ALTFLUXES COBRA TOOLBOX EXTENSION FOR FLUX VARIABILITY ANALYSIS OF STOICHIOMETRIC MODELS OF METABOLISM

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Abstract: *The size and number of available constraint-based genome scale metabolic reconstructions and models is rapidly growing. Flux Balance Analysis (FBA) is an important analysis for these models to investigate possible steady state conditions for particular constraints sets in the models. FBA is based on linear algebra and it returns only one feasible set of distribution of fluxes. In the model there is possible infinite number of unique solutions depending on constraints set in the model. The COBRA Toolbox is a powerful tool with expanding functionality for analysis of stoichiometric models. The COBRA Toolbox offers the function to calculate alternative flux rates for each reaction in the model, however there is a lack of visual representation of the obtained results. Therefore the AltFluxes extension for the COBRA Toolbox is developed to visualize the flux variability results in the graph. The AltFluxes represents the numerical results in more transparent structure in the MATLAB command window as well. Moreover, proposed function returns not only alternative fluxes for each reaction in the model, but the sets of the other fluxes of reactions in the extreme flux rates of corresponding reaction. The function AltFluxes is written in the MATLAB scripting language ensuring the cross-platform approach. It is an open source software under the GPL v3 license and available at http://www.biosystems.lv/altfluxes.*

Keywords: flux variability, COBRA Toolbox, stoichiometric model, FBA, MATLAB.

Introduction

There are several modeling approaches (Boolean networks (Lahdesmaki et al, 2006), Bayesian networks (Lahdesmaki et al, 2006) Time Petri Nets (Koch et al, 2005), Constraints-based stoichiometric models (Price et al, 2003), Dynamic models (Anesiadis et al, 2008), Neural models (Ławryńczuk, 2008) etc.) to investigate and simulate metabolism of biological organisms in silico. Each approach has advantages and disadvantages, and it serves for different purposes. Constraints-based stoichiometric models have got a high popularity in the field of metabolic engineering. Moreover metabolic network reconstructions have become an integral part of studying the metabolism of target organism (Thiele and Palsson, 2010). Constraint-based reconstruction and analysis approach is one of the beginning steps before dynamic modeling, where kinetic parameters are taken into account.

A rapidly growing and improving tool - the COBRA Toolbox (Schellenberger et al, 2011) is powerful software specially developed for wide range of simulation and analysis of the metabolic engineering tasks in the MATLAB environment (http://www.mathworks.com). There are already implemented a lot of algorithms to process on the constraint-based models for model debugging and analysis purposes (network gap filling, ^{13}C analysis, metabolic engineering, omics-guided analysis etc.). One of the key function in the metabolic engineering and so in the COBRA Toolbox is flux balance analysis (FBA) that shows if model is able to produce the products of interest according to objective function at steady state (Orth et al., 2010). The calculations are accomplished by linear programming and there are possible three result cases: (1) zero solutions, (2) one unique solution and (3) infinite number of feasible solutions, however FBA is able to return only one set of reactions fluxes. To check the uniqueness of distribution of fluxes it is necessary to perform flux variability analysis (FVA) which can find alternative solutions for the constrained solution space (Schellenberger et al, 2011).

Despite the wide functionality of the COBRA Toolbox, it still has a lack of user friendly interface that nowadays becomes to play a big role among almost any program user, especially for biologists. The *Paint4Net* toolbox is available for visualization of stoichiometric models of metabolism (Kostromins and Stalidzans, 2012). It is possible to see FBA results, however it does not visualize FVA results. Therefore the *AltFluxes* - extension for the COBRA Toolbox has been developed to deliver FVA functionality in combination with user friendly result representation and usage simplicity.

The *AltFluxes* returns FVA results in the MATLAB command window in form of four columns (Reaction name, Min flux, Max flux, Detailed view) and creates color graphs with flux rates of reactions. Furthermore the *AltFluxes* checks the model constraints and signalizes about potential mistake in case of equal lower and upper bound rates of a particular reaction that causes the limitation of solution space and could exclude the best FVA result.

Materials and methods

The COBRA Toolbox works in MATLAB that is already a long time one of the most popular engineering environments. A big advantage of MATLAB is platform independency, so the *AltFluxes* can be run on any platform where MATLAB is working. The *AltFluxes* is written in the MATLAB scripting language as open source software under the GPL v3 license.

The execution of the *AltFluxes* function is very easy with only two arguments: a stoichiometric model and a list of reactions for FVA, that could be as well as few or even all reactions that are present in the model. Before execution of the *AltFluxes* function it is necessary to set known constraints from literature and practical experiments or simulations in silico.

The first step *AltFluxes* do after execution is the reaction presence check, where it goes through the list of reactions of interest and checks if each reaction is present in the model. In case of absence of any reaction the calculations are stopped and informative message appears in the MATLAB command window. In case of uninterrupted calculation the *AltFluxes* stores all nonzero coefficients of the objective function in the temporary list and changes coefficients in the model to zeroes. The next step is the cycle through the list of reactions of interest, where the *AltFluxes* (1) changes the coefficient of the objective function of reaction of interest to one, (2) performs two optimizations with "min" and "max" flags of the COBRA Toolbox internal function *optimizeCbModel* and (3) set the coefficient of the objective function back to zero (see Fig.1).

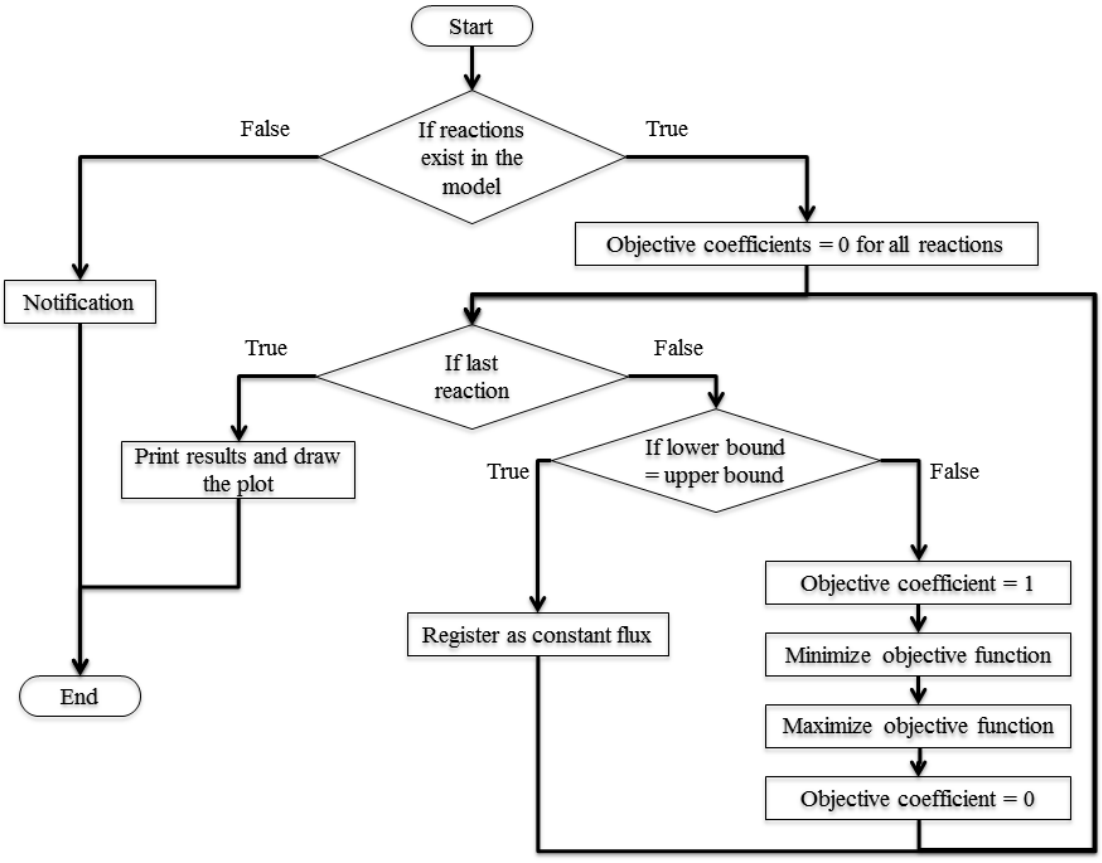


Fig. 1. **The algorithm of the *AltFluxes*.**

For those reactions where lower and upper bounds are equal the *AltFluxes* specially signalizes in the MATLAB command window as constant flux rates in the model (see Fig.2).

'R74'	[0]	[0]	{2x2 cell}
'R75'	'Const 0'	'Const 0'	[]
'R76'	[0]	[40]	{2x2 cell}
'R77'	[0]	[0]	{2x2 cell}
'R78'	[0]	[0]	{2x2 cell}
'R79'	[0]	[0]	{2x2 cell}
'R80'	[0]	[0]	{2x2 cell}
'R81'	[0]	[0]	{2x2 cell}
'R82'	'Const 0'	'Const 0'	[]
'R83'	[0]	[80]	{2x2 cell}
'R84'	[0]	[80]	{2x2 cell}
'R85'	[0]	[0]	{2x2 cell}
'R86'	[0]	[0]	{2x2 cell}
'R87'	[0]	[40]	{2x2 cell}
'R88'	[0]	[20]	{2x2 cell}
'R89'	[0]	[40]	{2x2 cell}
'R90'	[-40]	[0]	{2x2 cell}
'R91'	'Const 0'	'Const 0'	[]

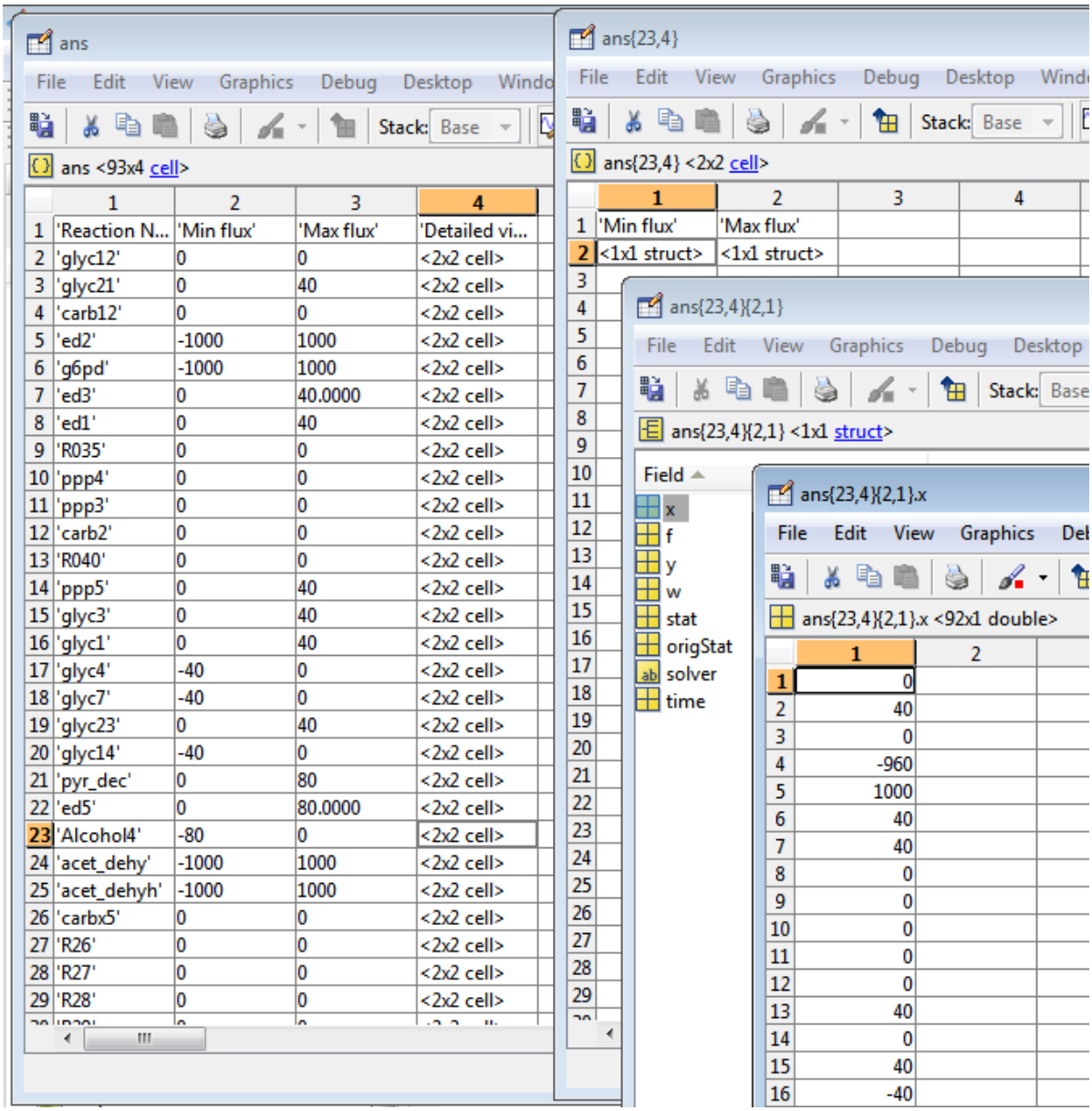
Fig. 2. **Fragment of the result of the *AltFluxes* function with constant flux rates.** Reactions *R75*, *R82* and *R91* are marked as constant with 0 flux rates set in the model.

The *AltFluxes* returns the FVA result in the MATLAB command window (see Fig. 2 and Fig. 3) and stores it in the MATLAB workspace as a variable for further analysis.

'Reaction Name'	'Min flux'	'Max flux'	'Detailed view'
'glyc12'	[0]	[0]	{2x2 cell}
'glyc21'	[0]	[40]	{2x2 cell}
'carb12'	[0]	[0]	{2x2 cell}
'ed2'	[-1000]	[1000]	{2x2 cell}
'g6pd'	[-1000]	[1000]	{2x2 cell}
'ed3'	[0]	[40.0000]	{2x2 cell}
'ed1'	[0]	[40]	{2x2 cell}
'R035'	[0]	[0]	{2x2 cell}
'ppp4'	[0]	[0]	{2x2 cell}
'ppp3'	[0]	[0]	{2x2 cell}
'carb2'	[0]	[0]	{2x2 cell}
'R040'	[0]	[0]	{2x2 cell}
'ppp5'	[0]	[40]	{2x2 cell}
'glyc3'	[0]	[40]	{2x2 cell}
'glyc1'	[0]	[40]	{2x2 cell}
'glyc4'	[-40]	[0]	{2x2 cell}
'glyc7'	[-40]	[0]	{2x2 cell}
'glyc23'	[0]	[40]	{2x2 cell}
'glyc14'	[-40]	[0]	{2x2 cell}
'pyr_dec'	[0]	[80]	{2x2 cell}
'ed5'	[0]	[80.0000]	{2x2 cell}
'Alcohol4'	[-80]	[0]	{2x2 cell}
'acet_dehy'	[-1000]	[1000]	{2x2 cell}
'acet_dehyh'	[-1000]	[1000]	{2x2 cell}

Fig. 3. **Fragment of the result of the *AltFluxes* function in the MATLAB command window.** The result is formed in four columns: Reaction Name, Min flux, Max flux and Detailed view.

The column "*Detailed view*" in the structure of the variable contains FBA results for extreme points (min and max) of every reaction of interest, however it can be again only one feasible flux distribution set from infinity (see Fig. 4).



	1	2	3	4
1	'Reaction N...	'Min flux'	'Max flux'	'Detailed vi...
2	'glyc12'	0	0	<2x2 cell>
3	'glyc21'	0	40	<2x2 cell>
4	'carb12'	0	0	<2x2 cell>
5	'ed2'	-1000	1000	<2x2 cell>
6	'g6pd'	-1000	1000	<2x2 cell>
7	'ed3'	0	40.0000	<2x2 cell>
8	'ed1'	0	40	<2x2 cell>
9	'R035'	0	0	<2x2 cell>
10	'ppp4'	0	0	<2x2 cell>
11	'ppp3'	0	0	<2x2 cell>
12	'carb2'	0	0	<2x2 cell>
13	'R040'	0	0	<2x2 cell>
14	'ppp5'	0	40	<2x2 cell>
15	'glyc3'	0	40	<2x2 cell>
16	'glyc1'	0	40	<2x2 cell>
17	'glyc4'	-40	0	<2x2 cell>
18	'glyc7'	-40	0	<2x2 cell>
19	'glyc23'	0	40	<2x2 cell>
20	'glyc14'	-40	0	<2x2 cell>
21	'pyr_dec'	0	80	<2x2 cell>
22	'ed5'	0	80.0000	<2x2 cell>
23	'Alcohol4'	-80	0	<2x2 cell>
24	'acet_dehy'	-1000	1000	<2x2 cell>
25	'acet_dehyh'	-1000	1000	<2x2 cell>
26	'carbx5'	0	0	<2x2 cell>
27	'R26'	0	0	<2x2 cell>
28	'R27'	0	0	<2x2 cell>
29	'R28'	0	0	<2x2 cell>

	1	2	3	4
1	'Min flux'	'Max flux'		
2	<1x1 struct>	<1x1 struct>		

	1	2
1	0	
2	40	
3	0	
4	-960	
5	1000	
6	40	
7	40	
8	0	
9	0	
10	0	
11	0	
12	0	
13	40	
14	0	
15	40	
16	-40	

Fig. 4. **The column "Detailed view" expanded for reaction *glyc12*.** Flux distribution for extreme value of "Min flux" is expanded.

In addition the *AltFluxes* generates the visual graphs where reactions are split by 30 per graph in case of large number of reactions of interest in the model. The example of *AltFluxes* visual results are shown in Figure 5, where the model of the central metabolism of bacteria Zymomonas mobilis is taken as study case. The spreadsheet file of the model can be found at http://www.biosystems.lv/altfluxes/sample_files.

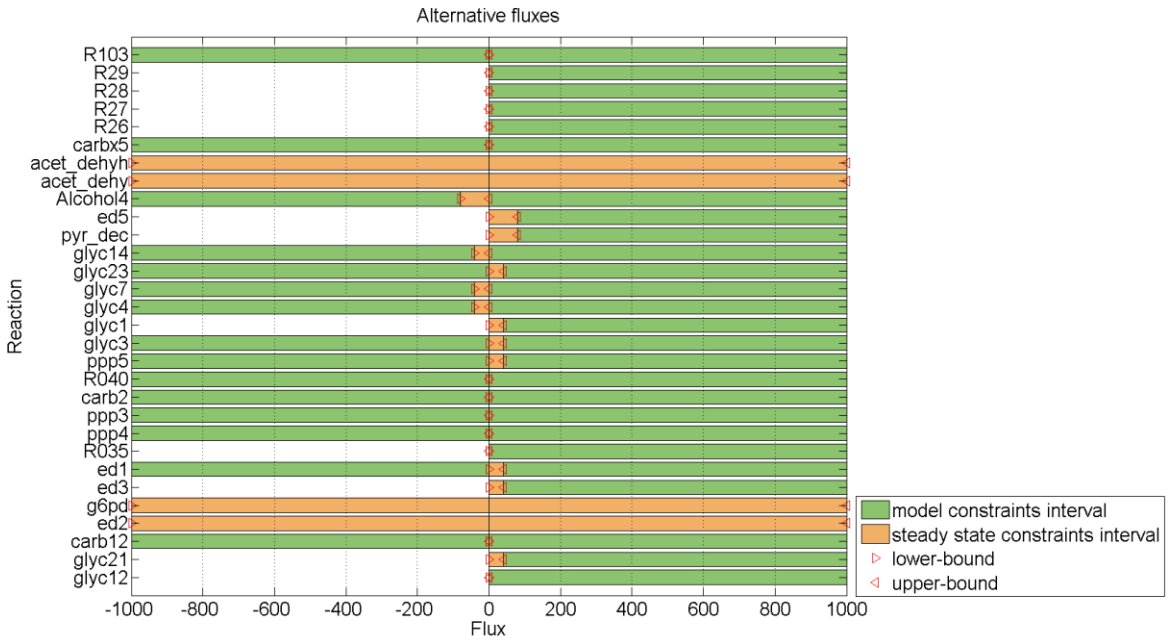


Fig. 5. **Part of the result of the *AltFluxes* function.** The first 30 of 92 total reactions in the model. The *AltFluxes* argument is set to perform FVA to all reaction in the model. The intervals of the constraints of the model are represented as green bars. The feasible steady state constraints intervals calculated by the *AltFluxes* function are represented as orange bars with triangles in extreme points of intervals.

Results and discussion

According to the *AltFluxes* the example model contains three constant flux reactions, where constraints are set to zero (see Fig. 2) that is definitely limiting the metabolism and possible solution space. However such constraints can be used to simulate gene knockouts (Orth et al., 2010) as well. Another case is constant nonzero flux rates. Although it can be a right approach for fixing particular flux rates of reactions of interest (Orth et al., 2010, supplementary tutorial), still it is worth to check if they are not a bottleneck reactions in the model.
Some reactions in the model can use tight interval or even single value for flux rates at steady state (see Fig. 5), but there are several reactions, which are running full speed as well. Later analysis has shown that clusters of high flux reactions actually are stoichiometrically balanced cycles (Thiele and Palsson, 2010), which are running even, when all substrates are cut down.
Useful information, which can be taken in to account in the future in the *AltFluxes*, is shadow prices and reduced costs (Orth et al., 2010, supplementary tutorial). Shadow prices are derivative of the objective function respecting to the exchange flux of a metabolite, and it shows how much the addition of particular metabolite will change the objective value. Reduced costs are the derivatives of the objective function respecting to an internal reaction with zero flux rate, showing how much each particular reaction affects the objective.

Conclusion

AltFluxes – the COBRA Toolbox extension for FVA of stoichiometric models of metabolism is developed. It is the open source product and it's functionality can be easily extended in the MATLAB scripting language. By default the COBRA Toolbox offers to perform FVA, but user must execute two separate functions (*fluxVariability*, *printLabeledData*) to get structured FVA results in the MATLAB command window, while the *AltFluxes* with it's function delivers FVA results not only in the MATLAB command window, but in graphical format as well in order to facilitate the analysis and debugging of the model of the target organism. In addition the *AltFluxes* returns the distribution of FBA fluxes for each extreme point of the reactions of interest. The *AltFluxes* functionality helps to identify potentially limiting reactions, where lower and upper bounds are equal in the model, by indicating them as constant rate reactions. Graphical representation of the constraints intervals of the model and FVA constraints intervals at steady state allows comparing them and determine potentially limiting flux bounds in the model as well as find stoichiometrically balances cycles running in the model without any substrates.

Acknowledgements

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References

Anesiadis, N., Cluett, W.R. and Mahadevan, R., 2008. Dynamic metabolic engineering for increasing bioprocess productivity. Metabolic engineering, 10(5), pp.255-66.

Koch I., Heiner M. and Popova-Zeugmann L., 2005. Time Petri Nets for Modelling and Analysis of Biochemical Networks, Fundamenta Informaticae, IOS Press, 67, pp.149–162.

Kostromins, A. and Stalidzans, E., 2012. Paint4Net: COBRA Toolbox extension for visualization of stoichiometric models of metabolism. BioSystems. http://dx.doi.org/10.1016/j.biosystems.2012.03.002

Lahdesmaki, H., Hautaniemi S., Shmulevich I. and Yli-Harja O., 2006. Relationships between probabilistic Boolean networks and dynamic Bayesian networks as models of gene regulatory networks, Signal Processing, Elsevier, 86, pp.814–834.

Ławryńczuk, M., 2008. Modelling and nonlinear predictive control of a yeast fermentation biochemical reactor using neural networks. Chemical Engineering Journal, 145(2), pp.290-307.

Orth, J.D., Thiele, I. and Palsson, B.Ø., 2010. What is flux balance analysis? Nature biotechnology, 28(3), pp.245-248.

Price N.D., Papin J.A., Schilling C.H. and Palsson B. Ø., 2003. Genome-scale microbial in silico models: the constraints-based approach, TRENDS in Biotechnology, Elsevier, Vol.21, No.4., pp.162-169.

Schellenberger, J., Que, R., Fleming, R.M.T., Thiele, I., Orth, J.D., Feist, A.M., Zielinski, D.C., Bordbar, A., Lewis, N.E., Rahmanian, S., Kang, J., Hyduke, D.R. and Palsson, B.Ø., 2011. Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0. Nature Protocols, 6(9), pp.1290-1307.

Thiele, I. and Palsson, B.Ø., 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. Nature protocols, 5(1), pp.93-121.