#### **CTox Test Set**

User Test 3: GEO(GSE64370)

# **Generate Gene Fold Factors**

### **Data Source**

This test set data comes from the Gene Expression Omnibus (GEO), an international repository for archiving and distributing the high throughput data. This data set (GSE64370) has four biological replicates for the liver tissue of female B6C3F1 mice [a cross between female C57BL/6 and male C3H] dosed via oral gavage for 21 days. The animals were exposed 8mg/kg bw/day furan in corn ild. And preserved in formalin for 18 hrs and 3 weeks, that were examined using RNA-seq (polyA-enrichment protocol).

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64370

Species: Mice Female Liver Tissue: 8mg/kg bw furan(or vehicle control)

Experimental Time Points and RNA-seq count data files:

Table 1

|                | 18-hr                                      | 3wk  |
|----------------|--|--|
| Control        | Liver_Control_FFPE18h_02_riboRNAseq        | Liver_Control_FFPE3wk_02_riboRNAseq        |
| Control        | Liver_Control_FFPE18h_03_riboRNAseq        | Liver_Control_FFPE3wk_03_riboRNAseq        |
| Control        | Liver_Control_FFPE18h_04_riboRNAseq        | Liver_Control_FFPE3wk_04_riboRNAseq        |
| Control        | Liver_Control_FFPE18h_06_riboRNAseq        | Liver_Control_FFPE3wk_06_riboRNAseq        |
| 8mkd-<br>Furan | Liver_8mkd-<br>furan_FFPE18h_41_riboRNAseq | Liver_8mkd-<br>furan_FFPE3wk_41_riboRNAseq |
| 8mkd-<br>Furan | Liver_8mkd-<br>furan_FFPE18h_42_riboRNAseq | Liver_8mkd-<br>furan_FFPE3wk_42_riboRNAseq |
| 8mkd-<br>Furan | Liver_8mkd-<br>furan_FFPE18h_43_riboRNAseq | Liver_8mkd-<br>furan_FFPE3wk_43_riboRNAseq |
| 8mkd-<br>Furan | Liver_8mkd-<br>furan_FFPE18h_44_riboRNAseq | Liver_8mkd-<br>furan_FFPE3wk_44_riboRNAseq |

The above renamed files allow easier understanding of the samples and their controls. The below table show the actual file names in GEO.

Table 2

|            | 18-hrs     | 3-wks      |
|------------|------------|------------|
| Control    | GSM1569635 | GSM1569643 |
| Control    | GSM1569636 | GSM1569644 |
| Control    | GSM1569637 | GSM1569645 |
| Control    | GSM1569638 | GSM1569646 |
| 8mkd-Furan | GSM1569641 | GSM1569647 |
| 8mkd-Furan | GSM1569642 | GSM1569648 |
| 8mkd-Furan | GSM1569643 | GSM1569649 |
| 8mkd-Furan | GSM1569644 | GSM1569650 |

# **Running The Test Case**

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

| Parameter Name | Suggested Value                        |
|----------------|--|
| Study name     | Female Mice Liver Tissue Furan Treated |
| Study info     | 18 hrs and 3wks                        |
| Source         | GEO                                    |

Select [Save and add/edit experiments]

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

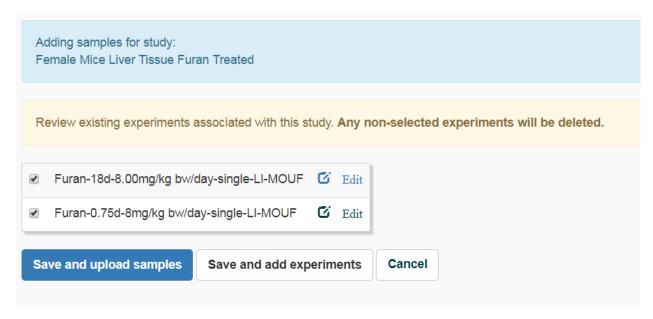
[Note: use [Save and add another] to reduce data entry between experiments]

| Parameter Name   | Exp 1 Values                             | Exp 2 Values                              |
|------------------|--|---|
| Tech             | RNASeq-Rnor_6.0.80                       | RNASeq-Rnor_6.0.80                        |
| Compound<br>Name | Furan                                    | Furan                                     |
| Dose             | 8  | 8   |
| Dose Unit        | mg/kg bw/day                             | mg/kg bw/day                              |
| Time             | 18-hrs                                   | 3-wks                                     |
| Tissue           | liver                                    | liver                                     |
| Organism         | Mice                                     | Mice                                      |
| Strain           | B6C3F1                                   | B6C3F1                                    |
| Gender           | Female                                   | Female                                    |
| Repeat Type      | single-dose                              | single-dose                               |
| Route            | diet                                     | diet                                      |
| Experiment name  | Furan-0.75d-8mg/kg bw/day-single-LI-MOUF | Furan-18d-8.00mg/kg bw/day-single-LI-MOUF |

[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 2 experiments are available for data upload. The checkbox list should include the 2 experiments that were created in above two steps of step 2. Click on [Save and upload samples] to proceed to the next step.

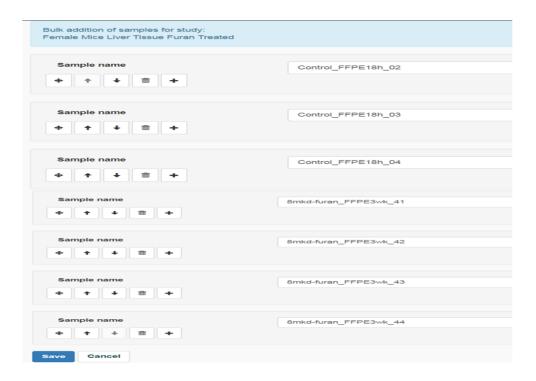


Click on [Save and upload samples] to proceed to the next step.

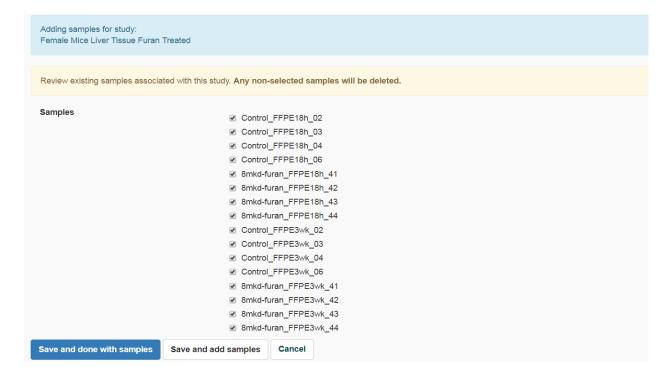
4. The study and the experiments will be listed. Click on the desired tab i.e. RNAseq File or Affy CEL Files. Upload the single tab delimited file containing all samples using [Choose File] option and then [Upload Files] and continue to the next step.



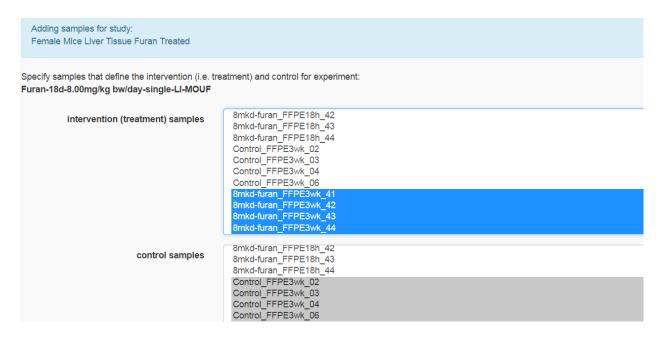
5. Confirm that the Gene Count data is available as desired for all the samples listed in the Table 1. Click on [Save].



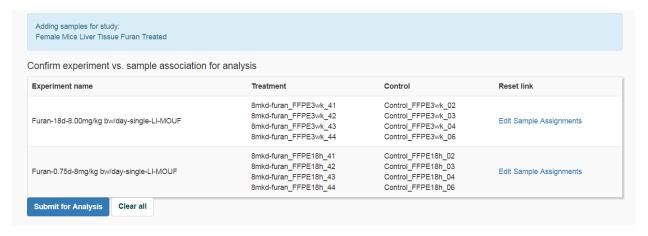
6. Again, confirm the samples list. Click on [Save and done with samples]



7. Next iterate through the each of the 2 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 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.

## Success

Submitted process for group fold changes using: experiments and 16 samples.

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

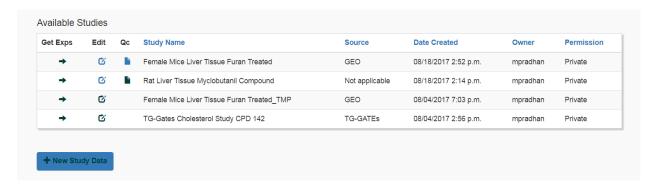
10. User receives an email on completion of analysis processes.

### Results

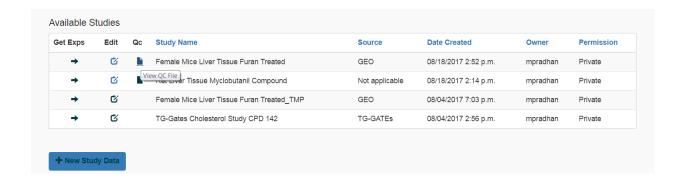
11. Click [Studies] on the tab.



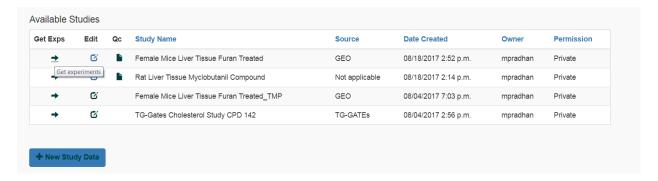
12. Study page [shows user studies]. Also, can see the Qc icon for the figures.



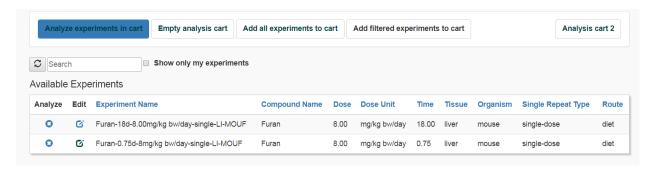
- 13. Click on the Qc icon and download the images. There are six images.
  - (i) Max counts for ALL genes-all samples
  - (ii) Clustering on ALL genes
  - (iii) Max counts for protein coding genes-all samples
  - (iv) Clustering on protein coding genes
  - (v) Filtered gene counts
  - (vi) Normalized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



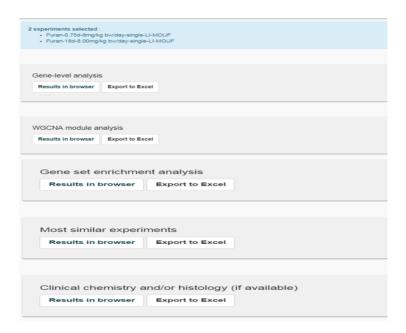
14. To analyze the experiments in the given study. Click on [Get Exps]



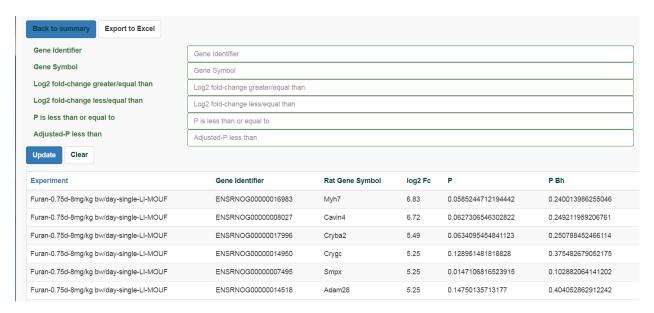
15. Select the experiments. Click [Analyze]. As the experiments are added a new pop-screen is displayed. After addition of all the experiments, click [Analyze experiments in cart]



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

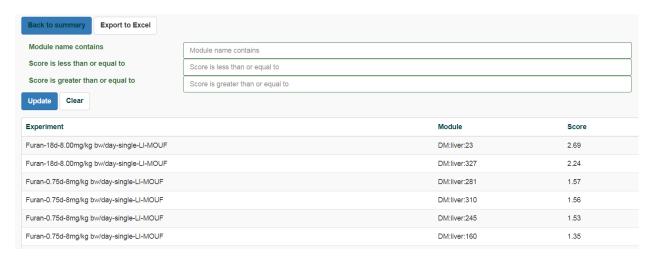


17. Gene-level analysis. Click [Results in browser]. Get the differentially expressed genes for experiments. They can be ranked based on the p-value, log2FC or PBh. Exporting to excel and further analysis can identify the unique and the common genes across the experiments After exporting or analysis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

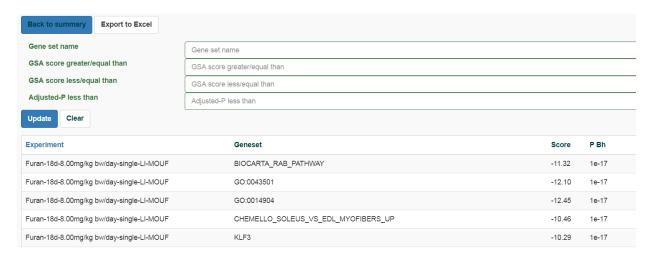


18. WCGNA analysis. Click [Results in browser].

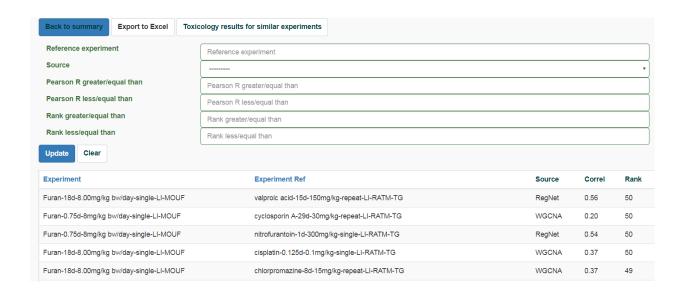
Get the WCGNA modules and rank them based on positive or negative scores. The data can be exported and analyzed for uniqueness and similarities or the module name. The modules can also be studied for their functional significance using the supplementary material. After exporting or analysis click [Back to summary] and return to the result page.



19. Gene Set Enrichment Analysis. Click [Results in browser]. Identify the gene sets highly correlated and with low p-values with the experiments. After exporting or analysis click [Back to summary] and return to the result page.



20. Most Similar Experiments. Click [Results in browser]. Identify the most similar experiments in the database to the input experiment based on the Source, Correl, Rank. After exporting or analysis click [Back to summary] and return to the result page.



21. Clinical chemistry and /or histology (if available)