The Metagenomic Binning Problem: Clustering Markov Sequences

Grant Greenberg
Electrical and Computer Engineering Department
University of Illinois at Urbana-Champaign
Champaign, IL, USA
gcgreen2@illinois.edu

Ilan Shomorony
Electrical and Computer Engineering Department
University of Illinois at Urbana-Champaign
Champaign, IL, USA
ilans@illinois.edu

Abstract—The goal of metagenomics is to study the composition of microbial communities, typically using high-throughput shotgun sequencing. In the metagenomic binning problem, we observe random substrings (called contigs) from a mixture of genomes and want to cluster them according to their genome of origin. Based on the empirical observation that genomes of different bacterial species can be distinguished based on their *tetranucleotide frequencies*, we model this task as the problem of clustering N sequences generated by M distinct Markov processes, where $M \ll N$. Utilizing the large-deviation principle for Markov processes, we establish the information-theoretic limit for perfect binning. Specifically, we show that the length of the contigs must scale with the inverse of the Chernoff Information between the two most similar species. Our result also implies that contigs should be binned using the conditional relative entropy as a measure of distance, as opposed to the Euclidean distance often used in practice.

I. Introduction

In the last decade, advances in high-throughput DNA sequencing technologies have allowed a vast amount of genomic data to be generated. Countless tasks such as genome assembly, RNA quantification, and genome-wide association studies have become a reality, opening up exciting new research directions within biology and medicine.

Significant attention has recently been given to the analysis of the human microbiome through *metagenomics* [1]. In metagenomics, a sample is taken from a microbial community, such as the human gut. The genetic material in the sample is then sequenced and analyzed to determine the microbial composition of the community. Recent research, including the Human Microbiome Project [2], has shown that the composition of the microbiome is a "snapshot" into an individual's overall health, providing great potential for personalized medicine.

Full reconstruction of the genomes in a metagenomic sample is generally infeasible due to insufficient coverage and high similarity across species [3]. In the typical analysis pipeline, the millions of reads obtained via high-throughput sequencing are used to create a much smaller number of contiguous sequences, known as *contigs*, by merging reads with large overlaps [4]. The set of resulting contigs typically make up only a small fraction of the full genomes of all species present in the sample and have no significant overlaps with each other.

Metagenomic binning is concerned with the following question: is it possible to group the resulting contigs based on the genome from which they were derived? Somewhat surprisingly, it has been shown that contigs belonging to the same species typically have similar sequence compositions. Specfically, it was empirically verified that the distribution of four-letter strings (e.g. $AGCG$) remains relatively constant across an entire bacterial genome [5]. Hence one can compute for each contig the *tetranucleotide frequency* (TNF) vector, and group together contigs with "similar" TNF vectors. Provided that the underlying TNF distributions are distinct enough, metagenomic binning can thus be performed. Based on this idea[1], many different algorithms and software packages have been proposed to perform metagenomic binning [3], [6].

The fact that the distribution of four-symbol strings is consistent throughout a given genome motivates the modeling of each genome as a *third*-order Markov process. Hence we assume that a contig is generated by one out of M distinct, *unknown* Markov processes $p_1, \ldots, p_M$ with equal probability, where each p_k corresponds to a certain species. In order to study the fundamental limits of this problem, we assume all N contigs have length L, and consider an asymptotic regime where $N \to \infty$ and the contig length grows slowly with the number of contigs (specifically we set $L = \bar{L} \log N$). Our goal is to characterize how large $\bar{L}$ needs to be in order to allow perfect binning with high probability.

To obtain our main result, we establish the equivalent of the Chernoff Information [7, Chapter 11.9] for Markov processes, which gives the error exponent for the Bayesian error probability when testing between two known Markov processes. This result, combined with a scheme to estimate the M Markov distributions, allows us to show that perfect binning is possible if and only if

$$\bar{L} > \frac{1}{\min_{i,j} C(p_k, p_\ell)},$$

where $C(p_k, p_\ell)$ is the Chernoff Information between p_k and p_ℓ. To estimate the unknown distributions, we consider building a graph where contigs whose empirical distributions are close are connected. We then show that, with high probability, M large cliques can be found, which can be used to find

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[1]Usually, in addition to the TNF vector, the read coverage of each contig is used as another feature to help with the clustering. However, in this paper, we focus on using the TNF alone.

estimates $\tilde{p}_k$, $k = 1, ..., M$, of the Markov distributions. Each contig $\mathbf{x}$ is then then placed in bin k given by

$$\underset{k \in \{1,...,M\}}{\arg\min} \; D_c\left(\hat{p}_\mathbf{x} \| \tilde{p}_k\right)$$

where $\hat{p}_\mathbf{x}$ is the empirical 4-symbol distribution of $\mathbf{x}$ and $D_c(\cdot\|\cdot)$ is the conditional relative entropy [7, Definition 2.65].

Our main result suggests that the optimal way to bin metagenomic contigs is to estimate the underlying TNF distribution and then bin contigs using the conditional relative entropy as a metric, as opposed to the commonly used Euclidean distance. By simulating contigs from real bacterial genomes, we show that this metric can lead to lower binning error probabilities.

The paper is organized as follows. In Section II we describe the problem formulation in detail and state our main result. In Section III we describe our achievability scheme and the main technical ingredients used to prove it, and in Section IV we describe the converse argument. In Section V we provide preliminary simulation results, and we conclude the paper with a discussion in Section VII.

II. PROBLEM STATEMENT

As shown in [5], the distribution of tetranucleotides (four-letter strings), tends to be stationary across a fixed bacterial genome. Hence, it is natural to assume that each of the M species in our sample was generated by a distribution p_i, $i = 1, ..., M$, over all possible tetranucleotides[2] $\{AAAA, AAAC, ..., TTTT\}$.

Let $\mathcal{P}$ be the $|\mathcal{X}|^4$-dimensional simplex where $\mathcal{X} = \{A, C, G, T\}$. Notice that not all distributions in $\mathcal{P}$ are valid tetranucleotide distributions, as the tetranucleotides in a sequence overlap with each other. Let $\widetilde{\mathcal{P}}$ be the set of all $p \in \mathcal{P}$ with $p(\mathbf{c}) > 0$, $\forall \mathbf{c} \in \mathcal{X}^4$, which in addition satisfy for all $\mathbf{a} \in \mathcal{X}^3$

$$\sum_{b \in \mathcal{X}} p(\mathbf{a}b) = \sum_{b \in \mathcal{X}} p(b\mathbf{a}). \tag{1}$$

Condition (1) guarantees that any $p \in \widetilde{\mathcal{P}}$ corresponds to the tetranucleotide distribution of a stationary third-order Markov chain. More precisely, for a given $p \in \widetilde{\mathcal{P}}$, we can let $p^{(3)}$ be the induced distribution over 3-letter strings; i.e.,

$$p^{(3)}(\mathbf{a}) = \sum_{b \in \mathcal{X}} p(\mathbf{a}b). \tag{2}$$

This uniquely determines a stationary Markov process with initial state distributed as $p^{(3)}$ and transition probabilities

$$p(b|\mathbf{a}) = \frac{p(\mathbf{a}b)}{p^{(3)}(\mathbf{a})}. \tag{3}$$

Hence we will model each species in the sample using a distribution $p_i \in \widetilde{\mathcal{P}}$. Notice that the constraint that $p_i(\mathbf{c}) > 0$ for any $\mathbf{c} \in \mathcal{X}^4$ guarantees that the resulting Markov processes are irreducible.

[2]In practical approaches, *reverse-complementary* tetranuclotides such as $ACAG$ and $CTGT$ are treated as the same tetranucleotide, but we ignore that fact for the sake of simplicity.

A. Metagenomic Binning Problem

We assume that we have M species in our sample (for a known M). Each species is modeled by a stationary third-order Markov process defined by $p_k \in \mathcal{P}$, for $k = 1, ..., M$. From this genomic mixture, we observe a set of N realizations $\mathcal{C} = \{\mathbf{x}_i\}_{i=1}^N$, which we call *contigs*. Each $\mathbf{x} \in \mathcal{C}$ is generated independently by first choosing a species $k \in \{1, ..., M\}$ uniformly at random, and then generating a length-L sequence according to p_k. We wish to determine which contigs originated from the same genome.

We point out that in real metagenomic experiments, the *coverage depth*, i.e. the expected number of contigs containing a specific nucleotide from one of the M genomes, is low. Hence, contigs will have no overlap with high probability, allowing us to model them as independent realizations of the different Markov processes in the sample.

B. Perfect Binning

The goal of the metagenomic binning problem is to cluster the N contigs into M "bins", where each bin k corresponds to a unique species with distribution p_k. More precisely, the goal is to find a decision rule $\delta_L : \mathcal{X}^L \rightarrow \{1, \ldots, M\}$ which correctly maps each contig to its respective genome bin.

For each $k \in \{1, \ldots, M\}$, let $\mathcal{C}_k$ be the set of contigs generated according to p_k. Perfect binning would be achieved if for every contig $\mathbf{x}$, $\delta(\mathbf{x})$ chooses the label of the distribution from which it was generated. However, we have the added difficulty that the distributions are unknown. As a result, we can only require the decision rule to be correct up to a consistent relabeling of species indices. Hence, the error event for a decision rule δ_L is

$$\mathcal{E}_{\delta_L} = \{\exists \mathbf{x} \in \mathcal{C}_k, \mathbf{y} \in \mathcal{C}_\ell, k \neq \ell : \delta(\mathbf{x}) = \delta(\mathbf{y})\}. \tag{4}$$

We would like to know under what circumstances we can perfectly bin all N contigs. In order to study the information-theoretic limits of this problem, we analyze an asymptotic regime, similar to [8], in which $N \rightarrow \infty$ and

$$L = \bar{L} \log N \tag{5}$$

where $\bar{L}$ is the "normalized contig length". This scaling forces the contig length to be small compared to the number of contigs and, as we will show, is a meaningful scaling for the asymptotic problem we consider. Intuitively, a larger value of $\bar{L}$ should allow one to bin a contig with higher accuracy. This asymptotic regime allows us to define when species are resolvable as follows:

Definition 1. *The M species with distributions $\{p_k\}_{k=1}^M$ are* **resolvable** *if there exists a sequence of decision rules $\{\delta_L\}$ such that $Pr(\mathcal{E}_{\delta_L}) \rightarrow 0$ as $N \rightarrow \infty$.*

C. Main Result

Interestingly, the fundamental limit of resolvability relies on the Chernoff Information, which we define next.

Definition 2. *For two Markov models p_k and p_ℓ and some $\lambda \in (0,1)$, let*

$$p_\lambda(\mathbf{c}) = \frac{p_k^{1-\lambda}(\mathbf{c})p_\ell^{\lambda}(\mathbf{c})}{\sum_{\mathbf{d}\in\mathcal{X}} p_k^{1-\lambda}(\mathbf{d})p_\ell^{\lambda}(\mathbf{d})}, \qquad (6)$$

*for every $\mathbf{c} \in \mathcal{X}^4$. The **Chernoff Information** for p_k and p_ℓ is given by*

$$C(p_k, p_\ell) = D_c(p_{\lambda^*} \| p_k) = D_c(p_{\lambda^*} \| p_\ell) \qquad (7)$$

where D_c is the conditional relative entropy and $\lambda = \lambda^*$ is chosen such that $D_c(p_{\lambda^*} \| p_k) = D_c(p_{\lambda^*} \| p_\ell)$.

The distribution p_λ traces a curve between p_k and p_ℓ and the Chernoff Information can be thought of as the distance to the midpoint of this curve from either distribution. Our main result establishes that the minimum normalized contig length, $\bar{L}$, depends exlusively on the minimum Chernoff Information between species distributions.

Theorem 1. *Let $C_{\min} \triangleq \min_{k,\ell} C(p_k, p_\ell)$. The species' distributions $\{p_k\}_{k=1}^{M}$ are resolvable if and only if*

$$\bar{L} > \frac{1}{C_{\min}}. \qquad (8)$$

Intuitively, this means that the contig lengths must be large enough to distinguish between the two closest distributions.

III. ACHIEVABILITY

The achievability proof of Theorem 1 is first shown in the form of an algorithm so as to highlight the algorithmic nature of metagenomic binning. Given a contig $\mathbf{x} \in \mathcal{C}$, we define the empirical fourth-order distribution of $\mathbf{x}$ as $\hat{p}_{\mathbf{x}}$ and we use d as the ℓ_1 distance between distributions, i.e. $d(p,q) = \sum_{\mathbf{a}\in\mathcal{X}^4} |p(\mathbf{a}) - q(\mathbf{a})|$.

Algorithm 1: Binning Contigs

Result: Decision Rule $\delta(\mathbf{x})$
Input: Contigs $\mathcal{C}$, Parameter $\alpha \in [0,1]$
begin
    $\mathcal{D} \longleftarrow$ sort ascending $\{d(\hat{p}_{\mathbf{x}}, \hat{p}_{\mathbf{y}}) : \mathbf{x}, \mathbf{y} \in \mathcal{C}\}$
    for ϵ *in* $\mathcal{D}$
        $\mathcal{G}_\epsilon \longleftarrow (V = \mathcal{C}, E_\epsilon = \{(\mathbf{x}, \mathbf{y}) : d(\hat{p}_{\mathbf{x}}, \hat{p}_{\mathbf{y}}) \leq \epsilon\})$
        if $\mathcal{G}_\epsilon$ has cliques $\{\mathcal{K}_k\}_{k=1}^{M}, |\mathcal{K}_k| \geq (1-\alpha)\frac{N}{M}$
            for $k \longleftarrow 1$ to M
                $\widetilde{p}_k \longleftarrow \frac{1}{|\mathcal{K}_k|} \sum_{\mathbf{x}\in\mathcal{K}_k} \hat{p}_{\mathbf{x}}$
            break
    for $\mathbf{x} \in \mathcal{C}$
        $\delta(\mathbf{x}) \longleftarrow \arg\min_{k\in\{1,...,M\}} D_c(\hat{p}_{\mathbf{x}} \| \widetilde{p}_k)$

The algorithm first estimates the species distributions by averaging the empirical distributions of the contigs in each clique, then bins the contigs based on the estimates. Note that the algorithm as described is not computationally efficient (specifically, finding large cliques), and is used only to establish the achievability of Theorem 1. The proof of Theorem 2 is provided in Section VI.

A. Estimating Distributions

Recall that $\mathcal{C}_k$ is the set of contigs generated by p_k. We expect the empirical distribution of the majority of contigs in $\mathcal{C}_k$ to be near p_k. To identify those "good" contigs, let

$$\mathcal{C}_{k,\epsilon} = \{\mathbf{x} \in \mathcal{C}_k : d(\hat{p}_{\mathbf{x}}, p_k) \leq \epsilon\}.$$

To prove that the distribution estimates $\{\tilde{p}_k\}_{k=1}^{M}$ are close to the true distributions $\{p_k\}_{k=1}^{M}$ (after proper reindexing), we will first show that once the algorithm identifies cliques $\mathcal{K}_1, ..., \mathcal{K}_M$, each clique is "pure" in the sense that it contains "good" contigs from only one species. We will let $d_{\min} \triangleq \min_{k\neq\ell} d(p_k, p_\ell)$ be the minimum ℓ_1 distance between any pair of the M species distributions.

Lemma 1. *If $\mathcal{K}_k$ is a clique in $\mathcal{G}_\epsilon$ for $\epsilon < \frac{d_{\min}}{2}$, then*

$$\sum_{\ell=1}^{M} \mathbb{1}\{\mathcal{K}_k \cap \mathcal{C}_{\ell,\epsilon/2} \neq \emptyset\} \leq 1, \qquad (9)$$

Lemma 1 establishes that, if Algorithm 1 finds M cliques of size $(1-\alpha)\frac{N}{M}$ in $\mathcal{G}_\epsilon$ for $\epsilon < \frac{d_{\min}}{2}$, then each clique contains contigs from at most one $\mathcal{C}_{\ell,\epsilon/2}$, $\ell = 1, ..., M$. In order to establish that these M cliques will exist in $\mathcal{G}_\epsilon$, we use the following lemma, which essentially says that, for N large enough, a large fraction of the contigs will be close to their respective generating distributions.

Lemma 2. *For $\epsilon > 0$, $k \in \{1, ..., M\}$, and N large enough,*

$$\Pr\left(|\mathcal{C}_{k,\epsilon/2}| < (1-\alpha)\frac{N}{M}\right) \leq \frac{1}{\alpha}e^{-\gamma\alpha^2 L}, \qquad (10)$$

where γ is a positive constant.

Fixing $\epsilon < \frac{d_{\min}}{2}$, Lemma 2 guarantees that for a reasonably chosen α, as long as N is large enough, we will have $|\mathcal{C}_{k,\epsilon/2}| \geq (1-\alpha)\frac{N}{M}$ for all $k = 1, ..., M$. Moreover, by the triangle inequality, any two contigs $\mathbf{x}, \mathbf{y} \in \mathcal{C}_{k,\epsilon/2}$ will be at a distance ϵ or less of each other and will thus have an edge between them. Hence, $\mathcal{C}_{k,\epsilon/2}$ forms a clique in $\mathcal{G}_\epsilon$.

Notice that $d_{\min}$ is not known, so the algorithm cannot restrict its search to $\epsilon < \frac{d_{\min}}{2}$. However, since the algorithm considers different values of ϵ in increasing order, for some $\epsilon < \frac{d_{\min}}{2}$, M cliques of size $(1-\alpha)\frac{N}{M}$ will exist with probability $1 - o(1)$. Lemma 1 will then guarantee that any cliques $\mathcal{K}_1, ..., \mathcal{K}_M$ that are found will be pure.

Consider a clique $\mathcal{K}_k$ and let ℓ be such that $\mathcal{K}_k \cap \mathcal{C}_{\ell,\epsilon/2} \neq \emptyset$. By Lemma 2, the fraction of "good" contigs in $\mathcal{K}_k$ will be

$$\frac{|\mathcal{K}_k \cap \mathcal{C}_{\ell,\epsilon/2}|}{|\mathcal{K}_k|} \geq 1 - \frac{N - M \cdot (1-\alpha)\frac{N}{M}}{(1-\alpha)\frac{N}{M}} = 1 - \frac{\alpha M}{1-\alpha} \quad (11)$$

with probability $1 - o(1)$. If we set $\alpha = \frac{1}{\log N}$, (11) converges to 1 and (10) converges to 0 as $N \to \infty$. Thus, with high probability, a vanishing fraction of the contigs in $\mathcal{K}_k$ does not belong to $\mathcal{C}_{\ell,\epsilon/2}$. Since distribution vectors are bounded, their impact on $\tilde{p}_k$ also vanishes, and we conclude that the distribution estimate $\tilde{p}_k = \frac{1}{|\mathcal{K}_k|} \sum_{\mathbf{x}\in\mathcal{K}_k} \hat{p}_{\mathbf{x}} \to p_\ell$ as $N \to \infty$.

B. Binning Contigs

In Section III-A, we established that we can construct arbitrarily accurate estimates $\{\tilde{p}_\ell\}_{\ell=1}^M$ of the underlying distributions $\{p_k\}_{k=1}^M$ as $N \to \infty$. Next we show that, binning the contigs based on the conditional relative entropy using these estimates achieves (8) in the limit.

Consider the hypothesis test between two Markov processes p_k and p_ℓ (i.e. true distributions, not estimates). Given prior probabilities π_k and π_ℓ, the Bayesian probability of error is

$$\pi_k \Pr(\text{choose } \ell | k \text{ true}) + \pi_\ell \Pr(\text{choose } k | \ell \text{ true})$$

for the decision rule on a contig generated by either p_k or p_ℓ.

Theorem 2. *Let $\mathcal{E}_{k,\ell}$ be the error event for the decision rule which minimizes the Bayesian probability of error. Then*

$$\lim_{L \to \infty} \frac{1}{L} \log \Pr(\mathcal{E}_{k,\ell}) = -C(p_k, p_\ell), \tag{12}$$

i.e., $C(p_k, p_\ell)$ is the optimal error exponent.

We point out that a similar characterization of this error exponent was recently provided by Moulin and Veeravalli [9, Chapter 10] (under the name of Chernoff divergence).

Now, suppose we replace the true distributions with our estimates. Theorem 3 shows that in the limit as $N \to \infty$, the optimal probability of error will be characterized by Chernoff information as in Thereom 2.

Theorem 3. *Suppose the estimates of p_k and p_ℓ are such that $d(\tilde{p}_k, p_k) < \epsilon_L$ and $d(\tilde{p}_\ell, p_\ell) < \epsilon_L$ where $\epsilon_L \to 0$ as $L \to \infty$. Let $\widetilde{\mathcal{E}}_{k,\ell}$ be the error event for the decision rule which uses the estimates $\tilde{p}_k$ and $\tilde{p}_\ell$. Then*

$$\lim_{L \to \infty} \frac{1}{L} \log \Pr(\widetilde{\mathcal{E}}_{k,\ell}) = -C(p_k, p_\ell) + g(\epsilon_L) \tag{13}$$

where the function $g(\epsilon_L) \to 0$ as $\epsilon_L \to 0$.

The proofs of Theorems 2 and 3 are given in Section VI.

For a given contig, the last step of Algorithm 1 can be thought of as $M-1$ binary hypothesis tests between the true distribution and each of the remaining distributions. Thus, we will use Theorem 2 to bound the overall error probability by considering the two closest distributions. Then

$$\Pr(\mathcal{E}_{\delta_L}) \leq \sum_{k=1}^M \sum_{\mathbf{x} \in \mathcal{C}_k} \sum_{\ell \neq k} \Pr(\widetilde{\mathcal{E}}_{k,\ell}) \tag{14}$$

$$\leq (M-1)N \max_{k \neq \ell} \Pr(\widetilde{\mathcal{E}}_{k,\ell}) \tag{15}$$

$$\leq M 2^{L\left(1/\bar{L} + \max_{k \neq \ell}(1/L) \log \Pr(\widetilde{\mathcal{E}}_{k,\ell})\right)} \tag{16}$$

where (14) follows from the union bound. By Theorem 3,

$$\max_{k \neq \ell} \frac{1}{L} \log \Pr(\widetilde{\mathcal{E}}_{k,\ell}) \to -\min_{k \neq \ell} C(p_k, p_\ell) + g(\epsilon_L)$$

$$= -C_{\min} + g(\epsilon_L)$$

as $N \to \infty$. Hence, if $\bar{L} > \frac{1}{C_{\min}}$, there exists an L_0 such that for $L > L_0$, $\bar{L} > \frac{1}{C_{\min} - g(\epsilon_L)}$ and thus, $\Pr(\mathcal{E}_{\delta_L}) \to 0$. This concludes the achievability proof of Theorem 1.

IV. CONVERSE

Without loss of generality, let p_1 and p_2 be such that $C_{\min} = C(p_1, p_2)$. Given the decision rule δ_L and a contig $\mathbf{x} \in \mathcal{C}$, let

$$\hat{\mathcal{E}}_{1,2,\mathbf{x}} = \{\mathbf{x} \in \mathcal{C}_1, \delta_L(\mathbf{x}) \neq 1\} \cup \{\mathbf{x} \in \mathcal{C}_2, \delta_L(\mathbf{x}) \neq 2\}$$

i.e. the event that $\mathbf{x}$ was generated by either p_1 or p_2 *and* incorrectly binned. Note that $\Pr(\widetilde{\mathcal{E}}_{1,2,\mathbf{x}}) \geq \frac{2}{M} \Pr(\mathcal{E}_{1,2})$. Then

$$\Pr(\mathcal{E}_{\delta_L}) \geq \Pr\left(\bigcup_{i=1}^N \hat{\mathcal{E}}_{1,2,\mathbf{x}_i}\right) = 1 - (1 - \Pr(\hat{\mathcal{E}}_{1,2,\mathbf{x}_1}))^N$$

$$\geq 1 - \left[\left(1 - \frac{2}{M}\Pr(\mathcal{E}_{1,2})\right)^{1/\Pr(\mathcal{E}_{1,2})}\right]^{N\Pr(\mathcal{E}_{1,2})}$$

$$\geq 1 - e^{-\frac{2}{M}N\Pr(\mathcal{E}_{1,2})} \tag{17}$$

where (17) follows from the bound $(1 - ap)^{1/p} \leq e^{-a}$ for $p \in [0,1], a \in \mathbb{R}$. We see that, if $\frac{2}{M}N\Pr(\mathcal{E}_{1,2}) \not\to 0$, then $\Pr(\mathcal{E}) \not\to 0$. Since

$$\frac{2}{M}N\Pr(\mathcal{E}_{1,2}) = \frac{2}{M}2^{L\left(1/\bar{L} + (1/L)\log\Pr(\mathcal{E}_{1,2})\right)},$$

then by Theorem 2, $\frac{2}{M}N\Pr(\mathcal{E}_{1,2}) \not\to 0$ when $\bar{L} \leq \frac{1}{C_{\min}}$. This concludes the converse proof for Theorem 1.

V. EXPERIMENTAL RESULTS

From the point of view of practical metagenomic binning algorithms, our main result suggests that:

1) the conditional relative entropy is a good metric for binning contigs,
2) the Chernoff information can be used as a measure of how difficult it is to distinguish two species.

In this section, we provide preliminary empirical evidence of these claims. To this end, we utilized several previously sequenced and assembled bacterial genomes, available at NCBI [10]. For each bacterial species, we computed its fourth-order distribution p_k (i.e., the overall tetranucleotide frequency vector), and were able to simulate contigs of a desired length L by randomly sampling length-L substrings from the genome.

In order to verify the usefulness of the conditional relative entropy and compare it to the Euclidean distance (used in state-of-the-art tools such as [3], [6]), we considered the following experiment: we generated random contigs from a species p_1 and then tested whether it was closer to species p_1 or to another species p_2 based on both the Euclidean distance and the conditional relative entropy. In Figure 1a, the conditional relative entropy metric[3] outperforms the Euclidean metric in the test between the species Alistipes Obesi and Megamonas Funiformi, as was the case for most pairs of species. We note, however, that in a select few pairs, the Euclidean metric gives the lower error.

Theorem 1 implies that the inverse of the Chernoff Information characterizes how long the contigs need to be in order for two species to be reliably distinguishable. In order to verify that, we considered different pairs of species p_k, p_ℓ. For each

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[3] $D_c(\cdot || \cdot)$ is not technically a metric as it is not symmetric.

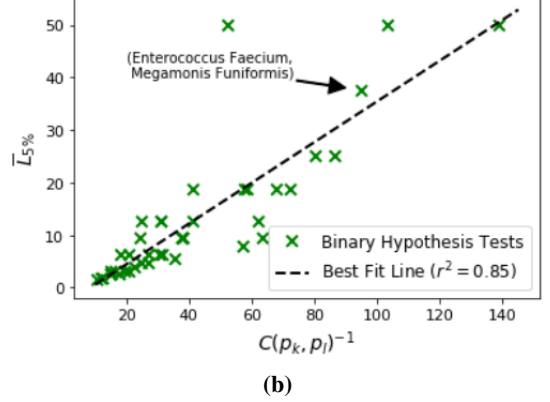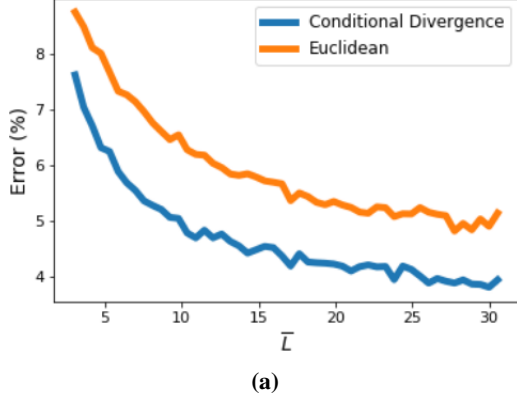**Fig. 1:** (a) Comparison of conditional divergence and Euclidean distance for a hypothesis test between Alistipes Obesi and Megamonas Funiformis; (b) Normalized contig length needed for 5% error ($\overline{L}_{5\%}$) vs the inverse of the Chernoff information for several pairs of species.

such pair, we computed the Chernoff Information $C(p_k, p_\ell)$ and $\overline{L}_{5\%}$, the minimum normalized contig length $\overline{L}$ required to guarantee a 5% error rate in a Bayesian hypothesis test between p_k and p_ℓ with equal priors. In Figure 1 (right), we plot $\overline{L}_{5\%}$ vs $C^{-1}(p_k, p_\ell)$ for many such pairs and observe a roughly linear relationship between these two quantities.

VI. Proofs

A. Proof of Lemma 1

Suppose by contradiction that $\mathbf{x}, \mathbf{y} \in K_j$, $\mathbf{x} \in \mathcal{C}_{k,\epsilon/2}$ and $\mathbf{y} \in \mathcal{C}_{\ell,\epsilon/2}$, for $k \neq \ell$. Then $d(\mathbf{x}, \mathbf{y}) < \epsilon$, and we have

$$
\begin{aligned}
d(p_k, p_\ell) &\leq d(p_k, \hat{p}_{\mathbf{x}}) + d(\hat{p}_{\mathbf{x}}, p_\ell) \\
&\leq d(p_k, \hat{p}_{\mathbf{x}}) + d(\hat{p}_{\mathbf{x}}, \hat{p}_{\mathbf{y}}) + d(\hat{p}_{\mathbf{y}}, p_l) \\
&< \epsilon/2 + \epsilon + \epsilon/2 < d_{\min},
\end{aligned}
$$

which is a contradiction to the definition of $d_{\min}$.

B. Proof of Lemma 2

Let $\mathcal{E}_k$ be the event of interest, $\left\{ |\mathcal{C}_{k,\epsilon/2}| < (1-\alpha)\frac{N}{M} \right\}$, and let $\mathcal{A}_k = \left\{ |C_k| < (1-\frac{\alpha}{2})\frac{N}{M} \right\}$. Note that we use $\frac{\alpha}{2}$ for $\mathcal{A}_k$ as opposed to α because we need $|C_k|$ to be larger than $|\mathcal{C}_{k,\epsilon/2}|$. By Hoeffding's inequality, $\Pr(\mathcal{A}_k) \leq e^{-N\frac{\alpha^2}{2M^2}}$. This means that, with high probability, p_k will generate enough contigs.

Let $\mathcal{F}_k$ be the set of distributions "far" from p_k:

$$
\mathcal{F}_k = \left\{ p \in \widetilde{\mathcal{P}} : d(p_k, p) \geq \frac{\epsilon}{2} \right\}. \tag{18}
$$

By a version of Sanov's theorem for Markov chains, given in Section VI, Theorem 5, for any $\mathbf{x} \in \mathcal{C}_k$,

$$
\Pr(\hat{p}_{\mathbf{x}} \in \mathcal{F}_k) \leq (L+1)^4 2^{-L D_c(p^* \| p_k)} \tag{19}
$$

where $p^* = \arg\inf_{p \in \mathcal{F}_k} D_c(p \| p_k)$; i.e., p^* is the distribution in $\mathcal{F}_k$ closest to p_k in conditional relative entropy. Notice that $\mathcal{E}_k$ occurs when more than $|C_k| - (1-\alpha)\frac{N}{M} + 1$ contigs

lie in $\mathcal{F}_k$, leaving an insufficient number of "good" contigs. Letting $\mathbf{x}_0 \in \mathcal{C}_k$ be some contig generated by p_k,

$$
\Pr(\mathcal{E}_k | \mathcal{A}_k^c) \tag{20}
$$

$$
= \Pr\left(\sum_{\mathbf{x} \in \mathcal{C}_k} \mathbb{1}\{\hat{p}_{\mathbf{x}} \in \mathcal{F}_k\} \geq |\mathcal{C}_k| - (1-\alpha)\frac{N}{M} + 1 \Big| \mathcal{A}_k^c \right) \tag{21}
$$

$$
\leq \sum_{i=1}^{N} \Pr\left(\mathbb{1}\{\hat{p}_{\mathbf{x}_0} \in \mathcal{F}_k\} \geq \frac{\alpha}{2}\frac{N}{M} \Big| \mathcal{A}_k^c \right) \tag{22}
$$

$$
\leq \frac{2M}{\alpha} \cdot \Pr(\hat{p}_{\mathbf{x}_0} \in \mathcal{F}_k | \mathcal{A}_k^c) \tag{23}
$$

where (22) follows from symmetry between contigs and (23) from Markov's inequality. Combining the probabilities,

$$
\Pr(\mathcal{E}_k) = \Pr(\mathcal{E}_k | \mathcal{A}_k^c)\Pr(\mathcal{A}_k^c) + \Pr(\mathcal{E}_k | \mathcal{A}_k)\Pr(\mathcal{A}_k) \tag{24}
$$

$$
\leq \frac{2M}{\alpha} \cdot \Pr(\hat{p}_{\mathbf{x}_0} \in \mathcal{F}_k | \mathcal{A}_k^c)\Pr(\mathcal{A}_k^c) + \Pr(\mathcal{A}_k) \tag{25}
$$

$$
\leq \frac{2M}{\alpha}(L+1)^4 2^{-L D_c(p^* \| p_k)} + e^{-N\frac{\alpha^2}{2M^2}} \tag{26}
$$

$$
\leq \frac{1}{\alpha} e^{-\gamma \alpha^2 L} \tag{27}
$$

where $\gamma > 0$ is some constant (guaranteed to exist for $\alpha \in [0, 1)$) such that (27) holds for large N.

C. Proof of Theorems 2 and 3

A contig is represented by a length-L sequence, $\mathbf{x} = (x_1, \ldots, x_L)$ over the alphabet $\mathcal{X}$, and is modeled as a third-order Markov process with fourth-order stationary distribution q. All four-letter combinations have a non-zero probability, i.e.

$$
\forall \mathbf{a} \in \mathcal{X}^4 : q^*(\mathbf{a}) > 0. \tag{28}
$$

This ensures that all Markov processes are irreducible and "well-behaved", following (2) and (3).

We define the *type* of $\mathbf{x}$ to be its empirical fourth-order distribution, denoted $\hat{p}_{\mathbf{x}}$. Let the set of all possible types of length-L sequences be $\mathcal{P}_L$. The cardinality of $\mathcal{P}_L$ is upper-bounded by $(L+1)^4$ as shown in [11]. The *type class*, T_L, of

a given type, $p \in \mathcal{P}_L$, is then defined as the set of all length-L sequences whose types are equal to p:

$$T_L(p) \triangleq \left\{ \mathbf{x} \in \mathcal{X}^L : \hat{p}_\mathbf{x} = p \right\} \tag{29}$$

To facilitate analysis, we use a *cyclical* Markov model, where three artificial transitions are added from the end of the sequence to the beginning. This model ensures that $\hat{p}_\mathbf{x}$ is *consistent*, similar to (1). Specifically for $\mathbf{a} \in \mathcal{X}^3$,

$$\sum_{b \in \mathcal{X}} \hat{p}_\mathbf{x}(\mathbf{a}b) = \sum_{b \in \mathcal{X}} \hat{p}_\mathbf{x}(b\mathbf{a}) \tag{30}$$

Note that this implies $\hat{p}_\mathbf{x} \in \widetilde{P}$ as defined in Section II. Furthermore, the third-order empirical distribution, $\hat{p}_\mathbf{x}^{(3)}$, and empirical conditional distribution $\hat{p}_\mathbf{x}^{(c)}$, can be derived from $\hat{p}_\mathbf{x}$ as follows.

$$\hat{p}_\mathbf{x}^{(3)}(\mathbf{a}) = \sum_{b \in \mathcal{X}} \hat{p}_\mathbf{x}(\mathbf{a}b) \tag{31}$$

and

$$\hat{p}_\mathbf{x}^{(c)}(b|\mathbf{a}) = \frac{\hat{p}_\mathbf{x}(\mathbf{a}b)}{\hat{p}_\mathbf{x}^{(3)}(\mathbf{a})} \tag{32}$$

for any $b \in \mathcal{X}$. We now use some Large Deviations theory to make an argument about the probability of error in the hypothesis test.

1) Large Deviations Principle: Vidyasagar [11] provides an extensive analysis of large deviations theory for Markov processes. Theorems 4 and 5, shown below, utilize this analysis, which allows us to make an argument about the probability of error for the subsequent hypothesis test. For the proofs of Theorems 4 and 5, the reader is referred to [11, Theorem 7] and [7, Chapter 11].

Vidyasagar proves that a Markov process $\mathbf{X} = (X_1, \ldots, X_L)$ with type p generated by q satisfies the large deviations property with rate function

$$I(p) = D_c(p\|q) \tag{33}$$

Here, D_c is the conditional relative entropy defined as the KL divergences averaged over $p^{(3)}$:

$$
\begin{aligned}
D_c(p\|q) &\triangleq \sum_{\mathbf{a} \in \mathcal{X}^3} p^{(3)}(\mathbf{a}) \sum_{b \in \mathcal{X}} p^{(c)}(b|\mathbf{a}) \log \left(\frac{p^{(c)}(b|\mathbf{a})}{q^{(c)}(b|\mathbf{a})} \right) \\
&= \sum_{\mathbf{a} \in \mathcal{X}^3, b \in \mathcal{X}} p(\mathbf{a}b) \log \frac{p(\mathbf{a}b)}{q(\mathbf{a}b)} \\
&\quad - \sum_{\mathbf{a} \in \mathcal{X}^3} p^{(3)}(\mathbf{a}) \log \frac{p^{(3)}(\mathbf{a})}{q^{(3)}(\mathbf{a})} \\
&= D(p\|q) - D\left(p^{(3)}\|q^{(3)}\right)
\end{aligned}
$$

Similarly, the conditional entropy can be written as

$$
\begin{aligned}
H_c(p) &\triangleq \sum_{\mathbf{a} \in \mathcal{X}^3} p^{(3)}(\mathbf{a}) \sum_{b \in \mathcal{X}} p^{(c)}(b|\mathbf{a}) \log p^{(c)}(b|\mathbf{a}) \\
&= H(p) - H\left(p^{(3)}\right)
\end{aligned}
$$

Theorem 4. *The probability of $\mathbf{x}$ under q depends only on its type $\hat{p}_\mathbf{x}$ and is given by*

$$q^L(\mathbf{x}) = 2^{-L[D_c(\hat{p}_\mathbf{x}\|q) + H_c(\hat{p}_\mathbf{x})] + \log \alpha} \tag{34}$$

where $\alpha = q^{(3)}(x_1, x_2, x_3)$, i.e. the initial state of $\mathbf{x}$.

Theorem 5 (Sanov's Theorem for Markov Processes). *Let $\mathbf{X} = (X_1, X_2, \ldots, X_L)$ be a Markov process q, and let $\mathcal{F} \subseteq \tilde{P}$ be a set of consistent probability distributions. The probability that the empirical distribution of $\mathbf{X}$ is contained in $\mathcal{F}$, denoted $q^L(\mathcal{F})$, is upper-bounded as*

$$q^L(\mathcal{F}) \leq |\mathcal{P}_L| \, 2^{-L D_c(p^*\|q) + \log \alpha} \tag{35}$$

where p^* is the information projection of q onto $\mathcal{F}$:

$$p^* = \arg\inf_{p \in \mathcal{F}} D_c(p\|q) \tag{36}$$

If, in addition, the closure of $\mathcal{F}$ is equal to the closure of its interior ($\bar{\mathcal{F}} = \bar{\mathcal{F}}^o$), then

$$\lim_{L \to \infty} \frac{1}{L} \log q^L(\mathcal{F}) = -D_c(p^*\|q) = -I(p^*) \tag{37}$$

2) Hypothesis Test: There are two candidate models for q: p_1 and p_2, where $p_1 \neq p_2$. We decide between the two hypotheses:

- $H_1: q = p_1$
- $H_2: q = p_2$

Let $\mathcal{P}_1$ and $\mathcal{P}_2$ be the decision regions for H_1 and H_2, respectively. The sets $\mathcal{P}_1$ and $\mathcal{P}_2$ form a disjoint union of $\tilde{\mathcal{P}}$. As a result, given any $\mathbf{x} \in \mathcal{X}^L$, the decision rule $\delta_L(\mathbf{x}) = H_1$ if $\hat{p}_\mathbf{x} \in \mathcal{P}_1$ and $\delta_L(\mathbf{x}) = H_2$ if $\hat{p}_\mathbf{x} \in \mathcal{P}_2$. The Bayesian probability of error P_e for the binary hypothesis test with priors π_1 and π_2 is given by

$$P_e = \pi_1 p_1^L(\mathcal{P}_2) + \pi_2 p_2^L(\mathcal{P}_1) \tag{38}$$

To minimize the error, the decision rule[4] uses a Neyman-Pearson test

$$\delta_L(\hat{p}_\mathbf{x}) = \begin{cases} H_1 & \text{if } \mathcal{L}(\mathbf{x}) \geq \frac{\pi_2}{\pi_1} \\ H_2 & \text{if } \mathcal{L}(\mathbf{x}) < \frac{\pi_2}{\pi_1} \end{cases} \tag{39}$$

where the likelihood ratio, $\mathcal{L}$, is defined as:

$$\mathcal{L}(\mathbf{x}) \triangleq \frac{\Pr(\mathbf{x}|H_1 \text{ true})}{\Pr(\mathbf{x}|H_2 \text{ true})} = \frac{p_1^L(\mathbf{x})}{p_2^L(\mathbf{x})} \tag{40}$$

Using Theorem 4, the normalized log-likelihood ratio is

$$
\begin{aligned}
\frac{1}{L} \log \mathcal{L}(\mathbf{x}) &= -\left[D_c(\hat{p}_\mathbf{x}\|p_1) + H_c(\hat{p}_\mathbf{x}) \right] + \frac{\log \alpha_1}{L} \\
&\quad + \left[D_c(\hat{p}_\mathbf{x}\|p_2) + H_c(\hat{p}_\mathbf{x}) \right] - \frac{\log \alpha_2}{L} \\
&= D_c(\hat{p}_\mathbf{x}\|p_2) - D_c(\hat{p}_\mathbf{x}\|p_1) + \frac{1}{L} \log \frac{\alpha_1}{\alpha_2}
\end{aligned}
$$

Again, α_1 and α_2 represent the probabilities of the initial states of $\mathbf{x}$ under p_1 and p_2, respectively. In reality, we are given only the estimates of the distributions and therefore, in the limit as

---

[4]The decision rule δ_L uses slightly overloaded notation with the decision rule for the main problem.

$N \to \infty$, the pseudo-optimal decision regions are equivalent to

$$\mathcal{P}_1 = \{p \in \widetilde{\mathcal{P}} : D_c\left(p\|\tilde{p}_2\right) - D_c\left(p\|\tilde{p}_1\right) \geq 0\} \quad (41)$$

$$\mathcal{P}_2 = \{p \in \widetilde{\mathcal{P}} : D_c\left(p\|\tilde{p}_2\right) - D_c\left(p\|\tilde{p}_1\right) < 0\} \quad (42)$$

Using the decision rule defined by these regions, we would like to characterize P_e. First, however, we must prove Lemmas 3 and 4, allowing for the use of Theorem 5.

Lemma 3. $\mathcal{P}_1$ and $\mathcal{P}_2$ are convex.

Proof. Let $p_a, p_b \in \mathcal{P}_1$ and let $p_c = \lambda p_a + (1-\lambda)p_b, \lambda \in (0,1)$ be a convex combination of p_a and p_b. Then

$$D_c\left(p_c\|\tilde{p}_2\right) - D_c\left(p_c\|\tilde{p}_1\right)$$
$$= \sum_{\mathcal{X}^4} p_c \log \frac{p_c^{(c)}}{\tilde{p}_2^{(c)}} - \sum_{\mathcal{X}^4} p_c \log \frac{p_c^{(c)}}{\tilde{p}_1^{(c)}}$$
$$= \sum_{\mathcal{X}^4} p_c \log \frac{\tilde{p}_1^{(c)}}{\tilde{p}_2^{(c)}}$$
$$= \lambda\left[D_c\left(p_a\|\tilde{p}_2\right) - D_c\left(p_a\|\tilde{p}_1\right)\right]$$
$$+ (1-\lambda)\left[D_c\left(p_b\|\tilde{p}_2\right) - D_c\left(p_b\|\tilde{p}_1\right)\right]$$
$$\geq 0$$

so $p_c \in \mathcal{P}_1$ and therefore $\mathcal{P}_1$ is a convex set. A similar argument can be made for the set $\mathcal{P}_2$. Note that since $\mathcal{P}_1$ and $\mathcal{P}_2$ are both convex, this implies that the boundary linearly divides the set of consistent fourth-order distributions, $\widetilde{\mathcal{P}}$. $\square$

Lemma 4.
$$\overline{\mathcal{P}_1} = \overline{\mathcal{P}_1^o} \quad and \quad \overline{\mathcal{P}_2} = \overline{\mathcal{P}_2^o} \quad (43)$$

Proof. The boundary between $\mathcal{P}_1$ and $\mathcal{P}_2$ consists of the set of distributions $p \in \widetilde{\mathcal{P}}$ for which $D_c\left(p\|\tilde{p}_2\right) - D_c\left(p\|\tilde{p}_1\right) = 0$. We see that $\tilde{p}_1$ does not lie on this boundary because

$$D_c\left(\tilde{p}_1\|\tilde{p}_2\right) - D_c\left(\tilde{p}_1\|\tilde{p}_1\right) = D_c\left(\tilde{p}_1\|\tilde{p}_2\right) > 0 \quad (44)$$

by the non-negativity of the KL-divergence. Furthermore, $\tilde{p}_1$ cannot lie on any other boundary of $\mathcal{P}_1$ because all of the elements of $\tilde{p}_1$ are nonzero for L large enough, which follows from (28).

Finally, we need to show that convexity and a non-empty interior imply (43). Take a point $p \in \overline{\mathcal{P}_1}$. Then either $p \in \mathcal{P}_1^o$ or $p \in \partial\mathcal{P}_1$, the boundary of $\mathcal{P}_1$. If $p \in \mathcal{P}_1^o$, then $p \in \overline{\mathcal{P}_1^o}$, trivially. If $p \in \partial\mathcal{P}_1$, we must prove that p is a limit point of $\mathcal{P}_1^o$. Since $\tilde{p}_1$ is an interior point of $\mathcal{P}_1$, then there exists an open ball U_1 centered at $\tilde{p}_1$ which is completely contained in $\mathcal{P}_1$. We define V_1 as the set of distributions that result from a convex combination of p and U_1

$$V_1 = \{\alpha U_1 + (1-\alpha)p : 0 < \alpha \leq 1\} \quad (45)$$

using Minkowski addition. The set V_1 clearly has non-zero volume (by Lebesgue measure) and all of its points are interior points of $\mathcal{P}_1$. Therefore there exists a sequence of interior points $\{p_t\}, p_t \in V_1$ such that $p_t \to p$. Thus $p \in \overline{\mathcal{P}_1^o}$.

A similar argument can be made for $\mathcal{P}_2$. Hence, the proof of Lemma 4 is complete. $\square$

Now, by Theorem 5, the error probabilities converge as

$$\lim_{L \to \infty} p_1^L(\mathcal{P}_2) = 2^{-LD_c(p_1^*\|p_1)} \quad (46)$$

and

$$\lim_{L \to \infty} p_2^L(\mathcal{P}_1) = 2^{-LD_c(p_2^*\|p_2)} \quad (47)$$

To find p_1^*, we maximize the error $p_1^L(\mathcal{P}_2)$, which is equivalent to minimizing $D_c\left(p_1^*\|p_1\right)$ in the exponent, subject to the constraint

$$D_c\left(p_1^*\|\tilde{p}_1\right) - D_c\left(p_1^*\|\tilde{p}_2\right) \geq 0 \quad (48)$$

This will yield the distribution $p_1^* \in \mathcal{P}_1$ that is closest to p_2. Using Lagrange multipliers, we have

$$J(p_1^*, \lambda, \nu) = \sum_{\mathcal{X}^4} p^* \log \frac{p^{*(c)}}{p_2^{(c)}} + \lambda p_1^* \log \frac{\tilde{p}_1^{(c)}}{\tilde{p}_2^{(c)}} + \nu p_1^* \quad (49)$$

Now we set the derivative to zero for a particular $\mathbf{a} \in \mathcal{X}^3, b \in \mathcal{X}$. Note that

$$\frac{\partial p^{(3)}}{\partial p(\mathbf{a}b)} = \frac{\partial}{\partial p(\mathbf{a}b)}\left[p(\mathbf{a}b) + \sum_{b' \neq b} p(\mathbf{a}b')\right] = 1$$

and therefore,

$$0 = \frac{\partial J}{\partial p_1^*(\mathbf{a}b)} = \log \frac{p_1^{*(c)}(b|\mathbf{a})}{p_1^{(c)}(b|\mathbf{a})} + \lambda \frac{\tilde{p}_1^{(c)}(b|\mathbf{a})}{\tilde{p}_2^{(c)}(b|\mathbf{a})} + \nu + 1 \quad (50)$$

which determines that the p_1^* which maximizes the error to be in the form

$$p^*(\mathbf{c}) = \frac{p_2^{1-\lambda}(\mathbf{c})p_1(\mathbf{c})^\lambda \left(\frac{p_2(\mathbf{c})}{\tilde{p}_2(\mathbf{c})} \frac{\tilde{p}_1(\mathbf{c})}{p_1(\mathbf{c})}\right)^\lambda}{\sum_{\mathbf{d} \in \mathcal{X}} p_2^{1-\lambda}(\mathbf{c})p_1(\mathbf{c})^\lambda \left(\frac{p_2(\mathbf{c})}{\tilde{p}_2(\mathbf{c})} \frac{\tilde{p}_1(\mathbf{c})}{p_1(\mathbf{c})}\right)^\lambda}. \quad (51)$$

where $\lambda = \lambda^*$ such that (48) holds with equality. Replacing the estimates with the true distributions, one can see that as $L \to \infty$, (51) converges to (6) and thus the proof of Theorem 2 is complete.

Now, since $d(p_2, \tilde{p}_2) < \epsilon_L$ and $d(p_1, \tilde{p}_1) < \epsilon_L$, we can find an L_0 such that for any $L > L_0$,

$$\frac{1}{1+\gamma} < \frac{p_1(\mathbf{c})}{\tilde{p}_1(\mathbf{c})}, \frac{\tilde{p}_1(\mathbf{c})}{p_1(\mathbf{c})}, \frac{p_2(\mathbf{c})}{\tilde{p}_2(\mathbf{c})}, \frac{\tilde{p}_2(\mathbf{c})}{p_1(\mathbf{c})} < 1+\gamma$$

where $\gamma \ll 1$ is an arbitrarily small positive constant. Then the divergence sum is upper-bounded as

$$D_c(p_1^*\|p_1) \quad (52)$$
$$= \sum_{\mathbf{c} \in \mathcal{X}^4} p_1^*(\mathbf{c}) \log \frac{p_1^*(\mathbf{c})}{p_1(\mathbf{c})} \quad (53)$$
$$< (1+\gamma)^{4\lambda} C(p_1, p_2) + (1+\gamma)^{4\lambda} \log(1+\gamma)^{2\lambda} \quad (54)$$

and lower-bounded as

$$D_c(p_1^*\|p_1) > \frac{C(p_1, p_2)}{(1+\gamma)^{4\lambda}} + \frac{\log(1+\gamma)^{-2\lambda}(1+\gamma)^{2\lambda}}{}$$

equation We denote the distance from $C(p_1, p_2)$ as $g(\gamma)$, which goes to 0 as $\gamma \to 0$. Therefore proof of Theorem 3 is complete.

VII. Discussion

In this paper, we modeled the metagenomic binning problem as the problem of clustering sequences generated by distinct Markov processes. While overly simplistic, this model allowed us to establish the Chernoff Information as a measure of how easy it is to distinguish contigs generated by two species.

The algorithm used to prove the achievability suggests that a good "metric" for binning is the conditional relative entropy between a contig and an estimate of a species TNF. Through simple experiments, we provided preliminary evidence that this metric often outperforms the Euclidean metric. However, it is unclear whether for real genomes one can reliably estimate the overall TNF of a genome.

A direction for future work includes verifying whether it is possible to achieve Theorem 1 without estimating the underlying TNF distributions using a community detection method, or more specifically, a stochastic block model. In a stochastic block model, communities (each representing the contigs from one species) form internal and external edges with set probabilities. The resulting fundamental limits under this model could provide some interesting insights. Another possibility is finding a computationally efficient version of Algorithm 1 through clique-finding heuristics.

In metagenomics, the read coverage, or *abundance*, is used to compare contigs in addition to the TNF. The abundance is essentially the number of reads which overlap to create a contig. Each nucleotide in a contig has an associated abundance based on the total overlap at that point, and typically the abundance is averaged over all nucleotides in a contig to create one feature. The fundamental limits of the metagenomic binning problem *including* the abundance (i.e. by assigning prior probabilities to each species) would be of potential interest to the metagenomics community.

Acknowledgements

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