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Sequenza-utils is The supporting python library for the sequenza R package.

Sequenza is a software for the estimation and quantification of purity/ploidy and copy number alteration in sequencing experiments of tumor samples. Sequenza-utils provide command lines programs to transform common NGS file format - such as BAM, pileup and VCF - to input files for the R package
1.1 Installation

The sequenza-utils code is hosted in BitBucket. Supported Python version are 2.7+, including python3, and pypy. Sequenza-utils can be installed either via the Python Package Index (PyPI) or from the git repository. The package uses the external command line tools samtools and tabix. For the package to function correctly such programs need to be installed in the system.

1.1.1 Latest release via PyPI

To install the latest release via PyPI using pip:

```
pip install sequenza-utils
```

1.1.2 Development version

To use the test suite for the package is necessary to install also bwa Using the latest development version directly from the git repository:

```
git clone https://bitbucket.org/sequenzatools/sequenza-utils
cd sequenza-utils
python setup.py test
python setup.py install
```
1.2 Command line interface

The sequenza-utils comprehends several programs. All programs are accessible from the sequenza-utils command line interface.

```
$ sequenza-utils --help
usage: sequenza-utils [-h] [-v] 
       {bam2seqz,gc_wiggle,pileup2acgt,seqz_binning,seqz_merge, 
        →snp2seqz} ...

Sequenza Utils is a collection of tools primarily design to convert bam, pileup and vcf files to seqz files, the format used in the sequenza R package

positional arguments:
  bam2seqz Process a paired set of BAM/pileup files (tumor and matching normal), and GC-content genome-wide information, to extract the common positions withA and B alleles frequencies
  gc_wiggle Given a fasta file and a window size it computes the GC percentage across the sequences, and returns a file in the UCSC wiggle format.
  pileup2acgt Parse the format from the samtools mpileup command, and report the occurrence of the 4 nucleotides in each position.
  seqz_binning Perform the binning of the seqz file to reduce file size and memory requirement for the analysis.
  seqz_merge Merge two seqz files
  snp2seqz Parse VCFs and other variant and coverage formats to produce seqz files

optional arguments:
  -h, --help     show this help message and exit
  -v, --verbose  Show all logging information

This is version 2.1.9999b1 - Favero Francesco - 22 February 2019
```

1.2.1 CG wiggle

```
$ sequenza-utils gc_wiggle
error: argument -f/--fasta is required
usage: sequenza-utils gc_wiggle -f FASTA [-o OUT] [-w WINDOW]

optional arguments:
  -f FASTA, --fasta FASTA  the fasta file. It can be a file name or "-" to use STDIN
  -o OUT       Output file "-" for STDOUT
  -w WINDOW    The window size to calculate the GC-content percentage
```
1.2.2 BAM/mpileup to seqz

```shell
$ sequenza-utils bam2seqz
error: argument -n/--normal is required
```

Usage:
```
usage: sequenza-utils bam2seqz [-p] [-n NORMAL -t TUMOR -gc GC [-F FASTA]
[ -o OUT] [-n2 NORMAL2] [-C CHR [CHR ...]]
[--parallel NPROC] [-S SAMTOOLS] [-T TABIX]
[ -q QLIMIT] [-f QFORMAT] [-N N] [--hom HOM]
[ --het HET] [--het_f HET_F]
```

Input/Output:
- Input and output files.
- `-p`, `--pileup` Use pileups as input files instead of BAMs.
- `-n NORMAL`, `--normal NORMAL` Name of the BAM/pileup file from the reference/normal sample.
- `-t TUMOR`, `--tumor TUMOR` Name of the BAM/pileup file from the tumor sample.
- `-gc GC` The GC-content wiggle file.
- `-F FASTA`, `--fasta FASTA` The reference FASTA file used to generate the intermediate pileup. Required when input are BAM.
- `-o OUT`, `--output OUT` Name of the output file. To use gzip compression name the file ending in .gz. Default STDOUT.
- `-n2 NORMAL2`, `--normal2 NORMAL2` Optional BAM/pileup file used to compute the depth.normal and depth-ratio, instead of using the normal BAM.

Genotype:
- Options regarding the genotype filtering.
  - `--hom HOM` Threshold to select homozygous positions. Default 0.9.
  - `--het HET` Threshold to select heterozygous positions. Default 0.25.
  - `--het_f HET_F` Threshold of frequency in the forward strand to trust heterozygous calls. Default -0.2 (Disabled, effective with values >= 0).

Subset indexed files:
- Option regarding samtools and bam indexes.
  - `-C CHR [CHR ...]`, `--chromosome CHR [CHR ...]` Argument to restrict the input/output to a chromosome or a chromosome region. Coordinate format is Name:start-end, e.g. chr17:7565097-7590856, for a particular region; e.g. chr17, for the entire chromosome. Chromosome names can checked in the BAM/pileup files and are depending on the FASTA reference used for alignment. Default behavior is to not selecting any chromosome.
  - `--parallel NPROC` Defines the number of chromosomes to run in parallel. The output will be divided in multiple files, one for each chromosome. The file name will be composed by the output argument (used as prefix) and a chromosome name given by the chromosome argument list. This imply that
both output and chromosome argument need to be set correctly.

-S SAMTOOLS, --samtools SAMTOOLS
Path of samtools exec file to access the indexes and compute the pileups. Default "samtools"

-T TABIX, --tabix TABIX
Path of the tabix binary. Default "tabix"

Quality and Format:
Options that change the quality threshold and format.

-q QLIMIT, --qlimit QLIMIT
Minimum nucleotide quality score for inclusion in the counts. Default 20.

-f QFORMAT, --qformat QFORMAT
Quality format, options are "sanger" or "illumina". This will add an offset of 33 or 64 respectively to the qlimit value. Default "sanger".

-N N
Threshold to filter positions by the sum of read depth of the two samples. Default 20.

1.2.3 Binning seqz

$ sequenza-utils seqz_binning
error: argument -s/--seqz is required
usage: sequenza-utils seqz_binning -s SEQZ [-w WINDOW] [-o OUT] [-T TABIX]

optional arguments:
-s SEQZ, --seqz SEQZ A seqz file.
-w WINDOW, --window WINDOW
Window size used for binning the original seqz file. Default is 50.
-o OUT Output file "-" for STDOUT
-T TABIX, --tabix TABIX
Path of the tabix binary. Default "tabix"

1.2.4 VCF to seqz

$ sequenza-utils snp2seqz
error: argument -v/--vcf is required
usage: sequenza-utils snp2seqz [-o OUTPUT] -v VCF -gc GC
[--vcf-depth VCF_DEPTH_TAG]
[--vcf-samples {n/t,t/n}]
[--vcf-alleles VCF_ALLELES_TAG]
[--preset {caveman,mutect,mpileup,strelka2_som}]
[--hom HOM] [--het HET] [--het_f HET_F] [-N N]
[-T TABIX]

Output:
Output arguments
-o OUTPUT, --output OUTPUT
Output file. For gzip compressed output name the file ending in .gz. Default STDOUT

Input:
Input files
-v VCF, --vcf VCF VCF input file
-gc GC The GC-content wiggle file

VCF:
Parsing option for the VCF file
--vcf-depth VCF_DEPTH_TAG
  Column separated VCF tags in the format column for the read depth for the normal and for the tumor. Default "DP:DP"
--vcf-samples {n/t,t/n}
  Order of the normal and tumor sample in the VCF column, choices are "n/t" or "t/n". Default "n/t"
--vcf-alleles VCF_ALLELES_TAG
  Column separated VCF tags in the format column for the alleles depth for the normal and for the tumor. Default "AD:AD"
--preset {caveman,mutect,mpileup,strelka2_som}
  Preset set of options to parse VCF from popular variant callers

Genotype:
Genotype filtering options
--hom HOM Threshold to select homozygous positions. Default 0.9
--het HET Threshold to select heterozygous positions. Default 0.25.
--het_f HET_F Threshold of frequency in the forward strand to trust heterozygous calls. Default -0.2 (Disabled, effective with values >= 0).

Programs:
Option to call and control externa programs
-T TABIX, --tabix TABIX
  Path of the tabix binary. Default "tabix"

Filters:
Filter output file by various parameters
-N N Threshold to filter positions by the sum of read depth of the two samples. Default 20.

1.2.5 Merge overlapping seqz

$ sequenza-utils seqz_merge
error: argument -1/--seqz1 is required
usage: sequenza-utils seqz_merge [-o OUTPUT] -1 S1 -2 S2 [-T TABIX]

1.2. Command line interface
1.3 User cookbook

In order to process BAM files and generate file index, the software samtools and tabix need to be installed in the system.

The package sequenza-utils includes several programs and it should support the generation of seqz files using commonly available input files, such as fasta, BAM and vcf files.

To write your own program using the sequenza-utils library, please refer to the API library interface

1.3.1 Generate GC reference file

The GC content source required to generate seqz files must be in the wiggle track format (WIG). In order to generate the wig file from any fasta file use the gc_wiggle program.

```bash
sequenza-utils gc_wiggle --fasta genome.fa.gz -w 50 -o genome_gc50.wig.gz
```

1.3.2 From BAM files

Normal and tumor BAM files

```bash
sequenza-utils bam2seqz --normal normal_sample.bam --tumor tumor_sample.bam \ 
  --fasta genome.fa.gz --gc genome_gc50.wig.gz --output sample.seqz.gz
```

Normal and tumor pileup files

```bash
sequenza-utils bam2seqz --normal normal_sample.pielup.gz \ 
  --tumor tumor_sample.pielup.gz --fasta genome.fa.gz \ 
  --gc genome_gc50.wig.gz --output sample.seqz.gz --pileup
```
Without normal, workaround

```
sequenza-utils bam2seqz --normal tumor_sample.bam --tumor tumor_sample.bam \
   --normal2 non_matching_normal_sample.bam --fasta genome.fa.gz \
   -gc genome_gc50.wig.gz --output sample.seqz.gz
```

1.3.3 Binning seqz, reduce memory

```
sequenza-utils seqz_binning --seqz sample.seqz.gz --window 50 \
   --o sample_bin50.seqz.gz
```

1.3.4 Seqz from VCF files

VCF files with DP and AD tags

```
sequenza-utils snp2seqz --vcf sample_calls.vcf.gz -gc genome_gc50.wig.gz \
   --output samples.seqz.gz
```

Mutect/Caveman/Strelka2 preset

```
sequenza-utils snp2seqz --vcf sample_calls.vcf.gz -gc genome_gc50.wig.gz \
   --preset mutect --output samples.seqz.gz
```
```
sequenza-utils snp2seqz --vcf sample_calls.vcf.gz -gc genome_gc50.wig.gz \
   --preset caveman --output samples.seqz.gz
```
```
sequenza-utils snp2seqz --vcf sample_calls.vcf.gz -gc genome_gc50.wig.gz \
   --preset strelka2_som --output samples.seqz.gz
```

1.3.5 Merge seqz files

Non overlapping calls (eg different chromosomes)

```
gzcat sample_chr1.seqz.gz sample_chr1.seqz.gz | \
   gawk '{if (NR!=1 && $1 != "chromosome") {print $0}}' | bgzip > \
   sample.seqz.gz
tabix -f -s 1 -b 2 -e 2 -S 1 sample.seqz.gz
```

Overlapping sample_calls

```
sequenza-utils seqz_merge --seqz1 sample_somatic.seqz.gz \
   --seqz2 sample_snps.seqz.gz --output samples.seqz.gz
```

1.3. User cookbook
2.1 API library interface

2.1.1 sequenza.izip

```python
class sequenza.izip.zip_coordinates(item1, item2)
    Merge two object that have coordinate chromosome/position. The format of the objects must be a tuple with (coordinates, data) where coordinate is a tuple with chromosome,position_start, position_end and data is a tuple with the data. The data of the two object will be merged for matching lines. For the first object only the start coordinate is taken into account.
```

```python
class sequenza.izip.zip_fast(item1, item2)
    Use the native implementation of the heapq algorithm to sort and merge files chromosome-coordinate ordered. It assumes that the two files are position ordered and both files have the same chromosome order. It differs from zip_coordinates by the fact that this return all the position present in both files, group together the lines present in both
```

2.1.2 sequenza.wig

```python
class sequenza.wig.Wiggle(wig)
    Read/write wiggle files as iterable objects.
```

```python
exception sequenza.wig.WiggleError(message)
```

2.1.3 sequenza.fasta

```python
class sequenza.fasta.Fasta(file, n=60)
    Creates an iterable with genomic coordinates from a fasta file
```
2.1.4 sequenza.pileup

sequenza.pileup.acgt (pileup, quality, depth, reference, qlimit=53, noend=False, nostart=False)
Parse the mpileup format and return the occurrence of each nucleotides in the given positions.

sequenza.pileup.pileup_acgt (pileup, quality, depth, reference, qlimit=53, noend=False, nostart=False)
Yet another version of the pileup parser. Used as a template for the C implementation, the old function still runs slightly faster, to my surprise...

2.1.5 sequenza.samtools

class sequenza.samtools.bam_mpileup (bam, fasta, q=20, Q=20, samtools_bin='samtools', regions=[])
Use samtools via subprocess and return an iterable object.

class sequenza.samtools.indexed_pileup (pileup, tabix_bin='tabix', regions=[])
Use tabix via subprocess to slice the pileup data and return an iterable object

sequenza.samtools.program_version (program)
Parse tabix or samtools help message in attempt to retrieve the software version: return format: [major, minor, *]

sequenza.samtools.tabix_seqz (file_name, tabix_bin='tabix', seq=1, begin=2, end=2, skip=1)
Index a seqz file with tabix

2.1.6 sequenza.seqz

sequenza.seqz.acgt_genotype (acgt_dict, freq_list, strand_list, hom_t, het_t, het_f, bases_list)
Return the alleles in the genotype

sequenza.seqz.unpack_data (data)
Unpack normal, tumor and gc info from the specific tuple structure and remove redundant information

2.1.7 sequenza.vcf

sequenza.vcf.vcf_headline_content (line)
Try to get the string enclosed by "< . . . >" in the VCF header

sequenza.vcf.vcf_parse (vcf_file, sample_order='n/t', field='FORMAT', depth=['DP', 'DP'], alleles=['AD', 'AD'], preset=None)
Parse the specified tags of a vcf file to retrieve total and per-allele depth information.
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