A SUBSPACE METHOD FOR THE DETECTION OF TRANSCRIPTION FACTOR BINDING SITES

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Abstract: Transcription Factor binding sites are short and degenerate sequences, located mostly at the promoter of the gene, where some proteins bind in order to regulate transcription. Locating these sequences is an important issue, and many experimental and computational methods have been developed. Algorithms to search binding sites are usually based on Position Specific Scoring Matrices (PSSM), where each position is treated independently. Mapping symbolical DNA to numerical sequences, a detector has been built with a Principal Component Analysis of the numerical sequences, taking into account covariances between positions. When a treatment of missing values is incorporated the Q-residuals detector, based on PCA, performs better than a PSSM algorithm. The performance on the detector depends on the estimation of missing values and the percentage of missing values considered in the model.

1 INTRODUCTION

The Central dogma of molecular biology establishes that information flows from DNA to RNA by means of a process called transcription, and then RNA is translated into proteins. Gene expression is highly regulated by complex mechanisms that involve both transcription and translation.

One of the most important mechanisms to regulate transcription is the binding of some proteins, transcription factors, to DNA specific sequences located mostly near the gene start site. These transcription factor binding sites (TFBS) are commonly short sequences (typically 5-20 bp), that show high variability without loss of function, although they are evolutionary conserved. In order to unravel the mechanisms involved in gene expression, finding and understanding the function of these sequences is a major challenge in biology.

In the last years there has been many computational and experimental advances in the discovery of TFBS (Elnitski et al., 2006), and that, together with the increasing availability of genome data, made it possible to develop TFBS databases like JASPAR (http://jaspar.binf.ku.dk)(Sandelin et al., 2004) or TRANSFAC (http://www.gene-regulation.com) (Wingender et al., 2000), and models to search for TFBS within genome data.

Although evidences that interdependences between nucleotides in TFBS exists (Bulyk et al., 2002), most of the methods used to model or search for binding sites in databases, are based on Position Specific Scoring Matrices (PSSM) methods (Stormo, 2000), which assume that each position in the binding site is independent. Some examples of algorithms using PSSM, are MAST (Bailey and Gribskov, 1998), based on the QFAST algorithm and available in Internet as part of the MEME suite (Bailey and Elkan, 2006) and MATCH (Kel et al., 2003), that uses information per sequence, in order to construct a PSSM.

A large body of knowledge exists for specific event detection in numerical sequences (signals). For this reason it may be interesting to translate symbolical DNA sequences into numerical sequences. This translation has been advocated by different authors with different methods, see for instance (Anastassiou,

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2001).
Last year we proposed a detector, based on a Principal Components Analysis of the numerical DNA sequences, using it to detect yeast and E. Coli TFBS within synthetic and chromosomic data. The scope of the project was to demonstrate that, even if the covariance is just a second order statistics, it can capture information of position interdependencies in binding sites, and, consequently, a detector can be built using that information. In this paper we extent the analysis of that detector and incorporate the treatment of missing values, comparing the results of our detector with a PSSM algorithm for real data using *S. Cerevisiae* TFBS and with MAST algorithm for synthetic data and *D.Melanogaster* TFBS.

2 MATERIALS AND METHODS

2.1 Data

In order to carry out models and subsequently search for binding sites in chromosomes, *Saccharomyces cerevisiae* and *Drosophila Melanogaster* TFBS have been extracted from the TRANSFAC public database, which contains data on transcription factors, their binding sites and regulated genes.
In the case of yeast TFBS, the information on the relative position to the gene has also been collected. Chromosome sequences of all the yeast genome and gene positions, belonging to genes regulated by transcription factors modeled, have been taken from the EMBL database (Baker et al., 2000).
For the analysis of Drosophila TFBS, 1923 promoter sequences have been collected between -499 and +100 relative to Transcription Start Site (TSS), from the Eukaryotic Promoter Database (Schmid et al., 2006) in order to build the background models used to simulate Drosophila DNA. In table 1, information on the TFBS used in this study is summed up, showing the number of sequences and nucleotides for each TF.

2.2 Preprocessing

2.2.1 Alignment

Sequences in TRANSFAC belonging to the same binding site do not have the same length. In order to analyze the TFBS is needed to align these sequences. To construct models for yeast TFBS, two different algorithms have been used, MUSCLE (Edgar, 2004) and CLUSTALW (Thompson et al., 1994). The reason to use different alignment methods is that they produce different matrices with significant differences in the detection of binding sites. The method giving a better result has been used in each case. To study D.Melanogaster TFBS, the sequences in TRANSFAC have been used as an input for the MEME program, which is a motif discovery that return motifs models. The aligned sequences of the MEME output have been used as an input for MAST and Q-residuals, to ensure that the comparison is done using the same sequences.
Due to the differences in length, when sequences are aligned gaps must appear. In some positions there are nucleotides present in some sequences but missing in others. These gaps (missing values) are located at the beginning and end of the sequences. Although insertions or deletions in intermediate positions are theoretically possible, they are not frequent, and alignments producing them have been discarded.

2.2.2 Conversion to Numerical Sequences

Once the sequences have been aligned, in order to perform a PCA, they must be converted to a rectangular matrix of numerical sequences. Two processes are needed. First translate symbolical DNA to numerical sequences, and then a treatment of the missing values.
The conversion from symbolical to numerical DNA used is that proposed for Silverman and Linske (Silverman and Linske, 1986), where each nucleotide is placed at the vertex of a regular tetrahedron. It is a conversion symmetric for all nucleotides, as it can be seen in figure 1. Each sequence of length M becomes a sequence of length $3 \times M$, concatenating numerical vectors corresponding to each nucleotide. Then, the N sequences belonging to the same transcription factor where arranged in a matrix form.

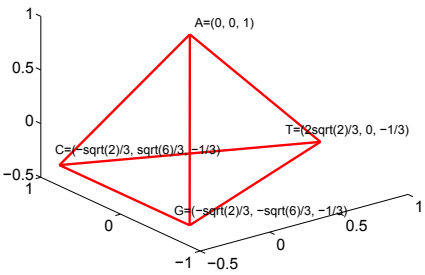


Figure 1: Schema to illustrate the numerical representation of DNA. Each nucleotide is placed in a vertex of a regular tetrahedron.

Table 1: Information about TFBS used, the alignment method, and the results using the different methods.

TF	Organism	Alignment	No M.V	PSSM	50%	all
ROX1	S. Cerevisiae	MUSCLE	8.1520	7.1040	X	X
ABF1	S. Cerevisiae	MUSCLE	7.1972	7.8609	9.5956	8.1868
MCM1	S. Cerevisiae	MUSCLE	3.5518	3.1236	4.7872	3.9900
Repr. CAR1	S. Cerevisiae	CLUSTALW	6.0748	7.6009	7.8240	X
MIG1	S. Cerevisiae	CLUSTALW	5.3507	4.7297	7.6454	4.4844
ADF1	D. Melanogaster	MEME	7.5159	8.7789	10.2449	X
Bcd	D. Melanogaster	MEME	5.7499	5.8511	5.9865	X

2.2.3 Missing Values Estimation

Three different treatments have been studied for the missing values. The first one is to omit missing values, taking into account only the positions where the nucleotide is known for all sequences. That is the common treatment when a construction of a DNA matrix is needed, for example in PSSM algorithms.
The second treatment consists in the assumption that missing values are nucleotides that do not affect the binding of a TF to that particular sequence, but that may be important in the sequences where the position is present. To use the information in the sequences where the nucleotide is present is need to estimate these missing values. The approach taken is that, using the nucleotides probability distribution in the genome, and the numerical conversion, the nucleotide can be located at the mean of the chromosome, as in equation 1

$$\overrightarrow{MV} = P(A)\overrightarrow{A} + P(C)\overrightarrow{C} + P(G)\overrightarrow{G} + P(T)\overrightarrow{T} \quad (1)$$

In order to do a more accurate estimation, a Bayesian Principal Components Analysis (BPCA) is performed. BPCA is a Bayesian estimation method for a probabilistic reformulation of PCA. It was first proposed by Bishop to choose automatically the number of principal components (Bishop, 1999), and later used in micro-array experiments to estimate missing values, showing a high accuracy (Oba et al., 2003). To implement this method we use the R PcaMethods package (Stacklies et al., 2007).
Using these techniques, different percentages of missing values can be estimated, keeping the number of nucleotides equal in all sequences in order to have a rectangular matrix.

2.3 Definition of the Subspace Method

2.3.1 Principal Components Analysis

Principal Components Analysis reduces the dimensionality of TFBS dataset while retaining as much as the variance present in the original data. This is achieved projecting the intercorrelated data into the subspace of eigenvectors retaining the maximum variance giving new variables which are uncorrelated. In equation 2, the PCA bilinear decomposition is defined. X is the TFBS numerical matrix, A is the projected data, called scores, B is the loading matrix, which define the subspace where data is projected and E is the error obtained from reducing the dimensionality.

$$X = AB^T + E \quad (2)$$

2.3.2 Q-residuals Detector

The detector has been built using the Q-residuals of the Principal Components model, calculated as in equation 3, where E is the error obtained modeling the binding sites. Q-residuals are the Euclidean distance from a given sequence to the subspace of principal components.

$$Q = EE^T \quad (3)$$

Most of the variance must be explained by the model. Q-residuals of sequences belonging to the modeled TFBS should be smaller than Q-residuals of random or other genomic sequences. Consequently, defining a threshold should be sufficient to distinguish between TFBS from other sequences.

2.4 Comparison

2.4.1 Comparison with MATCH within Real Data

To compare our detector to existing PSSM methods, showing that calculating interdependencies can lead to an improvement on the detection, we implement the MATCH algorithm, but taking into account the probability distribution of nucleotides in the yeast genome. The PSSM matrix is calculated as in equation 4, where f_{i,b_i} is the frequency of each

nucleotide in each position and $I(i)$ is the information vector. Then a Similarity Score for the sequence and the core (five first consecutive more conserved positions), are used to discriminate between TFBS as in the MATCH program (http://www.gene-regulation.com/pub/programs.html)

$$Score = \sum_{i=1}^{L} I(i) f_{i,b_i} \quad (4)$$

2.4.2 Comparison with MAST within Synthetic Data

A comparison with TFBS of a more complex organism has been done using the TFBS of *D. Melanogaster*. The background sequences of Drosophila have been simulated with a fourth-order Markov Model, constructed using the Drosophila promoter regions from the EPD and the Cosmo R package (Bembom et al., 2007). Drosophila DNA sequences have been simulated and each 1000 nucleotides a TFBS sequence has been inserted.
TFBS sequences (without alignment) have been used as the MEME input, and the aligned sequences from the MEME output have been the input for MAST and the Q-residuals detector, to ensure that the comparison using the same sequences, aligned the same way.

2.4.3 Comparison Method

Receiver Operating Characteristic (ROC) curves have been computed to compare the performance of the detectors, using a leave-one-out cross validation method. When comparison has been performed within real data, the sequences located at the chromosome have been omitted.
The Area Under Curve (AUC) has been calculated to show the accuracy of the different detectors. In the case of the MATCH algorithm, the Core Similarity Score has been fixed in its maximum value, in order to obtain the greatest AUC curve, varying just the Sequence Similarity Score threshold. In MAST comparison, different models have been constructed using MEME, and the model with best AUC has been compared to Q-residuals detector.
To have an accurate detector means to have an extremely small false positive rate, with an AUC near than one. Differences between detector become almost zero, even if they are significant. To avoid this problem, a new parameter has been defined in equation 5, which changes as the logarithm of AUC, making differences in performance more visible.

$$\alpha = -log(1 - AUC) \quad (5)$$

Not only PSSM methods have been compared to our detector, but AUC has also been used to compare between detectors taking into account different amounts of gaps and between the two methods to estimate missing values.

3 RESULTS

3.1 Comparison to PSSM Algorithms

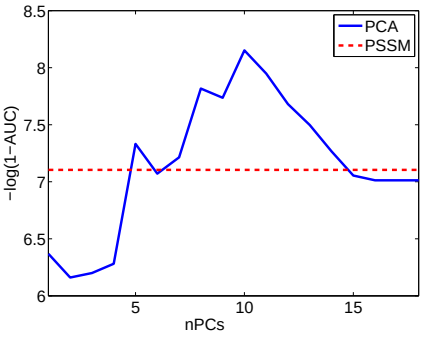
3.1.1 Comparison with MATCH within Real Data

Comparison to the MATCH algorithm has been done in three different cases. First when missing values have been omitted, then when only these positions where the nucleotides are present at least in a 50% of the sequences have been considered, and finally using all the positions available. The estimation of the missing values in the last two cases, has been the assumption that they are in the mean of the chromosome.
In figure 2, ROX1 and ABF1 binding sites have been studied for different number of principal components. ROX1 sequences have all the same length, no treatment of missing values is needed, and it can be seen in figure 2 a) that our detector has a better performance than the PSSM algorithm. In ABF1 binding sites, we need the treatment of missing values to outperform the PSSM algorithm. It can also be observed that estimating all the missing values leads to a worse detection than estimating just those present in at least half of the sequences.

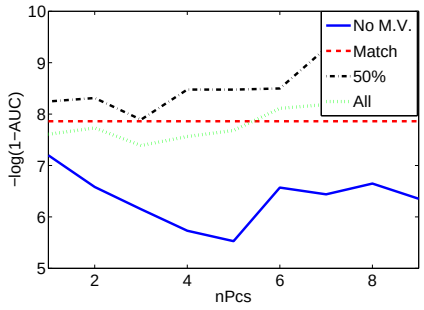
The same study has been done for different S. Cerevisiae binding sites, table 1 summarizes the results, confirming that, in some cases PSSM algorithm obtains better results when no missing values are considered but the treatment of missing values increases leads to best AUC than PSSM. In all cases, the estimation of all missing values, deteriorates the detector performance when is compared to the estimation of a percentage of M.V. A compromise between information and uncertainty introduced must be reached.

3.1.2 Comparison with MAST within Synthetic data

Comparison to MAST has been done for D. Melanogaster TFBS detection, when missing values are omitted and when a percentage of missing values has been estimated with the mean of the chromosome method. In figure 3 the results using MAST and Q-residuals detector have been shown in Bcd binding



(a) AUC ROX1



(b) AUC ABF1

Figure 2: AUC vs the number of PCs in ROX1 and ABF1, using different percentages of missing values.

sites and synthetic data. Q-residuals detector outperforms MAST algorithm when information available in at least 50% of the sequences is taken into account.

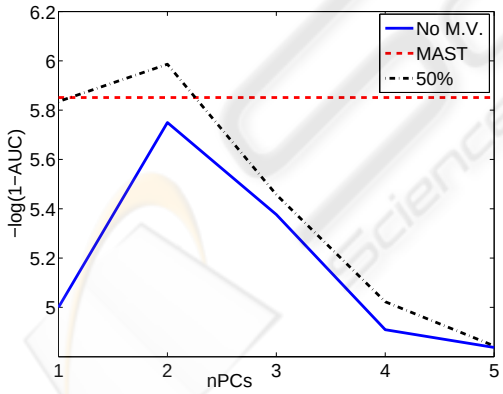


Figure 3: Comparison between Q-residuals detector and MAST algorithm for D.melanogaster TFBS and synthetic data. Q-residuals outperforms MAST when missing values are taken into account.

The same analysis has been done in Adf1 binding sites, and results are summarized in table 1, confirming that Q-residuals detector outperforms PSSM methods when missing values are taken into account.

3.2 BPCA Missing Value Estimation

Comparison between BPCA and the first approximation of the missing values in ABF1 is shown in figure 4, where the number of missing values has been increased from 0% to 12% just incorporating to the model positions missing in an increasing number of sequences. It can be first observed that the result without missing values estimation of BPCA is not the same as the result performing PCA, that is caused by the fact that in BPCA estimation of missing values vectors are not constrained to be orthogonal. Then it can also be seen that BPCA estimation lead to better results, for a small number of missing values considered, but then its results fall. Both BPCA and mean of the chromosome have a maximum AUC in a percentage of missing values equal to 2.19%.
The percentage of missing values in TFBS matrices, can be near 50% , and all of them are concentrated at the beginning and end of the sequences. It leads to positions where few nucleotides are present, and more than ten must be estimated. BPCA method needs information available in order to estimate missing values, when little information is available it becomes an unuseful method, leading to models where all loadings are 0 vectors. In that cases, the mean-of-the-chromosome method which needs no information, performs better.

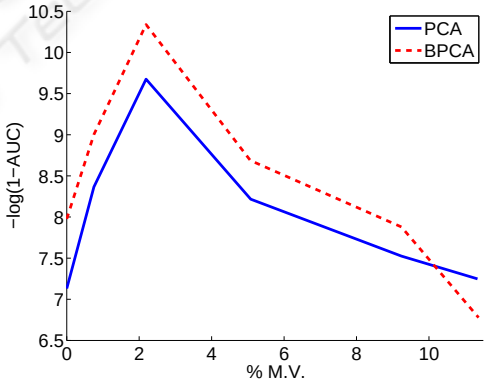


Figure 4: AUC comparison between BPCA and mean of the chromosome treatment of missing values in ABF1.

4 CONCLUSIONS

Performing a Principal Components Analysis of numerical TFBS has been demonstrated to be an effective method to detect TFBS within real and synthetic data, having always a better performance than PSSM methods when missing values treatment is incorporated. This demonstrates that covariance, in spite of

being just a second order statistics can capture TFBS information.

More information can be incorporated taken into account missing values of TFBS. When a treatment of missing values is incorporated the detector performance increases. When only that nucleotides present in at least 50% of the sequences are taken into account, the AUC is greater than when all gaps are present in the model. The reason is that gaps are placed in the beginning and end of the sequences, and in some positions we have almost no information available to construct a model. An equilibrium between information and uncertainty incorporated must be reached for each TFBS.

A more complex estimation of missing values, BPCA, has been proved to perform better when the percentage of missing values is low, but to fall quickly to worse results than the simple approximation to the mean of the chromosome, when more missing values are considered. BPCA fails when no information is available in a certain position because this method tries to estimate a value using the existing information.

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