Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM

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ABSTRACT

Summary: BWA-MEM is a new alignment algorithm for aligning sequence reads or assembly contigs against a large reference genome such as human. It automatically chooses between local and end-to-end alignments, supports paired-end reads and performs chimeric alignment. The algorithm is robust to sequencing errors and applicable to a wide range of sequence lengths from 70bp to a few megabases. For mapping 100bp sequences, BWA-MEM shows better performance than several state-of-art read aligners to date.

Availability and implementation: BWA-MEM is implemented as a component of BWA, which is available at http://github.com/h3-bwa

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1 INTRODUCTION

Most short-reads mappers for next-generation sequencing (NGS) data were developed when sequence reads were about 36bp in length. For 36bp reads, it is reasonable to require end-to-end alignment (i.e. every read base to be aligned to the reference) and to only report hits within certain hamming or edit distance. However, with emerging technologies and improved chemistry, NGS reads are not short any more, which poses new challenges to read alignment. For 100bp or longer reads, it becomes more important to allow long gaps under the affine-gap penalty and to report multiple non-overlapping local hits potentially caused by structural variations or misassemblies in the reference genome. Many short-read alignment algorithms are not applicable or not preferred for mapping longer reads. At the same time, although several mature algorithms exist for aligning capillary sequence reads, they are slow and lack features for analyzing large-scale NGS data. Fast moving NGS technologies keep pressing for the development of new alignment algorithms.

In this background, a few long-read alignment algorithms, notably including BWA-SW (Li and Durbin, 2010), Bowtie2 (Langmead and Salzberg, 2012), Cushaw2 (Liu and Schmidt, 2012) and GEM (Marco-Sola et al., 2012), have been developed. However, they all have some weakness. BWA-SW is slower than bowtie2 for 100bp reads at a comparable accuracy and less accurate then Cushaw2 at a comparable speed. Bowtie2 and Cushaw2 are slower for 600bp reads (see Results). While GEM is both fast and accurate for up to approximately 1000bp reads, it mandates end-to-end alignment and does not perform affine-gap alignment, which limits its uses for long-read alignment. Even for typical sequence reads ranged from 100bp to 1000bp in length, no mappers work well across the full spectrum. At the same time, the increasing read length not only calls for new alignment algorithms, but also opens the opportunity to de novo assembly which typically results in contigs ranged from several hundred base pairs to a few megabases. Very few algorithms are able to align sequences of such variable lengths at high accuracy and to be robust to translocations in the assembly. All these concerns motivated us to explore a new alignment algorithm.

2 METHODS

2.1 Aligning a single query sequence

2.1.1 Seeding and re-seeding BWA-MEM follows the canonical seed-and-extend paradigm. It initially seeds an alignment with supermaximal exact matches (SMEMs) using an algorithm we found previously (Li, 2012) (Algorithm 5), which essentially finds at each query position the longest exact match covering the position. However, occasionally the true alignment may not contain any SMEMs. To reduce mismappings caused by missing seeds, we introduce re-seeding. Suppose we have a SMEM of length $l$ with $k$ occurrences in the reference genome. If $l$ is too large (over 28bp by default), we re-seed with the longest exact matches that cover the middle base of the SMEM and occur at least $k+1$ times in the genome. Such seeds can be found by requiring a minimum occurrence in the original SMEM algorithm.

2.1.2 Chaining and chain filtering We call a group of seeds that are colinear and close to each other as a chain. We greedily chain the seeds while seeding and then filter out short chains that are largely contained in a long chain and are much worse than the long chain (by default, both 50% and 38bp shorter than the long chain). Chain filtering aims to reduce unsuccessful seed extension at a later step. Each chain may not always correspond to a final hit. Chains detected here do not need to be accurate.

2.1.3 Seed extension We rank a seed by the length of the chain it belongs to and then by the seed length. For each seed in the ranked list, from the best to the worst, we drop the seed if it is already contained in an alignment found before, or extend the seed with a banded affine-gap-penalty dynamic programming (DP) if it potentially leads to a new alignment. BWA-MEM’s seed extension differs from the standard seed extension in two aspects. Firstly, suppose at a certain extension step we come to reference position $x$ with the best extension score achieved at query position $y$, BWA-MEM will stop extension if the difference between the best extension score and the score at $(x, y)$ is larger than $Z + |x-y| \times P_{gapExt}$, where $P_{gapExt}$ is the gap extension penalty and $Z$ is an arbitrary cutoff. This heuristic avoids extension through a poorly aligned region with good flanking alignment. It is similar to the X-dropoff heuristic in BLAST (Altschul et al., 1997), but does not penalize long gaps in one of the reference or the query sequences.

Secondly, while extending a seed, BWA-MEM tries to keep track of the best extension score reaching the end of the query sequence. If the difference between the best score reaching the end and the best local alignment score is below a threshold, the local alignment will be rejected even if it has a higher score. BWA-MEM uses this strategy to automatically choose between local and end-to-end alignments. It may align through true variations towards the end of a read and thus reduces reference bias, while avoids introducing excessive mismatches and gaps which may happen when we force a chimeric read into an end-to-end alignment.
2.2 Paired-end mapping

2.2.1 Rescuing missing hits Like BWA (Li and Durbin, 2009), BWA-MEM processes a batch of reads at a time. For each batch, it estimates the means $\mu$ and the variance $\sigma^2$ of the insert size distribution from reliable single-end hits. For the top 100 hits (by default) of either end, if the mate is unmapped in a window $[\mu - 4\sigma, \mu + 4\sigma]$ from each hit, BWA-MEM performs SSE2-based Smith-Waterman alignment (SW; Farrar, 2007) for the mate within the window. The second best SW score is recorded to detect potential mismapping in a long tandem repeat. Hits found from both the single-sequence alignment and SW rescuing will be used for pairing.

2.2.2 Pairing Given the $i$-th hit for the first read and $j$-th hit for the second, BWA-MEM computes their distance $d_{ij}$ if the two hits are in the right orientation, or sets $d_{ij}$ to infinity otherwise. It scores the pair $(i, j)$ with $S_{ij} = S_i + S_j - \min\{-a \log_4 P(d_{ij}), U\}$, where $S_i$ and $S_j$ are the SW score of the two hits, respectively, $a$ is the matching score and $P(d)$ gives the probability of observing an insert size larger than $d$ assuming a normal distribution. $\log_4$ arises when we interpret SW score as odds ratio $O(d)$, where $U$ is a threshold that controls pairing: if $d_{ij}$ is small enough such that $-a \log_4 P(d_{ij}) < U$, BWA-MEM prefers to pair the two ends; otherwise it prefers the unpaired alignments. BWA-MEM chooses the pair $(i, j)$ that maximizes $S_{ij}$ as the final alignments for both ends. This pairing strategy jointly considers the alignment scores, the insert size and the possibility of chimeric read pairs.

3 RESULTS AND DISCUSSIONS

We evaluated the performance of BWA-MEM on simulated data together with NovoAlign (http://novocraft.com), GEM, Bowtie2, Cushaw2, SeqAlto (Mu et al., 2012), BWA-SW and BWA (Figure 1). On accuracy, NovoAlign is the best. BWA-MEM is close to NovoAlign for PE reads and is comparable to GEM and Cushaw2 for SE. On speed, BWA-MEM is similar to GEM and Bowtie2 for this data set, but is about 6 times as fast as Bowtie2 and Cushaw2 for a 650bp long-read data set.

We speculate BWA-MEM is more performant for longer reads firstly because of its advanced seeding algorithm, which identifies most standing seeds with one pass of the read, and secondly because of banded dynamic programming, which guarantees linear time complexity in the length of query sequences. BWA-MEM and BWA-SW are also able to identify chimeric reads, a crucial feature for contig alignment but lacked in most NGS long-read mappers. Our earlier paper (Li and Durbin, 2010) discussed this in details.

To evaluate the performance for even longer query sequences, we aligned the E. coli K-12 MG1655 strain (AC:NC_000913; 4.6Mb in length) against the 536 strain (AC:NC_008253) with both BWA-MEM and nucmer (Kurtz et al., 2004). Nucmer finished the alignment in 25 seconds, giving 105,505 substitution differences between the strains. BWA-MEM is slower. It finished the alignment in 131 seconds, reporting 104,321 substitutions of which 102,241 overlapping with the nucmer output. Manual examination of the substitutions unique to each aligner reveals that most of them fall in short regions with very high divergence. It is unclear which aligner is better. Note that although nucmer is faster in the evaluation, only BWA-MEM scales well to large genomes.

BWA-MEM is a fast and accurate aligner for sequence reads and is one of the few that work well for both 70bp reads and long sequences up to a few megabases. Technically, it is possible to make BWA-MEM even faster for long sequences by using SSE2-based banded DP and by restricting DP to regions not covered by a long exact match. Seeding is the bottleneck for short sequences, while banded DP is the bottleneck for long sequences.

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