

Package ‘dpcR’

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Type Package

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Description Analysis, visualisation and simulation of digital polymerase chain reaction (dPCR) (Burdukiewicz et al. (2016) <doi:10.1016/j.bdq.2016.06.004>). Supports data formats of commercial systems (Bio-Rad QX100 and QX200; Fluidigm BioMark) and other systems.

License GPL-3

URL <https://github.com/michbur/dpcR>

BugReports <https://github.com/michbur/dpcR/issues>

Depends R (>= 3.0.0), methods

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Maintainer Michal Burdukiewicz <michalburdukiewicz@gmail.com>

Collate 'AUCtest.R' 'BioradCNV.R' 'White.R' 'adpcr2panel.R' 'adpcr2ppp.R' 'binarize.R' 'classes.R' 'bind_dpcr.R' 'bioamp.R' 'calc_breaks.R' 'calc_coordinates.R' 'calc_lambda.R' 'compare_dens.R' 'count_test-class.R' 'create_dpcr.R' 'dPCRmethyl.R' 'ddpcRquant.R' 'df2dpcr.R' 'dpcR-package.R' 'dpcReport_gui.R' 'dpcr2df.R' 'dpcr_calculator.R' 'dpcr_density.R' 'dpcr_density_gui.R' 'dpcr_density_table.R'

'extract_run.R' 'fit_adpqr.R' 'fl.R' 'get_k_n.R' 'hsm.R'
 'limit_cq.R' 'many_peaks.R' 'modlist.R' 'moments.R' 'num2int.R'
 'pds.R' 'pds_raw.R' 'plot_distr.R' 'plot_panel.R'
 'plot_vf_circ.R' 'plot_vic_fam.R' 'print_summary.R' 'qdpcr.R'
 'qpcr2pp.R' 'qpcr_analyser.R' 'read_amp.R' 'read_dpcr.R'
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 'sim_adpqr.R' 'sim_ddpqr_bkm.R' 'sim_dpcr.R' 'simulations.R'
 'six_panels.R' 'summary_dpcr.R' 'test_counts.R'
 'test_counts_gui.R' 'test_panel.R' 'test_peaks.R'
 'test_pooled.R' 'valid_amp.R' 'y_val_conf.R'

Author Michal Burdukiewicz [cre, aut]
 (<<https://orcid.org/0000-0001-8926-582X>>),
 Stefan Roediger [aut] (<<https://orcid.org/0000-0002-1441-6512>>),
 Bart Jacobs [aut],
 Piotr Sobczyk [ctb],
 Andrej-Nikolai Spiess [ctb]

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Description

The dpcR package is a collection of functions for a digital Polymerase Chain Reaction (dPCR) analysis. dPCR comprises methods to quantify nucleic acids, copy number variations (CNV), homo- and heterozygosity, as well as rare mutations (including single nucleotide polymorphisms (SNP)). The chemical basis of dPCR is similar to conventional PCR but the reaction-mix is divided into hundredths to thousands of small compartments with parallel amplifications reactions. The analysis is based on counting the number of positive compartments and relating it to the total number of compartments by means of Poission statistics which enables an absolute quantification.

Details

The package includes plot functions, summary functions, data sets and simulations for dPCR and customizable GUI for droplet digital PCRs and array-based digital PCRs. We aim to include all statistical approaches published in peer-review literature and additional selected sources of expertise currently available. We intent to make these methods available to the scientific community in an open and cross-platform environment. Using the naming convention derived from the MIQE Guidelines for digital PCR, we hope to become a reference to a unified nomenclature in dPCR.

The package is primarily targeted at researchers who wish to use it with an existing technology or during the development of novel digital PCR systems. In addition the dpcR package provides interactive tools that can be used to better learn about digital PCR concepts and data interpretation.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

Maintainer: Michal Burdukiewicz <michalburdukiewicz@gmail.com>

References

Huggett J, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, Bustin SA *The Digital MIQE Guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments* Clinical Chemistry, 2013. 59(6): p.892-902.

Vogelstein B, Kinzler KW, *Digital PCR*. PNAS, 1999. 96(16): p. 9236-9241.

See Also

[qpcR.news](http://michbur.github.io/pcRuniveRsum/), <http://michbur.github.io/pcRuniveRsum/>.

Examples

```
adpcr <- sim_adpcr(m = 400, n = 765, times = 20, pos_sums = FALSE, n_panels = 1)
plot_panel(adpcr, col = "green")
pos_chambers <- sum(adpcr > 0)
dpcr_density(k = pos_chambers, n = 765)
```

adpcr-class

Class "adpcr" - end-point array digital PCR experiments

Description

A class specifically designed to contain results from end-point array digital PCR experiments. Data is represented as matrix, where each column describes different experiment. Type of data in all columns is specified in slot "type". Inherits from [dpcr](#).

Details

For more in-depth explanation of digital PCR data structure, see [dpcr](#).

Slots

col_names "character" vector naming columns in the array.

row_names "character" vector naming rows in the array.

row_id "integer" vector providing row indices of all runs.

col_id "integer" vector providing column indices of all runs.

panel_id "factor" naming the panel to which experiment belong.

Author(s)

Michal Burdukiewicz.

See Also

Data management: [adpcr2panel](#), [bind_dpcr](#), [extract_run](#).

Plotting: [plot_panel](#).

Tests: [test_panel](#).

Simulation: [sim_adpcr](#).

Real-time array digital PCR: [rtadpcr](#).

Droplet digital PCR: [dpcr](#).

Examples

```
rand_array <- sim_adpcr(400, 1600, 100, pos_sums = FALSE, n_panels = 5)
one_rand_array <- extract_run(rand_array, 1)
plot_panel(one_rand_array, 40, 40)
```

adpcr2panel

Convert adpcr object to array

Description

Converts [adpcr](#) object into the list of array-like matrices.

Usage

```
adpcr2panel(input, breaks = FALSE)
```

Arguments

input object of the [adpcr](#) class.
 breaks if TRUE, the data is divided into intervals.

Value

A named list of length equal to the number of arrays in the input. Each element is a single array in matrix-like form, where dimensions are set exactly as in case of the real plate. Names of the list corresponds to the names of assays ("tnp" data) or runs (any other type of [adpcr](#) data).

The matrices contain values from array, either integers (when `use_break` is FALSE) or characters (when `use_break` is TRUE).

Author(s)

Michal Burdukiewicz.

Examples

```
#generate data
ttest <- sim_adpcr(m = 400, n = 765, times = 20, pos_sums = FALSE,
                 n_panels = 3)
#convert object into three arrays
arrays <- adpcr2panel(ttest)
length(arrays)
#print an array
arrays[[1]]
```

adpcr2ppp

Convert adpcr to ppp

Description

Converts [adpcr](#) object to the list of [ppp.objects](#).

Usage

```
adpcr2ppp(input, marks = TRUE, plot = FALSE)
```

Arguments

input Object of the [adpcr](#) class containing data from one or more panels.
 marks If TRUE, marks values for non-empty partitions. See [ppp](#) for more in-depth description.
 plot If TRUE, array is plotted.

Details

Each array is independently converted by `ppp` function. marks attached to each point represent values contained by the `adpcr` object.

Value

A list containing objects with class `ppp.object` with the length equal to the number of arrays (minimum 1).

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

`ppp.object`, `ppp`.

Examples

```
many_panels <- sim_adpcr(m = 400, n = 765, times = 1000, pos_sums = FALSE,
                        n_panels = 5)

# Convert all arrays to ppp objects
adpcr2ppp(many_panels)

# Convert all arrays to ppp objects and get third plate
third_plate <- adpcr2ppp(many_panels)[[3]]

# Convert only third plate to ppp object
third_plate2 <- adpcr2ppp(extract_run(many_panels, 3))

# Check the class of a new object
class(third_plate2)

# It's a list with the length 1. The third plate is a first element on this
#list
class(third_plate2[[1]])
```

binarize

Binarize digital PCR data

Description

Transforms multinomial (number of molecules per partition) or continuous (fluorescence) digital PCR data to binary (positive/negative partition) format.

Usage

```
binarize(input)
```

Arguments

input object of the class `adpcr` or `dpcr` with one of following types: "ct", "fluo" or "nm".

Value

object of the class `adpcr` or `dpcr` (depending on input) with type "np".

Author(s)

Michal Burdukiewicz.

Examples

```
#adpcr object
rand_array <- sim_adpcr(200, 300, 100, pos_sums = FALSE, n_panels = 1)
binarize(rand_array)

#dpcr object
rand_droplets <- sim_dpcr(200, 300, 100, pos_sums = FALSE, n_exp = 1)
binarize(rand_droplets)
```

bind_dpcr-methods *Bind dpcr objects*

Description

A convenient wrapper around `cbind` and `rbind` tailored specially for binding multiple objects containing results from digital PCR experiments.

Usage

```
bind_dpcr(input, ...)
```

Arguments

input an object of class `adpcr` or `dpcr` or a list.
... objects of class `adpcr` or `dpcr`. See Details. If input is a list, ignored.

Details

In case of `adpcr` or `dpcr` objects, `bind_dpcr` works analogously to `cbind`, but without recycling. In case on unequal length, shorter objects will be filled in with additional NA values. The original length is always preserved in `n` slot.

`bind_dpcr` automatically names binded experiments using format `x.y`, where `x` is number of object passed to function and `y` is a number of experiment in a given object.

Value

An object of class `adpcr` or `dpcr`, depending on the input.

Note

Because `bind_dpcr` calls `do.call` function, binding together at the same time more than 500 objects can lead to 'stack overflow' error.

Author(s)

Michal Burdukiewicz

See Also

Opposite function: `extract_run`

Examples

```
bigger_array <- sim_adpcr(400, 765, 1000, pos_sums = FALSE, n_panels = 5)
smaller_array <- sim_adpcr(100, 700, 1000, pos_sums = FALSE, n_panels = 3)
bound_arrays <- bind_dpcr(bigger_array, smaller_array)

smaller_droplet <- sim_dpcr(m = 7, n = 20, times = 5, n_exp = 2)
bigger_droplet <- sim_dpcr(m = 15, n = 25, times = 5, n_exp = 4)
biggest_droplet <- sim_dpcr(m = 15, n = 35, times = 5, n_exp = 1)
bound_droplets <- bind_dpcr(smaller_droplet, bigger_droplet, biggest_droplet)
```

bioamp

A function to analyze plot the raw data from a Bio-Rad droplet digital PCR experiment

Description

`bioamp` is a function to plot and analyze the amplitude data of a Bio-Rad droplet digital PCR experiment.

Usage

```
bioamp(data = data, amp_x = 1, amp_y = 2, cluster = 3,
       robust = TRUE, plot = TRUE, stat = TRUE,
       xlab = "Assay 1 Amplitude", ylab = "Assay 2 Amplitude", ...)
```

Arguments

<code>data</code>	object of class what containing the amplitude data.
<code>amp_x</code>	is the first amplitude channel (x-axis).
<code>amp_y</code>	is the second amplitude channel (y-axis).
<code>cluster</code>	are the clusters of the plot. The number indicates the column of a table, which contains the cluster information.
<code>robust</code>	Is the method used to calculate the location (mean or median) and dispersion (standard deviation or median absolute deviation).
<code>plot</code>	logical, if TRUE, the plot is printed.
<code>stat</code>	logical, if TRUE, the statistics of the droplet digital PCR experiment are calculated.
<code>xlab</code>	x-label of the plot.
<code>ylab</code>	y-label of the plot.
<code>...</code>	other arguments passed to the <code>plot</code> function (see <code>plot.default</code> for details).

Author(s)

Stefan Roediger, Michal Burdukiewicz

Examples

```
par(mfrow = c(1,2))
bioamp(data = pds_raw[["D01"]], main = "Well D01", pch = 19)
bioamp(data = pds_raw[["D02"]], main = "Well D02", pch = 19)
par(mfrow = c(1,1))
```

BioradCNV

Copy number variation experiment

Description

Copy Number Variation of MYC Gene for seven patients measured using QX200 (Bio-Rad)

Format

An object of class `adpccr` with "tnp" type containing four runs from seven experiments (four runs per each experiment).

Author(s)

Robert M. Dorazio, Margaret E. Hunter.

Source

Dorazio RM, Hunter ME, *Statistical Models for the Analysis and Design of Digital Polymerase Chain Reaction (dPCR) Experiments*. Analytical Chemistry 2015. 87(21): p.10886-10893

Examples

```
data(BioradCNV)
summary(BioradCNV)
```

calc_coordinates	<i>Calculate Array Coordinates</i>
------------------	------------------------------------

Description

Calculates coordinates of points on plot to represent a digital PCR array.

Usage

```
calc_coordinates(array, half)
```

Arguments

array	A single array, as generated by adpcr2panel .
half	If left or right, every well is represented only by the adequate half of the rectangle.

Value

Returns two sets of coordinates of each microfluidic well: coords is a list of coordinates suitable for usage with functions from [graphics](#) package. The second element is a data frame of coordinates useful for users utilizing [ggplot2](#) package.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[plot_panel](#) - plots [adpcr](#) data. [adpcr2panel](#) - converts [adpcr](#) object to arrays.

compare_dens	<i>Plot and Compare Densities</i>
--------------	-----------------------------------

Description

Plot empirical and theoretical density of the result of the digital PCR experiment.

Usage

```
compare_dens(input, moments = TRUE, ...)
```

Arguments

input	object of class <code>dpcr</code> containing only one run.
moments	logical, if TRUE, both theoretical and empirical moments are printed on the plot.
...	other arguments passed to the <code>plot</code> function.

Author(s)

Michal Burdukiewicz.

See Also

[moments](#) is used to calculate moments of Poisson distribution.

Examples

```
adpcr_big <- sim_adpcr(m = 35, n = 40, times = 50, pos_sums = FALSE, n_panels = 1)
compare_dens(adpcr_big, moments = TRUE)
```

count_test	<i>Class "count_test"</i>
------------	---------------------------

Description

Objects of this class are created by [test_counts](#).

Usage

```
## S4 method for signature 'count_test'  
summary(object)  
  
## S4 method for signature 'count_test'  
coef(object)  
  
## S4 method for signature 'count_test'  
show(object)  
  
## S4 method for signature 'count_test,ANY'  
plot(x, aggregate = FALSE, nice = TRUE)
```

Arguments

object	of class count_test.
x	object of class count_test.
aggregate	logical, if TRUE experiments are aggregated according to their group.
nice	logical, if TRUE a more aesthetically pleasing (but harder to customize) version of the plot is created.

Details

In case of the aggregated plot, mean confidence intervals for groups are presented as dashed lines.

Methods (by generic)

- `summary`: Summary statistics of assigned groups.
- `coef`: Extract coefficients of groups.
- `show`: Print both `group_coef` and `test_res`.
- `plot`: plots mean number of molecules per partition and its confidence intervals.

Slots

group_coef "data.frame" containing experiments, groups to which they belong and calculated values of rate (λ).

test_res "matrix" containing result of multiple comparisons t-test.

model "character" name of GLM used to compare experiments.

Author(s)

Michal Burdukiewicz.

See Also

[test_counts](#).

create_dpcr	<i>Create dpcR object</i>
-------------	---------------------------

Description

Creates [adpcr](#) and [dpcr](#) objects from data.

Usage

```
create_dpcr(data, n, exper = "Experiment 1", replicate = NULL,
  assay = "Unknown", type, v = 1, uv = 0, threshold = NULL, adpcr,
  col_names = NULL, row_names = NULL, panel_id = NULL)
```

Arguments

data	a numeric vector or matrix of data from dPCR experiments. Data frames will be converted to matrices.
n	integer equal to number of partitions.
exper	The id of experiments.
replicate	The id of technical replicates.
assay	The name or id of assays.
type	Object of class "character" defining type of data. Could be "nm" (number of molecules per partition), "tnp" (total number of positive wells in the panel), "fluo" (fluorescence), "np" (status (positive (1) or negative(0)) of each droplet) or "ct" (threshold cycle).
v	The volume of partitions [nL].
uv	The volume uncertainty of partitions [nl].
threshold	numeric value giving the threshold above which droplet is counted as positive. Ignored if adpcr is TRUE.
adpcr	logical. If TRUE, function creates adpcr object. If FALSE, function creates dpcr object.
col_names	character vector of column names in array. Ignored if not adpcr.
row_names	character vector of row names in array. Ignored if not adpcr.
panel_id	factor vector of panel IDs (or names). Ignored if not adpcr.

Details

This constructor function assists in creation of objects used by other functions of the package. It is also responsible for checking the correctness of arguments.

A warning is prompted whenever any of arguments is converted to other type.

Value

An [adpcr](#) or [dpcr](#) object.

Note

create_dpcr is preferred to calling directly [new](#).
Currently only end-point measurements are supported.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

Streamlined, but more limited version: [df2dpcr](#)

Examples

```
# Droplet digital PCR example
sample_runs <- matrix(rpois(60, lambda = 1.5), ncol = 2)
ddpcr1 <- create_dpcr(sample_runs[,1], n = 30L,
  threshold = 1, type = "nm", adpcr = FALSE)
ddpcr2 <- create_dpcr(sample_runs[,2], n = 30L,
  threshold = 1, type = "nm", adpcr = FALSE)
plot_vic_fam(ddpcr1, ddpcr2)

# Array digital PCR example
sample_adpcr <- create_dpcr(rpois(765, lambda = 0.8), n = 765L,
  type = "nm", adpcr = TRUE)
plot_panel(sample_adpcr, 45, 17)
```

ddpcRquant

Quantify droplets

Description

Cluster raw data from QX100 and QX200 systems.

Usage

```
ddpcRquant(path, threshold.int = 0.9995, reps = 10, blocks = 150,
  threshold.manual = NULL)
```

Arguments

path	character path to directory with raw data.
threshold.int	numeric probability of the threshold quantile.
reps	numeric vector representing the number of replications.
blocks	numeric vector representing the number of blocks.

threshold.manual

if numeric, the value is used as the threshold. If NULL, the threshold is calculated automatically.

Value

dpcr object.

Note

This function is a modification of code was implemented using the code found on <http://www.ddpcrquant.ugent.be/>.

Author(s)

Wim Trypsteen, Matthijs Vynck, Jan De Neve, Pawel Bonczkowski, Maja Kiselinova, Eva Malatinkova, Karen Vervisch, Olivier Thas, Linos Vandekerckhove, Ward De Spiegelaere.

References

Trypsteen W, Vynck M, De Neve J, Bonczkowski P, Kiselinova M, Malatinkova E, Vervisch K, Thas O, Vandekerckhove L, De Spiegelaere W, *ddpcRquant: Threshold Determination for Single Channel Droplet Digital PCR Experiments*. Analytical and Bioanalytical Chemistry 2015. 407(19): p.5827-34.

df2dpcr

Convert data.frame to dpcr object

Description

Converts `data.frame` object to to `adpcr` or `dpcr` object. The resulting object will have "tnp" type.

Usage

```
df2dpcr(df)
```

Arguments

df data frame with specified column names. See Details.

Details

The data frame must have REDF structure. It means that data must contain following columns with exactly specified names:

experiment names of experiments

replicate indices of replicates

assay names of assays

k number of positive partitions
n total number of partitions
v volume of partition (nL)
uv uncertainty of partition's volume (nL)
threshold partitions with k equal or higher than threshold are treated as positive.

There are also one optional column:

panel_id indices of panels

If the additional column is present, the resulting object has [adpcr](#) type.

Value

An object of [adpcr](#) or [dpcr](#) type, depends on the presence of additional column with panel indices (see Details).

Author(s)

Michal Burdukiewicz, Stefan Roediger

See Also

Flexibly create [dpcr](#) objects: [create_dpcr](#) Inverse function: [dpcr2df](#)

Examples

```
dat <- data.frame(experiment = factor(rep(paste0("Experiment", 1L:2), 3)),
  replicate = c(1, 1, 2, 2, 3, 3),
  assay = "Assay1",
  k = c(55, 121, 43, 150, 70, 131),
  n = 765,
  v = 1,
  uv = 0)
df2dpcr(dat)
```

dpcr-class

Class "dpcr" - general digital PCR

Description

A class containing results of any digital PCR experiment. Type of data in all columns is specified in slot "type".

Details

Possible type values of dpcr objects:

1. "ct": cycle threshold of each partition,
2. "fluo": fluorescence of each partition,
3. "nm": number of molecules in each partition,
4. "np": status (positive (1) or negative(0)) of each partition,
5. "tnp": total number of positive partitions in the run (single value per each run, not per partition).

Digital PCR data is always a matrix, where columns and rows represent respectively runs and data points. For example, matrix with 2 columns and 765 rows means two runs with 765 data points each. In case of "tnp" data, each run is represented by only one measurement, the count of all positive partitions.

The number of partitions is defined in slot n. In the previous example, two runs have 765 data points, but they can have less detected partitions (for example some reads may be not available). In this case, the data point will have value NA.

The structure of dpcr class is described more deeply in the vignette.

Slots

.Data matrix data from digital PCR experiments. See Details.

n integer equal to the number of partitions in each run.

exper factor the id or name of experiments.

replicate factor the id or name of replicates.

assay factor the id or name of the assay.

v "numeric" volume of the partition [nL].

uv "numeric" uncertainty of the volume of the partition [nL].

threshold "numeric" value specifying the threshold. Partition with the value equal or bigger than threshold are considered positive.

type Object of class "character" defining type of data. See Details.

Note

This class represent the most general droplet-based digital PCR. In more specific cases, the user is directed to other classes: [adpcr](#), where results can be placed over a plate, [qdpcr](#) where digital assay is based on multiple qPCR experiments and [rtadpcr](#), where data points represent the status of partitions measured in the real time.

Author(s)

Michal Burdukiewicz.

Examples

```
dpcr_fluo <- sim_dpcr(m = 10, n = 20, times = 5, fluo = list(0.1, 0))  
plot(dpcr_fluo)
```

```
dpcr <- sim_dpcr(m = 10, n = 20, times = 5)
```

dpcr2df-methods	<i>Convert dpcr object to data frame</i>
-----------------	--

Description

Converts [adpcr](#) or [dpcr](#) object to [data.frame](#) object.

Usage

```
dpcr2df(input)
```

Arguments

input [adpcr](#) or [dpcr](#) object.

Value

data frame with 5 (if input was [dpcr](#)) or 8 columns (if input was [adpcr](#)).

Author(s)

Michal Burdukiewicz, Stefan Roediger

See Also

Inverse function: [df2dpcr](#)

Examples

```
dpcr2df(six_panels)
```

dpcReport

Digital PCR Report Graphical User Interface

Description

Launches graphical user interface that generates reports from digital PCR data.

Usage

```
dpcReport()
```

Warning

Any ad-blocking software may cause malfunctions.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

dPCRmethyl

Methylated human gDNA

Description

Results of dPCR experiment: serial dilutions (0.25, 0.50, 0.75, 1.00) of methylated human gDNA. For each concentration level, three samples were measured using QX100 (Bio-Rad).

Format

A `adpccr` object

Author(s)

Mario Menschikowski, Stefan Roediger, Michal Burdukiewicz

Source

Mario Menschikowski group / Institute of Clinical Chemistry and Laboratory Medicine, TU-Dresden, Dresden, Germany

Examples

```
summary(dPCRmethyl)
```

dpcr_density	<i>Calculate Density of Single dPCR Run</i>
--------------	---

Description

Calculates and plots the density of the number of positive molecules or the average number of molecules per partition. Can be used for both array digital PCR and droplet digital PCR.

Usage

```
dpcr_density(k, n, average = FALSE, methods = "wilson",
             conf.level = 0.95, plot = TRUE, bars = FALSE, ...)
```

Arguments

k	Total number of positive molecules.
n	Total number of partitions.
average	If TRUE, calculates density of the average number of molecules per partition. If FALSE, instead performs calculations for the total number of positive molecules.
methods	Method for calculating the confidence interval. Possible values are: "wilson", "agresti-coull", "exact", "prop.test", "profile", "lrt", "asymptotic", "bayes", "cloglog", "logit", "probit". Default value is "wilson". See Details.
conf.level	The level of confidence to be used in the confidence interval. Values from 0 to 1 and -1 to 0 are acceptable.
plot	If TRUE, plots density plot.
bars	plot on density plot bars for discrete values of lambda.
...	Additional arguments send to plot function.

Value

A data frame with one row containing bounds of the confidence intervals and a name of the method used to calculate them.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

References

Brown, Lawrence D., T. Tony Cai, and Anirban DasGupta. *Confidence Intervals for a Binomial Proportion and Asymptotic Expansions*. The Annals of Statistics 30, no. 1 (February 2002): 160–201.

See Also

Computation of confidence intervals: [binom.confint](#),

The browser-based graphical user interface for this function: [dpcr_density_gui](#).

Examples

```
# Calculate the average number of molecules per partition and show the area
# of the confidence interval (left plot) and the area within the
# confidence interval
par(mfrow = c(1,2))
dpcr_density(k = 25, n = 55, average = TRUE, methods = "wilson",
             conf.level = 0.95)
dpcr_density(k = 25, n = 55, average = TRUE, methods = "wilson",
             conf.level = -0.95)
par(mfrow = c(1,1))

# By setting average to FALSE the total number of positive molecules is
# calculated
dpcr_density(k = 25, n = 55, average = FALSE, methods = "wilson",
             conf.level = 0.95)
```

dpcr_density_gui

Digital PCR Density Graphical User Interface

Description

Launches graphical user interface that allows calculating density of positive partitions distribution.

Usage

```
dpcr_density_gui()
```

Warning

Any ad-blocking software may cause malfunctions.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[dpcr_density](#).

dpcr_density_table *Calculate Density of Multiple dPCR runs*

Description

Calculates the density of the number of positive molecules or the average number of molecules per partition of `dpcr` objects.

Usage

```
dpcr_density_table(input, average = FALSE, methods = "wilson",  
  conf.level = 0.95)
```

Arguments

<code>input</code>	an object of class <code>dpcr</code> .
<code>average</code>	If TRUE, calculates density of the average number of molecules per partition. If FALSE, instead performs calculations for the total number of positive molecules.
<code>methods</code>	Method for calculating the confidence interval. Possible values are: "wilson", "agresti-coull", "exact", "prop.test", "profile", "lrt", "asymptotic", "bayes", "cloglog", "logit", "probit". Default value is "wilson". See Details.
<code>conf.level</code>	The level of confidence to be used in the confidence interval. Values from 0 to 1 and -1 to 0 are acceptable.

Value

A list (with the length equal to the number of runs in `input`) of data frames containing densities and borders of confidence intervals.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[dpcr_density](#) for easy analysis and plots of single runs.

Examples

```
dens <- dpcr_density_table(six_panels)  
  
# create plot using ggplot2  
library(ggplot2)  
  
ggplot(dens[["Experiment2.2"]], aes(x = x, y = y)) +  
  geom_line() +
```

```
geom_area(aes(fill = !(conf_up | conf_low))) +  
scale_y_continuous("Density") +  
scale_fill_discrete("0.95 CI")
```

extract_dpcr

Extract Assays or Experiments

Description

Extract all runs belonging to specific assay or experiment(s) from a coded `dpcr` object while preserving all other attributes.

Usage

```
extract_dpcr(input, id_exper = NULL, id_assay = NULL)
```

Arguments

input	object of the class <code>adpcr</code> or <code>dpcr</code> .
id_exper	vector of indices or names of experiments. Must be NULL if <code>id_assay</code> is specified.
id_assay	vector of indices or names of assays. Must be NULL if <code>id_exper</code> is specified.

Value

The object of the input's class (`adpcr` or `dpcr`).

Note

The standard `Extract` operator `x[i]` treats `dpcr` objects as `matrix` and extracts values without preserving other attributes of the object.

Author(s)

Michal Burdukiewicz.

See Also

Extract run(s): `extract_run`.

Examples

```
#extract using only experiment's ID
extract_dpcr(six_panels, id_exper = 1)

#extract using assay name
extract_dpcr(six_panels, id_assay = "MYC")

#extract using multiple names
extract_dpcr(six_panels, id_exper = c("Experiment1", "Experiment2"))
```

extract_run	<i>Extract Digital PCR Run</i>
-------------	--------------------------------

Description

Extract runs from a coded [dpcr](#) object while preserving all other attributes.

Usage

```
extract_run(input, id)
```

Arguments

input	object of the class adpcr or dpcr .
id	vector of indices or names of runs.

Details

The `extract_run` function allows to choose one or more panels from an object of the [adpcr](#) or [dpcr](#) class and save it without changing other attributes. It is the most recommended method of extracting a subset from an array of panels, because it preserves class and structure of the object in contrary to standard operator [Extract](#).

Value

The object of the input's class ([adpcr](#) or [dpcr](#)).

Note

The standard [Extract](#) operator `x[i]` treats `dpcr` objects as `matrix` and extracts values without preserving other attributes of the object.

Author(s)

Michal Burdukiewicz.

See Also

Opposite function: [bind_dpcr](#) Extract multiple runs belonging to an experiment of assay: [extract_dpcr](#)

Examples

```
#sample extracting
panels <- sim_adpcr(10, 40, 1000, pos_sums = FALSE, n_panels = 50)
single_panel <- extract_run(panels, 5)
random_three <- extract_run(panels, sample.int(ncol(panels), 3))
all_but_one <- extract_run(panels, -5)

#the same for fluorescence data
fluos <- sim_dpcr(10, 40, 1000, pos_sums = FALSE, n_exp = 50,
                fluo = list(0.1, 0))
single_fluo <- extract_run(fluos, 5)
```

 limit_cq

Limit Cy0 values

Description

Calculates the Cq values of a qPCR experiment within a defined range of cycles. The function can be used to extract Cq values of a chamber based qPCR for conversion into a dPCR experiment. All Cq values are obtained by Second Derivative Maximum or by Cy0 method (Guescini et al. (2008)).

Usage

```
limit_cq(data, cyc = 1, fluo = NULL, Cq_range = c(1, max(data[cyc])),
        model = 15, SDM = TRUE, pb = FALSE)
```

Arguments

data	a dataframe containing the qPCR data.
cyc	the column containing the cycle data. Defaults to first column.
fluo	the column(s) (runs) to be analyzed. If NULL, all runs will be considered (equivalent of (1L:ncol(data))[-cyc]).
Cq_range	is a user defined range of cycles to be used for the determination of the Cq values.
model	is the model to be used for the analysis for all runs. Defaults to '15' (see pcrfit).
SDM	if TRUE, Cq is approximated by the second derivative method. If FALSE, Cy0 method is used instead.
pb	if TRUE, progress bar is shown.

Details

The Cq_range for this function can be defined by the user. The default is to take all amplification curves into consideration. However, under certain circumstances it is recommended to define a range. For example if amplifications are positive in early cycle numbers (less than 10).

Approximated second derivative is influenced both by how often interpolation takes place in each data interval and by the smoothing method used. The user is encouraged to seek optimal parameters for his data himself. See [inder](#) for details.

The calculation of the Cy0 value (equivalent of Cq) is based on a five-parameter function. From experience this function leads to good fitting and avoids overfitting of critical data sets. Regardless, the user is recommended to test for the optimal fitting function himself (see [mselect](#) for details).

Value

A data frame with two columns and number of rows equal to the number of runs analyzed. The column Cy0 contains calculated Cy0 values. The column in_range contains adequate logical constant if given Cy0 value is in user-defined Cq_range.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

References

Guescini M, Sisti D, Rocchi MB, Stocchi L & Stocchi V (2008) *A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition*. BMC Bioinformatics, 9: 326.

Ruijter JM, Pfaffl MW, Zhao S, et al. (2013) *Evaluation of qPCR curve analysis methods for reliable biomarker discovery: bias, resolution, precision, and implications*. Methods, San Diego Calif 59:32–46.

See Also

SDM method: [inder](#), [summary.der](#).

Cy0 method: [mselect](#), [efficiency](#).

Examples

```
library(qpcR)
test <- cbind( reps[1L:45, ], reps2[1L:45, 2L:ncol(reps2)], reps3[1L:45,
  2L:ncol(reps3)] )

# results.dPCR contains a column with the Cy0 values and a column with
# converted values.
Cq.range <- c(20, 30)
ranged <- limit_cq(data = test, cyc = 1, fluo = NULL,
  Cq_range = Cq.range, model = 15)
# Same as above, but without Cq.range
no_range <- limit_cq(data = test, cyc = 1, fluo = NULL, model = 15)
```

```
# Same as above, but only three columns
no_range234 <- limit_cq(data = test, cyc = 1, fluo = c(2:4), model = 15)
```

many_peaks	<i>Artificial data set with many peaks</i>
------------	--

Description

A set of data from an artificial droplet flow experiment. It contains various kinds of peaks - positive peaks, negative peaks and peak-like noise.

Format

A data frame with 493 observations on the following 2 variables.

t a numeric vector

F fluorescence value

Examples

```
data(many_peaks)
plot(many_peaks, type = "b")
```

moments-methods	<i>Calculate Moments of Poisson Distribution</i>
-----------------	--

Description

Computes moments of a Poisson distribution. The calculations are based on values of positive and total partitions or the theoretical lambda value.

Usage

```
moments(input)
```

Arguments

input a vector with two elements (the first element is treated as a number of positive partitions and the second as a number of total partitions) or a matrix with two columns (first columns contains numbers of positive partitions and the second total numbers of total partitions) or an object of class `dpcr` .

Value

A data frame with four columns: name of the experiment, name of the replicate, method of computation (theoretical or empirical), name of the moment and the value of the moment. The theoretical moments are computed using the lambda value and the empirical using the sample values.

Note

Four first moments of a Poisson distribution.

Mean : λ .

Variance: λ .

Skewness: $\sqrt{\lambda}$.

Kurtosis: $\frac{1}{\lambda}$.

Author(s)

Michal Burdukiewicz.

Examples

```
# moments for 100 positive partitions of 765 total partitions
moments(c(100, 765))
```

```
# calculate moments for an array digital PCR
moments(six_panels)
```

num2int

Convert numeric to integer

Description

Converts numeric values to positive integers with a warning.

Usage

```
num2int(x)
```

Arguments

x an numeric vector

Details

num2int uses [as.integer](#) functionality.

Examples

```
suppressWarnings(num2int(pi) == 3L)
```

pds

*Plasmid dilution series results***Description**

These are the results data from the pds_raw data as calculated by the Bio-Rad QX100 Droplet Digital PCR System.

Format

A data frame with 64 observations on the following 44 variables.

Well a factor with levels A01 to H04

ExptType a factor with levels Absolute Quantification

Experiment a factor with levels ABS

Sample a factor with levels B B + P 10^2 gDNA gDNA + P 10^0 gDNA + P 10^1 gDNA + P 10^{-1} gDNA + P 10^2 gDNA + P 10^3 gDNA + P 10^4

TypeAssay a factor with levels Ch1NTC Ch1Unknown Ch2NTC Ch2Unknown

Assay a factor with levels ileS styA

Status a factor with levels Manual

Concentration a numeric vector

TotalConfMax a logical vector

TotalConfMin a logical vector

PoissonConfMax a numeric vector

PoissonConfMin a numeric vector

Positives a numeric vector

Negatives a numeric vector

Ch1.Ch2. a numeric vector

Ch1.Ch2..1 a numeric vector

Ch1.Ch2..2 a numeric vector

Ch1.Ch2..3 a numeric vector

Linkage a numeric vector

AcceptedDroplets a numeric vector

CNV a logical vector

TotalCNVMax a logical vector

TotalCNVMin a logical vector

PoissonCNVMax a logical vector

PoissonCNVMin a logical vector

ReferenceCopies a logical vector

UnknownCopies a logical vector
Ratio a numeric vector
TotalRatioMax a logical vector
TotalRatioMin a logical vector
PoissonRatioMax a numeric vector
PoissonRatioMin a numeric vector
FractionalAbundance a numeric vector
TotalFractionalAbundanceMax a logical vector
TotalFractionalAbundanceMin a logical vector
PoissonFractionalAbundanceMax a numeric vector
PoissonFractionalAbundanceMin a numeric vector
ReferenceAssayNumber a numeric vector
TargetAssayNumber a numeric vector
MeanAmplitudeofPositives a numeric vector
MeanAmplitudeofNegatives a numeric vector
MeanAmplitudeTotal a numeric vector
ExperimentComments a logical vector
MergedWells a logical vector

Details

Setup: Duplex assay with constant amount of genomic DNA and six 10-fold dilutions of plasmid DNA with 4 replicates, ranging theoretically from $\sim 10^4$ to 10^{-1} copies/ micro L plus 4 replicates without plasmid DNA. Included are No-gDNA-control and No-template-control, 2 replicates each.

Annotation: FX.Y (X = dilution number, Y = replicate number). Hardware: Bio-Rad QX100 Droplet digital PCR system Details: Genomic DNA isolated from *Pseudomonas putida* KT2440. Plasmid is pCOM10-StyA::EGFP StyB [Jahn et al., 2013, *Curr Opin Biotechnol*, Vol. 24 (1): 79-87]. Template DNA was heat treated at 95 degree Celsius for 5 min prior to PCR. Channel 1, primers for genomic DNA marker ileS, Taqman probes (FAM labelled). Channel 2, primers for plasmid DNA marker styA, Taqman probes (HEX labelled).

Author(s)

Michael Jahn, Stefan Roediger, Michal Burdukiewicz

Source

Michael Jahn Flow cytometry group / Environmental microbiology Helmholtz Centre for Environmental Research - UFZ Permoserstrasse 15 / 04318 Leipzig / Germany phone +49 341 235 1318 michael.jahn [at] ufz.de / www.ufz.de

References

Jahn et al., 2013, *Curr Opin Biotechnol*, Vol. 24 (1): 79-87

Examples

```
summary(extract_run(read_QX100(pds), 1L:10))
```

pds_raw

Plasmid dilution series raw data

Description

Subset of raw data from the pds_raw data set as measured by the Bio-Rad QX100 Droplet Digital PCR System.

Format

A list of 3 data frames.

Well | ExptType | Experiment | Sample + Dilution step | TypeAssay | Assay

D01 | Absolute Quantification | ABS | gDNA + P 10⁴ | Ch1Unknown | ileS

D02 | Absolute Quantification | ABS | gDNA + P 10² | Ch1Unknown | ileS

C04 | Absolute Quantification | ABS | gDNA | Ch1Unknown | ileS

Details

The results can be calculated as by the Bio-Rad QX100 Droplet Digital PCR System are to be found in pds.

The results can be calculated as by the Bio-Rad QX100 Droplet Digital PCR System are to be found in pds.

Setup: Duplex assay with a constant amount of genomic DNA and six 10-fold dilutions of plasmid DNA with 4 replicates, ranging theoretically from ~ 10⁴ to 10⁻¹ copies/ micro L plus 4 replicates without plasmid DNA. Included are No-gDNA-control and No-template-control, 2 replicates each.

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#' Setup: Duplex assay with a constant amount of genomic DNA and six 10-fold dilutions of plasmid DNA with 4 replicates, ranging theoretically from ~ 10⁴ to 10⁻¹ copies/ micro L plus 4 replicates without plasmid DNA. Included are No-gDNA-control and No-template-control, 2 replicates each.

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primers for genomic DNA marker ileS, Taqman probes (FAM labelled). Channel 2, primers for plasmid DNA marker styA, Taqman probes (HEX labelled).

The full data are located at https://github.com/michbur/dpcR_data as QX100_rawdata.zip.

Author(s)

Michael Jahn, Stefan Roediger, Michal Burdukiewicz

Source

Michael Jahn Flow cytometry group / Environmental microbiology Helmholtz Centre for Environmental Research - UFZ Permoserstrasse 15 / 04318 Leipzig / Germany phone +49 341 235 1318 michael.jahn [at] ufz.de / www.ufz.de

References

Jahn et al., 2013, *Curr Opin Biotechnol*, Vol. 24 (1): 79-87

Jahn M, Vorpahl C, Tuerkowsky D, Lindmeyer M, Buehler B, Harms H, et al. Accurate Determination of Plasmid Copy Number of Flow-Sorted Cells using Droplet Digital PCR. *Anal Chem* 2014; 86:5969–76. doi:10.1021/ac501118v.

Examples

```
#str(pds_raw)
bioamp(data = pds_raw[["D02"]], main = "Well D02", pch = 19)
```

plot.qdpcr

Plot qdpcr objects

Description

An analytical plot describing relationship between the cycle number and the current value of Poisson mean. The plot can be used for quality control of process.

Arguments

x	is a <code>qdpcr</code> object.
mincyc	is the first cycle to start the plot from.
maxcyc	the last cycle for the plot.
rug	Adds a rug representation of the data to the plot.
digits	how many significant digits are to be used in plot.

Details

The rug parameter allows user to add density of the number of events to the plot.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

[qdpqr](#)

Examples

```
library(qpcR)
test <- cbind( reps[1L:45, ], reps2[1L:45, 2L:ncol(reps2)], reps3[1L:45,
  2L:ncol(reps3)] )

plot(qpcr2pp(data = test, cyc = 1, fluo = NULL, model = 15, delta = 5), rug = TRUE)
```

plot_panel

Plot Panel

Description

The `plot_panel` function takes objects of the class `adpqr` to enable customizable graphical representations of a chamber-based digital PCR experiments (e.g., Digital Array (R) IFCs (integrated fluidic circuits) of the BioMark (R) and EP1 (R)).

Usage

```
plot_panel(input, col = "red", legend = TRUE, half = "none",
  plot = TRUE, ...)
```

Arguments

<code>input</code>	object of the <code>adpqr</code> class.
<code>col</code>	A single color or vector of colors for each level of input.
<code>legend</code>	If <code>TRUE</code> , a built-in legend is added to the plot.
<code>half</code>	If <code>left</code> or <code>right</code> , every well is represented only by the adequate half of the rectangle.
<code>plot</code>	"logical", if <code>FALSE</code> , only plot data is returned invisibly.
<code>...</code>	Arguments to be passed to <code>plot</code> function.

Details

Currently, only objects containing `tnp` data can be plotted as a whole. For the any other type of the `adpcr` data, only just one column of data (one panel) can be plotted at the same time (see Examples how easily plot multipanel objects). Moreover the object must contain fluorescence intensities or exact number of molecules or the positive hits derived from the `Cq` values for each well. The `Cq` values can be obtained by custom made functions (see example in [dpcr_density](#)) or the yet to implement `"qpcr_analyser"` function from the `dpcR` package.

If the `col` argument has length one, a color is assigned for each interval of the input, with the brightest colors for the lowest values.

Value

Invisibly returns two sets of coordinates of each microfluidic well as per `calc_coordinates`: `coords` is a list of coordinates suitable for usage with functions from `graphics` package. The second element is a data frame of coordinates useful for users utilizing `ggplot2` package.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[extract_run](#) - extract experiments. [adpcr2panel](#) - convert `adpcr` object to arrays.

Examples

```
# Create a sample dPCR experiment with 765 elements (~> virtual compartments)
# of target molecule copies per compartment as integer numbers (0,1,2)
ttest <- sim_adpcr(m = 400, n = 765, times = 20, pos_sums = FALSE,
                 n_panels = 1)
# Plot the dPCR experiment results with default settings
plot_panel(ttest)

# Apply a two color code for number of copies per compartment
plot_panel(ttest, col = c("blue", "red"))

# plot few panels
ttest2 <- sim_adpcr(m = 400, n = 765, times = 40, pos_sums = FALSE,
                 n_panels = 4)
par(mfcol = c(2, 2))
four_panels <- lapply(1:ncol(ttest2), function(i)
  plot_panel(extract_run(ttest2, i), legend = FALSE,
            main = paste("Panel", LETTERS[i], sep = " ")))
par(mfcol = c(1, 1))

# two different channels
plot_panel(extract_run(ttest2, 1), legend = FALSE,
          half = "left")
par(new = TRUE)
plot_panel(extract_run(ttest2, 2), col = "blue",
```

```

        legend = FALSE, half = "right")

# plot two panels with every well as only the half of the rectangle
ttest3 <- sim_adpccr(m = 400, n = 765, times = 40, pos_sums = FALSE,
                   n_panels = 2)
par(mfcol = c(1, 2))
two_panels <- lapply(1:ncol(ttest3), function(i)
  plot_panel(extract_run(ttest3, i), legend = FALSE,
             main = paste("Panel", LETTERS[i], sep = " ")))
par(mfcol = c(1, 1))

```

plot_vic_fam	<i>Amplitude Plot VIC and FAM Channels of a Droplet Digital PCR Experiment</i>
--------------	--

Description

This function generates an amplitude plot of two fluorescence channels as found in droplet digital PCR.

Usage

```
plot_vic_fam(vic, fam, col_vic = "green", col_fam = "blue",
             circle = TRUE)
```

Arguments

vic	Amplitudes of the VIC channel - object of class <code>dpcr</code> .
fam	Amplitudes of the FAM channel - object of class <code>dpcr</code> .
col_vic	Color of the VIC channel.
col_fam	Color of the FAM channel.
circle	If TRUE circles are drawn, if FALSE not. If "numeric", specifies the radius of circles.

Details

Droplet digital PCR experiments consist of three steps (droplet generation, clonal amplification, droplet amplitude analysis). Typically 20000 nano-sized droplets are analyzed and separated into amplification-positive and amplification-negative droplets. An example of such system is the Bio-Rad QX100 and QX200 (Pinheiro et al. 2012). Such systems have applications in the detection of rare DNA target copies, the determination of copy number variations (CNV), detection of mutation, or expression analysis of genes or miRNA. Each droplet is analyzed individually using a virtual two-color detection system. The channels are treated separately but finally aligned (e.g., FAM and VIC or FAM and HEX).

Author(s)

Michal Burdukiewicz, Stefan Roediger.

References

Pinheiro, L.B., Coleman, V.A., Hindson, C.M., Herrmann, J., Hindson, B.J., Bhat, S., and Em-slie, K.R. (2012). *Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification*. Anal. Chem. 84, 1003 - 1011.

Examples

```
# Generate an amplitude plot for the first fluorescence channel (e.g., FAM)
fluos1 <- sim_dpqr(m = 16, n = 30, times = 100, pos_sums = FALSE, n_exp = 1,
  fluo = list(0.1, 0))

# Generate an amplitude plot for the second fluorescence channel (e.g., VIC)
fluos2 <- sim_dpqr(m = 16, n = 30, times = 100, pos_sums = FALSE, n_exp = 1,
  fluo = list(0.1, 0))

# Plot the amplitudes of both fluorescence channel in an aligned fashion
plot_vic_fam(fam = fluos1, vic = fluos2)

# Same as above but different colors
plot_vic_fam(fam = fluos1, vic = fluos2, col_vic = "red", col_fam = "yellow")

# Same as above without circles
plot_vic_fam(fam = fluos1, vic = fluos2, col_vic = "red", col_fam = "yellow", circle = FALSE)

# Generate two channels in one object and plot them
fluos_both <- sim_dpqr(m = 16, n = 30, times = 100, pos_sums = FALSE, n_exp = 2,
  fluo = list(0.1, 0))
plot_vic_fam(extract_run(fluos_both, 1), extract_run(fluos_both, 2))
```

qdpcr-class

Class "qdpcr"

Description

An object representing digital PCR reaction depicted as Poisson process. Inherits from [dpcr](#).

Slots

list("mu") "numeric" of the expected number of events in a defined interval.

list("C0") "numeric" the occurrence in a defined interval.

list("CT") "numeric" value of the "average time" between the occurrence of a positive reaction and another positive reaction.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

See Also

[plot.qdpcr](#),

qpcr2pp

qPCR to Poisson Process

Description

Describes qPCR as Poisson process.

Usage

```
qpcr2pp(data, cyc = 1, fluo = NULL, Cq_range = c(min(data[cyc]) + 6,
max(data[cyc]) - 6), model = 15, SDM = TRUE, NuEvents = 1,
delta = 1, exper = "qPCR1", replicate = 1, assay = "Unknown",
type = "np")
```

Arguments

data	a dataframe containing the qPCR data.
cyc	the column containing the cycle data. Defaults to first column.
fluo	the column(s) (runs) to be analyzed. If NULL, all runs will be considered (equivalent of (1L:ncol(data))[-cyc]).
Cq_range	is a user defined range of cycles to be used for the determination of the Cq values.
model	is the model to be used for the analysis for all runs. Defaults to '15' (see pcrfit).
SDM	if TRUE, Cq is approximated by the second derivative method. If FALSE, Cy0 method is used instead.
NuEvents	"number of expected events" within a time frame (interval).
delta	difference "time (cycles) points" e.g., Cycle 18 and 25.
exper	The id of experiments.
replicate	The id of technical replicates.
assay	The name or id of assays.
type	object of class "character" defining type of data. Could be "np" (status (positive (1) or negative(0)) of each droplet) or "ct" (threshold cycle).

Details

Selected platforms (e.g., Open Array) are real-time platforms. dPCR can be described by Poisson statistics. The function `qpcr2pp` takes a step further and interprets the dPCR as a Poisson process if it is analyzed as a "time" based process.

The dPCR Technology breaks fundamentally with the previous concept of nucleic acid quantification. dPCR can be seen as a next generation nucleic acid quantification method based on PCR. The key difference between dPCR and traditional PCR lies in the method of measuring (absolute) nucleic acids amounts. This is possible after "clonal DNA amplification" in thousands of small separated partitions (e.g., droplets, nano chambers). Partitions with no nucleic acid remain negative and the others turn positive. Selected technologies (e.g., OpenArray(R) Real-Time PCR System) monitor amplification reactions in the chambers in real-time. Cq values are calculated from the amplification curves and converted into discrete events by means of positive and negative partitions and the absolute quantification of nucleic acids is done by Poisson statistics.

PCR data derived from a qPCR experiment can be seen as a series of events over time. We define t_i as the time between the first $(i - 1)^{\text{st}}$ and the i^{th} event. Therefore, the time S_n is the sum of all t_i from $i = 1$ to $i = n$. This is the time to the n^{th} event. $S(t)$ is the number of events in $[0, t]$. This can be seen as a Poisson process. The Poisson statistics is the central theorem to random processes in digital PCR.

The function `qpcr2pp` is used to model random point events in time units (PCR cycles), such as the increase of signal during a qPCR reaction in a single compartment. A Poisson process can be used to model times at which an event occurs in a "system". The `qpcr2pp` (quantitative Real-Time PCR to Poisson process) function transforms the qPCR amplification curve data to quantification points (Cq), which are visualized as Poisson process. This functions helps to spot differences between replicate runs of digital PCR experiments. In ideal scenarios the `qpcr2pp` plots are highly similar.

This tool might help to spot differences between experiments (e.g., inhibition of amplification reactions, influence of the chip arrays). The qPCR is unique because the amplification of conventional qPCRs takes place in discrete steps (cycles: 1, 2 ... 45), but the specific Cq values are calculated with continuous outcomes (Cq: 18.2, 25.7, ...). Other amplification methods such as isothermal amplifications are time based and thus better suited for Poisson process.

Value

An object of `qdpcr` class.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

Examples

```
library(qpcr)
test <- cbind( reps[1L:45, ], reps2[1L:45, 2L:ncol(reps2)],
             reps3[1L:45, 2L:ncol(reps3)] )

# before interpolation qPCR experiment must be converted into dPCR
qpcrpp <- qpcr2pp(data = test, cyc = 1, fluo = NULL, Cq_range = c(20, 30),
                 model = 15, delta = 5)
```

```
summary(qpcrpp)
```

```
qpcr_analyser
```

```
qPCR Analyser
```

Description

Calculate statistics based on fluorescence. The function can be used to analyze amplification curve data from quantitative real-time PCR experiments. The analysis includes the fitting of the amplification curve by a non-linear function and the calculation of a quantification point (often referred to as Cp (crossing-point), Cq or Ct) based on a user defined method. The function can be used to analyze data from chamber based dPCR machines.

Arguments

<code>input</code>	a dataframe containing the qPCR data or a result of function <code>modlist</code> or an object of the class <code>adpqr</code> .
<code>cyc</code>	the column containing the cycle data. Defaults to first column.
<code>fluo</code>	the column(s) (runs) to be analyzed. If NULL, all runs will be considered. Use <code>fluo = 2</code> to chose the second column for example.
<code>model</code>	is the model to be used for the analysis for all runs. Defaults to '15' (see <code>pcrfit</code>).
<code>norm</code>	logical. Indicates if the raw data should be normalized within [0, 1] before model fitting.
<code>iter_tr</code>	<code>iter_tr</code> number of iteration to fit the curve.
<code>type</code>	is the method for the crossing point/threshold cycle estimation and efficiency estimation (<code>efficiency</code>). Defaults to 'Cy0' (<code>Cy0</code>).
<code>takeoff</code>	logical; if TRUE calculates the first significant cycle of the exponential region (takeoff point). See <code>takeoff</code> for details.

Details

The `qpcRanalyzer` is a functions to automatize the analysis of amplification curves from conventional quantitative real-time PCR (qPCR) experiments and is adapted for the needs in dPCR. This function calls instances of the `qpcR` package to calculate the quantification points (`cpD1`, `cpD2`, `Cy0` (default), `TOP` (optional)), the amplification efficiency, fluorescence at the quantification point (`Cq`), the absolute change of fluorescence and the take-off point (`TOP`). Most of the central functionality of the `qpcR` package is accessible. The user can assign concentrations to the samples. One column contains binary converted (`pos` (1) and `neg` (0)) results for the amplification reaction based on a user defined criteria (`Cq-range`, `fluorescence cut-off`, ...). `qpcr_analyser` tries to detect cases where an amplification did not take place or was impossible to analyze. By default `qpcr_analyser` analyses uses the `Cy0` as described in Guescini et al. (2008) for estimation of the quantification point since this method is considered to be better suited for many probe systems. By default a 5-parameter model is used to fit the amplification curves. As such `qpcr_analyser` is a function, which serves for preliminary data inspection (see Example section) and as input for other R functions from the `dpcR` package (e.g., `plot_panel`).

Value

A matrix where each column represents crossing point, efficiency, the raw fluorescence value at the point defined by type and difference between minimum and maximum of observed fluorescence. If takeoff parameter is TRUE, additional two column represents start and the end of the fluorescence growth.

Author(s)

Stefan Roediger, Andrej-Nikolai Spiess, Michal Burdukiewicz.

References

Ritz C, Spiess An-N, *qpcR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis*. *Bioinformatics* 24 (13), 2008.

Andrej-Nikolai Spiess (2013). qpcR: Modelling and analysis of real-time PCR data.
<https://CRAN.R-project.org/package=qpcR>

See Also

[modlist](#).

Examples

```
# Take data of guescini1 data set from the qpcR R package.
library(qpcR)
# Use the first column containing the cycles and the second column for sample F1.1.
data(guescini1)
qpcr_analyser(guescini1, cyc = 1, fluo = 2)

# Use similar setting as before but set takeoff to true for an estimation of
# the first significant cycle of the exponential region.
qpcr_analyser(guescini1, cyc = 1, fluo = 2, takeoff = TRUE)

# Use similar setting as before but use qpcr_analyser in a loop to calculate the results for the
# first four columns containing the fluorescence in guescini1
print(qpcr_analyser(guescini1, cyc = 1, fluo = 2:5, takeoff = TRUE))

# Run qpcr_analyser on the list of models (finer control on fitting model process)
models <- modlist(guescini1)
qpcr_analyser(models)
```

read_amp	<i>Read digital PCR amplitude raw data</i>
----------	--

Description

Reads digital PCR amplitude data.

Usage

```
read_amp(input, ext = NULL)
```

Arguments

input	name of the input file (character) or input object (data.frame).
ext	extension of the file ().

Details

The amplitude data means a compressed directory of amplification.

Value

An object of [adpccr](#).

Author(s)

Michal Burdukiewicz, Stefan Roediger

read_BioMark	<i>Read BioMark</i>
--------------	---------------------

Description

Reads digital PCR data from the BioMark (Fluidigm).

Usage

```
read_BioMark(input, ext = NULL, detailed = FALSE)
```

Arguments

input	name of the input file (character) or input object (data.frame).
ext	extension of the file ().
detailed	logical, if TRUE, the input file is processed as if it was 'Detailed Table Results'. In the other case, the expected input file structure is 'Summary Table Results'.

Value

An object of [adpcr](#) class.

Author(s)

Michal Burdukiewicz, Stefan Roediger

References

Dong, L. et al (2015). Comparison of four digital PCR platforms for accurate quantification of DNA copy number of a certified plasmid DNA reference material. *Scientific Reports*. 2015;5:13174.

See Also

See [read_dpcr](#) for detailed description of input files.

read_dpcr	<i>Read digital PCR data</i>
-----------	------------------------------

Description

Reads digital PCR data in various formats.

Usage

```
read_dpcr(input, format, ext = NULL, ...)
```

Arguments

input	name of the input file (character) or input object (data.frame).
format	of the file, for example: "raw", "QX100", "BioMark", "amp" (raw amplitude compressed using .zip).
ext	extension of the file ().
...	additional parameters for the appropriate function. For example, if format has value "raw", the additional parameter must be adpcr.

Details

Input files may have .csv, .xls or .xlsx extension. In case of Excel files with multiple sheets, only the first sheet will be analyzed.

Value

Always an object of [adpcr](#) or [dpcr](#) type.

Author(s)

Michal Burdukiewicz, Stefan Roediger

See Also

Read raw format files: [read_redf](#). Read BioMark format files: [read_BioMark](#). Read QX100 format files: [read_QX100](#). Read QX200 format files: [read_QX200](#).

read_QX100

Read QX100

Description

Reads digital PCR data from the QX100 Droplet Digital PCR System (Bio-Rad).

Usage

```
read_QX100(input, ext = NULL)
```

Arguments

input	name of the input file (character) or input object (data.frame).
ext	extension of the file ().

Value

An object of [adpccr](#) class.

Note

The volume and its uncertainty are taken from the literature (see references).

Author(s)

Michal Burdukiewicz, Stefan Roediger

References

Corbisier, P. et al (2015). DNA copy number concentration measured by digital and droplet digital quantitative PCR using certified reference materials. *Analytical and Bioanalytical Chemistry* 407, 1831-1840.

See Also

See [read_dpccr](#) for detailed description of input files.

Example of QX100 data: [pds](#).

`read_QX200`*Read QX200*

Description

Reads digital PCR data from the QX200 Droplet Digital PCR System (Bio-Rad).

Usage

```
read_QX200(input, ext = NULL)
```

Arguments

<code>input</code>	name of the input file (character) or input object (data.frame).
<code>ext</code>	extension of the file ().

Value

An object of `adpccr` class.

Note

The volume and its uncertainty are taken from the literature (see references).

Author(s)

Michal Burdukiewicz, Stefan Roediger

Source

Droplet Digital PCR Applications Guide, Rev. A, Bulletin 6407, Biorad, accessed on 28.10.2016, http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf.

References

Corbisier, P. et al (2015). DNA copy number concentration measured by digital and droplet digital quantitative PCR using certified reference materials. *Analytical and Bioanalytical Chemistry* 407, 1831-1840.

See Also

See `read_dpccr` for detailed description of input files.

`read_redf`*Read digital PCR raw data*

Description

Reads REDF (Raw Exchange Digital PCR format) data.

Usage

```
read_redf(input, ext = NULL)
```

Arguments

<code>input</code>	name of the input file (character) or input object (data.frame).
<code>ext</code>	extension of the file ().

Details

REDF (Raw Exchange Digital PCR format) data is preferably a .csv file with following columns:

experiment names of experiments

replicate indices of replicates

assay names of assays

k number of positive partitions

n total number of partitions

v volume of partition (nL)

uv uncertainty of partition's volume (nL)

threshold partitions with k equal or higher than threshold are treated as positive.

panel_id indices of panels

Column `panel_id` should be specified only in case of array-based dPCR.

Value

An object of `adpccr` or `dpcr` type, depends on the value of `adpccr` parameter.

Author(s)

Michal Burdukiewicz, Stefan Roediger

rename_dpcr	<i>Rename object</i>
-------------	----------------------

Description

Renames objects of class `adpcr` or `dpcr`.

Usage

```
rename_dpcr(x, exper = NULL, replicate = NULL, assay = NULL)
```

Arguments

<code>x</code>	an <code>adpcr</code> or <code>dpcr</code> object.
<code>exper</code>	a vector of new experiments' names. If <code>NULL</code> , experiments' names are not changed.
<code>replicate</code>	a vector of new replicates' ids. If <code>NULL</code> , replicates' names are not changed.
<code>assay</code>	a vector of new assays' names. If <code>NULL</code> , assays' names are not changed.

Details

The valid `exper`, `replicate` and `assay` names are factors. For the sake of convenience, this function converts other types to factors if it is possible.

<code>rtadpcr-class</code>	<i>Class "rtadpcr" - real-time array digital PCR experiments</i>
----------------------------	--

Description

A class designed to contain results from real-time array digital PCR experiments. Data is represented as matrix, where each column describes different measurement point (i.e. cycle number) and every row different partition.

Slots

list(".Data") "matrix" containing data from array. See Description.
: "matrix" containing data from array. See Description.
list("n") Object of class "integer" equal to the number of partitions.
: Object of class "integer" equal to the number of partitions.
list("type") Object of class "character" defining type of data.
: Object of class "character" defining type of data.

Author(s)

Michal Burdukiewicz.

See Also

End-point array digital PCR: [adpcr](#).

Droplet digital PCR: [dpcr](#).

Examples

```
#none
```

show-methods	<i>Methods for Function</i> show
--------------	----------------------------------

Description

Expands function [show](#) allowing showing objects of the class [adpcr](#) or [dpcr](#).

Arguments

object an object of class [dpcr](#).

Author(s)

Michal Burdukiewicz.

Examples

```
#array dpcr
ptest <- sim_adpcr(400, 765, 5, FALSE, n_panels = 1)
show(ptest)

#multiple experiments
ptest <- sim_adpcr(400, 765, 5, FALSE, n_panels = 5)
show(ptest)

#droplet dpcr - fluorescence
dropletf <- sim_dpcr(7, 20, times = 5, fluo = list(0.1, 0))
show(dropletf)

#droplet dpcr - number of molecules
droplet <- sim_dpcr(7, 20, times = 5)
show(droplet)
```


sim_adpcr

*Simulate Array Digital PCR***Description**

A function that simulates results of an array digital PCR.

Usage

```
sim_adpcr(m, n, times, n_panels = 1, dube = FALSE, pos_sums = FALSE)
```

Arguments

m	the total number of template molecules added to the plate. Must be a positive integer.
n	the number of chambers per plate. Must be a positive integer.
times	number of repetitions (see Details).
n_panels	the number of panels that are simulated by the function. Cannot have higher value than the times argument.
dube	if TRUE, the function is strict implementation of array digital PCR simulation (as in Dube et al., 2008). If FALSE, the function calculates only approximation of Dube's experiment. See Details and References.
pos_sums	if TRUE, function returns only the total number of positive (containing at least one molecule) chamber per panel. If FALSE, the functions returns a vector of length equal to the number of chambers. Each element of the vector represents the number of template molecules in a given chamber.

Details

The array digital PCR is performed on plates containing many microfluidic chambers with a randomly distributed DNA template, fluorescence labels and standard PCR reagents. After the amplification reaction, performed independently in each chamber, the chambers with the fluorescence level below certain threshold are treated as negative. From differences between amplification curves of positive chambers it is possible to calculate both total number of template molecules and their approximate number in a single chamber.

The function contains two implementations of the array digital PCR simulation. First one was described in Dube et al. (2008). This method is based on random distributing $m \times times$ molecules between $n \times times$ chambers. After this step, the required number of plates is created by the random sampling of chambers without replacement. The above method is used, when the dube argument has value TRUE.

The second method treats the total number of template molecules as random variable with a normal distribution $\mathcal{N}(n, 0.05n)$. The exact sum of total molecules per plate is calculated and randomly adjusted to the value of $m \times times$. The above method is used, when the dube argument has value FALSE. This implementation is much faster than previous one, especially for big simulations. The higher the value of the argument times, the simulation result is closer to theoretical calculations.

Value

If the `pos_sums` argument has value `FALSE`, the function returns a matrix with n rows and n_{panels} columns. Each column represents one plate. The type of such simulation would be "nm". If the `pos_sums` argument has value `TRUE`, the function returns a matrix with one row and n_{panels} columns. Each column contains the total number of positive chambers in each plate and type of simulation would be set as "tnp".

In each case the value is an object of the `adpccr` class.

Author(s)

Michal Burdukiewicz.

References

Dube S, Qin J, Ramakrishnan R, *Mathematical Analysis of Copy Number Variation in a DNA Sample Using Digital PCR on a Nanofluidic Device*. PLoS ONE 3(8), 2008.

See Also

[sim_dpccr](#).

Examples

```
# Simulation of a digital PCR experiment with a chamber based technology.
# The parameter pos_sums was altered to change how the total number of positive
# chamber per panel are returned. An alteration of the parameter has an impact
# in the system performance.
adpccr_big <- sim_adpccr(m = 10, n = 40, times = 1000, pos_sums = FALSE, n_panels = 1000)
adpccr_small <- sim_adpccr(m = 10, n = 40, times = 1000, pos_sums = TRUE, n_panels = 1000)
# with pos_sums = TRUE, output allocates less memory
object.size(adpccr_big)
object.size(adpccr_small)

# Mini version of Dube et al. 2008 experiment, full requires replic <- 70000
# The number of replicates was reduced by a factor of 100 to lower the computation time.
replic <- 700
dube <- sim_adpccr(400, 765, times = replic, dube = TRUE,
  pos_sums = TRUE, n_panels = replic)
mean(dube) # 311.5616
sd(dube) # 13.64159

# Create a barplot from the simulated data similar to Dube et al. 2008
bp <- barplot(table(factor(dube, levels = min(dube):max(dube))),
  space = 0)
lines(bp, dnorm(min(dube):max(dube), mean = 311.5, sd = 13.59)*replic,
  col = "green", lwd = 3)

# Exact Dube's method is a bit slower than other one, but more accurate
system.time(dub <- sim_adpccr(m = 400, n = 765, times = 500, n_panels = 500,
  pos_sums = TRUE))
```

```
system.time(mul <- sim_adpcr(m = 400, n = 765, times = 500, n_panels = 500,
  pos_sums = FALSE))
```

sim_dpcr

*Simulate Droplet Digital PCR***Description**

A function that simulates results of a droplet digital PCR.

Usage

```
sim_dpcr(m, n, times, n_exp = 1, dube = FALSE, pos_sums = FALSE,
  fluo = NULL)
```

Arguments

m	the total number of template molecules used in the experiment. Must be a positive integer.
n	the number of droplets per experiment. Must be a positive integer.
times	number of repetitions (see Details).
n_exp	the number of experiments that are simulated by the function. Cannot have higher value than the times argument.
dube	if TRUE, the function is strict implementation of digital PCR simulation (as in Dube et al., 2008). If FALSE, the function calculates only approximation of Dube's experiment. See Details and References.
pos_sums	if TRUE, function returns only the total number of positive (containing at least one molecule) chamber per panel. If FALSE, the function returns a vector of length equal to the number of chambers. Each element of the vector represents the number of template molecules in a given chamber.
fluo	if NULL, the function calculates number of molecules per well or total number of positive droplets. If list of two, the first argument defines smoothness of the fluorescence curve and second space between two consecutive measured droplets. Space must be a vector containing positive integers of the length n or 1.

Details

The function contains two implementations of the array digital PCR simulation. First one was described in Dube et al. (2008). This method is based on random distributing $m \times times$ molecules between $n \times times$ chambers. After this step, the required number of plates is created by the random sampling of chambers without replacement. The above method is used, when the dube argument has value TRUE.

The higher the value of the argument times, the simulation result is closer to theoretical calculations.

Value

If the `pos_sums` argument has value `FALSE`, the function returns matrix with n rows and n_{panels} columns. Each column represents one plate. The type of such simulation would be "nm". If the `pos_sums` argument has value `TRUE`, the function return matrix with one row and n_{panels} columns. Each column contains the total number of positive chambers in each plate and type of simulation would be set as "tnp".

In each case the value is an object of the `dpcr` class.

Note

Although Dube's simulation of digital PCR was developed for array digital PCR, it's also viable for simulating droplet-based methods.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[sim_adpcr](#).

Examples

```
#simulate fluorescence data
tmp_VIC <- sim_dpcr(m = 7, n = 20, times = 5, fluo = list(0.1, 0))
tmp_FAM <- sim_dpcr(m = 15, n = 20, times = 5, fluo = list(0.1, 0))
par(mfrow = c(2,1))
plot(tmp_VIC, col = "green", type = "l")
plot(tmp_FAM, col = "blue", type = "l")
summary(tmp_FAM)

summary(sim_dpcr(m = 7, n = 20, times = 5, n_exp = 5))
```

six_panels

Simulated Digital PCR data

Description

Simulated data from array-based digital PCR experiment (see [sim_adpcr](#)).

Format

An object of class `adpcr` containing six runs from three experiments (two runs per each experiment).

Examples

```
#code below was used to create six_panels data set
## Not run:
set.seed(1944)
adpcr1 <- sim_adpcr(m = 10, n = 765, times = 10000, pos_sums = FALSE, n_panels = 2)
adpcr2 <- sim_adpcr(m = 40, n = 765, times = 10000, pos_sums = FALSE, n_panels = 2)
adpcr2 <- rename_dpcr(adpcr2, exper = "Experiment2")
adpcr3 <- sim_adpcr(m = 100, n = 765, times = 10000, pos_sums = FALSE, n_panels = 2)
adpcr3 <- rename_dpcr(adpcr3, exper = "Experiment3")
six_panels_example <- bind_dpcr(adpcr1, adpcr2, adpcr3)
six_panels_example <- rename_dpcr(six_panels_example, assay = factor(rep(c("Chr4", "MYC"), 3)))

## End(Not run)
```

summary-methods

Methods for Function summary

Description

Expands function [summary](#) allowing printing summaries objects of the class [adpcr](#) to or [dpcr](#).

Arguments

object	object of class adpcr , dpcr or qdpqr .
print	if FALSE, no output is printed.

Details

The function prints a summary of the dPCR reaction, including k (number of positive chambers), n (total number of chambers), estimated lambda and concentration, as well as confidence intervals for the last two variables.

Value

The data frame with estimated values of lambda, m and corresponding confidence intervals.

Note

If [summary](#) is used on an object containing results of many experiments, all experiments would be independently summarized. Currently supported only for objects of class [adpcr](#).

Author(s)

Michal Burdukiewicz, Stefan Roediger.

References

Bhat S, Herrmann J, Corbisier P, Emslie K, *Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number*. Analytical and Bioanalytical Chemistry 2 (394), 2009.

Dube S, Qin J, Ramakrishnan R, *Mathematical Analysis of Copy Number Variation in a DNA Sample Using Digital PCR on a Nanofluidic Device*. PLoS ONE 3(8), 2008.

Examples

```
# array dpcr
# Simulates a chamber based digital PCR with m total number of template molecules
# and n number of chambers per plate and assigns it as object ptest of the class
# adpcr for a single panel. The summary function on ptest gets assigned to summ
# and the result with statistics according to Dube et al. 2008 and Bhat et al. 2009
# gets printed.
ptest <- sim_adpcr(m = 400, n = 765, times = 5, dube = FALSE, n_panels = 1)
summ <- summary(ptest) #save summary
print(summ)

# multiple experiments
# Similar to the previous example but with five panels
ptest <- sim_adpcr(m = 400, n = 765, times = 5, dube = FALSE, n_panels = 5)
summary(ptest)

# droplet dpcr - fluorescence
# Simulates a droplet digital PCR with m = 7 total number of template molecules
# and n = 20 number of droplets. The summary function on dropletf gives the
# statistics according to Dube et al. 2008 and Bhat et al. 2009. The fluo parameter
# is used to change the smoothness of the fluorescence curve and the space between
# two consecutive measured peaks (droplets).
dropletf <- sim_dpccr(m = 7, n = 20, times = 5, fluo = list(0.1, 0))
summary(dropletf)

# droplet dpcr - number of molecules
# Similar to the previous example but with five panels but without and modifications
# to the peaks.
droplet <- sim_dpccr(m = 7, n = 20, times = 5)
summary(droplet)

# Visualize the results of dropletf and droplet
# The curves of dropletf are smoother.
par(mfrow = c(1,2))
plot(dropletf, main = "With fluo parameter", type = "l")
plot(droplet, main = "Without fluo parameter", type = "l")
```

test_counts	<i>Test counts</i>
-------------	--------------------

Description

The test for comparing counts from two or more digital PCR experiments.

Usage

```
test_counts(input, model = "ratio", conf.level = 0.95)
```

Arguments

input	object of class <code>adpccr</code> or <code>dpcr</code> with "nm" type.
model	may have one of following values: binomial, poisson, prop, ratio. See Details.
conf.level	confidence level of the intervals and groups.

Details

`test_counts` incorporates two different approaches to models: GLM (General Linear Model) and multiple pair-wise tests. The GLM fits counts data from different digital PCR experiments using quasibinomial or quasipoisson [family](#). Comparisons between single experiments utilize Tukey's contrast and multiple t-tests (as provided by function [glht](#)).

In case of pair-wise tests, ([rateratio.test](#) or [prop.test](#)) are used to compare all pairs of experiments. The p-values are adjusted using the Benjamini & Hochberg method ([p.adjust](#)). Furthermore, confidence intervals are simultaneous.

Value

an object of class `count_test`.

Note

Mean number of template molecules per partition and its confidence intervals will vary depending on input.

Author(s)

Michal Burdukiewicz, Stefan Roediger, Piotr Sobczyk.

References

Bretz F, Hothorn T, Westfall P, *Multiple comparisons using R*. Boca Raton, Florida, USA: Chapman & Hall/CRC Press (2010).

See Also

Functions used by test_counts:

- [glm](#),
- [glht](#),
- [cld](#),
- [prop.test](#),
- [rateratio.test](#)

GUI presenting capabilities of the test: [test_counts_gui](#).

Examples

```
#be warned, the examples of test_counts are time-consuming
## Not run:
adpcr1 <- sim_adpcr(m = 10, n = 765, times = 1000, pos_sums = FALSE, n_panels = 3)
adpcr2 <- sim_adpcr(m = 60, n = 550, times = 1000, pos_sums = FALSE, n_panels = 3)
adpcr2 <- rename_dpcr(adpcr2, exper = "Experiment2")
adpcr3 <- sim_adpcr(m = 10, n = 600, times = 1000, pos_sums = FALSE, n_panels = 3)
adpcr3 <- rename_dpcr(adpcr3, exper = "Experiment3")

#compare experiments using binomial regression
two_groups_bin <- test_counts(bind_dpcr(adpcr1, adpcr2), model = "binomial")
summary(two_groups_bin)
plot(two_groups_bin)
#plot aggregated results
plot(two_groups_bin, aggregate = TRUE)
#get coefficients
coef(two_groups_bin)

#this time use Poisson regression
two_groups_pois <- test_counts(bind_dpcr(adpcr1, adpcr2), model = "poisson")
summary(two_groups_pois)
plot(two_groups_pois)

#see how test behaves when results aren't significantly different
one_group <- test_counts(bind_dpcr(adpcr1, adpcr3))
summary(one_group)
plot(one_group)

## End(Not run)
```

test_counts_gui

Compare digital PCR runs - interactive presentation

Description

Launches graphical user interface allowing multiple comparisons of simulated digital PCR reactions.

Usage

```
test_counts_gui()
```

Warning

Any ad-blocking software may be cause of malfunctions.

Author(s)

Michal Burdukiewicz, Stefan Roediger.

See Also

[test_counts.](#)

test_panel	<i>Dispersion Test for Spatial Point Pattern in Array dPCR Based on Quadrat Counts</i>
------------	--

Description

Performs a test of Complete Spatial Randomness for each plate. This function is a wrapper around [quadrat.test](#) function working directly on the objects of [adpccr](#).

Usage

```
test_panel(X, nx = 5, ny = 5, alternative = c("two.sided", "regular",
      "clustered"), method = c("Chisq", "MonteCarlo"), conditional = TRUE,
      nsim = 1999)
```

Arguments

X	Object of the adpccr class containing data from one or more panels.
nx	Number of quadrats in the x direction.
ny	Number of quadrats in the y direction.
alternative	character string (partially matched) specifying the alternative hypothesis.
method	character string (partially matched) specifying the test to use: either "Chisq" for the chi-squared test (the default), or "MonteCarlo" for a Monte Carlo test.
conditional	logical. Should the Monte Carlo test be conducted conditionally upon the observed number of points of the pattern? Ignored if method="Chisq".
nsim	The number of simulated samples to generate when method="MonteCarlo".

Details

Under optimal conditions, the point pattern of dPCR events (e.g., positive droplet & negative droplets) should be randomly distributed over a planar chip. This function verifies this assumption using chi-square or Monte Carlo test. Arrays with non-random patterns should be checked for integrity.

Value

A list of objects of class "htest" with the length equal to the number of plates (minimum 1).

Note

A similar result can be achieved by using [adpccr2ppp](#) and [quadrat.test](#). See Examples.

Author(s)

Adrian Baddeley, Rolf Turner, Michal Burdukiewicz, Stefan Roediger.

References

<http://www.spatstat.org/>

See Also

[quadrat.test](#).

Examples

```
many_panels <- sim_adpccr(m = 400, n = 765, times = 1000, pos_sums = FALSE,
                        n_panels = 5)
test_panel(many_panels)

#test only one plate
test_panel(extract_run(many_panels, 3))

#do test_panel manually
require(spatstat)
ppp_data <- adpccr2ppp(many_panels)
lapply(ppp_data, function(single_panel) quadrat.test(single_panel))
```

test_peaks	<i>Peak Test</i>
------------	------------------

Description

Detect, separate and count positive and negative peaks, as well as peak-like noise. Additionally, function calculates area of the peaks.

Arguments

x	a vector containing the abscissa values (e.g., time, position) OR an object of class <code>adpccr</code> .
y	a vector of fluorescence value.
threshold	a value, which defines the peak heights not to consider as peak.
noise_cut	a numeric value between 0 and 1. All data between 0 and noise_cut quantile would be considered noise in the further analysis.
savgol	logical value. If TRUE, Savitzky-Golay smoothing filter is used.
norm	logical value. If TRUE, data is normalised.
filter.q	a vector of two numeric values. The first element represents the quantile of the noise and the second one is the quantile of the negative peaks.

Details

The localization of peaks is determined by the `findpeaks` function. The area under the peak is calculated by integration of approximating spline.

Value

A list of length 2. The first element is a data frame containing: peak number, peak group (noise, negative, positive), position of the peak maximum, area under the peak, peak width, peak height, position of the peak and time resolution.

The second element contains smoothed data.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

References

Savitzky, A., Golay, M.J.E., 1964. Smoothing and Differentiation of Data by Simplified Least Squares Procedures. *Anal. Chem.* 36, 1627-1639.

Examples

```

data(many_peaks)
par(mfrow = c(3,1))
plot(many_peaks, type = "l", main = "Noisy raw data")
abline(h = 0.01, col = "red")

tmp.out <- test_peaks(many_peaks[, 1], many_peaks[, 2], threshold = 0.01, noise_cut = 0.1,
                    savgol = TRUE)
plot(tmp.out[["data"]], type = "l", main = "Only smoothed")
abline(h = 0.01, col = "red")
abline(v = many_peaks[tmp.out[["peaks"]][, 3], 1], lty = "dashed")

tmp.out <- test_peaks(many_peaks[, 1], many_peaks[, 2], threshold = 0.01, noise_cut = 0.1,
                    savgol = TRUE, norm = TRUE)
plot(tmp.out[["data"]], type = "l", main = "Smoothed and peaks detected")
abline(v = many_peaks[tmp.out[["peaks"]][, 3], 1], lty = "dashed")
for(i in 1:nrow(tmp.out$peaks)) {
  if(tmp.out$peaks[i, 2] == 1) {col = 1}
  if(tmp.out$peaks[i, 2] == 2) {col = 2}
  if(tmp.out$peaks[i, 2] == 3) {col = 3}
  points(tmp.out$peaks[i, 7], tmp.out$peaks[i, 6], col = col, pch = 19)
}

positive <- sum(tmp.out$peaks[, 2] == 3)
negative <- sum(tmp.out$peaks[, 2] == 2)
total <- positive + negative

```

test_pooled

Compare pooled digital PCR

Description

Estimates mean number of template molecules per partition and concentration of sample from pooled replicates of experiments.

Usage

```
test_pooled(input, conf.level = 0.05)
```

Arguments

input object of class `adpccr` or `dpcr`.
conf.level confidence level of the intervals and groups.

Value

data frame with the number of rows equal to the number of experiments (not runs). The unit of concentration is the number template molecules per nanoliter (nL).

Note

This function was implemented using the code in supplemental materials in Dorazio, 2015 (see References).

Author(s)

Robert M. Dorazio, Margaret E. Hunter.

References

Dorazio RM, Hunter ME, *Statistical Models for the Analysis and Design of Digital Polymerase Chain Reaction (dPCR) Experiments*. Analytical Chemistry 2015. 87(21): p.10886-10893

Examples

```
test_pooled(six_panels)
```

 White

Digitalized Data from a Fluidigm Array

Description

These are the results data from the White data as measured by the UT digital PCR on Fluidigm 12.765 digital Array. The data were digitized from a supplementary figure "1471-2164-10-116-S1.pdf" by White et al. (2009) BMC Genomics

Format

A dataframe with 9180 rows and 10 columns.

Position of an array in the figure 1471-2164-10-116-S1.pdf from White et al. (2009) BMC Genomics (e.g., 11 is the image in the first column and the first row, 24 is second column and fourth image)

Image_pos is the sample (e.g., "Ace 1:100") as described by White et al. (2009) BMC Genomics

X.1 Running index for *all* samples

Index Index within an array

Row Row within an array

Column Column within an array

Area is the area that was measured with "MicroArray Profile"

Min is the minimum intensity of an area that was measured with "MicroArray Profile"

Max is the maximum intensity of an area that was measured with "MicroArray Profile"

Mean is the mean intensity of an area that was measured with "MicroArray Profile"

Details

Setup: Experimental details were described by White et al. (2009) BMC Genomics. The digitalization of the figure was done with imageJ and the "MicroArray Profile" plugin by Bob Dougherty (rpd@optinav.com) and Wayne Rasband.

Annotation: See the White et al. (2009) BMC Genomics paper for details.

Author(s)

Stefan Roediger, Michal Burdukiewicz, White et al. (2009) BMC Genomics

Source

Data were digitalized from the supplement material (Additional file 1. dPCR analysis of mock library control.) "1471-2164-10-116-S1.pdf" by White et al. (2009) BMC Genomics

References

White RA, Blainey PC, Fan HC, Quake SR. Digital PCR provides sensitive and absolute calibration for high throughput sequencing. *BMC Genomics* 2009;10:116. doi:10.1186/1471-2164-10-116.

Dougherty B, Rasband W. MicroArray Profile ImageJ Plugin n.d. <http://www.optinav.com/imagej.html> (accessed August 20, 2015).

Examples

```
str(White)
par(mfrow = c(3,3))

White_data <- sapply(unique(White[["Image_position"]]), function(i)
  White[White[["Image_position"]] == i, "Mean"])

assays <- sapply(unique(White[["Image_position"]]), function(i)
  unique(White[White[["Image_position"]] == i, "Sample"]))

White_adpccr <- create_dpccr(White_data > 115, n = 765, assay = assays,
  type = "np", adpccr = TRUE)

White_k <- colSums(White_data > 115)

sapply(2:4, function(i) {
  plot_panel(extract_run(White_adpccr, i))

  # Create the ECDF of the image scan data to define
  # a cut-off for positive and negative partitions
  # Plot the ECDF of the image scan data and define a cut-off
  plot(ecdf(White_data[, i]), main = paste0("ECDF of Image Scan Data\n", assays[i]),
  xlab = "Grey value", ylab = "Density of Grey values")
  abline(v = 115, col = 2, cex = 2)
  text(80, 0.5, "User defined cut-off", col = 2, cex = 1.5)
```

```
# Plot the density of the dPCR experiment
dpcr_density(k = White_k[i], n = 765, bars = TRUE)
}
)

par(mfrow = c(1,1))
```

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