# An introduction of drawing genomic figures with *circlize*

Zuguang Gu <z.gu@dkfz.de>

September 1, 2014

### 1 Introduction

Since circos plots are mostly used in genomic research, the *circlize* package particularly provides functions which focus on genomic plots. These functions are synonymous to the basic circos graphical functions but expect special format of input data:

- circos.genomicTrackPlotRegion: create a new track and add graphics.
- circos.genomicPoints: low-level function, add points
- circos.genomicLines: low-level function, add lines
- circos.genomicRect: low-level function, add rectangles
- circos.genomicText: low-level function, add text
- circos.genomicLink: add links

The genomic functions are implemented by basic circos functions (e.g. circos.trackPlotRegion, circos.points), thus, you can customize your own plots by both genomic functions and basic circos functions.

# 2 Input data

Genomic circos functions expect input data as a data frame or a list of data frames in which there are at least three columns. The first column is genomic category (e.g. chromosome), the second column is the start positions in the genomic category and the third column is the end positions. Following columns are optional where numeric values or other related values are stored. Such data structure is known as *BED* format and is broadly used in genomic research.

circlize provides a simple function generateRandomBed which can generate random genomic data. Positions are uniformly generated from human genome. In the function, nr and nc are number of rows and numeric columns that users want. Please note nr are not exactly the same to the number of rows which are returned by the function. fun is a self-defined function to generate random values.

```
> bed = generateRandomBed()
> bed = generateRandomBed(nr = 200, nc = 4)
> bed = generateRandomBed(fun = function(k) runif(k))
```

# 3 Initialize with cytoband data

Similar as general circos plots, the first step is to initialize the plot with genomic categories. In most situations, genomic categories are measured by chromosomes. The easiest way is to used circos.initializeWithIdeogram:

```
> circos.initializeWithIdeogram()
```

By default, the function will initialize the plot with cytoband data of hg19. You can also use your own cytoband data by specifying the path of your cytoband file (no matter compressed or not) or providing your cytoband data as a data frame. An example for cytoband file is http://hgdownload.cse.ucsc.edu/goldenpath/hg19/database/cytoBand.txt.gz.

If you want to read cytoband data from file, please explicitly specify colClasses arguments and set the class of position columns as numeric. The reason is since positions are represented as integers, read.table would treat those numbers as integer by default. In initialization of circos plot, *circlize* needs to calculate the summation of all chromosome lengths. The sumation of such large integers would throw error of data overflow.

For simple use, users can also specify abbreviation of the species and the function will download cytoband file from UCSC server automatically (If it exists in UCSC).

```
> circos.initializeWithIdeogram(species = "hg18")
> circos.initializeWithIdeogram(species = "mm10")
```

By default, the function will use all chromosomes which are available in cytoband data to initialize the circos plot. Users can also choose a subset of chromosomes by specifying chromosome.index. Please note this argument is only used for subsetting but not for ordering.

```
> circos.initializeWithIdeogram(chromosome.index = c("chr1", "chr2"))
```

Initialization step is important for circos plot. It controls the order of chromosomes which is going to be shown on the circle. There are several ways to control the order. If cytoband is provided as a data frame, and if the first column is a factor, the order of chromosomes would be levels(cytoband[[1]]). If the first column is not a factor, the order of chromosomes would be unique(cytoband[[1]]). If sort.chr is set to TRUE, chromosomes will be sorted (first by numbers then by letters).

If cytoband is specified as a file path, or species is specified, the order of chromosomes depends on the original order in the source file.

circlize provides a function read.cytoband which can read/download and process cytoband data. In fact, circos.initializeWithIdeogram calls read.cytoband internally. Please refer to the help page of the function for more details.

```
> cytoband = read.cytoband()
> cytoband = read.cytoband(file)
> cytoband = read.cytoband(df)
> cytoband = read.cytoband(species)
```

After the intialization of the circos plot, the function will additionally create a track where there are genomic axes and chromosome names, and create another track where there is an ideogram. plotType can be used to control which graphics need to be plotted.

```
> circos.initializeWithIdeogram(plotType = c("axis", "labels"))
> circos.initializeWithIdeogram(plotType = NULL)
> # height of these pre-defined tracks can be set
> circos.initializeWithIdeogram(track.height = 0.05)
> circos.initializeWithIdeogram(ideogram.height = 0.05)
```

Similar as general circos plot, the layout of circos plot can be controlled by circos.par

```
> circos.par("start.degree" = 90)
> circos.initializeWithIdeogram()
> circos.clear()
> circos.par("gap.degree" = rep(c(2, 4), 11))
> circos.initializeWithIdeogram()
> circos.clear()
```

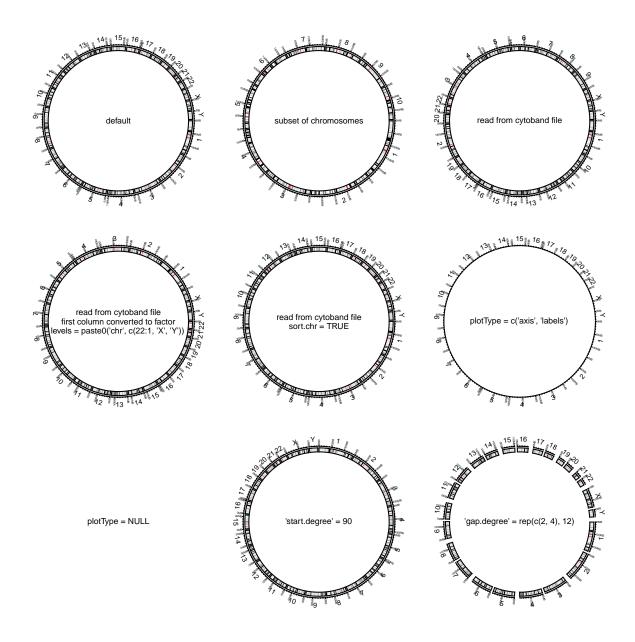


Figure 1: Different ways to initialize genomic circos plot

Please refer to figure 1 for examples of different ways to initialize genomic circos plot.

# 4 Customize ideogram

The default style of ideogram can be changed. If plotType is set to NULL, circos layout is only initialized but nothing is plotted. Then several new tracks can be created and new style of ideogram can be added by users. In the following example, we use different colors to represent chromosomes and change the style of chromosome names (figure 2).

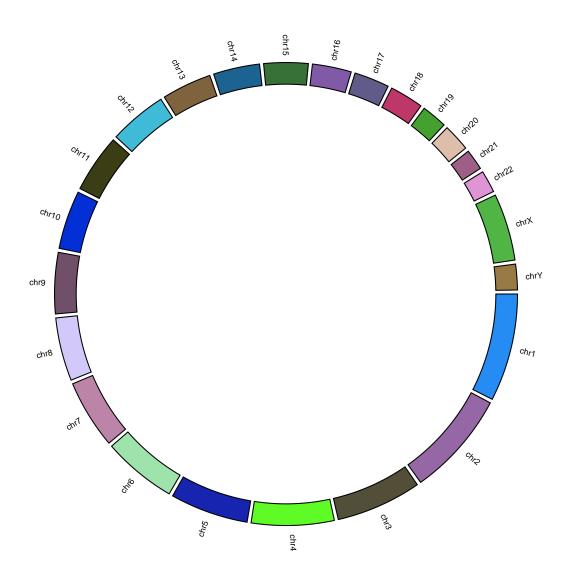


Figure 2: Customize ideogram

# 5 Initialize with general genomic category

Cytoband data is just a special case of genomic category. circos.genomicInitialize can initialize circos plot with any kind of genomic categories. In fact, circos.initializeWithIdeogram is implemented by circos.genomicInitialize. The input data for the function is a data frame with at least

three columns. The first column is genomic category (for cytoband data, it is chromosome name), and the next two columns are genomic positions in each genomic category. The range of each category will be inferred from the minimum position and the maximum position in corresponding category. In the following example, a circos plot is initialized with three genes.

```
> df = data.frame(
+    name = c("TP53", "TP63", "TP73"),
+    start = c(7565097, 189349205, 3569084),
+    end = c(7590856, 189615068, 3652765))
> circos.genomicInitialize(df)
```

Note it is not necessary that the record for each gene is one row.

As explained in previous section, the order of genomic categies is controlled by the first column of df which depends whether it is a factor or a simple vector. Alternative names can be assigned to each category and the order of alternative names correspond to the order of genomic categories.

```
> circos.genomicInitialize(df)
> circos.genomicInitialize(df, sector.names = c("tp53", "tp63", "tp73"))
> circos.genomicInitialize(df, plotType)
> circos.par(gap.degree = 2)
> circos.genomicInitialize(df)
```

Figure 3 initializes a circos plot with three genes and plots all alternative transcripts. The transcripts are drawn by circos.genomicRect which will be explained in following sections.

# 6 Create plotting regions

In following sections, chromosome will be used as the type of genomic category.

Similar as circos.trackPlotRegion, circos.genomicTrackPlotRegion also accepts a self-defined function panel.fun which is applied in every cell but with different form.

```
> circos.genomicTrackPlotRegion(data, panel.fun = function(region, value, ...) {
+ circos.genomicPoints(region, value, ...)
+ })
```

Inside panel.fun, users can use low-level genomic graphical functions to add basic graphics in each cell. panel.fun expects two arguments region and value. region is a data frame containing start position and end position in the current chromosome which is extracted from data. value is also a data frame which contains other columns in data. There should be a third arguments . . . which is mandatory and is used to pass user-invisible variables to inner functions.

Since circos.genomicTrackPlotRegion will create a new track, it needs values to calculate range of y-values to arrange data points. Users can either specify the index of numeric columns in data by numeric.column (named index or numeric index) or set ylim. If none of them are set, the function will try to look for all numeric columns in data (of course, excluding the first three columns), and set them as numeric.column.

### 6.1 Points

circos.genomicPoints is similar as circos.points. The difference is circos.genomicPoints expects a data frame containing genomic regions and a data frame containing values. The data column for plotting

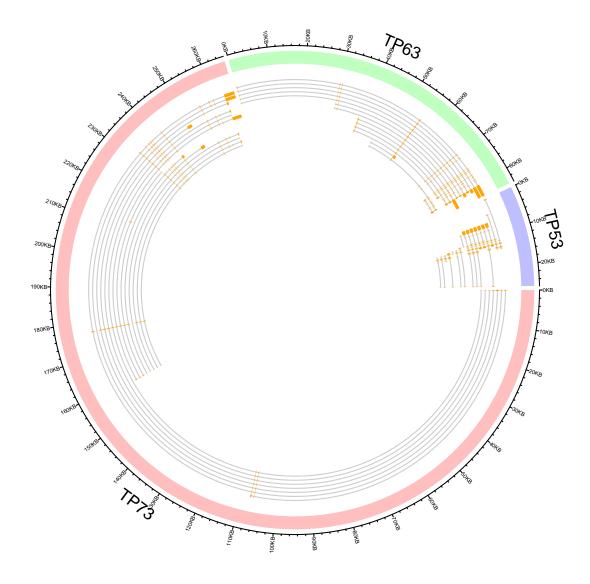


Figure 3: A circos plot with three genes

should be indicated by numeric.column. If the function is called inside circos.genomicTrackPlotRegion and users have been already set numeric.column in circos.genomicTrackPlotRegion, proper value of numeric.column will be passed to circos.genomicPoints through ... in panel.fun. Which means, you need to add ... as the final argument in circos.genomicPoints to pass such information into it. If numeric.column is not set, circos.genomicPoints will use all numeric columns detected in value.

```
> circos.genomicPoints(region, value, ...)
> circos.genomicPoints(region, value, numeric.column = c(1, 2))
> circos.genomicPoints(region, value, cex, pch)
> circos.genomicPoints(region, value, sector.index, track.index)
```

If there is only one numeric column, graphical parameters such as pch, cex can be of length one or number of rows of region. If there are more than one numeric columns specified, points for each numeric column will be added iteratively, and the graphical parameters should be either length one or number of numeric columns specified.

## 6.2 Lines

circos.genomicLines is similar as circos.lines. The setting of graphical parameters is similar as circos.genomicPoints.

```
> circos.genomicLines(region, value, ...)
> circos.genomicLines(region, value, numeric.column = c(1, 2))
> circos.genomicLines(region, value, lwd, lty = "segment")
> circos.genomicLines(region, value, area, baseline, border)
> circos.genomicLines(region, value, sector.index, track.index)
```

For lty, we additionally provide a new option segment by which each genomic interval will represent as a 'horizontal' line at corresponding value in value.

#### 6.3 Text

For circos.genomicText, the position of text can be specified either by numeric.column or a separated vector y. The labels of text can be specified either by labels.column or a vector labels.

```
> circos.genomicText(region, value, ...)
> circos.genomicText(region, value, y, labels)
> circos.genomicText(region, value, numeric.column, labels.column)
> circos.genomicText(region, value, facing, niceFacing, adj)
> circos.genomicText(region, value, sector.index, track.index)
```

## 6.4 Rectangle

For circos.genomicRect, the positions of top and bottom of the rectangles can be specified by ytop, ybottom or ytop.column, ybottom.column.

```
> circos.genomicRect(region, value, ytop = 1, ybottom = 0)
> circos.genomicRect(region, value, ytop.column = 2, ybottom = 0)
> circos.genomicRect(region, value, col, border)
```

One of the usage of circos.genomicRect is to plot heatmap on the circle. *circlize* provides a simple function colorRamp2 to interpolate colors. The arguments of colorRamp2 are break points and colors which correspond to the the break points. colorRamp2 returns a new function which can be used to generate new colors.

```
> col_fun = colorRamp2(breaks = c(-1, 0, 1), colors = c("green", "black", "red"))
> col_fun(c(-2, -1, -0.5, 0, 0.5, 1, 2))

[1] "#00FF00FF" "#00FF00FF" "#008000FF" "#000000FF" "#800000FF" "#FF0000FF"
[7] "#FF0000FF"
```

### 6.5 More details on circos.genomicTrackPlotRegion

The behavior of circos.genomicTrackPlotRegion and panel.fun will be different according to different input data and different settings.

### 6.5.1 Normal mode

If input data is a simple data frame, region in panel.fun would be a data frame containing start position and end position in the current chromosome which is extracted from input data. value is also a data frame which contains columns in input data excluding the first three columns. Index of proper numeric columns will be passed by .... So if users want to use such information, they need to pass ... to low-level genomic function such as circos.genoimcPoints as well.

```
> bed = generateRandomBed(nc = 2)
> circos.genomicTrackPlotRegion(bed, numeric.column = 4,
+ panel.fun = function(region, value, ...) {
```

```
+ circos.genomicPoints(region, value, ...)
+ circos.genomicPoints(region, value)
+ circos.genomicPoints(region, value, numeric.column = 1)
+ })
```

If input data is a list of data frames, panel.fun is applied on each data frame iteratively. Under such situation, region and value will contain corresponding data in the current data frame. The numeric index for the current data frame can be get by getI(...). For numeric.column argument if input data is a list of data frame, the length can only be one or the number of data frames, which means, there is only one numeric column that will be used in each data frame.

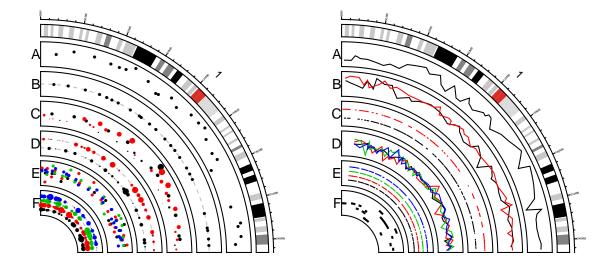
### 6.5.2 stack mode

circos.genomicTrackPlotRegion also support a stack mode. Under stack mode, ylim is re-defined inside the function. The y-axis will be splitted into several parts with equal height and graphics will be drawn on each 'horizontal' lines (y = 1, 2, ...).

Under stack mode, when input data is a single data frame containing one or more numeric columns, each numeric column defined in numeric.column will be treated as a single unit. ylim is re-defined to c(0.5, n+0.5) in which n is number of numeric columns. panel.fun will be applied iteratively on each numeric column. In each iteration, in panel.fun, region is still the genomic regions in current genomic category, but value only contains current numeric column plus all non-numeric columns. All low-level genomic graphical functions will be drawn on the 'horizontal line' y = i in which i is the index of current numeric column and the value of i can be obtained by getI(...).

When input data is a list containing data frames, each data frame will be treated as a single unit. The situation is quite similar as described previously. ylim is re-defined to c(0.5, n+0.5) in which n is number of data frames. panel.fun will be applied iteratively on each data frame. In each iteration, in panel.fun, region is still the genomic regions in current chromosome, and value contains columns in current data frame excluding the first three columns. Still, graphics by low-level genomic graphical functions will be added on the 'horizontal' lines.

Please see figure 4, for examples of using different settings in circos.genomicTrackPlotRegion.



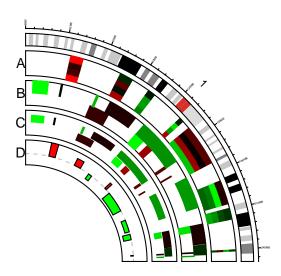


Figure 4: Topleft: Plotting points from A) a data frame with one numeric column; B) a data frame with one numeric column and under stack mode; C) a list of two data frames D) a list of two data frames under stack mode; E) a data frame with four numeric column; F) a data frame with four numeric column and under stack mode. Topright: Plotting lines in different ways. Plotting lines from A) a data frame with one numeric column; B) a list of two data frames C) a list of two data frames under stack mode; D) a data frame with four numeric column; E) a data frame with four numeric column and under stack mode. F) a data frame with one numeric column and 1ty is set to segment. Bottomleft: Plotting rectangles in different ways. Plotting lines from A) a data frame with four numeric column and under stack mode. B) a list of two data frames under stack mode; C) and D) adding rectangles with self-defined panel.fun.

#### 6.5.3 Mixed use of general circos functions

panel.fun is applied on each cell, which means, besides genomic functions, you can also use general circos functions to add more graphics. For example, some horizontal lines and texts are added to each cell and axes are put on top of each cell:

```
> circos.genomicTrackPlotRegion(bed, ylim = c(-1, 1),
+ panel.fun = function(region, value, ...) {
```

#### 6.6 links

circos.genomicLink expects two data frames and it will add links from genomic intervals in the first data frame to corresponding genomic intervals in the second data frame (figure 5).

```
> circos.genomicLink(bed1, bed2)
> circos.genomicLink(bed1, bed2, col)
```

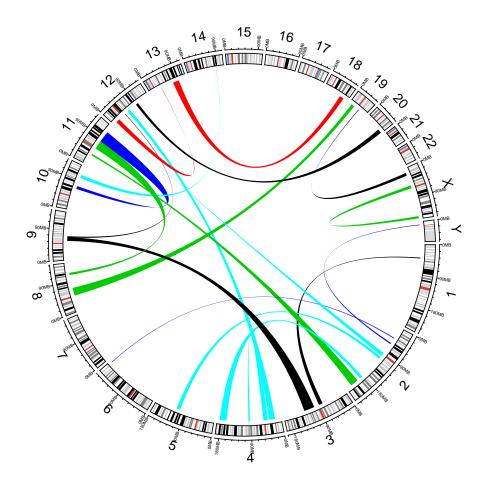


Figure 5: Add links from two sets of regions.

## 6.7 Highlight chromosomes

highlight.chromosome provides a simple way to highlight chromosomes. Just remember to use transparent filled colors. The position of the highlighted region and be fine-tuned by padding argument which are percentages of corresponding height and width in the highlighted region. (figure 6)

```
> highlight.chromosome("chr1")
> highlight.chromosome("chr1", track.index = c(2, 3))
> highlight.chromosome("chr1", col = NA, border = "red")
> highlight.chromosome("chr1", padding = c(0.1, 0.1, 0.1, 0.1))
```

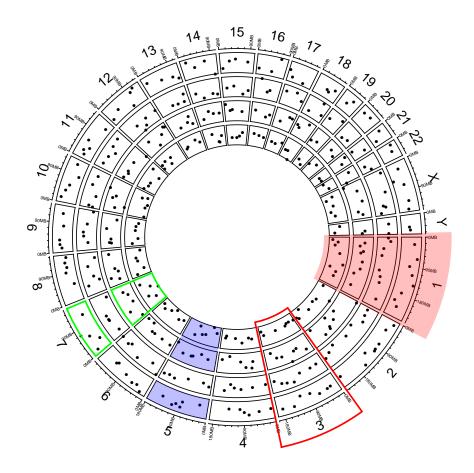


Figure 6: Highlight chromosomes.

# 7 High-level genomic functions

circlize implements several high-level functions which may help to visualize genomic data.

#### 7.1 Position transformation

This feature is experimental in current version, thus there may be errors when you using it.

There is one representative situation when genomic position transformation needs to be applied. For example, there are two sets of regions in a chromosome in which regions in one set regions are quite densely to each other and regions in other set are far from others. Heatmap or text is going to be drawn on the next track. If there is no position transformation, heatmap or text for those dense regions would be overlapped and hard to identify, also ugly to visualize. Thus, a way to transform original positions to new positions would help for the visualization.

Low-level genomic functions such as circos.genomicPoints all accept an argument posTransform to apply user-defined position transformation. Value for posTransform is a self-defined function which only accepts at least one argument: a data frame with two columns (start position and end position). There is only one requirement for position transformation: Number of rows of regions should be the

same before and after the transformation. In *circlize*, there already provides a position transformation function posTransform.default which distributes positions uniformly in current chromosome.

Since position transformation function is always executed inside low-level genomic graphical functions, You can use get.cell.meta.data to obtains meta information for the current chromosome.

Following code does the transformation. The points are plotted with the new transformed regions.

```
> circos.genomicTrackPlotRegion(data, panel.fun = function(region, value, ...) {
+ circos.genomicPoints(region, value, posTransform = posTransform.default, ...)
+ })
```

There is a function circos.genomicPosTransformLines which adds a line from untransformed regions to transformed regions. Note circos.genomicPosTransformLines will create a new track. In the function, direction controls whether the position transformation track is inside or outside the track which is created by circos.genomicPosTransformLines. Please see figure 7 for examples.

There is another position transformation function posTransform.text provided in *circlize* that can smartly position text on the circle. Normally, we don't want the text too away from the original position and also we want to get avoid of text overlapping. posTransform.text calculates the height of text and transform position properly. Since such text position transformation relies on font size of text and which track the text is in, the usage of posTransform.text is a little bit complex. Currently, posTransform.text only makes sense if it is called inside circos.genomicText and only works when facing is set to clockwise or reverse.clockwise.

In following example code, using posTransform.text is quite similar as posTransform.default:

```
> bed = generateRandomBed(nr = 400, fun = function(k) rep("text", k))
> circos.genomicTrackPlotRegion(bed, ylim = c(0, 1),
+ panel.fun = function(region, value, ...) {
+ circos.genomicText(region, value, y = 0, labels.column = 1,
+ facing = "clockwise", adj = c(0, 0.5), posTransform = posTransform.text,
+ cex = 0.8)
+ }, track.height = 0.1, bg.border = NA)
```

For the next track, position transformation lines are going to be plotted. Now code will be a little complex. Since such text position transformation relies on text settings (e.g. font size, font family) and track for the text, we need to go back to the track where text position transformation happens. Such information should be collected when plotting transformation lines. The solution by *circlize* is to save the real call by quote and pass it to posTransform argument. Inside circos.genomicPosTransformLines, posTransform.text will be called by panel.fun by eval, then text information will be recovered and the transformation will be calculated in the correct track.

```
> i_track = get.cell.meta.data("track.index") # previous track
> circos.genomicPosTransformLines(bed,
+ posTransform = quote(posTransform.text(region, y = 0, labels = value[[1]],
+ cex = 0.8, track.index = i_track)), direction = "outside"
+ )
```

If the track where text position is transformed is after the track where transformation lines are plotted, things will be more complicated. Since circos.genomicPosTransformLines needs information of the text, but at that moment, track which transforms text position has not be created. The solution is first creating a empty track for position transformation lines, then creating and plotting the position transformation track. Finally go back to the transformation line track to add transformation lines afterwards.

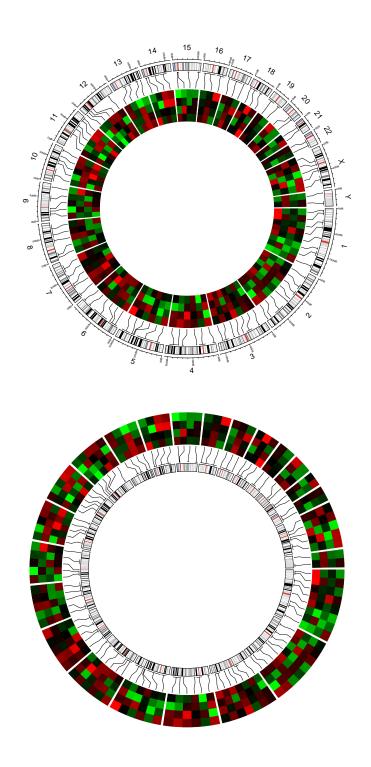


Figure 7: Transformation of genomic positions. Top: position transformed track is inside; Bottom: Position transformed track is outside.

Padding of text after transformation can be set through padding argument to adjust the space between two neighbouring text.

For examples and comparison between posTransform.default and posTransform.text when visualizing text, please refer to figure 8.

### 7.2 Genomic density and Railfall plot

circos.genomicDensity calculate how much a genomic window is covered by regions in bed. The input data can be a single data frame or a list of data frames.

```
> circos.genomicDensity(bed)
> circos.genomicDensity(bed, window.size = 1e6)
> circos.genomicDensity(bedlist)
```

Rainfall plot can be used to visualize distribution of regions. On the plot, y-axis corresponds to the distance to neighbour regions (log10-based). So if there is a drop-down on the plot, it means there is a cluster of regions at that area (figure 9). The input data can be a single data frame or a list of data frames.

```
> circos.genoimcRainfall(bed)
> circos.genoimcRainfall(bedlist, col = c("red", "green"))
```

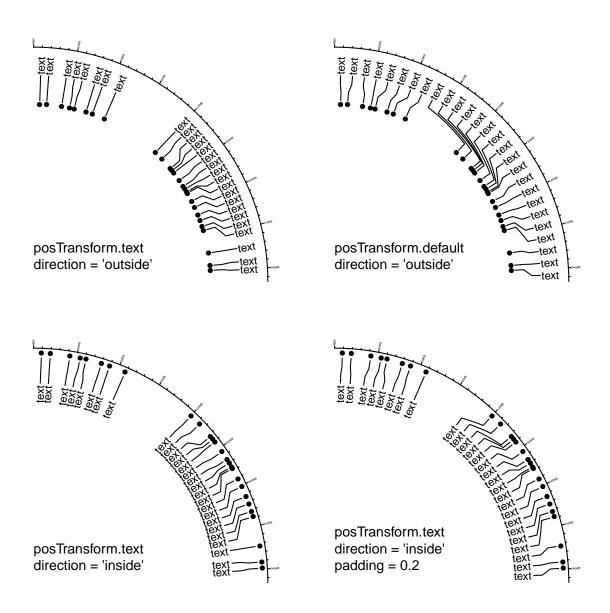


Figure 8: Transformation of text positions

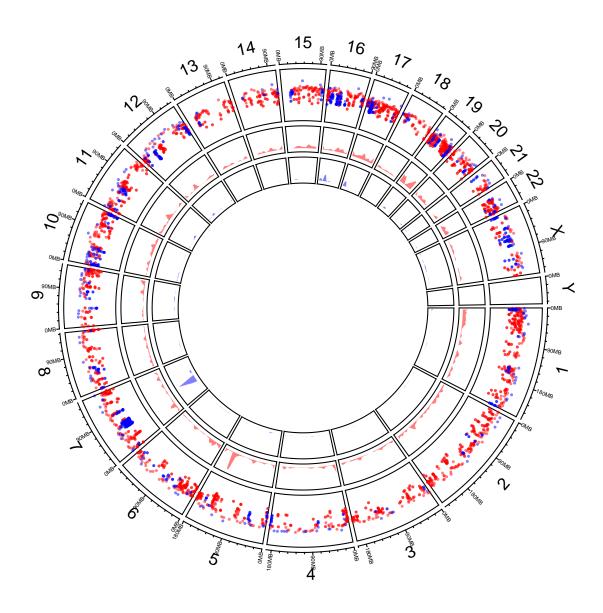


Figure 9: Rainfall plot and genomic density