

HiTC - Exploration of High Throughput 'C' experiments

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1 Introduction

Chromosome Capture Conformation (3C) was first introduced by [Dekker et al. \[2002\]](#) ten years ago. The 3C technique aims in detecting physical contact between pairs of genomic loci and is now widely used to detect intrachromosomal (cis) and interchromosomal (trans) interactions between genes and regulatory elements. The development of the 3C-based techniques has changed our vision of the nuclear organization (see [de Wit and de Laat \[2012\]](#) for a review).

With the development of high throughput analyses, and in particular second-generation sequencing, the 3C has been adapted to study in parallel physical interactions between many loci, and thus increase the scale at which interactions between genomic loci can be detected (4C - Circular 3C, [Simonis et al. \[2006\]](#), [Zhao et al. \[2006\]](#); 5C - 3C Carbone Copy, [Dostie et al. \[2006\]](#)). More recently, this technique was further extended to obtain detailed insights into the general three-dimensional arrangements of complete genomes (Hi-C, [Lieberman-Aiden et al. \[2009\]](#)).

While the use of high-throughput 'C' techniques is expected to increase in the coming years, it also creates some new statistical and bioinformatics challenges. In this way, publicly available bioinformatics tools, as well as clear analysis strategy are still lacking. The [my5C web browser](#) was proposed by [Lajoie et al. \[2009\]](#) to visualize, transform and analyze 5C data. However, the my5C webtool is targeted to end-users and biologists to prepare their 5C experiments and to handle their data but is not dedicated to the development of new statistical algorithms.

The *HiTC* R package has been developed to offer a bioinformatic environment to explore high-throughput 'C' data. One advantage of this package is that it operates within the open source Bioconductor framework, and thus, offers new opportunities for future development in this field. The current version of the package provides the basic visualization, transformation and normalization functions described in [Lajoie et al. \[2009\]](#), but also some new functionalities such as data import, new visualization functions, annotation and other data transformation. Our goal is also to provide

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a flexible basis for further development, aiming at the integration of new analysis algorithm that are being developed (Yaffe and Tanay [2011], Hu et al. [2012], Imakaev et al. [2012])

This document briefly describes how to use the *HiTC* R package. The package is built on the functionality of Bioconductor packages such as *IRanges* and *GenomicRanges*, and provides new classes and methods to handle with high-throughput 'C' data. It is especially suited to 5C and Hi-C data handling, but can also in principle be used for 4C, though specific needs of 4C users may be best met by *r3Cseq* R package.

Even if the 5C and Hi-C approaches are derived from the same 3C technique, strong differences in their protocol can also be noticed. While 5C enables analysis of interactions between many loci, it also required an extensive number of primers, which is not suitable for a genome-wide analysis as the Hi-C. Thus, the pre-processing of these two types of data is totally different with, for instance, two different mapping strategies.

If you use *HiTC* for analyzing your data, please cite:

- Servant N., Lajoie B.R., Nora E.P., Giorgetti L., Chen C., Heard E., Dekker J., Barillot E. (2012) HiTC : Exploration of High-Throughput 'C' experiments. *Bioinformatics*.

2 Getting started

The current version of the *HiTC* package was developed to work on processed 5C, Hi-C or other high-throughput 3C data.

The *HTCexp* (High-Throughput 'C' experiment) class aims at representing a single 'C' experiment, characterized by :

- An interaction map (i.e a *Matrix*)
- Two *GRanges* objects that describe each features of the interaction matrix, respectively, the x (i.e. columns) and y (i.e. rows) labels of the interaction matrix. Basically, in the context of 5C, these objects will be the forward and reverse primers, and for the Hi-C the binned genomic intervals.

Note that *HiTC* was not designed to process chromatin conformation capture from raw reads, but takes contact maps as input. In order to process Hi-C data from raw sequencing reads, you can use the HiC-Pro pipeline Servant et al. [2015] which is freely available at <https://github.com/nservant/HiC-Pro>. Data processed with HiC-Pro can then be loaded into the R environment using the `importC` function of *HiTC*.

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Whereas a 5C dataset can be composed of a single cis interaction map (i.e. *HTCexp* object), a complete Hi-C dataset is composed of a list of cis and trans interaction maps, characterized by the physical interactions of each pair of chromosomes. The *HTClist* class represents a list of *HTCexp* objects and provides dedicated methods and visualization functions.

```
> library(HiTC)
> showClass("HTCexp")

Class "HTCexp" [package "HiTC"]

Slots:

Name:  intdata      xgi      ygi
Class: Matrix GRanges GRanges

> showClass("HTClist")

Class "HTClist" [package "HiTC"]

Slots:

Name:  .Data
Class: list

Extends:
Class "list", from data part
Class "vector", by class "list", distance 2
Class "list_OR_List", by class "list", distance 2
Class "AssayData", by class "list", distance 2
Class "vector_OR_Vector", by class "list", distance 3
Class "vector_OR_factor", by class "list", distance 3
```

3 Working with 'C' Data

HTCexp or *HTClist* objects can be easily created using the dedicated constructors. Additional functions to import data from files are also available.

3.1 A simple example

```
> require(Matrix)
> ## Two genome intervals objects with primers informations
> reverse <- GRanges(seqnames=c("chr1","chr1"),
+                     ranges = IRanges(start=c(98831149, 98837507),
+                     end=c(98834145, 98840771),
```

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```
+             names=c("REV_2", "REV_4"))
> forward <- GRanges(seqnames=c("chr1", "chr1"),
+             ranges = IRanges(start=c(98834146, 98840772),
+             end=c(98837506, 98841227),
+             names=c("FOR_3", "FOR_5"))
> ## A matrix of interaction counts
> interac <- Matrix(c(8463, 7144, 2494, 8310), ncol=2)
> colnames(interac) <- c("REV_2", "REV_4")
> rownames(interac) <- c("FOR_3", "FOR_5")
> z <- HTCexp(interac, xgi=reverse, ygi=forward)
> detail(z)
```

HTC object

Focus on genomic region [chr1:98831149-98841227]

CIS Interaction Map

Matrix of Interaction data: [2-2]

2 genomic ranges from 'xgi' object

2 genomic ranges from 'ygi' object

Total Reads = 26411

Number of Interactions = 4

Median Frequency = 7727

Sparsity = 1

> ## Access to the slots

> x_intervals(z)

GRanges object with 2 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
REV_2	chr1	98831149-98834145	*
REV_4	chr1	98837507-98840771	*

seqinfo: 1 sequence from an unspecified genome; no seqlengths

> y_intervals(z)

GRanges object with 2 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
FOR_3	chr1	98834146-98837506	*
FOR_5	chr1	98840772-98841227	*

seqinfo: 1 sequence from an unspecified genome; no seqlengths

> intdata(z)

2 x 2 Matrix of class "dgeMatrix"

REV_2 REV_4

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```
FOR_3 8463 2494
FOR_5 7144 8310

> ## Methods
> range(z)

GRanges object with 1 range and 0 metadata columns:
      seqnames      ranges strand
      <Rle>        <IRanges> <Rle>
[1] chr1 98831149-98841227      *
-----
seqinfo: 1 sequence from an unspecified genome; no seqlengths

> isBinned(z)

[1] FALSE

> isIntraChrom(z)

[1] TRUE

> seqlevels(z)

[1] "chr1"
```

3.2 Import/Export Data

The HiTC package provides the `importC` and `exportC` functions to import/export data from a list file. Only non null values have been written in this file allowing an efficient storage of Hi-C (sparse) data. The format is defined as follows:

- A list file (tab-separated) with per line, the name of both interactors and the number of associated sequencing reads (i.e. I1 I2 Count1-2).
- The associated `BED` files describing the x and y intervals of the HTCexp object. For 5C experiment, it can be the forward and reverse primers location, whereas for Hi-C experiment, it can be a description of the genomic bins. The name of these intervals must match with the name of the interactors in the list file.

In addition, the package is fully compatible with the [my5C web browser](#). The interaction counts matrices can be imported/exported from a matrix file format. The matrix format summarizes all the information with genomic coordinates as row and column names (ex: HIC_bin1|hg18|chr14:1-999999). The row and column names are splitted to create the HTCexp object.

The *HiTC* package includes a sample of the Human Hi-C dataset ([GSE18199](#)) published by [Lieberman-Aiden et al. \[2009\]](#). The interaction map of chromosome 12 to 14 is used to illustrate the capabilities of the *HiTC* package to explore Hi-C data.

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```
> ## Load Lieberman et al. Chromosome 12 to 14 data (from GEO GSE18199)
> exDir <- system.file("extdata", package="HiTC")
> l <- sapply(list.files(exDir, pattern=paste("HIC_gm06690_"), full.names=TRUE),
+            import.my5C)
> hiC <- HTclist(l)
> show(hiC)

HTclist object of length 6
3 intra / 3 inter-chromosomal maps

> names(hiC)

[1] "chr12chr12" "chr12chr13" "chr12chr14" "chr13chr13" "chr13chr14"
[6] "chr14chr14"

> ## Methods
> ranges(hiC)

GRangesList object of length 6:
$chr12chr12
GRanges object with 1 range and 0 metadata columns:
      seqnames      ranges strand
      <Rle>      <IRanges> <Rle>
[1]    chr12 1-132349533      *
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

$chr12chr13
GRanges object with 2 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>      <IRanges> <Rle>
[1]    chr12 1-132349533      *
[2]    chr13 1-114142979      *
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

$chr12chr14
GRanges object with 2 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>      <IRanges> <Rle>
[1]    chr12 1-132349533      *
[2]    chr14 1-106368584      *
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

...
<3 more elements>
```

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```
> range(hiC)

GRanges object with 3 ranges and 0 metadata columns:
      seqnames      ranges strand
      <Rle>      <IRanges> <Rle>
[1]   chr12 1-132349533      *
[2]   chr13 1-114142979      *
[3]   chr14 1-106368584      *
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

> isBinned(hiC)

chr12chr12 chr12chr13 chr12chr14 chr13chr13 chr13chr14 chr14chr14
      TRUE      TRUE      TRUE      TRUE      TRUE      TRUE

> isIntraChrom(hiC)

chr12chr12 chr12chr13 chr12chr14 chr13chr13 chr13chr14 chr14chr14
      TRUE      FALSE      FALSE      TRUE      FALSE      TRUE

> isComplete(hiC)

[1] TRUE

> seqlevels(hiC)

[1] "chr12" "chr13" "chr14"

> summary(hiC)
```

	seq1	seq2	nbreads	nbinteraction	averagefreq	medfreq	sparsity
chr12chr12	chr12	chr12	1179512	17418	67.718	20	0.0153
chr12chr13	chr12	chr13	58509	12495	4.6826	4	0.1831
chr12chr14	chr12	chr14	57593	11535	4.9929	5	0.1894
chr13chr13	chr13	chr13	779070	9518	81.8523	28	0.2803
chr13chr14	chr13	chr14	41230	8455	4.8764	5	0.3129
chr14chr14	chr14	chr14	755139	7913	95.4302	30	0.3088

4 Quality Control

The first step after data pre-processing is a quality control to check whether the data are likely to reflect cis and/or trans chromosomal interactions rather than just random collisions. Quality control for the percentage of reads aligned to interchromosomal and intrachromosomal interactions is available, as well as distribution of the interaction frequency against the genomic distance between two loci, and simple statistics (see Figure 1).

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```
> par(mfrow=c(2,2))  
> CQC(hiC, winsize = 1e+06, dev.new=FALSE, hist.dist=FALSE)
```

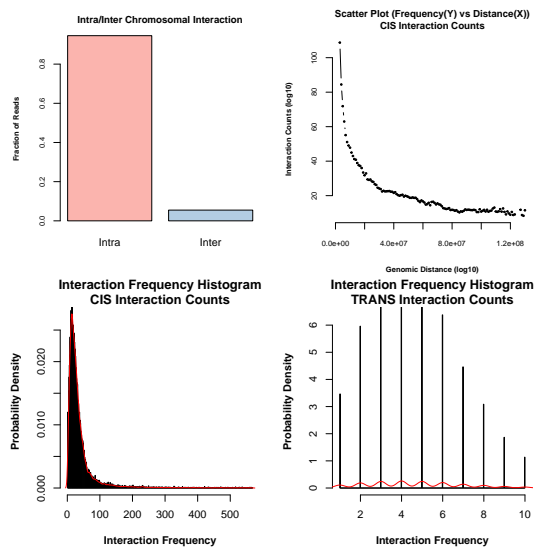


Figure 1: Quality Control of hiC data

From top-left to bottom-right : proportion of intra/inter chromosomal interactions, scatter-plot of interaction counts versus genomic distance between two loci, histogram of interaction counts for intra (CIS) and inter (TRANS) interactions, histogram of distances between two intrachromosomal loci.

5 HTCexp : single 'C' map experiment

5.1 Attached 5C data

The *HiTC* package includes a 5C dataset ([GSE35721](#)) published by [Nora et al. \[2012\]](#), from which we choose two different Mouse samples, male undifferentiated ES cells (E14, GSM873935) and male embryonic fibroblasts (MEF, GSM873924). This dataset is mainly used to describe the available functionalities of the package.

```
> ## Load Nora et al 5C dataset
> data(Nora_5C)
> show(E14)

HTClist object of length 1
1 intra / 0 inter-chromosomal maps

> show(MEF)

HTClist object of length 1
1 intra / 0 inter-chromosomal maps
```

5.2 Visualization of Interaction Maps

The interaction map represents the frequency at which each pair of restriction fragments have been ligated together during the 3C procedure. The goal is to visualize at once these counts for many pairs of restriction fragments across a large genomic region. Each entry in the matrix corresponds to a count information, i.e., number of times two restriction fragments have been sequenced as a pair.

In the *HiTC* package, the *HTCexp* object are represented as a triangle view (see [Figure 2](#)). This view is particularly useful for interaction maps comparison and alignment with genomic or epigenomic features on a small region. The *mapC* function proposes a list of options to play with data visualization, such as contrast, color, or trimming.

```
> mapC(E14$chrXchrX)
```

5.3 Data Transformation

5.3.1 Windowing

Each pixel of an interaction map can correspond either to a single restriction fragment, several restriction fragments or genomic intervals of any given size (and therefore various restriction fragment numbers). 5C allows assessing interaction frequencies for each pair of restriction fragments. The Hi-C protocol, on contrary, does not necessarily yields counts for every single pair of restriction fragments, especially when working with large genomes. Results are thus typically displayed for genomic bins of an arbitrary size.

To produce an interaction map, the genomic range of the display should be divided

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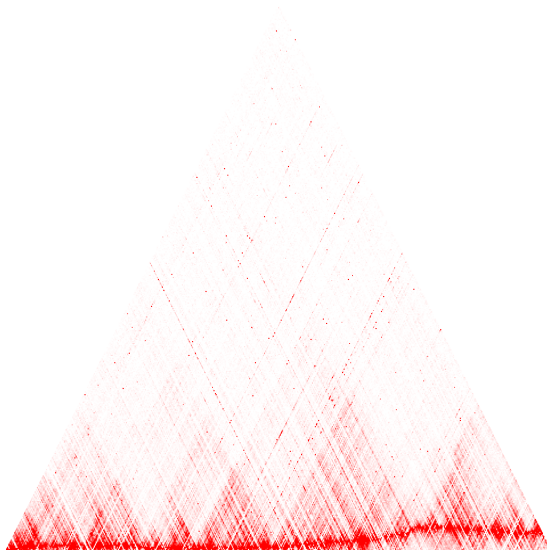


Figure 2: Visualization of *HTCexp* object (1)
Raw 5C interaction map of chromosome X.

into appropriately size loci. This size depends on the resolution desired for the analysis. For instance, 5C data can be visualized at the primers resolution, or segmented into 100Kb or 1Mb bins that can be partially overlap or not. Such binned interaction map is symmetrical around the diagonal. For the following example, we decided to focus on a subset of the original dataset (see Figure 3).

```
> ## Focus on a subset chrX:100295000:102250000
> E14subset<-extractRegion(E14$chrXchrX, c(1,2),
+                           chr="chrX", from=100295000, to=102250000)
> ## Binning of 5C interaction map
> E14subset.binned <- binningC(E14subset, binsize=100000, method="median", step=3)
> mapC(E14subset.binned)
```

5.3.2 Data Normalization

Due to the polymer nature of chromatin, at small genomic distances, pairs of restriction fragments that are close to each other in the linear genome will give higher signal than fragments that are further apart. Such property leads to strongest counts falling on the heatmap diagonal. When considering any given pair of restriction fragments, it is therefore informative to assess whether the observed counts are above what is expected given the genomic distance that separate them.

Different ways of normalization have been proposed. Here, we propose to estimate the expected interaction counts as presented in [Bau et al. \[2011\]](#). The expected value is the interaction frequency between two loci that one would expect based on a sole dependency on the genomic proximity of these fragments in the linear genome.

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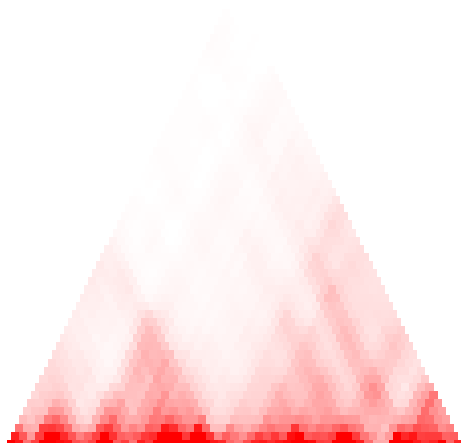


Figure 3: Visualization of *HTCexp* object (2)

Binned 5C interaction map of chrX:100295000-102250000.

This can be estimated using a Loess regression model (see Figure 4). Note that another model based on mean counts at each genomic distance can also be used (`method=mean`)

```
> ## Look at expected counts
> E14exp <- getExpectedCounts(E14subset.binned, method="loess", stdev=TRUE, plot=TRUE)
```

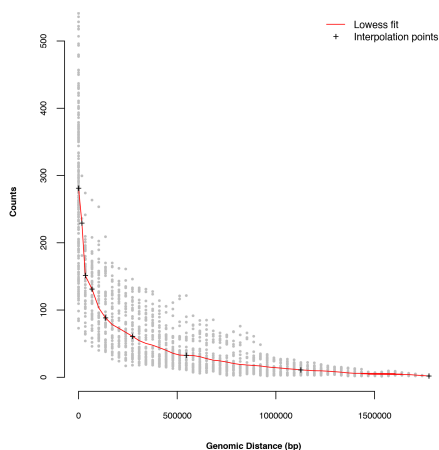


Figure 4: Estimation of expected count using a Loess smoothing

The crosses represent the interpolation points.

Interaction frequencies can be then normalized for distance by dividing the observed value by the expected value (`normPerExpected`). The variability between the interaction counts and the genomic distance between pairs of loci can be calculated if specified. These normalization methods can be easily applied using the methods `normPerReads` and `normPerExpected`.

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```
> E14norm.binned <- normPerExpected(E14subset.binned, method="loess", stdev=TRUE)
> mapC(E14norm.binned)
```

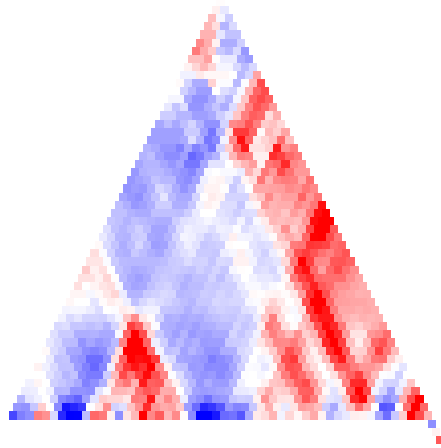


Figure 5: Normalized 5C data

Interaction map of data normalized from the background level of interactions.

5.3.3 Annotation of Interaction Maps

The *HiTC* package contains functions for visualizing genomic regions with interaction maps (see Figure 6). The annotation objects have to belong to the *GRanges* class, and can be loaded from *BED* files using the *rtracklayer* package. For instance, the following example displays the CTCF enriched regions (Kagey et al. [2010]) and RefSeq genes over the interaction map of the E14 sample.

```
> E14.binned <- binningC(E14$chrXchrX, binsize=100000, method="median", step=3)
> require(rtracklayer)
> gene <- import(file.path(exDir, "refseq_mm9_chrX_98831149_103425150.bed"),
+               format="bed")
> ctcf <- import(file.path(exDir, "CTCF_chrX_98892125_102969775.bed"),
+               format="bed")
> mapC(E14.binned,
+       tracks=list(RefSeqGene=gene, CTCF=ctcf),
+       maxrange=10)
```

5.4 Comparison of HTCexp objects

The *HiTC* package provides methods to perform simple operations on *HTCexp*, such as dividing, subtracting two objects or extracting a genomic region. It also proposes a graphical view to compare two 'C' experiments. In the following example, the MEF sample is compared to the E14 sample (see Figure 7).

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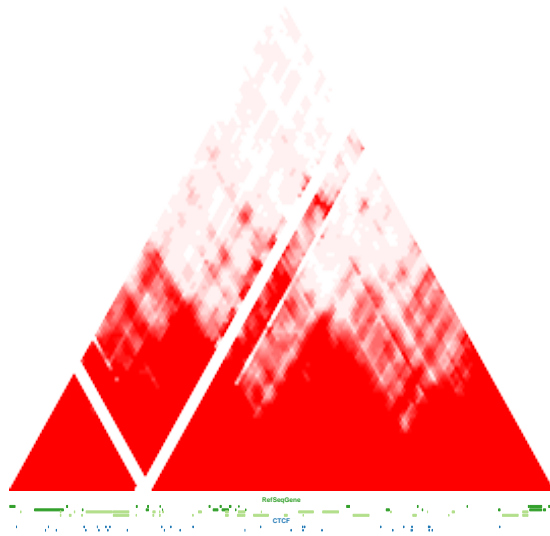


Figure 6: Visualization of interaction map and genomic annotations
CTCF enriched regions and RefSeq genes over the interaction map of the E14 sample.

```
> MEF.binned <- binningC(MEF$chrXchrX, binsize=100000, method="median", step=3)
> mapC(E14.binned, MEF.binned,
+     tracks=list(RefSeqGene=gene, CTCF=ctcf),
+     maxrange=10)
```

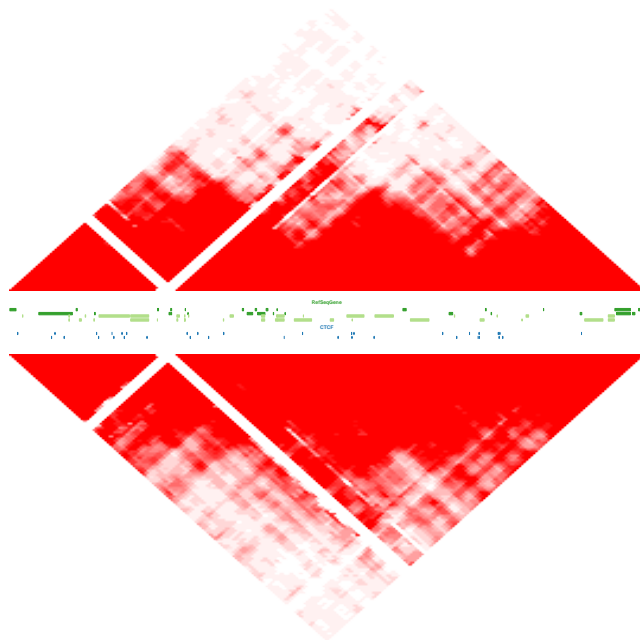


Figure 7: Comparison of interaction maps
Comparison of two binned interaction maps, and visualization with genomic annotations.

6 HTClist : Multiple 'C' experiments

Basically, 5C and Hi-C data can be described in the same way. Thus, most of the functions and methods described for the 5C data can be applied to the Hi-C data.

6.1 Visualization of Interaction Maps

The visualization of the *HTClist* is designed such as several interaction maps from the same experiment can be displayed together.

Therefore these data are typically displayed using two dimensional heatmaps of all cis/trans maps.

```
> mapC(hiC, maxrange=100)
```

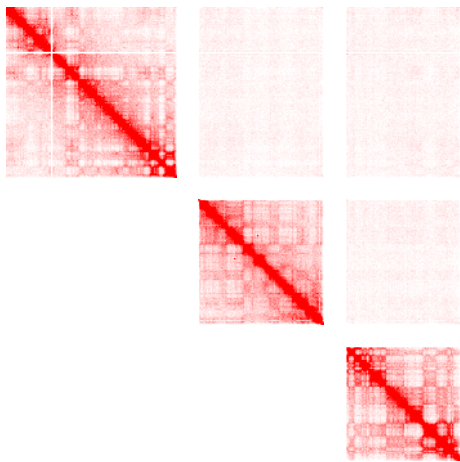


Figure 8: Visualization of a Hi-C dataset

Two dimensional heatmaps of the cis/trans maps from the Liberman-Aiden et al. dataset.

6.2 Hi-C analysis

In this section, we present how, using a few command lines, we can reproduce some analyses of the [Lieberman-Aiden et al. \[2009\]](#) paper (see Figures 9-11) on the chromosome 14, from visualization of maps to Principal Component Analysis (PCA).

```
> ## Extract region of interest and plot the interaction map
> hiC14 <- extractRegion(hiC$chr14chr14,
+                         chr="chr14", from=1.8e+07, to=106368584)
> mapC(HTClist(hiC14), maxrange=100)
```

```
> ## Data Normalization by Expected number of Counts
> hiC14norm <- normPerExpected(hiC14, method="loess")
> mapC(HTClist(hiC14norm), log.data=TRUE)
```

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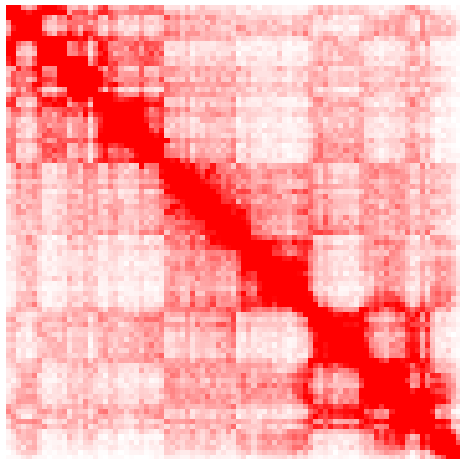


Figure 9: Hi-C interaction map of chromosome 14

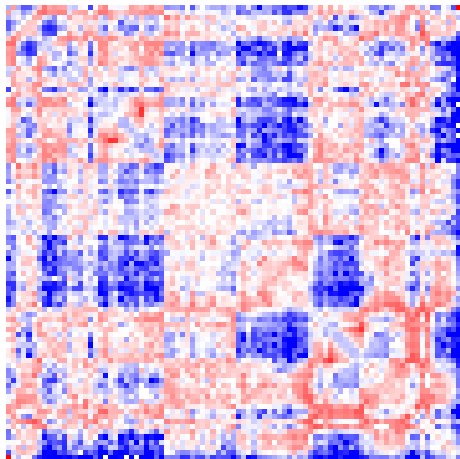


Figure 10: Interaction map of chromosome 14 normalized by the expected interaction counts

```
> ## Correlation Map of Chromosome 14
> #intdata(hiC14norm) <- as(cor(as.matrix(intdata(hiC14norm))), "Matrix")
> intdata(hiC14norm) <- HiTC:::sparseCor(intdata(hiC14norm))
> mapC(HTClist(hiC14norm), maxrange=1, minrange=-1,
+     col.pos=c("black", "red"), col.neg=c("blue", "black"))
```

```
> ## Principal Component Analysis
> pc <- pca.hic(hiC14, normPerExpected=TRUE, method="loess", npc=1)
> plot(start(pc$PC1), score(pc$PC1), type="h",
+     xlab="chr14", ylab="PC1vec", frame=FALSE)
>
```

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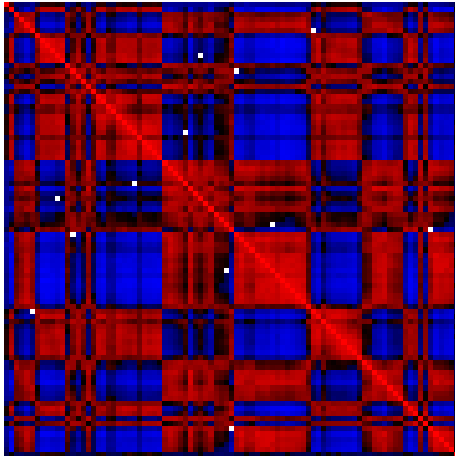


Figure 11: Correlation map of chromosome 14

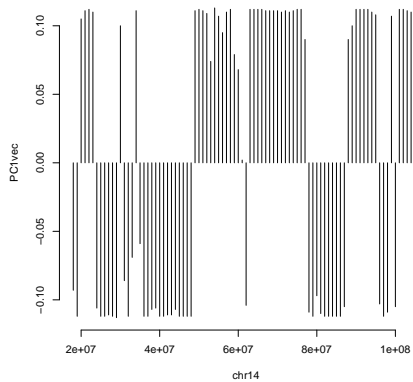


Figure 12: PCA analysis (Lieberman-Aiden et al.)

Results of the PCA (eigenvector), which reflect the compartmentalization inherent in the heatmap.

7 A word about speed and memory usage

In order to improve the run time on machines with multiple processors, some of the functions in the *HiTC* package have been implemented to make use of the functionality of the *parallel* package. If the options `mc.cores` is initialised before calling these functions, they will make use of `mclapply` instead of the normal `lapply`. Since the version 1.5.2 of the package, the interaction maps are now stored as *Matrix* object. In case of very high resolution data, such as the 20kb interaction maps published by Dixon et al. [2012], a sparse matrix representation is much more efficient in terms of memory usage. The memory requires by the HiTC package for high resolution Hi-C data is represented in the figure 13. However, in many cases, using *Matrix* objects instead of *matrix* objects is much more slower. Thus, for some functions such as `binningC`, the user can now set the `optimize.by` argument to "speed" or "memory". If set to "speed", the *Matrix* object is convert into a standard

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matrix class, thus taking much more memory during the execution of the function but being much faster. One can notice that for now, all the visualization functions are based on *matrix* object.

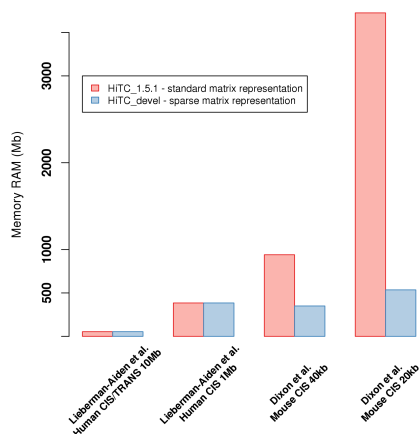


Figure 13: HiTC memory usage

Improvement of the memory usage of the HiTC package through the storage of sparse matrix (*Matrix*).

Package versions

This vignette was generated using the following package versions:

- R version 4.1.0 RC (2021-05-16 r80304), x86_64-apple-darwin17.0
- Running under: macOS Mojave 10.14.6
- Matrix products: default
- BLAS:
/Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.dylib
- LAPACK:
/Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: BSgenome 1.61.0, BSgenome.Hsapiens.UCSC.hg18 1.3.1000, BiocGenerics 0.39.0, Biostrings 2.61.0, GenomInfoDb 1.29.0, GenomicRanges 1.45.0, HiCDataHumanIMR90 1.11.0, HiTC 1.37.0, IRanges 2.27.0, Matrix 1.3-3, S4Vectors 0.31.0, XVector 0.33.0, rtracklayer 1.53.0
- Loaded via a namespace (and not attached): Biobase 2.53.0, BiocIO 1.3.0, BiocManager 1.30.15, BiocParallel 1.27.0, BiocStyle 2.21.0, DelayedArray 0.19.0, GenomInfoDbData 1.2.6, GenomicAlignments 1.29.0, MatrixGenerics 1.5.0, RColorBrewer 1.1-2, RCurl 1.98-1.3, Rsamtools 2.9.0, SummarizedExperiment 1.23.0, XML 3.99-0.6, bitops 1.0-7, compiler 4.1.0,

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crayon 1.4.1, digest 0.6.27, evaluate 0.14, grid 4.1.0, htmltools 0.5.1.1, knitr 1.33,
lattice 0.20-44, matrixStats 0.58.0, restfulr 0.0.13, rjson 0.2.20, rlang 0.4.11,
rmarkdown 2.8, rstudioapi 0.13, tools 4.1.0, xfun 0.23, yaml 2.2.1, zlibbioc 1.39.0

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