GenomicInteractions: An R/Bioconductor package for manipulating and investigating chromatin interaction data

Nathan Harmston¹,²*, Elizabeth Ing-Simmons¹†, Malcolm Perry¹†, Anja Barešić¹ and Boris Lenhard¹*

Abstract

Background: Precise quantitative and spatiotemporal control of gene expression is necessary to ensure proper cellular differentiation and the maintenance of homeostasis. The relationship between gene expression and the spatial organisation of chromatin is highly complex, interdependent and not completely understood. The development of experimental techniques to interrogate both the higher-order structure of chromatin and the interactions between regulatory elements has recently lead to important insights on how gene expression is controlled. The ability to gain these and future insights is critically dependent on computational tools for the analysis and visualisation of data produced by these techniques.

Results and conclusion: We have developed GenomicInteractions, a freely available R/Bioconductor package designed for processing, analysis and visualisation of data generated from various types of chromosome conformation capture experiments. The package allows the easy annotation and summarisation of large genome-wide datasets at both the level of individual interactions and sets of genomic features, and provides several different methods for interrogating and visualising this type of data. We demonstrate this package’s utility by showing example analyses performed on interaction datasets generated using Hi-C and ChIA-PET.

Background

Metazoan gene expression is controlled through the complex interplay of transcription factors, histone modifications and regulatory elements [1, 2] in threedimensional nuclear space [3]. Gene expression is typically regulated by both the gene’s core and proximal promoters and through the action of distal elements such as enhancers [4] and insulators [5]. Physical interactions between these elements and their cognate promoters are currently thought to be a major mechanism for quantitatively and spatiotemporally regulating gene expression [6]. The positioning of chromosomes in the nucleus [7–10] and the organisation of chromatin at multiple scales [11, 12] have important roles in controlling the dynamics and specificity of these interactions, although the mechanisms involved are not completely understood. Information on how the spatial organisation of chromosomes impacts the regulation of gene expression is becoming increasingly available due to the development of experimental techniques to interrogate this phenomenon in a genome-wide manner [13].

Chromosome conformation capture methods have been developed for investigating chromatin interactions at both the level of individual loci (i.e. 3C [14], 4C [15], 5C [16], T2C [17]) and genome-wide (i.e. Capture-C [18], Hi-C [19, 20], ChIA-PET [21]). These methods work by cross-linking regions of genomic DNA that are in close physical proximity and thereby allowing the identification of interactions between genomic loci by the capture and sequencing of these regions. ChIA-PET (Chromatin Interaction Analysis with Paired-End Tag sequencing) allows for the investigation of interactions that are mediated by or associated with a specific protein (e.g. PolII [21]) or histone modification (e.g. H3K4me2 [22]), which is accomplished by performing a chromatin-immunoprecipitation step after cross-linking. The resulting data can then be used to generate

* Correspondence: nathan.harmston@duke-nus.edu.sg; b.lenhard@imperial.ac.uk
† Equal contributors
¹ Computational Regulatory Genomics, MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London W12 0NN, UK
Full list of author information is available at the end of the article

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interaction maps or networks detailing chromatin interactions, focusing either on specific genes and elements or genome-wide.

These methods have provided insights into the 3D organisation of chromatin across multiple cell types and conditions. Most interactions between genomic regions occur within the same chromosome (cis-interactions), with only a small number of interactions occurring reproducibly between elements on different chromosomes (trans-interactions) [23]. Chromatin is organised into distinct topologically associated domains (TADs) [12], with regulatory elements and genes preferentially interacting within the same TAD, and at the larger scale TADs are organised into compartments of active/inactive chromatin [19]. Both genes and enhancers are promiscuous with respect to their interaction partners, with genes able to interact with multiple enhancers and, less frequently, enhancers able to regulate multiple promoters [24]. The interaction landscape of a promoter is often highly dynamic and cell-type specific [25], with changes in its interaction partners thought to play a role in regulating its expression during development and differentiation [26, 27]. These findings were made possible not only by advances in experimental techniques but also because of the development of statistical and computational methods for data processing, filtering, normalisation and visualisation [19, 28–33], and currently there is considerable work on developing new statistical methodologies for analysing this type of data [34, 35].

Here, we present GenomicInteractions, an R/Bioconductor [36] package for the manipulation, annotation and visualisation of various types of chromatin interaction data, e.g. Hi-C, ChIA-PET. The development of this software was motivated by the lack of a general platform to analyse and visualise chromatin interaction data. Existing analysis tools are mostly standalone packages (e.g. HOMER, ChIA-PET tool), which do not have interfaces to the popular R/Bioconductor tools for genomic data analysis. Current R/Bioconductor packages for chromatin interaction data are generally specialised for a specific data type (e.g. HiC: diffHiC [37], HiTC [38], GOTHic [39]; 4C: r3Cseq [32], Basic4CSeq [40], FourCSeq [41]). Most of these packages take BAM files as input and provide data processing and normalisation and visualisation functions. In contrast, GenomicInteractions can be used with any type of chromatin interaction data in a range of formats, and is designed for interactive data exploration and visualisation. The ability to import data from several formats and its integration with existing Bioconductor packages facilitates the integrative analysis of data from different experiments, for example combining ChIP-seq signal or gene expression data with interaction data. We describe the main features of this package and demonstrate its utility and novel features by analysing two different chromatin interaction datasets.

Implementation

GenomicInteractions is a publicly available Bioconductor package for the handling of chromatin interaction data. It follows the same naming conventions as core Bioconductor packages, such as GenomicRanges [42]. We provide vignettes detailing the use of GenomicInteractions in analysing both Hi-C and ChIA-PET data.

Interoperability and integration with other Bioconductor packages

Our package is designed to be as high-level as possible in order to allow its use in a wide range of analyses using different types of chromatin interaction data. Although the methods used to generate and process chromatin interaction data vary, the conceptual structure of the data is a series of pairs of genomic regions involved in the interactions (known as anchors) and data associated with each pair of regions e.g. supporting counts, p-value and false discovery rate (FDR). We define an S4 class, which encapsulates this structure and allows the easy manipulation and investigation of interactions stored within it. Anchor regions are stored as GenomicRanges objects, allowing individual anchors to be efficiently queried and annotated with relevant data and metadata. As with any analysis of biological data, the specific steps involved depend on the experimental design and on the biological questions being asked. However, most tasks can be grouped together and organised into a workflow structure (Fig. 1), regardless of how the data was generated originally.

Data import

The package can import chromatin interaction data stored in several formats, including the output from common processing tools [43], e.g. HOMER [28], ChIA-PET tool [30], and from standard formats, e.g. bed12, bedpe and BAM. This allows users to easily import data processed using existing tools, while also providing methods for directly manipulating aligned reads (e.g. merging interactions between predefined anchors, removing positional duplicates and determining thresholds for self-ligation events).

Determining self-ligation thresholds

The package contains implementations of two methods for calculating thresholds to separate reads into those that are the result from self-ligations versus those that arise from inter-ligations. This threshold can be identified by comparing the distribution of paired-end reads mapping to the same-strand against those aligning to different strands. The paired-end reads are binned by
distance and the ratio is calculated for each bin. A binomial test is available for testing whether this ratio is different from the expected 50:50 ratio in a specific bin. Additionally, we provide an implementation of the method described in Heidari et al. [44, 45], where the cut-off is determined by examining the strand distribution of reads which span over long distances.

Interaction summaries
We provide methods for creating various diagnostic plots (see Figs. 2 and 3), including visualising the distribution of distances spanned by the interactions, the proportion of cis- and trans-interactions in the dataset, and the number of reads supporting each interaction.

Annotation, interrogation of interacting regions
The package allows both interactions and genomic features/regions of interest to be annotated and examined easily. Each anchor region can be annotated with whether or not it overlaps a region of interest (which specifies the class of the anchor e.g. promoter) and an identifier specifying that region (e.g. a gene identifier). For example, this allows anchors to be annotated with which gene promoters, transcription factor binding peaks or chromatin states they overlap with. This in turn allows the extraction of all interactions that are between pairs of promoters (promoter:promoter interactions), or between other features of interest (e.g. promoter:enhancer or enhancer:enhancer interactions). A GenomicInteractions object can be queried and filtered based on user-defined criteria: for example, it is straightforward to subset the object to only contain interactions within or between specific chromosomes or specific features. Users can summarise interactions at the level of individual genomic features, identifying the total number of interactions a feature is involved in, or the number of other features with which it interacts. This makes it possible to identify gene promoters involved in many interactions with distal/enhancer regions, thus resolving promoter:enhancer interactions at complex loci with non-linear arrangement of genes and the regulatory elements that control them [27, 45].

Visualisation of interactions
The proportion of interactions between different classes of features can be calculated and visualised (Figs. 2 and 3). It is also possible to generate a virtual 4C viewpoint-style plot of all interactions involving a region(s) of interest, e.g. a specific promoter, or around a set of transcription factor binding sites. In addition, the package provides methods for visualising interactions and features within a defined genomic region by representing interactions between anchors as curves (Figs. 4 and 5) via integration with the Gviz visualisation library [46].

Data export
Finally, users can export their dataset to a variety of output formats for further analysis with other tools. We have provided methods for exporting a GenomicInteractions object to bed12 format, which can be used, for example, to visualise the interactions in the UCSC Genome Browser [47]. It is also possible to convert the interaction data into a graph format compatible with the igraph library [48], allowing the examination of data using network analysis approaches.
Investigating Hi-C data from mouse thymocytes

Here, we describe using GenomicInteractions to perform an example analysis of Hi-C data generated using mouse double positive (CD4+ CD8+) thymocytes [49] (GEO dataset GSE48763). All code and data required to reproduce this analysis can be found in Additional file 1. Two biological replicates, totalling about 203 M paired-end reads were aligned using bowtie [50]. Uniquely mappable reads were then pooled and processed using the HOMER software pipeline, to remove sources of noise and bias. This resulted in the identification of a set of 100 kb regions involved in significant interactions, taking both genomic distance and sequencing depth into account. GenomicInteractions has a built-in function to import data from the HOMER interaction file format.

This gives 74443 interactions at an FDR of 5%. Almost all (96.2%) of these are cis-chromosomal interactions, although many are long-range interactions across distances of more than 2 Mb. These properties can be quickly summarised using plotting functions provided in the package (Fig. 2a,c). Annotation of these interactions (as either promoters or distal elements) reveals that the majority are annotated as promoter:promoter interactions (Fig. 2b). This is partly due to the resolution of the Hi-C data; as the anchors are 100 kb, the chance that they will contain at least one promoter is high.

Figure 4 shows the interaction landscape around the 100 kb anchor that contains the promoter of the Cd4 gene. Cd4 is a cell surface protein that is a key cell identity marker for CD4+ CD8+ thymocytes. Its gene is highly expressed in these cells and is regulated by an intronic enhancer and multiple distal elements [51, 52]. Although the resolution of the data is not high enough to detect interactions within the Cd4 gene region, numerous interactions with both neighbouring 100 kb regions and distal regions on the same chromosome are apparent. The 100 kb region containing Cd4 also participates in at least one trans-chromosomal interaction (grey line, Fig. 4). These interactions could be investigated further using other chromosome conformation capture methods or DNA FISH.

Results

Usage examples

**Investigating Hi-C data from mouse thymocytes**

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Investigating ChIA-PET data from human K562 cells

K562 ChIA-PET data for PolII (8WG16) was taken from Li et al. [21] replicate 1 (GEO dataset GSE33664). This dataset has been processed using the ChIA-PET tool, with interactions supported by more than two PET counts and having an FDR < 0.05 considered as significant. All code and data required to replicate this analysis can be found in Additional file 2.

All interactions involving chrM were filtered from the dataset, resulting in 64554 unique interactions supported by 879351 PETs. The vast majority of interactions in this dataset occur in cis, with only 1% (637) occurring trans-chromosomally (Fig. 3a). There are 166 interactions which span more than 1 Mb, some of which show interactions between regions over 17 Mb apart. These super-long range interactions were removed from further analysis. Only a small number (N = 508) of remaining interactions appear to span distances longer than 500 kb (Fig. 3c).

In order to more accurately define the promoter region of a gene, the robust DPI promoter set generated from the FANTOM5 data [53] was used to propose the TSS of each gene. Only genes coding for proteins, long intergenic non-coding RNAs (lincRNAs) or microRNAs (miRNAs) were considered. Promoter regions were defined as +/- 2.5 kb around an Ensembl gene TSS, r (repressed region), e (enhancer or weak enhancer), t (transcribed region) and c (CTCF region). If an anchor lies within a region not covered by one of these annotations it was labelled as distal. The majority of interactions in this dataset appear to be between promoters and other promoters (N = 21694), with a large number of promoter:enhancer interactions (N = 4177) (see Fig. 3b). As expected [23], a number of enhancer:enhancer interactions were also observed (N = 1209).

Interaction data was summarised at the level of promoters, i.e. PET counts of all anchors overlapping the promoter regions of each gene have been summed together, which revealed the genes involved in the highest number of interactions genome-wide. 13215 of the 19358 genes examined were involved in some form of interaction as identified by ChIA-PET. The top ten
genes ranked by total number of promoter:enhancer interactions are shown in Table 1. Some of these genes have been previously found to play important roles in haematopoiesis and leukaemia pathogenesis, e.g. \textit{PIM1} [55], \textit{BCOR} [56], \textit{TNFRSF8} [57] and \textit{NR4A2} [58]. The number of promoters and enhancers that interact with each promoter was also calculated. In some cases, due to the close genomic proximity of some enhancers and promoters it was not possible to distinguish which individual enhancer or promoter an interaction was involved with.

\textit{NR4A2} (also known as Nurr1) is a member of the steroid orphan nuclear receptor transcription factor superfamily. It is essential in neurogenesis and the maintenance of dopaminergic neurons [59], plays a role in the activation of \textit{FOXP3} in regulatory T cells and in their differentiation and function [60] and has been associated with various types of cancer [61]. The interaction landscape of \textit{NR4A2} is shown in Fig. 5. The promoter of \textit{NR4A2} is involved in interactions with the promoter of its neighbouring gene \textit{GPD2} (located 93 kb away) and a promoter of the gene \textit{GALNT5} (located 910 kb away). It is interacting with five putative enhancers, four of which are located within 100 kb of the promoter of \textit{NR4A2}, with one located almost 900 kb away. This enhancer also has interactions with the promoter of \textit{GALNT5} and appears to be bound by a number of factors in K562 including GATA2, PML, TAL1 and BCL3, all of which have been implicated in the leukemia or other forms of cancer [62–64].

\textbf{Conclusions}

\textit{GenomicInteractions} provides a set of tools to import, manipulate, visualise and mine chromatin interaction data in R. The package has the potential to serve as a starting point for different types of analyses, providing the ability to ask relevant questions about the chromatin interactome using data generated from a variety of experimental techniques. In this paper, we have shown how \textit{GenomicInteractions} allows an end-user to reproducibly
Fig. 5 The interaction landscape of NR4A2 in K562 cells (hg19) as determined by ChIA-PET (chr2:156898860–158248860). See associated text for more details on processing. All identified interactions are shown in the top panel, with promoter:promoter interactions and promoter:enhancer interactions shown in the panels below. This gene is involved in interactions with the promoters of two nearby genes (GPD2 and GALNT5) and a small number of enhancers. The height of each curve corresponds to negative logarithm of the FDR for each interaction. Promoter:promoter interactions are shown in green, promoter:enhancer interactions in purple, promoter:distal interactions in orange and promoter:ctcf interactions are displayed in blue.

Table 1 Genes with the highest number of promoter:enhancer interactions in RNA Polymerase II ChIA-PET with 8WG16 antibody in human K562 cell line, replicate 1 [21], see associated text for more details on processing of this dataset

| Ensembl ID   | HGNC Symbol | Number of interactions | Number of promoter: promoter interactions | Number of promoters | Number of promoter: enhancer interactions | Number of enhancers |
|--------------|-------------|------------------------|------------------------------------------|-------------------|------------------------------------------|-------------------|
| ENSG00000177000 | MTHFR       | 934                    | 686                                      | 7                 | 233                                      | 9                 |
| ENSG0000011021 | CLCN6       | 901                    | 686                                      | 7                 | 208                                      | 9                 |
| ENSG00000183337 | BCOR       | 2585                   | 2444                                     | 1                 | 110                                      | 14                |
| ENSG00000137193 | PIM1        | 167                    | 60                                       | 1                 | 107                                      | 42                |
| ENSG0000007944 | MYLIP       | 721                    | 485                                      | 2                 | 97                                       | 38                |
| ENSG00000120949 | TNFRSF8     | 198                    | 91                                       | 1                 | 84                                       | 3                 |
| ENSG00000153234 | NR4A2       | 671                    | 541                                      | 2                 | 82                                       | 5                 |
| ENSG00000253276 | Ccdc17l     | 303                    | 186                                      | 3                 | 76                                       | 12                |
| ENSG00000116717 | GADD45A     | 537                    | 317                                      | 1                 | 73                                       | 12                |
| ENSG00000172216 | CebpB       | 414                    | 265                                      | 8                 | 65                                       | 21                |

A window size of 2.5 kb around TSSs, defined using the FANTOM5 robust tag cluster set, was used to define promoters. Enhancers and other chromatin states were defined using the Genome Segments K562 track. The number of interactions is calculated as the sum of the PET counts of all anchors overlapping the promoter region of a gene.
and efficiently perform analyses of two publicly available genome-wide chromatin interaction datasets. This allowed the identification and visualisation of regulatory elements that are interacting with a number of interesting genes, the identification of genes with the highest number of interactions and the characterisation of sets of those interactions. The package is available under a GPL-3 licence, and users and developers can easily extend the implemented functionality to match their specific analysis needs. In the future we are looking to extend this package with additional methods for normalising and processing the data, and expand the number of formats from which interaction data can be imported.

Availability and requirements

**GenomicInteractions** is a publicly available Bioconductor package available from http://bioconductor.org/packages/GenomicInteractions/. Documentation is available on the Bioconductor website, and we provide vignettes describing two example analyses using publicly available ChIA-PET and Hi-C data. We also maintain a public github repository (https://github.com/ComputationalRegulatoryGenomicsCL/GenomicInteractions), and invite the community to submit or request additional functionality to incorporate into this package. This package requires R > 3.0.1 and depends on several R/Bioconductor packages including Rsamtools, GenomicRanges, data.table, stringr, rtracklayer, ggplot2, gridExtra, igraph and Gviz.

All of the analyses and figures presented in the paper can be reproduced via the RMarkdown documents provided in the supplemental material using **GenomicInteractions** (version 1.3.6 available on Github), which is available (as version 1.4.0) in Bioconductor 3.2.

Additional files

Additional file 1: R script used to generate figures, tables and numbers used in the described analysis of Hi-C data. (RMD 7 kb)
Additional file 2: R script used to generate figures, tables and numbers used in the described analysis of ChIA-PET data. (RMD 14 kb)

Abbreviations

ChIA-PET: Chromatin interaction analysis with paired-end tag sequencing; TAD: Topologically associated domain; FANTOM: Functional annotation of the mammalian genome; PET: Paired-end tag; GEO: Gene expression omnibus; FDR: False discovery rate; T2C: Targeted chromatin capture; FISH: Fluorescence in situ hybridisation; DPI: Decomposition-based peak identification.

Competing interests

The authors declare no competing interests.

Authors’ contributions

NH and BL conceived the research. NH designed the software. NH, EI-S, MP and AB contributed to the development of software and documentation. MP and EG are responsible for the maintenance of the software. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Author details

1Computational Regulatory Genomics, MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London W12 ONN, UK. 2Program in Cardiovascular and Metabolic Disease, Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857, Singapore.

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References

1. Phillips-Cremins JE, Sauria MEG, Sanyal A, Gerasimova TI, Lajoie BR, Bell JSK, et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell. 2013;153:1281–95.
2. Harmston N, Lenhard B. Chromatin and epigenetic features of long-range gene regulation. Nucleic Acids Res. 2013;41:7185–99.
3. Gibcus JH, Dekker J. The hierarchy of the 3D genome. Mol Cell. 2013;49:773–82.
4. Sliyueva D, Stampfel G, Stark A. Transcriptional enhancers: From properties to genome-wide predictions. Nat Rev Genet. 2014;15:272–86.
5. Phillips-Cremins JE, Corces VG. Chromatin insulators: Linking genome organization to cellular function. Mol Cell. 2013;50:461–74.
6. Deng W, Lee J, Wang H, Miller J, Reik A, Gregory PD, et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell. 2012;149:1233–44.
7. Chambeyron S, Bickmore WA. Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes Dev. 2004;18:1119–30.
8. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature. 2008;453:948–51.
9. Dotter J, Stasiak A. The role of transcription factor-mediated interchromosomal contacts in the organization of nuclear architecture. Nucleic Acids Res. 2010;38:7410–21.
10. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, et al. Preferential associations between co-regulated genes reveal a transcriptional interactor in erythroid cells. Nat Genet. 2010;42:53–61.
11. Armano T, Sagi T, Tanabe H, Mizushina Y, Nakazawa H, Shiroishi T. Chromosomal dynamics at the Shh locus: Limb bud-specific differential regulation of competence and active transcription. Dev Cell. 2009;16:47–57.
12. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012;485:376–80.
13. Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nat Rev Genet. 2013;14:390.
14. Dekker J, Ripk R, Dekker M, Kleckner N. Capturing chromosome conformation. Science. 2002;295:1306–11.
15. Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, de Wit E, et al. Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). Nat Genet. 2006;38:1348–54.
16. Dostie J, Richmond TA, Arnaout RR, Lee WL, Honan TA, et al. Chromosome Conformation Capture Carbon Copy (3C), a massively parallel solution for mapping interactions between genomic elements. Genome Res. 2006;16:299–309.
17. Kokolos P, van de Wijken HJ, Kepper N, Zuin J, Brouwer RW, Kockx CE, et al. Targeted Chromatin Capture (T2C). A novel high resolution high
throughput method to detect genomic interactions and regulatory elements. Epigenetics Chromatin. 2014;7:10.

18. Hughes JR, Roberts N, McGowan S, Hay D, Giannoulou E, Lynch M, et al. Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. Nat Genet. 2014;46:205–12.

19. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009;326:289–93.

20. Belmont JM, McCord RP, Gubicz JH, Naumova N, Zhan Y, Dekker J. Hi-C: A comprehensive technique to capture the configuration of genomes. Methods. 2012;58:268.

21. Li G, Ruan X, Auerbach RK, Sandhur KS, Zheng M, Wang P, et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell. 2012;148:84–98.

22. Chepelev I, Wei G, Wangda D, Tang Q, Zhao K. Characterization of genome-wide enhancer-promoter interactions reveals co-expression of genome genes and modes of higher order chromatin organization. Cell Res. 2012;22:490–503.

23. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, et al. An oestrogen-receptor-alpha-bound human chromatin interactome. Nature. 2009;462:58–64.

24. Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature. 2013;503:290–4.

25. Sanjay A, Lajoie BR, Jain G, Dekker J. The long-range interaction landscape of gene promoters. Nature. 2012;489:109–13.

26. Stadhouders R, Thongjuea S, Andrieu-Soler C, Palstra R-J, Bryne JC, van den Hurvel J, et al. Dynamic long-range chromatin interactions control Myb proto-oncogene transcription during erythroid development. EMBO J. 2012;31:1886–99.

27. Zhang Y, Wong CH, Bimbbaum RA, Li G, Favaro R, Ngan CY, et al. Chromatin connectivity maps reveal dynamic promoter-enhancer-long-range associations. Nature. 2013;504:306–10.

28. Heinz S, Benner C, Spann N, Bertolino E, Lin Y-C, Laslo P, et al. Simple combinations of lineage-determination transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell. 2011;30:385–95.

29. Imakaev M, Rudolphi G, McCord RP, Naumova N, Goloborodko A, Lajoie BR, et al. Iterative correction of Hi-C data reveals hallmarks of chromosome organization. Nat Methods. 2012;9:999–1003.

30. Li G, Fullwood MJ, Xu H, Mulawadi FH, Velkov S, Vega V, et al. CHIA-PET tool for comprehensive chromatin interaction analysis with paired-end tag sequencing. Genome Biol. 2010;11:R22.

31. Lin YC, Benner C, Mansson R, Heinz S, Miyazaki K, Miyazaki M, et al. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. Nat Immunol. 2012;13:1196–204.

32. Thongjuea S, Stadhouders R, Grosfeld FG, Soler E, Lenhard B. r3Cseq: an R/Bioconductor package for the discovery of long-range genomic interactions from chromosome conformation capture and next-generation sequencing data. Nucleic Acids Res. 2013;41:e132.

33. Scales M, Jäger R, Migliorini G, Houston RS, Henion MV, VisPI-G: A web tool for producing multi-region, multi-track, multi-scale plots of genomic data. PloS One. 2014;9:e107497.

34. Ay F, Bailey TL, Noble WS. Statistical confidence estimation for Hi-C data reveals regulatory chromatin contacts. Genome Res. 2014;24:999–1011.

35. Paulsen J, Redland EA, Holden L, Holden M, Hovig E. A statistical model of CHIA-PET data for accurate detection of chromatin 3D interactions. Nucleic Acids Res. 2014;42:e143.

36. Gentelman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dubart S, et al. Bioconductor: Open software development for computational biology and bioinformatics. Genome Biol. 2004;5:R80.

37. Lun ATL, Smyth GK. dlFCHIC: A Bioconductor package to detect differential genomic interactions in Hi-C data. BMC Bioinformatics. 2015;16:258.

38. Servant N, Lajoie BR, Nora EP, Giorgetti L, Chen C-J, Heard E, et al. HTC: Exploration of high-throughput "C" experiments. Bioinformatics. 2012;28:2843–4.

39. Mifsud B, Martincorena I, Darbie E, Sugar R, Schoenfelder S, Fraser P, Luscombe N. GOTHIC, a simple probabilistic model to resolve complex bis and to identify real interactions in Hi-C data. bioRxiv 2015;023317. http://dx.doi.org/10.1101/023317.

40. Walter C, Schuetzmann D, Rosenbauer F, Dugas M. Basic4Cseq: An R/Bioconductor package for analyzing 4C-seq data. Bioinformatics. 2014;30:3268–9.

41. Klein FA, Pakzad T, Anders S, Ghiari-Helm Y, Furlong EM, Huber W. FourCseq: Analysis of 4C sequencing data. Bioinformatics. 2015;31(19):3085–91. btv335.

42. Lawrence M, Huber W, Pages H, Aboyoun P, Carlsson M, Gentleman R, et al. Software for computing and annotating genomic ranges. PLoS Comput Biol. 2013;9:e1003118.

43. Ay F, Noble WS. Analysis methods for studying the 3D architecture of the genome. Genome Biol. 2015;16:183.

44. Phanstiel DH, Boyle AP, Heidari N, Snyder MP. Mango: A bias-correcting CHA-PET analysis pipeline. Bioinformatics. 2015;31(19):3092–8. btv336.

45. Heidari N, Phanstiel DH, He C, Grubert F, Johananian F, Kasowski M, et al. Genome-wide map of regulatory interactions in the human genome. Genome Res. 2014;24:1905–17.

46. Hafner F, Durincik S, Ivanek R, Mueller A, Lianoglou S, Tan G, Parsons L: Gviz: Plotting Data and Annotation Information Along Genomic Coordinates. http://bioconductor.org/packages/2.14/bioc/html/Gviz.html.

47. Karolchik D, Barber GP, Casher J, Clawson H, Cline MS, Diekhans M, et al. The UCSC Genome Browser database: 2014 update. Nucleic Acids Res. 2014;42.Database Issue:D764–70.

48. Csardi G, Nepusz T. The igraph software package for complex network research. Interjel. 2006;40:Complex Systems:1695.

49. Seitan VC, Faure AJ, Zhan Y, McCord RP, Lajoie BR, Ing-Simmons E, et al. Cohesion-based chromatin interactions enable regulated gene expression within preexisting architectural compartments. Genome Res. 2013;23:2066–77.

50. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009;10:25.

51. McCready PM, Hansen RK, Burke SL, Sands JF. Multiple negative and positive cis-acting elements control the expression of the murine CD4 gene. Biochim Biophys Acta. 1997;1351:181–91.

52. Shen Y, Yue F, McCleary DF, Ye Z, Edall L, Kuan S, et al. A map of the cis-regulatory sequences in the mouse genome. Nature. 2002;488:116–20.

53. FANTOM Consortium and the RIKEN PMI and CLST (DGT). A promoter-level mammalian expression atlas. Nature. 2004;405:462.

54. Hoffman MM, Ernst J, Wilder SP, Kundaje A, Harris RS, Libbrecht M, et al. Integrative annotation of chromatin elements from ENCODE data. Nucleic Acids Res. 2013;41:827.

55. Decker S, Finter J, Forde AJ, Kissel S, Schwaller J, Mack TS, et al. PIM kinases are essential for chronic lymphocytic leukemia cell survival (PIM2/3) and CXCR4-mediated microenvironmental interactions (PIM1). Mol Cancer Ther. 2014;13:2313–25.

56. Damm F, Chnessa V, Nagata Y, Yoshida K, Scourlate LC, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood. 2013;122:1619–77.

57. Gatte V, Degani M, Gloghini A, De Iuliis A, Improtta S, Rossi FM, et al. CD30 ligand is frequently expressed in human hematopoietic malignancies of myeloid and lymphoid origin. Blood. 1997;90:2048–59.

58. Ramirez-Herrick AM, Mulligan SE, Sheehan AM, Conneely OM. Reduced NR4A gene dosage leads to mixed myelodysplastic/myelo proliferative neoplasms in mice. Blood. 2011;117(9):2681–90.

59. Kadkhodaei B, Ito T, Joodmarsi E, Mattsson B, Rouillard C, Krichmar J, et al. Nrun1 is required for maintenance of mature and adult dopaminergic neurons. J Neurosci. 2009;29:5259–52.

60. Sekiya T, Kashwagi I, Inoue N, Motta R, Horii S, Waldmann H, et al. The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of naive CD4+ T cells by inhibiting the transcriptional activity of E47/HEB. Cancer Cell. 2004;5:587–96.