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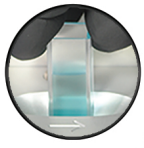

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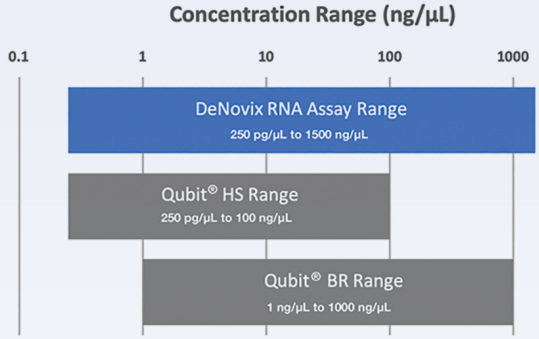

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Seeing with DNA

DNA SEQUENCING MICROSCOPY PROVIDES QUANTITATIVE AND SPATIAL GENE EXPRESSION INFORMATION, ALLOWING RESEARCHERS TO PROFILE AND UNDERSTAND DISEASE AT A NEW DEPTH.

By **Nathan Ni, PhD**

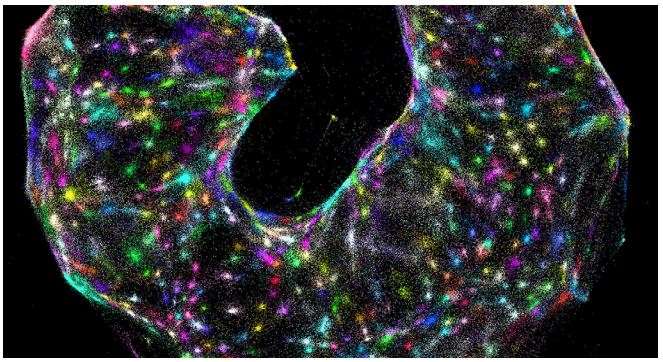
DNA sequencing microscopy first emerged in November 2018 with a flurry of manuscripts posted on pre-print servers. Each outlined similar but distinct methods for determining spatial gene expression information by sequencing and mapping biomolecule-bound DNA tags, giving researchers the potential to map genetic expression patterns.

The first of the three manuscripts came from Edward Marcotte's lab at the University of Texas at Austin[1], followed by Feng Zhang's group at the Broad Institute of MIT and Harvard[2] and then by Bjorn Högberg's team at the Karolinska Institute[3]. "We posted our pre-print[3] as soon as we saw [the other two]," said Ian Hoffecker from the Högberg lab, lead author of both the pre-print and the subsequent manuscript published in the *Proceedings of the National Academy of Sciences*[4]. "It was very exciting to be part of the birth of a new field."

Joshua Weinstein, the lead author of the Broad Institute study who is now at the University of Chicago, believes that DNA sequencing microscopy changes the landscape of life sciences imaging. His manuscript laid the foundation for a publication in *Cell*[5], the first peer-reviewed documentation of an image construction method that does not use light.

Biological imaging techniques typically involve looking inward from the outside, either by cutting a specimen open or using light to penetrate it. DNA sequencing microscopy creates another option: imaging specimens from the inside-out. "Through technologies like this, we finally have the opportunity to take a look at structures with uniquely specific mutational and morphological signatures," said Weinstein, "That really empowers us to be able to understand biology down to the cellular and subcellular level in a way that we couldn't before."

The three methods share some similarities. In general, DNA sequencing microscopy labels biomolecules of interest with unique DNA tags that interact with each other within the local environment. Researchers determine the nature and proximity of these interactions via sequencing, and this information is spatially mapped in silico. How this mapping occurs is where the methods diverge.



DNA sequencing microscopy image courtesy of Joshua Weinstein.

Hoffecker's method employs a topology-based reconstruction approach. A mesh of pixels forms from tagged DNA patches. When target molecules land on these pixels, scientists identify their positions relative to the pixels. This, combined with pixel positions relative to each other, makes reconstruction possible.

Weinstein's method, on the other hand, is a distance metric-based reconstruction approach, basically turning all of the molecules of interest within a specimen into a network of nodes. "Imagine that you were trying to reconstruct a map of the United States by getting every cell phone tower to ping one another," explained Weinstein, "DNA [sequencing] microscopy does precisely the same thing, but instead of towers, it uses the communications between individual molecules within the specimen."

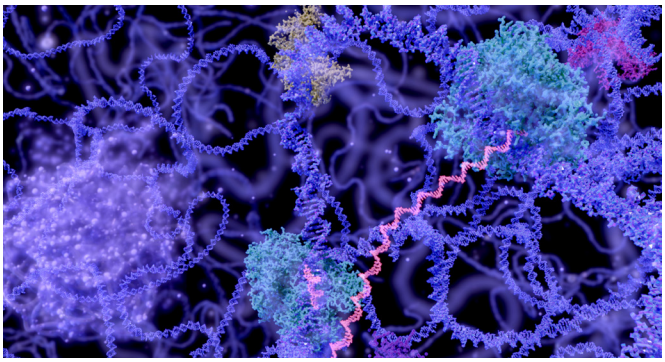
Finally, the method developed by Alexander Boulgakov and the Marcotte lab captures ligation events between spatially distributed barcoded oligonucleotides. They then catalog these iterative proximity ligation events and employ next-generation DNA sequencing to delineate relative positions.

For both Hoffecker and Weinstein, the biggest technical advantage of DNA sequencing microscopy is greater imaging bandwidth. Optical microscopy is restricted by whether two spatially overlapping signals can be distinguished from one another. DNA sequencing microscopy encodes all of its information within the sequence itself, and since the four bases of DNA spell out enormous unique sequences, researchers can distinguish up to 4^{100} different possibilities.

Naturally, variability is a potential problem when multiple methods are developed simultaneously. Weinstein holds similarity to experimental data as the gold standard for validation. Hoffecker takes a longer-term approach, concerned that immediately addressing standardization may curtail innovation. "The main priority is to explore the space of possibilities and find out what can and cannot be done," he said. "If at some point one technique is shown to be more versatile, practical, or powerful, then we may see some convergence toward standardization. But for now, we have only begun to scratch the surface of what is possible."

See references on page 6.

Poly(A) Tails Tell a Tale



A NEW SEQUENCING METHOD REVEALS THAT POLYADENYLATION MAY BE A MISNOMER.

By **Kathryn Loydall, PhD**

A method developed by Nikolaus Rajewsky's team from the Max Delbrück Center for Molecular Medicine revealed a number of cytosines in the mRNA poly(A) tails from a variety of organisms. The new method, full-length poly(A) and mRNA sequencing (FLAM-seq), is a rapid and simple method for sequencing entire mRNAs. It differs from other methods by sequencing full-length mRNA—including the poly(A) tails—as opposed to combining short-read sequences.

Polyadenylation—the addition of a poly(A) tail to mRNA—is a key step in the larger process of gene expression. Determining poly(A) tail length and sequence is therefore important to genetics researchers. Until now, researchers relied on short-read sequencing combined with advanced biochemical or computational approaches to estimate a broad range of tail lengths or determine terminal modifications of poly(A) tails. However, these approaches cannot determine the mRNA isoform of the sequenced tails. One established method, Isoform Sequencing (Iso-Seq™), employs long reads to sequence transcript isoforms from the 5' end up to their poly-A tails, but it does not include the tails.

"We wanted to go deeper than Iso-Seq by capturing the entire poly(A) tails of the transcript," said Ivano Legnini, coauthor of the paper, which was published in the journal *Nature Methods*.

To achieve this, the team copied each mRNA into a single strand of cDNA, which entailed adding a short stretch of guanosines and inosines to the tails. They then performed long-read sequencing. "The big challenge was to optimize the chemistry of our library preparation using chemically modified oligonucleotides," explained Jonathan Alles, coauthor.

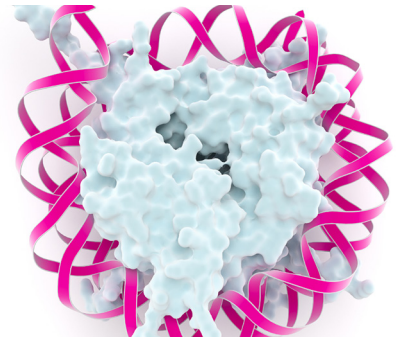
FLAM-seq helps to disentangle the effects of polyadenylation and poly(A) tail length. "We can easily check how long the poly(A) tails are that are attached to an RNA molecule, which also tells us exactly how this RNA molecule has been spliced and polyadenylated," Alles said. This facilitates data analysis, as researchers can omit complicated algorithms that assemble and infer how different RNA isoforms are expressed from short-read sequencing data. "We can now simply look at the full-length molecules we sequence and have this information," said Alles.

The new method works with any third-generation sequencer and incorporates the transcription start site, splicing pattern, the 3' end, and the poly(A) tail for each sequenced molecule. "We wanted to develop a tool that is accessible to the community and make things easier for labs less familiar with the details of methods development and implementation," explained Alles.

Poly(A) tails regulate translation and turnover of RNA, which cells can modify by adding other nucleotides in addition to adenosine. Using their new method, Rajewsky's team surprisingly found many cytosines in poly(A) tails in human cell lines, brain organoids, and *Caenorhabditis elegans* cells. The presence of non-A nucleotides within poly(A) tails can have substantial effects on tail shortening, which affects RNA turnover. "We don't know what the full consequence of these cytosines is on mRNAs yet," said Legnini. "We are using FLAM-seq to understand basically the dynamics of adenylation, deadenylation, and turnover of mRNA."

See references on page 6.

Good Things Come in Small Packages



A NEW HIGH THROUGHPUT HISTONE SEQUENCING METHOD ENABLES GENOME-WIDE ANALYSIS OF LOW-ABUNDANCE CELL POPULATIONS, PRESENTIN RESEARCHERS WITH A USEFUL METHOD FOR SCREENING PATIENT SAMPLES.

By **Niki Spahich, PhD**

Epigenetic changes such as histone modifications affect chromosome conformation, gene expression, and genome stability, which can lead to diseases including cancer[1]. Because epigenetic changes vary from person to person, researchers are devising strategies to study them on a genome-wide scale in individuals, with the hope of using this information for precision medicine. To that end, a team led by Chang Lu from Virginia Polytechnic Institute and State University recently developed a method that combines high throughput DNA sequencing with chromatin immunoprecipitation (ChIP) from small cell samples, such as those derived from patients[2].

"It's pretty exciting," said Alon Goren, associate professor at the University of California, San Diego, who was not involved in this study. "There has been excitement over the last 5-10 years for using microfluidics to do ChIP sequencing. It is good to see that they have this method up and running. They have some nice, clever solutions."

To find DNA sequences that interact with modified histones, researchers often couple ChIP with DNA sequencing. To perform a ChIP assay, a scientist digests chromatin and isolates DNA-bound histones using antibodies specific to a desired histone modification. Next, the researcher may use a variety of methods to identify the associated DNA.

Traditional ChIP-seq methods call for millions of cells, a requirement that hinders experiments on patient samples. To generate data that can be compared to reference tissue and cell epigenomes, researchers must purify patient samples into homogenous pools, which often decreases the experimental yield to levels not conducive to traditional ChIP-seq. "Once you can work with a small number of cells, that opens doors to look at patient samples, which do not yield a lot of cells," said Lu.

The new paper, published in *Nature Protocols*, provides instructions for constructing and using a device that performs microfluidic oscillatory washing-based chromatin immunoprecipitation, followed by next-generation sequencing (MOWChIP-seq). With just 100 cells, MOWChIP-seq reproducibly generates data similar in quality to other ChIP-seq assays that require 1-10 million cells. This method also results in better coverage of the epigenome compared to other ChIP-seq methods designed to sequence low numbers of cells.

"The difference between what we do and the conventional method is that we use a microfluidic device that conducts the immunoprecipitation in a very small micrometer chamber," said Lu.

The small device pushes digested chromatin samples through a bed of micrometer-sized immunomagnetic beads coupled to the histone-specific antibodies. Oscillatory washing decreases non-specific antibody binding prior to performing Illumina sequencing on the histone-bound DNA.

Lu's method improves upon a protocol he and his team published in 2015[3]. According to Lu, his updated device tightly packs a larger volumetric quantity of beads compared to standard protocols using microfuge tubes. Better bead packing increases the efficiency of histone-antibody binding as samples flow through the device. Both the dense bead bed and the efficient washing contribute to the method's success when using small cell numbers.

A major improvement in this method over the 2015 version is its ability to run eight samples at once. "Overall, it's a perfectly scalable technology. The reason why we presented eight assays in parallel in our paper is because that tends to be the kind of throughput needed by an individual lab," said Lu. "In the future, if you want this technology to work in a hospital, we can easily scale that up." Additionally, aside from a few steps involving the addition of reagents, this technology is automated, which reduces labor and human error while increasing reproducibility.

Goren recognized the potential for using methods like MOWChIP-seq for precision medicine by identifying aberrations during disease, finding markers of disease, or learning how the body responds to treatment. "It is fun for me to see how multiple groups are trying to push the boundaries [of epigenomics] in a lot of directions by implementing a variety of approaches," said Goren. At the same time, he wondered how complicated it would be for non-engineers to get the device up and running. Lu wanted the device to be accessible, so there are detailed instructions on how to build the machine in the new paper. However, he admits that academic researchers may need help from engineers or electricians initially.

Currently, how the epigenome changes during disease and the function of these changes is relatively unknown. Lu anticipated that other researchers would build his microfluidic device to enhance their own work. "Hopefully our technology will allow more people to work with biologically relevant materials and samples, and then we can generate more information about diseases," he said.

See references on page 6.

Article 1 – Seeing with DNA

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Article 2 – Poly(A) Tails Tell a Tale

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