

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.

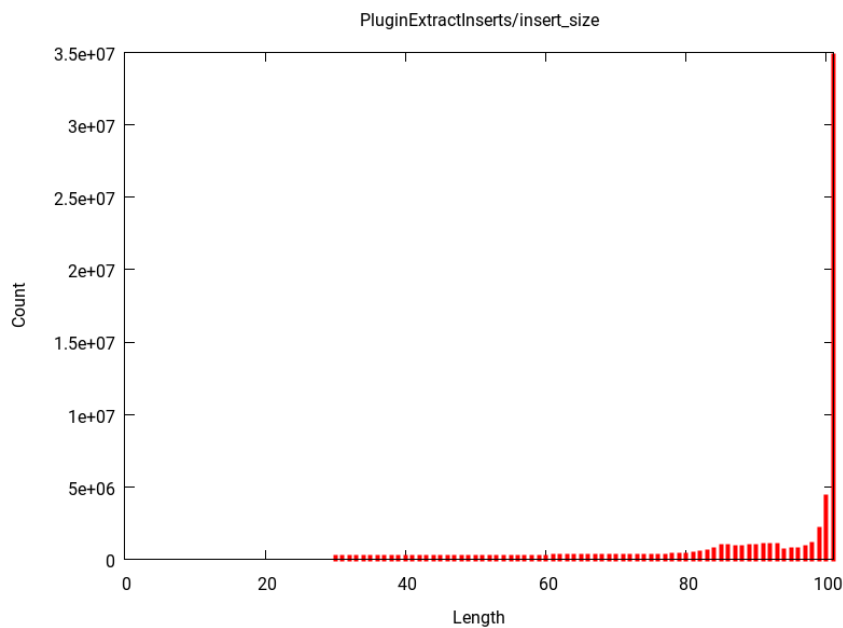


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	69742404
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	2001729
	Rejected	5847805
	Low complexity reads	45462
	Mode (bp)	91
	Mean (bp)	92.7
	Output paired reads	61847408
	Total output reads	63849137
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	462053
Cloning vector pKOHPRT complete sequence.	438821
Enterobacteria phage lambda	108901
Cloning vector pVLH/hsp	37878
Cloning vector pWormgate2, complete sequence.	32508

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

Adapters	sequences
TruSeq Universal Adapter	848481
Illumina Multiplexing Read2 Sequencing Primer	368557
ABI Solid3 Adapter A	200888
Illumina Single End Sequencing Primer	166633
ABI Solid3 GAPDH Reverse Primer	151223

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

Contaminants	sequences
rRNA_small_subunit_Metazoa_Myotis	67105
rRNA_long_subunit_Metazoa_Dasytus	66305
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	65042
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	47552
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	40417

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	739198635	Inf %	OK
Low Complexity	57969169	Inf %	OK
Poly T	24081933	Inf %	OK
Poly A	24484634	Inf %	OK
Contaminants	46141957	Inf %	OK
Adapters	87089588	Inf %	OK
Vectors	48002582	Inf %	OK
Indeterminations	224306	Inf %	OK
Inserts	5922187732	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	69742404
Output sequences	2001729
Rejected sequences	5847805
Output paired sequences	61847408
Total output sequences	63849137
Low complexity sequences	45462

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	5847805	8.385 %	OK
Short inserts	3632925	5.209 %	OK
Empty Inserts	1701720	2.440 %	rdW3
Contaminants	491518	0.705 %	OK
No Valid Inserts	12632	0.018 %	OK
Low Complexity	6232	0.009 %	OK
Indeterminations	2680	0.004 %	OK
Unexpected Vector	98	0.000 %	OK
Total rejected	5847805	8.385 %	OK

rdW3 Warning!, a 2.440 % 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