High-level S4 containers for HTS data

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High-level vs low-level

Small nb of high-level containers for HTS data (< 10).
Built on top of 100+ low-level containers!

High-level containers for HTS data

Covered in this presentation:

- `GRanges`
- `GRangesList`
- `GappedAlignments`
- `GappedAlignmentPairs`

Defined in the *GenomicRanges* package.

Not covered in this presentation:

- Defined in the *GenomicRanges* package: `SummarizedExperiment`
- Defined in the *ShortRead* package: `ShortRead`, `AlignedRead`

Low-level containers (most frequently seen, only)

Covered in this presentation:

- `Rle`
- `IRanges`
- `DataFrame`
- `CharacterList`, `IntegerList`
- `RleList`, `RleViews`, `RleViewsList`

Defined in the *IRanges* package.

Not covered in this presentation:

- In the *IRanges* package: `IRangesList`, `SplitDataFrameList`, `RangedData`, and many, many more...
- In the *Biostrings* package: `DNAString`, `DNAStringSet`

About the implementation

S4 classes (aka *formal* classes) –> relies heavily on the *methods* package.

Current implementation tries to provide an API that is as consistent as possible. In particular:

- The end-user should never need to use `new()`: a *constructor*, named as the container, is provided for each container. E.g. `GRanges()`.
- The end-user should never need to use `@` (aka *direct slot access*): slot *accessors* (*getters* and *setters*) are provided for each container. Not all getters have a corresponding setter!
- Standard functions/operators like `length()`, `names()`, `[`, `c()`, `[[`, `$`, etc... work almost everywhere and behave "as expected".
- Additional functions that work almost everywhere: `elementMetadata()`, `elementLengths()`, `seqinfo()`, etc...
- Consistent display (`show` methods).

Basic operations

Vector operations:

- Single-bracket subsetting: `[`
- Combining: `c()`
- Comparing: `==`, `!=`, `duplicated()`, `unique()`
- Ordering: `<=`, `>=`, `<`, `>`, `order()`, `sort()`, `rank()`

List operations:

- Double-bracket subsetting: `[[`
- `elementLengths()`, `unlist()`, `relist()`
- `lapply()`, `sapply()`, `endoapply()`
- `mendoapply()` (not covered in this presentation)

Basic operations (continued)

Ranges operations:

- `shift()`, `narrow()`, `resize()`, `flank()`
- `disjoin()`
- `range()`, `reduce()`, `gaps()`
- `union()`, `intersect()`, `setdiff()`
- `punion()`, `pintersect()`, `psetdiff()`, `pgap()`

Coercion methods: `as()` and all the *S3 forms* (`as.vector()`, `as.character()`, `as.factor()`, etc...)

Splitting: `split()`

Advanced operations

Coverage and slicing:

- `coverage()`
- `slice()`

Finding/counting overlaps:

- `findOverlaps()`
- `countOverlaps()`

and more...

`Rle` objects

`Rle`: Run Length Encoding

Supported basic operations

- *Vector operations*: **YES**
- *List operations*: **NO**
- *Ranges operations*: **NO**
- *Coercion methods*: **YES** (to atomic vector, factor, or `IRanges`)
- *Splitting*: **YES** (produces an `RleList` object)

Rle objects (continued)

```
> library(IRanges)
> set.seed(2012)
> rle1 <- Rle(sample(c(-0.9, 0), 20, replace=TRUE))
> rle1

numeric-Rle of length 20 with 12 runs
  Lengths:    1    1    1    7    1    1    1    2    1    1    2    1
  Values : -0.9    0 -0.9    0 -0.9    0 -0.9    0 -0.9    0 -0.9    0

> runLength(rle1)

 [1] 1 1 1 7 1 1 1 2 1 1 2 1

> runValue(rle1)

 [1] -0.9  0.0 -0.9  0.0 -0.9  0.0 -0.9  0.0 -0.9  0.0 -0.9  0.0

> as.vector(rle1)

 [1] -0.9  0.0 -0.9  0.0  0.0  0.0  0.0  0.0  0.0  0.0 -0.9  0.0 -0.9  0.0  0.0 -0.9
[17]  0.0 -0.9 -0.9  0.0

> rle1[c(TRUE, FALSE)]

numeric-Rle of length 10 with 5 runs
  Lengths:    2    3    2    2    1
  Values : -0.9    0 -0.9    0 -0.9
```

`Rle` objects (continued)

```
> sort(rle1)

numeric-Rle of length 20 with 2 runs
  Lengths:    7   13
  Values : -0.9    0

> rle1 * 50.1

numeric-Rle of length 20 with 12 runs
  Lengths:      1      1      1      7      1 ...      1      1      2      1
  Values : -45.09      0 -45.09      0 -45.09 ... -45.09      0 -45.09      0

> sum(rle1)

[1] -6.3

> cumsum(rle1)

numeric-Rle of length 20 with 7 runs
  Lengths:    2    8    2    3    2    1    2
  Values : -0.9 -1.8 -2.7 -3.6 -4.5 -5.4 -6.3

> cumsum(rle1) <= -4.2

logical-Rle of length 20 with 2 runs
  Lengths:    15     5
  Values : FALSE  TRUE

> rle1[cumsum(rle1) <= -4.2]

numeric-Rle of length 5 with 4 runs
  Lengths:    1    1    2    1
  Values : -0.9    0 -0.9    0
```

Rle objects (continued)

```
> rle2 <- Rle(c("ch1", "chMT", "ch1", "ch2", "chMT"), c(4, 2, 1, 5, 1))
> rle2

character-Rle of length 13 with 5 runs
  Lengths:      4      2      1      5      1
  Values :  "ch1" "chMT"  "ch1"  "ch2" "chMT"

> as.vector(rle2)

 [1] "ch1"  "ch1"  "ch1"  "ch1"  "chMT" "chMT" "ch1"  "ch2"  "ch2"  "ch2"  "ch2"
[12] "ch2"  "chMT"

> c(rle2, c("chMT", "chX"))

character-Rle of length 15 with 6 runs
  Lengths:      4      2      1      5      2      1
  Values :  "ch1" "chMT"  "ch1"  "ch2" "chMT"  "chX"
```

Rle objects (continued)

```
> runValue(rle2) <- factor(runValue(rle2))
> rle2

factor-Rle of length 13 with 5 runs
  Lengths:    4    2    1    5    1
  Values :  ch1 chMT  ch1  ch2 chMT
Levels(3): ch1 ch2 chMT

> runValue(rle2)

[1] ch1  chMT ch1  ch2  chMT
Levels: ch1 ch2 chMT

> as.vector(rle2)

 [1] "ch1"  "ch1"  "ch1"  "ch1"  "chMT" "chMT" "ch1"  "ch2"  "ch2"  "ch2"  "ch2"
[12] "ch2"  "chMT"

> as.factor(rle2)

 [1] ch1  ch1  ch1  ch1  chMT chMT ch1  ch2  ch2  ch2  ch2  ch2  chMT
Levels: ch1 ch2 chMT
```

Rle objects (continued)

```
> rle1 == 0

logical-Rle of length 20 with 12 runs
  Lengths:     1     1     1     7     1     1     1     2     1     1     2     1
  Values : FALSE  TRUE FALSE  TRUE FALSE  TRUE FALSE  TRUE FALSE  TRUE FALSE  TRUE

> as(rle1 == 0, "IRanges")

IRanges of length 6
    start end width
[1]     2   2     1
[2]     4  10     7
[3]    12  12     1
[4]    14  15     2
[5]    17  17     1
[6]    20  20     1
```

The purpose of the `IRanges` container is...

... to store a set of *integer ranges* (aka *integer intervals*).

- Each range can be defined by a *start* and an *end* value: both are included in the interval (except when the range is empty).
- The *width* of the range is the number of integer values in it: *width* = *end* - *start* + 1.
- *end* is always >= *start*, except for empty ranges where *end* = *start* - 1.

Supported basic operations

- *Vector operations*: **YES**
- *List operations*: **YES** (not covered in this presentation)
- *Ranges operations*: **YES**
- *Coercion methods*: **YES** (from logical or integer vector to `IRanges`)
- *Splitting*: **YES** (produces an `IRangesList` object)

IRanges objects (continued)

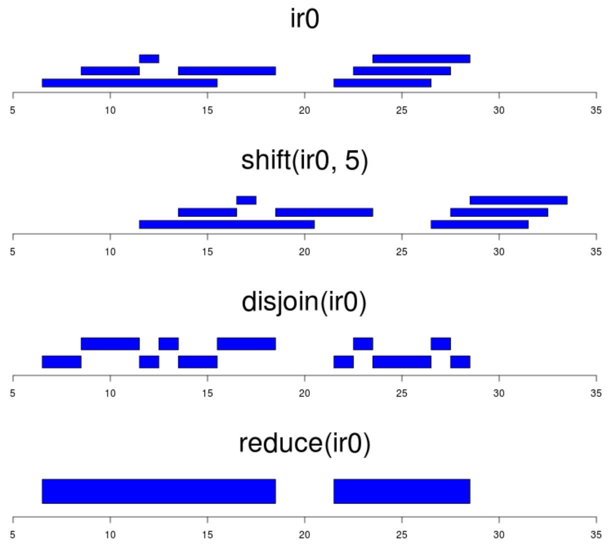
```
> ir1 <- IRanges(start=c(12, -9, NA, 12),
+                end=c(NA, 0, 15, NA),
+                width=c(4, NA, 4, 3))
> ir1  # "show" method not yet consistent with the other "show" methods (TODO)

IRanges of length 4
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  15     4
[4]    12  14     3

> start(ir1)

[1] 12 -9 12 12

> end(ir1)

[1] 15  0 15 14

> width(ir1)

[1]  4 10  4  3

> successiveIRanges(c(10, 5, 38), from=101)

IRanges of length 3
    start end width
[1]   101 110    10
[2]   111 115     5
[3]   116 153    38
```

IRanges objects (continued)

```
> ir1[-2]

IRanges of length 3
    start end width
[1]    12  15     4
[2]    12  15     4
[3]    12  14     3

> ir2 <- c(ir1, IRanges(-10, 0))
> ir2

IRanges of length 5
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  15     4
[4]    12  14     3
[5]   -10   0    11
```

```
> duplicated(ir2)

[1] FALSE FALSE  TRUE FALSE FALSE

> unique(ir2)

IRanges of length 4
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  14     3
[4]   -10   0    11

> sort(ir2)

IRanges of length 5
    start end width
[1]   -10   0    11
[2]    -9   0    10
[3]    12  14     3
[4]    12  15     4
[5]    12  15     4
```

Ranges operations



IRanges objects (continued)

```
> ir1

IRanges of length 4
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  15     4
[4]    12  14     3
```

```
> shift(ir1, -start(ir1))

IRanges of length 4
    start end width
[1]     0   3     4
[2]     0   9    10
[3]     0   3     4
[4]     0   2     3

> flank(ir1, 10, start=FALSE)

IRanges of length 4
    start end width
[1]    16  25    10
[2]     1  10    10
[3]    16  25    10
[4]    15  24    10
```

IRanges objects (continued)

```
> ir1

IRanges of length 4
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  15     4
[4]    12  14     3
```

```
> range(ir1)

IRanges of length 1
    start end width
[1]    -9  15    25

> reduce(ir1)

IRanges of length 2
    start end width
[1]    -9   0    10
[2]    12  15     4
```

```
> union(ir1, IRanges(-2, 6))

IRanges of length 2
    start end width
[1]    -9   6    16
[2]    12  15     4

> intersect(ir1, IRanges(-2, 13))

IRanges of length 2
    start end width
[1]    -2   0     3
[2]    12  13     2

> setdiff(ir1, IRanges(-2, 13))

IRanges of length 2
    start end width
[1]    -9  -3     7
[2]    14  15     2
```

IRanges objects (continued)

```
> ir3 <- IRanges(5:1, width=12)
> ir3

IRanges of length 5
    start end width
[1]     5  16    12
[2]     4  15    12
[3]     3  14    12
[4]     2  13    12
[5]     1  12    12
```

```
> ir2

IRanges of length 5
    start end width
[1]    12  15     4
[2]    -9   0    10
[3]    12  15     4
[4]    12  14     3
[5]   -10   0    11
```

```
> pintersect(ir3, ir2, resolve.empty="max.start")

IRanges of length 5
    start end width
[1]    12  15     4
[2]     4   3     0
[3]    12  14     3
[4]    12  13     2
[5]     1   0     0
```

IRanges objects (continued)

```
> ok <- c(FALSE, FALSE, TRUE, TRUE, TRUE, FALSE, FALSE, TRUE)
> ir4 <- as(ok, "IRanges")  # from logical vector to IRanges
> ir4

IRanges of length 2
    start end width
[1]     3   5     3
[2]     8   8     1

> as(which(ok), "IRanges")  # from integer vector to IRanges

IRanges of length 2
    start end width
[1]     3   5     3
[2]     8   8     1

> rle2[ir4]  # IRanges subscript

factor-Rle of length 4 with 3 runs
  Lengths:    2    1    1
  Values :  ch1 chMT  ch2
Levels(3): ch1 ch2 chMT
```

DataFrame objects

`DataFrame`: An S4 version of `data.frame` that can hold almost anything it its columns.

Supported basic operations

- All the `data.frame` operations. Just manipulate a `DataFrame` as a `data.frame`!
- *Coercion methods*: from almost anything to `DataFrame`, and from `DataFrame` to `data.frame`.
- *Splitting*: **YES** (produces a `SplitDataFrameList` object)

```
> library(Biostrings)
> dna <- DNAStringSet(c("AAA", "TGGATT", "CATTNGAGC", "TAATAG"))
> af <- alphabetFrequency(dna, baseOnly=TRUE)
> df <- DataFrame(dna, af)
> df

DataFrame with 4 rows and 6 columns
             dna         A         C         G         T     other
  <DNAStringSet> <integer> <integer> <integer> <integer> <integer>
1            AAA         3         0         0         0         0
2         TGGATT         1         0         2         3         0
3      CATTNGAGC         2         2         2         2         1
4         TAATAG         3         0         1         2         0

> df$G

[1] 0 2 2 1
```

DataFrame objects (continued)

```
> df$cds_id <- paste("CDS", 1:4, sep="")
> df$cds_range <- successiveIRanges(width(dna), from=51)
> df

DataFrame with 4 rows and 8 columns
              dna         A         C         G         T     other      cds_id
  <DNAStringSet> <integer> <integer> <integer> <integer> <integer> <character>
1             AAA         3         0         0         0         0        CDS1
2          TGGATT         1         0         2         3         0        CDS2
3       CATTNGAGC         2         2         2         2         1        CDS3
4          TAATAG         3         0         1         2         0        CDS4
  cds_range
  <IRanges>
1  [51, 53]
2  [54, 59]
3  [60, 68]
4  [69, 74]

> as.data.frame(df)

        dna A C G T other cds_id cds_range.start cds_range.end cds_range.width
1       AAA 3 0 0 0     0   CDS1              51            53               3
2    TGGATT 1 0 2 3     0   CDS2              54            59               6
3 CATTNGAGC 2 2 2 2     1   CDS3              60            68               9
4    TAATAG 3 0 1 2     0   CDS4              69            74               6
```

CharacterList objects

An S4 virtual class for representing a list of character vectors.

Exists in 2 flavors (i.e. 2 different internal representations):

- CompressedCharacterList
- SimpleCharacterList

```
> ccl <- CharacterList(one=c("aaa", "bb", "c"), two=c("dd", "e", "fff", "gggg"))
```

```
> ccl

CompressedCharacterList of length 2
[["one"]] aaa bb c
[["two"]] dd e fff gggg

> length(ccl)

[1] 2

> names(ccl)

[1] "one" "two"
```

```
> as.list(ccl)

$one
[1] "aaa" "bb"  "c"

$two
[1] "dd"   "e"    "fff"  "gggg"

> ccl[[2]]

[1] "dd"   "e"    "fff"  "gggg"
```

CharacterList objects (continued)

```
> toupper(ccl)

CompressedCharacterList of length 2
[["one"]] AAA BB C
[["two"]] DD E FFF GGGG

> elementLengths(ccl)  # fast version of sapply(ccl, length)

one two
  3   4

> unlist(ccl)  # insane! will be changed soon...

   one    one    one    two    two    two    two
 "aaa"   "bb"    "c"   "dd"    "e"  "fff" "gggg"

> unlist(ccl, use.names=FALSE)

[1] "aaa"  "bb"   "c"    "dd"   "e"    "fff"  "gggg"
```

IntegerList objects

An S4 virtual class for representing a list of integer vectors.

Exists in 2 flavors (i.e. 2 different internal representations):

- `CompressedIntegerList`
- `SimpleIntegerList`

```
> cil <- IntegerList(6:-2, 5, integer(0), 14:21)
> cil

CompressedIntegerList of length 4
[[1]] 6 5 4 3 2 1 0 -1 -2
[[2]] 5
[[3]] integer(0)
[[4]] 14 15 16 17 18 19 20 21

> cil * cil

CompressedIntegerList of length 4
[[1]] 36 25 16 9 4 1 0 1 4
[[2]] 25
[[3]] integer(0)
[[4]] 196 225 256 289 324 361 400 441
```

IntegerList objects (continued)

2 different ways to obtain the same result:

```
> cil * 100L - 2L

CompressedIntegerList of length 4
[[1]] 598 498 398 298 198 98 -2 -102 -202
[[2]] 498
[[3]] integer(0)
[[4]] 1398 1498 1598 1698 1798 1898 1998 2098
```

```
> relist(unlist(cil) * 100L - 2L, cil)

CompressedIntegerList of length 4
[[1]] 598 498 398 298 198 98 -2 -102 -202
[[2]] 498
[[3]] integer(0)
[[4]] 1398 1498 1598 1698 1798 1898 1998 2098
```

But the above trick would not work here:

```
> cumsum(cil)

CompressedNumericList of length 4
[[1]] 6 11 15 18 20 21 21 20 18
[[2]] 5
[[3]] numeric(0)
[[4]] 14 29 45 62 80 99 119 140
```

`RleList`, `RleViews` and `RleViewsList` objects

Typically seen when doing *Coverage and slicing*.

`RleList`: An S4 virtual class for representing a list of `Rle` objects. Exists in 2 flavors (i.e. 2 different internal representations):

- `CompressedRleList`
- `SimpleRleList`

`RleViews`: An S4 class for representing a set of *views* (i.e. ranges) defined on an `Rle` *subject*.

`RleViewsList`: An S4 virtual class for representing a list of `RleViews` objects. Exists only in 1 flavor: `SimpleRleViewsList`.

The purpose of the `GRanges` container is...

... to store a set of *genomic ranges* (aka *genomic regions* or *genomic intervals*).

- Like for `IRanges` objects, each range can be defined by a *start* and an *end* value.
- *start* and *end* are both 1-based positions relative to the 5' end of the plus strand of the chromosome (aka *reference sequence*), even when the range is on the minus strand.
- The *start* is the leftmost position and the *end* is the rightmost, even when the range is on the minus strand.
- Each range is assigned a chromosome name and a strand.

Supported basic operations

- *Vector operations*: **YES**
- *List operations*: **NO**
- *Ranges operations*: **YES**
- *Coercion methods*: to `RangedData` or `IRangesList` (both not covered in this presentation)
- *Splitting*: **YES** (produces a `GRangesList` object)

GRanges constructor

```
> library(GenomicRanges)
> gr1 <- GRanges(seqnames=rep(c("ch1", "chMT"), c(2, 4)),
+                ranges=IRanges(start=16:21, end=20),
+                strand=rep(c("+", "-", "*"), 2))
> gr1

GRanges with 6 ranges and 0 elementMetadata cols:
      seqnames    ranges strand
         <Rle> <IRanges>  <Rle>
  [1]      ch1  [16, 20]      +
  [2]      ch1  [17, 20]      -
  [3]     chMT  [18, 20]      *
  [4]     chMT  [19, 20]      +
  [5]     chMT  [20, 20]      -
  [6]     chMT  [21, 20]      *
  ---
  seqlengths:
    ch1 chMT
     NA   NA
```

GRanges accessors

```
> length(gr1)

[1] 6

> seqnames(gr1)

factor-Rle of length 6 with 2 runs
  Lengths:    2    4
  Values :  ch1 chMT
Levels(2): ch1 chMT

> ranges(gr1)

IRanges of length 6
    start end width
[1]    16  20     5
[2]    17  20     4
[3]    18  20     3
[4]    19  20     2
[5]    20  20     1
[6]    21  20     0
```

GRanges accessors (continued)

```
> start(gr1)

[1] 16 17 18 19 20 21

> end(gr1)

[1] 20 20 20 20 20 20

> width(gr1)

[1] 5 4 3 2 1 0

> strand(gr1)

factor-Rle of length 6 with 6 runs
  Lengths: 1 1 1 1 1 1
  Values : + - * + - *
Levels(3): + - *

> strand(gr1) <- c("-", "-", "+")
> strand(gr1)

factor-Rle of length 6 with 4 runs
  Lengths: 2 1 2 1
  Values : - + - +
Levels(3): + - *
```

GRanges accessors (continued)

```
> names(gr1) <- LETTERS[1:6]
> names(gr1)

[1] "A" "B" "C" "D" "E" "F"

> elementMetadata(gr1) <- DataFrame(score=11:16, GC=seq(1, 0, length=6))
> elementMetadata(gr1)

DataFrame with 6 rows and 2 columns
      score        GC
  <integer> <numeric>
1        11       1.0
2        12       0.8
3        13       0.6
4        14       0.4
5        15       0.2
6        16       0.0

> gr1

GRanges with 6 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  E     chMT  [20, 20]      - |        15       0.2
  F     chMT  [21, 20]      + |        16         0
  ---
  seqlengths:
    ch1 chMT
     NA   NA
```

GRanges accessors (continued)

```
> seqinfo(gr1)

Seqinfo of length 2
seqnames seqlengths isCircular genome
ch1              NA         NA   <NA>
chMT             NA         NA   <NA>

> seqlevels(gr1)

[1] "ch1"  "chMT"

> seqlengths(gr1)

 ch1 chMT
  NA   NA

> seqlengths(gr1) <- c(50000, 800)
> seqlengths(gr1)

  ch1  chMT
50000   800
```

Vector operations on GRanges objects

```
> gr1[c("F", "A")]

GRanges with 2 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  F     chMT  [21, 20]      + |        16         0
  A      ch1  [16, 20]      - |        11         1
  ---
  seqlengths:
     ch1  chMT
   50000   800

> gr1[strand(gr1) == "+"]

GRanges with 2 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  C     chMT  [18, 20]      + |        13       0.6
  F     chMT  [21, 20]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Vector operations on GRanges objects (continued)

```
> gr1 <- gr1[-5]
> gr1

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  F     chMT  [21, 20]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Vector operations on GRanges objects (continued)

```
> gr2 <- GRanges(seqnames="ch2",
+                ranges=IRanges(start=c(2:1,2), width=6),
+                score=15:13,
+                GC=seq(0, 0.4, length=3))
> gr12 <- c(gr1, gr2)
> gr12

GRanges with 8 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  F     chMT  [21, 20]      + |        16         0
         ch2  [ 2,  7]      * |        15         0
         ch2  [ 1,  6]      * |        14       0.2
         ch2  [ 2,  7]      * |        13       0.4
  ---
  seqlengths:
     ch1  chMT    ch2
   50000   800     NA
```

Vector operations on GRanges objects (continued)

```
> gr12[length(gr12)] == gr12

[1] FALSE FALSE FALSE FALSE FALSE  TRUE FALSE  TRUE

> duplicated(gr12)

[1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE  TRUE

> unique(gr12)

GRanges with 7 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  F     chMT  [21, 20]      + |        16         0
  6      ch2  [ 2,  7]      * |        15         0
  7      ch2  [ 1,  6]      * |        14       0.2
  ---
  seqlengths:
     ch1  chMT   ch2
   50000   800    NA
```

Vector operations on GRanges objects (continued)

```
> sort(gr12)

GRanges with 8 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  F     chMT  [21, 20]      + |        16         0
  D     chMT  [19, 20]      - |        14       0.4
  6      ch2  [ 1,  6]      * |        14       0.2
  7      ch2  [ 2,  7]      * |        15         0
  8      ch2  [ 2,  7]      * |        13       0.4
  ---
  seqlengths:
     ch1  chMT   ch2
   50000   800    NA
```

Ranges operations on GRanges objects

```
> gr2

GRanges with 3 ranges and 2 elementMetadata cols:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]      ch2    [2, 7]      * |        15         0
  [2]      ch2    [1, 6]      * |        14       0.2
  [3]      ch2    [2, 7]      * |        13       0.4
  ---
  seqlengths:
   ch2
    NA

> shift(gr2, 50)

GRanges with 3 ranges and 2 elementMetadata cols:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]      ch2  [52, 57]      * |        15         0
  [2]      ch2  [51, 56]      * |        14       0.2
  [3]      ch2  [52, 57]      * |        13       0.4
  ---
  seqlengths:
   ch2
    NA

> narrow(gr2, start=2, end=-2)

GRanges with 3 ranges and 2 elementMetadata cols:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]      ch2    [3, 6]      * |        15         0
  [2]      ch2    [2, 5]      * |        14       0.2
  [3]      ch2    [3, 6]      * |        13       0.4
  ---
  seqlengths:
   ch2
    NA
```

Ranges operations on GRanges objects (continued)

```
> gr1

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  F     chMT  [21, 20]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> resize(gr1, 12)

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [ 9, 20]      - |        11         1
  B      ch1  [ 9, 20]      - |        12       0.8
  C     chMT  [18, 29]      + |        13       0.6
  D     chMT  [ 9, 20]      - |        14       0.4
  F     chMT  [21, 32]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Ranges operations on GRanges objects (continued)

```
> gr1

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [16, 20]      - |        11         1
  B      ch1  [17, 20]      - |        12       0.8
  C     chMT  [18, 20]      + |        13       0.6
  D     chMT  [19, 20]      - |        14       0.4
  F     chMT  [21, 20]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> flank(gr1, 3)

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  A      ch1  [21, 23]      - |        11         1
  B      ch1  [21, 23]      - |        12       0.8
  C     chMT  [15, 17]      + |        13       0.6
  D     chMT  [21, 23]      - |        14       0.4
  F     chMT  [18, 20]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Ranges operations on GRanges objects (continued)

```
> gr3 <- shift(gr1, c(35000, rep(0, 3), 100))
> width(gr3)[c(3,5)] <- 117
> gr3

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> range(gr3)

GRanges with 3 ranges and 0 elementMetadata cols:
      seqnames      ranges strand
         <Rle>   <IRanges>  <Rle>
  [1]      ch1 [17, 35020]      -
  [2]     chMT [18,   236]      +
  [3]     chMT [19,    20]      -
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Ranges operations on GRanges objects (continued)

```
> gr3

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> disjoin(gr3)

GRanges with 6 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
         <Rle>      <IRanges>  <Rle>
  [1]      ch1 [   17,    20]      -
  [2]      ch1 [35016, 35020]      -
  [3]     chMT [   18,   119]      +
  [4]     chMT [  120,   134]      +
  [5]     chMT [  135,   236]      +
  [6]     chMT [   19,    20]      -
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Ranges operations on GRanges objects (continued)

```
> gr3

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> reduce(gr3)

GRanges with 4 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
         <Rle>      <IRanges>  <Rle>
  [1]      ch1 [   17,    20]      -
  [2]      ch1 [35016, 35020]      -
  [3]     chMT [   18,   236]      +
  [4]     chMT [   19,    20]      -
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Ranges operations on GRanges objects (continued)

```
> gr3

GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0
  ---
  seqlengths:
     ch1  chMT
   50000   800

> gaps(gr3)

GRanges with 10 ranges and 0 elementMetadata cols:
       seqnames         ranges strand
          <Rle>      <IRanges>  <Rle>
   [1]      ch1 [    1, 50000]      +
   [2]      ch1 [    1,    16]      -
   [3]      ch1 [   21, 35015]      -
   [4]      ch1 [35021, 50000]      -
   [5]      ch1 [    1, 50000]      *
   [6]     chMT [    1,    17]      +
   [7]     chMT [  237,   800]      +
   [8]     chMT [    1,    18]      -
   [9]     chMT [   21,   800]      -
  [10]     chMT [    1,   800]      *
  ---
  seqlengths:
     ch1  chMT
   50000   800
```

Splitting a GRanges object

```
> split(gr3, seqnames(gr3))

GRangesList of length 2:
$ch1
GRanges with 2 ranges and 2 elementMetadata cols:
    seqnames          ranges strand |     score        GC
       <Rle>       <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8

$chMT
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames     ranges strand | score  GC
  C     chMT [ 18, 134]      + |    13 0.6
  D     chMT [ 19,  20]      - |    14 0.4
  F     chMT [120, 236]      + |    16   0

---
seqlengths:
   ch1  chMT
 50000   800
```

Exercise 1

a. Load the *GenomicRanges* package.

b. Open the man page for the `GRanges` class and run the examples in it.

c. Shift the ranges in `gr` by 1000 positions to the right.

d. What method is called when doing `shift()` on a `GRanges` object? Find the man page for this method.

The purpose of the `GRangesList` container is...

... to store a list of *compatible* `GRanges` objects.

compatible means:

- they are relative to the same genome,
- AND they have the same columns in their `elementMetadata` slot.

Supported basic operations

- *Vector operations*: partially supported (no comparing or ordering)
- *List operations*: **YES**
- *Ranges operations*: partially supported (some operations like `disjoin()` or `gaps()` are missing but they could/will be added)
- *Coercion methods*: to `IRangesList` (not covered in this presentation)
- *Splitting*: **NO**

GRangesList constructor

```
> grl <- GRangesList(gr3, gr2)
> grl

GRangesList of length 2:
[[1]]
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

[[2]]
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

GRangesList accessors

```
> length(grl)

[1] 2
```

```
> seqnames(grl)

CompressedRleList of length 2
[[1]]
factor-Rle of length 5 with 2 runs
  Lengths:    2    3
  Values :  ch1 chMT
Levels(3): ch1 chMT ch2

[[2]]
factor-Rle of length 3 with 1 run
  Lengths:   3
  Values : ch2
Levels(3): ch1 chMT ch2
```

```
> strand(grl)

CompressedRleList of length 2
[[1]]
factor-Rle of length 5 with 4 runs
  Lengths: 2 1 1 1
  Values : - + - +
Levels(3): + - *

[[2]]
factor-Rle of length 3 with 1 run
  Lengths: 3
  Values : *
Levels(3): + - *
```

GRangesList accessors (continued)

```
> ranges(grl)

CompressedIRangesList of length 2
[[1]]
IRanges of length 5
    start   end width names
[1] 35016 35020     5     A
[2]    17    20     4     B
[3]    18   134   117     C
[4]    19    20     2     D
[5]   120   236   117     F

[[2]]
IRanges of length 3
    start end width names
[1]     2   7     6
[2]     1   6     6
[3]     2   7     6
```

```
> start(grl)

CompressedIntegerList of length 2
[[1]] 35016 17 18 19 120
[[2]] 2 1 2

> end(grl)

CompressedIntegerList of length 2
[[1]] 35020 20 134 20 236
[[2]] 7 6 7

> width(grl)

CompressedIntegerList of length 2
[[1]] 5 4 117 2 117
[[2]] 6 6 6
```

GRangesList accessors (continued)

```
> names(grl) <- c("TX1", "TX2")
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score       GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

GRangesList accessors (continued)

```
> elementMetadata(grl)$geneid <- c("GENE1", "GENE2")
> elementMetadata(grl)

DataFrame with 2 rows and 1 column
       geneid
  <character>
1       GENE1
2       GENE2

> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

GRangesList accessors (continued)

```
> seqinfo(grl)

Seqinfo of length 3
seqnames seqlengths isCircular genome
ch1           50000         NA   <NA>
chMT            800         NA   <NA>
ch2              NA         NA   <NA>
```

Vector operations on GRangesList objects

```
> grl[c("TX2", "TX1")]

GRangesList of length 2:
$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  1      ch2    [2, 7]      * |        15         0
  2      ch2    [1, 6]      * |        14       0.2
  3      ch2    [2, 7]      * |        13       0.4

$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames          ranges strand | score  GC
  A      ch1 [35016, 35020]      - |    11   1
  B      ch1 [   17,    20]      - |    12 0.8
  C     chMT [   18,   134]      + |    13 0.6
  D     chMT [   19,    20]      - |    14 0.4
  F     chMT [  120,   236]      + |    16   0

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

Vector operations on GRangesList objects (continued)

```
> c(grl, GRangesList(gr3))

GRangesList of length 3:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

[[3]]
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand | score  GC
  A      ch1 [35016, 35020]      - |    11   1
  B      ch1 [   17,    20]      - |    12 0.8
  C     chMT [   18,   134]      + |    13 0.6
  D     chMT [   19,    20]      - |    14 0.4
  F     chMT [  120,   236]      + |    16   0

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

List operations on GRangesList objects

```
> grl[[2]]

GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  1      ch2    [2, 7]      * |        15         0
  2      ch2    [1, 6]      * |        14       0.2
  3      ch2    [2, 7]      * |        13       0.4
  ---
  seqlengths:
     ch1  chMT   ch2
   50000   800    NA

> elementLengths(grl)

TX1 TX2
  5   3

> unlisted <- unlist(grl, use.names=FALSE)  # same as c(grl[[1]], grl[[2]])
> unlisted

GRanges with 8 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0
         ch2 [    2,     7]      * |        15         0
         ch2 [    1,     6]      * |        14       0.2
         ch2 [    2,     7]      * |        13       0.4
  ---
  seqlengths:
     ch1  chMT   ch2
   50000   800    NA
```

List operations on GRangesList objects (continued)

```
> grl100 <- relist(shift(unlisted, 100), grl)
> grl100

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35116, 35120]      - |        11         1
  B      ch1 [  117,   120]      - |        12       0.8
  C     chMT [  118,   234]      + |        13       0.6
  D     chMT [  119,   120]      - |        14       0.4
  F     chMT [  220,   336]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames     ranges strand | score  GC
  1      ch2 [102, 107]      * |    15   0
  2      ch2 [101, 106]      * |    14 0.2
  3      ch2 [102, 107]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

List operations on GRangesList objects (continued)

```
> grl100b <- endoapply(grl, shift, 100)
> grl100b

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35116, 35120]      - |        11         1
  B      ch1 [  117,   120]      - |        12       0.8
  C     chMT [  118,   234]      + |        13       0.6
  D     chMT [  119,   120]      - |        14       0.4
  F     chMT [  220,   336]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames     ranges strand | score  GC
  1      ch2 [102, 107]      * |    15   0
  2      ch2 [101, 106]      * |    14 0.2
  3      ch2 [102, 107]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA

> elementMetadata(grl100)

DataFrame with 2 rows and 0 columns

> elementMetadata(grl100b)

DataFrame with 2 rows and 1 column
       geneid
  <character>
1       GENE1
2       GENE2
```

Ranges operations on `GRangesList` objects

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

```
> shift(grl, 100)

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35116, 35120]      - |        11         1
  B      ch1 [  117,   120]      - |        12       0.8
  C     chMT [  118,   234]      + |        13       0.6
  D     chMT [  119,   120]      - |        14       0.4
  F     chMT [  220,   336]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames     ranges strand | score  GC
  1      ch2 [102, 107]      * |    15   0
  2      ch2 [101, 106]      * |    14 0.2
  3      ch2 [102, 107]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

`shift(grl, 100)` is equivalent to `endoapply(grl, shift, 100)`

Ranges operations on `GRangesList` objects (continued)

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

```
> flank(grl, 10)

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35021, 35030]      - |        11         1
  B      ch1 [   21,    30]      - |        12       0.8
  C     chMT [    8,    17]      + |        13       0.6
  D     chMT [   21,    30]      - |        14       0.4
  F     chMT [  110,   119]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames  ranges strand | score  GC
  1      ch2 [-8, 1]      * |    15   0
  2      ch2 [-9, 0]      * |    14 0.2
  3      ch2 [-8, 1]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

`flank(grl, 10)` is equivalent to `endoapply(grl, flank, 10)`

Ranges operations on `GRangesList` objects (continued)

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames          ranges strand |     score        GC
       <Rle>       <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

```
> range(grl)

GRangesList of length 2:
$TX1
GRanges with 3 ranges and 0 elementMetadata cols:
      seqnames      ranges strand
         <Rle>   <IRanges>  <Rle>
  [1]      ch1 [17, 35020]      -
  [2]     chMT [18,   236]      +
  [3]     chMT [19,    20]      -

$TX2
GRanges with 1 range and 0 elementMetadata cols:
      seqnames ranges strand
  [1]      ch2 [1, 7]      *

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

`range(grl)` is equivalent to `endoapply(grl, range)`

Ranges operations on `GRangesList` objects (continued)

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 elementMetadata cols:
    seqnames         ranges strand |     score        GC
       <Rle>      <IRanges>  <Rle> | <integer> <numeric>
  A      ch1 [35016, 35020]      - |        11         1
  B      ch1 [   17,    20]      - |        12       0.8
  C     chMT [   18,   134]      + |        13       0.6
  D     chMT [   19,    20]      - |        14       0.4
  F     chMT [  120,   236]      + |        16         0

$TX2
GRanges with 3 ranges and 2 elementMetadata cols:
    seqnames ranges strand | score  GC
  1      ch2 [2, 7]      * |    15   0
  2      ch2 [1, 6]      * |    14 0.2
  3      ch2 [2, 7]      * |    13 0.4

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

```
> reduce(grl)

GRangesList of length 2:
$TX1
GRanges with 4 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
         <Rle>      <IRanges>  <Rle>
  [1]      ch1 [   17,    20]      -
  [2]      ch1 [35016, 35020]      -
  [3]     chMT [   18,   236]      +
  [4]     chMT [   19,    20]      -

$TX2
GRanges with 1 range and 0 elementMetadata cols:
      seqnames ranges strand
  [1]      ch2 [1, 7]      *

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

`reduce(grl)` is equivalent to `endoapply(grl, reduce)`

Ranges operations on GRangesList objects (continued)

```
> grl2

GRangesList of length 2:
$TX1
GRanges with 1 range and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  C     chMT [18, 134]      + |        13       0.6

$TX2
GRanges with 1 range and 2 elementMetadata cols:
    seqnames ranges strand | score GC
  1      ch2 [2, 7]      * |    15  0

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA

> grl3

GRangesList of length 2:
[[1]]
GRanges with 1 range and 2 elementMetadata cols:
    seqnames    ranges strand |     score        GC
       <Rle> <IRanges>  <Rle> | <integer> <numeric>
  1     chMT [22, 130]      + |        13       0.6

[[2]]
GRanges with 1 range and 2 elementMetadata cols:
    seqnames ranges strand | score GC
  1      ch2 [2, 7]      * |    15  0

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

```
> psetdiff(grl2, grl3)

GRangesList of length 2:
$TX1
GRanges with 2 ranges and 0 elementMetadata cols:
      seqnames     ranges strand
         <Rle>  <IRanges>  <Rle>
  [1]     chMT [ 18,  21]      +
  [2]     chMT [131, 134]      +

$TX2
GRanges with 0 ranges and 0 elementMetadata cols:
     seqnames ranges strand

---
seqlengths:
   ch1  chMT   ch2
 50000   800    NA
```

The purpose of the `GappedAlignments` container is...

... to store a set of genomic alignments (aligned reads, typically).

The alignments can be loaded from a BAM file with `readGappedAlignments()`. By default, only the following information is loaded for each alignment:

- RNAME field: name of the reference sequence to which the query is aligned.
- strand bit (from FLAG field): strand in the reference sequence to which the query is aligned.
- CIGAR field: a string in the "Extended CIGAR format" describing the "gemoetry" of the alignment (i.e. locations of insertions, deletions and gaps). See the SAM Spec for the details.
- POS field: 1-based position of the leftmost mapped base.

In particular, the query sequences (SEQ) and qualities (QUAL) are not loaded by default.

Supported basic operations

- *Vector* operations: partially supported (no comparing or ordering)
- *List* operations: **NO**
- *Ranges* operations: only `narrow()` and `qnarrow()` (`GappedAlignments` specific) are supported
- *Coercion methods*: to `GRanges` or `GRangesList`
- *Splitting*: **NO**

GappedAlignments constructor

Typically not used directly!

```
> gal0 <- GappedAlignments(seqnames=Rle(c("ch1", "ch2"), c(3, 1)),
+                          pos=1L + 10L*0:3,
+                          cigar=c("36M", "20M3D16M", "20M703N16M", "14M2I20M"),
+                          strand=strand(c("+", "-", "-", "+")))
> gal0

GappedAlignments with 4 alignments and 0 elementMetadata cols:
      seqnames strand       cigar    qwidth     start       end     width      ngap
         <Rle>  <Rle> <character> <integer> <integer> <integer> <integer> <integer>
  [1]      ch1      +         36M        36         1        36        36         0
  [2]      ch1      -    20M3D16M        36        11        49        39         0
  [3]      ch1      -  20M703N16M        36        21       759       739         1
  [4]      ch2      +    14M2I20M        36        31        64        34         0
  ---
  seqlengths:
   ch1 ch2
    NA  NA
```

An N in the cigar indicates a gap (!= deletion).

`readGappedAlignments()`

```
> library(pasillaBamSubset)
> U1gal <- readGappedAlignments(untreated1_chr4())
> length(U1gal)

[1] 204355

> head(U1gal)

GappedAlignments with 6 alignments and 0 elementMetadata cols:
      seqnames strand       cigar    qwidth     start       end     width      ngap
         <Rle>  <Rle> <character> <integer> <integer> <integer> <integer> <integer>
  [1]     chr4      -         75M        75       892       966        75         0
  [2]     chr4      -         75M        75       919       993        75         0
  [3]     chr4      +         75M        75       924       998        75         0
  [4]     chr4      +         75M        75       936      1010        75         0
  [5]     chr4      +         75M        75       949      1023        75         0
  [6]     chr4      -         75M        75       967      1041        75         0
  ---
  seqlengths:
       chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
    23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

GappedAlignments accessors

```
> seqnames(U1gal)

factor-Rle of length 204355 with 1 run
  Lengths: 204355
  Values :   chr4
Levels(8): chr2L chr2R chr3L chr3R chr4 chrM chrX chrYHet

> table(as.factor(seqnames(U1gal)))

  chr2L   chr2R   chr3L   chr3R    chr4    chrM    chrX chrYHet
      0       0       0       0  204355       0       0       0

> strand(U1gal)

factor-Rle of length 204355 with 53763 runs
  Lengths:  2  3  3  1  2  2  4  1  4  2  2  1 ... 13  1 13  1 17  1 20  3  3 40  2
  Values :  -  +  -  +  -  +  -  +  -  +  -  + ...  -  +  -  +  -  +  -  +  -  +  -
Levels(3): + - *

> table(as.factor(strand(U1gal)))

     +      -      *
102101 102254      0

> head(cigar(U1gal))

[1] "75M" "75M" "75M" "75M" "75M" "75M"

> head(qwidth(U1gal))

[1] 75 75 75 75 75 75

> table(qwidth(U1gal))

    75
204355
```

GappedAlignments accessors (continued)

```
> head(start(U1gal))

[1] 892 919 924 936 949 967

> head(end(U1gal))

[1]  966  993  998 1010 1023 1041

> head(width(U1gal))

[1] 75 75 75 75 75 75

> head(ngap(U1gal))

[1] 0 0 0 0 0 0

> table(ngap(U1gal))

     0      1      2
184039  20169    147
```

```
> elementMetadata(U1gal)

DataFrame with 204355 rows and 0 columns

> seqinfo(U1gal)

Seqinfo of length 8
seqnames seqlengths isCircular genome
chr2L      23011544         NA   <NA>
chr2R      21146708         NA   <NA>
chr3L      24543557         NA   <NA>
chr3R      27905053         NA   <NA>
chr4        1351857         NA   <NA>
chrM          19517         NA   <NA>
chrX       22422827         NA   <NA>
chrYHet      347038         NA   <NA>
```

Loading additional information from the BAM file

```
> param <- ScanBamParam(what=c("flag", "mapq"), tag=c("NH", "NM"))
> U1gal <- readGappedAlignments(untreated1_chr4(),
+                                use.names=TRUE, param=param)
> U1gal[1:5]

GappedAlignments with 5 alignments and 4 elementMetadata cols:
                    seqnames strand       cigar    qwidth     start       end
                       <Rle>  <Rle> <character> <integer> <integer> <integer>
  SRR031729.3941844     chr4      -         75M        75       892       966
  SRR031728.3674563     chr4      -         75M        75       919       993
  SRR031729.8532600     chr4      +         75M        75       924       998
  SRR031729.2779333     chr4      +         75M        75       936      1010
  SRR031728.2826481     chr4      +         75M        75       949      1023
                        width      ngap |      flag      mapq        NH        NM
                    <integer> <integer> | <integer> <integer> <integer> <integer>
  SRR031729.3941844        75         0 |        16      <NA>         1         1
  SRR031728.3674563        75         0 |        16      <NA>         1         3
  SRR031729.8532600        75         0 |         0         3         2         2
  SRR031729.2779333        75         0 |         0         3         2         1
  SRR031728.2826481        75         0 |         0         1         3         2
  ---
  seqlengths:
       chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
    23011544 21146708 24543557 27905053  1351857    19517 22422827   347038

> any(duplicated(names(U1gal)))

[1] TRUE
```

Exercise 2

a. Find the SAM Spec online and investigate the meaning of predefined tags NH and NM.

b. Load BAM file untreated1_chr4.bam into a `GappedAlignments` object and subset this object to keep only the alignments satisfying the 2 following conditions:
   - The alignment corresponds to a query with a *unique alignment* (aka *unique match* or *unique hit*).
   - The alignment is a *perfect match* (i.e. no insertion, no deletion, no mismatch).

c. Do those alignments have gaps?

From `GappedAlignments` to `GRanges`

GAPS ARE IGNORED! That is, each alignment is converted into a *single* genomic range defined by the *start* and *end* of the alignment.

```
> as(U1gal, "GRanges")

GRanges with 204355 ranges and 0 elementMetadata cols:
                     seqnames             ranges strand
                        <Rle>          <IRanges>  <Rle>
   SRR031729.3941844     chr4       [ 892,  966]      -
   SRR031728.3674563     chr4       [ 919,  993]      -
   SRR031729.8532600     chr4       [ 924,  998]      +
   SRR031729.2779333     chr4       [ 936, 1010]      +
   SRR031728.2826481     chr4       [ 949, 1023]      +
   SRR031728.2919098     chr4       [ 967, 1041]      -
   SRR031729.2873401     chr4       [1035, 1109]      -
    SRR031728.343975     chr4       [1236, 1310]      -
   SRR031729.2496773     chr4       [1252, 1326]      +
                 ...      ...                ...    ...
  SRR031729.12776621     chr4 [1348265, 1348339]      +
   SRR031728.1130675     chr4 [1348268, 1348342]      +
   SRR031728.1263853     chr4 [1348268, 1348342]      +
   SRR031728.1270714     chr4 [1348268, 1348342]      +
   SRR031728.1789947     chr4 [1348268, 1348342]      +
   SRR031728.4528492     chr4 [1348268, 1348342]      +
   SRR031729.5150849     chr4 [1348268, 1348342]      +
   SRR031729.9070096     chr4 [1348449, 1348523]      -
   SRR031729.9070096     chr4 [1350124, 1350198]      -
  ---
  seqlengths:
       chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
   23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

From `GappedAlignments` to `GRangesList`

GAPS ARE NOT IGNORED! That is, each alignment is converted into one or more genomic ranges (one more range than the number of gaps in the alignment).

```
> U1grl <- as(U1gal, "GRangesList")
> U1grl

GRangesList of length 204355:
$SRR031729.3941844
GRanges with 1 range and 0 elementMetadata cols:
      seqnames     ranges strand
         <Rle>  <IRanges>  <Rle>
  [1]     chr4 [892, 966]      -

$SRR031728.3674563
GRanges with 1 range and 0 elementMetadata cols:
      seqnames     ranges strand
  [1]     chr4 [919, 993]      -

$SRR031729.8532600
GRanges with 1 range and 0 elementMetadata cols:
      seqnames     ranges strand
  [1]     chr4 [924, 998]      +


...
<204352 more elements>
---
seqlengths:
    chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
 23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

From GappedAlignments to GRangesList (continued)

One more range than the number of gaps in the alignment:

```
> all(elementLengths(U1grl) == ngap(U1gal) + 1)
[1] TRUE
```

The purpose of the `GappedAlignmentPairs` container is...

... to store a set of aligned *paired-end* reads.

- Implemented on top of the `GappedAlignments` class.
- The alignments can be loaded from a BAM file with `readGappedAlignmentPairs()`.
- `first(x)`, `last(x)`: extract the *first* and *last* ends in 2 separate `GappedAlignments` objects of the same length.

Supported basic operations

- *Vector* operations: partially supported (no comparing or ordering)
- *List* operations: **YES**
- *Ranges* operations: **NO**
- *Coercion methods*: to `GRanges` or `GRangesList`
- *Splitting*: **NO**

readGappedAlignmentPairs()

```
> library(pasillaBamSubset)
> U3galp <- readGappedAlignmentPairs(untreated3_chr4())
> length(U3galp)

[1] 75346

> head(U3galp)

GappedAlignmentPairs with 6 alignment pairs and 0 elementMetadata cols:
    seqnames strand :       ranges --       ranges
       <Rle>  <Rle> :    <IRanges> --    <IRanges>
[1]     chr4      + : [169,  205] -- [ 326,  362]
[2]     chr4      + : [943,  979] -- [1086, 1122]
[3]     chr4      + : [944,  980] -- [1119, 1155]
[4]     chr4      + : [946,  982] -- [ 986, 1022]
[5]     chr4      + : [966, 1002] -- [1108, 1144]
[6]     chr4      + : [966, 1002] -- [1114, 1150]
---
seqlengths:
    chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
 23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

GappedAlignmentPairs accessors

```
> head(first(U3galp))

GappedAlignments with 6 alignments and 0 elementMetadata cols:
      seqnames strand       cigar    qwidth     start       end     width      ngap
         <Rle>  <Rle> <character> <integer> <integer> <integer> <integer> <integer>
  [1]     chr4      +         37M        37       169       205        37         0
  [2]     chr4      +         37M        37       943       979        37         0
  [3]     chr4      +         37M        37       944       980        37         0
  [4]     chr4      +         37M        37       946       982        37         0
  [5]     chr4      +         37M        37       966      1002        37         0
  [6]     chr4      +         37M        37       966      1002        37         0
  ---
  seqlengths:
      chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
   23011544 21146708 24543557 27905053  1351857    19517 22422827   347038

> head(last(U3galp))

GappedAlignments with 6 alignments and 0 elementMetadata cols:
      seqnames strand       cigar    qwidth     start       end     width      ngap
         <Rle>  <Rle> <character> <integer> <integer> <integer> <integer> <integer>
  [1]     chr4      -         37M        37       326       362        37         0
  [2]     chr4      -         37M        37      1086      1122        37         0
  [3]     chr4      -         37M        37      1119      1155        37         0
  [4]     chr4      -         37M        37       986      1022        37         0
  [5]     chr4      -         37M        37      1108      1144        37         0
  [6]     chr4      -         37M        37      1114      1150        37         0
  ---
  seqlengths:
      chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
   23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

Currently, `readGappedAlignmentPairs()` drops pairs where the *first* and *last* ends have incompatible sequence names and/or strands (a rare situation).

GappedAlignmentPairs accessors (continued)

```
> seqnames(U3galp)

factor-Rle of length 75346 with 1 run
  Lengths: 75346
  Values :  chr4
Levels(8): chr2L chr2R chr3L chr3R chr4 chrM chrX chrYHet

> strand(U3galp)

factor-Rle of length 75346 with 18999 runs
  Lengths:   6  6  3  1  6  1  1  2  2  1  1  3 ...  3  2  3  1  2  1  5  6  2  7  3
  Values :   +  -  +  -  +  -  +  -  +  -  +  - ...  +  -  +  -  +  -  +  -  +  -  +
Levels(3): + - *

> head(ngap(U3galp))

[1] 0 0 0 0 0 0

> table(ngap(U3galp))

    0     1     2
72949  2291   106
```

From GappedAlignmentPairs to GRangesList

```
> U3grl <- as(U3galp, "GRangesList")
> U3grl

GRangesList of length 75346:
[[1]]
GRanges with 2 ranges and 0 elementMetadata cols:
      seqnames     ranges strand
         <Rle>  <IRanges>  <Rle>
  [1]     chr4 [169, 205]      +
  [2]     chr4 [326, 362]      +

[[2]]
GRanges with 2 ranges and 0 elementMetadata cols:
      seqnames       ranges strand
  [1]     chr4 [ 943,  979]      +
  [2]     chr4 [1086, 1122]      +

[[3]]
GRanges with 2 ranges and 0 elementMetadata cols:
      seqnames       ranges strand
  [1]     chr4 [ 944,  980]      +
  [2]     chr4 [1119, 1155]      +

...
<75343 more elements>
---
seqlengths:
    chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
 23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

From GappedAlignmentPairs to GRangesList (continued)

```
> U3grl[ngap(U3galp) != 0]

GRangesList of length 2397:
[[1]]
GRanges with 3 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
         <Rle>      <IRanges>  <Rle>
  [1]     chr4 [74403, 74435]      -
  [2]     chr4 [77050, 77053]      -
  [3]     chr4 [13711, 13747]      -

[[2]]
GRanges with 3 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
  [1]     chr4 [56932, 56968]      +
  [2]     chr4 [57072, 57083]      +
  [3]     chr4 [57142, 57166]      +

[[3]]
GRanges with 3 ranges and 0 elementMetadata cols:
      seqnames         ranges strand
  [1]     chr4 [56932, 56968]      +
  [2]     chr4 [57065, 57083]      +
  [3]     chr4 [57142, 57159]      +

...
<2394 more elements>
---
seqlengths:
    chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
 23011544 21146708 24543557 27905053  1351857    19517 22422827   347038
```

Coverage

```
> U1cvg <- coverage(U1grl)
>
```

Coverage (continued)

```
> mean(U1cvg)
   chr2L    chr2R    chr3L    chr3R     chr4     chrM     chrX  chrYHet
 0.00000  0.00000  0.00000  0.00000 11.33746  0.00000  0.00000  0.00000
> max(U1cvg)
  chr2L   chr2R   chr3L   chr3R    chr4    chrM    chrX chrYHet
      0       0       0       0    5627       0       0       0
```

Slicing the coverage

```
> U1sl <- slice(U1cvg, lower=10)
> U1sl

SimpleRleViewsList of length 8
names(8): chr2L chr2R chr3L chr3R chr4 chrM chrX chrYHet

> elementLengths(U1sl)

  chr2L   chr2R   chr3L   chr3R    chr4    chrM    chrX chrYHet
      0       0       0       0    1183       0       0       0

> head(U1sl$chr4)

Views on a 1351857-length Rle subject

views:
    start  end width
[1]  4936 5077   142 [11 12 12 13 13 14 16 16 17 18 18 18 18 19 19 19 19 19 ...]
[2]  5211 5245    35 [10 10 10 10 10 10 10 10 10 10 10 10 10 12 12 13 13 13 ...]
[3]  5334 5337     4 [10 10 10 10]
[4]  5736 5744     9 [10 10 10 10 10 10 10 10 10]
[5]  5752 5754     3 [10 10 10]
[6]  5756 5882   127 [10 11 11 11 11 11 11 11 11 11 11 11 11 11 11 12 12 13 ...]

> head(mean(U1sl$chr4))

[1] 23.88028 11.60000 10.00000 10.00000 10.00000 25.65354

> head(max(U1sl$chr4))

[1] 39 13 10 10 10 38
```

Finding/counting overlaps

A typical use case: count the number of *hits* (aka *overlaps*) per transcript.

Typical input:

- A BAM file with the aligned reads (*single-* or *paired-end*).
- Transcript annotations *for the same reference genome* that was used to align the reads.

Typical tools:

- `readGappedAlignments()` or `readGappedAlignmentPairs()` to load the reads in a `GappedAlignments` or `GappedAlignmentPairs` object.
- A `TranscriptDb` object containing the transcript annotations.
- The `exonsBy()` extractor (defined in the *GenomicFeatures* package) to extract the exons ranges grouped by transcript from the `TranscriptDb` object. The exons ranges are returned in a `GRangesList` object with 1 top-level element per transcript.
- The `findOverlaps()` and/or `countOverlaps()` functions.

Load the transcripts

```
> library(TxDb.Dmelanogaster.UCSC.dm3.ensGene)
> txdb <- TxDb.Dmelanogaster.UCSC.dm3.ensGene
> exbytx <- exonsBy(txdb, by="tx", use.names=TRUE)
> exbytx

GRangesList of length 23017:
$FBtr0089116
GRanges with 11 ranges and 3 elementMetadata cols:
       seqnames           ranges strand |   exon_id   exon_name exon_rank
          <Rle>        <IRanges>  <Rle> | <integer> <character> <integer>
   [1]     chr4 [251356, 251521]      + |         1        <NA>         1
   [2]     chr4 [252561, 252603]      + |         2        <NA>         2
   [3]     chr4 [252905, 253474]      + |         3        <NA>         3
   [4]     chr4 [254891, 254971]      + |         4        <NA>         4
   [5]     chr4 [255490, 255570]      + |         5        <NA>         5
   [6]     chr4 [257021, 257101]      + |         6        <NA>         6
   [7]     chr4 [257895, 258185]      + |         7        <NA>         7
   [8]     chr4 [260940, 261024]      + |         8        <NA>         8
   [9]     chr4 [263892, 264211]      + |         9        <NA>         9
  [10]     chr4 [264260, 264374]      + |        10        <NA>        10
  [11]     chr4 [265806, 266500]      + |        11        <NA>        11

...
<23016 more elements>
---
seqlengths:
     chr2L  chr2LHet     chr2R  chr2RHet ...   chrXHet   chrYHet      chrM
  23011544    368872  21146708   3288761 ...    204112    347038     19517
```

Single-end overlaps

```
> U1txhits <- countOverlaps(exbytx, U1grl)
> length(U1txhits)

[1] 23017

> head(U1txhits)

FBtr0089116 FBtr0300800 FBtr0300796 FBtr0300799 FBtr0300798 FBtr0300797
        439         527         533         476         533         523

> sum(U1txhits)  # total nb of hits

[1] 250802

> head(sort(U1txhits, decreasing=TRUE))

FBtr0089177 FBtr0089175 FBtr0089176 FBtr0112904 FBtr0289951 FBtr0089243
      20380       20380       20380        6018        5982        5979
```

Rough counting!

- More than 1 alignment per read can be reported in the BAM file (sometimes the same read hits the same transcript many times).
- A hit is counted even if it's not *compatible* with the splicing of the transcript.

Paired-end overlaps

```
> U3txhits <- countOverlaps(exbytx, U3grl)
> length(U3txhits)

[1] 23017

> head(U3txhits)

FBtr0089116 FBtr0300800 FBtr0300796 FBtr0300799 FBtr0300798 FBtr0300797
        194         195         197         192         197         197

> sum(U3txhits)  # total nb of hits

[1] 95587

> head(sort(U3txhits, decreasing=TRUE))

FBtr0089175 FBtr0089176 FBtr0089177 FBtr0112904 FBtr0289951 FBtr0089243
       6799        6799        6790        2617        2610        2609
```

Note that exons that fall within the *inter-read* gap are NOT considered to overlap.

Exercise 3

Use the *TxDb.Dmelanogaster.UCSC.dm3.ensGene* package and the result of Exercise 2 to count the number of *unique hits* per transcript, that is, the number of hits from reads with a *unique alignment*.

Final notes

Further developments:

- Some optimization to the pairing algorithm used by `readGappedAlignmentPairs()`.
- Convenience functions for extracting the *inter-read gap* and computing the *observed template length* (aka TLEN in BAM/SAM jargon).
- Facilities for detecting/counting hits (from *single-* or *paired-end* reads) that are *compatible* with the splicing of the transcript.
- On user request...

Resources:

- Vignettes in *GenomicRanges* (`browseVignettes("GenomicRanges")`).
- `GRanges`, `GRangesList`, `GappedAlignments`, and `GappedAlignmentPairs` man pages in *GenomicRanges*.
- SAMtools website: `http://samtools.sourceforge.net/`
- *Bioconductor* mailing lists: `http://bioconductor.org/help/mailing-list/`