# Handling genomic data using Bioconductor II: GenomicRanges and GenomicFeatures

# Motivating examples

- Genomic "Features" (e.g., genes, exons, CpG islands) on the genome are often represented as intervals, e.g., chromosome, start, end, strand.
  - A common task is to explore the overlaps of two types of features, for example, How CpG islands overlap promoters.
  - Sometimes one wants to obtain the intersect/union of two sets of intervals.
- To obtain a list of genes/exons for an organism.

Without Bioconductor you have to rely on your own scripts for these operations.

# Today's topics

- GenomicRanges: package dealing with genomic intervals (genes, CpG islands, binding sites, etc.)
  - Built on more general package IRanges for range data.
  - Provide a rich collection of functions for genomic interval operations.
- **GenomicFeatures**: package for transcript centric genomic annotations.

## IRanges package

- "The IRanges package is designed to represent sequences, ranges representing indices along those sequences, and data related to those ranges".
  - sequence: ordered finite collection of elements, such as a vector of integers. Not necessarily DNA sequence.
  - Consecutive indices can be represented as a range to save memory and computation, for example, instead of saving c(1,2,3,4,5), just save 1 and 5.

## Construct an object of IRanges

Provide start and end indices:

Or provide start and width of each range:

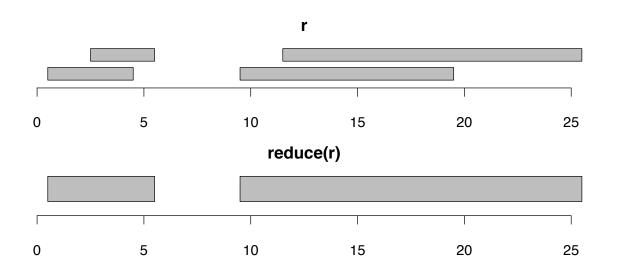
### Simple operations of an IRanges object

```
> length(r)
[1] 4
> start(r)
[1] 1 3 12 10
> end(r)
[1] 4 5 25 19
> width(r)
[1] 4 3 14 10
> r[1:2]
IRanges of length 2
   start end width
[1] 1 4
[2] 3 5
                3
> range(r)
IRanges of length 1
   start end width
[1] 1 25
               25
```

#### reduce

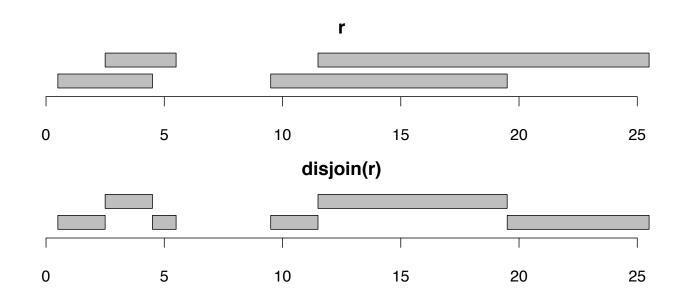
 Merge redundant ranges, and return the minimum non-overlapping ranges covering all the input ranges.

```
> reduce(r)
IRanges of length 2
    start end width
[1]    1    5    5
[2]    10    25    16
```



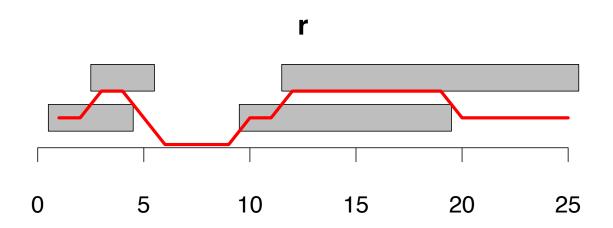
## disjoin

- Return a set of non-overlapping ranges satisfying:
  - the union of results is the same as the union of the inputs.
  - for every range in the result, it overlapping pattern with the input is constant.



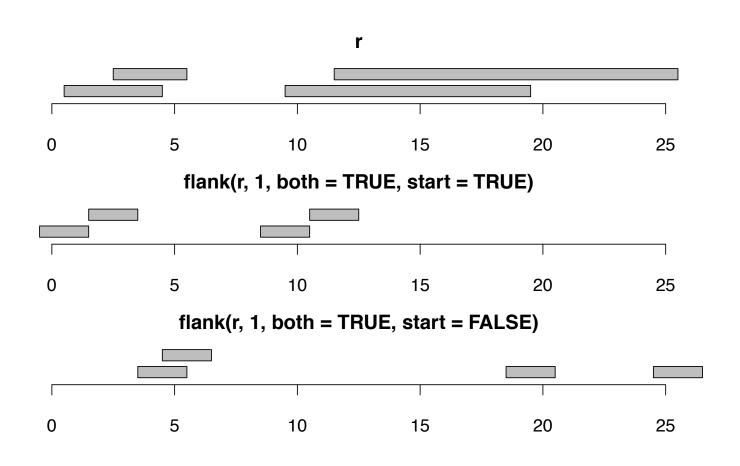
#### coverage

 Compute the coverage depth by the input ranges of each position.



## flank

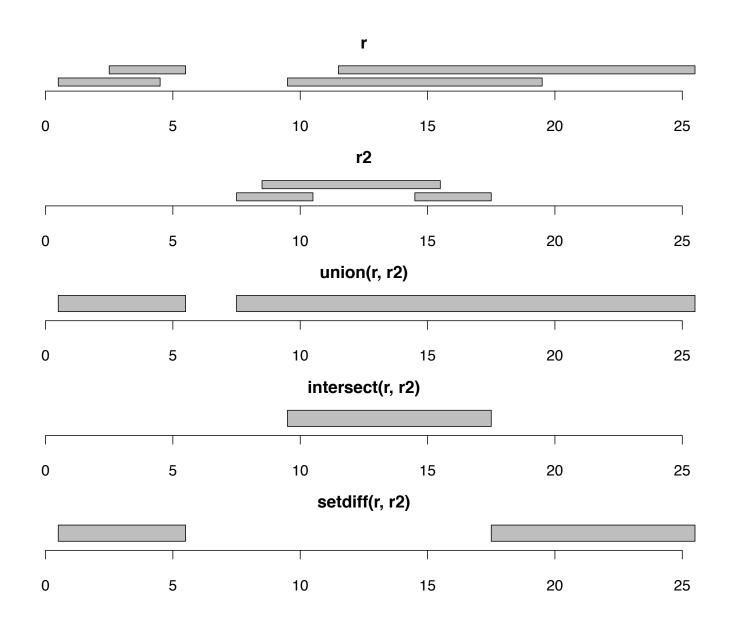
Create flanking ranges for each input range.



#### Operations on two IRanges objects

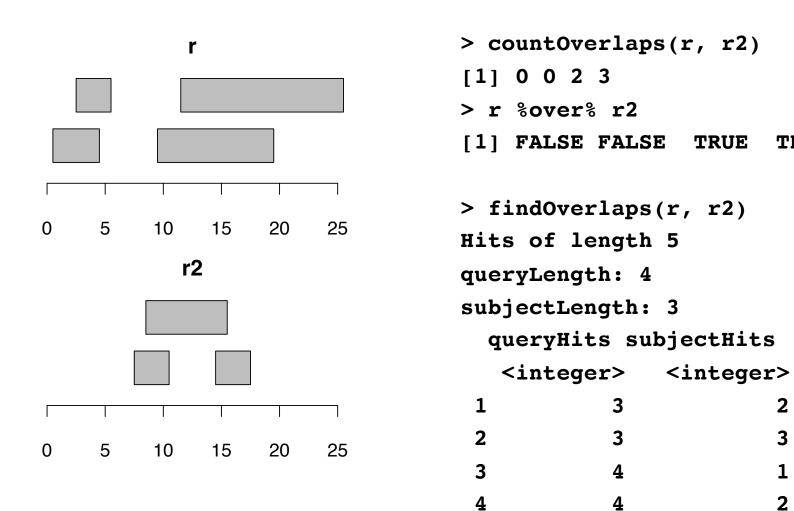
- Functions for different set operations of two lists of ranges:
  - union/intersect/setdiff.
  - countOverlaps: for a "query" and a "reference", count the number of ranges in reference overlapping each range in query.
  - findOverlaps: locating the overlapping ranges in reference for each range in query.

# **Set operations**



## overlaps

TRUE



## Rle: Run length encoding

- A simple data compression method to represent a long sequence in which consecutive elements often take the same value.
- Instead of saving the whole sequence, it stores the consecutive elements with the same value as a single value and count.

## Create Rle object

```
> x < - Rle(c(1,1,2,2,2))
> x
'numeric' Rle of length 5 with 2 runs
 Lengths: 2 3
 Values: 12
> x <- Rle(values=c(1,2), lengths=c(2,3))
> x
'numeric' Rle of length 5 with 2 runs
 Lengths: 2 3
 Values: 12
> as.numeric(x)
[1] 1 1 2 2 2
> x <- Rle(values=c("a","b","c"), lengths=c(2,3,4))
> x
'character' Rle of length 9 with 3 runs
 Lengths: 2 3 4
 Values : "a" "b" "c"
> as.character(x)
[1] "a" "a" "b" "b" "c" "c" "c" "c"
```

## Simple operations of Rle object

```
> x <- Rle(c(1,1,2,2,2))
> length(x)
[1] 5
> start(x)
[1] 1 3
> end(x)
[1] 2 5
> width(x)
[1] 2 3
> nrun(x)
[1] 2
> runLength(x)
[1] 2 3
```

## GenomicRanges package

- Designed to represent genomic intervals (genes, CpG islands, binding sites, etc.)
- Based on IRanges package and provide support for BSgenome, GenomicFeatures, etc.
- Contain three major classes:
  - GRanges: single interval range features: a set of genomic features that each has a single start and end locations.
  - GRangesList: multiple interval range features: each feature has multiple start/end locations. Ex: a transcript has multiple exons.
  - GappedAlignments: gapped alignments.

#### Create a *GRanges* object

#### Required fields:

chr1 chr2

NA

NA

- seqnames: Rle object for sequence name, e.g., the chromosome number.
- ranges: IRanges object for locations.

**Other fields**: strand, elementMetadata for other information.

```
> qr <- GRanges(segnames = Rle(c("chr1", "chr2"), c(2, 3)),
             ranges = IRanges(1:5, end = 6:10),
+
             strand = Rle(strand(c("-", "+", "+", "-")), c(1,1,2,1)),
+
             score = 1:5, GC = seq(1, 0, length = 5))
> gr
GRanges with 5 ranges and 2 elementMetadata values
            ranges strand |
   segnames
                                         GC
                              score
      <Rle> <IRanges> <Rle> | <integer> <numeric>
[1] chr1 [1, 6] - |
                                       1.00
[2] chr1 [2, 7] + |
                                       0.75
[3] chr2 [3, 8] + [3]
                                       0.50
                                       0.25
[4] chr2 [4, 9] + | 4
[5] chr2 [5, 10] - | 5
                                       0.00
seqlengths
```

#### **Operate on a GRanges object**

```
> length(gr)
[1] 5
> seqnames(gr)
'factor' Rle of length 5 with 2 runs
 Lengths:
             2
 Values : chr1 chr2
Levels(2): chr1 chr2
> start(gr)
[1] 1 2 3 4 5
> end(gr)
[1] 6 7 8 9 10
> ranges(gr)
IRanges of length 5
   start end width
[1]
       1
           6
[2]
       2 7
[3]
       3 8
                 6
       4 9
[4]
       5 10
[5]
```

```
> strand(gr)
'factor' Rle of length 5 with 3 runs
  Lengths: 1 3 1
 Values : - + -
Levels(3): + - *
> elementMetadata(gr)
DataFrame with 5 rows and 2 columns
                  GC
      score
  <integer> <numeric>
         1
                1.00
              0.75
         3 0.50
             0.25
              0.00
5
```

All other fields (besides seqnames, range and strands) need to be accessed by elementMetadata function, which returns other fields as a data frame.

## Subsetting and combining

```
> gr[1:2]
GRanges with 2 ranges and 2 elementMetadata values
   segnames
               ranges strand |
                                              GC
                                  score
      <Rle> <IRanges> <Rle> | <integer> <numeric>
[1]
       chr1
               [1, 6]
                                            1.00
[2]
       chr1 [2, 7] + |
                                            0.75
                                     2
seqlengths
chr1 chr2
  NA
       NA
> c(gr[1], gr[3])
GRanges with 2 ranges and 2 elementMetadata values
               ranges strand |
   segnames
                                              GC
                                  score
      <Rle> <IRanges> <Rle> | <integer> <numeric>
[1]
       chr1
               [1, 6]
                                             1.0
       chr2
               [3, 8]
                                             0.5
[2]
                          + |
seglengths
chr1 chr2
  NA
       NA
```

# Other utility functions

- Inherited from IRanges package. Most of the functions working for IRanges also works for GRanges:
  - single range functions: reduce/disjoin/flank/coverage/etc.
  - set operation: intersect/union/setdiff/gap.
  - overlap functions: findOverlap, countOverlap, match, etc.
- The results take into account the chromosome number and strand directions.

```
> coverage(gr)
SimpleRleList of length 2
$chr1
'integer' Rle of length 7 with 3 runs
 Lengths: 1 5 1
 Values : 1 2 1
$chr2
'integer' Rle of length 10 with 6 runs
 Lengths: 2 1 1 4 1 1
 Values : 0 1 2 3 2 1
> reduce(gr)
GRanges with 4 ranges and 0 elementMetadata values
   seqnames ranges strand |
      <Rle> <IRanges> <Rle> |
     chr1 [2, 7]
[1]
                         + |
[2] chr1 [1, 6] - |
[3] chr2 [3, 9] + [3]
[4] chr2 [5, 10] - |
```

```
> disjoin(qr)
GRanges with 6 ranges and 0 elementMetadata values
              ranges strand |
   segnames
      <Rle> <IRanges> <Rle> |
       chr1
[11]
             [2, 7]
                         + |
[2]
       chr1 [1, 6]
      chr2 [3, 3]
[3]
                         + |
[4] chr2 [4, 8]
                         + |
[5] chr2 [9, 9]
                         + |
             [5, 10]
       chr2
[6]
```

#### > flank(gr, 2)

GRanges with 5 ranges and 2 elementMetadata values ranges strand | GC segnames score <Rle> <IRanges> <Rle> | <integer> <numeric> chr1 [ 7, 8] [1] 1.00 - 1 1 [2] chr1 [ 0, 1] + | 2 0.75 3 0.50 [3] chr2 [ 1, 2] + | [4] chr2 [ 2, 3] 4 0.25 + | chr2 [11, 12] [5] 5 0.00

```
> gr1 <- GRanges(seqnames = Rle("chr1", 2),</pre>
                ranges=IRanges(start=c(1,10), end = c(5,15)))
> gr2 <- GRanges(seqnames = Rle("chr1", 1),</pre>
                ranges = IRanges(start=3, end = 12))
> union(gr1, gr2)
GRanges with 1 range and 0 elementMetadata values
             ranges strand |
   segnames
      <Rle> <IRanges> <Rle> |
[1] chr1 [1, 15]
> intersect(gr1, gr2)
GRanges with 2 ranges and 0 elementMetadata values
   segnames ranges strand |
      <Rle> <IRanges> <Rle> |
[1] chr1 [3, 5]
[2] chr1 [10, 12] * |
> setdiff(gr1, gr2)
GRanges with 2 ranges and 0 elementMetadata values
   segnames ranges strand |
      <Rle> <IRanges> <Rle> |
[1]
       chr1 [ 1, 2] * |
[2] chr1 [13, 15] * |
```

#### Overlapping between two GRanges object

findOverlaps: overlap queries.

 %over%: return TRUE/FALSE to indicate if each interval in object 1 overlaps any interval in object 2.

```
> gr1 %over% gr2
[1] TRUE TRUE
```

#### GRangesList: multiple interval range features

Basically a list of GRanges objects:

```
> GRangesList(gr1, gr2)
GRangesList of length 2
[[1]]
GRanges with 2 ranges and 0 elementMetadata values
              ranges strand |
   segnames
      <Rle> <IRanges> <Rle> |
[1] chr1 [1, 5]
[2] chr1 [10, 15] * |
[[2]]
GRanges with 1 range and 0 elementMetadata values
   segnames
              ranges strand |
      <Rle> <IRanges> <Rle> |
       chr1 [3, 12]
[1]
```

- Subsetting by [[]].
- Support sapply/lapply.

## **Summary of GenomicRanges**

- Provides flexible and efficient functions to operate on the intervals.
- Genomic interval are represented as GRanges object, which contains chromosome name in R1e, start/end positions as IRanges object.
- For second generation sequencing data (will be taught later), each sequence read can be represented as an interval, which makes many operations easier.

#### GenomicFeatures

- Retrieves and manages different genomic features from public databases (UCSC genome browser and BioMart).
- Provides more convenient access for genomic features than to manually download and read in text files.

## TxDb object

- Stores transcript annotations.
- Backed by a SQLite database.
- Three methods to create a new TxDb object:
  - makeTxDbFromUCSC to download from UCSC Genome browser.
  - makeTxDbFromBiomart to download from BioMart.
  - Use a GFF3 or GTF file containing transcript information with makeTxDbFromGFF.
  - makeTxDbPackageFromUCSC and makeTxDbPackageFromBiomart to make annotation packages for later use.

#### makeTxDbFromUCSC

#### > supportedUCSCtables()

tablenam	e trad	ck		sul	otrack
1	knownGene		UCSC	Genes	<na></na>
2	knownGeneOld8	Old	UCSC	Genes	<na></na>
3	knownGeneOld7	Old	UCSC	Genes	<na></na>
4	knownGeneOld6	Old	UCSC	Genes	<na></na>
5	knownGeneOld4	Old	UCSC	Genes	<na></na>
6	knownGeneOld3	Old	UCSC	Genes	<na></na>
7	ccdsGene			CCDS	<na></na>
8	xenoRefGene	Of	ther 1	RefSeq	<na></na>
9	vegaGene		Vega	Genes	Vega Protein Genes
10	vegaPseudoGene		Vega	Genes	Vega Pseudogenes
11	ensGene	Ens	sembl	Genes	<na></na>

#### Creating, saving and loading

```
> txdb
TxDb object:
# Db type: TxDb
# Supporting package: GenomicFeatures
# Data source: UCSC
# Genome: hg19
# Organism: Homo sapiens
# Taxonomy ID: 9606
# UCSC Table: knownGene
# UCSC Track: UCSC Genes
# transcript nrow: 82960
# exon nrow: 289969
# cds nrow: 237533
# Db created by: GenomicFeatures package from Bioconductor
> saveDb(txdb, file="hg19 knownGenes.sqlite")
> txdb = loadDb("hg19_knownGenes.sqlite")
```

#### makeTxDbPackageFromUCSC

- Directly make a package for the TxDb
- Need to provide some basic information for R package building (maintainer, author, version, etc.)
- Result is an R package in current directory.
- Need to install the package and then it can be used.

```
> makeTxDbPackageFromUCSC(
          maintainer="Hao Wu <hao.wu@emory.edu>",
          author="Hao Wu",
          version="1.0",
          genome="hq19",
          tablename="knownGene")
Download the knownGene table ... OK
Download the knownToLocusLink table ... OK
Extract the 'transcripts' data frame ... OK
Extract the 'splicings' data frame ... OK
Download and preprocess the 'chrominfo' data frame ... OK
Prepare the 'metadata' data frame ... OK
Make the TxDb object ... OK
Creating package in ./TxDb.Hsapiens.UCSC.hg19.knownGene
```

## **Retrieving features**

• Retrieve basic features: transcripts, exons.

> transcripts(txdb)

GRanges object with 82960 ranges and 2 metadata columns:

```
segnames
                          ranges strand | tx id
                                                  tx name
             <Rle>
                       <IRanges> <Rle> | <integer> <character>
                                              1 uc001aaa.3
   [1]
              chr1 [ 11874, 14409]
                                    + |
   [2]
              chr1 [ 11874, 14409]
                                             2 uc010nxq.1
                                    + |
   [3]
              chr1 [ 11874, 14409] + |
                                             3 uc010nxr.1
   [4]
              chr1 [ 69091, 70008] + | 4 uc001aal.1
                                              5 uc001aaq.2
   [5]
              chr1 [321084, 321115] + |
[82956] chrUn gl000237 [ 1, 2686]
                                    - 1
                                          82956 uc011mgu.1
[82957] chrUn gl000241 [20433, 36875]
                                    - 1
                                          82957
                                                uc011mgv.2
[82958] chrUn gl000243 [11501, 11530] + |
                                          82958
                                                uc011mgw.1
[82959] chrUn gl000243
                   [13608, 13637] + |
                                          82959
                                                uc022brq.1
[82960] chrUn gl000247
                    [ 5787, 5816]
                                          82960
                                                uc022brr.1
                                    - |
```

-----

> transcripts(txdb, filter=list(tx\_chrom="chr1"))
GRanges object with 7967 ranges and 2 metadata columns:

	seqnames		ranges	strand	١	tx_id	tx_name
	<rle></rle>		Ranges	<rle></rle>	١	<integer></integer>	<character></character>
[1]	chr1	[ 11874	4, 14409]	+	١	1	uc001aaa.3
[2]	chr1	[ 11874	4, 14409]	+	١	2	uc010nxq.1
[3]	chr1	[ 11874	4, 14409]	+	١	3	uc010nxr.1
[4]	chr1	[ 69091	L, 70008]	+	١	4	uc001aal.1
[5]	chr1	[321084	4, 321115]	+	١	5	uc001aaq.2
• • •					•		• • •
[7963]	chr1	[249144203, 2	249152264]	-	١	7963	uc031pta.1
[7964]	chr1	[249144203, 2	249152912]	-	١	7964	uc001ifb.2
[7965]	chr1	[249144203, 2	249153125]	-	١	7965	uc010pzr.2
[7966]	chr1	[249144203, 2	249153315]	-	١	7966	uc001ifc.2
[7967]	chr1	[249144203, 2	249153315]	-	I	7967	uc001iff.2

-----

> exons(txdb)

GRanges object with 289969 ranges and 1 metadata column:

	seqnames		ranges	strand	1	exon_id
	<rle></rle>	Ranges		<rle></rle>	١	<integer></integer>
[1]	chr1	[11874,	12227]	+	١	1
[2]	chr1	[12595,	12721]	+	١	2
[3]	chr1	[12613,	12721]	+	١	3
[4]	chr1	[12646,	12697]	+	1	4
[5]	chr1	[13221,	14409]	+	١	5
• • •					•	
[289965]	chrUn_gl000241	[35706,	35859]	_	١	289965
[289966]	chrUn_gl000241	[36711,	36875]	_	١	289966
[289967]	chrUn_gl000243	[11501,	11530]	+	1	289967
[289968]	chrUn_g1000243	[13608,	13637]	+	١	289968
[289969]	chrUn_gl000247	[ 5787,	5816]	_	١	289969

-----

## Retrieve by group

- Grouped features functions retrieve features grouped by other features (e.g., genes):
  - transcriptsBy, exonsBy, cdsBy, intronsByTranscript, fiveUTRsByTranscript, threeUTRsByTranscript.

```
> transcriptsBy(txdb, by="gene")
GRangesList object of length 23459:
$1
GRanges object with 2 ranges and 2 metadata columns:
                         ranges strand | tx id tx name
     segnames
       <Rle>
                      <IRanges> <Rle> | <integer> <character>
  [1] chr19 [58858172, 58864865] - | 70455 uc002qsd.4
  [2] chr19 [58859832, 58874214] - | 70456 uc002qsf.2
$10
GRanges object with 1 range and 2 metadata columns:
     seqnames
                         [1] chr8 [18248755, 18258723] + | 31944 uc003wyw.1
$100
GRanges object with 1 range and 2 metadata columns:
     segnames
                         ranges strand | tx id tx name
  [1] chr20 [43248163, 43280376] - | 72132 uc002xmj.3
<23456 more elements>
seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
> exonsBy(txdb, by="gene")
GRangesList object of length 23459:
$1
```

GRanges object with 15 ranges and 2 metadata columns:

exon_name	exon_id	I	strand	ranges		seqnames	
<character></character>	<integer></integer>	I	<rle></rle>	<iranges></iranges>		<rle></rle>	
<na></na>	250809	1	_	58858395]	[58858172,	chr19	[1]
<na></na>	250810	I	-	58859006]	[58858719,	chr19	[2]
<na></na>	250811	I	-	58860494]	[58859832,	chr19	[3]
<na></na>	250812	I	-	58862017]	[58860934,	chr19	[4]
<na></na>	250813	١	-	58862017]	[58861736,	chr19	[5]

\$10 GRanges object with 2 ranges and 2 metadata columns:

exon_name	_id	exon	1	strand	ranges		seqnames	
<na></na>	3603	113	١	+	18248855]	[18248755,	chr8	[1]
<na></na>	3604	113	ı	+	18258723]	[18257508,	chr8	[2]

. . .

<23457 more elements>

\_\_\_\_\_

```
> intronsByTranscript(txdb)
GRangesList object of length 82960:
$1
GRanges object with 2 ranges and 0 metadata columns:
     segnames ranges strand
       <Rle> <IRanges> <Rle>
  [1] chr1 [12228, 12612]
  [2] chr1 [12722, 13220] +
$2
GRanges object with 2 ranges and 0 metadata columns:
     seqnames ranges strand
  [1] chr1 [12228, 12594] +
  [2] chr1 [12722, 13402] +
$3
GRanges object with 2 ranges and 0 metadata columns:
     seqnames ranges strand
  [1] chr1 [12228, 12645] +
  [2] chr1 [12698, 13220] +
. . .
<82957 more elements>
seqinfo: 93 sequences (1 circular) from hg19 genome
```

## Retriving by overlaps

- transcriptsByOverlaps,
   exonsByOverlaps, cdsByOverlaps:
  - return a GRangesList object containing data about transcripts, exons, or coding sequences that overlap genomic coordinates specified by a GRanges object.
  - Useful for, for example, obtain a list of genes overlapping the binding sites of a TF.

> transcriptsByOverlaps(txdb, gr)
GRanges object with 5 ranges and 2 metadata columns:

tx_name	tx_id	strand	ranges	seqnames	
<character></character>	<integer></integer>	<rle>  </rle>	Ranges	<rle></rle>	
uc001abb.3	13	+	[568844, 568913]	l] chr1	[1]
uc021oeg.2	4095	-	[134773, 140566]	2] chr1	[2]
uc021oej.1	4096	- 1	[566093, 566115]	3] chr1	[3]
uc021oek.1	4097	-	[566135, 566155]	d] chr1	[4]
uc021oel.1	4098	- 1	[566240, 566263]	5] chr1	[5]

## A practical example

- Assume I have a list of TF binding sites in human genome hg19, How to obtain:
  - GC content (%G+%C) of each site.
  - percentage of gene promoters covered by the binding sites.

#### Steps:

- Load in BSgenome. Hsapiens. UCSC. hg19.
- 2. For each site, retrieve its DNA sequence (use Views to speed up).
- 3. Use alphabetFrequency to compute GC content.
- 4. Create GRanges object to represent the binding sites.
- 5. Retrieve gene locations using GenomicFeatures.
- 6. Create GRanges to represent all the gene promoters.
- 7. Use countOverlaps to analyze the overlap.

#### biomaRt

- R interface to the BioMart databases (http://www.biomart.org).
- Examples of BioMart databases are Ensembl, Uniprot and HapMap.
- Works similarly to GenomicFeatues, but slower since everything has to be retrieved from internet.
- More flexible: have connections with affy ID and GO annotation, etc.

#### Review

- We have introduced following useful Bioconductor package: GenomicRanges, GenomicFeatures.
- Use a combination of these and
   Biostrings/BSgenome, you can easily achieve most
   routine analysis works for bioinformatician.

#### After class:

- Review slides and rerun the R codes (on the class webpage).
- Install GenomicRanges and GenomicFeatures.