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Sequence analysis

The Sequence Alignment/Map format and SAMtools

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1INTRODUCTION

With the advent of novel sequencing technologies such as Illumina/Solexa, AB/SOLiD and Roche/454 (Mardis, 2008), a variety of new alignment tools (Langmead et al., 2009; Li et al., 2008) have been designed to realize efficient read mapping against large reference sequences, including the human genome. These tools generate alignments in different formats, however, complicating downstream processing. A common alignment format that supports all sequence types and aligners creates a well-defined interface downstream processing. A common alignment format that supports all sequence types and aligners creates a well-defined interface.

The Sequence Alignment/Map (SAM) format is designed to achieve this goal. It supports single- and paired-end reads and thus provides universal tools for processing read alignments. Availability: http://samtools.sourceforge.net

Contact: rd@sanger.ac.uk

2METHODS

2.1 The SAM format

2.1.1 Overview of the SAM format The SAM format consists of one header section and one alignment section. The lines in the header section start with character '@', and lines in the alignment section do not. All lines are TAB delimited. An example is shown in Figure 1b.

In SAM, each alignment line has 11 mandatory fields and a variable number of optional fields. The mandatory fields are briefly described in Table 1. They must be present but their value can be a '*' or a zero (depending on the field) if the corresponding information is unavailable. The optional fields are presented as key-value pairs in the format of TAG:TYPE:VALUE. They store extra information from the platform or aligner. For example, the '@RG' tag keeps the 'read group' information for each read. In combination with the '@RG' header lines, this tag allows each read to be labeled with metadata about its origin, sequencing center and library. The SAM format specification gives a detailed description of each field and the predefined TAGs.

2.1.2 Extended CIGAR The standard CIGAR description of pairwise alignment defines three operations: 'M' for match/mismatch, 'I' for insertion compared with the reference and 'D' for deletion. The extended CIGAR proposed in SAM added four more operations: 'S' for skipped bases on the reference, 'H' for soft clipping, 'R' for hard clipping and 'P' for padding. These support splicing, clipping, multi-part and padded alignments. Figure 1 shows examples of CIGAR strings for different types of alignments.

2.1.3 Binary Alignment/Map format To improve the performance, we designed a companion format Binary Alignment/Map (BAM), which is the binary representation of SAM and keeps exactly the same information as SAM. BAM is compressed by the BGZF library, a generic library developed by us to achieve fast random access in a zlib-compatible compressed file.

An example alignment of 112 Gbp of Illumina GA data requires 116 GB of disk space (1.0 byte per input base), including sequences, base qualities and all the meta information generated by MAQ. Most of this space is used to store the base qualities.

2.1.4 Sorting and indexing A SAM/BAM file can be unsorted, but sorting by coordinate is used to streamline data processing and to avoid loading extra alignments into memory. A position-sorted BAM file can be indexed. We combine the UCSC binning scheme (Kent et al., 2002) and simple linear indexing to achieve fast random retrieval of alignments overlapping a
Fig. 1. Example of extended CIGAR and the pileup output. (a) Alignments of one pair of reads and three single-end reads. (b) The corresponding SAM file. The ‘@SQ’ line in the header section gives the order of reference sequences. Notably, r001 is the name of a read pair. According to FLAG 163 (=1 + 2 + 32 + 128), the read mapped to position 7 is the second read in the pair (128) and regarded as properly paired (1 + 2); its mate is mapped to 37 on the reverse strand (32). Read r002 has three soft-clipped (unaligned) bases. The coordinate shown in SAM is the position of the first aligned base. The CIGAR string for this alignment contains a P (padding) operation which correctly aligns the inserted sequences. Padding operations can be absent when an aligner does not support multiple sequence alignment. The last six bases of read r003 map to position 9, and the first five to position 37 on the reverse strand (32). Read r004 has three soft-clipped (unaligned) bases. The coordinate shown in SAM is the position of the first aligned base. The CIGAR string for this alignment contains a P (padding) operation which correctly aligns the inserted sequences. Padding operations can be absent when an aligner does not support multiple sequence alignment. The last six bases of read r005 map to position 9, and the first five to position 37 on the reverse strand (32). Read r006 is aligned across an intron, indicated by the N operation. (c) Simplified pileup output by SAMtools. Each line consists of reference name, sorted coordinate, reference base, the number of reads covering the position and read bases. In the fifth field, a dot or a comma denotes a base identical to the reference; a dot or a capital letter denotes a base from a read mapped on the forward strand, while a comma denotes a base from a read mapped on the reverse strand.

Table 1. Mandatory fields in the SAM format

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QNAME</td>
<td>Query NAME of the read or the read pair</td>
</tr>
<tr>
<td>2</td>
<td>FLAG</td>
<td>Bitwise FLAG (pairing, strand, mate strand, etc.)</td>
</tr>
<tr>
<td>3</td>
<td>RMSQ</td>
<td>Reference sequence NAME</td>
</tr>
<tr>
<td>4</td>
<td>POS</td>
<td>1-Based leftmost POSition of clipped alignment</td>
</tr>
<tr>
<td>5</td>
<td>MAPQ</td>
<td>MAPping Quality (Phred-scaled)</td>
</tr>
<tr>
<td>6</td>
<td>CTGAR Extended CIGAR string (operations: MIDDSHIP)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>HRMN</td>
<td>Mate Reference NaMe (r’w’ if same as HRMN)</td>
</tr>
<tr>
<td>8</td>
<td>MDPOG</td>
<td>1-Based leftmost Mate POSition</td>
</tr>
<tr>
<td>9</td>
<td>ISEIZ</td>
<td>Inferred Insert SIZE</td>
</tr>
<tr>
<td>10</td>
<td>SEQ</td>
<td>Query SEQuence on the same strand as the reference</td>
</tr>
<tr>
<td>11</td>
<td>QUAL</td>
<td>Query QUALity (ASCII-33-Phred base quality)</td>
</tr>
</tbody>
</table>

SAMtools has two separate implementations, one in C and the other in Java, with slightly different functionality.

3 CONCLUSIONS

We designed and implemented a generic alignment format, SAM, which is simple to work with and flexible enough to keep most information from various sequencing platforms and read aligners. The equivalent binary representation, BAM, is compact in size and supports fast retrieval of alignments in specified regions. Using positional sorting and indexing, applications can perform stream-based processing on specific genomic regions without loading the entire file into memory. The SAM/BAM format, together with SAMtools, separates the alignment step from downstream analyses, enabling a generic and modular approach to the analysis of genomic sequencing data.

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Conflict of Interest: none declared.

REFERENCES


SAM and SAMtools

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