

# SEMIPs: Structural Equation Modeling of In silico Perturbations

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## Abstract

**Summary:** Structural Equation Modeling (SEM) is a statistical approach for studying complex cause-effect hypotheses in a “closed system” of latent (hidden) endogenous variables. SEM has been widely used in various fields involving perturbations and measurable outcomes. We developed an R Shiny application, termed “Structural Equation Modeling of In silico Perturbations (SEMIPs)” to aid in the transfer of perturbations in gene expression pathways from one system to another for determining casual inference of molecular interactions *in silico*. SEMIPs computes a two-sided t-statistic (T score) to rank signature gene activities for modeling. It implements a basic SEM model and then performs bootstrap random sampling for statistical significance. As a use case example for SEMIPs, we showed that putative direct downstream genes of the GATA2 transcription factor are sufficient to infer GATA2’s activities *in silico* for the conserved PGR-GATA2-SOX17 genetic network in the human uterine endometrium.

**Availability and implementation:** The SEMIPs Shiny app and source code are freely available at <https://github.com/NIEHS/SEMIPs> under the MIT license. SEMIPs is developed in R.

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**Supplementary information:** Supplementary data are available at *Bioinformatics* online.

## Introduction

Although gene expression data in public repositories provide a valuable resource for investigators to infer regulatory processes (Edgar et al. 2002), the variables of interest are not always directly measurable in a causal response model system. Moreover, it is challenging to test the knowledge obtained from experimental model systems in humans due to undetermined clinical outcomes and ethical considerations. Structural equation modeling (SEM) offers a statistical framework to make casual inferences about the causality of latent (hidden) endogenous variables in a system (Grace 2006). We were motivated to develop a Structural Equation Modeling of In silico Perturbations (SEMIPs) Shiny application to facilitate casual inference from *in silico* alterations of gene expression pathways. SEMIPs enables quantification of a projected activity metric (two-sided t-statistic, i.e. T score) calculated from gene expression activity upon exposure to a perturbation (Wu et al. 2015), thus allowing users to fit desired SEM models using selected endogenous and exogenous variables. This application also provides two different bootstrap random sampling procedures (elimination with or without replacement) for testing the significance of a model based a non-parametric distribution.

Previously, SEM was applied to gene expression data to evaluate an alteration of latent gene interactions that disrupts the progesterone receptor pathway in the uterus of pregnant mice and the model was then transferred *in silico* to a human reproductive system (Rubel et al. 2016). SEMIPs streamlines this process and allows bench scientists to perform the computations and analysis through a user-friendly interface.

## Implementation and usage

SEMIPs was written in R with the Shiny package (Rstudio 2014) that is known for its light weight web development framework with shiny-related features. The lavaan package (Rosseel 2018) was used for the SEM. The application requires modern multicore CPUs for the backend parallel processes. SEMIPs was developed under Linux CentOS7 and has been successfully tested on MacOS (v. 10.14.6) and Windows10. To install and run this application, users can follow the detailed instructions provided in the README.txt file.

As shown in Figure 1A, the SEMIPs workflow depicts a biological question initially tested in an animal model and then applied to a human system. Based on the SEM model, a presumed relationship can be tested in humans by determining the significance of the inference via a non-parametric bootstrap resampling framework. The resulting perturbed pathways can be eventually tested in the animal model. These workflow steps are shown within the dotted rectangle on the right side of Figure 1A.

The SEMIPs Shiny application has three main features. The first feature allows users to quantify the projected “regulator activity” of the gene of interest from a study in the form of T scores (Wu, S.P. et al 2015, Liu et al. 2019, Wetendorf et al. 2020). The “T Scores” tab (Figure. 1B) was designed in the main panel to conduct such an analysis. It requires two components: (1) A list of gene signature (in Entrez gene symbol format) obtained from a study of interest; and (2) A gene expression data matrix that consists of gene expression profiles in a given context. The application will conduct the analysis and produce inferred activity results reflected as T scores that can be used in subsequent downstream analyses. The second feature (the SEM tab) provides users a convenient SEM model fitting interface with the T scores. The users can hypothesize a 3-

node structural equation model by selecting the desired endogenous and exogenous variables. The tool reports model fitting statistics in a compressed (zipped) file. This feature also allows users to test a separate system by uploading their relevant dataset. The dataset requires the same format as the example data. The third feature (the bootstrap tab) assesses the potential impact from a perturbation on any downstream system. We implemented a two-class (elimination with or without replacement) bootstrap resampling for statistical inference, which eliminates unrelated signatures and provides statistical significance to the SEM fitting. For this feature, it is assumed that the users have successfully run a T score analysis. The users also need to enter the signatures associated with the downstream system of interest to evaluate. To improve the rigor of the statistical test, it is recommended to run the bootstrap a minimum of 1,000 times.

The application provides a user-friendly interface (Fig. 1B) with special features that are provided as separate tabs. The data needs to be in the same format as shown under the “Instructions” tab. Further details for running the application are provided under the “Instructions” tab.

## **Case study**

With the 634 putative GATA2 direct downstream genes, SEMIPs provided evidence to infer GATA2’s activities that permitted modeling fitting of the PGR-GATA2-SOX17 genetic network in the human endometrium (See the Supplemental information for details).

## **Conclusion**

The SEMIPs R Shiny app offers an easy to use *in silico* perturbation testing system with several advantages. First, it has capability to calculate response activities using large datasets representative of biological systems. Second, it leverages the power of SEM to test the

relationship among end points in a study and provides users with the flexibility for testing new hypotheses. Lastly, it integrates a non-parametric testing procedure for assessing statistical significance.

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