A PRELIMINARY STUDY ON THE DETECTION OF TRANSCRIPTION FACTOR BINDING SITES

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Abstract: Transcription starts when multiple proteins, known as transcription factors recognize and bind to transcription start site in DNA sequences. Since mutation in transcription factor binding sites are known to underlie diseases it remains a major challenge to identify these binding sites. Conversion from symbolic DNA to numerical sequences and genome data make it possible to construct a detector based on a numerical analysis of DNA binding sites. A subspace model for the TFBS is built. TFBS will show a very small distance to this particular subspace. Using this distance binding sites are distinguished from random sequences and from genome data.

1 INTRODUCTION

Understanding transcription is a major challenge in molecular biology, and knowledge of transcription factors binding sites is a prerequisite for a complete understanding of gene expression. Transcription factor binding sites (TFBS) are typically short sequences, shorter in eukaryotes than in prokaryotes, and degenerate. These sequences show variability without loss of function. TFBS are mostly located near the transcription start site, in the promoter of a gene, but in complex organisms, such as humans, can be located several kilobases away from it. Characteristics described above made the detection of TFBS challenging, although both experimental and computational methods have been developed (Bulyk, 2003).

Availability of genome collected data and large-scale gene expression experiments make it possible to devise a large number of algorithms for identification and prediction of transcription factor binding sites. Many algorithms use non-coding sequences of co-regulated genes from a single specie or non-coding sequences from orthologous genes from several related species for doing *de novo* motif discovery (Pavesi et al., 2004). Other algorithms use known transcription factors, to build a model and search binding sites in databases (Hannenhali, 2008).

Models of transcription factor binding sites can be based on consensus sequences, where each position is represented by the most common nucleotide on that position using the IUPAC alphabet. Whether a sequence belong or not to a transcription factor is determined by the mismatches to the consensus, like Weeder (Pavesi et al., 2001). Position weight matrices (PWM) provide a probabilistic representation of binding sites because they capture the relative preference for all four bases at each position. Among the models for discovery and finding of transcription factors based on PWM, some examples are Gibbs sampling (Neuwald et al., 1995) and MEME (Bailey and Elkan, 2006). There are also models that use Hidden Markov chains, which take into account interpositional dependence.

While genomic information is represented by character strings, it can also be mapped into a numerical sequence. Many conversions from the symbolic DNA sequences to numeric sequences have been proposed (Anastassiou, 2001; Cristea, 2005). Numerical representations can be used to analyze numerical DNA sequences for detecting transcription factor binding sites. In this paper a detector based on a subspace model of the TFBS is proposed. The sub-

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space is found by a Principal Component Analysis (PCA) of a training dataset containing known examples of TFBS. The structure of the sequences that are recognized by a specific transcription factor can be captured by its covariance, taking into account interpositional dependence. While PWM based methods use only the information regarding the frequency of each nucleotide at a given position, the development of methods that capture interpositional dependence opens new possibilities to improve detector performance.

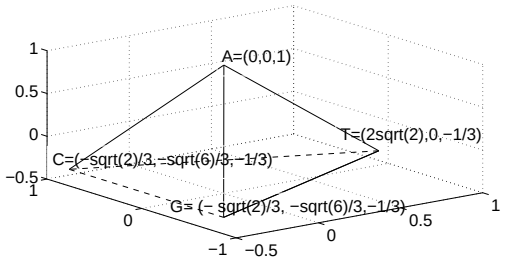


Figure 1: Schema to illustrate the numerical representation of DNA. Each nucleotide is placed in a vertex of a regular tetrahedron.

2 MATERIALS AND METHODS

The analysis has been done on four different groups of DNA sequences previously aligned. Each one is recognized by a transcription factor as a binding site. The first two groups of sequences come from Dr. Schneider data's (http://www.-lmmb.ncifcrf.gov/∼toms), and corresponds to the Dr. Thomas Schneider's work on the characterization of transcription factor binding sites (Schneider, 1997). The last two groups have been obtained using the TRANSFAC data base (http://www.generegulation.com/pub/databases.html), that contains data on transcription factors, their binding sites and the regulated genes. The last groups of sequences have been aligned using MUSCLE (Edgar, 2004). Table 1 summarizes the characteristics of these groups of sequences.

Not all the aligned sequences, corresponding to a certain transcription factor, have the same length, because of missing data at the extremes of some sequences. To carry out PCA sequences of the same length are needed, therefore data have been preprocessed and missing values have been omitted. Although many techniques to model missing data have been proposed, the present work only considers positions where the nucleotide is present for all sequences.

In order to perform or a PCA analysis conversion to numerical sequences is needed. Each nucleotide has been assigned to a vertex of a regular tetrahedron, so that nucleotides are symmetric among each others (Silverman and Linske, 1986). In figure 1, the position of each nucleotide in a tetrahedron is schematically shown.

Vectors corresponding to each nucleotide of a sequence have been concatenated to a vector, and the different sequences corresponding to a TFBS have been arranged in matrix form. The result obtained is a matrix with $3 \cdot$ Number of nucleotides columns and as many rows as the number of original sequences.

Principal component analysis performs a eigenanalysis of the covariance and permits to project the data into a subspace defined by the set of eigenvectors capturing the maximum variance. Equation 1, shows the PCA decomposition, where X is the DNA numerical matrix with dimensions $M \times 3N$ (where M is the number of sequences and N is the binding site length), A are the scores, B are the eigenvectors (loadings), and E the error. Dimensions of A are $M \cdot npc$ and dimensions of B correspond to $3N \cdot npc$, where npc represents the number of principal components in the model.

$$X = AB^T + E \tag{1}$$

In case of DNA sequences,the similarly distribution of the variance along all dimensions indicates the complexity of DNA data. In order to capture a great percentage of variance more dimensions have to be taken into account.

A detector has been created using Q-residuals, calculated using equation 2, where E is the distance orthogonal to the subspace defined by the principal components. Since most of the variance of transcription factor binding sites has been captured by the model, the Q-residuals of a sequence belonging to a binding site should be small, while random sequences should have higher Q-residuals, according to their symmetric variance in all the space. Defining a threshold on Q-residuals, TFBS can easily been distinguished from random sequences.

$$Q = EE^T \tag{2}$$

Models with different number of principal components have been built for each transcription factor and to demonstrate that binding sites have a structure 100,000 random sequences have been used as test data. To evaluate the detector we propose the use of Receiving Operating Characteristic (ROC) curves, which show the true positive rate (TP) against the false positive rate (FP). ROC curves have been computed in a range of principal components in order to

Table 1: Summary of the principal characteristics of the studied DNA sequences.

Transcription factor	Organism	number of bases	Sequences aligned
Argr	E. Coli	13	34
T7 symmetry	Plasmid T7	41	34
ROX1	S.cerevisae	12	20
Abf1	S.cerevisae	11	22

study the efficiency of the proposed detector. Studying the Area under ROC curve (AUC), helps to choose the ideal number of principal components for a given TFBS. The results have been validated using leave-one-out-cross-validation: a TFBS sequence has been removed and the remaining have been used to calculate a model. Then, this model has been used to distinguish the removed sequence from random sequences.

Finally, a more realistic data has been used like test data.*S.cerevisae* chromosomes 1 and 16, where Abf1 and ROX1 binding sites, respectively, are known to be. The model has been built without the binding sequences in the chromosome and leave one out has been used, to compute ROC curves in real data.

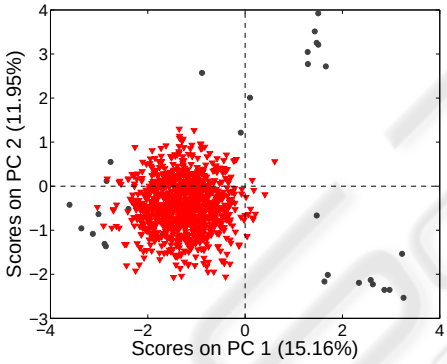


Figure 2: Scores in PCA 2 versus scores in PCA 1. T7 symmetry sequences are represented with circles and random sequences with triangles.

3 RESULTS

A first visualization of the data can be obtained representing the scores of the two first principal components. In figure 2, the scores of the first principal components in T7 symmetry binding sites are shown, for better visualization only 1000 random sequences are represented. It can be observed that binding sites have a structure different from random sequences.

In order to confirm that Q-residuals can be used in order to build a detector, the histograms of Q-residuals have been computed, for model data and 100,000 random sequences in each TFBS. In figure 3, the histogram has been represented, using 8 principal components and Argr binding sites. It is clearly shown than a threshold can be defined between random sequences and binding sites.

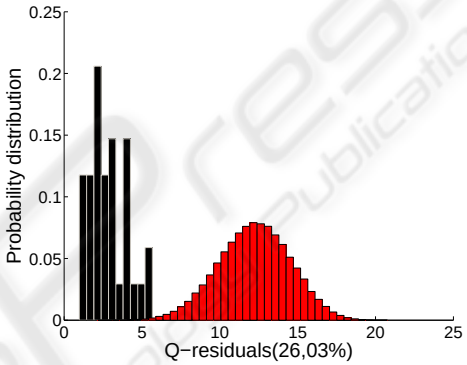


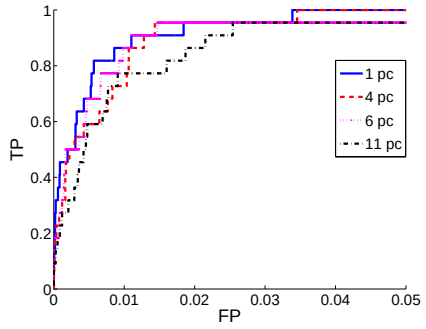
Figure 3: Q-residuals histogram of transcription factor binding, in black, and random sequences in red. A threshold can be defined to separate the two kinds of sequences

3.1 Detection Within Synthetic Data

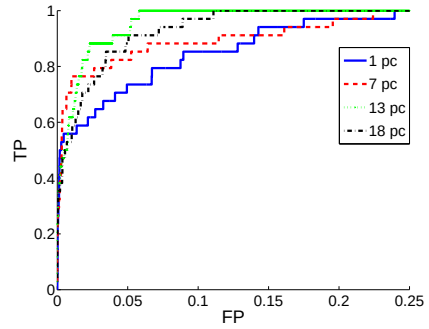
The ideal number of principal components can be chosen, as it is said above, using AUC. ROC curves for Abf1 and Argr in figure 4, show that this number is different for each TFBS. Increasing the number of principal components, in Argr binding sites, leads to better efficiency of the detector, but it exists a threshold and above it, the PCA starts capturing individual information of each sequence. Trying to increase more the number of principal components leads then, to worse efficient detectors. In Abf1 the ideal number of principal components is one, and using only two principal components decreases the AUC.

3.2 Detection Within a Real Genome

Next step on the validation of a the detector must be detect TFBS within the chromosome where they are known to be. Abf1 and ROX1 models are used to test the chromosome data, and figure 5 show that the result is even better than the detection in random sequences.



(a) ROC Abf1



(b) ROC ARGR

Figure 4: ROC curves for two different transcription factor binding sites.

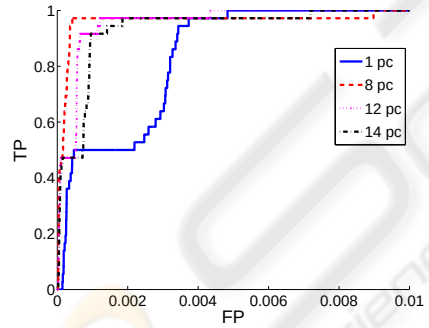


Figure 5: Detection of ROX1 binding site within the yeast chromosome 16 .

4 CONCLUSIONS

A detector that uses Q-residuals has been proposed to distinguish TFBS from random and genome sequences. ROC curves show that this detector can be used efficiently to find TFBS locations in random data, as a preliminary study of the accuracy of Q-residuals detectors. Increasing the number of principal components not always involves more accuracy in the detection, as it exists a ideal number of principal components that must be chosen for each TFBS.

Efficiency of the detector has also been proved using a complete chromosome where the Abf1 and ROX1 binding sites are known to be. The results have also been tested using a leave one out cross validation. Inside the genome the resolution of the detector is higher than in random sequences.

Detection in higher organisms and comparison between this detector and other detectors proposed in literature have to be developed in the future.

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