

## 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](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 or interventions. The original file names are detailed in the table below

	<b>4-day</b>	<b>8-day</b>	<b>15-day</b>	<b>29-day</b>
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)

<b>Parameter Name</b>	<b>Suggested Value</b>
<b>Study name</b>	TG-Gates Cholesterol Study CPD 142
<b>Study info</b>	4 High doses and 4 controls at 4 time points
<b>Source</b>	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
<b>Tech</b>	microarray-RG230A	microarray-RG230A	microarray-RG230A	microarray-RG230A
<b>Compound Name</b>	Cholesterol	Cholesterol	Cholesterol	Cholesterol
<b>Dose</b>	500	500	500	500
<b>Dose Unit</b>	mg/kg	mg/kg	mg/kg	mg/kg
<b>Time</b>	4	8	15	29
<b>Tissue</b>	liver	liver	liver	liver
<b>Organism</b>	rat	rat	rat	rat
<b>Strain</b>	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley	Sprague-Dawley
<b>Gender</b>	male	male	male	male
<b>Repeat Type</b>	single-dose	single-dose	single-dose	single-dose
<b>Route</b>	diet	diet	diet	diet
<b>Experiment name [Auto populate]</b>	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.

Adding samples for study:  
TG-Gates Cholesterol Study CPD 142-1

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

<input checked="" type="checkbox"/>	Cholesterol-29d-500mg/kg-single-LI-RATM	<a href="#">Edit</a>
<input checked="" type="checkbox"/>	Cholesterol-15d-500.00mg/kg-single-LI-RATM	<a href="#">Edit</a>
<input checked="" type="checkbox"/>	Cholesterol-8d-500.00mg/kg-single-LI-RATM	<a href="#">Edit</a>
<input checked="" type="checkbox"/>	Cholesterol-4d-500mg/kg-single-LI-RATM	<a href="#">Edit</a>

[Save and upload samples](#) [Save and add experiments](#) [Cancel](#)

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.

Adding experiments for study:  
TG-Gates Cholesterol Study CPD 142

Associated experiments:

- Cholesterol-29d-500mg/kg-single-LI-RATM
- Cholesterol-15d-500mg/kg-single-LI-RATM
- Cholesterol-8d-500mg/kg-single-LI-RATM
- Cholesterol-4d-500mg/kg-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 File **Affy CEL Files**

Upload multiple files, one per sample, which are either CEL files or a text file containing gene or probe identifiers in column 1 and intensities in column 2.  
The sample name will be the file name after removing file extension

multiple files, one per sample  
[Choose Files](#) 24 files

[Upload Files](#) [Cancel](#)

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

Bulk addition of samples for study:  
TG-Gates Cholesterol Study CPD 142

Sample name

4day\_c1



Sample name

4day\_c2



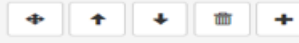
Sample name

4day\_c3



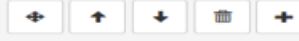
Sample name

29day\_c3



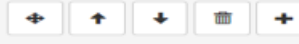
Sample name

29day\_h1



Sample name

29day\_h2



Sample name

29day\_h3



Save

Cancel

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

**Samples**

- ☒ 4day\_c1
- ☒ 4day\_c2
- ☒ 4day\_c3
- ☒ 4day\_h1
- ☒ 4day\_h2
- ☒ 4day\_h3
- ☒ 8day\_c1
- ☒ 8day\_c2
- ☒ 8day\_c3
- ☒ 8day\_h1
- ☒ 8day\_h2
- ☒ 8day\_h3
- ☒ 15day\_c1
- ☒ 15day\_c2
- ☒ 15day\_c3
- ☒ 15day\_h1
- ☒ 15day\_h2
- ☒ 15day\_h3
- ☒ 29day\_c1
- ☒ 29day\_c2
- ☒ 29day\_c3
- ☒ 29day\_h1
- ☒ 29day\_h2

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.

Specify samples that define the intervention (i.e. treatment) and control for experiment:  
Cholesterol-29d-500mg/kg-single-LI-RATM

intervention (treatment) samples

- 15day\_c2
- 15day\_c3
- 15day\_h1
- 15day\_h2
- 15day\_h3
- 29day\_c1
- 29day\_c2
- 29day\_c3
- 29day\_h1
- 29day\_h2
- 29day\_h3

control samples

- 15day\_c2
- 15day\_c3
- 15day\_h1
- 15day\_h2
- 15day\_h3
- 29day\_c1
- 29day\_c2
- 29day\_c3
- 29day\_h1
- 29day\_h2
- 29day\_h3

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.

Adding samples for study:  
TG-Gates Cholesterol Study CPD 142

Confirm experiment vs. sample association for analysis

Experiment name	Treatment	Control	Reset link
Cholesterol-29d-500mg/kg-single-LI-RATM	29day_h1	29day_c1	<a href="#">Edit Sample Assignments</a>
	29day_h2	29day_c2	
	29day_h3	29day_c3	
Cholesterol-15d-500mg/kg-single-LI-RATM	15day_h1	15day_c1	<a href="#">Edit Sample Assignments</a>
	15day_h2	15day_c2	
	15day_h3	15day_c3	
Cholesterol-8d-500mg/kg-single-LI-RATM	8day_h1	8day_c1	<a href="#">Edit Sample Assignments</a>
	8day_h2	8day_c2	
	8day_h3	8day_c3	
Cholesterol-4d-500mg/kg-single-LI-RATM	4day_h1	4day_c1	<a href="#">Edit Sample Assignments</a>
	4day_h2	4day_c2	
	4day_h3	4day_c3	

[Submit for Analysis](#) [Clear all](#)

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.

## Success

Submitted process for group fold changes using: experiments and **24** samples.

An email will be sent to [mpadhan@indianabiosciences.org](mailto:mpadhan@indianabiosciences.org) when the job completes and the information is available.

### Result Analysis

10. Click [Studies] on the tab.

IBRI Toxicogenomics Platform

User: mpadhan   Studies   Experiments   Samples   Log Out

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

Available Studies							
Get Exps	Edit	Qc	Study Name	Source	Date Created	Owner	Permission
→	✎		TG-Gates Cholesterol Study CPD 142	TG-GATES	08/18/2017 4:11 p.m.	mpradhan	Private
→	✎	📄	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
→	✎		TG-Gates Cholesterol Study CPD 142-tmp	TG-GATES	08/04/2017 2:56 p.m.	mpradhan	Private

[+ New Study Data](#)

12. Select the experiments for analysis. 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
		Cholesterol-29d-500mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	29.00	liver	rat	single-dose	diet
		Cholesterol-15d-500.00mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	29.00	liver	rat	single-dose	diet
		Cholesterol-8d-500.00mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	8.00	liver	rat	single-dose	diet
		Cholesterol-4d-500mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	4.00	liver	rat	single-dose	diet

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

Add Listed Experiments to Cart

Empty Analysis Cart

Analyze Experiments in Cart

Analysis Cart 4

Search

☐ Show only my experiments

Available Experiments

Analyze	Edit	Experiment Name	Compound Name	Dose	Dose Unit	Time	Tissue	Organism	Single Repeat Type	Route
<div></div>	<div></div>	Cholesterol-29d-500mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	29.00	liver	rat	single-dose	diet
<div></div>	<div></div>	Cholesterol-15d-500.00mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	29.00	liver	rat	single-dose	diet
<div></div>	<div></div>	Cholesterol-8d-500.00mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	8.00	liver	rat	single-dose	diet
<div></div>	<div></div>	Cholesterol-4d-500mg/kg-single-LI-RATM	Cholesterol	500.00	mg/kg	4.00	liver	rat	single-dose	diet



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.

4 experiments selected :

- Cholesterol-4d-500mg/kg-single-LI-RATM
- Cholesterol-8d-500.00mg/kg-single-LI-RATM
- Cholesterol-15d-500.00mg/kg-single-LI-RATM
- Cholesterol-29d-500mg/kg-single-LI-RATM

Access or Export Results		
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

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.

[← 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
Cholesterol-15d-500.00mg/kg-single-LI-RATM	140910_at	Msmo1	-2.12	4.51844750202858e-09	5.54335796372291e-05
Cholesterol-15d-500.00mg/kg-single-LI-RATM	29230_at	Sqle	-2.76	7.84511458211564e-09	5.54335796372291e-05
Cholesterol-15d-500.00mg/kg-single-LI-RATM	25427_at	Cyp51	-1.68	1.26975876867022e-08	5.98141030628252e-05
Cholesterol-15d-500.00mg/kg-single-LI-RATM	89784_at	Idl1	-1.87	3.75757864448201e-08	0.000106204202807639

16. WGCNA analysis. Click [Results in browser]. Get the WGCNA 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.

<a href="#">← Back to Results Summary Table</a> <a href="#">Export to Excel</a>		
Module name contains	<input type="text"/>	
Score is less than or equal to	<input type="text"/>	
Score is greater than or equal to	<input type="text"/>	
<a href="#">Update</a>	<a href="#">Clear</a>	
Experiment	Module	Score
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:10	1.69
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:240	1.59
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:37	1.41
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:209	1.39
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:282	1.37
Cholesterol-4d-500mg/kg-single-LI-RATM	DMiliver:37m	1.36

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.

<a href="#">← Back to Results Summary Table</a> <a href="#">Export to Excel</a>			
Gene set name	<input type="text"/>		
GSA score greater/equal than	<input type="text"/>		
GSA score less/equal than	<input type="text"/>		
Adjusted-P less than	<input type="text"/>		
<a href="#">Update</a>	<a href="#">Clear</a>		
Experiment	Geneset	Score	P Bh
Cholesterol-4d-500mg/kg-single-LI-RATM	RegNet:Nfe2	-13.47	1e-17
Cholesterol-4d-500mg/kg-single-LI-RATM	YU_MYC_TARGETS_UP	15.37	1e-17
Cholesterol-4d-500mg/kg-single-LI-RATM	RegNet:Pcgf6	11.04	1e-17
Cholesterol-4d-500mg/kg-single-LI-RATM	MANALO_HYPOXIA_DN	12.22	1e-17
Cholesterol-4d-500mg/kg-single-LI-RATM	SONG_TARGETS_OF_IE86_CMV_PROTEIN	10.18	1e-17

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.

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

Update

Clear

Reference experiment

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▼

Pearson R greater/equal than

Pearson R less/equal than

Rank greater/equal than

Rank less/equal than

Experiment	Experiment Ref	Source	Correl	Rank
Cholesterol-4d-500mg/kg-single-LI-RATM	1% cholesterol + 0.25% sodium cholate-4d-9999%-repeat-LI-RATM-TG	WGCNA	1.00	1
Cholesterol-15d-500.00mg/kg-single-LI-RATM	Cholesterol-15d-500mg/kg-single-LI-RATM	WGCNA	1.00	1
Cholesterol-29d-500mg/kg-single-LI-RATM	Cholesterol-29d-500mg/kg-single-LI-RATM	RegNet	1.00	1
Cholesterol-8d-500.00mg/kg-single-LI-RATM	Cholesterol-8d-500mg/kg-single-LI-RATM	WGCNA	1.00	1

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.

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

Result type

Result name

Group avg greater/equal than

Group avg less/equal than

Update

Clear

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Group avg greater/equal than

Group avg less/equal than

Experiment	Result Type	Result Name	Group Avg	Animal Details
olanzapine-5d-23mg/kg-repeat-LI-RATM-DM	Clinpath	TP %	3.39	6.00 (2), 6.30 (1)
olanzapine-5d-23mg/kg-repeat-LI-RATM-DM	Clinpath	T Bill %	18.52	0.20 (1), 0.30 (2)
olanzapine-5d-23mg/kg-repeat-LI-RATM-DM	Clinpath	Glu %	0.22	135.00 (1), 153.00 (1), 163.00 (1)
olanzapine-5d-23mg/kg-repeat-LI-RATM-DM	Clinpath	Chol %	16.48	66.00 (1), 81.00 (1), 88.00 (1)
olanzapine-5d-23mg/kg-repeat-LI-RATM-DM	Clinpath	AST %	17.16	102.00 (1), 127.00 (1), 97.00 (1)

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.

[← Back to Results Summary Table](#)

Result type

Result name

Group avg greater/equal than

Group avg less/equal than

Update

Clear

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Group avg greater/equal than

Group avg less/equal than

Experiment	Result Type	Result Name	Group Avg	Animal Details
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