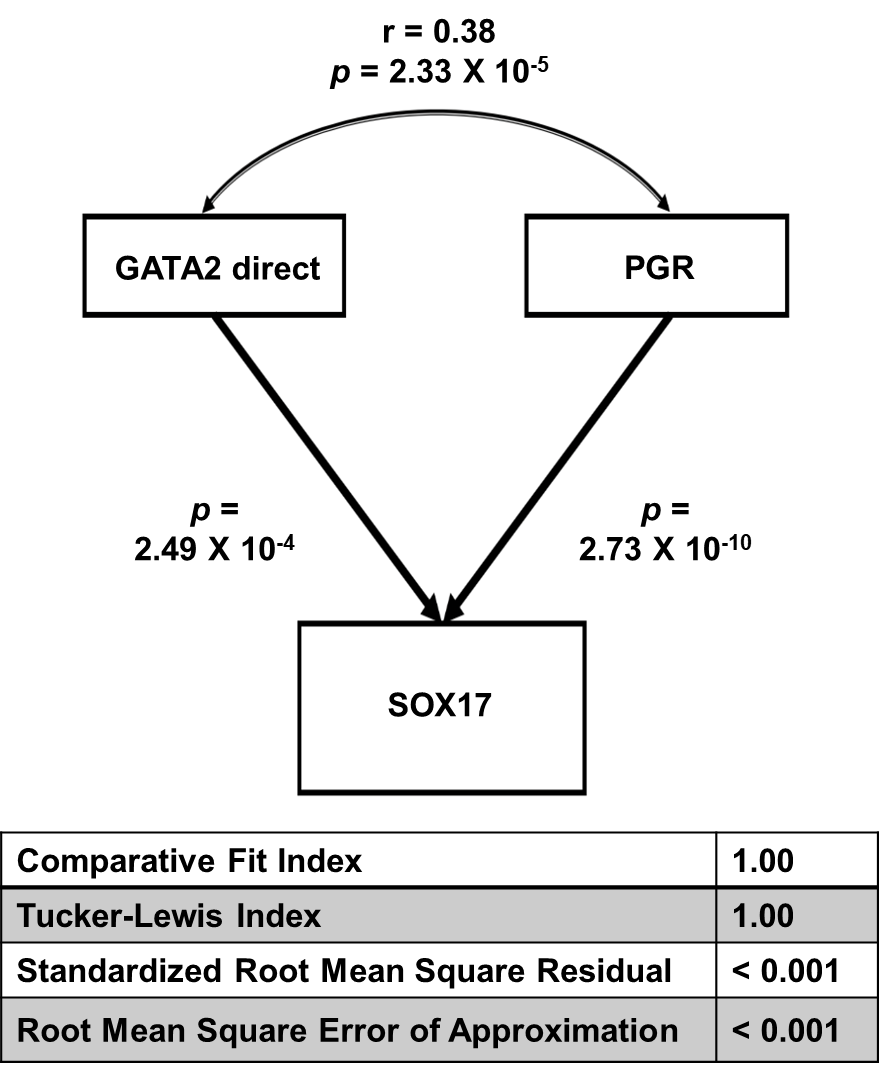
**SEMIPs: Structural Equation Modeling of In silico Perturbations**

**Overview**

This SEMIPs RShiny App allows the user to compute a two-sided t-statistic (T score) from gene expression data to infer the activities of genes of interest in a quantitative manner. This app also provides a 3-node model fitting function using the structural equation modeling to test the joint regulation of a target gene by two upstream regulators in a given data matrix *in silico*. In addition, for the hypothesis generation purpose, a two-way bootstrap method, elimination with replacement or elimination without replacement, is included in the app to examine the impact of removing genes that belong to the same signaling cascade from the downstream targets of the gene of interest. As an example, here we applied SEMIPs to evaluate latent gene interactions that mediate the progesterone signaling in the uterus for female fertility.

**A use case on T-score calculation**

Previously we demonstrated that the mouse gene signatures of GATA2 and PGR allows inference of the interaction between GATA2 and PGR for regulation of SOX17 expression in the human endometrial tissues (Rubel, C.A. 2016). The full GATA2 gene signature consists of both direct and indirect downstream genes of GATA2 in the uterus (Rubel, C.A. 2016). Since GATA2 is known as a transcription factor that occupies cis-acting elements and confers genomic actions, we hypothesize that expression levels of GATA2’s direct downstream targets reflect its activities *in silico.* Here the GATA2 direct downstream target is defined as a GATA2 regulated gene with GATA2 genome occupancy within 2-kilobase vicinity of the said gene’s transcription start site in the uterus (NCBI: GSE40659, Rubel, C.A. 2016). This stringent criterion identified 634 genes (Supplemental Table A), which is termed “GATA2 direct signature”. The GATA2 activity, as represented by the GATA2 direct signature in a T-score, was quantified by the SEMIPs app from gene expression data of the endometrium tissue for each individual human subject (NCBI: GSE58144, PMID: 26797113). T-scores for the uterine GATA2 in all 115 patients were calculated by the app with the GATA2 direct signature and the data matrix of NCBI: GSE58144 (Supplemental Table B). Similarly, T-scores for the uterine PGR (termed PGR signature) were obtained using the NCBI: GSE39920 dataset (Rubel, C.A. 2016) on the same data matrix via the application’s the T-score calculation function. To test whether the GATA2 direct signature allows model fitting of the 3-node PGR-GATA2-SOX17 genetic network, the application was fed with T-scores of GATA2 direct signature and PGR signature as exogenous variables and the SOX17 expression levels as the endogenous variable under the “SEM” function. The output data shows that, with GATA2 direct signature in place of the full gene signature, model significantly fits the NCBI: GSE58144 dataset with all proposed paths (Supplemental Figure A) and this model is considered not rejected by the human data. This finding suggests that the expression levels of GATA2 direct downstream targets, a subset of the full GATA2 regulated genes, can mathematically serve as surrogate reporters of the GATA2 activities in the human endometrium tissues, which supports our hypothesis. Results of this analysis not only reduce the number of reporter genes for GATA2 activities to 634, but also implicates possibilities of a further reduction with additional filtering criteria on the gene list. A small and manageable panel of markers for GATA2 activities could serve as future diagnostic tool for pregnancy failure (PMID: 20619403).



**Supplemental Figure A**. model fit statistics for joint regulation of the SOX17 gene expression levels by GATA2 and PGR activities in the NCBI: GSE58144 dataset using SEM. “GATA2 direct” depicts GATA2 activities that were derived from the GATA2 direct downstream targets.

Another feature of this app is a framework for hypothesis generation beyond simple model fitting. Under the Bootstrap tab of this app, we implemented a two-class (elimination with or without replacement) bootstrap resampling simulation for statistical inference (Supplemental Figure B). The overall concept is illustrated in the figure 1 by the left most four rectangles. The idea is that the model fitting results would be altered if a subset of genes that has a significant role in the genetic network is removed from the gene signature. Results of this function may aid prioritizing subsequent wet bench tests.

Diagram

Description automatically generated

**Supplemental Figure B**. A two-class (elimination with or without replacement) bootstrap resampling simulation. From the initial GATA2 significant gene list in the yellow rectangle, a same number of genes as that of the subset of genes (represented by the white oval shape inside the yellow rectangle) are eliminated either without replacement (left side) or with replacement of same number of “genes other than those in the subset” (right side). The resulting shrunk GATA2 or shrunk GATA2 and restored by the same number of irrelevant genes were tested in the SEM model. The simulation can be repeated for “number of bootstraps” to provide non-parametric empirical distribution for statistics testing.

**Future directions**

Although the SEMIPs web-application offers user-friendly *in silico* perturbation testing system with many useful features, it does have limitations. For example, there are more options in designing the Structural Equation Model to count for more variables or to include some hidden variables in order to solve more complicated hypotheses, these features are yet to be implemented in the future releases. Another R package -- MplusAutomation focuses on automating the SEM modeling currently done via a commercial Mplus software (Hallquist, M. et al, 2018) will be additional resource for us to implement more complicated model in an automated fashion. In addition, the two-class bootstrap analysis can only be conducted separately, integration of these into the SEMIPs methodology for formulation into a single test will be investigated for future design, development and implementation. As noted in the manuscript and mentioned previously, the SEMIPs app has been adopted by researchers in the field with a few papers published recently. We hope that it can serve a wider research community to address additional scientific questions.

**Supplemental Methods**

**Prepare the gene list**

The microarray gene expression experiment data was analyzed using The Partek Genomics Suite 7.17 software (Partek Inc., St. Louis, MO). The Robust Multichip Analysis (RMA) algorithm with quantile for normalization and log2 transformation was applied to generate signal values of all samples. The one-way ANOVA model was used to compare expression profiles from different groups. Differentially expressed genes (DGE) were defined using the filters of ANOVA unadjusted p value < 0.01 and absolute fold change >1.3. Putative GATA2 direct downstream genes are identified by HOMER (PMID: 20513432).

**The main steps to test the use case**

**Step 1.** To get the T-score. Users can launch the App and import the 634 genes list (Supplemental Table A) and HumanArray4Shiny comes with the App. Click the green “Go” button, the corresponding Tscore will be calculated and can be download (shown in Supplemental Figure T). We also provided this calculated Tscore in Supplemental Table Q.

**Step 2.** To construct the dataset. Users need to open the \_sampleDAT.txt under the “app\_installation\_dir/dataSEM/”, i.e. /Users/li11/myGit/SEMIPs/dataSEM, and append the new Tscore column from step 1 and name the header accordingly, we use “GATA2 Direct” in this use case. Please save the new file as “app\_installation\_dir/dataSEM/sampleDAT.txt”.

**Step 3.**  To run the SEM model. Users need to re-launch the app, under the SEM tab, select from the drop down list, select “GATA2 Direct”, “PGR\_act\_FC13\_P01”,and “SOX17\_lev” as show in Supplemental Figure U. Then the structural equation model will be fed accordingly. User can download the 3-node SEM image as well as the model fitting details in Supplemental Figure R.

Graphical user interface, table

Description automatically generated

**Supplemental Figure C**. An illustration to use the App to calculate T-score for Supplemental Table A.

Graphical user interface, text, application

Description automatically generated

**Supplemental Figure D**. An illustration to use the App to fit the structural equation model for Supplemental Table Q (GATA2 direct gene list). The fitting statistics can be downloaded by clicking the “Download Zip” button.

**References**

Hallquist, M. N. & Wiley, J. F. (2018). MplusAutomation: An R Package for Facilitating Large-Scale Latent Variable Analyses in Mplus Structural Equation Modeling, 1-18. doi: 10.1080/10705511.2017.1402334.

Liu, J., et al. (2019). "JNK(1/2) represses Lkb(1)-deficiency-induced lung squamous cell carcinoma progression." Nat Commun **10**(1): 2148.

Rubel, C. A., et al. (2016). "A Gata2-Dependent Transcription Network Regulates Uterine Progesterone Responsiveness and Endometrial Function." Cell Rep **17**(5): 1414-1425.

Wetendorf, M., et al. (2020). "Constitutive expression of progesterone receptor isoforms promotes the development of hormone-dependent ovarian neoplasms." Sci Signal **13**(652).