

RESEARCH ARTICLE

rasbhari: Optimizing Spaced Seeds for Database Searching, Read Mapping and Alignment-Free Sequence Comparison

Lars Hahn^{1*}, Chris-André Leimeister¹, Rachid Ounit², Stefano Lonardi², Burkhard Morgenstern^{1,3}

1 University of Göttingen, Department of Bioinformatics, Goldschmidtstr. 1, 37077 Göttingen, Germany, **2** University of California, Riverside, Department of Computer Science and Engineering, 900 University Ave. Riverside, CA 92521, USA, **3** University of Göttingen, Center for Computational Sciences, Goldschmidtstr. 7, 37077 Göttingen, Germany

* lhahn@biologie.uni-goettingen.de



OPEN ACCESS

Citation: Hahn L, Leimeister C-A, Ounit R, Lonardi S, Morgenstern B (2016) *rasbhari*: Optimizing Spaced Seeds for Database Searching, Read Mapping and Alignment-Free Sequence Comparison. PLoS Comput Biol 12(10): e1005107. doi:10.1371/journal.pcbi.1005107

Editor: Andreas Prlic, UCSD, UNITED STATES

Received: June 16, 2016

Accepted: August 11, 2016

Published: October 19, 2016

Copyright: © 2016 Hahn et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper or it is mentioned how to download it with a given http-link.

Funding: For BM, LH and CAL this work was funded by the budget of the Department of Bioinformatics, Institute of Microbiology and Genetics, University of Göttingen. Therefore there was no external funding. For SL this work was funded by the budget of the Department of Computer Science and Engineering, University of California, Riverside USA. For RO this work was funded by the graduate student support NSF IIS-1526742 and NSF IIS-1302134. The funders had

Abstract

Many algorithms for sequence analysis rely on word matching or word statistics. Often, these approaches can be improved if binary patterns representing *match* and *don't-care* positions are used as a filter, such that only those positions of words are considered that correspond to the *match positions* of the patterns. The performance of these approaches, however, depends on the underlying patterns. Herein, we show that the *overlap complexity* of a pattern set that was introduced by Ilie and Ilie is closely related to the *variance* of the number of matches between two evolutionarily related sequences with respect to this pattern set. We propose a modified hill-climbing algorithm to optimize pattern sets for database searching, read mapping and alignment-free sequence comparison of nucleic-acid sequences; our implementation of this algorithm is called *rasbhari*. Depending on the application at hand, *rasbhari* can either minimize the *overlap complexity* of pattern sets, maximize their *sensitivity* in database searching or minimize the *variance* of the number of pattern-based matches in alignment-free sequence comparison. We show that, for database searching, *rasbhari* generates pattern sets with slightly higher sensitivity than existing approaches. In our *Spaced Words* approach to alignment-free sequence comparison, pattern sets calculated with *rasbhari* led to more accurate estimates of phylogenetic distances than the randomly generated pattern sets that we previously used. Finally, we used *rasbhari* to generate patterns for short read classification with *CLARK-S*. Here too, the sensitivity of the results could be improved, compared to the default patterns of the program. We integrated *rasbhari* into *Spaced Words*; the source code of *rasbhari* is freely available at <http://rasbhari.gobics.de/>

no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Author Summary

We propose a fast algorithm to generate spaced seeds for database searching, read mapping and alignment-free sequence comparison. Spaced seeds—i.e. patterns of match and don't-care positions—are used by many algorithms for sequence analysis; designing optimal seeds is therefore an active field of research. In sequence-database searching, one wants to optimize sensitivity, i.e. the probability of finding a region of homology; this can be done by minimizing the so-called overlap complexity of pattern sets. In alignment-free DNA sequence comparison, the number N of pattern-based matches is used to estimate phylogenetic distances. Here, one wants to minimize the variance of N in order to obtain stable phylogenies. We show that for spaced seeds, the overlap complexity—and therefore the sensitivity in database searching—is closely related to the variance of N . Our algorithm can optimize the sensitivity, overlap complexity or the variance of N , depending on the application at hand.

Introduction

k -mers, i.e. words of length k , are used in many basic algorithms for biological sequence comparison. Word matches are used, for example, as *seeds* in the *hit-and-extend* approach to database searching and read mapping [1–3]. Here, fast algorithms are applied to find pairs of identical or similar words between two sequences. A slower but more sensitive alignment method is then used to extend these word pairs to both directions, to distinguish biologically relevant homologies from spurious word matches. In alignment-free sequence comparison, sequences are often represented by word-frequency vectors to estimate distances or similarities between them, e.g. as a basis for phylogeny reconstruction [4–8], see [9] for a review. Similarly, word statistics are used to classify DNA or protein sequences [10–12], for datamining [13] and for remote homology detection [14].

It is well known that many word-based approaches produce better results if *spaced words* or *seeds* are used instead of the previously used *contiguous* words or word matches. That is, for a pre-defined binary pattern P representing *match* and *don't-care* positions, one considers only those positions in a word of the same length that correspond to the *match* positions of P . Pattern-based word matching has been proposed for *hit-and-extend* database searching by Ma et al. [15], see also [16]. Spaced seeds are also routinely used in metagenome sequence clustering and classification [17, 18], protein classification [19], read mapping [20, 21], to find *anchor points* for multiple sequence alignment [22, 23] and for alignment-free phylogeny reconstruction [24]. Similarly, the *average common substring* approach to sequence comparison [25] could be improved by allowing for mismatches [26–30]. Brejova et al. extended the concept of *spaced seeds* to homologies among protein-coding regions [31] and introduced *vector seeds* [32]. In general, the advantage of pattern-based approaches is the fact that spaced-word occurrences at neighbouring sequence positions are statistically less dependent than occurrences of contiguous words [33, 34]. Often *sets* of patterns are used, instead of single patterns; such *multiple* spaced seeds are now a standard filtering step in homology searching [35, 36].

In pattern-based approaches, the underlying patterns of match and don't-care positions are of crucial importance for the quality of the results. Generally, non-periodic patterns are preferred since they minimize redundancies between overlapping words or word matches and lead to a more even distribution of matches. This increases the probability of obtaining a hit between two homologous sequences in database searching and leads to more stable distance estimates in phylogeny reconstruction. Noé and Martin [37] defined a *coverage criterion* for multiple spaced seeds and showed that this criterion is related to the *Hamming distance*

between two sequences. In the context of database searching, patterns or sets of patterns are often called *seeds*. (Originally, the word *seed* denoted a match of—contiguous or spaced—words between a query and a database sequence that would be extended to the left and to the right. But now *seed* often denotes the underlying pattern in pattern-based approaches).

In hit-and-extend database searching, the *sensitivity* of a pattern set is defined as the probability of finding at least one hit within a gap-free alignment of a given length L and probability p for a match between two residues. Since each hit is extended to a local alignment, the sensitivity is the proportion of homologies that will be found by a search program—under the above simple model of homology, and under the assumption that each extension of a hit in a homologous region will verify the homology. In database searching, the goal is thus to maximize the sensitivity of pattern sets.

Calculating the sensitivity of a pattern set is *NP-hard* [33]. The sensitivity can be approximated by dynamic programming [15, 38], but the run time of this algorithm is still exponential in the length of the pattern. In *PatternHunter II*, a *greedy* algorithm is used to find suitable patterns. In 2007, Ilie and Ilie introduced the *overlap complexity* of a pattern set and showed experimentally that—for a given number of patterns with a given length and number of match positions—minimizing the overlap complexity corresponds to maximizing the sensitivity in database searching [39]. In contrast to the sensitivity, however, the overlap complexity can be easily calculated. To find optimal pattern sets, Ilie and Ilie proposed a *hill-climbing algorithm* that minimizes the overlap complexity. They implemented their algorithm in a software tool called *SpEED* [40], which is several orders of magnitude faster than competing approaches and is now considered the state-of-the-art in seed optimization.

Recently, we proposed to use *spaced-word* frequencies instead of word frequencies for alignment-free sequence comparison [24, 41]. We showed that phylogenetic trees calculated from spaced-word frequencies are more accurate than trees calculated from contiguous-word frequencies. As in database searching, our results could be improved by using *multiple* patterns. In our original study, we used randomly generated multiple patterns of *match* and *don't-care* positions. In a follow-up paper, we studied the number N of spaced-word matches between two DNA sequences for a set of binary patterns [34]. Our data suggest that minimizing the variance of N for pattern sets improves alignment-free phylogeny reconstruction.

In this paper, we first show that the variance of the number N of spaced-word matches is closely related to the *overlap complexity* of the underlying set of patterns. We propose a modified hill-climbing algorithm that can be used to generate pattern sets, either with minimal variance of N , or with minimal overlap complexity, or with maximal sensitivity in database searching, depending on the application at hand. While the algorithm proposed in [39] iterates over all patterns P in a set \mathcal{P} of patterns and all pairs of positions in P to improve \mathcal{P} , we calculate for each pattern $P \in \mathcal{P}$ how much P contributes to the variance or overlap complexity, respectively, of \mathcal{P} . We then modify those patterns first that contribute most to the variance or complexity.

The implementation of our approach is called *rasbhari* (*Rapid Approach for Seed optimization Based on a Hill-climbing Algorithm that is Repeated Iteratively*). Experimental results show that pattern sets calculated with *rasbhari* have a slightly higher sensitivity in database searching than pattern sets calculated with *SpEED*, while the run time of both programs is comparable. In alignment-free sequence comparison, we obtain more accurate phylogenetic distances if we use *rasbhari* to minimize the variance of N for the underlying pattern sets, than we obtained with the randomly generated pattern sets that we previously used. In a third application, we used pattern sets generated with *rasbhari* in the program *CLARK-S* [18] for short read classification. The sensitivity of the classification could be improved in this way, while *rasbhari* is substantially faster than the method that is used by default for pattern generation in *CLARK-S*.

A earlier version of this paper has been published at the preprint server *arXiv* [42].

Methods

Overlap complexity

We consider sets $\mathcal{P} = \{P_1, \dots, P_m\}$ of binary patterns, where ℓ_r is the length of pattern P_r and $\ell = \max_r \ell_r$. That is, each P_r is a word of length ℓ_r over the alphabet $\{1, 0\}$. A '1' in a pattern P_r represents a *match* position, a '0' a *don't-care* position. For a single pattern P_r , the number of match positions is called its *weight* w . For simplicity, we assume that all patterns in a set \mathcal{P} have the same weight.

In [34], we considered for two patterns $P_r, P_{r'}$ and $s \in \mathbb{Z}$ the number $n(P_r, P_{r'}, s)$ of positions that are match positions of P_r or match positions of $P_{r'}$ (or both), if $P_{r'}$ is shifted by s positions to the right, relative to P_r . If s is negative, $P_{r'}$ is shifted to the left. For $P_r = 101011, P_{r'} = 111001$, for example, if $P_{r'}$ is shifted by 2 positions to the right, relative to P_r , then there are 6 positions (marked by asterisks below) that are match positions of P_r or $P_{r'}$. Thus, for $s = 2$, we have $n(P_r, P_{r'}, 2) = 6$:

$$\begin{array}{cccccccc}
 P_r : & 1 & 0 & 1 & 0 & 1 & 1 & \\
 P_{r'} : & & & 1 & 1 & 1 & 0 & 0 & 1 \\
 & & & * & * & * & * & * & * \\
 & & & & \$ & & \$ & &
 \end{array}$$

For the same situation, Ilie and Ilie [39] defined $\sigma[s] = \sigma_{r,r'}[s]$ as the number of positions where P_r and $P_{r'}$ have a match positions, such as the positions marked by '\$' above. In the above example one would therefore have $\sigma[2] = 2$. The *overlap complexity* (OC) of a set of patterns $\mathcal{P} = \{P_1, \dots, P_m\}$ is then defined in [39] as

$$\sum_{r \leq r'} \sum_{s=1-\ell_{r'}}^{\ell_r-1} 2^{\sigma_{r,r'}[s]} \tag{1}$$

Note that, since for any two patterns $P_r, P_{r'}$ and $s \in \mathbb{Z}$, the equality

$$\sigma_{r,r'}[s] = 2w - n(P_r, P_{r'}, s)$$

holds, the overlap complexity of a set \mathcal{P} can be written as

$$\sum_{r \leq r'} \sum_{s=1-\ell_{r'}}^{\ell_r-1} 2^{\sigma_{r,r'}[s]} = 2^{2w} \cdot \sum_{r \leq r'} \sum_{s=1-\ell_{r'}}^{\ell_r-1} (1/2)^{n(P_r, P_{r'}, s)} \tag{2}$$

Consequently, if we are looking at sets \mathcal{P} of m patterns with fixed weight w and lengths ℓ_r , then minimizing the overlap complexity of \mathcal{P} is equivalent to minimizing the sum

$$\sum_{r \leq r'} \sum_{s=1-\ell_{r'}}^{\ell_r-1} (1/2)^{n(P_r, P_{r'}, s)} \tag{3}$$

Ilie and Ilie showed experimentally that the OC is closely related to the sensitivity of a pattern set. More precisely, they showed that for pattern sets with a given number of patterns of given lengths and weight, minimizing the OC practically amounts to maximizing the sensitivity. Consequently, in order to find suitable pattern sets for hit-and-extend approaches in database searching, they proposed to search for pattern sets with minimal OC. The main advantage of this approach is the fact that the OC of a pattern set is much easier to calculate than its sensitivity.

By contrast, if a pattern P_r is shifted against itself, only shifts between 0 and $\ell_r - 1$ need to be considered, to avoid double counting of shifts, for example:

$$\begin{array}{rcccccccc}
 P_r : & 1 & 0 & 1 & 1 & & & 1 & 0 & 1 & 1 \\
 P_r : & 1 & 0 & 1 & 1 & , & \dots & , & & 1 & 0 & 1 & 1 \\
 s : & & & & 0 & & & & & & & & 3
 \end{array}$$

In [34], we ignored this fact and gave a slightly different estimate for $Var(N)$.

On the right-hand side of Eq (4), the first summand is the variance of the ‘homologous’ spaced-word matches (in a model without indels, these are spaced-word matches involving the same positions in both sequences), while the second summand comes from background matches. The relative weight of the background matches in Eq (4) depends on the match probability p and the sequence length L ; for $p \gg q$ and small L , the $Var(N)$ is dominated by the ‘homologous’ term, see Fig 1. Obviously, for large L , the background spaced-word matches dominate the ‘homologous’ ones, since the number of background matches grows quadratically with L , while the ‘homologous’ matches grow only linearly.

Note that, for L, ℓ and w fixed, minimizing the $Var(N)$ amounts to minimizing

$$\sum_{r \leq r'} \sum_{s \in R(r,r')} p^{n(P_r, P_{r'}, s)} + (L - \ell) \cdot \sum_{r \leq r'} \sum_{s \in R(r,r')} q^{n(P_r, P_{r'}, s)} \tag{5}$$

Comparison with Eq (2) shows that, in the special case of $p = 1/2$, the first summand of Eq (5) that corresponds to the homologous matches is almost identical with the overlap complexity defined by Ilie and Ilie (except for the range $R(r, r)$ in which a pattern P_r is shifted against itself). For sequences of moderate length, the overlap complexity can therefore be seen as an approximation to the variance of the number of spaced-word matches.

In any case, the overlap complexity and the $Var(N)$ for a set of pattern $\mathcal{P} = \{P_1, \dots, P_m\}$ both have the form

$$\sum_{r \leq r'} \alpha_{r,r'}(\mathcal{P}) \tag{6}$$

with

$$\alpha_{r,r'}(\mathcal{P}) = \begin{cases} \sum_{s=1-\ell_{r'}}^{\ell_r-1} 2^{\alpha_{r,r'}[s]} & (OC) \\ (L - \ell + 1) \sum_{s \in R(r,r')} (p^{n(P_r, P_{r'}, s)} + (L - \ell) \cdot q^{n(P_r, P_{r'}, s)}) & (Var) \end{cases} \tag{7}$$

Our optimization problem is therefore: for integers $m, \ell_1, \dots, \ell_m, w$, find a set \mathcal{P} of m patterns of lengths ℓ_1, \dots, ℓ_m and weight w that minimizes the sum Eq (6).

Hill-climbing algorithms to find sets of patterns with minimal $Var(N)$ or OC

Both *SpEED* and our new algorithm start with randomly generated pattern sets and use *hill-climbing* to gradually reduce the OC or $Var(N)$. If one wants to find a pattern set with maximal *sensitivity*, the sensitivity is calculated for the pattern set that is produced by this procedure (this step is omitted, of course, if *rasbhari* is used to minimize $Var(N)$ or OC). The whole procedure is repeated, and the pattern set with the overall highest sensitivity—or lowest $Var(N)$ or OC , respectively—is returned.

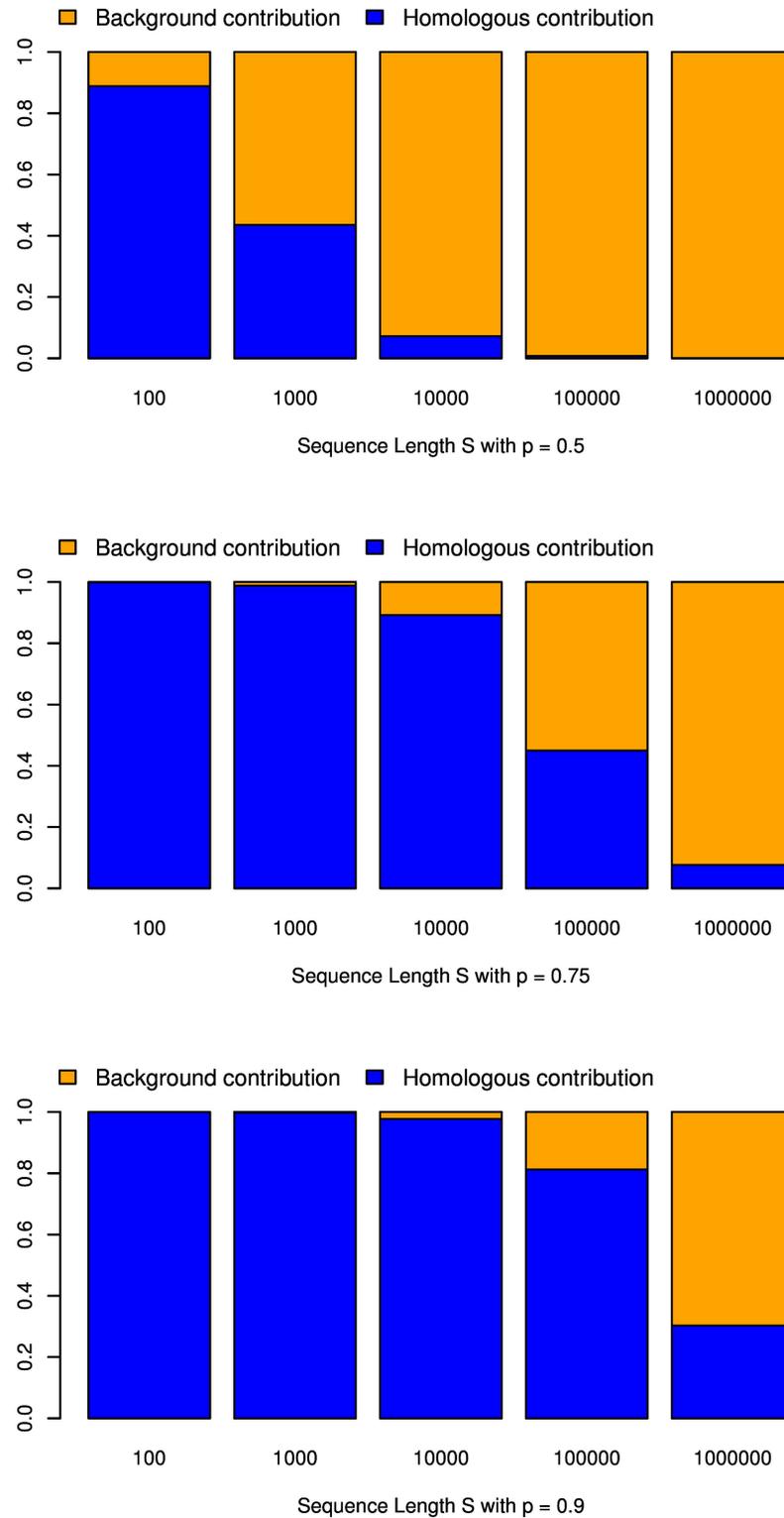


Fig 1. Homologue and background contribution to the variance of the number N of spaced-word matches. Contribution of the *homologue* and *background* variance to the total variance of the number N of spaced-word matches in eq (4) for different match probabilities p and sequence lengths L .

doi:10.1371/journal.pcbi.1005107.g001

Original hill-climbing algorithm. To improve the current pattern set \mathcal{P} , the hill-climbing algorithm implemented in *SpEED* looks at all triplets (r, i, j) where P_r is a pattern in \mathcal{P} , and i and j are a *match position* and a *don't-care position* in P_r , respectively. For each such triplet (r, i, j) , the algorithm considers the pattern set that would be obtained from \mathcal{P} by swapping i and j in P_r —i.e. by turning i into a don't-care and j into a match position. The *OC* is calculated for all pattern sets that can be obtained in this way, and the one with the lowest *OC* is selected as the next pattern set \mathcal{P} . This is repeated iteratively.

There are $O(m \cdot \ell^2)$ triplets (r, i, j) to be considered to modify the current pattern set \mathcal{P} . For each of these triplets, the *OC* is to be calculated for the pattern set that would be obtained by swapping i and j in P_r . To this end, the modified pattern P_r has to be compared to the $m - 1$ remaining patterns in \mathcal{P} which, for each pattern comparison, involves $O(\ell)$ shifts of two patterns against each other. In each shift, the number of common match positions is to be counted, which takes again $O(\ell)$ time. Thus, calculating the *OC* of the pattern set obtained by swapping two positions i and j in a pattern P_r takes $O(m \cdot \ell^2)$ time, so finding an optimal triplet (r, i, j) to determine the next pattern set takes $O(m^2 \cdot \ell^4)$ time. In *SpEED*, this step is repeated until the *OC* cannot be improved further, i.e. until a local minimum is reached. For the pattern set that is obtained by this hill-climbing routine, the sensitivity is calculated. This whole procedure is repeated 5,000 times, and finally the set with the best sensitivity is returned.

Modified hill-climbing algorithm. In our modified hill-climbing algorithm, we also swap a match position i with a don't-care position j in some pattern P_r in each step of the algorithm, and we evaluate the *OC* or *Var(N)* of the pattern set that would be obtained by this operation. However, instead of looking at *all* possible triplets (r, i, j) , we look at those patterns first that contribute most to the *OC* or *Var(N)*, respectively, of the current pattern set \mathcal{P} . The contribution

$$C_r = \sum_{r'} \alpha_{r,r'} \tag{8}$$

of a pattern $P_r \in \mathcal{P}$ to the *OC* or *Var(N)* of \mathcal{P} can be calculated as a by-product, whenever *OC* or *Var(N)* is calculated, with $\alpha_{r,r'}$ as in Eq (7). We then sort the patterns in $P_r \in \mathcal{P}$ according to the values C_r , and we process them in descending order of C_r , i.e. we look at those patterns first that contribute *most* to the *OC* or *Var(N)* of \mathcal{P} .

For the current pattern in the list, we randomly select a match position i and a don't-care position j . If swapping i and j does *not* improve the current pattern set, we move on to the next pattern in the list and proceed in the same way. This is repeated until we find a pattern where swapping the selected pair of random positions does improve \mathcal{P} . In this case, the modified pattern is accepted, all values C_r are updated, the patterns in \mathcal{P} are sorted accordingly, and we start again with the pattern P_r with maximum C_r . If we reach the last pattern in the list without obtaining any improvement, we start again with the first pattern, i.e. the pattern with the largest C_r , select new random positions i and j etc. Processing one pattern P_r in this way takes $O(m \cdot \ell^2)$ time, since we look only at one single pair (i, j) and calculate the *OC* or *Var(N)* of the pattern set that would be obtained by swapping i and j in P_r .

The hill climbing is continued until a user-defined number of pairs (i, j) have been swapped to improve the current pattern set; by default, 25,000 pairs are swapped. If we want to obtain a pattern set with maximal sensitivity, the described hill-climbing procedure is repeated 100 times, and for the pattern set with the lowest *OC* among the 100 obtained pattern sets, the sensitivity is calculated. To calculate the sensitivity, *rasbhari* uses program code from *SpEED*. Again, this whole process is repeated 5,000 times, so for a total of 5,000 pattern sets the sensitivity is calculated during one program run. This is similar to *SpEED*, but in *SpEED* the time-consuming sensitivity calculation is carried out after *one* round of hill climbing. By contrast,

we run our faster hill-climbing routine 100 times before we calculate the sensitivity for the *best* pattern set from these 100 runs. The final output of our program is the pattern set with the highest sensitivity from the 5,000 iterations.

The number m of patterns and their weight w are to be specified by the user. If $Var(N)$ is to be minimized for alignment-free sequence comparison, all patterns have the same length ℓ which is also to be specified by the user. If the sensitivity is to be maximized for database searching and read alignment, better results are achieved if the patterns in \mathcal{P} have different lengths. In this case, the maximum and minimum pattern lengths need to be specified. The program then selects lengths ℓ_1, \dots, ℓ_m that are evenly distributed between these extreme values.

Results

Sensitivity in database searching

To evaluate *rasbhari*, we first applied it to generate pattern sets, maximizing the *sensitivity* for database searching and read mapping. For the number m and weight w of the patterns and for the length H and match probability p of the homology regions, we used the parameter settings from *SHRiMP2* [43], *PatternHunter II* [38] and *BFAST* [44]. We compared it to the sensitivity of pattern sets obtained with *Iedera* [45], *SpEED* [40], *AcoSeeD* [46], *FastHC* and *MuteHC* [47] as published by the authors of these programs; the results of this comparison are shown in [Table 1](#). Here, the sensitivity values of *AcoSeeD* are *average* values over 10 program runs reported in [46].

If pattern sets with maximal sensitivity are to be found, and if the lengths ℓ_r of the patterns are small, the run time of *rasbhari* is comparable to *SpEED*. In this case, the most time-consuming step in both programs is to calculate the sensitivity of pattern sets which, by default, is done 5,000 times per program run in each of the two programs. For longer patterns, however, *SpEED* can be much slower since it carries out hill-climbing until a local minimum is reached and, as explained above, each single step in the hill-climbing procedure of *SpEED* takes $O(m^2 \cdot \ell^4)$ time. In contrast, *rasbhari* terminates this procedure after a given number of iteration steps, and it considers only a limited number of swaps of *match* and *don't-care* positions in one iteration step.

Alignment-free phylogeny reconstruction

Next, we wanted to know how alignment-free phylogeny reconstruction can be improved with *rasbhari*. To this end, we simulated pairs of DNA sequences and estimated the distances between them using the *Spaced Words* approach described in [34]. We then measured the accuracy of the distance estimates for different underlying pattern sets. First, we used *rasbhari* to minimize the *variance* of the number N of spaced-word matches between two sequences. Since there is no other method to minimize $Var(N)$, we compared the pattern sets from *rasbhari* with the randomly generated pattern sets that we previously used. The phylogenetic distances estimated with both types of pattern sets were compared to the 'real' distances between the sequences, i.e. the average number of substitutions per position. As test data, we generated nine data sets with 2,500 pairs of DNA sequences of length 100 kb each. The distances d of the sequence pairs ranged between 0.1 and 0.9 substitutions per position. For each program run, we used a set of $m = 3$ patterns of length 20 with 16 *match* and 4 *don't-care* positions. [Fig 2](#) shows the root mean square error of the estimated distances, compared to the 'real' distances d . The pattern sets generated with *rasbhari* were superior to the randomly generated pattern sets, especially for large distances.

Table 1. Sensitivity comparison of different programs.

<i>w</i>	<i>p</i>	<i>ledera</i>	<i>SpEED</i>	<i>AcoSeeD</i>	<i>FastHC</i>	<i>MuteHC</i>	<i>rasbhari</i>
SHRiMP2: 4 patterns (H = 50)							
10	0.75	90.6820	90.9098	90.9513	90.7312	92.6812	90.9614
	0.80	97.7586	97.8337	97.8521	97.7625	98.3836	97.8554
	0.85	99.7437	99.7569	99.7614	99.7431	99.8356	99.7618
11	0.75	83.2413	83.3793	83.4728	83.3068	83.4127	83.4679
	0.80	94.9350	94.9861	95.037	94.9453	95.0194	95.0386
	0.85	99.2189	99.2431	99.2478	99.2250	99.2486	99.2506
12	0.80	90.3934	90.5750	90.6328	90.4735	90.5820	90.6648
	0.85	98.0781	98.1589	98.1766	98.1199	98.1670	98.1824
	0.90	99.8773	99.8821	99.8853	99.8771	99.8836	99.8864
16	0.85	84.5795	84.8212	84.9829	84.6558	84.8764	84.969
	0.90	97.2806	97.4321	97.4712	97.3556	97.4460	97.5035
	0.95	99.9331	99.9388	99.9419	99.9347	99.9424	99.9441
18	0.85	72.1695	73.1664	73.27	72.9558		73.2209
	0.90	93.0442	93.7120	93.7778	93.6030		93.78
	0.95	99.6690	99.7500	99.7599	99.7399		99.7557
PatternHunterII: 16 patterns (H = 64)							
11	0.70	92.0708	93.2526		93.0585		93.4653
	0.75	98.3391	98.6882		98.6352		98.7573
	0.80	99.8366	99.8820		99.8750		99.8907
BFAST: 10 patterns (H = 50)							
22	0.85	60.1535	60.8127		60.0943		60.9919
	0.90	87.9894	88.5969		88.0426		88.8005
	0.95	99.2196	99.3659		99.2923		99.4099

Sensitivity of pattern sets in hit-and-extend database searching, calculated with different programs. Parameter settings for the number *m* and weight *w* of patterns, the length *H* of the gap-free homology region between query and database sequences and the match probability *p* in the homology regions, are taken from three popular programs *SHRiMP2*, *PatternHunter II* and *BFAST*. Sensitivity values from *rasbhari* were calculated using program code from *SpEED*; results of all other programs are taken from their respective publications.

doi:10.1371/journal.pcbi.1005107.t001

Read classification with *CLARK-S*

As a third test case, we used different pattern sets for *CLARK-S* [18, 48], a recently developed tool for short read classification. We evaluated the accuracy of *CLARK-S* with three underlying pattern sets, namely (A) with the patterns used by default in the program, (B) with patterns from *rasbhari* minimizing *overlap complexity* and (C) with patterns from *rasbhari* maximizing *sensitivity*. *CLARK-S* uses sets of *m* = 3 patterns of length *ℓ* = 31 and with a weight of *w* = 22. Since *SpEED* is too slow to generate pattern sets with long patterns, the authors of the program generated pattern sets for *CLARK-S* by exhaustively searching over all *single* patterns with *ℓ* = 31 and *w* = 22. If the first and the last position in the patterns are required to be *match positions*, this approach has to evaluate $\binom{29}{20} \approx 10^7$ possible patterns. The sensitivity of each of these patterns was calculated, and the three patterns with the highest sensitivity were selected. Note however, that maximizing the sensitivity of *single* patterns is only an approximation to finding a *set* of patterns with maximal *total* sensitivity.

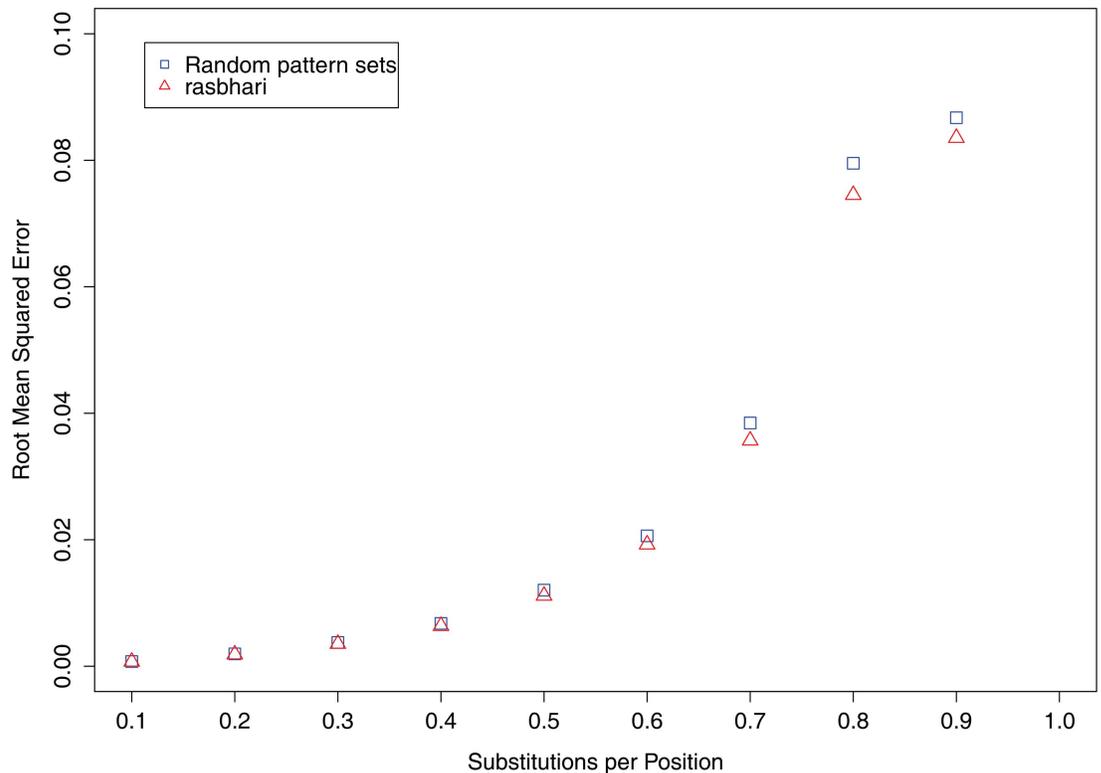


Fig 2. Accuracy of phylogenetic distance estimates based on different pattern sets. Nine sets of DNA sequence pairs were simulated with distances d between 0.1 and 0.9 substitutions per position. Distances were estimated based on the number N of spaced-word matches between them, using the alignment-free method published in [34]. We used two types of underlying pattern sets, (a) pattern sets generated with *rasbhari*, minimizing the variance of N , and (b) randomly generated pattern sets. The root mean square error of the estimated distances is plotted against the ‘real’ distances d .

doi:10.1371/journal.pcbi.1005107.g002

Fig 3 shows the default pattern set from *CLARK-S* and the two pattern sets generated by *rasbhari* as described. The exhaustive procedure used by *CLARK-S* took 2 hours to generate the pattern set. *rasbhari*, by contrast, calculated pattern sets with the same parameters within 7.54 seconds with the *slow* version where the *sensitivity* is calculated, and within 0.068 seconds with the *fast* version where the *overlap complexity* is maximized without considering the sensitivity explicitly. The slow version of *rasbhari* is thus around 480 times faster than the exhaustive procedure in *CLARK-S*, while the fast version is around 52,000 times faster. The theoretical sensitivity of the three pattern sets is 0.999771 for the default patterns from *CLARK-S*, 0.999811 for the *rasbhari* patterns with minimized overlap complexity and 0.999822 for the *rasbhari* patterns with maximized sensitivity.

To evaluate the classification accuracy of *CLARK-S* with these three pattern sets experimentally, we used five data sets from the literature, namely two sets, *HC1* and *HC2*, from the *MetaPhlan* project [49] and three sets, *simHC*, *simMC* and *simLC*, from the *FAMeS* databases [50]. For each of these data sets, we calculated *precision* and *sensitivity* of the classification at the species level as defined in [11]. That is, for a classification task where objects are to be assigned to classes, *precision* is defined as the fraction of correct assignments among the total number of assignments, while *sensitivity* is the ratio between the number of correct assignments and the number of objects to be classified. The two values are not the same since not every object is necessarily assigned to one of the classes; *precision* is always larger than or equal

(A)
 1111101101001110100111011101111
 1111011101110010111001011011111
 1111100101110110101100111011111

(B)
 1111010111010011001110111110111
 1110111011101111010010110011111
 1111101001011100111110101101111

(C)
 1111011110011010111110101011011
 1110101011101100110100111111111
 1111110101101011100111011001111

Fig 3. Pattern sets for short read classification. Pattern sets used for short read classification: (A) as used by default in CLARK-S, (B) generated with *rasbhari* minimizing overlap complexity and (C) generated with *rasbhari* maximizing sensitivity.

doi:10.1371/journal.pcbi.1005107.g003

to *sensitivity* since the denominator in the definition of precision is smaller or equal to the denominator in the definition of sensitivity. Since this definition of *sensitivity* refers to the ability of a program to correctly classify objects, it is not to be confused with the sensitivity in database searching as discussed above. Table 2 summarizes precision and sensitivity of CLARK-S with its default pattern set and with a pattern set generated by *rasbhari*.

Fig 4 shows how the overlap complexity (OC) of pattern sets produced by *rasbhari* depends on the number of iteration steps carried out in the hill-climbing algorithm. For a set of $m = 10$ patterns of length $\ell = 14$ and weight $w = 8$, a single run of the hill-climbing procedure converges after around 3,000 steps; for $m = 20$, $\ell = 44$, $w = 14$, it converges after around 80,000 steps. The OC is further improved if the hill-climbing procedure is run multiple times and the best result of these runs is used.

Table 2. Read classification with CLARK-S using different pattern sets.

Dataset	#reads	Default pattern set		rasbhari	
		Precision	Sensitivity	Precision	Sensitivity
HC1	999,998	97.69	90.36	97.69	90.44
HC2	999,991	96.45	88.11	96.45	88.18
simHC	116,771	97.20	90.53	97.20	90.54
simMC	97,495	98.75	95.09	98.73	95.09
simLC	114,457	98.29	94.26	98.28	94.25

Read classification with CLARK-S [18] with the default pattern set of the program and with the pattern set from *rasbhari* for the same parameter values, namely $n = 3$ patterns of length $\ell = 31$ and weight $w = 21$. Precision and sensitivity of the classification are reported at the species level for five data sets from the literature.

doi:10.1371/journal.pcbi.1005107.t002

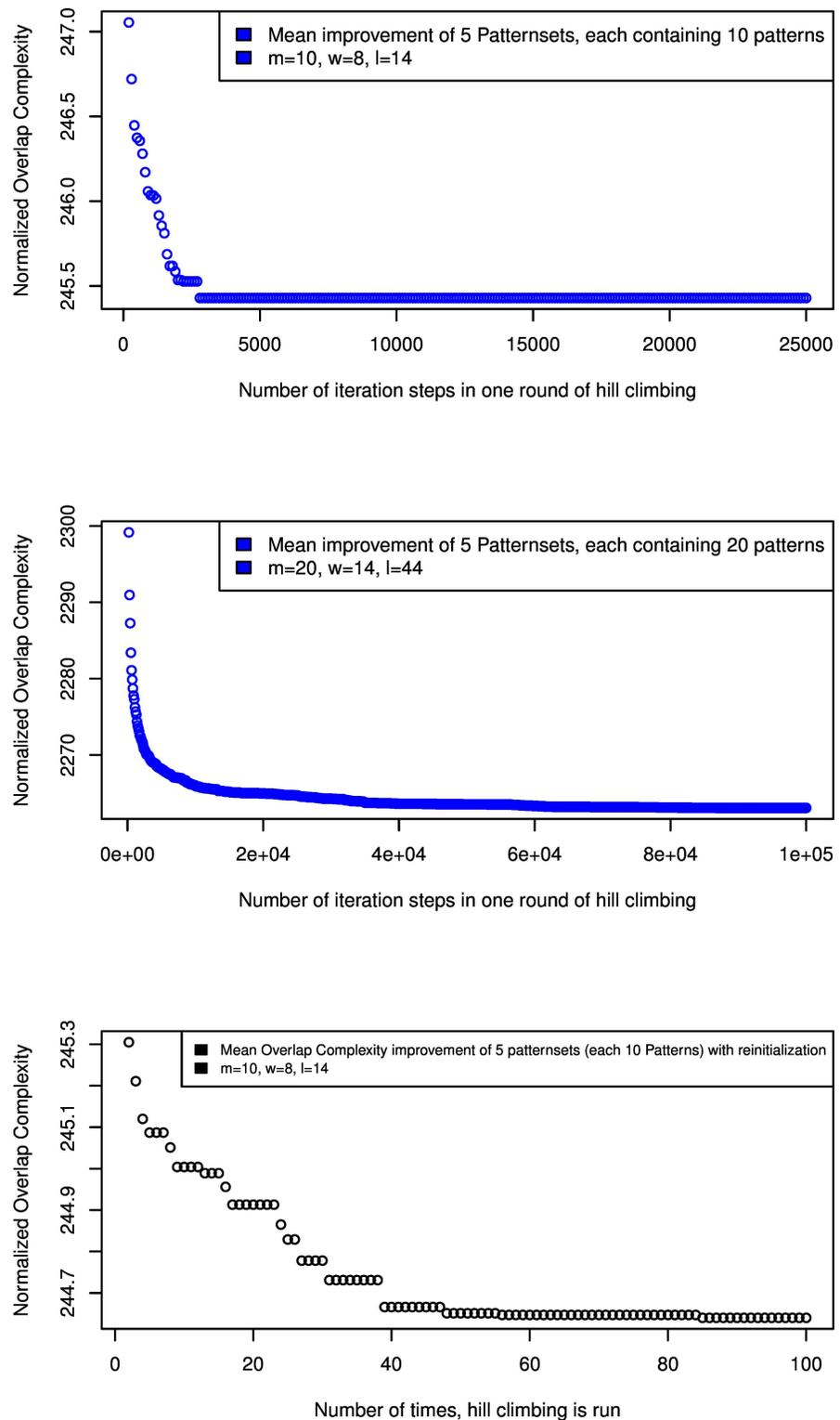


Fig 4. overlap complexity of pattern sets in the hill-climbing algorithm. Normalized overlap complexity (OC) of pattern sets depending on the number of iteration steps in our algorithm. The first two plots show how the OC is reduced in a single round of the hill-climbing algorithm for different parameters. For a set of $m = 10$ patterns of length $\ell = 14$ and weight $w = 8$, the algorithm converges after around 3,000 iteration steps of hill-climbing (upper plot); for a set of $m = 20$ patterns of length $\ell = 44$ and weight $w = 14$, it converges after around

80,000 steps (middle plot). The lower plot shows how the *OC* is improved if the hill-climbing algorithm is run multiple times and the best result of all runs is returned.

doi:10.1371/journal.pcbi.1005107.g004

In the previous section, we mentioned that the *OC* is related to the variance of the number N of spaced word matches. Comparison of eqs (5) and (2) showed that, in the special case where $p = 1/2$ and the contribution of the ‘background’ spaced-word matches is small, minimizing the *OC* is equivalent to minimizing the variance of N . In general, however, this is not the case, as the following example shows. We applied *rasbhari* to generate two sets of $m = 10$ patterns each, with length $\ell = 20$ and weight $w = 8$, one set by minimizing the *OC* and the other one by minimizing $Var(N)$. When generating the second set, we used a match probability of $p = 0.75$ and a sequence length of $L = 10,000$. The pattern set that we obtained when we minimized the *OC* had an *OC* of 11,116, the set for which we minimized $Var(N)$ had an *OC* of 11,195. Conversely, when we minimized $Var(N)$, we obtained a pattern set with a variance of 156,061, while the variance was 156,152 when we minimized the *OC*. It thus makes a difference which one of these two parameters is minimized.

Discussion

We developed a program called *rasbhari* to calculate sets of binary *patterns*—or *spaced seeds*, as they are often called—for read mapping, database searching and alignment-free sequence comparison. For sequence-homology searching, *rasbhari* optimizes the *sensitivity* of pattern sets, i.e. the probability of obtaining at least one hit between a query and a database sequence sharing a gap-free homology of a given length and with a given match probability between nucleotides. Since the sensitivity of a pattern set is expensive to calculate, our algorithm optimizes the *overlap complexity* of the produced pattern sets which is closely related to its sensitivity. We use a hill-climbing algorithm, similar to the one used in *SpEED*, to minimize the overlap complexity. Unlike *SpEED*, however, our algorithm does not calculate the overlap complexity of *all* neighbours of a current pattern set, but modifies those patterns first that contribute most to the overlap complexity of the current pattern set. To maximize the sensitivity in database searching, we calculate the sensitivity of the current pattern set after a certain number of iterations. We repeat this procedure and, finally, we pick the pattern set with the highest sensitivity in all iterations.

Since calculating the sensitivity is time consuming, *rasbhari* can alternatively minimize the overlap complexity alone, without calculating the sensitivity of pattern sets. This option is useful in situations where large pattern sets are needed for which it would take too long to calculate the sensitivity. As a third option, *rasbhari* can minimize the variance of the number N of spaced-word matches in alignment-free sequence comparison which is used by various methods to estimate phylogenetic distances between sequences. We could show that, mathematically, the variance of N has a similar form as the overlap complexity of a pattern set, so the same optimization algorithm can be used to minimize both of them.

In both homology searching and read classification, pattern sets generated by *rasbhari* are more sensitive than alternative pattern sets, so more homologies can be detected and more reads can be correctly classified. At first glance, the increase in sensitivity that we obtained seems moderate; as shown in [Table 1](#), the improvement is usually in the first or second digit after the decimal mark. In database searching and read mapping, however, even small improvements in sensitivity can lead to a large number of additional hits. Moreover, as these additional hits will be mostly in the ‘twilight zone’ of low sequence similarity, they may be of particular interest to the user.

In the context of read alignment, Ilie et al. pointed out that, with a 100-fold coverage of the human genome, a 1 percent improvement in pattern sensitivity would mean that 3 billion more nucleotides could be mapped [40], so the improvement that we achieved with *rasbhari* would still lead to tens or hundreds of millions of additionally mapped nucleotides. In database searching, the situation is similar. If we consider, for example, homology regions of length $H = 64$ with a match probability of $p = 0.8$ at the nucleotide level, then with $w = 11$, the sensitivity of *rasbhari* is improved by less than 0.01 percentage points compared to *SpEED*, see Table 1. Note, however, that these sensitivity values are already close to 100%, so the fraction of homologies that are *not* detected can be considerably reduced with the slight improvement in sensitivity obtained with *rasbhari*. In our example, the number of homologies that are *missed* is reduced by >7% if *rasbhari* is used instead of *SpEED*. With the same parameters, but with $p = 0.7$, the sensitivity of both programs is around 93%. Here, the number of missed homologies is still reduced by 3% with *rasbhari*, compared to *SpEED*.

For alignment-free sequence comparison, pattern sets produced by *rasbhari* lead to more accurate phylogenetic distances than the random pattern sets that we previously used. While this result may not be surprising, *rasbhari* is, to our knowledge, the first program that has been designed for this purpose and that can minimize the variance of the number of spaced-word matches. We therefore integrated *rasbhari* into our web server for alignment-free sequence comparison [41].

In read classification, the sensitivity of *CLARK-S* could be increased by 0.08 and 0.07 percentage points, respectively, for the largest data sets that we used, *HC1* and *HC2*. Each of these data sets contains around one million reads, so the improvement in sensitivity that we achieved with *rasbhari* means that 800 more reads from *HC1* and 700 more from *HC2* could be correctly classified by *CLARK-S*. This is remarkable, since the classification accuracy of *CLARK-S* is already very high, so it is hard to further improve the program. An interesting question in the context of *CLARK-S* is how the length and weight of the patterns influence its accuracy. So far, it was difficult to investigate this question systematically, since the exhaustive method that the program uses by default, is too time consuming. With the massive improvement in runtime that we achieved with *rasbhari*, it is now possible to systematically investigate how the accuracy of *CLARK-S* depends on the parameters of the underlying pattern sets.

In the hill-climbing procedure, our default of 25,000 iteration steps was sufficient to obtain stable results for the parameter settings that we used in our benchmark studies; we were unable to further improve these results by increasing the number of iterations. For different values of m , w , ℓ , p and H , however, it may be advisable to adapt the number of iteration steps. Fig 4 shows that, if the number of patterns or their length and weight are increased, a larger number of iteration steps can improve the results. The number of iterations within one round of hill climbing and the number of times the hill-climbing is carried out can be passed to *rasbhari* through the command line; the users can therefore adapt these parameter values for their particular applications if they do not want to use the default values of the program.

Acknowledgments

We would like to thank Laurent Noé for helpful discussions and for pointing out the similarity between the overlap complexity and the variance of the number of spaced-word matches. Lucian Ilie made useful comments on a previous version of this paper.

Author Contributions

Conceptualization: BM.

Formal analysis: CAL BM.

Investigation: LH RO SL.

Methodology: BM LH.

Project administration: BM

Software: LH.

Supervision: BM SL.

Validation: RO SL.

Visualization: LH CAL.

Writing – original draft: BM.

Writing – review & editing: BM RO SL.

References

1. Altschul SF, Gish W, Miller W, Myers EM, Lipman DJ. Basic Local Alignment Search Tool. *Journal of Molecular Biology*. 1990; 215:403–410. doi: [10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2) PMID: [2231712](https://pubmed.ncbi.nlm.nih.gov/2231712/)
2. Schbath S, Martin V, Zytnicki M, Fayolle J, Loux V, Gibrat JF. Mapping Reads on a Genomic Sequence: An Algorithmic Overview and a Practical Comparative Analysis. *Journal of Computational Biology*. 2012; 19:796–813. doi: [10.1089/cmb.2012.0022](https://doi.org/10.1089/cmb.2012.0022) PMID: [22506536](https://pubmed.ncbi.nlm.nih.gov/22506536/)
3. Hauswedell H, Singer J, Reinert K. Lambda: the local aligner for massive biological data. *Bioinformatics*. 2014; 30:i349–i355. doi: [10.1093/bioinformatics/btu439](https://doi.org/10.1093/bioinformatics/btu439) PMID: [25161219](https://pubmed.ncbi.nlm.nih.gov/25161219/)
4. Chor B, Horn D, Levy Y, Goldman N, Massingham T. Genomic DNA *k*-mer spectra: models and modalities. *Genome Biology*. 2009; 10:R108. doi: [10.1186/gb-2009-10-10-r108](https://doi.org/10.1186/gb-2009-10-10-r108) PMID: [19814784](https://pubmed.ncbi.nlm.nih.gov/19814784/)
5. Sims GE, Jun SR, Wu GA, Kim SH. Alignment-free genome comparison with feature frequency profiles (FFP) and optimal resolutions. *Proceedings of the National Academy of Sciences*. 2009; 106:2677–2682. doi: [10.1073/pnas.0813249106](https://doi.org/10.1073/pnas.0813249106) PMID: [19188606](https://pubmed.ncbi.nlm.nih.gov/19188606/)
6. Vinga S, Carvalho AM, Francisco AP, Russo LMS, Almeida JS. Pattern matching through Chaos Game Representation: bridging numerical and discrete data structures for biological sequence analysis. *Algorithms for Molecular Biology*. 2012; 7:10. doi: [10.1186/1748-7188-7-10](https://doi.org/10.1186/1748-7188-7-10) PMID: [22551152](https://pubmed.ncbi.nlm.nih.gov/22551152/)
7. Jun SR, Sims GE, Wu GA, Kim SH. Whole-proteome phylogeny of prokaryotes by feature frequency profiles: An alignment-free method with optimal feature resolution. *Proceedings of the National Academy of Sciences*. 2010; 107:133–138. doi: [10.1073/pnas.0913033107](https://doi.org/10.1073/pnas.0913033107) PMID: [20018669](https://pubmed.ncbi.nlm.nih.gov/20018669/)
8. Allman ES, Rhodes JA, Sullivant S. Statistically-Consistent *k*-mer Methods for Phylogenetic Tree Reconstruction. *arXiv:151101956 [q-bio.PE]*.
9. Vinga S. Editorial: Alignment-free methods in computational biology. *Briefings in Bioinformatics*. 2014; 15:341–342. doi: [10.1093/bib/bbu005](https://doi.org/10.1093/bib/bbu005) PMID: [24819825](https://pubmed.ncbi.nlm.nih.gov/24819825/)
10. Leslie CS, Eskin E, Cohen A, Weston J, Noble WS. Mismatch string kernels for discriminative protein classification. *Bioinformatics*. 2004; 20:467–476. doi: [10.1093/bioinformatics/btg431](https://doi.org/10.1093/bioinformatics/btg431) PMID: [14990442](https://pubmed.ncbi.nlm.nih.gov/14990442/)
11. Ounit R, Wanamaker S, Close TJ, Lonardi S. CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative *k*-mers. *BMC Genomics*. 2015; 16:236. doi: [10.1186/s12864-015-1419-2](https://doi.org/10.1186/s12864-015-1419-2) PMID: [25879410](https://pubmed.ncbi.nlm.nih.gov/25879410/)
12. Meinicke P. UProC: tools for ultra-fast protein domain classification. *Bioinformatics*. 2015; 31:1382–1388. doi: [10.1093/bioinformatics/btu843](https://doi.org/10.1093/bioinformatics/btu843) PMID: [25540185](https://pubmed.ncbi.nlm.nih.gov/25540185/)
13. Meinicke P, Tech M, Morgenstern B, Merkl R. Oligo kernels for datamining on biological sequences: a case study on prokaryotic translation initiation sites. *BMC Bioinformatics*. 2004; 5:169. doi: [10.1186/1471-2105-5-169](https://doi.org/10.1186/1471-2105-5-169) PMID: [15511290](https://pubmed.ncbi.nlm.nih.gov/15511290/)
14. Lingner T, Meinicke P. Remote homology detection based on oligomer distances. *Bioinformatics*. 2006; 22:2224–2231. doi: [10.1093/bioinformatics/btl376](https://doi.org/10.1093/bioinformatics/btl376) PMID: [16837522](https://pubmed.ncbi.nlm.nih.gov/16837522/)
15. Ma B, Tromp J, Li M. PatternHunter: faster and more sensitive homology search. *Bioinformatics*. 2002; 18:440–445. doi: [10.1093/bioinformatics/18.3.440](https://doi.org/10.1093/bioinformatics/18.3.440) PMID: [11934743](https://pubmed.ncbi.nlm.nih.gov/11934743/)

16. Califano A, Rigoutsos I. FLASH: a fast look-up algorithm for string homology. In: Computer Vision and Pattern Recognition, 1993. Proceedings CVPR'93., 1993 IEEE Computer Society Conference on; 1993. p. 353–359.
17. Břinda K, Sykulski M, Kucherov G. Spaced seeds improve *k*-mer-based metagenomic classification. *Bioinformatics*. 2015; 31:3584–3592. doi: [10.1093/bioinformatics/btv419](https://doi.org/10.1093/bioinformatics/btv419) PMID: [26209798](https://pubmed.ncbi.nlm.nih.gov/26209798/)
18. Ounit R, Lonardi S. Higher Classification Accuracy of Short Metagenomic Reads by Discriminative Spaced *k*-mers. In: Pop M, Touzet H, editors. Algorithms in Bioinformatics: 15th International Workshop, WABI 2015, Atlanta, GA, USA, September 10–12, 2015, Proceedings. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 286–295.
19. Onodera T, Shibuya T. The gapped spectrum kernel for support vector machines. In: Perner P, editor. Machine Learning and Data Mining in Pattern Recognition. vol. 7988 of Lecture Notes in Computer Science. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 1–15.
20. Rumble SM, Lacroute P, Dalca AV, Fiume M, Sidow A, Brudno M. SHRiMP: Accurate Mapping of Short Color-space Reads. *PLOS Computational Biology*. 2009; 5:e1000386+. doi: [10.1371/journal.pcbi.1000386](https://doi.org/10.1371/journal.pcbi.1000386) PMID: [19461883](https://pubmed.ncbi.nlm.nih.gov/19461883/)
21. Noé L, Gırdea M, Kucherov G. Designing efficient spaced seeds for SOLiD read mapping. *Advances in Bioinformatics*. 2010; 2010:1–12. doi: [10.1155/2010/708501](https://doi.org/10.1155/2010/708501) PMID: [20936175](https://pubmed.ncbi.nlm.nih.gov/20936175/)
22. Darling AE, Treangen TJ, Zhang L, Kuiken C, Messeguer X, Perna NT. Procrastination Leads to Efficient Filtration for Local Multiple Alignment. In: Bücher P, Moret BME, editors. Algorithms in Bioinformatics: 6th International Workshop, WABI 2006, Zurich, Switzerland, September 11–13, 2006. Proceedings. Berlin, Heidelberg: Springer Berlin Heidelberg; 2006. p. 126–137.
23. Darling AE, Mau B, Perna NT. progressiveMauve: Multiple Genome Alignment with Gene Gain, Loss and Rearrangement. *PLOS ONE*. 2010; 5:e11147+. doi: [10.1371/journal.pone.0011147](https://doi.org/10.1371/journal.pone.0011147) PMID: [20593022](https://pubmed.ncbi.nlm.nih.gov/20593022/)
24. Leimeister CA, Boden M, Horwege S, Lindner S, Morgenstern B. Fast alignment-free sequence comparison using spaced-word frequencies. *Bioinformatics*. 2014; 30:1991–1999. doi: [10.1093/bioinformatics/btu177](https://doi.org/10.1093/bioinformatics/btu177) PMID: [24700317](https://pubmed.ncbi.nlm.nih.gov/24700317/)
25. Ulitsky I, Burstein D, Tuller T, Chor B. The average common substring approach to phylogenomic reconstruction. *Journal of Computational Biology*. 2006; 13:336–350. doi: [10.1089/cmb.2006.13.336](https://doi.org/10.1089/cmb.2006.13.336) PMID: [16597244](https://pubmed.ncbi.nlm.nih.gov/16597244/)
26. Haubold B, Pierstorff N, Möller F, Wiehe T. Genome comparison without alignment using shortest unique substrings. *BMC Bioinformatics*. 2005; 6:123. doi: [10.1186/1471-2105-6-123](https://doi.org/10.1186/1471-2105-6-123) PMID: [15910684](https://pubmed.ncbi.nlm.nih.gov/15910684/)
27. Leimeister CA, Morgenstern B. *kmacs*: the *k*-mismatch average common substring approach to alignment-free sequence comparison. *Bioinformatics*. 2014; 30:2000–2008. doi: [10.1093/bioinformatics/btu331](https://doi.org/10.1093/bioinformatics/btu331) PMID: [24828656](https://pubmed.ncbi.nlm.nih.gov/24828656/)
28. Thankachan SV, Chockalingam SP, Liu Y, Krishnan A, Aluru S. A greedy alignment-free distance estimator for phylogenetic inference (extended abstract). In: Computational Advances in Bio and Medical Sciences (ICCBS), 2015 IEEE 5th International Conference on; 2015. p. 1–1.
29. Thankachan SV, Chockalingam SP, Liu Y, Apostolico A, Aluru S. ALFRED: a practical method for alignment-free distance computation. *Journal of Computational Biology, in press*. doi: [10.1089/cmb.2015.0217](https://doi.org/10.1089/cmb.2015.0217) PMID: [27138275](https://pubmed.ncbi.nlm.nih.gov/27138275/)
30. Thankachan SV, Apostolico A, Aluru S. A Provably Efficient Algorithm for the *k*-Mismatch Average Common Substring Problem. *Journal of Computational Biology*. 2016; doi: [10.1089/cmb.2015.0235](https://doi.org/10.1089/cmb.2015.0235) PMID: [27058840](https://pubmed.ncbi.nlm.nih.gov/27058840/)
31. Brejova B, Brown DG, Vinar T. Optimal spaced seeds for homologous coding regions. *Journal of Bioinformatics and Computational Biology*. 2004; 1:595–610. doi: [10.1142/S0219720004000326](https://doi.org/10.1142/S0219720004000326) PMID: [15290755](https://pubmed.ncbi.nlm.nih.gov/15290755/)
32. Brejova B, Brown DG, Vinar T. Vector seeds: an extension to spaced seeds. *Journal of Computer and System Sciences*. 2005; 70:364–380. doi: [10.1016/j.jcss.2004.12.008](https://doi.org/10.1016/j.jcss.2004.12.008)
33. Li M, Ma B, Zhang L. Superiority and complexity of the spaced seeds. In: Proceedings of the Seventeenth Annual ACM-SIAM Symposium on Discrete Algorithm. SODA'06. Philadelphia, PA, USA: Society for Industrial and Applied Mathematics; 2006. p. 444–453.
34. Morgenstern B, Zhu B, Horwege S, Leimeister CA. Estimating evolutionary distances between genomic sequences from spaced-word matches. *Algorithms for Molecular Biology*. 2015; 10:5. doi: [10.1186/s13015-015-0032-x](https://doi.org/10.1186/s13015-015-0032-x) PMID: [25685176](https://pubmed.ncbi.nlm.nih.gov/25685176/)
35. Frith MC, Noé L. Improved search heuristics find 20 000 new alignments between human and mouse genomes. *Nucleic Acids Research*. 2014; 42:e59. doi: [10.1093/nar/gku104](https://doi.org/10.1093/nar/gku104) PMID: [24493737](https://pubmed.ncbi.nlm.nih.gov/24493737/)
36. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. *Nature Methods*. 2015; 12:59–60. doi: [10.1038/nmeth.3176](https://doi.org/10.1038/nmeth.3176) PMID: [25402007](https://pubmed.ncbi.nlm.nih.gov/25402007/)

37. Noé L, Martin DEK. A coverage criterion for spaced seeds and its applications to SVM string-kernels and *k*-mer distances. *Journal of Computational Biology*. 2014; 12:947–963. doi: [10.1089/cmb.2014.0173](https://doi.org/10.1089/cmb.2014.0173) PMID: [25393923](https://pubmed.ncbi.nlm.nih.gov/25393923/)
38. Li M, Ma B, Kisman D, Tromp J. PatternHunter II: highly sensitive and fast homology search. *Journal of Bioinformatics and Computational Biology*. 2004; 02:417–439. doi: [10.1142/S0219720004000661](https://doi.org/10.1142/S0219720004000661) PMID: [15359419](https://pubmed.ncbi.nlm.nih.gov/15359419/)
39. Ilie L, Ilie S. Multiple spaced seeds for homology search. *Bioinformatics*. 2007; 23:2969–2977. doi: [10.1093/bioinformatics/btm422](https://doi.org/10.1093/bioinformatics/btm422) PMID: [17804438](https://pubmed.ncbi.nlm.nih.gov/17804438/)
40. Ilie L, Ilie S, Bigvand AM. SpEED: fast computation of sensitive spaced seeds. *Bioinformatics*. 2011; 27:2433–2434. doi: [10.1093/bioinformatics/btr368](https://doi.org/10.1093/bioinformatics/btr368) PMID: [21690104](https://pubmed.ncbi.nlm.nih.gov/21690104/)
41. Horwege S, Lindner S, Boden M, Hatje K, Kollmar M, Leimeister CA, et al. *Spaced words* and *kmacs*: fast alignment-free sequence comparison based on inexact word matches. *Nucleic Acids Research*. 2014; 42:W7–W11. doi: [10.1093/nar/gku398](https://doi.org/10.1093/nar/gku398) PMID: [24829447](https://pubmed.ncbi.nlm.nih.gov/24829447/)
42. Hahn L, Leimeister CA, Morgenstern B. *RasBhar*: optimizing spaced seeds for database searching, read mapping and alignment-free sequence comparison. arXiv:151104001 [q-bioGN]. 2015;.
43. David M, Dzamba M, Lister D, Ilie L, Brudno M. SHRIMP2: Sensitive yet Practical Short Read Mapping. *Bioinformatics*. 2011; 27:1011–1012. doi: [10.1093/bioinformatics/btr046](https://doi.org/10.1093/bioinformatics/btr046) PMID: [21278192](https://pubmed.ncbi.nlm.nih.gov/21278192/)
44. Homer N, Merriman B, Nelson SF. BFAST: an alignment tool for large scale genome resequencing. *PLOS ONE*. 2009; 4:e7767+. doi: [10.1371/journal.pone.0007767](https://doi.org/10.1371/journal.pone.0007767) PMID: [19907642](https://pubmed.ncbi.nlm.nih.gov/19907642/)
45. Kucherov G, Noé L, Roytberg M. A unifying framework for seed sensitivity and its application to subset seeds. *Journal of Bioinformatics and Computational Biology*. 2006; 4:553–569. doi: [10.1142/S0219720006001977](https://doi.org/10.1142/S0219720006001977) PMID: [16819802](https://pubmed.ncbi.nlm.nih.gov/16819802/)
46. Duc DD, Dinh HQ, Dang TH, Laukens K, Xuan HH. AcoSeeD: An ant colony optimization for finding optimal spaced seeds in biological sequence search. In: ANTS'12; 2012. p. 204–211.
47. Do PT, Tran-Thi CG. An improvement of the overlap complexity in the spaced seed searching problem between genomic DNAs. In: Proceedings of the 2nd National Foundation for Science and Technology Development Conference on Information and Computer Science (NICS); 2015. p. 271–276.
48. Ounit R, Lonardi S. Higher classification sensitivity of short metagenomic reads with CLARK-S. *Bioinformatics*. 2016; doi: [10.1093/bioinformatics/btw542](https://doi.org/10.1093/bioinformatics/btw542) PMID: [27540266](https://pubmed.ncbi.nlm.nih.gov/27540266/)
49. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nature Methods*. 2012; 9:811–814. doi: [10.1038/nmeth.2066](https://doi.org/10.1038/nmeth.2066) PMID: [22688413](https://pubmed.ncbi.nlm.nih.gov/22688413/)
50. Mavromatis K, Ivanova N, Barry K, Shapiro H, Goltsman E, McHardy AC, et al. Use of simulated data sets to evaluate the fidelity of metagenomic processing methods. *Nature Methods*. 2007; 4:495–500. doi: [10.1038/nmeth1043](https://doi.org/10.1038/nmeth1043) PMID: [17468765](https://pubmed.ncbi.nlm.nih.gov/17468765/)