COMPUTEL V0.3

User Manual

Contents

Introduction

Computel is R-based software for computation of mean telomere length from whole genome next generation sequencing (NGS) data.

Development

Computel has been developed by the members of the Bioinformatics Group at the Institute of Molecular Biology of the National Academy of Sciences of the Republic of Armenia (IMB NAS RA). You can visit the group's webpage at the following link: http://big.sci.am.

Citation

When using Computel in your research, please refer to the GitHub repository at https://github.com/lilit-nersisyan/computel.

License

Requirements

Computel v03 works for **Unix** systems (tested for Linux Ubuntu), refer to Computl v02 for Windows and Mac OS tested versions.

R 3.0.3 or higher should be installed on your system.

The following binaries are included in the Computel package, and should work without worries for installation (there is a plan B in case these binaries do not work for your system):

Bowtie2-2.1.0 (not 2.2.0!), http://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.1.0/

Samtools 0.1.19 or higher, source: http://sourceforge.net/projects/samtools/files/samtools/0.1.19/ the complied version for Linux is included in the computel.zip file).

Picard tools 1.108 SamtoFastq.jar, http://sourceforge.net/projects/picard/ .

Computel has been tested only on provided versions of software. It does not work with Bowtie2-2.2.0, and is not guaranteed to work with higher versions of Samtools and Picard.

Download and installation

Computel can be downloaded from GitHub at https://github.com/lilit-nersisyan/computel.

Download and extract the package into a local directory. The folder contains the needed scripts, binaries and files for checking proper setup. Do not change the relative location of the folders within the package.

Setting up samtools

The Precompiled Samtools binary is provided with the Computel package: if it works, then you're fine, you may skip the following text.

If it doesn't work on your system you may provide your own Samtools with the option –sam <path to Samtools>. Samtools source is available for download from http://www.htslib.org/. However, some of the samtools releases have a threshold of 8000 for maximum depth of coverage. This threshold should be increased for telomere length calculation, by adding the line "bam_mplp_set_maxcnt(mplp,1000000);" in the *bam2depth.c*, as indicated in the following patch:

```
Index: bam2depth.c
===================================================================
--- bam2depth.c     (revision 995)
+++ bam2depth.c     (working copy)
@@ -80,6 +80,7 @@

       // the core multi-pileup loop
       mplp = bam_mplp_init(n, read_bam, (void**)data); // initialization
+        bam_mplp_set_maxcnt(mplp,1000000); // set maxdepth to 1M
       n_plp = calloc(n, sizeof(int)); // n_plp[i] is the number of covering reads
from the i-th BAM
       plp = calloc(n, sizeof(void*)); // plp[i] points to the array of covering reads
(internal in mplp)
       while (bam_mplp_auto(mplp, &tid, &pos, n_plp, plp) > 0) { // come to the next
covered position
```

Running Computel from command line

The following lines demonstrate the input-output arguments and how to run Computel, the next sections will provide more detailed explanations on arguments and the algorithm.

Basic usage:

./computel.sh [options] {-1 <fq1> -2 <fq2> -3 <fq3> -o <o>}

Input:

<fq1> fastq file (the first pair or the only fastq file (for single end reads)
<fq2> fastq file (optional: the second pair of fastq files, if exists)
<fq3> fastq file (optional: the third pair of fastq files, if exists)
<o> output directory (optional: the default is computel_out)

Options (advanced):

<-proc> number of processors to be used (default: 4)
<-sam> samtools path (optional: the required samtools is located at computel's bin directory. Change this only if it doesn't work)

<-bowal> bowtie2-align path (optional: the bowtie2-align is located at computel's bin directory by default.)

<-bowb> bowtie2-build path (optional: the bowtie2-build is located at computel's bin directory by default.)

<-nchr> number of chromosomes in a haploid set (the default is 23)

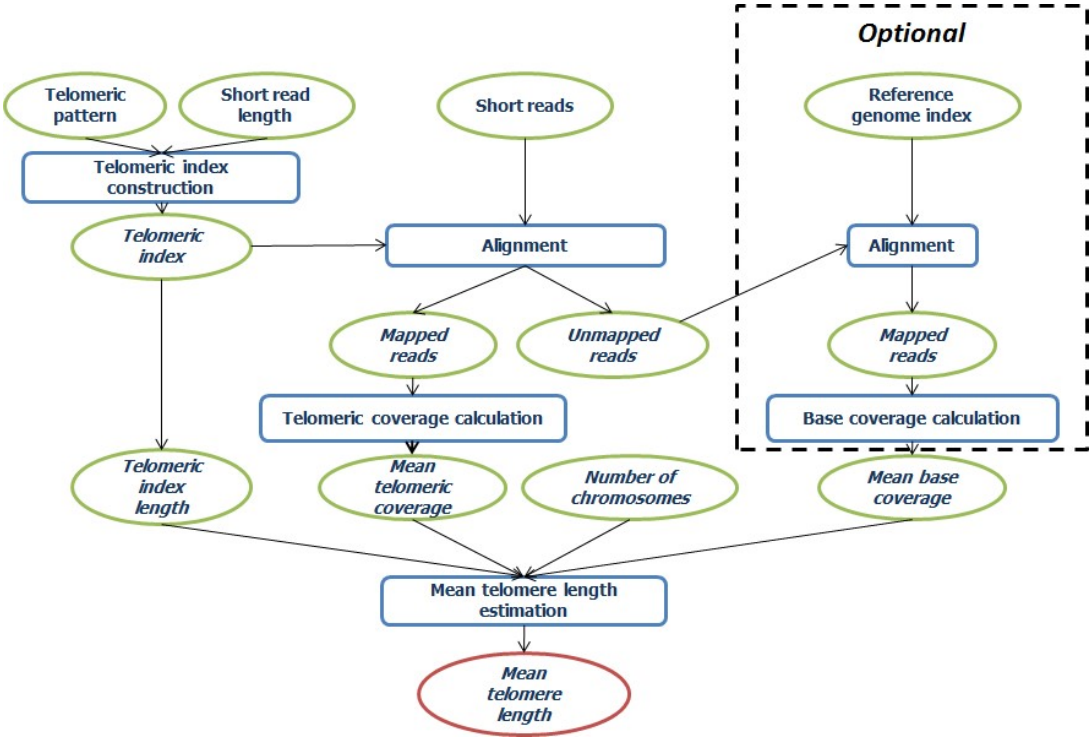
<-lgenome> whole genome length (the default is 3244610000)

<-pattern> telomere repeat pattern (the default is 'TTAGGG'; change this if you're using Computel for a non-human organism)

<-minseed> the min seed length (read length minus the number of flanking N's in the telomeric index; should be in the range [12-read.length]; This is a tested and carefully set parameter (defualt = 12); Change this only if you REALLY KNOW what you're doing!)

Algorithm description

The algorithm workflow is presented in the figure. For its detailed description, refer to the main paper [Nersisyan L, Arakelyan A (2015) Computel: Computation of Mean Telomere Length from Whole-Genome Next-Generation Sequencing Data. PLoS ONE 10(4): e0125201. doi:10.1371/journal.pone.0125201].



Input and advanced options

Fastq files:

Computel uses whole genome fastq files as input. If the reads are single end, usually only one fastq file is available (use only -1 <fq1> option and skip the rest). If the reads are paired-end there will be two or three fastq files (the third one contains unpaired reads), which can be supplied with -1 <fq1>, -2 <fq2> and -3 <fq3> options.

The output directory will contain the index and alignment files generated by Computel and the final output file (tel.length.xls). The directory may be specified to replace the default (/computel_out) with the option -o <outputdir>.

Options (advanced):

<-proc>: number of processors to be used for parallelization of the alignment process (default: 4)

<-sam>: samtools path. Specifying Samtools directory rather then using the default binary available in the Computel package should only be done if the latter doesn't work. Please, refer to the "Setting up samtools" section for details.

<-bowal>: bowtie2-align path. This is also optional: the bowtie2-align is located at computel's bin directory by default. Replace it only if the binary does not work.

<-bowb>: bowtie2-build path. This is also optional: the bowtie2-build is located at computel's bin directory by default. Replace it only if the binary does not work.

<-nchr>*: number of chromosomes in a haploid set (the default is 23 for human genomes). Use this for organisms other than humans or for special cases where the number of chromosomes is not a multiple of 23. Note, you might need to change the genome length accordingly.

<-lgenome>: whole genome length (the default is 3244610000 for humans)

<-pattern>: telomere repeat pattern (the default is 'TTAGGG'; change this if you're using Computel for a non-human organism). Note, this is the repeat pattern present at the 3' end (on the 5'-3' strand).

<-minseed>**: the min seed length (read length minus the number of flanking N's in the telomeric index; should be in the range [12-read.length]; This is a tested and carefully set parameter (defualt = 12); Change this only if you REALLY KNOW what you're doing!)

*Notes on number of haploid chromosomes

Telomere length is calculated with the formula:

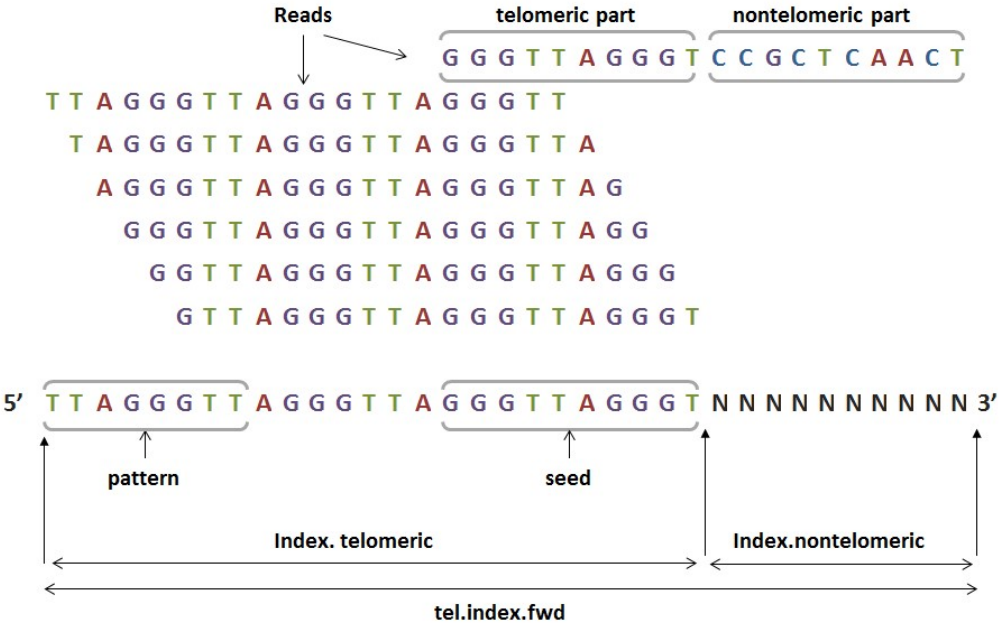
`(mean(`*`tel.cov/base.cov`*`))*(rl+pl-1) / (2*num.haploid.chr)`*`,`*

where *tel.cov* is coverage at telomeric index and *base.cov* is coverage at reference genome; *rl* is read length and *pl* is telomeric pattern length, *num.haploid.chr* is the number of haploid chromosomes.

The division by *num.haploid.chr* is for deriving at a mean value of telomere length for each chromosome end. The number 2 is to account for the two ends of each chromosome. Note that the ploidy of the genome will have no influence on the results, meaning that, adjusted for the rest of the parameters, the results will not differ for haploid, diploid and polyploid genomes.

**Min.seed

This is an advanced option and specifies the minimum number of telomeric repeat bases. More specifically, this value is used for building the telomeric index with trailing N bases of length (*read.length* - *min.seed*), which is the "index.nontelomeric" region of the index in the figure below.



If this option is omitted, the default value of 12 will be used. Increasing **min.seed** will increase specificity but decrease sensitivity of capturing telomeric reads, and vice-versa.
We believe that for the majority of biological cases, the default value is optimal.

Runing Computel from R

Open "computel.R" and change the configuration variables in the script as appropriate (see *Configuration options* section). The *scripts.dir* should point to the directory where the "pipeline.R", "validate.options.R" and "functions.R" scripts are located. Finally, run the script.

Configuration options

The following options should be specified before running Computel in R. These are given in the body of "computel.R" script in case of running Computel from R.

Value	**Example**	**Description**
#directories		
scripts.dir	D:/computel/	Path to directory containing "pipeline.R", "validate.options.R" and "functions.R" scripts.
bowtie.build.path	D:/bowtie2-2.1.0/bowtie2-build.exe	Path to bowtie2-build executable file or respective PATH variable, if applicable
bowtie.align.path	D:/bowtie2-2.1.0/bowtie2-align.exe	Path to bowtie2-align executable file or respective PATH variable, if applicable
samtools.path	D:/samtools-0.1.19/samtools.exe	Path to samtools executable file or respective PATH variable, if applicable
picard.samtofastq.jar	D:/picard-tools-1.108/SamToFastq.jar	Path to SamToFastq.jar or respective PATH variable, if applicable
#input_reads		
read.length	76	Number of bases in short reads. Default is *100*.
single	F	*T*-single-end reads, *F* - paired-end reads. Default is *T*.
IF paired-end reads		
fastq1	tel_reads1.fq	Path to first pair FASTQ file in case of paired-end reads (option *single* should be set to *F*)
fastq2	tel_reads2.fq	Path to second pair FASTQ file in case of paired-end reads (option *single* should be set to *F*)

IF single-end reads		
files.with.prefix	F	Specifies whether in single-end read FASTQ files are given with their prefix or as separate files
IF single-end reads AND files.with.prefix is F		
fastq	tel_reads.fq, tel_reads.fq1, tel_reads.fq2	Path to one or multiple FASTQ files (comma or tab separated) (option *single* should be set to *T* and *files.with.prefix* to *F*)
IF single-end reads AND files.with.prefix is T		
fastq.dir	./	Path to the directory where the reads are stored. (option *single* should be set to *T* and *files.with.prefix* to *T*)
fastq.prefix	tel_reads	Prefix of the reads to searched in the *fastq.dir* directory (option *single* should be set to *T* and *files.with.prefix* to *T*)
#algorithm_options		
pattern	TTAGGG	Sequence of single telomeric repeat. Default is *TTAGGG*.
num.haploid.chr	23	Number of chromosomes in haploid genome. Default is *23*.
min.seed	12	Minimum number of telomeric bases to be present in short reads for telomeric read alignment. Default is *12*.
mode.local	F	Mode of alignment. *T* - local, *F* - end-to-end. Default is *F.*
#base_coverage_calculation_options		
compute.base.cov	T	Specifies if the base coverage should be computed by read alignment to reference genome (*T*), or is supplied by the user (*F*). Default is F.
base.cov	5.4	Precalculated mean coverage at reference genome. Given

		if option *compute.base.cov* is *F.* Default is *1*.
base.index.pathtoprefix	base.index/base_index	Path to reference genome index directory and index prefix. Given if option *compute.base.cov* is *T.*
#output_options		
output.dir	output	Path to output directory. Default is *./output*
#system_options		
num.proc	3	Number of processors to be used during alignment. Default is *3.*
#additional_options		
quals	--phred33	Quality scores of reads. Default is "--phred33". Alternatives are: --phred33, --phred64 or --solexa-quals. **Caution:** be careful when specifying quality score values (see the text).
ignore.err	F	Specifies whether error messages thrown by system calls to bowtie should be ignored. Default is *F.* **Caution:** if set to *T,* wrong results may be generated by computel, if there were errors in alignment or index building (see text).

Notes on some configuration options

Single vs. paired-end reads

Generally, Computel performs equally well with paired-end and single-end reads. Therefore, it is also valid to treat all the FASTQ files as single-end (by setting the **single** option to *T*); and specify them by supplying multiple comma or space separated files (with **fastq** option) or supplying the directory and the prefix of the files (with **fastq.dir** and **fastq.prefix** options).

Read length

Caution: If the **read.length** option is omitted or set to a wrong value, Computel will not warn the user and the algorithm will run, however, a wrong value of telomere length estimate will be outputted.

Pattern

Telomeric repeat pattern is specified for the 3'-end of the chromosome. E.g. in case of human telomeres, the sequence at 3'-end it is TTAGGG, while the 5'-end has pattern CCCTAA.
If the pattern is omitted in the configuration file, the default value TTAGGG for human telomere repeats will be used. In case of a different study organism another pattern should be specified.

Number of haploid chromosomes

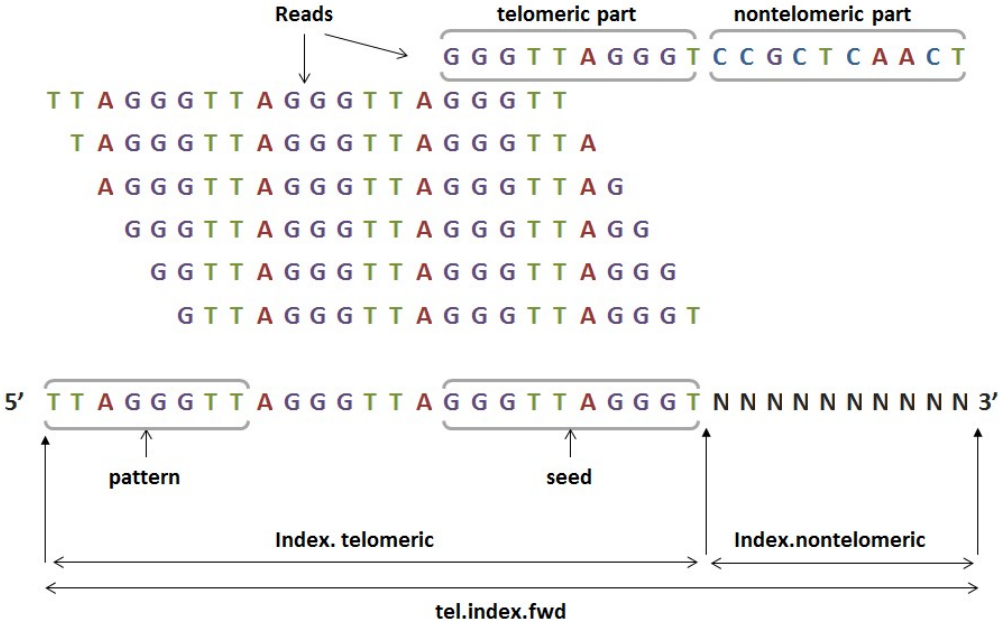
Telomere length is calculated with the formula:

(mean(*tel.cov*/*base.cov*))*(rl+pl-1) / (2*num.haploid.chr*),*

where *tel.cov* is coverage at telomeric index and *base.cov* is coverage at reference genome; *rl* is read length and *pl* is telomeric pattern length, *num.haploid.chr* is the number of haploid chromosomes.
The division by *num.haploid.chr* is for deriving at a mean value of telomere length for each chromosome end. The number 2 is to account for the two ends of each chromosome. Note that the ploidy of the genome will have no influence on the results, meaning that, adjusted for the rest of the parameters, the results will not differ for haploid, diploid and polyploid genomes.

Min.seed

This is an advanced option and specifies the minimum number of telomeric repeat bases. More specifically, this value is used for building the telomeric index with trailing N bases of length (*read.length* - *min.seed*), which is the "index.nontelomeric" region of the index in the figure below.



If this option is omitted, the default value of 12 will be used. Increasing **min.seed** will increase specificity but decrease sensitivity of capturing telomeric reads, and vice-versa.
We believe that for the majority of biological cases, the default value is optimal.

Alignment mode

When setting the **mode.local** value to T, the alignment of reads will be perfromed in Bowtie --local mode, instead of the default --end-to-end mode. Theoretically, local mode increases sensitivity of capturing telomeric reads. To our experience, for the majority of biological cases, the default --end-to-end mode is more accurate than the --local mode.

Base coverage calculation options

Alignment of the reads to reference genome is the most time consuming part of the algorithm. To our experience, the accuracy of the algorithm does not decrease if the base coverage is assessed beforehand with the formula

base.cov = (total number of reads)*(read length)/(total genome length)

and supplied to Computel with **base.cov** option. In that case, the **compute.base.cov** option should be set to *F*.

Note: If multiple FASTQ files from the same run are supplied to Computel as input, the **base.cov** is then equal to the summed/overall coverage at the reference.

If the user wants the algorithm to compute the base coverage with alignment, the user should provide the prebuilt reference genome index via the **base.index.pathtoprefix** option and set the **compute.base.cov** option to *F*.

quals

The quality scores can differ in various experimental settings. By default, computel will assume bowtie's default option for quality scores. Alternatively, the user may choose the quality scores to either '--phred33', '--phred64' or '--solexa-quals'. **Caution:** if **ignore.err** option is set to *T*, and the specified quality score values are wrong, bowtie may fail to run the alignment by throwing a warning message, but telomere computation will be performed with incomplete alignment and wrong results will be generated. Thus, the user should be careful when specifying quality score values.

ignore.err

If this option is set to *F,* computel will halt further execution, if it receives error messages while performing system calls to bowtie align or build. Setting this option to *T,* will make computel ignore the error messages and continue telomere length calculation, which may result in wrong result generation. Therefore, the user should pay attention to the error messages thrown to command line and R consoles, after getting the results of the calculation.

FAQ

The FAQ section will be generated once a question arises.

Feel free to ask questions and make suggestions by mailing to Lilit Nersisyan at l_nersisyan@mb.sci.am.