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Chapter 1

Getting started

1.1 Citation

```
citation("ggbio")
## To cite package 'ggbio' in publications use:
##
     Tengfei Yin, Dianne Cook and Michael Lawrence (2012): ggbio: an R
##
     package for extending the grammar of graphics for genomic data Genome
##
     Biology 13:R77
##
## A BibTeX entry for LaTeX users is
##
##
     @Article{,
       title = {ggbio: an R package for extending the grammar of graphics for genomic data},
       author = {Tengfei Yin and Dianne Cook and Michael Lawrence},
##
       journal = {Genome Biology},
       volume = \{13\},
       number = \{8\},
       pages = \{R77\},
       year = \{2012\},\
##
       publisher = {BioMed Central Ltd},
```

1.2 Introduction

ggbio is a Bioconductor package building on top of ggplot2(), leveraging the rich objects defined by Bioconductor and its statistical and computational power, it provides a flexible genomic visualization framework, extends the grammar of graphics into genomic data, try to delivers high quality, highly customizable graphics to the users.

What it features

- autoplot function provides ready-to-use template for Bioconductor objects and different types of data.
- flexible low level components to use grammar of graphics to build you graphics layer by layer.
- layout transformation, so you could generate circular plot, grandlinear plot, stacked overview more easily.
- flexible tracks function to bind any ggplot2(), ggbio based plots.

Chapter 2

Case study: building your first tracks

In this chapter, you will learn

- how to add ideogram track.
- How to add gene model track.
- how to add track for bam files to visualize coverage and mismatch summary.
- how to add track for vcf file to visualize the variants.

2.1 Add an ideogram track

Ideogram provides functionality to construct ideogram, check the manual for more flexible methods. We build genome *hg19*, *hg18*, *mm10*, *mm9* inside, so you don't have download it on the fly. When embed with tracks, ideogram show zoomed region highlights automatically. xlim has special function here, is too changed highlighted zoomed region on the ideogram.

```
library(ggbio)
p.ideo <- Ideogram(genome = "hg19")
p.ideo

chr1

library(GenomicRanges)
## special highlights instead of zoomin!
p.ideo + xlim(GRanges("chr2", IRanges(le8, le8+10000000)))</pre>
chr2
```

2.2 Add a gene model track

2.2.1 Introduction

Gene model track is one of the most frequently used track in genome browser, it is composed of genetic features CDS, UTR, introns, exons and non-genetic region. In *ggbio* we support three methods to make gene model track:

- OrganismDb object: recommended, support gene symbols and other combination of columns as label.
- TxDb object: don't support gene symbol labeling.
- GRangesList object: flexible, if you don't have annotation package available for the
 first two methods, you could prepare a data set parsed from gtf file, you can simply use
 it and plot it as gene model track.
- EnsDb object: supports gene symbol labeling, filtering etc.

2.2.2 Make gene model from OrganismDb object

OrganismDb object has a simpler API to retrieve data from different annotation resources, so we could label our transcripts in different ways

```
library(ggbio)
library(Homo.sapiens)
class(Homo.sapiens)

## [1] "OrganismDb"

## attr(,"package")

## [1] "OrganismDbi"

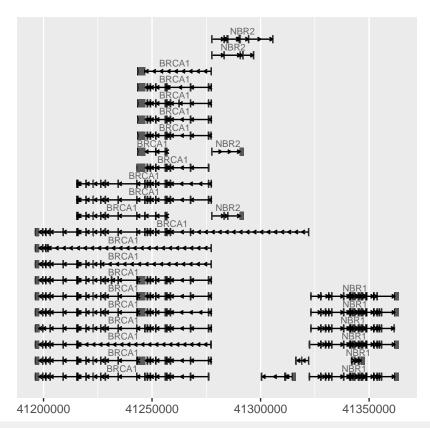
##

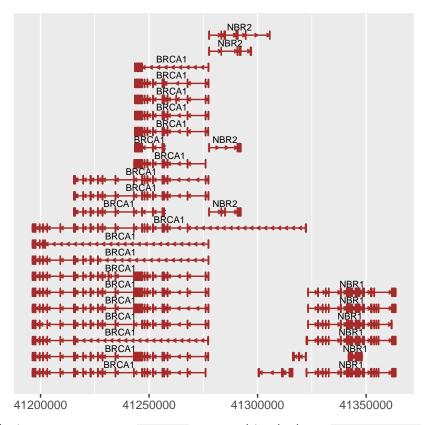
data(genesymbol, package = "biovizBase")

wh <- genesymbol[c("BRCA1", "NBR1")]

wh <- range(wh, ignore.strand = TRUE)

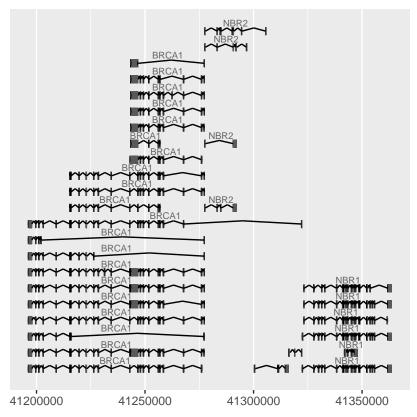
p.txdb <- autoplot(Homo.sapiens, which = wh)
p.txdb</pre>
```





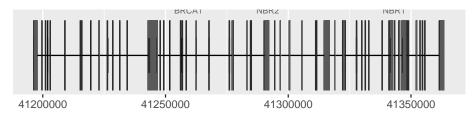
To change the intron geometry, use $\overline{gap.geom}$ to control it, check out $\overline{geom_alignment}$ for more control parameters.

autoplot(Homo.sapiens, which = wh, gap.geom = "chevron")



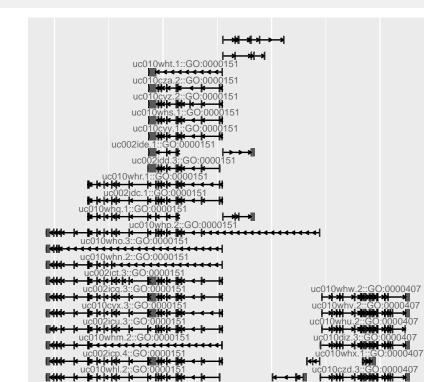
To collapse all features, use stat 'reduce'

```
autoplot(Homo.sapiens, which = wh, stat = "reduce")
```



Label could be turned off by setting it to FALSE, you could also use expression to make a flexible label combination from column names.

col	<pre>columns(Homo.sapiens)</pre>								
##	[1]	"ACCNUM"	"ALIAS"	"CDSCHROM"	"CDSEND"	"CDSID"			
##	[6]	"CDSNAME"	"CDSSTART"	"CDSSTRAND"	"DEFINITION"	"ENSEMBL"			
##	[11]	"ENSEMBLPROT"	"ENSEMBLTRANS"	"ENTREZID"	"ENZYME"	"EVIDENCE"			
##	[16]	"EVIDENCEALL"	"EXONCHROM"	"EXONEND"	"EXONID"	"EXONNAME"			
##	[21]	"EXONRANK"	"EXONSTART"	"EXONSTRAND"	"GENEID"	"GENENAME"			
##	[26]	"G0"	"GOALL"	"GOID"	"IPI"	"MAP"			
##	[31]	"OMIM"	"ONTOLOGY"	"ONTOLOGYALL"	"PATH"	"PFAM"			
##	[36]	"PMID"	"PROSITE"	"REFSEQ"	"SYMBOL"	"TERM"			
##	[41]	"TXCHROM"	"TXEND"	"TXID"	"TXNAME"	"TXSTART"			
##	[46]	"TXSTRAND"	"TXTYPE"	"UCSCKG"	"UNIGENE"	"UNIPROT"			



autoplot(Homo.sapiens, which = wh, columns = c("TXNAME", "GO"), names.expr = "TXNAME::GO")

2.2.3 Make gene model from TxDb object

41200000

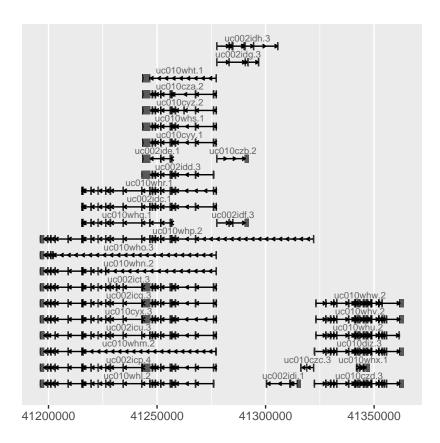
TxDb doesn't contain any gene symbol information, so we use tx_id as default for label.

41300000

41350000

```
library(TXDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
autoplot(txdb, which = wh)</pre>
```

41250000

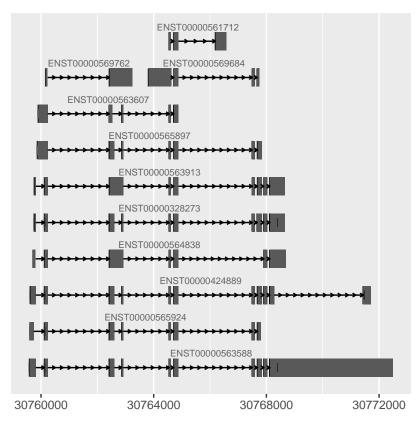


2.2.4 Make gene model from EnsDb object

An alternative source for gene models are the *EnsDb* objects from the *ensembldb* package that provide gene annotations provided from Ensembl. The *ensembldb* package provides a rich filtering system that allows to easily fetch specific information (genes/transcripts) from an *EnsDb*. The *EnsDb* objects provide gene symbol annotations in the column gene_name. Alternatively, we could use tx_id to label transcripts.

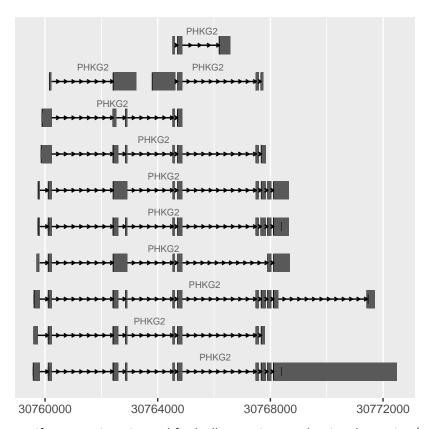
In the example below we plot the gene model of the gene PHKG2. We use a *GenenameFilter* to specify which gene we want to plot.

```
library(EnsDb.Hsapiens.v75)
ensdb <- EnsDb.Hsapiens.v75
autoplot(ensdb, GenenameFilter("PHKG2"))</pre>
```



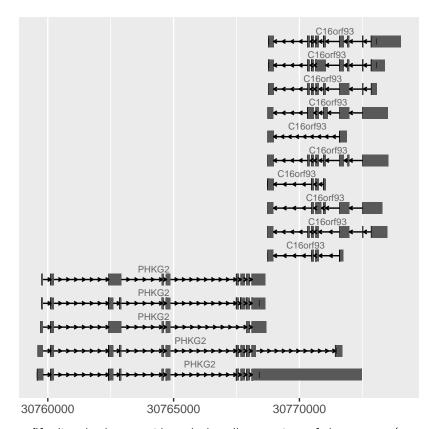
We can pass any filter class defined in the *AnnotationFilter* package with argument *which*. Alternatively we can combine filter classes using an *AnnotationFilterList* or we can pass a filter expression in form of a *formula*. Below we pass such a filter expression to the function.

```
autoplot(ensdb, ~ symbol == "PHKG2", names.expr="gene_name")
```



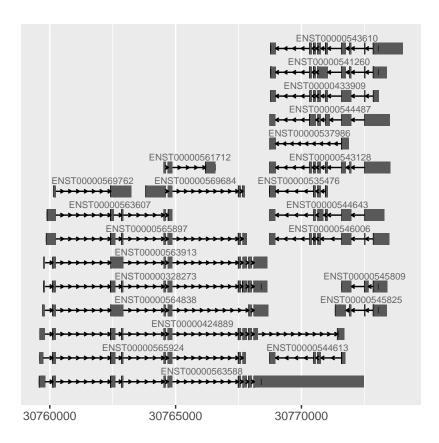
We could also specify a genomic region and fetch all transcripts overlapping that region (also partially, i.e. with a part of an intron or an exon).

```
## We specify "*" as strand, thus we query for genes encoded on both strands
gr <- GRanges(seqnames = 16, IRanges(30768000, 30770000), strand = "*")
autoplot(ensdb, GRangesFilter(gr), names.expr = "gene_name")</pre>
```



Also, we can spefify directly the gene ids and plot all transcripts of these genes (not only those overlapping with the region)

autoplot(ensdb, GeneIdFilter(c("ENSG00000196118", "ENSG00000156873")))



2.2.5 Make gene model from GRangesList object

Sometimes your gene model is not available as none of OrganismDb or TxDb object, it's may be stored in a table, you could simple parse it into a GRangeList object.

- each group indicate one transcripts
- names of group are shown as labels
- this object must has a column contains following key word: cds, exon, intron, and it's
 not case senstitive. use type to map this column. By default, we will try to parse 'type'
 column.

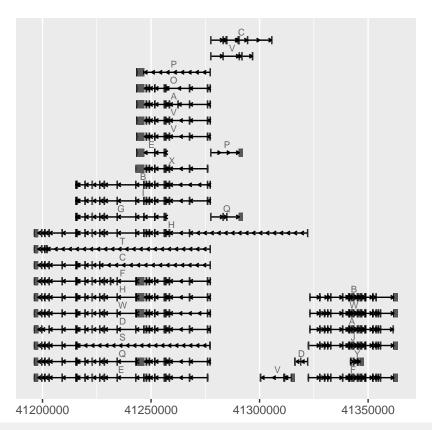
Let's make a sample GRangesList object which contains all information, and fake some labels.

```
library(biovizBase)
gr.txdb <- crunch(txdb, which = wh)
## change column to 'model'
colnames(values(gr.txdb))[4] <- "model"
grl <- split(gr.txdb, gr.txdb$tx_id)
## fake some randome names
names(grl) <- sample(LETTERS, size = length(grl), replace = TRUE)
grl
## GRangesList object of length 32:
## $Q</pre>
```

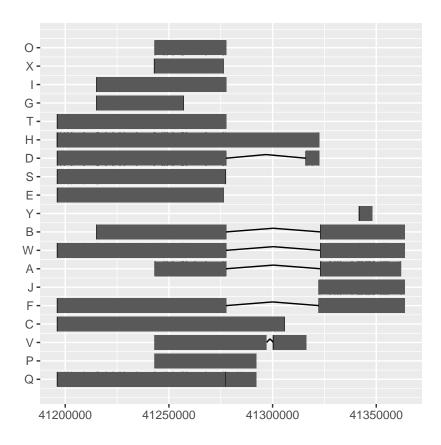
```
## GRanges object with 7 ranges and 4 metadata columns:
##
                            ranges strand |
        segnames
                                               tx_id
                                                        tx_name gene_id
                                                                            model
##
           <Rle>
                         <IRanges> <Rle> | <factor> <factor> <factor> <factor>
           chr17 41277600-41277787
##
     [1]
                                        + |
                                               61241 uc002idf.3
                                                                   10230
                                                                             exon
     [2]
           chr17 41283225-41283287
                                               61241 uc002idf.3
##
                                        + |
                                                                   10230
                                                                             exon
##
     [3]
           chr17 41284973-41285154
                                               61241 uc002idf.3
                                                                   10230
                                        + |
                                                                             exon
##
    [4]
           chr17 41290674-41292342
                                               61241 uc002idf.3
                                                                   10230
                                        + |
                                                                             exon
                                               61241 uc002idf.3
##
    [5]
           chr17 41277788-41283224
                                                                10230
                                        *
                                                                              gap
    [6]
           chr17 41283288-41284972
                                               61241 uc002idf.3
##
                                        *
                                                                  10230
                                                                              gap
##
     [7]
           chr17 41285155-41290673
                                        * |
                                               61241 uc002idf.3
                                                                   10230
                                                                              gap
##
  GRanges object with 3 ranges and 4 metadata columns:
##
##
        segnames
                            ranges strand | tx_id
                                                     tx_name gene_id model
##
           chr17 41277600-41277787
                                      + | 61242 uc010czb.2 10230 exon
     [1]
    [2]
           chr17 41290674-41292342
                                        + | 61242 uc010czb.2
                                                               10230 exon
    [3]
           chr17 41277788-41290673
                                        * | 61242 uc010czb.2
##
                                                               10230
                                                                       gap
##
## $V
  GRanges object with 9 ranges and 4 metadata columns:
##
        segnames
                            ranges strand | tx_id
                                                     tx_name gene_id model
##
           chr17 41277600-41277787
                                       + | 61243 uc002idg.3 10230
     [1]
                                                                      exon
##
    [2]
           chr17 41283225-41283287
                                        + | 61243 uc002idg.3
                                                               10230
                                                                      exon
##
    [3]
           chr17 41290674-41290939
                                        + | 61243 uc002idg.3
                                                             10230
                                                                      exon
                                        + | 61243 uc002idg.3
##
     [4]
           chr17 41291833-41292300
                                                               10230
                                                                      exon
##
    [5]
           chr17 41296745-41297125
                                       + | 61243 uc002idg.3 10230
                                                                      exon
##
    [6]
           chr17 41277788-41283224
                                        * | 61243 uc002idg.3 10230
                                                                       gap
           chr17 41283288-41290673
                                        * | 61243 uc002idg.3 10230
##
     [7]
                                                                       gap
##
     [8]
           chr17 41290940-41291832
                                        * | 61243 uc002idg.3
                                                               10230
                                                                       gap
##
    [9]
           chr17 41292301-41296744
                                       * | 61243 uc002idg.3
                                                               10230
                                                                       gap
##
## ...
## <29 more elements>
## -----
## seqinfo: 1 sequence from hg19 genome
```

We get our example data ready, it meets all requirements, to make it a gene model track it's pretty simple to use autoplot, but don't forget mapping because we changed our column names, asssume you store you model key words in column 'model'.

```
autoplot(grl, aes(type = model))
```



ggplot() + geom_alignment(grl, type = "model")



2.3 Add a reference track

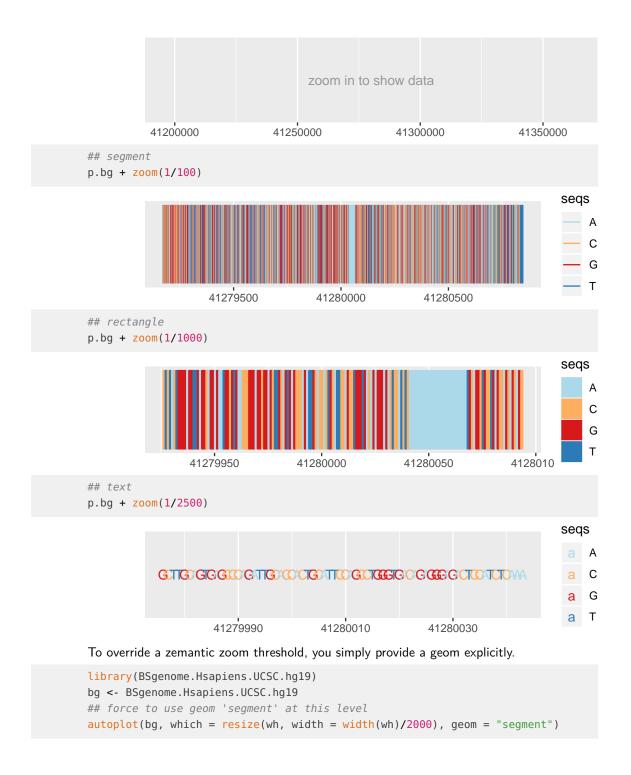
To add a reference track, we need to load a *BSgenome* object from the annotation package. You can choose to plot the sequence as *text*, *rect*, *segment*.

2.3.1 Semantic zoom

Here we introduce semantic zoom in *ggbio*, for some plots like reference sequence, we use pre-defined zoom level threshold to automatically assign geom to the track, unless the geom is explicitly specified. In the example below, when your region is too wide we show text 'zoom in to see text', when you zoom into different level, it shows you different details. **zoom** is a function we will introduce more in chapter 3 when we introduce more about navigation.

You can pass a zoom in factor into zoom function, if it's over 1 it's zooming out, if it's smaller than 1 it's zooming in.

```
library(BSgenome.Hsapiens.UCSC.hg19)
bg <- BSgenome.Hsapiens.UCSC.hg19
p.bg <- autoplot(bg, which = wh)
## no geom
p.bg</pre>
```



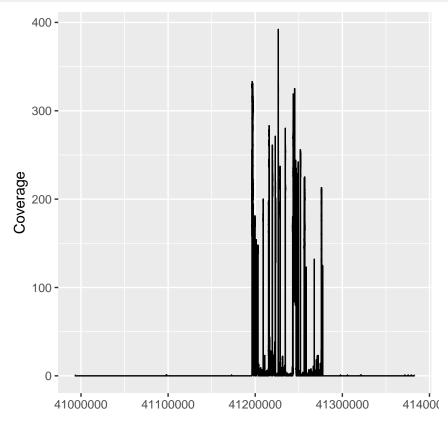
2.4 Add an alignment track

ggbio supports visuaization of alignemnts file stored in bam, autoplot method accepts

- bam file path (indexed)
- BamFile object
- GappedAlignemnt object

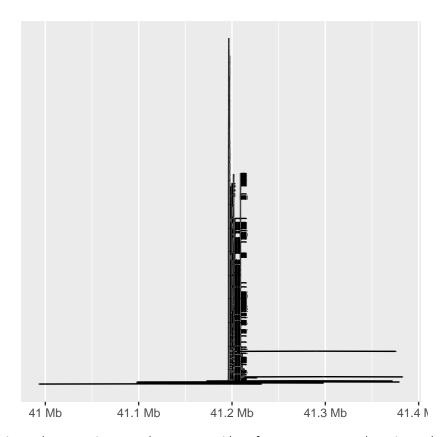
It's simple to just pass a file path to autoplot function, you can stream a chunk of region by providing 'which' parameter. Otherwise please use method 'estiamte' to show overall estiamted coverage.

```
fl.bam <- system.file("extdata", "wg-brcal.sorted.bam", package = "biovizBase")
wh <- keepSeqlevels(wh, "chr17")
autoplot(fl.bam, which = wh)</pre>
```



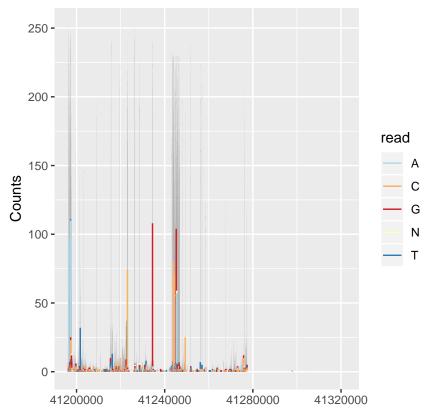
geom 'gapped pair' will show you alignments.

```
fl.bam <- system.file("extdata", "wg-brcal.sorted.bam", package = "biovizBase")
wh <- keepSeqlevels(wh, "chr17")
autoplot(fl.bam, which = resize(wh, width = width(wh)/10), geom = "gapped.pair")</pre>
```



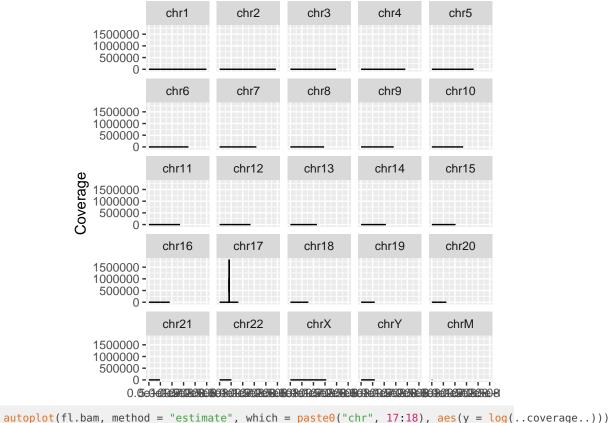
To show mismatch proportion, you have to provide reference sequence, the mismatched proportion is color coded in the bar chart.

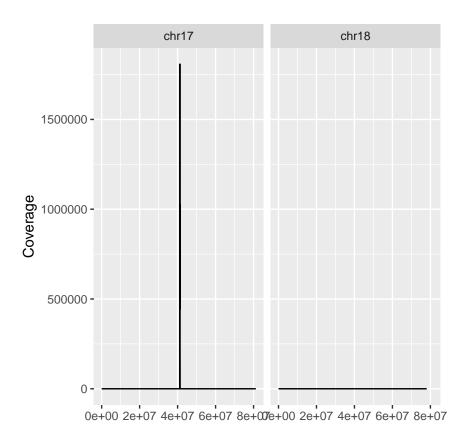
```
library(BSgenome.Hsapiens.UCSC.hg19)
bg <- BSgenome.Hsapiens.UCSC.hg19
p.mis <- autoplot(fl.bam, bsgenome = bg, which = wh, stat = "mismatch")
p.mis</pre>
```



To view overall estimated coverage distribution, please use method 'estiamte'. 'which' parameter also accept characters. And there is a hidden value called '..coverage..' to let you do simple transformation in aes().

autoplot(fl.bam, method = "estimate")





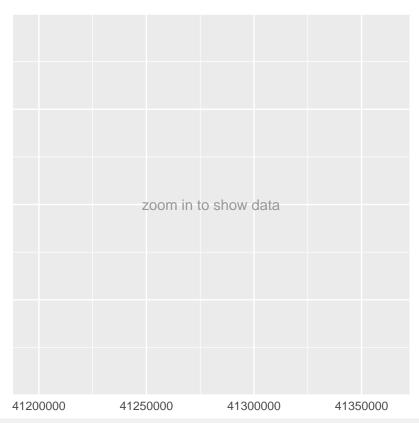
2.5 Add a variants track

This track is supported by semantic zoom.

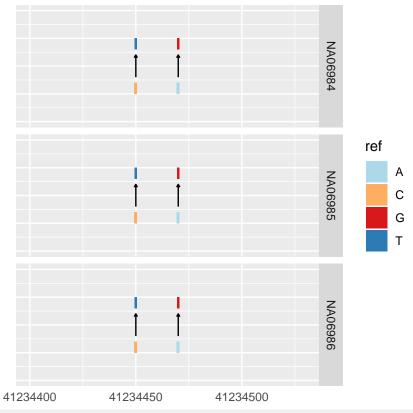
To view your variants file, you could

- Import it using package VariantAnntoation as VCF object, then use autoplot
- Convert it into *VRanges* object and use autoplot.
- Simply provide vcf file path in autoplot().

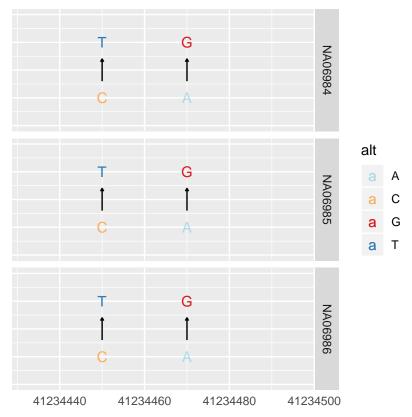
```
library(VariantAnnotation)
fl.vcf <- system.file("extdata", "17-1409-CEU-brca1.vcf.bgz", package="biovizBase")
vcf <- readVcf(fl.vcf, "hg19")
vr <- as(vcf[, 1:3], "VRanges")
vr <- renameSeqlevels(vr, value = c("17" = "chr17"))
## small region contains data
gr17 <- GRanges("chr17", IRanges(41234400, 41234530))
p.vr <- autoplot(vr, which = wh)
## none geom
p.vr</pre>
```



rect geom
p.vr + xlim(gr17)



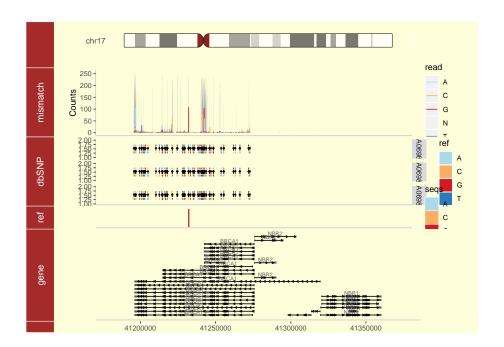
```
## text geom
p.vr + xlim(gr17) + zoom()
```



You can simply overide geom

```
autoplot(vr, which = wh, geom = "rect", arrow = FALSE)
```

2.6 Building your tracks



Chapter 3

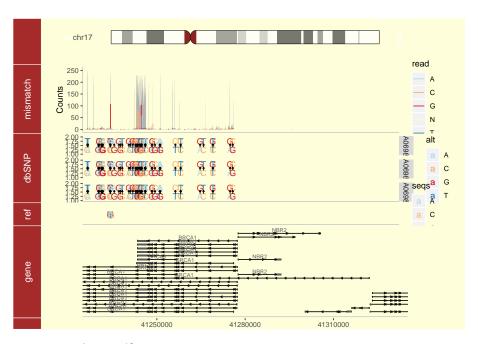
Simple navigation

We try to provide a simple navigation API for your plot, so you could zoom in and zoom out, or go through view chunks one by one.

- zoom: put a factor inside and you can zoom in or zoom out
- nextView: switch to next view
- prevView: switch to previous view

Navigation function also works for tracks plot too.

```
## zoom in
tks + zoom()
```



Try following command yourself.

```
## zoom in with scale
p.txdb + zoom(1/8)
## zoom out
p.txdb + zoom(2)
## next view page
p.txdb + nextView()
## previous view page
p.txdb + prevView()
```

Don't forget Ranges object (single row), so you could simply prepare a GRanges to store the region of interests and go through them one by one.

Chapter 4

Overview plots

Overview is a good way to show all events at the same time, give overall summary statiics for the whole genome.

In this chapter, we will introcue three different layouts that are used a lots in genomic data visualization.

4.1 how to make circular plots

4.1.1 Introduction

Circular view is a special layout in *ggbio*, this idea has been implemented in many different software, for example, the *Circos* project. However, we keep the grammar of graphics for users, so mapping variables to aesthetics is very easy, *ggbio* leverage the data structure defiend in *Bioconductor* to make this process as simple as possible.

4.1.2 Buidling circular plot layer by layer

Ok, let's start to process some raw data to the format we want. The data used in this study is from this a paper¹. In this tutorial, We are going to

¹http://www.nature.com/ng/journ

- 1. Visualize somatic mutation as segment.
- 2. Visualize inter, intro-chromosome rearrangement as links.
- 3. Visualize mutation score as point tracks with grid-background.
- 4. Add scale and ticks and labels.
- 5. To arrange multiple plots and legend. create multiple sample comparison.

All the raw data processed and stored in *GRanges* ready for use, you can simply load the sample data from *biovizBase*

```
data("CRC", package = "biovizBase")
```

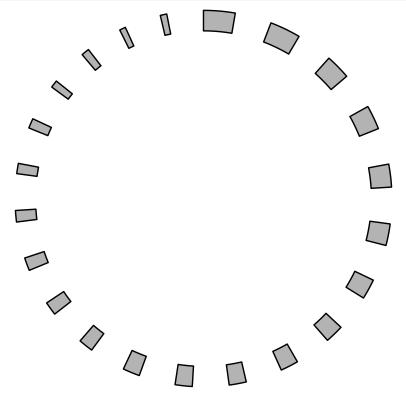
layout_circle is depreicated, because you have to set up radius and trackWidth manually with this function for creating circular plot.

We now present the new circle function, it accepts Granges object, and users don't have to specify radius, track width, you just add them one by one, it will be automatically created from innter circle to outside, unless you specify trackWidth and radius manually. To change default radius and trackWidth for all tracks, you simply put them in ggbio function.

- rule of thumb seqlengths, seqlevels and chromosomes names should be exactly the same.
- to use circle, you have to use ggbio constructor at the beginning instead of ggplot.

You can use autoplot to create single track easily like

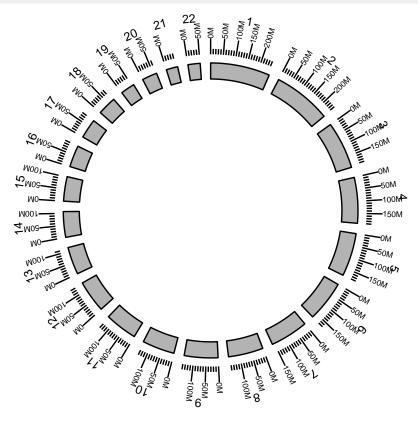
```
head(hg19sub)
## GRanges object with 6 ranges and 0 metadata columns:
##
         segnames
                        ranges strand
                    <IRanges> <Rle>
##
            <Rle>
##
     [1]
                1 1-249250621
##
     [2]
                2 1-243199373
                3 1-198022430
##
     [4]
                4 1-191154276
##
     [5]
                5 1-180915260
##
     [6]
                6 1-171115067
##
     seqinfo: 22 sequences from hg19 genome
##
autoplot(hg19sub, layout = "circle", fill = "gray70")
```



Hoever, the low level circle function leave you more flexibility to build circular plot one by one. Let's start to add tracks one by one.

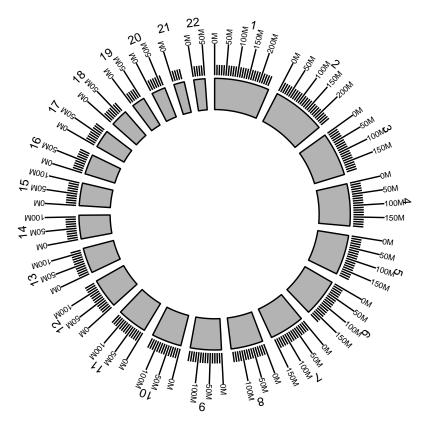
Let's use the same data to create ideogram, label and scale track, it layouts the circle by the order you created from inside to outside.

```
p <- ggbio() + circle(hg19sub, geom = "ideo", fill = "gray70") +
    circle(hg19sub, geom = "scale", size = 2) +
    circle(hg19sub, geom = "text", aes(label = seqnames), vjust = 0, size = 3)
p</pre>
```



To simply override the setting, you can do it globally in ggbio function or individually circle function by specifying parametters trackWidth and radius, you can also specify the global settin for buffer in between in ggbio like example below.

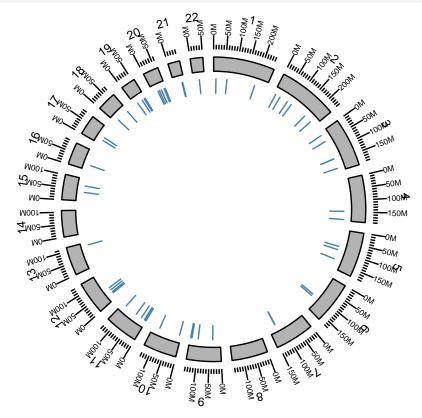
```
p <- ggbio(trackWidth = 10, buffer = 0, radius = 10) + circle(hg19sub, geom = "ideo", fill = "gray70") +
    circle(hg19sub, geom = "scale", size = 2) +
    circle(hg19sub, geom = "text", aes(label = seqnames), vjust = 0, size = 3)
p</pre>
```



Then we add a "rectangle" track to show somatic mutation, this will looks like vertical segments.

```
head(mut.gr)
## GRanges object with 6 ranges and 10 metadata columns:
         seqnames
                      ranges strand | Hugo_Symbol Entrez_Gene_Id
##
            <Rle> <IRanges> <Rle>
                                                         <integer> <factor>
                                          <factor>
##
                1 11003085
                                            TARDBP
                                                             23435
                                                                      Broad
     [1]
##
     [2]
                1 62352395
                                             INADL
                                                             10207
                                                                      Broad
##
     [3]
                1 194960885
                                               CFH
                                                              3075
                                                                      Broad
##
     [4]
                   10116508
                                              CYS1
                                                            192668
                                                                      Broad
##
     [5]
                2
                   33617747
                                           RASGRP3
                                                             25780
                                                                      Broad
##
                2 73894280
                                           C2orf78
                                                            388960
                                  +
                                                                      Broad
                       Strand Variant_Classification Variant_Type Reference_Allele
##
         NCBI_Build
          <integer> <factor>
##
                                             <factor>
                                                           <factor>
                                                                             <factor>
##
     [1]
                 36
                                                                SNP
                                                                                    G
                                             Missense
##
     [2]
                 36
                                             Missense
                                                                SNP
                                                                                    Т
     [3]
                 36
                                             Missense
                                                                SNP
                                                                                    G
##
##
     [4]
                  36
                                             Missense
                                                                SNP
                                                                                    C
##
                 36
                                                                SNP
                                                                                    C
     [5]
                                             Missense
##
                 36
                                             Missense
                                                                SNP
                                                                                    T.
##
         Tumor_Seq_Allele1 Tumor_Seq_Allele2
##
                   <factor>
                                      <factor>
##
     [1]
                          G
                                             Α
     [2]
                          Т
                                             G
##
                          G
##
     [3]
```

```
[4]
                          C
##
                          C
     [5]
                                             Т
##
                          Т
                                             C
##
     [6]
##
     seqinfo: 22 sequences from an unspecified genome
##
p <- ggbio() + circle(mut.gr, geom = "rect", color = "steelblue") +</pre>
    circle(hg19sub, geom = "ideo", fill = "gray70") +
    circle(hg19sub, geom = "scale", size = 2) +
 circle(hg19sub, geom = "text", aes(label = seqnames), vjust = 0, size = 3)
р
```



Next, we need to add some "links" to show the rearrangement, of course, links can be used to map any kind of association between two or more different locations to indicate relationships like copies or fusions. To create a suitable structure to plot, please use another *GRanges* to represent the end of the links, and stored as elementMetadata for the "start point" *GRanges*. Here we named it as "to.gr" and will be used later.

```
head(crc.gr)
## GRanges object with 6 ranges and 17 metadata columns:
##
         segnames
                      ranges strand | individual
                                                       str1
                                                                  class
                                                                             span
##
            <Rle> <IRanges> <Rle>
                                        <factor> <integer>
                                                              <factor> <numeric>
##
               18 56258628
                                            CRC-4
                                                                          2104165
     [1]
                                                          1 long_range
##
     [2]
               18
                   44496014
                                            CRC-4
                                                          1 long_range
                                                                         12947165
     [3]
               18
                   45023683
                                            CRC-4
                                                                         13356670
##
                                                          0 long_range
##
     [4]
                   52186319
                                            CRC-4
                                                               deletion
                                                                              268
```

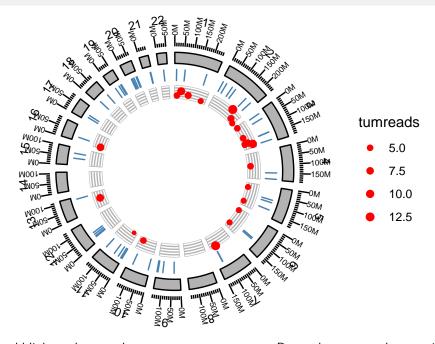
```
8 37328910
                                            CRC-4
                                                          0 inter_chr
                                                                              NaN
     [5]
##
     [6]
                8 35575394
                                  * |
                                            CRC-4
                                                          0 inter_chr
                                                                              NaN
##
          tumreads normreads
                                 gene1
                                           gene2
##
         <integer> <integer> <factor> <factor>
##
     [1]
               491
                            2
                                  MC4R
                                         ZCCHC2
##
     [2]
               265
                            0 KIAA0427
                                          CDH20
##
     [3]
               238
                            0
                                   DYM
                                         ZCCHC2
                94
                                          PXDNL
##
     [4]
                            0
                                 PXDNL
##
     [5]
                56
                                ZNF703
                                           PAK7
                            0
##
     [6]
                53
                            0
                                 UNC5D
                                        RALGAPB
##
                                             site1
##
                                         <factor>
##
                         IGR: 69Kb before MC4R(-)
     [1]
##
     [2] Intron of KIAA0427(+): 4Kb after exon 8
##
     [3]
            Intron of DYM(-): 14Kb after exon 13
     [4]
                      IGR: 208Kb before PXDNL(-)
##
     [5]
                      IGR: 344Kb before ZNF703(+)
##
##
     [6]
            Intron of UNC5D(+): 3Kb after exon 4
##
                                               site2
##
                                            <factor>
          Intron of ZCCHC2(+): 222bp before exon 4
##
     [1]
##
     [2]
                        IGR: 134Kb before CDH20(+)
##
     [3]
          Intron of ZCCHC2(+): 854bp before exon 9
##
     [4]
                        IGR: 208Kb before PXDNL(-)
              Intron of PAK7(-): 11Kb after exon 4
##
     [5]
##
     [6] Intron of RALGAPB(+): 839bp after exon 15
##
                                         fusion quality
                                                               score BPresult
##
                                       <factor> <numeric> <numeric> <integer>
##
     [1]
                                                         1
                                                                  491
                                               - 0.9944118 263.5191
                                                                              1
##
##
     [3] Protein fusion: in frame (ZCCHC2-DYM)
                                                                              1
##
     [4]
                                                                             -1
                                                         1
                                                                   94
##
     [5]
                                               - 0.9740215
                                                            54.54521
                                                                              1
     [6]
                                                                   53
                                                                              1
##
                               Antisense fusion
                                                         1
##
                   validation_result
                                             to.gr
                                                     rearrangements
##
                             <factor>
                                       <GRanges>
                                                        <character>
     [1] not_subjected_to_validation 18:58362793 intrachromosomal
##
     [2] not_subjected_to_validation 18:57443167 intrachromosomal
##
##
     [3]
                              somatic 18:58380361 intrachromosomal
     [4] not_subjected_to_validation 8:52186587 intrachromosomal
##
##
                              somatic 20:9561906 interchromosomal
##
     [6] not_subjected_to_validation 20:36595752 interchromosomal
##
     seginfo: 22 sequences from an unspecified genome
##
```

Here in this example, we use "intrachromosomal" to label rearrangement within the same chromosomes and use "interchromosomal" to label rearrangement in different chromosomes.

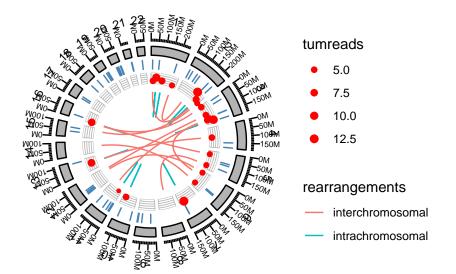
Get subset of links data for only one sample "CRC1"

```
gr.crc1 <- crc.gr[values(crc.gr)$individual == "CRC-1"]</pre>
```

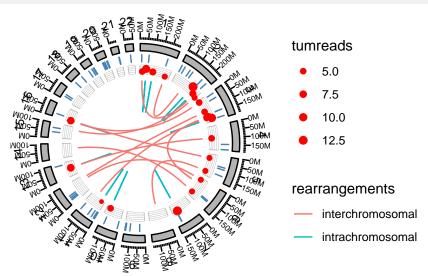
Ok, add a "point" track with grid background for rearrangement data and map 'y' to variable "score", map 'size' to variable "tumreads", rescale the size to a proper size range.



Finally, let's add links and map color to rearrangement types. Remember you need to specify 'linked.to' parameter to the column that contain end point of the data.



All those code could be simply constructed by following code



4.1.3 Complex arragnment of plots

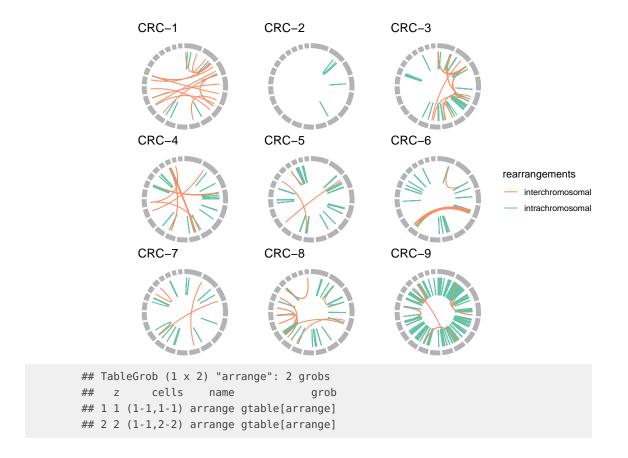
In this step, we are going to make multiple sample comparison, this may require some knowledge about package *grid* and *gridExtra*. We will introduce a more easy way to combine your graphics later after this.

We just want 9 single circular plots put together in one page, since we cannot keep too many tracks, we only keep ideogram and links. Here is one sample.

```
grl <- split(crc.gr, values(crc.gr)$individual)</pre>
## need "unit", load grid
library(grid)
crc.lst <- lapply(grl, function(gr.cur){</pre>
  print(unique(as.character(values(gr.cur)$individual)))
  cols <- RColorBrewer::brewer.pal(3, "Set2")[2:1]</pre>
  names(cols) <- c("interchromosomal", "intrachromosomal")</pre>
  p <- ggbio() + circle(gr.cur, geom = "link", linked.to = "to.gr",</pre>
                          aes(color = rearrangements)) +
                   circle(hg19sub, geom = "ideo",
                          color = "gray70", fill = "gray70") +
                   scale_color_manual(values = cols) +
                   labs(title = (unique(values(gr.cur)$individual))) +
                   theme(plot.margin = unit(rep(0, 4), "lines"))
})
## [1] "CRC-1"
## [1] "CRC-2"
## [1] "CRC-3"
## [1] "CRC-4"
## [1] "CRC-5"
## [1] "CRC-6"
## [1] "CRC-7"
## [1] "CRC-8"
## [1] "CRC-9"
```

We wrap the function in grid level to a more user-friendly high level function, called arrange GrobByParsingLegend. You can pass your ggplot2 graphics to this function , specify the legend you want to keep on the right, you can also specify the column/row numbers. Here we assume all plots we have passed follows the same color scale and have the same legend, so we only have to keep one legend on the right.

```
arrangeGrobByParsingLegend(crc.lst, widths = c(4, 1), legend.idx = 1, ncol = 3)
```



4.2 How to make grandlinear plots

4.2.1 Introduction

Let's use a subset of *PLINK* output (https://github.com/stephenturner/qqman/blob/master/plink.assoc.txt.gz) as our example test data.

```
snp <- read.table(system.file("extdata", "plink.assoc.sub.txt", package = "biovizBase"),</pre>
                  header = TRUE)
require(biovizBase)
gr.snp <- transformDfToGr(snp, seqnames = "CHR", start = "BP", width = 1)</pre>
head(gr.snp)
## GRanges object with 6 ranges and 10 metadata columns:
##
         seqnames
                                                                SNP
                              ranges strand |
                                                    CHR
##
            <Rle>
                          <IRanges> <Rle> | <integer>
                                                           <factor> <integer>
##
                4 10794096-10794099
                                                          rs9291494 10794096
     [1]
                                                      4
               14 55853742-55853755
##
     [2]
                                                     14
                                                          rs1152481 55853742
##
     [3]
                6 55188853-55188858
                                                      6
                                                         rs3134708 55188853
                    4146033-4146049
                                                          rs2325988
##
     [4]
               17
                                                      17
                                                                     4146033
               19 46089501-46089519
     [5]
                                                         rs8103444 46089501
```

```
[6]
                        107051695
                                       * | 1 rs12072065 107051695
                                                             Р
##
                               F_{-}U
                                         A2
                                                CHISQ
                                                                      0R
              A1
                      F_A
##
        <factor> <numeric> <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##
    [1]
                    0.3061
                             0.1341
                                          Α
                                                7.507 0.006147
##
    [2]
                    0.3542
                             0.2805
                                                1.103
                                                        0.2936
                                                                   1.407
               G
                                          Α
##
    [3]
               C
                     0.25
                             0.2875
                                          Α
                                               0.3135
                                                        0.5755
                                                                  0.8261
##
    [4]
               G
                   0.2551
                             0.2317
                                          Α
                                              0.1323
                                                        0.7161
                                                                   1.136
               C
                    0.398
                             0.2927
                                                                   1.597
##
    [5]
                                          Α
                                               2.175
                                                        0.1403
##
    [6]
               0
                        0
                                  0
                                          C
                                                 <NA>
                                                           <NA>
                                                                    <NA>
##
    seqinfo: 22 sequences from an unspecified genome; no seqlengths
## change the seqname order
require(GenomicRanges)
gr.snp <- keepSeqlevels(gr.snp, as.character(1:22))</pre>
seqlengths(gr.snp)
## 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
## need to assign seqlengths
data(ideoCyto, package = "biovizBase")
seqlengths(gr.snp) <- as.numeric(seqlengths(ideoCyto$hg18)[1:22])</pre>
## remove missing
gr.snp <- gr.snp[!is.na(gr.snp$P)]</pre>
## transform pvalue
values(gr.snp)$pvalue <- -log10(values(gr.snp)$P)</pre>
head(gr.snp)
## GRanges object with 6 ranges and 11 metadata columns:
##
        segnames
                           ranges strand | CHR
                                                         SNP
##
           <Rle>
                        <IRanges> <Rle> | <integer> <factor> <integer>
##
    [1]
              4 10794096-10794099
                                      *
                                                 4 rs9291494 10794096
    [2]
              14 55853742-55853755
                                                 14 rs1152481 55853742
                                      *
                                                 6 rs3134708 55188853
              6 55188853-55188858
##
    [3]
                                     * |
##
    [4]
              17
                 4146033-4146049
                                                 17 rs2325988
                                     * |
##
    [5]
              19 46089501-46089519
                                       *
                                                 19 rs8103444 46089501
               9 81517907-81517915
                                                  9
                                                       rs2591 81517907
##
    [6]
                                      * |
                                                CHISQ
##
              A1
                      F_A
                            F_U
                                         A2
                                                                      0R
##
        <factor> <numeric> <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
                                             7.507 0.006147
##
    [1]
               G
                   0.3061
                             0.1341
                                        Α
                                                                   2.848
                   0.3542
##
    [2]
               G
                             0.2805
                                          Α
                                                1.103
                                                        0.2936
                                                                   1.407
##
    [3]
               C
                     0.25
                             0.2875
                                          Α
                                               0.3135
                                                        0.5755
                                                                  0.8261
##
    [4]
               G
                   0.2551
                             0.2317
                                          A 0.1323
                                                        0.7161
                                                                  1.136
                                                2.175
##
    [5]
               C
                    0.398
                             0.2927
                                          Α
                                                        0.1403
                                                                   1.597
##
    [6]
                   0.1042 0.04878
                                          Τ
                                               1.872
                                                        0.1712
                                                                   2.267
##
                   pvalue
##
                <numeric>
##
    [1] 2.21133678687914
##
    [2] 0.532243948755967
    [3] 0.239954672034189
##
    [4] 0.145026326273583
##
    [5] 0.85294232897164
```

```
## [6] 0.766496239658866

## ------

## seqinfo: 22 sequences from an unspecified genome

## done
```

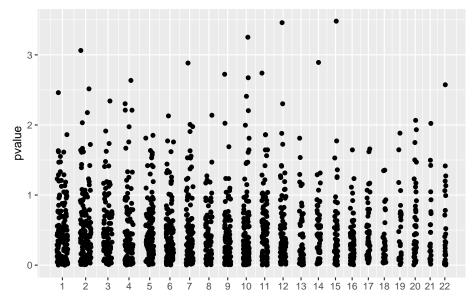
The data is ready, we need to pay attention

- if seqlengths is missing, we use data range, so the chromosome length is not accurate
- use seqlevel to control order of chromosome

4.2.2 Corrdinate genome

In autoplot, argument coord is just used to transform the data, after that, you can use it as common GRanges, all other geom/stat works for it.

```
autoplot(gr.snp, geom = "point", coord = "genome", aes(y = pvalue))
```



However, we recommend you to use more powerful function plotGrandLinear to generate manhattan plot introduced in next section.

4.2.3 Convenient plotGrandLinear function

For Manhattan plot, we have a function called plotGrandLinear. aes(y =) is required to indicate the y value, e.g. p-value.

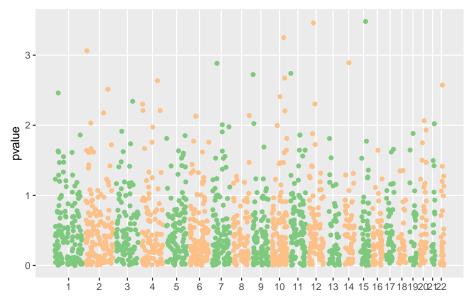
Color mapping is automatically figured out by ggbio following the rules

• if color present in aes(), like aes(color = seqnames), it will assume it's mapping to data column called 'seqnames'.

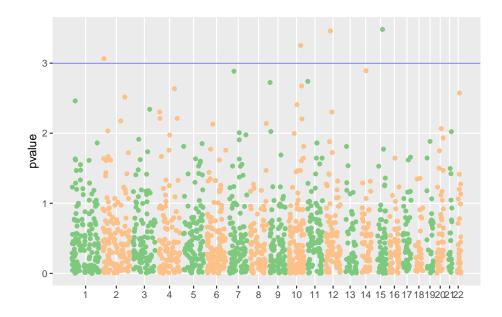
- if color is not wrapped in aes(), then this function will recylcle them to all chromosomes.
- if color is single character representing color, then just use one arbitrary color.

Let's test some examples for controling colors.

```
plotGrandLinear(gr.snp, aes(y = pvalue), color = c("#7fc97f", "#fdc086"))
```

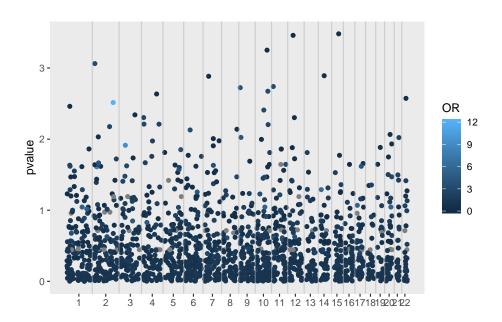


Let's add a cutoff line



Sometimes you use color to mapping other varibles so you may need a different to separate chromosomes.

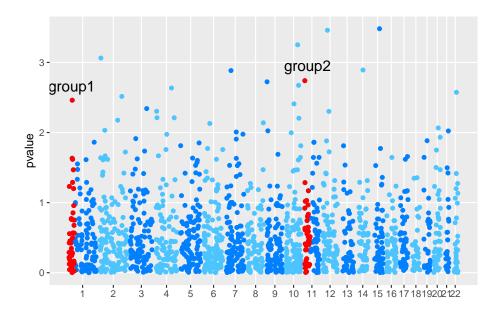
plotGrandLinear(gr.snp, aes(y = pvalue, color = OR), spaceline = TRUE, legend = TRUE)



4.2.4 How to highlight some points?

You can provide a highlight *GRanges*, and each row highlights a set of overlaped snps, and labeled by rownames or certain columns, there is more control in the function as parameters, with prefix highlight.*, so you could control color, label size and color, etc.

```
gro <- GRanges(c("1", "11"), IRanges(c(100, 2e6), width = 5e7))
names(gro) <- c("group1", "group2")
plotGrandLinear(gr.snp, aes(y = pvalue), highlight.gr = gro)</pre>
```



4.3 How to make stacked karyogram overview plots

4.3.1 Introduction

A karyotype is the number and appearance of chromosomes in the nucleus of a eukaryotic cell². It's one kind of overview when we want to show distribution of certain events on the genome, for example, binding sites for certain protein, even compare them across samples as example shows in this section.

²http://en.wikipedia.org/wiki/Kar

GRanges and Seqinfo objects are an ideal container for storing data needed for karyogram plot. Here is the strategy we used for generating ideogram templates.

- Althouth seqlengths is not required, it's highly recommended for plotting karyogram.
 If a GRanges object contains seqlengths, we know exactly how long each chromosome is, and will use this information to plot genome space, particularly we plot all levels included in it, NOT JUST data space.
- If a GRanges has no seqlengths, we will issue a warning and try to estimate the chromosome lengths from data included. This is NOT accurate most time, so please pay attention to what you are going to visualize and make sure set seqlengths before hand.

4.3.2 Create karyogram temlate

Let's first introduce how to use autoplot to generate karyogram graphic.

The most easy one is to just plot Seqinfo by using autoplot, if your *GRanges* object has seqinfo with seqlengths information. Then you add data layer later.

0 Mb

50 Mb

```
data(ideoCyto, package = "biovizBase")
autoplot(seqinfo(ideoCyto$hg19), layout = "karyogram")
                                                                   chr1
                                                                   chr2
                                                                   chr3
                                                                   chr4
                                                                   chr5
                                                                   chr6
                                                                   chr7
                                                                   chr8
                                                                   chr9
                                                                   chr10
                                                                   chr11
                                                                   chr12
                                                                   chr13
                                                                   chr14
                                                                   chr15
                                                                   chr16
                                                                   chr17
                                                                   chr18
                                                                   chr19
                                                                   chr20
                                                                   chr21
                                                                   chr22
                                                                   chrX
```

To show cytobands, your data need to have cytoband information, we stored some data for you, including hg19, hg18, mm10, mm9.

150 Mb

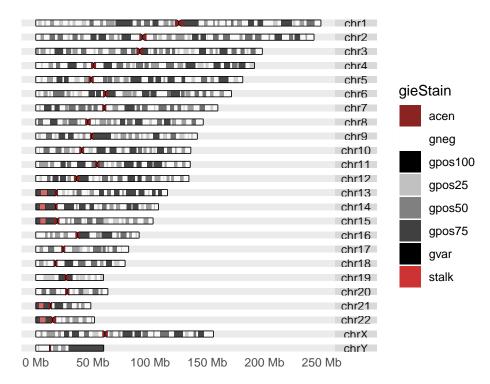
200 Mb

```
## turn on cytobands if present
biovizBase::isIdeogram(ideoCyto$hg19)
## [1] TRUE
autoplot(ideoCyto$hg19, layout = "karyogram", cytobands = TRUE)
```

100 Mb

chrY

250 Mb



To change order or only show a subset of the karyogram, you have to manipulate seqlevels, please check out manual for keepSeqlevels, seqlevels in *GenomicRanges* package for more information. Or you could read the example below.

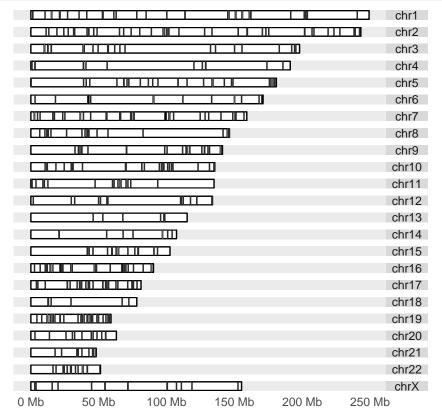
4.3.3 Add data on karyogram layout

If you have single data set stored as *GRanges* to show on a karyogram layout, autoplot function is enough for you to plot the data on it.

We use a default data in package *biovizBase*, which is a subset of RNA editing set in human. The data involved in this GRanges is sparse, so we cannot simply use it to make karyogram template, otherwise, the estimated chromosome lengths will be very rough and inaccurate. So what we need to do first is to *add seglength information to this object*.

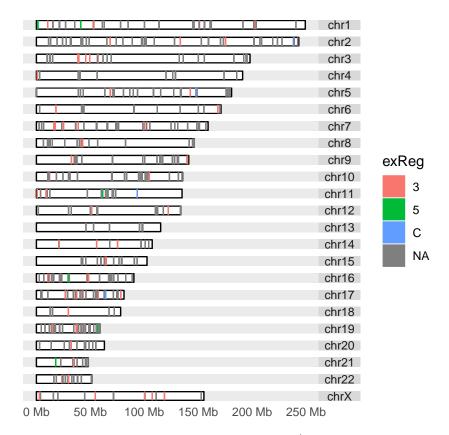
```
data(darned_hg19_subset500, package = "biovizBase")
dn <- darned_hg19_subset500</pre>
library(GenomicRanges)
seqlengths(dn)
    chr1 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19
                                                                          chr2 chr20
            NA
                   NA
                         NA
                                NA
                                      NA
                                             NA
                                                   NA
                                                         NA
                                                                NA
                                                                            NA
                                                                                   NA
                       chr4
                             chr5
                                          chr7
                                                 chr8
                                                       chr9
                                                             chrX
## chr21 chr22 chr3
                                    chr6
      NA
            NA
                   NA
                         NA
                                NA
                                      NA
                                             NA
                                                   NA
                                                         NA
                                                                NA
## add seqlengths
## we have seqlegnths information in another data set
seqlengths(dn) <- seqlengths(ideoCyto$hq19)[names(seqlengths(dn))]</pre>
## then we change order
```

```
dn <- keepSeqlevels(dn, paste0("chr", c(1:22, "X")))</pre>
seqlengths(dn)
                                                                                  chr8
##
        chr1
                   chr2
                             chr3
                                        chr4
                                                  chr5
                                                             chr6
                                                                       chr7
## 249250621 243199373 198022430 191154276 180915260 171115067 159138663 146364022
                  chr10
##
        chr9
                            chr11
                                       chr12
                                                 chr13
                                                            chr14
                                                                      chr15
                                                                                 chr16
## 141213431 135534747 135006516 133851895 115169878 107349540 102531392
                                                                             90354753
##
       chr17
                  chr18
                            chr19
                                       chr20
                                                 chr21
                                                            chr22
                                                                       chrX
    81195210
             78077248 59128983 63025520
                                              48129895
                                                        51304566 155270560
autoplot(dn, layout = "karyogram")
```



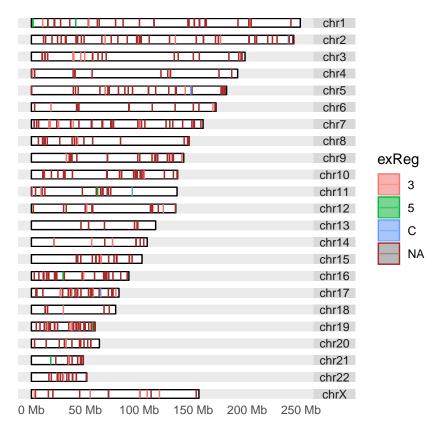
Then we take one step further, the power of *ggplot2* or *ggbio* is the flexible multivariate data mapping ability in graphics, make data exploration much more convenient. In the following example, we are trying to map a categorical variable 'exReg' to color, this variable is included in the data, and have three levels, '3' indicate 3' utr, '5' means 5' utr and 'C' means coding region. We have some missing values indicated as NA, in default, it's going to be shown in gray color, and keep in mind, since the basic geom(geometric object) is rectangle, and genome space is very large, so change both color/fill color of the rectangle to specify both border and filled color is necessary to get the data shown as different color, otherwise if the region is too small, border color is going to override the fill color.

```
## since default is geom rectangle, even though it's looks like segment
## we still use both fill/color to map colors
autoplot(dn, layout = "karyogram", aes(color = exReg, fill = exReg))
```



Or you can set the missing value to particular color yo u want (NA values is not shown on the legend).

```
## since default is geom rectangle, even though it's looks like segment
## we still use both fill/color to map colors
autoplot(dn, layout = "karyogram", aes(color = exReg, fill = exReg), alpha = 0.5) +
scale_color_discrete(na.value = "brown")
```



Well, sometimes we have too many values, we want to separate them by groups and show them at diffent height, below is a hack for that purpose and in next section, we will introduce a more flexible and general way to add data layer by layer.

Template chromosome y limits is [0, 10], that's why this hack works

4.3.4 Add more data using layout_karyogram function

In this section, a lower level function <code>layout_karyogram</code> is going to be introduced. This is convenient API for constructing karyogram plot and adding more data layer by layer. Function <code>ggplot</code> is just to create blank object to add layer on.

You need to pay attention to

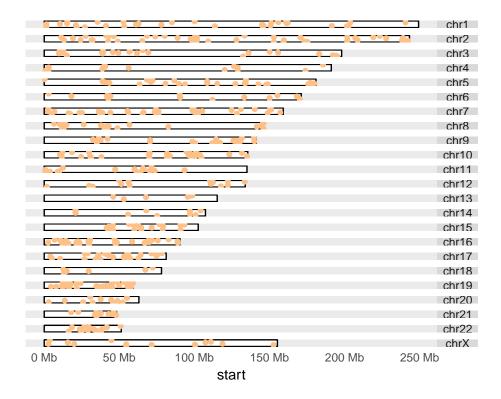
- when you add plots layer by layer, seqnames of different data must be the same to make sure the data are mapped to the same chromosome. For example, if you name chromosome following schema like chr1 and use just number 1 to name other data, they will be treated as different chromosomes.
- cannot use the same aesthetics mapping multiple time for different data. For example, if you have used aes(color =), for one data, you cannot use aes(color =) anymore for mapping variables from other add-on data, this is currently not allowed in ggplot2, even though you expect multiple color legend shows up, this is going to confuse people which is which. HOWEVER, color or fill without aes() wrap around, is allowed for any track, it's set single arbitrary color.
- Default rectangle y range is [0, 10], so when you add on more data layer by layer on existing graphics, you can use ylim to control how to normalize your data and plot it relative to chromosome space. For example, with default, chromosome space is plotted between y [0, 10], if you use ylim = c(10, 20), you will stack data right above each chromosomes and with equal width. For geom like 'point', which you need to specify 'y' value in aes(), we will add 5% margin on top and at bottom of that track.

Many times we overlay different datas sets, so let's break down the previous samples into 4 groups and treat them as different data and build them layer by layer, assign the color by hand. You could use ylim to control where they are ploted.

```
## prepare the data
dn3 <- dn.nona[dn.nona$exReg == '3']
dn5 <- dn.nona[dn.nona$exReg == '5']
dnC <- dn.nona[dn.nona$exReg == 'C']
dn.na <- dn[is.na(dn$exReg)]
## now we have 4 different data sets
autoplot(seqinfo(dn3), layout = "karyogram") +
    layout_karyogram(data = dn3, geom = "rect", ylim = c(0, 10/3), color = "#7fc97f") +
    layout_karyogram(data = dn5, geom = "rect", ylim = c(10/3, 10/3*2), color = "#beaed4") +
    layout_karyogram(data = dnC, geom = "rect", ylim = c(10/3*2, 10), color = "#fdc086") +
    layout_karyogram(data = dn.na, geom = "rect", ylim = c(10, 10/3*4), color = "brown")</pre>
```

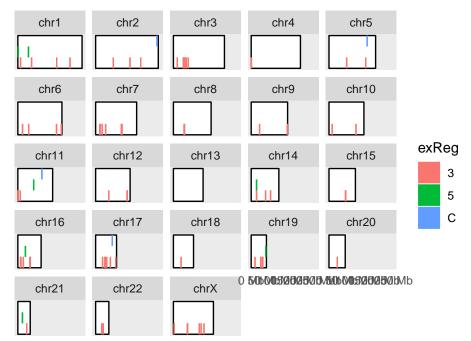
```
chr1
                                                     chr2
                                                     chr3
                                                     chr4
                                                     chr5
                                                     chr6
                                                     chr7
                                                     chr8
                                                     chr9
                                                     chr10
                                                     chr11
                                                     chr12
                                                     chr13
                                                     chr14
                                                     chr15
                                                     chr16
                                                     chr17
                                                     chr18
                                                     chr19
                                                     chr20
                                                     chr21
                                                     chr22
                                                    chrX
0 Mb
         50 Mb
                  100 Mb
                            150 Mb
                                     200 Mb
                                               250 Mb
                       start
```

What's more, you could even chagne the geom for those data



4.3.5 More flexible layout of karyogram

```
p.ylim + facet_wrap(~seqnames)
```



O **506005202500000520000052000052000052000**5000005

Chapter 5

Link ranges to your data

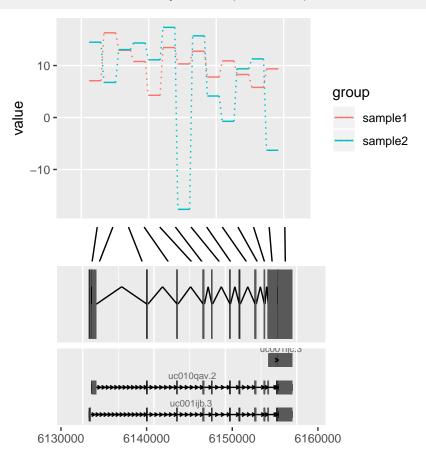
Plot GRanges object structure and linked to a even spaced paralell coordinates plot which represting the data in elementeMetadata.

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(ggbio)
data(genesymbol, package = "biovizBase")
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
model <- exonsBy(txdb, by = "tx")</pre>
model17 <- subsetByOverlaps(model, genesymbol["RBM17"])</pre>
exons <- exons(txdb)
exon17 <- subsetByOverlaps(exons, genesymbol["RBM17"])</pre>
## reduce to make sure there is no overlap
## just for example
exon.new <- reduce(exon17)
## suppose
values(exon.new)$sample1 <- rnorm(length(exon.new), 10, 3)</pre>
values(exon.new)$sample2 <- rnorm(length(exon.new), 10, 10)</pre>
values(exon.new)$score <- rnorm(length(exon.new))</pre>
values(exon.new)$significant <- sample(c(TRUE,FALSE), size = length(exon.new),replace = TRUE)</pre>
## data ready
exon.new
## GRanges object with 13 ranges and 4 metadata columns:
        segnames
                   ranges strand | sample1
                                                              sample2
                   <IRanges> <Rle> | <numeric>
          <Rle>
                                                            <numeric>
     [1] chr10 6130949-6131156 + | 7.0824594057456 14.4950794612897
     [2] chr10 6131309-6131934
                                 + | 16.2993120173228  6.75417925416136
                                 [3] chr10 6139011-6139151
                                 [4] chr10 6143234-6143350
     [5] chr10 6146894-6147060
                                 + | 4.27381907657722 11.1059925521905
         [9]
   [10]
         chr10 6155471-6155544
                                + | 10.8948095510498 -0.730753196306706
         chr10 6156012-6156110 + | 8.27815196098471 9.37217284666189
   [11]
          chr10 6156126-6157274 + | 5.79685247561179 11.2788341340633
    [12]
    [13]
           chr10 6157416-6159422
                                 + | 9.36487423975036 -6.30790027266965
```

```
##
                         score significant
##
                                  <logical>
                     <numeric>
            0.057026585377773
                                       TRUE
##
      [1]
##
           -0.556838987578142
                                      FALSE
##
      [3]
            -0.16908244006771
                                       TRUE
##
      [4]
           -0.769317291907936
                                       TRUE
##
      [5] -0.0167095639746955
                                       TRUE
##
                                       . . .
                                       TRUE
##
      [9]
            -1.07108720662701
##
     [10]
           0.0835202078948099
                                       TRUE
##
            0.561169399734248
                                       TRUE
     [11]
##
             2.89295289995145
                                       TRUE
           -0.778042015678011
                                      FALSE
##
     [13]
##
     seqinfo: 93 sequences (1 circular) from hg19 genome
##
```

Make the plots, you can pass a list of annotation tracks too.

```
p17 <- autoplot(txdb, genesymbol["RBM17"])
plotRangesLinkedToData(exon.new, stat.y = c("sample1", "sample2"), annotation = list(p17))</pre>
```



For more information, check the manual.

Chapter 6

Miscellaneous

Every plot object produced by ggplot2 is essentially a ggplot2 object, so you could use all the tricks you know with ggplot2 on ggbio plots too, including scales, colors, themes, etc.

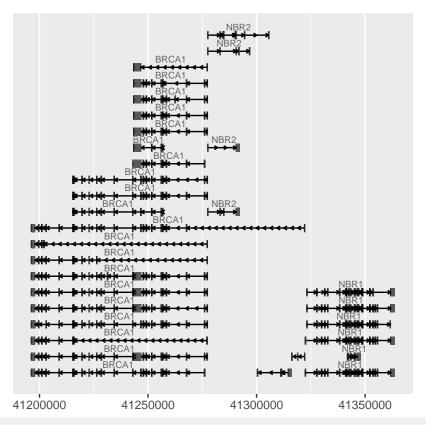
6.1 Themes

In ggbio, we developed some more themes to make things easier.

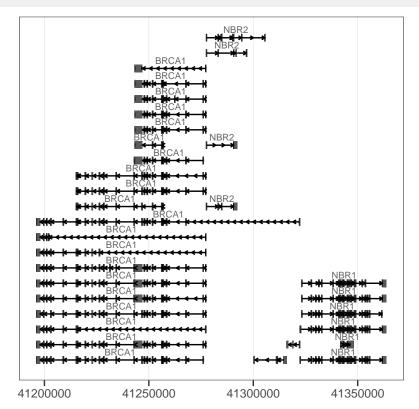
6.1.1 Plot theme

Plot level themes are like any other themes defined in ggplot2, simply apply it to a plot.

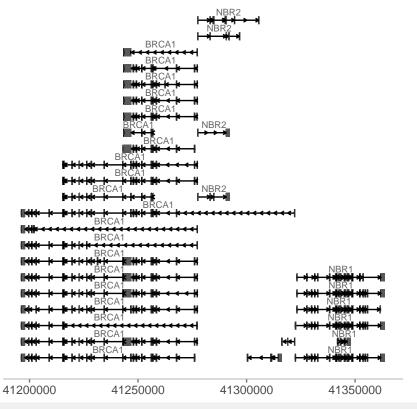
p.txdb



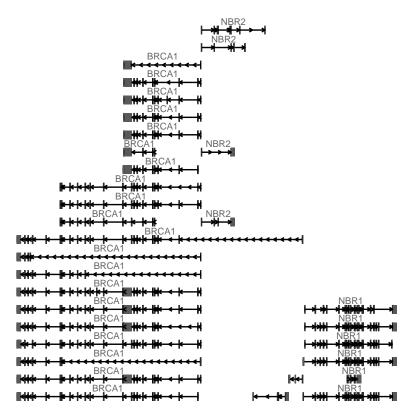
p.txdb + theme_alignment()



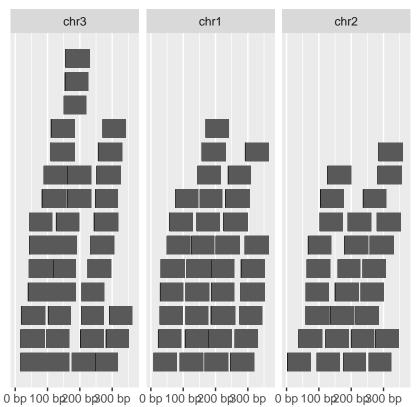
p.txdb + theme_clear()

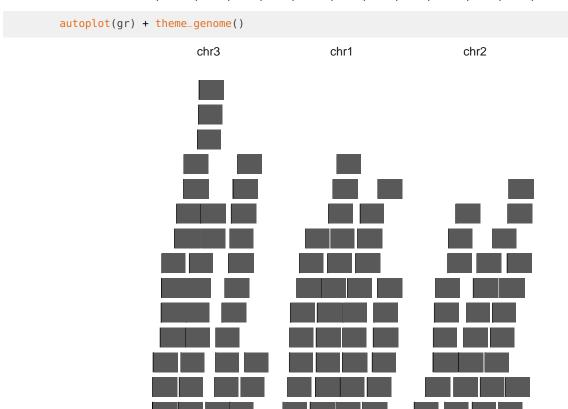


p.txdb + theme_null()



When you have multiple chromosomes encoded in seqnames, you could use theme_genome to make a 'fake' linear view of genome coordinates quickly by applying this theme, because it's not equal to chromosome lengths, it's simply





6.1.2 Track theme

Track level themes are more complex, it controls whole looking of the tracks, it's essentially a theme object with some attributes controlling the tracks appearance.

See how we make a template, you could customize in the same way

```
theme_tracks_sunset

## function (bg = "#fffedb", alpha = 1, ...)

## {

## res <- theme_clear(grid.x.major = FALSE, ...)

## attr(res, "track.plot.color") <- sapply(bg, scales::alpha,

## alpha)

## attr(res, "track.bg.color") <- bg

## attr(res, "label.text.color") <- "white"

## attr(res, "label.bg.fill") <- "#a52a2a"

## res

## }

## <bytecode: 0x575545e8>

## <environment: namespace:ggbio>
```

The attributes you could control is basically passed to tracks() constructor, including

label.bg.color	character
label.bg.fill	character
label.text.color	character
label.text.cex	numeric
label.text.angle	numeric
track.plot.color	character_OR_NULL
track.bg.color	character_OR_NULL
label.width	unit

Table 6.1: tracks attributes

Chapter 7

Session Information

```
sessionInfo()
## R version 3.5.1 Patched (2018-07-12 r74967)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.5 LTS
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.8-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.8-bioc/R/lib/libRlapack.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8
                                 LC_NUMERIC=C
                              LC_COLLATE=C
## [3] LC_TIME=en_US.UTF-8
## [5] LC_MONETARY=en_US.UTF-8 LC_MESSAGES=en_US.UTF-8
                               LC_NAME=C
## [7] LC_PAPER=en_US.UTF-8
## [9] LC_ADDRESS=C
                                 LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
## attached base packages:
## [1] grid stats4
                           parallel stats graphics grDevices utils
## [8] datasets methods
## other attached packages:
## [1] VariantAnnotation_1.28.0
## [2] Rsamtools_1.34.0
## [3] SummarizedExperiment_1.12.0
## [4] DelayedArray_0.8.0
## [5] BiocParallel_1.16.0
## [6] matrixStats_0.54.0
## [7] BSgenome.Hsapiens.UCSC.hg19_1.4.0
## [8] BSgenome_1.50.0
## [9] rtracklayer_1.42.0
## [10] Biostrings_2.50.0
## [11] XVector_0.22.0
## [12] biovizBase_1.30.0
```

```
## [13] EnsDb.Hsapiens.v75_2.99.0
## [14] ensembldb_2.6.0
## [15] AnnotationFilter_1.6.0
## [16] Homo.sapiens_1.3.1
## [17] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
## [18] org.Hs.eg.db_3.7.0
## [19] GO.db_3.7.0
## [20] OrganismDbi_1.24.0
## [21] GenomicFeatures_1.34.0
## [22] AnnotationDbi_1.44.0
## [23] Biobase_2.42.0
## [24] GenomicRanges_1.34.0
## [25] GenomeInfoDb_1.18.0
## [26] IRanges_2.16.0
## [27] S4Vectors_0.20.0
## [28] ggbio_1.30.0
## [29] ggplot2_3.1.0
## [30] BiocGenerics_0.28.0
## [31] knitr_1.20
## loaded via a namespace (and not attached):
                                 bitops_1.0-6
## [1] ProtGenerics_1.14.0
                                                           bit64_0.9-7
## [4] RColorBrewer_1.1-2
                                 progress_1.2.0
                                                           httr_1.3.1
## [7] rprojroot_1.3-2
                                 tools_3.5.1
                                                           backports_1.1.2
## [10] R6_2.3.0
                                 rpart_4.1-13
                                                           Hmisc_4.1-1
## [13] DBI_1.0.0
                                 lazyeval_0.2.1
                                                           colorspace_1.3-2
## [16] nnet_7.3-12
                                 withr_2.1.2
                                                           tidyselect_0.2.5
## [19] gridExtra_2.3
                                 prettyunits_1.0.2
                                                           GGally_1.4.0
## [22] curl_3.2
                                 bit_{-}1.1-14
                                                           compiler_3.5.1
## [25] graph_1.60.0
                                 htmlTable_1.12
                                                           labeling_0.3
## [28] scales_1.0.0
                                 checkmate_1.8.5
                                                           RBGL_1.58.0
## [31] stringr_1.3.1
                                 digest_0.6.18
                                                           foreign_0.8-71
## [34] rmarkdown_1.10
                                 base64enc_0.1-3
                                                           dichromat_2.0-0
## [37] pkgconfig_2.0.2
                                 htmltools_0.3.6
                                                           highr_0.7
## [40] htmlwidgets_1.3
                                 rlang_0.3.0.1
                                                           rstudioapi_0.8
## [43] RSQLite_2.1.1
                                 bindr_0.1.1
                                                           acepack_1.4.1
## [46] dplyr_0.7.7
                                 RCurl_1.95-4.11
                                                           magrittr_1.5
## [49] GenomeInfoDbData_1.2.0
                                 Formula_1.2-3
                                                           Matrix_1.2-14
## [52] Rcpp_0.12.19
                                 munsell_0.5.0
                                                           stringi_1.2.4
## [55] yaml_2.2.0
                                 zlibbioc_1.28.0
                                                           plyr_1.8.4
## [58] blob_1.1.1
                                 crayon_1.3.4
                                                           lattice_0.20-35
## [61] splines_3.5.1
                                 hms_{-}0.4.2
                                                           pillar_1.3.0
## [64] reshape2_1.4.3
                                 biomaRt_2.38.0
                                                           XML_3.98-1.16
## [67] glue_1.3.0
                                 evaluate_0.12
                                                           latticeExtra_0.6-28
## [70] data.table_1.11.8
                                 BiocManager_1.30.3
                                                           gtable_0.2.0
## [73] purrr_0.2.5
                                 reshape_0.8.8
                                                           assertthat_0.2.0
## [76] survival_2.43-1
                                 tibble_1.4.2
                                                           GenomicAlignments_1.18.0
## [79] memoise_1.1.0
                                 bindrcpp_0.2.2
                                                           cluster_2.0.7-1
## [82] BiocStyle_2.10.0
```