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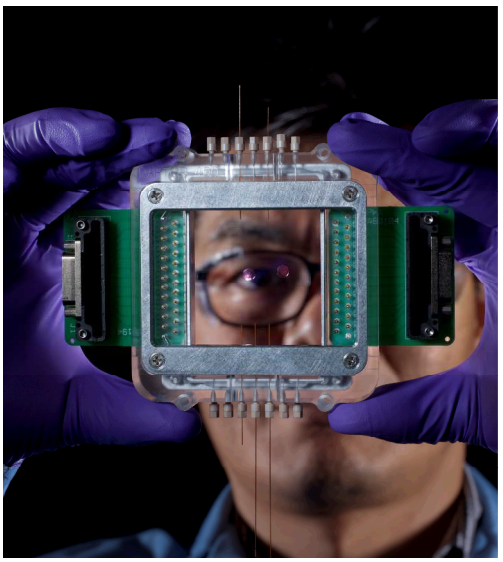
Exceptional service in the national interest

Sandia National Laboratories

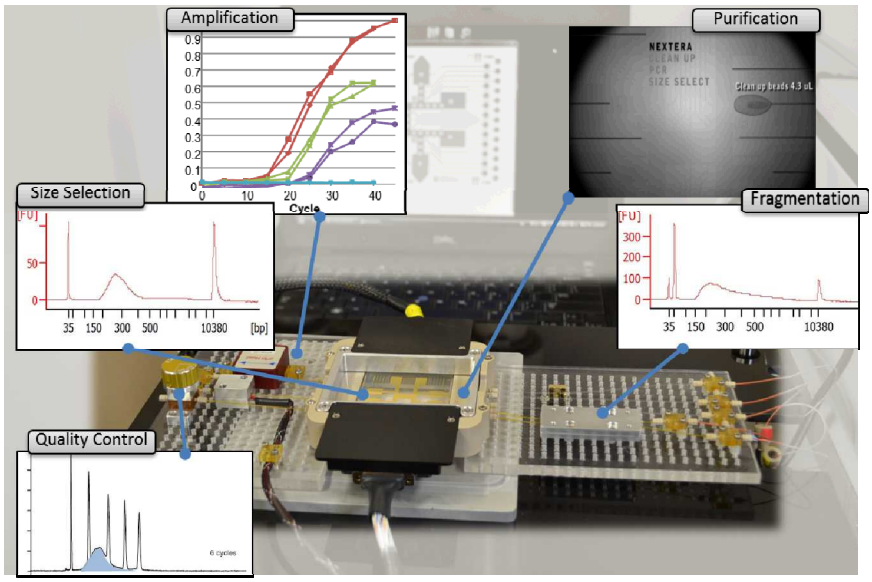
The Rapid Threat Organism Recognition (RapTOR) System

Rapid advances in biotechnology and the increasing efficiency of global transportation networks virtually guarantee that the United States will face devastating disease outbreaks caused by pathogens intentionally or accidentally introduced into the population. There is a clear need in public health for tools to identify and characterize emerging or novel engineered pathogens to guide rapid response and to develop diagnostic tests, treatments, and vaccines. Recent revolutionary breakthroughs in second-generation sequencing (SGS) have enabled an approach to generate the type of information needed to identify and characterize novel emerging, or even man-made pathogens. With SGS technology, it is now possible to rapidly sequence entire genomes and characterize gene expression of organisms within days at an affordable cost.



The RapTOR system rapidly and automatically applies suppressive molecular biology methods, next generation sequencing and bioinformatics to identify and characterize unknown pathogens in clinical samples, thereby enabling a faster and more effective public and military health response ***The system is designed to provide sample-to-answer results within 24 hours by increasing the sequencing hits on an unknown pathogen by 100-fold.***

Sandia has developed a nucleic acid sample preparation platform to interface directly with SGS to detect unknown pathogens by enriching pathogen nucleic acids sequences and suppressing host background to maximize the sensitivity of state-of-the-art SGS. This Automated Molecular Biology (AMB) platform has a unique design in that it implements digital microfluidics to function as a central hub for interfacing multiple lab-on-a-chip sample processing modules for consistent and contamination-free preparation of clinically-derived DNA samples. The AMB platform is able to carry out a diverse series of benchtop-like steps at a scale adapted to handling small, but precious, samples for DNA manipulations, but with far greater speed and efficiency than at the benchtop.



The most time-consuming and error prone steps associated with Illumina SGS is library preparation. The standard 6-hour benchtop protocols require microgram quantities of DNA, multiple reactions, numerous between-step washings, and bias inducing thermal amplification. We are integrating a library prep method that offers significant advantages over traditional approaches by eliminating inefficient nebulization, ligation, and clean-up steps. Transposition simultaneously fragments the DNA and adds the appropriate Illumina priming adapters in a single reaction step—reducing the preparation time to just minutes. Additionally, up to 12 different barcodes can be incorporated into the reaction to increase throughput and multiplexing. We have demonstrated transposase mediated fragmentation adapted to microfluidic scale. We have also demonstrated that we can analyze the fragmentation products without PCR amplification by concentrating reaction products at the interface of a photopolymerized membrane embedded in a microchannel followed by an on-chip gel electrophoretic separation.

We have developed an in-house automated bioinformatic system for analyzing SGS sequence data from samples processed by AMB and knowledge extraction tools for data interpretation and improving experimental design. Our system includes a sample tracking feature which integrates specimen, sample processing, and sequencing metadata, an automated OmniSeq sequence analysis pipeline which identifies and bins host sequences and performs phylogenetic analyses of background microbiome and pathogen sequences, and software tools to characterize the effectiveness of suppression including artifactual analysis, hit counting, and quantitative comparison and visualization.

RapTOR

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