

## ***OptiCon*** ***version 1.0***

### **Overview**

Complex diseases are caused by multiple deregulated pathways. To achieve an effective and lasting treatment, multiple pathways need to be targeted in combination. Current combination therapies are developed mainly based on targets of existing drugs, which only represent a small portion of the human proteome. For *de novo* identification of combinatorial therapeutic targets, systematic identification of synergistic key regulators of disease conditions represents an effective strategy. We introduce a network controllability-based method, OptiCon, to identify synergistic regulators as candidate targets for combination therapy. These regulators jointly exert maximal control over deregulated genes but minimal control over unperturbed genes in a disease. Therefore, modulating these regulators maximizes the likelihood of correcting gene deregulation and minimizes the likelihood of side effect. In a broader sense, OptiCon represents a general framework for systemic identification of synergistic regulators underlying a cellular state transition. OptiCon is implemented using MATLAB.

### **I. Input files**

OptiCon requires three tab-delimited text files as the input, “DScore.txt”, “GeneExpression.txt”, “RecurMutant\_entrez.txt”.

1) “**DScore.txt**” contains data in two tab-delimited columns. Each row includes a gene identifier (first column) and its corresponding p-value of differential expression (second column) under two conditions (e.g. diseased vs. healthy). The gene identifier is based on Entrez Gene ID with a prefix “En\_”. Duplicated gene identifiers are not allowed. Place this file in the “OptiCon/” directory.

“OptiCon/InputExample/DScore.txt” shows an example for lung cancer. The p-value was computed using RNA-Seq data generated from tumor tissues and matched normal tissues in 57 lung adenocarcinoma patients. P-value was adjusted for multiple testing using the method of Benjamini and Hochberg. RNA-Seq data were downloaded from the Genomic Data Commons (GDC, <https://gdc-portal.nci.nih.gov/>).

2) “**GeneExpression.txt**” contains data in a matrix format. Rows represent genes and columns represent samples. Each entry in the matrix contains a gene expression value in a specific sample. Duplicated gene identifiers are not allowed. Place this file into the “OptiCon/” directory.

“OptiCon/InputExample/GeneExpression.txt” shows an example of gene expression data in lung cancer. Data source is the same as above.

3) **“RecurMutant\_entrez.txt”** contains the list of genes (based on Entrez gene IDs) that are known to harbor recurrent somatic mutations in the cancer type of interest. In the example file, the mutation data was obtained from the Catalogue of Somatic Mutations in Cancer (COSMIC) database. Duplicated gene identifiers are not allowed. Place this file in the “OptiCon/output/” directory. “OptiCon/InputExample/RecurMutant\_entrez.txt” shows an example for lung cancer.

## II. Specifying the absolute paths of MATLAB and Python programs

In the five files below, specify the absolute paths of executable MATLAB and Python programs in your linux cluster.

- 1) “OptiCon/GreedySearch\_input.sh”
- 2) “OptiCon/gen\_m\_sh\_qsubFiles.py”
- 3) “OptiCon/output/OptiCon\_output.sh”
- 4) “OptiCon/output/Gen\_SynScoNull.sh”
- 5) “OptiCon/output/gen\_m\_sh\_qsubFiles\_randNull.py”

## III. Running OptiCon

1) Copy the “OptiCon/” directory to your working space and set the current working directory to “/Your/path/OptiCon/”. Run the bash script “GreedySearch\_input.sh”. It is recommended to use the “qsub” command on a linux cluster.

Outputs: MAT-files, MATLAB scripts and bash scripts necessary for Step 2.

(Tips: Before running this step, please change the value of "e (epsilon)" in the "comp\_w.m" file to a Pearson Correlation Coefficient value that corresponds to a two-tailed p-value of 0.05 based on the total number of gene expression samples. A useful link for this computation:

<http://www.danielsoper.com/statcalc/calculator.aspx?id=44>)

2) Set the current working directory to “/Your/path/OptiCon/” and run the bash script “QSUB.sh” that is generated by Step 1 (do NOT use "qsub").

Outputs: MAT-files necessary for following steps.

(Tips: Hundreds of jobs are submitted to your linux cluster in this step and each job will generate a MAT-file “finTherapTar\_relax\*.mat”.)

3) Set the current working directory to “/Your/path/OptiCon/output/” and run the bash script “Gen\_SynScoNull.sh” (do NOT use "qsub") to generate a null distribution

of synergy scores based on 10 million randomly selected gene pairs from the gene regulatory network.

Outputs: 200 “randSynScore\*.mat” files, which collectively constitute a null distribution of synergy scores that will be used to calculate empirical p-values for identified synergistic gene pairs in Step 4.

(Tips: 200 jobs are submitted to your HPC cluster in this step and each job will generate a MAT-file “randSynScore\*.mat”.)

4) Set the current working directory to “/Your/path/OptiCon/output/” and run (or “qsub”, recommended) the bash script “OptiCon\_output.sh”.

## IV. Output

The output file, “OCN\_pairs.txt”, contains a list of optimal control node pairs (i.e. synergistic key regulators) ranked based on their synergy scores. Original empirical p-values and multiple-testing-corrected p-values are also included.

## Appendix

1000 structural control configurations (SCCs) of our constructed gene regulatory network are provided in the “OptiCon/” folder (CF\_\*.mat). If you want to identify synergistic key regulators using a customized directed network, files in the OptiCon/Gen\_SCCs/ can be used to generate a given number of SCCs of your customized network.

- 1) Format your network data into a tab-delimited file “MyGeneNetwork.txt”. Each row represents a directed edge from the node in the first column to the node in the second column.
- 2) Specify the number of SCCs you want to generate in the Row 7 of the file “gen\_diffSCCs.cpp”.
- 3) Pre-define the maximum number of nodes in your customized network in the Row 9 of the file “gen\_diffSCCs.cpp”.
- 4) In the file “OptiCon/Gen\_SCCs/gen\_SCCs.sh”, specify the absolute path of executable MATLAB program in your linux cluster.
- 5) Set the current working directory to “Your/path/OptiCon/Gen\_SCCs/” and run (or “qsub”, recommended) the bash script “gen\_SCCs.sh”.
- 6) Run the OptiCon using the four steps described above.

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