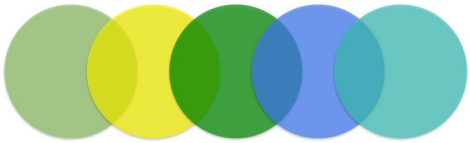
omics.data.edu.au

How to exploring differences in gene expression levels between two bacterial strains

STEP	Task
1	Go to http://omics.data.edu.au/use/
2	Launch the platform and log in
3	*Add the "ABPRI-Data" and "Degust" apps to the toolbar.*
5	Open the "ABPRI-Data" app.
6	Log-into the "ABPRI-Data" app using the username and password given to you (you will need to enter **omics** as the domain).
Steps 7-10 are to locate a file containing differential gene expression level data for two strains of Klebsiella pneumoniae (AJ218 and KPC2 (6 replicates of each)). This file has been generated by the ABRPI consortium.	
7	Search for "processed" "transcriptomic" data from "Klebsiella pneumoniae".
8	Send the resultant data file to the GenomeSpace part of the OMICs platform.
9	Locate the data you just sent to GenomeSpace.
10	Download the file onto your desktop.
The next steps are to import the file just downloaded into Degust, a differential gene expression exploration, analysis and visualisation tool.	
11	Launch Degust by clicking on the Degust app you added to the GenomeSpace toolbar in step 4. Degust is a public server and log-in is not necessary.
12	Upload the counts file to the Degust server that you have previously downloaded.
The counts file has 19 columns. Bowtie2 and featureCounts were used respectively by the ABPRI bioinformaticians for mapping and generating the quantification information in this file.	

Column Header	Description
GeneID	A project-specific gene identifier used in the ABPRI project (and common across the genomics and transcriptomics analysis)



Chr	chromosome/plasmid identifier in the species (Klebsiella pneumoniae)
Start	Gene start position on the chromosome sequence
End	Gene end position on the chromosome sequence
Strand	Strand (+ or -)
Length	Gene length
25861_PE_230bp_SEP_AGRF_CA3FUANXX_CTGAAGCT-CAGGACGT	Counts of gene expression in BPA sample **25861** (K. pneumoniae, strain **AJ218**)
g25862_PE_230bp_SEP_AGRF_CA3FUANXX_TAATGCG C-CAGGACGT	Counts of gene expression in BPA sample **25862** (K. pneumoniae, strain **AJ218**)
25863_PE_230bp_SEP_AGRF_CA3FUANXX_CGGCTATG-CAGGACGT	Counts of gene expression in BPA sample **25863** (K. pneumoniae, strain **AJ218**)
25864_PE_230bp_SEP_AGRF_CA3FUANXX_TCCGCGAA-CAGGACGT	Counts of gene expression in BPA sample **25864** (K. pneumoniae, strain **AJ218**)
25865_PE_230bp_SEP_AGRF_CA3FUANXX_TCTCGCGC-CAGGACGT	Counts of gene expression in BPA sample **25865** (K. pneumoniae, strain **AJ218**)
25866_PE_230bp_SEP_AGRF_CA3FUANXX_AGCGATAG-CAGGACGT	Counts of gene expression in BPA sample **25866** (K. pneumoniae, strain **AJ218**)
25867_PE_230bp_SEP_AGRF_CA3FUANXX_ATTACTCG-CAGGACGT	Counts of gene expression in BPA sample **25867** (K. pneumoniae, strain **KPC2**)
25868_PE_230bp_SEP_AGRF_CA3FUANXX_TCCGGAGA-CAGGACGT	Counts of gene expression in BPA sample **25868** (K. pneumoniae, strain **KPC2**)
25869_PE_230bp_SEP_AGRF_CA3FUANXX_CGCTCATT-CAGGACGT	Counts of gene expression in BPA sample **25869** (K. pneumoniae, strain **KPC2**)
25870_PE_230bp_SEP_AGRF_CA3FUANXX_GAGATTCC-CAGGACGT	Counts of gene expression in BPA sample **25870** (K. pneumoniae, strain **KPC2**)
25871_PE_230bp_SEP_AGRF_CA3FUANXX_ATTCAGAA-CAGGACGT	Counts of gene expression in BPA sample **25871** (K. pneumoniae, strain **KPC2**)
25872_PE_230bp_SEP_AGRF_CA3FUANXX_GAATTCGT-CAGGACGT	Counts of gene expression in BPA sample **25872** (K. pneumoniae, strain **KPC2**)
Annotation	Gene symbol, EC_number (when known)

13	Configure Degust as follows: **Name**: Enter a name for your analysis **Format Type:** TSV

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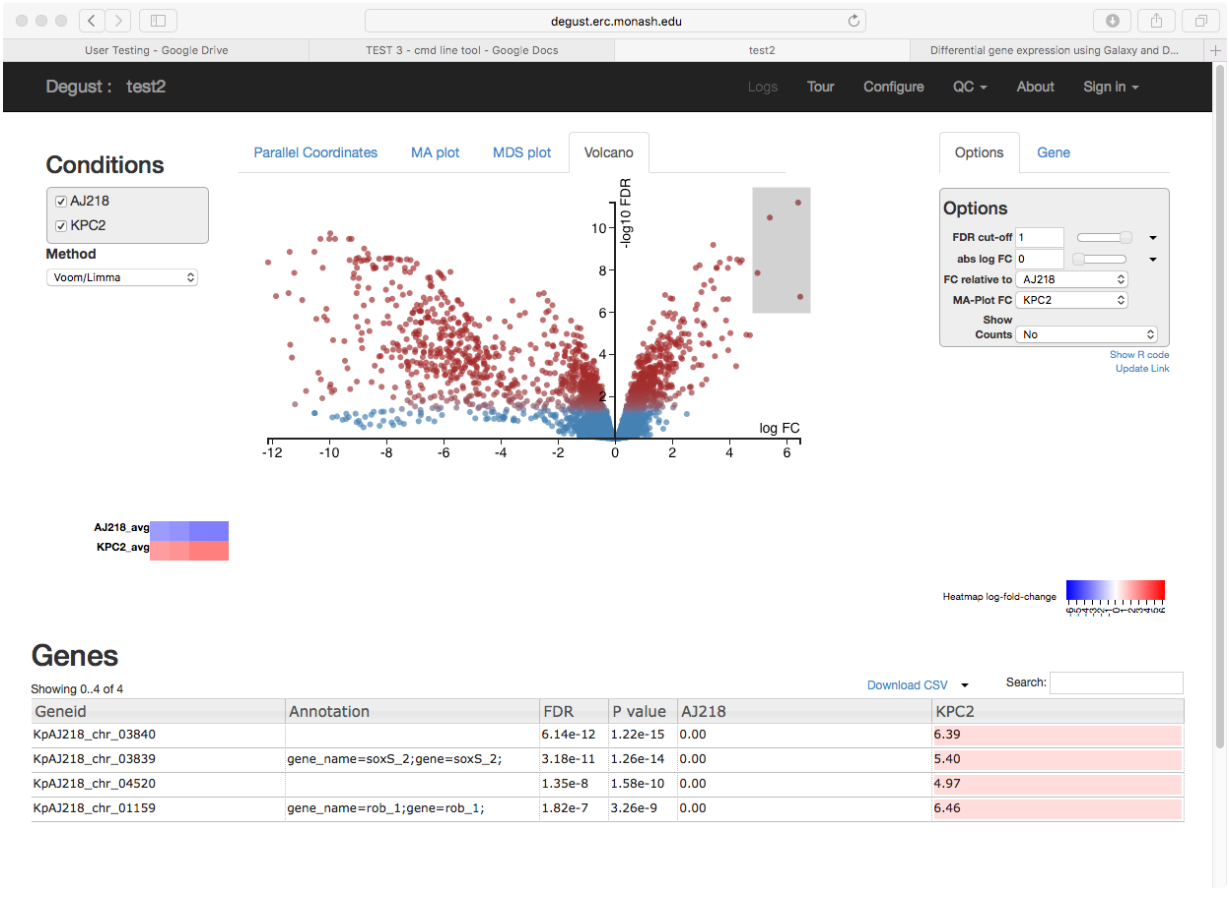
	Info Columns: select GeneID and Annotation **EC Number Column:** Leave blank **Gene Link Column:** Leave blank **Gene link URL:** Leave blank **Analyse server side:** Check the box **Min gene read count:** 10 **Min gene CPM:** Leave Blank
14	Define the two conditions (which are strains in this case) and replicates by clicking on 'Add condition.
15	Name the first condition as **AL218**, and select the columns starting with numbers `25861-25866` as the 6 replicates.
16	Name the second condition as **KPC2**, and select the columns starting with numbers `25867-25872` as the 6 replicates .
17	Click on View. The most over/underexpressed genes in one strain relative to the other can be interactively explored using various tools (e.g. the volcano plot as shown below)



If you need further assistance, please contact us at omicsdataservices@lists.unimelb.edu.au