FastqCleaner: an interactive Bioconductor application for quality-control, filtering and trimming of FASTQ files

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Abstract

Background

Exploration and processing of FASTQ files are the first steps in state-of-the-art data analysis workflows of Next Generation Sequencing (NGS) platforms. The large amount of data generated by these technologies has put a challenge in terms of rapid analysis and visualization of sequencing information. Recent integration of the R data analysis platform with web visual frameworks has stimulated the development of user-friendly, powerful, and dynamic NGS data analysis applications.

Results

This paper presents *FastqCleaner,* a Bioconductor visual application for both quality-control (QC) and pre-processing of FASTQ files. The interface shows diagnostic information for the input and output data and allows to select a series of filtering and trimming operations in an interactive framework. *FastqCleaner* combines the technology of Bioconductor for NGS data analysis with the data visualization advantages of a web environment.

Conclusions

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FastqCleaner is an user-friendly, offline-capable tool that enables access to advanced Bioconductor infrastructure. The novel concept of a Bioconductor interactive application that can be used without the need for programming skills, makes *FastqCleaner* a valuable resource for NGS data analysis.

Keywords

Bioconductor, FASTQ, Next generation sequencing, R, Shiny, User-friendly tool, Visualization, Web app

Background

The advent of Next Generation Sequencing (NGS) technologies has revolutionized genomics, transcriptomics and epigenomics research [1, 2]. The large amount of genetic information produced by these instruments requires suitable data handling and exploration methods. For most common platforms, FASTQ files are the raw starting material for subsequent analyses. A portion of the reads can include adapters or contaminants, the quality of the sequences becomes generally lower towards the end of the reads, and ambiguous base calls may be present. The correction of these and other artifacts are important steps that should be performed before using sequencing reads for mapping or assembly.

Bioconductor [3] is a widely used repository based on the R [4] programming language, containing tools devoted to the analysis of high-throughput genomic data. The massive use of these tools is, however, limited by the learning curve that users need to go through to work with customized code routines. Recently, R integration with web tools, in particular JavaScript APIs, has dramatically increased the potential of R to produce more interactive and dynamic experiences of data analysis. This integration is promissory to promote the adoption of R by many researchers for whom learning a programming language has proven to be a prohibitive investment of time and effort.

Here we present *FastqCleaner*, an R package with an offline-capable web application for QC, trimming and filtering of FASTQ files. The tool combines Bioconductor libraries for data analysis and the dynamism of a web application for data visualization.

Implementation

Application overview

FastqCleaner offers the following features:

1) Implementation of a local, offline-capable and user-friendly web interface

2) Processing of Single-Read (SR) and Paired-End (PE) files

3) Dynamic analysis of the input and output files, for customizable sampling size of reads

4) Interactive, dynamical exploration and visualization of the data, using cutting-edge technology based on JavaScript and CSS3

5) Cross-platform (running in Linux, Mac-OSX and Windows)

6) Open source, under GNU GPL (>= 2) license

Program architecture

FastqCleaner was developed in R and is distributed as an R package. Data processing is controlled via R functions, that can be also accessed as normal functions from the R console (Additional file 1). These programs make extensive use of the Bioconductor packages *IRanges* [5], *Biostrings* [6] and *ShortRead* [7]. For speed improvement of the routines, C++ code was implemented in R using the *Rcpp* API [8]. The web interface included in the package was developed with *Shiny* [9], using JavaScript code written via the *jQuery* API, and CSS3.

Design

FastqCleaner takes compressed or uncompressed SR or PE files as input (Fig. 1). It accepts files with qualities in both Phred+33 and Phred+64 encoding, detecting Sanger, Solexa and Illumina 1.3+, 1.5+, and > 1.8+ formats. Input files can be processed through a set of independent filters based on either one of the following two principles: 1) *Remotion of a subset of reads that do not meet a given criterion*. This group of filters can remove: a) reads with unknown bases (Ns), b) low complexity sequences, c) duplicated reads, d) reads with length below a threshold quality value, and e) reads with an average quality below a threshold value. 2) *Trimming of individual reads*. This group of filters can trim: a) full and partial adapters, b) 5' regions below a predefined quality threshold, and c) 3' or 5' regions for a fixed nucleotide length. The adapter trimming algorithm extends the methodology of the *trimLRPatterns* function of *Biostrings,* designed to trim on the flanks of reads. For this purpose, *FastqCleaner* includes the *adapter_filter* function, a wrapper of *Biostrings* matching tools. The function is able to trim both adapters present on the flanks or within reads (Fig. 2). Several parameters can be passed to modify the behavior of the tool. These parameters allow, for example, to select a different threshold for the number of mismatches, to take into account the presence of indels, etc.

For SR files, *FastqCleaner* sequentially processes a block of reads and writes the resulting post-processed block into the corresponding output file. For PE files, the program uses in each cycle a two-step procedure: first, a block of forward and another of reverse reads are separately processed as in the SR case, and then only those reads present in both post-processed blocks are written into the corresponding output files.

Availability

The application and a tutorial are available in Bioconductor at http://bioconductor.org/packages/FastqCleaner/

Installation

The application can be installed following the instructions detailed at http://bioconductor.org/packages/FastqCleaner/

Launching the application

The application can be launched with the following commands in the R console:

```
> library("FastqCleaner")
> launch_fqc()
```

Optionally, when the application is used in RStudio (versions 0.99.878 or higher), a button that allows the direct launch of the application with a single click can be found in the addins menu (Fig. 3).

Results and Discussion

The web interface with its three main tabs is described in Fig. 4. The first tab (Fig. 4A) shows the file-selection menu, the available filters, and the run/reset buttons. The selection of files and filters represents the starting point in the *FastqCleaner* workflow. The second tab (Fig. 4B) shows the sequential operations performed on reads after processing. This information consists in the names of the input and output files, and a summary of informative statistics of the reads that passed the filter. The third tab (Figs. 4C, D) shows tables and interactive plots for data diagnostic. Plots can be constructed for both input (original data) and output (post-processed) files. A table with the most frequent k-mers can also be visualized.

A comparison of the package with other applications is shown in Table 1. Benchmarking results indicated an excellent performance of *FastqCleaner* in comparison with other pre-processing tools in

114 terms of elapsed time. Analysis of SR pre-processing (Fig. 5) showed that these tools can be divided
115 into three groups, in function of significant differences observed in processing speed for routine
116 operations (Tukey HSD test, $p < 0.001$ for all the three pairwise comparisons). The slowest were
117 *cutadapt* and *FASTX-Toolkit* (group 1), while *AdapterRemoval* and *Trimmomatic* (group 2) were the
118 fastest. *FastqCleaner* showed an intermediate performance, comparable to *Skewer* and *FLEXBAR*
119 (group 3). In PE mode, benchmarking of PE pre-processing operations (Fig. 6) showed that
120 *FastqCleaner* significantly outperforms all other tools for routine operations (Tukey HSD test, $p <$
121 0.001 for pairwise comparisons of *FastqCleaner* vs other individual applications).
122

123 **Conclusions**

124 *FastqCleaner* is a tool with a rich and interactive cutting-edge graphical interface for pre-processing
125 and exploration of SR and PE FASTQ files. Comparison with other available programs in a typical pre-
126 processing scenario of adapter trimming and length filtering, showed an excellent performance of the
127 application for both SR and PE real datasets. The application is made available as an open source
128 license. Coding experience is not required for its use, and is therefore particularly useful for users who
129 are unfamiliar with R programming. Furthermore, all processing happens locally in the user's computer
130 (even if the computer is disconnected from the network), making *FastqCleaner* amenable to run in
131 environments where data confidentiality prevents uploading of files to the cloud.

132 In essence, *FastqCleaner*'s dual capability facilitates both access to the underlying state-of-art
133 Bioconductor infrastructure and to dynamic graphical visualizations in a 100% client-side friendly web
134 environment. This makes *FastqCleaner* a novel technological advance for the analysis of Next
135 Generation Sequencing data.
136

Methods

In order to assess the performance of *FastqCleaner*, we have compared the package with other available pre-processing tools in benchmark tests: *AdapterRemoval* 2.2.2 [10], *cutadapt* 1.14 [11], *FASTX-Toolkit* 0.0.13 [12], *FLEXBAR* 3.0.3 [13], *Skewer* 0.2.2 [14] and *Trimmomatic* 0.36 [15]. The tests (Additional file 2) were conducted for adapter removal and length filtering using SR and PE files, with 22 replicates of each tests for statistical analysis of performance. Processing conditions were standardized by disabling compression of output files and using a single thread. In addition, pre-processing in *FastqCleaner* was performed using a chunk size of 10,000 reads per cycle. For SR processing, we downloaded from SRA the dataset SRR014966**,** with 14.3 M reads of 36 bp. For PE processing, we downloaded the dataset SRR330569 with 27 M reads of 101 bp. Benchmark tests were conducted in R using a laptop with Linux, a 2.20GHz Intel Core i7 CPU and 16GB of 1600MHz RAM (Additional file 2).

Additional files

Additional file 1: PDF version of the online tutorial.

Additional file 2: R script used in this work for benchmark testing.

Additional file 3: Source code of *FastqCleaner* (zip file)

List of abbreviations

NGS: Next Generation Sequencing

SR: Single-Reads

PE: Paired-End Reads

Availability and requirements

Project name: FastqCleaner

Project home page: http://bioconductor.org/packages/FastqCleaner/

Operating system(s): Platform independent

Programming language: R, C++, HTML, JavaScript, CSS3

License: GNU GPL (>= 2)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

FastqCleaner is freely available from its Bioconductor home page at http://bioconductor.org/packages/FastqCleaner/ under a GNU GPL (>= 2) license. *FastqCleaner* can be launched on any system that has R installed. An online tutorial is available at the package home page. A PDF version of this tutorial is included as supplemental material (Additional file 1).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LGR designed and developed the R package. FA contributed to the improvement of the original design. LGR, FA and DOS wrote the manuscript and tested the package. DOS and FA supervised the project. All authors read and approved the final manuscript.

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Captions to Figures

Fig. 1 Graphical representation of a typical workflow with *FastqCleaner*, showing the initial selection of FASTQ file(s), processing, and generation of output(s). Diagnostic interactive plots can be constructed for both input and output files. Circular arrows indicate halfway points in the workflow, where different configurations can be selected to re-run the program from there.

Fig. 2 Examples for adapter trimming. Pictures show the relative position of an adapter and a read, and the expected result after processing with the *adapter_filter* function of *FastqCleaner*. Dotted lines indicate the portion of the read that will be removed. Arrows show the direction along the read used for

205 the program to seek for matches. If one or more matches are found, the function trims the longest
206 subsequence, that contains the matching region plus the rest of the read, in the corresponding trimming
207 direction. *A*: partial adapter on the right + right-trimming of anchored adapter. *B*: partial adapter on the
208 left + left-trimming of anchored adapter. *C*: partial adapter within read + right-trimming. *D,E*: full
209 match between an adapter and a portion of the read + left- (*D*) or right- (*E*) trimming. *F*: multiple
210 matches for a same adapter + left-trimming.
211
212 **Fig. 3** RStudio addins menu, showing the button to launch the *FastqCleaner* application.
213
214 **Fig. 4** Web interface of the *FastqCleaner* application. *A*: first tab, showing an example where a file and
215 a filter are selected. *B*: second tab, showing the processes performed after running the program. *C*: third
216 tab, showing the analysis of the data, in this case for the input FASTQ file. The plot shows the base
217 composition of the sequences. *D*: fourth tab, showing a table with the frequency and the sequence of
218 each duplicated read.
219
220 **Fig. 5** Boxplots for elapsed time (in seconds) for SR adapter trimming and read length filtering.
221
222 **Fig. 6** Boxplots for elapsed time (in seconds) for PE adapter trimming and read length filtering.
223 FASTX-Toolkit is not capable to pre-process PE reads, and hence it is not shown in the plot.
224

225 ## References

226 1. Koboldt D, Steinberg K, Larson D, Wilson R, Mardis E. The next-generation sequencing revolution
227 and its impact on genomics. Cell 2013; 155: 27–38.

2. Tripathi R, Sharma P, Chakraborty P, Varadwaj P. Next-generation sequencing revolution through big data analytics. Front Life Sci. 2016; 9: 119–149.

3. Huber W, Carey V, Gentleman R, Anders S, Carlson M, Carvalho B, Bravo H, Davis S, Gatto L, Girke T, Gottardo R. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods. 2015; 12: 115–121.

4. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2017.

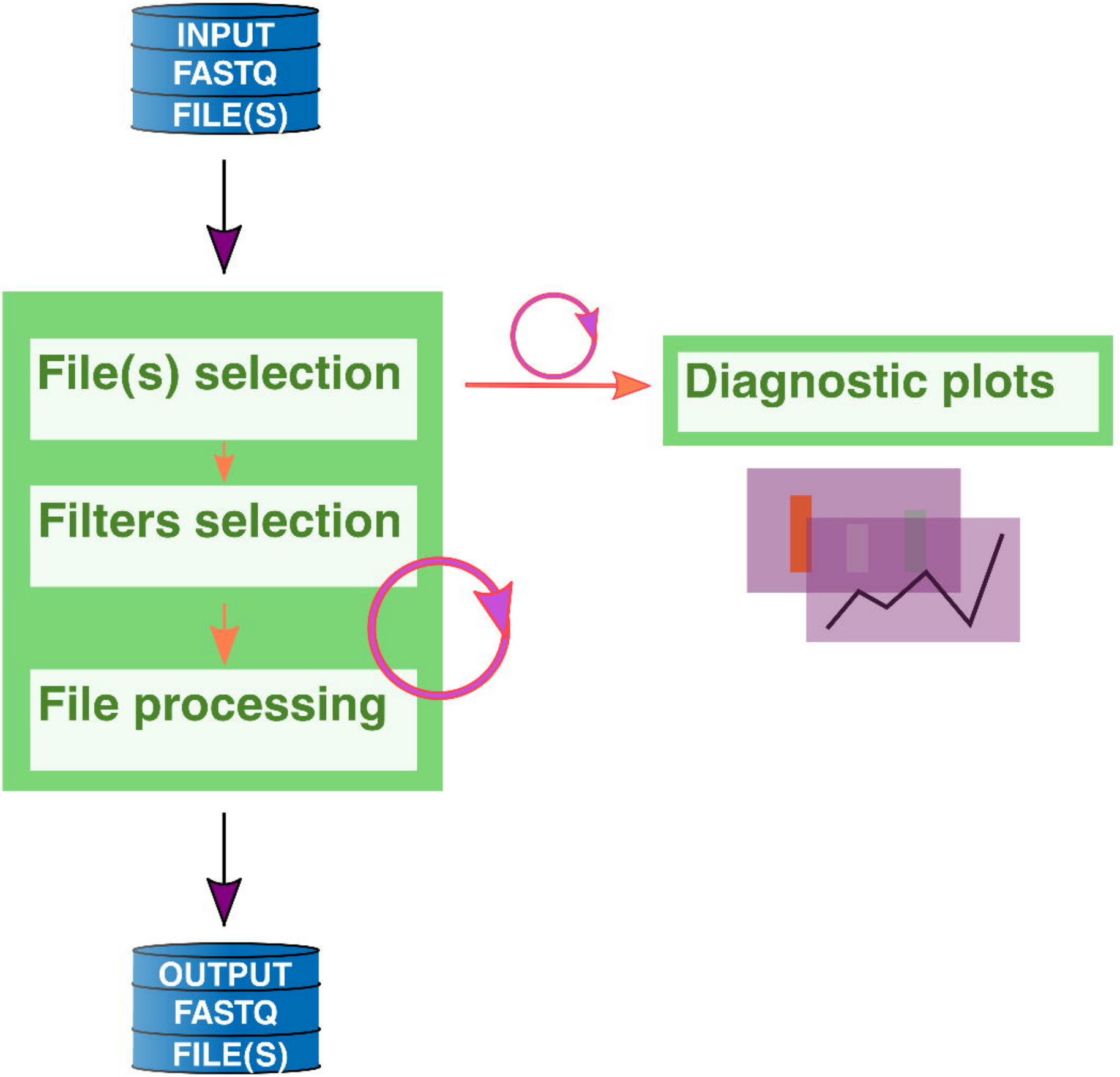
5. Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan M, Carey V. Software for Computing and Annotating Genomic Ranges. PLoS Comput Biol. 2013; 9:e1003118.

6. Pagès H, Aboyoun P, Gentleman R and DebRoy S. Biostrings: String objects representing biological sequences, and matching algorithms. R package version 2.44.2. 2017.

7. Morgan M, Anders S, Lawrence M, Aboyoun P, Pages H, Gentleman R. ShortRead: a Bioconductor package for input, quality assessment and exploration of high-throughput sequence data. Bioinformatics 2009; 25: 2607–2608.

8. Eddelbuettel D, Romain F, Allaire J, Chambers J, Bates D, Ushey K. Rcpp: Seamless R and C++ integration. J Stat SoftW. 2011; 40: 1-18.

252 9. Chang W, Cheng J, Allaire JJ, Xie Y, McPherson J. shiny: Web Application Framework for R. R
253 package version 0.14.2. 2016. https://CRAN.R-project.org/package=shiny
254
255 10. Schubert M, Stinus L, and Ludovic O. AdapterRemoval v2: rapid adapter trimming, identification,
256 and read merging. BMC R Notes. 2016; 9: 88.
257
258 11. Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads.
259 EMBnet journal. 2011; 17:10.
260
261 12. Hannon G. FASTX Toolkit. http://hannonlab.cshl.edu/fastx_toolkit/index.html. Accessed
262 September 5 2017.
263
264 13. Dodt M, Roehr J, Ahmed R, Dieterich C. FLEXBAR—flexible barcode and adapter processing for
265 next-generation sequencing platforms. Biology 2012; 1: 895-905.
266
267 14. Jiang H, Lei R, Ding SW, Zhu S. Skewer: a fast and accurate adapter trimmer for next-generation
268 sequencing paired-end reads. BMC bioinformatics 2014; 15: 182.
269
270 15. Bolger A, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data.
271 Bioinformatics 2014; 30: 2114-2120.


INPUT
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FILE(S)
File(s) selection
Filters selection
File processing
Diagnostic plots
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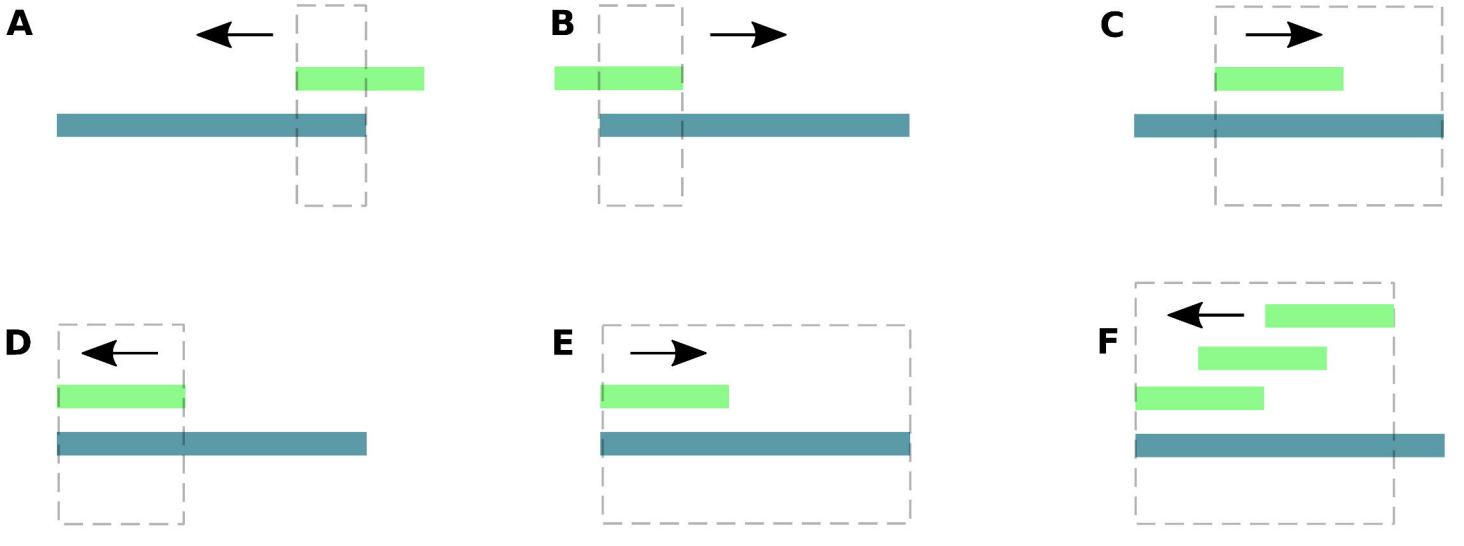
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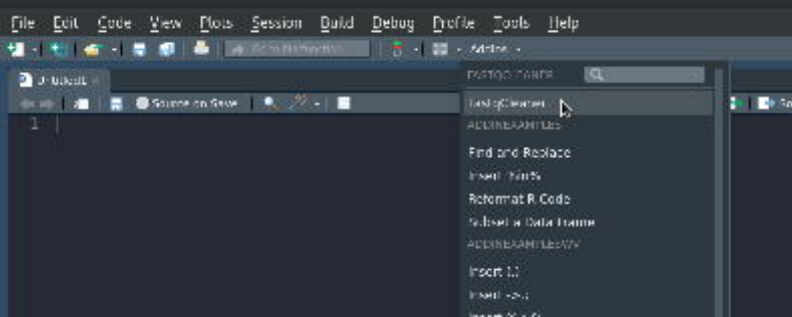
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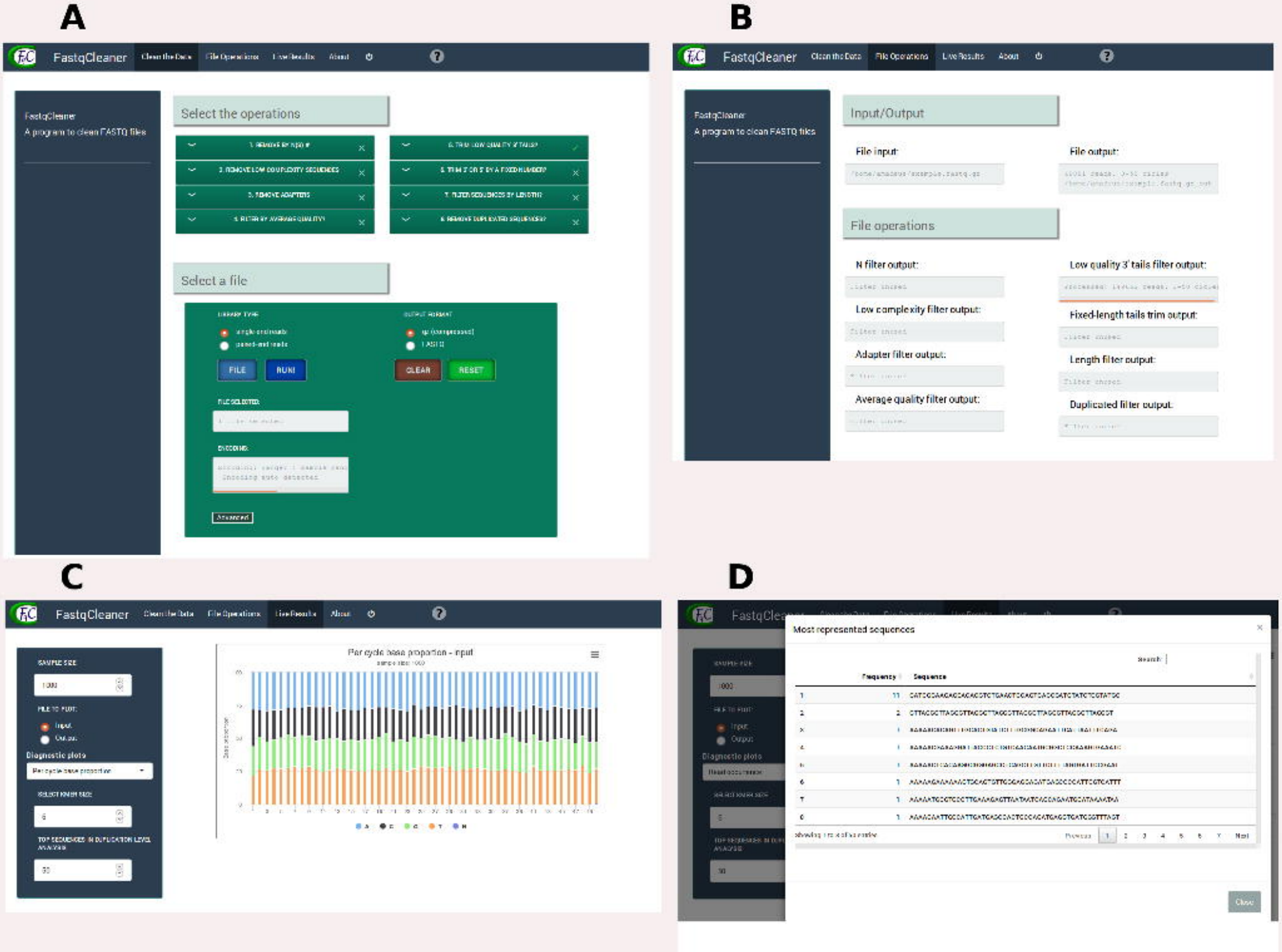
A
FastqCleaner
Clean the Data
File Operations
Live Results
About
FastqCleaner
A program to clean FASTQ files
Select the operations
Select a file
FILE
RUN!
CLEAR
RESET
Advanced
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File output:
File operations
N filter output:
Low complexity filter output:
Adapter filter output:
Average quality filter output:
Low quality 3' tails filter output:
Fixed-length tails trim output:
Length filter output:
Duplicated filter output:
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Input
Output
Diagnostic plots
Per cycle base proportion
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TOP SEQUENCES IN DUPLICATION LEVEL ANALYSIS
Per cycle base proportion - input
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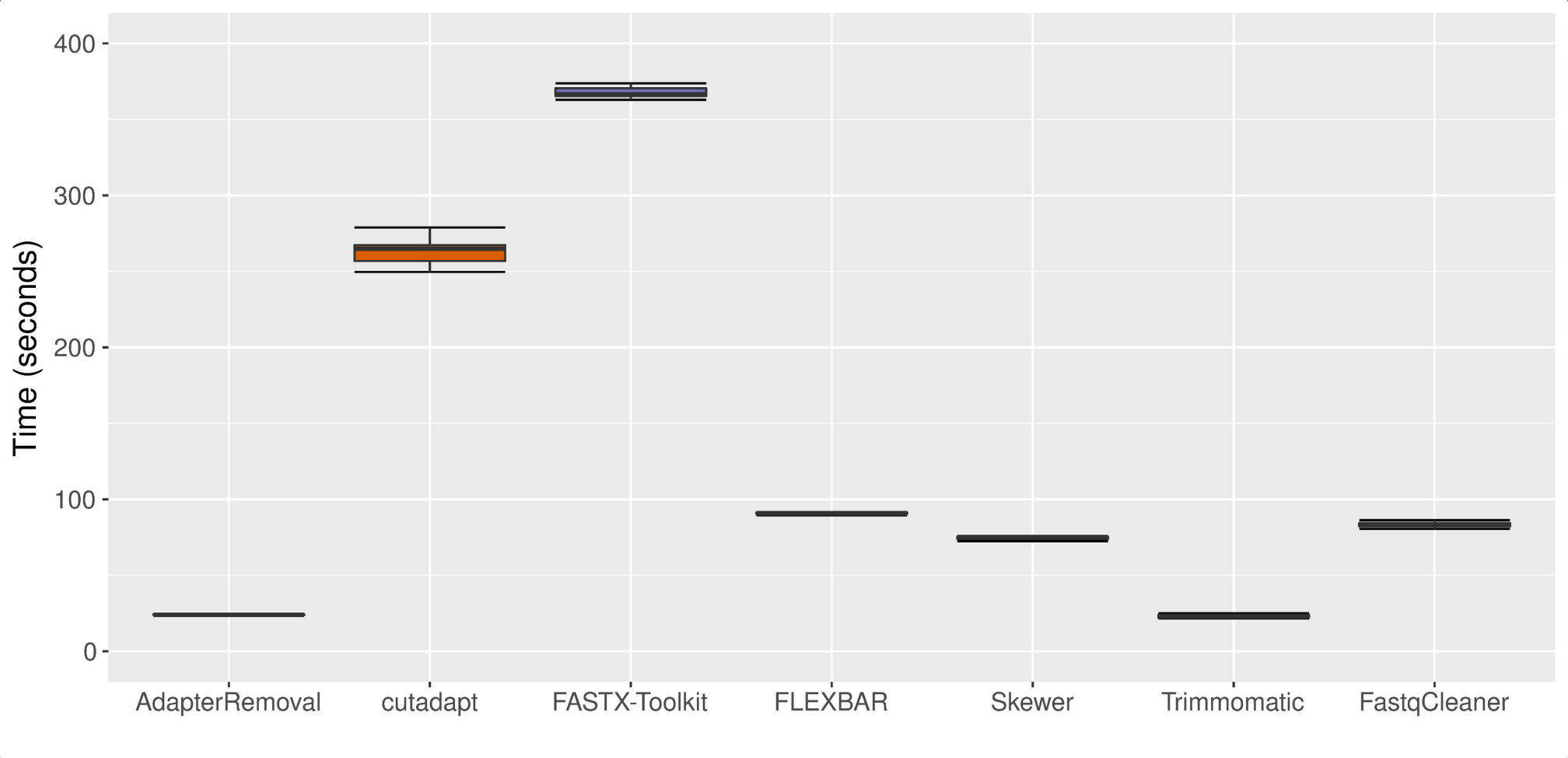
Most represented sequences
Frequency
Sequence
Close


Time (seconds)
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300
200
100
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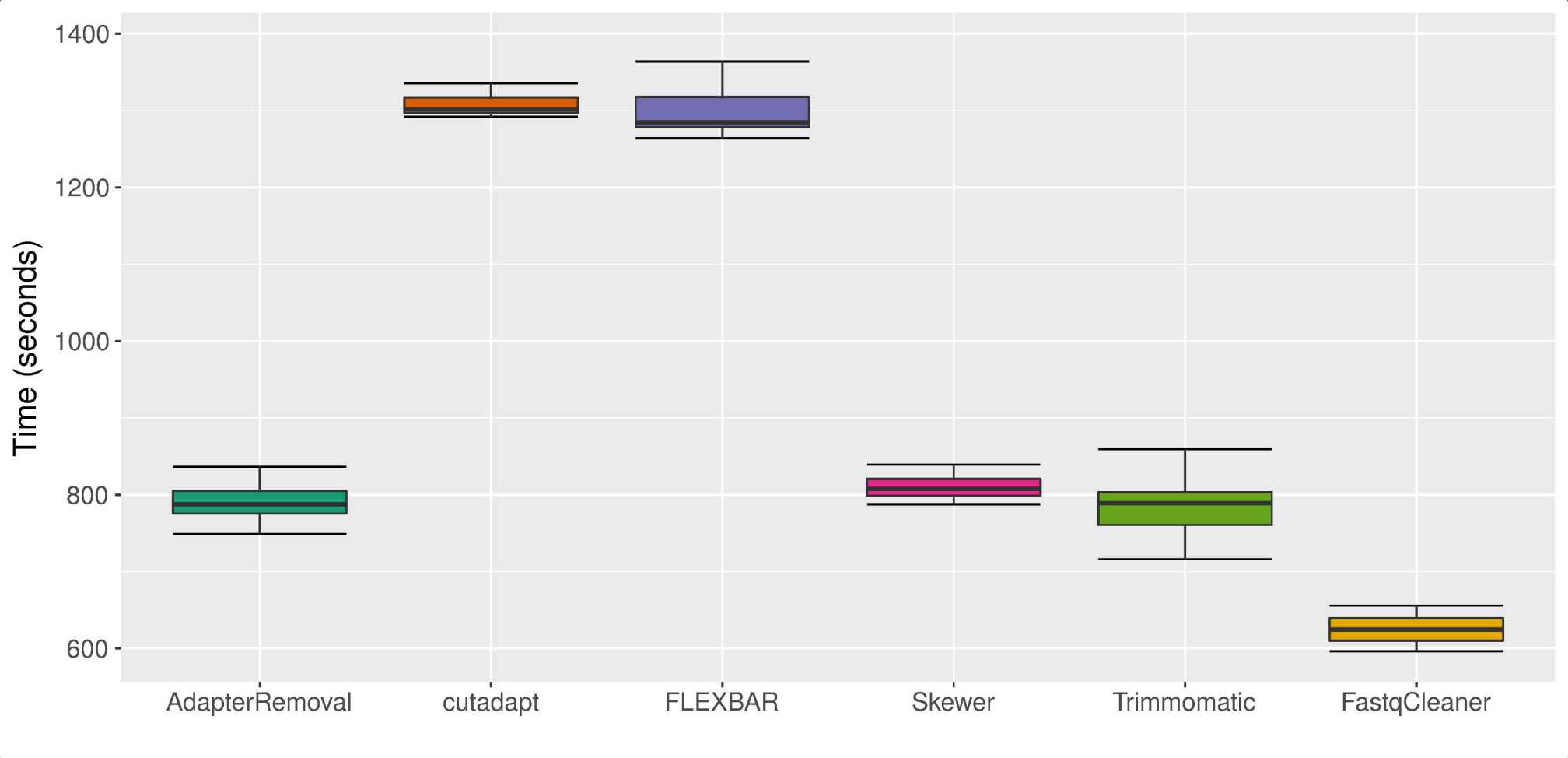
AdapterRemoval
cutadapt
FASTX-Toolkit
FLEXBAR
Skewer
Trimmomatic
FastqCleaner


Time (seconds)
1400
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1000
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AdapterRemoval
cutadapt
FLEXBAR
Skewer
Trimmomatic
FastqCleaner