# Supplementary materials for GROD

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# Supplementary Tables

**Supplementary Table 1.** Summary of ground truth GRN resources used in this study.

|  |  |  |
| --- | --- | --- |
| Ground Truth | Source | Link |
| STRING | STRING | https://string-db.org/ |
| Cell-type Non specific ChIP-seq | TRRUST | https://www.grnpedia.org/trrust/ |
| RegNetwork | http://www.regnetworkweb.org/ |
| DoRothEA | https://saezlab.github.io/dorothea/index.html |
| Cell type specific ChIP-seq | ChIP-Atlas | https://chip-atlas.org/peak\_browser |
| ESCAPE | http://www.maayanlab.net/ESCAPE/download.php |
| ChEA | https://maayanlab.cloud/Harmonizome/dataset/CHEA+Transcription+Factor+Targets |
| hTFtarget | hTFtarget | http://bioinfo.life.hust.edu.cn/hTFtarget#!/ |

**Supplementary Table 2.** Summary of the scRNA-seq datasets used in this GRN inference.

|  |  |  |  |
| --- | --- | --- | --- |
| Dataset | # of cells | # of genes | GEO |
| Human embryonic stem cells (hESC)[1] | 759 | 17735 | GSE75748 |
| Human mature hepatocytes (hHep)[2] | 426 | 11515 | GSE81252 |
| Mouse embryonic stem cells (mESC)[3] | 422 | 18385 | GSE98664 |
| Erythroid lineages mouse hematopoietic stem cells (mHSC-E)[4] | 1072 | 4762 | GSE81682 |
| Lymphoid lineages mouse hematopoietic stem cells (mHSC-L)[4] | 848 | 4762 | GSE81682 |
| Granulocyte-macrophage lineages mouse hematopoietic stem cells (mHSC-GM)[4] | 890 | 4762 | GSE81682 |
| Mouse dendritic cells (mDC)[5] | 384 | 7371 | GSE48968 |

**Supplementary Table 3.** Summary of GRN inference results for all the methods in terms of AUROC.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | mESC | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GROD | **0.5553** | 0.5104 | 0.5795 | **0.5679** | 0.5177 | **0.6072** | **0.5725** | 0.5029 | **0.5964** |
| DeepSEM | 0.5531 | 0.4983 | **0.5856** | 0.5553 | 0.5043 | 0.5894 | 0.5393 | 0.5150 | 0.5783 |
| NGM | 0.5003 | 0.5001 | 0.5002 | 0.5001 | 0.5001 | 0.5005 | 0.5001 | 0.5000 | 0.5000 |
| CUTS | 0.4971 | 0.5011 | 0.4982 | 0.5017 | 0.5001 | 0.5001 | 0.4802 | 0.4859 | 0.5059 |
| LEAP | 0.5069 | 0.5089 | 0.4942 | 0.5029 | 0.5068 | 0.4910 | 0.5002 | 0.3808 | 0.5671 |
| PIDC | 0.5427 | 0.4998 | 0.5663 | 0.5431 | 0.5014 | 0.5720 | 0.5400 | 0.4177 | 0.5654 |
| SINCERITIES | 0.5006 | **0.5219** | 0.5277 | 0.4991 | 0.5202 | 0.5271 | 0.4727 | **0.5184** | 0.4732 |
| SCRIBE | 0.5224 | 0.5218 | 0.5433 | 0.5176 | **0.5268** | 0.5352 | 0.4794 | 0.4872 | 0.4825 |
| SCODE | 0.5342 | 0.4972 | 0.5435 | 0.5288 | 0.5033 | 0.5370 | 0.5244 | 0.4287 | 0.5517 |
| GRNBOOST2 | 0.5408 | 0.5181 | 0.5733 | 0.5408 | 0.5190 | 0.5680 | 0.5536 | 0.4961 | 0.5872 |
| SINGE | 0.4978 | 0.4970 | 0.4977 | 0.4976 | 0.4968 | 0.4969 | 0.5015 | 0.4992 | 0.4981 |
|  | mHSC-E | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GROD | **0.6192** | **0.5287** | **0.6961** | 0.6215 | **0.5458** | **0.7112** | 0.6057 | 0.6520 | **0.7700** |
| DeepSEM | 0.6130 | 0.5092 | 0.6731 | 0.5940 | 0.5317 | 0.6490 | 0.5841 | **0.6627** | 0.6805 |
| NGM | 0.5351 | 0.5007 | 0.5844 | 0.5447 | 0.5035 | 0.5995 | 0.5334 | 0.4802 | 0.5627 |
| CUTS | 0.5131 | 0.4982 | 0.5123 | 0.5234 | 0.4978 | 0.5192 | 0.5495 | 0.4747 | 0.5557 |
| LEAP | 0.6184 | 0.5222 | 0.6087 | **0.6361** | 0.4893 | 0.6076 | **0.6495** | 0.4419 | 0.6880 |
| PIDC | 0.6040 | 0.4769 | 0.6806 | 0.6118 | 0.4672 | 0.6892 | 0.6170 | 0.4308 | 0.7500 |
| SINCERITIES | 0.4909 | 0.5149 | 0.4544 | 0.4932 | 0.5190 | 0.4301 | 0.4963 | 0.5720 | 0.4230 |
| SCRIBE | 0.5585 | 0.5209 | 0.5886 | 0.5677 | 0.5119 | 0.5890 | 0.5710 | 0.4871 | 0.5981 |
| SCODE | 0.5060 | 0.4921 | 0.5296 | 0.5204 | 0.5285 | 0.5099 | 0.4729 | 0.5396 | 0.4296 |
| GRNBOOST2 | 0.6049 | 0.5216 | 0.6472 | 0.5869 | 0.5036 | 0.6393 | 0.5754 | 0.4912 | 0.6700 |
| SINGE | 0.4621 | 0.4689 | 0.4619 | 0.4809 | 0.4850 | 0.4786 | 0.4674 | 0.5039 | 0.4638 |
|  | mHSC-L | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GROD | 0.5922 | 0.5370 | 0.6936 | 0.5832 | 0.5362 | **0.7030** |  |  |  |
| DeepSEM | 0.5755 | 0.5493 | 0.6676 | 0.5989 | 0.5666 | 0.6716 |  |  |  |
| NGM | 0.5288 | 0.4829 | 0.6441 | 0.5034 | 0.5064 | 0.5665 |  |  |  |
| CUTS | 0.5064 | 0.4971 | 0.6125 | 0.4912 | 0.5008 | 0.5293 |  |  |  |
| LEAP | 0.5512 | **0.5705** | 0.5938 | 0.5504 | **0.5822** | 0.6010 |  |  |  |
| PIDC | **0.6027** | 0.4769 | 0.6556 | **0.6028** | 0.4906 | 0.6731 |  |  |  |
| SINCERITIES | 0.4803 | 0.5479 | 0.4749 | 0.4813 | 0.5424 | 0.4749 |  |  |  |
| SCRIBE | 0.5400 | 0.5125 | 0.5701 | 0.5495 | 0.5287 | 0.5754 |  |  |  |
| SCODE | 0.5185 | 0.4746 | 0.5430 | 0.5159 | 0.4880 | 0.5378 |  |  |  |
| GRNBOOST2 | 0.5948 | 0.5071 | **0.6937** | 0.5679 | 0.5235 | 0.6728 |  |  |  |
| SINGE | 0.4849 | 0.4855 | 0.4887 | 0.4745 | 0.4940 | 0.4998 |  |  |  |
|  | mHSC-GM | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GRODE | **0.6513** | **0.5432** | 0.7302 | 0.6340 | 0.5115 | 0.7277 | 0.6490 | 0.5197 | 0.7726 |
| DeepSEM | 0.6447 | 0.5225 | 0.7285 | 0.6046 | **0.5354** | 0.6979 | 0.6322 | **0.6125** | 0.7214 |
| NGM | 0.5977 | 0.5070 | 0.6502 | 0.5892 | 0.5062 | 0.6672 | 0.6222 | 0.5067 | 0.6741 |
| CUTS | 0.4980 | 0.4951 | 0.5048 | 0.4966 | 0.4929 | 0.5222 | 0.5443 | 0.4801 | 0.5554 |
| LEAP | 0.6383 | 0.4755 | 0.6553 | **0.6804** | 0.4657 | 0.7004 | **0.6753** | 0.3921 | 0.7290 |
| PIDC | 0.6158 | 0.4846 | **0.7344** | 0.6257 | 0.4776 | **0.7373** | 0.6548 | 0.4866 | **0.7803** |
| SINCERITIES | 0.5410 | 0.5126 | 0.5129 | 0.5423 | 0.5010 | 0.5100 | 0.5637 | 0.5044 | 0.4985 |
| SCRIBE | 0.5837 | 0.4893 | 0.5679 | 0.5943 | 0.4913 | 0.5856 | 0.5823 | 0.4828 | 0.5886 |
| SCODE | 0.5260 | 0.4955 | 0.4998 | 0.5241 | 0.5025 | 0.5018 | 0.5477 | 0.5318 | 0.4857 |
| GRNBOOST2 | 0.6396 | 0.5065 | 0.7190 | 0.6319 | 0.4984 | 0.7106 | 0.6322 | 0.4835 | 0.7289 |
| SINGE | 0.4880 | 0.4933 | 0.5015 | 0.4899 | 0.4988 | 0.4952 | 0.4919 | 0.4942 | 0.4949 |
|  | mDC | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GRODE | **0.5796** | 0.4942 | **0.5936** | **0.5623** | 0.5021 | **0.5837** | **0.6041** | 0.5068 | **0.6016** |
| DeepSEM | 0.5633 | 0.5006 | 0.5750 | 0.5615 | 0.4735 | 0.5626 | 0.5743 | 0.4982 | 0.5878 |
| NGM | 0.4992 | 0.4989 | 0.4980 | 0.5001 | 0.4999 | 0.4999 | 0.5000 | 0.5000 | 0.5000 |
| CUTS | 0.4992 | 0.5036 | 0.5020 | 0.5073 | 0.4981 | 0.5032 | 0.5081 | 0.5175 | 0.5043 |
| LEAP | 0.5179 | 0.4476 | 0.5133 | 0.5303 | 0.4293 | 0.5259 | 0.5706 | **0.6514** | 0.5624 |
| PIDC | 0.5301 | 0.4966 | 0.5352 | 0.5308 | 0.5059 | 0.5438 | 0.5455 | 0.5112 | 0.5460 |
| SINCERITIES | 0.5342 | 0.5415 | 0.5194 | 0.5219 | 0.5405 | 0.5115 | 0.5112 | 0.5105 | 0.5087 |
| SCRIBE | 0.4944 | 0.4913 | 0.5182 | 0.5115 | 0.4975 | 0.5237 | 0.4845 | 0.5263 | 0.4921 |
| SCODE | 0.5064 | **0.5481** | 0.5053 | 0.5093 | **0.5450** | 0.5044 | 0.5447 | 0.4680 | 0.5078 |
| GRNBOOST2 | 0.5212 | 0.4959 | 0.5309 | 0.5181 | 0.5064 | 0.5301 | 0.5363 | 0.5269 | 0.5467 |
| SINGE | 0.4991 | 0.5068 | 0.4892 | 0.5013 | 0.4961 | 0.4906 | 0.5002 | 0.4360 | 0.4890 |
|  | hESC | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GRODE | **0.5294** | 0.5058 | 0.6168 | **0.5336** | 0.4969 | **0.6349** | 0.5020 | 0.5152 | 0.6216 |
| DeepSEM | 0.5255 | 0.5856 | 0.6099 | 0.5214 | 0.5819 | 0.6025 | 0.4727 | **0.5352** | 0.6381 |
| NGM | 0.5205 | 0.5166 | 0.5165 | 0.5056 | 0.5329 | 0.5227 | **0.5362** | 0.5012 | 0.5141 |
| CUTS | 0.5173 | 0.5064 | 0.5045 | 0.5210 | 0.5066 | 0.4999 | 0.5342 | 0.5096 | 0.5142 |
| LEAP | 0.5102 | 0.4958 | 0.4898 | 0.5138 | 0.4927 | 0.4938 | 0.4049 | 0.4623 | 0.5383 |
| PIDC | 0.5107 | 0.4945 | **0.6200** | 0.5106 | 0.4982 | 0.6281 | 0.4606 | 0.4798 | **0.6915** |
| SINCERITIES | 0.5016 | 0.5792 | 0.5057 | 0.4996 | **0.5828** | 0.4937 | - | - | - |
| SCRIBE | 0.4739 | 0.5075 | 0.5547 | 0.4732 | 0.5053 | 0.5515 | 0.4562 | 0.5070 | 0.5331 |
| SCODE | 0.4959 | **0.5899** | 0.4594 | 0.4824 | 0.5452 | 0.4793 | 0.4223 | 0.4707 | 0.4927 |
| GRNBOOST2 | 0.4851 | 0.4812 | 0.5816 | 0.4884 | 0.4871 | 0.5782 | 0.4779 | 0.4862 | 0.5968 |
| SINGE | 0.5076 | 0.4926 | 0.4948 | 0.5061 | 0.4920 | 0.4970 | 0.5124 | 0.5034 | 0.4952 |
|  | hHep | | | | | | | | |
|  | 500\_tf | | | 1000\_tf | | | 1000 | | |
|  | Non-Spec | Spec | STR | Non-Spec | Spec | STR | Non-Spec | Spec | STR |
| GRODE | **0.5531** | 0.5218 | 0.6107 | 0.5548 | 0.5220 | **0.6452** | **0.5837** | 0.5183 | **0.7091** |
| DeepSEM | 0.5376 | **0.5456** | 0.6151 | 0.5429 | **0.5697** | 0.6005 | 0.5542 | 0.5420 | 0.6856 |
| NGM | 0.5115 | 0.4990 | 0.5200 | 0.5116 | 0.4987 | 0.5144 | 0.5273 | 0.5130 | 0.5344 |
| CUTS | 0.5169 | 0.4852 | 0.5360 | 0.5244 | 0.5038 | 0.5051 | 0.5218 | 0.4940 | 0.5072 |
| LEAP | 0.5432 | 0.4897 | 0.5489 | 0.5508 | 0.4897 | 0.5566 | 0.4837 | 0.4880 | 0.6557 |
| PIDC | 0.5116 | 0.5139 | **0.6193** | **0.5566** | 0.5059 | 0.6124 | 0.5742 | **0.5442** | 0.6933 |
| SINCERITIES | 0.4833 | 0.4761 | 0.4922 | 0.4845 | 0.4775 | 0.4977 | 0.5433 | 0.5100 | 0.5334 |
| SCRIBE | 0.4548 | 0.5263 | 0.5507 | 0.4508 | 0.5191 | 0.5421 | 0.4988 | 0.5053 | 0.5089 |
| SCODE | 0.5343 | 0.5069 | 0.5086 | 0.5444 | 0.5124 | 0.5221 | 0.5252 | 0.4779 | 0.5121 |
| GRNBOOST2 | 0.4924 | 0.5157 | 0.5770 | 0.4913 | 0.5120 | 0.5627 | 0.5637 | 0.5221 | 0.6007 |
| SINGE | 0.4961 | 0.4995 | 0.4950 | 0.4962 | 0.4992 | 0.4981 | 0.4959 | 0.4803 | 0.4929 |

**Supplementary Table 4.**

Summary of default network parameters setting for GRODE.

|  |  |
| --- | --- |
| **Hyper-parameters** | **value** |
|  | 50 |
|  | 150 |
|  | 200 |
|  | 0.1 |
|  | 10 |
|  | 5 |
| **Batch\_size** | 128 |
| **Learning\_rate for data prediction** | 0.001 → 0.00001 |
| **Learning\_rate for graph learner** | 0.0001 → 0.00001 |

Summary of weight hyperparameters setting in various experiments.

|  |  |  |  |
| --- | --- | --- | --- |
| **dataset** | **task** |  |  |
| **mESC** | non\_celltype\_GRN | 100 | 1 |
| celltype\_GRN | 0.01 | 1 |
| **mHSC-E** | non\_celltype\_GRN | 1 | 10 |
| celltype\_GRN | 0.001 | 1 |
| **mHSC-L** | non\_celltype\_GRN | 0.1 | 10 |
| celltype\_GRN | 1 | 1 |
| **mHSC-GM** | non\_celltype\_GRN | 0.1 | 10 |
| celltype\_GRN | 10 | 1 |
| **mDC** | non\_celltype\_GRN | 100 | 1 |
| celltype\_GRN | 0.1 | 1 |
| **hESC** | non\_celltype\_GRN | 100 | 1 |
| celltype\_GRN | 0.01 | 1 |
| **hHep** | non\_celltype\_GRN | 100 | 1 |
| celltype\_GRN | 0.01 | 1 |
| **Chu** | imputation | 0.01 | 1 |
| **Hayashi** | imputation | 0.01 | 1 |
| **Klein** | imputation | 1 | 1 |
| **Yanagida** | imputation | 1 | 1 |

# Supplementary Notes

**Supplementary Notes 1.** Brief introduction and running details about GRN inference algorithms used in this study.

|  |  |
| --- | --- |
| Method | Description |
| DeepSEM[7] | To capture the causality, DeepSEM uses a structural equation model(SEM) that is an auto-regression model based on the beta-VAE framework to explicitly model the regulatory relationships among genes and obtain biologically meaningful representation of scRNA-seq data. |
| NGM[8] | It’s a score-based structure learning algorithm based on penalized Neural Ordinary Differential Equations. It uses least squares optimization with adaptive regularization schemes consistently which can recovers directed graphs of local independencies in systems of stochastic differential equations. |
| CUTS[9] | CUTS is a neural Granger causal discovery algorithm to jointly impute unobserved data points and build causal graphs, via plugging in two mutually boosting modules in an iterative framework. It designs a Delayed Supervision Graph Neural Network (DSGNN) to construct irregular data and then builds a causal adjacency matrix with imputed data under sparse penalty. |
| LEAP[10] | LEAP operates on pseudotime-ordered data to determine the Pearson’s correlation of normalized mapped-read counts within fixed-size temporal windows at various time lags. The relation score for each gene pair is the highest Pearson’s correlation observed among all considered lag values. As the computed correlations are not symmetrical, this method can generate directed networks. |
| PIDC[11] | PIDC utilize multivariate information theory, Partial Information Decomposition, to explore the statistical dependencies between triplets of genes. Specifically, for each gene pair, x and y, considering a third gene, z, the method divides pairwise mutual information of x and y into a redundant and a unique component. It computes the ratio of unique component and mutual information. The sum of this ratio over all other genes z is the proportional unique contribution between x and y. Finally, we use the threshold to identify the most important interactions for each gene. |
| SCRIBE[12] | The key concept is conditional restricted direct information. It quantifies the mutual information between the past state (expression values) of a regulator gene and the current state of a target gene, conditioned on the target gene's state at the previous time point. This algorithm helps eliminate edges that represent indirect effects. |
| SCODE[13] | Linear ordinary differential equations is used to model how a regulatory network contributes to gene expression dynamics. SCODE relies on a specific equation which can be estimated using linear regression. |
| SINCERITIES[14] | This method analyzes time-stamped transcriptional data by calculating the temporal changes in gene expression. It measures the distance between the marginal distributions of gene expression levels at consecutive time points using the Kolmogorov-Smirnov statistic. To infer regulatory connections between transcription factors (TFs) and target genes, the approach utilizes Granger causality. It leverages the changes in TF gene expression within a time window to predict how the expression distributions of target genes shift in the subsequent time window. |
| GRNBOOST2[15] | GRNBoost2 is an extension version of GENIE3 designed for large-scale dataset. It employed a regression model to identify the most significant regulators for each gene. It use stochastic gradient boosting machine regression along with early stopping regularization to achieve high efficiency. |
| SINGE[16] | Considering irregular time distribution, SINGE used kernel-based Granger causality regression. SINGE performs multiple regressions, one for each set of input parameters, and aggregates the resulting predictions using a modified Borda method. |

**Supplementary Notes 2.** Detailed introduction of data preprocessing process.

Firstly, we performed scaling and log transformation on the raw expression data to achieve normalization. We then filtered out genes that were expressed in fewer than 10\% of the cells. Next, we applied PCA on the dataset and computed the pseudotime using Slingshot[6]. Additionally, we used a general additive model implemented in the 'gam' package in R to calculate the variance along with its corresponding P-value.

After performing data preprocessing, we obtained three files: expression data, pseudotime for each cell, and gene variance. Before feeding the data into the model, we choose a strategy to select highly variable genes and then order the expression data based on pseudotime. Furthermore, we normalize genes once more to ensure that GROD can effectively simulate gene changes, and we can optionally interpolate data if there is a zero.

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