

# Package ‘Rsubread’

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**Title** Subread sequence alignment and counting for R

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**Description** Rsubread is a toolbox developed for the analyses of second and third generation sequencing data. It can be used for read mapping, read counting, SNP calling, structural variant detection and gene fusion discovery. It can be applied to all major sequencing technologies and it is suitable for the analysis of both short and long reads.

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align	<i>Align sequence reads to a reference genome via seed-and-vote</i>
-------	---

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## Description

align function can align both DNA and RNA sequencing reads. Subjunc is an RNA-seq aligner and it reports full alignment of each read (align reports partial alignment for exon spanning reads).

## Usage

```
align(
# index for reference sequences
index,

# input reads and output
readfile1,
readfile2=NULL,
type="rna",
input_format="gzFASTQ",
output_format="BAM",
output_file=paste(as.character(readfile1),"subread",output_format,sep="."),

# offset value added to Phred quality scores of read bases
phredOffset=33,

# thresholds for mapping
nsubreads=10,
TH1=3,
TH2=1,
maxMismatches=3,

# unique mapping and multi-mapping
unique=FALSE,
nBestLocations=1,

# indel detection
```

```
indels=5,
complexIndels=FALSE,

# read trimming
nTrim5=0,
nTrim3=0,

# distance and orientation of paired end reads
minFragLength=50,
maxFragLength=600,
PE_orientation="fr",

# number of CPU threads
nthreads=1,

# read group
readGroupID=NULL,
readGroup=NULL,

# read order
keepReadOrder=FALSE,
sortReadsByCoordinates=FALSE,

# color space reads
color2base=FALSE,

# dynamic programming
DP_GapOpenPenalty=-1,
DP_GapExtPenalty=0,
DP_MismatchPenalty=0,
DP_MatchScore=2,

# detect structural variants
detectSV=FALSE,

# gene annotation
useAnnotation=FALSE,
annot.inbuilt="mm10",
annot.ext=NULL,
isGTF=FALSE,
GTF.featureType="exon",
GTF.attrType="gene_id",
chrAliases=NULL)

subjunc(
# index for reference sequences
index,

# input reads and output
readfile1,
readfile2=NULL,
input_format="gzFASTQ",
```

```
output_format="BAM",
output_file=paste(as.character(readfile1),"subjunc",output_format,sep="."),

# offset value added to Phred quality scores of read bases
phredOffset=33,

# thresholds for mapping
nsubreads=14,
TH1=1,
TH2=1,
maxMismatches=3,

# unique mapping and multi-mapping
unique=FALSE,
nBestLocations=1,

# indel detection
indels=5,
complexIndels=FALSE,

# read trimming
nTrim5=0,
nTrim3=0,

# distance and orientation of paired end reads
minFragLength=50,
maxFragLength=600,
PE_orientation="fr",

# number of CPU threads
nthreads=1,

# read group
readGroupID=NULL,
readGroup=NULL,

# read order
keepReadOrder=FALSE,
sortReadsByCoordinates=FALSE,

# color space reads
color2base=FALSE,

# dynamic programming
DP_GapOpenPenalty=-1,
DP_GapExtPenalty=0,
DP_MismatchPenalty=0,
DP_MatchScore=2,

# detect all junctions including gene fusions
reportAllJunctions=FALSE,
```

```
# gene annotation
useAnnotation=FALSE,
annot.inbuilt="mm10",
annot.ext=NULL,
isGTF=FALSE,
GTF.featureType="exon",
GTF.attrType="gene_id",
chrAliases=NULL)
```

### Arguments

index	character string giving the basename of index file. Index files should be located in the current directory.
readfile1	a character vector including names of files that include sequence reads to be aligned. For paired-end reads, this gives the list of files including first reads in each library. File format is FASTQ/FASTA by default. See <code>input_format</code> option for more supported formats.
readfile2	a character vector giving names of files that include second reads in paired-end read data. Files included in <code>readfile2</code> should be in the same order as their mate files included in <code>readfile1</code> . NULL by default.
type	a character string or an integer giving the type of sequencing data. Possible values include <code>rna</code> (or 0; RNA-seq data) and <code>dna</code> (or 1; genomic DNA-seq data such as WGS, WES, ChIP-seq data etc.). Character strings are case insensitive.
input_format	character string specifying format of read input files. <code>gzFASTQ</code> by default (this also includes FASTQ, FASTA and gzipped FASTA formats). Other supported formats include SAM and BAM. Character values are case insensitive.
output_format	character string specifying format of output file. BAM by default. Acceptable formats include SAM and BAM.
output_file	a character vector specifying names of output files. By default, names of output files are set as the file names provided in <code>readfile1</code> added with an suffix string.
phredOffset	numeric value added to base-calling Phred scores to make quality scores (represented as ASCII letters). Possible values include 33 and 64. By default, 33 is used.
nsubreads	numeric value giving the number of subreads extracted from each read.
TH1	numeric value giving the consensus threshold for reporting a hit. This is the threshold for the first reads if paired-end read data are provided.
TH2	numeric value giving the consensus threshold for the second reads in paired-end data.
maxMismatches	numeric value giving the maximum number of mis-matched bases allowed in the alignment. 3 by default. Mis-matches found in soft-clipped bases are not counted.
unique	logical indicating if only uniquely mapped reads should be reported. A uniquely mapped read has one single mapping location that has less mis-matched bases than any other candidate locations. By default, multi-mapping reads will be reported in addition to uniquely mapped reads. Number of alignments reported for each multi-mapping read is determined by the <code>nBestLocations</code> parameter.
nBestLocations	numeric value specifying the maximal number of equally-best mapping locations that will be reported for a multi-mapping read. 1 by default. The allowed value is between 1 to 16 (inclusive). In the mapping output, 'NH' tag is used

	to indicate how many alignments are reported for the read and ‘HI’ tag is used for numbering the alignments reported for the same read. This argument is only applicable when unique option is set to FALSE.
indels	numeric value giving the maximum number of insertions/deletions allowed during the mapping. 5 by default.
complexIndels	logical indicating if complex indels will be detected. If TRUE, the program will try to detect multiple short indels that occurs concurrently in a small genomic region (indels could be as close as 1bp apart).
nTrim5	numeric value giving the number of bases trimmed off from 5’ end of each read. 0 by default.
nTrim3	numeric value giving the number of bases trimmed off from 3’ end of each read. 0 by default.
minFragLength	numeric value giving the minimum fragment length. 50 by default.
maxFragLength	numeric value giving the maximum fragment length. 600 by default.
PE_orientation	character string giving the orientation of the two reads from the same pair. It has three possible values including fr, ff and rf. Letter f denotes the forward strand and letter r the reverse strand. fr by default (ie. the first read in the pair is on the forward strand and the second read on the reverse strand).
nthreads	numeric value giving the number of threads used for mapping. 1 by default.
readGroupID	a character string giving the read group ID. The specified string is added to the read group header field and also be added to each read in the mapping output. NULL by default.
readGroup	a character string in the format of tag:value. This string will be added to the read group (RG) header in the mapping output. NULL by default.
keepReadOrder	logical indicating if the order of reads in the BAM output is kept the same as that in the input file. Reads from the same pair are always placed next to each other no matter this option is specified or not.
sortReadsByCoordinates	logical indicating if reads will be sorted by their mapping locations in the mapping output. This option is applicable for BAM output only. A BAI index file will also be generated for each BAM file so the generated BAM files can be directly loaded into a genome browser such as IGB or IGV. FALSE by default.
color2base	logical. If TRUE, color-space read bases will be converted to base-space bases in the mapping output. Note that the mapping itself will still be performed at color-space. FALSE by default.
DP_GapOpenPenalty	a numeric value giving the penalty for opening a gap when using the Smith-Waterman dynamic programming algorithm to detect insertions and deletions. The Smith-Waterman algorithm is only applied for those reads which are found to contain insertions or deletions. -1 by default.
DP_GapExtPenalty	a numeric value giving the penalty for extending the gap, used by the Smith-Waterman algorithm. 0 by default.
DP_MismatchPenalty	a numeric value giving the penalty for mismatches, used by the Smith-Waterman algorithm. 0 by default.
DP_MatchScore	a numeric value giving the score for matches used by the Smith-Waterman algorithm. 2 by default.

<code>detectSV</code>	logical indicating if structural variants (SVs) will be detected during read mapping. See below for more details.
<code>reportAllJunctions</code>	logical indicating if all discovered junctions will be reported. This includes exon-exon junctions and also gene fusions. Presence of donor/receptor sites is not required when <code>reportAllJunctions</code> is TRUE. This option should only be used for RNA-seq data.
<code>useAnnotation</code>	logical indicating if gene annotation information will be used in the mapping. FALSE by default.
<code>annot.inbuilt</code>	a character string specifying an in-built annotation used for read summarization. It has four possible values including "mm10", "mm9", "hg38" and "hg19", corresponding to the NCBI RefSeq annotations for genomes 'mm10', 'mm9', 'hg38' and 'hg19', respectively. "mm10" by default. See <a href="#">featureCounts</a> function for more details on the in-built annotations.
<code>annot.ext</code>	A character string giving name of a user-provided annotation file or a data frame including user-provided annotation data. If the annotation is in GTF format, it can only be provided as a file. If it is in SAF format, it can be provided as a file or a data frame. The provided annotation file can be a uncompressed or gzip compressed file. If an annotation is provided via <code>annot.ext</code> , the <code>annot.inbuilt</code> parameter will be ignored. See <a href="#">featureCounts</a> function for more details about this parameter.
<code>isGTF</code>	logical indicating if the annotation provided via the <code>annot.ext</code> argument is in GTF format or not. FALSE by default. This option is only applicable when <code>annot.ext</code> is not NULL.
<code>GTF.featureType</code>	a character string denoting the type of features that will be extracted from a GTF annotation. "exon" by default. This argument is only applicable when <code>isGTF</code> is TRUE.
<code>GTF.attrType</code>	a character string denoting the type of attributes in a GTF annotation that will be used to group features. "gene_id" by default. The grouped features are called meta-features. For instance, a feature can be an exon and several exons can be grouped into a gene (meta-feature). This argument is only applicable when <code>isGTF</code> is TRUE.
<code>chrAliases</code>	a character string providing the name of a comma-delimited text file that includes aliases of chromosome names. This file should contain two columns. First column contains names of chromosomes included in the SAF or GTF annotation and second column contains corresponding names of chromosomes in the reference genome. No column headers should be provided. Also note that chromosome names are case sensitive. This file can be used to match chromosome names between the annotation and the reference genome.

## Details

The `align` function is an R wrapper for the Subread aligner (Liao et al., 2013) that uses a novel mapping paradigm called "seed-and-vote". Subread is general-purpose aligner that can be used to align both genomic DNA-seq reads and RNA-seq reads. The `subjunc` function is an R wrapper for the Subjunc aligner (Liao et al., 2013) that is designed for mapping RNA-seq reads only.

The main difference between `subjunc` and `align` is that `subjunc` reports discovered exon-exon junctions and it also performs full alignments for every read including exon-spanning reads. `align` reports the largest mappable regions for each read.

align and subjunc can be run with a full index or a gapped index (see [buildindex](#) for more details). Maximum mapping speed is achieved when a one-block full index is used for mapping.

subjunc requires the presence of donor and receptor sites when calling exon-exon junctions. It can detect up to four junction locations in a junction read.

The detectSV parameter in align function can be used to detect structural variants (SVs) in genomic DNA sequencing data. The reportAllJunctions parameter in subjunc function can be used for the detection of SVs in RNA-seq data, as well as the detection of non-canonical junctions that do not have the canonical donor/receptor sites. For each library, breakpoints detected from SV events are saved to a file with suffix name '.breakpoints.txt', which includes chromosomal coordinates of SV breakpoints and numbers of supporting reads. The BAM/SAM output includes extra fields to describe the complete alignments of breakpoint-containing reads. For a breakpoint-containing read, mapping of its major sequence segment is described in the main fields of BAM/SAM output whereas mapping of its minor sequence segment, which does not map along with the major segment due to the presence of a breakpoint, is described in the extra fields including 'CC'(Chr), 'CP'(Position), 'CG'(CIGAR) and 'CT'(strand). Note that each breakpoint-containing read occupies only one row in BAM/SAM output.

### Value

Return a data.frame object that includes mapping statistics for each library. Read mapping results are saved to BAM or SAM files in the current working directory. For subjunc, BED files including discovered exon-exon junctions are also written to the current working directory.

### Author(s)

Wei Shi and Yang Liao

### References

Yang Liao, Gordon K Smyth and Wei Shi. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Research, 41(10):e108, 2013.

### Examples

```
# Build an index for the artificial sequence included in file 'reference.fa'.
library(Rsubread)
ref <- system.file("extdata", "reference.fa", package="Rsubread")
buildindex(basename="./reference_index", reference=ref)

# align a sample read dataset ('reads.txt') to the sample reference
reads <- system.file("extdata", "reads.txt.gz", package="Rsubread")
align.stat <- align(index="./reference_index", readfile1=reads, output_file="./Rsubread_alignment.BAM", phredO
```

---

atgcContent

*Calculate percentages of nucleotides A, T, G and C in a sequencing read datafile*

---

### Description

Calculate percentages of nucleotides A, T, G and C



**Usage**

```
atgcContent(filename, basewise=FALSE)
```

**Arguments**

filename	character string giving the name of input FASTQ/FASTA file
basewise	logical. If TRUE, nucleotide percentages will be calculated for each base position in the read across all the reads. By default, percentages are calculated for the entire dataset.

**Details**

Sequencing reads could contain letter "N" besides "A", "T", "G" and "C". Percentage of "N" in the read dataset is calculated as well.

The basewise calculation is useful for examining the GC bias towards the base position in the read. By default, the percentages of nucleotides in the entire dataset will be reported.

**Value**

A named vector containing percentages for each nucleotide type if basewise is FALSE. Otherwise, a data matrix containing nucleotide percentages for each base position of the reads.

**Author(s)**

Zhiyin Dai and Wei Shi

---

buildindex	<i>Build index for a reference genome</i>
------------	---

---

**Description**

An index needs to be built before read mapping can be performed. This function creates a hash table index for the reference genome, which can then be used by Subread and Subjunc aligners for read alignment.

**Usage**

```
buildindex(basename, reference, gappedIndex=FALSE, indexSplit=FALSE, memory=8000,
TH_subread=100, colorspace=FALSE)
```

**Arguments**

basename	character string giving the basename of created index files.
reference	character string giving the name of a FASTA-format file that includes sequences of all chromosomes and contigs.
gappedIndex	logical indicating if a gapped index or a full index will be built. A gapped index contains 16mers (subreads) that are extracted every three bases from a reference genome, whereas a full index contains subreads extracted from every chromosomal location of a genome. The index contains a hash table, which includes sequences of subreads and their corresponding chromosomal locations. Default value of this argument is FALSE (ie. a full index is built).

<code>indexSplit</code>	logical indicating if an index can be split into multiple blocks. The block size is determined by value of parameter <code>memory</code> . FALSE by default (ie. a single-block index is generated).
<code>memory</code>	numeric value specifying the amount of memory (in megabytes) used for storing the index during read mapping. 8000 MB by default. Note that this option is ignored when <code>indexSplit</code> is FALSE.
<code>TH_subread</code>	numeric value specifying the threshold for removing highly repetitive subreads (16bp mers). 100 by default. Subreads will be excluded from the index if they occur more than threshold number of times in the genome.
<code>colospace</code>	logical. If TRUE, a color space index will be built. Otherwise, a base space index will be built.

### Details

This function generates a hash-table index for a reference genome, in which keys are subreads (16mers) and values are their chromosomal locations in the reference genome. The built index can then be used by Subread ([align](#)) and [subjunc](#) aligners to map reads(Liao et al. 2013).

Highly repetitive subreads (or uninformative subreads) are excluded from the hash table so as to reduce mapping ambiguity. `TH_subread` specifies the maximal number of times a subread is allowed to occur in the reference genome to be included in hash table.

When an index is split into multiple blocks, only one block will be loaded into memory at any time during read mapping. Therefore the size of memory used during mapping can be controlled. The more memory is used in mapping, the faster the mapping speed. Generating a one-block full index (eg. setting both `gappedIndex` and `indexSplit` to FALSE) will enable the maximum mapping speed to be achieved.

Once an index is built, it can be re-used in each mapping.

Sequences of reference genomes can be downloaded from public databases. For instance, primary assembly of human genome GRCh38/hg38 or mouse genome GRCm38/mm10 can be downloaded from the GENCODE database via the following links:

[ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode\\_human/release\\_28/GRCh38.primary\\_assembly.genome.fa.gz](ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_28/GRCh38.primary_assembly.genome.fa.gz)

[ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode\\_mouse/release\\_M18/GRCm38.primary\\_assembly.genome.fa.gz](ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_mouse/release_M18/GRCm38.primary_assembly.genome.fa.gz)

### Value

No value is produced but index files are written to the current working directory.

### Author(s)

Wei Shi and Yang Liao

### References

Yang Liao, Gordon K Smyth and Wei Shi. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Research*, 41(10):e108, 2013.

## Examples

```
# Build an index for the artificial sequence included in file 'reference.fa'
library(Rsubread)
ref <- system.file("extdata","reference.fa",package="Rsubread")
buildindex(basename="./reference_index",reference=ref)
```

---

createAnnotationFile    *Create an annotation file from a GRanges object, suitable for featureCounts()*

---

## Description

Any of `rtracklayer::import.bed('samplesubjunc.bed')`, `unlist(spliceGraph(TxDb))`, `transcripts(TxDb)`, `exons(TxDb)`, or `features(FDB)` will produce a `GRanges` object containing usable features for read counting.

This function converts a suitably streamlined `GRanges` object into annotations which can then be used by `featureCounts()` to quickly count aligned reads.

The `GRanges` object must contain an `elementMetadata` column named 'id'.

## Usage

```
createAnnotationFile(GR)
write.Rsubread(GR)
```

## Arguments

GR                    The `GRanges` object to convert to an `Rsubread` annotation file

## Value

A data frame with five columns named `GeneID`, `Chr`, `Start`, `End` and `Strand`.

## Author(s)

Tim Triche, Jr. and Wei Shi

## Examples

```
## Not run:
library(TxDb.Hsapiens.UCSC.hg19.lincRNAsTranscripts)
hg19LincRNAs <- transcripts(TxDb.Hsapiens.UCSC.hg19.lincRNAsTranscripts)
names(values(hg19LincRNAs)) <- gsub('tx_id', 'id', names(values(hg19LincRNAs)))
annot_for_featureCounts <- createAnnotationFile(hg19LincRNAs)

## End(Not run)
```

---

detectionCall                      *Determine detection p values for each gene in an RNA-seq dataset*

---

**Description**

Use GC content adjusted background read counts to determine the detection p values for each gene

**Usage**

```
detectionCall(dataset, species="hg", plot=FALSE)
```

**Arguments**

dataset	a character string giving the filename of a SAM format file, which is the output of read alignment.
species	a character string specifying the species. Options are hg and mm.
plot	logical, indicating whether a density plot of detection p values will be generated.

**Value**

A data frame which includes detection p values and annotation information for each genes.

**Author(s)**

Zhiyin Dai and Wei Shi

---

detectionCallAnnotation                      *Generate annotation data used for calculating detection p values*

---

**Description**

This is for internal use only.

**Usage**

```
detectionCallAnnotation(species="hg", binsize=2000)
```

**Arguments**

species	character string specifying the species to analyse
binsize	binsize of intergenic region

**Details**

This is an internal function and should not be called by users directly.

It takes as input the annotation files produced by [processExons](#) function, calculates GC percentages for each exon of genes and also for intergenic regions and add GC info into the annotations. The new annotation data are then saved to files which can be used by [detectionCall](#) function for calling absolutely expressed genes.

**Value**

Two annotation files, which contain GC content for exons of genes and for intergenic regions respectively, are written to the current working directory. This function returns a NULL object.

**Author(s)**

Zhiyin Dai and Wei Shi

---

exactSNP

*exactSNP - an accurate and efficient SNP caller*

---

**Description**

Measure background noises and perform Fisher's Exact tests to detect SNPs.

**Usage**

```
exactSNP(readFile, isBAM=FALSE, refGenomeFile, SNPAnnotationFile=NULL,
outputFile=paste(readFile, ".exactSNP.VCF", sep=""), qvalueCutoff=12, minAllelicFraction=0,
minAllelicBases=1, minReads=1, maxReads=1000000, minBaseQuality=13, nTrimmedBases=3, nthreads=1)
```

**Arguments**

- |                    |   |
|--------------------|---|
| readFile           | a character string giving the name of a file including read mapping results. This function takes as input a SAM file by default. If a BAM file is provided, the isBAM argument should be set to TRUE.   |
| isBAM              | logical indicating if the file provided via readFile is a BAM file. FALSE by default.   |
| refGenomeFile      | a character string giving the name of a file that includes reference sequences (FASTA format).  |
| SNPAnnotationFile  | a character string giving the name of a VCF-format file that includes annotated SNPs (the file can be uncompressed or gzip compressed). Such annotation can be downloaded from public databases such as dbSNP. Incorporating known SNPs into SNP calling has been found to be helpful. However note that the annotated SNPs may or may not be called for the sample being analyzed. |
| outputFile         | a character string giving the name of the output file to be generated by this function. The output file includes all the reported SNPs (in VCF format). It includes discovered indels as well.  |
| qvalueCutoff       | a numeric value giving the q-value cutoff for SNP calling at sequencing depth of 50X. 12 by default. The q-value is calculated as $-\log_{10}(p)$ , where p is the p-value yielded from the Fisher's Exact test. Note that this function automatically adjusts the q-value cutoff for each chromosomal location according to its sequencing depth, based on this cutoff.            |
| minAllelicFraction | a numeric value giving the minimum fraction of allelic bases out of all read bases included at a chromosomal location required for SNP calling. Its value must be within 0 and 1. 0 by default.   |

minAllelicBases	an integer giving the minimum number of allelic (mis-matched) bases a SNP must have at a chromosomal location. 1 by default.
minReads	an integer giving the minimum number of mapped reads a SNP-containing location must have (ie. the minimum coverage). 1 by default.
maxReads	an integer specifying the maximum depth a SNP-containing location is allowed to have. 1000000 by default. Any location having number of mapped reads higher than this threshold will not be considered for SNP calling. This option is useful for removing PCR artefacts.
minBaseQuality	a numeric value giving the minimum base quality score (Phred score) read bases should satisfy before being used for SNP calling. 13 by default (corresponding to base calling p value of 0.05). Read bases with quality scores less than 13 will be excluded from analysis.
nTrimmedBases	a numeric value giving the number of bases trimmed off from each end of the read. 3 by default.
nthreads	a numeric value giving the number of threads/CPU's used. 1 by default.

### Details

This function takes as input a SAM/BAM format file, measures local background noise for each chromosomal location and then performs Fisher's exact tests to find statistically significant SNPs.

This function implements a novel algorithm for discovering SNPs. This algorithm is comparable with or better than existing SNP callers, but it is fast more efficient. It can be used to call SNPs for individual samples (ie. no control samples are required). Detail of the algorithm is described in a manuscript which is currently under preparation.

### Value

No value is produced but but a VCF format file is written to the current working directory. This file contains detailed information for discovered SNPs including chromosomal locations, reference bases, alternative bases, read coverages, allele frequencies and p values.

### Author(s)

Yang Liao and Wei Shi

---

featureCounts	<i>featureCounts: a general-purpose read summarization function</i>
---------------	---

---

### Description

This function assigns mapped sequencing reads to genomic features

### Usage

```
featureCounts(files,
# annotation
annot.inbuilt="mm10",
annot.ext=NULL,
```

```
isGTFAnnotationFile=FALSE,
GTF.featureType="exon",
GTF.attrType="gene_id",
GTF.attrType.extra=NULL,
chrAliases=NULL,

# level of summarization
useMetaFeatures=TRUE,

# overlap between reads and features
allowMultiOverlap=FALSE,
minOverlap=1,
fracOverlap=0,
fracOverlapFeature=0,
largestOverlap=FALSE,
nonOverlap=NULL,
nonOverlapFeature=NULL,

# Read extension and reduction
readExtension5=0,
readExtension3=0,
read2pos=NULL,

# multi-mapping reads
countMultiMappingReads=TRUE,

# fractional counting
fraction=FALSE,

# long reads
isLongRead=FALSE,

# read filtering
minMQS=0,
splitOnly=FALSE,
nonSplitOnly=FALSE,
primaryOnly=FALSE,
ignoreDup=FALSE,

# strandness
strandSpecific=0,

# exon-exon junctions
juncCounts=FALSE,
genome=NULL,

# parameters specific to paired end reads
isPairedEnd=FALSE,
requireBothEndsMapped=FALSE,
checkFragLength=FALSE,
minFragLength=50,
maxFragLength=600,
```

```

countChimericFragments=TRUE,
autosort=TRUE,

# number of CPU threads
nthreads=1,

# read group
byReadGroup=FALSE,

# report assignment result for each read
reportReads=NULL,
reportReadsPath=NULL,

# miscellaneous
maxMOp=10,
tmpDir=".",
verbose=FALSE)

```

### Arguments

<code>files</code>	a character vector giving names of input files containing read mapping results. The files can be in either SAM format or BAM format. The file format is automatically detected by the function.
<code>annot.inbuilt</code>	a character string specifying an in-built annotation used for read summarization. It has four possible values including "mm10", "mm9", "hg38" and "hg19", corresponding to the NCBI RefSeq annotations for genomes 'mm10', 'mm9', 'hg38' and 'hg19', respectively. "mm10" by default. The in-built annotation has a SAF format (see below).
<code>annot.ext</code>	A character string giving name of a user-provided annotation file or a data frame including user-provided annotation data. If the annotation is in GTF format, it can only be provided as a file. If it is in SAF format, it can be provided as a file or a data frame. See below for more details about SAF format annotation. If an annotation file is provided, it can be uncompressed or gzip compressed. Note that <code>annot.ext</code> will override <code>annot.inbuilt</code> if both provided.
<code>isGTFAnnotationFile</code>	logical indicating whether the annotation provided via the <code>annot.ext</code> argument is in GTF format or not. FALSE by default. This option is only applicable when <code>annot.ext</code> is not NULL.
<code>GTF.featureType</code>	a character string giving the feature type used to select rows in the GTF annotation which will be used for read summarization. "exon" by default. This argument is only applicable when <code>isGTFAnnotationFile</code> is TRUE. Feature types can be found in the third column of a GTF annotation.
<code>GTF.attrType</code>	a character string giving the attribute type in the GTF annotation which will be used to group features (eg. exons) into meta-features (eg. genes). "gene_id" by default. This argument is only applicable when <code>isGTFAnnotationFile</code> is TRUE. Attributes can be found in the ninth column of a GTF annotation.
<code>GTF.attrType.extra</code>	a character vector specifying extra GTF attribute types that will also be included in the counting output. These attribute types will not be used to group features. NULL by default.



chrAliases	a character string giving the name of a chromosome name alias file. This should be a two-column comma-delimited text file. Chromosome name aliases included in this file are used to match chr names in annotation with those in the reads. First column in the file should include chr names in the annotation and second column should include chr names in the reads. Chr names are case sensitive. No column header should be included in the file.
useMetaFeatures	logical indicating whether the read summarization should be performed at the feature level (eg. exons) or meta-feature level (eg. genes). If TRUE, features in the annotation (each row is a feature) will be grouped into meta-features, using their values in the GeneID column in the SAF-format annotation file or using the GTF.attrType attribute in the GTF-format annotation file, and then reads will be assigned to the meta-features instead of the features. See below for more details.
allowMultiOverlap	logical indicating if a read is allowed to be assigned to more than one feature (or meta-feature) if it is found to overlap with more than one feature (or meta-feature). FALSE by default.
minOverlap	integer giving the minimum number of overlapped bases required for assigning a read to a feature (or a meta-feature). For assignment of read pairs (fragments), number of overlapping bases from each read in the same pair will be summed. If a negative value is provided, then a gap of up to specified size will be allowed between read and the feature that the read is assigned to. 1 by default.
fracOverlap	numeric giving minimum fraction of overlapping bases in a read that is required for read assignment. Value should be within range [0,1]. 0 by default. Number of overlapping bases is counted from both reads if paired end. Both this option and minOverlap option need to be satisfied before a read can be assigned.
fracOverlapFeature	numeric giving minimum fraction of bases included in a feature that is required for overlapping with a read or a read pair. Value should be within range [0,1]. 0 by default.
largestOverlap	If TRUE, a read (or read pair) will be assigned to the feature (or meta-feature) that has the largest number of overlapping bases, if the read (or read pair) overlaps with multiple features (or meta-features).
nonOverlap	integer giving the maximum number of bases in a read (or a read pair) that are allowed not to overlap the assigned feature. NULL by default (ie. no limit is set).
nonOverlapFeature	integer giving the maximum number of non-overlapping bases allowed in a feature during read assignment. NULL by default (ie. no limit is set).
readExtension5	integer giving the number of bases extended upstream from 5' end of each read. 0 by default.
readExtension3	integer giving the number of bases extended downstream from 3' end of each read. 0 by default.
read2pos	Specifying whether each read should be reduced to its 5' most base or 3' most base. It has three possible values: NULL, 5 (denoting 5' most base) and 3 (denoting 3' most base). The default value is NULL. With the default value, the whole read is used for summarization. When read2pos is set to 5 (or 3), read summarization will be performed based on the 5' (or 3') most base position. If this option is used together with readExtension5 or readExtension3, a read will be extended first and then reduced to a single base.

countMultiMappingReads	logical indicating if multi-mapping reads/fragments should be counted, TRUE by default. 'NH' tag is used to located multi-mapping reads in the input BAM/SAM files.
fraction	logical indicating if fractional counts are produced for multi-mapping reads and/or multi-overlapping reads. FALSE by default. See below for more details.
isLongRead	logical indicating if input data contain long reads. This option should be set to TRUE if counting Nanopore or PacBio long reads.
minMQS	integer giving the minimum mapping quality score a read must satisfy in order to be counted. For paired-end reads, at least one end should satisfy this criteria. 0 by default.
splitOnly	logical indicating whether only split alignments (their CIGAR strings contain letter 'N') should be included for summarization. FALSE by default. Example split alignments are exon-spanning reads from RNA-seq data. useMetaFeatures should be set to FALSE and allowMultiOverlap should be set to TRUE, if the purpose of summarization is to assign exon-spanning reads to all their overlapping exons.
nonSplitOnly	logical indicating whether only non-split alignments (their CIGAR strings do not contain letter 'N') should be included for summarization. FALSE by default.
primaryOnly	logical indicating if only primary alignments should be counted. Primary and secondary alignments are identified using bit 0x100 in the Flag field of SAM/BAM files. If TRUE, all primary alignments in a dataset will be counted no matter they are from multi-mapping reads or not (ie. countMultiMappingReads is ignored).
ignoreDup	logical indicating whether reads marked as duplicates should be ignored. FALSE by default. Read duplicates are identified using bit 0x400 in the FLAG field in SAM/BAM files. The whole fragment (read pair) will be ignored if paired end.
strandSpecific	an integer vector indicating if strand-specific read counting should be performed. Length of the vector should be either 1 (meaning that the value is applied to all input files), or equal to the total number of input files provided. Each vector element should have one of the following three values: 0 (unstranded), 1 (stranded) and 2 (reversely stranded). Default value of this parameter is 0 (ie. unstranded read counting is performed for all input files).
juncCounts	logical indicating if number of reads supporting each exon-exon junction will be reported. Junctions are identified from those exon-spanning reads in input data. FALSE by default.
genome	a character string giving the name of a FASTA-format file that includes the reference sequences used in read mapping that produced the provided SAM/BAM files. NULL by default. This argument should only be used when juncCounts is TRUE. Note that providing reference sequences is optional when juncCounts is set to TRUE.
isPairedEnd	logical indicating if paired-end reads are used. If TRUE, fragments (templates or read pairs) will be counted instead of individual reads. FALSE by default.
requireBothEndsMapped	logical indicating if both ends from the same fragment are required to be successfully aligned before the fragment can be assigned to a feature or meta-feature. This parameter is only applicable when isPairedEnd is TRUE.
checkFragLength	logical indicating if the two ends from the same fragment are required to satisfy the fragment length criteria before the fragment can be assigned to a feature or

	meta-feature. This parameter is only applicable when <code>isPairedEnd</code> is TRUE. The fragment length criteria are specified via <code>minFragLength</code> and <code>maxFragLength</code> .
<code>minFragLength</code>	integer giving the minimum fragment length for paired-end reads. 50 by default.
<code>maxFragLength</code>	integer giving the maximum fragment length for paired-end reads. 600 by default. <code>minFragLength</code> and <code>maxFragLength</code> are only applicable when <code>isPairedEnd</code> is TRUE. Note that when a fragment spans two or more exons, the observed fragment length might be much bigger than the nominal fragment length.
<code>countChimericFragments</code>	logical indicating whether a chimeric fragment, which has its two reads mapped to different chromosomes, should be counted or not. TRUE by default.
<code>autosort</code>	logical specifying if the automatic read sorting is enabled. This option is only applicable for paired-end reads. If TRUE, reads will be automatically sorted by their names if reads from the same pair are found not to be located next to each other in the input. No read sorting will be performed if there are no such reads found.
<code>nthreads</code>	integer giving the number of threads used for running this function. 1 by default.
<code>byReadGroup</code>	logical indicating if read counting will be performed for each individual read group. FALSE by default.
<code>reportReads</code>	output detailed read assignment results for each read (or fragment if paired end). The detailed assignment results can be saved in three different formats including CORE, SAM and BAM (note that these values are case sensitive). Default value of this option is NULL, indicating not to report assignment results for reads. CORE format represents a tab-delimited text file that contains four columns including read name, status (assigned or the reason if not assigned), number of targets and target list. A target is a feature or a meta-feature. Items in the target lists is separated by comma. If a read is not assigned, its number of targets will be set as -1. For each input file, a text file is generated and its name is the input file name added with <code>‘.featureCounts’</code> . When SAM or BAM format is specified, the detailed assignment results will be saved to SAM and BAM format files. Names of generated files are the input file names added with <code>‘.featureCounts.sam’</code> or <code>‘.featureCounts.bam’</code> . Three tags are used to describe read assignment results: XS, XN and XT. Tag XS gives the assignment status. Tag XN gives number of targets. Tag XT gives comma separated target list.
<code>reportReadsPath</code>	a character string specifying the directory where files including detailed assignment results are saved. If NULL, the results will be saved to the current working directory.
<code>maxMOp</code>	integer giving the maximum number of ‘M’ operations (matches or mis-matches) allowed in a CIGAR string. 10 by default. Both ‘X’ and ‘=’ operations are treated as ‘M’ and adjacent ‘M’ operations are merged in the CIGAR string.
<code>tmpDir</code>	a character string specifying the directory under which intermediate files are saved (later removed). By default, current working directory is used.
<code>verbose</code>	logical indicating if verbose information for debugging will be generated. This may include information such as unmatched chromosomes/contigs between reads and annotation.

## Details

featureCounts is a general-purpose read summarization function, which assigns to the genomic features (or meta-features) the mapped reads that were generated from genomic DNA and RNA sequencing.

This function takes as input a set of files containing read mapping results output from a read aligner (e.g. `align` or `subjunc`), and then assigns mapped reads to genomic features. Both SAM and BAM format input files are accepted.

featureCounts accepts two annotation formats: SAF (Simplified Annotation Format) and GTF/GFF formats. Specification of GTF/GFF format can be found at <https://genome.ucsc.edu/FAQ/FAQformat.html>. SAF is a simplified annotation format and below shows an example of this format:

```
GeneID Chr Start End Strand
497097 chr1 3204563 3207049 -
497097 chr1 3411783 3411982 -
497097 chr1 3660633 3661579 -
100503874 chr1 3637390 3640590 -
100503874 chr1 3648928 3648985 -
100038431 chr1 3670236 3671869 -
...
```

SAF annotation includes the following five required columns: GeneID, Chr, Start, End and Strand. The GeneID column includes identifiers of features. These identifiers can be integer or character string. The Chr column includes chromosome names of features and these names should match the chromosome names included in the provided SAM/BAM files. The Start and End columns include start and end coordinates of features, respectively. Both start and end coordinates are inclusive. The Strand column indicates the strand of features ("+" or "-"). Column names in a SAF annotation should be the same as those shown in the above example (case insensitive). Columns can be in any order. Extra columns are allowed to be added into the annotation.

In-built annotations, which were generated based on NCBI RefSeq gene annotations, are provided to facilitate convenient read summarization. We provide in-built annotations for the following genomes: "hg38", "hg19", "mm10" and "mm9". The content of in-built annotations can be accessed via the `getInBuiltAnnotation` function. These annotations have a SAF format.

The in-built annotations are a modified version of NCBI RefSeq gene annotations. We downloaded the RefSeq gene annotations from NCBI ftp server (eg. RefSeq annotation for mm10 was downloaded from [ftp://ftp.ncbi.nlm.nih.gov/genomes/M\\_musculus/ARCHIVE/BUILD.38.1/mapview/seq\\_gene.md.gz](ftp://ftp.ncbi.nlm.nih.gov/genomes/M_musculus/ARCHIVE/BUILD.38.1/mapview/seq_gene.md.gz)). We then used these annotations to create our in-built annotations. For each gene, we used its CDS (coding DNA sequence) and UTR (untranslated) regions provided in the original annotation to construct a list of exons, by merging and concatenating all CDSs and UTRs belonging to the same gene. Exons within each gene include all chromosomal bases included in the original CDS and UTR regions, but they include each base only once (they might be included multiple times in the original CDSs and UTRs). Also, exons within the same gene do not overlap with each other.

Users may provide an external annotation for read summarization via the `annot.ext` argument. If the external annotation is in SAF format, it can be provided as either a `data.frame` or a tab-delimited text file with proper column names included. If it is in GTF/GFF format, it should be provided as a file only (and `isGTFAnnotationFile` should be set to TRUE).

featureCounts function uses the `GTF.attrType` attribute in a GTF/GFF annotation to group features to form meta-features when performing read summarization at meta-feature level.

The argument `useMetaFeatures` specifies whether read summarization should be performed at feature level or at meta-feature level. A feature represents a continuous genomic region and a

meta-feature is a group of features. For instance, an exon is a feature and a gene comprising one or more exons is a meta-feature. To assign reads to meta-features, featureCounts firstly groups into meta-features the features that have the same gene identifiers. featureCounts looks for gene identifiers in GeneID column of a SAF annotation or by using GTF .attrType attribute in a GTF/GFF annotation. Then for each read featureCounts searches for meta-features that have at least one feature that overlaps with the read. A read might be found to overlap with more than one feature within the same meta-feature (eg. an exon-spanning read overlaps with more than one exon from the same gene), however this read will still be counted only once for the meta-feature.

RNA-seq reads are often summarized to meta-features to produce read counts for genes. Further downstream analysis can then be carried out to discover differentially expressed genes. Feature-level summarization of RNA-seq data often yields exon-level read counts, which is useful for investigating alternative splicing of genes.

featureCounts provides multiple options to count multi-mapping reads (reads mapping to more than one location in the reference genome). Users can choose to ignore such reads by setting countMultiMappingReads to FALSE, or fully count every alignment reported for a multi-mapping read by setting countMultiMappingReads to TRUE (each alignment carries 1 count), or count each alignment fractionally by setting both countMultiMappingReads and fraction to TRUE (each alignment carries  $1/x$  count where  $x$  is the total number of alignments reported for the read).

featureCounts also provides multiple options to count multi-overlapping reads (reads overlapping with more than one meta-feature when conducting meta-feature-level summarization or overlapping with more than one feature when conducting feature-level summarization). Users can choose to ignore such reads by setting allowMultiOverlap to FALSE, or fully count them for each overlapping meta-feature/feature by setting allowMultiOverlap to TRUE (each overlapping meta-feature/feature receives a count of 1 from a read), or assign a fractional count to each overlapping meta-feature/feature by setting both allowMultiOverlap and fraction to TRUE (each overlapping meta-feature/feature receives a count of  $1/y$  from a read where  $y$  is the total number of meta-features/features overlapping with the read).

If a read is both multi-mapping and multi-overlapping, then each overlapping meta-feature/feature will receive a fractional count of  $1/(x*y)$  if countMultiMappingReads, allowMultiOverlap and fraction are all set to TRUE.

When isPairedEnd is TRUE, fragments (pairs of reads) instead of reads will be counted. featureCounts function checks if reads from the same pair are adjacent to each other (this could happen when reads were for example sorted by their mapping locations), and it automatically reorders those reads that belong to the same pair but are not adjacent to each other in the input read file.

## Value

A list with the following components:

counts	a data matrix containing read counts for each feature or meta-feature for each library.
counts_junction (optional)	a data frame including primary and secondary genes overlapping an exon junction, position information for the left splice site ('Site1') and the right splice site ('Site2') of a junction (including chromosome name, coordinate and strand) and number of supporting reads for each junction in each library. Both primary and secondary genes overlap at least one of the two splice sites of a junction. Secondary genes do not overlap more splice sites than the primary gene. When the primary and secondary genes overlap same number of splice sites, the gene with the smallest leftmost base position is selected as the primary gene. For each junction, no more than one primary gene is reported but there might be more

	than one secondary genes reported. If a junction does not overlap any genes, it has a missing value in the fields of primary gene and secondary gene. Note that this data frame is only generated when <code>juncCounts</code> is TRUE.
<code>annotation</code>	a data frame with six columns including GeneID, Chr, Start, End and Length. When read summarization was performed at feature level, each row in the data frame is a feature and columns in the data frame give the annotation information for the features. When read summarization was performed at meta-feature level, each row in the data frame is a meta-feature and columns in the data frame give the annotation information for the features included in each meta feature except the Length column. For each meta-feature, the Length column gives the total length of genomic regions covered by features included in that meta-feature. Note that this length will be less than the sum of lengths of features included in the meta-feature when there are features overlapping with each other. Also note the GeneID column gives Entrez gene identifiers when the in-built annotations are used.
<code>targets</code>	a character vector giving sample information.
<code>stat</code>	a data frame giving numbers of unassigned reads and the reasons why they are not assigned (eg. ambiguity, multi-mapping, secondary alignment, mapping quality, fragment length, chimera, read duplicate, non-junction and so on), in addition to the number of successfully assigned reads for each library.

**Author(s)**

Wei Shi and Yang Liao

**References**

Yang Liao, Gordon K Smyth and Wei Shi. `featureCounts`: an efficient general-purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7):923-30, 2014.

**See Also**

[getInBuiltAnnotation](#)

**Examples**

```
## Not run:
library(Rsubread)

# Summarize SAM format single-end reads using built-in RefSeq annotation for mouse genome mm10:
featureCounts(files="mapping_results_SE.sam",annot.inbuilt="mm10")

# Summarize single-end reads using a user-provided GTF annotation file:
featureCounts(files="mapping_results_SE.sam",annot.ext="annotation.gtf",
isGTFAnnotationFile=TRUE,GTF.featureType="exon",GTF.attrType="gene_id")

# Summarize single-end reads using 5 threads:
featureCounts(files="mapping_results_SE.sam",nthreads=5)

# Summarize BAM format single-end read data:
featureCounts(files="mapping_results_SE.bam")

# Perform strand-specific read counting (strandSpecific=2 if reversely stranded):
featureCounts(files="mapping_results_SE.bam",strandSpecific=1)
```

```
# Summarize paired-end reads and counting fragments (instead of reads):
featureCounts(files="mapping_results_PE.bam",isPairedEnd=TRUE)

# Count fragments satisfying the fragment length criteria, eg. [50bp, 600bp]:
featureCounts(files="mapping_results_PE.bam",isPairedEnd=TRUE,
checkFragLength=TRUE,minFragLength=50,maxFragLength=600)

# Count fragments that have both ends successfully aligned without checking the fragment length:
featureCounts(files="mapping_results_PE.bam",isPairedEnd=TRUE,requireBothEndsMapped=TRUE)

# Exclude chimeric fragments from fragment counting:
featureCounts(files="mapping_results_PE.bam",isPairedEnd=TRUE,countChimericFragments=FALSE)

## End(Not run)
```

---

findCommonVariants      *Finding the common variants among all input VCF files*

---

## Description

The common variants (inc. SNPs and indels) among all the input files are found. A data frame containing these common variants is returned. The data frame has a similar format as VCF files.

## Usage

```
findCommonVariants(VCF_files)
```

## Arguments

VCF\_files      a character vector giving the names of VCF format files.

## Details

This function loads all variants (SNPs and indels) from the input VCF files, and find the common variants that are reported in all the VCF files. If a variant record in a input VCF file has multiple alternative sequences (split by ‘;’), each alternative sequence is treated as a single variant. Two variants in two VCF files are the same only if their genomic locations, their reference sequences, their alternative sequences and their variant types are identical.

This function currently does not support other types of variants other than SNPs and indels.

There are eight columns in the returned data frame: chromosome name, position, identity, reference sequence, alternative sequence, quality, filter and extra information. The input may have more columns; these columns are not included in the data frame. If the identity, the quality, the filter and the extra information for the same variant are different among the input VCF files, those information associated with the lowest quality value of this variant among the VCF files is reported in the resulted data frame. For example, if an SNP on chrX:12345 (A=>G) is reported in all the three input VCF files, and the quality scores in the three VCF files are 100, 10, 50 respectively, the identity, the quality, the filter and the extra information in the second VCF file are reported in the resulted data frame for this SNP.

**Value**

A data frame containing the common variants among all the input VCF files is returned. The first eight columns are: chromosome name, position, identity, reference sequence, alternative sequence, quality, filter and extra information.

If there are not any common variants, this function returns an NA value.

**Author(s)**

Yang Liao and Wei Shi

**Examples**

```
## Not run:
# finding the common variants between to input VCF files: a.vcf and b.vcf
library(Rsubread)
findCommonVariants(c('a.vcf', 'b.vcf'))

## End(Not run)
```

---

 flattenGTF

*Flatten features provided in a GTF annotation*


---

**Description**

Overlapping features in a GTF annotation, such as overlapping exons from the same gene, are collapsed into a single feature that covers all overlapping features.

**Usage**

```
flattenGTF(GTFfile, GTF.featureType = "exon", GTF.attrType = "gene_id")
```

**Arguments**

GTFfile	a character string giving the name of a GTF file as input.
GTF.featureType	a character string giving the feature type used to select rows in a GTF annotation. "exon" by default. Feature types can be found in the third column of a GTF annotation.
GTF.attrType	a character string giving the attribute type in a GTF annotation which will be used to group features. "gene_id" by default. Attributes can be found in the ninth column of a GTF annotation.

**Details**

This function locates features in a GTF annotation via `GTF.featureType` and then groups them into meta-features via `GTF.attrType`. Default values of these two parameters will make exons be identified and then grouped into genes.

If there is overlap found between features belonging to the same meta-feature, this function will merge all overlapping features to form a single larger feature that covers all overlapping features. Output of this function is a SAF format annotation which can be fed to [featureCounts](#) function for read counting. Description to SAF format annotation can also be found in [featureCounts](#).



**Value**

A data.frame including a SAF format annotation.

**Author(s)**

Yang Liao and Wei Shi

**See Also**

[featureCounts](#)

---

getInBuiltAnnotation *Retrieve in-built annotations provided by featureCounts function*

---

**Description**

Retrieve an in-built annotation and save it to a data frame

**Usage**

```
getInBuiltAnnotation(annotation="mm10")
```

**Arguments**

annotation a character string specifying the in-built annotation to be retrieved. It has four possible values including mm10, mm9, hg38 and hg19, corresponding to the NCBI RefSeq annotations for genomes 'mm10', 'mm9', 'hg38' and 'hg19', respectively. mm10 by default.

**Details**

The [featureCounts](#) read summarization function provides in-built annotations for conveniently summarizing reads to genes or exons, and this function allows users to have access to those in-built annotations.

For more information about these annotations, please refer to the help page for [featureCounts](#) function.

**Value**

A data frame with five columns including GeneID, Chr, Start, End and Strand.

**Author(s)**

Wei Shi

**See Also**

[featureCounts](#)

## Examples

```
library(Rsubread)
x <- getInBuiltAnnotation("hg38")
x[1:5,]
```

---

processExons	<i>Obtain chromosomal coordinates of each exon using NCBI annotation</i>
--------------	--

---

## Description

This is for internal use.

## Usage

```
processExons(filename="human_seq_gene.md", species="hg")
```

## Arguments

filename	a character string giving the name of input .md file (NCBI annotation file)
species	a character string specifying the species

## Details

This is an internal function and should not be called by users directly.

It processes the NCBI mapview annotation data downloaded from the following links: (these annotations include chromosomal coordinates of UTR and CDS regions of genes).

[ftp://ftp.ncbi.nlm.nih.gov/genomes/H\\_sapiens/mapview/seq\\_gene.md.gz](ftp://ftp.ncbi.nlm.nih.gov/genomes/H_sapiens/mapview/seq_gene.md.gz)

[ftp://ftp.ncbi.nlm.nih.gov/genomes/M\\_musculus/mapview/seq\\_gene.md.gz](ftp://ftp.ncbi.nlm.nih.gov/genomes/M_musculus/mapview/seq_gene.md.gz)

This function finds the chromosomal coordinates of intergenic regions (regions between neighbouring genes) and then outputs them to a file. It also outputs to a file chromosomal coordinates of exons of genes by concatenating UTRs with CDSs and merging overlapping CDSs within each gene. The generated annotation files will then be used by [detectionCallAnnotation](#) function to produce annotation data required by [detectionCall](#) function.

## Value

Two annotation files are written to the current working directory. This function returns a NULL object.

## Author(s)

Zhiyin Dai and Wei Shi

---

promoterRegions	<i>Generate annotation for promoter region of genes</i>
-----------------	---

---

### Description

Create a SAF data.frame of genewise promoter regions

### Usage

```
promoterRegions(annotation="mm10", upstream=3000L, downstream=2000L)
```

### Arguments

annotation	a character string giving the name of a genome with built-in annotation or a SAF format gene annotation. If using built-in annotation, the character string should be one of the following: mm10, mm9, hg38 or hg19. They correspond to the NCBI RefSeq annotations for genomes 'mm10', 'mm9', 'hg38' and 'hg19', respectively. mm10 by default.
upstream	an integer giving the number of upstream bases that will be included in the promoter region generated for each gene. These bases are taken immediately upstream (5' end) from transcriptional start site of each gene.
downstream	an integer giving the number of downstream bases that will be included in the promoter region generated for each gene. These bases are taken immediately downstream (3' end) from transcriptional start site of each gene.

### Details

This function takes as input a SAF format gene annotation and produces a SAF format annotation that contains chromosomal coordinates of promoter region of each gene. See [featureCounts](#) for definition of the SAF format.

Built-in annotations can be accessed via function [getInBuiltAnnotation](#).

### Value

A SAF format data.frame with columns GeneID, Chr, Start, End and Strand.

### Author(s)

Gordon K Smyth

### See Also

[featureCounts](#), [getInBuiltAnnotation](#)

### Examples

```
library(Rsubread)
x <- promoterRegions()
head(x)
```

---

propmapped	<i>Calculate the proportion of mapped reads/fragments in SAM/BAM files</i>
------------	--

---

### Description

Number of mapped reads/fragments will be counted and fraction of such reads/fragments will be calculated.

### Usage

```
propmapped(files, countFragments=TRUE, properlyPaired=FALSE, verbose=FALSE)
```

### Arguments

files	a character vector giving the names of SAM/BAM format files. Format of input files is automatically determined by the function.
countFragments	logical, indicating whether reads or fragments (read pairs) should be counted. If TRUE, fragments will be counted when paired-end read data are provided. This function automatically detects if the data are single end or paired end. For single end data, each read is treated as a fragment and therefore the value of this parameter should be set to TRUE.
properlyPaired	logical, indicating if only properly paired reads will be counted. This is only applicable for paired end data. FALSE by default.
verbose	display verbose information.

### Details

This function uses the FLAG field in the SAM/BAM to look for mapped reads and count them. Reads/fragments, which have more than one reported location, will be reported only once.

When counting single end reads, counting reads has the same meaning with counting fragments (the results are identical).

### Value

A data frame containing the total number of reads, number of mapped reads and proportion of mapped reads for each library.

### Author(s)

Wei Shi and Yang Liao

### Examples

```
# build an index using the sample reference sequence provided in the package
# and save it to the current directory
library(Rsubread)
ref <- system.file("extdata", "reference.fa", package="Rsubread")
buildindex(basename="./reference_index", reference=ref)

# align the sample read data provided in this packge to the sample reference
```

```
# and save the mapping results to the current directory
reads <- system.file("extdata","reads.txt.gz",package="Rsubread")
align(index="./reference_index",readfile1=reads,output_file="./Rsubread_alignment.BAM")

# get the percentage of successfully mapped reads
propmapped("./Rsubread_alignment.BAM")
```

---

qualityScores	<i>Extract quality score data in a sequencing read dataset</i>
---------------	--

---

## Description

Extract quality strings and convert them to Phred scores

## Usage

```
qualityScores(filename, input_format="gzFASTQ", offset=33, nreads=10000)
```

## Arguments

filename	character string giving the name of an input file containing sequence reads.
input_format	character string specifying format of the input file. gzFASTQ (gzipped FASTQ) by default. Acceptable formats include gzFASTQ, FASTQ, SAM and BAM. Character string is case insensitive.
offset	numeric value giving the offset added to the base-calling Phred scores. Possible values include 33 and 64. By default, 33 is used.
nreads	numeric value giving the number of reads from which quality scores are extracted. 10000 by default.

## Details

Quality scores of read bases are represented by ASCII characters in next-gen sequencing data. This function extracts the quality characters from each base in each read and then converts them to Phred scores using the provided offset value (`offset`).

If the total number of reads in a dataset is  $n$ , then every  $n/nreads$  read is extracted from the input data.

## Value

A data matrix containing Phred scores for read bases. Rows in the matrix are reads and columns are base positions in each read.

## Author(s)

Wei Shi, Yang Liao and Zhiyin Dai

## Examples

```
library(Rsubread)
reads <- system.file("extdata","reads.txt.gz",package="Rsubread")
x <- qualityScores(filename=reads,offset=64,nreads=1000)
x[1:10,1:10]
```

---

removeDupReads	<i>Remove sequencing reads which are mapped to identical locations</i>
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---

### Description

Remove reads which are mapped to identical locations, using mapping location of the first base of each read.

### Usage

```
removeDupReads(SAMfile, threshold=50, outputFile)
```

### Arguments

SAMfile	a character string giving the name of a SAM format input file.
threshold	a numeric value giving the threshold for removing duplicated reads, 50 by default. Reads will be removed if they are found to be duplicated equal to or more than threshold times.
outputFile	a character string giving the base name of output files.

### Details

This function uses the mapping location of first base of each read to find duplicated reads. Reads are removed if they are duplicated more than threshold number of times.

### Value

A SAM file including the remaining reads after duplicate removal.

### Author(s)

Yang Liao and Wei Shi

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repair	<i>Re-order paired-end reads to place reads from the same pair next to each other</i>
--------	---

---

### Description

Fast re-ordering of paired-end reads using read names and mapping locations.

### Usage

```
repair(inFiles, inFormat="BAM", outFiles=paste(inFiles, "repair", sep="."),
addDummy=TRUE, fullData=TRUE, compress=FALSE, nthreads=8)
```

**Arguments**

<code>inFiles</code>	a character vector giving names of input files. These files are typically location-sorted BAM files.
<code>inFormat</code>	a character string specifying format of input files. Supported formats include BAM and SAM.
<code>outFiles</code>	a character string giving names of output files. Re-ordered reads are saved to BAM-format files.
<code>addDummy</code>	logical indicating if a dummy read will be added to each singleton read which has a missing pair in the input. TRUE by default.
<code>fullData</code>	logical indicating if sequences and base-calling quality scores of reads will be included in the output. TRUE by default.
<code>compress</code>	logical indicating if compression should be turned on when generating BAM output. FALSE by default.
<code>nthreads</code>	a numeric value giving number of CPU threads. 8 by default.

**Details**

This function takes as input paired-end BAM or SAM files, re-orders reads to make reads from the same pair be adjacent to each other and then outputs the re-ordered reads into BAM files.

The function makes use of both read names and mapping information of reads (eg. mapping coordinates) to identify reads belonging to the same pair. This makes sure that all paired-end reads are correctly re-ordered, especially those multi-mapping read pairs that include more than one reported alignment in the input.

The BAM files produced by this function are compatible with [featureCounts](#), meaning that no read re-ordering will be performed when providing these files to [featureCounts](#).

**Value**

No value is produced but BAM files with re-ordered reads are written to the current working directory.

**Author(s)**

Wei Shi and Yang Liao

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RsubreadUsersGuide      *View Rsubread Users Guide*

---

**Description**

Users Guide for Rsubread and Subread

**Usage**

```
RsubreadUsersGuide()
```

**Details**

The Subread/Rsubread Users Guide provides detailed description to the functions and programs included in the Subread and Rsubread software packages. It also includes case studies for analyzing next-gen sequencing data.

The Subread package is written in C and it can be downloaded from <http://subread.sourceforge.net>. The Rsubread package provides R wrappers functions for many of the programs included in Subread package.

**Value**

Character string giving the file location.

**Author(s)**

Wei Shi

**See Also**

[vignette](#)

---

sam2bed

*Convert a SAM format file to a BED format file*

---

**Description**

SAM to BED conversion

**Usage**

```
sam2bed(samfile,bedfile,readlen)
```

**Arguments**

samfile	character string giving the name of input file. Input format should be in SAM format.
bedfile	character string giving the name of output file. Output file is in BED format.
readlen	numeric value giving the length of reads included in the input file.

**Details**

This function converts a SAM format file to a BED format file, which can then be displayed in a genome browser like UCSC genome browser, IGB, IGV.

**Value**

No value is produced but a BED format file is written to the current working directory. This file contains six columns including chromosomal name, start position, end position, name('.'), mapping quality score and strandness.

**Author(s)**

Wei Shi



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sublong	<i>Align long sequence reads to a reference genome via seed-and-vote</i>
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---

### Description

This function aligns DNA-seq reads, generated by long-read sequencing technologies such as Nanopore and PacBio sequencers, to a reference genome.

### Usage

```
sublong(  
  index,  
  readFiles,  
  outputFiles,  
  outputFormat="BAM",  
  nthreads=1  
)
```

### Arguments

index	a character vector giving the basename of index files. Index files should be located in the current directory. The provided index should be a full index and also it should have only one block. See <code>buildindex</code> for index building options.
readFiles	a character vector giving the names of input files that contain long sequence reads. FASTQ and gzipped FASTQ formats are both accepted.
outputFiles	a character vector specifying the names of output files that contain read mapping results.
outputFormat	a character string specifying the format of output files. BAM by default. Acceptable formats include SAM and BAM.
nthreads	an integer giving the number of threads used for mapping. 1 by default. Note that when more than one thread is used, the order of reads might be changed in the output.

### Details

sublong is designed for the mapping of long reads. It performs full alignment of reads by performing seed-and-vote mapping followed by a bounded dynamic programming procedure. sublong is able to map reads as long as millions of bases.

sublong is extremely fast. It takes less than 10 minutes to complete the mapping of more than 100,000 long reads generated from Nanopore MinION ultra-long sequencing protocol.

The number of CIGAR operations (eg. insertion and deletion) reported for a long read may exceed the limit on the total number of operations allowed in a CIGAR string (up to 65,535 operations in a CIGAR string in BAM output and up to 99,900 operations in a CIGAR string in SAM output). If this limited is exceeded, the read will be soft clipped.

### Author(s)

Yang Liao and Wei Shi

**Examples**

```
library(Rsubread)
ref <- system.file("extdata", "reference.fa", package="Rsubread")
buildindex(basename="./full_index", reference=ref, gappedIndex=FALSE, indexSplit=FALSE)
reads <- system.file("extdata", "longreads.txt.gz", package="Rsubread")
sublong("./full_index", reads, "./Long_alignment.BAM", nthreads=4)
```

txUnique

*Count number of bases unique to each transcript***Description**

For each transcript, number of unique bases and total number of bases will be reported.

**Usage**

```
txUnique(GTF_Annotation_File, Feature_Type = "exon", Gene_ID_Attribute = "gene_id", Transcript_ID
```

**Arguments**

GTF\_Annotation\_File

character string giving the name of a GTF file containing the transcript-level annotations.

Feature\_Type

character string specifying the type of annotations. Only the annotations of the given type will be loaded from the GTF file. exon by default. The third column in the GTF file contains the type of the annotation in each line

Gene\_ID\_Attribute

character string specifying the attribute name in the 9-th column in the GTF file for the gene identifier. gene\_id by default.

Transcript\_ID\_Attribute

character string specifying the attribute name in the 9-th column in the GTF file for the transcript identifier. transcript\_id by default.

**Details**

This function compares the transcripts belonging to the same gene and then counts the number of bases unique to each transcript. It also reports the total number of bases for each transcript. When a transcript is found to contain overlapping exons, the overlapping exons will be merged and each overlapping base will be counted only once.

**Value**

A data matrix in which each row represents a transcript. The matrix includes four columns: gene identifier, transcript identifier, number of bases unique to each transcript and total number of bases each transcript has.

**Author(s)**

Yang Liao and Wei Shi

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