Roswell Biotechnologies Harnesses Molecular Electronics for Chip-Based DNA Sequencing

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NEW YORK (GenomeWeb) – Roswell Biotechnologies of San Diego is developing a semiconductor chip-based single-molecule sequencing technology that relies on molecular electronics. The technology, dubbed Electronic Nano-Device Sequencing (ENDSeq), measures changes in current that occur when a polymerase that is part of an integrated circuit incorporates nucleotides into a growing DNA strand. The company believes that the platform will be able to sequence a human genome at 30x coverage within an hour at a user cost of $100 and plans to test the technology with early-access customers at the end of this year.

Besides large-scale human whole-genome sequencing, the platform could serve as a "data reader" for DNA-based digital data storage systems.

Roswell plans to describe the technology and show initial data in a journal publication early this year and is considering presenting it at an upcoming conference.

The company, which recently moved into a 10,000-square-foot facility near the University of California, San Diego campus, became operational in 2015 and currently has about 15 employees and a handful of consultants. So far, Roswell has secured about $6 million in funding from private investors and is currently raising on the order of $15 million from institutional and strategic investors in a Series A round that it expects to close very soon. That funding will allow the company to improve the technology and make it more robust in preparation for a commercial launch.

Roswell was founded in 2014 by genomics industry veterans Paul Mola, the company's president and CEO, and Barry Merriman, its CSO. Both were previously involved with the development or commercialization of several other sequencing technologies, including Life Technologies' (now Thermo Fisher Scientific's) SOLiD and Ion Torrent platforms and Roche's Genia. More recently, they worked together at Human Longevity, seeking business partnerships for population-based sequencing projects. However, they found that the current $1,000 genome was still cost-prohibitive for large-scale sequencing efforts, prompting them to return to sequencing technology development.

Roswell's technology is based on molecular electronics, which inserts individual molecules into electronic circuits that can be integrated into semiconductor chips.

One of the field's conceptual founders is Ari Aviram, a former scientist at the IBM Watson Research Center and an advisor to the company, and Roswell licenses fundamental intellectual property he helped develop.

Another pioneer in the field, James Tour, is a member of Roswell's scientific advisory board. Tour, a professor of chemistry, computer science, materials science, and nanoengineering at Rice University, created some of the first molecular electronic devices in the late 1990s. Another SAB member is Harvard's George Church, who has been involved in many other sequencing technologies.

Roswell has developed a nanosensor that can measure the incorporation of nucleotides by a polymerase. The sensor can be integrated into a complementary metal oxide semiconductor (CMOS) chip and is compatible with chip mass manufacturing

Ion Torrent was the first sequencing technology to rely on semiconductor chips — Illumina's recently launched iSeq platform and Genia's nanopore approach are also chip-based — but Roswell is taking a different tack. "When we came across Ion Torrent, it was clear to us that at least their approach was right; it was the first initiative to fully deploy sequencing chemistry on a chip," Mola said. "Roswell is now the realization of that vision, where you have the appropriate sensor that can really take advantage of scaling on CMOS, which these other technologies are not able to leverage fully."

In Roswell's approach, a single DNA polymerase is tethered to a molecule, for example a carbon nanotube or a piece of DNA, that is part of an electronic circuit. When the polymerase binds a DNA template and starts synthesizing DNA, the current flow through the circuit changes with each nucleotide incorporated, creating a signal that is base-specific and can also detect base modifications.

"We really have the polymerase wired into the circuit, and you are directly electrically monitoring what it does," Merriman said. "This actually gives you a much more precise signal of what base is being incorporated than you get from nanopore-based approaches to interrogating DNA."
The current levels are similar to those used in nanopore sequencing, on the order of picoamps, and the company has so far found no negative effect of the current on polymerase activity.

In 2015, researchers led by Philip Collins and Gregory Weiss at the University of California, Irvine, published a proof-of-concept study in the *Journal of the American Chemical Society* in which they measured the incorporation of various nucleotide analogs by a DNA polymerase using a nanocircuit. However, according to Roswell, that approach had poor resolution and was not scalable.

Roswell's nanosensor circuits rely on self-assembly of the molecular parts and can be manufactured with great precision, Merriman said. The company also has various strategies to ensure that a single polymerase is bound to each sensor.In addition, the firm has shown that it can discriminate between different bases. "This is very non-theoretical," Merriman said. "This has all been experimentally demonstrated quite thoroughly, and we do sequencing-type runs, with all four bases having distinct signals."Roswell currently has 10 prototype instruments in its lab and hopes to scale up to 50 when new funding comes in. Its current chips have eight sensors each that can record data in parallel.

The goal is to scale up to chips with millions of sensors. "Those are also entirely non-theoretical," Merriman said. "We already have 1,000-sensor chips in our lab that are true CMOS integrated circuit chips that we put these constructs on."Roswell is about to have its third-generation chip fabricated and plans to develop three more generations after this, which should get it to a chip with 10 million sensors by the end of this year.

The sequencing workflow with Roswell's platform is expected to be very simple, involving just a few minutes of set-up time, and its chips will be single-use. "There is no library prep, no other sample prep," Mola said.

Based on Roswell's projections — including an estimated 30 percent yield of usable sensors per chip — the final system will be able to sequence a human genome at 30x coverage within an hour, at a cost of

$100 to customers, with reads up to 10 kilobases in length and single-read accuracy of at least 99.9 percent. There would still be room for improvement from there. "We fully believe there will be $10 genomes in less than 10 years," Merriman said.

Read accuracy will be limited by the intrinsic accuracy of the polymerase, which tends to make mistakes every 10,000 bases or so. Sequencing speed will be determined by the speed of the polymerase, on the order of 5 to 100 bases per second, and the current plan is to use natural nucleotides.

The next step will be to scale up development, "mostly from the perspective of running many more experiments to fully develop the chemistry, lock the chemistry, have it reproducible and consistent," Mola said. "It's a numbers game — the more experiments you can run, [the faster] you can start to lock things down and optimize them, and that's where we are now."

The plan is to have a system ready for testing by early-access users in the fourth quarter and to start commercializing the system in 2019.

Roswell has filed or licensed more than 40 patent applications or patents so far in an attempt to secure molecular electronics for sequencing and other applications. "This is a whole new area of technology, so we are patenting very broadly and have a very clear strategy to lock up this molecular electronics approach," Merriman said.

The main application Roswell plans for its sequencer is human whole-genome sequencing, but it is also considering other uses, for example as a reader for DNA-based digital data storage. In addition, biomolecular sensing applications of the platform are possible, for example antibody binding assays or next-generation microarrays, which could be developed in a mobile format. "These are all opportunities that we will continue to explore as we go," Mola said.