

Interview

Studying Genome Interaction and Regulation Networks in Three Dimensions

Dr. Yijun Ruan discusses how paired-end-tag (PET) strategies are unlocking the secrets of the DNA world with the help of Illumina's Genome Analyzer_{IIx}.

INTRODUCTION

It started with the creation of a new tool—paired-end-tag or PET—as an alternative to serial analysis of gene expression (SAGE) to identify protein binding sites in DNA. Over five years later, the technology developed by Dr. Yijun Ruan and his team at the Genome Institute of Singapore (GIS) has matured. Leveraging the unique features and high-throughput power of Illumina's Genome Analyzer_{IIx}, PET technology is answering a number of basic genome biology questions from how cancer cells progress to how stem cells maintain their unique properties.

i: What sparked the development of PET technology and what are its unique capabilities?

Our research is focused on elucidating the structures and dynamics of all functional DNA elements in complex genomes through transcriptome characterization and genome interrogation. To facilitate that understanding, we developed the PET sequencing strategy in which short and paired tags are extracted from the ends of long DNA fragments for ultra-high-throughput sequencing. PET sequences can be accurately mapped to a reference genome, thus demarcating the genomic boundaries of PET-represented DNA fragments and revealing the identities of target DNA elements.

The exclusive advantage of the PET technology is its ability to uncover linkages between the two ends of DNA fragments. Using this unique feature, unconventional fusion transcripts, genome structural variations, and even molecular interactions between distant genomic elements can be unraveled.

The PET sequencing strategy is being used to answer many questions regarding nuclear processes. Using different PET applications, different types of nucleic acids can be analyzed, such as RNA for transcriptome analysis (RNA-PET), DNA for genome structure variation and genome sequence assembly (DNA-PET), manipulated DNA fragments, such as chromatin immunoprecipitation-enriched DNA for mapping transcription factor binding sites (ChIP-PET), and proximity-ligated DNA for chromatin interaction analyses (ChIA-PET)¹.

i. What types of studies have you conducted?

We've been using RNA-PET to study transcriptomes with a focus to identify fusion genes², and DNA-PET to study genome rearrangements in cancer cells. In pursuing our core interests on transcription regulation, we've used ChIP-PET to map the binding sites of a number of transcription factors that are important in cancer biology, such as p53, and enable embryonic stem



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cells to maintain their pluripotency³. When we started ChIP-PET, the field was dominated by ChIP-chip, a microarray-based approach to study transcription factor binding. At the time, people doubted that any sequencing alternative would be viable. Our ChIP-PET studies demonstrated that DNA sequencing could be better. Soon after, when Illumina's tag sequencing platform became available, the whole field embraced it. Illumina's technology is contributing to the overwhelming success that we are now enjoying. We are happy to be among the first to lead the way in this new field.

Moving forward, we realized that many transcription factors actually have binding sites that are distant from gene promoters. This motivated us to develop the ChIA-PET method to study whole-genome chromatin interactions involved in transcription regulation. We are successfully using ChIA-PET to map the chromatin interaction networks mediated by estrogen receptor α (ER- α) in human cancer cells⁴. Before our work, chromatin interactions and their impact on transcription regulation had never been investigated in a genome-wide manner. As a result of our ER- α research, we believe that chromatin interactions are a primary mechanism for regulating the transcriptome in mammalian genomes.

i: What has been the overall impact of using Illumina's Genome Analyzer_{ix}?

Illumina's Genome Analyzer_{ix} enables us to continue pushing the envelope in developing unique technologies to study nuclear processes and helps us to identify which methods are the best. When we began our research programs in 2002, we realized that efficient paired-end sequencing would provide the two-point reference that we needed to define the relationship between distant genomic elements in the transcriptome. Clearly, being able to do

this on a genome-wide basis was imperative. Because of the protein packing within the nucleus, we also needed a system that would provide deep sequencing. The Genome Analyzer_{ix} met these needs and more, enabling our technologies to be powerful and seamless. So far we've used it to generate hundreds of libraries for various PET analyses.

i: What are the next steps in your research?

The ChIA-PET strategy has really opened up a new way of performing analysis to study transcription regulation networks. In our first ChIA-PET study, we looked at a very specific estrogen receptor and were able to identify a network of transcription interactions and its three-dimensional architecture. We'd now like to look at a number of transcription factors under many different conditions and, at different points in time (before, during, and after drug treatment), to see the dynamic changes that occur. At that point we'll be analyzing the three-dimensional interactions of thousands of genes, a virtual village in the global nucleus.

We expect that our ChIA-PET strategy, with its whole-genome approach for unbiased and *de novo* discovery of long-range chromatin interactions, will become an established method for studying genome interaction and regulation networks in three dimensions.

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ADDITIONAL INFORMATION

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