

Package ‘sangeranalyseR’

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Type Package

Title sangeranalyseR: a suite of functions for the analysis of Sanger sequence data in R

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Description This package builds on sangerseqR to allow users to create contigs from collections of Sanger sequencing reads. It provides a wide range of options for a number of commonly-performed actions including read trimming, detecting secondary peaks, and detecting indels using a reference sequence. All parameters can be adjusted interactively either in R or in the associated Shiny applications. There is extensive online documentation, and the package can outputs detailed HTML reports, including chromatograms.

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'MethodSangerAlignment.R' 'MethodSangerContig.R'
 'MethodSangerRead.R' 'MethodShared.R' 'MethodsQualityReport.R'
 'ShinySangerAlignmentServer.R' 'ShinySangerAlignmentUI.R'
 'ShinySangerContigServer.R' 'ShinySangerContigUI.R'
 'ShinyServerModule.R' 'UtilitiesFunc.R'
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ChromatogramParam-class
<i>ChromatogramParam</i>

Description

An S4 class storing chromatogram related inputs in a SangerRead S4 object.

Slots

- baseNumPerRow It defines maximum base pairs in each row. The default value is 100.
- heightPerRow It defines the height of each row in chromatogram. The default value is 200.
- signalRatioCutoff The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.
- showTrimmed The logical value storing whether to show trimmed base pairs in chromatogram. The default value is TRUE.

Author(s)

Kuan-Hao Chao

Examples

```
Chromatogram <- new("ChromatogramParam",
  baseNumPerRow = 100,
  heightPerRow = 200,
  signalRatioCutoff = 0.33,
  showTrimmed = TRUE)
```

generateReport	<i>Method generateReport</i>
----------------	------------------------------

Description

A method which generates final reports of the SangerRead, SangerContig, and SangerAlignment instance.

Usage

```
generateReport(
  object,
  outputDir = NULL,
  includeSangerContig = TRUE,
  includeSangerRead = TRUE,
  colors = "default",
  ...
)
```

Arguments

object	A SangerRead, SangerContig, or SangerAlignment S4 instance.
outputDir	The output directory of the generated HTML report.
includeSangerContig	The parameter that decides whether to include SangerContig level report. The value is TRUE or FALSE and the default is TRUE.
includeSangerRead	The parameter that decides whether to include SangerRead level report. The value is TRUE or FALSE and the default is TRUE.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.
...	Further generateReportSR, generateReportSC, and generateReportSA related parameters.

Value

A SangerRead, SangerContig, or SangerAlignment object.

Author(s)

Kuan-Hao Chao

Examples

```

data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
generateReport(sangerReadFData)
generateReport(sangerReadFData, colors="cb_friendly")
generateReport(sangerContigData)
generateReport(sangerContigData, colors="cb_friendly")
generateReport(sangerAlignmentData)
generateReport(sangerAlignmentData, colors="cb_friendly")
## End(Not run)

```

generateReportSA	<i>Method generateReportSA</i>
------------------	--------------------------------

Description

Method generateReportSA

Usage

```

generateReportSA(
  object,
  outputDir = NULL,
  includeSangerContig = TRUE,
  includeSangerRead = TRUE,
  colors = "default",
  ...
)

```

Arguments

object	A SangerAlignment S4 instance.
outputDir	The output directory of the generated HTML report.
includeSangerContig	The parameter that decides whether to include SangerContig level report. The value is TRUE or FALSE and the default is TRUE.
includeSangerRead	The parameter that decides whether to include SangerRead level report. The value is TRUE or FALSE and the default is TRUE.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.
...	Further generateReportSA-related parameters.

Value

The output absolute path to the SangerAlignment’s HTML file.

Examples

```
data(sangerAlignmentData)
## Not run:
generateReportSA(sangerAlignmentData)
## End(Not run)
```

generateReportSC	<i>Method generateReportSC</i>
------------------	--------------------------------

Description

Method generateReportSC

Usage

```
generateReportSC(
  object,
  outputDir = NULL,
  includeSangerRead = TRUE,
  colors = "default",
  ...
)
```

Arguments

object	A SangerContig S4 instance.
outputDir	The output directory of the generated HTML report.
includeSangerRead	The parameter that decides whether to include SangerRead level report. The value is TRUE or FALSE and the default is TRUE.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.
...	Further generateReportSC-related parameters.

Value

The output absolute path to the SangerContig’s HTML file.

Examples

```
data(sangerContigData)
## Not run:
generateReportSC(sangerContigData)
## End(Not run)
```

generateReportSR	<i>Method generateReportSR</i>
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Description

Method generateReportSR

Usage

```
generateReportSR(object, outputDir = NULL, colors = "default", ...)
```

Arguments

object	A SangerRead S4 instance.
outputDir	The output directory of the generated HTML report.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.
...	Further generateReportSR-related parameters.

Value

The output absolute path to the SangerRead's HTML file.

Examples

```
data(sangerReadFData)
## Not run:
generateReportSR(sangerReadFData)
## End(Not run)
```

launchApp	<i>Method launchApp</i>
-----------	-------------------------

Description

A method which launches Shiny application of the SangerContig and SangerAlignment instance.

Usage

```
launchApp(object, outputDir = NULL, colors = "default")
```

Arguments

object	A SangerContig or SangerAlignment S4 instance.
outputDir	The output directory of the saved new SangerContig or SangerAlignment S4 instance.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

A SangerContig or SangerAlignment object.

Author(s)

Kuan-Hao Chao

Examples

```
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
launchApp(sangerContigData)
launchApp(sangerContigData, colors="cb_friendly")
launchApp(sangerAlignmentData)
launchApp(sangerAlignmentData, colors="cb_friendly")
## End(Not run)
```

launchAppSA	<i>Method launchAppSA</i>
-------------	---------------------------

Description

Method launchAppSA

Usage

```
launchAppSA(object, outputDir = NULL, colors = "default")
```

Arguments

object	A SangerAlignment S4 instance.
outputDir	The output directory of the saved new SangerAlignment S4 instance.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

A shiny.appobj object.

Examples

```
data(sangerAlignmentData)
## Not run:
launchAppSA(sangerAlignmentData)
## End(Not run)
```

launchAppSC	<i>Method launchAppSC</i>
-------------	---------------------------

Description

Method launchAppSC

Usage

```
launchAppSC(object, outputDir = NULL, colors = "default")
```

Arguments

object	A SangerContig S4 instance.
outputDir	The output directory of the saved new SangerContig S4 instance.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

A shiny.appobj object.

Examples

```
data(sangerContigData)
## Not run:
launchAppSC(sangerContigData)
## End(Not run)
```

MakeBaseCalls

Method MakeBaseCalls

Description

Method MakeBaseCalls

Usage

```
MakeBaseCalls(object, signalRatioCutoff = 0.33)
```

Arguments

object	A SangerRead S4 instance.
signalRatioCutoff	The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.

Value

A SangerRead instance.

Examples

```
data(sangerReadFData)
MakeBaseCalls(sangerReadFData, signalRatioCutoff = 0.22)
```

ObjectResults-class	<i>ObjectResults</i>
---------------------	----------------------

Description

An S4 class storing results related inputs in a SangerRead, SangerContig, and SangerAlignment S4 object.

Slots

printLevel

Author(s)

Kuan-Hao Chao

Examples

```
objectResults <- new("ObjectResults",
  creationResult = TRUE,
  errorMessages = character(0),
  errorTypes    = character(0),
  warningMessages = character(0),
  warningTypes  = character(0),
  readResultTable = data.frame(),
  printLevel     = "SangerRead")
```

qualityBasePlot	<i>Method qualityBasePlot</i>
-----------------	-------------------------------

Description

Method qualityBasePlot

Usage

```
qualityBasePlot(object)
```

Arguments

object A QualityReport or SangerRead S4 instance

Value

A quality plot.

Examples

```
data(qualityReportData)
data(sangerReadFData)
qualityBasePlot(qualityReportData)
qualityBasePlot(sangerReadFData)
```

QualityReport-class	<i>QualityReport</i>
---------------------	----------------------

Description

An S4 class storing quality related inputs and results in a SangerRead S4 object.

Slots

TrimmingMethod The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.

M1TrimmingCutoff The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.

M2CutoffQualityScore The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.

M2SlidingWindowSize The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.

qualityPhredScores The Phred quality scores of each base pairs after base calling.

qualityBaseScores The probability of incorrect base call of each base pairs. They are calculated from qualityPhredScores.

rawSeqLength The number of nucleotides of raw primary DNA sequence.

trimmedSeqLength The number of nucleotides of trimmed primary DNA sequence.

trimmedStartPos The base pair index of trimming start point from 5' end of the sequence.

trimmedFinishPos The base pair index of trimming finish point from 3' end of the sequence.

rawMeanQualityScore The mean quality score of the primary sequence after base calling. In other words, it is the mean of qualityPhredScores.

trimmedMeanQualityScore The mean quality score of the trimmed primary sequence after base calling.

rawMinQualityScore The minimum quality score of the primary sequence after base calling.

trimmedMinQualityScore The minimum quality score of the trimmed primary sequence after base calling.

remainingRatio The remaining sequence length ratio after trimming.

Author(s)

Kuan-Hao Chao

Examples

```

inputFilePath <- system.file("extdata/", package = "sangeranalyseR")
A_chloroticaFFN <- file.path(inputFilePath,
                             "Allolobophora_chlorotica",
                             "ACHLO",
                             "Ach1_ACHLO006-09_1_F.ab1")
sangerReadF <- new("SangerRead",
                   inputSource      = "ABIF",
                   readFeature      = "Forward Read",
                   readFileName     = A_chloroticaFFN,
                   geneticCode      = GENETIC_CODE,
                   TrimmingMethod   = "M1",
                   M1TrimmingCutoff = 0.0001,
                   M2CutoffQualityScore = NULL,
                   M2SlidingWindowSize = NULL,
                   baseNumPerRow    = 100,
                   heightPerRow     = 200,
                   signalRatioCutoff = 0.33,
                   showTrimmed      = TRUE)
"@"(sangerReadF, QualityReport)

```

QualityReport-class-qualityBasePlot
qualityBasePlot

Description

A QualityReport method which creates quality base interactive plot.

Usage

```

## S4 method for signature 'QualityReport'
qualityBasePlot(object)

```

Arguments

object A QualityReport S4 instance.

Value

A quality plot.

Examples

```

data("qualityReportData")
## Not run:
qualityBasePlot(qualityReportData)
## End(Not run)

```

QualityReport-class-updateQualityParam
updateQualityParam

Description

A QualityReport method which updates quality base interactive plot.

Usage

```
## S4 method for signature 'QualityReport'
updateQualityParam(
  object,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL
)
```

Arguments

object	A QualityReport S4 instance.
TrimmingMethod	The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.

Value

A QualityReport instance.

Examples

```
data("qualityReportData")
updateQualityParam(qualityReportData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 30,
  M2SlidingWindowSize = 15)
```

qualityReportData	<i>QualityReport instance</i>
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Description

QualityReport instance

Usage

data(qualityReportData)

Author(s)

Kuan-Hao Chao

readTable	<i>Method readTable</i>
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Description

Method readTable

Usage

readTable(object, indentation = 0, ...)

Arguments

- | | |
|-------------|---|
| object | A SangerRead, SangerContig, or SangerAlignment S4 instance. |
| indentation | The indentation for different level printing |
| ... | Further generateReportSR-related parameters. |

Value

None.

Examples

```
data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
readTable(sangerReadFData)
readTable(sangerContigData)
readTable(sangerAlignmentData)

## End(Not run)
```

SangerAlignment	<i>SangerAlignment</i>
-----------------	------------------------

Description

the wrapper function for SangerAlignment

Usage

```
SangerAlignment(
  printLevel = "SangerAlignment",
  inputSource = "ABIF",
  processMethod = "REGEX",
  ABIF_Directory = NULL,
  FASTA_File = NULL,
  REGEX_SuffixForward = NULL,
  REGEX_SuffixReverse = NULL,
  CSV_NamesConversion = NULL,
  geneticCode = GENETIC_CODE,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow = 100,
  heightPerRow = 200,
  signalRatioCutoff = 0.33,
  showTrimmed = TRUE,
  refAminoAcidSeq = "",
  minReadsNum = 2,
  minReadLength = 20,
  minFractionCall = 0.5,
  maxFractionLost = 0.5,
  acceptStopCodons = TRUE,
  readingFrame = 1,
  processorsNum = 1
)
```

Arguments

inputSource	The input source of the raw file. It must be "ABIF" or "FASTA". The default value is "ABIF".
ABIF_Directory	The parent directory of all of the reads contained in ABIF format you wish to analyse. In SangerAlignment, all reads in subdirectories will be scanned recursively.
FASTA_File	If inputSource is "FASTA", then this value has to be the name of the FASTA file; if inputSource is "ABIF", then this value is "" by default.

REGEX_SuffixForward	The suffix of the filenames for forward reads in regular expression, i.e. reads that do not need to be reverse-complemented. For forward reads, it should be "_F.ab1".
REGEX_SuffixReverse	The suffix of the filenames for reverse reads in regular expression, i.e. reads that need to be reverse-complemented. For reverse reads, it should be "_R.ab1".
CSV_NamesConversion	The file path to the CSV file that provides read names that follow the naming regulation. If inputSource is "FASTA", then users need to prepare the csv file or make sure the original names inside FASTA file are valid; if inputSource is "ABIF", then this value is NULL by default.
geneticCode	Named character vector in the same format as GENETIC_CODE (the default), which represents the standard genetic code. This is the code with which the function will attempt to translate your DNA sequences. You can get an appropriate vector with the getGeneticCode() function. The default is the standard code.
TrimmingMethod	TrimmingMethod The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.
baseNumPerRow	It defines maximum base pairs in each row. The default value is 100.
heightPerRow	It defines the height of each row in chromatogram. The default value is 200.
signalRatioCutoff	The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.
showTrimmed	The logical value storing whether to show trimmed base pairs in chromatogram. The default value is TRUE.
refAminoAcidSeq	An amino acid reference sequence supplied as a string or an AAString object. If your sequences are protein-coding DNA sequences, and you want to have frameshifts automatically detected and corrected, supply a reference amino acid sequence via this argument. If this argument is supplied, the sequences are then kept in frame for the alignment step. Fwd sequences are assumed to come from the sense (i.e. coding, or "+") strand. The default value is "".

<code>minReadsNum</code>	The minimum number of reads required to make a consensus sequence, must be 2 or more. The default value is 2.
<code>minReadLength</code>	Reads shorter than this will not be included in the readset. The default 20 means that all reads with length of 20 or more will be included. Note that this is the length of a read after it has been trimmed.
<code>minFractionCall</code>	Minimum fraction of the sequences required to call a consensus sequence for <code>SangerContig</code> at any given position (see the <code>ConsensusSequence()</code> function from DECIPHER for more information). Defaults to 0.75 implying that 3/4 of all reads must be present in order to call a consensus.
<code>maxFractionLost</code>	Numeric giving the maximum fraction of sequence information that can be lost in the consensus sequence for <code>SangerContig</code> (see the <code>ConsensusSequence()</code> function from DECIPHER for more information). Defaults to 0.5, implying that each consensus base can ignore at most 50 percent of the information at a given position.
<code>acceptStopCodons</code>	The logical value TRUE or FALSE. TRUE (the default): keep all reads, regardless of whether they have stop codons; FALSE: reject reads with stop codons. If FALSE is selected, then the number of stop codons is calculated after attempting to correct frameshift mutations (if applicable).
<code>readingFrame</code>	1, 2, or 3. Only used if <code>accept.stop.codons == FALSE</code> . This specifies the reading frame that is used to determine stop codons. If you use a <code>refAminoAcidSeq</code> , then the frame should always be 1, since all reads will be shifted to frame 1 during frameshift correction. Otherwise, you should select the appropriate reading frame.
<code>processorsNum</code>	The number of processors to use, or NULL (the default) for all available processors.
<code>minFractionCallSA</code>	Minimum fraction of the sequences required to call a consensus sequence for <code>SangerAlignment</code> at any given position (see the <code>ConsensusSequence()</code> function from DECIPHER for more information). Defaults to 0.75 implying that 3/4 of all reads must be present in order to call a consensus.
<code>maxFractionLostSA</code>	Numeric giving the maximum fraction of sequence information that can be lost in the consensus sequence for <code>SangerAlignment</code> (see the <code>ConsensusSequence()</code> function from DECIPHER for more information). Defaults to 0.5, implying that each consensus base can ignore at most 50 percent of the information at a given position.

Value

A `SangerAlignment` instance.

Author(s)

Kuan-Hao Chao

Examples

```

rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, "Allolobophora_chlorotica", "RBNII")
REGEX_SuffixForward <- "_[0-9]*_F.ab1$"
REGEX_SuffixReverse <- "_[0-9]*_R.ab1$"
sangerAlignment <- SangerAlignment(
  inputSource          = "ABIF",
  ABIF_Directory       = parentDir,
  REGEX_SuffixForward  = REGEX_SuffixForward,
  REGEX_SuffixReverse  = REGEX_SuffixReverse,
  refAminoAcidSeq = "SRQWLFSTN HKDIGTLYFIFGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHA FIMIFFMVMPI MIGGFC",
  TrimmingMethod       = "M1",
  M1TrimmingCutoff     = 0.0001,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize  = NULL,
  baseNumPerRow        = 100,
  heightPerRow         = 200,
  signalRatioCutoff    = 0.33,
  showTrimmed          = TRUE,
  processorsNum        = 2)

```

SangerAlignment-class *SangerAlignment*

Description

An S4 class containing SangerContigs lists and contigs alignment results which corresponds to a final alignment in Sanger sequencing.

Slots

objectResults This is the object that stores all information of the creation result.

inputSource The input source of the raw file. It must be "ABIF" or "FASTA". The default value is "ABIF".

processMethod The method to create a contig from reads. The value is "REGEX" or "CSV". The default value is "REGEX".

ABIF_Directory If inputSource is "ABIF", then this value is the path of a parent directory storing all reads in ABIF format you want to analyse. If inputSource is "FASTA", then this value has to be NULL by default.

FASTA_File If inputSource is "FASTA", then this value has to be the path to a valid FASTA file ; if inputSource is "ABIF", then this value has to be NULL by default.

REGEX_SuffixForward The suffix of the filenames for forward reads in regular expression, i.e. reads that do not need to be reverse-complemented. For forward reads, it should be "_F.ab1".

REGEX_SuffixReverse The suffix of the filenames for reverse reads in regular expression, i.e. reads that need to be reverse-complemented. For reverse reads, it should be "_R.ab1".

CSV_NamesConversion The file path to the CSV file that provides read names, directions, and their contig groups. If `processMethod` is "CSV", then this value has to be the path to a valid CSV file; if `processMethod` is "REGEX", then this value has to be NULL by default.

geneticCode Named character vector in the same format as `GENETIC_CODE` (the default), which represents the standard genetic code. This is the code with which the function will attempt to translate your DNA sequences. You can get an appropriate vector with the `getGeneticCode()` function. The default is the standard code.

refAminoAcidSeq An amino acid reference sequence supplied as a string or an `AAString` object. If your sequences are protein-coding DNA sequences, and you want to have frameshifts automatically detected and corrected, supply a reference amino acid sequence via this argument. If this argument is supplied, the sequences are then kept in frame for the alignment step. Fwd sequences are assumed to come from the sense (i.e. coding, or "+") strand. The default value is "".

contigList A list storing all `SangerContigs` S4 instances.

contigsConsensus The consensus read of all `SangerContig` S4 instances in `DNASTring` object.

contigsAlignment The alignment of all `SangerContig` S4 instances with the called consensus sequence in `DNASTringSet` object. Users can use `BrowseSeqs()` to view the alignment.

contigsTree A phylo instance returned by `bionj` function in `ape` package. It can be used to draw the tree.

Author(s)

Kuan-Hao Chao

Examples

```
## Simple example
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, 'Allolobophora_chlorotica', 'ACHLO')
my_aligned_contigs <- new("SangerAlignment",
                          ABIF_Directory = parentDir,
                          REGEX_SuffixForward = "_[0-9]*_F.ab1$",
                          REGEX_SuffixReverse = "_[0-9]*_R.ab1$")

rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, 'Allolobophora_chlorotica', 'ACHLO')
CSV_NamesConversion <- file.path(rawDataDir, "ab1", "SangerAlignment", "names_conversion.csv")
sangerAlignment <- new("SangerAlignment",
                       processMethod = "CSV",
                       ABIF_Directory = parentDir,
                       CSV_NamesConversion = CSV_NamesConversion)

## Input From ABIF file format (Regex)
REGEX_SuffixForward <- "_[0-9]*_F.ab1$"
REGEX_SuffixReverse <- "_[0-9]*_R.ab1$"
sangerAlignment <- new("SangerAlignment",
                      printLevel = "SangerAlignment",
                      inputSource = "ABIF",
                      processMethod = "REGEX",
```

```

        FASTA_File           = NULL,
        CSV_NamesConversion  = NULL,
        ABIF_Directory       = parentDir,
        REGEX_SuffixForward  = REGEX_SuffixForward,
        REGEX_SuffixReverse  = REGEX_SuffixReverse,
        TrimmingMethod       = "M1",
        M1TrimmingCutoff     = 0.0001,
        M2CutoffQualityScore = NULL,
        M2SlidingWindowSize  = NULL,
        baseNumPerRow        = 100,
        heightPerRow         = 200,
        signalRatioCutoff    = 0.33,
        showTrimmed          = TRUE,
        refAminoAcidSeq = "SRQWLFSTNHKDIGTLYFIGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI MIGGF
        minReadsNum          = 2,
        minReadLength        = 20,
        minFractionCall      = 0.5,
        maxFractionLost      = 0.5,
        geneticCode           = GENETIC_CODE,
        acceptStopCodons     = TRUE,
        readingFrame         = 1,
        processorsNum        = 2)

## Input From ABIF file format (Csv three column)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, 'Allolobophora_chlorotica', 'ACHLO')
CSV_NamesConversion <- file.path(rawDataDir, "ab1", "SangerAlignment",
"names_conversion_all.csv")
sangerAlignment <- new("SangerAlignment",
        inputSource          = "ABIF",
        processMethod        = "CSV",
        ABIF_Directory       = parentDir,
        CSV_NamesConversion  = CSV_NamesConversion,
        refAminoAcidSeq = "SRQWLFSTNHKDIGTLYFIGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI MIGGF
        TrimmingMethod       = "M1",
        M1TrimmingCutoff     = 0.0001,
        M2CutoffQualityScore = NULL,
        M2SlidingWindowSize  = NULL,
        baseNumPerRow        = 100,
        heightPerRow         = 200,
        signalRatioCutoff    = 0.33,
        showTrimmed          = TRUE,
        processorsNum        = 2)

## Input From FASTA file format (No Csv - Regex)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
fastaFN <- file.path(rawDataDir, "fasta",
        "SangerAlignment", "Sanger_all_reads.fa")
REGEX_SuffixForwardFa <- "_[0-9]*_F$"
REGEX_SuffixReverseFa <- "_[0-9]*_R$"
sangerAlignmentFa <- new("SangerAlignment",
        inputSource          = "FASTA",
        processMethod        = "REGEX",

```

```

FASTA_File           = fastaFN,
REGEX_SuffixForward  = REGEX_SuffixForwardFa,
REGEX_SuffixReverse   = REGEX_SuffixReverseFa,
refAminoAcidSeq = "SRQWLFSTNHKDIGTLYFIGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIIMIGG
processorsNum         = 2)

## Input From FASTA file format (Csv three column method)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
fastaFN <- file.path(rawDataDir, "fasta",
                     "SangerAlignment", "Sanger_all_reads.fa")
CSV_NamesConversion <- file.path(rawDataDir, "fasta",
                                 "SangerAlignment", "names_conversion.csv")
sangerAlignmentFa <- new("SangerAlignment",
                          inputSource      = "FASTA",
                          processMethod    = "CSV",
                          FASTA_File       = fastaFN,
                          CSV_NamesConversion = CSV_NamesConversion,
                          refAminoAcidSeq = "SRQWLFSTNHKDIGTLYFIGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIIMIGG
                          processorsNum     = 2)

```

SangerAlignment-class-generateReportSA
generateReportSA

Description

A SangerAlignment method which generates final reports of the SangerContig instance.

Usage

```

## S4 method for signature 'SangerAlignment'
generateReportSA(
  object,
  outputDir,
  includeSangerContig = TRUE,
  includeSangerRead = TRUE,
  colors
)

```

Arguments

object	A SangerAlignment S4 instance.
outputDir	The output directory of the generated HTML report.
includeSangerContig	The parameter that decides whether to include SangerContig level report. The value is TRUE or FALSE and the default is TRUE.
includeSangerRead	The parameter that decides whether to include SangerRead level report. The value is TRUE or FALSE and the default is TRUE.

colors A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

The output absolute path to the SangerAlignment's HTML file.

Examples

```
data("sangerAlignmentData")
## Not run:
generateReportSA(sangerAlignmentData)
generateReportSA(sangerAlignmentData, colors="cb_friendly")
## End(Not run)
```

SangerAlignment-class-launchAppSA
launchAppSA

Description

A SangerAlignment method which launches Shiny app for SangerAlignment instance.

Usage

```
## S4 method for signature 'SangerAlignment'
launchAppSA(object, outputDir = NULL, colors = "default")
```

Arguments

object A SangerAlignment S4 instance.

outputDir The output directory of the saved new SangerContig S4 instance.

colors A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

A shiny.appobj object.

Examples

```
data("sangerAlignmentData")
RShinySA <- launchAppSA(sangerAlignmentData)
RShinySA <- launchAppSA(sangerAlignmentData, colors="cb_friendly")
```

SangerAlignment-class-updateQualityParam
updateQualityParam

Description

A SangerAlignment method which updates QualityReport parameter for each the SangerRead instance inside SangerAlignment.

Usage

```
## S4 method for signature 'SangerAlignment'
updateQualityParam(
  object,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  processorsNum = NULL
)
```

Arguments

object	A SangerAlignment S4 instance.
TrimmingMethod	The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.
processorsNum	The number of processors to use, or NULL (the default) for all available processors.

Value

A SangerAlignment instance.

Examples

```
data("sangerAlignmentData")
## Not run:
updateQualityParam(sangerAlignmentData,
                   TrimmingMethod      = "M2",
                   M1TrimmingCutoff    = NULL,
                   M2CutoffQualityScore = 40,
                   M2SlidingWindowSize = 15)
## End(Not run)
```

```
SangerAlignment-class-writeFastaSA
      writeFastaSA
```

Description

A SangerAlignment method which writes sequences into Fasta files.

Usage

```
## S4 method for signature 'SangerAlignment'
writeFastaSA(
  object,
  outputDir = NULL,
  compress = FALSE,
  compression_level = NA,
  selection = "all"
)
```

Arguments

object	A SangerAlignment S4 instance.
outputDir	The output directory of generated FASTA files.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.
selection	This value can be all, contigs_alignment, contigs_unalignment or all_reads. It generates reads and contigs FASTA files.

Value

The output directory of FASTA files.

Examples

```
data("sangerAlignmentData")
writeFastaSA(sangerAlignmentData)
```

sangerAlignmentData	<i>SangerAlignment instance</i>
---------------------	---------------------------------

Description

SangerAlignment instance

Usage

```
data(sangerAlignmentData)
```

Author(s)

Kuan-Hao Chao

sangeranalyseR	<i>sangeranalyseR-package</i>
----------------	-------------------------------

Description

sangeranalyseR-package

SangerContig	<i>SangerContig</i>
--------------	---------------------

Description

the wrapper function for SangerContig

Usage

```

SangerContig(
  printLevel = "SangerContig",
  inputSource = "ABIF",
  processMethod = "REGEX",
  ABIF_Directory = NULL,
  FASTA_File = NULL,
  REGEX_SuffixForward = NULL,
  REGEX_SuffixReverse = NULL,
  CSV_NamesConversion = NULL,
  contigName = NULL,
  geneticCode = GENETIC_CODE,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow = 100,
  heightPerRow = 200,
  signalRatioCutoff = 0.33,
  showTrimmed = TRUE,
  refAminoAcidSeq = "",
  minReadsNum = 2,
  minReadLength = 20,
  minFractionCall = 0.5,
  maxFractionLost = 0.5,
  acceptStopCodons = TRUE,
  readingFrame = 1,
  processorsNum = 1
)

```

Arguments

- | | |
|---------------------|--|
| inputSource | The input source of the raw file. It must be "ABIF" or "FASTA". The default value is "ABIF". |
| ABIF_Directory | The parent directory of all of the reads contained in ABIF format you wish to analyse. In SangerContig, all reads must be in the first layer in this directory. |
| FASTA_File | If inputSource is "FASTA", then this value has to be the name of the FASTA file; if inputSource is "ABIF", then this value is "" by default. |
| REGEX_SuffixForward | The suffix of the filenames for forward reads in regular expression, i.e. reads that do not need to be reverse-complemented. For forward reads, it should be "_F.ab1". |
| REGEX_SuffixReverse | The suffix of the filenames for reverse reads in regular expression, i.e. reads that need to be reverse-complemented. For reverse reads, it should be "_R.ab1". |
| CSV_NamesConversion | The file path to the CSV file that provides read names that follow the naming regulation. If inputSource is "FASTA", then users need to prepare the csv file |

	or make sure the original names inside FASTA file are valid; if inputSource is "ABIF", then this value is NULL by default.
contigName	The contig name of all the reads in ABIF_Directory.
geneticCode	Named character vector in the same format as GENETIC_CODE (the default), which represents the standard genetic code. This is the code with which the function will attempt to translate your DNA sequences. You can get an appropriate vector with the getGeneticCode() function. The default is the standard code.
TrimmingMethod	TrimmingMethod The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.
baseNumPerRow	It defines maximum base pairs in each row. The default value is 100.
heightPerRow	It defines the height of each row in chromatogram. The default value is 200.
signalRatioCutoff	The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.
showTrimmed	The logical value storing whether to show trimmed base pairs in chromatogram. The default value is TRUE.
refAminoAcidSeq	An amino acid reference sequence supplied as a string or an AAString object. If your sequences are protein-coding DNA sequences, and you want to have frameshifts automatically detected and corrected, supply a reference amino acid sequence via this argument. If this argument is supplied, the sequences are then kept in frame for the alignment step. Fwd sequences are assumed to come from the sense (i.e. coding, or "+") strand. The default value is "".
minReadsNum	The minimum number of reads required to make a consensus sequence, must be 2 or more. The default value is 2.
minReadLength	Reads shorter than this will not be included in the readset. The default 20 means that all reads with length of 20 or more will be included. Note that this is the length of a read after it has been trimmed.
minFractionCall	Minimum fraction of the sequences required to call a consensus sequence for SangerContig at any given position (see the ConsensusSequence() function from DECIPHER for more information). Defaults to 0.75 implying that 3/4 of all reads must be present in order to call a consensus.

maxFractionLost	Numeric giving the maximum fraction of sequence information that can be lost in the consensus sequence for SangerContig (see the ConsensusSequence() function from DECIPHER for more information). Defaults to 0.5, implying that each consensus base can ignore at most 50 percent of the information at a given position.
acceptStopCodons	The logical value TRUE or FALSE. TRUE (the default): keep all reads, regardless of whether they have stop codons; FALSE: reject reads with stop codons. If FALSE is selected, then the number of stop codons is calculated after attempting to correct frameshift mutations (if applicable).
readingFrame	1, 2, or 3. Only used if accept.stop.codons == FALSE. This specifies the reading frame that is used to determine stop codons. If you use a refAminoAcidSeq, then the frame should always be 1, since all reads will be shifted to frame 1 during frameshift correction. Otherwise, you should select the appropriate reading frame.
processorsNum	The number of processors to use, or NULL (the default) for all available processors.

Value

A SangerContig instance.

Author(s)

Kuan-Hao Chao

Examples

```
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, "Allolobophora_chlorotica", "ACHLO")
contigName <- "Ach1_ACHLO006-09"
REGEX_SuffixForward <- "_F.ab1"
REGEX_SuffixReverse <- "_R.ab1"
sangerContig <- SangerContig(
  inputSource          = "ABIF",
  ABIF_Directory       = parentDir,
  contigName           = contigName,
  REGEX_SuffixForward  = REGEX_SuffixForward,
  REGEX_SuffixReverse  = REGEX_SuffixReverse,
  refAminoAcidSeq = "SRQWLFSTNHKDIGTLYFIFGAWAGMVGTSLSILIRAEIGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIIMIGGFN
  TrimmingMethod       = "M2",
  M1TrimmingCutoff     = NULL,
  M2CutoffQualityScore = 20,
  M2SlidingWindowSize = 10,
  baseNumPerRow        = 100,
  heightPerRow         = 200,
  signalRatioCutoff    = 0.33,
  showTrimmed          = TRUE,
  processorsNum        = 2)
```

SangerContig-class	<i>SangerContig</i>
--------------------	---------------------

Description

An S4 class containing forward and reverse SangerRead lists and alignment, consensus read results which corresponds to a contig in Sanger sequencing.

Slots

objectResults This is the object that stores all information of the creation result.

inputSource The input source of the raw file. It must be "ABIF" or "FASTA". The default value is "ABIF".

processMethod The method to create a contig from reads. The value is "REGEX" or "CSV". The default value is "REGEX".

ABIF_Directory If inputSource is "ABIF", then this value is the path of a parent directory storing all reads in ABIF format you want to analyse. If inputSource is "FASTA", then this value has to be NULL by default.

FASTA_File If inputSource is "FASTA", then this value has to be the path to a valid FASTA file ; if inputSource is "ABIF", then this value has to be NULL by default.

REGEX_SuffixForward The suffix of the filenames for forward reads in regular expression, i.e. reads that do not need to be reverse-complemented.

REGEX_SuffixReverse The suffix of the filenames for reverse reads in regular expression, i.e. reads that need to be reverse-complemented.

CSV_NamesConversion The file path to the CSV file that provides read names, directions, and their contig groups. If processMethod is "CSV", then this value has to be the path to a valid CSV file; if processMethod is "REGEX", then this value has to be NULL by default.

contigName The contig name of all the reads in ABIF_Directory.

geneticCode Named character vector in the same format as GENETIC_CODE (the default), which represents the standard genetic code. This is the code with which the function will attempt to translate your DNA sequences. You can get an appropriate vector with the getGeneticCode() function. The default is the standard code.

forwardReadList The list of SangerRead S4 instances which are all forward reads.

reverseReadList The list of SangerRead S4 instances which are all reverse reads.

minReadsNum The minimum number of reads required to make a consensus sequence, must be 2 or more. The default value is 2.

minReadLength Reads shorter than this will not be included in the readset. The default 20 means that all reads with length of 20 or more will be included. Note that this is the length of a read after it has been trimmed.

refAminoAcidSeq An amino acid reference sequence supplied as a string or an AAString object. If your sequences are protein-coding DNA sequences, and you want to have frameshifts automatically detected and corrected, supply a reference amino acid sequence via this argument.

If this argument is supplied, the sequences are then kept in frame for the alignment step. Fwd sequences are assumed to come from the sense (i.e. coding, or "+") strand. The default value is "".

minFractionCall Minimum fraction of the sequences required to call a consensus sequence for SangerContig at any given position (see the ConsensusSequence() function from DECIPHER for more information). Defaults to 0.75 implying that 3/4 of all reads must be present in order to call a consensus.

maxFractionLost Numeric giving the maximum fraction of sequence information that can be lost in the consensus sequence for SangerContig (see the ConsensusSequence() function from DECIPHER for more information). Defaults to 0.5, implying that each consensus base can ignore at most 50 percent of the information at a given position.

acceptStopCodons The logical value TRUE or FALSE. TRUE (the default): keep all reads, regardless of whether they have stop codons; FALSE: reject reads with stop codons. If FALSE is selected, then the number of stop codons is calculated after attempting to correct frameshift mutations (if applicable).

readingFrame 1, 2, or 3. Only used if accept.stop.codons == FALSE. This specifies the reading frame that is used to determine stop codons. If you use a refAminoAcidSeq, then the frame should always be 1, since all reads will be shifted to frame 1 during frameshift correction. Otherwise, you should select the appropriate reading frame.

contigSeq The consensus read of all SangerRead S4 instances in DNASTring object.

alignment The alignment of all SangerRead S4 instances with the called consensus sequence in DNASTringSet object. Users can use BrowseSeqs() to view the alignment.

differencesDF A data frame of the number of pairwise differences between each read and the consensus sequence, as well as the number of bases in each input read that did not contribute to the consensus sequence. It can assist in detecting incorrect reads, or reads with a lot of errors.

distanceMatrix A distance matrix of genetic distances (corrected with the JC model) between all of the input reads.

dendrogram A list storing cluster groups in a data frame and a dendrogram object depicting the distance.matrix. Users can use plot() to see the dendrogram.

indelsDF If users specified a reference sequence via refAminoAcidSeq, then this will be a data frame describing the number of indels and deletions that were made to each of the input reads in order to correct frameshift mutations.

stopCodonsDF If users specified a reference sequence via refAminoAcidSeq, then this will be a data frame describing the number of stop codons in each read.

secondaryPeakDF A data frame with one row for each column in the alignment that contained more than one secondary peak. The data frame has three columns: the column number of the alignment; the number of secondary peaks in that column; and the bases (with IUPAC ambiguity codes representing secondary peak calls) in that column represented as a string.

Author(s)

Kuan-Hao Chao

Examples

```
## Simple example
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, "Allolobophora_chlorotica", "RBNII")
contigName <- "Ach1_RBNII384-13"
REGEX_SuffixForward <- "_[0-9]*_F.ab1$"
REGEX_SuffixReverse <- "_[0-9]*_R.ab1$"
sangerContig <- new("SangerContig",
                    ABIF_Directory      = parentDir,
                    contigName          = contigName,
                    REGEX_SuffixForward = REGEX_SuffixForward,
                    REGEX_SuffixReverse = REGEX_SuffixReverse)

## forward / reverse reads match error
## Input From ABIF file format (Regex)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, "Allolobophora_chlorotica", "ACHLO")
contigName <- "Ach1_ACHLO006-09"
REGEX_SuffixForward <- "_[0-9]*_F.ab1$"
REGEX_SuffixReverse <- "_[0-9]*_R.ab1$"
sangerContig <- new("SangerContig",
                    inputSource          = "ABIF",
                    processMethod        = "REGEX",
                    ABIF_Directory      = parentDir,
                    contigName          = contigName,
                    REGEX_SuffixForward = REGEX_SuffixForward,
                    REGEX_SuffixReverse = REGEX_SuffixReverse,
                    refAminoAcidSeq = "SRQWLFSTNHKDIGTLYIFGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI MIGGFGN",
                    TrimmingMethod      = "M1",
                    M1TrimmingCutoff    = 0.0001,
                    baseNumPerRow       = 100,
                    heightPerRow        = 200,
                    signalRatioCutoff    = 0.33,
                    showTrimmed          = TRUE,
                    minReadsNum         = 2,
                    processorsNum       = 2)

## Input From ABIF file format (Csv three column method)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
parentDir <- file.path(rawDataDir, "Allolobophora_chlorotica", "RBNII")
CSV_NamesConversion <- file.path(rawDataDir, "ab1", "SangerContig", "names_conversion_2.csv")
sangerContig <- new("SangerContig",
                    inputSource          = "ABIF",
                    processMethod        = "CSV",
                    ABIF_Directory      = parentDir,
                    CSV_NamesConversion = CSV_NamesConversion,
                    contigName          = "Ach1_RBNII384-13",
                    refAminoAcidSeq = "SRQWLFSTNHKDIGTLYIFGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI MIGGFGN",
                    TrimmingMethod      = "M1",
                    M1TrimmingCutoff    = 0.000001,
                    baseNumPerRow       = 100,
                    heightPerRow        = 200,
```



```

        signalRatioCutoff      = 0.33,
        showTrimmed            = TRUE,
        processorsNum          = 2)

## Input From FASTA file format (Regex)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
fastaFN <- file.path(rawDataDir, "fasta",
                     "SangerContig", "Ach1_ACHL0006-09.fa")
contigName <- "Ach1_ACHL0006-09"
REGEX_SuffixForwardFa <- "_[0-9]*_F$"
REGEX_SuffixReverseFa <- "_[0-9]*_R$"
sangerContigFa <- new("SangerContig",
                      inputSource      = "FASTA",
                      processMethod    = "REGEX",
                      FASTA_File       = fastaFN,
                      contigName       = contigName,
                      REGEX_SuffixForward = REGEX_SuffixForwardFa,
                      REGEX_SuffixReverse = REGEX_SuffixReverseFa,
                      refAminoAcidSeq  = "SRQWLFSTNHKDIGTLYIFGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIMI",
                      processorsNum    = 2)

## Input From FASTA file format (Csv - Csv three column method)
rawDataDir <- system.file("extdata", package = "sangeranalyseR")
fastaFN <- file.path(rawDataDir, "fasta",
                     "SangerContig", "Ach1_ACHL0006-09.fa")
CSV_NamesConversion <- file.path(rawDataDir, "fasta", "SangerContig", "names_conversion_1.csv")
sangerContigFa <- new("SangerContig",
                      inputSource      = "FASTA",
                      processMethod    = "CSV",
                      FASTA_File       = fastaFN,
                      CSV_NamesConversion = CSV_NamesConversion,
                      contigName       = "Ach1_ACHL0006-09",
                      refAminoAcidSeq  = "SRQWLFSTNHKDIGTLYIFGAWAGMVGTSLSILIRAE LGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIMI",
                      processorsNum    = 2)

```

SangerContig-class-generateReportSC
generateReportSC

Description

A SangerContig method which generates final reports of the SangerContig instance.

Usage

```

## S4 method for signature 'SangerContig'
generateReportSC(
  object,
  outputDir,

```

```

    includeSangerRead = TRUE,
    colors,
    navigationAlignmentFN = NULL
  )

```

Arguments

<code>object</code>	A SangerContig S4 instance.
<code>outputDir</code>	The output directory of the generated HTML report.
<code>includeSangerRead</code>	The parameter that decides whether to include SangerRead level report. The value is TRUE or FALSE and the default is TRUE.
<code>colors</code>	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.
<code>navigationAlignmentFN</code>	The internal parameter passed to HTML report. Users should not modify this parameter on their own.

Value

The output absolute path to the SangerContig's HTML file.

Examples

```

data("sangerContigData")
## Not run:
generateReportSC(sangerContigData)
generateReportSC(sangerContigData, colors="cb_friendly")
## End(Not run)

```

SangerContig-class-launchAppSC
launchAppSC

Description

A SangerContig method which launches Shiny app for SangerContig instance.

Usage

```

## S4 method for signature 'SangerContig'
launchAppSC(object, outputDir = NULL, colors = "default")

```

Arguments

object	A SangerContig S4 instance.
outputDir	The output directory of the saved new SangerContig S4 instance.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

Value

A shiny.appobj object.

Examples

```
data("sangerContigData")
RShinySC <- launchAppSC(sangerContigData)
RShinySC <- launchAppSC(sangerContigData, colors="cb_friendly")
```

SangerContig-class-readTable
readTable

Description

A SangerContig method which generates summary table for SangerContig instance

Usage

```
## S4 method for signature 'SangerContig'
readTable(object, indentation = 0)
```

Arguments

object	A SangerContig S4 instance.
indentation	The indentation for different level printing.

Value

None

Examples

```

data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
readTable(sangerReadFData)
readTable(sangerContigData)
readTable(sangerAlignmentData)

## End(Not run)

```

SangerContig-class-updateQualityParam
updateQualityParam

Description

A SangerContig method which updates QualityReport parameter for each the SangerRead instance inside SangerContig.

Usage

```

## S4 method for signature 'SangerContig'
updateQualityParam(
  object,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  processorsNum = NULL
)

```

Arguments

object	A SangerContig S4 instance.
TrimmingMethod	The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.

M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.
processorsNum	The number of processors to use, or NULL (the default) for all available processors.

Value

A SangerContig instance.

Examples

```
data("sangerContigData")
## Not run:
updateQualityParam(sangerContigData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
## End(Not run)
```

SangerContig-class-writeFastaSC
writeFastaSC

Description

A SangerContig method which writes sequences into Fasta files.

Usage

```
## S4 method for signature 'SangerContig'
writeFastaSC(
  object,
  outputDir = NULL,
  compress = FALSE,
  compression_level = NA,
  selection = "all"
)
```

Arguments

object	A SangerContig S4 instance.
outputDir	The output directory of generated FASTA files.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.

- compression_level This parameter will be passed to writeXStringSet function in Biostrings package.
- selection This value can be all, reads_alignment, reads_unalignment or contig. It generates reads and the contig FASTA files.

Value

The output directory of FASTA files.

Examples

```
data("sangerContigData")
writeFastaSC(sangerContigData)
```

sangerContigData	<i>SangerContig instance</i>
------------------	------------------------------

Description

SangerContig instance

Usage

```
data(sangerContigData)
```

Author(s)

Kuan-Hao Chao

SangerRead	<i>SangerRead</i>
------------	-------------------

Description

the wrapper function for SangerRead

Usage

```

SangerRead(
  printLevel = "SangerRead",
  inputSource = "ABIF",
  readFeature = "",
  readFileName = "",
  fastaReadName = NULL,
  geneticCode = GENETIC_CODE,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow = 100,
  heightPerRow = 200,
  signalRatioCutoff = 0.33,
  showTrimmed = TRUE
)

```

Arguments

inputSource	The input source of the raw file. It must be "ABIF" or "FASTA". The default value is "ABIF".
readFeature	The direction of the Sanger read. The value must be "Forward Read" or "Reverse Read".
readFileName	The filename of the target ABIF file.
fastaReadName	If inputSource is "FASTA", then this value has to be the name of the read inside the FASTA file; if inputSource is "ABIF", then this value is "" by default.
geneticCode	Named character vector in the same format as GENETIC_CODE (the default), which represents the standard genetic code. This is the code with which the function will attempt to translate your DNA sequences. You can get an appropriate vector with the getGeneticCode() function. The default is the standard code.
TrimmingMethod	TrimmingMethod The read trimming method for this SangerRead. The value must be "M1" (the default) or "M2". M1 is the modified Mott's trimming algorithm that can also be found in Phred/Phrap and Biopython. M2 is like trimmomatic's sliding window method.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.

baseNumPerRow	It defines maximum base pairs in each row. The default value is 100.
heightPerRow	It defines the height of each row in chromatogram. The default value is 200.
signalRatioCutoff	The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.
showTrimmed	The logical value storing whether to show trimmed base pairs in chromatogram. The default value is TRUE.

Value

A SangerRead instance.

Author(s)

Kuan-Hao Chao

Examples

```
inputFilePath <- system.file("extdata/", package = "sangeranalyseR")
A_chloroticaFdFN <- file.path(inputFilePath,
                              "Allolobophora_chlorotica",
                              "ACHLO",
                              "Ach1_ACHLO006-09_1_F.ab1")

sangerRead <- SangerRead(
  printLevel          = "SangerRead",
  inputSource         = "ABIF",
  readFeature         = "Forward Read",
  readFileName        = A_chloroticaFdFN,
  geneticCode         = GENETIC_CODE,
  TrimmingMethod      = "M1",
  M1TrimmingCutoff    = 0.0001,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow       = 100,
  heightPerRow        = 200,
  signalRatioCutoff    = 0.33,
  showTrimmed         = TRUE)
```

SangerRead-class

SangerRead

Description

An S4 class extending sangerseq S4 class which corresponds to a single ABIF file in Sanger sequencing.

Examples

```

sangerReadF <- new("SangerRead",
  readFeature      = "Forward Read",
  readFileName     = A_chloroticaFFN)

## Input From ABIF file format
# Forward Read
A_chloroticaFFN <- file.path(inputFilesPath,
  "Allolobophora_chlorotica",
  "ACHLO",
  "Ach1_ACHLO006-09_1_F.ab1")
sangerReadF <- new("SangerRead",
  printLevel      = "SangerRead",
  inputSource      = "ABIF",
  readFeature      = "Forward Read",
  readFileName     = A_chloroticaFFN,
  fastaReadName    = NULL,
  geneticCode      = GENETIC_CODE,
  TrimmingMethod   = "M1",
  M1TrimmingCutoff = 0.0001,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow    = 100,
  heightPerRow     = 200,
  signalRatioCutoff = 0.33,
  showTrimmed      = TRUE)

# Reverse Read
A_chloroticaRFN <- file.path(inputFilesPath,
  "Allolobophora_chlorotica",
  "ACHLO",
  "Ach1_ACHLO006-09_2_R.ab1")
sangerReadR <- new("SangerRead",
  inputSource      = "ABIF",
  readFeature      = "Reverse Read",
  readFileName     = A_chloroticaRFN,
  geneticCode      = GENETIC_CODE,
  TrimmingMethod   = "M1",
  M1TrimmingCutoff = 0.0001,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  baseNumPerRow    = 100,
  heightPerRow     = 200,
  signalRatioCutoff = 0.33,
  showTrimmed      = TRUE)

## Input From FASTA file format
# Forward Read
inputFilesPath <- system.file("extdata/", package = "sangeranalyseR")
A_chloroticaFFNfa <- file.path(inputFilesPath,
  "fasta",
  "SangerRead",
  "Ach1_ACHLO006-09_1_F.fa")

```

```

readNameFfa <- "Ach1_ACHL0006-09_1_F"
sangerReadFfa <- new("SangerRead",
                     inputSource      = "FASTA",
                     readFeature      = "Forward Read",
                     readFileName     = A_chloroticaFFNfa,
                     fastaReadName    = readNameFfa,
                     geneticCode      = GENETIC_CODE)

# Reverse Read
A_chloroticaRFNfa <- file.path(inputFilesPath,
                               "fasta",
                               "SangerRead",
                               "Ach1_ACHL0006-09_2_R.fa")
readNameRfa <- "Ach1_ACHL0006-09_2_R"
sangerReadRfa <- new("SangerRead",
                     inputSource      = "FASTA",
                     readFeature      = "Reverse Read",
                     readFileName     = A_chloroticaRFNfa,
                     fastaReadName    = readNameRfa,
                     geneticCode      = GENETIC_CODE)

```

SangerRead-class-generateReportSR
generateReportSR

Description

A SangerRead method which generates final reports of the SangerRead instance.

Usage

```

## S4 method for signature 'SangerRead'
generateReportSR(
  object,
  outputDir,
  colors,
  navigationContigFN = NULL,
  navigationAlignmentFN = NULL
)

```

Arguments

object	A SangerRead S4 instance.
outputDir	The output directory of the generated HTML report.
colors	A vector for users to set the colors of (A, T, C, G, else). There are three options for users to choose from. 1. "default": (green, blue, black, red, purple). 2. "cb_friendly": ((0, 0, 0), (199, 199, 199), (0, 114, 178), (213, 94, 0), (204, 121, 167)). 3. Users can set their own colors with a vector with five elements.

navigationContigFN

The internal parameter passed to HTML report. Users should not modify this parameter on their own.

navigationAlignmentFN

The internal parameter passed to HTML report. Users should not modify this parameter on their own.

Value

The output absolute path to the SangerRead's HTML file.

Examples

```
data("sangerReadFData")
## Not run:
generateReportSR(sangerReadFData, "~/Documents")
generateReportSR(sangerReadFData, colors="cb_friendly")
## End(Not run)
```

SangerRead-class-MakeBaseCalls

MakeBaseCalls

Description

A SangerRead method which does base calling on SangerRead instance

Usage

```
## S4 method for signature 'SangerRead'
MakeBaseCalls(object, signalRatioCutoff = 0.33)
```

Arguments

object A SangerRead S4 instance.

signalRatioCutoff

The ratio of the height of a secondary peak to a primary peak. Secondary peaks higher than this ratio are annotated. Those below the ratio are excluded. The default value is 0.33.

Value

A SangerRead instance.

Examples

```
data("sangerReadFData")
newSangerReadFData <- MakeBaseCalls(sangerReadFData, signalRatioCutoff = 0.22)
```

SangerRead-class-qualityBasePlot
qualityBasePlot

Description

A SangerRead method which creates quality base interactive plot.

Usage

```
## S4 method for signature 'SangerRead'  
qualityBasePlot(object)
```

Arguments

object A SangerRead S4 instance.

Value

A quality plot.

Examples

```
data("sangerReadFData")  
## Not run:  
qualityBasePlot(sangerReadFData)  
## End(Not run)
```

SangerRead-class-readTable
readTable

Description

A SangerRead method which generates summary table for SangerRead instance

Usage

```
## S4 method for signature 'SangerRead'  
readTable(object, indentation = 0)
```

Arguments

object A SangerRead S4 instance.
indentation The indentation for different level printing.

Value

None

Examples

```

data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
readTable(sangerReadFData)
readTable(sangerContigData)
readTable(sangerAlignmentData)

## End(Not run)

```

SangerRead-class-updateQualityParam
updateQualityParam

Description

A SangerRead method which updates QualityReport parameter inside the SangerRead.

Usage

```

## S4 method for signature 'SangerRead'
updateQualityParam(
  object,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL
)

```

Arguments

object	A SangerRead S4 instance.
TrimmingMethod	The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.

M2SlidingWindowSize

The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.

Value

A SangerRead instance.

Examples

```
data("sangerReadFData")
updateQualityParam(sangerReadFData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
```

SangerRead-class-writeFastaSR
writeFastaSR

Description

A SangerRead method which writes the sequence into Fasta files.

Usage

```
## S4 method for signature 'SangerRead'
writeFastaSR(
  object,
  outputDir = NULL,
  compress = FALSE,
  compression_level = NA
)
```

Arguments

object	A SangerRead S4 instance.
outputDir	The output directory of the generated FASTA file.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.

Value

The output absolute path to the FASTA file.

Examples

```
data("sangerReadFData")
writeFastaSR(sangerReadFData)
```

sangerReadFData	<i>SangerRead instance</i>
-----------------	----------------------------

Description

SangerRead instance

Usage

```
data(sangerReadFData)
```

Author(s)

Kuan-Hao Chao

updateQualityParam	<i>Method updateQualityParam</i>
--------------------	----------------------------------

Description

Method updateQualityParam

Usage

```
updateQualityParam(
  object,
  TrimmingMethod = "M1",
  M1TrimmingCutoff = 1e-04,
  M2CutoffQualityScore = NULL,
  M2SlidingWindowSize = NULL,
  ...
)
```


Arguments

object	A QualityReport, SangerRead, SangerContig, or SangerAlignment S4 instance.
TrimmingMethod	The read trimming method for this SangerRead. The value must be "M1" (the default) or 'M2'.
M1TrimmingCutoff	The trimming cutoff for the Method 1. If TrimmingMethod is "M1", then the default value is 0.0001. Otherwise, the value must be NULL.
M2CutoffQualityScore	The trimming cutoff quality score for the Method 2. If TrimmingMethod is 'M2', then the default value is 20. Otherwise, the value must be NULL. It works with M2SlidingWindowSize.
M2SlidingWindowSize	The trimming sliding window size for the Method 2. If TrimmingMethod is 'M2', then the default value is 10. Otherwise, the value must be NULL. It works with M2CutoffQualityScore.
...	Further updateQualityParam-related parameters.

Value

A QualityReport, SangerRead, SangerContig, or SangerAlignment instance.

Examples

```

data(qualityReportData)
data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
updateQualityParam(qualityReportData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
updateQualityParam(sangerReadFData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
updateQualityParam(sangerContigData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
updateQualityParam(sangerAlignmentData,
  TrimmingMethod      = "M2",
  M1TrimmingCutoff    = NULL,
  M2CutoffQualityScore = 40,
  M2SlidingWindowSize = 15)
## End(Not run)

```

writeFasta

Method writeFasta

Description

A method which writes FASTA files of the SangerRead, SangerContig, and SangerAlignment instance.

Usage

```
writeFasta(
  object,
  outputDir = NULL,
  compress = FALSE,
  compression_level = NA,
  selection = "all"
)
```

Arguments

object	A SangerRead, SangerContig, or SangerAlignment S4 instance.
outputDir	The output directory of generated FASTA files.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.
selection	This parameter will be passed to writeFastaSC or writeFastaSA.

Value

A SangerRead, SangerContig, or SangerAlignment object.

Author(s)

Kuan-Hao Chao

Examples

```
data(sangerReadFData)
data(sangerContigData)
data(sangerAlignmentData)
## Not run:
writeFasta(sangerReadFData)
writeFasta(sangerContigData)
```

```
writeFasta(sangerAlignmentData)
## End(Not run)
```

writeFastaSA	<i>Method writeFastaSA</i>
--------------	----------------------------

Description

Method writeFastaSA

Usage

```
writeFastaSA(
  object,
  outputDir = NULL,
  compress = FALSE,
  compression_level = NA,
  selection = "all"
)
```

Arguments

object	A SangerAlignment S4 instance.
outputDir	The output directory of generated FASTA files.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.
selection	This value can be all, contigs_alignment, contigs_unalignment or all_reads. It generates reads and contigs FASTA files.

Value

The output directory of FASTA files.

Examples

```
data(sangerAlignmentData)
writeFastaSA(sangerAlignmentData)
```

writeFastaSC	<i>Method writeFastaSC</i>
--------------	----------------------------

Description

Method writeFastaSC

Usage

```
writeFastaSC(  
  object,  
  outputDir = NULL,  
  compress = FALSE,  
  compression_level = NA,  
  selection = "all"  
)
```

Arguments

object	A SangerContig S4 instance.
outputDir	The output directory of generated FASTA files.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.
selection	This value can be all, reads_alignment, reads_unalignment or contig. It generates reads and the contig FASTA files.

Value

The output directory of FASTA files.

Examples

```
data(sangerContigData)  
writeFastaSC(sangerContigData)
```

writeFastaSR	<i>Method writeFastaSR</i>
--------------	----------------------------

Description

Method writeFastaSR

Usage

```
writeFastaSR(  
  object,  
  outputDir = NULL,  
  compress = FALSE,  
  compression_level = NA  
)
```

Arguments

object	A SangerRead S4 instance.
outputDir	The output directory of the generated FASTA file.
compress	Like for the save function in base R, must be TRUE or FALSE (the default), or a single string specifying whether writing to the file is to use compression. The only type of compression supported at the moment is "gzip". This parameter will be passed to writeXStringSet function in Biostrings package.
compression_level	This parameter will be passed to writeXStringSet function in Biostrings package.

Value

The output absolute path to the FASTA file.

Examples

```
data(sangerReadFData)  
writeFastaSR(sangerReadFData)
```

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