

# MetaPar: Metagenomic Sequence Assembly via Iterative Reclassification

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**Abstract**—We introduce a parallel algorithmic architecture for metagenomic sequence assembly, termed *MetaPar*, which allows for significant reductions in assembly time and consequently enables the processing of large genomic datasets on computers with low memory usage. The gist of the approach is to iteratively perform read (re)classification based on phylogenetic marker genes and assembler outputs generated from random subsets of metagenomic reads. Once a sufficiently accurate classification within genera is performed, de novo metagenomic assemblers (such as Velvet or IDBA-UD) or reference based assemblers may be used for contig construction. We analyze the performance of *MetaPar* on synthetic data consisting of 15 randomly chosen species from the NCBI database [18] through the effective gap and effective coverage metrics.

## I. INTRODUCTION

Metagenomics is a scientific discipline devoted to the study of complex microbial samples in the environment. Unlike classical genomics, where one is faced with the task of processing samples corresponding to one organism, metagenomics is concerned with samples that consist of a mixture of genetic material of different species and strains of bacteria or viruses [11] within a host. With raw data file sizes capable of exceeding hundreds of gigabytes, metagenomic data poses significant new challenges in Big Data signal processing and analysis since genomic assembly is computationally difficult even for single species analysis [11], [10], [8].

Although significant progress was made in the last few years on developing new methods for metagenome assembly [14], [4], [8], [15], the problem of accurate metasequence profiling remains wide open. To hasten the progress of this new discipline, InnoCentive recently launched a special competition under the auspices of the U.S. Defense Threat Reduction Agency (DTRA) in metagenomic de novo assembly. Specialized access to powerful computers and clusters were given to all active participants for processing relatively small amounts of data (tens of gigabytes). In the absence of such strong computational support, large metagenomic assembly appears to be infeasible. One way to mitigate this issue is to break down the metagenomic data into smaller subsets that may be processed independently and in parallel, using modest computational power. This is the gist of the assembly approach *MetaPar* for parallel metagenomic assembly that we proceed to describe in the remainder of the paper.

*MetaPar* mitigates the need for computationally demanding full metagenomic assembly by using an iterative two-stage read classification technique. In the first stage, the microbial identification tool *MetaPhyler* [6] is used for providing a rough profile of organisms present in the metagenomic mix. By aligning the reads to *all genomes* of organisms within the identified genera via *Bowtie2* [3], one obtains a rough partition of the reads into subgroups. Reads within different identified subgroup are assembled in parallel, producing contigs that may be run through BLAST (Basic Local Alignment Search Tool) [1] to verify the accuracy of the classifier. After this first classification

step, some reads may remain unaligned, and require alternative means of processing.

Two options may be pursued in the second round of classification, depending on the number of unaligned reads. If the number of reads is prohibitively large so as not to allow one-pass assembly with a standard metagenomic assembler, the reads are *randomly* partitioned into subgroups small enough to be assembled. All assemblies are performed in parallel. Unaligned reads are iteratively reassigned between assemblers until no changes in the assembled contigs are reported or until a maximum number of iterations is reached. On the other hand, if the number of reads is small enough to allow for one pass assembly, the same procedure as outlined for the initial step is performed. Related ideas involving dynamic classification of reads were described in [15], but for the purpose of *single genome* assembly. *MetaPar* is a simple parallel (and distributable) metagenomic assembler which is able to take advantage of improvements in standard de novo metagenomic assemblers and reference-based assembly, in contrast to recent distributed and parallel assemblers such as Ray Meta [21].

The paper is organized as follows. In Section II, we provide a step-by-step description of the *MetaPar* algorithm. In Section III, we demonstrate the performance of the method on synthetic Illumina sequencer data, using a randomly selected set of 15 bacterial organisms. We also compute and list the effective coverage and gaps in *MetaPar* alignments to the identified species' genomes.

## II. AN ALGORITHMIC SOLUTION FOR PARALLEL METAGENOMIC ASSEMBLY

The following terminology is used throughout the paper. When describing a living organism, we will refer to several taxonomy levels, listed from most general to most specific: life, domain, kingdom, phylum, class, order, family, genus, and species. Each organism has a *genome*, which is a sequence of *bases* over a four letter alphabet. A *read* is a *substring* of a genome or a chromosome (a part of a genome), generated through some sequencing system. The *coverage* of a base in a genome equals the number of reads that contain the base. *Assembly* refers to the process of overlapping reads – suffix to prefix – in order to reconstruct the original sequence from which the reads came from, or in order to reconstruct sufficiently long substrings of the genome, termed *contigs*. *Alignment* refers to mapping reads onto a given genome.

The metagenome assembly problem may be formulated as follows: given a mixture of reads from genomes of different species providing sufficiently high coverage, reconstruct the original genomes as accurately as possible via some computationally plausible assembly method. Many different assembly methods for metagenomic data were developed in the past few years, using greedy algorithms, reference-based approaches, algorithms based on deBruijn graphs and Eulerian path searches, and many other techniques [7]. Although the accuracy of the aforementioned assembly methods is high for small metagenomic samples, it quickly deteriorates when the metagenomic data contains fragments of a large number of species. Even more important is the fact that the complexity of most assembly methods

grows exponentially with the number of species, leading to poor performance scaling with sample size. As a result, large metagenomic samples require powerful computers for assembly. This raises the natural question of parallelizing the assembly process.

We next outline the parallel *MetaPar* assembly algorithm that allows for assembling metagenomes containing several hundred species suitable for commodity computers with 16-48+ GB RAM. The running time of all components, aside from calls to standard assemblers, grows linearly with the size of the metagenomic sample. The block diagram of the algorithm is depicted in Figure 1. Note that some steps in the algorithm are implemented only if the metasample (metagenomic sample) is very large, since in that case, direct assembly is computationally infeasible. The proposed algorithm uses certain techniques related to the authors' MCUIUC algorithm for compression, described in [20].

- **Step 1 (Level I Identification):** The first step of the iterative procedure is to remove as many reads that can be associated with known species from the original sample before running the assembly process. Such a filtering procedure is expected to produce significantly reduced metasamples for subsequent assembly. Filtering is achieved by passing the metasample through a taxonomic identifier, such as *MetaPhyler* [6]<sup>1</sup>. The gist of the approach in [6] is that almost every genomic substring of length exceeding 20 is unique to a species or a genus. *MetaPhyler* scans through the reads to identify such substrings and links them to a sequenced species. Identification usually amounts to specifying the genera of organisms, and the abundances of the marker sequences. Due to identification errors, the output of the taxonomic classifier contains both false-positive and false-negative results. Selection of the identified genera used for read removal can depend on factors such as the number of identified organisms, genome lengths and identifier abundance. Simulation on synthetic data involving 15, 30 and  $\geq 60$  species was used to determine simple abundance thresholds, as described in the next section.

- **Step 2 (Level I Partitioning of the Dataset):**

- 1) Once a group of genera of interest is identified in Step 1, representative reference genomes for alignment of reads are selected. Selection is accomplished by using complete genomes of all species within the identified genera in an alignment procedure performed via *Bowtie2* [3]. *Bowtie2* is designed for ultra-fast alignment of short reads to long genomes, and its complexity scales roughly linearly with the length of the genomes and number of reads (*Bowtie2*, along with BLAST [1], is one of the most frequently used alignment algorithms). Given that not all correct genera may have been found by *MetaPhyler*, some reads will be reported as unaligned. Unaligned in this context refers to not having sufficient sequence similarity to any substrings of the chosen reference genomes. The percentage of unaligned reads heavily depends on the size of the metagenome, the number of species involved, as well as the number of identifiers of the species used in the identification software.
- 2) **Step 3 (Level II Identification):** Given that *MetaPhyler* may miss identifying a large number of species present in the sample, and that consequently *Bowtie2*-based partitioning may leave a large fraction of reads unclassified, additional identification procedures are needed. Two different procedures are employed based on the volume of the unaligned reads. If the size of the unaligned metagenome is relatively small, metagenomic assembly based on *Velvet*, *SOAP deNovo* or *IDBA-UD* [8] is used. If the unaligned metagenome is prohibitively large to be assembled by existing assemblers, the unaligned read set is partitioned into an appropriate number of *random subsets* (The largest number of subsets we needed to run on any dataset was eight.). The subsets are processed in parallel by independent assemblers that produce contigs, which can be run through

<sup>1</sup>Very recently, a new approach to species identification was described in [2] that may outperform *MetaPhyler*. Given that comparing identification software packages is beyond the scope of the paper, we only report results for the more commonly used *MetaPhyler* package.

TABLE I. A RANDOMLY SELECTED SET OF 15 SPECIES USED TO ILLUSTRATE THE OPERATING PRINCIPLES OF *MetaPar*.

#	Genus	Species
1	Acidilobus	Acidilobus_saccharovorans_345_uid51395
2	Alcanivorax	Alcanivorax_borkumensis_SK2_uid58169
3	Bacteroides	Bacteroides_fragilis_YCH46_uid58195
4	Borrelia	Borrelia_garinii_NMJW1_uid177081
5	Corynebacterium	Corynebacterium_pseudotuberculosis_119_uid159673
6	Enterobacter	Enterobacter_asburiae_LF7a_uid72793
7	Frankia	Frankia_Cc13_uid58397
8	Halomicrobium	Halomicrobium_mukohataei_DSM_12286_uid59107
9	Helicobacter	Helicobacter_pylori_Shi417_uid162205
10	Lactobacillus	Lactobacillus_amylovorus_GRL1118_uid160233
11	Mobiluncus	Mobiluncus_curtisii_ATCC_43063_uid49695
12	Mycoplasma	Mycoplasma_arthritis_158L3_1_uid58005
13	Odoribacter	Odoribacter_splanchnicus_DSM20712_uid63397
14	Prevotella	Prevotella_denticola_F0289_uid65091
15	Psychroflexus	Psychroflexus_torquis_ATCC700755_uid54205

BLAST to identify additional reference genomes for alignment with *Bowtie2*. Given that the partitioning of the dataset is random, many reads may appear as stand-alone contigs at the output of the assembler, and are treated as unaligned reads that need to be re-classified.<sup>2</sup>

- 3) **Step 4 (Iterative Re-classification):** Reads that remain unaligned after the described three steps are processed iteratively through Step 3, as long as the number of unaligned reads is higher than a certain threshold or until a maximum number of iterations is executed.

### III. WORKING EXAMPLE

Metagenomic samples have vastly different sizes [10], and the exact number of iterations performed in the assembly process, as well as the exact number of parallel read classes used depends on the metagenomic file size. For example, for the "CO182: Coal cuttings from Coal bed Methane well site" sample [19], one has to run eight or more instances of a conventional assembler such as IDBA-UD – in parallel on several machines, or sequentially on one machine – with about 5.3 GB of reads per assembler. The algorithms were executed on computers equipped with dual Intel Xeon E5630 processors (16 threads) and 48 GB RAM in order to not exhaust the available memory at the 20 kmer level for the modified deBruijn graph search. This problem is further exacerbated with sequencing technologies that produce "long reads" (>128 bases in the case of IDBA-UD) which require additional data to be maintained for reads during assembly. In many cases, it is not feasible to increase available memory beyond a certain point per machine. Each part required ~140 CPU hours to assemble, which while relatively low is not the critical constraint due to available memory. On the other hand, for most synthetic metagenomes including roughly 15 species, only one assembler is needed. All computations other than the running of parallel assemblers were performed on a computer equipped with an Intel Core i5 3470 and 16 GB of memory, and were primarily I/O and CPU limited rather than memory limited. In the former case (CO182), *MetaPhyler* only identified genera accountable for 40% of the metareads, while it identified more than 70% of reads in the small synthetic samples. Due to space limitations, we illustrate the performance and the steps of the *MetaPar* algorithm on a small synthetic sample involving 15 species, and defer the analysis of real metagenomic samples to the full version of the paper.

#### A. Simulating the Metagenomic Sample

Species were randomly selected from the NCBI microbial genome database available at [18]. A selected group of 15 organisms is listed in Table I. Of the chosen species, *Frankia* has the longest genome with 5,511,253 bps (base pairs), while *Mycoplasma arthritis* has the shortest genome with 832,175 bps. For each species, we selected the FASTA file containing the complete genome and generated paired

<sup>2</sup>As already mentioned, a means for parallelizing *single genome* assembly that shares some of the classification ideas outlined in this step was first reported in [15].



TABLE III. UP TO 9 BIGGEST SPECIES FILES PER GENUS ASSIGNED BY BOWTIE AFTER THE FIRST ITERATION.

#	Genus	Accession # and Description
1	Alcanivorax	NC_008260 (Alcanivorax borkumensis SK2)
2	Bacteroides	NC_006347 (Bacteroides fragilis YCH46) NC_016776 (Bacteroides fragilis 638R) NC_003228 (Bacteroides fragilis NCTC 9343)
3	Borrelia	NC_018747 (Borrelia garinii NMJW1) NC_017717 (Borrelia garinii BgVir) NC_006156 (Borrelia garinii PBI)
4	Corynebacterium	NC_017303 (Corynebacterium pseudotuberculosis I19) NC_017031 (Corynebacterium pseudotuberculosis P54B96) NC_017462 (Corynebacterium pseudotuberculosis 267) NC_017306 (Corynebacterium pseudotuberculosis 42/02-A) NC_017305 (Corynebacterium pseudotuberculosis PAT10) NC_017301 (Corynebacterium pseudotuberculosis C231) NC_017300 (Corynebacterium pseudotuberculosis 1002) NC_016781 (Corynebacterium pseudotuberculosis 3/99-5) NC_014329 (Corynebacterium pseudotuberculosis FRC41)
5	Enterobacter	NC_015968 (Enterobacter asburiae LF7a)
6	Frankia	NC_007777 (Frankia sp. CcL3)
7	Helicobacter	NC_017739 (Helicobacter pylori Shi417)
8	Lactobacillus	NC_017470 (Lactobacillus amylovorus GRL1118) NC_015214 (Lactobacillus acidophilus 30SC) NC_014724 (Lactobacillus amylovorus GRL 1112)
9	Mobiluncus	NC_014246 (Mobiluncus curtisii ATCC 43063)
10	Mycoplasma	NC_011025 (Mycoplasma arthritidis 158L3-1)
11	Prevotella	NC_015311 (Prevotella denticola F0289)

TABLE IV. TABLE OF EFFECTIVE COVERAGE & GAP FOR REFERENCE-BASED ASSEMBLY WITH BOWTIE2

Genus	Effective Coverage	Effective Gap
Acidilobus	99.866	5.5
Alcanivorax	99.863	2
Bacteroides	99.947	0
Borrelia	99.863	3
Corynebacterium	99.867	2
Enterobacter	99.867	1
Frankia	99.869	1
Halomicrobium	99.866	1
Helicobacter	99.867	4
Lactobacillus	99.866	4
Mobiluncus	99.867	4
Mycoplasma	99.865	0
Odiobacter	99.751	0
Prevotella	99.861	2
Psychroflexus	99.856	1

The utility of the N50 value for assessing assembler performance is questionable, since it does not convey important information about what percentage of the length of underlying genomes is actually covered by the contigs and to what extent. This is especially true for reference based assembly. To mitigate this problem, we introduced two performance measures, termed the *effective average coverage* and the *effective gap*. The effective coverage measures the average number of times a base in the genome is covered by the longest matches in each read aligned via *Bowtie2* without errors. Similarly, the effective gap measures the number of bases that were not covered by the longest matches contained in each read aligned via *Bowtie2*. The effective gap and effective coverage for the given example are listed in Table IV.

As can be seen from the table, the effective coverage is very large, exceeding 99.751% for all metasample organisms. The observed gaps are extremely small, with the worst performance observed for the genera *Acidilobus*. A quick look at Table III reveals that the selected organism in this genus was not identified by *Metaphyer* and may have consequently had many substrings shared by other organisms. This may be a plausible explanation for read misclassification and consequently high effective gap.

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#### IV. CONCLUSIONS

We have described a new parallelizable framework for metagenomic assembly in which the computational time for most steps grow linearly in time with the size of the metagenomic sample. The algorithm identifies genera and species in order to use reference based assembly to reduce the amount of standard de novo assembly required. Performance was illustrated on a synthetic sample of 15 species. Further work includes designing schemes for efficient partitioning of reads akin to TIGER, incorporation of phylogenetic aligners and incorporation of other classifiers for reads.

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