

An Introduction to *SimFFPE*

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1 Introduction

The NGS (Next-Generation Sequencing) reads from FFPE (Formalin-Fixed Paraffin-Embedded) samples contain numerous artificial chimeric reads. These reads are derived from the combination of two single-stranded DNA (ss-DNA) fragments with short reverse complementary sequences. The combined ss-DNA may come from adjacent or distant regions. The *SimFFPE* package simulates these artificial reads as well as normal reads for FFPE samples. 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 performed for desired simulation results, and multi-threading can help reduce the runtime.

2 Input

The essential inputs for the simulation include a FASTA file of the reference genome, a Phred score profile matrix to simulate Phred scores based on the position on the reads, and a DataFrame or GenomicRanges object representing the target regions (optional). The Phred score profile can be estimated from existing BAM files using `calcPhredScoreProfile` function (two available examples for Phred score profile are stored in the 'extdata' directory of *SimFFPE* package).

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

```
> ## Example Phred score profile with 100 read length
> PhredScoreProfilePath <- system.file("extdata", "PhredScoreProfile1.txt",
+                                     package = "SimFFPE")
> PhredScoreProfile <- as.matrix(read.table(PhredScoreProfilePath, skip = 1))
> colnames(PhredScoreProfile) <- read.table(PhredScoreProfilePath,
+                                           nrow = 1,
+                                           colClasses = "character")
> #
> ## Example Phred score profile with 150 read length
>
> PhredScoreProfilePath2 <- system.file("extdata", "PhredScoreProfile2.txt",
+                                       package = "SimFFPE")
> PhredScoreProfile2 <- as.matrix(read.table(PhredScoreProfilePath2, skip = 1))
> colnames(PhredScoreProfile2) <- read.table(PhredScoreProfilePath2,
+                                             nrow = 1,
+                                             colClasses = "character")
>
```

The FASTA file of reference genome can be read in as *DNAStringSet* with `readDNAStringSet` function from *Biostrings* package. The reference genome example file consists of small regions of 24 chromosomes from human hg19 reference genome.

```
> referencePath <- system.file("extdata", "example.fasta", package = "SimFFPE")
> reference <- readDNAStringSet(referencePath)
> reference

DNAStringSet object of length 24:
      width seq                      names
[1] 30000 AGACTAACATGGA...TCCTTTCTTTCC 1 dna 20000001:20...
[2] 30000 ACATTTCCATTG...GTAGCGGGGCA 2 dna 20000001:20...
[3] 30000 TGTTTACACATT...TGCCCAAACTT 3 dna 20000001:20...
[4] 30000 GTTTAACGATCTA...TGTCGTCTGCCT 4 dna 20000001:20...
[5] 30000 CCACTTATCTTGT...AGGTGTTTGCTA 5 dna 20000001:20...
...
[20] 30000 TCAGTTTGGGAGG...AATCTCCTTTAG 20 dna 20000001:2...
[21] 30000 CCCTTCTCCTATC...TAAATACTCAA 21 dna 20000001:2...
[22] 30000 TGGAAGGTGGG...GAAATATTTGTT 22 dna 20000001:2...
[23] 30000 AGAATGATGGCT...AGGCTCTGAAGA X dna 20000001:20...
[24] 30000 ATGGTATTTGGGA...CAAAAAGGAATG Y dna 20000001:20...
>
```

To simulate reads of certain regions, a *DataFrame* or *GenomicRanges* object representing the target regions is required (not required when simulating reads on the whole genome / several chromosomes / large regions). The *DataFrame* representing the target regions should have three columns, which indicate chromosomes, start positions and end positions respectively (one-based coordinate).

```
> regionPath <- system.file("extdata", "regionsSim.txt", package = "SimFFPE")
> targetRegions <- read.table(regionPath)
```

3 Simulation

The simulation includes three steps: 1) Simulate artificial chimeric reads derived from the combination of two ss-DNA segments from adjacent regions on the chromosome. 2) Simulate artificial chimeric reads derived from the combination of two ss-DNA from distant regions (distant regions on the same chromosome, or any regions on different chromosomes). 3) Simulate reads derived from normal sequences. You can also skip any of these steps in the simulation. There are two functions which can be used for the simulation: `readSimFFPE` and `targetReadSimFFPE`.

3.1 Read simulation functions

To simulate reads on whole genome, or several chromosomes, or large regions, please use the `readSimFFPE` function:

```
> ## Simulate reads of the first three sequences of the reference genome
>
> sourceSeq <- reference[1:3]
> outFile1 <- paste0(tempdir(), "/sim1")
> readSimFFPE(sourceSeq, referencePath, PhredScoreProfile2, outFile1,
+             overwrite = TRUE, coverage = 80, readLen = 150,
+             enzymeCut = TRUE, threads = 4)
> #
> ## Simulate reads of defined regions on the first two sequences of
> ## the reference genome
>
> sourceSeq2 <- DNASTringSet(lapply(reference[1:2], function(x) x[1:10000]))
> outFile2 <- paste0(tempdir(), "/sim2")
> readSimFFPE(sourceSeq2, referencePath, PhredScoreProfile2, outFile2,
+             overwrite = TRUE, coverage = 80, readLen = 150, enzymeCut = TRUE)
>
```

To simulate reads on whole exome or targeted regions please use the `targetReadSimFFPE` function:

```
> outFile3 <- paste0(tempdir(), "/sim3")
> targetReadSimFFPE(referencePath, PhredScoreProfile, targetRegions, outFile3,
+                  coverage = 120, readLen = 100, meanInsertLen = 180,
+                  sdInsertLen = 50, enzymeCut = FALSE)
>
```

Additional information can be found on the help pages for the `readSimFFPE` function and the `targetReadSimFFPE` function.

3.2 Fine-tuning of the simulation

Fine-tuning of the simulation is achievable by the adjustments of some parameters of the function `readSimFFPE` and `targetReadSimFFPE`. You can simulate reads in smaller regions during fine-tuning to save the runtime. To illustrate the impact of some of these parameters, screenshots from IGV tools are used (see Figure 1, 2 and 3). These parameters include:

- 1) enzymeCut: Simulate enzymatic fragmentation. With this fragmentation method, chimeric read pairs with improper pair orientations might be mapped to exactly the same location on the reference genome (see Figure 1).
- 2) chimericRatio: Proportion of artificial chimeric reads. The higher the value, the greater the proportion of improper paired reads as shown in Figure 1 and 2, the smaller the proportion of proper paired reads as shown in Figure 3, and the larger the proportion of proper paired reads with soft-clips as shown in Figure 3.
- 3) localMatchRatio: Proportion of chimeric reads that are formed by the combination of two adjacent ss-DNA. The higher the value, the greater the proportion of reads with improper pair orientations as shown in Figure 1, and the smaller the proportion of reads with their mates mapped to different chromosomes as shown in Figure 2.
- 4) sameStrandProb: Probability of seed and target coming from the same DNA strand in adjacent ss-DNA combination simulation. The higher the value, the greater the proportion of reads with RR and LL orientations in all reads with improper pair orientations as shown in Figure 1.
- 5) spikeWidth: The width of chimeric read spike used in the simulation of distant ss-DNA combinations. As shown in Figure 3, some regions are enriched in reads with paired reads mapped to other chromosomes, and some others are scarce. The lengths of these regions are of similar scale, and the parameter "spikeWidth" is used to simulate this length.
- 6) sameTarRegionProb: Probability of neighboring seeds to search targets in same random region for distant ss-DNA combination simulation. The larger the value, the more the false positive translocation variants.
- 7) highNoiseRate and highNoiseProb: The noise rate for each base in noisy reads and the proportion of these noisy reads. These very noisy reads as well as less noisy reads are shown in Figure 3.

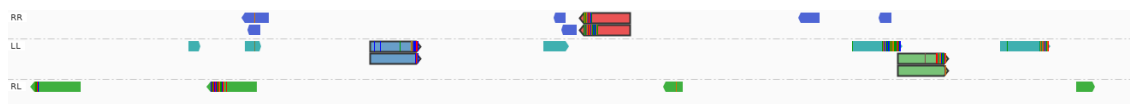


Figure 1: Simulated reads with improper pair orientations

These read pairs are mapped in RR, LL, or RL orientations. Black-framed reads are paired reads that are mapped to the same position (enzymatic fragmentation).

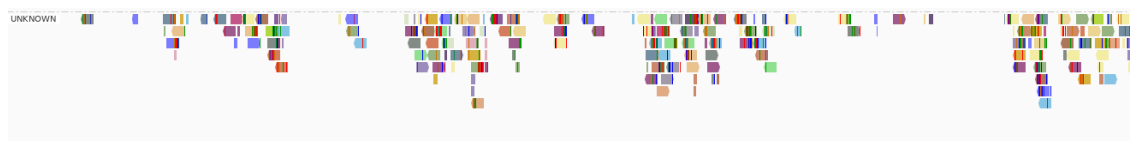


Figure 2: Simulated reads with mate reads mapped to different chromosomes

The different read colors indicate the different chromosomes that their mate reads mapped to.

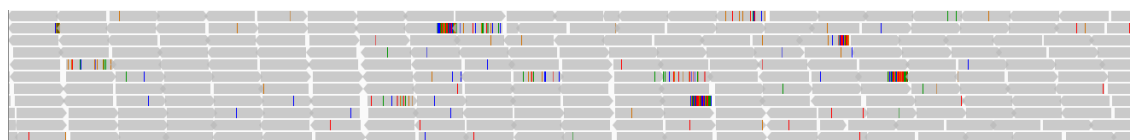


Figure 3: Simulated reads mapped in proper pair

Mismatches and soft-clips are shown as colored vertical lines in reads.

A Session info

```
> packageDescription("SimFFPE")

Package: SimFFPE
Type: Package
Title: NGS Read Simulator for FFPE Tissue
Version: 0.99.32
Author: Lanying Wei
Maintainer: Lanying Wei <lanying.wei@uni-muenster.de>
Description: This package simulates artifact chimeric
             reads specifically generated in next-generation
             sequencing (NGS) process of formalin-fixed
             paraffin-embedded (FFPE) tissue.
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: master
git_last_commit: 71d6a0e
git_last_commit_date: 2020-03-30
Date/Publication: 2020-04-07
Built: R 4.0.0; ; 2020-04-08 05:14:06 UTC; unix

-- File: /private/tmp/RtmpxUTDw3/Rinst114b77c9157da/SimFFPE/Meta/package.rds
> sessionInfo()

R version 4.0.0 alpha (2020-04-05 r78150)
Platform: x86_64-apple-darwin17.0 (64-bit)
Running under: macOS Mojave 10.14.6

Matrix products: default
BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib

locale:
[1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats4    parallel  stats     graphics  grDevices
[6] utils     datasets  methods   base

other attached packages:
[1] SimFFPE_0.99.32    Biostrings_2.55.7
```

```
[3] XVector_0.27.2      IRanges_2.21.8
[5] S4Vectors_0.25.15   BiocGenerics_0.33.3

loaded via a namespace (and not attached):
[1] Rcpp_1.0.4.6          pillar_1.4.3
[3] compiler_4.0.0        BiocManager_1.30.10
[5] GenomeInfoDb_1.23.16  bitops_1.0-6
[7] iterators_1.0.12      tools_4.0.0
[9] zlibbioc_1.33.1       digest_0.6.25
[11] lifecycle_0.2.0       tibble_3.0.0
[13] evaluate_0.14         pkgconfig_2.0.3
[15] rlang_0.4.5           foreach_1.5.0
[17] cli_2.0.2             yaml_2.2.1
[19] xfun_0.12             GenomeInfoDbData_1.2.2
[21] dplyr_0.8.5           knitr_1.28
[23] vctrs_0.2.4           tidyselect_1.0.0
[25] glue_1.4.0            R6_2.4.1
[27] fansi_0.4.1           BiocParallel_1.21.2
[29] rmarkdown_2.1         purrr_0.3.3
[31] magrittr_1.5          ellipsis_0.3.0
[33] Rsamtools_2.3.7       codetools_0.2-16
[35] htmltools_0.4.0       GenomicRanges_1.39.3
[37] assertthat_0.2.1      BiocStyle_2.15.6
[39] RCurl_1.98-1.1        doParallel_1.0.15
[41] truncnorm_1.0-8       crayon_1.3.4
```