Systems biology

Fast and accurate inference of gene regulatory networks through robust precision matrix estimation

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Abstract

Motivation: Transcriptional regulation mechanisms allow cells to adapt and respond to external stimuli by altering gene expression. The possible cell transcriptional states are determined by the underlying gene regulatory network (GRN), and reliably inferring such network would be invaluable to understand biological processes and disease progression.

Results: In this article, we present a novel method for the inference of GRNs, called PORTIA, which is based on robust precision matrix estimation, and we show that it positively compares with state-of-the-art methods while being orders of magnitude faster. We extensively validated PORTIA using the DREAM and MERLIN datasets as benchmarks. In addition, we propose a novel scoring metric that builds on graph-theoretical concepts.

Availability and implementation: The code and instructions for data acquisition and full reproduction of our results are available at https://github.com/AntoinePassemiers/PORTIA-Manuscript. PORTIA is available on PyPI as a Python package (portia-grn).

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Transcriptional regulation is a crucial mechanism that allows cells to adapt to changing environmental conditions and respond to external stimuli (Sławek and Arditi, 2013) by dynamically modulating their gene expression. Transcription factors (TFs) play a central role in this behaviour by regulating their own expression and the one of their downstream target genes (TGs) (Aibar et al., 2017), effectively constituting complex gene regulatory networks (GRNs) that underlie the possible transcriptional states of each cell (Aibar et al., 2017). Although gene expression is also impacted by higher-level epigenomic regulation mechanisms (chromatin accessibility and DNA methylation) (Klemm et al., 2019), TFs have the most relevant role. For this reason, elucidating the structure of GRNs is crucial for understanding both physiological cell processes and pathological mechanisms (Ruyssinck et al., 2014). A deeper understanding of GRNs could indeed open possibilities for the treatment of complex diseases such as cancer (Plaisier et al., 2016; Chen et al., 2014).

Inferring GRNs from experimental gene expression data is a non-trivial challenge that poses several major issues due to the noisiness, scarcity and complexity of the available data (Gardner and Faith, 2005) which cause the severe under-determination of the problem (Ruyssinck et al., 2014). Moreover, gene expression data are heterogeneous, since they can be acquired from different experimental methods, such as (i) wild-type measurement (naturally occurring expression levels), (ii) time series (multiple observations obtained after initial perturbation of the system), (iii) multifactorial perturbation data (simultaneous perturbation of multiple genes), or from more controlled experiments such as (iv) transient gene knock-downs (KD) and (v) homozygous gene knock-outs (KO). In the first case, a gene’s expression is reduced through genetic techniques performed at RNA level (e.g. RNAi). In knock-out experiments, both copies of the same gene are made non-functional (e.g. through nonsense mutations or null mutations that result in a complete loss-of-function). Each type of experiment leads to data with certain peculiarities. For example, time series allow causal analysis of GRNs (Geurts et al., 2018), but are scarce and can suffer from design issues such as the need for cell synchronization over time (Bar-Joseph et al., 2012). KO experiments are very informative as they show how the intervention on a gene affects the rest of the network. The causal relationship is captured using the so-called null-mutant Z-score (Prill et al., 2010). However, such approach requires knocking out each gene separately, making the whole procedure time-consuming and expensive. KD experiments are less controlled than the latter, since interventions are often performed at transcriptional level. Finally, multifactorial data are the least expensive, as they allow the study of multiple regulatory genes in parallel, but are less informative than data collected through other techniques. KO, KD and multifactorial data are all steady-state measurements obtained after initial perturbation of the system.

Because of its complexity, the analysis of this data requires algorithmic methods. Various computational methods for the
In this article, we describe PORTIA, a novel algorithm for GRN inference based on machine learning methods such as Random Forest (Breiman, 2001) or gradient boosting (Friedman, 2001) have been used. GENIE3 (Irrthum et al., 2010) and its time-series-targeted variant dynGENIE3 (Geurts et al., 2018) build on the former, while ENNET (Lachmann et al., 2013) and GRNBoost (Aibar et al., 2017) on the latter. PLSNET (Guo et al., 2016) and TIGRESS (Hauy et al., 2012) rely instead on partial least squares and least angle regression, respectively. Finally, NIMFEI (Ryuissinck et al., 2014) generalizes the task of feature selection by combining multiple models, like random forests, elastic-net and support vector machines.

In this article, we describe PORTIA, a novel algorithm for GRN inference based on power transforms and covariance matrix inversion. In our vision, a key aspect of GRN inference is the need to discriminate direct from indirect (e.g. transitive) correlations. Our work has thus been conceptually inspired by Direct Coupling Analysis methods used in the field of protein contact prediction (Jones et al., 2012; Baldassi et al., 2014), but several major adaptations were necessary to transfer these concepts to the GRN inference task.

We benchmarked PORTIA on the widely used DREAM datasets, as well as the more recent MERLIN-P datasets, showing that it performs very well with state-of-the-art models, while being orders of magnitude faster. We also analysed the potential causes of its mis-predictions, showing that beyond performance, the topology of GRNs inferred by PORTIA differs from other methods. Finally, we propose a novel and more informative scoring metric for the GRN inference task, based on these graph-theoretical concepts. PORTIA is freely available at: https://github.com/AntoinePassemiers/PORTIA.

2 Materials and methods

2.1 Datasets

We benchmarked our method on GRN inference datasets provided in the editions 3, 4 and 5 of the DREAM (Dialogue for Reverse Engineering Assessments and Methods) challenge. All datasets are available on the website https://www.synapse.org/ and are widely used in literature as common benchmark within the GRN inference community. In addition, we evaluated our method on the more recent MERLIN-P datasets (Siahpirani and Roy, 2017).

From DREAM, we considered the In Silico Size 100 dataset, and we refer to it as DREAM3 for short. From DREAM4, we considered the In Silico Size 100 (we call it DREAM4 from now on) and the In Silico Size 100 Multifactorial (called DREAM4MF) datasets. Each of these datasets contains five different in silico gene networks generated with GeneNetWeaver (Schaffer et al., 2011). Except for DREAM4MF, the datasets comprise time series, gene KO and gene KD experiments.

From the DREAM5 challenge, we adopted the Network Inference Challenge dataset (called DREAM5 from now on), composed of one in silico network generated with GeneNetWaver, as well as two in vivo networks from Escherichia coli and Saccharomyces cerevisiae. Despite being two well-studied organisms, the drawback of evaluating on these real networks is the incompleteness of the experimentally determined regulatory interactions. Inferred networks are being evaluated on these verified interactions, even though they might constitute a subset of the actual network. DREAM5 consists of a combination of multifactorial data, sparse time series and very few KO experiments. It is worthy of note that DREAM5 is the only DREAM challenge for which a list of potential TFs was provided. Network sizes, as well as the number of measurements and other statistics, are provided in Supplementary Table S1.

We further validated PORTIA on datasets from the MERLIN-P study. Three types of yeast expression datasets are considered: natural variation (NatVar), knock-out (KO) and response to stress (StressResp). Each of the GRNs inferred from these datasets was evaluated with three different goldstandard networks (Siahpirani and Roy, 2017). Finally, performance on two natural variation datasets from human lymphoblastoid cell lines (LCL) was assessed, based on the same goldstandard network derived from regulator perturbation data (Cusanovich et al., 2014).

2.2 PORTIA: a method for Gaussian modelling of GRNs

In this section, we describe PORTIA, our algorithm for GRN inference. The full pipeline is shown in Figure 1. We represent each target dataset as a gene expression matrix \( X \in \mathbb{R}^{n \times m} \) composed of \( n \) expression measurements and \( m \) genes. For the DREAM5 and MERLIN-P datasets, a list \( L \) of potential TFs is provided. If such a list is not available, we consider by default that every gene \( j \) is in \( L \). This is equivalent to assuming that the GRN can be any sub-graph of the complete graph.

Gene expression levels generally have an asymmetric (skewed) distribution, and are governed by non-linear regulatory relationships. For this reason, Gaussian modelling might not be suitable, unless a prior non-linear transformation of the data is performed beforehand. We thus processed the input data \( X \) by applying a power transform on each gene (each column) individually, ensuring that the relationship between gene expressions becomes more linear, and the joint distribution Gaussian-like. Namely, we assume that the relationship between the expression of two genes \( i \) and \( j \) before processing is non-linear but monotonic [i.e. a positive change in the expression of a TF always induces a positive (negative) change in the expression of a TG if the sign of the regulatory relationship is positive (negative), and vice versa.] More specifically, each column \( X_j \) is transformed using the Box–Cox transform (Box and Cox, 1964), a general-purpose (monotonic) power transform:

\[
Y_j = \begin{cases} 
\frac{X_j^{\lambda_j} - 1}{\lambda_j} & \text{if } \lambda_j \neq 0 \\
\log X_j & \text{otherwise}
\end{cases}
\]

where \( \lambda \) is a parameter vector found by maximum likelihood estimation. Power transforms are usually applied on each column of the data matrix \( X \) independently. However, enforcing the marginals to be Gaussian does not guarantee that the joint distribution will be Gaussian too. For this reason, we propose an extension of our approach, called etePORTIA, that jointly optimizes the parameters of the power transforms. We described it in Supplementary Material.

In vivo data usually contain more genes than experimental measurements and thus the sample covariance matrix \( S \) is likely to be rank-deficient. To overcome this problem, we perform a shrinkage estimation of the covariance matrix. Let \( D = I \otimes S \in \mathbb{R}^{m \times m} \) be a diagonal matrix, where \( I \) is the identity matrix and \( \otimes \) the Hadamard
2.3 Modelling edge directionality

Due to the undirected nature of Gaussian Graphical Models, the approach described in the previous section will a priori not perform well unless it is supplemented with directional information. For this reason, we considered an additional step (fourth step in Fig. 1), using three sources of directional information that do not impact the speed of the whole inference process, namely the expression data itself, the list of potential TFs and null-mutant Z-scores computed from KO experiments. However, all these information might not necessarily be available in all contexts.

First, we re-weighted each score $M_{ij}$ with weight $B_i / \max(B_i, B_j)$, where $B_i$ is the $i$th coefficient of linear regression for the prediction of the $i$th gene's expression. We propose a way to solve the $m$ linear regression problems in quadratic time, and provide the details in Supplementary Material.

In Equation 2, the relation between TF $i$ and TG $j$ is discarded if $i \notin L$ (where $L$ is the list of TFs). Sidelining of such relations poses an issue only if undiscovered TFs are missing from $L$. Precisely to limit this risk in the framework of the DREAM5 challenge, sensitivity was privileged over specificity in the TF selection process. Assuming there are no false negatives among potential TFs, $L$ is a great source of directional hints, as $x\%$ of regulatory links can be discarded when $L$ contains $1 - x\%$ of the total network size.

Another way of taking into account the directionality of regulatory relationships, is to rely on null-mutant Z-scores, similarly to the method described in Greenfield et al. (2010). We propose to compute these scores as follows:

$$Z_{ij} = \frac{|X_{ij}^{\text{knockout}} - \mu_i|}{\sigma_i} \quad \text{if KOs available for genes } i \text{ and } j$$

(3)

where $Z$ is the median of all Z-scores across all KO experiments, $X_{ij}^{\text{knockout}}$ are the expression levels for KO experiment of gene $i$, $\mu_i$ is the average expression level of TG $j$ across all KO experiments, and $\sigma_i$ the standard deviation obtained similarly. We discarded experiments involving multiple knocked-out genes, to avoid dealing with the ambiguity caused by these genes being confounders. When multiple KO experiments are available for the same TF, then Z-scores are averaged across experiments. Calculation of these Z-scores is a principled way of approximating the causal effect of a gene KO on all other genes, since it quantifies the deviation of the expression of all genes (but one) after KO from the background noise. Since Z-scores, when they can be computed, are highly informative about causal relationships in GRNs, we will also report their performance as a standalone baseline method in the Section 3.

The final score matrix $\mathbb{M}$ predicted by PORTIA is obtained as follows: $\mathbb{M}_i = M_{ij}Z_j$. We squared the elements in $Z$ to attach a higher degree of importance to the top scores derived from interventional data. For low values in $Z$, interventional and observational data have approximately equal importance. Because squaring is a scale-dependent operation, $Z$ is first $L^2$-normalized.

Also, we performed a post-processing step on $\mathbb{M}$, where each row is multiplied by its standard deviation. This step is of great importance as it allows high scores to stand out of the background noise. Indeed, because GRNs are likely to be scale-free networks, where most genes are regulated by a few hubs (Liu and Hu, 2019), noise. Indeed, because GRNs are likely to be scale-free networks, where most genes are regulated by a few hubs (Liu and Hu, 2019), detecting these hubs would enable the accurate prediction of many regulatory links at once. Therefore, we used standard deviation as a proxy for the node centrality of these hub genes.

Finally, the sign of each regulatory link can be simply reported as the sign of the corresponding element in the precision matrix. Indeed, because genes are assumed to be co-expressed in a monotonic manner, their expression correlates either positively or negatively.

2.4 Performance assessment

We used the DREAMTools Python package (Cokelaer et al., 2015) to compare reconstructed networks to the goldstandard networks...
Table 1. AUROC, AUPR and overall scores of different GRN inference methods, evaluated on the five networks from DREAM3

| Method        | Net1 AUROC | Net2 AUROC | Net3 AUROC | Net4 AUROC | Net5 AUROC | Overall score (no KO) | Overall score  |
|---------------|------------|------------|------------|------------|------------|----------------------|----------------|
| ARACNe-AP     | 0.021      | 0.563      | 0.030      | 0.555      | 0.039      | 0.581                | 2.475          | 2.975          |
| GENIE3        | 0.019      | 0.602      | 0.014      | 0.552      | 0.021      | 0.532                | 0.621          | 1.289          |
| PLSNET        | 0.018      | 0.541      | 0.029      | 0.526      | 0.044      | 0.674                | 2.742          | 4.835          |
| TIGRESS       | 0.050      | 0.760      | 0.051      | 0.692      | 0.045      | 0.628                | 8.128          | 8.131          |
| ENNET         | 0.382      | 0.887      | 0.593      | 0.926      | 0.347      | 0.866                | 5.372          | 78.413         |
| Z-scores      | 0.692      | 0.913      | 0.854      | 0.963      | 0.576      | 0.887                | 142.938        |                |
| PORTIA        | 0.726      | 0.956      | 0.826      | 0.986      | 0.512      | 0.888                | 3.492          | 144.029        |
| etePORTIA     | 0.728      | 0.956      | 0.832      | 0.986      | 0.516      | 0.888                | 3.398          | 144.373        |
When $L$ contains all $m$ genes, it clearly appears that PORTIA is orders of magnitude faster than other state-of-the-art methods. As expected, the end-to-end version etePORTIA is comparatively slower since the estimated covariance matrix has to be factorized at each iteration until convergence is reached.

4 Discussion

4.1 A novel GRN inference evaluation metric based on the underlying causal structures

The hypothesis behind PORTIA’s development is that GRN inference methods need to reliably filter out indirect correlations from predicted relations, analogously to direct coupling-based protein contact prediction methods (Dunn et al., 2008; Jones et al., 2012). However, standard metrics like AUROC or AUPR are not sufficient to fully characterize to what extent the disentanglement of direct and indirect correlations occurs, especially when computed on the whole gene network. Therefore, we looked at the causes of mispredictions from a graph-theoretic perspective.

For each network, we categorised the false-positive (FP) cases according to the causal structure of the sub-network wherein the regulatory link is predicted. Next, we associated a relevance score with each category and computed a metric reflecting the overall relevance of the inferred network. In graphs illustrated in Figure 2b, plain arrows correspond to existing relations in the goldstandard and dashed arrows marked with a cross correspond to regulatory

| Method  | Net1 AUROC | Net2 AUROC | Net3 AUROC | Net4 AUROC | Net5 AUROC | Net1 AUPR | Net2 AUPR | Net3 AUPR | Net4 AUPR | Net5 AUPR |
|---------|------------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-----------|
| ARACNe-AP | 0.052 | 0.614 | 0.073 | 0.601 | 0.096 | 0.630 | 0.063 | 0.614 | 0.080 | 0.650 | 10.086 |
| GENIE3 | 0.105 | 0.835 | 0.101 | 0.766 | 0.182 | 0.821 | 0.113 | 0.807 | 0.128 | 0.821 | 1.840 |
| PLSNET | 0.055 | 0.765 | 0.058 | 0.704 | 0.083 | 0.740 | 0.073 | 0.746 | 0.059 | 0.712 | 10.046 |
| TIGRESS | 0.090 | 0.807 | 0.072 | 0.695 | 0.162 | 0.797 | 0.099 | 0.748 | 0.107 | 0.765 | 24.873 |
| ENNET | 0.462 | 0.894 | 0.384 | 0.853 | 0.455 | 0.880 | 0.418 | 0.867 | 0.312 | 0.853 | 23.886 |
| Z-scores | 0.407 | 0.898 | 0.357 | 0.806 | 0.383 | 0.818 | 0.318 | 0.838 | 0.141 | 0.769 | — |
| PORTIA | 0.613 | 0.932 | 0.504 | 0.890 | 0.438 | 0.869 | 0.472 | 0.888 | 0.292 | 0.840 | 13.271 |
| etePORTIA | 0.619 | 0.935 | 0.514 | 0.889 | 0.437 | 0.869 | 0.462 | 0.889 | 0.286 | 0.846 | 14.418 |

Table 2. AUROC, AUPR and overall scores of different GRN inference methods, evaluated on the five networks from DREAM4

| Method  | Net1 AUROC | Net2 AUROC | Net3 AUROC | Net4 AUROC | Net5 AUROC | Net1 AUPR | Net2 AUPR | Net3 AUPR | Net4 AUPR | Net5 AUPR |
|---------|------------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-----------|
| ARACNe-AP | 0.119 | 0.602 | 0.086 | 0.568 | 0.163 | 0.655 | 0.131 | 0.645 | 0.124 | 0.627 | 17.520 |
| GENIE3 | 0.156 | 0.750 | 0.153 | 0.726 | 0.229 | 0.764 | 0.217 | 0.788 | 0.191 | 0.795 | 37.008 |
| PLSNET | 0.110 | 0.716 | 0.265 | 0.828 | 0.227 | 0.796 | 0.208 | 0.819 | 0.186 | 0.780 | 44.155 |
| TIGRESS | 0.159 | 0.751 | 0.156 | 0.698 | 0.228 | 0.765 | 0.214 | 0.779 | 0.224 | 0.755 | 36.426 |
| ENNET | 0.179 | 0.725 | 0.262 | 0.802 | 0.287 | 0.816 | 0.296 | 0.821 | 0.282 | 0.831 | 52.543 |
| PORTIA | 0.137 | 0.693 | 0.139 | 0.706 | 0.230 | 0.773 | 0.229 | 0.778 | 0.144 | 0.725 | 32.819 |
| etePORTIA | 0.138 | 0.706 | 0.151 | 0.704 | 0.237 | 0.774 | 0.230 | 0.778 | 0.155 | 0.729 | 34.050 |

Table 3. AUROC, AUPR and overall scores of different GRN inference methods, evaluated on the five networks proposed in the DREAM4 in silico network challenge, size 100 multifactorial networks

Fig. 2. (a) Top scores predicted by PORTIA on networks from four different datasets. X-axis is the ranking of the gene pair, Y-axis is the score of the gene pair (in log-scale) and the presence of a bar at position $i$ indicates that reporting the corresponding $i$th pair as a regulatory link would result in a FP, and its colour indicates the causal structure of the sub-network wherein the FP occurs, as illustrated in (b). (b) Colour legend for (a). (c) Average normalized discounted cumulative gain (NDCG) of each method on each dataset, measured in percentage. (d) Matrix symmetry of the inferred and goldstandard networks from the DREAM4MF dataset
relations that are (erroneously) present in the inferred network. We grouped all possible cases into categories, sorted by decreasing order of relevance:

- **True positive**: A gene directly regulates another gene.
- **Chain**: A gene indirectly regulates another gene.
- **D-connected genes** that fall in none of the two previous categories: The two genes are either part of a fork (they are indirectly regulated by the same TF) or a reversed chain (indirect regulatory relation predicted in the wrong direction)
- **D-separated genes**: This category is composed of colliders (2 TFs regulating the same gene) and undirected links (remaining cases). Two genes A and B are d-separated if there is no TF C regulating both of them.
- **Spurious, etc.**: No indirect causal relationship can justify the presence of a FP. We refer to spurious correlations as correlations that cannot be attributed to anything causal, including indirect effects (forks, chains, etc.), regardless of the directionality of regulatory links in the goldstandard networks. However, it is likely that many FPs will fall in this category within in vivo networks (networks from DREAM5 and MERLIN+P) due to the fact that our knowledge of these networks is still incomplete.

We propose a variant of the Normalized Discounted Cumulative Gain (NDCG), taking into account the relevance of each prediction, as an evaluation metric for quantifying the information content of a reconstructed GRN. Details about its implementation are provided in Supplementary Materials. A ready-to-use Python implementation of NDCG is available as part of the portia-grn Python package. NDCG scores, averaged across all networks in a same dataset, are reported in Figure 2c for each dataset. It can be noted that PORTIA and its end-to-end variant etePORTIA outperform other methods on DREAM3 (except the baseline Z-scores), DREAM4, DREAM5 and all yeast goldstandard networks from MERLIN+P.

### 4.2 The causes of misprediction are dataset-dependent

Beyond NDCG scores, finer analysis reveals the different difficulties that GRN inference tools are facing. The causal structures wherein false positive (FPs) occur not only depend on the inference tool itself, but also on the dataset, as shown in Figure 2a for PORTIA. Each bar indicates a FP among the top-scoring predictions, and its colour relates to the causal structure (given by panel b of the same figure) that is the most likely to justify the presence of such FP.

#### 4.3 Intervenational data better contribute to the accurate reconstruction of GRNs than observational data

Causal relationships cannot be inferred from observations alone, and require either assumptions or additional information.
collected through interventions (Pearl, 2009). From the perspective of causal calculus, gene KO experiments are valuable interventions as they allow sampling from $P(X|do(X_i = 0))$, and null-mutant Z-scores are simple approximations of how dissimilar this distribution is from $P(X)$. Expressions containing the $do(\cdot)$ operator are used to formalize causal relationships, but they can be evaluated only with the aid of experimental interventions, such as null mutations (complete loss-of-function of a gene). In particular, $do(X_i = 0)$ refers to gene $i$ not being expressed due to a KO mutation.

To empirically show the relevance of KO data, we removed it from the datasets, when applicable. Overall scores were reported for DREAM3, DREAM4 and DREAM5 as an extra column in Tables 1, 2 and 4, respectively. In addition, full performance comparison with AUPIRs and AUROCis is shown in Supplementary Tables S13–S15. A strong loss of performance was noted for PORTIA, etPortIA, GENIE3 and ENNET on DREAM3 and DREAM4. Surprisingly, we even observed a loss of 44.7% of the overall score on DREAM5 (from 75.425 to 41.691) for PORTIA, despite the fact that single-gene KOs were given for only 1.1% of the genes on average. The significant performance drop of ENNET can also be attributed to its modelling, as it also relies on the genes on average. The significant performance drop of ENNET shows the importance of interventional data in the discovery of causal relationships, even when such data are sparse, and even when their modelling is implicit.

Finally, a slight improvement of TIGRESS can be systematically observed after removal of KO data, from 8.151 to 8.128 on DREAM3, from 24.723 to 24.873 on DREAM4 and from 31.803 to 31.914 on DREAM5. TIGRESS outperforms PORTIA in such settings on DREAM3 and DREAM4, however, this poses questions about the scalability of its accuracy with respect to the availability of interventional data. Indeed, the performance of GRN inference tools can reasonably be expected to scale with the elucidation of real networks.

5 Conclusion

In this article, we presented PORTIA, a fast and accurate tool devised for inferring GRNs from heterogeneous gene expression datasets. Our method positivly compares with state-of-the-art approaches, while being at least one order of magnitude faster. In addition, we proposed a novel scoring metric for the evaluation of inference approaches, while being at least one order of magnitude faster. In total, we showed that our method positively compares with state-of-the-art and general-purpose metrics like AUROC or AUPR. Finally, we highlighted the explicit (e.g. ENNET, PORTIA) and implicit (e.g. GENIE3, PLSNET) dependence of GRN inference tools on KO experiments, suggesting that the performance of some methods (e.g. GENIE3) is not solely driven by the sophistication of their modelling.

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