

**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:

```
> r <- IRanges(start=c(1,3,12, 10), end=c(4, 5, 25, 19))
> r
IRanges of length 4
      start end width
[1]      1   4     4
[2]      3   5     3
[3]     12  25    14
[4]     10  19    10
```

- Or provide start and width of each range:

```
> r <- IRanges(start=c(1,3,12, 10), width=c(4, 3, 14, 10))
> r
IRanges of length 4
      start end width
[1]      1   4     4
[2]      3   5     3
[3]     12  25    14
[4]     10  19    10
```

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

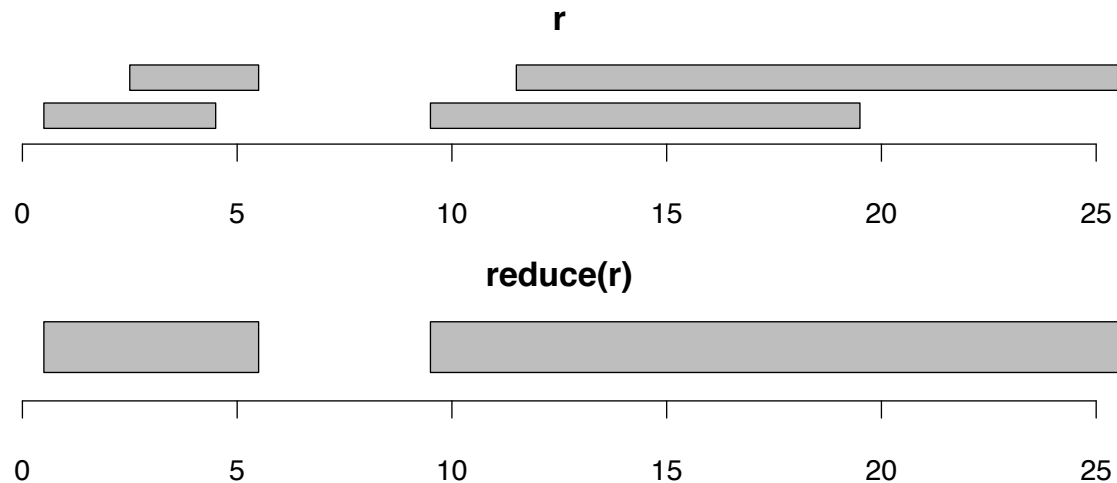
reduce

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

```
> reduce(r)
```

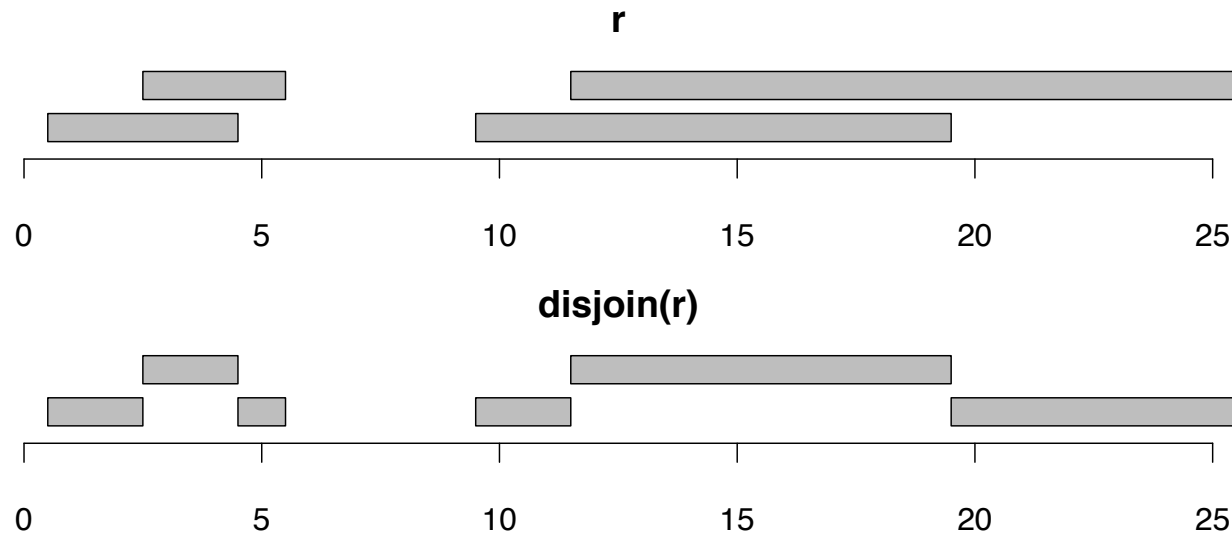
```
IRanges of length 2
```

	start	end	width
[1]	1	5	5
[2]	10	25	16



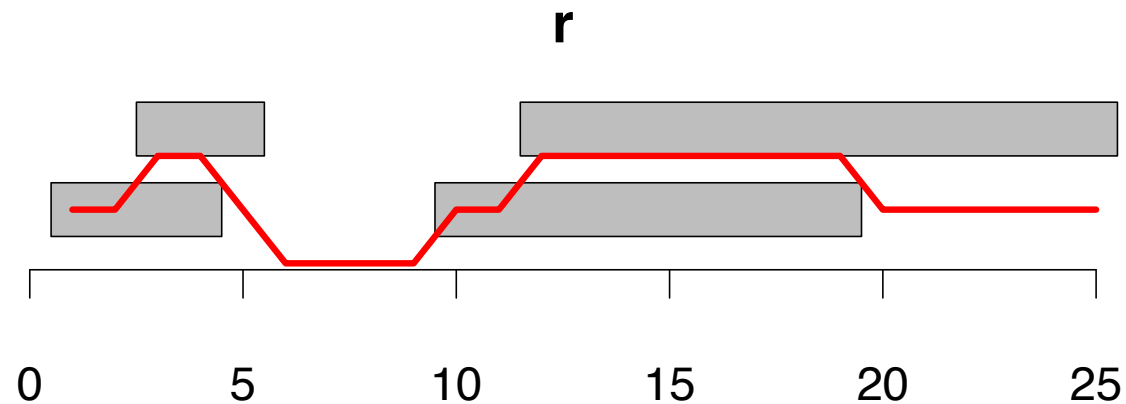
disjoin

- 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, its overlapping pattern with the input is constant.



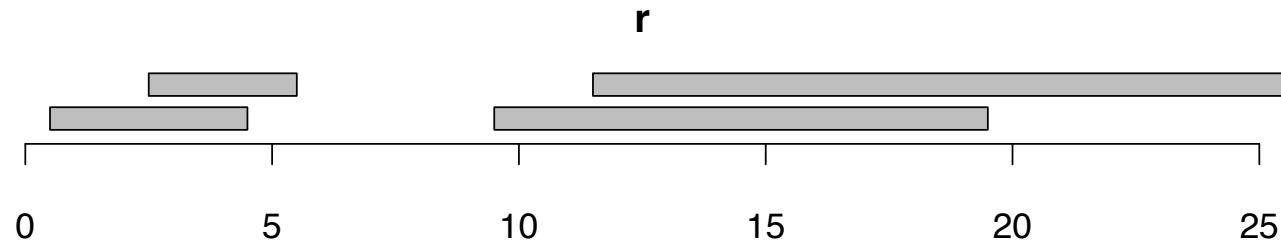
coverage

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

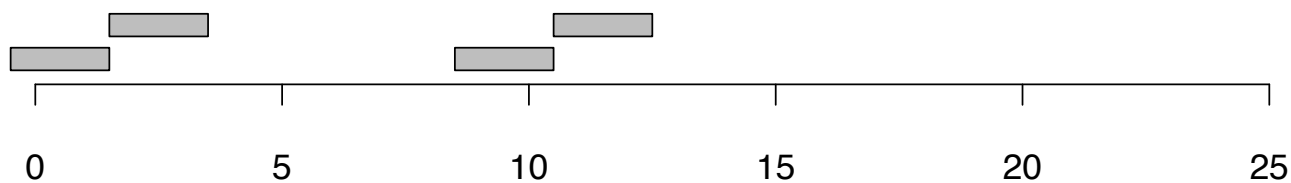


flank

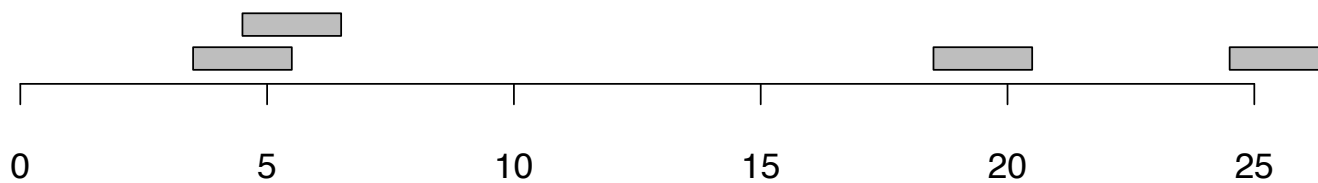
- Create flanking ranges for each input range.



flank(r, 1, both = TRUE, start = TRUE)



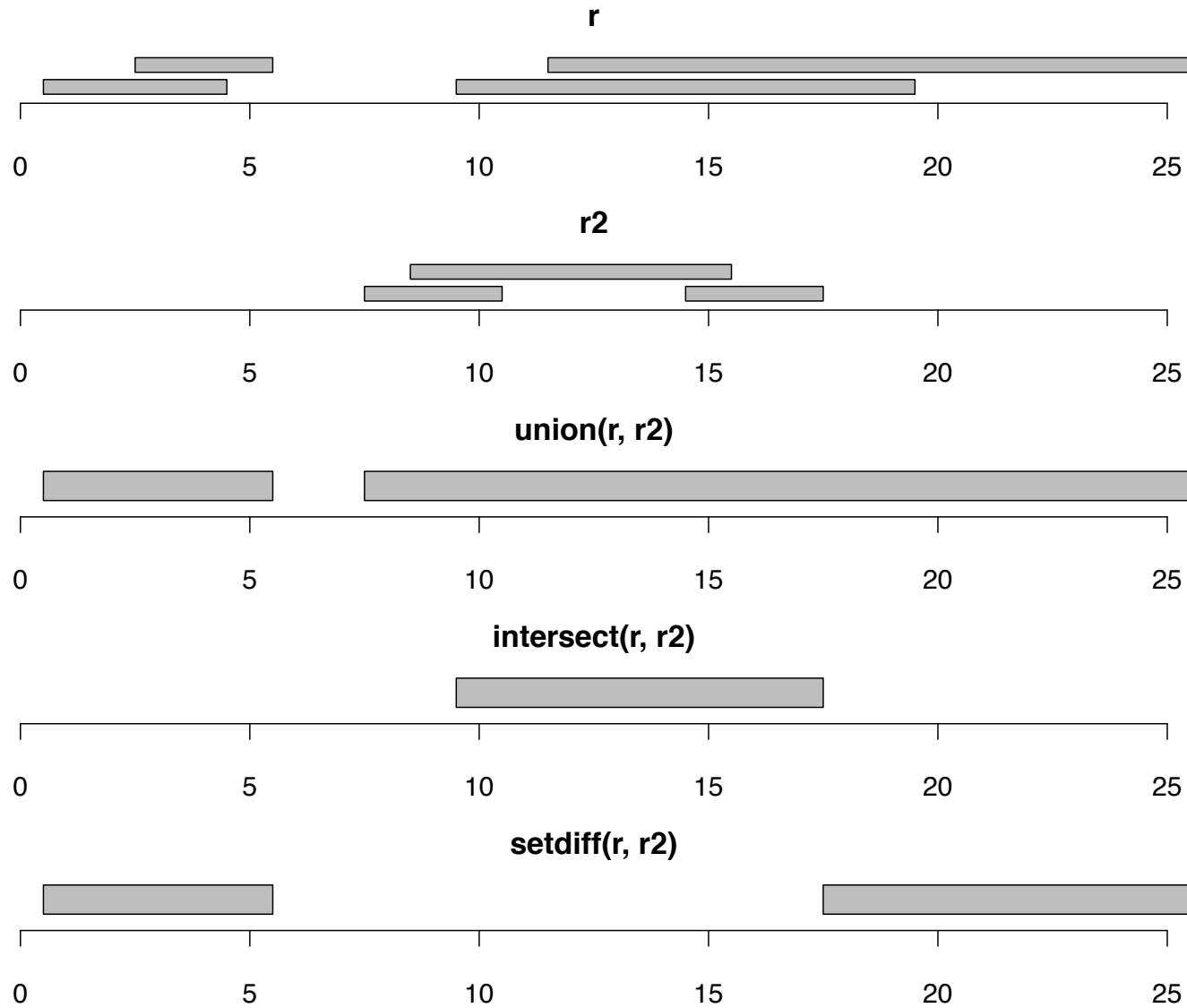
flank(r, 1, both = TRUE, start = FALSE)



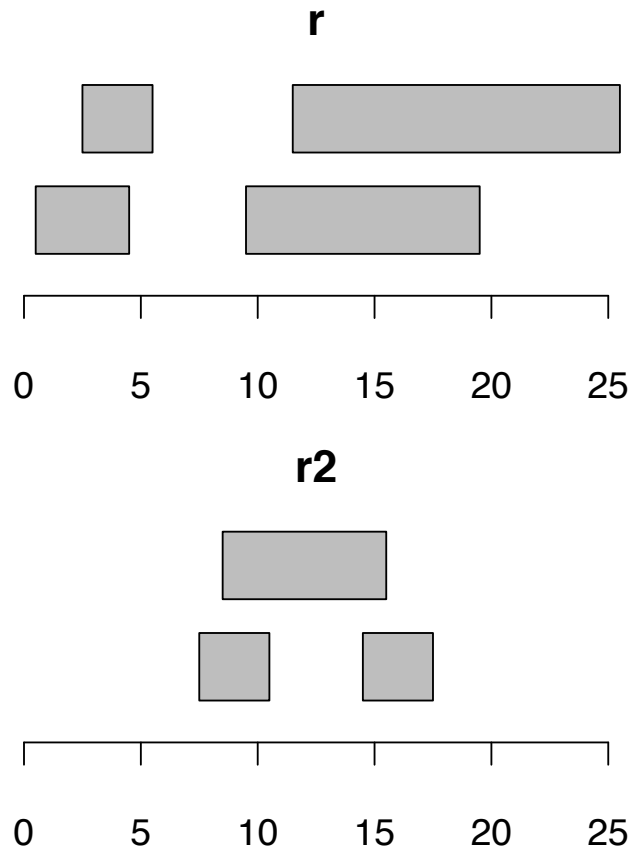
Operations on two IRanges objects

- 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



```
> countOverlaps(r, r2)
[1] 0 0 2 3
> r %over% r2
[1] FALSE FALSE TRUE TRUE
```

```
> findOverlaps(r, r2)
Hits of length 5
queryLength: 4
subjectLength: 3
  queryHits subjectHits
    <integer>    <integer>
1           3           2
2           3           3
3           4           1
4           4           2
5           4           3
```

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 : 1 2
> x <- Rle(values=c(1,2), lengths=c(2,3))
> x
'numeric' Rle of length 5 with 2 runs
  Lengths: 2 3
  Values : 1 2
> 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" "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:

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

Other fields: strand, elementMetadata for other information.

```
> gr <- GRanges(seqnames = 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

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
[1]	chr1	[1, 6]	-	1	1.00
[2]	chr1	[2, 7]	+	2	0.75
[3]	chr2	[3, 8]	+	3	0.50
[4]	chr2	[4, 9]	+	4	0.25
[5]	chr2	[5, 10]	-	5	0.00

seqlengths

chr1 chr2

NA NA

Operate on a GRanges object

```
> length(gr)
[1] 5
> seqnames(gr)
'factor' Rle of length 5 with 2 runs
  Lengths:      2      3
  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     6
[2]     2   7     6
[3]     3   8     6
[4]     4   9     6
[5]     5  10     6
```

```

> 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
      score      GC
  <integer> <numeric>
1          1      1.00
2          2      0.75
3          3      0.50
4          4      0.25
5          5      0.00

```

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
```

	seqnames	ranges	strand		score	GC
	<Rle>	<IRanges>	<Rle>		<integer>	<numeric>
[1]	chr1	[1, 6]	-		1	1.00
[2]	chr1	[2, 7]	+		2	0.75

```
seqlengths
```

```
chr1 chr2
```

```
NA NA
```

```
> c(gr[1], gr[3])
```

```
GRanges with 2 ranges and 2 elementMetadata values
```

	seqnames	ranges	strand		score	GC
	<Rle>	<IRanges>	<Rle>		<integer>	<numeric>
[1]	chr1	[1, 6]	-		1	1.0
[2]	chr2	[3, 8]	+		3	0.5

```
seqlengths
```

```
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>	
[1]	chr1	[2, 7]	+	
[2]	chr1	[1, 6]	-	
[3]	chr2	[3, 9]	+	
[4]	chr2	[5, 10]	-	

```
> disjoint(gr)
```

GRanges with 6 ranges and 0 elementMetadata values

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[2, 7]	+	
[2]	chr1	[1, 6]	-	
[3]	chr2	[3, 3]	+	
[4]	chr2	[4, 8]	+	
[5]	chr2	[9, 9]	+	
[6]	chr2	[5, 10]	-	

```
> flank(gr, 2)
```

GRanges with 5 ranges and 2 elementMetadata values

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


```

> gr1 <- GRanges(seqnames = Rle("chr1", 2),
+               ranges=IRanges(start=c(1,10), end = c(5,15)))
> gr2 <- GRanges(seqnames = Rle("chr1", 1),
+               ranges = IRanges(start=3, end = 12))
> union(gr1, gr2)

```

GRanges with 1 range and 0 elementMetadata values

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[1, 15]	*	

```

> intersect(gr1, gr2)

```

GRanges with 2 ranges and 0 elementMetadata values

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[3, 5]	*	
[2]	chr1	[10, 12]	*	

```

> setdiff(gr1, gr2)

```

GRanges with 2 ranges and 0 elementMetadata values

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[1, 2]	*	
[2]	chr1	[13, 15]	*	

Overlapping between two GRanges object

- `findOverlaps`: overlap queries.

```
> findOverlaps(gr1, gr2)
```

```
An object of class "RangesMatching"
```

```
Slot "matchMatrix":
```

	query	subject
[1,]	1	1
[2,]	2	1

```
Slot "DIM":
```

```
[1] 2 1
```

- `%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
```

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[1, 5]	*	
[2]	chr1	[10, 15]	*	

```
[[2]]
```

```
GRanges with 1 range and 0 elementMetadata values
```

	seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>	
[1]	chr1	[3, 12]	*	

- 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 Rle, 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 convenient access for genomic features, compared 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()
```

	tablename	track	subtrack
1	knownGene	UCSC Genes	<NA>
2	knownGeneOld8	Old UCSC Genes	<NA>
3	knownGeneOld7	Old UCSC Genes	<NA>
4	knownGeneOld6	Old UCSC Genes	<NA>
5	knownGeneOld4	Old UCSC Genes	<NA>
6	knownGeneOld3	Old UCSC Genes	<NA>
7	ccdsGene	CCDS	<NA>
8	xenoRefGene	Other RefSeq	<NA>
9	vegaGene	Vega Genes	Vega Protein Genes
10	vegaPseudoGene	Vega Genes	Vega Pseudogenes
11	ensGene	Ensembl Genes	<NA>
...			

Creating, saving and loading

```
> txdb=makeTxDbFromUCSC(genome="hg19",  
                        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


```
> 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="hg19",  
    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:
```

	seqnames	ranges	strand	tx_id	tx_name
	<Rle>	<IRanges>	<Rle>	<integer>	<character>
[1]	chr1	[11874, 14409]	+	1	uc001aaa.3
[2]	chr1	[11874, 14409]	+	2	uc010nxq.1
[3]	chr1	[11874, 14409]	+	3	uc010nxr.1
[4]	chr1	[69091, 70008]	+	4	uc001aal.1
[5]	chr1	[321084, 321115]	+	5	uc001aaq.2
...
[82956]	chrUn_g1000237	[1, 2686]	-	82956	uc011mgu.1
[82957]	chrUn_g1000241	[20433, 36875]	-	82957	uc011mgv.2
[82958]	chrUn_g1000243	[11501, 11530]	+	82958	uc011mgw.1
[82959]	chrUn_g1000243	[13608, 13637]	+	82959	uc022brq.1
[82960]	chrUn_g1000247	[5787, 5816]	-	82960	uc022brr.1

```
-----
```

```
seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
> transcripts(txdb, filter=list(tx_chrom="chr1"))
```

```
GRanges object with 7967 ranges and 2 metadata columns:
```

	seqnames	ranges	strand	tx_id	tx_name
	<Rle>	<IRanges>	<Rle>	<integer>	<character>
[1]	chr1	[11874, 14409]	+	1	uc001aaa.3
[2]	chr1	[11874, 14409]	+	2	uc010nxq.1
[3]	chr1	[11874, 14409]	+	3	uc010nxr.1
[4]	chr1	[69091, 70008]	+	4	uc001aal.1
[5]	chr1	[321084, 321115]	+	5	uc001aaq.2
...
[7963]	chr1	[249144203, 249152264]	-	7963	uc031pta.1
[7964]	chr1	[249144203, 249152912]	-	7964	uc001ifb.2
[7965]	chr1	[249144203, 249153125]	-	7965	uc010pzs.2
[7966]	chr1	[249144203, 249153315]	-	7966	uc001ifc.2
[7967]	chr1	[249144203, 249153315]	-	7967	uc001iff.2

```
-----
```

```
seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
> exons(txdb)
```

```
GRanges object with 289969 ranges and 1 metadata column:
```

	seqnames	ranges	strand	exon_id
	<Rle>	<IRanges>	<Rle>	<integer>
[1]	chr1	[11874, 12227]	+	1
[2]	chr1	[12595, 12721]	+	2
[3]	chr1	[12613, 12721]	+	3
[4]	chr1	[12646, 12697]	+	4
[5]	chr1	[13221, 14409]	+	5
...
[289965]	chrUn_g1000241	[35706, 35859]	-	289965
[289966]	chrUn_g1000241	[36711, 36875]	-	289966
[289967]	chrUn_g1000243	[11501, 11530]	+	289967
[289968]	chrUn_g1000243	[13608, 13637]	+	289968
[289969]	chrUn_g1000247	[5787, 5816]	-	289969

```
-----
```

```
seqinfo: 93 sequences (1 circular) from hg19 genome
```

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:
```

	seqnames	ranges	strand	tx_id	tx_name
	<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	ranges	strand	tx_id	tx_name
[1]	chr8	[18248755, 18258723]	+	31944	uc003wyw.1

```
$100
```

```
GRanges object with 1 range and 2 metadata columns:
```

	seqnames	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:
```

	seqnames	ranges	strand		exon_id	exon_name
	<Rle>	<IRanges>	<Rle>		<integer>	<character>
[1]	chr19	[58858172, 58858395]	-		250809	<NA>
[2]	chr19	[58858719, 58859006]	-		250810	<NA>
[3]	chr19	[58859832, 58860494]	-		250811	<NA>
[4]	chr19	[58860934, 58862017]	-		250812	<NA>
[5]	chr19	[58861736, 58862017]	-		250813	<NA>
...

```
$10
```

```
GRanges object with 2 ranges and 2 metadata columns:
```

	seqnames	ranges	strand		exon_id	exon_name
[1]	chr8	[18248755, 18248855]	+		113603	<NA>
[2]	chr8	[18257508, 18258723]	+		113604	<NA>

```
...
```

```
<23457 more elements>
```

```
-----
```

```
seqinfo: 93 sequences (1 circular) from hg19 genome
```

```
> intronsByTranscript(txdb)
```

```
GRangesList object of length 82960:
```

```
$1
```

```
GRanges object with 2 ranges and 0 metadata columns:
```

	seqnames	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
```

Retrieving 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.

```
> gr=GRanges(seqnames = Rle("chr1", 2),
              ranges=IRanges(start=c(100000,500000),
                             end = c(200000,600000)))
```

```
> transcriptsByOverlaps(txdb, gr)
```

GRanges object with 5 ranges and 2 metadata columns:

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

seqinfo: 93 sequences (1 circular) from hg19 genome

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:
 1. 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 GenomicFeatures, 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`.