

Range-based containers in *Bioconductor*

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Introduction

IRanges objects

- Constructor and accessors
- Vector operations
- Range-based operations

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- Splitting a GRanges object

GRangesList objects

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- Coercion to GRanges or GRangesList

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- Coverage and slicing
- Finding/counting overlaps

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Range-based containers in *Bioconductor*

Implemented and documented in the *IRanges* package:

- ▶ `IRanges`

Implemented and documented in the *GenomicRanges* package:

- ▶ `GRanges`
- ▶ `GRangesList`
- ▶ `GAlignments`
- ▶ `GAlignmentPairs`
- ▶ `GAlignmentsList` (not covered in this presentation)

About the implementation

S4 classes (a.k.a. *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 `@` (a.k.a. *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: `mcols()`, `elementLengths()`, `seqinfo()`, etc...
- ▶ Consistent display (`show methods`).

Basic operations

Vector operations

Operate on *vector-like* objects

(e.g. on Rle, IRanges, GRanges, DNASTringSet, etc... objects)

- ▶ Accessors: `length()`, `names()`, `mcols()`
- ▶ Single-bracket subsetting: `[`
- ▶ Combining: `c()`
- ▶ Splitting/relisting: `split()`, `relist()`
- ▶ Comparing: `==`, `!=`, `match()`, `%in%`, `duplicated()`, `unique()`
- ▶ Ordering: `<=`, `>=`, `<`, `>`, `order()`, `sort()`, `rank()`

Coercion methods

- ▶ `as()`
- ▶ S3-style form: `as.vector()`, `as.character()`, `as.factor()`, etc...

List operations

Operate on *list-like* objects^a

(e.g. on IRangesList, GRangesList, DNASTringSetList, etc... objects)

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

^a*list-like* objects are also *vector-like* objects

Range-based operations

Range-based operations operate on *range-based* objects

(e.g. on IRanges, IRangesList, GRanges, GRangesList, etc... objects)

Intra range transformations

shift(), narrow(), flank(), resize()

Coverage and slicing

coverage(), slice()

Inter range transformations

disjoin(), range(), reduce(), gaps()

Finding/counting overlapping ranges

findOverlaps(), countOverlaps()

Range-based set operations

union(), intersect(), setdiff(),
punion(), pintersect(), psetdiff(),
pgap()

Finding the nearest range neighbor

nearest(), precede(), follow()

and more...

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Resources

The purpose of the IRanges container is...

... to store a set of *integer ranges* (a.k.a. *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 $\geq start$, except for empty ranges (a.k.a. zero-width ranges) where $end = start - 1$.

Supported operations

- ▶ *Vector operations*: **YES** (splitting/relisting produces an IRangesList object)
- ▶ *List operations*: **YES** (not covered in this presentation)
- ▶ *Coercion methods*: **YES** (from logical or integer vector to IRanges)
- ▶ *Range-based operations*: **YES**

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IRanges constructor and accessors

```
> library(IRanges)
> 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 accessors (continued)

```
> names(ir1) <- LETTERS[1:4]
> names(ir1)

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

> mcols(ir1) <- DataFrame(score=11:14, GC=seq(1, 0, length=4))
> mcols(ir1)

DataFrame with 4 rows and 2 columns
  score      GC
<integer> <numeric>
1      11 1.0000000
2      12 0.6666667
3      13 0.3333333
4      14 0.0000000

> ir1

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

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Vector operations on IRanges objects

```
> ir1[-2]

IRanges of length 3
  start end width names
[1]  12  15   4   A
[2]  12  15   4   C
[3]  12  14   3   D

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

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

```
> duplicated(ir2)

[1] FALSE FALSE  TRUE FALSE FALSE

> unique(ir2)

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

```
> order(ir2)

[1] 5 2 4 1 3

> sort(ir2)

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

```
> 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.data.frame(ir4)

  start end width
1     3  5     3
2     8  8     1
```

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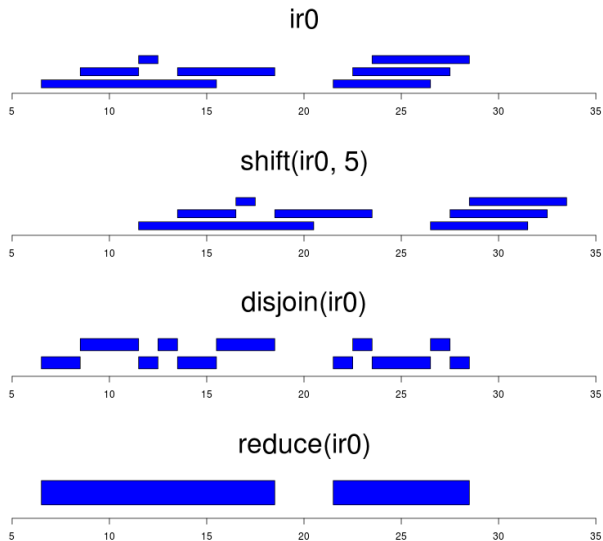
Advanced operations

- Coverage and slicing

- Finding/counting overlaps

Resources

Range-based operations on IRanges objects



Range-based operations on IRanges objects (continued)

```
> ir1
```

```
IRanges of length 4
```

	start	end	width	names
[1]	12	15	4	A
[2]	-9	0	10	B
[3]	12	15	4	C
[4]	12	14	3	D

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

```
IRanges of length 4
```

	start	end	width	names
[1]	0	3	4	A
[2]	0	9	10	B
[3]	0	3	4	C
[4]	0	2	3	D

```
> flank(ir1, 10, start=FALSE)
```

```
IRanges of length 4
```

	start	end	width	names
[1]	16	25	10	A
[2]	1	10	10	B
[3]	16	25	10	C
[4]	15	24	10	D

Range-based operations on IRanges objects (continued)

```
> ir1
```

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

```
> 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
```

Range-based operations on 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 names
[1]    12  15     4     A
[2]    -9   0    10     B
[3]    12  15     4     C
[4]    12  14     3     D
[5]   -10   0    11
```

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

```
IRanges of length 5
  start end width names
[1]    12  15     4     A
[2]     4   3     0     B
[3]    12  14     3     C
[4]    12  13     2     D
[5]     1   0     0
```

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Resources

The purpose of the GRanges container is...

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

- ▶ Like for IRanges objects, each range can be defined by a *start* and an *end* value.
- ▶ In addition, each range is also assigned a chromosome name and a strand.
- ▶ *start* and *end* are both **1-based** positions relative to the 5' end of the plus strand of the chromosome (a.k.a. *reference sequence*), even when the range is on the minus strand.
- ▶ So the *start* is always the leftmost position and the *end* the rightmost, even when the range is on the minus strand.
- ▶ As a consequence, **if a genomic range represents a gene on the minus strand, the gene "starts" (biologically speaking) at the end of it.**

Supported operations

- ▶ *Vector operations*: **YES** (splitting/relicing produces a GRangesList object)
- ▶ *List operations*: **NO**
- ▶ *Coercion methods*: to IRangesList (not covered in this presentation)
- ▶ *Range-based operations*: **YES**

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GRanges constructor

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

GRanges with 6 ranges and 0 metadata columns:

	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"

> mcols(gr1) <- DataFrame(score=11:16, GC=seq(1, 0, length=6))
> mcols(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 metadata columns:
  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
```

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Vector operations on GRanges objects

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

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

	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 metadata columns:
```

	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 metadata columns:

	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 metadata columns:

	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 metadata columns:

	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

seqlengths:

ch1	chMT	ch2
50000	800	NA

Vector operations on GRanges objects (continued)

```
> sort(gr12)
```

```
GRanges with 8 ranges and 2 metadata columns:
```

	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
	ch2	[1, 6]	*	14	0.2
	ch2	[2, 7]	*	15	0
	ch2	[2, 7]	*	13	0.4

```
---
```

```
seqlengths:
```

	ch1	chMT	ch2
	50000	800	NA

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Range-based operations on GRanges objects

```
> gr2
```

```
GRanges with 3 ranges and 2 metadata columns:
```

	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 metadata columns:
```

	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 metadata columns:
```

	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
```

Range-based operations on GRanges objects (continued)

```
> gr1
```

```
GRanges with 5 ranges and 2 metadata columns:
```

	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 metadata columns:
```

	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
```

Range-based operations on GRanges objects (continued)

```
> gr1
```

```
GRanges with 5 ranges and 2 metadata columns:
```

	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 metadata columns:
```

	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
```

Range-based 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 metadata columns:

	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	[121, 237]	+	16	0

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

```
> range(gr3)
```

GRanges with 3 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	ch1	[17, 35020]	-
[2]	chMT	[18, 237]	+
[3]	chMT	[19, 20]	-

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

Range-based operations on GRanges objects (continued)

```
> gr3
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121,  237] + |      16        0
---
seqlengths:
  ch1 chMT
50000  800

> disjoint(gr3)
GRanges with 6 ranges and 0 metadata columns:
  seqnames      ranges strand
   <Rle>        <IRanges> <Rle>
[1]   ch1 [  17,   20] -
[2]   ch1 [35016, 35020] -
[3]  chMT [  18,  120] +
[4]  chMT [ 121,  134] +
[5]  chMT [ 135,  237] +
[6]  chMT [  19,   20] -
---
seqlengths:
  ch1 chMT
50000  800
```

Range-based operations on GRanges objects (continued)

```
> gr3
```

```
GRanges with 5 ranges and 2 metadata columns:
```

	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	[121, 237]	+	16	0

```
---
```

```
seqlengths:
```

	ch1	chMT
	50000	800

```
> reduce(gr3)
```

```
GRanges with 4 ranges and 0 metadata columns:
```

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	ch1	[17, 20]	-
[2]	ch1	[35016, 35020]	-
[3]	chMT	[18, 237]	+
[4]	chMT	[19, 20]	-

```
---
```

```
seqlengths:
```

	ch1	chMT
	50000	800

Range-based operations on GRanges objects (continued)

```
> gr3
```

```
GRanges with 5 ranges and 2 metadata columns:
```

	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	[121, 237]	+	16	0

```
---
```

```
seqlengths:
```

ch1	chMT
50000	800

```
> gaps(gr3)
```

```
GRanges with 10 ranges and 0 metadata columns:
```

	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	[238, 800]	+
[8]	chMT	[1, 18]	-
[9]	chMT	[21, 800]	-
[10]	chMT	[1, 800]	*

```
---
```

```
seqlengths:
```

ch1	chMT
50000	800

Introduction

IRanges objects

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- Vector operations
- Range-based operations

GRanges objects

- Constructor and accessors
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- Range-based operations

Splitting a GRanges object

GRangesList objects

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GAlignments objects

- Constructor and accessors
- Coercion to GRanges or GRangesList

GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

Splitting a GRanges object

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

```
GRangesList of length 2:
```

```
$ch1
```

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

	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 metadata columns:
```

	seqnames	ranges	strand	score	GC
C	chMT	[18, 134]	+	13	0.6
D	chMT	[19, 20]	-	14	0.4
F	chMT	[121, 237]	+	16	0

```
---
```

```
seqlengths:
```

ch1	chMT
50000	800

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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 metadata columns (accessible with the `mcols()` accessor).

Supported operations

- ▶ *Vector operations*: **partially supported** (no splitting/relisting, no comparing or ordering)
- ▶ *List operations*: **YES**
- ▶ *Coercion methods*: to IRangesList (not covered in this presentation)
- ▶ *Range-based operations*: **partially supported** (some operations like `gaps()` are missing but they could/will be added)

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GRangesList constructor

```
> gr1 <- GRangesList(gr3, gr2)
> gr1
```

GRangesList of length 2:

[[1]]

GRanges with 5 ranges and 2 metadata columns:

	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	[121, 237]	+	16	0

[[2]]

GRanges with 3 ranges and 2 metadata columns:

	seqnames	ranges	strand	score	GC
	ch2	[2, 7]	*	15	0
	ch2	[1, 6]	*	14	0.2
	ch2	[2, 7]	*	13	0.4

seqlengths:

	ch1	chMT	ch2
	50000	800	NA

GRangesList accessors

```
> length(grl)
```

```
[1] 2
```

```
> seqnames(grl)
```

```
RleList 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)
```

```
RleList 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)
IRangesList 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]  121  237   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)
IntegerList of length 2
[[1]] 35016 17 18 19 121
[[2]]  2 1 2

> end(grl)
IntegerList of length 2
[[1]] 35020 20 134 20 237
[[2]]  7 6 7

> width(grl)
IntegerList 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 metadata columns:

	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	[121, 237]	+	16	0

\$TX2

GRanges with 3 ranges and 2 metadata columns:

	seqnames	ranges	strand	score	GC
	ch2	[2, 7]	*	15	0
	ch2	[1, 6]	*	14	0.2
	ch2	[2, 7]	*	13	0.4

seqlengths:

ch1	chMT	ch2
50000	800	NA

GRangesList accessors (continued)

```
> mcols(grl)$geneid <- c("GENE1", "GENE2")
> mcols(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 metadata columns:
  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 [ 121, 237] + |         16         0

$TX2
GRanges with 3 ranges and 2 metadata columns:
  seqnames ranges strand | score GC
  ch2 [2, 7] * | 15 0
  ch2 [1, 6] * | 14 0.2
  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>

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GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

Vector operations on GRangesList objects

```
> grl[c("TX2", "TX1")]  
GRangesList of length 2:  
$TX2  
GRanges with 3 ranges and 2 metadata columns:  
  seqnames      ranges strand |      score      GC  
  <Rle> <IRanges> <Rle> | <integer> <numeric>  
    ch2      [2, 7]   * |         15         0  
    ch2      [1, 6]   * |         14         0.2  
    ch2      [2, 7]   * |         13         0.4  
  
$TX1  
GRanges with 5 ranges and 2 metadata columns:  
  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 [ 121, 237] + |         16         0  
  
---  
seqlengths:  
  ch1  chMT  ch2  
50000  800  NA
```

Vector operations on GRangesList objects (continued)

```
> c(gr1, GRangesList(gr3))

GRangesList of length 3:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121,  237] + |         16         0

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

[[3]]
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121,  237] + |         16         0

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

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GAlignments objects

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GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

List operations on GRangesList objects

```
> gr1[[2]]  
GRanges with 3 ranges and 2 metadata columns:  
  seqnames   ranges strand |   score   GC  
  <Rle> <IRanges> <Rle> | <integer> <numeric>  
    ch2    [2, 7]   * |      15     0  
    ch2    [1, 6]   * |      14    0.2  
    ch2    [2, 7]   * |      13    0.4  
---  
seqlengths:  
  ch1 chMT ch2  
50000  800  NA  
  
> elementLengths(gr1)  
TX1 TX2  
  5   3  
  
> unlisted <- unlist(gr1, use.names=FALSE) # same as c(gr1[[1]], gr1[[2]])  
> unlisted  
GRanges with 8 ranges and 2 metadata columns:  
  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 [ 121, 237] + |      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 metadata columns:
      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 [ 221, 337]     + |      16      0

$TX2
GRanges with 3 ranges and 2 metadata columns:
      seqnames      ranges strand | score GC
      ch2 [102, 107]    * | 15 0
      ch2 [101, 106]    * | 14 0.2
      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 metadata columns:
  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 [ 221,  337]   + |      16     0

$TX2
GRanges with 3 ranges and 2 metadata columns:
  seqnames      ranges strand |   score   GC
   <Rle>      <IRanges> <Rle> | <integer> <numeric>
ch2 [102, 107]   * |      15     0
ch2 [101, 106]   * |      14    0.2
ch2 [102, 107]   * |      13    0.4

---
seqlengths:
  ch1  chMT  ch2
50000  800   NA

> mcols(grl100)

DataFrame with 2 rows and 0 columns

> mcols(grl100b)

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

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GAlignments objects

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GAlignmentPairs objects

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- Coercion to GRangesList

Advanced operations

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- Finding/counting overlaps

Resources

Range-based operations on GRangesList objects

```
> gr1

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121, 237] + |         16         0

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

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

```
> shift(gr1, 100)

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 221, 337] + |         16         0

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

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

`shift(gr1, 100)` is equivalent to `endoapply(gr1, shift, 100)`

Range-based operations on GRangesList objects (continued)

```
> gr1

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121, 237] + |         16         0

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

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

```
> flank(gr1, 10)

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 111, 120] + |         16         0

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

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

`flank(gr1, 10)` is equivalent to `endoapply(gr1, flank, 10)`

Range-based operations on GRangesList objects (continued)

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121,   237]   + |         16         0

$TX2
GRanges with 3 ranges and 2 metadata columns:
  seqnames ranges strand | score GC
   ch2 [2, 7] * | 15 0
   ch2 [1, 6] * | 14 0.2
   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 metadata columns:
  seqnames      ranges strand
   <Rle>        <IRanges> <Rle>
 [1]    ch1 [17, 35020]   -
 [2]   chMT [18,  237]   +
 [3]   chMT [19,   20]   -

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

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

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

Range-based operations on GRangesList objects (continued)

```
> grl

GRangesList of length 2:
$TX1
GRanges with 5 ranges and 2 metadata columns:
  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 [ 121, 237] + |      16      0

$TX2
GRanges with 3 ranges and 2 metadata columns:
  seqnames ranges strand | score GC
   ch2 [2, 7] * | 15 0
   ch2 [1, 6] * | 14 0.2
   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 metadata columns:
  seqnames      ranges strand
   <Rle>        <IRanges> <Rle>
[1]    ch1 [ 17, 20] -
[2]    ch1 [35016, 35020] -
[3]   chMT [ 18, 237] +
[4]   chMT [ 19, 20] -

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

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

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

Range-based operations on GRangesList objects (continued)

```
> grl2

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

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

---
seqlengths:
  ch1 chMT ch2
50000 800 NA

> grl3

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

[[2]]
GRanges with 1 range and 2 metadata columns:
  seqnames ranges strand | score GC
  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 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
  [1]      chMT [ 18, 21]   +
  [2]      chMT [131, 134]   +

$TX2
GRanges with 0 ranges and 0 metadata columns:
  seqnames ranges strand

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

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- Range-based operations

GRanges objects

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- Range-based operations
- Splitting a GRanges object

GRangesList objects

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- Vector operations
- List operations
- Range-based operations

GAlignments objects

- Constructor and accessors
- Coercion to GRanges or GRangesList

GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

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

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

The alignments can be loaded from a BAM file with `readGAlignments()`. 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 "geometry" 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 operations

- ▶ *Vector* operations: **partially supported** (no splitting/relisting, no comparing or ordering)
- ▶ *List* operations: **NO**
- ▶ *Ranges* operations: only `narrow()` and `qnarrow()` (`GAlignments` specific) are supported
- ▶ *Coercion methods*: to `GRanges` or `GRangesList`

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- Constructor and accessors**
- Coercion to GRanges or GRangesList

GAlignmentPairs objects

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- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

GAlignments constructor

Typically not used directly!

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

GAlignments with 4 alignments and 0 metadata columns:

	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).

```
readGAlignments()
```

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

```
[1] 204355
```

```
> head(U1gal)
```

```
GAlignments with 6 alignments and 0 metadata columns:
```

	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

GAlignments 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
```

GAlignments 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
```

```
> mcols(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 <- readGAlignments(untreated1_chr4(),
+                           use.names=TRUE, param=param)
> U1gal[1:5]
```

GAlignments with 5 alignments and 4 metadata columns:

	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
```

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GAlignments objects

- Constructor and accessors
- Coercion to GRanges or GRangesList**

GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

From GAlignments 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(Uigal, "GRanges")  
GRanges with 204355 ranges and 0 metadata columns:  
      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] +  
...  
SRR031728.1789947 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 GAlignments 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).

```
> U1gr1 <- as(U1gal, "GRangesList")
> U1gr1

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

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

$SRR031729.8532600
GRanges with 1 range and 0 metadata columns:
      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 GAlignments to GRangesList (continued)

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

```
> all(elementLengths(U1gr1) == ngap(U1gal) + 1)
[1] TRUE
```

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- Range-based operations

GAlignments objects

- Constructor and accessors
- Coercion to GRanges or GRangesList

GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

The purpose of the GAlignmentPairs container is...

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

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

Supported operations

- ▶ *Vector* operations: **partially supported** (no splitting/relisting, no comparing or ordering)
- ▶ *List* operations: **YES**
- ▶ *Ranges* operations: **NO**
- ▶ *Coercion methods*: to GRanges or GRangesList

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GRanges objects

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- Splitting a GRanges object

GRangesList objects

- Constructor and accessors
- Vector operations
- List operations
- Range-based operations

GAlignments objects

- Constructor and accessors
- Coercion to GRanges or GRangesList

GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources


```
readGAlignmentPairs()
```

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

```
[1] 75346
```

```
> head(U3galp)
```

```
GAlignmentPairs with 6 alignment pairs and 0 metadata columns:
```

```
      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

GAlignmentPairs accessors

```
> head(first(U3galp))

Alignments with 6 alignments and 0 metadata columns:
  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))

Alignments with 6 alignments and 0 metadata columns:
  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, `readGAlignmentPairs()` drops pairs where the *first* and *last* ends have incompatible sequence names and/or strands (a rare situation).

GAlignmentPairs 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
```

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GAlignmentPairs objects

- Constructor and accessors
- Coercion to GRangesList**

Advanced operations

- Coverage and slicing
- Finding/counting overlaps

Resources

From GAlignmentPairs to GRangesList

```
> U3gr1 <- as(U3galp, "GRangesList")
> U3gr1

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

[[2]]
GRanges with 2 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>    <IRanges> <Rle>
 [1]   chr4 [ 943,  979]     +
 [2]   chr4 [1086, 1122]     +

[[3]]
GRanges with 2 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>    <IRanges> <Rle>
 [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 GAlignmentPairs to GRangesList (continued)

```
> U3gr1[ngap(U3galp) != 0]

GRangesList of length 2397:
[[1]]
GRanges with 3 ranges and 0 metadata columns:
      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 metadata columns:
      seqnames      ranges strand
   <Rle>         <IRanges> <Rle>
[1]   chr4 [56932, 56968]     +
[2]   chr4 [57072, 57083]     +
[3]   chr4 [57142, 57166]     +

[[3]]
GRanges with 3 ranges and 0 metadata columns:
      seqnames      ranges strand
   <Rle>         <IRanges> <Rle>
[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

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Coverage

```
> U1cvg <- coverage(U1gr1)
> U1cvg

RleList of length 8
$chr2L
integer-Rle of length 23011544 with 1 run
  Lengths: 23011544
  Values :      0

$chr2R
integer-Rle of length 21146708 with 1 run
  Lengths: 21146708
  Values :      0

$chr3L
integer-Rle of length 24543557 with 1 run
  Lengths: 24543557
  Values :      0

$chr3R
integer-Rle of length 27905053 with 1 run
  Lengths: 27905053
  Values :      0

$chr4
integer-Rle of length 1351857 with 122061 runs
  Lengths: 891 27 5 12 13 45 5 ... 3 106 75 1600 75 1659
  Values : 0 1 2 3 4 5 4 ... 6 0 1 0 1 0

...
<3 more elements>
```

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

```
> U1s1 <- slice(U1cvg, lower=10)
> U1s1

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

> elementLengths(U1s1)

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

> head(U1s1$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 ...]
[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 11 11 11 12 12 13 ...]

> head(mean(U1s1$chr4))

[1] 23.88028 11.60000 10.00000 10.00000 10.00000 25.65354

> head(max(U1s1$chr4))

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

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Finding/counting overlaps

A typical use case: count the number of *hits* (a.k.a. *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

- ▶ `readGAlignments()` or `readGAlignmentPairs()` to load the reads in a `GAlignments` or `GAlignmentPairs` 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 29173:

\$FBtr0300689

GRanges with 2 ranges and 3 metadata columns:

	seqnames	ranges	strand	exon_id	exon_name	exon_rank
	<Rle>	<IRanges>	<Rle>	<integer>	<character>	<integer>
[1]	chr2L	[7529, 8116]	+	1	<NA>	1
[2]	chr2L	[8193, 9484]	+	3	<NA>	2

\$FBtr0300690

GRanges with 3 ranges and 3 metadata columns:

	seqnames	ranges	strand	exon_id	exon_name	exon_rank
[1]	chr2L	[7529, 8116]	+	1	<NA>	1
[2]	chr2L	[8193, 8589]	+	2	<NA>	2
[3]	chr2L	[8668, 9484]	+	5	<NA>	3

\$FBtr0330654

GRanges with 2 ranges and 3 metadata columns:

	seqnames	ranges	strand	exon_id	exon_name	exon_rank
[1]	chr2L	[7529, 8116]	+	1	<NA>	1
[2]	chr2L	[8229, 9484]	+	4	<NA>	2

...

<29170 more elements>

seqlengths:

Single-end overlaps

```
> U1txhits <- countOverlaps(exbytx, U1gr1)
> length(U1txhits)

[1] 29173

> head(U1txhits)

FBtr0300689 FBtr0300690 FBtr0330654 FBtr0309810 FBtr0306539 FBtr0306536
           0           0           0           0           0           0

> sum(U1txhits) # total nb of hits

[1] 284609

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

FBtr0308296 FBtr0089175 FBtr0089176 FBtr0112904 FBtr0289951 FBtr0089243
    20399         20330         20330         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, U3gr1)
> length(U3txhits)

[1] 29173

> head(U3txhits)

FBtr0300689 FBtr0300690 FBtr0330654 FBtr0309810 FBtr0306539 FBtr0306536
           0           0           0           0           0           0

> sum(U3txhits) # total nb of hits

[1] 106947

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

FBtr0308296 FBtr0089175 FBtr0089176 FBtr0112904 FBtr0289951 FBtr0089243
      6806       6791       6791       2617       2610       2609
```

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

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Resources

- ▶ Vignettes in the *GenomicRanges* package (`browseVignettes("GenomicRanges")`).
- ▶ `GRanges`, `GRangesList`, `GAlignments`, and `GAlignmentPairs` man pages in the *GenomicRanges* package.
- ▶ SAMtools website: <http://samtools.sourceforge.net/>
- ▶ *Bioconductor* mailing lists: <http://bioconductor.org/help/mailing-list/>

Where to look next

- ▶ `summarizeOverlaps()` function in the *GenomicRanges* package for counting overlaps between reads and genomic features, and resolve reads that overlap multiple features.

THANKS!