# Package 'SimFFPE'

May 16, 2025

Type Package	
Title NGS Read Simulator for FFPE Tissue	
Version 1.21.0	
Description The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artifact chimeric reads (ACRS), which can lead to false positive structural variant calls. These ACRs are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary regions (SRCRs). This package simulates these artifact chimeric reads as well as normal reads for FFPE samples on the whole genome / several chromosomes / large regions.	
License LGPL-3	
Encoding UTF-8	
Depends Biostrings	
Imports dplyr, foreach, doParallel, truncnorm, GenomicRanges, IRanges, Rsamtools, parallel, graphics, stats, utils, methods	
Suggests BiocStyle	
biocViews Sequencing, Alignment, MultipleComparison, SequenceMatching, DataImport	
git_url https://git.bioconductor.org/packages/SimFFPE	
git_branch devel	
git_last_commit 1a970ef	
git_last_commit_date 2025-04-15	
Repository Bioconductor 3.22	
Date/Publication 2025-05-15	
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SimFFPE-package

NGS Read Simulator for FFPE Tissue

## **Description**

The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artifact chimeric reads (ACRS), which can lead to false positive structural variant calls. These ACRs are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary regions (SRCRs). This package simulates these artifact chimeric reads as well as normal reads for FFPE samples on the whole genome / several chromosomes / large regions.

#### **Details**

This package was not yet installed at build time.

The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artifact chimeric reads (ACRs), which can lead to false positive structural variant calls. These ACRs are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary regions (SRCR). This package simulates these artifact chimeric reads as well as normal reads for FFPE samples. To simplify the simulation, the genome is divided into small windows, and SRCRs are found within the same window (adjacent ss-DNA combination) or between different windows (distant ss-DNA simulation). For adjacent ss-DNA combination events, the original genomic distance between and strands of two combined SRCRs are also simulated based on real data. The simulation can cover whole genome, or several chromosomes, or large regions, or whole exome, or targeted regions. It also supports enzymatic / random fragmentation and paired-end / single-end sequencing simulations. Fine-tuning can be achieved by adjusting the parameters, and multi-threading is surported. Please check the package vignette for the guidance of fine-tuning Index: This package was not yet installed at build time.

There are three available functions for NGS read simulation of FFPE samples:

- 1. calcPhredScoreProfile: Calculate positional Phred score profile from BAM file for read simulation.
- 2. readSimFFPE: Simulate artifact chimeric reads on whole genome, or several chromosomes, or large regions.
- 3. targetReadSimFFPE: Simulate artifact chimeric reads in exonic / targeted regions.

#### Author(s)

```
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Maintainer: Lanying Wei <a href="https://orcid.org/0000-0002-4281-8017">lttps://orcid.org/0000-0002-4281-8017</a>)
```

## See Also

```
calcPhredScoreProfile, readSimFFPE, targetReadSimFFPE
```

## **Examples**

calcPhredScoreProfile 3

```
colnames(PhredScoreProfile) <-</pre>
    strsplit(readLines(PhredScoreProfilePath)[1], "\t")[[1]]
referencePath <- system.file("extdata", "example.fasta", package = "SimFFPE")</pre>
reference <- readDNAStringSet(referencePath)</pre>
## Simulate reads of the first three sequences of the reference genome
sourceSeg <- reference[1:3]</pre>
outFile1 <- paste0(tempdir(), "/sim1")</pre>
readSimFFPE(sourceSeq, referencePath, PhredScoreProfile, outFile1,
            coverage = 80, enzymeCut = TRUE, threads = 2)
## Simulate reads for targeted regions
bamFilePath <- system.file("extdata", "example.bam", package = "SimFFPE")</pre>
regionPath <- system.file("extdata", "regionsBam.txt", package = "SimFFPE")</pre>
regions <- read.table(regionPath)</pre>
PhredScoreProfile <- calcPhredScoreProfile(bamFilePath, targetRegions = regions)
regionPath <- system.file("extdata", "regionsSim.txt", package = "SimFFPE")</pre>
targetRegions <- read.table(regionPath)</pre>
outFile <- paste0(tempdir(), "/sim2")</pre>
targetReadSimFFPE(referencePath, PhredScoreProfile, targetRegions, outFile,
                   coverage = 80, readLen = 100, meanInsertLen = 180,
                   sdInsertLen = 50, enzymeCut = FALSE)
```

calcPhredScoreProfile Estimate Phred score profile for FFPE read simulation

## **Description**

Calculate Phred score profile from the entire BAM file or reads in subsampled regions.

## Usage

```
calcPhredScoreProfile(bamFilePath, mapqFilter = 0, maxFileSize = 1,
targetRegions = NULL, subsampleRatio = NA, subsampleRegionLength = 1e+05,
disableSubsampling = FALSE, threads = 1)
```

## **Arguments**

bamFilePath	BAM file to be processed.
mapqFilter	Filter for mapping quality. Reads with mapping quality below this value will be excluded from calculation.
maxFileSize	The maximum file size (in GB) that allows processing of the entire BAM file. If disableSubsampling is set to false, BAM file larger than this size will be subsampled for calculation.
targetRegions	A DataFrame or GenomicRanges object representing target regions for calculation. Use it for targeted sequencing / WES data, or when you need to manually select subsampled regions (set disableSubsampling to true in this case). If it is

> a DataFrame, the first column should be the chromosome, the second the start position and the third the end position. Please use one-based coordinate systems (the first base should be marked with 1 but not 0).

subsampleRatio Subsample ratio. Together with subsampleRegionLength to determine subsampled regions. When subsampleRatio is not given, it will be assigned the value of maxFileSize divided by the input BAM file size. Range: 0 to 1.

subsampleRegionLength

Length of each subsampled region. Unit: base pair (bp).

disableSubsampling

Force to use the entire BAM file for calculation when set to true.

threads

Number of threads used. Multi-threading can speed up the process.

#### **Details**

Calculate positional Phred score profile from the entire BAM file or reads in subsampled regions. A Phred score profile will be returned, which can then be used in read simulation.

#### Value

A matrix will be returned. Each row of the matrix represents a position in the read (from begin to end), and each column the Phred quality score of base-calling error probabilities. The value in the matrix represents the positional Phred score proportion.

#### Author(s)

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#### See Also

```
SimFFPE, readSimFFPE, targetReadSimFFPE
```

#### **Examples**

```
bamFilePath <- system.file("extdata", "example.bam", package = "SimFFPE")</pre>
regionPath <- system.file("extdata", "regionsBam.txt", package = "SimFFPE")</pre>
regions <- read.table(regionPath)</pre>
PhredScoreProfile <- calcPhredScoreProfile(bamFilePath, targetRegions = regions)
```

readSimFFPE

Simulate normal and artifact chimeric reads in NGS data of FFPE samples for whole genome / several chromosomes / large regions

## **Description**

NGS data from FFPE samples contain numerous artifact chimeric reads. These chimeric reads are formed through the combination of two single-stranded DNA (ss-DNA) with short reverse complementary regions (SRCR). This function simulates these artifact chimeric reads as well as normal reads for FFPE samples on the whole genome, or several chromosomes, or large regions. To simplify the simulation, the genome is divided into small windows, and SRCRs are found within the same window (adjacent ss-DNA combination) or between different windows (distant ss-DNA simulation).

#### **Usage**

readSimFFPE(sourceSeq, referencePath, PhredScoreProfile, outFile, coverage, readLen=150, meanInsertLen=250, sdInsertLen=80, enzymeCut=FALSE, chimericProp=0.1, sameChrProp=0.43, windowLen=5000, adjChimProp=0.63, sameStrandProp=0.65, meanLogSRCRLen=1.8, sdLogSRCRLen=0.55, maxSRCRLen=32, meanLogSRCRDist=4.7, sdLogSRCRDist=0.35, distWinLen=5000, spikeWidth = 1500, betaShape1=0.5, betaShape2=0.5, sameTarRegionProb=0, adjFactor=1.65, distFactor=1.65, chimMutRate=0.003, noiseRate=0.0015, highNoiseRate=0.08, highNoiseProp=0.01, pairedEnd=TRUE, prefix="SimFFPE", threads=1, adjChimeric=TRUE, distChimeric=TRUE, normalReads=TRUE, overWrite=FALSE)

#### **Arguments**

sourceSeq A DNAStringSet object of DNA sequences used for simulation. It can cover the

entire reference genome or selected chromosomes or chromosome regions.

referencePath Path to the reference genome.

PhredScoreProfile

A matrix representing the positional Phred score proportion. Each row of the matrix represents a position in the read (from begin to end), and each column the Phred quality score of base-calling error probabilities. The profile can be calculated from BAM file using the calcPhredScoreProfile function.

outFile Output file path for the FASTQ file with simulated reads. Please include the

name of the output file without extension, e.g. "/tmp/sim1".

coverage Coverage of the simulation.

readLen Read length of the simulation.

meanInsertLen Mean insert length for the simulation (normally distributed).

sdInsertLen Standard deviation of the insert length for simulation (normally distributed).

enzymeCut Simulate enzymatic fragmentation if it is set to true, otherwise simulate random

fragmentation.

chimeric Prop Proportion of artifact chimeric fragments (chimeric fragments / chimeric or nor-

mal fragments). Range: 0 to 1.

sameChrProp Proportion of artifact chimeric fragments that are derived from the combination

of two ss-DNA coming from the same chromosome. Range: 0 to 1.

windowLen The window length used in adjacent ss-DNA combination simulation. To simu-

late adjacent ss-DNA combinations, input DNA sequences are divided into small windows of equal size, and short reverse complementary regions are searched within the same window to form artifact chimeric fragments. Unit: base pair

(bp).

adjChimProp Proportion of adjacent ss-DNA combinations among same chromosomal ss-

DNA combinations. Range: 0 to 1.

sameStrandProp Proportion of same-strand ss-DNA combinations among adjacent ss-DNA com-

binations . For paired end sequencing, the larger the proportion, the greater the proportion of improperly paired reads with LL / RR pair orientation, and the

smaller the proportion with RL pair orientation. Range: 0 to 1.

meanLogSRCRLen Mean of log scaled length of the short reverse complementary regions (SRCR) in

artifact chimeric fragments. SRCRs links two ss-DNA together, yielding artifact chimeric fragments. The length of SRCR follows a log-normal distribution. See

rlnorm for more details. Unit: base pair (bp).

sdLogSRCRLen Standard deviation of log scaled length of the short reverse complementary re-

gions.

maxSRCRLen Maximum length of the short reverse complementary regions. Unit: base pair

meanLogSRCRDist

Mean of log scaled original genomic distance of the short reverse complementary regions(SRCR) in artifact chimeric fragments. SRCRs links two ss-DNA together, yielding artifact chimeric fragments. The distance of SRCR is the original genomic distance between the two short reverse complementary segments, which follows a log-normal distribution in simulation. For log-normal distribution, see rlnorm for more details. Unit: base pair (bp).

sdLogSRCRDist Standard deviation of log scaled original genomic distance of the short reverse complementary regions(SRCR) in artifact chimeric fragments.

distWinLen The window length used in distant ss-DNA simulation. To simulate distant ss-DNA combinations, the short reverse complementary regions(SRCR) are searched between different windows. Unit: base pair (bp).

> The width of chimeric read spike used to simulate distant ss-DNA combinations. In real FFPE samples, the chimeric reads formed by distant DNA combination are unevenly distributed along the chromosome. Some regions are enriched in these reads while some others are scarce. The length of these regions are of similar scale; therefore, a defined width is used for simulation. Suggested range: 1500-2000. Unit: base pair (bp).

Shape parameter a of beta distribution used to model the unevenly distributed distant ss-DNA combinations. The number of seeds in each "spike" follows a "U" shaped beta distribution. Use this parameter to adjust the shape of the curve. See rbeta for more details. Range: 0-1.

Shape parameter b of beta distribution used to model the unevenly distributed distant ss-DNA combinations. The number of seeds in each "spike" follows a "U" shaped beta distribution. Use this parameter to adjust the shape of the curve. See rbeta for more details. Range: 0-1.

sameTarRegionProb

Probability of two distant ss-DNA combination events coming from the same two different windows.

Increase this value if the number of simulated adjacent chimeric reads is smaller than expected (sameChrProp \* adjChimProp), decrease if the opposite is true.

Increase this value if the number of simulated distant chimeric reads is smaller than expected, decrease if the opposite is true.

Mutation rate for each base in chimeric fragments. In the chimeric fragment formation process, biological-level errors might occur and lead to mutations on these artifact fragments. For all four basic types of nucleotides, the substitution probability is set equal. Range: 0-0.75.

Noise rate for each base in reads. This is used for sequencing-level errors. The probability is set equal for all four basic types of nucleotides. Range: 0-0.75.

A second noise rate for each base in reads. In some real sequencing data, some reads are much more noisy than others. This parameter can be used for this situation. Range: 0-0.75.

Proportion of reads to be simulated with highNoiseRate other than noiseRate. Range: 0-1.

spikeWidth

betaShape1

betaShape2

adjFactor

distFactor

chimMutRate

noiseRate

highNoiseProp

highNoiseRate

pairedEnd Simulate paired end sequencing when set to true. prefix Prefix for read names. When reads from different runs of simulation have to be merged, please make sure that they have different prefixes. threads Number of threads used. Multi-threading can speed up the process. adjChimeric Generate reads from adjacent ss-DNA combinations if it is set to true. If it is set to false, skip this process. distChimeric Generate reads from distant ss-DNA combinations if it is set to true. If it is set to false, skip this process. normalReads Generate reads from normal fragments if it is set to true. If it is set to false, skip this process. overWrite Overwrite the file if file with the same output path exists and it is set to true. If file with same output path exists and it is set to false, reads will be appended to the existing file.

#### **Details**

The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artifact chimeric reads (ACRS), which can lead to false positive structural variant calls. These ACRs are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary regions (SRCR). This function simulates these artifact chimeric reads as well as normal reads for FFPE samples on the whole genome / several chromosomes / large regions. To simplify the simulation, the genome is divided into small windows, and SRCRs are found within the same window (adjacent ss-DNA combination) or between different windows (distant ss-DNA simulation). For adjacent ss-DNA combination events, the original genomic distance between and strands of two combined SRCRs are also simulated based on real data. In the output fastq file, reads are distinguished by prefixes "adjChimeric", "distChimeric" and "Normal" in their names. The parameter PhredScoreProfile can be calculated by the function calcPhredScoreProfile. To simulate whole exome sequencing (WES) or targeted sequencing, please use the function targetReadSimFFPE.

#### Value

NULL. Reads will be written to the output FASTQ file.

#### Note

When fine-tuning is needed, simulate reads from certain areas / chromosomes instead of the entire genome to save the run-time. Please check the package vignette for the guidance of fine-tuning.

## Author(s)

Lanying Wei <a href="mailto:lanying.wei@uni-muenster.de">lanying.wei@uni-muenster.de</a>

## See Also

 ${\tt SimFFPE, calcPhredScoreProfile, targetReadSimFFPE}$ 

## **Examples**

```
colnames(PhredScoreProfile) <-</pre>
    strsplit(readLines(PhredScoreProfilePath)[1], "\t")[[1]]
referencePath <- system.file("extdata", "example.fasta", package = "SimFFPE")</pre>
reference <- readDNAStringSet(referencePath)</pre>
## Simulate reads of the first three sequences of reference genome
sourceSeg <- reference[1:3]</pre>
outFile1 <- paste0(tempdir(), "/sim1")</pre>
readSimFFPE(sourceSeq, referencePath, PhredScoreProfile, outFile1,
            enzymeCut = FALSE, coverage=80, threads = 2)
## Simulate reads of defined regions on the first two sequences of reference
## genome
sourceSeq2 <- DNAStringSet(lapply(reference[1:2], function(x) x[1:10000]))</pre>
outFile2 <- paste0(tempdir(), "/sim2")</pre>
readSimFFPE(sourceSeq2, referencePath, PhredScoreProfile, outFile2,
            coverage = 80, enzymeCut = TRUE, threads = 1)
## Simulate reads of defined regions on the second and the third sequence of
## reference genome and merge them with existing reads (a different prefix is
## needed)
sourceSeq3 <- DNAStringSet(lapply(reference[2:3], function(x) x[1:10000]))</pre>
readSimFFPE(sourceSeg3, referencePath, PhredScoreProfile, outFile2,
            prefix = "simFFPE2", coverage = 80, enzymeCut = TRUE,
            threads = 1, overWrite = FALSE)
```

target Read Sim FFPE

Simulate normal and artifact chimeric reads in NGS data of FFPE samples for exonic / targeted regions

## Description

NGS data from FFPE samples contain numerous artifact chimeric reads. These chimeric reads are formed through the combination of two single-stranded DNA (ss-DNA) with short reverse complementary regions (SRCR). This function simulates these artifact chimeric reads as well as normal reads for FFPE samples within defined regions. To simplify the simulation, the genome is divided into small windows, and SRCRs are found within the same window (adjacent ss-DNA combination) or between different windows (distant ss-DNA simulation).

#### Usage

targetReadSimFFPE(referencePath, PhredScoreProfile, targetRegions, outFile, coverage, readLen=150, meanInsertLen=250, sdInsertLen=80, enzymeCut=FALSE, padding=50, minGap=5, chimericProp=0.1, sameChrProp=0.43, windowLen=5000, adjChimProp=0.63, sameStrandProp=0.65, meanLogSRCRLen=1.8, sdLogSRCRLen=0.55, maxSRCRLen=32, meanLogSRCRDist=4.7, sdLogSRCRDist=0.35, distWinLen=5000, spikeWidth=1500, betaShape1=0.5, betaShape2=0.5, sameTarRegionProb=0,

adjFactor = 1.3, distFactor = 1.3, chimMutRate=0.003, noiseRate=0.0015, highNoiseRate=0.08, highNoiseProp=0.01, pairedEnd=TRUE, prefix="SimFFPE", threads=1, adjChimeric=TRUE, distChimeric=TRUE, normalReads=TRUE, overWrite=FALSE)

## **Arguments**

referencePath Path to the reference genome.

PhredScoreProfile

A matrix representing the positional Phred score proportion. Each row of the matrix represents a position in the read (from begin to end), and each column the Phred quality score of base-calling error probabilities. The profile can be calculated from BAM file using the calcPhredScoreProfile function.

targetRegions A DataFrame or GenomicRanges object representing the exonic / targeted re-

gions to simulate. If it is a DataFrame, the first column should be the chromosome, the second the start position and the third the end position. Please use one-based coordinate systems (the first base should be marked with 1 but not 0).

outFile Output file path for the FASTQ file with simulated reads. Please include the

name of the output file without extension, e.g. "/tmp/sim1".

coverage Coverage of the simulation.

readLen Read length of the simulation.

meanInsertLen Mean insert length for the simulation (normally distributed).

sdInsertLen Standard deviation of the insert length for simulation (normally distributed).

enzymeCut Simulate enzymatic fragmentation if it is set to true, otherwise simulate random

fragmentation.

padding Length of padding of input target regions. The padding length will be added to

both sides of target regions. Range: natural numbers. Unit: base pair (bp).

minGap Minimal allowed length of gap between target regions. Regions with a gap

smaller than this value will be merged. If this value is not given, the value of input readLen will be used. Range: natural numbers. Unit: base pair (bp).

chimericProp Proportion of artifact chimeric fragments (chimeric fragments / chimeric or nor-

mal fragments). Range: 0 to 1.

sameChrProp Proportion of artifact chimeric fragments that are derived from the combination

of two ss-DNA coming from the same chromosome. Range: 0 to 1.

windowLen The window length used in adjacent ss-DNA combination simulation. To simu-

late adjacent ss-DNA combinations, input DNA sequences are divided into small windows of equal size, and short reverse complementary regions are searched within the same window to form artifact chimeric fragments. Unit: base pair

(bp).

adjChimProp Proportion of adjacent ss-DNA combinations among same chromosomal ss-

DNA combinations. Range: 0 to 1.

sameStrandProp Proportion of same-strand ss-DNA combinations among adjacent ss-DNA com-

binations. For paired end sequencing, the larger the proportion, the greater the proportion of improperly paired reads with LL / RR pair orientation, and the

smaller the proportion with RL pair orientation. Range: 0 to 1.

meanLogSRCRLen Mean of log scaled length of the short reverse complementary regions (SRCR) in

artifact chimeric fragments. SRCRs links two ss-DNA together, yielding artifact chimeric fragments. The length of SRCR follows a log-normal distribution. See

rlnorm for more details. Unit: base pair (bp).

sdLogSRCRLen Standard deviation of log scaled length of the short reverse complementary re-

gions.

maxSRCRLen Maximum length of the short reverse complementary regions. Unit: base pair

meanLogSRCRDist

Mean of log scaled original genomic distance of the short reverse complementary regions(SRCR) in artifact chimeric fragments. SRCRs links two ss-DNA together, yielding artifact chimeric fragments. The distance of SRCR is the original genomic distance between the two short reverse complementary segments, which follows a log-normal distribution in simulation. For log-normal distribution, see rlnorm for more details. Unit: base pair (bp).

sdLogSRCRDist Standard deviation of log scaled original genomic distance of the short reverse complementary regions(SRCR) in artifact chimeric fragments.

distWinLen The window length used in distant ss-DNA simulation. To simulate distant ss-DNA combinations, the short reverse complementary regions(SRCR) are searched between different windows. Unit: base pair (bp).

The width of chimeric read spike used to simulate distant ss-DNA combinations. In real FFPE samples, the chimeric reads formed by distant DNA combination are unevenly distributed along the chromosome. Some regions are enriched in these reads while some others are scarce. The length of these regions are of similar scale; therefore, a defined width is used for simulation. Suggested range:

1500-2000. Unit: base pair (bp).

Shape parameter a of beta distribution used to model the unevenly distributed distant ss-DNA combinations. The number of seeds in each "spike" follows a "U" shaped beta distribution. Use this parameter to adjust the shape of the curve. See rbeta for more details. Range: 0-1.

Shape parameter b of beta distribution used to model the unevenly distributed distant ss-DNA combinations. The number of seeds in each "spike" follows a "U" shaped beta distribution. Use this parameter to adjust the shape of the curve.

See rbeta for more details. Range: 0-1.

sameTarRegionProb

Probability of two distant ss-DNA combination events coming from the same two different windows.

Increase this value if the number of simulated adjacent chimeric reads is smaller than expected (sameChrProp \* adjChimProp), decrease if the opposite is true.

Increase this value if the number of simulated distant chimeric reads is smaller distFactor

than expected, decrease if the opposite is true.

Mutation rate for each base in chimeric fragments. In the chimeric fragment formation process, biological-level errors might occur and lead to mutations on these artifact fragments. For all four basic types of nucleotides, the substitution

probability is set equal. Range: 0-0.75.

Noise rate for each base in reads. This is used for sequencing-level errors. The noiseRate probability is set equal for all four basic types of nucleotides. Range: 0-0.75.

highNoiseRate A second noise rate for each base in reads. In some real sequencing data, some reads are much more noisy than others. This parameter can be used for this

situation. Range: 0-0.75.

Proportion of reads to be simulated with highNoiseRate other than noiseRate.

Range: 0-1.

spikeWidth

betaShape1

betaShape2

adjFactor

chimMutRate

highNoiseProp

pairedEnd Simulate paired end sequencing when set to true. prefix Prefix for read names. When reads from different runs of simulation have to be merged, please make sure that they have different prefixes. threads Number of threads used. Multi-threading can speed up the process. adjChimeric Generate reads from adjacent ss-DNA combinations if it is set to true. If it is set to false, skip this process. distChimeric Generate reads from distant ss-DNA combinations if it is set to true. If it is set to false, skip this process. normalReads Generate reads from normal fragments if it is set to true. If it is set to false, skip this process. overWrite Overwrite the file if file with the same output path exists and it is set to true. If file with same output path exists and it is set to false, reads will be appended to

## **Details**

The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artifact chimeric reads (ACRs), which can lead to false positive structural variant calls. These ACRs are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary regions (SRCRs). This function simulates these artifact chimeric reads as well as normal reads for FFPE samples within defined regions. To simplify the simulation, the genome is divided into small windows, and SRCRs are found within the same window (adjacent ss-DNA combination) or between different windows (distant ss-DNA simulation). For adjacent ss-DNA combination events, the original genomic distance between and strands of two combined SRCRs are also simulated based on real data. In the output fastq file, reads are distinguished by prefixes "adjChimeric", "distChimeric" and "Normal" in their names. The parameter PhredScoreProfile can be calculated by the function calcPhredScoreProfile. To simulate whole genome sequencing (WGS) or to simulate reads on several large regions / full chromosomes, please use the function readSimFFPE.

#### Value

NULL. Reads will be written to the output FASTQ file.

the existing file.

#### Note

When fine-tuning is needed, simulate reads from part of the regions instead of all the target regions to save the runtime. Please check the package vignette for the guidance of fine-tuning.

## Author(s)

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## See Also

```
{\tt SimFFPE, calcPhredScoreProfile, readSimFFPE}
```

## **Examples**

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