Gene Tagging and the Data Hiding Rate

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Abstract — We analyze the maximum number of ways in which one can intrinsically tag a very particular kind of digital asset: a gene, which is just a DNA sequence that encodes a protein. We consider gene tagging under the most relevant biological constraints: protein encoding preservation with and without codon count preservation. We show that our finite and deterministic combinatorial results are asymptotically—as the length of the gene increases—particular cases of the stochastic Gel’fand and Pinsker capacity formula for communications with side information at the encoder, which lies at the foundations of data hiding theory. This is because gene tagging is a particular case of DNA watermarking.

Keywords — Gene tagging, DNA watermarking, combinatorial analysis, Gel’fand and Pinsker capacity.

I Introduction

The advent of the biotechnological age has made it possible to tag genes—the molecular units of heredity in living organisms. Gene tagging refers to marking genes in unique, distinct ways, usually in order to enable tracking applications. The most fundamental strategy to tag a gene, which is essentially a digital sequence, is to modify it in such a way that it is possible to distinguish it later from the original gene (and from other differently tagged versions of it). This has sometimes been termed DNA watermarking, and a number of methods to digitally tag genes have been proposed over the last years (see [1, 2, 3]). DNA watermarking constraints are biological, and thus very different from the imperceptibility constraints typically used in watermarking of multimedia assets. The most important constraint is that the full biological functionality of the gene must always be preserved, so that the tagged gene may be reintroduced in a living being by means of recombinant DNA techniques and still remain fully operative. This has actually been achieved with living organisms by Arita and Ohashi [2] and by Heider and Barnekow [3].

In this paper we analyze the maximum number of ways in which a given gene can be tagged, relying on finite and deterministic combinatorial analyses. Furthermore, we show that the asymptotics of the information rates derived from these deterministic analyses coincide with the stochastic limiting rates furnished by the Gel’fand and Pinsker formula for the capacity of communications with side information at the encoder. This is not surprising given the fact that gene tagging is a special data hiding problem. This type of link between the asymptotics of deterministic combinatorics and probabilistic information-theoretical amounts is related to previous results, which we discuss towards the end of this paper.

II Basic Concepts and Notation

Calligraphic letters (X) denote sets; |X| is the cardinality of X. Boldface Roman letters (x) denote row vectors, x = [x₁, ⋯, xₙ]. A Roman letter that appears in uppercase (X) and in lowercase (x) denotes a random variable and a realization of it, respectively. p(X = x), or just p(x) when unambiguous from the context, is the probability mass function or distribution of X. X can also refer to its distribution, depending on the context. E(X) is the expectation of the random variable X and H(X) its entropy. I(X; Y) = H(X) − H(X|Y) is the mutual information between X and Y. Log-
II Gene Tagging

From the previous introduction, it is clear that any particular gene can be written as a vector of codons \( \mathbf{x} = [x_1, x_2, \ldots, x_n] \), where \( x_i \in \mathcal{X}^3 \) is the \( i \)-th codon in the gene. In this section we will obtain the tagging rates associated to \( \mathbf{x} \). A tagging rate is just the number of bits per codon needed to represent one of the possible tags. We will consider two relevant cases: 1) the basic case in which the tagged gene \( \mathbf{y} \) is just constrained to translate into the same protein as \( \mathbf{x} \), and 2) the codon count preservation case, in which \( \mathbf{y} \) additionally preserves a biologically meaningful feature of \( \mathbf{x} \) known as codon bias, that will be described in Section b). The codon count for a given codon \( x \in \mathcal{X}^3 \) is \( n_x = \sum_{i=1}^{n} 1 \{x_i = x\} \), that is, the number of times that it appears in gene \( \mathbf{x} \). Clearly, the sum of the codon counts for all possible genes has to be equal to the length of the gene, that is, \( \sum_{x \in \mathcal{X}^3} n_x = n \).

a) Primary Structure Preservation Case

Since each codon can be replaced by any other synonymous codon without altering the translation of the gene into a protein, the number \( m \) of different DNA sequences \( \mathbf{y} \) with the same primary structure as \( \mathbf{x} \) is

\[
m = \prod_{i=1}^{n} |S_{\xi(x_i)}| = \prod_{x' \in \mathcal{X}'} |S_{x'}|^{n_x} \tag{3}
\]

This is because if a codon \( x \) represents the amino acid \( x' = \xi(x) \) then we have \( |S_{x'}| \) alternatives to choose from. Using expression (3), the tagging rate for that gene can be expressed as \( \tilde{R} = \frac{1}{n} \log m \) bits/codon, that is

\[
\tilde{R} = \frac{1}{n} \sum_{x' \in \mathcal{X}'} \left( \sum_{x \in S_{x'}} n_x \right) \log |S_{x'}| \text{ bits/codon} \tag{4}
\]

If we now approximate \( p(x') \approx \frac{1}{n} \sum_{x \in S_{x'}} n_x \) for large \( n \), then the tagging rate (4) can in turn be approximated as \( R \approx \tilde{R} = \sum_{x' \in \mathcal{X}'} p(x') \log |S_{x'}| \), that is, the asymptotic approximation is

\[
\tilde{R} = E(\log |S_{X'|}) \text{ bits/codon}. \tag{5}
\]

In the particular case in which codons are uniformly distributed, one has that \( p(x') = |S_{x'}|/|\mathcal{X}'|^3 \) and (5) becomes

\[
\tilde{R}_{\text{unif}} = H(\mathbf{X}) - H(\mathbf{X}') \text{ bits/codon}. \tag{6}
\]

That is, in this case the tagging rate can be approximated by the difference of discrete entropies between the codon and the amino acid distributions of the gene. We will see that this particular
result is parallel to the general result in the following section. However it must be noted that codons are never uniformly distributed in nature.

b) Codon Count Preservation Case

Tagging a gene with the preservation of its primary structure as the sole constraint—as we have done in the previous section, and as assumed in and [1], [2] and [3]—is risky from a biological point of view. Despite the fact that \( m \) tagged genes have the exact same protein translation as the original gene \( \mathbf{x} \), many of those possible \( \mathbf{Y} \) sequences may have codon counts very different to that of \( \mathbf{x} \). This is because, for each amino acid \( x \) in the original gene \( \mathbf{x} \), there are never uniformly distributed in nature. Tagging a gene with the preservation of its primary structure and codon count as the original gene \( \mathbf{x} \) is an instance of data hiding, or information transmission with side information available at the encoder and the channel output, then the maximum transmission rate is given by

\[
C = \max_{p(\mathbf{U} \mid \mathbf{X})} I(\mathbf{Z} ; \mathbf{U}) - I(\mathbf{U} ; \mathbf{X}) \text{ bits/symbol},
\]

where \( U \) is an auxiliary random variable that must be determined for each particular problem, and the channel input \( Y \) (the information-carrying signal) is a deterministic function of \( U \) and \( X \), that is, \( Y = e(U, X) \). Also \( Y \) must be close to \( X \) according to some problem-dependent distortion constraint.

In the gene tagging setting the side information at the encoder is an amino acid \( X' = \xi(\mathbf{X}) \) corresponding to a codon \( \mathbf{X} \) of the original gene, which as we will see completely determines the channel.
state. The auxiliary variable will be denoted as \( U \), because we will see later that it can be seen as taking codon values. The basic information-carrying signal is the tagged codon \( Y \). We do not need to consider a channel output, since we are not considering any distortions on the tagged gene in the combinatorial analysis, and therefore \( I(Z; U) = H(Y) \). The fundamental distortion constraint is just the equality \( \xi(Y) = X' \), which guarantees that the tagged codon always stands for the same amino acid as the original codon. Since \( Y = \epsilon(U, X') \), the cardinality of the support set of \( U \) is only \(|S_U|\) since \( U \) is an auxiliary variable.

One can then define the tagged codon variable as \( Y' = U \) without loss of generality (although there are actually \(|S_U|\) equivalent choices for the encoder, from the achievable rate viewpoint), and thus guarantee that the genetic distortion constraint is fulfilled. Therefore the variable \( Y \), which represents the distribution of the tagged gene, is equal to \( U \) in what follows.

Focusing next our attention on the subtracting mutual information in (12) see that \( H(X'|U) = 0 \), because given a codon there is no uncertainty about the amino acid that it represents. Then we may particularize (12) for gene tagging as

\[
C = \max_{p(u|x')} H(U) - H(X') \text{ bits/codon.}
\]  

It must be remarked that the tagging rates in the combinatorial analyses (4) and (8) in Section III concern finite and deterministic genetic sequences. As such, they are always upper bounded by the rates given by the more general formula (13), that is, \( R_c < R \leq C \). This is because this formula concerns stochastic rather than deterministic signals, and it is a limit which, in general, can only be attained asymptotically as the number of channel uses tends to infinity. However, as we will see next, the asymptotic approximations (5) and (11) of these combinatorial analyses are able to achieve \( C \).

### a) Primary Structure Preservation Case

In this case we just have to carry out the maximization in (13). Using the chain rule of the entropy we can write

\[
H(U, X') = H(U) + H(X'|U) = H(X') + H(U|X').
\]

As \( H(X'|U) = 0 \), this implies that (13) can be put as

\[
C = \max_{p(u|x')} H(U|X').
\]

One can see now that the maximization of

\[
H(U|X') = \sum_{x' \in X'} p(x') H(U|x')
\]

is achieved when \( H(U|x') \) is maximum for all \( x' \), which implies that \( p(u|x') \) be uniform for all \( x' \).

Then

\[
H(U|x') = \log |S_{x'}| \text{ and consequently}
C = E(\log |S_{X'}|) = \tilde{R}.
\]

### b) Codon Count Preservation Case

Since (13) contemplates the preservation of the primary structure already, additionally preserving the codon count just amounts to enforcing \( p(u|x') = p(x|x') \), that is, keeping the codon bias found in the original gene in the tagged gene. Therefore in this case no maximization of (13) is needed, or possible. Hence \( U \) has to be distributed as \( X \), and it follows that

\[
C_c = H(X) - H(X') = \tilde{R}_c.
\]

Due to the additional constraint \( C_c \leq C \). As \( Y = U \), note that the codon count preservation constraint is akin to a steganographic constraint in data hiding, that is, Cachin’s criterion for perfect steganography [7].

### V Discussion and Conclusions

Figure 1 shows a comparison of the combinatorial rates with and without codon count preservation against the Gel’fand and Pinsker capacity when \( X \) is uniformly distributed, for varying gene length \( n \). In this case \( C = C_c = 1.7819 \) bits/codon. We can see that \( R \approx C \) even for small \( n \), whereas \( R_c \rightarrow C \) as \( n \rightarrow \infty \). Some results for real genes are also given in Table 1, showing that with real genes \( \tilde{R}_c \approx C_c \) and that preserving the codon bias does not necessarily entail an important tagging rate decrease with respect to the unconstrained rate \( C \). The results in Table 1 use the empirical amino acid distribution and codon bias distributions of the corresponding genes, obtained from GenBank using the accession numbers provided.

It is interesting to mention that the connections that we have given between asymptotic combinatorial analyses and side-informed achievable rates
Table 1: Tagging rates (bits/codon) for some real genes

can actually be seen as an informal application of the method of types [8]. For instance, a well
known such connection is the following one: take a Bernoulli random variable $X$ and assume that
$n$ independent outcomes of this binary variable have resulted in $k$ ones. The binomial coefficient
gives the number $m$ of different ways in which such a situation can happen, that is, $m = \binom{n}{k} = \frac{n!}{k!(n-k)!}$. Using the Stirling’s approximation of the factorial we can describe these $m$ possibilities using the rate $R = \frac{1}{n} \log m$ bits/outcome, that is

$$R \approx \tilde{R} = -\frac{k}{n} \log \frac{k}{n} - \frac{(n-k)}{n} \log \frac{(n-k)}{n}.$$  \hfill (18)

Now, as we did in Section III, for large $n$ we can approximate $k/n \approx p(X = 1)$ and $(n-k)/n \approx p(X = 0)$, which yields the approximation $R \approx H(X)$, or, equivalently, $\binom{n}{k} \approx 2^{nH(X)}$, that is, the combinatorial analysis can be asymptotically approximated using the entropy of the random variable that generates the deterministic outcomes. A more rigorous analysis actually shows that $\binom{n}{k} \leq 2^{nH(X)}$ [8].

To conclude, the work described here should mainly be seen as a theoretical contribution for the application of data hiding to an unusual kind of host. Our analysis might also be able to inform future practical tagging strategies in biotechnological applications. As an example, we may point out the recent suggestion by Jupiter et al. of tagging infectious agents manipulated in biotechnological laboratories, in order to enable tracking and liability determination in case of leaks [9].

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References


