

# 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.

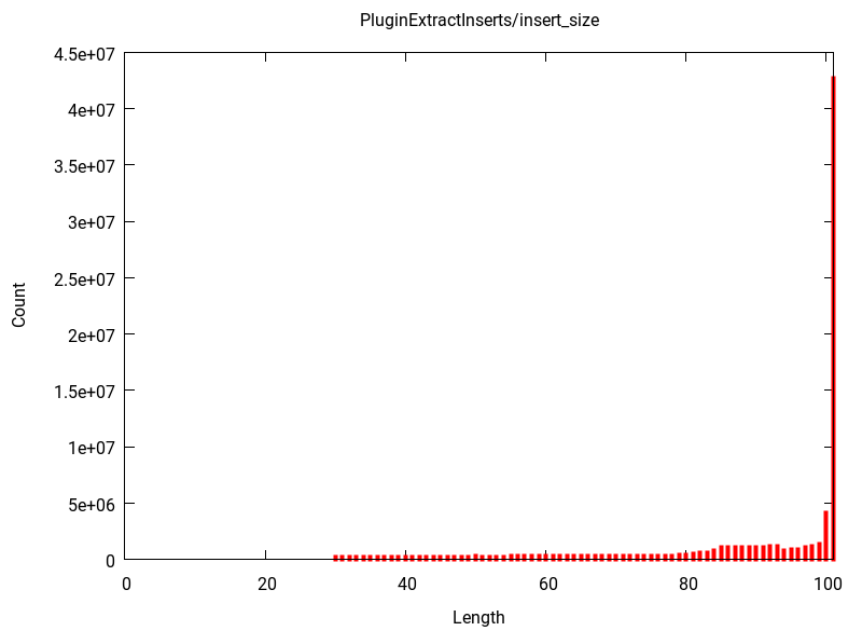


Figure 1: Size distribution of the output sequences. Short sequences ( $< \text{min\_insert\_size\_trimmed}$ ) were removed. [PluginExtractInserts\_insert\_size.png]

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	87996806
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	3278326
	Rejected	10100091
	Low complexity reads	65131
	Mode (bp)	91
	Mean (bp)	91.3
	Output paired reads	74553258
	Total output reads	77831584
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	457653
Cloning vector pKOHPRT complete sequence.	437458
Enterobacteria phage lambda	133460
Cloning vector pVLH/hsp	47542
Cloning vector pWormgate2, complete sequence.	40899

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

Adapters	sequences
TruSeq_Universal_Adapter	332607
ABI_Solid3_Adapter_A	249480
ABI_Solid3_GAPDH_Reverse_Primer	195645
Illumina_Single_End_Adapter_1	181190
ABI_Solid3_GAPDH_Forward_Primer	173381

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

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	145250
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	123504
rRNA_long_subunit_Metazoa_Dasytus	118181
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	91775
Aspergillus niger CBS 513.88 clone An03	74970

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1242855481	Inf %	OK
Low Complexity	70901711	Inf %	OK
Poly T	30945603	Inf %	OK
Poly A	31962395	Inf %	OK
Contaminants	81099204	Inf %	OK
Adapters	86181816	Inf %	OK
Vectors	53137146	Inf %	OK
Indeterminations	265917	Inf %	OK
Inserts	7110103144	Inf %	iW1

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

## 4 Rejected reads

Input sequences	87996806
Output sequences	3278326
Rejected sequences	10100091
Output paired sequences	74553258
Total output sequences	77831584
Low complexity sequences	65131

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	10100091	11.478 %	OK
Short inserts	6418962	7.295 %	OK
Empty Inserts	2803793	3.186 %	rdW3
Contaminants	852695	0.969 %	rdW4
No Valid Inserts	17279	0.020 %	OK
Low Complexity	6919	0.008 %	OK
Indeterminations	307	0.000 %	OK
Unexpected Vector	136	0.000 %	OK
Total rejected	10100091	11.478 %	OK

**rdW3 Warning!, a 3.186 % of your sequences are empty (without an insert)**

**rdW4 Warning!, a 0.969 % of your sequences are from a contaminant organism or from organelles**

## 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