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Starcode: an exact algorithm for sequence clustering

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ABSTRACT

Motivation: The increasing throughput of sequencing technologies offers new applications and challenges for computational biology. One such application is the use of random barcodes to trace and quantify transcripts or lineages in experimental setups. The high error rate of modern sequencers calls for additional post-processing techniques capable of detecting and reverting the misreads. However, in the absence of a reference population, the problem amounts to performing a pairwise comparison of all the barcodes, which is unfeasible for excessive computationally complexity.

Results: Here we address this problem and describe an exact algorithm to determine which pairs of sequences lie within a given Levenshtein distance. The matched pairs are merged into clusters represented by a canonical sequence. The effiency of starcode is attributable to the poucet search, a novel implementation of the Needleman-Wunsch algorithm performed on the nodes of a trie. On the task of clustering random barcodes, starcode outperforms sequence clustering algorithms in both speed and precision. We further show that starcode can also be used to identify enriched motifs in DNA and RNA sequences.

Availability and implementation: The C source code is available at http://github.com/gui11aume/starcode.

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1 INTRODUCTION

Sequence clustering is the process of grouping similar biological sequences. It has been traditionally applied to identify related protein families and to reduce sequence redundancy in databases. Recently, the advent of high throughput sequencing has created additional needs for efficient clustering algorithms, in particular because of the high error rate of such technologies. For instance, the Illumina platform (Margulies *et al.*, 2005) shows a 1-2% error rate consisting of substitutions near the 3' end of the read (Dohm *et al.*, 2008; Nakamura *et al.*, 2011). The PacBio platform shows a 15% error rate that mostly corresponds to insertions and deletions (Eid *et al.*, 2009). As a consequence, the same sequence is often decoded in different ways, which artificially increases the diversity of the output.

Sequencing errors can be discovered by mapping the reads onto a reference, if it is available. When the sequences are random or drawn from an unknown reference, clustering is the best option to tell real from spurious reads. One such case is the use of random barcodes to track cells or transcripts (Schepers *et al.*, 2008; Akhtar *et al.*, 2013). Sequencing errors will create erroneous barcodes that have to be reverted to the original sequence. Hands on experience with real datasets shows that this step becomes limiting when the number of unique sequences is high. In search for a solution to this problem, we realized that heuristic approaches rely on assumptions that may not hold as technologies evolve. We therefore set out to find an exact algorithm.

The first task of clustering is a matching phase where closely related barcodes are paired, similarly to linked nodes on a graph. The second task is the clustering proper, where communities in this graph are merged. We called our algorithm “starcode”, in reference to the star shape of the graph formed by the barcodes in the same cluster. The first version of the algorithm had the same performance regardless of the order in which the input sequences were processed, because it did not exploit data structuring of any kind. Exploiting the prefix redundancy of alphabetically sorted sequences allowed us to avoid unnecessary recomputations and gain speed. This is the rationale behind the poucet search algorithm at the heart of the matching step.

Here we describe the starcode algorithm and we benchmark it against existing software that perform related tasks. We show that starcode is both faster and more precise than the alternatives, achieving perfect clustering on ideal datasets. We further show that starcode can be used to identify enriched motifs in large datasets such as bacterial genomes or protein-DNA/protein-RNA interaction experiments.

2 METHODS

2.1 Inexact string matching using tries

The matching method of starcode is based on a variation of the Needleman-Wunsch (NW) algorithm (Needleman and Wunsch, 1970). In the original algorithm (Figure 1a), the Levenshtein distance between two sequences is found by applying a recurrence relation throughout a matrix of mn terms, where m and n are the respective sequence lengths. The complexity of this dynamic programming approach is $O(mn)$.

In many instances, the only information of interest is to find out whether the sequences are d-neighbors (separated by a distance less than or equal to a threshold d). In that case, the complexity then reduces to $O(d\min(m,n))$ as described below (Ukkonen, 1995). Instead of initializing the margins of the matrix and computing all the terms, the matrix is initialized as shown on Figure 1b and only the terms around the diagonal are computed. If the

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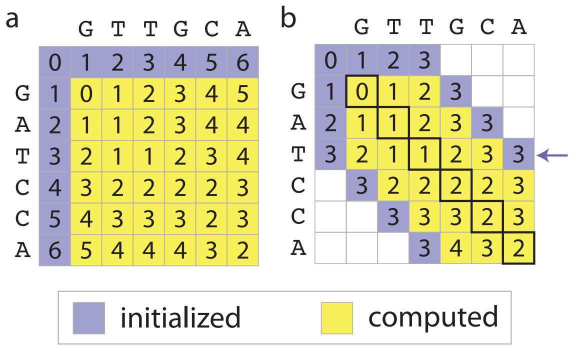


Fig. 1. Needleman-Wunsch (NW) alignment. **a** Alignment of GTTGCA and GATCCA using the NW alignment matrix. The margins (purple) are initialized and the cells of the matrix (yellow) are computed from left to right and from top to bottom by the following dynamic programming algorithm. The value in each cell is computed from the values of the neighboring top, left and top-left cells. Denoting a, b and c these respective terms, the value in the cell of coordinates (i, j) is computed as $\min(a + 1, b + 1, c)$ if the i-th letter from the first sequence is identical to the j-th letter from the second, or $\min(a + 1, b + 1, c + 1)$ if the letters are different. The Levenshtein distance between the two sequences is found in the bottom right cell. **b** Lower complexity algorithm to determine whether GTTGCA and GATCCA are 2-neighbors. The values in purple cells are set during initialization. The dynamic programming algorithm proceeds as above, with the difference that it is interrupted if the value of a diagonal cell (bold borders) is larger than 2. The values in purple cells are not always the same as their equivalent in **a** (purple arrow), but the values in the yellow cells are nevertheless identical. The values of the white cells are never computed, which contributes to making the algorithm less complex.

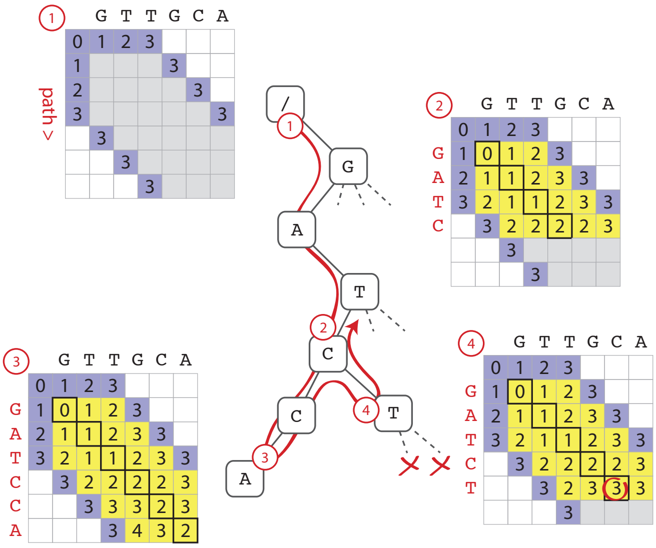


Fig. 2. Trie query with NW alignment. Each sequence of the index is a path in the trie. The query GTTGCA is written at the top of a NW matrix, which is initialized as shown on Figure 1b. The trie is traversed by a depth-first search (red path) from the root. At each depth, the node added to the path is written on the left of the NW matrix and the row is computed. Checkpoints from 1 to 4 (circled red numbers) show the state of the NW matrix as the search proceeds. The node labeled 3 is a leaf and thus corresponds to a 2-neighbor of the query. The search path then backtracks to the node labeled 2 and the last rows of the NW are erased. The search path then goes to the node labeled 4, in which case the newly computed diagonal cell exceeds the threshold (circled in red). Even if this node has children, they are not visited (red crosses).

computation of a diagonal term yields a value greater than d, the distance is known to be greater than d and the process is halted. Otherwise, the bottom-right term indicates the actual distance between the sequences, as in the NW algorithm.

This method can be used for inexact matching of sequences against a reference set indexed as a prefix tree, also known as a trie (Ukkonen, 1995). The terms of the matrix are updated row-wise as a depth-first search traverses the trie from the root, as illustrated in Figure 2. Every time a node is visited, the row corresponding to its depth is recomputed. If the threshold value d is exceeded for a diagonal term, the Levenshtein distance for all the downstream sequences is also necessarily greater than d. Therefore, no more hits are to be discovered in this path and the depth-first search backtracks to the parent node. When the process halts, every tail node (corresponding to a sequence of the database) on the path of this search is a d-neighbor of the query. This method is efficient because it eliminates large areas of the search space, and because the NW alignment of the query with each prefix of the database is computed only once.

2.2 The poucet search algorithm

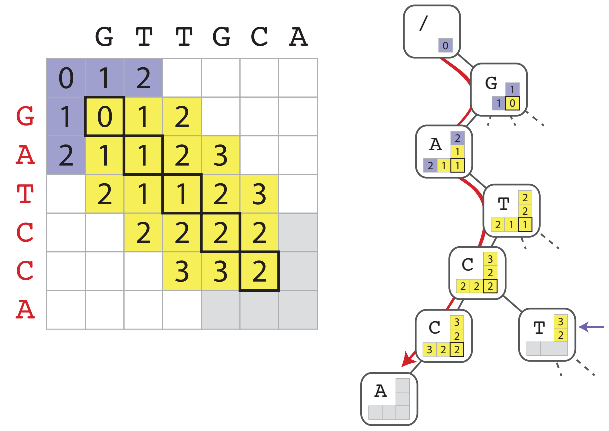
This strategy can be further improved. Notice that if two consecutive queries share a prefix of length k, the succession of computations up to the k-th row of the NW matrix will be exactly the same in both queries. To take advantage of this property, the input sequences are sorted alphabetically in order to maximize the prefix sharing. The main idea of the algorithm is to store the computational intermediates in the nodes of the trie and use them later to resume the computation in the next query. This way, the search can start at depth k whenever a query shares a prefix of such length with the previous one.

However, storing the rows of the NW matrix in the nodes, as suggested by the approach described in the previous section is not optimal. At depth equal to the length of the shared prefix, say k, the terms on the right of the diagonal were computed using characters that belong to the previous query. Since they also depend on the terms computed at lower depth, the search cannot restart at depth k. This issue is solved by storing in each node a combination of row and column terms that form an angle shape, looking like a horizontally flipped L character as shown on Figure 3. This way, the computation intermediates stored in a node of depth k depend only on the previous characters of the query. Using this technique, the search can effectively restart at a depth equal to the length of the shared prefix with the previous query, thereby averting the need to recompute the first terms of the NW alignments.

In the fairy tale “Le Petit Poucet”, the hero seeds white pebbles for his older brothers to find their way home, which is reminiscent of the way queries pave the way for the next in this algorithm. We therefore called this search algorithm “poucet”.

2.3 Lookup search

If the Levenshtein distance of two sequences of length L is less than or equal to τ, there must exist a perfect match between them of at least $k = \lfloor L/(\tau + 1)\rfloor$ nucleotides. We take advantage of this property to build a lookup table containing all such k-mers of the sequences already present in the trie. When a new sequence is queried, all its subsequences are extracted and queried against the lookup table. If none of the subsequences are found, the minimum distance between the query and all the sequences in the trie is necessarily greater than τ, and the trie search can therefore be omitted. This lookup is



		G	T	T	G	C	A
	0	1	2				
G	1	0	1	2			
A	2	1	1	2	3		
T		2	1	1	2	3	
C			2	2	2	2	
C				3	3	2	
A							

Fig. 3. Poucet search algorithm. The algorithm proceeds with the same principles as shown on Figure 2 with the difference that the NW matrix is not updated row-wise, but along horizontally flipped L shapes. As the depth-first search proceeds, these values are stored in the nodes of the trie. Only the nodes at the top contain initialized values; for the other nodes, the values at the border are implicitly known to be 3. Since the values in the vertical part of the flipped L are the same for every child of the same node, they are computed only once (purple arrow). The values in the grey cells will be computed as the search path (red) visits the node. Storing the intermediates in the nodes allows the next query to restart at depth k if it shares a common prefix of length k with the current query.

particulary useful for long query sequences (see 3.1), where k is large and the probability of an exact match between subsequences is small unless they are actually related, i.e. they belong to the same cluster. With this method, the absence of match is detected at the computational cost of several table lookups, compared to the higher cost of measuring the Levenshtein distance between long sequences.

In general, however, $L/(\tau+1)$ is not an integer. To deal with this complication, we divide the query into $\tau{+}2$ subsequences. The first $k{+}1$ are called *words* and have a target length $(L-\tau)/(\tau+1)$. The last subsequence is called *tail* and has length τ. The length of the words is computed by integer division, and the remainder R is distributed equally among the last R words. As a result, the words have a length that differs by at most 1, with an aggregated length equal to $L-\tau$.

Since two exact words at different positions in the sequence shall not represent a lookup match, we store and query the words against $k+1$ distinct lookup tables. In order to allow insertions and deletions, the lookup search must also query shifted versions of each word. In the worst matching case, only one word is found and the mismatches are evenly distributed in the query sequence. In such case, the maximum number of cumulative insertions/deletions at the i-th word is $i-1$. Hence, the i-th word has to be shifted and queried against the lookup table $2(i-1)+1$ times to cover all the possible cases. The last word will be queried $2\tau+1$ times, including τ shifts on the right and τ shifts on the left. For the latter shift we make use of the τ nucleotides stored in the tail. The total number of lookups per sequence is $(\tau+1)^2$.

2.4 Seek and construct

To reduce the size of the search space, we use a dynamic "seek and construct" approach whereby queries are processed meanwhile the trie is built. In other words, each sequence is matched against the trie before it is inserted. To illustrate why the trie does not need to contain all the sequences upon query, assume that two sequences A and B are d-neighbors. A is processed first. Since B is not yet inserted, A yields no hit. It is then inserted in the trie. At the time B is processed, A is a hit for the query and the match A-B is discovered. This approach guarantees that every hit is discovered, while maintaining the trie as "thin" as possible, thereby reducing the search time. The whole matching process is summarized in the pseudocode shown in Algorithms 1 and 2.

Algorithm 1 Starcode algorithm

```
Define: $\tau$
Variables: $seed$, $start = 0$, $height$, $seq$, $trie$, $lastseq$, $k$
Containers: $hits$, $pebbles$
READ sequence file
$height \leftarrow$ DETERMINE maximum sequence length
PAD sequences up to $height$
SORT sequences alphabetically
$k \leftarrow$ COMPUTE lookup word lengths
$trie \leftarrow$ CREATE an empty trie of height $height$
INSERT root node of $trie$ in $pebbles$ at depth 0
for all sequences do
    $seq \leftarrow$ GET next sequence
    if at least one $k$-mer of $seq$ is in the lookup table then
        $seed \leftarrow$ LENGTH of shared prefix between current and next sequence
        $start \leftarrow$ LENGTH of shared prefix between $seq$ and $lastseq$
        CLEAR hits
        CLEAR $pebbles$ at depth $> start$
        for all $pebbles$ at depth $start$ do
            $node \leftarrow$ GET next node from $pebbles$
            call POUCET($seq$, $node$, $seed$, $hits$, $pebbles$)
        end for
        PROCESS $hits$ and LINK matches to $seq$
        $lastseq \leftarrow seq$
    end if
    INSERT $seq$ path in $trie$
    INSERT $seq$ $k$-mers into the lookup table
end for
```

2.5 Parallelization

To parallelize the search, queries are separated into contiguous blocks after sorting. The matching algorithm proceeds in two phases. During the first phase, an independent trie is created and filled with the sequences of its associated block using the seek and construct process described above. In the second phase, all the other blocks of sequences are queried against but not inserted into each trie built in the first phase. If the queries are segregated into N blocks, the first phase consists of N seek and construct jobs, whereas the second consists of $N(N-1)/2$ query jobs. Since the jobs show little dependence on each other, the matching algorithm can be efficiently parallelized provided N is larger than the number of independent threads.

2.6 Clustering

Starcode implements a multi-purpose clustering algorithm called "sphere clustering" (Akhtar *et al.*, 2013), and a message passing algorithm (MacKay, 2002) tailored for the task of clustering random barcodes. In sphere clustering, barcodes are sorted by frequency of occurrence and each barcode, starting from the most frequent, can claim its d-neighbors that were not already claimed.

In message passing clustering, read counts are distributed equally among the closest neighbors of each barcode only if they are at least 5 times more

Algorithm 2 Poucet search algorithm

```
procedure POUCET($query, node, seed, hits, pebbles$):
    COMPUTE $node$-specific column following NW                ▷ Fig.1
    for all $child$ nodes in $node$ do
        COMPUTE $child$-specific row following NW              ▷ Fig.1
        COMPUTE center value using row and column              ▷ Fig.1
        if center value $> \tau$ then                          ▷ Mismatches exceeded.
            continue with next $child$
        end if
        if $node$ depth = $height$ then                        ▷ Hit found.
            SAVE $node$ sequence in $hits$
            continue with next $child$
        end if
        if $node$ depth $\leq$ $seed$ then
            SAVE $node$ in $pebbles$ at current depth
        end if
        call poucet($query, child, seed, hits, pebbles$)
    end for
end procedure
```

frequent. The remaining sequences at the end of the process are considered canonical, and their associated count is the estimated cluster size. A barcode is assigned to a cluster if all its read counts are eventually given to the corresponding canonical barcode. The barcodes for which the read counts are split between different canonical barcodes are not assigned to any cluster. The reason for imposing a factor 5 or larger in order to transfer the read counts is that barcodes with similar frequencies are not likely derived from each other through sequencing errors. More likely they are either unrelated, or they both derive from another more abundant barcode.

3 RESULTS

3.1 Performance

We measured basic performance and scalability metrics of starcode on a dataset of pseudo random sequences (Figure 4). The standard configuration consists of a set of 1,000,000 sequences of length 40 running on 1 thread and with a maximum Levenshtein distance of 3. To test the scalability as a function of a single parameter, only the parameter under study was modified whereas the others were kept constant.

Figure 4a shows the running time of starcode as a function of the number of input sequences n. In double logarithmic scale the trend is a straight line with slope 1.7, which suggests that the running time complexity of starcode is approximately $O(n^{1.7})$. Figure 4b shows that the running time grows exponentially as a function of the maximum Levenshtein distance used for clustering. This is a common feature of exact algorithms based on trie search. As this parameter increases, the poucet search bails out at a deeper average depth in a trie that fans out exponentially. As a function of the sequence length, the running time first increases but then plummets and stays low (Figure 4c). Beyond a certain length, the lookup search algorithm starts to be efficient, and most of the queries are resolved without the need for trie-search. Finally, we show the scalability of starcode with increasing number of threads in Figure 4d. The search algorithm is fully parallel and the relative performance increases linearly up to 8 threads. The bending observed thereafter has two sources. The first is that the input reading and clustering steps are brief but not parallel, the second is that there is insufficient memory bandwidth to satisfy the increased demand of memory accesses due to hardware limitations.

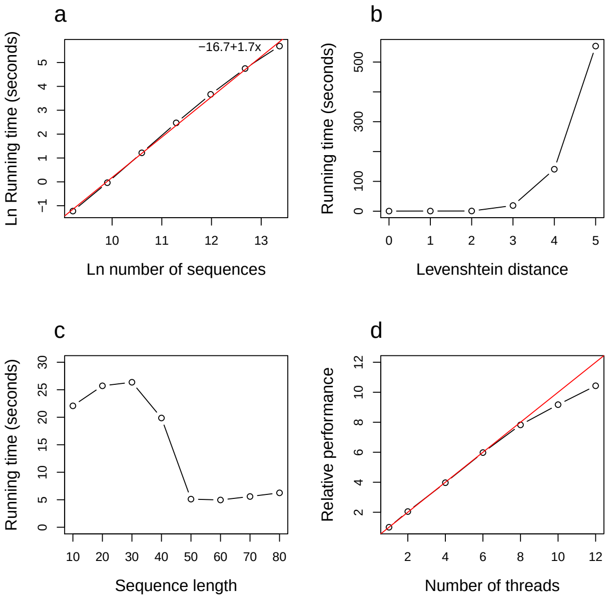


Fig. 4. Scalability. a. Logarithm of the running time versus the logarithm of the number of sequences to be clustered. b. Running time as a function of the clustering distance. c. Running time versus length of the input sequences. d. Relative performance increase for different number of parallel threads.

3.2 Benchmark against sequence clustering algorithms

Sequence clustering is routinely used to curate databases of non redundant nucleotide or protein sequences. We benchmarked starcode against the two popular sequence clustering algorithms CD-HIT (Fu *et al.*, 2012) and USEARCH (Edgar, 2010).

We generated 1 million random 40-mers duplicated 47 times. For each 40-mer, we also inserted 3 additional mutant sequences to the pool by sampling again 3 nucleotides at random positions. The probability that such a mutant sequence is within a distance 3 of any other 40-mer is of the order of 10^{-12} and can be neglected. Each cluster thus consists of 50 sequences, of which 3 have a Levenshtein distance 3 from the canonical representant (referred to as the centroid in clustering terms).

All software were set to run on a 6-core single-processor Intel Xeon E5-2620 system with 48 GB of DDR3-RAM at 1333 Mhz. Using 12 threads, starcode clustered the shuffled 50 million sequences in 5 minutes and 3 seconds without a single error (the output consisted of 1 million clusters of size 50). There is no obvious way to compare exact algorithms with heuristic algorithms such as CD-HIT and USEARCH. We decided to allocate the heuristics 6 minutes on the same dataset (20% additional time) and use the parameters giving the best accurary under this constraint. However, we could not find a combination of parameters such that either of them could terminate in less than 6 minutes. To simplify the task, we extracted the unique sequences from the input file, bringing the number down to 4,000,000 and followed the same rationale. We could not find any set of parameters such that USEARCH could

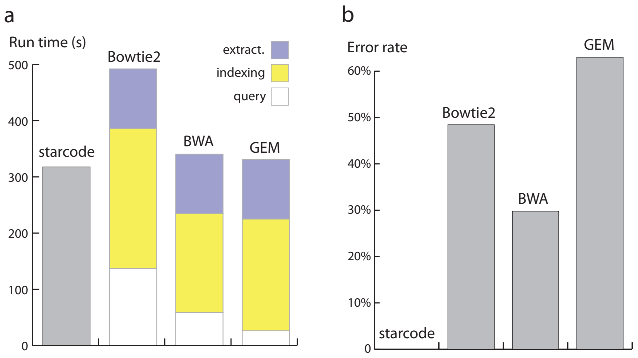


Fig. 5. Benchmark of starcode against short read mappers. **a** Running time compared to Bowtie2, BWA and GEM on the dataset described in the text. The running time is decomposed as extraction of unique sequences (purple), Burrows-Wheeler indexing (yellow) and query proper (white). The extraction was performed with the Linux `sort` command, so the time is the same in every case. **d** Error rate compared to Bowtie2, BWA and GEM, expressed as the proportion of matches with a single hit. This is an underestimate of the true error rate.

process this input in less than 6 minutes (because of poor multi-threading performance), but CD-HIT could perform the task when run as shown in Table 1. In such conditions, it identified 2,798,656 clusters, indicating that the clustering was not nearly complete.

Table 1. Software execution parameters (benchmark 1)

starcode	`-d3 -t12 -s`
CD-HIT 4.6.1	`cd-hit-est -n 7 -c .925 -T 12 -M 0 -r 0 -A 40`

We next benchmarked starcode on the biological dataset used by Fu *et al.* (2012). Because USEARCH imposes a memory limit on the size of the input file, we clustered the forward reads on 95% identity from one lane only (reference ERR011089), adding up to 4,189,859 reads, each 75 nucleotides long. Using 12 threads, starcode identified 4,111,111 clusters in 6 minute 19 seconds. Allocating up to 7 minutes 30 seconds to CD-HIT and USEARCH, we selected the parameters giving highest accuracy. In such conditions (see Table 2) CD-HIT identified 4,126,352 clusters while USEARCH could not be run in the allocated time. These results indicate that starcode achieves good performance on clustering high throughput sequencing reads.

Table 2. Software execution parameters (benchmark 2)

starcode	`-d4 -t12 -s`
CD-HIT 4.6.1	`cd-hit-est -n 8 -c .95 -T 12 -M 0 -r 0 -A 75`

3.3 Benchmark against short read mappers

A potential strategy to cluster short sequences is to use Burrows-Wheeler transform-based short read mappers to match a set of sequences against itself. Once matches are available, several community detection algorithms can be used to identify the clusters. We benchmarked starcode against the short read mappers Bowtie2 (Langmead and Salzberg, 2012), BWA (Li and Durbin, 2009) and GEM (Marco-Sola *et al.*, 2012). Short read mappers do not perform clustering, so we only evaluated their performance on the matching problem.

For this test, we used the first dataset described above, in which every sequence has at least 2 distinct matches at a distance 3 (mutant sequences from the same cluster may be at a distance up to 6 from each other, but their distance to the centroid is always 3). This means that we can use matches with a single hit (every read trivially matches itself) as a lower bound on the inaccuracy. We used the 4,000,000 unique 40-mers to build an index that was later queried with the same 40-mers using parameters shown in Table 3.

Table 3. Software execution parameters (benchmark 3)

Bowtie2 indexer	`bowtie2-build --quiet`
Bowtie2 2.1.0	`-f -a -p 12 --no-hd --very-sensitive`
BWA indexer	`bwa index -a is`
BWA 0.7.9a	`mem -t 12 -a -k1 -B0`
GEM indexer	`gem-indexer -T 12`
GEM 1.423	`-e3 -s4 -T12 --granularity 100000`

Figure 5a shows the running time of starcode along with the decomposition of the running time for the three mappers. Note that the time to extract unique sequences is always the same because we used the Linux `sort -u` command in every case. The running time of starcode is slightly lower than short read mappers when all steps ares taken into account. Figure 5b shows the lower bound on the error rate, as estimated by the proportion of single hits. BWA was found to be the most accurate aligner with a lower bound of 31% sequences with a single match. This measure is an underestimate of the true error rate, but given that starcode is an exact algorithm, a more accurate measure is not necessary: starcode can compete with the short read aligners on the matching problem, and it provides a precision that none of the other tools can offer.

3.4 Clustering TRIP barcodes

In the course of setting up the TRIP technology in our laboratory (Akhtar *et al.*, 2013), we realized the need to develop efficient algorithms to cluster similar sequences. Briefly, the principle of TRIP (Thousands of Reporters Integrated in Parallel) is to tag reporter transcripts with random barcodes and measure the abundance of barcodes in the RNA as a proxy for gene expression. There is no reference to match aberrant barcodes against, because the tagging sequences are unknown. Instead, barcodes are matched against each other and clustered by similarity to infer canonical sequences.

We tested the efficiency of starcode on the TRIP dataset from Akhtar *et al.* (2013). In the experiment labeled mPGKA, we identified 24.1 million (91%) barcode-containing reads out of 26.6 million, consisting of approximately 223,000 and 220,000 unique barcode sequences for PCR replicates 1 and 2, respectively. Following the authors, we kept only the barcodes with at least 5 counts and performed clustering as described: “First we sorted barcodes according to their counts. Then, for each barcode (starting from the most frequent one), we identified and removed all its mutant versions, defined as barcodes within a Hamming distance of 2." We implemented this method, here on referred to as the "sequential algorithm" with the function call `stringDist(method='lv', maxDist=2)` from R the package `stringDist` (R Core Team, 2014). When replacing the Hamming distance by the Levenshtein distance, starcode produced exactly the same output as the sequential algorithm. The running time of starcode averaged over the replicates was 2.90 seconds, *versus* more than 3 minutes 40 seconds, which represents a 75-fold speedup.

The performance of the sequential algorithm relies on the arbitrary exclusion of reads with less than 5 counts, the main purpose of which is to reduce the computational burden. When all barcodes were kept, the average running time of starcode was 10.27 seconds, *versus* $>$ 6 hours for the sequential algorithm. Using the Hamming distance, as the authors originally suggested, decreased the average running time of the sequential algorithm to about 3 hours and 30 minutes (and the output differed from that of starcode). Note that in all the cases mentioned above, starcode was run with a single core to compare the algorithms based on similar computer resources. In conclusion, the barcode clustering problem can be simplified by various tricks, but starcode brings down the running time to nearly instantaneous, and thereby obviates the need for such arbitrary heuristics.

3.5 Identifying enriched sequence motifs

Sequence motifs are thought to play an important role in DNA metabolism. Key regulators, such as transcription factors, nucleosomes and non coding RNAs have sequence preferences targeting them to the sites where they act. Identifying those sequences is a way to pinpoint the regulators and the mechanisms they are involved in. However, the sequence motifs are not strictly identical at different sites, hence they are better identified by inexact matching. This problem becomes computationally difficult for long motifs (above 12-13 nucleotides) because of the combinatorial scaling. But as motifs become longer, the problem of identifying abundant inexact matches becomes similar to barcode clustering. We reasoned that starcode could also be used for the task of identifying biologically meaningful sequence motifs.

We set up a test based on the meningitis-causing agent *Neisseria meningitidis*. The genome of this bacterium is interspersed with a frequent 12 bp sequence known as DNA uptake sequence (Smith *et al.*, 1999). We extracted the 12-mers from both orientations of the 2.19 Mb genome, yielding 4.39 million 12-mers, consisting of 2.77 million unique sequences. Clustering the 12-mers with starcode within a Levenshtein distance of 2 took less than 45 seconds with 12 threads. We identified the known DNA uptake sequence of *Neisseria meningitidis* (ATGCCGTCTGAA) as the most abundant 12-mer, with 1466 exact and 2096 inexact hits. This result testifies to the fact that starcode can be used to identify biologically relevant motifs in bacterial genomes.

To test starcode on another application, we used the RNA-protein interaction data produced by RNAcompete (Ray *et al.*, 2009). The mammalian splicing factor SRSF1 is known to bind RNA GA-rich motifs, but there is some disagreement about the motif that it recognizes (Pandit *et al.*, 2013). For each replicate of the human SRSF1 in the RNAcompete dataset, we replaced the microarray signals by their rank and extracted the 10-mers from the microarray probes. The 10-mers were given a score equal to the rank of the probe they belong, and enriched motifs were found using the sphere clustering of starcode with maximum Levenshtein distance 2. The score of the most enriched 10-mer is thus the sum of the ranks of all 10-mers within this distance. Clustering the 6.3 million extracted 10-mers with 12 threads took about 20 seconds for each replicate. The most enriched 10-mers were AGGACACGGA, AGGACACGGA, AGGACGGAGG, AGGACGGAGG, AGGACACGGA and AGGATACAGG. Except for the last replicate, the motifs consist of AGGAC and GGA, with a spacer of variable length. This suggests that the binding of SRSF1 to RNA may involve a spacer sequence, which would explain the disagreement between the motifs derived from 6-mers or 7-mers.

4 DISCUSSION AND CONCLUSION

Through the parallel poucet search algorithm, starcode implements an exact sequence clustering algorithm that can be faster than popular heuristics. By design, starcode is tailored to process high throughput sequencing data on multi-core platforms. Our benchmark shows that starcode achieves perfect clustering on short random sequences in less time than what is considered acceptable for heuristic searches. We also show that starcode outperforms next generation read mappers in this context. The software tools used for this benchmark are usually not used for barcode or random sequence clustering. In this respect, starcode fills a need arising from the development of barcoding technologies.

The speed of starcode also makes it useful for other clustering tasks, such as identifying enriched motifs in microbial genomes and in experimental data. Here we have given two examples of such applications. In the first, we recover a known enriched 12-mer in the genome of *Neisseria meningitidis*. In the second, we recover the motif of the human RNA binding protein SRSF1 and notice that it seems to consist of two halves separated by a linker. This hypothesis is consistent with the fact that SRSF1 binds RNA through two consecutive RNA Recognition Motifs (RRM) that are known to bind 3-4 nucleotides in a row(Daubner *et al.*, 2013). The Levenshtein distance, which incorporates insertions and deletions is more likely to capture bi-partite binding motifs than position weight matrix representations. The use of a clustering method to tackle this problem is unusual, but it it illustrates the potential advantages of distance-based approaches.

The current version of starcode has been primarily optimized for speed. The memory footprint depends on the number of sequences to cluster (because the sequences of the input set are loaded in memory as a set of of tries) and on the mean number of matches per sequence. Every match is stored in memory until the clustering phase, which may represent a large overhead if the dataset is dense. As counterintuitive as it may seem, long queries will usually impose a lower memory footprint because the matches between the sequences are more sparse. We have shown that the running time will also be shorter thanks to the lookup table search (Figure 4c).

Perhaps the most surprising element of this study is that an exact algorithm can compete with extremely fast heuristics. This will no longer be true when clustering divergent sequences because the Levenshtein distance will have to be increased, leading to exponentially longer running times (Figure 4b). However, for the imporant practical case that the divergence is driven by sequencing errors, starcode illustrates that there is still room for algorithmic innovations that can outperform heuristics. The idea of the poucet search seems simple in retrospect, yet it is a powerful way to tap into the data structuration provided by string sorting. This principle could find some applications in algorithms used in various fields.

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