**Instructions/ descriptions**

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**0. Basic software installation**

1. Install MATLAB (2020a or newer)
2. Install Gurobi version 9.52 or higher (https://www.gurobi.com/). Free academic licenses can be obtained.
3. Add “cobratoolbox” and “gurobi” folders to your MATLAB path

**1. Reconstruction of tissue-specific genome-scale metabolic models for *Drosophila***

1. Go to ‘\1\_making\_tissue\_models’ folder.
2. Run ‘script\_1\_makeTissueSpecificGEMs.m’. This will create transcripts in data structure format.
3. Run ‘script\_2\_run\_tINITyml\_multiple\_specific.m’. This will create automatic tissue-specific genome-scale metabolic models for 32 tissues. The raw data files for the pseudo-bulk single-nuclei RNA-seq data can be found in ‘files’ folder.
4. For metabolic subsystem analysis, run ‘script\_3\_graph\_compareModel\_all.m’. This will create a heatmap that compares metabolic subsystem coverage across tissues (ref. Fig. 1c-d).
5. For metabolic functional analysis, run ‘script\_4\_graph\_compareGEM\_fns\_all.m’. This will create a spy figure describing tissues’ ability to complete metabolic takss (ref. Fig. 1e).
6. **Linking EC numbers to GEMs**
7. Go to ‘\2\_EC\_annotataion\_link’ folder.
8. Run ‘A\_xxx.m’ to ‘D\_xxx.m’ in an alphabetical order. A series of running these manuscript will allow EC annotations linked to Fruitfl1, making a Fruitfly3. The interim results will be stored in each folder. The result is summarized in ‘\D\updatedECINfo.xlsx’.
9. **Enzyme abundance prediction pipeline**
10. Go to ‘\3\_protein\_abundance\_prediction’ folder.
11. Run ‘A\_xxx.m’ to ‘D\_xxx.m’ in an alphabetical order. A series of running these manuscripts will enable estimation of enzyme abundance based on RNA-seq database. The whole fly RNA-seq database and protein parameters can be found in files folder. The interim results are found in each folder. The resulting figures shown in the supplementary Figure 2 are generated by running ‘B\_xxx.m’ and ‘D\_xxx.m’ manuscripts.
12. **Vmax calculation for whole fly or tissues**
13. Go to ‘\4\_Vmax\_calculation’ folder
14. Go to either ‘\whole\_fly’ or ‘\tissue\_specific’ folders for calculation of interest.
15. Run ‘A\_xxx.m’ to ‘F\_xxx.m’ in a alphabetical order. A series of running these manuscripts will enable calculation of V­max for whole fly or for individual tissues. The resulting figures shown in Figure 3 are created by running the ‘F\_xxx.m’ manuscript.

**5. Flux balance analysis**

1. Go to ‘\5\_fluxAnalysis’ folder
2. Script 1 and 2 are to prepare the data.
3. Run ‘script3\_fluxAnalz.m’ to perform flux analysis for pathways of interest.
4. Run ‘script4\_xxx.m’ to append the resulting flux balance analysis for all tissues. This will create a matrix with reactions and flux values for each tissue.
5. Run ‘script5\_1\_run\_heatmap\_subsys.m’ to create heatmap based for flux balance analysis results. The script neglects reactions with NaN values and show only those that contain at least some flux for at least one tissue.

**6. Flux variability analysis**

1. Go to ‘\6\_flux\_variability\_analysis’ folder
2. Run ‘A\_xxx.m’ to ‘B\_xxx.m’ for flux variability analysis of each individual model and update the reaction bounds to the models. Since flux variability analysis takes longer, it is recommended to use a web-based cloud server to run the codes, if applicable. Running flux variability analysis may take from 20 minute or longer, depending on computers.
3. Run ‘C\_xxx.m’ to graph the flux variability results. The code will generate horizontal bar plots for specific pathway of interest. Each bar graph is fixed with the reaction/enzyme and each rows represent the tissues.
4. Run ‘D\_1\_xxx.m’ to generate a summary of flux variability analysis and pathway variability analysis. The summary is written in ‘output.xlsx’ file. In ‘subsystem’ tab, the tissues are ranked based on the higher pathway flux variability (pfv) for each metabolic subsystem (columns title). The subsystem name can be found in ‘SubSysOrig’ tab. In the ‘score’ tab, the tissues with ranking are shown. The ranking in each array represents tissues found in ‘Subsystem’ tab. In ‘flux’ tab, the row pathway flux variability values are written for each tissue across different metabolic subsystems. In ‘scoreSum’ tab, all the scores across metabolic subsystems for each tissues are summed. The lower the score, the higher pathway flux variability ranking the tissues made. In ‘FVS’ tab, the normalized pfv (across the tissues) is shown. In ‘tissues’ tab, the tissue information can be found.
5. Run ‘D\_2\_xxx.m’ to make the heatmap for pathway flux variability results.

**7. Flux analysis for high sugar diet**

1. Go to ‘\7\_flux\_analysis\_high\_sugar\_diet’ folder.
2. Run ‘A\_xxx.m’ to ‘B\_xxx.m’ for flux balance and variability analysis. This will allow muscle-GEM to perform flux balance analysis based on constraints made to represent normal sugar diet and high sugar diet.
3. Run ‘C\_xxx\_NSD.m’ and ‘C\_xxx\_HSD.m’ to perform flux sampling analysis for each NSD and HSD models made from 2. These will create random sampling results for 10,000 runs.
4. Run ‘D\_xxx\_analysis/m’ for flux analysis for pathway of interest. The results can be found in ‘\D\swarm’ folder. For instance, for glucose, genes, reactions, equations, median, interquartile ranges, max, min, 25 and 75 % percentile values can be found. The summary excel sheet describes the mean values based on random sampling analysis and the corresponding relative change values are found between NSD and HSD. the ctr represents NSD condition, while exp represents the HSD condition. These values were used for generating a manual flux map as shown in figure 5.i.
5. Run ‘D\_xxx\_ specificRxn.m’ to make histogram for specific reaction of interest. The results are found in ‘\D\swarm\_spec’ folder. This manuscript will also generate a summary excel sheet.
6. Run ‘E\_1\_xxx.m’ to run a pathway flux index analysis for HSD condition. The resulting excel file is found in ‘\E\_1\_PFI\_table’ folder. The summary excel sheet is similar to section 6.4. Instead of showing all tissues, the excel file compares the resulting PFI analysis for NSD and HSD. In ‘flux’ tab, the pathway flux index values are described.
7. Run ‘E\_2\_xxx\_PFI.m’ to determine the relative PFI between HSD and NSD conditions. the resulting relative PFI can be found in the ‘\E\_2\_PFI’ folder. The first column represents the tissues and the second column represents the relative PFI values sorted from low to high. The empty arrays represent no relPFI values. The resulting graph found in Fig 6.b is generated through this code.