An overview of the girafe package

J. Toedling, C. Ciaudo, O. Voinnet, E. Heard, E. Barillot

April 26, 2022

1 Introduction

The intent of package girafe is to facilitate the functional exploration of the alignments of multiple reads¹ from next-generation sequencing (NGS) data to a genome.

It extends the functionality of the Bioconductor (Gentleman et al., 2004) packages *ShortRead* (Morgan et al., 2009) and *genomeIntervals*.

> library("girafe")
> library("RColorBrewer")

If you use *girafe* for analysing your data, please cite:

• J Toedling, C Ciaudo, O Voinnet, E Heard and E Barillot (2010) girafe – an R/Bioconductor package for functional exploration of aligned next-generation sequencing reads. *Bioinformatics*, 26(22):2902-3.

Getting help

If possible, please send questions about girafe to the Bioconductor mailing list.

See http://www.bioconductor.org/docs/mailList.html

Their archive of questions and responses may prove helpful, too.

2 Workflow

We present the functionality of the package *girafe* using example data that was downloaded from the Gene Expression Omnibus (GEO) database (Edgar et al., 2002, GSE10364). The example data are Solexa reads of 26 nt length derived from small RNA profiling of mouse oocytes. The data has previously been described in Tam et al. (2008).

¹The package has been developed for analysing single-end reads (fragment libraries) and does not support mate-pair reads yet.

```
> exDir <- system.file("extdata", package="girafe")
> ### load object describing annotated mouse genome features:
> load(file.path(exDir, "mgi_gi.RData"))
```

2.1 Adapter trimming

We load reads that include parts of the adapter sequence.

To removing adapter sequences, we use the function trimAdapter, which relies on the pairwiseAlignment function from the *Biostrings* package. The adapter sequence was obtained from the GEO page of the data.

```
> adapter <- "CTGTAGGCACCATCAAT"
> ra23.na <- trimAdapter(ra23.wa, adapter)
> show(ra23.na)

class: ShortReadQ
length: 1000 reads; width: 23 cycles
```

2.2 Importing aligned reads

The reads have been mapped to the mouse genome (assembly mm9) using the alignment tool Bowtie alignment tool (Langmead et al., 2009).

The resulting tab-delimited map file can be read into an object of class AlignedRead using the function readAligned. Both, this class and this function, are defined in the Bioconductor package ShortRead.

```
> exA <- readAligned(dirPath=exDir, type="Bowtie",
+ pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
> show(exA)

class: AlignedRead
length: 1689 reads; width: 23 cycles
chromosome: chr14 chr17 ... chr3 chr14
```

position: 115443405 13011859 ... 68813840 62250772

strand: + + ... + -

alignQuality: NumericQuality

alignData varLabels: similar mismatch

The object of class AlignedRead can be converted into an object of class AlignedGenomeIntervals, which is the main class of the girafe package.

```
> exAI <- as(exA, "AlignedGenomeIntervals")
> organism(exAI) <- "Mm"</pre>
```

For alignments in BAM format (Li et al., 2009), there is an alternative way of importing the data. The function agiFromBam can be used to directly create *AlignedGenomeIntervals* objects from indexed and sorted BAM files, making use of functionalities in the *Rsamtools* package.

2.3 Exploring the aligned reads

```
> show(exAI)
```

```
1,286 genome intervals with 1,689 aligned reads on 22 chromosome(s). Organism: \mbox{Mm}
```

Which chromosomes are the intervals located on?

> table(seqnames(exAI))

```
chr1 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19
                                                                       chr2
                                                                             chr3
 112
        50
               60
                     53
                            65
                                  74
                                         59
                                               48
                                                      47
                                                            43
                                                                   19
                                                                         87
                                                                                62
      chr5
                                chr9 chrMT
chr4
            chr6
                   chr7
                          chr8
                                             chrX
                                                    chrY
  57
        52
               82
                     51
                            57
                                  69
                                          5
                                              132
```

A subset of the intervals on a specific chromosome can be obtained using subsetting via '['.

> detail(exAI[seqnames(exAI)=="chrMT"])

	start	end	${\tt seq_name}$	strand	reads	${\tt matches}$	sequence
1	964	986	chrMT	+	1	1	${\tt GTTTATGAGAGGAGATAAGTTGT}$
2	11613	11635	chrMT	+	10	2	AAGAAAGATTGCAAGAACTGCTA
3	11613	11635	chrMT	+	1	2	AAGCAAGATTGCAAGAACTGCTA
4	11613	11635	chrMT	+	1	2	AAGAACGATTGCAAGAACTGCTA
5	11613	11635	chrMT	+	1	3	AAGAAAGATTGCAAGAACTGTTA

Finally, what is the number of aligned <u>reads</u> per chromosome?

```
> summary(exAI)
```

```
Number of aligned reads per chromosome:
 chr1 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19
                                                                           chr2
                                                                                 chr3
                                                                44
  139
          51
               105
                       56
                              83
                                    131
                                           68
                                                  61
                                                         48
                                                                      19
                                                                            106
                                                                                   107
       chr5
                                                       chrY
 chr4
              chr6
                     chr7
                            chr8
                                  chr9 chrMT
                                                chrX
   61
          52
               107
                       57
                              70
                                    102
                                           14
                                                 206
                                                          2
```

2.4 Processing the aligned intervals

Sometimes, users may wish to combine certain aligned intervals. One intention could be to combine aligned reads at exactly the same position, which only differ in their sequence due to sequencing errors. Another objective could be to combine overlapping short reads that may be (degradation) products of the same primary transcript. The function reduce combines a set of aligned intervals into a single aligned interval, if the intervals:

- are on the same strand,
- are overlapping (or contained in each other) or directly adjacent to each other AND
- have the same read match specificity (see below)

Read match specificity By the read match specificity $r(I_i)$ of an interval I_i , we refer to the total number of valid alignments of reads that have been aligned to I_i , i.e. the total numbers of intervals with the same reads aligned in the whole genome (or other set of reference sequences). If $r(I_i) = 1$, the reads that were aligned to the interval I_i have no other valid alignments in the whole genome, i.e. the interval I_i is the unique match position of these reads. If $r(I_i) = 2$, the reads that were aligned to the interval I_i have exactly one other valid alignment to another interval I_j , $j \neq i$. The match specificity is stored in the matches slot of objects of the class AlignedGenomeIntervals.

We first demonstrate the reduce using a toy example data object.

```
> D <- AlignedGenomeIntervals(
       start=c(1,3,4,5,8,10,11), end=c(5,5,6,8,9,11,13),
       chromosome=rep(c("chr1", "chr2", "chr3"), c(2,2,3)),
+
       strand=c("-","-","+","+","+","+","+"),
+
       sequence=c("ACATT","ACA","CGT","GTAA","AG","CT","TTT"),
       reads=rep(1,7), matches=c(rep(1,6),3))
> detail(D)
  start end seq_name strand reads matches sequence
          5
      1
                chr1
                                  1
                                          1
                                               ACATT
1
2
      3
          5
                chr1
                                 1
                                          1
                                                 ACA
```

3	4	6	chr2	+	1	1	CGT
4	5	8	chr2	+	1	1	GTAA
5	8	9	chr3	+	1	1	AG
6	10	11	chr3	+	1	1	CT
7	11	13	chr3	+	1	3	TTT

Calling the reduce method on these example data results in the following:

> detail(reduce(D))

```
start end seq_name strand reads matches sequence
          5
                 chr1
                                  2
                                                ACATT
      4
          8
                                  2
                                                CGTAA
                 chr2
                                           1
3
                                  2
                                                 AGCT
      8
         11
                 chr3
                                           1
4
     11
         13
                                  1
                                           3
                                                  TTT
                 chr3
```

Note that the two last intervals still show overlap. However, the last interval is a non-unique match position of the respective reads (matches= 3), in contrast to the other intervals.

Here is another example using the data introduced above.

```
> S \leftarrow exAI[seqnames(exAI) == "chrX" \& matches(exAI) == 1L \& exAI[,1] > 1e8] > detail(S)
```

	start	end	seq_name	strand	reads	matches	sequence
1	100768450	100768472	chrX	-	1	1	ATATAATACAACCTGCTAACTGT
2	101311567	101311589	chrX	-	18	1	TGAGGTTGGTGTACTGTGTGTGG
3	101311567	101311589	chrX	-	12	1	TGAGGTTGGTGTACTGTGTGA
4	101311567	101311589	chrX	_	2	1	TGAGGTTGGTGTACTGTGTGTGT
5	101311567	101311589	chrX	_	1	1	TGACGTTGGTGTACTGTGTGA
6	101311567	101311589	chrX	_	1	1	TGAGGTTGGTGTACTGTGTGCGG
7	148346896	148346918	chrX	+	4	1	TGAGGTAGTAGATTGTATAGTTT

Calling the reduce method on these data leads to the following result:

> detail(reduce(S))

sequence	${\tt matches}$	reads	strand	seq_name	end	start	
ATATAATACAACCTGCTAACTGT	1	1	_	chrX	100768472	100768450	1
TGAGGTTGGTGTACTGTGTGTG	1	34	_	chrX	101311589	101311567	2
TGAGGTAGTAGATTGTATAGTTT	1	4	+	chrX	148346918	148346896	3

Notice that the reads that match the same segment of the X chromosome differ in their last base. However, since most of the reads have a 'G' as final letter, the combined aligned interval als has a 'G' as the last letter.

The additional argument method="exact" can be specified if you want to solely combine intervals that have exactly the same start and stop position (but may have reads of slightly different sequence aligned to them). Consider the following example:

```
> S2 <- exAI[seqnames(exAI)=="chr11" & matches(exAI)==1L & exAI[,1]>8e7] > detail(S2)
```

	start	end	seq_name	strand	reads	matches	sequence
1	86397621	86397643	chr11	-	20	1	TAGCTTATCAGACTGATGTTTAC
2	86397621	86397643	chr11	-	1	1	TAGATTATCAGACTGATGTTTAC
3	86397621	86397643	chr11	-	2	1	TAGCTTATCAGACTGATGTTCAC
4	88515338	88515360	chr11	-	1	1	GGTGCAGGGAGCGCCAGTGTCTC
5	96178500	96178522	chr11	+	2	1	TACCCTGTAGATCCGAATTTTTG
6	96178501	96178523	chr11	+	1	1	ACCCTGTAGATCCGAATTTGTGA
7	108873196	108873218	chr11	_	1	1	AGTGCGGTAACGCGACCGCTACC

> detail(reduce(S2, method="exact"))

```
end seq_name strand reads matches
                                                                      sequence
      start
  86397621
             86397643
                                           23
                                                    1 TAGCTTATCAGACTGATGTTTAC
1
                          chr11
  88515338
             88515360
                                                    1 GGTGCAGGGAGCGCCAGTGTCTC
2
                          chr11
                                            1
3
                                            2
  96178500
             96178522
                          chr11
                                     +
                                                    1 TACCCTGTAGATCCGAATTTTTG
  96178501
             96178523
                          chr11
                                            1
                                                    1 ACCCTGTAGATCCGAATTTGTGA
                                                    1 AGTGCGGTAACGCGACCGCTACC
5 108873196 108873218
                                            1
                          chr11
```

Notice that the 6th aligned interval in S2 is only shifted by 1 nt from the 5th one. By default, the function reduce would merge them into one aligned genome interval. However, when method="exact" is specified, these two intervals are not merged since they are not at exactly the same position. There are additional methods for restricting the merging to intervals with the same 5'- and 3'-ends (specify method="same5" and method="same3", respectively).

2.5 Visualising the aligned genome intervals

The package girafe contains functions for visualising genomic regions with aligned reads.

```
> plot(exAI, mgi.gi, chr="chrX", start=50400000,
+ end=50410000, show="minus")
```

See the result in Figure 1.

Chromosome chrX coordinate [bp]

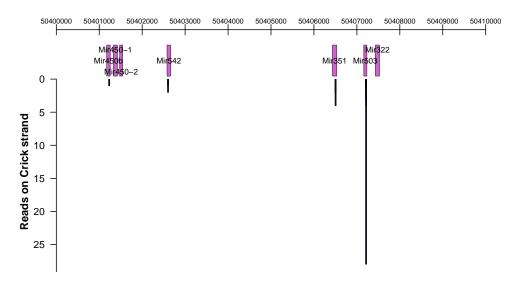


Figure 1: A 10-kb region on the mouse X chromosome. Reads aligned to the Watson strand in this region would be shown above the chromosome coordinate axis with the annotation of genome elements in this region, while reads aligned to the Crick strand are shown below. In the region shown, there are only intervals with aligned reads on the Crick strand, and these four intervals overlap with annotated microRNA positions.

Note that the annotation of genome elements (as shown in Figure 1) has to be supplied to the function. Here the object mgi.gi contains most annotated genes and ncRNAs for the mouse genome (assembly: mm9). This object has been created beforehand² and it is of class $Genome_intervals_stranded$, a class defined in package genomeIntervals.

2.6 Summarising the data using sliding windows

The data can be searched for regions of defined interest using a sliding-window approach implemented in the function perWindow. For each window, the number of intervals with aligned reads, the total number of reads aligned, the number of unique reads aligned, the fraction of intervals on the Plusstrand, and the higher number of aligned reads at a single interval within the window are reported.

```
> exPX <- perWindow(exAI, chr="chrX", winsize=1e5, step=0.5e5)
> head(exPX[order(exPX$n.overlap, decreasing=TRUE),])
```

²See the script prepareAnnotation.R in the package scripts directory for an example of how to create such an object.

943	chrX 503	91103	50491102	18	55	18	0	28
1960	chrX 1012	41103	101341102	5	34	5	0	18
1961	chrX 1012	91103	101391102	5	34	5	0	18
1216	chrX 640	41103	64141102	4	5	4	0	2
1215	chrX 639	91103	64091102	3	4	3	0	2
	first		last					
942	50401220	5040	07226					
943	50401220	5040	07226					
1960	101311567	1013	11589					
1961	101311567	1013	11589					
1216	64049984	640	92296					
1215	64049984	640	67192					

2.7 Exporting the data

The package *girafe* also contains methods for exporting the data into tab-delimited text files, which can be uploaded to the UCSC genome browser³ as 'custom tracks'.

Currently, there are methods for exporting the data in 'bed' format and 'bedGraph' format, either writing intervals from both strands into one file or into two separate files. Details about these track formats can be found at the UCSC genome browser web pages.

```
> export(exAI, con="export.bed",
+ format="bed", name="example_reads",
+ description="Example reads",
+ color="100,100,255", visibility="pack")
```

Additional arguments to the export function, besides object, con, and format, are treated as attributes for the track definition line, which specifies details concerning how the data should be visualised in the genome browser.

Users may also wish to consult the Bioconductor package *rtracklayer* for data transfer and direct interaction between R and the UCSC genome browser.

2.8 Overlap with annotated genome features

Next, we determine the degree of overlap of the aligned reads with annotated genomic elements. In this example, the annotated genome elements are provided as an object of class $Genome_intervals_stranded^4$. This objects needs to be created beforehand. See the script prepareAnnotation.R in the package scripts directory⁵ for an example of how to create such an object.

³http://genome.ucsc.edu

⁴Objects of class Genome intervals and AlignedGenomeIntervals are also allowed.

⁵system.file("scripts", package="girafe")

```
> exOv <- interval_overlap(exAI, mgi.gi)</pre>
```

How many elements do read match positions generally overlap?

> table(listLen(ex0v))

```
0 1 2 12
815 340 130 1
```

What are the 12 elements overlapped by a single match position?

```
> mgi.gi$ID[ex0v[[which.max(listLen(ex0v))]]]
```

```
[1] "Pcdha1" "Pcdha2" "Pcdha3" "Pcdha4" "Pcdha5" "Pcdha6" "Pcdha7" [8] "Pcdha8" "Pcdha9" "Pcdha10" "Pcdha11" "Pcdha12"
```

And in general, what kinds of annotated genome elements are overlapped by reads?

> (tabOv <- table(as.character(mgi.gi\$type)[unlist(exOv)]))</pre>

gene	lincRNA	miRNA	ncRNA	pseudogene	rRNA	${\tt snoRNA}$
238	15	297	19	28	10	1
tRNA						
4						

We display these overlap classes using a pie chart.

```
> my.cols <- brewer.pal(length(tabOv), "Set3")
> pie(tabOv, col=my.cols, radius=0.88)
```

See the result in Figure 2.

Note that function interval.overlap only determines whether two intervals are overlapping by at least one base. For restricting the result to intervals overlapping by at least a certain number of bases or by a fraction of the interval's length, see the function fracOverlap.

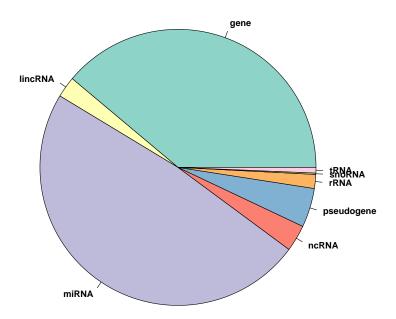


Figure 2: Pie chart showing what kinds of genome elements are overlapped by aligned reads. Note that the proportions of the pie chart are given by the proportions among all annotated genome elements that have ≥ 1 reads mapped to them and not by the total numbers of reads mapped to elements of that class, in which case the proportion of the miRNA class would be significantly larger.

3 Memory usage

At the moment, *girafe* and the packages that it depends on, retain all the information concerning the read alignments in memory. This allows quick access to and swift operations on the data, but may limit the package's usability on machines with low amounts of RAM.

The step with the highest RAM requirements is importing the alignments and saving them as objects of the AlignedRead class using the functionality in package ShortRead. Usually, objects of the AlignedGenomeIntervals class are created starting from AlignedRead objects and the AlignedRead objects can safely be discarded after this step. Since the data is summarised in that process, AlignedGenomeIntervals objects require about 10–100 times less memory than the original AlignedRead object⁶. We recommend that the import of the alignments and the generation of the AlignedGenomeIntervals are performed using a separate script which only needs to be called once on a machine with sufficient RAM.

⁶e.g., an *AlignedRead* object for holding 10⁶ reads of length 36 bp aligned to the mouse reference genome occupies about 1.4 Gb in RAM but is processed into an *AlignedGenomeIntervals* object of size 66.7 Mb

A suggestion for limiting memory usage is to perform the read alignments and import of the results in batches of a few million reads each. The batch-wise result *AlignedGenomeIntervals* objects can later be combined using the basic R function "c", the standard way of combining objects, optionally followed by calls of the reduce function.

For alignments in SAM/BAM format, the *Samtools* software suite (Li et al., 2009) as well as the Bioconductor package *Rsamtools* allow the user to access and import only selected subsets of the data, which also leads to a lower memory footprint. For details, please refer to the documentation of these packages.

Finally, while the processing of AlignedRead objects is the principal way of generating AlignedGenomeIntervals objects, there is also a convenience function called

AlignedGenomeIntervals, which can be used to create these objects from simpler objects in the work space, such as data read in using basic R functions such as scan. This convenience function may be easier to use for importing and processing the data in manageable chunks.

When following these suggestions, most operations with the girafe package should be possible on a machine with 4 Gb of RAM, and we have not so far encountered a situation that requires more than 12 Gb (state as of the end of 2009). However, increased throughput of sequencing machines and longer reads will lead to increased memory requirements. Future developments of this and other NGS-related Bioconductor packages will therefore likely concern ways to reduce the memory footprint. One idea is to make use of packages like ff, which provide ways of swapping data from RAM to flat files on the hard disk, while still allowing fast and direct access to the data.

4 A word about speed

For improving the run time on machines with multiple processors, some of the functions in the *girafe* package have been implemented to make use of the functionality in the *parallel* package. If *parallel* has been attached and initialised before calling these functions, the functions will make use of mclapply instead of the normal lapply function. The number of cores to be used in parallel is determined by the mc.cores option (see the example below).

For example, if *parallel* is functional on a given system⁷, there should be an obvious speed improvement in the following code example.

```
> library("parallel")
> options("mc.cores"=4) # adjust to your machine
> exAI.R <- reduce(exAI, mem.friendly=TRUE)</pre>
```

5 Links to other Bioconductor packages

The girafe package is mostly built upon the interval notation and implementation provided by the packages intervals and genome Intervals. Functions from the ShortRead package (Morgan et al.,

⁷The mclapply function currently does not support Windows operating systems.

2009) are used for importing the data, and *Biostrings* provides help for working with the read nucleotide sequences. *girafe* also makes limited use of the *Rle* and *IRanges* classes defined in the *IRanges* package. Furthermore, the data can be converted between object classes defined in *girafe* and *IRanges*.

We note that many of the interval operations in *girafe* can also be performed using classes and functions defined in the *IRanges* package. However, the scope of the packages is slightly different. While *IRanges* is meant to be a generic infrastructure package of the Bioconductor project, the aim of *girafe* is to provide a single, comparatively lightweight, object class for working with reads aligned to the genome, the *AlignedGenomeIntervals*. This class and its methods allow easy access to such data and facilitate standard operations and workflows.

There is some overlap in functionality between girafe, IRanges, GenomicRanges and tracklayer. The range of interactions between these packages and new Bioconductor packages related to next-generation sequencing is likely to increase over the releases. Our aim is to provide users with a broad range of alternatives for selecting the classes and functions that are most suited for their workflows.

Package versions

This vignette was generated using the following package versions:

- R version 4.2.0 RC (2022-04-21 r82226), x86_64-pc-linux-gnu
- Running under: Ubuntu 20.04.4 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.16-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.16-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, stats4, utils
- Other packages: AnnotationDbi 1.59.0, Biobase 2.57.0, BiocGenerics 0.43.0, BiocParallel 1.31.0, Biostrings 2.65.0, GenomeInfoDb 1.33.0, GenomicAlignments 1.33.0, GenomicRanges 1.49.0, IRanges 2.31.0, MatrixGenerics 1.9.0, RColorBrewer 1.1-3, Rsamtools 2.13.0, S4Vectors 0.35.0, ShortRead 1.55.0, SummarizedExperiment 1.27.0, XVector 0.37.0, genomeIntervals 1.53.0, girafe 1.49.0, intervals 0.15.2, matrixStats 0.62.0, org.Mm.eg.db 3.15.0
- Loaded via a namespace (and not attached): DBI 1.1.2, DelayedArray 0.23.0, GenomeInfoDbData 1.2.8, KEGGREST 1.37.0, Matrix 1.4-1, R6 2.5.1, RCurl 1.98-1.6, RSQLite 2.2.12, Rcpp 1.0.8.3, bit 4.0.4, bit64 4.0.5, bitops 1.0-7, blob 1.2.3, cachem 1.0.6, cli 3.3.0, compiler 4.2.0, crayon 1.5.1, fastmap 1.1.0, httr 1.4.2, hwriter 1.3.2.1, jpeg 0.1-9, lattice 0.20-45, latticeExtra 0.6-29, memoise 2.0.1, parallel 4.2.0, pkgconfig 2.0.3, png 0.1-7, rlang 1.0.2, tools 4.2.0, vctrs 0.4.1, zlibbioc 1.43.0

Acknowledgements

Many thanks to Nicolas Servant, Valérie Cognat, Nicolas Delhomme, and especially Patrick Aboyoun for suggestions and feedback on the package. Special thanks to Julien Gagneur and Richard Bourgon for writing *genomeIntervals* and for rapidly answering all my questions regarding the package.

The plotting functions in package *girafe* are largely based on the function plotAlongChrom and its auxiliary functions from package *tilingArray*, most of which were written by Wolfgang Huber.

Funding: This work was supported by the Institut Curie, INCa "GepiG".

References

- R. Edgar, M. Domrachev, and A. E. Lash. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*, 30(1):207–210, Jan 2002.
- R. C. Gentleman, V. J. Carey, D. J. Bates, B. M. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A. J. Rossini, G. Sawitzki, C. Smith, G. K. Smyth, L. Tierney, Y. H. Yang, and J. Zhang. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biology*, 5:R80, 2004.
- B. Langmead, C. Trapnell, M. Pop, and S. L. Salzberg. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, 10(3):R25, 2009.
- H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. D. and. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16):2078–9, Aug 2009.
- M. Morgan, S. Anders, M. Lawrence, P. Aboyoun, H. Pages, and R. Gentleman. ShortRead: a Bioconductor package for input, quality assessment and exploration of high-throughput sequence data. *Bioinformatics*, 25(19):2607–2608, Oct 2009.
- O. H. Tam, A. A. Aravin, P. Stein, A. Girard, E. P. Murchison, S. Cheloufi, E. Hodges, M. Anger, R. Sachi-danandam, R. M. Schultz, and G. J. Hannon. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature*, 453(7194):534–538, May 2008.
- J. Toedling, C. Ciaudo, O. Voinnet, E. Heard, and E. Barillot. girafe an R/Bioconductor package for functional exploration of aligned next-generation sequencing reads. *Bioinformatics*, 26(22):2902–2903, Nov 2010.