Better, Faster, Cheaper

Columbia University and the New York Genome Center Scientists Create Software that Improves Accuracy of Human DNA Matching

By Camille Mojica Rey, Ph.D.

Few gadgets in modern science are more intriguing than a hand-held, USB compatible, real-time DNA sequencer. The possibility of sequencing anyone, anywhere, and anytime for just about any reason is tantalizing. Making that a reality, however, is difficult when the current portable technology has an inaccuracy rate that is an order of magnitude higher than the genetic differences between human beings. That has made the holy grail of on-the-spot DNA fingerprinting impossible.

Until Now

In November, a team of scientists from Columbia University and the New York Genome Center (NYGC) reported in the journal *eLife* that they were able to verify the identity of a human DNA sample within three minutes of sequencing, with 99.9% confidence, using software they created for use with the MinION portable DNA sequencer.

"We thought: 'If we can make this work, it would democratize DNA fingerprinting," said Sophie Zaaijer, Ph.D., lead author of the study. Zaaijer is a former member of the NYGC and is now a member of the Runway Startup Postdoc Program at the Jacobs Technion-Cornell Institute.

The technique, called MinION Sketching, can quickly and accurately identify people and cell lines using DNA. The technology puts field forensics by non-scientists, such as law enforcement agents, within reach. But, the most immediate application for its use, the study's authors say, is cell-line authentication, which could potentially save billions in preclinical research dollars.

"MinION Sketching is possible and immediately applicable for cell-line authentication in labs," Zaaijer said. And it is greatly needed. A 2015 analysis published in the online journal *PLoS Biology* found that the prevalence of irreproducible preclinical research exceeds 50% in the United States

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alone, costing approximately $28 billion in waste. Preclinical research can take years, during which times labeling errors and accidental contamination are real possibilities. Without thorough authentication, the wrong cell lines may be listed in scientific publication, spreading incorrect information through the scientific community, Zaaijer said. "This eventually results in preclinical labs not being able to translate their findings to the treatment of patients."

Cell-Line Authentication

Currently, few scientists spend the time and money required to send samples to a lab equipped to validate their cell lines, said Yaniv Erlich, Ph.D., senior author of the study. Erlich is a computer science professor at Columbia University and an adjunct core member at NYGC.

"In this era of instant video streaming, online conversations with people on the other side of the globe, and searching Google records in a fraction of second, it is frustrating to stall a competitive research program for two to three weeks just to verify a cell line," he said.

MinION Sketching, on the other hand, allows researchers to conduct cell line authentication in one afternoon. "We hope that the short time and DIY approach will reduce the burden and facilitate authentication," Erlich said.

Neville Sanjana, a CRISPR biologist, is excited to make MinION Sketching a regular part of the work done in his lab. "There are very few techniques right now for cell-line authentication," said Sanjana, a member at NYGC and assistant professor at NYU's department of biology who was not involved in the research.

Sanjana said MinION Sketching is a step in the right direction for the field of clinical research. "This technique is one that is fast and more accurate than anything we have now. Cell authentication is really something that everyone should be doing."

For the first time, the affordability of the MinION makes eventual widespread adoption of cell line authentication possible. The MinION is priced at $1,000, with little additional costs to a laboratory already equipped to work with cancer cell lines. The Sketching code is available for free at github.com, and a link can be found in the online paper.

Creating a DNA Sketch

But, just how does MinION Sketching work? The entire workflow involved in reidentification of human samples involves collecting the sample, extracting the DNA, creating a library of strands to be sequenced, and sequencing using the MinION, which was created by Oxford Nanopore. The MinION is a nanopore sequencer containing a membrane embedded with nanopore proteins that are just over a billionth of a millimeter wide. A steady ion current runs through the membrane. As single strands of DNA pass through the pores, each nucleotide (A, G, T, and C) is identified by the way its unique shape interrupts the ion flow.

The MinION generates readouts in real time of random pieces of DNA, while the Sketching software analyzes a selected number of single nucleotide polymorphisms, or SNPs, using a Bayesian algorithm. These variants make each person unique. "Every time a DNA read comes out, we are screening in semi–real time for variants that are informative and update the posterior probability. That gives us more strokes of the sketch, and we get better resolution of the match," Zaaijer said. Matches are made by comparisons to a database of known sequences. Researchers looking to use this method can download a SNP database, or, ideally, create their own.

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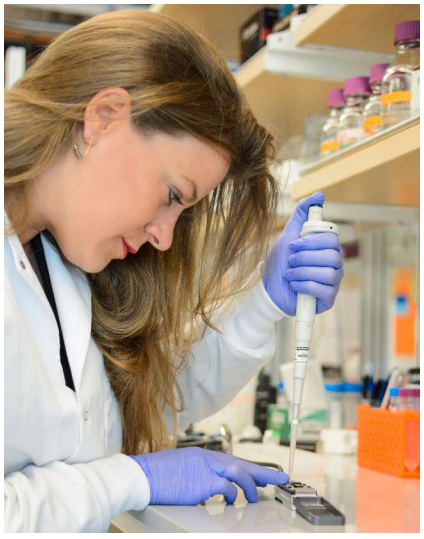
> **— Neville Sanjana,** New York Genome Center and NYU Department of Biology

In the paper, the researchers describe verifying their method by matching a strain of leukemic cells after three minutes of sequencing by the MinION and comparing it against a reference file in the Cancer Cell Line Encyclopedia database. The sketch required 91 SNPs to reach 99.9% accuracy. The researchers then contaminated that line with another and ran the test, again. The method correctly rejected a match if the contamination level climbed above 25%.

This rapid procedure, along with the affordability of the MinION sequencer, promises to make cell-line authentication what it should be: standard procedure in every research lab. "No one wants to waste time and reagents working on the wrong cells," Sanjana said. "At the right price, every lab will adopt this." Zaaijer added it also has the potential for a long list of clinical applications. For example, it would take less than an hour before a surgery to verify the correct organ is being transplanted to a patient. Currently, this verification takes a minimum of 24 hours. Likewise, it could be used in diagnostics in which patient samples are tested at various time points, making them more prone to mix-ups. "The speed of the MinION Sketching makes clinical sample authentication a game changer."



Sophie Zaaijer, Ph.D., of the Runway Startup Postdoc Program at the Jacobs Technion-Cornell Institute was the lead author of a study that could "democratize DNA fingerprinting."