

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.

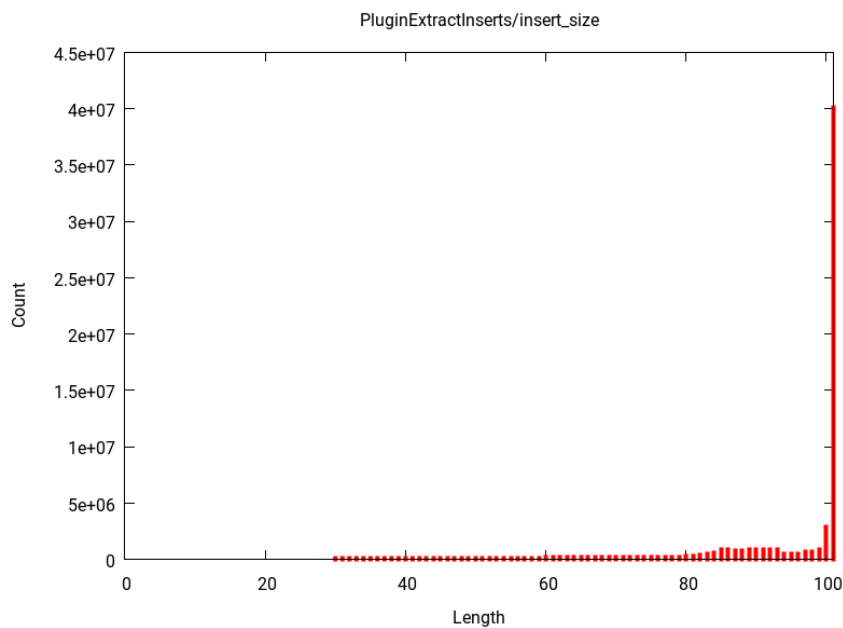


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	67313994
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	1563022
	Rejected	4238339
	Low complexity reads	35275
	Mode (bp)	91
	Mean (bp)	94.1
	Output paired reads	61477358
	Total output reads	63040380
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	401126
Cloning vector pKOHPRT complete sequence.	375721
Enterobacteria phage lambda	104583
Cloning vector pVLH/hsp	38480
Cloning vector pJAZZ-KA	36045

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

Adapters	sequences
TruSeq Universal Adapter	507979
Illumina Multiplexing Read2 Sequencing Primer	192488
ABI Solid3 Adapter_A	188854
ABI Solid3 GAPDH Reverse Primer	144897
Illumina Single End Adapter_1	137009

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

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	82848
rRNA_long_subunit_Metazoa_Dasytus	78198
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	77703
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	59289
Aspergillus niger CBS 513.88 clone An03	49474

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	523018944	Inf %	OK
Low Complexity	54141459	Inf %	OK
Poly T	22934780	Inf %	OK
Poly A	24553805	Inf %	OK
Contaminants	52766767	Inf %	OK
Adapters	76946902	Inf %	OK
Vectors	44335341	Inf %	OK
Indeterminations	208575	Inf %	OK
Inserts	5934429979	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	67313994
Output sequences	1563022
Rejected sequences	4238339
Output paired sequences	61477358
Total output sequences	63040380
Low complexity sequences	35275

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	4238339	6.296 %	OK
Short inserts	2523558	3.749 %	OK
Empty Inserts	1136906	1.689 %	rdW3
Contaminants	557929	0.829 %	rdW4
No Valid Inserts	12937	0.019 %	OK
Low Complexity	5939	0.009 %	OK
Indeterminations	985	0.001 %	OK
Unexpected Vector	85	0.000 %	OK
Total rejected	4238339	6.296 %	OK

rdW3 Warning!, a 1.689 % of your sequences are empty (without an insert)

rdW4 Warning!, a 0.829 % of your sequences are from a contaminant organism or from organelles

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