

Supplementary Materials for

LogBTF: Gene regulatory network inference using Boolean threshold network model from single-cell gene expression data

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1. The proof of Theorem 2.1

Theorem 2. 1. *The inequality $w_1l_1 + w_2l_2 + \dots + w_Nl_N \geq \theta_i$ in*

$$x_i(t+1) = f_i(x_1(t), x_2(t), \dots, x_N(t)) = \begin{cases} 1, & w_1l_1 + w_2l_2 + \dots + w_Nl_N \geq \theta_i, \\ 0, & \text{otherwise,} \end{cases} \quad (\text{S1})$$

is equivalent to $\theta_1^i x_1(j) + \theta_2^i x_2(j) + \dots + \theta_N^i x_N(j) + \theta_0^i \geq 0$ in

$$\text{logit}(\pi_j^i) = \log\left(\frac{\pi_j^i}{1 - \pi_j^i}\right) = \theta_1^i x_1(j) + \theta_2^i x_2(j) + \dots + \theta_N^i x_N(j) + \theta_0^i. \quad (\text{S2})$$

under the given update scheme

$$\text{Update scheme} := \begin{cases} w_k = \theta_k^i \text{ and } l_k = x_k, & \text{if } \theta_k^i > 0, \\ w_k = 0, & \text{if } \theta_k^i = 0, \\ w_k = -\theta_k^i \text{ and } l_k = \bar{x}_k, & \text{if } \theta_k^i < 0. \end{cases} \quad (\text{S3})$$

Proof. For a fixed node x_i , if its status at the next time point will be evolved to 1, i.e., the Boolean threshold function takes the “True” state, then it holds

$$w_1l_1 + w_2l_2 + \dots + w_Nl_N \geq \theta_i. \quad (\text{S4})$$

According to Equation (S3), Equation (S4) can be written as

$$\sum_{k \in \{k|k \in [1,N], \theta_k^i > 0\}} \theta_k^i x_k - \sum_{k \in \{k|k \in [1,N], \theta_k^i < 0\}} \theta_k^i \bar{x}_k \geq -\theta_0^i - \sum_{k \in \{k|k \in [1,N], \theta_k^i < 0\}} \theta_k^i, \quad (\text{S5})$$

Since $1 - x_k = \bar{x}_k$, so we have

$$\sum_{k \in \{k|k \in [1,N], \theta_k^i > 0\}} \theta_k^i x_k - \sum_{k \in \{k|k \in [1,N], \theta_k^i < 0\}} \theta_k^i (1 - x_k) \geq -\theta_0^i - \sum_{k \in \{k|k \in [1,N], \theta_k^i < 0\}} \theta_k^i. \quad (\text{S6})$$

Clearly, by a simple operation, Equation (S6) becomes

$$\theta_1^i x_1(j) + \theta_2^i x_2(j) + \dots + \theta_N^i x_N(j) + \theta_0^i \geq 0. \quad (\text{S7})$$

The proof is thus completed. □

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2. The details of all datasets and preprocessing implementation

2.1. Datasets

First, one artificial Boolean value dataset with a standard gene regulatory network is generated to evaluate the applicability, robustness and accuracy of our proposed LogBTF method.

Then, fifteen simulated datasets were generated using GeneNetWeaver (GNW) (Schaffter et al., 2011), a well-known simulation tool to generate gene expression data and “gold standard” networks for evaluating different GRN inference methods (Chen & Mar, 2018). On one hand, noise-free “bulk” gene expression data with the corresponding source network (gold standard) from real *E.coli* and *Yeast* species are generated. Namely, there are three types of available datasets according to the number of genes (size 10, size 50 and size 100). Each type contains five *insilico* networks (*Ecoli1*, *Ecoli2*, *Yeast1*, *Yeast2* and *Yeast3*) with 21 time points with the same step. On the other hand, the aim of this study is to test the applicability of network inference methods to single-cell data, while drop-out events are one of the most important features of single-cell data. Therefore, we artificially induced the dropout data with excessive zero counts to the data generated from GNW to mimic the characteristics of single-cell experimental data (Chen & Mar, 2018), which is widely used to benchmark numerous GRN inference methods (Dibaeinia & Sinha, 2020). Specifically, for each gene, we cluster its expression across cell samples into two clusters using K -means algorithm. For each sample, if the gene’s expression was closer to the smaller cluster center, it would be replaced according to a Binomial probability of 0.5 (i.e., inducing drop-out where the resulting value was now either 0 or the original data point). Additionally, the distributions of gene expression value for both simulated single-cell data and simulated bulk gene expression data used in this work are shown in Figure S1, and their corresponding networks are shown in Figure S2.

Finally, we collected three retrieved real single-cell gene expression datasets: Matsumoto (Treutlein et al., 2016), hHEP (Camp et al., 2017) and LMPP (Hamey et al., 2017). The first raw dataset is real Matsumoto scRNA-Seq data obtained by examining direct reprogramming from mouse embryonic fibroblast (MEF) into myocytes at days 0, 2, 5 and 22. This dataset contains 373 cells with corresponding pseudo-temporal information and expression dynamics. The second real specific human mature hepatocytes (hHEP) data, derived from a scRNA-seq experiment that induces pluripotent stem cells (iPSCs) to differentiate into hepatocyte-like cells in two-dimensional culture, is used as the fourth dataset in this paper. In this dataset, it contains 425 scRNA-seq measurements from multiple time points: days 0, 6, 8, 14 and 21, and the pseudo-time was obtained using Slingshot with cells measured on day 0 as the starting cluster and the cells measured on 21 (mature hepatocytes) as the ending cluster. The last dataset we used is the data of transforming single hematopoietic stem cells (HSCs) into lymphoid-primed multipotent progenitors (LMPP). In this dataset, cells belonging to this branch were ordered into the pseudo-time differentiation trajectories to allow investigation of gene expression dynamics during HSCs differentiation. By the way, these three datasets with their ground truth networks can be acquired from the listed references.

The details of all datasets used in our experiments are illustrated in Table S1, in which we use SIGN to characterize the gold standard whether with signed edges (SIGN = 1, activation or inhibition) or not (SIGN = 0).

Table S1 The details of all datasets used in our experiments, where ‘–’ indicates that the value is set artificially.

Experiment	Dataset	Time point	# of nodes	Gold standard	Reference
Boolean dataset and network	Artificial data	–	9	SIGN = 1	–
Simulated single-cell dataset and <i>insilico</i> networks	Ecoli1 data	21	Size10, Size50, Size100	SIGN = 1	(Schaffter et al., 2011)
	Ecoli2 data	21	Size10, Size50, Size100	SIGN = 1	
	Ecoli3 data	21	Size10, Size50, Size100	SIGN = 1	
	Yeast1 data	21	Size10, Size50, Size100	SIGN = 1	
	Yeast2 data	21	Size10, Size50, Size100	SIGN = 1	
Real scRNA-seq dataset with pseudo-time and ground truth network	Matsumoto data	373	100	SIGN = 0	(Treutlein et al., 2016)
	hHEP data	425	948	SIGN = 0	
	LMPP data	531	31	SIGN = 1	

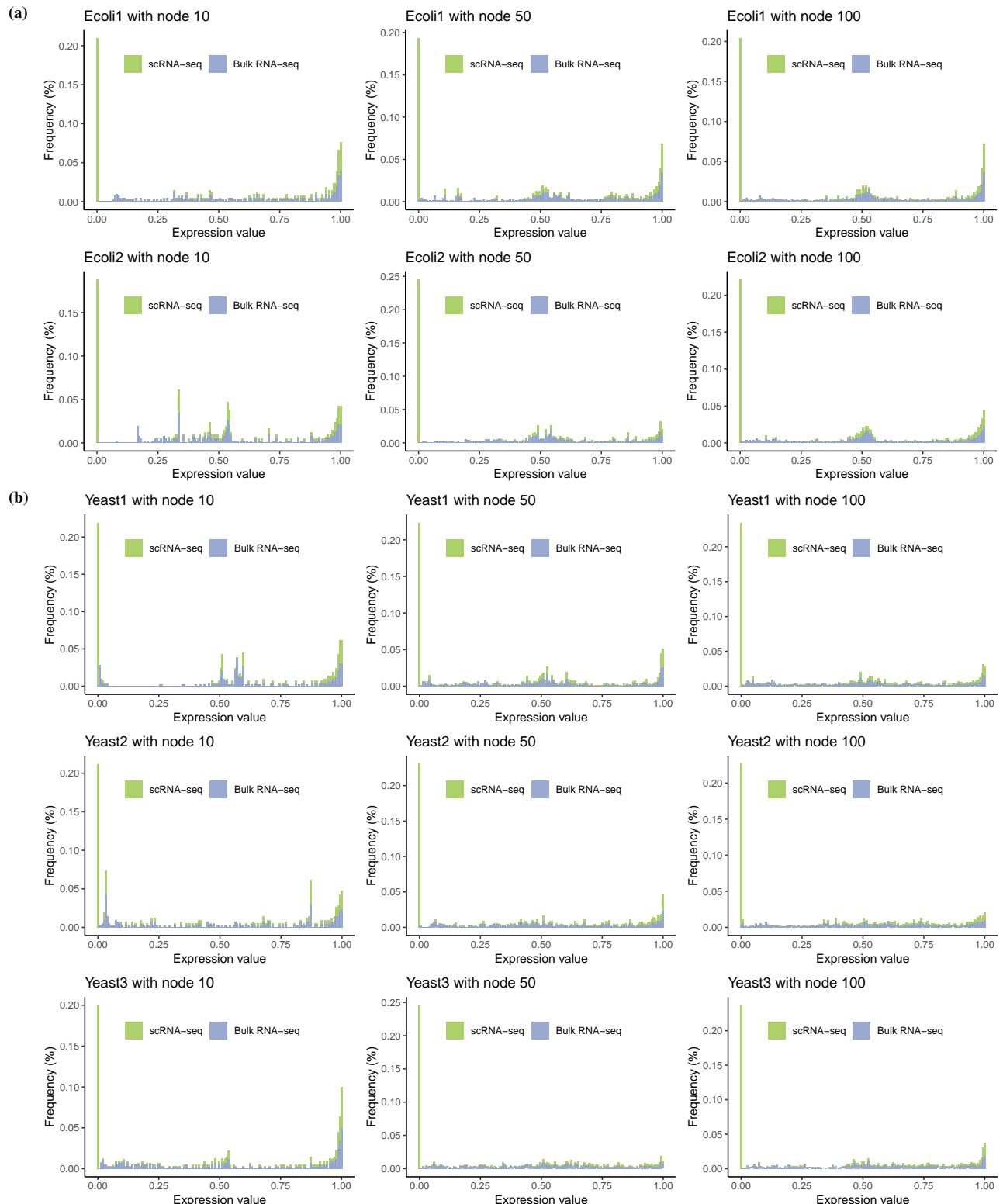


Figure S1 The distributions of gene expression value for both simulated single-cell and simulated “bulk” data. (a) Six *insilico* datasets for Ecoli and (b) Nine *insilico* datasets for Yeast, generated from two 10-gene, two 50-gene and two 100-gene networks.

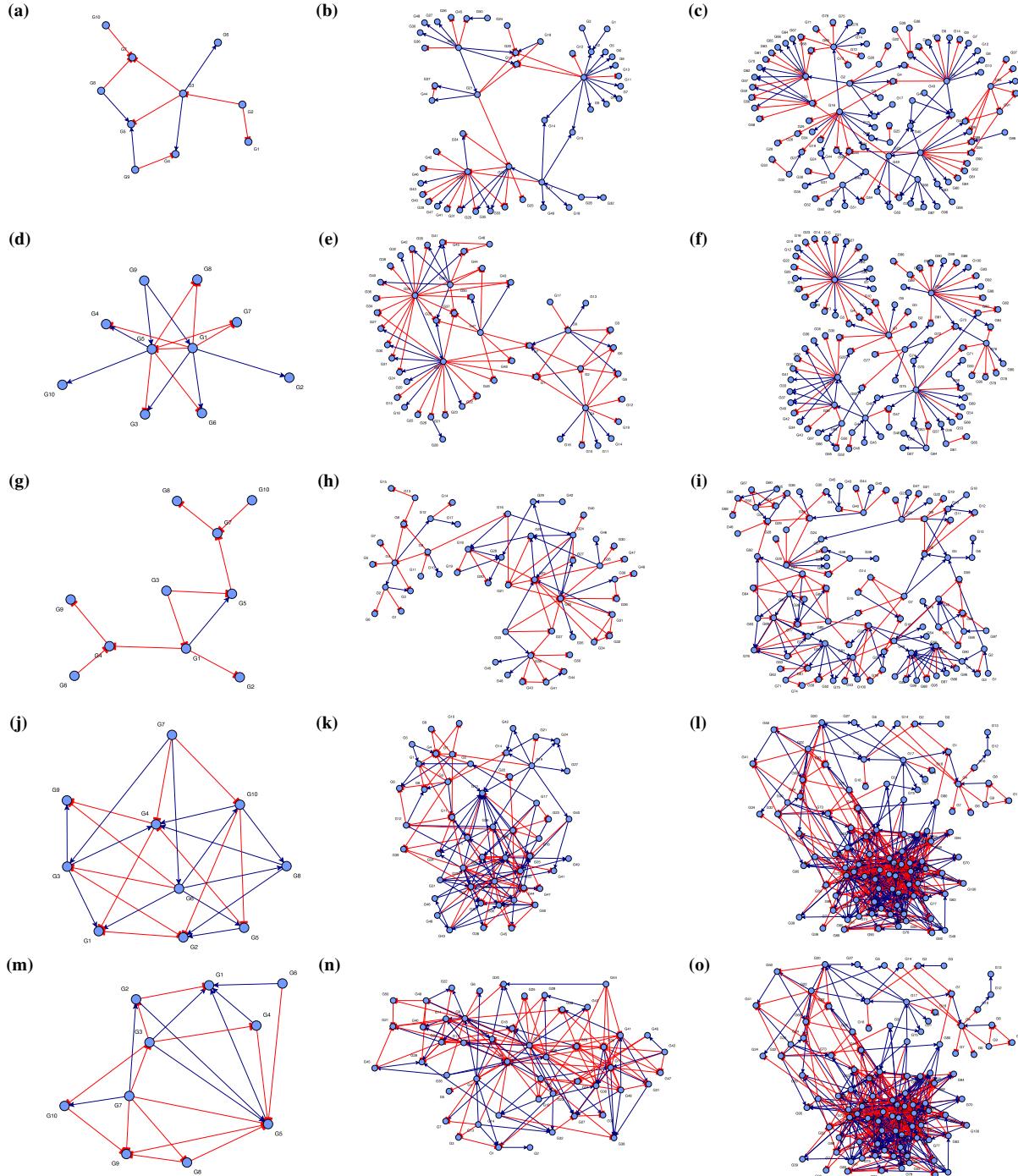


Figure S2 The ground truth networks of fifteen simulated (single-cell and “bulk”) gene expression datasets. Three networks of in silico datasets from 10-gene, 50-gene and 100-gene for Ecoli 1 (a)-(c); Ecoli 2 (d)-(f); Yeast 1 (g)-(i); Yeast 2 (j)-(l) and Yeast 3 (m)-(o).

2.2. Data preprocessing

Our LogBTF method needs the state of each gene to be quantified as 0 (false/not expressed) or 1 (true/expressed). For the simulated or real-world single-cell expression data, no imputation is required, all dropouts are set to 0 and all non-zero counts are set to 1s regardless of the expression level (Qiu, 2020). For the simulated or real-world bulk expression data, we first preprocess the original gene expression data by binarization. For each gene, its expression data at all time points can be seen as a one-dimensional vector, data binarization is the process of converting continuous data attribute values into finite interval sets, that is, 0 or 1 in Boolean modeling. Specifically, we apply K -means algorithm to split the given data into two clusters with $K = 2$ in an unsupervised way (Müssel et al., 2016). First, it randomly picks two initial cluster centers from the original data and then assigns the remaining points to the closest cluster center. Then, it updates this cluster progress by calculating the mean of the data in each cluster during several consecutive cycles, redefines the cluster centers to these mean values and reassigned all data points to the closest updated cluster center by minimizing the sum over the squared distance of the data points to their respective cluster center, i.e.,

$$\sum_{j=1}^2 \sum_{x_i \in \text{Cluster}_j} (x_i - x_j^*)^2,$$

where Cluster_j is the j -th cluster, x_i is one value of the input one-dimensional gene expression vector, x_j^* is the center of the j -th cluster. Finally, the values belonging to the cluster with the smaller center are set to 0, and the values belonging to the larger center are set to 1.

3. Framework

Figure S3 illustrates the framework of the LogBTF method for inferring GRNs from single-cell gene expression data. LogBTF method consists of the following two steps: (i) for single-cell gene expression data with corresponding pseudo-time series information, we binarized the continuous values of gene expression into Boolean values (0 or 1); (ii) based on the binarized data, the Boolean threshold network is constructed using logistic regression with potential regularization term. Here, we respectively evaluate our method by comparing the inferred gene expression states and the reconstructed GRN with the original states and gold standard. The consistency between Boolean trajectory generated by the inferred network and the binarized time series gene expression data is characterized by the dynamical accuracy (DyAcc) metric. The consistency between inferred GRN and the ground-truth GRN is characterized by the structural accuracy (StAcc) metric.

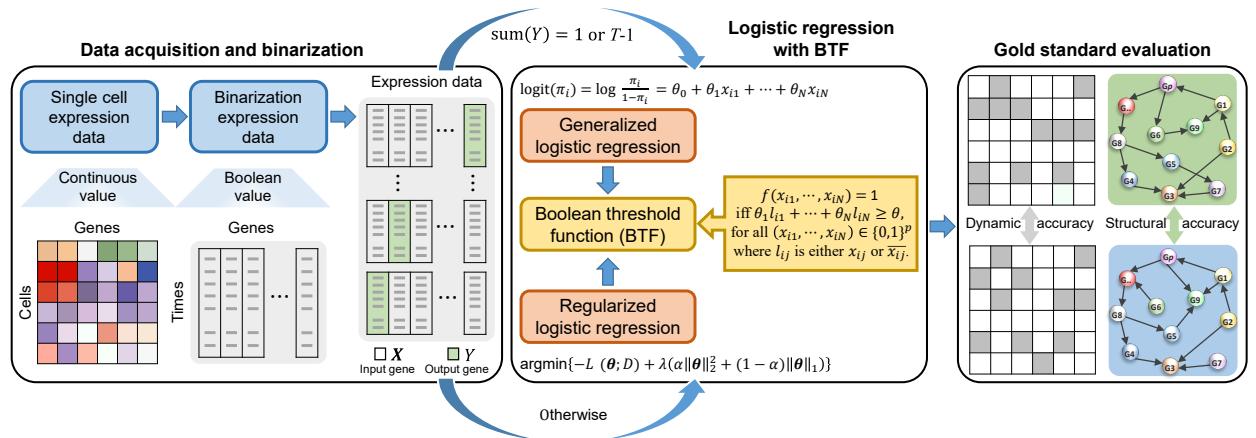


Figure S3 The framework of LogBTF method for gene regulatory network inference from single-cell gene expression data.

4. Selection of tuning parameters

The selection of optimal tuning parameters for our method is vital in performing inferring GRNs. Without loss of generality, for $\theta_i = \arg \min \{-\mathcal{L}(\theta_i | \mathcal{D}_i) + \lambda [\alpha \|\theta_i\|_1 + (1 - \alpha) \|\theta_i\|_2^2]\}$, we set α to a fixed value of 0.5 (Li & Liu, 2020), and then implement the K -fold cross-validation (CV) procedure in combination with search algorithms to obtain the optimal tuning parameter λ (James et al., 2013). At first, the whole dataset is randomly split into K folds with approximately equal sample sizes, and each fold contains the corresponding responding input genes at t time point and output genes at $t + 1$ time point. Then, the $K - 1$ folds data constitutes the training dataset used to train and fit the Boolean threshold network model, and the remaining fold as the test dataset used to evaluate the inference performances of the trained model by calculating the mean squared error (MSE):

$$\text{MSE} = \frac{1}{T-1} \sum_{j=1}^{T-1} [y^i(j) - f_i(x_1(j), x_2(j), \dots, x_N(j))]^2 \quad (\text{S8})$$

for each value of λ over the a range of different λ values. The procedure is performed K times and finally results in K estimates of the test error. The final K -fold CV estimate value is defined as the average value of all MSE errors shown in Equation (S8), that is,

$$\text{CV}(\lambda) = \frac{1}{K} \sum_{i=1}^K \text{MSE}_i. \quad (\text{S9})$$

Finally, the optimal choice of regularization parameter λ can be obtained by maximizing Equation (S9). The choice of K depends on the size of the training dataset (Liang et al., 2013). In our experiments, if the network size is relatively small, we set $K = N$ and perform leave-one-out cross-validation (LOOCV) to fit the Boolean threshold network model N times. LOOCV uses almost all data, which guarantees the bias of the model to be smaller. But if the network size is extremely large, the computational cost of LOOCV can be expensive. If the network size is more than 500, we set $K = 10$ to conduct typical 10-fold CV to save the running times (James et al., 2013).

5. Optimization of network topology

The binarized Boolean value data obtained by the K -means algorithm may suffer from the multi-collinearity problem if the number of time points is small. For example, the binarized data of two different genes are same or differ by a minus sign. In this case, using logistic regression to estimate the parameters of the Boolean threshold network model, it may happen that only one gene is selected from a group of genes with high correlation (positive or negative correlation), so that the coefficient of the selected gene is a real number, but the coefficients of other genes are labeled “NA”. Obviously, in this case, we cannot determine whether the selected gene is a true regulator.

To overcome the multi-collinearity problem and detect reliable regulatory relationships, we propose an optimization strategy based on knowledge of the perturbation design matrix (Seçilmiş et al., 2022) as follows: First, we generate a normal distribution matrix with mean $\mu = 0$ and very small variance σ , and the dimension of this random matrix is the same as the binarized input matrix we have obtained. Then, a new input matrix is obtained by adding the random matrix and the binarized input matrix. Based on the newly obtained input matrix, we apply our LogBTF method to estimate the regression coefficient $\hat{\theta}_i$ of the i -th target gene,

$$\hat{\theta}_i = (\hat{\theta}_1^i, \hat{\theta}_2^i, \dots, \hat{\theta}_N^i, \hat{\theta}_0^i)^\top, \quad i \in [1, N], \quad (\text{S10})$$

Using the above method, the multi-collinearity problem is solved but the estimated value $\hat{\theta}_i$ we obtained is noisy, and if the resulting regression coefficients are used directly for network inference, some redundant edges are generated. In order to optimize the network topology, we remove the influence of the added tiny random perturbation matrix by setting the coefficient whose absolute value is less than the given σ value to 0. The remaining no-zero elements in the coefficient $\hat{\theta}_i$ are used to infer the potential gene regulatory work. Finally, we normalize the regulatory coefficient $\hat{\theta}_i$ of each gene as follows:

$$\hat{\theta}_i \leftarrow \frac{\hat{\theta}_i}{\|\hat{\theta}_i\|_\infty}, \quad (\text{S11})$$

where $\|\hat{\theta}_i\|_\infty = \max_{1 \leq k \leq N} |\hat{\theta}_k^i|$ is the L_∞ -norm. Thus, the contribution of all regulatory genes to the given target gene has a standard scale, with the strongest regulatory relationship being assigned 1, and the weakest being 0.

6. Evaluation criteria of inference

6.1. Dynamical accuracy metrics

Most of the existing algorithms for reconstructing BNs cannot accurately give Boolean function during each update status (Liu et al., 2021; Barman & Kwon, 2020; Schwab et al., 2021; Barman & Kwon, 2018). However, the LogBTF method can represent the corresponding Boolean threshold function for each gene. The logistic regression coefficient-based Boolean threshold function can be applied to evaluate the dynamical accuracy (DyAcc) by comparing the Boolean trajectory generated by the inferred network and the binarized time-series gene expression data.

Let $\tilde{x}_i(t)$ be the predicted Boolean value of gene x_i at time t in the inferred Boolean threshold network, we define the DyAcc of an inferred network using the average gene-wise consistency/similarity between the Boolean trajectories of the observed gene expression $x_i(t)$ and the estimated gene expression $\tilde{x}_i(t)$ over all N genes as follows:

$$\text{DyAcc} = \frac{1}{N(T-1)} \sum_{i=1}^N \sum_{t=2}^T \mathcal{I}(x_i(t) = \tilde{x}_i(t)), \quad (\text{S12})$$

where $\mathcal{I}(\cdot)$ is the indicator function as follows:

$$\mathcal{I}(\cdot) = \begin{cases} 1, & x_i(t) = \tilde{x}_i(t), \\ 0, & \text{otherwise.} \end{cases} \quad (\text{S13})$$

6.2. Structural accuracy metrics

Mathematically speaking, the gold standard or ground-truth GRN can be represented by a directed graph G with a gene set V and an edge set E , i.e., $G = (V, E)$. Correspondingly, the inferred GRN by Boolean threshold network model can also be characterized using $\tilde{G} = (\tilde{V}, \tilde{E})$. An important evaluation metric for the inference performance of a method is measuring the consistency/similarity between graph G and graph \tilde{G} . As usual, we compute the numbers of true positive (TP) edges, true negative (TN) edges, false positive (FP) edges and false negative (FN) edges by comparing the regulatory edges on a graph G and graph \tilde{G} respectively. Structural accuracy (StAcc) is the ratio of correct predictions out of all predictions as follows:

$$\text{StAcc} = \frac{TP + TN}{TP + TN + FP + FN}. \quad (\text{S14})$$

Especially, when considering GRN with signed edges, a true positive prediction refers to the correct prediction of an edge and its sign.

6.3. Other evaluation criteria

Moreover, we also use the following measures: recall (Recal, $TP/(TP + FN)$), precision (Pre, $TP/(TP + FP)$), false positive rates (FPR, $FP/(FP + TN)$), F -measure ($2TP/(2TP + FP + FN)$) and the area under the ROC curve (AUC) (Bradley, 1997) to evaluate the inferring performance of LogBTF and other inferring methods, including SINCERITIE (Papili Gao et al., 2018) (regression-based method), GRISLI (Aubin-Frankowski & Vert, 2020) and SCODE (Matsumoto et al., 2017) (ordinary differential equation methods), GENIE3 (Huynh-Thu et al., 2010) and TIGRESS (Haury et al., 2012) (miscellaneous methods), ARACNE (Margolin et al., 2006) and CLR (Faith et al., 2007) (correlation-based methods). At the same time, we assess the performance of LogBTF by evaluating the areas under the receiver operating characteristic (AUROC) (Hanley & McNeil, 1982) and the precision-recall curve (AUPR). For the GRISLI method, we apply the source MATLAB code, which directly outputs the AUROC and AUPR values. It is worth noting that SCODE, GENIE3, TIGRESS, ARACNE and CLR methods cannot give a certain number of inferred edges. In order to guarantee a fair comparison, we select the same number of regulatory edges with the top κ edges from the ranked list output of SCODE, GENIE3, TIGRESS, ARACNE and CLR methods, where κ is the number of links inferred from the LogBTF method. On the other hand, the SINCERITIE method can infer the number of edges. In summary, the differences between LogBTF and seven alternative gene regulatory network inference methods are shown in Table S2.

Table S2 The comparison with other network inferring models.

Method	Dlink	SIGN	InferBas	DyAcc	StAcc	Update rule	Gene status	# of in-degree	# of RegShips	Computational complexity
CLR	✗	✗	CorCoef	✗	T-based	✗	✗	✗	✗	$O(N^2)$
ARACNE	✗	✗	CorCoef	✗	T-based	✗	✗	✗	✗	$O(N^3)$
TIGRESS	✓	✗	Weights	✗	T-based	✗	✗	✗	✗	$O(RN^{3/2}T), R = 1000$
GENIE3	✓	✗	Weights	✗	T-based	✗	✗	✗	✗	$O(RN^{3/2}T \log T), R = 1000$
SCODE	✓	✗	CoefMat	✗	T-based	✗	✗	✗	✗	$O(RNT + N^2)$
GRISLI	✓	✗	CoefMat	✗	—	✗	✗	✗	✗	—
SINCERITIES	✓	✓	RegCoef	✗	✓	✗	✗	✓	✓	—
LogBTFs	✓	✓	RegCoef	✓	✓	✓	✓	✓	✓	—

*Abbreviation: Dlink – Directed links; InferBas – Inference basis; CorCoef – Correlation coefficients; CoefMat – Coefficient Matrix; RegCoef – Regression coefficients; T-based – Threshold-based; RegShips – regulatory relationships. *The Computational complexity of gene regulatory network inference methods with respect to: N – the number of genes; R – the number of iterations; T – the number of time points.

7. Results on artificial Boolean data

7.1. Ground truth and inferred Boolean threshold network

The Boolean threshold network corresponding to the Boolean network in Equations (12) is shown in Figure S4.

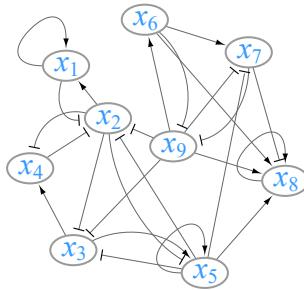


Figure S4 The synthetic Boolean threshold network, where each node x_i has a state value of 1 (expressed) or 0 (not expressed). The directed links with ‘ \rightarrow ’ represent a positive regulation (activating) from x_i to x_j , and the directed links with ‘ \dashv ’ represent a negative regulation (inhibiting).

By employing the generalized linear regression (un-penalized logistic regression) model, we obtained the inferred Boolean threshold network as follows:

$$\begin{aligned}
 x_1 : & [56.7x_1 + 56.7x_2 \geq 28.4], \\
 x_2 : & [54.7\bar{x}_1 + 54.7\bar{x}_4 + 54.7\bar{x}_5 + 54.7\bar{x}_9 \geq 191.8], \\
 x_3 : & [56.6\bar{x}_2 + 56.6\bar{x}_5 + 56.6\bar{x}_9 \geq 141.7], \\
 x_4 : & [56.7\bar{x}_2 + 56.7x_3 \geq 85.1], \\
 x_5 : & [56.6\bar{x}_2 + 56.6x_3 + 56.6x_5 \geq 141.7], \\
 x_6 : & [57.1x_9 \geq 28.6], \\
 x_7 : & [56.6\bar{x}_5 + 113.7x_6 + 56.6\bar{x}_9 \geq 85.1], \\
 x_8 : & [53.6x_5 + 53.6x_6 + 216.2\bar{x}_7 + 162x_8 + 53.6x_9 \geq 188.5], \\
 x_9 : & [56.7\bar{x}_6 + 56.7\bar{x}_7 \geq 85].
 \end{aligned} \tag{S15}$$

Here all coefficients are kept to one decimal place in this experiment for the convenience of representation.

7.2. Inference accuracy results under random sampling

In order to test the high accuracy or effectiveness of the LogBTF method, we randomly sample a certain percentage (abbreviated as “percent”) of data from the 512 observations, in which each group of data is randomly sampled ten times. We calculated the average AUC value of the gene expression status obtained by the Boolean threshold network

model. At the same time, we calculated the minimum sample size required to infer the state of each gene at different time points when AUC=1, and the results are shown in Figure S5. Here we only give the sample size required to infer two consecutive AUC values of 1 for each node. We consider this percentage to be the minimum number of samples required to correctly infer the state of the node. From Figure S5, we see that it only needs 2% data to infer the expression state of x_6 that evolves completely. For gene x_8 , although it may need approximate 30% data to reach its inferred AUC value equal to 1, we can see that its AUC value is already very close to 1 when the percentage is around 10%. The other seven genes also show that our method does not require data at all time points to make accurate predictions about the dynamic expression behavior of genes.

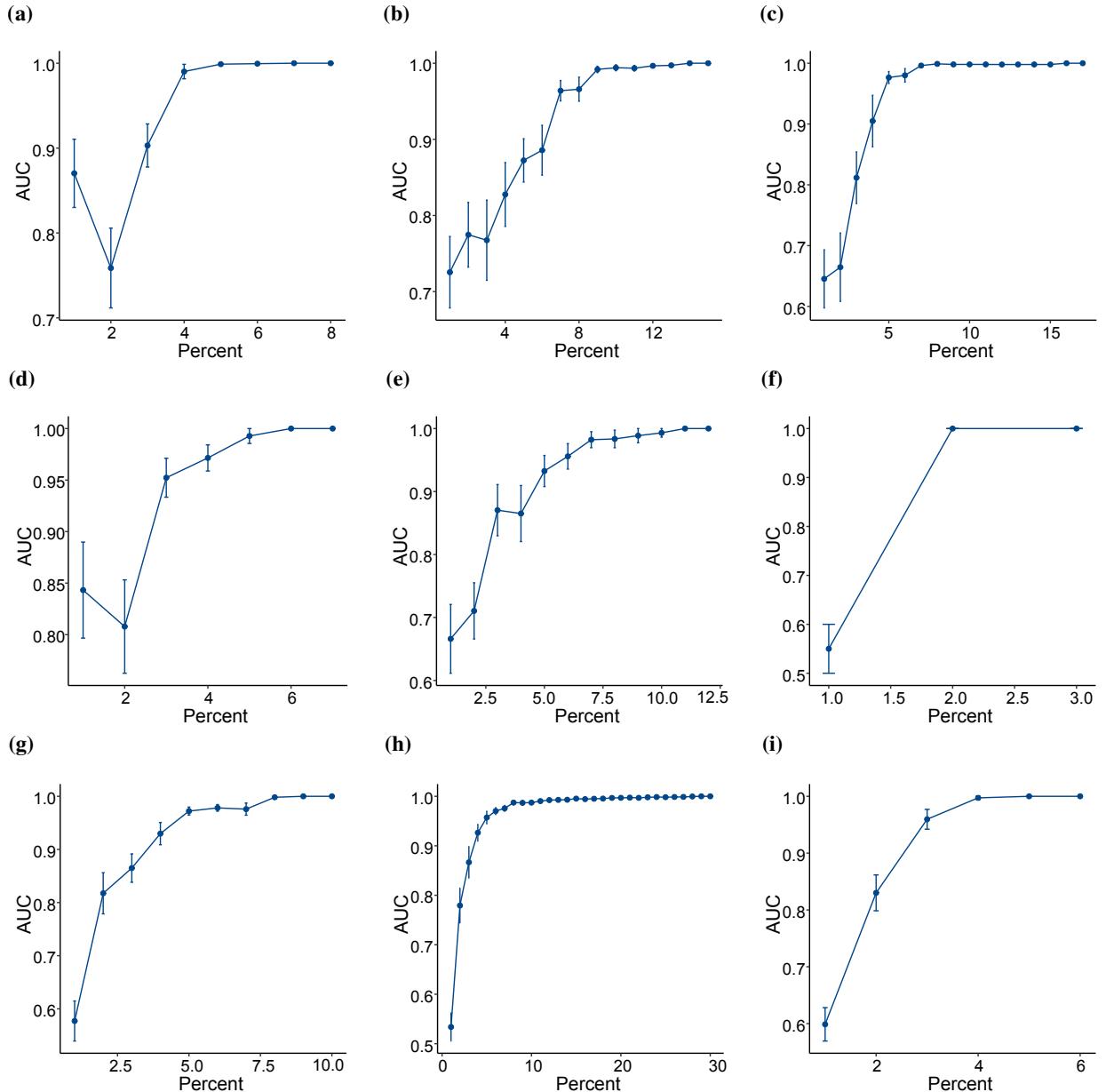


Figure S5 The AUC value of ten times random sampling under different percentages, where the polyline drawing ends if the AUC value of each node did not change with the increase of the sampling percentage. (a) x_1 . (b) x_2 . (c) x_3 . (d) x_4 . (e) x_5 . (f) x_6 . (g) x_7 . (h) x_8 . (i) x_9 .

8. Results on simulated single-cell data

In this experiment, we use the leave-one-out cross-validation (LOOCV) method (James et al., 2013) to select the optimal tuning parameter and investigate the performance of LogBTF method. Figure S6 shows the experiment results. We can see that the StAcc values of LogBTF are larger than 0.77 when the network sizes are 50 and 100. Clearly, LogBTF method shows strong superiority when the network size is 100, compared with size 10 and size 50, both from single experimental results and mean value. Furthermore, our LogBTF method performs well both for directed GRN inference ($SIGN = 1$) and undirected internship inference ($SIGN=0$), and the larger the number of nodes in the network, the higher the StAcc value of our method.

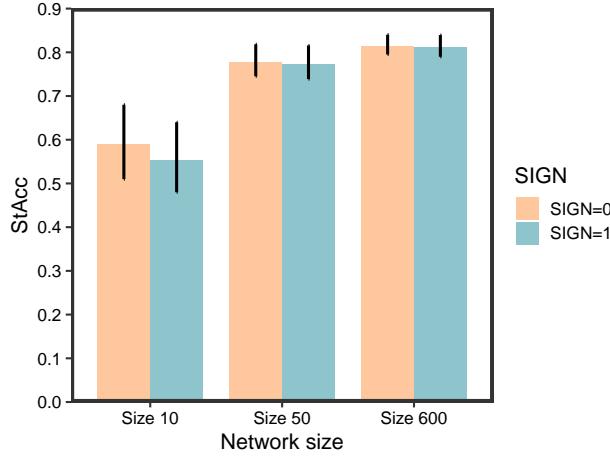


Figure S6 The StAcc value of our LogBTF method on three types of simulated single-cell datasets with different sizes.

All experimental results, including AUROC, AUPR, StAcc, Recal, Pre, FPR and F-measure), are available in Table S3 - Table S6.

Table S3 The detailed experimental results on simulated single-cell datasets under $SIGN=1$.

Size	Dataset	Method	Mean AUC	DyAcc	AUROC	AUPR	StAcc	Recal	Pre	FPR	F-measure
10	Ecoli1	LogBTF	0.952	0.960	0.674	0.199	0.620	0.818	0.200	0.404	0.321
		SINCERITIES	-	-	0.309	0.005	0.580	0.000	0.000	0.383	0.000
	Ecoli2	LogBTF	0.994	0.990	0.489	0.141	0.520	0.500	0.125	0.477	0.200
		SINCERITIES	-	-	0.410	0.055	0.570	0.182	0.056	0.382	0.085
	Yeast1	LogBTF	0.950	0.995	0.490	0.063	0.480	0.571	0.075	0.527	0.133
		SINCERITIES	-	-	0.339	0.005	0.620	0.000	0.000	0.333	0.000
50	Ecoli1	LogBTF	0.926	0.935	0.509	0.222	0.500	0.765	0.220	0.554	0.342
		SINCERITIES	-	-	0.522	0.283	0.570	0.348	0.222	0.364	0.271
	Yeast2	LogBTF	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.354	0.379
		SINCERITIES	-	-	0.361	0.068	0.520	0.105	0.061	0.383	0.077
	Yeast3	LogBTF	0.995	0.995	0.501	0.063	0.790	0.163	0.062	0.167	0.090
		SINCERITIES	-	-	0.475	0.036	0.806	0.093	0.042	0.145	0.058
100	Ecoli1	LogBTF	0.987	0.986	0.516	0.020	0.822	0.203	0.015	0.170	0.027
		SINCERITIES	-	-	0.451	0.004	0.855	0.055	0.004	0.136	0.008
	Ecoli2	LogBTF	0.993	0.989	0.509	0.019	0.794	0.211	0.012	0.199	0.022
		SINCERITIES	-	-	0.471	0.006	0.863	0.065	0.005	0.128	0.010
	Yeast1	LogBTF	0.990	0.988	0.504	0.023	0.839	0.153	0.016	0.150	0.029
		SINCERITIES	-	-	0.464	0.006	0.880	0.026	0.004	0.106	0.007
Yeast2	LogBTF	0.992	0.989	0.485	0.029	0.805	0.140	0.029	0.171	0.048	
		SINCERITIES	-	-	0.472	0.018	0.850	0.056	0.017	0.121	0.026
	Yeast3	LogBTF	0.994	0.993	0.471	0.034	0.790	0.114	0.033	0.175	0.051
		SINCERITIES	-	-	0.471	0.023	0.838	0.044	0.020	0.119	0.028

Table S4 The detailed experimental results on simulated single-cell datasets with size 10 under SIGN=0.

Size	Dataset	Method	AUROC	AUPR	StAcc	Recal	Pre	FPR	F-measure
10	<i>Ecoli1</i>	LogBTF	0.674	0.200	0.620	0.818	0.200	0.404	0.321
		SCODE	0.634	0.138	0.110	1.000	0.110	1.000	0.198
		GRISLI	0.410	0.096	—	—	—	—	—
		SINCERITIES	0.450	0.132	0.600	0.250	0.056	0.370	0.091
		GENIE3	0.469	0.416	0.580	0.636	0.156	0.427	0.250
		TREGRESS	0.541	0.519	0.620	0.818	0.200	0.404	0.321
		ARACNE	0.000	0.555	0.110	1.000	0.110	1.000	0.198
10	<i>Ecoli2</i>	LogBTF	0.565	0.233	0.550	0.600	0.188	0.459	0.286
		SCODE	0.451	0.126	0.150	1.000	0.150	1.000	0.261
		GRISLI	0.539	0.199	—	—	—	—	—
		SINCERITIES	0.480	0.120	0.590	0.308	0.111	0.368	0.163
		GENIE3	0.489	0.501	0.590	0.733	0.229	0.435	0.349
		TREGRESS	0.433	0.424	0.550	0.600	0.188	0.459	0.286
		ARACNE	0.000	0.575	0.150	1.000	0.150	1.000	0.261
10	<i>Yeast1</i>	LogBTF	0.574	0.162	0.510	0.700	0.132	0.511	0.222
		SCODE	0.489	0.115	0.100	1.000	0.100	1.000	0.182
		GRISLI	0.570	0.121	—	—	—	—	—
		SINCERITIES	0.483	0.090	0.650	0.300	0.097	0.311	0.146
		GENIE3	0.350	0.322	0.470	0.500	0.094	0.533	0.159
		TREGRESS	0.350	0.322	0.470	0.500	0.094	0.533	0.159
		ARACNE	0.111	0.556	0.200	1.000	0.111	0.889	0.200
10	<i>Yeast2</i>	LogBTF	0.603	0.351	0.580	0.840	0.356	0.507	0.500
		SCODE	0.490	0.236	0.250	1.000	0.250	1.000	0.400
		GRISLI	0.375	0.227	—	—	—	—	—
		SINCERITIES	0.517	0.264	0.570	0.348	0.222	0.364	0.271
		GENIE3	0.350	0.501	0.480	0.640	0.271	0.573	0.381
		TREGRESS	0.390	0.548	0.520	0.720	0.305	0.547	0.429
		ARACNE	0.294	0.586	0.450	0.840	0.292	0.680	0.433
10	<i>Yeast3</i>	CLR	0.319	0.590	0.470	0.840	0.300	0.653	0.442
		LogBTF	0.695	0.464	0.680	0.682	0.375	0.321	0.484
		SCODE	0.507	0.223	0.220	1.000	0.220	1.000	0.361
		GRISLI	0.497	0.234	—	—	—	—	—
		SINCERITIES	0.406	0.155	0.540	0.190	0.121	0.367	0.148
		GENIE3	0.402	0.352	0.540	0.364	0.200	0.410	0.258
		TREGRESS	0.495	0.473	0.620	0.545	0.300	0.359	0.387
10	<i>Yeast3</i>	ARACNE	0.363	0.317	0.500	0.318	0.167	0.449	0.219
		CLR	0.338	0.261	0.480	0.227	0.125	0.449	0.161

Table S5 The detailed experimental results on simulated single-cell datasets with size 50 under SIGN=0.

Size	Dataset	Method	AUROC	AUPR	StAcc	Recal	Pre	FPR	F-measure
50	<i>Ecoli1</i>	LogBTF	0.583	0.086	0.762	0.355	0.038	0.227	0.069
		SCODE	0.508	0.024	0.025	1.000	0.025	1.000	0.048
		GRISLI	0.512	0.024	—	—	—	—	—
		SINCERITIES	0.495	0.023	0.814	0.157	0.019	0.172	0.033
		GENIE3	0.427	0.074	0.750	0.113	0.012	0.233	0.022
		TREGRESS	0.490	0.161	0.758	0.274	0.029	0.230	0.053
		ARACNE	0.495	0.169	0.755	0.290	0.031	0.233	0.056
50	<i>Ecoli2</i>	CLR	0.470	0.143	0.745	0.242	0.025	0.242	0.045
		LogBTF	0.603	0.065	0.760	0.415	0.058	0.228	0.102
		SCODE	0.583	0.050	0.033	1.000	0.033	1.000	0.064
		GRISLI	0.526	0.041	—	—	—	—	—
		SINCERITIES	0.467	0.023	0.838	0.065	0.015	0.138	0.024
		GENIE3	0.442	0.104	0.744	0.159	0.022	0.237	0.039
		TREGRESS	0.479	0.158	0.746	0.256	0.035	0.237	0.062
50	<i>Yeast1</i>	ARACNE	0.476	0.151	0.748	0.244	0.034	0.235	0.060
		CLR	0.432	0.091	0.741	0.134	0.019	0.239	0.033
		LogBTF	0.539	0.053	0.818	0.247	0.046	0.163	0.077
		SCODE	0.520	0.039	0.031	1.000	0.031	1.000	0.060
		GRISLI	0.493	0.028	—	—	—	—	—
		SINCERITIES	0.459	0.019	0.822	0.058	0.010	0.156	0.018
		GENIE3	0.476	0.098	0.812	0.143	0.027	0.167	0.045
50	<i>Yeast2</i>	TREGRESS	0.504	0.135	0.815	0.208	0.038	0.165	0.065
		ARACNE	0.305	0.274	0.408	0.506	0.026	0.596	0.050
		CLR	0.500	0.135	0.809	0.208	0.037	0.172	0.063
		LogBTF	0.518	0.072	0.745	0.250	0.072	0.221	0.112
		SCODE	0.493	0.069	0.064	1.000	0.064	1.000	0.120
		GRISLI	0.533	0.068	—	—	—	—	—
		SINCERITIES	0.454	0.040	0.797	0.056	0.021	0.159	0.031
50	<i>Yeast3</i>	GENIE3	0.490	0.189	0.746	0.256	0.074	0.221	0.114
		TREGRESS	0.483	0.181	0.743	0.244	0.070	0.223	0.108
		ARACNE	0.368	0.291	0.494	0.488	0.062	0.505	0.110
		CLR	0.479	0.183	0.733	0.250	0.068	0.234	0.107
		LogBTF	0.530	0.097	0.795	0.225	0.094	0.162	0.132
		SCODE	0.423	0.059	0.069	1.000	0.069	1.000	0.129
		GRISLI	0.503	0.069	—	—	—	—	—
50	<i>Yeast3</i>	SINCERITIES	0.463	0.044	0.803	0.058	0.025	0.148	0.035
		GENIE3	0.489	0.151	0.788	0.173	0.072	0.166	0.102
		TREGRESS	0.497	0.163	0.790	0.191	0.079	0.165	0.112
		ARACNE	0.446	0.240	0.633	0.364	0.072	0.347	0.121
		CLR	0.469	0.124	0.780	0.133	0.055	0.171	0.077

Table S6 The detailed experimental results on simulated single-cell datasets with size 100 under SIGN=0.

Size	Dataset	Method	AUROC	AUPR	StAcc	Recal	Pre	FPR	F-measure
100	<i>Ecoli1</i>	LogBTF	0.523	0.027	0.822	0.216	0.016	0.170	0.029
		SCODE	0.539	0.013	0.013	1.000	0.013	1.000	0.025
		GRISLI	0.515	0.014	—	—	—	—	—
		SINCERITIES	0.442	0.006	0.855	0.019	0.001	0.136	0.003
		GENIE3	0.498	0.112	0.822	0.200	0.015	0.170	0.027
		TREGRESS	0.535	0.159	0.824	0.288	0.021	0.170	0.039
		ARACNE	0.510	0.137	0.810	0.248	0.017	0.183	0.032
		CLR	0.517	0.138	0.821	0.248	0.018	0.172	0.033
100	<i>Ecoli2</i>	LogBTF	0.546	0.029	0.795	0.277	0.017	0.199	0.031
		SCODE	0.539	0.013	0.013	1.000	0.013	1.000	0.025
		GRISLI	0.507	0.012	—	—	—	—	—
		SINCERITIES	0.468	0.008	0.863	0.057	0.005	0.128	0.009
		GENIE3	0.433	0.050	0.791	0.084	0.005	0.201	0.009
		TREGRESS	0.496	0.134	0.791	0.244	0.014	0.202	0.027
		ARACNE	0.500	0.172	0.753	0.319	0.016	0.242	0.030
		CLR	0.496	0.133	0.791	0.244	0.014	0.203	0.027
100	<i>Yeast1</i>	LogBTF	0.528	0.033	0.840	0.199	0.022	0.149	0.040
		SCODE	0.541	0.018	0.017	1.000	0.017	1.000	0.033
		GRISLI	0.485	0.016	—	—	—	—	—
		SINCERITIES	0.455	0.008	0.880	0.007	0.001	0.107	0.002
		GENIE3	0.505	0.110	0.840	0.187	0.021	0.149	0.037
		TREGRESS	0.492	0.094	0.839	0.157	0.017	0.150	0.031
		ARACNE	0.379	0.287	0.490	0.548	0.018	0.511	0.034
		CLR	0.488	0.091	0.837	0.151	0.017	0.151	0.030
100	<i>Yeast2</i>	LogBTF	0.531	0.056	0.809	0.229	0.052	0.168	0.085
		SCODE	0.545	0.051	0.039	1.000	0.039	1.000	0.075
		GRISLI	0.465	0.037	—	—	—	—	—
		SINCERITIES	0.456	0.019	0.848	0.015	0.004	0.122	0.007
		GENIE3	0.478	0.110	0.803	0.152	0.035	0.171	0.056
		TREGRESS	0.479	0.113	0.802	0.157	0.036	0.172	0.058
		ARACNE	0.349	0.289	0.461	0.522	0.038	0.541	0.070
		CLR	0.474	0.109	0.798	0.152	0.034	0.176	0.055
100	<i>Yeast3</i>	LogBTF	0.514	0.063	0.795	0.200	0.064	0.170	0.097
		SCODE	0.482	0.052	0.055	1.000	0.055	1.000	0.104
		GRISLI	0.517	0.061	—	—	—	—	—
		SINCERITIES	0.468	0.033	0.837	0.035	0.016	0.119	0.022
		GENIE3	0.466	0.109	0.787	0.129	0.041	0.174	0.063
		TREGRESS	0.498	0.156	0.794	0.203	0.065	0.172	0.098
		ARACNE	0.358	0.296	0.475	0.512	0.054	0.527	0.097
		CLR	0.489	0.145	0.789	0.187	0.058	0.176	0.089

9. Results on synthetic bulk gene expression data

9.1. Case 1: SIGN = 1

In this experiment, we also use the LOOCV method (James et al., 2013) to select the optimal tuning parameter and predict the expression state of each gene on each dataset to investigate the performance of LogBTF in terms of predictive AUC value. Figure S7 shows the experiment results. Figure S7(a) shows that the means of the AUC value and DyAcc value on 15 datasets are both larger than 0.98. Considering that the SINCERITIES method is also functional in evaluating the inferred network from the aspect of activating and inhibitive regulations between regulator and target genes, we compare the StAcc and AUROC indexes of LogBTF with SINCERITIES.

From Figure S7(b), one can see that the StAcc values of LogBTF are larger than SINCERITIES in most cases when the network size is small, but the mean StAcc value is smaller than SINCERITIES. While as the network size increases, our LogBTF method shows strong superiority when compared with SINCERITIES, both from single experimental results and mean value. Furthermore, the larger the number of nodes in the network, the higher the StAcc value of our method. Figure S7(c) shows the results of the AUROC value comparing the LogBTF with the SINCERITIES method on three types of datasets of different sizes. We can see that our proposed LogBTF method performs better than SINCERITIES for all network sizes. Also, there seems to be a trend that when the number of nodes/genes in the network increases, the difference between these two methods gradually decreases (under the premise that LogBTF is better than SINCERITIES).

9.2. Case 2: SIGN = 0

When omitting the activation or inhibition functions, we only study the regulatory relationships and the regulator/target roles among genes, i.e., SIGN=0. In this case, the signature of regression or correlation coefficient does not work, which means that all coefficients can be taken as the measure like weights. Thus these six network inference methods have a standard benchmark for making comparisons. For more reliable performance validation, we further compare LogBTF with five methods, including SINCERITIES that we just discussed, GENIE3, TIGRESS, ARACNE

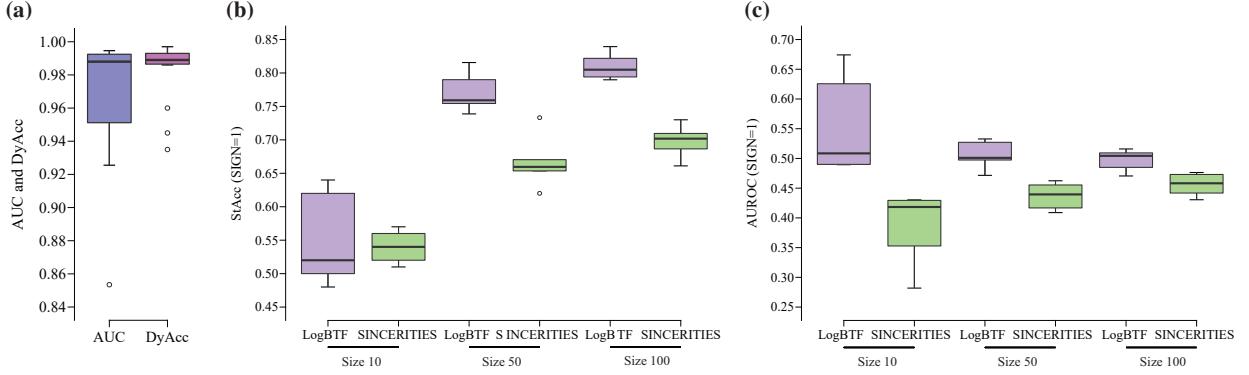


Figure S7 The prediction results of LogBTF method on DREAM3 change dataset in the case of SNGN=1. (a) The AUC and DyAcc values on 15 datasets in DREAM3 change. (b) The StAcc and (c) AUROC comparison of LogBTF and SINCERITIES methods on three types of datasets of different sizes.

and CLR. As we all know, the inferred interactions/links of four methods, except SINCERITIES, require a cut-off value to truncate the weight or correlation coefficient to ensure comparability with our proposed method. In the experiments, we determine the cut-off threshold value corresponding to the number κ of edges predicted by our method by arranging all edges' weights or correlations in descending order. The comparing results of StAcc, AUROC and Pre indexes on all available datasets are shown in Figure S8.

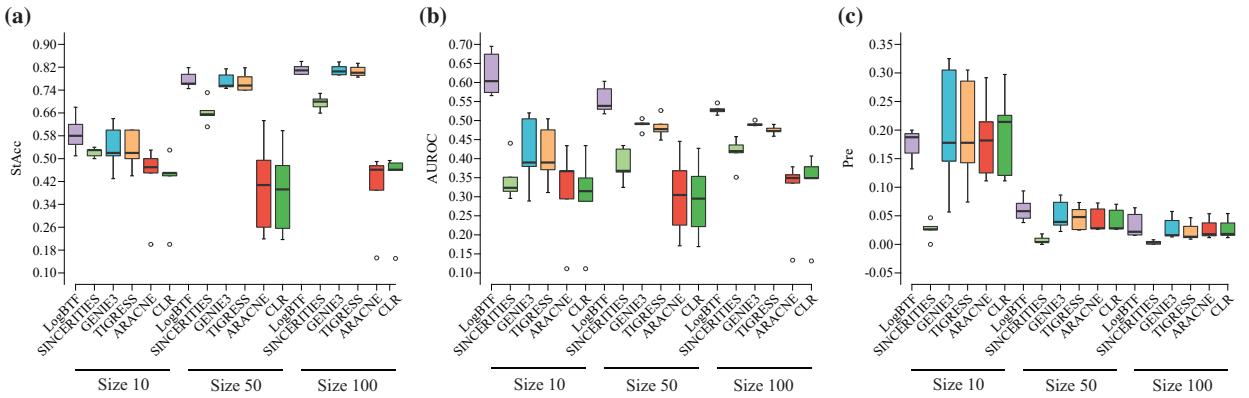


Figure S8 The comparing results of LogBTF with the other five methods on three type datasets with different gene sizes from DREAM3 change in the case of SNGN=0. (a) The StAcc values. (b) The AUROC value. (c) Pre value.

Figure S8(a) shows the StAcc values among all methods, where the LogBTF method achieves the best results regardless of the network size of the dataset. In particular, when the size of network nodes is relatively large (such as size 100), our method has the largest mean StAcc and the smallest standard deviation in five datasets experiments. Figure S8(b) displays that our proposed method is significantly superior to the other five comparable methods in terms of AUROC evaluation, regardless of the size of the network. Figure S8(c) gives the Pre values comparing the inferred network relationship with sourced gold standards. In the five datasets with 10 genes, the LogBTF method achieves worse Pre values and whose mean Pre value is also lower than the CLR method, but it should be noted that whose standard deviation is very small compared with GENIE3, TIGRESS, ARACNE and CLR methods. And, this situation is also comfortable for the case of size 50 and size 100. As for SINCERITIES, it obtains the smallest standard deviation while its Pre values are the worst among all methods on all 15 datasets. All experimental results, including other estimation criteria (such as Recal, FPR and F-measure), are available in Table S7. They reflect the stability and applicability of the LogBTF method for large-scale networks.

Table S7 The detailed experimental results on simulated “bulk” gene expression datasets.

# of genes	Dataset	Method	# of min genes	# of max genes	# of regulatory relationships	Mean AUC	DyAcc	AUROC	AUPR	StAcc	Recal	Pre	FPR	F_- measure	No activate or inhibit function (SIGN = 0)				F_- measure				
															Activate or inhibit function (SIGN = 1)								
10	Ecoli	LogBTIF	3	6	45	0.952	0.960	0.674	0.199	0.620	0.818	0.200	0.404	0.321	0.674	0.200	0.620	0.818	0.250	0.046	0.404	0.321	
		SINCER-GENIE3	2	6	45	0.957	0.960	0.282	0.095	0.510	0.000	0.000	0.457	0.000	0.440	0.084	0.000	0.530	0.250	0.047	0.446	0.078	
		TIGRESS	45	45	45	0.994	0.990	0.489	0.141	0.520	0.500	0.125	0.477	0.200	0.401	0.468	0.600	0.727	0.178	0.416	0.416	0.286	
	ARACNE	CLR	45	45	45	0.994	0.990	0.489	0.141	0.520	0.500	0.125	0.477	0.200	0.401	0.468	0.600	0.727	0.178	0.416	0.416	0.286	
		LogBTIF	4	7	48	0.950	0.995	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.551	0.551	0.209	
		SINCER-GENIE3	2	6	48	0.953	0.953	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.573	0.573	0.203	
Yeast1	Ecoli2	LogBTIF	2	8	53	0.950	0.995	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.536	0.536	0.286	
		SINCER-GENIE3	3	5	53	0.948	0.948	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.536	0.536	0.286	
		TIGRESS	48	48	48	0.994	0.990	0.489	0.141	0.520	0.500	0.125	0.477	0.200	0.401	0.468	0.600	0.736	0.178	0.416	0.416	0.286	
	ARACNE	CLR	48	48	48	0.994	0.990	0.489	0.141	0.520	0.500	0.125	0.477	0.200	0.401	0.468	0.600	0.736	0.178	0.416	0.416	0.286	
		LogBTIF	2	7	53	0.950	0.995	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.536	0.536	0.286	
		SINCER-GENIE3	3	5	53	0.948	0.948	0.490	0.063	0.480	0.571	0.075	0.527	0.133	0.349	0.399	0.401	0.401	0.125	0.536	0.536	0.286	
Yeast2	Ecoli	LogBTIF	53	53	53	0.926	0.935	0.509	0.222	0.500	0.765	0.220	0.554	0.342	0.323	0.402	0.179	0.351	0.522	0.522	0.800	0.214	0.518
		CLR	53	59	59	0.926	0.935	0.509	0.222	0.500	0.765	0.220	0.554	0.342	0.323	0.402	0.179	0.351	0.522	0.522	0.800	0.214	0.518
		SINCER-GENIE3	2	6	59	0.926	0.935	0.509	0.222	0.500	0.765	0.220	0.554	0.342	0.323	0.402	0.179	0.351	0.522	0.522	0.800	0.214	0.518
	TIGRESS	ARACNE	59	59	59	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.554	0.379	0.390	0.548	0.520	0.720	0.305	0.547	0.547	0.429	
		CLR	59	59	59	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.554	0.379	0.390	0.548	0.520	0.720	0.305	0.547	0.547	0.429	
		LogBTIF	2	5	59	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.554	0.379	0.390	0.548	0.520	0.720	0.305	0.547	0.547	0.429	
Yeast3	Ecoli	LogBTIF	2	5	59	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.554	0.379	0.390	0.548	0.520	0.720	0.305	0.547	0.547	0.429	
		SINCER-GENIE3	2	32	59	0.853	0.945	0.626	0.335	0.640	0.611	0.275	0.554	0.379	0.390	0.548	0.520	0.720	0.305	0.547	0.547	0.429	
		TIGRESS	40	40	40	0.429	0.429	0.560	0.112	0.560	0.200	0.125	0.550	0.154	0.351	0.520	0.503	0.640	0.591	0.325	0.346	0.349	0.375
	ARACNE	CLR	40	40	40	0.429	0.429	0.560	0.112	0.560	0.200	0.125	0.550	0.154	0.351	0.520	0.503	0.640	0.591	0.325	0.346	0.349	0.375
		LogBTIF	40	40	40	0.429	0.429	0.560	0.112	0.560	0.200	0.125	0.550	0.154	0.351	0.520	0.503	0.640	0.591	0.325	0.346	0.349	0.375
		SINCER-GENIE3	40	40	40	0.429	0.429	0.560	0.112	0.560	0.200	0.125	0.550	0.154	0.351	0.520	0.503	0.640	0.591	0.325	0.346	0.349	0.375
50	Ecoli	LogBTIF	3	23	576	0.990	0.991	0.527	0.032	0.759	0.259	0.024	0.230	0.044	0.583	0.086	0.762	0.355	0.038	0.227	0.069	0.069	
		SINCER-GENIE3	5	26	576	0.920	0.976	0.576	0.997	0.533	0.030	0.754	0.284	0.032	0.233	0.058	0.603	0.065	0.762	0.355	0.038	0.227	0.069
		TIGRESS	45	789	576	0.970	0.997	0.533	0.417	0.011	0.670	0.157	0.010	0.319	0.019	0.491	0.172	0.752	0.280	0.065	0.320	0.069	
		ARACNE	576	576	576	0.994	0.990	0.533	0.417	0.011	0.670	0.157	0.010	0.319	0.019	0.491	0.172	0.752	0.280	0.065	0.320	0.069	
		CLR	4	20	585	0.970	0.997	0.533	0.417	0.011	0.670	0.157	0.010	0.319	0.019	0.491	0.172	0.752	0.280	0.065	0.320	0.069	
		LogBTIF	4	25	585	0.970	0.997	0.533	0.417	0.011	0.670	0.157	0.010	0.319	0.019	0.491	0.172	0.752	0.280	0.065	0.320	0.069	
		SINCER-GENIE3	3	25	585	0.970	0.997	0.533	0.417	0.011	0.670	0.157	0.010	0.319	0.019	0.491	0.172	0.752	0.280	0.065	0.320	0.069	
		TIGRESS	45	417	585	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		ARACNE	45	417	585	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		CLR	45	417	585	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		LogBTIF	4	16	577	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		SINCER-GENIE3	3	23	577	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		TIGRESS	45	417	577	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		ARACNE	45	417	577	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		CLR	45	417	577	0.988	0.987	0.472	0.039	0.739	0.167	0.043	0.226	0.068	0.518	0.072	0.745	0.256	0.068	0.320	0.069		
		LogBTIF	4	16	546	0.995	0.995	0.501	0.440	0.027	0.733	0.099	0.027	0.226	0.043	0.520	0.097	0.755	0.274	0.069	0.320	0.069	
		SINCER-GENIE3	5	16	546	0.995	0.995	0.501	0.440	0.027	0.733	0.099	0.027	0.226	0.043	0.520	0.097	0.755	0.274	0.069	0.320	0.069	
		TIGRESS	45	417	546	0.995	0.995	0.501	0.440	0.027	0.733	0.099	0.027	0.226	0.043	0.520	0.097	0.755	0.274	0.069	0.320	0.069	
		ARACNE	45	417	546	0.995	0.995	0.501	0.440	0.027	0.733	0.099	0.027	0.226	0.043	0.520	0.097	0.755	0.274	0.069	0.320	0.069	
		CLR	45	417	546	0.995	0.995	0.501	0.440	0.027	0.733	0.099	0.027	0.226	0.043	0.520	0.097	0.755	0.274	0.069	0.320	0.069	

# of genes	Dataset	Method	# of min genes	# of max genes	# of regulatory relationships	Activate or inhibit function (SIGN = 1)						No activate or inhibit function (SIGN = 0)										
						Mean AUC	DyAcc	AUROC	AUPR	StAcc	Recal	Pre	FPR	F_- measure	AUROC	AUPR	StAcc	Recal	Pre	FPR	F_- measure	
100	Ecoli1	LogBTF	2	43	1708	0.987	0.986	0.516	0.020	0.822	0.203	0.015	0.170	0.027	0.523	0.027	0.822	0.216	0.016	0.170	0.029	
		SINCER-GENIE3	4	49	2833	0.983	0.986	0.476	0.005	0.710	0.118	0.004	0.285	0.008	0.458	0.005	0.709	0.012	0.000	0.286	0.001	
		TIGRESS			1708										0.501	0.117	0.822	0.208	0.015	0.170	0.028	
		ARACNE			1708										0.490	0.104	0.820	0.184	0.013	0.172	0.025	
		CLR			1708										0.390	0.381	0.744	0.015	0.615	0.015	0.030	
		LogBTF	4	34	1996	0.993	0.989	0.509	0.019	0.794	0.211	0.012	0.199	0.022	0.546	0.029	0.795	0.277	0.017	0.199	0.031	
Ecoli2	Ecoli2	SINCER-GENIE3	4	50	3335	0.996	0.993	0.431	0.005	0.661	0.200	0.005	0.335	0.011	0.251	0.004	0.659	0.14	0.000	0.336	0.001	
		TIGRESS			1996										0.488	0.120	0.794	0.218	0.013	0.199	0.025	
		ARACNE			1996										0.480	0.112	0.792	0.202	0.012	0.201	0.023	
		CLR			1996										0.431	0.133	0.849	0.012	0.856	0.023	0.023	
		LogBTF	5	29	1497	0.990	0.988	0.504	0.023	0.839	0.153	0.016	0.150	0.029	0.528	0.029	0.840	0.199	0.022	0.149	0.040	
		SINCER-GENIE3	3	36	2602	1497	0.998	0.458	0.008	0.730	0.150	0.008	0.262	0.015	0.420	0.008	0.729	0.063	0.003	0.263	0.006	
Yeast1	Yeast1	TIGRESS			1497										0.487	0.087	0.839	0.145	0.016	0.150	0.029	
		ARACNE			1497										0.459	0.054	0.834	0.084	0.009	0.154	0.017	
		CLR			1497										0.379	0.387	0.490	0.548	0.018	0.511	0.034	
		LogBTF	4	35	1700	0.992	0.989	0.485	0.029	0.805	0.140	0.029	0.171	0.048	0.579	0.026	0.566	0.018	0.517	0.035	0.035	
		SINCER-GENIE3	4	46	2743	1700	0.998	0.442	0.017	0.702	0.132	0.016	0.279	0.028	0.446	0.019	0.700	0.072	0.008	0.281	0.014	
		TIGRESS			1700										0.491	0.128	0.805	0.183	0.042	0.169	0.068	
Yeast2	Yeast2	ARACNE			1700										0.471	0.102	0.801	0.139	0.032	0.173	0.051	
		CLR			1700										0.349	0.289	0.461	0.522	0.038	0.541	0.070	
		LogBTF	4	38	2789	1718	0.994	0.993	0.471	0.034	0.790	0.114	0.033	0.175	0.051	0.514	0.063	0.795	0.200	0.064	0.170	0.097
		SINCER-GENIE3	3	42	1718	1718	0.994	0.993	0.473	0.030	0.687	0.135	0.023	0.286	0.039	0.436	0.024	0.682	0.033	0.005	0.290	0.009
		TIGRESS			1718										0.489	0.141	0.793	0.180	0.058	0.171	0.087	
		ARACNE			1718										0.473	0.121	0.786	0.149	0.047	0.177	0.071	
Yeast3	Yeast3	CLR			1718										0.358	0.296	0.475	0.512	0.054	0.527	0.097	
		LogBTF	4	34	2789	1718	0.994	0.993	0.471	0.034	0.790	0.114	0.033	0.175	0.051	0.514	0.063	0.795	0.200	0.064	0.170	0.097
		SINCER-GENIE3	3	42	1718	1718	0.994	0.993	0.473	0.030	0.687	0.135	0.023	0.286	0.039	0.436	0.024	0.682	0.033	0.005	0.290	0.009
		TIGRESS			1718										0.473	0.121	0.786	0.149	0.047	0.177	0.071	
		ARACNE			1718										0.349	0.296	0.475	0.512	0.054	0.527	0.097	
		CLR			1718										0.349	0.307	0.459	0.534	0.054	0.545	0.098	

10. Results on real single-cell data

10.1. Matsumoto and hHEP data

Figure S9 shows the comparison results of AUROC and computational time of LogBTF with the other six methods on hHEP dataset, where LogBTF method behaves with higher AUROC values than those of the other six methods and SINCERITIES shows a second-best AUROC. The detailed comparison results of LogBTF with the other seven methods on Matsumoto and hHEP dataset are shown in Table S8. Regarding StAcc, SINCERITIES performs best in both Matsumoto and specific hHEP datasets. All other methods are worse than those two methods in StAcc. For GRISLI, Aubin-Frankowski & Vert (2020) has stressed that the performance of GRISLI method is impacted by two main parameters and the choice of the parameters is a difficult question. Considering that hHEP includes nearly one thousand genes, here we take $R = L = 10$ in its source code for saving runtimes. In particular, ARACNE and CLR are two inference algorithms based on spearman estimator/correlation, but the inferred results on these two large real networks are very different. Thus, ARACNE seems not appropriate in the case of scRNA-seq data with pseudo-time, as mentioned in prior work that it is not for the time series data (Cantone et al., 2009). In particular, we note that GRISLI and TIGRESS are not suitable for large-scale network inference analysis due to the expensive computational time, though they can also obtain satisfactory inference results. Clearly, our LogBTF method performs better on both Matsumoto and hHEP scRNA-seq datasets.

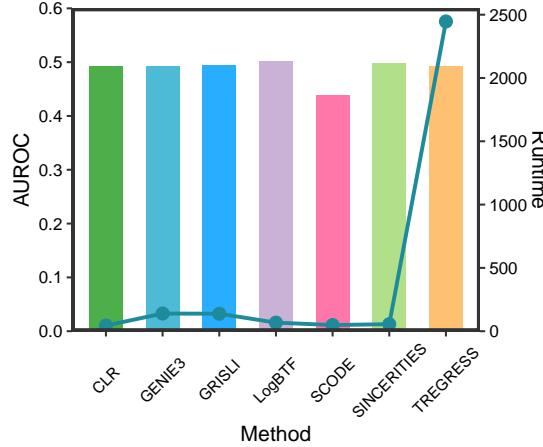


Figure S9 The performance comparison among different methods.

Table S8 The performance comparison of inferring GRNs on two real scRNA-seq datasets under SIGN=0, where the ‘-’ represents that the evaluation values are not available using the other inferring seven methods.

Dataset	Method	Mean AUC	DyAcc	AUROC	AUPR	StAcc	Recal	Pre	FPR	F-measure	Time (min)
Matsumoto scRNA-seq data (373*100)	LogBTF	0.577	0.697	0.507	0.072	0.755	0.218	0.070	0.207	0.106	11.741
	GRISLI	-	-	0.502	0.426	-	-	-	-	-	14.256
	SCODE	-	-	0.478	0.063	0.067	1.000	0.067	1.000	0.125	0.249
	SINCERITIES	-	-	0.491	0.064	0.778	0.156	0.059	0.178	0.086	7.502
	GENIE3	-	-	0.483	0.170	0.755	0.218	0.070	0.207	0.106	3.911
	TIGRESS	-	-	0.492	0.186	0.754	0.245	0.077	0.210	0.117	150.604
	ARACNE	-	-	0.000	0.533	0.067	1.000	0.067	1.000	0.125	0.049
Specific hHEP scRNA-seq data (425*948)	CLR	-	-	0.481	0.167	0.754	0.213	0.068	0.207	0.104	0.048
	LogBTF	0.708	0.731	0.501	0.012	0.950	0.043	0.012	0.040	0.019	25.174
	GRISLI	-	-	0.495	0.013	-	-	-	-	-	95.494
	SCODE	-	-	0.438	0.009	0.011	1.000	0.011	1.000	0.022	5.93
	SINCERITIES	-	-	0.498	0.010	0.959	0.026	0.009	0.031	0.014	12.893
	GENIE3	-	-	0.493	0.023	0.950	0.028	0.008	0.040	0.012	97.019
	TIGRESS	-	-	0.492	0.022	0.948	0.027	0.007	0.041	0.011	2403.995
	ARACNE	-	-	0.000	0.506	0.011	1.000	0.011	1.000	0.022	1.934
	CLR	-	-	0.493	0.023	0.949	0.027	0.008	0.040	0.012	1.896

10.2. LMPP data

For the LMPP scRNA-seq dataset, LogBTF method infers 306 regulatory relationships, shown in Figure S10.

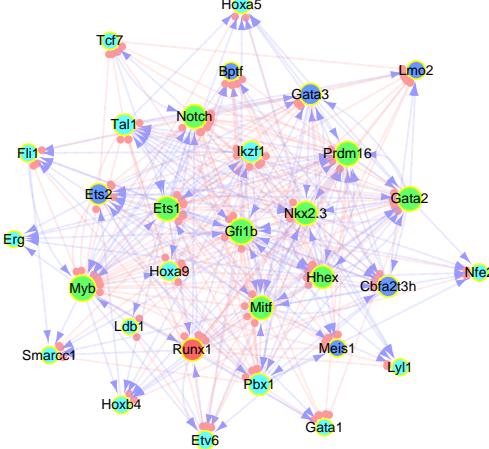


Figure S10 The network topology of reconstructed GRN that reflects the differentiation of HSCs into LMPP, where the node's size is proportional to its degree (sum of out-degree and in-degree). The genes with the top 15 activations are colored in blue, the genes with the top 10 inhibitions are colored in red, and the genes with both high activation and high inhibitions regulation relationships are marked in green (9 in total).

10.3. NOA enrichment results

The functional enrichment analysis results (P -value < 0.05) enriched by NOA are listed in Table S9.

11. Discussion

Many existing gene regulatory network inference algorithms may suffer from the over-fitting problem, which means that the inferred in-degree tends to be larger than the real in-degree of the nodes (Liu et al., 2021). However, our LogBTF method embeds regularized logistic regression into the Boolean threshold network model to automatically make regulatory link selection when fitting the input gene using input gene expression data. LogBTF takes regulatory relationship inference for each gene as an embedded feature selection process and successfully handles the over-fitting problem.

Most recently developed inference methods that need to determine the maximum in-degree of each gene considering the sparsity of networks. For example, Liu et al. fixed the maximum in-degree as five for each gene when applying a Boolean network model to real-world gene regulatory network inference problems (Liu et al., 2021). Barman and Kwon set the maximum number of regulatory genes as three-fifths of the total number of all genes for the Boolean network inference to save the search cost of the neural network (Barman & Kwon, 2020). Barman and Kwon also constrained the maximum in-degree for the target gene to reduce the search space of the genetic algorithm when conducting the Boolean network inference problems (Barman & Kwon, 2018). In contrast, our proposed LogBTF method does not need to set the maximum in-degree of each target node in advance when inferring gene regulatory network using the Boolean network model. LogBTF method automatically determines the number of regulatory genes according to the best-found solution to achieve a sparse regulatory network and perform the four levels (I, II, III and IV), as discussed in our Introduction section, of gene regulatory network inference problems.

In addition, the LogBTF method is an interpretable network inference method, where the regulatory rules are represented in Boolean threshold functions. Namely, for the normalized coefficients $\hat{\theta}_i$, the ratio of the number of zero elements to the number of all components determines the sparsity of the inferred regulatory relationship among all genes, the value of no-zero element (large or small) determines the regulatory strength (strong or weak) between the regulator genes and the target gene, and the sign of non-zero element (positive/negative) determines whether the regulation is activated or inhibited.

Table S9 The functional enrichment analysis results enriched by NOA (Wang et al., 2011).

GO: term	P-value	Corrected P-value	R	T	G	O	Term name
GO:0048518	2.00E-06	3.50E-04	379	177	91	62	positive regulation of biological process
GO:0010468	1.50E-05	0.0026	379	177	325	166	regulation of gene expression
GO:0010556	1.50E-05	0.0026	379	177	325	166	regulation of macromolecule biosynthetic process
GO:0019219	1.50E-05	0.0026	379	177	325	166	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0019222	1.50E-05	0.0026	379	177	325	166	regulation of metabolic process
GO:0031323	1.50E-05	0.0026	379	177	325	166	regulation of cellular metabolic process
GO:0031326	1.50E-05	0.0026	379	177	325	166	regulation of cellular biosynthetic process
GO:0045449	1.50E-05	0.0026	379	177	325	166	regulation of transcription
GO:0051171	1.50E-05	0.0026	379	177	325	166	regulation of nitrogen compound metabolic process
GO:0060255	1.50E-05	0.0026	379	177	325	166	regulation of macromolecule metabolic process
GO:0080090	1.50E-05	0.0026	379	177	325	166	regulation of primary metabolic process
GO:0009889	1.50E-05	0.0026	379	177	325	166	regulation of biosynthetic process
GO:0048513	5.50E-05	0.0094	379	177	91	59	organ development
GO:0048522	5.80E-05	0.0099	379	177	78	52	positive regulation of cellular process
GO:0010557	7.50E-04	0.1291	379	177	55	37	positive regulation of macromolecule biosynthetic process
GO:0010604	7.50E-04	0.1291	379	177	55	37	positive regulation of macromolecule metabolic process
GO:0010628	7.50E-04	0.1291	379	177	55	37	positive regulation of gene expression
GO:0031325	7.50E-04	0.1291	379	177	55	37	positive regulation of cellular metabolic process
GO:0031328	7.50E-04	0.1291	379	177	55	37	positive regulation of cellular biosynthetic process
GO:0045935	7.50E-04	0.1291	379	177	55	37	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0045941	7.50E-04	0.1291	379	177	55	37	positive regulation of transcription
GO:0051173	7.50E-04	0.1291	379	177	55	37	positive regulation of nitrogen compound metabolic process
GO:0009891	7.50E-04	0.1291	379	177	55	37	positive regulation of biosynthetic process
GO:0009893	7.50E-04	0.1291	379	177	55	37	positive regulation of metabolic process
GO:0034641	0.0016	0.2842	379	177	276	142	cellular nitrogen compound metabolic process
GO:0034645	0.0016	0.2842	379	177	276	142	cellular macromolecule biosynthetic process
GO:0043170	0.0016	0.2842	379	177	276	142	macromolecule metabolic process
GO:0044237	0.0016	0.2842	379	177	276	142	cellular metabolic process
GO:0044238	0.0016	0.2842	379	177	276	142	primary metabolic process
GO:0044249	0.0016	0.2842	379	177	276	142	cellular biosynthetic process
GO:0044260	0.0016	0.2842	379	177	276	142	cellular macromolecule metabolic process
GO:0006139	0.0016	0.2842	379	177	276	142	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0006350	0.0016	0.2842	379	177	276	142	transcription
GO:0006807	0.0016	0.2842	379	177	276	142	nitrogen compound metabolic process
GO:0008152	0.0016	0.2842	379	177	276	142	metabolic process
GO:0090304	0.0016	0.2842	379	177	276	142	nucleic acid metabolic process
GO:0009058	0.0016	0.2842	379	177	276	142	biosynthetic process
GO:0009059	0.0016	0.2842	379	177	276	142	macromolecule biosynthetic process
GO:0006357	0.0019	0.3249	379	177	91	55	regulation of transcription from RNA polymerase II promoter
GO:0051239	0.0023	0.4083	379	177	78	48	regulation of multicellular organismal process
GO:0045893	0.0033	0.5708	379	177	36	25	positive regulation of transcription, DNA-dependent
GO:0045944	0.0033	0.5708	379	177	36	25	positive regulation of transcription from RNA polymerase II promoter
GO:0051254	0.0033	0.5708	379	177	36	25	positive regulation of RNA metabolic process
GO:0032502	0.0049	0.848	379	177	210	111	developmental process
GO:0051094	0.0055	0.9563	379	177	10	9	positive regulation of developmental process
GO:0048568	0.008	1	379	177	15	12	embryonic organ development
GO:0048869	0.0092	1	379	177	66	40	cellular developmental process
GO:0022603	0.0099	1	379	177	6	6	regulation of anatomical structure morphogenesis
GO:0045765	0.0099	1	379	177	6	6	regulation of angiogenesis
GO:0050793	0.0185	1	379	177	66	39	regulation of developmental process
GO:0030154	0.0232	1	379	177	55	33	cell differentiation

References

- Aubin-Frankowski, P.-C., & Vert, J.-P. (2020). Gene regulation inference from single-cell rna-seq data with linear differential equations and velocity inference. *Bioinformatics*, 36, 4774–4780. <https://doi.org/10.1093/bioinformatics/btaa576>.
- Barman, S., & Kwon, Y.-K. (2018). A boolean network inference from time-series gene expression data using a genetic algorithm. *Bioinformatics*, 34, i927–i933. <https://doi.org/10.1093/bioinformatics/bty584>.
- Barman, S., & Kwon, Y.-K. (2020). A neuro-evolution approach to infer a boolean network from time-series gene expressions. *Bioinformatics*, 36, i762–i769. <https://doi.org/10.1093/bioinformatics/btaa840>.
- Bradley, A. P. (1997). The use of the area under the roc curve in the evaluation of machine learning algorithms. *Pattern Recognition*, 30, 1145–1159. [https://doi.org/10.1016/S0031-3203\(96\)00142-2](https://doi.org/10.1016/S0031-3203(96)00142-2).
- Camp, J. G., Sekine, K., Gerber, T., Loeffler-Wirth, H., Binder, H., Gac, M., Kanton, S., Kageyama, J., Damm, G., Seehofer, D. et al. (2017). Multi-lineage communication regulates human liver bud development from pluripotency. *Nature*, 546, 533–538. <https://doi.org/10.1038/nature22796>.
- Cantone, I., Marucci, L., Iorio, F., Ricci, M. A., Belcastro, V., Bansal, M., Santini, S., Di Bernardo, M., Di Bernardo, D., & Cosma, M. P. (2009). A yeast synthetic network for in vivo assessment of reverse-engineering and modeling approaches. *Cell*, 137, 172–181. <https://doi.org/10.1016/j.cell.2009.01.055>.
- Chen, S., & Mar, J. C. (2018). Evaluating methods of inferring gene regulatory networks highlights their lack of performance for single cell gene expression data. *BMC Bioinformatics*, 19, 1–21. <https://doi.org/10.1186/s12859-018-2217-z>.
- Dibaenia, P., & Sinha, S. (2020). Sergio: a single-cell expression simulator guided by gene regulatory networks. *Cell Systems*, 11, 252–271. <https://doi.org/10.1016/j.cels.2020.08.003>.
- Faith, J. J., Hayete, B., Thaden, J. T., Mogno, I., Wierzbowski, J., Cottarel, G., Kasif, S., Collins, J. J., & Gardner, T. S. (2007). Large-scale mapping and validation of escherichia coli transcriptional regulation from a compendium of expression profiles. *PLoS Biology*, 5, e8. <https://doi.org/10.1371/journal.pbio.0050008>.
- Hamey, F. K., Nestorowa, S., Kinston, S. J., Kent, D. G., Wilson, N. K., & Göttgens, B. (2017). Reconstructing blood stem cell regulatory network models from single-cell molecular profiles. *Proceedings of the National Academy of Sciences, USA*, 114, 5822–5829. <https://doi.org/10.1073/pnas.1610609114>.
- Hanley, J. A., & McNeil, B. J. (1982). The meaning and use of the area under a receiver operating characteristic (roc) curve. *Radiology*, 143, 29–36. <https://doi.org/10.1148/radiology.143.1.7063747>.
- Haury, A.-C., Mordelet, F., Vera-Licona, P., & Vert, J.-P. (2012). Tigress: trustful inference of gene regulation using stability selection. *BMC Systems Biology*, 6, 1–17. <https://doi.org/10.1186/1752-0509-6-145>.
- Huynh-Thu, V. A., Irrthum, A., Wehenkel, L., & Geurts, P. (2010). Inferring regulatory networks from expression data using tree-based methods. *PloS One*, 5, e12776. <https://doi.org/10.1371/journal.pone.0012776>.
- James, G., Witten, D., Hastie, T., & Tibshirani, R. (2013). *An introduction to statistical learning* volume 112. Springer. <https://link.springer.com/content/pdf/10.1007/978-1-0716-1418-1.pdf>.
- Li, L., & Liu, Z.-P. (2020). Biomarker discovery for predicting spontaneous preterm birth from gene expression data by regularized logistic regression. *Computational and Structural Biotechnology Journal*, 18, 3434–3446. <https://doi.org/10.1016/j.csbj.2020.10.028>.
- Liang, Y., Liu, C., Luan, X.-Z., Leung, K.-S., Chan, T.-M., Xu, Z.-B., & Zhang, H. (2013). Sparse logistic regression with a l1/2 penalty for gene selection in cancer classification. *BMC Bioinformatics*, 14, 1–12. <https://doi.org/10.1186/1471-2105-14-198>.
- Liu, X., Wang, Y., Shi, N., Ji, Z., & He, S. (2021). Gapore: Boolean network inference using a genetic algorithm with novel polynomial representation and encoding scheme. *Knowledge-Based Systems*, 228, 107277. <https://doi.org/10.1016/j.knosys.2021.107277>.
- Margolin, A. A., Nemenman, I., Basso, K., Wiggins, C., Stolovitzky, G., Favera, R. D., & Califano, A. (2006). Aracne: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. In *BMC Bioinformatics* (pp. 1–15). BioMed Central volume 7. <https://doi.org/10.1186/1471-2105-7-S1-S7>.
- Matsumoto, H., Kiryu, H., Furusawa, C., Ko, M. S., Ko, S. B., Gouda, N., Hayashi, T., & Nikaido, I. (2017). Scode: an efficient regulatory network inference algorithm from single-cell rna-seq during differentiation. *Bioinformatics*, 33, 2314–2321. <https://doi.org/10.1093/bioinformatics/btx194>.
- Müssel, C., Schmid, F., Blätte, T. J., Hopfensitz, M., Lausser, L., & Kestler, H. A. (2016). Bitrina—multiscale binarization and trinarization with quality analysis. *Bioinformatics*, 32, 465–468. <https://doi.org/10.1093/bioinformatics/btv591>.
- Papili Gao, N., Ud-Dean, S. M., Gandrillon, O., & Gunawan, R. (2018). Sincerities: inferring gene regulatory networks from time-stamped single cell transcriptional expression profiles. *Bioinformatics*, 34, 258–266. <https://doi.org/10.1093/bioinformatics/btx575>.
- Qiu, P. (2020). Embracing the dropouts in single-cell rna-seq analysis. *Nature Communications*, 11, 1–9. <https://doi.org/10.1038/s41467-020-14976-9>.
- Schaffter, T., Marbach, D., & Floreano, D. (2011). Genenetweaver: in silico benchmark generation and performance profiling of network inference methods. *Bioinformatics*, 27, 2263–2270. <https://doi.org/10.1093/bioinformatics/btr373>.
- Schwab, J. D., Ikonomi, N., Werle, S. D., Weidner, F. M., Geiger, H., & Kestler, H. A. (2021). Reconstructing boolean network ensembles from single-cell data for unraveling dynamics in the aging of human hematopoietic stem cells. *Computational and Structural Biotechnology Journal*, 19, 5321–5332. <https://doi.org/10.1016/j.csbj.2021.09.012>.
- Seçilmiş, D., Hillerton, T., Tjärnberg, A., Nelander, S., Nordling, T. E., & Sonnhammer, E. L. (2022). Knowledge of the perturbation design is essential for accurate gene regulatory network inference. *Scientific Reports*, 12, 1–12. <https://doi.org/10.1038/s41598-022-19005-x>.
- Treutlein, B., Lee, Q. Y., Camp, J. G., Mall, M., Koh, W., Shariati, S. A. M., Sim, S., Neff, N. F., Skotheim, J. M., Wernig, M. et al. (2016). Dissecting direct reprogramming from fibroblast to neuron using single-cell rna-seq. *Nature*, 534, 391–395. <https://doi.org/10.1038/nature18323>.
- Wang, J., Huang, Q., Liu, Z.-P., Wang, Y., Wu, L.-Y., Chen, L., & Zhang, X.-S. (2011). Noa: a novel network ontology analysis method. *Nucleic Acids Research*, 39, e87–e87. <https://doi.org/10.1093/nar/gkr251>.