

Introduction

FILLME

Notation

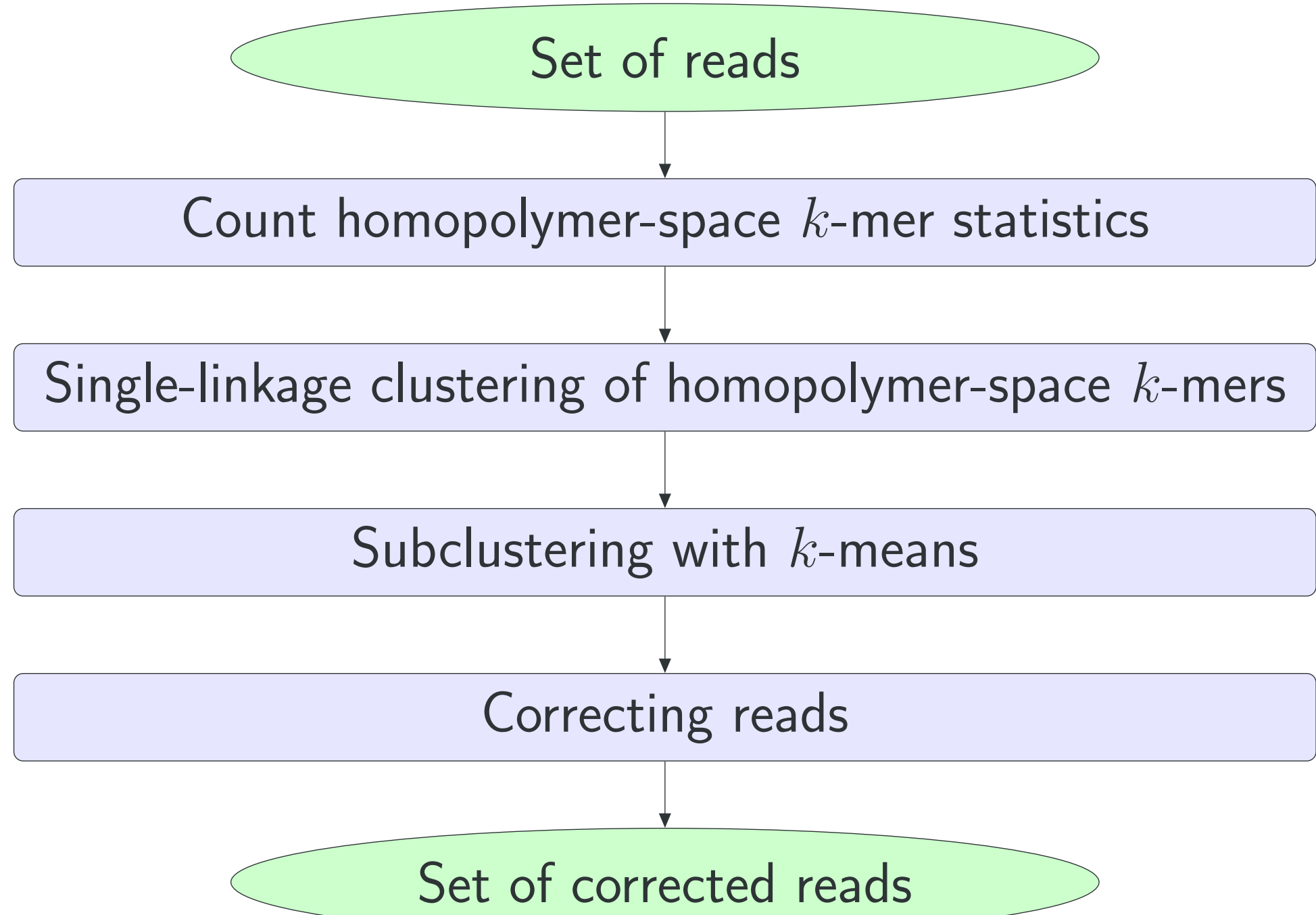
Let \mathcal{N} denote the nucleotide alphabet $\{A, C, G, T\}$. By definition, put $\mathcal{H} = \mathcal{N} \times \mathbb{N}^+$.

We call an element of the alphabet \mathcal{H} a *homopolymer run*, and an element of \mathcal{H}^k a *homopolymer-space k -mer*.

We use $x[k]$ to denote k -th element of a sequence, using zero-based indexing; $x[k \dots l]$ to denote a subsequence $x[k]x[k+1] \dots x[l]$; finally, length of x is denoted by $|x|$.

Distance between $i, j \in \mathcal{H}^k$ is defined as the minimum number of 1-base insertions/deletions/mismatches needed to align common part of i and j . It is denoted by $\text{dist}(i, j)$.

HammerIT workflow



Pairwise distance calculation

We use 5-base lookahead to compute distance between k -mers in homopolymer-space. Helper table stores precomputed values of

$$H_k : \mathcal{N}^k \times \mathcal{N}^k \rightarrow \{\text{Insertion, Deletion, Mismatch}\}, \quad k = 1, 2, 3, 4, 5.$$

The chosen value of 5 is a trade-off between accuracy and speed.

(Mapped reads from B7-295.bam dataset were used for training.)

G A G T A C A C T G T C G T C G
G T G T A C A T G T C G A T G C

$$H_5(\text{AGTAC}, \text{TGTAC}) = \text{Mismatch}$$

$$H_5(\text{CTGTG}, \text{TGTG}) = \text{Deletion}$$

$$H_3(\text{TCG}, \text{ATG}) = \text{Mismatch}$$

$$H_2(\text{CG}, \text{TG}) = \text{Mismatch}$$

Algorithm

Input: $x, y \in \mathcal{N}^+$ — homopolymer-space k -mers in nucleotide alphabet
Output: dist — approximate distance between x and y

```

pos.x ← 0; pos.y ← 0; dist ← 0;
while pos.x < |x| and pos.y < |y| do
  if x[pos.x] = y[pos.y] then
    pos.x ← pos.x + 1; pos.y ← pos.y + 1;
  else
    k ← min(5, |x| - pos.x, |y| - pos.y);
    adjust pos.x and pos.y according to
      H_k(x[pos.x .. pos.x + k - 1], y[pos.y .. pos.y + k - 1]);
    dist ← dist + 1;
  
```

IonTorrent error profile

Corrected flow signal intensities are available in BAM files produced by versions of Ion Torrent Suite prior to 3.4. Called homopolymer length is obtained as corrected flow signal intensity rounded to the nearest integer.

We have studied flow signal intensity distributions around insertion/deletion sites. File B7-295.bam, downloaded from Ion Community website, contained 4.6M insertions, 5.0M deletions, and 1.5M mismatches.

Overwhelming majority of errors turned out to be insertions/deletions of length 1, occurring when flow signal intensity is approximately halfway between two adjacent integers.

Figure 1: Flow signal intensities at insertion sites

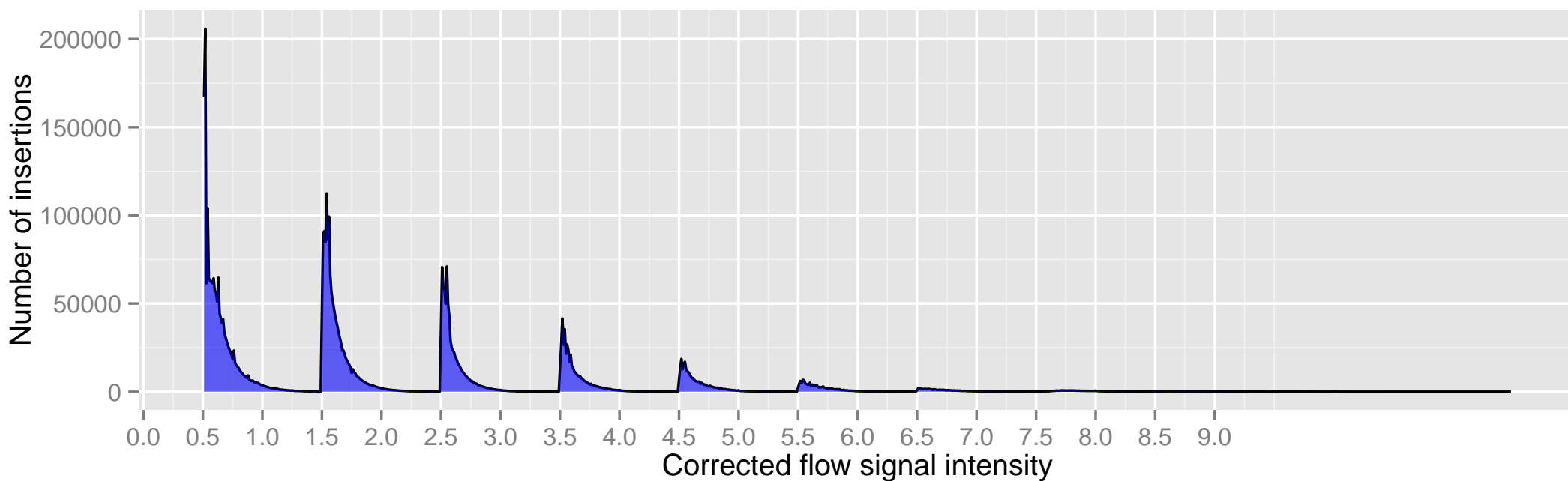
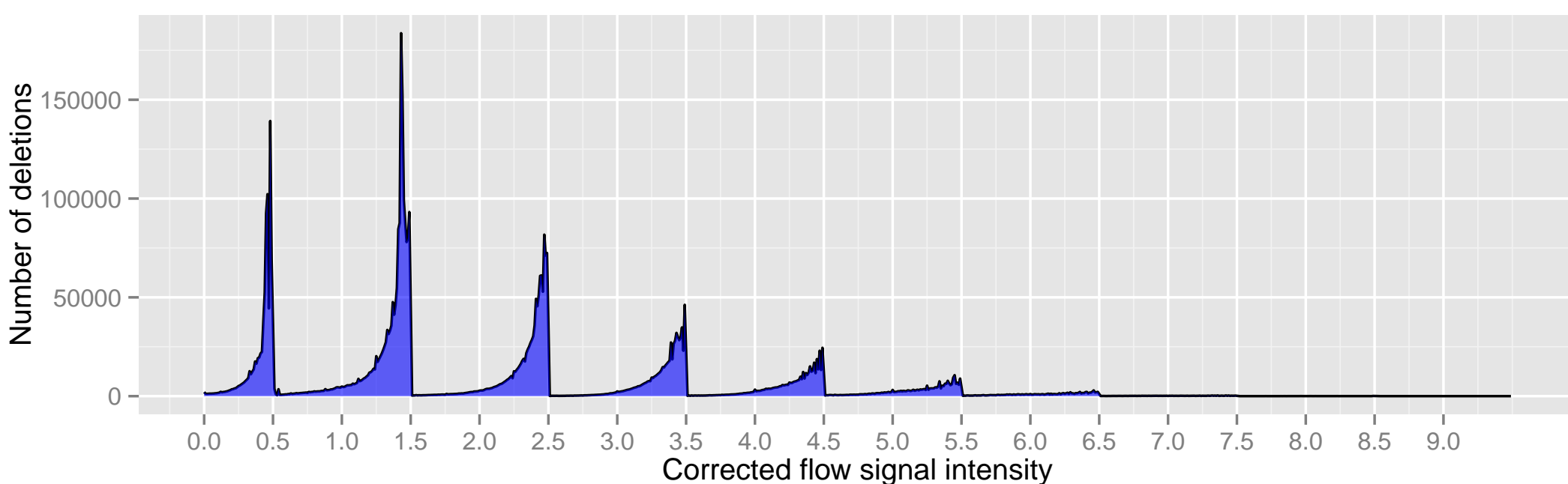


Figure 2: Flow signal intensities at deletion sites



More detailed analysis of errors in IonTorrent data can be found in the article “Shining a Light on Dark Sequencing: Characterising Errors in Ion Torrent PGM Data” (*PLoS Comput Biol* 9(4))

Homopolymer-space k -mer clustering

The starting point is single-linkage clustering of homopolymer-space k -mers. Two homopolymer-space k -mers belong to the same cluster if the distance between their nucleotide representations does not exceed one.

We reduce quadratic time requirements of the naive algorithm by noticing that if distance between two k -mers is less or equal to one, they share a common substring of length at least $\lfloor k/2 \rfloor$. This allows us to group k -mers into smaller blocks sharing a substring, and then use the quadratic algorithm for each block.

In order to detect all pairs of connected k -mers with such grouping, ranges $(0 \dots \lfloor k/2 \rfloor - 1), (1 \dots \lfloor k/2 \rfloor), \dots, (\lfloor (k+1)/2 \rfloor \dots k-1)$ are to be examined. Despite of this, we use only the first and the last of the ranges, to speed up the clustering step. This increases the number of *singletons* — clusters consisting of only one k -mer, usually erroneous — but has little impact on correction performance because of adjacent read k -mers making much larger contribution into the consensus scores (the number of singleton occurrences is usually low).

$$\text{partition}(\mathcal{K}, i, j) = \{B_s : \bigcup_{s \in \mathcal{H}^{j-i+1}} B_s = \mathcal{K}, \forall x \in B_s \ x[i \dots j] = s\}$$

Algorithm

```

kmers ← homopolymer-space k-mers seen in the data
components ← {{k} : k ∈ kmers}
blocksL ← partition(kmers, 0, ⌊k/2⌋ - 1)
blocksR ← partition(kmers, ⌊(k+1)/2⌋, k - 1)
for each block in blocksL, blocksR do
  for each i ∈ block do
    for each j ∈ block do
      if dist(i, j) ≤ 1 then
        join the components to which i and j belong
  
```

Error reduction results

We evaluated HammerIT on 6 publicly available datasets, using the same pipeline as the authors of the recently published article “Updating benchtop sequencing performance comparison” (*Nature Biotechnology*, v. 31, no. 4). In that article, error rate in four Ion Torrent datasets has been assessed. We used the same data plus two extra datasets from 314v2 chip, which recently became available on Ion Community Portal.

Indel/mismatch error rates were calculated for uniquely mapped reads before and after correction. For each dataset, correction was done in two ways. In the first setup, trimming was done for read ends that couldn’t be corrected due to lack of good k -mers, while in the second one such read ends were preserved in the output. Relative change in read coverage after correction stayed within 0.4% in all cases.

Figure 3: Error rates before and after correction

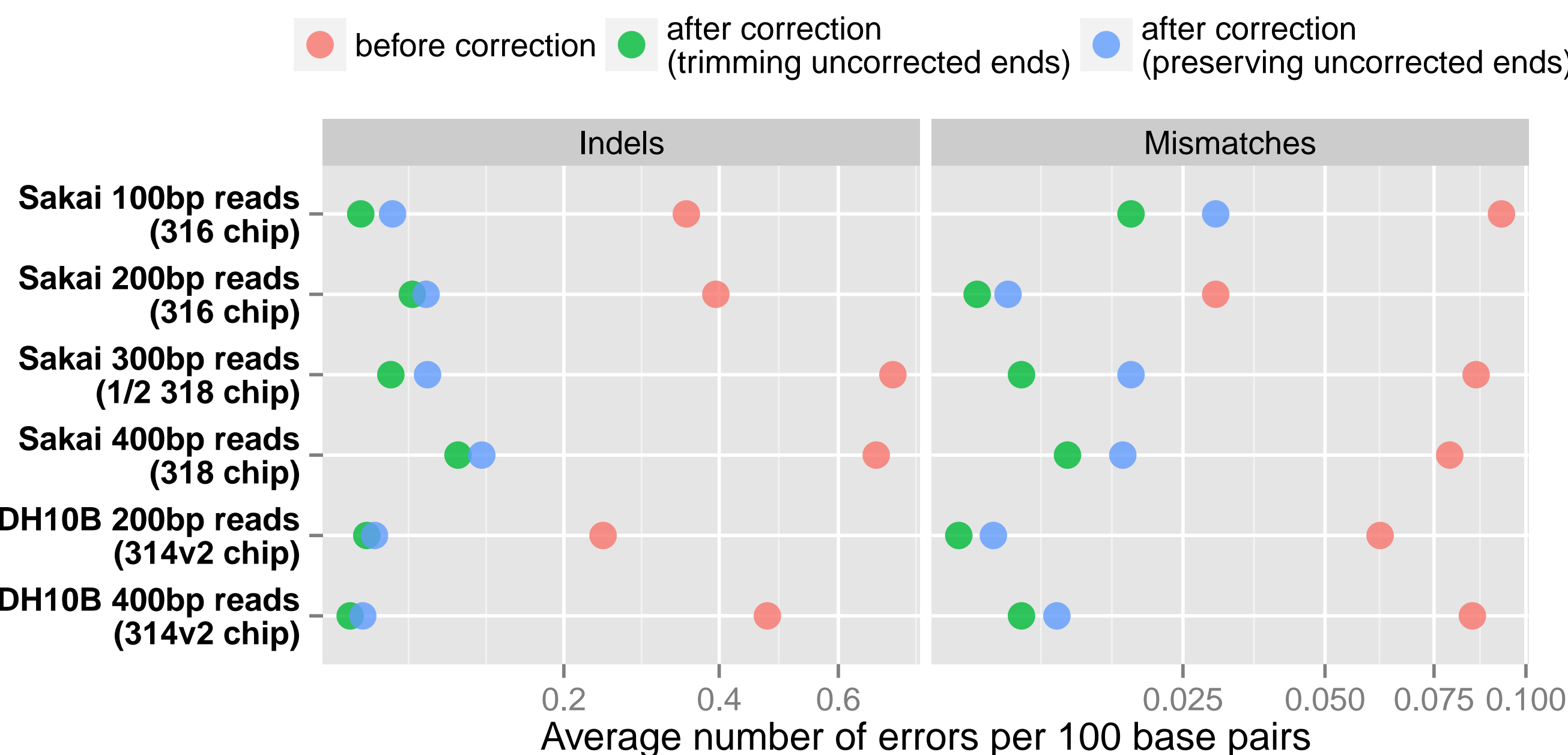
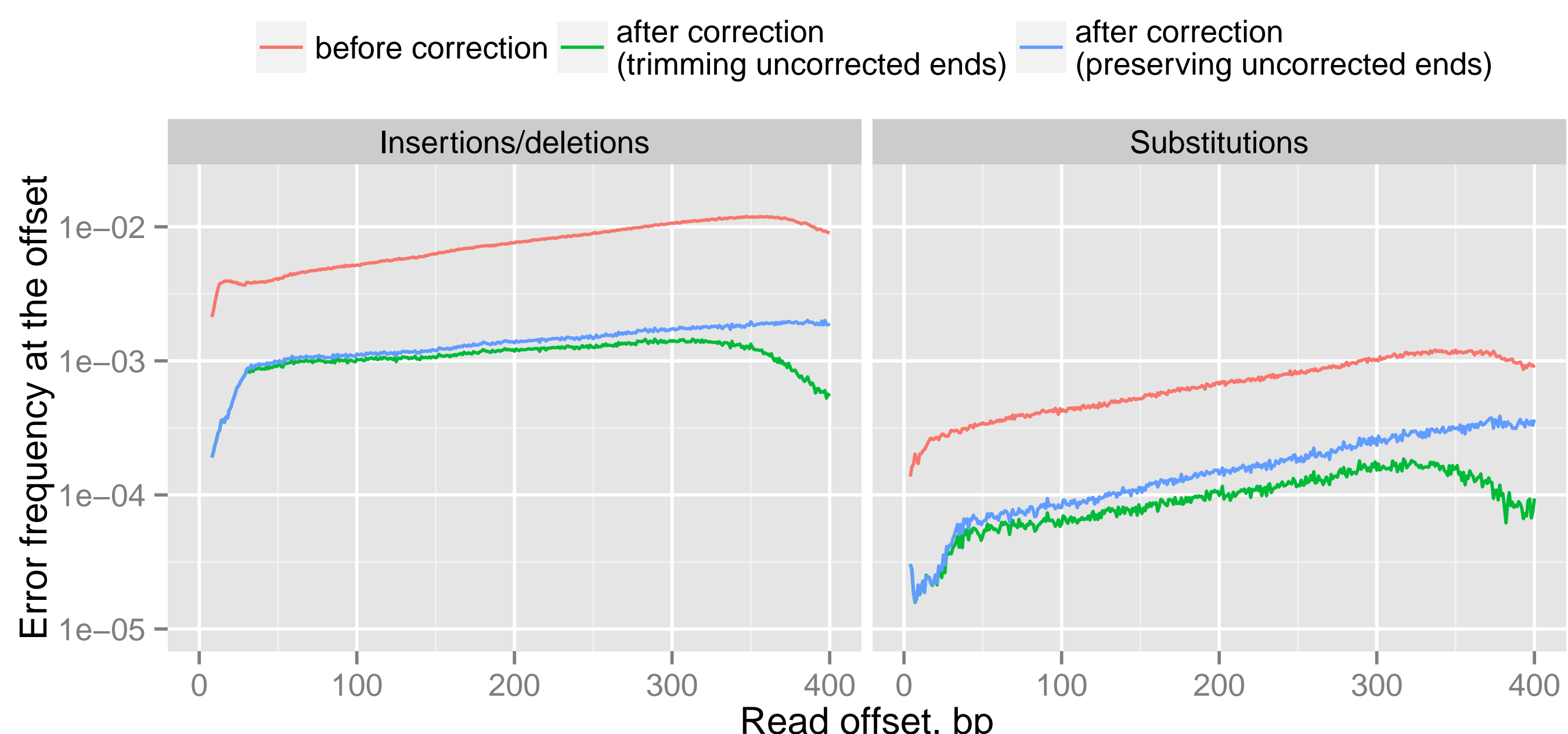


Figure 4: Error reduction by read position for Sakai 400bp reads



Subclustering

A cluster obtained from the initial process may contain $m \geq 2$ homopolymer-space k -mers from the genome. In this case, we split the cluster into m subclusters by running k -means algorithm on it.

The subtle question is how to determine m . Currently we just set it to be the number of the cluster elements with quality within machine epsilon of 1, where *quality* of $x \in \mathcal{H}^k$ is defined as $\Pr(x \text{ is genomic})$. For the set of reads $\mathcal{R} \subset \mathcal{N}^+$ the quality of $x \in \mathcal{H}^k$ is computed as

$$\prod_{r \in \mathcal{R}} \prod_{\substack{0 \leq m \leq |r| - |x'| \\ r[m \dots m + |x'| - 1] = x'}} \prod_{m \leq n < m + |x'|} \Pr(r[n] \text{ is correct}),$$

where $x' \in \mathcal{N}^+$ is the sequence of nucleotides in x .

Typical cluster

16-mer	n	qual.
GTGTACATGTCGATGC	113	1.00
GTGTACATGTCGATGT	23	0.90
GTGTACATGCCGATGCT	7	0.80
GTGTACATGTCGTCGCT	6	0.33
GTGTACATGTCGATGCT	6	0.31
CTTGATCATGTCGATGC	6	0.26
GTGTACATGTCGCTGC	4	0.28
GTGTACATGTCGATGCG	4	0.12
GTGTACATGTCGATGCT	4	0.09
GTGTACATGTCGATGCT	3	0.41
GTGTACATGTCGATGCT	3	0.14
GTGTACATGTCGATGCT	3	0.12
GTGTACATGTCGATGCT	3	0.10
GTGTACATGCCGATGCA	2	0.28
GAGTACACTGTCGCTG	2	0.04
GTGTACATGTCGATGA	2	0.03
GTGTATACATGTCGCTG	1	0.37
GTGTACATGTCGATGCT	1	0.32
GTGTACACTGTCGCTA	1	0.25
GTGTACACTGTCGCTA	1	0.13
GTGTACATGTCGATGCT	1	0.10
GTGTACATGTCGATGCT	1	0.09
CAGTACACTGTCGCTA	1	0.08
GTGTACATGCCGATGCT	1	0.08
GTGTACATGTCGAGCCG	1	0.08
GTGTACATGTCGAGGTC	1	0.08
GAGTACACACACTCTG	1	0.06
GTGTACATGTCGATGCT	1	0.05
GTGTACATGTCGATGCT	1	0.04
GTGTACATGTCGATGCT	1	0.04
GTGTACATGCCGCTGC	1	0.04
GTGTACATGTCGATGCT	1	0.03
GTGTACATGTCGATGCT	1	0.03
ATGTACGCTGTAGCTG	1	0.03
CAGTACACTGTAGCTG	1	0.03
GTGTACATGTCGATGCT	1	0.02
GTGTACATGTCGATGCT	1	0.02
GTGTACATGTCGATGCT	1	0.02
GTGTATACATGTCATG	1	0.01
CAGTACACTGTGCTG	1	0.01
GTGTACATGTCGATGCT	1	0.01
GTGTACATGTCGATGCT	1	0.01
GTGTACATGTCGATGCT	1	0.01
GTGTACATGTCGATGCT	1	0.01
ATGTACGCTGTAGCTG	1	0.01
ATGTACGCTGTAGCTG	1	0.01
GTGTACATGTCGATGCT	1	0.01
GTGTACATGTCGAGAG	1	0.00

Error correction algorithm

for each read from the dataset **do**
(Producing contiguous corrected parts of read)
for each homopolymer-space k mer from the read **do**
 $\text{center} \leftarrow$ center of the cluster to which k mer belongs;
 if center quality is more than user-specified threshold **and**
 center bases agree with the previous center **then**
 include center into consensus score calculation;
else
 yield new corrected part from current consensus;
 trim homopolymer runs with low consensus score from ends;
 estimate its position on the read;
 reset consensus table and start a new part;

(Combining corrected parts)

while there are two or more parts **do**
 $\text{curr} \leftarrow$ first part; $\text{next} \leftarrow$ second part;
 align last 8 homopolymer runs of curr against the read;
 align first 8 homopolymer runs of next against the read;
 if there is a gap on the read between the two parts **then**
 copy read homopolymer runs as is;
else
 select homopolymer runs with higher consensus score
 from the intersection of the two parts;
 replace curr and next with the combined part;
(Optionally, attaching uncorrected end)
 align last 8 runs of the last chunk against the read sequence;
 append read homopolymer runs after the last aligned run.