

# FAMetA application workflow

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## LipidMS Overview

FAMetA is an R-based tool aimed to the analysis of fatty acids (FA) metabolism. It allows the estimation of FA import (I), de novo synthesis (S), fractional contribution of  $^{13}\text{C}$ -tracers (D0, D1, D2), elongation (E) and desaturation (Des) based on  $^{13}\text{C}$  mass isotopologue distributions. Next figure summarizes the basis of FAMetA analysis:

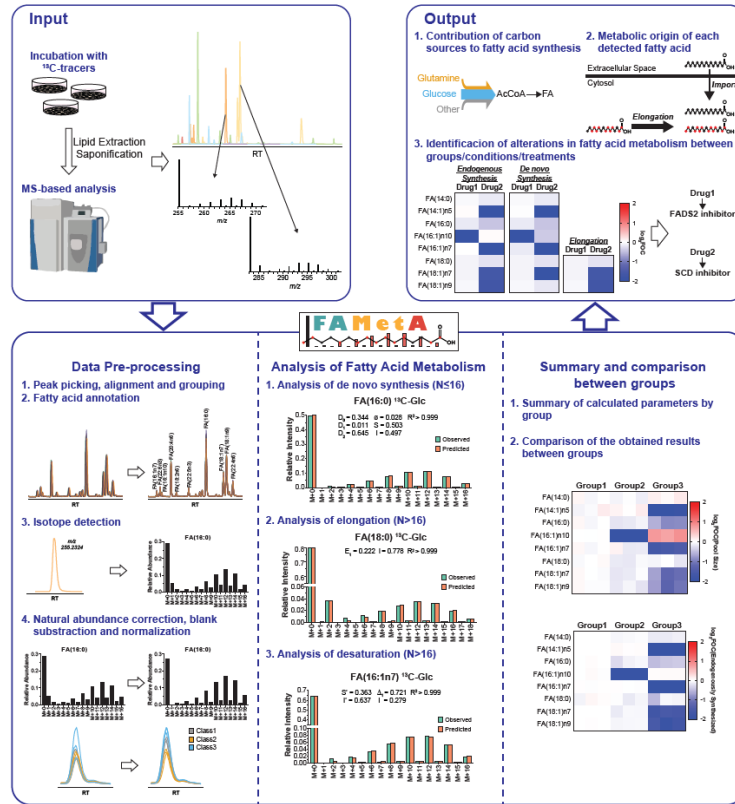


Figure 1: FAMetA abstract

## Web access

FAMetA application can be accessed through our website at [www.fameta.es](http://www.fameta.es).

## Files conversion

To start the MS analysis raw files need to be converted into mzXML format (you can use any software such as MSConvert from proteowizard) and then, FAMetA can be run.

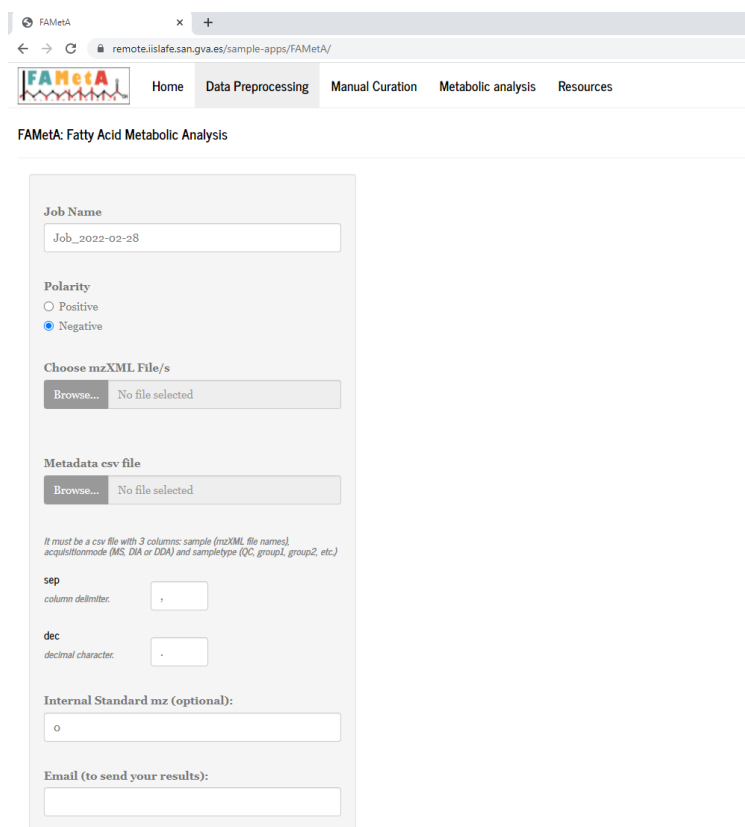
## Example data files

Some example files and scripts (for R environment) can be downloaded ([here](#)).

## Data Preprocessing

First step in FAMetA workflow consists on preprocessing your data with LipidMS R-package. At this tab, mzXML files and a metadata csv file are required. Metadata file must have 3 columns: sample (mzXML file names), acquisitionmode (MS, group1, group2, etc.). An example can be found at the [samples.csv](#) file ([here](#)). Once all files have been uploaded, preprocessing parameters must be tuned.

After data preprocessing has been performed, an email will be sent with the FA annotation results.



The screenshot shows the 'Data Preprocessing' tab of the FAMetA application. The browser address bar shows the URL 'remote.isiiafe.san.gva.es/sample-apps/FAMetA/'. The navigation menu includes 'Home', 'Data Preprocessing' (active), 'Manual Curation', 'Metabolic analysis', and 'Resources'. The main heading is 'FAMetA: Fatty Acid Metabolic Analysis'. The form contains the following fields:

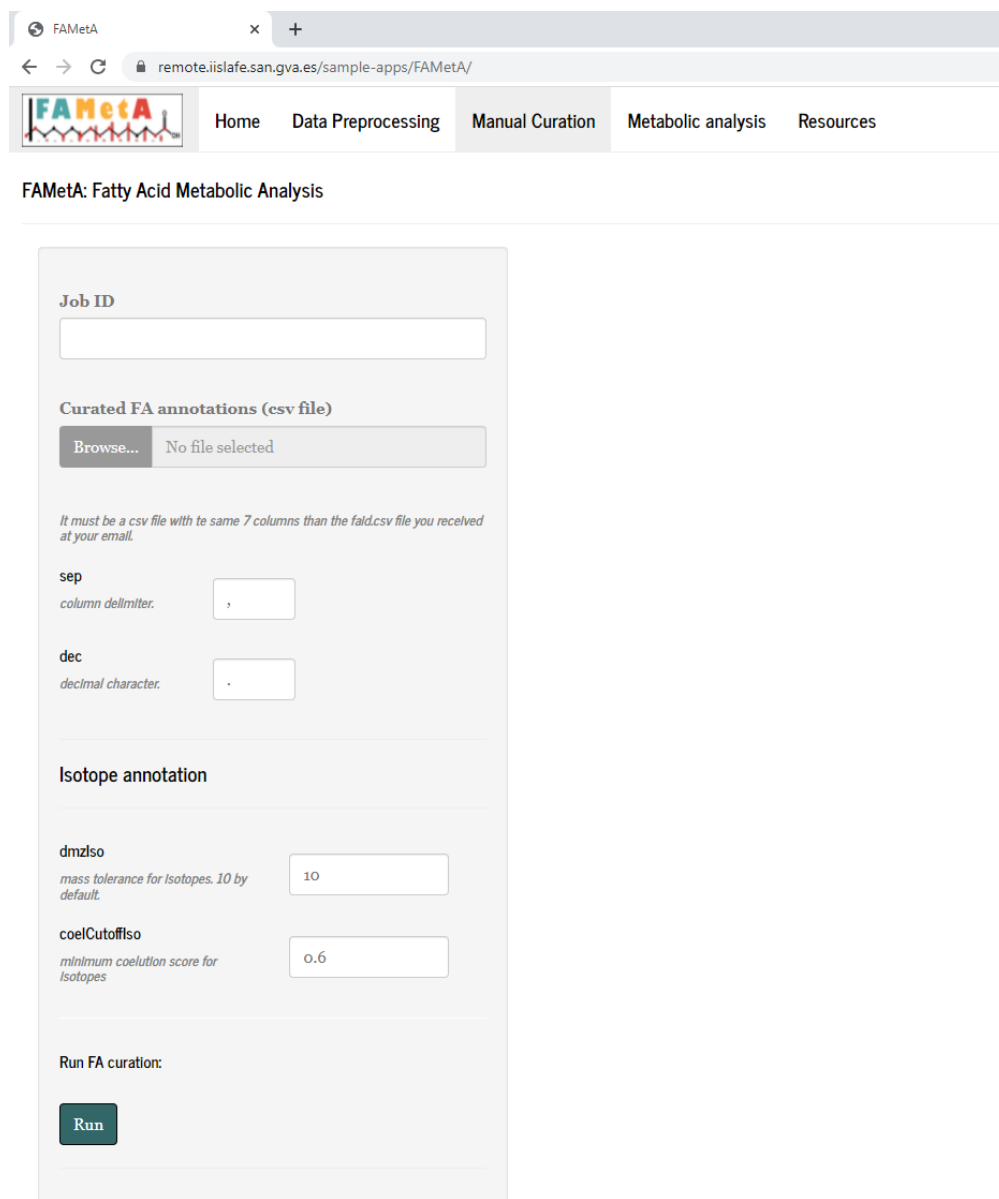
- Job Name:** A text input field with the value 'Job\_2022-02-28'.
- Polarity:** Radio buttons for 'Positive' and 'Negative'. 'Negative' is selected.
- Choose mzXML File/s:** A 'Browse...' button and a status 'No file selected'.
- Metadata csv file:** A 'Browse...' button and a status 'No file selected'.
- Help text:** 'It must be a csv file with 3 columns: sample (mzXML file names), acquisitionmode (MS, DIA or DDA) and sampletype (QC, group1, group2, etc.)'.
- sep:** A text input field with a comma ',' as the column delimiter.
- dec:** A text input field with a period '.' as the decimal character.
- Internal Standard mz (optional):** A text input field with the value '0'.
- Email (to send your results):** An empty text input field.

Figure 2: Data preprocessing tab of FAMetA app

## Manual Curation

Automatic FA annotations can be modified by editing the csv file received by email: removing rows of unwanted FA, modifying the initial and end retention times, or adding new rows with missing compounds. Unique compound names with the nomenclature “FA(16:1)n7”, where n7 (omega-7) indicates the last double bond position, are required to differentiate between FA isomers. For any unknown positions, letters x, y and z are allowed (i.e. FA(16:1)nx). The internal standards for later normalization can also be added at this point to a new row by indicating IS in the compound name column. An example file with curated annotations can be found at (here).

Once FA annotations have been curated,  $^{13}\text{C}$  isotopologues for each FA will be searched and mass isotopologue distributions will be sent by email.



The screenshot shows the FAMetA web application interface. The browser address bar displays "remote.iislafe.san.gva.es/sample-apps/FAMetA/". The navigation menu includes "Home", "Data Preprocessing", "Manual Curation" (which is the active tab), "Metabolic analysis", and "Resources". The main heading is "FAMetA: Fatty Acid Metabolic Analysis".

The "Manual Curation" form contains the following sections:

- Job ID:** A text input field.
- Curated FA annotations (csv file):** A file selection area with a "Browse..." button and the text "No file selected". Below this, a note states: "It must be a csv file with te same 7 columns than the fald.csv file you received at your email."
- sep:** A label "column delimiter." followed by a text input field containing a comma (`,`).
- dec:** A label "decimal character." followed by a text input field containing a period (`,`).
- Isotope annotation:**
  - dmzIso:** A label "mass tolerance for isotopes. 10 by default." followed by a text input field containing "10".
  - coelCutoffIso:** A label "minimum coelution score for isotopes" followed by a text input field containing "0.6".
- Run FA curation:** A section with a green "Run" button.

Figure 3: Manual Curation tab of FAMetA app

## Metabolic Analysis

Finally, FA analysis can be performed. To this end, a previous data correction is required which will run four different steps (all of them are optional): data correction for natural  $^{13}\text{C}$  abundance using the accucor algorithm, data normalization with internal standards, blank subtraction and external normalization. Then, the actual FA metabolism analysis can be performed.

Figure 4: Metabolic Analysis tab of FAMetA app

Once you receive your results, check S and D2 parameters. In case you use inhibitors that decrease S below the confidence interval, D2 parameter can be misestimated. To avoid this problem fix D2 values using your control group (you can replace misestimated values for the mean value of the control group from palmitic acid results preferentially). To this end, a new row on the first row of your fadata.csv file can be added to fix D2 values and run the analysis again. An example can be found at the [examplefadata\\_withD2.csv](#) file (here).

If you have any further questions, please do not hesitate to contact us at: [maribel\\_alcoriza@iislafes.es](mailto:maribel_alcoriza@iislafes.es) or [maribel\\_alcoriza@hotmail.com](mailto:maribel_alcoriza@hotmail.com)