
# ChIPpeakAnno

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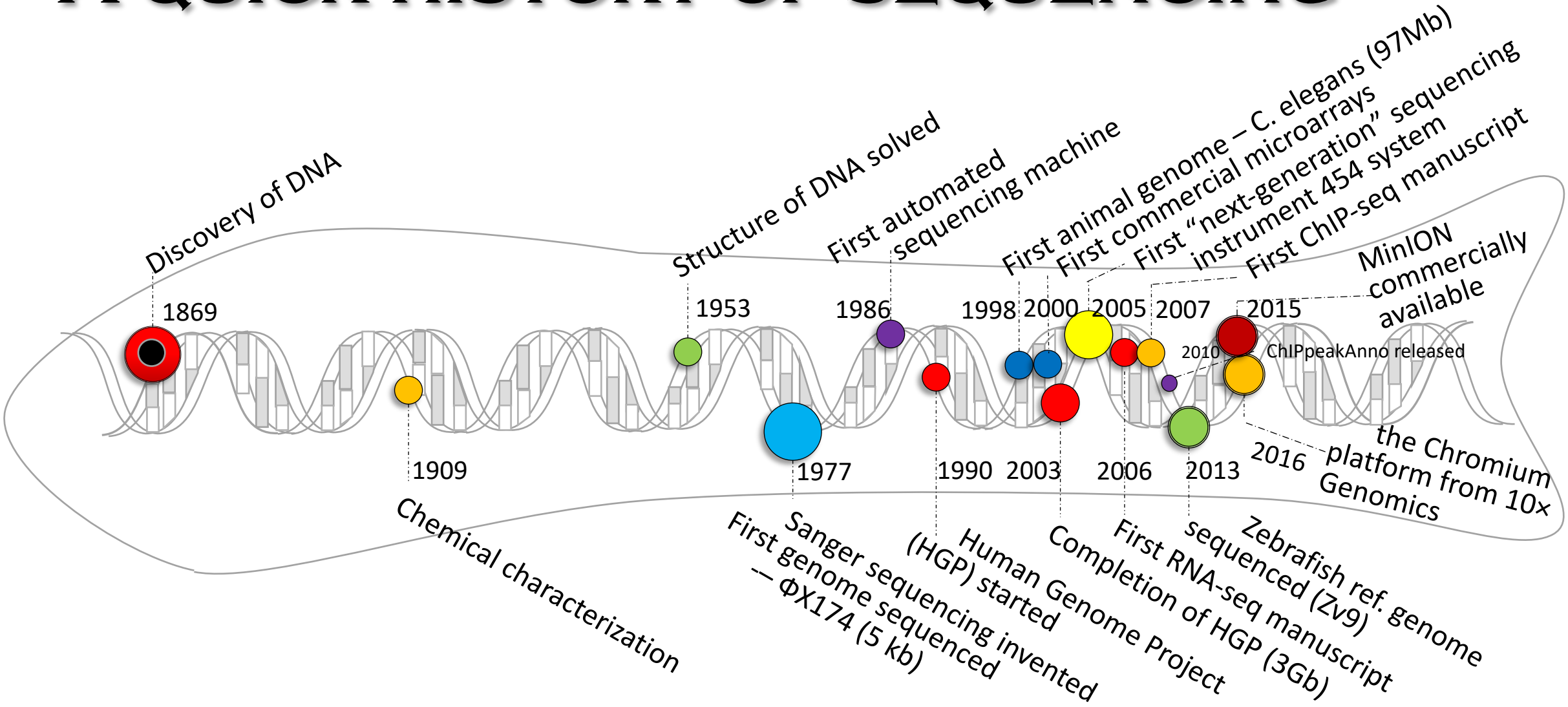
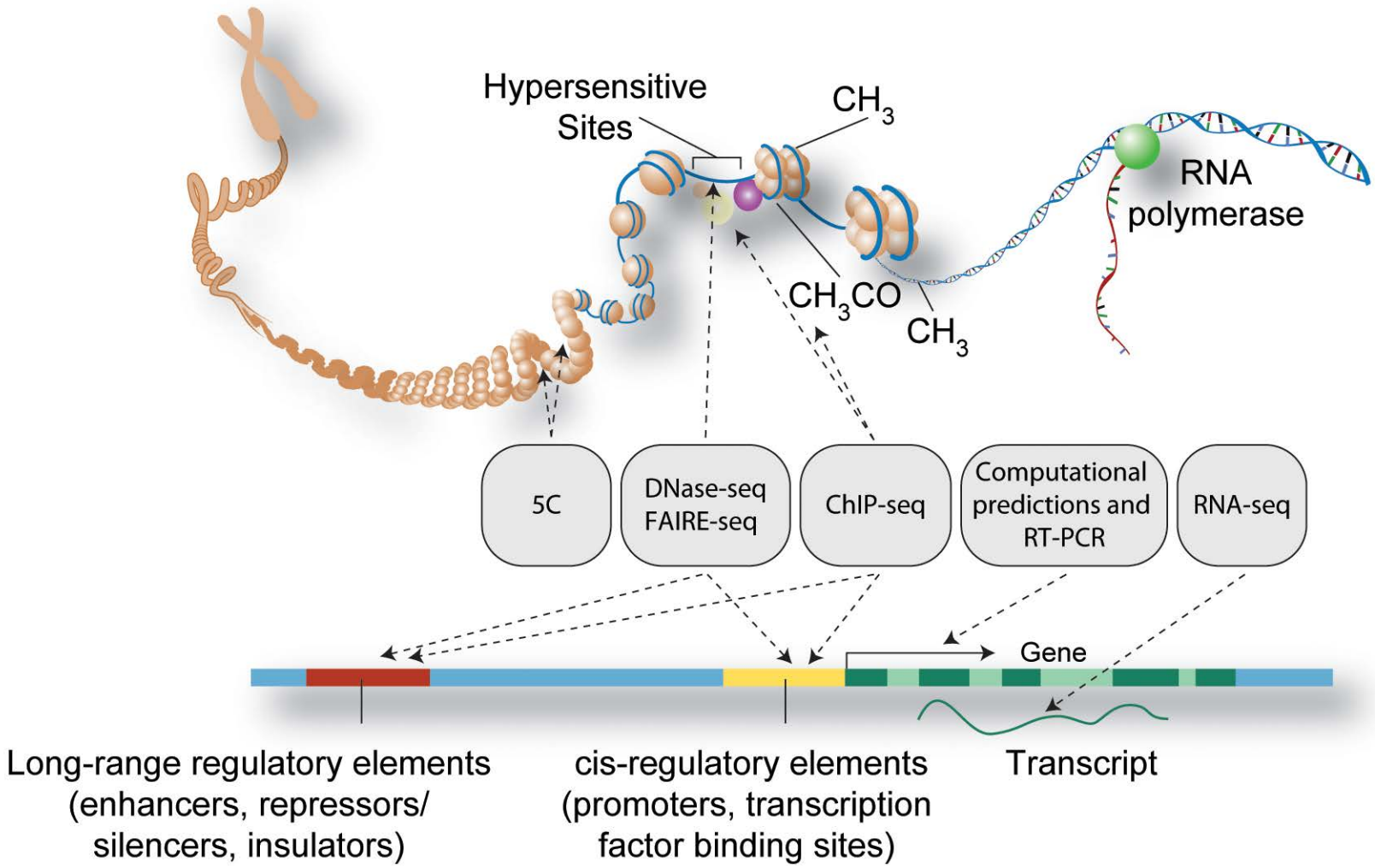
# Install the workshop pkg

```
## set the working directory,
## replace "~/Downloads/ATACseqQCworkshop" by your path
wd <- "~/Downloads/workshop2020"
dir.create(wd)
setwd(wd)
library(BiocManager)
install("jianhong/workshop2020", build_vignettes = TRUE)
vignette("ChIPpeakAnno", package="workshop2020")
```

https://github.com/jianhong/workshop2020
https://bioconductor.org/packages/ChIPpeakAnno
Slides:
https://github.com/jianhong/workshop2020/blob/master/inst/extdata/ChIPpeakAnno_workshop2020.pdf

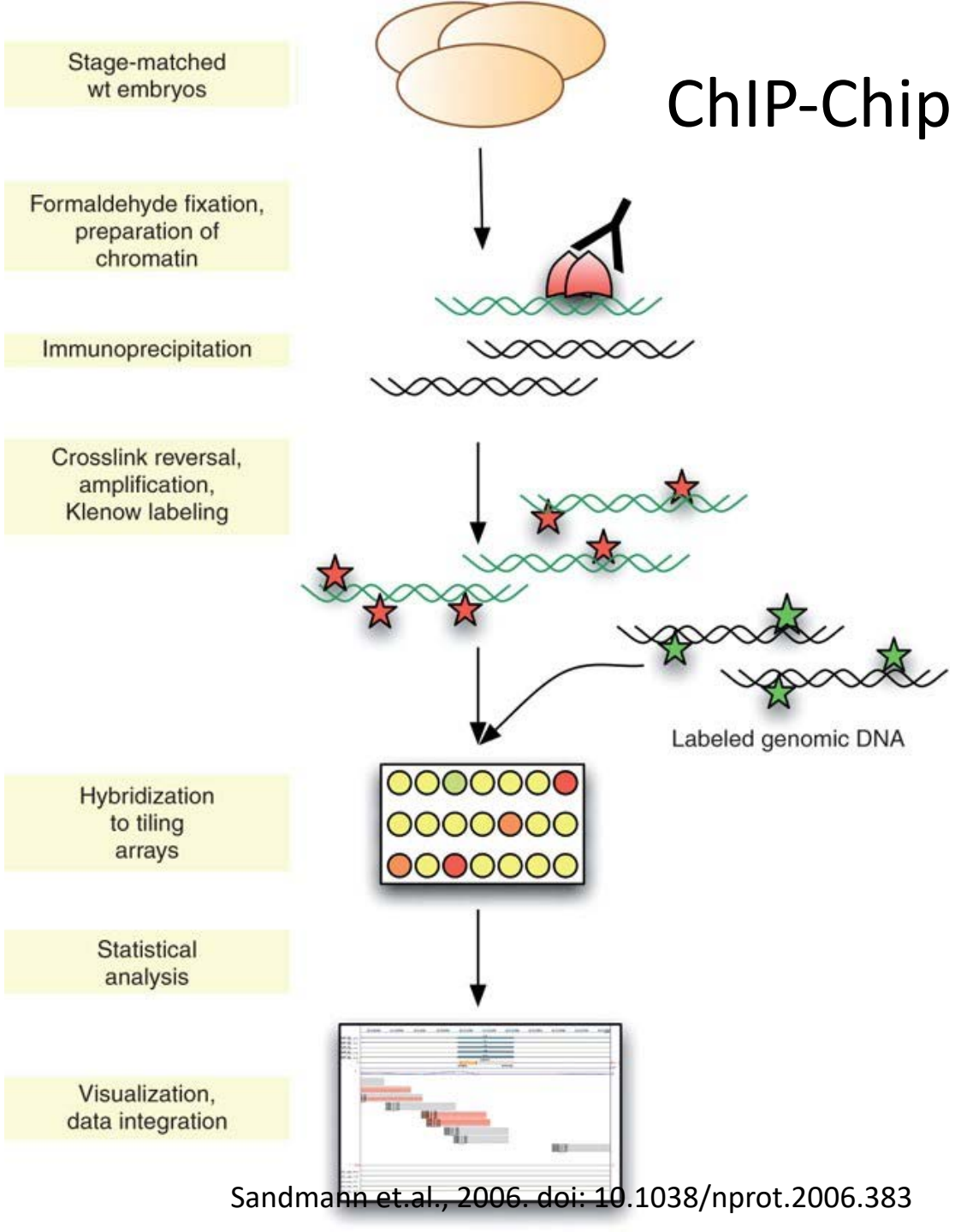
# A QUICK HISTORY OF SEQUENCING





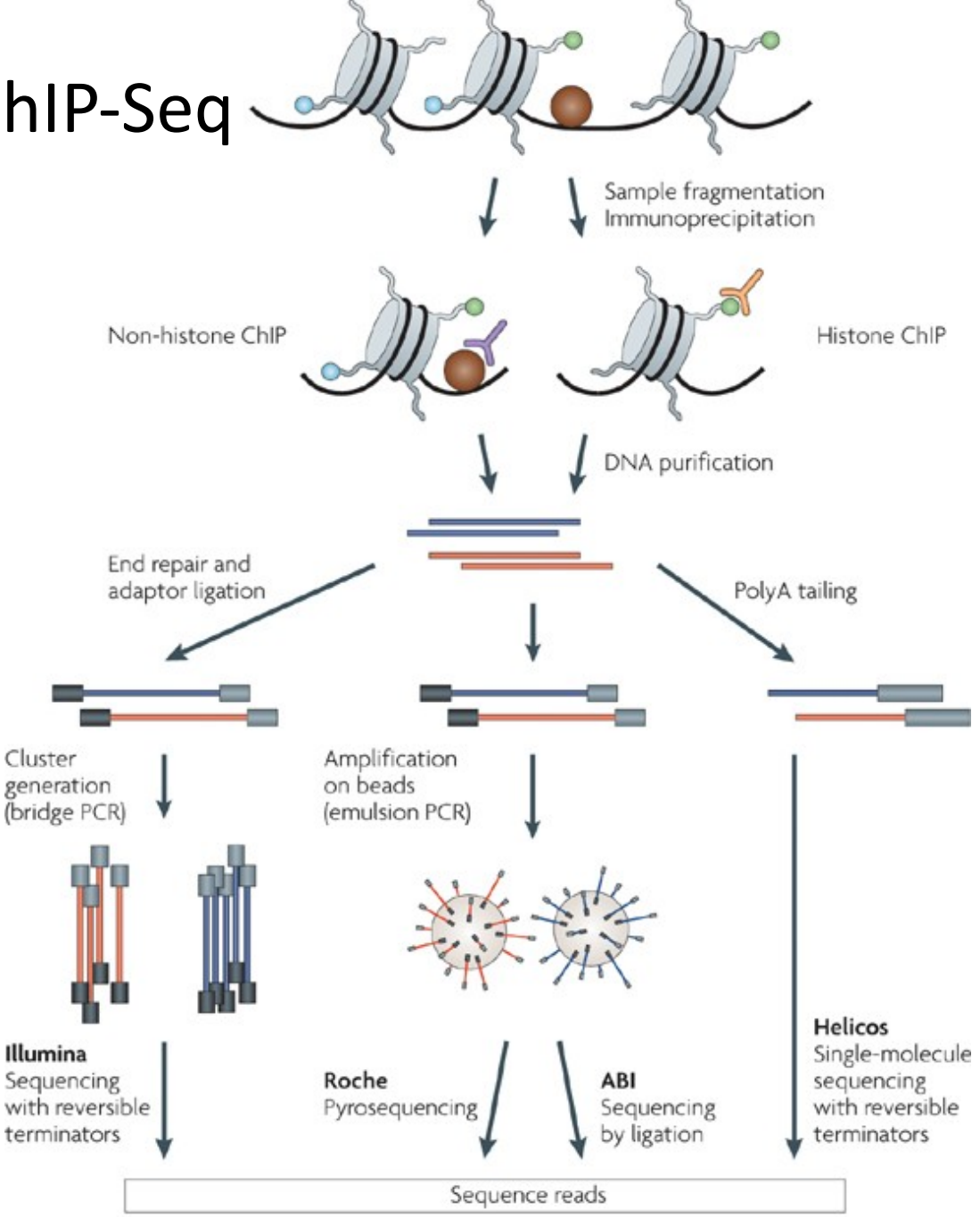

The ENCODE Project Consortium 2011. doi: 10.1371/journal.pbio.1001046

# ChIP-Chip




Sandmann et.al., 2006. doi: 10.1038/nprot.2006.383

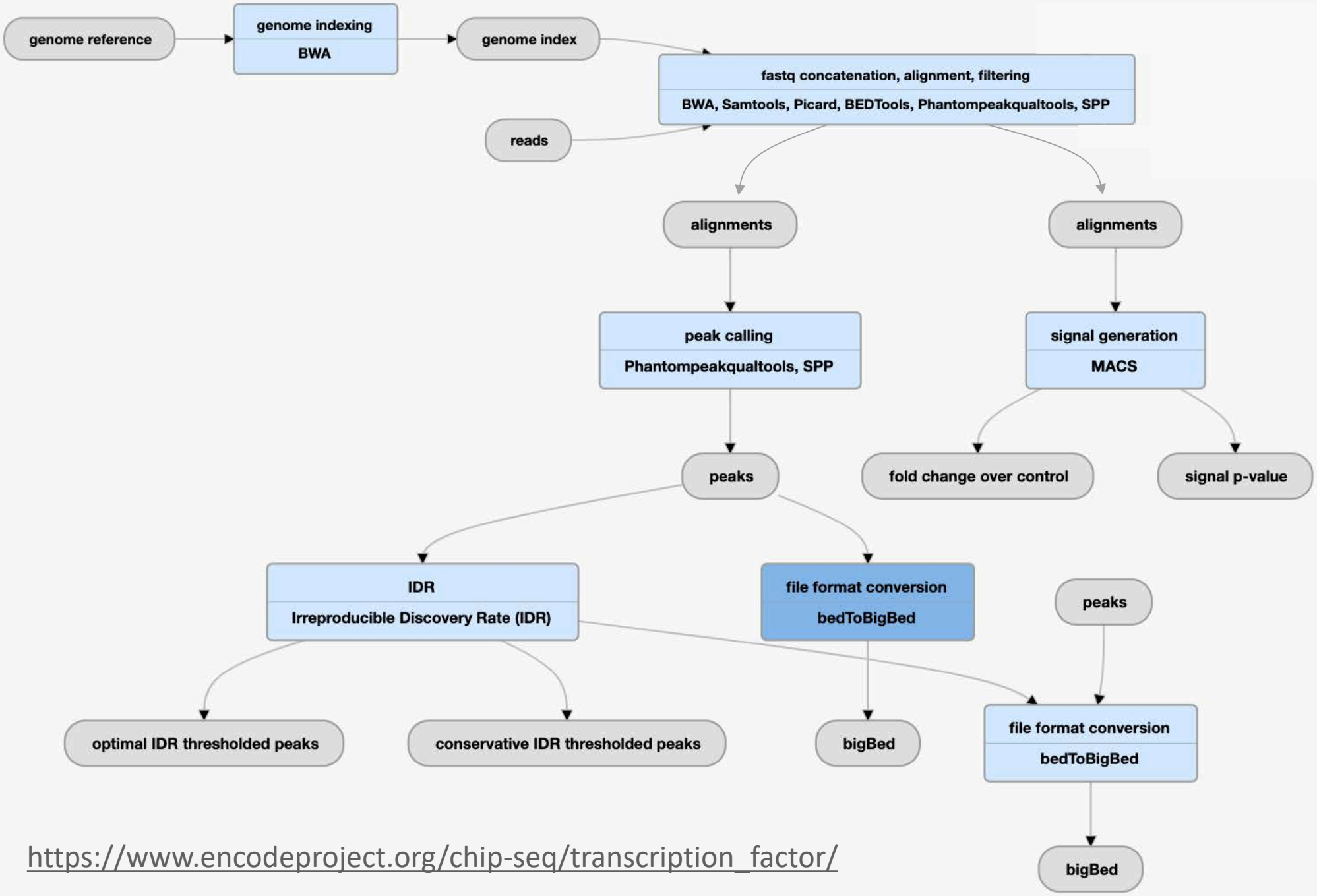
# ChIP-Seq




Park 2009. doi:10.1038/nrg2641

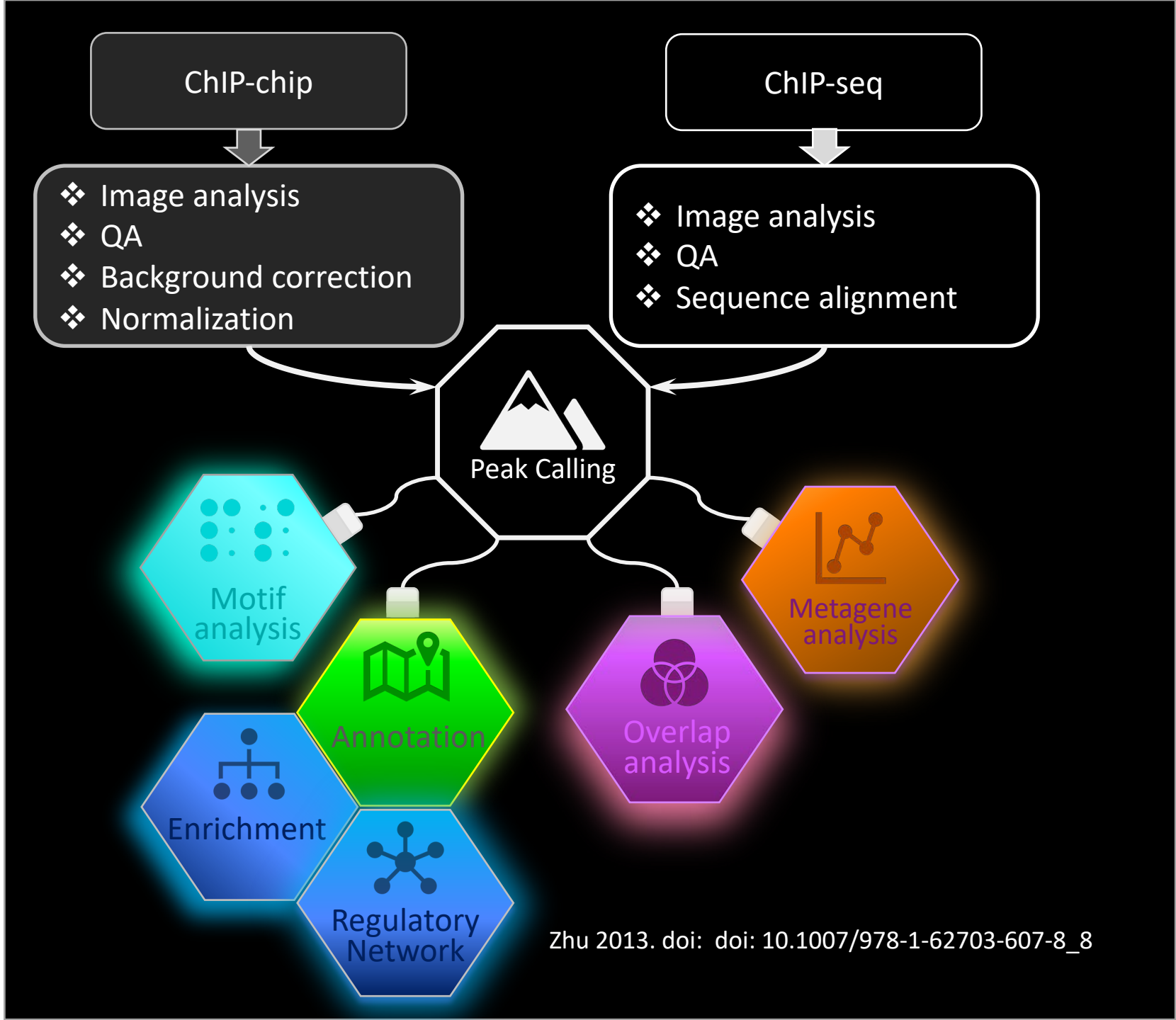
Nature Reviews | Genetics

# ChIP-seq Data Processing Pipeline from ENCODE project



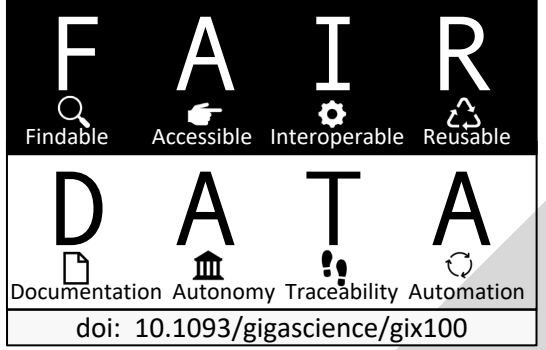

https://www.encodeproject.org/chip-seq/transcription_factor/

# Downstream peak calling




Zhu 2013. doi: doi: 10.1007/978-1-62703-607-8_8

# WHY WE NEED AN ANNOTATION TOOL LIKE ChIPpeakAnno?




## Reproducibility

The ChIPpeakAnno pkg (PKG in short) is trackable by git version control system. By providing the accurate package versions and annotation resources, the accuracy of an experimental claim can be checked by complete following to the protocol in the published analysis.

## Transparency

PKG is open source software. All the source code are accessible from Bioconductor website or GitHub with detailed documentation. There is no hidden step when you publish the annotation method with proper descriptions when citing PKG.

## Efficiency

The license of PKG is GPL (>=2). It can be easily re-used with minimal modified or extended to achieve new functionalities. This is particularly effective because the full documentation and multiple vignettes are available.

## Credibility

PKG accepts comments, questions and challenges via Bioconductor support website, email, and GitHub comments. Over 8 years continuous development, PKG become strong and informative to avoid general mistakes in annotation.

## Flexibility

PKG provide plenty of parameters to meet the requirements of different annotation context. The implementation of new function is also available on demand.

## Feasibility

With well documented functions and vignettes, PKG is feasible for biologists with base R program skills. It is easy to get support from the authors via Bioconductor support website and personal communications.

# Main function list

## I/O: toGRanges

The *toGRanges* function Convert UCSC BED format and its variants, such as GFF, or user defined dataset such as MACS output file to GRanges

## Annotation: annotatePeakInBatch

The *annotatePeakInBatch* function will obtain the distance to nearest/given-range TSS, miRNA, and /or exons for a list of peak.

## Set operation: findOverlapsOfPeaks

The *findOverlapsOfPeaks* function will findthe overlapped peaks among two or more (less than five) set of peaks.

## Enrichment: getEnrichedGO

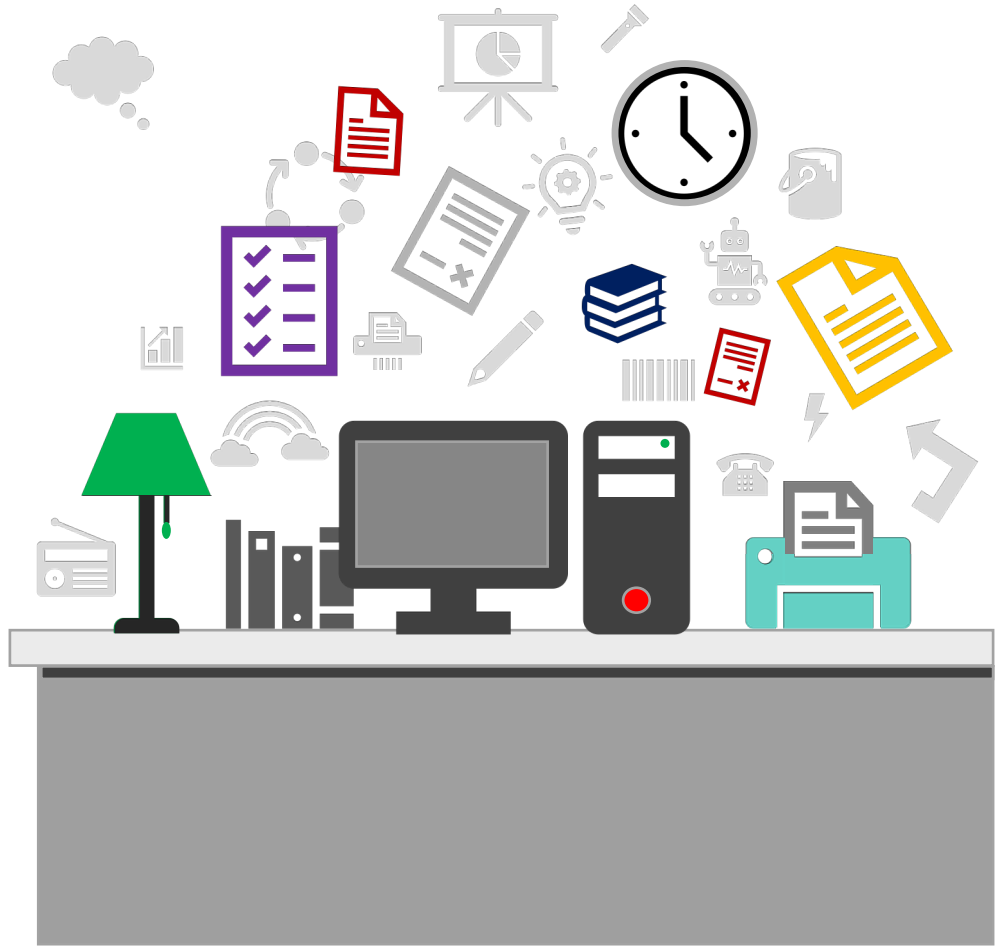
The *getEnrichedGO* function obtain the enriched gene ontology (GO) terms that near the peaks.

## Metagene: featureAlignedExtendSignal

The *featureAlignedExtendSignal* function obtain extract signals in given ranges from bam files of DNA-seq. For RNA-seq, use *featureAlignedSignal* instead.

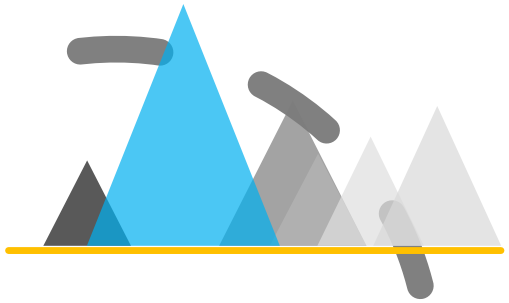
## Motif search: summarizePatternInPeaks

The *summarizePatternInPeaks* function Output a summary of the occurrence of each pattern in the sequences



# Steps of annotation by ChIPpeakAnno

The functions, *toGRanges*, *annotatePeakInBatch*, and *addGeneIDs* in the **ChIPpeakAnno**, make the annotation of ChIP-Seq peaks streamlined into four major steps:



Read peak data
with *toGRanges*

Generate annotation data
with *toGRanges*

Annotate peaks
with *annotatePeakInBatch*

Add additional
information
with *addGeneIDs*

# 0-BASED VS. 1-BASED COORDINATES SYSTEMS
# CLOSED VS. HALF-OPEN COORDINATES SYSTEMS

0/1

**0-based vs. 1-based coordinates systems**

0-based coordinates system start counting from 0 where 1-based coordinates system start counting from 1.

1/)

**Closed vs. Half-open coordinates systems**

A coordinates system use two number to indicate start and end position.

Closed coordinates: [start, end] == start ≤ x ≤ end, where width = end – start + 1

Half-open means half-closed-half-open coordinates: [start, end) == start ≤ x < end, where width = end – start

∞

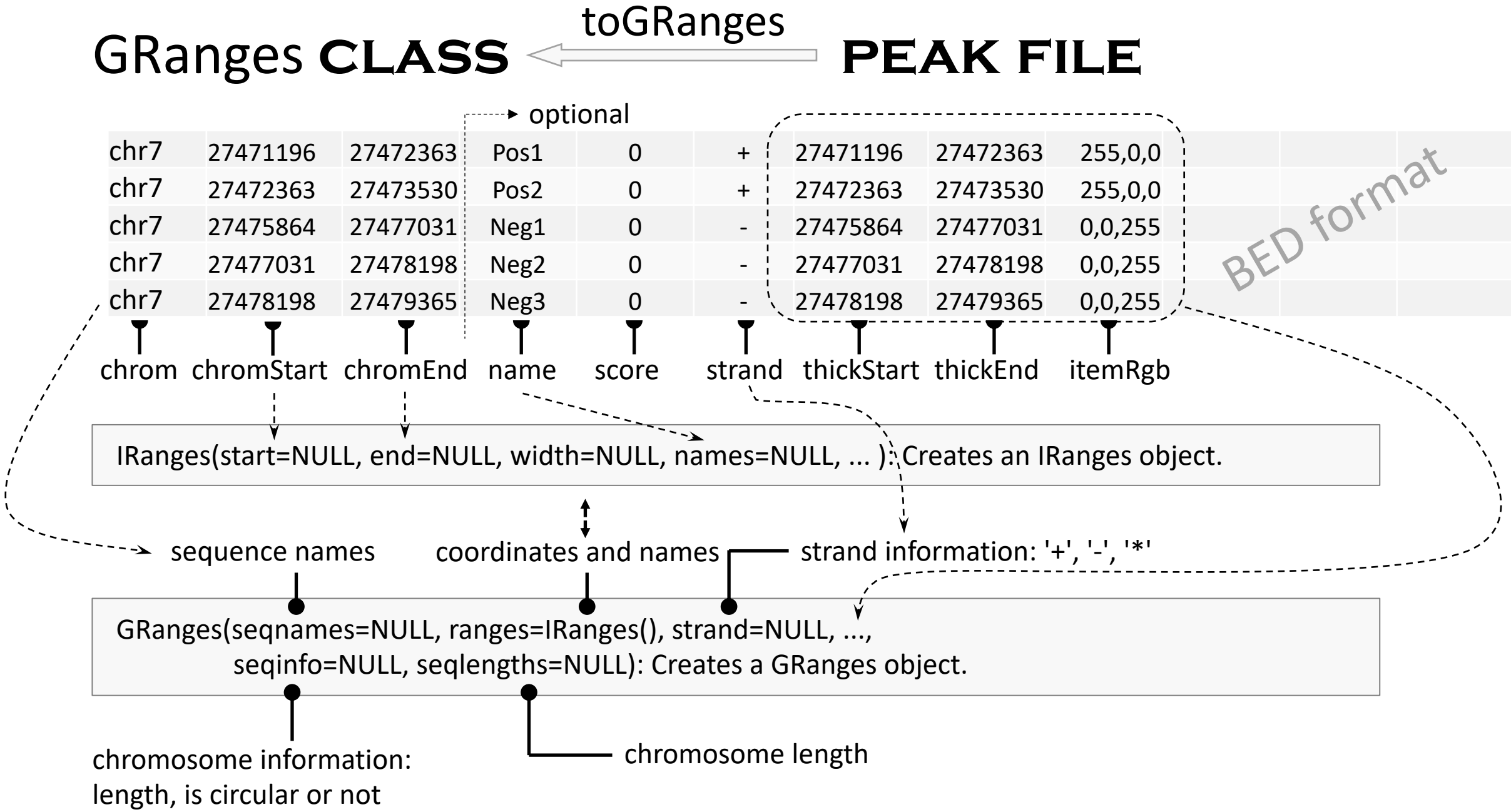
Most of 0-based coordinates system use half-open coordinates, where 1-based coordinates system use closed coordinates.

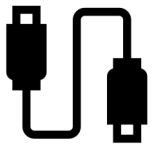
**1-based coordinates system**: SAM, VCF, GFF/GTF and Wiggle formats.

**0-based coordinates system**: BAM, BCFv2, BED, and PSL formats.

IRanges/GRanges are using **closed** coordinates system. There is no definition about 0-based or 1-based coordinates for IRanges/GRanges ( IRanges(0, 0) ). The *rtracklayer* package treat it as **1-based** coordinates and *ChIPpeakAnno* follows this rule.

https://genome.ucsc.edu/FAQ/FAQformat.html


GRanges CLASS
toGRanges
PEAK FILE
optional
chr7 27471196 27472363 Pos1 0 + 27471196 27472363 255,0,0
chr7 27472363 27473530 Pos2 0 + 27472363 27473530 255,0,0
chr7 27475864 27477031 Neg1 0 - 27475864 27477031 0,0,255
chr7 27477031 27478198 Neg2 0 - 27477031 27478198 0,0,255
chr7 27478198 27479365 Neg3 0 - 27478198 27479365 0,0,255
BED format
chrom chromStart chromEnd name score strand thickStart thickEnd itemRgb
IRanges(start=NULL, end=NULL, width=NULL, names=NULL, ... ): Creates an IRanges object.
sequence names
coordinates and names
strand information: '+', '-', '*'
GRanges(seqnames=NULL, ranges=IRanges(), strand=NULL, ...,
seqinfo=NULL, seqlengths=NULL): Creates a GRanges object.
chromosome information:
length, is circular or not
chromosome length



# INPUT DATA FOR ANNOTATION, *toGRanges*

```r
## the sample file is included in ChIPpeakAnno package.
## chage the file path into your own file path to handle your data
path <- system.file("extdata", "Tead4.broadPeak", package="ChIPpeakAnno")
## toGRanges is overlaoded method,
## by define the correct file format to import the file in correct coordinates
peaks <- toGRanges(path, format="broadPeak")
## see top 2 lines of the imported peaks.
## the imported peaks will be packaged into GRanges object
head(peaks, n=2)
```

```
## GRanges object with 2 ranges and 4 metadata columns:
##             seqnames        ranges strand |     score signalValue    pValue
##                <Rle>     <IRanges>  <Rle> | <integer>   <numeric> <numeric>
##   peak12338     chr2 175473-176697      * |       206      668.42        -1
##   peak12339     chr2 246412-246950      * |        31      100.23        -1
##               qValue
##             <numeric>
##   peak12338        -1
##   peak12339        -1
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

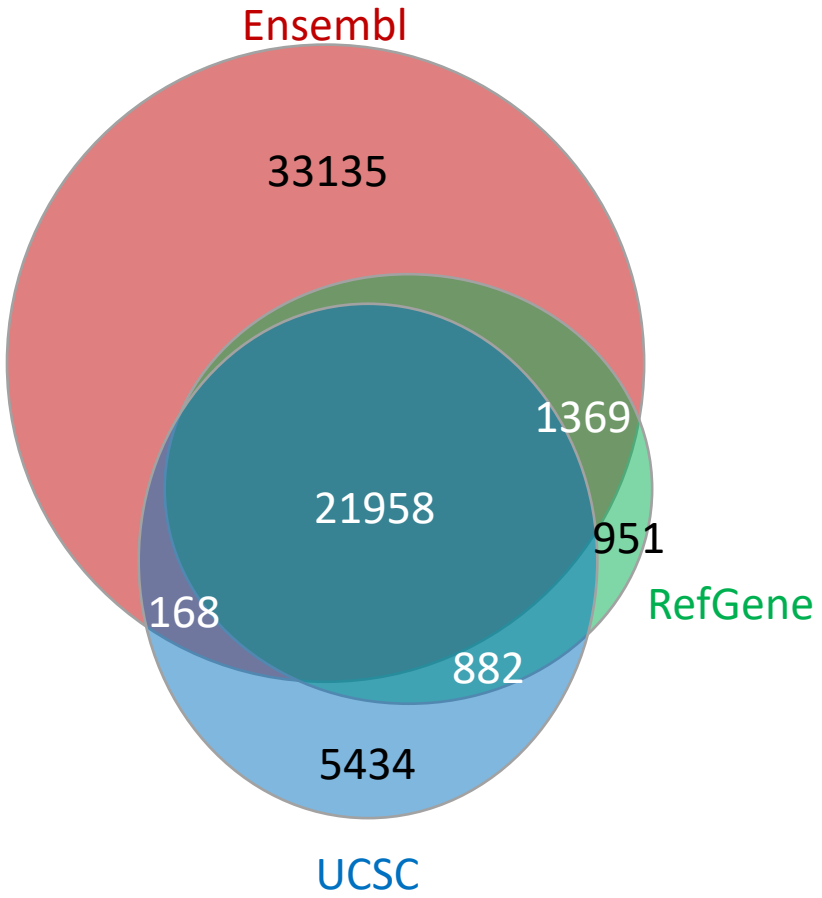
# Important concepts in annotation

**Annotation**: Computational process of attaching biologically relevant information to a given data.

**Assembly**: Computational reconstruction of a longer sequence from smaller sequence reads.

**Genome assembly**: A computational representation of the sequence of a haploid genome, representative of a species or strain.

# Ensembl, RefSeq and UCSC annotations




The overlap and intersection among RefGene, UCSC(hg19), and Ensembl (GRCh37) annotations

Zhao et.al., 2015. doi: 10.1186/s12864-015-1308-8

**Ensembl**: annotated by genebuild pipeline + manually annotated by the HAVANA team

**UCSC**: also called "Known Gene", is available only on assemblies before hg38. It was built with UCSC gene predictor which uses protein, EST and cDNA annotations.

**RefSeq**: most stringent. RefSeq also have additional sequences independent of the genome assembly. This has the important implication that the position of genome variants are harder to map to RefSeq transcripts.

For **RNA-seq**:

Less complex genome annotations, such as RefGene, are preferable for reproducible and robust gene expression estimates.

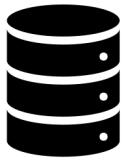
However, to discover and explain unknown biological mechanisms, more comprehensive and complex genome annotations are necessary, such as Ensembl.

Wu et.al., 2013. doi: 0.1186/1471-2105-14-S11-S8

# Genome assembly versions

| SPECIES | UCSC VERSION | RELEASE DATE | Ensembl VERSION | RELEASE DATE | ASSEMBLY NAME |
|---|---|---|---|---|---|
| Human | hg38 | Dec. 2013 | GRCh38 | P13: Sep. 2019 (>=V98); P12: Apr. 2018 (>=V92, <=V97); P10: Mar. 2017 (>=V88, <=V91); P7: Jul. 2016 (>=V85, <=V87); P5: Dec. 2015 (>=V83, <=V84); P3: Jul. 2015 (>=V81, <=V82); P2: Mar. 2015 (>=V79, <=V80); Aug. 2014 (>=V76, <=V78); | Genome Reference Consortium GRCh38 |
| | hg19 | Feb. 2009 | GRCh37 | P13: Dec. 2013 (>=V74, <=V75); P12: Sep. 2013 (=V73); P11: Jun. 2013 (=V72); P10: Apr. 2013 (=V71); P8: Jul. 2012 (>=V68, <=V70); P7: May 2012 (=V67); P6: Feb. 2012 (=V66); P5: Sep. 2011 (>=V64, <=V65); P3: Jun. 2011 (=V63); Jul. 2009 (>=V55, <=V62); | Genome Reference Consortium GRCh37 |
| Mouse | mm10 | Dec. 2011 | GRCm38 | P6: Apr. 2018 (>=V92); P5: Dec. 2016 (>=V87, <=V91); P4: Jul. 2015 (>=V81, <=V86); P3: Dec. 2014 (>=V78, <=V80); P2: Dec. 2013 (>=V74, <=V77); P1: Jan. 2013 (>=V70, <=V73); Jul. 2012 (>=V68, <=V69) | Genome Reference Consortium GRCm38 |
| | mm9 | Jul. 2007 | NCBIM37 | Oct. 2007(>=V47) | NCBI Build 37 |
| Zebrafish | danRer11 | May 2017 | GRCz11 | Apr. 2018 (>=V92) | Genome Reference Consortium GRCz11 |
| | danRer10 | Sep. 2014 | GRCz10 | May 2015 (>=V80, <=V91) | Genome Reference Consortium GRCz10 |
| D. melanogaster | dm6 | Aug. 2014 | BDGP6 | Mar. 2015 (>=V79) | BDGP Release 6 + ISO1 MT |
| | dm3 | Apr. 2006 | BDGP5 | NA | BDGP Release 5 |
| C. elegans | ce11 | Feb. 2013 | WBcel235 | Apr. 2013 (>=V71) | C. elegans Sequencing Consortium WBcel235 |
| | ce10 | Oct. 2010 | WS220 | Feb. 2011 (>=V61, <=V66) | WormBase v. WS220 |

https://genome.ucsc.edu/FAQ/FAQreleases.html
https://useast.ensembl.org/info/website/archives/assembly.html

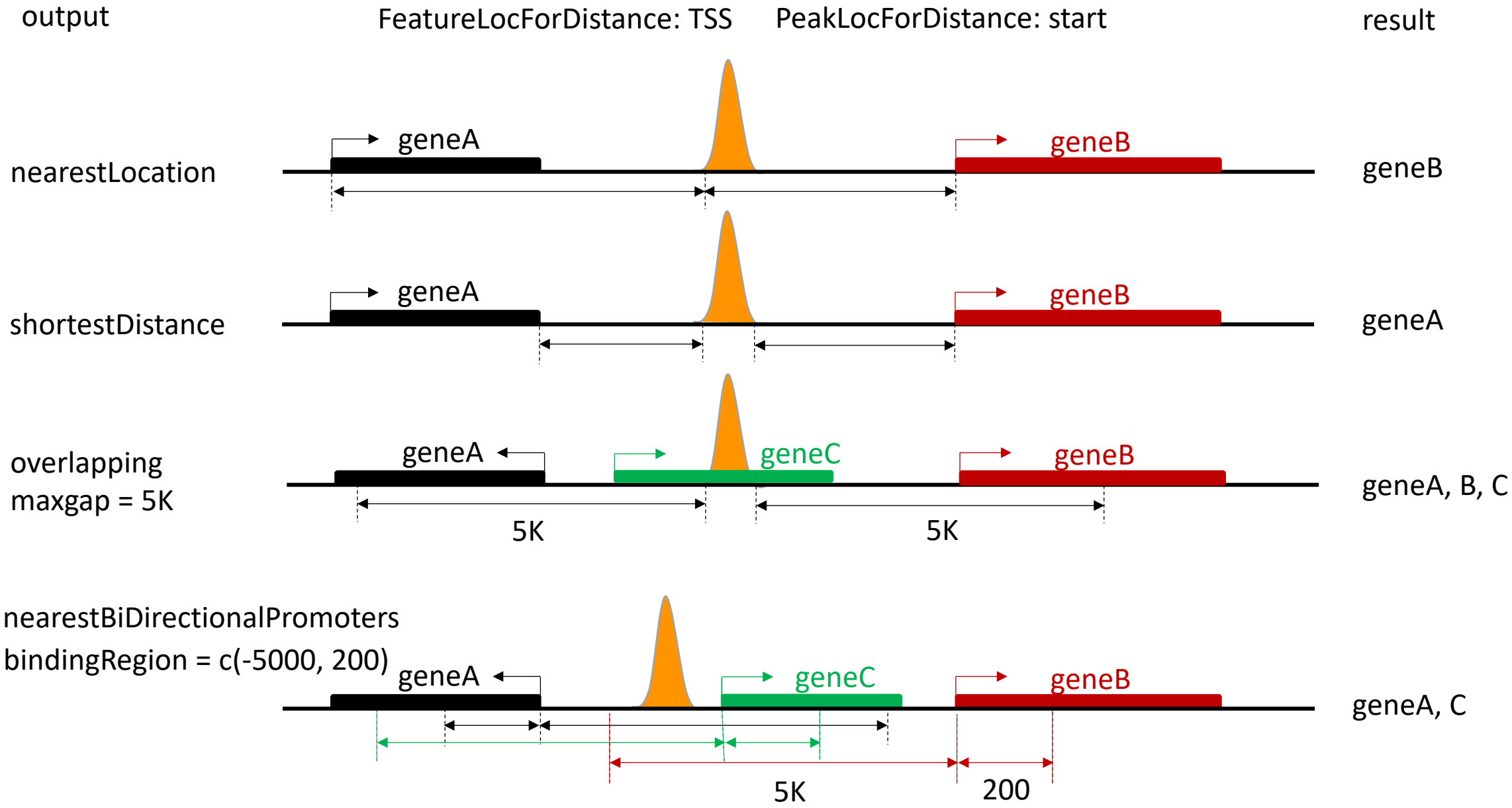


# Prepare annotation data, *toGRanges*

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
annoData <- toGRanges(TxDb.Hsapiens.UCSC.hg19.knownGene, feature="gene")
annoData[1]
```

```
## GRanges object with 1 range and 0 metadata columns:
##       seqnames              ranges  strand
##          <Rle>           <IRanges>   <Rle>
##   1     chr19 58858172-58874214          -
##   -------
##   seqinfo: 93 sequences (1 circular) from hg19 genome
```

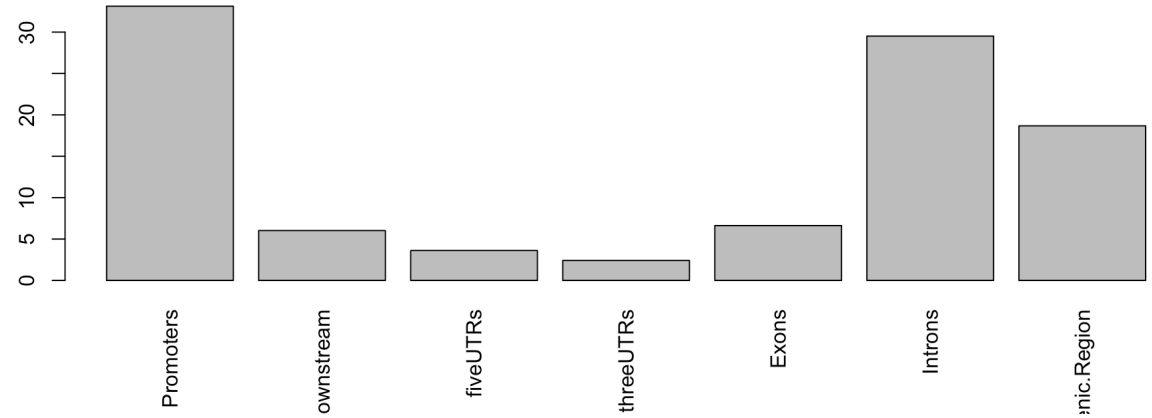
# ANNOTATE BY annotatePeakInBatch

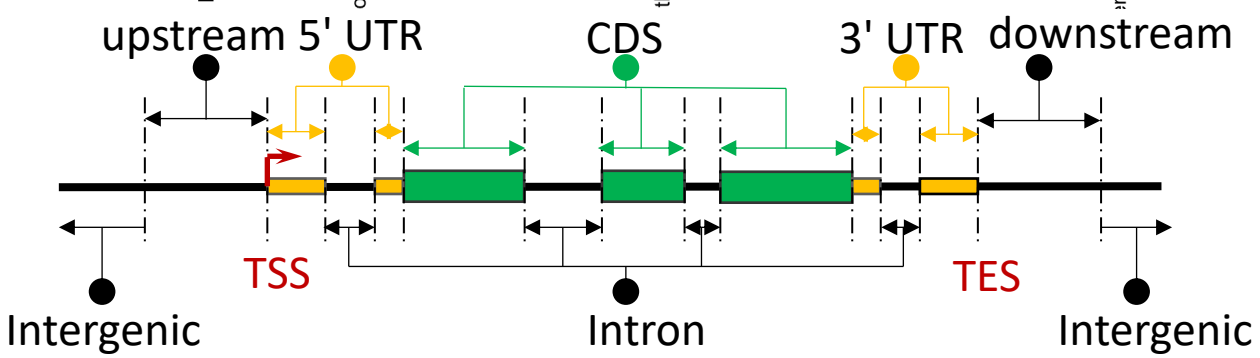


# Select annotation method

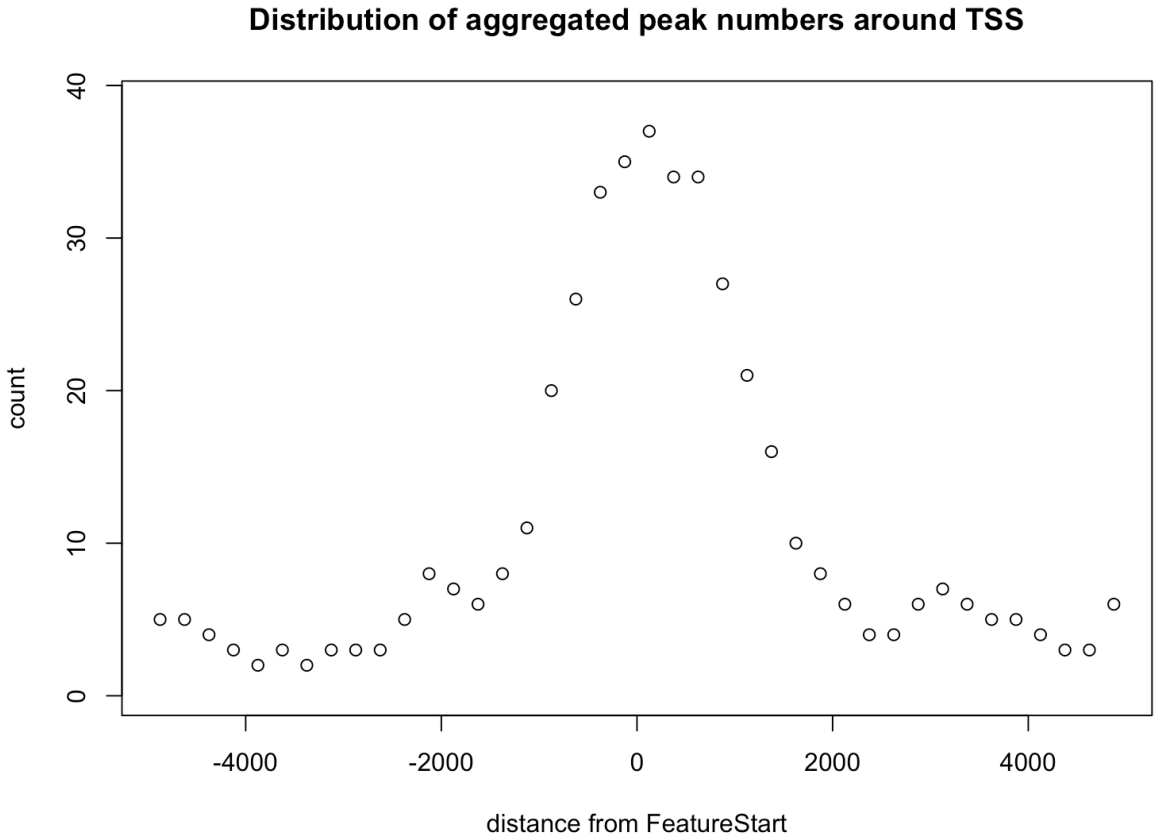
Visualize binding site distribution relative to features

```
aCR<-assignChromosomeRegion(overlaps,
          nucleotideLevel=FALSE,
           precedence=c("Promoters",
"immediateDownstream", "fiveUTRs", "threeUTRs",
"Exons", "Introns"),
          TxDb=TxDb.Hsapiens.UCSC.hg19.knownGene)
barplot(aCR$percentage, las=3)
```




```
binOverFeature(overlaps, annotationData=annoDataTxDb,
                radius=5000, nbins=20, FUN=length,
                errFun=0, ylab="count",
                main="Distribution of aggregated peak numbers around TSS")
```



# Annotate data by nearest features

```
## keep the seqnames in the same style
if(!identical(seqlevelsStyle(peaks), seqlevelsStyle(annoDataTxDb))){
 seqlevelsStyle(peaks) <- seqlevelsStyle(annoDataTxDb)[1]
}
## do annotation by nearest TSS of UCSC hg19 annotations
annoTxDb <- annotatePeakInBatch(peaks, AnnotationData=annoDataTxDb)
head(annoTxDb, n=1)
```

```
## GRanges object with 1 ranges and 13 metadata columns:
##                     seqnames        ranges strand |     score signalValue
##                        <Rle>     <IRanges>  <Rle> | <integer>   <numeric>
##   peak12338.26751      chr2 175473-176697      * |       206      668.42
##                       pValue    qValue        peak     feature start_position
##                    <numeric> <numeric> <character> <character>      <integer>
##   peak12338.26751         -1        -1   peak12338       26751         218136
##                    end_position feature_strand insideFeature distancetoFeature
##                       <integer>    <character>   <character>         <numeric>
##   peak12338.26751       264810                -    downstream             89337
##                    shortestDistance fromOverlappingOrNearest
##                           <integer>              <character>
##   peak12338.26751             41439          NearestLocation
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

# Add additional annotation, *addGeneIDs*

```
annoTxDb <- addGeneIDs(annoTxDb, orgAnn="org.Hs.eg.db", feature_id_type="entrez_id", IDs2Add=c("symbol"))
head(annoTxDb, n=2)
```

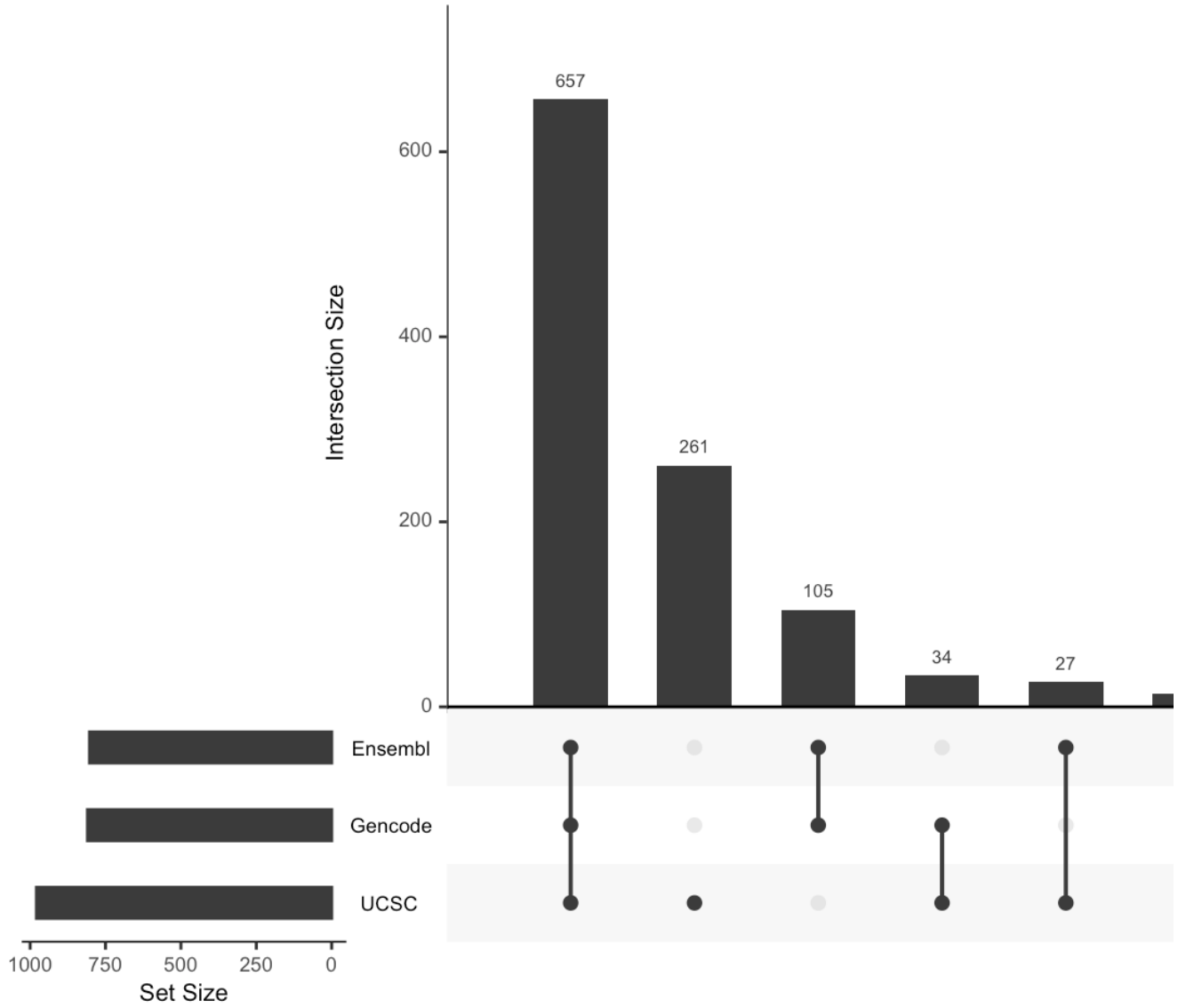
Use "entrez_id" for TxDb annotations;
Use ”ensemble_gene_id" for EnsDb annotations;

```
## GRanges object with 2 ranges and 14 metadata columns:
##                    seqnames        ranges strand |     score signalValue
##                       <Rle>     <IRanges>  <Rle> | <integer>   <numeric>
##   peak12338.26751     chr2 175473-176697      * |       206      668.42
##   peak12339.26751     chr2 246412-246950      * |        31      100.23
##                      pValue    qValue        peak     feature start_position
##                   <numeric> <numeric> <character> <character>      <integer>
##   peak12338.26751        -1        -1   peak12338       26751         218136
##   peak12339.26751        -1        -1   peak12339       26751         218136
##                   end_position feature_strand insideFeature distancetoFeature
##                      <integer>    <character>   <character>         <numeric>
##   peak12338.26751       264810              -    downstream             89337
##   peak12339.26751       264810              -        inside             18398
##                   shortestDistance fromOverlappingOrNearest      symbol
##                          <integer>             <character> <character>
##   peak12338.26751            41439          NearestLocation      SH3YL1
##   peak12339.26751            17860          NearestLocation      SH3YL1
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

# GOTO VIGNETTE

# Annotations with different annotation source

As a conclusion, annotate with different annotation resources, even the other parameter keep same, the annotations will be different from each other. To improve the reproducibility, accuracy of an annotation source should be provided.




upset plot of common gene symbols among annotations by Ensembl, UCSC and Gencode features.

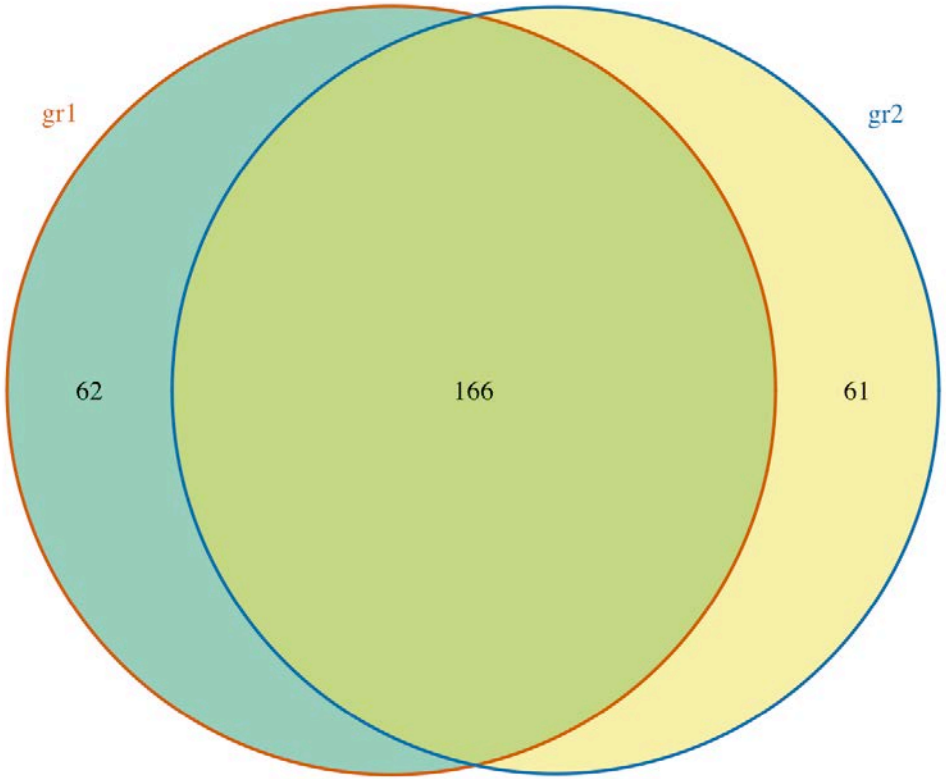
# Find overlaps for replicates, *findOverlapsOfPeaks*

```
## import peaks from a bed file
bed <- system.file("extdata", "MACS_output.bed", package="ChIPpeakAnno")
gr1 <- toGRanges(bed, format="BED", header=FALSE)
## import peaks from a gff file
gff <- system.file("extdata", "GFF_peaks.gff", package="ChIPpeakAnno")
gr2 <- toGRanges(gff, format="GFF", header=FALSE, skip=3)
## find overlaps for replicates
ol <- findOverlapsOfPeaks(gr1, gr2, connectedPeaks = "keepAll")
head(ol$peaklist[["gr1///gr2"]], n=2)
```

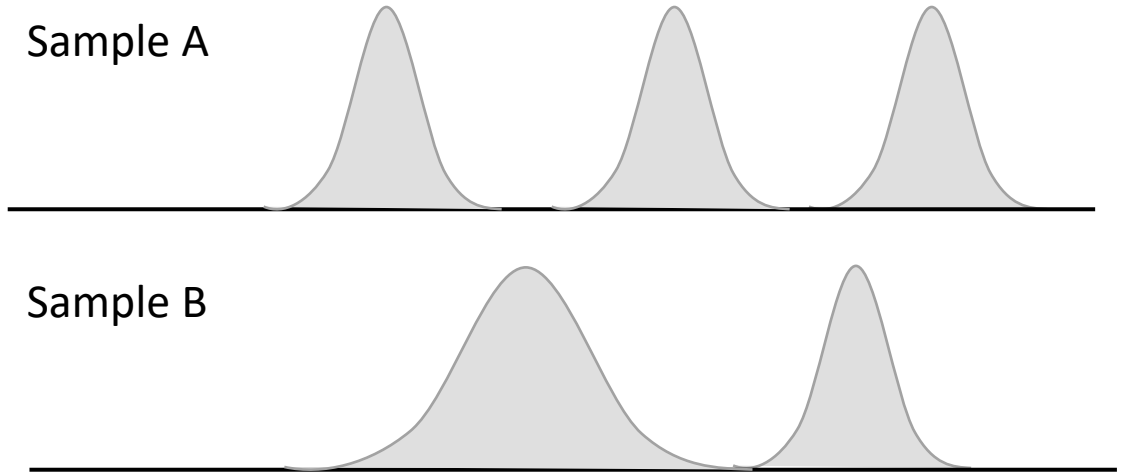
```
## GRanges object with 2 ranges and 2 metadata columns:
##       seqnames          ranges strand |                              peakNames
##          <Rle>       <IRanges>  <Rle> |                        <CharacterList>
##   [1]     chr1 713791-715578      * | gr1__MACS_peak_13,gr2__001,gr2__002
##   [2]     chr1 724851-727191      * |          gr2__003,gr1__MACS_peak_14
##   -------
##   seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

# Find overlaps for replicates, *findOverlapsOfPeaks*

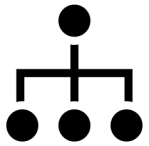
```
makeVennDiagram(ol, fill=c("#009E73", "#F0E442"),
                col=c("#D55E00", "#0072B2"),
                cat.col=c("#D55E00", "#0072B2"))
```




connectedPeaks:
"min" : minimal involved peaks → 2
"merge" : 1
"keepAll": keep all the original counts for each list while the final counts will be same as "min"



# GOTO VIGNETTE



# Enrichment: Obtain enriched GO terms and Pathways

Use *getEnrichedGO* to obtain a list of enriched GO terms with annotated peaks. For pathway analysis, please use function *getEnrichedPATH* with reactome or KEGG database.

```
getEnrichedGO(annotatedPeak, orgAnn,
              feature_id_type="ensembl_gene_id",
              maxP=0.01, minGOterm=10,
              multiAdjMethod=NULL,
              condense=FALSE,
              removeAncestorByPval=NULL,
              keepByLevel=NULL)
```

The *getEnrichedGO* function obtain enriched gene ontology (GO) terms based on the features near the enriched peaks using GO.db package and GO gene mapping package such as org.Hs.db.eg to obtain the GO annotation and using hypergeometric test (phyper) and multtest package for adjusting p-values.

```
getEnrichedPATH(annotatedPeak, orgAnn,
                pathAnn,
                feature_id_type="ensembl_gene_id",
                maxP=0.01,
                minPATHterm=10,
                multiAdjMethod=NULL)
```

The *getEnrichedPATH* function obtain enriched PATH that are near the peaks using path package such as reactome.db and path mapping package such as org.Hs.db.eg to obtain the path annotation and using hypergeometric test (phyper) and multtest package for adjusting p-values.

# GOTO VIGNETTE

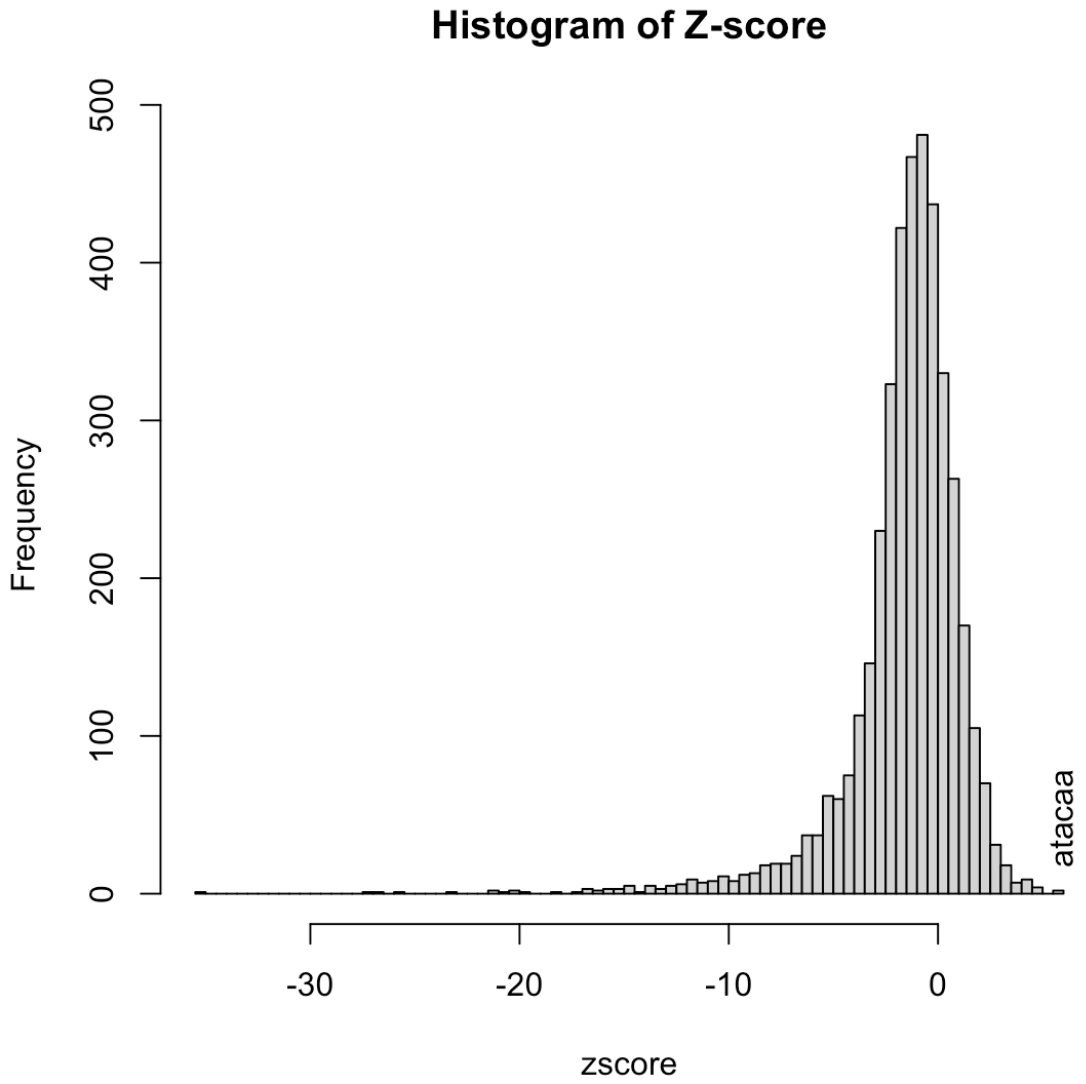
# Motif search: Output a summary of consensus in the peaks

There are multiple methods to get the consensus in the peaks:

1. output the fasta file by the *getAllPeakSequence* function and search the motif by the 3rd program such as homer, MEME, and so on.

2. test the pre-defined consensus patterns to see if target consensus are enriched or not by the *summarizePatternInPeaks* function.

3. calculate the z-scores of all combinations of oligonucleotide in a given length by Markove chain by the *oligoSummary* function.



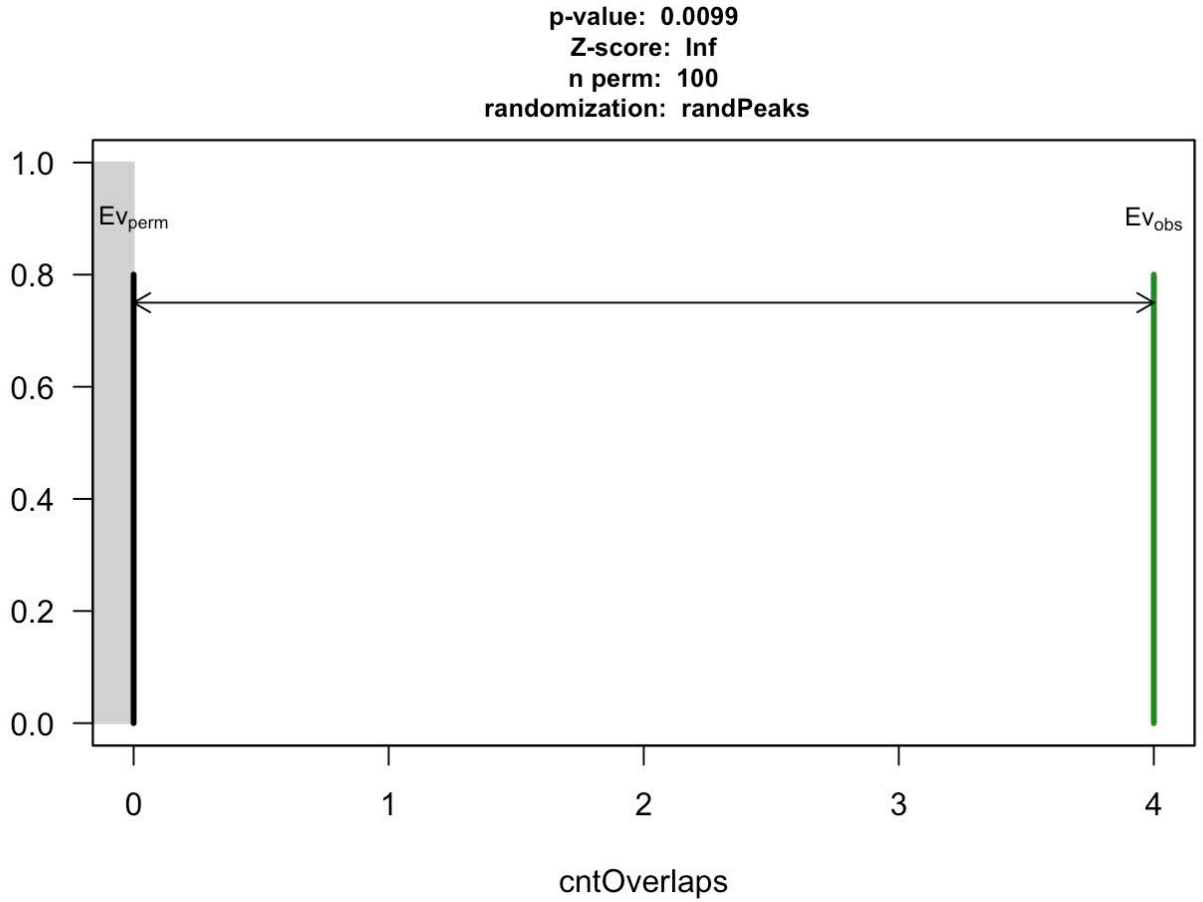
# GOTO VIGNETTE

# Determine if there is a significant overlap among multiple sets of peaks

*makeVennDiagram*: The p.value is calculated by **hypergeometric test** to determine whether the peaks or features are overlapped significantly. The number of all potential binding sites is required.

*peakPermTest*: Performs a **permutation test** to see if there is an association between two given peak lists.

```
## $p.value
##      TAF Tead4 YY1          pval
## [1,]   0     1   1  1.000000e+00
## [2,]   1     0   1 2.904297e-258
## [3,]   1     1   0  8.970986e-04
##
## $vennCounts
##      TAF Tead4 YY1 Counts count.TAF count.Tead4
## [1,]   0     0   0    849         0           0
## [2,]   0     0   1    621         0           0
## [3,]   0     1   0   2097         0        2097
## [4,]   0     1   1    309         0         310
## [5,]   1     0   0     59        59           0
## [6,]   1     0   1    166       172           0
## [7,]   1     1   0      8         8           8
## [8,]   1     1   1    476       545         537
## attr(,"class")
## [1] "VennCounts"
```



# GOTO VIGNETTE

# Save annotation results

The annotation results can be saved in XLS file format using **WriteXLS** package to avoid the gene name errors that can be inadvertently introduced when opened by Excel.

```
library(WriteXLS)
WriteXLS(as.data.frame(unname(overlaps.anno)), "anno.xls")
```

gene names | internal date format | default date format | gene names | internal date format | default date format | gene names | internal date format | default date format

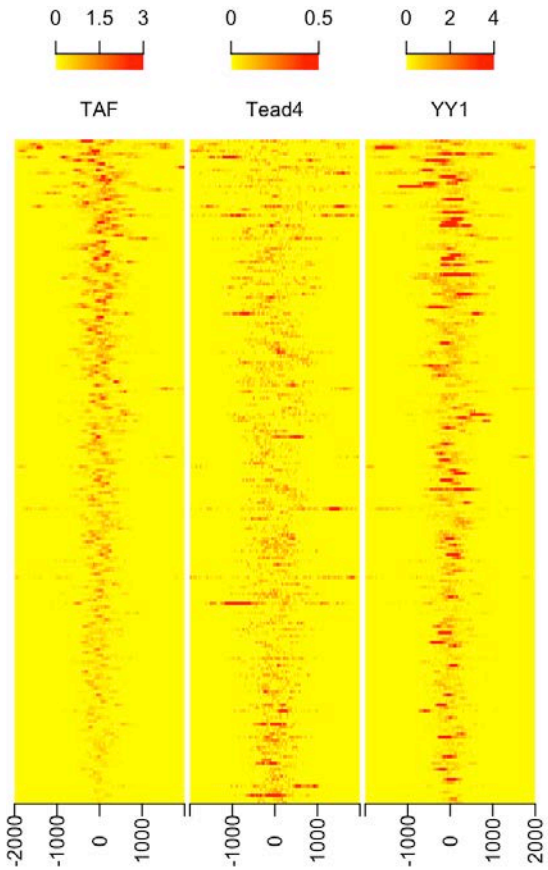
excel.gene2date.xls

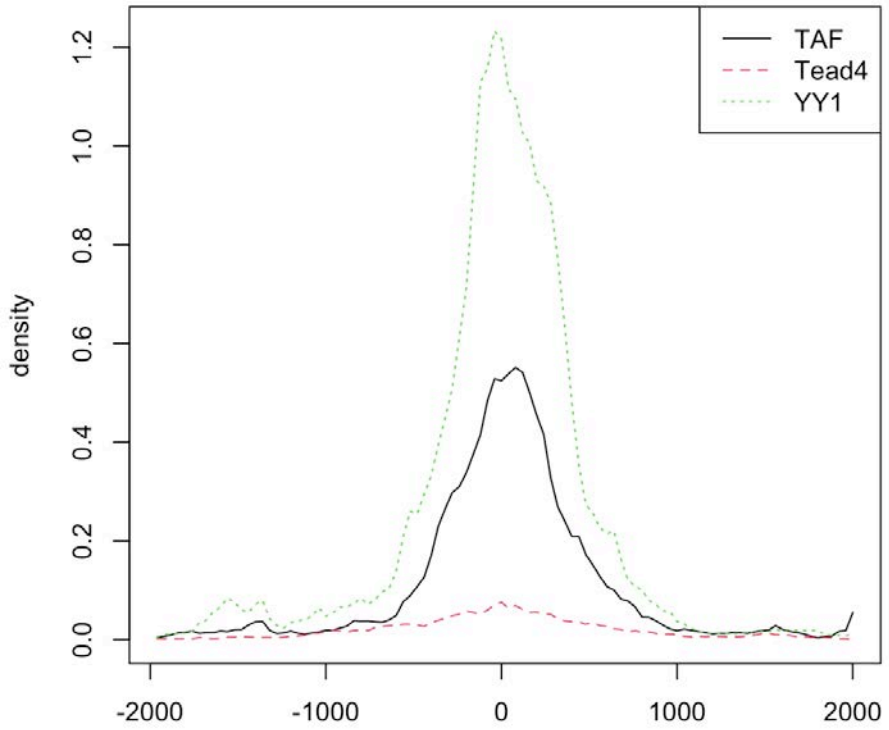
| | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | APR-1 | 35885 | 1-Apr | | OCT-1 | 36068 | 1-Oct | | SEP2 | 36039 | 2-Sep |
| 2 | APR-2 | 35886 | 2-Apr | | OCT-2 | 36069 | 2-Oct | | SEP3 | 36040 | 3-Sep |
| 3 | APR-3 | 35887 | 3-Apr | | OCT-3 | 36070 | 3-Oct | | SEP4 | 36041 | 4-Sep |
| 4 | APR-4 | 35888 | 4-Apr | | OCT-4 | 36071 | 4-Oct | | SEP5 | 36042 | 5-Sep |
| 5 | APR-5 | 35889 | 5-Apr | | OCT-6 | 36073 | 6-Oct | | SEP6 | 36043 | 6-Sep |
| 6 | DEC-1 | 36129 | 1-Dec | | OCT1 | 36068 | 1-Oct | | SEPT1 | 36038 | 1-Sep |
| 7 | DEC-2 | 36130 | 2-Dec | | OCT11 | 36078 | 11-Oct | | SEPT2 | 36039 | 2-Sep |
| 8 | DEC1 | 36129 | 1-Dec | | OCT2 | 36069 | 2-Oct | | SEPT3 | 36040 | 3-Sep |
| 9 | DEC2 | 36130 | 2-Dec | | OCT3 | 36070 | 3-Oct | | SEPT4 | 36041 | 4-Sep |
| 10 | MAR1 | 35854 | 1-Mar | | OCT4 | 36071 | 4-Oct | | SEPT5 | 36042 | 5-Sep |
| 11 | MAR2 | 35855 | 2-Mar | | OCT6 | 36073 | 6-Oct | | SEPT6 | 36043 | 6-Sep |
| 12 | MAR3 | 35856 | 3-Mar | | OCT7 | 36074 | 7-Oct | | SEPT7 | 36044 | 7-Sep |
| 13 | NOV1 | 36099 | 1-Nov | | SEP-1 | 36038 | 1-Sep | | SEPT8 | 36045 | 8-Sep |
| 14 | NOV2 | 36100 | 2-Nov | | SEP-2 | 36039 | 2-Sep | | SEPT9 | 36046 | 9-Sep |
| 15 | | | | | SEP1 | 36038 | 1-Sep | | | | |

Sheet1 Sheet2

Ready Sum=0 SCRL CAPS NUM

Zeeberg et.al., 2004. doi: 10.1186/1471-2105-5-80




# Metagene analysis for given features/peaks

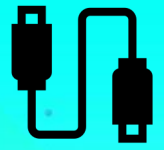
The *featureAlignedExtendSignal* function obtain extract signals in given ranges from bam files of DNA-seq. For RNA-seq, use *featureAlignedSignal* instead.
Function featureAlignedHeatmap and featureAlignedDistribution can be used to visualize and compare the binding patterns of raw signals of multiple ChIP-Seq experiments.

# GOTO VIGNETTE

# ChIPpeakAnno CAN...

## Annotation: annotatePeakInBatch

The *annotatePeakInBatch* function will obtain the distance to nearest/given-range TSS, miRNA, and /or exons for a list of peak.



## I/O: toGRanges

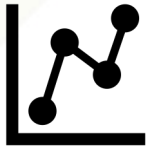
The *toGRanges* function Convert UCSC BED format and its variants, such as GFF, or user defined dataset such as MACS output file to GRanges

## Enrichment: getEnrichedGO/PATH

The *getEnrichedGO* and *getEnrichedPATH* function obtain the enriched gene ontology (GO) terms that near the peaks.

## Set operation: findOverlapsOfPeaks

The *findOverlapsOfPeaks* function will findthe overlapped peaks among two or more (less than five) set of peaks.

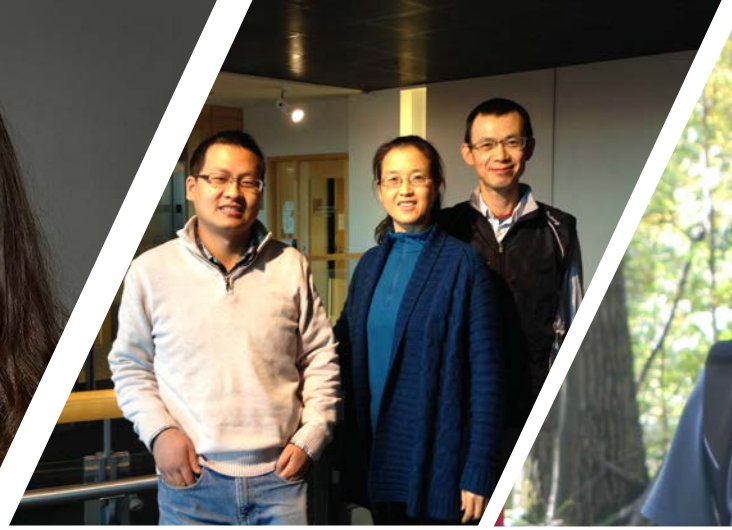


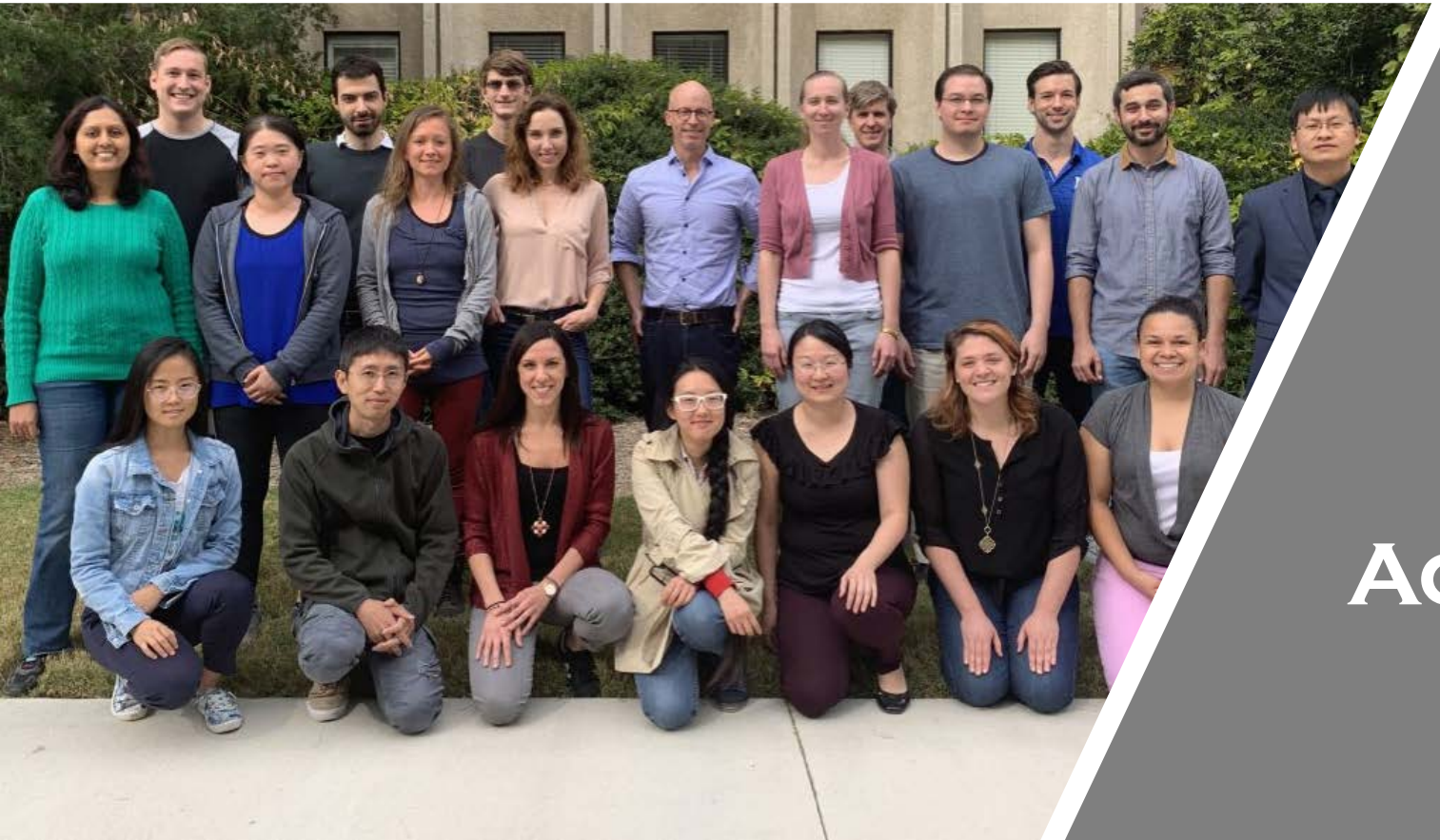
## Metagene: featureAlignedExtendSignal

The *featureAlignedExtendSignal* function obtain extract signals in given ranges from bam files of DNA-seq. For RNA-seq, use *featureAlignedSignal* instead.

## Motif search: oligoSummary

The *summarizePatternInPeaks* and *oligoSummary* function Output a summary of the occurrence of each pattern or all combinations of oligonucleotide in the sequences

# Acknowledgement