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Guest Editor's Introduction

Methods for mapping three-dimensional genome architecture

It is becoming increasingly clear that DNA-templated processes are influenced by the three-dimensional (3D) conformation and spatial organization of the genome. In eukaryotes, gene transcription is controlled by the packaging of chromatin within intricate loops that fold into a hierarchy of higher-order structures [1,2]. Chromatin loops bring distal genomic elements into spatial proximity and facilitate long-range communication between them. These loops are formed between enhancers and gene promoters that are located tens or even hundreds of kilobases apart [3–6] or, on a smaller scale, between gene promoters and terminators [7–12] or gene coding and regulatory regions [13–15]. Such loops are then assembled into topologically associating domains (TADs) that ensure gene-specific regulation by preventing enhancers from promiscuously activating promoters [1,16,17]. Perturbation of TAD integrity can cause cancer [18] or developmental disorders [19], underscoring the importance of chromosome topology and genome organization in the proper regulation of gene transcription.

While chromatin loops and chromosome topology facilitate proper regulation of transcription, spatial positioning of genes inside the nucleus is also important. Genes may adopt distinct 3D locations that modulate their expression. They may interact with the nuclear envelope [20–23] or nucleolus [24,25], or cluster within nuclear substructures enriched in transcriptional activation [26–28] or repression machinery [29,30].

A combination of microscopy-based and molecular assays has been applied to study genome architecture and its organization. Fluorescence microscopy and its derivative techniques such as immunofluorescence, fluorescence *in situ* hybridization (FISH) and fluorescent reporter-operator system (FROS) have enabled visualization of chromosomal territories [31], gene re-positioning and association with the nuclear envelope [21,22,32], transcription factories [33–35] and biomolecular condensates [36]. Molecular techniques such as chromosome conformation capture (3C) and its genomic variants (4C, 5C, Hi-C, ChIA-PET), DNA adenine methyltransferase identification (DamID), and RNA tagging and recovery of associated proteins (RNA TRAP) have been useful in mapping long-range *cis*- [3–6] and *trans*- [27,37] chromatin interactions. They have also enabled identification of higher-order chromatin structures such as chromatin compartments [38], TADs [10,39–41] and Lamin-associated domains (LADs) [42]. This special issue on *Methods for Mapping Three-Dimensional Genome Architecture* provides a compendium of research and review articles reporting improved versions of these existing techniques – as well as a few novel ones – for studying 3D genome architecture and organization.

The advent and application of 3C in *Saccharomyces cerevisiae* (budding yeast) has uncovered important features of its genome [41,43–47]. Nonetheless, mapping short-range interactions in this organism using a typical 3C assay has always been a challenge. In this issue, Chowdhary et al describe a modified and highly sensitive version of 3C, termed TaqI-3C, that detects stimulus-dependent chromatin contacts between genomic loci located as close as 500 bp [48]. Use of a 4 bp restriction cutter, Taq I, substantially enhances resolution, as does incorporation of a number of technical controls, the most important of which accounts for the variation in digestion efficiency of individual restriction sites within chromatin. Application of the technique has led to the discovery of enhancer (upstream activation sequence) – promoter looping, regulatory region – gene coding region "crumpling", and most striking, activator-specific *cis*- and *trans*-contacts between transcriptionally induced genes [15,49].

A major limitation of the locus-specific 3C assay is that it is constrained by throughput, and capturing multiple genomic interactions can be labor intensive. Chromosome conformation capture-on-chip (4C) [50] and its derivative techniques [51,52] enables high-throughput mapping of interactions between a locus of interest and all other genomic regions by either deep sequencing or microarray. However, these methods require PCR amplification that can introduce a bias in the readout. 4C-seq, an improved version, can correct for this and other biases, and at the same time offer much higher resolution [53]. In this issue, Krijger and co-workers provide a detailed protocol for processing 4C-seq libraries using improved PCR and purification strategies that makes their technique more efficient and reproducible [54]. In addition, the authors provide a novel computational pipeline to process 4C-seq data directly from FASTQ files to widely used output formats and then to standard peak-calling genome browsers for visualization of 3D contact maps. This step-by-step guide should allow any user of this method to generate high-quality 4C-seq libraries and process and interpret their data.

In contrast to 4C, Hi-C renders an unbiased, comprehensive map of all possible interactions occurring within the genome [55]. Hi-C is fundamentally similar to 3C, but is a genome-wide method that offers much higher coverage of both *cis* and *trans* interactions, including those that occur over large genomic distances. Hi-C has become a prominent tool for studying genome architecture and organization; as a consequence, efforts are continuously being made to enhance its resolution and sensitivity. Papantonis and colleagues have described a cross-linking-free variant of Hi-C, called intrinsic Hi-C (iHi-C), that can detect native chromatin folding patterns inside living cells [56]. Although formaldehyde has been widely used in many molecular procedures, including 3C and 3C-based methods, its *in vivo* effects are not insignificant and its use might confound data interpretation. iHi-C is performed in the absence of chemical fixation and thus corrects for biases due to crosslinking [56]. In this issue, Mizi et al present an updated version of intrinsic Hi-C, iHi-C 2.0, that corrects for an additional limitation due to inefficient blunt-ended ligation of biotinylated DNA fragments [57]. It also permits analysis of samples with limiting cell counts and offers improved signal to noise ratios compared with typical

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Hi-C methods.

In situ Hi-C is a technique that involves formaldehyde crosslinking of cells and restriction digestion of isolated nuclei. In this issue, Huang and co-workers describe Bridge linker-Alul-Tn5 Hi-C (BAT Hi-C) [58], a method that represents a significant improvement over conventional *in situ* Hi-C in terms of both simplicity and cost associated with library preparation.

Hi-C-generated libraries have a high combinatorial complexity that arises from the large number of theoretically possible ligation products. Therefore, great sequencing depth would, in principle, be required to map all ligation combinations, a costly endeavor. To overcome this problem, Golov and colleagues have developed Chromatin Target Ligation Enrichment (C-TALE), a technique that uses a solution hybridization-based approach to enrich for the region of interest and its interacting regions [59]. C-TALE is a cost-efficient method that can be used for mapping fine-scale chromatin contacts sometimes missed by typical Hi-C methods and offers increased signal-to-noise ratios.

A typical Hi-C data set is an ensemble of interaction frequencies obtained from a population of cells. Therefore, it cannot detect cell-to-cell heterogeneity in chromatin structure that may exist due to cell cycle variation or epigenetic variegation, or due to the process of transcription itself being dynamic and stochastic. Therefore, to apply Hi-C to single cells, Ramani et al developed a method that they term single-cell combinatorial indexed Hi-C (sci-Hi-C) [60]. The sci-Hi-C procedure applies combinatorial cellular indexing that enables high-throughput mapping of chromatin interactions in a statistically significant number of single cells. This procedure utilizes an in-silico sorting method to separate cells from a mixed population, eliminating the need for any special devices to physically sort the cells. A step-by-step guide to sci-Hi-C and a working protocol is included in this issue [61].

While Hi-C and its related methods can provide an unbiased map of chromatin contacts, these may not reveal the functional relevance of such interactions. However, Chromatin Interaction Analysis by Paired-End Tag sequencing (ChIA-PET) might, as it combines chromatin immunoprecipitation of the protein of interest and proximity ligation of protein-bound chromatin fragments [62]. ChIA-PET has been applied to map chromatin contacts formed between regions bound by RNA Polymerase II [5], a gene-specific transcription factor (estrogen receptor α) [62], and architectural proteins including cohesin and CTCF [63]. Therefore, there is a widespread interest in utilizing both ChIA-PET and Hi-C for their individual applications in mapping 3D genome architecture and organization. In this issue, Capurso and colleagues provide a comprehensive review of computational tools available for processing and viewing both Hi-C and ChIA-PET data [64].

All currently available 3C-based methods rely on proximity-based ligation of chromatin fragments and most, if not all, provide a composite average of interaction frequencies of the cell population. Thus, most of these methods cannot measure simultaneous interactions that may occur between more than two genomic regions. However, two recently developed techniques, split-pool recognition of interactions by tag extension (SPRITE) [24] and Trac-looping [65], can do so, and in a ligation-independent manner. In this issue, McKay and colleagues provide a detailed comparison of these two techniques as well as discuss promising developments in the field of 3D genomics [66].

As neither SPRITE, Trac-looping, nor any of the 3C-based methods discussed above can completely resolve concerns associated with molecular assays, a useful complement is microscopy-based analysis. In this context, single-molecule imaging can be utilized to provide real-time snippets of 3D genome organization. In this issue, Wollman and co-workers use the spatial localization of a DNA-bound protein as a proxy for determining the position of an underlying genomic locus [67]. Although this approach is constrained by the type and identity of a DNA-bound factor, it can provide context-dependent snapshots of the 3D genome. Also, when combined with other imaging and molecular analyses, single-molecule tracking data can resolve complex features of the 3D genome in millisecond timescales.

Taken together, the papers of this special issue present a palette of powerful techniques for mapping chromatin topology and genome organization. While the focus is on eukaryotic cells, there is no reason why the methods described here cannot be used to query 3D genome architecture in all three kingdoms of life.

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