WoPPER Tutorial 5

Helicobacter pylori

(24/03/2017)

Introduction

This tutorial will show you the analysis of a strand-specific RNA-seq experiment on *Helicobacter pylori*, an organism with one chromosome, using a custom annotation file, using the separated strand analysis option.

For performing this tutorial, you will need to download the GED and the custom annotation 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 two files should be named:

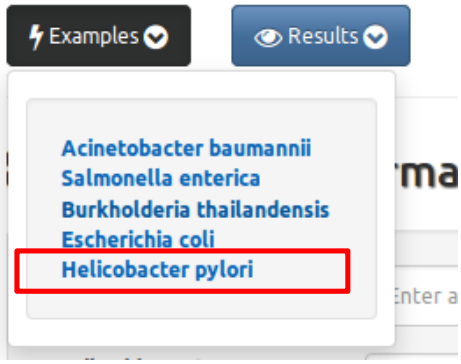
- "**GEDfile_RNAseq_StrandSpec_Dataset_Acinetobacter-baumanii-ATCC17978.txt**"
- "**HP_G27_GCF_000021165.1_ASM2116v1_genomic.gff**"

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
5		*Helicobacter pylori*	RNA-Seq	1	Custom	Yes			

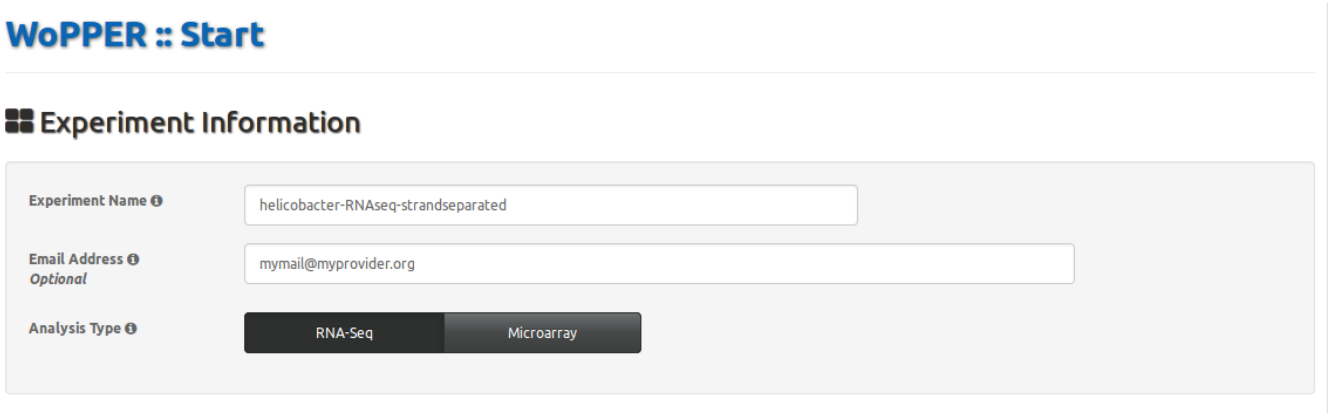
2. Selecting "Helicobacter pylori" from the "Examples" drop-down menu available at the top of WoPPER "Start" page



Step-by-step procedure

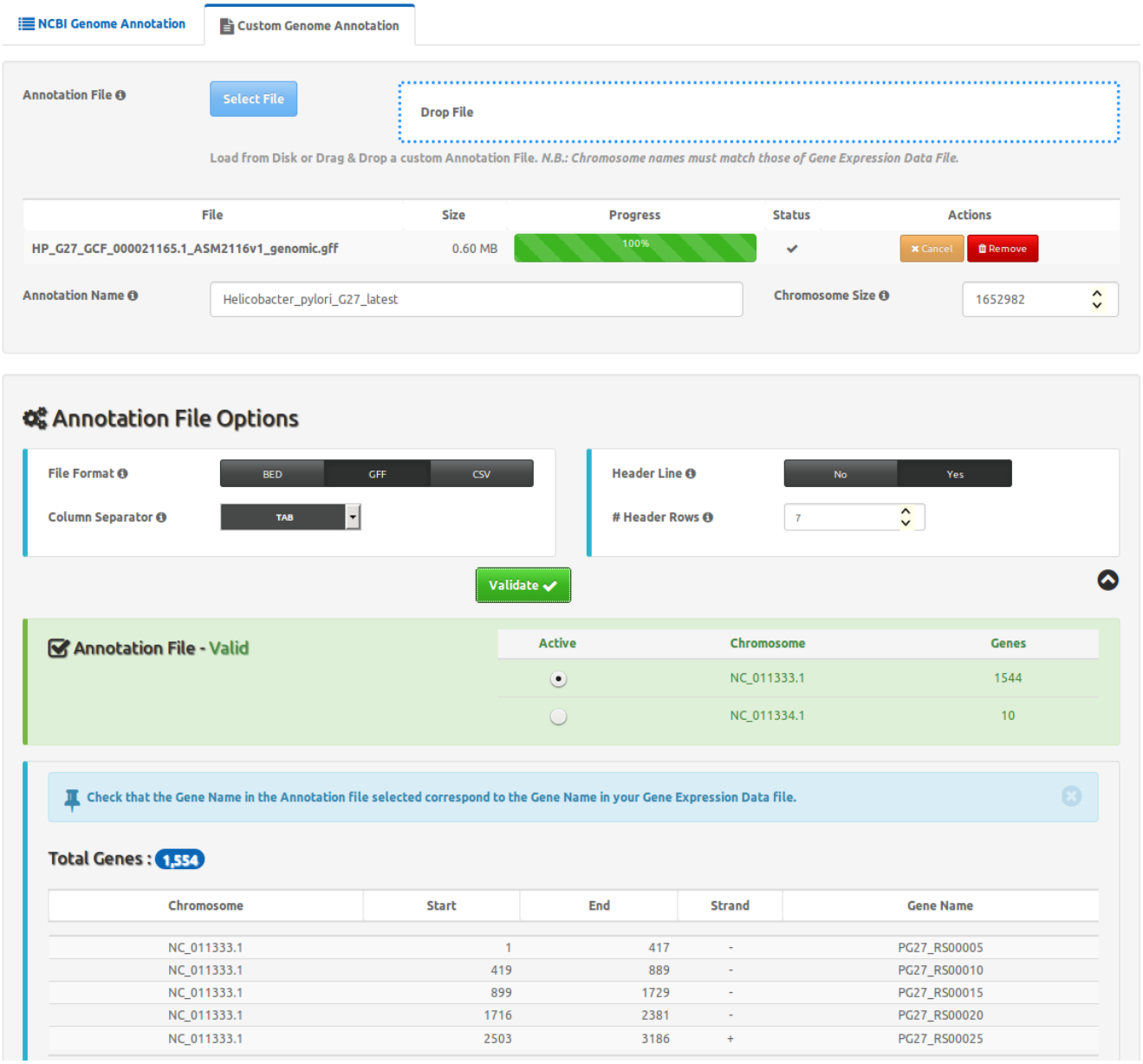
Step 1: Insert Experiment information

- Fill in the "Experiment Name" field with a suitable name for your experiment
- Fill in the "Email address" field 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



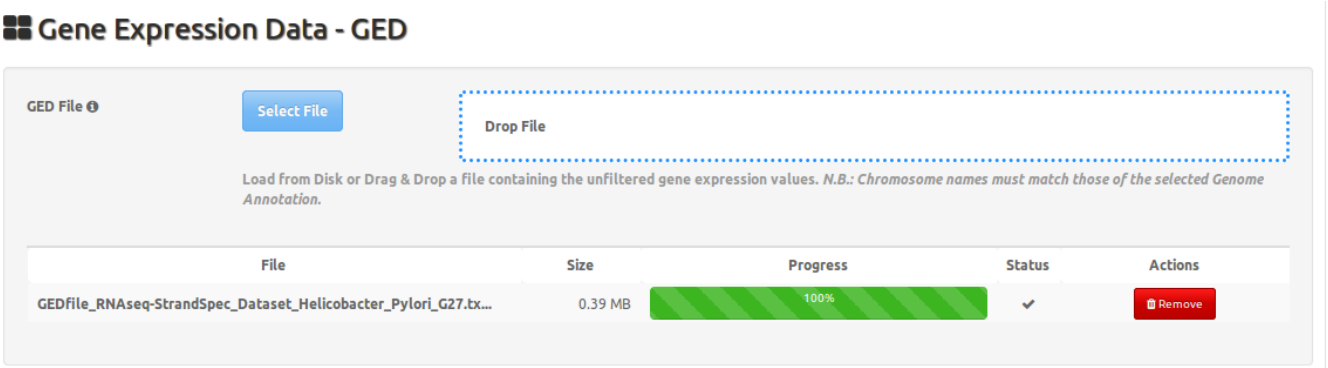
Step 2: Select Genome Annotation

- Select the "Custom Genome Annotation" tab
- Click on "Select file" button and load file named "HP_G27_GCF_000021165.1_ASM2116v1_genomic.gff" (alternatively, you can drag'n'drop the same file into the area named "Drop File")
- Set the "Annotation Name" and "Chromosome size" as indicated in screenshot below
- In "Annotation File Options", set:
  - "File format" to GFF
  - "Column Separator" to "TAB"
  - "Header Line" to "Yes"
  - "#Header Rows" to 7
- Click on "Validate" button to see the selected genome annotation
- In the green box, select Chromosome NC_011333.1, corresponding to the main genome (the other "chromosome" is a plasmid)



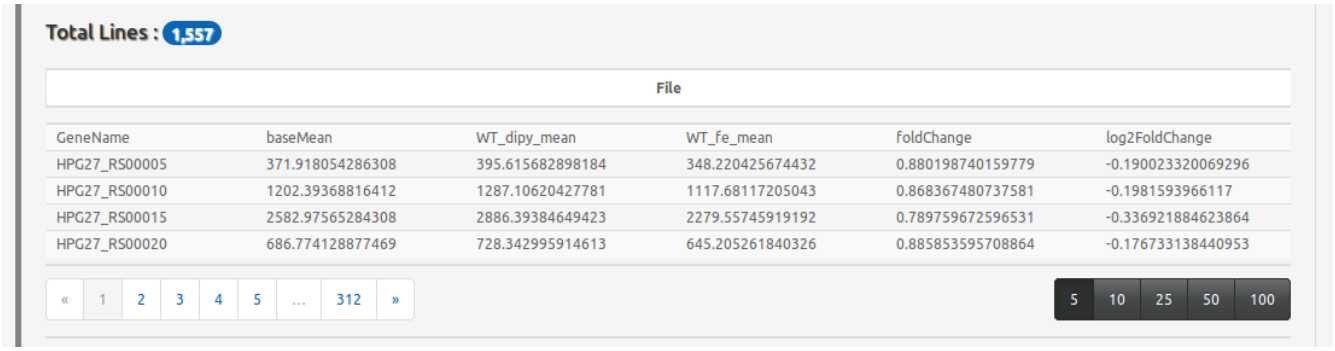
Step 3: Load Gene Expression Data

- Click on the "Select file" button and load the file named "GEDfile_RNAseq-StrandSpec_Dataset_Helicobacter_Pylori_G27.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

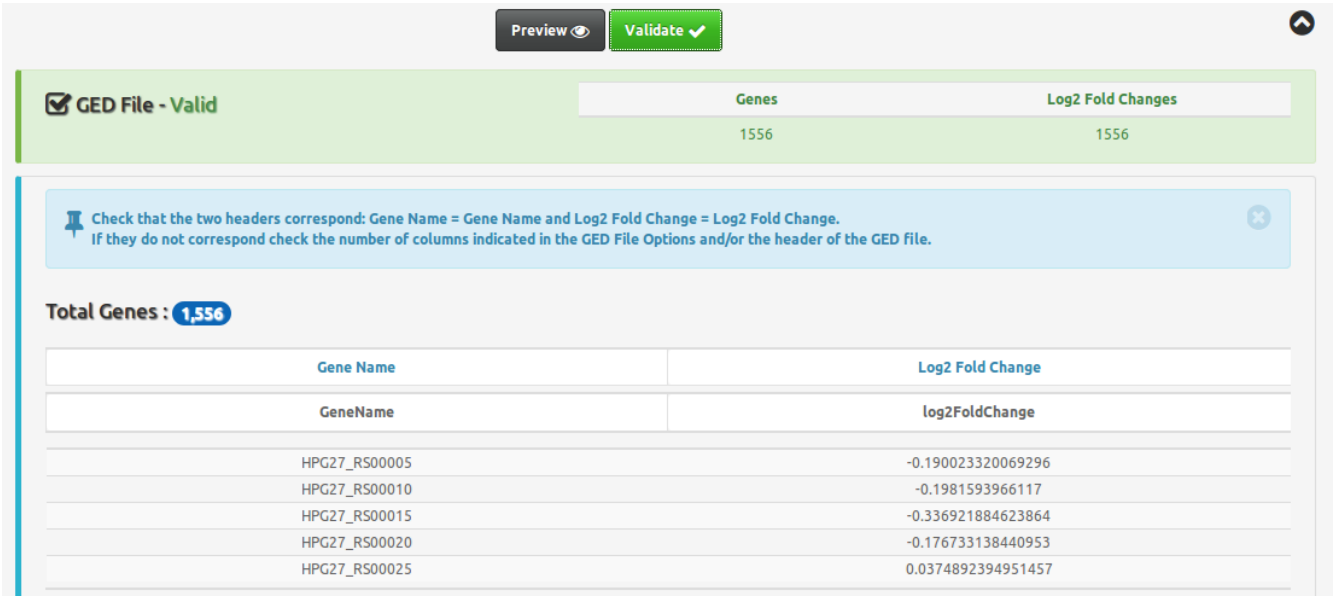
- Click on "Preview" button to check the GED file content and columns



- Click on the "Validate" button

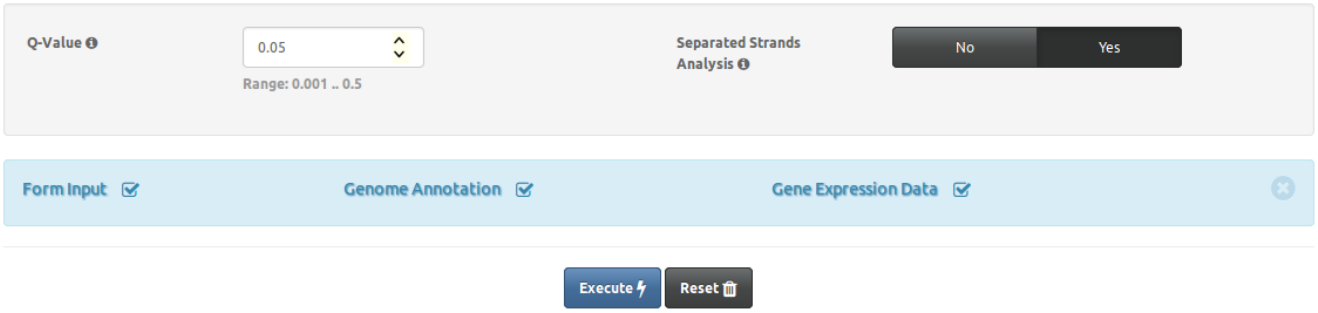
Step 4: Check Gene Expression Data

- 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



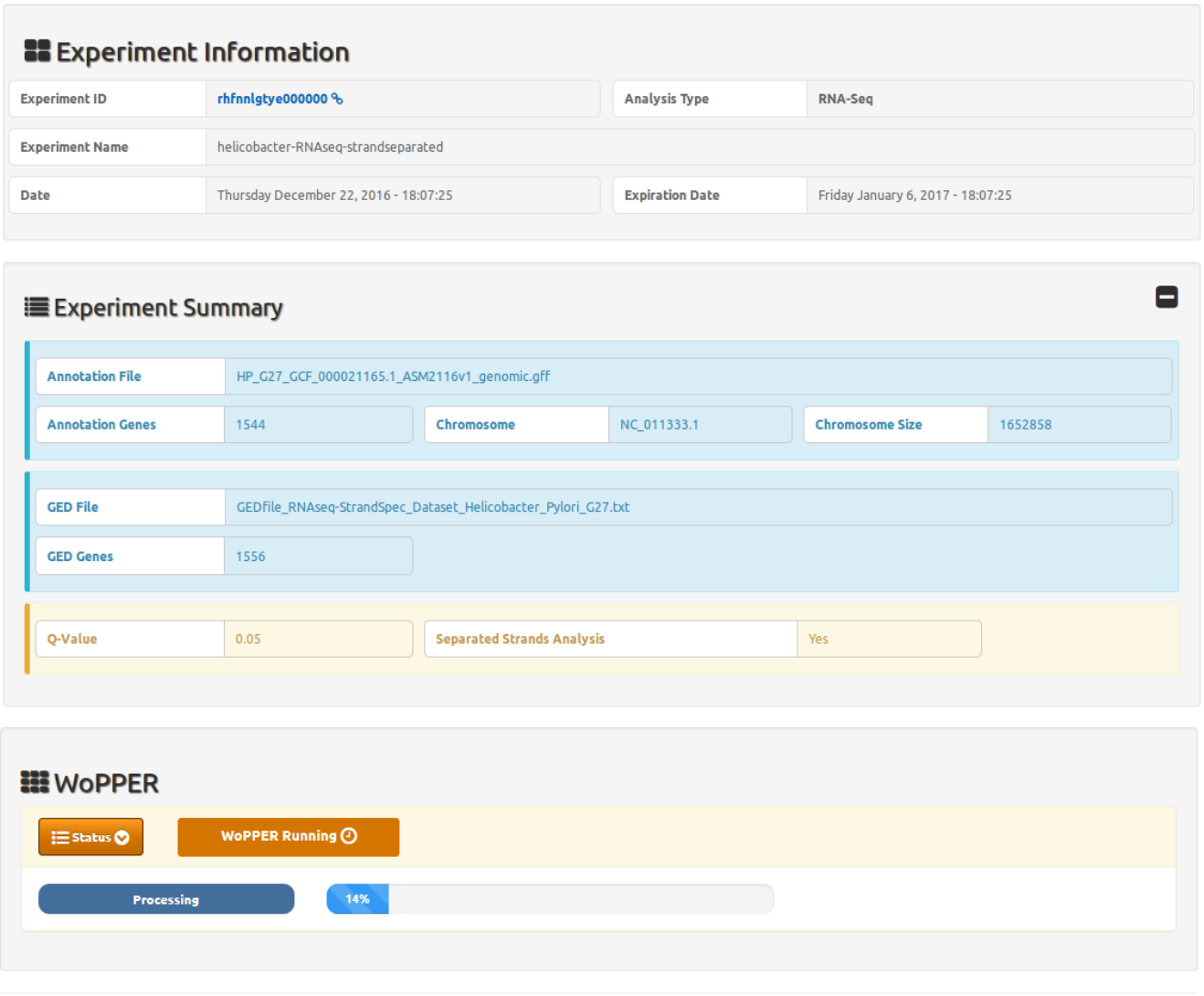
Step 5: Q-value and Separated Strands Analysis

- 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


Experiment Information
Experiment ID
rhfnnlgtye000000
Analysis Type
RNA-Seq
Experiment Name
helicobacter-RNAseq-strandseparated
Date
Thursday December 22, 2016 - 18:07:25
Expiration Date
Friday January 6, 2017 - 18:07:25
Experiment Summary
Annotation File
HP_G27_GCF_000021165.1_ASM2116v1_genomic.gff
Annotation Genes
1544
Chromosome
NC_011333.1
Chromosome Size
1652858
GED File
GEDfile_RNAseq-StrandSpec_Dataset_Helicobacter_Pylori_G27.txt
GED Genes
1556
Q-Value
0.05
Separated Strands Analysis
Yes
WoPPER
Status
WoPPER Running
Processing
14%

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 : 56

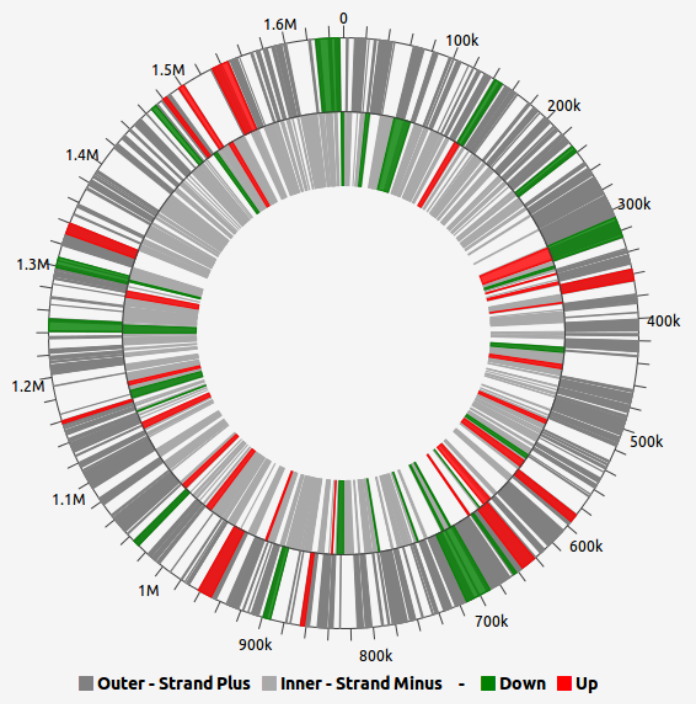
Clear filtering | Clear sorting | Columns

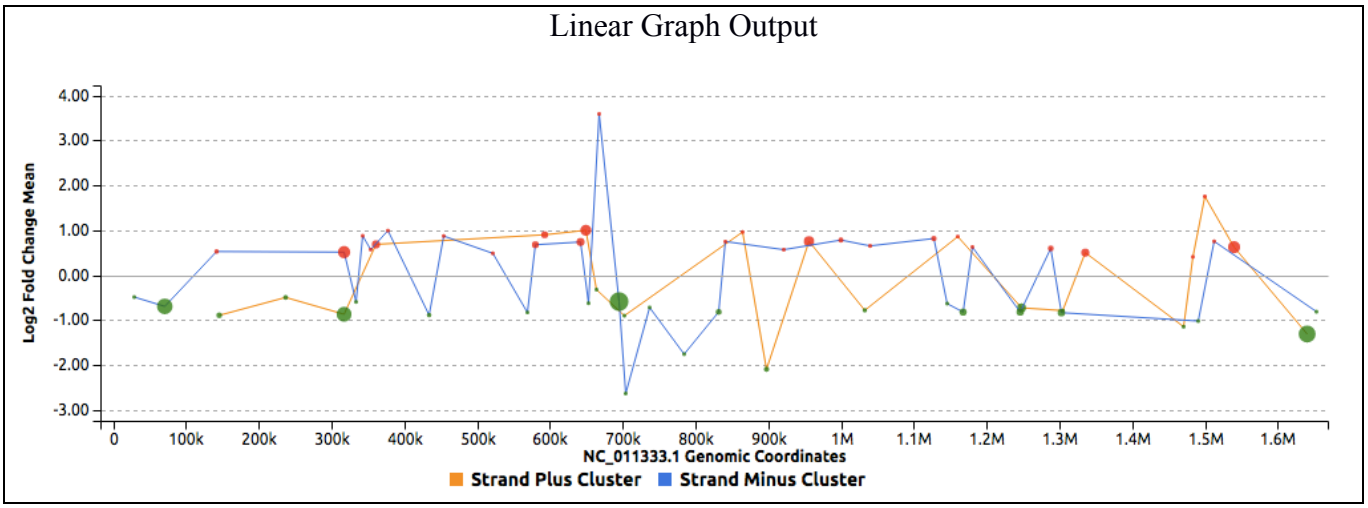
ID	Cluster Start	Cluster End	Cluster Width	# Genes	Genes in Cluster	Strand	Log2 FC Mean	Log2 FC SD	Expression Trend
1	26584	30779	4195	3	HPG27_RS00145 HPG27_RS00160 HPG27_RS00165	-	-0.4911	0.6523	↓
2	60714	80384	19670	11	HPG27_RS00300 HPG27_RS00355 HPG27_RS00360 HPG27_RS00365 HPG27_RS00370 HPG27_RS00375 HPG27_RS00380 HPG27_RS00385 HPG27_RS00390 HPG27_RS00395 HPG27_RS00400	-	-0.6993	0.6079	↓
3	138789	144713	5924	5	HPG27_RS00670 HPG27_RS00675 HPG27_RS00690 HPG27_RS00695 HPG27_RS00700	-	0.5241	0.2803	↑
4	141569	148950	7381	4	HPG27_RS00680 HPG27_RS00685 HPG27_RS00710 HPG27_RS00715	+	-0.8986	0.7947	↓
5	233009	239233	6224	5	HPG27_RS01140 HPG27_RS01160 HPG27_RS01165 HPG27_RS01170 HPG27_RS01175	+	-0.4996	0.1997	↓

« 1 2 3 4 5 ... 12 »

5 10 25 50 100

Circular Plot Output





Congratulations! You have successfully completed WoPPER Tutorial 5

Conclusion

Once you have completed running WoPPER Tutorial 5, 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
5		*Helicobacter pylori*	RNA-Seq	1	Custom	Yes			