

SeqTrimNext

Statistics of pre-processing

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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.

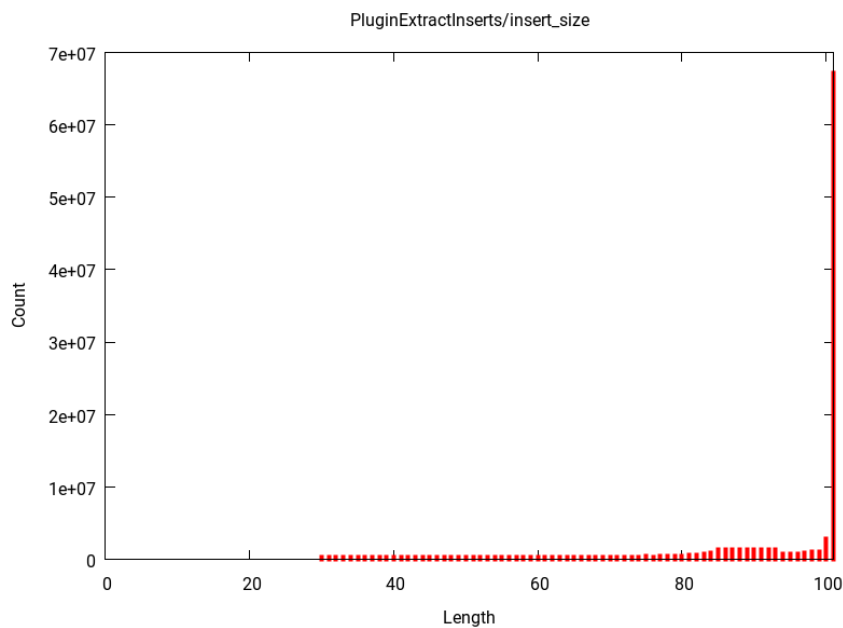


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	115502326
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	3935260
	Rejected	10484488
	Low complexity reads	68610
	Mode (bp)	91
	Mean (bp)	92.8
	Output paired reads	101013968
	Total output reads	104949228
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	686418
Cloning vector pKOHPRT complete sequence.	662403
Enterobacteria phage lambda	163832
Cloning vector Mini-Tn4001PStetM, complete sequence	56104
Cloning vector pVLH/hsp	50984

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

Adapters	sequences
ABISolid3_Adapter_A	326897
TruSeq_Universal_Adapter	272793
ABISolid3_GAPDH_Reverse_Primer	262971
Illumina_Single_End_Adapter_1	257110
ABISolid3_GAPDH_Forward_Primer	226370

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	131290
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	109164
rRNA_small_subunit_Metazoa_Myotis	106285
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	80763
Aspergillus niger CBS 513.88 clone An03	70587

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1291172716	Inf %	OK
Low Complexity	82582427	Inf %	OK
Poly T	36572848	Inf %	OK
Poly A	40489742	Inf %	OK
Contaminants	75612784	Inf %	OK
Adapters	107435844	Inf %	OK
Vectors	72492166	Inf %	OK
Indeterminations	77990	Inf %	OK
Inserts	9745457789	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	115502326
Output sequences	3935260
Rejected sequences	10484488
Output paired sequences	101013968
Total output sequences	104949228
Low complexity sequences	68610

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	10484488	9.077 %	OK
Short inserts	6951278	6.018 %	OK
Empty Inserts	2697087	2.335 %	rdW3
Contaminants	806640	0.698 %	OK
No Valid Inserts	20829	0.018 %	OK
Low Complexity	7840	0.007 %	OK
Indeterminations	671	0.001 %	OK
Unexpected Vector	143	0.000 %	OK
Total rejected	10484488	9.077 %	OK

rdW3 Warning!, a 2.335 % 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