User Manual for ML-Flux (metabolicflux.org)

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1. Introduction

Machine learning flux (ML-Flux) is hosted on www.metabolicflux.org as the first online web tool for quantitative metabolic flux analysis. ML-Flux is trained from five universal metabolic models for users to choose from. Training across a wide coverage of biologically relevant flux ranges and over 20 different ^{13}C and ^{2}H tracers enable broad user applicability. The resulting ML-Flux model is constructed to be able to take variable input sizes depending on the metabolites measured and tracing experiments conducted. As such, we offer two major predictive capabilities: 1) labeling pattern inference of unmeasured metabolites and 2) rapid prediction of metabolic fluxes.

2. Overview of Metabolic Models Available

The www.metabolicflux.org website offers five different universal metabolic models to choose from:

2.1 Simple Toy Model

This simple test model represents a basic metabolic network for instructional use that simulates labeling patterns 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 [1,2-$^{13}C_2$]-glucose and [5-D]-glucose tracers.

2.3 Glycolysis

The glycolysis model represents the entire glycolysis pathway. The model simulates isotope labeling patterns using [1,2-$^{13}C_2$]-glucose and [5-D]-glucose tracers.

2.4 Glycolysis & Pentose Phosphate Pathway (PPP)

This model combines the glycolysis and the pentose phosphate pathway (GlyPPP) by simulating isotope labeling patterns using 24 different ^{13}C-glucose tracers.

2.5 Central Carbon Metabolism (CCM)

The CCM model represents a central carbon metabolic network by simulating isotope labeling patterns using 10 most cited ^{13}C-glucose tracers. The tracers used either unlabeled or [U-$^{13}C_5$]-glutamine for a total of 20 unique tracer combinations.

2.6 Isotope tracers used for each metabolic model

Tracer experiments used for ML training for each metabolic model							
Model	**Simple**	[1-$^{13}C_1$]-A	[2-$^{13}C_1$]-A	[1,2-$^{13}C_2$]-A	[3-$^{13}C_1$]-A	[1,3-$^{13}C_2$]-A	[2,3-$^{13}C_2$]-A
	Upper glycolysis	[1,2-$^{13}C_2$]-glucose					
	Glycolysis	[1,2-$^{13}C_2$]-glucose [5-D]-glucose					
	Glycolysis + PPP	[1,2,3-$^{13}C_3$]-glucose	[1,2-$^{13}C_2$]-glucose	[1-$^{13}C_1$]-glucose	[2,3,4,5,6-$^{13}C_5$]-glucose	[2,3-$^{13}C_2$]-glucose	[2-$^{13}C_1$]-glucose
		[3,4-$^{13}C_2$]-glucose	[3-$^{13}C_1$]-glucose	[4,5,6-$^{13}C_3$]-glucose	[4,5-$^{13}C_2$]-glucose	[4-$^{13}C_1$]-glucose	[5-$^{13}C_1$]-glucose
		[6-$^{13}C_1$]-glucose	[U-$^{13}C_6$]-glucose	[2,5,6-$^{13}C_3$]-glucose	[1,5,6-$^{13}C_3$]-glucose	[1,2,4-$^{13}C_3$]-glucose	[2,4-$^{13}C_2$]-glucose
		[1,4-$^{13}C_2$]-glucose	[4,6-$^{13}C_2$]-glucose	[2,5-$^{13}C_2$]-glucose	[1,3-$^{13}C_2$]-glucose	[5,6-$^{13}C_2$]-glucose	[1,6-$^{13}C_2$]-glucose
	CCM	[U-$^{13}C_6$]-glucose [^{12}C]-glutamine	[^{12}C]-glucose [U-$^{13}C_5$]-glutamine	[1-$^{13}C_1$]-glucose [^{12}C]-glutamine	[1-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	[2-$^{13}C_1$]-glucose [^{12}C]-glutamine	
		[2-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	[1,2-$^{13}C_2$]-glucose [^{12}C]-glutamine	[1,2-$^{13}C_2$]-glucose [U-$^{13}C_5$]-glutamine	[6-$^{13}C_1$]-glucose [^{12}C]-glutamine	[6-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	
		[1,6-$^{13}C_2$]-glucose [^{12}C]-glutamine	[1,6-$^{13}C_2$]-glucose [U-$^{13}C_5$]-glutamine	[3-$^{13}C_1$]-glucose [^{12}C]-glutamine	[3-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	[5-$^{13}C_1$]-glucose [^{12}C]-glutamine	
		[5-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	[4-$^{13}C_1$]-glucose [^{12}C]-glutamine	[4-$^{13}C_1$]-glucose [U-$^{13}C_5$]-glutamine	[5,6-$^{13}C_2$]-glucose [^{12}C]-glutamine	[5,6-$^{13}C_2$]-glucose [U-$^{13}C_5$]-glutamine	

Table 1. Summary of isotope tracers used for each metabolic model

3. Setting up the Input Spreadsheet

Before utilizing the website, it is necessary for the user to prepare the input spreadsheet with essential parameters. An example template is provided downloadable from the website (ML-Flux_Input_Template.xlsx). At a minimum, two sheets are mandatory:

3.1 Input Sheet

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.

	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).

3.2 Tracer Name Sheet

The “Tracer name” sheet (ex: U-13C), 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.

3.2.1 Metabolite and reaction nomenclature

Each metabolic model uses its own nomenclature and abbreviations for metabolic reactions as shown in the Figure 1. In the provided template file (ML-Flux_Input_Template.xlsx), there is an additional sheet named “MetaboliteNames” that describes how each metabolite should be named for input

3.3 Additional Sheets

Any additional sheets may be included to the .xlsx file without impacting flux prediction

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.

4.2 Uploading the Spreadsheet

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

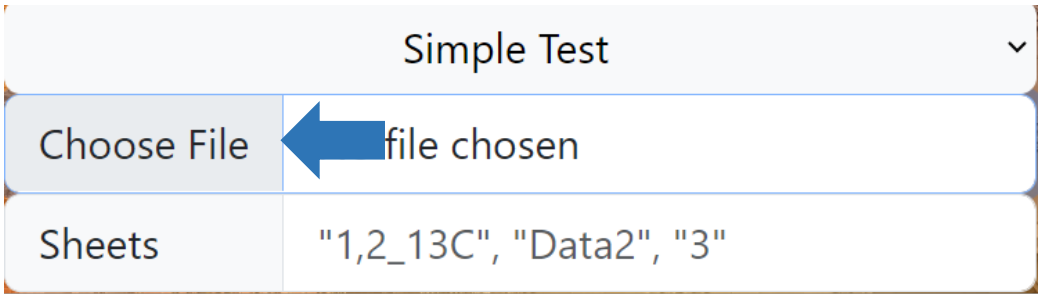

Figure 2. “Choose File” Icon

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.

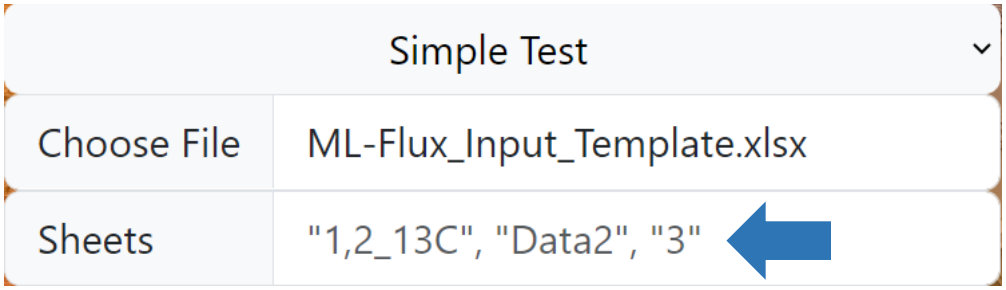

Figure 3. “Sheets” Input

4.4 Analyzing results

After clicking on the Output Fluxes icon, the web tool will provide downloaded a .csv titled “result.csv”. In the result file will be a vector of fluxes formatted for each row.

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 parklab@g.ucla.edu for further assistance.