

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 ENSG00000197049	1_12_703729	+
2	1	559774	559874	101	1_41_559455 ENSG00000212678	1_41_559455	+
3	1	556660	556760	101	1_93_556427 ENSG00000212875	1_93_556427	+
4	1	1041646	1041746	101	1_11_1041174 ENSG00000131591	1_11_1041174	-
5	1	1270239	1270339	101	1_14_1269014 ENSG00000107404	1_14_1269014	-
6	1	926058	926158	101	1_20_925025 ENSG00000188290	1_20_925025	-
		feature	start_position	end_position	insideFeature	distancetoFeature	
1		ENSG00000197049	711183	712376	upstream	-7298	
2		ENSG00000212678	559619	560165	inside	155	
3		ENSG00000212875	556317	557859	inside	343	
4		ENSG00000131591	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.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))
> 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
12	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", "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,-1,1,1,1,1,1))
> 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	+
5	Site2	2	t2	2010898	2011108	+	2010897	2010997	+
10	Site7	2	t8	201089	201299	-	201089	201089	+
6	Site3	3	t3	2496700	2496920	+	2496704	2496804	+
7	Site4	4	t4	3075866	3076166	+	3075869	3075969	+
4	Site12	5	t17	167888600	167888999	+	167889600	167893599	+
8	Site5	5	t5	3123260	3123470	+	3123260	3123360	+
2	Site10	6	t15	1565000	1565399	+	1563000	1565199	+
3	Site11	6	t16	1569400	1571199	+	1569800	1573799	+
9	Site6	6	t6	3857500	3857780	+	3857501	3857601	+
11	Site8	6	t13	1549800	1550599	+	1543200	1555199	+
12	Site9	6	t14	1554400	1560799	+	1557200	1560599	+

	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	+
2	2	2010897	2010997	101	Site2	+
3	2	201089	201089	1	Site7	+

```

4    3  2496704  2496804  101 Site3    +
5    4  3075869  3075969  101 Site4    +
6    5 167889600 167893599 4000 Site12  +
7    5  3123260  3123360  101 Site5    +
8    6  1563000  1565199  2200 Site10  +
9    6  1569800  1573799  4000 Site11  +
10   6  3857501  3857601  101 Site6    +
11   6  1543200  1555199 12000 Site8    +
12   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     2  201089  201299   211   t8     -
4     3 2496700 2496920   221   t3     +
5     4 3075866 3076166   301   t4     +
6     5 167888600 167888999  400  t17    +
7     5 3123260 3123470   211   t5     +
8     6 1565000 1565399   400  t15    +
9     6 1569400 1571199  1800  t16    +
10    6 3857500 3857780   281   t6     +
11    6 1549800 1550599   800  t13    +
12    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)

```

`$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 +

```

9	Site7	2	t8	201089	201299	-	201089	201089	+
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	+
10	Site8	6	t13	1549800	1550599	+	1543200	1555199	+
11	Site9	6	t14	1554400	1560799	+	1557200	1560599	+

\$MergedPeaks

RangedData with 11 rows and 0 value columns across 6 spaces

	space	ranges	
	<factor>	<IRanges>	
TF1-Site1-TF2-t1	1	[967654, 967869]	
TF1-Site2-TF2-t2	2	[2010897, 2011108]	
TF1-Site7-TF2-t8	2	[201089, 201299]	
TF1-Site3-TF2-t3	3	[2496700, 2496920]	
TF1-Site4-TF2-t4	4	[3075866, 3076166]	
TF1-Site5-TF2-t5	5	[3123260, 3123470]	
TF1-Site10-TF2-t15	6	[1563000, 1565399]	
TF1-Site11-TF2-t16	6	[1569400, 1573799]	
TF1-Site6-TF2-t6	6	[3857500, 3857780]	
TF1-Site8-TF2-t13	6	[1543200, 1555199]	
TF1-Site9-TF2-t14	6	[1554400, 1560799]	

\$Peaks1withOverlaps

RangedData with 11 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]	+
Site10	6	[1563000, 1565199]	+
Site11	6	[1569800, 1573799]	+
Site6	6	[3857501, 3857601]	+
Site8	6	[1543200, 1555199]	+
Site9	6	[1557200, 1560599]	+

\$Peaks2withOverlaps

RangedData with 11 rows and 1 value column across 6 spaces

	space	ranges	strand
	<factor>	<IRanges>	<character>
t1	1	[967659, 967869]	+
t2	2	[2010898, 2011108]	+
t8	2	[201089, 201299]	-
t3	3	[2496700, 2496920]	+
t4	4	[3075866, 3076166]	+
t5	5	[3123260, 3123470]	+
t15	6	[1565000, 1565399]	+
t16	6	[1569400, 1571199]	+
t6	6	[3857500, 3857780]	+
t13	6	[1549800, 1550599]	+
t14	6	[1554400, 1560799]	+

[1] 12

[1] 17

\$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
1 GO:0000187
2 GO:0002573
3 GO:0002702
4 GO:0002761
5 GO:0002763
6 GO:0006213

      go.term
1      activation of MAPK activity
2 myeloid leukocyte differentiation
```



```

3 positive regulation of production of molecular mediator of immune response
4         regulation of myeloid leukocyte differentiation
5         positive regulation of myeloid leukocyte differentiation
6         pyrimidine nucleoside metabolic process

```

```

1
2
3
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                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 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 me
4 The portion of the nuclear lumen proximal to the inner nuclear me
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 ENSG00000218550 1 [1300503, 1300603] | 1_14_1300250
1_41_559455 ENSG00000212678 1 [ 559774,  559874] | 1_41_559455
1_93_556427 ENSG00000212875 1 [ 556660,  556760] | 1_93_556427
1_14_1300250 ENSG00000175756 1 [1300503, 1300603] | 1_14_1300250
1_41_559455 ENSG00000209350 1 [ 559774,  559874] | 1_41_559455
1_93_556427 ENSG00000209349 1 [ 556660,  556760] | 1_93_556427
      strand      feature start_position
<character> <character> <numeric>
1_14_1300250 ENSG00000218550 + ENSG00000218550 1303907
1_41_559455 ENSG00000212678 + ENSG00000212678  559619
1_93_556427 ENSG00000212875 + ENSG00000212875  556317
1_14_1300250 ENSG00000175756 - ENSG00000175756 1298973
1_41_559455 ENSG00000209350 - ENSG00000209350  557859
1_93_556427 ENSG00000209349 - ENSG00000209349  556239
end_position insideFeature distancetoFeature
<numeric> <character> <numeric>
1_14_1300250 ENSG00000218550 1304275 upstream -3354
1_41_559455 ENSG00000212678  560165 inside 205
1_93_556427 ENSG00000212875  557859 inside 393
1_14_1300250 ENSG00000175756 1300443 upstream -110
1_41_559455 ENSG00000209350  557930 upstream -1894
1_93_556427 ENSG00000209349  556304 upstream -406
shortestDistance fromOverlappingOrNearest
<numeric> <character>
1_14_1300250 ENSG00000218550 3304 NearestStart
1_41_559455 ENSG00000212678 155 NearestStart

```

```

1_93_556427 ENSG00000212875          343          NearestStart
1_14_1300250 ENSG00000175756          60          NearestStart
1_41_559455 ENSG00000209350         1844          NearestStart
1_93_556427 ENSG00000209349          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"                "GGNCCCK"
[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	ENSG00000202254	1 [100272800, 100272900]	1_11_100272487
1_11_108905539	ENSG00000186086	1 [108906025, 108906125]	1_11_108905539
1_11_110106925	ENSG00000065135	1 [110107266, 110107366]	1_11_110106925
1_11_110679983	ENSG00000197106	1 [110680468, 110680568]	1_11_110679983
1_11_110681677	ENSG00000197106	1 [110682124, 110682224]	1_11_110681677
1_11_110756560	ENSG00000116396	1 [110756822, 110756922]	1_11_110756560
	strand	feature	start_position
	<character>	<character>	<numeric>
1_11_100272487	ENSG00000202254	1 ENSG00000202254	100257218
1_11_108905539	ENSG00000186086	1 ENSG00000186086	108918435
1_11_110106925	ENSG00000065135	1 ENSG00000065135	110091233
1_11_110679983	ENSG00000197106	1 ENSG00000197106	110693108
1_11_110681677	ENSG00000197106	1 ENSG00000197106	110693108
1_11_110756560	ENSG00000116396	1 ENSG00000116396	110753965
	end_position	insideFeature	distancetoFeature
	<numeric>	<character>	<numeric>
1_11_100272487	ENSG00000202254	100257309 downstream	15582
1_11_108905539	ENSG00000186086	109013624 upstream	-12410
1_11_110106925	ENSG00000065135	110136975 inside	16033
1_11_110679983	ENSG00000197106	110744824 upstream	-12640
1_11_110681677	ENSG00000197106	110744824 upstream	-10984
1_11_110756560	ENSG00000116396	110776666 inside	2857

```
shortestDistance fromOverlappingOrNearest
```

		<numeric>	<character>
1_11_100272487	ENSG00000202254	15491	NearestStart
1_11_108905539	ENSG00000186086	12310	NearestStart
1_11_110106925	ENSG00000065135	16033	NearestStart
1_11_110679983	ENSG00000197106	12540	NearestStart
1_11_110681677	ENSG00000197106	10884	NearestStart
1_11_110756560	ENSG00000116396	2857	NearestStart

		symbol
		<factor>
1_11_100272487	ENSG00000202254	NA
1_11_108905539	ENSG00000186086	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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9. N. L. Johnson, S. Kotz and A. W. Kemp (1992) *Univariate Discrete Distributions*, Second Edition. New York: Wiley
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4 Session Info

```
> sessionInfo()
```

```
R version 2.15.3 (2013-03-01)
```

```
Platform: x86_64-unknown-linux-gnu (64-bit)
```

```
locale:
```

```
[1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
[3] LC_TIME=en_US.UTF-8      LC_COLLATE=C
[5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
[7] LC_PAPER=C                LC_NAME=C
[9] LC_ADDRESS=C             LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
```

```
attached base packages:
```

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

```
other attached packages:
```

```
[1] ChIPpeakAnno_2.6.1          limma_3.14.4
[3] org.Hs.eg.db_2.8.0         GO.db_2.8.0
[5] RSQLite_0.11.2             DBI_0.2-5
[7] AnnotationDbi_1.20.7       BSgenome.Ecoli.NCBI.20080805_1.3.17
[9] BSgenome_1.26.1           GenomicRanges_1.10.7
[11] Biostrings_2.26.3          IRanges_1.16.6
[13] multtest_2.14.0           Biobase_2.18.0
[15] biomaRt_2.14.0            BiocGenerics_0.4.0
[17] VennDiagram_1.5.1
```

```
loaded via a namespace (and not attached):
```

```
[1] MASS_7.3-23      RCurl_1.95-4.1  XML_3.95-0.2    parallel_2.15.3
[5] splines_2.15.3  stats4_2.15.3   survival_2.37-4 tools_2.15.3
```