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Editorial

Joint Imaging and Sequencing of Protein-DNA Interactions in Single Cells Using a Microfluidic Approach

Nicolas satura

University of California

The epigenome is made up of protein-DNA interactions that include interactions with histone proteins, transcription factors, DNA (de)methylases, and chromatin remodelling complexes, among other things. These interactions allow the nucleus's static DNA sequence to dynamically execute various gene expression programmes that form the cell's identity and behaviour. Methods for measuring protein-DNA interactions have proven important for understanding the epigenome, but much of what we know so far has come from bulk cell population experiments. These bulk methods can miss essential epigenomic processes that occur in small numbers of dividing cells, such as those that affect embryo formation, developmental diseases, stem cell differentiation, and certain cancers, because they require large numbers of cells.

Bulk methods, as a result, can miss essential biological heterogeneity within a tissue. It's also difficult to combine large amounts of biochemical data with imaging data, which inherently provides knowledge about single cells and can show the spatial position of protein-DNA interactions within living cells' nuclei. The need for high-sensitivity single-cell methods for measuring protein-DNA interactions is highlighted by these limitations. Immunoaffinity purification, in which protein-DNA complexes are physically separated using a high-affinity antibody against the protein, then purified by washing and de-complexed so that the interacting DNA can be amplified and measured, is the most common method for mapping protein-DNA interactions.

The most commonly used of these techniques is chromatin immunoprecipitation sequencing (ChIP-seq), which has served as the foundation for a number of large-scale epigenome mapping projects. One disadvantage of ChIP-seq is that fragile protein-DNA complexes must withstand shearing or digestion of the surrounding DNA, as well as several intermediate washing and purification steps, before being amplified and sequenced.

When using a small amount of starting material, this results in a lack of sensitivity. Recent immunoaffinity-based approaches have reduced ChIP-high seq's input requirements, but they only recover a few interactions in small groups of cells or single cells. DNA adenine methyltransferase identification (DamID), an alternative approach for probing protein-DNA interactions, relies on a kind of "chemical recording" of protein-DNA interactions onto the DNA itself, rather than physical separation of protein-DNA complexes (as in ChIP-seq).

DNA adenine methyltransferase, a small E. coli enzyme, is used in this process (Dam). Dam deposits methyl groups near protein-DNA contacts at the N6 positions of adenine bases (m6A) within GATC sequences when genetically fused to the protein of interest (which occur once every 270 bp on average across the human genome). That is, m6A marks are left at the GATC sites in the protein's trail wherever it touches DNA in the genome.

In eukaryotic cells that do not methylate (or demethylate) adenines, these m6A marks are extremely stable. Dam expression had little impact on gene expression in a human cell line, and its m6A marks were found to be passed down to daughter cells, halving in quantity per generation after Dam was inactivated..