DNA Sequencing by Synthesis Using Fluorescent, Raman, and Nanopore Detectable Tagged Nucleotides

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Abstract

DNA sequencing is a fundamental tool in biological and medical research. High-throughput DNA sequencing is especially important for the paradigm of precision medicine. The approach that has dominated the second generation of sequencing technologies is sequencing by synthesis (SBS), whereby the DNA sequence is determined as each nucleotide adds to the growing strand of DNA in the polymerase reaction. This is made possible by the presence of detectable tags that distinguish the 4 bases of DNA (A, C, G and T). In the Ju laboratory, we have pioneered the development of the SBS approach using a wide variety of tagged nucleotides.

• Sets of nucleotides with molecular tags detectable by mass spectrometry
• Sets of nucleotides with fluorescent tags detectable by optical methods
• Sets of nucleotides with molecular tags detectable by surface enhanced Raman spectroscopy (SERS)
• Sets of nucleotides with polymeric tags detectable at single molecule level by their effect on ion transport through nanopores

In this poster, we present some of our work on the latter 3 methods. The fluorescent SBS approach (upper center) underpins the most popular second generation sequencing platform. SERS-SBS (upper right) may offer very high sensitivity. The Nanopore-SBS real-time single molecule approach (lower left) offers the opportunity to achieve very long sequence reads at low cost and high speed.

Fluorescence SBS Using Nucleotide Reversible Terminators (NRTs)

In our SERS-SBS approach, nucleotides were modified with 3'-azidomethyl moiety to:
• temporarily terminate polymerase reaction after incorporation
• serve as the reporter group with distinct fluorescent signature

Each SBS cycle consists of incorporation, detection, and cleavage of the dyes and 3' terminators using a polymerase. This process is monitored using a 2D Raman microscope. The SERS measurements show that the Raman signals of 3'-N3-dNTPs display enhanced Raman signal at the expected Raman shift.

Raman Measurements of NRTs on SERS Substrate

In our SERS-SBS approach, nucleotides were modified with 3'-azidomethyl moiety to:
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• serve as the reporter group with distinct fluorescent signature

We carried out proof of principle studies demonstrating the ability to conduct SBS using a variety of novel tagged nucleotide analogues. Depending on the chemical structure of these tags, the specific nucleotide added to the growing strand can be detected optically (fluorescence based SBS), by Raman spectroscopy (SERS-SBS), or electronically at single molecule level in real time (Nanopore-SBS).

With our multidisciplinary team of organic chemists, molecular biologists, biochemists, chemical engineers and bioinformaticians, we continue to further develop these methods in our laboratory, with the aim of enhancing accuracy, lowering cost, and reducing time to obtain sequences for a wide variety of biomedical and biological applications (e.g., whole genome and whole exome sequencing, disease-specific diagnostic sequencing and SNP genotyping, single cell RNA expression studies, and metagenomic sequencing of the human microbiome). Other lab projects at various stages of development include new technologies for characterizing the epigenetic landscape (e.g., methylated and hydroxymethylated cytosines) and for detecting non-DNA biomarkers.

**Conclusions and Other Projects**

**Instrumentation and SERS Substrate**

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**Ju Laboratory Publications for Material Discussed in This Poster**


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