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 exists 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.

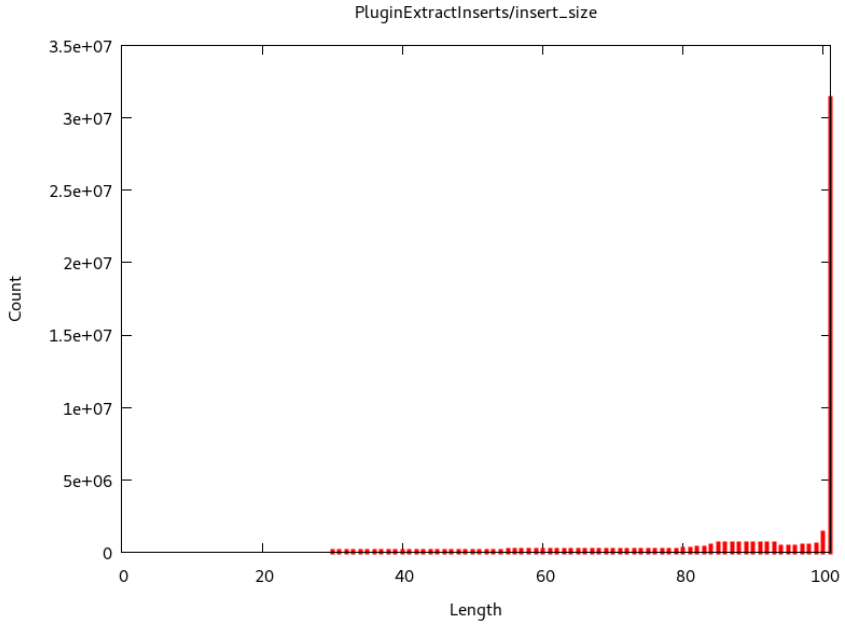

Figure 1: Size distribution of the output sequences. Short sequences (< `min_insert_size_trimmed`) were removed. [PluginExtractInserts_insert_size.png]

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	51278158
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	1557998
	Rejected	3539459
	Low complexity reads	27629
	Mode (bp)	91
	Mean (bp)	93.6
	Output paired reads	46153072
	Total output reads	47711070
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	229794
Cloning vector pKOHPRT complete sequence.	212204
Enterobacteria phage lambda	65365
Cloning vector Mini-Tn4001PStetM, complete sequence	22779
pBPV cloning vector	17334

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

Adapters	sequences
TruSeq_Universal_Adapter	174347
ABI_Solid3_Adapter_A	150379
ABI_Solid3_GAPDH_Reverse_Primer	139741
ABI_Solid3_GAPDH_Forward_Primer	115945
Illumina_Single_End_Adapter_1	114873

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

Contaminants	sequences
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	57660
rRNA_long_subunit_Metazoa_Dasypus	31004
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	29895
Aspergillus niger CBS 513.88 clone An15	29016
rRNA_small_subunit_Metazoa_Myotis	26695

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	468163997	Inf %	OK
Low Complexity	37425951	Inf %	OK
Poly T	12507525	Inf %	OK
Poly A	14434659	Inf %	OK
Contaminants	27649343	Inf %	OK
Adapters	47082869	Inf %	OK
Vectors	26830051	Inf %	OK
Indeterminations	47128	Inf %	OK
Inserts	4466318641	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	51278158
Output sequences	1557998
Rejected sequences	3539459
Output paired sequences	46153072
Total output sequences	47711070
Low complexity sequences	27629

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	3539459	6.902 %	OK
Short inserts	2467080	4.811 %	OK
Empty Inserts	754237	1.471 %	rdW3
Contaminants	307237	0.599 %	OK
No Valid Inserts	6478	0.013 %	OK
Low Complexity	3423	0.007 %	OK
Indeterminations	961	0.002 %	OK
Unexpected Vector	43	0.000 %	OK
Total rejected	3539459	6.902 %	OK

rdW3 Warning!, a 1.471 % 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