Reconstruction of Gene Regulatory Networks using Multiple Datasets

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ABSTRACT

Motivation: Laboratory gene regulatory data for a species are sporadic. Despite the abundance of gene regulatory network algorithms that employ single data sets, few algorithms can combine the vast but disperse sources of data and extract the potential information. With a motivation to compensate for this shortage, we developed an algorithm called GENEREF that can accumulate information from multiple types of data sets in an iterative manner, with each iteration boosting the performance of the prediction results.

Results: The algorithm is examined extensively on data extracted from the quintuple DREAM4 networks. Many single-dataset algorithms and one multi-dataset algorithm were compared to test the performance of the algorithm. Results show that GENEREF surpasses non-ensemble state-of-the-art multi-perturbation algorithms on the selected networks and is competitive to present multiple-dataset algorithms. Specifically, it outperforms dynGENIE3 and is on par with iRatNet. Also, we argued that a scoring method solely based on the AUPR criterion would be more trustworthy than the traditional score.

Availability: The Python implementation along with the data sets and results can be downloaded from github.com/msaremi/GENEREF

1 Introduction

Gene Regulatory Networks (GRNs) model the crucial interactional patterns that control the molecular machinery in the cells of an organism¹. With the advent of high-throughput technologies, simultaneous expression data from several genes have been available for many species and many algorithms have been proposed that can reconstruct GRNs from these data. Though algorithms perform quite well on artificial data sets, the shortcomings the algorithms encounter when dealing with laboratory data, keeps deciphering GRN data an open challenge in the field of systems biology. Multiplicity and variety of genomic data sets gathered for a species provide an opportunity to develop algorithms that employ multiple data sets to extract more information and yield more accurate results in the real world challenges. In this paper, we provide an algorithm, named GENEREF (GEne NEtwork inference with REgularized Forests), that brings together the idea of boosting in machine learning² and the capability of feature scoring in decision trees into a single model. The model is capable of using multiple types of data sets for the task of GRN reconstruction in arbitrary orders.

There are typically two types of transcriptome data that are used to reconstruct GRNs: Steady-state data and time series data. The steady-state data are obtained by applying simultaneous perturbations on a number of genes and measuring the expressions after the network reaches a steady state. Data of this type is plentiful. In the case of multiple perturbations, the system can be corresponding to profiles acquired from different donors or biological replicates. The time series data on the other hand can in principle be more informative, since they capture the dynamic behaviour of system throughout the time. Despite the temporal information, they are less available due to the capturing and timing difficulties in the process. The systematical issues that arise with regards to this type of data sets include rarity of fairly uniform cell populations to sample over time, de-synchronization of cells during the experiment, and high costs of dense sampling of the population³.

In the realm of steady state data, ensemble methods based on regressor trees have shown to be one of the most effective approaches to extract the relation between the genes in a GRN⁴–⁷, with GENIE3 being the best performer in the DREAM4 challenge⁸. The DREAM4 challenge measures performance of variant algorithms on the reverse engineering of five steady state in silico networks. GENIE3 divides a problem with g genes into g sub-problems and proceeds to solve each problem by the intrinsic feature ranking property of random forests or extra trees. This work can be thought of as a multi-iteration algorithm where each iteration contains an extension to the GENIE3 algorithm applied on a data set. Unlike GENIE3, it can exploit multiple data sets and is less prone to over-fitting because of the regularization mechanisms it employs.
An advent algorithm that we will consider as the competing algorithm with our work is dynGENIE3. It is another extension to GENIE3, that introduces the differential equations governing the gene expression relationships to the typical tree-based ensemble methods used in GENIE3. The differential equations are formed based on the assumption that the transcription rate of the target gene is a function of the expression levels of all other genes and the decay rate of the products of the target gene itself. Compared to dynGENIE3, two advantages of our algorithm are that it does not depend on the decay rate parameters included in the differential equations and it can be extended to use different types of steady state data (e.g. double knock-out and multi-perturbation)

In sum, GENEREF is a highly scalable method that consistently increases the performance of the base algorithm (GENIE3), and outperforms the present multi-perturbation GRN algorithms. The algorithm also receives partly better scores as the competing algorithm (dynGENIE3) when using the typical scoring method. Moreover, GENEREF drastically outperforms dynGENIE3 when a new scoring method based only on the AUPR metric is used.

In what follows we will introduce our method. This paper also encompasses the evaluation of our algorithm on the in silico DREAM4 networks in comparison to other network reverse-engineering methods.

2 Methods
The problem of GRN reconstruction can be summarized as initially inferring rankings for the potential regulatory links from the data and eventually applying a threshold on these rankings to obtain a predicted network. GENEREF—along with many other algorithms in the field—deals only with the former issue and puts the latter aside. In what follows in this section, we provide the details of problem and the preliminary structures of the algorithm, along with its definition.

2.1 Network Inference with Tree-based Methods
Tree-based algorithms belong to the group of GRN inference methods that find the regulatory confidence between every two genes. This is done by finding a regression that predicts the expression of one gene based on the others. The basic idea is to decompose the problem of finding the regulatory links in $g$ genes into $g$ sub-problems. Each sub-problem will then be a regression problem in which the goal is to find the best prediction of the target gene based on the expression values of all the other genes.

Generally, tree-based methods—be them random forest or extra trees— are non-parametric algorithms that implement the idea of regression algorithms using regression trees: For each gene $G_j$ ($j = 1, ... , g$), a sub-problem is defined. In the $j$-th sub-problem, the expression values of the $j$-th gene will be considered as the target values and the expression values of all other genes will express the feature values. A regression is then solved for each problem using a tree-based method.

Trees have the innate capability of calculating feature importance scores. As the importance of the $i$-th feature in the $j$-th sub-problem gives an estimation about the certainty of the existence of an edge from gene $G_i$ to $G_j$, the tree-based algorithms merge these scores measured for each sub-problem to produce a final ranking for all edges in the network.

In a random forest setup, the average of the importance values of all features for a tree is close to the variance of the output variable. Therefore, to make the feature importances in all sub-problems homogeneous, the expression levels of all genes are typically normalized before the data is provided to the algorithm
d. In our work, we applied this pre-processing step for each data set before it is fed to a random forest.

2.2 Regularized Random Forests
Regularized Random Forests (RRFs) are an extension to the typical random forests that are superior to the regular forests in handling the issue of feature redundancy. When used as a feature selection technique, RRFs can run with the same setup as regular forests. Firstly introduced in 11, RRFs addressed the feature redundancy issue in the problem of feature selection by setting a penalty against adding new features to the currently selected features set and trying to maintain a minimal feature set.

In the original RRF framework, consider a feature selection problem with $g$ features represented as the feature set $F = \{ z_j | j = 1, ... , g \}$. Trees in a forest are formed in a top-down manner, selecting one feature and a split point for each node that is being added. The selected feature and its split point will determine the importance of each feature after the construction of all trees. As a tree in the forest is built, there will be more and more features based on which the learning data is split. We name the set of these features $F_S$. In every node of each tree, the node is configured based on the improvement function as follows:

$$\text{Improvement}_R(z_j, R) = \begin{cases} \lambda \text{Improvement}(z_j, R) & z_j \notin F_S \\ \text{Improvement}(z_j, R) & \text{otherwise}. \end{cases}$$

where $R$ is the node being configured and $\lambda$ is a regularization parameter in the range $[0, 1)$. Improvement(.) is the function used in regular random forests and in the case of a regression problem, can be any of the Mean Squared Error ($\text{MSE}$) or Mean
Absolute Error (MAE) gains. The Improvement function is tested for a randomly selected set of features and their randomly selected split points and the feature with the maximum improvement will be assigned to the node:

\[ z_R = \arg \max_{z_j} \{ \text{Improvement}_R(z_j, R) \} \]

(2)

where \( z_R \) is the feature assigned to node \( R \). \( F_S \) is then updated by the insertion of \( z_R \). It can be seen that as \( \lambda \) shrinks in equation 1, the tree biases more towards selecting features that are already in the selected feature set, and hence will be more picky about choosing features in computed nodes. By choosing the proper value for \( \lambda \), it will therefore become possible to omit the redundant features from the final selected features using an importance score.

After the construction of the whole forest, the relative importance of a feature \( z_j \) is measured by averaging the values of the Improvement function over all the nodes split by that feature:

\[ \text{Importance}(z_j) = \frac{1}{|N_{z_j}|} \sum_{R \in N_{z_j}} \text{Improvement}_R(z_j, R) \]

(3)

where \( N_{z_j} \) is the set of all nodes in the whole forest with the \( z_j \) feature. These importance measurements guide the procedure of feature selection in the RRF.

Empirical data indicate that GRNs are sparse networks with in-degree distribution concentrated around the mean connectivity. We speculate that the prediction performance of such networks can be enhanced using regularization methods. The regularization also reduces the probability of over-fitting. Based on these grounds, we utilize the same idea of RRFs and use an algorithm similar to the guided regularized random forests proposed by 16. We propose an iterative algorithm that uses various sets of learning data to reconstruct the final regulatory network. Each of these data sets can be seen as the output of an experiment. This work also inherits the decomposition approach used in many GRN reverse engineering algorithms like GENIE3 and TIGRESS. On each iteration, the algorithm picks a data set and uses the regularization parameters obtained from the previous iteration to guide a random forest. The network edges are at the end ranked based on the final feature importance values.

2.3 Problem Definition

Suppose that we want to discover the regulatory links between \( g \) genes \((G_1, \ldots, G_g)\) of a certain species. Also, consider there to be \( M \) learning data sets \( X_i \), \((l = 1, \ldots, M)\) of that species. Each data set represents a set of gene expression profiles obtained from a corresponding experiment. The \( l\)-th data set is comprised of \( N_l \) gene expression profiles. Each gene profile contains the expression values of all of the \( g \) genes.

Here, we consider two types of experiments: steady state experiments and time-series experiments. To differentiate between these types of data sets, we superscribe them with appropriate abbreviations. In our case, every arbitrary data set \( X_i \) is either represented as \( X_i^{SS} \) if it is a steady state data set, or as \( X_i^{TS} \) if it is a time-series data set.

For \( X_i^{SS} \), each element of the set equates the snapshot of all values of the genes at the same time after applying perturbations to a randomly selected subset of genes (i.e., randomly changing the basal expression of those genes) and waiting until the network reaches a steady state condition. The data set will have the following states:

\[ X_i^{SS} = \{ x_i(e_1), x_i(e_2), \ldots, x_i(e_{N_l}) \} \]

(4)

where \( x_i(e_l) \) is a vector containing the expression levels of all \( g \) genes at the \( i\)-th state in the data set:

\[ x_i(e_l) = [x_{i1}(e_l), x_{i2}(e_l), \ldots, x_{ig}(e_l)]^T \]

(5)

where \( x_{ij}(e_l) \) contains the expression level of gene \( G_j \) on state \( e_l \) in the \( l\)-th data set.

In a time series data set, \( X_i^{TS} \), interventions are applied to a subset of genes, and while their causal effect is propagated through the GRN, the expression of all genes are probed simultaneously at specific time points. Each element \( x_i(t_l) \) in such a data set contains the values of all genes at the \( i\)-th time point of the experiment:

\[ X_i^{TS} = \{ x_i(t_1), x_i(t_2), \ldots, x_i(t_{N_l}) \} \]

(6)

where \( x_i(t_l) \) contains the expression levels of all \( g \) genes at time \( t_i \) (\( i < j \) indicates \( t_i < t_j \)):

\[ x_i(t_l) = [x_{i1}(t_l), x_{i2}(t_l), \ldots, x_{ig}(t_l)]^T \]

(7)
For each multi-factorial perturbation learning data, a learning problem composed of \( g \) sub-problems can be constructed, such that in the \( j \)-th sub-problem, the \( j \)-th gene values become the target and all the other values constitute the input features. Every sub-problem is parallel to a regression problem where the goal is to find functions \( f_{l,j}(\cdot) \) that minimizes the errors \( \varepsilon_{l,j} \) in the following equation:

\[
x_{l,j}(e_i) = f_{l,j}(x_{l,-j}(e_i)) + \varepsilon_{l,j}(i), \quad \forall i,
\]

and

\[
\varepsilon_{l,j} = \sum_{i=1}^{N_l} \varepsilon_{l,j}(i)
\]

Here, \( x_{l,-j}(e_i) \) is the expression value of all genes in the \( i \)-th state in the \( l \)-th data set except for the \( j \)-th gene.

Time-series learning data can be treated similarly, with the difference that the causal effect of the regulators appear after a time delay. Hence, the expression of each gene will be a function of the expression of all genes (possibly including the same gene) in a previous time point. This is equivalent an auto-regressive model. We use the same expression as equation 8 with the exception that the input features will be chosen from a previous time point \( t_{l-k} \) (\( k > 0 \)):

\[
x_{l,j}(t_i) = f_{l,j}(x_{l}(t_{i-k})) + \varepsilon_{l,j}(i), \quad \forall i
\]

These regression sub-problems can be solved by the regular random forests or the guided RRFs within which the importance of a feature will be measured using equation 3. When the guided RRFs are used, the regularization parameters should be provided as introduced in sub-section 2.4.

### 2.4 The GENEREF framework

The goal of a multi-dataset algorithm is to combine available data sets in a plausible manner to produce a network as similar to the ground-truth network as possible. Here, we represent the ground-truth network by an adjacency matrix \( W_{g \times g} \). Our algorithm, GENEREF, produces confidence matrices, \( W_l \ (l \in \{1, \ldots, M\}) \), that are of the same size as \( W \). The elements of the last confidence matrix, \( W_M \), are expected to show how certainly the corresponding edges will be appearing on the actual network. The goal of GENEREF is therefore to produce a \( W_M \) on which the elements corresponding to the real connections of the network get higher values than the others.

GENEREF works in an iterative manner: On each iteration it takes a pre-processed data set \( X_l \) and provides a set of regressors (here, the regular or regularized random forests) with it. Figure 1 shows the overall structure of the algorithm. There are \( M \) data sets in total. The data sets can be of either type. They can also be repeated, i.e., a data set can be fed to the regressors on several iterations. In principle, the algorithm accepts any order of data sets.

On each iteration, GENEREF picks a data set and decomposes it to \( g \) new data sets, each of which is represented as a separate sub-problem to be solved by a regular or regularized random forest. On the first iteration, a set of regular random forests are trained with the decompositions of data set \( X_1 \). Consider sub-problem \( j \) on this iteration. The random forest regressor solves this sub-problem and provides importance scores for the features. We represent these scores as a vector \( \hat{w}_{1,j} = [\hat{w}_{1,j,1}, \ldots, \hat{w}_{1,j,i}, \ldots, \hat{w}_{1,j,g}]^T \) where \( \hat{w}_{1,j,i} \) is the importance score for the \( i \)-th feature in the \( j \)-th sub-problem of data set \( X_1 \) and is calculated based on equation 3. Note that in the steady state configuration, the \( j \)-th feature is not present and therefore \( \hat{w}_{1,j,j} = 0 \). However, in a time-series data set, the \( j \)-th feature can be preserved.

Up to this stage, our algorithm parallels GENIE3. GENIE3 generates a \( \hat{W} \) matrix by simply merging the column vectors \( \hat{w}_{1,j}, \) and represents it as the final edge confidence matrix. The issue with this approach is that since the sub-problems have been solved independently, feature importance values will not be comparable across the columns and cannot be joined without further modification. Therefore, a modulation phase is needed on the columns before joining them. GENEREF does this by applying the following function on each column:

\[
\hat{w}'_{1,j} = \frac{\hat{w}_{1,j} - \min(\hat{w}_{1,j})}{\max(\hat{w}_{1,j}) - \min(\hat{w}_{1,j})} + \epsilon,
\]

where \( \epsilon \) is a small constant. An ideal regressor is expected to assign the highest possible value (i.e. 1) to the “relevant” features and the least possible value (i.e. 0) to the “irrelevant” ones. By diversifying the values on each column to these extents, the modulation phase helps the potential links on various columns to be treated equally.

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1The pre-processing is done as in 4.
Figure 1. Folded data flow diagram of GENEREF. On the $l$-th iteration, the $l$-th data set $X_l$ and the $l$-th regularization matrix $P_l$ are fed to a random forest and the $l$-th confidence matrix $\hat{W}_l$ will be produced. The next level’s regularization matrix $P_{l+1}$ is then constructed based on $\hat{W}_l$.

The edge confidence matrix $\hat{W}_1$ is constructed by merging the vectors $\hat{w}_{l,j}'$, $j = 1, ..., g$:

$$\hat{W}_1 = [\hat{w}_{1,1}', ..., \hat{w}_{1,g}']$$

(12)

On each of the next iterations, GENEREF exploits the next data set. The feature importance values are computed using the regularized random forest. However, since on these iterations GENEREF has already acquired knowledge about which features are potentially more important using the previous learning data, it uses this knowledge to regularize the random forests. To this end, GENEREF uses a vector of regularization values for each sub-problem. We represent these vectors by $p_{l,i} = [\rho_{1,i,1}, ..., \rho_{1,i,g}]^T$ ($i \in \{1, ..., g\}$), where $\rho_{1,i,j}$ is the regularization value of the $j$-th feature in the $i$-th sub-problem on iteration $l$. The $p_{l,i}$ vectors form the $l$-th iteration’s regularization matrix using the following equation:

$$P_l = [p_{l,1}, ..., p_{l,g}]$$

(13)

The details about how the regularization parameters are computed are skipped for now and will be provided in sub-section 2.4.1. After these parameters are calculated for the current iteration, the RRF computes the improvement function for each node in the forest using equation 14 and retrieves the new feature importance scores.

$$\text{Improvement}_{R}(z_{i,j}, \hat{X}) = \rho_{i,j} \text{Improvement}_{R}(z_{i,j}, \hat{X})$$

(14)

Here, $z_{i,j}$ is equivalent to the feature corresponding to the $j$-th gene in the $i$-th sub-problem.

GENEREF provides a $\hat{W}_l$ matrix for each iteration in the same way as the second iteration. Once the algorithm finishes iteration $M$, it returns the final edge confidence matrix $\hat{W}_M$. Every element $\hat{w}_{M,i,j}$ on this matrix, shows the certainty that an edge exists from gene $G_i$ to gene $G_j$. Please refer to algorithm 1 for the concise definition of this method. Standard performance criteria, as described in section 3, can be used to evaluate this matrix by comparing it to the adjacency matrix $W$.

### 2.4.1 Regularization parameters

The regularization parameters matrix on the $l$-th iteration of GENEREF, $P_l$, is calculated using the edge confidence matrix values obtained in iteration $(l-1)$ in the algorithm, $\hat{W}_l$. The more the certainty of the presence of an edge, the more potentially important that edge will be, and therefore it should be penalized less.

Although the confidence values provide an ordering for the importance of each edge, there is no guarantee that their values depict the relative importance of the edges. In fact, using the random forest regressors is not a proper means to get accurate regularization values. Instead, we remove the relative importance values while keeping their ordering. The following function does this.

$$\omega_{l,i,j} = \frac{1}{g^2 - 1} \sum_{m,n \in \{1, ..., g\}} \delta(\hat{w}_{l,m,n} < \hat{w}_{l,i,j})$$

(15)
where $\delta$ is the indicator function. The operation helps us make sure that the whole domain of the mapping takes its effect evenly at each iteration. This gives a full control of how we want to take into account different edges based on their importance.

It has been reported that GRN’s have fast decreasing in-degree distributions\(^\text{18}\). This means that for each node there are only a few regulators. Also, it has been shown that GRN’s typically have few global regulators\(^\text{18–20}\). It is therefore expected that a few links should be preserved as the important ones and all the others should be left as unimportant. The important ones are preferably those with the highest important values.

For the mapping, we chose a simple, yet fairly flexible function – the Kumaraswamy cumulative distribution function (CDF)\(^\text{21}\). This extension of the power function, ensures selecting the few global regulators if proper parameters are set. However, other functions can be used too. The Kumaraswamy CDF alters the importance by two constant shape parameters $\alpha$ and $\beta$:

$$P_{l+1} = 1 - \left(1 - (\Omega_l)^{\alpha}\right)^{\beta}.$$  \hfill (16)

Both $\alpha$ and $\beta$ range from 0 to 1. Roughly speaking, greater $\alpha$ values extend the domain of non-regulatory edges, while greater $\beta$ values favour preserving a broader domain for regulatory links. When $\alpha \to 0$, all features are treated equally, there will be no regularization, and the RRF treats all features as equally important.

### 2.5 Availability

Python implementation of GENEREF is available on github\(^\text{22}\).

## 3 Results

### 3.1 Data selection and performance evaluation

In this work we chose the DREAM4 networks as the base gold-standard networks from which the data sets were generated. The DREAM4 data sets\(^\text{23}\) are available as part of the DREAM4 challenge\(^\text{8}\) and consist of five in silico networks. Each network has 100 genes and is generated from specific gene modules chosen to mimic networks in two model species Escherichia coli and Saccharomyces cerevisiae.

We used the GeneNetWeaver (GNW) program\(^\text{24}\) as the tool to generate the data sets from the gold-standard networks. For each network, a multi-perturbation and a time series data set were generated. Both networks were produced using the recommended settings in DREAM4. As in the DREAM4 challenge, internal noise based on stochastic differential equations and a mix of normal and log-normal noise as the measurement noise were added to each network.

### Algorithm 1: The GENEREF algorithm.

begin
  \textbf{inputs} : Number of data sets $M$
  \textit{Set of all data sets} $\{X_l|l=1,...,M\}$
  \textbf{output} : The edge confidence values matrix $\hat{W}_M$

  \textbf{begin}
  \textbf{Set} $l = 1$
  \textbf{while} $l < M$ \textbf{do}
  \begin{itemize}
    \item \textbf{Take} $X_l$ as the current data set and normalize it
    \begin{itemize}
      \item if $l = 1$ then
        \begin{itemize}
          \item \textbf{Compute} $\hat{w}_{l,j}$’s $(j = 1,...,g)$ from $X_l$ using the regular random forest
        \end{itemize}
      \item else
        \begin{itemize}
          \item \textbf{Compute} $\hat{w}_{l,j}$’s $(j = 1,...,g)$ from $X_l$ and $P_l$ using the regularized random forest
        \end{itemize}
    \end{itemize}
    \textbf{Apply} the modulation phase on $\hat{w}_{l,j}$’s and produce $\hat{w}'_{l,j}$’s $(j = 1,...,g)$ using equation 11
    \textbf{Construct} $\hat{W}_l$ by merging $\hat{w}'_{l,j}$’s $(j = 1,...,g)$ using equation 12
    \begin{itemize}
      \item if $l = M$ then
        \begin{itemize}
          \item \textbf{return} $\hat{W}_l$ as the final scorings
        \end{itemize}
    \end{itemize}
    \textbf{end}
    \textbf{Compute} the regularization parameters matrix $P_{l+1}$ using equation 16
    \textbf{end}
  \end{itemize}
  \textbf{end}

end
Figure 2. The AUROC and AUPR of 100 thousand evaluations of a random predictor used on a network with 100 genes and 176 edges (Network 1 from DREAM4).

To evaluate the results from the algorithm, each final edge confidence matrix ($\hat{W}_m$) is compared to the corresponding gold-standard network. Two evaluation metrics were considered: AUROC and AUPR. The p-values of the metrics—which are the probability that a random reconstruction algorithm produces an equal or greater value than the metric—were calculated based on the methods introduced in 25. Based on the p-values, the prediction performance metric for a single network can be defined as:

$$\text{score}(k) = -\frac{1}{2} \log_{10} [p_{\text{AUPR}}(k) \times p_{\text{AUROC}}(k)]$$  \hspace{1cm} (17)$$

where $p_{\text{AUPR}}(k)$ and $p_{\text{AUROC}}(k)$ are respectively the AUPR p-value of the $k$-th network and the AUROC p-value of the $k$-th network. The overall score of the algorithm over all $C$ networks is determined by averaging the scores obtained for each network:

$$\text{overall-score} = \frac{1}{C} \sum_{k=1}^{C} \text{score}(k)$$  \hspace{1cm} (18)$$

Our observations suggest that the AUROC and AUOR scores do not show uncorrelatedness to a confidential extent on sparse networks on the scale of DREAM4's. Refer to figure 2 as an example, where the random AUROC and AUPR values of Network 1 in DREAM4 are depicted and a lack of complete correlatedness can be inferred. On the other hand, it has been shown that the AUPR metric is more informative than the AUROC in classification problems with skewed class distributions as in biological regulatory networks and the DREAM426. Considering these two facts, we determined to introduce a second score metric too:

$$\text{score}_{\text{AUPR}}(k) = -\log_{10} [p_{\text{AUPR}}(k)] ,$$  \hspace{1cm} (19)$$

and similar to equation 18, the overall score for $\text{score}_{\text{AUPR}}$ will be calculated using the following equation:

$$\text{overall-score}_{\text{AUPR}} = \frac{1}{C} \sum_{k=1}^{C} \text{score}_{\text{AUPR}}(k)$$  \hspace{1cm} (20)$$

3.2 Parameter selection

There are two parameters in GENEREF that should be kept in consideration. They are the $\alpha$ and $\beta$ parameters that are the shape parameters of the mapping applied on the confidence matrices to obtain the feature importance matrices. Though the parameter can differ from one iteration in the algorithm to another, we choose to keep it constant throughout the algorithm for the sake of simplicity.

We used the five DREAM4 networks to examine the influence of these parameters in the performance of the algorithm. We applied a grid search with 13-by-13 logarithmically uniform intervals over the range of $[2^{-2}, 2^7]$ for $\alpha$ and $[2^{-7}, 2^2]$ for $\beta$. 

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Results show that the $\alpha$ and $\beta$ parameters clearly affect the performance of the network and with the values approximately in the range of $(\alpha, \beta) \in [(2^3, 2^{-4}), (2^6, 2^{-2})]$ it performs strongly. Also, we used 5-fold cross validation on the five networks to eliminate the parameters and get an overall assessment for the performance that is comparable to present state-of-the-art methods.

3.3 Evaluation of the competitive methods

Among the competing algorithms are those that utilize only one type of data sets: SVR\(^2\) (which uses support vector regressors) and its ensemble variant E-SVR; TIGRES\(^9\), which applies the linear regression equation directly to the problem; GENIE3\(^4\) and its time-lagged variant tl-GENIE3, which are roughly equivalent to one iteration of GENEREF; and NIMEFI (GENIE3+E-SVR)\(^2\), an ensemble method that combines predictions from GENIE3 and E-SVR. In order to have a fairer comparison, we also included dynGENIE3\(^7\), which utilizes both types of data sets, and iRafNet\(^6\), which guides the training of a data set using an other data set. Both GENIE3 and dynGENIE3 are tree-based ensemble methods, with dynGENIE3 taking into account the ordinary differential equations acting in both the steady state and time series data sets. iRafNet, is another tree based algorithm that applies prior knowledge extracted from other data set during the training of random forests on the main data set. Unlike GENEREF, that alters the Improvement function, iRafNet uses the prior weights to alter the distribution of feature selection probability during the construction of the trees.

The scores reported for SVR, E-SVR, TIGRESS, and NIMEFI were taken from 28. We ran our own implementations for GENIE3, tl-GENIE3, and GENEREF. In order to obtain the results for dynGENIE3, the original implementation was run on our data sets. The scoring metrics of iRafNet were calculated using the AUROC and AUPR values reported in 6.

Performance evaluation on the DREAM4 networks

As GENEREF can be launched with different arrangement of data sets, here we evaluate the influence of the number of the data sets and their types. We tested GENEREF with different combination of three data sets: a multi-factorial perturbation steady state data set, a multi-perturbation time-series data set, and a single knock-out steady state data set. For the sake of abbreviation, we call them respectively MF, TS, and KO. All data sets were generated using the GNW application, with the default settings. This means that all the data sets were generated with the same settings as the DREAM4 contest. We call an ordering of the data sets Network 1, Network 2, Network 3, Network 4, Network 5.

![Figure 3](image-url)  

**Figure 3.** The effect of the modulation phase on the performance of the first iteration of GENEREF. 10 experiments for each model were done in order to obtain the robustness values.
sets a configuration. Based on the number of data sets that GENEREF was trained on, the configurations that we used for our experiments can be divided into three categories:

- **GENEREF(MF), GENEREF(TS), and GENEREF(KO):** On each of these configurations, only one data set was exploited. Note that the GENEREF algorithm in these configurations is equivalent to GENIE3 or tl-GENIE3 with the exception that GENEREF applies a modulation phase.

- **GENEREF(KO+TS), GENEREF(TS+MF), and GENEREF(KO+MF):** In this set of configurations, only two of the data sets were used. For example, GENEREF(TS+MF) trains on the MF data set, using the regularization matrix attained from the TS data set.

- **GENEREF(KO+TS+MF):** This configuration utilizes all the three data sets, in the indicated order, to infer the GRN.

Unless explicitly stated for an experiment, other orderings of the data sets were set aside.

As our first experiment, we evaluated the influence of the modulation phase on GENEREF. The evaluation was done only using the first category of configurations, i.e., one-level GENEREF trained on the three data sets. To get a proper evaluation, we compared each GENEREF run with the corresponding configuration of the GENIE3 algorithm. Figure 3 shows the comparison of the robustness of these two algorithms. In order to evaluate the robustness, 10 data sets for each of the 5 networks were generated and the corresponding score and \( \text{score}_{\text{AUPR}} \) values were measured. The figure depicts an obvious improvement on every one of the 5 networks.

We also examined the robustness of our algorithm based on the number of iterations it was run. The same data sets were used. Figure 4 compares the robustness of all three categories of configurations. On each network, the first three box and whisker plots show the algorithm run on only one data set. The next three plots, depict the robustness of the two-dataset configurations of the algorithm. The last plot belongs to a run with three data sets. Although not universal, a general pattern of enhancement is observed when more data sets are added to the algorithm. Except for Network 2 with negligible improvement, all other data sets have a noticeable improvement when the third category of configurations is used in comparison to the second category. The improvement is in terms of both score and \( \text{score}_{\text{AUPR}} \).

Along with the robustness evaluation, we directly tested the influence of the number of iterations on the performance of GENEREF. Figure 5 compares the score and \( \text{score}_{\text{AUPR}} \) criteria of GENEREF versus the number of iterations. Here, instead of using the aforementioned categories, all \( M \)-permutations of the three data sets were tested for the \( M \)-iteration algorithm. This eliminates the effect of the order of data sets and gives an expectation of improvement based on \( M \). An exceptionless improvement is observed as the number of iteration increases from 1 to 3.

To further evaluate our method, we also performed a Mann-Whitney test that compares four configurations of the algorithm with GENIE3 trained on the KO data set, GENIE3 trained on the MF data set, tl-GENIE3 trained on the TS data set, and dynGENIE3 trained on both the TS and MF data sets. These are the only algorithms that we managed to test directly. The results are shown in table 1, which compares the overall-score and overall-score\( \text{AUPR} \) metrics. As can be seen, the U-statistic marginally depend on the score metric. While GENEREF outperforms the other corresponding algorithms when using either score, the difference is more considerable when using the \( \text{score}_{\text{AUPR}} \) metric. Also, based on the U-statistic, it can be concluded again that modulation phase has improved the overall performance of the algorithm.

For the last experiment, we compared the performance of various algorithms. We compared our algorithm to seven other methods: SVR, E-SVR, GENIE3, NIMEFI, iRafNet, and dynGENIE3. The results can be found on table 2. To have a fair comparison, we only compared those configurations of GENEREF that utilized the same data set as the algorithms in comparison. The competing algorithms use one of the four groups of data sets. SVR, E-SVR, TIGRESS, NIMEFI and TIGRESS only use the MF data set. tl-GENIE3 works only based on the TS data set. iRafNet trains on the TS data set based on the information obtained from the KO data set. dynGENIE3 merges the MF and TS data sets and then performs the inference. None of the competing algorithms were run on all of the three data sets. Although the KO and MF data sets can hypothetically

|                         | overall-score | overall-score\( \text{AUPR} \) |
|-------------------------|---------------|-------------------------------|
| tl-GENIE3(TS) vs. GENEREF(TS) | 0.686         | 0.794                         |
| GENIE3(KO) vs. GENEREF(KO)    | 0.780         | 0.862                         |
| GENIE3(MF) vs. GENEREF(MF)    | 0.982         | 1.000                         |
| dynGENIE3(TS&MF) vs. GENEREF(TS+MF) | 0.648   | 0.768                         |

Table 1. The U statistic values derived from the Mann-Whitney test for comparison of GENEREF to the base algorithms. Number on a cell shows how probably the GENEREF algorithm performs better than the competing algorithm.
Two metrics were used for the comparisons: score and score$_{AUPR}$. With any of the two metrics, GENEREF has the highest hit-count in every group of data sets. It also has the highest overall score and score$_{AUPR}$ among all the algorithms. When the information from the MF and TS data sets is used GENEREF outperforms dynGENIE3 in all cases. iRafNet is the only algorithm that has higher values in more than one of the networks when the KO and TS have been used. It also has the higher overall performance in terms of both score and score$_{AUPR}$. However, after the third data set (the MF data set) comes into play, the overall performance of GENEREF goes higher than that of iRafNet.

4 Discussion

In this paper, we introduced GENEREF, an iterative algorithm that can derive regulatory information between genes from various types of data sets. The evaluation was done on the synthetic data set networks and although GENEREF does not systematically acquire the best performance when compared to other competitive algorithms, it has the highest overall scores when all the three data sets are used. Therefore, we conclude that GENEREF performs on par with other algorithms that utilize multiple data sets.

On the one hand, the diversity of existing data sets stems from the ongoing process of generating data from different kinds of experiments in laboratories around the world. Because of the widespread prevalence in production of data sets, it seems necessary to have advanced multi-dataset algorithms. Because our algorithm exploits the data sets in an incremental manner, it also has the advantage that new data sets can be fed to the algorithm without the need to reutilize the previously used data sets.

On the other hand, our observations reveal that, on our synthetic data sets, as the number of data sets increased, the improvement in the performance increase of adding the next data set plummeted. We hypothesize that this reduction can have
Figure 5. Average score and score_{AUPR} on the DREAM4 networks based on the number of iterations in GENEREF. For $M \in \{1, 2, 3\}$ iterations, the average was taken on all $M$-permutations of the three data sets (the KO, TS, and MF data sets). All configurations with repetitive data sets have been dismissed.

been induced by at least one of these two scenarios: Firstly, if the data sets contain redundant information, the data sets that are on the close-to-final iterations will have less new information to reveal. The other problem that can arise when dealing with linear algorithms is that the patterns learned on previous iterations can start to fade out. In our case, the previously inferred regulatory links may vanish when new data sets are learned. This problem can be more prominent with the regulatory links that are detected only through surgical interventions (e.g. single knock-out/knock-down experiments). More experiments will be needed to reveal the exact cause of the plummeting improvement phenomenon.

It is recommended that data sets that can be merged are not considered as separate learning data and are fed to the algorithm as a single data set, even if multiple times. The recommendation applies to all multi-perturbation data sets and time series data sets with the same configuration (e.g. those with identical time gaps and profiled in similar laboratory conditions). The reason is, as it is argued in [16], in a tree node with a small number of instances, RRF is likely to select a feature not strongly relevant, and therefore performs poorly if the number of data records falls dramatically. However, further experiments to test the validity of this hypothesis might be required.

Although, GENEREF principally outperforms both of the base algorithms, i.e. GENIE3 and GENIE3 (time-lagged), it cannot be concluded that it is the case for all GRNs. The results on the five networks are very data-dependent. The difference in the performance can be explained as a result of various aspects of the networks that control the regulatory relationship between the genes. Further investigations might be needed to determine the influential parameters impacting the performance of the algorithm. Also, to investigate more deeply the performance of GENEREF, we plan to test it on a real-world data set in the future.

Similar to the AdaBoost algorithm [29], on the conceptual level GENEREF can be thought of as machine learning meta algorithm that can exploit various regressors into a single model. Though, we used only random forests as our regressors, other regressor algorithms, including TIGRESS and dynGENIE3, can take this place. The combination and replacement of other regression algorithms in the meta algorithm will be an other topic for exploration in our future work.

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| Metric | Data sets | Algorithm   | Net 1 | Net 2 | Net 3 | Net 4 | Net 5 | Overall |
|--------|-----------|-------------|-------|-------|-------|-------|-------|---------|
|        | MF        | SVR         | 2.46  | 5.61  | 6.76  | 5.39  | 3.08  | 4.66    |
|        |           | E-SVR       | 21.58 | 45.76 | 41.16 | 38.68 | 37.30 | 34.09   |
|        |           | TIGRESS     | 26.70 | 37.52 | 41.03 | 37.85 | 36.65 | 33.55   |
|        |           | NIMEFI      | 27.45 | 44.60 | 48.15 | 44.71 | 44.60 | 41.90   |
|        |           | GENIE3      | 27.17 | 34.02 | 44.30 | 43.03 | 38.21 | 34.95   |
|        |           | GENEREF(MF) | 32.56 | 36.14 | 53.42 | 51.09 | 46.92 | 44.03   |
|        | TS        | tl-GENIE3   | 22.50 | 8.94  | 30.32 | 23.93 | 21.27 | 21.39   |
|        |           | GENEREF(TS) | 30.08 | 10.11 | 39.96 | 34.42 | 26.57 | 28.23   |
|        | KO, TS    | iRafNet     | 76.46 | 53.54 | 64.11 | 57.47 | 33.25 | 56.97   |
|        |           | GENEREF(TS+KO) | 53.21 | 38.67 | 67.49 | 59.37 | 43.60 | 52.47   |
|        | MF, TS    | dynGENIE3   | 39.75 | 15.96 | 54.49 | 58.27 | 38.36 | 41.37   |
|        | MF, TS, KO| GENEREF(TS+MF) | 52.76 | 39.06 | 69.98 | 63.14 | 51.05 | 55.20   |
|        | MF, TS, KO| GENEREF(TS+KO+MF) | 73.61 | 38.21 | 71.55*| 73.43*| 59.26*| 63.21*  |

Table 2. Comparison of algorithms using various scoring metrics. The best results in each dataset group are highlighted in boldface. The best total results are marked with an asterisk.

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5 **Author contributions statement**

M.S. performed the experiments and analyzed the results. M.S. and M.A. reviewed the manuscript.

6 **Additional information**

Mr. Saremi declares no competing interests. Dr. Amirmazlaghani, who has supervised this thesis, has received compensation as an assistant professor at Amirkabir University of Technology for her supervision.