Improvement of the Accuracy of Mapping by Composing Alleles

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Background

Genome analyses using short reads which are generated by the high-throughput sequencer begin by mapping reads to the target genome. Therefore, they are sensitive to mapping results and high accuracy mapping is crucial. Ideal mapping results correctly identify the original location of each read. However, conventional mapping tools cause multireads and unmapped reads, that is, the mapping accuracy is insufficient. One reason for causing those reads is that only one genome sequence is used as the reference, even if the target organisms are polyploidy. That means both alleles are not treated equally and reads originating from a non-reference allele are more susceptible to mismapping when aligned to the reference allele, which contains at least one mismatch (in the case of SNPs) or gap (in the case of indels) [1].

Methods

We propose a novel mapping method that takes alleles into account. The inputs are a sequence data set Read of l-mer short reads and the reference genome Ref. Using these inputs, allele sequences are constructed and Read is remapped to the allele sequences. The output is the remapping result Remap. This concept is presented in Figure 1. In order to explain our method, we use the following functions:

- **classify(m):** classify the mapping result into three classes: uniq, multi and unmapped, which is a set of unique, multi and unmapped reads.
- **map(r, Ref):** map read r to a reference sequence Ref.
- **detect(p, Rs, Ref):** query whether a position p is a SNP using read set Rs and reference sequence Ref. If p is a SNP, this function returns snp. If p is not a SNP, this function returns notsnp.
- **base(m, p):** query which types of bases the read of mapping result m has at position p. If base(m, p) is A, T, G, or C, then the returned value is a, t, g, or c, respectively. If m does not include p, then NULL is returned.

Step 1: Map reads set to the reference genome

Read is mapped to the reference genome Ref.

Firstmap = map(Read, Ref)

The mapping result is classified into three subsets:

- \( F_{\text{uniq}} \) = \{ m | m \in \text{Firstmap} \land \text{classify}(m) = \text{uniq} \}
- \( F_{\text{multi}} \) = \{ m | m \in \text{Firstmap} \land \text{classify}(m) = \text{multi} \}
- \( F_{\text{unmap}} \) = \{ m | m \in \text{Firstmap} \land \text{classify}(m) = \text{unmap} \}

Step 2: Detect SNP positions

We use an SNP detection tool given two input data sets, \( F_{\text{uniq}} \) and Ref.

\( S_{\text{pos}} = \{ s | s \in \text{all positions} \land \text{detect}(s, F_{\text{uniq}}, \text{Ref}) = \text{snp} \} \)

The set \( S_{\text{pos}} \) of detected SNP positions satisfies the following condition:

\[ s_i < s_j \text{ for all } s_i, s_j \in S_{\text{pos}} (0 \leq i < j < |S_{\text{pos}}|) \]

Step 3: Construct allele sequences

This step is composed of the following four sub-steps.

1) Classify the SNP positions

According to read length l, \( S_{\text{pos}} \) is classified into \( S_1 \) which is SNP position subsets. Because, If adjacent \( s_i \) and \( s_{i+1} \) are separated by more than l, the base pairs of \( (s_i, s_{i+1}) \) cannot be determined. A set of \( S_1 \) is \( S_{\text{set}} \).

\[ S_i = \{ s_j, s_{j+1} | s_j, s_{j+1} \in S_{\text{pos}} \land (s_{j+1} - s_j) \leq l \} \]

\[ S_{\text{set}} = \{ S_0, S_1, S_2, \ldots | S_i \cap S_j = \emptyset \} \]

2) Generate a graph for each subset of SNP positions

A weighted directed graph \( G_i = (V_i, E_i, c_e) \) is generated for a subset \( S_i = \{ s_{j_1}, s_{j_1+1}, \ldots, s_{j_{i-1}} \} \) using \( F_{\text{uniq}} \). An overview of this step is shown in Figure 2.

\[ V_i = \{ v_{j(b)} | s_j \in S_i \land \text{base}(m, s_j) = b \} \]

\[ E_i = \{ (v_{j(b_1)}, v_{j+1(b_2)}) \land [v_{j(b_1)}, v_{j+1(b_2)}] \}

\[ c_e(v_{j(b)}) = \begin{cases} 1 & \text{if } m \in F_{\text{uniq}} \land \text{base}(m, s_j) = b \\ \text{NULL} \end{cases} \]

\[ c_e(e) = \begin{cases} 1 & \text{if } m \in F_{\text{uniq}} \land \text{base}(m, s_j) = b_1 \land \text{base}(m, s_{j+1}) = b_2 \\ \text{NULL} \end{cases} \]

If there is a mapping result m with base b at position \( s_j \), then a node \( v_{j(b)} \) is generated. The weight of the node is the ratio of m with base b at position \( s_j \) to m covering position \( s_j \). If there is a mapping result m with both \( b_1 \) at \( s_j \) and \( b_2 \) at \( s_{j+1} \), then a left-right direction edge \( (v_{j(b_1)}, v_{j+1(b_2)}) \) is generated. The
weight of this edge is the ratio of \( m \) with both \( b_i \) at \( s_i \) and \( b_{j+1} \) at \( s_{j+1} \) to \( m \) covering both \( s_i \) and \( s_{j+1} \).

3) Search two adopted paths for each graph
The permutation of nodes on a path corresponds to the permutation of SNP position bases of one allele sequence. Then two adopted paths are searched in each graph. The searching method is to assign a score to each path and to select the 1st and 2nd largest score paths. The score of a path

\[
p = (v_i(v_{i+1}b_{i+1})v_{j-1}(b_{j-1})v_{j}b_{j})
\]

defined as follows:

\[
\text{score}(p) = \prod_{k=i}^{j} w(v_k(b_k)) \prod_{k=i}^{j-1} w(v_k(b_k)v_{k+1}(b_{k+1}))
\]

4) Construct allele sequences
The 1st and 2nd largest score paths are

\[
p_1 = (v_i(v_{i+1}b_{i+1})v_{j-1}(b_{j-1})v_{j}b_{j})
\]

\[
p_2 = (v_i(v_{i+1}b_{i+1})v_{j-1}(b_{j-1})v_{j}b_{j})
\]

then, the two permutations of SNP position bases are defined as follows:

\[
ale_{i_1} = (b_i, b_{i+1}, \ldots, b_{i-1}, b_j)
\]

\[
ale_{i_2} = (b_i, b_{i+1}, \ldots, b_{j-1}, b_j)
\]

After determining all base patterns of \( S_{\text{set}} = \{ S_0, S_1, S_2, \ldots \} \), using the reference genome \( \text{REF} \), two allele sequences are constructed. The SNP position bases of \( \text{REF} \) are replaced to \( ale_{i_1} \) (0 ≤ \( i < |S_{\text{set}}| \)), and the corresponding sequence is labeled \( \text{REF}_1 \). In the same manner, \( \text{REF}_2 \) is constructed using \( ale_{i_2} \) (0 ≤ \( i < |S_{\text{set}}| \)).

**Step 4: Remap a sequence data set to allele sequences**

Reads are classified into three categories using the first mapping result, \( F_{\text{m uniq}}, F_{\text{m multi}}, \) and \( F_{\text{m u n map}} \).

\[
R_{\text{uniq}} = \{ (r \mid m \in F_{\text{m uniq}} \land m = \text{map}(r, \text{REF}) \}
\]

\[
R_{\text{multi}} = \{ (r \mid m \in F_{\text{m multi}} \land m = \text{map}(r, \text{REF}) \}
\]

\[
R_{\text{unmap}} = \{ (r \mid m \in F_{\text{m u n map}} \land m = \text{map}(r, \text{REF}) \}
\]

Then, \( R_{\text{multi}} \) and \( R_{\text{unmap}} \) are remapped to two allele sequences, \( \text{REF}_1 \) and \( \text{REF}_2 \).

\[
\text{Remap} = \text{map}(R_{\text{multi}} \cup R_{\text{unmap}}, \text{REF}_1 \cup \text{REF}_2)
\]

**Results**

We performed two experiments in order to verify the effectiveness of our method. RNA-seq of human female (ERS025099) and a human genome (hg19) are used as the input. We used Bowtie mapping tool [2] and the SAMtools SNP detection tool [3]. Bowtie ran with two parameter ‘-k 2’ and ‘-sam’. SAMtools ran under the default condition.

**Experiment 1:** Count the number of SNPs and graphs
This experiment aimed to verify the constructed allele sequences. First, we mapped RNA-seq to the human reference genome using Bowtie and classified the mapping results. The number of unique reads, multireads and unmapped reads were 48,809,028, 13,266,096, and 15,118,069 respectively. Second, we detected the SNP positions using SAMtools. The number of detected SNP positions was 9,004 (coverage ≥ 30). Third, we generated graphs that correspond to the detected SNP positions. As a result, 8,151 graphs were created. This result indicates that proposed method is able to construct allele sequences because it detected 9,004 SNP positions and 710 graphs had two or more SNP positions were generated.

**Experiment 2:** Remap reads to two allele sequences
This experiment aimed to demonstrate that our method is more accurate than conventional methods. Using 8,151 graphs in the Experiment 1, we constructed two allele sequences and remapped multireads and unmapped reads to two allele sequences. The result shows 219,453 (0.28%) reads were additionally uniquely mapped. The result indicates that our method can reduce non-uniquely mapped reads, and consequently it contributes to more accurate mapping.

**Conclusions**

We proposed a method that takes alleles into account. In the experiments our method constructed allele sequences and remapped reads to those sequences. As a result, multireads and unmapped reads were reduced, that is, mapping accuracy could be improved.

**References**


**Figure 1 Overview of the proposed method**

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