

# Package ‘ddCt’

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**Title** The ddCt Algorithm for the Analysis of Quantitative Real-Time PCR (qRT-PCR)

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**Author** Jitao David Zhang, Rudolf Biczok, and Markus Ruschhaupt

**Description** The Delta-Delta-Ct (ddCt) Algorithm is an approximation method to determine relative gene expression with quantitative real-time PCR (qRT-PCR) experiments. Compared to other approaches, it requires no standard curve for each primer-target pair, therefore reducing the working load and yet returning accurate enough results as long as the assumptions of the amplification efficiency hold. The ddCt package implements a pipeline to collect, analyse and visualize qRT-PCR results, for example those from TaqMan SDM software, mainly using the ddCt method. The pipeline can be either invoked by a script in command-line or through the API consisting of S4-Classes, methods and functions.

**Maintainer** Jitao David Zhang <jitao\_david.zhang@roche.com>

**biocViews** GeneExpression, DifferentialExpression,  
MicrotitrePlateAssay, qPCR

**License** LGPL-3

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**Imports** Biobase (>= 1.10.0), RColorBrewer (>= 0.1-3), xtable, lattice,  
BiocGenerics

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|               |                                 |
|---------------|---------------------------------|
| barploterrbar | <i>Barplot with error bars.</i> |
|---------------|---------------------------------|

---

Description

Barplot with error bars.

Usage

```
barploterrbar(y, yl, yh, barcol="orange", errcol="black", horiz=FALSE,
w=0.2,theCut=NULL,columnForDiffBars=TRUE,cex.axis =
par("cex.axis"),zeroForNA=TRUE,legend=FALSE,groups = NULL, order=FALSE, ...)
```

Arguments

|        |                                          |
|--------|------------------------------------------|
| y      | Numeric vector.                          |
| yl     | Numeric vector of same length as y.      |
| yh     | Numeric vector of same length as y.      |
| barcol | Color of the bars.                       |
| errcol | Color of the error bars.                 |
| horiz  | Logical. As in <a href="#">barplot</a> . |
| w      | Size of the error bar ticks.             |
| theCut | The cut value                            |

|                                |                                                                                    |
|--------------------------------|------------------------------------------------------------------------------------|
| <code>columnForDiffBars</code> | Whether the matrix should be transposed (by default the rows are for diff bars)    |
| <code>zeroForNA</code>         | Draw 0 instead of NA                                                               |
| <code>cex.axis</code>          | Axis font cex                                                                      |
| <code>legend</code>            | Should a legend be plotted ?                                                       |
| <code>groups</code>            | a factor - if specified the bars are colored according to the group they belong to |
| <code>order</code>             | plot sample values in descending order                                             |
| <code>...</code>               | Further arguments that get passed on to <a href="#">barplot</a> .                  |

**Details**

The function calls [barplot](#) with `y` and decorates it with error bars according to `yl` and `yh`.

**Value**

The function is called for its side effect, producing a plot.

**Author(s)**

Markus Ruschhaupt, Florian Hahne

**See Also**

[barplot](#)

**Examples**

```
y <- matrix(runif(80), ncol=5)
ym <- apply(y, 2, mean)
dy <- apply(y, 2, sd)*2/sqrt(nrow(y))
barploterrbar(ym, ym-dy, ym+dy, barcol="#0000c0", errcol="orange")
```

---

ddCtAbsolute

*absolute quantification for Taqman data*


---

**Description**

absolute quantification for Taqman data

**Usage**

```
ddCtAbsolute(raw.table, addData, type = "mean", ADD = -30.234, DIV = -1.6268, sampleInformation = NULL,
```

**Arguments**

|                   |                                                                                                                                                     |
|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| raw.table         | data frame. It must contain columns with the following names: 'Ct', 'Sample', 'Detector', 'Platename'. The column 'Ct' must contain numeric values. |
| addData           | add data                                                                                                                                            |
| type              | character of length 1. 'mean' or 'median' - which method should be used for the aggregation of the replicates                                       |
| ADD               | Add constant                                                                                                                                        |
| DIV               | Div constant                                                                                                                                        |
| sampleInformation | if specified it must be an object of class phenoData with a column named 'Sample'.                                                                  |
| toZero            | boolean - if there is only one replication should the error be treated as zero ? (only if 'type' is mean)                                           |
| filename          | character of length 1. The name of the file the warnings should be stored in.                                                                       |

**Value**

A an object of class `eSet`. The assayData has the following components: exprs, error, Ct, Ct.error, Difference, number\\_NA, number, Plate.

**Author(s)**

Markus Ruschhaupt <mailto:m.ruschhaupt@dkfz.de>

**References**

~put references to the literature/web site here ~

---

ddCtExpression-class    *ddCt Expression*

---

**Description**

This class is a subclass of ExpressionSet and represents objects which are produced by the ddCt algorithm in the ddCtExpression method

**Extends**

Class ExpressionSet, directly. Class eSet, by class "ExpressionSet", distance 2. Class VersionedBiobase, by class "ExpressionSet", distance 3. Class Versioned, by class "ExpressionSet", distance 4.

**Methods**

**Ct** signature(object = "ddCtExpression"): returns the Ct value of this ddCtExpressionobject

**CtErr** signature(object = "ddCtExpression"): returns the error number of the Ct value of this ddCtExpressionobject

**dCt** signature(object = "ddCtExpression"): returns the dCt value of this ddCtExpressionobject

**dCtErr** signature(object = "ddCtExpression"): returns the error number of the dCt value of this ddCtExpressionobject

**ddCt** signature(object = "ddCtExpression"): returns the ddCt value of this ddCtExpressionobject

**ddCtErr** signature(object = "ddCtExpression"): returns the error number of the ddCt value of this ddCtExpressionobject

**level** signature(object = "ddCtExpression"): returns the levels in this ddCtExpressionobject

**levelErr** signature(object = "ddCtExpression"): returns the error number of the levels in this ddCtExpressionobject

**numberCt** signature(object = "ddCtExpression"): returns the Ct number of this ddCtExpressionobject

**numberNA** signature(object = "ddCtExpression"): returns the NA number of this ddCtExpressionobject

**elist** signature(object = "ddCtExpression"): returns a data frame which represents this expression object

**elistWrite** signature(object = "ddCtExpression", file = "character"): writes ddCtExpression object into a file

**Author(s)**

Rudolf Biczok <mailto:r.biczok@dkfz.de>

**See Also**

[SDMFrame](#): reader for SDM files [elist](#), [elistWrite](#): utility functions for ddCtExpression objects  
[ddCtExpression](#): the method which invokes the ddCt algorithm

**Examples**

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                                package="ddCt"))

## call ddCtExpression method to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                        calibrationSample="Sample1",
                        housekeepingGenes=c("Gene1", "Gene2"))

## use getter methods
ddCt(result)
ddCtErr(result)
```

---

ddCtExpression-methods

*Apply the ddCt algorithm for a given data set*

---

**Description**

Apply the ddCt algorithm for a given data set

**Arguments**

|                    |                                                                                                                                                                              |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object             | SDMFrame Data object which holds a data set containing columns with the following names: 'Ct','Sample','Detector','Platenname'. The column 'Ct' must contain numeric values. |
| algorithm          | character. Name of the calibration samples.                                                                                                                                  |
| warningStream      | character of length 1. The name of the file the warnings should be stored in.                                                                                                |
| calibrationSample  | character. Name of the calibration samples.                                                                                                                                  |
| housekeepingGenes  | character. Name of the housekeeping genes.                                                                                                                                   |
| type               | character of length 1. 'mean' or 'median' - which method should be used for the aggregation of the replicates                                                                |
| sampleInformation  | if specified it must be an object of class phenoData with a column named 'Sample'.                                                                                           |
| toZero             | boolean - if there is only one replication should the error be treated as zero ? (only if 'type' is mean)                                                                    |
| efficiencies       | n.V.                                                                                                                                                                         |
| efficiencies.error | n.V.                                                                                                                                                                         |

**Value**

A an object of class [ddCtExpression](#).

**usage**

```
ddCtExpression(object, warningStream = "warning.output.txt", algorithm="ddCt" calibrationSample, housekeepingGenes, type="mean", sampleInformation=NULL, toZero=TRUE, efficiencies = NULL, efficiencies.error = NULL)
```

**Methods**

**object = "InputFrame"** An object of [InputFrame](#), constructed with the method [InputFrame](#)

**Author(s)**

Rudolf Biczok <mailto:r.biczok@dkfz.de>

**References**

Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta -Delta C(T)) Method. KJ Livak and TD Schmittgen, Methods, Vol. 25, No. 4. (December 2001), pp. 402-408

**See Also**

[InputFrame](#): reader for SDM files [ddCtExpression](#): representation for ddCt calculated expressions

**Examples**

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt", package="ddCt"))

## call ddCtExpression method from class SDMFrame
## to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                          calibrationSample="Sample1",
                          housekeepingGenes=c("Gene1", "Gene2"))

result
```

---

elistWrite-methods

*Write ddCtExpression object into data frame or files*

---

**Description**

ddCtExpression object contains a list of matrices as the results of [ddCt](#) method. elist combines these lists into one data frame, and elistWrite writes the data frame into file.

summary is a wrapper for the elist method

**Usage**

```
elist(object,...)
summary(object,...)
elistWrite(object,file,...)
```

**Arguments**

|        |                                             |
|--------|---------------------------------------------|
| object | an <a href="#">ExpressionSet</a> object.    |
| file   | output file.                                |
| ...    | additional arguments passed to write.table. |

**Details**

elist is a wrapper to `as(object, "data.frame")` function.

**Value**

A data frame or output file.

**Author(s)**

Jitao David Zhang <jitao\_david.zhang@roche.com>

**Examples**

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt", package="ddCt"))

## call ddCtExpression method from class SDMFrame
## to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                        calibrationSample="Sample1",
                        housekeepingGenes=c("Gene1", "Gene2"))

## call elist
elistResult <- elist(result)
elistResult
```

---

errBarchart-methods      *Draw barchart of relative expression level with error-bars*

---

**Description**

Draw barchart (with error-bars) of relative expression level represented in `ddCtExpression` object. The barchart is implemented as grid plot by `lattice` package, where each panel represents one sample and the relative expression values of detectors (as well as their standard errors) are depicted as bars.

Detectors which are not determined are marked by grey ND.

Two types of figures are supported: either condition on samples (`by="Sample"`) or on detectors (`by="Detector"`).

**Methods**

**object = "ddCtExpression", by="character"** An object of `ddCtExpression`, constructed with the method `ddCtExpression`

---

```
errBarchartParameter-class  
      Class "errBarchartParameter"
```

---

## Description

Parameter object for errBarchart

## Objects from the Class

Objects can be created by calls of the form `new("errBarchartParameter", ...)`. So far the object is only internally used, but in the near future it will be exported.

## Slots

**exprsUndeterminedLabel:** Object of class "character", specifying the text label when the expression level is 'Undetermined'

## Methods

**exprsUndeterminedLabel** signature(object = "errBarchartParameter"): getting the text label when the expression level is 'Undetermined'

**show** signature(object = "errBarchartParameter"): print method

## Note

So far it is only internally used

## Author(s)

Jitao David Zhang <jitao\_david.zhang@roche.com>

## Examples

```
## Internally used  
## param <- new("errBarchartParameter")  
## exprsUndeterminedLabel(param)
```

---

`getDir`*Auxillary functions for the executive scripts*

---

**Description**

`getDir` creates a directory in case it does not exist and returns the directory name.

**Usage**

```
getDir(dir, ...)
```

**Arguments**

|                  |                                                       |
|------------------|-------------------------------------------------------|
| <code>dir</code> | Directory name                                        |
| <code>...</code> | Other parameters passed to <a href="#">dir.create</a> |

**Details**

Auxillary functions

**Value**

`getDir` returns the directory name

**Author(s)**

Jitao David Zhang <jitao\_david.zhang@roche.com>

**Examples**

```
getDir(tempdir())
```

---

`InputFrame`*Build an InputFrame from a ReaderClass or a data frame*

---

**Description**

Generally an `InputFrame` is built from a `ReaderClass` (e.g. [InputReader](#)), or a `data.frame`. See the example below for building an object from a valid `data.frame`.

**Usage**

```
InputFrame(object)
```

**Arguments**

|                     |                                                                        |
|---------------------|------------------------------------------------------------------------|
| <code>object</code> | A <code>data.frame</code> with three columns: Sample, Detector, and Ct |
|---------------------|------------------------------------------------------------------------|

**Value**

A object of class InputFrame

**Author(s)**

Jitao David Zhang [mailto:jitao\\_david.zhang@roche.com](mailto:jitao_david.zhang@roche.com)

**Examples**

```
testDf <- data.frame(Sample=rep(paste("Sample", 1:3), each=2),
  Detector=rep(paste("Gene", 1:2), 3),
  Ct=30+rnorm(6))
testInputFrame <- InputFrame(testDf)
```

---

InputFrame-class

*Working with SDM files*


---

**Description**

The class InputFrame provides core functionalities to read gene and sample information from SDM files and calculate them with a ddCt algorithm.

The function InputFrame reads the data given in the columns 'Detector', 'Sample' and 'Ct' of the specified SDM output files and stores them as a data.frame. An additional column including the respective filename is added.

**Slots**

**coreData:** Object of class "data.frame": Holds all the required data extracted from the SDM file

**files:** Object of class "character" contains the source SDM files

**Methods**

**[,][,\$** signature(x = "InputFrame"): primitive accessors. Returns an object of InputFrame-class with the subset data.

**names** signature(x = "InputFrame"): returns the column names in this SDM object

**ddCtExpression** signature(object = "InputFrame"): runs a ddCt algorithm with this SDM object and returns a object of class [ddCtExpression](#)

**fileNames** signature(object="InputFrame"): returns the source SDM file names.

**detectorNames** signature(object = "InputFrame"): returns the detector names in this SDM object

**detectorNames<-** signature(object = "InputFrame", value = "character"): replaces the detector names in this SDM object

**sampleNames** signature(object = "InputFrame"): returns the sample names in this SDM object

**sampleNames**<- signature(object = "InputFrame", value = "character"): replaces the sample names in this SDM object

**uniqueDetectorNames** signature(object = "InputFrame"): returns a vector of unique detector names in this SDM object

**uniqueDetectorNames**<- signature(object = "InputFrame", target = "missing", value = "character"): replaces all detector names given by the 'names' attribute in 'value' with new detector names

**uniqueDetectorNames**<- signature(object = "InputFrame", target = "character", value = "character"): replaces all detector names given by 'target' with new detector names

**uniqueSampleNames**<- signature(object = "InputFrame", target = "missing", value = "character"): replaces all sample names given by the 'names' attribute in 'value' with new sample names

**uniqueSampleNames**<- signature(object = "InputFrame", target = "character", value = "character"): replaces all sample names given by 'target' with new sample names

**uniqueSampleNames** signature(object = "InputFrame"): returns a vector of unique sample names in this SDM object

**removeSample** signature(object = "InputFrame", sample="character"): removes the sample(s) specified from the InputFrame object

**replaceDetector** signature(object = "InputFrame", target="character", value="character"): replace the detectors equal to the target with the value. Both target and value can be vectors of the same length, then the replace takes place iteratively.

**replaceSample** signature(object = "InputFrame", target="character", value="character"): replace the samples equal to the target with the value. Both target and value can be vectors of the same length, then the replace takes place iteratively.

**show** signature(object="InputFrame"): pretty print of the InputFrame instance.

**rightCensoring** signature(object="InputFrame", threshold="numeric"): Right censoring the Ct value, which targets the data points above a certain value (threshold). High Ct values (higher than 40 or 45 by the rule of thumb) are often not accurate and may indicate too weak expression. The function performs the right censoring on the data and set the value above the threshold as NA (by default) or a given value. See the example.

**coreData** signature(object="InputFrame"): returns the data frame read from SDM file.

**coreData**<- signature(object="InputFrame"): replace the data frame read from SDM file.

**Ct** signature(object="InputFrame"): returns the Ct value of the SDM file.

**Ct** signature(object="InputFrame", value="numeric"): replace the Ct value in the object with the new values, and return the object.

### Author(s)

Rudolf Biczok <mailto:r.biczok@dkfz.de>, Jitao David Zhang <mailto:jitao\_david.zhang@roche.com>

### See Also

[SDMFrame](#) function reads in data from SDM files. Data from SDM files is used to construct [ddCtExpression](#) objects to analyze differential expression.

**Examples**

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                                package="ddCt"))

## you can also write
## sampdat <- new("SDMFrame",system.file("extdata", "Experiment1.txt",
##                                       package="ddCt"))

## use the getter methods
sampleNames(sampdat)

## or the overloaded primitive accessors
sampdat[1:3,"Sample"]

## see all unique samples
uniqueSampleNames(sampdat)

## replace all sample names 'Sample1' and 'Sample2' in sampdat
## with 'NewSample1' and 'NewSample2'
uniqueSampleNames(sampdat,c("Sample1","Sample2")) <- c("NewSample1","NewSample2")
uniqueSampleNames(sampdat)

## or use this syntax to replace the gene names
uniqueDetectorNames(sampdat) <- c(Gene1="NewGene1", Gene2="NewGene2")
uniqueDetectorNames(sampdat)

## remove sample or detector
removeSample(sampdat, "Sample1")
removeDetector(sampdat, "Gene1")

## replace sample or detector
replaceSample(sampdat, "Sample1", "Sample0")
replaceDetector(sampdat, "Gene1", "PLCG1")

## right censoring the data
rightCensoring(sampdat, 35)
rightCensoring(sampdat, 35, 35)
```

---

|                   |                     |
|-------------------|---------------------|
| InputReader-class | Class "InputReader" |
|-------------------|---------------------|

---

**Description**

Abstract factory for data input

**Objects from the Class**

A virtual Class: No objects may be created from it.

**Slots**

files: Input files

colmap: Column mapping

**Author(s)**

Rudolf Biczok and Jitao David Zhang

**Examples**

```
showClass("InputReader")
```

---

|                  |                                 |
|------------------|---------------------------------|
| QuantStudioFrame | <i>Read QuantStudio file(s)</i> |
|------------------|---------------------------------|

---

**Description**

Read QuantStudio file(s)

**Usage**

```
QuantStudioFrame(file)  
readQuantStudio(file)
```

**Arguments**

|      |                               |
|------|-------------------------------|
| file | Character vector of filenames |
|------|-------------------------------|

**Details**

This function reads the data given in the QuantStudio output files

**Value**

A object of class [SDMFrame](#)

**Author(s)**

Jitao David Zhang [mailto:jitao\\_david.zhang@roche.com](mailto:jitao_david.zhang@roche.com)

**Examples**

```
sampdat <- QuantStudioFrame(system.file("extdata", c("QuantStudio_File1.txt", "QuantStudio_File2.txt"), package="removeNTC-methods"))

## use the getter methods
sampleNames(sampdat)

## or the overloaded primitive accessors
sampdat[1:3,"Sample"]

## see all unique samples
uniqueSampleNames(sampdat)

## replace all sample names 'Sample1' and 'Sample2' in sampdat
## with 'NewSample1' and 'NewSample2'
uniqueSampleNames(sampdat,c("Sample1","Sample2")) <- c("NewSample1","NewSample2")
uniqueSampleNames(sampdat)

## or use this syntax to replace the gene names
uniqueDetectorNames(sampdat) <- c(Hs00559368_m1="Gene1", Hs02576168_g1="Gene2")
uniqueDetectorNames(sampdat)
```

---

|                   |                           |
|-------------------|---------------------------|
| removeNTC-methods | <i>Remove NTC samples</i> |
|-------------------|---------------------------|

---

**Description**

NTC stands for Non-template controls. This method remove the NTC samples from the input object.

**Methods**

signature(object = "ddCtExpression") An object hat has been analyzed with the ddCt method  
signature(object = "InputFrame") An input object

---

|                         |                                            |
|-------------------------|--------------------------------------------|
| replaceVectorByEquality | <i>REPLACE ITEMS OF VECTOR BY EQUALITY</i> |
|-------------------------|--------------------------------------------|

---

**Description**

The function replces (or updates) the items of a given vector by checking the equality with the target parameter. If found, the item will be replaced by the value parameter. The length of both target and value must be the same and could be longer than 1, in which case the replace will be iterated.

**Usage**

```
replaceVectorByEquality(vector, target, value)
```

**Arguments**

|        |                                                                                                             |
|--------|-------------------------------------------------------------------------------------------------------------|
| vector | A vector to be replaced. The items of the vector must be atom types, since the equality is checked by '=='. |
| target | targets to be replaced, could be either single or a vector                                                  |
| value  | values to be replaced at the positions of targets, must be of the same length of target                     |

**Details**

A warning will be prompted if any item in the target cannot be found

**Value**

A vector of the same length as the parameter vector

**Author(s)**

Jitao David Zhang

**See Also**

== for checking equality.

**Examples**

```
vector <- c("java", "perl", "python", "c#")
replaceVectorByEquality(vector, target="c#", value="c/c++")
replaceVectorByEquality(vector, target=c("c#", "perl"), value=c("c/c++", "R"))
```

---

SDMFrame

*Read an SDM file*


---

**Description**

Read an SDM file: Data Output File for SDS, Version 2.1

**Usage**

```
SDMFrame(file)
readSDM(file)
```

**Arguments**

|      |                               |
|------|-------------------------------|
| file | Character vector of filenames |
|------|-------------------------------|

## Details

This function reads the data given in the columns 'Detector', 'Sample' and 'Ct' of the specified SDM output file(s) and stores them as a data.frame. An additional column including the respective file-name is added.

This function is a wrapper for the SDMFrame constructor

## Value

A object of class `SDMFrame`

## Author(s)

Rudolf Biczok <mailto:r.biczok@dkfz.de>

## Examples

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                                package="ddCt"))

## you can also write
## sampdat <- new("SDMFrame",system.file("extdata", "Experiment1.txt",
##                                         package="ddCt"))

## or with
## sampdat <- readSDM(system.file("extdata", "Experiment1.txt",
##                                 package="ddCt"))

## use the getter methods
sampleNames(sampdat)

## or the overloaded primitive accessors
sampdat[1:3,"Sample"]

## see all unique samples
uniqueSampleNames(sampdat)

## replace all sample names 'Sample1' and 'Sample2' in sampdat
## with 'NewSample1' and 'NewSample2'
uniqueSampleNames(sampdat,c("Sample1","Sample2")) <- c("NewSample1","NewSample2")
uniqueSampleNames(sampdat)

## or use this syntax to replace the gene names
uniqueDetectorNames(sampdat) <- c(Gene1="NewGene1", Gene2="NewGene2")
uniqueDetectorNames(sampdat)
```

---

|                 |                                                                 |
|-----------------|-----------------------------------------------------------------|
| write.htmltable | <i>Write a data frame into an html table within a html page</i> |
|-----------------|-----------------------------------------------------------------|

---

**Description**

Write a 'data.frame' into an html table within a html page

**Usage**

```
write.htmltable(x, file, title = "", sortby = NULL, decreasing = TRUE, open = "wt")
```

**Arguments**

|            |                                                             |
|------------|-------------------------------------------------------------|
| x          | 'data.frame'                                                |
| file       | character. File name.                                       |
| title      | character. Title of html page                               |
| sortby     | character. Name of column by which to sort the table rows   |
| decreasing | logical. Should the sort order be increasing or decreasing? |
| open       | character. This argument is passed onto 'file'              |

**Value**

The function is called for its side effect: writing a file

**Author(s)**

Wolfgang Huber

---

|                   |                                                     |
|-------------------|-----------------------------------------------------|
| writeSimpleTabCsv | <i>Write a data frame into a tab delimited file</i> |
|-------------------|-----------------------------------------------------|

---

**Description**

Write a 'data.frame' into a tab delimited file (not quoted and no-row-name TSV file)

**Usage**

```
writeSimpleTabCsv(x, file, ...)
```

**Arguments**

|      |                                               |
|------|-----------------------------------------------|
| x    | 'data.frame'                                  |
| file | character. File name.                         |
| ...  | Additional arguments passed onto the function |

**Value**

The function is called for its side effect: writing a file

**Author(s)**

Wolfgang Huber

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