

# Blini: lightweight nucleotide sequence search and derePLICATION

Amit Lavon<sup>1</sup>

<sup>1</sup>University of California, Irvine, CA, USA

24 June 2025

## Abstract

Blini is a tool for quick lookup of nucleotide sequences in databases, and for quick derePLICATION of sequence collections. It is meant to help clean and characterize large collections of assembled contigs or long sequences that would otherwise be too big to search with online tools, or too demanding for a local machine to process. Benchmarks on simulated data demonstrate that it is faster than existing tools and requires less RAM, while preserving search and clustering accuracy.

## Introduction

Metagenomes are collections of genetic material from various organisms, which are often not initially known. Characterizing the taxonomic makeup of a sample involves searching its contents in large databases in order to find which organism matches each nucleotide sequence. Assembled sequences can reach lengths of millions of bases, making alignment-based search methods too cumbersome. Such big queries are often outsourced to powerful cloud-based services such as BLAST (Altschul et al. 1990). In recent years, k-mer-based algorithms were introduced that enabled efficient searching in large datasets on local machines. Mash distance (Ondov et al. 2016) introduced an alignment-free estimation formula for average nucleotide identity between sequences, making sequence comparison linear. Sourmash (Brown and Irber 2016) uses fractional min-hashing in order to create small representations of large sequences, which allow for efficient searching and comparison. The LinClust clustering algorithm (Steinegger and Söding 2018) uses k-mer matching to reduce the number of pairwise comparisons and achieve linear scaling with the size of the input.

While each of these techniques provides value on its own, processing datasets of tens or hundreds of thousands of genomes can still take many hours on a local machine. Here, insights from previous techniques are combined to create a unified tool that can search and replicate big datasets quickly using estimated identity or containment.

## Results

### Algorithm

#### Fingerprinting with k-mers

Blini uses constant-length subsequences (k-mers) to create fingerprints for sequences. It uses the fractional min-hashing technique, similarly to Sourmash (Brown and Irber 2016). A sliding window of length  $k$  goes over the sequence and hashes each canonical  $k$ -long subsequence. This collection of hashes is often called the sequence's *sketch*. The lower  $1/s$  hashes are retained, for an input scale parameter  $s$ . A high  $s$  means fewer hashes used in downstream calculations, trading accuracy for better CPU and RAM performance.

Once k-mer hashes are extracted, the sequence is discarded and only its sketch is used for downstream operations. These sketches can be saved to files and reused.

### **Similarity estimation**

Blini uses Mash distance (Ondov et al. 2016) to estimate average nucleotide identity (ANI) between sequences. This formula translates the Jaccard similarity between two k-mer sets to an estimation of the ANI between the original sequences. For containment matching, the hashes of the query sequence are compared against their intersection with the hashes of the reference sequence.

### **Search**

The first step of searching is indexing the reference dataset. After the reference sequences are fingerprinted, the 25% lowest hashes are used for indexing. The index is a mapping from hash value to a list of sequence identifiers of the reference sequences that had that hash in their fingerprints. The number 25% was chosen as a sweet spot between saving memory and retaining enough information for the search. As an optimization, hashes with a single reference sequence are kept in a separate ‘singletons’ map. Since in practice most of the index elements are singletons, this helps reduce RAM consumption and garbage collection times.

In the second stage, each query sequence is read and fingerprinted. The hash values are looked up in the index, and candidate reference sequences are fetched. Then, the query sequence is compared against each candidate sequence using Mash distance, and matches that pass the similarity threshold are reported.

### **Clustering**

The clustering (dereplication) procedure follows a similar scheme to LinClust (Steinegger and Söding 2018). Sequences are indexed and ordered from the longest to the shortest. Then, going by that order, each sequence is searched for using the search procedure. Matches that pass the similarity threshold are joined with the query sequence and are considered a cluster. These matches are then removed from the search loop’s candidates. This clustering procedure does not produce inter-cluster distances for hierarchy generation.

## **Performance evaluation**

### **Search - small**

The search function was tested on RefSeq’s viral reference (Pruitt, Tatusova, and Maglott 2007). Blini was compared against Sourdash and MMseqs. 100 viral genomes were randomly selected for the test. The algorithms were then run on the 100 genomes as queries, and the original database as reference. Each algorithm was expected to match each genome to its source in the database. In a second run, random SNPs were introduced to 1% of the genomes’ bases, and the same test was rerun. For each test, the number of matches with sequences other than the query’s source was also measured. The searches were run against an index of the reference dataset, created by each tool.

All three tools were able to match all 100 queries with their sources in the database (Figure 1a). The number of non-source matches was 824 and 712 in Blini, 865 and 660 in Sourdash, and 3143 and 3019 in MMseqs, in the raw and mutated datasets, respectively (Figure 1b).

Run-time was measured for searching the 100 sequences sequentially. Blini and MMseqs were executed once and searched for all the queries in one run, while Sourdash had to be executed once for each individual query. Each run was repeated five times and the average run-time is reported. Blini completed the run in 0.5 seconds, Sourdash completed the run in 126 seconds, and MMseqs completed the run in 151 seconds (Figure 1c). The times shown here do not include reference-preprocessing time.

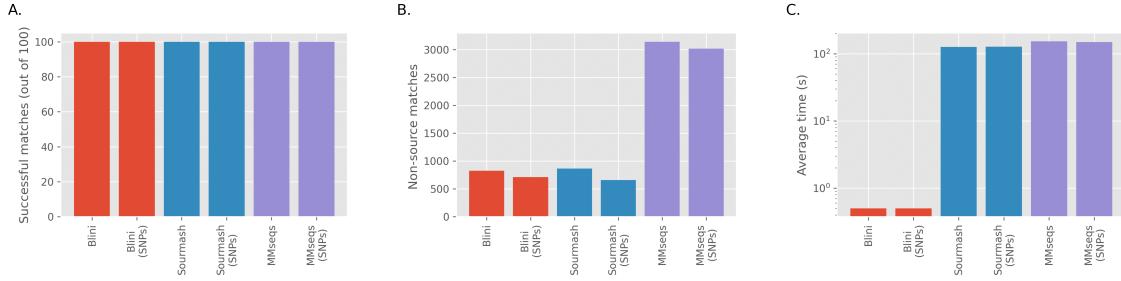


Figure 1: Search results for the viral dataset. Each tool was run on 100 randomly chosen viral genomes, to find them in the original dataset. (A) shows how many of the 100 genomes were correctly mapped to their source in the database. (B) shows how many additional matches were found in addition to the 100 chosen genomes. (C) shows the search times for the 100 queries together.

### Search - big

To test the search function on a large dataset, the bacterial contigs from (Pasolli et al. 2019) were used. This 10GB dataset contains 934K contigs from almost 5K bacterial species. Each of the compared tools was run to create an index of the dataset.

The simulated query dataset consisted of 100K random fragments of length 10K bases, sampled uniformly from the bacterial contigs. Each fragment was mutated with random SNPs in 0.1% of its bases. Blini, Sourmash and MMseqs were run on the query dataset, to search it in the bacterial reference. Because of the long search times, only Blini was run on the full set of queries, while the other tools were run on one or ten queries out of the 100K.

MMseqs took longer than 30 minutes to search for a single query, and was therefore terminated prematurely. Sourmash was run on one query and on ten queries and took 31 seconds per query. Blini took 6 seconds for one and ten queries, and 25 seconds for the entire set of 100K queries (Figure 2). This means a throughput of 5100 queries per second after the 6 seconds of loading the reference index. Blini matched all 100K queries with their correct source in the reference, with 2444 additional non-source matches (false-positives).

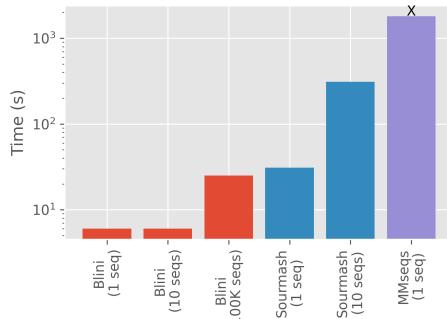


Figure 2: Search times for the bacterial dataset. Each tool was run on randomly chosen 10 kilo-base fragments from a 10GB bacterial dataset. MMseqs is marked with an X because it was stopped manually before it could finish running.

## Clustering

The clustering function was tested on two simulated datasets created from the 100 chosen genomes of the previous test. In one dataset each sequence had multiple counterparts with random SNPs. In the second dataset random fragments were extracted from each root sequence. In the SNPs dataset, each of the 100 original sequences had another 100 mutated counterparts. Each counterpart had random SNPs in 1% of its bases. In the fragments dataset, each of the 100 original sequences had 300 random fragments extracted from it, of length of at least 1000 bases. The algorithms were expected to group each sequence with its mutated counterparts or with its fragments. Performance was evaluated using the Adjusted Rand-Index (ARI). Blini's *scale* refers to the fraction of k-mers considered for the operation. Scale 50 means that 1/50 of k-mers were used.

In the SNPs dataset, both Blini and MMseqs achieved an ARI between 0.999 and 1.0, except for Blini with scale 200 which achieved an ARI of 0.997 (Figure 3b). Blini created 100, 100, 101 and 110 clusters using scales 25, 50, 100 and 200 respectively. MMseqs created 103 clusters (Figure 3a). Blini took on average 10.5 seconds, and MMseqs took 46 seconds with one thread, and 14 seconds with four threads (Figure 3b). In terms of memory, Blini had a maximal memory footprint of 255, 129, 65, and 38 MB using scales 25, 50, 100 and 200 respectively. MMseqs had a maximal memory footprint of 3072 MB (Figure 3c).

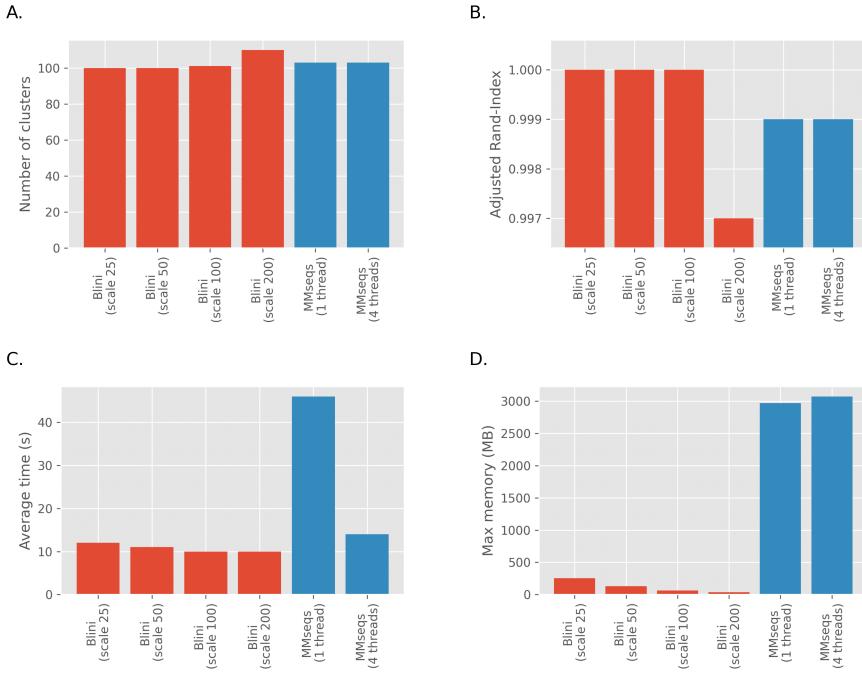


Figure 3: Clustering results for the SNPs dataset. Each of the 100 viral genomes from the search benchmark was used to create 100 mutant sequences with SNPs in 1% of their bases. The tools were run on this collection of 10100 genomes and were expected to cluster them into 100 groups, corresponding to the original genomes.

In the fragments dataset, MMseqs achieved an ARI of 1.0 while Blini achieved an ARI of 0.999, 0.999, 0.998 and 0.989 with scales 25, 50, 100 and 200 (Figure 4b). Blini grouped the dataset into 100, 104, 135 and 386 clusters, while MMseqs grouped the dataset into 101 clusters (Figure 4a). Blini took on average 20 seconds, and MMseqs took 80 seconds with one thread, and 24 seconds with four threads (Figure 4c). In terms of memory, Blini had a maximal memory footprint of 462, 233, 119, and 67 MB using scales 25, 50, 100 and 200 respectively. MMseqs had a maximal memory footprint of 5632 MB (Figure 4d).

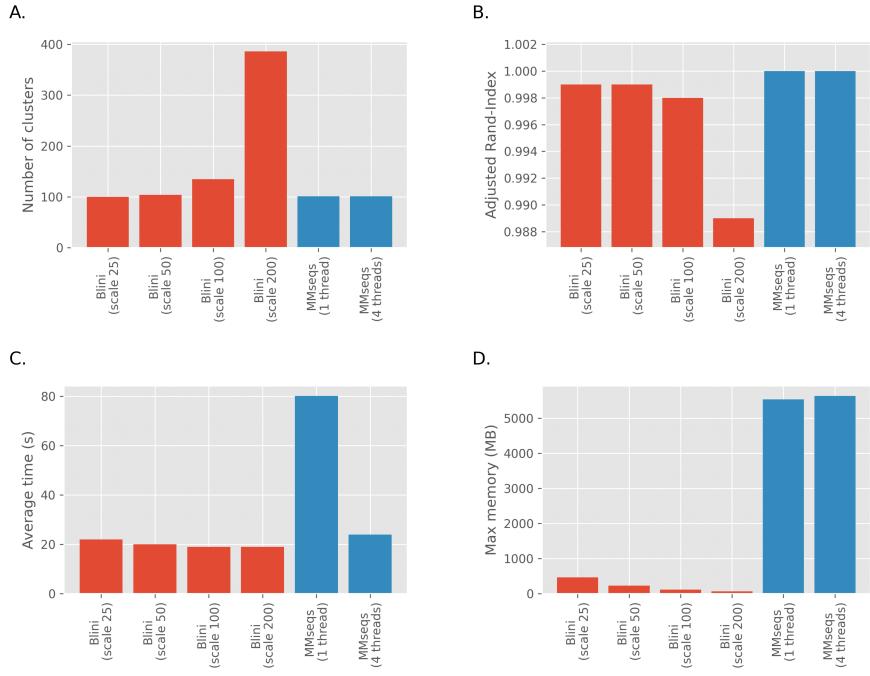


Figure 4: Clustering results for the fragments dataset. Each of the 100 viral genomes from the search benchmark was used to create 300 random fragments of length 1000 bases and above. The tools were run on this collection of 30100 genomes and were expected to cluster them into 100 groups, corresponding to the original genomes.

## Discussion

This work introduces a new technique for nucleotide sequence search and dereplication. The algorithm incorporates insights from several developments in the field, specifically around the use of k-mers for sequence search and average nucleotide identity (ANI) approximation, combined with a minimalist implementation, resulting in a scalable and easy to use utility.

The performance benchmarks shown here demonstrate an over 5000 queries-per-second throughput on a 10GB reference dataset, while existing implementations took 30 seconds or over 30 minutes for a single query. For clustering, the speedup was 4x in comparison to existing implementations, as well as a reduced memory footprint, while clustering accuracy was maintained.

One drawback is that Blini does not perform alignment, and instead uses an approximation for ANI. This can be observed in Figure 3a-b, where clustering accuracy decreased as the *scale* parameter increased beyond the recommended value for that dataset (see below). This approach trades some accuracy for scalability.

The *scale* parameter controls the ratio of k-mers used in the procedure, and it can be tweaked. While higher scale values reduce CPU and RAM consumption, they also affect the minimal length of sequences the algorithm would be effective for. Blini is designed to work on sequences ~20 times longer than the selected *scale* value. For the default scale of 100, sequences shorter than 2000 are likely to be falsely missed. This can be seen in Figure 3a-b, where a decrease in clustering accuracy was observed for scale >50 with fragments of size 1000. While the scale can be tweaked, this tool might not be suitable for short reads.

In summary, this is a new algorithm for search and dereplication of large-scale sequence collections. Blini significantly reduces the barrier to search and clustering tasks that would otherwise require huge computing power to perform, while maintaining result accuracy. For resource-constrained settings, Blini enables search and clustering that would otherwise be impossible due to time and memory constraints. For large systems such as cloud-based search servers, Blini can reduce query costs significantly and open the door for increased bandwidth. The tool is optimized for ease of use and does not require installing additional software. Pre-sketched databases can be easily created, reused and shared between research groups.

## Software Availability

Code and runnable binaries are available for Linux, Windows and MacOS at: <https://github.com/fluhus/blini/releases>  
Feedback on the tool or on this manuscript is welcome at: <https://github.com/fluhus/blini/discussions>

## References

- Altschul, Stephen F, Warren Gish, Webb Miller, Eugene W Myers, and David J Lipman. 1990. "Basic Local Alignment Search Tool." *Journal of Molecular Biology* 215 (3): 403–10.
- Brown, C Titus, and Luiz Irber. 2016. "Sourmash: A Library for MinHash Sketching of DNA." *Journal of Open Source Software* 1 (5): 27.
- Ondov, Brian D, Todd J Treangen, Páll Melsted, Adam B Mallonee, Nicholas H Bergman, Sergey Koren, and Adam M Phillippy. 2016. "Mash: Fast Genome and Metagenome Distance Estimation Using MinHash." *Genome Biology* 17: 1–14.
- Pasolli, Edoardo, Francesco Asnicar, Serena Manara, Moreno Zolfo, Nicolai Karcher, Federica Armanini, Francesco Beghini, et al. 2019. "Extensive Unexplored Human Microbiome Diversity Revealed by over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle." *Cell* 176 (3): 649–62.
- Pruitt, Kim D, Tatiana Tatusova, and Donna R Maglott. 2007. "NCBI Reference Sequences (RefSeq): A Curated Non-Redundant Sequence Database of Genomes, Transcripts and Proteins." *Nucleic Acids Research* 35 (suppl\_1): D61–65.

Steinegger, Martin, and Johannes Söding. 2018. "Clustering Huge Protein Sequence Sets in Linear Time." *Nature Communications* 9 (1): 2542.