Corrected 19 February 2016; see full text.

cause they had to mash up the brain tissue to extract the barcodes, Zador says. "It was a brain-in-a-blender approach," R. Jacob Vogelstein, the IARPA program manager who runs the project in McLean, Virginia, says.

The companion technique, developed by Church, solves that issue by allowing Zador to read the barcodes without the need to extract them from the brain. The technique, called FISSEQ, applies washes of different fluorescent molecules to the tissue. Each molecule links to specific nucleotides, so successive washes cause each "letter" in a barcode to light up one at a time, like a series of brightly colored Christmas lights. An optical microscope captures the sequence: If a barcode in a given cell is ATGGCG, for example, the sequence of colors might be red-yellow-green-green-cyan-green, Zador says. When barcodes from two different cells show up within a synapse's distance of each other, a computer program infers that those two neurons are connected, Zador says.

Zador and Church predict their joint technique can extract the connectivity of a cubic millimeter of mouse brain tissue in days or weeks—not the months or years typical of electron microscopy. But hurdles remain, Zador says. Injecting the viruses does not guarantee that 100% of neurons receive only one unique barcode apiece. To address that problem, Church is now working on a way to genetically engineer mice to express random RNA sequences, barcodes, in their neurons during development.

Though he supports the general direction of the project, Karel Svoboda, a neuroscientist at Janelia Research Campus in Ashburn, Virginia, is skeptical that the barcoding strategy or its main competitor, improved electron microscopy, can fulfill IARPA's lofty ambitions. He suspects the mapping will contain too many errors. And Svoboda questions the premise of MICrONS. Given that most important brain functions are distributed across several brain regions, devising useful or fundamental neural computations based on the connection maps of a cubic millimeter of cortex is "a pipe dream, in my opinion," he says.

Vogelstein is more optimistic. If the brain has stereotypical rules that govern cortical circuitry, a cubic millimeter is "a sufficiently large chunk" of brain tissue that it should give a good picture of how neurons are wired to perform complex computations. "We believe and hope that there is this modularity," he says—indeed, "all of neuroscience is banking on it." If not? MICrONS may be the first to produce "solid evidence" of that disappointing fact, Vogelstein says. ■

GENOMICS

Pocket DNA sequencers make real-time diagnostics a reality

Advances in accuracy of nanopore sequencing help pave the way for on-the-spot DNA tests

By **Elizabeth Pennisi**

Not so long ago, sophisticated DNA sequencing required massive equipment and lots of time and money. Now, relatively cheap, pocket-sized devices are on the verge of giving real-time sequencing abilities to the masses. These so-called nanopore sequencers, produced so far by a single company, have suffered from poor accuracy. But this month, researchers reported that the instruments passed an important field test, conducting on-the-spot sequencing of viruses isolated from patients during last year's Ebola epidemic in West Africa. In the lab, meanwhile, other researchers are tweaking sample preparation and data analysis to boost the devices' accuracy and speed. Real-time analyses of pathogens and the rest of life are within reach, they say.

Ecologists, public health officials, epidemiologists, food safety officials, and many others may reap the benefits. Nanopore sequencing "is a point of departure in the way DNA is sequenced on this planet," says Mark Akeson, a molecular biologist at the University of California, Santa Cruz, who developed some of the technology that makes this approach possible and who consults with and holds stock in Oxford Nanopore Technologies, the U.K. company that is commercializing the technology. "It's democratizing sequencing."

> *"There's going to be one of these things in everyone's lab."*
>
> **Justin O'Grady**, University of East Anglia

To date, most sequencing works by building a DNA strand complementary to the one being sequenced. The building blocks, or bases, must be chemically tagged so that they can be identified as they are added one-by-one to the new strand, and the technique yields many short stretches of sequence that have to be pieced together. The nanopore approach instead reads the bases more directly, as a single strand of DNA is pulled through a microscopic pore. Each base interrupts an ionic current in the pore in a distinctive way that reveals its identity. The technique allows DNA strands thousands of bases long to be decoded in a single pass, without the delay and effort needed to piece together many short reads.

The idea is more than 25 years old, and it has been 4 years since Oxford Nanopore announced that it had used its prototype nanopore sequencer, called MinION, to decipher the DNA of a virus (*Science*, 4 May 2012, p. 534). Yet academic researchers have complained that the sequencer did a poor job of naming the bases. "The first thing everyone knows about nanopore [sequencing] is that it's not very accurate at the per-read level," says Rory Bowden, a genomicist at the Oxford Genomics Centre in the United Kingdom. To make the correct "call" for each base, the nanopore data had to be combined with conventional sequencing data pulled from databases (*Science*, 21 February 2014, p. 829).

Over about the past 2 years, hundreds of labs have been trying out the MinION, some through an early access program run by the company, and have made steady improvements. Last month, for example, a team led by Niranjan Nagarajan, a computational biologist at the Genome Institute of Singapore, reported a way to improve accuracy without modifying the sequencing process. His group uses the MinION to identify bacteria in a sample of, say, skin or stool. To distinguish bacterial species, researchers sequence all copies of a ribosomal gene called *16S* in each sample, as each species has a unique version. But conventional sequencing methods yield just parts of the gene, sometimes not enough for a positive identification. The MinION can capture more, or all, of the gene, which should make species identification more precise—if the sequences are accurate enough.

To increase accuracy, Nagarajan's strategy is to treat the DNA with a chemical that makes each *16S* gene form a circle. Then he adds a special DNA-replicating enzyme that copies the circular DNA, creating strings of the same DNA repeated over and over. When

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This pocket sequencer, used here during the Ebola outbreak in West Africa, read viral genomes from human samples.

PHOTO: EUROPEAN MOBILE LAB/TOMMY TRENCHARD

each string goes through the MinION's pore, that *16S* gene is sequenced multiple times. About six repetitions are enough to guarantee each base is accurately identified, Nagarajan and his colleagues reported on 27 January in a paper posted on bioRxiv, a preprint repository.

The recent Ebola fieldwork depended on a different strategy for improving the MinION's accuracy. Nicholas Loman, a microbial genomicist at the University of Birmingham in the United Kingdom, and his colleagues realized that they could extract further clues to a base's identity from the change in ionic current as the base moves through the pore. "There is yet more data encoded in the electrical signature," notes Winston Timp, a biomedical engineer at Johns Hopkins University in Baltimore, Maryland; for example, each base's signature is influenced by the bases on either side of it. With a new computer program for analyzing these "squiggles," developed by Jared Simpson from the Ontario Institute for Cancer Research in Toronto, Canada, and Loman's graduate student Joshua Quick, the team managed to sequence a bacterium in the lab with nanopore data alone.

They then took their sequencer to West Africa, where they successfully sequenced 148 Ebola virus genomes from patients, the group reported in the 3 February issue of *Nature*. Even under field conditions, they could complete a genome in 24 hours. "It means we can start taking public health measures based on genomic data," says microbiologist Andy Kilianski, a National Research Council fellow at the U.S. Army Edgewood Chemical Biological Center in Gunpowder, Maryland.

Matthew Loose, a developmental and computational biologist at the University of Nottingham in the United Kingdom, is tweaking the data analysis as well, but his goal is to reduce the time wasted sequencing unnecessary DNA. In 2014, Oxford Nanopore suggested that the MinION could kick a piece of DNA out of a pore before it was fully sequenced by reversing the pore current. Loose and his colleagues have now worked out a way to predict from the first 250 bases moving through a pore whether that piece likely has already been sequenced, as they reported 3 February in a paper posted on bioRxiv. Sequencing "will be faster in the end because every single read that you get, you want," says Justin O'Grady, a microbiologist at the University of East Anglia in Norwich, U.K. Loose, who described his group's latest work last week at the annual Advances in Genome Biology and Technology meeting in Orlando, Florida, hopes to get the number of bases needed to reject a strand down to 64 or even 32.

Several groups are working on other ways to increase efficiency of nanopore sequencing for diagnostics by speeding up DNA sample preparation and sequence analysis. That's important because "with Ebola, we knew what we were looking for," says Oxford Nanopore molecular biologist Daniel Turner. "With [a typical] infection, you have no idea." Kilianski's team has in the works a MinION-based test that could go into the field and diagnose in a matter of hours RNA virus infections, including corona, dengue, Ebola, chikungunya, and Zika.

And O'Grady and his team can now pinpoint the cause of urinary tract infections in 4 hours, which will enable physicians to prescribe pathogen-specific antibiotics instead of broad-spectrum ones. "For me, nanopore is the only sequencing technology that gets us into the timeframe for actionable clinical diagnostics," he says.

Ideally, such tests should take less than an hour, O'Grady says. The latest MinION in the works could help; it passes 350 bases through the pore a second, up from 70. An even newer machine, the PromethION, integrates many more pores than MinION and will be 280 times more powerful, Oxford Nanopore says. That may help address another drawback of nanopore sequencing: low throughput—the base-by-base output is fast, and the reads long enough to quickly assemble small microbial genomes, but the overall amount of DNA that can be read by each MinION is fairly limited, which puts sequencing whole human genomes out of reach. It may also help for samples such as human blood, which have so much human DNA that the rarer pathogen's genetic material stands a good chance of being missed unless the sequencer can quickly and comprehensively process a sample.

The improvements all point in one direction, says O'Grady, who has been given free access to Oxford Nanopore's machine but has no financial stake in the company: "There's going to be one of these things in everyone's lab." And one day, perhaps in everyone's pocket, adds Camilla Ip of the Oxford Genomics Centre, who helped coordinate a multicenter evaluation of the MinION. "It will be like the mobile phone," she predicts. ■