KmeRtone User Manual

Contents

Installation

```
# Install from CRAN
install.packages('kmeRtone')

# Install directly from GitHub
devtools::install_github('SahakyanLab/kmeRtone', ref = 'master')
```

Alternatively, download and install using the latest release files from here.

Overview of `kmeRtone` operations

`KmeRtone` contains many modules. The core module (SCORE) calculates the z-score of k-meric enrichment and depletion. Briefly, the input source are case coordinates for the DNA-related phenomenon under study (e.g. DNA damage, DNA binding, DNA breakage, etc.) and a reference to the chromosome-separated FASTA files. `KmeRtone` calculates the k-mer z-score for every k-mer sequence and generates a table of all k-mer sequences and their associated z-scores. Here, the resulting z-scores indicate how enriched ($z \gg 1$) or depleted ($z \ll 1$) a given k-mer sequence is under the studied phenomenon.

`kmeRtone` Input Flags - Overview

Here, we highlight some of the key arguments as input to the `kmeRtone` function. Please refer to the documentation of the function for further details on the required and optional arguments.

1. **Case coordinate**

Flag	Class	Description
case.coor.path	`<character>`	A path to a **folder** containing chromosome-separated genomic coordinates or chromosome-combined BED files. This flag is ignored when case.coor is not NULL.
case	`<genomic.coordinate>`	A pre-loaded `<genomic.coordinate>` class object..

2. **Genome**

Flag	Class	Description
genome.name	`<character>`	Available: "hg19" or "hg38". User's own genome name.
genome.path	`<character>`	A path to a user's **folder** containing chromosome-separated fasta files. Default is `NULL`. The file name must be the name of chromosome.
genome	`<genome>`	Pre-loaded `<genome>` class object. Default is `NULL`. The two flags above are ignored when this is used.

3. **Case characteristics**

Flag	Class	Description
strand.sensitive	`<bool>`	Does strand polarity matter?
single.case.length	`<int>`	Default is `NULL` for unspecified/varied length.

Flag	Class	Description
case.pattern	`<character>`	Default is `NULL` for no pattern.

4. **Case coordinate operation**

Flag	Class	Description
rm.case.kmer.overlaps	`<bool>`	Default is `TRUE`. This is important to remove neighbouring effect.
merge.replicates	`<bool>`	Default is `TRUE`. When merging replicates, duplicated coordinates coming from different replicates are removed.
k	`<int>`	Length of k-mer
ctrl.rel.pos	`<character>`	Position of control regions relative to the case positions. Input is a vector of length two: `c(from, to)`

5. **Other module flags**

Flag	Class	Description
kmer.table	`<data.table>`	Pre-loaded k-mer table with calculated score. Default is `NULL`.

6. **kmeRtone module**

Flag	Class	Description
module	`<character>`	Available module: “score”, “tune”, “explore”, “evolution”, “genic element”, “cancer”, etc.

7. **Other**

Flag	Class	Description
ncpu	`<int>`	Number of CPU cores. Default is 1.
output.path	`<character>`	A path to an output **folder**. Default is “data/”

kmeRtone Input Flags - Additional Description

Flag	Description
single.case.length	The case length unit is number of nucleotide. In an event where case happens in between two nucleotide e.g. DNA breakage, the case.length is 2 nt.
case.coor.path	Three situations can happen. (1) A folder containing a BED file. A second or more BED files indicates a presence of replicates. (2) A folder containing chromosome-separated files. The file name must be the name of chromosome. (3) A folder containing sub-folders of chromosome-separated files, indicating a presence of replicates. In situation (2) and (3), the coordinates must be a 1-based index due to `R` language conventions. Alternatively, user can specify this with the `case.coor.1st.idx` argument

kmeRtone Objects

kmeRtone introduce two class objects: `<genome>` and `<genomic.coordinate>`

1. `<genome>`

kmeRtone comes with two pre-built `<genome>`: hg19 and hg38. The `<genome>`s are saved as uncompressed RDS binary object for fast loading. `print.genome` function is built to print the `<genome>` object. It will show the genome name (e.g. hg19) and genome length by chromosome. The default `base::print` showing the very long sequence will crash the R console.

`<genome>` is an S3-class object with the following contents:

```
$seq
 named <character> vector
   chr1              chr2             ...
 c(AACTCGTACC......, ACGTTGGTTC....)  ...

$chr.names
 <character> vector
 c(chr1, chr2, ...)

$length
 <character> vector
 c(2947924, 2093123, ...)

$name
 <character>
 hg19
```

2. `<genomic.coordinate>`

`<genomic.coordinate>` is an S3-class object. The reason for building this class is to reduce data redundancy in genomic coordinate table (e.g. repeated number of chromosome name and unnecessary column end when case length is fixed). It also helps with organisation of kmeRtone configuration (e.g. k-mer size, case length, etc.) as the `<genomic.coordinate>` object will carry and contain those information. It utilises `<data.table>` to use its inherent feature to update by reference (instead of memory copy) for genomic coordinate table and coordinate status (case vs. k-mer coordinate). This will help to reduce memory (RAM) consumption and keep track what the coordinates refer to (whether the case itself or k-mer). The contents of the `<genomic.coordinate>` object are as follow:

```
$chr1
 <data.table>
   start strand ...
 1:    12      +
 2:    16      +
 3:   499      -
 ...

$chr2 .__C__.externalptr

$chr3 ...
```

```
$chr... ...

$chr.names
 <character> vector
 c(chr1, chr2, ...)

$status
 <data.table> single row
   is.kmer
 1:    TRUE

$case.length
 <character>
 2

$case.pattern
 <character> vector
 c(CT, TT, ...)
```

Code Convention

- Table column name is written in lowercase and snake_case.
- Function name is written in camelCase. The function filename if it is saved will be the same like the function name except for workflow functions which begin with capital case corresponds to their module letter.
- Module workflow code begins with a function calling (left-aligned) and ends with variable assignment (right-aligned).
- Workflow boolean is designed to make it natural to read in English e.g. `if(coor$status$is.kmer)` or `if(coor$is.strand.sensitive)`.
- Looping uses singular and plural as variable name i.e. `for (chr.name in chr.names)`.
- The code finish at a standard column number 80 for better viewing.
- This symbol <> refers to R class object e.g. `<character>`

Quick examples

Below we will demonstrate various capabilities of the default kmeRtone `SCORE` function to quantify the k-meric enrichment and depletion.

Single-nucleotide resolution case coordinates: non-specific patterns

Suppose we expose a DNA sequence to ionisation radiation and record the positions that are broken. For example, the A-T bond is broken between positions 10,000-10,001 on chromosome 1. Here, we are working with single-nucleotide resolution data, where specific bonds are broken between two adjacent nucleotides. Below we show an example simulation of generating files that can be used by `kmeRtone`.

The chromosomes and start positions are randomly sampled with a `width` of 2 as in our simulated example, we are interested in the breakage phenomenon between adjacent two nucleotides. The `width` depends on your DNA phenomenon under study. For instance, if you are working with DNA damages, where, for instance, the thymine base at position 50,000 on chromosome 1 is damaged, then the `width` variable is 1 and the `single.case.len` is also 1. For the DNA breakage example, the `width` variable is 2, hence the `single.case.len` is also 2. Finally, save the results as chromosome-separated files in your desired folder.

```
library(data.table)
library(kmeRtone)
temp_dir <- tempdir()

#' 1. Randomly generate genomic positions and save results
dir.create("./data", showWarnings = FALSE)

set.seed(1234)
temp_files <- character(22)
for(chr in 1:22){
    genomic_coor <- data.table(
        seqnames = paste0("chr", chr),
        start = sample(
            x = 10000:100000,
            size = 1000,
            replace = FALSE
        ),
        width = 2
    )

    f <- file.path(temp_dir, paste0("chr", chr, ".csv"))
    fwrite(genomic_coor, f)
    temp_files[chr] <- f
}

#' 2. Run kmeRtone `score` function
kmeRtone::kmeRtone(
    case.coor.path = temp_dir,
    genome.name = "hg19",
    strand.sensitive = FALSE,
    k = 2,
    ctrl.rel.pos = c(80, 500),
    case.pattern = NULL,
    single.case.len = 2,
```

```
    output.dir = temp_dir,
    module = "score",
    rm.case.kmer.overlaps = FALSE,
    merge.replicate = TRUE,
    kmer.table = NULL,
    verbose = TRUE
)
```

To run a k-meric enrichment and depletion analysis with `kmeRtone` on this simulated dataset, run the below function. Here, we specified `k` as 4, thus, the z-scores are calculated for each 4-mer sequence.

You will need to be conscious on the specified control range from which you sample the negative control k-mer population. If it's too close to the case regions, you may have too much of a sequence-context bias from the case region that may influence your negative control population, even though the coordinates are not overlapping. Similarly, if it's too far from the case regions, you may not capture enough of the local sequence variation and only capture the broad sequence influence. One of the advantages of `KmeRtone` is the inherent flexibility that comes with the choice of the control regions, giving the user full control of which genomic regions to start and stop the sampling. This gives you more accurate statistical testing than simply comparing the case regions to the overall genome-wide average. With that said, unless you know what range makes sense, we otherwise recommend that you experiment with different ranges and make an informed decision based on this. For now, we will stick to the default values.

Please refer to the documentation of the function for further details on the required and optional arguments. Please note, that your results will differ depending on which `ctrl.rel.pos` range you use, whether you have a specific `case.pattern` of interest, whether to remove overlapping case k-mers in case regions with `rm.case.kmer.overlaps`, whether to merge replicates or treat them separately with `merge.replicate`, and more.

```
#' 2. Run kmeRtone `score` function
kmeRtone::kmeRtone(
    case.coor.path = temp_dir,
    genome.name = "hg19",
    strand.sensitive = FALSE,
    k = 4,
    ctrl.rel.pos = c(80, 500),
    case.pattern = NULL,
    single.case.len = 2,
    output.dir = temp_dir,
    module = "score",
    rm.case.kmer.overlaps = FALSE,
    merge.replicate = TRUE,
    kmer.table = NULL,
    verbose = TRUE
)
```

The above should generate the below output. The results are saved in the path you specified in the `output.dir` argument.

```
------------------------------------------------------------
                Extraction of Case K-mers
------------------------------------------------------------
Extracting 4-mers from chr1.............DONE! -- 3.63 secs
Extracting 4-mers from chr2.............DONE! -- 3.39 secs
Extracting 4-mers from chr3.............DONE! -- 2.66 secs
```

```
Extracting 4-mers from chr4.............DONE! -- 2.39 secs
Extracting 4-mers from chr5.............DONE! -- 2.46 secs
Extracting 4-mers from chr6.............DONE! -- 2.16 secs
Extracting 4-mers from chr7.............DONE! -- 2.19 secs
Extracting 4-mers from chr8.............DONE! -- 1.86 secs
Extracting 4-mers from chr9.............DONE! -- 1.94 secs
Extracting 4-mers from chr10............DONE! -- 1.73 secs
Extracting 4-mers from chr11............DONE! -- 1.92 secs
Extracting 4-mers from chr12............DONE! -- 1.8 secs
Extracting 4-mers from chr13............DONE! -- 1.39 secs
Extracting 4-mers from chr14............DONE! -- 1.43 secs
Extracting 4-mers from chr15............DONE! -- 1.14 secs
Extracting 4-mers from chr16............DONE! -- 1.17 secs
Extracting 4-mers from chr17............DONE! -- 1.26 secs
Extracting 4-mers from chr18............DONE! -- 1.41 secs
Extracting 4-mers from chr19............DONE! -- 0.76 secs
Extracting 4-mers from chr20............DONE! -- 0.73 secs
Extracting 4-mers from chr21............DONE! -- 0.54 secs
Extracting 4-mers from chr22............DONE! -- 0.56 secs

Total time taken: 38.86 secs
-------------------------------------------------------------
               Extraction of Control K-mers
-------------------------------------------------------------
Building control regions of chr1........DONE! -- 3.97 secs
Building control regions of chr2........DONE! -- 3.31 secs
Building control regions of chr3........DONE! -- 3.72 secs
Building control regions of chr4........DONE! -- 2.58 secs
Building control regions of chr5........DONE! -- 3.13 secs
Building control regions of chr6........DONE! -- 2.26 secs
Building control regions of chr7........DONE! -- 2.34 secs
Building control regions of chr8........DONE! -- 1.95 secs
Building control regions of chr9........DONE! -- 2 secs
Building control regions of chr10.......DONE! -- 1.78 secs
Building control regions of chr11.......DONE! -- 2.03 secs
Building control regions of chr12.......DONE! -- 1.93 secs
Building control regions of chr13.......DONE! -- 1.81 secs
Building control regions of chr14.......DONE! -- 1.48 secs
Building control regions of chr15.......DONE! -- 1.15 secs
Building control regions of chr16.......DONE! -- 1.05 secs
Building control regions of chr17.......DONE! -- 1.02 secs
Building control regions of chr18.......DONE! -- 1.19 secs
Building control regions of chr19.......DONE! -- 0.69 secs
Building control regions of chr20.......DONE! -- 0.76 secs
Building control regions of chr21.......DONE! -- 0.57 secs
Building control regions of chr22.......DONE! -- 0.56 secs

Total time taken: 41.42 secs
Extracting 4-mers from chr1.............DONE! -- 3.3 secs
Extracting 4-mers from chr2.............DONE! -- 3.31 secs
Extracting 4-mers from chr3.............DONE! -- 2.56 secs
Extracting 4-mers from chr4.............DONE! -- 2.65 secs
Extracting 4-mers from chr5.............DONE! -- 2.36 secs
```

```
Extracting 4-mers from chr6.............DONE! -- 2.43 secs
Extracting 4-mers from chr7.............DONE! -- 2.19 secs
Extracting 4-mers from chr8.............DONE! -- 1.94 secs
Extracting 4-mers from chr9.............DONE! -- 2.02 secs
Extracting 4-mers from chr10............DONE! -- 1.85 secs
Extracting 4-mers from chr11............DONE! -- 1.82 secs
Extracting 4-mers from chr12............DONE! -- 1.95 secs
Extracting 4-mers from chr13............DONE! -- 1.42 secs
Extracting 4-mers from chr14............DONE! -- 1.24 secs
Extracting 4-mers from chr15............DONE! -- 1.18 secs
Extracting 4-mers from chr16............DONE! -- 1.25 secs
Extracting 4-mers from chr17............DONE! -- 0.99 secs
Extracting 4-mers from chr18............DONE! -- 0.93 secs
Extracting 4-mers from chr19............DONE! -- 0.69 secs
Extracting 4-mers from chr20............DONE! -- 0.75 secs
Extracting 4-mers from chr21............DONE! -- 0.89 secs
Extracting 4-mers from chr22............DONE! -- 0.54 secs

Total time taken: 38.5 secs
-------------------------------------------------------------
            Calculation of K-mer Susceptibility
-------------------------------------------------------------
The 4-mer scores are saved at {temp_dir}/score_4-mer.csv

FINISH! Total time taken: 1.98 mins
```

Single-nucleotide resolution case coordinates: specific patterns

Suppose we expose a DNA sequence to UV-light and record the pyrimidine-pyrimidone (6-4) photoproduct and cyclobutane pyrimidine dimers as done in this paper (Hu et al. 2017). We can retrieve the deposited data through the GEO repository (Edgar, Domrachev, and Lash 2002) under the accession identifier GSE98025. For the purposes of this demonstration, we shall download one of the samples: `NHF1_CPD_10J_48h_A` from GSM2585697.

```
#!/bin/bash
wget -O UV_data.bed.gz https://ftp.ncbi.nlm.nih.gov/geo/samples/GSM2585nnn/GSM2585697/suppl/GSM2585697%

gunzip UV_data.bed.gz

head UV_data.bed
```

which should show the below as output.

```
chr1    10108   10118   +
chr1    10163   10173   +
chr1    10194   10204   -
chr1    10275   10285   -
chr1    10299   10309   -
chr1    10342   10352   -
chr1    10346   10356   +
chr1    10357   10367   +
chr1    10377   10387   -
chr1    10397   10407   -
```

As per the GEO repository, "the Bed files contain genomic locations of damages of the most common two dinucleotides at the damage sites for (6-4)PP and CPD. Each interval length is 10 nt, and the pyrimidine dimer is located at the 4-5th positions".

`KmeRtone` requires you to have column headers for it to work. For the purposes of this demonstration, we will import this data into `R` using the `fread` function from the `data.table` library, and label the four columns as the following: `seqnames`, `start`, `end`, and `strand`.

```
library(data.table)
library(kmeRtone)

df <- data.table::fread("UV_data.bed")
data.table::setnames(df, c("seqnames", "start", "end", "strand"))

print(head(df))
```

which should show the below as output.

```
   seqnames start   end strand
     <char> <int> <int> <char>
1:     chr1 10108 10118      +
2:     chr1 10163 10173      +
3:     chr1 10194 10204      -
4:     chr1 10275 10285      -
5:     chr1 10299 10309      -
6:     chr1 10342 10352      -
```

The GEO repository outlined that the dimer is located at the 4-5th position. Before proceeding with the `kmeRtone` analysis, we shall briefly verify this with the below commands.

```
library(BSgenome.Hsapiens.UCSC.hg19)
hg19 <- BSgenome.Hsapiens.UCSC.hg19

library(plyranges)
df_granges <- plyranges::as_granges(df)

print(head(df_granges))
```

which should show the below as output.

```
GRanges object with 6 ranges and 0 metadata columns:
      seqnames      ranges strand
         <Rle>   <IRanges>  <Rle>
  [1]     chr1 10108-10118      +
  [2]     chr1 10163-10173      +
  [3]     chr1 10194-10204      -
  [4]     chr1 10275-10285      -
  [5]     chr1 10299-10309      -
  [6]     chr1 10342-10352      -
  -------
  seqinfo: 25 sequences from an unspecified genome; no seqlengths
```

Next, we visualise the top 10 genomic sequences.

```
library(Biostrings)
sequences <- Biostrings::getSeq(hg19, df_granges[1:10])

print(sequences)
```

which should show the below as output.

```
DNAStringSet object of length 10:
     width seq
 [1]    11 CAACCCTAACC
 [2]    11 TAACCCTAACC
 [3]    11 AGGGTTAGGGT
 [4]    11 AGGGTTAGGGT
 [5]    11 GGGGTTGGGGT
 [6]    11 AGGGTTAGGGT
 [7]    11 TAACCCTACCC
 [8]    11 TAACCCTAACC
 [9]    11 AGGGTTAGGGT
[10]    11 AGGGTTAGGGG
```

We can see from the above print statement, that, indeed, the middle two nucleotides are of the CT or TT motifs.

Again, we will split this data.table into chromosome-separated files. We will only focus on the 22 autosomes, but you can use the X and Y chromosomes too, if available. Here, we save it as `csv` files since we are already operating on it with data.table, however, if you are using `plyranges` or similar libraries, you may continue using the `bed` file format.

```
temp_dir <- tempdir()
for(chr in 1:22){
    data.table::fwrite(
        df[seqnames == paste0("chr", chr)],
        paste0(temp_dir, "/chr", chr, ".csv")
    )
}
```

To run a k-meric enrichment and depletion analysis with `kmeRtone` on this publicly-available dataset, run the below function. Here, we specified `k` as 4, thus, the z-scores are calculated for each 4-mer sequence. The same negative control range as described previously applies here too. For now, we will stick to the default values.

We set the `single.case.len` argument to 2 as this retrieved dataset specifically records the pyrimidine dimer at the 4-5th positions. We also set the `case.pattern` argument to CT as we know these UV-induced damaged sites are of the CT motif, and we are specifically interested in the k-meric enrichment and depletion in the context of this CT motif. We have data recording the damaged site on both the plus and minus strand, hence, we set the `strand.sensitive` argument to $TRUE$.

Please refer to the documentation of the function for further details on the required and optional arguments. Please note, that your results will differ depending on which `ctrl.rel.pos` range you use, whether to remove overlapping case k-mers in case regions with `rm.case.kmer.overlaps`, whether to merge replicates or treat them separately with `merge.replicate`, and more.

```r
#' 2. Run kmeRtone `score` function
kmeRtone::kmeRtone(
    case.coor.path = temp_dir,
    genome.name = "hg19",
    strand.sensitive = FALSE,
    k = 4,
    ctrl.rel.pos = c(80, 500),
    case.pattern = c("CT","TT"),
    single.case.len = 2,
    output.dir = temp_dir,
    module = "score",
    rm.case.kmer.overlaps = FALSE,
    merge.replicate = TRUE,
    kmer.table = NULL,
    verbose = TRUE
)
```

The above should generate the below output. The results are saved in the path you specified in the `output.dir` argument.

```
------------------------------------------------------------
                Extraction of Case K-mers
------------------------------------------------------------
Extracting 4-mers from chr1.............DONE! -- 8.54 secs
Extracting 4-mers from chr2.............DONE! -- 10.65 secs
Extracting 4-mers from chr3.............DONE! -- 8.45 secs
Extracting 4-mers from chr4.............DONE! -- 8.82 secs
Extracting 4-mers from chr5.............DONE! -- 9.62 secs
Extracting 4-mers from chr6.............DONE! -- 6.77 secs
Extracting 4-mers from chr7.............DONE! -- 6.77 secs
Extracting 4-mers from chr8.............DONE! -- 6.73 secs
Extracting 4-mers from chr9.............DONE! -- 5.24 secs
Extracting 4-mers from chr10............DONE! -- 6.38 secs
Extracting 4-mers from chr11............DONE! -- 5.94 secs
Extracting 4-mers from chr12............DONE! -- 6.11 secs
Extracting 4-mers from chr13............DONE! -- 4.82 secs
Extracting 4-mers from chr14............DONE! -- 5.32 secs
Extracting 4-mers from chr15............DONE! -- 3.04 secs
Extracting 4-mers from chr16............DONE! -- 2.86 secs
Extracting 4-mers from chr17............DONE! -- 2.38 secs
Extracting 4-mers from chr18............DONE! -- 3.04 secs
Extracting 4-mers from chr19............DONE! -- 1.83 secs
Extracting 4-mers from chr20............DONE! -- 2.1 secs
Extracting 4-mers from chr21............DONE! -- 1.64 secs
Extracting 4-mers from chr22............DONE! -- 1.15 secs

Total time taken: 1.97 mins
------------------------------------------------------------
               Extraction of Control K-mers
------------------------------------------------------------
Building control regions of chr1........DONE! -- 8.69 secs
Building control regions of chr2........DONE! -- 9.24 secs
Building control regions of chr3........DONE! -- 7.22 secs
Building control regions of chr4........DONE! -- 6.85 secs
```

```
Building control regions of chr5........DONE! -- 6.59 secs
Building control regions of chr6........DONE! -- 6.18 secs
Building control regions of chr7........DONE! -- 5.93 secs
Building control regions of chr8........DONE! -- 6.24 secs
Building control regions of chr9........DONE! -- 5.1 secs
Building control regions of chr10.......DONE! -- 5.02 secs
Building control regions of chr11.......DONE! -- 4.99 secs
Building control regions of chr12.......DONE! -- 6.32 secs
Building control regions of chr13.......DONE! -- 3.53 secs
Building control regions of chr14.......DONE! -- 3.46 secs
Building control regions of chr15.......DONE! -- 3.15 secs
Building control regions of chr16.......DONE! -- 3.09 secs
Building control regions of chr17.......DONE! -- 3.02 secs
Building control regions of chr18.......DONE! -- 2.7 secs
Building control regions of chr19.......DONE! -- 2.16 secs
Building control regions of chr20.......DONE! -- 2.24 secs
Building control regions of chr21.......DONE! -- 1.41 secs
Building control regions of chr22.......DONE! -- 1.47 secs

Total time taken: 1.75 mins
Extracting 4-mers from chr1.............DONE! -- 29.23 secs
Extracting 4-mers from chr2.............DONE! -- 34.54 secs
Extracting 4-mers from chr3.............DONE! -- 24.51 secs
Extracting 4-mers from chr4.............DONE! -- 19.64 secs
Extracting 4-mers from chr5.............DONE! -- 18.15 secs
Extracting 4-mers from chr6.............DONE! -- 17.05 secs
Extracting 4-mers from chr7.............DONE! -- 15.26 secs
Extracting 4-mers from chr8.............DONE! -- 13.53 secs
Extracting 4-mers from chr9.............DONE! -- 12.37 secs
Extracting 4-mers from chr10............DONE! -- 13.15 secs
Extracting 4-mers from chr11............DONE! -- 13.85 secs
Extracting 4-mers from chr12............DONE! -- 15.26 secs
Extracting 4-mers from chr13............DONE! -- 10.26 secs
Extracting 4-mers from chr14............DONE! -- 8.96 secs
Extracting 4-mers from chr15............DONE! -- 8.36 secs
Extracting 4-mers from chr16............DONE! -- 7.45 secs
Extracting 4-mers from chr17............DONE! -- 8.99 secs
Extracting 4-mers from chr18............DONE! -- 7.3 secs
Extracting 4-mers from chr19............DONE! -- 5.66 secs
Extracting 4-mers from chr20............DONE! -- 5.73 secs
Extracting 4-mers from chr21............DONE! -- 2.79 secs
Extracting 4-mers from chr22............DONE! -- 4.39 secs

Total time taken: 4.94 mins
------------------------------------------------------------
            Calculation of K-mer Susceptibility
------------------------------------------------------------
The 4-mer scores are saved at {temp_dir}/score_4-mer.csv

FINISH! Total time taken: 8.66 mins
```

To explore the results, we run the below command with the resulting k-mer score table displayed after.

```
scores <- data.table::fread(paste0(temp_dir, '/score_4-mers.csv'))
print(scores)
```

This highlights one of the key advantages of the `kmeRtone` software. It is highly flexible, where the user can specify the case k-mer patterns (CT and TT patterns in this example) and quantify the corresponding k-mer enrichment and depletion z-scores in the context of these patterns. As a result, the k-mer table only includes k-mer sequences with a CT and TT motif centrla to the k-mer. This functionality allows for a more comprehensive approach to understanding the functional implications of specific DNA sequences on a genomic scale.

```
      kmer    case  control    case_skew  control_skew           z
    <char>   <int>    <int>        <num>         <num>       <num>
 1:   ACTA  172188  7164905  0.093850907  0.0013140439  -734.98933
 2:   ACTC  199682  8271891  0.020853157  0.0003088774  -790.02857
 3:   ACTG  183576 10687722  0.019664880 -0.0003821207  -968.57235
 4:   ACTT  941818 10809720  0.067322986 -0.0005173122  -291.95047
 5:   ATTA 1542837 12150674  0.072150849 -0.0007008665   102.75058
 6:   ATTC  752161 10080861  0.051631233 -0.0019745337  -399.40541
 7:   ATTG  798686  9024810  0.022557050  0.0010810200  -253.89860
 8:   ATTT 3936871 19988383  0.068213310 -0.0034174350  1073.66234
 9:   CCTA  178612  6457019  0.060130338 -0.0005428201  -669.85683
10:   CCTC  276033 11817164  0.011255901 -0.0016657127  -956.94049
11:   CCTG  233643 14169493 -0.005311522  0.0001087548 -1128.42319
12:   CCTT 1097911 10403912  0.043268535 -0.0027333949  -110.26574
13:   CTTA  885911  7917213  0.068468503 -0.0005101543   -42.91182
14:   CTTC  762270 10692993  0.050546394 -0.0028651473  -443.96560
15:   CTTG  716068 10115452  0.027653798 -0.0015493129  -436.14909
16:   CTTT 2981906 15085303  0.058601445 -0.0034241937   934.47375
17:   GCTA  150920  6088128  0.061197986  0.0002365259  -670.99816
18:   GCTC  160017  7727359  0.016404507 -0.0028991794  -791.18668
19:   GCTG  217984 12044290 -0.049434821  0.0006109119 -1020.65321
20:   GCTT  654241  8471154  0.045541322 -0.0010154461  -342.77052
21:   GTTA  359542  6274672  0.083912311 -0.0006272838  -441.29095
22:   GTTC  277604  6793487  0.056656244 -0.0020132518  -585.89553
23:   GTTG  360434  7178590 -0.004616657  0.0009609129  -528.67826
24:   GTTT 1583482 11779426  0.057679216 -0.0028104935   177.10006
25:   TCTA  682672  8806301  0.063099116 -0.0017163847  -347.10919
26:   TCTC  910395 13001127  0.027013549 -0.0023806398  -504.67948
27:   TCTG  777519 13250152  0.026007081 -0.0018381676  -632.59802
28:   TCTT 2413768 14131267  0.058897955 -0.0043706626   602.27398
29:   TTTA 3904809 16155176  0.061480344 -0.0025543516  1497.59726
30:   TTTC 2550044 15498635  0.036584467 -0.0042352762   558.16449
31:   TTTG 2891034 15209799  0.023224908 -0.0030150957   850.09419
32:   TTTT 9497245 30534758  0.063873576 -0.0065422493  3271.57838
      kmer    case  control    case_skew  control_skew           z
```

ChIP-sequencing peaks and related profiling assays spanning large genomic regions

Suppose we have a bed file that represents chromatin profiling assays with ChIP-seq. Here, the data spans larger genomic regions than the aforementioned single-nucleotide examples. We will use one of the many publicly-available ChIP-seq dataset form the ENCODE project (The ENCODE Project Consortium 2012). While the below is just an example, you can replace it with your dataset of interest.

```
#!/bin/bash
wget -O chipseq_example.bed.gz https://www.encodeproject.org/files/ENCFF579UXQ/@@download/ENCFF579UXQ.b

gunzip chipseq_example.bed.gz

head chipseq_example.bed
```

which should show the below as output.

```
chr1    10008   10200   .   0   .   0.719818    -1  -1  75
chr1    10360   10520   .   0   .   0.331552    -1  -1  75
chr1    16160   16320   .   0   .   0.597667    -1  -1  75
chr1    17430   17531   .   0   .   0.148326    -1  -1  75
chr1    29320   29417   .   0   .   0.209401    -1  -1  75
chr1    104940  105073  .   0   .   0.431891    -1  -1  75
chr1    180920  181200  .   0   .   0.462428    -1  -1  75
chr1    181380  181580  .   0   .   0.745993    -1  -1  75
chr1    183220  183380  .   0   .   0.287927    -1  -1  75
chr1    183700  183900  .   0   .   0.335915    -1  -1  75
```

`KmeRtone` requires you to have column headers for it to work. Therefore, we follow the same process as in the previous section.

```
library(data.table)
library(kmeRtone)

df <- data.table::fread("chipseq_example.bed")
df <- df[, .(V1, V2, V3)]
data.table::setnames(df, c("seqnames", "start", "end"))

print(head(df))
```

which should show the below as output.

```
   seqnames  start    end
     <char>  <int>  <int>
1:     chr1  10008  10200
2:     chr1  10360  10520
3:     chr1  16160  16320
4:     chr1  17430  17531
5:     chr1  29320  29417
6:     chr1 104940 105073
```

Again, we will split this data.table into chromosome-separated files. We will only focus on the 22 autosomes, but you can use the X and Y chromosomes too, if available.

```
temp_dir <- tempdir()
for(chr in 1:22){
    data.table::fwrite(
        df[seqnames == paste0("chr", chr)],
        paste0(temp_dir, "/chr", chr, ".csv")
    )
}
```

To run a k-meric enrichment and depletion analysis with `kmeRtone` on this publicly-available dataset, run the below function. Here, we specified `k` as 4, thus, the z-scores are calculated for each 4-mer sequence.

The same negative control range as described previously applies here too. For now, we will stick to the default values. Please note, we set the `single.case.len` argument to *NULL* as these chromatin profiling assays tend to have variable widths.

Please refer to the documentation of the function for further details on the required and optional arguments. Please note, that your results will differ depending on which `ctrl.rel.pos` range you use, whether you have a specific `case.pattern` of interest, whether to remove overlapping case k-mers in case regions with `rm.case.kmer.overlaps`, whether to merge replicates or treat them separately with `merge.replicate`, and more.

```
#' 2. Run kmeRtone `score` function
kmeRtone::kmeRtone(
    case.coor.path = temp_dir,
    genome.name = "hg19",
    strand.sensitive = FALSE,
    k = 4,
    ctrl.rel.pos = c(80, 500),
    case.pattern = NULL,
    single.case.len = 2,
    output.dir = temp_dir,
    module = "score",
    rm.case.kmer.overlaps = FALSE,
    merge.replicate = TRUE,
    kmer.table = NULL,
    verbose = TRUE
)
```

The above should generate the below output. The results are saved in the path you specified in the `output.dir` argument.

```
------------------------------------------------------------
                Extraction of Case K-mers
------------------------------------------------------------
Extracting 4-mers from chr1.............DONE! -- 3.62 secs
Extracting 4-mers from chr2.............DONE! -- 3.81 secs
Extracting 4-mers from chr3.............DONE! -- 2.99 secs
Extracting 4-mers from chr4.............DONE! -- 2.87 secs
Extracting 4-mers from chr5.............DONE! -- 2.67 secs
Extracting 4-mers from chr6.............DONE! -- 2.59 secs
Extracting 4-mers from chr7.............DONE! -- 2.37 secs
Extracting 4-mers from chr8.............DONE! -- 2.13 secs
Extracting 4-mers from chr9.............DONE! -- 2.03 secs
Extracting 4-mers from chr10............DONE! -- 2.06 secs
Extracting 4-mers from chr11............DONE! -- 2.09 secs
Extracting 4-mers from chr12............DONE! -- 2.04 secs
Extracting 4-mers from chr13............DONE! -- 1.6 secs
Extracting 4-mers from chr14............DONE! -- 1.37 secs
Extracting 4-mers from chr15............DONE! -- 1.37 secs
Extracting 4-mers from chr16............DONE! -- 1.38 secs
Extracting 4-mers from chr17............DONE! -- 1.2 secs
Extracting 4-mers from chr18............DONE! -- 1.12 secs
Extracting 4-mers from chr19............DONE! -- 0.91 secs
```

```
Extracting 4-mers from chr20...........DONE! -- 1.01 secs
Extracting 4-mers from chr21...........DONE! -- 0.57 secs
Extracting 4-mers from chr22...........DONE! -- 0.64 secs

Total time taken: 42.93 secs
-------------------------------------------------------------
               Extraction of Control K-mers
-------------------------------------------------------------
Building control regions of chr1........DONE! -- 3.78 secs
Building control regions of chr2........DONE! -- 3.86 secs
Building control regions of chr3........DONE! -- 3.17 secs
Building control regions of chr4........DONE! -- 2.91 secs
Building control regions of chr5........DONE! -- 2.8 secs
Building control regions of chr6........DONE! -- 2.7 secs
Building control regions of chr7........DONE! -- 2.54 secs
Building control regions of chr8........DONE! -- 2.28 secs
Building control regions of chr9........DONE! -- 2.2 secs
Building control regions of chr10.......DONE! -- 2.21 secs
Building control regions of chr11.......DONE! -- 2.28 secs
Building control regions of chr12.......DONE! -- 2.18 secs
Building control regions of chr13.......DONE! -- 1.61 secs
Building control regions of chr14.......DONE! -- 1.63 secs
Building control regions of chr15.......DONE! -- 1.47 secs
Building control regions of chr16.......DONE! -- 1.46 secs
Building control regions of chr17.......DONE! -- 1.37 secs
Building control regions of chr18.......DONE! -- 1.18 secs
Building control regions of chr19.......DONE! -- 1.08 secs
Building control regions of chr20.......DONE! -- 0.98 secs
Building control regions of chr21.......DONE! -- 0.67 secs
Building control regions of chr22.......DONE! -- 0.74 secs

Total time taken: 45.26 secs
Extracting 4-mers from chr1............DONE! -- 4.76 secs
Extracting 4-mers from chr2............DONE! -- 4.32 secs
Extracting 4-mers from chr3............DONE! -- 3.63 secs
Extracting 4-mers from chr4............DONE! -- 3.22 secs
Extracting 4-mers from chr5............DONE! -- 3.28 secs
Extracting 4-mers from chr6............DONE! -- 3.22 secs
Extracting 4-mers from chr7............DONE! -- 2.94 secs
Extracting 4-mers from chr8............DONE! -- 2.66 secs
Extracting 4-mers from chr9............DONE! -- 2.45 secs
Extracting 4-mers from chr10...........DONE! -- 2.68 secs
Extracting 4-mers from chr11...........DONE! -- 2.7 secs
Extracting 4-mers from chr12...........DONE! -- 2.69 secs
Extracting 4-mers from chr13...........DONE! -- 1.74 secs
Extracting 4-mers from chr14...........DONE! -- 1.84 secs
Extracting 4-mers from chr15...........DONE! -- 1.65 secs
Extracting 4-mers from chr16...........DONE! -- 1.65 secs
Extracting 4-mers from chr17...........DONE! -- 1.83 secs
Extracting 4-mers from chr18...........DONE! -- 1.23 secs
Extracting 4-mers from chr19...........DONE! -- 1.27 secs
Extracting 4-mers from chr20...........DONE! -- 1.28 secs
Extracting 4-mers from chr21...........DONE! -- 0.79 secs
```

```
Extracting 4-mers from chr22.............DONE! -- 0.9 secs

Total time taken: 53.02 secs
------------------------------------------------------------
            Calculation of K-mer Susceptibility
------------------------------------------------------------
The 4-mer scores are saved at {temp_dir}/score_4-mer.csv

FINISH! Total time taken: 2.35 mins
```

References

Edgar, Ron, Michael Domrachev, and Alex E Lash. 2002. "Gene Expression Omnibus: NCBI Gene Expression and Hybridization Array Data Repository." *Nucleic Acids Research*, January. https://doi.org/10.1093/nar/30.1.207.

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The ENCODE Project Consortium. 2012. "An Integrated Encyclopedia of DNA Elements in the Human Genome." *Nature*, September. https://doi.org/10.1038/nature11247.