

SeqTrimNext

Statistics of pre-processing

Plataforma Andaluza de Bioinformática
Universidad de Málaga

April 23, 2020

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.

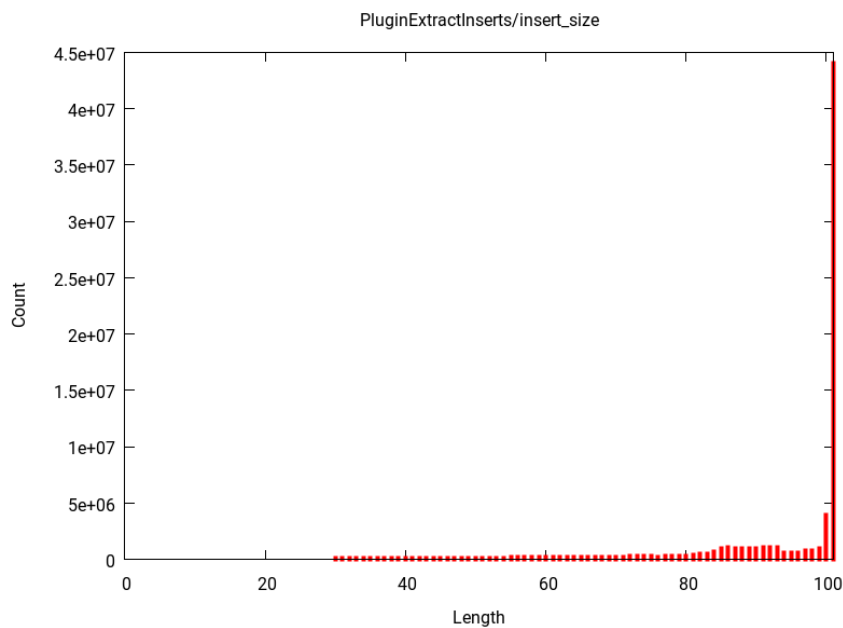


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	77966754
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	1861089
	Rejected	6332510
	Low complexity reads	46201
	Mode (bp)	91
	Mean (bp)	93.9
	Output paired reads	69726954
	Total output reads	71588043
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	408650
Cloning vector pKOHPRT complete sequence.	390306
Enterobacteria phage lambda	111120
Illumina PCR Primer	74561
Cloning vector pVLH/hsp	47374

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

Adapters	sequences
TruSeq Universal Adapter	1529723
Illumina Multiplexing Read2 Sequencing Primer	588709
Illumina Single End Sequencing Primer	220144
TruSeq Adapter Index 9	219110
ABI Solid3 Adapter A	214917

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

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	148008
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	103849
rRNA_long_subunit_Metazoa_Dasytus	91014
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	81796
rRNA_small_subunit_Metazoa_Equus	75611

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	675209406	Inf %	OK
Low Complexity	62504719	Inf %	OK
Poly T	22372803	Inf %	OK
Poly A	24615582	Inf %	OK
Contaminants	70417951	Inf %	OK
Adapters	166564241	Inf %	OK
Vectors	52583451	Inf %	OK
Indeterminations	240822	Inf %	OK
Inserts	6724981734	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	77966754
Output sequences	1861089
Rejected sequences	6332510
Output paired sequences	69726954
Total output sequences	71588043
Low complexity sequences	46201

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	6332510	8.122 %	OK
Short inserts	3528238	4.525 %	OK
Empty Inserts	2043610	2.621 %	rdW3
Contaminants	742182	0.952 %	rdW4
No Valid Inserts	10448	0.013 %	OK
Low Complexity	6480	0.008 %	OK
Indeterminations	1428	0.002 %	OK
Unexpected Vector	124	0.000 %	OK
Total rejected	6332510	8.122 %	OK

rdW3 Warning!, a 2.621 % of your sequences are empty (without an insert)

rdW4 Warning!, a 0.952 % 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