

1 A survey of mapping algorithms in the long-reads 2 era

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12 — Abstract —

13 It has been ten years since the first publication of a method dedicated entirely to mapping third-
14 generation sequencing long-reads. The unprecedented characteristics of this new type of sequencing
15 data created a shift, and methods moved on from the *seed-and-extend* framework previously used for
16 short reads to a *seed-and-chain* framework due to the abundance of seeds in each read. As a result,
17 the main novelties in proposed long-read mapping algorithms are typically based on alternative seed
18 constructs or chaining formulations. Dozens of tools now exist, whose heuristics have considerably
19 evolved with time. The rapid progress of the field, synchronized with the frequent improvements of
20 data, does not make the literature and implementations easy to keep up with. Therefore, in this
21 survey article, we provide an overview of existing mapping methods for long reads with insights into
22 algorithmic details.

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29 **1** Introduction

30 With the introduction of PacBio long-read sequencing and later Oxford Nanopore Technologies
31 emerged a need for mapping long and noisy sequencing reads. The data proposed new
32 computational challenges of mapping millions of sequences, initially at expected error rates
33 of 10-20%. From the start, authors noticed that the seed-and-extend paradigm used in short-
34 read mapping was not practical for long-reads. First, seed-and-extend would usually rely on
35 a single match before extending, while long-reads required multiple consistent matches along
36 the read to be confidently mapped. Second, the extending part, which relies on alignment
37 algorithms with quadratic time complexity, had to be avoided given the combined length and
38 the frequent insertions and deletions in such data. Early on, the computational problem was
39 compared to whole-genome alignment, with the additional complexity of high error rates.
40 Such observations lead to the novel *seed-and-chain* paradigm for mapping long-reads (see
41 Figure 1). However, the first long-read alignment algorithms using older seeding techniques

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42 designed for generic sequence alignment (e.g., BLAST) were not time-competitive in their
43 throughput compared to short-read mappers. Thus, sketching and subsampling techniques
44 imported from comparative genomics started to appear in this domain.

45 Recently, specific sub-problems in the mapping domain have been identified and investig-
46 ated, such as partial and gapped alignment of reads for structural variant discovery, aligning
47 reads in repetitive regions or from non-reference alleles to correct loci, and other applications
48 such as spliced-mapping of RNA reads. These specific problems require and motivate novel
49 algorithmic solutions. In this survey article, we give an overview of the techniques that have
50 been proposed over the last decade for mapping long reads to genomes.

51 **2** Definitions and state-of-the-art of tools

52 2.1 Preliminaries

53 In this article we restrain ourselves to the problem of mapping a sequence shorter or equal
54 to a genome (a read) to a reference genome. We further assume that the reads come from a
55 genome that is closely related to the reference genome, such as from the same organism or a
56 closely related species.

57 Let $q = (q_1, \dots, q_l)$ be the read sequence of size l and $t = (t_1, \dots, t_n)$ the sequence of the
58 reference region of size n . Let $\Sigma = \{A, C, G, T\}$ and $\Sigma_+ = \{A, C, G, T, -\}$ be two alphabets,
59 x and y strings are defined on Σ . Let $f : \Sigma_+^* \rightarrow \Sigma^*$ be a transform that maps a string to its
60 subsequence with all "-" characters removed. An alignment is a pair of strings $(q', t') \in \Sigma_+^2$
61 such that:

- 62 1. $|q'| = |t'| = S$
- 63 2. $f(q') = q$ and $f(t') = t$
- 64 3. $(q'[i], t'[i]) \neq (-, -)$, for $0 \leq i < S$

65 Many alignments exist for a given pair of strings, in theory, the methods described
66 hereafter aim at finding *good* alignments, i.e. alignments that optimize some distance
67 between the pair of strings. The distance is computed using score functions which give rules
68 on the characters pairing.

69 With *read mapping*, we mean the procedure to find a read's location on the reference
70 genome. Typically, long-read mapping is performed by seeding and chaining the seeds into
71 high-scoring regions on the genome. In this study, a *read alignment* implies both that the
72 read has been mapped to a location, and that a pairwise alignment between the read and the
73 genome at the mapped location has been performed. Algorithms exist to compute optimal
74 semi-global pairwise alignments with respect to a score function. However, their complexity
75 in $\mathcal{O}(n \times l)$, disqualifies them in the context of handling big data such as sequencing data.
76 Therefore, methods of the literature use heuristics to perform read mapping on a reference.
77 They do not guarantee to find the optimal solution.

78 In our survey, we discuss read mapping to a genome sequence. We will use the terms
79 *query* for a read and *reference* to denote the genome.

80 2.2 Overview of fundamental ideas

81 To our knowledge, the first mapper explicitly written for long-reads was BLASR [12], although
82 short-reads mappers had been adapted for the long-read usage [37, 41, 47]. While solutions
83 specialized for either Nanopore [5] or PacBio [28] characteristics appeared, most modern
84 mappers work for both technologies with adapted parameters. BLASR presented itself as an

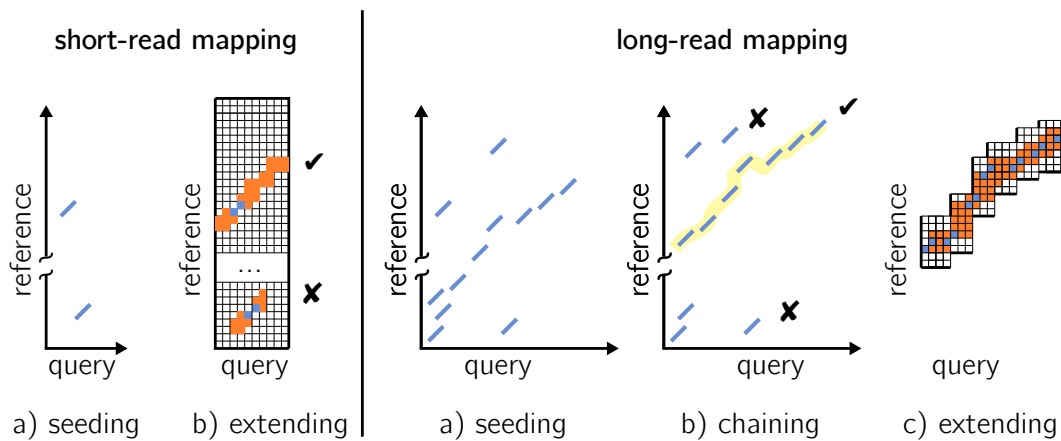


Figure 1 Differences in the main steps between short-read mapping (left) and long-read mapping (right). *Query* denotes the read and *reference* denotes a genome region. Mainly, short-read approaches extend (orange parts) from a single anchor (in blue) on the whole read length while long-read approaches gather multiple anchors, and chain (yellow line) them in for a candidate extending procedure that is done between pairs of anchors.

85 approach descending from both genome to genome alignment methods (such as MUMmer [16])
86 and short-read mappers. The paper contains seminal ideas used in modern long-read mappers
87 such as the seed-and-chain paradigm.

88 **Seeding** Seeding is the first operation in the heuristics used by mapping techniques.

89 ► **Definition 1.** A *seed* is a subsequence extracted from the query or the reference.

90 The purpose of seeding is to find relatively small matching segments between the query
91 and the reference that serves as markers for reference regions that potentially are similar to
92 the read. The reason seeding is used is that it is typically computationally efficient to find
93 matching seeds that can narrow down regions of interest compared to, *e.g.*, global alignment
94 of the read to the reference. As we will see in Section 3.1, seeds can be of different nature.
95 Seeding relates to pattern matching, although in sequence bioinformatics, practically all
96 approaches work under the paradigm which indexes the reference and query the index to find
97 matches. The underlying assumption is that once the index is created, it can be used several
98 times to map different query sets. To save space, reference indexes can be in a compressed
99 form. Once matches are found, a second operation aims at finding sets of concordantly
100 ordered seeds between the query and the reference (*chaining*; section 3.3 and to "fill the gaps"
101 between seeds as well as providing the final nucleotide level alignment (*extension*; section 4).
102 Seeding was quickly identified as a critical phase in long-read mapping, which led to novel
103 proposals [49, 42, 71].

104 **Sketching and subsampling** An important idea for seeding is *sketching* that was introduced
105 in MHAP, a long-read overlap finder implemented in an assembly algorithm [7]. Although long
106 read mappers had already been proven faster than alignment approaches [71], the rationale
107 was to improve the time efficiency of the long-read mapping problem in comparison to the
108 throughput of the second generation sequencing mappers. Sketching consists of compressing
109 the information of a set (here a set of k -mers) into a fixed-length vector (a sketch) of
110 representative elements called fingerprints. By comparing two sketches, one can approximate

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111 a similarity estimation of the two sets quickly and independently of their initial set sizes.
112 Several approaches exist [9, 54, 14]. MHAP relied on sketching with a MinHash approach.
113 MinHash [9] is a sketching technique based on locally sensitive hashing, which produces an
114 unbiased estimator for the Jaccard distance between two sets by selecting a subsample for
115 each set and comparing them in a very efficient way. Thus, MHAP overcame a space limitation
116 of BLASR which would index the whole reference. The type of matches (exact, fixed-size)
117 induced by MHAP's approach also allowed to perform rapid queries. An important limitation
118 of MHAP was that the sampling technique gave no guarantee to uniformly cover the query's
119 sequence. This led to the development of subsampling techniques which have been adapted
120 to approximate distances between sequences, starting with `minimap` [42]. Seeding is still an
121 active research area of long-read mapping with several recent developments [35, 70, 22, 60].
122 Sketching and subsampling are discussed in Section 3.1.2.

123 **Chaining** A key intuition is that in short-reads mapping, the extending procedure could
124 start after finding a single shared seed between the query and the reference, called anchors
125 (for details on techniques related to the previous sequencing generation, we refer the reader
126 to a methodological survey of short-read mapping [3]).

127 ► **Definition 2.** An *anchor* is a matching seed between the query and the reference. It is
128 represented by a pair of coordinates on the query and the reference.

129 In long-read mapping, the length of the reads and the short seed length used due to the
130 initial high long-read error rates can lead to a large number of seed matches. It is therefore
131 necessary to reduce the search space by selecting subsequences of ordered anchors (chains).

132 ► **Definition 3.** Let $\mathcal{A} = [a_0, a_1, \dots, a_k]$ be an list of anchors defined by their coordinates
133 on the reference and the query. A *chain* is a subsequence of \mathcal{A} of length $c \leq k$. A *colinear*
134 *chain* is a subsequence of \mathcal{A} in which anchors are sorted by such that if $i < j$, a_j is above
135 and to the right of a_i in the (reference, query) plane.

136 Drawing inspiration from genome-wide mapping, BLASR introduced a chaining step which
137 aims at selecting high-scoring chains from a set of candidate chains. Chaining allows to reduce
138 the final step of a long-read aligner (the base level extension) to alignment of sub-regions
139 between ordered anchors in chains. Chaining in long-reads has been solved using various
140 dynamic programming procedures [71, 61, 43]. In particular, the continuous work effort put
141 in `minimap2` [42, 43, 44] in both seeding and chaining processes made it a baseline for many
142 other tools' development.

143 While this survey covers the genomic mapping aspects, other important contributions
144 have dealt with adapted procedures in the case of long-read RNA mapping [53, 65, 50, 74],
145 and structural variant identification [68, 48, 24, 73], or other specialized problems [55]. Other
146 related research focused on read-to-read overlap detection [20, 75]², or alignment-free/pseudo-
147 mapping approaches [33, 13]. Finally, here we describe algorithmic solutions working on the
148 nucleotide sequence, but raw signal mappers for Nanopore long-reads is also an active area
149 of research [29, 76, 38].

² and the unpublished DALIGNER <https://github.com/thegenemyers/DALIGNER>

150 **3 A survey of algorithmic steps**

151 **3.1 Seeding almost always uses sampled, exact, fixed-length matches**

152 Seeding is the procedure that consists in collecting a set \mathcal{S} of seeds from the reference, then
153 finding matches between the query's seeds and \mathcal{S} . In order to find matches efficiently, \mathcal{S} is
154 stored using an index data-structure. In the following we detail the different types of seeds
155 that can be encountered and Figure 2 illustrates some of the approaches that have been
156 proposed.

157 **3.1.1 k -mers**

158 Substrings of length k , or k -mers, are perhaps the most commonly used seed in bioinformatics.
159 Such seeds can be extracted from the reference and stored for queries with little computational
160 cost. This makes k -mers popular in mapping and alignment applications that require high-
161 performance to scale for millions to billions of reads. A k -mer seed can be indexed by using
162 a hash function to produce an integer value (usually as a 32 or 64-bit integer), which is then
163 added to a hash table. This makes indexing of k -mers computationally cheap, provided that
164 the hash function and hash table implementations are efficient. Methods to efficiently hash
165 k -mers have been proposed [56], which uses the previous k -mers hash value to compute the
166 next one using a rolling hash function.

167 Both a strength and a weakness with k -mers are that if a k -mer match is found, it is
168 guaranteed to be exact. While it is desirable to produce matches only to identical regions, a
169 downside is that mutations will "destroy" the k -mers in the region. This has been studied
170 theoretically in [6] where the authors derived analytical expressions for the mean and variance
171 of regions without matches for a given mutation rate.

172 **3.1.2 k -mer subsampling techniques**

173 As any two consecutive k -mers share most of their sequence and are therefore mostly
174 redundant, we could reduce the memory overhead and query time without losing much of
175 the information if not all adjacent or nearby k -mers were stored. In the following, we present
176 different methods that allow picking a subsample of representative k -mers as seeds. These
177 approaches have proven their efficiency at reducing drastically the number of objects to index
178 while keeping high sensitivity and specificity for matches.

179 **No distance guarantee between seeds: sketching** Sketching gives typically no guarantee
180 of distance between two k -mer representatives, which means that a very large gap can appear
181 between two consecutive selected k -mers. An early work [7] bases its long-read mapping
182 strategy on MinHash sketching by using a total ordering on the k -mers' hashes (see (a) in
183 Figure 2), and keeping minimal hashes in the ordering (representing their k -mers). Related to
184 read mapping, it was used to perform genome-length sequences alignment-free mapping [33]
185 and to find read-to-read overlaps in long-read assembly [69]. However, fixed-size sketches do
186 not adapt well to different read lengths since the number of fingerprints remains constant for
187 any distinct k -mer number. Because of this, two similar regions from sequences of different
188 sizes will not automatically have the same representative, which is a desired property for
189 seeding. Therefore this approach was later replaced by other subsampling strategies in
190 following papers.

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191 **Distance guaranteed between seeds** On the contrary, subsampling techniques have been
192 proposed to guarantee that for a certain amount of consecutive k -mers, at least one will
193 be selected. The first k -mer subsampling technique proposed in the context of long-read
194 mapping was *minimizers* [62]. In our framework, minimizers are sampled k -mers given
195 two parameters m and w . Given the set k -mers starting in a window $[m, m + w - 1]$ of w
196 positions on the sequence, a minimizer is the minimal value over this set (and therefore the
197 k -mer associated with this minimal value) (see (b) in Figure 2). Minimizers are produced by
198 extracting a minimizer in each window $w \in [0, |S| - w + 1]$ over a sequence S . The techniques
199 used for assigning values to k -mers are discussed in section 3.2.1. Minimizers are agnostic
200 to their relative abundance over a sequence. Different optimizations have been proposed
201 to reduce the density of sampled minimizers in some regions. Weighted minimizers [35]
202 implement a procedure to select k -mers of variable rareness. In order for k -mers from
203 highly repetitive regions not to be as likely as others to be selected, it first counts k -mers,
204 and downweights frequently occurring ones. Then it takes this weight into account for the
205 hashing procedure. Other subsampling techniques include syncmers [18] and minimally
206 overlapping words (MOW) [23]. The first was used in the context of long-read mapping [70]
207 in an alternative implementation of `minimap2` and even more recently in [60]³. For their
208 construction, syncmers use s -mers of size $k - s + 1$ ($s < k$) occurring within k -mers (see (c)
209 in Figure 2, and Supplementary Figure S1 for an illustrated difference with the minimizers).
210 The k -mer is selected if its smallest (in the sense of an ordering, typically on hashes) s -mer
211 meets some criteria. An example criteria is that the s -mer appear at position p within the
212 k -mer ($1 \leq p < k - s + 1$). By construction, syncmers tend to produce a more even spacing
213 between sampled seeds while still allowing a distance guarantee.

214 **Context dependency of subsampling techniques** Minimizers are generated through a *win-*
215 *nowing* procedure which compares all k -mers of a given window. The choice of representative
216 k -mer in a given window depends on the window's k -mer content. This property has been
217 called *context dependency* [70]. On the contrary, syncmers can be described as context-free
218 since each k -mer's capacity to be selected is independent. Being context-free implies better
219 conservation of the overall sampled region under mutations. Indeed, context-dependent
220 representatives can tend to be broken over several consecutive windows because of the k -mers
221 propagating an error. Finally, other aspects can be considered, such as the related density [70]
222 (informally, the expected number of selected k -mer over the total number of k -mers), or the
223 deviation of minimizer-based strategies from the initial unbiased Jaccard estimator [6].

224 3.1.3 Fuzzy seeds handling substitutions

225 Due to read errors and SNPs between the reference and sequenced organism, it is in many
226 scenarios desired that a seed match between the query and the reference even if the seed
227 contains a substitution. Put differently, we would want similar k -mers to hash to identical
228 hash values. A hash function that would produce identical hash values for similar but not
229 necessarily identical inputs is usually referred to as a locality-sensitive hash function. We
230 will refer to seeds produced under such methods as fuzzy or inexact seeds.

231 Several methods to produce inexact seeds have been described. Perhaps the most common
232 one is spaced-seeds. Within a spaced-seed, some positions are required to match (called

³ <https://github.com/bluenote-1577/os-minimap2> and https://github.com/Shamir-Lab/syncmer_mapping

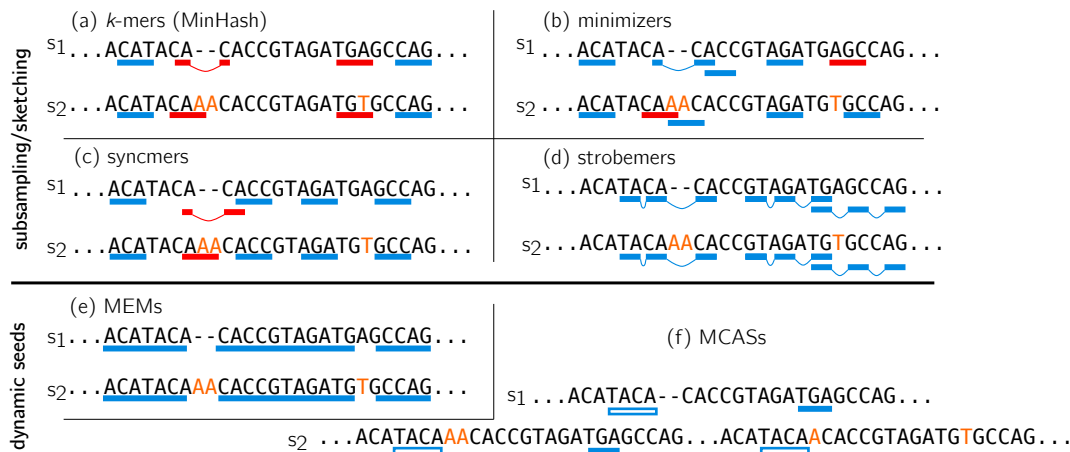


Figure 2 Overview of major seeding techniques used in long-read mapping. The figure presents informally which bases will be selected (underlined) given the technique. For the sake of simplicity, we are not consistent with a hash pattern (for instance lexicographical order) when selecting the seeds in the different panels. A more comprehensive example following a pattern is presented in Supplementary Figure S1.

We use two related sequences s_1 and s_2 which differ from a (A/T) substitution and a AA insertion in s_2 (in orange) to show the possible differences in selected bases (underlined in blue or red) due to mutations/errors. (a) k -mer seeds of length 3 selected with MinHash. k -mers have no distance guarantee and are picked based on having minimal hash value in total ordering of the hashes. (b) Minimizers picked with $k = 3$ a window size of w . Minimizers has a maximum distance guarantee given by w but has no minimal distance guarantee and may therefore subsample densely in some regions. (c) A subset of strobemers consisting of three *strobes* (short k -mers) are illustrated. The first strobe is picked at the seed start position and the remaining strobes are selected in windows downstream from the start strobe. (d) Syncmers selected with $k = 3$, s and the condition for selection are not detailed. Syncmers are context-free and respect a distance guarantee which tends to create pairs of evenly spaced seeds. (e) MEMs computed as exact matches until reaching a position that breaks the exactness. (f) MCAS. s_1 remain the same than in other panels, s_2 now contains two copies of a repeat, each has accumulated different mutations. A blue bordered region gives an example of a substring which is not a MCAS: it is repeated in the two copies. The blue-filled underlined region is a MCAS.

233 fixed positions) while the remaining positions can be ignored (called wildcards or don't care
 234 positions). Within a k -mer, fixed positions can be selected to be wildcards by applying
 235 particular masks on the k -mer's bases [32]. A problem with spaced-seeds is to find a fixed-
 236 position profile to minimize the overlap of the fixed positions in the seeds [31]. Although the
 237 computation of good spaced-seeds has been optimized [32], constructing good spaced-seeds
 238 profiles requires extra computational work compared to k -mers and is therefore slower to
 239 compute, and in practice, multiple different seeds are used [46] to increase sensitivity, which
 240 requires storing multiple hash tables. Another limitation with fuzzy seeds for substitutions
 241 is that seeds will, just as for k -mers, not match over indels.

242 While fuzzy-seeds handling substitutions have been used e.g. in metagenome short-read
 243 classification [10] and permutation-based seeds were implemented for short-read mapping [40],
 244 few of long-read mapping algorithms implement them. As indels are a frequent source of
 245 variability on long-reads, the computations to construct these seeds may not be worth the
 246 trade-off in increased sensitivity. An exception to this is a recent seeding mechanism [22],
 247 where the authors use a variant of SimHash [14](an alternative locality sensitive hashing to

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248 MinHash) to construct fuzzy seeds over subsampled k -mers using the minimizer technique [62].
249 The authors showed read alignment can be improved both in terms of speed and accuracy by
250 integrating their seeds into `minimap2` [43].

251 3.1.4 Fuzzy seeds handling indels

252 A common source of errors and biological variation is short insertions and deletions. Neither
253 the exact seeds nor the fuzzy seeds discussed so far are designed to match over such variability.
254 Traditionally, matching over indels has typically been solved not by a single query of a fuzzy
255 seed, but instead involved queries of a few short k -mers at a close occurring distance which are
256 then inferred as a matching region. While several queries in a nearby region usually provide
257 gold standard sequence similarity queries [4, 36], it comes at a significant computational cost.
258 Along the same vein [71] proposed to index one so-called spaced k -mer as a seed in each
259 position of the reference and would, query three different seeds for each position in the query
260 (representing a mismatch, a deletion of length one, and a mismatch and a one nucleotide
261 insertion). This design was motivated by overcoming the frequent substitutions and short
262 indels present in third-generation sequencing techniques, but would only handle indels of
263 one nucleotide (we provide details on this scheme in Supplementary Figures S2 and S3).
264 Earlier, there have been works to handle higher error rates with so-called covering template
265 families [26] that can guarantee a match up to any error rate e . Naturally, with higher e ,
266 more seeds need to be indexed and queried and it becomes computationally prohibitive to
267 use such seeding.

268 To remove the overhead of post-processing of nearby seeds [4, 36] or multiple queries [71]
269 per indexed reference seed, one can instead link the k -mers up into a seed before storing
270 it in the index. Such indexing has been favorable in the long-reads era where indels are
271 frequent. One proposed method is to join two nearby minimizers into a seed. Joining nearby
272 minimizers is usually a relatively cheap computation as the minimizers constitute a subset of
273 the positions on the reference. Such a seeding technique has been used for long-read overlap
274 detection for both genome assembly [15] and error correction [66]. While such indexing is
275 relatively fast and matches regions over indels, the joining of nearby minimizers implies that
276 if some minimizer(s) are destroyed due to mutations in a region, all of the seeds in that
277 region will be destroyed. Put another way, nearby seeds share the same information (in the
278 form of a shared minimizer). Therefore, alternative approaches such as strobemers [63] (see
279 (d) in Figure 2) have been described, where the goal has been to reduce the information
280 between closeby seeds by linking k -mers at seemingly random positions within a window.
281 Such pseudorandom linking implies that if one seed is destroyed due to a mutation, a nearby
282 seed may still match. Strobemers have shown effective at finding matches between long-reads
283 and for long-read mapping [63], and have been used in short-read alignment programs [64]
284 but they come at an increased computational cost to joining neighboring minimizers.

285 Another way to alleviate the issue that mutations will destroy consecutive seeds in the
286 neighboring minimizers technique is to apply the SimHash technique on strobemers instead
287 of k -mers [22]. Such seeds were used for long-read overlap detection [22] and the authors
288 show that for the highest quality long-reads (PacBio HiFi), such seeds can speed up long-read
289 overlap detection by an order of magnitude or more while retaining the same downstream
290 level assembly accuracy.

291 3.1.5 Dynamic seeds

292 Previously discussed seeds share the characteristic that they can all be produced and inserted
293 in a hash table, and consequently, only require a single lookup. This is typically fast and,
294 hence, popular to use in long-read alignment algorithms. The downside is that if a seed is
295 different in a region between the reference and the query (e.g., due to an error), there is no
296 way to alternate the seeds in this region at alignment time. There are however other types
297 of constructs, that we here refer to as dynamic seeds, that can be computed on the fly at the
298 mapping step, and then used as seeds downstream in the read alignment algorithm.

299 **Maximal Exact Matches** Maximal exact matches (MEMs) [16] are matches between a
300 query and reference sequence that cannot be extended in any direction on the query or
301 reference without destroying the match (see (e) in Figure 2). These are typically produced
302 by first identifying a k -mer match, and then an extension process is applied. MEMs are
303 guaranteed to be an exact match between the query and the reference and are bounded below
304 by length k but do not have an upper threshold for seed size. As there can typically exist
305 many MEMs, a subset of MEMs that has a unique location on both the query and reference
306 is sometimes considered. MEMs or similar approaches have been used in one of the earlier
307 long-read alignment programs (e.g., BWA-MEM) [41, 12] and for long-read splice alignment [65],
308 but these seeds are more computationally expensive to compute and are typically slower
309 than single-query seed-based algorithms.

310 **Anchors from minimal confidently alignable substrings (MCASs)** If a query was sampled
311 from a repetitive region in the reference, one may likely find several clusters of anchors
312 across the reference. Further dynamic programming operations to decipher the true origin
313 region of the query are typically costly or even unfeasible if too many copies have to be
314 considered. Even in the case a query is located on the reference, it might be attributed to the
315 wrong copy because of the sequencing errors. A recent contribution [34] proposed a solution
316 for handling seeding in repetitive regions. The procedure finds smallest subsequences that
317 *uniquely* match (MCASs) between the query and the reference (see (f) in Figure 2). There
318 can be as many as the query length in theory. In practice, the more the repeats are divergent,
319 the shortest the MCASs since a base pertaining to a single copy is more likely to be met.
320 MCASs are computed using an alignment procedure, which means that *uniquely* matched
321 must be understood as a relative property. For each position on a query, the best and
322 second-best alignment scores are compared, and a substring is considered uniquely matched if
323 the difference between the scores is above a threshold. It is interesting to bound the maximal
324 size of MCASs, both for performance purposes and because they may become less specific as
325 to their size increase. Fixed-size, exact match anchors (minimizers) are then extracted from
326 MCAS regions.

327 3.2 Implementation of the seeding step

328 3.2.1 Seeds transformations before indexing

329 Originally, minimizers use a lexicographical ordering. However, in our four base alphabet,
330 this can tend to select sequences starting with long alphabetically smaller runs such as
331 "AAA...". Random hash functions assigning each k -mer a value between 0 and a maximum
332 integer are preferred [67].

333 Oxford Nanopore reads are known for accumulating errors in homopolymers, typically
334 adding/removing a base in a stretch of a single nucleotide. Sequences can be homopolymer-

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335 compressed before finding k -mers. Homopolymers longer than a size s are reduced to a single
336 base, then k -mers are computed over the compressed sequence. For instance, for $s = 3$, $k = 4$,
337 an original sequence ATTTTGAAAACC is compressed to ATGACC, and the final k -mers
338 are ATGA, TGAC, GACC. This procedure allows finding more anchors while indexing fewer
339 k -mers/minimizers. Homopolymer compression is ubiquitous in long-read mappers.

340 In regions of low complexity (e.g. ATATATA, CCCCC) the standard minimizer procedure
341 keeps all minimal k -mers in windows. It is then possible for two k -mers to get the minimal
342 value and to be selected, which tends to over-sample repetitive k -mers. A *robust winnowing*
343 procedure is proposed in [35], which avoids the over-sampling effect by selecting fewer copies
344 of a k -mer, but increases the context dependency phenomenon.

345 3.2.2 Hash tables prevail for seed indexing

346 Indexing of fixed size is usually done using hash tables (although FM-indexes for k -mers
347 exist [8]). In the context of subsampling, invertible hash functions have been a key asset for
348 using minimizers as k -mers representatives. In other words, a hash value is associated with
349 one and only one k -mer, and the k -mer sequence can be retrieved from the hash value (using
350 reciprocal operations). This choice allows a very fast k -mer/minimizer correspondence but
351 is costly as it implies that the fingerprints of the hash table are not compressed (which is
352 mitigated by the subsampling). Minimizers are then used to populate a hash table, which
353 associates them to their position(s) in the reference and their strand information (usually
354 hashed seeds are canonical k -mers: the smallest lexicographic sequence between the original
355 k -mer and its reverse complement).

356 Variable-length seeds are indexed in full-text data structures (suffix arrays, FM-index),
357 which allow to find and count arbitrarily long queries in the reference. They have been
358 used in the first versions of long-read mappers. Variable-length seeds type can be longer to
359 query in the structure, while hashed matches are queried in constant time. Since minimizers
360 represent fixed-length k -mers, hash table solutions mainly prevail.

361 3.2.3 Seeds selection at the query

362 In [43], it is proposed to select all minimizers from the reference during the indexing phase
363 (although the latest versions include the weighted k -mers and robust winnowing heuristics),
364 and to soft mask some representative k -mers at the query. The procedure simply avoids
365 k -mers seen too many times according to a fixed cutoff. The authors noticed that in cases
366 where a query is sampled from a repetitive region, such a procedure prevents it to be seeded.
367 Therefore, an update was proposed [44], which detects if low occurrence k -mers are too
368 far away in a query, and in this case, allows sampling minimizers in the repetitive region
369 in between (and keeps some of the lowest possible occurrences among these minimizers).
370 Techniques that use longer fuzzy seeds (e.g., strobemers) [22] reduce the number of masked
371 regions, although it comes at the cost of sensitivity. Another approach [61] computes a new
372 set of minimizers on the targeted reference region in order to obtain finer candidate chains,
373 in particular in repeated or low complexity regions.

374 **3.3 Chaining is dominated by dynamic programming with concave gap**
375 **score functions**

376 **3.3.1 A dynamic programming problem**

377 Once the reference's seeds are indexed, a set of seeds is extracted from the query and looked
378 up in the index to find anchors. Anchors' positions on the query and reference are stored,
379 as well as the forward/reverse information. Instead of directly extending the alignment
380 between anchors, as it is done in short-read mapping, a step of chaining is added and meant
381 to accelerate further extensions. Chaining acts as a filter and a guide for smaller extensions
382 that need to be realized only between selected anchor pairs. Without it, too many extension
383 procedures, most of which would be dead-ends, would have to be started.

384 In an ideal case, there is a unique way of ordering anchors by ascending Cartesian
385 positions in the (*reference, query*) space, which passes by all the anchors. In practice, some
386 anchors are spurious, others correspond to repeated regions and yield different possible chains.
387 Moreover, over parameters have to be taken into account. Thus, methods optimize different
388 aspects (also illustrated in Figure 3):

389 A1) Do not allow anchors which are not ascending either by the anchors' start or end
390 coordinates in both the query and reference (see first case in Figure 3).

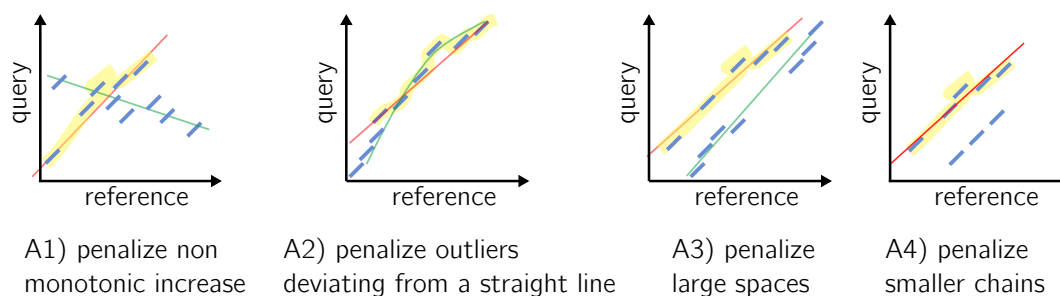
391 A2) Avoid discrepancies in diagonals between anchors (second case in Figure 3).

392 A3) Do not allow large spaces between consecutive anchors of the chain (see third case in
393 Figure 3).

394 A4) Favor the longest possible anchor chain (fourth case in Figure 3).

395 A5) If inexact matches in seeds are possible, find a series of anchors ensuring a minimal
396 Levenshtein distance between the query and the reference.

397 The problem of finding an optimal chain using non-overlapping anchors has been called
398 the *local chaining problem* [1], although in this application anchors can overlap. The score
399 $f(i + 1)$ represents the cost of appending an anchor a_{i+1} to a_i to the chain. This score is
400 often called the *gap score* in the literature, though it includes other constraints, as described
401 above. The chaining problem for long reads seeks to find an optimal colinear chain with a
402 positive gap score.



■ **Figure 3** An illustration of the different constraint taken into account in the gap score functions. The reference axis shows a genome region of interest where anchors were found, not the whole reference. A1–A4 correspond to items in the text in section 3.3.1. Anchors are showed in blue. The selected chain with respect to the described constraint is highlighted in yellow and a line approximately passing by its anchors is showed in red. The line passing by the longest chain is showed in green.

403 Mainly, methods use either a two-step approach: 1-find rough clusters of seeds as putative
404 chains, followed by 2-find the best scored chain among the selected clusters; or work in

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405 a single pass and apply a custom dynamic programming solution to find the best anchor
406 chain. We can start by noting that one of the first mappers dedicated to long-reads solved
407 a global chaining problem to determine a chain of maximum score, by fixing starting and
408 ending points (anchors) such that their interval is roughly the size of the query [12]. Such an
409 approach would easily discard long gaps and spaces in alignments.

410 3.3.2 Chaining in two steps

411 **Clusters of seeds are found through single-linkage in 2D space** The two-step approaches
412 rely on a first clustering step. Although it tends to be replaced by single-step chaining (see
413 Section 3.3.3), in the following we describe the fundamental ideas of the clustering. Methods
414 first find rough clusters of anchors by considering a discrete (*reference, query*) position
415 space. In this space, an anchor realizing a perfect match is a line of the size of the seed.
416 This line should have a 45-degree angle, which also corresponds to the main diagonal of a
417 (*reference, query*) alignment matrix. The same idea stands for a set of anchors. However,
418 because of insertions and deletions, each small line materializing an anchor may not be on
419 the exact same diagonal, thus realizing approximate lines in the (*reference, query*) space. A
420 method from image processing has been proposed to find approximate lines in this space:
421 the *Hough transform* [17], which makes it possible to detect imperfect straight lines in 2D
422 space. Contrary to linear regression which would output the best line explained by the
423 anchor distribution, here an arbitrary number of straight lines can be output and considered
424 (see Supplementary Figure S4 for an illustration). Hough transform or other similar anchor
425 grouping algorithms ([61] proposes to delineate fine-grained clusters in order to increase the
426 chaining specificity in repeated regions) all can be assimilated to single-linkage clustering in
427 2D space, which finds groups of anchors placed roughly on the same diagonal.

428 **Anchor chaining using longest subsequences of anchors** The previous clustering techniques
429 aim at finding lines in groups of anchors that can be approximately colinear. To determine
430 truly colinear chains, a subset of anchors can be ordered by finding a longest increasing
431 subsequence (LIS) of anchors. Let each anchor be mapped to $1 \dots n$ integers. The LIS
432 problem consists in finding a longest increasing subsequence from a permutation P of the set
433 $\{1, 2, \dots, c\}$, which can be solved in $\mathcal{O}(c \times \log(c))$.

434 In the case of exact fuzzy seeds, inexact matches are to be dealt with on top of the
435 initial increasing chain problem. Indeed, one wants to obtain the closest base-wise anchor
436 chain. In this case, the problem is converted to LCS $_k$ (longest common subsequence in at
437 least k -length substrings). Note that there is a correspondence between LIS and LCS. The
438 LIS of P is the LCS between P and the sequence $(1, 2, \dots, c)$. In both cases, neither the
439 longest nor the increasing requirements are sufficient to find correct anchor chains: they lack
440 definitions for other constraints, such as distance between anchors or the possibility to allow
441 large gaps. They are complemented with heuristics or replaced by more recent approaches in
442 Section 3.3.3. In addition, several methods use graphs built over anchors as backbones to
443 the chaining and alignment steps [73, 49, 71] (one approach is described in the Appendix).
444 Because they would fail to take into account distances between anchors, these methods have
445 been replaced by dynamic programming approaches relying on gap score functions.

446 3.3.3 Chaining in a single step: gap score functions

447 The main drawback of the approaches previously described in 3.3.2 is that though large
448 spaces between two anchors of a pair must be avoided, some spaces correspond to gaps in

449 the alignment and can be kept. In order to deal concurrently with these two problems, most
450 recent methods drop the two-step clustering and LIS to directly apply a custom dynamic
451 programming solution. It is globally the same spirit as LIS, but integrates a more fine-
452 grained gap penalty solution. It defines a cost function that grants a maximum penalty for
453 non-monotonic increasing seed chains.

454 **Concave gap functions** The cost function is designed to handle the gaps induced by frequent
455 indels in the data. Intuitively, it is likely that indels happen in clusters of n positions rather
456 than at n independent positions in the chain because some regions on the query might be
457 particularly spurious, or because of local repeats on the reference. Therefore, the same cost
458 is not attributed to opening a gap and extending a gap, thus a linear gap function does
459 not fit. The choice of gap functions which are concave (verifying the *Quadrangle Inequality*)
460 improves the time complexity by using the *wider is worse* strategy [25, 21]. In practice,
461 these concave gap functions are affine, a set of affine functions, or a combination of affine
462 and log functions, as proposed in [43]. We chose to present `minimap2`'s [43] gap functions in
463 Figure 4 as they are adopted without modifications in most current papers (with the recent
464 exception of [61]). Chains are built by aggregating close anchors of smaller coordinates to
465 the current anchor by penalizing the shifts compared to the main diagonal. In Figure 4,
466 Panel 4a presents how the set of possible anchors to prolong the chain is selected. Panel 4b
467 illustrates the dynamic function's parameters. The complete description of the functions is
468 available in the Appendix.

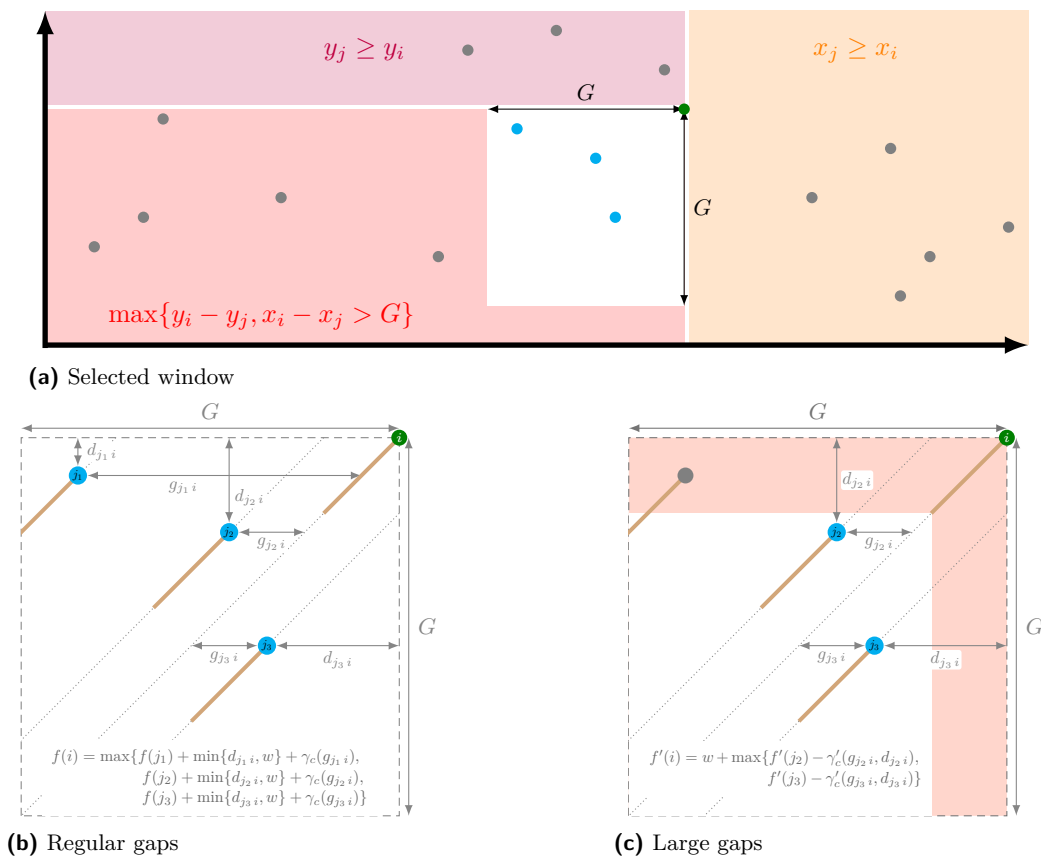
469 Heuristics are applied to rapidly drop a dynamic programming procedure in regions
470 that are unlikely to align and to avoid $O(c^2)$ worst cases. Based on empirical results, these
471 heuristics mostly check if seeds are not separated by too large regions and drop the chaining
472 procedure if the score becomes too low.

473 **Solutions for large gaps** Noticing that [43]'s original approach would be failing in large
474 gaps, one contribution [61] proposed techniques to perform dynamic programming with a
475 family of concave functions by relying on a previous work [21] (built on a prior clustering
476 step as described in 3.3.2). Recently, [43] integrated a solution designed for mapping long
477 structural variants in pangenomic graphs [45]. Its recent versions entail a cost function for
478 regular gaps, and a long gap patching procedure. Then it chooses the cheapest solution to
479 move on to the alignment step. The gap patching procedure uses a linear gap cost so that it
480 has a higher long-gap opening cost in comparison to the regular procedure but at a cheaper
481 extending cost. The chaining with a linear function is solved with a range minimum query
482 (RMQ) algorithm using a balanced binary search tree [1, 59]. It allows to solve the linear
483 chaining in $\mathcal{O}(c \times \log(c))$. Although this time complexity can be improved in $\mathcal{O}(c)$ by using
484 range maximum queue [11], the implemented algorithm is more costly than the solution for
485 regular gaps, which is preferred if possible. Panel 4c in Figure 4 illustrates the dynamic
486 function for large gaps.

487 3.3.4 Mapping quality scores have been adapted for ranking chains

488 The described methods may deliver a set of chains that satisfies the chaining score threshold.
489 To choose among the candidates and decide the final location, chains can then be categorized
490 into primary/secondary chains. Chains with a sufficient score are ranked from highest to
491 lowest score. Primary chains are those with the highest scores which do not overlap with
492 another ranked chain for the most of their length. Secondary chains are others. Mapping
493 quality, which is a measure that had been introduced to assess short-reads mapping, is

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■ **Figure 4** Outline of chaining of `minimap2`. Figure a shows for an anchor (in green) the selected region (in white, G is the gap threshold) to find available anchors to continue chaining (in blue). Figures b and c give respectively the dynamic programming functions for regular and large gaps size. Anchors are shown as segments ending with green or blue dots with the same color as in Figure a. Besides, for the large gap size (Figure c), to improve the complexity, the anchors do not overlap (available anchors are not in the red zone). d_{ji} represents the smallest "distance" between the two anchors (but is not really a distance by definition), w is the minimizer window size, g_{ji} is the gap length, and the γ functions are the concave gap functions.

494 redefined for long-reads with slight variations according to articles. It reports, for chains,
 495 whether the primary is very far in terms of score from the best secondary, and if it is long
 496 enough.

497 **4** Extension step and final alignment computation

498 **Extension step** In order to allow gaps, the methods rely on local alignment between
 499 pairs of successive anchors using classical algorithms [27, 57] derived from Needleman and
 500 Wunsch [58]. They are based on alignment matrices, which aggregate the base-wise alignment
 501 scores from the two prefixes (top left of the matrix) to the two suffixes (bottom right).

502 To compute the scores and report them in a matrix, affine cost functions allow to allocate
 503 different penalties for opening and extending gaps and therefore can favor short or long
 504 gaps. More precisely, such algorithms use pairs of affine gap score functions and choose the
 505 cheapest cost between the scoring for short gaps (i.e. less costly to open, costly to extend),

506 and the scoring for long gaps (i.e., more costly to open, cheap to extend). Allowing long gaps
507 has a drastic negative impact on the alignment efficiency because more cells in the alignment
508 matrix have to be considered.

509 **Heuristics for speed-up and quality enhancement** Therefore, alignment is commonly
510 accelerated through vectorization, using single instruction multiple data (SIMD) sets of
511 instructions, which increase the computational throughput by passing simultaneously several
512 matrix cells for the processors to evaluate. Second, practical alignment implementation
513 relies on banded alignment, which, simply put, bounds the alignment matrix in a band of
514 size ℓ around the top-left – bottom-right diagonal. Inspired from BLAST’s X-drop [4], [43]
515 implements a Z-drop procedure. X-drop quits extending the alignment if the maximum score
516 reached at some point when aligning the prefix drops by more than X. Z-drop adds the
517 possibility not to drop the extension during large gaps.

518 Due to sequencing errors, some spurious anchors main remain in a chain, which can
519 lead to a suboptimal alignment. At the alignment step, [43] chooses to remove anchors that
520 produce an insertion and a deletion at the same time ($>10\text{bp}$) or that lead to a long gap at
521 the extremity of a chain. Another solutions [12] involves to re-compute a chain with novel
522 anchors computed on a window that comprises the alignment.

523 **5 Future directions**

524 On top of mentioned novel seeding techniques bringing new properties concerning their
525 coverage of the seeded sequence and robustness to errors and mutations (syncmers, strobe-
526 mers [70, 60, 22]), we can expect to see advances in the chaining and extending parts in the
527 coming months.

528 Indeed, the usage of *diagonal-transition algorithms* which was initially define for edit
529 distance [72, 39, 30] has been reactivated recently for the gap-affine model with the wavefront
530 alignment algorithm (WFA, including [52, 51, 19]). More precisely, instead of using dynamic
531 programming on the adjacent cells, WFA transposes the optimization problem on the
532 diagonals and the score. In particular, WFA has the potential to make computation faster
533 for similar sequences and large gaps (by setting the score accordingly and adapting the
534 scoring). A current result shows that we can exploit the massive parallel capabilities of
535 modern GPU devices to accelerate this wavefront alignment algorithm [2]. Currently, different
536 implementations exist that have been tested on long reads [52]⁴, although no dedicated
537 long-read mapper integrates them yet.

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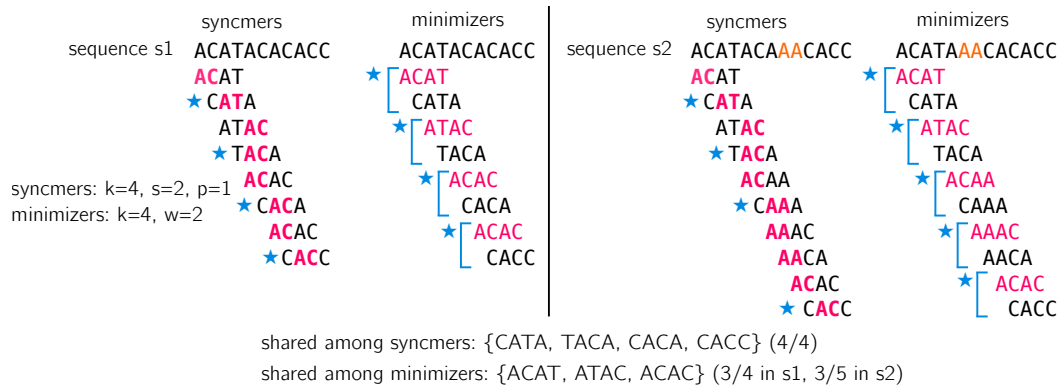
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731 Appendix

732 **Details on subsampling techniques** In Supplementary Figure S1, we present an example of the difference in the selected bases between a minimizer approach and a syncmer approach.



733

734 **Figure S1** Usage syncmers and minimizers for comparing two similar sequences. Sequences s1 and s2 differ by a AA insertion in orange in S2. We show how selected syncmers and minimizers do not produce the same sets of representative k -mers and therefore yield different fractions of shared k -mers between s1 and s2. The k -mer size is 4, the s -mers in syncmers (smallest showed in pink, we choose the lexicographic order) are of size 2, and in this example we require that the smallest s -mer appears at the first position of the k -mer. Minimizers have windows of size 2, materialized in blue, with the minimizer in pink. The selected k -mers are highlighted using a blue star.

733

734 **Graphmap's indexing strategy for fuzzy seeds** Graphmap builds two hash indexes from
 735 two types of shapes, called 6-1-6 and 4-1-4-1-4. As shown in Supplementary Figure S2,
 736 for each position is seeded (no subsampling). A seeded key corresponds to the subsequence
 737 at a given position of the reference when applying the shape mask: each *don't care* (*)
 738 base is skipped.

739 Then, for each read in the query, several lookup keys (for mismatch, deletion and insertion)
 740 are built from a shape (Supplementary Figure S3). To that extent, the lookup key treats the
 741 *don't care* base in three different ways. The mis(match) shape has the same behaviour as the
 742 indexed key, i.e., the *don't care* base are skipped. The insertion shape skips two bases: the
 743 initial *don't care* base and the base next to it. Finally the deletion shape will simply build
 744 the key and keep all the base including the *don't care* base. In total, for a number d of *don't*
 745 *care* base, 3^d different keys are built per shape.

746 **Graphmap's backbone graph for LCSk** Because of the possible spurious matches that
 747 occur because of the ambiguous bases, Graphmap's fuzzy seeds require more treatments to
 748 find proper chains. A first step after seeding finds groups of anchors representing longer
 749 shared subsequences between the query and the reference, on which is applied LCSk. Anchors
 750 are placed in a vertex-centric positional graph of k -mers, in which k -mers in both sequences
 751 appear, and share an arc if they are directly consecutive (or consecutive up to a distance
 752 parameter)⁵. Most weighted paths of anchors (i.e. supported by the query and the reference)
 753 are found in this graph and output as shared subsequences. After the LCSk pass, a L1 linear

⁵ NB: this is different from a de Bruijn graph since nodes with similar contents can be repeated

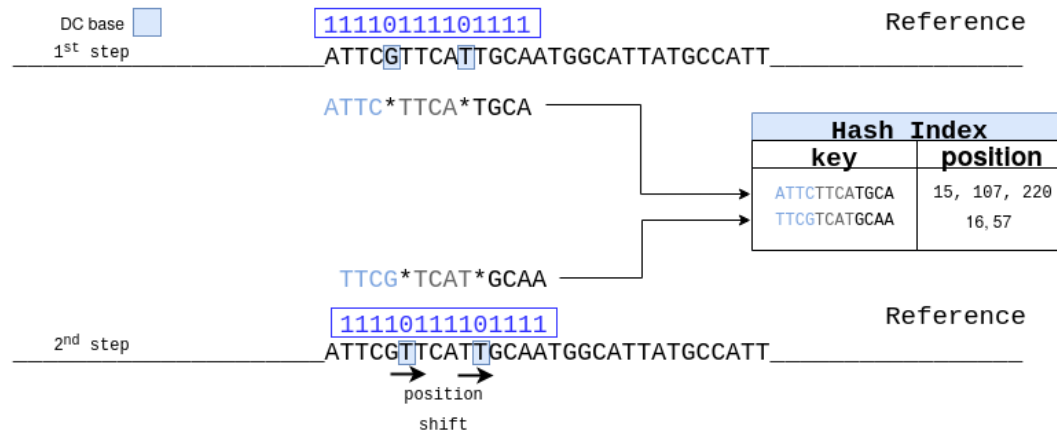


Figure S2 Indexing scheme for fuzzy seeds allowing indels and substitutions in Graphmap. In the figure the shape 4.1.4.1.4 is represented. The zeros represent the don't care positions of the shape. The shape is then applied for each position of the genome. The substring built from the shape is used as key inside a hash index. Each key will correspond to one or more positions on the reference.

754 regression step is applied to fit a straight line with a 45 degree slope and remove outliers,
 755 especially in the beginning and end of the chain (see case 4 in Figure 3 in the main text).
 756 Note that other methods use graphs built over anchors as backbones to the chaining and
 757 alignment steps without fuzzy seeds [73, 49].

758 Minimap2's complete formula for regular and large gaps size

759 ■ For regular gaps size:

$$760 \quad f(i) = \max\left\{ \max_{\substack{i > j \geq 1 \\ x_i - G < x_j \leq x_i \\ y_i - G < y_j < y_i}} f(j) + \min\{d_{ji}, w\} - \gamma_r(g_{ji}, w) \right\}$$

761 The property $x_i - G \leq x_j \leq x_i$ and $y_i - G \leq y_j < y_i$ is equivalent to $y_j < y_j, x_j \leq x_i$
 762 and $e_{ji} < G$.

763 ■ For large gaps size:

$$764 \quad f'(i) = \max_{\substack{i > j \geq 1 \\ x_i - G < x_j \leq x_i - w \\ y_i - G \leq y_j \leq y_i - w}} f'(j) + w - \gamma_l(g_{ji}, d_{ji})$$

765 where

$$d_{ji} = \min\{y_i - y_j, x_i - x_j\}$$

Smaller "distance" between the two anchors. This is not really a distance by definition : for $(x_i, y_i) = (-n, 0)$, $(x_j, y_j) = (0, 0)$ and $(x_k, y_k) = (0, n)$, we have $d_{ij} + d_{jk} = 0 < n = d_{ij}$.

$$766 \quad e_{ji} = \max\{y_i - y_j, x_i - x_j\}$$

Discrete Chebyshev distance between the two anchors

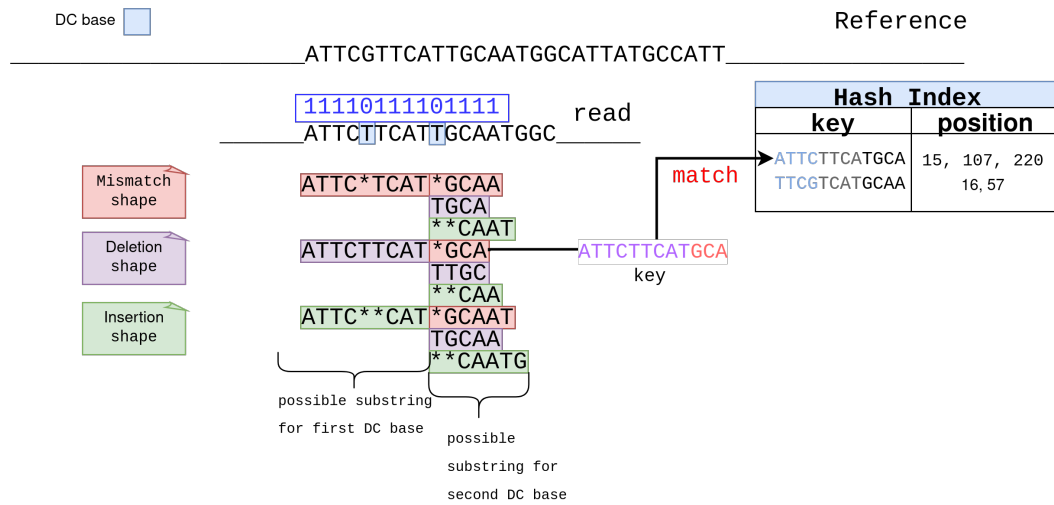
$$g_{ji} = |(y_i - y_j) - (x_i - x_j)|$$

Gap length (or Manhattan distance between the diagonals passing by the two anchors)

$$\gamma_r(g) = 0.01 \times w \times g + 0.5 \log_2 g$$

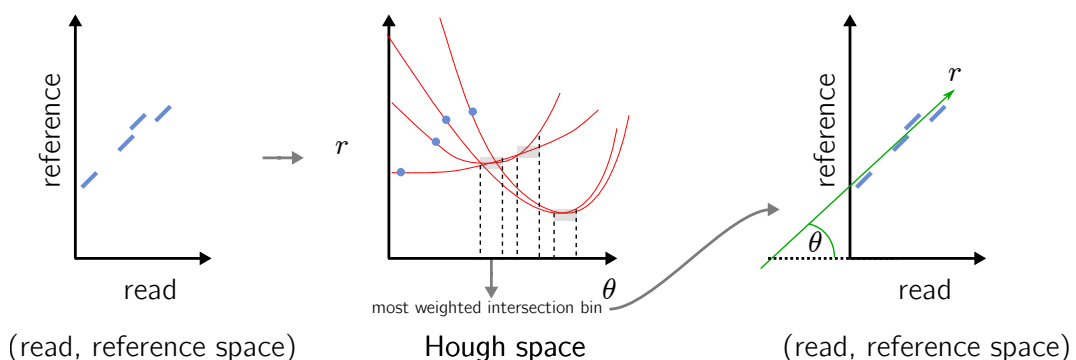
$$\gamma_l(g, d) = c_1 \times g + c_2 \times d + \log_2 g \quad \text{where } c_1 \text{ and } c_2 \text{ are parameters}$$

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■ **Figure S3** Query in Graphmap, different possible sequences can be matched using a single key. As we can see, there are three types of look-up shapes, and each of them is used to reconstruct a different substring. Each type corresponds to three phenomena that can occur with errors in sequencing, namely substitution, substitution + 1 insertion, and substitution + 1 deletion. Here, two don't care bases are present and nine substrings can be obtained. In this example the substring obtained from the substitution + insertion shape and the mismatch leads to a match with the reference.

767 **Hough transform principle** Applying the Hough transform means going from the $S_1 =$
 768 (*query, reference*) space to the Hough S_2 space of coordinates. If a line ($y = ax + b$) exists
 769 in S_1 , it is a point of coordinates (a, b) in S_2 (practically, polar coordinates are used for
 770 technical reasons). All possible lines intersecting a point in S_1 can be translated in S_2 as
 771 a sine wave. Multiple anchors give multiple points in S_2 , and the intersection of possible
 772 sinusoids intersecting the different points in S_2 correspond to a line roughly intersecting
 773 the initial anchors in S_1 . The Hough space is rasterized, and by counting and weighing the
 774 possible solutions in S_2 , lines can be deduced in S_1 . Contrary to linear regression which
 775 would output the best line explained by the seed distribution in S_1 , here an arbitrary number
 776 of straight lines can be output and considered (see Supplementary Figure S4 for an overview
 of the steps).



■ **Figure S4** An overview of the Hough transform steps.