

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.

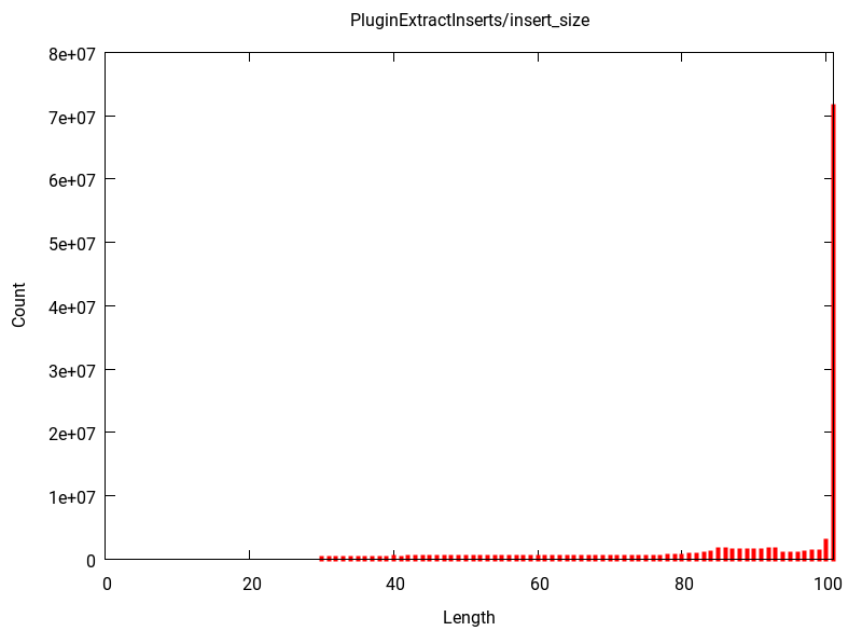


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	123775806
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	4202358
	Rejected	11100406
	Low complexity reads	84574
	Mode (bp)	91
	Mean (bp)	92.7
	Output paired reads	108388468
	Total output reads	112590826
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	863916
Cloning vector pKOHPRT complete sequence.	852409
Enterobacteria phage lambda	187724
Cloning vector pVLH/hsp	67537
Cloning vector pWormgate2, complete sequence.	58089

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

Adapters	sequences
ABI_Solid3_Adapter_A	351749
ABI_Solid3_GAPDH_Reverse_Primer	281628
Illumina_Single_End_Adapter_1	261148
ABI_Solid3_GAPDH_Forward_Primer	257335
TruSeq_Universal_Adapter	256914

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

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	107048
rRNA_long_subunit_Metazoa_Dasytus	101180
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	97763
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	80151
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	70214

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1377963707	Inf %	OK
Low Complexity	103638864	Inf %	OK
Poly T	44238037	Inf %	OK
Poly A	46817640	Inf %	OK
Contaminants	73229709	Inf %	OK
Adapters	114378687	Inf %	OK
Vectors	86975267	Inf %	OK
Indeterminations	84143	Inf %	OK
Inserts	10442089060	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	123775806
Output sequences	4202358
Rejected sequences	11100406
Output paired sequences	108388468
Total output sequences	112590826
Low complexity sequences	84574

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	11100406	8.968 %	OK
Short inserts	7320718	5.914 %	OK
Empty Inserts	2953409	2.386 %	rdW3
Contaminants	790438	0.639 %	OK
No Valid Inserts	24016	0.019 %	OK
Low Complexity	10939	0.009 %	OK
Indeterminations	724	0.001 %	OK
Unexpected Vector	162	0.000 %	OK
Total rejected	11100406	8.968 %	OK

rdW3 Warning!, a 2.386 % 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