NetID setup

This section provides step-by-step instructions to set up the environment to run NetID algorithm in a local computer. A Windows system is recommended. Typical install time on a "normal" desktop computer is within a few hours.

(1) Software installation

Install R, Rstudio, Rtools40, ILOG CPLEX Optimization Studio (CPLEX), preferably at default location.

R (4.0.3) : https://www.r-project.org/

Rstudio: https://rstudio.com/products/rstudio/download/

Rtools40 (used for R 4.0.0 and newer): https://cran.r-project.org/bin/windows/Rtools/ow

CPLEX (12.10): https://www.ibm.com/academic/technology/data-science

You need to add R and Rtools40 to Environmental Variables PATH, with instruction provided at the end.

(2) Download code

Option 1. Using git (recommended).

(a) Install git

https://support.rstudio.com/hc/en-us/articles/200532077?version=1.3.1093&mode=desktop

(b) In Rstudio, go to File -> New project -> Version control -> Git, enter https://github.com/LiChenPU/NetID.git for URL, select a subdirectory, and create project.

(c) You should be able to see all files in place under your selected subdirectory. Use pull option to check for latest updates.

Option 2. Download files from github

Go to website https://github.com/LiChenPU/NetID, hit the green “code” button, select download zip, and unzip files.

(3) Package dependency installation

Most of the dependent packages can be installed by running the R script NetID_packages.R in the “get started” folder. See troubleshoot section for possible errors.

The package, cplexAPI, connecting R to CPLEX, requires additional installation steps.

(a) Go to website: https://cran.r-project.org/web/packages/cplexAPI/index.html, look for Package source, and download cplexAPI_1.4.0.tar.gz. In the same page, look for Materials, and open the INSTALL link.

(b) Unzip the folder cplexAPI to the desktop, follow the installation guide to modify the file Makevars.win.

> Note: Replace "\" in the Makevars.win file into "/" in order for R to recognize the path.
>
> For example, the -I"${CPLEX_STUDIO_DIR}\cplex\include" should be replaced with path such as
>
> -I"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/include"
>
> The -L"${CPLEX_STUDIO_LIB}" should be replaced with path such as
>
> -L"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/bin/x64_win64"

(c) In command line, run line below to build package, change ${Username} to actual name.

R CMD build --no-build-vignettes --no-manual --md5 "C:\Users\${Username}\Desktop\cplexAPI"

A new package cplexAPI_1.4.0.tar.gz will be built under the default path (for example, C:\Users\${Username})

(d) In command line, run line below to install package.

R CMD INSTALL --build --no-multiarch .\cplexAPI_1.4.0.tar.gz

If you see “DONE (cplexAPI)”, then the package installation is successful.

Note: if error occurs relating to “__declspec(dllimport deprecated)”, you need to go to C:\Program Files\IBM\ILOG\CPLEX_Studio1210\cplex\include\ilcplex (or other installation path), open the file “cpxconst.h”, go to the line indicated in the error message or search for “__declspec(dllimport deprecated)”, add “_” to “__declspec(dllimport deprecated)”, making it to “__declspec(dllimport_deprecated)”. Save file and repeat step (IV).

(e) To take a short venture using CPLEX in R, refer to “Package cplexAPI – Quick Start” in the link https://cran.r-project.org/web/packages/cplexAPI/index.html.

Using NetID

This section will use yeast negative-mode dataset and mouse liver negative-mode dataset as examples to walk through the NetID workflow.

Yeast negative-mode dataset

In the Sc_neg folder, file raw_data.csv is the output from Elmaven recording MS information, and is the input file for NetID. MS2 is not collected for this dataset.

(1) Running the code

(a) Open code folder -> NetID_run_script.R.

(b) In the # Setting path #### section, set work_dir as "../Sc_neg/".

(c) In the # Read data and files #### section, set filename as “raw_data.csv”, set MS2_folder as “”.

(d) Keep all other parameters as default, and run all lines.

(2) Expected output

(a) In the console, error message should not occur. If optimization step is successful, you will see messages in the following format.

"Optimization ended successfull - integer optimal, tolerance - OBJ_value = 2963.71 (bestobjective - bestinteger) / (1e-10 + |bestinteger|) = 0.000048268"

95.74 sec elapsed

(b) Three files will be generated in the Sc_neg folder. Expected run time on a "normal" desktop computer should be within an hour.

“NetID_output.csv” contains the annotation information for each peak.

“NetID_output.RData” contains node, edge and network information. The file will be used for network visualization in Shiny R app.

“.RData” records the environmental information after running codes. The file is mainly used for development and debugging.

Run your own dataset

(1) Prepare MS1 input data

(a) File conversion. Use software ProteoWizard40 (version 3.0.11392) to convert LC-MS raw data files (.raw) into mzXML format. A command line script specifies the conversion parameter. Assuming the raw data are in D:/MS data/test. Type in the scripts below.

D:

cd D:/MS data/test

"C:\Program Files\ProteoWizard\ProteoWizard 3.0.11392\msconvert.exe" *.raw --filter "peakPicking true 1-" --simAsSpectra --srmAsSpectra --mzXML

If ProteoWizard is installed in location other than "C:\Program Files\ProteoWizard\ProteoWizard 3.0.11392\msconvert.exe", specify your path to where you can find the msconvert.exe file.

Expected outputs will be .mzXML files from .raw data.

(b) El-MAVEN (version 7.0) is used to generate a peak table containing m/z, retention time, intensity for peaks. Detailed guides for peak picking can be found in https://elucidatainc.github.io/ElMaven/faq/

After peak picking and a peak table tab has shown up, click export to CSV. Choose “export all groups”. In the pop-up saved window, choose format “Groups Summary Matrix Format Comma Delimited”. Save to the desired path.

(c) Under the NetID folder, create a new folder NetID_test, copy the csv file from step (b) into the folder, and change the filename into raw_data.csv

(2) Prepare MS2 input data

NetID currently utilizes targeted MS2 data for better MS2 quality, and will incorporate data-dependent MS2 data in the future.

(a) **Prepare MS2 inclusion list.** For targeted MS2 analysis, from the peak list generated in step 1, select the peaks (m/z, RT) that you want to perform MS2, and arrange them into multiple csv files that will serve as the inclusion lists to set up the PRM method on Thermo QExactive instrument. Instruction can be found in https://proteomicsresource.washington.edu/docs/protocols05/PRM_QExactive.pdf

Note: Arrange the parent ions so as to avoid to perform many PRMs at same time. An example is shown below with the start and End time set as RT-1.5 and RT+1.5 (min) to have good chromatogram coverage.

Mass [m/z]	Formula [M]	Formula type	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	(N)CE type	MSX ID	Comment
498.93039					Negative	0.456	3.456	30	NCE		ID=116
721.57831					Negative	0.733	3.733	30	NCE		ID=773
403.09219					Negative	1.058	4.058	30	NCE		ID=1446
211.08781					Negative	1.201	4.201	30	NCE		ID=1989
328.28586					Negative	1.401	4.401	30	NCE		ID=2444
149.07196					Negative	1.587	4.587	30	NCE		ID=2780
151.07631					Negative	2.689	5.689	30	NCE		ID=3585
335.22299					Negative	2.701	5.701	30	NCE		ID=3865
143.04614					Negative	4.069	7.069	30	NCE		ID=4493
88.987938					Negative	5.666	8.666	30	NCE		ID=5389
283.10352					Negative	6.917	9.917	30	NCE		ID=5906
202.10851					Negative	8.789	11.789	30	NCE		ID=6400
160.02507					Negative	10.267	13.267	30	NCE		ID=7124
216.08766					Negative	11.445	14.445	30	NCE		ID=7644
125.00095					Negative	11.954	14.954	30	NCE		ID=8245

230.12581					Negative	12.894	15.894	30	NCE		ID=10103

(b) **Instrument setup.** Set up the QExactive instrument so that it contains both "Full MS" and "PRM" scan events. For PRM setup, use the above file as inclusion list to perform targeted MS2 analysis. We typically use the following setting for MS2 analysis: resolution 17500, AGC target 1e6, Maximum IT 500 ms, isolation window 1.5 m/z. For a total of 1500 parent ions and 15 parent ions for each method, it requires a total of 100 runs, or ~42 hours using a 25-min LC method.

(c) **MS2 file conversion.** RawConverter (version 1.2.0.1, http://fields.scripps.edu/rawconv/) is used to convert the .raw file into mzXML file that contains MS2 information. Keep the default parameters except setting "Environment Type" as Data Independent, and "Output Formats" as mzXML.

(d) **MS2 reading and cleaning.** A matlab code is used for MS2 reading and cleaning, which can be found in CodeOcean as a published capsule (https://codeocean.com/capsule/1048398/tree/v1). The csv files from (a) paired with the MS2 data files in mzXML format from (c) are the required input data. Refer to capsule description and 'readme.md' file for more details of how the code works. In Brief,

(i) Prepare filename. Filenames for both csv and mzXML files should be named as "prefixNNN", where prefix is the given file name and NNN is the 3 digits number in continuous order (e.g. 'M001.csv', 'M002.csv',… and 'M001.mzXML', 'M002.mzXML',… in the '/data' folder).

(ii) Duplicate the capsule to your own account so you can edit and use the capsule. Upload your own files and remove the previous files in '/data' folder.

(iii) Specify the prefix and the range of numbers at the beginning section of the main code 'Main_example.m'.

(iv) Set the main code as file to run in Code Ocean using the dropdown menu next to main code.

(v) Click reproducible run to perform the batch processing.

(vi) The resulting output files in .xlsx format with the same filenames will appear in the timeline. Each xlsx file contains multiple tabs of cleaned MS2 spectra. The names of the tabs correspond to the row numbers of the csv file specifying the individual parent peak information.

(e) **Save files to folders.** Back to the NetID_test folder, create a new folder "MS2", download all xlsx files from (d) into the folder.

(3) Running the code

(a) Open code folder -> NetID_run_script.R.

(b) In the # Setting path #### section, set work_dir as "../NetID_test/".

(c) In the # Read data and files #### section,

set filename as "raw_data.csv", set MS2_folder as "MS2".

set LC_method to specify column to read for the retention time of known standards. (In folder NetID -> dependent -> known_library.csv, update the retention time info as needed.)

set ion_mode as -1 if negative ionization data is loaded, and 1 if positive ionization data loaded.

(d) Keep all other parameters as default, and run all lines.

Note 1: If other El-MAVEN version was used, check the "raw_data.csv" for the column number where the first sample is located, and specify that in the NetID_run_script.R file. For example, In El-MAVEN (version 7.0), first_sample_col_num is set at 15 as default. If El-MAVEN (version 12.0) is used, first_sample_col_num should be set at 16.

Note 2: for more advanced uses, scoring and other parameters can be edited in NetID_function.R and NetID_run_script.R. Read the manuscript method section for detailed explanation on parameters.

(4) Expected output

Similar to the demo file, the console will print out message indicating optimization step is successful, and three files "NetID_output.csv", "NetID_output.RData" and ".RData" will be generated in the NetID_test folder.

NetID visualization in Shiny R app

This section provides instruction to visualize and explore NetID output results in the interactive Shiny R app. A 21-inch or larger screen is recommended for best visualization.

(1) Run the Shiny R app.

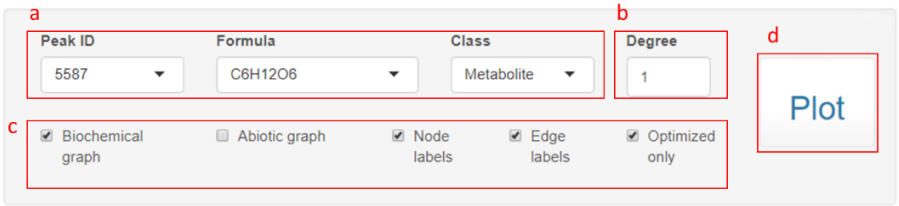
(a) Open code folder -> R_shiny_App.R.
(b) In the # Read in files #### section, set datapath as "../Sc_neg/".
(c) Keep all other parameters as default, and run all lines.
(d) A Shiny app will pop up.

(2) Search peak of interest (top half of Shiny app).

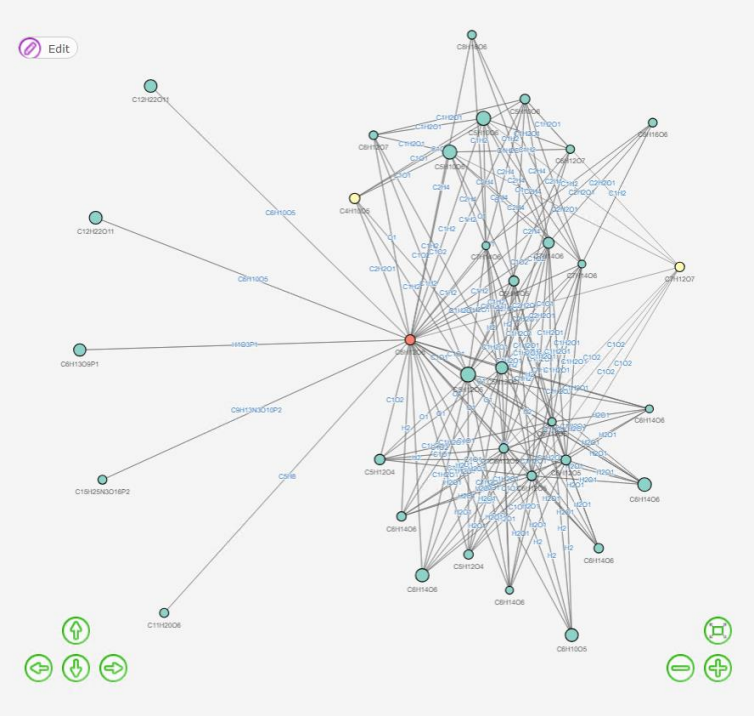
(a) On the left panel, you can enter a m/z or a formula to search your peak of interest. For example, 180.0631 or C6H12O6 will automatically update the data table on the right. Enter 0 to restore full list for the data table.
(b) Change ionization and ppm window to adjust calculated m/z.
(c) On the right, you can explore the peak list in an interactive data table, including global text search on top right, specifying ranges for numeric column or searching text within character columns, ranking each column etc.

(3) Visualize network (bottom left of Shiny app).

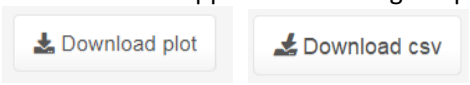
(a) Peak ID, formula and class determines the center node for the network graph. Peak ID will be automatically updated by the first line in the data table if a m/z or formula is given. Alternatively, you can manually enter Peak ID.
(b) The degree parameter controls how far the network expands from the center node. Degree 1 means only nodes directly connected to the center node will be shown and degree 2 means nodes connected to degree 1 will be shown, etc.
(c) Biochemical graph shows biochemical connections. Abiotic graph shows abiotic connections. Node labels and Edge labels determines if the graph show node or edge labels. Optimized only determines whether to show only the optimal annotations or all possible annotations in the network.
(d) When setting parameters, hit plot to see the network graph.



(e) A sample network graph is shown below (a different center node may give less complicated graph). You may edit the nodes or edges (top left), move figures with the arrow buttons (bottom left), and zoom in/out or center figure (bottom right).



(f) You can use the “Download plot” button to download a html webpage to visualize the network graph independent of the Shiny app, and the “Download csv” button to download the information of the nodes in the network. The download buttons will appear after hitting the plot button. Note: edits within the Shiny app will not go into the html file.



(4) Explore possible structures (bottom right of Shiny app).

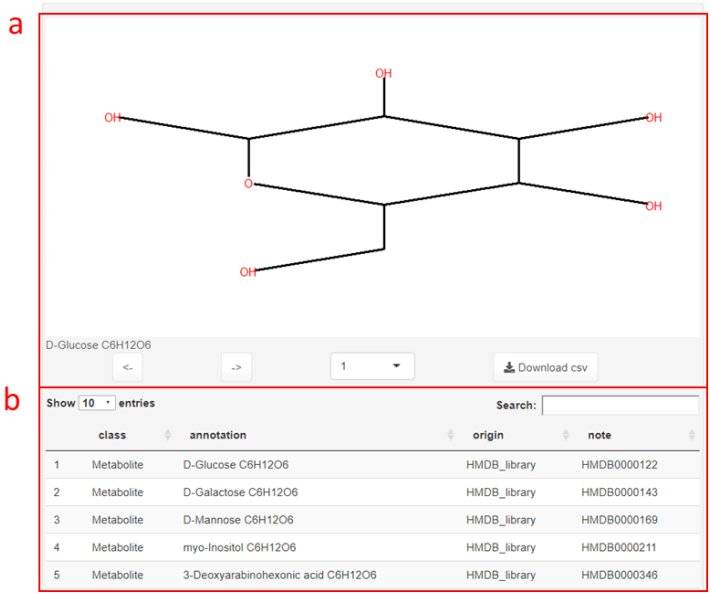
A figure + data table is provided to explore structures of the selected node in the network graph.

(a) The figure shows the chemical structure of the annotated metabolites. If the node is annotated as a putative metabolite, only the known parts of the putative metabolite will be shown.

Scroll left or right, or select the entry number, to visualize different annotations. Right click and select to save image.

(b) In the data table, class has 3 possible entries: Metabolite if it is documented in database such as HMDB library; Putative metabolite if it is transformed from a metabolite through a biotransformation edge; and Artifact if it is transformed by an abiotic edge.

Use the download button to download the data table.



	class	annotation	origin	note
1	Metabolite	D-Glucose C6H12O6	HMDB_library	HMDB0000122
2	Metabolite	D-Galactose C6H12O6	HMDB_library	HMDB0000143
3	Metabolite	D-Mannose C6H12O6	HMDB_library	HMDB0000169
4	Metabolite	myo-Inositol C6H12O6	HMDB_library	HMDB0000211
5	Metabolite	3-Deoxyarabinohexonic acid C6H12O6	HMDB_library	HMDB0000346

Troubleshooting

Failing to install package lc8
Reinstall the packages “devtools” and “digest”.

Cannot find cplexAPI even if the installation seems successful
Check R version used in RStudio to see if cplexAPI is installed under the same R version library. Which R library cplexAPI goes to depends on the R path specified in Environment Variables.

Add R to PATH
(I) Go to Environment Variables:
search PATH in windows -> open edit Environment Variables -> Environment Variables
or
control panel -> system and security -> System -> Advanced system Settings (on your left) -> Advanced -> Environment Variables
(II) In the lower Panel select the “Path Variable” and select Edit, add the R path (C:\Program Files\R\R-4.0.3\bin\x64, if installed at default location) to the Path Variable.
(III) You may need to restart computer for the R path to take effect.

Add Rtools40 to PATH
Option 1.
Add the path “C:\Rtools\bin” to the “Path Variable” in Environment Variables
Option 2.
Run the line in R
writeLines('PATH="${RTOOLS40_HOME}\\usr\\bin;${PATH}"', con = "~/.Renviron")
Use the line below in R console to check for successfully adding Rtools40
Sys.which("make")
Expected output: ## "C:\\rtools40\\usr\\bin\\make.exe"