

# GenomicRanges HOWTOs

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## 1 Introduction

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### 1.1 Purpose of this document

This document is a collection of *HOWTOs*. Each *HOWTO* is a short section that demonstrates how to use the containers and operations implemented in the *GenomicRanges* and related packages (*IRanges*, *Biostrings*, *Rsamtools*, *GenomicAlign*

[ments](#), [BSgenome](#), and [GenomicFeatures](#)) to perform a task typically found in the context of a high throughput sequence analysis.

Unless stated otherwise, the *HOWTOs* are self contained, independent of each other, and can be studied and reproduced in any order.

## 1.2 Prerequisites and additional recommended reading

We assume the reader has some previous experience with *R* and with basic manipulation of *GRanges*, *GRangesList*, *Rle*, *RleList*, and *DataFrame* objects. See the “An Introduction to Genomic Ranges Classes” vignette located in the [GenomicRanges](#) package (in the same folder as this document) for an introduction to these containers.

Additional recommended readings after this document are the “Software for Computing and Annotating Genomic Ranges” paper [Lawrence et al. (2013)] and the “Counting reads with summarizeOverlaps” vignette located in the [GenomicAlignments](#) package.

To display the list of vignettes available in the [GenomicRanges](#) package, use `browseVignettes("GenomicRanges")`.

## 1.3 Input data and terminology used across the HOWTOs

In order to avoid repetition, input data, concepts and terms used in more than one *HOWTO* are described here:

- **The [pasillaBamSubset](#) data package:** contains both a BAM file with single-end reads (`untreated1_chr4`) and a BAM file with paired-end reads (`untreated3_chr4`). Each file is a subset of chr4 from the “Pasilla” experiment.
 

```
> library(pasillaBamSubset)
> untreated1_chr4()
[1] "/home/biocbuild/bbs-3.1-bioc/R/library/pasillaBamSubset/extdata/untreated1_chr4.bam"
> untreated3_chr4()
[1] "/home/biocbuild/bbs-3.1-bioc/R/library/pasillaBamSubset/extdata/untreated3_chr4.bam"
See ?pasillaBamSubset for more information.
> ?pasillaBamSubset
```
- **Gene models and TxDb objects:** A *gene model* is essentially a set of annotations that describes the genomic locations of the known genes, transcripts, exons, and CDS, for a given organism. In *Bioconductor* it is typically represented as a *TxDb* object but also sometimes as a *GRanges* or *GRangesList* object. The [GenomicFeatures](#) package contains tools for making and manipulating *TxDb* objects.

# 2 HOWTOs

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## 2.1 How to read single-end reads from a BAM file

As sample data we use the [pasillaBamSubset](#) data package described in the introduction.

```
> library(pasillaBamSubset)
> un1 <- untreated1_chr4() # single-end reads
```

Several functions are available for reading BAM files into *R*:

```
readGAlignments()
readGAlignmentPairs()
readGAlignmentsList()
scanBam()
```

`scanBam` is a low-level function that returns a list of lists and is not discussed further here. See `?scanBam` in the [Rsamtools](#) package for more information.

Single-end reads can be loaded with the `readGAlignments` function from the [GenomicAlignments](#) package.

```
> library(GenomicAlignments)
> gal <- readGAlignments(un1)
```

Data subsets can be specified by genomic position, field names, or flag criteria in the `ScanBamParam`. Here we input records that overlap position 1 to 5000 on the negative strand with `flag` and `cigar` as metadata columns.

```
> what <- c("flag", "cigar")
> which <- GRanges("chr4", IRanges(1, 5000))
> flag <- scanBamFlag(isMinusStrand = TRUE)
> param <- ScanBamParam(which=which, what=what, flag=flag)
> neg <- readGAlignments(un1, param=param)
> neg
```

GAlignments object with 37 alignments and 2 metadata columns:

	seqnames	strand	cigar	qwidth	start	end
	<Rle>	<Rle>	<character>	<integer>	<integer>	<integer>
[1]	chr4	-	75M	75	892	966
[2]	chr4	-	75M	75	919	993
[3]	chr4	-	75M	75	967	1041
...	...	...	...	...	...	...
[35]	chr4	-	75M	75	4997	5071
[36]	chr4	-	75M	75	4998	5072
[37]	chr4	-	75M	75	4999	5073

  

	width	njunc	flag	cigar
	<integer>	<integer>	<integer>	<character>
[1]	75	0	16	75M
[2]	75	0	16	75M
[3]	75	0	16	75M
...	...	...	...	...
[35]	75	0	16	75M
[36]	75	0	16	75M
[37]	75	0	16	75M

```
-----
seqinfo: 8 sequences from an unspecified genome
```

Another approach to subsetting the data is to use `filterBam`. This function creates a new BAM file of records passing user-defined criteria. See `?filterBam` in the [Rsamtools](#) package for more information.

## 2.2 How to read paired-end reads from a BAM file

As sample data we use the [pasillaBamSubset](#) data package described in the introduction.

```
> library(pasillaBamSubset)
> un3 <- untreated3_chr4() # paired-end reads
```

Paired-end reads can be loaded with the `readGAlignmentPairs` or `readGAlignmentsList` function from the [GenomicAlignments](#) package. These functions use the same mate pairing algorithm but output different objects.

Let's start with `readGAlignmentPairs`:

```
> un3 <- untreated3_chr4()
> gapairs <- readGAlignmentPairs(un3)
```

The `GAlignmentPairs` class holds only pairs; reads with no mate or with ambiguous pairing are discarded. Each list element holds exactly 2 records (a mated pair). Records can be accessed as the `first` and `last` segments in a template or as `left` and `right` alignments. See `?GAlignmentPairs` in the [GenomicAlignments](#) package for more information.

```
> gapairs
```

```
GAlignmentPairs object with 75346 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]
...   ...      ... ..
[75344] chr4      + : [1348217, 1348253] -- [1348215, 1348251]
[75345] chr4      + : [1349196, 1349232] -- [1349326, 1349362]
[75346] chr4      + : [1349708, 1349744] -- [1349838, 1349874]
```

```
-----
seqinfo: 8 sequences from an unspecified genome
```

For readGAlignmentsList, mate pairing is performed when asMates is set to TRUE on the BamFile object, otherwise records are treated as single-end.

```
> galist <- readGAlignmentsList(BamFile(un3, asMates=TRUE))
```

GAlignmentsList is a more general 'list-like' structure that holds mate pairs as well as non-mates (i.e., singletons, records with unmapped mates etc.) A mates\_status metadata column (accessed with mcols) indicates which records were paired.

```
> galist
```

```
GAlignmentsList object of length 96636:
```

```
[[1]]
```

```
GAlignments object with 2 alignments and 0 metadata columns:
```

```
      seqnames strand cigar qwidth start end width njunc
[1]   chr4      +   37M     37   169 205   37    0
[2]   chr4      -   37M     37   326 362   37    0
```

```
[[2]]
```

```
GAlignments object with 2 alignments and 0 metadata columns:
```

```
      seqnames strand cigar qwidth start end width njunc
[1]   chr4      +   37M     37   946 982   37    0
[2]   chr4      -   37M     37   986 1022  37    0
```

```
[[3]]
```

```
GAlignments object with 2 alignments and 0 metadata columns:
```

```
      seqnames strand cigar qwidth start end width njunc
[1]   chr4      +   37M     37   943 979   37    0
[2]   chr4      -   37M     37  1086 1122  37    0
```

```
...
```

```
<96633 more elements>
```

```
-----
```

```
seqinfo: 8 sequences from an unspecified genome
```

Non-mated reads are returned as groups by QNAME and contain any number of records. Here the non-mate groups range in size from 1 to 9.

```
> non_mates <- galist[unlist(mcols(galist)$mate_status) == "unmated"]
```

```
> table(elementLengths(non_mates))
```

```
   1    2    3    4    5    6    7    8    9
18191 2888   69   60    7    8    2    1    1
```

## 2.3 How to read and process a big BAM file by chunks in order to reduce memory usage

A large BAM file can be iterated through in chunks by setting a `yieldSize` on the `BamFile` object. As sample data we use the `pasillaBamSubset` data package described in the introduction.

```
> library(pasillaBamSubset)
> un1 <- untreated1_chr4()
> bf <- BamFile(un1, yieldSize=100000)
```

Iteration through a BAM file requires that the file be opened, repeatedly queried inside a loop, then closed. Repeated calls to `readGAlignments` without opening the file first result in the same 100000 records returned each time.

```
> open(bf)
> cvg <- NULL
> repeat {
+   chunk <- readGAlignments(bf)
+   if (length(chunk) == 0L)
+     break
+   chunk_cvg <- coverage(chunk)
+   if (is.null(cvg)) {
+     cvg <- chunk_cvg
+   } else {
+     cvg <- cvg + chunk_cvg
+   }
+ }
> close(bf)
> cvg

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 ... 106  75 1600  75 1659
  Values :   0   1   2   3   4   5 ...   0   1   0   1   0

...
<3 more elements>
```

## 2.4 How to compute read coverage

The “read coverage” is the number of reads that cover a given genomic position. Computing the read coverage generally consists in computing the coverage at each position in the genome. This can be done with the `coverage()` function.

As sample data we use the [pasillaBamSubset](#) data package described in the introduction.

```
> library(pasillaBamSubset)
> un1 <- untreated1_chr4() # single-end reads
> library(GenomicAlignments)
> reads1 <- readGAlignments(un1)
> cvg1 <- coverage(reads1)
> cvg1

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 ... 106 75 1600 75 1659
  Values : 0 1 2 3 4 5 ... 0 1 0 1 0

...
<3 more elements>

Coverage on chr4:
> cvg1$chr4

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

Average and max coverage:
> mean(cvg1$chr4)
[1] 11.33746
> max(cvg1$chr4)
[1] 5627
```

Note that `coverage()` is a generic function with methods for different types of objects. See `?coverage` for more information.

## 2.5 How to find peaks in read coverage

ChIP-Seq analysis usually involves finding peaks in read coverage. This process is sometimes called “peak calling” or “peak detection”. Here we’re only showing a naive way to find peaks in the object returned by the `coverage()` function. *Bioconductor* packages *BayesPeak*, *bumphunter*, *Starr*, *CexoR*, *exomePeak*, *RIPSeeker*, and others, provide sophisticated peak calling tools for ChIP-Seq, RIP-Seq, and other kind of high throughput sequencing data.

Let’s assume `cvg1` is the object returned by `coverage()` (see previous *HOWTO* for how to compute it). We can use the `slice()` function to find the genomic regions where the coverage is greater or equal to a given threshold.

```
> chr4_peaks <- slice(cvg1$chr4, lower=500)
> chr4_peaks
```

Views on a 1351857-length Rle subject

views:

	start	end	width	
[1]	86849	87364	516	[ 525  538  554  580  583  585  589 ...]
[2]	87466	87810	345	[4924 4928 4941 4943 4972 5026 5039 ...]
[3]	340791	340798	8	[508 512 506 530 521 519 518 501]
[4]	340800	340885	86	[500 505 560 560 565 558 564 559 555 ...]
[5]	348477	348483	7	[503 507 501 524 515 513 512]
[6]	348488	348571	84	[554 554 559 552 558 553 549 550 559 ...]
[7]	692512	692530	19	[502 507 508 518 520 522 524 526 547 ...]
[8]	692551	692657	107	[ 530  549  555  635  645  723  725 ...]
[9]	692798	692800	3	[503 500 503]
...	...	...	...	...
[34]	1054306	1054306	1	[502]
[35]	1054349	1054349	1	[501]
[36]	1054355	1054444	90	[510 521 525 532 532 539 549 555 557 ...]
[37]	1054448	1054476	29	[502 507 516 517 508 517 525 528 532 ...]
[38]	1054479	1054482	4	[504 503 506 507]
[39]	1054509	1054509	1	[500]
[40]	1054511	1054511	1	[502]
[41]	1054521	1054623	103	[529 521 529 530 524 525 547 540 536 ...]
[42]	1054653	1054717	65	[520 519 516 528 526 585 591 589 584 ...]

```
> length(chr4_peaks) # nb of peaks
```

```
[1] 42
```

The weight of a given peak can be defined as the number of aligned nucleotides that belong to the peak (a.k.a. the area under the peak in mathematics). It can be obtained with `sum()`:

```
> sum(chr4_peaks)
```

[1]	1726347	1300700	4115	52301	3575	51233	10382	95103
[9]	1506	500	2051	500	5834	10382	92163	500
[17]	88678	1512	500	11518	14514	5915	3598	7821
[25]	511	508	503	500	1547	8961	43426	22842
[33]	503	502	501	51881	15116	2020	500	502
[41]	67010	40496						

## 2.6 How to retrieve a gene model from the UCSC genome browser

See introduction for a quick description of what *gene models* and *TxDb* objects are. We can use the `makeTranscriptDbFromUCSC()` function from the *GenomicFeatures* package to import a UCSC genome browser track as a *TxDb* object.

```
> library(GenomicFeatures)
> ### Internet connection required! Can take several minutes...
> txdb <- makeTxDbFromUCSC(genome="sacCer2", tablename="ensGene")
```

See `?makeTxDbFromUCSC` in the *GenomicFeatures* package for more information.

Note that some of the most frequently used gene models are available as *TxDb* packages. A *TxDb* package consists of a pre-made *TxDb* object wrapped into an annotation data package. Go to [http://bioconductor.org/packages/release/BiocViews.html#\\_\\_\\_TxDb](http://bioconductor.org/packages/release/BiocViews.html#___TxDb) to browse the list of available *TxDb* packages.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
> txdb
```

*TxDb* object:

```
# Db type: TxDb
# Supporting package: GenomicFeatures
# Data source: UCSC
# Genome: hg19
# Organism: Homo sapiens
# UCSC Table: knownGene
# Resource URL: http://genome.ucsc.edu/
# Type of Gene ID: Entrez Gene ID
# Full dataset: yes
# miRBase build ID: GRCh37
# transcript_nrow: 82960
# exon_nrow: 289969
# cds_nrow: 237533
# Db created by: GenomicFeatures package from Bioconductor
# Creation time: 2015-03-19 13:55:51 -0700 (Thu, 19 Mar 2015)
# GenomicFeatures version at creation time: 1.19.32
# RSQLite version at creation time: 1.0.0
# DBSCHEMAVERSION: 1.1
```

Extract the transcript coordinates from this gene model:

```
> transcripts(txdb)
```

GRanges object with 82960 ranges and 2 metadata columns:

	seqnames	ranges	strand	tx_id
	<Rle>	<IRanges>	<Rle>	<integer>
[1]	chr1	[11874, 14409]	+	1
[2]	chr1	[11874, 14409]	+	2
[3]	chr1	[11874, 14409]	+	3
...	...	...	...	...
[82958]	chrY	[27607404, 27607432]	-	78805
[82959]	chrY	[27635919, 27635954]	-	78806
[82960]	chrY	[59358329, 59360854]	-	78807
	tx_name			
	<character>			
[1]	uc001aaa.3			
[2]	uc010nxq.1			
[3]	uc010nxr.1			



```

...      ...
[82958] uc004fwz.3
[82959] uc022cpd.1
[82960] uc011ncc.1
-----
seqinfo: 93 sequences (1 circular) from hg19 genome

```

## 2.7 How to retrieve a gene model from Ensembl

See introduction for a quick description of what *gene models* and *TxDb* objects are. We can use the `makeTranscriptDbFromBiomart()` function from the *GenomicFeatures* package to retrieve a gene model from the Ensembl Mart.

```

> library(GenomicFeatures)
> ### Internet connection required! Can take several minutes...
> txdb <- makeTxDbFromBiomart(biomart="ensembl",
+                             dataset="hsapiens_gene_ensembl")

```

See `?makeTxDbFromBiomart` in the *GenomicFeatures* package for more information.

Note that some of the most frequently used gene models are available as *TxDb* packages. A *TxDb* package consists of a pre-made *TxDb* object wrapped into an annotation data package. Go to [http://bioconductor.org/packages/release/BiocViews.html#\\_\\_\\_TxDb](http://bioconductor.org/packages/release/BiocViews.html#___TxDb) to browse the list of available *TxDb* packages.

```

> library(TxDb.Athaliana.BioMart.plantsmart22)
> txdb <- TxDb.Athaliana.BioMart.plantsmart22
> txdb

```

*TxDb* object:

```

# Db type: TxDb
# Supporting package: GenomicFeatures
# Data source: BioMart
# Organism: Arabidopsis thaliana
# Resource URL: www.biomart.org:80
# BioMart database: plants_mart_22
# BioMart database version: ENSEMBL PLANTS 22 (EBI UK)
# BioMart dataset: athaliana_eg_gene
# BioMart dataset description: Arabidopsis thaliana genes (TAIR10 (2010-09-TAIR10))
# BioMart dataset version: TAIR10 (2010-09-TAIR10)
# Full dataset: yes
# miRBase build ID: NA
# transcript_nrow: 41671
# exon_nrow: 171013
# cds_nrow: 147494
# Db created by: GenomicFeatures package from Bioconductor
# Creation time: 2014-09-26 11:23:54 -0700 (Fri, 26 Sep 2014)
# GenomicFeatures version at creation time: 1.17.17
# RSQLite version at creation time: 0.11.4
# DBSCHEMAVERSION: 1.0

```

Extract the exon coordinates from this gene model:

```

> exons(txdb)

```

GRanges object with 171013 ranges and 1 metadata column:

	seqnames	ranges	strand	exon_id
	<Rle>	<IRanges>	<Rle>	<integer>
[1]	1	[3631, 3913]	+	1

```

      [2]      1   [3996, 4276]    + |      2
      [3]      1   [4486, 4605]    + |      3
      ...      ...      ...      ...
[171011]    Pt [137869, 137940]    - |    171011
[171012]    Pt [144921, 145154]    - |    171012
[171013]    Pt [145291, 152175]    - |    171013
-----
seqinfo: 7 sequences (1 circular) from an unspecified genome

```

## 2.8 How to load a gene model from a GFF or GTF file

See introduction for a quick description of what *gene models* and *TxDb* objects are. We can use the `makeTranscriptDbFromGFF()` function from the [GenomicFeatures](#) package to import a GFF or GTF file as a *TxDb* object.

```

> library(GenomicFeatures)
> gff_file <- system.file("extdata", "GFF3_files", "a.gff3",
+                          package="GenomicFeatures")
> txdb <- makeTxDbFromGFF(gff_file, format="gff3")
> txdb

TxDb object:
# Db type: TxDb
# Supporting package: GenomicFeatures
# Data source: /home/biocbuild/bbs-3.1-bioc/R/library/GenomicFeatures/extdata/GFF3_files/a.gff3
# Organism: NA
# miRBase build ID: NA
# Genome: NA
# transcript_nrow: 488
# exon_nrow: 1268
# cds_nrow: 1258
# Db created by: GenomicFeatures package from Bioconductor
# Creation time: 2015-09-21 19:13:17 -0700 (Mon, 21 Sep 2015)
# GenomicFeatures version at creation time: 1.20.4
# RSQLite version at creation time: 1.0.0
# DBSCHEMAVERSION: 1.1

```

See `?makeTxDbFromGFF` in the [GenomicFeatures](#) package for more information.

Extract the exon coordinates grouped by gene from this gene model:

```

> exonsBy(txdb, by="gene")

GRangesList object of length 488:
$Solyc00g005000.2
GRanges object with 2 ranges and 2 metadata columns:
      seqnames      ranges strand | exon_id      exon_name
      <Rle>      <IRanges> <Rle> | <integer> <character>
[1] SL2.40ch00 [16437, 17275]    + |      1 Solyc00g005000.2.1.1
[2] SL2.40ch00 [17336, 18189]    + |      2 Solyc00g005000.2.1.2

$Solyc00g005020.1
GRanges object with 3 ranges and 2 metadata columns:
      seqnames      ranges strand | exon_id      exon_name
[1] SL2.40ch00 [68062, 68211]    + |      3 Solyc00g005020.1.1.1
[2] SL2.40ch00 [68344, 68568]    + |      4 Solyc00g005020.1.1.2
[3] SL2.40ch00 [68654, 68764]    + |      5 Solyc00g005020.1.1.3

```

```
$Solyc00g005040.2
GRanges object with 4 ranges and 2 metadata columns:
      seqnames      ranges strand | exon_id      exon_name
 [1] SL2.40ch00 [550920, 550945]   + |      6 Solyc00g005040.2.1.1
 [2] SL2.40ch00 [551034, 551132]   + |      7 Solyc00g005040.2.1.2
 [3] SL2.40ch00 [551218, 551250]   + |      8 Solyc00g005040.2.1.3
 [4] SL2.40ch00 [551343, 551576]   + |      9 Solyc00g005040.2.1.4
...
<485 more elements>
-----
seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

## 2.9 How to retrieve a gene model from AnnotationHub

When a gene model is not available as a *GRanges* or *GRangesList* object or as a *Bioconductor* data package, it may be available on [AnnotationHub](#). In this *HOWTO*, will look for a gene model for *Drosophila melanogaster* on [AnnotationHub](#). Create a 'hub' and then filter on *Drosophila melanogaster*:

```
> library(AnnotationHub)
> ### Internet connection required!
> hub <- AnnotationHub()
> hub <- subset(hub, hub$species=='Drosophila melanogaster')
```

There are 87 files that match *Drosophila melanogaster*. If you look at the metadata in hub, you can see that the 7th record represents a *GRanges* object from UCSC

```
> length(hub)
[1] 198
> head(names(hub))
[1] "AH79" "AH80" "AH81" "AH82" "AH83" "AH84"
> head(hub$title, n=10)
 [1] "Drosophila_melanogaster.BDGP5.69.cdna.all.fa"
 [2] "Drosophila_melanogaster.BDGP5.69.dna.toplevel.fa"
 [3] "Drosophila_melanogaster.BDGP5.69.dna_rm.toplevel.fa"
 [4] "Drosophila_melanogaster.BDGP5.69.dna_sm.toplevel.fa"
 [5] "Drosophila_melanogaster.BDGP5.69.ncrna.fa"
 [6] "Drosophila_melanogaster.BDGP5.69.pep.all.fa"
 [7] "Assembly"
 [8] "GDP Insertions"
 [9] "BAC End Pairs"
[10] "FlyBase Genes"
> ## then look at a specific slice of the hub object.
> hub[7]
AnnotationHub with 1 record
# snapshotDate(): 2015-08-26
# names(): AH6789
# $dataprovder: UCSC
# $species: Drosophila melanogaster
# $rdataclass: GRanges
```

```
# $title: Assembly
# $description: GRanges object from on UCSC track ĀćāČñĚIJAssemblyĀćāČñāĎć
# $taxonomyid: 7227
# $genome: dm3
# $sourcetype: UCSC track
# $sourceurl: rtracklayer://hgdownload.cse.ucsc.edu/goldenpath/dm3/d...
# $sourcelastmodifieddate: NA
# $sourcesize: NA
# $tags: gold, UCSC, track, Gene, Transcript, Annotation
# retrieve record with 'object[["AH6789"]]'
```

So you can retrieve that dm3 file as a GRanges like this:

```
> gr <- hub[[names(hub)[7]]]
> summary(gr)

Length Class      Mode
 37688 GRanges      S4
```

The metadata fields contain the details of file origin and content.

```
> metadata(gr)

$trackLine
track name=gold description=gold visibility=1 url="NA"
```

Split the *GRanges* object by gene name to get a *GRangesList* object of transcript ranges grouped by gene.

```
> txbygn <- split(gr, gr$name)
```

You can now use *txbygn* with the *summarizeOverlaps* function to prepare a table of read counts for RNA-Seq differential gene expression.

Note that before passing *txbygn* to *summarizeOverlaps*, you should confirm that the *seqlevels* (chromosome names) in it match those in the BAM file. See *?renameSeqlevels*, *?keepSeqlevels* and *?seqlevels* for examples of renaming *seqlevels*.

## 2.10 How to annotate peaks in read coverage

[coming soon...]

## 2.11 How to prepare a table of read counts for RNA-Seq differential gene expression

Methods for RNA-Seq gene expression analysis generally require a table of counts that summarize the number of reads that overlap or 'hit' a particular gene. In this *HOWTO* we count with the *summarizeOverlaps* function from the *GenomicAlignments* package and create a count table from the results.

Other packages that provide read counting are *Rsubread* and *easyRNASeq*. The *parathyroidSE* package vignette contains a workflow on counting and other common operations required for differential expression analysis.

As sample data we use the *pasillaBamSubset* data package described in the introduction.

```
> library(pasillaBamSubset)
> un1 <- untreated1_chr4() # single-end reads
```

*summarizeOverlaps* requires the name of a BAM file(s) and a *gene model* to count against. See introduction for a quick description of what a *gene models* is. The gene model must match the genome build the reads in the BAM file were aligned to. For the *pasilla* data this is dm3 *Dmelanogaster* which is available as a *Bioconductor* package. Load the package and extract the exon ranges grouped by gene:

```
> library(TxDb.Dmelanogaster.UCSC.dm3.ensGene)
> exbygene <- exonsBy(TxDb.Dmelanogaster.UCSC.dm3.ensGene, "gene")
```

exbygene is a *GRangesList* object with one list element per gene in the gene model.

summarizeOverlaps automatically sets a yieldSize on large BAM files and iterates over them in chunks. When reading paired-end data set the singleEnd argument to FALSE. See ?summarizeOverlaps for details regarding the count modes and additional arguments.

```
> library(GenomicAlignments)
> se <- summarizeOverlaps(exbygene, un1, mode="IntersectionNotEmpty")
```

The return object is a SummarizedExperiment with counts accessible with the assays accessor:

```
> class(se)
[1] "SummarizedExperiment"
attr(,"package")
[1] "GenomicRanges"
> head(table(assays(se)$counts))
      0      1      2      3      4      5
15593    1     3     1     4     1
```

The count vector is the same length as exbygene:

```
> identical(length(exbygene), length(assays(se)$counts))
[1] TRUE
```

A copy of exbygene is stored in the se object and accessible with rowRanges accessor:

```
> rowRanges(se)
GRangesList object of length 15682:
$FBgn0000003
GRanges object with 1 range and 2 metadata columns:
      seqnames      ranges strand | exon_id exon_name
      <Rle>        <IRanges> <Rle> | <integer> <character>
[1]   chr3R [2648220, 2648518]   + |    45123      <NA>

$FBgn0000008
GRanges object with 13 ranges and 2 metadata columns:
      seqnames      ranges strand | exon_id exon_name
[1]   chr2R [18024494, 18024531]   + |    20314      <NA>
[2]   chr2R [18024496, 18024713]   + |    20315      <NA>
[3]   chr2R [18024938, 18025756]   + |    20316      <NA>
...
[11]  chr2R [18059821, 18059938]   + |    20328      <NA>
[12]  chr2R [18060002, 18060339]   + |    20329      <NA>
[13]  chr2R [18060002, 18060346]   + |    20330      <NA>
```

```
...
<15680 more elements>
```

```
-----
seqinfo: 15 sequences (1 circular) from dm3 genome
```

Two popular packages for RNA-Seq differential gene expression are *DESeq* and *edgeR*. Tables of counts per gene are required for both and can be easily created with a vector of counts. Here we use the counts from our *SummarizedExperiment* object:

```
> library(DESeq)
> deseq <- newCountDataSet(assays(se)$counts, rownames(colData(se)))
> library(edgeR)
> edger <- DGEList(assays(se)$counts, group=rownames(colData(se)))
```

## 2.12 How to summarize junctions from a BAM file containing RNA-Seq reads

As sample data we use the *pasillaBamSubset* data package described in the introduction.

```
> library(pasillaBamSubset)
> un1 <- untreated1_chr4() # single-end reads
> library(GenomicAlignments)
> reads1 <- readGAlignments(un1)
> reads1
```

GAlignments object with 204355 alignments and 0 metadata columns:

	seqnames	strand	cigar	qwidth	start	end
	<Rle>	<Rle>	<character>	<integer>	<integer>	<integer>
[1]	chr4	-	75M	75	892	966
[2]	chr4	-	75M	75	919	993
[3]	chr4	+	75M	75	924	998
...	...	...	...	...	...	...
[204353]	chr4	+	75M	75	1348268	1348342
[204354]	chr4	-	75M	75	1348449	1348523
[204355]	chr4	-	75M	75	1350124	1350198

  

	width	njunc
	<integer>	<integer>
[1]	75	0
[2]	75	0
[3]	75	0
...	...	...
[204353]	75	0
[204354]	75	0
[204355]	75	0

-----

seqinfo: 8 sequences from an unspecified genome

For each alignment, the aligner generated a CIGAR string that describes its "geometry", that is, the locations of insertions, deletions and junctions in the alignment. See the SAM Spec available on the SAMtools website for the details (<http://samtools.sourceforge.net/>).

The `summarizeJunctions()` function from the *GenomicAlignments* package can be used to summarize the junctions in `reads1`.

```
> junc_summary <- summarizeJunctions(reads1)
> junc_summary
```

GRanges object with 910 ranges and 3 metadata columns:

	seqnames	ranges	strand	score	plus_score
	<Rle>	<IRanges>	<Rle>	<integer>	<integer>
[1]	chr4	[ 5246, 11972]	*	3	1
[2]	chr4	[10346, 10637]	*	1	1
[3]	chr4	[27102, 27166]	*	13	11
...	...	...	...	...	...
[908]	chr4	[1333752, 1346734]	*	1	0
[909]	chr4	[1334150, 1347141]	*	1	1

```

[910] chr4 [1334557, 1347539] * | 1 0
      minus_score
      <integer>
      [1] 2
      [2] 0
      [3] 2
      ... ..
[908] 1
[909] 0
[910] 1
-----

```

```
seqinfo: 8 sequences from an unspecified genome
```

See `?summarizeJunctions` in the [GenomicAlignments](#) package for more information.

## 2.13 How to get the exon and intron sequences of a given gene

The exon and intron sequences of a gene are essentially the DNA sequences of the introns and exons of all known transcripts of the gene. The first task is to identify all transcripts associated with the gene of interest. Our sample gene is the human TRAK2 which is involved in regulation of endosome-to-lysosome trafficking of membrane cargo. The Entrez gene id is '66008'.

```
> trak2 <- "66008"
```

The [TxDb.Hsapiens.UCSC.hg19.knownGene](#) data package contains the gene model corresponding to the UCSC 'Known Genes' track.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
```

The transcript ranges for all the genes in the gene model can be extracted with the `transcriptsBy` function from the [GenomicFeatures](#) package. They will be returned in a named *GRangesList* object containing all the transcripts grouped by gene. In order to keep only the transcripts of the TRAK2 gene we will subset the *GRangesList* object using the `[[` operator.

```
> library(GenomicFeatures)
> trak2_txs <- transcriptsBy(txdb, by="gene")[[trak2]]
> trak2_txs
```

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

```

      seqnames          ranges strand |      tx_id      tx_name
      <Rle>             <IRanges> <Rle> | <integer> <character>
[1] chr2 [202241930, 202316319] - | 12552 uc002uyb.4
[2] chr2 [202259851, 202316319] - | 12553 uc002uyc.2
-----

```

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

`trak2_txs` is a *GRanges* object with one range per transcript in the TRAK2 gene. The transcript names are stored in the `tx_name` metadata column. We will need them to subset the extracted intron and exon regions:

```
> trak2_tx_names <- mcols(trak2_txs)$tx_name
> trak2_tx_names
```

```
[1] "uc002uyb.4" "uc002uyc.2"
```

The exon and intron genomic ranges for all the transcripts in the gene model can be extracted with the `exonsBy` and `intronsByTranscript` functions, respectively. Both functions return a *GRangesList* object. Then we keep only the exon and intron for the transcripts of the TRAK2 gene by subsetting each *GRangesList* object by the TRAK2 transcript names.

Extract the exon regions:

```
> trak2_exbytx <- exonsBy(txdb, "tx", use.names=TRUE)[trak2_tx_names]
> elementLengths(trak2_exbytx)
```

```
uc002uyb.4 uc002uyc.2
      16      8
```

... and the intron regions:

```
> trak2_inbytx <- intronsByTranscript(txdb, use.names=TRUE)[trak2_tx_names]
> elementLengths(trak2_inbytx)
```

```
uc002uyb.4 uc002uyc.2
      15      7
```

Next we want the DNA sequences for these exons and introns. The `getSeq` function from the [Biostrings](#) package can be used to query a [BSgenome](#) object with a set of genomic ranges and retrieve the corresponding DNA sequences.

```
> library(BSgenome.Hsapiens.UCSC.hg19)
```

Extract the exon sequences:

```
> trak2_ex_seqs <- getSeq(Hsapiens, trak2_exbytx)
> trak2_ex_seqs
```

DNASTringSetList of length 2

```
["uc002uyb.4"] GCTGGGAGAGTGGCTCTCCTTTGGCTTCCCAATTGTGTGGGGGCTGCCATT...
["uc002uyc.2"] GCTGGGAGAGTGGCTCTCCTTTGGCTTCCCAATTGTGTGGGGGCTGCCATT...
```

```
> trak2_ex_seqs[["uc002uyb.4"]]
```

A DNASTringSet instance of length 16

width seq

```
[1] 247 GCTGGGAGAGTGGCTCTCCTTTGGCTTCC...CGGACGACAGAGGATGCCGAACCACTCCA
[2] 290 GTCATGACTGTCCAAAGTATGATAATCAC...CAATCACAGAGACTCGGAGAGCATCACTG
[3] 195 ATGTCTGCTCCAATGAGGATCTCCCTGAA...CCTTGCTGAAGAGACTTCCGTTACATGA
... ..
[14] 267 GATCACAACTCTGTATCACTGGCAGCAG...CATTACTTCAGCAGGTGGACCAGTTACAG
[15] 106 TTGCAACCGCCAAACCCAGAAAGTGCCTG...CCCTCTGACATCACTCAGTTACCCCCAG
[16] 4012 CTCTGGGTTCCCTTATTATCCTGTGGAA...TTAATAAACATGAGTAGCTGAATTTCA
```

```
> trak2_ex_seqs[["uc002uyc.2"]]
```

A DNASTringSet instance of length 8

width seq

```
[1] 247 GCTGGGAGAGTGGCTCTCCTTTGGCTTCCC...CGGACGACAGAGGATGCCGAACCACTCCA
[2] 290 GTCATGACTGTCCAAAGTATGATAATCACA...CAATCACAGAGACTCGGAGAGCATCACTG
[3] 195 ATGTCTGCTCCAATGAGGATCTCCCTGAA...CCTTGCTGAAGAGACTTCCGTTACATGA
[4] 77 TTCTAGGCACAGACAGGGTGGAGCAGATGA...TCGACATGGTTACACATCTCCTGGCAGAG
[5] 117 AGGGATCGTGATCTGGAACCTCGCTGCTCGA...AGGAGCAATTGGGACAAGCCTTTGATCAA
[6] 210 GTTAATCAGCTGCAGCATGAGCTATGCAAG...AGAAGAGAATATGGCTCTTCGATCCAAG
[7] 79 GCTTGTACATAAAGACAGAACTGTTACC...GCTTGTGACGACTGTGTTAAAGAACTTC
[8] 317 GTGAAACAAATGCTCAGATGTCCAGAATGA...AGATATCATGAATAAATACTTTCAAGTCA
```

... and the intron sequences:

```
> trak2_in_seqs <- getSeq(Hsapiens, trak2_inbytx)
> trak2_in_seqs
```

DNASTringSetList of length 2

```
["uc002uyb.4"] GTAAGAGTGCCTGGGAAATCTGGGGCCTCACTTCTTCTCAGCTATATTTT...
["uc002uyc.2"] GTGAGTATTAACATATTCTCTTTGTACCTTTTTGGACAATTCTTTGGTAGG...
```



```
> trak2_in_seqs[["uc002uyb.4"]]
A DNASTringSet instance of length 15
width seq
[1] 2892 GTAAGAGTGCCTGGGAAATCTGGGGCCTC...GTCTCCCACTTTTTTTTTTTTTTTTAAAG
[2] 2001 GTGAGAAGAGTGTCTGGTTGAATATGGTA...TGTATTTGCTCCCTAAAAATCTATTTTCAG
[3] 1218 GTAATAAATCAGTAAGGGCCCTTACTAAG...TTCCCTTCCTTTGTTTTGCATATTCAG
... ..
[13] 6308 GTGAGTATTTTTTTACTCTTTTAGTTG...CTATAAATAGTTGTTTTTAACTATATTAG
[14] 12819 GTAAGTCCAGTTAATAAATATTGAAGTG...GATTCATTTACATAGACTCTCCTCTTAG
[15] 30643 GTGAGTAAGCTGTCCGCGCAGAACCCGAA...GTTCTAGTCACTTGATGTTTTTGTTTTAG

> trak2_in_seqs[["uc002uyc.2"]]
A DNASTringSet instance of length 7
width seq
[1] 2057 GTGAGTATTAACATATTCTCTTTTGTACCT...AATTTAAAAAATTTTTTTTGTCTTCCAAG
[2] 564 GTACGTTCAACCTAATTGCCATTTTCCTTT...ATTGTCACATACTGATTTTTTTCTTGAAG
[3] 1022 GTAAGCCTTTGATCAAATGTCTGCAGTATG...CATGAAAATCAAGCATTTTATATGGACAG
[4] 1524 GTAGGAATATCTTTTCTTTCTCCAGTACAA...AAGAAAAGGTGATTTGGTATTTTAAACAG
[5] 6308 GTGAGTATTTTTTTACTCTTTTAGTTTGT...CTATAAATAGTTGTTTTTAACTATATTAG
[6] 12819 GTAAGTCCAGTTAATAAATATTGAAGTGC...GATTCATTTACATAGACTCTCCTCTTAG
[7] 30643 GTGAGTAAGCTGTCCGCGCAGAACCCGAAC...GTTCTAGTCACTTGATGTTTTTGTTTTAG
```

## 2.14 How to get the CDS and UTR sequences of genes associated with colorectal cancer

In this *HOWTO* we extract the CDS and UTR sequences of genes involved in colorectal cancer. The workflow extends the ideas presented in the previous *HOWTO* and suggests an approach for identifying disease-related genes.

### 2.14.1 Build a gene list

We start with a list of gene or transcript ids. If you do not have pre-defined list one can be created with the [KEGG.db](#) and [KEGGgraph](#) packages. Updates to the data in the [KEGG.db](#) package are no longer available, however, the resource is still useful for identifying pathway names and ids.

Create a table of KEGG pathways and ids and search on the term 'cancer'.

```
> library(KEGG.db)
> pathways <- toTable(KEGGPATHNAME2ID)
> pathways[grep("cancer", pathways$path_name, fixed=TRUE),]

  path_id      path_name
299 05200 Pathways in cancer
300 05210 Colorectal cancer
302 05212 Pancreatic cancer
303 05213 Endometrial cancer
305 05215 Prostate cancer
306 05216 Thyroid cancer
309 05219 Bladder cancer
312 05222 Small cell lung cancer
313 05223 Non-small cell lung cancer
```

Use the "05210" id to query the KEGG web resource (accesses the currently maintained data).

```
> library(KEGGgraph)
> dest <- tempfile()
> retrieveKML("05200", "hsa", dest, "internal")
```

The suffix of the KEGG id is the Entrez gene id. The `translateKEGGID2GeneID` simply removes the prefix leaving just the Entrez gene ids.

```
> crids <- as.character(parseKGML2DataFrame(dest)[,1])
> crgenes <- unique(translateKEGGID2GeneID(crids))
> head(crgenes)

[1] "1630" "836" "842" "1499" "51384" "54361"
```

### 2.14.2 Identify genomic coordinates

The list of gene ids is used to extract genomic positions of the regions of interest. The Known Gene table from UCSC will be the annotation and is available as a *Bioconductor* package.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
```

If an annotation is not available as a *Bioconductor* annotation package it may be available in [AnnotationHub](#). Additionally, there are functions in [GenomicFeatures](#) which can retrieve data from UCSC and Ensembl to create a TxDb. See `?makeTxDbFromUCSC` for more information.

As in the previous *HOWTO* we need to identify the transcripts corresponding to each gene. The transcript id (or name) is used to isolate the UTR and coding regions of interest. This grouping of transcript by gene is also used to re-group the final sequence results.

The `transcriptsBy` function outputs both the gene and transcript identifiers which we use to create a map between the two. The map is a `CharacterList` with gene ids as names and transcript ids as the list elements.

```
> txbygene <- transcriptsBy(txdb, "gene")[crgenes] ## subset on colorectal genes
> map <- relist(unlist(txbygene, use.names=FALSE)$tx_id, txbygene)
> map
```

```
IntegerList of length 239
[["1630"]] 64962 64963 64964
[["836"]] 20202 20203 20204
[["842"]] 4447 4448 4449 4450 4451 4452
[["1499"]] 13582 13583 13584 13585 13586 13587 13589
[["51384"]] 29319 29320 29321
[["54361"]] 4634 4635
[["7471"]] 46151
[["7472"]] 31279 31280
[["7473"]] 63770
[["7474"]] 16089 16090 16091 16092
...
<229 more elements>
```

Extract the UTR and coding regions.

```
> cds <- cdsBy(txdb, "tx")
> threeUTR <- threeUTRsByTranscript(txdb)
> fiveUTR <- fiveUTRsByTranscript(txdb)
```

Coding and UTR regions may not be present for all transcripts specified in `map`. Consequently, the subset results will not be the same length. This length discrepancy must be taken into account when re-listing the final results by gene.

```
> txid <- unlist(map, use.names=FALSE)
> cds <- cds[names(cds) %in% txid]
> threeUTR <- threeUTR[names(threeUTR) %in% txid]
> fiveUTR <- fiveUTR[names(fiveUTR) %in% txid]
```

Note the different lengths of the subset regions.

```
> length(txid) ## all possible transcripts
```

```
[1] 1045
```

```
> length(cds)
```

```
[1] 960
```

```
> length(threeUTR)
```

```
[1] 919
```

```
> length(fiveUTR)
```

```
[1] 947
```

These objects are GRangesLists with the transcript id as the outer list element.

```
> cds
```

GRangesList object of length 960:

\$2043

GRanges object with 6 ranges and 3 metadata columns:

	seqnames	ranges	strand	cds_id	cds_name
	<Rle>	<IRanges>	<Rle>	<integer>	<character>
[1]	chr1	[113010160, 113010213]	+	6055	<NA>
[2]	chr1	[113033633, 113033703]	+	6056	<NA>
[3]	chr1	[113057496, 113057716]	+	6058	<NA>
[4]	chr1	[113058762, 113059039]	+	6060	<NA>
[5]	chr1	[113059743, 113060007]	+	6061	<NA>
[6]	chr1	[113062902, 113063131]	+	6062	<NA>

  

	exon_rank
	<integer>
[1]	1
[2]	2
[3]	3
[4]	4
[5]	5
[6]	6

\$2044

GRanges object with 4 ranges and 3 metadata columns:

	seqnames	ranges	strand	cds_id	cds_name
[1]	chr1	[113057590, 113057716]	+	6059	<NA>
[2]	chr1	[113058762, 113059039]	+	6060	<NA>
[3]	chr1	[113059743, 113060007]	+	6061	<NA>
[4]	chr1	[113062902, 113063131]	+	6062	<NA>

  

	exon_rank
[1]	2
[2]	3
[3]	4
[4]	5

\$2045

GRanges object with 5 ranges and 3 metadata columns:

	seqnames	ranges	strand	cds_id	cds_name
[1]	chr1	[113051885, 113052066]	+	6057	<NA>
[2]	chr1	[113057496, 113057716]	+	6058	<NA>

```
[3] chr1 [113058762, 113059039] + | 6060 <NA>
[4] chr1 [113059743, 113060007] + | 6061 <NA>
[5] chr1 [113062902, 113063131] + | 6062 <NA>
  exon_rank
[1] 1
[2] 2
[3] 3
[4] 4
[5] 5

...
<957 more elements>
-----
seqinfo: 93 sequences (1 circular) from hg19 genome
```

### 2.14.3 Extract sequences from BSgenome

The BSgenome packages contain complete genome sequences for a given organism.

Load the BSgenome package for homo sapiens.

```
> library(BSgenome.Hsapiens.UCSC.hg19)
> genome <- BSgenome.Hsapiens.UCSC.hg19
```

Use `extractTranscriptSeqs` to extract the UTR and coding regions from the BSgenome. This function retrieves the sequences for an any GRanges or GRangesList (i.e., not just transcripts like the name implies).

```
> threeUTR_seqs <- extractTranscriptSeqs(genome, threeUTR)
> fiveUTR_seqs <- extractTranscriptSeqs(genome, fiveUTR)
> cds_seqs <- extractTranscriptSeqs(genome, cds)
```

The return values are DNASTringSet objects.

```
> cds_seqs

A DNASTringSet instance of length 960
  width seq names
[1] 1119 ATGTTGGATGGCCTTGGGA...TGGCTGGACCAAACCTGA 2043
[2] 900 ATGCGTTCAGTGGGCGAG...TGGCTGGACCAAACCTGA 2044
[3] 1176 ATGCTGAGACCGGGTGGT...TGGCTGGACCAAACCTGA 2045
...
[958] 681 ATGTTACGACAAGATTCC...CACAATGAATCAACGTAG 78103
[959] 768 ATGAGTGGAAAGGTGACC...CACAATGAATCAACGTAG 78104
[960] 600 ATGAGTGGAAAGGTGACC...CACAATGAATCAACGTAG 78105
```

Our final step is to collect the coding and UTR regions (currently organized by transcript) into groups by gene id. The `relist` function groups the sequences of a DNASTringSet object into a DNASTringSetList object, based on the specified `skeleton` argument. The `skeleton` must be a list-like object and only its shape (i.e. its element lengths) matters (its exact content is ignored). A simple form of `skeleton` is to use a partitioning object that we make by specifying the size of each partition. The partitioning objects are different for each type of region because not all transcripts had a coding or 3' or 5' UTR region defined.

```
> lst3 <- relist(threeUTR_seqs, PartitioningByWidth(sum(map %in% names(threeUTR))))
> lst5 <- relist(fiveUTR_seqs, PartitioningByWidth(sum(map %in% names(fiveUTR))))
> lstc <- relist(cds_seqs, PartitioningByWidth(sum(map %in% names(cds))))
```

There are 239 genes in `map` each of which have 1 or more transcripts. The table of element lengths shows how many genes have each number of transcripts. For example, 47 genes have 1 transcript, 48 genes have 2 etc.

```
> length(map)
[1] 239
> table(elementLengths(map))
 1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 21 30
47 48 46 22 17 18 10  4  3  3  5  3  1  1  1  1  4  1  2  1  1
```

The lists of DNA sequences all have the same length as `map` but one or more of the element lengths may be zero. This would indicate that data were not available for that gene. The tables below show that there was at least 1 coding region available for all genes (i.e., none of the element lengths are 0). However, both the 3' and 5' UTR results have element lengths of 0 which indicates no UTR data were available for that gene.

```
> table(elementLengths(lstc))
 1  2  3  4  5  6  7  8  9 10 11 12 14 15 16 17 18 30
48 54 49 20 17 16  8  5  5  3  1  2  3  1  2  1  3  1
> table(elementLengths(lst3))
 0  1  2  3  4  5  6  7  8  9 11 12 13 14 15 16 17 18 30
 2 49 56 47 19 18 13  9  5  8  2  2  2  1  1  2  1  1  1
> names(lst3)[elementLengths(lst3) == 0L] ## genes with no 3' UTR data
[1] "2255" "8823"
> table(elementLengths(lst5))
 0  1  2  3  4  5  6  7  8  9 10 11 12 14 15 16 17 18 30
 3 48 52 49 19 17 16  8  5  5  3  2  2  3  1  1  1  3  1
> names(lst5)[elementLengths(lst5) == 0L] ## genes with no 5' UTR data
[1] "2255" "27006" "8823"
```

## 2.15 How to create DNA consensus sequences for read group 'families'

The motivation for this *HOWTO* comes from a study which explored the dynamics of point mutations. The mutations of interest exist with a range of frequencies in the control group (e.g., 0.1% - 50%). PCR and sequencing error rates make it difficult to identify low frequency events (e.g., < 20%).

When a library is prepared with Nextera, random fragments are generated followed by a few rounds of PCR. When the genome is large enough, reads aligning to the same start position are likely descendant from the same template fragment and should have identical sequences.

The goal is to eliminate noise by grouping the reads by common start position and discarding those that do not exceed a certain threshold within each family. A new consensus sequence will be created for each read group family.

### 2.15.1 Sort reads into groups by start position

Load the BAM file into a `GAlignments` object.

```
> library(Rsamtools)
> bamfile <- system.file("extdata", "ex1.bam", package="Rsamtools")
> param <- ScanBamParam(what=c("seq", "qual"))
> library(GenomicAlignments)
> gal <- readGAlignments(bamfile, use.names=TRUE, param=param)
```

Use the `sequenceLayer` function to *lay* the query sequences and quality strings on the reference.

```

> qseq <- setNames(mcols(gal)$seq, names(gal))
> qual <- setNames(mcols(gal)$qual, names(gal))
> qseq_on_ref <- sequenceLayer(qseq, cigar(gal),
+                             from="query", to="reference")
> qual_on_ref <- sequenceLayer(qual, cigar(gal),
+                              from="query", to="reference")

```

Split by chromosome.

```

> qseq_on_ref_by_chrom <- splitAsList(qseq_on_ref, seqnames(gal))
> qual_on_ref_by_chrom <- splitAsList(qual_on_ref, seqnames(gal))
> pos_by_chrom <- splitAsList(start(gal), seqnames(gal))

```

For each chromosome generate one GRanges object that contains unique alignment start positions and attach 3 metadata columns to it: the number of reads, the query sequences, and the quality strings.

```

> gr_by_chrom <- lapply(seqlevels(gal),
+ function(seqname)
+ {
+   qseq_on_ref2 <- qseq_on_ref_by_chrom[[seqname]]
+   qual_on_ref2 <- qual_on_ref_by_chrom[[seqname]]
+   pos2 <- pos_by_chrom[[seqname]]
+   qseq_on_ref_per_pos <- split(qseq_on_ref2, pos2)
+   qual_on_ref_per_pos <- split(qual_on_ref2, pos2)
+   nread <- elementLengths(qseq_on_ref_per_pos)
+   gr_mcols <- DataFrame(nread=unname(nread),
+                         qseq_on_ref=unname(qseq_on_ref_per_pos),
+                         qual_on_ref=unname(qual_on_ref_per_pos))
+   gr <- GRanges(Rle(seqname, nrow(gr_mcols)),
+                 IRanges(as.integer(names(nread)), width=1))
+   mcols(gr) <- gr_mcols
+   seqlevels(gr) <- seqlevels(gal)
+   gr
+ })

```

Combine all the GRanges objects obtained in (4) in 1 big GRanges object:

```

> gr <- do.call(c, gr_by_chrom)
> seqinfo(gr) <- seqinfo(gal)

```

'gr' is a GRanges object that contains unique alignment start positions:

```

> gr[1:6]

```

GRanges object with 6 ranges and 3 metadata columns:

	seqnames	ranges	strand	nread	qseq_on_ref
	<Rle>	<IRanges>	<Rle>	<integer>	<DNAStrngSetList>
[1]	seq1	[ 1, 1]	*	1	CACTAGTGGCTCATTGTAATGTGTGGTTTAACTCG
[2]	seq1	[ 3, 3]	*	1	CTAGTGGCTCATTGTAATGTGTGGTTTAACTCGT
[3]	seq1	[ 5, 5]	*	1	AGTGGCTCATTGTAATGTGTGGTTTAACTCGTCC
[4]	seq1	[ 6, 6]	*	1	GTGGCTCATTGTAATTTTTTGTTTTAACTCTTCTCT
[5]	seq1	[ 9, 9]	*	1	
[6]	seq1	[13, 13]	*	2	



### 2.15.2 Remove low frequency reads

For each start position, remove reads with and under-represented sequence (e.g. threshold = 20% for the data used here which is low coverage). A unique number is assigned to each unique sequence. This will make future calculations easier and a little bit faster.

```
> qseq_on_ref <- mcols(gr)$qseq_on_ref
> tmp <- unlist(qseq_on_ref, use.names=FALSE)
> qseq_on_ref_id <- relist(match(tmp, tmp), qseq_on_ref)
```

Quick look at 'qseq\_on\_ref\_id': It's an IntegerList object with the same length and "shape" as 'qseq\_on\_ref'.

```
> qseq_on_ref_id
IntegerList of length 1934
[[1]] 1
[[2]] 2
[[3]] 3
[[4]] 4
[[5]] 5
[[6]] 6 7
[[7]] 8
[[8]] 9
[[9]] 10 11
[[10]] 12
...
<1924 more elements>
```

Remove the under represented ids from each list element of 'qseq\_on\_ref\_id':

```
> qseq_on_ref_id2 <- endoapply(qseq_on_ref_id,
+   function(ids) ids[countMatches(ids, ids) >= 0.2 * length(ids)])
```

Remove corresponding sequences from 'qseq\_on\_ref':

```
> tmp <- unlist(qseq_on_ref_id2, use.names=FALSE)
> qseq_on_ref2 <- relist(unlist(qseq_on_ref, use.names=FALSE)[tmp],
+   qseq_on_ref_id2)
```

### 2.15.3 Create a consensus sequence for each read group family

Compute 1 consensus matrix per chromosome:

```
> split_factor <- rep.int(seqnames(gr), elementLengths(qseq_on_ref2))
> qseq_on_ref2 <- unlist(qseq_on_ref2, use.names=FALSE)
> qseq_on_ref2_by_chrom <- splitAsList(qseq_on_ref2, split_factor)
> qseq_pos_by_chrom <- splitAsList(start(gr), split_factor)
> cm_by_chrom <- lapply(names(qseq_pos_by_chrom),
+   function(seqname)
+     consensusMatrix(qseq_on_ref2_by_chrom[[seqname]],
+       as.prob=TRUE,
+       shift=qseq_pos_by_chrom[[seqname]]-1,
+       width=seqlengths(gr)[[seqname]]))
> names(cm_by_chrom) <- names(qseq_pos_by_chrom)
```

'cm\_by\_chrom' is a list of consensus matrices. Each matrix has 17 rows (1 per letter in the DNA alphabet) and 1 column per chromosome position.

```
> lapply(cm_by_chrom, dim)
```



```
$seq1
[1] 18 1575
```

```
$seq2
[1] 18 1584
```

Compute the consensus string from each consensus matrix. We'll put "+" in the strings wherever there is no coverage for that position, and "N" where there is coverage but no consensus.

```
> cs_by_chrom <- lapply(cm_by_chrom,
+   function(cm) {
+     ## need to "fix" 'cm' because consensusString()
+     ## doesn't like consensus matrices with columns
+     ## that contain only zeroes (e.g., chromosome
+     ## positions with no coverage)
+     idx <- colSums(cm) == 0L
+     cm[ "+", idx] <- 1
+     DNASTring(consensusString(cm, ambiguityMap="N"))
+   })
```

The new consensus strings.

```
> cs_by_chrom
```

```
$seq1
1575-letter "DNASTring" instance
seq: NANTAGNNNCTCANTTTAAANNTTNTTTTTN...AATNATANNTTNTTNTTTNTCTGNAC+++++
```

```
$seq2
1584-letter "DNASTring" instance
seq: ++++++...NNNANANANANCTNNA+++++
```

## 2.16 How to compute binned averages along a genome

In some applications, there is the need to compute the average of a variable along a genome for a set of predefined fixed-width regions (sometimes called "bins"). One such example is coverage. Coverage is an `RleList` with one list element per chromosome. Here we simulate a coverage list.

```
> library(BSgenome.Scerevisiae.UCSC.sacCer2)
> set.seed(22)
> cov <- RleList(
+   lapply(seqlengths(Scerevisiae),
+     function(len) Rle(sample(-10:10, len, replace=TRUE))),
+   compress=FALSE)
> head(cov, 3)
```

RleList of length 3

```
$chrI
```

```
integer-Rle of length 230208 with 219146 runs
```

```
Lengths: 1 1 1 1 1 1 1 ... 1 1 1 1 1 1
Values : -4 -1 10 0 7 5 2 ... 4 -2 -8 1 -10 -8 -10
```

```
$chrII
```

```
integer-Rle of length 813178 with 774522 runs
```

```
Lengths: 1 1 1 1 1 1 1 ... 1 1 1 2 2 1 1
Values : -3 -6 -7 -3 9 -4 -10 ... -3 -4 -5 2 -2 -8 0
```

```
$chrIII
integer-Rle of length 316617 with 301744 runs
  Lengths:  1  1  1  1  1  1  1 ...  1  1  1  1  1  1  1
  Values  :  2 -3 -6  5  9  5  3 ...  4 -7 -10 -5 -10 -1 -3
```

Use the `tileGenome` function to create a set of bins along the genome.

```
> bins1 <- tileGenome(seqinfo(Scerevisiae), tilewidth=100,
+                    cut.last.tile.in.chrom=TRUE)
```

We define the following function to compute the binned average of a numerical variable defined along a genome.

Arguments:

```
'bins': a GRanges object representing the genomic bins.
        Typically obtained by calling tileGenome() with
        'cut.last.tile.in.chrom=TRUE'.
'numvar': a named RleList object representing a numerical
           variable defined along the genome covered by 'bins', which
           is the genome described by 'seqinfo(bins)'.
'mcolname': the name to give to the metadata column that will
            contain the binned average in the returned object.
```

The function returns 'bins' with an additional metadata column named 'mcolname' containing the binned average.

```
> binnedAverage <- function(bins, numvar, mcolname)
+ {
+   stopifnot(is(bins, "GRanges"))
+   stopifnot(is(numvar, "RleList"))
+   stopifnot(identical(seqlevels(bins), names(numvar)))
+
+   ## A version of viewMeans() that pads "out of limits" views with zeros.
+   viewMeans2 <- function(v) {
+     means <- viewMeans(v)
+     w0 <- width(v)
+     w1 <- width(trim(v))
+     means <- means * w1 / w0
+     means[w0 != 0L & w1 == 0L] <- 0
+     means
+   }
+
+   bins_per_chrom <- split(ranges(bins), seqnames(bins))
+   means_list <- lapply(names(numvar),
+     function(seqname) {
+       v <- Views(numvar[[seqname]], bins_per_chrom[[seqname]])
+       viewMeans2(v)
+     })
+   new_mcol <- unsplit(means_list, as.factor(seqnames(bins)))
+   mcols(bins)[[mcolname]] <- new_mcol
+   bins
+ }
```

Compute the binned average for 'cov':

```
> bins1 <- binnedAverage(bins1, cov, "binned_cov")
> bins1
```

GRanges object with 121639 ranges and 1 metadata column:

```
  seqnames      ranges strand |      binned_cov
   <Rle>      <IRanges> <Rle> |      <numeric>
```

```

[1] chrI [ 1, 100] * | -0.66
[2] chrI [101, 200] * | -0.05
[3] chrI [201, 300] * | -1.56
...
[121637] 2micron [6101, 6200] * | -0.25
[121638] 2micron [6201, 6300] * | -0.54
[121639] 2micron [6301, 6318] * | -0.4444444444444444
-----

```

seqinfo: 18 sequences (2 circular) from sacCer2 genome

The bin size can be modified with the `tilewidth` argument to `tileGenome`. For additional examples see `?tileGenome`.

### 3 Session Information

---

R version 3.2.2 (2015-08-14)

Platform: x86\_64-pc-linux-gnu (64-bit)

Running under: Ubuntu 14.04.3 LTS

locale:

```

[1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
[3] LC_TIME=en_US.UTF-8      LC_COLLATE=C
[5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
[7] LC_PAPER=en_US.UTF-8     LC_NAME=C
[9] LC_ADDRESS=C             LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

```

attached base packages:

```

[1] stats4      parallel    stats      graphics  grDevices  utils
[7] datasets   methods     base

```

other attached packages:

```

[1] BSgenome.Scerevisiae.UCSC.sacCer2_1.4.0
[2] KEGGgraph_1.26.0
[3] graph_1.46.0
[4] XML_3.98-1.3
[5] KEGG.db_3.1.2
[6] RSQLite_1.0.0
[7] DBI_0.3.1
[8] BSgenome.Hsapiens.UCSC.hg19_1.4.0
[9] BSgenome_1.36.3
[10] rtracklayer_1.28.10
[11] edgeR_3.10.2
[12] limma_3.24.15
[13] DESeq_1.20.0
[14] lattice_0.20-33
[15] locfit_1.5-9.1
[16] AnnotationHub_2.0.4
[17] TxDb.Athaliana.BioMart.plantsmart22_3.0.0
[18] TxDb.Hsapiens.UCSC.hg19.knownGene_3.1.2
[19] TxDb.Dmelanogaster.UCSC.dm3.ensGene_3.1.2
[20] GenomicFeatures_1.20.4
[21] AnnotationDbi_1.30.1
[22] Biobase_2.28.0

```

```
[23] GenomicAlignments_1.4.1
[24] Rsamtools_1.20.4
[25] Biostrings_2.36.4
[26] pasillaBamSubset_0.6.0
[27] XVector_0.8.0
[28] GenomicRanges_1.20.8
[29] GenomeInfoDb_1.4.2
[30] IRanges_2.2.7
[31] S4Vectors_0.6.6
[32] BiocGenerics_0.14.0
```

loaded via a namespace (and not attached):

```
[1] genefilter_1.50.0          VariantAnnotation_1.14.13
[3] splines_3.2.2             htmltools_0.2.6
[5] interactiveDisplayBase_1.6.1 survival_2.38-3
[7] BiocParallel_1.2.21      RColorBrewer_1.1-2
[9] lambda.r_1.1.7           stringr_1.0.0
[11] zlibbioc_1.14.0          futile.logger_1.4.1
[13] geneplotter_1.46.0       biomaRt_2.24.1
[15] httpuv_1.3.3             BiocInstaller_1.18.4
[17] curl_0.9.3               Rcpp_0.12.1
[19] xtable_1.7-4             annotate_1.46.1
[21] mime_0.4                 BiocStyle_1.6.0
[23] digest_0.6.8            stringi_0.5-5
[25] shiny_0.12.2             grid_3.2.2
[27] tools_3.2.2             bitops_1.0-6
[29] magrittr_1.5            RCurl_1.95-4.7
[31] futile.options_1.0.0     httr_1.0.0
[33] R6_2.1.1
```

## References

---

Michael Lawrence, Wolfgang Huber, Hervé Pagès, Patrick Aboyoun, Marc Carlson, Robert Gentleman, Martin T. Morgan, and Vincent J. Carey. Software for computing and annotating genomic ranges. *PLOS Computational Biology*, 4(3), 2013.