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Constitution Analysis

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ABSTRACT
Motivation:
Results: https://github.com/someone/rich.
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1 INTRODUCTION

The genome is targeted by a sophisticated and highly coordinated series of molecular events. Among these events, aberrant DNA methylation patterns in human malignancy De *et al.* (2013), somatic retrotransposition in human cancers Lee *et al.* (2012), AID-dependent chromosomal translocations (Klein, 2011) and HIV integration (Cohn, 2014), which arrives throughout DNA, are not randomly distributed but instead associated with chromosomal regions and contributes to disrupt the integrity of the genome and human disease.

As result, these regions represents a genomic context in which are associate with multiple underlying mechanisms. The motif-based sequence analysis is the starting point to aim potential binding site of cis-regulatory elements associated. Nevertheless, the inherent low signal/noise ratio in sequence-based motif discovery is a limitation to detect a nucleic acid sequence pattern that has some biological significance. Moreover, these events may recognise a structural feature, rather than a specific sequence motif.

Our work has some intersection whit the computation of 'enrichment p-values' considered in GO analysis. We may include the references Huang *et al.* (2009), Rivals *et al.* (2007) (just one!) or any other if you know a better alternative. We may like to mention the paper by Bailey and Machanick (2012) because it also considers a test for enrichment (although it is restricted to ChiP-seq peaks, and somehow different).

However, how exactly the pattern nucleotide composition could influence the selection of target site selection are not well understood. To further characterize at a genome-wide scale these regions, we introduce a new method (*k-enrich*) to provide a quantitative measure of the differential spectra of k-mers (DNA 'words' of length k) throughout target DNA.

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2 METHOD

Let $\mathcal{A} = \{\mathsf{A}, \mathsf{C}, \mathsf{G}, \mathsf{T}\}$ and $\mathcal{S} \in \mathcal{A}^\ell$, be a given specific string of length $\ell \geq 1$. In what follows, we describe a method to study the profile of $\mathcal{S}$ along a region of interest such as those defined by viral insertion or retrotranslocation hotspots. This provides the means to asses the significance of a differently distributed profiles along two functionally defined regions. We specialise to viral insertion hotspots as described by Silva *et al.* (2014), but the scope is clearly not restricted to this particular application.

Let $h = \{h_1, \ldots, h_n\}$ bet a set of viral insertion hotspots, namely a set of DNA segments characterized by having a substantially high density of viral insertion events. Let w be the length of the longest of such segments. The segments $h_1, \ldots, h_n$ are aligned with respect to their central base and then extended at both ends to have length w. Next, consider the partition of resulting set of segments into k evenly spaced bins of length $\ell = w/k$. Denote by h_{ij}, $1 \leq j \leq k$, the jth bin of the ith segment. Consider now the set $r = \{r_1, \ldots, r_n\}$ of segments of width w that are either at the left or at the right of any one segment in h. Likewise, let r_{ij}, $1 \leq i \leq n$, $1 \leq j \leq k$, be the matrix formed by bins of length ℓ that result by partitioning the elements of r. For any $j = 1, \ldots, k-1$ and $i = 1, \ldots, n$, let ξ_{ij} and η_{ij} be the following Bernoulli random variables

$$\xi_{ij} = \begin{cases} 1, & \text{if } \mathcal{S} \in h_{ij} \text{ and } \mathcal{S} \notin h_{i,j+1} \\ 1, & \text{if } \mathcal{S} \in h_{ij} \text{ and } \mathcal{S} \in h_{i,j+1} \\ 0, & \text{otherwhise} \end{cases},$$

$$\eta_{ij} = \begin{cases} 1, & \text{if } \mathcal{S} \in r_{ij} \text{ and } \mathcal{S} \notin r_{i,j+1} \\ 1, & \text{if } \mathcal{S} \in r_{ij} \text{ and } \mathcal{S} \in r_{i,j+1} \\ 0, & \text{otherwhise} \end{cases}.$$

Set $\xi_{ik} = 1$ if $\mathcal{S} \notin h_{i,k-1}$ and $\mathcal{S} \in h_{i,k-1}$, and $\xi_{ik} = 0$ otherwhise. Similarly define η_{ik} by using the information in r_{ik}. Finaly, let

$$\xi_j = \sum_{i=1}^{n} \xi_{ij}, \qquad \eta_j = \sum_{i=1}^{n} \eta_{ij}.$$

The variables ξ_j and η_j, $1 \leq j \leq k$, count the number of times that the string $\mathcal{S}$ occurs along of a hotspot region and of a reference region respectively.

The basic question we like to address is wether the distribution profile of the string $\mathcal{S}$ is significatively different along a typical

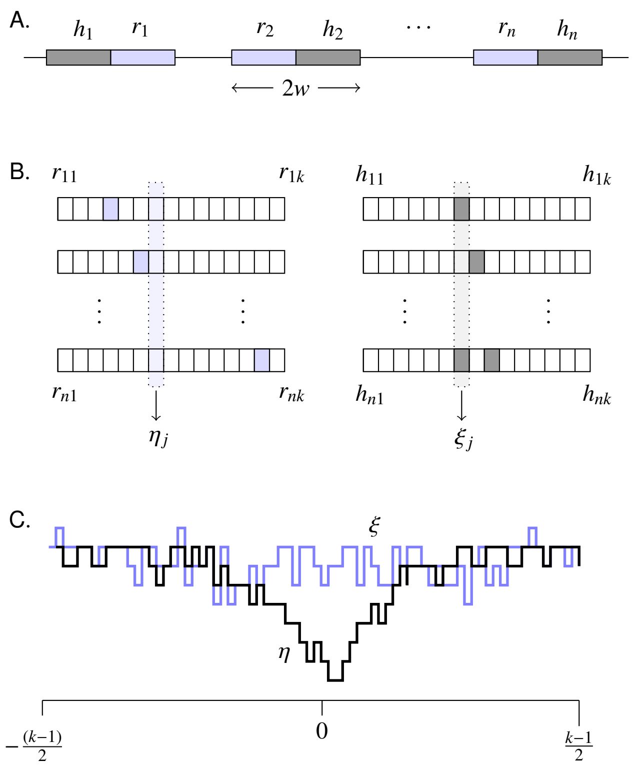

Fig. 1. A: Input data segments $h_1, \ldots, h_n$ containing the occurence of a string $\mathcal{S}$ and reference segments $r_1, \ldots, r_n$. B: r_{ij} and h_{ij} matrizes of counts for a particular realization of the random variables η_{ij}, ξ_{ij}. C: Profile distribution for the occurence of $\mathcal{S}$ along a hotspost and a reference region.

hotspot region and a reference region. This may be assessed by considering the following $2 \times k$ contingency table

$$\begin{bmatrix} \xi_1 & \xi_2 & \ldots & \xi_k \\ \eta_1 & \eta_2 & \ldots & \eta_k \end{bmatrix},$$

obtained by merging the two vectors of counts ξ_j and η_j. Provided the number of counts in each of the cells of this table is sufficiently large, the significance of a differential profile can be determined by using Pearson's X^2 statistic, which is distributed according to a χ^2 density with $k-1$ degrees of freedom. Other alternatives for the large sample case exist, see for instance Read and Cressie (1988), but we do not pursue this further here. It is well known that this procedure can give a poor approximation when several cells present low counts (smaller than 10). This may be the case in the current setting when analysing the profile distribution of longer strings with $\ell \geq 10$ or even smaller but rarely occuring strings. In these situations the significance for a differential profile is more appropriately determined by using Fisher's exact test, see for instance Agresti (2012). The computations necessary to derive a p-value are not feasible because of the large number of contingency tables that have to be considered as a reference set when k is large. The significance may however be approximately computed by considering a permutation test using the method in Patefield (1981). We found that R's implementation via `fisher.test` takes only few secconds for relatively large tables with $k = 1000$.

We provide examples for the two scenarios just mentioned by considering strings formed by a single base and strings defined by longer motivs with $\ell = 15$. The former provides an example where the X^2 statistic is a appropriate and the latter one that is amenable to the analysis with Fisher's exact test.

2.1 TC-Seq libraries

The TC-Seq datasets analyzed here are those described by Klein *et al.* (2011). These are deposited at Sequence Read Archive (SRA, `http://www.ncbi.nlm.nih.gov/sra`) under accession numbers SRA061477. These datasets are from two different translocation libraries: (i) a library from activated B cells infected with AID-expressing retrovirus (denoted hereafter as AID^{rv}) and (ii) a library from AID-deficient B cells (denoted as AID$^{-/-}$). Three set of curately hotspots were defined from these samples: (i) 59 physiological hotspots in Ig-genes (AID^{rv} and AID^{wt} samples), (ii) 157 off-target hotspots (in non Ig-genes from AID^{rv} and AID^{wt} samples) and iii) 34 hotspots from AID-deficient B cells.

3 RESULTS & DISCUSSION

We have applied our method to further understand the genomic complexity at recurrent translocations hotspots locus induced by activation-induced cytidine deaminase (AID). Recurrent chromosomal translocations are associated with hematopoietic malignancies such as leukemia and lymphoma and with some sarcomas and carcinomasNussenzweig and Nussenzweig (2010).

Although AID specifically targets the immunoglobulin genes loci (IgH, Igl and Igλ), it also targets a array of non-immunoglobulin genes. The[1] and how nucleotide composition could impact the formation of translocations require better understanding. In this work we examine the landscape of k-mers across the physiological and off-targets translocation hotspots from AID^{wt} and AID^{rv} samples and, translocation hotsptots from AID^{ko} sample.

The results obtained by analyzing the three hotspot sets (Section 2.1) are presented in Figure X using 1-mer (A-mer, C-mer, G-mer and T-mer) and 2-mer (CG-mer). By apply our method, we detected remarkably differences between the translocation hotspots across nucleotide composition for each sample.

The Figures Xa and Xb exhibit 1-mer inrichment throughout of hotspots (C and G nucleotides goes up and A and T nucleotides goes down, see Table S1 for *pvalues*), but only in the physiological targets (Figure Xa) the enrichment is sharply in the middle of hotspots while in the off-targets is broad around the center of hotspots. Interestingly, when we search for 2-mer (CG-mer), off-targets hostpots is marked by a high degree of CG-mer ($pvalue$ = 4.67179250921589e-136) . This effect in off-targets AID supports the theoretical mechanism for CpG-type Double Strand Breakage proposed in Tsai *et al.* (2008), when a slippage event between the top and bottom strand would place the CpG within a loop, thereby making it vulnerable to AID.

According to the Figure Xc, we can not observe specific preference across hotspots from AID^{ko} sample, although occurs nucleotide composition enrichment for $A|C|G|T$-mer (see Table S1 for p-values).

[1] STILL WRITING...

Following these observations, these findings strongly suggest that either $C|G$-mer or CG-mer are markers for distinct sequence-dependent mechanisms that attract the AID under physiological and overexpressed levels of AID respectively.

4 CONCLUSION

We propose a standalone method (*k-enrich*) to calculate enrichment distribution of k-mer throughout of target DNA. We use a few examples to demonstrate that *k-enrich* provide a way to investigate the genomic complexity, although our method can be applied to any nominal variable data.

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