**How to use FOCuS toolbox**

**Requirements**

1. COBRA Toolbox (<http://opencobra.sourceforge.net/openCOBRA/Welcome.html>)
2. A desktop computer having required hardware and software to run MATLAB
3. MATLAB (MathWorks, <http://www.mathworks.com/>)
4. libSBMLprogramming library (<http://www.sbml.org>)
5. SBMLToolbox for MATLAB
6. Gurobi Optimizer (<https://www.gurobi.com>)

**Steps to run FOCuS**

Install COBRA Toolbox

Follow the below link for instruction (As on 23rd February 2017)

http://benheavner.com/systemsbio/index.php?title=Installing\_COBRA\_toolbox\_for\_MATLAB

Change directory to Cobra in MATLAB

Unzip the FOCuS folder as a subfolder in Cobra

Read a stoichiometric model/genome-scale metabolic model (GSMM) (eg. iAF1260 or iMM904) in standard format using readCbModel command

**Preprocessing step**

After deploying the model, preprocessing is carried out using indexing () function.

Open the indexing function and modify the xlswrite file path (Line number: 133) as per the local directory and choice of xlsx file name

Example for acetate index file

xlswrite('C:\Users\....\MATLAB\cobra\FOCUS\acetate\_index.xlsx',index)

Example for indexing acetate flux maximization using iAF1260:

Syntax call for indexing () from MATLAB command prompt is >>

indexing(model,'Ec\_biomass\_iAF1260\_core\_59p81M','EX\_ac(e)',-10,-18.5,8.39)

Store the output index in an excel file inside the local directory.

Here the following file is shown as an example

acetate\_index.xlsx

**FOCuS Sectioning step**

After finishing the indexing for a specific metabolite to be maximized, the FOCuS\_Section () function is used for sectioning and reducing the search space.

First step is to change the xlsread file path (Line No: 68) to call the index file

Example for sectioning acetate index

ind=xlsread('C:\Users\....\MATLAB\cobra\test\_codes\FOCUS\acetate\_index.xlsx')

Syntax call for FOCuS\_Section () from MATLAB command prompt is >>

FOCuS\_Section(model\_in,'Ec\_biomass\_iAF1260\_core\_59p81M','EX\_ac(e)',-10,-18.5,8.39,0.1)

Store the output reduce in an excel file inside the local directory.

xlswrite('C:\Users\...\MATLAB\cobra\FOCuS\acetate\_iter1.xlsx', reduced\_section)

This *acetate\_iter1* becomes the index file for second iteration which can be stored as *acetate\_iter2.xlsx* so on so forth until reasonable reduction in the size of GSMM is achieved.

**FOCuS Single Sectioning step**

After reducing the size of GSMM, the FOCuS\_Single\_Section () function is used to arrive at an optimal solution

First step is to change the xlsread file path (Line No: 61) to call the final search index file which is arrived from FOCuS\_Section () function

ind=xlsread('C:\Users\...\MATLAB\cobra\FOCUS\final\_search\_index.xlsx')

Syntax call for FOCuS\_Single\_Section () from MATLAB command prompt is >>

FOCuS\_Single\_Section(model\_in,'Ec\_biomass\_iAF1260\_core\_59p81M','EX\_ac(e)',-10,-18.5,8.39,0.1)

The output will be seen at the MATLAB command prompt after the successfully running this function.

Focus sectioning result can also be coupled to Optknock program as described in the manuscript.

The results for acetate from iAF1260 and tyrosine from iMM904 are provided for reference.

In case of iAF1260, a total of 6 iterations were carried as it is relatively big GSMM, whereas for tyrosine only 3 iterations were sufficient. The excel files shows the progression of size reduction for these metabolites using FOCuS\_Section () function.