## LipidIN Installation and Launch Guide

After downloading the **LipidIN** project from GitHub, navigate to the LipidIN GUI folder. Open the file 'code for launch UI.R' using Rstudio.

**How to install R and RStudio:** https://rstudio-education.github.io/hopr/starting.html; https://blog.csdn.net/W_chuanqi/article/details/123626811.

**How to install Rtools:** https://cran.rstudio.com/bin/windows/Rtools/rtools40.html.

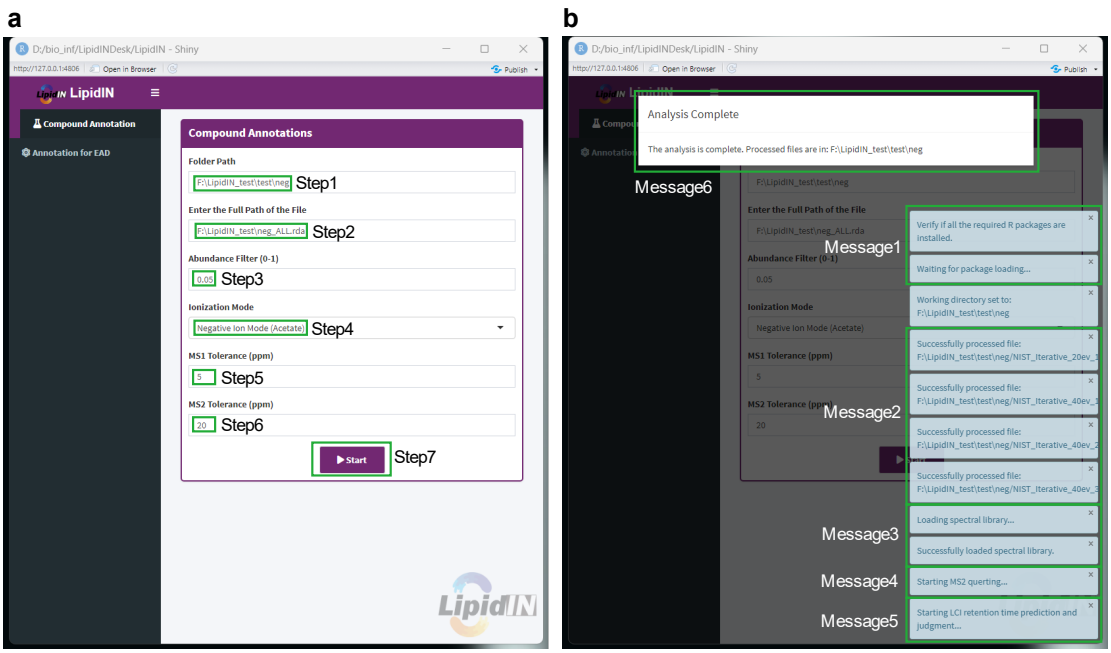
1. **Check Required Packages**: Lines 1–10 of the script are used to check whether the required R packages are installed. Select these lines, then click **Run** in the top-right corner of your R console. Wait for the necessary packages to be installed.
2. **Modify File Path**: Once all the dependencies are installed, update the file path on **line 15** of the script. Replace the placeholder file path with the path to the downloaded 'LipidIN_2.0.0.1.tar.gz' file.
3. **Install LipidIN:** After updating the file path, run **lines 12–19** to install LipidIN. During the installation process, a pop-up window will prompt you to confirm the installation of private packages. Click **Yes** to proceed.
4. **Launch the LipidIN UI**: Finally, run **lines 22–23** of the script. This will launch the LipidIN user interface.

## Parameter input and precautions

1. **Step 1: Input mzML File Path**: In the first input field of the UI, enter the file path of the mzML file you wish to annotate. If necessary, you can use the **MSConvert** software to convert your files into the mzML format. The default parameters of MSConvert are sufficient for this conversion, and additional operations such as centralization are not required. Please ensure that there are no spaces or special characters in the file path.
2. **Step 2: Input Spectral Library Path**: In the second input field, provide the file path to the spectral library to be used for annotation. Note that the spectral library must be in **RDA format**. For instructions on

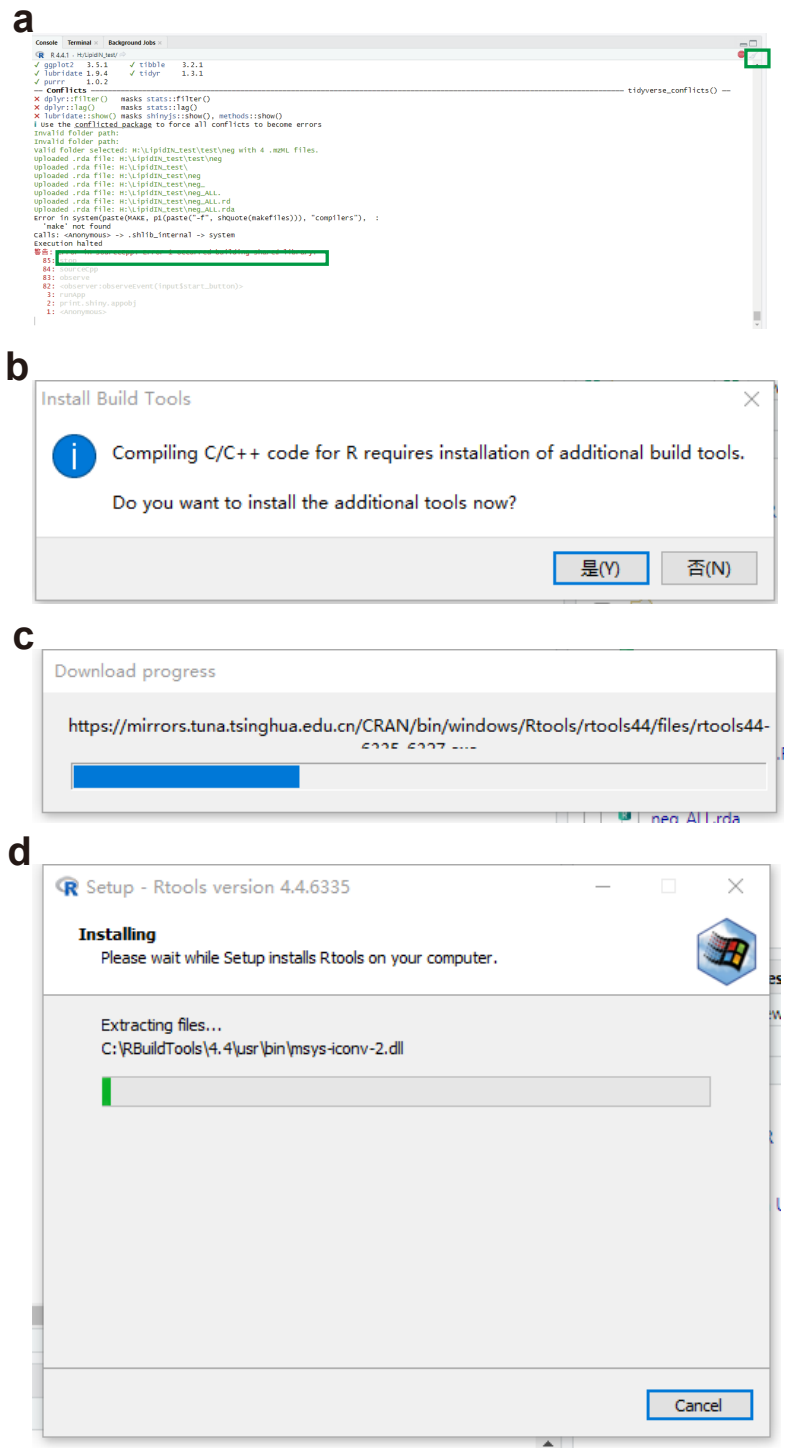
converting an MSP-format spectral library to RDA format, refer to the guide: How to Convert Your MSP Format Spectral Library to RDA Format (https://github.com/LinShuhaiLAB/LipidIN/tree/main/How%20to%20Convert%20Your%20MSP%20Format%20Spectral%20Library%20to%20RDA%20Format). Alternatively, you can use the pre-compiled RDA spectral library available on our GitHub repository: LipidIN/LipidIN 4-level hierarchical library/pos_ALL.rda. As with the mzML file path, ensure that the spectral library path does not contain spaces or special characters.

3. **Step 3: Select Filtering Threshold**: Choose a filtering threshold to exclude peaks with intensities lower than a certain percentage of the maximum intensity in the spectrum. Recommended values for this threshold are **0.01**, **0.05**, or **0.1**.
4. **Step 4: Select Ionization Mode:** Select the ionization mode for your analysis. The available options are: Positive ionization mode, Negative ionization mode ([M+HCOO]-) and Negative ionization mode ([M+$CH_3COO$]-).
5. **Step 5: Set Mass Tolerance for Precursor Ions**: Specify the mass-to-charge ratio (m/z) tolerance for precursor ions during spectral library matching. Input the desired tolerance value in this step.
6. **Step 6: Set Mass Tolerance for Fragment Ions**: Specify the mass-to-charge ratio (m/z) tolerance for fragment ions during spectral library matching. Input the desired tolerance value in this step.
7. **Step 7: Start the Annotation Process**: After verifying the inputs for all the steps above, click the **Start** button to initiate the lipid annotation process.




## Package Loading and Installation

After clicking "Run," the first message (Message 1) will indicate that the system is checking for missing packages and loading them. If you are running the script for the first time, this process might take longer, as it will automatically install RTools if necessary. Please be patient during this step.




The installation process is as follows: 1. When encountering the error message **"Error in SourceCpp"**, it indicates that **Rtools** needs to be installed. 2. Click the red dot in the upper-right corner of the image to terminate the LipidIN operation. 3. Afterward, an installation prompt for **Rtools** will appear. 4. Click **Yes** to begin the download and installation process.

1. **File Format Conversion**: Once all required packages are loaded,

the second message (Message 2) will notify you that LipidIN is converting mzML files to rda files. If you encounter errors at this step, please ensure the following:Your mzML files contain MS2 spectra, and the input file path does not contain spaces or special characters.

2. **Library Loading**: After file conversion, the system will load the spectral library, which will be indicated by the third message (Message 3). This step can take a significant amount of time, especially if your spectral library contains millions of entries.
3. **Spectral Matching**: Once the library is loaded, spectral matching will begin, and the fourth message (Message 4) will appear. This process is typically very fast. If it becomes stuck or an error occurs, check the format and content of your spectral library for accuracy.
4. **Lipid Retention Time Analysis**: After spectral matching, the system will perform lipid retention time prediction, indicated by the fifth message (Message 5). This step takes approximately 1–2 minutes.
5. **Completion**: Finally, when all annotation processes are complete, the system will display the sixth message (Message 6).