ELMER v.2: an R/Bioconductor package to reconstruct gene regulatory networks from DNA methylation and transcriptome profiles

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Abstract

Motivation: DNA methylation has been used to identify functional changes at transcriptional enhancers and other cis-regulatory modules (CRMs) in tumors and other disease tissues. Our R/Bioconductor package ELMER (Enhancer Linking by Methylation/Expression Relationships) provides a systematic approach that reconstructs altered gene regulatory networks (GRNs) by combining enhancer methylation and gene expression data derived from the same sample set.

Results: We present a completely revised version 2 of ELMER that provides numerous new features including an optional web-based interface and a new Supervised Analysis mode to use predefined sample groupings. We show that Supervised mode significantly increases statistical power and identifies additional GRNs and associated Master Regulators, such as SOX11 and KLF5 in Basal-like breast cancer.

Availability and implementation: ELMER v.2 is available as an R/Bioconductor package at http://bioconductor.org/packages/ELMER/.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Motivated by the identification of transcription factor binding sites (TFBSs), enhancers and other cis-regulatory modules (CRMs) from DNA methylation data in tumor samples (Berman et al., 2012; Hovestadt et al., 2014; Johann et al., 2016), and the strong association between DNA methylation and target gene expression in tumors (Aran et al., 2013; Aran and Hellman, 2013), we previously developed an R/Bioconductor package ELMER (Enhancer Linking by Methylation/Expression Relationships) to infer regulatory element landscapes and GRNs from cancer methylomes (Yao et al., 2015). ELMER version 1 has been adopted by other groups (Dhingra et al., 2017; Malta et al., 2018; Mishra and Guda, 2017), and remains the only publicly available software tool to use matched DNA methylation and expression profiles to reconstruct TF networks (reviewed in Teschendorff and Relton, 2017). Other tools such as TENET (Rhee, 2016) and RegNetDriver (Dhingra et al., 2017) have incorporated ELMER principles and code into cancer network analysis.

We present here a substantially re-written ELMER v. 2 (Fig. 1A) that implements new features and improvements including:
2 Feature highlights

2.1 Supervised versus Unsupervised mode

ELMER first identifies Differentially Methylated CpGs (DMCs) occurring at distal (non-promoter) probes (Step 1), then searches for downstream gene targets for each DMC (Step 2), and finally identifies Master Regulator TFs based on enriched binding motifs and TF expression (Step 3), as shown in Supplementary Figure S1. ELMER v. 1 identified DMCs by comparing methylation in all cancer versus non-cancer samples, while the subsequent steps used correlation between methylation and expression in the n% of tumors with the most extreme methylation values (default, n = 20). The rationale was that any particular GRN might only be altered in a subset of tumors with a specific molecular phenotype, which would not always be known a priori. While 20% was an arbitrary definition, we found this to be a useful exploratory strategy given the heterogeneity of cancer molecular phenotypes.

In ELMER v. 2, we continue to support this original Unsupervised strategy. However, we have found many practical use cases where the group structure is known in advance, and a Supervised search strategy is preferable. This is especially true for “case-control” experimental designs such as treated versus untreated samples. The major difference is that in Supervised mode, all samples must be contained in one of the two comparison groups, whereas Unsupervised mode still uses only the n% most extreme.

To compare Supervised versus Unsupervised modes, we used ELMER v. 2.4.3 to analyze TCGA BRCA (Breast Invasive Carcinoma) data (Supplementary Figs S2–S15 and Supplementary Tables S2–S3). When considering enhancer-gene pairing, Supervised mode had greater statistical power (Fig. 1B), and identified more enhancer-gene pairs overall when molecular subtypes were pre-defined using the PAM50 molecular subtypes (Ciriello et al., 2015)( Supplementary Fig. S3). Specifically, Supervised mode not only re-identified most of the results obtained by Unsupervised mode, but also generated many additional subtype specific enhancer-gene pairs. This comparison suggests that while Unsupervised mode can serve as a useful exploratory tool when sample subtype is unknown a priori, Supervised mode offers greater statistical power when sample subtype is pre-defined.

While it is very difficult to directly assess the false positive rates of Supervised versus Unsupervised analyses, we gained insight into
the question by comparing ELMER-predicted enhancer-gene links to pairs identified using PolII looping (ChIA-PET) in Luminal type MCF7 cells (Li et al., 2012). This analysis showed that while all of the Luminal-specific Supervised analyses produced pairs that were enriched in ChIA-PET loops (compared to randomized ELMER data), the pairs from the Unsupervised analysis were more strongly enriched based on both Precision and Recall values (Supplementary Fig. S8). For heterogeneous patient samples composed of multiple subtypes, it thus appears that Unsupervised and Supervised analyses can offer complementary merits, with Unsupervised analysis displaying a higher false negative rate, but a lower false positive rate. It is recommended to run both Supervised and Unsupervised analyses, as we demonstrated here, to gain maximum insights. This approach is discussed more below in the context of the Master Regulators identified.

2.2 Functional interpretation of chromatin states
While ELMER v.1 was limited to analyze only probes overlapping known enhancers, ELMER v.2 analyzes all distal probes, and thus it is now important to provide a functional interpretation of the resulting regions. We perform a chromatin state enrichment analysis using states automatically downloaded from the http://StateHub.org database, a publicly-available resource that integrates histone modification and other publicly-available epigenomic data for over 1000 different human samples (Coetzee et al., 2018). Enrichment of these states is calculated against a randomly sampled background set drawn from the same distal probe set used as input. We used ELMER v.2 to perform this state enrichment analysis for the BRCA dataset, yielding insights into the cell-type specificity of the genomic regions identified (Fig. 1C and Supplementary Fig. S6). The strongest enrichment was for active enhancer and promoter states having cell-type specificity for MCF7, a Luminal Breast Cancer cell line.

2.3 Motif enrichment analysis and identification of Master Regulator TFs
The final step of ELMER identifies enriched TF binding motifs within candidate regulatory regions, followed by correlation with TF expression to identify upstream Master Regulators (Supplementary Fig. S1). ELMER v. 1 used a hand-curated selection of 145 TF motifs, which were grouped into binding domain families manually. We re-implemented these sections in ELMER v. 2 to use publicly available databases for these steps, making the package more comprehensive and easier to update in future versions. ELMER v. 2 uses 771 human binding models from HOCOMOCO v11 (Kulakovskiy et al., 2018). Each of these is associated with one or more of 1639 transcription factors defined in Lambert et al. (2018), which are grouped into 82 different binding domain families and 331 subfamilies using the TFClass database (Wingender et al., 2018). We use the Fisher’s exact test and Benjamini-Hochberg multiple hypothesis correction to compare the frequency of each motif flanking the

Fig. 2. (A) List of all Master Regulators TFs identified in pairwise Supervised analyses between all PAM50 subtypes (left 15 columns) and an Unsupervised analysis (the right-most column). Each row is a Master Regulator TF, with expression vs. TFBS methylation FDR values color-coded in the corresponding analysis. TFs were clustered based on binary values (Jaccard dissimilarity), and four TF clusters were identified. TFs that were ranked among top five most significant hits were highlighted on the right. (B–D) Scatter plots showing TFBS probe methylation and expression of example TFs from different subtypes: FOXA1 from Luminal (B), OSR1 from Normal-like (C), and SOX11 from Basal-like (D)
positive CpG probes to a background defined by all distal probes on the array, plotting the top hits as odds ratios with 95% confidence intervals (Supplementary Fig. S15).

For each enriched motif, we then calculate a mean DNA methylation value for all probes having a motif instance within ±250 bp, and correlate this value to each of the 1639 TFs in our database. This helps to distinguish between different members of the same TF family, which often have nearly indistinguishable binding motifs. For instance, in the BRCA analysis, the most highly enriched motif corresponded to FOXA2, but this Master Regulator (MR) analysis showed the likely family member to be FOXA1 (Fig. 1D), which has been extensively validated as a MR in luminal subtypes of breast cancer (Meyer and Carroll, 2012; Nakshatri and Badve, 2009).

In order to directly compare the results of Supervised and Unsupervised modes, we performed a Supervised analysis for each pair of known PAM50 molecular subtypes (Ciriello et al., 2015) (Fig. 2, Supplementary Table S3). Luminal-specific analyses successfully identified almost all of the MR TFs obtained by the Unsupervised analysis. More importantly, Supervised modes identified many additional MR TFs. For example, the Basal-specific analyses identified several factors that have been recently been described as functional in BRCA, including SOX11 (Shepherd et al., 2016) and KLF5 (Ben-Porath et al., 2008).

3 Conclusions and future directions
ELMER v.2 has been substantially re-written based on Bioconductor standards and user needs. The new Supervised mode significantly improves the comparisons of two homogeneous groups, such as treated versus untreated, mutant versus wildtype, etc. For heterogeneous groups, we showed that Unsupervised and Supervised analyses can have complementary strength. Showcasing TCGA BRCA data, we used PAM50 (which was originally defined by unsupervised clustering of tumor expression data) for subtype definitions, but any multi-omic unsupervised clustering method can used, depending on what data types are available.

In addition to the new Supervised mode, our improved TF analysis identified additional known and novel Master Regulators candidates in TCGA BRCA analyses. ELMER v.2 has only been tested on data from Illumina methylation arrays, which cover only 5-15% of all enhancer regions based on whole-genome bisulfite sequencing (WGBS). While ELMER does not currently support WGBS due to lack of sufficient test data, the number of WGBS datasets is quickly growing, and we expect the same basic ELMER approach will scale well in the future to take advantage of this more comprehensive data type.

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