Tox21 Enricher
Setup Guide

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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. There is limited toxicological information for many of these chemicals, which makes risk assessment difficult or 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 a set of 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 built using the R Shiny framework. 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. Setup

II.1. Getting a Copy of Tox21 Enricher

The Tox21 Enricher code for both the server-side and client-side applications may be found at https://github.com/hurlab/tox21enricher. Assuming you have cloned the repository into a folder called "/home/user/tox21enricher/" on your machine, server-side code can be found in `/home/user/tox21enricher/` and client-side code can be found in `/home/user/tox21enricher/tox21enricher/`.

II.2. Server Application Setup

There are three main components to the Tox21 Enricher server-side utilities: the Plumber API, the request queue, and the database. This installation guide assumes you are using a *nix environment, preferably Ubuntu 20.04 LTE.

II.2.a. Server Configuration

Assuming you have cloned the code to /home/user/tox21enricher/, the server-side code's configuration file can be found at /home/user/tox21enricher/config.yml. This configuration file has two namespaces, `tox21enricher` and `tox21enricher-queue`, for the **tox21enricher database** and the **tox21queue database**, respectively.

The importance of each of the variables in this configuration file is explained below:

- **driver** – The database driver. This should always be set to `"Postgres"`.
- **server** – The location of the Tox21 Enricher database in the form `<host_ip>:<port>/<database_name>`. For example, if you are hosting the database on the same machine at the default Postgres port of 5432 with a database name of "tox21enricher", this should be set to `"127.0.0.1:5432/tox21enricher"`. For the tox21queue database, this should be `"127.0.0.1:5432/tox21queue"`.
- **host** – The name or IP address of the machine where the database is hosted. If you are hosting the database on the same machine, this should be set to `"127.0.0.1"` or `"localhost"`.
- **uid** – The username of the Postgres user that owns the tox21enricher database for the tox21enricher namespace / the username of the Postgres user that owns the tox21queue database for the tox21enricher-queue namespace.
- **pwd** – The password of the Postgres user that owns the tox21enricher database for the tox21enricher namespace / the password of the Postgres user that owns the tox21queue database for the tox21enricher-queue namespace.
- **port** – The port that the database is listening on. If you set up PostgreSQL to use the default port, this should be set to `"5432"`.
- **database** – The name of the Tox21 Enricher database on the Postgres server. This will be `"tox21enricher"` for the tox21enricher namespace and `"tox21queue"` for the tox21enricher-queue namespace.
- **appversion** – The current version of the application. This is used to know which version of the Tox21 Enricher manual to serve to users.

- **appdir** – The location of the server-side project files. This should be wherever you cloned the repository to (i.e., `“/home/user/tox21enricher/”`).
- **python** – The location of the Python installation **in the RDKit environment** (i.e., `“/home/user/anaconda3/envs/my-rdkit-env/bin/python3.6”`).
- **cores** – The number of cores available to use when using multicore processing. This is initially set to 4.

II.2.b. Setting Up the Plumber API

The Plumber API serves as the primary point of contact between the Tox21 Enricher client application and the other components of the Tox21 Enricher server-side utilities. Requests to perform enrichment and to access the application's database are processed through the Plumber API via HTTP requests. The Plumber API should ideally run as a daemon on the same machine as the queue and the database.

To set up the Plumber API:

1. If they do not already exist, create an Input/ and Output/ directory (note the capital letters) in the project's root directory. Assuming you have cloned the code to /home/user/tox21enricher, these directories should be created at /home/user/tox21enricher/Input and /home/user/tox21enricher/Output. If these directories already exist when you cloned the project, delete the README.md files in them.
2. Download and install R from the appropriate CRAN mirror: https://cran.r-project.org/mirrors.html.
3. Download and install an OpenJDK Java distribution from https://openjdk.java.net/ or through SDKMAN! (https://sdkman.io/).
4. Install a few necessary packages using `apt-get update && apt-get install`:
   - curl, libcurl4-openssl-dev, and libxml2-dev (all needed for cURL)
   - libbz2-dev
   - libpcre2-dev
   - libpq-dev
   - libssl-dev
   - libz-dev
   - r-base-dev
5. Use `install.packages('package_name',dependencies=TRUE)` to install the necessary R libraries for the API:
   - config
   - future
   - ggplot2
   - httr
   - parallel
   - plumber
   - plyr
   - pool
   - promises
   - reticulate
   - rjson
   - RPostgreSQL
   - stringr
   - tidyverse
   - uuid
6. Ensure you have properly updated the server's configuration file.
7. Ensure the Tox21 Enricher PostgreSQL server is running and listening on an open port.

8. Open the startPlumberAPI.sh script in a text editor and set `port=9000` to whatever port you want the API to listen on.
9. Ensure the startPlumberAPI.sh script has executable permissions by running `chmod +x /home/user/tox21enricher.startPlumberAPI.sh`.
10. Run the API with the command `/home/user/tox21enricher.startPlumberAPI.sh`.

II.2.c. Setting Up the Queue

The queue is an Rscript that continually observes the database to see if any enrichment requests have been submitted and either performs enrichment analysis or fetches corresponding annotations for the inputted chemicals.

To set up the queue:

1. If they do not already exist, create an Input/ and Output/ directory (note the capital letters) in the project's root directory. Assuming you have cloned the code to /home/user/tox21enricher, these directories should be created at /home/user/tox21enricher/Input and /home/user/tox21enricher/Output. If these directories already exist when you clone the project, delete the README.md files in them.
2. Download and install R from the appropriate CRAN mirror: https://cran.r-project.org/mirrors.html.
3. Download and install an OpenJDK Java distribution from https://openjdk.java.net/ or through SDKMAN! (https://sdkman.io/).
4. Install a few necessary packages using `apt-get update && apt-get install`:
    - curl, libcurl4-openssl-dev, and libxml2-dev (all needed for cURL)
    - libbz2-dev
    - libpcre2-dev
    - libpq-dev
    - libssl-dev
    - libz-dev
    - r-base-dev
5. Use `install.packages('package_name',dependencies=TRUE)` to install the necessary R libraries for the API:
    - config
    - future
    - ggplot2
    - httr
    - parallel
    - plumber
    - plyr
    - pool
    - promises
    - rjson
    - RPostgreSQL
    - stringr

- tidyverse
- uuid

6. Ensure you have properly updated the server's configuration file.
7. Ensure the Tox21 Enricher PostgreSQL server is running and listening on an open port.
8. Ensure the startQueue.sh script has executable permissions by running `chmod +x /home/user/tox21enricher.startQueue.sh`.
9. Run the queue with the command `/home/user/tox21enricher.startQueue.sh`.

II.2.d. Setting Up the Database

Tox21 Enricher uses a PostgreSQL database to store chemical and annotation data. Specifically, the application uses the PostgreSQL cartridge for RDKit (https://www.rdkit.org/docs/index.html).

To install RDKit and the PostgreSQL database:

1. Download and install RDKit, following the directions at https://www.rdkit.org/docs/Install.html.
2. This will require you to download and install the Conda package manager and Python.
3. Install and initialize the RDKit PostgreSQL cartridge, following the directions at https://www.rdkit.org/docs/Install.html#installing-and-using-postgresql-and-the-rdkit-postgresql-cartridge-from-a-conda-environment.
4. Make sure the PostgreSQL server is running by using `<conda_folder>/envs/my-rdkit-env/bin/pg_ctl -D /<data_folder>/`.
5. Obtain a backup or copy of the Tox21 Enricher database. Instructions for setting up the database from scratch can be found in **Section IV**.
6. Create a schema called "tox21enricher" and either:
    a. Log into it by using `<conda_folder>/envs/my-rdkit-env/bin/psql tox21enricher`. Run the command `\i /<path_to_database_backup>/` from within Postgres to restore the database from the backup.
    b. Use the pg_restore utility to restore the database from backup by running from the command line using `<conda_folder>/envs/my-rdkit-env/bin/pg_restore /<path_to_database_backup>/`.
7. Obtain a backup or copy of the Tox21 Enricher queue database. Instructions for setting up the database from scratch can be found in **Section IV**.
8. Create a schema called "tox21queue" and either:
    a. Log into it by using `<conda_folder>/envs/my-rdkit-env/bin/psql tox21queue`. Run the command `\i /<path_to_database_backup>/` from within Postgres to restore the database from the backup.
    b. Use the pg_restore utility to restore the database from backup by running from the command line using `<conda_folder>/envs/my-rdkit-env/bin/pg_restore /<path_to_database_backup>/`.

II.3. Client Application Setup

The Tox21 Enricher client application provides a graphical user interface for performing enrichment analysis and interacting with the data stored in the Tox21 Enricher database. The client application may be run on the same machine as the server-side utilities and exposed to the internet so that other remote clients may use the application via a web browser. Otherwise, the client application may also be run directly on the client machine as a standalone executable and connect remotely to the server utilities running on a remote host. To access the Tox21 Enricher client application via an internet browser, your browser must have JavaScript enabled for the application to load properly.

II.3.a. Client Configuration

Assuming you have cloned the code to /home/user/tox21enricher/, the server-side code's configuration file can be found at /home/user/tox21enricher/tox21enricher/config.yml. This configuration file is notably simpler than the configuration file used for the Tox21 Enricher server. The client configuration file has only one namespace, `tox21enricher-client`.

The importance of the two variables in this configuration file is explained below:

- **host** – The name or IP address of the machine where the Tox21 Enricher Plumber API is hosted. If you are hosting the API on the same machine, this should be set to `"127.0.0.1"` or `"localhost"`.
- **port** – The port that the Tox21 Enricher Plumber API is listening on. By default, this is set to `"9000"`.

II.3.b. Setting Up the Client Application (Standalone)

The standalone client application will be available in a future update.

II.3.c. Setting Up the Client Application (Web Application)

The Tox21 Enricher client application is a Shiny (https://www.rstudio.com/products/shiny/) application and thus can be hosted on a machine and accessed from a remote client via an internet browser.
To set up the client application as a web application:

1. If they do not already exist, create the `<project_root>/tox21enricher/www/docs` and `<project_root>/tox21enricher/www/local/`. Assuming you have cloned the code to /home/user/tox21enricher, these directories should be created at /home/user/tox21enricher/tox21enricher/www/. If these directories already exist when you cloned the project, delete the README.md files in them.
2. Download and install R from the appropriate CRAN mirror: https://cran.r-project.org/mirrors.html.
3. Download and install an OpenJDK Java distribution from https://openjdk.java.net/ or through SDKMAN! (https://sdkman.io/).
4. Install a few necessary packages using `apt-get update && apt-get install`:
   - libbz2-dev
   - libgdal-dev
   - libpcre2-dev

    - libpq-dev
    - libudunits2-dev
    - libz-dev
    - r-base-dev
5. Use `install.packages('package_name',dependencies=TRUE)` to install the necessary R libraries for the API:
    - bslib
    - catmaply
    - CePa
    - config
    - dplyr
    - DT
    - future
    - ggVennDiagram
    - httr
    - igraph
    - igraphdata
    - parallel
    - plotly
    - pool
    - promises
    - rclipboard
    - rjson
    - RPostgreSQL
    - shiny
    - shinyBS
    - shinycssloaders
    - shinydashboard
    - shinyjs
    - shinythemes
    - stringr
    - uuid
    - visNetwork
6. Ensure you have properly updated the client application's configuration file.
7. Launch the application according to the **Running in a Separate Process** section in the instructions here: https://shiny.rstudio.com/articles/running.html.
8. Open the port that the client application runs on. By default, this is a random port, but you can configure the application to run on the same port every time by specifying a specific port for the `port` argument of the `shiny::runApp()` function as described in the documentation here: https://shiny.rstudio.com/reference/shiny/1.0.1/runApp.html.

III. Deployment

The server components of Tox21 Enricher can be deployed using Docker. A Docker container is the recommended tool for hosting a publicly available Plumber API, as described here: https://www.rplumber.io/articles/hosting.html.

III.1. Configuring and Building the Docker Images

Before you can host the Tox21 Enricher server code, you must create three necessary Docker images: **tox21enricher/plumber,** **tox21enricher/queue**, and **tox21enricher/database**. These images should be configured to suit your needs before you attempt to build them:

1. Install Docker following the directions on the official website: https://docs.docker.com/get-docker/.
2. Navigate to the project root folder for Tox21 Enricher (wherever you cloned the repository to). Assuming you have cloned the repository into a folder called "/home/user/tox21enricher/" on your machine, this should be `/home/user/tox21enricher/`.
3. Make sure the file `<project_root>/config.yml` is set up to match the login, host, and port information of the Tox21 Enricher database in its Docker container.
    a. The host name should be whatever the name of the database service is as specified in the docker-compose.yml file (by default this is "db").
    b. Make sure you configure these values for both the Plumber API and the queue.
4. Make sure you have **Anaconda** installed with Python, RDKit, and its PostgreSQL cartridge as described in ***Section II.2.d. Setting Up the Database***. The recommended location for this installation is (on a *nix system) `/home/username/anaconda3/`.
5. Make sure Tox21 Enricher's **Input/** and **Output/** directories exist. These will be treated as shared volumes among the Docker containers. Because these directories are written to by the queue and API scripts from within their respective containers, we must give these directories special permissions using the commands:
    a. `sudo chown -R 1001 <path_to_input_dir>/Input/ && sudo chmod -R 750 <path_to_input_dir>/Input/` for the Input directory.
    b. `sudo chown -R 1001 <path_to_output_dir>/Output/ && sudo chmod -R 750 <path_to_output_dir>/Output/` for the Output directory.
6. Configure the **tox21enricher/plumber** image:
    a. Make sure there is a **Dockerfile** called "Dockerfile.plumber" in this folder. By default, this should already be in the folder if you have cloned the project directly from the GitHub repository.
    b. Open the Dockerfile in any text editor and follow the directions in the file to configure the necessary values.
        i. You will have to come up with a username and password for the user inside the container that you want to run the API process.
        ii. Make sure the file `<project_root>/docs/Tox21Enricher_Manual_vX.X.pdf`

matches the correct version number of the manual you want to display to users.

iii. Make sure the file `<project_root>/docs/Tox21Enricher_Setup_vX.X.pdf` matches the correct version number of the manual you want to display to users.

c. Make sure the following files exist at the correct locations:
   i. <project_root>/requirements-src.R
   ii. <project_root>/config.yml
   iii. <project_root>/plumber.R
   iv. <project_root>/HClusterLibrary/ **(this is a directory)**
   v. <project_root>/calcReactiveGroups.py
   vi. <project_root>/docs/<user_manual>
   vii. <project_root>/docs/<setup_guide>

d. From within the project root folder, run the command `docker build -t tox21enricher/plumber -f Dockerfile.plumber .`. Note the period at the end of the command, which signifies that Docker should use the current directory as a context.

7. Configure the **tox21enricher/database** image:
   a. From the project root folder, navigate to the `tox21enricher-database/` directory.
   b. Make sure there is a **Dockerfile** called “Dockerfile.database” in this folder. By default, this should already be in the folder if you have cloned the project directly from the GitHub repository.
   c. Open the Dockerfile in any text editor and follow the directions in the file to configure the necessary values. You will have to come up with a username and password for the user inside the container that you want to run the API process.
   d. Make sure the following files exist at the correct locations:
      i. <project_root>/tox21enricher-database/tox21db_tox21enricher.bak **(This should be a backup of the *tox21enricher* database, specifically named like this and ideally created with the *pg_dump* utility.)**
      ii. <project_root>/tox21enricher-database/tox21db_tox21queue.bak **(This should be a backup of the *tox21queue* database, specifically named like this and ideally created with the *pg_dump* utility.)**
      iii. <project_root>/tox21enricher-database/entrypoint.sh
   e. From within the project root folder, run the command `docker build -t tox21enricher/database -f Dockerfile.database .`. Note the period at the end of the command, which signifies that Docker should use the current directory as a context.
8. Navigate to the **HClusterLibrary** folder. By default, this should be at `<project_root>/HClusterLibrary/`. Make sure the files **clusterLinux64** and **hclimage-o.jar** have run permissions by running the commands `RUN chmod +x <project_root>/HClusterLibrary/clusterLinux64` and `RUN chmod +x <project_root>/HClusterLibrary/hclimage-o.jar`.

9. Install Docker Compose following the directions on the official website: https://docs.docker.com/compose/install/.
10. Navigate back to the project root folder and make sure the file **docker-compose.yml** is in that folder. By default, this should already be in the folder if you have cloned the project directly from the GitHub repository.
11. Open **docker-compose.yml** in any text editor and make sure you correctly configure the paths for the shared volumes.
12. If testing, you can uncomment lines 29 and 45 in docker-compose.yml that state `network_mode: host` and comment out lines 25-28 and 41-44. This will allow the services to run attached to the host's network which may make it easier to test the Docker setup.
13. Make sure that the paths on lines 37-39 and 51-53 match the locations of the Input, Output, and Anaconda directories on the host machine, respectively.
14. The Docker containers can now be started through Docker Compose by using the command `docker-compose up`. This will start the Tox21 Enricher Plumber API, queue, and database.

Additional notes:

- The Docker images needed for Tox21 Enricher are built on a few existing Docker images. You may need to install each image using `docker pull <image_name>` before you can build its respective Tox21 Enricher image. ***These dependencies may change in a future version of Tox21 Enricher***. The images required are:
  - **dockercloud/haproxy:1.2.1** (needed for load balancing using HAProxy)
  - **mcs07/postgres-rdkit** (needed for database)
  - **rstudio/plumber** (needed for Plumber API)
- You may need superuser permissions to run `docker-compose up` and `docker build`.
- **Due to a bug currently under investigation, support for the Tox21 Enricher queue Docker container has been temporarily removed. To deploy the queue, you will have to simply run the startQueue.sh script on the host machine and make sure the settings in the config.yml file match those of the Tox21 Enricher database.**

III.2. Setting Up the Load Balancer

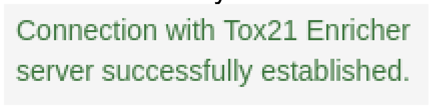
By default, Tox21 Enricher as deployed using Docker Compose does not enable its load balancing system. However, load balancing using **HAProxy** is implemented and can easily be enabled by changing a few lines in the **docker-compose.yml** configuration file. Using the load balancer is the recommended approach for deploying Tox21 Enricher. Not using the load balancer is only recommended for testing purposes.

1. Navigate to the project root folder for Tox21 Enricher (wherever you cloned the repository to). Assuming you have cloned the repository into a folder called "/home/user/tox21enricher/" on your machine, this should be `/home/user/tox21enricher/`.
2. Make sure the file **docker-compose.yml** is in that folder.
3. Open docker-compose.yml in any text editor and:
   a. Un-comment lines 9 – 19 to define the HAProxy service.
   b. Edit line 42 (port mapping for the tox21enricher service) and change the port value left of the colon to a port range formatted like `N-M`, where `N` is the first port in the range and `M` is `N+(<number_of_API_instances_desired> - 1)`. For example, if you want 3 instances of the API running concurrently, you could change the port value to `7998-8000`.
   c. Comment out lines 43 – 44 as we no longer want to expose the ports that the API instance(s) will be listening on.
   d. Comment out lines 29 and 45 that state `network_mode: host` as we do not want the services to run on the host network.
4. The Docker containers can now be started through Docker Compose by using the command `docker-compose up --scale tox21enricher=N`. Where `N` is the number of concurrently running instances of the Tox21 Enricher API.

III.3. Testing Connectivity

To connect an instance of the Tox21 Enricher client application to the API running on a remote server, follow these steps:

1. Navigate to the project root folder for Tox21 Enricher (wherever you cloned the repository to). Assuming you have cloned the repository into a folder called "/home/user/tox21enricher/" on your machine, this should be `/home/user/tox21enricher/`.
2. Navigate to the client application's folder at `<project_root>/tox21enricher/` and assure the configuration file, **config.yml**, exists.
3. Open config.yml and navigate to the **tox21enricher-client** namespace, under which you should see the **host** and **port** variables.
4. Set **host** to the address of the remote server hosting the Tox21 Enricher API.
5. Set **port** according to the situation:
    a. If you are running Docker Compose on the host network:
        i. Set port to the port the Tox21 Enricher will run on. By default, this is 8000 when deploying through the tox21enricher/plumber Docker container.
    b. If you are NOT running Docker Compose on the host network AND if you are NOT using the HAProxy load balancer:
        i. Set port to the port on the left of the colon on line 42 of docker-compose.yml (the mapped port for the **tox21enricher** service).
    c. If you are NOT running Docker Compose on the host network AND if you ARE using the HAProxy load balancer:
        i. Set port to the port on the left of the colon on line 17 of docker-compose.yml (the mapped port for the **lb** service).
6. Make sure your firewall allows connections on the specified port.
7. Run the client application. If the following success message appears in the sidebar, then the client application has successfully connected to the remote server.



Connection with Tox21 Enricher server successfully established.

8. Alternatively, you may use the cURL command `curl <server_address>:<port>/ping` to ping the Tox21 Enricher API. If your machine can connect to the remote server, this will return a response of `[true]`. This is the same API endpoint that is called by the client application when it is checking if it can connect to the Tox21 Enricher API (Step 7).

IV. Database Specifications and Maintenance

This section will detail the specifications of the Tox21 Enricher database. It will also explain how to periodically update the data stored in the database.

IV.1. Building the Database from Scratch

The Tox21 Enricher database is made up of two schemas: **tox21enricher** and **tox21queue**. **tox21enricher** stores read-only data relating to the chemicals in the Tox21 dataset, while **tox21queue** contains a few writable tables that store information about submitted requests.

To build the Tox21 Enricher database from scratch, follow these specifications:

1. First, create the role that will own the **tox21enricher** database.
2. Next, create the tox21enricher database and set the owner to the user created in step 1.
3. Create the following tables and functions:
    a. annotation_class

Name	**Type**	**Constraints**
annoclassid	INTEGER	NOT NULL, AUTO INCREMENT, PRIMARY KEY
annoclassname	VARCHAR(50)	NOT NULL
firsttermid	INTEGER	NOT NULL
lasttermid	INTEGER	NOT NULL
numberoftermids	INTEGER	NOT NULL
baseurl	VARCHAR(255)	NOT NULL
annotype	VARCHAR(100)	
annogroovyclassname	VARCHAR(100)	
annodesc	VARCHAR(7000)	
networkcolor	VARCHAR(11)	

    b. annotation_detail

Name	**Type**	**Constraints**
annotermid	INTEGER	NOT NULL, AUTO INCREMENT, PRIMARY KEY
annoclassid	INTEGER	NOT NULL, FOREIGN KEY REFERENCES annotation_class(annoclassid)
annoterm	VARCHAR(7000)	NOT NULL

    c. annotation_matrix

Name	**Type**	**Constraints**
casrn	VARCHAR(15)	NOT NULL
name	VARCHAR(500)	NOT NULL
annotation	VARCHAR(40000)	

    d. annotation_matrix_terms

Name	**Type**	**Constraints**
id	INTEGER	
term	VARCHAR(7000)	

e. annoterm_pairwise

Name	Type	Constraints
pairwise	INTEGER	NOT NULL, AUTO INCREMENT, PRIMARY KEY
term1uid	INTEGER	NOT NULL
term2uid	INTEGER	NOT NULL
term1size	INTEGER	NOT NULL
term2size	INTEGER	NOT NULL
common	INTEGER	NOT NULL
union	INTEGER	NOT NULL
jaccardindex	DOUBLE	NOT NULL
pvalue	DOUBLE	NOT NULL
qvalue	DOUBLE	NOT NULL

f. chemical_detail

Name	Type	Constraints
casrnuid	INTEGER	NOT NULL, AUTO INCREMENT, PRIMARY KEY
casrn	VARCHAR(15)	NOT NULL
testsubstance_chemname	VARCHAR(500)	NOT NULL
molecular_formular	VARCHAR(50)	
iupac_name	VARCHAR(1000)	
inchis	VARCHAR(1000)	
inchikey	VARCHAR(30)	
smiles	VARCHAR(600)	
smiles_qsar_ready	VARCHAR(600)	
dtxsid	VARCHAR(20)	
stoichiometric_ratio	VARCHAR(10)	
cid	VARCHAR(20)	
mol_formula	VARCHAR(200)	
mol_weight	DOUBLE	
dtxrid	VARCHAR(20)	

g. fps_2

Name	Type	Constraints
casrn	VARCHAR(15)	
torsionbv	BFP	
mfp2	BFP	
ffp2	BFP	

h. mols_2

Name	Type	Constraints
casrn	VARCHAR(15)	
m	MOL	
cyanide	INTEGER	
isocyanate	INTEGER	
aldehyde	INTEGER	
epoxide	INTEGER	

i. term2casrn_mapping

Name	Type	Constraints
term2casrnmappinguid	INTEGER	NOT NULL, AUTO INCREMENT, PRIMARY KEY
annotermid	INTEGER	NOT NULL
annoclassid	INTEGER	NOT NULL
casrnuid_id	INTEGER	NOT NULL

j. get_mfp2_neighbors

Description
CREATE OR REPLACE FUNCTION public.get_mfp2_neighbors(smiles text) RETURNS TABLE(casrn character varying, m cstring, similarity double precision, cyanide integer, isocyanate integer, aldehyde integer, epoxide integer) LANGUAGE sql STABLE AS $function$ select casrn,mol_to_smiles(m),tanimoto_sml(morganbv_fp(mol_from_smiles($1::cstring)),mfp2) as similarity,cyanide,isocyanate,aldehyde,epoxide from fps_2 join mols_2 using (casrn) where morganbv_fp(mol_from_smiles($1::cstring))%mfp2 order by morganbv_fp(mol_from_smiles($1::cstring))<%>mfp2; $function$

4. Revoke all privileges on every table in this database for the user created in step 1.
5. Grant only the SELECT privilege on each table in the database for the user created in step 1.
6. Next, create the role that will own the **tox21queue** database.
7. Next, create the **tox21queue** database and set the owner to the user created in the previous step.

8. Create the following tables and functions:
    a. queue

Name	Type	Constraints
mode	VARCHAR(15)	
uuid	VARCHAR(50)	NOT NULL, PRIMARY KEY
annoselectstr	VARCHAR(1500)	
cutoff	INTEGER	
finished	INTEGER	DEFAULT=0
index	INTEGER	NOT NULL, AUTO INCREMENT
error	VARCHAR(500)	
cancel	INTEGER	NOT NULL, DEFAULT=0

    b. status

Name	Type	Constraints
step	INTEGER	NOT NULL, DEFAULT=0
uuid	VARCHAR(50)	NOT NULL, FOREIGN KEY REFERENCES queue(uuid)
setname	VARCHAR(500)	

    c. transaction

Name	Type	Constraints
original_mode	VARCHAR(15)	
mode	VARCHAR(15)	NOT NULL
uuid	VARCHAR(40)	NOT NULL, PRIMARY KEY
annotation_selection_string	VARCHAR(1000)	
cutoff	INTEGER	NOT NULL
input	VARCHAR(5000)	
original_names	VARCHAR(5000)	
reenrich	VARCHAR(5000)	
colors	VARCHAR(200)	
timestamp_posted	VARCHAR(50)	NOT NULL, DEFAULT =CURRENT_DATE
timestamp_started	VARCHAR(50)	DEFAULT='not started'
timestamp_finished	VARCHAR(50)	DEFAULT='incomplete'
cancel	INTEGER	NOT NULL, DEFAULT=0

9. Revoke all privileges on every table in this database for the queue-owning user.
10. Grant only the SELECT, INSERT, and UPDATE privileges on each table in the database for the queue-owning user.

IV.2. Updating the Database

It will sometimes be necessary to update the database used by Tox21 Enricher. Included in the application's GitHub repository under the `/<project_root>/tox21enricher-database/update/` directory are several scripts that must be run to properly update the database.

The following instructions must be run to ensure the database update proceeds smoothly.

1. Navigate to the `/<project_root>/tox21enricher-database/update/` folder.
2. Make sure that three subdirectories, **Annotation**, **ReOrganized**, and **tmp**, all exist in this directory. If they do not, create them. **Note the capitalization of the names**.
3. Make sure the files **Tox21_CASRN_Names.anno** and **Tox21_CASRN_Names_Full.anno** exist in the **Annotation** folder. They should already be in this folder if you cloned the project from the GitHub repository.
4. Place your annotation files in the **Annotation** folder. There are a few specifications these files need to follow:
    a. You must place ALL annotation files in the **Annotation** folder, not just the new/updated files.
    b. Each line must be structured like this:
    `<CASRN>	semicolon and; space; separated; list; of; annotations`
    Note that the CASRN and annotations should be tab-separated, and each annotation should be separated by a semicolon AND a space. The last annotation on each line should be followed immediately by the newline character ("\n") and NO semicolon.
    c. Each file should be named like this:
    `DrugMatrix_EXAMPLE_NAME.txt`
    Note that each file must be prefixed with "DrugMatrix_", class names should be in all capital letters, and multiple-word names must be split by single underscores ("_").
5. Run the first four scripts using Perl. Note that some or all of these scripts may take hours to days to complete, depending on processing speed, memory, and the number of cores available.
    a. Run the **first** script to reorganize the annotation data using the command `perl 01_ReOrganize_Annotation_Data_v2.1-20161003.pl`.
    b. Run the **second** script to perform the Fisher exact test and create most of the database files using the command `perl 02_Split-perform_Fisher-Exact-Test.pl`.
    c. Run the **third** script to create the final pairwise calculation file using the command `perl 03_Merge_Pvalues-and-calculate-qvalues.pl`.
    d. Run the **fourth** script to create the initial annotation matrix files using the command `perl 04_Create_complete_annotation_matrix_v1.0_11182020.pl`.
6. Run the next two scripts using Python.
    a. Run the **fifth** script to create the file **annotation-matrix-names.sql** which should store the names and IDs of each annotation in the matrix using the

    command `python 05_Create_annotation-matrix-names.py`.
    b. Run the **sixth** script to create the file **newmatrix.sql** which should store the new annotation matrix using the command `python 06_Create_annotation-matrix.py`.
7. The **seventh** script is optional and should only be run if you are updating the **mols_2** table. In most cases, you can ignore this script.
8. Before you can import the generated text files into the PostgreSQL database, a little bit of formatting needs to be done:
    a. The headers (first line) of each file must be deleted. The easiest way to do this is by running the **remove_headers.sh** bash script. Ensure this script has execute permissions before running.
    b. The table_annotation_class_v2.1.txt file needs to be padded with placeholder data that will be overwritten with a later script. To do this, run the **pad_annotation_class.py** script with Python.
9. You should now be able to import the files into the database. First, ensure that all database tables exist. They should be:
    a. annotation_class
    b. annotation_detail
    c. annotation_matrix
    d. annotation_matrix_terms
    e. annoterm_pairwise
    f. chemical_detail
    g. fps_2
    h. mols_2
    i. term2casrn_mapping

    The *italicized* tables should not have to be updated.
10. You can use the PostgreSQL command `COPY <table_name> FROM '/path_to_file/file.txt';` to fill each table with the data from the respective text files. You should only copy from the files:
    a. table_annotation_class_v2.1.txt
    b. table_annotation_detail_v2.1.txt
    c. table_annoterm_pairwise_v2.1_FINAL.txt
    d. table_chemical_detail_v2.1.txt
    e. term2casrn_mapping_v2.1.txt
11. After filling in the above tables, run the eighth script, `08_Add_additional_info_annotation_class.sql`, with PostgreSQL to finish filling in the annotation_class table.
12. To fill the annotation_matrix and annotation_matrix_terms tables, run the **annotation-matrix-names.sql** and **newmatrix.sql** scripts.

V. Common Issues

Both the client and server code for Tox21 Enricher are still in continued development, so there are likely issues that may arise when trying to either perform certain tasks or set up the application. Known bugs in the code and planned features are documented on the project's GitHub repository at: https://github.com/hurlab/tox21enricher/issues. Other known issues not necessarily related to the code itself are documented in this section of the manual.

V.1. Setup Issues

These are known issues that you may experience when attempting to set up Tox21 Enricher.

- **Problem installing RDKit PostgreSQL cartridge** – Even if you successfully install RDKit using Conda, Conda may fail to install the RDKit PostgreSQL cartridge due to package version conflicts. A workaround for this is to create a new Conda environment using `conda create -n <environment_name>`, delete or move the contents of the new environment's `/bin/` directory, install the RDKit PostgreSQL cartridge to that environment, then finally move the newly installed files from the new environment's `/bin/` directory into the RDKit environment's /bin/ directory.
- **Problem installing "CePa" package – missing dependencies** – The **CePa** R package requires two dependency packages called **Rgraphviz** and **graph**. These cannot be installed with `install.packages()`. You must first install the **BiocManager** R package and then run `BiocManager::install("Rgraphviz")` and `BiocManager::install("graph")`.
- **Potential conflict with Python versions when using the "reticulate" R package** – The Tox21 Enricher API code file (plumber.R) sets the environment variable **RETICULATE_PYTHON** to the **python** path defined in the application's config.yml file. This may cause a conflict if you have another application running on the same machine using the **reticulate** package with a different version of Python. A fix or workaround for this may be implemented in a future update.

END OF THE SETUP GUIDE