NOTE: Due to the location of the position, the applicate must either be a US Citizen, US Greencard holder, or have legally resided in the US for 3 of the last 5 years.

Project: Evaluation of MinION and GRidION for bacterial genome closing using long reads sequencing

Research Mentor: Narjol Gonzalez-EScalona, Ph.D.

Location: FDA/CFSAN/ORS/DMS, 5001 Campus Drive, College Park MD 20740

Whole genome sequencing is an essential tool for tracing which bacteria have contaminated foods and identifying whether these bacteria pose serious public health threats because they carry virulence factors. Closing bacterial genomes provide a comprehensive view of their genetic composition that allows the generation of: 1) high quality reference genomes for source tracking during a foodborne outbreak investigation, 2) understanding long-term evolution of foodborne pathogens, 3) new insights in drug resistance and transmission of mobile elements carrying antimicrobial resistance markers, and 4) information about the contribution of DNA modification on pathogenesis. Currently, the only instrument that can reliably complete that task is the Pacific Bioscience (PacBio) Sequencer. Unfortunately, this equipment is expensive, requires specialized laboratories, needs significant quantities of DNA, and produce reads of around 30 kilo base(kb) in size at a time, making it challenging to correctly assemble the genomes of bacteria and the plasmids they may carry if they have high repetitive regions (e.g. *E. coli*). We need accessible technology that can produce longer reads so we can quickly help solve these problems, we propose to evaluate a relatively new alternative to SMRT sequencing, the MinION (Oxford Nanopore), which needs much less DNA to run, and can produce much longer reads. The MinION has been in testing for several years, is much less expensive, can be carried in one hand, and can be operated by regular lab staff. We will test the efficiency and accuracy of the MinION on strains of *Escherichia coli* and *Clostridium botulinum*.

We would like to sequence 200 selected strains (100 STECs *E. coli* from diverse serotypes and 100 *C. botulinum* representing groups I (proteolytic serotypes A-G) and II (non-proteolytic), isolated from both clinical and food samples) using an alternative platform: the MinION and GridION nanopore sequencer (Oxford Nanopore, Oxford, UK). Our goal is to develop a pipeline that could be used by other FDA researchers to perform real-time calling for sequences of interest, suitable for metagenomics, studies of antimicrobial resistance and virulence, and the detection/identification of BoTN toxins. We believe that such a MinION-based pipeline can help reduce the cost of closing genomes and simultaneously enable more laboratories to perform such analyses, since it would eliminate barriers posed by cumbersome technologies that require specialized bioinformatics.

Scope of Work:

The ORISE participant shall perform the following tasks during the above-specified period including:

1. Test and evaluation of MinION and GridION using system for sequencing complete bacterial genomes and to develop a pipeline to analyze the output data as an alternative to the PacBio for rapid detection of virulence markers (Ecoli), antimicrobial resistance features (Ecoli), and toxin genes (Cbot) among food isolates and shotgun metagenomics samples.
2. Test the effectiveness of new MinION sequencing kits and flow cells using 8 genomes already been sequenced by PacBio or Sanger methods.
3. Data captured in reports and presentation in meetings.
4. Perform experiments as designed by PI
5. Maintain a detailed report and present to PI
6. Maintain the MiSeq and MinION laboratory, inventories of chemicals and reagents.
7. Assist PI in growing cultures, inoculating food products with bacterial cultures, and extracting DNA using various protocols.
8. Participate in writing of publications.
9. We will evaluate the MinION nanopore system for sequencing complete bacterial genomes and to develop a pipeline to analyze the output data as an alternative to the PacBio for rapid detection of virulence markers

(Ecoli), antimicrobial resistance features (Ecoli), and toxin genes (Cbot) among food isolates and shotgun metagenomics samples.

10. Evaluate the utility of MinION nanopore sequencing for analysis of metagenomic samples and determine its detection limit.

Requirements:

1. ***MUST BE A*** U.S. Citizen, U.S. Greencard holder or legal resident of the U.S. for 3 of the last 5 years
2. **MUST be able to** successful complete a background security investigation and a security risk assessment will be required for all appointees. Appointment to the position is subject to favorable background adjudication. In addition, the selected applicant must maintain required suitability assessments and favorable background investigations as a condition of continued employment. Applicants are also advised that all information concerning qualifications is subject to investigation. Applicants will be issued an Official job offer letter after a favorable background security investigation and security risk assessment has been completed.
3. **MUST** have at least a Bachelor's degree with a concentration in an applicable field of study
4. At least (3) months of sequencing experience
5. At least (2) years of microbiology or molecular biology active lab experience

Duration: 12 months, extendable for an additional year depending of performance.

Period of Performance: June 30th, 2019 – June 29, 2020.

If interested in applying, please send a copy of your CV or resume to Narjol.Gonzalez-Escalona@fda.hhs.gov . Please also include: (3) professional references with contact information and a letter of interest.