

# Package ‘riboSeqR’

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**Type** Package

**Title** Analysis of sequencing data from ribosome profiling experiments.

**Description** Plotting functions, frameshift detection and parsing of sequencing data from ribosome profiling experiments.

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riboSeqR-package	<i>Analysis of sequencing data from ribosome profiling experiments.</i>
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### Description

Plotting functions, frameshift detection and parsing of sequencing data from ribosome profiling experiments.

### Details

Package:	riboSeqR
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Further information is available in the following vignettes:

riboSeqR    riboSeqR (source, pdf)

### Author(s)

Thomas J. Hardcastle

Maintainer: Thomas J. Hardcastle <tjh48@cam.ac.uk>

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filterHits	<i>Filters framecalled data based on the mean number of hits observed across a replicate group.</i>
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### Description

If no ribosomal footprints of the correct lengths (and frames) are seen at a coding sequence in any replicate group, this sequence is unlikely to be translated (and is therefore likely to be uninteresting). This function filters out these coding sequences, leaving only those with a minimum number of hits in at least one replicate group, and a minimum number of unique sequences aligning in at least one replicate group (to exclude single stacks of sequenced reads passing the filtering).

**Usage**

```
filterHits(fCs, lengths = 27, frames, hitMean = 10, unqhitMean = 1,  
ratioCheck = TRUE, fS)
```

**Arguments**

fCs	riboCoding object defining the number of ribosome footprint reads observed over a set of coordinates, their lengths, and their frame relative to coding start sites.
lengths	The lengths of ribosome footprint reads to be used in filtering.
frames	If given, the frames of the ribosome footprint reads to be used in filtering. Should be of equal length to the 'lengths' parameter - see examples.
hitMean	The mean number of hits within a replicate group that should be observed to pass filtering.
unqhitMean	The mean number of unique sequences within a replicate group that should be observed to pass filtering. This parameter is intended to avoid cases where a coding sequence is deemed to be expressed based on a few highly expressed sequences.
ratioCheck	Checks the ratios of expected phase to maximal phase within the putative coding sequence. See Details.
fS	The output of 'frameCounting' function. Required if 'ratioCheck = TRUE'.

**Details**

Frames can be given as a single vector (which specifies the frames used for all lengths of footprints, or as a list, specifying the frame for each length given in 'lengths'.

For highly translated coding regions, the small proportion of out-of-phase reads may cause overlapping but out-of-phase putative coding regions to pass this filtering step. If 'ratioCheck = TRUE', filterHits identifies those cases where the phase with the maximum number of reads (the maximal phase) is not the expected phase for that putative coding region. If the ratio of reads in the expected phase to maximal phase does not significantly (Chi-squared test with significance threshold of 0.05) exceed that ratio observed for all coding regions, the putative coding region is filtered out.

**Value**

riboCoding object containing the filtered coding sequences and the associated numbers of ribosome footprint reads.

**Author(s)**

Thomas J. Hardcastle

**See Also**

frameCounting

**Examples**

```

#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                 "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", "M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
fS <- readingFrame(rC = fCs, lengths = 27:28)

# filter coding sequences. 27-mers are principally in the 0-frame,
# 28-mers are principally in the 2-frame relative to coding start (see
# readingFrame function).

ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(0, 2),
                  hitMean = 50, unqhitMean = 10, fS = fS)

```

---

findCDS

*Parses a transcriptome file looking for start/stop codons in frame.*


---

**Description**

Looks in the fastaFile for defined start and stop codons in frame with one another. Reports the discovered coordinates as a GRanges object with a 'frame' value.

**Usage**

```
findCDS(fastaFile, startCodon = c("ATG"), stopCodon = c("TAG", "TAA", "TGA"))
```

**Arguments**

fastaFile	Fasta file of transcriptome sequences.
startCodon	Vector of possible start codons. Defaults to "ATG".
stopCodon	Vector of possible stop codons. Defaults to c("TAG", "TAA", "TGA").

**Value**

A GRanges object.

**Author(s)**

Thomas J. Hardcastle

**Examples**

```
datadir <- system.file("extdata", package = "riboSeqR")
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                   startCodon = c("ATG"),
                   stopCodon = c("TAG", "TAA", "TGA"))
```

---

frameCounting	<i>Counts aligned reads within coding sequence regions by frame and footprint size, splitting by frame and footprint size.</i>
---------------	--

---

**Description**

Ribosome footprint sequencing reads aligning within coding sequence regions may align in the same frame (relative to start codon) as the coding sequence, or frame shifted by 1 or 2 frames. This function calls the number of aligning reads within the coding sequence, split by frame and footprint size.

**Usage**

```
frameCounting(riboDat, fastaCDS, lengths = 25:30)
```

**Arguments**

riboDat	A riboData object containing the ribosome footprints to be counted.
fastaCDS	A GenomicRanges object containing the coordinates of the coding sequences.
lengths	Lengths of ribosome footprints to be included in the riboData object.

**Value**

A riboCoding object.

**Author(s)**

Thomas J. Hardcastle

**See Also**

riboData

**Examples**

```

#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                 "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", "M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

```

---

lengthDist

*A function plotting the density of lengths in a riboData object.*


---

**Description**

riboData object contain data on multiple lengths of sequenced reads. This function plots the density of those lengths.

**Usage**

```
lengthDist(riboDat, add = FALSE, legend = NULL, ...)
```

**Arguments**

riboDat	riboData object to be plotted
add	Should the curve be added to an existing plot, or a new plot drawn?
legend	Legend text, if given.
...	Additional arguments to be passed to 'plot' or 'legend'.

**Author(s)**

Thomas J. Hardcastle

**Examples**

```
#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", " M"))

lengthDist(riboDat)
```

---

plotCDS	<i>Plots average ribosome footprint alignment to coding sequences at 5' and 3' ends.</i>
---------	--

---

**Description**

For each sample, the average (normalised by translation abundance over transcript) of the ribosome footprints of a given length alignments at the 5' and 3' ends of all specified transcripts beginning at each base relative to coding start/end are plotted. The bases are colour coded relative to start codon.

**Usage**

```
plotCDS(coordinates, riboDat, lengths = 27, min5p = -20, max5p = 200,
min3p = -200, max3p = 20, cap, main = "", plot = TRUE)
```

**Arguments**

coordinates	Coordinates (as a GRanges object) of the coding sequences.
riboDat	<a href="#">riboData</a> object containing ribosome footprint data.
lengths	Lengths of footprints to be plotted. May be given as a vector, in which case multiple plots will be produced.
min5p	The distance upstream of the translation start to be plotted.
max5p	The distance downstream of the translation start to be plotted.
min3p	The distance upstream of the translation end to be plotted.
max3p	The distance downstream of the translation end to be plotted.
cap	If given, caps the height of plotted values.
main	Title of the plot.
plot	Should the acquired matrix of mean expression be plotted? Defaults to TRUE.

**Value**

Invisibly returns lists of lists of matrices containing weighted averages plotted for each sample/length combination.

**Author(s)**

Thomas J. Hardcastle

**Examples**

```
#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                 "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", "M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
fS <- readingFrame(rC = fCs, lengths = 27:28)

# filter coding sequences. 27-mers are principally in the 0-frame,
# 28-mers are principally in the 2-frame relative to coding start (see
# readingFrame function).

ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(0, 2),
                  hitMean = 50, unqhitMean = 10, fS = fS)

plotCDS(coordinates = ffCs@CDS, riboDat = riboDat, lengths = 27)
```

---

plotTranscript

*Plots ribosome footprint abundance and mRNA coverage (if available) for a specific transcript.*

---

**Description**

Abundances of ribosomal footprints of a given size class are plotted on a transcript. The footprints are colour coded according to the first base of the transcript, and not any coding start site, to allow for multiple coding start sites on a given transcript. Coding regions may simultaneously be plotted and colour coded under the same scheme.



**Usage**

```
plotTranscript(transcript, coordinates, annotation, riboData, length =  
27, frameShift = 0, cap, riboScale, rnaScale, xlim, main, note = "", ...)
```

**Arguments**

transcript	The name of the transcript to be plotted.
coordinates	A GRanges object containing any coding regions on the transcript.
annotation	A GRanges object containing annotated coding coordinates to be plotted as bars above the figure.
riboData	A <a href="#">riboData</a> object containing the ribosome footprint (and optionally, RNA-seq) data.
length	Size class of ribosome footprint data to be plotted.
frameShift	Frameshift for the ribosome footprint data. See Details.
cap	Cap on the largest value that will be plotted as an abundance of the ribosome footprint data.
riboScale	Scale to be used on the ribosome footprint axis.
rnaScale	Scale to be used on the RNA-seq coverage axis.
xlim	Limits of the bases of the transcript to be plotted (i.e., the x-axis). If missing, the full transcript will be plotted.
main	Optional title for the plot.
note	Additional note to be added to plot titles (in addition to transcript and sample names).
...	Additional arguments to be passed to plotting function.

**Details**

The `readingFrame` value allows the colour-coding of the ribosome footprints to be shifted so that the colours of the coding sequences match the colours of the ribosome footprint data. E.g., if 28-mers are predominantly in frame 2 relative to coding start, a value of `readingFrame=2` will ensure that 28-mers in a coding region will take the same colour as that coding region if they are in the correct relative frame.

**Value**

NULL; plotting function.

**Author(s)**

Thomas J. Hardcastle

**Examples**

```

#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", " M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
fS <- readingFrame(rC = fCs, lengths = 27:28)

# filter coding sequences. 27-mers are principally in the 0-frame,
# 28-mers are principally in the 2-frame relative to coding start (see
# readingFrame function).

ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(0, 2),
                  hitMean = 50, unqhitMean = 10, fS = fS)

plotTranscript("CUFF.37930.1", coordinates = ffCs@CDS,
              riboData = riboDat, length = 27, cap = 200)

```

---

readingFrame

*Analyses frame called ribosome footprint data within coding sequences and identifies likely frame-shift of different length ribosome footprint reads.*

---

**Description**

Ribosome footprint data data can be used to identify the frame-shift relative to start codon of the different n-mers. For each readlength specified, the sum of alignments in the different frames is shown, together with the maximum likelihood frame.

**Usage**

```

readingFrame(coordinates, riboDat, rC, lengths = 26:30)
plotFS(fS, lengths, legend.text = c("Frame 0", "Frame 1", "Frame 2"), ...)

```

**Arguments**

coordinates	Coordinates of transcripts. Need not be given if a riboCoding object is specified in 'rC'.
riboDat	A riboData object containing the ribosome footprint data. Need not be given if a riboCoding object is specified in 'rC'.
rC	A riboCoding object.
lengths	Lengths of reads to be analysed for frame-shift, or to be plotted. May be omitted in plotting, in which case all lengths will be plotted.
fS	The output of the readingFrame function, to be plotted.
legend.text	Text for legend.
...	Additional arguments to be passed to barplot function.

**Value**

A matrix giving the number of aligned reads in each frame for each length, and the maximum likelihood frame.

**Author(s)**

Thomas J. Hardcastle

**See Also**

[frameCounting](#)

**Examples**

```
#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", "M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
rCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
fS <- readingFrame(rC = rCs, lengths = 27:28)
plotFS(fS)
```

---

readRibodata                      *Reads ribosomal and (optionally) rna data from alignment files.*

---

### Description

Reads BAM files, or flat text files (which may be compressed) containing strand, transcript name, start and sequence information for each alignment.

### Usage

```
readRibodata(riboFiles, rnaFiles, columns = c(strand = 1, seqname = 2,
start = 3, sequence = 4), zeroIndexed = TRUE, header = FALSE, replicates, seqnames)
```

### Arguments

riboFiles	Filenames of ribosomal alignments.
rnaFiles	Filenames of RNA alignments.
columns	Columns of alignment files containing strand, transcript (seqname) name, start of alignment, and sequence. Ignored for BAM files.
zeroIndexed	Are the alignments zero-indexed (i.e., the first base in a sequence is 0). Defaults to TRUE, which will result in an adjustment to 1-indexed data.
header	Does the alignment file have a header line? Defaults to FALSE.
replicates	Replicate information for the files.
seqnames	Transcript (seqname) names to be read into the object.

### Details

If a filename ends in '.bam' or '.BAM' it will be assumed that the file is a BAM file and processed accordingly. Otherwise, it will be treated as a flat text file.

### Value

riboData object.

### Author(s)

Thomas J. Hardcastle

### Examples

```
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT", "M", "M"))
```

---

riboCoding-class	Class "riboCoding"
------------------	--------------------

---

**Description**

The riboCoding class contains a set of coordinates defining coding sequences, a set of replicate data for the experimental samples, an array of ribosome footprint abundances for each coding sequence split by size class and frame, and a similar array describing the abundance of unique sequences aligning within each coding sequence.

**Slots**

**CDS:** Object of class "GRanges" defining the coordinates of coding sequences.

**hits:** An array describing the abundances of ribosome footprints, split by size class and frame (relative to coding start) for each of the coding sequences defined in the 'CDS' slot.

**unqHits:** An array describing the abundances of unique sequences of ribosome footprints, split by size class and frame (relative to coding start) for each of the coding sequences defined in the 'CDS' slot.

**replicates:** A factor defining the replicate structure of the samples for which data are held. Replicate samples will have the same level in this factor.

**Methods**

Methods '[' and 'show' are defined for this class.

**Author(s)**

Thomas J. Hardcastle

---

riboData-class	Class "riboData"
----------------	------------------

---

**Description**

The riboData class contains a list of GRanges objects containing ribosome footprint alignment data, a factor defining the replicate structure of the samples involved, and (optionally) a list of GRanges objects containing RNA-seq alignment data (paired with the ribosome footprint data). It will generally be created by the 'readRibodata' function and not directly by the user.

**Slots**

**riboGR:** List of "GRanges" objects (one for each sequenced sample) describing the alignments of ribosomal footprint data to a transcriptome.

**rnaGR:** List of "GRanges" objects (one for each sequenced sample, paired with 'riboGR' slot) describing the alignments of RNA-seq data to a transcriptome.

**replicates:** A factor defining the replicate structure of the samples for which data are held. Replicate samples will have the same level in this factor.

**Methods**

No methods are currently defined for this class.

**Author(s)**

Thomas J. Hardcastle

---

rnaCounts	<i>Extracts mRNA counts from a riboDat object for a set of coding sequence coordinates.</i>
-----------	---

---

**Description**

Takes mRNA count data from riboDat object, maps them to coding sequences specified in GRanges object, and counts the total number of hits. This is a crude approach intended to quickly produce comparable data to ribosome footprint counts. More sophisticated alternatives, addressing coverage variation, isoforms, multireads &c. have been widely described in the literature on mRNA-seq analyses.

**Usage**

```
rnaCounts(riboDat, CDS)
```

**Arguments**

riboDat	A <a href="#">riboData</a> object containing the RNA-seq alignments.
CDS	A GRanges object defining the coordinates of the coding sequences for which to acquire counts.

**Details**

The count data thus acquired can be compared to counts of ribosomal footprint data through a beta-binomial analysis (see vignette) to discover differential translation.

**Value**

A matrix containing count data for the RNA-seq libraries.

**Author(s)**

Thomas J. Hardcastle

**See Also**

[sliceCounts](#)

**Examples**

```

#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
"MT", "MT"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                   startCodon = c("ATG"),
                   stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
fS <- readingFrame(rC = fCs, lengths = 27:28)

# filter coding sequences. 27-mers are principally in the 0-frame,
# 28-mers are principally in the 2-frame relative to coding start (see
# readingFrame function).

ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(0, 2),
                  hitMean = 50, unqhitMean = 10, fS = fS)

# Extract counts of RNA hits from riboCount data.
rnaCounts <- rnaCounts(riboDat, ffCs@CDS)

```

---

sliceCounts

*Slices out count data from riboCoding object for use in differential translation analyses.*

---

**Description**

For any given coding sequence, multiple lengths of reads in various frames (relative to coding start) may align. This function extracts specific size-classes and frames of ribosome footprint reads and sums them to give a single value for each coding sequence and each sequencing library, for use in downstream analysis.

**Usage**

```
sliceCounts(rC, lengths = 27, frames)
```

**Arguments**

rC	A <a href="#">riboCoding</a> object containing the coordinates of the coding sequences and the number of ribosomal footprint reads of various classes to be found in each.
lengths	Lengths of ribosome footprints to inform count data.
frames	Frames of ribosome footprints (relative to coding start site). If omitted, all frames are used.

**Details**

Frames can be given as a single vector (which specifies the frames used for all lengths of footprints, or as a list, specifying the frame for each length given in 'lengths'.

The count data thus acquired can be compared to counts of RNA-seq data through a beta-binomial analysis (see vignette) to discover differential translation.

**Value**

A matrix containing counts of ribosomal footprint matches to coding sequences specified in riboCoding object 'rC'.

**Author(s)**

Thomas J. Hardcastle

**See Also**

[rnaCounts](#)

**Examples**

```
#ribosomal footprint data
datadir <- system.file("extdata", package = "riboSeqR")
ribofiles <- paste(datadir,
                  "/chlamy236_plus_deNovo_plusOnly_Index", c(17,3,5,7), sep = "")
rnafiles <- paste(datadir,
                 "/chlamy236_plus_deNovo_plusOnly_Index", c(10,12,14,16), sep = "")

riboDat <- readRibodata(ribofiles, rnafiles, replicates = c("WT", "WT",
" M", " M"))

# CDS coordinates
chlamyFasta <- paste(datadir, "/rsem_chlamy236_deNovo.transcripts.fa", sep = "")
fastaCDS <- findCDS(fastaFile = chlamyFasta,
                  startCodon = c("ATG"),
                  stopCodon = c("TAG", "TAA", "TGA"))

# frame calling
fCs <- frameCounting(riboDat, fastaCDS)

# analysis of frame shift for 27 and 28-mers.
```



```
fS <- readingFrame(rC = fCs, lengths = 27:28)

# filter coding sequences. 27-mers are principally in the 0-frame,
# 28-mers are principally in the 2-frame relative to coding start (see
# readingFrame function).

ffCs <- filterHits(fCs, lengths = c(27, 28), frames = list(0, 2),
                  hitMean = 50, unqhitMean = 10, fS = fS)

# Extract counts of ribosomal footprints from riboCount data.

riboCounts <- sliceCounts(ffCs, lengths = c(27, 28), frames = list(0,
2))
```

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