

# 10 things (maybe) you didn't know about GenomicRanges, Biostrings, and Rsamtools

Hervé Pagès  
hpages@fredhutch.org

June 2016

## 1. Inner vs outer metadata columns

```
> mcols(grl)$id <- paste0("ID", seq_along(grl))
> grl
```

GRangesList object of length 3:

\$gr1

GRanges object with 1 range and 2 metadata columns:

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
[1]	Chrom2	[3, 6]	+	5	0.45

\$gr2

GRanges object with 2 ranges and 2 metadata columns:

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[ 7,  9]	+	3	0.3
[2]	Chrom1	[13, 15]	-	4	0.5

\$gr3

GRanges object with 2 ranges and 2 metadata columns:

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[1, 3]	-	6	0.4
[2]	Chrom2	[4, 9]	-	2	0.1

-----

seqinfo: 2 sequences from an unspecified genome; no seqlengths

## 1. Inner vs outer metadata columns

```
> mcols(grl) # outer mcols
```

```
DataFrame with 3 rows and 1 column
```

```
      id
<character>
1      ID1
2      ID2
3      ID3
```

```
> mcols(unlist(grl, use.names=FALSE)) # inner mcols
```

```
DataFrame with 5 rows and 2 columns
```

```
      score      GC
<integer> <numeric>
1         5     0.45
2         3     0.30
3         4     0.50
4         6     0.40
5         2     0.10
```

## 2. invertStrand()

Works out-of-the-box on any object that has a strand() getter and setter ==>  
no need to implement specific methods.

```
> gr
```

```
GRanges object with 10 ranges and 2 metadata columns:
```

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
a	chr2	[1, 10]	-	1	1
b	chr2	[2, 10]	+	2	0.8888888888888889
c	chr2	[3, 10]	+	3	0.7777777777777778
.	...	...	...	...	...
h	chr3	[ 8, 10]	+	8	0.2222222222222222
i	chr3	[ 9, 10]	-	9	0.1111111111111111
j	chr3	[10, 10]	-	10	0

```
-----
```

```
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

## 2. invertStrand()

```
> invertStrand(gr)
```

```
GRanges object with 10 ranges and 2 metadata columns:
```

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
a	chr2	[1, 10]	+	1	1
b	chr2	[2, 10]	-	2	0.8888888888888889
c	chr2	[3, 10]	-	3	0.7777777777777778
.	...	...	...	...	...
h	chr3	[ 8, 10]	-	8	0.2222222222222222
i	chr3	[ 9, 10]	+	9	0.1111111111111111
j	chr3	[10, 10]	+	10	0

```
-----
```

```
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

## 2. invertStrand()

```
> gr1
```

```
GRangesList object of length 3:
```

```
$gr1
```

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

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
[1]	Chrom2	[3, 6]	+	5	0.45

```
$gr2
```

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

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[7, 9]	+	3	0.3
[2]	Chrom1	[13, 15]	-	4	0.5

```
$gr3
```

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

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[1, 3]	-	6	0.4
[2]	Chrom2	[4, 9]	-	2	0.1

```
-----
```

```
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

## 2. invertStrand()

```
> invertStrand(gr1)
```

```
GRangesList object of length 3:
```

```
$gr1
```

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

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
[1]	Chrom2	[3, 6]	-	5	0.45

```
$gr2
```

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

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[ 7,  9]	-	3	0.3
[2]	Chrom1	[13, 15]	+	4	0.5

```
$gr3
```

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

	seqnames	ranges	strand	score	GC
[1]	Chrom1	[1, 3]	+	6	0.4
[2]	Chrom2	[4, 9]	+	2	0.1

```
-----
```

```
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

### 3. extractList()

Extract groups of elements from a vector-like object and return them in a list-like object.

```
> cvg <- Rle(c(0L, 2L, 5L, 1L, 0L), c(10, 6, 3, 4, 15))
> cvg
```

```
integer-Rle of length 38 with 5 runs
```

```
Lengths: 10 6 3 4 15
```

```
Values : 0 2 5 1 0
```

```
> i <- IRanges(c(16, 19, 9), width=5, names=letters[1:3])
> i
```

```
IRanges object with 3 ranges and 0 metadata columns:
```

	start	end	width
	<integer>	<integer>	<integer>
a	16	20	5
b	19	23	5
c	9	13	5



### 3. extractList()

```
> extractList(cvg, i)
RleList of length 3
$a
integer-Rle of length 5 with 3 runs
  Lengths: 1 3 1
  Values : 2 5 1

$b
integer-Rle of length 5 with 2 runs
  Lengths: 1 4
  Values : 5 1

$c
integer-Rle of length 5 with 2 runs
  Lengths: 2 3
  Values : 0 2
```

### 3. extractList()

`i` can be an `IntegerList` object:

```
> i <- IntegerList(c(25:20), NULL, seq(from=2, to=length(cvg), by=2))  
> i
```

```
IntegerList of length 3
```

```
[[1]] 25 24 23 22 21 20
```

```
[[2]] integer(0)
```

```
[[3]] 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
```

```
> extractList(cvg, i)
```

```
RleList of length 3
```

```
[[1]]
```

```
integer-Rle of length 6 with 2 runs
```

```
Lengths: 2 4
```

```
Values : 0 1
```

```
[[2]]
```

```
integer-Rle of length 0 with 0 runs
```

```
Lengths:
```

```
Values :
```

```
[[3]]
```

```
integer-Rle of length 19 with 5 runs
```

```
Lengths: 5 3 1 2 8
```

```
Values : 0 2 5 1 0
```

#### 4. 'with.revmap' arg for reduce() and (now) disjoint()

```
> ir
```

IRanges object with 6 ranges and 2 metadata columns:

	start	end	width		id	score
	<integer>	<integer>	<integer>		<character>	<integer>
[1]	11	13	3		a	3
[2]	12	14	3		b	2
[3]	13	15	3		c	1
[4]	2	4	3		d	0
[5]	7	9	3		e	-1
[6]	6	8	3		f	-2

```
> ir2 <- reduce(ir, with.revmap=TRUE)
```

```
> ir2
```

IRanges object with 3 ranges and 1 metadata column:

	start	end	width		revmap
	<integer>	<integer>	<integer>		<IntegerList>
[1]	2	4	3		4
[2]	6	9	4		6,5
[3]	11	15	5		1,2,3

#### 4. 'with.revmap' arg for reduce() and disjoint()

```
> revmap <- mcols(ir2)$revmap  
> extractList(mcols(ir)$id, revmap)
```

CharacterList of length 3

```
[[1]] d  
[[2]] f e  
[[3]] a b c
```

```
> extractList(mcols(ir)$score, revmap)
```

IntegerList of length 3

```
[[1]] 0  
[[2]] -2 -1  
[[3]] 3 2 1
```

```
> mcols(ir2) <- DataFrame(id=extractList(mcols(ir)$id, revmap),  
+                          score=extractList(mcols(ir)$score, revmap))  
> ir2
```

IRanges object with 3 ranges and 2 metadata columns:

	start	end	width		id	score
	<integer>	<integer>	<integer>		<CharacterList>	<IntegerList>
[1]	2	4	3		d	0
[2]	6	9	4		f,e	-2,-1
[3]	11	15	5		a,b,c	3,2,1

## 5. Zero-width ranges

`findOverlaps/countOverlaps` support zero-width ranges.

```
> sliding_query <- IRanges(1:6, width=0)
> sliding_query
```

IRanges object with 6 ranges and 0 metadata columns:

	start	end	width
	<integer>	<integer>	<integer>
[1]	1	0	0
[2]	2	1	0
[3]	3	2	0
[4]	4	3	0
[5]	5	4	0
[6]	6	5	0

```
> countOverlaps(sliding_query, IRanges(3, 4))
```

```
[1] 0 0 0 0 0 0
```

But you have to specify `minoverlap=0` for this to work (default is 1).

```
> countOverlaps(sliding_query, IRanges(3, 4), minoverlap=0)
```

```
[1] 0 0 1 1 1 0
```

## 6. Biostrings::replaceAt()

Perform multiple substitutions at arbitrary positions in a set of sequences.

```
> library(Biostrings)
> library(hgu95av2probe)
> probes <- DNASTringSet(hgu95av2probe)
> probes

A DNASTringSet instance of length 201800
      width seq
[1]      25 TGGCTCCTGCTGAGGTCCCCTTTCC
[2]      25 GGCTGTGAATTCCTGTACATATTTTC
[3]      25 GCTTCAATTCATTATGTTTTAATG
...      ...
[201798] 25 TTCTGTCAAAGCATCATCTCAACAA
[201799] 25 CAAAGCATCATCTCAACAAGCCCTC
[201800] 25 GTGCTCCTTGTC AACAGCGCACCCA
```

## 6. Biostrings::replaceAt()

Replace 3rd and 4th nucleotides by pattern `--+-`.

```
> replaceAt(probes, at=IRanges(3, 4), value="--+-")
```

```
A DNASTringSet instance of length 201800
```

```
width seq
```

```
[1] 27 TG--+-TCCTGCTGAGGTCCCCTTTCC
```

```
[2] 27 GG--+-GTGAATTCCTGTACATATTTC
```

```
[3] 27 GC--+-CAATTCATTATGTTTTAATG
```

```
... ..
```

```
[201798] 27 TT--+-GTCAAAGCATCATCTCAACAA
```

```
[201799] 27 CA--+-GCATCATCTCAACAAGCCCTC
```

```
[201800] 27 GT--+-TCCTTGTC AACAGCGCACCCA
```

## 6. Biostrings::replaceAt()

If supplied pattern is empty, then performs deletions.

```
> replaceAt(probes, at=IRanges(3, 4), value="")
```

```
A DNASTringSet instance of length 201800
```

```
width seq
```

```
[1] 23 TGCCTGCTGAGGTCCCCTTCC
```

```
[2] 23 GGGTGAATTCCTGTACATATTC
```

```
[3] 23 GCCAATTCCATTATGTTTAAATG
```

```
...
```

```
[201798] 23 TTGTCAAAGCATCATCTCAACAA
```

```
[201799] 23 CAGCATCATCTCAACAAGCCCTC
```

```
[201800] 23 GTTCCTTGTC AACAGCGCACCCA
```



## 6. Biostrings::replaceAt()

If `at` is a zero-width range, then performs insertions.

```
> replaceAt(probes, at=IRanges(4, 3), value="--+-")
```

```
A DNASTringSet instance of length 201800
```

```
width seq
```

```
[1] 29 TGG--+-CTCCTGCTGAGGTCCCCTTTCC
```

```
[2] 29 GGC--+-TGTGAATTCCTGTACATATTTCC
```

```
[3] 29 GCT--+-TCAATTCATTATGTTTTAATG
```

```
...
```

```
[201798] 29 TTC--+-TGTCAAAGCATCATCTCAACAA
```

```
[201799] 29 CAA--+-AGCATCATCTCAACAAGCCCTC
```

```
[201800] 29 GTG--+-CTCCTTGTC AACAGCGCACCCA
```

## 6. Biostrings::replaceAt()

Use it in combination with `vmatchPattern` to replace all the occurrences of a given pattern with another pattern:

```
> midx <- vmatchPattern("VCGTT", probes, fixed=FALSE)
> replaceAt(probes, at=midx, value="-++-")
```

A DNASTringSet instance of length 201800

```
      width seq
[1]      25 TGGCTCCTGCTGAGGTCCCCTTTCC
[2]      25 GGCTGTGAATTCCTGTACATATTTC
[3]      25 GCTTCAATTCATTATGTTTTAATG
...      ...
[201798] 25 TTCTGTCAAAGCATCATCTCAACAA
[201799] 25 CAAAGCATCATCTCAACAAGCCCTC
[201800] 25 GTGCTCCTTGTC AACAGCGCACCCA
```

## 7. GRanges as a subscript

```
> cvg <- RleList(chr1=101:120, chr2=2:-8, chr3=31:40)
> gr
```

GRanges object with 10 ranges and 2 metadata columns:

	seqnames	ranges	strand	score	GC
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>
a	chr2	[1, 10]	-	1	1
b	chr2	[2, 10]	+	2	0.8888888888888889
c	chr2	[3, 10]	+	3	0.7777777777777778
.	...	...	...	...	...
h	chr3	[ 8, 10]	+	8	0.2222222222222222
i	chr3	[ 9, 10]	-	9	0.1111111111111111
j	chr3	[10, 10]	-	10	0

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

## 7. GRanges as a subscript

```
> cvg[gr]
RleList of length 10
$chr2
integer-Rle of length 10 with 10 runs
  Lengths: 1 1 1 1 1 1 1 1 1 1
  Values : 2 1 0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 9 with 9 runs
  Lengths: 1 1 1 1 1 1 1 1 1
  Values : 1 0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 8 with 8 runs
  Lengths: 1 1 1 1 1 1 1 1
  Values : 0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 7 with 7 runs
  Lengths: 1 1 1 1 1 1 1
  Values : -1 -2 -3 -4 -5 -6 -7

$chr1
integer-Rle of length 6 with 6 runs
  Lengths: 1 1 1 1 1 1
  Values : 105 106 107 108 109 110

...
<5 more elements>
```

## 8. BSgenomeViews objects

```
> library(BSgenome.Mmusculus.UCSC.mm10)
> genome <- BSgenome.Mmusculus.UCSC.mm10
> library(TxDb.Mmusculus.UCSC.mm10.knownGene)
> txdb <- TxDb.Mmusculus.UCSC.mm10.knownGene
> ex <- exons(txdb, columns=c("exon_id", "tx_name", "gene_id"))
> v <- Views(genome, ex)
```

## 8. BSgenomeViews objects

```
> v
```

```
BSgenomeViews object with 257665 views and 3 metadata columns:
```

	seqnames	ranges	strand	dna
	<Rle>	<IRanges>	<Rle>	<DNAStringSet>
[1]	chr1	[4807893, 4807982]	+	[GCACTGTCCG...CACGCGCG]
[2]	chr1	[4808455, 4808486]	+	[GTTATTTTC...GAGATACAGG]
[3]	chr1	[4828584, 4828649]	+	[GCATGGATGG...GTCCACATGC]
...	...	...	...	...
[257663]	chrUn_JH584304	[56986, 57151]	-	[GTTGTACTTT...CCTGAGCAGG]
[257664]	chrUn_JH584304	[58564, 58835]	-	[CTGTGGTCCT...CAGAGAAATG]
[257665]	chrUn_JH584304	[59592, 59689]	-	[TCTCTGCTGC...GCCTTCTCAG]
	exon_id	tx_name	gene_id	
	<integer>	<CharacterList>	<CharacterList>	
[1]	1	uc007afg.1,uc007afh.1	18777	
[2]	2	uc007afg.1,uc007afh.1	18777	
[3]	3	uc007afg.1,uc007afh.1	18777	
...	...	...	...	
[257663]	257663	uc029xhj.1	66776	
[257664]	257664	uc029xhj.1,uc029xho.1	66776	
[257665]	257665	uc029xhi.1,uc029xho.1	66776	

```
-----  
seqinfo: 66 sequences (1 circular) from mm10 genome
```

## 8. BSgenomeViews objects

```
> af <- alphabetFrequency(v, baseOnly=TRUE)
> head(af)
```

	A	C	G	T	other
[1,]	12	36	30	12	0
[2,]	7	5	9	11	0
[3,]	20	18	14	14	0
[4,]	12	11	9	16	0
[5,]	23	14	17	17	0
[6,]	24	10	17	23	0

## 9. Pile-up statistics on a BAM file with Rsamtools::pileup()

```
> library(Rsamtools)
> library(RNAseqData.HNRNPC.bam.chr14)
> fl <- RNAseqData.HNRNPC.bam.chr14_BAMFILES[1]
> sbp <- ScanBamParam(which=GRanges("chr14", IRanges(1, 53674770)))
> pp <- PileupParam(distinguish_nucleotides=FALSE,
+                  distinguish_strands=FALSE,
+                  min_mapq=13,
+                  min_base_quality=10,
+                  min_nucleotide_depth=4)
> res <- pileup(fl, scanBamParam=sbp, pileupParam=pp)
```



## 9. Pile-up statistics on a BAM file with Rsamtools::pileup()

```
> dim(res)
```

```
[1] 248441      4
```

```
> head(res)
```

	seqnames	pos	count	which_label
1	chr14	19681651	4	chr14:1-53674770
2	chr14	19681655	4	chr14:1-53674770
3	chr14	19681657	4	chr14:1-53674770
4	chr14	19681658	4	chr14:1-53674770
5	chr14	19681661	4	chr14:1-53674770
6	chr14	19681662	4	chr14:1-53674770

## 10. Merging 2 GRanges objects (added this week)

```
> x
```

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

	seqnames	ranges	strand	score	a1	a2
	<Rle>	<IRanges>	<Rle>	<numeric>	<integer>	<numeric>
[1]	chr1	[ 1, 1000]	*	0.45	5	6
[2]	chr2	[2000, 3000]	*	<NA>	7	8

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
> y
```

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

	seqnames	ranges	strand	score	b1	b2
	<Rle>	<IRanges>	<Rle>	<numeric>	<integer>	<numeric>
[1]	chr2	[ 150, 151]	*	0.7	0	1
[2]	chr1	[ 1, 10]	*	0.82	5	-2
[3]	chr2	[2000, 3000]	*	0.1	1	1

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

## 10. Merging 2 GRanges objects

```
> merge(x, y)
```

```
GRanges object with 1 range and 5 metadata columns:
```

	seqnames	ranges	strand	score	a1	a2	b1
	<Rle>	<IRanges>	<Rle>	<numeric>	<integer>	<numeric>	<integer>
[1]	chr2	[2000, 3000]	*	0.1	7	8	1
	b2						
	<numeric>						
[1]	1						

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

## 10. Merging 2 GRanges objects

```
> merge(x, y, all=TRUE)
```

```
GRanges object with 4 ranges and 5 metadata columns:
```

	seqnames	ranges	strand	score	a1	a2	b1
	<Rle>	<IRanges>	<Rle>	<numeric>	<integer>	<numeric>	<integer>
[1]	chr1	[ 1, 10]	*	0.82	<NA>	<NA>	5
[2]	chr1	[ 1, 1000]	*	0.45	5	6	<NA>
[3]	chr2	[ 150, 151]	*	0.7	<NA>	<NA>	0
[4]	chr2	[2000, 3000]	*	0.1	7	8	1

```
b2
```

```
<numeric>
```

[1]	-2
[2]	<NA>
[3]	1
[4]	1

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```