

Package ‘PoDCall’

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Type Package

Title Positive Droplet Calling for DNA Methylation Droplet Digital PCR

Version 1.8.0

Description Reads files exported from 'QuantaSoft' containing amplitude values from a run of ddPCR (96 well plate) and robustly sets thresholds to determine positive droplets for each channel of each individual well. Concentration and normalized concentration in addition to other metrics is then calculated for each well. Results are returned as a table, optionally written to file, as well as optional plots (scatterplot and histogram) for both channels per well written to file. The package includes a shiny application which provides an interactive and user-friendly interface to the full functionality of PoDCall.

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Encoding UTF-8

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Depends R (>= 4.3)

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importAmplitudeData	<i>importAmplitudeData</i>
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Description

importAmplitudeData

Usage

```
importAmplitudeData(dataDirectory)
```

Arguments

`dataDirectory` Path to directory containing Quantasoft amplitude files from one 96 well plate. Since well coordinates are used as identifiers, files in this directory should all be from the same 96 well plate. Furthermore, there should be no other files than the amplitude files from a well plate in the directory.

Value

The function returns a list of dataframes named with the well ID and contains the amplitude values from the corresponding well.

Examples

```
# Path to example data files included in PoDCall
path <- system.file("extdata", "Amplitudes/", package="PoDCall")

# Read in data files
dataList <- importAmplitudeData(dataDirectory=path)
```

importSampleSheet	<i>importSampleSheet</i>
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Description

Function that takes a path to a .csv-file containing information about the samples that correspond to the uploaded amplitude files. This file must contain the following columns: Well, Sample, Target-Type and Target. A character vector with well IDs must also be provided, which is used to match rows in sample sheet to amplitude files

Usage

```
importSampleSheet(sampleSheet = NULL, well_id = NULL)
```

Arguments

sampleSheet	Path to sample sheet file containing information about samples.
well_id	Character vector with well IDs corresponding to uploaded amplitude files.

Value

A matrix with columns for sample ID, target assay and control assay.

Examples

```
## Path to example sample sheet included in PoDCall
path <- system.file("extdata", "Sample_names.csv", package="PoDCall")

## Select wells to get information for
well_id <- c("A04", "B04", "D04")

## Get information for selected wells
sampleSheet <- importSampleSheet(sampleSheet=path, well_id=well_id)
```

podcallChannelPlot	<i>podcallChannelPlot</i>
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Description

Function that calls podcallScatterplot and podcallHistogram and draws a plot with both scatter plot and histogram.

Usage

```
podcallChannelPlot(channelData, thr, channel, plotId = NULL)
```

Arguments

channelData	Amplitude values from one channel of a well.
thr	The threshold set for channel of a well.
channel	The channel the amplitude values belong to.
plotId	A character string with title for the plot

Value

A gtable with scatterplot and histogram

Examples

```
## Get path to data
path <- system.file("extdata", "Amplitudes/", package="PoDCall")

## Read in data
data <- importAmplitudeData(path)
data("thrTable")

## Get name of first list element and use as well ID
well_id <- names(data)[1]

## Set channel to plot
channel <- 1

## Get threshold for well_id and channel 1 (see ?thrTable)
thr <- thrTable[well_id, "thr_target"]

podcallChannelPlot(channelData=data[[well_id]][[channel]], thr, channel)
```

Description

Wrapper function that provide a complete workflow for the functionality of PoDCall. It takes path to amplitude files and sample sheet (optional), and parameters for setting threshold as input. Calls functions that read in data from files, sets threshold for each channel per well, calculates concentrations and optionally makes scatter plot and histogram for each channel per well. Results are returned as a table, optionally written to file. Plots will be written to file in a results directory if argument plots is set to TRUE.

Usage

```
podcallDdpcr(dataDirectory,
             sampleSheetFile=NULL,
             B=200,
             Q=9,
             refwell=1,
             ch2=TRUE,
             resultsToFile=FALSE,
             plots=FALSE,
             resPath=NULL)
```

Arguments

dataDirectory	Path to directory containing QuantaSoft amplitude files from one 96 well plate. Since well coordinates are used as identifiers, files in this directory should all be from the same 96 well plate. Furthermore, there can be no other files than the amplitude files from a well plate in the directory.
sampleSheetFile	File (optional) containing sample information from ddPCR experiment. This file must be a comma separated file containing the following columns: Well, Sample, TargetType and Target.
B	The number of permutations used for the Likelihood Ratio Test (default=200)
Q	A parameter for calling outliers (default=9)
refwell	reference well to calculate the shift in baseline (default=1)
ch2	Logical argument to denote channel 2 amplitudes (default=TRUE)
resultsToFile	Should results be written to file(.csv)? (default=FALSE)
plots	Should plots be created and written to file? (default=FALSE)
resPath	Optional argument to provide results directory path (default=NULL)

Value

The function returns a table (data frame) with thresholds, droplet counts, concentration and normalized concentration. The table is optionally written to a .csv-file and plots for both channels per well can be written to files.

Examples

```
## Paths to data and sample sheet
dataPath <- system.file("extdata", "Amplitudes/", package="PoDCall")
ssPath <- system.file("extdata", "Sample_names.csv", package="PoDCall")

## Run PodCall
podcallResults <- podcallDdpcr(dataDirectory=dataPath,
                              sampleSheetFile=ssPath,
                              B=100)
```

podcallHistogram *podcallHistogram*

Description

Function that make a histogram of amplitude values from one channel of a well with threshold indicated by a vertical line.

Usage

```
podcallHistogram(channelData, thr, channel, plotId = NULL)
```

Arguments

channelData	Amplitude values from one channel of a well.
thr	The threshold set for channel of a well.
channel	The channel the amplitude values belong to.
plotId	A character string with title for the plot

Value

A histogram of amplitude values from a channel from a well with a line indicating the set threshold.

Examples

```
# Get path to data
path <- system.file("extdata", "Amplitudes/", package="PoDCall")

# Read in data
data <- importAmplitudeData(path)
data("thrTable")

# Get name of first list element and use as well ID
well_id <- names(data)[1]

# Set channel to plot
channel <- 1

# Get threshold for well_id and channel 1 (see ?thrTable)
thr <- thrTable[well_id, "thr_target"]

histogram <- podcallHistogram(channelData=data[[well_id]][[channel]],
                             thr,
                             channel)
```

podcallMultiplot *podcallMultiplot*

Description

A function that returns faceted scatterplots for multiple wells suitable for comparison of wells.

Usage

```
podcallMultiplot(plateData, thresholds, channel)
```

Arguments

plateData	A list containing data frames with amplitude values from selected wells that is to be compared. One data frame per well.
thresholds	A vector containing the thresholds for the selected wells
channel	What channel to plot: 1 or 2

Value

Faceted scatterplot with line indicating threshold. One facet per selected well.

Examples

```
## Set path to data
path <- system.file("extdata", "Amplitudes/", package="PoDCall")

## Read in data files
data <- importAmplitudeData(path)
data("thrTable")

## Create plot using threshold from thrTable, see ?thrTable
plot <- podcallMultiplot(plateData=data,
                        thresholds=thrTable[names(data), ],
                        channel=1)
```

podcallScatterplot *podcallScatterplot*

Description

Function that make a scatterplot of amplitude values from one channel of a well with threshold indicated by a horizontal line.

Usage

```
podcallScatterplot(channelData, thr, channel, plotId = NULL)
```

Arguments

channelData	Amplitude values from one channel of a well.
thr	The threshold set for channel of a well.
channel	The channel the amplitude values belong to.
plotId	A character string with title for the plot

Value

A scatterplot of all droplets from a channel from a well with a line indicating the set threshold.

Examples

```
# Get path to data
path <- system.file("extdata", "Amplitudes/", package="PoDCall")

# Read in data
data <- importAmplitudeData(path)
data("thrTable")

# Get name of first list element and use as well ID
well_id <- names(data)[1]

# Set channel to plot
channel <- 1

# Get threshold for well_id and channel 1 (see ?thrTable)
thr <- thrTable[well_id, "thr_target"]

scatterplot <- podcallScatterplot(channelData=data[[well_id]][[channel]],
                                  thr,
                                  channel)
```

podcallShiny

PoDCall shiny launcher

Description

This function launches the PoDCall shiny app in a web browser

Usage

```
podcallShiny()
```


Value

Does not return anything, but launches PoDCall shiny app

Examples

```
## Not run:  
podcallShiny()  
  
## End(Not run)
```

podcallThresholds *podcallThresholds*

Description

Function sets threshold per channel per well and calculates concentrations. Results are returned as a data frame.

Usage

```
podcallThresholds(plateData,  
                  nchannels=c(1,2)[2],  
                  B=200,  
                  Q=9,  
                  refWell=1,  
                  updateProgress=NULL)
```

Arguments

- plateData List of data frames with amplitude data from a 96 well plate
- nchannels Number of channels used in the experiment (default=2)
- B Number of permutations for the Likelihood Ratio Test (LRT) (default=200)
- Q Parameter for outlier calling (default=9)
- refWell reference well to calculate the shift in baseline (default=1)
- updateProgress function to update progress bar in shiny app (default=NULL)

Value

A table with results and metrics, one row per well.

Examples

```
## Path to example data
dataPath <- system.file("extdata", "Amplitudes/", package="PoDCall")

## Read in example data
dataList <- importAmplitudeData(dataDirectory=dataPath)

## Set thresholds
thresholds <- podcallThresholds(plateData=dataList,
                                B=100)
```

 thrTable

PoDCall Example Threshold Table

Description

A data.frame that contains the results of running PodCall with the amplitude data files included in the package. For testing and running of examples. See vignette for more detailed description about columns.

Usage

```
data("thrTable")
```

Format

A data.frame with 13 columns, which are:

sample_id Sample ID
thr_target Threshold channel 1 (target assay)
thr_ctrl Threshold channel 2 (control assay)
pos_dr_target Positive droplets target
pos_dr_ctrl Positive droplets control
tot_droplets Total droplets
c_target Concentration target
c_ctrl Concentration control
c_norm_4Plex Normalized concentration based on 4Plex control
c_norm_sg Normalized concentration based on single gene control
q Parameter Q for calling outliers
target_assay Target assay
ctrl_assay Control assay
ref_well Reference well used to set threshold

Source

In-house cell-line experiment.

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