

# Package ‘qsvaR’

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**Title** Generate Quality Surrogate Variable Analysis for Degradation Correction

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**Description** The qsvaR package contains functions for removing the effect of degradation in rna-seq data from postmortem brain tissue. The package is equipped to help users generate principal components associated with degradation. The components can be used in differential expression analysis to remove the effects of degradation.

**License** Artistic-2.0

**URL** <https://github.com/LieberInstitute/qsvaR>

**BugReports** <https://support.bioconductor.org/t/qsvaR>

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|                    |                                      |
|--------------------|--------------------------------------|
| degradation_tstats | <i>Degradation time t-statistics</i> |
|--------------------|--------------------------------------|

---

**Description**

These t-statistics are derived from the degradation timepoints data built into qsvaR. They are the results from multiple models where we determined the association of transcripts with degradation time adjusting for brain region (so parallel degradation effects across brain regions). They are used for plotting in DEqual().

**Format**

A data.frame() with the t statistics for degradation time. The rownames() are the GENCODE transcript IDs.

**See Also**

[DEqual](#)

**Description**

A DEqual plot compares the effect of RNA degradation from an independent degradation experiment on the y axis to the effect of the outcome of interest. They were originally described by Jaffe et al, PNAS, 2017 <https://doi.org/10.1073/pnas.1617384114>. Other DEqual versions are included in Collado-Torres et al, Neuron, 2019 <https://doi.org/10.1016/j.neuron.2019.05.013>. This function compares your t-statistics of interest computed on transcripts against the t-statistics from degradation time adjusting for the six brain regions from degradation experiment data used for determining `rse_tx`.

**Usage**

```
DEqual(
  DE,
  deg_tstats = qsvaR::degradation_tstats,
  show.legend = TRUE,
  show.cor = c("caption", "corner-top", "corner-bottom", "none"),
  font.size = 12,
  cor.size = font.size/2,
  cor.label = "cor: "
)
```

**Arguments**

|                          |                                                                                                                                                                                                                                       |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>DE</code>          | a <code>data.frame()</code> with a column "t" containing the t-statistics from Differential Expression, typically generated with <code>limma::topTable()</code> . <code>rownames(DE)</code> must have transcript Ensembl/Gencode IDs. |
| <code>deg_tstats</code>  | an optional <code>data.frame()</code> with a column "t" containing t-statistics resulted from a degradation experiment. Default is the internal <code>qsvaR::degradation_tstats</code> from the package authors.                      |
| <code>show.legend</code> | logical (default TRUE) to show legend in the plot                                                                                                                                                                                     |
| <code>show.cor</code>    | specify where to show the correlation value. Can be one of "caption", "corner-top", "corner-bottom", or "none".                                                                                                                       |
| <code>font.size</code>   | numeric value to set the base font size of the plot                                                                                                                                                                                   |
| <code>cor.size</code>    | numeric (default <code>font.size/2</code> ) to set the font size for the correlation text                                                                                                                                             |
| <code>cor.label</code>   | character (default "cor: ") to set the text preceding the correlation value                                                                                                                                                           |

**Value**

a `ggplot` object of the DE t-statistic vs the DE statistic from degradation

## Examples

```
## Random differential expression t-statistics for the same transcripts
## we have degradation t-statistics for in `degradation_tstats`.
set.seed(101)
random_de <- data.frame(
  t = rt(nrow(degradation_tstats), 5),
  row.names = sample(
    rownames(degradation_tstats),
    nrow(degradation_tstats)
  )
)

## Create the DEqual plot
DEqual(random_de)
```

---

getDegTx

---

*Obtain expression matrix for degraded transcripts*


---

## Description

This function is used to obtain a [RangedSummarizedExperiment-class](#) of transcripts and their expression values #' These transcripts are selected based on a prior study of RNA degradation in postmortem brain tissues. This object can later be used to obtain the principle components necessary to remove the effect of degradation in differential expression.

## Usage

```
getDegTx(
  rse_tx,
  sig_transcripts = select_transcripts(),
  assayname = "tpm",
  verbose = TRUE
)
```

## Arguments

|                 |                                                                                                                             |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------|
| rse_tx          | A <a href="#">RangedSummarizedExperiment-class</a> object containing the transcript data desired to be studied.             |
| sig_transcripts | A character() vector of transcripts that should be associated with degradation, expected to be present in rownames(rse_tx). |
| assayname       | character string specifying the name of the assay desired in rse_tx                                                         |
| verbose         | specify if the function should report how many model transcripts were matched                                               |

## Value

A [RangedSummarizedExperiment-class](#) object.

**Examples**

```
degTx <- getDegTx(rse_tx)
```

---

getPCs

*PCs from transcripts*


---

**Description**

This function returns the pcs from the obtained RangedSummarizedExperiment object of selected transcripts

**Usage**

```
getPCs(rse_tx, assayname = "tpm")
```

**Arguments**

|           |                                                                       |
|-----------|-----------------------------------------------------------------------|
| rse_tx    | Ranged Summarizeed Experiment with only transcripts selected for qsva |
| assayname | character string specifying the name of the assay desired in rse_tx   |

**Value**

prcomp object generated by taking the pcs of degraded transcripts

**Examples**

```
getPCs(rse_tx, "tpm")
```

---

get\_qsvs

*Generate matrix of qsvs*


---

**Description**

Using the pcs and the k number of components be included, we generate the qsva matrix.

**Usage**

```
get_qsvs(qsvPCs, k)
```

**Arguments**

|        |                                                                   |
|--------|-------------------------------------------------------------------|
| qsvPCs | prcomp object generated by taking the pcs of degraded transcripts |
| k      | number of qsvs to be included.                                    |

**Value**

matrix with k principal components for each sample.

**Examples**

```
qsv <- getPCs(rse_tx, "tpm")
get_qsvs(qsv, 2)
```

---

|        |                                                                             |
|--------|-----------------------------------------------------------------------------|
| k_qsvs | <i>Apply num.sv algorithm to determine the number of pcs to be included</i> |
|--------|-----------------------------------------------------------------------------|

---

**Description**

Apply num.sv algorithm to determine the number of pcs to be included

**Usage**

```
k_qsvs(rse_tx, mod, assayname)
```

**Arguments**

|           |                                                                                                                 |
|-----------|-----------------------------------------------------------------------------------------------------------------|
| rse_tx    | A <a href="#">RangedSummarizedExperiment-class</a> object containing the transcript data desired to be studied. |
| mod       | Model Matrix with necessary variables the you would model for in differential expression                        |
| assayname | character string specifying the name of the assay desired in rse_tx                                             |

**Value**

integer representing number of pcs to be included

**Examples**

```
## First we need to define a statistical model. We'll use the example
## rse_tx data. Note that the model you'll use in your own data
## might look different from this model.
mod <- model.matrix(~ mitoRate + Region + rRNA_rate + totalAssignedGene + RIN,
  data = colData(rse_tx)
)

## To ensure that the results are reproducible, you will need to set a
## random seed with the set.seed() function. Internally, we are using
## sva::num.sv() which needs a random seed to ensure reproducibility of the
## results.
set.seed(20230621)
k_qsvs(rse_tx, mod, "tpm")
```

---

|                    |                                                                    |
|--------------------|--------------------------------------------------------------------|
| normalize_tx_names | <i>Remove version number from Gencode/Ensembl transcript names</i> |
|--------------------|--------------------------------------------------------------------|

---

**Description**

This function removes the Gencode/ENSEMBL version from the transcript ID, while protecting \_PAR\_Y suffixes if present

**Usage**

```
normalize_tx_names(txnames)
```

**Arguments**

|         |                                                           |
|---------|-----------------------------------------------------------|
| txnames | A character() vector of GENCODE or ENSEMBL transcript IDs |
|---------|-----------------------------------------------------------|

**Value**

A character() vector of transcript names without versioning

**Examples**

```
ensIDs <- normalize_tx_names(rownames(rse_tx))
```

---

|      |                                                             |
|------|-------------------------------------------------------------|
| qSVA | <i>A wrapper function used to perform qSVA in one step.</i> |
|------|-------------------------------------------------------------|

---

**Description**

A wrapper function used to perform qSVA in one step.

**Usage**

```
qSVA(rse_tx, sig_transcripts = select_transcripts(), mod, assayname)
```

**Arguments**

|                 |                                                                                                                             |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------|
| rse_tx          | A <a href="#">RangedSummarizedExperiment-class</a> object containing the transcript data desired to be studied.             |
| sig_transcripts | A character() vector of transcripts that should be associated with degradation, expected to be present in rownames(rse_tx). |
| mod             | Model Matrix with necessary variables the you would model for in differential expression.                                   |
| assayname       | character string specifying the name of the assay desired in rse_tx                                                         |

**Value**

matrix with k principal components for each sample

**Examples**

```
## First we need to define a statistical model. We'll use the example
## rse_tx data. Note that the model you'll use in your own data
## might look different from this model.
mod <- model.matrix(~ mitoRate + Region + rRNA_rate + totalAssignedGene + RIN,
  data = colData(rse_tx)
)

## To ensure that the results are reproducible, you will need to set a
## random seed with the set.seed() function. Internally, we are using
## sva::num.sv() which needs a random seed to ensure reproducibility of the
## results.
set.seed(20230621)
qSVA(rse_tx = rse_tx, mod = mod, assayname = "tpm")
```

---

rse\_tx

---

*Example of RSE object with RNA-seq transcript quantification data*


---

**Description**

This data is a [RangedSummarizedExperiment-class](#) with transcript quantification data stored in an "tpm" assay. It is used to demonstrate the use of qsvaR in bulk RNA-seq data.

**Format**

A [RangedSummarizedExperiment-class](#)

**See Also**

[getPCs](#) [k\\_qsvs](#) [getDegTx](#) [qSVA](#)

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select\_transcripts

---

*Select transcripts associated with degradation*


---

**Description**

Helper function to select which experimental model(s) will be used to generate the qSVs. Degradation-associated transcripts are derived in four different models ([transcripts](#)). The cell\_component parameter controls whether the models with cell-type proportions are included. This function extract the top top\_n transcripts found to be significant in each considered model, then returns the union of transcripts across all considered models. Up to 10,000 transcripts are available to select from each model prior to taking the union.

**Usage**

```
select_transcripts(top_n = 1000, cell_component = FALSE)
```

**Arguments**

**top\_n** An integer(1) specifying how many significant transcripts to extract from each model prior to taking a union across models.

**cell\_component** A logical(1). If FALSE, only include transcripts from the main and interaction models (see `main_model` and `int_model` here: [transcripts](#)). If TRUE, additionally include main and interaction models that include cell-type proportions (a total of 4 models).

**Value**

A character() with the transcript IDs.

**Examples**

```
## Default set of transcripts associated with degradation
sig_transcripts <- select_transcripts()
length(sig_transcripts)
head(sig_transcripts)

## Select more transcripts if desired
length(select_transcripts(top_n = 5000))
```

---

transcripts

*Transcripts for Degradation Models*


---

**Description**

This object is a list of four tibbles where each element corresponds to the top 10,000 transcripts (by significance) and their adjusted p-values for a given degradation model. The `main_model` model is a linear model modelling expression against a sample's degradation time, with brain region as a covariate. The `int_model` model is similar but includes an interaction term with degradation time and brain region. The `cell_main_model` and `cell_int_model` models are like the respective `main_model` and `int_model` models, but including cell-type fractions from deconvolution as linear terms.

**Usage**

```
transcripts
```

**Format**

A list() of tibble()s containing the transcripts and adjusted p-values selected by each model. Each string is a GENCODE transcript IDs.

**See Also**[select\\_transcripts](#)

---

|                |                                                                                         |
|----------------|-----------------------------------------------------------------------------------------|
| which_tx_names | <i>Check validity of transcript vectors and return a vector matching indexes in tx1</i> |
|----------------|-----------------------------------------------------------------------------------------|

---

**Description**

This function is used to check if tx1 and tx2 are GENCODE or ENSEMBL transcript IDs and return an integer vector of tx1 transcript indexes that are in tx2.

**Usage**

```
which_tx_names(txnames, sig_tx)
```

**Arguments**

|         |                                                                      |
|---------|----------------------------------------------------------------------|
| txnames | A character() vector of GENCODE or ENSEMBL transcript IDs.           |
| sig_tx  | A character() vector of GENCODE or ENSEMBL signature transcript IDs. |

**Value**

A integer() vector of txnames transcript indexes in sig\_tx.

**Examples**

```
sig_tx <- select_transcripts(cell_component = TRUE)
whichTx <- which_tx_names(rownames(rse_tx), sig_tx)
```

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