Filtering read alignments in BAM format

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Abstract

RNA-seq data has become an important source of information for tasks such as differential expression analysis and transcript quantification. Given that this new technology produces millions of such short-reads, bespoke methods and tools are required to process such big amounts of information. Furthermore, the introduction of the Sequence AlignMent Format (SAM) Li et al. (2009) has meant that many of the state-of-the-art alignment tools (Bowtie, GMAP, etc.) now produce outputs in such format or in its binary version, aka BAM.

This note documents "filterBAM", a program designed to clean single and paired RNA-seq reads. The filter is based on filterPSL, a perl script written by Prof. Mario Stanke. Both filterPSL and filterBam are designed for the cleaning of data that will subsequently be applied to the gene prediction problem. Nevertheless, it should be possible to modify rather easily, if it is to be applied to a different type of application. filterBam is written in C++ and makes use of the Bamtools API Barnett et al. (2011).

1 Main features

filterBam is a C++ code that cleans alignment files in BAM format that is based on filterPSL, a Perl routine written by Prof. Mario Stanke for PSL files. filterBAM includes the following filtering options:

- Screens out unmapped alignments.
- Screens out alignments that do not comply with a pre-defined coverage level (default=80%).
- Screens out alignments that do not comply with a pre-defined percentage of identity (default=92%).
- Screeens out alignments whose insert gaps do not comply with a pre-defined distance (default=10).
- Filters in two modalities: single and paired-end reads, expecting paired-queries to be given the suffixes "/1", "/2" or ".f", ".r".
- When in paired-read mode, it optionally writes to separate files a prospective list of common target genes and of pairedness coverage.
- The filter should work fine for filtering data coming from 454 and Illumina but not for SOLiD technology.

The subsequent sections of this document describe how the filtering is done to single or paired-end reads.

2 Single reads

Operation of the single-read filter is described in a step by step basis because the paired-read filter works in a very similar way. Figure 1 below shows the schematics of the operation of the filter. Each read is processed independently, so assumming a set of reads $i \in 1, \ldots, N$, the filter processes independently each of them one at a time.

Firstly, the filter checks read i is mapped or not. This is easily done by means of checking SAM field number 2, the alignment FLAG, and more specifically bit 0×4, which is the only source of reliable information to determine whether a read is mapped or not Li et al. (2009). This is achieved by using the $isMapped()$ methods of BamTools. Unmapped reads are dropped, while mapped reads continue further processing. A counter keeps track of the number of unmapped reads that were dropped.

As a second step, reads that passed the mapping test are appended with two additional but temporary string-tags. Tag "co" and tag "pi" are added to the binary alignment by the $addTag()$ method of BamTools. "co" stands for *coverage* and is a measure of the amount of reads located at a given genomic position. "pi" stands for *percentage identity* and is a measure of the number of basis that correctly identify a genomic position. Estimation of the coverage is done according to Equation 2, whilst estimation of the percentage identity is done following Equation 3.

If the estimated coverage value for read i is less than that of minCover, the read will be dropped and a counter keeping track of such types of drops will be updated. In a similar way, if the value of percId for read i, is less than that specified by minId, the read will be dropped and the corresponding counter will be updated. Default values for $minCover = 80$ and $percId = 92$ might be modified by using the options '–minCover ¡value¿' and '–minId ¡value¿'.

An optional value, the number of inserts to the base reference (baseInsert), is computed optionally if the '–noIntrons' option is used. The number of insertions to the reference is computed through the application of Equation 4. This filter depends on the insertLimit value that has been specified, and which by default has a value of 10. The insertLimit parameter might be modifed by applying the '–insertLimit ¡value¿' option.

Once this filters have been applied: mapping filter, coverage filter, percentage identity filter and intron filter, a set of reads will be dropped out. From the remaining reads that satisfied such filter criteria, the information from coverage and percentage identity will be combined into a single figure, a *score* that will help in determining which reads are worth keeping and which not.

2.1 Uniq and Best criteria

Filtering by 'unique' or 'best' criteria is mutually exclusive, as Figure 1 shows. After passing the basic filtering scheme already described, all the remaining alignments that belong to a common query are scored through the function

$$\text{score} = \text{percId} + \text{coverage}. \tag{1}$$

The score is added as an optional tag to each alignment by means of the "addTag()" method of BamTools. Afterwards, this group of alignments is sorted by its scored value; as illustrated in Table 1 below.

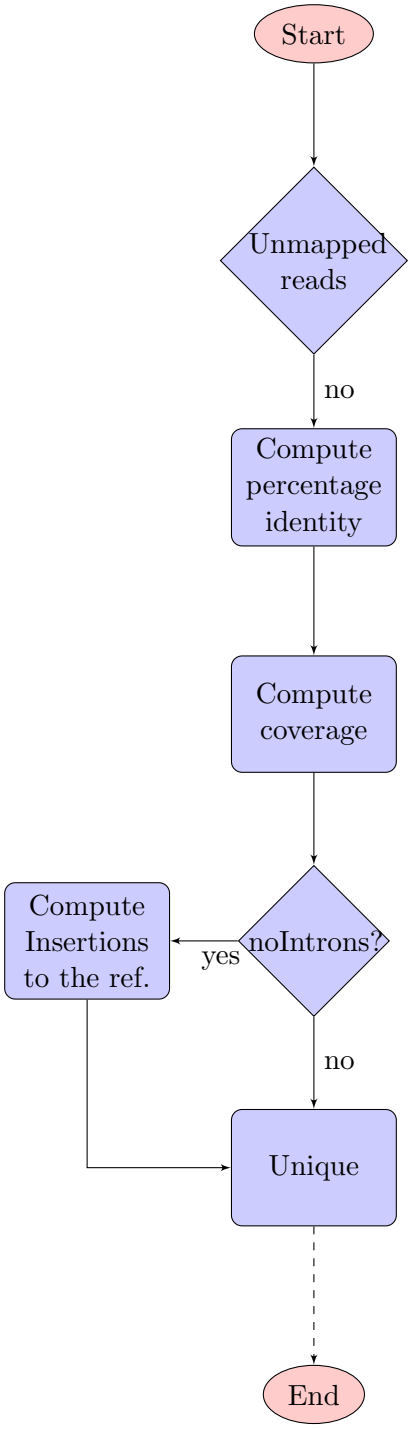
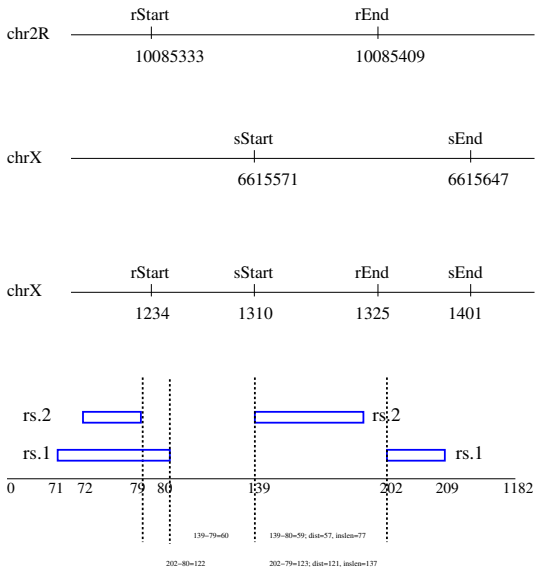


Figure 1: Flow diagram of the operation of the single-read filter

r2/1 147 chr17 27698730 37 33M1I16M = 27698611 -168	Field10	Field11	XT:A:U NM:i:1 SM:i:37 AM:i:0 X0:i:1 X1:i:0 XM:i:0 XO:i:1 XG:i:1 M
r2/1 99 chr17 20320141 36 43M1I6M = 20320287 196	Field10	Field11	XT:A:R NM:i:3 SM:i:0 AM:i:0 X0:i:2 X1:i:0 XM:i:2 XO:i:1 XG:i:1 MI
r2/1 99 chr19 1365 60 5M1I44M = 8297 187	Field10	Field11	XT:A:U NM:i:1 SM:i:37 AM:i:37 X0:i:1 X1:i:0 XM:i:0 XO:i:1 XG:i:1 M
r2/1 83 chr17 8038459 60 45M1I4M = 8038315 -193	Field10	Field11	XT:A:U NM:i:2 SM:i:37 AM:i:37 X0:i:1 X1:i:0 XM:i:1 XO:i:1 XG:i:1 M
r2/1 99 chr17 24524224 60 19M2I29M = 24524392 218	Field10	Field11	XT:A:U NM:i:3 SM:i:37 AM:i:23 X0:i:1 X1:i:0 XM:i:1 XO:i:1 XG:i:2 M
r2/1 163 chr17 30676705 37 26M2I20M2S = 30676860 205	Field10	Field11	XT:A:M NM:i:2 SM:i:37 AM:i:37 XM:i:0 XO:i:1 XG:i:2 MD:Z:46
r2/1 99 chr17 16894328 29 26M1I23M = 16894487 209	Field10	Field11	XT:A:R NM:i:3 SM:i:0 AM:i:0 X0:i:4 X1:i:0 XM:i:2 XO:i:1 XG:i:1 MI
r2/1 147 chr17 5031883 60 24M1I25M = 5031761 -171	Field10	Field11	XT:A:U NM:i:2 SM:i:37 AM:i:37 X0:i:1 X1:i:0 XM:i:1 XO:i:1 XG:i:1 M
r2/1 83 chr18 1 0 10M1I39M = 24 -206	Field10	Field11	XT:A:R NM:i:1 SM:i:0 AM:i:0 X0:i:2 X1:i:0 XM:i:0 XO:i:1 XG:i:1 MI

Table 1: Some SAM alignments showing added tags: percId, coverage and score



The difference between the 'unique' and 'best' criteria is that the former will select only the top-scored alignment and will write it into file. Table Y below shows the same set of alignments as in 1 after ranking. According to the 'uniq' criterion, only the top-ranked alignmnent is elligible to be preserved. However, in case a group of alignments happen to share the same score, filterBam checks whether such alignments are similar.

2.2 Similarity function

3 Paired reads

4 Bamtools

Bamtools is a C++ wrapper API of the more well-known Samtools software. The latest version of Bamools is 2.0 and is available on the website `https://github.com/pezmaster31/bamtools/downloads`.

5 Test data

We have generated two data sets to show filterBam in operation.

6 Compilation

Note that the flag "-std=c++0" has been used given that some of the functionalities of the filter require some of the newest features of GNU's g++ compiler. This and future versions of the software have been tested on Ubuntu's g++ version 4.4.3.

7 How to run

A run that will let pass most, if not all, readings:

./filterBam input.bam output.bam –minCover 0 –minId 0 –insertLimit 10000000 –nointrons

Note: that all options are provided at the very end.

Make sure to link with the "-lz" and "-libbamtools.a" flags on; where -lz refers to the ZLIB library, and libbamtools.a to the static bamtools library included in the software distribution. An example of how to compile and link follows:

1. g++ -I**$BAMTOOLS**/include -g -std=c++0x -c filterBam.cc -o filterBam.o
2. g++ -g -std=c++0x filterBam.o -o filterBam **$BAMTOOLS**/lib/libbamtools.a -lz

where **$BAMTOOLS** is the path where Bamtools was installed.

8 Coverage, percent of identity and insert length

The coverage is computed as the sum of the alignment matches (sequence matches or mismatches) and the insertions to the reference. Both figures, alignment matches and insertions to the reference, correspond to CIGAR string operations M and I, respectively. Thus the following is done

$$\text{coverage} = \frac{\sum \text{CIGAR}\,(M, I)}{qLength} \tag{2}$$

An approximation to the percentage of identity is given by computing the query length and subtracting the so-called edit distance to the reference (tag "NM" in SAM jargon), i.e.

$$\text{percId} = \frac{qLength - \text{Tag}(NM)}{qLength} \tag{3}$$

The length of inserts is estimated by summing CIGAR operations "M" and "I", which correspond to alingment matches and deletions from the reference. In other words, we do the following

$$\text{InsertSize} = \frac{\sum \text{CIGAR}\,(D, I)}{qLength} \tag{4}$$

References

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D. Barnett, E. Garrison, A. Quinlan, M. Strmberg, G. Marth. BamTools: a C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics*, 27(12):1691-1692, 2011.