Engineering Advancements in 3D Genomics: Hi-C

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The genome in the mammalian nucleus comprises several 3D interactions that are pivotal in the functionality of the cell. These interactions are multitudinous, often comprising of near infinite loci-to-loci relationships. The introduction of Hi-C technology has allowed scientists to identify and map these relationships in the complete nuclear genome (Van Berkum et al., 2010). As the first unbiased approach evaluating chromatin conformation, Hi-C has led researchers to unearth an array of gene regulation mechanisms (Guide to Understanding, 2020). Hi-C has been pivotal in disease research, specifically uncovering gene fusion in pediatric cancers and gene dysregulation in neurodegenerative diseases (Dileep et at., 2023; Schmitt et al., 2024).

Prior to Hi-C, three key methods were employed for the small-scale analysis of genetic structure: 3C, 4C, and 5C. In the 3C technique, a single pair of chromosome loci (1 x 1) can be contrasted (Lieberman-Aiden et al., 2009). 4C utilizes inverse PCR to determine the interactions between one locus and all other loci (1 x all), while 5C utilizes multiplex PCR to contrast all interactions within a collection of about 1000 loci (~1000 x ~1000) (Lieberman-Aiden et al., 2009). These developmental stages in 3D genomics lacked a comprehensive approach to chromatin architecture and focused on pinpoint attributes of nucleic genome structure. In 2009, Erez Lieberman-Aiden and his colleagues at the Broad Institute developed a new method that does not specify a limited number of loci, but rather, the entire nuclear genome (all x all) (Guide to Understanding, 2020). This refinement has led to a modern understanding of 3D-nuclear genome architecture.

The methodology of Hi-C combines both wet-lab and computational methods to determine the architecture of nuclear chromatin. Cells are initially placed in a formaldehyde fixing solution, crosslinking DNA-DNA interactions (Guide to Understanding, 2020). DNA is then

lysed and subsequently fragmented with the restriction endonuclease HindIII (Belton et al., 2012). After fragmentation, the 5' overhangs are filled in and labeled with a biotinylated nucleic acid (Van Berkum et al., 2010). Blunt-end ligation is performed at the site of the biotinylated nucleic acid (the ligation junction) to create a hybrid DNA strand. The crosslinking is reversed, and the samples are sheared (Guide to Understanding, 2020). Streptavidin beads are bound to these linear fragments, as they maintain a high affinity for biotin. This allows for the separation of biotinylated and unwanted DNA fragments (Belton et al., 2012). Once purified, the samples contain ligated DNA fragments that were physically adjacent in the original chromatin structure (Belton et al., 2012). These fragments are sequenced and computationally analyzed to visualize spatial adjacency in the genome. The output of such analysis can be visualized in heatmaps and 3D architectural tools (Van Berkum et al., 2010).

Hi-C is advantageous in detecting "all-to-all" DNA interactions, however, it may not be effective in the precise study of individual "loci-to-loci" interactions (as in 3C) or other molecular interactions (Kumar et al., 2021). ChIA-PET, for example, analyzes specific protein-DNA interactions that may be targeted by researchers (Fullwood et al., 2009). Today, Hi-C has been further refined to address some of these shortcomings. One refinement known as "Capture Hi-C" maintains similar methodology but allows for a more focused analysis of specific target regions of nuclear chromatin (Mifsud et al., 2015).

As the field of genomics migrates toward single-cell and higher resolution 3D genome mapping, the need for increasingly accurate technology remains. The development of Single-cell Hi-C exists but is only emerging from a state of infancy (Zhou et al., 2019).

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