Technology could upend DNA sequencing for diagnosing certain DNA mutations

January 27 2021

From left, postdoctoral scholar Andrey Mikheykin, Ph.D., Jason Reed, Ph.D., and postdoctoral fellow Sean Koebley, Ph.D., worked together on the study. Credit: John Wallace, VCU Massey Cancer Center

Doctors are increasingly using genetic signatures to diagnose diseases

and determine the best course of care, but using DNA sequencing and other techniques to detect genomic rearrangements remains costly or limited in capabilities. However, an innovative breakthrough developed by researchers at Virginia Commonwealth University Massey Cancer Center and the VCU Department of Physics promises to diagnose DNA rearrangement mutations at a fraction of the cost with improved accuracy.

Led by VCU physicist Jason Reed, Ph.D., the team developed a technique that combines a process called digital polymerase chain reaction (dPCR) with high-speed atomic force microscopy (HSAFM) to create an image with such nanoscale resolution that users can measure differences in the lengths of genes in a DNA sequence. These variations in gene length, known as polymorphisms, can be key to accurately diagnosing many forms of cancer and neurological diseases.

A study detailing the method was recently published in the journal *ACS Nano*, and the research team reported their results at the annual meetings for the Association of Molecular Pathology and the American Society of Hematology. Previous research detailing the HSAFM technology was described by VCU Massey Cancer Center in 2017.

"The technology needed to detect DNA sequence rearrangements is expensive and limited in availability, yet medicine increasingly relies on the information it provides to accurately diagnose and treat cancers and many other diseases," says Jason Reed, Ph.D., member of the Cancer Biology research program at VCU Massey Cancer Center and associate professor in the Department of Physics at the VCU College of Humanities and Sciences. "We've developed a system that combines a routine laboratory process with an inexpensive yet powerful atomic microscope that provides many benefits over standard DNA sequencing for this application, at a fraction of the cost."

dPCR uses the DNA polymerase enzyme to exponentially clone samples of DNA or RNA for further experimentation or analysis. The sample is then placed on an atomically flat plate for inspection using HSAFM, which drags an extremely sharp microscopic stylus similar to the needle on a record player across the sample to create precise measurements at a molecular level. The technique was adapted by Reed's team to use optical lasers, like those in a DVD player, to process samples at a rate thousands of times faster than typical atomic force microscopy. The researchers then developed computer code to trace the length of each DNA molecule.

The team claims that each dPCR reaction costs less than $1 to scan using their technique.

To demonstrate the clinical utility of the process, Reed partnered with Amir Toor, M.D., hematologist-oncologist and member of the Developmental Therapeutics research program at Massey, and Alden Chesney, M.D., associate professor of pathology in the Department of Pathology at the VCU School of Medicine. Together, they compared Reed's technique to the current standard test to diagnose DNA length polymorphisms in the FLT3 gene in patients with acute myeloid leukemia. Patients with these mutations typically have a more aggressive disease and poor prognosis when compared to patients without the mutation.

Reed's technique accurately identified FLT3 gene mutations in all samples and matched the results of the current gold standard test (LeukoStrat CDx FLT3 Mutation Assay) in measuring the lengths of the gene segments. However, unlike the current test, Reed's analysis also reports the variant allele fraction (VAF). The VAF can show whether the mutation is inherited and allows the detection of mutations that could potentially be missed by the current test.

"We chose to focus on FLT3 mutations because they are difficult to diagnosis, and the standard assay is limited in capability," says Reed. "We plan to continue developing and testing this technology in other diseases involving DNA structural mutations. We hope it can be a powerful and cost-effective tool for doctors around the world treating cancer and other devastating diseases driven by DNA mutations."

More information: Sean R. Koebley et al, Digital Polymerase Chain Reaction Paired with High-Speed Atomic Force Microscopy for Quantitation and Length Analysis of DNA Length Polymorphisms, *ACS Nano* (2020). DOI: 10.1021/acsnano.0c05897

Provided by Virginia Commonwealth University