

USER MANUAL

1. File transformation from CNA format to other formats.

If you already have the file of reactions in a Cell Net Analyzer (CNA) format, then import this file to an Excel spreadsheet. To do this, use the following sequence of commands which will appear along successive windows, namely:

Data → Get External Data → From Text → look in (select directory and file to be imported) while using the option **“show all files “(*.*) → import → delimited option → next → Tab delimited (option) → “general format” option → “finish”**.

Once the Excel file was obtained, you have to manually delete the columns placed at the right of the symbol of end of reaction (|). That is, you have to retain only the first column (ID), and the second ones (Reactions). Save the file with the name you want, but in **xls format**. To use it, simply upload it to the web service on our site. You will recover immediately a file containing the following outputs:

- A map of interactions in Cytoscape, BiNom and Diva compatible formats.
- A map of interactions useful to be used with software M-finder.
- A list of equivalence between metabolites and numerical codes for M-Finder.

Important

Note that for the case of the software Cytoscape and Binom, the resulting output file can be used directly without further transformations.

For the case of the software Diva, first you must delete manually the central column corresponding to the interactions. Thus, the resulting file can be able to be run on-line at the website of the program (<http://www.bioinf.med.uni-goettingen.de/services/diva/>).

Regarding M-Finder program, keep in mind that this program only uses numeric codes instead of literal names, and also requires a specific ordering of the fields (source, target, type of interaction) (see for example Milo et al (2002) in <http://www.weizmann.ac.il/mcb/UriAlon/Papers/networkMotifs/sma.html>).

Hence, you must still perform two additional operations to be able of using the obtained output file with this program:

- a) First, select and cut the second column (interactions). Thereafter, copy it immediately to the right of the column of products. Remove now resulting empty column so that columns appear adjacent substrates and products.
- b) By using the commands “find” and “replace” of Excel, transform the column of interactions in a column of numerical codes. For example, it could be the following: activation = **1**, inhibition = **2**. If you are not interested in establishing the type of interaction, but only to declare its existence, you can put the entire column as 1.

For operational details of M-Finder program visit the site <http://www.weizmann.ac.il/mcb/UriAlon/groupNetworkMotifSW.html>.

2. Obtention of Cell Net Analyzer files by an alternative way

In our experience, the manual process of data entry into CellNet Analyzer is not very practical if you have to deal with a large network. For these cases we recommend the following procedure:

- a) Generate by the normal CNA procedure the file of “metabolites” but uniquely including there the compounds involved in the first reaction only.
- b) Generate by the CNA procedure the file of “reactions” that corresponds to the first reaction uniquely.
- c) Open the file of metabolites generated by using some editor such as MatLab, WordPad, or Block of Notes, and copy its content to an Excel spreadsheet that will be also called as metabolites.xls.
- d) Repeat the procedure in (c) with the generated file for the reactions, pasting it into another sheet Excel spreadsheet, called as reactions.xls.
- e) First, we will concentrate on completing the file of reactions. You have to type all model reactions (except the first) along successive rows of the Excel file. As shown in equation (1), each row of the Excel spreadsheet must respect the structure of fields required by CNA program, namely:

<reaction ID> <reaction equation |” <Par1><Par2><Par3>.....<Par9> (1)

Note that except for the first reaction, each field of the reactions should occupy a column of the Excel spreadsheet. From the bar indicating the end of reaction (|), you must specify nine fields or parameters (see Manual Net Cell Analyzer for details). For example, while the reactions themselves vary from row to row, the pattern of parameters would be practically the same, except when inputs are declared. For example, a typical model reaction in Excel could be written as:

R1 CdhE(gen) + CITED2(n) + !SNAIL(c) = CdhE(c) | # 0 0 0 100 100 1 3 1

- f) Once the reactions of the model have been written, you have to save your model in two formats, as **reactions.txt** (Tabulated) file, and as a **reactions.xls** file.
- g) The reactions.txt file can then be copied directly into the file of reactions generated within the CNA, which contained uniquely the first reaction. You have to save the complete file again as the file called as “**reactions**”, being located within the folder of CNA..
- h) On the other hand, the file called as **reactions.xls** can be uploaded to our web site. Immediately, you will recover a file containing several outputs. From them, you have to

inspect the list of compounds participants (first column of the table of equivalence between cell and M-Net Analyzer Finder).

i) From the web service output file copy the first column of the table metabolites equivalence in the "**metabolites.xls**". From there, complete the file following the field structure given by the manual of Cell Net Analyzer. For example, for the case showed above, the list of metabolites should be:

CdhE(gen)	CdhE(gen)	1	0	100	100	1	3
CITED2(n)	CITED2(n)	NaN	0	100	100	1	3
SNAIL(c)	SNAIL(c)	NaN	0	100	100	1	3
CdhE(c)	CdhE(c)	NaN	0	100	100	1	3

j) Finally, transform the Excel file "**metabolitos.xls**" in a tabulated text file called **metabolites.txt**. Copy the content of these last file within the file of metabolites previously generated from the Cell Net Analyzer, and save again as "metabolites" within the folder of CNA.

k) You now have all the files required to run your program logic simulation by Cell Net Analyzer. However, if you decide to apply multi-valued logic criteria with gradual inhibitions, you can go to the site of the **MUVAL program** (<http://calsys.informatik.uni-rostock.de/muval11/index.html>). In this site you are available tutorials and web facilities to implement these criteria in such a way that you can achieve these type of results by logic modeling but still using Cell Net Analyzer software.