RIPSeeker: a statistical package for identifying protein-associated transcripts from RIP-seq experiments

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1 Introduction

Ribonucleoprotein (RNP) immunoprecipitation (IP) followed by high-throughput sequencing (RIP-seq) has recently been developed to discover genome-wide RNA transcripts that interact with a protein or protein complex. RIP-seq is similar to both RNA-seq and ChIP-seq, but presents unique properties and challenges. Currently, no statistical tool is dedicated to RIP-seq analysis. We developed *RIPSeeker*, an HMM-based R package for *de novo* RIP peak predictions. *RIPSeeker* infers and discriminate RIP peaks from background or control (if available) using two-state HMM with negative binomial emission probability followed by statistical tests for model confidence. To evaluate the performance of *RIPSeeker*, we used the published RIP-seq data for PRC2 and also generated in-house data for CCNT1. Under an equal footing, *RIPSeeker* compares competitively with ChIP-seq and transcript-based methods in predicting canonical protein-associated transcripts with high statistical confidence. Importantly, *RIPSeeker* has the ability to model reads on plus and minus strand separately, which allows identification of strand-specific noncoding RNA (e.g., antisense transcripts). Comparing to the published methods, *RIPSeeker* is adaptive to a larger dynamical range of peaks suitable for detecting the entire transcripts with various lengths rather than the punctuated binding sites.

While *RIPSeeker* is specifically tailored for RIP-seq data analysis, it also provides a suite of bioinformatics tools integrated within this self-contained software package comprehensively addressing issues ranging from post-alignments processing to visualization and annotation. In addition, a rule-based approach is provided as an additional function named `rulebaseRIPSeek` for user to obtain RPKM/FPKM (and fold-change) for the gene/transcripts expressions in RIP (and control) based on automatically retrieved online Ensembl/UCSC annotation given single or paired-end alignments. This vignette provides a guide to the most common application of *RIPSeeker* package.

2 *RIPSeeker* overview

Figure 3 in the manuscript (in press) for peer-review presents the workflow of *RIPSeeker*. The input for RIPSeeker is a list of read alignments in BAM/BED/SAM format. Map-

ping reads to the reference genome can be performed by any RNA-seq aligner such as TopHat **?**. After post-processing the alignment input by `combineAlignGals`, RIPSeeker first stratifies the genome into bins of automatically selected (`selectBinSize`) or a fixed user-defined size. Each bin may contain more than one aligned read. Multiple bins may together correspond to a single RNA transcript that binds to the protein of interest. Thus, these bins when treated as individual observations are not independent identically distributed (*i.i.d.*) and need to be treated as dependent events. Hidden Markov model (HMM) provides a sensible and efficient way to probabilistically model the dependence between sequential events through hidden variables (**??**). The adaptation of HMM is inspired by HPeak, which was specifically designed for ChIP-seq (**?**).

As an overview, RIPSeeker consists of two major steps: probabilistic inference of RIP regions (`mainSeek`) and significance test for the inferred RIP regions from HMM (`seekRIP`). In the first step, we apply a two-state HMM to model the background and RIP distributions (or emission probabilities) of RIP-seq read counts as negative binomial distributions, which has been shown by **?** to be a more realistic parametric model than Gaussian and Poisson models. The parameters of HMM are learned from the data by expectation-maximization (EM). The intermediate quantities required in the EM iterations are efficiently computed using forward-backward algorithm. After the optimization, Viterbi algorithm is applied to derive the most probable sequence of hidden states, which encodes whether each region is background (1) or RIP (2) across the genome. The consecutive RIP bins are merged into a single RIP region. In the second step, we compute the statistical significance of each RIP region with or without a control library based on the posterior probabilities derived directly from the HMM.

RIPSeeker is able to detect strand-specific RIP regions by running the same workflow on either plus and minus strand separately, making use of the strand-specific information retained in the original RIP-seq protocol (**??**). In addition, RIPSeeker takes advantage of modern computational architecture equipped with multiple processors by treating each chromosome as an independent thread and computing multiple threads in parallel using `mclapply` from *multicore* R package. Thus, the most time consuming step such as HMM inference operates on per-chromosome basis each running on a separate CPU core. The parallel computing is much more computationally and memory efficient than computing the entire genome all at once by treating it as a single concatenated sequence. *RIPSeeker* has numerous other features including disambiguating multihits (i.e., reads mapped to multiple loci with `disambiguateMultihits`), automatic annotation of RIP regions (`annotateRIP`), GO enrichment analysis (`annotateRIP`), and UCSC visualization (`viewRIP`).

Although the ultimate goal is to predict RIP regions, each step or component in the workflow has been implemented as a stand-alone function. Please referred to MATERIALS AND METHODS in the manuscript (may not be available during peer-review) for the underlying algorithms and the help manual for their usage:

```
> library(RIPSeeker)

> ?RIPSeeker
```

3 RIP-seq data

3.1 PRC2 in mouse embrynoic stem cell

Only a few RIP-seq datasets are available. In this tutorial, we will make use of part of the dataset from **?** (GEO accession: GSE17064). The data was generated to identify transcripts phycially associated with Ezh2 (a PRC2 unique subunit) in mouse embryonic stem cell (mESC). A negative control was also generated from mutant mESC depleted of Ezh2 (Ezh2 -/-) (MT). Here we will use the RIP data consisting of two techincal replicates (code ID: SRR039210-1, SRR039212) and the MT control (code ID: SRR039214). Notably, the library construction and *strand-specific* sequencing generated sequences from the opposite strand of the PRC2-bound RNA (**?**), consequently, each read will be treated as if it were reverse complemented. After the quality control (QC) and alignments (**Quality Control of Raw Read Library** and **Alignment of Filtered RIP-seq Read Library to Reference Genome** in Supplementary Datafor the manuscript), the technical replicates were merged, resulting in 1,022,474 and 208,445 reads mapped to unique loci of the mouse reference genome (mm9 build) for RIP and control, respectively. To make the demonstration and the package size more managable, only the alignments to chromosome X (chrX) were extracted to generate the test data stored in the package. The full data (containing all of the processed alignments for the test data and another biological replicate SRR039213) are available as a Bioconductor data package *RIPSeekerData*. User can try the same command below on the full dataset.

```
> biocLite("RIPSeekerData")
> library(RIPSeekerData)
> extdata.dir <- system.file("extdata", package="RIPSeekerData")
> bamFiles <- list.files(extdata.dir, "\\.bam$",
+                        recursive=TRUE, full.names=TRUE)
> bamFiles <- grep("PRC2/", bamFiles, value=TRUE)
```

3.2 CCNT1 in HEK293

The data for CCNT1 and GFP control were generated in-house in two experiments. The pilot experimental data contain 5,853 and 4,556 uniquely mapped read after the stringent QC for the CCNT1 and GFP control, respectively. Same as in the PRC2 data, the reads came from the second strand of the cDNA synthesis opposite to the original RNA strand. The non-strand-specific library from the second screen has deeper coverage with 26,859 and 45,024 uniquely aligned reads under QC for CCNT1 and GFP, respectively. CCNT1 is known to only associate with *RN7SK*, which can be used as an empirical measurement of senstivitiy and specificity of *RIPSeeker*. The data are in *RIPSeekerData* package.

3.3 User-supplied data

For the following example, user may replace the `extdata.dir` with the directory containing their own alignment data and change the `cNAME` to point to the specific control (if applicable).

```
> # Retrieve system files
> # OR change it to the extdata.dir from the code chunk above
```

```
> # to get RIP predictions on the full alignment data
> extdata.dir <- system.file("extdata", package="RIPSeeker")
> bamFiles <- list.files(extdata.dir, "\\.bam$",
+                        recursive=TRUE, full.names=TRUE)
> bamFiles <- grep("PRC2", bamFiles, value=TRUE)
> # RIP alignment for Ezh2 in mESC
> ripGal <- combineAlignGals(grep(pattern="SRR039214", bamFiles, value=TRUE, in
+                            reverseComplement=TRUE, genomeBuild="mm9")
> # Control RIP alignments for mutant Ezh2 -/- mESC
> ctlGal <- combineAlignGals(grep(pattern="SRR039214", bamFiles, value=TRUE, in
+                            reverseComplement=TRUE, genomeBuild="mm9")
> ripGal

GAlignments object with 31054 alignments and 1 metadata column:
                    seqnames strand       cigar    qwidth     start       end
                       <Rle>  <Rle> <character> <integer> <integer> <integer>
  SRR039210.4322179     chrX      +         36M        36   3000326   3000361
  SRR039210.5524106     chrX      -         36M        36   3001293   3001328
  SRR039210.5069294     chrX      -         36M        36   3002790   3002825
  SRR039210.1476279     chrX      -         36M        36   3016328   3016363
   SRR039210.711491     chrX      +         36M        36   3021161   3021196
                ...      ...    ...         ...       ...       ...       ...
  SRR039211.1427139     chrX      -         36M        36 166443604 166443639
   SRR039211.447965     chrX      -         36M        36 166445241 166445276
  SRR039211.1352670     chrX      -         36M        36 166446255 166446290
   SRR039211.918096     chrX      -         36M        36 166446315 166446350
    SRR039211.67451     chrX      -         36M        36 166446621 166446656
                        width     njunc | uniqueHit
                    <integer> <integer> | <logical>
  SRR039210.4322179        36         0 |     FALSE
  SRR039210.5524106        36         0 |     FALSE
  SRR039210.5069294        36         0 |     FALSE
  SRR039210.1476279        36         0 |      TRUE
   SRR039210.711491        36         0 |     FALSE
                ...       ...       ... .       ...
  SRR039211.1427139        36         0 |     FALSE
   SRR039211.447965        36         0 |     FALSE
  SRR039211.1352670        36         0 |     FALSE
   SRR039211.918096        36         0 |     FALSE
    SRR039211.67451        36         0 |      TRUE
  -------
  seqinfo: 22 sequences from mm9 genome

> ctlGal

GAlignments object with 8276 alignments and 1 metadata column:
                    seqnames strand       cigar    qwidth     start       end
                       <Rle>  <Rle> <character> <integer> <integer> <integer>
  SRR039214.4949641     chrX      +         36M        36   3028539   3028574
  SRR039214.5310910     chrX      -         35M        35   3039791   3039825
  SRR039214.4625677     chrX      -         36M        36   3084567   3084602
```

```
SRR039214.1854227     chrX      +         36M        36   3139109   3139144
SRR039214.5753635     chrX      +         36M        36   3139131   3139166
              ...      ...    ...         ...       ...       ...       ...
 SRR039214.904524     chrX      -         32M        32 166445788 166445819
SRR039214.2473565     chrX      -         21M        21 166445960 166445980
 SRR039214.576581     chrX      -         36M        36 166446074 166446109
SRR039214.3756853     chrX      -         36M        36 166446781 166446816
SRR039214.2347055     chrX      +         36M        36 166650104 166650139
                      width     njunc | uniqueHit
                  <integer> <integer> | <logical>
SRR039214.4949641        36         0 |     FALSE
SRR039214.5310910        35         0 |     FALSE
SRR039214.4625677        36         0 |     FALSE
SRR039214.1854227        36         0 |     FALSE
SRR039214.5753635        36         0 |     FALSE
              ...       ...       ... .       ...
 SRR039214.904524        32         0 |     FALSE
SRR039214.2473565        21         0 |     FALSE
 SRR039214.576581        36         0 |     FALSE
SRR039214.3756853        36         0 |     FALSE
SRR039214.2347055        36         0 |      TRUE
-------
seqinfo: 22 sequences from mm9 genome
```

Note from the commands above that the RIP alignment files corresponding to the technical replicates are combined into a single *GAlignments* object(**?**). The `uniqueHit` column from the output above indicate whether the read mapped to a single locus or multiple loci. The latter is commonly referred as the "multihits". The ambiguous alignments arise from the repetitive elements or gene duplication events in the mammalian genomes. By default, the unique and multihits are flagged with binary `TRUE` and `FALSE` value in column `uniqueHit`, respectively. In the later step, each multihit will be assigned by the function `disambiguateMultihits` to a unique locus based on the posterior probabilities of the loci of being at the background or read enriched states of the two-state HMM (**Disambiguate multihits using posterior decoding from HMM** in the manuscript).

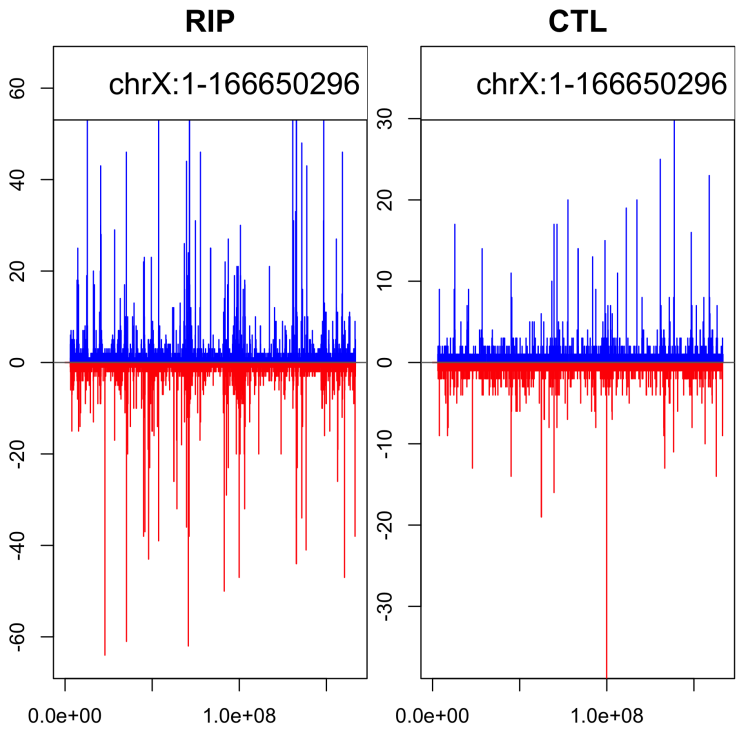
4 RIP-seq analysis

4.1 High-level visuzliation of alignments at chromosomal level

After processing the alignment files, we could first visualize the alignments by calling `plotStrandedCoverage` to get a rough idea about the alignment quality and where the reads aggregate within each chromosome. `plotStrandedCoverage` first bins each chromosome by nonoverlapping windows of fixed size (1 kb in the example below) and plots the number of alignments fall within these bins across the whole chromosome. The function is implemented based on the vignette *GenomicRanges Use Cases* from the *GenomicRanges* package (**?**).

```
> ripGR <- as(ripGal, "GRanges")
> ripGRList <- split(ripGR, seqnames(ripGR))
```

```
> # get only the nonempty chromosome
> ripGRList <- ripGRList[elementNROWS(ripGRList) != 0]
> ctlGR <- as(ctlGal, "GRanges")
> ctlGRList <- GRangesList(as.list(split(ctlGR, seqnames(ctlGR))))
> ctlGRList <- ctlGRList[lapply(ctlGRList, length) != 0]
> binSize <- 1000
> #c(bottom, left, top, right)
> par(mfrow=c(1, 2), mar=c(2, 2, 2, 0) + 0.1)
> tmp <- lapply(ripGRList, plotStrandedCoverage, binSize=binSize,
+               xlab="", ylab="", plotLegend=TRUE,
+               legend.cex=1.5, main="RIP", cex.main=1.5)
> tmp <- lapply(ctlGRList, plotStrandedCoverage, binSize=binSize,
+               xlab="", ylab="", plotLegend=TRUE,
+               legend.cex=1.5, main="CTL", cex.main=1.5)
```



Here we first converted the *GAlignments* object `ripGal` and `ctlGal` to *GenomicRangesList* to have each list item represented by a chromosome. We then `lapply` the function to all nonempty chromosomes, which in our case is *chrX* for both RIP (left) and control (right). RIP represents the pooled alignments of the two technical replicates. Read count on + and - strand are displayed as blue and red bars on the positive and negative y-axis, respectively. Read counts are drawn to scale within a chromosome. Unlike ChIP-seq on double-stranded DNA, no symmetry is observed for the peak for the strand-specific sequencing data. However, considerable noise is observed within the Ezh2 -/- mutant library (right panel), which ideally should not have any aligned reads. This may also imply considerable noise within the wild type library and thus a high false discovery rate if the RIP regions were simply determined based on read counts. Please refer to Figure S3 and S4 in Supplementary Datafor similar RIP-seq

results on all chromosomes, which motivated the development of *RIPSeeker*.

4.2 `ripSeek`: the all-in-one function

The front-end main function `ripSeek` is in many cases the only function that users need to run to get the RIP predictions and most of the relevant information out of the alignment data. It requires a few important parameter settings:

```
> # specify control name
> cNAME <- "SRR039214"
> # output file directory
> outDir <- file.path(getwd(), "RIPSeeker_vigenette_example_PRC2")
> # Parameters setting
> binSize <- NULL      # set to NULL to automatically determine bin size
> minBinSize <- 10000  # min bin size in automatic bin size selection
> maxBinSize <- 10100         # max bin size in automatic bin size selection
> multicore <- TRUE                 # use multicore
> strandType <- "-"                 # set strand type to minus strand
> biomart <- "ENSEMBL_MART_ENSEMBL"           # use archive to get ensembl 65
> biomaRt_dataset <- "mmusculus_gene_ensembl" # mouse dataset id name
> host <- "dec2011.archive.ensembl.org"       # use ensembl 65 for annotation f
> goAnno <- "org.Mm.eg.db"                    # GO annotation database
> ################ run main function ripSeek to predict RIP ###############
> seekOut.PRC2 <- ripSeek(bamPath = bamFiles, cNAME = cNAME,
+                     reverseComplement = TRUE, genomeBuild = "mm9",
+                     strandType = strandType,
+                     uniqueHit = TRUE, assignMultihits = TRUE,
+                     rerunWithDisambiguatedMultihits = TRUE,
+                     binSize=binSize, minBinSize = minBinSize,
+                     maxBinSize = maxBinSize,
+                     biomart=biomart, host=host,
+                     biomaRt_dataset = biomaRt_dataset, goAnno = goAnno,
+                     multicore=multicore, outDir=outDir)
```

- `bamPath` saves the paths to the bam files;
- `cNAME` is a character string that distinguishes the control from the RIP among the `bamFiles`;
- `reverseComplement=TRUE` indicates that the reads were sequenced from the opposite strand of the original RNA molecule such that the original strand signs of the alignments are switched (i.e. + to -, - to +);
- `genomeBuild="mm9"` specifiies the fact that the previous mouse genome (mm9/NCBIM37) build was used as reference for the alignments;
- `strandType` is set to "-" to search for transcripts only on the minus strand of chromosomes such as Xist, which is known to physically interact with PRC2 **?**.
- `uniqueHit = TRUE` requires training HMM with only the unique hits.

- `assignMultihits = TRUE` enables `disambiguateMultihits` to assign each multihit to a unique locus based on the posterior probabilitites derived from HMM.
- `rerunWithDisambiguatedMultihits = TRUE` tells RIPSeeker to retrain the HMM using the dataset with disambiguated multihits.
- `binSize = NULL` enables automatic bin size selection by the routine `selectBinSize`.
- `minBinSize=10000, maxBinSize=12000` For demonstration puprose, we set the lower and upper bounds of the search space for the optimal bin size to 10 kb and 12 kb, respectively. Such choice may suffice if one just wants to quickly look for some known long noncoding RNA (lncRNA) such as Xist (22,843 nt) or Tsix (53,441 nt).
- `biomart, host, biomaRt_dataset, goAnno` are set to enable automatic online annotation of RIP predictions via `annotateRIP`, which makes use of the functions from *ChIPpeakAnno* (**?**) and *biomaRt* (**??**). Since the genome we used for the alignments is mm9 not the most recent build mm10, we need to retreive the archive annotation from Ensembl by setting the `host` and `biomart`. If the user uses the most recent assembly as reference genome for the alignments, then `biomart` should be set to `"ensembl"` and `host` may be omitted.
- `multicore = TRUE` enables parallel computing on each chromosome separately. It will greatly improve the computation time when used on a cluster.
- `outDir` the output directory to save the results (See **Value** section in `?ripSeek`)

4.3 `ripSeek` outputs

The main function `ripSeek` returns a *list* which comprises four items outlined below. The user may find `annotatedRIPGR` the most useful. In most cases, the other three items can be considered as intermediate results.

```
> names(seekOut.PRC2)
> df <- elementMetadata(seekOut.PRC2$annotatedRIPGR$sigGRangesAnnotated)
> # order by eFDR
> df <- df[order(df$eFDR), ]
> # get top 100 predictions
> df.top100 <- head(df, 100)
> head(df.top100)
> # examine known PRC2-associated lncRNA transcripts
> df.top100[grep("Xist", df$external_gene_id), ]
```

- `mainSeekOutputRIP`A (inner) list containing three items:
  - `nbhGRList` A *GRangesList* object of the HMM trained parameters for each chromosome on RIP;
  - `alignGal, alignGalFiltered` *GAlignments* objects of the RIP alignment outputs from `combineAlignGals` and `disambiguateMultihits`, respectively. The former may contain multiple alignments due the same reads whereas the latter contains a one-to-one mapping from read to alignment after disambiguating the multihits;

- `mainSeekOutputCTL` Same as `mainSeekOutputRIP` but for the control library;
- `RIPGRList` The peaks in *GRangesList*. Each list item represents the RIP peaks on a chromosome accompanied with statistical scores including (read) count, logOddScore, pval, pvalAdj, eFDR for the RIP and control (if available).
- `annotatedRIPGR` A *list* containing two items:
  - `sigGRangesAnnotated`*GRanges* containing peaks and their scores (same as in `RIPGRList`) accompanied with genomic information retrieved from Ensembl database;
  - `enrichedGO`A *data.frame* containing enriched GO terms associated with the RIP peaks.

In addition, the (annotated) peaks and enriched GO annotations are saved in `outDir` as text files in tab-delimted (viewable with Excel) or GFF3 (General Feature Format 3) formats (`?rtracklayer:io` for details for the formats.).

```
> list.files(outDir)
```

File (1) and (4) can be imported to a dedicated genome browser such as Integrative Genomic Viewer (IGV) **?** to visualize and interact with the putative RIP regions accompanied with all of the scores. Files (2) and (3) provide the most detailed information directly viewable in Excel. File (5) stores all of the intermediate results as `RData` for future reference or retrieval (with `load` command).

4.4 Visualization with UCSC browser

In addition, we could visualize the results using `viewRIP`, which launches online UCSC genome browser with uploaded custom track corresponding to the loci of RIP regions and scores (RIPScore, -log10(p-value), -log10(q-value), or -log10(eFDR)) generated from `ripSeek`. This task is accomplished seamlessly within the R console by making use of the available functions from *rtracklayer* **?**

```
> viewRIP(seekOut.PRC2$RIPGRList$chrX,
+         seekOut.PRC2$mainSeekOutputRIP$alignGalFiltered,
+         seekOut.PRC2$mainSeekOutputCTL$alignGalFiltered, scoreType="eFDR")
```

4.5 Compute RPKM and fold-change of RIP over control for known genes or transcripts

User may want to know the abundance of all of the known coding or noncoding genes or transcripts in their RIP libraries and how their expressions compare with the control. Given a list of single-end or paired-end read alignment files in BAM/SAM/BED format, the function `computeRPKM` computes the read counts and normalized read counts as expression of annotated transcripts in the unit of "reads per kilobase of exon per million mapped reads" (RPKM) or "fragments per kilobase of exon per million mapped reads" (FPKM), respectively.[1] Furthermore, `rulebaseRIPSeek` computes

[1]The "fragments" refers to the long fragment sequneced on both ends by the short read pairs.

the fold-change difference of gene expression in terms of RPKM/FPKM between the RIP and control libraries. `rulebaseRIPSeek` reports putative RIP genes/transcripts if their RPKM expression and the ratio of RPKM[RIP]/RPKM[control] (on either + or - strand) are above t_1 and t_2. By default, $t_1 = 0.4$ and $t_2 = 3.0$, consistent with the thresholds applied in original method **?**.

```
> # Retrieve system files
> extdata.dir <- system.file("extdata", package="RIPSeeker")
> bamFiles <- list.files(extdata.dir, ".bam$", recursive=TRUE, full.names=TRUE)
> bamFiles <- grep("PRC2", bamFiles, value=TRUE)
> # use biomart
> txDbName <- "biomart"
> biomart <- "ENSEMBL_MART_ENSEMBL"          # use archive to get ensembl 65
> dataset <- "mmusculus_gene_ensembl"
> host <- "dec2011.archive.ensembl.org"         # use ensembl 65 for annotation
> # compute transcript abundance in RIP
> ripRPKM <- computeRPKM(bamFiles=grep(pattern="SRR039214", bamFiles, value=TRU
+                        dataset=dataset, moreGeneInfo=TRUE, justRPKM=FALSE,
+                        idType="ensembl_transcript_id", txDbName=txDbName,
+                        biomart=biomart, host=host, by="tx")
> # compute transcript abundance in RIP and control as well as
> # foldchnage in RIP over control
> rulebase.results <- rulebaseRIPSeek(bamFiles=bamFiles, cNAME=cNAME, myMin=1,
+                                     featureGRanges=ripRPKM$featureGRanges,
+                                     biomart=biomart, host=host, dataset=datas
> head(ripRPKM$rpkmDF)
> df <- rulebase.results$rpkmDF
> df <- df[order(df$foldchange, decreasing=TRUE), ]
> # top 10 transcripts
> head(df, 10)
```

5 RIP-seq analysis on CCNT1 data

As promised earlier, we will apply `ripSeek` to the CCNT1 data and annotate the *de novo* putative RIP regions with the latest online Ensembl annotation based on human genome reference NCBI37/hg19.

```
> # Retrieve system files
> biocLite("RIPSeekerData")
> extdata.dir <- system.file("extdata", package="RIPSeekerData")
> bamFiles <- list.files(extdata.dir, "\\.bam$",
+                        recursive=TRUE, full.names=TRUE)
> bamFiles <- grep("CCNT1/firstscreen", bamFiles, value=TRUE)
> # specify control name
> cNAME <- "GFP"
> # output file directory
> outDir <- file.path(getwd(), "RIPSeeker_vigenette_example_CCNT1")
> # Parameters setting
> binSize <- 10000     # automatically determine bin size
> minBinSize <- NULL         # min bin size in automatic bin size selection
```

```
> maxBinSize <- NULL         # max bin size in automatic bin size selection
> multicore <- TRUE                 # use multicore
> strandType <- "+"                 # set strand type to minus strand
> biomart <- "ensembl"           # use archive to get ensembl 65
> biomaRt_dataset <- "hsapiens_gene_ensembl" # human dataset id name
> goAnno <- "org.Hs.eg.db"                    # GO annotation database
> ################ run main function ripSeek to predict RIP ###############
> seekOut.CCNT1 <- ripSeek(bamPath = bamFiles, cNAME = cNAME,
+                     reverseComplement = TRUE, genomeBuild = "hg19",
+                     strandType = strandType,
+                     uniqueHit = TRUE, assignMultihits = TRUE,
+                     rerunWithDisambiguatedMultihits = TRUE,
+                     binSize=binSize, minBinSize = minBinSize,
+                     maxBinSize = maxBinSize,
+                     biomart=biomart, goAnno = goAnno,
+                     biomaRt_dataset = biomaRt_dataset,
+                     multicore=multicore, outDir=outDir)
> df <- elementMetadata(seekOut.CCNT1$annotatedRIPGR$sigGRangesAnnotated)
> # order by eFDR
> df <- df[order(df$eFDR), ]
> # get top 100 predictions
> df.top20 <- head(df, 20)
> # examine known PRC2-associated lncRNA transcripts
> df.top20[grep("RN7SK", df$external_gene_id)[1], ]
> list.files(outDir)

> viewRIP(seekOut.CCNT1$RIPGRList$chr6, seekOut.CCNT1$mainSeekOutputRIP$alignGa

> # use biomart
> txDbName <- "biomart"
> biomart <- "ensembl"
> dataset <- "hsapiens_gene_ensembl"
> # compute transcript abundance in RIP
> ripRPKM <- computeRPKM(bamFiles=bamFiles[1],
+                        dataset=dataset, moreGeneInfo=TRUE, justRPKM=FALSE,
+                        idType="ensembl_transcript_id", txDbName=txDbName,
+                        biomart=biomart, by="tx")
> # compute transcript abundance in RIP and control as well as
> # foldchnage in RIP over control
> rulebase.results <- rulebaseRIPSeek(bamFiles=bamFiles, cNAME=cNAME, myMin=1,
+                                     featureGRanges=ripRPKM$featureGRanges,
+                                     biomart=biomart, dataset=dataset)
> head(ripRPKM$rpkmDF)
> df <- rulebase.results$rpkmDF
> df <- df[order(df$foldchange, decreasing=TRUE), ]
> # top 10 transcripts
> head(df, 10)
```

6 Session Info

```
> sessionInfo()

R version 3.3.0 RC (2016-04-26 r70550)
Platform: x86_64-apple-darwin13.4.0 (64-bit)
Running under: OS X 10.9.5 (Mavericks)

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

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

other attached packages:
 [1] RIPSeeker_1.12.0           rtracklayer_1.32.0
 [3] GenomicAlignments_1.8.0    Rsamtools_1.24.0
 [5] Biostrings_2.40.0          XVector_0.12.0
 [7] SummarizedExperiment_1.2.0 Biobase_2.32.0
 [9] GenomicRanges_1.24.0       GenomeInfoDb_1.8.0
[11] IRanges_2.6.0              S4Vectors_0.10.0
[13] BiocGenerics_0.18.0

loaded via a namespace (and not attached):
[1] XML_3.98-1.4       bitops_1.0-6       zlibbioc_1.18.0    BiocParallel_1.6.0
[5] tools_3.3.0        RCurl_1.95-4.8
```