SOFTWARE

Enrichment Depletion Logo plots

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Abstract

Background:
Sequence logo plots have developed into a standard graphical tool for identifying sequence motifs in DNA, RNA or protein sequences, largely because of its ease of interpretation and the visual appeal. However standard logo plots tend to be biased towards highlighting enrichment of symbols, thereby occasionally missing out on finer motif patterns.

Results:
In this article, we propose a new logo representation that highlights both enrichment as well as depletion of symbols at each position, resulting in a more parsimonious visualization. We show the benefits of this representation over the standard information content based logo plot through applications in displaying transcription factor binding site motifs, protein sequence alignments and mutational signature profiles.

Conclusion:
We present an easy-to-use and highly customizable R package *Logolas* that allows the user to plot such enrichment depletion logo plots where the characters in the logo plot can be any string symbol, consisting of alphabets, numerics, punctuations, dots, dashes etc.

Keywords: Logo plots; Enrichment Depletion; EDLogo; String symbols

Background

Ever since their introduction in early 90's by Schneider and Stephens [1], sequence logo plots have found extensive use in identifying short conserved patterns, also called sequence motifs, in multiple alignment of DNA, RNA and protein sequences. In a standard logo plot, for each position in the aligned sequences, symbols representing characters in the sequence are stacked on top of each other with their relative heights being proportional to the positional frequencies of the characters and the stack height per position determined by the information content at that position. Several packages in R such as *seqLogo* [2] (exclusive to DNA, RNA sequence alignment), *RWebLogo* (Wagih 2014), *ggseqlogo* (Wagih 2017) [3] and web servers like *WebLogo* (Crooks et al 2004) [4], *Seq2Logo* (Thomsen and Nielsen 2012) [5], *iceLogo* (Coalert el al 2009) [6] etc have been developed for sequence logo visualization of aligned DNA, RNA and protein sequences.

The standard sequence logo visualization based on Information Content tends to primarily highlight the enrichment of the symbols (bases or amino acids) at each position. Though *seq2Logo* allows the user to plot position specific scores that account for both enrichment and depletion, the representation is not parsimonious [5].

We introduce here a logo visualization package, *Logolas*, which allows the user to highlight both the enrichment as well as the depletion of symbols in a logo plot, but in a parsimonious and visually appealing way. We call this representation the *Enrichment Depletion Logo* or *EDLogo* plot. Additionally most logo plotting softwares are mainly limited in their applications to DNA, RNA and protein sequence alignment compositional data. *Logolas* provides the user the flexibility to plot logos for any alphanumeric strings and not just English alphabets as in standard packages, which extends the applicability of logo plots to more generic compositional data with string labels. In this article, we demonstrate various applications of the *EDLogo* representation and also highlight several features of the *Logolas* package.

Implementation

In **Supplementary Figure 1**, we illustrate the main intuition behind the *EDLogo* plot. Say for a specific position in a set of aligned DNA sequences, the relative frequencies are $p = (p_A, p_C, p_G, p_T) = (0.33, 0.33, 0.33, 0.01)$. A standard logo will show three equally high symbols A, C, G stacked vertically along the positive Y axis with T at the bottom having negligible height. The standard sequence logo plot is biased towards highlighting base enrichments. So, when this position is flanking by highly enriched bases as in panel (a), its stack height would be relatively much smaller compared to that of the neighboring positions which would make the depletion of T even harder to see (panel (b)). *EDLogo* provides an alternative parsimonious and perhaps more meaningful representation by highlighting for the said position, the depletion of T along the negative Y axis. This representation is designed to highlight large enrichments as well as large depletions, as observed in panel (c). We present the algorithm behind computing the enrichment and depletion of characters for the *EDLogo* representation below.

Let $p_n = (p_{n1}, p_{n2}, \ldots, p_{nB})$ be the position weights (normalized position frequencies of aligned sequences) of the characters at position n and $q_n = (q_{n1}, q_{n2}, \ldots, q_{nB})$ be the corresponding background probabilities. Typically we encounter q_n to be same for all positions n ($q_n \equiv q$).

We first define a score vector f_n for each n.

$$f_{nb} = \log_2 \frac{p_{nb} + \epsilon}{q_{nb} + \epsilon} - median\left(\left\{\log_2 \frac{p_{nb} + \epsilon}{q_{nb} + \epsilon} : b = 1, 2, \ldots, B\right\}\right) \tag{1}$$

where ϵ is a thresholding parameter controlling the effect of small position weight values p_{nb}. The default choice of ϵ is 0.1. We next compute

$$f^+_{nb} = f_{nb}\mathbf{I}(f_{nb} \geq 0) \qquad\qquad f^-_{nb} = f_{nb}\mathbf{I}(f_{nb} < 0) \tag{2}$$

where $\mathbf{I}$ is the indicator function. The above formulation ensures that for a base b, one of f^+_{nb} and f^-_{nb} is zero.

For each position n, we plot the f^+_{nb} values along the positive Y axis and the f^-_{nb} values along the negative Y axis. For a base b enriched at position n, f^+_{nb} value will

be large resulting in large size of the symbol for base b in the positive Y axis of the *EDLogo* plot. For a base b depleted at position n, f^{-}_{nb} value will be large resulting large size of the symbol for base b in the negative Y axis of the *EDLogo* plot.

Results

Sequence logos have been used extensively in visualizing transcription factor binding motifs (TFBSs). Though base enrichment is usually the more prevalent feature in most positions of the binding motif, some transcription factors tend to show depletion of bases at specific positions. In Figure 1(panel (A)), we present the standard logo and the *EDLogo* representation of the Early B cell factor 1 disc 1 (EBF1-disc1) transcription factor. Not only does the sequence motif show strong depletion signals of bases G and C at the center of the sequence but the depletion is also a part of the palindrome TCCCg - cGGGA, where lowercase letters stand for depletion and uppercase case letters stand for enrichment of characters. Note that this depletion signal is hard to see in the standard logo plot (panel (A) *left*) because it is flanked by strong enrichments, but the *EDLogo* representation (panel (A) *right*) shows it clearly. In **Supplementary Figure 2**, we present the *EDLogo* representation of all the members of the EBF1 family and besides EBF1-disc1, the depletion of G and C at the center of the palindromic sequence is observed also in EBF1 - known3 and EBF1 - known4.

In Figure 1 panel (B), we compare the *EDLogo* plot (*right*) with the PSSM profile representation (*left*) of the binding sequence of the Bacterial transcription activator, effector binding domain protein PF06445 (motif 4, Start=153 Length=8). The PSSM visualization is a common alternative to standard logo plots for protein sequences. But here it is evident that the *EDLogo* representation is much more parsimonious and interpretable than the PSSM representation. The main reason behind the sparse visualization in *EDLogo* is due to the median adjustment in determining the enrichment and depletion patterns (see Implementation). Both the position weight matrix (PWM) and position specific scoring matrix (PSSM) for this protein have been fetched from 3PFDB webpage `http://caps.ncbs.res.in/3pfdb/` [7] [8].

An important feature of *Logolas* is that the user can plot logos for any alphanumeric strings . An example application of this feature is in visualizing mutational signature profiles. Each mutation signature is usually represented by the type of C and T mutations, ($C \rightarrow T$, $C \rightarrow A$, $C \rightarrow G$, $T \rightarrow A$, $T \rightarrow C$, $T \rightarrow G$) flanked by bases to the left and right. The A and G mutations are clubbed with C and T mutations above to avoid strand bias. In Figure 1 panel (C), we compare the standard sequence logo (*left*) and the *EDLogo* representations (*right*) of the mutational signature profile of lymphoma B cell mutations in Alexandrov et al [9]. We observe that it is much easier to identify the depletion of G on the right flanking base (possibly occurring due to methylated CpG sites being less prone to mutation) in the *EDLogo* plot compared to the standard sequence logo plot. In **Supplementary Figure 3**, we compare the *EDLogo* representation with the *pmsignature* representation due to Shiraishi et al [10] for the same B cell lymphoma mutation type example as in

Figure 1 panel (C) and it is evident that the *EDLogo* plot depicts the overall features of the logo plot way more clearly. In **Supplementary Figure 4**, we present the *EDLogo* representations of all 27 cancer mutation signature profiles reported in Figure 4 of Shiraishi et al [10].

Discussion

Besides the approach shown in Implementation, *Logolas* allows the user to use other options for computing the enrichment and depletion levels of each character in a position of the aligned sequences. We discuss these options in greater detail under Supplementary Methods. In **Supplementary Figure 5** and **Supplementary Figure 6**, we present the different types of *EDLogo* representation for the transcription factor EBF1-disc1 and the protein PF06445 example in Figure 1. The *EDLogo* representation generates heights of the bases along the positive and negative Y-axes representing enrichment and depletion respectively, which can be used as scores for these downstream analysis - like motif matching, comparing motif patterns, regulatory SNP detection etc (see packages *DiffLogo* [11], *motifStack* [12], *atSNP* [13]).

We have shown an application of string logos in *Logolas* in displaying mutational signature profiles in Figure 1 panel (C). We present another string logo based example in **Supplementary Figure 7**. In this figure, we present the standard and *EDLogo* plots for the composition of different types of histone modifications at sites that overlap with an intergenic sequence, intron, exon, gene start and gene end for a lymphoblastoid cell line, GM06990 using ChIP-chip data (Koch et al [14]). The characters here are the histone mark names - for example H3K4ME1 - which are alphanumeric strings.

Besides the *EDLogo* representation and string symbols features, *Logolas* provides many other customizable features - different fill and border styles for enriched and depleted symbols in *EDLogo* plot, different approaches of calculating stack heights for a standard logo plot (Renyi entropy at differet scales, Shannon entropy, relative frequnecy based plot), plotting logos in multiple panels and also combining logo plots with external ggplot2 graphics. The example applications of these features are presented in https://kkdey.github.io/Logolas-pages/.

The Logolas package is currently released on Bioconductor (https://bioconductor.org/packages/release/bioc/html/Logolas.html) and is also under active development on Github (https://github.com/kkdey/Logolas).

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Competing interests
The authors declare that they have no competing interests.

Author's contributions
KKD and MS conceived the idea. KKD implemented the package. KKD and DX tested Logolas on the data applications. KKD, DX and MS wrote the manuscript.

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Figures

Figure 1 EDLogo representation and its performance comparison to standard logo plots. We present a comparative study of the *EDLogo* representation with respect to the standard logo plots, through three examples. In the first example (panel (A)), we compare the *EDLogo* representation (*right*) with the standard information content based logo plot (*left*) for modeling the transcription factor binding site of EBF1-disc1 transcription factor. We observe that the *EDLogo* plot identifies the depletion in the middle of the sequence much better than the standard plot. In panel (B), we compare the *EDLogo* plot with the position specific scoring matrix (PSSM) plot of the binding sequence of the Bacterial transcription activator, effector binding domain protein PF06445 (motif 4, Start=153 Length=8). We observe that the *EDLogo* representation is much more visually parsimonious and detailed than the PSSM plot. In panel (C), we present the standard logo and the *EDLogo* representation of the mutational signature profile of the all mutations in lymphoma B cells, where the data is due to Alexandrov et al 2013 [9]. We observe that the depletion of G to the right of the mutation - possibly occurring due to methylated CpG sites being less prone to mutation - much more clearly in the *EDLogo* representation compared to the standard logo plot.

Supplementary Figures
S1 Fig. **Illustration of the EDLogo representation.** We present an illustration of how *EDLogo* representation accounts for depletion signal and provides a more informative visualization of the sequence motif. In panel (a), we present a position weight matrix with the position weight vector at the second position having a depletion of T, but is flanked by enrichments around it. In panel (b), we present the corresponding standard logo plot representation of the PWM matrix in panel (a). The signal at the second position gets swamped by the bias towards enrichment signals flanking it. In panel (c), we present the *EDLogo* representation of the PWM matrix, where both the enrichment signals as well as the depletion signal at position 2 are clearly observed.

S2 Fig. **EDlogo representation of the members of the EBF1 family of transcription factors**: We present the *EDlogo* representation for the binding sites of 6 transcription factors in the EBF1 family. EBF1-known4 and EBF1-disc1, and also to some extent EBF1-known3 seem to show the depletion of G and C in the middle of the binding site. The PWM data for all the transcription factors have been obtained from the ENCODE TF Chip-seq datasets and are hosted on the webpage http://compbio.mit.edu/encode-motifs/ [15].

S3 Fig. **Comparison of Logolas EDLogo plot with pmsignature representation for cancer mutation signatures**: We compare the *EDLogo* plot representation and the *pmsignature* representation due to Shiraishi et al (2015) [10] for mutation signature profile of lymphoma B cell from Alexandrov et al 2013 [9]. The position 0 corresponds to the mutation. Positions -1 and -2 correspond to the the two left flanking bases with respect to the mutation. Positions 1 and 2 correspond to the the two right flanking bases with respect to the mutation. Clearly, *EDLogo* representation shows the depletion of G at the right flanking base more clearly and is more interpretable and visually appealing in highlighting the overall mutation signature patterns compared to the*pmsignature* plot.

S4 Fig. **EDLogo plots for the mutational signature profiles for 27 clusters in Shiraishi et al (2015)**: We present the *EDlogo* representations (ratio) method for the 27 cluster signature profiles obtained from fitting a grade of membership model on the cancer mutational signature data across 30 cancer types by Shiraishi et al (2015) [10]. This plot is an alternative logo plot based representation of Figure 4 in Shiraishi et al (2015) [10].

S5 Fig. **Different options for EDLogo representation - TFBS example**: We present the visualizations corresponding to the different options of the *EDLogo* plot for the EBF1-disc1 transcription factor. The details of the methods behind the various options of *EDLogo* are presented in Supplementary Methods.

S6 Fig. **Different options for EDLogo representation - Protein example**: We present the visualizations corresponding to the different options of the *EDLogo* plot for the binding sequence of the Bacterial transcription activator, effector binding domain protein PF06445 (motif 4, Start=153 Length=8).

S7 Fig. **Example applications of string logo plots.** We present the standard and the *EDlogo* plot representations of the composition of histone modification types that overlap with an intergenic region, intron, exon, gene start or gene end for the lymphoblastoid cell line GM06990 as reported in Koch et al 2007 [14]. These plots use alphanumeric string symbols as characters in the plot - a special feature of the package *Logolas*.

Supplementary Methods

Here we discuss the additional options for creating stacks of symbols in the *EDLogo* plots. We call the method discussed in the Implementation section of the main text as the *log* approach. Some other other options for *EDLogo* plots are *log-odds*, *ratio* and their information content based counterparts namely *ic-log*, *ic-log-odds*, *ic-ratio*.

Let p_n be the position weights of the symbols at position n and q_n be the background probabilities at that position. We define the score vector f_n for the *log-odds* and *ratio* approaches.

- *log-odds* approach

$$f_{nb} = \log_2 \frac{p_{nb}/(1-p_{nb}) + \epsilon}{q_{nb}/(1-q_{nb}) + \epsilon} - median\left(\left\{\log_2 \frac{p_{nb}/(1-p_{nb}) + \epsilon}{q_{nb}/(1-q_{nb}) + \epsilon} : b = 1, 2, \ldots, B\right\}\right) \tag{3}$$

- *ratio* approach

$$f_{nb} = \frac{p_{nb} + \epsilon}{q_{nb} + \epsilon} - median\left(\left\{\frac{p_{nb} + \epsilon}{q_{nb} + \epsilon} : b = 1, 2, \ldots, B\right\}\right) \tag{4}$$

As in the *log* approach, we define $f^+_{nb} = f_{nb}\mathbf{I}(f_{nb} \geq 0)$ and $f^-_{nb} = f_{nb}\mathbf{I}(f_{nb} < 0)$ where $\mathbf{I}$ is the indicator function. For the *ic* based approaches (*ic-log*, *ic-log-odds* and *ic-ratio*), we additionally compute the information content for each position $IC(n)$ and then redefine the f^+_{nb} and f^-_{nb} scores

$$f^+_{nb} \leftarrow IC(n) \times \frac{f^+_{nb}}{\sum_b \left(f^+_{nb} + f^-_{nb}\right)} \qquad f^-_{nb} \leftarrow IC(n) \times \frac{f^-_{nb}}{\sum_b \left(f^+_{nb} + f^-_{nb}\right)} \tag{5}$$

These f^+_{nb} values are plotted along the positive Y axis, while the f^-_{nb} values are plotted along the negative Y axis. In terms of performance, the *log* and *log-odds* tend to highlight the depletion signal more. On the other hand, all the *ic* based options - *ic-log*, *ic-log-odds* and *ic-ratio* are slightly biased towards the enrichment signal.