

# The ChIPpeakAnno user's guide

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## 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

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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 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, gene end 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       | +      |
| 2 | 1     | 559774           | 559874         | 101          | 1_41_559455  | ENSG000000212678 | 1_41_559455       | +      |
| 3 | 1     | 556660           | 556760         | 101          | 1_93_556427  | ENSG000000212875 | 1_93_556427       | +      |
| 4 | 1     | 1041646          | 1041746        | 101          | 1_11_1041174 | ENSG000000131591 | 1_11_1041174      | -      |
| 5 | 1     | 1270239          | 1270339        | 101          | 1_14_1269014 | ENSG000000107404 | 1_14_1269014      | -      |
| 6 | 1     | 926058           | 926158         | 101          | 1_20_925025  | ENSG000000188290 | 1_20_925025       | -      |
|   |       | 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 | ENSG00000107404                           | 1260522      | 1274623 | inside   | 4384 |
| 6 | ENSG00000188290                           | 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.human.GRCh37, TSS.mouse.NCBIM37, TSS.mouse.GRCm38, TSS.rat.RGSC3.4, TSS.rat.Rnor\_5.0, TSS.zebrafish.Zv8, and TSS.zebrafish.Zv9 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))
> 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  | +      | t1      |
| 2  | 2     | 2010897   | 2010997   | 101   | Site2 t2   | Site2  | +      | t2      |
| 3  | 2     | 201089    | 201089    | 1     | Site7 t8   | Site7  | -      | t8      |
| 4  | 3     | 2496704   | 2496804   | 101   | Site3 t3   | Site3  | +      | t3      |
| 5  | 4     | 3075869   | 3075969   | 101   | Site4 t4   | Site4  | +      | t4      |
| 6  | 5     | 167889600 | 167893599 | 4000  | Site12 t17 | Site12 | +      | t17     |
| 7  | 5     | 3123260   | 3123360   | 101   | Site5 t5   | Site5  | +      | t5      |
| 8  | 6     | 1563000   | 1565199   | 2200  | Site10 t15 | Site10 | +      | t15     |
| 9  | 6     | 1569800   | 1573799   | 4000  | Site11 t16 | Site11 | +      | t16     |
| 10 | 6     | 3857501   | 3857601   | 101   | Site6 t6   | Site6  | +      | t6      |
| 11 | 6     | 1543200   | 1555199   | 12000 | Site8 t13  | Site8  | +      | t13     |

|    | 6              | 1557200      | 1560599        | 3400 | Site9             | t14 | Site9 | + | 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 BED2RangedData and GFF2RangedData 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","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","5"), strand=c(1,1,1,1,1,1,-1,-1,-1,-1,-1,1,1,1,1))
> t1 =findOverlappingPeaks(peaks1, peaks2, maxgap=1000, minoverlap =20,select="first", NameOfPeaks1="TF1", NameOfPeaks2="TF2", and
>
```

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  | +      | 967654    | 967754  | +       |
| 4  | Site2  | 2   | t2  | 2010898   | 2011108 | +      | 2010897   | 2010997 | +       |
| 5  | Site3  | 3   | t3  | 2496700   | 2496920 | +      | 2496704   | 2496804 | +       |
| 6  | Site4  | 4   | t4  | 3075866   | 3076166 | +      | 3075869   | 3075969 | +       |
| 7  | Site5  | 5   | t5  | 3123260   | 3123470 | +      | 3123260   | 3123360 | +       |
| 2  | Site10 | 6   | t15 | 1565000   | 1565399 | +      | 1563000   | 1565199 | +       |
| 3  | Site11 | 6   | t16 | 1569400   | 1571199 | +      | 1569800   | 1573799 | +       |
| 8  | Site6  | 6   | t6  | 3857500   | 3857780 | +      | 3857501   | 3857601 | +       |
| 9  | Site8  | 6   | t13 | 1549800   | 1550599 | +      | 1543200   | 1555199 | +       |
| 10 | Site9  | 6   | t14 | 1554400   | 1560799 | +      | 1557200   | 1560599 | +       |

  

|    | overlapFeature | shortestDistance |
|----|----------------|------------------|
| 1  | overlapStart   | 5                |
| 4  | overlapStart   | 1                |
| 5  | inside         | 4                |
| 6  | inside         | 3                |
| 7  | inside         | 0                |
| 2  | overlapStart   | 199              |
| 3  | overlapEnd     | 400              |
| 8  | inside         | 1                |
| 9  | includeFeature | 4600             |
| 10 | 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  | 3     | 2496700 | 2496920 | 221   | TF1-Site3-TF2-t3   |
| 4  | 4     | 3075866 | 3076166 | 301   | TF1-Site4-TF2-t4   |
| 5  | 5     | 3123260 | 3123470 | 211   | TF1-Site5-TF2-t5   |
| 6  | 6     | 1563000 | 1565399 | 2400  | TF1-Site10-TF2-t15 |
| 7  | 6     | 1569400 | 1573799 | 4400  | TF1-Site11-TF2-t16 |
| 8  | 6     | 3857500 | 3857780 | 281   | TF1-Site6-TF2-t6   |
| 9  | 6     | 1543200 | 1555199 | 12000 | TF1-Site8-TF2-t13  |
| 10 | 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  | +      |
| 2  | 2     | 2010897 | 2010997 | 101   | Site2  | +      |
| 3  | 3     | 2496704 | 2496804 | 101   | Site3  | +      |
| 4  | 4     | 3075869 | 3075969 | 101   | Site4  | +      |
| 5  | 5     | 3123260 | 3123360 | 101   | Site5  | +      |
| 6  | 6     | 1563000 | 1565199 | 2200  | Site10 | +      |
| 7  | 6     | 1569800 | 1573799 | 4000  | Site11 | +      |
| 8  | 6     | 3857501 | 3857601 | 101   | Site6  | +      |
| 9  | 6     | 1543200 | 1555199 | 12000 | Site8  | +      |
| 10 | 6     | 1557200 | 1560599 | 3400  | Site9  | +      |

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    | +      |
| 2  | 2     | 2010898 | 2011108 | 211   | t2    | +      |
| 3  | 3     | 2496700 | 2496920 | 221   | t3    | +      |
| 4  | 4     | 3075866 | 3076166 | 301   | t4    | +      |
| 5  | 5     | 3123260 | 3123470 | 211   | t5    | +      |
| 6  | 6     | 1565000 | 1565399 | 400   | t15   | +      |
| 7  | 6     | 1569400 | 1571199 | 1800  | t16   | +      |
| 8  | 6     | 3857500 | 3857780 | 281   | t6    | +      |
| 9  | 6     | 1549800 | 1550599 | 800   | t13   | +      |
| 10 | 6     | 1554400 | 1560799 | 6400  | t14   | +      |

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, minoverlap = 1,
+ select= "first", NameOfPeaks1="TF1", NameOfPeaks2="TF2"))$Peaks1withOverlap,
+ peaks3, maxgap=1000, minoverlap = 1, select="first", NameOfPeaks1="TF1TF2", NameOfPeaks2="TF3")$Peaks1withOverlap
```

RangedData with 7 rows and 1 value column across 6 spaces

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

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, minoverlap =1, totalTest=100, cex = 1, counts.col = "red",useFeature=FALSE)
```

```
$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, "test.fa")
```

## 2.4 Task 4: 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 |  |
| 2 | GO:0002573 |  |
| 3 | GO:0002702 |  |
| 4 | GO:0002761 |  |
| 5 | GO:0002763 |  |
| 6 | GO:0006213 |  |
|   |            | activation of MAPK activity  |
|   |            | myeloid leukocyte differentiation  |
|   |            | positive regulation of production of molecular mediator of immune response |
|   |            | regulation of myeloid leukocyte differentiation                            |
|   |            | positive regulation of myeloid leukocyte differentiation                   |
|   |            | pyrimidine nucleoside metabolic process                                    |

|   |  |   |
|---|--|---|
| 1 |  |   |
| 2 |  | The process whereby a relatively unspec |
| 3 |  | Any process that act                    |

```

4
5
6 The chemical reactions and pathways involving any pyrimidine nucleoside, one of a family of organic molecules consisting of a
  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
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

      Definition
1 Functions to initiate or regulate RNA polymerase II transcription.
2 Functions to initiate or regulate RNA polymerase II transcription by binding an enhancer region of DNA.
3 Catalysis of the reaction: a nucleoside cyclic phosphate + H2O = a nucleoside phosphate.
4 Catalysis of the reaction: nucleoside 3',5'-cyclic phosphate + H2O = nucleoside 5'-phosphate.
5 Catalysis of the transfer of a prenyl group from one compound (donor) to another (acceptor).
6 Combining with a cytokine to initiate a change in cell activity.
  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
1 GO:0005811 lipid particle
2 GO:0005942 phosphoinositide 3-kinase complex
3 GO:0016363 nuclear matrix
4 GO:0034399 nuclear periphery

      Definition
1 Any particle of coalesced lipids in the cytoplasm of a cell. May include associated pr

```

```

2 A complex containing a heterodimer of a catalytic subunit and a regulatory (adaptor) subunit of any phosphoinositide 3-kinase
3                                     The dense fibrillar network lying on the inner side of the nuclear membrane
4                                     The portion of the nuclear lumen proximal to the inner nuclear membrane

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

```

## 2.5 Task 5: Find peaks with bi-directional promoters

Here is an example to find peaks with bi-directional promoters and output percent of peaks near bi-directional promoters.

```

> data(myPeakList)
> data(TSS.human.NCBI36)
> annotatedBDP = peaksNearBDP(myPeakList[1:10,], AnnotationData=TSS.human.NCBI36,
+ MaxDistance=5000,PeakLocForDistance = "middle",
+ FeatureLocForDistance = "TSS")
> annotatedBDP$peaksWithBDP

RangedData with 6 rows and 9 value columns across 1 space
      space      ranges |      peak
      <factor>      <IRanges> | <character>
1_14_1300250 ENSG000000218550      1 [1300503, 1300603] | 1_14_1300250
1_41_559455 ENSG000000212678      1 [ 559774,  559874] | 1_41_559455
1_93_556427 ENSG000000212875      1 [ 556660,  556760] | 1_93_556427
1_14_1300250 ENSG000000175756      1 [1300503, 1300603] | 1_14_1300250
1_41_559455 ENSG000000209350      1 [ 559774,  559874] | 1_41_559455
1_93_556427 ENSG000000209349      1 [ 556660,  556760] | 1_93_556427
      strand      feature start_position
      <character>      <character>      <numeric>
1_14_1300250 ENSG000000218550      + ENSG000000218550      1303907
1_41_559455 ENSG000000212678      + ENSG000000212678      559619
1_93_556427 ENSG000000212875      + ENSG000000212875      556317
1_14_1300250 ENSG000000175756      - ENSG000000175756      1298973
1_41_559455 ENSG000000209350      - ENSG000000209350      557859
1_93_556427 ENSG000000209349      - ENSG000000209349      556239
      end_position insideFeature distancetoFeature
      <numeric>      <character>      <numeric>
1_14_1300250 ENSG000000218550      1304275      upstream      -3354
1_41_559455 ENSG000000212678      560165      inside      205
1_93_556427 ENSG000000212875      557859      inside      393
1_14_1300250 ENSG000000175756      1300443      upstream      -110
1_41_559455 ENSG000000209350      557930      upstream      -1894
1_93_556427 ENSG000000209349      556304      upstream      -406
      shortestDistance fromOverlappingOrNearest
      <numeric>      <character>
1_14_1300250 ENSG000000218550      3304      NearestStart
1_41_559455 ENSG000000212678      155      NearestStart
1_93_556427 ENSG000000212875      343      NearestStart
1_14_1300250 ENSG000000175756      60      NearestStart
1_41_559455 ENSG000000209350      1844      NearestStart
1_93_556427 ENSG000000209349      356      NearestStart

> c(annotatedBDP$percentPeaksWithBDP, annotatedBDP$n.peaks, annotatedBDP$n.peaksWithBDP)

[1] 0.3 10.0 3.0

```

## 2.6 Task 6: Output a summary of motif occurrence in the peaks.

Here is an example to search the peaks for the motifs in examplepattern.fa file.

```
> peaks = RangedData(IRanges(start=c(100, 500), end=c(300, 600), names=c("peak1", "peak2")),
+ space=c("NC_008253", "NC_010468"))
> filepath =system.file("extdata", "examplePattern.fa", package="ChIPpeakAnno")
> library(BSgenome.Ecoli.NCBI.20080805)
> summarizePatternInPeaks(patternFilePath=filepath, format="fasta", skip=0L, BSgenomeName=Ecoli, peaks=peaks)

      n.peaksWithPattern n.totalPeaks Pattern
[1,] "0"                "2"            "GGNCCK"
[2,] "1"                "2"            "AACCNM"
```

## 2.7 Task 7: Add other IDs to annotated peaks or enrichedGO

Here is an example to add gene symbol to annotated peaks .

```
> data(annotatedPeak)
> library(org.Hs.eg.db)
> addGeneIDs(annotatedPeak[1:6,], "org.Hs.eg.db", c("symbol"))
```

Adding symbol ... done

prepare output ... done

RangedData with 6 rows and 10 value columns across 24 spaces

|                |                  | space            | ranges                   | peak              |
|----------------|------------------|------------------|--------------------------|-------------------|
|                |                  | <factor>         | <IRanges>                | <character>       |
| 1_11_100272487 | ENSG000000202254 | 1                | [100272800, 100272900]   | 1_11_100272487    |
| 1_11_108905539 | ENSG000000186086 | 1                | [108906025, 108906125]   | 1_11_108905539    |
| 1_11_110106925 | ENSG000000065135 | 1                | [110107266, 110107366]   | 1_11_110106925    |
| 1_11_110679983 | ENSG000000197106 | 1                | [110680468, 110680568]   | 1_11_110679983    |
| 1_11_110681677 | ENSG000000197106 | 1                | [110682124, 110682224]   | 1_11_110681677    |
| 1_11_110756560 | ENSG000000116396 | 1                | [110756822, 110756922]   | 1_11_110756560    |
|                |                  | strand           | feature                  | start_position    |
|                |                  | <character>      | <character>              | <numeric>         |
| 1_11_100272487 | ENSG000000202254 | 1                | ENSG000000202254         | 100257218         |
| 1_11_108905539 | ENSG000000186086 | 1                | ENSG000000186086         | 108918435         |
| 1_11_110106925 | ENSG000000065135 | 1                | ENSG000000065135         | 110091233         |
| 1_11_110679983 | ENSG000000197106 | 1                | ENSG000000197106         | 110693108         |
| 1_11_110681677 | ENSG000000197106 | 1                | ENSG000000197106         | 110693108         |
| 1_11_110756560 | ENSG000000116396 | 1                | ENSG000000116396         | 110753965         |
|                |                  | end_position     | insideFeature            | distancetoFeature |
|                |                  | <numeric>        | <character>              | <numeric>         |
| 1_11_100272487 | ENSG000000202254 | 100257309        | downstream               | 15582             |
| 1_11_108905539 | ENSG000000186086 | 109013624        | upstream                 | -12410            |
| 1_11_110106925 | ENSG000000065135 | 110136975        | inside                   | 16033             |
| 1_11_110679983 | ENSG000000197106 | 110744824        | upstream                 | -12640            |
| 1_11_110681677 | ENSG000000197106 | 110744824        | upstream                 | -10984            |
| 1_11_110756560 | ENSG000000116396 | 110776666        | inside                   | 2857              |
|                |                  | shortestDistance | fromOverlappingOrNearest |                   |
|                |                  | <numeric>        | <character>              |                   |
| 1_11_100272487 | ENSG000000202254 | 15491            | NearestStart             |                   |
| 1_11_108905539 | ENSG000000186086 | 12310            | NearestStart             |                   |
| 1_11_110106925 | ENSG000000065135 | 16033            | NearestStart             |                   |
| 1_11_110679983 | ENSG000000197106 | 12540            | NearestStart             |                   |
| 1_11_110681677 | ENSG000000197106 | 10884            | NearestStart             |                   |
| 1_11_110756560 | ENSG000000116396 | 2857             | NearestStart             |                   |
|                |                  | symbol           |                          |                   |
|                |                  | <factor>         |                          |                   |
| 1_11_100272487 | ENSG000000202254 | NA               |                          |                   |
| 1_11_108905539 | ENSG000000186086 | NBPF6            |                          |                   |

```

1_11_110106925 ENSG00000065135    GNAI3
1_11_110679983 ENSG00000197106    SLC6A17
1_11_110681677 ENSG00000197106    SLC6A17
1_11_110756560 ENSG00000116396    KCNC4

> addGeneIDs(annotatedPeak$feature[1:6], "org.Hs.eg.db", c("symbol"))

Adding symbol ... done
prepare output ... done
  ensembl_gene_id  symbol
1 ENSG00000065135    GNAI3
2 ENSG00000116396    KCNC4
3 ENSG00000197106    SLC6A17
4 ENSG00000186086    NBPF6
5 ENSG00000202254    <NA>

```

### 3 References

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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 3.1.2 (2014-10-31)
Platform: x86_64-apple-darwin13.4.0 (64-bit)

```

locale:

[1] C/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8

attached base packages:

[1] stats4 parallel grid stats graphics grDevices utils  
[8] datasets methods base

other attached packages:

|                          |                                       |
|--------------------------|---------------------------------------|
| [1] org.Hs.eg.db_3.0.0   | BSgenome.Ecoli.NCBI.20080805_1.3.1000 |
| [3] BSgenome_1.34.0      | rtracklayer_1.26.2                    |
| [5] GenomicRanges_1.18.3 | ChIPpeakAnno_2.16.4                   |
| [7] AnnotationDbi_1.28.1 | GenomeInfoDb_1.2.3                    |
| [9] Biobase_2.26.0       | RSQLite_1.0.0                         |
| [11] DBI_0.3.1           | Biostrings_2.34.1                     |
| [13] XVector_0.6.0       | IRanges_2.0.1                         |
| [15] S4Vectors_0.4.0     | BiocGenerics_0.12.1                   |
| [17] biomaRt_2.22.0      | VennDiagram_1.6.9                     |

loaded via a namespace (and not attached):

|                      |                         |                        |
|----------------------|-------------------------|------------------------|
| [1] BBmisc_1.8       | BatchJobs_1.5           | BiocParallel_1.0.0     |
| [4] GO.db_3.0.0      | GenomicAlignments_1.2.1 | GenomicFeatures_1.18.2 |
| [7] MASS_7.3-35      | RCurl_1.95-4.5          | Rsamtools_1.18.2       |
| [10] XML_3.98-1.1    | base64enc_0.1-2         | bitops_1.0-6           |
| [13] brew_1.0-6      | checkmate_1.5.0         | codetools_0.2-9        |
| [16] digest_0.6.6    | fail_1.2                | foreach_1.4.2          |
| [19] iterators_1.0.7 | limma_3.22.1            | multtest_2.22.0        |
| [22] sendmailR_1.2-1 | splines_3.1.2           | stringr_0.6.2          |
| [25] survival_2.37-7 | tools_3.1.2             | zlibbioc_1.12.0        |