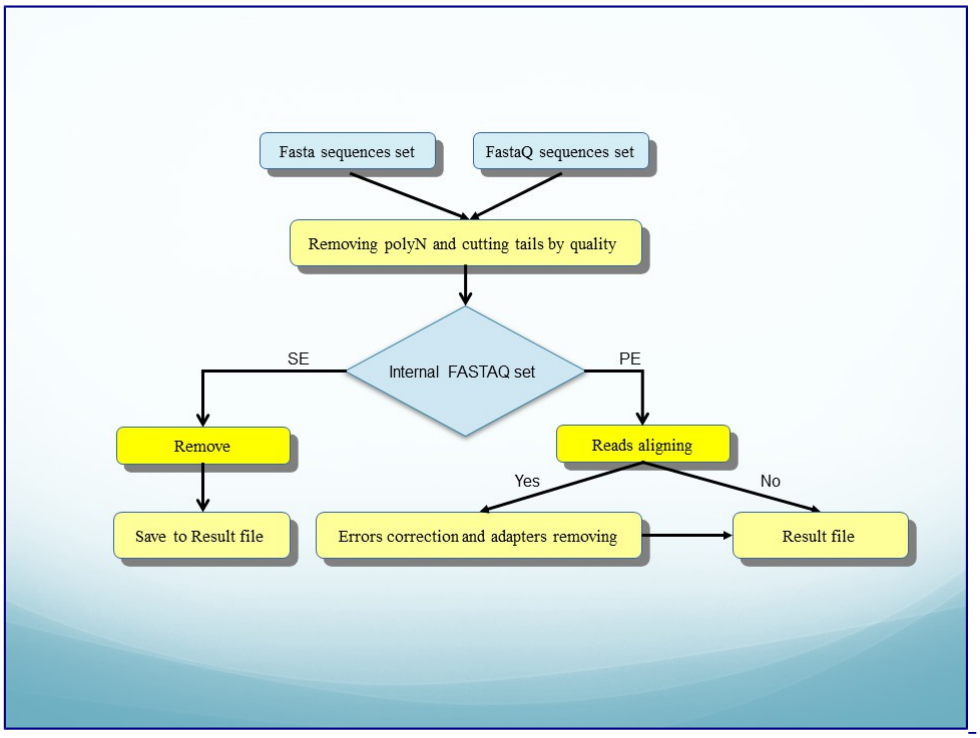
Adapter_Trim



Contents

SYNOPSIS

Try to determinate adapters sequences in FASTQ pair-ends reads. Result will be printed in stdout.

```
./adapter_trim SRR330569_1.fastq SRR330569_2.fastq -ifastq - phread33
-PE -o:adapter_trim.cfg  -analyze -j:8
```

Scan FASTQ pair-ends reads and remove adapters. Source reads are in two files. Result will be saved to one file in FASTA format.

```
./adapter_trim SRR519624_1.fastq SRR519624_2.fastq -ifastq -phread33
-PE -o:adapter_trim.cfg -adapters_trim
-adpt1_seq:AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCGTCTTCTGCT
TG
-adpt2_seq:AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
-min_read_flen:0 -min_read_slen:0 -to_one_file -to_fasta -j:7
```

Remove polyN and low quality tails from reads in FASTQ format. Result will be saved to two files in FASTQ format.

```
./adapter_trim SRR519624_1.fastq SRR519624_2.fastq -ifastq - phread33
```

```
-PE -o:adapter_trim.cfg -cut_polyN -cut_qual -cut_qual_level:15
-to_fastq -to_two_files
```

DESCRIPTION

adapter_trim is a newest program for the preparation of short sequences (reads) sets for further analysis. The adapter_trim main goal is removing adapters from reads, but you can use it for the task of searching and removing polyN tails and cutting sequence by quality.

BUILD

Run the *_buid_all.sh script in the ./build folder
Assembled programs are to be placed to the ./bin folder

COMMANDS AND OPTIONS

Define adapters sequences . More than one adapter sequences can be use for each set of reads.

```
   -adpt1_seq:AGATC...GA   Read1 adapter sequence, may be set more
than once

   -adpt2_seq:GGACC...TA   Read2 adapter sequence, may be set more
than once
```

Input files format. . You can specify only one file for single ends reads. There are two valid variants for "MP" and "PE" reads:

1. Reads are separated in two files. In this case first read from pair must be placed in one file and second read in other file. Obviously the sequence number of reads from one pair must be equal.
2. All reads in one file. In this case sequences of paired reads are supposed to be in order (even/odd).

```
    -PE                     Input file(s) is the "PE" reads (default).
    -MP                     Input file(s) is the "MP" reads.
    -SE                    Input file is the "SE" (Single ends) reads.
```

Input files parameters

```
    -fasta                    input file(s) are in FASTA format.
(Default)
    -fastq                     input file(s) are in FASTQ format.
```

You are need to specify FASTQ version by one of this options:

```
    -phread33          Sanger and Illumina 1.8+ version format
    -phread64          Illumina 1.3+ and Illumina 1.5+ version format
```

For FASTA format each nucleotide have fixed quality. You can change this value by -def_fasta_qua: option.

```
-def_fasta_qua:XX           Default quality for input file(s) in FASTA
format. Numeric value (Default is 20)
```

Select mode . You must set at least one mode in command line, otherwise no any actions will be done,

```
    -adapters_trim           Adapter trimmer mode. Search and remove
adapters.
    -cut_polyN               Cut plyN mode. Search and remove polyN
```

```
tails.
    -cut_qual                Cut by quality mode. Search and remove
tails with bad quality. Use it with -cut_qual_level: option.
    -cut_qual_level:XX       Numerical value (0-40) for cut by quality
threshold. (Default is 0)
```

Multiprocessing .

```
    -j:XX                    Number of processes for multiprocessing
regime.
```

Output files parameters.

```
    -no_save_name            Skip name of read. Name will be replaced by
number. C
    -to_fasta                Save result in FASTA format.
    -to_fastq                Save result in FASTQ format.
    -to_one_file             Save result reads pairs in single file.
Read1 will be odd and read2 will be even.
    -to_two_files            Save result reads pairs in differ file. So
one file from pair will be saved in file with suffix      ".1" and
other in file with suffix  ".2". Single reads will be saved in file
with suffix  ".0".

    -no_qual_limiter         Allow store quality values larger than 40.;
    -join                    Join overlapped paired reads in one
sequence.
```

adaper_trim can sort source base to multiply files. You can regulate this behavior by next options:

```
    -min_read_flen:XXX       Minimal length of long reads.  Reads
shorter than XXX will be placed in base of short reads. Default is 55.
    -min_read_slen:XXX       Minimal length of short reads. Reads
shorter than XXX will be skipped. Default 15.
```

Set both of this in 0 and all result will be placed in one or two (according **-to_two_files** or **-to_one_file** options) file(s).

Result destination

```
     -ad_trim_path:path      Destination folder.
     -ad_base_name:name      Prefix for output files. If it set the
basename will be replaced by given name.
```

Advanced options

```
    -analyze                   Try to analyze source set of reads and
offer  the most probable variants of adapters. It`s strongly
recommended to repeat analyze procedure after first run, with using
the sequences which were found after the first run as seed (see the
sample).
     -adapter_len_max:XX       Use not greater then XX nucleotides
from given adapters.
     -store_quality            For FASTA output. Save quality string
in FASTA name.
     -ad_max_pass:XX           While align - gaps length sum in
adapter must be less then XX.
     -read_max_pass:XX         While align - gaps length sum in read
must be less then XX.
     -max_gap_len:XX           Maximum single gap length must be less
then XX.
     -cut_agressivity:XX       Aggressivity level for all types of
cutting.(1...inf, default 2.2).
     -cut_hole:XX              Length of "bad" regions for cutting.
```

```
          -mp_cross_only            Process only overlapped MP reads.
          -adpt1_shift:val          Shift adapter in read1. Use 0 (default)
     if unsure.
          -adpt2_shift:val          Shift adapter in read2. Use 0 (default)
     if unsure.
```

SAMPLE

First run – search a seed.

```
> ./adapter_trim SRR1611127_1.fastq SRR1611127_2.fastq -ifastq -phread33 -PE
-o:adapter_trim.cfg -analyze -j:8


Resolve Default adapter1
Resolve Default adapter2
First File.
Second File.
Done... Execution time: 1.638169644 sec.

---------------- Read 1 Adapter. ----------------------------------
>Read 1 Adapter.
AGATCGGAAGAGCACACGTCTGAACTC
It may be one of...
>Illumina Multiplexing PCR Primer 2.01
AGATCGGAAGAGCACACGTCTGAACTC CAGTCAC
>Illumina Multiplexing Index Sequencing Primer
AGATCGGAAGAGCACACGTCTGAACTC CAGTCAC
>Illumina Multiplexing Read2 Sequencing Primer
AGATCGGAAGAGCACACGTCTGAACTC CAGTCAC

...

---------------- Read 2 Adapter. ----------------------------------
>Read 2 Adapter.
AGATCGGAAGAGCGTCGTGTAGGGAAAGA
It may be one of...
>TruSeq Universal Adapter
AGATCGGAAGAGCGTCGTGTAGGGAAAGA GTGTAGATCTCGGTGGTCGCCGTATCATT
>Illumina Single End PCR Primer 1
AGATCGGAAGAGCGTCGTGTAGGGAAAGA GTGTAGATCTCGGTGGTCGCCGTATCATT
...
```

Second run – analyze with the seed using.

```
> ./adapter_trim SRR1611127_1.fastq SRR1611127_2.fastq -ifastq -phread33 -PE
-o:adapter_trim.cfg -analyze -j:8 -adpt1_seq:AGATCGGAAGAGCACACGTCTGAACTC
-adpt2_seq:AGATCGGAAGAGCGTCGTGTAGGGAAAGA
Resolve user defined adapter1
Resolve user defined adapter2
First File.
Second File.
Done... Execution time: 1.848383758 sec.

---------------- Read 1 Adapter. ----------------------------------
>Read 1 Adapter.
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCACTGATCTCGTATGCCGTCTTCTGCTTGAA
It may be one of...
>TruSeq Adapter, ATTCACTG
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCACTGATCTCGTATGCCGTCTTCTGCTTG

---------------- Read 2 Adapter. ----------------------------------
>Read 2 Adapter.
```

```
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATTAAAAAAAAAA
It may be one of...
>TruSeq Universal Adapter
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
>Illumina Single End PCR Primer 1
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
>Illumina Paried End PCR Primer 1
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
>Illumina Multiplexing PCR Primer 1.01
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

Trimming

```
> ./adapter_trim SRR1611127_1.fastq SRR1611127_2.fastq -ifastq -phread33 -PE
-o:adapter_trim.cfg -adapters_trim
-adpt1_seq:AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCACTGATCTCGTATGCCGTCTTCTGCTTG
-adpt2_seq:AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
-min_read_flen:0 -min_read_slen:0 -to_one_file -to_fasta -j:8
```

TRIMMING ACCURACY

We try to compare results of adapter trimming by adapter_trim and by skewer (http://sourceforge.net/projects/skewer/files/) programs with using the same adapter sequences. For the source data the reads of Arabidopsis thaliana was used. The results of adapter trimming was aligned to full genome. Quality indicators of alignment were used as a quality measure for trimming results.

Skewer

```
command line:
./skewer-0.1.123-linux-x86_64 -l 0 -r 0.3 -x adapter1.fa -y
adapter2.fa SRR519624_1.fastq SRR519624_2.fastq
```

Total reads 34683594.
Reads aligned with homology > 95% 27122070 (78.199%)
Total nucleotides 3462985140.
Summary alignment length 2670451726.
Matched nucleotides 2622437780 (75.728%)

adapter_trim

```
command line:
./adapter_trim SRR519624_1.fastq SRR519624_2.fastq -PE -ifastq
-phread33 -o:adapter_trim.cfg
-adpt1_seq:AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCGTCTTCTGCT
TG
-adpt2_seq:AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
-min_read_flen:0 -min_read_slen:0 -to_one_file -to_fasta
-store_quality  -j:7 -adapters_trim
```

Total reads 34683594
Reads aligned with homology > 95% 27149366 (78.277%)
Total nucleotides 3461498047
Summary alignment length 2707527341
Matched nucleotides 2640217552 (76.274%)

Thus it can be seen that the **adapter_trim** performs better clean quality.

LICENSE AND CITATION

adapter_trim is a free for academic usage. Please contact to softberry@softberry.com in otherwise.