

Interpreting RNAseq Mapping results (Part 2: Loading data generated by the pathogen portal's RNAseq pipeline in the Genome Browser) Exercise 5

For this exercise we will be using:

<http://pathogenportal.org>
<http://microsporidiadb.org>

1. Explore the results of the RNA-sequence pipeline. What files were generated? To view contents of any of the results, click on the eye icon () next to the file name.

!!! important note – do not click on the icon next to the file called “Tophat2 on data 1 and data 3: accepted_hits” – this file is huge and will not display but rather will download the contents to your computer.

TopHat generates four files: insertions, deletions, splice junctions and accepted hits. The accepted hits file is the BAM file (binary alignment map). Note that many alignment programs will generate a file called a SAM file (sequence alignment map) which is a table including text of the alignment and mapping. However, for viewing results in a sequence browser like GBrowse, the file needs to be converted into the binary formatted (BAM) – you do not have to worry about this for this exercise.

Cufflinks generates three files: gene expression, transcript expression and assembled transcripts. The gene expression and transcript expression files for our purposes should be identical since EuPathDB genomes do not have separate genes and transcripts. These files include the FPKM values for each gene in the genome analyzed – in this case *Encephalitozoon cuniculi* ECII.

2. Share your accepted hits files. Click on the drop down menu for your project and select the option “share or publish”.

10: Cufflinks Eukaryotic on data 7: assembled transcripts			
9: Cufflinks Eukaryotic on data 7: transcript expression			
8: Cufflinks Eukaryotic on data 7: gene expression			
7: Tophat2 on data 1 and data 3: accepted hits			
6: Tophat2 on data 1 and data 3: splice junctions			
5: Tophat2 on data 1 and data 3: deletions			
4: Tophat2 on data 1 and data 3: insertions			
3: ftp://ftp.ddbj.nig.ac.jp/ddbj_databases/dra/fastq/SRA061/SRA061150/SRX229331/SRR769606_1.fastq			
1: ftp://ftp.ddbj.nig.ac.jp/ddbj_databases/dra/fastq/SRA061/SRA061150/SRX229331/SRR769606_2.fastq			

	9	0 Tags	3.0 GB	4 days ago	4 days ago	current project
Switch						
View		0 Tags	0 bytes	4 days ago	4 days ago	
Share or Publish						
Rename	2	0 Tags	2.0 GB	4 days ago	4 days ago	
Delete		0 Tags	0 bytes	6 days ago	6 days ago	
<input type="checkbox"/> Babesia_bovis	8	0 Tags Accessible, Published	853.0 MB	Jun 15, 2012	May 12, 2013	
For 0 selected projects:		<input type="button" value="Rename"/>	<input type="button" value="Delete"/>			

On the next page select the option: Make History Accessible and Publish



Once your project is published other people can access it by going to "Published Projects" section under the Shared data menu option in the Galaxy menu bar.



3. Load your BAM data into GBrowse. Click on your "Tophat2 on data 2 and data 1: accepted_hits" in your project history panel. This will show you information about the file including a link to display data in microsporidiadb – click on the link.

4. Load the assembled transcript data. Essentially use a similar procedure as above.



