

Package ‘qckitfastq’

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Type Package

Title FASTQ Quality Control

Version 1.24.0

Description Assessment of FASTQ file format with multiple metrics including quality score, sequence content, overrepresented sequence and Kmers.

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adapter_content	<i>Creates a sorted from most frequent to least frequent abundance table of adapters that are found to be present in the reads at greater than 0.1% of the reads. If output_file is selected then will save the entire set of adapters and counts. Only available for macOS/Linux due to dependency on C++14.</i>
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Description

Creates a sorted from most frequent to least frequent abundance table of adapters that are found to be present in the reads at greater than 0.1% of the reads. If output_file is selected then will save the entire set of adapters and counts. Only available for macOS/Linux due to dependency on C++14.

Usage

```
adapter_content(infile, adapter_file = system.file("extdata",
  "adapters.txt", package = "qckitfastq"), output_file = NA)
```

Arguments

infile	the path to a gzipped FASTQ file
adapter_file	Path to adapters.txt file. Default from package.
output_file	File to save data frame to. Default NA.

Value

Sorted table of adapters and counts.

Examples

```
if(.Platform$OS.type != "windows") {  
  infile <- system.file("extdata", "test.fq.gz",  
    package = "qckitfastq")  
  adapter_content(infile)[1:5]  
}
```

calc_adapter_content	<i>Compute adapter content in reads. This function is only available for macOS/Linux.</i>
----------------------	---

Description

Compute adapter content in reads. This function is only available for macOS/Linux.

Usage

```
calc_adapter_content(infile, adapters)
```

Arguments

infile	filepath to fastq sequence
adapters	filepath to adapters

Value

map object with adapter names as the key and the number of times the adapters appears in the reads as the value

Examples

```
if(.Platform$OS.type != "windows") {  
  adapter_file <- system.file("extdata", "adapters.txt", package = "qckitfastq")  
  infile <- system.file("extdata", "test.fq.gz", package = "qckitfastq")  
  content <- calc_adapter_content(infile, adapter_file)  
}
```

calc_format_score	<i>Calculate score based on Illumina format</i>
-------------------	---

Description

Calculate score based on Illumina format

Usage

```
calc_format_score(score, score_format)
```

Arguments

score	An ascii quality score from the fastq
score_format	The illumina format

Value

a string as with the best guess as to the illumina format

Examples

```
calc_format_score("A", "Sanger")
```

calc_over_rep_seq	<i>Calculate sequece counts for each unique sequence and create a table with unique sequences and corresponding counts</i>
-------------------	--

Description

Calculate sequece counts for each unique sequence and create a table with unique sequences and corresponding counts

Usage

```
calc_over_rep_seq(infile, min_size = 5L, buffer_size = 1000000L)
```

Arguments

infile	A string giving the path for the fastqfile
min_size	An int for thresholding over representation
buffer_size	An int for the number of lines to keep in memory

Value

calculate overrepresented sequence count

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
calc_over_rep_seq(infile)[seq_len(5)]
```

dimensions	<i>Extract the number of columns and rows for a FASTQ file using seqTools.</i>
------------	--

Description

Extract the number of columns and rows for a FASTQ file using seqTools.

Usage

```
dimensions(fseq, sel)
```

Arguments

fseq	an object that is the read result of the seq.read function
sel	'reads' for #reads/rows, 'positions' for #positions/columns

Value

a numeric value of the number of reads or the number of positions

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz",
  package = "qckitfastq")
fseq <- seqTools::fastq(infile, k=6)
dimensions(fseq, "reads")
```

find_format	<i>Gets quality score encoding format from the FASTQ file. Return possibilities are Sanger(Illumina1.8), Solexa(Illumina1.0), Illumina1.3, and Illumina1.5. This encoding is heuristic based and may not be 100 since there is overlap in the encodings used, so it is best if you already know the format.</i>
-------------	---

Description

Gets quality score encoding format from the FASTQ file. Return possibilities are Sanger(Illumina1.8), Solexa(Illumina1.0), Illumina1.3, and Illumina1.5. This encoding is heuristic based and may not be 100 since there is overlap in the encodings used, so it is best if you already know the format.

Usage

```
find_format(infile, reads_used)
```

Arguments

`infile` A string giving the path for the fastq file
`reads_used` int, the number of reads to use to determine the encoding format.

Value

A string denoting the read format. Possibilities are Sanger, Solexa, Illumina1.3, and Illumina1.5.

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
find_format(infile, 100)
```

GC_content	<i>Calculates GC content percentage for each read in the dataset.</i>
------------	---

Description

Calculates GC content percentage for each read in the dataset.

Usage

```
GC_content(infile, output_file = NA)
```

Arguments

`infile` the object that is the path to the FASTQ file
`output_file` File to write results to. Default NA.

Value

Data frame with read ID and GC content of each read.

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz",
  package = "qckitfastq")
head(GC_content(infile))
```

gc_per_read	<i>Calculate GC nucleotide sequence content per read of the FASTQ gzipped file</i>
-------------	--

Description

Calculate GC nucleotide sequence content per read of the FASTQ gzipped file

Usage

```
gc_per_read(infile)
```

Arguments

infile	A string giving the path for the fastqfile
--------	--

Value

GC content percentage per read

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
gc_per_read(infile)[1:10]
```

kmer_count	<i>Return kmer count per sequence for the length of kmer desired</i>
------------	--

Description

Return kmer count per sequence for the length of kmer desired

Usage

```
kmer_count(infile, k, output_file = NA)
```

Arguments

infile	the object that is the path to gzipped FASTQ file
k	the length of kmer
output_file	File to save plot to. Default NA.

Value

kmers counts per sequence

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz",
  package = "qckitfastq")
km<-kmer_count(infile,k=4)
km[1:20,1:10]
```

overrep_kmer	<i>Generate overrepresented kmers of length k based on their observed to expected ratio at each position across all sequences in the dataset. The expected proportion of a length k kmer assumes site independence and is computed as the sum of the count of each base pair in the kmer times the probability of observing that base pair in the data set, i.e. $P(A)count_in_kmer(A)+P(C)count_in_kmer(C)+...$ The observed to expected ratio is computed as $\log_2(obs/exp)$. Those with $obsexp_ratio > 2$ are considered to be overrepresented and appear in the returned data frame along with their position in the sequence.</i>
--------------	--

Description

Generate overrepresented kmers of length k based on their observed to expected ratio at each position across all sequences in the dataset. The expected proportion of a length k kmer assumes site independence and is computed as the sum of the count of each base pair in the kmer times the probability of observing that base pair in the data set, i.e. $P(A)count_in_kmer(A)+P(C)count_in_kmer(C)+...$ The observed to expected ratio is computed as $\log_2(obs/exp)$. Those with $obsexp_ratio > 2$ are considered to be overrepresented and appear in the returned data frame along with their position in the sequence.

Usage

```
overrep_kmer(infile, k, output_file = NA)
```

Arguments

infile	path to gzipped FASTQ file
k	the kmer length
output_file	File to save plot to. Default NA.

Value

Data frame with columns: Position (in read), Obsexp_ratio, & Kmer

Examples

```
infile <-system.file("extdata", "test.fq.gz",
  package = "qckitfastq")
overrep_kmer(infile,k=4)
```

overrep_reads	<i>Sort all sequences per read by count.</i>
---------------	--

Description

Sort all sequences per read by count.

Usage

```
overrep_reads(infile, output_file = NA)
```

Arguments

infile	Path to gzipped FASTQ file.
output_file	File to save data frame to. Default NA.

Value

Table of sequences sorted by count.

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz",
  package = "qckitfastq")
overrep_reads(infile)[1:5,]
```

per_base_quality	<i>Compute the mean, median, and percentiles of quality score per base. This is returned as a data frame.</i>
------------------	---

Description

Compute the mean, median, and percentiles of quality score per base. This is returned as a data frame.

Usage

```
per_base_quality(infile, output_file = NA)
```

Arguments

infile	Path to a gzipped FASTQ file
output_file	File to write results in CSV format to. Default NA.

Value

A dataframe of the mean, median and quantiles of the FASTQ file

Author(s)

Wenyue Xing, <wenyue_xing@brown.edu>

August Guang, <august_guang@brown.edu>

Examples

```
per_base_quality(system.file("extdata", "10^5_reads_test.fq.gz",
  package = "qckitfastq"))
```

per_read_quality	<i>Compute the mean quality score per read.</i>	per_read_quality
------------------	---	------------------

Description

Compute the mean quality score per read. per_read_quality

Usage

```
per_read_quality(infile, output_file = NA)
```

Arguments

infile	Path to FASTQ file
output_file	File to write plot to. Will not write to file if NA. Default NA.

Value

Data frame of mean quality score per read

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
prq <- per_read_quality(infile)
```

plot_adapter_content *Creates a bar plot of the top 5 most present adapter sequences.*

Description

Creates a bar plot of the top 5 most present adapter sequences.

Usage

```
plot_adapter_content(ac_sorted, output_file = NA)
```

Arguments

ac_sorted Sorted table of adapters and counts.
output_file File to save data frame to. Default NA.

Value

Barplot of top 5 most frequent adapter sequences.

Examples

```
if(.Platform$OS.type != "windows") {  
  infile <- system.file("extdata", "test.fq.gz", package = "qckitfastq")  
  ac_sorted <- adapter_content(infile)  
  plot_adapter_content(ac_sorted)  
}
```

plot_GC_content *Generate mean GC content histogram.*

Description

Generate mean GC content histogram.

Usage

```
plot_GC_content(gc_df, output_file = NA)
```

Arguments

gc_df the object that is the GC content vectors generated from GC content function
output_file File to write plot to. Will not write to file if NA. Default NA.

Value

A histogram of mean GC content.

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
gc_df<-GC_content(infile)
plot_GC_content(gc_df)
```

plot_outliers	<i>Determine how to plot outliers. Heuristic used is whether their obsexp_ratio differs by more than 1 and whether they fall into the same bin or not. If for 2 outliers, obsexp_ratio differs by less than .4 and they are in the same bin, then combine into a single plotting point. NOT FULLY FUNCTIONAL</i>
---------------	--

Description

Determine how to plot outliers. Heuristic used is whether their obsexp_ratio differs by more than 1 and whether they fall into the same bin or not. If for 2 outliers, obsexp_ratio differs by less than .4 and they are in the same bin, then combine into a single plotting point. NOT FULLY FUNCTIONAL

Usage

```
plot_outliers(overkm, top_num)
```

Arguments

overkm	data frame with columns pos, obsexp_ratio, and kmer that has already been reordered by descending obsexp_ratio
top_num	number of most overrepresented kmers to plot. Default is 5.

Value

currently 0 as function is not fully working.

plot_overrep_kmer	<i>Create a box plot of the log2(observed/expected) ratio across the length of the sequence as well as top overrepresented kmers. Only ratios greater than 2 are included in the box plot. Default is 20 bins across the length of the sequence and the top 2 overrepresented kmers, but this can be changed by the user.</i>
-------------------	---

Description

Create a box plot of the log2(observed/expected) ratio across the length of the sequence as well as top overrepresented kmers. Only ratios greater than 2 are included in the box plot. Default is 20 bins across the length of the sequence and the top 2 overrepresented kmers, but this can be changed by the user.

Usage

```
plot_overrep_kmer(overkm, bins = 20, top_num = 2, output_file = NA)
```

Arguments

overkm	data frame with columns pos, obsexp_ratio, and kmer
bins	number of intervals across the length of the sequence
top_num	number of most overrepresented kmers to plot
output_file	File to write plot to. Will not write to file if NA. Default NA.

Value

A box plot of the $\log_2(\text{observed/expected ratio})$ across the length of the sequence

Examples

```
infile <- system.file("extdata", "test.fq.gz",
  package = "qckitfastq")
over_km <- overrep_kmer(infile,k=4)
plot_overrep_kmer(over_km)
```

plot_overrep_reads	<i>Plot the top 5 sequences</i>
--------------------	---------------------------------

Description

Plot the top 5 sequences

Usage

```
plot_overrep_reads(overrep_reads, output_file = NA)
```

Arguments

overrep_reads	the table that sorts the sequence content and corresponding counts in descending order
output_file	File to save plot to. Will not write to file if NA. Default NA.

Value

plot of the top 5 overrepresented sequences

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
overrep_df <- overrep_reads(infile)
plot_overrep_reads(overrep_df)
```

plot_per_base_quality *Generate a boxplot of the per position quality score.*

Description

Generate a boxplot of the per position quality score.

Usage

```
plot_per_base_quality(per_base_quality, output_file = NA)
```

Arguments

per_base_quality
a data frame of the mean, median and quantiles of sequence quality per base. Most likely generated with the 'per_base_quality' function.

output_file File to save plot to. Will not write to file if NA. Default NA.

Value

A boxplot of per position quality score distribution.

Examples

```
pbq <- per_base_quality(system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq"))
plot_per_base_quality(pbq)
```

plot_per_read_quality *Plot the mean quality score per sequence as a histogram. High quality sequences are those mostly distributed over 30. Low quality sequences are those mostly under 30.* plot_per_read_quality

Description

Plot the mean quality score per sequence as a histogram. High quality sequences are those mostly distributed over 30. Low quality sequences are those mostly under 30. plot_per_read_quality

Usage

```
plot_per_read_quality(prq, output_file = NA)
```

Arguments

prq Data frame from per_read_quality function

output_file File to write plot to. Will not write to file if NA. Default NA.

Value

Plot of mean quality score per read

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
prq <- per_read_quality(infile)
plot_per_read_quality(prq)
```

plot_read_content	<i>Plot the per position nucleotide content.</i>
-------------------	--

Description

Plot the per position nucleotide content.

Usage

```
plot_read_content(read_content, output_file = NA)
```

Arguments

read_content	Data frame produced by read_content function.
output_file	File to save plot to. Will not write to file if NA. Default NA.

Value

ggplot line plot of all nucleotide content including A, T, G, C and N

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
fseq <- seqTools::fastqq(infile,k=6)
read_content <- read_content(fseq)
plot_read_content(read_content)
```

plot_read_length	<i>Plot a histogram of the number of reads with each read length.</i>
------------------	---

Description

Plot a histogram of the number of reads with each read length.

Usage

```
plot_read_length(read_len, output_file = NA)
```

Arguments

read_len	Data frame of read lengths and number of reads with that length.
output_file	File to save plot to. Default is NA, i.e. do not write to file.

Value

A histogram of the read length distribution.

Author(s)

Wenyue Xing, <wenyue_xing@brown.edu>, August Guang, <august_guang@brown.edu>

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
fseq <- seqTools::fastq(infile,k=6)
read_len <- read_length(fseq)
plot_read_length(read_len)
```

qual_score_per_read	<i>Calculate the mean quality score per read of the FASTQ gzipped file</i>
---------------------	--

Description

Calculate the mean quality score per read of the FASTQ gzipped file

Usage

```
qual_score_per_read(infile)
```

Arguments

infile	A string giving the path for the fastqfile
--------	--

Value

mean quality per read

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
qual_score_per_read(infile)$q50_per_position[1:10]
```

read_base_content	<i>Compute nucleotide content per position for a single base pair. Wrapper function around seqTools.</i>
-------------------	--

Description

Compute nucleotide content per position for a single base pair. Wrapper function around seqTools.

Usage

```
read_base_content(fseq, content)
```

Arguments

fseq	a seqTools::fastq object
content	nucleotide. Options are "A","T","G","C","N"(either capital or lower case)

Value

Nucleotide sequence content per position.

Author(s)

Wenyue Xing, <wenyue_xing@brown.edu>, August Guang <august_guang@brown.edu>

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
fseq <- seqTools::fastq(infile,k=6)
read_base_content(fseq,"A")
```

read_content	<i>Compute nucleotide content per position. Wrapper function around seqTools.</i>
--------------	---

Description

Compute nucleotide content per position. Wrapper function around seqTools.

Usage

```
read_content(fseq, output_file = NA)
```

Arguments

fseq	a seqTools::fastqq object
output_file	File to write results in CSV format to. Will not write to file if NA. Default NA.

Value

Data frame of nucleotide sequence content per position

Examples

```
infile <- system.file("extdata", "10^5_reads_test.fq.gz", package = "qckitfastq")
fseq <- seqTools::fastqq(infile,k=6)
read_content(fseq)
```

read_length	<i>Creates a data frame of read lengths and the number of reads with that read length.</i>
-------------	--

Description

Creates a data frame of read lengths and the number of reads with that read length.

Usage

```
read_length(fseq, output_file = NA)
```

Arguments

fseq	a seqTools object produced by seqTools::fastqq on the raw FASTQ file
output_file	File to save data frame to. Default NA.

Value

Data frame of read lengths and number of reads with that length.

Examples

```
infile <- system.file("extdata", "test.fq.gz",
  package = "qckitfastq")
fseq <- seqTools::fastqq(infile, k=6)
read_len <- read_length(fseq)
```

run_all	<i>Will run all functions in the qckitfastq suite and save the data frames and plots to a user-provided directory. Plot names are supplied by default.</i>
---------	--

Description

Will run all functions in the qckitfastq suite and save the data frames and plots to a user-provided directory. Plot names are supplied by default.

Usage

```
run_all(infile, dir)
```

Arguments

infile	Path to gzipped FASTQ file
dir	Directory to save results to

Value

Generate files from all functions

Examples

```
infile <- system.file("extdata", "test.fq.gz",
  package = "qckitfastq")
testfolder <- tempdir()
run_all(infile, testfolder)
```

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