biobambam: tools for read pair collation based algorithms on BAM files

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Abstract

Background: Sequence alignment data is often ordered by coordinate (id of the reference sequence plus position on the sequence where the fragment was mapped) when stored in BAM files, as this simplifies the extraction of variants between the mapped data and the reference or of variants within the mapped data. In this order paired reads are usually separated in the file, which complicates some other applications like duplicate marking or conversion to the FastQ format which require to access the full information of the pairs.

Results: In this paper we introduce biobambam, an API for efficient BAM file reading supporting the efficient collation of alignments by read name without performing a complete resorting of the input file and some tools based on this API performing tasks like marking duplicate reads and conversion to the FastQ format.

Conclusions: In comparison with previous approaches to problems involving the collation of alignments by read name like the BAM to FastQ or duplication marking utilities in the Picard suite the approach of biobambam can often perform an equivalent task more efficiently in terms of the required main memory and run-time.

Background

The SAM (Sequence Alignment/Matching) and BAM (Binary Alignment/Matching) file formats have become the standard formats for storing sequence data which was obtained through high throughput
sequencing and alignment of the resulting data to a reference genome. Both formats were introduced as part of the SAMtools package (cf. [1]). SAM is a human readable text format whereas BAM is a more compact and compressed binary format. The current specification of the formats is available at [2]. These files can be used for many applications including the detection of variants between the contained data and a reference, sequencing quality control and long term storage. Many programs have been created for the alignment of sequencing reads to reference sequences including SSAHA [3], BWA [4,5], Bowtie [6,7], SOAP [8,9] and SMALT [10] and most of the recently published aligners are either capable of generating SAM or BAM output or come with a script for converting their output to SAM or BAM. Most sequence data produced at the time being is sequenced as paired end reads. Short linear DNA templates are sequenced from both ends. This produces a pair of reads for each template. Both ends of the pair are assigned the same read name in the resulting data files thus providing the information that both ends are most likely within a certain expected distance in the underlying genome. This information aids in correctly aligning the resulting short sequences to a reference or assembling the fragments to a new draft reference. In the data obtained from a sequencer the pairs are usually collated in some form, either the two ends of a pair directly follow each other in a file or appear in an equivalent position in two separate files such that each of the two holds only the information for one of the two ends. The order of reads aligned to a reference which is most suitable for calling variants between the reads and the reference or within the reads is however the one resulting from sorting the data by coordinate (id of the reference sequence plus position on the sequence where the fragment was mapped). Thus many SAM and BAM files are processed in this order. There are however some applications which require the complete information from each pair. This includes the conversion of BAM files to a FastQ format suitable for realignment or a de novo assembly for an alternative detection of variants (see e.g. [11]) as well as the marking of duplicate reads. It is thus useful to have a quick, easy to use and reliable way of collating reads from a SAM/BAM file by their name without needing to resort to a full sorting of the file by read names. For the application of duplicate marking it is in addition desirable to keep the order after collation as close to the coordinate sorted order as possible, as clusters of reads pairs mapped to the same coordinates need to be detected. In this paper we present biobambam, a C++ API for efficient read name collation in BAM file and two tools bamtofastq and bammarkduplicates based on this API. These tools are more efficient in terms of runtime and memory usage than previous tools solving equivalent tasks like the SamToFastq and MarkDuplicates modules in Picard (see [12]).
Implementation

The biobambam package is split into two parts. The front-end tools `bamtofastq` and `bammarkduplicates` show-casing some applications of fast collation of alignments by name can be found in the biobambam source package (cf. [13]). The implementation of the collation code and the BAM file input and output routines are part of the larger `libmaus` project (see [14]), which also contains some supporting data structures.

There are various code bases and APIs available for SAM and BAM file input and output, including SAMtools (C), SeqAn (C++, cf. [15]) and Bio-samtools (Ruby, cf. [16]). We use our own implementation for reading BAM files, which can be found in the `libmaus` project (C++, [14]). The `libmaus` project also contains various supporting data structures which we use, including the biobambam API in its namespace `libmaus::bambam`. The front-end programs can be found in the biobambam project. The tools can easily be extended to handle the newer CRAM format (cf. [17]) via the `io` part of the Staden package (cf. [18, 19]) which contains the Gap5 software (see [20]). So far this extension has been implemented for the `bamtofastq` program, which is capable of transforming CRAM files to FastQ.

In the following we will first describe the algorithms and data structures used to for collating alignments by their name. Subsequently we will present the API making the functionality available to other users.

Algorithms and Data Structures for Collation by Read Name

Although the BAM file format can store alignments in any order, most BAM files will either have the alignments collated by the corresponding read names or will contain the alignments sorted by their coordinates on the reference the reads were aligned to. The first case will commonly appear as the output of alignment programs or if raw FastQ files coming from a sequencer are converted to the BAM format without aligning the contained reads to any reference. In this setting the output of the alignments in an order collated by read name to another format offering the same or less information is very simple. In the second case a straight-forward but often inefficient way is to first sort the input BAM file by query name using tools like SAMtools or Picard and then resort to a conversion as employed in the first case, as a BAM file sorted by query name will have the alignments collated by read name. For a BAM file sorted by alignment coordinates collating the alignments by read name can often be done more efficiently by observing that while the alignments paired by read name will commonly not be consecutive in the file, they are in most cases close together. If we denote the average coverage of a coordinate on the reference by $d$
(i.e. each coordinate is covered by \(d\) reads/alignments on average), the average absolute template length of a pair with both ends mapped to the same sequence by \(t\) (e.g. the absolute value of the distance between the mapping positions of the 5′ ends for Illumina paired-end reads) and the read length by \(l\), then we would expect the distance between two such ends in the BAM file to be \(\frac{d}{2}(t - l)\) on average. The mean number of read ends starting at each position on the forward strand is \(\frac{d}{2}\) and the distance between the two starting points on the forward strand is \(t - l\). Mapping the data from the whole human genome sequencing study ERP001231 (cf. [21]) to the human genome (GRCh37, see [22]) using the SMALT aligner (see [10]) for instance, we observe an average sequencing depth of \(d = 45\) with an average template length of \(t = 324\) at a mean read length of \(l = 101\) (100 base pairs were sequenced at one end of the templates and 102 from the other end). According to our formula this implies an average number of about 99 alignments between the two alignments of one pair in a BAM file containing the aligned reads. The actual median we observe in the file is 107. Due to some improperly mapped pairs in the file the weighted average value we see is not a meaningful number. Thus for the average case it would be sufficient to use any type of data structure which allows fast insertion, deletion and lookup of alignments by read name for a small set of alignments.

One such data structure would be a hash table with collision resolution by separate chaining. In practice however we see cases where some read ends stay in this hash table for an extended time when we process a BAM file sorted by coordinates from start to end. This may happen for reads where the two ends map to different chromosomes (split reads). There are also often regions in a genome where the sequencing depth is much higher than on average, which can lead to a drastic increase in the amount of memory required to store the hash table at certain points. Instead of using a hash table with collision resolution we use a hash table \(H\) of fixed size \(h\) without collision resolution. If there is a collision because two alignments with different names are assigned the same hash value, then the alignment previously in the hash table is removed from the table and inserted into a list \(L\) of fixed size \(l\) of alignments to be handled later. Each time the list \(L\) runs out of space we sort the alignments in \(L\) by read name. This sorting may yield some new pairs, which we extract before storing the unpaired alignments still in \(L\) in a temporary file and emptying the list \(L\). As soon as all alignments have been read from the source BAM file we move all the alignments remaining in \(H\) over to the list \(L\) and in the end flush the list \(L\) by sorting the remaining elements by name, extracting the resulting pairs and writing the remaining unpaired alignments to another temporary file. As all the temporary files are sorted by name, we can easily merge the files together to obtain a stream of alignments that is sorted by read name. In this stream it is again simple to detect and
The collation process uses several layers of data structures for handling alignments. This includes the hash table $H$ (see Figure 2), the overflow list $L$ (see Figure 3), a set of temporary files $T_i$ and a merged list $M$ produced from the $T_i$.

output pairs. A diagram of this data flow can be seen in Figure 1. Using this kind of setup we are able to quickly process most of the reads which have both ends close together in the BAM file while avoiding the use of excessive amounts of main memory to handle those pairs which are not close together.

To avoid the overhead resulting from the allocation of a small block of memory for each single alignment, we implement the hash table $H$ and the list $L$ in the following way. The hash table $H$ is implemented as a fixed size character array $R$ which we use as a ring buffer, an array $P$ of integers and a B-tree $B$. $P$ is the actual hash table storing pointers into $R$, $R$ is used to store alignments as uncompressed BAM entries and $B$ contains the starting positions of all alignments currently stored in $R$. A pointer $r$ which is initially set to 0 marks the current position in $R$. When a name $q$ is to be searched in $H$, then we first compute the hash value $h$ of the name and check whether position $P[h]$ in $R$ designates the start of an alignment and the stored alignment has the name $q$. An alignment with hash value $h$ can be erased from $H$ by first removing $P[h]$ from $B$ and then setting $P[h]$ to a special value marking a free position in $P$. To insert a new alignment with hash value $h$ into $H$ we first need to make sure there is sufficient space. If $P[h]$ is used, then the currently stored alignment for $h$ needs to be moved to $L$ and erased from $H$. Then we possibly need to remove more alignments from $H$ until the difference between the current insert pointer $r$ and the next higher value in $B$ (considered in a circular way as $R$ is a ring buffer) contains sufficient space to store the new alignment. As soon as sufficient space is available, we can copy the alignment data to position $r$ in $R$, insert $r$ into $B$, store $P[h] = r$ and advance $r$ by the length of the alignment data we have just stored. Figure 2 visualises the components of the hash table $H$. We store the list $L$ as a byte array. The alignment
The ring buffer $R$ stores alignment data. In the picture it contains three alignments $A_i, A_j$ and $A_k$. The insert pointer $r$ is situated just after the alignment $A_j$. The hash table $P$ stores pointers into $R$, where the position of the respective pointers is given by a hash value computed from the name of the stored alignment. The B-tree $B$ stores the starting positions of alignments in $R$ in sorted order.

Alignments are inserted from the start of the array. In the picture $A_0, A_1, \ldots, A_4$ are contained. Pointers to the respective starting positions are inserted from the end of the byte array.

The list runs full if we are no longer able to add the next alignment in the same way as the ones already stored. Figure 3 shows a list $L$ containing 5 alignments $A_0, A_1, \ldots, A_4$. A full list can be sorted by keeping the alignment data in place and reordering the pointers at the end of the byte array. Storing $H$ and $L$ in this way requires a very small amount of memory allocation and freeing operations for handling large sets of alignments.

**Results and Discussion**

**Using libmaus/biobambam: a short introduction**

In the following we will describe how to obtain, compile and use the libmaus-API for collating reads extracted from BAM files by name and how to use the tools `bamtofastq` and `bammarkduplicates`. 
Installation

The installation of libmaus and biobambam is as fairly straight-forward process on a recent Linux system. Both are based on the standard GNU autoconf/automake based build system. The latest source tarballs are available at https://github.com/gt1/libmaus/tags and https://github.com/gt1/biobambam/tags respectively. After unpacking the tarballs the packages can be compiled using

```bash
autoreconf -i -f
./configure --prefix=$HOME/libmaus && make install
```

for libmaus and subsequently

```bash
autoreconf -i -f
./configure --with-libmaus=$HOME/libmaus --prefix=$HOME/biobambam && make install
```

for the biobambam front-end tools. Using these commands the tools bamtofastq and bammarkduplicates will be installed in the directory $HOME/biobambam/bin.

The installation on recent versions of Ubuntu Linux (11.10 and newer) is particularly easy, as both packages can be installed from LaunchPad as binaries:

```bash
sudo add-apt-repository ppa:gt1/staden-io-lib-current
sudo add-apt-repository ppa:gt1/libmaus
sudo add-apt-repository ppa:gt1/biobambam
sudo apt-get install libmaus-dev biobambam
```

This will place the new tools in the /usr/bin directory. The LaunchPad version comes with support for converting CRAM files to FastQ via the Staden package's io_lib.

libmaus can be compiled on MacOS X in way very similar to the one shown for Linux. On non recent versions of the MacOS X development environment this will require the boost libraries (see [23]).
Compiling programs using libmaus

The compiler and linker flags necessary for using libmaus can be obtained using the pkg-config tool. If libmaus is not installed in a system directory via LaunchPad, then pkg-config needs to be informed of it’s location via the PKG_CONFIG_PATH environment variable. An example for the bash shell is

   export PKG_CONFIG_PATH=$HOME/libmaus/lib/pkgconfig:$PKG_CONFIG_PATH

The compilation flags can then be obtained using

   pkg-config --cflags --libs libmaus

A sample program can thus be compiled using

   c++ source.cpp -o binary 'pkg-config --cflags --libs libmaus'

Including read name collating BAM input in libmaus in source code

For including the name collating BAM input functionality of libmaus in other C++ source code, the respective definitions need to be made available using

   #include <libmaus/bambam/CircularHashCollatingBamDecoder.hpp>
   #include <libmaus/util/TempFileRemovalContainer.hpp>
   using namespace libmaus::bambam;
   using namespace libmaus::util;
   using namespace std;
   typedef BamCircularHashCollatingBamDecoder collator_type;
   typedef collator_type::alignment_ptr_type alignment_ptr_type;

The first two lines include header files from libmaus. The next five lines simplify notation in the following.

The collating input class can then be instantiated using

   collator_type C(cin,"tmpfile");
to read from the standard input channel cin. The second argument specifies the name of the file used to write alignments out to disk when the list \( L \) described above overflows. The temporary file can be removed after all alignments have been extracted from the input stream. For the sake of convenience this can also be done automatically using

\[
\text{TempFileRemovalContainer::addTempFile("tmpfile");}
\]

After the instantiation of the collator object pairs can be extracted using

\[
\text{pair<alignment_ptr_type,alignment_ptr_type> P;}
\]

\[
\text{while ( C.tryPair(P) )}
\]

\[
\text{if ( P.first && P.second )}
\]

\[
\text{\{}
\]

\[
\text{/* process pair */}
\]

\[
\text{\cout << "Found pair with name " << P.first->getName() << endl;}
\]

\[
\text{\}}
\]

The function \text{tryPair} of the collator class tries to extract pairs from the input BAM file. It returns \text{true} if any data could be extracted. The pair \( P \) will contain two pointers to alignments if this extraction was succesful. In case there are single or orphan reads in the input one of the two pointers may be a null pointer (an orphan read is a read end such that the other end is missing from the file). A list with accessor functions for alignments with their respective return types is shown in Table 1. Header information like the length and name of reference sequences can be obtained by calling methods of the header object in the collation class.

\[
\text{BamHeader const & header = C.getHeader();}
\]

Some methods and return types of the \text{BamHeader} class can be found in Table 2.

\textbf{A sample program for converting BAM to FastQ}

The following code is a complete program for converting an input BAM file to FastQ while keeping only complete pairs.

\[
\text{#include <libmaus/bambam/CircularHashCollatingBamDecoder.hpp>}
\]

\[
\text{#include <libmaus/util/TempFileRemovalContainer.hpp>}
\]
### Alignment accessor functions

| Name of function | Return type | Description |
|------------------|-------------|-------------|
| getName()        | string      | alignment name |
| getLReadName()   | integer     | length of read name |
| getRefID()       | integer     | id of reference sequence this end was mapped to |
| getPos()         | integer     | position on reference sequence this end was mapped to |
| getNextRefID()   | integer     | id of reference sequence other end was mapped to |
| getNextPos()     | integer     | position on reference sequence other end was mapped to |
| getFlags()       | integer     | alignment flags |
| isPaired()       | bool        | true if read was paired in sequencing |
| isProper()       | bool        | true if template is mapped as a proper pair |
| isMapped()       | bool        | true if this end is mapped |
| isMateMapped()   | bool        | true if other end is mapped |
| isReverse()      | bool        | true if this end is mapped to the reverse strand |
| isMateReverse()  | bool        | true if other end is mapped to the reverse strand |
| isRead1()        | bool        | true if this end is the first read of the pair |
| isRead2()        | bool        | true if this end is the second read of the pair |
| isSecondary()    | bool        | true if this alignment is not the primary one |
| isQCFail()       | bool        | true if alignment has failed quality control |
| isDup()          | bool        | true if alignment is duplicate of another |
| getLseq()        | integer     | length of query sequence |
| getRead()        | string      | query sequence |
| getReadRC()      | string      | reverse complement of query sequence |
| getQual()        | string      | quality string |
| getQualRC()      | string      | reverse quality string |
| getMapQ()        | integer     | mapping quality for this end |
| getNCigar()      | integer     | number of cigar operations |
| getCigarFieldOpAsChar(i) | character | i’th cigar operator as character |
| getCigarFieldLength(i) | integer    | i’th cigar operation length |
| getTlen()        | integer     | inferred template length |
| getAuxAsString("tagname") | string   | content of auxiliary field with id tagname |
| formatFastQ()    | string      | alignment converted to a FastQ entry |

Table 1: Accessor methods of the alignment class in **libmaus**

### BAM header accessor functions

| Name of function | Return type | Description |
|------------------|-------------|-------------|
| getRefIDName(i)  | string      | name of i’th reference sequence |
| getRefIDLength(i)| integer     | length of i’th reference sequence |
| getNumRef()      | integer     | number of reference sequences |
| getVersion()     | string      | BAM format version number |
| getSortOrder()   | string      | sort order of the BAM file |

Table 2: Accessor methods of the BAM header class in **libmaus**
using namespace libmaus::bambam;
using namespace libmaus::util;
using namespace std;

int main()
{
    typedef BamCircularHashCollatingBamDecoder collator_type;
    typedef collator_type::alignment_ptr_type alignment_ptr_type;

    /* remove temporary file at program exit */
    string const tmpfilename = "tmpfile";
    TempFileRemovalContainer::addTempFile(tmpfilename);

    /* set up collator object */
    collator_type C(cin,tmpfilename);
    pair<alignment_ptr_type,alignment_ptr_type> P;

    /* read alignments */
    while ( C.tryPair(P) )
    { /* if we have a pair, then print both ends as FastQ */
        if ( P.first && P.second )
        {
            cout << P.first->formatFastq();
            cout << P.second->formatFastq();
        }
    }
}

Note that the source code of the bamtofastq program in biobambam is somewhat more complicated because it offers more options (different input formats like SAM and CRAM, handling of single and orphan reads, etc.) and introduces a few small syntactic nuances (for instance a reusable buffer for the conversion of
alignments to FastQ) to increase performance further. The interested reader is referred to the respective source code (cf. [13]).

**Performance Comparison of bamtofastq and Picard**

All benchmarks for this paper were run on a PC using an Intel Core i7-2700K processor running at a frequency of 3.5 GHz and equipped with 16GB of memory. While this is a quad core CPU, the benchmarks were run using only 1 thread per job. Temporary files were stored on a fast solid state type drive (SSD). The machine was running version 13.04 (Raring Ringtail) of Ubuntu Linux. libmaus and biobambam were compiled using the version of the C++ compiler delivered with the operating system (GCC version 4.7.3). We compare against version 1.91 of the Picard suite. We have downloaded the binary distribution of Picard from SourceForge and run the programs using Oracle’s Java (Java SE 7u21). For evaluating the performance of our approach for converting BAM to FastQ in comparison with Picard we have used the following data sets, each stored in a single BAM file:

1. The low depth human data set HG00096 (see [24]) from the 1000 genomes project (cf. [25]) with a depth median value of 4. We have used the BAM file as provided by the project, the size of the file is 15GB. The median of the distance between the two ends of a template in the file is 5 (due to some outliers the weighted average is 96220). Both programs were able to handle the data set with a heap size of about 256MB. The runtime was 305s (5m5s) for *bamtofastq* and 1182s (19m42s) for Picard’s SamToFastq component, i.e. *bamtofastq* was faster by a factor of 3.87. None of the two programs gained significantly by using more main memory.

2. The high depth human data set ERP001231 (see [21]) with a median depth value of 45. This data was downloaded as FastQ and mapped to the human reference [22] using the SMALT aligner [10]. The resulting BAM file was sorted by coordinate using SAMtools. The median of the distance between the two ends of a template in the file is 107 but due to some very pronounced peaks and outliers in the distribution the weighted average is $1.4 \cdot 10^7$. The size of the resulting sorted BAM file is 95GB. *bamtofastq* was able to handle the file in 2661s (44m21s) seconds using its default memory setting of 256MB of heap space. Using more main memory resulted in only slightly better performance, the runtime dropped to 42m28s for 816MB of heap space and did not decrease further for 1451MB. Picard ran out of memory on the file when provided with 5GB and less of main memory. It took 30270s (8h24m) given 6GB of main memory. By increasing the amount of memory to 10GB
the runtime was reduced to 9870s (2h44m). Due to the modest drop in run time from 9GB (2h47m) to 10GB we did not measure the run time for more than 10GB. These numbers give bamtofastq a runtime which is between 3.78 and 11.88 times faster than Picard’s MarkDuplicates. The high runtime of Picard using 6GB of main memory suggests that the employed memory management gets very inefficient when the program uses most of the provided memory. For 5GB it fails after a run time of more than 7 hours.

3. The high depth E. coli data set from the study SRP017681 (see [26]) with a median depth value of 879. This data was also downloaded as FastQ and mapped to the respective reference genome (see [27]) using SMALT. The resulting BAM file was sorted by coordinate using SAMtools. The sorted BAM file has a size of 44GB. The median of the distance between the two ends of a template in the file is 158240, the weighted average is \(1.7 \times 10^7\). bamtofastq is able to handle this file in 2689s (44m49s) using 256MB of main memory. In this memory setting a large amount of reads need to be handled by resorting to temporary files on secondary storage because of the high depth of the input data. Due to this effect the runtime decreases to 1720s (28m40s) when we let the program use 1.45G of memory by increasing the size of the hash table \(H\). Picard fails with an out of memory type error when given 8GB of main memory. Using 9GB of memory it processes the file in 4h7m. Increasing the main memory given to 10G and 11G decreases the run time to 2h25m and 2h6m respectively. Thus bamtofastq is depending on the memory settings between 2.8 and 8.6 times faster than Picard while using significantly less memory.

Picard uses Java’s HashMap class and keeps each end in this hash table until the other end of the read is found in the file. This explains its high memory requirements. The performance is also low due to frequent object allocation and implicit deallocation (garbage collection) processes, in particular when the memory used is close to the memory given. bamtofastq can handle all the given files easily with its default small memory footprint. In particular it does not require the user to adjust the input parameters to process any of the files.

**Marking Duplicate Alignments**

Large sets of sequenced reads often contain reads or read pairs which are not unique, i.e. such reads and read pairs which map to the same coordinates on a given reference genome. This may happen for several reasons including artefacts of library preparation (e.g. duplication by PCR), sequencing artefacts (e.g.
optical duplicates) or just by chance as the selection of sequenced molecules is usually a random process. For some data sets to number of duplicate reads can be very high. In the E. coli data set SRP017681 mentioned above (see [26]) for instance more than 90% of the reads are duplicates. The presence of duplicates can significantly influence downstream analysis. Thus the detection and marking of duplicates is an important step in the analysis of sequenced data. The Picard tool suite contains a program for marking duplicates in BAM files. We will not describe the algorithmic procedure employed in detail here but only provide a rough description of how it works. First the program constructs a list \( L_P \) of aligned pairs and a list of aligned single ended reads and orphans \( L_S \). Both lists are sorted by coordinates, where the sorting of the list \( L_P \) is lexicographic in the coordinates of the two ends (i.e. the pairs are first sorted by the leftmost mapping end and then those which have the same leftmost mapping position are sorted by the mapping coordinate of the other end). In this sorted order it is very simple to partition the lists \( L_P \) and \( L_S \) into subsets of read pairs and single reads respectively which map to the same coordinates. In each such subset a single element with the highest score computed from the base qualities of the reads is selected as representant and the other elements are considered and marked as duplicates. In addition the current code also considers single ended reads and orphans as duplicates if they map to the same coordinate as one end of a mapped pair. The read name collation approach we present in this paper can be used as a building block for the construction of the list \( L_P \). Based on this we have implemented our own version of a duplication marking tool \texttt{bammarkduplicates}, which in its first release mimics the behaviour of Picard’s MarkDuplicates tools. In addition to the read name collation machinery it uses a structure very similar to the list \( L \) above (see also Figure 3) for sorting fragments of the lists \( L_P \) and \( L_S \) before those fragments are written to secondary memory. This gives \texttt{bammarkduplicates} a very stable and predictable memory usage profile. This is different from Picard’s MarkDuplicates tool, which uses a large amount of main memory for some data sets featuring high coverage in some regions or as a whole and thus is harder to handle in automated sequencing pipelines, as it sometimes requires manual intervention due to out of memory type errors.

We have evaluated the performance of \texttt{bammarkduplicates} in comparison to Picard’s MarkDuplicates for the same BAM files as we have used above for the BAM to FastQ evaluation.

1. As can be expected the low depth data set HG00096 can efficiently be handled by both programs using 256MB of heap space. None of the two programs benefit from being allowed to use more memory. \texttt{bammarkduplicates} handles the file in 36m49s and MarkDuplicate in 42m42s. Thus on this
data set \texttt{bammarkduplicates} is faster by a factor of 1.16.

2. \texttt{bammarkduplicates} processes the file obtained by mapping the reads from ERP001231 as described above in 5h28m using 281MB of main memory. It does not benefit from using more memory. Picard is not able to handle the file using 512MB of RAM. With 768MB it runs for 5h37m. The run time for 2GB and 3GB is about the same at 5h30m, thus we assume that further increasing the amount of given RAM will not result in better performance. The run time on this data set is thus about the same for both programs, while \texttt{bammarkduplicates} uses less memory.

3. The BAM file obtained by mapping the reads from the study SRP017681 as stated above is handled by \texttt{bammarkduplicates} in time 2h45m using 416MB of main memory. The run time gradually decreases to 2h20m when the main memory provided is increased to 2.56GB. Picard’s MarkDuplicates tool is not capable of handling the file given 3GB of memory; it aborts with an out of memory type error. Given 4GB it processes the file in time 2h29m. Further increasing the main memory threshold to 10GB decreases its run time to 2h23m. Thus the run time of the two tools again is similar with a tendency of \texttt{bamtofastq} being slightly faster for an equivalent amount of given main memory. \texttt{bammarkduplicates} is capable of handling the file using significantly less memory than Picard.

\textbf{Conclusions}

In this paper we have presented efficient algorithms and data structures for name collated BAM file input. We have provided an implementation of these in libmaus, an open source programming library for C++. As part of the biobambam package we have developed two tools \texttt{bamtofastq} and \texttt{bammarkduplicates} based on the API. These are often faster than their counter parts in Picard and use significantly less main memory.

\textbf{Competing interests}

The authors declare that they have no competing interests.

\textbf{Authors contributions}

GT wrote the code, ran the tests and benchmarks and wrote the paper. SL contributed to the testing and provided patches for making the bammarkduplicates tool more compatible with Picard’s MarkDuplicates module.
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Availability and requirements

Project name: biobambam/libmaus

Operating systems: Linux and MacOS X

Programming language: C++

Other requirements: none

License: GPL3

Any restrictions to use by non-academics: none

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