



BioC2011: ChIPpeakAnno Practical

July 29th 2011

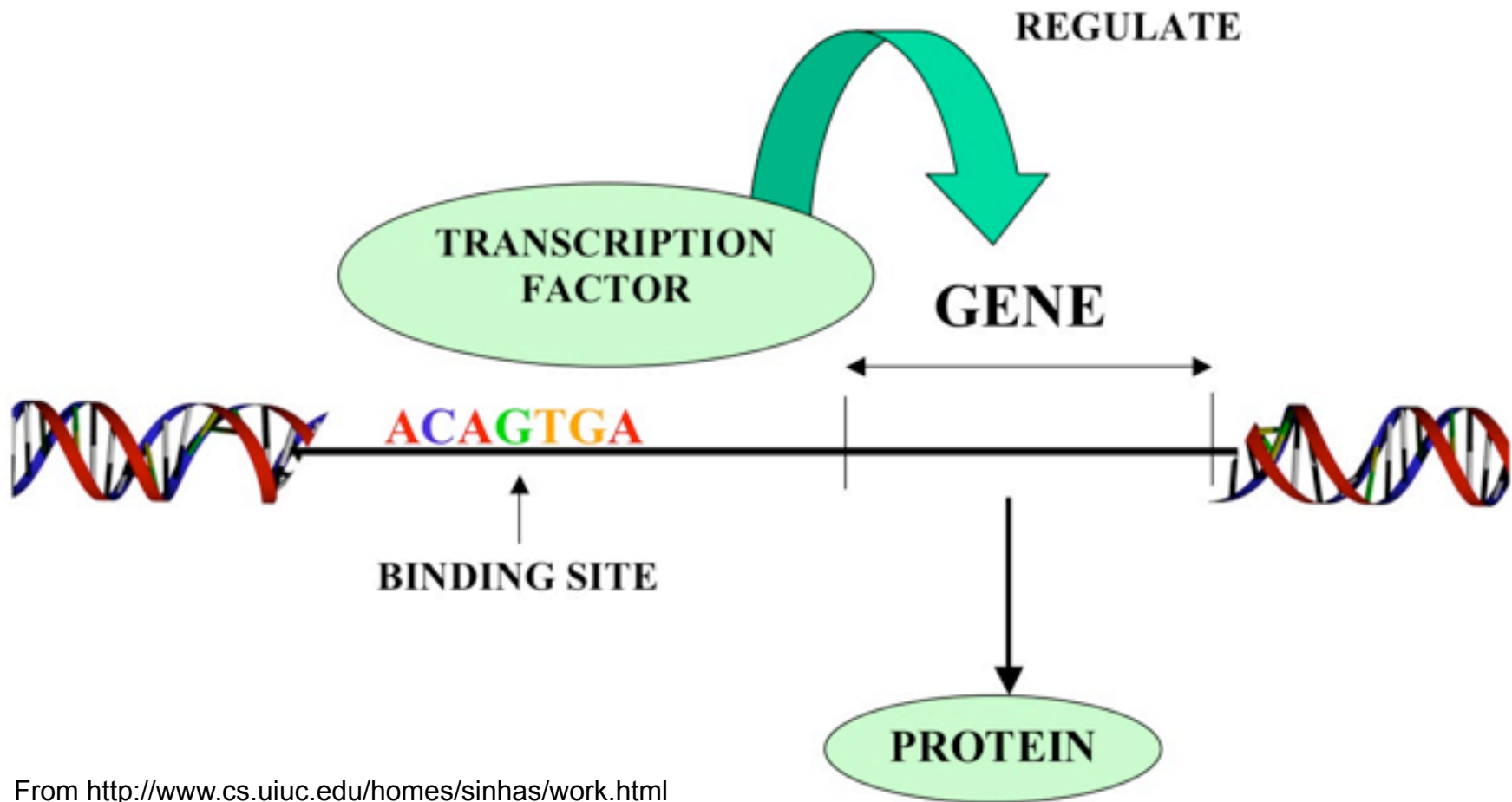
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THE HEART OF MASSACHUSETTS



Outline

- Motives
- Functionality
- Dependency
- Installation
- Demo
- Get help and Reference
- FAQ



From <http://www.cs.uiuc.edu/homes/sinhas/work.html>

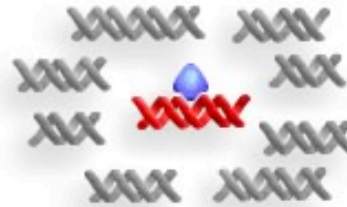
- ChIP (Chromatin immunoprecipitation)

Procedure

- From <http://www.bio.brandeis.edu/haberlab>



DNA-binding proteins are crosslinked to DNA with formaldehyde in vivo.



Isolate the chromatin. Shear DNA along with bound proteins into small fragments.



Bind antibodies specific to the DNA-binding protein to isolate the complex by precipitation. Reverse the cross-linking to release the DNA and digest the proteins.



Use PCR to amplify specific DNA sequences to see if they were precipitated with the antibody.



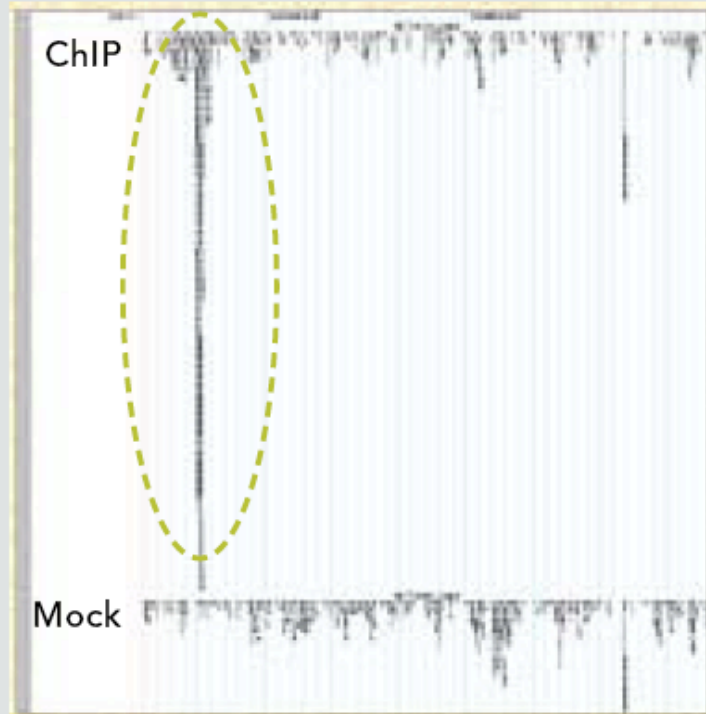
HIGH-THROUGHPUT IDENTIFICATION OF DNA BINDING SITES

- ChIP-seq
 - ChIP followed by high-throughput sequencing
- ChIP-chip
 - ChIP followed by genome tiling array analysis

PEAK IDENTIFICATION

- [Johnson DS, Mortazavi A, Myers RM, Wold B: Genome-wide mapping of in vivo protein-DNA interactions. Science 2007, 316\(5830\):1497-1502.](#)
- [Fejes AP, Robertson G, Bilenky M, Varhol R, Bainbridge M, Jones SJ: FindPeaks 3.1: a tool for identifying areas of enrichment from massively parallel short-read sequencing technology. Bioinformatics 2008, 24\(15\):1729-1730.](#)
- [Ji H, Jiang H, Ma W, Johnson DS, Myers RM, Wong WH: An integrated software system for analyzing ChIP-chip and ChIP-seq data. Nat Biotechnol 2008, 26\(11\):1293-1300.](#)
- [Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nussbaum C, Myers RM, Brown M, Li W et al: Model-based analysis of ChIP-Seq \(MACS\). Genome Biol 2008, 9\(9\):R137.](#)
- [Zhang ZD, Rozowsky J, Snyder M, Chang J, Gerstein M: Modeling ChIP sequencing in silico with applications. PLoS Comput Biol 2008, 4\(8\):e1000158.](#)
- [Sharon E, Lubliner S, Segal E: A feature-based approach to modeling protein-DNA interactions. PLoS Comput Biol 2008, 4\(8\):e1000154.](#)
- [Rozowsky J, Euskirchen G, Auerbach RK, Zhang ZD, Gibson T, Bjornson R, Carriero N, Snyder M, Gerstein MB: PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls. Nat Biotechnol 2009, 27\(1\):66-75.](#)
- [Zhang X. et al., PICS: Probabilistic Inference for ChIP-seq. Biometrics 2010](#)
- [Cairns J. et al., BayesPeak—an R package for analysing ChIP-seq data. Bioinformatics 2011, 27 \(5\): 713-714.](#)
- [Salmon-Divon M. et al., PeakAnalyzer: Genome-wide annotation of chromatin binding and modification loci. BMC Bioinformatics 2010, 11:415](#)

FIGURE 1: CHIP-SEQ TAGS IN BED FORMAT, DISPLAYED IN UCSC BROWSER



Example of custom tracks submitted in BED format (upper track is from ChIP sample and lower track is from mock control sample). The peak on the left in the ChIP sample (green circle) is significant. However, the peak on the right side is detected in both the ChIP and mock samples and is not significant.

From <http://www.illumina.com>

CHIPPEAKANNO

- Batch annotate enriched peaks
 - ChIP-seq
 - ChIP-chip
 - cap analysis of gene expression (CAGE)
 - SNP
 - any experiments resulting in a large number of enriched genomic regions

OUTPUT OF PEAK CALLING ALGORITHMS

- A file with at least the chromosome coordinate information if not in standard BED or GFF file format.
 - MACS (<http://liulab.dfci.harvard.edu/MACS/>) outputs BED file.
 - ChipSeq Peak Finder (http://woldlab.caltech.edu/html/chipseq_peak_finder) outputs BED file.
 - CisGenome (<http://www.biostat.jhsph.edu/~hji/cisgenome/>) for analyzing both ChIP-chip and ChIP-seq outputs cod file format which is a text file format, e.g., peak1 chr1 1000 2000 +
 - SSSRS for analyzing ChIP-seq dataset (<http://www.rajajothi.com/sissrs/>) outputs a text file with chr binding-site-start-position binding-site-end-position NumTags [Fold] [p-value]
 - NimbleScan software for analyzing ChIP-chip dataset output gff file.
 - QuEST (<http://mendel.stanford.edu/sidowlab/downloads/quest/>) outputs a text file with the first three columns containing name of the peak, chromosome and start-end, e.g., R-2
chr11 47556767-47557549)

FUNCTIONALITY

- Find the nearest genes for each set of peaks.
- Find all genes within a certain distance from the peaks.
- Identify enriched Gene Ontology (GO) terms associated with adjacent genes of the peaks.
- Label peaks with any annotation of interest
 - a dataset from the literature
 - CpG island
 - conserved element
 - histone modification marks
- Determine the significance of overlap and drawing Venn diagrams to visualize the extent of the overlap
 - binding sites among replicates
 - binding sites among transcription factors within a complex
 - binding sites among different experiments such as yours and the ones in literature
- Retrieve genomic sequences flanking putative binding sites
 - for motif discovery
 - for cloning
 - for PCR amplification

NEW FUNCTIONALITY

- Find the peaks with bi-directional promoters with summary statistics (\geq version 1.9.6).
- Summarize the occurrence of motifs in peaks (\geq version 1.9.6).
- The function `makeVennDiagram` supports 4-way venn diagram (\geq 1.9.5).
- Enriched Gene Ontology (GO) terms are annotated with a list of genes which can be traced back to peaks (\geq 1.9.5).

DEPENDENCY

- IRanges
 - Provides infrastructure for representing and manipulating sets of integer ranges, and implements algorithms for range-based calculations, matching and searching
- BSgenome
 - Supplies infrastructure for efficiently representing, accessing and analyzing whole genome
- Biostrings
 - Implements functions for pattern matching, sequence alignment and string manipulation
- GO.db
 - A set of annotation maps describing the entire Gene Ontology assembled using data from GO
- biomaR
 - Provides an R interface to a collection of databases implementing the BioMart software suite
- Gplots
 - Various R programming tools for plotting data
- Multtest
 - Non-parametric bootstrap and permutation re-sampling-based multiple testing procedures



INSTALLATION

Install R-2.13.1

Windows: [http://cran.fhcrc.org/bin/windows/
base/](http://cran.fhcrc.org/bin/windows/base/)

OS X: <http://cran.fhcrc.org/bin/macosx/>

Source (Linux): [http://cran.fhcrc.org/
sources.html](http://cran.fhcrc.org/sources.html)

INSTALLATION

The gplots package can be obtained at <http://cran.r-project.org/web/packages/gplots/index.html>

All the other dependent packages can be installed from R as:

```
source("http://bioconductor.org/biocLite.R")
biocLite(c("IRanges", "Biostring",
           "BSgenome", "biomaRt", "GO.db", "multtest",
           "limma", "org.Hs.eg.db", "BSgenome.Ecoli.NCBI.
           20080805"))
```

ChIPpeakAnno can be installed from R as:

```
source("http://bioconductor.org/biocLite.R")
biocLite("ChIPpeakAnno")
```



INSTALLATION FOR THE LAB

- Download and install R-2.14 <http://www.bioconductor.org/help/course-materials/2011/BioC2011/>
- `source("http://bioconductor.org/BioC2011.R");`
- `pkgInstall(c("ChIPpeakAnno", "biomaRt", "multtest", "IRanges", "Biostrings", "BSgenome", "BSgenome.Ecoli.NCBI.20080805", "GO.db", "org.Hs.eg.db", "limma", "gplots"))`
- `biocLite("multtest", type="source")`

MAIN FUNCTIONS

annotatePeakInBatch

- Find the nearest genes for each set of peaks
- Find all genes within a certain distance from the peaks
- Label peaks with any annotation of interest
 - a dataset from the literature
 - CpG island
 - conserved element

```
annotatePeakInBatch(myPeakList, mart, featureType = c("TSS",  
"miRNA", "Exon"), AnnotationData, output=c("nearestStart", "overlapping",  
"both"), multiple=c(FALSE, TRUE), maxgap=0, PeakLocForDistance =  
c("start", "middle", "end"), FeatureLocForDistance = c("TSS",  
"middle", "start", "end", geneEnd))
```

PARAMETERS

- **myPeakList**
 - Peaks as RangedData
- **mart**
 - used if AnnotationData not supplied, a mart object, see useMart of bioMaRt package for details
- **featureType**
 - used if AnnotationData not supplied, TSS, miRNA or exon
- **AnnotationData**
 - annotation data obtained from getAnnotation or customized annotation of class RangedData. If not supplied, then annotation will be obtained from biomaRt automatically
- **output**
 - nearestStart (default): will output the nearest features calculated as peak start - feature start (feature end if feature resides at minus strand);
 - overlapping: will output overlapping features with maximum gap =maxgap between peak range and feature range
 - both: will output all the nearest features, in addition, will output any features that overlap the peak that is not the nearest features.
- **Multiple:** not applicable when output is nearestStart. TRUE: output multiple overlapping features for each peak. FALSE: output at most one overlapping feature for each peak
- **Maxgap:** Non-negative integer. Intervals with a separation of maxgap or less are considered to be overlapping



MORE PARAMETERS

- PeakLocForDistance
 - Specify the location of peak for calculating distance, i.e., middle means using middle of the peak to calculate distance to feature, start means using start of the peak to calculate the distance to feature. To be compatible with previous version, by default using start
- FeatureLocForDistance
 - Specify the location of feature for calculating distance, i.e., start/end/middle means using start/end/middle of the feature to calculate the distance to feature, TSS means using start of feature when feature is on plus strand and using end of feature when feature is on minus strand, geneEnd means using end of feature when feature is on plus strand and using start of feature when feature is on minus strand. To be compatible with previous version, by default using TSS

OUTPUT

- RangedData
 - start: the start position of the peak
 - end : the end position of the peak
 - space: the chromosome location where the peak is located
 - Feature: id of the feature such as ensembl gene ID
 - start_position: start position of the feature such as gene
 - end_position: end position of the feature such as the gene
 - shortestDistance: the shortest distance from either end of peak to either end the feature.
 - fromOverlappingOrNearest
 - NearestStart: indicates this feature's start (feature's end for features at minus strand) is closest to the peak start
 - Overlapping: indicates this feature overlaps with this peak although it is not the nearest feature start
 - strand
 - 1 or + for positive strand and -1 or - for negative strand where the feature is located
- insideFeature:
 - upstream: peak resides upstream of the feature;
 - downstream: peak resides downstream of the feature;
 - inside: peak resides inside the feature;
 - overlapStart: peak overlaps with the start of the feature;
 - overlapEnd: peak overlaps with the end of the feature;
 - includeFeature: peak include the feature entirely
- distancetoFeature
 - distance to the nearest feature. By default, the distance is calculated as the distance between the start of the binding site and the TSS. Please refer to PeakLocForDistance and FeatureLocForDistance for details



DEMO

**EXAMPLE 1: FINDING THE NEAREST GENE AND THE DISTANCE TO
THE TRANSCRIPTION START SITE OF THE NEAREST GENE.**

```
library(ChIPpeakAnno)
data(myPeakList)
data(TSS.human.GRCh37)
annotatedPeak = annotatePeakInBatch (myPeakList[1:6,],
  AnnotationData = TSS.human.GRCh37)
#The annotated peaks can be saved as an Excel file for biologists to
  view easily.
write.table(as.data.frame(annotatedPeak),
  file="annotatedPeakList.xls", sep="\t", row.names=FALSE)
```

Plot the distribution of the peaks relative to the TSS

Gives a birds-eye view of the peak distribution relative to the genomic features of interest.

```
data(annotatedPeak)
```

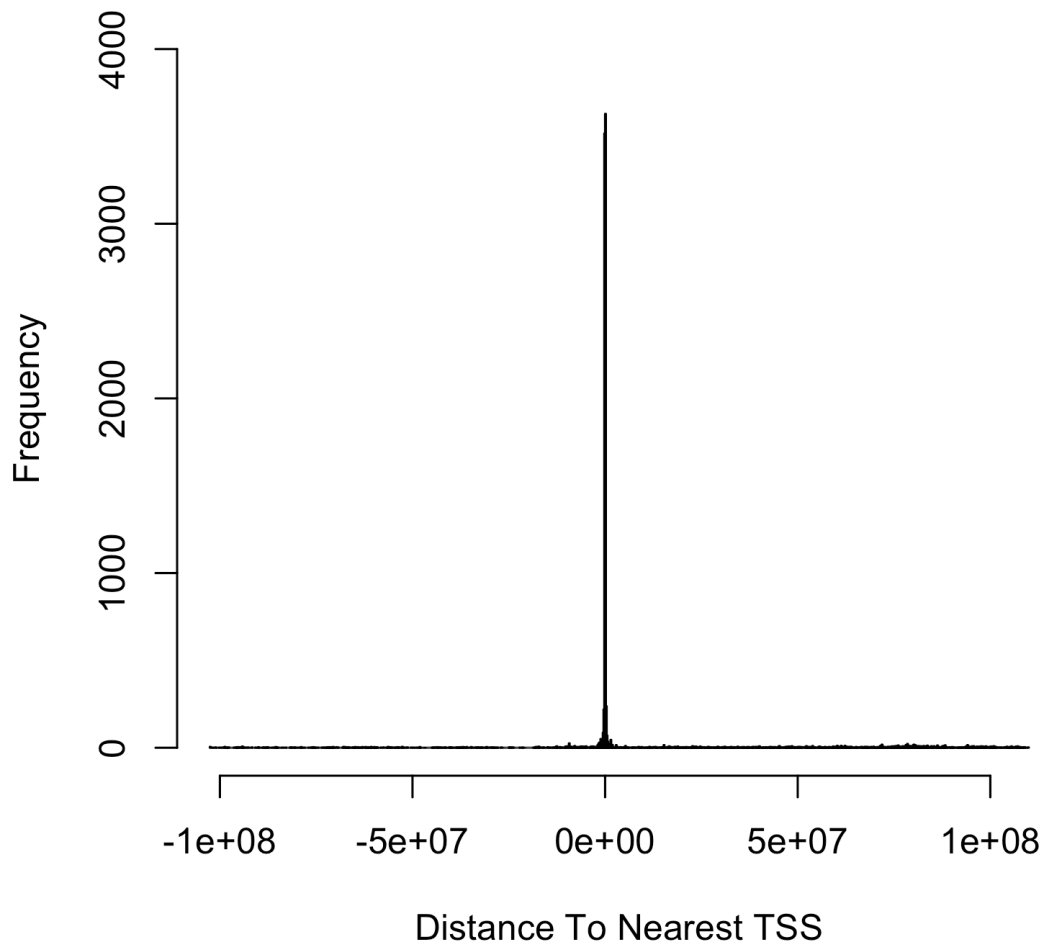
```
y = annotatedPeak$distancetoFeature[!is.na(annotatedPeak$distancetoFeature)  
& annotatedPeak$fromOverlappingOrNearest == "NearestStart"]
```

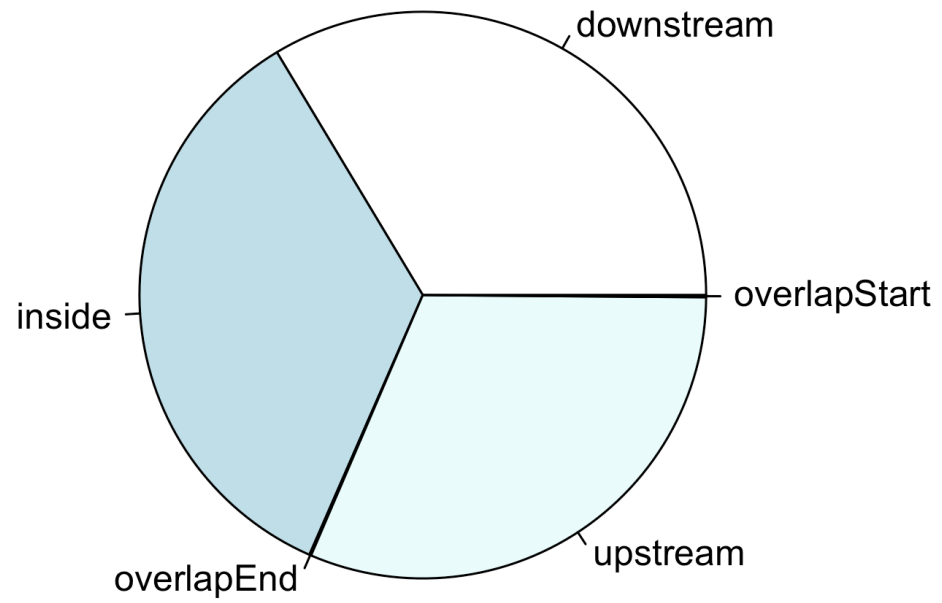
```
hist(y, xlab="Distance To Nearest TSS", main="", breaks=1000,  
xlim=c(min(y)-100, max(y)+100))
```

```
temp = as.data.frame(annotatedPeak)
```

```
y = annotatedPeak$distancetoFeature[!is.na(annotatedPeak$distancetoFeature)  
& annotatedPeak$fromOverlappingOrNearest == "NearestStart" &  
abs(annotatedPeak$distancetoFeature) < 10000]
```

```
pie(table(temp[as.character(temp$fromOverlappingOrNearest) == "Overlapping" |  
(as.character(temp$fromOverlappingOrNearest) == "NearestStart" & !temp$peak  
%in% temp[as.character(temp$fromOverlappingOrNearest) == "Overlapping", ]  
$peak) ,]$insideFeature))
```



Obtain annotation on-line using *getAnnotation*

```
mart = useMart(biomart="ensembl",  
              dataset="hsapiens_gene_ensembl")  
  
#Annotation = getAnnotation(mart, featureType="TSS")  
Annotation = getAnnotation(mart, featureType="miRNA")  
as.data.frame(Annotation)[1:10,]
```

To obtain annotation with other genomic features, it is necessary to change the *featureType* (e.g., "exon" for exon, "miRNA" for miRNA, "5utr" for 5' utr, "3utr" for 3' utr, and "ExonPlusUtr" for Exon plus utr).

Example 2: Label the peaks from your experiment with a list of peaks in the literature

```
myexp =  
RangedData(IRanges(start=c(1543200,1557200,1563000,1569800,  
167889600,100,1000), end=c(1555199,1560599,1565199,1573799,  
167893599,200,1200), names=c("p1","p2","p3","p4","p5","p6", "p7")),  
strand=as.integer(1),space=c(6,6,6,6,5,4,4))  
literature =  
RangedData(IRanges(start=c(1549800,1554400,1565000,1569400,16  
7888600,120,800),  
end=c(1550599,1560799,1565399,1571199,167888999,140,1400),  
names=c("f1","f2","f3","f4","f5","f6","f7")), strand=c(1,1,1,1,1,-1,-1),  
space=c(6,6,6,6,5,4,4))  
annotatedPeak1= annotatePeakInBatch(myexp, AnnotationData =  
literature, output="both", maxgap=1000, multiple=TRUE)  
pie(table(as.data.frame(annotatedPeak1)$insideFeature))  
as.data.frame(annotatedPeak1)
```

Example 2 – Cont. Different Parameter Setting

```
annotatedPeak1= annotatePeakInBatch(myexp,  
AnnotationData = literature, output="overlapping",  
maxgap=1000, multiple=TRUE)  
as.data.frame(annotatedPeak1)  
annotatedPeak1= annotatePeakInBatch(myexp,  
AnnotationData = literature, output="nearestStart",  
PeakLocForDistance ="middle",  
FeatureLocForDistance="middle")  
as.data.frame(annotatedPeak1)
```

BED2RangedData and GFF2RangedData

```
test.bed = data.frame(cbind(chrom = c("4", "6"),  
chromStart=c("100", "1000"),chromEnd=c("200", "1100"),  
name=c("peak1", "peak2")))  
test.rangedData = BED2RangedData(test.bed)  
as.data.frame(annotatePeakInBatch(test.rangedData,  
AnnotationData = literature))  
test.GFF = data.frame(cbind(seqname = c("chr4", "chr4"),  
source=rep("Macs", 2), feature=rep("peak", 2), start=c("100",  
"1000"), end=c("200", "1100"), score=c(60, 26), strand=c(1, 1),  
frame=c(".", 2), group=c("peak1", "peak2")))  
test.rangedData = GFF2RangedData(test.GFF)  
as.data.frame(annotatePeakInBatch(test.rangedData,  
AnnotationData = literature))
```

BED2RangedData and GFF2RangedData

```
test.bed = data.frame(cbind(chrom = c("4", "6"),  
chromStart=c("100", "1000"),chromEnd=c("200", "1100"),  
name=c("peak1", "peak2")))  
test.rangedData = BED2RangedData(test.bed)  
as.data.frame(annotatePeakInBatch(test.rangedData,  
AnnotationData = literature))  
test.GFF = data.frame(cbind(seqname = c("chr4", "chr4"),  
source=rep("Macs", 2), feature=rep("peak", 2), start=c("100",  
"1000"), end=c("200", "1100"), score=c(60, 26), strand=c(1, 1),  
frame=c(".", 2), group=c("peak1", "peak2")))  
test.rangedData = GFF2RangedData(test.GFF)  
as.data.frame(annotatePeakInBatch(test.rangedData,  
AnnotationData = literature))
```

EXAMPLE 3: DETERMINE THE SIGNIFICANCE OF THE OVERLAPPING AND VISUALIZE THE OVERLAP AS A VENN DIAGRAM AMONG DIFFERENT DATASETS.

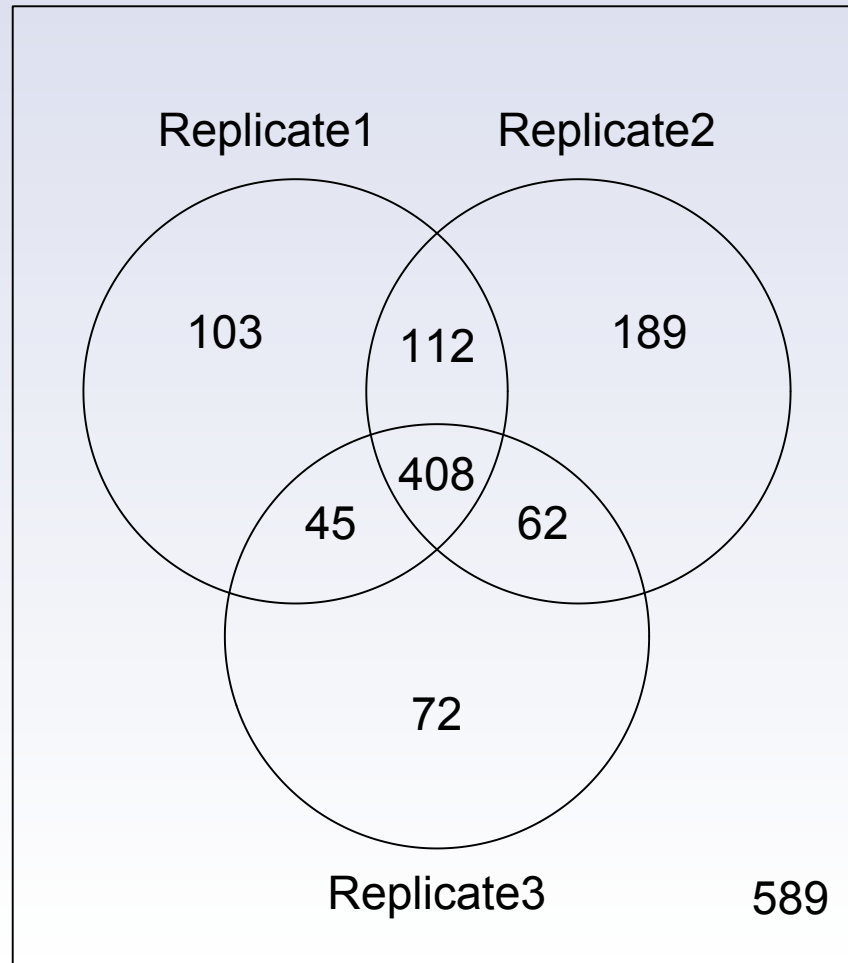
Overlap Significance Testing and Visualization

```
data(Peaks.Ste12.Replicate1)
```

```
data(Peaks.Ste12.Replicate2)
```

```
data(Peaks.Ste12.Replicate3)
```

```
makeVennDiagram(RangedDataList(Peaks.Ste12.Repli  
cate1, Peaks.Ste12.Replicate2,  
Peaks.Ste12.Replicate3), NameOfPeaks =  
c("Replicate1", "Replicate2", "Replicate3"), maxgap =  
0, totalTest = 1580)
```



Combine the Overlapping Peaks Across Replicates

MergedPeaks =

```
findOverlappingPeaks(findOverlappingPeaks(Peaks.Ste12.Replicate1, Peaks.Ste12.Replicate2, maxgap = 0, multiple = F, NameOfPeaks1 = "R1", NameOfPeaks2 = "R2")$MergedPeaks, Peaks.Ste12.Replicate3, maxgap = 0, multiple = F, NameOfPeaks1 = "R1R2", NameOfPeaks2 = "R3")$MergedPeak
```

```
as.data.frame(MergedPeaks)
```

**EXAMPLE 4: OBTAIN THE SEQUENCES AROUND THE BINDING
SITES FOR PCR AMPLIFICATION OR MOTIF DISCOVERY**

Obtain Genomic DNA sequences

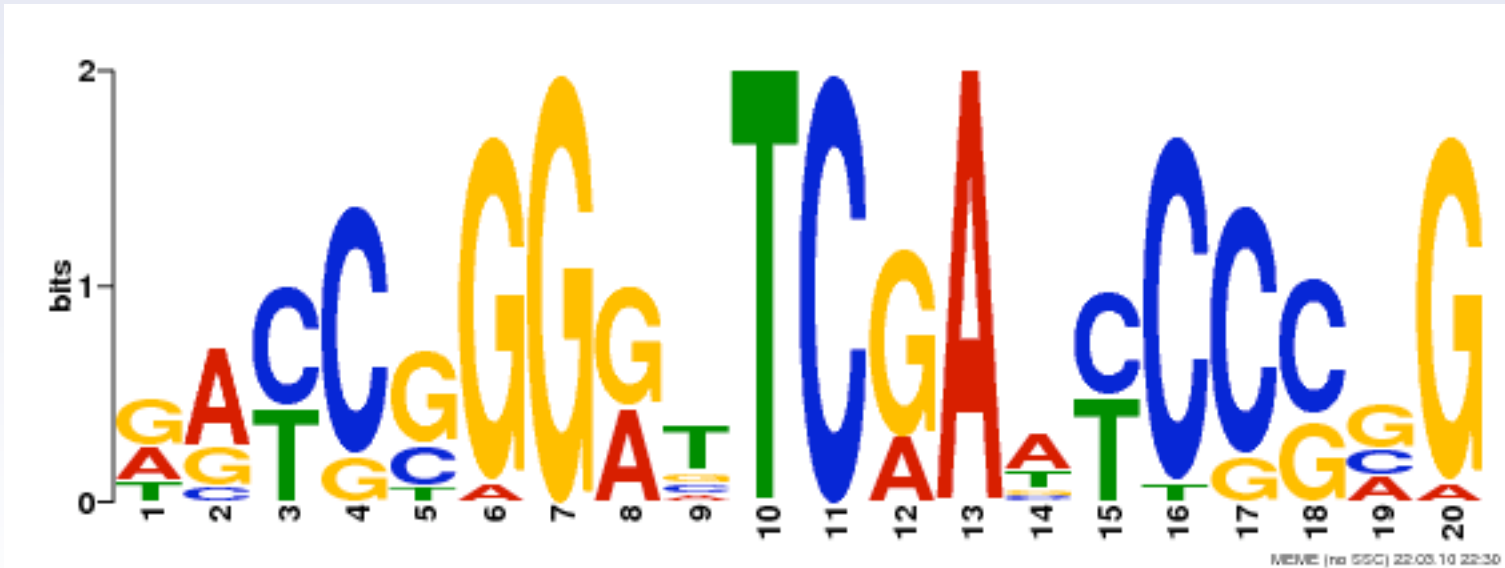
```
peaks = RangedData(IRanges(start = c(100, 500), end = c(300, 600), names = c("peak1", "peak2")), space = c("NC_008253", "NC_010468"))
```

```
library(BSgenome.Ecoli.NCBI.20080805)
```

```
peaksWithSequences = getAllPeakSequence(peaks, upstream = 100, downstream = 100, genome = Ecoli)
```

#To convert the sequences to a common FASTA file format, the following function is called.

```
write2FASTA(peaksWithSequences, file="test.fa", width=50)  
available.genomes()
```



EXAMPLE 5: OBTAIN ENRICHED GO TERMS NEAR THE PEAKS

Obtain Enriched GO

```
data(annotatedPeak)
```

```
library(org.Hs.eg.db)
```

```
enrichedGO <- getEnrichedGO (annotatedPeak[1:6,],  
  orgAnn="org.Hs.eg.db", maxP=0.1, multiAdj =TRUE,  
  minGOterm=1, multiAdjMethod="BH")
```

Parameters

maxP is the maximum p-value required to be considered to be significant

multiAdj indicates whether to apply multiple hypothesis testing adjustment

minGOterm is the minimum count in a genome for a GO term to be included

multiAdjMethod is the multiple testing procedure to be applied

orgAnn is the organism specific GO annotation (<http://www.bioconductor.org/packages/release/data/annotation/> for additional org.xx.eg.db packages)

Additional Parameters

annotatedPeak: RangedData with a feature column or a character vector of feature_ids with type of feature_id_type
feature_id_type: ensembl_gene_id, refseq_id, gene_symbol or entrez_id

Caveat

Not all species specific GO annotation package are Enrez ID centric

`org.At.tair.db` (Arabidopsis) is TAIR ID centric

`org.Sc.sgd.db` (Yeast) is orf centric

Work around:

set `annotatedPeak` to a character vector of species-specific IDs

set `feature_type_id` as `entrez_id`

```
enrichedGO.Arab <- getEnrichedGO (tarIDs, feature_id_type="entrez_id",  
orgAnn="org.At.tair.db", maxP=0.05, multiAdj =TRUE, minGOterm=10,  
multiAdjMethod="BH")
```

```
enrichedGO.Cse4 <- getEnrichedGO (orfs, feature_id_type="entrez_id",  
orgAnn="org.Sc.sgd.db", maxP=0.05, multiAdj =TRUE, minGOterm=5,  
multiAdjMethod="BH")
```

NEW FEATURES AND FUTURE PLAN

- New Features

- Find the peaks with bi-directional promoters with summary statistics (\geq version 1.9.6).
- Summarize the occurrence of motifs in peaks (\geq version 1.9.6).
- The function `makeVennDiagram` supports 4-way venn diagram (\geq 1.9.5).
- Enriched Gene Ontology (GO) terms are annotated with a list of `entrez_ids` (\geq 1.9.5).

- Future Plan

- De novo motif discovery in the peaks probably interfacing with `rGADEM`, `BCRANK` and `MotIV`
- Add other gene IDs such as gene symbol to `annotatedPeaks` and enriched GO annotation in condensed/expanded format

NEW FEATURES

- Find the peaks with bi-directional promoters with summary statistics (\geq version 1.9.6).
 - `data(myPeakList)`
 - `temp = peaksNearBDP(myPeakList[1:10,],
AnnotationData=TSS.human.GRCh37, MaxDistance=5000,
PeakLocForDistance="middle", FeatureLocForDistance = "TSS")`
 - `c(temp$percentPeaksWithBDP, temp$n.peaksWithBDP, temp
$n.peaks)`
 - `temp$peaksWithBDP`
- Summarize the occurrence of motifs in peaks (\geq version 1.9.6).
 - `filePath = system.file("extdata", "examplePattern.fa",
package="ChIPpeakAnno")`
 - `summarizePatternInPeaks(patternFilePath=filePath, skip=0L,
format="fasta", BSgenomeName=Ecoli, peaks=peaks,
outfile="testExample10.xls", append=FALSE)`

MORE NEW FEATURES

- The function `makeVennDiagram` supports 4-way venn diagram ($\geq 1.9.5$).
 - `makeVennDiagram(RangedDataList(Peaks.Ste12.Replicate1, Peaks.Ste12.Replicate2, Peaks.Ste12.Replicate3, rbind(Peaks.Ste12.Replicate1,Peaks.Ste12.Replicate2)), NameOfPeaks = c("Replicate1","Replicate2","Replicate3", "Replicate12"), maxgap = 0, totalTest = 1580)`
- Enriched Gene Ontology (GO) terms are annotated with a list of `entrez_ids` ($\geq 1.9.5$)

REFERENCE AND HELP

- ?ChIPpeakAnno in a R session
- browseVignettes("ChIPpeakAnno")
- Zhu LJ*, Gazin C, Lawson ND, Pages H, Lin SM, Lapointe DS and Green MR. (2010) [* denotes corresponding author]
ChIPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. BMC Bioinformatics 2010, 11:237.

FREQUENTLY ASKED QUESTIONS

- makeVennDiagram
 - <https://stat.ethz.ch/pipermail/bioconductor/2010-June/033941.html>
 - <https://stat.ethz.ch/pipermail/bioconductor/2010-November/036540.html>
 - <http://comments.gmane.org/gmane.science.biology.informatics.conductor/32345>
 - <https://stat.ethz.ch/pipermail/bioconductor/attachments/20100905/bb689e19/attachment.pl>
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/29476>
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/30115>
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/35629>
 - <http://comments.gmane.org/gmane.science.biology.informatics.conductor/34765>

FREQUENTLY ASKED QUESTIONS

- **getEnrichedGO**
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/35033>
 - <http://comments.gmane.org/gmane.science.biology.informatics.conductor/35870>
 - <https://stat.ethz.ch/pipermail/bioconductor/2010-June/033909.html>
 - <https://stat.ethz.ch/pipermail/bioconductor/2011-January/037220.html>
 - <http://www.mentby.com/julie-zhu-2/concerning-your-response-to-error-message-working-with-chippeakanno.html>
- **annotatePeakInBatch and BED2RangedData**
 - <http://answerpot.com/showthread.php?1310386-about+ChIPpeakAnno>
 - <http://www.mail-archive.com/bioc-sig-sequencing@r-project.org/msg01024.html>
 - <http://comments.gmane.org/gmane.science.biology.informatics.conductor/28493>
 - <https://stat.ethz.ch/pipermail/bioconductor/2011-January/037435.html>
 - <https://stat.ethz.ch/pipermail/bioconductor/2011-March/038302.html>
 - <https://stat.ethz.ch/pipermail/bioconductor/2011-March/038125.html>
 - <http://blog.gmane.org/gmane.science.biology.informatics.conductor>
- **findOverlappingPeaks**
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/32880>
 - <https://stat.ethz.ch/pipermail/bioconductor/2011-January/037484.html>
 - <http://permalink.gmane.org/gmane.science.biology.informatics.conductor/36036>

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