Sequence analysis

Fast and accurate short read alignment with Burrows–Wheeler transform

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ABSTRACT

Motivation: The enormous amount of short reads generated by the new DNA sequencing technologies call for the development of fast and accurate read alignment programs. A first generation of hash table-based methods has been developed, including MAQ, which is accurate, feature rich and fast enough to align short reads from a single individual. However, MAQ does not support gapped alignment for single-end reads, which makes it unsuitable for alignment of longer reads where indels may occur frequently. The speed of MAQ is also a concern when the alignment is scaled up to the resequencing of hundreds of individuals.

Results: We implemented Burrows-Wheeler Alignment tool (BWA), a new read alignment package that is based on backward search with Burrows–Wheeler Transform (BWT), to efficiently align short sequencing reads against a large reference sequence such as the human genome, allowing mismatches and gaps. BWA supports both base space reads, e.g. from Illumina sequencing machines, and color space reads from ABI SOLiD machines. Evaluations on both simulated and real data suggest that BWA is ∼10–20× faster than MAQ, while achieving similar accuracy. In addition, BWA outputs alignment in the new standard SAM (Sequence Alignment/Map) format. Variant calling and other downstream analyses after the alignment can be achieved with the open source SAMTools software package.

Availability: http://maq.sourceforge.net
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1 INTRODUCTION

The Illumina/Solexa sequencing technology typically produces 50–200 million 32–100 bp reads on a single run of the machine. Mapping this large volume of short reads to a genome as large as human poses a great challenge to the existing sequence alignment programs. To meet the requirement of efficient and accurate short read mapping, many new alignment programs have been developed. Some of these, such as Eland (Cox, 2007, unpublished material), RMAP (Smith et al., 2008), MAQ (Li et al., 2008a), ZOOM (Lin et al., 2008), SeqMap (Jiang and Wong, 2008), CloudBurst (Schatz, 2009), NovoAlign (http://www.novocraft.com), ReSEQ (http://code.google.com/p/re-seq), Mosaik (http://bioinformatics.bc.edu/marthlab/Mosaik) and BFAST (http://genome.ucla.edu/bfast), hash the genome. These programs can be easily parallelized with multi-threading, but they usually require large memory to build an index for the human genome. In addition, the iterative strategy frequently introduced by these software may make their speed sensitive to the sequencing error rate. The third category includes slider (Malnisky et al., 2009) which does alignment by merge-sorting the reference subsequences and read sequences.

Recently, the theory on string matching using Burrows–Wheeler Transform (BWT) (Burrows and Wheeler, 1994) has drawn the attention of several groups, which has led to the development of SOAPv1 (Li et al., 2008b), PASS (Campagna et al., 2009), MOM (Eaves and Gao, 2009), ProbaseMatch (Jung Kim et al., 2009), NovoAlign (http://www.novocraft.com), ReSEQ (http://code.google.com/p/re-seq), Mosaik (http://bioinformatics.bc.edu/marthlab/Mosaik) and BFAST (http://genome.ucla.edu/bfast), hash the genome. These programs can be easily parallelized with multi-threading, but they usually require large memory to build an index for the human genome. In addition, the iterative strategy frequently introduced by these software may make their speed sensitive to the sequencing error rate. The third category includes slider (Malnisky et al., 2009) which does alignment by merge-sorting the reference subsequences and read sequences.

2 METHODS

2.1 Prefix trie and string matching

The prefix trie for string X is a tree where each edge is labeled with a symbol and the string concatenation of the edge symbols on the path from a leaf to
2.2 Burrows–Wheeler transform

Let \( \Sigma \) be an alphabet. Symbol \( S \) is not present in \( \Sigma \) and is lexicographically smaller than all the symbols in \( \Sigma \). A string \( X = a_0 \ldots a_{n-1} \) is always ended with symbol \( S \) (i.e. \( a_{n-1} = S \)) and this symbol only appears at the end. Let \( X[i] = a_i, i = 0, 1, \ldots, n-1 \), be the \( i \)-th symbol of \( X \), \( X[i] = a_i \) a substring and \( X[i] = X[n-1] \) a suffix of \( X \). Suffix array \( S \) of \( X \) is a permutation of the integers \( 0 \ldots n-1 \) such that \( S(i) \) is the start position of the \( i \)-th smallest suffix.

The BWT of \( X \) is defined as \( B[i] = S(i) \) when \( S(i) = 0 \) and \( B[i] = |X[S(i)-1]| \) otherwise. We also define the length of string \( X \) as \( |X| \) and therefore \( |X| = |B| = n \). Figure 2 gives an example on how to construct BWT and suffix array.

The algorithm shown in Figure 2 is quadratic in time and space. However, this is not necessary. In practice, we usually construct the suffix array first and then generate BWT. Most algorithms for constructing suffix array require at least \( n \log_2 n \) bits of working space, which amounts to 12 GB for human genome. Recently, Hon et al. (2007) gave a new algorithm that uses \( n \) bits of working space and only requires \(< 1 \) GB memory at peak time for constructing the BWT of human genome. This algorithm is implemented in BWT-SW (Lam et al., 2008). We adapted its source code to make it work with BWA.

2.3 Suffix array interval and sequence alignment

If string \( W \) is a substring of \( X \), the position of each occurrence of \( W \) in \( X \) will occur in an interval in the suffix array. This is because all the suffixes that have \( W \) as prefix are sorted together. Based on this observation, we define:

\[
R(W) = \min \{ k : W \text{ is the prefix of } X[k] \} \quad (1)
\]

\[
R(W) = \max \{ k : W \text{ is the prefix of } X[k] \} \quad (2)
\]

In particular, if \( W \) is an empty string, \( R(W) = 0 \) and \( R(W) = n-1 \). The interval \( [R(W), R(W)] \) is called the suffix interval of \( W \) and the set of positions of all occurrences of \( W \) in \( X \) is \( \{ S(k) : R(W) \leq k \leq R(W) \} \). For example in Figure 2, the SA interval of string ‘go’ is \([1,2] \). The suffix array values in this interval are 3 and 0 which give the positions of all the occurrences of ‘go’.

Knowing the intervals in suffix array we can get the positions. Therefore, sequence alignment is equivalent to searching for the SA intervals of substrings of \( X \) that match the query. For the exact matching problem, we can find only one such interval; for the inexact matching problem, there may be many.

2.4 Exact matching: backward search

Let \( C(a) \) be the number of symbols in \( X[0\ldots n-2] \) that are lexicographically smaller than \( a \in \Sigma \) and \( O(a,i) \) the number of occurrences of \( a \) in \( B[i] \). Ferragina and Manzini (2000) proved that if \( W \) is a substring of \( X \):

\[
\hat{R}(a\{W\}) = C(a)+O(a, R(W) - 1) + 1 \quad (3)
\]

\[
\hat{R}(a\{W\}) = C(a)+O(a, R(W)) \quad (4)
\]

and that \( \hat{R}(a\{W\}) \subseteq R(a\{W\}) \) if and only if \( \{W\} \) is a substring of \( X \). This result makes it possible to test whether \( W \) is a substring of \( X \) to count the occurrences of \( W \) in \( O(|W|) \) time by iteratively calculating \( \hat{R} \) and \( R \) from the end of \( W \). This procedure is called backward search.

It is important to note that Equations (3) and (4) actually realize the top-down traversal on the suffix trie of \( X \) given that we can compute the SA interval of a child node in constant time if we know the interval of its parent. In this sense, backward search is equivalent to exact string matching on the prefix trie, but without explicitly putting the trie in the memory.
Precalculation:
Calculate BWT string B for reference string X
Calculate array C(·) and O(·,·) from B
Calculate BWT string B′ for the reverse reference
Calculate array O′(·,·) from B′

Procedures:

\[
\text{INEXACTSEARCH}(W; z)
\]

\[
\text{CALCULATED}(W)
\]

\[
\text{return} \quad \text{INEXRECUR}(W, |W| - 1, z, 1, |X| - 1)
\]

\[
\text{CALCULATED}(W)
\]

\[
k \leftarrow 1
\]

\[
l \leftarrow |X| - 1
\]

\[
z \leftarrow 0
\]

\[
\text{for} \quad i = 0 \text{ to } |W| - 1 \text{ do}
\]

\[
k \leftarrow C(W[i]) + O(W[i], k - 1) + 1
\]

\[
l \leftarrow C(W[i]) + O(W[i], l) + 1
\]

\[
\text{if} \quad k > l \text{ then}
\]

\[
l \leftarrow |X| - 1
\]

\[
z \leftarrow z + 1
\]

\[
D(k) \leftarrow z
\]

\[
\text{INEXRECUR}(W, i, z, k, l)
\]

\[
\text{if} \quad z < D(0)
\]

\[
\text{return} \quad \emptyset
\]

\[
\text{if} \quad i = 0
\]

\[
\text{return} \quad [k, l]
\]

\[
\text{I} \leftarrow \text{I} \cup \text{INEXRECUR}(W, i, z - 1, k, l)
\]

\[
\text{for} \quad b \in \{A, C, G, T\} \text{ do}
\]

\[
k \leftarrow C(b) + O(b, k - 1) + 1
\]

\[
l \leftarrow C(b) + O(b, l) + 1
\]

\[
\text{if} \quad k \leq l \text{ then}
\]

\[
I \leftarrow I \cup \text{INEXRECUR}(W, i, z - 1, k, l)
\]

\[
\text{if} \quad b = W[i] \text{ then}
\]

\[
I \leftarrow I \cup \text{INEXRECUR}(W, i, z, k, l)
\]

\[
\text{else}
\]

\[
I \leftarrow I \cup \text{INEXRECUR}(W, i - 1, z - 1, k, l)
\]

\[
\text{return} \quad I
\]

Fig. 3. Algorithm for inexact search of SA intervals of substrings that match W. Reference X is SA terminated, while W is ACGGT terminated. Procedure \text{INEXACTSEARCH}(W; z) returns the SA intervals of substrings that match W with no more than z differences (mismatches or gaps); \text{INEXRECUR}(W, i, z, k, l) recursively calculates the SA intervals of substrings that match W[i], with no more than z differences on the condition that suffix W[i:] matches interval [k, l]. Lines started with asterisk are for insertions and deletions from X, respectively. D(0) is the lower bound of the number of differences in string W[0, i].

2.5 Inexact matching: bounded traversal/backtracking

Figure 3 gives a recursive algorithm to search for the SA intervals of substrings of X that match the query string W with no more than z differences (mismatches or gaps). Essentially, this algorithm uses backward search to sample distinct substrings from the genome. This process is bounded by the \text{D}(i) array where \text{D}(i) is the lower bound of the number of differences in W[0, i]. The better \text{D} is estimated, the smaller the search space and the more efficient the algorithm is. A naive bound is achieved by setting \text{D}(i) = 0 for all i, but the resulting algorithm is clearly exponential in the number of differences and would be less efficient.

\[
\text{CALCULATED}(W)
\]

\[
z \leftarrow 0
\]

\[
j \leftarrow 0
\]

\[
\text{for} \quad i = 0 \text{ to } |W| - 1 \text{ do}
\]

\[
\text{if} \quad W[j, i] \text{ is not a substring of X then}
\]

\[
z \leftarrow z + 1
\]

\[
j \leftarrow i + 1
\]

\[
D(i) \leftarrow z
\]

Fig. 4. Equivalent algorithm to calculate D(0).

The \text{CALCULATED} procedure in Figure 3 gives a better, though not optimal, bound. It is conceptually equivalent to the one described in Figure 4, which is simpler to understand. We use the BWT of the reverse (not complemented) reference sequence to test if a substring of W is also a substring of X. Note that to do this test with BWT string # alone would make \text{CALCULATED} an \(O(|W|^3)\) procedure, rather than \(O(|W|)\) as is described in Figure 3.

To understand the role of D, we come back to the example of searching for \(W = \text{GOOGOL}\) in \(X = \text{GOOGOL}\) (Fig. 1). If we set \(D(0) = 0\) for all i and disallow gaps (removing the two star lines in the algorithm), the call graph of \text{INEXRECUR}, which is a tree, effectively mimics the search route shown as the dashed line in Figure 1. However, with \text{CALCULATED}, we know that \(D(0) = 0\) and \(D(1) = D(2) = 1\). We can then avoid descending into the ‘o’ and ‘O’ subtrees in the prefix trie to get a much smaller search space.

The algorithm in Figure 3 guarantees to find all the intervals allowing maximum \(z\) differences. It is complete in theory, but in practice, we also made various modifications. First, we pay different penalties for mismatches, gap opens and gap extensions, which is more realistic to biological data. Second, we use a heap-like data structure to keep partial hits rather than using recursion. The heap-like structure is prioritized on the alignment score of the partial hits to make BWA always find the best intervals first. The reverse complemented read sequence is processed at the same time. Note that the recursion described in Figure 3 effectively mimics a depth-first search (DFS) on the prefix trie, while BWA implements a breadth-first search (BFS) using this heap-like data structure. Third, we adopt an iterative strategy: if the top interval is repetitive, we do not search for suboptimal intervals by default; if the top interval is unique and has \(z\) differences, we only search for hits with up to \(z + 1\) differences. This iterative strategy accelerates BWA while retaining the ability to generate mapping quality. However, this also makes BWA’s speed sensitive to the mismatch rate between the reads and the reference because finding hits with more differences is usually slower. Fourth, we allow to set a limit on the maximum allowed differences in the first few tens of base pairs on a read, which we call the \text{seed} sequence. Given 70 bp simulated reads, alignment with maximum two differences in the 32 by seed is 2.5× faster than without seeding. The alignment error rate, which is the fraction of wrong alignments out of confident mappings in simulation (see also Section 3.2), only increases from 0.08% to 0.11%. Seeding is less effective for shorter reads.

2.6 Reducing memory

The algorithm described above needs to load the occurrence array \(O\) and the suffix array \(S\) in the memory. Fortunately, we can reduce the memory by only storing a small fraction of the \(O\) and \(S\) arrays, and calculating the rest on the fly. BWT-SW (Lam et al., 2008) and Bowtie (Langmead et al., 2009) use a similar strategy which was first introduced by Ferragina and Manzini (2000).

Given a genome of size \(n\), the occurrence array \(O(·,·)\) requires \(4n(\log_2 n)\) bits as each integer takes \((\log_2 n)\) bits and there are \(4n\) of them in the array. In practice, we store in memory \(O(k, k)\) for \(k\) that is a factor of 128 and calculate the rest of elements using the BWT string \(B\). When we use two bits to represent a nucleotide, \(B\) takes 2n bits. The memory for backward search is
We tried 2 million 32 bp reads and did not see any reads mapped to poly-N

For \( k \) in BWA, we only store in memory \( S \) bp, configuration, for 15–37 bp reads, 2% uniform base error rate may contain differences more than the genome. Additionally, a few hundred megabyte of memory is required for a region, merge/sort alignments, get single nucleotide polymorphism (SNP) and indel calls and visualize the alignment.

BWA is distributed under the GNU General Public License (GPL). Documentations and source code are freely available at the MAQ web site: http://maq.sourceforge.net.

2.8 Mapping SOLiD reads

For SOLiD reads, BWA converts the reference genome to dinucleotide ‘color’ sequence and builds the BWT index for the color genome. Reads are mapped in the color space where the reverse complement of a sequence is the same as the reverse, because the complement of a color is itself. For SOLiD paired-end mapping, a read pair is said to be in the correct orientation if either of the two scenarios is true: (i) both ends mapped to the forward strand of the genome with the R3 read having smaller coordinate; and (ii) both ends mapped to the reverse strand of the genome with the R1 read having smaller coordinate. Smith-Waterman alignment is also done in the color space. After the alignment, BWA decodes the color read sequences to the nucleotide sequences using dynamic programming. Given a nucleotide reference subsequence \( h_2 \ldots h_{n+1} \) and a color read sequence \( c_2 \ldots c_{l+1} \) mapped to the subsequence, BWA infers a nucleotide sequence \( \hat{h}_2 \ldots \hat{h}_{l+1} \) such that it minimizes the following objective function:

\[
\sum_{i=1}^{l+1} q' \left( 1 - b_{i,h} \right) - q \left( 1 - \delta_{c_i,h_i} \right)
\]

where \( q' \) is the Phred-scaled probability of a mutation, \( q \) is the Phred quality of color \( c_i \) and function \( g(b') = g(b) \) gives the color corresponding to the two adjacent nucleotides \( b \) and \( b' \). Essentially, we pay a penalty \( q' \) if \( h_1 \neq \hat{h}_1 \) and a penalty \( q \) if \( c_1 \neq g(h_1, \hat{h}_1) \). This optimization can be done by dynamic programming because the best decoding beyond position \( i \) only depends on the choice of \( \hat{h}_i \). Let \( f(\hat{h}_i) \) be the best decoding score up to \( i \). The iteration equations are

\[
f(\hat{h}_{i+1}) = q' \left( 1 - b_{i+1,h} \right) + q \left( 1 - \delta_{c_{i+1},h_{i+1}} \right)
\]

BWA approximates bases qualities as follows. Let \( \hat{c}_i = g(h_i, \hat{h}_i) \). The \( i \)-th base quality \( \hat{q}_i \), \( i = 2 \ldots l \), is calculated as:

\[
\hat{q}_i = \begin{cases} 
q_i + q' & \text{if } c_{i-1} = \hat{c}_{i-1} \text{ and } c_{i-1} = \hat{c}_i \\
q_i - q & \text{if } c_{i-1} = \hat{c}_{i-1} \text{ but } c_{i-1} \neq \hat{c}_i \\
q_{i-1} + q' & \text{if } c_{i-1} = \hat{c}_i \text{ and } c_{i-1} \neq \hat{c}_{i-1} \\
q_{i-1} - q & \text{if } c_{i-1} = \hat{c}_{i-1} \text{ but } c_{i-1} \neq \hat{c}_i \\
0 & \text{otherwise}
\end{cases}
\]

BWA outputs the sequence \( h_2 \ldots h_{l} \) and the quality \( \hat{q}_2 \ldots \hat{q}_{l+1} \) as the final result for SOLiD mapping.

3 RESULTS

3.1 Implementation

We implemented BWA to do short read alignment based on the WRF of the reference genome. It performs gapped alignment for single-end reads, supports paired-end mapping, generates mapping quality and gives multiple hits if required. The default output alignment format is SAM (Sequence Alignment/Map format). Users can use SAMTools (http://samtools.sourceforge.net) to extract alignments in a region, merge/sort alignments, get single nucleotide polymorphism (SNP) and indel calls and visualize the alignment.

BWA is distributed under the GNU General Public License (GPL).
3.2 Evaluated programs

To evaluate the performance of BWA, we tested additional three alignment programs: MAQ (Li et al., 2008a), SOAPv2 (http://soap.genomics.org.cn) and Bowtie (Langmead et al., 2009). MAQ indexes reads with a hash table and scans through the genome. It is the software package we developed previously for large-scale read mapping. SOAPv2 and Bowtie are the other two BWT-based short read aligners that we are aware of. The latest SOAP-2.1.7 (Li et al., unpublished data) uses 2way-BWT (Lam et al., unpublished data) for alignment. It tolerates more mismatches beyond the 35 bp seed sequence and supports gapped alignment limited to one gap open. Bowtie (version 0.9.2) deploys a similar algorithm to BWA. Nonetheless, it does not reduce the search space by bounding the search with \( D(k) \), but by cleverly doing the alignment for both original and reverse read sequences to bypass unnecessary searches towards the root of the prefix trie. By default, Bowtie performs a DFS on the prefix trie and stops when the first qualified hit is found. Thus, it may miss the best inexact hit even if its seeding strategy is disabled. It is possible to make Bowtie perform a BFS by applying \( \text{–best} \) at the command line, but this makes Bowtie slower. Bowtie does not support gapped alignment at the moment.

All the four programs, including BWA, randomly place a repetitive read across the multiple equally best positions. As we are mainly interested in confident mappings in practice, we need to rule out repetitive hits. SOAPv2 gives the number of equally best hits of a read. Only unique mappings are retained. We also ask SOAPv2 to limit the possible gap size to at most 3 bp. We run Bowtie with the command-line option \( \text{–best k 2} \), which renders Bowtie to output the top two hits of a read. We discard a read alignment if the second best hit contains the same number of mismatches as the best hit. MAQ and BWA generate mapping qualities. We use mapping quality threshold 1 for MAQ and 10 for BWA to determine confident mappings. We use different thresholds because we know that MAQ’s mapping quality is underestimated, while BWA’s is overestimated.

Table 1. Evaluation on simulated data

<table>
<thead>
<tr>
<th>Program</th>
<th>Single-end</th>
<th>Paired-end</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (s)</td>
<td>Conf (%)</td>
</tr>
<tr>
<td>Bowtie-32</td>
<td>1271</td>
<td>79.0</td>
</tr>
<tr>
<td>MAQ-32</td>
<td>10977</td>
<td>81.0</td>
</tr>
<tr>
<td>SOAP-32</td>
<td>256</td>
<td>78.6</td>
</tr>
<tr>
<td>Bowtie-70</td>
<td>1726</td>
<td>86.3</td>
</tr>
<tr>
<td>MAQ-70</td>
<td>19728</td>
<td>91.0</td>
</tr>
<tr>
<td>SOAP-70</td>
<td>317</td>
<td>90.3</td>
</tr>
<tr>
<td>bowtie-125</td>
<td>1966</td>
<td>88.0</td>
</tr>
<tr>
<td>MAQ-125</td>
<td>3021</td>
<td>93.0</td>
</tr>
<tr>
<td>BWA-125</td>
<td>17506</td>
<td>92.7</td>
</tr>
<tr>
<td>SOAP-125</td>
<td>555</td>
<td>91.5</td>
</tr>
</tbody>
</table>

One million pairs of 32, 70 and 125 bp reads, respectively, were simulated from the human genome with 0.09% SNP mutation rate, 0.01% indel mutation rate and 2% uniform sequencing base error rate. The insert size of 32 bp reads is drawn from a normal distribution \( N(70,25) \), and of 70 and 125 bp reads from \( N(300,50) \). CPU time in seconds on a single core of a 2.5 GHz Xeon ES420 processor (Time), percent confidently mapped reads (Conf) and percent erroneous alignments out of confident mappings (Err) are shown in the table.

BWA and MAQ, BWA is 6–18 \( \times \) faster, depending on the read length. MAQ’s speed is not affected by read length because internally it treats all reads as 128 bp. It is possible to accelerate BWA by not checking suboptimal hits similar to what Bowtie and SOAPv2 are doing. However, calculating mapping quality would be impossible in this case and we believe generating proper mapping quality is useful to various downstream analyses such as the detection of structural variations.

On memory, SOAPv2 uses 5.4 GB. Both Bowtie and BWA uses 2.3 GB for single-end mapping and about 5 GB for paired-end, larger than MAQ’s memory footprint 1 GB. However, the memory usage of all the three BWT-based aligners is independent of the number of reads to be aligned, while MAQ’s is linear in it. In addition, all BWT-based aligners support multi-threading, which reduces the memory per CPU core on a multi-core computer. On modern computer servers, memory is not a practical concern with the BWT-based aligners.

3.4 Evaluation on real data

To assess the performance on real data, we downloaded about 12.2 million pairs of 51 bp reads from European Read Archive (ACERR000589). These reads were produced by Illumina for NA12750, a male included in the 1000 Genomes Project (http://www.1000genomes.org). Reads were mapped to the human genome NCBI build 36. Table 2 shows that almost all confident mappings from MAQ and BWA exist in consistent pairs although MAQ gives fewer confident alignments. A slower mode of BWA (no seeding; searching for suboptimal hits even if the top hit is a repeat) did even better. In that mode, BWA confidently mapped 89.2% of all reads in 6.3 hours with 99.2% of confident mappings in consistent pairs.

In this experiment, SOAPv2 would be twice as fast with both percent confident mapping (Conf) and percent paired (Paired)
The 12.2 million read pairs were mapped to the human genome. CPU time in hours on the MAQ package with additional features. SAMtools. BWA plus SAMtools provides most of functionality of to take the advantage of the downstream analyses implemented in tend to contain indels. BWA outputs alignment in the SAM format reads, which is increasingly important when reads get longer and alignment accuracy. It supports gapped alignment for single-end is an order of magnitude faster than MAQ while achieving similar 4 DISCUSSION

dropping by 1% if gapped alignment was disabled. In contrast, BWA is 1.4 times as fast when it performs ungapped alignment only. But even with BWT-based gapped alignment disabled, BWA is still able to recover many short indels with Smith–Waterman alignment given paired-end reads. We also obtained the chicken genome sequence (version 2.1) and aligned these 12.2 million read pairs against a human–chicken hybrid reference sequence. The percent confident mappings is almost unchanged in comparison to the human-only alignment. As for the number of reads mapped to the chicken genome, Bowtie mapped 2640, BWA 2942, MAQ 3005 and SOAPv2 mapped 4531 reads to the wrong genome. If we consider that the chicken sequences take up one-quarter of the human–chicken hybrid reference, the alignment error rate for BWA is about 0.06% (=2942×4/12.2M×0.889). Note that such an estimate of the alignment error rate may be underestimated because wrongly aligned human reads tend to be related to repetitive sequences in human and to be mapped back to the human sequences. The estimate may also be overestimated due to the presence of highly conservative sequences and the incomplete assembly of human or misassembly of the chicken genome.

If we want fewer errors, the mapping quality generated by BWA and MAQ allows us to choose alignments of higher accuracy. If we increased the mapping quality threshold in determining a confident hit to 25 for BWA, 86.4% of reads could be aligned confidently with 1927 reads mapped to the chicken genome, outperforming Bowtie in terms of both percent confident mappings and the number of reads mapped to the wrong genome.

4 DISCUSSION

For short read alignment against the human reference genome, BWA is an order of magnitude faster than MAQ while achieving similar alignment accuracy. It supports gapped alignment for single-end reads, which is increasingly important when reads get longer and tend to contain indels. BWA outputs alignment in the SAM format to take the advantage of the downstream analyses implemented in SAMtools. BWA plus SAMtools provides most of functionality of the MAQ package with additional features. In comparison to speed, memory and the number of mapped reads, alignment accuracy is much harder to evaluate on real data as we do not know the ground truth. In this article, we used three criteria for evaluating the accuracy of an aligner. The first criterion, which can only be evaluated with simulated data, is the combination of the number of confident mappings and the alignment error rate out of the confident mappings. Note that the number of confident mappings alone may not be a good criterion: we can map more at the cost of accuracy. The second criterion, which is the combination of the number of aligned reads and the number of reads mapped in consistent pairs, works on real data on the assumption that the mapping information from the experiment is correct and that structural variations are rare. Although this criterion is related to the way an aligner defines pair ‘consistency’, in our experience it is highly informative if the pairing parameters are set correctly. The third criterion is the fraction of reads mapped to the wrong reference sequence if we intentionally add reference sequences from a diverged species.

Although in theory BWA works with arbitrarily long reads, its performance is degraded on long reads especially when the sequencing error rate is high. Furthermore, BWA always requires the full read to be aligned, from the first base to the last one (i.e. global with respect to reads), but longer reads are more likely to be interrupted by structural variations or misassemblies in the reference genome, which will fail BWA. For long reads, a possibly better solution would be to divide the read into multiple short fragments, align the fragments separately with the algorithm described above and then join the partial alignments to get the full alignment of the read.

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REFERENCES


