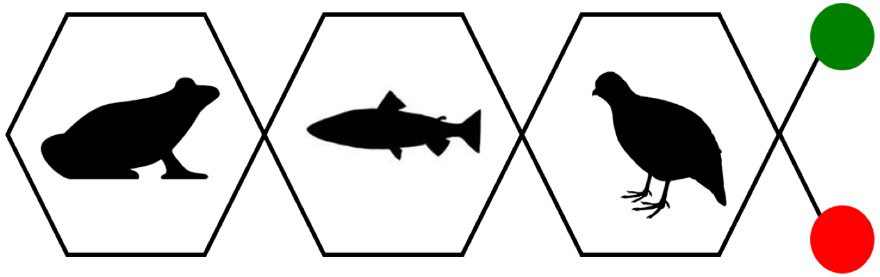
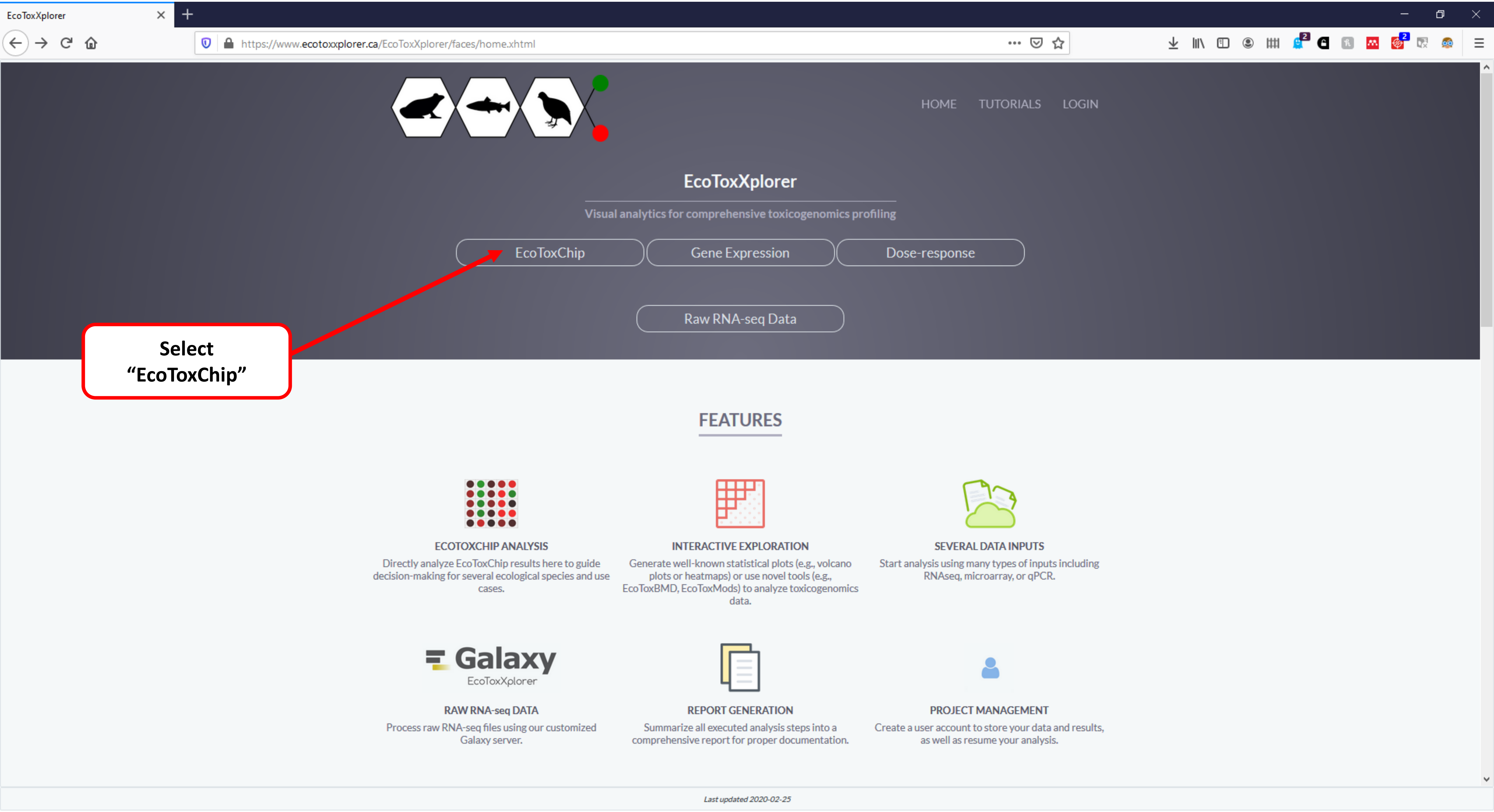


Tutorial #1

EcoToxChip Module



EcoToxXplorer

Visual analytics for comprehensive toxicogenomics profiling

FEATURES

ECOTOXCHIP ANALYSIS

Directly analyze EcoToxChip results here to guide decision-making for several ecological species and use cases.

INTERACTIVE EXPLORATION

Generate well-known statistical plots (e.g., volcano plots or heatmaps) or use novel tools (e.g., EcoToxBMD, EcoToxMods) to analyze toxicogenomics data.

SEVERAL DATA INPUTS

Start analysis using many types of inputs including RNAseq, microarray, or qPCR.

RAW RNA-seq DATA

Process raw RNA-seq files using our customized Galaxy server.

REPORT GENERATION

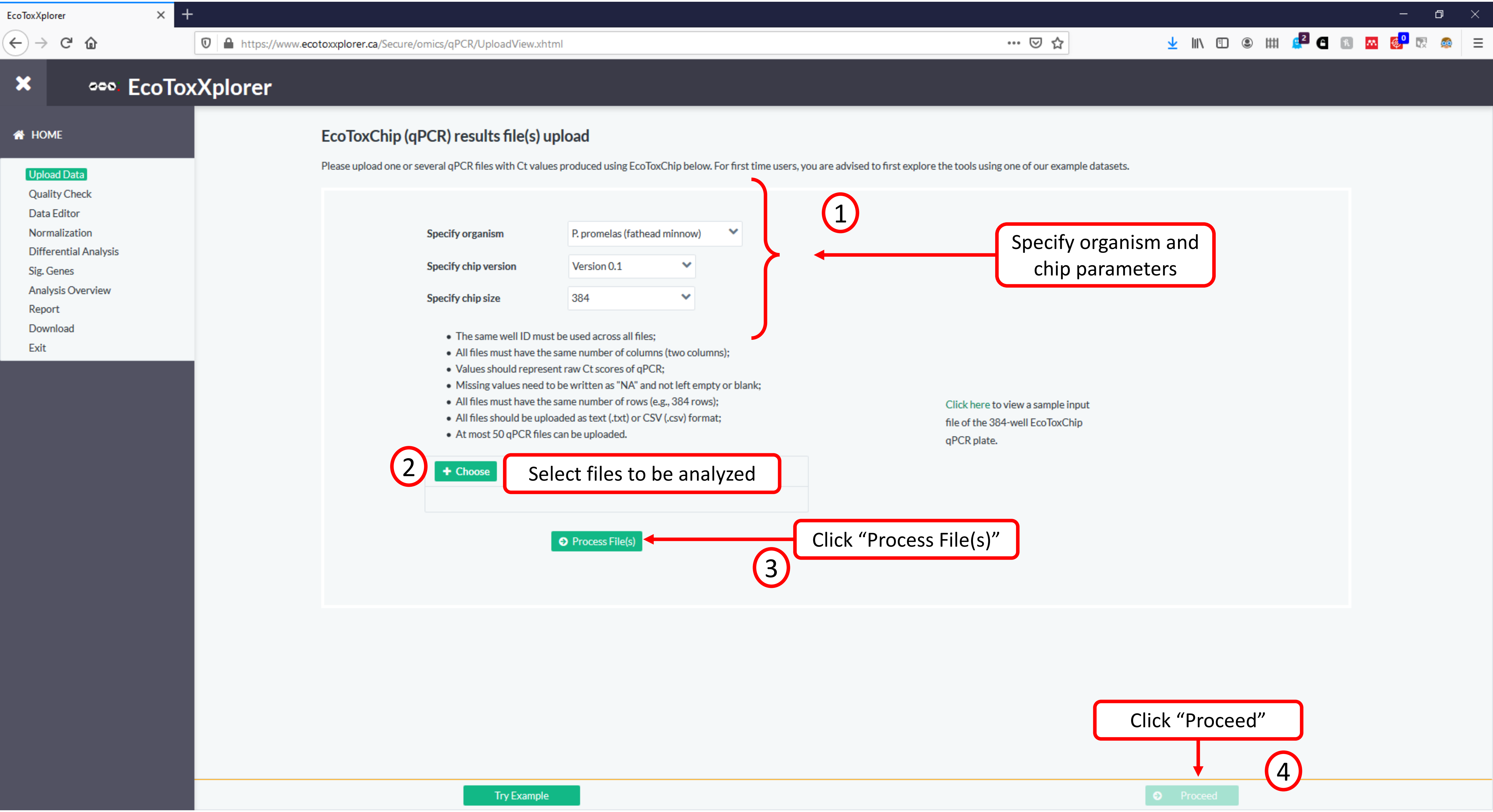
Summarize all executed analysis steps into a comprehensive report for proper documentation.

PROJECT MANAGEMENT

Create a user account to store your data and results, as well as resume your analysis.

Last updated 2020-02-25

Select "EcoToxChip"



EcoToxChip (qPCR) results file(s) upload

Please upload one or several qPCR files with Ct values produced using EcoToxChip below. For first time users, you are advised to first explore the tools using one of our example datasets.

Specify organism P. promelas (fathead minnow)

Specify chip version Version 0.1

Specify chip size 384

- The same well ID must be used across all files;
- All files must have the same number of columns (two columns);
- Values should represent raw Ct scores of qPCR;
- Missing values need to be written as "NA" and not left empty or blank;
- All files must have the same number of rows (e.g., 384 rows);
- All files should be uploaded as text (.txt) or CSV (.csv) format;
- At most 50 qPCR files can be uploaded.

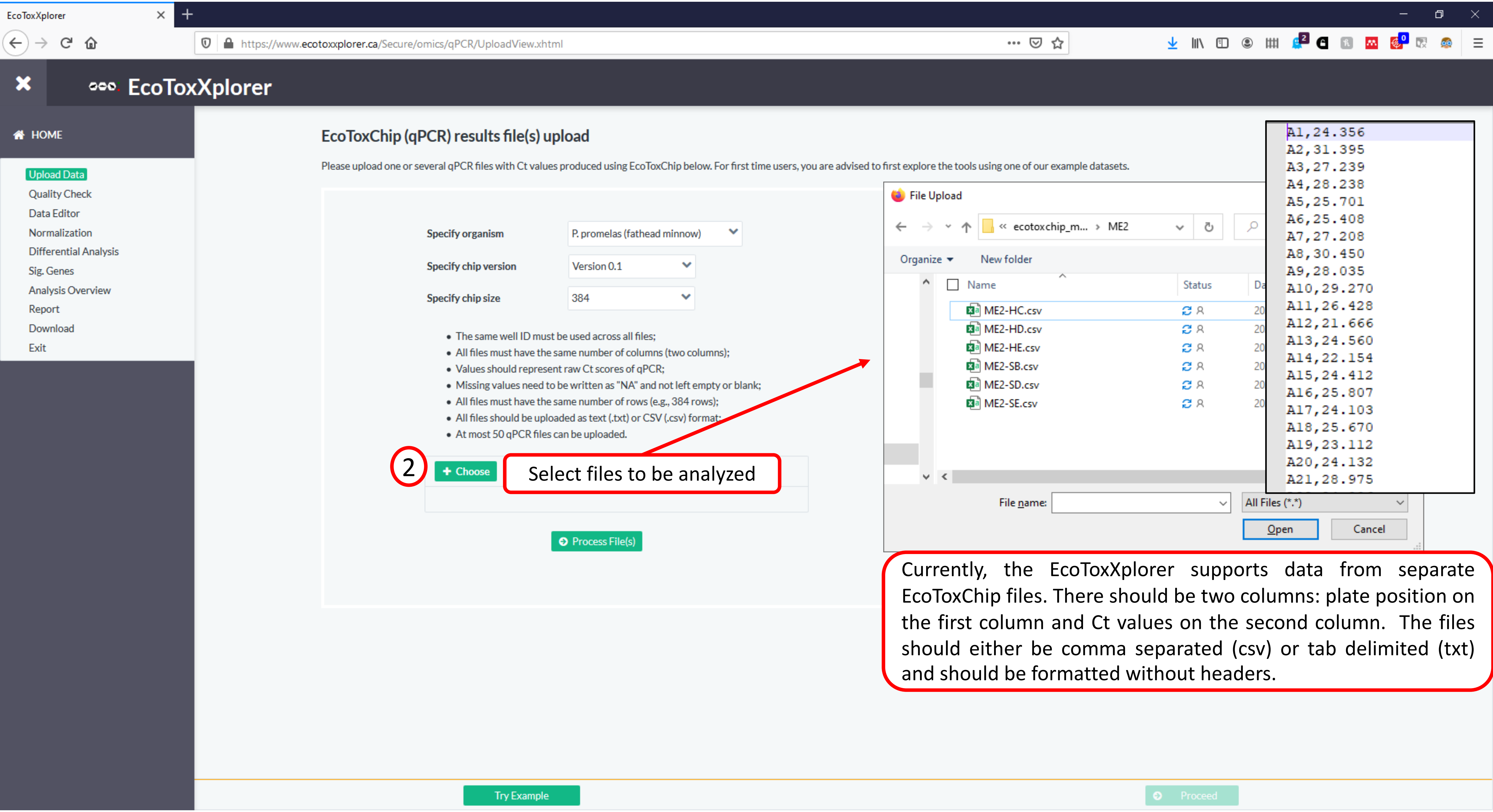
Click here to view a sample input file of the 384-well EcoToxChip qPCR plate.

① Specify organism and chip parameters

② Select files to be analyzed

③ Click “Process File(s)”

④ Click “Proceed”



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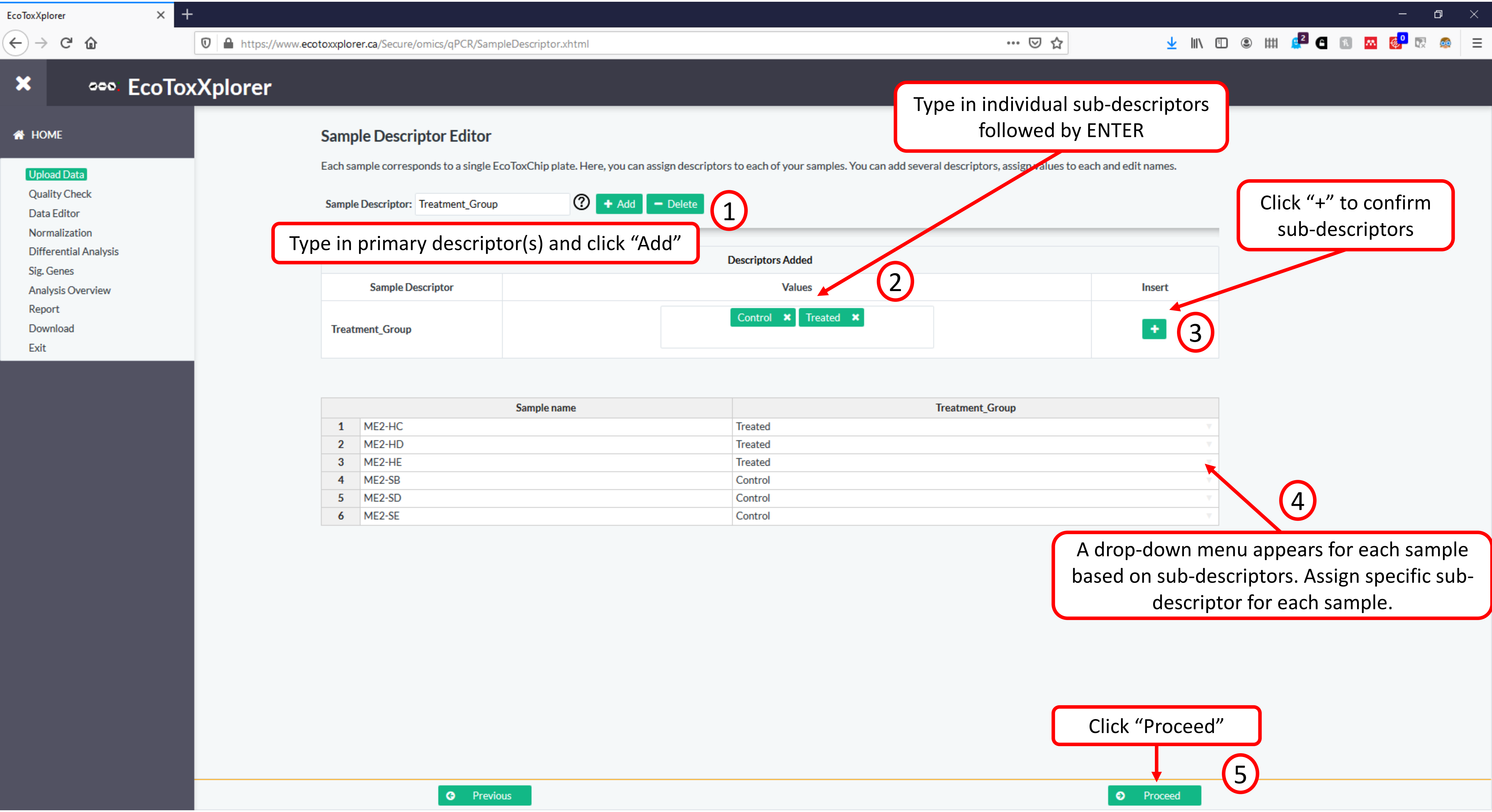
- All files should be uploaded as text (.txt) or CSV (.csv) format;
- At most 50 qPCR files can be uploaded.

ME2-HC.csv
ME2-HD.csv
ME2-HE.csv
ME2-SB.csv
ME2-SD.csv
ME2-SE.csv

```
A1,24.356
A2,31.395
A3,27.239
A4,28.238
A5,25.701
A6,25.408
A7,27.208
A8,30.450
A9,28.035
A10,29.270
A11,26.428
A12,21.666
A13,24.560
A14,22.154
A15,24.412
A16,25.807
A17,24.103
A18,25.670
A19,23.112
A20,24.132
A21,28.975
```

② Select files to be analyzed

Currently, the EcoToxXplorer supports data from separate EcoToxChip files. There should be two columns: plate position on the first column and Ct values on the second column. The files should either be comma separated (csv) or tab delimited (txt) and should be formatted without headers.



Sample Descriptor Editor

Each sample corresponds to a single EcoToxChip plate. Here, you can assign descriptors to each of your samples. You can add several descriptors, assign values to each and edit names.

Sample Descriptor: Treatment_Group

Descriptors Added

Sample Descriptor	Values	Insert
Treatment_Group	Control ✖ Treated ✖	+

	Sample name	Treatment_Group
1	ME2-HC	Treated
2	ME2-HD	Treated
3	ME2-HE	Treated
4	ME2-SB	Control
5	ME2-SD	Control
6	ME2-SE	Control

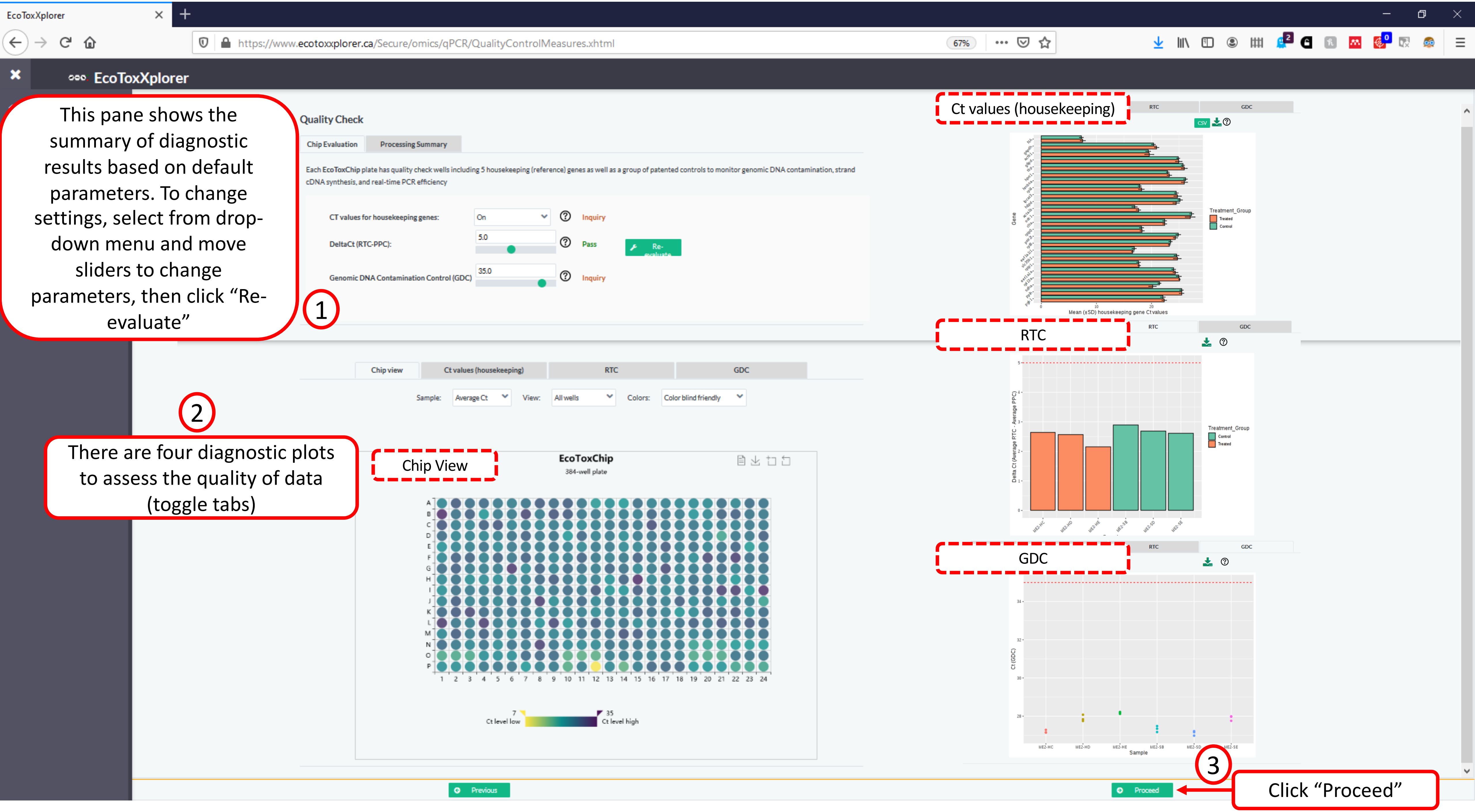
1 Type in primary descriptor(s) and click "Add"

2 Type in individual sub-descriptors followed by ENTER

3 Click "+" to confirm sub-descriptors

4 A drop-down menu appears for each sample based on sub-descriptors. Assign specific sub-descriptor for each sample.

5 Click "Proceed"



This pane shows the summary of diagnostic results based on default parameters. To change settings, select from drop-down menu and move sliders to change parameters, then click "Re-evaluate"

①

Quality Check

Chip Evaluation | Processing Summary

Each EcoToxChip plate has quality check wells including 5 housekeeping (reference) genes as well as a group of patented controls to monitor genomic DNA contamination, strand cDNA synthesis, and real-time PCR efficiency

CT values for housekeeping genes: On ⓘ Inquiry

DeltaCt (RTC-PPC): 5.0 ⓘ Pass Re-evaluate

Genomic DNA Contamination Control (GDC) 35.0 ⓘ Inquiry

②

There are four diagnostic plots to assess the quality of data (toggle tabs)

Sample: Average Ct View: All wells Colors: Color blind friendly

Chip View

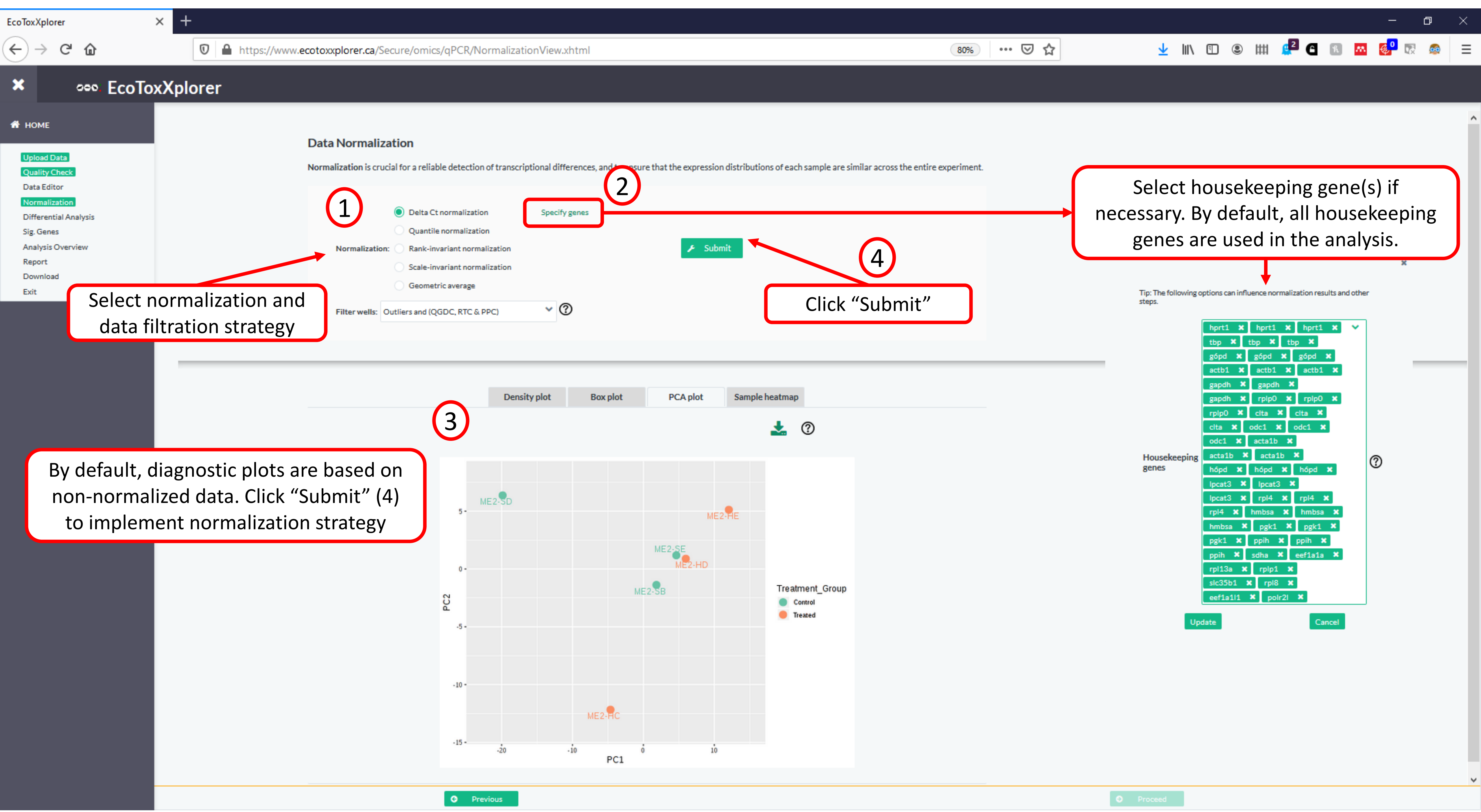
Ct values (housekeeping)

RTC

GDC

③

Click "Proceed"



Data Normalization

Normalization is crucial for a reliable detection of transcriptional differences, and to ensure that the expression distributions of each sample are similar across the entire experiment.

Normalization:
- ● Delta Ct normalization
- ○ Quantile normalization
- ○ Rank-invariant normalization
- ○ Scale-invariant normalization
- ○ Geometric average

Filter wells: Outliers and (QGDC, RTC & PPC)

1

Select normalization and data filtration strategy

2

Select housekeeping gene(s) if necessary. By default, all housekeeping genes are used in the analysis.

4

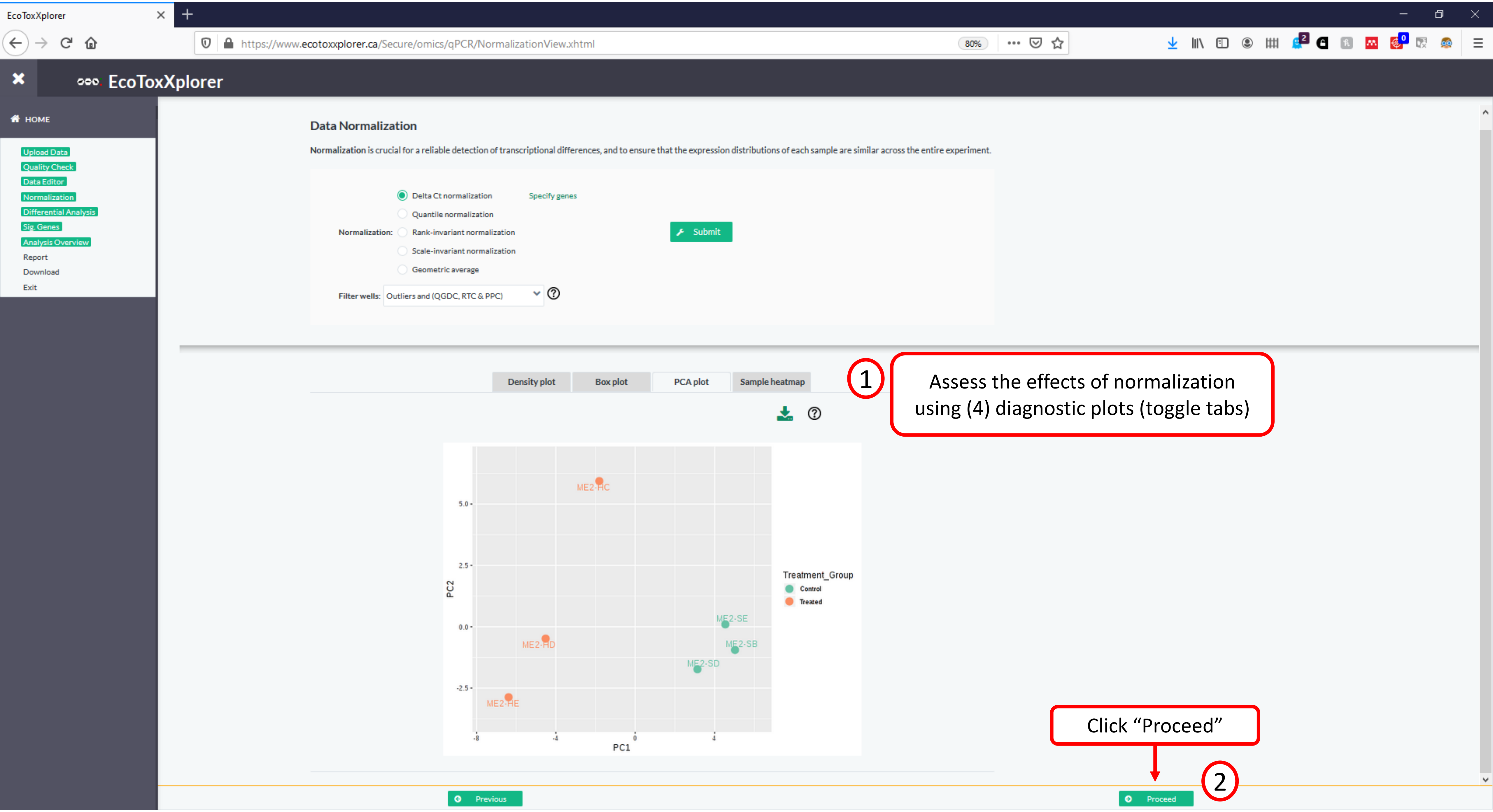
Click “Submit”

3

By default, diagnostic plots are based on non-normalized data. Click “Submit” (4) to implement normalization strategy

Tip: The following options can influence normalization results and other steps.

Housekeeping genes: hprt1, hprt1, hprt1, tbp, tbp, tbp, g6pd, g6pd, g6pd, actb1, actb1, actb1, gapdh, gapdh, gapdh, rplp0, rplp0, rplp0, clta, clta, clta, odc1, odc1, odc1, acta1b, acta1b, acta1b, h6pd, h6pd, h6pd, lpcat3, lpcat3, lpcat3, rpl4, rpl4, rpl4, hmbsa, hmbsa, hmbsa, pgk1, pgk1, pgk1, ppih, ppih, ppih, sdha, eef1a1a, rpl13a, rplp1, slc35b1, rpl8, eef1a1l1, polr2l



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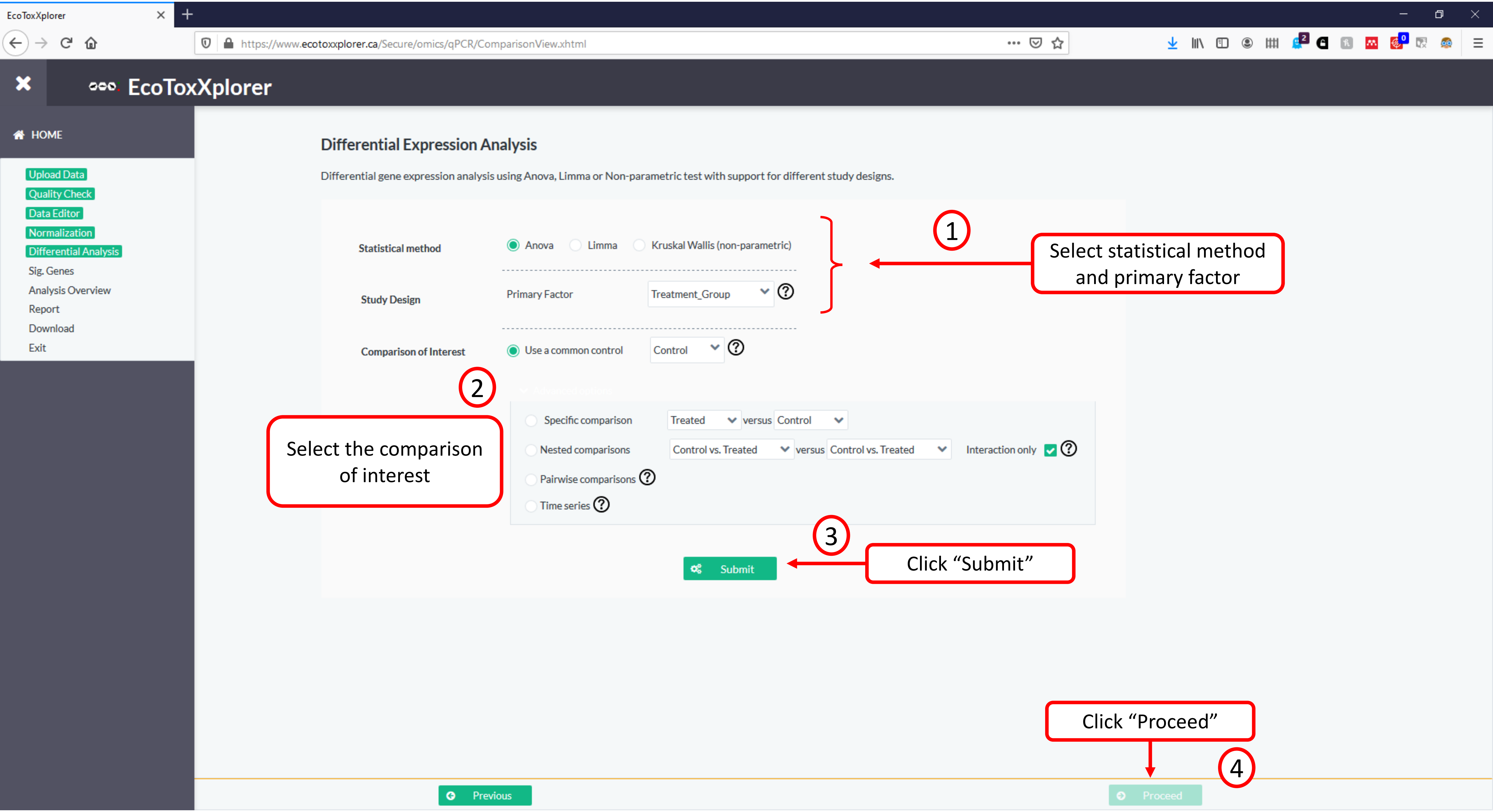
- ○ Scale-invariant normalization
- ○ Geometric average

Filter wells: Outliers and (QGDC, RTC & PPC)

① Assess the effects of normalization using (4) diagnostic plots (toggle tabs)

Click “Proceed”

②



Differential Expression Analysis

Differential gene expression analysis using Anova, Limma or Non-parametric test with support for different study designs.

Statistical method ● Anova ○ Limma ○ Kruskal Wallis (non-parametric)

Study Design Primary Factor: Treatment_Group

Comparison of Interest ● Use a common control: Control

Advanced options

○ Specific comparison: Treated versus Control

○ Nested comparisons: Control vs. Treated versus Control vs. Treated — Interaction only ☑

○ Pairwise comparisons

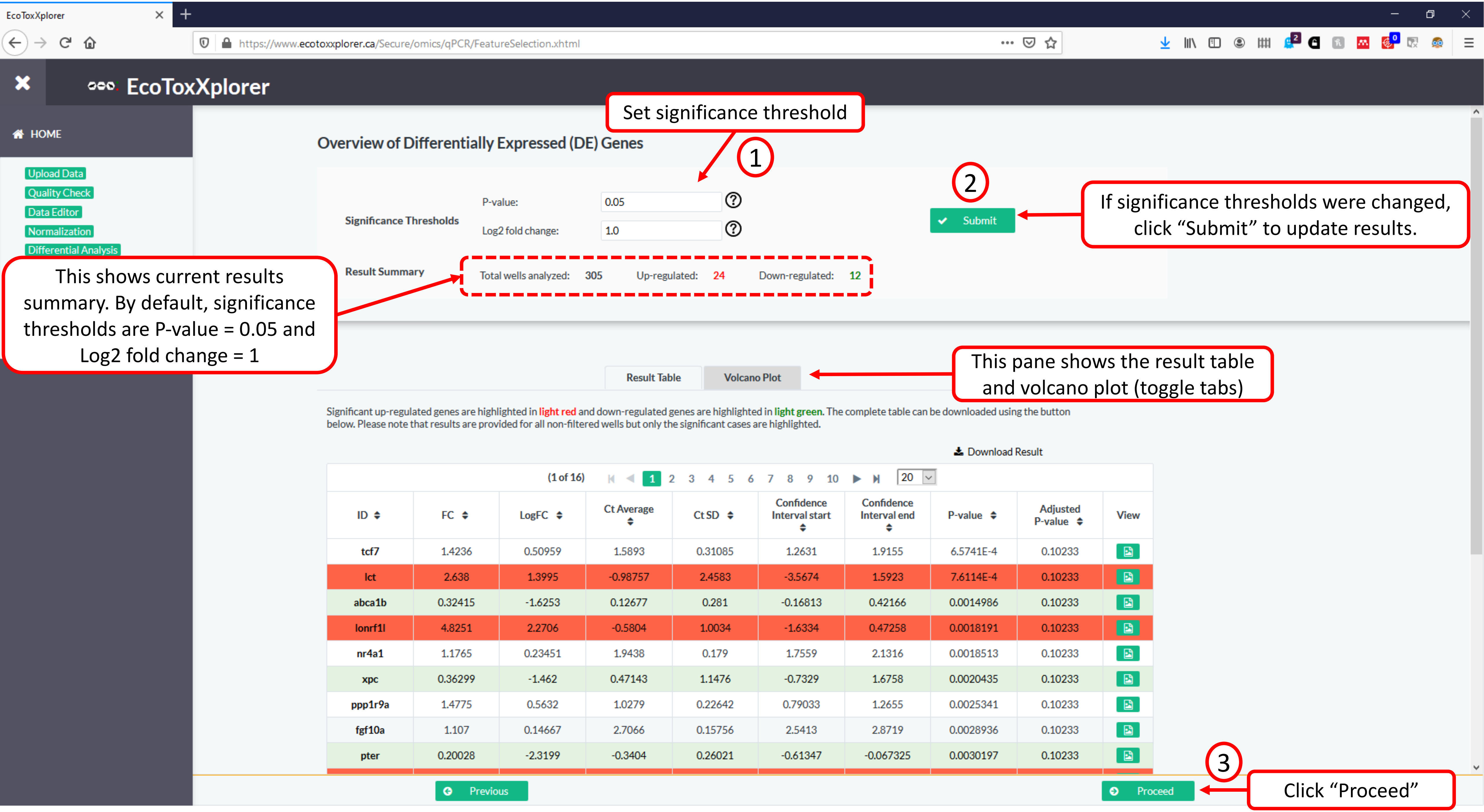
○ Time series

① Select statistical method and primary factor

② Select the comparison of interest

③ Click “Submit”

④ Click “Proceed”



Overview of Differentially Expressed (DE) Genes

Significance Thresholds

P-value: 0.05

Log2 fold change: 1.0

✓ Submit

Result Summary

Total wells analyzed: 305 Up-regulated: 24 Down-regulated: 12

Set significance threshold

①

②

If significance thresholds were changed, click “Submit” to update results.

This shows current results summary. By default, significance thresholds are P-value = 0.05 and Log2 fold change = 1

Result Table | Volcano Plot

This pane shows the result table and volcano plot (toggle tabs)

Significant up-regulated genes are highlighted in light red and down-regulated genes are highlighted in light green. The complete table can be downloaded using the button below. Please note that results are provided for all non-filtered wells but only the significant cases are highlighted.

Download Result

(1 of 16) 1 2 3 4 5 6 7 8 9 10 20

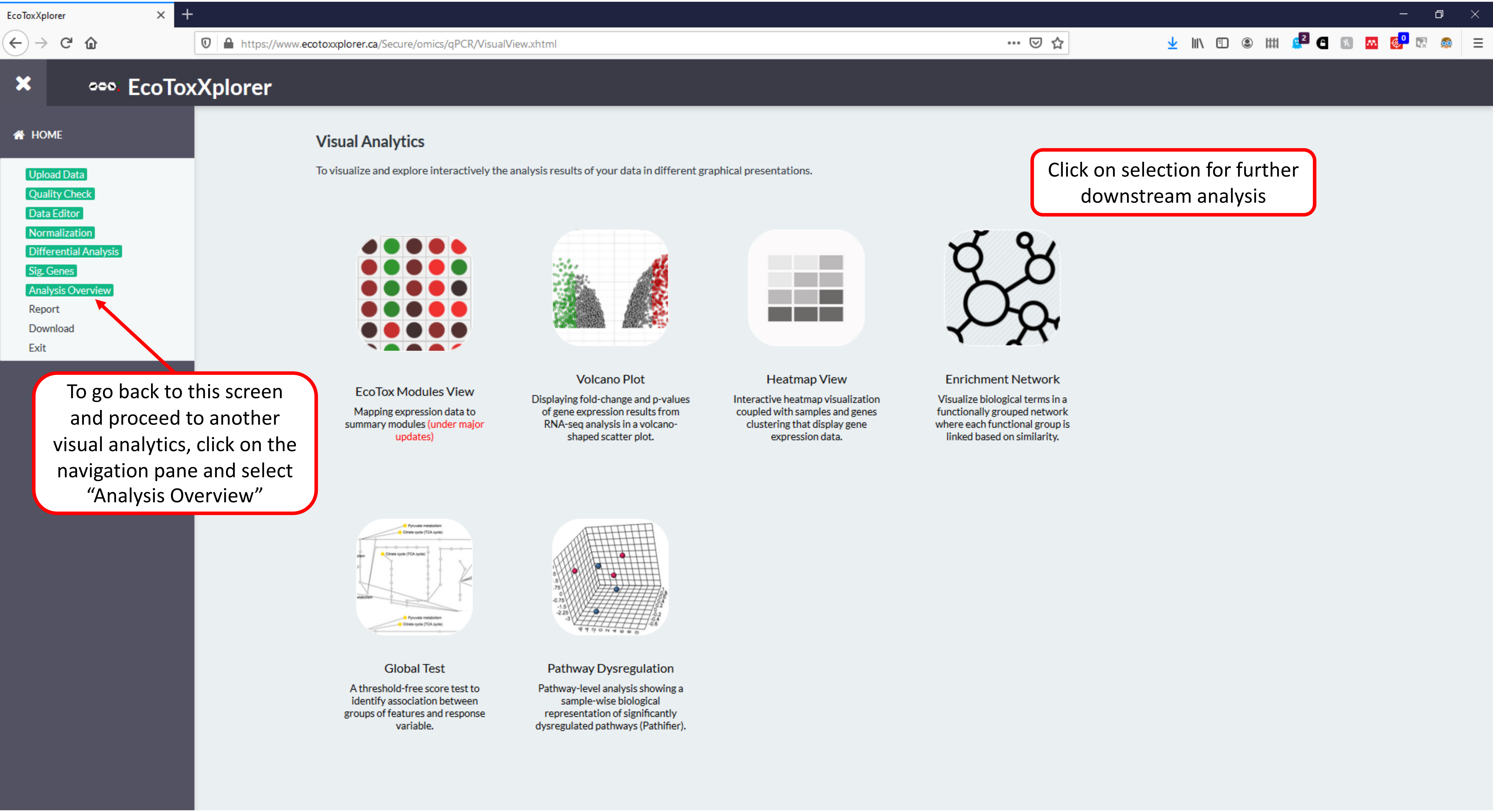
ID	FC	LogFC	Ct Average	Ct SD	Confidence Interval start	Confidence Interval end	P-value	Adjusted P-value	View
tcf7	1.4236	0.50959	1.5893	0.31085	1.2631	1.9155	6.5741E-4	0.10233	
lct	2.638	1.3995	-0.98757	2.4583	-3.5674	1.5923	7.6114E-4	0.10233	
abca1b	0.32415	-1.6253	0.12677	0.281	-0.16813	0.42166	0.0014986	0.10233	
lonrf1l	4.8251	2.2706	-0.5804	1.0034	-1.6334	0.47258	0.0018191	0.10233	
nr4a1	1.1765	0.23451	1.9438	0.179	1.7559	2.1316	0.0018513	0.10233	
xpc	0.36299	-1.462	0.47143	1.1476	-0.7329	1.6758	0.0020435	0.10233	
ppp1r9a	1.4775	0.5632	1.0279	0.22642	0.79033	1.2655	0.0025341	0.10233	
fgf10a	1.107	0.14667	2.7066	0.15756	2.5413	2.8719	0.0028936	0.10233	
pter	0.20028	-2.3199	-0.3404	0.26021	-0.61347	-0.067325	0.0030197	0.10233	

Previous

Proceed

③

Click “Proceed”



Visual Analytics

To visualize and explore interactively the analysis results of your data in different graphical presentations.

EcoTox Modules View

Mapping expression data to summary modules (under major updates)

Volcano Plot

Displaying fold-change and p-values of gene expression results from RNA-seq analysis in a volcano-shaped scatter plot.

Heatmap View

Interactive heatmap visualization coupled with samples and genes clustering that display gene expression data.

Enrichment Network

Visualize biological terms in a functionally grouped network where each functional group is linked based on similarity.

Global Test

A threshold-free score test to identify association between groups of features and response variable.

Pathway Dysregulation

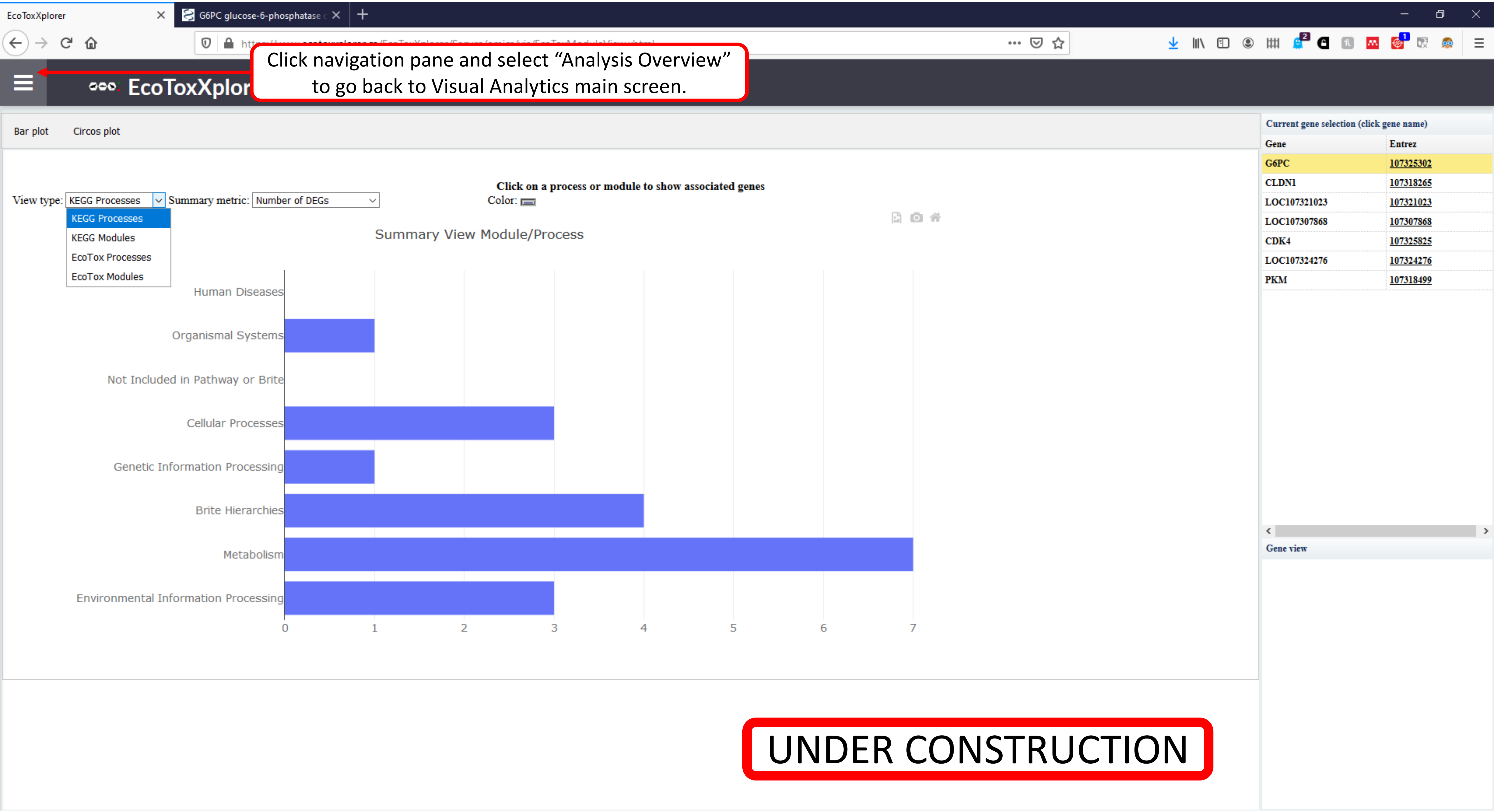
Pathway-level analysis showing a sample-wise biological representation of significantly dysregulated pathways (Pathifier).

Click on selection for further downstream analysis

To go back to this screen and proceed to another visual analytics, click on the navigation pane and select “Analysis Overview”

Ecotox Module

(under construction)



Click navigation pane and select “Analysis Overview” to go back to Visual Analytics main screen.

Click on a process or module to show associated genes

View type: KEGG Processes

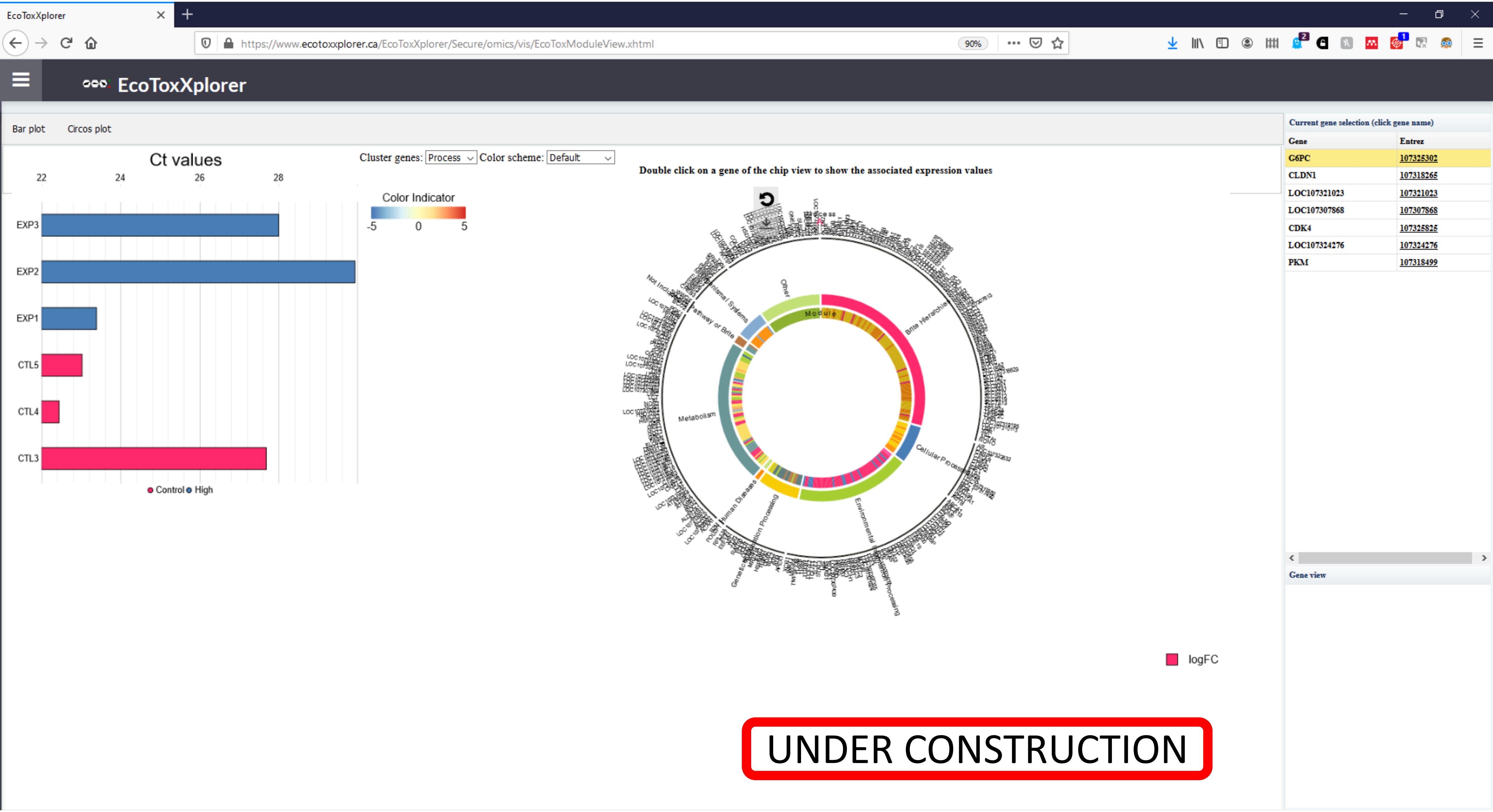
Summary metric: Number of DEGs

Color:

Current gene selection (click gene name)

Gene	Entrez
G6PC	107325302
CLDN1	107318265
LOC107321023	107321023
LOC107307868	107307868
CDK4	107325825
LOC107324276	107324276
PKM	107318499

UNDER CONSTRUCTION



Cluster genes: Process

Color scheme: Default

Double click on a gene of the chip view to show the associated expression values

Current gene selection (click gene name)

Gene	Entrez
G6PC	107325302
CLDN1	107318265
LOC107321023	107321023
LOC107307868	107307868
CDK4	107325825
LOC107324276	107324276
PKM	107318499

UNDER CONSTRUCTION

Gene Set Enrichment

Query: Sig. All

Database: GO:BP

☐	Pathway	Hits	P-value

Displaying 1 to 30 of 30 items

Gene View

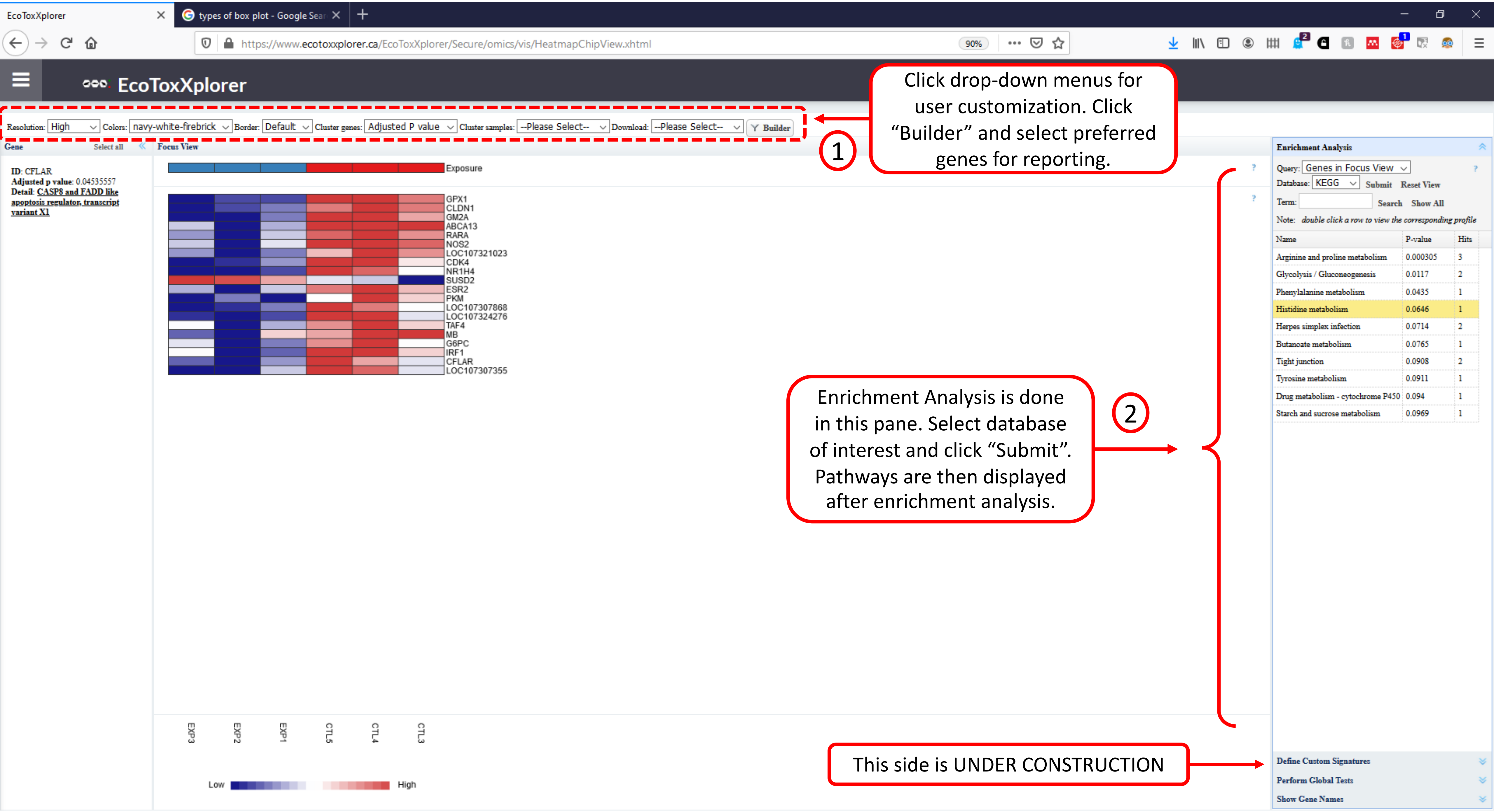
Interactive Volcano Plot

Double-click on a point to view, or select an area to explore (mouse-drag).

UNDER CONSTRUCTION

The interactive volcano plot shows the scatterplot based on set statistical significance.

Select a gene to display response across treatment groups



Resolution: High
Colors: navy-white-firebrick
Border: Default
Cluster genes: Adjusted P value
Cluster samples: --Please Select--
Download: --Please Select--
Builder

ID: CFLAR
Adjusted p value: 0.04535557
Detail: CASP8 and FADD like apoptosis regulator, transcript variant X1

Enrichment Analysis

Query: Genes in Focus View
Database: KEGG Submit Reset View
Term: Search Show All
Note: *double click a row to view the corresponding profile*

Name	P-value	Hits
Arginine and proline metabolism	0.000305	3
Glycolysis / Gluconeogenesis	0.0117	2
Phenylalanine metabolism	0.0435	1
Histidine metabolism	0.0646	1
Herpes simplex infection	0.0714	2
Butanoate metabolism	0.0765	1
Tight junction	0.0908	2
Tyrosine metabolism	0.0911	1
Drug metabolism - cytochrome P450	0.094	1
Starch and sucrose metabolism	0.0969	1

① Click drop-down menus for user customization. Click “Builder” and select preferred genes for reporting.

② Enrichment Analysis is done in this pane. Select database of interest and click “Submit”. Pathways are then displayed after enrichment analysis.

This side is UNDER CONSTRUCTION

Linking: Kappa Statistics

Database: GO:BP

Submit

☐	Pathway	Expected	Hits	P-value
☐	response to xenobiotic stimulus	0.00573	1	0.00572
☐	microtubule cytoskeleton organiza	0.00764	1	0.00762
☐	lipid transport	0.0197	1	0.0196
☐	apoptotic process	0.0382	1	0.0377
☐	carbohydrate metabolic process	0.0752	1	0.073
☐	proteolysis	0.0968	1	0.0932
☐	transport	0.291	1	0.259
☐	glucose metabolic process	0.653		0.503

Page 1 of 1

Gene View

Response to xenobiotic stimulus

vtg1

1

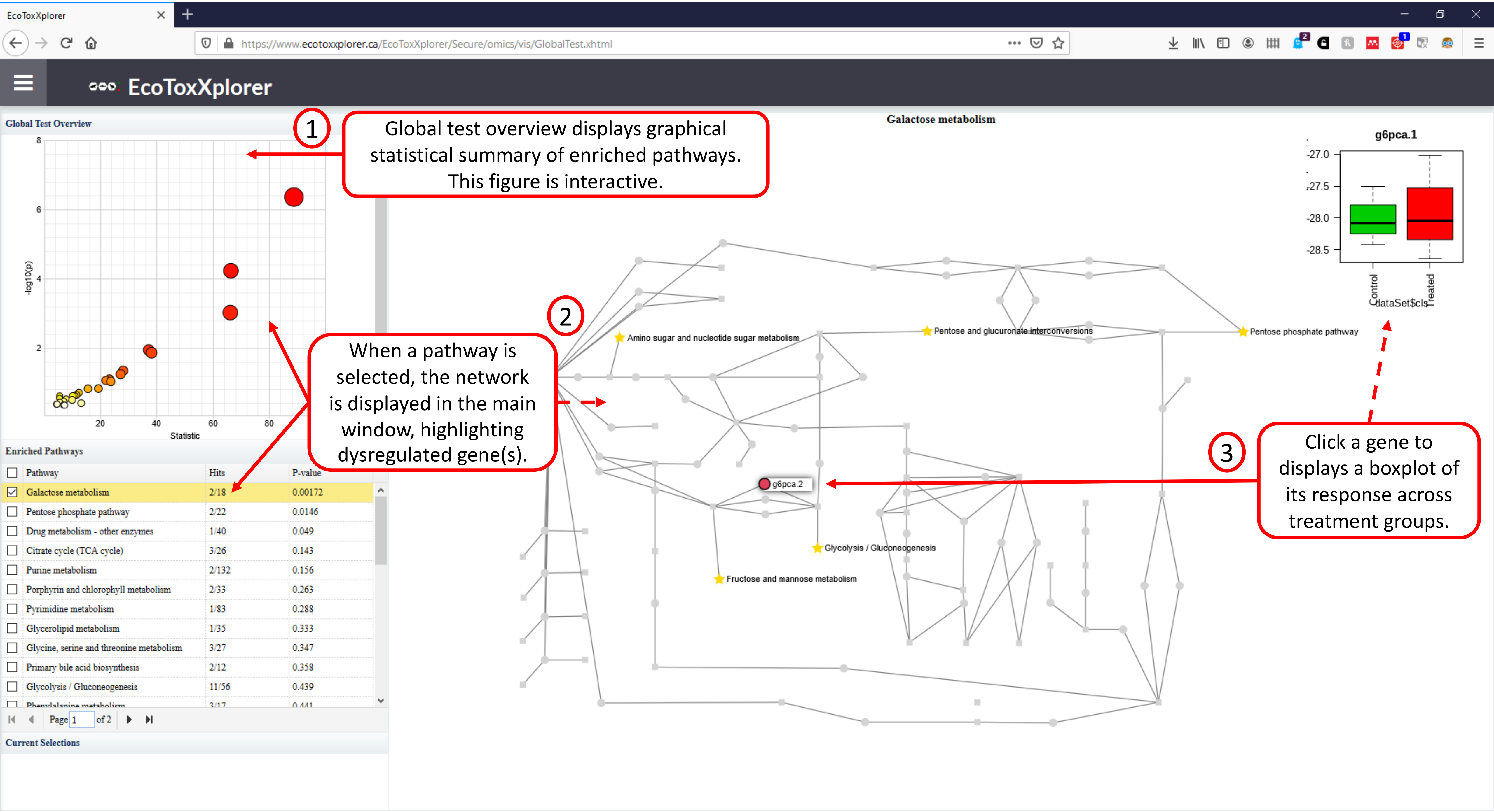
Network Enrichment allows interactive view of enriched pathways. Select database of interest and click “Submit” to show enriched pathways.

2

Select a pathway to zoom in and center the network to that specific node.

3

Click a node to display the associated gene(s) in the Gene View. Clicking the gene opens a new tab showing gene information from public databases (NCBI).



① Global test overview displays graphical statistical summary of enriched pathways. This figure is interactive.

② When a pathway is selected, the network is displayed in the main window, highlighting dysregulated gene(s).

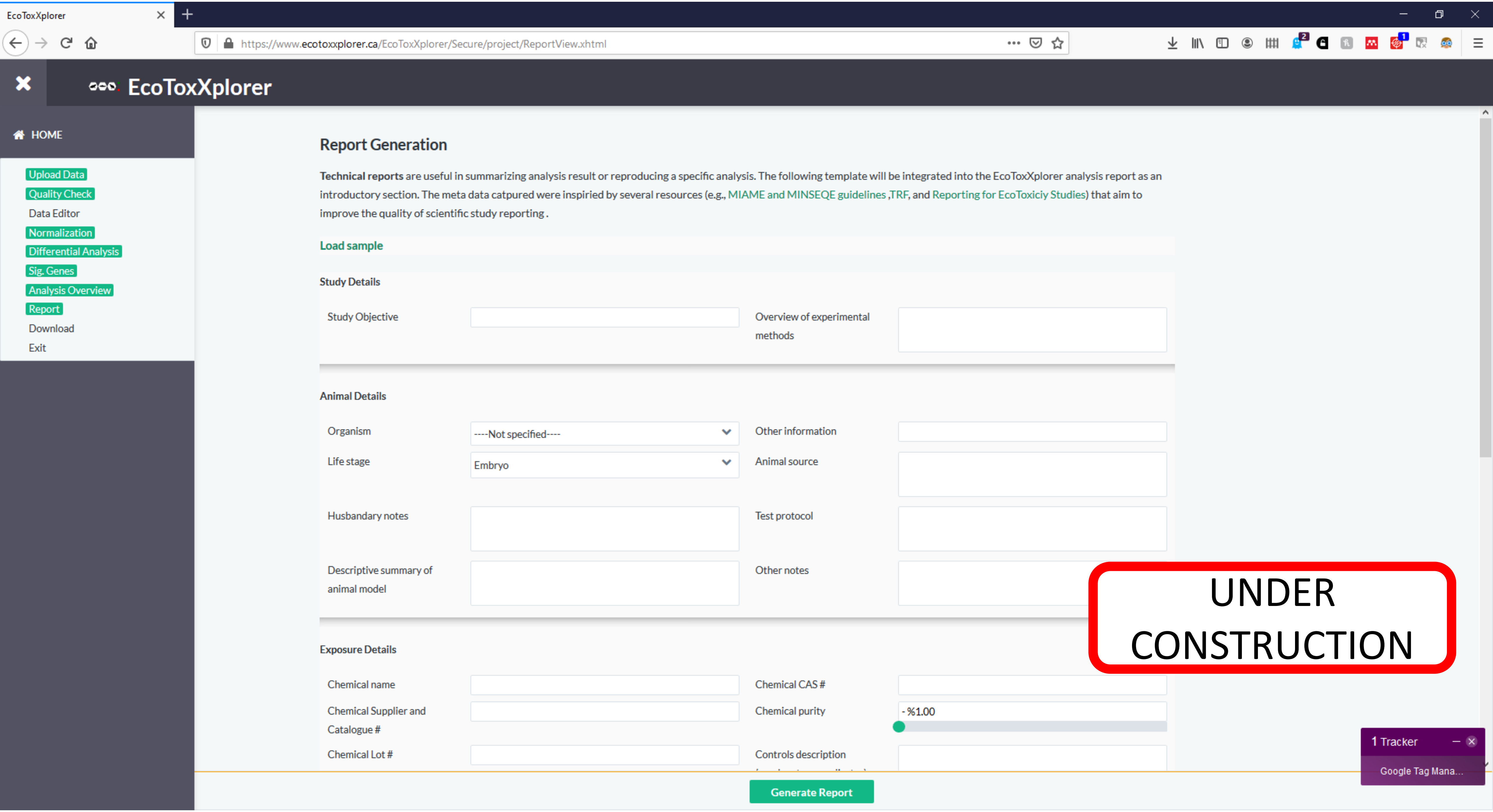
③ Click a gene to displays a boxplot of its response across treatment groups.

Enriched Pathways

☐	Pathway	Hits	P-value
☑	Galactose metabolism	2/18	0.00172
☐	Pentose phosphate pathway	2/22	0.0146
☐	Drug metabolism - other enzymes	1/40	0.049
☐	Citrate cycle (TCA cycle)	3/26	0.143
☐	Purine metabolism	2/132	0.156
☐	Porphyrin and chlorophyll metabolism	2/33	0.263
☐	Pyrimidine metabolism	1/83	0.288
☐	Glycerolipid metabolism	1/35	0.333
☐	Glycine, serine and threonine metabolism	3/27	0.347
☐	Primary bile acid biosynthesis	2/12	0.358
☐	Glycolysis / Gluconeogenesis	11/56	0.439
☐	Phenylalanine metabolism	3/17	0.441

Pathway Dysregulation

(under construction)



Report Generation

Technical reports are useful in summarizing analysis result or reproducing a specific analysis. The following template will be integrated into the EcoToxXplorer analysis report as an introductory section. The meta data catpured were inspiried by several resources (e.g., MIAME and MINSEQE guidelines ,TRF, and Reporting for EcoToxiciy Studies) that aim to improve the quality of scientific study reporting .

Load sample

Study Details

Study Objective

Overview of experimental methods

Animal Details

Organism: ----Not specified----

Other information

Life stage: Embryo

Animal source

Husbandary notes

Test protocol

Descriptive summary of animal model

Other notes

Exposure Details

Chemical name

Chemical CAS #

Chemical Supplier and Catalogue #

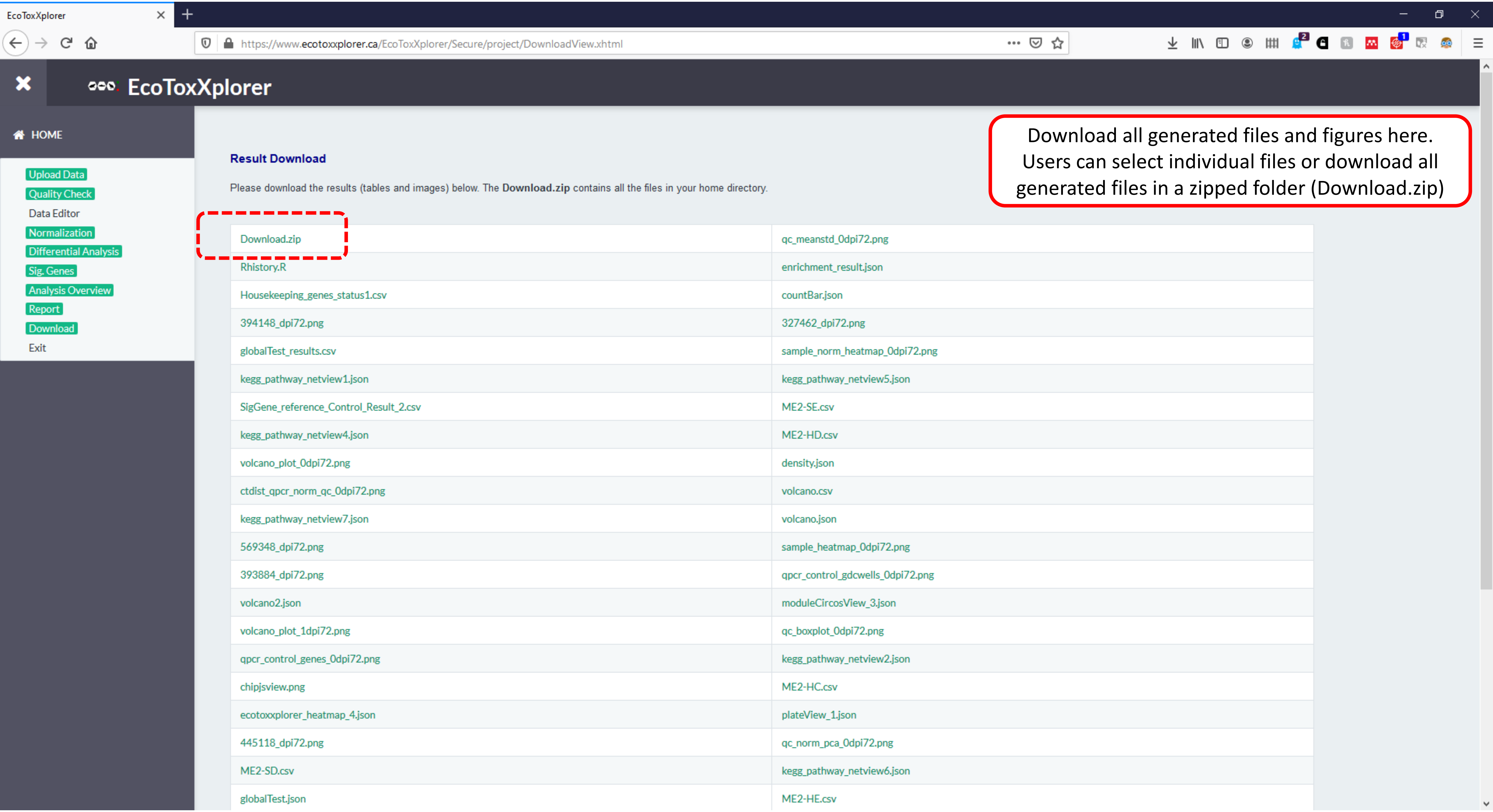
Chemical purity: - %1.00

Chemical Lot #

Controls description

UNDER CONSTRUCTION

Download all generated files and figures here. Users can select individual files or download all generated files in a zipped folder (Download.zip)

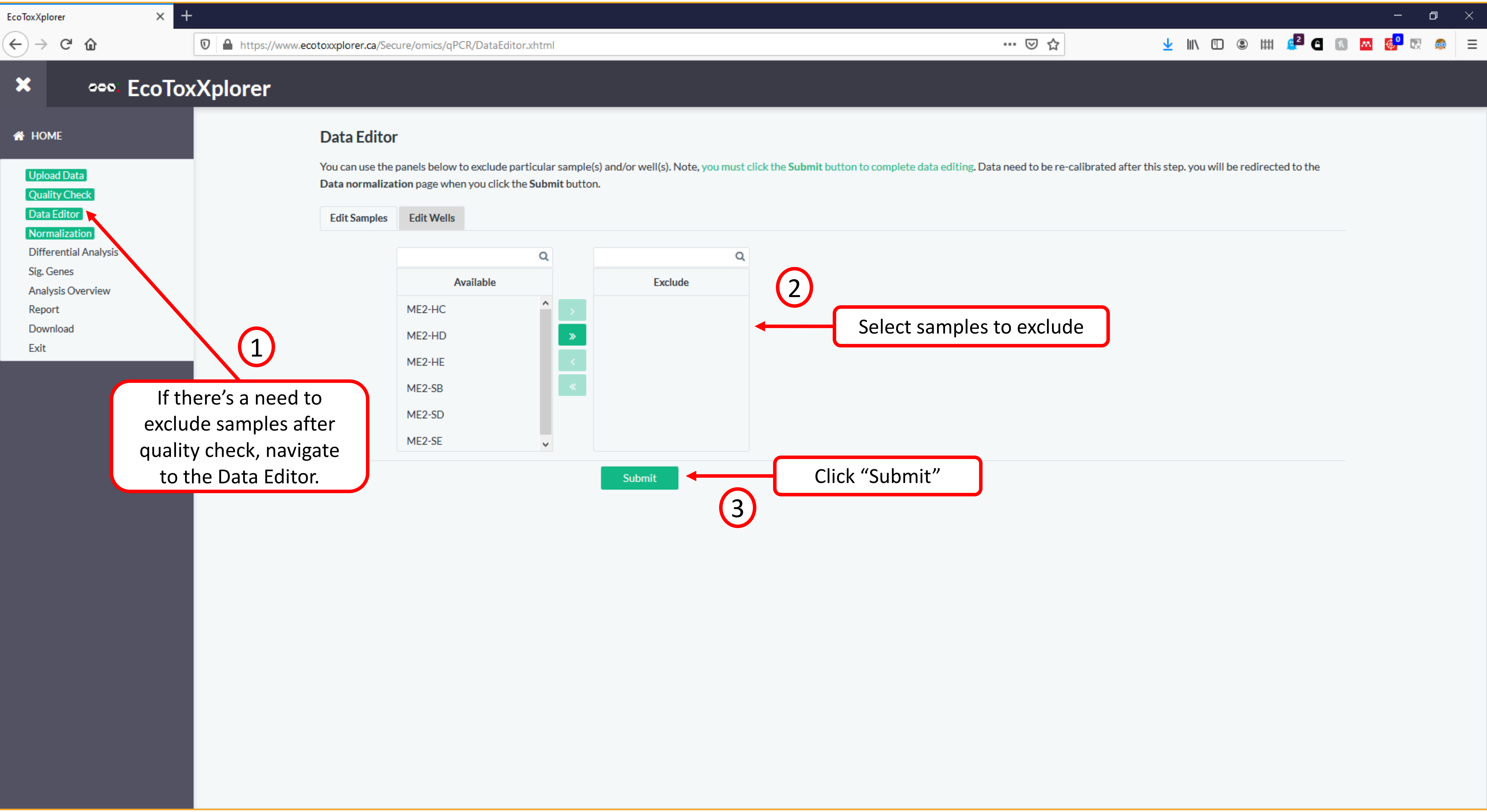


Result Download

Please download the results (tables and images) below. The **Download.zip** contains all the files in your home directory.

Download.zip	qc_meanstd_0dpi72.png
Rhistory.R	enrichment_result.json
Housekeeping_genes_status1.csv	countBar.json
394148_dpi72.png	327462_dpi72.png
globalTest_results.csv	sample_norm_heatmap_0dpi72.png
kegg_pathway_netview1.json	kegg_pathway_netview5.json
SigGene_reference_Control_Result_2.csv	ME2-SE.csv
kegg_pathway_netview4.json	ME2-HD.csv
volcano_plot_0dpi72.png	density.json
ctdist_qpcr_norm_qc_0dpi72.png	volcano.csv
kegg_pathway_netview7.json	volcano.json
569348_dpi72.png	sample_heatmap_0dpi72.png
393884_dpi72.png	qpcr_control_gdcwells_0dpi72.png
volcano2.json	moduleCircosView_3.json
volcano_plot_1dpi72.png	qc_boxplot_0dpi72.png
qpcr_control_genes_0dpi72.png	kegg_pathway_netview2.json
chipjsview.png	ME2-HC.csv
ecotoxxplorer_heatmap_4.json	plateView_1.json
445118_dpi72.png	qc_norm_pca_0dpi72.png
ME2-SD.csv	kegg_pathway_netview6.json
globalTest.json	ME2-HE.csv

Appendix



Data Editor

You can use the panels below to exclude particular sample(s) and/or well(s). Note, you must click the **Submit** button to complete data editing. Data need to be re-calibrated after this step. you will be redirected to the **Data normalization** page when you click the **Submit** button.

① If there's a need to exclude samples after quality check, navigate to the Data Editor.

② Select samples to exclude

③ Click "Submit"

Questions?

Contact us at

https://www.ecotoxxplorer.ca/doc/ContactView.xhtml