

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.

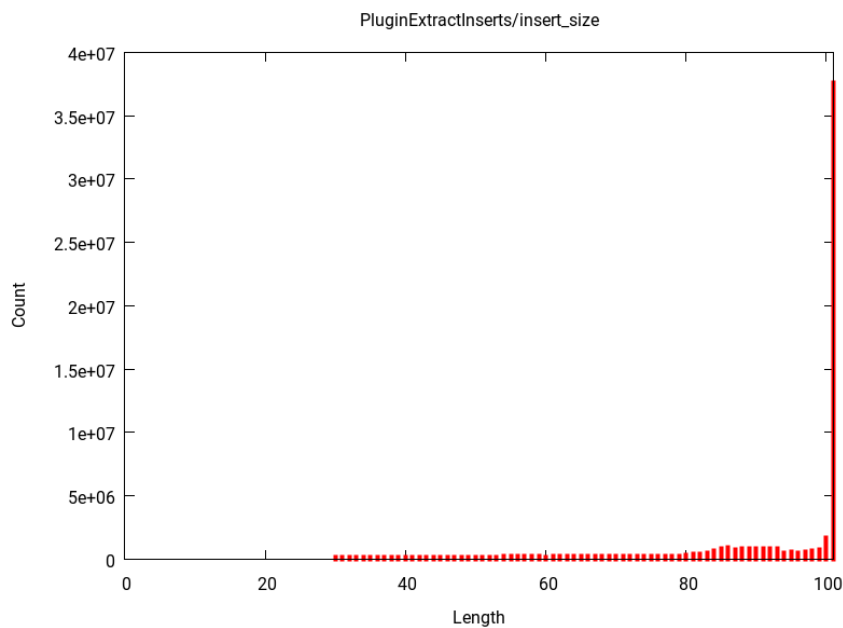


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	74668630
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	7003638
	Rejected	10874221
	Low complexity reads	129527
	Mode (bp)	91
	Mean (bp)	91.0
	Output paired reads	56661244
	Total output reads	63664882
Linkers:		

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

Vectors	sequences
Cloning vector pKOHPR7 complete sequence.	455723
Cloning vector pAAV-MCS, complete sequence.	452881
Enterobacteria phage lambda	105412
Cloning vector pVLH/hsp	48569
Cloning vector pWormgate2, complete sequence.	39895

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

Adapters	sequences
ABI_Solid3_Adapter_A	203373
Illumina_Single_End_Adapter_1	147671
ABI_Solid3_GAPDH_Reverse_Primer	144160
ABI_Solid3_Adapter_B	137599
TruSeq_Universal_Adapter	136331

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	40926
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	37403
rRNA_small_subunit_Metazoa_Myotis	36993
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	32190
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	26139

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1111640135	Inf %	OK
Low Complexity	64071312	Inf %	OK
Poly T	35496479	Inf %	OK
Poly A	33602068	Inf %	OK
Contaminants	29066457	Inf %	OK
Adapters	59957595	Inf %	OK
Vectors	47641312	Inf %	OK
Indeterminations	186011560	Inf %	OK
Inserts	5804080464	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	74668630
Output sequences	7003638
Rejected sequences	10874221
Output paired sequences	56661244
Total output sequences	63664882
Low complexity sequences	129527

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	10874221	14.563 %	OK
Short inserts	6158279	8.247 %	rdW2
Empty Inserts	2585100	3.462 %	rdW3
No Valid Inserts	1790897	2.398 %	rdW5
Contaminants	314629	0.421 %	OK
Indeterminations	16532	0.022 %	OK
Low Complexity	8680	0.012 %	OK
Unexpected Vector	104	0.000 %	OK
Total rejected	10874221	14.563 %	OK

rdW2 Warning!, a 8.247 % of your sequences are too short

rdW3 Warning!, a 3.462 % of your sequences are empty (without an insert)

rdW5 Warning!, a 2.398 % of your sequences are no valid sequences

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