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
```

```
GappedAlignments with 1022474 alignments and 1 elementMetadata col:
                    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
  SRR039210.4605170     chr1      +         36M        36   3044715   3044750
   SRR039210.192768     chr1      +         36M        36   3044735   3044770
  SRR039210.2879118     chr1      -         36M        36   3084493   3084528
  SRR039210.4235512     chr1      -         36M        36   3096432   3096467
                ...      ...    ...         ...       ...       ...       ...
   SRR039212.891425     chrY      +         36M        36   2671088   2671123
  SRR039212.3401215     chrY      -         36M        36   2779787   2779822
  SRR039212.1732007     chrY      -         36M        36   2786112   2786147
  SRR039212.2698603     chrY      -         36M        36   2790755   2790790
  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      ngap   | 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
  SRR039210.4605170        36         0   |     FALSE
   SRR039210.192768        36         0   |     FALSE
  SRR039210.2879118        36         0   |      TRUE
  SRR039210.4235512        36         0   |     FALSE
                ...       ...       ... ...       ...
   SRR039212.891425        36         0   |      TRUE
  SRR039212.3401215        36         0   |      TRUE
  SRR039212.1732007        36         0   |     FALSE
  SRR039212.2698603        36         0   |     FALSE
  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
  ---
  seqlengths:
        chr1     chr10     chr11     chr12 ...      chrM      chrX      chrY
   197195432 129993255 121843856 121257530 ...     16299 166650296  15902555

> ripGal.PRC2.rip.biorep2
```

```
GappedAlignments with 442030 alignments and 1 elementMetadata col:
                    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
  SRR039213.3227516     chr1      -         36M        36   3260107   3260142
   SRR039213.500139     chr1      -         36M        36   3315792   3315827
  SRR039213.5727812     chr1      +         36M        36   3375268   3375303
  SRR039213.1995540     chr1      -         36M        36   3377020   3377055
                ...      ...    ...         ...       ...       ...       ...
  SRR039213.1257286     chrY      +         36M        36   2552665   2552700
  SRR039213.2809240     chrY      +         36M        36   2552818   2552853
   SRR039213.984990     chrY      -         36M        36   2578603   2578638
  SRR039213.2291969     chrY      +         36M        36   2620190   2620225
  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      ngap   | 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
  SRR039213.3227516        36         0   |      TRUE
   SRR039213.500139        36         0   |     FALSE
  SRR039213.5727812        36         0   |     FALSE
  SRR039213.1995540        36         0   |      TRUE
                ...       ...       ... ...       ...
  SRR039213.1257286        36         0   |      TRUE
  SRR039213.2809240        36         0   |      TRUE
   SRR039213.984990        36         0   |     FALSE
  SRR039213.2291969        36         0   |     FALSE
  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
  ---
  seqlengths:
        chr1     chr10     chr11     chr12 ...      chrM      chrX      chrY
   197195432 129993255 121843856 121257530 ...     16299 166650296  15902555

> ripGal.PRC2.ctl
```

```
GappedAlignments with 208445 alignments and 1 elementMetadata col:
                    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
  SRR039214.6766928     chr1      +         20M        20   3205149   3205168
  SRR039214.1435988     chr1      +         20M        20   3215634   3215653
  SRR039214.6803807     chr1      -         20M        20   3218132   3218151
   SRR039214.924205     chr1      -         20M        20   3240974   3240993
                ...      ...    ...         ...       ...       ...       ...
  SRR039214.5091435     chrY      -         33M        33   2389144   2389176
  SRR039214.6303207     chrY      +         24M        24   2396570   2396593
    SRR039214.78345     chrY      -         36M        36   2423421   2423456
  SRR039214.3249178     chrY      -         36M        36   2511336   2511371
  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      ngap   | 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
  SRR039214.6766928        20         0   |     FALSE
  SRR039214.1435988        20         0   |     FALSE
  SRR039214.6803807        20         0   |     FALSE
   SRR039214.924205        20         0   |     FALSE
                ...       ...       ... ...       ...
  SRR039214.5091435        33         0   |     FALSE
  SRR039214.6303207        24         0   |     FALSE
    SRR039214.78345        36         0   |     FALSE
  SRR039214.3249178        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
  ---
  seqlengths:
        chr1     chr10     chr11     chr12 ...      chrM      chrX      chrY
   197195432 129993255 121843856 121257530 ...     16299 166650296  15902555
```

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=TR
> 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
```

```
GappedAlignments with 10409 alignments and 1 elementMetadata col:
                        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
   5:2208:15004:81202:Y     chr1      +         21M        21   1265828
  5:2202:18872:165183:Y     chr1      +         21M        21   1543351
   5:2105:20933:71037:Y     chr1      +         21M        21   1850623
   5:2105:20933:71037:Y     chr1      +         21M        21   1850632
                    ...      ...    ...         ...       ...       ...
   5:2106:19901:19101:Y     chrY      +         20M        20  58984179
    5:2203:8767:99765:Y     chrY      +         21M        21  58984179
  5:2203:20933:146500:Y     chrY      +         21M        21  58994691
  5:2106:16657:195579:Y     chrY      +         21M        21  58994694
   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      ngap   | 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
   5:2208:15004:81202:Y   1265848        21         0   |     FALSE
  5:2202:18872:165183:Y   1543371        21         0   |     FALSE
   5:2105:20933:71037:Y   1850643        21         0   |     FALSE
   5:2105:20933:71037:Y   1850652        21         0   |     FALSE
                    ...       ...       ...       ... ...       ...
   5:2106:19901:19101:Y  58984198        20         0   |      TRUE
    5:2203:8767:99765:Y  58984199        21         0   |      TRUE
  5:2203:20933:146500:Y  58994711        21         0   |     FALSE
  5:2106:16657:195579:Y  58994714        21         0   |      TRUE
   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
  ---
  seqlengths:
        chr1     chr10     chr11     chr12 ...      chrM      chrX      chrY
   249250621 135534747 135006516 133851895 ...     16571 155270560  59373566

> ripGal.CCNT1.ctl

GappedAlignments with 5853 alignments and 1 elementMetadata col:
                        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
   5:2208:15004:81202:Y     chr1      +         21M        21   1265828
  5:2202:18872:165183:Y     chr1      +         21M        21   1543351
   5:2105:20933:71037:Y     chr1      +         21M        21   1850623
   5:2105:20933:71037:Y     chr1      +         21M        21   1850632
                    ...      ...    ...         ...       ...       ...
   5:1105:20223:70248:Y     chrY      +         22M        22  58995993
  5:2203:11089:104750:Y     chrY      +         21M        21  58995993
  5:2204:11266:100807:Y     chrY      +         20M        20  58995993
  5:2106:15607:153947:Y     chrY      -         21M        21  59128395
   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      ngap   | 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
   5:2208:15004:81202:Y   1265848        21         0   |     FALSE
  5:2202:18872:165183:Y   1543371        21         0   |     FALSE
   5:2105:20933:71037:Y   1850643        21         0   |     FALSE
   5:2105:20933:71037:Y   1850652        21         0   |     FALSE
                    ...       ...       ...       ... ...       ...
   5:1105:20223:70248:Y  58996014        22         0   |      TRUE
  5:2203:11089:104750:Y  58996013        21         0   |      TRUE
  5:2204:11266:100807:Y  58996012        20         0   |     FALSE
  5:2106:15607:153947:Y  59128415        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
  ---
  seqlengths:
        chr1     chr10     chr11     chr12 ...      chrM      chrX      chrY
   249250621 135534747 135006516 133851895 ...     16571 155270560  59373566
```

3 Session Info

```
> sessionInfo()
```

```
R version 2.15.1 (2012-06-22)
Platform: i386-apple-darwin9.8.0/i386 (32-bit)

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

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

other attached packages:
[1] RIPSeeker_0.99.0    Rsamtools_1.8.5     Biostrings_2.24.1
[4] rtracklayer_1.16.3  GenomicRanges_1.8.9 IRanges_1.14.4
[7] BiocGenerics_0.2.0

loaded via a namespace (and not attached):
[1] BSgenome_1.24.0 RCurl_1.91-1    XML_3.9-4       bitops_1.0-4.1
[5] stats4_2.15.1   tools_2.15.1    zlibbioc_1.2.0
```

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.