

Zero-Waste Algorithm 2

(ZWA2)

User manual

January 2024, version 1

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ZWA2 methodology description

Zero-Waste Algorithm 2 (ZWA2) is a context-based trimming bioinformatics tool, especially designed for viral metagenomics, which allows Next Generation Sequencing (NGS) read decontamination based on any given reference sequence(s). ZWA2 incorporates ready-to-use well-established bioinformatics software to detect and dissect partially mapped reads (chimeric reads) by specifically removing the moieties, which align to the given reference sequence(s). The clean output reads enhance de novo assembly performance, increasing the availability of reads for more accurate and more efficacious de novo virus genome assembly. The concept behind the ZWA2 pipeline is outlined in Figure 1.

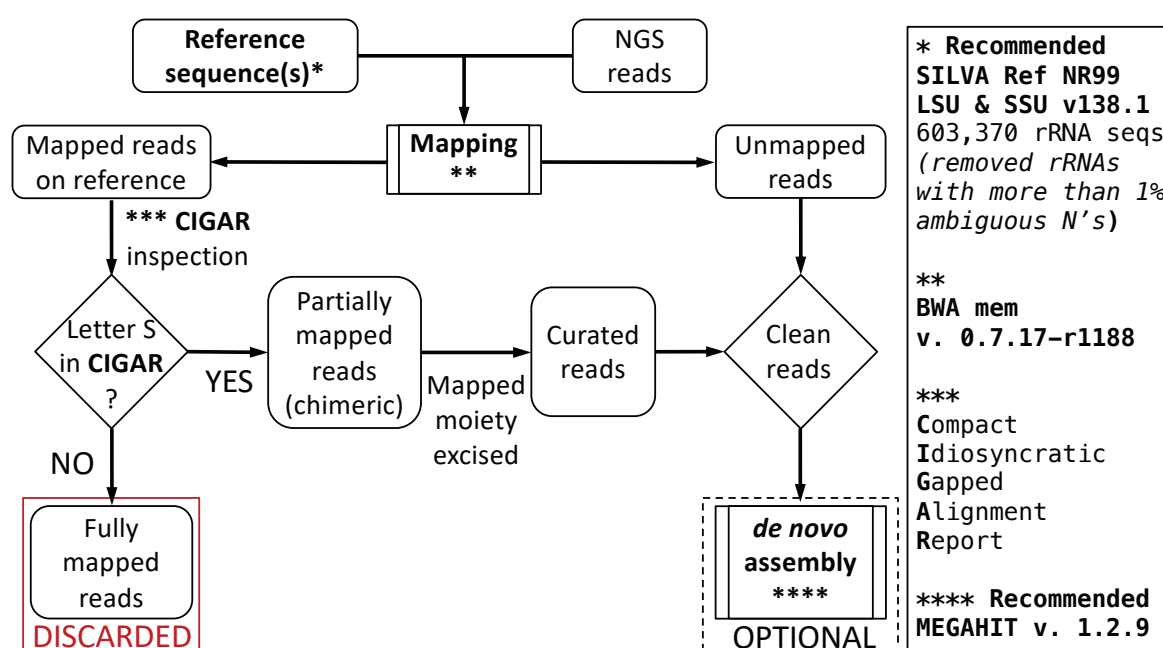


Figure 1

Important note: ZWA2 focuses on the identification and decontamination of partially mapped (chimeric) NGS reads on any given reference sequence(s). For optimum decontamination, we highly recommend downloading and inputting [our custom SILVA ribosomal database \(RiDB\)](#) as reference file, containing 603370 seqs of 16S, 18S, 23S and 28S rRNAs from a wide variety of *Archaea*, *Bacteria* and *Eukarya* organisms. Noteworthy, *de novo* assembly of the clean output reads after decontamination is optional, and therefore is not included in the main ZWA2 pipeline or the program's prerequisites. However, the user may separately perform *de novo* assembly on the clean output reads using the software of preference, with [MEGAHIT *de novo* assembler](#) being highly recommended.

ZWA2 standalone application (offline)

Installation

The ZWA2 standalone application is a Bash shell script distributed for Linux and MacOS systems and may be executed directly after making the downloaded ZWA2.sh file executable (e.g. command 'chmod +x'). The prerequisites of ZWA2 ([bwa 0.7.17](#) and [samtools 1.13](#)) will be verified for installation upon ZWA2 execution and if not installed, they will be downloaded automatically by the program.

Execution

The parameters of the ZWA2 standalone application are summarized in the following table:

Required arguments	Description	Deployment
-i <string>	directory of INPUT NGS READS file (.fastq, .fq or .gz extension)	Full
-r <string>	directory of INPUT REFERENCE file (.fasta, .fa, .fna, .fsta or .gz extension)	Full
-m <string>	directory of MAPPED NGS READS on REFERENCE file (.bam extension)	Partial
-o <string>	directory of OUTPUT folder	Full & Partial
Optional arguments	Description	Deployment
-l <integer>	alignment stringency value (default value 30 <30 loose, >30 stringent)	Full
-u <string>	directory of UNMAPPED NGS READS on INPUT REFERENCE file (.bam, .fastq, .fq or .gz extension)	Partial
-t	run ZWA2 on a test dataset to verify installation	TEST MODE

Run examples

In order to better comprehend the use and output of the ZWA2 standalone application, we highly recommend inputting the `-t` flag only the first time you run it so as to deploy ZWA2 on a small test dataset, which also verifies the installation of all prerequisites and downloads them automatically if needed.

ZWA2 test mode run example:

```
./ZWA2.sh -t
```

For non-test run, the ZWA2 standalone application may be fully or partially deployed upon execution depending on the available user input files. In the case of ZWA2 full deployment, the directories of NGS reads (FASTQ format) and appropriate reference file (FASTA format) must be provided as arguments after the `-i` and `-r` flags respectively, in order to be able to perform all necessary alignments. The user also has the ability to adjust the mapping sensitivity of the incorporated BWA software by passing the desired level of alignment stringency as an integer number after the `-l` flag (default value 30 | <30 loose, >30 stringent).

Full ZWA2 deployment run examples:

```
./ZWA2.sh -i reads.fastq -r ref.fasta -o ./
./ZWA2.sh -i reads.fq.gz -r ref.fasta.gz -o ./
./ZWA2.sh -i reads.fq.gz -r ref.fasta.gz -l 40 -o ./
```

For the alternative and faster partial deployment of ZWA2, in which the user may have already carried out the desired alignment with the mapping software of preference, the directory of a BAM file may only be provided after the `-m` flag, instead of FASTQ and FASTA files. Alongside the input BAM file, the user may optionally pass the output unmapped reads of the performed alignment in FASTQ or BAM format after the `-u` flag, for later use by the algorithm.

Partial ZWA2 deployment run examples:

```
./ZWA2.sh -m mapped.bam -o ./
./ZWA2.sh -m mapped.bam -u unmapped.bam -o ./
./ZWA2.sh -m mapped.bam -u unmapped.fq.gz -o ./
```

Output

ZWA2 outputs 2 files, which are: i) the clean reads after processing as a GZIPPED FASTQ file with the suffix "*ZWA2_cleaned.fastq.gz*", and ii) a detailed cleaning report file named "*ZWA2_cleaning_report.out.gz*". The generated GZIPPED FASTQ file contains all the clean reads by ZWA2 and may be used separately for *de novo* assembly or other downstream analysis. The generated report file is a multi-column file, which provides further information and details on the cleaning performed by ZWA2, in the following format:

Column header	Description
Read_ID	Unique read sequence identifier
RefID	Unique reference sequence identifier
CIGAR	Compact Idiosyncratic Gapped Alignment Report string
Read_seq	Complete read sequence
Read_seqlength	Total read sequence length
Mapped_seq	Mapped sequence of read
Mapped_seq_length	Mapped sequence length of read
Mapped_start	Mapping start position of read
Mapped_end	Mapping end position of read
Left_unmapped_seq_start	New start position of read sequence after left-side trimming
Left_unmapped_seq_end	New end position of read sequence after left-side trimming
Left_unmapped_seq	New read sequence after left-side trimming
Left_unmapped_seq_quality	New read sequence quality after left-side trimming
Left_unmapped_seqlength	New read sequence length after left-side trimming
Right_unmapped_seq_start	New start position of read sequence after right-side trimming
Right_unmapped_seq_end	New end position of read sequence after right-side trimming
Right_unmapped_seq	New read sequence after right-side trimming
Right_unmapped_seq_quality	New read sequence quality after right-side trimming
Right_unmapped_seqlength	New read sequence length after right-side trimming

An overall summary of the ZWA2 analysis is also provided at the end of the generated report file, which has the following line-by-line format:

Line header	Description
BWA alignment stringency	Alignment stringency value of BWA (default value 30 <30 loose, >30 stringent)
Total input reads	Total number of user input reads
Total unmapped reads	Total number of unmapped reads
Total mapped reads	Total number of mapped reads
Fully mapped reads	Total number of fully mapped reads only
Fully mapped reads / Total mapped reads	Percentage of the total number of fully mapped reads to total number of mapped reads
Partially mapped (chimeric) reads	Total number of chimeric reads only
Partially mapped (chimeric) reads / Total mapped reads (%)	Percentage of the total number of chimeric reads to total number of mapped reads
Average mapped bases	Numnber of average mapped bases in chimeric reads
Average mapped bases / Average read length (%)	Percentage of the number of average mapped bases in chimeric reads to average length of chimeric reads
ZWA2 cleaned chimeric reads	Number of chimeric reads cleaned by ZWA2
ZWA2 cleaned chimeric reads / Chimeric reads (%)	Percentage of the number of chimeric reads cleaned by ZWA2
Total clean reads (Unmapped + ZWA2 cleaned)	Total number of clean reads (equal to the sum of unmapped reads + chimeras cleaned by ZWA2)
ZWA2 discarded chimeric reads	Number of chimeric reads discarded by ZWA2
ZWA2 discarded chimeric reads / Chimeric reads (%)	Percentage of the number of chimeric reads discarded by ZWA2 to total number of chimeric reads
Total discarded reads (Fully mapped+ZWA2 discarded)	Total number of discarded reads (equal to the sum of fully mapped reads + chimeras discarded by ZWA2)
Execution time (seconds)	Total execution time of ZWA2 (wall clock run time)

ZWA2 web-based application (online)

Apart from the ZWA2 standalone application, the user may utilize the [ZWA2 online tool](#), which does not require the installation of any software but solely the provision of the appropriate input files according to the following 3 steps:

A) Deployment method selection: Similarly to the standalone application, the user may choose to fully or partially deploy ZWA2 depending on the available input through our online platform. If NGS reads and reference files are available in FASTQ and FASTA formats respectively, then the user should choose “Method 1” as displayed in **Figure 2**, which corresponds to full ZWA2 deployment. Alternatively, if the user has already performed the desired alignment between the NGS reads and reference file of preference, then “Method 2” should be selected, which stands for the faster partial deployment of ZWA2, with the sole input of the appropriate BAM file.

ZWA2 upload method selection

Method 1	?	Full ZWA2 deployment
Method 2	?	Partial ZWA2 deployment

Figure 2

B) Input files upload: Clicking on the ZWA2 deployment method of preference, redirects the user to the upload webpage. Upon selection of full ZWA2 deployment (Method 1), the webpage displays three different upload options to choose from, for the necessary FASTQ and FASTA files separately. These upload options, as displayed in **Figure 3**, are:

1. selection of FASTQ or FASTA input file from a prompt file dialog,
2. submission of a valid SRA accession number (in the case of FASTQ input) or selection from a dropdown menu list of recommended reference files (in the case of FASTA input), or
3. provision of the appropriate link address, where the FASTQ or FASTA input file is stored.

ZWA2 upload page

Upload input NGS reads file (supported formats FASTQ or GZ)








1 <input type="radio"/> Select from file dialog  <input type="button" value="Choose file"/> No file chosen	2 <input type="radio"/> SRA accession number from NCBI SRA database  <input type="text"/>	3 <input type="radio"/> Direct download link address from Filetransfer  <input type="text"/>
Upload reference file (supported formats FASTA or GZ)		
1 <input type="radio"/> Select from file dialog  <input type="button" value="Choose file"/> No file chosen	2 <input type="radio"/> Select from a list of recommended references  SILVA SSU+LSU rRNA (v138.1) 	3 <input type="radio"/> Direct download link address from Filetransfer  <input type="text"/>
<input type="button" value="Submit"/>		

Figure 3

As regards to the partial ZWA2 deployment (Method 2), there are two available upload options, as displayed in **Figure 4**, which are:

1. selection of the necessary BAM input file from a prompt file dialog, or
2. provision of the appropriate link address, where the necessary BAM input file is stored.

ZWA2 upload page

Upload BAM file (supported formats BAM)



1 <input type="radio"/> Select from file dialog  <input type="button" value="Choose file"/> No file chosen	2 <input type="radio"/> Direct download link address from Filetransfer  <input type="text"/>
<input type="button" value="Submit"/>	

Figure 4

Submission of the required input files triggers the upload process and redirects to a new webpage, where the user is informed about the upload progression in real time. In case of upload failure, the user is redirected automatically back to the upload webpage after clicking "OK" on the prompted warning message.

- C) ZWA2 implementation and output:** After successful upload of the appropriate input files, the back-end script execution of ZWA2 begins and the user is informed about its progression in real time as displayed in **Figure 5**. Upon ZWA2 run completion, an overall summary is displayed at the current webpage, along with a download link corresponding to a zipped folder containing the clean reads and generated report files by ZWA2.

ZWA2 execution result

Running ZWA2 now, please wait...

ZWA2

Executing ZWA2...

Verifying installation of ZWA2 prerequisites (bwa & samtools), please wait...

Checking for 0.7.17 version of bwa...

bwa 0.7.17 correctly installed !!!

Checking for 1.13 version of samtools...

samtools 1.13 correctly installed !!!

Performing default BWA alignment, please wait...

Alignment results

Total reads: 100

Mapped reads: 0 [80 (80.00%) fully mapped + 20 (20.00%) partially mapped/chimeric]

Unmapped reads: 0

Cleaning chimeric, please wait...

Cleaning results

Discarded reads: 0 (0.00% of chimeric reads)

Cleaned reads: 20 (100.00% of chimeric reads)

ZWA clean reads: 20

ZWA2 script completed successfully !!!

[Download ZWA2 files](#)

Download link of ZWA2 output files

Real time ZWA2 report

Figure 5