National Bioinformatics Week 2010 Introduction to using Bioconductor for High Throughput Sequencing Analysis

Winter Genomics and Instituto de Biotecnología
LCG Leonardo Collado Torres
lcollado@wintergenomics.com – lcollado@ibt.unam.mx

August 4th, 2010

Open R

Options:

- Type R on the terminal window
- Open emacs and then use `alt+X` followed by `R`

To quit R type:

```
> q()
```

and choose `no`

Get Help

- On a package:

```
> help(package = "pkgName")
```

- On a function, for example q:

```
> `?`(q)
> args(q)
```

- Find functions:

```
> apropos("session")
```

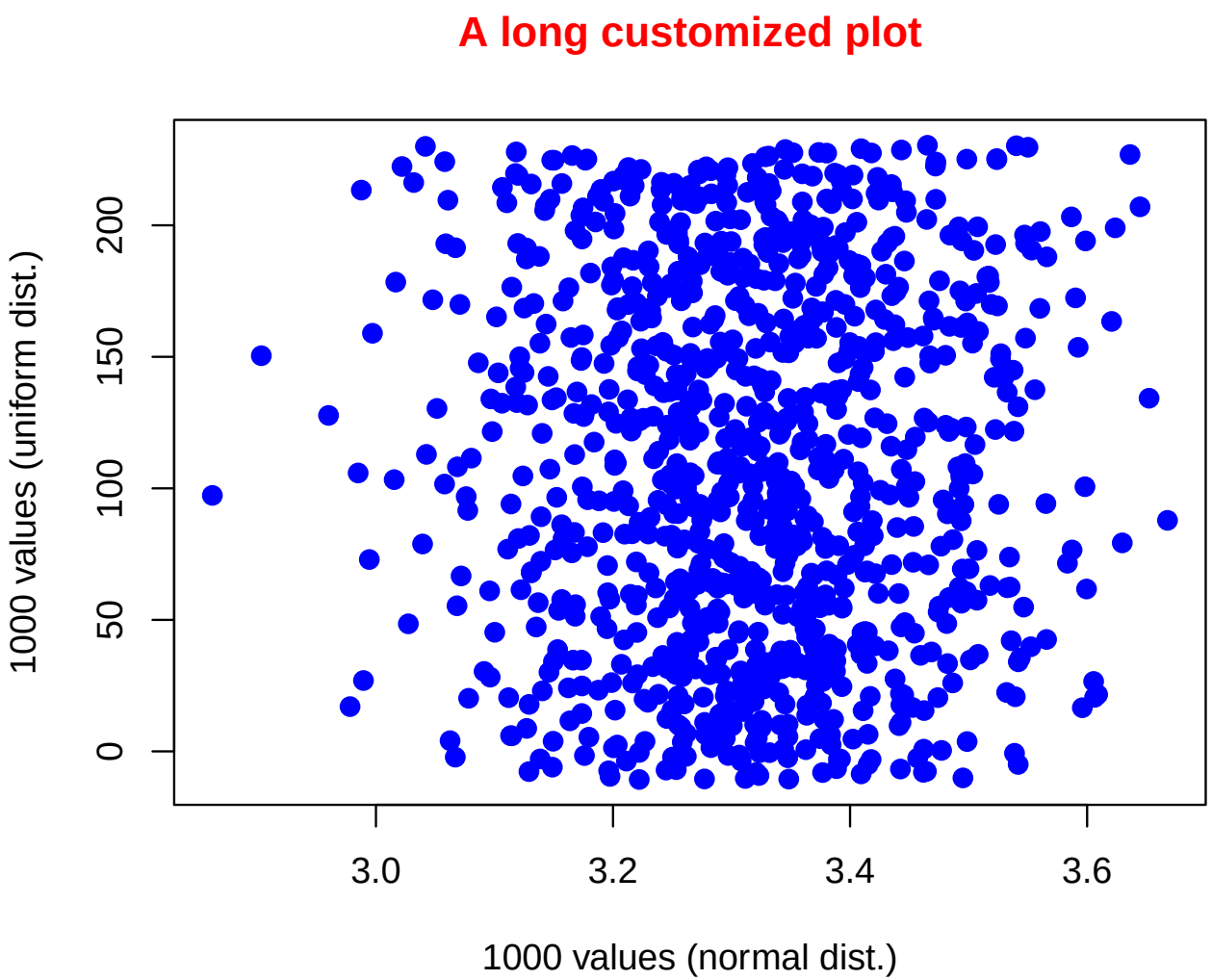
```
[1] "sessionData"
[2] "sessionInfo"
[3] "setSessionTimeLimit"
```

Use the lab files

- Whenever you see R code, instead of typing it yourself you should copy paste it from the `.R` file into your R session.
- This will save you a lot of time!

```
> plot(rnorm(1000, mean = 3.3128,
+     sd = 0.123), runif(1000, -10.74,
+     231), type = "p", lwd = 2,
+     col = "blue", main = "A long customized plot",
+     col.main = "red", pch = 19,
+     xlab = "1000 values (normal dist.)",
+     ylab = "1000 values (uniform dist.)")
```

Use the lab files



Install today's packages

- Use the following code assuming that you have R version 2.11 or higher[1]:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite(c("Rsamtools", "GenomicRanges",
+     "ChIPpeakAnno"))
```

[1] These should be installed on the server.

Illumina Tech

- Let's look at a tech summary for the Illumina platform.
- And some specs on the Genome Analyzer IIx.

Hmm...

- Surely the story seems nice enough. It ends with a *align data, compare to a reference, and identify sequence differences*.
- The story just begins once we get the FASTQ files!!!
- Managing and analyzing the millons of reads is not a simple task.

Closer to reality

These are just some steps you might need to do in a workflow:

- Check the quality of your data (quality values, nucleotide frequencies)
- Filter out unwanted sequences
  1. Adaptors: illumina, protocol specific, ...
  2. Low quality: lots of Ns, low phred values
- Trim sequences
- Choose appropriate programs / parameters for aligning (or assembling) your reads.
- Choose how you'll find peaks (ChIP-seq), differentially expressed genes (RNA-seq), etc.
- Develop some specific algorithms for *your* data.

These are non trivial decisions!

Work frameworks

To analyze HTS data you have plenty of options :)

- **HTSeq** Python package: overview. It follows a *read centric* workflow.
- Buy a license from commercial packages such as NextGENe or CLC bio.
- Write custom scripts in Perl, Python, Java, C, ...
- Use **Bioconductor**'s packages (**R** language/environment)

Why do we use BioC?

- Open source code and development.
- There is a very active community behind. Lots of useful packages available.
- R is strong in statistics and visualizing data.
- *Vignette* files offer excellent examples on how to combine functions.
- By using this framework, integration is a great side bonus!
- Promotes reproducible research :)

Disadvantages:

- Pretty steep learning curve.
- Staying updated is a challenge.

Browsing Bioconductor

`http://bioconductor.org/`

- As of July 28th, it has a new look! :)
- Basic categories on the main page, more choices at the bottom.
- Probably the best way to find packages useful for you is to use the BiocViews.
  Go to software, assay tech., high throughput seq or click here.
- Workflow pages are also useful, such as the HTS one.

So what is available?

- I/O packages such as Rsamtools and ShortRead.
- Infrastructure packages (mostly ranged based) such as GenomicRanges, IRanges, genomeIntervals, ...
- Tools for integrating data such as biomaRt.
- Packages for visualizing data (in R or the UCSC browsers): GenomeGraphs, rtracklayer
- Analysis-specific packages like DESeq, edgeR, ChIPpeakAnno, ...

Package Documentation

- Once you find a package of interest, you can get overall documentation on its webpage.
- Lets look at the info on GenomicRanges
- We can find who are the authors, how to install it, **vignette** files that exemplify how to use and integrate the functions it provides and other details (dependencies, . . .).
- Alternatively, if you have installed GenomicRanges we can use:

```
> help(package = "GenomicRanges")
> browseVignettes(package = "GenomicRanges")
```

- So, which BioC package does GenomicRanges depend on?

Advanced help

Let's assume that you have a specific problem and you have already:

- Browsed the help main page for clues.
- Googled key words.
- Checked that you have the latest R version[2] and BioC installed.

Then you might seriously consider asking in the mailing lists. Remember to include your session information!!

[2] Every April and October new releases are made public.

For today:

- Rsamtools
  One of the latest I/O packages :)
- GenomicRanges
  Very useful for containing your data. Plus it's memory efficient.
- ChIPpeakAnno
  Useful in ChIP-seq analysis plus it's a small tool box.

Loading

- For any session that you want to use GenomicRanges, you'll need to load it with the library function.

```
> library(GenomicRanges)
```

GRanges

- The basic container is a GRanges object. Lets create one:

```
> gr <- GRanges(seqnames = Rle(c("chr",
+     "plasmid", "chr"), c(3, 4,
+     3)), ranges = IRanges(11:20,
+     end = 91:100, names = toupper(head(letters,
+         10))), strand = Rle(strand(c("+",
+     "*", "-", "+", "-")), c(2,
+     1, 3, 3, 1)), score = round(runif(10,
+     1, 100)), GC = 46:55)
```

Our gr object

- Lets look at it:

```
> gr
```

```
GRanges with 10 ranges and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr [11,  91]      + |        44
B      chr [12,  92]      + |        59
C      chr [13,  93]      * |        88
D  plasmid [14,  94]      - |        81
E  plasmid [15,  95]      - |        85
F  plasmid [16,  96]      - |        78
G  plasmid [17,  97]      + |        43
H      chr [18,  98]      + |        69
I      chr [19,  99]      + |        77
J      chr [20, 100]      - |        31
         GC
  <integer>
A        46
```

Our gr object

```
B         47
C         48
D         49
E         50
F         51
G         52
H         53
I         54
J         55

seqlengths
     chr plasmid
      NA      NA
```

Details on gr

Woah! Lots of info! Lets look at gr object a bit closer

- Yes, gr is a GRanges object

```
> class(gr)
```

```
[1] "GRanges"
attr(,"package")
[1] "GenomicRanges"
```

- An Run length encoded object (Rle) is useful when you repeat values.

```
> Rle(c("chr", "plasmid", "chr"),
+     c(3, 4, 3))
```

Details on gr

```
'character' Rle of length 10 with 3 runs
  Lengths:         3         4         3
  Values :     "chr" "plasmid"     "chr"
```

- Let's look at how we made the IRanges object inside gr

```
> head(letters, 2)

[1] "a" "b"

> toupper(head(letters, 2))

[1] "A" "B"

> IRanges(11:20, end = 91:100, names = toupper(head(letters,
+     10)))
```

Details on gr

```
IRanges of length 10
     start end width names
[1]     11  91    81     A
[2]     12  92    81     B
[3]     13  93    81     C
[4]     14  94    81     D
[5]     15  95    81     E
[6]     16  96    81     F
[7]     17  97    81     G
[8]     18  98    81     H
[9]     19  99    81     I
[10]    20 100    81     J
```

- Rles are great for strand information!

```
> Rle(strand(c("+", "*", "-", "+",
+     "-")), c(2, 1, 3, 3, 1))
```

Details on gr

```
'factor' Rle of length 10 with 5 runs
  Lengths: 2 1 3 3 1
  Values : + * - + -
Levels(3): + - *
```

- `> round(runif(10, 1, 100))`

```
 [1] 23 52 71 77 60  1 47  6 76 10
```

`> 46:55`

```
 [1] 46 47 48 49 50 51 52 53 54 55
```

Data extractors

Once we have a GRanges we can extract subsets of the informations:

- Replicon names:

  > *seqnames(gr)*

```
'factor' Rle of length 10 with 3 runs
  Lengths:       3       4       3
  Values :     chr plasmid     chr
Levels(2): chr plasmid
```

- The actual ranges:

  > *ranges(gr)*

Data extractors

```
IRanges of length 10
      start end width names
[1]      11  91    81     A
[2]      12  92    81     B
[3]      13  93    81     C
[4]      14  94    81     D
[5]      15  95    81     E
[6]      16  96    81     F
[7]      17  97    81     G
[8]      18  98    81     H
[9]      19  99    81     I
[10]     20 100    81     J
```

- Strand info:

Data extractors

```
> strand(gr)
```

```
'factor' Rle of length 10 with 5 runs
  Lengths: 2 1 3 3 1
  Values : + * - + -
Levels(3): + - *
```

- Specific custom variables:

```
> values(gr)[, "GC"]
```

```
 [1] 46 47 48 49 50 51 52 53 54 55
```

- Ranges names:

```
> names(gr)
```

```
 [1] "A" "B" "C" "D" "E" "F" "G" "H" "I"
[10] "J"
```

Data extractors

- The total number of ranges:

```
> length(gr)
```

```
[1] 10
```

- Modify the length of the replicons:

```
> seqlengths(gr) <- c(4e+06, 1e+05)
```

- Length of the ranges:

```
> width(gr)
```

```
 [1] 81 81 81 81 81 81 81 81 81 81
```

Manipulating GRanges

These are some useful functions / operations for manipulating a GRanges object:

- Divide into several objects:

```
> split(gr)
```

```
GRangesList of length 10
$A
GRanges with 1 range and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr  [11, 91]      + |        44
         GC
  <integer>
A        46

$B
GRanges with 1 range and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
```

Manipulating GRanges

```
B      chr  [12, 92]      + |        59
         GC
  <integer>
B        47

$C
GRanges with 1 range and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
C      chr  [13, 93]      * |        88
         GC
  <integer>
C        48

...
<7 more elements>


seqlengths
```

Manipulating GRanges

```
     chr plasmid
 4000000  100000
```

```
> split(gr)[[9]]
```

```
GRanges with 1 range and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
I      chr  [19, 99]      + |        77
         GC
  <integer>
I        54

seqlengths
     chr plasmid
 4000000  100000
```

- Subset select ranges:

```
> gr[1:2]
```

Manipulating GRanges

```
GRanges with 2 ranges and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr  [11, 91]      + |        44
B      chr  [12, 92]      + |        59
         GC
  <integer>
A        46
B        47

seqlengths
     chr plasmid
 4000000  100000
```

- Reverse:

Manipulating GRanges

```
> rev(gr[1])

GRanges with 1 range and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr  [11, 91]      + |        44
         GC
  <integer>
A        46

seqlengths
     chr plasmid
 4000000  100000
```

- Get the upstream regions of our ranges:

Manipulating GRanges

```
> flank(gr, 10)[1:2]
```

```
GRanges with 2 ranges and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr   [1, 10]      + |        44
B      chr   [2, 11]      + |        59
         GC
  <integer>
A        46
B        47

seqlengths
     chr plasmid
 4000000  100000
```

Manipulating GRanges

- Move our ranges:

```
> shift(gr, 5)[1:2]
```

```
GRanges with 2 ranges and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr  [16, 96]      + |        44
B      chr  [17, 97]      + |        59
         GC
  <integer>
A        46
B        47

seqlengths
```

Manipulating GRanges

```
     chr plasmid
 4000000  100000
```

- Resize them:

```
> resize(gr, 1)[1:2]
```

```
GRanges with 2 ranges and 2 elementMetadata values
  seqnames    ranges strand |     score
     <Rle> <IRanges>  <Rle> | <numeric>
A      chr  [11, 11]      + |        44
B      chr  [12, 12]      + |        59
         GC
  <integer>
A        46
B        47
```

Manipulating GRanges

```
seqlengths
     chr plasmid
 4000000  100000
```

- Compact overlapping ranges:

```
> reduce(gr)
```

```
GRanges with 5 ranges and 0 elementMetadata values
    seqnames    ranges strand |
       <Rle> <IRanges>  <Rle> |
[1]      chr [11,  99]      + |
[2]      chr [20, 100]      - |
[3]      chr [13,  93]      * |
[4]  plasmid [17,  97]      + |
```

Manipulating GRanges

```
  [5]  plasmid [14,  96]      - |

seqlengths
     chr plasmid
 4000000  100000
```

- Find the gaps:

  > gaps(gr)[1:2]

```
GRanges with 2 ranges and 0 elementMetadata values
    seqnames          ranges strand |
       <Rle>       <IRanges>  <Rle> |
[1]      chr [  1,       10]      + |
[2]      chr [100, 4000000]      + |
```

Manipulating GRanges

```
seqlengths
     chr plasmid
 4000000  100000
```

- And most importantly, find the coverage!

```
> coverage(gr)

SimpleRleList of length 2
$chr
'integer' Rle of length 4000000 with 13 runs
  Lengths:      10       1 ... 3999900
  Values :       0       1 ...       0

$plasmid
'integer' Rle of length 100000 with 9 runs
```

Manipulating GRanges

```
Lengths:    13     1 ...     1 99903
Values :     0     1 ...     1     0
```

BioC I/O packages

- ShortRead was the first one for HTS data.
  It's great for reading Illumina output files (export, fastq), alignments from short read aligners such as Bowtie, Eland, ...[3]
- With the surge of short read aligners, a group decided to create a unified format. The SAM format:
  `http://samtools.sourceforge.net/`
  The format is detailed at
  `http://samtools.sourceforge.net/SAM1.pdf` and
  Rsamtools is the BioC package.
- Basically most tools now use the SAM format and its brother, the BAM format (binary files, less HD space!).
- Caveats...

[3] Definitely check the *qa* function if you use ShortRead!

scanBAM

- scanBAM is the main function in this package!
- To read in a file you need to specify some parameters. For example, data from select ranges.
- Lets look at the example:

```
> library(Rsamtools)
> which <- RangesList(seq1 = IRanges(1000,
+     2000), seq2 = IRanges(c(100,
+     1000), c(1000, 2000)))
> what <- c("rname", "strand", "pos",
+     "qwidth", "seq")
> param <- ScanBamParam(which = which,
+     what = what)
```

scanBAM

- Now we have all the pieces we need to read in a BAM file. Lets add the file path.

```
> which
```

```
SimpleRangesList of length 2
$seq1
IRanges of length 1
    start  end width
[1]  1000 2000  1001

$seq2
IRanges of length 2
    start  end width
[1]   100 1000   901
[2]  1000 2000  1001
```

scanBAM

```
> bamFile <- system.file("extdata",
+     "ex1.bam", package = "Rsamtools")
```

- And finally read the BAM file:

```
> bam <- scanBam(bamFile, param = param)
```

Understanding our bam object

- Careful! Don't **print** the bam object since it is actually a list:

```
> class(bam)
```

```
[1] "list"
```

```
> names(bam)
```

```
[1] "seq1:1000-2000" "seq2:100-1000"
[3] "seq2:1000-2000"
```

- In the list, we have one element per each range we specified. Each of those elements is another list:

```
> class(bam[[1]])
```

```
[1] "list"
```

```
> names(bam[[1]])
```

Understanding our bam object

```
[1] "rname"  "strand" "pos"    "qwidth"
[5] "seq"
```

- As we can see, we have one element per every variable we read in (specified in our *what* object).

```
> sapply(bam[[1]], class)
```

```
       rname       strand
    "factor"     "factor"
         pos      qwidth
   "integer"    "integer"
         seq
"DNAStringSet"
```

To a DataFrame

- Probably an easier to use class is the DataFrame[4]
- The transformation involves more complicated code:

```
> lst <- lapply(names(bam[[1]]),
+     function(elt) {
+         do.call(c, unname(lapply(bam,
+             "[[", elt)))
+     })
> names(lst) <- names(bam[[1]])
> df <- do.call("DataFrame", lst)
> head(df, 2)
```

To a DataFrame

```
DataFrame with 2 rows and 5 columns
      rname    strand       pos
  <integer> <integer> <integer>
1         1         1       970
2         1         1       971
     qwidth
  <integer>
1        35
2        35
                                 seq
                         <DNAStringSet>
1 TATTAGGAAATGCTTTACTGTCATAACTATGAAGA
2 ATTAGGAAATGCTTTACTGTCATAACTATGAAGAG
```

[4] It isn't a *data.frame*!!!

THE advantage of BAM files

- BAM files are not only binary files, but they are indexed.
- Meaning that we can quickly read a subset of our aligned data!
- If you set na19240url to `ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/data/NA19240/alignment/NA19240.chrom6.SLX.maq.SRP000032.2009_07.bam` you can do the reading the following data subset:

```
> which <- GRanges(seqnames = "6",
+     ranges = IRanges(1e+05, 110000))
> param <- ScanBamParam(which = which)
> na19240bam <- scanBam(na19240url,
+     param = param)
```

THE advantage of BAM files

- Rsamtools also includes functions for dealing with multiple BAM files.

Overview

- It's one of the newest packages for ChIP-seq workflows.
- It integrates functionality across several packages.
- Basic idea: compare a set of ranges with annotation ranges, find the closest ones and make tests.
- I find it to be a very interesting tool box :)

A quick demo

- Lets load the example data:

```
> library(ChIPpeakAnno)
> data(myPeakList)
> data(TSS.human.GRCh37)
> head(myPeakList, 2)
```

```
RangedData with 2 rows and 0 value columns across 24 spaces
                  space
            <character>
1_93_556427           1
1_41_559455           1
                   ranges |
                <IRanges> |
1_93_556427 [556660, 556760] |
1_41_559455 [559774, 559874] |
```

```
> head(TSS.human.GRCh37, 2)
```

A quick demo

```
RangedData with 2 rows and 2 value columns across 72 spaces
                     space
                 <character>
ENSG00000223972           1
ENSG00000227232           1
                      ranges |
                   <IRanges> |
ENSG00000223972 [11874, 14412] |
ENSG00000227232 [14363, 29570] |
                    strand
                 <integer>
ENSG00000223972         1
ENSG00000227232        -1


ENSG00000223972 DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 like 10 [S
ENSG00000227232                  WAS protein family homolog 5 pseudogene [S
```

- Using annotatePeakInBatch we can associate the two types of ranges:

A quick demo

```
> annotatedPeak = annotatePeakInBatch(myPeakList[1:6,
+     ], AnnotationData = TSS.human.GRCh37)
> head(annotatedPeak, 2)
```

```
RangedData with 2 rows and 9 value columns across 1 space
                                      space
                                <character>
1_14_1269014 ENSG00000169962            1
1_11_1041174 ENSG00000131591            1
                                       ranges
                                    <IRanges>
1_14_1269014 ENSG00000169962 [1270239, 1270339]
1_11_1041174 ENSG00000131591 [1041646, 1041746]
                                    |
                                    |
1_14_1269014 ENSG00000169962        |
1_11_1041174 ENSG00000131591        |
                                      peak
                                 <character>
1_14_1269014 ENSG00000169962 1_14_1269014
```

A quick demo

```
1_11_1041174 ENSG00000131591 1_11_1041174
                                  strand
                                <character>
1_14_1269014 ENSG00000169962           1
1_11_1041174 ENSG00000131591          -1
                                     feature
                                 <character>
1_14_1269014 ENSG00000169962 ENSG00000169962
1_11_1041174 ENSG00000131591 ENSG00000131591
                                start_position
                                     <numeric>
1_14_1269014 ENSG00000169962        1266694
1_11_1041174 ENSG00000131591        1017198
                                end_position
                                   <numeric>
1_14_1269014 ENSG00000169962      1270686
1_11_1041174 ENSG00000131591      1051741
                                insideFeature
                                  <character>
1_14_1269014 ENSG00000169962         inside
```

A quick demo

```
1_11_1041174 ENSG00000131591        inside
                              distancetoFeature
                                      <numeric>
1_14_1269014 ENSG00000169962                3545
1_11_1041174 ENSG00000131591               10095
                              shortestDistance
                                      <numeric>
1_14_1269014 ENSG00000169962                347
1_11_1041174 ENSG00000131591               9995
                              fromOverlappingOrNearest
                                       <character>
1_14_1269014 ENSG00000169962              NearestStart
1_11_1041174 ENSG00000131591              NearestStart
```

- Export to Excel[5]

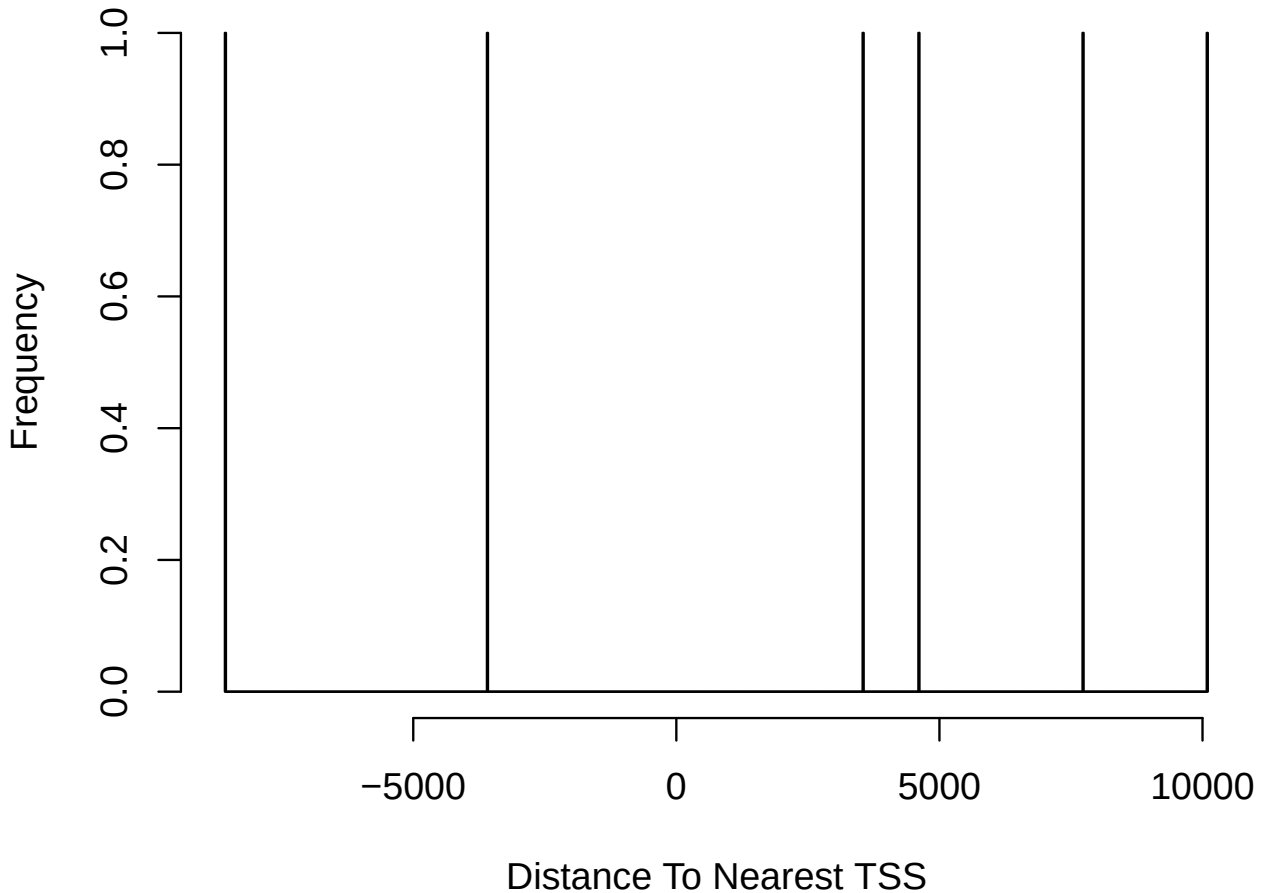
```
> write.table(as.data.frame(annotatedPeak),
+     file = "annotatedPeakList.xls",
+     sep = "\t", row.names = FALSE)
```

[5] Though it might be faster to manipulate in R :)

Peak distribution distance to your annotation

```
> 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))
```

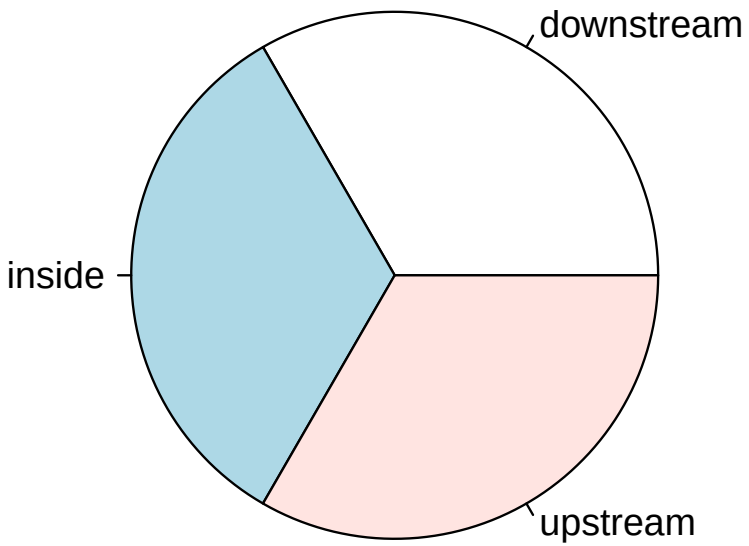
Peak distribution distance to your annotation



And a look at the genomic regions

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

And a look at the genomic regions



A second example

- Say that you have 3 replicates and you want to check how significant is the overlap between the peaks and visualize it as a **Venn** diagram.
- Lets load the example data.

```
> data(Peaks.Ste12.Replicate1)
> data(Peaks.Ste12.Replicate2)
> data(Peaks.Ste12.Replicate3)
> head(Peaks.Ste12.Replicate1, 2)
```

A second example

```
RangedData with 2 rows and 1 value column across 16 spa
         space         ranges |
   <character>      <IRanges> |
22        chr1 [67961, 70254] |
23        chr1 [67961, 70254] |
      strand
   <integer>
22          1
23          1
```

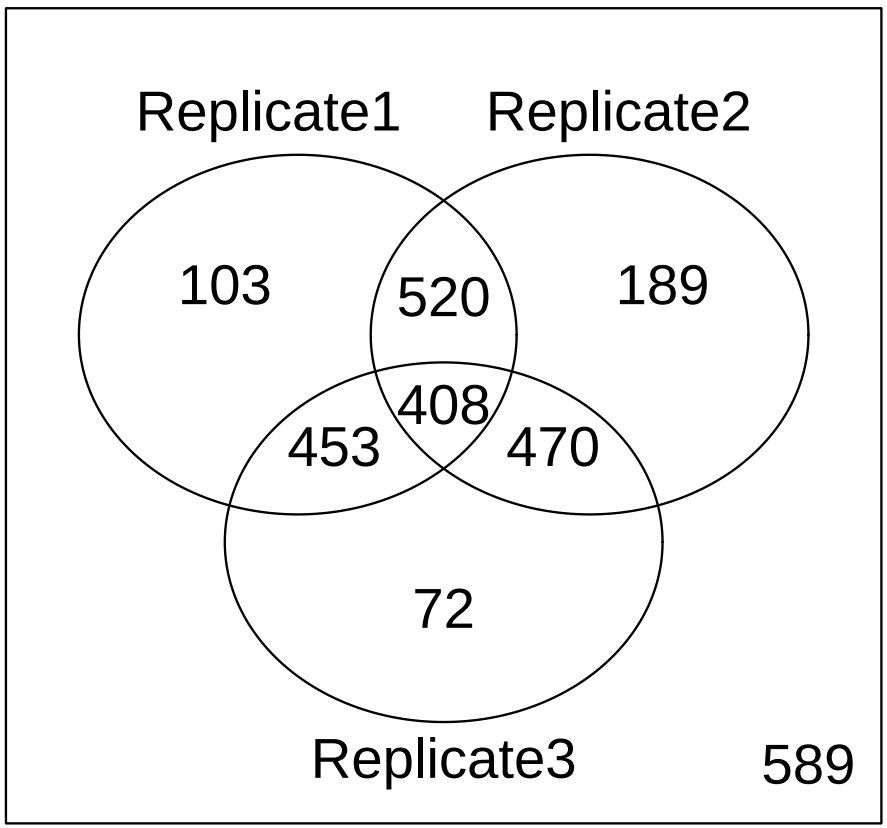
Venn diagram

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

$p.value.1vs2
[1] 6.803956e-91

$p.value.1vs3
[1] 4.54906e-107

$p.value.2vs3
[1] 1.853842e-85

$vennCounts
     Replicate1 Replicate2 Replicate3
[1,]          0          0          0
[2,]          0          0          1
[3,]          0          1          0
```

Venn diagram

```
[4,]          0          1          1
[5,]          1          0          0
[6,]          1          0          1
[7,]          1          1          0
[8,]          1          1          1
     Counts
[1,]    589
[2,]     72
[3,]    189
[4,]    470
[5,]    103
[6,]    453
[7,]    520
[8,]    408
attr(,"class")
[1] "VennCounts"
```

Venn diagram



Other useful tools

- BED / GFF import
- Get annotation from a public database using biomaRt
- Get the sequences nearby interesting peaks to do motif discovery
- Get GO terms and test for significant enrichment
- Find the peak distance to other features in custom ways.

Session Information

```
> sessionInfo()

R version 2.11.1 (2010-05-31)
x86_64-pc-linux-gnu

locale:
 [1] LC_CTYPE=en_US.utf8
 [2] LC_NUMERIC=C
 [3] LC_TIME=en_US.utf8
 [4] LC_COLLATE=en_US.utf8
 [5] LC_MONETARY=C
 [6] LC_MESSAGES=en_US.utf8
 [7] LC_PAPER=en_US.utf8
 [8] LC_NAME=C
 [9] LC_ADDRESS=C
[10] LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.utf8
[12] LC_IDENTIFICATION=C

attached base packages:
```

Session Information

```
[1] stats     graphics  grDevices
[4] utils     datasets  methods
[7] base

other attached packages:
 [1] ChIPpeakAnno_1.4.1
 [2] limma_3.4.4
 [3] org.Hs.eg.db_2.4.1
 [4] GO.db_2.4.1
 [5] RSQLite_0.9-2
 [6] DBI_0.2-5
 [7] AnnotationDbi_1.10.2
 [8] BSgenome.Ecoli.NCBI.20080805_1.3.16
 [9] BSgenome_1.16.5
[10] multtest_2.5.14
[11] Biobase_2.8.0
[12] biomaRt_2.4.0
[13] Rsamtools_1.0.7
[14] Biostrings_2.16.9
[15] GenomicRanges_1.0.7
```

Session Information

```
[16] IRanges_1.6.11

loaded via a namespace (and not attached):
[1] MASS_7.3-7      RCurl_1.4-3
[3] splines_2.11.1  survival_2.35-8
[5] tools_2.11.1    XML_3.1-0
```