Pscan: finding over-represented transcription factor binding site motifs in sequences from co-regulated or co-expressed genes

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ABSTRACT

The first step in gene expression, transcription, is modulated by the interaction of transcription factors with their corresponding binding sites on the DNA sequence. Pscan is a software tool that scans a set of sequences (e.g. promoters) from co-regulated or co-expressed genes with motifs describing the binding specificity of known transcription factors and assesses which motifs are significantly over- or under-represented, providing thus hints on which transcription factors could be common regulators of the genes studied, together with the location of their candidate binding sites in the sequences. Pscan does not resort to comparisons with orthologous sequences and experimental results show that it compares favorably to other tools for the same task in terms of false positive predictions and computation time. The website is free and open to all users and there is no login requirement. Address: http://www.beaconlab.it/pscan.

INTRODUCTION

The first step in gene expression, transcription, is mediated and regulated by transcription factors (TFs), that bind DNA in a sequence specific manner on transcription factor binding sites (TFBSs), usually located near the transcription start site (TSS) of genes (i.e. in the promoter region), but also in distal elements like enhancers or silencers. Several studies aimed at the characterization of the DNA binding specificity of TFs have been performed, from earlier studies able to identify single binding sites to large scale genome-wide experiments like chromatin immunoprecipitation coupled with genome tiling micro-arrays or next-generation sequencing. Once a set of sites experimentally known to be recognized by a given TF has been collected, they can be used to build a motif, describing and generalizing the binding specificity of the TF. Since the sites have usually the same size, a common approach is to align them and to build a profile [or position specific weight matrix (1)], representing the frequency with which each nucleotide appears at each position of the alignment. Several profiles are nowadays available in dedicated databases like TRANSFAC (2) or JASPAR (3) and can be employed to scan genomic sequences to find novel candidate sites for the TF (1).

A typical computational issue is deciding, given a profile, if and when a nucleotide sequence can be considered a valid instance of the TFBSs modeled by the profile itself. Redundancy yields information, and while reliable predictions on a single sequence are nearly impossible without further considerations, analyses on sets of sequences (e.g. promoters) coming from co-regulated or co-expressed sequences are more likely to produce meaningful results. The rationale is that most of the genes should be the target of the same TF(s) and their promoters should contain a number of binding sites for them significantly higher than some suitably computed expected number that would be obtained from a collection of unrelated genes or some random background model. This is the general strategy implemented in web-based tools like OTFBS (4) and ASAP (5).

Given a set of motif profiles, and a typical input consisting of a set of sequences (e.g. promoters) from genes co-regulated or co-expressed, a 'likelihood' score can be computed (1), expressing how well each oligo of the input sequences fits the descriptors and thus predict TFBSs locations. The main issue at this point is setting suitable likelihood thresholds for 'yes or no' decisions. Setting high-thresholds increases specificity at the price of low sensitivity, and vice versa, setting low-thresholds yields too many false positives (4–7). Other than setting matrix-specific thresholds, another possible way to circumvent this problem is presented in a very recent tool called PASTAA (8), in which rather than on a selected

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When good sites can be found only in a subset of the input, the average computed on whole sequences, especially in cases of enrichment in orthologous sequences for TFBSs; and while this strategy is successful in cases like muscle-specific gene expression, in others we have TFs for which only a limited number of TFBSs is conserved (e.g. between human and mouse), and also when two orthologous genes are targeted by the same TFs they do not show conserved TFBSs that can be singled out by inspecting genomic alignments.

The usual drawback of methods like Pscan (with or without orthologous sequences) lies in the high number of predictions that can be considered as ‘false positive’, at least when compared with estimates one could derive by using directly the P-values associated with the results. We thoroughly assessed this point for Pscan, by building random promoter sets changing the size of the set (from 5 to 200 genes) and sequence length. Clearly, any significant motif reported with P-value lower than a given threshold in a random set should be regarded as a false positive. Once defined how many promoters we want in the set and their length, by building n sets of sequences in this way we should observe about np times that the profile has yielded P-value lower than a given threshold p. Figure 1 shows the average false positive

![Figure 1](image_url)

**Figure 1.** Experimental false positive rate for JASPAR vertebrate matrices at different P-value thresholds on random collections of human promoters (from 50 to 500 with respect to the TSS) of different size (from 5 to 200 sequences).
rate (in this case the ratio between false positives and the overall number of tests performed) observed for vertebrate JASPAR profiles on sets of human promoters of length 500. For each motif and each set size we performed 1000 random runs. It can be clearly seen how in our case the observed false positive rate virtually matches the estimate for different P-value thresholds, regardless of the number of input sequences, with also no significant difference for different profiles (data not shown). Remarkably this observation is true also for small sequence sets, from 5 to 20 genes, sample sizes for which employing the z-test is not always advised by literature. Changing the length of the promoters did not produce significantly different results.

As a comparison, a similar test performed in (12) for the TFM_Explorer algorithm—although on longer sequences filtered by phylogenetic footprinting—led authors to suggest using P-value thresholds between $10^{-8}$ and $10^{-6}$ to maintain a false positive rate of 0.1. For the oPOSSUM algorithm, in which two different measures of significance are used, a P-value of 0.01 has a false positive rate around 0.2–0.3 (changing according to input set size), that is reduced to about 0.1–0.15 when coupled with further filtering based on a z-score (10). Thus, the P-value associated with the results by Pscan provides a more intuitive way of interpreting the results and of assessing the actual significance of the enrichment of the motifs, keeping the false positive rate easily under control. It should be kept in mind, however, that typically collections of dozens or hundreds of profiles are employed in analyses of this kind. If we assume without loss of generality that for each motif profile an independent test is performed, then we need to keep the familywise error rate below a given threshold. In other words, if we try 100 profiles on a given sequence set by using a significant P-value threshold of 0.01, then we can expect one profile to have a P-value lower than 0.01 purely due to chance. The simplest solution to account for this problem is to use a Bonferroni corrected threshold of $p/m$ to maintain the same significance threshold of $p$, where $m$ is the number of profiles used. More involved methods can be anyway used, like the Holm–Bonferroni or Benjamini–Hockberg procedures.

THE USER INTERFACE

User input

In the current implementation Pscan performs analyses with human, mouse, Drosophila, Arabidopsis and yeast sequences and motifs. The user interface is shown in Figure 2. Users have to input a list of gene IDs in the text box and choose source organism, promoter region with respect to the TSS of the genes, and profile set to be employed in the analysis. If one wants to perform an analysis on a set of human and mouse orthologous genes (see Supplementary Data and the online help page). Then, users have to specify the promoter region they want to investigate, with respect to the TSSs of the genes, for example from $-450$ to $+50$ or $-950$ to $+50$ or $-200$ to $+50$. We advise users to select regions encompassing also a sequence downstream of the TSS, since functional TFBSs are often found also here.

Given gene IDs and the promoter region selected, the corresponding sequences are automatically retrieved by the server. Finally, users have two choices: employing for the analysis the profiles already available in a given database (the interface now includes the matrices available at JASPAR, the familial binding profile collection of JASPAR and the public release of TRANSFAC), or uploading a file containing their own matrices. In the latter case, an upload dialog box appears (see the online help for the format in which matrices have to be uploaded). For example, if users have at their disposal the matrices available in the subscription-only version of...
TRANSFAC, they can upload them and use for their analyses, since they will not be made public or shared with other users. Another possible application is to use Pscan to assess the significance of a motif output by a de novo motif discovery algorithm for which ‘false positive’ results are very often an issue ([16]; see also Supplementary Data). Clicking the ‘Run!’ button, starts the computation and possible error/warning messages are displayed in the text box directly below the button.

Output
The result of the computation will appear in the middle column of the page, together with a small image (the ‘heatmap’) on the top right corner. The output shows the ranking of the profiles selected according to their $z$-test $P$-value (see Supplementary Data). An example is shown in Figure 3. At the top of the column there is also a link for downloading the results in text format as well as the number of matrices used to analyze the sequences, suitable for computing corrected $P$-value thresholds for assessing the significance of the results.

By clicking on a profile name, users can open a dedicated page showing further details (Figure 4), and in particular the matrix itself (with its ‘sequence logo’ at the bottom), its information content and links to its database entry as well as to the ID (PMID) of the PubMed entry describing its generation (in case of user-submitted matrices these two latter pieces of information are missing). A simple graphic representation shows the average matching value of the matrix on the sequences analyzed compared to the average matching value and standard deviation on the whole promoter set (same set of regions with respect to the TSS as selected) of the same organism. Under these fields the interface reports $P$-value, Bonferroni corrected $P$-value (the $z$-test $P$-value multiplied by the number of profiles employed in the analysis), with mean and standard deviation for the matching value of the matrix in the current input set. Next to this, an input mask allowing users to compare the results just obtained with the results that came for the same matrix on a different sequence set (see Supplementary Data and the online help pages).

Furthermore, by clicking on the ‘Report Occurrences’ button at the bottom of the ‘Matrix Info’ table users can retrieve, for each gene submitted, the best matching oligo in the respective promoter, as well as its score (from 0 to 1, see Supplementary Data) and its position relative to the annotated TSS. Occurrences are sorted according to their score, so to have an immediate idea of which genes are more likely to be actual targets of the TF corresponding to the profile. The ‘Text Results’ button allows for the download of this occurrence table in text format. On the bottom right hand of the page two diagrams appear, showing the distribution of the location of the best occurrences in the promoter (with score higher than the genome-wide mean, above) and the scores of the best occurrences (below). Predictions are also colored according to their matching score (red-high).

The ‘heatmap’ image shows intuitively in a microarray-like fashion the contribution of each input gene to the $z$-score of each matrix. Red spots (with proportional color intensity) correspond to positive contributions (e.g. scores higher than the genome-wide mean), vice versa green spots (black spots are around the average genome-wise score of the matrix itself).

To restore the interface to the initial settings, users can click the ‘Reset’ button located below the input text box.

**CONCLUSIONS**

Pscan is a software tool that scans promoter sequences from co-regulated or co-expressed genes, looking for
over- or under-represented motifs describing the binding specificity of known TFs, thus providing quick hints on which factors could be responsible for the patterns of expression observed, or vice versa seem to be avoided (with \( P \)-values nearing 1). The user interface is simple and immediate, and results can be obtained in a few seconds or minutes (in case users submit their own motifs, the computation takes longer since background genome-wide scores have to be computed as well). More involved analyses are nevertheless possible, from intergenic regions or 3'UTR sequences, and so on, for which users are welcome to download the standalone version that permits to build customized background models as well as the input of FASTA sequences. The interface will be updated anytime new descriptors and matrices are made available, and also by including novel species and updated gene and promoter annotations.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

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