

# Package ‘bambu’

June 1, 2020

**Type** Package

**Title** Reference-guided isoform reconstruction and quantification for long read RNA-Seq data

**Version** 0.1.0

**Description** Multi-sample transcript discovery and quantification using long read RNA-Seq data.

**License** GPL-3

**Encoding** UTF-8

**LazyData** true

**Depends** R(>= 3.5.0),  
data.table(>= 1.1.8),  
dplyr,  
SummarizedExperiment(>= 1.1.6),  
GenomicRanges,  
BiocManager,  
GenomicFeatures,  
ggplot2

**Suggests** knitr,  
rmarkdown,  
fs,  
testthat,  
ComplexHeatmap,  
circlize,  
ggbio,  
RColorBrewer,  
gridExtra

**Enhances** parallel

## SystemRequirements

**biocViews** FeatureExtraction,  
GeneExpression,  
GeneExpressionWorkflow,  
GenomeAnnotation,  
ImmunoOncology,  
Normalization,

RNASeq,  
Regression,  
Sequencing,  
Software,  
Transcription,  
Transcriptomics

**bugReports** <https://github.com/GoekeLab/bambu/issues>

**URL** <https://github.com/GoekeLab/bambu>

**RoxygenNote** 7.1.0

**LinkingTo** Rcpp,  
RcppArmadillo,  
RcppProgress

**Imports** S4Vectors(>= 0.22.1),  
IRanges,  
GenomicAlignments,  
glmnet,  
Rsamtools,  
Rcpp,  
RcppArmadillo,  
RcppProgress,  
progress

**VignetteBuilder** knitr,  
rmarkdown

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bambu	<i>long read isoform reconstruction and quantification</i>
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Description

This function takes bam file of genomic alignments and performs isoform recontruction and gene and transcript expression quantification. It also allows saving of read class files of alignments, extending provided annotations, and quantification based on extended annotations. When multiple samples are provided, extended annotations will be combined across samples to allow comparison.

**Usage**

```

bambu(
  reads = NULL,
  readClass.file = NULL,
  readClass.outputDir = NULL,
  annotations = NULL,
  genomeSequence = NULL,
  stranded = FALSE,
  ncore = 1,
  yieldSize = NULL,
  isoreParameters = NULL,
  emParameters = NULL,
  extendAnnotations = TRUE,
  verbose = FALSE
)

```

**Arguments**

**reads** A string or a vector of strings specifying the paths of bam files for genomic alignments, or a [BamFile](#) object or a [BamFileList](#) object (see [Rsamtools](#)).

**readClass.file** A string or a vector of strings specifying the read class files that are saved during previous run of [bambu](#).

**readClass.outputDir** A string variable specifying the path to where read class files will be saved.

**annotations** A [TxDb](#) object or A [GRangesList](#) object obtained by [prepareAnnotations](#) or [prepareAnnotationsFromGTF](#).

**genomeSequence** A fasta file or a [BSGenome](#) object.

**ncore** specifying number of cores used when parallel processing is used, defaults to 1.

**yieldSize** see [Rsamtools](#).

**isoreParameters**

A list of controlling parameters for isoform reconstruction process:

- **prefix** specifying prefix for new gene Ids (`genePrefix.number`), defaults to empty
- **remove.subsetTx** indicating whether filter to remove read classes which are a subset of known transcripts(), defaults to TRUE
- **min.readCount** specifying minimum read count to consider a read class valid in a sample, defaults to 2
- **min.readFractionByGene** specifying minimum relative read count per gene, highly expressed genes will have many high read count low relative abundance transcripts that can be filtered, defaults to 0.05
- **min.sampleNumber** specifying minimum sample number with minimum read count, defaults to 1
- **min.exonDistance** specifying minum distance to known transcript to be considered valid as new, defaults to 35
- **min.exonOverlap** specifying minimum number of bases shared with annotation to be assigned to the same gene id, defaults 10 base pairs

**emParameters** A list of controlling parameters for quantification algorithm estimation process:

- **maxiter** specifying maximum number of run iterations, defaults to 10000.
- **bias** specifying whether to correct for bias, defaults to FALSE.
- **conv** specifying the coverage threshold control, defaults to 0.0001.

**extendAnnotations** A logical variable indicating whether annotations are to be extended for quantification.

**verbose** A logical variable indicating whether processing messages will be printed.

## Details

Main function

## Value

A list of two SummarizedExperiment object for transcript expression and gene expression.

## Examples

```
## Not run:
## =====
## More stringent new gene/isoform discovery: new isoforms are identified with at least 5 read count in 1 sample
## Increase EM convergence threshold to 10-6
seOutput <- bambu(reads, annotationGrangesList,
  genomeSequence, isoreParameters = list(min.readCount=5),
  emParameters = list(conv = 10-6))

## End(Not run)
```

---

plot.bambu

*plot.bambu*

---

## Description

plotSEOutput

## Usage

```
## S3 method for class 'bambu'
plot(
  se,
  group.variable = NULL,
  type = c("annotation", "pca", "heatmap"),
  gene_id = NULL,
  transcript_id = NULL
)
```

**Arguments**

se	An summarized experiment object obtained from <a href="#">bambu</a> or <a href="#">transcriptToGene</a> .
group.variable	Variable for grouping in plot, has be to provided if choosing to plot PCA.
type	plot type variable, a values of annotation for a single gene with heatmap for isoform expressions, pca, or heatmap, see <a href="#">details</a> .
gene_id	specifying the gene_id for plotting gene annotation, either gene_id or transcript_id has to be provided when type = "annotation".
transcript_id	specifying the transcript_id for plotting transcript annotation, either gene_id or transcript_id has to be provided when type = "annotation"

**Details**

[type](#) indicates the type of plots to be plotted. There are two types of plots can be chosen, PCA or heatmap.

**Value**

A heatmap plot for all samples

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prepareAnnotations	<i>prepare annotations from txdb object</i>
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**Description**

Function to prepare tables and genomic ranges for transcript reconstruction using a txdb object

**Usage**

```
prepareAnnotations(txdb)
```

**Arguments**

txdb	a <a href="#">TxDb</a> object
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**Value**

A [GrangesList](#) object

**Examples**

```
## Not run:
library(TxDb.Hsapiens.UCSC.hg38.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg38.knownGene
prepareAnnotations(txdb)

## End(Not run)
```

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prepareAnnotationsFromGTF

*Prepare annotation granges object from GTF file into a GRangesList object*

---

### Description

Prepare annotation granges object from GTF file

### Usage

```
prepareAnnotationsFromGTF(file)
```

### Arguments

file                    a GTF file

---

readFromGTF

*convert a GTF file into a GRangesList*

---

### Description

Outputs GRangesList object from reading a GTF file

### Usage

```
readFromGTF(file)
```

### Arguments

file                    a `.gtf` file

### Value

grlist a [GRangesList](#) object, with two columns

- TXNAME specifying prefix for new gene Ids (genePrefix.number), defaults to empty
- GENEID indicating whether filter to remove read classes which are a subset of known transcripts(), defaults to TRUE

---

transcriptToGeneExpression	
	<i>transcript to gene expression</i>

---

**Description**

Reduce transcript expression to gene expression

**Usage**

```
transcriptToGeneExpression(se)
```

**Arguments**

se	a summarizedExperiment object from <a href="#">bambu</a>
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writeBambuOutput	<i>Write bambu results to GTF and transcript/gene-count files</i>
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**Description**

Outputs a GTF file, transcript-count file, and gene-count file from bambu

**Usage**

```
writeBambuOutput(se, path)
```

**Arguments**

se	a <a href="#">SummarizedExperiment</a> object from <a href="#">bambu</a>
path	the destination of the output files (gtf, transcript counts, and gene counts)

**Value**

The function will generate three files, a [.gtf](#) file for the annotations, two [.txt](#) files for transcript and gene counts respectively.

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writeToGTF	<i>write GRangeslist into GTF file</i>
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**Description**

Write annotation GRangesList into a GTF file

**Usage**

```
writeToGTF(annotation, file, geneIDs = NULL)
```

**Arguments**

annotation	a <a href="#">GRangesList</a> object
file	the output gtf file name
geneIDs	an optional dataframe of geneIDs (column 2) with the corresponding transcriptIDs (column 1)

**Value**

gtf a GTF dataframe



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