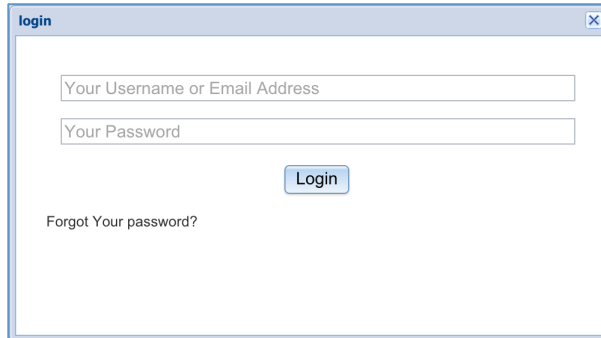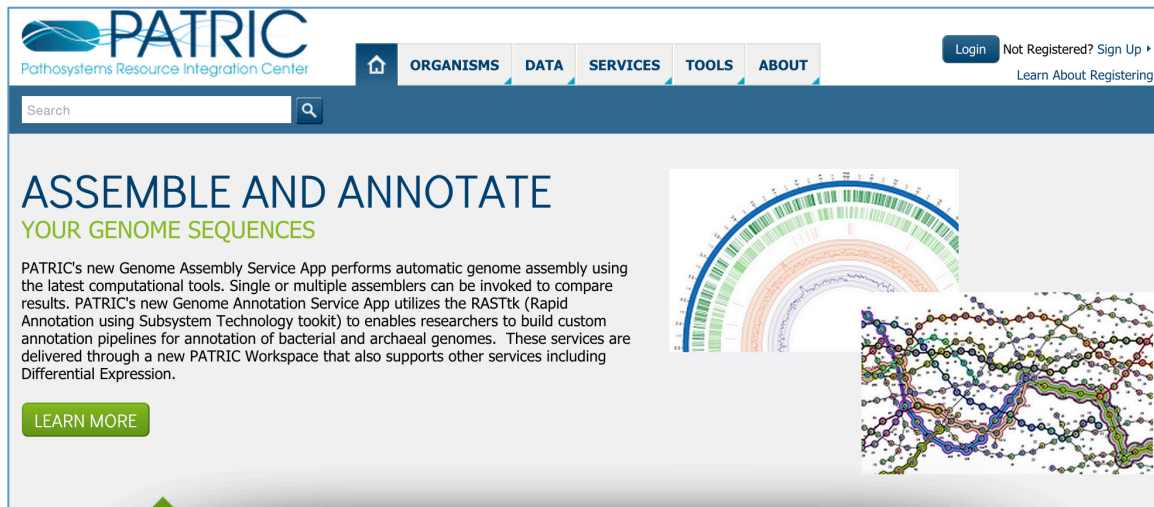# RNA-Seq Analysis at PATRIC

The following step-by-step workflow is intended to help you learn how to use the RNA-Seq Analysis Service at PATRIC

1. **Go to the PATRIC website and log in to your user account.**
   - https://www.patricbrc.org/

2. **Download sample RNA-Seq raw data files from the PATRIC FTP site**
   - Go to ftp://ftp.patricbrc.org/workshop/RNAseq_a_baumannii_subsample/
   - Download six sample read files (*.fq.gz) from an Acinetobacter baumannii RNA-Seq experiment to your desktop.

Index of /workshop/RNAseq_a_baumannii_subsample/

Name	Size	Date Modified
[parent directory]		
COL_75_R1.fq.gz	40.9 MB	6/15/15, 4:23:00 PM
COL_75_R2.fq.gz	40.1 MB	6/15/15, 4:25:00 PM
MERO_75_R1.fq.gz	41.7 MB	6/12/15, 6:25:00 AM
MERO_75_R2.fq.gz	41.0 MB	6/12/15, 6:25:00 AM
MHB_R1.fq.gz	36.6 MB	6/12/15, 6:25:00 AM
MHB_R2.fq.gz	36.6 MB	6/12/15, 6:25:00 AM
ref_genome_1310581.3.txt	42 B	6/12/15, 6:25:00 AM

3. Upload Acinetobacter baumannii RNA-Seq read files to your private workspace.

- Go to your workspace home by clicking on "WORKSPACE HOME" on the top right corner.

Welcome mshukla
Not mshukla? Logout »
My Account »

WORKSPACE: HOME ▸
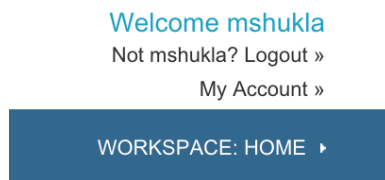
- Create a new folder called RNA-Seq by clicking on ADD FOLDER icon on the top right corner and typing the folder name "RNA-Seq" in the popup box.

UPLOAD   ADD FOLDER

Create Folder   x

RNA-Seq

Cancel   Create Folder
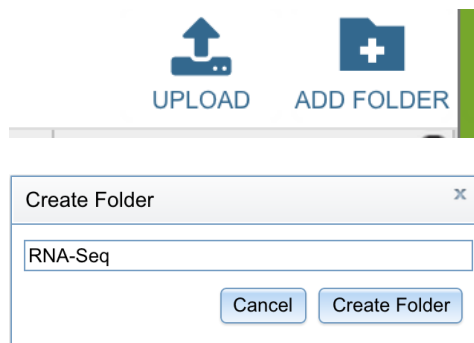
- Now, navigate to the RNA-Seq folder by clicking on the folder name.

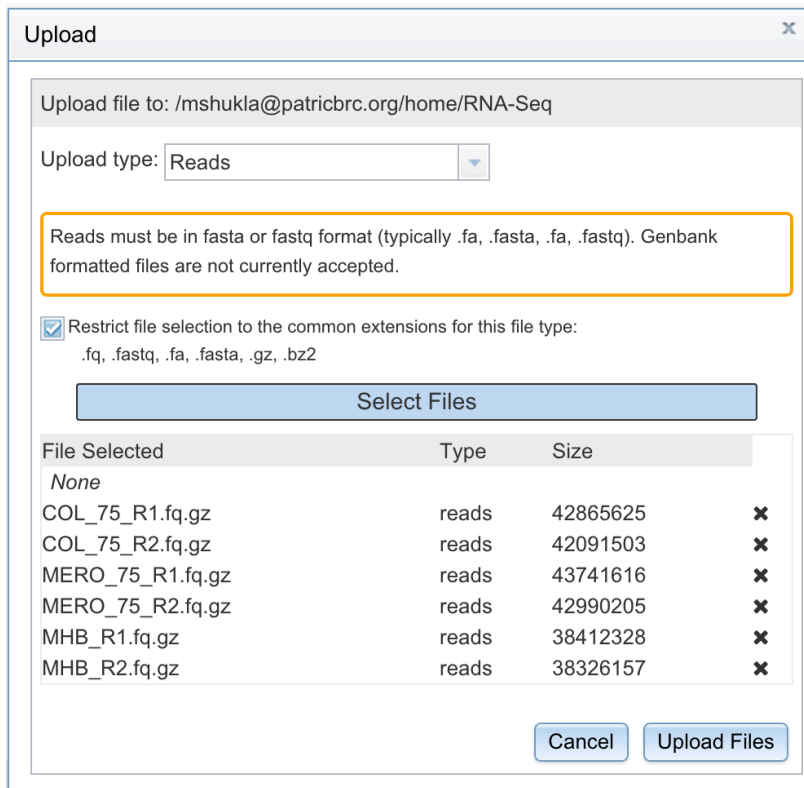RNA-Seq   mshukla   6/15/15, 12:50 PM

- Now, lick on the UPLOAD icon in the top right corner.

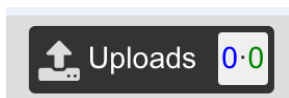- In the file uploader popup window, select file type as "Reads.
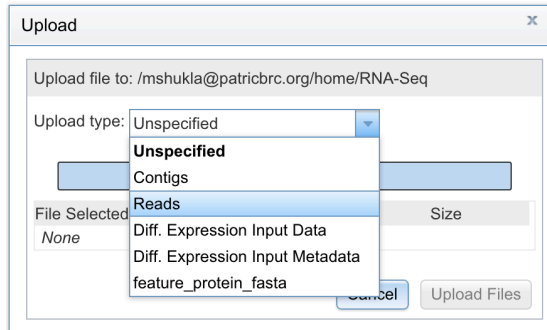
- Now, click on "Select Files", select the six RNA-Seq read files from your computer and click Upload Files button.

- Check the progress of file uploads in bottom right corner.

- When the file upload is complete, you should see all six files loaded in your RNA-Seq folder. Check the size of each file to make sure the file it loaded correctly. If the file size is zero, delete the file and try to upload it again.

	Name	Size	Owner	Created
⬆	Parent Folder			
📄	COL_75_R1.fq.gz	42.9 MB	mshukla	6/15/15, 12:53 PM
📄	COL_75_R2.fq.gz	42.1 MB	mshukla	6/15/15, 12:53 PM
📄	MERO_75_R1.fq.gz	43.7 MB	mshukla	6/15/15, 1:01 PM
📄	MERO_75_R2.fq.gz	43.0 MB	mshukla	6/15/15, 12:53 PM
📄	MHB_R1.fq.gz	38.4 MB	mshukla	6/15/15, 12:53 PM
📄	MHB_R2.fq.gz	38.3 MB	mshukla	6/15/15, 12:53 PM

4. **Submit a job to RNA-Seq Analysis Service.**

- From the top navigation bar, under Services, select RNA-Seq.
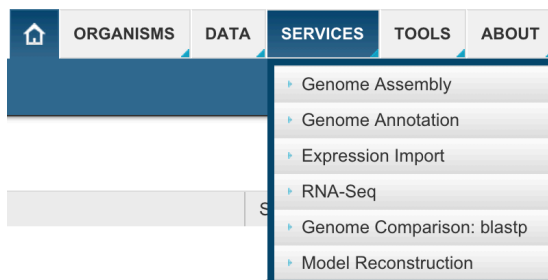
- It will take you to the submission form for the RNA-Seq Analysis service.

RNA-Seq Analysis
Align reads, assemble transcripts, measure/test expression.

Parameters ⓘ

STRATEGY
Rockhopper ▾

Target Genome
▼ e.g. Mycobacterium tuberculosis H37Rv ▾

OUTPUT FOLDER
▾ 📁

OUTPUT NAME
Output Name

Groups/Conditions ⓘ

◯ OFF
Condition Name ⊕

Paired read library ⓘ ⊕

READ FILE 1
▾ 📁

READ FILE 2
▾ 📁

CONDITION
Condition Name ▾

Single read library ⊕

READ FILE
▾ 📁

CONDITION
Condition Name ▾

Selected libraries ⓘ
Place read files here using the arrow buttons.

Reset   Submit

- Select strategy for performing RNA-Seq analysis. There are two different strategies available at this point: Rockhopper and Tuxedo. For the purpose of this tutorial, select Rockhopper.

STRATEGY
Rockhopper ▾

- Select a Target Genome that you want to user to align RNA-Seq reads. For this example, type and select Acinetobacter baumanni AYE genome.

Target Genome
▼ Acinetobacter baumannii AYE ▾

Acinetobacter baumannii AYE VEB [509173.8]
Acinetobacter baumannii UH8907 [1398988.3]
Acinetobacter baumannii UH9907 [1398991.3]
Acinetobacter baumannii UH9007 [1398989.3]
Acinetobacter baumannii SDF [509170.6]

- Now select output folder "RNA-Seq "from the drop down. Provide output name, say, Acinetobacter Baumannii AR.

- Now, using the Groups / Conditions panel, define conditions represented in the given RNA-Seq experiment. For our experiment, there are three conditions: MHB (Mueller Hilton Broth, ni treatment), MERO (Meropenem treatment), and COL (Polymyxin-B treatment).

- Now, upload paired-end read libraries. Click on the dropdown menu for read file 1 and read file 2. You should see the read files you uploaded earlier listed in the dropdown menu. Select the paired read files for one of the conditions, say, MHB. Also, select corresponding condition from the dropdown menu. Then, click on the arrow at the top right corner, to add the files to Selected libraries panel.

- Using the same procedure above, add paired libraries for the two AR treatments.

- At this point, we have filled out all information necessary to submit the job. Click the Submit button.

- Notice the green bar that indicates the job has been added to the queue.

- You can review the progress and status of the job by clicking on the Jobs panel in the bottom right corner.

- It takes you to the job list, where you can see all the jobs you have submitted and their status. The RNA-Seq job you just submitted should be listed at the top. The page will be refreshed automatically every few seconds to show the latest status of the job.

Status	Submit	App	Output Name
queued	6/15/15, 1:35 PM	RNASeq	Acinetobacter baumannii AR

- When the job starts being processed, its status will change to "In-progress".

| in-progress | 6/15/15, 7:17 AM |

- When the job is complete, its status will change to "Complete".

| completed | 6/15/15, 1:35 PM |

5. View/download the RNA-Seq Analysis Results.

- Click on the completed job, which will take you to the job result page.
- Here, you can see the details of programs and parameters used to process the job.

mshukla / home / RNA-Seq / Acinetobacter baumannii AR

Job Result

Start time	6/15/15, 2:14 PM
End time	6/15/15, 2:18 PM
Run time	4m35s
Parameters	{"single_end_libs":[],"experimental_conditions":["MHB","COL","MERO"],"strand_specific":"1","output_file":"Acinetobacter baumannii AR","output_path":"/mshukla@patricbrc.org/home/RNA-Seq","paired_end_libs":[{"read2":"/scratch/tmp/RsbrhSK8eL/MHB_R2.fq.gz","read1":"/scratch/tmp/RsbrhSK8eL/MHB_R1.fq.gz","condition":1}, {"read2":"/scratch/tmp/RsbrhSK8eL/COL_75_R2.fq.gz","read1":"/scratch/tmp/RsbrhSK8eL/COL_75_R1.fq.gz","condition":2}, {"read2":"/scratch/tmp/RsbrhSK8eL/MERO_75_R2.fq.gz","read1":"/scratch/tmp/RsbrhSK8eL/MERO_75_R1.fq.gz","condition":3}],"reference_genome_id":"509173.8","recipe":"Rockhopper"}

- This page also provides all the results and output files generated by the RNA-Seq Analysis service.

Result Files

	Filename	Type	File size
⬇	Rockhopper_509173.8_operons.txt	txt	61.8 kB
⬇	Rockhopper_509173.8_transcripts.txt	txt	500.1 kB
⬇	Rockhopper_COL_75.bam	bam	98.9 MB
⬇	Rockhopper_MERO_75.bam	bam	100.2 MB
⬇	Rockhopper_MHB.bam	bam	89.5 MB
⬇	Rockhopper_summary.txt	txt	11.9 kB
⬇	Rockhopper_509173.8_gene_exp.gmx	diffexp_input_data	154.8 kB

- You can download a result file by clicking on the download icon in front of the file name. Download the differential expression input data file.

⬇	Rockhopper_509173.8_gene_exp.gmx	diffexp_input_data	154.8 kB

- You can open the file using any text utility application to view its contents. Note that file shows differential expression of genes in three different comparisons: COL|MHB, MERO|MHB, and MERO|COL.

```
Gene ID COL|MHB MERO|MHB      MERO|COL
fig|509173.8.peg.1       0.000    0.000    0.000
fig|509173.8.peg.2       0.000    0.000    0.000
fig|509173.8.peg.3       0.474    0.903    0.429
fig|509173.8.peg.4       0.000    0.000    0.000
fig|509173.8.peg.5       0.000    0.000    0.000
fig|509173.8.peg.6       0.000    0.000    0.000
fig|509173.8.peg.7       6.644    0.000   −6.644
fig|509173.8.peg.8       0.511    0.350   −0.160
fig|509173.8.peg.9       0.000    0.000    0.000
fig|509173.8.peg.10      1.308    0.441   −0.868
fig|509173.8.peg.11      1.939    0.918   −1.021
fig|509173.8.peg.12      0.838    0.105   −0.733
fig|509173.8.peg.13      1.669    0.561   −1.108
fig|509173.8.peg.14      0.000    0.000    0.000
fig|509173.8.peg.15      0.000    0.000    0.000
fig|509173.8.peg.16      0.000    0.000    0.000
fig|509173.8.peg.17      0.000    0.000    0.000
fig|509173.8.peg.18      0.000    0.000    0.000
```