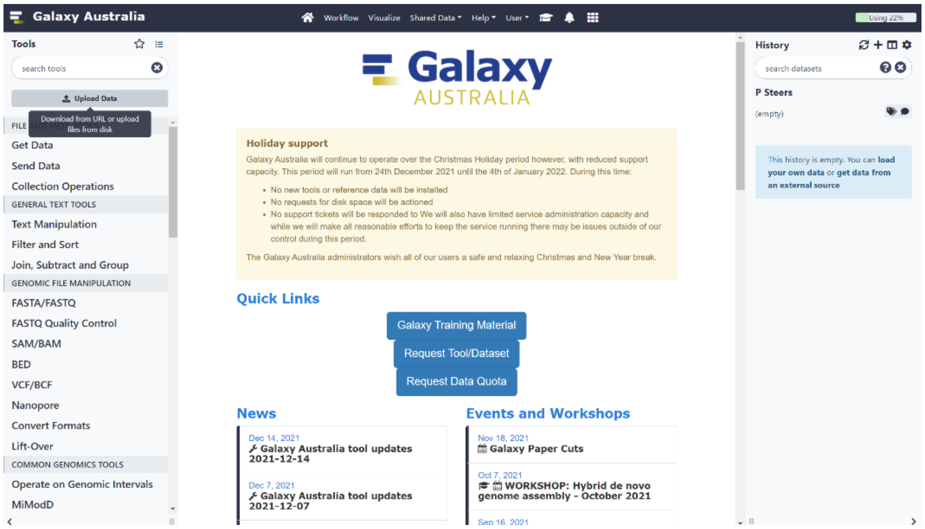
Chapter 2

Upload and Prepare Data

2.1 Import fastq files

2.1.1 Upload

Firstly we upload the files to the Galaxy History. Follow figures below.



Holiday support

Galaxy Australia will continue to operate over the Christmas Holiday period however, with reduced support capacity. This period will run from 24th December 2021 until the 4th of January 2022. During this time:

- No new tools or reference data will be installed
- No requests for disk space will be actioned
- No support tickets will be responded to We will also have limited service administration capacity and while we will make all reasonable efforts to keep the service running there may be issues outside of our control during this period.

The Galaxy Australia administrators wish all of our users a safe and relaxing Christmas and New Year break.

Dec 14, 2021
Galaxy Australia tool updates 2021-12-14

Dec 7, 2021
Galaxy Australia tool updates 2021-12-07

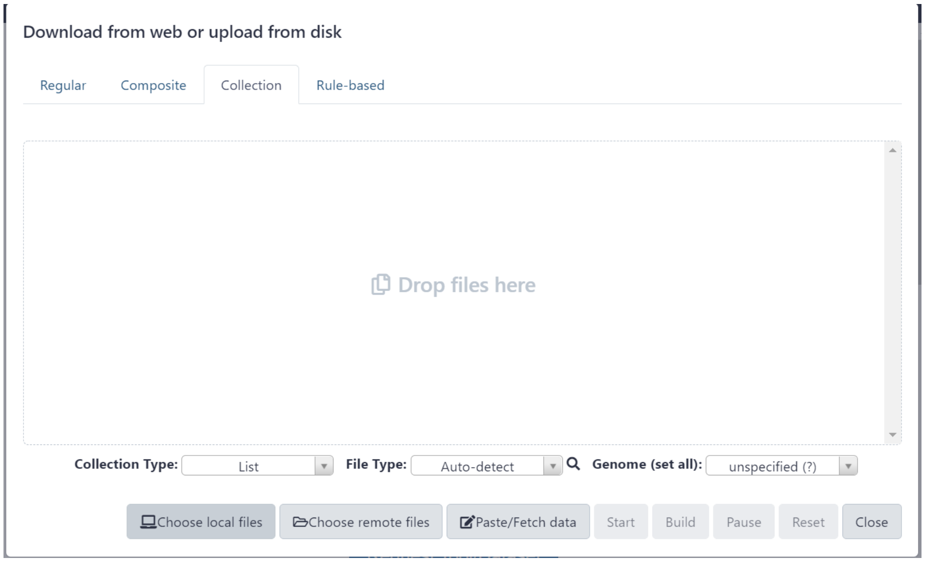
Nov 18, 2021
Galaxy Paper Cuts

Oct 7, 2021
WORKSHOP: Hybrid de novo genome assembly - October 2021

Sep 16, 2021

This history is empty. You can **load your own data** or **get data from an external source**

Figure 2.1: Click Upload Data button



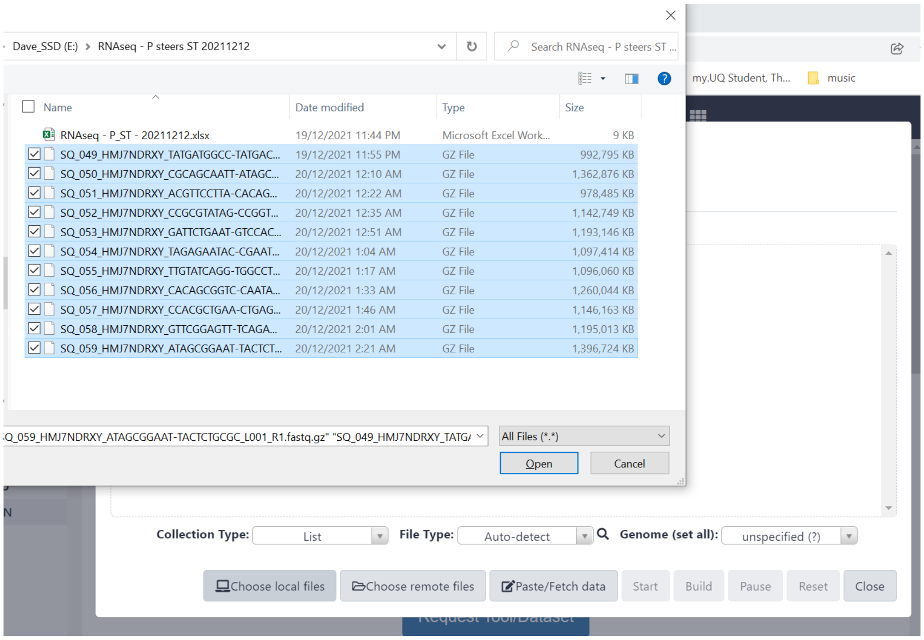
Download from web or upload from disk

Collection Type: List

File Type: Auto-detect

Genome (set all): unspecified (?)

Figure 2.2: Select ‘Collection’ from the top ribbon and click ‘Choose local files’ button



Dave_SSD (E:) › RNAseq - P steers ST 20211212

Name	Date modified	Type	Size
RNAseq - P_ST - 20211212.xlsx	19/12/2021 11:44 PM	Microsoft Excel Work...	9 KB
☑ SQ_049_HMJ7NDRXY_TATGATGGCC-TATGAC...	19/12/2021 11:55 PM	GZ File	992,795 KB
☑ SQ_050_HMJ7NDRXY_CGCAGCAATT-ATAGC...	20/12/2021 12:10 AM	GZ File	1,362,876 KB
☑ SQ_051_HMJ7NDRXY_ACGTTCCTTA-CACAG...	20/12/2021 12:22 AM	GZ File	978,485 KB
☑ SQ_052_HMJ7NDRXY_CCGCGTATAG-CCGGT...	20/12/2021 12:35 AM	GZ File	1,142,749 KB
☑ SQ_053_HMJ7NDRXY_GATTCTGAAT-GTCCAC...	20/12/2021 12:51 AM	GZ File	1,193,146 KB
☑ SQ_054_HMJ7NDRXY_TAGAGAATAC-CGAAT...	20/12/2021 1:04 AM	GZ File	1,097,414 KB
☑ SQ_055_HMJ7NDRXY_TTGTATCAGG-TGGCCT...	20/12/2021 1:17 AM	GZ File	1,096,060 KB
☑ SQ_056_HMJ7NDRXY_CACAGCGGTC-CAATA...	20/12/2021 1:33 AM	GZ File	1,260,044 KB
☑ SQ_057_HMJ7NDRXY_CCACGCTGAA-CTGAG...	20/12/2021 1:46 AM	GZ File	1,146,163 KB
☑ SQ_058_HMJ7NDRXY_GTTCGGAGTT-TCAGA...	20/12/2021 2:01 AM	GZ File	1,195,013 KB
☑ SQ_059_HMJ7NDRXY_ATAGCGGAAT-TACTCT...	20/12/2021 2:21 AM	GZ File	1,396,724 KB

;Q_059_HMJ7NDRXY_ATAGCGGAAT-TACTCTGCGC_L001_R1.fastq.gz" "SQ_049_HMJ7NDRXY_TATG/

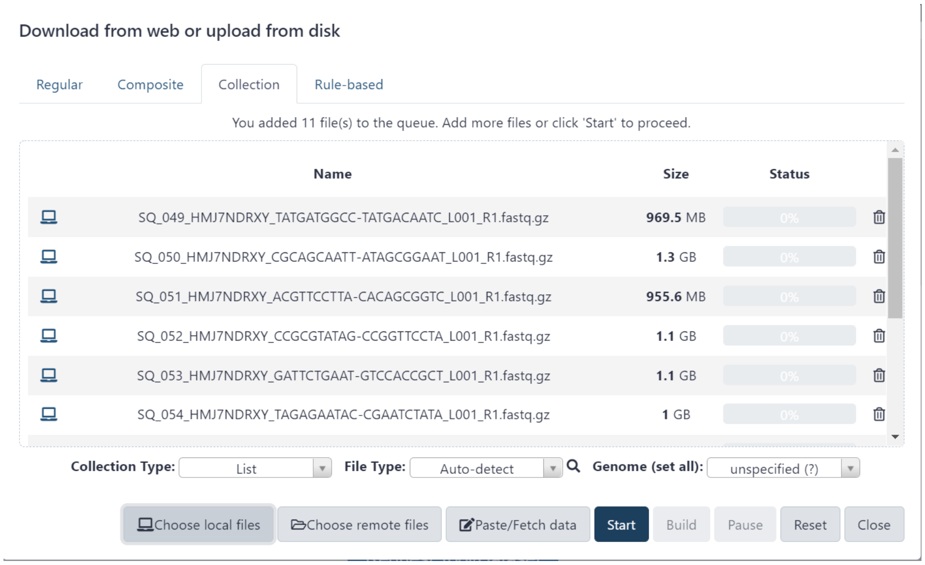
All Files (*.*)

Collection Type: List

File Type: Auto-detect

Genome (set all): unspecified (?)

Figure 2.3: Navigate to the files to upload and highlight them all using Shift or Ctrl keys to help, then click ‘Open’



Download from web or upload from disk

You added 11 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Status
SQ_049_HMJ7NDRXY_TATGATGGCC-TATGACAATC_L001_R1.fastq.gz	**969.5** MB	0%
SQ_050_HMJ7NDRXY_CGCAGCAATT-ATAGCGGAAT_L001_R1.fastq.gz	**1.3** GB	0%
SQ_051_HMJ7NDRXY_ACGTTCCTTA-CACAGCGGTC_L001_R1.fastq.gz	**955.6** MB	0%
SQ_052_HMJ7NDRXY_CCGCGTATAG-CCGGTTCCTA_L001_R1.fastq.gz	**1.1** GB	0%
SQ_053_HMJ7NDRXY_GATTCTGAAT-GTCCACCGCT_L001_R1.fastq.gz	**1.1** GB	0%
SQ_054_HMJ7NDRXY_TAGAGAATAC-CGAATCTATA_L001_R1.fastq.gz	**1** GB	0%

Collection Type: List **File Type:** Auto-detect **Genome (set all):** unspecified (?)

Figure 2.4: Click ‘Start’ button to begin uploading files. Keep this website open until it is finished.

2.1.2 Add files to a ‘collection’

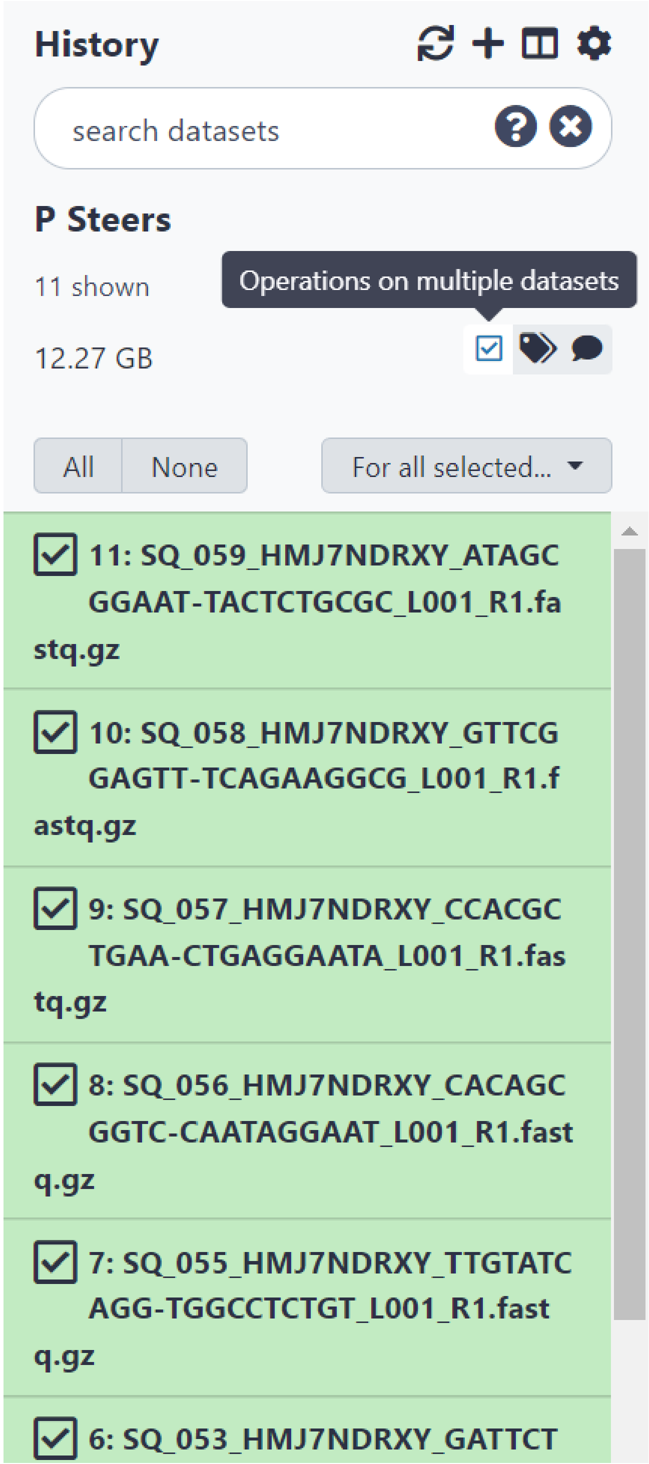
Once the files have uploaded they will appear in the History pane. Next, we add them to a ‘collection’, which is basically just a list of files. It allows all files to be parsed through a workflow one by one. For example, if a tool was used on a collection, then each item in the list/collection would invoke its own job while all output is kept within a collection in the history.

2.2 Rename Files

This step renames the files to more meaningful and user friendly names.

To do this, a new file is imported with the old names and the new names. This should be a tab delimited .txt file with 2 columns of data.

The 1st column has original name (which is likely to be filename) and the 2nd column has new names. E.g. original filename might be `SQ_049_HMJ7NDRXY_TATGATGGCC-TATGACAATC_L001_R1.fastq.gz` whereas new name could be changed to include treatment information such as `ST_811_P5_L001`. It is important that the ”_L” number is included at the end, even if there is not multiple lanes per sample. This will be dealt with by the “Step 1” workflow. See section 2.2.



P Steers

11 shown

12.27 GB

☑ 11: SQ_059_HMJ7NDRXY_ATAGCGGAAT-TACTCTGCGC_L001_R1.fastq.gz

☑ 10: SQ_058_HMJ7NDRXY_GTTCGGAGTT-TCAGAAGGCG_L001_R1.fastq.gz

☑ 9: SQ_057_HMJ7NDRXY_CCACGCTGAA-CTGAGGAATA_L001_R1.fastq.gz

☑ 8: SQ_056_HMJ7NDRXY_CACAGCGGTC-CAATAGGAAT_L001_R1.fastq.gz

☑ 7: SQ_055_HMJ7NDRXY_TTGTATCAGG-TGGCCTCTGT_L001_R1.fastq.gz

☑ 6: SQ_053_HMJ7NDRXY_GATTCT

Figure 2.5: Select ‘Operations on multiple datasets’ then select all items to add to collection

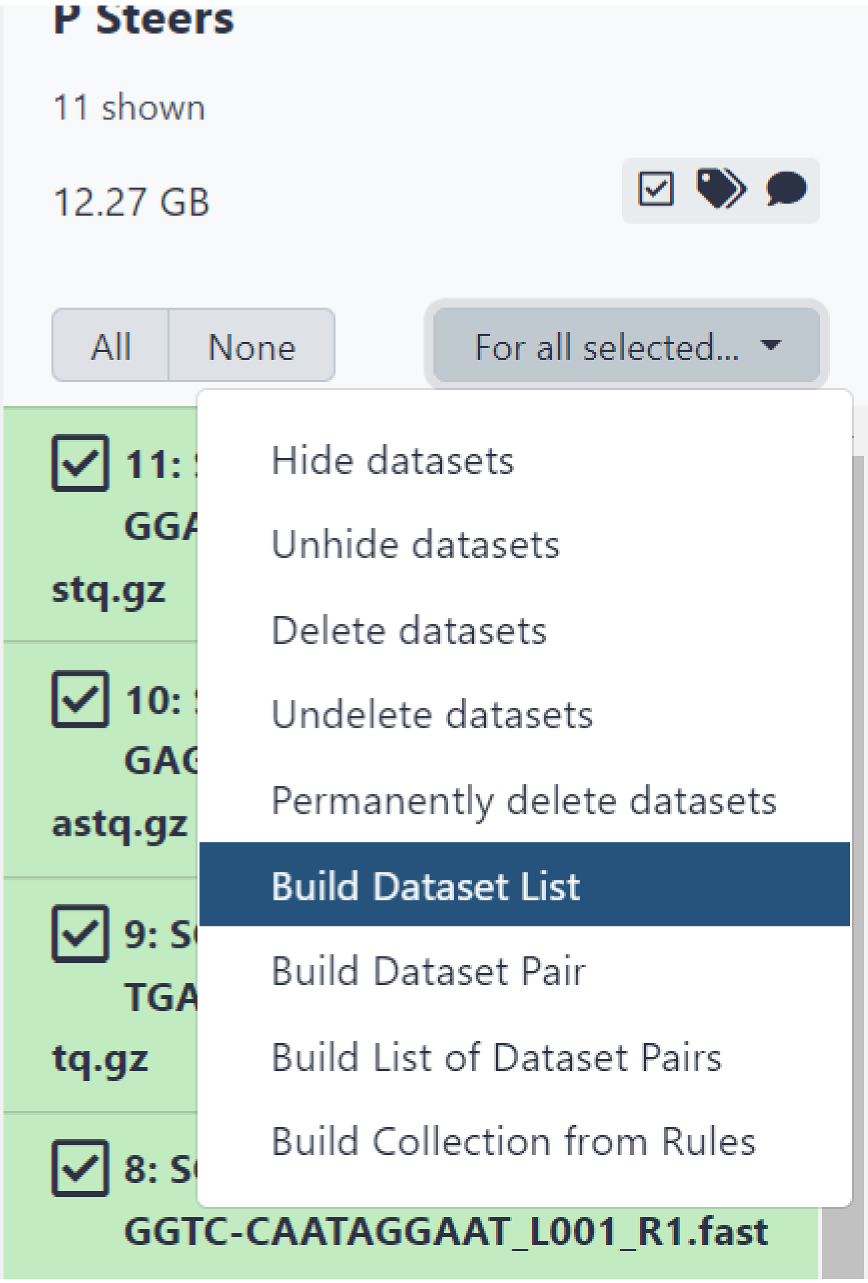
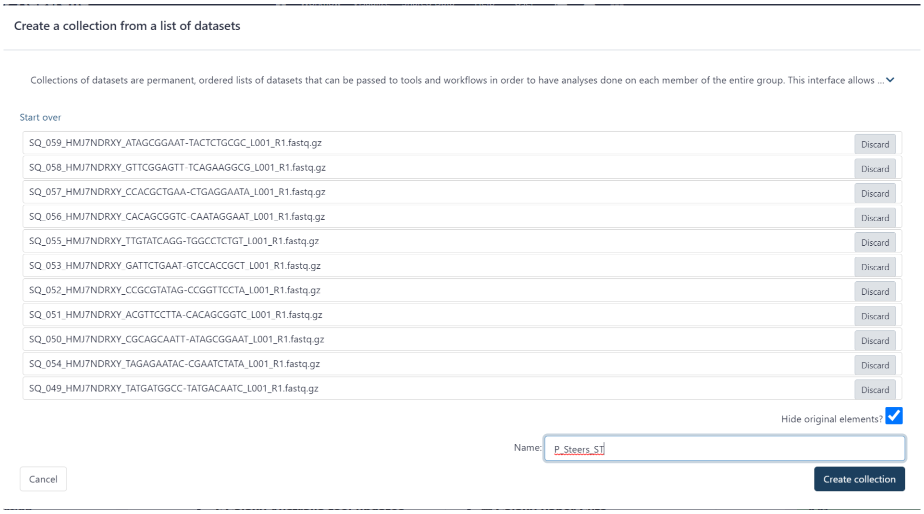


Figure 2.6: Click ‘For all selected’ then ’Build dataset list’



Create a collection from a list of datasets

Collections of datasets are permanent, ordered lists of datasets that can be passed to tools and workflows in order to have analyses done on each member of the entire group. This interface allows ...

SQ_059_HMJ7NDRXY_ATAGCGGAAT-TACTCTGCGC_L001_R1.fastq.gz	Discard
SQ_058_HMJ7NDRXY_GTTCGGAGTT-TCAGAAGGCG_L001_R1.fastq.gz	Discard
SQ_057_HMJ7NDRXY_CCACGCTGAA-CTGAGGAATA_L001_R1.fastq.gz	Discard
SQ_056_HMJ7NDRXY_CACAGCGGTC-CAATAGGAAT_L001_R1.fastq.gz	Discard
SQ_055_HMJ7NDRXY_TTGTATCAGG-TGGCCTCTGT_L001_R1.fastq.gz	Discard
SQ_053_HMJ7NDRXY_GATTCTGAAT-GTCCACCGCT_L001_R1.fastq.gz	Discard
SQ_052_HMJ7NDRXY_CCGCGTATAG-CCGGTTCCTA_L001_R1.fastq.gz	Discard
SQ_051_HMJ7NDRXY_ACGTTCCTTA-CACAGCGGTC_L001_R1.fastq.gz	Discard
SQ_050_HMJ7NDRXY_CGCAGCAATT-ATAGCGGAAT_L001_R1.fastq.gz	Discard
SQ_054_HMJ7NDRXY_TAGAGAATAC-CGAATCTATA_L001_R1.fastq.gz	Discard
SQ_049_HMJ7NDRXY_TATGATGGCC-TATGACAATC_L001_R1.fastq.gz	Discard

Hide original elements? ☑

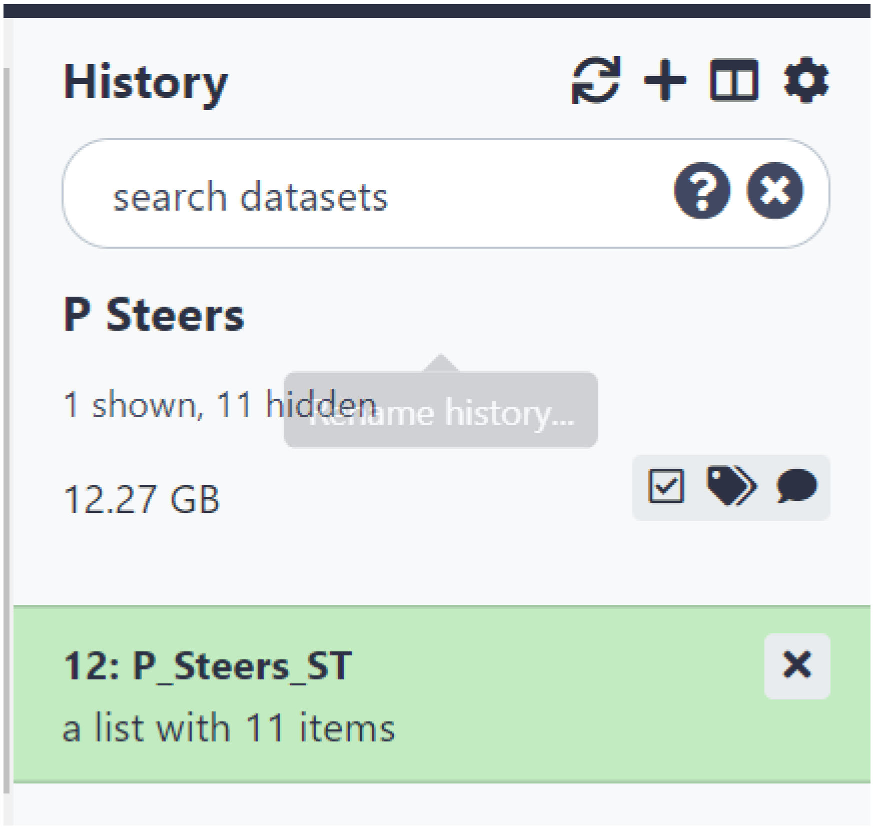
Name: P_Steers_ST

Figure 2.7: Type a name for the list and click ‘Create collection’ button

Use the ‘upload data’ feature of galaxy to import the .txt file to to the History. It should look something like 2.9.

This .txt file can be made in Excel, or it can be done much quicker using a simple R script. The following is an example script:

```
# list file names and create table with new file names for use in galaxy's "Relabel li
library(tidyverse)
library(data.table)

#list the names of the fastq files in the working directory
file_list <- list.files(path = "E:/RNAseq - P steers ST 20211212/", pattern = ".fastq"

#list .csv files to select the .csv file that contains sample information
files_csv <- list.files(path = "E:/RNAseq - P steers ST 20211212/", pattern = ".csv")

#selects the correct .csv file, in this case there is only 1 file anyway, but the corr
selected_file <- files_csv[1]
message(paste("Selected file -", selected_file))

#import the selected file, making sure no columns have the same name
sample_IDs <- fread(file = paste0("E:/RNAseq - P steers ST 20211212/",selected_file)) %
  as_tibble(.name_repair = "unique") %>%
  mutate(`Steer ID` = as.character(`Steer ID`))


#As the file names have a set structure, it can be split by the _ character
```



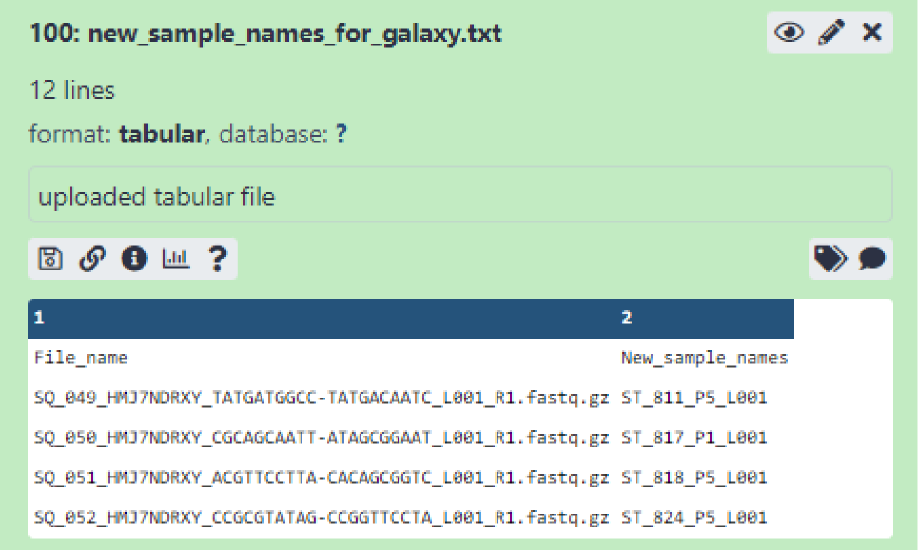
P Steers

1 shown, 11 hidden

12.27 GB

12: P_Steers_ST
a list with 11 items

Figure 2.8: All files should now be in a collection in the History pane.



100: new_sample_names_for_galaxy.txt

12 lines

format: **tabular**, database: **?**

uploaded tabular file

1	2
File_name	New_sample_names
SQ_049_HMJ7NDRXY_TATGATGGCC-TATGACAATC_L001_R1.fastq.gz	ST_811_P5_L001
SQ_050_HMJ7NDRXY_CGCAGCAATT-ATAGCGGAAT_L001_R1.fastq.gz	ST_817_P1_L001
SQ_051_HMJ7NDRXY_ACGTTCCTTA-CACAGCGGTC_L001_R1.fastq.gz	ST_818_P5_L001
SQ_052_HMJ7NDRXY_CCGCGTATAG-CCGGTTCCTA_L001_R1.fastq.gz	ST_824_P5_L001

Figure 2.9: Example of text file imported to History with original and new filenames

```
df_file_names_split <- str_split(file_list, pattern = "_", simplify = TRUE) %>% as.data

#In this example, the 'key' column is the RNAseq_ID column. So this will be modified t
sample_IDs <-
  sample_IDs %>%
  mutate(RNAseq_ID_2 = str_remove(RNAseq_ID, "SQ_"),
         Treatment2 = str_remove(Treatment, '-'))

#join the sampleID annotations to this dataframe, concat required columns for new name
new_names <- df_file_names_split %>%
  left_join(sample_IDs, by = c("V2" = "RNAseq_ID_2")) %>%
  mutate(new_sample_names = str_c(`Tissue type`,`Steer ID`, Treatment2, V5, sep = "_")
  magrittr::use_series(new_sample_names) #select only the required column as a list (n

#create new table with only old file names and new names
new_name_table <-
  data.frame("File_name" = file_list, "New_sample_names" = new_names)

#export to working directory, as tab delim
fwrite(new_name_table, file = "E:/RNAseq - P steers ST 20211212/new_sample_names_for_ga
```

A note on multiple lanes

The output from Illumina sequencing is sometimes provided in multiple files, each corresponding to a 'Lane' on the sequencer. It would be easier to ask the lab to provide the output as a single file, which can be computed using the `--no-lane-splitting` option from Illumina's `bcl2fastq` program. However, it can also be handled in Galaxy. If there are multiple files, it is best practice to run FastQC on each individual file, as there is a chance that one file could be corrupt or you may identify a bias for one particular 'Lane'. If they are ok, then these files can be concatenated together before proceeding with all further steps.

This is described further in _________. Make sure the new names generated in 2.2 have a format that includes "_L". The protocol relies on there being a '_L' in the name for it to find the lane number. The rest of the name before the '_L' should be the same.

E.g. the following three files would be concatenated together by the "Step 1" workflow:

- 2139_Stage 2_Fast_L008
- 2139_Stage 2_Fast_L007
- 2139_Stage 2_Fast_L006

2.3 Import ENSEMBL files

In this step we need to import the required fastq file for sequence alignment and a gtf file for gene annotation. These can be uploaded directly to Galaxy via a URL. To find the required files, navigate to http://ftp.ensembl.org in a browser.

For sheep (ovis_aries) these might be:

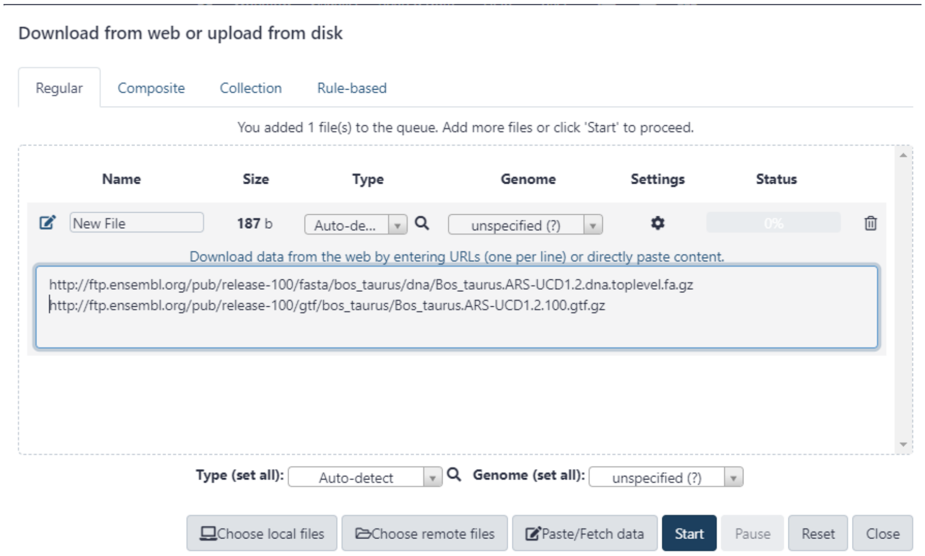
- http://ftp.ensembl.org/pub/release-100/fasta/ovis_aries/dna/Ovis_aries.Oar_v3.1.dna.toplevel.fa.gz
- http://ftp.ensembl.org/pub/release-100/gtf/ovis_aries/Ovis_aries.Oar_v3.1.100.gtf.gz

For cattle (bos_taurus) these might be:

- http://ftp.ensembl.org/pub/release-100/fasta/bos_taurus/dna/Bos_taurus.ARS-UCD1.2.dna.toplevel.fa.gz
- http://ftp.ensembl.org/pub/release-100/gtf/bos_taurus/Bos_taurus.ARS-UCD1.2.100.gtf.gz

Note that unmasked files are used here (i.e. use files without `_rm` or `_sm`).

To upload these to Galaxy, use the 'Paste/Fetch data' button in the 'Upload data' dialogue box on Galaxy.



Download from web or upload from disk

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

Name	Size	Type	Genome	Settings	Status
New File	187 b	Auto-de...	unspecified (?)	⚙	0%

Download data from the web by entering URLs (one per line) or directly paste content.

```
http://ftp.ensembl.org/pub/release-100/fasta/bos_taurus/dna/Bos_taurus.ARS-UCD1.2.dna.toplevel.fa.gz
http://ftp.ensembl.org/pub/release-100/gtf/bos_taurus/Bos_taurus.ARS-UCD1.2.100.gtf.gz
```

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

Figure 2.10: Paste each URL on a new line to upload directly from ENSEMBL to Galaxy.

Once executed, 2 new files will appear in the History, each named as the URL entered. This can take some time to finish as they are large files.

2.3.1 Uncompress .gtf file

These files are actually .gz files, which means they are compressed. Normally, this is automatically handled by Galaxy but does not currently work for the .gtf file when using it with the `STAR Aligner` in this workflow. Therefore, use the tool https://usegalaxy.org.au/root?tool_id=CONVERTER_gz_to_uncompressed to uncompress the .gtf file before proceeding.

Chapter 3

Mutliple Lane files

The output from Illumina sequencing is sometimes provided in multiple files, each corresponding to a 'Lane' on the sequencer. It would be easier to ask the lab to provide the output as a single file, which can be computed using the `--no-lane-splitting` option from Illumina's `bcl2fastq` program. However, it can also be handled in Galaxy.

If there are multiple files, it is best practice to run FastQC on each individual file, as there is a chance that one file could be corrupt or you may identify a bias for one particular 'Lane'. If they are ok, then these files can be concatenated together before proceeding with all further steps. There are 2 workflows that will be used in Galaxy, with the first designed to work with each individual lane file and the second requiring 1 file per sample. This can be done by following these steps:

1. 'Apply Rule to Collection' tool
2. Input collection is the list with the re-labelled data
3. Press Edit button
4. There should be 1 column titled 'A' with the names of the files (the re-labelled names set previously)

Make new columns with grouping information:

5. Press +Column -> Using a regular expression
6. Select 'Create column matching expression groups'
7. Paste the following code into the Regular Expression box: `(.*?)_L(.*)`
8. Set the number of groups to 2
9. Press Apply

Set columns as identifiers for grouping:

10. Press +Rules -> Add / Modify Column Definitions
11. Press +Add Definition -> List Identifiers
12. Select column B, then click on ‘Assign another column’ and select C
13. Press Apply
14. Press Save, then Execute the job

This outputs a nested list to the history. The number of items in the list should match the number of samples/animals, and then each sample in the list should contain the number of individual files (e.g. 2 or 3 files).

To then join this datasets together, the tool ‘Collapse Collection’ will work in the background to be the same as using the ‘concatenate datasets tail-to-head’ tool to concatenate the files individually. Although not clear in the tool’s description, if it is provided a nested list, it will collapse the lowest level of groups together, in this case it is the Column C from above, which was the individual lanes. It should output a new list with the same number of files as the number of samples/animals. The names should be the names defined in Column B above: e.g. 2139_Stage 2_Fast:

1. Open Collapse Collection tool
2. Choose to use a dataset collection, not an individual file
3. Select the nested list from above
4. Execute with all other default settings