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

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

$gr2
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       7-9      + |         3       0.3
  [2]   Chrom1     13-15      - |         4       0.5
  -------
  seqinfo: 2 sequences from an unspecified genome; no seqlengths

$gr3
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       1-3      - |         6       0.4
```

1. *Inner* vs *outer* metadata columns

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

```
DataFrame with 3 rows and 1 column
              id
    <character>
gr1         ID1
gr2         ID2
gr3         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  1.000000
  b     chr2      2-10      + |         2  0.888889
  c     chr2      3-10      + |         3  0.777778
  .      ...       ...    ... .       ...       ...
  h     chr3      8-10      + |         8  0.222222
  i     chr3      9-10      - |         9  0.111111
  j     chr3        10      - |        10  0.000000
  -------
  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  1.000000
  b     chr2      2-10      - |         2  0.888889
  c     chr2      3-10      - |         3  0.777778
  .      ...       ...    ... .       ...       ...
  h     chr3      8-10      - |         8  0.222222
  i     chr3      9-10      + |         9  0.111111
  j     chr3        10      + |        10  0.000000
  -------
  seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

2. invertStrand()

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

$gr2
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       7-9      + |         3       0.3
  [2]   Chrom1     13-15      - |         4       0.5
  -------
  seqinfo: 2 sequences from an unspecified genome; no seqlengths

$gr3
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       1-3      - |         6       0.4
  [2]   Chrom2       4-9      - |         2       0.1
```

2. invertStrand()

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

$gr2
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       7-9      - |         3       0.3
  [2]   Chrom1     13-15      + |         4       0.5
  -------
  seqinfo: 2 sequences from an unspecified genome; no seqlengths

$gr3
GRanges object with 2 ranges and 2 metadata columns:
      seqnames    ranges strand |     score        GC
         <Rle> <IRanges>  <Rle> | <integer> <numeric>
  [1]   Chrom1       1-3      + |         6       0.4
  [2]   Chrom2       4-9      + |         2       0.1
```

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) disjoin()

```
> 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 disjoin()

```
> 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 1 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 0 1 0 0
```

6. Biostrings::replaceAt()

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

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

```
DNAStringSet object of length 201800:
          width seq
      [1]    25 TGGCTCCTGCTGAGGTCCCCTTTCC
      [2]    25 GGCTGTGAATTCCTGTACATATTTC
      [3]    25 GCTTCAATTCCATTATGTTTTAATG
      ...   ... ...
 [201798]    25 TTCTGTCAAAGCATCATCTCAACAA
 [201799]    25 CAAAGCATCATCTCAACAAGCCCTC
 [201800]    25 GTGCTCCTTGTCAACAGCGCACCCA
```

6. Biostrings::replaceAt()

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

```
> replaceAt(probes, at=IRanges(3, 4), value="-++-")
DNAStringSet object of length 201800:
          width seq
      [1]    27 TG-++-TCCTGCTGAGGTCCCCTTTCC
      [2]    27 GG-++-GTGAATTCCTGTACATATTTC
      [3]    27 GC-++-CAATTCCATTATGTTTTAATG
      ...   ... ...
 [201798]    27 TT-++-GTCAAAGCATCATCTCAACAA
 [201799]    27 CA-++-GCATCATCTCAACAAGCCCTC
 [201800]    27 GT-++-TCCTTGTCAACAGCGCACCCA
```

6. Biostrings::replaceAt()

If supplied pattern is empty, then performs deletions.

```
> replaceAt(probes, at=IRanges(3, 4), value="")
DNAStringSet object of length 201800:
          width seq
      [1]    23 TGTCCTGCTGAGGTCCCCTTTCC
      [2]    23 GGGTGAATTCCTGTACATATTTC
      [3]    23 GCCAATTCCATTATGTTTTAATG
      ...   ... ...
 [201798]    23 TTGTCAAAGCATCATCTCAACAA
 [201799]    23 CAGCATCATCTCAACAAGCCCTC
 [201800]    23 GTTCCTTGTCAACAGCGCACCCA
```

6. Biostrings::replaceAt()

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

```
> replaceAt(probes, at=IRanges(4, 3), value="-++-")
DNAStringSet object of length 201800:
          width seq
      [1]    29 TGG-++-CTCCTGCTGAGGTCCCCTTTCC
      [2]    29 GGC-++-TGTGAATTCCTGTACATATTTC
      [3]    29 GCT-++-TCAATTCCATTATGTTTTAATG
      ...   ... ...
 [201798]    29 TTC-++-TGTCAAAGCATCATCTCAACAA
 [201799]    29 CAA-++-AGCATCATCTCAACAAGCCCTC
 [201800]    29 GTG-++-CTCCTTGTCAACAGCGCACCCA
```

6. Biostrings::replaceAt()

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

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

```
DNAStringSet object of length 201800:
          width seq
      [1]    25 TGGCTCCTGCTGAGGTCCCCTTTCC
      [2]    25 GGCTGTGAATTCCTGTACATATTTC
      [3]    25 GCTTCAATTCCATTATGTTTTAATG
      ...   ... ...
 [201798]    25 TTCTGTCAAAGCATCATCTCAACAA
 [201799]    25 CAAAGCATCATCTCAACAAGCCCTC
 [201800]    25 GTGCTCCTTGTCAACAGCGCACCCA
```

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  1.000000
  b     chr2      2-10      + |         2  0.888889
  c     chr2      3-10      + |         3  0.777778
  .      ...       ...    ... .       ...       ...
  h     chr3      8-10      + |         8  0.222222
  i     chr3      9-10      - |         9  0.111111
  j     chr3        10      - |        10  0.000000
  -------
  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 447558 views and 3 metadata columns:
                seqnames          ranges strand                       dna |
                   <Rle>       <IRanges>  <Rle>            <DNAStringSet> |
       [1]           chr1 3073253-3074322      + [AAGGAAAGAG...TAGAGAAATG] |
       [2]           chr1 3102016-3102125      + [GTGCTTGCTT...ACAAAAATAT] |
       [3]           chr1 3252757-3253236      + [TTCTTCTGTG...TACCTTCAAT] |
       ...            ...             ...    ...                       ... .
  [447556] chrUn_JH584304     58564-58835      - [CTGTGGTCCT...CAGAGAAATG] |
  [447557] chrUn_JH584304     58564-59690      - [CTCTCTGCTG...CAGAGAAATG] |
  [447558] chrUn_JH584304     59592-59667      - [AGCTGTCCCG...GCCTTCTCAG] |
            exon_id             tx_name         gene_id
          <integer>      <CharacterList> <CharacterList>
       [1]         1 ENSMUST00000193812.1
       [2]         2 ENSMUST00000082908.1
       [3]         3 ENSMUST00000192857.1
       ...       ...                 ...             ...
  [447556]    447556 ENSMUST00000179505.7           66776
  [447557]    447557 ENSMUST00000178343.1           66776
  [447558]    447558 ENSMUST00000179505.7           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,] 376 160 206 328     0
[2,]  45  20  20  25     0
[3,] 138 105  86 151     0
[4,]  28  14  30  29     0
[5,]  57  39  20  33     0
[6,] 208 258 204 256     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] 248409      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      * |      0.70         0         1
  [2]     chr1      1-10      * |      0.82         5        -2
  [3]     chr2 2000-3000      * |      0.10         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      * |      0.70      <NA>        NA         0
  [4]     chr2 2000-3000      * |      0.10         7         8         1
             b2
      <numeric>
  [1]        -2
  [2]        NA
  [3]         1
  [4]         1
  -------
  seqinfo: 2 sequences from an unspecified genome; no seqlengths
```