Used parameters for method application on medium and late TE stage discrimination

Here, we provide more information about the parameters used for each step of the method to discriminate the medium and late trophectoderm stages in our paper.

1 PKN reconstruction

We used 438 transcription factor (TF) genes involved in human embryonic development as input for pyBRAvo software to reconstruct a PKN. These TF genes were identified through SCENIC [1] analysis of scRNAseq data, and their list can be found on the GitHub repository. Queries were made on Pathway Commons v.13 [2], excluding miRTarBase, MSigDB, and CTD databases to remove miRNA and toxicogenomics interactions. The exploration depth parameter was fixed to 2, *i.e.* up to 2 levels upstream of the initial TFs. Only gene transcription events were queried, yielding a PKN of 327 nodes and 475 edges, with only 28 of the 438 initial TFs found in the database (see supplementary material on our GitHub repository). We then reduced the network to 191 nodes (84 input genes, 27 intermediate genes, 14 readout genes, and 66 complexes) and 285 edges, limited to genes measured in scRNAseq data and complexes linked to these genes.

2 Experimental design construction

To generate the pseudo-perturbations, we fix the k value to 10. We ran the program on a computer cluster comprising 160 CPUs and 1.5 To of RAM for 65 hours to obtain 20 pseudo-perturbations.

3 BNs inference

We used the generated experimental design combined with the reduced PKN to infer BNs specific to medium and late TE using the Caspo software. Caspo proposes BNs that match the PKN topology and have an optimal (minimal) mean square error (MSE) between the Boolean prediction of readout nodes (given the Boolean input states) and their experimental measurement.

To restrict the number of Boolean functions of type "AND" that a node can receive to 2, we set (i) $length = 2$, and to fix the optimal MSE fitness tolerance, we set (ii) $fit = 0.001$. Essentially, larger fitness values result in further exploration from the optimal BN.

References

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[2] Rodchenkov, I., Babur, O., Luna, A., Aksoy, B.A., Wong, J.V., Fong, D., Franz, M., Siper, M.C., Cheung, M., Wrana, M., Mistry, H., Mosier, L., Dlin, J., Wen, Q., O'Callaghan, C., Li, W., Elder, G., Smith, P.T., Dallago, C., Cerami, E., Gross, B., Dogrusoz, U., Demir, E., Bader, G.D., Sander, C.: Pathway Commons 2019 Update: integration, analysis and exploration of pathway data. Nucleic Acids Research **48**(D1), D489–D497 (10 2019)