

SeqTrimNext

Statistics of pre-processing

Plataforma Andaluza de Bioinformática
Universidad de Málaga

April 22, 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.

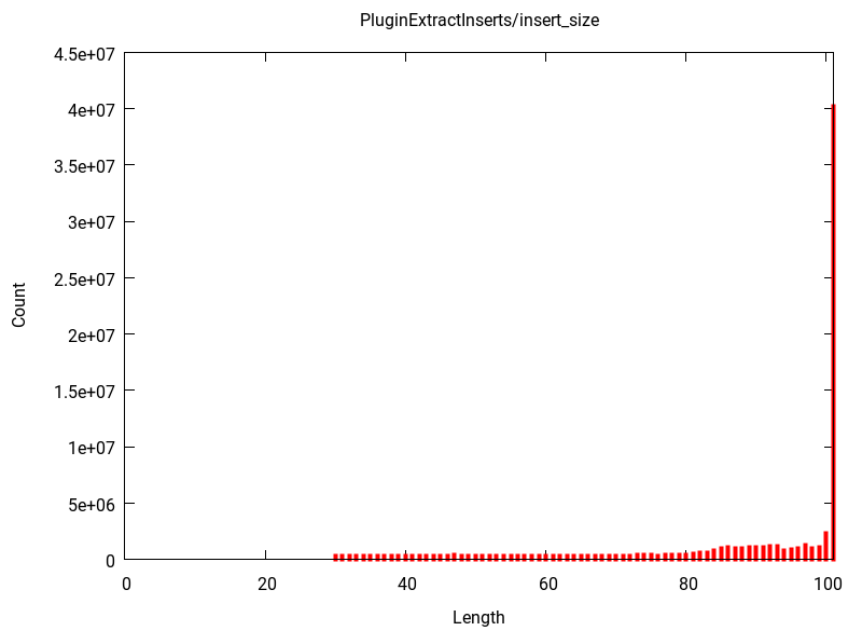


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	89309594
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	5994263
	Rejected	13250104
	Low complexity reads	94853
	Mode (bp)	91
	Mean (bp)	89.1
	Output paired reads	69970374
	Total output reads	75964637
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	531653
Cloning vector pKOHPRT complete sequence.	523760
Enterobacteria phage lambda	128894
Cloning vector pVLH/hsp	44702
Cloning vector pWormgate2, complete sequence.	40994

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

Adapters	sequences
ABISolid3_Adapter_A	252769
TruSeq_Universal_Adapter	201657
Illumina_Single_End_Adapter_1	186437
ABISolid3_GAPDH_Reverse_Primer	185712
ABISolid3_GAPDH_Forward_Primer	173711

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	60231
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	53509
rRNA_small_subunit_Metazoa_Myotis	50032
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	43157
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	36646

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1662814362	Inf %	OK
Low Complexity	62440594	Inf %	OK
Poly T	33124812	Inf %	OK
Poly A	34234901	Inf %	OK
Contaminants	38475262	Inf %	OK
Adapters	81061450	Inf %	OK
Vectors	56634444	Inf %	OK
Indeterminations	3763328	Inf %	OK
Inserts	6775960305	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	89309594
Output sequences	5994263
Rejected sequences	13250104
Output paired sequences	69970374
Total output sequences	75964637
Low complexity sequences	94853

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	13250104	14.836 %	OK
Short inserts	8829432	9.886 %	rdW2
Empty Inserts	3960691	4.435 %	rdW3
Contaminants	417697	0.468 %	OK
Indeterminations	18340	0.021 %	OK
No Valid Inserts	16981	0.019 %	OK
Low Complexity	6852	0.008 %	OK
Unexpected Vector	111	0.000 %	OK
Total rejected	13250104	14.836 %	OK

rdW2 Warning!, a 9.886 % of your sequences are too short

rdW3 Warning!, a 4.435 % 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