**Nanopore DNA Sequencing by Synthesis**

Author: Parker Balcom   
Major: Animal Science  
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

**Key Words:**

DNA sequencing, nanopore array, sequencing by synthesis, DNA polymerase ternary complex, oligonucleotide based polymers

**Abstract: DNA sequencing has become more prevalent in the past couple years since it’s discovery in the 1970’s. The potential to use information on a person’s genome to reveal an individual’s tendency for diseases has become significant and can lead to personalized and precise medicine. Therefore, people have been trying to discover new DNA sequencing methods that can be fast, accurate, and cost-effective. Nanopore DNA sequencing by synthesis may be the solution to this problem. Sequencing by synthesis uses polymer tags that are identifiable, attach to nucleotides, and enter a nanopore during enzyme-catalyzed DNA synthesis. Scientists have already proved this method works well, but are trying to further develop this approach by using nucleotides tagged with oligonucleotide based polymers and a DNA polymerase ternary complex attached to a nanopore. This new approach to sequencing by synthesis showed very promising results but still needs to be perfected if it is to one day be used in the biological and medical fields.**

**Introduction**

DNA sequencing is the method of determining the complete order of nucleotides in a DNA molecule. Frederick Sanger is credited with developing the first method of sequencing DNA back in the 1970’s, a process now known as the Sanger method. Since the discovery, scientists have realized the significance of the information on a person’s genome. It can reveal an individual’s tendency for diseases and can lead to personalized and precise medicine. In the more recent years, scientists have been interested in using nanopores to distinguish the bases of DNA to develop a faster and cheaper method of DNA sequencing.

A nanopore is a tunnel-like protein and in the core is a hollow tube that is a few nanometers in diameter. Nanopores can be inserted onto membranes, and once a potential is applied across the membrane, they can be used to identify molecules that enter the nanopores by measuring the distinctive disruptions in current. There are a couple different kinds of nanopore sequencing methods that are being developed.

**Recent Progress**

One type of nanopore sequencing is strand sequencing. Strand sequencing is characterized by feeding an unzipped DNA strand into a nanopore in which the bases of the DNA are identified by disruptions in the current. This method requires decoding numerous bases that pass through the channel and a solution to slow down the DNA entering the nanopore. Another type of nanopore sequencing is called exonuclease-based nanopore sequencing, in which nucleotides are cleaved from a DNA strand by an enzyme and are captured and passed through the nanopore. A drawback of this method is that it requires the nucleotides to be released close to the pore to ensure its capture and the entry has to be slow enough to get a valid current measure. Also, both of these techniques rely on being able to distinguish the disruption currents among the four nucleotide bases. The nanopore sequencing approach that will be discussed in this paper is called nanopore sequencing by synthesis (SBS). SBS uses polymer tags that are identifiable, attach to nucleotides, and enter the nanopore during enzyme-catalyzed DNA synthesis. These synthetic polymer tags offer an advantage because they are designed to produce distinctive and readily available current signatures for sequence determination. Also, attaching a polymerase onto the nanopore guarantees enough time for the polymer tags to be captured and read before the DNA polymerase catalytic cycle is completed.

Scientists have already demonstrated the nanopore SBS method works. They attached one of four different-sized PEG tags onto the terminal phosphate of each nucleotide, and these nucleotides were inserted correctly by DNA polymerase. Each tag was released as polyphosphate byproduct during the phosphoryl transfer step of the DNA polymerase reaction. A polymerase molecule was attached to the nanopore to try and make sure each tag was measured in order and allow fast capture into the nanopore. The different lengths of the PEG tag produced different measured pore current levels, which allowed them to differentiate each tag and enabled them to identify each nucleotide involved.

Other published experiments using streptavidin-bound 3’-biotinylated oligonucleotides proved that it is possible to capture oligonucleotides in nanopores and measure the current levels. So to further develop the nanopore SBS approach, scientists tested the use of nucleotides tagged with oligonucleotide based polymers to perform nanopore SBS. They decided to mimic the streptavidin experiments by using a DNA polymerase ternary complex instead of the streptavidin-tag complex. The DNA polymerase ternary complex would hold on to the nucleotides while the current is being measured. This would allow for the tags to be measured before the phosphoryl transfer step, rather than after, when the tags are released from the nucleotides as a byproduct.

**Discussion**

After synthesizing numerous tags and testing their performance, four tags were chosen that fabricated reproducible and distinct nanopore currents with minimum background. The tags were made of oligodeoxynucleotides, some of which had modified phosphodiester building blocks. To protect the tags from exonuclease activity, they were treated with 3’-phospho-propanol. Next, a polymerase molecule had to be engineered to attach directly to the heptameric alpha-HL nanopore. To form the polymerase ternary complex, a synthetic primer/template was added to the pore-polymerase complex.

For the sequencing experiments, a buffer solution and each of the four tagged nucleotides was added to the polymerase-nanopore chip to start the SBS procedure. The buffer solution contained 150 mM KCl, 3mM SrCl2, and 3 mM MgCl2. The disruption currents were measured by adding 80-120 mV across the membrane. During the first couple of experiments, a tag was sometimes captured numerous times in a row, (which is called “stuttering”), before the sequence of the next tag was detected. This is thought to be caused by the tagged nucleotide being captured and released repeatedly by the polymerase. After tinkering with the experimental conditions, they found a method to reduce stuttering. By applying 100 mV across the membrane, adding 150 mM KCl, using 3 mM MgCl2 on the cis side of the membrane, and using 3 mM SrCl2 on the trans side of the membrane, it resulted in increased speed of nucleotide captures and less stutter.

These experiments show that tagging nucleotides with oligonucleotide based polymers and having them captured and fed through a nanopore by a DNA polymerase ternary complex can have the bases readily distinguishable in a matter of microseconds. Although the complete accuracy of the last experiments were not perfect, SBS has parameters that can be improved further and is a step in the right direction. As a whole, this sequencing method offers advantages over the other nanopore sequencing methods. Enhanced and cost-effective DNA sequencing can develop copious amounts of information about inherited diseases, cancers, and creates huge potential for therapeutics and diagnostics. Once improved upon, DNA SBS could offer the solution to DNA sequencing that the biological and medicine fields are looking for.

**References**

Carl F, et al. “Real-time single-molecule electronic DNA sequencing by

synthesis using polymer-tagged nucleotides on a nanopore

array”. (2016).