



# The PATRIC RNASeq Service

Presented by  
Andrew Warren



# Executing the Service

1. Reads are aligned to a reference genome
2. Counts are normalized across experiments
3. Transcripts are assembled and transcript boundaries are identified
4. Transcript abundance is quantified
5. Tests for differential gene expression are performed
6. Operon structures are predicted
7. Results are formatted for visualization in a genome browser

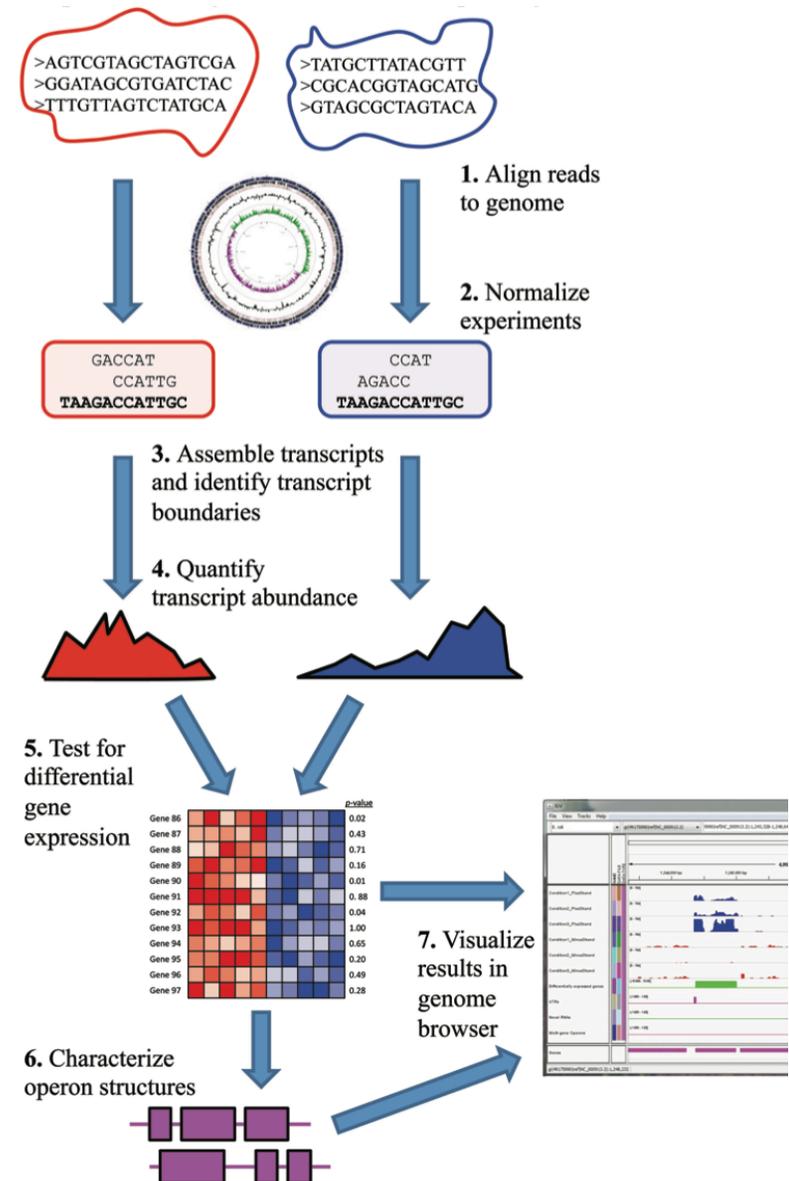


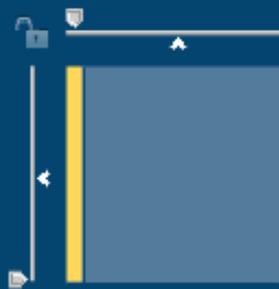
Table Heatmap

339 genes found

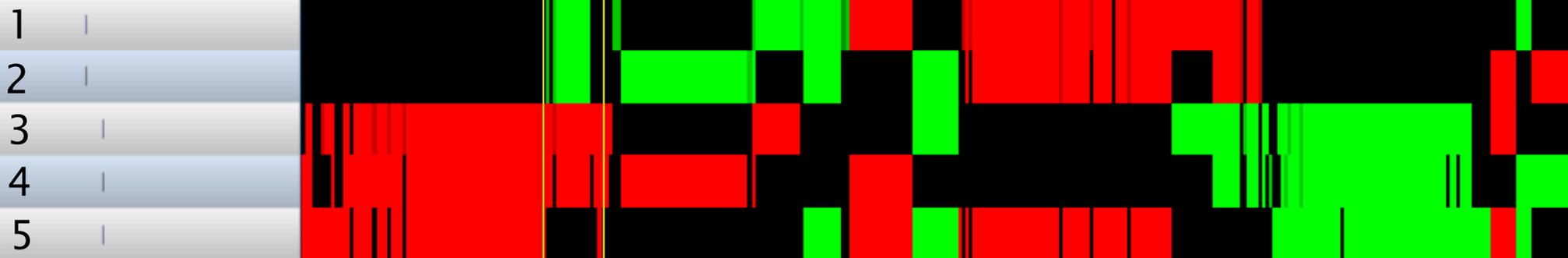
Flip Axis Heatmap Color Cluster Advanced Clustering Show Significant Genes

x: Gene  
y: Compar

↑ Red-Black-Green ↓  
↑ Red-White-Blue ↓

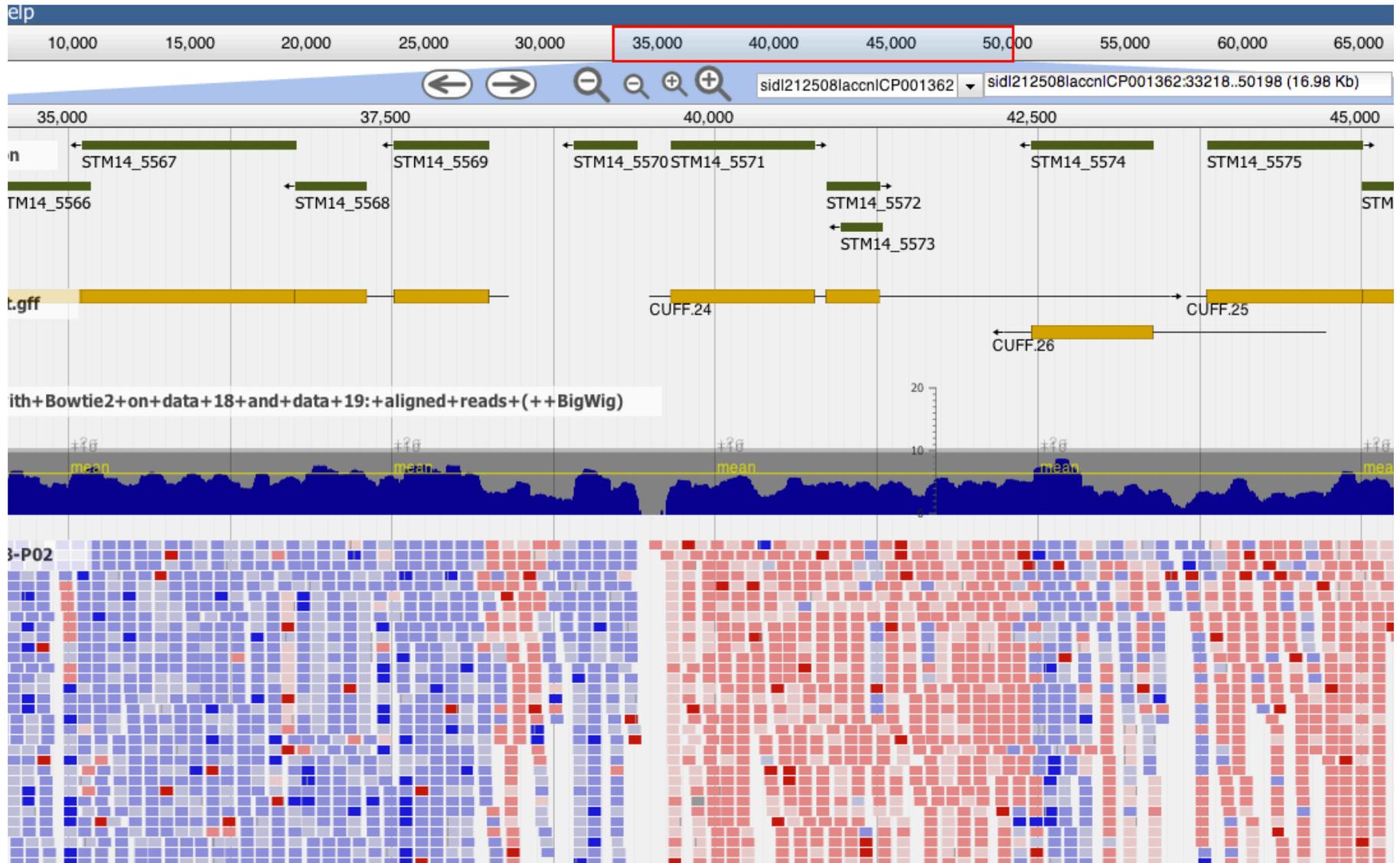


Gene



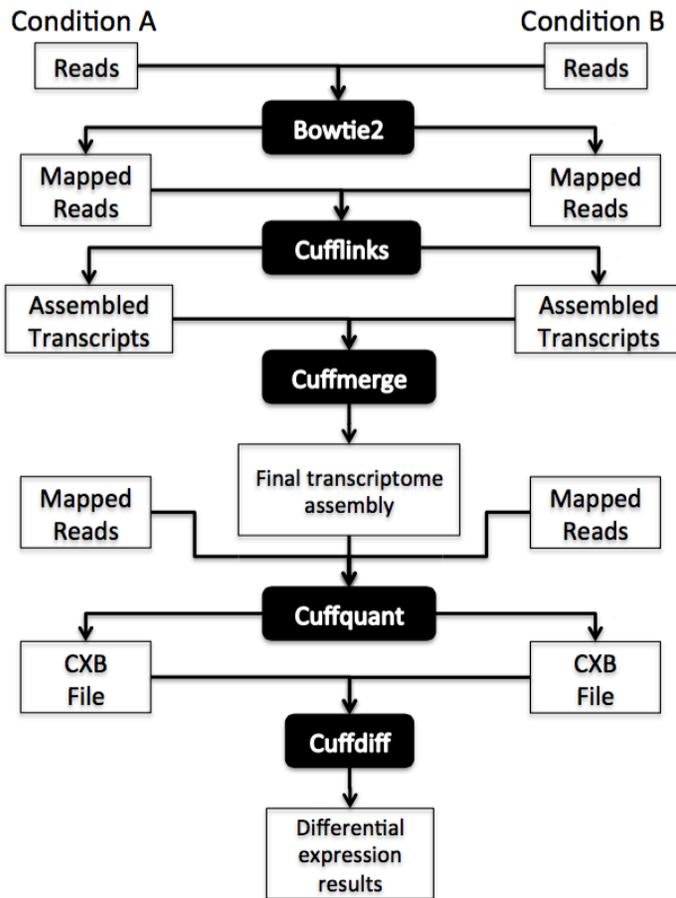
1. Mero 25 vs MHB
2. Mero 75 vs MHB
3. Mero 25 vs MHB-NaCl
4. Mero 75 vs MHB-NaCl
5. MHB vs MHB-NaCl

# Mapping RNA Reads to a Reference



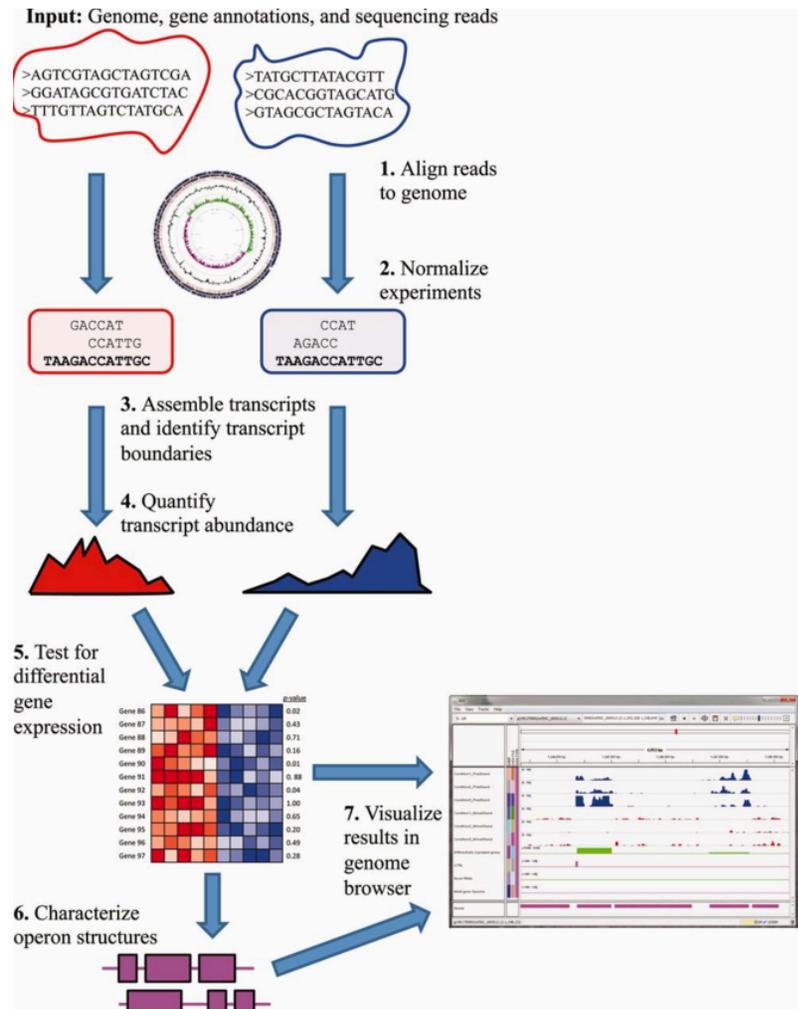
# RNA-seq strategies

## Tuxedo Pipeline



[1] C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D. R. Kelley, H. Pimentel, S. L. Salzberg, J. L. Rinn, and L. Pachter, "Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks," *Nature Protocols*, vol. 7, no. 3, pp. 562-578, Mar. 2012.

[2] R. McClure, D. Balasubramanian, Y. Sun, M. Bobrovskyy, P. Sumbly, C. A. Genco, C. K. Vanderpool, and B. Tjaden, "Computational analysis of bacterial RNA-Seq data." [Online]. Available: <http://nar.oxfordjournals.org>. [Accessed: 15-Apr-2016].

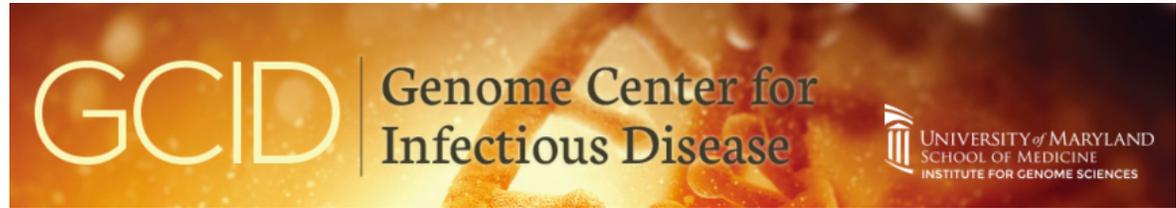


# Setting up a Submission to the PATRIC RNA Seq Service

- Upload data into a private workspace
- Select a strategy from two current strategies
  - Rockhopper (developed for bacteria)
  - Tuxedo (developed for eukaryotes but can be used on bacteria)
- Select a reference genome for mapping reads
  - Can be a public PATRIC genome
  - Can be one of your private genomes in your workspace
- Create or select an output folder in your workspace
- Create conditions and associate them to sets of reads

# RNA-seq in response to antibiotic stress

Bioproject: PRJNA234525



## Identification of transcriptional pathways associated with antibiotic stress in *Acinetobacter baumannii*

Once they reached an OD600 of 0.4, the cultures to receive antibiotic treatment were amended with 25% or 75% of the approximate MIC value of one antibiotic (meropenem, ciprofloxacin, amikacin sulfate, or polymyxin-B)

Transcriptome or Gene expression	
BioProject accession	Organism
<a href="#">PRJNA234519</a>	<a href="#">Acinetobacter baumannii 1000160</a>
<a href="#">PRJNA234520</a>	<a href="#">Acinetobacter baumannii 1207552</a>
<a href="#">PRJNA234521</a>	<a href="#">Acinetobacter baumannii 1428368</a>
<a href="#">PRJNA234522</a>	<a href="#">Acinetobacter baumannii 1457504</a>
<a href="#">PRJNA234523</a>	<a href="#">Acinetobacter baumannii 1564232</a>
<a href="#">PRJNA234524</a>	<a href="#">Acinetobacter baumannii 1592897</a>
<a href="#">PRJNA234516</a>	<a href="#">Acinetobacter baumannii 34654</a>
<a href="#">PRJNA234517</a>	<a href="#">Acinetobacter baumannii 478810</a>
<a href="#">PRJNA234518</a>	<a href="#">Acinetobacter baumannii 983759</a>

David Rasko

Institute for Genome Sciences/Microbiology and Immunology

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1432601>

<http://www.ncbi.nlm.nih.gov/bioproject/234524>

# RNA-seq in response to antibiotic stress

## RNA-Seq Analysis

Align reads, assemble transcripts, measure/test expression.

**Parameters** ⓘ  
STRATEGY  
Rockhopper  
Target Genome  
Acinetobacter baumannii 34654  
OUTPUT FOLDER  
Acinetobacter baumannii 34654  
OUTPUT NAME  
A\_baumannii\_34654\_COL\_BR2

**Groups/Conditions** ⓘ  
ON  
MHB-NaCl  
MHB-NaCl  
COL75  
COL25  
MHB

**Paired read library** ⓘ  
READ FILE 1  
SRR1184591\_1.fastq.gz  
READ FILE 2  
SRR1184591\_2.fastq.gz  
CONDITION  
MHB-NaCl

**Single read library** ⓘ  
READ FILE  
CONDITION  
Condition Name

**Selected libraries** ⓘ  
Place read files here using the arrow buttons.  
P(SRR11..tq.gz, SRR11..tq.gz)  
P(SRR11..tq.gz, SRR11..tq.gz)  
P(SRR11..tq.gz, SRR11..tq.gz)  
P(SRR11..tq.gz, SRR11..tq.gz)

Reset Submit

The conditions are:

MHB = Mueller Hinton Broth and represents “normal” growth conditions

MHB-NaCl = Mueller Hinton Broth and 200mM NaCl. This should invoke an osmotic stress response

COL25 = 25% MIC of polymyxin-b

COL75 = 75% MIC of polymyxin-b

The reference genome is

Acinetobacter baumannii 34654, a public genome in PATRIC

There are 45,065,646 genes in UniprotKB-GOA with MF annotations.  
Of those 22,433,281 genes have at least one leaf annotation.  
There are 76,239,509 non-redundant MF annotations.  
Of those 30,294,070 are leaves.

# Specific Contrasts

Services

## 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.

**Contrasts** ⓘ

Reset Submit

# Results

- ▶ Different strategies produce different results
- ▶ Both strategies produces this information
  - Transcripts
  - Operons
  - Alignments
  - Differential expression matrix

Result Files

	Filename	Type	File size
⬇	Rockhopper_1310581.3_transcripts.txt	txt	636.0 kB
⬇	Rockhopper_1310581.3_operons.txt	txt	77.3 kB
⬇	Rockhopper_SRR1184575_1.bam	bam	5.2 GB
⬇	Rockhopper_SRR1184591_1.bam	bam	4.9 GB
⬇	Rockhopper_SRR1184651_1.bam	bam	5.4 GB
⬇	Rockhopper_SRR1184656_1.bam	bam	4.3 GB
⬇	Rockhopper_summary.txt	txt	260.1 kB
⬇	Rockhopper_1310581.3_gene_exp.gmx	diffexp_input_data	256.9 kB

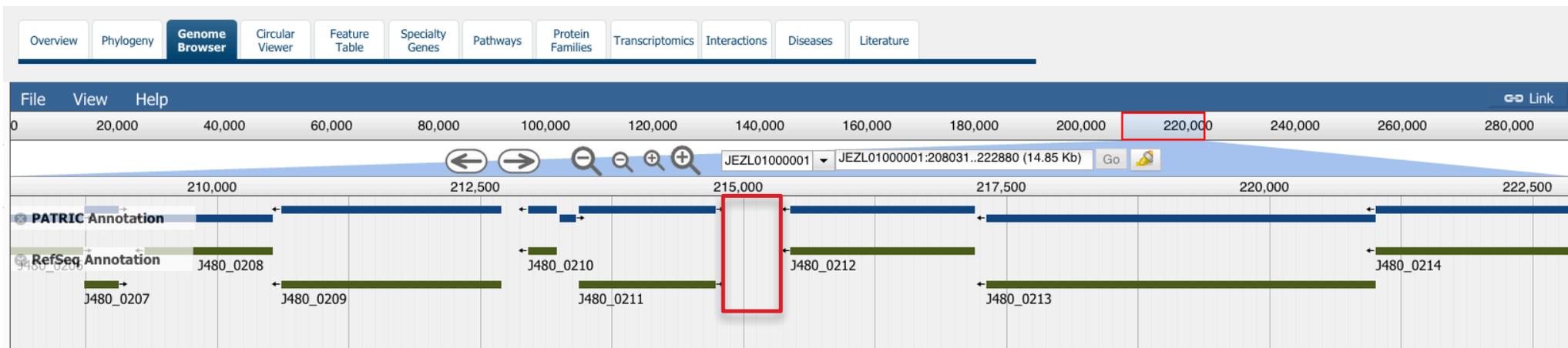
# Transcripts

- The output contains structural information
  - Transcription start and stop
  - Translation start and stop
- The output contains functional information
  - Gene product, gene synonyms
- The output contains cross references to the PATRIC databases
  - Contig identifiers and gene identifiers
- The output contains relative abundance measures of the transcripts
- The output contains q-values for the differentially expressed transcripts

# Visualizing Transcript Data in the Genome Browser

- Using the genome finder, locate the reference genome
- Filter the reference genome using the Contig ID
- Using the genome browser, zoom in on the area of interest

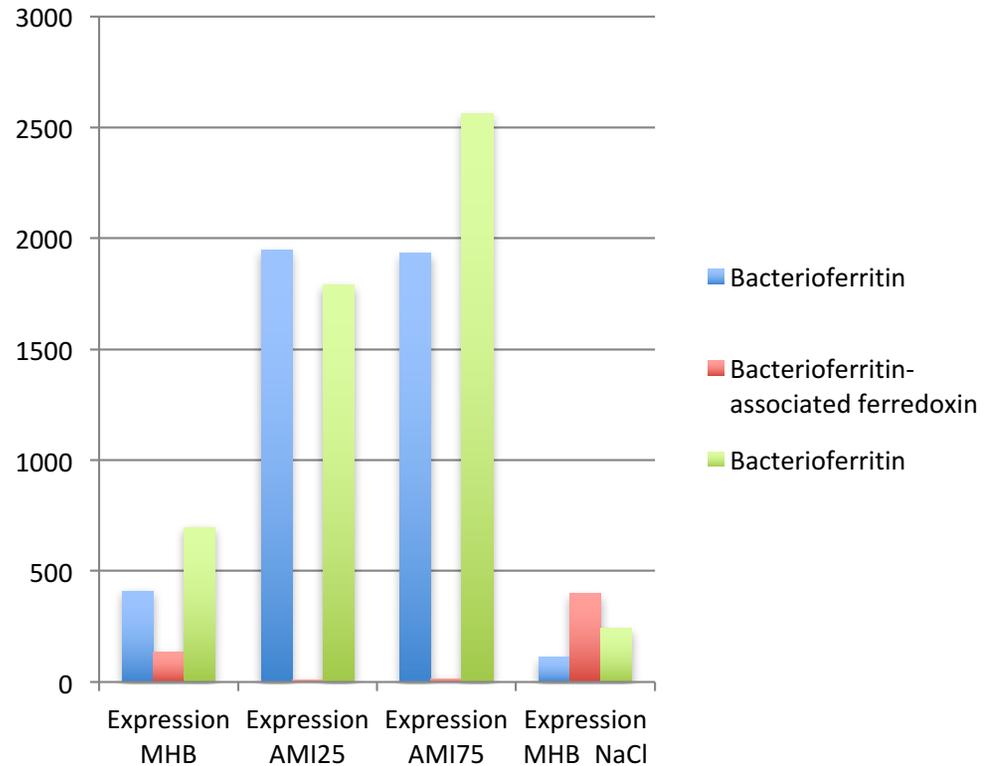
	Contig	Transcription Start	Translation Start	Translation Stop	Transcription Stop	Synonym	Product
	JEZL01000001	214905			214780	predicted RNA	-
	JEZL01000001	215347			215243	predicted RNA	-



# Work with the Results Off-line

- Data is easily downloaded
- Easy to import into MS-Excel

Normalized Counts



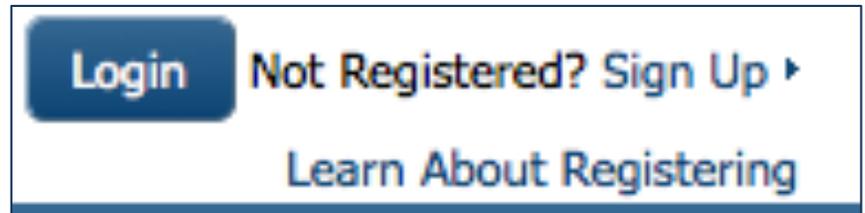
Product	qValue MHB vs AMI25	qValue MHB vs AMI75	qValue MHB vs MHB_NaCl	qValue AMI25 vs AMI75	qValue AMI25 vs MHB_NaCl	qValue AMI75 vs MHB_NaCl
Bacterioferritin	6.23E-03	3.92E-03	1	1	2.06E-33	1.46E-30
Bacterioferritin-associated ferredoxin	3.14E-11	5.73E-04	1	1	5.14E-11	1.50E-02
Bacterioferritin	7.32E-01	7.11E-02	1	1	8.17E-04	2.72E-09

# Integrate the Results with the Public Data in PATRIC

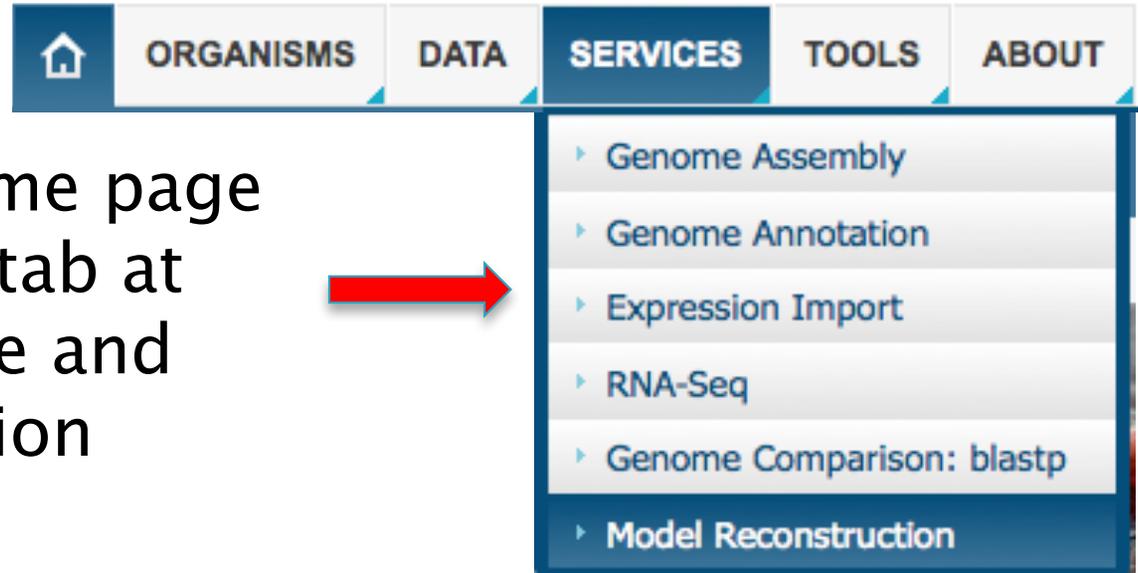
- ▶ The Differential Expression Import Service transforms and integrates differential expression data for viewing in PATRIC
- ▶ The data can be generated by the PATRIC RNA Seq service using any of the strategies
- ▶ The data can be generated using other chip based technologies using external software
- ▶ Differential expression objects are created in your workspace
- ▶ Differential expression objects contain expression data from different conditions

# PATRIC Expression Import

- ▶ Login to the PATRIC website at [www.patricbrc.org](http://www.patricbrc.org)



- ▶ On the PATRIC home page open the Services tab at the top of the page and select the Expression Import service



## Differential Expression Import

Transform differential expression data for viewing on PATRIC

### Experiment Data

EXPERIMENT DATA FILE

Rockhopper\_1310581.3\_gene\_exp.c 

EXPERIMENT TYPE

Transcriptomics 

### Optional Metadata

METADATA FILE

34654\_COL\_BR2\_metadata.xlsx 

### Experiment Information

EXPERIMENT TITLE

polymyxin-b treatment of A. baumannii 34654

EXPERIMENT DESCRIPTION

A. baumannii 34654 was treated with various le

ORGANISM NAME

Acinetobacter baumannii 34654 

PUBMED ID

*Optional*

OUTPUT FOLDER

Experiments 

Reset

Submit

Search



**PATRIC\_Workshop / home / Experiments**



DETAIL

Name	Size	Owner	Created
↑ Parent Folder			
 A baumannii 34654 treated with polymyxin-b	3.3 kB	PATRIC_Workshop	6/20/15, 11:22 AM
 A baumannii 34654 treated with meropenem	3.3 kB	PATRIC_Workshop	6/23/15, 8:00 AM
 A baumannii 34654 treated with ciprofloxacin	3.3 kB	PATRIC_Workshop	6/23/15, 8:01 AM
 A baumannii 34654 treated with amikacin sulfate	3.3 kB	PATRIC_Workshop	6/20/15, 11:25 AM



## PATRIC\_Workshop / home / Experiments / A baumannii 34654 treated with amikacin sulfate

Platform Organism: Acinetobacter baumannii 34654 Pubmed ID: Undefined

Genes Mapped/Genes Total: 4183/4183 Samples: 6

Title	Genes	Significant Genes (Log Ratio)	Significant Genes (Z Score) <span>+</span>
AMI25 MHB	4183	96	845
AMI75 AMI25	4183	39	130
AMI75 MHB	4183	105	889
MHB_NaCl AMI25	4183	165	165
MHB_NaCl AMI75	4183	167	167
MHB_NaCl MHB	4183	210	210

# Using Differential Expression Data at PATRIC

- You can select different conditions to compare
- Genes in the reference genome are displayed in a table containing
  - Product descriptions
  - Number of comparisons
  - How many comparisons the gene was up regulated
  - How many comparisons the gene was down regulated
- The table can be filtered using the log ratio or Z-score
  - This reduces the set of genes being displayed to those passing the filtering criteria
- The table can be filtered using text terms such as “transcription”

# View and Search using Heatmaps



**PATRIC\_Workshop / home / Experiments / A baumannii 34654 treated with meropenem**

Platform Organism: Acinetobacter baumannii 34654 Pubmed ID: Undefined

Genes Mapped/Genes Total: 4183/4183 Samples: 6



DETAIL



GENES

Title	Genes	Significant Genes (Log Ratio)	Significant Genes (Score)
MERO25 MHB	4183	95	701
MERO75 MERO25	4183	34	92
MERO75 MHB	4183	82	581
MHB_NaCl MERO25	4183	97	917
MHB_NaCl MERO75	4183	124	1037
MHB_NaCl MHB	4183	138	1186

Overview

Taxonomy

Phylogeny

Genome  
ListFeature  
TableSpecialty  
Genes

Pathways

Transcriptomics

Interactions

Diseases

Literature

## Transcriptomics Gene List

The gene list below provides details about gene regulation across a given set of experiments and comparisons. The gene list can be filtered based on regulation within each comparison as well as by locus tag and keyword. To learn more, see our [Transcriptomics Gene List FAQs](#).

**Filter By** <<

**Table** | Heatmap

**5 comparison(s)**

			Source	Title	Strain
<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	me	MERO25 MHB	
<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	me	MERO75 MHB	
<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	me	MHB_NaCl MER...	
<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	me	MHB_NaCl MER...	
<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	me	MHB_NaCl MHB	

Filter by Genome :

Filter by one or more keywords or locus tags

e.g. VBIescCol129921\_0001, Transcription factor

Filter by |Log Ratio|:  ▾

Filter by |Z-score|:  ▾

**Filter**

**339 genes found**

Workspace

Add Feature(s)

View

FASTA DNA

FASTA Protein

Download

Table ▾

FASTA ▾

Tools

Pathway Summary Multiple Seq Alignment

MAP IDs to... ▾

	Genome Name ▲	PATRIC ID	RefSeq Loc	Alt Locus T.	Gene Symb	Product Description	Comparisor	Up	Down
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		FIG00350452: hypot...	5	4	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>	piIV	Type IV fimbrial biog...	5	2	2
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Bacterioferritin-assoc...	5	3	2
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Bacterioferritin	5	2	3
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		hypothetical protein	5	1	1
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		amine oxidase, flavin...	5	0	3
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		TcuA: flavoprotein u...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		TcuC: integral memb...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Fatty acid desaturase	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Transcriptional regul...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		hypothetical protein	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		FIG00351518: hypot...	5	5	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Alpha,alpha-trehalos...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>	otsB	Trehalose-6-phosph...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Transcriptional regul...	5	3	0
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		Outer membrane rec...	5	3	2
<input type="checkbox"/>	Acinetobacter baum...	<a href="#">fig 1310...</a>	fig 1310...	<a href="#">VBIACiB...</a>		hypothetical protein	5	1	1

Thank you