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Master Thesis

以核苷酸 *k* 聚體頻度分類序列

Sequence Classification Based on *k*-mer Frequencies

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國立臺灣大學碩士學位論文
口試委員會審定書

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Sequence Classification Based on *k*-mer Frequencies

本論文係陳泓宇君（R06945024）在國立臺灣大學生醫電子與資訊學研究所完成之碩士學位論文，於民國 108 年 7 月 26 日承下列考試委員審查通過及口試及格，特此證明

口試委員：[illegible]（指導教授）

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所　　長：[illegible]

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摘要

序列分類在計算生物學的許多研究中是一個在研究初期就需要解決之問題，有許多方法被研發出來計算此問題，但隨著高通量定序技術的發展，需要計算的資料量也大幅增加，導致許多現有方法已無法在能取得的計算資源及可接受的時間內完成計算。以核苷酸 k 聚體為基礎的演算法就是其中一種，目前已有不少方法可以快速且準確的完成分類，但卻需要大量的計算空間，因此無法在一般個人電腦中完成計算。

在本篇論文中，我們提出一個以核苷酸 k 聚體為基礎的演算法，在時間上與現有方法相當，在空間上則避免現有方法中儲存上的冗餘性而做出改善。為進一步降低所需記憶體空間，我們提出一個分割架構，此架構除了可以減少所需空間，也適合平行化以縮短計算所需時間。

關鍵字：序列分類、環境基因體學、基因體學、$\boldsymbol{k}$ 聚體、免序列比對、序列特徵、演算法

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Abstract

Sequence classification is a preliminary step in many researches of computational biology. There are a variety of methods proposed to compute this problem. However, with the development of high-throughput sequencing technologies, the datasets of sequencing data are getting much larger. As a result, many existing methods cannot accomplish this task with limited computational resource and acceptable time. The *k*-mer based algorithms are some of these methods. Most of them could finish the classification fast and accurately, but they need large computational space, which is not available in common personal computers.

In this thesis, we propose a *k*-mer based algorithm. The time complexity of our algorithm is comparable to those of the existing methods, while we make an improvement in space usage by avoiding the redundancy of storing the *k*-mers. To further reduce the memory usage, we propose a partitioning strategy. In addition to the reduction in memory usage, the algorithm under this partitioning structure can be highly parallelized to improve performance.

Keywords: sequence classification, metagenomics, genomics, *k*-mer, alignment-free, sequence signature, algorithm

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Chapter 1

Introduction

A substring of length *k* in a given string, usually a sequencing read, is called a *k*-mer in bioinformatics. For example, given a sequencing read ACGGTTC, all 3-mers in this read are ACG, CGG, GGT, GTT, and TTC. With the development of high-throughput sequencing technologies, the studies of *k*-mers are getting more and more important because many computational methods use *k*-mers to analyze the sequencing reads and datasets. In other words, *k*-mer is a fundamental unit for many methods [2, 25, 28, 29, 32, 62].

The number of the occurrences of each *k*-mer in a given dataset is called *k*-mer frequency or *k*-mer spectrum, and the problem of computing *k*-mer frequencies is called *k*-mer counting. The *k*-mers with high frequencies can be regarded as features of a dataset. In contrast, the *k*-mers with low frequencies, such as *k*-mers occurring only one time, which are called singleton *k*-mers, are often generated by sequencing errors. There are many methods and applications making use of *k*-mer counting. For instance, genome assembly [4, 19, 41], estimation of genome size [57], read correction [23], repeat detection [8, 20, 26, 27, 34], sequence alignment [14] and comparison of genomes [9, 42, 64].

k-mer counting is conceptually simple, but it is difficult to be both fast and memory-efficient. In particular, the sizes and the amounts of datasets nowadays are increasing rapidly. Some traditional *k*-mer counting tools cannot complete the task of large datasets or large *k* in reasonable time and space. A naïve implementation of *k*-mer counting is to

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use a simple hash table, where keys are the *k*-mers and values are the counts. However, when there are many distinct *k*-mers, the hash table should be very large, which is often larger than given memory space, to avoid collisions. As a result, some studies make efforts to implement more space-efficient data structures for *k*-mer counting. There are also some studies trying to solve this problem by using disk space instead, which is a trade-off between performance and space because disk I/O operations are much slower. There are many latest researches [5, 18] about *k*-mer counting.

k-mers are widely used in metagenomics in recent years. For example, signature *k*-mers representing members of a certain protein family can be used to annotate metagenomes [15] and *k*-mer frequencies can be used in binning metagenomic contigs [1]. An important application is the classification of sequences. In some researches, the sequences to be classified belong to the same metagenome, and the researchers want to know which metagenome it is. In some other researches, the researchers want to determine the identities of the species in the sequenced sample. A solution common to these two problems is to compare the given sequences with sequences of known origins. *k*-mer frequencies can be very helpful in both cases. With analyzing and comparing the *k*-mer frequencies of the given sequences of the same metagenome/species to the *k*-mer frequencies of the sequences of other determined metagenomes/species, we can get the dissimilarities and then infer the relationships between different datasets [6, 13, 45, 50].

Considering the properties of *k*-mers and the fact that the computation of *k*-mer frequencies is getting faster and uses much lower memory space than before, the sequence comparison based on *k*-mer frequencies is promising. Some of the comparison methods mentioned above can use the output of the *k*-mer counting tools as input, while some methods develop their own methods to count *k*-mers. CLARK [45] is a fast classification method based on *k*-mer frequencies which takes files containing *k*-mer frequencies as input. Nevertheless, the memory usage of CLARK is too large for common personal computers. There is a variant of CLARK trying to solve this problem, but the accuracy gets lower. In this thesis, we propose the algorithms which not only overcome this issue but also improve the performance.

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1.1 Motivation

The classification of datasets is an essential task in the preliminary stage of many researches. An intuitive approach is to measure the dissimilarities between datasets. Sequence alignment is a traditional solution in taxonomy. Through analysing the results of the alignment, the evolutionary relationships between object datasets and reference datasets could be inferred. However, object datasets usually consist of sequencing reads, which are not suitable for alignment, and the assembly of reads from unknown origins is a difficult task. Therefore, alignment-free methods are proposed. One category of these alignment-free methods is based on *k*-mers. Some properties of *k*-mers, which are described in Section 2.2, make *k*-mers appropriate for comparing datasets. Some *k*-mer based methods compute the pairwise distances between *k*-mer spectra of datasets to measure the dissimilarities. Nonetheless, pairwise comparisons are time-consuming.

An approach to avoid pairwise comparisons between datasets is to find the signature *k*-mers of each dataset and classify the datasets according to these signature *k*-mers. The criteria of finding the signature *k*-mers is another critical issue. The *k*-mers with highest frequencies may be candidates, but *k*-mers occurring many times in one dataset could also occur many times in other datasets just because they are common in nucleotide sequences. CLARK provides an intuitive and efficient approach to solve this problem. It finds the *k*-mers specific to each target dataset and classifies the object datasets accordingly. The disadvantage of CLARK is the large memory usage. As a result, we try to improve this method by reducing memory usage.

1.2 Problem Description

Given *p* object datasets to be classified and *n* target datasets of known origins, determine which target is the most likely origin of each object (Figure 1.1).

The datasets could be metagenomic or genomic sequences as long as all the sequences in the object and target datasets are grouped into datasets at the same level. It is also feasible to construct the datasets with the sequences grouped at species or genus level.

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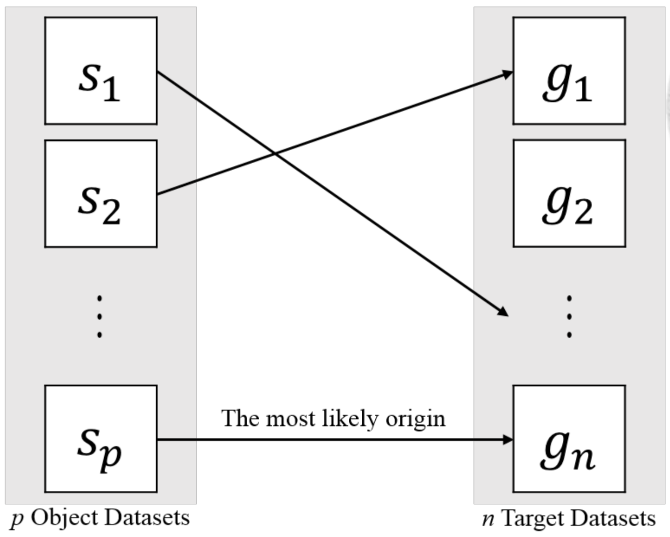

Figure 1.1: Sequence classification problem

In traditional methods, the definition of "the most likely origin" is the target with the smallest taxonomic dissimilarity. However, these methods usually need complete reference databases to compute the dissimilarities and they are computationally costly. Consequently, many researchers attempt to find methods based on other data instead of the contents of the sequences. Some proposed methods make use of *k*-mer frequencies. There are some existing *k*-mer based methods which could solve this problem fast. In this thesis, we focus on how to solve this problem fast and accurately on the basis of *k*-mer frequencies with limited memory space.

1.3 Main Results

There is a fast and accurate method, CLARK, resolving this problem based on *k*-mer frequencies. However, it uses large RAM space during the computation. In this thesis, we try to reduce the memory usage such that this task could be computed with common personal computers. We propose algorithms which avoid the redundancy of storing *k*-mers in CLARK. The time complexities of these algorithms are the same as CLARK's, while they should be faster than CLARK in practice. We also propose a partitioning strategy to further reduce the memory usage.

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1.4 Organization of the Thesis

- Chapter 2 - Related Work

  In this chapter, we summarize some related work about *k*-mer counting tools and *k*-mer based comparative metagenomics methods. The algorithms of some *k*-mer counting tools provide good ideas to improve classification methods. In addition, we conclude some researches indicating that *k*-mers have some good properties such that they are suitable for comparing datasets.

- Chapter 3 - Methods

  In this chapter, we propose algorithms which overcome the issue of large memory usage. We describe the algorithms, analyze them and compare them with the existing *k*-mer based classification method, CLARK. In addition, we propose a partitioning strategy to further reduce the memory usage of the purposed algorithms. The algorithms with this strategy can be highly parallelized.

- Chapter 4 - Conclusion

  In this chapter, we conclude the proposed algorithms and the partitioning strategy. Besides, we describe the direction of future work.

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Chapter 2

Related Work

2.1 *k*-mer Counting Tools

Although there are many studies based on *k*-mer counting, early ones only consider it as a preliminary step and describe it sketchily. Tallymer [26] is the first tool designed specifically for *k*-mer counting. Rather than hash table, this tool is based on suffix array. Meryl is a *k*-mer counting tool from the Celera assembler [41] package, which uses a sorting-based approach. However, these tools are not efficient enough to deal with large datasets.

Jellyfish [39] uses a multithreaded, lock-free hash table. Users have to pre-specify the memory size for the hash table to use. Once the hash table is full, the intermediary *k*-mer counts are saved to disk and merged to the final results later. Its current versions are still used commonly in recent studies.

BFCounter [40] points out that more than half of the observed *k*-mers even in preprocessed datasets are singletons, which can be weeded out as wrong data caused by sequencing error and thus should not be inserted into the data structure for counting. BFCounter uses a two-pass method. In the first pass, it uses a Bloom filter [7] to filter out the *k*-mers which occur only one time. The Bloom filter is an approximate membership query (AMQ) data structure. An AMQ data structure maintains a compact and probabilistic representation of a set or multiset, so it could generate false positives during querying if there are collisions. The *k*-mers that present more than once are inserted

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into a hash table. Since the result of a query to the Bloom filter is probably a false positive, there may be some *k*-mers occurring only once inserted into the hash table. In the second pass, it re-iterates over all the reads and counts the *k*-mers which are inserted in the hash table in the first pass using a hash table to get the exact frequencies. BFCounter uses less memory but much more time than Jellyfish. The difference in speed is mainly due to the two-pass method. It is feasible for BFCounter to do the counting in the first pass and omit the second pass to obtain approximate *k*-mer frequencies.

Jellyfish and BFCounter mainly rely on memory, and usually need dozens of gigabytes of memory, which are not available in most personal computers. Relatively, it is much easier to get sufficient capacity from disks. Consequently, some studies try to develop disk-based *k*-mer counting tools, such as DSK [51] and KMC [11, 12, 24]. DSK relies on hash tables. Different from Jellyfish, it processes the *k*-mers in several iterations. In each iteration, only a partition of *k*-mers classified according to their hash values are saved to their corresponding lists in the disk and then the lists are read from the disk to insert into a hash table for counting. Users can set the target memory usage size and the target disk space size. The numbers of iterations and lists are calculated accordingly, so it is guaranteed that the size of hash tables would not exceed the target memory usage size.

KMC [11] is similar to DSK in concept. The major difference is that KMC has developed a scheme of parallel algorithm. The whole process can be divided into two phases. In distribution phase, the *k*-mers are partitioned into several bins on the basis of their prefixes, sorted and compacted. The bins are then stored into disk as files. In sorting phase, the files are read from the disk. *K*-mers are uncompacted, sorted and counted.

Turtle [54] uses a method similar to BFCounter. It uses a pattern-blocked Bloom filter [49], which reduces the number of cache misses by restricting the locations to store a *k*-mer in the filter, to filter out singleton *k*-mers. The *k*-mers occurring more than once are stored in an array. By repeatedly sorting and compaction, users can get the array of these *k*-mers with their counts.

KAnalyze [3] implements a modified merge sort algorithm to count *k*-mers, and the algorithm could be divided into two components: split component and merge component.

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The split component reads a set of *k*-mers into an array and sorts the array with a dual-pivot quicksort algorithm. Then, it counts the *k*-mers by traversing the array, writes the *k*-mers with their counts to a file in disk and fills the array with the next set of *k*-mers. The merge component reads the files and accumulates the counts.

khmer [63] uses an AMQ data structure, count-min sketch [10], to count the *k*-mers. Different from other methods, khmer does not store *k*-mers in the data structure. To increment the count of a *k*-mer, it uses a hash function to get the hash value and determines the locations to be updated in the hash tables of the count-min sketch accordingly. To retrieve the count of a *k*-mer, the hash value is computed and the minimum count among the counts in all hash tables is returned. However, there are miscounts in the results of khmer because the count-min sketch could generate false positives. khmer provides a way to systematically trade larger memory usage for lower false positive rate.

There is high redundancy in above methods. Consecutive *k*-mers share $k-1$ symbols, but they are processed and stored as *k*-mers not relevant at all. MSPKmerCounter [30] introduces minimizers [52, 53] to the *k*-mer counting problem to reduce the redundancy. KMC 2 [12] refines the minimizers to signatures, which fit the parallel scheme of KMC better. In distribution phase, it partitions super *k*-mers consisting of *k*-mers sharing the same signatures into bins according to their signatures. In sorting phase, it breaks down super *k*-mers and counts them in an approach similar to the method in KMC. KMC 3 [24] follows the same scheme as KMC 2 and makes some improvements in details.

KCMBT [37] counts *k*-mers on the basis of multiple burst tries [21, 56]. It inserts *k*-mers into burst tries and traverses the tries to get the final *k*-mer frequencies after inserting all the *k*-mers. KCMBT constructs 4^a burst tries, where a is the prefix length for indexing the tries, to reduce the space for storing *k*-mers.

Gerbil [16] uses a disk-based and parallel approach similar to KMC 2. It is divided into two phases as well: distribution phase and counting phase. In distribution phase, it uses minimizers to split the input data into several smaller temporary files which are stored in the disk. They experimentally evaluated various ordering strategies of minimizers, and they found the strategy, signatures, used by KMC 2 is a good choice for most datasets. In

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counting phase, the temporary files are re-read from the disk. After splitting super *k*-mers into *k*-mers. Gerbil uses the hash table approach to count the *k*-mers and solves collisions via quadratic hashing. In addition, Gerbil puts emphasis on algorithm engineering. It points out several details implemented to gain high performance and utilizes GPUs to speed up the counting phase. Gerbil can support the counting of *k*-mers for large *k* of large datasets, which cannot be finished efficiently by KMC 2 and not supported by DSK.

Squeakr [47] uses counting quotient filters [46] to count the *k*-mers. The counting quotient filter (CQF) is a novel AMQ data structure, but the collisions can be avoided by adjusting the size of hash function and the size of data to be stored. Squeakr extracts the *k*-mers from input data and inserts them into a local CQF in each thread. The data in the local CQFs are then inserted into a global CQF to get the final approximate results. It is possible for Squeakr to get exact results by adjusting the CQFs as mentioned above, and it is called Squeakr-exact.

There is a benchmark study [38] of the *k*-mer counting tools. They find that KMC3, DSK and Gerbil are the most flexible and efficient. (Squeakr is not assessed in this study.)

It seems that the sizes of the output files of *k*-mer counting tools would be very large when *k* is large because of the redundancy of *k*-mers. Conceptually, if all possible *k*-mers occur, there would be 4^k *k*-mers stored in the file with their counts. In practice, there would not be so many *k*-mers in the output files because (1) some *k*-mers hardly occur due to the molecular structure of nucleic acids, (2) *k*-mers are counted in canonical form (the lexicographically smaller one among the *k*-mer and its reverse complement) because when a *k*-mer occurs, there must be its reverse complement occurring on the other strand, and (3) many *k*-mer counting tools filter out singleton *k*-mers in default, and these singleton *k*-mers usually account for a large proportion of the *k*-mers. In addition, in most *k*-mer counting tools and applications based on *k*-mers, the *k*-mers are stored in binary form. A is encoded as 00, C as 01, G as 10 and T (U) as 11 such that each four bases of a *k*-mer can be stored in one byte. Take KMC3 for example, it filters out *k*-mers occurring less than 2 times in default and stores the *k*-mers in binary form. To compact the sizes of output files with reducing the redundancy of *k*-mers, it divides the output into prefix file (.kmc_pre)

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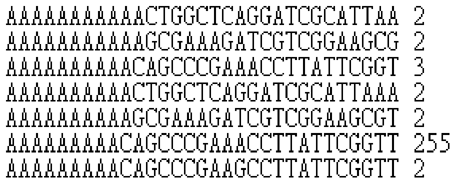
and suffix file (.kmc_suf). We compute the *k*-mer spectra of some datasets with KMC3 to show that *k*-mer spectrum is a succinct representation of sequence datasets (Table 2.1). However, it is a lossy representation. There is no information of *k*-mer position kept in the spectrum.

Organism	Genome length	Dataset	FASTQ files size	Gzipped files size	Output files size with/without filtering
E. coli	5	SRR5002442	3.71	1.24	0.14/1.35
C. elegans	102	DRR008444	19.62	5.95	0.78/1.71
F. vesca	214	SRA020125	9.52	3.38	2.22/5.43

Table 2.1: **Sizes of datasets and KMC3 output files.** $k = 31$. FASTQ and gzipped files are the datasets, and most *k*-mer counting tools could take both of these formats as inputs. Genome lengths are in Mbases according to http://www.ncbi.nlm.nih.gov/genome/. File sizes are in Gbytes (1 Gbyte = 10^9 bytes). For output files with filtering, we set KMC3 to filter out *k*-mers occurring less than 2 times. The datasets were downloaded from https://www.ebi.ac.uk/ena.

KMC3 provides a tool to convert output files to human readable *k*-mer spectrum files. The *k*-mer spectrum contains the *k*-mers and their frequencies (Figure 2.1).



```
AAAAAAAAAAACTGGCTCAGGATCGCATTAA 2
AAAAAAAAAAAGCGAAAGATCGTCGGAAGCG 2
AAAAAAAAAACAGCCCGAAACCTTATTCGGT 3
AAAAAAAAAACTGGCTCAGGATCGCATTAAA 2
AAAAAAAAAAGCGAAAGATCGTCGGAAGCGT 2
AAAAAAAAACAGCCCGAAACCTTATTCGGTT 255
AAAAAAAAACAGCCCGAAGCCTTATTCGGTT 2
```

Figure 2.1: Human readable *k*-mer spectrum file

2.2 *k*-mer Based Metagenomic Comparison and Classification Methods

A traditional method to determine the origin of a set of sequences is sequence alignment with reference sequences. But this method is not feasible when the reference databases are not complete. The lack of reference databases especially exists in metagenomics [22]. As a result, some researchers proposed *de novo* methods. A method [31] measures dissimilarities between datasets by marker genes and cluster them, but they are computationally

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expensive and leave lots of the reads unused. Therefore, another method [61] compare read contents directly with BLAST [2]. However, these methods cannot scale up to large datasets.

k-mer based methods are introduced into this issue in recent years. In [6], it states that "*k*-mers are a natural unit for comparing communities:

- sufficiently long *k*-mers are usually specific of a genome [17],
- *k*-mer frequency is linearly related to genome's abundance [60],
- *k*-mer aggregates organisms with very similar *k*-mer composition without need for a classification of those organisms [58]."

There is also a research [13] comparing *k*-mer based distances and taxonomic distances based on assignation against reference databases. They find that *k*-mer based distances are well correlated to taxonomic distances. Additionally, the *k*-mer based distances overcome the incompleteness issue of reference databases.

Compareads [36], Commet [35] and another method [55] compute the similarities between datasets based on the number of shared *k*-mers. MetaFast [59] and Mash [43] compute pairwise similarity matrices using feature vectors of *k*-mer composition.

Simka [6] computes varieties of distances between multiple datasets based on *k*-mer frequencies. To compute distances between multiple datasets simultaneously, the *k*-mer frequencies of all the datasets are needed. If we attempt to compute distances after finishing the counting of the frequencies of all the *k*-mers in datasets, we have to record a matrix of size $W \times N$, where W is the number of distinct canonical *k*-mers and N is the number of datasets. When W and N are large, this matrix would require a large amount of space. To avoid this demand for large space, Simka develops an efficient multiset *k*-mer counting algorithm (MKC) and applies some ecological distances which are additive over *k*-mers. To compute these distances, Simka only has to compute the distances using a part of the *k*-mers in each step, and aggregates the results of all the parts to get final distances (Figure 2.2). After extracting and storing the canonical representation of each *k*-mer, MKC separates the *k*-mers into a fixed amount of partitions. Each partition is

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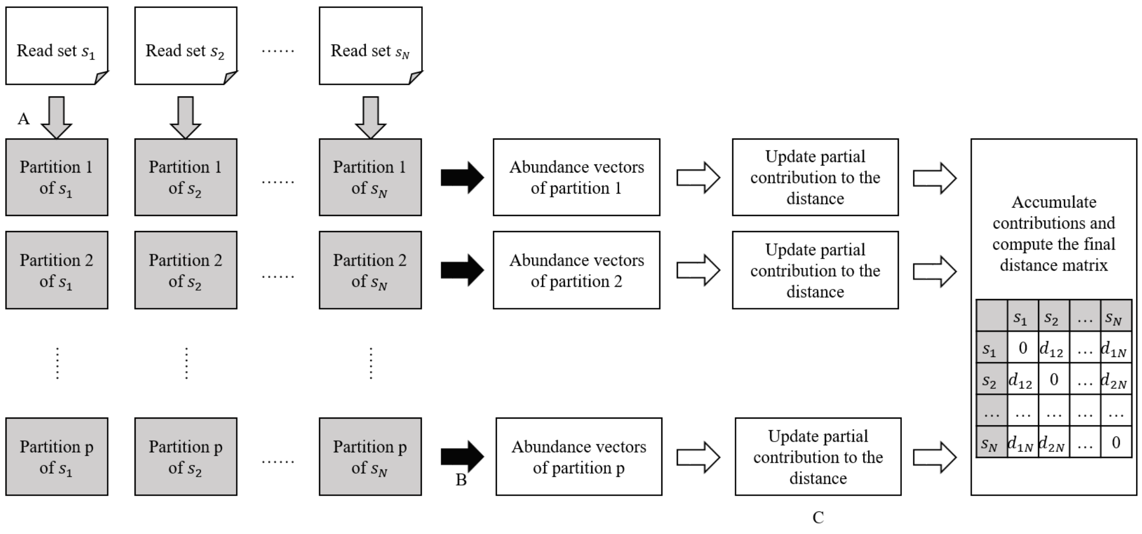
then sorted, counted and stored as files in disk independently. Thus the files associated to the same partition contain a specific subset of *k*-mers common to all datasets. With this partitioning strategy, Simka only takes a part of the counts at a time to compute the distances. Moreover, this approach is suitable for parallelization.



	s_1	s_2	…	s_N
s_1	0	d_{12}	…	d_{1N}
s_2	d_{12}	0	…	d_{2N}
…	…	…	…	…
s_N	d_{1N}	d_{2N}	…	0

Figure 2.2: **Workflow of Simka.** (A) The gray arrows represent the sorting count processes of MKC. Each process outputs *p* partitions of sorted *k*-mer counts. (B) The black arrows represent the merging count processes of MKC. Each process merges the counts in *N* partitions of a common subset of *k*-mers and outputs abundance vectors of these *k*-mers. (C) Simka uses the abundance vectors to update independent contributions to the distance. In final step, it accumulates contributions to compute the final distance matrix.

CLARK [45] classifies the datasets using discriminative *k*-mers. It builds a large index hash table containing the *k*-mer spectra of all target datasets and removes the *k*-mers occurring in more than one dataset. The remaining *k*-mers are discriminative (target-specific) *k*-mers which could be regarded as representatives of corresponding datasets because each of these *k*-mers exist in the dataset uniquely. As a result, the target-specific *k*-mer sets of all the target datasets are obtained (Figure 2.3 (A) Index Construction Phase). To classify an object dataset, for each *k*-mer in the dataset CLARK queries the index hash table to check if this *k*-mer matches a target-specific *k*-mer of a dataset. If so, it is called a "hit." After querying all the *k*-mers, the object is assigned to the target dataset having the highest number of hits (Figure 2.3 (B) Classification Phase). CLARK offers two modes of execution. The full mode outputs the top two target assignments, confidence score

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of the first assignment and the number of hits against all the targets. The default mode stops querying for an object as soon as there is one target collecting half of possible hits and only outputs assignments. There is a variant, CLARK-*E*, significantly accelerating the computation of classification while maintaining high precision and sensitivity. It only queries non-overlapping *k*-mers and assigns the object to the first target that obtains a hit.

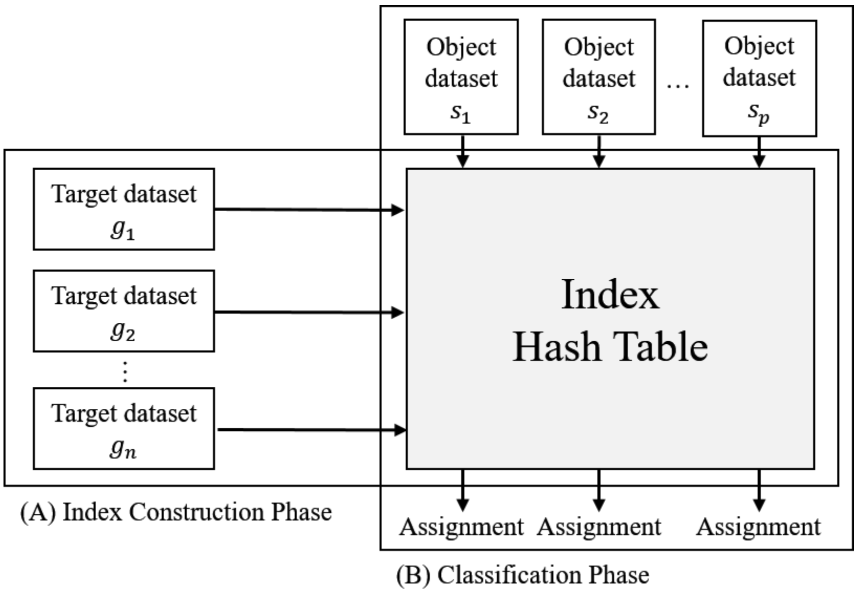

Figure 2.3: Workflow of CLARK

Besides high accuracy and speed, the major advantage of CLARK is that it provides an intuitive approach to find the signature *k*-mer sets. However, it uses large memory space while constructing the index hash table. For example, the RAM peak usage of the database construction of 2,752 bacterial genomes is 164.1 GB, which is not available in some workstations and most personal computers. There is another variant, CLARK-*l*, designed for machines with limited amounts of RAM. CLARK-*l* constructs a smaller hash table and smaller discriminative *k*-mer sets. It uses smaller *k* and samples a fraction of *k*-mers of each target datasets to build the index. In the experiment mentioned above, the RAM peak usage of CLARK-*l* is only 3.8 GB. Nevertheless, the sensitivity and precision of CLARK-*l* is much lower in some cases. A method called CLARK-*S* [44] was proposed later to improve the sensitivity of CLARK on the basis of the idea of spaced seed [33]. Compared to CLARK, its memory usage for classification is even larger and its running time is much longer.

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Chapter 3

Methods

CLARK provides an intuitive and efficient approach to find out the discriminative *k*-mer sets of target datasets and classify object datasets. However, there are some redundant computations in the algorithm of CLARK which may lead to large memory usage. We have devised an algorithm which removes the redundancy of CLARK.

In the algorithm of CLARK, the part using the most memory space is the index hash table. There are 4^k possible *k*-mers in the *k*-mer spectrum of a dataset, so the *k*-mer spectrum is a vector of dimension 4^k. To avoid too many collisions, CLARK builds large hash table and uses separate chaining. It simply inserts the IDs for all the targets containing a certain *k*-mer to the list of this *k*-mer. When the *k*-mer is common in many datasets, the list would be long and take up lots of space. In fact, the *k*-mer existing in more than one dataset would be removed afterwards. As a result, it is a waste of computation resource to store the same *k*-mer of all the datasets containing this *k*-mer in the hash table. In addition, there are some values stored in the hash table not necessary in the algorithm, so we also modify the data structure of the *k*-mer storing in the hash table.

3.1 Algorithm

The algorithms in this thesis focus on the construction of the index hash table and the classification of object datasets. The computation of *k*-mer frequencies can be finished fast and memory-efficiently using the *k*-mer counting tools mentioned in related work.

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The inputs of the whole algorithm are (1) the *k*-mer spectra of target datasets, (2) the *k*-mer spectra of object datasets, and optionally (3) a minimum number of occurrences if user wants to remove *k*-mers occurring less than a certain amount of times.

In this algorithm, the data structure of the *k*-mers stored in the index hash table contains (1) the *k*-mer, (2) the ID of the target dataset, and (3) the count of the *k*-mer in the dataset. In fact, the count could be replaced with a Boolean variable because the frequency would not be used afterwards. We describe the algorithm with the count for convenience. Assuming that the input *k*-mer spectra are counted in canonical form (Figure 3.1 arrow A) and stored in binary form (Figure 3.1 arrow B), we use a hash table the same as that designed in CLARK, a hash table of size L with separate chaining. The hash function h is defined as $h(l) = l \mod L$, where l is the value of the *k*-mer. With this hash function, we store the value l/L in the bucket $h(l)$ of the hash table (Figure 3.1 arrow C). It is trivial to get the original *k*-mer from the value stored in the hash table. The value of L is set to a power of 2 such that the modulo operation and division operation could be done easily with dividing the binary form of a *k*-mer into two parts and taking the second part as hash value and the first part as the value to store in the element .

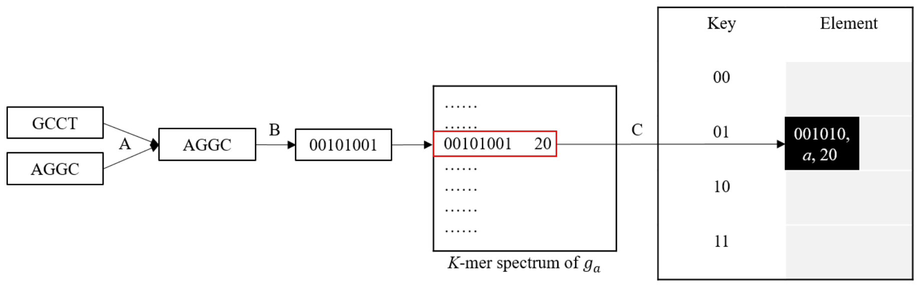

Figure 3.1: From a *k*-mer to an element in the hash table

In Algorithm 1, we describe the method of building the index hash table. The algorithm attempts to store all the *k*-mers into the hash table. If user has specified a minimum number of occurrences, the *k*-mers with frequencies lower than this number should not be stored in the index. This examination is useful especially for the datasets with low sequencing quality because most of the *k*-mers with low frequencies arise from sequencing errors. When storing *k*-mers in each dataset into the hash table, the algorithm would attempt to store identical *k*-mers from different datasets into the same bucket of the hash table based

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on the hash function. Rather than the exact frequency of each *k*-mer, what is crucial in this algorithm is whether a *k*-mer occurs in only one dataset. Based on this observation, when the algorithm tries to store a *k*-mer into a bucket with the same *k*-mer from a different dataset already stored in it, this new *k*-mer should be ignored. On the other hand, the *k*-mer which has been stored in the bucket cannot be used as a target-specific *k*-mer either, but it would not be removed because it is kept as a token to record that this *k*-mer exists in at least two datasets. The algorithm sets the count of the *k*-mer to 0, representing that this *k*-mer is merely kept as a token, so it would be removed afterwards and not included in the target-specific set. For convenience of understanding the algorithm, we check whether the count of the stored *k*-mer is equal to 0 in Algorithm 1. In practice, we could set the count to 0 without checking to save the time of checking the condition of the if statement. After processing all the *k*-mers from all the datasets, there is only one element stored in the index hash table for each distinct *k*-mer. For each element, the value of count is either 0, representing this *k*-mer should be removed, or a positive integer, representing the frequency of this *k*-mer in corresponding dataset. After removing the *k*-mers with counts equal to 0, the algorithm saves the index hash table in disk.

Algorithm 1 BuildIndex

```
Input: $k$-mer spectra $T(g_c)$ of $n$ target datasets $(g_c)_{1 \le c \le n}$
 1: create an empty hash table $H$
 2: for $c = 1$ to $n$ do
 3:     for each $k$-mer $km$ with frequency $cnt_c$ in $T(g_c)$ do
 4:         if $cnt_c \geq min_{occur}$ then
 5:             if there is $(km, i, cnt_i) \in H$ then
 6:                 if $cnt_i = 0$ then
 7:                     do nothing
 8:                 else
 9:                     $cnt_i = 0$
10:             else
11:                 insert $(km, c, cnt_c)$ in $H$
12: for each $(km, i, cnt_i) \in H$ do
13:     if $cnt_i = 0$ then
14:         remove this element
15: store the index hash table in disk
```

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After constructing the index hash table, we can use it to classify the object datasets. In Algorithm 2, we describe the method to classify object datasets. It is conceptually the same as the algorithm of the classification part of CLARK's algorithm. We modify it such that the algorithm fits the structure that we propose later and keep the computation of the statistics which are computed in CLARK. Using the same hash function, the algorithm checks whether each *k*-mer in the *k*-mer spectra of object datasets exists in the target-specific *k*-mer set of a target dataset easily. If so, it is a hit and the algorithm counts the hits by adding the frequency of this *k*-mer in the object dataset to the counter of corresponding target dataset. After counting all the hits, we calculate the statistic γ. $\mid T(s_l) \mid$ is the total number of *k*-mers in object dataset *l*. γ indicates the proportion of *k*-mers which hit the target-specific *k*-mer sets of target datasets. If $\gamma = 0$, it means that none of the *k*-mers in the object dataset hits target datasets and the algorithm cannot classify this object. Otherwise, the algorithm finds out the targets with the highest and second-highest numbers of hits and computes the confidence score of the assignment to the highest target accordingly.

Algorithm 2 Classify

Input: index hash table H; n: the number of target datasets; k-mer spectra $T(s_l)$ of p object datasets $(s_l)_{1 \leq l \leq p}$

```
1:  for $l$ = 1 to $p$ do
2:      declare n integer $b_1, b_2, ..., b_n = 0$
3:      for each $k$-mer $km$ with frequency $cnt_l$ in $T(s_l)$ do
4:          if there is $(km, i, cnt_i) \in H$ then
5:              $b_i = b_i + cnt_l$
6:      $\gamma = \sum_{t=1}^{n} \frac{b_t}{|T(s_l)|}$
7:      if $\gamma = 0$ then
8:          output $l$, "not assigned"
9:      else
10:         $m_1 = \arg\max\{b_1, b_2, ..., b_n\}$
11:         $m_2 = \arg\max\{\{b_1, b_2, ..., b_n\} - \{b_{m_1}\}\}$
12:         $confidence = \frac{b_{m_1}}{b_{m_1} + b_{m_2}}$
13:         output $l, b_1, b_2, ..., b_n, \gamma, m_1, m_2, confidence$
```

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3.2 Analysis of the Algorithms

In this section, we analyze and compare our algorithms with the algorithm of CLARK. To analyze the space complexity, we analyze the peak memory usage.

In index construction phase, the peak usages of both algorithms occur when the algorithms finish processing all the *k*-mers of target datasets. CLARK simply inserts all the *k*-mers of target datasets into the index hash table, so the number of elements inserted into the hash table is equal to the total number of the distinct *k*-mers in the datasets. Conceptually, there are 4^k total possible *k*-mers in a dataset. In fact, for each dataset of genome length *g*, there are $O(g)$ distinct *k*-mers because there are $s - k + 1$ *k*-mers in a sequence of length s. When the value of *k* is large, *g* is much smaller than 4^k. For example, when we set *k* to 31, 4^{31} is 4.6×10^{18}, while the length of the largest known genome is 1.5×10^{11} bp [48]. For all target datasets of total genome length G_T, there are $O(G_T)$ distinct *k*-mers. On the other hand, when *k* is small, there are $O(n \times 4^k)$ distinct *k*-mers. As a result, there are $O(\min(G_T, n \times 4^k))$ distinct *k*-mers in general. Considering the size of the hash table L, the space complexity of CLARK is $O(\max(L, \min(G_T, n \times 4^k)))$. In our algorithm, for each distinct *k*-mer, we store at most one element in the hash table. However, the worst case is that the *k*-mers of each target dataset are distinct in all the datasets, so we should store the same number of *k*-mers in the hash table as CLARK. As a result, the space complexities of our algorithm and CLARK are the same. But there are many repetitive *k*-mers in general, and our algorithm saves the space for storing these *k*-mers. Suppose that a *k*-mer occurs in *r* datasets on average, CLARK stores *r* times as many *k*-mers as as we do.

In classification phase, after removing the elements of identical *k*-mers from different datasets in CLARK, the peak usages of both algorithms are the same. The space complexities of both algorithms in this phase are equal to the space complexity of the completely built index hash table, which only contains target-specific *k*-mers. The worst case is the same as the case mentioned above, and all the *k*-mers are target-specific *k*-mers. Consequently, the space complexity of this phase is the same as the complexity of the index construction phase (Table 3.1).

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Index Construction	Classification	Total
$O(\max(L, \min(G_T, n \times 4^k)))$	$O(\max(L, \min(G_T, n \times 4^k)))$	$O(\max(L, \min(G_T, n \times 4^k)))$

Table 3.1: Space complexity of both algorithms

The time complexities of our algorithm and CLARK's algorithm are the same because the structure is basically the same. In index construction phase, the operations performing on each *k*-mer could be done in constant time. There are $O(\min(G_T, n \times 4^k))$ *k*-mers in total, so the time complexity of this phase is $O(\min(G_T, n \times 4^k))$. In classification phase, for each object dataset of genome size *g*, it takes $O(\min(g, 4^k))$ time to count the hits of the dataset. The declaration of integers, the search of the targets with highest numbers of hits and the computation of γ take $O(n)$ time. In general, $O(\min(g, 4^k))$ is much larger than *n*. So for *p* object datasets of total genome length G_O, it takes $O(\min(G_O, p \times 4^k))$ time (Table 3.2).

Index Construction	Classification	Total
$O(\min(G_T, n \times 4^k))$	$O(\min(G_O, p \times 4^k))$	$O(\min(G_T, n \times 4^k) + \min(G_O, p \times 4^k))$

Table 3.2: Time complexity of both algorithms

Although the time complexities of both algorithms are the same, our algorithm should be faster than CLARK's algorithm in practice. CLARK inserts all the *k*-mers in the hash table and then removes most of them, and we prevent this waste by only storing the first occurring *k*-mers.

Note that the peak memory usage of our algorithm is close to the peak memory usage in the classification phase of CLARK because the elements in the index hash table are the same. In the experiment of 2,752 bacterial genomes mentioned in the previous chapter, the RAM peak usage of classification phase of CLARK is 70.1 GB, which is still large for personal computers. In the next section, we provide a strategy to further reduce the memory usage.

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3.3 Partitioning Strategy

Inspired by the idea of MKC in [6], we propose a structure based on a partitioning strategy to further reduce the peak memory usage of our algorithm. In the algorithm, it stores all the *k*-mers of all the target datasets into a hash table, resulting in a hash table of large size. However, when the algorithm searches for a hit of a *k*-mer of an object dataset, at most one bucket containing corresponding *k*-mer is needed. That is, we only need to obtain the bucket containing this element in the hash table. Conceptually, when we want to query a *k*-mer, we can get the key value of the *k*-mer using the hash function and load the bucket of this key value from disk. With this approach, the classification phase could be completed within small memory space. Nonetheless, it would give rise to excessive disk accesses, causing a large increase of running time.

To make a trade-off between memory usage and performance, we partition all the *k*-mers into q parts according to their lexicographical order. The value of q is adjustable based on the specification of each machine. Some *k*-mer counting tools print the *k*-mers with their frequencies in lexicographical order in output files while some tools don't. If not, we sort the *k*-mers in the files as a preliminary task. It is easy to partition the sorted *k*-mers such that a partition contains a specific subset of *k*-mers common to all datasets. In index construction phase, we construct an index hash table independently for each partition. We take the same partition of each file to construct the same hash table of this partition (Figure 3.2). We use the same algorithm to construct the index hash tables of the partitions. In classification phase, we partition the *k*-mers with the same rule. To classify an object dataset, we count the hits of each partition of *k*-mers with the index of the partition (Figure 3.3).

This partitioning strategy can be applied to our algorithm with small modification (Algorithm 3). With this partitioning strategy, we only have to load a hash table of a partition in memory at a time during the construction and the classification of the partition. The space complexity of the original index hash table in our algorithm is $O(\max(L, \min(G_T, n \times 4^k)))$. With partitioning the *k*-mers into q parts, this can be reduced to $O(\frac{\max(L,\min(G_T,n\times 4^k))}{q})$. Users could adjust the value of q to fit the RAM size

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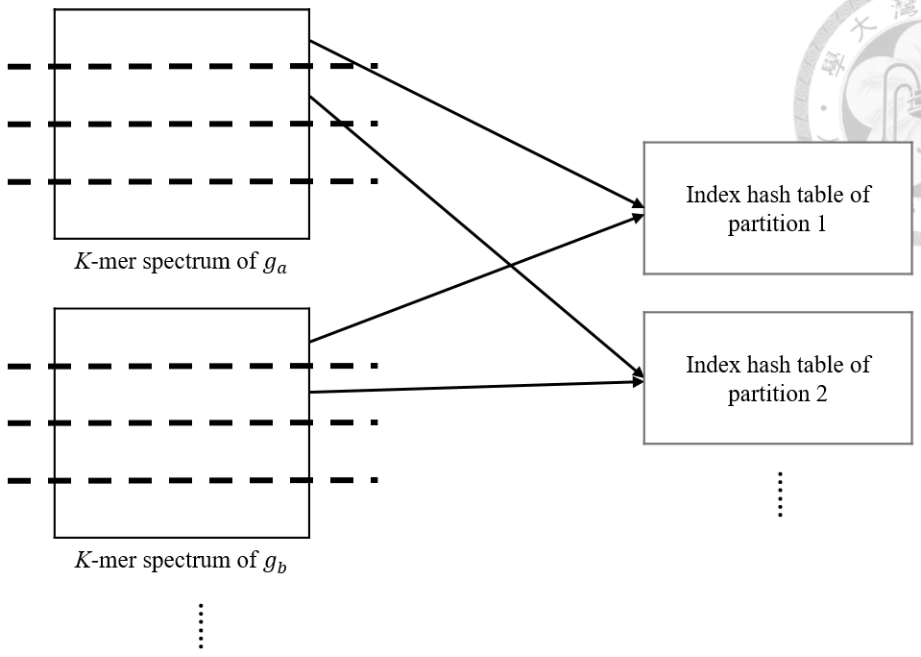
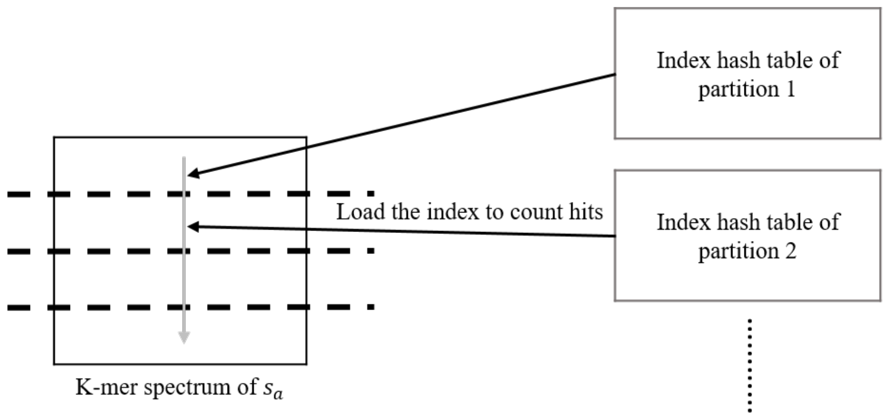


Index hash table of partition 1
Index hash table of partition 2
K-mer spectrum of g_a
K-mer spectrum of g_b

Figure 3.2: The construction of index hash tables with partitioning strategy



Index hash table of partition 1
Load the index to count hits
Index hash table of partition 2
K-mer spectrum of s_a

Figure 3.3: The classification of an object dataset with partitioning strategy

of the machine. The number of *k*-mers processed in total is the same as that in the original structure without partitioning, so the time complexity is still $O(\min(G_T, n \times 4^k) + \min(G_O, p \times 4^k))$. However, the execution time in practice would increase as the number of partitions increases because of the relatively time-consuming disk accesses of storing and loading the index hash tables.

Another advantage of this partitioning strategy is that the algorithm with this strategy can be highly parallelized. For example, in index construction phase, each thread takes a partition of the *k*-mers in all the target datasets to construct the index hash table of the partition simultaneously (Figure 3.4). In classification phase, each thread takes a

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Algorithm 3 AlgorithmWithPartitioning

```
Input: $q$; $k$-mer spectra $T(g_c)$ of $n$ target datasets $(g_c)_{1 \leq c \leq n}$; $k$-mer spectra $T(s_l)$ of $p$
    object datasets $(s_l)_{1 \leq l \leq p}$
 1: for each $T(x_y)$ do
 2:     partition $T(x_y)$ into $q$ parts $(T(x_y)_m)_{1 \leq m \leq q}$
 3: for $m$ = 1 to $q$ do
 4:     run BuildIndex($(T(g_c)_m)_{1 \leq c \leq n}$) to get index hash table $H_m$ and store it in disk
 5: for $l$ = 1 to $p$ do
 6:     declare n integer $b_1, b_2, ..., b_n = 0$
 7:     for $m$ = 1 to $q$ do
 8:         load $H_m$ from disk
 9:         for each $k$-mer $km$ with frequency $cnt_l$ in $T(s_l)_m$ do
10:             if there is $(km, i, cnt_i) \in H_m$ then
11:                 $b_i = b_i + cnt_l$
12:     $\gamma = \sum_{t=1}^{n} \frac{b_t}{|T(s_l)|}$
13:     if $\gamma = 0$ then
14:         output $l$, "not assigned"
15:     else
16:         $m_1 = \arg\max\{b_1, b_2, ..., b_n\}$
17:         $m_2 = \arg\max\{\{b_1, b_2, ..., b_n\} - \{b_{m_1}\}\}$
18:         $confidence = \frac{b_{m_1}}{b_{m_1} + b_{m_2}}$
19:         output $l, b_1, b_2, ..., b_n, \gamma, m_1, m_2, confidence$
```

partition of the *k*-mers in the object dataset and loads the index of the partition to count the hits. After all the threads finish counting, the counts are accumulated to get the final results (Figure 3.5). Consequently, for machines with large RAM size, we can improve the performance of index construction and classification instead of reducing the memory usage.

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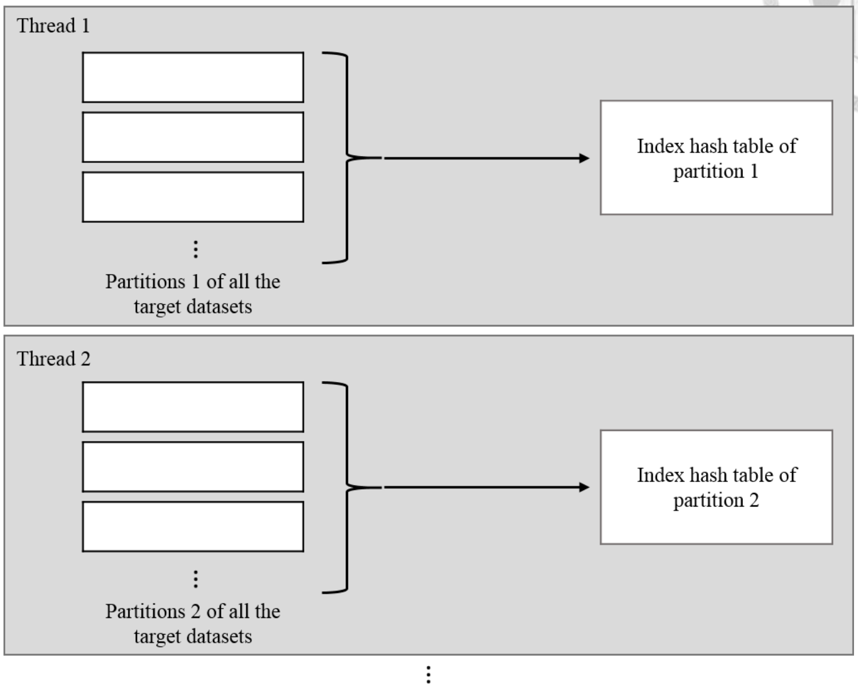
Thread 1
Partitions 1 of all the target datasets
Index hash table of partition 1
Thread 2
Partitions 2 of all the target datasets
Index hash table of partition 2

Figure 3.4: Index construction phase with multithreading

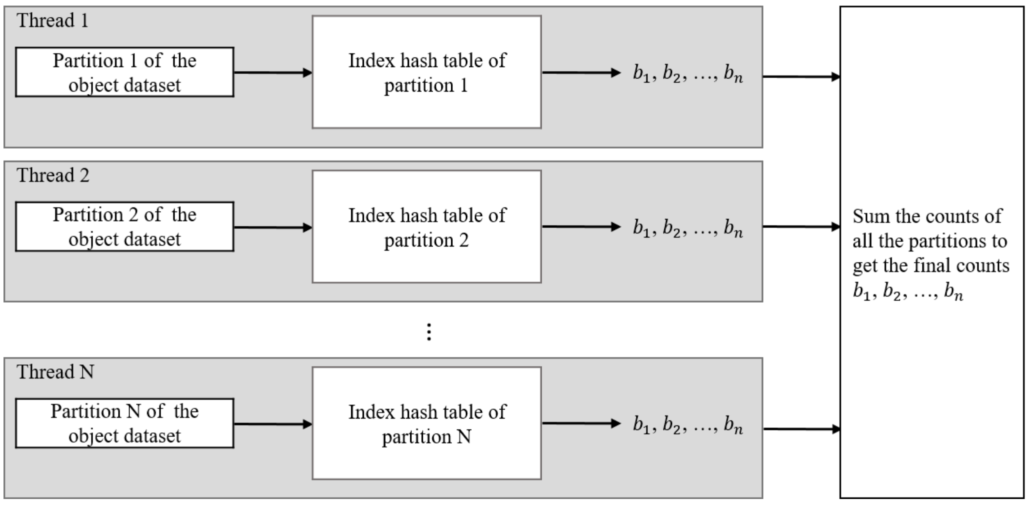
Thread 1
Partition 1 of the object dataset
Index hash table of partition 1
$b_1, b_2, \ldots, b_n$
Thread 2
Partition 2 of the object dataset
Index hash table of partition 2
$b_1, b_2, \ldots, b_n$
Thread N
Partition N of the object dataset
Index hash table of partition N
$b_1, b_2, \ldots, b_n$
Sum the counts of all the partitions to get the final counts $b_1, b_2, \ldots, b_n$

Figure 3.5: Classification phase with multithreading

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Chapter 4

Conclusion

In this thesis, we propose an algorithm with the space complexity $O(\max(L, \min(G_T, n \times 4^k)))$, where L is the size of the hash table, G_T is the total genome length of target datasets, *n* is the number of target datasets and *k* is the length of the *k*-mers. This is the same as the space complexity of CLARK, but we save large space by avoiding the redundancy of storing *k*-mers in CLARK. However, the RAM peak usage is still too large for common personal computers. To solve this problem, we propose a partitioning strategy which can be applied to our algorithm. The space complexity would be $O(\frac{\max(L, \min(G_T, n \times 4^k))}{q})$ if we partition the *k*-mers into *q* parts. The algorithm under this partitioning structure can be highly parallelized. For machines with sufficient RAM, we can improve the performance rather than reducing memory usage.

In theoretical analysis, our algorithm is not only more memory-efficient but also faster than CLARK. Nevertheless, we do not have the experimental data of practical memory usage and performance of the algorithms. The implementation of these algorithms is a direction of future work. In implementation, parallelization is another important issue. The partitioning rule based on lexicographical order is intuitive and efficient, but it may lead to some partitions with lots of *k*-mers and some partitions with few *k*-mers. This imbalance of partition sizes could reduce the performance of the parallelization scheme. Therefore, the partitioning policy is a crucial factor influencing the effectiveness of parallelization.

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