

The ChIPpeakAnno user's guide

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Contents

1	Introduction	1
2	Examples of using ChIPpeakAnno	2
2.1	Task 1: Find the nearest feature such as gene and the distance to the feature such as the transcription start site (TSS) of the nearest gene	2
2.2	Task 2: Obtain overlapping peaks for potential transcription factor complex and determine the significance of the overlapping and generate Venn Diagram	4
2.3	Task 3: Obtain sequences surrounding the peaks for PCR validation or motif discovery	7
2.4	Task 3: Obtain enriched gene ontology (GO) terms near the peaks	7
3	References	10
4	Session Info	10

1 Introduction

Chromatin immunoprecipitation (ChIP) followed by high-throughput tag sequencing (ChIP-seq) and ChIP followed by genome tiling array analysis (ChIP-chip) become more and more prevalent high throughput technologies for identifying the binding sites of DNA-binding proteins in a genome-wide bases. A number of algorithms have been published to facilitate the identification of the binding sites of the DNA-binding proteins of interest. The identified binding sites in the list of peaks are usually converted to BED or WIG file format to be loaded to UCSC genome browser as custom tracks for investigators to view the proximity to various genomic features such as genes, exons and conserved elements. However, clicking through the genome browser could be a daunting task for the biologist if the number of peaks gets large or the peaks spread widely across the genome. Here we have developed a

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Bioconductor package called ChIPpeakAnno to facilitate the batch annotation of the peaks identified from either ChIP-seq or ChIP-chip experiments. We have implemented functionality to find the nearest gene, exon, miRNA or custom features supplied by users such as most conserved elements and other transcription factor binding sites leveraging IRanges. Since the genome annotation gets updated from time to time, we have leveraged the *biomaRt* package from Bioconductor to retrieve the annotation data on the fly if the annotation of interest is available via the *biomaRt* package. The users also have the flexibility to pass their own annotation data as RangedData or pass in annotation data from *GenomicFeatures*. We have also leveraged *BSgenome* and *biomaRt* package on implementing functions to retrieve the sequences around the peak identified for peak validation. To understand whether the identified peaks are enriched around genes with certain GO terms, we have implemented GO enrichment test in ChIPpeakAnno package leveraging the hypergeometric test **phyper** in *stats* package and integrated with Gene Ontology (GO) annotation from *GO.db* package and multiplicity adjustment functions from *multtest* package.

2 Examples of using ChIPpeakAnno

2.1 Task 1: Find the nearest feature such as gene and the distance to the feature such as the transcription start site (TSS) of the nearest gene

We have a list of peaks identified from ChIP-seq or ChIP-chip experiments and we would like to retrieve the nearest gene and distance to the corresponding gene transcription start site. We have retrieved all the genomic locations of the genes for human genome as TSS.human.NCBI36 data package for repeated use with function `getAnnotation`, now we just pass the annotation to the `annotatePeakInBatch` function.

```
> library(ChIPpeakAnno)
> data(myPeakList)
> data(TSS.human.NCBI36)
> annotatedPeak = annotatePeakInBatch(myPeakList[1:6, ], AnnotationData = TSS.human.NCBI36)
> as.data.frame(annotatedPeak)
```

	space	start	end	width	names	peak	strand
1	1	703885	703985	101	1_12_703729 ENSG000000197049	1_12_703729	1
2	1	559774	559874	101	1_41_559455 ENSG000000212678	1_41_559455	1
3	1	556660	556760	101	1_93_556427 ENSG000000212875	1_93_556427	1
4	1	1041646	1041746	101	1_11_1041174 ENSG000000131591	1_11_1041174	-1
5	1	1270239	1270339	101	1_14_1269014 ENSG000000107404	1_14_1269014	-1
6	1	926058	926158	101	1_20_925025 ENSG000000188290	1_20_925025	-1

	feature	start_position	end_position	insideFeature	distancetoFeature
1	ENSG000000197049	711183	712376	upstream	-7298
2	ENSG000000212678	559619	560165	inside	155
3	ENSG000000212875	556317	557859	inside	343
4	ENSG000000131591	1007061	1041341	upstream	-305
5	ENSG000000107404	1260522	1274623	inside	4384
6	ENSG000000188290	924208	925333	upstream	-725

	shortestDistance	fromOverlappingOrNearest
1	7198	NearestStart
2	155	NearestStart

3	343	NearestStart
4	305	NearestStart
5	4284	NearestStart
6	725	NearestStart

To annotate the peaks with other genomic feature, you will need to call function `getAnnotation` with `featureType`, e.g., “Exon” for finding the nearest exon, and “miRNA” for finding the nearest miRNA, “5utr” or “3utr” for finding the overlapping 5 prime UTR or 3 prime UTR. Please refer to `getAnnotation` function for more details.

We have presented the examples using human genome as annotation source. To annotate your data with other species, you will need to pass to the function `getAnnotation` the appropriate dataset for example, `drerio_gene_ensembl` for zebrafish genome, `mmusculus_gene_ensembl` for mouse genome and `rnorvegicus_gene_ensembl` for rat genome. For a list of available biomaRt and dataset, please refer to the *biomaRt* package documentation (Durinck S. et al., 2005). For fast access, in addition to TSS.human.NCBI36, TSS.mouse.NCBIM37, TSS.rat.RGSC3.4 and TSS.zebrafish.Zv8 are included as annotation data packages.

You could also pass your own annotation data into the function `annotatePeakInBatch`. For example, if you have a list of transcription factor binding sites from literature and are interested in obtaining the nearest binding site of the transcription factor and distance to it for the list of peaks.

```
> myPeak1 = RangedData(IRanges(start = c(967654, 2010897, 2496704,
+ 3075869, 3123260, 3857501, 201089, 1543200, 1557200, 1563000,
+ 1569800, 167889600), end = c(967754, 2010997, 2496804, 3075969,
+ 3123360, 3857601, 201089, 1555199, 1560599, 1565199, 1573799,
+ 167893599), names = c("Site1", "Site2", "Site3", "Site4",
+ "Site5", "Site6", "Site7", "Site8", "Site9", "Site10", "Site11",
+ "Site12")), space = c("1", "2", "3", "4", "5", "6", "2",
+ "6", "6", "6", "6", "5"))
> TFbindingSites = RangedData(IRanges(start = c(967659, 2010898,
+ 2496700, 3075866, 3123260, 3857500, 96765, 201089, 249670,
+ 307586, 312326, 385750, 1549800, 1554400, 1565000, 1569400,
+ 167888600), end = c(967869, 2011108, 2496920, 3076166, 3123470,
+ 3857780, 96985, 201299, 249890, 307796, 312586, 385960, 1550599,
+ 1560799, 1565399, 1571199, 167888999), names = c("t1", "t2",
+ "t3", "t4", "t5", "t6", "t7", "t8", "t9", "t10", "t11", "t12",
+ "t13", "t14", "t15", "t16", "t17")), space = c("1", "2",
+ "3", "4", "5", "6", "1", "2", "3", "4", "5", "6", "6", "6",
+ "6", "6", "5"), strand = c(1, 1, 1, 1, 1, 1, -1, -1, -1,
+ -1, -1, -1, 1, 1, 1, 1, 1))
> annotatedPeak2 = annotatePeakInBatch(myPeak1, AnnotationData = TFbindingSites)
> pie(table(as.data.frame(annotatedPeak2)$insideFeature))
> as.data.frame(annotatedPeak2)
```

	space	start	end	width	names	peak	strand	feature
1	1	967654	967754	101	Site1 t1	Site1	1	t1
2	2	2010897	2010997	101	Site2 t2	Site2	1	t2
3	2	201089	201089	1	Site7 t8	Site7	-1	t8
4	3	2496704	2496804	101	Site3 t3	Site3	1	t3
5	4	3075869	3075969	101	Site4 t4	Site4	1	t4
6	5	167889600	167893599	4000	Site12 t17	Site12	1	t17
7	5	3123260	3123360	101	Site5 t5	Site5	1	t5
8	6	1563000	1565199	2200	Site10 t15	Site10	1	t15
9	6	1569800	1573799	4000	Site11 t16	Site11	1	t16
10	6	3857501	3857601	101	Site6 t6	Site6	1	t6
11	6	1543200	1555199	12000	Site8 t13	Site8	1	t13

	6	1557200	1560599	3400	Site9 t14	Site9	1	t14
	start_position	end_position			insideFeature		distancetoFeature	
1	967659	967869			overlapStart		-5	
2	2010898	2011108			overlapStart		-1	
3	201089	201299			inside		210	
4	2496700	2496920			inside		4	
5	3075866	3076166			inside		3	
6	167888600	167888999			downstream		1000	
7	3123260	3123470			inside		0	
8	1565000	1565399			overlapStart		-2000	
9	1569400	1571199			overlapEnd		400	
10	3857500	3857780			inside		1	
11	1549800	1550599			includeFeature		-6600	
12	1554400	1560799			inside		2800	
	shortestDistance				fromOverlappingOrNearest			
1		5			NearestStart			
2		1			NearestStart			
3		0			NearestStart			
4		4			NearestStart			
5		3			NearestStart			
6		601			NearestStart			
7		0			NearestStart			
8		199			NearestStart			
9		400			NearestStart			
10		1			NearestStart			
11		4600			NearestStart			
12		200			NearestStart			

Both BED format and GFF format are common file format that provides a flexible way to define the peaks and annotations as the data lines. Therefore, conversion functions `RfunctionBED2RangedData` and `RfunctionGFF2RangedData` were implemented for converting these data format to `RangedData` before calling `annotatePeakInBatch`

Once you annotated the peak list, you can plot the distance to nearest feature such as TSS.

2.2 Task 2: Obtain overlapping peaks for potential transcription factor complex and determine the significance of the overlapping and generate Venn Diagram

Here is an example of obtaining overlapping peaks with maximum gap 1kb for two peak ranges.

```
> peaks1 = RangedData(IRanges(start = c(967654, 2010897, 2496704,
+ 3075869, 3123260, 3857501, 201089, 1543200, 1557200, 1563000,
+ 1569800, 167889600), end = c(967754, 2010997, 2496804, 3075969,
+ 3123360, 3857601, 201089, 1555199, 1560599, 1565199, 1573799,
+ 167893599), names = c("Site1", "Site2", "Site3", "Site4",
+ "Site5", "Site6", "Site7", "Site8", "Site9", "Site10", "Site11",
+ "Site12")), space = c("1", "2", "3", "4", "5", "6", "2",
+ "6", "6", "6", "6", "5"), strand = as.integer(1))
> peaks2 = RangedData(IRanges(start = c(967659, 2010898, 2496700,
+ 3075866, 3123260, 3857500, 96765, 201089, 249670, 307586,
+ 312326, 385750, 1549800, 1554400, 1565000, 1569400, 167888600),
+ end = c(967869, 2011108, 2496920, 3076166, 3123470, 3857780,
+ 96985, 201299, 249890, 307796, 312586, 385960, 1550599,
+ 1560799, 1565399, 1571199, 167888999), names = c("t1",
+ "t2", "t3", "t4", "t5", "t6", "t7", "t8", "t9", "t10",
```

```
+      "t11", "t12", "t13", "t14", "t15", "t16", "t17")), space = c("1",
+      "2", "3", "4", "5", "6", "1", "2", "3", "4", "5", "6", "6",
+      "6", "6", "6", "5"), strand = c(1, 1, 1, 1, 1, 1, -1, -1,
+      -1, -1, -1, -1, 1, 1, 1, 1, 1))
> t1 = findOverlappingPeaks(peaks1, peaks2, maxgap = 1000, multiple = F,
+      NameOfPeaks1 = "TF1", NameOfPeaks2 = "TF2")
```

Here is a list of overlapping peaks with maximum gap 1kb and a pie graph describing the distribution of relative position of peaks1 to peaks2 for overlapping peaks.

```
> overlappingPeaks = t1$OverlappingPeaks
> overlappingPeaks
```

	TF1	chr	TF2	TF2_start	TF2_end	strand	TF1_start	TF1_end	strand1
1	Site1	1	t1	967659	967869	1	967654	967754	1
5	Site2	2	t2	2010898	2011108	1	2010897	2010997	1
10	Site7	2	t8	201089	201299	-1	201089	201089	1
6	Site3	3	t3	2496700	2496920	1	2496704	2496804	1
7	Site4	4	t4	3075866	3076166	1	3075869	3075969	1
4	Site12	5	t17	167888600	167888999	1	167889600	167893599	1
8	Site5	5	t5	3123260	3123470	1	3123260	3123360	1
2	Site10	6	t15	1565000	1565399	1	1563000	1565199	1
3	Site11	6	t16	1569400	1571199	1	1569800	1573799	1
9	Site6	6	t6	3857500	3857780	1	3857501	3857601	1
11	Site8	6	t13	1549800	1550599	1	1543200	1555199	1
12	Site9	6	t14	1554400	1560799	1	1557200	1560599	1

	overlapFeature	shortestDistance
1	overlapStart	5
5	overlapStart	1
10	inside	0
6	inside	4
7	inside	3
4	downstream	601
8	inside	0
2	overlapStart	199
3	overlapEnd	400
9	inside	1
11	includeFeature	4600
12	inside	200

```
> pie(table(overlappingPeaks$overlapFeature))
```

Here is the merged overlapping peaks, which can be used to obtain overlapping peaks with another TF binding sites from a protein complex.

```
> as.data.frame(t1$MergedPeaks)
```

	space	start	end	width	names
1	1	967654	967869	216	TF1-Site1-TF2-t1
2	2	2010897	2011108	212	TF1-Site2-TF2-t2
3	2	201089	201299	211	TF1-Site7-TF2-t8
4	3	2496700	2496920	221	TF1-Site3-TF2-t3
5	4	3075866	3076166	301	TF1-Site4-TF2-t4
6	5	167888600	167893599	5000	TF1-Site12-TF2-t17
7	5	3123260	3123470	211	TF1-Site5-TF2-t5
8	6	1563000	1565399	2400	TF1-Site10-TF2-t15
9	6	1569400	1573799	4400	TF1-Site11-TF2-t16
10	6	3857500	3857780	281	TF1-Site6-TF2-t6
11	6	1543200	1555199	12000	TF1-Site8-TF2-t13
12	6	1554400	1560799	6400	TF1-Site9-TF2-t14

Here is the peaks in peaks1 that overlaps with peaks in peaks2

```
> as.data.frame(t1$Peaks1withOverlaps)
```

	space	start	end	width	names	strand
1	1	967654	967754	101	Site1	1
2	2	2010897	2010997	101	Site2	1
3	2	201089	201089	1	Site7	1
4	3	2496704	2496804	101	Site3	1
5	4	3075869	3075969	101	Site4	1
6	5	167889600	167893599	4000	Site12	1
7	5	3123260	3123360	101	Site5	1
8	6	1563000	1565199	2200	Site10	1
9	6	1569800	1573799	4000	Site11	1
10	6	3857501	3857601	101	Site6	1
11	6	1543200	1555199	12000	Site8	1
12	6	1557200	1560599	3400	Site9	1

Here is the peaks in peaks2 that overlap with peaks in peaks1

```
> as.data.frame(t1$Peaks2withOverlaps)
```

	space	start	end	width	names	strand
1	1	967659	967869	211	t1	1
2	2	2010898	2011108	211	t2	1
3	2	201089	201299	211	t8	-1
4	3	2496700	2496920	221	t3	1
5	4	3075866	3076166	301	t4	1
6	5	167888600	167888999	400	t17	1
7	5	3123260	3123470	211	t5	1
8	6	1565000	1565399	400	t15	1
9	6	1569400	1571199	1800	t16	1
10	6	3857500	3857780	281	t6	1
11	6	1549800	1550599	800	t13	1
12	6	1554400	1560799	6400	t14	1

The `findOverlappingPeaks` function can be repeatedly called to obtain for example, the peaks in peaks1 that overlap with peaks in both peaks2 and peaks3.

```
> peaks3 = RangedData(IRanges(start = c(967859, 2010868, 2496500,
+   3075966, 3123460, 3851500, 96865, 201189, 249600, 307386),
+   end = c(967969, 2011908, 2496720, 3076166, 3123470, 3857680,
+   96985, 201299, 249890, 307796), names = c("p1", "p2",
+   "p3", "p4", "p5", "p6", "p7", "p8", "p9", "p10")), space = c("1",
+   "2", "3", "4", "5", "6", "1", "2", "3", "4"), strand = c(1,
+   1, 1, 1, 1, 1, -1, -1, -1, -1))
> findOverlappingPeaks(findOverlappingPeaks(peaks1, peaks2, maxgap = 1000,
+   multiple = F, NameOfPeaks1 = "TF1", NameOfPeaks2 = "TF2"))$Peaks1withOverlap,
+   peaks3, maxgap = 1000, multiple = F, NameOfPeaks1 = "TF1TF2",
+   NameOfPeaks2 = "TF3")$Peaks1withOverlap
```

RangedData with 7 rows and 1 value column across 6 spaces

	space	ranges	strand
<character>	<IRanges>	<character>	
Site1	1 [967654, 967754]		1
Site2	2 [2010897, 2010997]		1
Site7	2 [201089, 201089]		1
Site3	3 [2496704, 2496804]		1
Site4	4 [3075869, 3075969]		1
Site5	5 [3123260, 3123360]		1
Site6	6 [3857501, 3857601]		1

Venn Diagram can be generated by the following function call with p-value that indicates whether the extent of overlapping is significant.

```
> makeVennDiagram(RangedDataList(peaks1, peaks2), NameOfPeaks = c("TF1",
+ "TF2"), maxgap = 0, totalTest = 100, cex = 1, counts.col = "red")

$p.value
[1] 9.837922e-10

$vennCounts
      TF1 TF2 Counts
[1,]    0    0     82
[2,]    0    1      6
[3,]    1    0      1
[4,]    1    1     11
attr(,"class")
[1] "VennCounts"
```

2.3 Task 3: Obtain sequences surrounding the peaks for PCR validation or motif discovery

Here is an example of obtaining sequences surrounding the peak intervals including 20 bp upstream and downstream sequence.

```
> peaks = RangedData(IRanges(start = c(100, 500), end = c(300,
+ 600), names = c("peak1", "peak2")), space = c("NC_008253",
+ "NC_010468"))
> library(BSgenome.Ecoli.NCBI.20080805)
> peaksWithSequences = getAllPeakSequence(peaks, upstream = 20,
+ downstream = 20, genome = Ecoli)
```

You can easily convert the obtained sequences into fasta format for motif discovery by calling the function `write2FASTA`.

```
> write2FASTA(peaksWithSequences)

>peak1
GGTTACCTGCCGTGAGTAAATTAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGA
CAGATAAAATACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGG
TAACGGTGCGGGCTGACGCTACAGGAACACAGAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGG
T
>peak2
AAATCTAACCAACTGGCGCGCGCGGGCTCGCCAGGTGGCGGATAACCTGGCGGTGCCTATAACCCGTTGTTCTTTA
TGGCGGCACGGTCTGGGTAAACTCACCTGCTGCATGCGGTGGGTAACGGCATTATGGCG
```

2.4 Task 3: Obtain enriched gene ontology (GO) terms near the peaks

Once you have obtained the annotated peak data from the example above, you can also use the function `getEnrichedGO` to obtain a list of enriched gene ontology (GO) terms using hypergeometric test.

```
library(org.Hs.eg.db)
enrichedGO = getEnrichedGO (annotatedPeak, orgAnn = "org.Hs.eg.db", maxP =
0.01, multiAdj = TRUE, minGOTerm = 10, multiAdjMethod = "BH" )
```

Please note that `org.Hs.eg.db` is the GO gene mapping for Human, for other organisms, please refer to <http://www.bioconductor.org/packages/release/data/annotation/> for additional `org.xx.eg.db` packages.

```
> data(enrichedGO)
```

Here is a list of enriched GO biological process for myPeakList dataset.

```
> enrichedGO$bp[1:6, ]
```

	go.id	go.term
1	GO:0000187	activation of MAPK activity
2	GO:0002573	myeloid leukocyte differentiation
3	GO:0002702	positive regulation of production of molecular mediator of immune response
4	GO:0002761	regulation of myeloid leukocyte differentiation
5	GO:0002763	positive regulation of myeloid leukocyte differentiation
6	GO:0006213	pyrimidine nucleoside metabolic process

```
1
2
3
4
5
6 The chemical reactions and pathways involving any pyrimidine nucleoside, one of a fami
```

	Ontology	count.InDataset	count.InGenome	pvalue	totaltermInDataset
1	BP	17	65	0.001673400	85892
2	BP	19	81	0.004192510	85892
3	BP	4	10	0.005921074	85892
4	BP	13	50	0.004712934	85892
5	BP	8	22	0.001277580	85892
6	BP	4	10	0.005921074	85892

	totaltermInGenome
1	644151
2	644151
3	644151
4	644151
5	644151
6	644151

Here is a list of enriched GO molecular functions for myPeakList dataset.


```
> enrichedGO$mf[1:6, ]
```

	go.id	go.term
1	GO:0003702	RNA polymerase II transcription factor activity
2	GO:0003705	RNA polymerase II transcription factor activity, enhancer binding
3	GO:0004112	cyclic-nucleotide phosphodiesterase activity
4	GO:0004114	3',5'-cyclic-nucleotide phosphodiesterase activity
5	GO:0004659	prenyltransferase activity
6	GO:0004896	cytokine receptor activity

```

1           Functions to initiate or regulate RNA polymerase
2 Functions to initiate or regulate RNA polymerase II transcription by binding an enhanc
3           Catalysis of the reaction: a nucleoside cyclic phosphate + H2O = a nucl
4           Catalysis of the reaction: nucleoside 3',5'-cyclic phosphate + H2O = nucleos
5           Catalysis of the transfer of a prenyl group from one compound (donor) to an
6           Combining with a cytokine to initiate a change

```

	Ontology	count.InDataset	count.InGenome	pvalue	totaltermInDataset
1	MF	39	214	0.0065818928	29657
2	MF	11	29	0.0001003699	29657
3	MF	9	26	0.0007622170	29657
4	MF	9	25	0.0005282939	29657
5	MF	9	23	0.0002346785	29657
6	MF	16	66	0.0027160003	29657

```
totaltermInGenome
```

```

1           235991
2           235991
3           235991
4           235991
5           235991
6           235991

```

Heres is a list of enriched GO cellular components for myPeakList dataset.

```
> enrichedGO$cc
```

	go.id	go.term
1	GO:0005811	lipid particle
2	GO:0005942	phosphoinositide 3-kinase complex
3	GO:0016363	nuclear matrix
4	GO:0034399	nuclear periphery

```

1           Any particle of coalesced lipids in the cytoplasm
2 A complex containing a heterodimer of a catalytic subunit and a regulatory (adaptor) s

```

```

3                                     The dense fibrillar network 1
4                                     The portion of the nuclea
  Ontology count.InDataset count.InGenome      pvalue totaltermInDataset
1      CC              5          15 0.006685158          45317
2      CC              4          11 0.007074546          45317
3      CC             12          49 0.005607016          45317
4      CC             12          52 0.009516449          45317
  totaltermInGenome
1          365523
2          365523
3          365523
4          365523

```

3 References

1. Y. Benjamini and Y. Hochberg (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Statist. Soc. B. Vol. 57: 289-300.
2. Y. Benjamini and D. Yekutieli (2001). The control of the false discovery rate in multiple hypothesis testing under dependency. Annals of Statistics. Accepted.
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4. S. Dudoit, J. P. Shaffer, and J. C. Boldrick (Submitted). Multiple hypothesis testing in microarray experiments.
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4 Session Info

```

> sessionInfo()

R version 2.10.1 Patched (2009-12-14 r50738)
i386-apple-darwin8.11.1

```

locale:

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

attached base packages:

[1] tools stats graphics grDevices utils datasets methods

[8] base

other attached packages:

[1] ChIPpeakAnno_1.2.15

limma_3.2.3

[3] org.Hs.eg.db_2.3.6

GO.db_2.3.5

[5] RSQLite_0.8-4

DBI_0.2-5

[7] AnnotationDbi_1.8.2

BSgenome.Ecoli.NCBI.20080805_1.3.16

[9] BSgenome_1.14.2

Biostrings_2.14.12

[11] IRanges_1.4.16

multtest_2.3.2

[13] Biobase_2.6.1

biomaRt_2.2.0

loaded via a namespace (and not attached):

[1] MASS_7.3-5 Rcurl_1.3-1 XML_2.8-1 splines_2.10.1

[5] survival_2.35-8