

# Visualisation, transformations and arithmetic operations for grouped genomic intervals

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## **Abstract**

The soGGi package provides tools to summarise sequence data, genomic signal and motif occurrence over grouped genomic intervals as well to perform complex subsetting, arithmetic operations and transformations on these genomic summaries prior to visualisation.

As with other Bioconductor packages such as CoverageView and seqPlots, soGGi plots average signal across groups of genomic regions. soGGi provides flexibility in both its data acquisition and visualisation. Single or paired-end BAM files, bigWigs, rleLists and PWM matrices can be provided as input alongside a GRanges object or BED file location. soGGi can plot summaries across actual size and normalised features such as genomic intervals of differing length (as with genes). The use of normalised size plots for genes can however obscure high resolution events around the TSS. To address this, combination plots can be created within soGGi allowing for fine detail at the edges of normalised regions.

Arithmetic operation and transformations can be easily performed on soGGi objects allowing for rapid operations between profiles such as the subtraction of input signal or quantile normalisation of replicates within a group.

soGGi integrates the ggplot2 package and add functionality to rapidly subset, facet and colour profiles by their overlaps with GenomicRanges objects or grouping by metadata column IDs.

The plotting, arithmetic and transformation functions within soGGi allow for rapid evaluation of groups of genomic intervals and provide a toolset for user-defined analysis of these summaries over groups.

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## 1 Standard workflow

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### 1.1 The soggi function

The `regionPlot()` function is used to summarise signal, reads or PWM occurrence over grouped genomic intervals. Input can be BAM, bigWig or a PWM matrix and the regions to summarise over a character string of path to BED file or a GRanges object. The full set of soggi function arguments can be found in help pages.

In this example, signal coverage is summarised from a BAM file using the defaults. The default style of region plot is to produce a normalised by size region plot.

```
library(soGGi)
chipExample <- regionPlot("pathToBAM/mybam.bam", myGRangesObject, format="bam")
```

A pre-computed data set is included in the package containing averages profiles created with command above for DNase, Pol2, H3k9ac and H3k3me3. The object itself contains all counts along interval region windows in assays slots and information of the samples in metadata slot accessible by `assays()` and `metadata()` respectively.

```
library(soGGi)
data(chipExampleBig)
chipExampleBig
```

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```
## class: ChIPprofile
## dim: 201 300
## metadata(2): names AlignedReadsInBam
## assays(10): ' ' ' ... ' ' '
## rownames: NULL
## rowData names(5): name biotype Feature giID giID.1
## colnames(300): Start-1 Start-2 ... End+99 End+100
## colData names(0):
```

This object contains 10 sets of profiles for 200 genes. The object can be subset using `[[` to select samples of interest.

```
chipExampleBig[[1]]

## class: ChIPprofile
## dim: 201 300
## metadata(2): names AlignedReadsInBam
## assays(1): ' '
## rownames: NULL
## rowData names(5): name biotype Feature giID giID.1
## colnames(300): Start-1 Start-2 ... End+99 End+100
## colData names(0):

chipExampleBig$highdnase

## class: ChIPprofile
## dim: 201 300
## metadata(2): names AlignedReadsInBam
## assays(1): ' '
## rownames: NULL
## rowData names(5): name biotype Feature giID giID.1
## colnames(300): Start-1 Start-2 ... End+99 End+100
## colData names(0):
```

Similarly profile objects can be concatenated or bound together using `c` and `rbind`.

```
c(chipExampleBig[[1]],chipExampleBig[[2]])

## class: ChIPprofile
## dim: 201 300
## metadata(2): names AlignedReadsInBam
## assays(2): ' ' '
## rownames: NULL
```

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```
## rowData names(5): name biotype Feature giID giID.1
## colnames(300): Start-1 Start-2 ... End+99 End+100
## colData names(0):

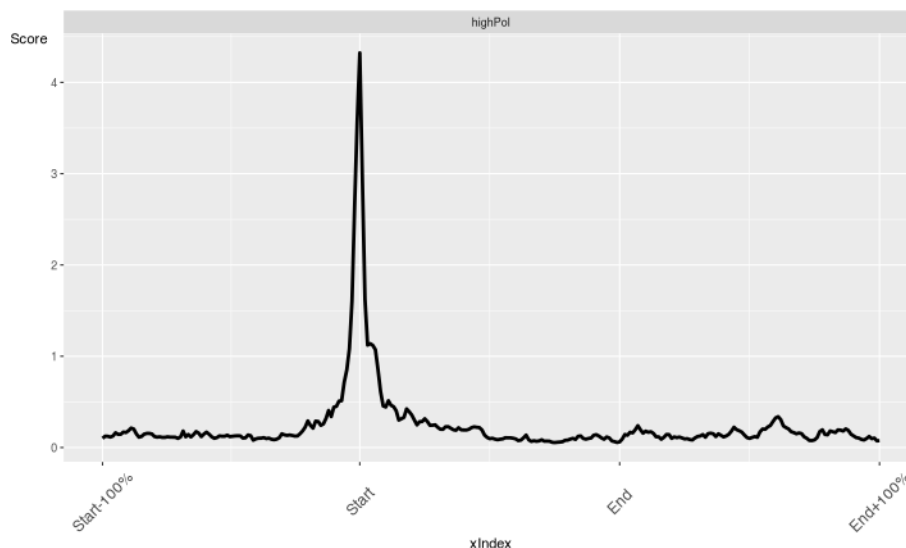
rbind(chipExampleBig[[1]],chipExampleBig[[2]])

## class: ChIPprofile
## dim: 402 300
## metadata(2): names AlignedReadsInBam
## assays(1): ''
## rownames: NULL
## rowData names(5): name biotype Feature giID giID.1
## colnames(300): Start-1 Start-2 ... End+99 End+100
## colData names(0):
```

### 1.2 Plotting profiles

The `plotRegion()` function is used to produce profile plots. `plotRegion()` uses `ggplot2` to generate plots and so returned object can be highly customisable using `ggplot2` methods.

```
plotRegion(chipExampleBig[[3]])
```

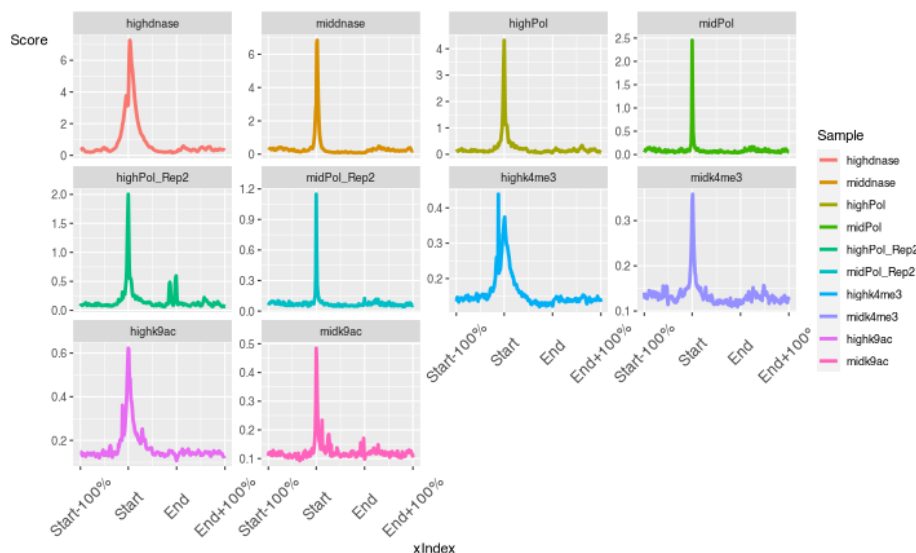


**Figure 1: Example profile plot.** The plot generated by `plotRegion()` function on a single sample `soGGi` object. The x-axis shows the normalised length and the y-axis shows the coverage in windows. A Clear peak around the TSS can be observed for this Pol2 ChIP-seq profile.

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When dealing with objects with multiple samples, the arguments `groupBy` and `colourBy` specify whether to facet or colour by Sample/Group respectively.

```
library(ggplot2)
plotRegion(chipExampleBig, colourBy="Sample", groupBy="Sample", freeScale=TRUE)
```



**Figure 2: Multi-Sample profile plot.** Here multiple samples are plotted simultaneously. Enrichment around TSS can be seen for all profiles with H3k9ac showing more enrichment in the gene body.

Here some samples can be seen to be noisy. Windsorisation can be applied when plotting using the `outliers` argument.

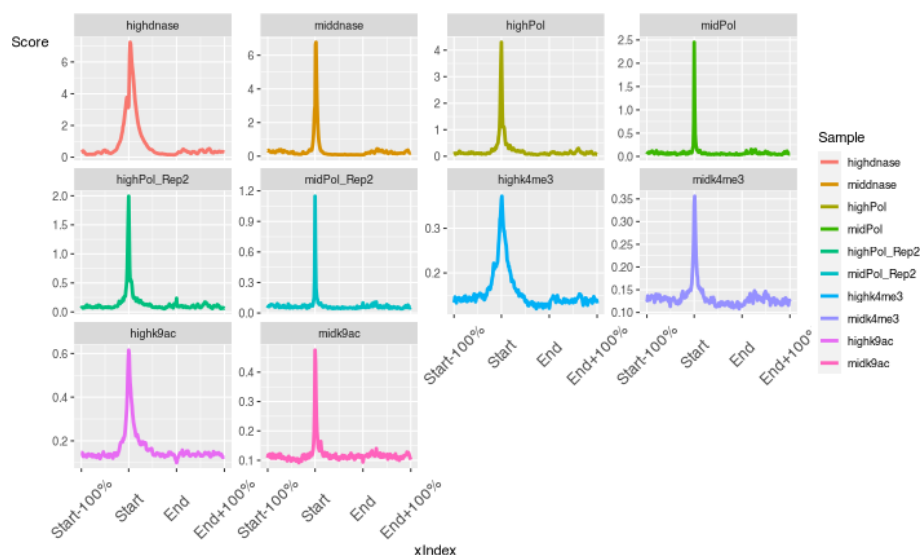
```
library(ggplot2)
plotRegion(chipExampleBig, colourBy="Sample", outliers=0.01, groupBy="Sample", freeScale=TRUE)
```

The `plotRegion()` can also be used to group genomic intervals while plotting using the `gts` argument. The `gts` argument either takes a `GRanges` object or a list of character vectors and the `summariseBy` argument to specify metadata to use.

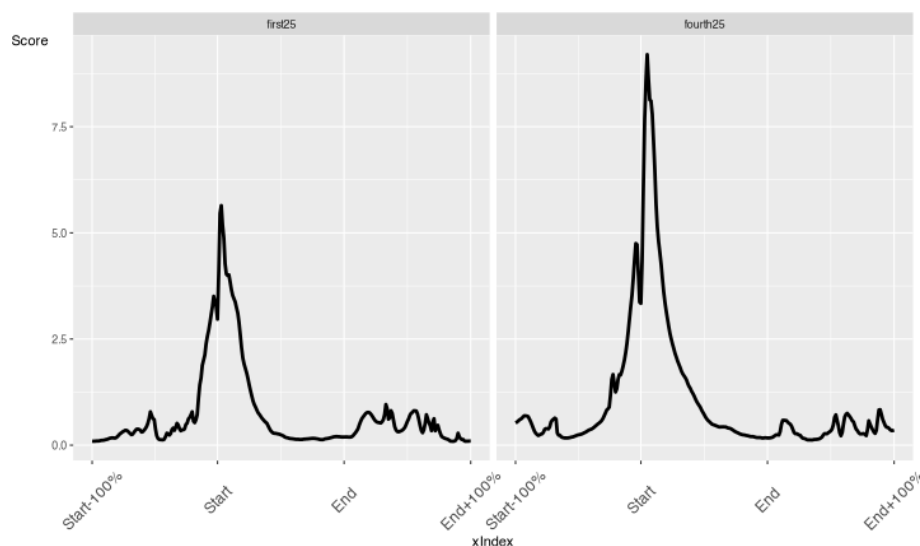
```
library(GenomicRanges)
subsetsCharacter <- list(first25 = (as.vector(rowRanges(chipExampleBig[[1]])$name[1:25])),
subsetsGRanges <- GRangesList(low=(rowRanges(chipExampleBig[[1]])[1:25]), high=rowRanges(chipExampleBig[[1]])[1:25])

plotRegion(chipExampleBig[[1]], gts=subsetsCharacter, summariseBy = "name")
plotRegion(chipExampleBig[[1]], gts=subsetsGRanges)
```

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**Figure 3: Multi-Sample profile plot with windsorisation.** This multi-sample plot has windsorisation applied to outliers. The resulting plot is smoother than that seen in figure 2.



**Figure 4: DNase grouped genomic intervals plot.** This plots shows the plot for DNase signal across normalised regions with separate profiles for each group of genomic intervals defined in gts. .

## 2 Transformations and arithmetic operations

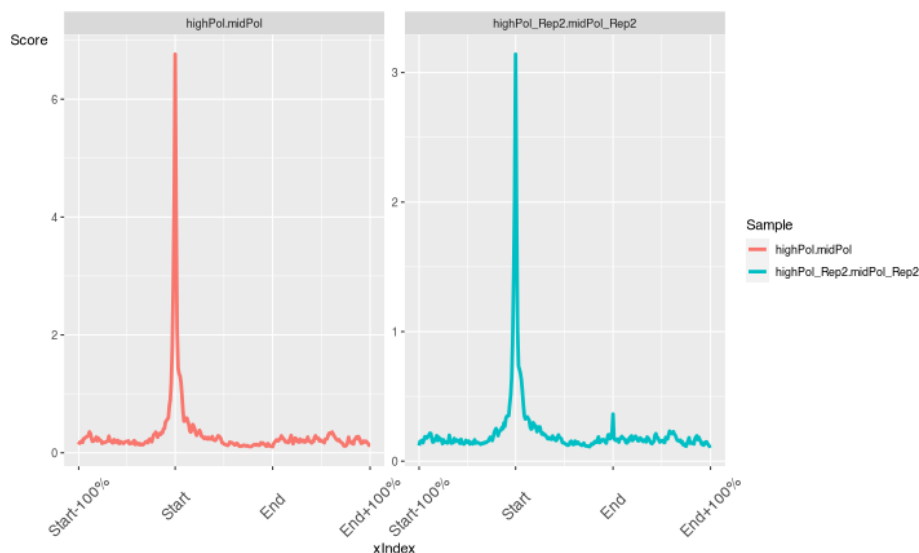
### 2.1 Simple arithmetic operations on grouped profiles

Common arithmetic operations and tranformations can be used with soGGi profile objects allowing for further analysis post summarisation and iteratively over visualisations.

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Here we summarise RNApol2 high and low and compare between replicates.

```
pol_Profiles <- c((chipExampleBig$highPol+chipExampleBig$midPol)
, (chipExampleBig$highPol_Rep2+chipExampleBig$midPol_Rep2))
plotRegion(pol_Profiles,colourBy="Sample",outliers=0.01, groupBy="Sample", freeScale=TRUE)
```



**Figure 5: Plotting arithmetic results.** This plot demonstrates the ability to plot the profiles generated by the results of arithmetic operations. Here data is combined within replicate number and results plotted.

Common normalisations, log transformations and other mathematical functions such as `mean()` are also implemented to allow for the comparison within and between profiles.

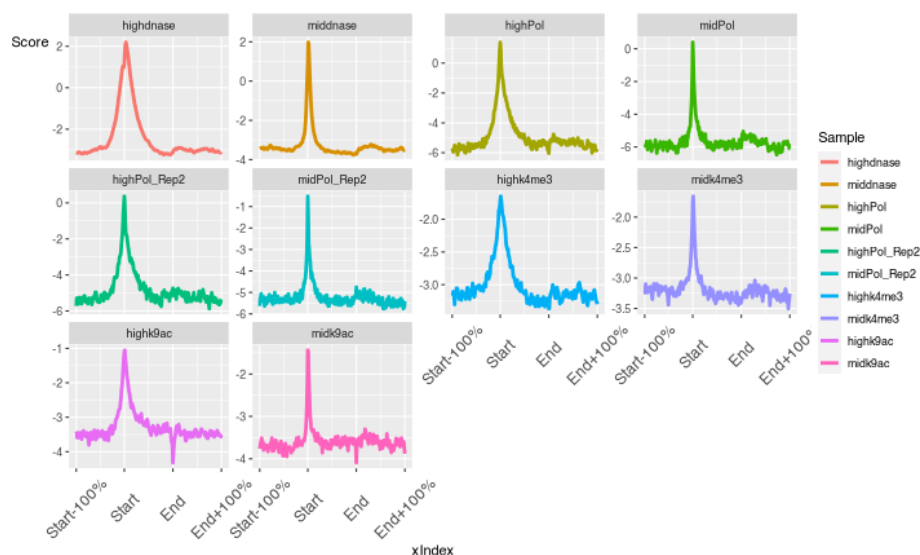
In this example the profiles are log<sub>2</sub> transformed with zeros being assigned the minimum value for that region.

```
log2Profiles <- log2(chipExampleBig)
plotRegion(log2Profiles,colourBy="Sample",outliers=0.01, groupBy="Sample", freeScale=TRUE)
```

From this log<sub>2</sub> transformed data we can look at the difference between Pol2 profiles

```
log2Polhigh <- mean(log2Profiles$highPol, log2Profiles$highPol_Rep2)
log2Polmid <- mean(log2Profiles$midPol, log2Profiles$midPol_Rep2)
diffPol <- log2Polhigh-log2Polmid
```

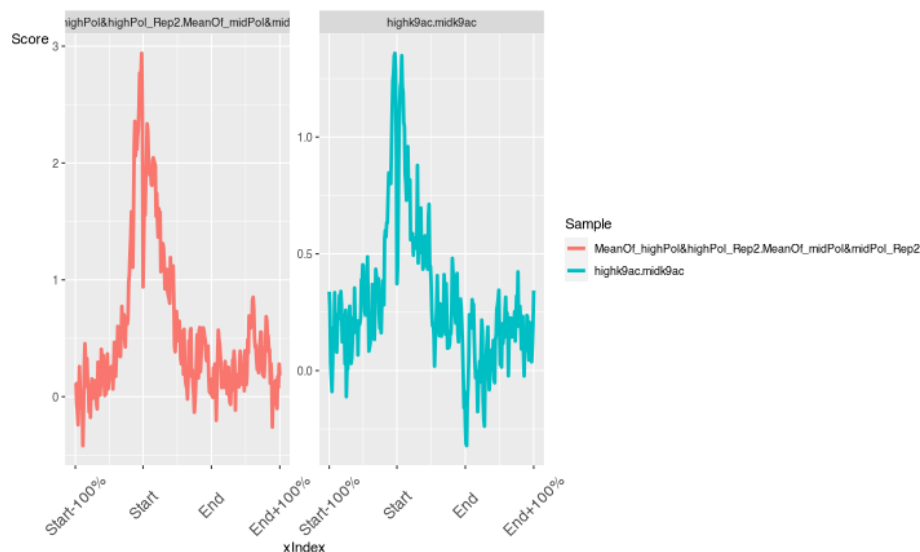
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**Figure 6: Plotting log<sub>2</sub> transformed profiles.** This plots shows the resulting profiles after log<sub>2</sub> transformation of ChIP-data across antibodies.

```
diffh3k9ac <- log2Profiles$highk9ac-log2Profiles$midk9ac
```

```
plotRegion(c(diffPol,diffh3k9ac),colourBy="Sample",outliers=0.01, groupBy="Sample", freeSc
```



**Figure 7: Plotting differentials.** In this plot the log<sub>2</sub> difference between high and low samples for H3k9ac and Pol2 replicates is shown.

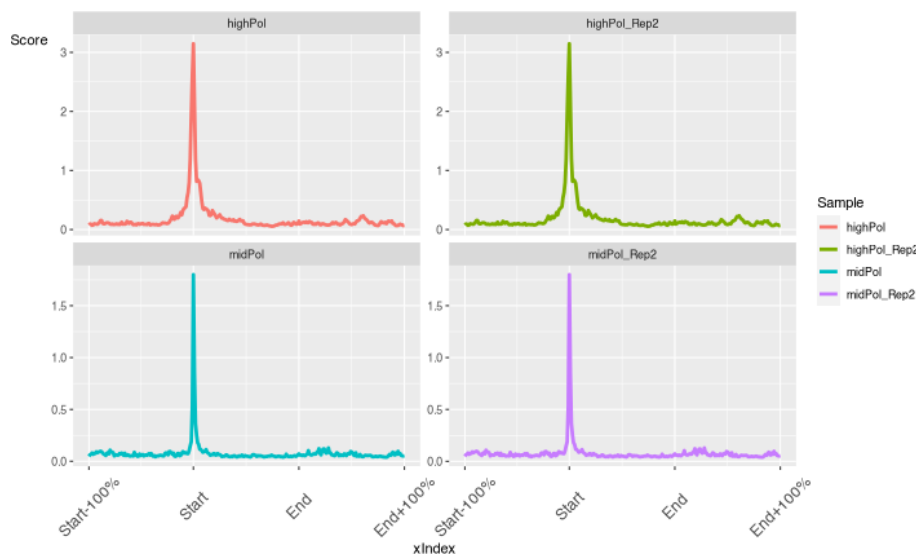


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Quantile normalisation of allow windows in regions between samples can allow for better visual comparison of changes between conditions when dealing with larger numbers of replicates. Here for demonstration we apply it two samples but with real data higher sample numbers would be recommended.

```
normHighPol <- normalise(c(chipExampleBig$highPol, chipExampleBig$highPol_Rep2), method="quantile")
normMidPol <- normalise(c(chipExampleBig$midPol, chipExampleBig$midPol_Rep2), method="quantile")

normPol <- c(normHighPol$highPol, normHighPol$highPol_Rep2, normMidPol$midPol, normMidPol$midPol_Rep2)
plotRegion(normPol, colourBy="Sample", outliers=0.01, groupBy="Sample", freeScale=TRUE)
```



**Figure 8: Quantile normalisation.** In this toy example, data has been quantiled normalised within groups and the results plotted. This demonstrates the uniformity in data following quantile normalisation.

### 3 Creating GRanges combinations for plotting

A common operation in the analysis of summaries over genomic intervals is to compare between different sets of grouped genomic intervals. soGGi includes helper functions to deal with grouped genomic intervals.

The `groupByOverlaps()` function creates all combinations of grouped genomic intervals from GRangesLists and so is useful to evaluate how summaries change over subclasses of genomic intervals (such as over co-occurring peak sets)

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The `findconsensusRegions()` and `summitPipeline()` functions identifies consensus regions between GRanges objects and re-summits consensus region sets respectively. This approach has been previously implemented to identify reproducible peak sets between biological replicates.

### 3.1 Grouping genomic interval sets and plotting results

In this example, two antibodies for the transcription factor Ikaros are used to plot the signal over common and unique peaks for each antibody. First the peaks sets are defined using `groupByOverlaps()`

```
data(ik_Example)
ik_Example

## $ha
## GRanges object with 200 ranges and 2 metadata columns:
##           seqnames      ranges strand |           ID      Score
##           <Rle>         <IRanges> <Rle> |         <factor> <factor>
##    [1]      chr1    5218713-5219618      * |   MACS_peak_4    60.84
##    [2]      chr1    6372427-6373665      * |   MACS_peak_7   438.68
##    [3]      chr1    6456792-6457633      * |   MACS_peak_9    96.87
##    [4]      chr1    7080191-7080946      * |   MACS_peak_15   57.82
##    [5]      chr1    9225736-9227873      * |   MACS_peak_17  166.57
##    ...      ...                ...      ... .         ...      ...
##   [196]     chr1  36765816-36767310      * |   MACS_peak_130  127.4
##   [197]     chr1  36822798-36824995      * |   MACS_peak_131 1387.29
##   [198]     chr1  36995290-36996196      * |   MACS_peak_132   73.84
##   [199]     chr1  37044729-37045925      * |   MACS_peak_135  193.63
##   [200]     chr1  37356201-37357028      * |   MACS_peak_138   66.39
##   -----
##   seqinfo: 21 sequences from an unspecified genome; no seqlengths
##
## $endo
## GRanges object with 200 ranges and 2 metadata columns:
##           seqnames      ranges strand |           ID      Score
##           <Rle>         <IRanges> <Rle> |         <factor> <factor>
##    [1]      chr1    4695718-4695917      * |   MACS_peak_2    98.76
##    [2]      chr1    4775045-4775288      * |   MACS_peak_3   100.91
##    [3]      chr1    4775403-4775658      * |   MACS_peak_4    50.4
##    [4]      chr1    4775664-4776101      * |   MACS_peak_5   150.6
##    [5]      chr1    4797674-4798084      * |   MACS_peak_6   651.89
##    ...      ...                ...      ... .         ...      ...
##   [196]     chr1  24740900-24741101      * |   MACS_peak_211  301.48
```

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```
## [197] chr1 30929613-30929871 * | MACS_peak_220 106.95
## [198] chr1 30930510-30931164 * | MACS_peak_221 1604.65
## [199] chr1 31005552-31005763 * | MACS_peak_222 174.71
## [200] chr1 31006685-31007192 * | MACS_peak_223 529.42
## -----
## seqinfo: 21 sequences from an unspecified genome; no seqlengths

peakSetCombinations <- groupByOverlaps(ik_Example)
peakSetCombinations

## $endo
## GRanges object with 100 ranges and 1 metadata column:
##      seqnames      ranges strand | grangesGroups
##      <Rle>        <IRanges> <Rle> |      <factor>
## [1] chr1 4695718-4695917 * |      endo
## [2] chr1 4775045-4775288 * |      endo
## [3] chr1 4775403-4775658 * |      endo
## [4] chr1 4775664-4776101 * |      endo
## [5] chr1 4797674-4798084 * |      endo
## ...      ...      ...      ...      ...
## [96] chr1 21534723-21534898 * |      endo
## [97] chr1 21635845-21636030 * |      endo
## [98] chr1 21705049-21705198 * |      endo
## [99] chr1 21739213-21739804 * |      endo
## [100] chr1 21757663-21757886 * |      endo
## -----
## seqinfo: 21 sequences from an unspecified genome; no seqlengths
##
## $ha
## GRanges object with 137 ranges and 1 metadata column:
##      seqnames      ranges strand | grangesGroups
##      <Rle>        <IRanges> <Rle> |      <factor>
## [1] chr1 5218713-5219618 * |      ha
## [2] chr1 6372427-6373665 * |      ha
## [3] chr1 6456792-6457633 * |      ha
## [4] chr1 7080191-7080946 * |      ha
## [5] chr1 9225736-9227873 * |      ha
## ...      ...      ...      ...      ...
## [133] chr1 89527991-89530046 * |      ha
## [134] chr1 89768074-89769026 * |      ha
## [135] chr1 89837445-89838176 * |      ha
## [136] chr1 90598333-90599360 * |      ha
## [137] chr1 91540499-91541383 * |      ha
```

## Visualisation, transformations and arithmetic operations for grouped genomic intervals

```
## -----
## seqinfo: 21 sequences from an unspecified genome; no seqlengths
##
## $`ha-endo`
## GRanges object with 63 ranges and 1 metadata column:
##      seqnames      ranges strand | grangesGroups
##      <Rle>         <IRanges> <Rle> |      <factor>
## [1]      chr1  4847117-4848878      * |      ha-endo
## [2]      chr1  5072908-5073864      * |      ha-endo
## [3]      chr1  6204023-6205748      * |      ha-endo
## [4]      chr1  6252357-6253568      * |      ha-endo
## [5]      chr1  6395852-6396924      * |      ha-endo
## ...      ...      ...      ...      ...
## [59]     chr1 24012021-24013019      * |      ha-endo
## [60]     chr1 24684410-24686139      * |      ha-endo
## [61]     chr1 24740575-24741381      * |      ha-endo
## [62]     chr1 30929409-30931590      * |      ha-endo
## [63]     chr1 31005112-31007518      * |      ha-endo
## -----
## seqinfo: 21 sequences from an unspecified genome; no seqlengths
```

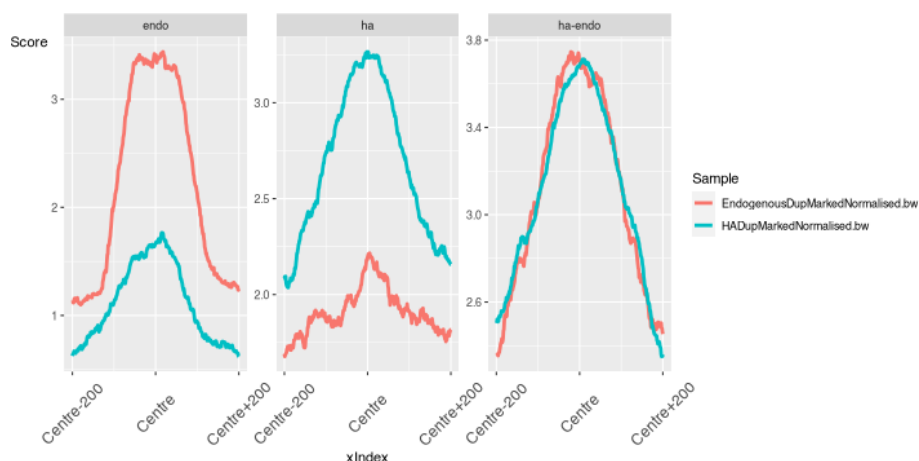
The output from `groupByOverlaps()` can then be used to subset precomputed profiles of HA and Endogenous ChIP signal. Here we apply a  $\log_2$  transformation to the data before plotting and cleaning up profile with windsorisation

```
data(ik_Profiles)
ik_Profiles

## class: ChIPprofile
## dim: 4800 401
## metadata(2): names AlignedReadsInBam
## assays(2): ' '
## rownames: NULL
## rowData names(3): ID Score giID
## colnames(401): Point_Centre-200 Point_Centre-199 ... Point_Centre199
##   Point_Centre200
## colData names(0):

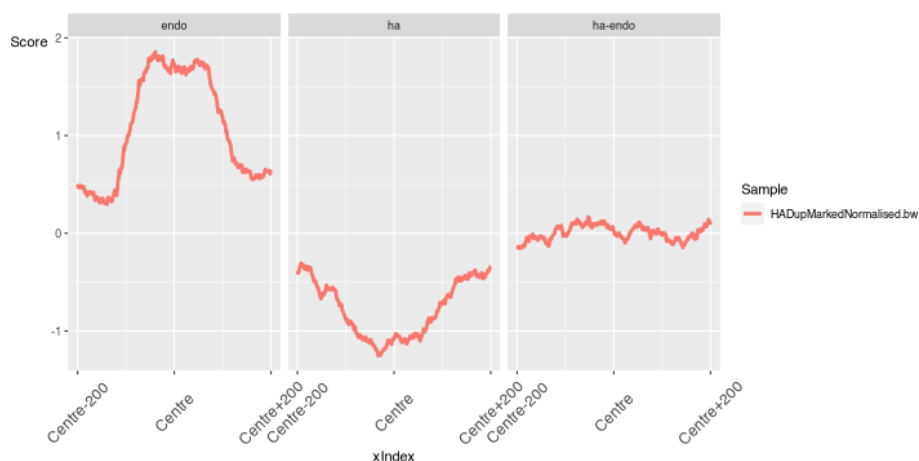
log2Ik_Profiles <- log2(ik_Profiles)
plotRegion(log2Ik_Profiles,outliers=0.01,gts=peakSetCombinations, groupBy="Group",colourBy:
plotRegion(log2Ik_Profiles[[1]] - log2Ik_Profiles[[2]] ,outliers=0.01,gts=peakSetCombinations)
```

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**Figure 9: Signal over common and unique Ikaros peaks.** This plot shows that, as expected, common and unique peaks show different profiles for the Ikaros antibodies.

This confirms that common and unique peaksets have different levels of the separate antibody signals. This can be better demonstrated by subtracting to signal sets from each other and re-plotting over groups as seen in the final example.



**Figure 10: Differential Ikaros profiles over common and unique sets.** The plot of difference in log2 HA and Endogenous Ikaros signal over peaks shows the expected difference in Ikaros antibody signal and uniformity of signal of common peaks.

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```
toLatex(sessionInfo())
```

- R version 4.3.0 RC (2023-04-13 r84266), aarch64-apple-darwin20
- Locale: C/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Time zone: America/New\_York
- TZcode source: internal
- Running under: macOS Monterey 12.6.1
- Matrix products: default
- BLAS:  
/Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
- LAPACK:  
/Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib  
; LAPACK version 3.11.0
- Base packages: base, datasets, grDevices, graphics, methods, stats, stats4, utils
- Other packages: Biobase 2.60.0, BiocGenerics 0.46.0, GenomInfoDb 1.36.0, GenomicRanges 1.52.0, IRanges 2.34.0, MatrixGenerics 1.12.0, S4Vectors 0.38.1, SummarizedExperiment 1.30.1, ggplot2 3.4.2, knitr 1.42, matrixStats 0.63.0, soGGi 1.32.0
- Loaded via a namespace (and not attached): BiocIO 1.10.0, BiocManager 1.30.20, BiocParallel 1.34.1, BiocStyle 2.28.0, Biostrings 2.68.0, DelayedArray 0.26.2, GenomInfoDbData 1.2.10, GenomicAlignments 1.36.0, Matrix 1.5-4, R6 2.5.1, RColorBrewer 1.1-3, RCurl 1.98-1.12, Rcpp 1.0.10, Rsamtools 2.16.0, S4Arrays 1.0.1, ShortRead 1.58.0, XML 3.99-0.14, XVector 0.40.0, bitops 1.0-7, chipseq 1.50.0, cli 3.6.1, codetools 0.2-19, colorspace 2.1-0, compiler 4.3.0, crayon 1.5.2, deldir 1.0-6, digest 0.6.31, dplyr 1.1.1, evaluate 0.20, fansi 1.0.4, farver 2.1.1, fastmap 1.1.1, generics 0.1.3, glue 1.6.2, grid 4.3.0, gtable 0.3.3, highr 0.10, htmltools 0.5.5, hwriter 1.3.2.1, interp 1.1-4, jpeg 0.1-10, labeling 0.4.2, lattice 0.21-8, latticeExtra 0.6-30, lifecycle 1.0.3, magick 2.7.4, magrittr 2.0.3, munsell 0.5.0, parallel 4.3.0, pillar 1.9.0, pkgconfig 2.0.3, plyr 1.8.8, png 0.1-8, preprocessCore 1.62.1, reshape2 1.4.4, restfulr 0.0.15, rjson 0.2.21, rlang 1.1.0, rmarkdown 2.21, rtracklayer 1.60.0, scales 1.2.1, stringi 1.7.12, stringr 1.5.0, tibble 3.2.1, tidyselect 1.2.0, tools 4.3.0, utf8 1.2.3, vctrs 0.6.1, withr 2.5.0, xfun 0.38, yaml 2.3.7, zlibbioc 1.46.0