

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 oil. 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-Furan	Liver_8mkd-furan_FFPE18h_41_riboRNAseq	Liver_8mkd-furan_FFPE3wk_41_riboRNAseq
8mkd-Furan	Liver_8mkd-furan_FFPE18h_42_riboRNAseq	Liver_8mkd-furan_FFPE3wk_42_riboRNAseq
8mkd-Furan	Liver_8mkd-furan_FFPE18h_43_riboRNAseq	Liver_8mkd-furan_FFPE3wk_43_riboRNAseq
8mkd-Furan	Liver_8mkd-furan_FFPE18h_44_riboRNAseq	Liver_8mkd-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 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.

Adding samples for study:
Female Mice Liver Tissue Furan Treated

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

- Furan-18d-8.00mg/kg bw/day-single-LI-MOUF [Edit](#)
- Furan-0.75d-8mg/kg bw/day-single-LI-MOUF [Edit](#)

Save and upload samples Save and add experiments Cancel

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.

Adding experiments for study:
Female Mice Liver Tissue Furan Treated

Associated experiments:

- Furan-18d-8.00mg/kg bw/day-single-LI-MOUF
- Furan-0.75d-8mg/kg bw/day-single-LI-MOUF

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 File** Affy 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

Gene_counts.txt

Upload Files Cancel

5. 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:
Female Mice Liver Tissue Furan Treated

Sample name	Control_FFPE18h_02
Sample name	Control_FFPE18h_03
Sample name	Control_FFPE18h_04
Sample name	8mkd-furan_FFPE3wk_41
Sample name	8mkd-furan_FFPE3wk_42
Sample name	8mkd-furan_FFPE3wk_43
Sample name	8mkd-furan_FFPE3wk_44

Save Cancel

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

Adding samples for study:
Female Mice Liver Tissue Furan Treated

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

Samples

- Control_FFPE18h_02
- Control_FFPE18h_03
- Control_FFPE18h_04
- Control_FFPE18h_06
- 8mkd-furan_FFPE18h_41
- 8mkd-furan_FFPE18h_42
- 8mkd-furan_FFPE18h_43
- 8mkd-furan_FFPE18h_44
- Control_FFPE3wk_02
- Control_FFPE3wk_03
- Control_FFPE3wk_04
- Control_FFPE3wk_06
- 8mkd-furan_FFPE3wk_41
- 8mkd-furan_FFPE3wk_42
- 8mkd-furan_FFPE3wk_43
- 8mkd-furan_FFPE3wk_44

Save and done with samples Save and add samples Cancel

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].

Adding samples for study:
Female Mice Liver Tissue Furan Treated

Specify samples that define the intervention (i.e. treatment) and control for experiment:
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF

intervention (treatment) samples

- 8mkd-furan_FFPE18h_42
- 8mkd-furan_FFPE18h_43
- 8mkd-furan_FFPE18h_44
- Control_FFPE3wk_02
- Control_FFPE3wk_03
- Control_FFPE3wk_04
- Control_FFPE3wk_06
- 8mkd-furan_FFPE3wk_41
- 8mkd-furan_FFPE3wk_42
- 8mkd-furan_FFPE3wk_43
- 8mkd-furan_FFPE3wk_44

control samples

- 8mkd-furan_FFPE18h_42
- 8mkd-furan_FFPE18h_43
- 8mkd-furan_FFPE18h_44
- Control_FFPE3wk_02
- Control_FFPE3wk_03
- Control_FFPE3wk_04
- Control_FFPE3wk_06

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.

Adding samples for study:
Female Mice Liver Tissue Furan Treated

Confirm experiment vs. sample association for analysis

Experiment name	Treatment	Control	Reset link
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	8mkd-furan_FFPE3wk_41 8mkd-furan_FFPE3wk_42 8mkd-furan_FFPE3wk_43 8mkd-furan_FFPE3wk_44	Control_FFPE3wk_02 Control_FFPE3wk_03 Control_FFPE3wk_04 Control_FFPE3wk_06	Edit Sample Assignments
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	8mkd-furan_FFPE18h_41 8mkd-furan_FFPE18h_42 8mkd-furan_FFPE18h_43 8mkd-furan_FFPE18h_44	Control_FFPE18h_02 Control_FFPE18h_03 Control_FFPE18h_04 Control_FFPE18h_06	Edit Sample Assignments

Submit for Analysis **Clear all**

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.

Available Studies

Get Exps	Edit	Qc	Study Name	Source	Date Created	Owner	Permission
→	✎	📄	Female Mice Liver Tissue Furan Treated	GEO	08/18/2017 2:52 p.m.	mpradhan	Private
→	✎	📄	Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpradhan	Private
→	✎	📄	Female Mice Liver Tissue Furan Treated_TMP	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](#)

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

Available Studies

Get Exps	Edit	Qc	Study Name	Source	Date Created	Owner	Permission
→			Female Mice Liver Tissue Furan Treated	GEO	08/18/2017 2:52 p.m.	mpradhan	Private
→			Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpradhan	Private
→			Female Mice Liver Tissue Furan Treated_TMP	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]

Available Studies

Get Exps	Edit	Qc	Study Name	Source	Date Created	Owner	Permission
→			Female Mice Liver Tissue Furan Treated	GEO	08/18/2017 2:52 p.m.	mpradhan	Private
→			Rat Liver Tissue Myclobutanil Compound	Not applicable	08/18/2017 2:14 p.m.	mpradhan	Private
→			Female Mice Liver Tissue Furan Treated_TMP	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]. As the experiments are added a new pop-screen is displayed. After addition of all the experiments, click [Analyze experiments in cart]

[Analyze experiments in cart](#)

[Empty analysis cart](#)

[Add all experiments to cart](#)

[Add filtered experiments to cart](#)

[Analysis cart 2](#)



Search

Show only my experiments

Available Experiments

Analyze	Edit	Experiment Name	Compound Name	Dose	Dose Unit	Time	Tissue	Organism	Single Repeat Type	Route
		Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	Furan	8.00	mg/kg bw/day	18.00	liver	mouse	single-dose	diet
		Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	Furan	8.00	mg/kg bw/day	0.75	liver	mouse	single-dose	diet

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.

2 experiments selected :

- Furan-0.75d-8mg/kg bw/day-single-LI-MOUF
- Furan-18d-8.00mg/kg bw/day-single-LI-MOUF

Gene-level analysis

[Results in browser](#) [Export to Excel](#)

WGCNA module analysis

[Results in browser](#) [Export to Excel](#)

Gene set enrichment analysis

[Results in browser](#) [Export to Excel](#)

Most similar experiments

[Results in browser](#) [Export to Excel](#)

Clinical chemistry and/or histology (if available)

[Results in browser](#) [Export 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.

[Back to summary](#) [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
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000016983	Myh7	6.83	0.0585244712194442	0.240013986255046
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000008027	Cavin4	6.72	0.0627306546302822	0.249211959206761
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000017996	Cryba2	5.49	0.0634095454841123	0.250788452466114
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000014950	Crygc	5.25	0.128951481818828	0.375482679052175
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000007495	Smpx	5.25	0.0147106816523915	0.102882064141202
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	ENSRNOG00000014518	Adam28	5.25	0.14750135713177	0.404052862912242

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.

Back to summary
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
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	DM:liver:23	2.69
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	DM:liver:327	2.24
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	DM:liver:281	1.57
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	DM:liver:310	1.56
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	DM:liver:245	1.53
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	DM:liver:160	1.35

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.

Back to summary
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
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	BIOCARTA_RAB_PATHWAY	-11.32	1e-17
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	GO:0043501	-12.10	1e-17
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	GO:0014904	-12.45	1e-17
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	CHEMELLO_SOLEUS_VS_EDL_MYOFIBERS_UP	-10.46	1e-17
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	KLF3	-10.29	1e-17

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.

Back to summary
Export to Excel
Toxicology results for similar experiments

Reference experiment

Source

Pearson R greater/equal than

Pearson R less/equal than

Rank greater/equal than

Rank less/equal than

Reference experiment

----- ▾

Pearson R greater/equal than

Pearson R less/equal than

Rank greater/equal than

Rank less/equal than

Update
Clear

Experiment	Experiment Ref	Source	Correl	Rank
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	valproic acid-15d-150mg/kg-repeat-LI-RATM-TG	RegNet	0.56	50
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	cyclosporin A-29d-30mg/kg-repeat-LI-RATM-TG	WGCNA	0.20	50
Furan-0.75d-8mg/kg bw/day-single-LI-MOUF	nitrofurantoin-1d-300mg/kg-single-LI-RATM-TG	RegNet	0.54	50
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	cisplatin-0.125d-0.1mg/kg-single-LI-RATM-TG	WGCNA	0.37	50
Furan-18d-8.00mg/kg bw/day-single-LI-MOUF	chlorpromazine-8d-15mg/kg-repeat-LI-RATM-TG	WGCNA	0.37	49

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