

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 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.

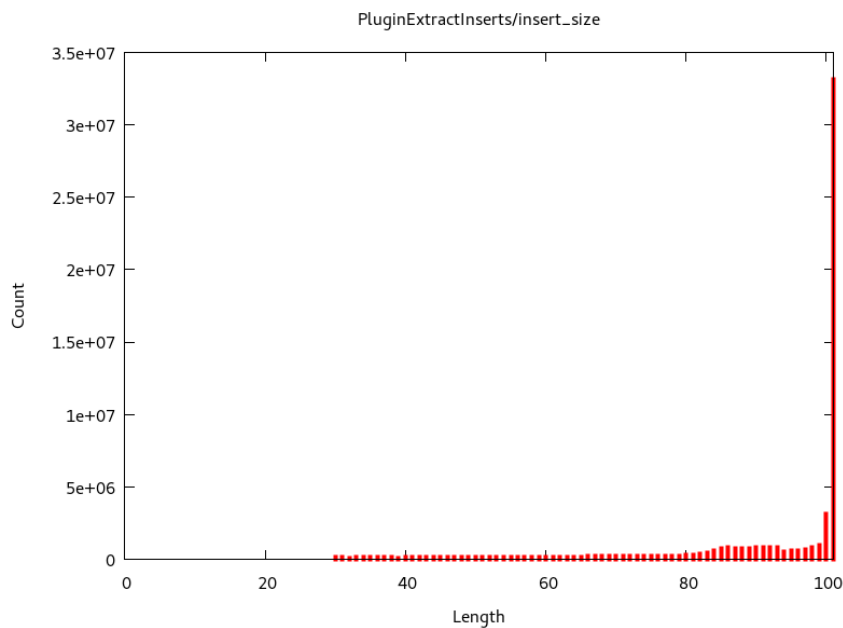


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	62580618
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	1964641
	Rejected	5001642
	Low complexity reads	38923
	Mode (bp)	91
	Mean (bp)	92.6
	Output paired reads	55575412
	Total output reads	57540053
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	303195
Cloning vector pKOHPRT complete sequence.	289710
Enterobacteria phage lambda	96003
Cloning vector pVLH/hsp	32745
Cloning vector pWormgate2, complete sequence.	26926

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

Adapters	sequences
TruSeq_Universal_Adapter	430705
ABI_Solid3_Adapter_A	175565
Illumina_Multiplexing_Read2_Sequencing_Primer	165850
ABI_Solid3_GAPDH_Reverse_Primer	144234
ABI_Solid3_GAPDH_Forward_Primer	127095

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	47744
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	45139
rRNA_small_subunit_Metazoa_Myotis	44181
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	43138
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	34209

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	665603368	Inf %	OK
Low Complexity	49794090	Inf %	OK
Poly T	19610566	Inf %	OK
Poly A	21243251	Inf %	OK
Contaminants	34513370	Inf %	OK
Adapters	72309128	Inf %	OK
Vectors	37016167	Inf %	OK
Indeterminations	205793	Inf %	OK
Inserts	5329489852	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	62580618
Output sequences	1964641
Rejected sequences	5001642
Output paired sequences	55575412
Total output sequences	57540053
Low complexity sequences	38923

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	5001642	7.992 %	OK
Short inserts	3240239	5.178 %	OK
Empty Inserts	1373537	2.195 %	rdW3
Contaminants	371267	0.593 %	OK
No Valid Inserts	10503	0.017 %	OK
Low Complexity	5191	0.008 %	OK
Indeterminations	812	0.001 %	OK
Unexpected Vector	93	0.000 %	OK
Total rejected	5001642	7.992 %	OK

rdW3 Warning!, a 2.195 % 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