Supplementary Materials

1 Preliminaries

We first briefly review some indexing data-structures: suffix array (Section 1.1) and Burrows-Wheeler Transform (Section 1.2). Then, a modified index is described in Section 1.3 that enables us to efficiently extract exact match hits of some fixed length strings.

1.1 The Suffix Array

Let $T[1..n]$ be a genome of length n where the nucleotides are represented by characters taken from the alphabet $\Sigma = \{a, c, g, t\}$. We assume that a special character \$ appears at the end of T, and it is assumed to be lexicographically smaller than all the characters in Σ. The suffix array $SA_T[1..n]$ of T is obtained by sorting the suffixes of T in lexicographically increasing order, and setting $SA_T[i]$ to be the starting position of the suffix in the i^{th} position in this sorted list. We will call each entry in SA_T as an SA_T-value.

Let P be a string. Suppose $SA_T[i]$ and $SA_T[j]$ are the lexicographically smallest and largest suffixes respectively, having P as a prefix. We define the interval $[i, j]$ as the SA_T range of P. The length of the SA_T range of P is $j - i + 1$. The entries $SA[i + k]$ for $k = 0, .., j - i$ gives all the locations in T where P occurs. Note that if the SA_T-range of P happens to be an interval of the form $[i, i]$, P occurs at a unique location in T.

1.2 The Burrows-Wheeler Transform

The Burrows-Wheeler transform (BWT) of T is a permutation of T derived from SA_T. Given SA_T, the BWT of T, denoted by B_T, is obtained by the formula $B_T[i] = T[SA_T[i] - 1]$ for $SA_T[i] > 1$; and $B_T[0] = \$$. BWT is highly compressible and it can be stored using $nH_k + o(n)$-bit space. BWT is a method suitable for indexing large genomes. It requires around 750MB for a genome as large as hg19.

With the help of some auxiliary data structures, BWT enables us to compute the SA range of a pattern using backward search.

Lemma 1.1 (Backward Search) *Consider a genome T. Given the BWT B_T and an auxiliary data structure of size $o(n \log n)$ bits. Let $[i, j]$ be the SA_T range of P. Then, the SA_T range of yP can be computed in constant time.*

Proof Let $C(y)$ be the total number of characters in string T that are lexicographically smaller than y. $C(y)$ for all $y \in \Sigma$ can be stored in $O(\log n)$ bits.

Let Occ_S be a function such that, for any $y \in \mathcal{A}$ and $1 \leq i \leq n$, $Occ_T(y, i)$ is the number of occurrences of y in $B_T[1..i]$ and $Occ(y, 0) = 0$. As shown in [**?**], we can store the Occ_T data-structure using $o(n \log n)$-bit space while allowing constant time access to any entry $Occ_T(y, i)$.

If $[i, j]$ is the SA_T range of P, [?] showed that the SA_T range of yP is $[C(y) + Occ_T(y, i1) + 1, C(y) + Occ_T(y, j)]$. Since the functions $C()$ and $Occ_T()$ can be computed in $O(1)$ time, the lemma follows. ∎

Furthermore, the following useful lemma can be shown to be true.

Lemma 1.2 (BWT Inversion) *Given the permutation of $T[i]$ under the BWT, and the auxiliary data structures in lemma 1.1, the permutation of $T[i+1]$ under the BWT can be obtained in $O(1)$ time.*

We can find the SA_T range of any pattern P by starting off with the empty string, whose SA_T range is $[1, n]$, and searching backward the characters of P using Lemma 1.1. This is called the backward search for P in T. If P does not exist in T, the result of the backward search is not a valid interval.

If the SA_T range of P is a valid interval $[i, j]$, the following lemma enables us to retrieve $SA_T[k]$ for each $k = i, \ldots, j$ in $O((j - i + 1)\kappa)$ time.

Lemma 1.3 *Given an $O((n \log n)/\kappa)$-bit auxiliary data-structure, for any k, $SA_T[k]$ can be computed in $O(\kappa)$ time.*

Proof As a pre-processing step, we sample and store each $SA_T[i]$, where i is a multiple of some constant integer κ. We can compute an arbitrary $SA_T[k]$ using this sampling and B_T. If k is a multiple of κ, $SA_T[k]$ can be found from the sampling. Otherwise, starting at $l = k$, by repeatedly applying Lemma 1.2 we count the number of steps s' needed to arrive at the first point where l' is a multiple of κ. Then $SA_T[k]$ is given by $SA_T[s] - s'$. This procedure needs on average $\kappa/2$ applications of the lemma. ∎

We choose $\kappa = O(\log n)$ in practice. In summary, using the BWT of T, we can store the index using $nH_k + o(n \log n)$ bits and enable us to find all hits of a pattern P using $O(|P| + occ \log n)$ time where occ is the number of hits of P in T.

If an SA_T-range $[i, j]$ is given, we can find all locations corresponding to it by simply using Lemma 1.3 to calculate $SA_T[k]$ for $i \leq l \leq j$. However, this basic method of converting SA_T ranges to locations can be time consuming, and we will next describe how to improve this step.

First we will describe how to efficiently convert an SA_T-value to the corresponding genomic location on the fly while performing a backward search with the following lemma.

Lemma 1.4 *Let P be a substring of T. When performing backward search for P, if the SA_T-range corresponding to $P[1..l]$ is in the form $[\kappa i, \kappa i]$, Then P occurs uniquely at the location $SA[\kappa i] - (|P| - l)$.*

Since $SA_T[\kappa i]$ is already sampled, if any backward search satisfies this condition, we can obtain the location of P in constant time as soon as the search terminates. For sufficiently long P, we can expect P to occur uniquely in T and to satisfy the above condition.

1.3 Retrieving Hits for a Fixed Length Pattern

Although BWT is space-efficient and can find the exact hits of a pattern, we need an average of $O(\log n)$ time to retrieve each hit. When the list of hits is long, it is time consuming to retrieve all hits.

We usually need to find hits of patterns having some fixed length l when mapping reads. Below, we describe a simple data-structure that will speedup the retrieval of such hits.

The basic observation is that, when l is long (say, $> \log_{|\Sigma|} n$), the SA_T-range for a length-l pattern is usually short. In fact, assuming the genome sequence is a random string, we can show that the expected size of the SA_T-range for a length-($\log_{|\Sigma|} n$) pattern is 1. If we don't store SA_T values for patterns whose SA_T-ranges are short, the data-structure will be small enough.

On the other hand, if the SA_T-range of a length l pattern is too long, the pattern is more likely to be extracted from low complexity or repeat regions. Since such patterns tend to be noisy, we will not use those hits.

Based on these two observations, our data-structure stores the SA_T values for patterns whose SA_T-ranges are of size between δ and D ($\delta < D$) as follows. Let $\mathcal{P} = \{P_1, \ldots, P_r\}$ be the set of length-l substrings of T whose number of hits in T are between δ and D. Let $[s_k, e_k]$ be the SA_T-ranges of P_k for $k = 1, 2, \ldots, r$. Note that the SA_T-ranges of all these patterns do not overlap. We define the reduced suffix array RSA_T to be an integer array formed by retaining only the SA_T values in $[s_k, e_k]$ for all $k = 1, \ldots, r$. SA_T values belonging to each $[s_k, e_k]$ are kept sorted in their positional order. Furthermore, we also store two arrays $S[1..r]$ and $SUM[1..r]$ where $S[k] = s_k$ and $SUM[k] = \sum_{i=1}^{k-1}(e_i - s_i + 1) + 1$. We denote our data-structure as $L_{T,l,\delta,D}$, and it consists of the BWT of T, RSA_T, S and SUM.

Figure 1 shows an example. Consider the string "$T = cccctgcggggccg\$$". The string contains 2-mers cc, cg, ct, gc, gg and tg. The BWT of T is "$tccgccgc\$gggccg$". Only the 2-mers cc, gg and cg appear more than once in T. Figure 1(b) lists down the occurrences of each of these in T sorted by their location, along with the SA-ranges. To construct $L_{T,2,2,10}$, we need to consider all 2-mers that appear at least twice and at most ten times, and the required data can be found in Figure 1(c). In the Figure, the SA-ranges are sorted by their starting position. Then, these starting positions are used as keys to the list of sorted locations belonging to the corresponding SA-ranges. If we are to construct $L_{T,2,2,3}$, then the entry for cc will be removed as it contains more than 3 entries. Table 1 shows that $L_{T,l,\delta,D}$ is space-efficient in practice, for a large genome like hg19 and for $\delta = 4$ and $D = 30,000$.

Now, given $L_{T,l,\delta,D}$ and the SA_T-range $[i, j]$ of a pattern P of length l, we can use algorithm SA_to_Loc in Figure 2 to get all of its occurrences in T efficiently. If P occurs at most δ times, or more than D times, we use the method given in Lemma 1.3 to obtain each $SA_T[i], \ldots, SA_T[j]$. Otherwise, we use $L_{T,l,\delta,D}$ to find all occurrences.

l-mer size	Size of $L_{T,l,\delta,D}$
18	2.5 GB
25	1.6 GB
30	495 MB
37	361 MB
68	244 MB
75	199 MB
90	140 MB
100	116 MB

Table 1: Size of the data structure $L_{T,l,\delta,D}$ for different values of l, where T is the hg19 genome, $\delta = 4$ and $D = 30,000$.

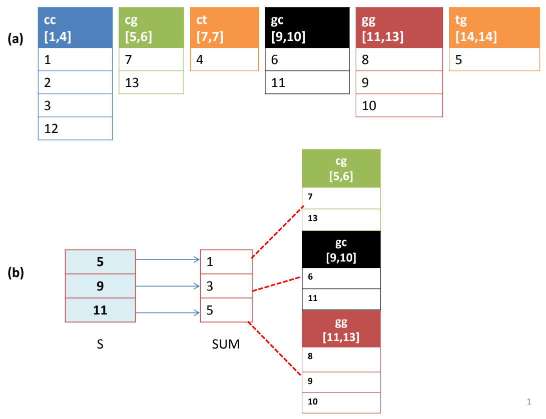


Figure 1: Illustration of the data structure for fast decoding of SA ranges for an example string $T = CCCCTGCGGGGCCG\$$. (a) shows the sorted positions of every non-unique 2-mers in the string. The label on top of of each list indicates the 2-mer and its SArange. (b) Data structure $L_{T,2,2,10}$ that indexes each 2-mer by the starting position of each SA-Range. The 2-mers TG and CT are not indexed as they occur uniquely in T. The 2-mers CC and GG do not appear as they appear more than twice. See Section 1.3 for details.

2 Bat-Align Mapping Strategy

Consider a genome T and a read R. This section describes the mapping strategy of Bat-Align. Our strategy first finds a set of candidate mapping locations (called hits) of R in T. The set of candidate hits is found by incrementally searching for the most likely combination of mismatches and indels. Based on the quality information and Smith-Waterman alignment, the best location is determined among them. Precisely, for every read R, our method performs three steps:

```
SA_to_Loc([i, j], L_{T,l,δ,D})

if $(j-i) > \delta$ and $(j-i) \le D$ then
    Binary search $S$ for the key $i$;
    if $S[k] == i$ then
        return $RSA[SUM[k]], \ldots, RSA[SUM[k+(j-i)]-1]$;
    else
        return $\emptyset$;
    end if
else
    $H = \emptyset$;
    for $k = i, .., j$ do
        $L$=location corresponding to $k$ found by inverting $BWT$.
        $H = H \cup L$
    end for
    return $H$;
end if
```

Figure 2: Algorithm $SA\_to\_Loc([i, j], L_{T,l,\delta,D})$ to convert SA_T-ranges to locations.

1. Finding candidate hits: This step identifies the hits of R that minimize the number of mismatches and indels using two strategies Reverse alignment and Deep scan;
2. Rescore and select the best hit: This step uses the quality scores and Smith Waterman algorithm to rescore the hits and identify the best hit.
3. Calculate the mapQ score of the best hit and report it.

In the following subsections, we will describe these three steps.

2.1 Finding Candidate Hits

Given a read R, this step aims to incrementally find the hits of R with the most likely combination of mismatches and indels. Its algorithm *Gen_Hits* is illustrated in Figure 3. we define a function F such that $F(i) = (p_i, q_i)$, where i, p_i and q_i are non-negative integers representing the number of mismatches and indels in an alignment respectively.The algorithm *Gen_Hits* incrementally tries to map R allowing $F(i)$ mismatch/indel combinations for $i = 0, 1.., M$. We call this method of incremental scanning using F as Reverse alignment. If $F(k)$ is the first such successful mismatch/indel combination, and there are multiple hits, we return all these hits. If there is a unique hit and $k < M$, we return all hits having the mismatch/indel combinations $F(k)$ and $F(k+1)$. We call this step the Deep scanning step.

In real-life, the likelihood of an indel in a genome is an order of magnitude less than that of a SNP and the likelihood of finding multiple indels becomes

```
Gen_Hits$(R, G, F, M)$

for $i = 0$ to $M$ do
    $H_1$=Hits of $R$ in $G$ having $F(i)$ mismatch/indel combinations.
    if $H_1$ is not empty then
        if $H_1$ contains unique hit and $i < M$ then
            $H_2$=Hits of $R$ having $F(i+1)$ mismatch/indel combinations.
            return $H_1 \cup H_2$.
        else
            return $H_1$
        end if
    end if
end for
```

Figure 3: Algorithm *Gen_Hits* to generate a candidate set of hits for examination

small as the length of R becomes small. If the sequencing is ideal and without any errors, we can expect mismatches in the read R to appear at a rate equal to the expected number of SNPs in a segment of length $|R|$ in G. However, empirical studies show that, for Illumina and SOLiD, the majority of mismatch errors occur due to sequencing errors. A general heuristic for such platforms is to set the mismatches in a read due to sequencing errors and SNPs to be about 4-5% of the read length. Furthermore, indels occur at a frequency of around 1 out 10k bases. Based on these figures, for a read of length around 75bp, we can set one indel and four mismatches as a reasonable upper bound for the number of indels and mismatches to be allowed in a mapping. For the default mode of enumerating candidate hits, We set $F^{-1}(n_1, n_2) < F^{-1}(n_1+1, n_2)$ for $n_1 \leq 4$ and $F^{-1}(5, 0) < F^{-1}(0, 1)$.

We use efficient BWT-based methods to enumerate hits corresponding to $F(i)$. With the restriction stated above, i.e. assuming we allow only one indel, we have two cases where $F(i)$ is of the form $(p_i, 0)$ or $(p_i, 1)$. When $F(i) = (p_i, 0)$, i.e. when only mismatches are allowed in R, we use the algorithm *BatMis* [**?**]. *BatMis* is a BWT-based algorithm that can be used to solve the k-mismatch problem efficiently. The following section describes the algorithm used to handle the case of a read having one indel in detail.

2.1.1 Finding Indel Hits.

When $F(i) = (p_i, 1)$, we allow p_i mismatches along with an indel. The algorithm *Find_Indel* is described in Figure 4. To find these hits, there are two cases. The first case is that the indel appears in one half of the read. Then the half of the read that does not contain the indel must contain at most p_i mismatches only. We identify hits in T having at most p_i mismatches with one half of R as

```
1:  $Find\_Indel(R, G, p_i)$
2:  {/* Case 1: Indel appears in one half of the read */}
3:  $X_1$= The set of $p_i$ mismatch hits of $R[1..|R|/2]$ in $G$.
4:  $H_1$= The set of hits having $p_i$ mismatches and one indel obtained by Smith-
    Waterman extension of hits in $X_1$.
5:  $X_2$= The set of $p_i$ mismatch hits of $R[|R|/2 + 1..|R|]$ in $G$.
6:  $H_2$= The set of hits having $p_i$ mismatches and one indel obtained by Smith-
    Waterman extension of hits in $X_2$.
    {/* Case 2: Indel is not completely contain in either half of the read */}
7:  for $j = 0$ to $p_i$ do
8:      $X_j$= The set of hits of $R[1..l]$ in $G$ having $j$ mismatches.
9:      $Y_j$= The set of hits of $R[|R| - l + 1..|R|]$ in $G$ having $j$ mismatches.
10: end for
11: for $j = 0$ to $p_i$ do
12:     $H = H \cup \{$ Hits in $X_j$ and $Y_{p_i - j}$ that are within $|R| - 2l + d$ bases from
        each other.$\}$
13: end for
14: $Hits$ = The set of hits in $H$ that can be extended to have $p_i$ mismatches
    and one indel.
15: return $H_1 \cup H_2 \cup Hits$.
```

Figure 4: Algorithm *Find_Indel* to find hits having one indel.

seeds and perform Smith-Waterman extension to recover locations having p_i mismatches and one indel for the whole read R.

The second case is that the indel is not completely contained in either half but is in the middle of the read, $Find\_Indel2$ is used. It will find $0, .., p_i$ mismatch hits of each l-mer suffix and prefix of R using the $BatMis$ algorithm. Then it will find the suffix and prefix locations that are at most $|R| - 2l + d$ bases apart using the method described in Section 2.1.2. Here, the value for l should be determined based on the maximum size of an insertion that is allowed. These potential hit locations are further examined by aligning against R with the Smith-Waterman algorithm, and those hits with p_i mismatches and one indel are reported.

2.1.2 Efficiently Pairing Two Patterns.

Given two patterns P_1 and P_2 of length l, we say that they can be paired if the two patterns can be found within a distance d of each other, with P_1 having the least genomic co-ordinate. Let $Occ_T(P)$ denote the number of times the pattern P occurs in T. We will describe now how to efficiently find pairings of P_1 and P_2 when $L_{T,l,\delta,D}$ is given and when $Occ_T(P_1) < D$ and $Occ_T(P_2) < D$.

1. If $Occ_T(P_1) < \delta$ and $Occ_T(P_2) < \delta$, we find all locations of P_1 and P_2. Then these locations can be cross checked to obtain all pairings of P_1 and P_2.

2. If $Occ_T(P_1) < \delta$ and $Occ_T(P_2) \geq \delta$, we obtain all the locations of P_2 from $L_{T,l,\delta,D}$. Since these are sorted by their position, we can check to see if any occurrence of P_1 can be paired with an occurrence of P_2 by performing a binary search.

3. If $Occ_T(P_1) \geq \delta$ and $Occ_T(P_2) < \delta$, the above procedure can be modified by looking up all the occurrences of P_1 from $L_{T,l,\delta,D}$.

4. If $Occ_T(P_1) \geq \delta$ and $Occ_T(P_2) \geq \delta$, all the occurrences of P_1 and P_2 are obtained from $L_{T,l,\delta,D}$. These two lists are merge sorted. If an occurrence of P_1 is followed by an occurrence of P_2, and they satisfy the distance constraint, we report them.

2.2 Reporting Hit.

Sequencing data usually produces a quality score that indicates the reliability of a base call. If the probability of a base call at position i being correct is $P(i)$, the quality score $Q(i)$ assigned to location i is given by the equation $P(i) = 1 - 10^{-Q(i)/10}$. Assuming that there is no bias to a particular set of nucleotides when a base gets miscalled with another nucleotide, then the probability of a base being miscalled at location i can be calculated by the formula $1 - P(i)/3$. For a given alignment, we compute an alignment score based on an affine scoring scheme. The score for a match or a mismatch at $R[i]$ is the phred scaled value of $P(i)$. When indels are present, positive gap open and extend values are chosen.

From Section 2.1, a candidate set X of mapping locations can be obtained for each read. Next, for each alignment in X we calculate the alignment score. If a unique alignment exists, it is reported. Otherwise, if the difference of alignment scores between the two smallest alignment scores is larger than some suitable cutoff(set as 10), the alignment with the smallest score is reported.

2.2.1 Increasing Sensitivity and Accuracy.

In Section 2.1 we described our basic criteria for finding a suitable set of hits. This criteria will miss enumerating the correct hit under three cases.(1) The read contains mismatches and indels outside the scope the algorithm is set to handle. (2) The read contains only m mismatches, but have a multiple mapping having less than m mismatches or a unique mapping having less than $m - 1$ mismatches.(3) A read containing indels with m mismatches might get mapped as pure mismatch hit or as an indel hit having less than m mismatches. We use several methods to recover these three kinds of hits.

During the stage described in Section 2.1.1, we might recover possible hits having more than the number of mismatches and indels specified by the basic algorithm. Instead of discarding them we will add them to the pool of candidate hits. If a hit found in the mismatch scanning stage turns out to have an indel when the full-read extension is done, an indel scan is forced. If the best alignment score happens to be larger than that of a single indel hit, again an indel scan is forced. Finally, for reads having more than one mismatch, the indel scanning is performed if the average quality of bases having a mismatch is higher than that of the average base quality of the read. We allow one high quality mismatch to consider the possibility of a SNP.

2.2.2 Making the Algorithms Faster.

We can use some strategies to speed-up the algorithm. These will result in a loss of accuracy and sensitivity. When extending the candidate hits along the full read at the indel stage. Instead of extending all the hits, we terminate the extension process, if for K successive steps, the extension has not resulted in an improvement in the alignment score. Furthermore, the extension can be limited to those hits already having an indel when an indel scan is forced.

The methods in Sections 2.2.1 and 2.2.2 has been used in various combinations to produce several modes of scanning that trades speed with accuracy and sensitivity.

2.3 Calculating Mapping Quality

The mapping quality is calculated as a measure to indicate the accuracy of the alignment, ranging from 0 (indicate a mapping without any confidence) to a value between 1 and 60, indicating the lowest and highest confidences for a hit that can be reported unambiguously. The mapping quality takes into account factors including the sequencing quality, the likelihood of the alignment appearing in the reference genome and the likelihood of the hit appearing by chance. We give an affine gap score S_{ref} to the final alignment to measure the likelihood of the sequence appearing in the reference. During the alignment stage, we count how many alignments S_{multi} the first half of the read will have when 0 mismatches are allowed. A large value for S_{multi} indicates a higher chance that the read will align by merely by chance. The final score S_{prior} uses quality information to factor in the prior probability of the read aligning at the reported location as explained below.

Let A_{top} be the best alignment returned by the aligner. Then we can calculate the prior probability to be

$$p(A_{top}|G,R) = \frac{p(R|G,A_{top})}{\sum_{i=1}^{|G|} p(R|G,x)},$$

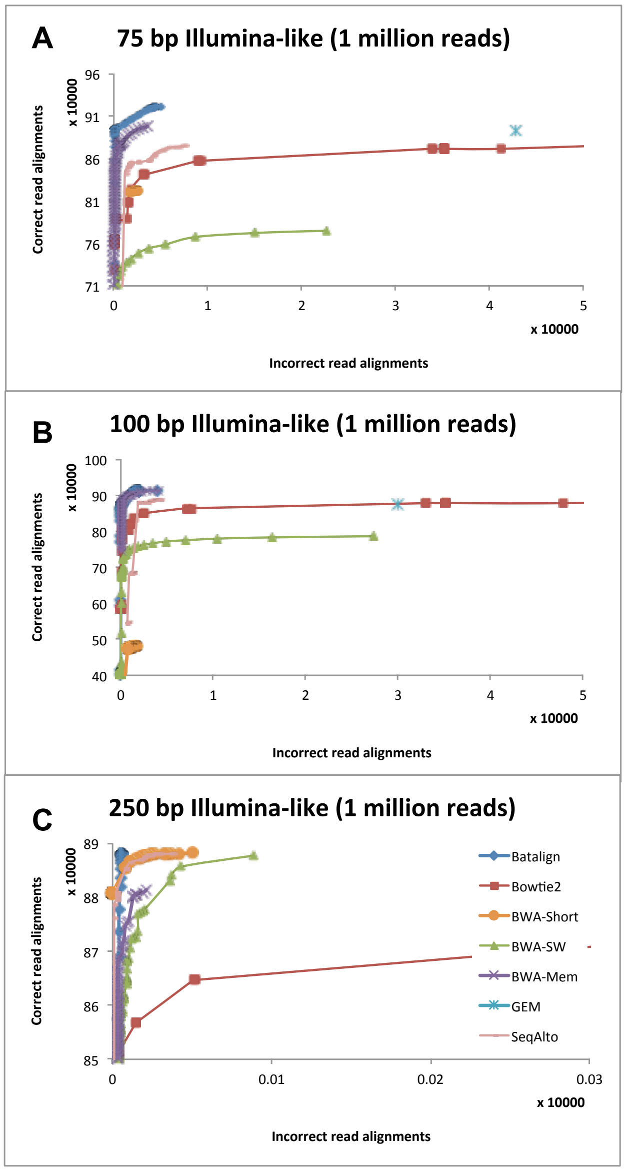
where $p(R|G,A_j)$ is the chance of R aligning at position A_{top}. A higher value of $p(R|G,A_{top})$ indicates a higher prior probability. For mismatch hits, $p(R|G,A_j) = 10^{-(\sum_i Q_i)/10}$, where Q_i are the quality scores at the mismatched bases. We set $S_{prior} = p(R|G,A_{top})$.

The final mapping score is calculated by the formula $60-k_1S_{ref}-k_2\min(10,S_{multi})+k_3S_{prior}$, where k_1,k_2 and k_3 are three positive constants that are empirically determined.

3. Evaluation on simulated reads

3.1 ART-simulated reads

We have simulated three dataset to evaluate the performance of the various methods in the main article. The mappings were stratified accordingly to the mapQ that the corresponding program assigns to them. The results were plotted with cumulative number of alignments, in order of decreasing mapQ, and were used to plot Supplementary Figure 3.1.



Supplementary Figure 3.1. Cumulative alignments stratified by decreasing mapQ as reported by their respective aligners in the form of ROC graphs. S.Figure A/B/C for 75/100/250 bp reads.

Similar to the experiments performed in GEM's paper, we also validated the top 10 hits reported by each method. The complete breakdown of this validation by the first (or best) alignment, as ordered by their respective aligner, can be found in Supplementary Table 3.1. From this table, we can see that BatAlign has reported the most number of correct hits as top-ranked hits across the 3 datasets of various read-lengths. It was only for the total correct hits on the 250 bp dataset that BatAlign is second to GEM by a small margin of < 0.01%. Upon inspection on the extra reads which GEM has reported, BatAlign found them to be repetitive and it was by chance that these hits were not represented as any of the top 10 hits for these reads.

Supplementary Table 3.1. Number of correct hits ordered by the rank that each aligner reported.

Rank	75bp dataset										
	#Correct hits										Sum of
Aligner	1	2	3	4	5	6	7	8	9	10	correct hits
BatAlign	933560	11604	732	622	745	335	146	116	90	68	948018
Bowtie2	866410	10873	4095	1541	551	314	192	142	112	77	884307
BWA-SW	786309	2	0	0	0	0	0	0	0	0	786311
BWA-Mem	897581	-	-	-	-	-	-	-	-	-	897581
GEM	893162	13603	5243	2231	1074	688	555	375	323	272	917526
BWA-Short	834519	10008	3535	1226	408	160	83	52	43	26	850060
Seqalto	885208	5692	1712	723	306	164	102	63	33	32	894035

Rank	100bp dataset										
	#Correct hits										Sum of
Aligner	1	2	3	4	5	6	7	8	9	10	correct hits
BatAlign	924272	7599	941	728	851	332	182	108	101	63	935177
Bowtie2	866310	8685	2833	948	254	110	69	42	23	5	879279
BWA-SW	794661	7	0	0	0	0	0	0	0	0	794668
BWA-Mem	912562	-	-	-	-	-	-	-	-	-	912562
GEM	875333	10327	3533	1445	638	377	283	193	163	178	892470
BWA-Short	484558	5207	1747	662	211	112	79	48	20	13	492657
Seqalto	890336	1821	515	194	91	44	38	11	3	8	893061

Rank	250bp dataset										
	#Correct hits										Sum of
Aligner	1	2	3	4	5	6	7	8	9	10	correct hits
BatAlign	894799	6394	732	824	225	175	107	98	53	50	903457
Bowtie2	892350	6245	1269	346	184	131	83	62	49	34	900753
BWA-SW	894395	1	0	0	0	0	0	0	0	0	894396
BWA-Mem	881193	-	-	-		-	-	-	-	-	881193
GEM	895450	5999	1203	319	201	116	92	79	50	46	903555
BWA-Short	894658	5615	800	67	0	0	0	0	0	0	901140
Seqalto	894661	3775	296	77	41	33	14	9	9	9	898924

3.2 Simulated Indel-abberrant reads

We have also simulated a new read class – Indel-abberrant datasets for analyzing the performance of the various programs on mapping indel-aberrant reads. We have tested various programs on read set with indel ranging from 1bp to 8bp long; each with 1 million 75 bp reads. For each of the indel-lengths, we further classified the simulated-variant into insertions or deletion class. Supplementary Table 3.2 detailed on the calculated F-measures in the main paper.

Supplementary Table 3.2. Results on mapping Indel-abberrant datasets by various programs

Methods	Delete-aberrant		
	Correct hits	Wrong hits	F-measure
BatAlign	856069	5365	92.0%
Bowtie2	819007	70844	86.7%
BWA-Short	764699	2580	86.5%
BWA-SW	719140	22798	82.6%
GEM	833640	38252	89.1%
SeqAlto	813325	7593	89.3%
BWA-Mem	833994	3746	90.8%

Methods	Insert-aberrant		
	Correct hits	Wrong hits	F-measure
BatAlign	855681	5586	91.9%
Bowtie2	818760	70977	86.7%
BWA-Short	764275	2674	86.5%
BWA-SW	718737	23051	82.5%
GEM	833295	38395	89.0%
SeqAlto	812779	7526	89.3%
BWA-Mem	833396	3891	90.7%

3.3 Reads from a RSVsim rearranged genome

WGSIM was used to simulate 2x5Mx100bp reads from hg19-chr21 for the analysis of alignment-performance of the compared methods in this section. Reads were simulated with the default of 2% sequencing errors and 1% polymorphism and aligned back to the original UCSC hg19 reference genome. Variants are called using Samtools and Vcftools.

We realized that different affine gap penalty scores used by different aligners could result in different clipping lengths of the reads in their corresponding alignments. Clipping will omit possible coverage above sites of polymorphic variants even when their alignments correct reflect the origin of the sequenced read. In order to minimise this bias, we only consider variants that are not within 50 bp of each other.

Below, we tabulated the F-measures of the called variants from the alignments of their respective aligners at various depth-cutoff.

Supplementary Table 3.3. F-measures of called SNVs and Indels from WGSIM-simulated reads at various depth-cutoff thresholds.

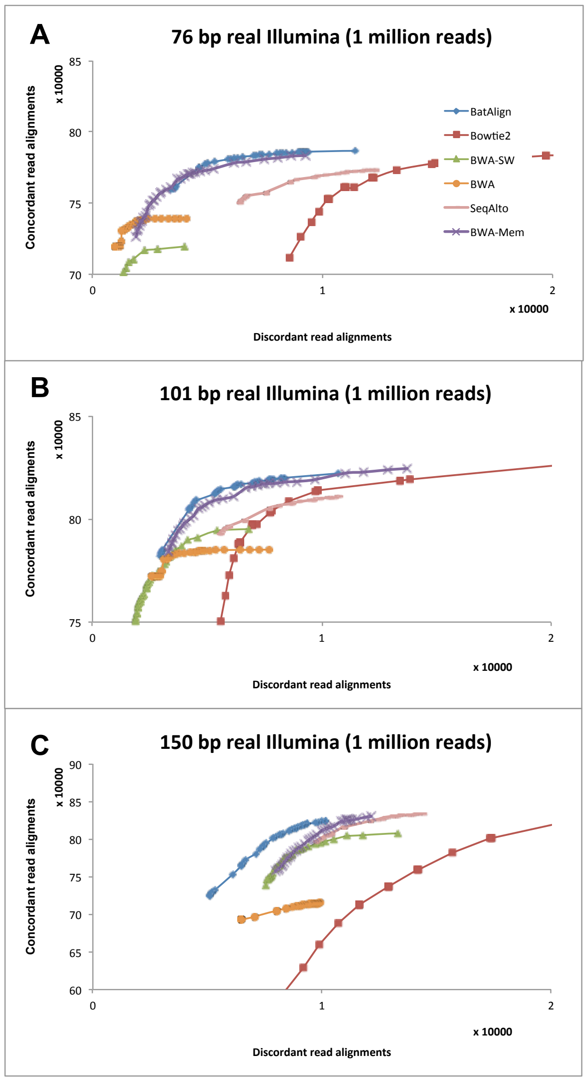
Methods	F-measures of SNV-calling (at various depth-cutoff)		
	2	5	10
BatAlign	**99.4%**	**99.4%**	**99.3%**
Bowtie2	98.0%	98.4%	96.0%
BWA-Short	99.0%	99.0%	98.9%
BWA-SW	98.5%	98.5%	98.3%
GEM	96.6%	95.6%	94.3%
SeqAlto	99.0%	99.0%	98.9%
BWA-Mem	99.1%	99.1%	99.0%

Methods	F-measures of Indel-calling (at various depth-cutoff)		
	2	5	10
BatAlign	**86.3%**	**86.3%**	86.2%
Bowtie2	74.9%	77.4%	73.5%
BWA-Short	84.6%	84.7%	84.2%
BWA-SW	85.0%	85.0%	84.9%
GEM	62.4%	62.2%	61.6%
SeqAlto	84.2%	84.2%	84.1%
BWA-Mem	**86.3%**	**86.3%**	**86.3%**

4 Evaluation on real reads

4.1 1 million reads data sets

We have downloaded three dataset, DRR000614, SRR315803 and SRR850313 to evaluate the performance of the various methods in the main article. From each of the dataset, 1 million read were extracted and mapped by the various stated programs. The mappings were stratified accordingly to the mapQ which the corresponding program assigned to them. The results were plotted with cumulative number of alignments, in order of decreasing mapQ of the 'head' alignment, in Supplementary Figure 4.1.



Supplementary Figure 4.1. Cumulative concordant & discordant alignments stratified in order of decreasing mapQ as reported by their respective aligners in the form of ROC graphs.

4.2 Evaluation on life-sized data

For real WGS data, we have only used the primary, non-secondary alignment reported for each read from the compared methods. On WGS data, we realized that BreakDancer might miss an SV if read_1 is a breakpoint-spanning read. In this case, either the primary or the supplementary alignment will form a concordant read-pair alignment with read_2 and can elude the discovery of a structural variant. 800 bp insert size from ERP001196's 11T, de-multiplexed from 4 lanes, were used as the discovery set for SNV-callings. Results of the callings, with variant scores >=30, were tabulated in Supplementary Table 4.1 and 4.2.

Supplementary Table 4.1. Comparison on the number of SNVs recalled from published and validated SNVs of Patient 11T at various depth cutoff. Best results are in bold.

Methods	Intersect with published 11T data (with different depth-cutoff)		
	2	5	10
BatAlign	**45**	**42**	**37**
Bowtie2	0	0	0
BWA	42	40	35
BWA-SW	44	41	36
GEM	43	41	35
SeqAlto	1	1	0
BWA-Mem	**45**	**42**	**37**

Supplementary Table 4.2. Total number of SNVs called from data of Patient 11T at various depth-ctRutoff.

Methods	Total SNVs called at various depth cutoff		
	2	5	10
BatAlign	2984901	2839346	2207313
Bowtie2	3003669	2506634	1098618
BWA	3052128	2939975	2372334
BWA-SW	2798359	2668373	2063522
GEM	4015855	3837328	3075882
SeqAlto	237425	172190	123160
BWA-Mem	3159183	3029818	2439042

The library used had ~16X in sequencing depth. A total of 80 validated SVs were [27] used for this comparison.

5 Parameters used for all compared programs

Single-end Read Settings

Bat-Align (Default)
```
./penguin –g <INDEX> -q <INPUT > -o <OUTPUT>
```

Bat-Align (Fast)
```
./penguin –g <INDEX> -q <INPUT > -o <OUTPUT> --mode=vfast --swlimit=10
```

Bat-Align (Turbo)
```
./penguin –g <INDEX> -q <INPUT > -o <OUTPUT> --boost=5 --mode= vfast --swlimit=10
```

Bowtie2
```
./bowtie2 --local -x <INDEX> -U <INPUT > -S <OUTPUT>
```

BWA-SW
```
./bwa bwasw –f <OUTPUT> <INDEX> <INPUT>
```

GEM
```
./gem-mapper –q offset-33 –I <INDEX> -i <INPUT> -o <OUTPUT.gem>
./gem-2-sam –I <INDEX> -i <INPUT.gem> -o <OUTPUT> -q offset-33
```

BWA-short
```
./bwa aln <INDEX> <INPUT>  >  <SAI>
./bwa samse –f <OUTPUT> <INDEX> <SAI> <INPUT>
```

SeqAlto
```
./seqalto_basic align <INDEX> -1 <INPUT>  >  <OUTPUT>
```

BWA-Mem
```
./bwa mem -M <INDEX> <INPUT>
```

To investigate best (or first) correct alignments of the various methods, we had to input additional options for the programs. We listed the options for the programs which needed it to report 10 multi-hits in its output below. This was used in the comparisons of multi-hits in *Evaluation on ART-simulated reads* of the main paper.

Bat-Align used "--tophits 10". Bowtie2 used "-k 10". BWA-short used "-n 10". BWA-Mem used "-a". The other programs prints multi-hits by default and the number, which they can report, cannot be restricted manually by parameter-input.

Paired-end mapping

Bat-Align (Default)
```
./penguin –g <INDEX> -q <INPUT1 > -q <INPUT2 > -o <OUTPUT>
```

Bowtie2
```
./bowtie2 --local -x <INDEX> -1 <INPUT1> -2 <INPUT2>  -S <OUTPUT>
```

BWA-SW
```
./bwa bwasw –f <OUTPUT> <INDEX> <INPUT1> <INPUT2>
```

GEM
```
./gem-mapper –q offset-33 –I <INDEX> -1 <INPUT1> -2 <INPUT2> -o <OUTPUTtmp> -p
./gem-2-sam –I <INDEX> -i <OUTPUTtmp>.gem -o <OUTPUT> -q offset-33
```

BWA-short
```
./bwa aln <INDEX> <INPUT1>  >  <SAI1>
./bwa aln <INDEX> <INPUT2>  >  <SAI2>
./bwa sampe –f <OUTPUT> <INDEX> <SAI1> <SAI2> <INPUT1> <INPUT2>
```

SeqAlto
```
./seqalto_basic align <INDEX> -1 <INPUT1> -2 <INPUT2> >  <OUTPUT>
```

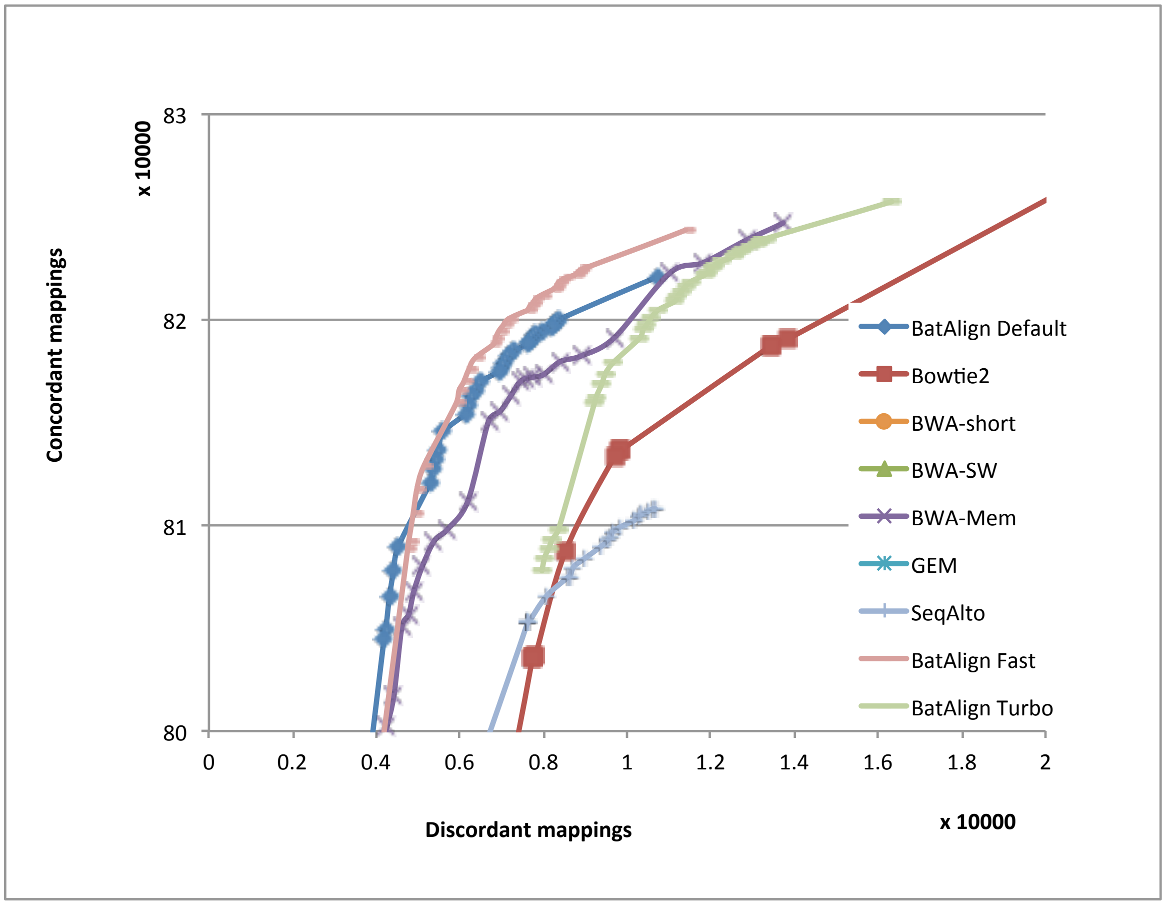
BWA-Mem
```
./bwa mem -M<INDEX> <INPUT1> <INPUT2>
```

6 Evaluation on running time

6.1 ROC curves of Bat-Align (different modes)

We tabulated the mapping runtimes from all the programs Table 5 of the main article. The various modes of Bat-Align have attained a better ROC curves as shown below. All the discussed modes of Bat-Align in the main article have also achieved competitive running times as compared to the other programs.

Supplementary Figure 6.1. ROC curves plotted from different modes of Bat-Align and other methods on 1 millions reads extracted from SRR315803.


x 10000
Concordant mappings
Discordant mappings
x 10000
BatAlign Default
Bowtie2
BWA-short
BWA-SW
BWA-Mem
GEM
SeqAlto
BatAlign Fast
BatAlign Turbo