1 Introduction

One of the central cellular processes underlying development is transcriptional regulation. During development, changes in transcription factor activity induce chromatin modifications, chromatin remodelling and ultimately a differential recruitment of the basal transcriptional machinery[1]. Modelling the dynamics of gene regulation is therefore essential to better understand why a cellular dynamic processes progresses through several steps, and what goes wrong in the case of disease.

The dynamics of gene regulation has classically been studied using time series data[2]. When dynamic processes progress asynchronously, such as in hematopoiesis, time series data are usually obtained by sorting different transition states and assessing bulk gene expression and transcription factor binding within the population[3, 4, 5, 6]. Alternatively, time series data can also be generated by synchronizing the dynamic process between cells. However, issues with time-resolution, heterogeneity and good in vivo synchronization models can often limit the predictive power of the dynamic models of gene regulation which can be constructed[2].

One of the main advantages of single-cell transcriptomics is the ability to quantify the exact cellular state of thousands of cells per experiment. The intercellular heterogeneity caused by naturally occurring biological stochasticity [7] can be exploited to predict regulatory interactions between transcription factors (TFs) and their target genes. The computational tools that infer gene regulatory networks (GRNs) from omics datasets are called network inference (NI) methods.

Several studies have highlighted how some regulatory interactions can be very dynamic while others show evidence of being static during consecutive developmental stages[8, 9]. Since regulatory interactions are context-dependent[10], attempting to create an accurate model of those processes by inferring a static regulatory network may have limited relevance. Case-wise NI methods[1] avoid predicting a static GRN and instead infer one GRN per cell (or per sample, for bulk omics data). The case-wise GRNs – or 'case-wise regulomes' – are analysed similarly to single-cell transcriptomics data; for example by clustering, inferring trajectories, or finding differentially activated interactions.

In order to compute a case-wise GRN for a single sample, Kuijjer et al.[11] and Liu et al.[12] employ similar strategies, namely by computing the difference of computing a static GRN for all the cases, and computing a static GRN for all the cases minus one. Since this procedure needs to be repeated for every case in the dataset, and because NI methods are already amongst the most computationally intensive analyses to perform on omics data, this methodology is not applicable for large omics datasets. Another case-wise NI method, SCENIC[13] infers case-wise GRNs by first inferring a static GRN using GENIE3[14]. GENIE3 is a static NI method which uses Random Forests[15] feature importance scores to prioritise candidate regulators for a particular target gene. SCENIC then post-processes the static GRN to determine whether an interaction is enriched for particular cases, resulting in a case-wise GRN. In

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[1]Case-wise NI is sometimes also called sample-specific NI or case-specific NI.

short, while several case-wise NI methods thus already exist, their implementation consisted of post-processing a static GRN to arrive at a case-wise GRN.

In this work, we introduce `bred`, the first 'true' case-wise NI method.

`bred` is an extension of GENIE3[14] in that it uses the case-wise feature importance[16] score rather than the regular Random Forest feature importance scores. In addition to predicting regulatory strength of an interaction, `bred` also predicts the regulatory effect of an interaction – that is, whether a regulator activates or represses the target. We demonstrate `bred` by applying it on a single-cell dataset of 25'000 hematopoietic cells from the Tabula Muris project[17], and to a collection of 15'000 bulk omics samples from The Cancer Genome Atlas project[18].
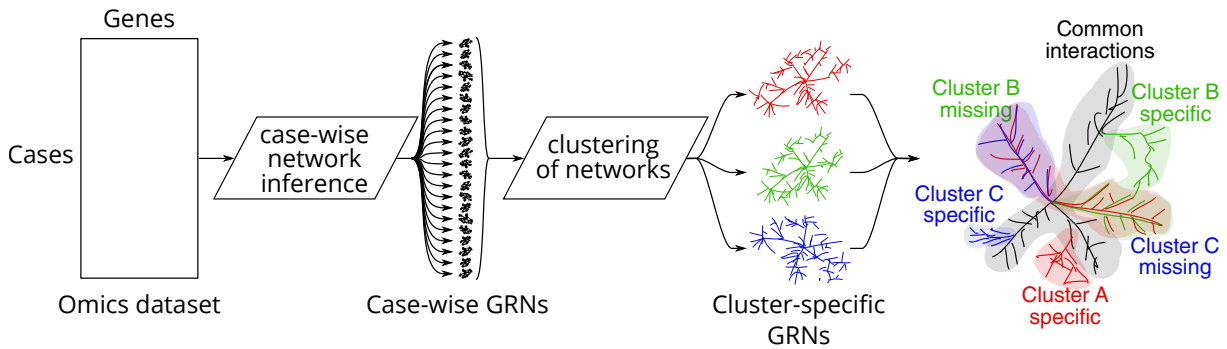
2 Results

Figure 1: **A schematic overview of the workings of the `bred` algorithm.**

3 Discussion

4 Methods

4.1 Building the case-wise GRNs

Having to infer a static GRN (Figure 4A) can be reduced to a simpler problem, namely for every target T, predict which of the potential regulators regulate T (Figure 4B). This simplification allowed GENIE3[14] to use Random Forest's[15] feature importance scores for inferring GRNs. Namely, a Random Forest is trained to predict the expression of a target gene of interest from the expression of potential regulators. The resulting Random Forest inherently allows to extract a feature importance score by observing the effect of each regulator in making a good prediction for the target expression.

We make the same simplification in order to build case-wise GRNs, also using Random Forests to compute the feature importance scores. A Random Forest consists of K trees, each of which produces feature importance scores, and the feature importance scores of a forest is simply the mean feature importance scores of each of the trees.
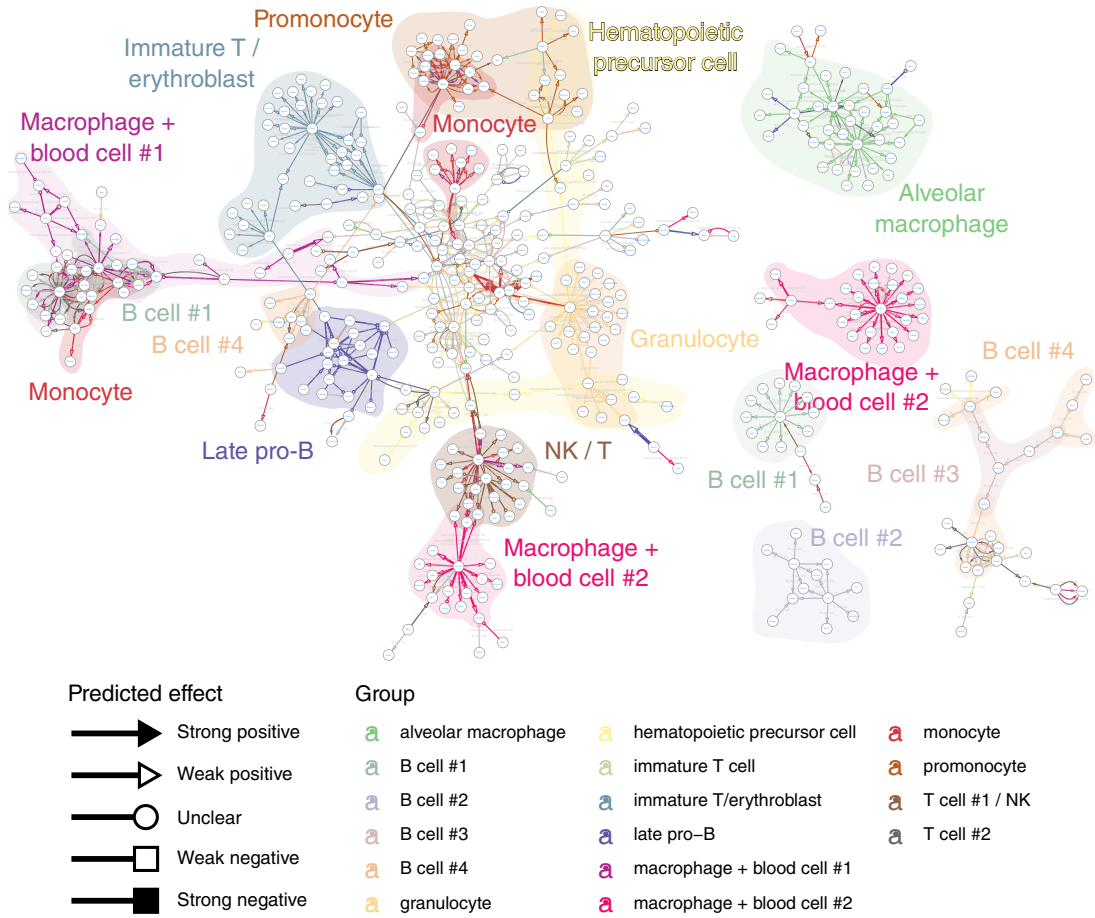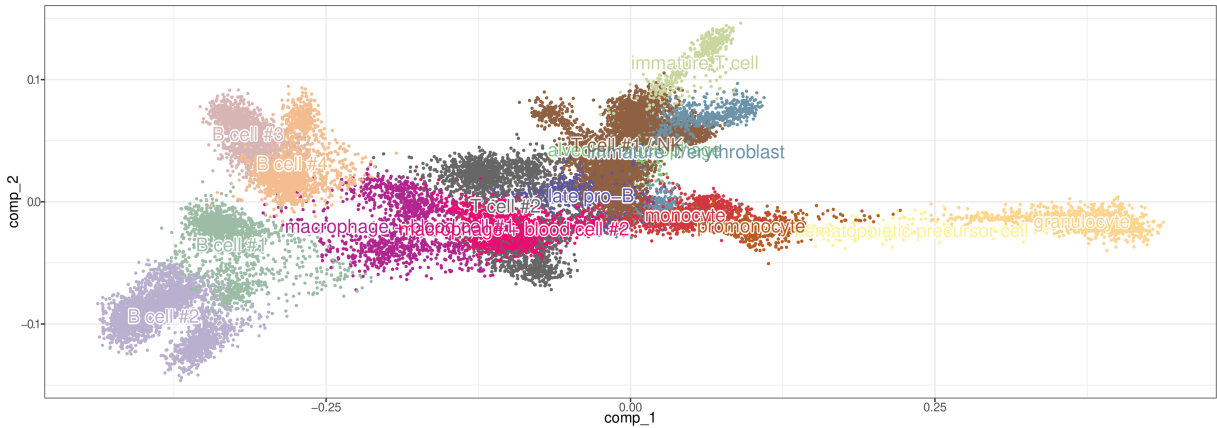
Figure 2: Hematopoietic cells from the Tabula Muris project.

Computing the case-wise feature importances of a tree consists of the following 8 steps (Figure 5). The 'randomness' of a Random Forest is due to only using a subset of the samples in the dataset in order to build a single decision tree. The samples are split into two groups, the 'in-bag' data and the 'out-of-bag' data (Figure 5A). A decision tree[19] is trained on the in-bag expression of the potential regulators in trying to predict the in-bag target gene expression (Figure 5B). The target expression of the out-of-bag samples is predicted using the

decision tree (Figure 5C), and the squared error between the real and target expression is computed (Figure 5D). For each sample in the out-of-bag set, this vector represents how well the decision tree was able to predict the expression of the target gene.

The next few steps are repeated for every potential regulator R_i. Within the out-of-bag samples, the expression of R_i is randomly shuffled. The target expression of the out-of-bag samples is again calculated (Figure 5F), as well as the squared error between the real target expression and the predicted expression is calculated (Figure 5G). The importance of regulator R_i for an out-of-bag sample S_j is defined as the increase in squared error between the predicted target expression and the real target expression, after perturbing the expression of R_i (Figure 5H).
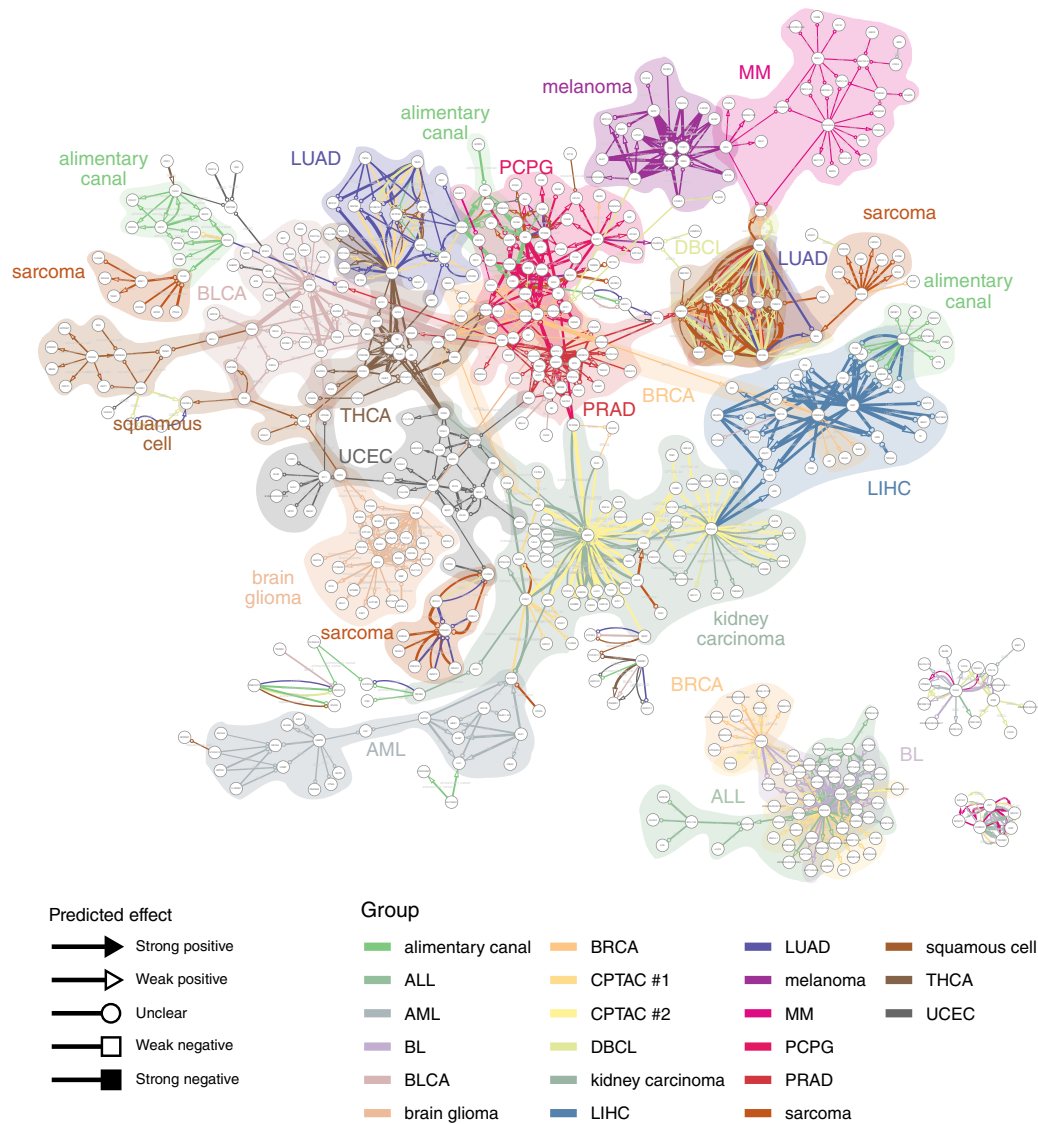
Steps F-G are repeated for every potential regulator R_i. By aggregating all of the feature importance scores over all the samples, regulators and targets, we obtain an M-by-N-by-P tensor[2].

A moderately-sized dataset could contain $M = 10'000$ samples, $N = 2'000$ regulators, and $P = 10'000$ target genes. Due to memory constrains, only interactions with an average importance value (across all samples) higher than a minimum threshold are retained.

4.2   Downstream analysis of case-wise GRNs

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[2]This is the origin of the name of the method, `bred`.

A

Predicted effect

→ Strong positive
▷ Weak positive
○ Unclear
□ Weak negative
■ Strong negative

Group

- alimentary canal
- ALL
- AML
- BL
- BLCA
- brain glioma
- BRCA
- CPTAC #1
- CPTAC #2
- DBCL
- kidney carcinoma
- LIHC
- LUAD
- melanoma
- MM
- PCPG
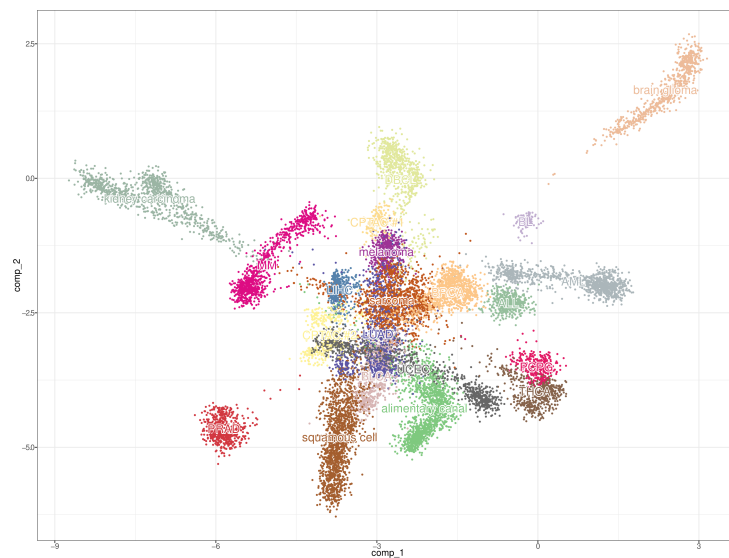- PRAD
- sarcoma
- squamous cell
- THCA
- UCEC

B

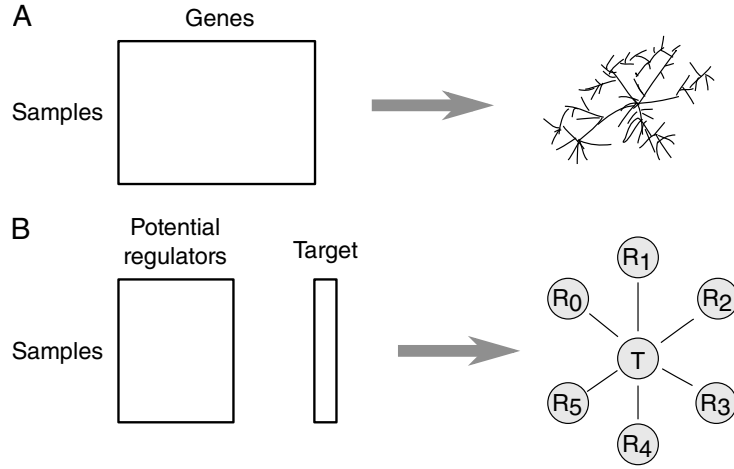Figure 3: The Cancer Genome Atlas.

Figure 4: A: Inferring a gene regulatory network from an omics dataset can be reduced to a simpler problem. **B:** Given the expression of a target of interest and a set of potential regulators, predict which regulators regulate the target gene.
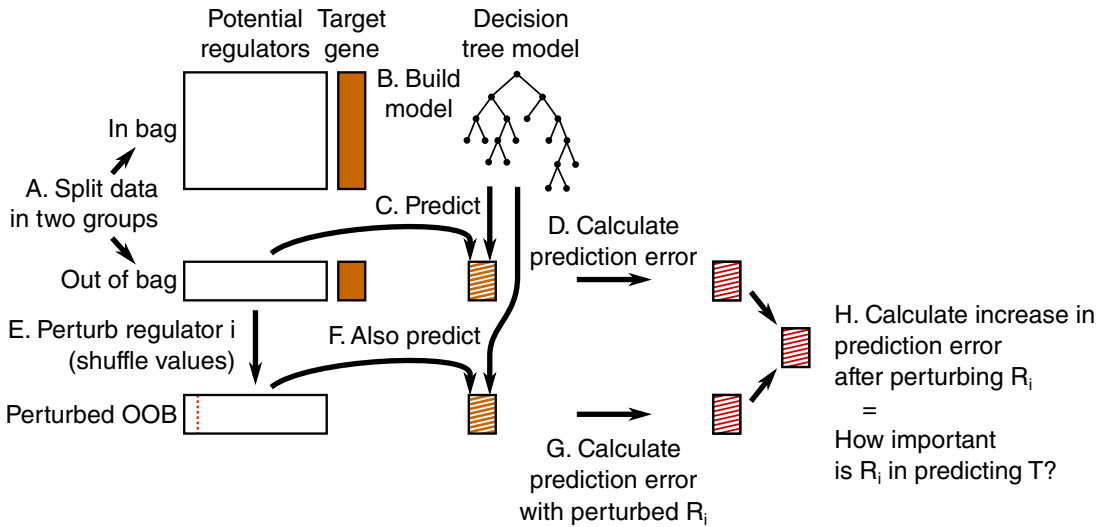

Figure 5: Calculating the feature importance score for one decision tree and one target consists of 8 distinct steps. A: Randomly split the data into two groups, the in-bag data and the out-of-bag data. **B:** The in-bag data is used to train a decision tree to try to predict the expression of the target gene from the expression values of the regulators. **C:** The decision tree is used to predict the gene expression of the target gene of the out-of-bag samples. **D:** Sample-specific squared error values are computed. **E:** Repeat steps E-H for every regulator R_i. Perturb the expression of regulator R_i in the out-of-bag samples. **F:** Again predict the gene expression of the target gene with the perturbed expression values. **G:** Again compute the sample-specific squared error values. **H:** The difference between the prediction error on the perturbed dataset versus the prediction error on the unperturbed is the importance in R_i in predicting T

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