Sequence analysis

A tree-based approach for motif discovery and sequence classification

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ABSTRACT

Motivation: Pattern discovery algorithms are widely used for the analysis of DNA and protein sequences. Most algorithms have been designed to find overrepresented motifs in sparse datasets of long sequences, and ignore most positional information. We introduce an algorithm optimized to exploit spatial information in sparse-but-populous datasets.

Results: Our algorithm Tree-based Weighted-Position Pattern Discovery and Classification (T-WPPDC) supports both unsupervised pattern discovery and supervised sequence classification. It identifies positionally enriched patterns using the Kullback–Leibler distance between foreground and background sequences at each position. This spatial information is used to discover positionally important patterns. T-WPPDC then uses a scoring function to discriminate different biological classes. We validated T-WPPDC on an important biological problem: prediction of single nucleotide polymorphisms (SNPs) from flanking sequence. We evaluated 672 separate experiments on 120 datasets derived from multiple species. T-WPPDC outperformed other pattern discovery methods and was comparable to the supervised machine learning algorithms. The algorithm is computationally efficient and largely insensitive to dataset size. It allows arbitrary parameterization and is embarrassingly parallelizable.

Conclusions: T-WPPDC is a minimally parameterized algorithm for both pattern discovery and sequence classification that directly incorporates positional information. We use it to confirm the predictability of SNPs from flanking sequence, and show that positional information is a key to this biological problem.

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1 INTRODUCTION

Pattern discovery algorithms have been widely used in bioinformatics to analyze recurrent groups of symbols, such as DNA and protein sequences. Existing methods can be divided into two classes based on their underlying approach: probabilistic versus deterministic. Probabilistic methods maximize the likelihood between a motif pattern model and a background pattern model. Gibbs sampling and its variants are the prototypical probabilistic methods (Bailey and Elkan, 1995; Lawrence et al., 1993). Some deterministic methods construct candidate patterns from a given pattern length and alphabet size [e.g. Oligo-Analysis/Dyad-Analysis (van Helden et al., 2000) and YMF (Sinha and Tompa, 2003)], some enumerate possible patterns from given sequences [e.g. MOPAC (Ganesh et al., 2003)], while others use tree structures [e.g. Weeder (Pevesi et al., 2004)] or other mathematical approaches [e.g. Projection (Buhler and Tompa, 2002)]. Despite this methodological diversity, there are three issues not well-addressed by the existing algorithms.

First, positional variability in sequences is typically ignored. Most widely used pattern discovery methods focus on finding high-frequency sequences independent of their location, primarily representing patterns with position-specific scoring matrices (Sinha and Tompa, 2003) or Markov models (Thijs et al., 2002). However, these techniques assume equal occurrence probabilities at all positions within a sequence. This assumption does not hold for many biological datasets (Birney et al., 2007). A recent method Amadeus uses a localization score to estimate whether the occurrences of the motif tend to cluster at specific distance from the transcription start site (Linhart et al., 2008); however, they focused on the specific distance or a range of sequences, not all the positions of each sequence. Second, it is unclear how to handle the numerous, low-information content motifs that occur in biological datasets. The presence of such motifs is a major reason for the low accuracy of current pattern discovery methods (Linhart et al., 2008; Tompa et al., 2005). Third, current algorithms focus on analyzing sparse datasets comprising a small number of long sequences. The advent of next-generation genome sequencing has led to much populous, short-sequence data.

To address these issues, we developed a new pattern discovery algorithm called Tree-based Weighted-Position Pattern Discovery and Classification (T-WPPDC). T-WPPDC first applies Kullback–Leibler distance between foreground and background sequences to determine a weight for each sequence position. It next integrates this spatial data to discover positionally important patterns. Such patterns are used to classify sequences. Moreover, the tree structure used by T-WPPDC allows handling of different
pattern lengths, sequence lengths and alphabet sizes. In addition, the algorithm is embarrassingly parallel. Another tree structure method, Weeder (Pevsni et al., 2004), applies a suffix tree to spell the sequences. Our method differs from Weeder since trees in T-WPPDC do not only hold the possible candidate of motifs, but also the positional weights. Furthermore, our method is used for classification.

We tested T-WPPDC on an important biological problem: predicting single nucleotide polymorphisms (SNPs). SNPs are single base pair variations in the genome, and are likely the most common form of genetic variation. On average, 1 out of every 1000 bp may be SNPs (Schafaer and Hawkins, 1997). The function of most SNPs remains unclear; especially those not associated with changes in protein sequence. Genome-wide linkage analyses have implicated a large number of SNPs in disorders ranging from Crohn’s disease (Vilani et al., 2009) to cancer (Houlston et al., 2008) and to quantitative traits such as height (Suzuki et al., 2009). Many groups are attempting to predict the functional effects of individual SNPs (Li et al., 2010; Ribas et al., 2006), and this problem has grown in importance with the advent of cheap genome-sequencing (Hudson et al., 2002).

In contrast, less research has focused on the causes of SNPs: why do they occur where they do? Zhao studied two SNPs databases such as Celera’s CgsSNP and RefSNP and found that natural selection influences patterns of genome variations (Zhao et al., 2003). Another study described a map with 1.42 million SNPs and showed that SNPs have been historically passed on across generations (Sachidanandam et al., 2001). However, recent study has discovered the effect sequence position (Zhang and Zhao, 2004) and our previous work also characterized sequence-based determinants of SNPs (Yan et al., 2007). Surprisingly, we were able to predict SNPs from flanking sequences alone using machine-learning methods. Pattern discovery methods failed at this task, with near-chance prediction accuracies (Yan et al., 2007).

To further examine this question, we tested T-WPPDC on the DNA sequences flanking known SNPs. Information is unevenly distributed in these sequences, with many motifs showing positional bias. Moreover, some biological factors such as natural selection, non-uniform experimentation and stochasticity interfere with sequence-based SNP analysis. T-WPPDC is able to select informative motifs despite these factors and shows superior performance relative to existing pattern discovery methods.

2 MATERIALS AND METHODS

2.1 Datasets

We evaluated SNP datasets from different species: (i) human chromosomes 21 and 22 and (ii) human and mouse chromosome X (which have a high degree of synteny). Both SNP datasets were generated as described previously (Yan et al., 2007), with minor modifications. Repeat-masked human genome sequence (build_hg18), and mouse genome sequences (mm8) and SNP annotations were downloaded from the University of California, Santa Cruz (UCSC) genome browser database (Karolichik et al., 2003) and dbSNP Build126 for human and mouse. The SNP sequence is combined with equal length of 5′ and 3′ flanking sequence from chromosomes 21 and 22 for human, chromosome X for human and mouse, parsed by a Perl script (v5.8.8). We create a negative control sequence for each SNP by randomly selecting a base of the same reference allele from the same chromosome. To avoid SNPs under strong selection, we applied four criteria to both positive and negative sequences: first, SNPs within 5000 bp of any known exon (RefSeq annotation of February 8, 2007) were excluded. Second, SNPs with discordant UCSC and dbSNP allele annotations were excluded. Third, non-SNP polymorphisms (e.g. indels) and SNPs with unknown strandedness were excluded. Fourth, SNPs with ambiguous or repeat-masked bases in their 5′ or 3′ flanking regions were excluded. These criteria removed two-thirds of SNPs. For each of the 12 SNP alleles, our dataset contains equal numbers of true positives (TP) and true negatives (TN). It is possible that some randomly selected negative controls are novel polymorphisms but this contamination is predicted to be under 0.1% (Yan et al., 2007). For method evaluation, we divided each allele dataset into equal-sized training and testing groups. Combined, there are 120 datasets are used, 72 datasets for chromosome 21 and 22 with symmetrical flanking sequences of 50, 100 and 150 bp (12 alleles × training/testing × 3 flanking lengths) and 48 datasets for chromosome X (12 alleles × training/testing × 2 species), which leads to 672 experiments. (72 × 4 pattern lengths × 48 × 2 cross-species × 4 pattern lengths). Our final chromosomes 21 and 22 human datasets contain 50 bp sequences (average number of sequences = SD: 5435 ± 4114), 100 bp sequences (4939 ± 3750) and 150 bp sequences (4518 ± 3434). Our final chromosome X datasets contain 50 bp sequences with 4188 ± 2703 for human and 9461 ± 6465 for mouse (shown in Supplementary Table S1).

2.2 Tree-based weighted position pattern discovery and classification (T-WPPDC)

T-WPPDC is designed to combine positional information and a novel scoring system to identify maximally predictive patterns. It uses a tree structure to greatly reduce algorithmic complexity. First, Tree Construction builds trees from training sequences. Each node of the tree holds a value representing the likelihood of a given pattern at a given sequence position. Second, T-WPPDC calculates the Kulback–Leibler (−) Distance (Kullback, 1987, Kullback and Leibler, 1951) for each tree to measure positional differences. Third, Leaf Selection introduces a new scoring system that identifies high information content patterns to handle the low-signal motifs. Fourth, T-WPPDC scans through each test sequence and performs Sequence Classification.

Here are the notations used in this section. T-WPPDC requires two sets of sequences, foreground and background. To simplify the description, we use class A as foreground and B as background in this section. Letters N and L represent sequence number and length, respectively. We study DNA sequences, therefore, the alphabet is M ∈ {A,C,G,T} and number of letters in the alphabet M is 4. As with most pattern discovery methods, the length of a pattern P, called K, must be fixed prior to analysis. We use l to describe a particular sequence, and lowercase letters, i and j represent a position within a particular sequence.

2.2.1 Tree construction T-WPPDC first constructs trees for the two sequence classes A and B. We scan all patterns with a K-width window from [l, l + K] in all sequences and use patterns at the l-th position construct tree-A(l) and tree-B(l). Therefore, for the training class A sequences, we built Forest-A of (l = K + 1) A-trees.

Tree Construction Rules:

• A node of a tree l has M children.
• A child has exactly one parent.
• Each child corresponds to an element of M.
• A node holds an alphabet, score V, and a pointer to its child.
• The depth of tree is the pattern length K.
• There are L − K + 1 trees in each Forest.
• Tree-size depends on M (alphabet size) and K (pattern length).

One can generate a l-bp pattern by traversing the tree from the root node to the node at depth l (Supplementary Fig. S1). The score in each node at depth K, as shown LR(l,P), in Formula 1, represents how likely this K-bp pattern P is to be found at position l in class A. Identical patterns in different
trees may have different score. This process is repeated identically on class B to build Forest-B with (L − K + 1)−trees.

\[
\text{LR}_A(P, i) = \log \left( \frac{\text{Number of } K \text{-bp pattern } P \text{ at } i \text{th position in sequence set } A}{\text{Total number of sequences in } A} \right)
\]  

(1)

2.2.2 Kullback-Leibler distance T-WPPDC uses Kullback-Leibler distance (K-L distance) to measure positional difference between class A and B. The K-L distance usually measures the distance from a true probability distribution \( p \) to a target probability distribution \( q \). (Kullback, 1987; Kullback and Leibler, 1951), but can also be viewed as the information content between two distributions. \( \text{KL}(A_i | B) \) is the distance between distributions A and B at position \( i \) (0 ≤ i ≤ L):

\[
\text{KL}(A_i | B) = \sum_{m=1}^{M} A_{im} \log (A_{im}/B_{im})
\]  

(2)

where \( A_{im} \) (or \( B_{im} \)) represents the probability of alphabet \( m \) at position \( i \) in class A (or B). Because K-L distance is asymmetric, \( \text{KL}(A_i | B) \) and \( \text{KL}(B_i | A) \) can be defined as the sum of the \( \text{KL}(A_i | B) \) and \( \text{KL}(B_i | A) \). Each position in the sequence has an individual KLD, giving L distinct KLDs. To multiply the log, we aggregate the log frequency value (Formula 1) by \( \text{KLD} \) for each node at class A:

\[
\text{VA}(i, j) = \sum_{L} \text{LR}_A(P, i) \times \text{KLD}(i + K)
\]  

(3)

where \( \text{VA}(i, j) \) represents the node value at depth \( j \) for tree \( i \) in class A. The same process is repeated identically on each node on class B. In other words, the value at each node is the sum of its parents and its own weighted value. These weighted values are the product of the normalized log frequency ratio and the K-L distance. Intuitively, summing the parent weighted scores reflects the influence of neighboring positions. The weighted value for a node at depth \( j \) for tree \( i \) reflects the likelihood that this \( j \)-bp pattern occurs at position \( j \). Therefore, each leaf node collects the sum of all its ancestors and every node in a tree holds values reflecting the likelihood that a specific pattern occurs at this position. This design allows handling of arbitrary pattern lengths.

2.2.3 Leaf selection As noted above, some patterns with low information simply reflect noise in the dataset and do not aid in discrimination. T-WPPDC is designed to select high signal patterns. Filtering based on signal strength trades sensitivity for increased specificity. The difference between corresponding leaf nodes from Forest-A and Forest-B (i.e. \( \text{VA}(i, j) − \text{VB}(i, j) \)) shows how well the pattern distinguishes A from B. If this difference is positive, it is overrepresented in class A. The larger this difference, the more informative a pattern is. By altering \( \epsilon \) we can change the sensitivity-specificity trade-off. Theoretically, \( \epsilon \geq 0 \) and should be set small enough to determine the optimal performance. Performance varies with different values of \( \epsilon \) and T-WPPDC defines \( \epsilon \) with equally spaced points in the range \( [\text{VA}(i, j) − \text{VB}(i, j)] \), shown in Formula 4. To optimize \( \epsilon \), one can preset \( n \) and choose the local/global optimal performance accordingly. When \( \epsilon \) is zero leaf selection is disabled, meaning that all leaves are used for classification.

\[
\epsilon = \frac{|\text{VA}(i, j) − \text{VB}(i, j)|}{n}
\]  

(4)

2.2.4 Sequence classification As in the training procedure, a K-width window is applied to a test sequence, L. The matching pattern at each position is selected from Forest-A and Forest-B. A final score, \( A(i) \), is calculated as the sum of all K-bp patterns in \( i \), as below in Formula 5. Patterns that do not meet the selection threshold, \( \epsilon \), will not contribute. This process is repeated on Forest-B to generate \( B(i) \). The sequence \( i \) then is classified to the class with the larger score. The detailed workflow is shown in Figure 1.

\[
A(i) = \sum_{\text{pattern in } B} \text{VA}(i, K)
\]  

(5)
they tested on 156 yeast ChIP-chip datasets (Bailey et al., 2010). The second group developed an approach called LocalMotif that applies three scoring schemes (Narang et al., 2010). Both PSP-MEME and LocalMotif require prior information on the motif, with PSP-MEME assuming OOPS (one motif per sequence) or ZOOPS models (zero or one motif per sequence) and not supporting ANR models (arbitrary number of motifs per sequence). LocalMotif requires the number of motifs to output, the number of candidates and the weights for three scoring schemes. Most of these parameters are unknown and must be estimated. Moreover, LocalMotif involves an exhaustive enumeration strategy, causing exponential complexity with pattern length, while T-WPPDC has polynomial complexity with pattern length \((K)\). \(\text{GN} \times (L-K) \times \log K\). Aside from these algorithmic details, T-WPPDC is the only one of these techniques that can do both motif discovery and sequence classification.

2.5 Pattern discovery analyses

To evaluate the performance of T-WPPDC, we used three publicly available pattern discovery methods: BioProspector (version 2004) (Liu et al., 2001) and Oligo (v2.5.1) (Yan et al., 2000) with RandomForests and LocalMotif (Narang et al., 2010). We ran each method (including T-WPPDC) using default parameter settings. Oligo returned all possible motifs for analysis \((n=1^M)^P\). We ranked the motifs by \(Z\)-score and selected the top 20 overrepresented (or underrepresented) motifs. The value 20 was selected to allow these methods to slightly exceed the number of motifs identified by BioProspector.

First, BioProspector, Oligo and LocalMotif were run separately on the foreground (true positive, TP) and background (true negative, TN) datasets. BioProspector and LocalMotif return both consensus motifs and position weight matrices. Oligo only returns consensus motifs. Second, motifs from Oligo found identically in the TP and TN sets were excluded. Third, a Perl script (v5.8.7) was used to scan through each test sequence for motifs found only in the TP or TN training datasets. If a motif occurred exactly in the test sequence one or more times, then the sequence was predicted to have the same class (SNP or non-SNP) as the motif. Fourth, the actual and predicted classes for all test sequences were used to generate two-way tables giving the number of TPs, TNs, false-positives (FPs) and false-negatives (FNs). Fifth, overall accuracy was calculated from this two-way table in the standard way \(\text{TP} + \text{TN}/(\text{TP} + \text{TN} + \text{FP} + \text{FN})\). Sixth, these steps were repeated with inversion of the testing and training datasets. Seventh, position weight matrices from BioProspector and LocalMotif were analyzed by using logistic regression analysis (Wasserman and Fickett, 1998). Finally, the entire procedure was repeated separately for each pattern discovery algorithm for sequence lengths of 50, 100 and 150bp and pattern lengths in \([3,6]\) for Oligo and LocalMotif and \([4,6]\) for BioProspector (BioProspector has a minimum pattern length of 4bp). The pattern discovery methods were run on two Linux GUM 2.6.15-29-64-bit servers with identical hardware/software configuration, and compiled with GCC (v4.0.3). Logistic regression analysis was run using Weka (v3.6-2).

2.6 Machine-learning analyses

Two machine-learning algorithms were employed for comparison: Random Forests (RF) and K-nearest neighbors (KNN). These methods are well-established, minimally parameterized techniques representative of diverse fields of machine learning. RF is a non-metric classification technique (Breiman, 2001) that uses an ensemble of decision trees. KNN is a standard non-parametric classification method (Duda et al., 2001). Machine-learning analyses were performed largely as described previously (Yan et al., 2007). Each base of flanking sequence was expanded into four predictor variables, one for each nucleotide, and was assigned a binary value from the sequence. Machine-learning methods were implemented in the R statistical environment, v2.11.1 with RandomForests in the RandomForest package (v4.5.18) and KNN in the class package (v7.2-36). RF contained 1000 trees and \(K\) was set to 51 for KNN analysis, based on our previous analysis (Yan et al., 2007).

2.7 Data visualization and statistical analyses

All plots and statistical analyses were generated in the R statistical environment (v2.11.1) using the lattice (v0.19-11) and latticeExtra (v0.6-14) packages. To evaluate the relationship between SNP position and weight \(w\), we employed Spearman’s rank-order correlation using the absolute value of the position as Spearman’s correlation has minimal assumptions: (i) two variables are ordinal, interval or ratio; and (ii) there is a monotonic relationship between variables. As such, it can be used as a non-parametric test. Statistical analysis of differences in means was determined using unpaired, two-tailed Wilcoxon signed-rank test, due to its minimal assumptions: (i) the differences of two observations are assumed to be independent; and (ii) directional comparisons can be given. Both these assumptions are met in the datasets used here. To improve visualization, weights were scaled for plotting as \(\frac{w}{\text{min}}\) (i.e. \(\log_2{|\text{IC}_\text{scaled} = \frac{w}{\text{min}}|}\)). Similarly, information content values were scaled as: \(\text{IC}_\text{scaled} = 0.3 \times \log_2{|\text{IC}_\text{scaled} = \frac{w}{\text{min}}|}\). (0.3 is chosen for better visualization). These operations are monotonic and only alter the centering of the distributions.

Pattern discovery methods CPU running time was loess-smoothed with a span of 0.2 (0.2 is chosen for the best smoothing fit).

3 RESULTS

3.1 Distribution of the K-L distance

Our previous work showed that classification methods greatly outperform pattern discovery algorithms in predicting SNPs from flanking sequences (Yan et al., 2007). Machine-learning methods can incorporate positional information, and so to assess the importance of this factor, we performed an information content analysis. For all SNPs that met our stringent inclusion criteria (Section 2.1), we extracted 50bp of flanking sequence centered on the SNP. We selected an equal number of 50bp sequences flanking non-SNP positions on the same chromosome. For each position and for each of the 12 SNP alleles, we calculated the mutual information as:

\[
\text{IC}_{\text{pos}} = \sum_{a} A_b \log_2 \frac{A_b}{B_b}
\]

Here \(b\) is the nucleotide base \(\{A,C,G,T\}\) and \(A_b\) \((B_b)\) is the frequency of base \(b\) at the current position from sequence \(A\) (or \(B\)). In our analysis, we set sequence set \(A\) as the TPs and \(B\) as the TNs. Figure 2 shows that positions close to the SNP (position 0) are generally more informative than those further away. However, some positions that are quite distal to the SNP itself carry information.

To incorporate this information directly into pattern discovery, we developed T-WPPDC, which uses the K-L distance (Kullback, 1987; Kullback and Leibler, 1951). K-L distance captures positional dependencies in information content (Supplementary Fig. S2). For most \((10/12)\) alleles, the distance of a position to the SNP is proportional to the K-L distance (i.e. Spearman’s rank-order correlation is significant, \(P < 0.05\)). This holds true for flanking sequences of 50, 100 and 150bp from human chromosomes 21, 22 and X.

As noted above, each leaf node holds a value that represents the likelihood that a specific pattern occurs at this position. As shown in Figure 3, the Foreground scores come from the leaf values from Forest-A (representing the Foreground class) and Background scores are from the leaf values of Forest-B (representing the Background class) (Fig. 3). Symmetric scores indicate little difference between
motifs from foreground and those from background. Most SNPs have symmetric score distributions except C/T and G/A SNPs (see Supplementary Figs 3–5), which shows that the C/T and G/A are more different from background. This might explain the better prediction performance of C/T and G/A alleles that is discussed below.

3.2 Prediction accuracy

T-WPPDC was evaluated by selecting half of each dataset for training and half for testing/validation. The training and testing datasets were then inverted and the procedure repeated (i.e. 2-fold cross-validation). We used sequences of lengths 50, 100 and 150 bp for SNPs from human chromosomes 21, 22 and X. We also performed four species-specific tests: Hs-Hs (train and test on human sequences), Mm-Mm (train and test on mouse sequences), Hs-Mm (train on human sequences and test on mouse sequences) and Mm-Hs (train on mouse sequences and test on human sequences). We used four different pattern lengths of 3–6 bp. In total, we conducted 672 experiments: 288 experiments on chromosome 21/22 and 384 experiments on chromosome X. We focus on overall prediction accuracy [(TP + TN)/(TP + TN + FP + FN)], but report multiple metrics (Supplementary Tables S2 and S3).

T-WPPDC with leaf selection off consistently outperforms existing pattern discovery methods. First, the overall prediction accuracy is $55.5 \pm 3.6\%$ when threshold $\epsilon = 0$ (Fig. 4 for chromosome 21/22 and Supplementary Fig. S6 for chromosome X). This is a statistically significant improvement over Oligo, BioProspector and LocalMotif (Oligo: $50.0 \pm 0.8\%$, $P = 5.61 \times 10^{-81}$; BioProspector: $50.8 \pm 0.9\%$, $P = 1.46 \times 10^{-72}$; LocalMotif: $51.3 \pm 1.2\%$, $P = 1.39 \times 10^{-70}$). We are unable to detect a difference between T-WPPDC in this naïve mode and KNN, a fully supervised method ($54.9 \pm 2.9\%$, $P = 0.61$), but remains lower than that of RF ($58.9 \pm 3.8\%$, $P = 2.66 \times 10^{-4}$).

These modest prediction accuracies suggest that not all SNPs are predictable because of non-sequence factors like natural selection. To demonstrate this, we exploited the fact that not all patterns carry equivalent information. Patterns with similar frequencies in TP and TN cases are mostly noise and have little independent predictive capacity. Enabling leaf selection helps to removes these patterns. The performance varies with threshold stringency (i.e. $\epsilon$ value), so we executed T-WPPDC at 20 equally spaced point (i.e. $n = 20$ in Formula 4) (Fig. 5). We chose the last point prior to any decline as the optimum position. Leaf-selection improves median accuracy, at the expense of increased SDs. In this mode, it classifies ~40% of sequences (Supplementary Fig. 57) and outperforms KNN on the Chromosome X ($P = 2.00 \times 10^{-73}$) and RF on Chromosome 21/22 ($P = 0.05$) datasets. T-WPPDC with leaf selection also is statistically indistinguishable from performance of KNN in the Chromosome 21/22 datasets ($P = 0.24$) and RF in Chromosome X ($P = 0.69$) datasets. We note that these analyses are not precisely comparable as we did not exploit the potential of RF or KNN to prioritize sequences based on vote numbers.
Fig. 4. Accuracy comparisons across all methods. (Data: chromosome 21/22) A boxplot of algorithms evaluated in this study (from left to right: pattern discovery method-OLIGO, BIO, LocalMotif, classification method-KNN, T-WPPDC with leaf selection off option, RF). The y-axis is the overall prediction accuracy \[(TP + TN)/(TP + TN + FP + FN)\], ranging from 0.47 to 0.67. T-WPPDC with leaf selection off includes all the patterns for prediction. A \(P\)-value is calculated by Wilcoxon signed-rank test, unpaired, two sided (OLIGO, Oligo; BIO, BioProspector; LocalMotif, LocalMotif).

To determine if prediction accuracy is a function of dataset characteristics, we analyzed sequence length, pattern length, SNP allele and species (Fig. 6). Shorter sequence (Fig. 6A) and pattern lengths (Fig. 6B) perform better than longer ones. For example, patterns with 3 bp perform on average 12% better than those with 6 bp long. More datasets should be examined in the future to explicitly test this observation. In all datasets, the C/T and G/A alleles have the best prediction accuracy (15–16% better than the overall median performance, \(P = 8.68 \times 10^{-9}\) for C/T and \(P = 1.98 \times 10^{-9}\) for G/A, Fig. 6C), reflecting the powerful (and known) influence of the bases immediately adjacent to the SNP for these alleles. We also noticed that interspecies performance is significantly better than the cross-species test (\(P = 2.77 \times 10^{-15}\) for Hs species test, mean accuracy for Hs-Hs: 61.0%, Hs-Mm: 58.6%; and \(P = 4.82 \times 10^{-9}\) for Mm species test, mean accuracy for Mm-Mm: 62.4%, Mm-Hs: 56.8%, Fig. 6D), which suggests that patterns are partially conserved across species.

3.3 CPU performance
T-WPPDC is an efficient algorithm with \(O(N \times L \times \log K)\) running time. Of our 672 experiments, 95% finished within 2 min on a Linux GNU 2.6.15-29-AMD64 server with Intel® Xeon® x5355 2.66 GHc CPUs and 16 GB RAM. T-WPPDC runs, on average, 39.7% faster than Oligo (\(P = 0.01\)) and 46.5% faster than BioProspector (\(P = 1.33 \times 10^{-31}\)). Figure 7 demonstrates that T-WPPDC is able to provide a lower bound of CPU running time (solid line), suggesting it is less sensitive to data size.

Fig. 5. Prediction accuracy (sequence length: 50 bp, SNPs: C/T, training from chromosome 21/22 training sequences and testing from chromosome 21/22 testing sequences). The x-axis shows the range of the threshold \(\epsilon\). The y-axis shows the prediction accuracy. Size for each dot represents the coverage rate, range from 0.1% to 100%, which means the numbers of sequences are identified. The point at \(\epsilon = 0\) represents no leaf-selection.

Fig. 6. Prediction accuracy impact from species, pattern length, SNPs and datatype (leaf selection on). (A–C) Shows the performance impact from different sequence length, pattern length and SNPs from chromosome 21/22. (D) Shows the performance impact from different species from chromosome X. Hs-Mm: intraspecies tests, Hs-Mm/Mm-Hs: cross-species tests.

4 CONCLUSIONS AND FUTURE WORK
4.1 Conclusions
We have developed a new pattern discovery algorithm, T-WPPDC, which identifies positional biases between two classes of sequences. We optimized search processes with tree structures, which dynamically improve the computation complexity and allow superior parallelization. By incorporating the pattern selection, T-WPPDC is able to reach the optimal classification performance.
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