The advent of next generation DNA sequencing (NGS) technology represents a quantum leap in the field of genetic analysis: what once required a decade-long, multibillion dollar Human Genome Project can now be reproduced in 1-2 weeks time for less than $5,000. Despite advances in sequencing technology, upstream library (sample) preparation protocols, which require numerous sample processing steps and hours of hands-on laboratory time, have not benefited from comparable increases in speed or efficiency. While automation of the library preparation process can help overcome this widely recognized bottleneck, current approaches rely on large and expensive pipetting robots designed for use in dedicated high-throughput sequencing facilities. To fully realize the promise of next generation sequencing for more ubiquitous, individualized, decentralized applications such as personalized genomic medicine, point-of-care diagnostics, public health screening, and DNA forensics, technologies automating NGS sample preparation must also become more affordable and accessible. To address this need, our Sandia team has developed an Automated Molecular Biology (AMB) system enabling the cost-effective automation of complex protocols like NGS library preparation and other labor-intensive bioanalytical procedures and processes.

The heart of the AMB system is a unique droplet-based digital microfluidic (DMF) platform which functions as a central hub for the distribution and routing of samples and reagents. Digital microfluidic technology uses electrostatic and electrowetting forces to manipulate microliter-scale droplets sandwiched between closely spaced, hydrophobically coated substrates patterned with individually addressable electrodes. These devices enable discrete droplet movement and droplet operations such as merging, splitting, mixing, and aliquotting to be performed at scales much smaller than what can be conventionally achieved. Accordingly, our DMF platform functions instead as a sample distribution and reagent interface hub and fills a role equivalent to that of a pipetting robot in a high-throughput laboratory automation workflow, but at a fraction of the size, cost, and complexity.

The central innovation of our digital microfluidic platform was the development of a custom manifold frame providing self-aligning registration of top and bottom DMF substrates to achieve the precise tolerances required for reliable DMF operation. The open architecture of the frame enables access to the interior of the DMF device by in-plane transfer capillaries. As shown in the figure, this capillary interface allows liquid to be transferred to and from the DMF device with nanoliter precision using external syringe pumps, providing not only a method for coupling the DMF platform to external modules, but also the means to execute a variety of advanced on-platform operations including serial dilution, droplet subsampling, chaotic mixing, fraction collection and sorting, magnetic bead manipulations, and sample archiving.
The Automated Molecular Biology system is completed by integrating this adaptable digital microfluidic platform, its high-voltage control electronics, supporting syringe pumps, and functional submodules with a PC-based user interface enabling coordinated control and script-based automation of all sample preparation operations. We estimate that the full AMB platform including all supporting hardware and software will cost less than $3,000 per unit.

APPLICATIONS ENABLED BY THE AMB PLATFORM

PUBLIC HEALTH—RAPID THREAT RECOGNITION SYSTEM

Targeting NGS sample preparation for public health applications as part of the RapTOR System, our Sandia team set out to harness the inherent flexibility and scalability of digital microfluidics central hub to format nucleic acids for sequencing unknown pathogens by enriching informative nucleic acid sequences (those derived from the pathogen) and suppressing background DNA (those from the host) to maximize the sensitivity-of-the-art NGS. While the AMB system has already attracted significant interest from industry leaders, our team’s greatest success to date has been the end-to-end execution of the full NGS library sample preparation protocol using the AMB platform and the subsequent sequencing of the resulting library on an Illumina MiSeq Personal Sequencing System. This milestone represents the first example of a non-robotic, microfluidic system providing a viable path toward the full automation of NGS sample preparation. Coupled directly to a MiSEQ sequencer, fully automated, and including in situ library validation, the AMB system is expected to execute the process in less than half the time required for the bench-top protocol (2+ hours) and with less than 1/10th the total DNA (<5 ng). Owing to the unique benefits of our digital microfluidic hub platform, the Sandia Automated Molecular Biology system offers an unparalleled combination of operational flexibility, functional capability, efficiency, cost-effectiveness, and sample-to-answer simplicity, paving the way for the new era of personalized genomics.

FORENSICS—BATTLEFIELD AUTOMATED DNA ANALYSIS AND SAMPLING SYSTEM

Customizing the AMB platform into a fieldable system will enable warfighters with minimal forensic training to effectively collect and rapidly process DNA samples in the field, minimizing opportunities for samples to be contaminated or misdirected while maximizing the timeliness and utility of the analytical result for tactical decision-making and intelligence gathering.

GLOBAL BIOSURVEILLANCE—ADVANCE DIAGNOSTIC AND SAMPLING PLATFORM

The key to preventing an outbreak before it goes global is to establish a biosurveillance network that effectively reaches even the most remote regions and provides a network-integrated, location-appropriate diagnostic capability. Leveraging the AMB platform into the field can extend biosurveillance activities beyond centralized laboratory facilities for a deployable rapid-response diagnostic platform and provide a safer method to process infected samples in the field for analysis.