

# Package ‘HTSeqGenie’

April 12, 2018

**Imports** BiocGenerics (>= 0.2.0), S4Vectors (>= 0.9.25), IRanges (>= 1.21.39), GenomicRanges (>= 1.23.21), Rsamtools (>= 1.8.5), Biostrings (>= 2.24.1), chipseq (>= 1.6.1), hwriter (>= 1.3.0), Cairo (>= 1.5.5), GenomicFeatures (>= 1.9.31), BiocParallel, parallel, tools, rtracklayer (>= 1.17.19), GenomicAlignments, VariantTools (>= 1.7.7), GenomeInfoDb, SummarizedExperiment, methods

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**License** Artistic-2.0

**Title** A NGS analysis pipeline.

**Type** Package

**LazyLoad** yes

**Author** Gregoire Pau, Jens Reeder

**Description** Libraries to perform NGS analysis.

**Version** 4.8.0

**Depends** R (>= 3.0.0), gmapR (>= 1.8.0), ShortRead (>= 1.19.13), VariantAnnotation (>= 1.8.3)

**Suggests** TxDb.Hsapiens.UCSC.hg19.knownGene, LungCancerLines, org.Hs.eg.db

**RoxygenNote** 5.0.1

**NeedsCompilation** no

## R topics documented:

analyzeVariants . . . . .	2
annotateVariants . . . . .	2
buildGenomicFeaturesFromTxDb . . . . .	3
callVariantsGATK . . . . .	4
checkGATKJar . . . . .	4
detectRRNA . . . . .	5
excludeVariantsByRegions . . . . .	5
gatk . . . . .	6
generateSingleGeneDEs . . . . .	7
getRRNAIds . . . . .	7
getTabDataFromFile . . . . .	8
hashCoverage . . . . .	8

hashVariants . . . . .	9
hashVector . . . . .	9
HTSeqGenie . . . . .	10
isSparse . . . . .	11
markDuplicates . . . . .	11
markDups . . . . .	12
realignIndels . . . . .	13
realignIndelsGATK . . . . .	13
runPipeline . . . . .	14
runPipelineConfig . . . . .	15
setupTestFramework . . . . .	16
TP53GenomicFeatures . . . . .	16
vcfStat . . . . .	17
wrap.callVariants . . . . .	17
writeVCF . . . . .	18

**Index** **19**

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analyzeVariants	<i>Calculate and process Variants</i>
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**Description**

Calculate and process Variants

**Usage**

analyzeVariants()

**Value**

Nothing

**Author(s)**

Jens Reeder

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annotateVariants	<i>Annotate variants via vep</i>
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**Description**

Annotate variants via vep

**Usage**

annotateVariants(vcf.file)

**Arguments**

vcf.file            A character vector pointing to a VCF (or gzipped VCF) file

**Value**

Path to a vcf file with variant annotations

**Author(s)**

Jens Reeder

---

`buildGenomicFeaturesFromTxDb`

*Build genomic features from a TxDb object*

---

**Description**

Build genomic features from a TxDb object

**Usage**

```
buildGenomicFeaturesFromTxDb(txdb)
```

**Arguments**

`txdb`            A TxDb object.

**Value**

A list named list of GRanges objects containing the biological entities to account for.

**Author(s)**

Gregoire Pau

**Examples**

```
## Not run:  
library("TxDb.Hsapiens.UCSC.hg19.knownGene")  
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene  
genomic_features <- buildGenomicFeaturesFromTxDb(txdb)  
  
## End(Not run)
```

callVariantsGATK      *Variant calling via GATK*

---

**Description**

Call variants via GATK using the pipeline framework. Requires a GATK compatible genome with a name matching the alignment genome to be installed in 'path.gatk\_genome'

**Usage**

```
callVariantsGATK(bam.file)
```

**Arguments**

bam.file      Path to bam.file

**Value**

Path to variant file

**Author(s)**

Jens Reeder

---

checkGATKJar      *Check for the GATK jar file*

---

**Description**

Check for the GATK jar file

**Usage**

```
checkGATKJar(path = getOption("gatk.path"))
```

**Arguments**

path      Path to the GATK jar file

**Value**

TRUE if tool can be called, FALSE otherwise

---

`detectRRNA`*Detect rRNA Contamination in Reads*

---

**Description**

Returns a named vector indicating if a read ID has rRNA contamination or not

**Usage**

```
detectRRNA(lreads, remove_tmp_dir = TRUE, save_dir = NULL)
```

**Arguments**

<code>lreads</code>	A list of ShortReadQ objects
<code>remove_tmp_dir</code>	boolean indicating whether or not to delete temp directory of gsnap results
<code>save_dir</code>	Save directory

**Details**

Given a genome and fastq data, each read in the fastq data is aligned against the rRNA sequences for that genome

**Value**

a named logical vector indicating if a read has rRNA contamination

**Author(s)**

Cory Barr

---

`excludeVariantsByRegions`*Filter variants by regions*

---

**Description**

Filter variants by regions

**Usage**

```
excludeVariantsByRegions(variants, mask)
```

**Arguments**

<code>variants</code>	Variants as Vranges, GRanges or VCF object
<code>mask</code>	region to mask, given as GRanges

**Details**

This function can be used to filter variants in a given region, e.g. low complexity and repeat regions

**Value**

The filtered variants

**Author(s)**

Jens Reeder

---

gatk

*gatk*

---

**Description**

Run a command from the GATK

**Usage**

```
gatk(gatk.jar.path = getOption("gatk.path"), method, args, maxheap = "4g")
```

**Arguments**

gatk.jar.path	Path to the gatk jar file
method	Name of the gatk method, e.g. UnifiedGenotyper
args	additional args passed to gatk
maxheap	Maximal heap space allocated for java, GATK recommends 4G heap for most of its apps

**Details**

Execute the GATK jar file using the method specified as arg. Stops if the command executed fails.

**Value**

0 for success, stops otherwise

**Author(s)**

Jens Reeder

---

```
generateSingleGeneDERs
    generateSingleGeneDERs
```

---

**Description**

Generate DEXSeq-ready exons

**Usage**

```
generateSingleGeneDERs(txdb)
```

**Arguments**

txdb                    A transcript DB object

**Details**

generateSingleGeneDERs() generates exons by: 1) disjoining the whole exon set 2) keeping only the exons of coding regions 3) keeping only the exons that belong to unique genes

**Value**

single gene DERs

---

```
getRRNAIds                    Detect reads that look like rRNA
```

---

**Description**

Detect reads that look like rRNA

**Usage**

```
getRRNAIds(file1, file2 = NULL, tmp_dir, rRNADb)
```

**Arguments**

file1                    FastQ file of forward reads  
file2                    FastQ of reverse reads in paired-end sequencing, NULL otherwise  
tmp\_dir                    temporary directory used for storing the gsnap results  
rRNADb                    Name of the rRNA sequence database. Must exist in the gsnap genome directory

**Value**

IDs of reads flagged as rRNA

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getTabDataFromFile	<i>Load tabular data from the NGS pipeline result directory</i>
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---

**Description**

Load tabular data from the NGS pipeline result directory

**Usage**

```
getTabDataFromFile(save_dir, object_name)
```

**Arguments**

save_dir	A character string containing an NGS pipeline output directory.
object_name	A character string containing the regular expression matching a filename in dir_path

**Value**

A data frame.

---

hashCoverage	<i>Hashing function for coverage</i>
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**Description**

Hashing function for coverage

**Usage**

```
hashCoverage(cov)
```

**Arguments**

cov	A SimpleRleList object
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**Value**

A numeric

**Author(s)**

Gregoire Pau



---

hashVariants	<i>Hashing function for variants</i>
--------------	--------------------------------------

---

**Description**

Hashing function for variants

**Usage**

```
hashVariants(var)
```

**Arguments**

var	A GRanges object
-----	------------------

**Value**

A numeric

**Author(s)**

Gregoire Pau

---

hashVector	<i>Hashing function for vector</i>
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---

**Description**

Hashing function for vector

**Usage**

```
hashVector(x)
```

**Arguments**

x	A vector
---	----------

**Value**

A numeric

**Author(s)**

Gregoire Pau

## Description

The HTSeqGenie package is a robust and efficient software to analyze high-throughput sequencing experiments in a reproducible manner. It supports the RNA-Seq and Exome-Seq protocols and provides: quality control reporting (using the ShortRead package), detection of adapter contamination, read alignment versus a reference genome (using the gmapR package), counting reads in genomic regions (using the GenomicRanges package), and read-depth coverage computation.

## Package content

To run the pipeline:

- runPipeline

To access the pipeline output data:

- getTabDataFromFile

To build the genomic features object:

- buildGenomicFeaturesFromTxDb
- TP53GenomicFeatures

## Examples

```
## Not run:
## build genome and genomic features
tp53Genome <- TP53Genome()
tp53GenomicFeatures <- TP53GenomicFeatures()

## get the FASTQ files
fastq1 <- system.file("extdata/H1993_TP53_subset2500_1.fastq.gz", package="HTSeqGenie")
fastq2 <- system.file("extdata/H1993_TP53_subset2500_2.fastq.gz", package="HTSeqGenie")

## run the pipeline
save_dir <- runPipeline(
  ## input
  input_file=fastq1,
  input_file2=fastq2,
  paired_ends=TRUE,
  quality_encoding="illumina1.8",

  ## output
  save_dir="test",
  prepend_str="test",
  overwrite_save_dir="erase",

  ## aligner
  path.gsnap_genomes=path(directory(tp53Genome)),
  alignReads.genome=genome(tp53Genome),
  alignReads.additional_parameters="--indel-penalty=1 --novelsplicing=1 --distant-splice-penalty=1",
```

```

    ## gene model
    path.genomic_features=dirname(tp53GenomicFeatures),
    countGenomicFeatures.gfeatures=basename(tp53GenomicFeatures)
  )

  ## End(Not run)

```

---

isSparse	<i>isSparse</i>
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---

### Description

Check coverage for sparseness

### Usage

```
isSparse(cov, threshold = 0.1)
```

### Arguments

cov	A cov object as SimpleRleList
threshold	Fraction of number of runs over total length

### Details

Some Rle related operations become very slow when they are dealing with data that violates their sparseness assumption. This method provides an estimate about whether the data is dense or sparse. More precisely it checks if the fraction of the number of runs over the total length is smaller than a threshold

### Value

Boolean whether this object is dense or sparse

### Author(s)

Jens Reeder

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markDuplicates	<i>markDuplicates</i>
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---

### Description

Mark duplicates in bam

### Usage

```
markDuplicates(bamfile, outfile = NULL, path = getOption("picard.path"))
```

**Arguments**

bamfile	Name of input bam file
outfile	Name of output bam file
path	Full path to MarkDuplicates jar

**Details**

Use MarkDuplicates from PicardTools to mark duplicate alignments in bam file.

**Value**

Path to output bam file

**Author(s)**

Jens Reeder

---

markDups

*markDups*

---

**Description**

Mark duplicates in pipeline context

**Usage**

markDups()

**Details**

High level function call to mark duplicates in the analyzed.bam file of a pipelin run.

**Value**

Nothing

**Author(s)**

Jens Reeder

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realignIndels	<i>realignIndels</i>
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---

**Description**

Realign indels in pipeline context

**Usage**

```
realignIndels()
```

**Details**

High level function call to realign indels in the analyzed.bam file using GATK

**Value**

Nothing

**Author(s)**

Jens Reeder

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realignIndelsGATK	<i>Realign indels via GATK</i>
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---

**Description**

Realign indels using the GATK tools RealignerTargetCreator and IndelRealigner. Requires a GATK compatible genome with a name matching the alignment genome to be installed in 'path.gatk\_genome'

**Usage**

```
realignIndelsGATK(bam.file)
```

**Arguments**

bam.file	Path to bam.file
----------	------------------

**Details**

Since GATKs IndelRealigner is not parallelized, we run it in parallel per chromosome.

**Value**

Path to realigned bam file

**Author(s)**

Jens Reeder

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runPipeline	<i>Run the NGS analysis pipeline</i>
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---

**Description**

Run the NGS analysis pipeline

**Usage**

```
runPipeline(...)
```

**Arguments**

... A list of parameters. See the vignette for details.

**Details**

This function starts the pipeline. It first preprocesses the input FASTQ reads, align them, count the read overlaps with genomic features and compute the coverage. See the vignette for details.

**Value**

The path to the NGS output directory.

**Author(s)**

Jens Reeder, Gregoire Pau

**See Also**

TP53Genome, TP53GenomicFeatures

**Examples**

```
## Not run:
## build genome and genomic features
tp53Genome <- TP53Genome()
tp53GenomicFeatures <- TP53GenomicFeatures()

## get the FASTQ files
fastq1 <- system.file("extdata/H1993_TP53_subset2500_1.fastq.gz", package="HTSeqGenie")
fastq2 <- system.file("extdata/H1993_TP53_subset2500_2.fastq.gz", package="HTSeqGenie")

## run the pipeline
save_dir <- runPipeline(
  ## input
  input_file=fastq1,
  input_file2=fastq2,
  paired_ends=TRUE,
  quality_encoding="illumina1.8",

  ## output
  save_dir="test",
  prepend_str="test",
```

```
    overwrite_save_dir="erase",

    ## aligner
    path.gsnap_genomes=path(directory(tp53Genome)),
    alignReads.genome=genome(tp53Genome),
    alignReads.additional_parameters="--indel-penalty=1 --novelsplicing=1 --distant-splice-penalty=1",

    ## gene model
    path.genomic_features=dirname(tp53GenomicFeatures),
    countGenomicFeatures.gfeatures=basename(tp53GenomicFeatures)
)

## End(Not run)
```

---

runPipelineConfig      *Run the NGS analysis pipeline*

---

## Description

Run the NGS analysis pipeline from a configuration file

## Usage

```
runPipelineConfig(config_filename, config_update)
```

## Arguments

config\_filename      Path to a pipeline configuration file

config\_update      A list of name value pairs that will update the config parameters

## Details

This is the launcher function for all pipeline runs. It will do some preprocessing steps, then aligns the reads, counts overlap with genomic Features such as genes, exons etc and applies a variant caller.

## Value

Nothing

## Author(s)

Jens Reeder, Gregoire Pau

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setupTestFramework	<i>setup test framework</i>
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---

**Description**

setup test framework

**Usage**

```
setupTestFramework(config.filename, config.update = list(),
  testname = "test", package = "HTSeqGenie", use.TP53Genome = TRUE)
```

**Arguments**

config.filename	configuration file
config.update	update list of config values
testname	name of test case
package	name of package
use.TP53Genome	Boolean indicating the use of the TP53 genome as template config

**Value**

the created temp directory

---

TP53GenomicFeatures	<i>Demo genomic features around the TP53 gene</i>
---------------------	---

---

**Description**

Build the genomic features of the TP53 demo region

**Usage**

```
TP53GenomicFeatures()
```

**Details**

Returns a list of genomic features (gene, exons, transcripts) annotating a region of UCSC hg19 sequence centered on the region of the TP53 gene, with 1 Mb flanking sequence on each side. This is intended as a test/demonstration to run the NGS pipeline in conjunction with the LungCancerLines data package.

**Value**

A list of GRanges objects containing the genomic features

**Author(s)**

Gregoire Pau



**See Also**

TP53Genome, buildGenomicFeaturesFromTxDb, runPipeline

---

vcfStat                      *Compute stats on a VCF file*

---

**Description**

Compute stats on a VCF file

**Usage**

```
vcfStat(vcf.filename)
```

**Arguments**

vcf.filename      A character pointing to a VCF (or gzipped VCF) file

**Value**

A numeric vector

**Author(s)**

Gregoire Pau

---

wrap.callVariants      *Variant calling*

---

**Description**

Call Variants in the pipeline framework

**Usage**

```
wrap.callVariants(bam.file)
```

**Arguments**

bam.file              Aligned reads as bam file

**Details**

A wrapper around VariantTools callVariant framework.

**Value**

Variants as Vranges

**Author(s)**

Jens Reeder

---

`writeVCF`*writeVCF*

---

**Description**

Write variants to VCF file

**Usage**

```
writeVCF(variants.vranges, filename)
```

**Arguments**

<code>variants.vranges</code>	Genomic Variants as VRanges object
<code>filename</code>	Name of vcf file to write

**Value**

VCF file name

**Author(s)**

Jens Reeder

# Index

## \*Topic **package**

HTSeqGenie, [10](#)

analyzeVariants, [2](#)

annotateVariants, [2](#)

buildGenomicFeaturesFromTxDb, [3](#)

callVariantsGATK, [4](#)

checkGATKJar, [4](#)

detectRRNA, [5](#)

excludeVariantsByRegions, [5](#)

gatk, [6](#)

generateSingleGeneDEs, [7](#)

getRRNAIds, [7](#)

getTabDataFromFile, [8](#)

hashCoverage, [8](#)

hashVariants, [9](#)

hashVector, [9](#)

HTSeqGenie, [10](#)

isSparse, [11](#)

markDuplicates, [11](#)

markDups, [12](#)

realignIndels, [13](#)

realignIndelsGATK, [13](#)

runPipeline, [14](#)

runPipelineConfig, [15](#)

setupTestFramework, [16](#)

TP53GenomicFeatures, [16](#)

vcfStat, [17](#)

wrap.callVariants, [17](#)

writeVCF, [18](#)