

# RIP-seq datasets for testing RIPSeeker package

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## 1 PRC2 Datasets

The RIP-seq data from Zhao et al. [2010] for Ezh2 (a PRC2 unique subunit) in mouse embryonic stem cell (mESC) were downloaded from Gene Expression Omnibus (GEO) (GSE17064). Briefly, there are in total five datasets. Two datasets correspond to the non-specific and specific negative controls using the antibody IgG and mutant mESC depleted of Ezh2 (Ezh2  $-/-$ ) (MT), respectively. Only the specific negative control is used in our test. The two and one remaining datasets correspond to the libraries constructed from two biological replicates of the wild type mESC. Notably, the library construction and *strand-specific* sequencing generated sequences from the opposite strand of the PRC2-bound RNA Zhao et al. [2010], consequently, each read was treated as if it were reverse complemented. After the quality control (QC) and alignments (?? and ?? in Supplementary Data), the technical replicates were merged, resulting in three test files - RIP-biorep1, RIP-biorep2, and CTL with 1,022,474, 442,030, and 208,445 reads mapped to unique loci of the mouse reference genome (mm9 build) (Table ??).

```
> library(RIPSeeker)
> extdata.dir <- system.file("extdata", package="RIPSeekerData")
> bamFiles <- list.files(extdata.dir, "\\*.bam$",
+                         recursive=TRUE, full.names=TRUE)
> bamFiles.PRC2 <- grep("PRC2/", bamFiles, value=TRUE)
> # import, process, and convert BAM data to GappedAlignments object
> # using function combineAlignGals
>
> # PRC2
> PRC2.rip <- grep(pattern="SRR039214", bamFiles.PRC2, value=TRUE, invert=TRUE)
> PRC2.rip.biorep1 <- PRC2.rip[grep(pattern="SRR039213", PRC2.rip, invert=TRUE)]
> PRC2.rip.biorep2 <- PRC2.rip[grep(pattern="SRR039213", PRC2.rip, invert=FALSE)]
> PRC2.ctl <- grep(pattern="SRR039214", bamFiles, value=TRUE, invert=FALSE)
> ripGal.PRC2.rip.biorep1 <- combineAlignGals(PRC2.rip.biorep1,
+                                             reverseComplement=TRUE, genomeBuild="mm9")
> ripGal.PRC2.rip.biorep2 <- combineAlignGals(PRC2.rip.biorep2,
+                                             reverseComplement=TRUE, genomeBuild="mm9")
> ripGal.PRC2.ctl <- combineAlignGals(PRC2.ctl,
+                                    reverseComplement=TRUE, genomeBuild="mm9")
> ripGal.PRC2.rip.biorep1
```

GAlignments object with 1022474 alignments and 1 metadata column:

	seqnames	strand	cigar	qwidth	start	end
	<Rle>	<Rle>	<character>	<integer>	<integer>	<integer>
SRR039210.2697764	chr1	+	36M	36	3038896	3038931
SRR039210.4759331	chr1	-	36M	36	3043067	3043102
SRR039210.5363123	chr1	+	36M	36	3043067	3043102
SRR039210.4785683	chr1	+	36M	36	3044642	3044677
SRR039210.5440116	chr1	+	36M	36	3044658	3044693
...	...	...	...	...	...	...
SRR039212.2286434	chrY	+	36M	36	2851672	2851707
SRR039212.5775845	chrY	+	20M	20	2854110	2854129
SRR039212.2698603	chrY	+	36M	36	2865319	2865354
SRR039212.1732007	chrY	+	36M	36	2870093	2870128
SRR039212.6081906	chrY	+	36M	36	2888278	2888313
	width	njunc		uniqueHit		
	<integer>	<integer>		<logical>		
SRR039210.2697764	36	0		TRUE		
SRR039210.4759331	36	0		TRUE		
SRR039210.5363123	36	0		FALSE		
SRR039210.4785683	36	0		TRUE		
SRR039210.5440116	36	0		TRUE		
...	...	...	...	...		
SRR039212.2286434	36	0		TRUE		
SRR039212.5775845	20	0		TRUE		
SRR039212.2698603	36	0		FALSE		
SRR039212.1732007	36	0		FALSE		
SRR039212.6081906	36	0		TRUE		

-----  
seqinfo: 22 sequences from mm9 genome

> ripGal.PRC2.rip.biorep2

GAlignments object with 442030 alignments and 1 metadata column:

	seqnames	strand	cigar	qwidth	start	end
	<Rle>	<Rle>	<character>	<integer>	<integer>	<integer>
SRR039213.2654515	chr1	-	36M	36	3044590	3044625
SRR039213.1340316	chr1	+	36M	36	3101886	3101921
SRR039213.5984066	chr1	+	36M	36	3165185	3165220
SRR039213.1775423	chr1	+	36M	36	3204806	3204841
SRR039213.1617846	chr1	+	36M	36	3226837	3226872
...	...	...	...	...	...	...
SRR039213.4441161	chrY	+	36M	36	2623680	2623715
SRR039213.4469893	chrY	+	36M	36	2681865	2681900
SRR039213.1027267	chrY	-	36M	36	2787416	2787451
SRR039213.5937961	chrY	+	20M	20	2854110	2854129
SRR039213.5666673	chrY	+	36M	36	2860460	2860495
	width	njunc		uniqueHit		
	<integer>	<integer>		<logical>		
SRR039213.2654515	36	0		FALSE		
SRR039213.1340316	36	0		FALSE		

```

SRR039213.5984066      36      0      |      TRUE
SRR039213.1775423      36      0      |      TRUE
SRR039213.1617846      36      0      |      TRUE
...
SRR039213.4441161      36      0      |      FALSE
SRR039213.4469893      36      0      |      FALSE
SRR039213.1027267      36      0      |      FALSE
SRR039213.5937961      20      0      |      TRUE
SRR039213.5666673      36      0      |      FALSE
-----
seqinfo: 22 sequences from mm9 genome

> ripGal.PRC2.ctf

GAlignments object with 208445 alignments and 1 metadata column:
      seqnames strand      cigar      qwidth      start      end
      <Rle>   <Rle> <character> <integer> <integer> <integer>
SRR039214.3256146    chr1      +      20M        20    3062094    3062113
SRR039214.4450026    chr1      -      20M        20    3095085    3095104
SRR039214.4200528    chr1      -      20M        20    3095086    3095105
SRR039214.4467447    chr1      -      36M        36    3161652    3161687
SRR039214.3463161    chr1      -      36M        36    3180311    3180346
...
SRR039214.5888680    chrY      -      22M        22    2606354    2606375
SRR039214.2883579    chrY      +      20M        20    2611734    2611753
SRR039214.2387301    chrY      -      20M        20    2648262    2648281
SRR039214.435163     chrY      +      33M        33    2779415    2779447
SRR039214.2969488    chrY      +      20M        20    2854110    2854129
      width      njunc      | uniqueHit
      <integer> <integer> | <logical>
SRR039214.3256146    20      0      | FALSE
SRR039214.4450026    20      0      | FALSE
SRR039214.4200528    20      0      | FALSE
SRR039214.4467447    36      0      | TRUE
SRR039214.3463161    36      0      | FALSE
...
SRR039214.5888680    22      0      | FALSE
SRR039214.2883579    20      0      | FALSE
SRR039214.2387301    20      0      | FALSE
SRR039214.435163     33      0      | FALSE
SRR039214.2969488    20      0      | FALSE
-----
seqinfo: 22 sequences from mm9 genome

```

## 2 CCNT1 Datasets

The data for CCNT1 were generated from two RIP-seq experiments. The pilot experiment generated 775,582 and 773,785 strand-specific raw reads, and 5,853 and 4,556 uniquely mapped read remain after the stringent QC for the CCNT1 and GFP control RIP RNA libraries, 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 1,647,641 and 2,369,271 raw reads, and 26,859 and 45,024 uniquely aligned reads under QC for CCNT1 and GFP, respectively (Table ??). Since the two experiments were performed with slightly different protocols, we treated them as two separate biological replicates for the following analyses.

```
> library(RIPSeeker)
> extdata.dir <- system.file("extdata", package="RIPSeekerData")
> bamFiles <- list.files(extdata.dir, "\\*.bam$",
+                         recursive=TRUE, full.names=TRUE)
> bamFiles.CCNT1 <- grep("CCNT1/", bamFiles, value=TRUE)
> # import, process, and convert BAM data to GappedAlignments object
> # using function combineAlignGals
>
> CCNT1.rip <- grep(pattern="humanCCNT1", bamFiles.CCNT1, value=TRUE, invert=TRUE)
> CCNT1.ctl <- grep(pattern="humanGFP", bamFiles.CCNT1, value=TRUE, invert=TRUE)
> ripGal.CCNT1.rip <- combineAlignGals(CCNT1.rip,
+                                     reverseComplement=TRUE, genomeBuild="hg19")
> ripGal.CCNT1.ctl <- combineAlignGals(CCNT1.ctl,
+                                     reverseComplement=TRUE, genomeBuild="hg19")
> ripGal.CCNT1.rip
```

GAlignments object with 10409 alignments and 1 metadata column:

	seqnames	strand	cigar	qwidth	start
	<Rle>	<Rle>	<character>	<integer>	<integer>
5:2106:4142:3430:Y	chr1	+	21M	21	918006
5:2108:3248:41912:Y	chr1	-	22M	22	1101224
5:1103:12850:21621:Y	chr1	+	20M	20	1186368
5:1203:17240:152389:Y	chr1	+	21M	21	1186368
5:2202:17340:164011:Y	chr1	+	21M	21	1201404
...	...	...	...	...	...
5:2204:14312:62539:Y	chrY	+	20M	20	58994694
5:2103:1434:12137:Y	chrY	+	22M	22	58995993
5:2105:15255:188637:Y	chrY	+	20M	20	58995993
5:2205:10179:8240:Y	chrY	+	21M	21	58995993
5:2203:8878:67831:Y	chrY	-	20M	20	59128396
	end	width	njunc	uniqueHit	
	<integer>	<integer>	<integer>		<logical>
5:2106:4142:3430:Y	918026	21	0		FALSE
5:2108:3248:41912:Y	1101245	22	0		FALSE
5:1103:12850:21621:Y	1186387	20	0		FALSE
5:1203:17240:152389:Y	1186388	21	0		FALSE
5:2202:17340:164011:Y	1201424	21	0		FALSE
...	...	...	...	...	...
5:2204:14312:62539:Y	58994713	20	0		FALSE
5:2103:1434:12137:Y	58996014	22	0		TRUE
5:2105:15255:188637:Y	58996012	20	0		FALSE
5:2205:10179:8240:Y	58996013	21	0		FALSE
5:2203:8878:67831:Y	59128415	20	0		FALSE

```

-----
seqinfo: 25 sequences from hg19 genome

> ripGal.CCNT1.ct1

GAlignments object with 5853 alignments and 1 metadata column:
      seqnames strand      cigar    qwidth    start
      <Rle>   <Rle> <character> <integer> <integer>
5:2106:4142:3430:Y chr1      +      21M         21    918006
5:2108:3248:41912:Y chr1      -      22M         22   1101224
5:1103:12850:21621:Y chr1      +      20M         20   1186368
5:1203:17240:152389:Y chr1      +      21M         21   1186368
5:2202:17340:164011:Y chr1      +      21M         21   1201404
...
5:1105:18196:33270:Y chrY      -      20M         20   59128396
5:2108:3344:10035:Y chrY      -      20M         20   59128397
5:1103:9654:115236:Y chrY      -      22M         22   59342111
5:1105:17962:142486:Y chrY      -      21M         21   59342112
5:2208:14704:146696:Y chrY      -      20M         20   59342113
      end      width    njunc    | uniqueHit
      <integer> <integer> <integer> | <logical>
5:2106:4142:3430:Y    918026      21      0 | FALSE
5:2108:3248:41912:Y   1101245      22      0 | FALSE
5:1103:12850:21621:Y   1186387      20      0 | FALSE
5:1203:17240:152389:Y   1186388      21      0 | FALSE
5:2202:17340:164011:Y   1201424      21      0 | FALSE
...
5:1105:18196:33270:Y   59128415      20      0 | FALSE
5:2108:3344:10035:Y   59128416      20      0 | FALSE
5:1103:9654:115236:Y   59342132      22      0 | FALSE
5:1105:17962:142486:Y   59342132      21      0 | FALSE
5:2208:14704:146696:Y   59342132      20      0 | FALSE
-----
seqinfo: 25 sequences from hg19 genome

```

### 3 Session Info

```

> sessionInfo()

R version 3.1.1 Patched (2014-09-25 r66681)
Platform: x86_64-apple-darwin10.8.0 (64-bit)

locale:
[1] en_US.UTF-8/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  utils      datasets
[8] methods   base

other attached packages:

```

```

[1] RIPSeeker_1.5.0          rtracklayer_1.26.1      GenomicAlignments_1.2.0
[4] Rsamtools_1.18.0         Biostrings_2.34.0       XVector_0.6.0
[7] GenomicRanges_1.18.1     GenomeInfoDb_1.2.0      IRanges_2.0.0
[10] S4Vectors_0.4.0          BiocGenerics_0.12.0

```

loaded via a namespace (and not attached):

```

[1] base64enc_0.1-2          BatchJobs_1.4           BBmisc_1.7             BiocParallel_1.0.
[5] bitops_1.0-6             brew_1.0-6             checkmate_1.5.0        codetools_0.2-9
[9] DBI_0.3.1                digest_0.6.4           fail_1.2               foreach_1.4.2
[13] iterators_1.0.7          RCurl_1.95-4.3         RSQLite_0.11.4         sendmailR_1.2-1
[17] stringr_0.6.2            tools_3.1.1            XML_3.98-1.1           zlibbioc_1.12.0

```

## References

Jing Zhao, Toshiro K Ohsumi, Johnny T Kung, Yuya Ogawa, Daniel J Grau, Kavitha Sarma, Ji Joon Song, Robert E Kingston, Mark Borowsky, and Jeannie T Lee. Genome-wide Identification of Polycomb-Associated RNAs by RIP-seq. *Molecular Cell*, 40(6):939–953, December 2010.