HTSQualC is a Flexible and One-Step Quality Control Software for High-throughput Sequencing Data Analysis

Renesh Bedre[1], Carlos Avila[2], Kranthi Mandadi[1,3*]

[1]Texas A&M AgriLife Research and Extension Center, Texas A&M University, Weslaco, TX, USA

[2]Department of Horticultural Science, Texas A&M University, College Station, TX, USA

[3]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA

Running title: Quality Control of High-throughput Sequencing Data

*corresponding author: kkmandadi@tamu.edu

Keywords: High-throughput sequencing (HTS), Quality control, Parallel computing, Software tool, CyVerse, Nextflow

ABSTRACT

Use of high-throughput sequencing (HTS) has become indispensable in life science research. Raw HTS data contains several sequencing artifacts, and as a first step it is imperative to remove the artifacts for reliable downstream bioinformatics analysis. Although there are multiple stand-alone tools available that can perform the various quality control steps separately, availability of an integrated tool that can allow one-step, automated quality control analysis of HTS datasets will significantly enhance handling large number of samples parallelly. Here, we developed HTSQualC, a stand-alone, flexible, and easy-to-use software for one-step quality control analysis of raw HTS data. HTSQualC can evaluate HTS data quality and perform filtering and trimming analysis in a single run. We evaluated the performance of HTSQualC for conducting batch analysis of HTS datasets with 322 samples with an average ~1M (paired end) sequence reads per sample. HTSQualC accomplished the QC analysis in ~3 hours in distributed mode and ~31 hours in shared mode, thus underscoring its utility and robust performance. In addition to command-line execution, we integrated HTSQualC into the free, open-source, CyVerse cyberinfrastructure resource as a GUI interface, for wider access to experimental biologists who have limited computational resources and/or programming abilities.

INTRODUCTION

Advancements in high throughput sequencing (HTS) technologies transformed biological research. HTS largely replaced conventional low-throughput Sanger-based sequencing technologies for genome-scale studies. Multiple genome sequencing approaches (DNA-seq, RAD-seq, GBS, AgSeq) are being used to study genetic variations, discovery of novel genes, high-throughput genotyping, biomarker discovery, and precision medicine [1-5]. Similarly, transcriptome-level sequencing approaches (RNA-seq) allows determining the steady-state expression of genes, identification of novel transcripts and isoforms, alternatively splicing patterns, polymorphisms, gene co-expression networks, allele-specific expressions, and long non-coding RNAs (lincRNA) [6-9]. Illumina's benchtop and production-scale sequencers, which are by far the most widely used HTS platforms, can generate up to 1 and 20 billion sequence reads per run, respectively. This sequencing output is expected to rapidly increase due to further advancements in the Illumina technologies. As such advanced bioinformatics software tools are necessary to accelerate the analysis and efficiently manage the large volumes of data generated by the Illumina sequencing platforms.

In all HTS experiments, a critical first step is raw data quality assessment, filtering and trimming. HTS raw data often contains sequences of poor quality along with adapter or primer contaminations, and uncalled bases (N), which if not removed can significantly hamper the downstream bioinformatics analysis leading erroneous conclusions [10-12]. Several standalone software tools are available for quality control analysis, trimming, and filtering of HTS data such as FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), NGS QC [13], FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), NGS QCbox [14], Trimmomatic [15], fastp [16], and QC-Chain [11]. However, most of them have limitations. For instance, FastQC performs only quality check of the data, and does not filter or trim of raw sequences (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Conversely, FASTX-Toolkit although is equipped to perform quality filtering does not support parallel computing to handle large-scale batch analysis. Other software tools such as NGS QC, QC-Chain, Trimmomatic, and NGS QCbox have limited features for quality filtering, handles few samples at a time, dependent on other open-source software tools, and have a need to be run separately for different quality control features. Even though fastp has advantages over other tools, it does not support batch analysis [16]. With HTS becoming more accessible and affordable, it is crucial to develop a

flexible and integrative tool that can not only perform thorough quality control analysis, but can handle several hundred samples parallelly, with the least number of data handling steps.

Here, we present HTSQualC, which is an open-source and easy-to-use quality control analysis software tool for cleaning raw HTS datasets generated from Illumina sequencing platforms. HTSQualC is a flexible, one-step quality control software tool and can handle large number of samples. HTSQualC integrates filtering and trimming modules for single and paired end HTS data and supports parallel computing for batch quality control analysis. In addition to the quality filtering and trimming analysis, HTSQualC generates statistical summaries and visualization to assess the quality of the HTS datasets. HTSQualC can be used as command-line interface (CLI) as well as GUI. The GUI is available through CyVerse [17,18] Discovery Environment (https://cyverse.org/).

MATERIALS AND METHODS

Implementation

HTSQualC is a standalone open-source command-line software developed using Python 3 for quality control analysis of HTS data generated from Illumina sequencing platforms. The current HTSQualC version (v1.1) was developed specifically for Illumina generated FASTQ datasets, however, it could be utilized with other sequencing platforms given the input is FASTQ and supports same quality formats as Illumina. We focused on Illumina mainly because it is the most-widely utilized platform. We will continue development and subsequent versions of HTSQualC will be released to expand its use to other platforms such as PacBio and/or Nanopore Sequencing. At its core, HTSQualC consists of two main modules that are intended to filter and trim single and paired-end sequence datasets. Both modules were implemented using parallel computation to increase the performance of quality control analysis by allocating the input workload to the multiple CPUs. By default, there are only two CPUs, however this number can be changed as per user preferences. To some extent, HTSQualC works similar to MapReduce where it splits the large sequence file into smaller chunks, distributes the input data to multiple CPUs, and combines the input from each process to produce a final output.

HTSQualC checks quality issues in the raw HTS datasets and performs the quality filtering and/or trimming in a single run for removing low-quality bases, adapter contamination, and uncalled bases (N) as per the settings of the user. The entire quality control can be completed

125 in a single input command. By default, HTSQualC filters out the sequence reads with Phred
126 quality score < 20. We did not add the feature of duplicate reads removal in HTSQualC as this
127 feature is directly related to the gene quantification and estimation of expression [7]. A flowchart
128 HTSQualC analysis is shown in Fig. 1.
129 HTSQualC can handle batch analysis of multiple sequencing datasets at a time. It accepts
130 FASTQ file format as input and produces FASTQ or FASTA file format as output. HTSQualC
131 accepts the GZ compressed FASTQ files and also provides an option to output GZ compressed
132 FASTQ file. HTSQualC also generates summary statistics and visualization outputs for the
133 filtered cleaned HTS datasets. All the outputs by default are saved in the same directory
134 containing the raw RNA-seq input datasets. HTSQualC was primarily designed to run on the
135 Linux and Mac operating systems as command-line interface CLI (Fig. 2), however, it can also
136 run on the Windows operating systems using virtual machine. In addition, we made HTSQualC
137 publicly available as GUI (Fig. 2) on CyVerse [17,18], which would be useful for experimental
138 biologists with minimal bioinformatics training. CyVerse is an open-access, scalable,
139 comprehensive, data analysis and management infrastructure for large-scale data analysis
140 developed for life science research. CyVerse can be accessed through world-wide-web, and users
141 can register for free at https://user.cyverse.org/register. To use the HTSQualC as GUI on
142 CyVerse, it is necessary to have CyVerse account. Additionally, we have also provided the
143 docker image and Nextflow template for running the HTSQualC.
144
145 **RESULTS AND DISCUSSION**
146 **Case studies**
147 To evaluate the performance of HTSQualC for quality control analysis, we analyzed several
148 datasets using a 64 GB RAM and 20 CPUs computing node (Intel 2.5 GHz IvyBridge processor)
149 of Texas A&M High Performance Research Computing Center (HPRC) (http://hprc.tamu.edu/).
150 First, we analyzed a single-end raw RNA-seq dataset generated on Illumina HiSeq 2000 platform
151 corresponding to cotton, a dicot plant (BioProject accession PRJNA275482 and SRA ID:
152 SRR1805340, Table 1) [19,20]. HTSQualC analysis was performed with the default parameters.
153 HTSQualC automatically detected the Illumina sequence quality variant and filtered out the
154 reads with Phred quality score < 20. In total, HTSQualC filtered out ~5 M reads (Fig.3 and
155 Supplementary File 1A).

156 Second, we evaluated multiple paired-end raw RNA-seq datasets generated on Illumina
157 HiScanSQ platform corresponding to sugarcane, a monocot plant (BioProject accession
158 PRJNA291816 and SRA IDs: SRR2165176, SRR2165177, SRR2165178) [6,21]. Initially, we ran
159 HTSQualC on one paired-end dataset (SRR2165176) using default parameters (Table 1).
160 HTSQualC was able to analyze the Illumina sequence quality variants and filtered out the reads
161 with Phred quality score < 20 (Supplementary File 1B). For instance, the HTSQualC filtered out
162 the ~250 K sequence reads which were below the quality threshold (Supplementary File 1B).
163 Next, we ran the HTSQualC with customized parameters for filtering adapter sequences, quality
164 thresholds, and uncalled bases. In total, HTSQualC filtered out ~451 K reads and trimmed ~20 K
165 reads (Supplementary File 1C). All the HTSQualC commands used for performing the above
166 analyses are provided in the README file.
167 In addition to quality control analysis, we also evaluated the processing time and batch
168 handling of HTSQualC using default parameters. HTSQualC took ~9 min to perform the quality
169 filtering analysis of ~9 M paired-end sequence reads, and ~40 min for ~82 M single-end
170 sequence reads with 18 CPUs (Table 1).
171 Lastly, we analyzed 322 paired-end genotyping-by-sequencing (GBS) datasets
172 corresponding to tomato [5]. These datasets had ~1M (×2) sequence reads per sample. For this
173 experiment, we also compared parallel computing in shared vs. distributed mode with 18 CPUs
174 per computing node using Nextflow. We used a single HPRC node for shared mode and several
175 HPRC nodes for distributed mode. We were able to analyze the 322 datasets in ~1855 min (~31
176 hours) and ~157 min (~3 hours) in the shared and distributed computing modes, respectively
177 (Table 1).
178
179 **Advantages and disadvantages of HTSQualC over existing quality control analysis**
180 **software**
181 We compared the advantages of HTSQualC with prevailing quality control analysis tools such as
182 the FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), NGS QC [13], QC
183 Chain [11], FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), fastp [16] and NGS QCbox [14]
184 on various important quality control features (Table 2). Many of the current tools are not as
185 integrated as HTSQualC (Table 2).

For instance, programs such as FastQC only provides the quality control statistics of the raw HTS data and cannot be used for quality filtering. The FASTX-Toolkit does quality filtering, but the various trimming, filtering options are not integrated, and each analysis must be performed separately. The NGS QCbox comes close, however only allows sequence read trimming based on a quality threshold for specific window size and it is highly dependent on other open-source software tools for quality control analysis [14]. NGS QC allows filtering and trimming of low-quality reads similar to FASTX-Toolkit, however these software tools are independent and must be run separately. The fastp provides single run quality control analysis of FASTQ files similar to HTSQualC but does not offer key features such as batch quality control analysis, automatic sequence quality format detection, etc. The fastp does offer other features which are not included in HTSQualC such as poly tail trimming, UMI preprocessing, and basic support for the long-read sequence data. Hence, in this context, the two tools can be complementary to each other for a range of applications.

The removal of sequence reads containing uncalled bases (N) is critical to improve the accuracy of the sequence reads [22]. HTSQualC has this feature of filtering the sequence reads based on the content of the uncalled bases (N). Although NGS QC allows this analysis, it is not integrated, and must be run separately [13]. In addition to overcoming several of these limitations, HTSQualC supports common features with NGS QC and NGS QCbox such as allowing inputs in the compressed GZIP file format for seamless input of large datasets (Table 2). Furthermore, similar to NGS QC, HTSQualC has an integrated function to automatically detect the FASTQ quality variants (Table 2). Only HTSQualC provides a parameter to output the cleaned FASTQ data in FASTA format, avoiding using additional file format conversion tools. Lastly, in addition to the command line execution, we integrated HTSQualC as a graphic user interface that is accessible freely for everyone to access through CyVerse.

We compared the running times of HTSQualC with FastQC, FASTX-Toolkit, and fastp with default settings for quality filtering Among these three tools, fastp took the least amount of time (2 minutes), followed by FastQC (3 minutes) and HTSQualC (24 minutes). The HTSQualC took longer as it is developed in Python 3 (interpreted language), which is slower than C/C++ and Java, while FASTX-Toolkit took the longest time (33 minutes) mainly because it does not support parallel computation. When we compared the memory consumption, the fastp (~420 MB) and HTSQualC (~818 MB) outperformed FastQC (1.55 GB). Because HTSQualC works in

217 the same way as MapReduce, it will require more storage because of the numerous input and
218 output processes. Furthermore, as HTSQualC is nearing completion, it automatically deletes the
219 intermediate files, reducing further manual steps required to clear the intermediate files. We
220 could not evaluate NGS QC toolkit, QC chain, and NGS QCbox as these tools were not available
221 or currently not maintained anymore.
222 We also evaluated the output of HTSQualC and fastp with default settings for quality
223 filtering. For quality filtering, HTSQualC and fastp have different algorithmic implementations.
224 For instance, by default, fastp performs the quality filtering on Phred quality score and discards
225 the sequencing reads where certain percentages of bases have Phred quality <15 (<97% base call
226 accuracy), whereas HTSQualC discards the reads which have average Phred quality <20 (<99%
227 base call accuracy). With default settings, fastp filtered out ~115K reads, whereas HTSQualC
228 filtered out 4256 reads on an Illumina dataset containing ~25 M reads. It is advisable to keep the
229 base call with high Phred quality (>20 or >30) to minimize calling false-positive variant calls [23].
230 HTSQualC also offers more data outputs, such as the sequence quality format, minimum,
231 maximum, and mean read lengths, and average Phred quality values of filtered and unfiltered
232 reads, which are not included in the fastp output (Supplementary File 2). The output details are
233 provided in Supplementary File 2.
234
235 **CONCLUSION**
236 HTSQualC is an open-source, integrated, and easy-to-use software designed for one-step quality
237 control visualization and quality filtering of raw HTS data generated using the Illumina
238 sequencing platforms. The flexiblility to detect and remove the low-quality sequences, adapter or
239 primer contamination, uncalled bases, in a single run, greatly enhances the automation of HTS
240 data analysis projects. Because HTSQualC can be implemented by parallel computing, it enables
241 batch handling of large number (>300) of datasets. In addition to the command line interface,
242 HTSQualC is available as graphic user interface that is accessible freely through CyVerse. The
243 later should significantly facilitate its use among biologists without much prior bioinformatics or
244 command line computing experience.
245
246
247

248 **REFERENCES**

249

250 1 Edwards, D. & Batley, J. Plant genome sequencing: applications for crop improvement.
251 *Plant Biotechnol. J.* **8**, 2-9, doi:10.1111/j.1467-7652.2009.00459.x (2010).
252 2 Bolger, M. E. *et al.* Plant genome sequencing - applications for crop improvement. *Curr.*
253 *Opin. Biotechnol.* **26**, 31-37, doi:10.1016/j.copbio.2013.08.019 (2014).
254 3 Suwinski, P. *et al.* Advancing personalized medicine through the application of whole
255 exome sequencing and big data analytics. *Front. Genet.* **10**, 49,
256 doi:10.3389/fgene.2019.00049 (2019).
257 4 Awika, H. O. *et al.* Developing Growth-Associated Molecular Markers Via High-
258 Throughput Phenotyping in Spinach. *Plant Genome-Us* **12**, doi:ARTN 190027
259 10.3835/plantgenome2019.03.0027 (2019).
260 5 Kandel, D. R., Bedre, R. H., Mandadi, K. K., Crosby, K. & Avila, C. A. Genetic diversity
261 and population structure of tomato (*Solanum lycopersicum*) germplasm developed by
262 Texas A&M breeding programs. *Am. J. Plant Sci.* **10**, 1154-1180 (2019).
263 6 Bedre, R. *et al.* Genome-wide alternative splicing landscapes modulated by biotrophic
264 sugarcane smut pathogen. *Sci. Rep.* **9**, 8876, doi:10.1038/s41598-019-45184-1 (2019).
265 7 Zhou, Q., Su, X., Jing, G., Chen, S. & Ning, K. RNA-QC-chain: comprehensive and fast
266 quality control for RNA-Seq data. *BMC Genomics* **19**, 144, doi:10.1186/s12864-018-
267 4503-6 (2018).
268 8 Bedre, R., Irigoyen, S., Petrillo, E. & Mandadi, K. K. New era in plant alternative
269 splicing analysis enabled by advances in high-throughput sequencing (HTS)
270 technologies. *Front. Plant Sci.* **10**, doi:10.3389/fpls.2019.00740 (2019).
271 9 Xu, W. *et al.* Differential expression networks and inheritance patterns of long non-
272 coding RNAs in castor bean seeds. *Plant J.* **95**, 324-340, doi:10.1111/tpj.13953 (2018).
273 10 Conesa, A. *et al.* A survey of best practices for RNA-seq data analysis. *Genome Biol.* **17**,
274 13, doi:10.1186/s13059-016-0881-8 (2016).
275 11 Zhou, Q., Su, X., Wang, A., Xu, J. & Ning, K. QC-Chain: fast and holistic quality control
276 method for next-generation sequencing data. *PLoS One* **8**, e60234,
277 doi:10.1371/journal.pone.0060234 (2013).
278 12 Trivedi, U. H. *et al.* Quality control of next-generation sequencing data without a
279 reference. *Front. Genet.* **5**, 111, doi:10.3389/fgene.2014.00111 (2014).
280 13 Patel, R. K. & Jain, M. NGS QC Toolkit: a toolkit for quality control of next generation
281 sequencing data. *PLoS One* **7**, e30619, doi:10.1371/journal.pone.0030619 (2012).
282 14 Katta, M. A., Khan, A. W., Doddamani, D., Thudi, M. & Varshney, R. K. NGS-QCbox
283 and Raspberry for Parallel, Automated and Rapid Quality Control Analysis of Large-
284 Scale Next Generation Sequencing (Illumina) Data. *PLoS One* **10**, e0139868,
285 doi:10.1371/journal.pone.0139868 (2015).
286 15 Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina
287 sequence data. *Bioinformatics* **30**, 2114-2120, doi:10.1093/bioinformatics/btu170 (2014).
288 16 Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor.
289 *Bioinformatics* **34**, i884-i890, doi:10.1093/bioinformatics/bty560 (2018).
290 17 Goff, S. A. *et al.* The iPlant collaborative: Cyberinfrastructure for plant biology. *Front*
291 *Plant Sci* **2**, doi:10.3389/fpls.2011.00034 (2011).

18 Merchant, N. *et al.* The iPlant Collaborative: Cyberinfrastructure for Enabling Data to Discovery for the Life Sciences. *PLoS Biol.* **14**, e1002342, doi:10.1371/journal.pbio.1002342 (2016).
19 Bedre, R. *et al.* Genome-wide transcriptome analysis of cotton (*Gossypium hirsutum* L.) identifies candidate gene signatures in response to aflatoxin producing fungus *Aspergillus flavus*. *PLoS One* **10**, e0138025, doi:10.1371/journal.pone.0138025 (2015).
20 Bedre, R. *Genome-wide Transcriptome Analysis of Cotton (Gossypium hirsutum L.) to Identify Genes in Response to Aspergillus flavus Infection, and Development of RNA-Seq Data Analysis Pipeline* Ph.D. thesis, Louisiana State University, (2016).
21 Schaker, P. D. *et al.* RNAseq transcriptional profiling following whip development in sugarcane smut disease. *PLoS One* **11**, e0162237, doi:10.1371/journal.pone.0162237 (2016).
22 Minoche, A. E., Dohm, J. C. & Himmelbauer, H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and genome analyzer systems. *Genome Biol.* **12**, R112, doi:10.1186/gb-2011-12-11-r112 (2011).
23 Illumina, I. Quality scores for next-generation sequencing. *Technical Note: Informatics* **31** (2011).

Acknowledgements

We thank Upendra Devisetty, Reetu Tuteja, and Sarah Roberts for their assistance with installing HTSQualC at CyVerse DE, which was made possible through CyVerse's External Collaborative Partnership program. We acknowledge support of Texas A&M High Performance Research Computing Center (http://hprc.tamu.edu/) resources and sequencing support of the Texas A&M AgriLife Genomics and Bioinformatics Service (https://txgen.tamu.edu/). We also acknowledge support of Louisiana State University Agricultural Center and Louisiana State University High Performance Computing resources (http://www.hpc.lsu.edu/) for supporting early stages of the HTSQualC development as part of Ph.D. research of RB. This work was supported in part by funds from Texas A&M AgriLife Research Insect-vectored Disease Seed Grant (114190-96210) to KM, Foundation for Food and Agricultural Research New Innovator Award (2018-534299) and USDA-NIFA (2018-70016-28198, HATCH 1023984) awards to KM.

Author contributions

RB developed the software, platform and conducted the analysis. CA, and KM supervised the study, data analysis and interpretation. All authors have read, reviewed, and approved the manuscript.

Competing interests

329 All authors declare no competing interests.

330

331 **Availability**

332 HTSQualC software, Docker image and Nextflow template are available for download at
333 https://github.com/reneshbedre/HTSQualC and graphical user interface (GUI) is available at
334 CyVerse Discovery Environment (DE) (https://cyverse.org/). Documentation is available at
335 https://reneshbedre.github.io/blog/htseqqc.html and https://cyverse-htseqqc-cyverse-
336 tutorial.readthedocs-hosted.com/en/latest/. The HTSQualC is also available on Anaconda cloud
337 (https://anaconda.org/bioconda/htsqualc) and can be installed using biconda channel.

338

339 **Figure legends**

340 **Fig. 1**: **Flowchart of HTSQualC analysis.** HTSQualC includes two main modules for quality
341 control analysis of single and paired-end HTS datasets generated from Illumina sequencing
342 platforms. HTSQualC filters and trims the raw HTS datasets to remove low-quality bases,
343 adapter or primer contamination, and uncalled bases to generate high-quality datasets for
344 downstream bioinformatics analysis.

345

346 **Fig. 2: Command-line interface (CLI) and Graphic-user interface (GUI) of HTSQualC.**
347 HTSQualC can be launched as CLI or GUI modules. For CLI, the HTSQualC need to installed
348 on a local system, whereas GUI is preinstalled and ready to use online at CyVerse
349 (https://cyverse.org/).

350

351 **Fig. 3: The sequence quality evalaution of HTS datatsets performed by HTSQualC.** A) The
352 sequence quality distribution among the raw (unfiltered) and cleaned (filtered) sequence data,
353 B) The distribution of percenatges of sequence reads with quality score, C) The percentage GC
354 content distribution among the raw and cleaned sequence data, D) The content of nucleotide
355 bases in raw and cleaned sequence data.

Table 1: Summary of the quality control analysis of single and paired-end datasets with single and multiple samples performed using HTSQualC CLI.

SRA Accession or Samples	Read Type (# Samples)	# Sequence reads (File size in GB[a])	Read length (bp)	# CPUs (Parallel computing)	Run time (Min)
SRR2165176	Paired (1)	8,583,424×2 (5.6)	100	18 (Shared)	9
SRR2165176 SRR2165177 SRR2165178	Paired (3)	8,583,424×2 (5.6) 9,282,222×2 (6) 9,918,081×2 (6.4)	100 100 100	18 (Shared)	28
SRR1805340	Single (1)	82,059,811 (27)	100	18 (Shared)	40
Tomato GBS[b]	Paired (322)	~ 1,000,000×2 (206)	150	18 (Shared) 18 (Distributed)	1855 157

[a]Combined file size of two files for paired-end sequence reads

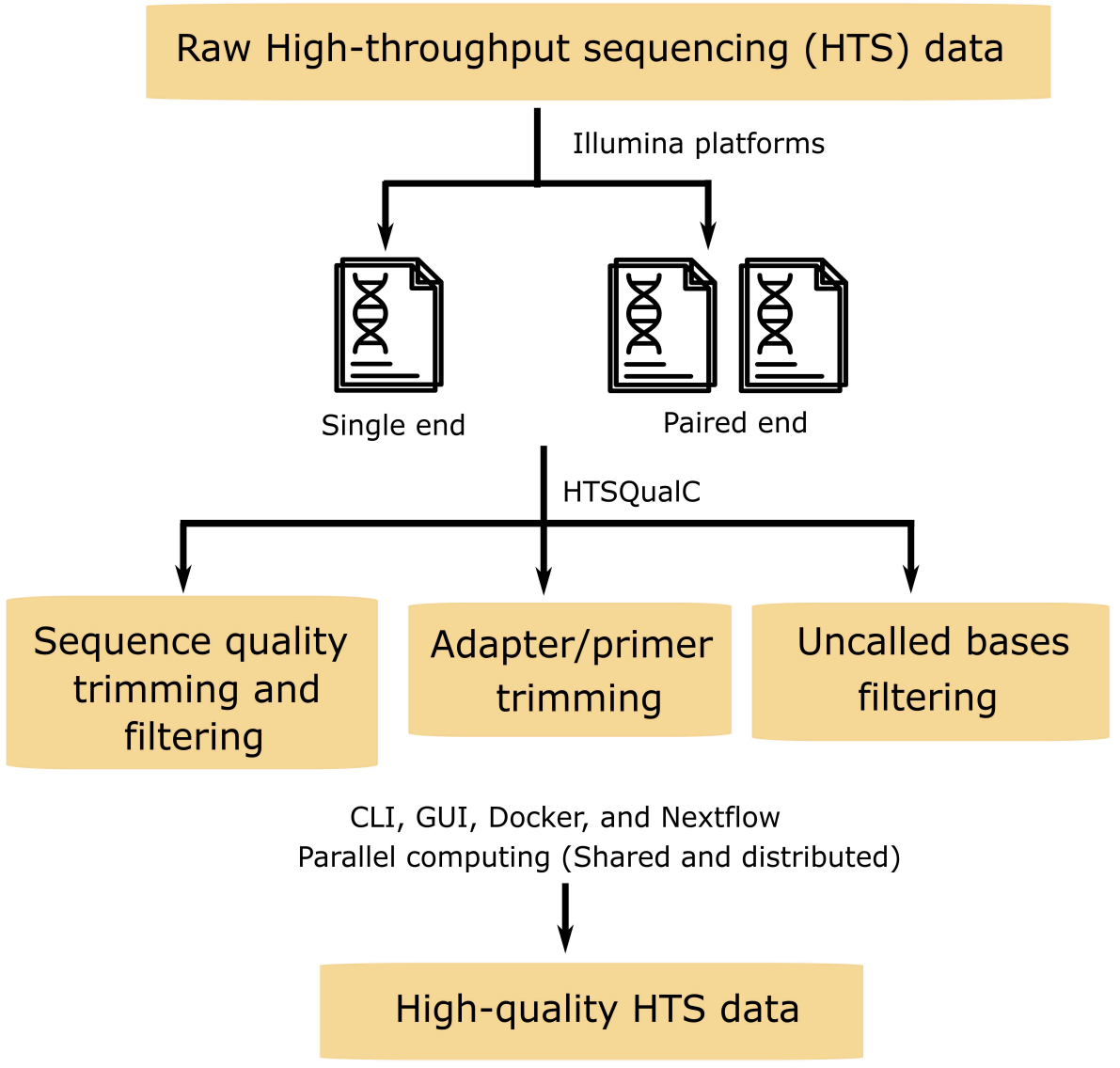
[b]322 tomato genotypes were sequenced using low-coverage whole genome sequencing by Illumina HiSeq 4000 [5]. The number of sequences reads are average of all the datasets.

Table 2: The key sequence quality features comparison of HTSQualC with other leading and equivalent quality control software tools.

Features	HTSQualC	FastQC[a]	NGS QC	QC-Chain	FASTX-Toolkit	NGS QCbox	fastp
Low quality filtering	Yes	Yes	Yes	Yes	Yes	No	Yes
Uncalled bases (N)	Yes	No	Yes*	No	No	No	No
Adapter or primer trimming	Yes	Yes	Yes	Yes	Yes	No	Yes
Multiple adapter support	Yes	Yes	Yes	Yes	No	No	Yes
Minimum read length filtering	Yes	No	No	No	No	Yes	Yes
Low quality trimming	Yes	No	Yes*	Yes	No	Yes	Yes
Paired-end support	Yes	No	Yes	Yes	No	Yes	Yes
Paired-end sequence order	Yes	No	Yes	Yes	No	Yes	Yes
Visualization	Yes	Yes	Yes	Yes	Yes	Yes	
Parallel computing	Yes	Yes	Yes	Yes	No	Yes	Yes
Automatic quality variant detection	Yes	No	Yes	No	No	No	No
FASTA Output	Yes	No	No	No	No	No	No
Programming	Python 3	Java	PERL	C++	C	C	C/C++
GZIP FASTQ input	Yes	No	Yes	No	No	Yes	Yes
Multiple sample support (batch analysis)	Yes	Yes	Yes	No	No	Yes	No
GitHub	Yes	Yes	No	No	Yes	Yes	Yes

[a]FastQC only provides quality metric and does not filter or trim HTS data

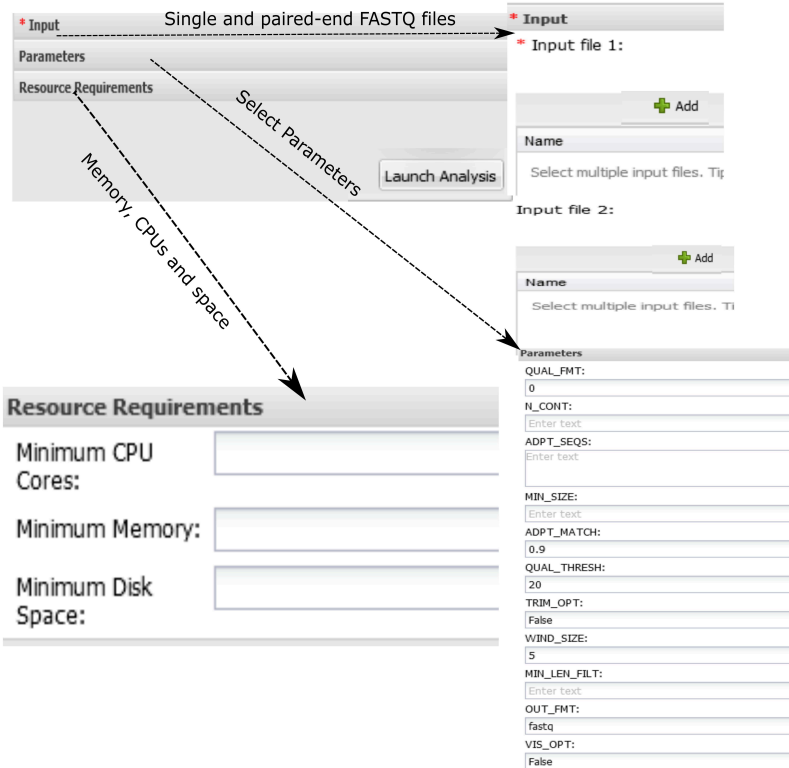
*Separate tools for given features

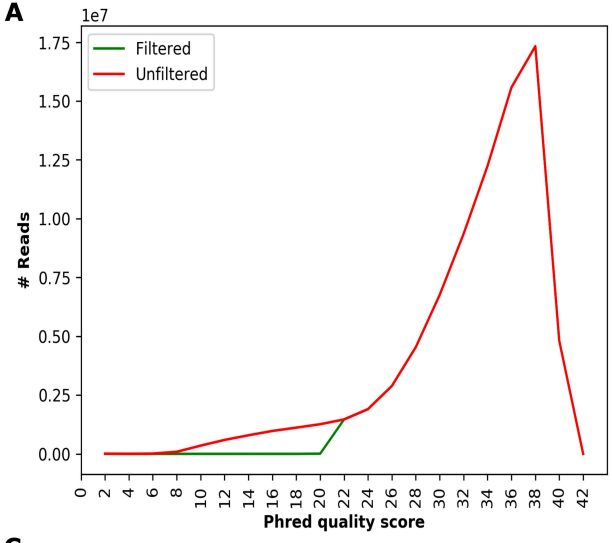

Raw High-throughput sequencing (HTS) data
Illumina platforms
Single end
Paired end
HTSQualC
Sequence quality trimming and filtering
Adapter/primer trimming
Uncalled bases filtering
CLI, GUI, Docker, and Nextflow
Parallel computing (Shared and distributed)
High-quality HTS data

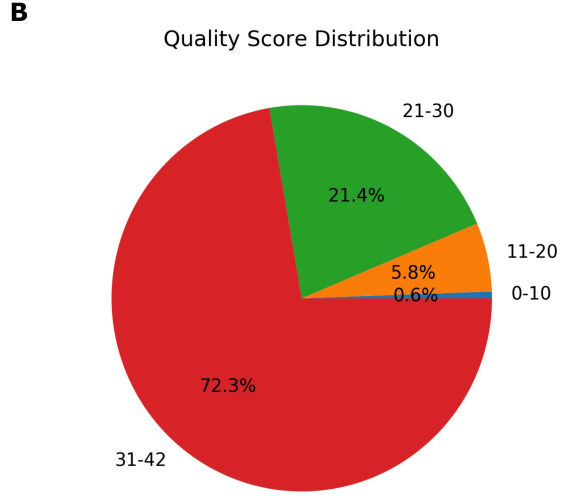
Command-line interface (CLI)

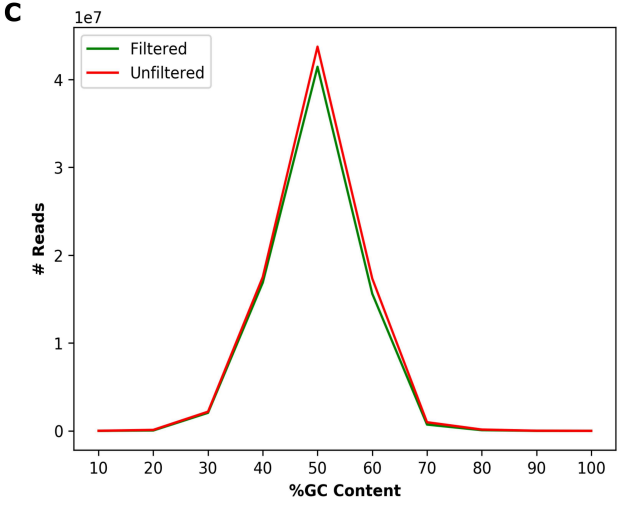
```
usage: filter.py [-h] [-a INPUT_FILES_1 [INPUT_FILES_1 ...]]
                 [-b INPUT_FILES_2 [INPUT_FILES_2 ...]] [-c QUAL_FMT]
                 [-e N_CONT] [-f ADPT_SEQS] [-d MIN_SIZE] [-g ADPT_MATCH]
                 [-i QUAL_THRESH] [-n TRIM_OPT] [-p WIND_SIZE]
                 [-r MIN_LEN_FILT] [-q CPU] [-m OUT_FMT] [-v VIS_OPT]
                 [--version]

Quality control analysis of single and paired-end sequence data

optional arguments:
  -h, --help            show this help message and exit
  -a INPUT_FILES_1 [INPUT_FILES_1 ...], --p1 INPUT_FILES_1 [INPUT_FILES_1 ...]
                        Single end input files or left files for paired-end
                        data (.fastq, .fq). Multiple sample files must be
                        separated by comma or space
  -b INPUT_FILES_2 [INPUT_FILES_2 ...], --p2 INPUT_FILES_2 [INPUT_FILES_2 ...]
                        Right files for paired-end data (.fastq, .fq).
                        Multiple files must be separated by comma or space
  -c QUAL_FMT, --qfmt QUAL_FMT
                        Quality value format [1= Illumina 1.8, 2= Illumina
                        1.3,3= Sanger]. If quality format not provided, it
                        will automatically detect based on sequence data
  -e N_CONT, --nb N_CONT
                        Filter the reads containing given % of uncalled bases
                        (N)
  -f ADPT_SEQS, --adp ADPT_SEQS
                        Trim the adapter and truncate the read sequence
                        (multiple adapter sequences must be separated by
                        comma)
  -d MIN_SIZE, --msz MIN_SIZE
                        Filter the reads which are lesser than minimum size
  -g ADPT_MATCH, --per ADPT_MATCH
                        Truncate the read sequence if it matches to adapter
                        sequence equal or more than given percent (0.0-1.0)
                        [default=0.9]
  -i QUAL_THRESH, --qthr QUAL_THRESH
                        Filter the read sequence if average quality of bases
                        in reads is lower than threshold (1-40) [default:20]
  -n TRIM_OPT, --trim TRIM_OPT
                        If trim option set to True, the reads with low quality
                        (as defined by option --qthr) will be trimmed instead
                        of discarding [True|False] [default: False]
  -p WIND_SIZE, --wsz WIND_SIZE
                        The window size for trimming (5->3) the reads. This
                        option should always set when -trim option is defined
                        [default: 5]
  -r MIN_LEN_FILT, --mlk MIN_LEN_FILT
                        Minimum length of the reads to retain after trimming
  -q CPU, --cpu CPU     Number of CPU [default:2]
  -m OUT_FMT, --ofmt OUT_FMT
                        Output file format (fastq/fasta) [default:fastq]
  -v VIS_OPT, --no-vis VIS_OPT
                        No figures will be produced [True|False]
                        [default:False]
  --version             show program's version number and exit
```

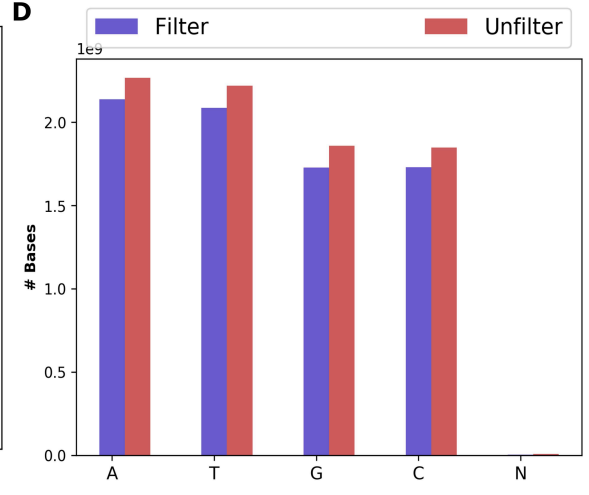
Graphical user interface (GUI) using CyVerse




A
1e7
Filtered
Unfiltered
Reads
Phred quality score


B
Quality Score Distribution
21-30
21.4%
11-20
5.8%
0-10
0.6%
72.3%
31-42


C
1e7
Filtered
Unfiltered
Reads
%GC Content


D
Filter
Unfilter
1e9
Bases
A
T
G
C
N