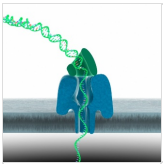

Oct 06, 2020

Introduction to Nanopore

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[1]UCSC

1 Works for me This document is published without a DOI.

UCSC BME 22L

ABSTRACT

We want students to have an excellent foundation and knowledge of nanopore sequencing. This lab will provide students with the understanding of how to sequence DNA and process and then analyze the sequence data. The workflow will be covered in detail and includes: sample collection -> sample prep -> library prep -> sequencing -> data collection/ analysis.

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ABSTRACT

We want students to have an excellent foundation and knowledge of nanopore sequencing. This lab will provide students with the understanding of how to sequence DNA and process and then analyze the sequence data. The workflow will be covered in detail and includes: sample collection -> sample prep -> library prep -> sequencing -> data collection/ analysis.

Introduction to Nanopore Sequencing

Goal of Lab

We want students to have an excellent foundation and knowledge of nanopore sequencing. This lab will provide students with an

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understanding of how to sequence DNA and process and then analyze the sequence data. The workflow will be covered in detail and includes: sample collection -> sample prep -> library prep -> sequencing -> data collection/ analysis.

Learning Objectives

Students will learn:

- About Nanopore Sequencing and the different protocols used for library preparation
- How to load the sample into a flongle
- About different devices and technologies that utilize the nanopore for sequencing
- How to analyze and interpret nanopore sequencing data

Safety

This safety sheet has been designed to provide you with the tips and guidelines to follow in order to maintain the utmost safety when doing this experiment. This lab will not include you using the actual Minion to sequence DNA, so there is not much that will pose a harm to you in this lab. However, it is still necessary to know the necessary guidelines to maintain safety when conducting a nanopore sequencing experiment.

In the library preparation process, numerous reagents are utilized. Because they are being used in such small quantities they can't harm you but one should **always wear PPE**. It is best to treat both the biological sample and the reagents you are working with as potentially hazardous to minimize danger and contamination. Make sure that each reagent is stored at its specified temperature according to the safety data sheet. You can find these sheets at the site, store.nanoporetech.com. The SDS will also indicate how these should be stored and contained.

When the MiniON device is plugged in and being used, it is required that you pay attention to the adjacent symbols to help the person using it to maintain the utmost safety. The hot surface symbol will appear shortly before and after the MinION is in use. Whenever this icon is visible, one should know that they should avoid direct contact with the device as it can be extremely hot and may cause injury. There are also reagents that come with the kit. Some of these may be irritants and have been labeled with a symbol indicating that they are hazardous. Whenever there is a reagent with such a label, make sure to handle it with care and avoid the reagent contacting your skin

Tips and Hazards

- Overloading the flow cell with too much water, buffer or sample can lead to an overflow of the waste compartment of the device. Absorbent material should be used to absorb the sample and buffer which will come out through the waste port. All materials should be disposed of in line with local regulations for biological waste.
- Make sure that when loading the sample or buffer into the flow cell that you aren't introducing any air bubbles into the device as a bubble can damage the pores of the cell.
- **IN CASE OF EMERGENCY, SWITCH OFF THE OXFORD NANOPORE TECHNOLOGIES DEVICE AT THE MAINS POWER SOURCE AND UNPLUG THE POWER CABLE FROM THE DEVICE.**

Background

Next-Generation Sequencing (NGS) has completely revolutionized the way we view the field of genomics and molecular biology. With the recent development of these technologies, we now have the ability to get whole genomes of information in mere hours. There are numerous NGS technologies, such as Illumina, Roche 454, and Ion Torrent Sequencing. However, the NGS tech that you will learn how to operate is nanopore sequencing.

Nanopore sequencing utilizes electrophoresis to pull nucleic acids through a protein pore. As a strand of DNA is pulled through a nanopore by an imposed electrical field, the DNA base sequence generates a sequence-dependent change in ionic current. The magnitude of the ionic current depends on the diameter of the pore being used to sequence, the concentration of electrolytes surrounding the pore, and the DNA or RNA that is occupying the pore. The sample is passed through the pore will cause characteristic changes to this current, which can then be interpreted to give us the genetic sequence of our sample.

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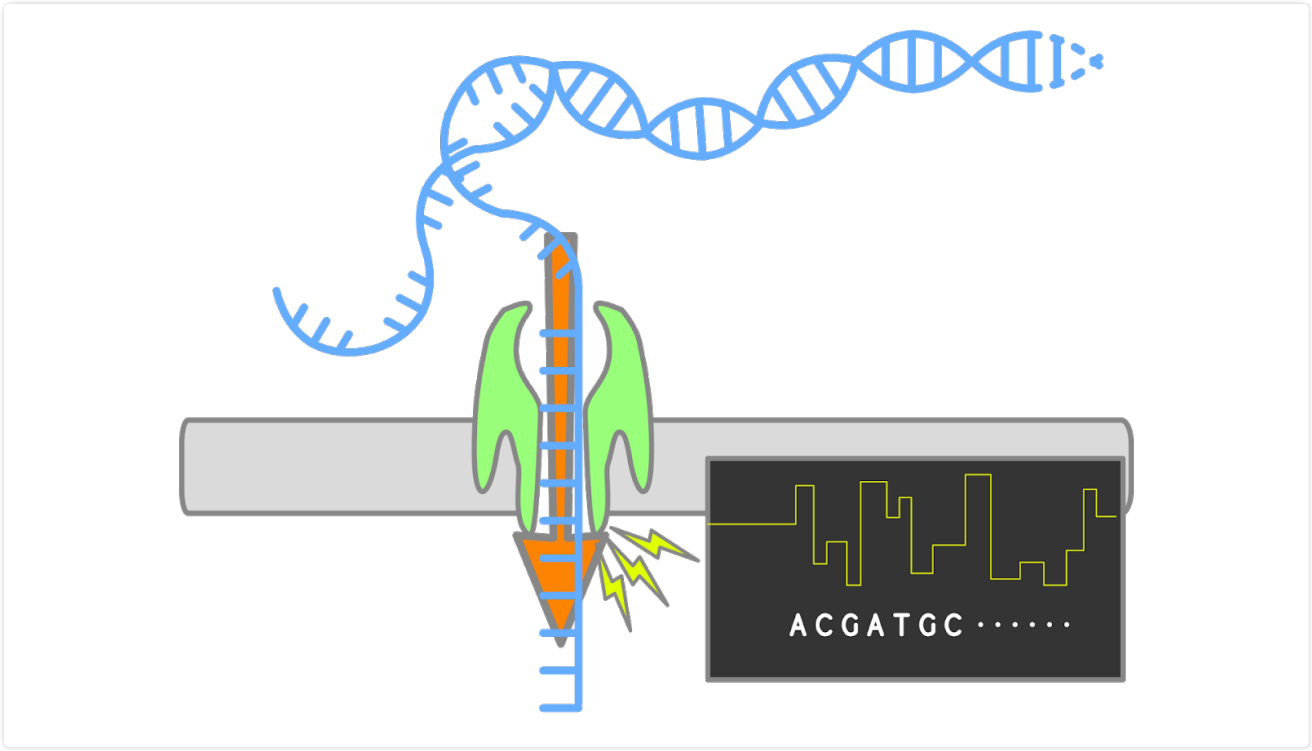

As mentioned earlier, the nanopore utilizes a protein pore in order to pass a strand of DNA through a membrane. There have been different types of pores that scientists have tested for optimal efficiency and accuracy of sequencing. The structure of later generation pores has even been modified by site-directed mutagenesis in an effort to optimize sequencing accuracy. An early protein pore was alpha-hemolysin (aHL).

This transmembrane pore resembles a hollowed-out mushroom and has a "large" vestibule opening at the top and 3 sites that contribute to discrimination between each base sequenced. Mycobacterium smegmatis porin A (MspA) is a second-generation protein pore that's been studied and has been presumed to be a more advantageous pore as compared to aHL because of it's improved sequencing accuracy. The natural version of this protein needed to be altered because it contained a core of 3 negatively charged aspartic acids so they changed these to 3 neutrally charged asparagines. This mutated version of MspA has increased the specificity of identifying bases tenfold.

There are also pores that don't rely on a protein's properties but that of a metal or metal alloy substrate to generate a solid-state nanopore (substrate with a nanometer-sized hole) for DNA or RNA translocation and base detection. These Solid State nanopores sense base sequences using different methods. One approach for base detection involves tunneling current which is the phenomenon where electrons are forced past a barrier they typically wouldn't be able to pass through. As bases pass through the pore, the tunneling current detector (in the form of a scanning probe microscope) is used to sense individual bases in a nucleic acid strand and provide sequence information.

Did you know?


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The Concept for nanopore sequencing was first conceptualized right here at UCSC! In the early 1990s, UCSC professor David Deamer, Harvard professor Daniel Branton, and Mark Akeson pioneered the early concepts of nanopore sequencing, using an aHL (alpha-hemolysin) pore. Currently, the nanopore group at UCSC, led by Professor Mark Akeson, in collaboration with the company Oxford Nanopore Technologies, and the National Institutes of Health to expand the capabilities of nanopore sequencing.

Key Things to know

- **MinION:** The MinION is a small, relatively cheap($1,000) sequencer developed by the company Oxford Nanopore Technologies. This device is capable of taking up to 30 GB worth of data and has an unlimited read length making it an ideal mode of sequencing for someone targeting long reads. It also has the ability to allow the user to see their reads get sequenced in real-time.
- **Flongle:** An adapter compatible with the MinION which enables direct sequencing of smaller samples. They are $90 per flow cell. Can hold up to 2GB of data.
- **MinKNOW:** Software designed to help process information from the nanopore. Can show base calling in real-time and can produce fasta files of the sequence.
- **Sequence Adapter:** Adapter sequences ligated to the ends of the DNA strands. These adapters are essential in aiding the loading of the strand to the motor protein as well as facilitating strand capture.
- **Enzyme Motor Protein:** An enzyme motor that controls the translocation speed of nucleotides through the nanopore. Once a strand is passed through, the motor protein detaches and then searches for the next strand to bring through the pore.
- **Nanopore Reader:** The nanoscale hole that nucleotides get passed through. It is through this hole that the passage of different nucleotides disrupts the ionic current in different ways. These fluctuations in the current are then interpreted by an algorithm that will tell us the sequence.

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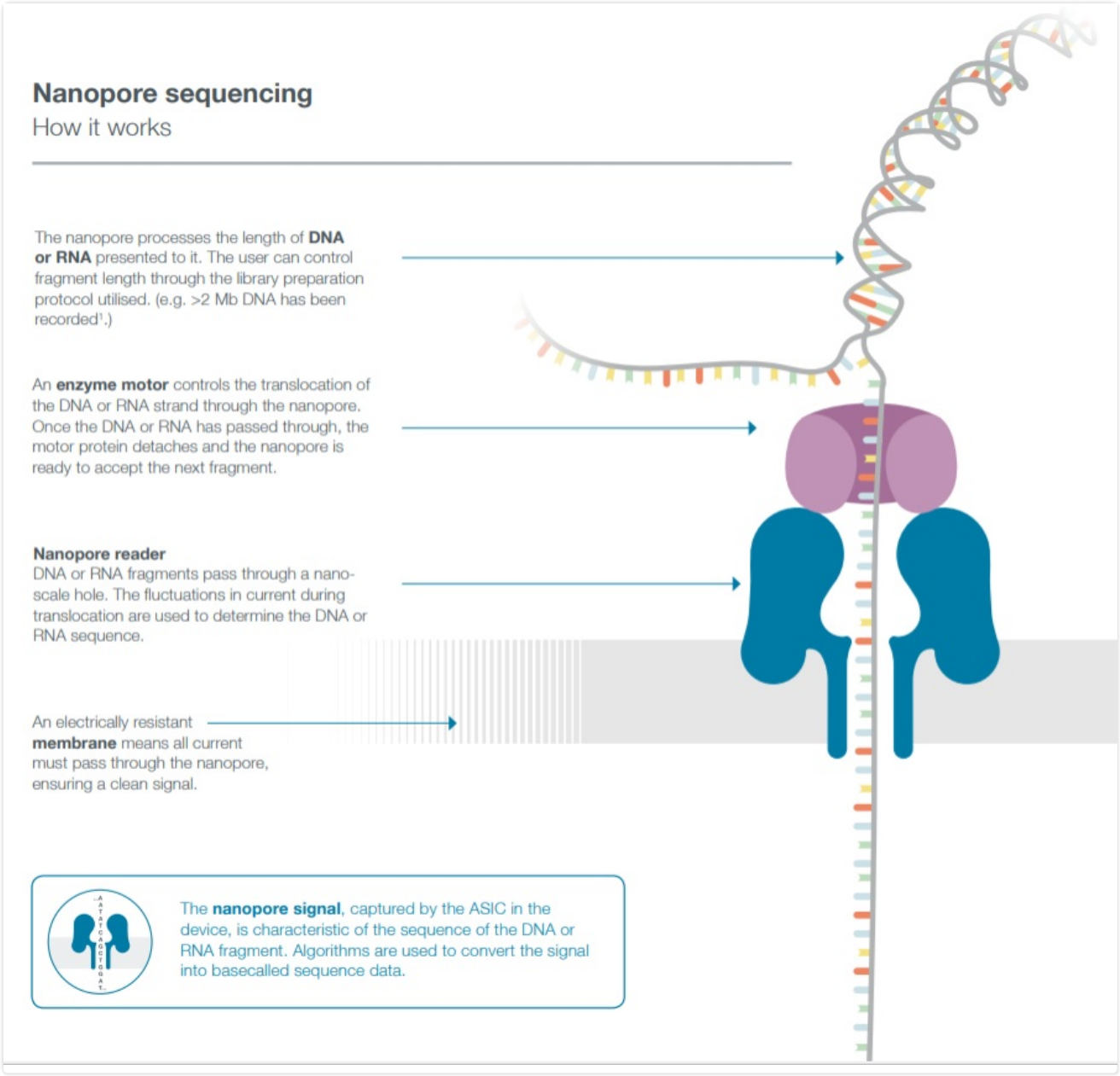

Resources

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