TCP Test Set

User Test 1: TG-GATEs

Generate Gene Fold Factors

Data Source

This test set comes from Open TG-GATEs [cpd 142] which used Affymetrix chip Rat 230A arrays (GPL3240)and six weeks old male Sprague-Dawley rats for both experiments and a single dose study design.

[TODO: Need to find reference to data and confirm field values]

http://toxico.nibiohn.go.jp/open-tggates/english/common/screen4/compound?compound_id=00161&design_name=Rat230_2&organ_id=ORGA0010&test_type=in+vivo

Species: Rat Tissue: Liver Compound: 1% cholesterol + 0.25% sodium cholate

Experimental Time Points and CEL files:

	4-day	**8-day**	**15-day**	**29-day**
Control	4day_c1.CEL	8d_c1.CEL	15d_c1.CEL	29d_c1.CEL
Control	4day_c2.CEL	8d_c2.CEL	15d_c2.CEL	29d_c2.CEL
Control	4day_c3.CEL	8d_c3.CEL	15d_c3.CEL	29d_c3.CEL
High Dose	4day_h1.CEL	8d_h1.CEL	15d_h1.CEL	29d_h1.CEL
High Dose	4day_h2.CEL	8d_h2.CEL	15d_h2.CEL	29d_h2.CEL
High Dose	4day_h3.CEL	8d_h3.CEL	15d_h3.CEL	29d_h3.CEL

These have been renamed for the test case for clarity from original file names in TG-Gates as to allow easier identification of which samples goes with which experiment and whether they are controls of interventions. The original file names are detailed in the table below

	4-day	8-day	15-day	29-day
Control	003017905028	003017906004	003017906010	003017906016
Control	003017905029	003017906005	003017906011	003017906018
Control	003017905030	003017906006	003017906012	003017906029
High Dose	003017906001	003017906007	003017906013	003017906019
High Dose	003017906002	003017906008	003017906014	003017906020
High Dose	003017906003	003017906009	003017906015	003017906021

Running The Test Case

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

Parameter Name	Suggested Value
Study name	TG-Gates Cholesterol Study CPD 142
Study info	4 High doses and 4 controls at 4 time points
Source	TG-Gates

Select [Save and add/edit experiments]

2. Generate in succession, 4 experiments relating to the 4 different dose endpoints with the data similar to below.

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

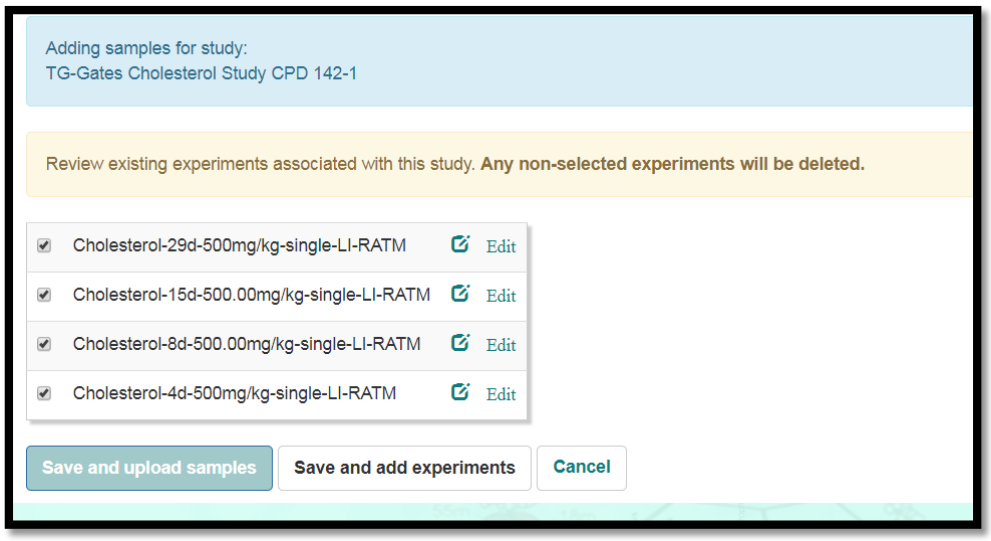
For the first experiment the file "GPL3240.txt" needs to be updated to allow selection of "microarray-RG230A"

Parameter Name	Exp 1 Values	Exp 2 Values	Exp 3 Values	Exp 4 Values
Tech	microarray-RG230A	microarray-RG230A	microarray-RG230A	microarray-RG230A
Compound Name	Cholesterol	Cholesterol	Cholesterol	Cholesterol
Dose	500	500	500	500
Dose Unit	mg/kg	mg/kg	mg/kg	mg/kg
Time	4	8	15	29
Tissue	liver	liver	liver	liver
Organism	rat	rat	rat	rat
Strain	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley
Gender	male	male	male	male
Repeat Type	single-dose	single-dose	single-dose	single-dose
Route	diet	diet	diet	diet
Experiment name [Auto populate]	Cholesterol-4d-500mg/kg-single-LI_RATM	Cholesterol-8d-500mg/kg-single-LI-RATM	Cholesterol-15d-500mg/kg-single-LI-RATM	Cholesterol-29d-500mg/kg-single-LI-RATM

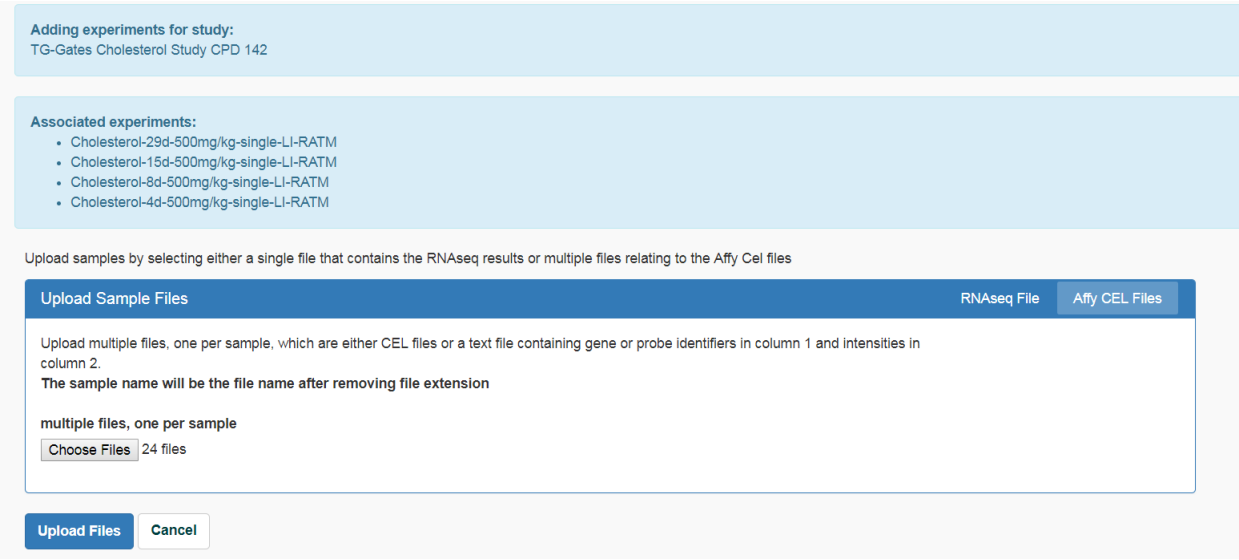
[Note] Add the first experiment details and then click on [Save and Add Another]. Add the second experiment details and then again click on [Save and Add Another], continue till all the experiments are added.

Finally, select [Save and Upload Samples]

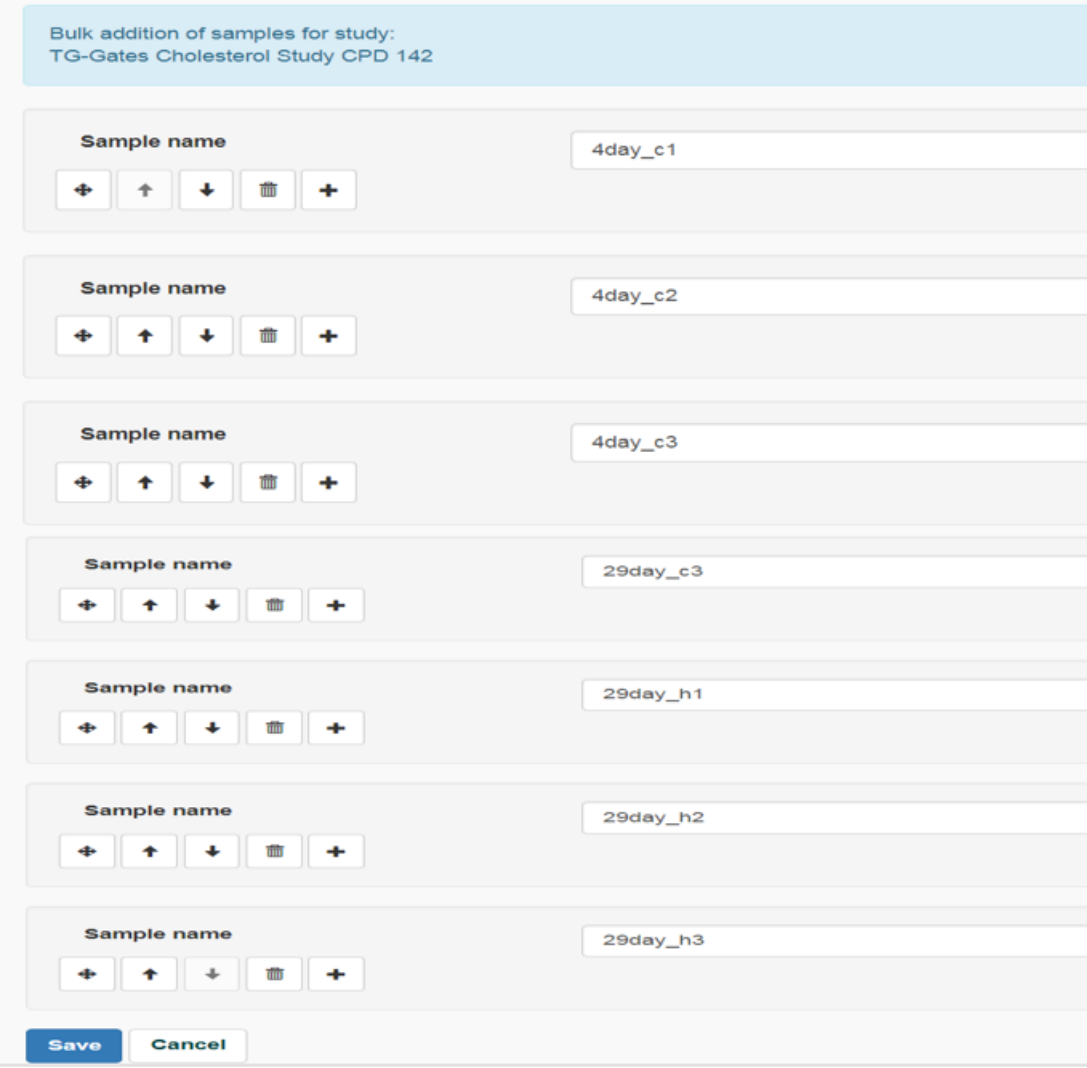
3. Confirm that the 4 experiments are available for data upload. The checkbox list should include the 4 experiments that were created in the above step 2 related to the study created in step 1. Click on [Save and upload samples] to proceed to the next step.



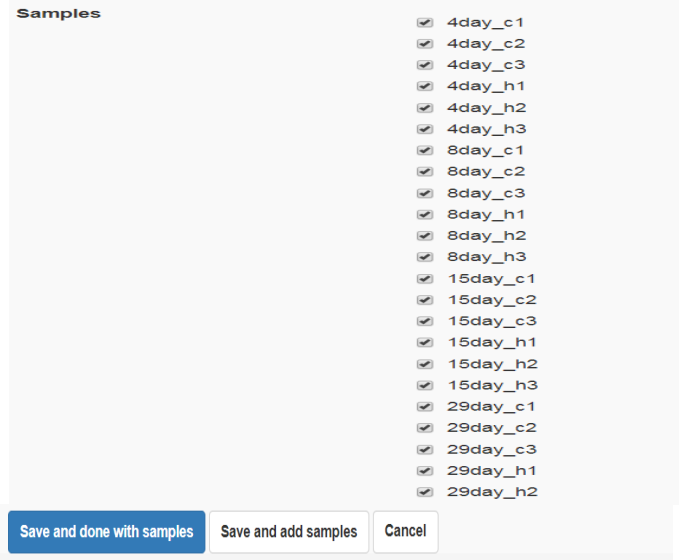
4. The study and the experiments will be listed. Click on appropriate tab [RNAseqFile] or [Affy Cel Files]. Click on the "Multiple files" [Choose Files] and then click [Upload Files] and upload the 24 CEL files.



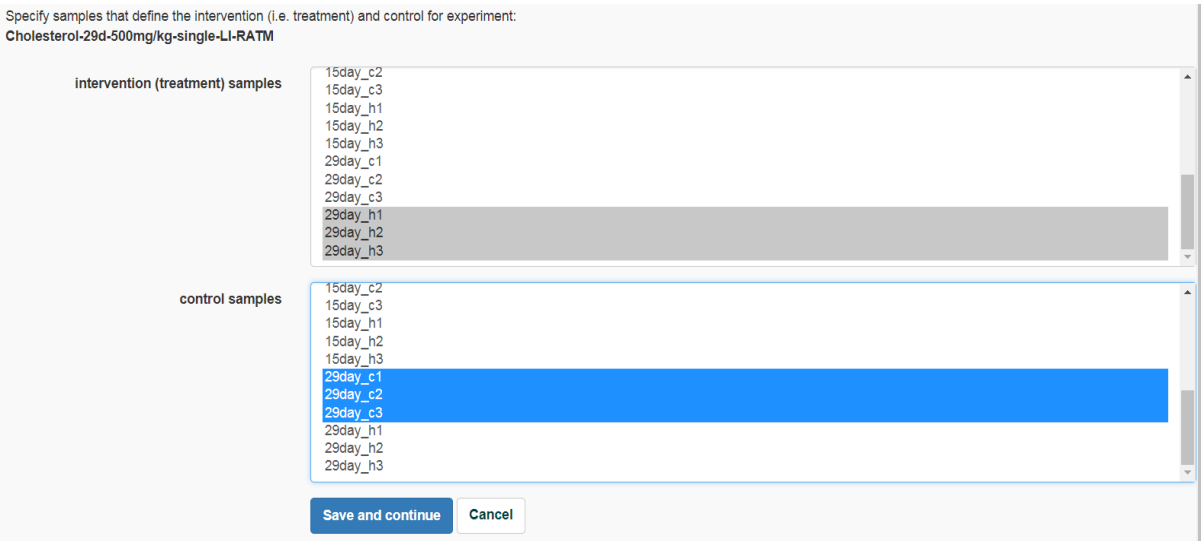
5. Confirm the 24 CEL files. Click[Save]



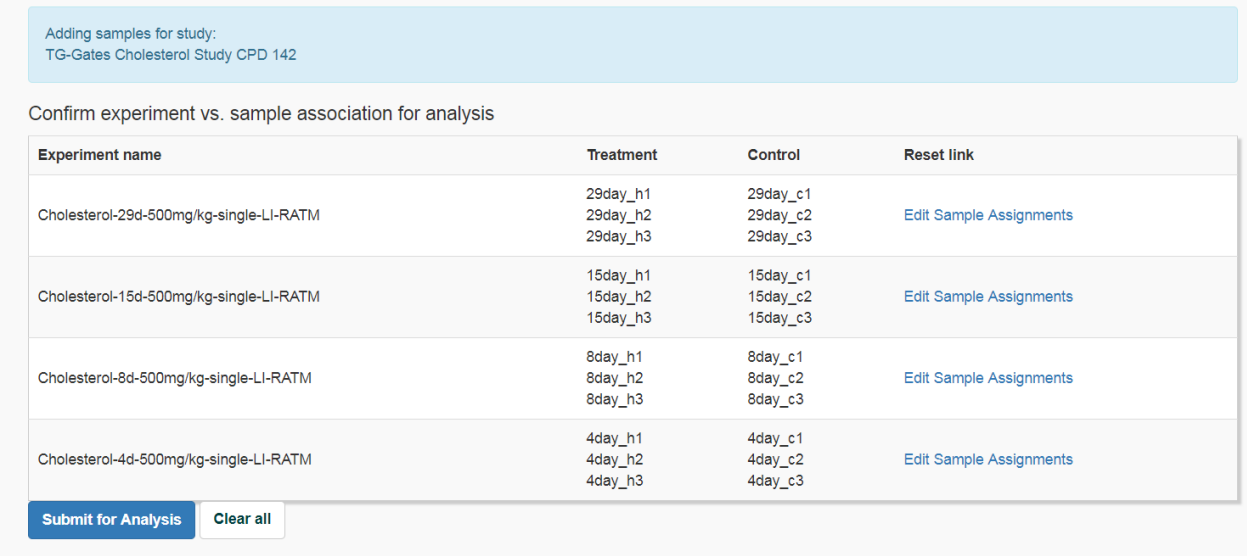
6. Review/Confirm the samples list again i.e. 24 CEL files. Click [Save and done with samples].



7. Next iterate through each of the 4 experiments and assist (highlight) the appropriate controls and interventions for each of the experiments. Click [Save and Continue] till all the experiments are annotated with their control and intervention.

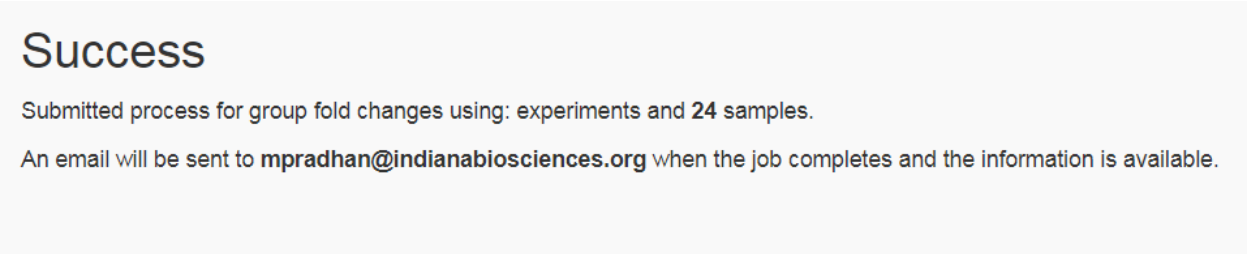


8. Confirm that the sample files are associated with the correct experiment and have the appropriate I/C (intervention or control) designation. Click [Submit for Analysis] to move on to the next step to perform the calculations to generate the group fold factors.



9. If submission 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.

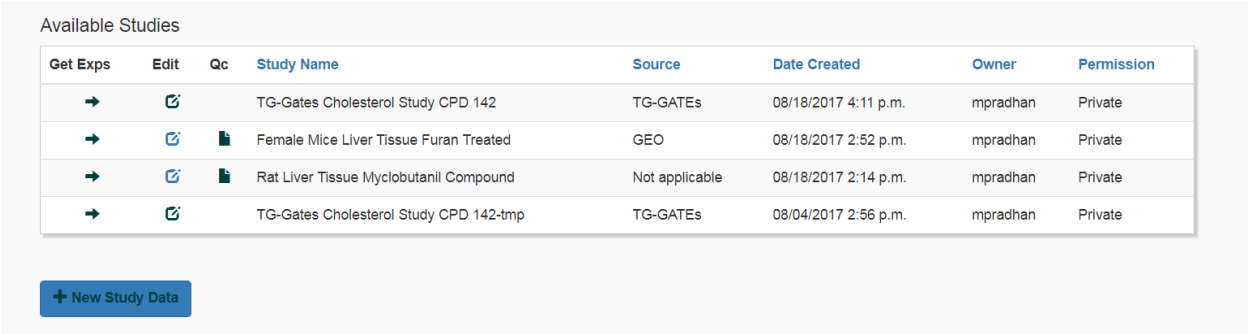
Depending on the experiments and number of samples, the analysis will complete in 5 to 30 minutes.



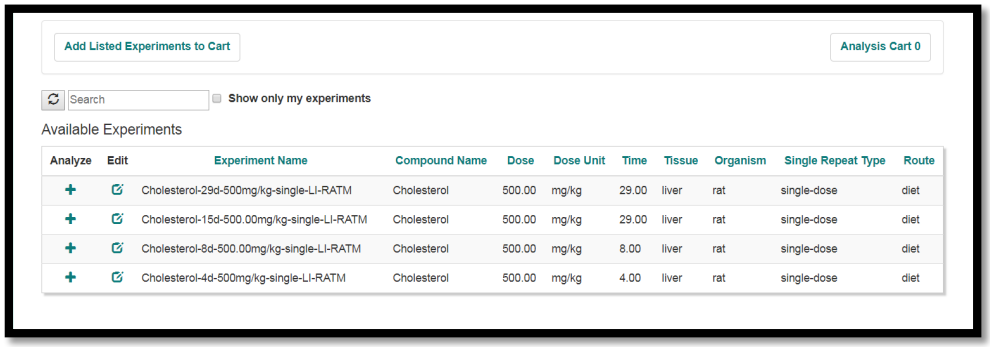
Result Analysis

10. Click [Studies] on the tab.

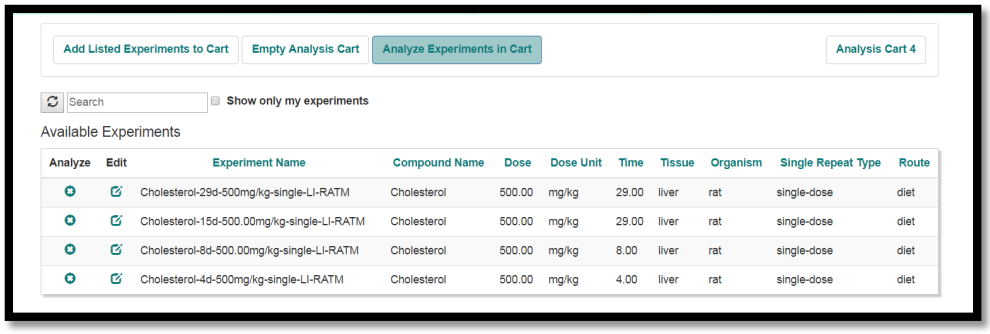
11. Click [Studies], will show all the user studies. Select the experiment. Click [Get Exps].



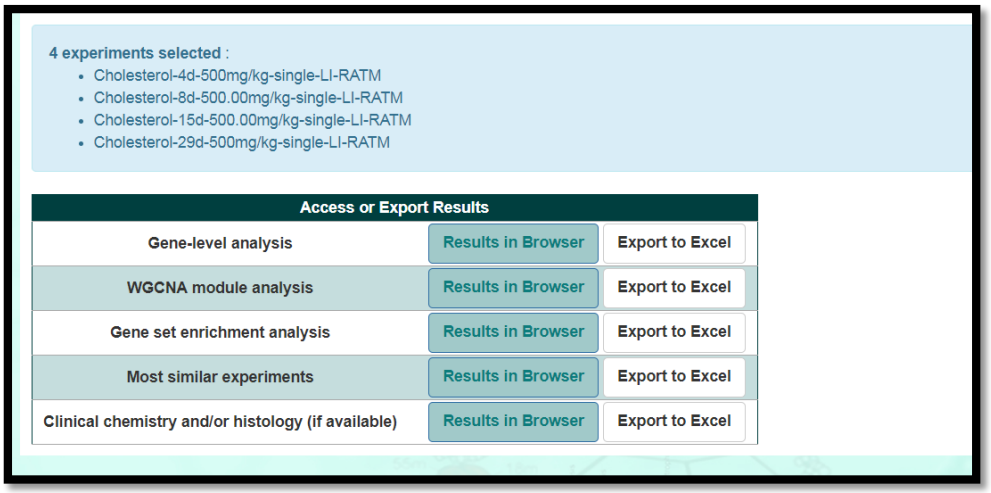
12. Select the experiments for analysis. Click [Analyze].



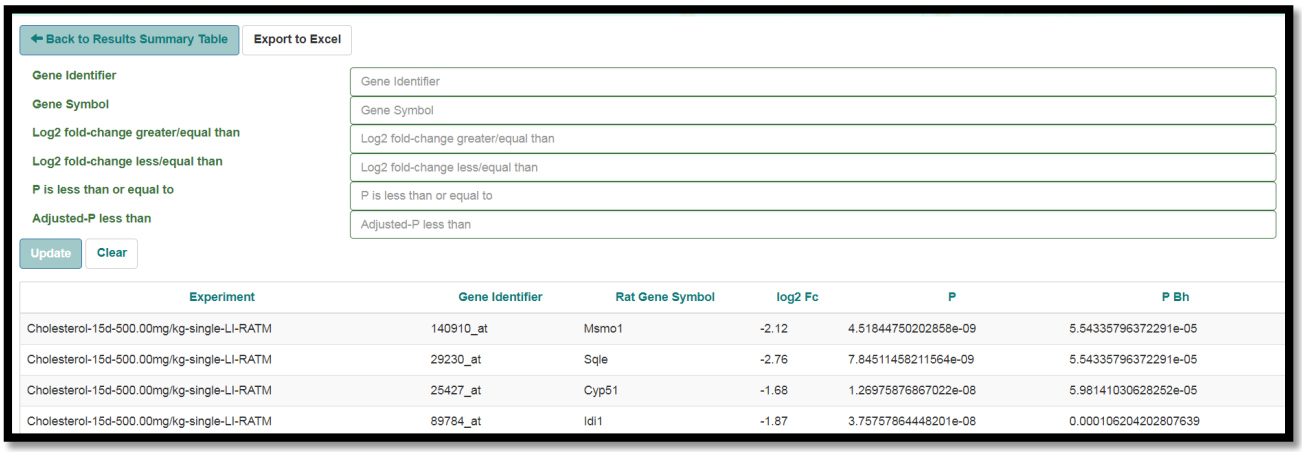
13. As the experiments are added a new pop-screen is displayed. After addition of all the experiments, click [Analyze Experiments in Cart].



14. 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 analysis files to excel or analyze the results in browser.

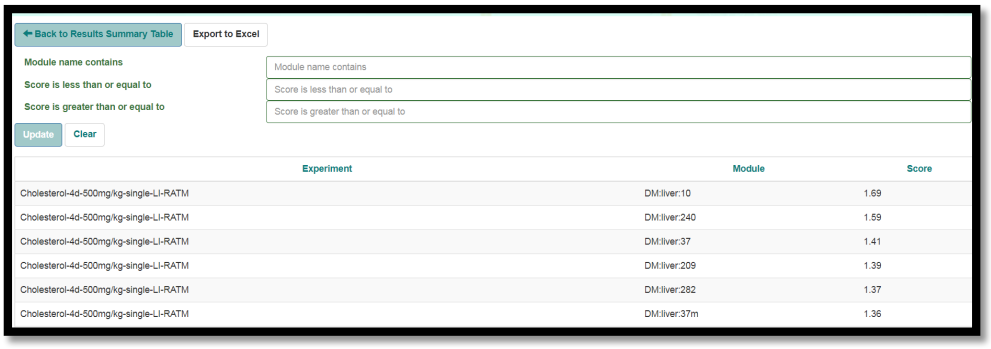


15. Gene-level analysis. Click [Results in browser]. Get the differentially expressed genes for experiments. They can be arranged based on the p-value, log2FC or PBh or gene Identifier or rat gene symbol. Exporting to excel and further analysis can identify the unique and the common genes across the experiments. The GSEA can be analyzed on the UI or data can be exported on to the Excel. Click [Back to Results Summary Table] will return to the results page.

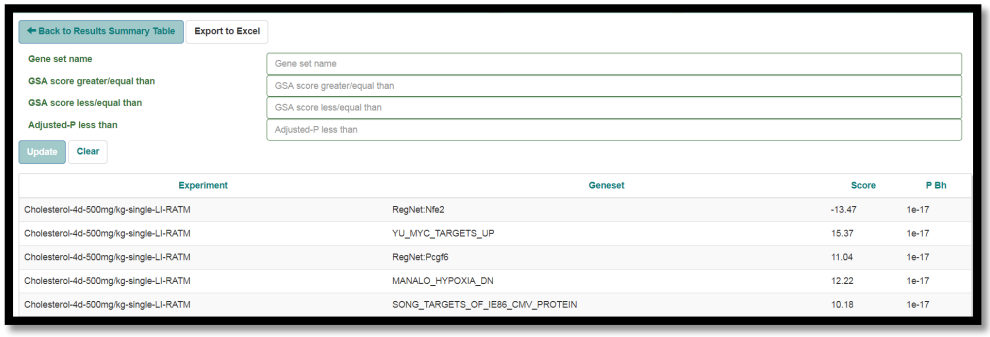


16. WCGNA analysis. Click [Results in browser].
Get the WCGNA modules and rank them based on positive or negative scores. The data can be exported and analyzed for uniqueness and similarities. The modules can also be studied for their

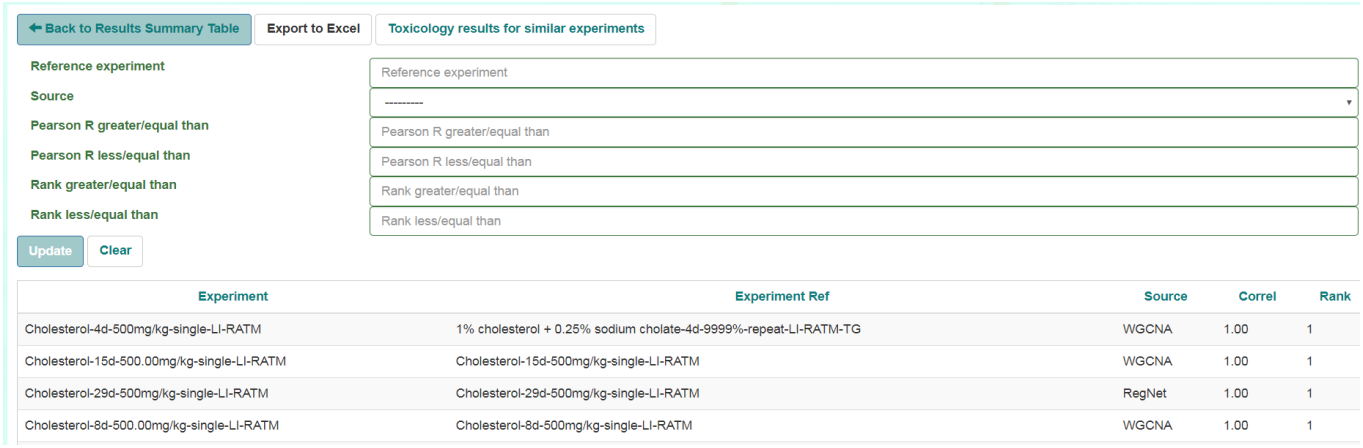
functional significance using the supplementary material. The modules can be analyzed on the server or exported to excel based on their Module or Score. Click [Back to Results Summary Table] will return to results page.



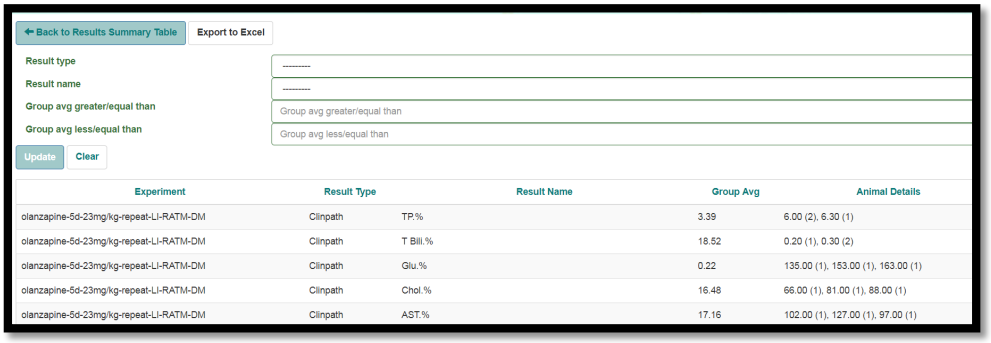
17. Gene Set Enrichment Analysis. Click [Results in browser]. Identify the gene sets highly correlated based on the Geneset, score and P Bh. Click [Back to Results Summary Table] will return to results page.



18. Most Similar Experiments. Click [Results in browser]. Identify the most similar experiments in the database to the input experiments. Click [Back to Results Summary Table] will return to the results page.



Click [Toxicology results for similar experiments], will identify the most significant toxicology parameters. Click [Back to Results Summary Table] will return to the results page.



19. Click [Clinical Chemistry and /or histology] will give the histology/clinical analysis. Click [Back to Results Summary Table] will return to the results page.