

CTox Test Set

User Test 2: DAS

Generate Gene Fold Factors

Data Source

This test set data comes in-house repository of DAS. This data set has four biological replicates for the liver tissue of Rats. The animals were exposed to different dosage of Myclobutanil, a conazole class fungicide.

URL:

Species: Rate Liver Tissue: Myclobutanil

Experimental Time Points and RNA-seq count data files:

Table 1

	Acetone	Clo (cod liver oil)	PB(phenobarbital)
Control	2377_cont_diet	2377_cont_diet	2377_cont_diet
Control	2378_cont_diet	2378_cont_diet	2378_cont_diet
Control	2379_cont_diet	2379_cont_diet	2379_cont_diet
Control	2380_cont_diet	2380_cont_diet	2380_cont_diet
Control	2381_cont_diet	2381_cont_diet	2381_cont_diet
Intervention	2382_acet_diet	2427_Clo	2432_PB
Intervention	2383_acet_diet	2428_Clo	2433_PB
Intervention	2384_acet_diet	2429_Clo	2434_PB
Intervention	2385_acet_diet	2430_Clo	2435_PB
Intervention	2386_acet_diet	2431_Clo	2436_PB

Running The Test Case

1. Generate a new study using the following data (or similar)

Parameter Name	Suggested Value
Study name	Rat Liver Tissue Myclobutanil Compound
Study info	3 comparisons- Acetone, CLO and PB
Source	DAS in house

Select [Save and add/edit experiments]

2. Generate in succession, 3 experiments relating to the 3 different dose endpoints with the data similar to below.

[Note: use [Save and add another] to reduce data entry between experiments]

Parameter Name	Exp1 Values	Exp 2 Values	Exp 3 Values
Tech	RNASeq-Rnor_6.0.80	RNASeq-Rnor_6.0.80	RNASeq-Rnor_6.0.80
Compound Name	Acetone-Myclobutanil	Clo-Myclobutanil	PB-Myclobutanil
Dose	5	5	5
Dose Unit	micrograms/day	micrograms/day	micrograms/days
Time	1	1	1
Tissue	liver	liver	liver




Parameter Name	Exp1 Values	Exp 2 Values	Exp 3 Values
Organism	Rat	Rat	Rat
Strain	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley
Gender	Male	Male	Male
Repeat Type	single-dose	single-dose	single-dose
Route	diet	diet	diet
Experiment name	Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	Clo-Myclobutanil-1d-5micrograms/day-single-LI-RATM	PB-Myclobutanil-1d--5micrograms/day-single-LI-RATM

[Note]: Add the first experiment details and then click on [Save and Add Another]. Add the second experiment details and then again click on [Save and Add Another], continue till all the experiments are added. Then Select [Save and Upload Samples]

- Confirm that the 3 experiments are available for data upload. Click on [Save and upload samples] to proceed to the next step.

Adding samples for study:
Rat Liver Tissue Myclobutanil Compound

Review existing experiments associated with this study. **Any non-selected experiments will be deleted.**

- ☒ PB-Myclobuta-1.00d-5.00micrograms/day-single-LI-RATM  Edit
- ☒ Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM  Edit
- ☒ Acetone-Mycl-1d-5micrograms/day-single-LI-RATM  Edit

Save and upload samples

Save and add experiments

Cancel

- The study and the experiments will be listed. Click on the desired tab i.e. RNAseq File or Affy CEL Files. Upload the single tab delimited file containing all samples using [Choose File] option and then [Upload Files] and continue to the next step.

Adding experiments for study:
Rat Liver Tissue Myclobutanil Compound

Associated experiments:

- PB-Myclobuta-1.00d-5.00micrograms/day-single-LI-RATM
- Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM
- Acetone-Mycl-1d-5micrograms/day-single-LI-RATM

Upload samples by selecting either a single file that contains the RNAseq results or multiple files relating to the Affy Cel files

Upload Sample Files

RNAseq FileAffy CEL Files

Upload a single delimited (comma or tab) containing gene or probe identifiers in first column and samples in additional columns;
the column header will be the sample name

single file containing all samples

Choose File

No file chosen

Upload Files

Cancel

- Confirm that the Gene Count data is available as desired for all the samples listed in the Table 1. Click on [Save].

Bulk addition of samples for study:
Rat Liver Tissue Myclobutanil Compound

Sample name

2377_conf_diet

Sample name

2378_conf_diet

Sample name

2379_conf_diet

Sample name

2433_PB

Sample name

2434_PB

Sample name

2435_PB

Sample name

2436_PB

Save

Cancel

6. Again, review the samples list. Click on [Save and done with samples]

Review existing samples associated with this study. Any non-selected samples will be deleted.

Samples

- ☒ 2377_cont_diet
- ☒ 2378_cont_diet
- ☒ 2379_cont_diet
- ☒ 2380_cont_diet
- ☒ 2381_cont_diet
- ☒ 2382_acet_diet
- ☒ 2383_acet_diet
- ☒ 2384_acet_diet
- ☒ 2385_acet_diet
- ☒ 2386_acet_diet
- ☒ 2427_Clo
- ☒ 2428_Clo
- ☒ 2429_Clo
- ☒ 2430_Clo
- ☒ 2431_Clo
- ☒ 2432_PB
- ☒ 2433_PB
- ☒ 2434_PB
- ☒ 2435_PB
- ☒ 2436_PB

Save and done with samples **Save and add samples** **Cancel**

7. Next iterate through the each of the 3 experiments and assist (highlight) the appropriate controls and interventions for each of the experiments. Click on [Save and continue].

Success

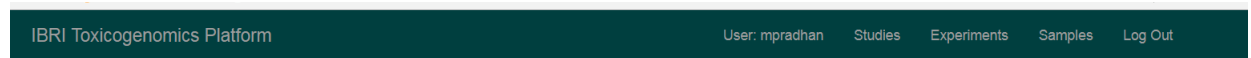
Submitted process for group fold changes using: experiments and 30 samples.

An email will be sent to mpadhan@indianabiosciences.org when the job completes and the information is available.

10. User receives an email on completion of analysis process.

Results

11. Click [Studies] on the app tab



12. Study page [shows user studies]. Also, can see the Qc icon for the figures.

Available Studies							
Get Exps	Edit	Qc	Study Name	Source	Date Created	Owner	Permission
→	✎	📊	Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpadhan	Private
→	✎	📊	Female Mice Liver Tissue Furan Treated	GEO	08/04/2017 7:03 p.m.	mpadhan	Private
→	✎	📊	TG-Gates Cholesterol Study CPD 142	TG-GATES	08/04/2017 2:56 p.m.	mpadhan	Private

[+ New Study Data](#)

13. Click on the Qc icon and down load the images. There are six images.

- (i) Max counts for ALL genes- all samples
- (ii) Clustering on ALL genes
- (iii) Max counts for protein coding genes – all samples
- (iv) Clustering on protein coding genes
- (v) Filtered Gene Counts
- (vi) Normalized Gene Counts

IBRI Toxicogenomics Platform
User: mpradhan
Studies
Experiments
Log Out

Available Studies

Get Exps	Edit	QC	Study Name	Source	Date Created	Owner	Permission
→	✎	📄	Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpradhan	Private
→	✎	📄 <small>View QC File</small>	Mice Liver Tissue Furan Treated	GEO	08/04/2017 7:03 p.m.	mpradhan	Private
→	✎		TG-Gates Cholesterol Study CPD 142	TG-GATES	08/04/2017 2:56 p.m.	mpradhan	Private

+ New Study Data

14. To analyze the experiments in the given study. Click on [Get Exps]

IBRI Toxicogenomics Platform
User: mpradhan
Studies
Experiments
Log Out

Available Studies

Get Exps	Edit	QC	Study Name	Source	Date Created	Owner	Permission
→	✎	📄	Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpradhan	Private
→ <small>Get experiments</small>	✎		Female Mice Liver Tissue Furan Treated	GEO	08/04/2017 7:03 p.m.	mpradhan	Private
→	✎		TG-Gates Cholesterol Study CPD 142	TG-GATES	08/04/2017 2:56 p.m.	mpradhan	Private

+ New Study Data

15. Select the experiments. Click [Analyze].

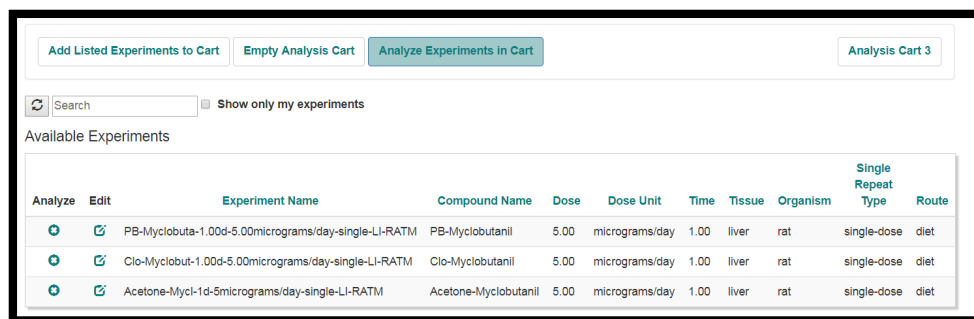
Add Listed Experiments to Cart
Analysis Cart 0

Search
☐ Show only my experiments

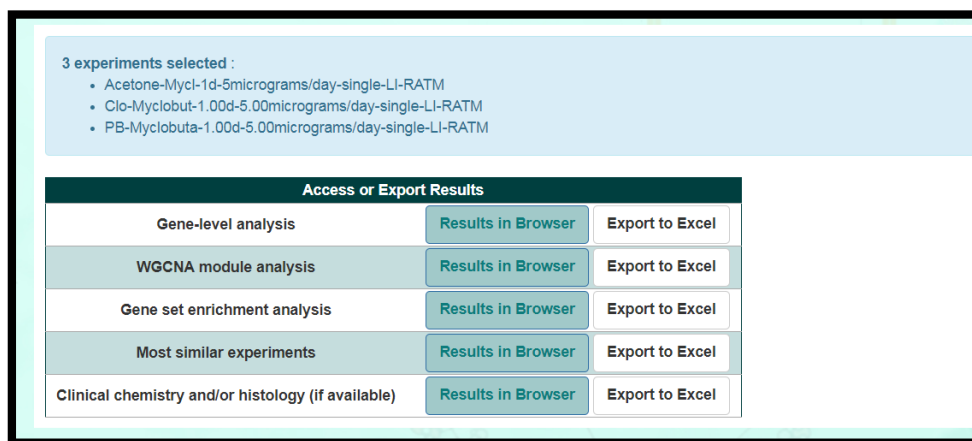
Available Experiments

Analyze	Edit	Experiment Name	Compound Name	Dose	Dose Unit	Time	Tissue	Organism	Single Repeat Type	Route
+	✎	PB-Myclobuta-1.00d-5.00micrograms/day-single-LI-RATM	PB-Myclobutanil	5.00	micrograms/day	1.00	liver	rat	single-dose	diet
+	✎	Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	Clo-Myclobutanil	5.00	micrograms/day	1.00	liver	rat	single-dose	diet
+	✎	Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	Acetone-Myclobutanil	5.00	micrograms/day	1.00	liver	rat	single-dose	diet

16. As the experiments are added a new pop-screen is displayed. After addition of all the experiments, click [Analyze Experiments in Cart].



17. In the results page, there will be icons for Gene-level analysis, WGCNA module Analysis, Gene set enrichment analysis, Most similar experiments and clinical chemistry and/or histology. User can export the individual analysis file to excel.



18. Gene-level analysis. Click [Results in browser]. Get the differentially expressed genes for experiments. They can be ranked based on the p-value, log2FC or PBh. Exporting to excel and further analysis can identify the unique and the common genes across the experiments. After exporting or analysis click [Back to Results Summary Table] and return to the result page.

[← Back to Results Summary Table](#)
[Export to Excel](#)

Gene Identifier
Gene Symbol
Log2 fold-change greater/equal than
Log2 fold-change less/equal than
P is less than or equal to
Adjusted-P less than

[Update](#)
[Clear](#)

Experiment	Gene Identifier	Rat Gene Symbol	log2 Fc	P	P Bh
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG00000001770	Ehhadh	5.34	2.94826943938125e-303	3.90586735329229e-299
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG00000006997	App	3.07	5.29873345197356e-287	7.01976207717459e-283
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG000000002948	Abcc3	4.71	6.94212403282034e-271	4.59846296920771e-267
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG000000007862	Acat1	2.35	7.87047946791821e-247	5.21340559954902e-243
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG000000012181	Lpl	5.15	2.42942501624219e-175	6.4370045230353e-172
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG000000009597	Cyp4a1	4.46	5.95869748389025e-159	1.31568040444297e-155

19. WCGNA analysis. Click [Results in browser].

Get the WCGNA modules and rank them based on positive or negative scores or Module Name Contains. The data can be exported and analyzed for uniqueness and similarities. The modules can also be studied for their functional significance using the supplementary material. After analysis or exporting to Excel, click [Back to Results Summary Table] to return to the results page.

[← Back to Results Summary Table](#)
[Export to Excel](#)

Module name contains
Score is less than or equal to
Score is greater than or equal to

[Update](#)
[Clear](#)

Experiment	Module	Score
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	DM.liver:285	3.99
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	DM.liver:159	3.53
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	DM.liver:159	3.07
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	DM.liver:277	2.31
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	DM.liver:85	2.28
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	DM.liver:293	2.26

20. Gene Set Enrichment Analysis. Click [Results in browser]. Identify the gene sets that are highly scored with low p-values. After analysis or exporting to Excel, click [Back to Results Summary Table] to return to the results page.

[← Back to Results Summary Table](#)
[Export to Excel](#)

Gene set name
GSA score greater/equal than
GSA score less/equal than
Adjusted-P less than

[Update](#)
[Clear](#)

Experiment	Geneset	Score	P Bh
Clo-Myclobut-1.00d-5.00micrograms/day-single-Li-RATM	GO.0004300	9.52	1e-17
Clo-Myclobut-1.00d-5.00micrograms/day-single-Li-RATM	RegNet.Creb3/3	11.60	1e-17
Clo-Myclobut-1.00d-5.00micrograms/day-single-Li-RATM	MOOTHA_MITOCHONDRIA	8.85	1e-17
Clo-Myclobut-1.00d-5.00micrograms/day-single-Li-RATM	RegNet.Mtrf	8.73	1e-17
Acetone-Mycl-1d-5micrograms/day-single-Li-RATM	RegNet.Rab24	9.54	1e-17

21. Most Similar Experiments. Click [Results in browser]. Identify the most similar experiments in the database to the input experiment based on the Source, Correl or Rank. Click [Back to Results Summary Table] to return to the results page.



22. Click [Toxicology results for similar experiments]