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.

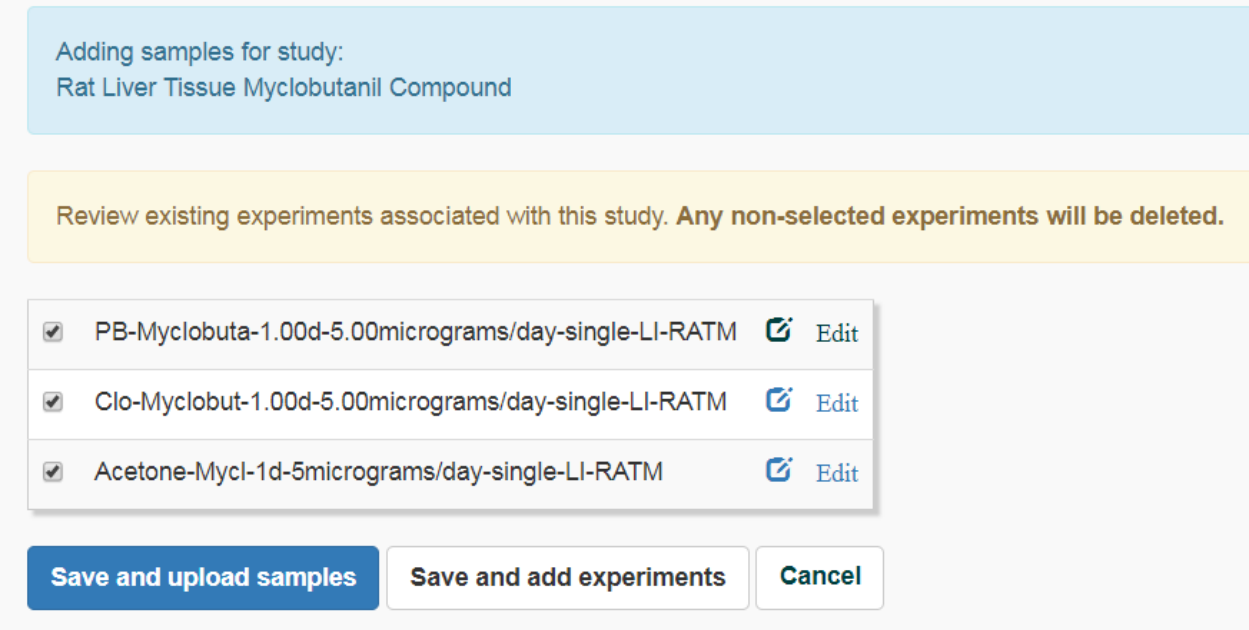
[Note: use **[Save and add another]** to reduce data entry between experiments]

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

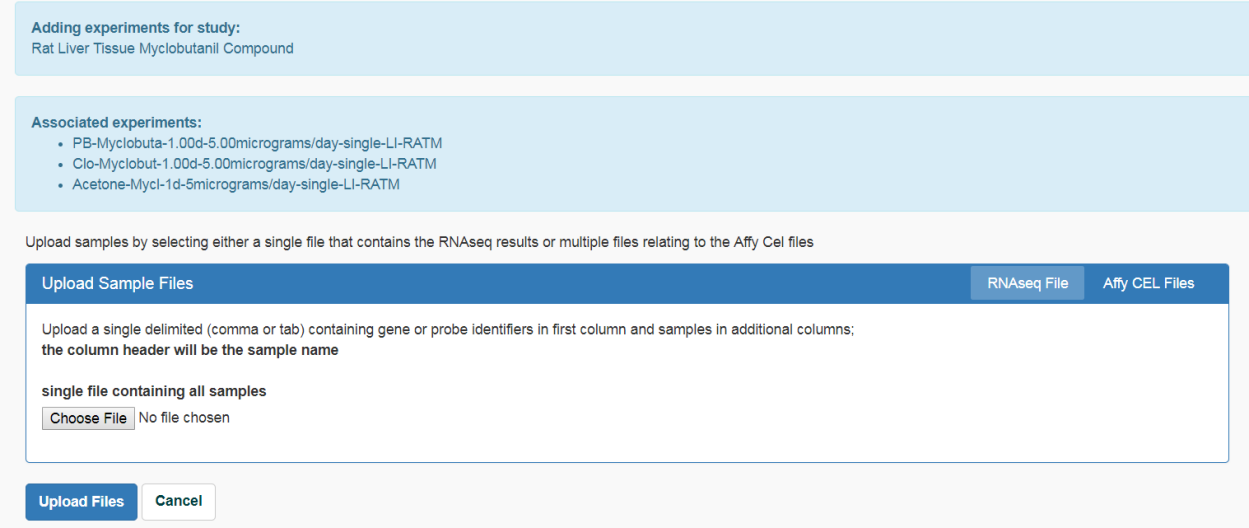
Parameter Name	Exp1 Values	Exp 2 Values	Exp 3 Values
Organism	Rat	Rat	Rat
Strain	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley
Gender	Male	Male	Male
Repeat Type	single-dose	single-dose	single-dose
Route	diet	diet	diet
Experiment name	Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	Clo-Myclobutanil-1d-5micrograms/day-single-LI-RATM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

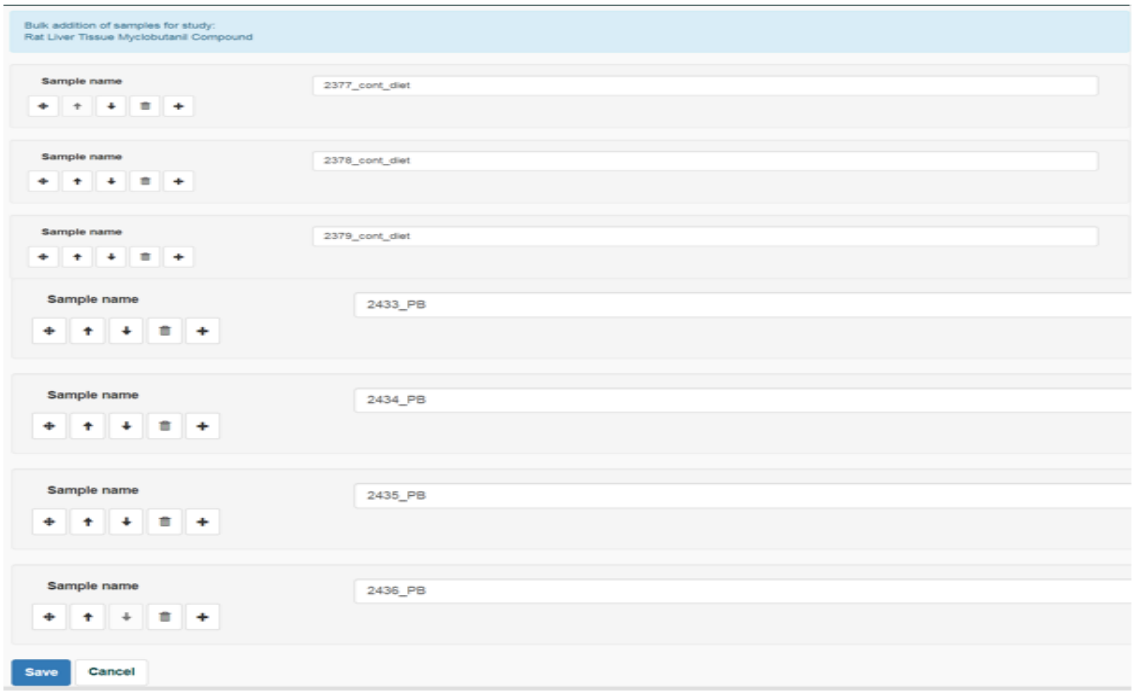
3. Confirm that the 3 experiments are available for data upload. Click on [Save and upload samples] to proceed to the next step.



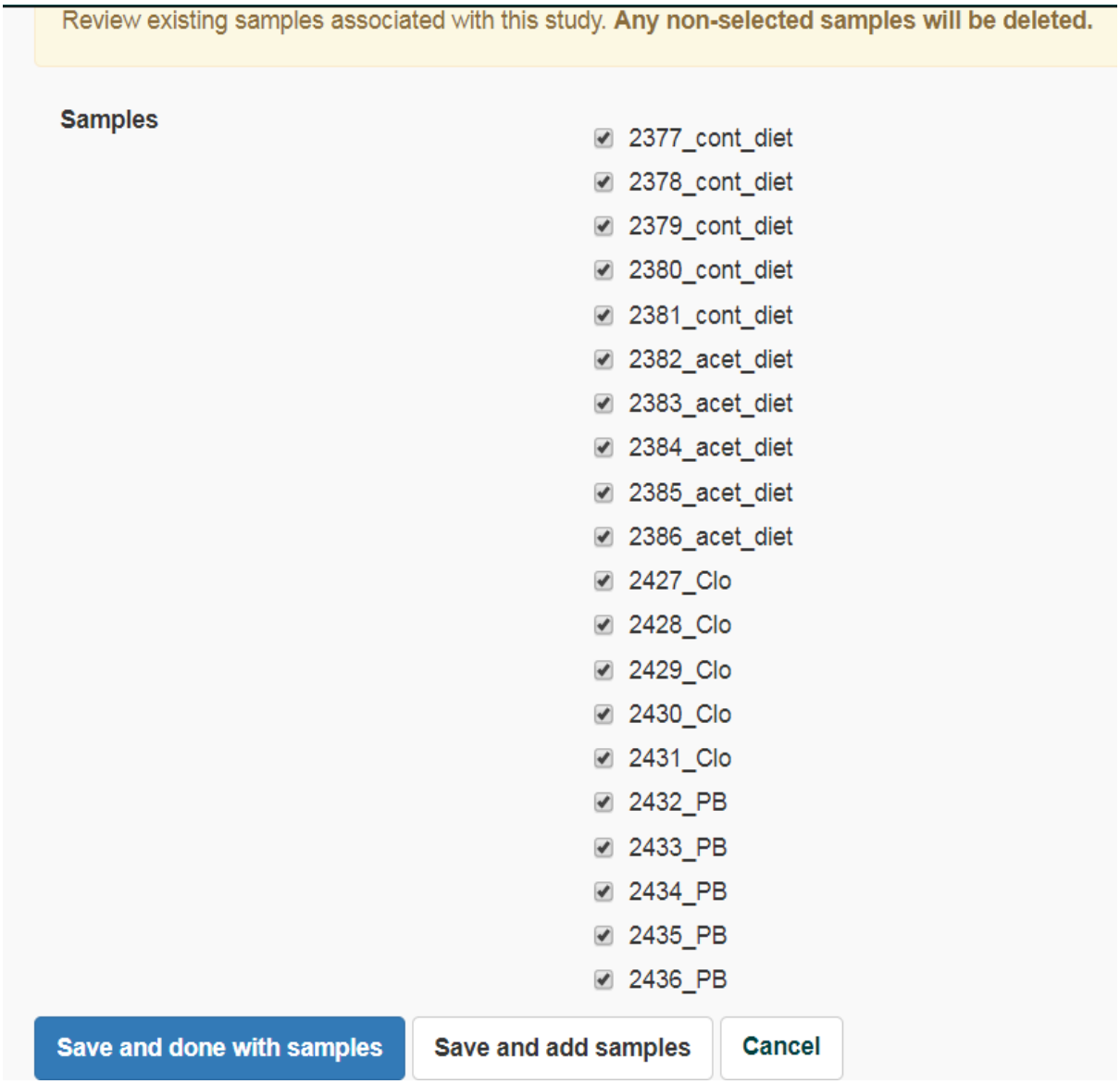
4. The study and the experiments will be listed. Click on the desired tab i.e. RNAseq File or Affy CEL Files. Upload the single tab delimited file containing all samples using [Choose File] option and then [Upload Files] and continue to the next step.



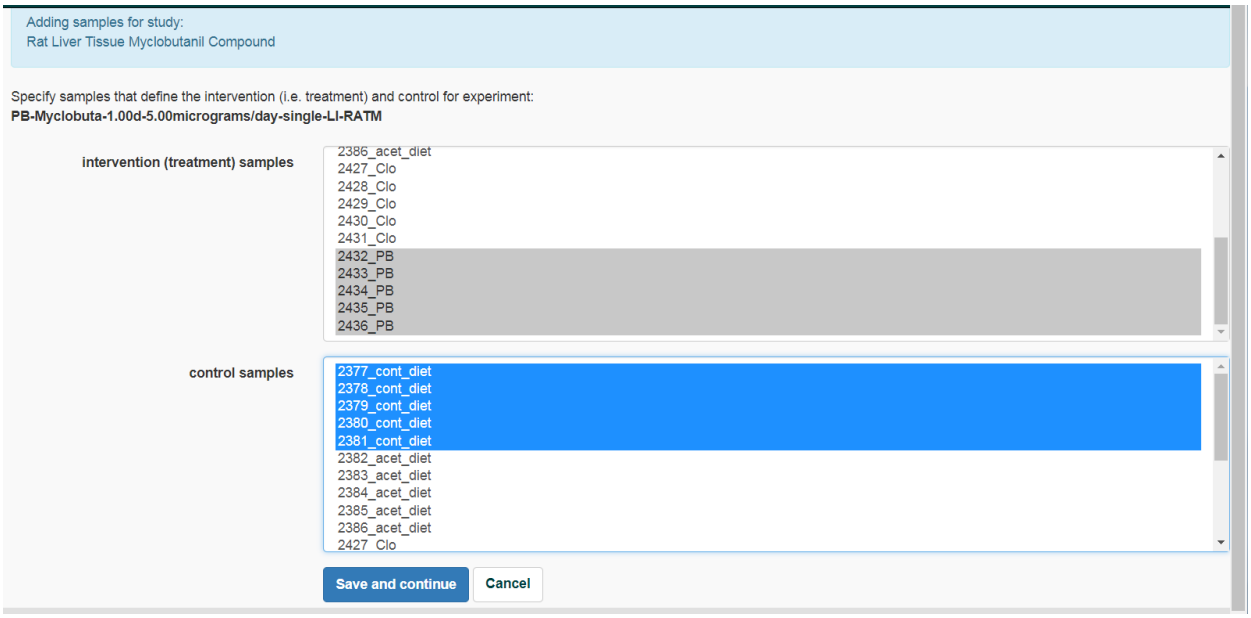
5. Confirm that the Gene Count data is available as desired for all the samples listed in the Table 1. Click on [Save].



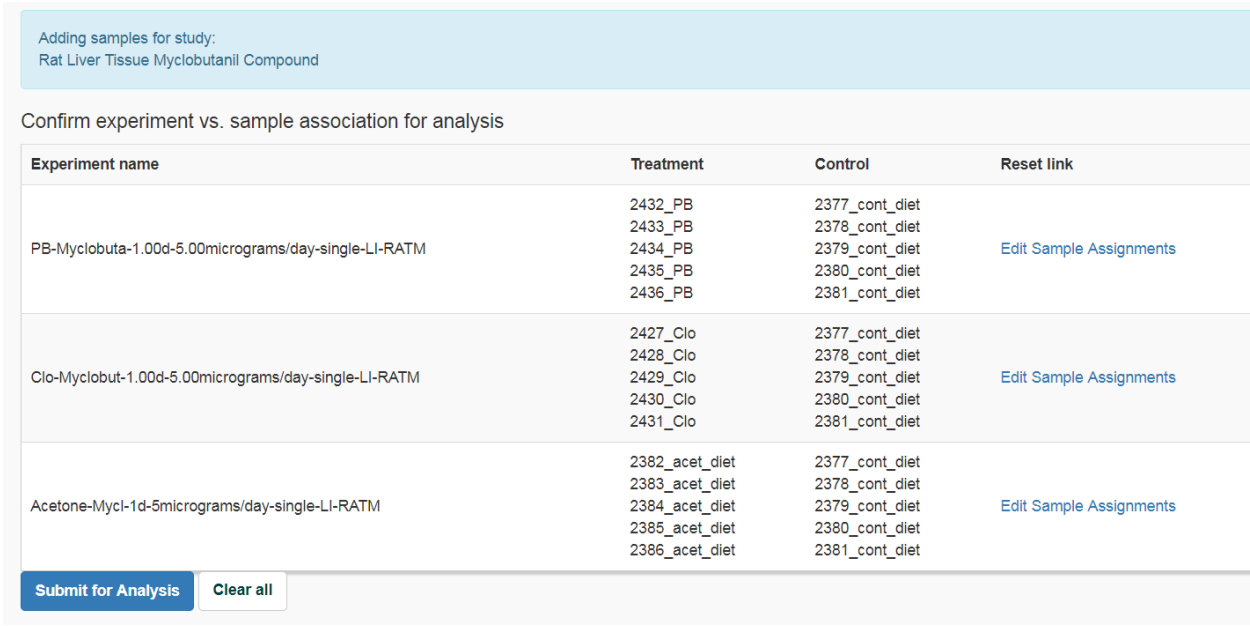
6. Again, review the samples list. Click on [Save and done with samples]



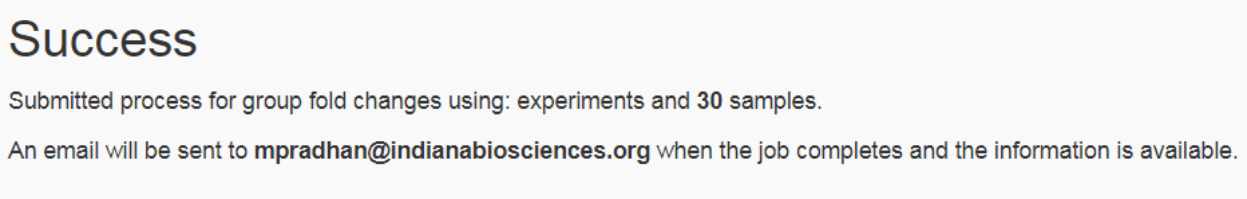
7. Next iterate through the each of the 3 experiments and assist (highlight) the appropriate controls and interventions for each of the experiments. Click on [Save and continue].



8. Confirm that the sample files are associated with the correct experiment and have the appropriate I/C (intervention or control) designation. Click [Submit for Analysis] to move to the next step to perform the calculations to generate the group fold factors.



9. If the submission process is correct, an output message as SUCCESS is printed and on completion of the fold change task an email with the link to results is received.

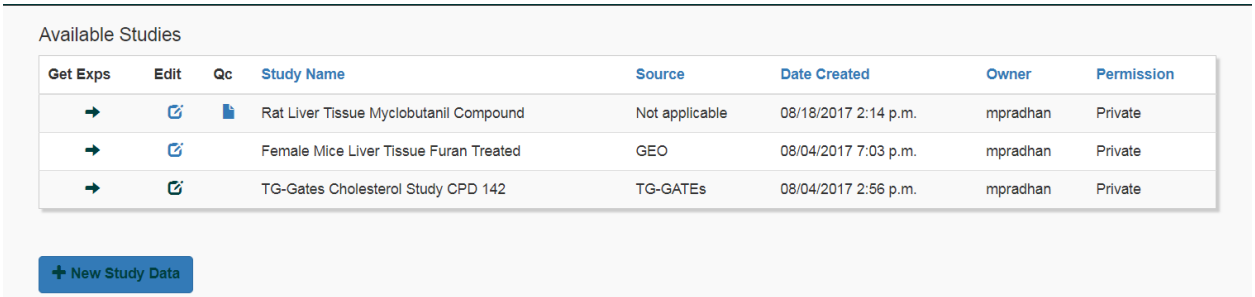


10. User receives an email on completion of analysis process.

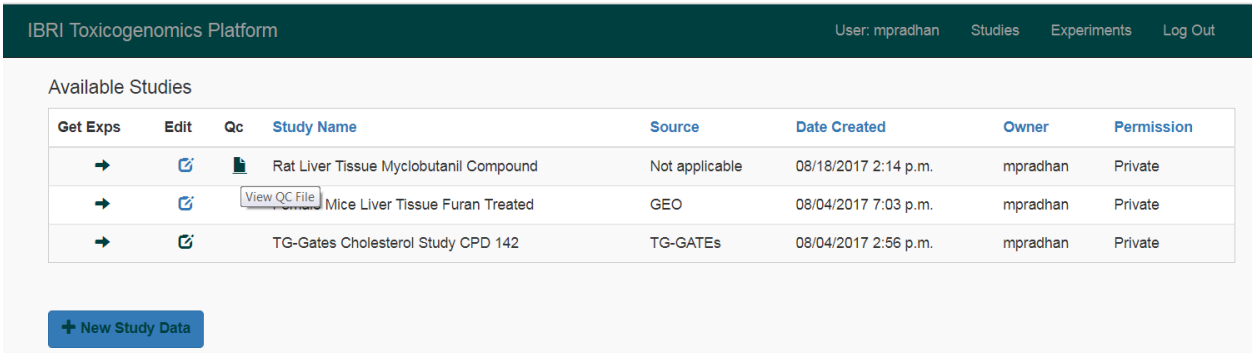
Results

11. Click [Studies] on the app tab

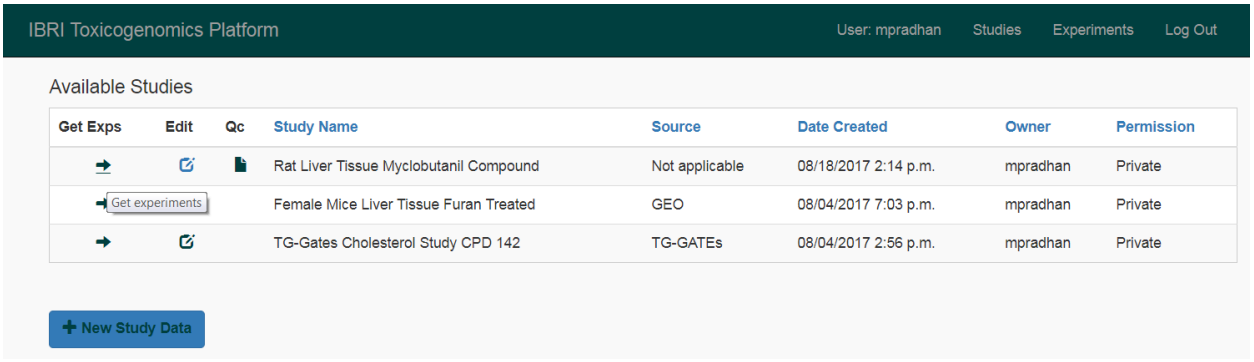
12. Study page [shows user studies]. Also, can see the Qc icon for the figures.



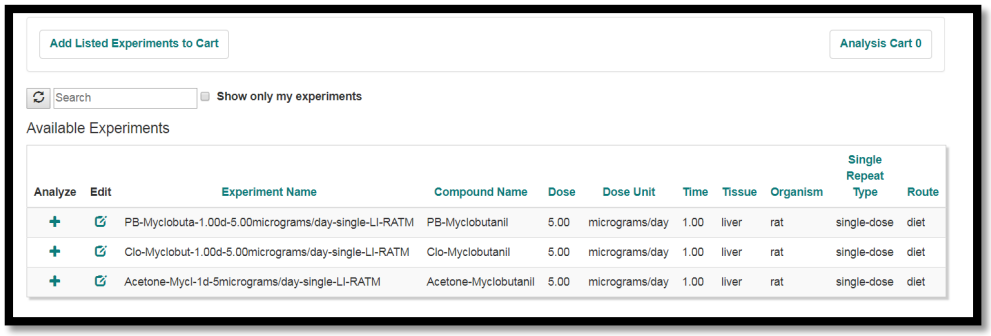
13. Click on the Qc icon and down load the images. There are six images.
    (i) Max counts for ALL genes- all samples
    (ii) Clustering on ALL genes
    (iii) Max counts for protein coding genes – all samples
    (iv) Clustering on protein coding genes
    (v) Filtered Gene Counts
    (vi) Normalized Gene Counts



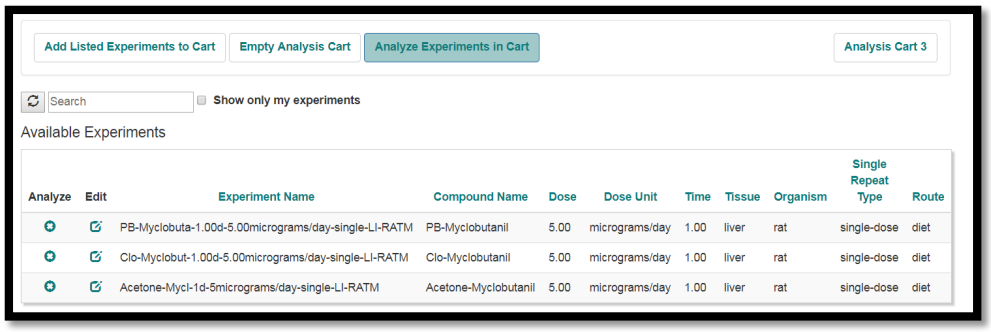
14. To analyze the experiments in the given study. Click on [Get Exps]



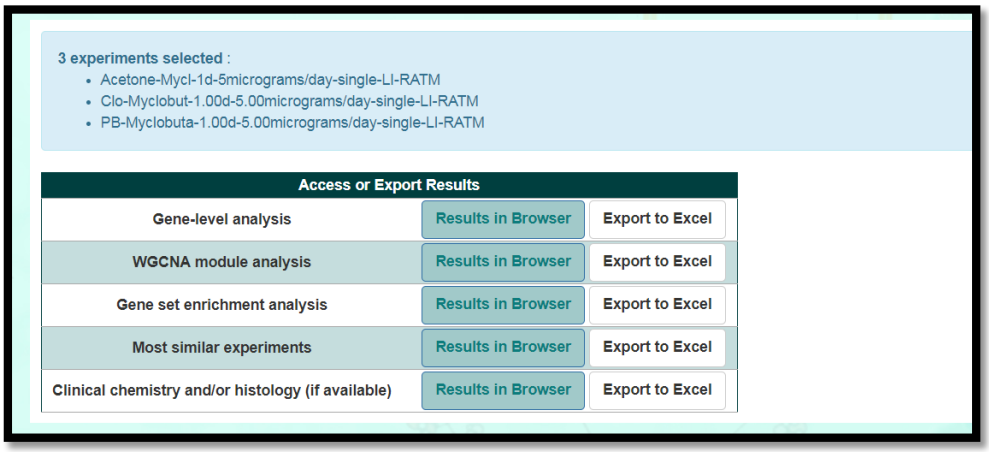
15. Select the experiments. Click [Analyze].



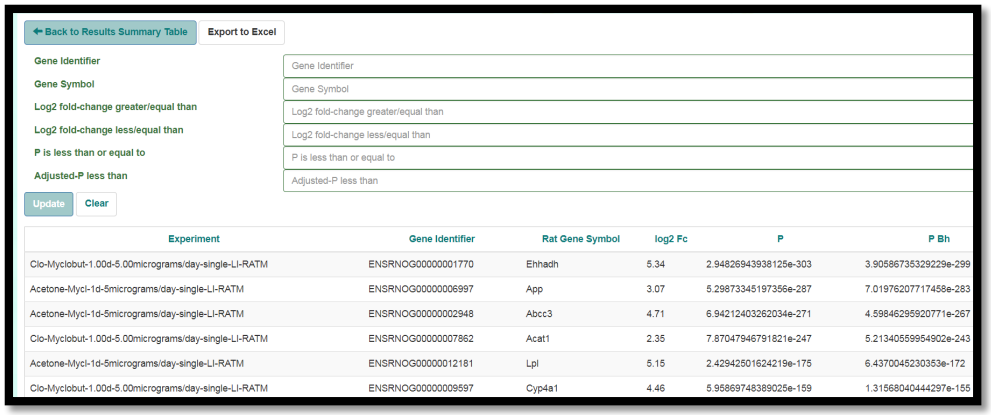
16. As the experiments are added a new pop-screen is displayed. After addition of all the experiments, click [Analyze Experiments in Cart].



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.

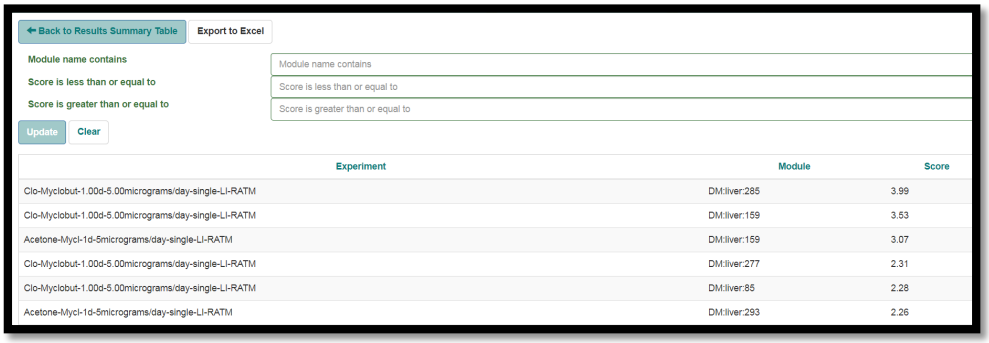


18. Gene-level analysis. Click [Results in browser]. Get the differentially expressed genes for experiments. They can be ranked based on the p-value, log2FC or PBh. Exporting to excel and further analysis can identify the unique and the common genes across the experiments. After exporting or analysis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



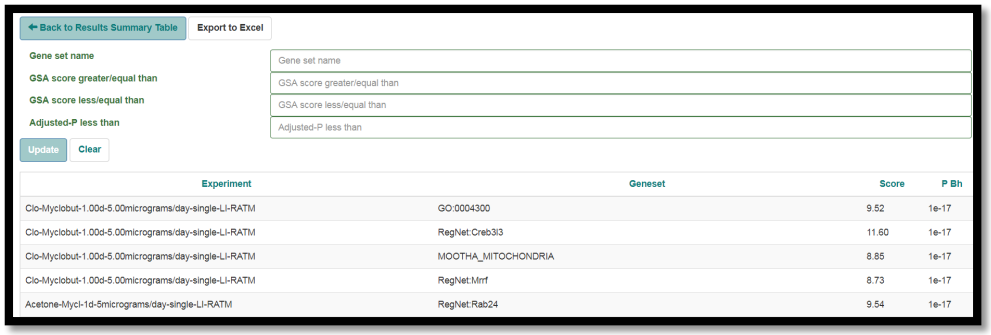
Experiment	Gene Identifier	Rat Gene Symbol	log2 Fc	P	P Bh
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG00000001770	Ehhadh	5.34	2.94826943938125e-303	3.90586735329229e-299
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG00000006997	App	3.07	5.29873345197356e-287	7.01976207717458e-283
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG00000002948	Abcc3	4.71	6.94212403262034e-271	4.59846295920771e-267
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG00000007862	Acat1	2.35	7.87047946791821e-247	5.21340559954902e-243
Acetone-Mycl-1d-5micrograms/day-single-LI-RATM	ENSRNOG00000012181	Lpl	5.15	2.42942501624219e-175	6.4370045230353e-172
Clo-Myclobut-1.00d-5.00micrograms/day-single-LI-RATM	ENSRNOG00000009597	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.



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.



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]