Tox21 Enricher

User's Manual

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http://hurlab.med.und.edu/Tox21Enricher

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I. Introduction to Tox21 Enricher

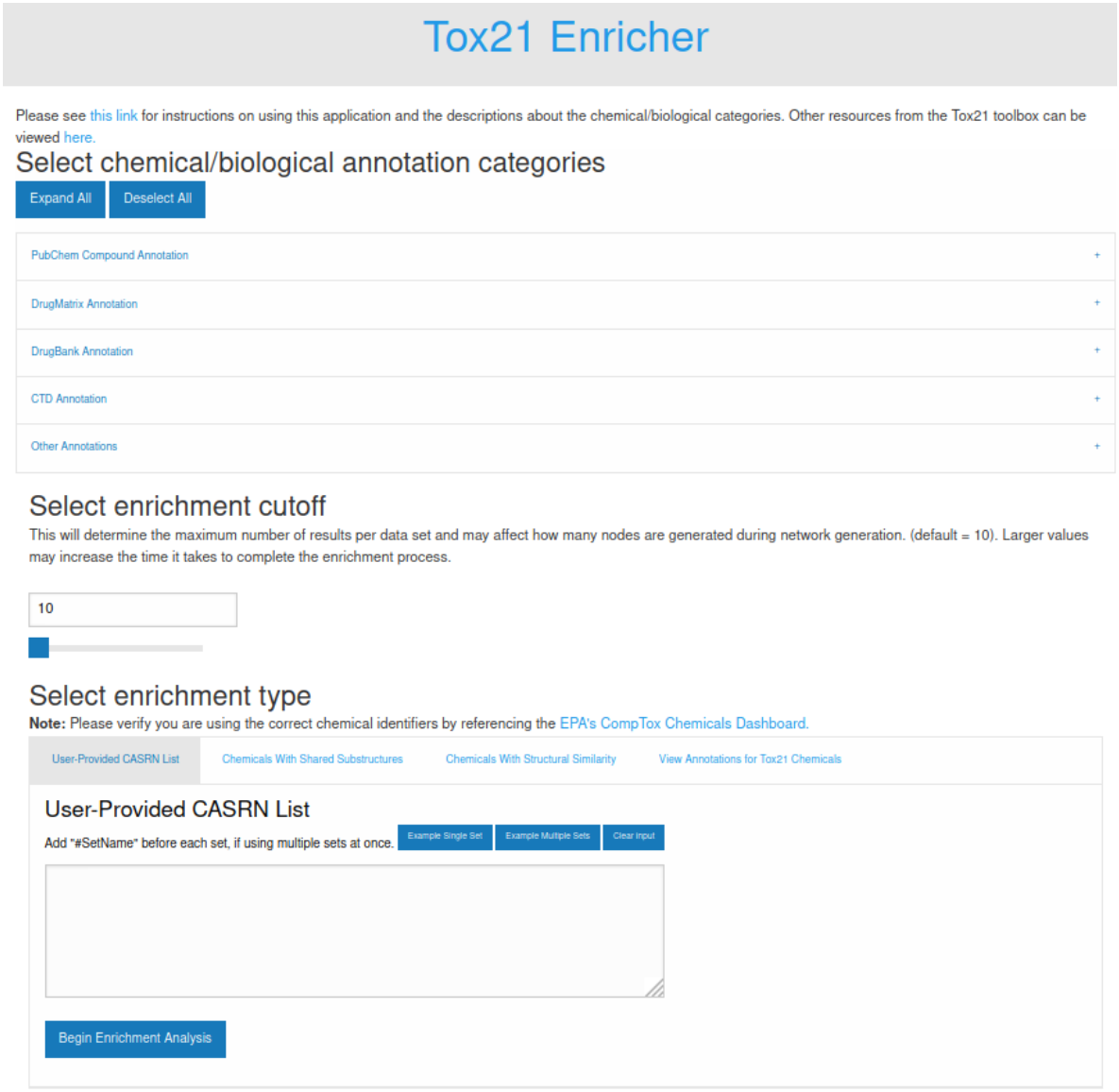
Humans are exposed to tens of thousands of chemicals that are used in daily life, some at levels that may pose a health risk. For many of these chemicals, there is limited toxicological information which makes risk assessment impossible. The United States Toxicology Testing in the 21st Century (Tox21) program was established to develop more efficient and human relevant toxicity assessment methods. The Tox21 program is currently screening over 10,000 chemicals, the Tox21 10K library, using quantitative high-throughput screening (qHTS) of assays that measure effects on toxicity pathways. To date, more than 70 assays have yielded >12 million concentration-response curves by Tox21 researchers. To efficiently apply these data for identifying potential hazardous compounds and for informing further toxicological testing, the United States National Toxicology Program (NTP) has developed several web applications (Tox21 Toolbox: http://ntp.niehs.nih.gov/tbox/), including tools for data visualization (Tox21 Curve Browser) and exploration (Tox21 Activity Profiler).

One critical usage of this dataset is to perform chemical-relational analysis based on the patterns of activity across the Tox21 assays and then to use nearest neighbor-based prediction to infer the toxicological properties of untested chemicals via their association with tested chemicals. One approach to inferring the specific properties is to perform chemical annotation enrichment of chemical neighborhoods.

Here, we present Tox21 Enricher, a web-based chemical annotation enrichment tool for Tox21 assay data. Tox21 Enricher identifies significantly over-represented chemical biological annotations among sets of chemicals (neighborhoods), which facilitates the identification of the toxicological properties and mechanisms in the chemical sets.

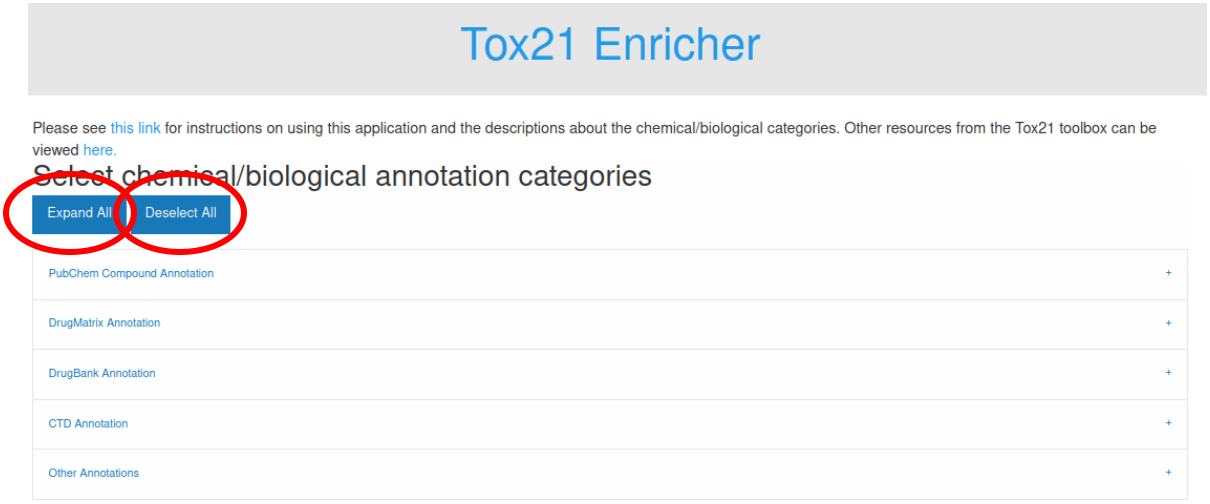
II. Landing Page

Upon visiting the Tox21 Enricher's web page, you will see the landing page.

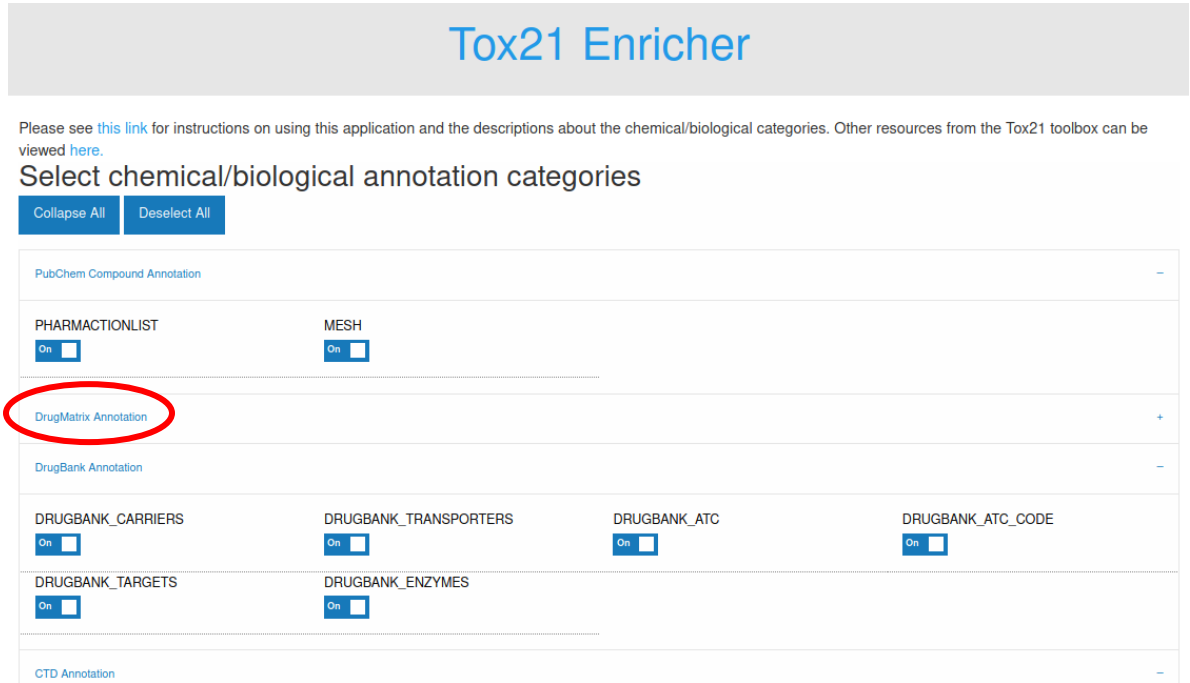


II.1. Selecting Annotation Categories

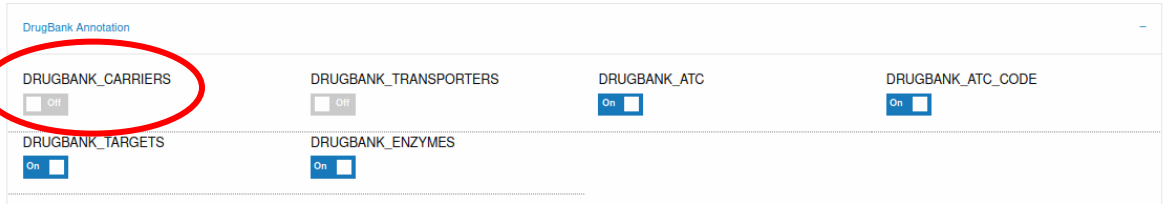
This page has buttons for expanding the annotation category group accordions and deselecting/selecting all enrichment categories. These buttons are circled in the image below.



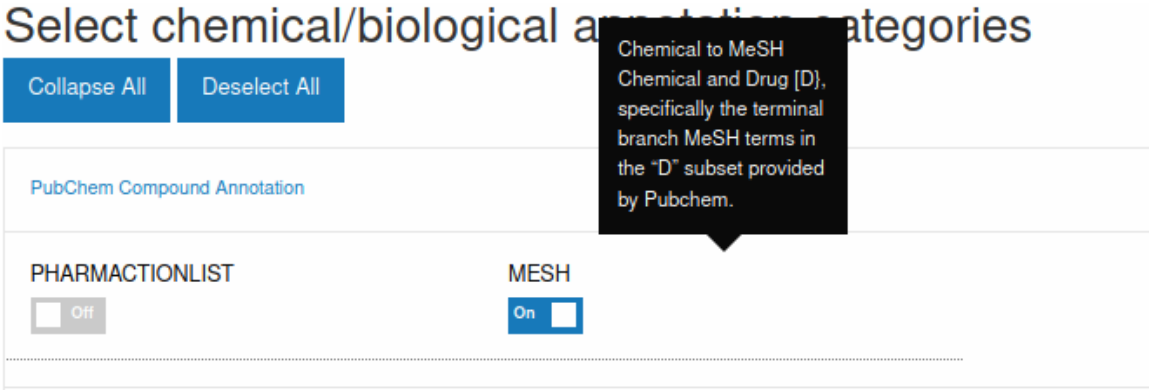
Once the “Expand All” button is clicked, each of the annotation category accordion tabs will expand to show the categories they contain. Each accordion tab may also be expanded/collapsed individually as shown below.



Each annotation class within the accordion tabs can be interacted with. Clicking on the toggle switch for an annotation class will set it to either “On” and “Off.” When a class is set to “On,” Tox21 Enricher will include results from that annotation class, if applicable. When a class is set to “Off,” Tox21 Enricher will ignore results from that class. By default, all annotation classes are set to “On,” *except* for CTD_GOFAT_BIOPROCESS as running enrichment analysis processes with these annotations is very time intensive.

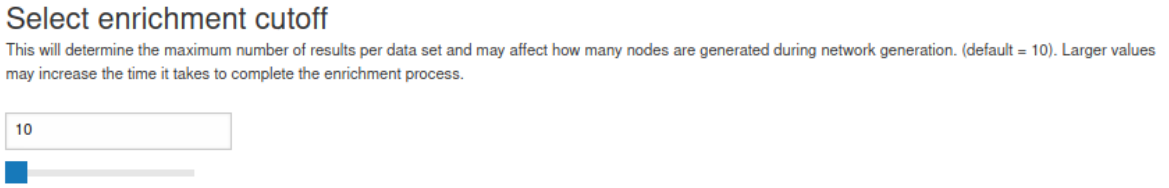


Additionally, hovering over each annotation class will display a tooltip with a brief description of the class.



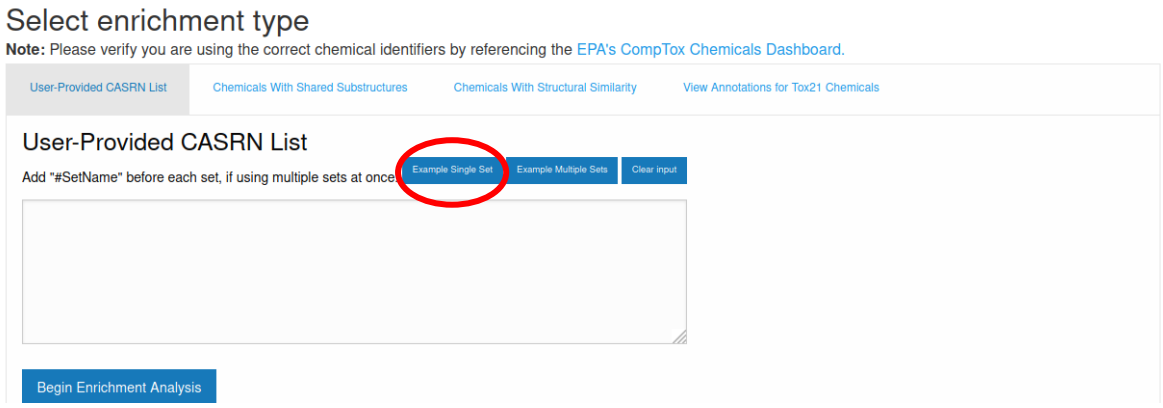
II.2. Selecting Enrichment Cutoff Value

After selecting the appropriate annotation categories, the next step is specifying the enrichment cutoff value. This value determines the maximum number of enrichment results displayed per data set submitted. By extension, this will also determine the maximum number of nodes displayed during network generation. By default, this is set to a minimum of 10 and can be increased to a maximum of 50.



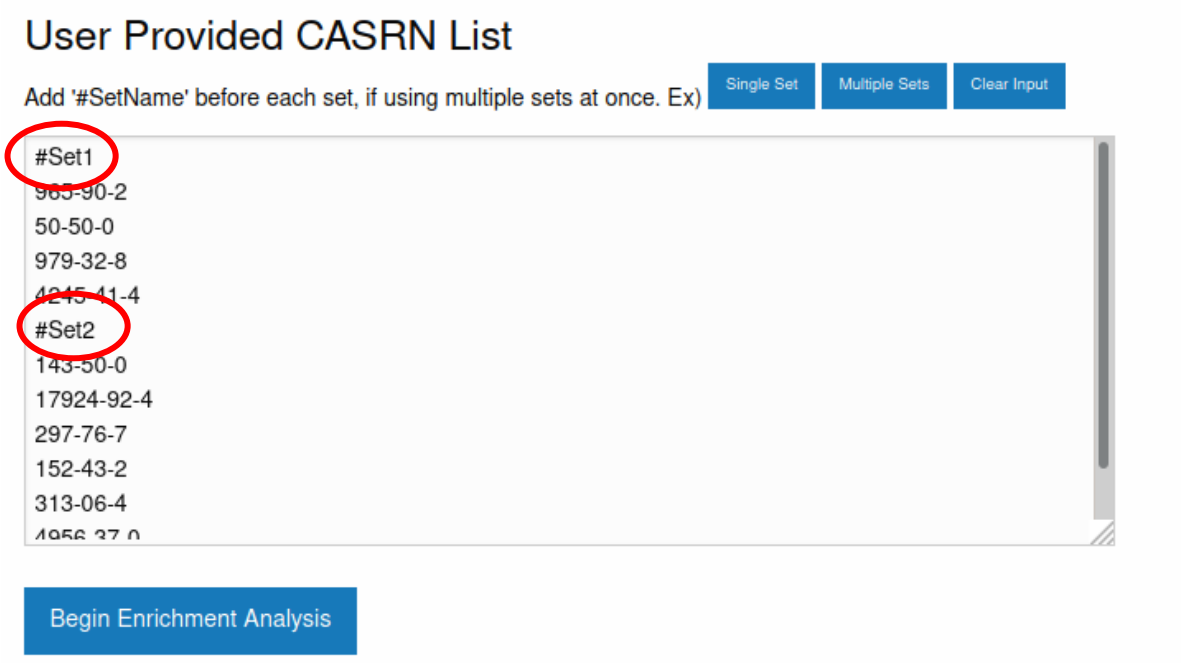
II.3. Selecting Enrichment Analysis Type

After selecting the cutoff value, the final step is selecting the enrichment analysis type and providing input for it. This input can take the form of CASRNs, SMILES, or InChI strings, depending on enrichment type. Each option has a text area for input. Each option also has buttons above their respective text areas that will populate the text areas with example input. The “Clear Input” button will clear the text in the box.



II.3.a. Enrich From User Provided CASRN List

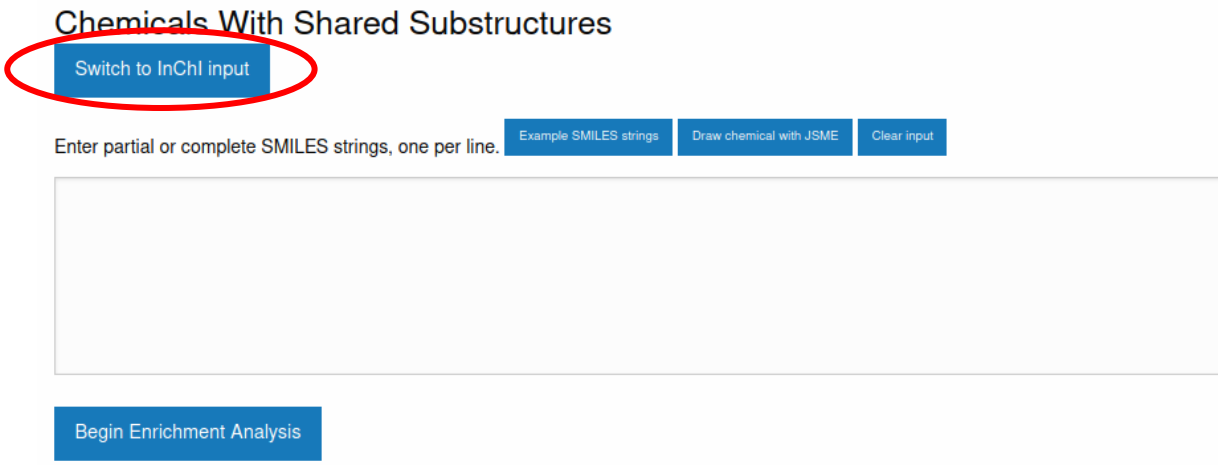
This enrichment analysis type accepts only CASRN strings as input (one CASRN per line). Multiple sets may be independently enriched at the same time. To enrich multiple sets, separate lines of CASRN strings with *unique* set names of the form “#SetName.”



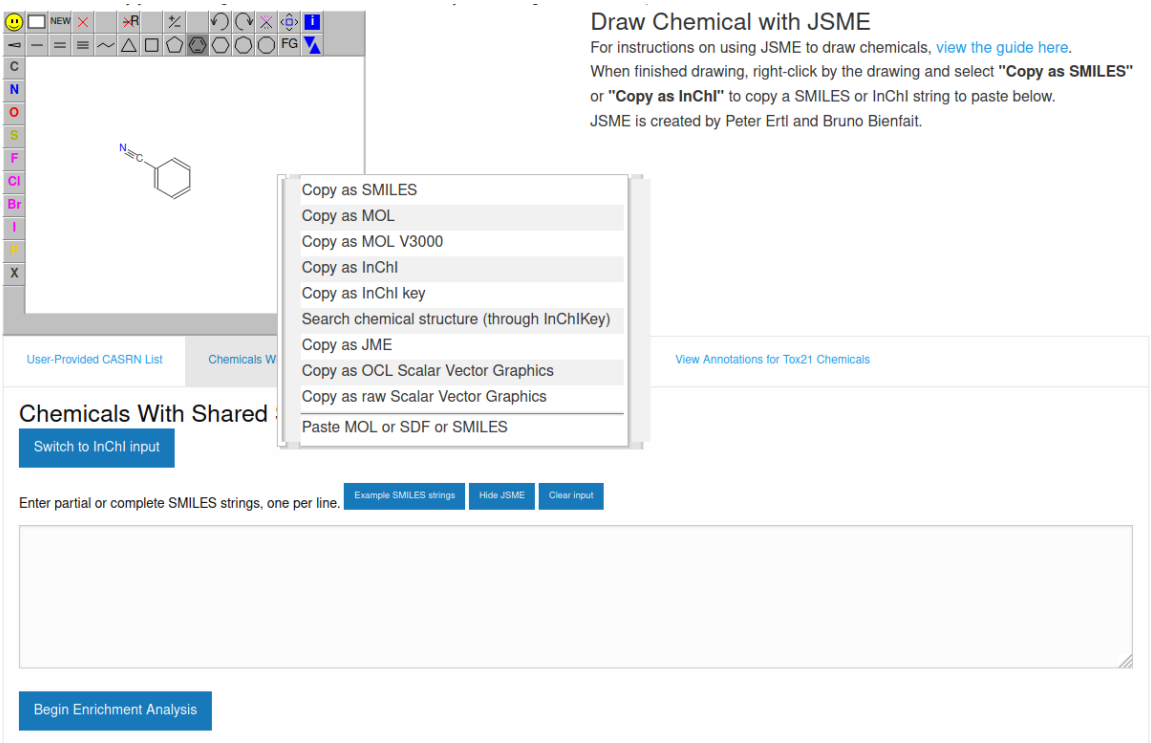
Before performing enrichment from a user provided CASRN list, please cross-reference your CASRN inputs with those in the EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) to ensure accuracy during the enrichment analysis.

II.3.b. Enrich From Chemicals With Shared Substructures

This enrichment analysis type accepts either SMILES or InChI strings (one per line). Each string is treated as its own data set and will be enriched independently of any other sets provided. By default, this enrichment is in SMILES mode. Clicking the "Switch to InChI input" button will switch to InChI mode, which allows the enrichment to accept InChI strings.

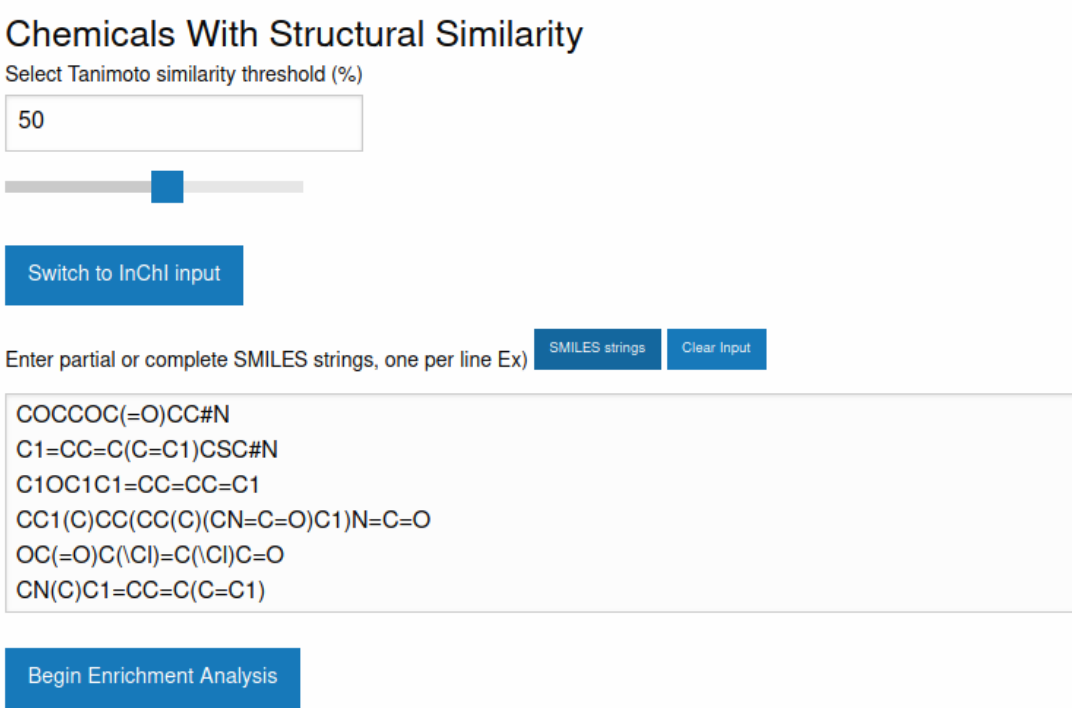


Clicking the "Draw chemical with JSME" button will open the JSME Molecule Editor by Peter Ertl and Bruno Bienfait. Here, we can draw a molecule using the applet's tools. When we are finished drawing, we can right-click in the whitespace of the canvas and select "Copy as SMILES" or "Copy as InChI" and paste in the appropriate input box.

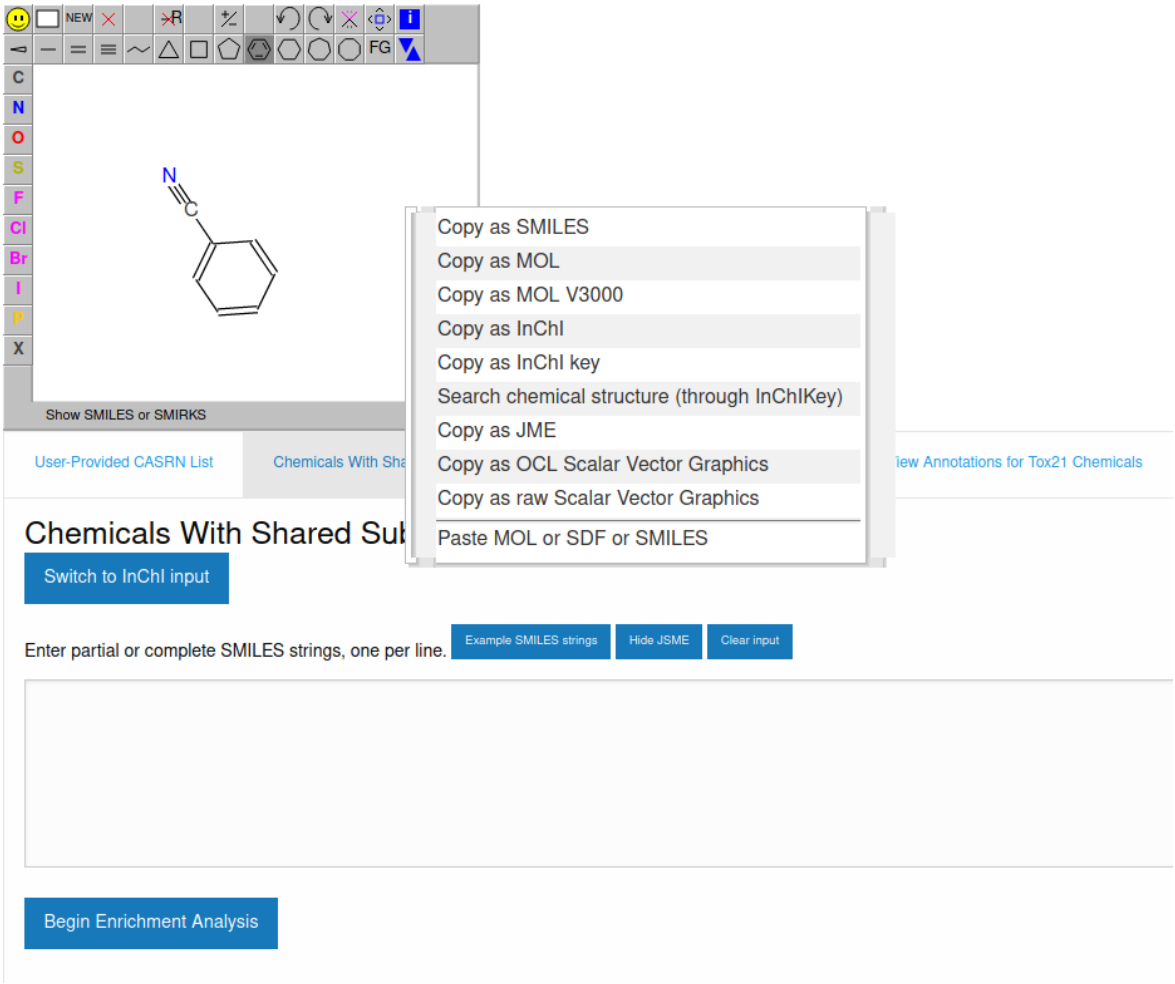


II.3.c. Enrich From Chemicals With Structural Similarity

This enrichment analysis type accepts either SMILES or InChI strings (one per line). Each string is treated as its own data set and will be enriched independently of any other sets provided. By default, this enrichment is in SMILES mode. Clicking the “Switch to InChI input” button will switch to InChI mode, which allows the enrichment to accept InChI strings. The Tanimoto similarity threshold may be specified using the slider controls to set the strictness of the search. By default, this is set to 50%.



Clicking the “Draw chemical with JSME” button will open the JSME Molecule Editor by Peter Ertl and Bruno Bienfait. Here, we can draw a molecule using the applet’s tools. When we are finished drawing, we can right-click in the whitespace of the canvas and select “Copy as SMILES” or “Copy as InChI” and paste in the appropriate input box.


NEW
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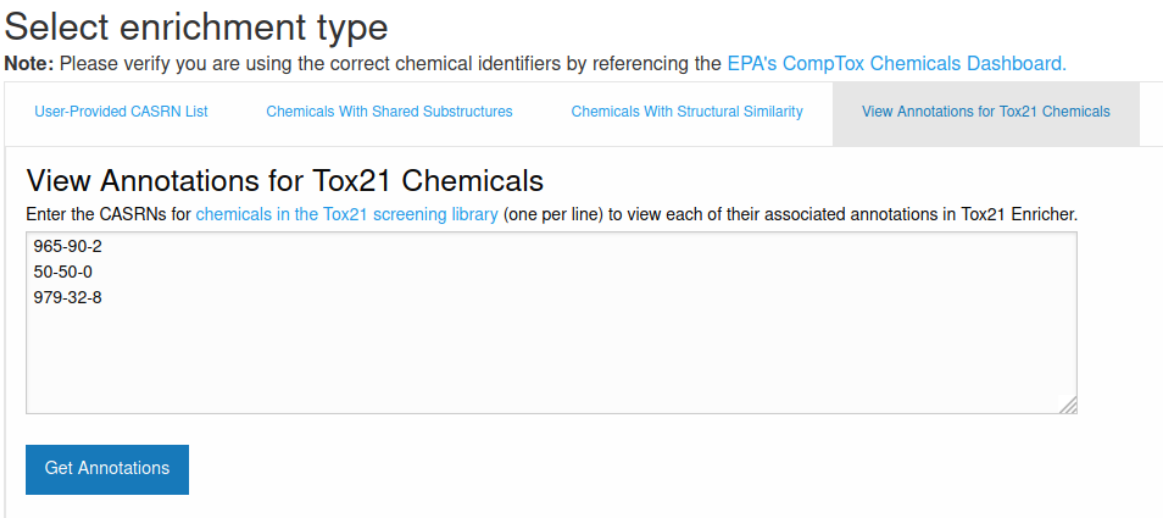
P
X
N
C
Show SMILES or SMIRKS
Copy as SMILES
Copy as MOL
Copy as MOL V3000
Copy as InChI
Copy as InChI key
Search chemical structure (through InChIKey)
Copy as JME
Copy as OCL Scalar Vector Graphics
Copy as raw Scalar Vector Graphics
Paste MOL or SDF or SMILES
User-Provided CASRN List
Chemicals With Sha
iew Annotations for Tox21 Chemicals
Chemicals With Shared Sub
Switch to InChI input
Enter partial or complete SMILES strings, one per line.
Example SMILES strings
Hide JSME
Clear input
Begin Enrichment Analysis

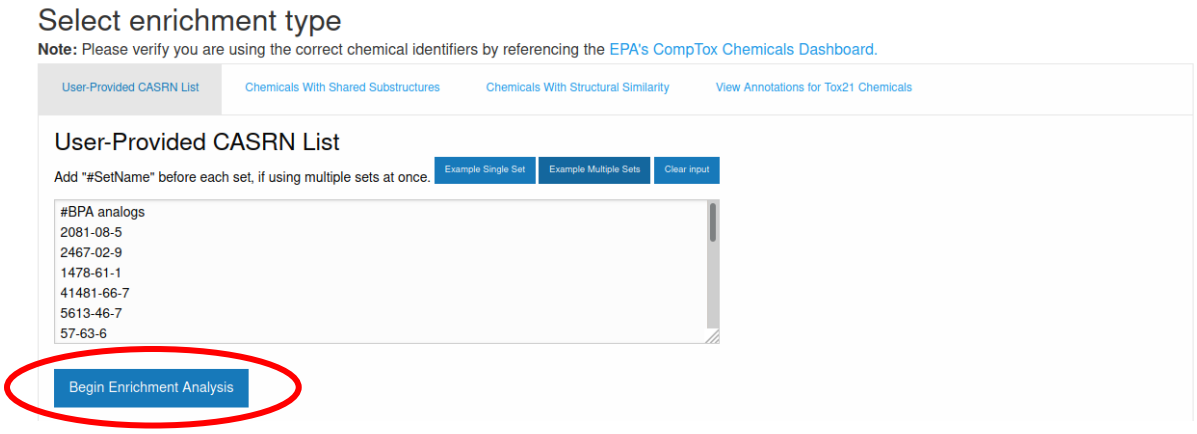
II.3.d. View Annotations for Tox21 Chemicals

This function does not actually perform enrichment analysis; rather, it returns a list of every annotation in the Tox21 database that is associated with each given chemical. Like the "User-Provided CASRN List" enrichment type, this enrichment analysis type accepts only CASRN strings as input (one CASRN per line). Each CASRN is treated as its own set. This mode will not provide any annotations for a CASRN if that chemical is not present in the Tox21 screening library. To view all the chemicals in the Tox21 screening library, click the "chemicals in the Tox21 screening library" link which opens the page https://comptox.epa.gov/dashboard/chemical_lists/TOX21SL in a new window.



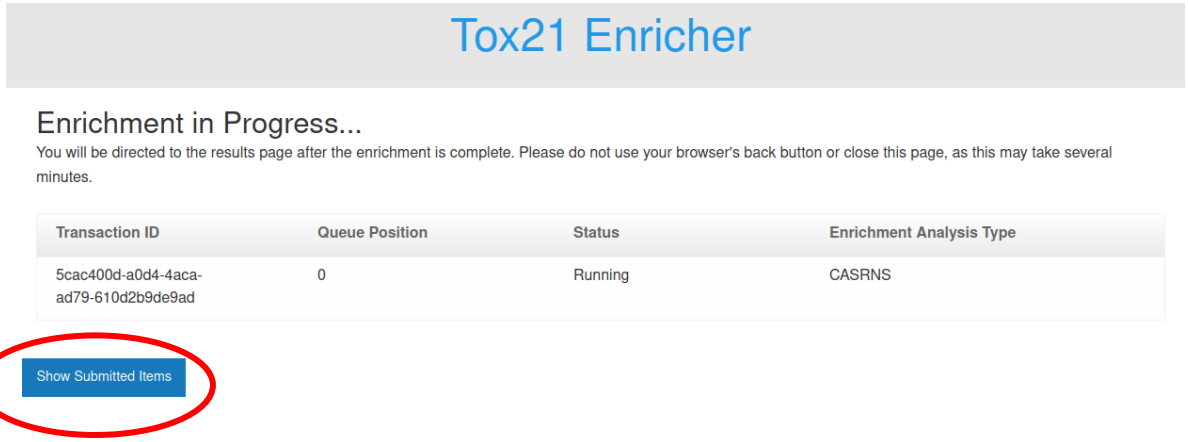
II.4. Performing Enrichment

After input has been entered, it is time to begin enrichment. This can be done by clicking on the appropriate “Begin Enrichment Analysis” button for whichever input you are using. The button that is clicked will use the data in its respective text area, so you must ensure you are using the right button. This can be seen using the multi-set CASRN example input below.



III. Waiting Page

After submitting data for enrichment analysis, the waiting page will be loaded. Do not press the “Back” or “Reload” browser buttons at this point, as this will prevent Tox21 Enricher from providing the enrichment results correctly. This page displays a transaction ID assigned to our submitted enrichment, its position in the application’s queue, its running status, and its enrichment analysis type. Clicking the “Show Submitted Items” button will display a list of the chemicals we submitted for this enrichment analysis.



III.1. Transaction ID

The transaction ID is a UUID assigned to each enrichment analysis process. This ID is for Tox21 Enricher to keep track of the enrichment requests it receives. This is not the same as the ID for the result set of the process: this is a separate ID.

III.2. Queue Position

Tox21 Enricher can only simultaneously process a maximum of 5 enrichment analysis processes. Therefore, only transactions in queue positions 0-4 may be running at any given time. Enrichment analysis processes in positions 5 and greater must wait for a currently running process to terminate.

III.3. Status

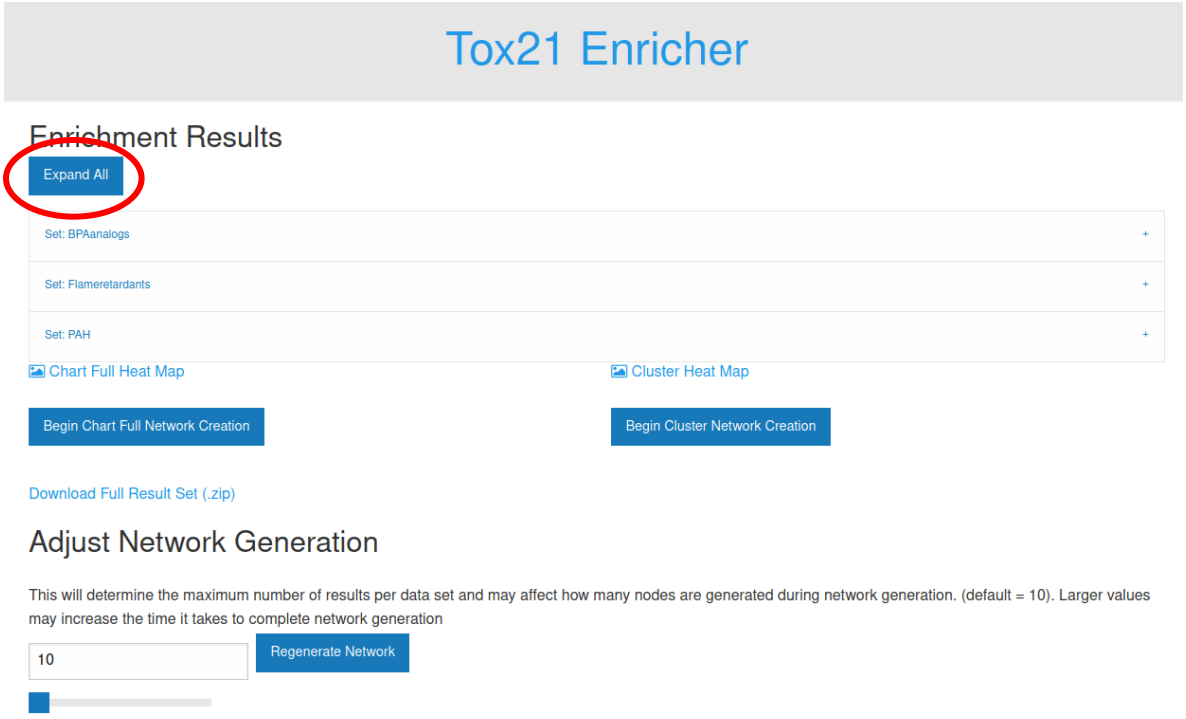
Enrichment processes may have one of four different statuses at any given time:

- **Initializing** – The enrichment analysis request is being processed.
- **Running** – The enrichment analysis is currently running.
- **Waiting** – The enrichment analysis is in a queue position in 5 or greater and is waiting for a running process to terminate.
- **Complete** – The enrichment analysis is complete and will shortly redirect the user to the results page.

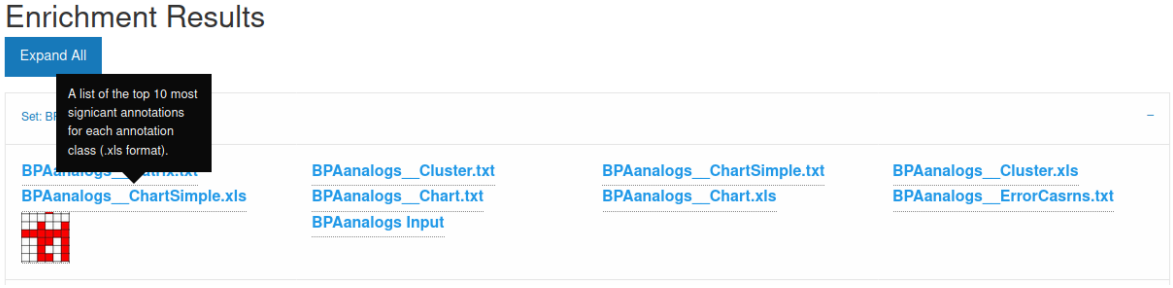
IV. Results Page

IV.1. Enrichment Results

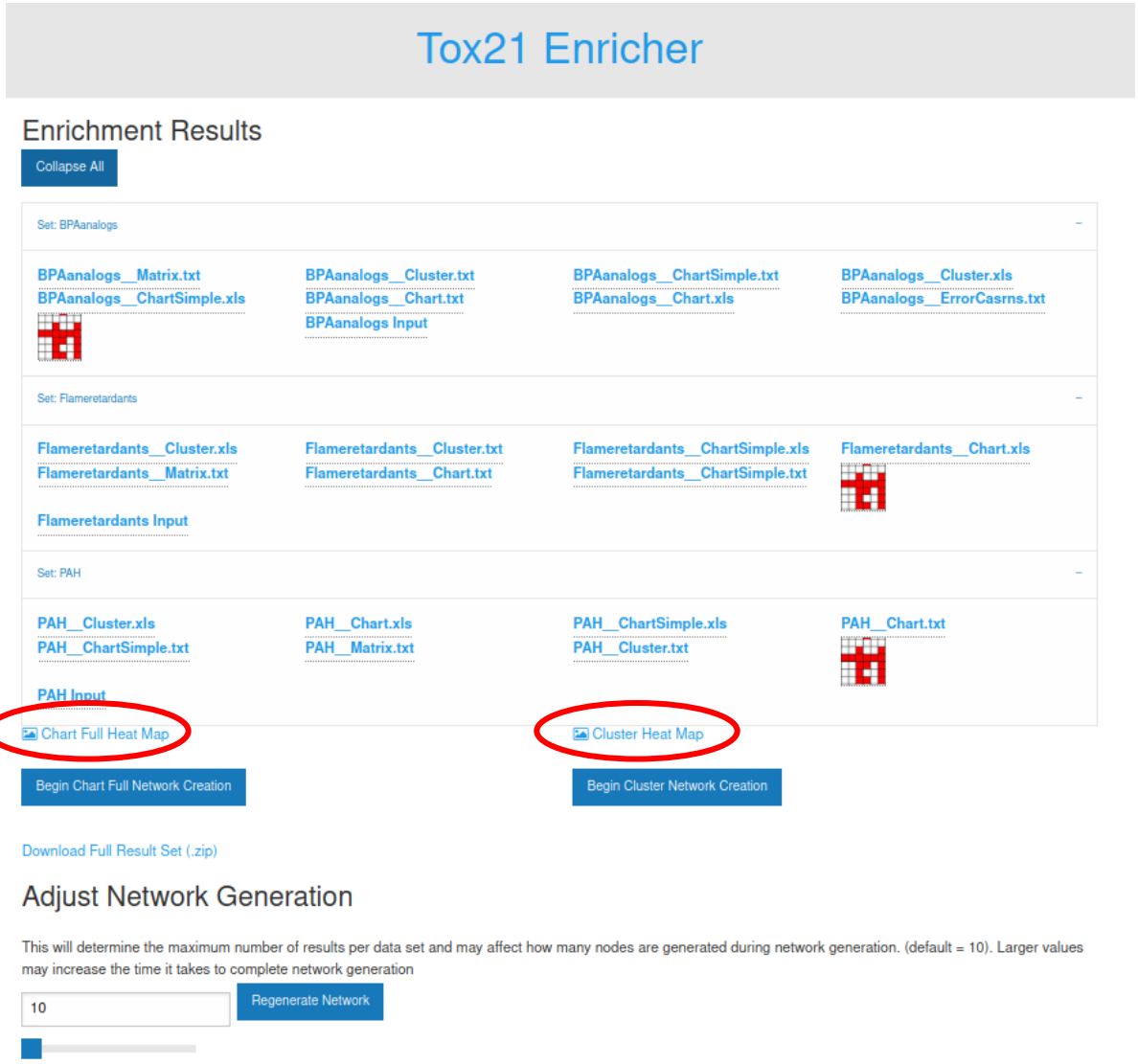
Once enrichment is complete, a results page will be loaded. Like the landing page, results on this page will be placed in collapsed accordion tabs. Each accordion tab can be clicked on to expand it, or the “Expand All” button may be used, like on the landing page. This button is highlighted in the screenshot below.



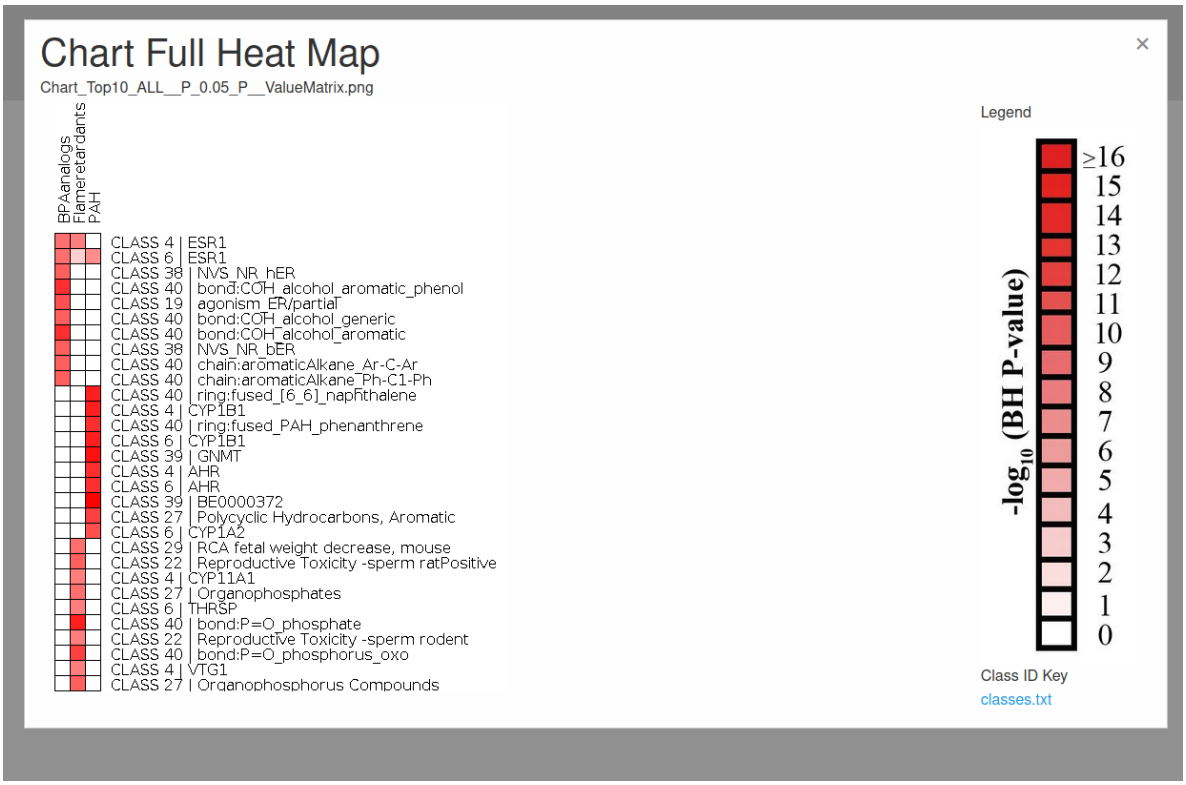
The results shown above are that of our multi-set input that we submitted in our landing page example. Hovering over any of the items in the result sets will display a tooltip describing what the item is.



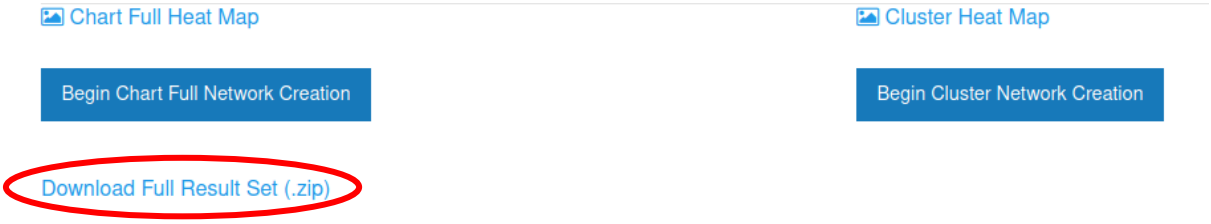
In the screenshot below, we can see the results for each set expanded. The cluster and chart full heat map image links can also be seen highlighted.



Clicking on one of the heatmap image links will display the corresponding heatmap as seen in the screenshot below.

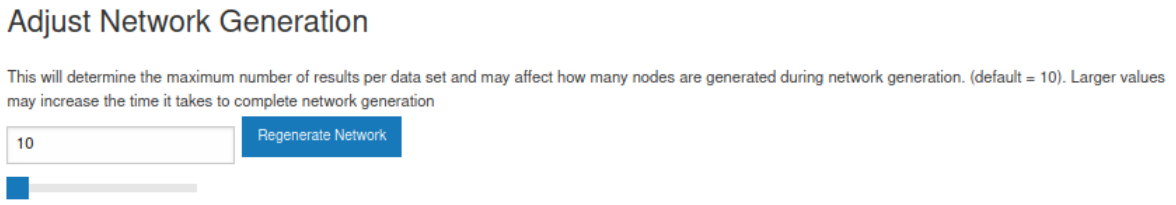


All the enrichment result files can be downloaded by clicking the “Download Full Result Set (.zip)” link, which will prompt us to download the zipped archive, **tox21enricher.zip**.

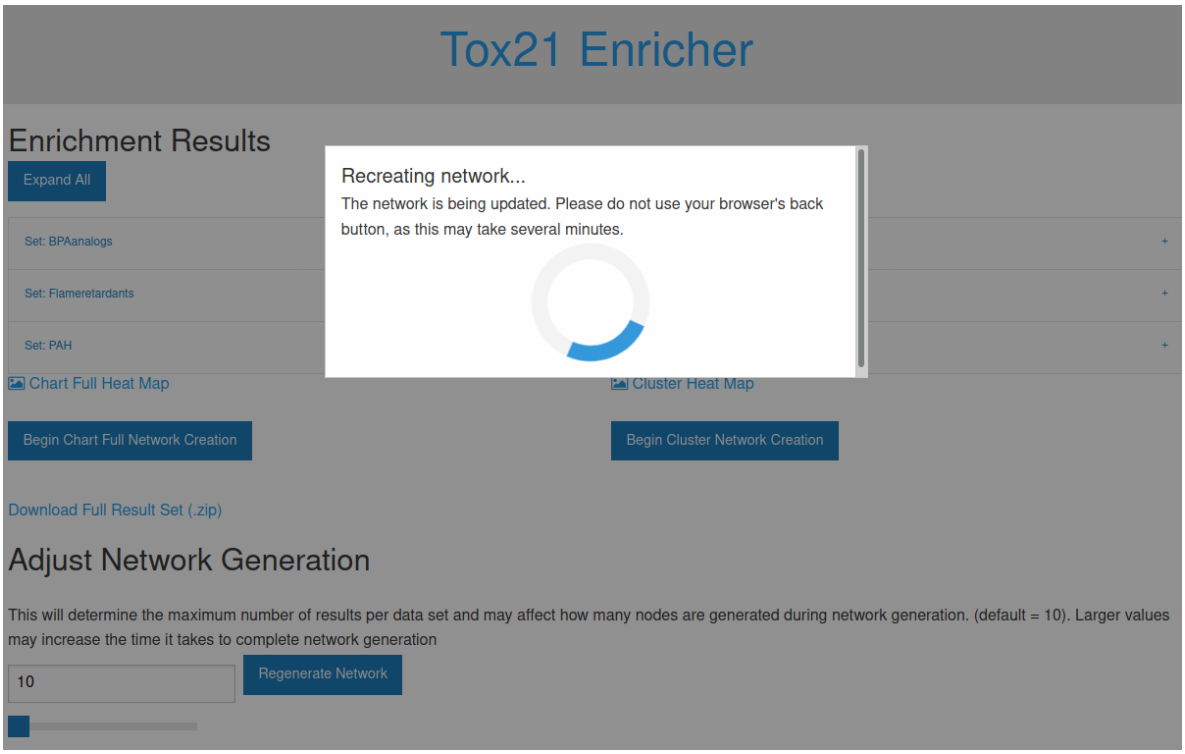


IV.2. Adjusting Network Generation

The slider controls can be used to re-perform enrichment with a different cutoff value. Changing this value may change the number of results displayed in the heatmaps and number of nodes in the network.

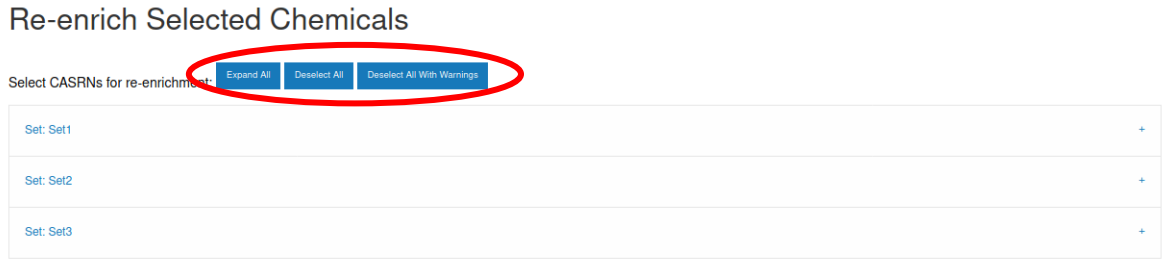


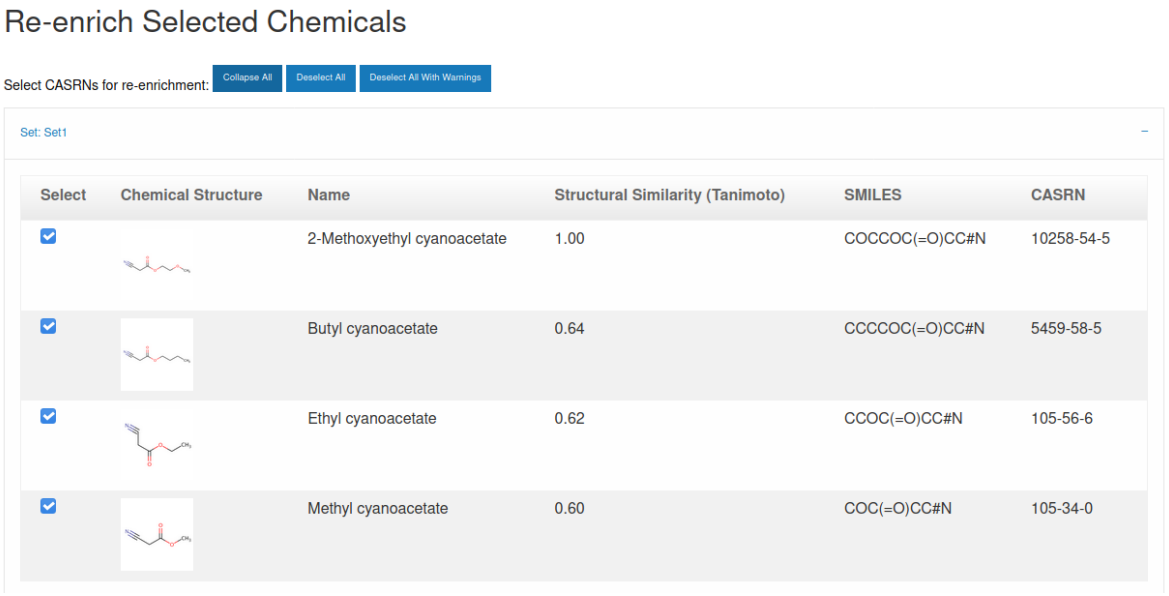
Clicking the “Regenerate Network” button will start the regeneration process. Do not press the “Back” or “Reload” browser buttons at this point, as this will prevent Tox21 Enricher from providing the new enrichment results correctly.



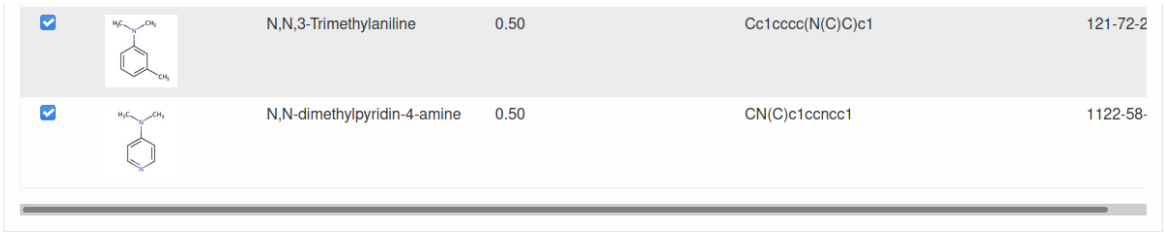
IV.3. Re-Enriching Selected Chemicals

After performing either the **Chemicals With Shared Substructures** or **Chemicals With Structural Similarity** enrichment analysis types, the “Re-enrich Selected Chemicals” table will appear on the results page. Here, we can see a list of all the CASRNs associated with the SMILES or InChI strings we submitted. By default, all chemicals in the table are selected. By clicking the “Expand All” button, we can view all the tabs like on the landing page. By clicking the “Deselect All” button, all the chemicals in the table will be deselected. The “Deselect All With Warnings” button only appears if any of the result chemicals produces a “reactive substructures” warning (discussed later). This button will only select/deselect the chemicals flagged with this warning.

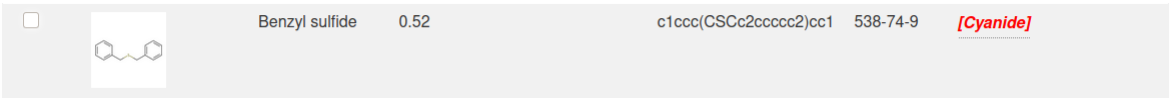




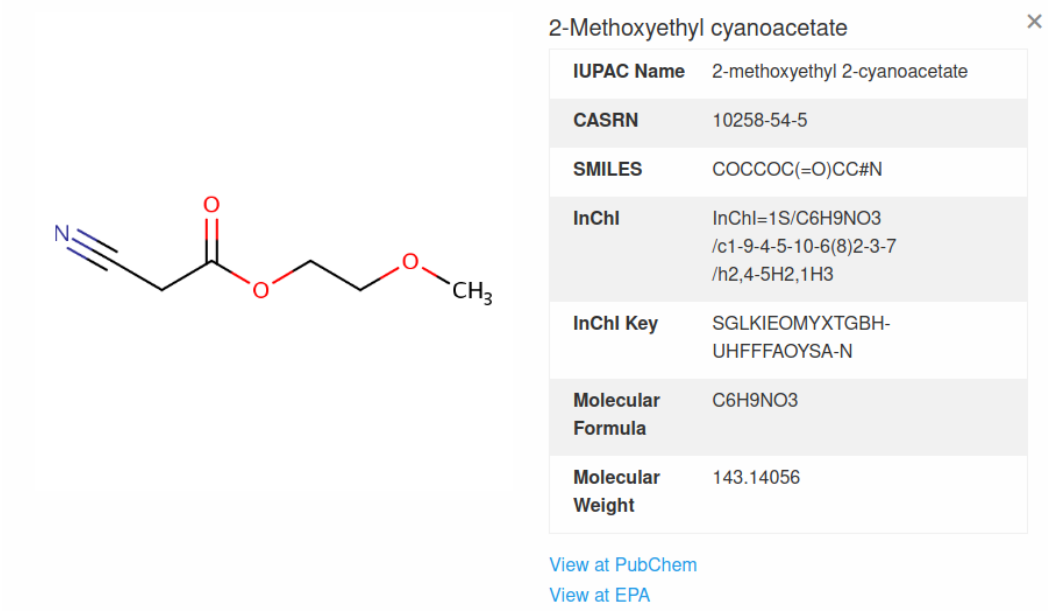
By clicking the “Re-Enrich Selected Chemicals” button, a new enrichment analysis is performed using all the selected chemicals. This enrichment analysis is functionally identical to the **User Provided CASRN List** enrichment type and clicking the button will show the waiting page until the enrichment process completes.



Occasionally, a result chemical may have a “reactive structures” warning. A chemical is flagged with this if it either contains a known hyperreactive structural group, but the user-submitted chemical does not, or if the user-submitted chemical contains a known hyperreactive structural group while the result chemical does not. It is recommended that you deselect chemicals with this warning and perform re-enrichment on your submitted data.



The chemical structure image for a chemical can be clicked to show additional information for the chemical. The “View at PubChem” link will take you to the chemical’s entry (if it exists) at PubChem (https://pubchem.ncbi.nlm.nih.gov/) in a new tab. The “View at EPA” link will take you to the chemical’s entry (if it exists) at the EPA’s CompTox Chemicals Dashboard https://comptox.epa.gov/dashboard/) in a new tab.



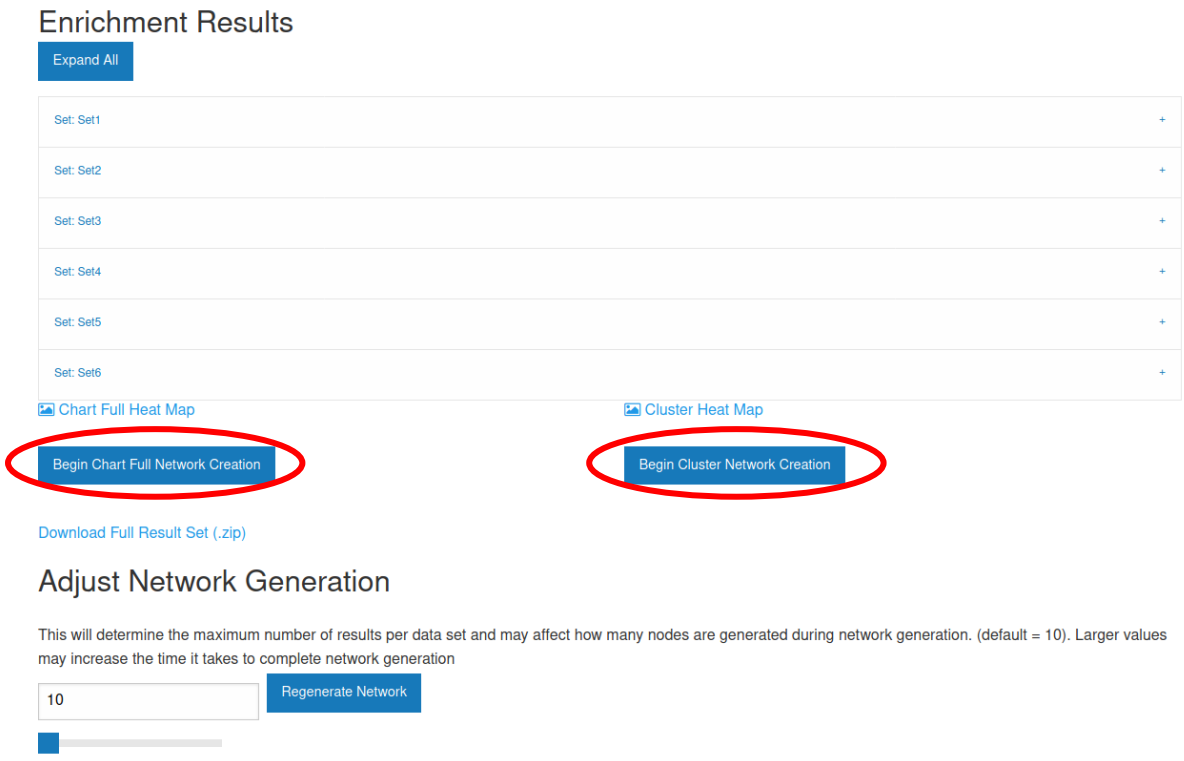
IV.4. Viewing Annotations for Given Chemicals

If the “View Annotations for Tox21 Chemicals” enrichment type was chosen, the results page will look different than if we performed a full enrichment analysis.

If a chemical is in the Tox21 screening library and has annotations in the Tox21 database, it will produce a text file called “<CASRN name>__Annotations.txt.” Clicking on this file will display a list of the annotations for the chemical. If a chemical does not have annotations or is not in the screening library, it will produce a file called “<CASRN name>__ErrorCasrns.txt.” Clicking the “Download Full Result Set (.zip)” link will prompt you to download the results in a zipped archive called **tox21enricher.zip**.

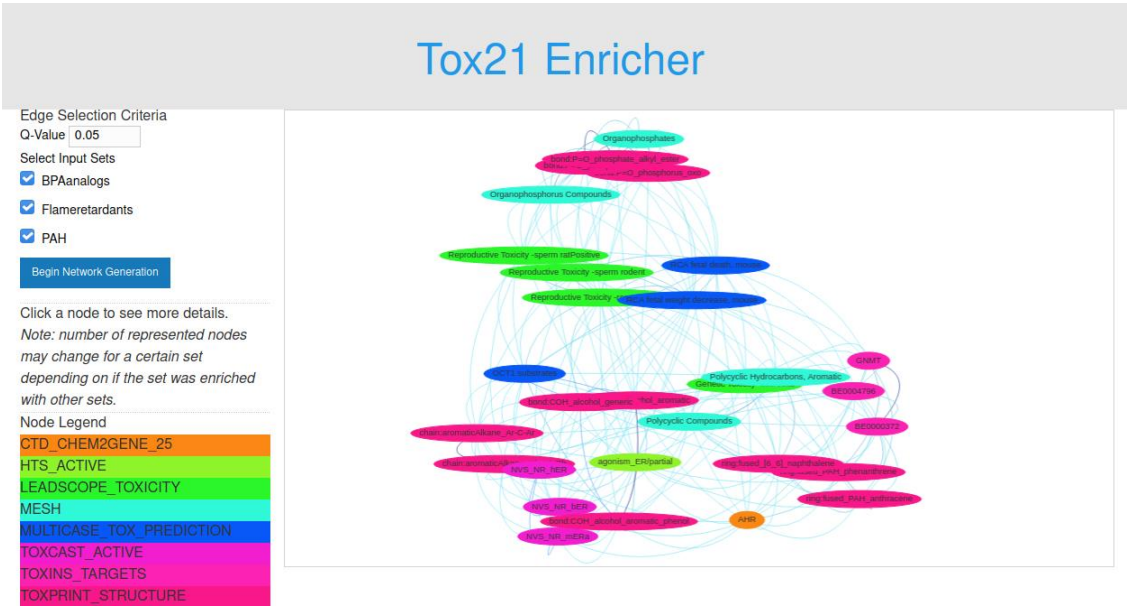
IV.5. Creating the Networks

Next, we can click either the “Begin Chart Full Network Creation” button or the “Begin Cluster Network Creation” button. These buttons can be seen highlighted below.

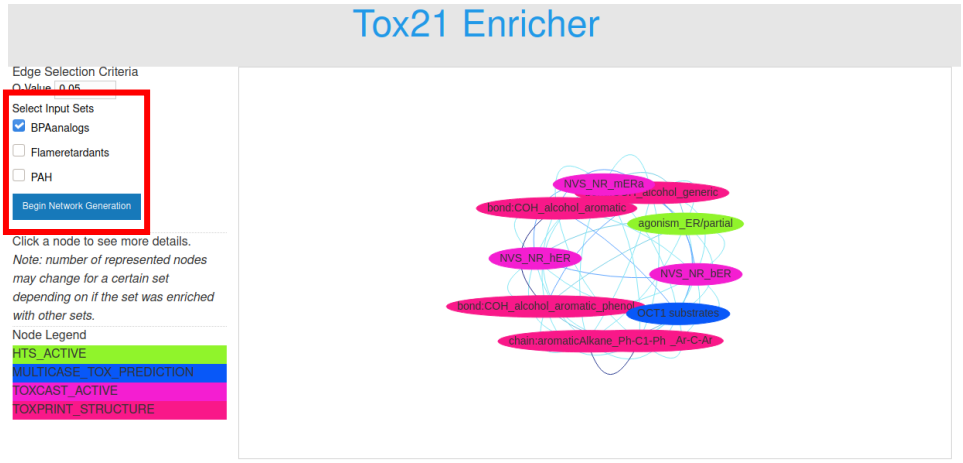


V. Network Page

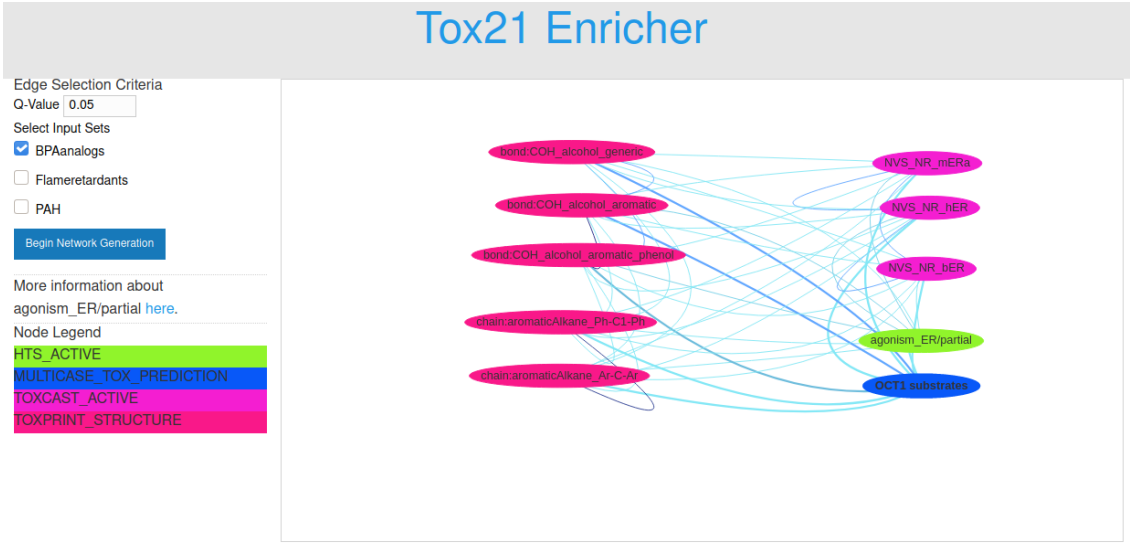
After clicking either of the network creation buttons, you will see the network page as shown below. The left side of this page is where we have edge selection criteria, input set select for node data to visualize, and the color key for the current network. In the screenshot below, we are using the Chart Full data for our network. The nodes correspond to significantly enriched annotations and the edges indicates that there is significant overlap between the two annotations in terms of chemical contents. The edge color gradient indicates the degree of overlap based on Jaccard index. **Note that the network may take some time to load depending on the number of nodes featured**.



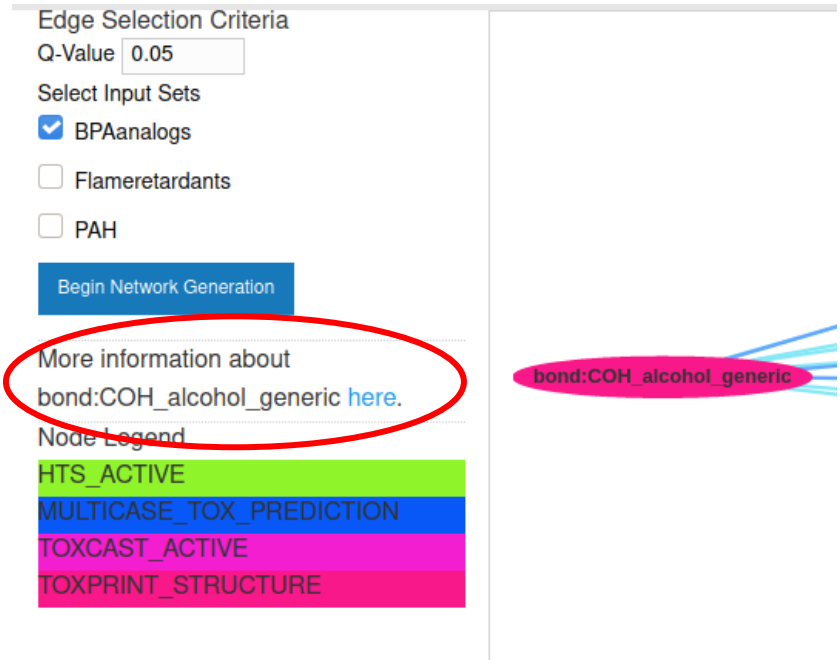
To view a subset of the network, select/deselect sets on the left and click the “Begin Network Generation” button.



Clicking and dragging a node allows the user to move it around in the network box.



Clicking on a node will also present a prompt with a link to get more details about the annotation's class.



VI. References

B. Bienfait and P. Ertl, JSME: a free molecule editor in JavaScript, J. Cheminformatics 5:24 (2013)

END OF THE USER'S MANUAL