

# Package ‘ORFquant’

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**Title** Splice-aware Quantification of ORF translation

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## Description

SaTAnn is a method that annotates and quantifies translation at the single ORF level using Ribo-seq data.

**Depends** rtracklayer, BSgenome, devtools, Biostrings, GenomicFeatures, foreach, doMC, multitaper, GenomicAlignments, GenomicFiles, reshape2, ggplot2, cowplot, grid, BiocGenerics, knitr, gridExtra, rmarkdown

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annotate_ORFs	<i>Annotate detected ORFs in transcript and genome space</i>
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**Description**

This function annotates quantified ORFs with respect to other detected ORFs and annotated ones, in both genome and transcript space.

**Usage**

```
annotate_ORFs(results_ORFs, Annotation, genome_sequence, region,  
              genetic_code)
```

**Arguments**

results_ORFs	Full list of detected ORFs, from select_quantify_ORFs
Annotation	Rannot object containing annotation of CDS and transcript structures (see prepare_annotation_files)
genome_sequence	BSgenome object
region	genomic region being analyzed
genetic_code	GENETIC_CODE table to use

**Details**

As multiple transcripts can contain the same ORF, all the transcript and transcript biotypes are indicated, with a preference for protein\_coding transcripts in the "compatible" columns (to be conservative when assessing translation of non-protein coding transcripts). Such compatibility is also output considering the most upstream start codon for that ORF.

Splice features of each orf is annotated with respect to the longest coding transcripts and to the highest translated ORF in that gene.

Variants in N or C terminus of the translated proteins are also indicated (Beta).

ORF annotation with respect to the annotated transcript is also indicated, as follows:

novel: no ORF annotated in the transcript.

ORF\_annotated: same exact ORF as annotated.

N\_extension: N terminal extension.  
 N\_truncation: N terminal extension.  
 uORF: upstream ORF.  
 overl\_uORF: upstream overlapping uORF.  
 NC\_extension: N and C termini extension.  
 dORF: downstream ORF.  
 overl\_dORF: downstream overlapping ORF.  
 nested\_ORF: nested ORF.  
 C\_truncation: C terminal truncation.  
 C\_extension: C terminal extension.

As transcript-specific annotation can be misleading due to a plethora of different transcripts, it is important to distinguish ORFs also on the basis of their overlap with known CDS regions. ORF annotation with respect to the entire set of CDS exon for the analyzed genomic regions is indicated as follows:

novel: No CDS region is annotated in the entire region.  
 novel\_Upstream: ORF is upstream of annotated CDS regions (does not overlap).  
 novel\_Downstream: ORF is downstream of annotated CDS regions (does not overlap).  
 novel\_Internal: genomic location of the ORF is present between the start of the first, and the end of the last CDS region (does not overlap).  
 exact\_start\_stop: Same start and end locations.  
 Alt5\_start: Different start region, upstream.  
 Alt3\_start: Different start region, downstream.  
 Alt5\_stop: Different end region, upstream.  
 Alt3\_stop: Different end region, downstream.

Another layer of annotation is performed by checking the position of the ORF stop codon with respect to the last exon-exon junction.

## Value

Exon structure of detected ORF including possible missing exons from reference, together with a spl\_type column including the annotation for each exon (e.g. alternative acceptors or donor).

Additional columns are added to the ORFs\_tx object:

compatible\_with: Set of transcript ids possibly containing the entire ORF structure.  
 compatible\_biotype: Compatible transcript biotype; if a protein coding transcript can contain the ORF, this is set to protein\_coding.  
 compatible\_tx: One selected compatible transcript (preference if protein\_coding).  
 compatible\_ORF\_id\_tr: ORF\_id\_tr id if selecting the compatible transcript.  
 compatible\_with\_longest: Same as compatible\_with but using the most upstream start codon.  
 compatible\_ORF\_id\_tr\_longest: Same as compatible\_ORF\_id\_tr but using the most upstream start codon .  
 ref\_id: transcript\_id of the transcript used to annotate splicing (longest) .  
 ref\_id\_maxORF: ORF\_id\_tr of the ORF used to annotated splicing (most translated of the gene).  
 NC\_protein\_isoform: Annotation of possible N or C termini variant (when transcript is protein\_coding) .  
 ORF\_category\_Tx: ORF annotation with respect to ORF position in the transcript .

ORF\_category\_Tx\_compatible: ORF annotation with respect to ORF position in the transcript, using the compatible\_ORF\_id\_tr .

ORF\_category\_Gen: ORF annotation with respect to its genomic position .

NMD\_candidate: TRUE or FALSE, depending on the presence of an additional exon-exon junction downstream the stop codon.

Distance\_to\_lastExEx: Distance (in nt) between the last exon-exon junction and the stop codon.

### Author(s)

Lorenzo Calviello, <calviello.l.bio@gmail.com>

### See Also

[select\\_quantify\\_ORFs](#), [annotate\\_splicing](#)

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annotate_splicing	<i>Annotate splice features of detected ORFs</i>
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### Description

This function detects usage of different exons and exonic boundaries of one ORF with respect to a reference ORF.

### Usage

```
annotate_splicing(orf_gen, ref_cds)
```

### Arguments

orf_gen	Exon structure of a detected ORF
ref_cds	Exon structure of a reference ORF

### Details

each exon is aligned to the closest one to match acceptor and donor sites, or to annotate missing exons. 5ss and 3ss indicate exon 5' and 3', respectively. CDS\_spanning indicates retained intron; missing\_CDS indicates no overlapping exon (missed or included); monoCDS indicates a single-exon ORF; firstCDS and lastCDS indicate first CDS exon or last CDS exon.

### Value

Exon structure of detected ORF including possible missing exons from reference, together with a spl\_type column including the annotation for each exon (e.g. alternative acceptors or donor).

### Author(s)

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [annotate\\_ORFs](#)

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calc_orf_pval	<i>Collect ORF Ribo-seq statistics</i>
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**Description**

This function calculates statistics for the analysis of P\_sites profiles for each ORF

**Usage**

```
calc_orf_pval(ORFs, P_sites_rle, P_sites_uniq_rle, P_sites_uniq_mm_rle,
              cutoff = 0.5, tapers = 24, bw = 12)
```

**Arguments**

ORFs	Set of detected ORFs
P_sites_rle	Rle signal of P_sites along the transcript
P_sites_uniq_rle	Rle signal of uniquely mapping P_sites along the transcript
P_sites_uniq_mm_rle	Rle signal of uniquely mapping P_sites with mismatches along the transcript
cutoff	cutoff of average in-frame signal for each codon in the ORF. Defaults to .5
tapers	Number of tapers to use in the multitaper analysis. Defaults to 24
bw	time_bw parameter to use in the multitaper analysis. Defaults to 12

**Details**

Number of P\_sites (uniquely mapping or all), frame percentage and multitaper test statistics are collected for each ORF. The parameter space for the multitaper analysis was explored in the RiboTaper paper.

**Value**

Set of detected ORFs, including info about the possible longest ORF for that frame.

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [get\\_orfs](#), [take\\_Fvals\\_spect](#)

---

`create_ORFquant_html_report`*Create an html report summarizing ORFquant results*

---

**Description**

This function creates an html report showing summary statistics for ORFquant-detected ORFs.

**Usage**

```
create_ORFquant_html_report(input_files, input_sample_names, output_file)
```

**Arguments**

<code>input_files</code>	Character vector with full paths to plot files (*ORFquant_plots_RData) generated with <code>plot_ORFquant_results</code> . Must be of same length as <code>input_sample_names</code> .
<code>input_sample_names</code>	Character vector containing input names. Must be of same length as <code>input_files</code> .
<code>output_file</code>	String; full path to html report file.

**Details**

This function creates the html report visualizing final ORFquant results.

Input are two lists of the same length:

- a) `input_files`: list of full paths to one or multiple input files (\*ORFquant\_plots\_RData files generated with `plot_ORFquant_results`) and
- b) `input_sample_names`: list of corresponding names describing the file content (these are used as names in the report).

For the report, a RMarkdown file is rendered as html document, saved as `output_file`.

**Value**

The function saves the html report file with the file path `output_file`.

**Author(s)**

Lorenzo Calviello, <calviello.bio@gmail.com>

**See Also**

[plot\\_ORFquant\\_results](#), [run\\_ORFquant](#)

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detect_readthrough	<i>Analyzed translation on possible readthrough regions (beta)</i>
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## Description

This function uses the multitaper method to look for readthrough translation

## Usage

```
detect_readthrough(results_orf, P_sites, P_sites_uniq, P_sites_uniq_mm,  
  genome_sequence, annotation, genetic_code_table, cutoff_fr_ave = 0.5,  
  uniq_signal = F)
```

## Arguments

results_orf	Full list of detected ORFs, from select_quantify_ORFs and annotate_ORFs
P_sites	GRanges object with P_sites positions
P_sites_uniq	GRanges object with uniquely mapping P_sites positions
P_sites_uniq_mm	Rle signal of uniquely mapping P_sites with mismatches along the transcript
genome_sequence	BSgenome object
annotation	Rannot object containing annotation of CDS and transcript structures (see prepare_annotation_files)
genetic_code_table	GENETIC_CODE table to use
cutoff_fr_ave	cutoff parameter for the calc_orf_pval functions
uniq_signal	Use only signal from uniquely mapping reads? Defaults to FALSE.

## Details

The function looks for stop-stop pairs after the stop codon of the detected ORF

## Value

GRanges object with the set of translated readthrough regions

## Author(s)

Lorenzo Calviello, <calviello.l.bio@gmail.com>

## See Also

[detect\\_translated\\_orfs](#), [select\\_quantify\\_ORFs](#), [annotate\\_ORFs](#), [get\\_reathr\\_seq](#)

---

detect\_translated\_orfs

*Detect actively translated ORFs*


---

## Description

This function detects translated ORFs

## Usage

```
detect_translated_orfs(selected_txs, genome_sequence, annotation, P_sites,
  P_sites_uniq, P_sites_uniq_mm, genomic_region, genetic_code,
  all_starts = T, nostarts = F, start_sel_cutoff = NA,
  start_sel_cutoff_ave = 0.5, cutoff_fr_ave = 0.5, uniq_signal = F)
```

## Arguments

selected_txs	set of selected transcripts, output from select_txs
genome_sequence	BSgenome object
annotation	Rannot object containing annotation of CDS and transcript structures (see prepare_annotation_files)
P_sites	GRanges object with P_sites positions
P_sites_uniq	GRanges object with uniquely mapping P_sites positions
P_sites_uniq_mm	GRanges object with uniquely mapping (with mismatches) P_sites positions
genomic_region	GRanges object with genomic coordinates of the genomic region analyzed
genetic_code	GENETIC_CODE table to use
all_starts	get_all_starts parameter for the get_orfs function
nostarts	Stop_Stop parameter for the get_orfs function
start_sel_cutoff	cutoff parameter for the select_start function
start_sel_cutoff_ave	cutoff_ave parameter for the select_start function
cutoff_fr_ave	cutoff parameter for the calc_orf_pval functions
uniq_signal	Use only signal from uniquely mapping reads? Defaults to FALSE.

## Details

A set of transcripts, together with genome sequence and Ribo-signal are analyzed to extract translated ORFs



**Value**

A list with transcript coordinates, exonic coordinates and statistics for each ORF exonic bin and junction(from select\_txs).

The value for each column is as follows:

ave\_pct\_fr: average percentage of in-frame reads for each codon in the ORF pct\_fr: percentage of in-frame reads in the ORF ave\_pct\_fr\_st: average percentage of in-frame reads per each codon between the selected start codon and the next candidate one pct\_fr\_st: percentage of in-frame reads between the selected start codon and the next candidate one longest\_ORF: GRanges coordinates for the longest ORF with the same stop codon pval: P-value for the multitaper F-test at 1/3 using the ORF P\_sites profile pval\_uniq: P-value for the multitaper F-test at 1/3 using the ORF P\_sites profile (only uniquely mapping reads) P\_sites\_raw: Raw number of P\_sites mapping to the ORF P\_sites\_raw\_unique: Uniquely mapping P\_sites mapping to the ORF ORF\_id\_tr: ORF id containing <tx\_id>\_<start>\_<end> Protein: AAString sequence of the translated protein region: Genomic coordinates of the analyzed region gene\_id: gene\_id for the corresponding analyzed transcript gene\_biotype: gene biotype for the corresponding analyzed transcript gene\_name: gene name for the corresponding analyzed transcript transcript\_id: transcript\_id for the corresponding analyzed ORF transcript\_biotype: transcript biotype for the corresponding analyzed ORF

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[select\\_txs](#), [get\\_orfs](#), [take\\_Fvals\\_spect](#), [select\\_start](#), [prepare\\_annotation\\_files](#)

---

from_tx_togen	<i>Map transcript coordinates to genomic coordinates</i>
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**Description**

This function uses the mapFromTranscripts function to switch between transcript and genomic coordinates

**Usage**

```
from_tx_togen(ORFs, exons, introns)
```

**Arguments**

ORFs	Set of detected ORFs from the calc_orf_pval function
exons	exonic regions of the analyzed transcripts, as a GRangesList object
introns	intronic regions of the analyzed transcripts, as a GRangesList object

**Value**

exonic coordinates for each ORF.

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[mapFromTranscripts](#)

---

get\_orfs

*Find ATG-starting ORFs in a sequence*

---

**Description**

This function loads the annotation created by the `prepare_annotation_files` function

**Usage**

```
get_orfs(tx_name, sequence, get_all_starts = T, Stop_Stop = F,  
        scores = c(1, 0.5), genetic_code_table)
```

**Arguments**

tx_name	transcript_id
sequence	DNAString object containing the sequence of the transcript
get_all_starts	Output all possible start codons? Defaults to TRUE
Stop_Stop	Find Stop-Stop pairs (no defined start codon)? Defaults to FALSE
scores	Deprecated
genetic_code_table	GENETIC_CODE table to use

**Value**

GRanges object containing coordinates for the detected ORFs

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#)

---

get\_ps\_fromsplicemin    *Offset spliced reads on minus strand*

---

**Description**

This function calculates P-sites positions for spliced reads on the minus strand

**Usage**

```
get_ps_fromsplicemin(x, cutoff)
```

**Arguments**

x	a GAlignments object with a cigar string
cutoff	number representing the offset value

**Value**

a GRanges object with offset reads

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[prepare\\_for\\_ORFquant](#)

---

get\_ps\_fromspliceplus    *Offset spliced reads on plus strand*

---

**Description**

This function calculates P-sites positions for spliced reads on the plus strand

**Usage**

```
get_ps_fromspliceplus(x, cutoff)
```

**Arguments**

x	a GAlignments object with a cigar string
cutoff	number representing the offset value

**Value**

a GRanges object with offset reads

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[prepare\\_for\\_ORFquant](#)

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get_reathr_seq	<i>Extract possible readthrough sequences (beta)</i>
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**Description**

This function extracts readthrough regions for subsequent analysis

**Usage**

```
get_reathr_seq(tx_name, orf, sequence, genetic_code)
```

**Arguments**

tx_name	transcript_id
orf	transcript-level ORF coordinates
sequence	DNASTring object containing the sequence of the transcript
genetic_code	GENETIC_CODE table to use

**Details**

The function looks for stop-stop pairs after the stop codon of the detected ORF

**Value**

GRanges object with the set of possible readthrough sequences

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [select\\_quantify\\_ORFs](#)

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load_annotation	<i>Load genomic features and genome sequence</i>
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---

**Description**

This function loads the annotation created by the prepare\_annotation\_files function

**Usage**

```
load_annotation(path)
```

**Arguments**

path	Full path to the *Rannot R file in the annotation directory used in the prepare_annotation_files function
------	---

**Value**

introduces a GTF\_annotation object and a genome\_seq object in the parent environment

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[prepare\\_annotation\\_files](#)

---

ORFquant	<i>Detection, quantification and annotation of translated ORFs in a genomic region</i>
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---

**Description**

This function detects, quantifies and annotates actively translated ORF in a genomic region

**Usage**

```
ORFquant(region, for_ORFquant, genetic_code_region,  
  orf_find.all_starts = T, orf_find.nostarts = F,  
  orf_find.start_sel_cutoff = NA, orf_find.start_sel_cutoff_ave = 0.5,  
  orf_find.cutoff_fr_ave = 0.5, orf_quant.cutoff_cums = NA,  
  orf_quant.cutoff_pct = 2, orf_quant.cutoff_P_sites = NA,  
  unique_reads = F)
```

**Arguments**

region            GRanges object with genomic coordinates of the genomic region analyzed  
 for\_ORFquant    "for\_ORFquant" Robject containing P\_sites positions and junction reads  
 genetic\_code\_region    GENETIC\_CODE table to use  
 orf\_find.all\_starts    get\_all\_starts parameter for the detect\_translated\_orfs function  
 orf\_find.nostarts    Stop\_Stop parameter for the detect\_translated\_orfs function  
 orf\_find.start\_sel\_cutoff    cutoff parameter for the detect\_translated\_orfs function  
 orf\_find.start\_sel\_cutoff\_ave    cutoff\_ave parameter for the detect\_translated\_orfs function  
 orf\_find.cutoff\_fr\_ave    cutoff parameter for the detect\_translated\_orfs function  
 orf\_quant.cutoff\_cums    cutoff\_cums parameter for the select\_quantify\_ORFs function  
 orf\_quant.cutoff\_pct    cutoff\_pct parameter for the select\_quantify\_ORFs function  
 orf\_quant.cutoff\_P\_sites    cutoff\_P\_sites parameter for the select\_quantify\_ORFs function  
 unique\_reads    Use only signal from uniquely mapping reads? Defaults to FALSE.

**Details**

A set of transcripts, together with genome sequence and Ribo-signal are analyzed to extract translated ORFs

**Value**

A list containing transcript coordinates, exonic coordinates and annotation for each ORF.

The description for each list object is as follows:

ORFs\_tx: transcript coordinates of the detected ORFs.  
 ORFs\_gen: genomic (exon) coordinates of the detected ORFs.  
 ORFs\_feat: list of ORF features together with mapping reads and uniqueness.  
 ORFs\_txs\_feats: list of transcript features present in the genomic region, together with mapping reads and uniqueness.  
 ORFs\_sp1\_feat\_longest: splicing annotation for each ORF exon, with respect to the longest annotated coding transcript for each gene.  
 ORFs\_sp1\_feat\_maxORF: splicing annotation for each ORF exon, with respect to the most translated ORF in each gene.  
 selected\_txs: character vector containing the transcript ids of the selected transcripts.  
 ORFs\_readthroughs: (Beta) transcript coordinates of the detected ORFs readthroughs.

**Author(s)**

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**See Also**

[select\\_txs](#), [detect\\_translated\\_orfs](#), [select\\_quantify\\_ORFs](#), [annotate\\_ORFs](#), [detect\\_readthrough](#)

---

plot\_ORFquant\_results *Plot general statistics about ORFquant results*

---

**Description**

This function produces a series of plots and statistics about the set ORFs called by ORFquant compared to the annotation. IMPORTANT: Use only on transcriptome-wide ORFquant results. See run\_ORFquant

**Usage**

```
plot_ORFquant_results(for_ORFquant_file, ORFquant_output_file,
  annotation_file, coverage_file_plus = NA, coverage_file_minus = NA,
  output_plots_path = NA, prefix = NA)
```

**Arguments**

for_ORFquant_file	path to the "for_ORFquant" file containing P_sites positions and junction reads
ORFquant_output_file	Full path to the "_final_ORFquant_results" RData object output by ORFquant. See run_ORFquant
annotation_file	Full path to the *Rannot R file in the annotation directory used in the prepare_annotation_files function
coverage_file_plus	Full path to a Ribo-seq coverage (no P-sites but read coverage) bigwig file (plus strand), as the ones created by RiboseQC
coverage_file_minus	Full path to a Ribo-seq coverage (no P-sites but read coverage) bigwig file (minus strand), as the ones created by RiboseQC
output_plots_path	Full path to the directory where plots in .pdf format are stored.
prefix	prefix appended to output filenames

**Value**

the function exports a RData object (\*ORFquant\_plots\_RData) containing data to produce all plots, and produces different QC plots in .pdf format. The plots created are as follows:

ORFs\_found: Number of ORF categories detected per gene biotype.  
 ORFs\_found\_pct\_tr: Distribution of ORF\_pct\_P\_sites ( ORFs\_found\_ORFs\_pM: Distribution of ORFs\_pM (ORFs per Million, similar to TPM) for different ORF categories and gene biotypes.  
 ORFs\_found\_len: Distribution of ORF length for different ORF categories and gene biotypes.  
 ORFs\_genes: Number of detected ORFs per gene.  
 ORFs\_genes\_tpm: Gene level TPM values, plotted by number of ORFs detected.  
 ORFs\_maxiso: Number of genes plotted against the percentages of gene translation of their most translated ORF.  
 ORFs\_maxiso\_tpm: Gene level TPM values, plotted against the percentages of gene translation of their most translated ORF.  
 Sel\_txs\_genes: Number of genes plotted against the number of selected transcripts.  
 Sel\_txs\_genes\_tpm: Gene level TPM values, plotted against the number of selected transcripts.  
 Sel\_txs\_genes\_pct: Percentages of annotated transcripts per gene, plotted against the number of selected transcripts.  
 Sel\_txs\_bins\_juns: Percentages of covered exonic bins or junctions, using all annotated transcripts, coding transcripts only, or the set of selected transcripts.  
 Meta\_splicing\_coverage: Aggregate signal of Ribo-seq coverage and normalized ORF coverage across different splice sites combinations, with different mixtures of translated overlapping ORFs.

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[run\\_ORFquant](#)

---

prepare\_annotation\_files

*Prepare comprehensive sets of annotated genomic features*

---

**Description**

This function processes a gtf file and a twobit file (created using faToTwoBit from ucsc tools: <http://hgdownload.soe.ucsc.edu/admin/exe/> ) to create a comprehensive set of genomic regions of interest in genomic and transcriptomic space (e.g. introns, UTRs, start/stop codons). In addition, by linking genome sequence and annotation, it extracts additional info, such as gene and transcript biotypes, genetic codes for different organelles, or chromosomes and transcripts lengths.

**Usage**

```
prepare_annotation_files(annotation_directory, twobit_file, gtf_file,
  scientific_name = "Homo.sapiens", annotation_name = "genc25",
  export_bed_tables_TxDb = T, forge_BSgenome = T, create_TxDb = T)
```



## Arguments

annotation_directory	The target directory which will contain the output files
twobit_file	Full path to the genome file in twobit format
gtf_file	Full path to the annotation file in GTF format
scientific_name	A name to give to the organism studied; must be two words separated by a ".", defaults to Homo.sapiens
annotation_name	A name to give to annotation used; defaults to genc25
export_bed_tables_TxDb	Export coordinates and info about different genomic regions in the annotation_directory? It defaults to TRUE
forge_BSgenome	Forge and install a BSgenome package? It defaults to TRUE
create_TxDb	Create a TxDb object and a *Rannot object? It defaults to TRUE

## Details

This function uses the `makeTxDbFromGFF` function to create a TxDb object and extract genomic regions and other info to a \*Rannot R file; the `mapToTranscripts` and `mapFromTranscripts` functions are used to map features to genomic or transcript-level coordinates. GTF file must contain "exon" and "CDS" lines, where each line contains "transcript\_id" and "gene\_id" values. Additional values such as "gene\_biotype" or "gene\_name" are also extracted. Regarding sequences, the twobit file, together with input scientific and annotation names, is used to forge and install a BSgenome package using the `forgeBSgenomeDataPkg` function.

The resulting GTF\_annotation object (obtained after running `load_annotation`) contains:

txs: annotated transcript boundaries.  
txs\_gene: GRangesList including transcript grouped by gene.  
seqinfo: indicating chromosomes and chromosome lengths.  
start\_stop\_codons: the set of annotated start and stop codon, with respective transcript and gene\_ids. `representative_mostcommon`, `representative_boundaries` and `representative_5len` represent the most common start/stop codon, the most upstream/downstream start/stop codons and the start/stop codons residing on transcripts with the longest 5'UTRs  
cds\_txs: GRangesList including CDS grouped by transcript.  
introns\_txs: GRangesList including introns grouped by transcript.  
cds\_genes: GRangesList including CDS grouped by gene.  
exons\_txs: GRangesList including exons grouped by transcript.  
exons\_bins: the list of exonic bins with associated transcripts and genes.  
junctions: the list of annotated splice junctions, with associated transcripts and genes.  
genes: annotated genes coordinates.  
threeutrs: collapsed set of 3'UTR regions, with corresponding gene\_ids. This set does not overlap CDS region.  
fiveutrs: collapsed set of 5'UTR regions, with corresponding gene\_ids. This set does not overlap CDS region.

ncIsof: collapsed set of exonic regions of protein\_coding genes, with corresponding gene\_ids. This set does not overlap CDS region.

ncRNAs: collapsed set of exonic regions of non\_coding genes, with corresponding gene\_ids. This set does not overlap CDS region.

introns: collapsed set of intronic regions, with corresponding gene\_ids. This set does not overlap exonic region.

intergenicRegions: set of intergenic regions, defined as regions with no annotated genes on either strand.

trann: DataFrame object including (when available) the mapping between gene\_id, gene\_name, gene\_biotypes, transcript\_id and transcript\_biotypes.

cds\_txs\_coords: transcript-level coordinates of ORF boundaries, for each annotated coding transcript. Additional columns are the same as as for the start\_stop\_codons object.

genetic\_codes: an object containing the list of genetic code ids used for each chromosome/organelle. see GENETIC\_CODE\_TABLE for more info.

genome\_package: the name of the forged BSgenome package. Loaded with load\_annotation function.

stop\_in\_gtf: stop codon, as defined in the annotation.

### Value

a TxDb file and a \*Rannot files are created in the specified annotation\_directory. In addition, a BSgenome object is forged, installed, and linked to the \*Rannot object

### Author(s)

Lorenzo Calviello, <calviello.l.bio@gmail.com>

### See Also

[load\\_annotation](#), [forgeBSgenomeDataPkg](#), [makeTxDbFromGFF](#), [run\\_ORFquant](#).

---

prepare\_for\_ORFquant    *Prepare the "for\_ORFquant" file*

---

### Description

Prepare the "for\_ORFquant" file

### Usage

```
prepare_for_ORFquant(annotation_file, bam_file,
  path_to_rl_cutoff_file = NA, chunk_size = 5e+06,
  path_to_P_sites_plus_bw = NA, path_to_P_sites_minus_bw = NA,
  path_to_P_sites_uniq_plus_bw = NA,
  path_to_P_sites_uniq_minus_bw = NA,
  path_to_P_sites_uniq_mm_plus_bw = NA,
  path_to_P_sites_uniq_mm_minus_bw = NA, dest_name = NA)
```

**Arguments**

annotation_file	Full path to the annotation file (*Rannot)
bam_file	Full path to the bam file
path_to_rl_cutoff_file	path to the rl_cutoff_file file specifying in 3 columns the read lengths, cutoffs and compartments ("nucl" for standard chromosomes)
chunk_size	the number of alignments to read at each iteration, defaults to 5000000, increase when more RAM is available
path_to_P_sites_plus_bw	path to a bigwig file containing P_sites positions on the plus strand
path_to_P_sites_minus_bw	path to a bigwig file containing P_sites positions on the minus strand
path_to_P_sites_uniq_plus_bw	(Optional) path to a bigwig file containing uniquely mapping P_sites positions on the plus strand
path_to_P_sites_uniq_minus_bw	(Optional) path to a bigwig file containing uniquely mapping P_sites positions on the minus strand
path_to_P_sites_uniq_mm_plus_bw	(Optional) path to a bigwig file containing uniquely mapping (with mismatches) P_sites positions on the plus strand
path_to_P_sites_uniq_mm_minus_bw	(Optional) path to a bigwig file containing uniquely mapping (with mismatches) P_sites positions on the minus strand
dest_name	prefix to use for the output files. Defaults to same as bam_file (appends "for_ORFquant" to its filename)

**Details**

This function uses a list of pre-determined read lengths, cutoffs and compartments to calculate P\_sites positions.

Alternatively, bigwig files containing P\_sites position for each strand can be specified. Optional bigwig files for uniquely mapping P\_sites position (with and without mismatches) can be specified to obtain more statistics on the ORFquant-identified ORFs

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[run\\_ORFquant](#)

---

run_ORFquant	<i>Run the ORFquant pipeline</i>
--------------	----------------------------------

---

## Description

This wrapper function runs the entire ORFquant pipeline

## Usage

```
run_ORFquant(for_ORFquant_file, annotation_file, n_cores,
  prefix = for_ORFquant_file, gene_name = NA, gene_id = NA,
  genomic_region = NA, write_temp_files = T, write_GTF_file = T,
  write_protein_fasta = T, interactive = T,
  stn.orf_find.all_starts = T, stn.orf_find.nostarts = F,
  stn.orf_find.start_sel_cutoff = NA,
  stn.orf_find.start_sel_cutoff_ave = 0.5,
  stn.orf_find.cutoff_fr_ave = 0.5, stn.orf_quant.cutoff_cums = NA,
  stn.orf_quant.cutoff_pct = 2, stn.orf_quant.cutoff_P_sites = NA,
  unique_reads_only = F, canonical_start_only = T)
```

## Arguments

for_ORFquant_file	REQUIRED - path to the "for_ORFquant" file containing P_sites positions and junction reads
annotation_file	REQUIRED - path to the *Rannot R file in the annotation directory used in the prepare_annotation_files function
n_cores	REQUIRED - number of cores to use
prefix	prefix to use for the output files. Defaults to same as for_ORFquant_file (appends to its filename)
gene_name	character vector of gene names to analyze.
gene_id	character vector of gene ids to analyze
genomic_region	GRanges object with genomic regions to analyze
write_temp_files	write temporary files. Defaults to TRUE
write_GTF_file	write a GTF files with the ORF coordinates. Defaults to TRUE
write_protein_fasta	write a protein fasta file. Defaults to TRUE
interactive	should put R object in global environment? Defaults to TRUE
stn.orf_find.all_starts	orf_find.all_starts parameter for the ORFquant function
stn.orf_find.nostarts	orf_find.nostarts parameter for the ORFquant function

```

stn.orf_find.start_sel_cutoff
    orf_find.start_sel_cutoff parameter for the ORFquant function
stn.orf_find.start_sel_cutoff_ave
    orf_find.start_sel_cutoff_ave parameter for the ORFquant function
stn.orf_find.cutoff_fr_ave
    orf_find.cutoff_fr_ave parameter for the ORFquant function
stn.orf_quant.cutoff_cums
    orf_quant.cutoff_cums parameter for the ORFquant function
stn.orf_quant.cutoff_pct
    orf_quant.cutoff_pct parameter for the ORFquant function
stn.orf_quant.cutoff_P_sites
    orf_quant.cutoff_P_sites parameter for the ORFquant function
unique_reads_only
    Use only signal from uniquely mapping reads? Defaults to FALSE.
canonical_start_only
    Use only the canonical start codon (no alternative initiation codons)? Defaults
    to TRUE.

```

### Details

A set of transcripts, together with genome sequence and Ribo-signal are analyzed to extract translated ORFs

### Value

A set of output files containing transcript coordinates, exonic coordinates and annotation for each ORF, including optional GTF and protein fasta files.

The description for each list object is as follows:

```

tmp_ORFquant_results: (Optional) RData object file containing the entire set of results for each
genomic region.
final_ORFquant_results: RData object file containing the final ORFquant results, see ORFquant.
Protein_sequences.fasta: (Optional) Fasta file containing the set of translated proteins .
Detected_ORFs.gtf: GTF file containing coordinates of the detected ORFs.

```

In addition, new columns are added in the ORFs\_tx file:

ORFs\_pM: number of P\_sites for each ORF, divided by ORF length and summing up to a million (akin to TPM).

### Author(s)

Lorenzo Calviello, <calviello.l.bio@gmail.com>

### See Also

[prepare\\_annotation\\_files](#), [load\\_annotation](#), [ORFquant](#)

---

select\_quantify\_ORFs    *Select and quantify ORF translation*

---

## Description

This function selects a subset of detected ORFs and quantifies their translation

## Usage

```
select_quantify_ORFs(results_ORFs, P_sites, P_sites_uniq,
  cutoff_cums = NA, cutoff_pct = 2, cutoff_P_sites = NA,
  optimiz = FALSE, scaling = TRUE, uniq_signal = F)
```

## Arguments

results_ORFs	Full list of detected ORFs, from detect_translated_ORFs
P_sites	GRanges object with P_sites positions
P_sites_uniq	GRanges object with uniquely mapping P_sites positions
cutoff_cums	cutoff to select ORFs until <x> percentage of total gene translation. Defaults to 99
cutoff_pct	minimum percentage of total gene translation for an ORF to be selected. Defaults to 1
cutoff_P_sites	minimum number of P_sites assigned to the ORF to be selected. Defaults to 10
optimiz	(Beta) should numerical optimization (minimizing distance between observed coverage and expected coverage) be used to quantify ORF translation? Defaults to FALSE
scaling	Additional scaling value taking into account total signal on the detected ORFs to adjust quantification estimates (recommended). Defaults to TRUE
uniq_signal	Use only signal from uniquely mapping reads? Defaults to FALSE.

## Details

ORFs are first selected using the same method as in the select\_txs function, but using ORF features (ORF structures are treated as transcript structures).

Ribo-seq coverage (reads/length) on bins and junctions (set to a length of 60) is used to derive a scaling factor (0-1) for each ORF, which indicates how much of the ORF coverage can be assigned to such ORF (1 when no other ORF is present). When no unique features are present on an ORF, an adjusted scaling value is calculated subtracting coverage expected from a ORF with a unique feature. When no unique features are present on any ORF, scaling values are calculated assuming uniform coverage on each ORF.

ORFs are then further filtered to exclude lowly translated ORFs and quantification/selection is reiterated until no ORF is further filtered out. Percentage of total gene translation and length-adjusted quantification estimates are produced. More details about the quantification procedure can be found in the ORFquant manuscript.

Additional columns are added to the ORFs\_tx object:  
P\_sites: P\_sites\_raw value from detect\_translated\_ORFs divided by the ORF scaling value.  
ORF\_pct\_P\_sites: Percentage of gene translation output for the ORF, derived using P\_sites values.  
ORF\_pct\_P\_sites\_pN: Percentage of gene translation output (adjusted by length) for the ORF, derived using P\_sites values.  
unique\_features\_reads: initial number of reads on each unique ORF feature. NA when no unique feature is present.  
adj\_unique\_features\_reads: final number of reads on each unique ORF feature after the ORF filtering/quantification procedure. NA when no unique feature is present.  
scaling\_factors: Set of 3 scaling factors assigned to the ORF using initial unique ORF features, after adjusting for the presence of ORFs with no unique features, and final scaling factor after correcting for total Ribo-seq coverage on the gene.

**Value**

modified results\_ORFs object with the selected ORFs including quantification estimates.

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [select\\_txs](#)

---

select_start	Select start codon
--------------	--------------------

---

**Description**

This function selects the start codon for ORFs in the same transcript

**Usage**

```
select_start(ORFs, P_sites_rle, cutoff = NA, cutoff_ave = 0.5)
```

**Arguments**

ORFs	Set of detected ORFs
P_sites_rle	Rle signal of P_sites along the transcript
cutoff	cutoff of total in-frame signal between start codons (sensitive to outliers). Defaults to NA
cutoff_ave	cutoff for frequency of in-frame codons between two start codons (less sensitive to outliers). Defaults to .5

**Details**

ORFs are divided based on stop codon and Ribo-seq signal between start codons is used to select one.  
When more than cutoff\_ave fraction of codons is in-frame between two candidate start codons, the most upstream is selected.

**Value**

Set of detected ORFs, including info about the possible longest ORF for that frame.

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [get\\_orfs](#)

---

select_txs	<i>Select a subset of transcripts with Ribo-seq data</i>
------------	--

---

**Description**

This function flattens all annotated transcript structures and uses Ribo-seq to select a subset of transcripts.

**Usage**

```
select_txs(region, annotation, P_sites, P_sites_uniq, junction_counts,
           uniq_signal = F)
```

**Arguments**

region	genomic region being analyzed
annotation	Rannot object containing annotation of CDS and transcript structures (see prepare_annotation_files)
P_sites	GRanges object with P_sites positions
P_sites_uniq	GRanges object with uniquely mapping P_sites positions
junction_counts	GRanges object containing Ribo-seq counts on the set of annotated junctions
uniq_signal	Use only signal from uniquely mapping reads? Defaults to FALSE.

**Details**

Features (bins and junctions) are divided into shared and unique features, and into with support and without support (with or without reads mapping). A set of logical rules filters out transcripts with internal features with no support and no unique features with reads. More specific details can be found in the ORFquant manuscript.



**Value**

GRanges object with the set of counts on each exonic bin and junctions, together with the list of selected transcripts

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[prepare\\_annotation\\_files](#)

---

take_Fvals_spect	<i>Extract output from multitaper analysis of a signal</i>
------------------	--

---

**Description**

This function uses the multitaper tool to extract F-values and multitaper spectral coefficients

**Usage**

```
take_Fvals_spect(x, n_tapers, time_bw, slepians_values)
```

**Arguments**

x	numeric signal to analyze
n_tapers	n of tapers to use
time_bw	time_bw parameter
slepians_values	set of calculated slepian functions to use in the multitaper analysis

**Details**

Values reported correspond to the closest frequency to 1/3 (same parameters as in RiboTaper). Padding to a minimum length of 1024 is performed to increase spectral resolution.

**Value**

two numeric values representing the F-value for the multitaper test and its corresponding spectral coefficient at the closest frequency to 1/3

**Author(s)**

Lorenzo Calviello, <calviello.l.bio@gmail.com>

**See Also**

[detect\\_translated\\_orfs](#), [spec.mtm](#), [dpss](#)

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