New strategies to improve minimap2 alignment accuracy
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ABSTRACT
Summary: We present several recent improvements to minimap2, a versatile pairwise aligner for nucleotide sequences. Now minimap2 v2.22 can more accurately map long reads to highly repetitive regions and align through insertions or deletions up to 100kb by default, addressing major weakness in minimap2 v2.18 or earlier.

Availability and implementation: https://github.com/lh3/minimap2
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1 INTRODUCTION
Minimap2 (Li, 2018) is widely used for mapping long sequence reads and assembly contigs. Jain et al. (2020) found minimap2 v2.18 or earlier occasionally misaligned reads from highly repetitive regions as minimap2 ignored seeds of high occurrence. They also noticed minimap2 may misplace reads with structural variations (SVs) in such regions. These misalignments have been a pressing issue in the advent of temolere-to-telemore human assembly (Miga et al., 2020). Meanwhile, old minimap2 was unable to efficiently align long insertions/deletions (INDELS) and often breaks an alignment around variable-number tandem repeats (VNTRs). This has inspired new chaining algorithms (Li et al., 2020; Ren and Chaisson, 2021) which are not integrated into minimap2. Here we will describe recent efforts implemented in v2.19 through v2.22 to improve mapping results.

2 METHODS
2.1 Rescuing high-occurrence k-mers
Minimap2 keeps all k-mer minimizers (Roberts et al., 2004) during indexing. Its original implementation only selected low-occurrence minimizers during mapping. The cutoff is a few hundred for mapping long reads against a human genome. If a read harbors only a few or even no low-occurrence minimizers, it will fail chaining due to insufficient anchors.

To resolve this issue, we implemented a new heuristic to add additional minimizers. Suppose we are looking at two adjacent low-occurrence k-mers located at position x₁ and x₂, respectively. If |x₁ − x₂| ≥ 500, minimap2 v2.22 additionally selects |x₁ − x₂|/500 minimizers of the lowest occurrence among minimizers between x₁ and x₂. We use a binary heap data structure to select minimizers of the lowest occurrence in this interval. This strategy adds necessary anchors at the cost of increasing total alignment time by a few percent on real data.

2.2 Aligning through longer INDELS
The original minimap2 may fail to align long INDELS due to its chaining heuristics. Briefly, minimap2 applies dynamic programming (DP) to chain minimizer anchors. This is a quadratic algorithm, slow for chaining contigs. For acceptable performance, the original minimap2 uses a 500bp band by default, which means a gap longer than 500bp will stop chaining. To align through longer gaps, older minimap2 implemented a long-join heuristic as follows. If there is an INDEL longer than 500bp and the two chains around the INDEL have no overlaps on either the query or the reference sequence, minimap2 may join the two short chains later. This heuristic may fail around VNTRs because short chains often have overlaps in VNTRs. More subtly, minimap2 may escape the inner DP loop early, again for performance, if the chaining result is not improved for 50 iterations. When there is a copy number change in a long segmental duplication, the early escape may break around the event even if users specify a large band.

In minigraph (Li et al., 2020), we developed a new chaining algorithm that finds up to 1kb INDELS with DP-based chaining and goes through longer INDELS with a subquadratic algorithm (Abouelhoda and Ohlebusch, 2003). We ported the same algorithm to minimap2 for contig mapping. For long-read mapping, the minigraph algorithm is slower. Minimap2 v2.22 still uses the DP-based algorithm to find short chains and then invokes the minigraph algorithm to rechain anchors in these short chains. The rechaining step achieves the same goal as long-join but is more reliable because it can resolve overlaps between short chains. The old long-join heuristic has since been removed.

2.3 Properly mapping long reads with SVs
The original minimap2 ranks an alignment by its Smith-Waterman score and outputs the best scoring alignment. However, when there are SVs on the read, the best scoring alignment is sometimes not the correct alignment. Jain et al. (2020) resolved this dilemma by altering the mapping algorithm.

In our view, this problem is rooted in improper scoring: affine-gap penalty over-penalizes a long INDEL that was often evolutionarily created in one event. We should not penalize a SV by a function linear in the SV length. Minimap2 v2.22 instead rescres an alignment with the following scoring function. Suppose an alignment consists of M matching bases, N substitutions and G gap opens, we empirically score the alignment with

\[ S = M - \frac{N + G}{2d} - \frac{G}{\log_2(1 + g_i)} \]

where \( g_i \geq 1 \) is the length of the i-th gap and

\[ d = \max \left\{ \frac{N + G}{M + N + G}, 0.02 \right\} \]

It approximates per-base sequence divergence except with the smallest value set to 2%. As an analogy to affine-gap scoring, the matching score in our scheme is 1, the mismatch and gap open penalties are both 1/2d and the gap extension penalty is a logarithm function of the gap length. Our scoring gives a long SV a much milder penalty. In terms of time complexity, scoring an alignment is linear in the length of the alignment. The time spent on rescoring is negligible in practice.

3 RESULTS
We evaluated minimap2 v2.22 along with v2.18, Winnowmap2 v2.03 and Ira v1.3.2 (Table I). Both versions of minimap2 achieved high mapping accuracy on simulated Nanopore reads (sim-map). Winnowmap2 aligned more reads at mapping quality 10 or higher (mapQ10). However, it may occasionally assign a high mapping quality to a read with multiple identical best alignments. This reduced its mapping accuracy.

In lack of ground truth for real data, we took Winnowmap2 mapping as ground truth to evaluate other mappers (winno-cmp in Table I). Out of 1,378,092 reads with mapQ10 alignments by Winnowmap2, minimap2 v2.22 could map all of them. 118 reads, less than 0.01% of all reads, were mapped differently by v2.22. 51 of them have multiple identical best alignments. We believe these are more likely to be Winnowmap2 errors. Most of the remaining...
Table 1. Evaluation of minimap2 v2.22

| Benchmark         | Metric            | v2.22  | v2.18  | Winno | lra |
|-------------------|-------------------|--------|--------|-------|-----|
| sim-map           | % mapped reads at Q10 | 97.9   | 97.6   | 99.0  | 97.3 |
| sim-map           | err. rate at Q10 (phredQ) | 52     | 52     | 38    | 24  |
| winno-cmp         | rate of diff. (phredQ) | 41     | 37     | N/A   | 18  |
| sim-sv            | % false negative rate | 0.5    | 2.0    | 0.5   | 1.4 |
| sim-sv            | % false discovery rate | 0.0    | 0.1    | 0.0   | 0.1 |
| real-sv-1k        | % false negative rate | 7.3    | 20.0   | 13.0  | N/A |
| real-sv-1k        | % false discovery rate | 2.7    | 2.4    | 2.7   | N/A |

In [sim-map], 152,713 reads were simulated from the CHM13 telomere-to-telomere assembly v1.1 (AC: GCA009914755.3) with pbsim2 \(^*\) and simulated with PBSIM2: a simulator for long-read sequencers with a novel generative model of quality scores. BioRxiv. Ono, Y. et al. (2021). To see if minimap2 v2.22 could improve long INDEL alignment, we ran dipcall on contig-to-reference alignments and focused on INDELS longer than 1kb (real-sv-1k). v2.22 is more sensitive at comparable specificity, confirming its advantage in more contiguous alignment. Ira is supposed to handle long INDELS well, too. However, we could not get dipcall to work well with Ira, so did not report the numbers.

Minimap2 spends most computing time on base alignment. As recent improvements in v2.22 incur little additional computing and do not change the base alignment algorithm, the new version has similar performance to older versions. It is consistently faster than Winnowmap2 by several times. Sometimes simple heuristics can be as effective as more sophisticated yet slower solutions.

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**REFERENCES**

Abouelhoda, M. I. and Ohlebusch, E. (2003). A local chaining algorithm and its applications in comparative genomics. In *Algorithms in Bioinformatics*, Third International Workshop, WABI 2003, Budapest, Hungary, September 15-20, 2003, Proceedings, pages 1–16.

Harpak, A. et al. (2017). Frequent nonallelic gene conversion on the human lineage and its effect on the divergence of gene duplicates. *Proc Natl Acad Sci U S A*, 114:12779–12784.

Jain, C. et al. (2020a). Weighted minimizing sampling improves long read mapping. *Bioinformatics*, 36:i111–i118.

Jeffares, D. C. et al. (2017). Transient structural variations have strong effects on quantitative traits and reproductive isolation in fission yeast. *Nat Commun*, 8:14061.

Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, 34:3094–3100.

Li, H. et al. (2018). A synthetic-diploid benchmark for accurate variant-calling evaluation. *Nat Methods*, 15(8):595–597.

Li, H. et al. (2020). The design and construction of reference pangenome graphs with minigraph. *Genome Biol.*, 21:265.

Miga, K. H. et al. (2020). Telomere-to-telomere assembly of a complete human X chromosome. *Nature*, 585:79–84.

Ono, Y. et al. (2021). PBSSM2: a simulator for long-read sequencers with a novel generative model of quality scores. *Bioinformatics*, 37:589–595.

Ren, J. and Chaisson, M. J. P. (2021). Ira: A long read aligner for sequences and contigs. *PLoS Comput Biol*, 17:e1009078.

Roberts, M. et al. (2004). Reducing storage requirements for biological sequence comparison. *Bioinformatics*, 20:3363–9.

Sedlazeck, F. J. et al. (2018). Accurate detection of complex structural variations using single-molecule sequencing. *Nat Methods*, 15:461–468.

Zook, J. M. et al. (2020). A robust benchmark for detection of germline large deletions and insertions. *Nat Biotechnol*, 38:1347–1355.