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**FASA (Fatty Acid Source Analysis)**

Originally described in “Argus, J.P., Wilks, M.Q., Zhou, Q.D., et al., Development and Application of FASA, a Model for Quantifying Fatty Acid Metabolism Using Stable Isotope Labeling, Cell Reports (2018), https://doi.org/10.1016/j.celrep.2018.11.041". See this manuscript for additional detail.

**Requirements:**

1. Install MATLAB R2018a. FASA was tested on this version of MATLAB, but it is possible that it would be compatible with other versions.
2. Add the downloaded FASA folder to the MATLAB directory on your computer.

**Usage:**

1. Create a new data folder for the current analysis (e.g., “Example\_Analysis”).
2. Inside this new data folder, create the input .xls file (not .xlsx) containing the isotopologue data to be analyzed (e.g., “Example Data.xls”).
   1. Isotopologue data requirements.
      1. In utilizing FASA, an isotope of carbon (13C) must be used as the label atom.
      2. FASA has been tested using isotopologue data collected from methyl esters of 14-, 16-, 18-, 20-, 22-, and 24-carbon fatty acid species. When isotopologue data comes from fatty acid methyl esters (FAMEs):
         1. All non-carbon natural isotope abundances must be corrected for before application of FASA.
         2. All carbon natural isotope abundances (for label and natural carbons) can be corrected for before application of FASA or accounted for during the application of FASA.
         3. Note that if all carbon natural isotope abundances are corrected for before using FASA, user handles q and e (see below for definitions) should be should be set at a small, positive, non-zero number (e.g. 1e-20) and 1, respectively, when applying FASA.
   2. See “Example Data.xls” and instructions below for appropriate formatting.
      1. Each sheet represents data from a single fatty acid species for all samples.
      2. Each sheet name must be unique and NOT contain these characters ": \ / ? \* [ or ]".
      3. There must be at least two sheets in the .xls file.
      4. In each sheet…
         1. Column A = Sample names (one sample per row). Note that the sample names and order should be consistent for all sheets.
         2. Column B through Column B+N+1 = area under the curve (AUC) values for molecular ion isotopologues M+0 through M+N+1. N = number of carbons in the fatty acid species (14, 16, 18, 20, 22, or 24). Note that for all sample rows, all cells in column B through column B+N+1 must contain 0 or a positive number (no blanks).
         3. Column B+N+2 = Sample code. 1 = labeled sample to model; 0 = unlabeled sample to model *q* only (optional); and -1 = sample to ignore (optional). Note that the sample code for each individual sample should be consistent for all sheets. N = number of carbons in the fatty acid species (14, 16, 18, 20, 22, or 24).
            1. *q* = 13C abundance in natural metabolites. See Argus, JP et al., 2018 for additional information.
         4. Column B+N+3 through column B+N+12 = *A priori* parameter values for *D0*, *D1*, *D2*, *S*, *I*, *IE1*, *IE2*, *IE3*, *IE4*, and *IE5*. “-1” indicates the parameters are not fixed *a priori*. If a value between 0 and 1 (inclusive) is entered, that parameter for that sample will be fixed at the entered value. N = number of carbons in the fatty acid species (14, 16, 18, 20, 22, or 24).
            1. *D0*, *D1*, and *D2* represent the relative abundance of lipogenic acetyl-CoAs containing 0, 1, or 2 13Cs. See Argus, JP et al, 2018 for additional information.
            2. *S*, *I*, and *IEn* represent the relative abundance of fatty acids in a given fatty acid pool that were “synthesized”, “imported”, or “imported-elongated *n* times”. See Argus, JP et al, 2018 for additional information.
3. Copy “FASA\_User.m” from the “Source\_Code” folder to the new data folder and rename appropriately (e.g., “FASA\_User\_Example.m”).
4. Open renamed FASA\_User file (e.g., “FASA\_User\_Example.m”) in MATLAB.
5. Choose “Add to Path => Selected Folder and Subfolders” for the downloaded FASA folder.
6. Choose desired settings for user handles.
   1. sheets\_to\_model = The sheets from your input .xls file that you want to analyze with FASA. “sheets\_to\_model” is a vector – for example, [1:13] would run sheets 1 through 13, while [1,3,5:10] would run sheets 1, 3, and 5 through 10.
   2. cutoff = If an isotopologue AUC value is equal to or less than this number, it will be included in the total AUC of that fatty acid, but will otherwise not contribute to the cost function of the fitting.
   3. q = 13C abundance in natural metabolites. Possible values: 0 ≤ q ≤ 1. If q = 0, the algorithm will determine *q* from unlabeled samples (“sample code” = 0). To fix *q* at “0”, use a small, positive, non-zero number such as 1e-20.
   4. e = 13C enrichment for each labeled metabolite used. Possible values: 0 ≤ e ≤ 1. See Argus, JP et al, 2018 for additional information.
   5. assume\_no\_label\_diffusion = Determines whether or not label diffusion is permitted during modeling of the lipogenic acetyl-CoA pool. When assume\_no\_label\_diffusion = 0, label diffusion is permitted. FASA iterates *D0* and *D1* with *D2* = 1-*D0*-*D1*. When assume\_no\_label\_diffusion = 1, label diffusion is not permitted. FASA iterates *D0*, with *D1* and *D2* being calculated from *D0*. This constrains the modeling of the lipogenic acetyl-CoA pool from 2 to 1 actively iterated parameter, reflecting how isotopomer spectral analysis (ISA) models the lipogenic acetyl-CoA pool. Note that if FASA is constrained to disallow label diffusion, all labeled metabolites must be uniformly labeled. See Argus, JP et al., 2018 for additional information.
   6. MC\_successes = The number of Monte Carlo replicates you wish to perform for each sample. For parameters *S*, *I,* *IE1*, *IE2*, *IE3*, *IE4*, and *IE5*: In each Monte Carlo replicate, starting parameter values are random unless fixed *a priori* by the user. For parameters *D0*, *D1*, and *D2*: In each Monte Carlo replicate, starting parameter values for *D0*, *D1*, *D2*, are .425, .025, and .55 (respectively) unless the user has fixed these parameters *a priori*.
7. Ensure that the new data folder (e.g., “Example\_Analysis”) is the current MATLAB folder.
8. Run the code; when prompted, select the .xls containing the isotopologue data to be analyzed (e.g. “Example Data.xls”).
9. Output files:
   1. Results-FASA-XXX.xls = Best fit FASA output values in .xls form. XXX = data input file name. See “Results-FASA-Example Data.xls” and description below for additional information.
      1. Each sheet represents data from a single fatty acid species for all samples.
      2. Values presented for each sample come from the Monte Carlo replicate with the sum of squared errors (SSE) closest to zero.
      3. In each sheet…
         1. Column A = Sample names (one sample per row).
         2. Column B through column O = Values for *q*, *e*, *D*, 1-*D*, *D0*, *D1*, *D2*, *S*, *I*, *IE1*, *IE2*, *IE3*, *IE4*, and *IE5*.
            1. *D* represents the percent contribution of 13C-labeled metabolites to the lipogenic acetyl-CoA pool. FASA’s calculation of *D* is valid when all labeled metabolites are uniformly labeled. See Argus, JP et al, 2018 for additional information.
         3. Column P = -SSE
         4. Column Q through Q+N+1 = Normalized modeled isotopologue abundance values. N = number of carbons in the fatty acid species (14, 16, 18, 20, 22, or 24).
   2. MCStats-xYYY-XXX.xls = FASA output values for each Monte Carlo replicate in .xls form. XXX = data input file name, YYY = sheet name. See “MCStats-x200-Example Data.xls”, “MCStats-x220-Example Data.xls”, and the description below for additional information.
      1. Each sheet represents the Monte Carlo replicate results from a single sample.
      2. Row 2 contains the Monte Carlo replicate with the SSE closest to 0.
      3. Rows 3-4 contain the mean and variance of the results from all Monte Carlo replicates.
      4. Rows 5 and beyond contain the results of each Monte Carlo replicate.
      5. Column B contains the -SSE values.
      6. Columns C and beyond represent modeled parameters *D0*, *D1*, *D2*, *S*, *I*, *IE1*, *IE2*, *IE3*, *IE4*, and *IE5*
   3. xYYY - SampleZZZ- Mod3.png = Isotopologue distribution graph in .png form. YYY = sheet name, ZZZ = sample number (determined by row order in input .xls).
   4. xYYY - SampleZZZMod3.fig = Isotopologue distribution graph in .fig form. YYY = sheet name, ZZZ = sample number (determined by row order in input .xls).
10. If rerunning an input data file, create a new folder (see Usage Step 1) to avoid conflicting and/or overwritten output files.