

# SeqTrimNext

## Statistics of pre-processing

Plataforma Andaluza de Bioinformática  
Universidad de Málaga

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# 1 Output Files

SeqTrimNext provides several files, the most interesting ones are in the following directories:

- `output_files`
  - `output.less`, containing an extensive information about the trimming of each sequence. It can be visualised on terminal using the command `less -R`.
  - `used_params.txt`, containing the complete set of parameters used for execution of SeqTrimNext with your data
  - `rejected.txt`, containing a list of rejected sequences together with the reason for their removal.
  - `initial_stats.json`, containing statistics for raw sequences.
  - `stats.json`, containing the statistics of the cleaning process.
  - There is a collection of `folders` that gather sequences with the same MID; each folder contains a `sequences` file (in FASTQ format) with useful reads. There may also exist a file with reads containing low complexity regions. If you want to reconstruct a SFF with the useful segment of each pre-processed read, use `sff_info` file in combination with the original SFF file for the `sfffile` tool.
- `graphs`
  - `size_stats.png`, a graph with the distribution of read lengths in raw data (see Fig. ??).
  - `qualities.png`, a graph to inspect read qualities in raw data (see Fig. ??).
  - `PluginExtractInserts_insert_size.png`, a graph with the distribution of read lengths after SeqTrimNext pre-processing (see Fig. 1).
  - There are other graphs (mostly bar plots) that illustrate the quality of pre-processed reads. All are in PNG format.
- `latex`
  - It is provided as a compressed file `latex.zip` containing all “.tex” files required to compile this document. Graphs are taken from the `graph` folder

# 2 Relevant parameters

In this section, the relevant parameters used in your experiment are shown. Full information about the parameters can be obtained from file `used_params.txt`

## 2.1 General

Plugins applied to every sequence, separated by commas. Order is important

1. PluginIndeterminations
2. PluginFindPolyAt
3. PluginAbAdapters
4. PluginUserContaminants
5. PluginContaminants
6. PluginVectors
7. PluginLowQuality
8. PluginLowComplexity
9. PluginExtractInserts

Remove duplicated (clonal) sequences (using CD-HIT 454)

```
remove_clonality: false
```

Minimum insert size for every trimmed sequence

```
min_insert_size_trimmed: 30
```

Minimum insert size for each end of paired-end reads; true paired-ends have both single-ends longer than this value

```
min_insert_size_paired: 40
```

Seqtrim version

```
seqtrim_version: 2.0.67
```

```
min_sequence_size_raw:
```

## 2.2 Quality

Minimum quality value for every nucleotide

```
min_quality: 20
```

```
window_width:
```

## 2.3 Contaminants

Blast E-value used as cut-off when searching for contaminations

```
blast_evalue_contaminants: 1.0e-10
```

Minimum required identity (%) for a reliable contamination

```
blast_percent_contaminants: 85
```

Minimum hit size (nt) for considering a true contamination

```
min_contam_seq_presence: 40
```

Genus of input data: contaminations belonging to this genus will be ignored

```
genus:
```

Is a contamination considered a source of sequence rejection? (setting to false will only trim contaminated sequences instead of rejecting the complete read)

```
contaminants_reject: true
Path for contaminants database
    contaminants.fasta
    cont_ribosome.fasta
```

### 3 Pre-processing statistics

Next figure is equivalent to Figure ?? but using output reads (useful sequences). The mode is expected to decrease but the shape of the plot should be similar.

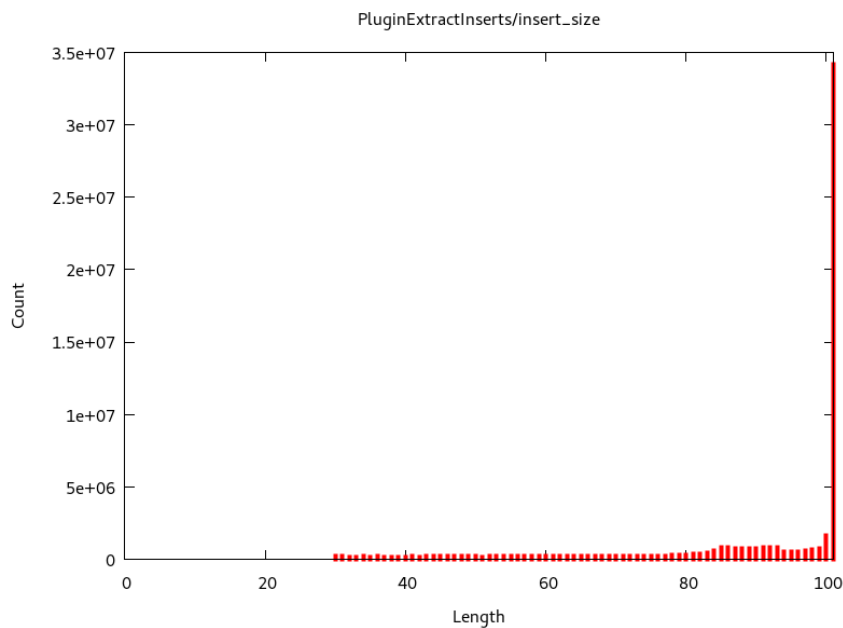


Figure 1: Size distribution of the output sequences. Short sequences ( $< \text{min\_insert\_size\_trimmed}$ ) were removed. [PluginExtractInserts\_insert\_size.png]

Summary statistics of the SeqTrimNext analysis. Be careful and read all warnings that are indicating concerns about your data. In the files `initial_stats.json` and `stats.json` can be found a full statistics of your data and SeqTrimNext pre-processing

Input reads:	total	69558844
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	6655676
	Rejected	10644852
	Low complexity reads	104952
	Mode (bp)	91
	Mean (bp)	90.7
	Output paired reads	52153364
	Total output reads	58809040
Linkers:		

Table 1: List of the most frequent Vectors found among your reads

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	393965
Cloning vector pKOHPRT complete sequence.	392040
Enterobacteria phage lambda	96551
Cloning vector pVLH/hsp	39534
Cloning vector pWormgate2, complete sequence.	32831

Table 2: List of the most frequent Adapters found among your reads

Adapters	sequences
ABISolid3_Adapter_A	193291
TruSeq_Universal_Adapter	154797
ABISolid3_GAPDH_Reverse_Primer	143789
Illumina_Single_End_Adapter_1	138266
ABISolid3_GAPDH_Forward_Primer	127901

Table 3: List of the most frequent Contaminants found among your reads

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	49930
rRNA_long_subunit_Metazoa_Dasytus	41949
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	41339
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	30155
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	30059

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1098779342	Inf %	OK
Low Complexity	51591975	Inf %	OK
Poly T	28636146	Inf %	OK
Poly A	27978441	Inf %	OK
Contaminants	30537737	Inf %	OK
Adapters	56785895	Inf %	OK
Vectors	42603574	Inf %	OK
Indeterminations	172248492	Inf %	OK
Inserts	5340988985	Inf %	iW1

**iW1 Warning!, only Inf % of nucleotides are useful**

## 4 Rejected reads

Input sequences	69558844
Output sequences	6655676
Rejected sequences	10644852
Output paired sequences	52153364
Total output sequences	58809040
Low complexity sequences	104952

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	10644852	15.303 %	OK
Short inserts	6067798	8.723 %	rdW2
Empty Inserts	2572752	3.699 %	rdW3
No Valid Inserts	1654054	2.378 %	rdW5
Contaminants	327784	0.471 %	OK
Indeterminations	15571	0.022 %	OK
Low Complexity	6803	0.010 %	OK
Unexpected Vector	90	0.000 %	OK
Total rejected	10644852	15.303 %	OK

**rdW2 Warning!, a 8.723 % of your sequences are too short**

**rdW3 Warning!, a 3.699 % of your sequences are empty (without an insert)**

**rdW5 Warning!, a 2.378 % of your sequences are no valid sequences**

## References

- [1] Falgueras et al. SeqTrim: a high-throughput pipeline for preprocessing any type of sequence reads. *BMC Bioinformatics* 11:38 (2010)
- [2] Weizhong Li & Adam Godzik. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* (2006) 22:1658-9

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