Package 'IONiseR'

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Title Quality Assessment Tools for Oxford Nanopore MinION data **Version** 2.31.0

Description IONiseR provides tools for the quality assessment of Oxford Nanopore MinION data. It extracts summary statistics from a set of fast5 files and can be used either before or after base calling. In addition to standard summaries of the read-types produced, it provides a number of plots for visualising metrics relative to experiment run time or spatially over the surface of a flowcell.

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'Methods-subsetting.R' 'fast5Readers.R' 'fast5Status.R'

'fast5readers_summary.R' 'fast5utilities.R' 'fastqProcessing.R'

'plotting_kmers.R' 'plotting_layout.R'

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base(Called Extract baseCalled slot	

Description

This generic function accesses the baseCalled slot stored in an object derived from the Fast5Summary class.

Usage

baseCalled(x)

channelActivityPlot 3

Arguments

Х

Object of class Fast5Summary

Value

A data.frame with 6 columns

Examples

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   baseCalled( s.typhi.rep2 )
}
```

 ${\tt channelActivityPlot}$

Visualise a specified metric over all channels over time.

Description

Plots a line for each fast 5 file, arranged by channel and experiment time when the signal was being recorded. The colour of each line can be specified by the user to reflect any metric they wish. The intention of the plot is to investigate trends that may appear at specific time points, or influence a subset of channels.

Usage

```
channelActivityPlot(summaryData, zScale = NULL, zAverage = TRUE)
```

Arguments

summaryData Object of class Fast5Summary.

zScale A data.frame containg two columns. The first must be labelled 'id' and cor-

respond to id field present in all slots in summaryData. The second column should contain data pertaining to that reads that you wish to be represented on

the coloured z-axis.

zAverage Logical indicating if a bar showing the mean across all channel for the choosen

zScale should be shown on the plot. Defaults to TRUE.

Value

Returns an object of class gg representing the plot.

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Examples

```
if( require(minionSummaryData) ) {
   require(dplyr)
   data(s.typhi.rep3, package = 'minionSummaryData')
   ## we will plot the median event signal for each read on z-axis
   z_scale = select(eventData(s.typhi.rep3), id, median_signal)
   channelActivityPlot( s.typhi.rep3, zScale = z_scale )
}
```

channelHeatmap

Create layout plot of flowcell

Description

Creates a plot representing the layout of a MinION flow cell. Each circle represents an individual channel with the intensity relecting a specified sequencing metric. This function is a more generalised version of layoutPlot, allowing the user to map any value the like on the channel layout.

Usage

```
channelHeatmap(data, zValue)
```

Arguments

data A data frame. Should have at least two columns, one of which has the name

'channel'.

zValue Character string specifying the name of the column to be used for the colour

scaling.

Value

Returns an object of gg representing the plot.

```
library(dplyr)
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   ## calculate and plot the mean number of events recorded by each channel
   avgEvents <- left_join(readInfo(s.typhi.rep2), eventData(s.typhi.rep2), by = 'id') %>%
   group_by(channel) %>%
   summarise(mean_nevents = mean(num_events))
   channelHeatmap(avgEvents, zValue = 'mean_nevents')
}
```

eventData 5

eventData

Extract eventData slot

Description

This generic function accesses the eventData slot stored in an object derived from the Fast5Summary class.

Usage

```
eventData(x)
```

Arguments

Х

Object of class Fast5Summary

Value

A data frame with 5 columns

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  eventData( s.typhi.rep2 )
}
```

Fast5Summary-class

An S4 class for summarised data from a MinION sequencing run

Description

An S4 class for summarised data from a MinION sequencing run

Usage

```
## $4 method for signature 'Fast5Summary'
length(x)

## $4 method for signature 'Fast5Summary'
readInfo(x)

## $4 method for signature 'Fast5Summary'
eventData(x)

## $4 method for signature 'Fast5Summary'
```

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```
baseCalled(x)
## S4 method for signature 'Fast5Summary'
fastq(x)
## S4 method for signature 'Fast5Summary,ANY,ANY,ANY'
x[i]
## S4 method for signature 'Fast5Summary'
fastqTemplate(x)
## S4 method for signature 'Fast5Summary'
fastqComplement(x)
## S4 method for signature 'Fast5Summary'
fastq2D(x)
```

Arguments

- x Object of class Fast5Summary
- i Vector defining index to subset by.

Value

An object of class Fast5Summary

Methods (by generic)

- length: Returns the number of files read during creation of the object
- readInfo: Returns readInfo data.frame
- eventData: Returns eventData data.frame
- baseCalled: Returns baseCalled data.frame
- fastq: Returns ShortReadQ object stored in fastq slot.
- [: Subset object and return an object of the same class.
- fastqTemplate: Returns ShortReadQ object containing only template reads
- fastqComplement: Returns ShortReadQ object containing only complement reads
- fastq2D: Returns ShortReadQ object containing only 2D reads

Slots

readInfo Object of class tibble. Contains five columns:

- id an integer key that allows use to match entries in the separate slots of this object.
- file Basename of the fast5 file the data was read from.
- read Read number from channel.
- channel channel.

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• mux - Specific pore that was used within the four that are assigned to a single channel. Should be in the range 1-4, but if this isn't available it will be 0.

rawData Object of class tibble. Intended to hold raw signal data although reading this is currently not implemented in IONiseR.

eventData Object of class tibble. Holds summary of events data prior to base calling. Contains five columns:

- id an integer key that allows use to match entries in the separate slots of this object.
- start time time in seconds after the run started that this reading began.
- duration time in seconds the reading lasted.
- num_events the number of events that were recorded as part of this reading.
- median_signal median of the recorded signals for this set of events.

baseCalled Object of class tibble. For the most part contains similar data to the @eventData slot, the base called data is derived from it.

- id an integer key that allows use to match entries in the separate slots of this object.
- start_time time in seconds after the run started that this reading began.
- duration time in seconds the reading lasted.
- num_events the number of events that were recorded as part of this reading.
- strand can be either 'template' or 'complement'
- full_2D boolean value specifying whether the read forms part of a 2D pair. If TRUE the FASTQ data for the template, complement and 2D read will be available in the @fastq slot.

fastq Object of class ShortReadQ. This slot contains all reads (template, complement and 2D). The read names take the form NUM_STRAND, where NUM matches with the id column in the other slots and STRAND indicates whether the read is template, complement or 2D.

versions A list intended to store the version of IONiseR that was used to create the object. (May be extended in the future to include the version of MinKNOW that the original fast5 files were processed, if this can be determined accurately.)

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  length( s.typhi.rep2 )
}
```

fast5toFastq

Extract FASTQ files from fast5 files

Description

This function provides direct access to the FASTQ entries held within fast5 files. If you are only interested in getting hold of the base called reads, and don't require any raw-signal or event information, use this function. Given a vector of fast5 files, the FASTQ entries will be combined and up to three gzip compressed FASTQ will be created - one for each of the template, complement and 2D strands depending upon what is available in the input files.

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Usage

```
fast5toFastq(files, strand = "all", fileName = NULL, outputDir = NULL,
ncores = 1)
```

Arguments

files Character vector of fast5 files to be read.

Strand Character vector specifying the strand to extract. Can take any combination of the following options: "template", "complement", "2D", "all", "both".

Stem for the name of the names of the output file names. The appropriate strand will be appended to each file e.g. fileName_complement.fq.gz or fileName_template.fq.gz

outputDir Directory output files should be written to.

Specify the number of CPU cores that should be used to process the files. Currently this seems to be more IO bound than CPU, so there is little benefit achieved

by using a high number of cores.

Value

No value returned. Run for the side effect of writing the FASTQ files to disk.

Examples

```
## Not run:
fast5files <- list.files('/foo/bar/', pattern = '.fast5$')
summaryData <- readFast5Summary(fast5files)
## End(Not run)</pre>
```

fastq

Extract fastq slot

Description

This generic function accesses the fastq slot stored in an object derived from the Fast5Summary class.

Usage

```
fastq(x)
```

Arguments

Х

Object of class Fast5Summary

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Value

A ShortReadQ object

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  fastq( s.typhi.rep2 )
}
```

fastq2D

Extract 2D reads

Description

This generic function accesses the fastq slot stored in an object derived from the Fast5Summary class, and returns only the 2D reads.

Usage

```
fastq2D(x)
```

Arguments

Χ

Object of class Fast5Summary

Value

A ShortReadQ object

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   fastq2D( s.typhi.rep2 )
}
```

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fastqComplement

Extract complement reads

Description

This generic function accesses the fastq slot stored in an object derived from the Fast5Summary class, and returns only the complement reads.

Usage

```
fastqComplement(x)
```

Arguments

Х

Object of class Fast5Summary

Value

A ShortReadQ object

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  fastqComplement( s.typhi.rep2 )
}
```

fastqTemplate

Extract template reads

Description

This generic function accesses the fastq slot stored in an object derived from the Fast5Summary class, and returns only the template reads.

Usage

```
fastqTemplate(x)
```

Arguments

Χ

Object of class Fast5Summary

Value

A ShortReadQ object

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Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  fastqTemplate( s.typhi.rep2 )
}
```

IONiseR

IONiseR: A package for assessing quality of MinION data

Description

IONiseR provides tools for the quality assessment of Oxford Nanopore MinION data. It extracts summary statistics from a set of fast5 files and can be used either before or after base calling. In addition to standard summaries of the read-types produced, it provides a number of plots for visualising metrics relative to experiment run time or spatially over the surface of a flowcell.

layoutPlot

Create layout plot of flowcell

Description

Creates a plot representing the layout of a MinION flow cell. Each circle represents an idividual channel with the intensity relecting the total kilobases of sequence produced. This only considers reads marked as template or complement, 2D reads are ignored as they are generated from the former two.

Usage

```
layoutPlot(summaryData, attribute = NULL)
```

Arguments

```
summary \texttt{Data} \qquad Object \ of \ class \ \textbf{Fast5Summary}.
```

attribute Character string indicating what to plot. Currently accepted values are: "nreads", "kb", "signal".

Value

Returns an object of gg representing the plot.

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   layoutPlot( s.typhi.rep2, attribute = 'nreads' )
   layoutPlot( s.typhi.rep2, attribute = 'kb' )
}
```

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muxHeatmap	Create layout plot of flowcell
------------	--------------------------------

Description

Creates a plot representing the layout of a MinION flow cell. Each circle represents an individual channel with the intensity relecting a specified sequencing metric. This function is a more generalised version of layoutPlot, allowing the user to map any value the like on the channel layout.

Usage

```
muxHeatmap(data, zValue)
```

Arguments

data A data.frame. Should have at least two columns, one of which has the name

'channel'.

zValue Character string specifying the name of the column to be used for the colour

scaling.

Value

Returns an object of gg representing the plot.

plotActiveChannels Plot the number of active channels for each minute of run time

Description

Plot the number of active channels for each minute of run time

Usage

```
plotActiveChannels(summaryData)
```

Arguments

summaryData Object of class Fast5Summary.

Value

Returns an object of class gg representing the plot.

plotBaseProductionRate 13

Examples

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   plotActiveChannels( s.typhi.rep2 )
}
```

plotBaseProductionRate

Plot the mean rate at which bases are recorded

Description

For each read, the ratio between the total number of bases called in the read (template and complement strand, but not 2D composite) and the time spent in the pore is calculated. This is then plotted against the time the read entered the pore, allow us to assess whether the rate at which callable bases are read changes during the experiment run time.

Usage

```
plotBaseProductionRate(summaryData)
```

Arguments

```
summaryData Object of class Fast5Summary.
```

Details

This is likely very similar to plotEventRate, although one may find that large number of events occur that can not be base called, resulting in a difference between these two plots.

Value

Returns an object of class gg representing the plot.

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  plotBaseProductionRate( s.typhi.rep2 )
}
```

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plotCurrentByTime

View changes in signal against run time.

Description

Plots the median recorded current for each fast5 file against the time at which the recording began.

Usage

```
plotCurrentByTime(summaryData)
```

Arguments

```
summaryData Object of class Fast5Summary.
```

Value

Returns an object of class gg representing the plot.

Examples

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   plotCurrentByTime( s.typhi.rep2 )
}
```

plotEventRate

Plot the mean rate at which events occur

Description

For each read, the ratio between the number of events comprising the read and the time spent in the pore is calculated. This is then plotted against the time the read entered the pore, allow us to assess whether the rate at which events occur changes during the experiment run time.

Usage

```
plotEventRate(summaryData)
```

Arguments

```
summaryData Object of class Fast5Summary.
```

Value

Returns an object of class gg representing the plot.

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  plotEventRate( s.typhi.rep2 )
}
```

plotKmerFrequencyCorrelation

Display correlation between pentemer proportions in two time windows

Description

Plots

Usage

```
plotKmerFrequencyCorrelation(summaryData, kmerLength = 5,
  groupedMinutes = 10, only2D = TRUE)
```

Arguments

summaryData Object of class Fast5Summary.

kmerLength Specifies the length of kmers to compare. Defaults to 5 given the current pentamer reading nature of the nanopores.

groupedMinutes Defines how many minutes each grouping of reads spans.

only2D Logical. If TRUE kmers are computed for only full 2D reads. If FALSE 2D reads are ignored and all available template and complement strands are used.

Value

Returns an object of class gg representing the plot.

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep3, package = 'minionSummaryData')
   plotKmerFrequencyCorrelation( s.typhi.rep3, only2D = FALSE )
}
```

Description

Plot the accumulation of reads over the duration of the experiment.

Usage

```
plotReadAccumulation(summaryData)
```

Arguments

```
summaryData Object of class Fast5Summary.
```

Value

Returns an object of class gg representing the plot.

Examples

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   plotReadAccumulation( s.typhi.rep2 )
}
```

plotReadCategoryCounts

Plot the proportion of template, complement and 2D reads found a dataset.

Description

Generates a bar plot showing the breakdown of read types found in a set of fast5 files. There is a strict hierarchy to the types of read that can be found in a fast5 file. A full 2D read requires both a complement and template strand to have been read correctly. Similarly, a complement strand can only be present if the template was read successfully. Finally, you can encounter a file containing now called bases on either strand. Here we visualise the total number of fast5 files, along with the counts containing each of the categories above. For an ideal dataset all four bars will be the same height. This is unlikely, but the drop between bars can give some indication of data quality.

Usage

```
plotReadCategoryCounts(summaryData)
```

plotReadCategoryQuals

Arguments

```
summaryData Object of class Fast5Summary.
```

Value

Returns an object of class gg representing the plot.

Examples

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   plotReadCategoryCounts( s.typhi.rep2 )
}
```

plotReadCategoryQuals Visualise the mean base quality of each read

Description

Generates a box plot showing the mean base quality for each read, broken down into the three categories of read type that can be found in a fast5 file.

Usage

```
plotReadCategoryQuals(summaryData)
```

Arguments

```
summaryData Object of class Fast5Summary.
```

Value

Returns an object of class gg representing the plot.

```
if( require(minionSummaryData) ) {
   data(s.typhi.rep2, package = 'minionSummaryData')
   plotReadCategoryQuals( s.typhi.rep2 )
}
```

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```
plotReadTypeProduction
```

View changes in signal against run time.

Description

Plots the median recorded current for each fast5 file against the time at which the recording began.

Usage

```
plotReadTypeProduction(summaryData, groupedMinutes = 10)
```

Arguments

```
summaryData Object of class Fast5Summary.

groupedMinutes Integer specifying how many minutes of runtime should be grouped together.
```

Value

Returns an object of class gg representing the plot.

Examples

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  plotReadTypeProduction( s.typhi.rep2 )
}
```

readFast5Log

Read the log entry from a single fast5 file

Description

Basecalling procedures performed on fast5 files generally leave a log file entry recording how far through the pipeline the file proceeded. This function will extract this information as a single string. It can be printed in a more readable format using the cat function.

Usage

```
readFast5Log(file)
```

Arguments

file

Character vector of fast5 file to be read.

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Value

Character vector containing the log information. NULL if no log is found.

Examples

```
fast5file <- system.file('extdata', 'example.fast5', package = "IONiseR")
log <- readFast5Log(fast5file)
cat(log)</pre>
```

readFast5Summary

Read summary data from fast5 files.

Description

Reads one or more fast5 files and collects summary information about them.

Usage

```
readFast5Summary(files)
```

Arguments

files

Character vector of fast5 files to be read.

Details

Currently this function assumes all files passed to it come from the same sequencing run. It makes no effort to check for alternative file names or the like. If files from multiple runs are passed to it they will be collated together and any analysis performed on them will represent the mixture of both experiments.

Value

Object of class Fast5Summary

```
## Not run:
fast5files <- list.files('/foo/bar/', pattern = '.fast5$')
summaryData <- readFast5Summary(fast5files)
## End(Not run)</pre>
```

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readInfo

 ${\it Extract\ readInfo\ slot}$

Description

This generic function accesses the readInfo slot stored in an object derived from the Fast5Summary class.

Usage

```
readInfo(x)
```

Arguments

Х

Object of class Fast5Summary

Value

A data.frame with 5 columns

```
if( require(minionSummaryData) ) {
  data(s.typhi.rep2, package = 'minionSummaryData')
  readInfo( s.typhi.rep2 )
}
```

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