

User Manual for ML-Flux Website

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1. Introduction

The ML-Flux website (www.metabolicflux.org) is the first ever software web tool for comprehensive metabolic flux analysis (MFA). This software can train from five universal metabolic models, where the user can choose the specific model for both imputing unmeasured labeling and determining fluxes in the dropdown menu. We offer two major predictive capabilities for quick and easy quantification of metabolic fluxes: labeling pattern inference of unmeasured metabolites, and rapid prediction of fluxes similar to C-MFA.

2. Overview of Metabolic Models Available

The www.metabolicflux.org website offers five different universal metabolic models to train networks. The five models are described below:

2.1 Simple Test

The simple test model represents a basic metabolic network, where it simulates isotope labeling patterns for metabolite using all possible tracers with the exclusion of the trivial set (fully labeled or fully unlabeled).

2.2 Upper Glycolysis

The upper glycolysis model focuses on the upper part of the glycolysis pathway. This model simulates isotope labeling patterns using only the [1,2- $^{13}\text{C}_2$]-glucose and [5-D]-glucose tracers.

2.3 Glycolysis

The glycolysis model represents the entire glycolysis pathway. The model simulates isotope labeling patterns using the [1,2- $^{13}\text{C}_2$]-glucose and [5-D]-glucose tracers.

2.4 Glycolysis & Pentose Phosphate Pathway (PPP)

This model combines the glycolysis and the PPP by simulating isotope labeling patterns using 24 different ^{13}C -glucose tracers. The tracers used either unlabeled or [U- $^{13}\text{C}_5$]-glutamine for a total of 20 unique tracer combinations.

2.5 Central Carbon Metabolism (CCM)

The CCM model represents a comprehensive CCM metabolism network by simulating isotope labeling patterns using 10 most cited ^{13}C -glucose tracers. The tracers used either unlabeled or [U- $^{13}\text{C}_5$]-glutamine for a total of 20 unique tracer combinations.

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2.6 Model Attributes Overview for Five Models

Model Attribute	Simple	Upper Glycolysis	Glycolysis ($^{13}\text{C}^2\text{H}$)	Glycolysis + PPP	Mammalian (CCM)
Model Description	Simple Antoniewicz network (https://doi.org/10.1016/j.ymben.2006.09.001)	Upper glycolysis only using [1,2- $^{13}\text{C}_2$]glucose	Glycolysis using [1,2- $^{13}\text{C}_2$ 5-D]glucose	Glycolysis and PPP using the 24 most cited ^{13}C glucose tracers	Mamalian central carbon metabolism using the 10 most cited ^{13}C glucose tracers with and without uniformly labeled ^{13}C glutamine
Number of Metabolites	5	5	24 (11 C labeled and 13 H labeled)	18	30
Length of MID per metabolite (based off the largest MID in the model)	4	7	8	8	8
Number of Tracers	6	1	1	24	20 (10 glucose tracers and 2 glutamine configurations)
Length of input vector	120	35	192	3456	4800
Length of output vector (free net and exchange fluxes)	5	7	18	33	55
Number of fluxes in full set (net + exchange)	14	7	40	72	104

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Table 1. Summary of Model Attributes

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3. Setting up the Input Spreadsheet

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Before utilizing the website, it is necessary for the user to prepare the input spreadsheet with essential parameters. Please follow these steps; we are utilizing the example .xml file (ML-Flux_Input_Template.xlsx):

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3.1 Spreadsheet Structure

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The input file should be .xml file, where the file has two mandatory sheets: **input** and

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“Tracer name” (ex: **U- ^{13}C**).

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3.1.1 Input Sheet

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The **input** sheet has information on labeling configuration of each input, where the sheet contains each row per tracer as shown in Table 1 below.

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	A	B	C	D
1	"inpute metabolite name"+"_"+"TracerDesignation"	Labeling of atom 1 (1 or 0, 1 being labeled)	Labeling of atom 2 (1 or 0, 1 being labeled)	Labeling of atom 3 (1 or 0, 1 being labeled)
2				
3				
4				

Table 2. Input Sheet. The first column contains “input metabolite name” + “_” + “Tracer Designation”. The next columns have labeling of atoms (1 or 0, 1 being labeled).

3.1.2 Tracer Name Sheet

The “Tracer name” sheet (ex: $U-^{13}C$), there should be one sheet per experiment with all metabolite labeling from the experiment in this sheet.

	A	B	C	D
1	"Metabolite name"+"_"+"Tracer designation"	M+0 replicate 1	M+0 replicate 2	M+0 replicate 3
2		M+1 replicate 1	M+1 replicate 2	M+1 replicate 3
3		M+2 replciate 1	M+2 replciate 2	M+2 replciate 3
4		M+3 replicate 1	M+3 replicate 2	M+3 replicate 3

Table 3. Tracer Name Sheet. The first column includes “Metabolite name” + “_” + “Tracer designation”. The next columns have ex: M+0 replicate 1.

3.1.3 Additional Sheets

Any additional sheets may be included to the .xml file.

4. Using www.metabolicflux.org

Now that we have the input spreadsheet ready, follow these steps to simulate fluxes in www.metabolicflux.org.

4.1 Choosing the Model

The model is defaulted to choose “Simple Model”. To choose different models, click on the dropdown menu to find the model you want among the five universal models as shown below.

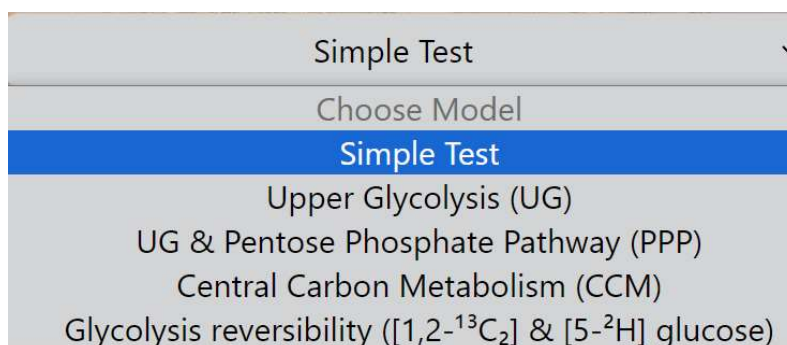


Figure 1. Dropdown Menu for Models

4.2 Uploading the Spreadsheet

After choosing the model, click on “Choose File” under the dropdown menu to upload the prepared .xml file from part 3.

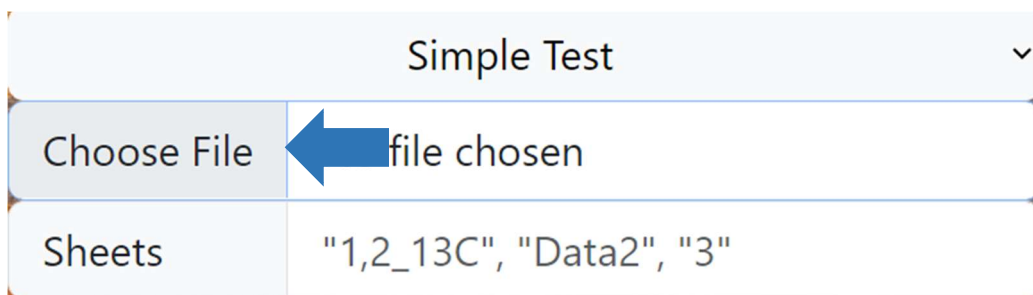


Figure 2. “Choose File” Icon

4.3 Inputting Sheet Names

On the right side of sheets, write the sheet names like the example given. Write quotation marks for the names and separate the names with commas.

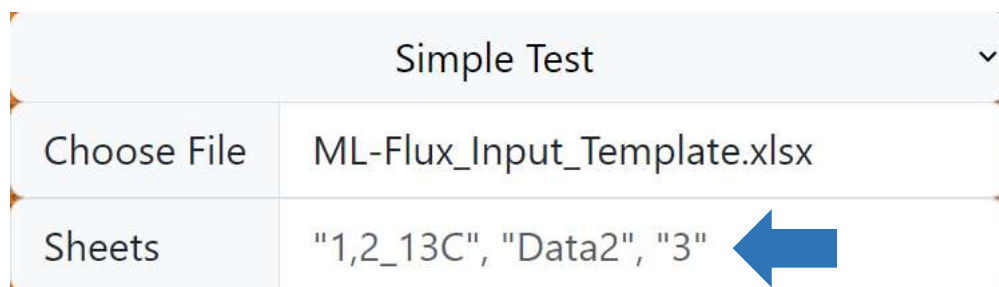


Figure 3. “Sheets” Input

4.4 Analyzing results

After clicking on the **Output Fluxes** icon, the web tool will utilize neural network training on the backend to retrieve the result. The webpage will automatically download a .dat file when the simulation is over. In the result file, the resulting arrays of fluxes and labels are formatted for each row to contain single simulation labeling data. This is ordered by metabolite, the isotope tracer used, and the mass isotope distribution (MID).

4.5 Troubleshooting

When you run into an error page, please reconfirm that the spreadsheet is formatted as shown in part 3, and the sheet names are identical in the spreadsheet. Contact jop@ucla.edu for further assistance.