

# SeqTrimNext

## Statistics of pre-processing

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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.

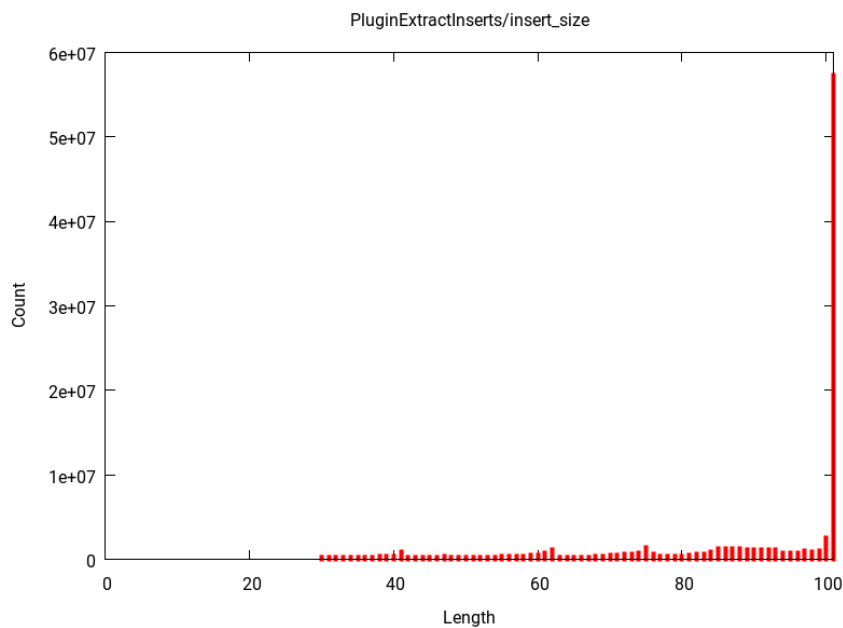


Figure 1: Size distribution of the output sequences. Short sequences ( $< \text{min\_insert\_size\_trimmed}$ ) were removed. [PluginExtractInserts\_insert\_size.png]

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	111448444
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	4514819
	Rejected	11790066
	Low complexity reads	68701
	Mode (bp)	91
	Mean (bp)	90.1
	Output paired reads	95074858
	Total output reads	99589677
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	621833
Cloning vector pKOHPRT complete sequence.	599141
Enterobacteria phage lambda	142152
Cloning vector Mini-Tn4001PStetM, complete sequence	71010
Cloning vector pVLH/hsp	46055

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

Adapters	sequences
TruSeq_Universal_Adapter	312062
ABI_Solid3_Adapter_A	294285
Illumina_Single_End_Adapter_1	245428
ABI_Solid3_GAPDH_Reverse_Primer	235289
ABI_Solid3_GAPDH_Forward_Primer	201476

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	120804
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	104467
rRNA_small_subunit_Metazoa_Myotis	97123
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	74818
Aspergillus niger CBS 513.88 clone An03	62038

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1666597527	Inf %	OK
Low Complexity	69590488	Inf %	OK
Poly T	35811651	Inf %	OK
Poly A	39268445	Inf %	OK
Contaminants	65350075	Inf %	OK
Adapters	97248265	Inf %	OK
Vectors	65606823	Inf %	OK
Indeterminations	100629	Inf %	OK
Inserts	8979696483	Inf %	iW1

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

## 4 Rejected reads

Input sequences	111448444
Output sequences	4514819
Rejected sequences	11790066
Output paired sequences	95074858
Total output sequences	99589677
Low complexity sequences	68701

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	11790066	10.579 %	OK
Short inserts	7696117	6.906 %	OK
Empty Inserts	3373948	3.027 %	rdW3
Contaminants	692770	0.622 %	OK
No Valid Inserts	18833	0.017 %	OK
Low Complexity	7691	0.007 %	OK
Indeterminations	578	0.001 %	OK
Unexpected Vector	129	0.000 %	OK
Total rejected	11790066	10.579 %	OK

**rdW3 Warning!, a 3.027 % of your sequences are empty (without an insert)**

## 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

Thanks you for use SeqTrimNext! Send us any comment to scbi support