

A computational pipeline for whole genome sequencing analysis of *Mycobacterium tuberculosis* complex isolates

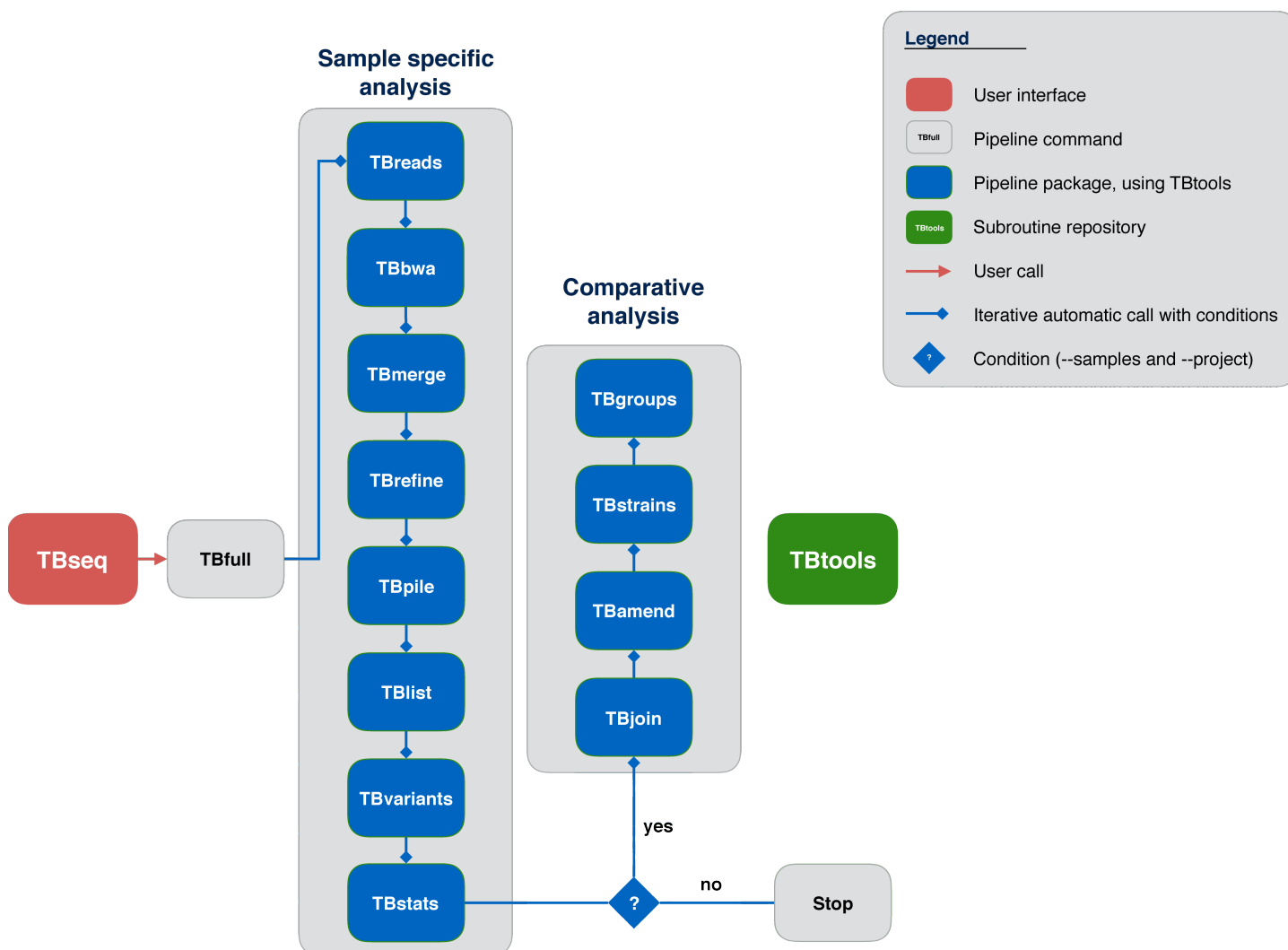
TBseq

[DESCRIPTION]

TBseq is a semi-automated pipeline for mapping, variant calling and detection of resistance mediating variants as well phylogenetic variants in user provided *Mycobacterium tuberculosis* samples. The pipeline consists of open source software for read mapping (SAMTOOLS, BWA, SAMBAMBA), base call recalibration and refinement (PICARD, GATK) and variant calling (GATK, SAMTOOLS). TBseq has a modular architecture that allows to repeat specific tasks (e.g. mapping, refinement, variant calling, strain Identification, etc.).

TBseq is able to perform a joined variant analysis from user provided samples. This is especially useful in transmission chain analysis. Phylogenetic SNPs are used to create FastA formatted files (.fasta). This files can be used to reconstruct minimum spanning trees (MS) or phylogenetic trees (NJ or ML). All additional output is written into files with tabular delimited file format (.tab). This format is readable with Microsoft Excel or Excel-like programs.

[OVERVIEW]



[SYNOPSIS]

You do not need to support TBseq with a path to your read files. Just make sure that your read files are located in a folder where you want to execute TBseq! The basic execution of TBseq on the command-line looks like this:

TBseq [OPTIONS] [VALUES]

In the following chapter, you will be introduced to the OPTIONS and the corresponding VALUES that you can use in TBseq.

[OPTIONS & VALUES]

[OPTIONS]

[VALUES]

--step

TBseq has a modular architecture. This ensures that you do not need to repeat the whole pipeline, if something went wrong. You can execute the full pipeline or start with certain pipeline steps. However, if you choose a specific pipeline step, make sure that you have the file dependencies for this step. The OPTION **--step** is essential and requires a VALUE! In the following, all possible VALUES are described:

TBfull

VALUE of **--step** for executing the whole pipeline. If you choose this VALUE, make sure that you setup all other OPTIONS to your appropriate VALUES (see OPTIONS below).

TBreads

VALUE of **--step** for merging read files (.fastq or .fastq.gz). TBseq is designed to operate on raw Illumina output by default. However, you can also use TBseq with preprocessed reads. **TBreads** is the first logical step within the pipeline. Depending on your sequencing machine, two, four or eight files (.fastq or .fastq.gz) will be generated for one sample. This step merges, compresses and renames multiple files (fastq or .fastq.gz) into one (single-end) or two (paired-end) file(s), depending on your input. It is recommended that your read files follow a **six field** naming scheme! This fields are separated by underscores within the file name:

[SampleID]_[LibID]_[Source]_[RunID]_[Read-length]_[Direction]

The **[SampleID]** is a unique sample identifier. If your analysis has more than one sample, make sure that this identifier is unique across samples! The **[LibID]** is an identifier for your used sequencing library. This identifier should be unique across your samples and should start with "lib"! For example, a **[LibID]** could look like "lib1". The **[Source]** is an optional field, describing from which sequencer the sample is coming from (e.g. hiseq, miseq, nextseq, torrent, etc.). You can provide an optional **[Source]** field for your naming scheme with the OPTION **--machine** (see OPTIONS below). The **[RunID]** is also an optional field and can be set with the OPTION **--run** (see OPTIONS below). If you have multiple sequencing runs for the same sample, it is useful to distinguish between them. This can help to detect samples coming from a sequencing run with a systematic error. The **[Read-length]** field supports the pipeline with the read length of the appropriate sample. We recommend **not** to clip or trim your reads. Put this field directly after the **[RunID]** field. For the statistics calculation it is necessary to put the correct read length! The **[Direction]** field is an **essential** field and indicates, if reads are forward (R1) or reverse (R2) in orientation. If single-end sequencing was performed, an unidirectional (R0) should be used. This field **must be** the last field in your naming scheme! In summary, the file names for your read files should contain at least the following fields: **[SampleID]_[LibID]_[Direction]**

Input

[SampleID]_[LibID]_[Direction].fastq.gz

Output

[SampleID]_[LibID]_[Source]_[RunID]_[Read-length]_[Direction].fastq.gz

TBbwa

VALUE of **--step** for mapping read files (.fastq.gz) to a reference genome, using BWA. On default, TBseq uses the *M. tuberculosis* H37Rv genome as a reference. BWA is executed with a default VALUE setting. After mapping, files (.sam) are converted into binary mapping files (.bam). The mapping is sorted, indexed and putative PCR duplicates are removed, using the program SAMTOOLS. Wherever possible, multi-threading is activated by the user provided VALUES for the OPTION **--threads** (see OPTIONS below).

Input

[SampleID]_[LibID]_[Source]_[RunID]_[Read-length]_[Direction].fastq.gz

Output

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bam

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bai

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bamlog

TBmerge

VALUE of **--step** for merging multiple files (.bam) from the same sample and sequencing library, using the program SAMBAMBA. Sometimes a sample is sequenced more than one time. This may be due to low coverage in a first try. Important is that the sample did not change in time! Meaning, if you analyse a sample over time than you should use a different sample identifier (e.g. SampleA_T1, SampleA_T2, etc.)! This logic merges mappings, if multiple sequencing runs were performed for the same sample and library. It improves mapping quality for samples. SAMBAMBA is executed with default values.

Input

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bam

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bai

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bamlog

Output

Bam/[SampleID]_[LibID]_multi_[DATE]_[Read-length].bam

Bam/[SampleID]_[LibID]_multi_[DATE]_[Read-length].bai

Bam/[SampleID]_[LibID]_multi_[DATE]_[Read-length].mergelog

TBrefine

VALUE of **--step** for realignment around insertions and deletions (indels) and base call recalibration, using the program GATK. The GATK program uses default VALUES with the exception of:

--downsample_to_coverage 10000

--defaultBaseQualities 12

--maximum_cycle_value 600

--noOriginalAlignmentTags

This internal VALUE setting for GATK ensures that you can also use Ion Torrent read files. For the Base call recalibration, a set of known MTB resistance SNPs is used, if you take *Mycobacterium tuberculosis* H37Rv as a reference genome. If not, this step will be skipped. The calibration list is stored in the directory "var/res/Base_Calibration_List.vcf" of the package.

Input

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bam

Output

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.bam

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.bai

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.bamlog

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.grp

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.intervals

TBpile

VALUE of **--step** for creating pileup file(s) (.mpileup) from refined mapping file(s) (.gatk.bam), using the program SAMTOOLS. The SAMTOOLS program uses default VALUES with the exception of:

-B
-A

see: <http://www.htslib.org/doc/samtools.html> for more information about this VALUE setting. TBseq needs to create this file format in order to perform all downstream analysis.

Input

GATK_Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.bam

Output

Mpileup/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.mpileup

Mpileup/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.mpileuplog

TBlist

VALUE of **--step** for creating position list(s) from pileup file(s) (.gatk.mpileup). The position list consists of 21 columns, representing the frequency of nucleotide compositions for each genomic position. This step can be executed with a user provided VALUE of the OPTION **--threads** (see below) and a different VALUE of the OPTION **--minbqual**. The columns of the output are:

Pos	Indicates the genome position
Insindex	An index for reporting insertion sites. 0 means the level of the reference genome
RefBase	The nucleotide found for the reference genome at this position
As	The forward read frequency of the nucleotide adenine
Cs	The forward read frequency of the nucleotide cytosine
Gs	The forward read frequency of the nucleotide guanine
Ts	The forward read frequency of the nucleotide thymine
Ns	The forward read frequency of ambiguous nucleotides
GAPs	The forward read frequency of a GAP
as	The reverse read frequency of the nucleotide adenine
cs	The reverse read frequency of the nucleotide cytosine
gs	The reverse read frequency of the nucleotide guanine
ts	The reverse read frequency of the nucleotide thymine
ns	The reverse read frequency of ambiguous nucleotides
gaps	The reverse read frequency of a gap
Aqual	Number of adenine nucleotides having a higher or equal phred 20 score
Cqual	Number of cytosine nucleotides having a higher or equal phred 20 score
Gqual	Number of guanine nucleotides having a higher or equal phred 20 score
Tqual	Number of thymine nucleotides having a higher or equal phred 20 score
Nqual	Number of ambiguous nucleotides having a higher or equal phred 20 score
GAPqual	Number of GAPs having a higher or equal phred 20

Input

Mpileup/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk.mpileup

Output

Position_Tables/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_table.tab

TBvariants

VALUE of **--step** for variant calling from position list(s). This step is able to use five user provided thresholds for variant calling. On default, an allele is called based on majority rule. First, positions are only considered, if at least four forward reads (**--mincovf 4**) and second, four reverse reads (**--mincovr 4**) support an allele. Third, at least four reads must show a phred score ≥ 20 (**--miphred20 4**). Fourth, the majority allele must be present with a frequency of 75%

or higher (**--mifreq 75**). If not, the allele is indicated as ambiguous. Fifth, the module can be executed with the OPTIONS **--all_vars**, **--snp_vars** and **--lowfreq_vars** (see OPTIONS below). Your setting of this OPTIONS will be visible at the end of the output files as a binary string (e.g. "outmode100" means that you activated **--all_vars** but not **--snp_vars** or **--lowfreq_vars**).

Input

Position_Tables/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_table.tab

Output

Called/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_uncovered_[mincovf]_[mincovr]_[minfreq]_[minphred20]_[all_vars][snp_vars][lowfreq_vars].tab
 Called/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_variants_[mincovf]_[mincovr]_[minfreq]_[minphred20]_[all_vars][snp_vars][lowfreq_vars].tab

TBstats

VALUE of **--step** for basic mapping statistics and called variant statistics, using SAMTOOLS flagstat. This step creates a tabular delimited file "Mapping_and_Variant_Statistics.tab". The file stores all sample statistics and is updated instantly, if you use different samples at different time points. The columns of the output are:

Date	The date of TBseq execution
SampleID	The analysed sample
LibraryID	There used library for the sequencing
Source	The used sequencing machine
Run	The sequencing run number
Total Reads	The total amount of sequenced reads
Mapped Reads (%)	The amount of reads mapped
Theoretical Coverage	The theoretical coverage if all reads have mapped
Real Coverage	The real coverage
(Theoretical / Real)	A ratio between theoretical and real coverage
log₂ (Theoretical / Real)	There logarithm to the base 2 of the ratio
Genome Size	The size of the reference genome
Genome GC	The GC content of the reference genome
(Any) Total Bases (%)	Number of bases used for the called variants
(Any) GC-Content	GC content calculated from the reads used for the called variants
(Any) Coverage mean / median	Coverage mean and median calculated from the reads used for the called variants
(Unambiguous) Total Bases (%)	Number of unambiguous bases used for the called variants
(Unambiguous) GC-Content	GC content calculated from the unambiguous reads used for the called variants
(Unambiguous) Coverage mean / median	Coverage mean and median calculated from the unambiguous reads used for the called variants
SNPs	Number of SNPs found
Deletions	Number of deletions found
Insertions	Number of insertions found
Uncovered	Uncovered positions
Substitutions (including Stop Codons)	Number of substitutions within genes

Input

Bam/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].bam
 Position_Tables/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_table.tab

Output

Statistics/Mapping_and_Variant_Statistics.tab

TBjoin

VALUE of **--step** for creating a joint SNP analysis of user provided samples. First, a scaffold of all variant positions is built from variant files of your provided samples (**--samples**). The samples need to be provided in a tabular delimited file with **[SampleID]** in column 1 and **[LibID]**

in column 2. You also need to provide a project name (**--project**) for file naming. Second, variants are recalculated with the OPTION **--all_vars**. The output is a table of concatenated variant files from user provided samples. The first line within the tabular delimited file is a sample header line. The second line describes the joint analysis for variant positions and is separated to joint fields and sample specific fields:

Joint fields

#Position	Genome position with a variant in at least one of the samples.
Insindex	An index for reporting insertion sites. 0 means the level of the genome. If Insindex > 0, then at least one sample showed an insertion at this position.
Ref	The reference allele present at this position
Gene	A gene ID is indicated, if the position is within a gene
GeneName	A gene name is indicated, if available
Annotation	The product of the gene
Sample specific fields	
Type	The type of the variant. Possible values are "none, SNP, GAP"
Allele	The allele that was present for a sample at this position
CovFor	The forward coverage at this position
CovRev	The reverse coverage at this position
Qual20	The number of nucleotides having a phred score above 20
Freq	The frequency of the allele
Cov	The coverage at this position
Subst	The quality of a non-synonymous substitution, if the SNP occurs in a gene

Input

```

samples.txt
--project
Called/[SampleID]_[LibID]_[Source]_[RunID]_[Read-
length].gatk_position_variants_[mincovf]_[mincovr]_[minfreq]_[minphred20]_[all_vars][snp_vars][lowfreq_vars].tab
Position_Tables/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_table.tab

```

Output

```

Joint/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples.tab
Joint/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples.log

```

TBamend

VALUE of **--step** for amending joint variant tables. This module is able to use three user provided thresholds. First, you need to provide a project name with **--project** to find the joint variant file. Second, only variants that are unambiguous in 95% of all samples are reported (**--unambig 95**) on default. Third, SNPs that occur in a distance of 12 nucleotides are excluded in order to reduce false positive calls (**--window 12**). SNPs in repetitive regions or nested within a resistance gene are excluded. Positions not passing this criteria are reported in the "removed" output.

Input

```

samples.txt
--project
Joint/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples.tab

```

Output

```

Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended.tab
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo.tab
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window].tab
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window]_removed.tab
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_plainIDs.fasta
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window]_plainIDs.fasta
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo.fasta
Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window]_removed.fasta

```

TBstrains

VALUE of **--step** for lineage classification based on phylogenetic SNP maps (1,2). This module creates a tabular delimited file within the "Classification" directory. Within this file, the majority lineage of a sequenced sample is reported. This file is updated instantly, if you use different samples at different time points.

Input

Position_Tables/[SampleID]_[LibID]_[Source]_[RunID]_[Read-length].gatk_position_table.tab

Output

Classification/Strain_Classification.tab

TBgroups

VALUE of **--step** for grouping samples based on SNP distances. This module is able to use one user provided threshold for SNP distances. Strains are grouped together, if they are not more than 12 SNPs apart from each other (**--distance 12**). If they are more than 12 SNPs apart from each other, a new group is formed. This module enables to distinguish between groups of samples via SNP distance patterns and creates a distance matrix.

Input

Amend/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window].tab

Output

Groups/[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window].matrix

Groups/

[PROJECT]_joint_[mincovf]_[mincovr]_[minfreq]_[minphred20]_samples_amended_[unambig]_phylo_[window]_[distance].groups

--continue

If a module was chosen with the **--step** OPTION, the **--continue** OPTION ensures that the pipeline will continue with downstream modules. You do not need to set this OPTION, if you start the analysis with the **--step TBfull** VALUE.

--samples

This OPTION requires a user sample file (e.g. samples.txt) as a VALUE. The file must be a two column file. Column 1 should be your **[SampleID]**. Column 2 should be your **[LibID]**. **TBjoin** requires this file!

--project

This OPTION takes a project name for the steps **TBjoin**, **TBamend** and **TBgroups**. If you do not support a project name, **[NONE]** is used as a default value.

--ref

This OPTION sets the reference genome for the read mapping. You can choose between *M. abscessus* CIP-104536T, *M. chimaera* DSM44623, *M. fortuitum* CT6 and *M. tuberculosis* H37Rv. On default, *M. tuberculosis* H37Rv will be used for the mapping.

--machine

This OPTION sets the field **[Source]** for the read file naming scheme. On default, the OPTION is set to the VALUE **[NGS]**. The VALUE is ignored, if your read files have already a six field naming scheme.

--run

This OPTION sets the field **[RunID]** for the read file naming scheme. On default, the option is set to **[RUN]**. The VALUE is ignored, if your read files have already a six field naming scheme.

--readlen

This OPTION sets the field **[Read-length]** for the read file naming scheme. **IMPORTANT:** the logic **TBstats** requires this field! Make sure you provide the correct read length. If not, statistics will be calculated wrong. On, default the OPTION is set to 151.

--all_vars

This OPTION is used in **TBvariants**, **TBstats**, **TBjoin** and **TBstrains**. On default, the OPTION is set to 0. The OPTION has influence on the variant output. If you set this OPTION, every position will be reported ignoring, if the position passes the filter criteria, is uncovered, is a reference allele or is coming from an insertion. Important is that low frequency alleles are only reported, if **—lowfreq_vars** is supported in addition. If not, only majority alleles are reported.

--snp_vars

This OPTION is used in **TBvariants**, **TBstats**, **TBjoin** and **TBstrains**. On default, the OPTION is set to 0. The OPTION has influence on the variant output. If you set this OPTION, only SNPs will be reported in the variant output file.

--lowfreq_vars

This OPTION is used in **TBvariants**, **TBstats**, **TBjoin** and **TBstrains**. On default, the OPTION is set to 0. The OPTION has influence on the variant output. If you set this OPTION, TBseq will consider alternative low frequency variants at positions where a majority reference allele is found. This is useful for analysing subpopulations within a sample (e.g mixed infection).

--minbqual

This OPTION is used in **TBlist**. On default, the OPTION is set to 13. The OPTION sets a threshold for the mapping quality. Bases covering a position are only considered, if the quality is greater or equal this VALUE.

--mincovf

This OPTION is used in **TBvariants**, **TBjoin**, **TBamend** and **TBstrains**. On default, the OPTION is set to 4. The OPTION sets a minimum forward read coverage threshold. Alleles must have a forward coverage of this VALUE or higher to be considered.

--mincovr

This OPTION is used in **TBvariants**, **TBjoin**, **TBamend** and **TBstrains**. On default, the OPTION is set to 4. The OPTION sets a minimum reverse read coverage threshold. Alleles must have a reverse coverage of this value or higher to be considered.

--minphred20

This OPTION is used in **TBvariants**, **TBjoin**, **TBamend** and **TBstrains**. On default, the OPTION is set to 4. The OPTION sets a minimum read coverage with a phred 20 quality score. A user provided number of reads must show a phred quality above or equal 20 for a certain position to be considered.

--minfreq

This OPTION is used in **TBvariants**, **TBjoin**, **TBamend** and **TBstrains**. On default, the OPTION is set to 75. The OPTION sets a minimum frequency for the majority allele. Only majority alleles with this frequency or higher are indicated as unambiguous. Within the module **TBstrains**, this OPTION will have an effect on lineage classification quality. If all phylogenetic SNPs have a frequency of this VALUE and are covered 10-fold, then lineage classification will result in a "good" quality, otherwise in a "bad" quality for that sample.

--unambig

This OPTION is used in **TBamend**. On default, the OPTION is set to 95. The option sets a minimum percentage of samples that need to show the called variant as unambiguous. If less than this percentage of samples have an unambiguous variant call at that position, the position will not be reported in the amended joint variant table.

--window

This OPTION is used in **TBamend**. On default, the OPTION is set to 12. The OPTION sets a window size in which the algorithm scans for multiple SNPs. If more than one SNP occurs within this window, SNP positions will not be reported in the amended joint variant table.

--distance

This OPTION is used in **TBgroups**. On default, the OPTION is set to 12. The OPTION sets a SNP distance that is used to classify samples into groups of samples, using a single linkage approach. If SNP distances between samples are less or equal this VALUE, they are grouped together. If not, a new group is formed. We recommend to execute **TBgroups** twice, with **--distance 5** and **--distance 12**.

--quiet

This OPTION turns off the display logging function and will report the logging only in a file, called "TBseq_[DATE]_[USER].log".

--threads

This OPTION is used in **TBbwa**, **TBmerge**, **TBrefine**, **TBpile** and **TBlist**. On default, the OPTION is set to 1. The OPTION sets the maximum number of CPUs to use within the pipeline. You can use more than one core in order to execute the pipeline faster. We recommend to use 8 cores, if your system provides it.

--help

This OPTION will show you all available OPTION and VALUE of TBseq on the display. Use this OPTION, if you are unsure about the OPTION and VALUES you can use in TBseq.

[EXAMPLES]

TBseq --step TBfull

Will execute the whole pipeline with default values. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen. The log file is located where you executed the pipeline.

TBseq --step TBbwa --continue --threads 8

Recommended, if read file naming scheme is correct. The pipeline starts with the read mapping module and continues after finishing this module. The system will use 8 cores whenever possible. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen.

TBseq --step TBlist --threads 8

This example uses **TBlist** with 8 threads. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen. To execute this module, you need to have a finished **TBpile** output.

TBseq --step TBjoin --sample samples.txt --project TEST

This example uses **TBjoin** with default VALUE setting. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen. To execute this module, you need to have a finished **TBlist** and **TBvariants** output.

TBseq --step TBstrains

This example uses **TBstrains** with default VALUE setting. To execute this module, you need to have a finished **TBlist** output. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen.

TBseq --step TBvariants --mincovf 10 --mincovr 10 --minfreq 80 --minphred20 10 --outmode 2

This example uses **TBvariants** with a modified VALUE setting for variant calling. **TBvariants** will output only SNP positions. All log output is written into a file called "TBseq_[DATE]_[USER].log" and on screen. To execute this module, you need to have a finished **TBlist** output.

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[REQUIREMENTS]

Perl: perl 5, version 18, subversion 2 (v5.18.2) or higher

Java: openjdk version "1.8.0_91" or higher

TBseq uses the following CPAN modules:

- MCE (v1.810)
- Statistics::Basic (v1.6611)
- FindBin (v1.51)
- Cwd (v3.62)
- Getopt::Long (v2.49)
- File::Copy (v2.31)
- List::Util (v1.47)
- Exporter (v5.72)

- vars (v1.03)
- lib (v0.63)
- strict (v1.11)
- warnings (v1.36)

TBseq uses the following third party software:

- bwa (v0.7.15)
- GenomeAnalysisTK (v3.6)
- IGVTools (v2.3.88)
- picard (v2.7.1)
- sambamba (v0.6.5)
- samtools (v1.3.1)

[INSTALLATION]

1. Download TBseq from github:

https://github.com/TaKohl/TBseq_source/releases

2. Extract TBseq

3. Move TBseq into /usr/local directory (needs sudo):

```
mv [PATH_TO_YOUR_DOWNLOADED_TBSEQ] /usr/local/
```

Or move TBseq into a directory where you have write permission:

```
mkdir -p /home/$USER/bin
```

```
mv [PATH_TO_YOUR_DOWNLOADED_TBSEQ] /home/$USER/bin/
```

4. Create a symbolic link to TBSeq.pl within /usr/local/bin:

```
ln -s /usr/local/TBseq/TBseq.pl /usr/local/bin/TBseq
```

Or in the alternative directory:

```
ln -s /home/$USER/bin/TBseq/TBseq.pl /home/$USER/bin/TBseq
```

5. Install modules via CPAN by typing in the command-line:

```
cpan
```

6. Install the modules by typing:

```
install MCE
```

```
install Statistics::Basic
```

```
install FindBin
```

```
install Cwd
```

```
install Getopt::Long
```

```
install File::Copy
```

```
install List::Util
```

```
install Exporter
```

```
install vars
```

```
install lib
```

```
install strict
```

```
install warnings
```

7. If third party programs (BWA and SAMTOOLS) in TBseq/opt/ are not working, try to re-compile them. The re-compiled executables MUST be located within the appropriate folders.

```
./configure --prefix = [PATH_TO_YOUR_TBSEQ]/opt/bwa_0.7.15/
```

```
./configure --prefix = [PATH_TO_YOUR_TBSEQ]/opt/samtools_1.3.1/
```

Tested on ubuntu 16.04 LTS.

[HOMEPAGE AND SOURCE REPOSITORY]

TBseq on github: https://github.com/TaKohl/TBseq_source/

Research center Borstel: <http://www.fz-borstel.de/cms/en/science/start.html>

[References]

1. Homolka, S. et al. High resolution discrimination of clinical *Mycobacterium tuberculosis* complex strains based on single nucleotide polymorphisms. PLoS One 7, e39855 (2012).
2. Coll, F. et al. A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains. Nature Communications 5, 4812 (2014).