

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.

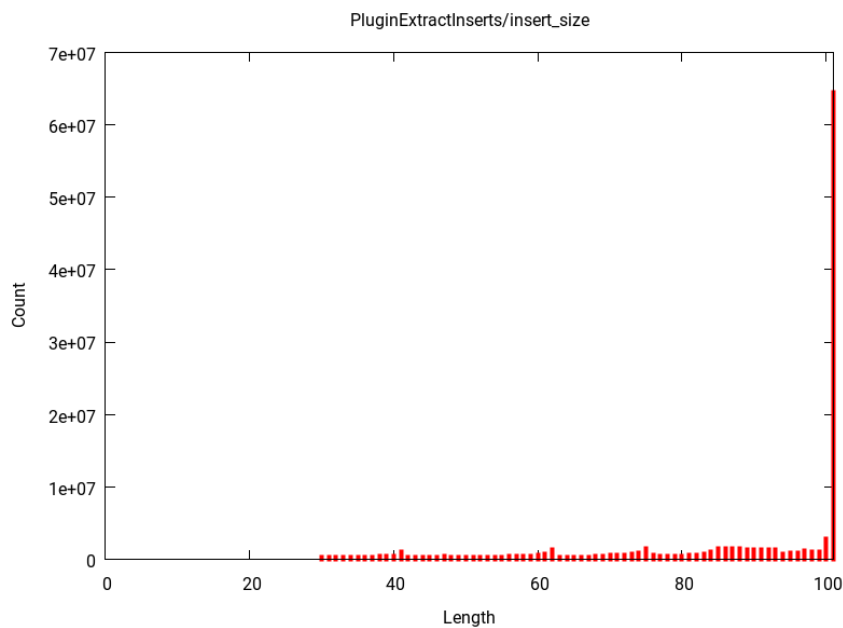


Figure 1: Size distribution of the output sequences. Short sequences ($< \text{min_insert_size_trimmed}$) were removed. [PluginExtractInserts_insert_size.png]

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	126634348
	Smallest read (bp)	101
	Largest read (bp)	101
	Mode (bp)	0
	Mean (bp)	0.0
Output results:	total	5299781
	Rejected	14624246
	Low complexity reads	86401
	Mode (bp)	91
	Mean (bp)	90.0
	Output paired reads	106623920
	Total output reads	111923701
Linkers:		

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

Vectors	sequences
Cloning vector pAAV-MCS, complete sequence.	692264
Cloning vector pKOHPRT complete sequence.	691640
Enterobacteria phage lambda	161940
Cloning vector pVLH/hsp	55029
Cloning vector pWormgate2, complete sequence.	50642

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

Adapters	sequences
ABISolid3_Adapter_A	342302
TruSeq_Universal_Adapter	294077
ABISolid3_GAPDH_Reverse_Primer	279041
Illumina_Single_End_Adapter_1	278125
ABISolid3_GAPDH_Forward_Primer	244406

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

Contaminants	sequences
rRNA_long_subunit_Metazoa_Dasytus	136986
rRNA_small_subunit_Metazoa_Myotis	121089
Saccharomyces cerevisiae S288c chromosome XII, complete sequence	120777
Schizosaccharomyces pombe 972h- chromosome III, complete sequence	86674
Podospora anserina S mat+ unordered scaffolds, whole genome shotgun sequence	77868

Table 4: Summary of nucleotides removed in every plugin.

Plugin	Nucleotides	Percent	Warnings
Low Quality	1990239438	Inf %	OK
Low Complexity	87299411	Inf %	OK
Poly T	43275963	Inf %	OK
Poly A	46651214	Inf %	OK
Contaminants	83909133	Inf %	OK
Adapters	110444745	Inf %	OK
Vectors	74326964	Inf %	OK
Indeterminations	117286	Inf %	OK
Inserts	10084238617	Inf %	iW1

iW1 Warning!, only Inf % of nucleotides are useful

4 Rejected reads

Input sequences	126634348
Output sequences	5299781
Rejected sequences	14624246
Output paired sequences	106623920
Total output sequences	111923701
Low complexity sequences	86401

Table 5: Summary of reads removed in every plugin.

Case	Number of sequences	Percent	Warnings
	14624246	11.548 %	OK
Short inserts	9349265	7.383 %	OK
Empty Inserts	4346867	3.433 %	rdW3
Contaminants	895796	0.707 %	OK
No Valid Inserts	22388	0.018 %	OK
Low Complexity	9093	0.007 %	OK
Indeterminations	684	0.001 %	OK
Unexpected Vector	153	0.000 %	OK
Total rejected	14624246	11.548 %	OK

rdW3 Warning!, a 3.433 % 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