BV-BRC Test Report

A22. Service – RNA-seq Analysis

Item to test	RNA-seq Analysis Service using transcriptomic read sets
URL	https://www.bv-brc.org/app/Rnaseq
Prerequisites	Transcriptomic read sets in workspace as fastq files
References	https://www.bv-brc.org/docs/quick_references/services/rna_seq_analysis_service.html https://www.bv-brc.org/docs/tutorial/rna_seq/rna_seq.html
Tester(s)	Rebecca Wattam, Maulik Shukla
Test date	08-May-2022 (follow-up from original test)
Test result	**Partial Success (Bugs reported)**

Overview

- Test the RNA-seq Analysis using exemplar transcriptomic reads sets.
- Test input options, i.e., single-end / paired-end read files.
- Test different strategies, i.e. Tuxedo and HTSeq.
- Test differential and non-differential analysis options.
- For each job submitted, verify successful completion of the job, presence of output files, their content and format.
- Review differential expression results using interactive gene list and heatmap viewer.

Test Data

Dataset	**Rational**	**Input Format**	**Input**
Acinetobacter baumannii AMR treatments	Workshop example	Fastq file	MHB_R1.fq.gz, MHB_R2.fq.gz COL_R1.fq.gz, COL_R2.fq.gz MERO_R1.fq.gz, MERO_R2.fq.gz

- All test datasets and corresponding job results are available in the following public workspace: https://www.bv-brc.org/workspace/BVBRC@patricbrc.org/BVBRC%20Tests/RNA-seq%20Analysis

Test Results

- Most jobs completed successfully. However, some jobs failed with errors.
- All jobs that completed successfully, resulted in expected output files in corresponding job output directory, including fastqc results, bam files, read counts, fpkm and tpm values for each sample, and a TPM summary matrix.
- For differential expression option, the results included differential expression values as log fold change and z scores.
- The interactive gene list allowed filtering of the genes based on log ratio, z-score, and up or down regulation of genes in various contrasts.

- The heatmap provided visual representation of the differential expression results and clustering helped identify genes with similar expression patters a cross one or more samples.
- All test datasets and corresponding job results are available in the following public workspace: https://www.bv-brc.org/workspace/BVBRC@patricbrc.org/BVBRC%20Tests/RNA-seq%20Analysis
- Below are a series of screenshots showing successful completion of the jobs, availability of the result files in the workspace, interactive gene list and heatmap.

Parameters

STRATEGY: HTSeq-DESeq

TARGET GENOME: Acinetobacter baumannii 34654

OUTPUT FOLDER: RNA-seq Analysis

OUTPUT NAME: Output Name

Groups/Conditions

ON

MHB

- MHB ●
- MERO 🟥
- COL ●

Paired read library

READ FILE 1: f

READ FILE 2: MHB_R2.fq.gz

The value entered is not valid.

CONDITION: MHB

Selected libraries

Place read files here using the arrow buttons.

- P(MHB_R1.fq.gz, MHB_R2.fq.gz) ●
- P(COL_7...fq.gz, COL_7...fq.gz) ●
- P(MERO_...fq.gz, MERO_...fq.gz) 🟥

Single read library

READ FILE:

CONDITION: Condition Name

SRA run accession

SRR ACCESSION: SRR

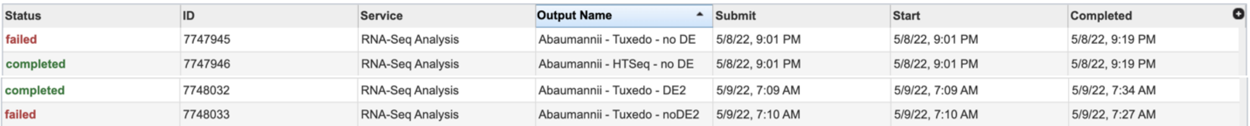
CONDITION: Condition Name

Contrasts

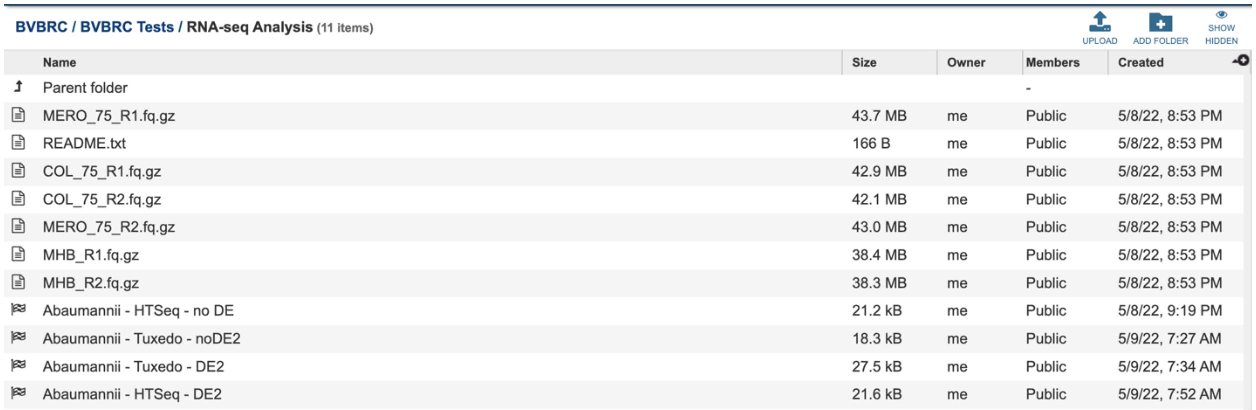
CONDITION 1: Condition Name

CONDITION 2: Condition Name

CONDITION 1		CONDITION 2	
MERO	🟥	MHB	●
COL	●	MHB	●



Status	ID	Service	Output Name	Submit	Start	Completed
failed	7747945	RNA-Seq Analysis	Abaumannii - Tuxedo - no DE	5/8/22, 9:01 PM	5/8/22, 9:01 PM	5/8/22, 9:19 PM
completed	7747946	RNA-Seq Analysis	Abaumannii - HTSeq - no DE	5/8/22, 9:01 PM	5/8/22, 9:01 PM	5/8/22, 9:19 PM
completed	7748032	RNA-Seq Analysis	Abaumannii - Tuxedo - DE2	5/9/22, 7:09 AM	5/9/22, 7:09 AM	5/9/22, 7:34 AM
failed	7748033	RNA-Seq Analysis	Abaumannii - Tuxedo - noDE2	5/9/22, 7:10 AM	5/9/22, 7:10 AM	5/9/22, 7:27 AM



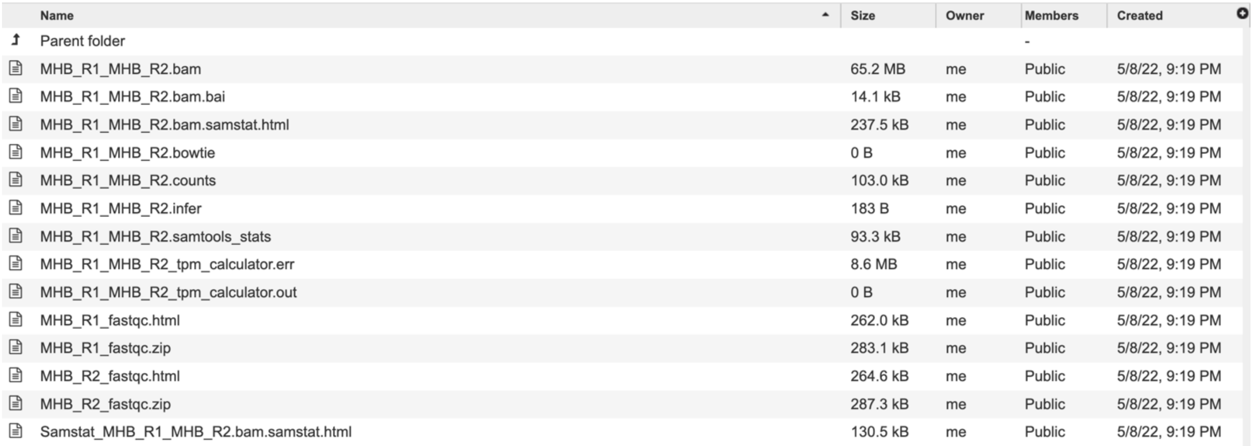
BVBRC / BVBRC Tests / RNA-seq Analysis (11 items)

Name	Size	Owner	Members	Created
Parent folder			-	
MERO_75_R1.fq.gz	43.7 MB	me	Public	5/8/22, 8:53 PM
README.txt	166 B	me	Public	5/8/22, 8:53 PM
COL_75_R1.fq.gz	42.9 MB	me	Public	5/8/22, 8:53 PM
COL_75_R2.fq.gz	42.1 MB	me	Public	5/8/22, 8:53 PM
MERO_75_R2.fq.gz	43.0 MB	me	Public	5/8/22, 8:53 PM
MHB_R1.fq.gz	38.4 MB	me	Public	5/8/22, 8:53 PM
MHB_R2.fq.gz	38.3 MB	me	Public	5/8/22, 8:53 PM
Abaumannii - HTSeq - no DE	21.2 kB	me	Public	5/8/22, 9:19 PM
Abaumannii - Tuxedo - noDE2	18.3 kB	me	Public	5/9/22, 7:27 AM
Abaumannii - Tuxedo - DE2	27.5 kB	me	Public	5/9/22, 7:34 AM
Abaumannii - HTSeq - DE2	21.6 kB	me	Public	5/9/22, 7:52 AM

- Non-DE analysis results

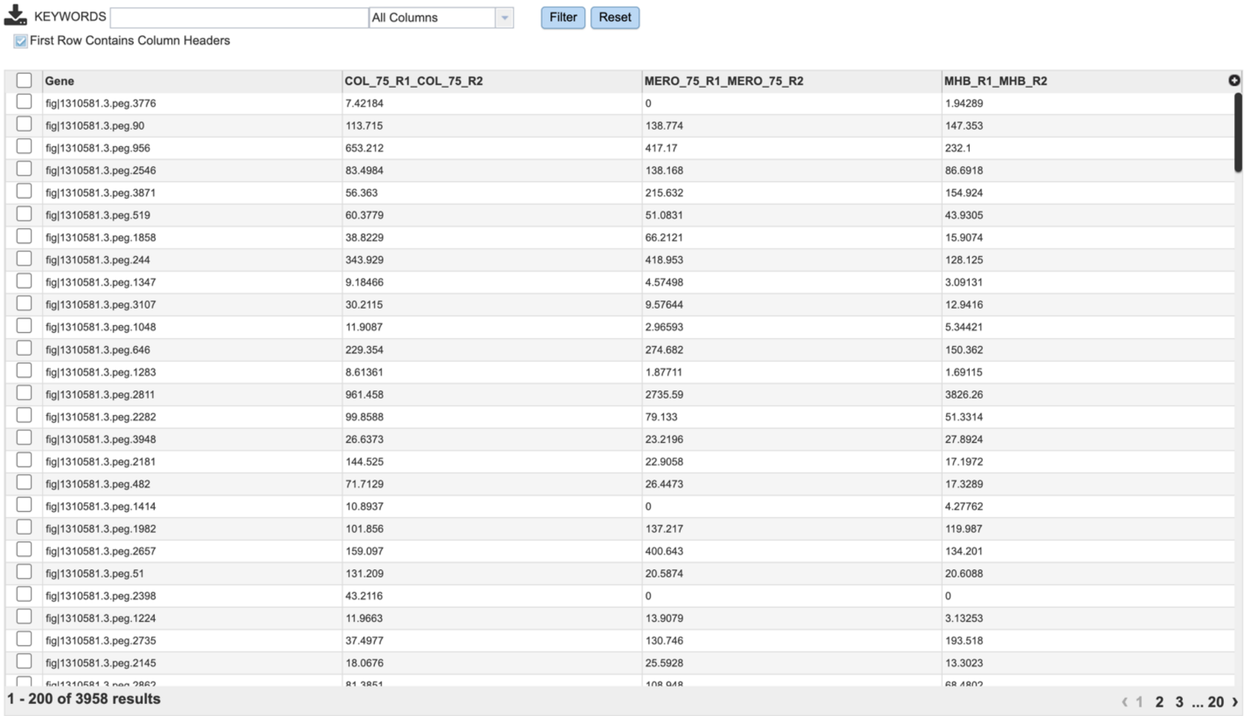
	Name	Size	Owner	Members	Created
↑	Parent folder			-	
📁	1310581.3		me	Public	5/8/22, 9:18 PM
🗎	Metadata.txt	97 B	me	Public	5/8/22, 9:18 PM
🗎	Pipeline.txt	7.6 kB	me	Public	5/8/22, 9:18 PM
📁	report_images		me	Public	5/8/22, 9:18 PM

	Name	Size	Owner	Members	Created
↑	Parent folder			-	
🗎	1310581.3.htseq.gene_counts.tsv	123.2 kB	me	Public	5/8/22, 9:19 PM
🗎	1310581.3.htseq.tpms.tsv	177.5 kB	me	Public	5/8/22, 9:19 PM
🗎	1310581.3_report.html	779.3 kB	me	Public	5/8/22, 9:19 PM
📁	TPMCalculator_Output		me	Public	5/8/22, 9:19 PM
🗎	library_geometry.txt	119 B	me	Public	5/8/22, 9:19 PM
📁	results		me	Public	5/8/22, 9:18 PM



	Name	Size	Owner	Members	Created
↑	Parent folder			-	
🗎	MHB_R1_MHB_R2.bam	65.2 MB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.bam.bai	14.1 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.bam.samstat.html	237.5 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.bowtie	0 B	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.counts	103.0 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.infer	183 B	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2.samtools_stats	93.3 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2_tpm_calculator.err	8.6 MB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2_tpm_calculator.out	0 B	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_fastqc.html	262.0 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_fastqc.zip	283.1 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R2_fastqc.html	264.6 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R2_fastqc.zip	287.3 kB	me	Public	5/8/22, 9:19 PM
🗎	Samstat_MHB_R1_MHB_R2.bam.samstat.html	130.5 kB	me	Public	5/8/22, 9:19 PM

	Name	Size	Owner	Members	Created
↑	Parent folder			-	
🗎	COL_75_R1_COL_75_R2_genes.ent	254.9 kB	me	Public	5/8/22, 9:19 PM
🗎	COL_75_R1_COL_75_R2_genes.out	438.2 kB	me	Public	5/8/22, 9:19 PM
🗎	COL_75_R1_COL_75_R2_genes.uni	254.8 kB	me	Public	5/8/22, 9:19 PM
🗎	MERO_75_R1_MERO_75_R2_genes.ent	252.0 kB	me	Public	5/8/22, 9:19 PM
🗎	MERO_75_R1_MERO_75_R2_genes.out	433.3 kB	me	Public	5/8/22, 9:19 PM
🗎	MERO_75_R1_MERO_75_R2_genes.uni	251.9 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2_genes.ent	248.9 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2_genes.out	427.6 kB	me	Public	5/8/22, 9:19 PM
🗎	MHB_R1_MHB_R2_genes.uni	248.9 kB	me	Public	5/8/22, 9:19 PM



☑ First Row Contains Column Headers

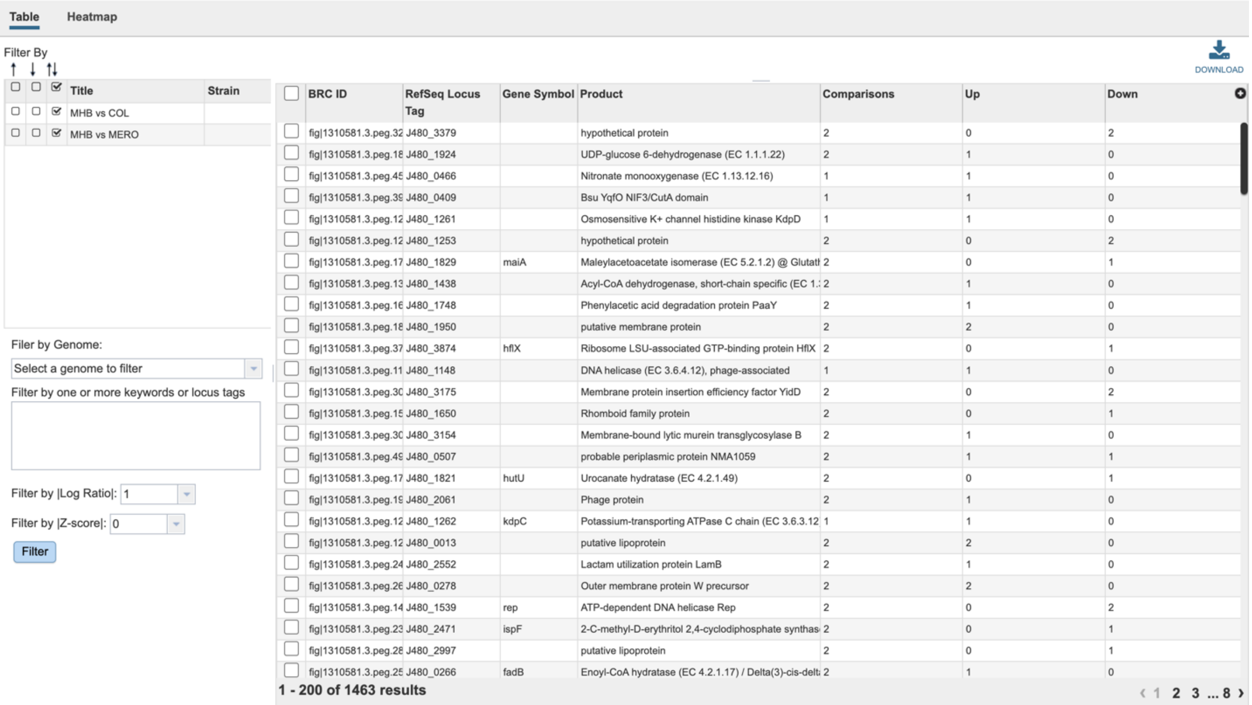
☐	Gene	COL_75_R1_COL_75_R2	MERO_75_R1_MERO_75_R2	MHB_R1_MHB_R2
☐	fig|1310581.3.peg.3776	7.42184	0	1.94289
☐	fig|1310581.3.peg.90	113.715	138.774	147.353
☐	fig|1310581.3.peg.956	653.212	417.17	232.1
☐	fig|1310581.3.peg.2546	83.4984	138.168	86.6918
☐	fig|1310581.3.peg.3871	56.363	215.632	154.924
☐	fig|1310581.3.peg.519	60.3779	51.0831	43.9305
☐	fig|1310581.3.peg.1858	38.8229	66.2121	15.9074
☐	fig|1310581.3.peg.244	343.929	418.953	128.125
☐	fig|1310581.3.peg.1347	9.18466	4.57498	3.09131
☐	fig|1310581.3.peg.3107	30.2115	9.57644	12.9416
☐	fig|1310581.3.peg.1048	11.9087	2.96593	5.34421
☐	fig|1310581.3.peg.646	229.354	274.682	150.362
☐	fig|1310581.3.peg.1283	8.61361	1.87711	1.69115
☐	fig|1310581.3.peg.2811	961.458	2735.59	3826.26
☐	fig|1310581.3.peg.2282	99.8588	79.133	51.3314
☐	fig|1310581.3.peg.3948	26.6373	23.2196	27.8924
☐	fig|1310581.3.peg.2181	144.525	22.9058	17.1972
☐	fig|1310581.3.peg.482	71.7129	26.4473	17.3289
☐	fig|1310581.3.peg.1414	10.8937	0	4.27762
☐	fig|1310581.3.peg.1982	101.856	137.217	119.987
☐	fig|1310581.3.peg.2657	159.097	400.643	134.201
☐	fig|1310581.3.peg.51	131.209	20.5874	20.6088
☐	fig|1310581.3.peg.2398	43.2116	0	0
☐	fig|1310581.3.peg.1224	11.9663	13.9079	3.13253
☐	fig|1310581.3.peg.2735	37.4977	130.746	193.518
☐	fig|1310581.3.peg.2145	18.0676	25.5928	13.3023
☐	fig|1310581.3.peg.2862	81.3851	108.948	68.4802

1 - 200 of 3958 results

- Differential Expression Results

Name	Size	Owner	Members	Created
Parent folder			-	
1310581.3		me	Public	5/9/22, 7:33 AM
1310581.3.bed	1.3 MB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.1.bt2	5.6 MB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.2.bt2	1.0 MB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.3.bt2	953 B	me	Public	5/9/22, 7:33 AM
1310581.3.fna.4.bt2	1.0 MB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.fai	3.4 kB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.rev.1.bt2	5.6 MB	me	Public	5/9/22, 7:33 AM
1310581.3.fna.rev.2.bt2	1.0 MB	me	Public	5/9/22, 7:33 AM
1310581.3.gff	1.2 MB	me	Public	5/9/22, 7:33 AM
Abaumannii - Tuxedo - DE2_diffexp	1.6 kB	me	Public	5/9/22, 7:33 AM
Metadata.txt	86 B	me	Public	5/9/22, 7:33 AM
Pipeline.txt	6.3 kB	me	Public	5/9/22, 7:33 AM

Name	Size	Owner	Members	Created
Parent folder			-	
COL		me	Public	5/9/22, 7:34 AM
MERO		me	Public	5/9/22, 7:33 AM
MHB		me	Public	5/9/22, 7:33 AM
bias_params.info	196.2 kB	me	Public	5/9/22, 7:33 AM
cds.count_tracking	12 B	me	Public	5/9/22, 7:33 AM
cds.diff	115 B	me	Public	5/9/22, 7:33 AM
cds.fpkm_tracking	91 B	me	Public	5/9/22, 7:33 AM
cds.read_group_tracking	115 B	me	Public	5/9/22, 7:33 AM
cds_exp.diff	124 B	me	Public	5/9/22, 7:33 AM
contrasts.txt	41 B	me	Public	5/9/22, 7:34 AM
diff_exp_params.json	333 B	me	Public	5/9/22, 7:33 AM
gene_exp.diff	959.1 kB	me	Public	5/9/22, 7:33 AM
gene_exp.gmx	96.2 kB	me	Public	5/9/22, 7:33 AM
genes.count_tracking	306.6 kB	me	Public	5/9/22, 7:33 AM
genes.fpkm_tracking	574.4 kB	me	Public	5/9/22, 7:33 AM
genes.read_group_tracking	519.0 kB	me	Public	5/9/22, 7:33 AM
gtf_manifest.txt	193 B	me	Public	5/9/22, 7:33 AM
isoform_exp.diff	124 B	me	Public	5/9/22, 7:33 AM
isoforms.count_tracking	343.8 kB	me	Public	5/9/22, 7:33 AM
isoforms.fpkm_tracking	725.2 kB	me	Public	5/9/22, 7:33 AM
isoforms.read_group_tracking	605.2 kB	me	Public	5/9/22, 7:33 AM
library_geometry.txt	108 B	me	Public	5/9/22, 7:33 AM
merged_annotation		me	Public	5/9/22, 7:33 AM
promoters.diff	399.9 kB	me	Public	5/9/22, 7:33 AM
read_groups.info	363 B	me	Public	5/9/22, 7:33 AM



Filter By

☐	☐	☑	Title	Strain
☐	☐	☑	MHB vs COL	
☐	☐	☑	MHB vs MERO	

Filer by Genome: Select a genome to filter

Filter by one or more keywords or locus tags

Filter by |Log Ratio|: 1

Filter by |Z-score|: 0

☐	BRC ID	RefSeq Locus Tag	Gene Symbol	Product	Comparisons	Up	Down
☐	fig|1310581.3.peg.32	J480_3379		hypothetical protein	2	0	2
☐	fig|1310581.3.peg.18	J480_1924		UDP-glucose 6-dehydrogenase (EC 1.1.1.22)	2	1	0
☐	fig|1310581.3.peg.45	J480_0466		Nitronate monooxygenase (EC 1.13.12.16)	1	1	0
☐	fig|1310581.3.peg.39	J480_0409		Bsu YqfO NIF3/CutA domain	1	1	0
☐	fig|1310581.3.peg.12	J480_1261		Osmosensitive K+ channel histidine kinase KdpD	1	1	0
☐	fig|1310581.3.peg.12	J480_1253		hypothetical protein	2	0	2
☐	fig|1310581.3.peg.17	J480_1829	maiA	Maleylacetoacetate isomerase (EC 5.2.1.2) @ Glutatl	2	0	1
☐	fig|1310581.3.peg.13	J480_1438		Acyl-CoA dehydrogenase, short-chain specific (EC 1.:	2	1	0
☐	fig|1310581.3.peg.16	J480_1748		Phenylacetic acid degradation protein PaaY	2	1	0
☐	fig|1310581.3.peg.18	J480_1950		putative membrane protein	2	2	0
☐	fig|1310581.3.peg.37	J480_3874	hflX	Ribosome LSU-associated GTP-binding protein HflX	2	0	1
☐	fig|1310581.3.peg.11	J480_1148		DNA helicase (EC 3.6.4.12), phage-associated	1	1	0
☐	fig|1310581.3.peg.30	J480_3175		Membrane protein insertion efficiency factor YidD	2	0	2
☐	fig|1310581.3.peg.15	J480_1650		Rhomboid family protein	2	0	1
☐	fig|1310581.3.peg.30	J480_3154		Membrane-bound lytic murein transglycosylase B	2	1	0
☐	fig|1310581.3.peg.49	J480_0507		probable periplasmic protein NMA1059	2	1	1
☐	fig|1310581.3.peg.17	J480_1821	hutU	Urocanate hydratase (EC 4.2.1.49)	2	0	1
☐	fig|1310581.3.peg.19	J480_2061		Phage protein	2	1	0
☐	fig|1310581.3.peg.12	J480_1262	kdpC	Potassium-transporting ATPase C chain (EC 3.6.3.12	1	1	0
☐	fig|1310581.3.peg.12	J480_0013		putative lipoprotein	2	2	0
☐	fig|1310581.3.peg.24	J480_2552		Lactam utilization protein LamB	2	1	0
☐	fig|1310581.3.peg.26	J480_0278		Outer membrane protein W precursor	2	2	0
☐	fig|1310581.3.peg.14	J480_1539	rep	ATP-dependent DNA helicase Rep	2	0	2
☐	fig|1310581.3.peg.23	J480_2471	ispF	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthas	2	0	1
☐	fig|1310581.3.peg.28	J480_2997		putative lipoprotein	2	0	1
☐	fig|1310581.3.peg.25	J480_0266	fadB	Enoyl-CoA hydratase (EC 4.2.1.17) / Delta(3)-cis-delt	2	1	0

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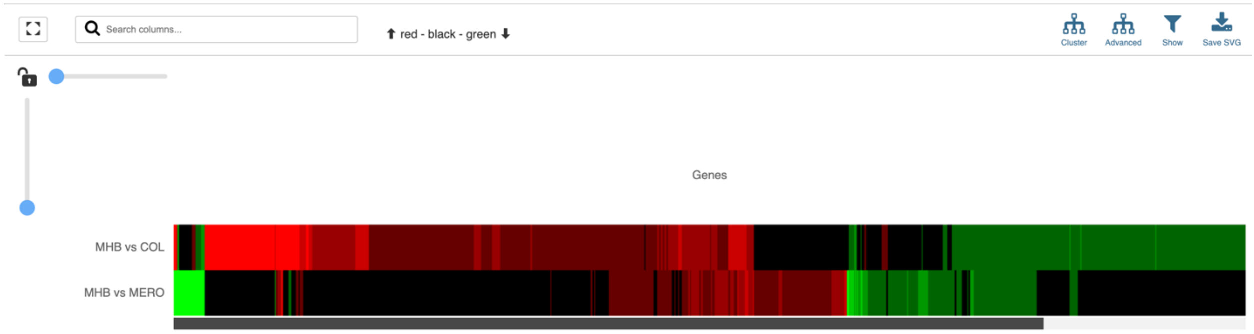

Note

- Issue: When Tuxedo – No DE recipe is selected, the service produces expected results. However, job is marked as failed.
- Resolution: The bug has been identified and being fixed. It will be deployed to production in the next release.

References

- RNA-seq Analysis Service Quick Reference Guide
- RNA-seq Analysis Service Tutorial