LipiDex Testing

Datasets for LipiDex validation

For testing, we provide a collection of chromatographic peak table generated by Compound Discoverer 2.0 (Thermo Scientific) and the associated Mascot Generic Format files from LC-MS/MS analysis of human Hap1 cells.

Procedures for testing LipiDex

The Java source code and executables for LipiDex are made available through https://github.com/coongroup/LipiDex. Experimental filed for testing are provided in the folder Multi-Tissue_Dataset\Hap1. To test the software on your system, please follow the instructions below. Additional animated tutorials are also available via the GitHub Wiki https://github.com/coongroup/LipiDex/wiki.

Installation of LipiDex and required programs

1. Move the entire LipiDex folder to a suitable folder on your computer.
2. Download and install the most recent version of Java via https://java.com/en/download/.
3. To open LipiDex, open the “LipiDex.jar” file.

Searching MS/MS Spectra for Lipid IDs

1. Open the *Spectrum Searcher* window from either the main menu or the top toolbar.
2. Select the “Add” button and navigate to the downloaded “Example Data \Hap1” folder.
3. Select all .mgf files in the folder and click “Open”.
4. In the “Available Libraries” table, select the check boxes next to the “LipiDex_HCD_Acetate”, “LipiDex_HCD_Hydroxy”, and “LipiDex_HCD_ULCFA” libraries.
5. Under the “MS/MS Search Parameters” heading, update the parameter fields according to **Table 1**:

Table 1

Parameter	Value
MS1 Search Tolerance	0.01 Th
MS2 Search Tolerance	0.01 Th
Mac Search Results Returned	1
MS2 Low Mass Cutoff	61.00 Th

6. Select “Search Spectra”.
7. MS/MS search results are written in the same directory as the input files and with “_Results.csv” appended to the input file as its name.

Identifying Lipid Chromatographic Peaks

1. Open the *Peak Finder* window from either the main menu or the top toolbar.
2. Under the “Select peak table type” heading, select “Compound Discoverer”.
3. Under the “Aligned Peak Table (.csv)” heading, select “Add”.
4. Navigate to the Example Data \Hap1 folder and select “Hap1_Aligned.csv”.
5. Select “Open” to add the file address to window.
6. Under the “Unaligned Peak Table (.csv)” heading, select “Add”.
7. Navigate to the Example Data \Hap1 folder and select “Hap1_Unaligned.csv”.
8. Select “Open” to add the file address to window.
9. Under the heading “Upload MS/MS result files” select “Load Files”. This will open a new “Results Uploader” window.
10. Under the heading “Select data acquisition type” heading, select the “Separate Polarity Analysis” radio button.
11. Under the heading “Upload .csv result files from Spectrum Searcher”, select “Add Files”.
12. Navigate to the “Test Files” folder and select all of the .csv files with “_Results.csv” in the filename.
13. Select “Open” to add the files to the MS/MS file window.
14. Under the “File ID” column, populate the cells with the File ID numbers corresponding to the **Table 2**:

Table 2

File Name	File ID
Hap1_Pos_1	1
Hap1_Pos_2	2
Hap1_Pos_3	3
Hap1_Neg_1	1
Hap1_Neg_2	2
Hap1_Neg_3	3

15. Select “Upload”.
16. Update all parameter fields according to **Table 3**:

Table 3

Parameter	Value

Min. Lipid Spectral Purity	75%
Min. MS2 Search Dot Prod.	500
Min MS2 Search Rev. Dot Product	700
FWHM Window Multiplier	2
Max. Mass Difference	15 ppm
Adduct/Dimer Filtering	TRUE
In-source Fragment Filtering	TRUE
Max. RT M.A.D. Factor	3.5
Feature Found in n Files	2

17. Select “Identify Chromatographic Peaks”.
18. Upon completion all result files are written to the same directory as the peak tables
19. The filtered peak table is written to Final_Results.csv, the unfiltered peak table is written to Unfiltered_Results.csv, and sample-specific metrics are written to Sample_Information.csv.
20. After LipiDex has finished processing, all results can be compared to the included result files in the directory Example Data\Hap1\Results for Comparison

Hardware and Software Requirements:

Test dataset:

- Hardware
  - 3 GB RAM, minimum 1 GB free memory
- Software
  - Any of the following operating systems: Windows Vista/7/8/8.1/10, Linux or Mac OS
  - A text editor
  - Java 8 or higher

Experimental Datasets

- Hardware
  - Minimum 8 GB RAM, minimum 2 GB free memory
- Software
  - Any of the following operating systems: Windows Vista/7/8/8.1/10, Linux or Mac OS
  - A text editor
  - Java 8 or higher

Estimates of Time Required for Testing

Test dataset

- Identifying lipids from MS/MS spectra will take no more than 2 minutes to set up the analysis and 30 seconds to execute.
- Mapping identifications to chromatographic features will take no more than 5 minutes to set up the analysis and 5 minutes to execute.

Experimental Datasets

- Identifying lipids from MS/MS spectra will take no more than 2 minutes to set up the analysis and ~ 1 second per MS/MS file to execute.
- Mapping identifications to chromatographic features will take no more than 5 minutes to set up the analysis. Please refer to Table 4 for the estimated required memory (RAM) and processing times for various dataset sizes. For larger datasets, more memory must be allocated to Java. For Windows systems, follow the instructions on the LipiDex wiki in the "Allocating Additional Memory to Java" tutorial.

Table 4

Number of Files	Estimated RAM Required (GB)	Estimated Processing Time (min)
5	3	5
10	5	10
50	8	20
100	10	60
1000	25	480