

MAGNET: Understanding and Improving the Accuracy of Genome Pre-Alignment Filtering

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Abstract: *In the era of high throughput DNA sequencing (HTS) technologies, calculating the edit distance (i.e., the minimum number of substitutions, insertions, and deletions between a pair of sequences) for billions of genomic sequences is the computational bottleneck in today's read mappers. The shifted Hamming distance (SHD) algorithm proposes a fast filtering strategy that can rapidly filter out invalid mappings that have more edits than allowed. However, SHD shows high inaccuracy in its filtering by admitting invalid mappings to be marked as correct ones. This wastes the execution time and imposes a large computational burden. In this work, we comprehensively investigate four sources that lead to the filtering inaccuracy. We propose MAGNET, a new filtering strategy that maintains high accuracy across different edit distance thresholds and data sets. It significantly improves the accuracy of pre-alignment filtering by one to two orders of magnitude. The MATLAB implementations of MAGNET and SHD are open source and available at: <https://github.com/BilkentCompGen/MAGNET>.*

Index Terms: *High throughput DNA sequencing, read mapping, read alignment, false positives.*

1. INTRODUCTION

Until today, it remains challenging to sequence the entire DNA molecule as a whole. As a workaround, High throughput DNA sequencing (HTS) technologies are used to sequence random fragments (called *short reads*, which are 75-300 base-pairs long) of copies of the original molecule. The biggest challenge with these technologies is the use of these short reads to construct the *complete* genome sequence (~3.2 billion base-pairs for human genome), as these reads do not have any

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information about which part of genome they come from. During this process, called *read mapping*, each read is mapped to a reference genome based on the similarity between the read and “candidate” locations in that reference genome (like solving a jigsaw puzzle). The similarity measurement, called *alignment* or *verification*, is formulated as an approximate string matching problem and solved using quadratic-time dynamic programming algorithms such as Levenshtein's edit distance [1]. The main goal of these algorithms is to find out the minimum number of edits needed to make the read exactly match the reference segment [1]. Common edits include substitutions, insertions, and deletions. If the number of edits (called edit distance) is greater than a user-defined *edit distance threshold* (usually less than 5% of the read length [2-4]), then the mapping is considered to be invalid (i.e., the read does not match the segment at seed location) and thus is rejected. Calculating the edit distance for billions of sequences incurs significant computational burden [4-6]. Given that understanding complex diseases such as autism and cancer requires sequencing hundreds of thousands to millions of genomes [7, 8], the long execution time of today's read mappers can severely hinder such studies.

A wide variety of algorithms have been proposed to efficiently calculate the edit distance of sequences and filter out invalid mappings. Most existing algorithms can be divided into two main approaches: (1) accelerating the dynamic programming algorithms, (2) developing filtering heuristics that eliminate some of the invalid mappings (especially the ones that contain far more edits than allowed) before the verification step. Of the first approach, the classical dynamic programming algorithms such as Smith-Waterman [9], Levenshtein's edit distance [1], and Needleman-Wunsch [10] are the most accurate algorithms but they are computationally expensive as they require a quadratic running time. Subsequently, they were improved by computing only some necessary regions of the dynamic programming matrix rather than the entire matrix (e.g., Ukkonen [11]). They also can be accelerated by exploiting bit-parallelism in their implementations (e.g., Myers [12], SeqAn [13], SWPS3

[14], and hardware accelerated Smith-Waterman algorithm such as GPU-based [15] and FPGA-based [16]). The second approach to accelerate alignment verification is to incorporate a *filtering technique* within the read mapper and before the verification step. This filter is responsible for quickly excluding invalid mappings in an early stage (i.e., as a pre-alignment step) to reduce the number of locations that must be verified via dynamic programming. There are several existing filtering techniques such as Adjacency Filtering from FastHASH [6] and the Shifted Hamming Distance (SHD) [4].

We select SHD as the main focus of our analytical study, since it outperforms the Adjacency Filter in terms of speed and accuracy [4, 17]. It also maintains multiple independent bit-vectors (called shifted Hamming masks and explained in Section 2) that makes it suitable for parallel implementation. These filtering heuristics do *not* replace the verification step. Hence, they should be able to eliminate enough of the invalid mappings to be worthwhile (to compensate the computation overhead introduced by the filtering technique). One limitation with SHD is that it introduces inaccuracy in the filtering mechanism, allowing invalid mappings to pass the filter as false positives. A high number of false positives is undesirable, as these invalid mappings incur additional computational burden (they are unnecessarily examined *twice*, by *both* the pre-alignment and the alignment steps).

In this paper, our goal is to provide a detailed analysis of the false positive sources of the state-of-the-art alignment filter, SHD, aiming at eliminating them and boosting the performance of existing and future read mappers. To the best of our knowledge, this is the first paper to comprehensively assess the filtering inaccuracy of the SHD algorithm and provide recommendations for desirable improvements. The contributions of this paper are as follows:

- We provide a detailed investigation of four potential false positive sources of the state-of-the-art alignment filter, SHD.
- We show that processing the short matches (i.e., less than three matches) between two genomic sequences is not efficient, as they exhibit an unpredictable (random-like) and highly irregular behavior. Instead, future alignment filters should pay more attention to the *long, exact* matches shared by the sequences. Based on our observation, we build MAGNET, an intelligent filter that accurately detects all *long, exact* matches shared between two genome sequences.
- We quantify the false positives and true negatives of MAGNET and SHD using real data sets. We also experimentally demonstrate that incorporating long-match-awareness into the design of a pre-alignment filter can greatly improve the filtering accuracy.

2. OVERVIEW OF SHIFTED HAMMING DISTANCE

To provide a proper analysis of the false positive rate of SHD, in this section, we describe the SHD algorithm [4] and provide an example to illustrate how it works. SHD is a filter specifically developed to accelerate the alignment verification procedure in read mapping. SHD implements a filtering strategy that is inspired by the *pigeonhole principle*. That is, if E items are put into $E+1$ boxes, then one or more boxes would be empty. This principle can be applied in the context of sequence alignment, as follows: if two reads differ by E edits, then they should share at least a single identical subsequence (i.e., free of edits) among $E+1$ non-overlapping subsequences, where E is the edit distance threshold. This is due to the fact that the E edits would result in dividing the read into $E+1$ identical subsequences in accordance with their correspondences in the reference, as explained in Fig. 1. The more edits involved between two sequences, the less contiguous stretches of exact matches they share.

However, due to insertions and deletions, these identical subsequences might not be *perfectly* aligned and might be slightly shifted. Each insertion (or deletion) can shift multiple trailing bases to the right direction (or the left direction). SHD realigns the identical subsequences by incrementally shifting the read sequence against the reference sequence. SHD first calculates the base-pair-wise XOR between the two sequences. Then, it performs E incremental shifts to the right direction to detect any read that has at most E deletions, where E is the edit distance threshold. Similarly, SHD also performs another E incremental shifts to the left direction to detect any read that has at most E insertions. After each shift, SHD calculates the base-pair-wise XOR between the read and the reference and stores the result in a shifted Hamming mask. In total, SHD generates $2E+1$ shifted Hamming masks regardless the source of the edit.

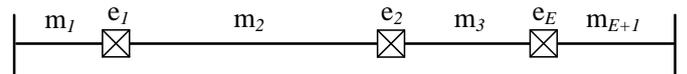


Fig. 1: Random edit distribution in a read sequence. The edits (e_1, e_2, \dots, e_E) act as dividers resulting in several identical subsequences (m_1, m_2, \dots, m_{E+1}) between the read and the reference.

Identical subsequences are then identified in each mask as a streak of continuous '0's. SHD ANDs all shifted Hamming masks together with the idea that all '0's in the individual Hamming masks propagate to the final bit-vector, thereby preserving the information of individual matching subsequences. SHD calculates the number of edits by counting the number of '1's in the

final bit-vector. As SHD uses a bitwise AND operation, a zero at any position in the $2E+1$ Hamming masks leads to a '0' in the resulting final bit-vector at the same position. Hence, even if some Hamming masks show a mismatch at that position, a zero in some other masks leads to a match ('0') at the same position. This tends to *underestimate* the actual number of edits and eventually causes some incorrect mappings to pass. To fix this issue, SHD proposes the so-called *speculative removal of short-matches* (SRS) before ANDing the masks, which flips short streaks of '0's in each mask into '1's such that they do *not* mask out '1's in other Hamming masks. The number of zeros to be amended (SRS threshold) is set by default to two. That is, bit streams such as 101, 1001

are replaced with 111 and 1111, respectively. Amending short streaks of '0's to '1's could cause correct mappings to be *mistakenly* filtered out, as it may produce multiple ones in the final bit-vector. To avoid this possibility, SHD always underestimates the number of edits from streaks of '1's. If there are four or three consecutive '1's in the final bit-vector, SHD counts them as a single edit. Thus, the total number of edits reported by SHD could be smaller than the real number of edits. In Fig. 2, we provide an example of a candidate alignment with all masks that are generated by the SHD algorithm. A segment of consecutive matches in one-step right-shifted mask indicates that there is a single deletion that occurred in the read sequence.



Fig. 2: An example of an alignment with all its generated masks, where the edit distance threshold (E) is set to 3. The green highlighted subsequences are part of the correct alignment. The red highlighted bit in the final bit-vector is a wrong alignment provided by SHD. The correct alignment (highlighted in yellow) shows that there are three substitutions and a single deletion, while SHD detects only two substitutions and a single deletion.

3. ON THE FALSE POSITIVES OF SHD

In this section, we investigate the potential sources of false positives that are introduced by the state-of-the-art filter, SHD [4]. We also provide examples that illustrate each of these sources of false positives. Adding an additional fast filtering heuristic before the verification step in a read mapper can be beneficial. But, such a filter can be easily worthless if it allows a high number of incorrect mappings to pass the filter. Even though the false positives that pass SHD are discarded later by the verification step (as the verification step has zero false positive rate), they can dramatically increase the running time of the read mapping by causing work to be done on a read by both the filtering step as well as the verification step. Below, we describe four major sources of false

positives that are introduced by the filtering strategy of SHD.

A. Leading and Trailing Zeros

The first source of false positives in SHD is the streaks of zeros that are located at any of the two ends of each bit-vector. Hence we refer to them as leading and trailing zeros. These streaks of zeros can be in two forms: (1) the vacant bits that are caused by shifting the read against the reference segment and (2) the streaks of zeros that are not vacant bits. As we mentioned earlier, SHD generates $2E+1$ masks using arithmetic left-shift and arithmetic right-shift operations. For both the left and right directions, the right-most and the left-most vacant bits, respectively, are filled with '0's. The number of vacant zeros depends on the number of shifted steps for

Rather than searching for all long non-overlapping subsequences, our algorithm recursively solves the subproblems until the number of the subsequences found in the first step exceeds $E+1$ or there are no more subproblems of size greater than or equal to a single bit. Each subproblem stores its solution (i.e., the longest identical subsequence) in the same final bit-vector that is shared by all subproblems.

Step 4: Once after the termination, MAGNET counts the occurrence of '1's in the final bit-vector. If their total number is equal or less than the edit distance threshold, E , then the mapping is considered to be valid. Likewise, if the total number of edits is sufficiently large (i.e. greater than a lower bound of edits), then the filter considers the mapping to be invalid and rejects it. In Fig. 8, we provide an example of how our filter works. Each '1' in the final bit-vector precisely reveals that there is an edit at its corresponding location of the Hamming mask.

With the help of our accuracy analysis of SHD (Section 3), we propose and incorporate long-match-awareness into the design of our filter. We get rid of the first three sources of false positives: (1) the leading and trailing zeros, (2) random zeros, and (3) conservative counting. In the next section, we investigate the impact of addressing these three sources on the false positive rate.

5. EVALUATION

In this section, we evaluate the false positive rate, true negative rate, and execution time of our proposed filter, MAGNET, against the best-performing previous filter, SHD [4]. As defined in previous work [17], the false positive rate is the fraction of incorrect mappings that are accepted by the filter out of all mappings, and the true negative rate is the fraction of incorrect mappings that are rejected by the filter out of all mappings. We always want to minimize the false positive rate and maximize the true negative rate. We implement both filters in MATLAB R2015b. We use a MATLAB implementation (nwalgn [18]) of the Needleman-Wunsch algorithm [10] to benchmark the two filters as this algorithm has a false positive rate of 0%. We use a popular seed-and-extend mapper, mrFAST [19], to retrieve all potential mappings (read-reference pairs) from five sets, each containing about 4 million reads of length 100 base-pairs, from the 1000 Genomes Project Phase I [8].

False Positive Rate. In Fig. 9, we show the false positive rates of SHD and our proposed filter, across different edit distance thresholds. We configure mrFAST to generate from each read set the first half million read-reference pairs that have no more than 5 edits. On average, SHD produces a false positive rate of 20%, which is significantly higher (on average 20x) than that of our MAGNET filter.

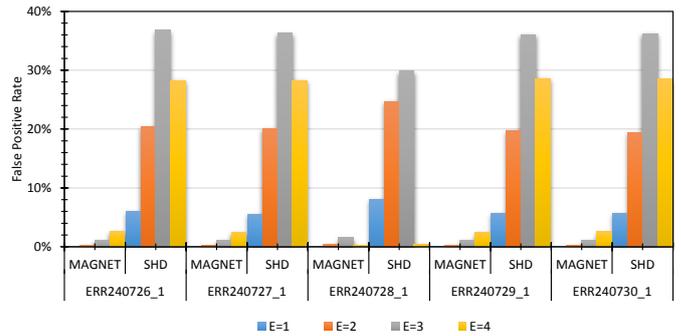


Fig. 9: The false positive rates of our MAGNET filter and SHD across different edit distance thresholds and read sets. The pairs are configured to have at most 5 edits.

In Fig. 10, we reconfigure mrFAST for an edit distance threshold of 7 (generated pairs have at most 7 edits). This enables us to measure the effectiveness of both filters when there are incorrect mappings that have a few more edits than the allowed threshold. We note that the number of false positives of SHD increases by at least 10%, while our filter still maintains a very low rate of false positives (<4%).

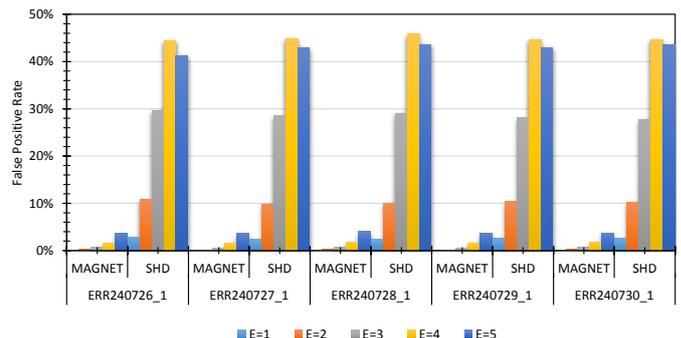


Fig. 10: The false positive rates of our MAGNET filter and SHD across different edit distance thresholds and read sets (configured to have at most 7 edits).

We now evaluate the false positive rate of MAGNET and SHD using the first 30 million pairs produced by mrFAST when the data set ERR240727_1 mapped to the human genome. We configure mrFAST to produce pairs that have at most 20 edits. Unlike the previous experiment, this configuration enables us to evaluate the false positive rate when the pairs have far more edits than the edit distance threshold. Fig. 11 demonstrates that SHD is more accurate in examining the edit-rich mappings than low-edit mappings. However, we find that MAGNET is very effective and superior to SHD in both situations (edit-rich and low-edit mappings). SHD falsely identifies potential mappings much more (15x - 100x,

depending on the data and edit distance threshold used) than our filter.

We conclude that building an intelligent filter that is aware of all long matches is worthwhile and doing so significantly improves the accuracy of alignment filtering by at least an order of magnitude compared to the best previous filtering mechanism.

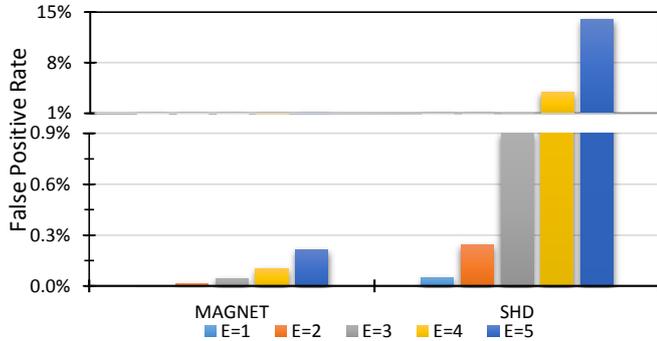


Fig. 11: The false positive rates of MAGNET and SHD across different edit distance thresholds and using edit-rich mappings (having at most 20 edits).

True Negative Rate. Next, we evaluate fraction of incorrect mappings that are rejected out of all rejected mappings, by both filters. We use in this experiment the first one million pairs that have at most 7 edits, produced by mrFAST when two data sets (ERR240726_1 and ERR240727_1) are mapped to the human genome. Fig. 12 shows that our filter rejects a significant fraction of incorrect mappings (e.g., up to 96%) and thus avoids expensive computations required by the verification step (dynamic programming). MAGNET rejects up to 20x more incorrect mappings than SHD. We conclude that our filtering strategy is more robust than SHD in handling invalid mappings. It boosts the overall performance by rejecting most of the incorrect mappings while at the same time providing a minimal false positive rate.

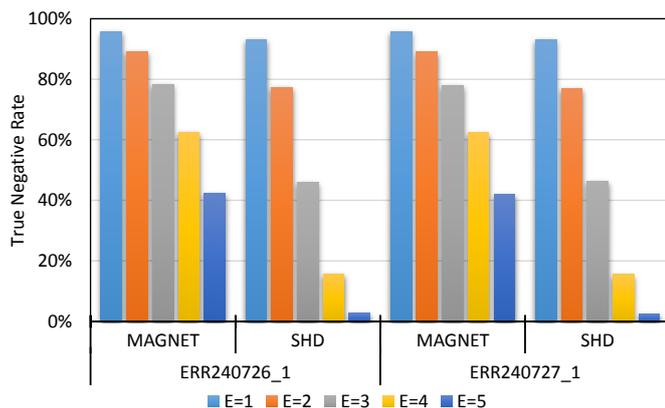


Fig. 12: The true negative rates of MAGNET and SHD with different edit distance thresholds using one million mappings with at most 7 edits.

Execution time. We now evaluate the execution time of our filter compared to the MATLAB implementation of the best existing filter, SHD, across different edit distance thresholds. We use the MATLAB Profiler [20] to track the execution time of both filters. We configure the Profiler to monitor the execution time based on the *performance clocking* option. Fig. 13 shows that as edit distance threshold increases, the execution time of both filters also increases. This is due to the fact that the number of Hamming masks is proportional to the edit distance threshold used and hence it requires more computations to be performed. We also find that MAGNET requires up to 1.4x more time than SHD to examine the first one million pairs that have at most 7 edits, produced by mrFAST when the data set ERR240726_1 is mapped to the human genome.

We conclude that our proposed filter, MAGNET, is extremely accurate, but this accuracy comes at the expense of a small increase in execution time. We believe this tradeoff is reasonable as examining the rejected mappings by a fast filter is much cheaper than having them verified by quadratic-time dynamic programming algorithms.

Note that the original SHD algorithm is implemented using Intel SSE instructions that provides significant performance improvements, especially for bit-parallel algorithms. We will explore in our future research how we can further improve the speed of our filtering strategy, to compete with the SIMD-implementation of SHD, using hardware accelerators (e.g., GPUs and FPGAs), multithreading, or SIMD instructions. In this work, we comprehensively evaluate the accuracy of the state-of-the-art alignment filter and mainly focus on addressing the sources of its filtering inaccuracy.

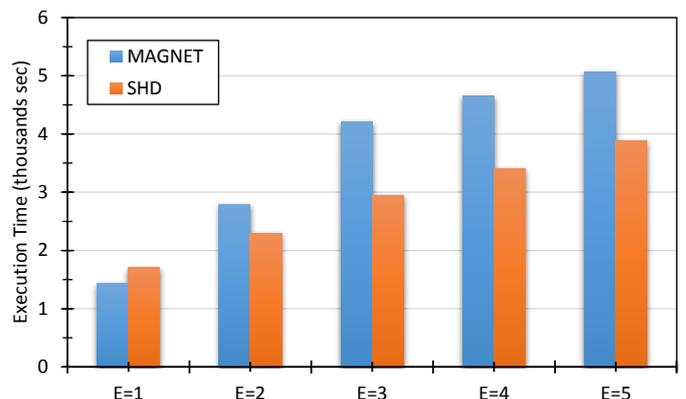


Fig. 13: Execution time performance of MAGNET and SHD under different edit distance thresholds.

6. CONCLUSION

In this paper, we comprehensively investigate four inaccuracy sources that make the state-of-the-art

alignment filtering algorithm, Shifted Hamming Distance (SHD), highly ineffective in examining potential mappings in read mapping for genome analysis. We propose MAGNET, a new filtering strategy that eliminates these sources and significantly improves the accuracy of pre-alignment with a minimal false positive rate. In our experiments, we show that MAGNET correctly detects invalid mappings much better than SHD (i.e., we see reductions in the false positive rates as high as 15x - 100x, depending on the data and edit distance threshold used). We also show that MAGNET is able to reject up to 20x more incorrect mappings than SHD at the expense of a slight increase in the execution time. We believe that MAGNET is the most accurate pre-alignment filter in literature today, As such, we hope that our filtering strategy inspires researchers to adopt it and improve its implementation aiming at building an even faster yet extremely accurate pre-alignment filter.

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