

GENOVA: explore the Hi-C's

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Abstract

The increase in interest for Hi-C methods in the chromatin community has led to a need for more user-friendly and powerful analysis methods. The few currently available software packages for Hi-C do not allow a researcher to quickly summarize and visualize their data. An easy to use software package, which can generate a comprehensive set of publication-quality plots, would allow researchers to swiftly go from raw Hi-C data to interpretable results. Here, we present **GENome Organisation Visual Analytics** (GENOVA): a software suite to perform in-depth analyses on various levels of genome organisation, using Hi-C data. GENOVA facilitates the comparison between multiple datasets and supports the majority of mapping-pipelines.

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1 Loading data

```
# devtools::install_github("robinweide/GENOVA", ref = 'dev')
library(GENOVA)
```

1.1 Data structures of input

GENOVA expects two input files: the signal- and the index-file. Signal-files have three columns (bin1, bin2, contactCount) and index-files have four (chromosome, start, end, bin). These are the default output of the Hi-C mapping pipeline HiC-Pro (Servant et al. 2015), where they are called *.matrix and *.bed. The files are expected to be genome-wide and may be corrected with ICE-normalisation.

1.2 Recommended resolutions

To ensure computational strain and time is kept to a minimum, we recommend different resolutions for different functions (table 1). More experienced users are free to deviate, while keeping in mind that these datasets are quite memory-heavy (table 2).

Table 1: Recommended resolutions

These will provide optimal resource/result tradeoffs.

Function	Resolution
APA	10kb-20kb
ATA	10kb-40kb
cisTotal.perChrom	500kb-1Mb
chromosomeMatrix	500kb-1Mb
RCP	40kb-500kb
intra.inter.TAD.contacts	20kb - 40kb
PE-SCAn	20kb-40kb
hic.matrixplot	$\frac{\text{width in bp of window}}{500}$
centromere.telomere.analysis	40kb
.compartment.plot	100kb

Table 2: Memory footprints of objects loaded into R

Experiment	Contacts	10kb	40kb	100kb	1Mb
Hap1 (Haarhuis et al. 2017)	433.5M	2.9Gb	1.7Gb	1.1Gb	0.1Gb
iPSC (Krijger et al. 2016)	427.9M	3.1Gb	1.9Gb	1.0Gb	53.1MB

1.3 construct.experiment

Every Hi-C experiment will be stored in an experiment-object. This is done by invoking the `construct.experiment` function. Inside, several sanity checks will be performed, data is normalised to the total number of reads and scaled to a billion reads (the default value of the `BPscaling`-option). For this example, we are going to use the Hi-C maps of WT and ΔWAPL Hap1 cells from Haarhuis et al. (2017). Since the genome-wide analyses do not need very high-resolution data, we will construct both 10kb, 40kb and 1Mb resolution experiment-objects.

```
Hap1_WT_10kb <- construct.experiment(ignore.checks = T, # time-saver for vignette,
                                         signalPath = 'data/WT_10000_iced.matrix',
                                         indicesPath = 'data/WT_10000_abs.bed',
                                         name = "WT",
                                         color = "black")

Hap1_WAPL_10kb <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/WAPL_10000_iced.matrix',
                                         indicesPath = 'data/WAPL_10000_abs.bed',
                                         name = "WAPL",
                                         color = "red")

Hap1_SCC4_10kb <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/SCC4_10kb_iced.matrix',
                                         indicesPath = 'data/SCC4_10kb_abs.bed',
                                         name = "SCC4",
                                         color = "green")

Hap1_WT_40kb <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/WT_40000_iced.matrix',
                                         indicesPath = 'data/WT_40000_abs.bed',
                                         name = "WT",
                                         color = "black")

Hap1_WAPL_40kb <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/WAPL_40000_iced.matrix',
                                         indicesPath = 'data/WAPL_40000_abs.bed',
                                         name = "WAPL",
                                         color = "red")

Hap1_WT_1MB <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/WT_1000000_iced.matrix',
                                         indicesPath = 'data/WT_1000000_abs.bed',
                                         name = "WT", centromeres = centromeres,
                                         color = "black")

Hap1_WAPL_1MB <- construct.experiment(ignore.checks = T,
                                         signalPath = 'data/WAPL_1000000_iced.matrix',
                                         indicesPath = 'data/WAPL_1000000_abs.bed',
                                         name = "WAPL",
                                         centromeres = centromeres,
```

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```
color = "red")
```

Several functions rely on centromere-information. You can add this in the form of a BED-like three-column data.frame when constructing the experiment-object.¹ If not present, the centromeres will be empirically identified by searching for the largest stretch of no coverage on a chromosome.

```
centromeres = read.delim('data/hg19_cytobandAcen.bed',
                        sep = '\t',
                        h = F,
                        stringsAsFactors = F)
head(centromeres)
##      V1      V2      V3
## 1 chr1 121500000 128900000
## 2 chr10 38000000 42300000
## 3 chr11 51600000 55700000
## 4 chr12 33300000 38200000
## 5 chr13 16300000 19500000
## 6 chr14 16100000 19100000
```

¹Please make sure that the chromosome-names match.

The resulting object has several slots. *ICE* and *ABS* are the signal- and index-data.tables, resp., and *RES* is the automatically determined resolution of the Hi-C data. The *NAME*, *COL* and *COMM* are user-provided metadata vectors, where the latter is a free-from slot to store comments and/or output of different functions. The amount of contacts in the *ICE* data.table is likely different from the input-data, because it is scaled to a fixed number of reads (which can be set with the `BPscaling`-option in `construct.experiment`).

```
## List of 10
## $ ICE :Classes 'data.table' and 'data.frame': 105110621
##   obs. of 3 variables:
##   ..$ V1: int [1:105110621] 1 1 ...
##   ..$ V2: int [1:105110621] 1 16 ...
##   ..$ V3: num [1:105110621] 275 ...
##   ...- attr(*, ".internal.selfref")=<externalptr>
##   ...- attr(*, "sorted")= chr [1:2] "V1" ...
## $ ABS :'data.frame': 77404 obs. of 4 variables:
##   ..$ V1: chr [1:77404] "chrM" ...
##   ..$ V2: int [1:77404] 0 0 ...
##   ..$ V3: int [1:77404] 16571 40000 ...
##   ..$ V4: int [1:77404] 1 2 ...
## $ NAME : chr "WT"
## $ RES : num 40000
## $ CHRS : chr [1:25] "chrM" ...
## $ COL : chr "black"
## $ COMM : NULL
## $ MASK : logi(0)
## $ CENTROMERES: NULL
## $ RMCHROM : logi FALSE
```

1.4 Juicebox

We added a convenience script **juicerToGENOVA.py**, to load files from Juicerbox (.hic files). This allows for a fast conversion to signal- and index-files from, for example, data from Sanborn et al.(2015):

```
# Convert data from Sanborn et al. normalised at 10kb resolution:
juicerToGenova.py -C ucsc.hg19_onlyRealChromosomes.noChr.chromSizes \
-JT ~/bin/juicer/AWS/scripts/juicebox_tools.7.0.jar \
-H ~/Downloads/Sanborn_Hap1_combined_30.hic \
-R 10000 \
-force TRUE \
-norm KR \
-o Sanborn_Hap1_combined_30.hic_10kb_KR
```

2 Genome-wide analyses

A good place to start your analyses are some functions on a genome-wide level. We can assess the quality of the library, identify translocations and generate contact probability (aka scaling or interaction decay plots).

2.1 Cis-quantification

Work by the group of Amos Tanay showed that the expected amount of intra-chromosomal contacts is the range of 90 to 93 percent (Olivares-Chauvet et al. 2016). Assuming that any extra inter-chromosomal contacts are due to debris/noise, the user might aspire to get the *cis*-percentages as close to 90% as possible. To compute the percentage of per-chromosome *cis*-contacts, we simply provide `cisTotal.perChrom` with the `exp`-object of interest. It will produce a boxplot of the percentages *cis* per chromosome and draw a red line with the genome-wide percentage (figure 1). If you assign a variable to the output of this function, you will also get a list with the underlying data.

```
cisChrom_out <- cisTotal.perChrom( Hap1_WT_1MB )
```

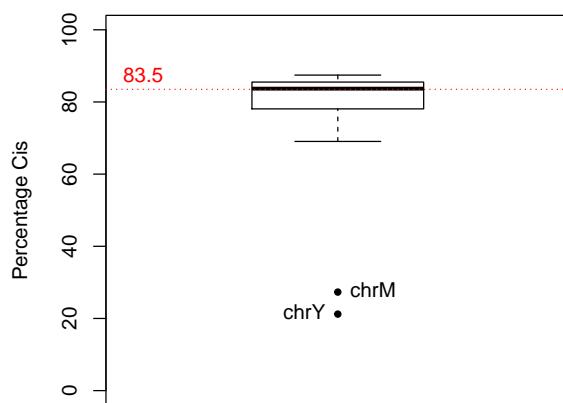


Figure 1: Fraction of cis-contacts per chromosome

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Using the underlying data stored in the variable `cisChrom_out`, we can also inspect the results per chromosome more closely. The list has two entries: a data.frame with the per-chromosome percentages (`perChrom`) and the genome-wide percentage (`genomeWide`). Invoking `plot` will provide a nice overview of the percentages *cis* (figure 2).

```
plot( cisChrom_out$perChrom, las=2 )
abline( h = cisChrom_out$genomeWide, col = 'red' )
```

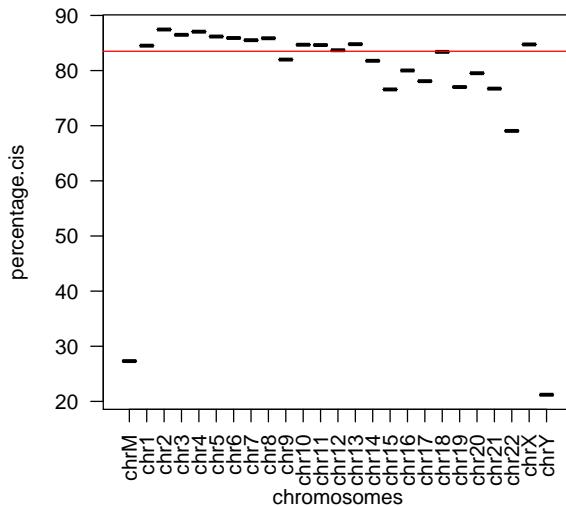


Figure 2: Fraction of cis-contacts per chromosome

Chromosomes 9, 15, 19 & 22 have translocations, which therefore appear to have more trans-contacts, but which in reality are cis-contacts.

2.2 chromosome plots

Hi-C has been shown to be a powerful data-source to detect chromosomal rearrangements (Harewood et al. 2017). To find possible translocations, we can plot the genome-wide enrichment of interactions between all combinations of chromosomes. The values in the matrix are $\log_{10}(\text{observed}/\text{expected})$. The Hap1 cell line has two known translocations, which we can easily see in the resulting plot (figure 3). To narrow-in on this location, you could use the `trans.compartment.plot`-function (discussed below).

```
# Lets remove mitochondrial and Y-chromosomal contacts
chromosomeMatrix(Hap1_WT_1MB, remove = c("chrM", "chrY"))
```

2.3 RCP

The Relative Contact Probability computes the contact probability as a function of genomic distance, as described in (Lieberman-Aiden and Berkum 2009). This can be computed for a specific set of chromosomes or genome-wide. To be able to ignore centromeric contacts (which have a aberrant RCP), centromeric information is need. This is taken from the experiment-object or found emperically by comparing trans-interactions.

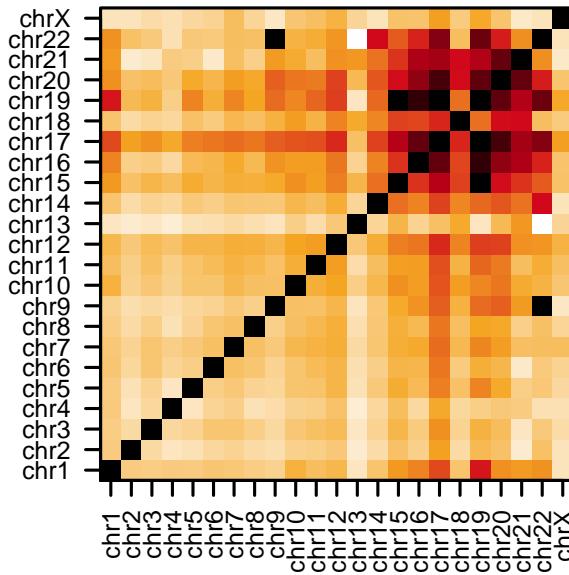


Figure 3: Chromosome matrix

The two known translocations of Hap1 cells are easily identified (15-19 & 9-22).

```
RCP_out135 <- RCP(experimentList = list(Hap1_WT_40kb, Hap1_WAPL_40kb),
                     chromsToUse = c('chr1', 'chr3', 'chr5'))
##
```

The user can decide to plot the RCP per chromosome. If the data is sparse, a LOESS-smoothing could be convenient. It takes the color and name from the experiment-objects. If we look at the resulting plot, we can see that the $\Delta WAPL$ has more interactions in the $[\pm 800kb, \pm 2Mb]$ range (figure 4). The sizes of TADs are fall into this range, so a next step could be to dive into the TAD-specific analyses (discussed below). Moreover, the $\Delta WAPL$ has less interactions in the far-*cis* range ($[10Mb, 100Mb]$): A- and B-compartments are often of these sizes, so a next step could be to look more into compartmentalisation with `cis.compartment.plot` or `trans.compartment.plot`, for example.

```
# Plot RCP: per-chromosome
visualise.RCP.ggpplot(RCPdata = RCP_out135,
                      smooth = T, # use a LOESS smoothing
                      combine = F) # Don't merge data from all chromosomes
```

2.3.1 combined

It is also possible to combine all available data into a genome-wide RCP-plot (figure 5). The average probabilities are then plotted, with optional errorbars for the standard error of the mean.

```
# Plot RCP: combined
visualise.RCP.ggpplot(RCPdata = RCP_out135,
                      smooth = F, # do not use a LOESS smoothing
                      combine = T) # Merge data from all chromosomes
```

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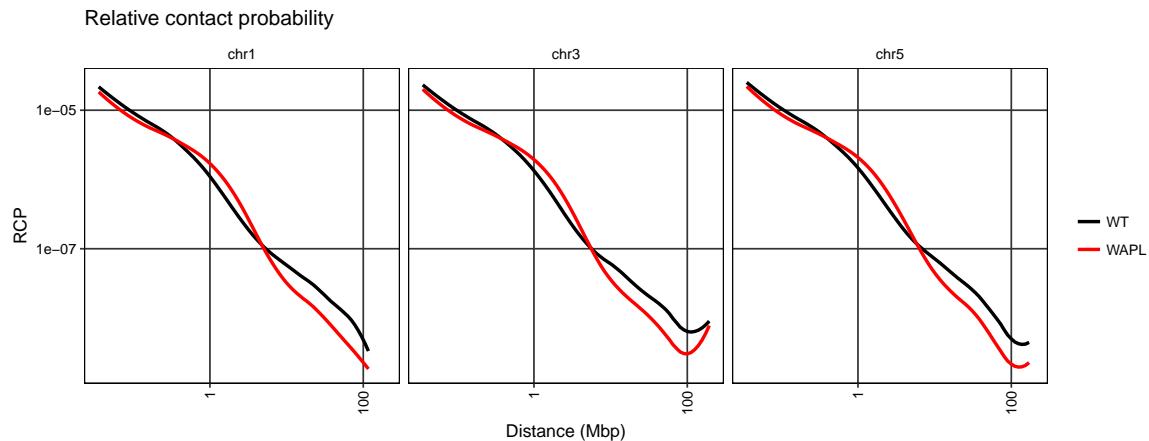


Figure 4: RCP

Every facet shows the RCP of one chromosome.

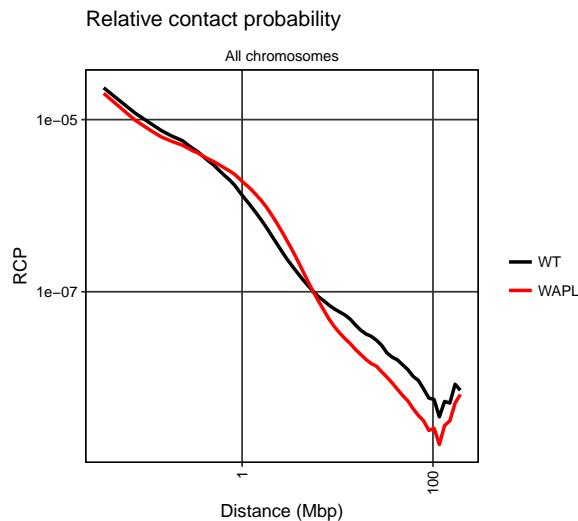


Figure 5: RCP

All data combined in one plot.

2.3.2 regions

But what if you want to compare the contact probabilities of specific regions, like Cohesin- or CTCF-bound regions? For this, we added the possibility to add a list of BED-data_frames to the `bedList`-argument. Under the hood, we perform a standard per-arm RCP (thus still enabling users to also set the `chromsToUse`-parameter), whereafter we filter out Hi-C bins that do not have entries in the dataframe(s) of `bedList`. The same plot-function can be used: different BED-files will have different line-types. The fact that we use linetype for the `bedList` entries, allows us to still use multiple samples in `experimentList`, as shown in figure 6. But if you only provide one experiment-object, we will use different line-colours of the different BEDs.

```
CTCF = read.delim('data/CTCF_WT_motifs.bed', h = F)
SMC1 = read.delim('data/SMC1_WT_peaks.narrowPeak', h = F)
```

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```
RCP_out = RCP(experimentList = list(Hap1_WT_40kb ),
               bedList = list("CTCF" = CTCF,
                             'Cohesin' = SMC1),
               chromsToUse = c('chr1','chr3', 'chr5'))

visualise.RCP.ggplot(RCPdata = RCP_out)
```

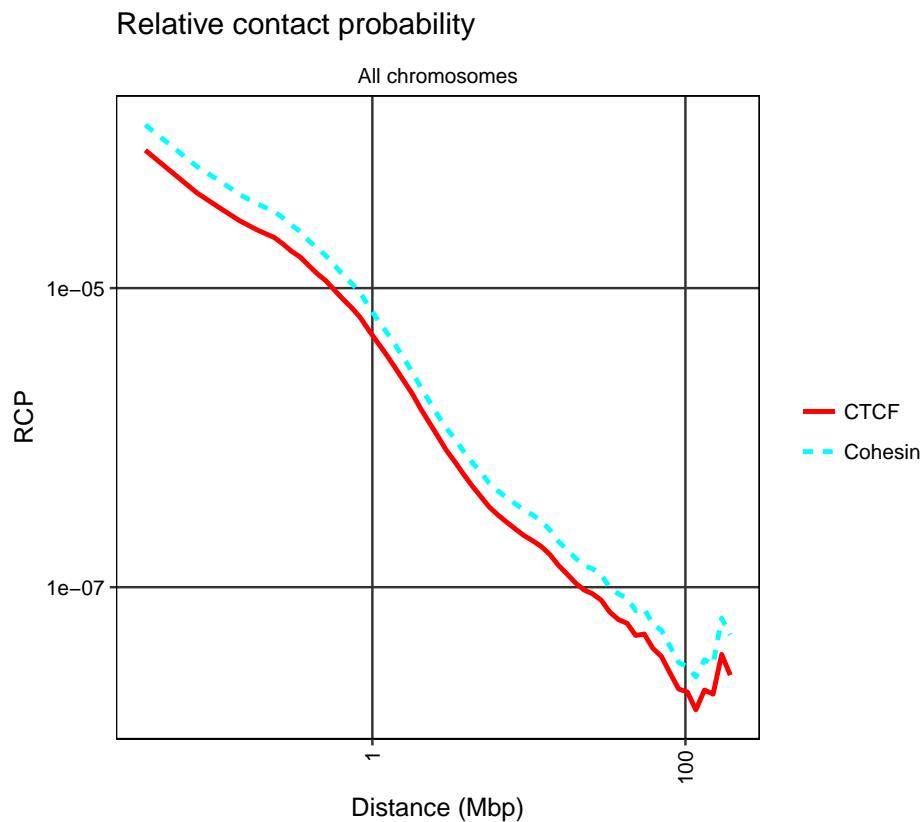


Figure 6: RCP with BEDs

We can also add BEDs as sites to compute the RCP.

2.4 A- and B-compartments

```
H3K27acPeaks = read.delim('data/H3K27ac_WT.narrowPeak', h = F)
CS = read.delim('/DATA/oidBackup/WAPL_Project/Hi-C/analysis/PCA/PCA/100kb/WT.PC1.bedGraph', h = F, skip = 1)
saddle_WT = saddleBins(exp = Hap1_WT_40kb,
                       ChIP = H3K27acPeaks, CS = CS,
                       chromsToUse = paste0('chr', 1:15),
                       nBins = 50,
                       verbose = T)

saddle_WAPL = saddleBins(exp = Hap1_WAPL_40kb,
                        ChIP = H3K27acPeaks, CS = CS,
```

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```
chromsToUse = paste0('chr', 1:15),  
nBins = 50,  
verbose = F)
```

2.4.1 Saddle-plot

```
visualise.saddle(SBoutList = list(saddle_WT, saddle_WAPL),  
crossLines = T,  
addText = T,  
zlim = c(-0.5,0.5),  
EVlim = c(-50,50))
```

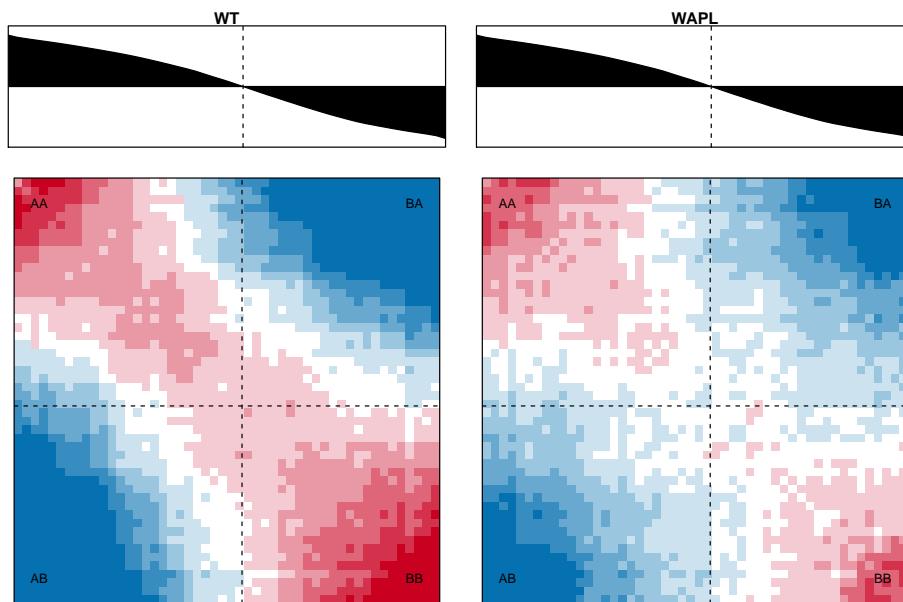


Figure 7: A saddle-plot

2.4.2 Compartment-strength

```
visualise.compartmentStrength(list(saddle_WT,  
saddle_WAPL))
```

```
visualise.compartmentStrength(list(saddle_WT,  
saddle_WAPL), showInteractions = T)
```

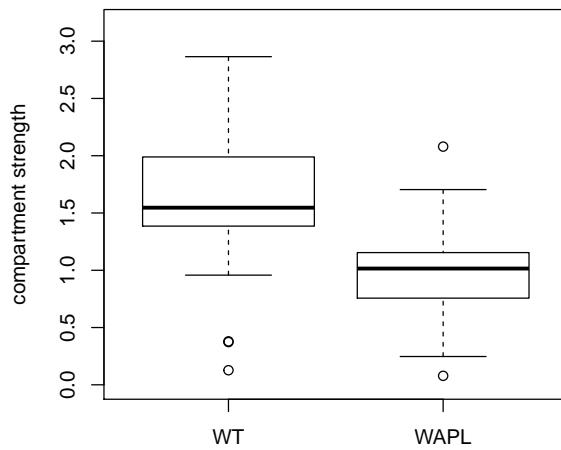


Figure 8: The per-arm compartment strength

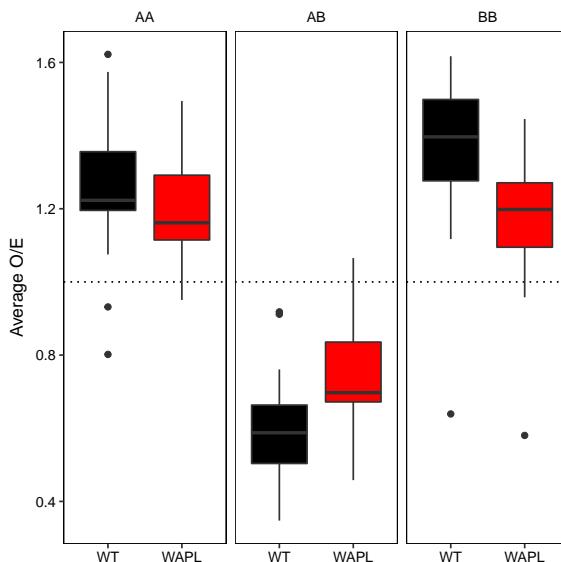


Figure 9: The per-arm compartment strength

3 Interaction plots

GENOVA has several plotting-functions for genomic loci. `cis.compartment.plot` and `trans.compartment.plot` provide an easy way to plot whole chromosome arms, including compartmentalisation-score tracks. For more zoomed-in plots `hic.matrixplot` can be used. This function also allows rich annotation and between-experiment comparison possibilities. All functions try to guess the most appropriate color-scale limits, but finer control of this can be gotten by setting the `cut.off`-argument.

3.1 cis-interactions

The compartmentalisation of the chromatin into A and B was already described in the original Hi-C paper (Lieberman-Aiden and Berkum 2009). Several papers have described the loss of compartmentalisation when the Cohesin complex is stabilised (Haarhuis et al. 2017, @Wutz2017, @Gassler2017). To view this interesting level of chromatin organisation, we can use `cis.compartment.plot`. With this, we can plot one arm of a chromosome with the compartment-score plotted above. To infer which compartment is A (viewed as the active state) and which is B, we can add a BED-data.frame of ChIP-seq peaks from active histone marks (e.g. H3K27ac, H3K4me1). In figure 10 you can see the resulting plots, where you can see that the checkerboard-pattern in the matrix and the amplitude of the compartment-score are diminished in the WAPL-knockout.

```
H3K27ac_peaks = read.delim('data/H3K27ac_WT.narrowPeak', h = F)

cis.compartment.plot(exp = Hap1_WT_40kb,
                     chrom = 'chr14',
                     arm = 'q',
                     cs.lim = 1.75, # max compartment-score
                     cut.off = 15,
                     chip = H3K27ac_peaks)

cis.compartment.plot(exp = Hap1_WAPL_40kb,
                     chrom = 'chr14',
                     arm = 'q',
                     cs.lim = 1.75,
                     cut.off = 15,
                     chip = H3K27ac_peaks)
```

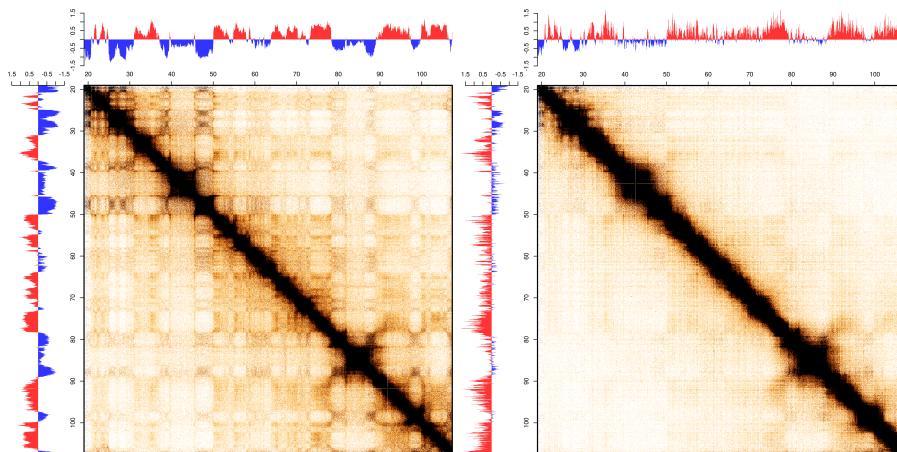


Figure 10: Cis compartment plot: WT vs WAPL

Stabilised Cohesin-mediated loops by WAPL-knockout leads to loss of compartments.

The compartment-score is calculated by performing an eigenvector decomposition on the correlation-matrix of the expected over expected matrix. To view this O/E matrix, we can set the `obs.exp`-option to TRUE. This view gives a visually better view of the A- and B-compartments (figure 11).

```
cis.compartment.plot(exp = Hap1_WT_40kb,
                     chrom = 'chr20',
                     arm = 'q',
                     cut.off = 1,
                     obs.exp = T,
                     chip = H3K27ac_peaks)
```

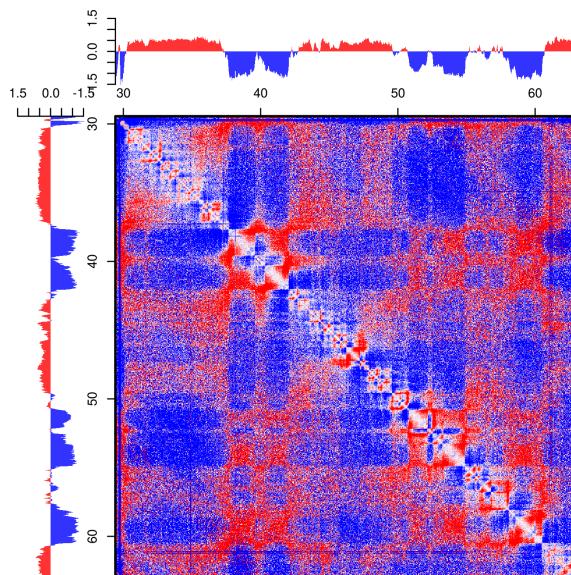
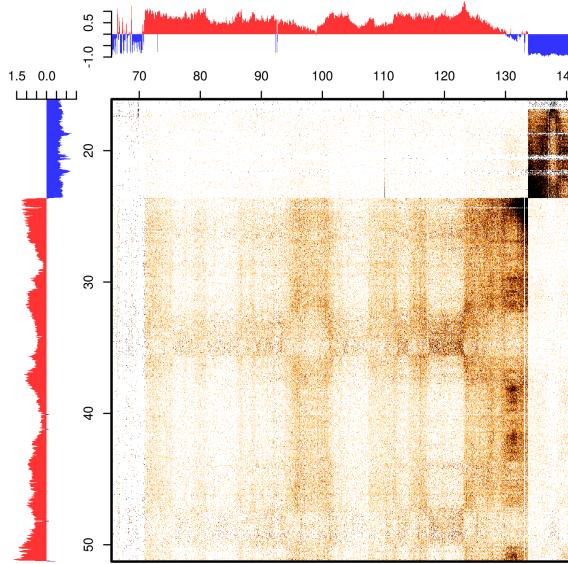


Figure 11: Cis compartment plot
Observed over expected.

3.2 *trans*-interactions

As could be seen above, A-compartments interact more with other A-compartments and the same is true for B-compartments. However, is the same true for *trans*? The function `trans.compartment.plot` will allow the user to plot a trans-matrix (i.e. a matrix of the arms of two different chromosomes) along with the respective *cis* compartment-scores. This function could also be used to investigate chromosomal translocations: the 9q;22q translocation can be clearly seen if we use this function, as in figure 12.

```
trans.compartment.plot(exp = Hap1_WT_40kb,
                      chrom1 = 'chr9',
                      arm1 = 'q',
                      chrom2 = 'chr22',
                      arm2 = 'q',
                      cut.off = 10,
                      chip = H3K27ac_peaks)
```

**Figure 12: Trans compartment plot**

The t(9q;22q) translocation is easily identified.

3.3 matrix plots

To produce richly annotated zoomed-in (i.e. max 10Mb) plots of specific regions, we use the `hic.matrixplot` function. In this, we can use one or two experiment objects: two can be shown either in diff-mode (the difference between the two) or upper/lower triangle mode. TAD- and loop-annotations can be added, as well as bigwig- and bed-tracks. Moreover, genemodel-files can be added. In this section, we will build up to a final, fully annotated, matrix from a humble one-experiment plot (figure 13).

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7',
               start = 25e6,
               end=30e6,
               cut.off = 50) # upper limit of contacts
```

3.3.1 two experiments

Adding a second experiment will give us the option of `coplot`, which can be `dual` (default) or `diff`. The first shows `exp1` in the upper triangle and `exp2` in the lower. `Exp1` is subtracted from `exp2` in `diff`-mode: red is therefore more contacts in `exp2` and blue denotes more contacts in `exp1` (figure 14).

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               exp2 = Hap1_WAPL_10kb,
               chrom = 'chr7',
               start = 25e6,
               end=30e6,
               cut.off = 50) # upper limit of contacts
```

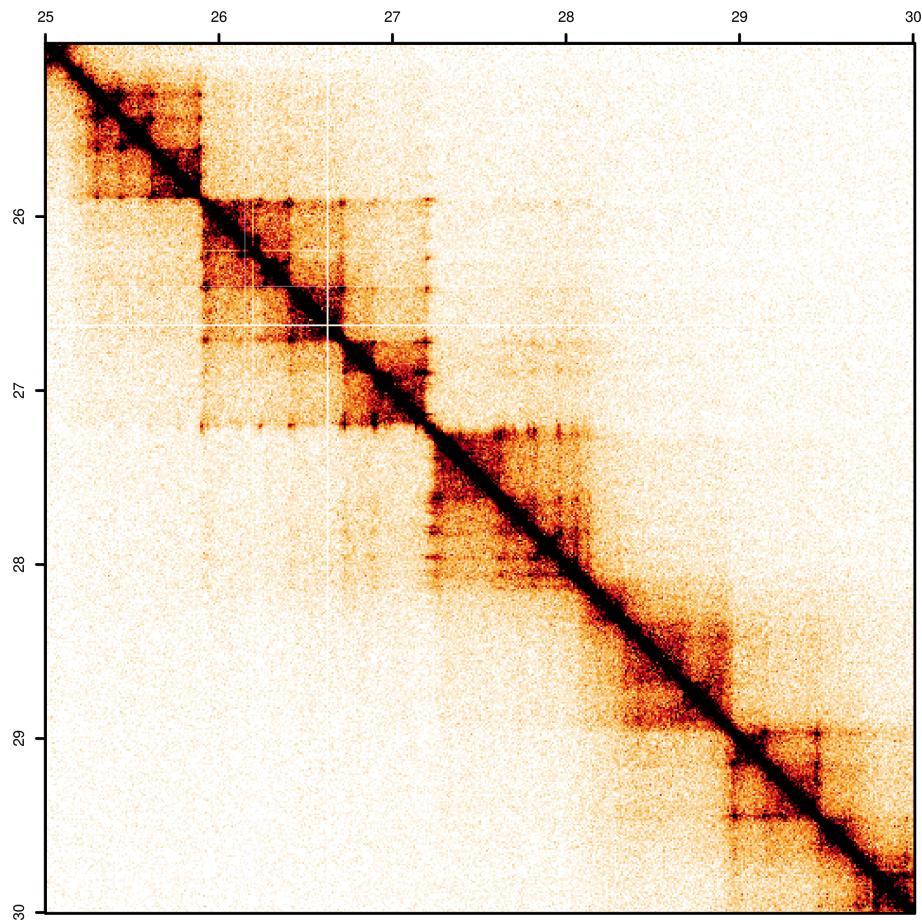


Figure 13: Hi-C matrixplot

Simplest example: one experiment, no annotation

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               exp2 = Hap1_WAPL_10kb,
               coplot = 'diff',
               chrom = 'chr7',
               start = 25e6,
               end=30e6, # upper limit of contacts
               cut.off = 25)
```

3.3.2 TADs and loops

It can be very useful to annotate the matrix with the positions of TADs and loops: take, for example, the situation where these structures are altered in a knockout for example. We are going to use the TAD- and loop-calls of WT Hap1 20-kb matrices from Haarhuis et al. (2017), generated with HiCseg (Lévy-Leduc et al. 2014).

Lets load some TAD- and loop-annotations:

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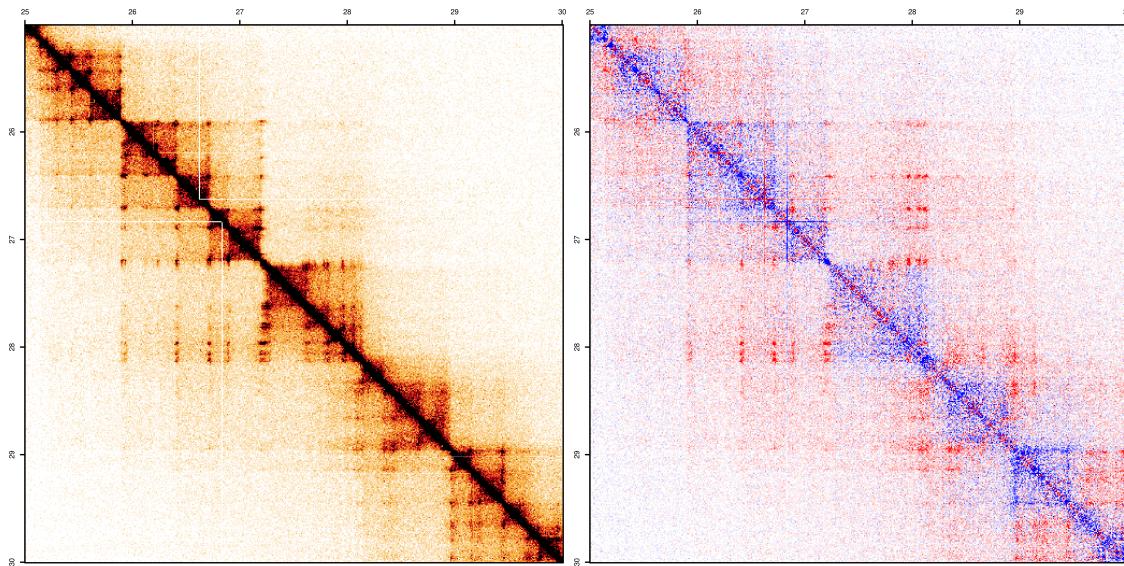


Figure 14: Hi-C matrixplot with two experiments: dual vs diff mode

The extended loops in the WAPL knockout are easily seen at around 28Mb in the lower triangle in dual-mode (left panel) and as red points in diff-mode (right panel).

```
WT_TADs = read.delim('data/WT_hicseg_TADs.bed', h = F)
WT_Loops = read.delim('data/WT_HICCUPS.bedpe', h = F, skip = 1)
sanborn2015_Loops = read.delim('data//GSE74072_Hap1_HICCUPS_looplist.txt.gz')
```

Add them to the plot by using the `tad-` and `loops-`arguments. Both can be plotted in one or both of the triangles and colored as wished (figure 15). Since loops are very small in a hic-matrixplot, they will be fully overlapped by the loop-annotations. To overcome this, we expand the annotations with a fixed bp using `loops.resize`. This will lead to a more box-like annotation surrounding the loop. If you have multiple loop- or tad-dataframes, you can provide a list of them: `tads = WT_TADs` for one dataframe or `tads = list(WT_TADs, KO_TADs)` for multiples, for example. All other arguments (e.g. type, color) will be recycled if only one is given.

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7',
               start = 25e6,
               end=30e6,
               loops = WT_Loops, # see APA
               loops.color = 'blue', # purple loops
               loops.type = 'upper', # only plot in upper triangle
               loops.resize = 20e3, # expand for visibility
               tads = WT_TADs, # see ATA
               tads.type = 'lower', # only plot in lower triangle
               tads.color = '#ffd92f', # green TAD-borders
               cut.off = 25) # upper limit of contacts

hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7',
               start = 25e6,
```

```
end=30e6,
loops = list(WT_Loops, sanborn2015_Loops), # see APA
loops.color = c('blue','green'), # purple loops
loops.type = c('upper','lower'), # only plot in upper triangle
loops.resize = c(20e3,20e3), # expand for visibility
tads = WT_TADs, # see ATA
tads.type = 'lower', # only plot in lower triangle
tads.color = '#ffd92f', # green TAD-borders
cut.off = 25) # upper limit of contacts
```

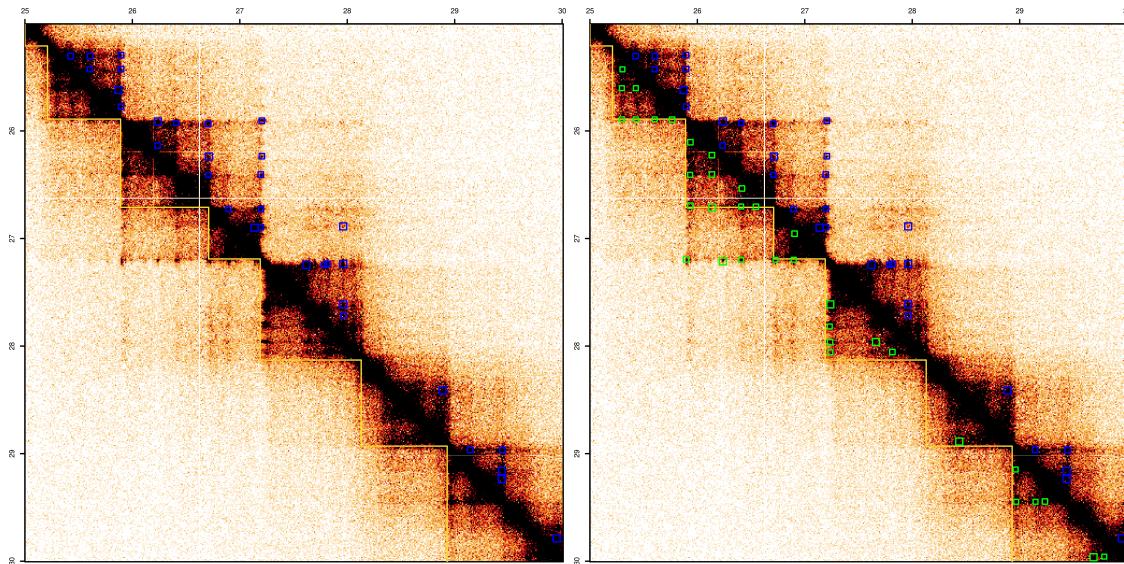


Figure 15: Hi-C matrixplot: TAD- and loop-annotations

Left: one loops-dataframe, right: a list of two dataframes from Haarhuis et al. (2017) and Sanborn et al. (2015).

3.3.3 BigWigs and BEDs

Manipulation of CTCF-binding sites can result in loss or gain of loops and/or TADs (Wit et al. 2015). If one is interested in the effects of a knockout on the binding of a protein in combination with changes in interaction frequencies, adding ChIP-seq signal or -peaks to the matrix can be very helpfull. Two tracks above and two tracks to the left can be added. These can be either BED-like data.frames or the paths the .bw files. For example, lets load a BED6-file (chrom, start, end, name, score, and strand²) of CTCF-motifs under CTCF-ChIP peaks. The argument type can be set to either *triangle* or *rectangle*: triangle is nice to use if you want to look at the orientation of the BED-entries (figure 16). If you only have a three column BED, then the output will always be *rectangle*.

²<https://genome.ucsc.edu/FAQ/FAQformat.html>

```
CTCF = read.delim('data/CTCF_WT_motifs.bed', h = F)
SMC1 = read.delim('data/SMC1_WT_peaks.narrowPeak', h = F)
```

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Table 3: A data.frame holding a standard BED6 format

V1	V2	V3	V4	V5	V6
chr1	237749	237768	GCAGCACCAGGTGGCAGCA	1412	+
chr1	714180	714199	CGGCCACCAGTAGGCAGCG	1428	-
chr1	793458	793477	CCACCAGCAGGTGGCCTCC	1160	-

Moreover, we can use a bigwig (.bw) file to plot a track. For this example, we are using a SMC1 ChIP-seq track from (Haarhuis et al. 2017). We need the `bigwrig` package, which is easily installed from github using `devtools::install_github()`. The `yMax` argument is handy if you want to compare bigwig-tracks: it lets you set the y-axis maximum.

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7', start = 26.75e6, end=28.5e6,
               loops = WT_Loops, # see APA
               loops.color = '#998ec3', # purple loops
               loops.type = 'upper', # only plot in upper triangle
               loops.resize = 20e3, # expand for visibility
               type = 'triangle',
               chip = list('data/SMC1_WT.bw', # inner top
                           CTCF),# outer-top
               symmAnn = F, # place annotations also on left side
               cut.off = 65) # upper limit of contacts
```

3.3.4 Genes

(Dixon et al. 2012) showed that housekeeping-genes are enriched in the vicinity of TAD-borders. Another interesting question could be whether differentially expressed genes are also found near TAD-borders or binding sites of specific proteins when studying a knockout. These type of questions can be tackled by adding the appropriate gene-models to `hic.matrixplot`. To do this, we make use of the data.fame, where each row is an exon from a gene. There are several ways to get this. One of the easiest is to use biomart to get exon-coordinates. This can be done with the biomaRt-package or via the web-based service. For this example, we downloaded data of all exons from the Ensembl biomart and plotted both the BED-file and the genes (figure 17).

```
# features downloaded:
## Gene stable ID & Transcript stable ID & Chromosome/scaffold name &
## Transcript start (bp) & Transcript end (bp) & Exon region start (bp) &
## Exon region end (bp) & Strand
martExport = read.delim('data/mart_export.txt.gz', stringsAsFactors = F)
colnames(martExport) = c('ENSG','ENST','chrom' , # change column names
                       'txStart' , 'txEnd' ,
                       'exonStart' , 'exonEnd' , 'strand')
martExport$chrom = gsub(martExport$chrom, # add chr-prefix
                       pattern = '^',
                       replacement = 'chr')
martExport$strand = ifelse(martExport$strand == 1, '+', "-") # 1/-1 to +/-
```

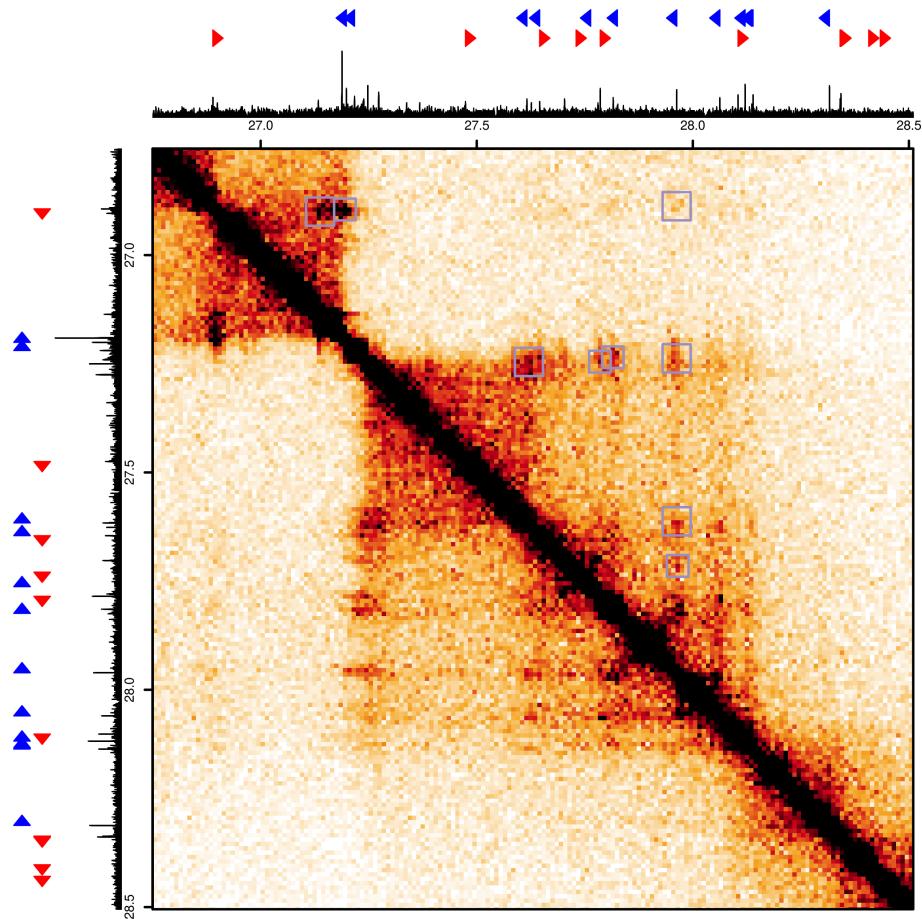


Figure 16: Hi-C matrixplot: ChIPseq

A BED-file of CTCF-sites is plotted at the top and a coverage-track of SMC1 ChIP-seq is plotted beneath this. The symmAnn-option leads to the same tracks being plotted on the left.

Table 4: A data.frame holding the needed columns for plotting genes

chrom	txStart	txEnd	exonStart	exonEnd	strand
chr1	44457280	44462200	44457519	44457676	+
chr1	44457280	44462200	44457280	44457418	+
chr1	44457280	44462200	44457884	44458059	+
chr1	44457280	44462200	44458195	44458311	+
chr1	44457280	44462200	44459559	44459636	+

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7', start = 26.75e6, end=28.5e6,
               genes = martExport,
               cut.off = 65) # upper limit of contacts
```

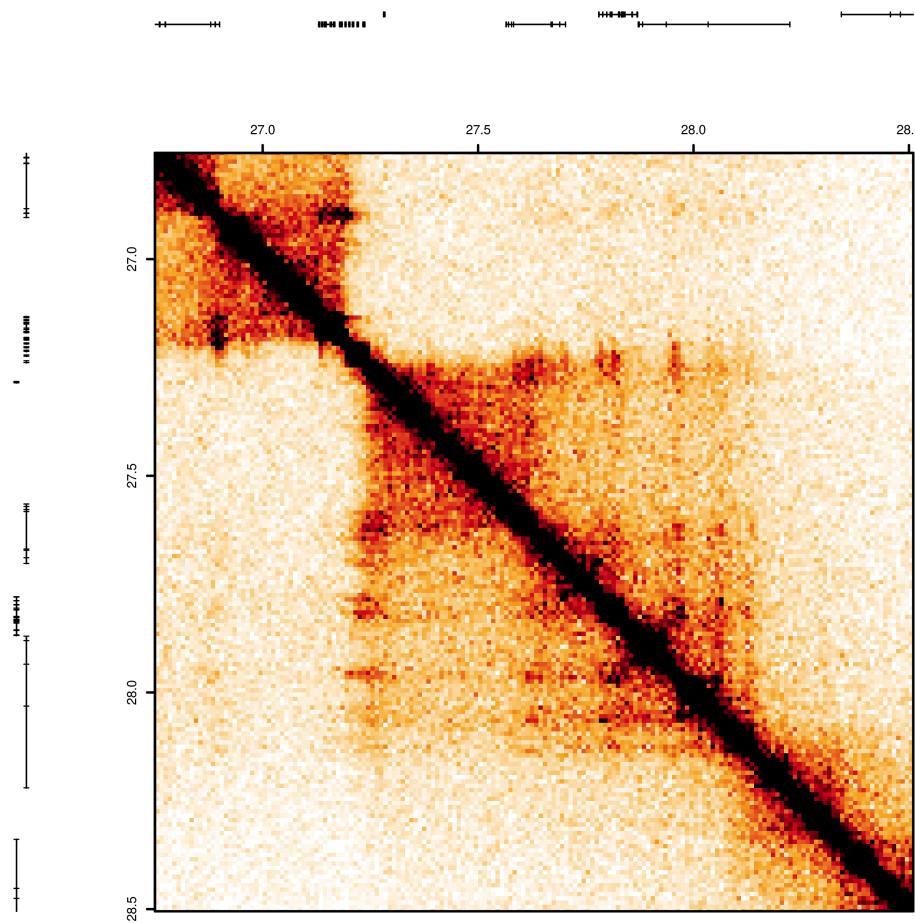


Figure 17: Hi-C matrixplot: genes

3.3.5 Everthing together

Finally, we can combine all these options in one. This may be complete overkill, but it could be quite handy. In this example, we can see that most TAD-borders and loop-anchors have clear SMC1- and CTCF-signal (figure 18). Both these are expected to be found at these locations according to the *chromatin extrusion model*. Moreover, we can also see that the CTCF-orientation of the upstream and downstream loop-anchor are forward and reverse, resp. This *convergent rule* is a known feature of loops (de Wit et al. 2015).

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7',
               start = 25e6,
               end=28.5e6,
               loops = WT_Loops, # see APA
               loops.color = '#998ec3', # purple loops
               loops.type = 'upper', # only plot in upper triangle
               loops.resize = 20e3, # expand for visibility
               genes = martExport,
               bed.col = 'black',
               chip = list('data/SMC1_WT.bw', # inner-top
```

```

SMC1, # outer-top
'data/SMC1_WT.bw', # inner-left
CTCF), # outer-left
tads = WT_TADs, # see ATA
tads.type = 'lower', # only plot in lower triangle
tads.color = '#91cf60', # green TAD-borders
cut.off = 50) # upper limit of contacts

```

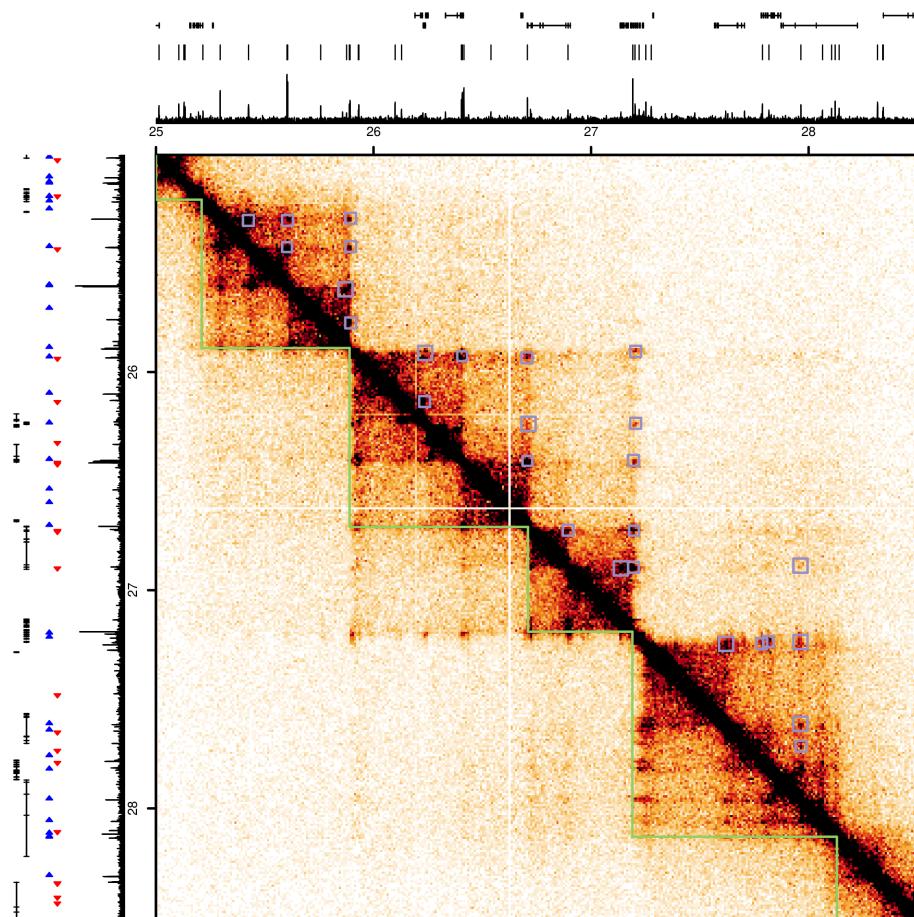


Figure 18: Hi-C matrixplot: a complex case

Loops and TADs are annotated within the Hi-C matrix. On the top annotation-bar, we have plotted the ChIP-seq signal and peaks of SMC1. On the left annotation-bar are the ChIP-seq signal and peaks (with orientation) of CTCF. Genes are plotted on both annotation-bars.

4 TADs

Topologically Associated Domains (TADs) are $\pm 0.8 - 2 Mb$ regions, which are seen as triangles in the matrix: regions that have more interactions within than outside. GENOVA has a repertoire of functions to generate and analyse TADs. First, we will use the insulation score to

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call TADs and compare the strength of TAD-borders between samples. Next, we will explore ATA to analyse aggregates of TADs. Finally, wil investigate wheter TADs interact with their neighbouring TADs.

4.1 Insulation

To estimate the strength of TAD-borders, we can look at the insulation-score (Crane et al. 2015). At a TAD-border, this score reaches a local minimum: the lower the score, the stronger the insulation. We can generate this for a specific sliding-window size with `genome.wide.insulation`. The choice of window-size is quite tricky, since smaller will be sensitive to very local effect (i.e. mapping-errors, loops), while too big windows will lead a an underrepresentation. Luckily, we can generate a domainogram of a range of window-sizes for a specific genomic region with `insulation.domainogram`.

4.1.1 Domainogram

To make a domainogram, we simply choose our experiment and our region of interest³. The window-size is directly proportional to the amount of Hi-C bins.

```
layout(matrix(c(1,2,3), nrow = 1, ncol = 3), widths = c(5,1,0.1) )
insulation.domainogram(Hap1_WT_10kb,
                        'chr7',
                        25.5e6,
                        30e6,
                        window.size1 = 1,
                        window.size2 = 101,
                        step = 2)
cols = c("#f03b20", "#ffeda0", "white", "#31a354")
color.bar(colorRampPalette(cols)(100), -1, nticks = 5)
```

³The colorbar is there to get you acquainted with this type of plot.

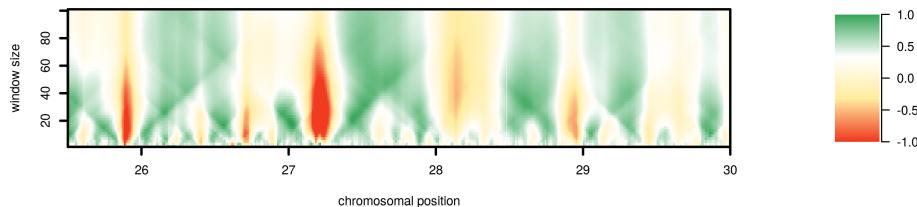


Figure 19: Insulation domainogram

Insulation-hotspots can be identified in red, which are regions with a very negative score.

A nice feature of `hic.matrixplot` is that if you use it without plotting anything on the sides (i.e. genes and/or ChIP-tracks), you can insert other plots. This allows us to plot the domainogram directly under the matrix, making it very easy to compare the insulation with the actual data (figure 20).

```
hic.matrixplot(exp1 = Hap1_WT_10kb,
               chrom = 'chr7',
               start = 25e6,
               end=29e6,
               tads = WT_TADs, # see ATA
```

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```
tads.type = 'upper', # only plot in lower triangle
tads.color = '#91cf60', # green TAD-borders
cut.off = 25, # upper limit of contacts
skipAnn = T) # skip the outside annotation

insulation.domainogram(Hap1_WT_10kb,
    'chr7',
    25e6,
    29e6,
    window.size1 = 1,
    window.size2 = 111,
    step = 2,
    axes = F)
```

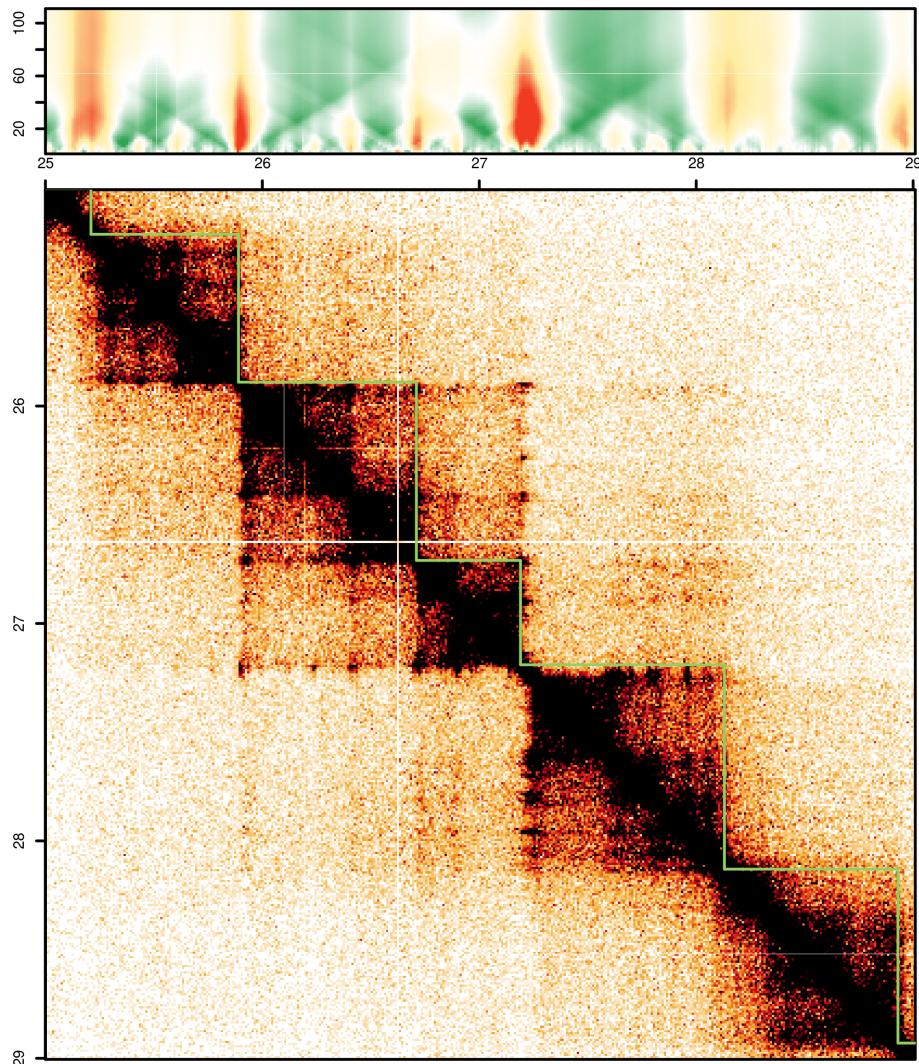


Figure 20: Insulation domainogram with Hi-C matrix

The insulation-hotspots are the sites where HiC-seg has called a TAD-border.

4.1.2 Computing the insulation score

To get the genome-wide insulation score in .bedgraph-format,⁴ we provide the `genome.wide.insulation` with an experiment-object and the window-size of choice. As can be seen in the domainogram above, at $W = 25$ we will catch the majority of the hotspots, while limiting the amount of noise.

⁴BED3 + signal column

```
Hap1_WT_10kb_insulation = genome.wide.insulation(hic = Hap1_WT_10kb,
                                                    window.size = 25)
Hap1_SCC4_10kb_insulation = genome.wide.insulation(hic = Hap1_SCC4_10kb,
                                                    window.size = 25)
```

4.1.3 Insulation-heatmap

We can align the border-strength of TADs in multiple samples to a specific BED-file, to compare “*borderness*” of feature. For example, let’s use the TAD-borders from (Haarhuis et al. 2017). In figure 21 we can see that the average signal drops at the border (which is to be expected) and that this is a genome-wide feautre, as we see in the heatmap.

```
insulation.heatmap_out = insulation.heatmap(
    insulationList = list(WT = Hap1_WT_10kb_insulation,
                          SCC4 = Hap1_SCC4_10kb_insulation ),
    bed = WT_TADs,
    zlim = c(-.5, 0.25), # zlim.
    profileZlim = c(-.75, -.1) # zlim for the profile
)
```

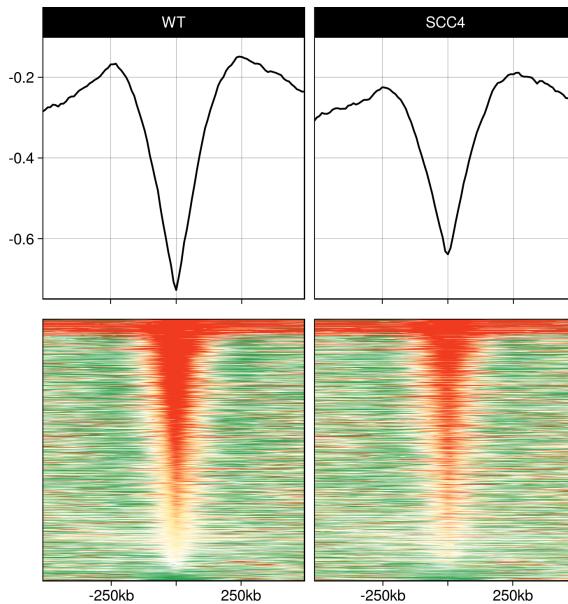


Figure 21: Insulation heatmap

The upper panel shows the average score. Each row is a TAD-border in the lower panel.

4.2 Call TADs

Use the domainogram to choose a good window size:

```
Hap1_WT_10kb$INSULATION= Hap1_WT_10kb_insulation  
  
TADcalls = insulation.callTAD(Hap1_WT_10kb,BEDCOLOR = "#7ec0ee")  
  
hic.matrixplot(exp1 = Hap1_WT_10kb,  
               chrom = 'chr7',  
               start = 25e6,  
               end=29e6,  
               tads = TADcalls, # see ATA  
               tads.type = 'lower', # only plot in lower triangle  
               tads.color = '#91cf60', # green TAD-borders  
               cut.off = 25) # upper limit of contacts
```

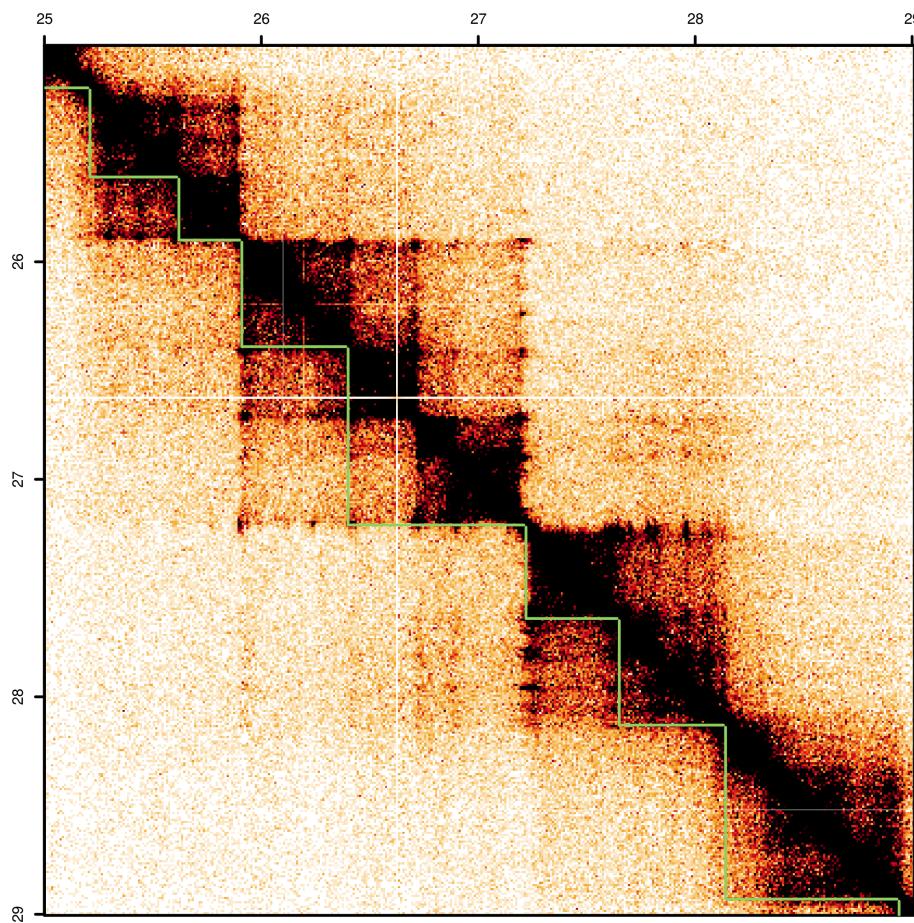


Figure 22: TADs called within GENOVA

4.3 ATA

```
TADcalls = WT_TADs
ATA_Hap1_WT <- ATA(experiment = Hap1_WT_10kb, verbose = F,
                     tad.bed = TADcalls)

ATA_Hap1_WAPL <- ATA(experiment = Hap1_WAPL_10kb, verbose = F,
                      tad.bed = TADcalls)
```

We can use `visualise.ATA.ggplot` to combine the ATA-results.

```
visualise.ATA.ggplot(stackedlist = list('WT' = ATA_Hap1_WT,
                                         'WAPL' = ATA_Hap1_WAPL), # a named list
                      title = "Hap1 Hi-C vs WT TADs",
                      zlim1 = c(0,26),
                      zlim2 = c(-5,5),
                      focus = 1) # which entry to use as comparison
```

Hap1 Hi-C vs WT TADs

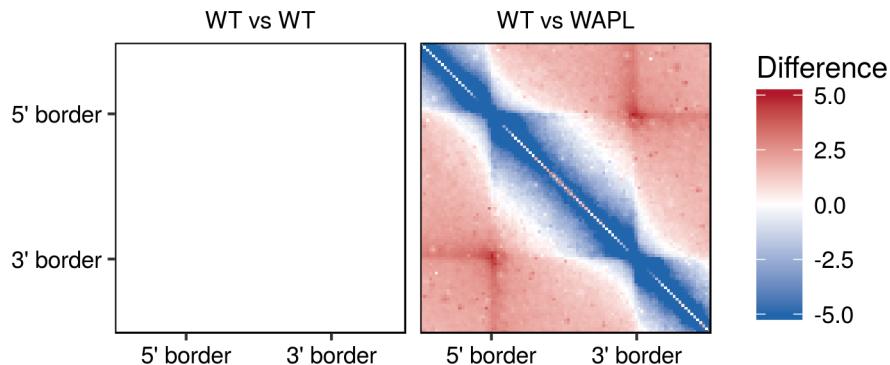
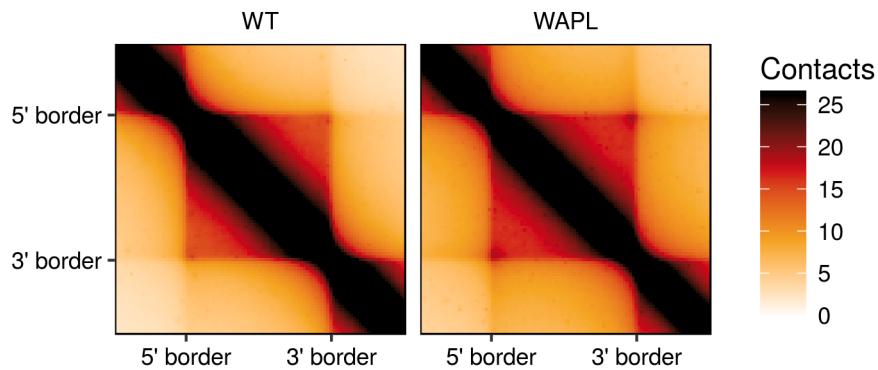


Figure 23: ATA

In the WAPL-knockout, we see a decrease of contacts within the TAD, but an increase at the corner.

4.4 TAD+N

```
TAD_N_WT    <- intra.inter.TAD.contacts(TAD = WT_TADs,
                                         max.neighbor = 10,
                                         exp = Hapl_WT_10kb)
TAD_N_WAPL <- intra.inter.TAD.contacts(TAD = WT_TADs,
                                         max.neighbor = 10,
                                         exp = Hapl_WAPL_10kb)
```

We can compute the enrichment of contacts between TADs with the differential.TAD.dotplot-function.

```
differential.TAD.dotplot(exp1 = TAD_N_WT, # denominator
                           exp2 = TAD_N_WAPL) # numerator
```

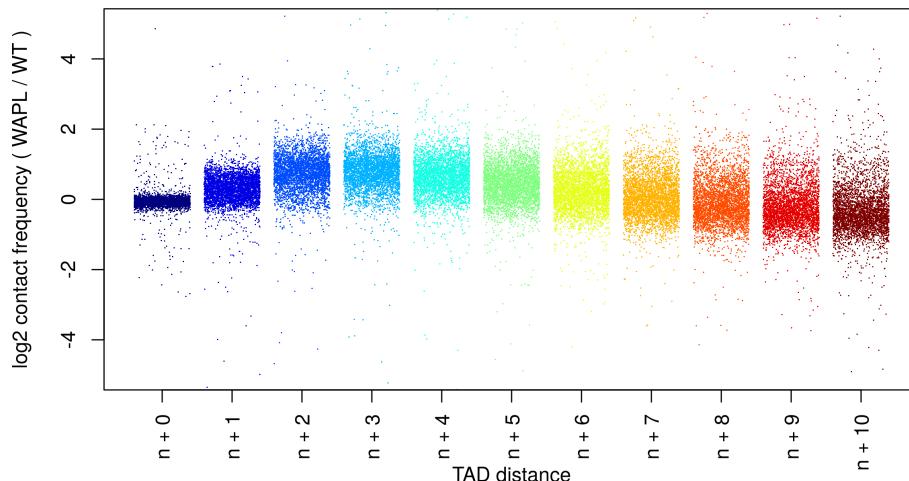


Figure 24: Differential TAD-analysis

Experiment 2 (WAPL) has more interactions between neighbouring TADs compared to wild type.

Or show it as a scatterplot. With differential.TAD.scatterplot, you can choose to add a diagonal line with line = T. Furthermore, you can choose to zoom in by allData == F.

```
par(mfrow = c(1,2), pty = 's')
differential.TAD.scatterplot(exp1 = TAD_N_WT, # x
                             exp2 = TAD_N_WAPL,
                             allData = T,
                             main = 'allData == T') # y
differential.TAD.scatterplot(exp1 = TAD_N_WT, # x
                             exp2 = TAD_N_WAPL,
                             allData = F,
                             main = 'allData == F') # y
```

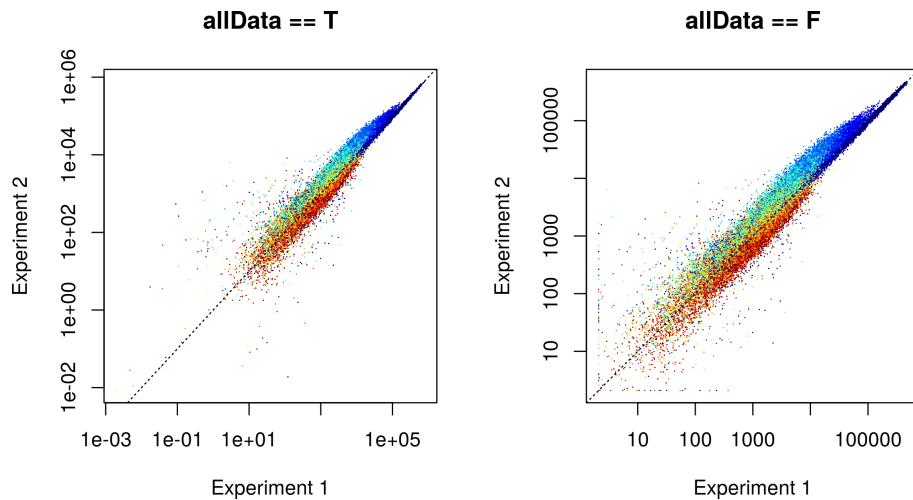


Figure 25: Differential TAD-analysis: scatterplot

Experiment 2 (WAPL) has more interactions between neighbouring TADs compared to wild type.

5 Loops

For this section, we are using the extended loops from Haarhuis et al. (2017). These are the anchor-combinations of the merged loop-calls of WT Hap1 5-, 10- and 20-kb matrices, generated with HICCUPS (Rao et al. 2014).

```
WT_Loops_extended = read.delim('data/WT_3Mb_extended_loops.bed', h = F)
```

Table 5: A data.frame holding a standard BEDPE format

Columns 1-3 are describe the 5' anchor, columns 4-6 describe the 3' anchor.

V1	V2	V3	V4	V5	V6
chr11	875000	900000	chr11	2020000	2025000
chr11	875000	900000	chr11	2162500	2187500
chr11	875000	900000	chr11	2020000	2025000
chr11	875000	900000	chr11	2020000	2030000
chr11	875000	900000	chr11	1940000	1945000

5.1 APA

Explain smallthreshold

```
APA_Hap1_WT_extended <- APA(experiment = Hap1_WT_10kb,
                                loop.bed = WT_Loops_extended)

APA_Hap1_WAPL_extended <- APA(experiment = Hap1_WAPL_10kb,
                                 loop.bed = WT_Loops_extended)
```

We can use `visualise.APA.ggplot` to combine the APA-results.

```
visualise.APA.ggplot(APAlist = list('WT' = APA_Hap1_WT_extended,
                                    'WAPL' = APA_Hap1_WAPL_extended), # a named list
                      title = "Hap1 Hi-C vs extended loops",
                      zTop = c(0,9.5), # set the zlims of the upper row
                      zBottom = c(-5,5),
                      focus = 1) # which item in APAlist to use as comparison
```

Hap1 Hi-C vs extended loops

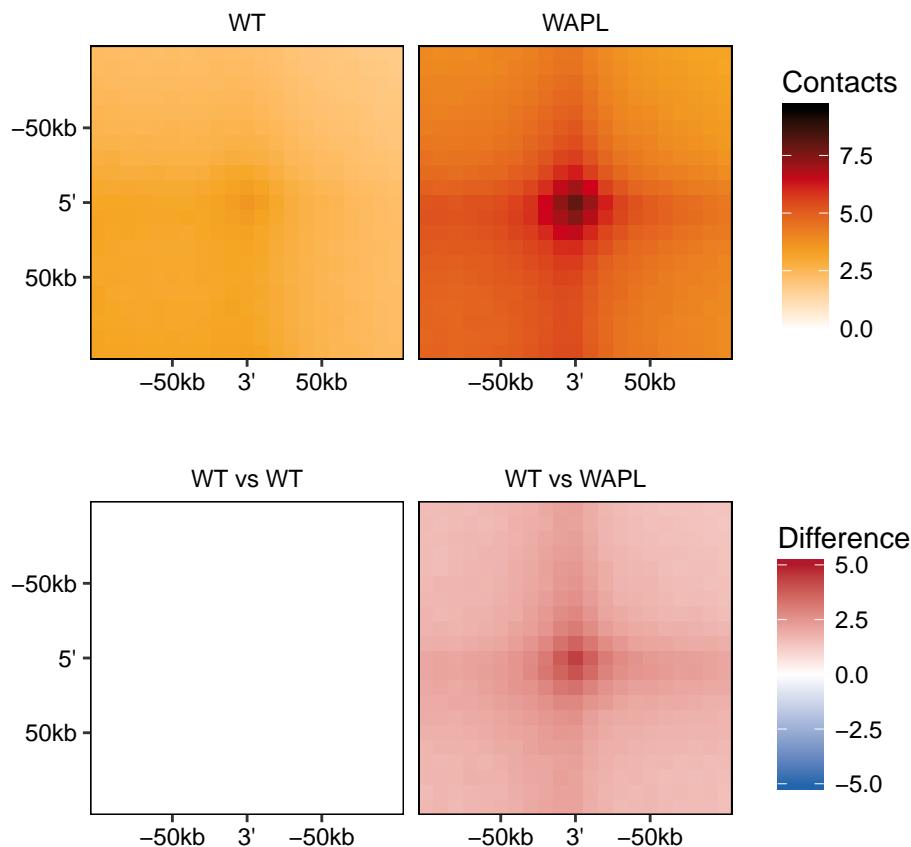


Figure 26: APA

In the WAPL-knockout, we see an increase of contacts at the loop.

6 Far-cis interactions

6.1 PE-SCAn

Some regulatory features, like super-enhancers come together in 3D-space. To test this, we implemented PE-SCAn. Here, the enrichment of interaction-frequency of all pairwise combinations of given regions is computed. The background is generated by shifting all regions by a fixed distance (1Mb: can be changed with the `shift`-argument).

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```
superEnhancers = read.delim('data/homerSuperEnhancers.txt',
                            h = F,
                            comment.char = "#")
```

Table 6: A data.frame holding the output of homer's findPeaks -style super

V1	V2	V3	V4	V5	V6
chr16-182	chr16	73074453	73092750	+	2572.8
chr12-14931	chr12	122219417	122249906	+	2532.3
chr2-1474	chr2	133025386	133026123	+	2523.7
chr11-4061	chr11	797422	842970	+	2227.4
chr15-2899	chr15	89158155	89165379	+	2087.3

The basic visualisation is comparable to ATA and APA: the first row shows the enrichment of all included samples, while the bottom row shows the difference.

```
WT_PE_OUT = PESCA(n(exp = Hap1_WT_40kb, bed = superEnhancers[,2:4])
visualise.PESCA(ggplot(PESCAlist = list(WT = WT_PE_OUT),
                      resolution = 40e3,
                      smooth = F))
```

Another way of looking at the PE-SCAn results, is to make a perspective plot. Here, the enrichment is encoded as the z-axis.

```
RES = 40e3 # resolution of the Hi-C
persp(list(x = seq(-1*(RES*10),(RES*10), length.out = 21)/1e6, # x-ticks (MB)
           y = seq(-1*(RES*10),(RES*10), length.out = 21)/1e6, # y-ticks (MB)
           z = WT_PE_OUT), # PE-SCAn out
       phi = 25, # colatitude
       theta = 40, # rotation
       col="#92c5de", # color of the surface
       shade=0.4, # how much shading
       xlab="",
       ylab="",
       zlab="",
       cex.axis = .6,
       ticktype="detailed",
       border=NA,
       zlim = c(min(c(WT_PE_OUT)),
                 max(c(WT_PE_OUT))))
```

6.2 centromere.telomere.analysis

```
centromere.telomere.analysis
```

```
draw.centromere.telomere We saw a enriched signal between chromosomes 15 and 19. We can wh
```

```
out1519 = centromere.telomere.analysis(Hap1_WT_40kb, chrom.vec = c('chr15', 'chr19'))
draw.centromere.telomere(out1519)
```

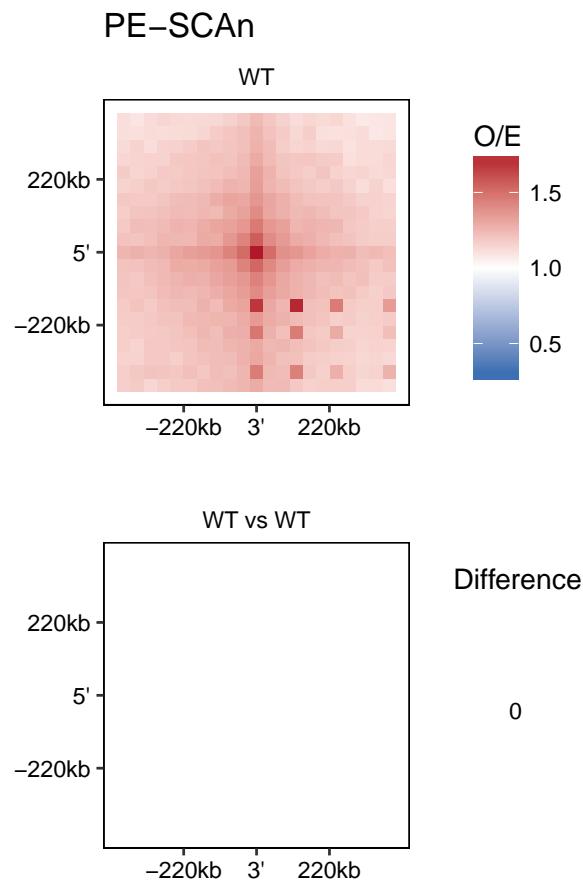


Figure 27: PE-SCAn

There is a pairwise enrichment of contacts between Superenhancers, compared to shifted regions in the WT.

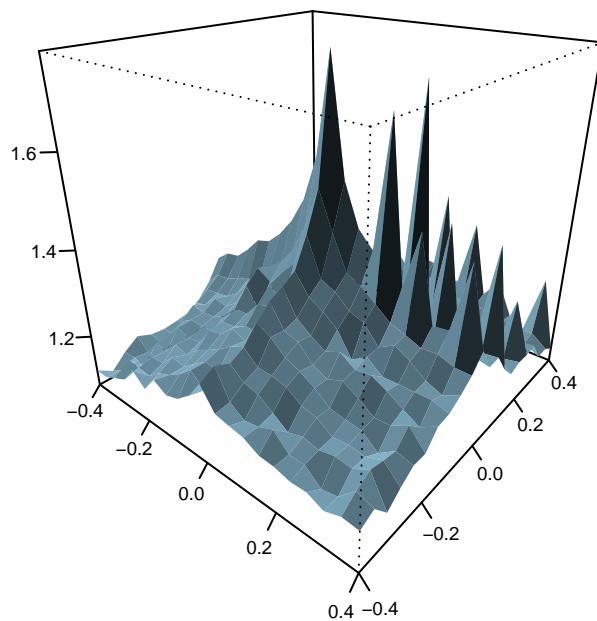


Figure 28: PE-SCAn perspective plot

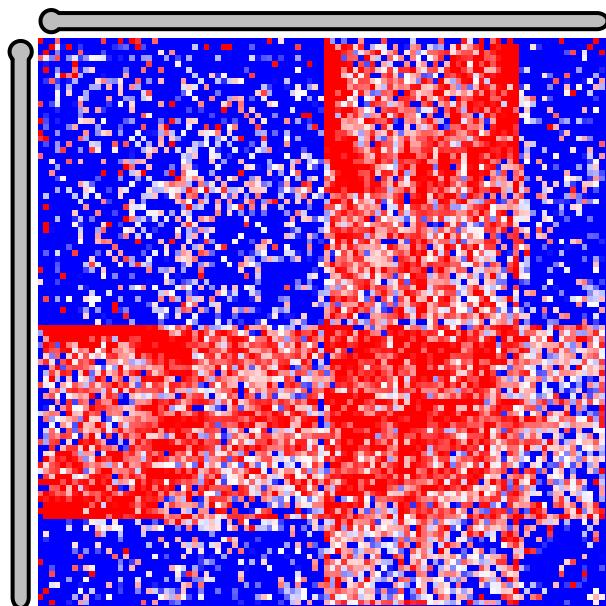


Figure 29: Centromere-telomere plot of chromosomes 15 and 19

7 To-do

For the next version, the following will be added/fixed:

- write `visualise.PESCAN.persp`
- write `findBadBin`
- optimise the time-v-nBin problem in `saddleBins`

Please post questions, comments and rants on [our github issue tracker](#).

8 Session info

```
## R version 3.4.3 (2017-11-30)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.4 LTS
##
## Matrix products: default
## BLAS: /usr/lib/openblas-base/libblas.so.3
## LAPACK: /usr/lib/libopenblas-p0.2.18.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=en_US.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=en_US.UTF-8      LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8         LC_NAME=C
## [9] LC_ADDRESS=C                 LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8   LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics    grDevices  utils      datasets   methods    base
##
## other attached packages:
## [1] reshape2_1.4.3  bigrwig_0.1.0  ggplot2_2.2.1  bindrcpp_0.2
## [5] GENOVA_0.9.95 BiocStyle_2.6.1
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.16     compiler_3.4.3    pillar_1.2.1
## [4] plyr_1.8.4       bindr_0.1.1       tools_3.4.3
## [7] digest_0.6.12    evaluate_0.10.1   tibble_1.4.2
## [10] gtable_0.2.0     pkgconfig_2.0.1   rlang_0.2.0
## [13] yaml_2.1.18     xfun_0.1        stringr_1.2.0
## [16] dplyr_0.7.4      knitr_1.20      rprojroot_1.3-2
## [19] grid_3.4.3       glue_1.2.0       data.table_1.10.4-3
## [22] R6_2.2.2        rmarkdown_1.9    bookdown_0.7
## [25] magrittr_1.5     backports_1.1.2   scales_0.4.1
## [28] codetools_0.2-15 htmltools_0.3.6   assertthat_0.2.0
## [31] colorspace_1.3-2 labeling_0.3     stringi_1.1.5
## [34] lazyeval_0.2.0    munsell_0.4.3
```

References

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