More Codon Usage Bias

Scaled $\chi^2$

$\chi^2$ measure. In statistics, the $\chi^2$ statistic computes how different the distribution of values is from a uniform distribution.

Let $d = c_1 \ldots c_N$ be a DNA string in nucleotide alphabet, and let $L$ be the total number of codons in $d$ that are not Methionine or Tryptophan (the number does include the stop codons though).

Let $a$ be a $k$-degenerate amino acid, and let $L_a$ be the total number of codons coding for $a$ in $d$. Let $O_1, \ldots, O_k$ be the number of occurrences of the $k$ different codons for $a$ in $d$ ($O_1 + \ldots + O_k = L_a$).

$\chi^2$ statistic for codon usage bias. The $\chi^2$ value of codon usage bias for the amino acid $a$ in DNA string $d$ is computed as follows [2, 1]:

$$\chi_a^2 = \sum_{i=1}^{k} \frac{(O_i - E)^2}{E},$$

where

$$E = \frac{L_a}{k}$$

is the expected number of codon occurrences assuming no bias.

Scaled $\chi^2$. A scaled $\chi^2$ statistic for codon usage bias for the amino acid $a$ in DNA string $d$ is

$$\hat{\chi}_a^2 = \frac{\chi_a^2}{L}.$$

Range. Smaller values of $\chi^2$ and scaled $\chi^2$ mean little or no codon usage bias. Larger values mean larger bias.
Other Means of Evaluating DNA.

Information Entropy

**Information Entropy.** Consider a set $S = S_1 \cup S_2 \cup \ldots \cup S_k$ of items, where $S_i \cap S_j = \emptyset$ when $i \neq j$.

As $Pr(s \in S_i)$ we denote the probability that a randomly chosen element $s \in S$ will be from set $S_i$.

$$Pr(s \in S_i) = \frac{|S_i|}{|S|}.$$ 

The entropy of the set $S$ w.r.t. the partition $S_1, \ldots, S_k$ is defined as follows:

$$entropy(S) = -\sum_{i=1}^{k} Pr(s \in S_i) \cdot \log_2(Pr(s \in S_i)).$$

*Entropy* is measured in bits.

*(Note: In this computation, we assume that $0 \cdot \log_2(0) = 0$.)

**Properties of entropy.** The entropy of a homogenous dataset in which $|S_i| = \frac{|S|}{k}$ for all sets $S_i \log_2 k$, i.e., the number of bits necessary to represent $k$.

$$entropy(S) = -\sum_{i=1}^{k} \frac{1}{k} \cdot \log_2 \left( \frac{1}{k} \right) = -\log_2 \left( \frac{1}{k} \right) \cdot \sum_{i=1}^{k} \frac{1}{k} = \log_2 k$$

The entropy of a dataset where only one set $S_i$ of the $k$ sets is non-empty is 0.

$$entropy(S) = -\sum_{i=1}^{k-1} 0 \cdot \log_2 0 - 1 \cdot \log_2 1 = 0.$$ 

**Entropy measures the impurity of data.** The higher the *entropy*, the more impure the data is.

For DNA sequence analysis, information entropy is used in the following way. Let $d = c_1 \ldots c_N$ be a DNA sequence fragment in a nucleotide alphabet. Let $N_A$, $N_T$, $N_C$ and $N_G$ be the total numbers of occurrence of nucleotides A, T, C and G respectively in $d$ ($N_A + N_T + N_C + N_G = N$). Then the entropy of the sequence $d$ is computed as follows:

$$entropy(d) = -\left( \frac{N_A}{N} \cdot \log_2 \left( \frac{N_A}{N} \right) + \frac{N_C}{N} \cdot \log_2 \left( \frac{N_C}{N} \right) + \frac{N_T}{N} \cdot \log_2 \left( \frac{N_T}{N} \right) + \frac{N_G}{N} \cdot \log_2 \left( \frac{N_G}{N} \right) \right).$$

High entropy in a DNA sequence means higher sequence complexity. Lower entropy means lower sequence complexity. (The simplest DNA sequence is one that consists of a single nucleotide).

**Proposition.** Let $d$ be a DNA sequence and $\hat{d}$ be its reverse compliment. Then

$$entropy(d) = entropy(\hat{d}).$$
Proof. Let $N_A, N_T, N_C, N_G$ be the numbers of occurrences of A, T, C and G in $d$, and $\bar{N}_A, \bar{N}_T, \bar{N}_C, \bar{N}_G$ be the numbers of occurrences of A, T, C, G in $d$. Since $d$ is a reverse complement of $\hat{d}$:

$$N_A = \bar{N}_T; \quad N_T = \bar{N}_A;$$

$$N_C = \bar{N}_G; \quad N_G = \bar{N}_C.$$ 

Using these equalities and plugging the values into the entropy formula, we obtain the desired result.

**Gene Content**

**Gene content** of DNA is the name of a collection of measures that evaluate the frequency and the size of genes, their components (introns and exons) and the intergenic regions (regions of DNA between genes).

**Notation.** Let $d = c_1 \ldots c_N$ be a DNA string in a nucleotide alphabet. Let $d = d_1 e_{i_{11}} e_{12} i_{12} \ldots e_{1s} d_2 \ldots d_k g_{k1} \ldots e_{k_s} d_{k+1}$, where

1. $d_s$ are non-coding intragenic regions, $e_{ij}$ are exons, i.e., coding regions
2. $e_{ij}$ is the $j$th exon of $l$th gene in $d$
3. $i_{ij}$ are intron, i.e., non-coding DNA regions separating exons of the same gene.

Let

$$N_e = |e_{11}| + |e_{12}| + \ldots |e_{k_s}|$$

be the number of base pairs in the exons,

$$N_o = |d_1| + |d_2| + \ldots + |d_{k+1}|$$

be the number of base pairs in the non-coding intragenic regions,

$$N_i = |i_{11}| + |i_{12}| + \ldots |i_{k_s-1}|$$

be the number of base pairs in all introns,

$$N_{nc} = N_o + N_i$$

be the total number of non-coding base pairs in the DNA fragment, and, finally,

$$N_g = N_e + N_i$$

be the total length of genes in the DNA fragment\(^1\).

In density computations, both genes expressed on the top and the bottom strands are considered: so $e_{lj}$'s in the notation above refer to all coding regions from both strands.

We let $k$ represent the total number of genes in $d$ and $q$ be the total number of exons.

The following measures are used for tracking gene density.

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\(^1\) Note the difference between the notions of "length of a gene" and "length of all exons of a gene".
**Average gene size.** The average gene size, $Avg_g(d)$ is computed as follows:

$$Avg_g = \frac{N_e + N_i}{k} = \frac{N_g}{k}.$$ 

**Average coding DNA sequence (CDS) size.** The average coding DNA sequence size, $Avg_c(d)$, only counts exon lengths in computations:

$$Avg_c(d) = \frac{N_e}{k}.$$ 

**Average exon size.** The average exon size, $Avg_e(d)$, uses $q$, the total number of exons, in the denominator:

$$Avg_e(d) = \frac{N_e}{q}.$$ 

**Average intron size.** A gene with $l$ exons contains $l-1$ introns. A DNA sequence with $k$ genes and $q$ exons has $p = q - k$ introns. The average intron size, $Avg_i(d)$, is computed as follows:

$$Avg_i(d) = \frac{N_i}{p} = \frac{N_i}{q - k}.$$ 

**Nucleotides to genes ratio.** This measure, denoted $ratio(d)$ is defined as

$$ratio(d) = \frac{N}{k}.$$ 

Usually, it is measured in Kilo-base pairs per gene, so the exact computation would be:

$$ratio(d) = \frac{N}{1000 \cdot k}.$$ 

$$Avg_g = \frac{N_e + N_i}{N_e + N_i + N_{nc}} = \frac{N_g}{N_g + N_{nc}} = \frac{N_g}{N}.$$ 

**Gene nucleotide fraction.** The gene nucleotide fraction of $d$, denoted $density_g(d)$, is the percent of base pairs in the genes:

$$frac_g(d) = \frac{N_e + N_i}{N_e + N_i + N_{nc}} = \frac{N_g}{N_g + N_{nc}} = \frac{N_g}{N}.$$ 

**Coding nucleotide fraction.** The coding nucleotide fraction of $d$ is the percent of base pairs in the exons:

$$frac_c(d) = \frac{N_e}{N_e + N_i + N_{nc}} = \frac{N_e}{N}.$$
**Exon density.** The exon density of \( d \) is the total number of coding regions (exons) in \( d \) divided by the length of \( d \):

\[
\text{density}_e(d) = \frac{q}{N}.
\]

**Gene density.** The gene density of \( d \) is the total number of genes in \( d \) divided by the length of \( d \):

\[
\text{density}_g(d) = \frac{k}{N}.
\]

Since genes/coding regions typically span 1 – 10 Kbp (Kilo-base pairs), density is usually expressed as the number of genes per 10Kbp, 100Kbp, or 1Mbp. Alternatively, a reciprocal value can quantify average gene spacing: DNA length in nucleotides divided by the number of genes present:

\[
\text{spacing}(d) = \frac{N}{k}.
\]

**Relative gene coverage.** This measure, denoted \( \text{coverage}_g(d) \), is the ratio of the average gene size and the total length of the DNA sequence:

\[
\text{coverage}_g(d) = \frac{\text{Avg}_g(d)}{N} = \frac{N_2}{k \cdot N}.
\]

**Relative exon coverage.** Same as relative gene coverage, but for exons only:

\[
\text{coverage}_e(d) = \frac{\text{Avg}_e(d)}{N} = \frac{N_e}{k \cdot N}.
\]

**References**
