WoPPER Tutorial 1

Acinetobacter baumannii

(23/03/2017)

Introduction

This tutorial will show you the analysis of an RNA-seq experiment on *Acinetobacter baumannii*, an organism with one chromosome using the NCBI annotation of the genome, using the separated strand analysis option.

For performing this tutorial you will need to download the GED file for this specific experiment from the "**Tutorials**" section of WoPPER.

Tutorials

#	Descriptions	Organism	Type	# Chr	Annotation	Separated Strands	GED Files
1		*Acinetobacter baumannii*	RNA-Seq	1	NCBI	Yes	
2		*Salmonella enterica*	RNA-Seq	1	NCBI	No	
3		*Burkholderia thailandensis*	RNA-Seq	2	NCBI	Yes	
4		*Escherichia coli*	Microarray	1	NCBI	No	
5		*Helicobacter pylori*	RNA-Seq	1	Custom	Yes	

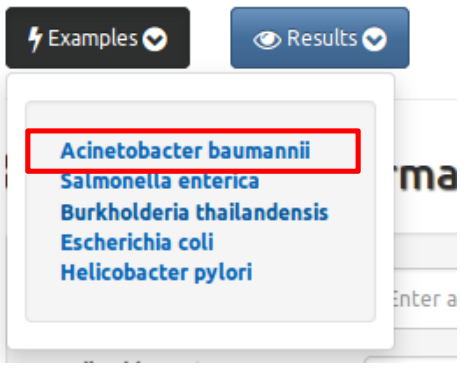
The file should be named: "**GEDfile_RNAseq_StrandSpec_Dataset_Acinetobacter-baumanii-ATCC17978.txt**".

An example on how the Input form should appear once completely filled in with the necessary files and parameters can be activated:

1. Clicking on the corresponding radio-button in "Examples" column of the "Tutorials" page

#	Descriptions	Organism	Type	# Chr	Annotation	Separated Strands	GED Files	Results	Examples
1		*Acinetobacter baumannii*	RNA-Seq	1	NCBI	Yes			

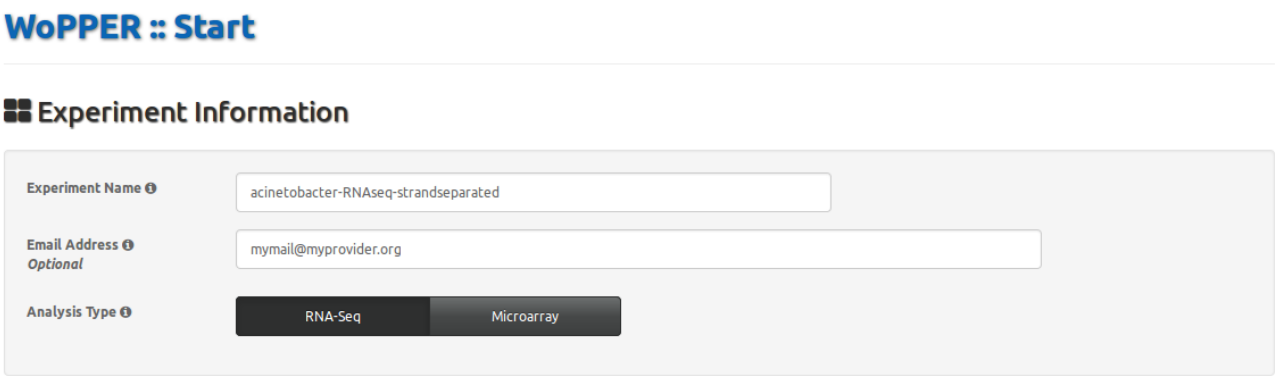
2. Selecting "Acinetobacter baumannii" from the "Examples" drop-down menu available at the top of WoPPER "Start" page



Step-by-step procedure

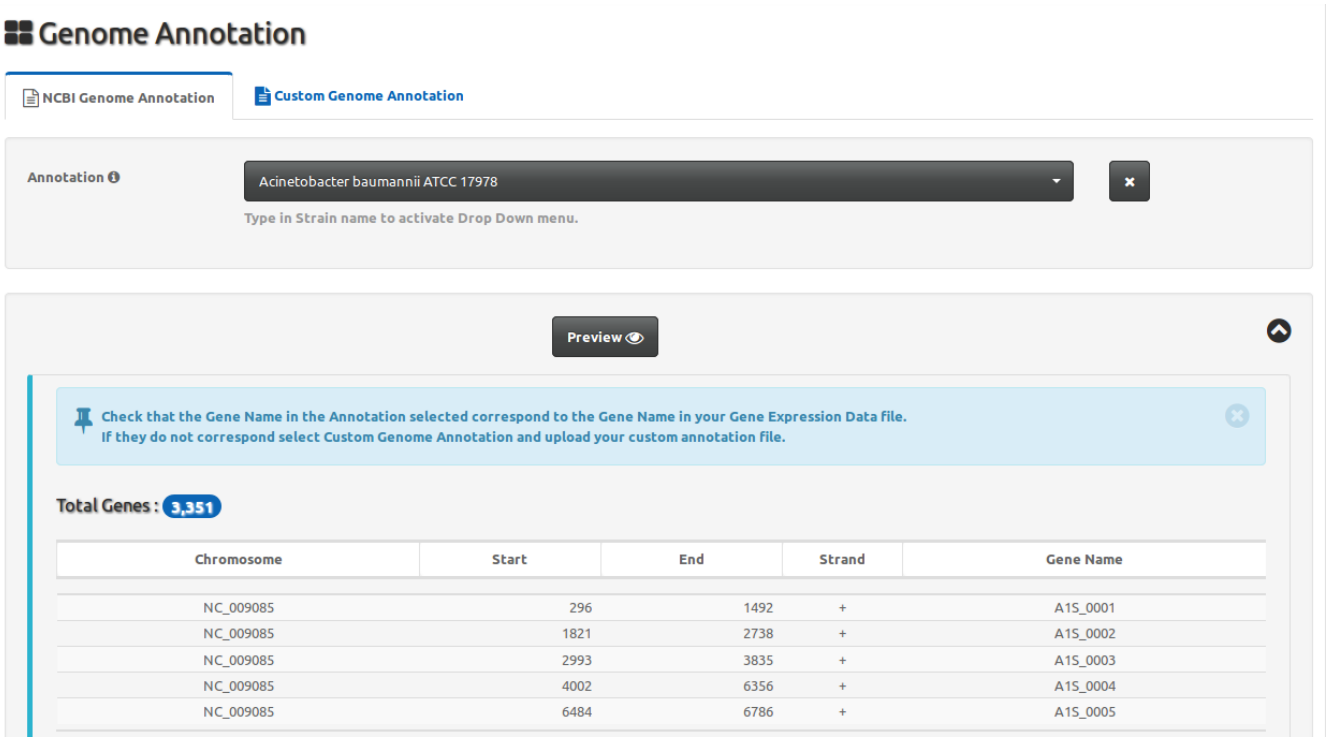
Step 1: Insert Experiment information

- Fill in the "Experiment Name" field with a suitable name for your experiment
- Fill in the "Email address" with your preferred email address. An email pointing to the results page for your experiment will be sent to this address
- Select RNA-seq as the Analysis Type



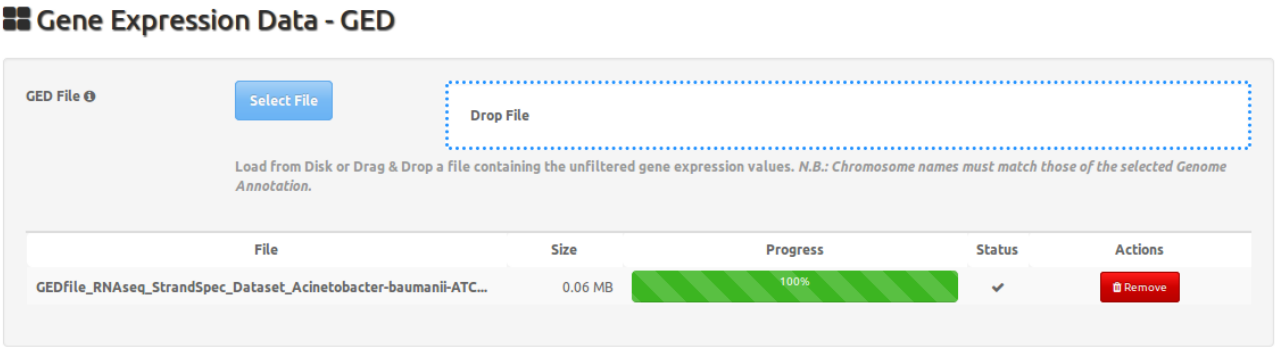
Step 2: Select Genome Annotation

- Select the "NCBI Genome Annotation" tab
- Type in "Acineto" in then web form to start the auto-fill based on a search on the internal database of bacterial genomes
- From the drop-down menu, select "Acinetobacter baumannii ATCC 17978" (please, make sure to select the main genome and not those annotated as "plasmid pAB1" or "plasmid pAB2")
- Click on the "Preview" button to see the selected genome annotation

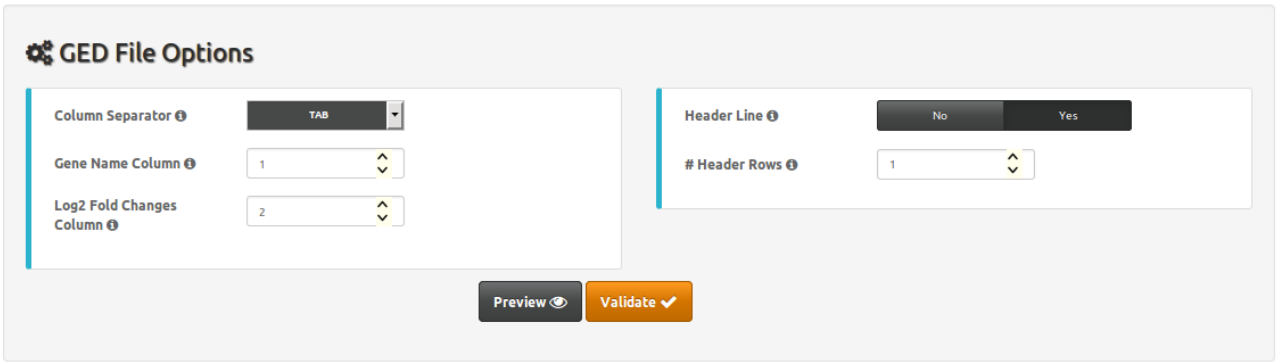


Step 3: Load Gene Expression Data

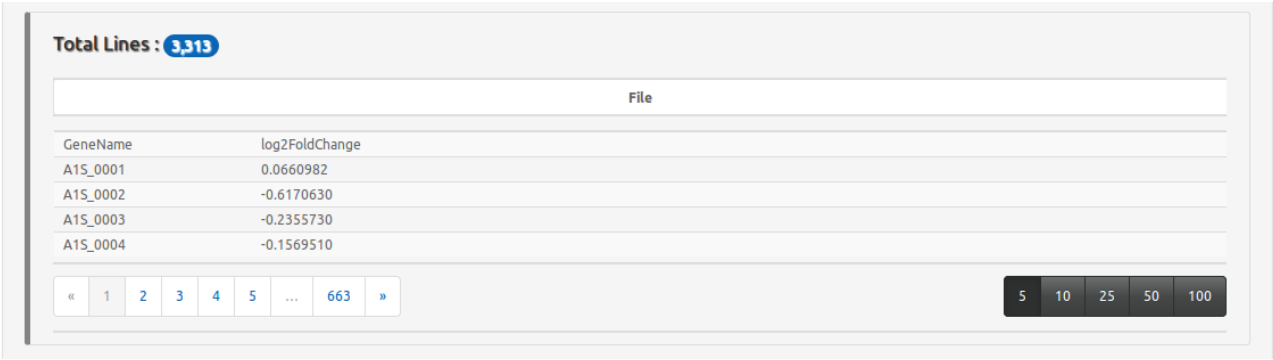
- Click on the "Select file" button and load the file named "GEDfile_RNAseq_StrandSpec_Dataset_Acinetobacter-baumanii-ATCC17978.txt" (alternatively, you can drag and drop the same file into the area named "Drop File")



- In the "GED File Options" section, select the values as in the reported screenshot



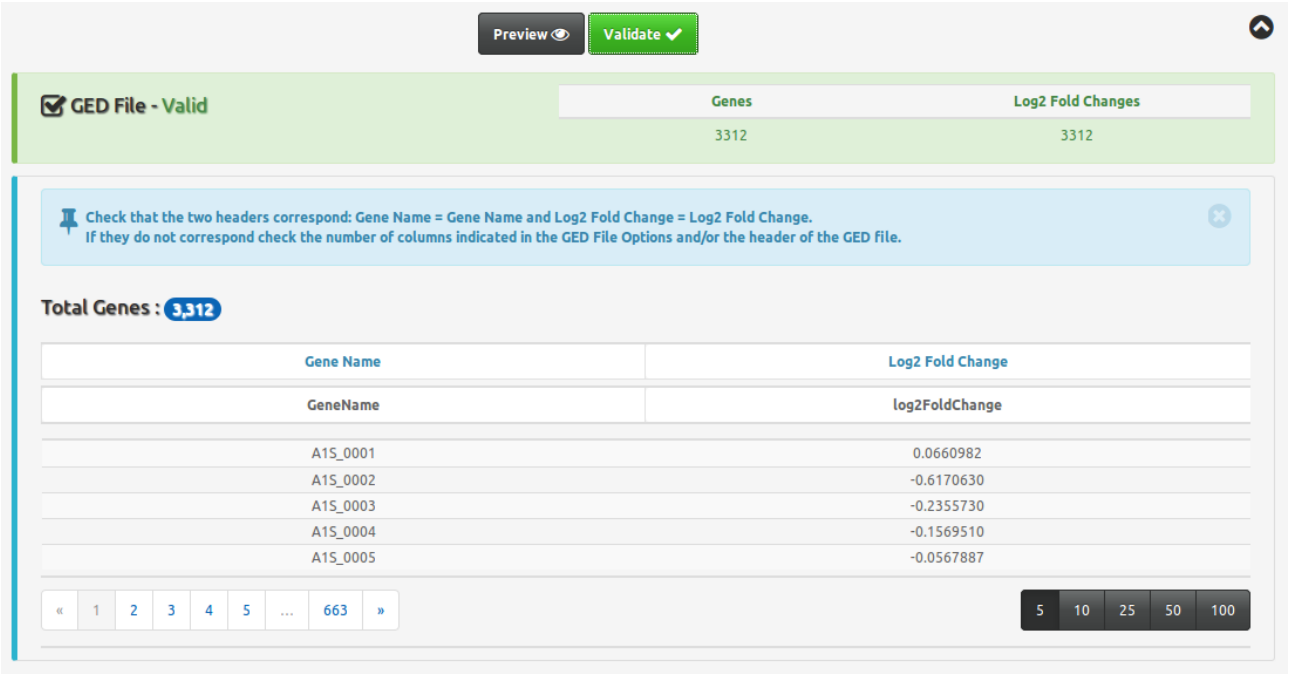
- Click on “Preview” button to check the GED file content and columns



- Click on the “Validate” button

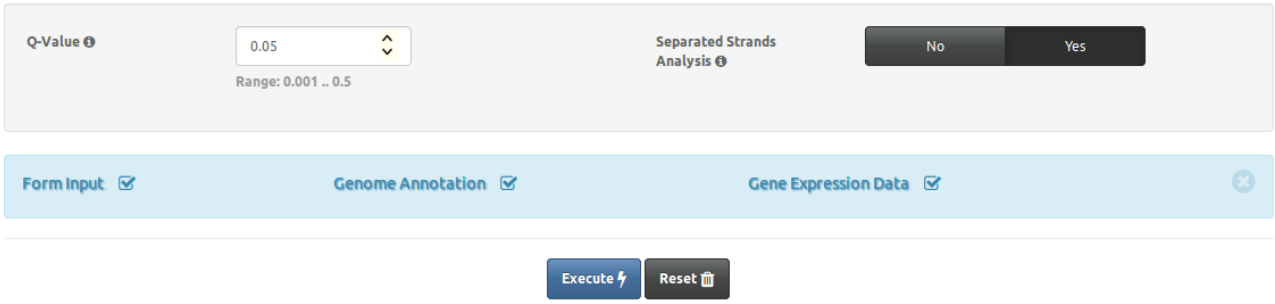
Step 4: Check Gene Expression Data

- Check that the box “GED File” is shaded in green and has a “Valid” flag. The numbers reported under “Genes” and “Log2 Fold Changes” must be the same
- Check that the two headers correspond (i.e.: the right columns were selected as those containing gene name and Log2 fold change information)
- Note that the “Gene Name” field contains the same values as the field of the same name in Genome Annotation section



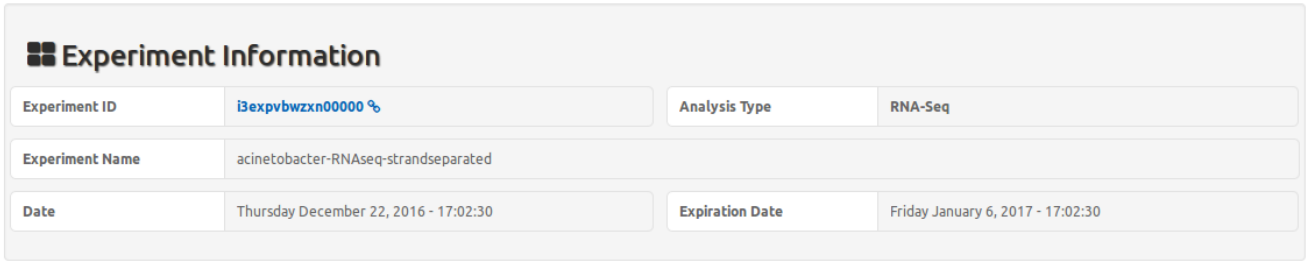
Step 5: Q-value and Separated Strands Analysis

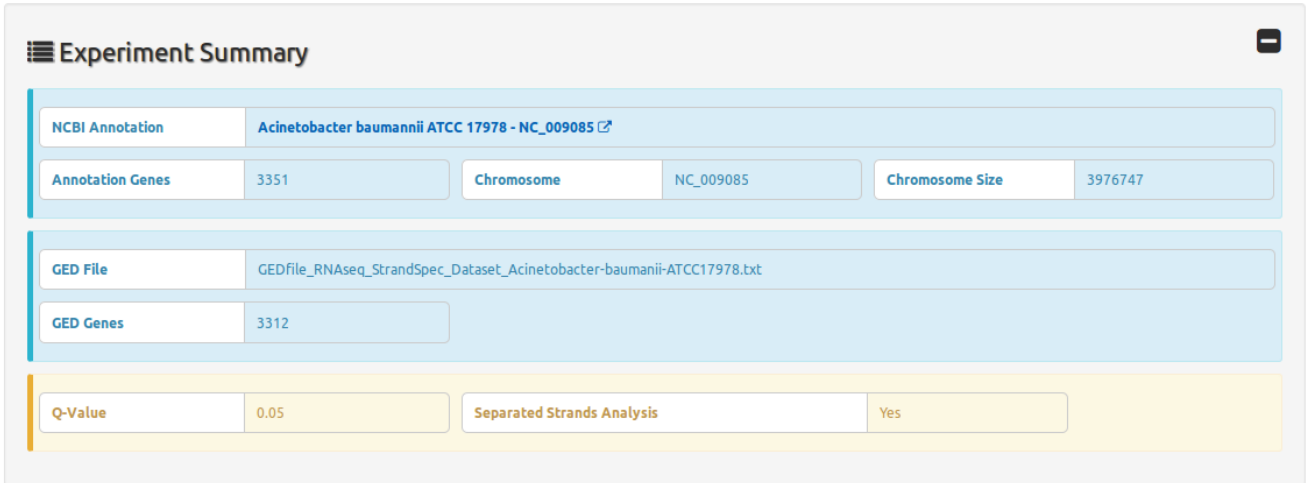
- Enter a q-value of “0.05” (default) or one in the range 0.001-0.5
- Select “Yes” button for “Separated strands analysis” selector
- Check that all check boxes in the light blue box are ☑
- Click the “Execute” button

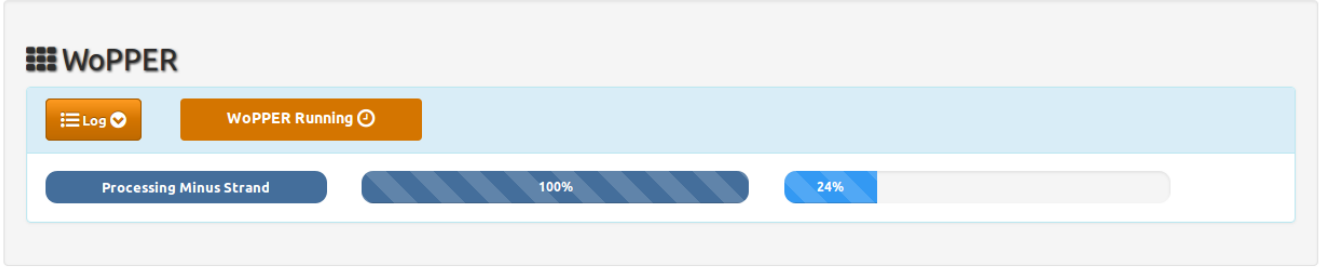


Step 6: Monitor the progress

- "Experiment Information" and "Experiment Summary" contain all the information about the current experiment, including the parameters and the overall number of genes to be tested
- "Experiment ID" contains the unique identifier of the WoPPER job, which can be bookmarked and used for redirection to the results page once WoPPER has finished analyzing the data.
- The progress bar updates regularly as the analysis proceeds







Step 7: Check the output

Once WoPPER has completed its execution, you can have a look at the different outputs, which should appear as follows:

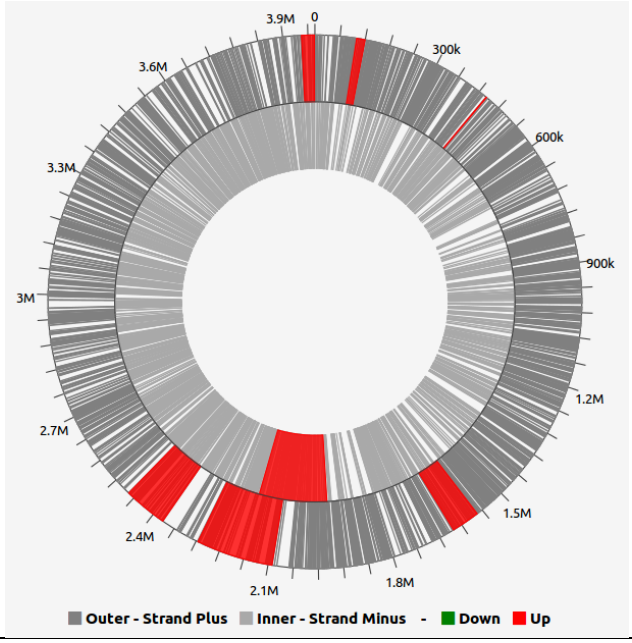
Tabular Output

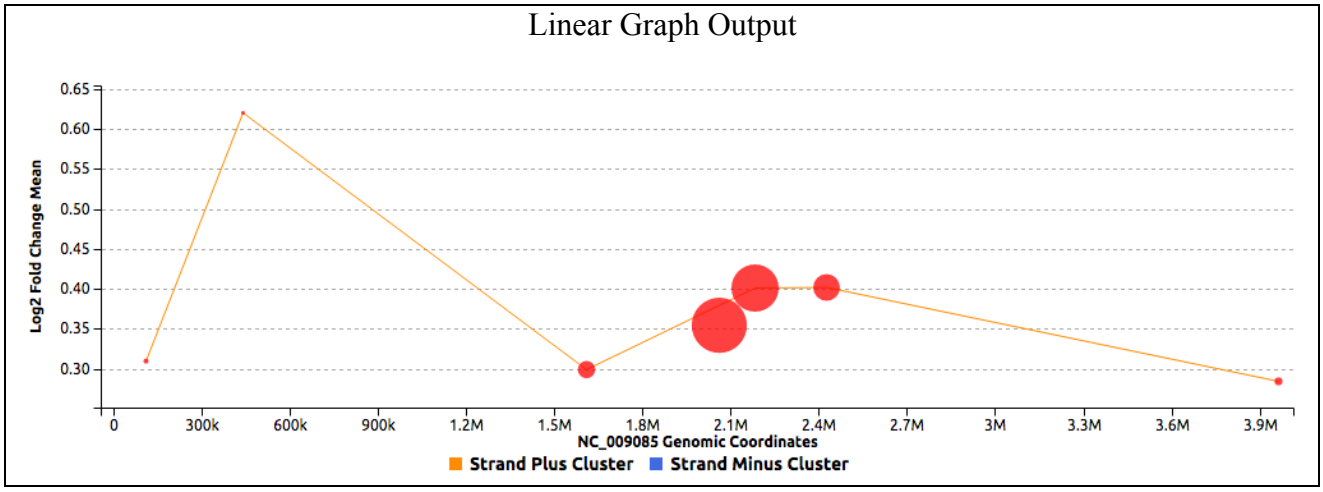
Total Clusters : 7

Clear filtering | Clear sorting | Columns

ID	Cluster Start	Cluster End	Cluster Width	# Genes	Genes in Cluster	Strand	Log2 FC Mean	Log2 FC SD	Expression Trend
1	99481	119928	20447	12	A1S_0080 A1S_0086 A1S_0088 A1S_0089 A1S_0090 A1S_0091 A1S_0093 A1S_0095 A1S_0096 A1S_0097 A1S_0098 A1S_0099	+	0.3097	0.4665	
2	440746	442179	1433	3	A1S_0406 A1S_0407 A1S_0408	+	0.6198	0.4666	
3	1574634	1642800	68166	36	A1S_1345 A1S_1346 A1S_1347 A1S_1348 A1S_1349 A1S_1350 A1S_1351 A1S_1352 A1S_1354 A1S_1356 A1S_1358 A1S_1359 A1S_1360 A1S_1361 A1S_1362 A1S_1363 A1S_1364 A1S_1365 A1S_1367 A1S_1371 A1S_1377 A1S_1378 A1S_1379 A1S_1380 A1S_1382 A1S_1385 A1S_1386 A1S_1389 A1S_1391 A1S_1393 A1S_1394 A1S_1395 A1S_1396 A1S_1397 A1S_1398 A1S_1399	+	0.2992	0.5051	
4	1950317	2166513	216196	101	A1S_1674 A1S_1677 A1S_1678 A1S_1679 A1S_1682 A1S_1687 A1S_1695 A1S_1697 A1S_1698 A1S_1706 A1S_1707 A1S_1712 A1S_1714 A1S_1716 A1S_1724 A1S_1726 A1S_1729 A1S_1730 A1S_1731 A1S_1732 A1S_1733 A1S_1735 A1S_1739 A1S_1740 A1S_1746 A1S_1747 A1S_1750 A1S_1751 A1S_1752 A1S_1756 A1S_1761 A1S_1762 A1S_1763 A1S_1764 A1S_1765 A1S_1766 A1S_1767 A1S_1768 A1S_1769 A1S_1770 A1S_1771 A1S_1772 A1S_1773 A1S_1774 A1S_1775 A1S_1776 A1S_1777 A1S_1778 A1S_1779 A1S_1780 A1S_1781 A1S_1782 A1S_1784 A1S_1785 A1S_1786 A1S_1787 A1S_1788 A1S_1790 A1S_1791 A1S_1792 A1S_1793 A1S_1798 A1S_1802 A1S_1803 A1S_1805 A1S_1807 A1S_1811 A1S_1812 A1S_1813 A1S_1814 A1S_1815 A1S_1816 A1S_1817 A1S_1818 A1S_1819 A1S_1820 A1S_1821 A1S_1827 A1S_1830 A1S_1831	-	0.3545	0.6170	

Circular Plot Output





Congratulations! You have successfully completed WoPPER Tutorial 1

Conclusion

Once you have completed running WoPPER Tutorial 1, you can have a look also to the pre-computed results page. This page should contain exactly the same results and outputs you have just obtained.

Pre-computed results can be viewed clicking on the corresponding radio-button in "Results" column of the "Tutorials" page

#	Descriptions	Organism	Type	# Chr	Annotation	Separated Strands	GED Files	Results	Examples
1		*Acinetobacter baumannii*	RNA-Seq	1	NCBI	Yes			