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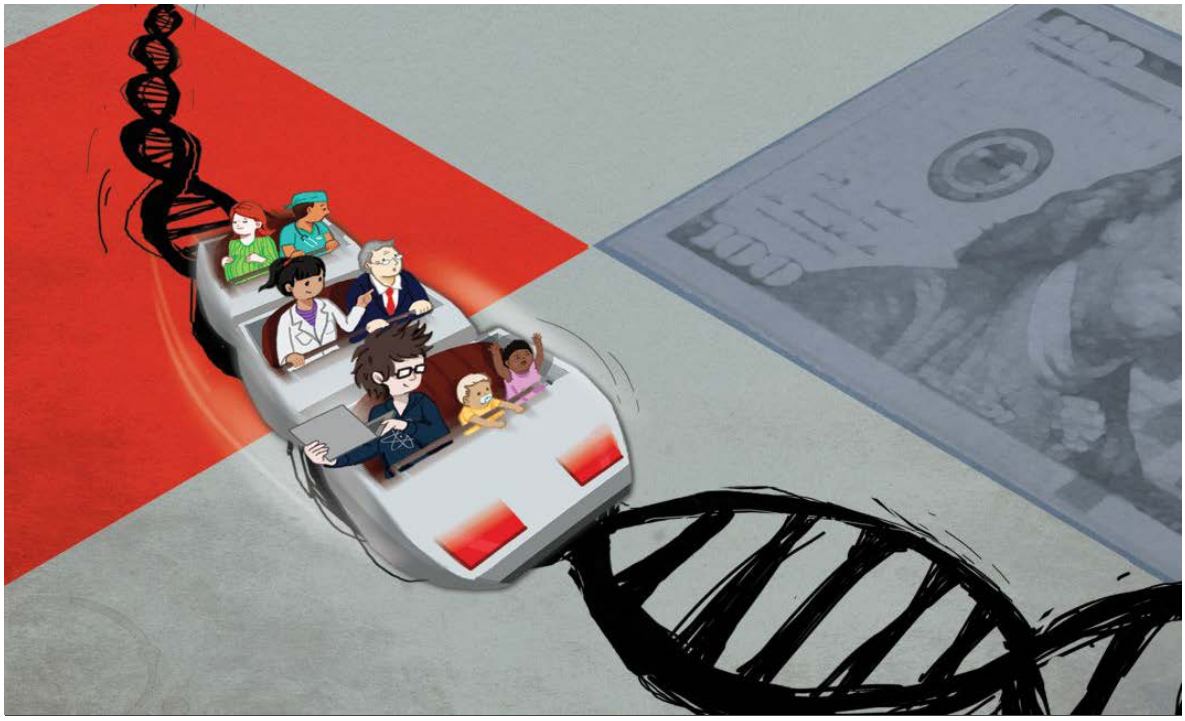

All Aboard the Genome Express

Is a new generation of DNA sequencing technology about to hit the fast track?

By Julianna LeMieux - January 11, 2019

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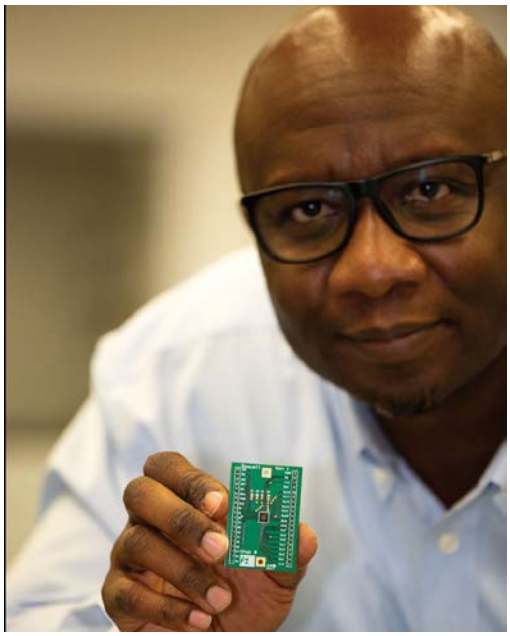
When you approach the San Diego headquarters of Roswell Biotechnologies, you see a company that blends into its office park environs and is, to all appearances, as down to earth as any of its neighbors. A bit of greenery. A plain façade. A nifty logo. Once you cross Roswell's threshold, however, you'll gather that the company has ambitions that are, well, out of this world. Affixed to the lobby wall is a framed copy of an old newspaper's front page. The headline story? The landing of a weather balloon

near Roswell, New Mexico—a mundane event that sparked imaginative theories about alien spacecraft and UFOs.



Roswell Biotechnologies' co-founder and CEO Paul Mola holds the company's first-generation integrated CMOS chip, which is shown here as part of an auxiliary board.

Other displays, including prototype sequencing instruments, establish that while Roswell is run by earthlings, it intends to become "disruptive," a word that is used liberally by Paul Mola, the company's co-founder, president, and CEO. A four-year-old company, Roswell believes that it offers a winning technology, one that can dramatically reduce the costs of sequencing whole genomes.

The cost targets announced by whole-genome sequencing companies are becoming increasingly aggressive. Not long ago, these companies vied to deliver the $1000 genome. Lately, they've become bolder, promising $100 and even $10 genomes. As they hurtle down the cost-competitive track, however, sequencing companies should brace themselves for abrupt curves just ahead. Soon, users of sequencing technology may place less emphasis on generating enormous amounts of data quickly and inexpensively. Instead, they may start demanding sequencing technology that incorporates more powerful interpretive tools, supports embedded applications, or tolerates adverse environments.

Speeding past one cost barrier after another

The Roswell technology is called Electronic Nano-Device Sequencing, or ENDSeq™. It incorporates single-molecule nanosensors in a scalable semiconductor chip format. According to Roswell's Barry Merriman, PhD, another company co-founder and chief scientific officer, ENDSeq will reduce the cost of genome sequencing by one to two orders of magnitude. Similar claims have been made by Francis deSouza, CEO at Illumina. When Illumina introduced its latest system, the Novaseq™, in January 2017, the company issued a press release predicting that the system's new and scalable

sequencing architecture will enable a $100 genome. But is a $10 genome, let alone a $100 genome, a promise that can be kept?

A cautious “yes” might seem appropriate, given that whole-genome sequencing is already passing the $1000 barrier.

According to Ewan Birney, PhD, director of EMBL-EBI in Cambridge, U.K., “Genome sequencing is routine in the same way the U.S. Navy routinely lands planes on aircraft carriers. Yes, a good, organized crew does this routinely, but it is complex and surprisingly easy to screw up.” Given that perspective, which new sequencing technology will arise that maintains the accuracy, length, speed, and usability to drop the price by another one or two orders of magnitude?

The 800-pound sequencing gorilla

By every conventional measure, the dominant sequencing company is Illumina. It enjoys the highest market value, and the company has, by its own estimation, generated more than 90% of all DNA sequence data collected to date.

Illumina enjoyed a growth spurt in November 2018 when it announced the acquisition of Pacific Biosciences (PacBio), one of its chief rivals, for $1.2 billion. The deal attracted the notice of industry veteran Keith Robison, PhD, who writes the Omics! Omics! blog. According to Robison, PacBio technology is “the work of certified geniuses.” The company specializes in long reads, whereas Illumina specializes in short reads. Robison says that by combining Illumina’s muscle and PacBio’s brains, the acquisition may lead to advancements that would almost certainly not have happened at PacBio alone.

Now that PacBio is in Illumina’s hands, another long-read specialist, Oxford Nanopore Technologies, stands out as Illumina’s most established competitor. According to Nathaniel Pearson, PhD, the founder of Root, a company that rewards blood and marrow donor volunteers with insight from their own HLA genes, nanopore technology is “fantastic and noisy—like punk rock.”

David Smith, PhD, professor of laboratory medicine and pathology at the Mayo Clinic, dreams of an Oxford Nanopore platform that can combine its ultra-long reads (some users have reported contiguous reads in excess of 2 million bases) with 99% accuracy—a fantasy, Smith admits, but he adds that he never thought that the current technology would be possible.

Unlike the lab instruments sold by Illumina, Oxford Nanopore's platforms have portability. "[Our MinION platform] is the world's best-selling NGS sequencer, now outstripping Illumina's MiSEQ®," asserts Clive G. Brown, Oxford Nanopore's chief technology officer. Costing just $1000 for a starter pack, the MinION's small size and long reads have democratized genomic sequencing.

Smith tells *GEN* that "the genomic revolution is coming like a storm," quoting Eric Green, MD, PhD, director of the National Human Genome Research Institute, as follows: "In the next 5 years, 50 million people will have their whole genomes sequenced—maybe more." This statement highlights a challenge that may concern startups such as Roswell even more than the challenge posed by Illumina's dominance. That is, startups are in a race against time. In 5 or 10 years, it may be too late for a new technology to compete. Smith notes that BGI (a Chinese company formerly known as the Beijing Genomics Institute) is perhaps best positioned to compete with Illumina, but this challenger is also up against the clock.

Rade Drmanac, PhD, chief scientific officer and co-founder of Complete Genomics, a BGI company tells *GEN* that BGI's focus over the next 5–10 years, is to "scale, scale, scale" to "bring this technology to the highest possible level" and to make accurate sequencing affordable. Yongwei Zhang, PhD, the chief operating officer of Complete Genomics, adds that the company plans to build on top of its own genomics platform, the MGI platform, to develop other omics-based technologies.

The core of all MGI sequencing platforms is the DNBseq™ nanoarray technology called DNA Nanoballs, which BGI has been refining since Complete Genomics' acquisition in 2013. Zhang adds without hesitation that the technology will make it possible to sequence a genome for $100 within the next 5–10 years.

His confidence rests on MGI's latest platform, the MGIseq T7, which is named for its target output of 7 terabytes per day (although it currently produces 6 TB per day). Johan Christiaanse, director of marketing for BGI, tells *GEN* that BGI used to be the biggest user of the Illumina platform. However, in the past 18 months, BGI has transferred roughly three-quarters of its sequencing services business to the DNA Nanoballs platform and will be the first recipient of the high-capacity MGIseq T7.

Will the instruments manage to become competitive in an already mature market? Zhang responds that DNA Nanoballs technology is the only technology that can deliver all four items of speed, length, accuracy, and cost. Zhang suggests that BGI recognizes Illumina's dominance of the field and is realistic that market adoption will take time.

Shawn Baker, PhD, a consultant at SanDiegOmics.com who has spent his career entrenched in the world of genomic sequencing, tells *GEN* that BGI's technology is not especially innovative. "If you take a 30,000 foot view," he maintains, "it is fundamentally the same as Illumina's with a lower price tag." That said, he adds that if BGI implements its product development and marketing well, it will put pricing pressure on Illumina, referencing the age-old marketing motto, "never compete on price."

Baker predicts that when BGI enters the U.S. market, the company will introduce products at very competitive prices—maybe even 50% cheaper than Illumina—while pushing hard on the value lever. "Illumina has really high margins," he points out, "and can reduce its prices if it wants to." Lower prices, in his opinion, would be a great thing for the sequencing market. Baker notes that, aside from BGI, Oxford Nanopore may be the one to watch as far as driving the price of sequencing down, saying that the company has a fairly convincing case that it can be as cheap as Illumina, or even cheaper.

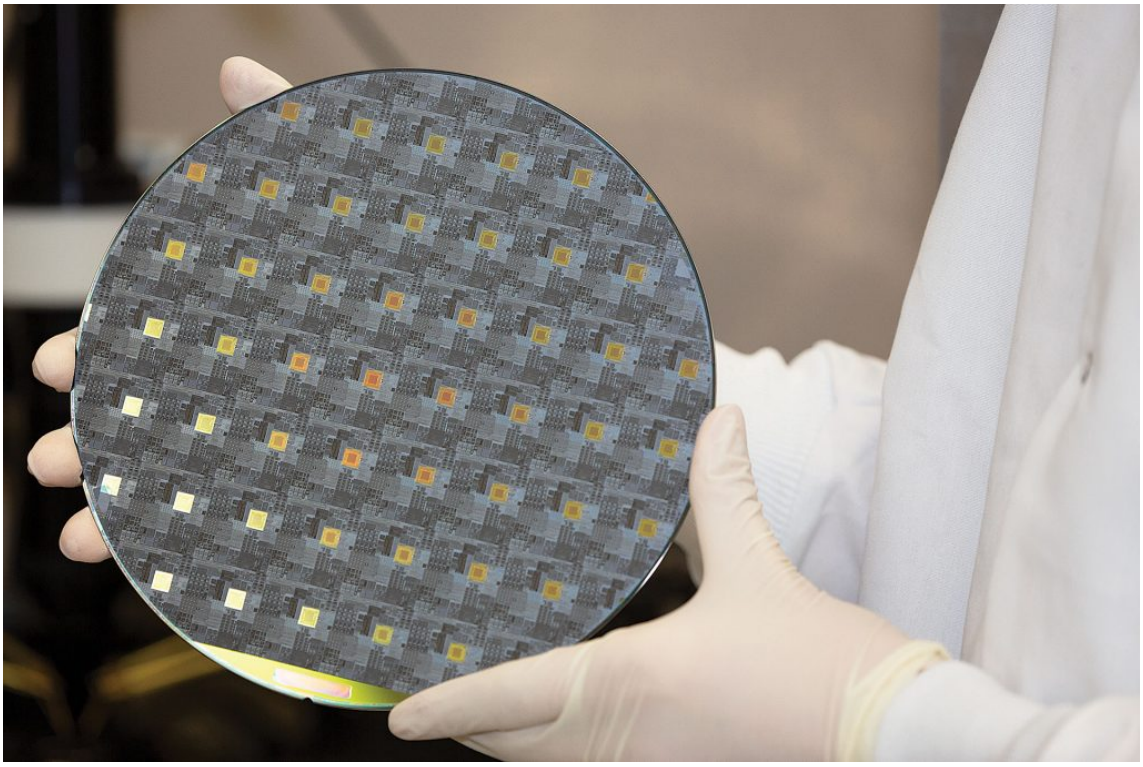
Will molecular electronics disrupt sequencing?

Paul Mola is convivial, self-effacing, and so enthusiastic about his company's work that he indulges in some fairly hyperbolic claims. For example, Mola claims that Roswell will provide super-comprehensive, super-long reads while supporting phasing, going

through repeats, and discerning epigenetic marks at a fraction of the cost currently charged by the company's competitors.

Roswell, however, has yet to publish a peer-reviewed publication and has not yet sequenced even bacterial genomes. Of course, big ideas and dramatic, headline-grabbing claims are nothing new to the genome sequencing field. For example, PacBio's launch in 2008 was accompanied by promises of the "15-minute genome" within 5 years—a claim that had little chance of being realized.

Roswell's platform tethers a polymerase into a nanosocket, as part of an electronic circuit with a molecular wire of some kind. As the polymerase incorporates nucleotides by executing an opening-and-closing motion that resembles a finger-and-thumb motion, it moves charged groups on the surface with respect to the wire, changing the scattering of electrons. A current spike occurs each time a nucleotide is incorporated, contributing to a sequence-specific signal pattern. The sensor is integrated into a complementary metal-oxide semiconductor (CMOS) chip. (Such chips are also used in Illumina's iSeq platform and Thermo Fisher Scientific's Ion Torrent technology).

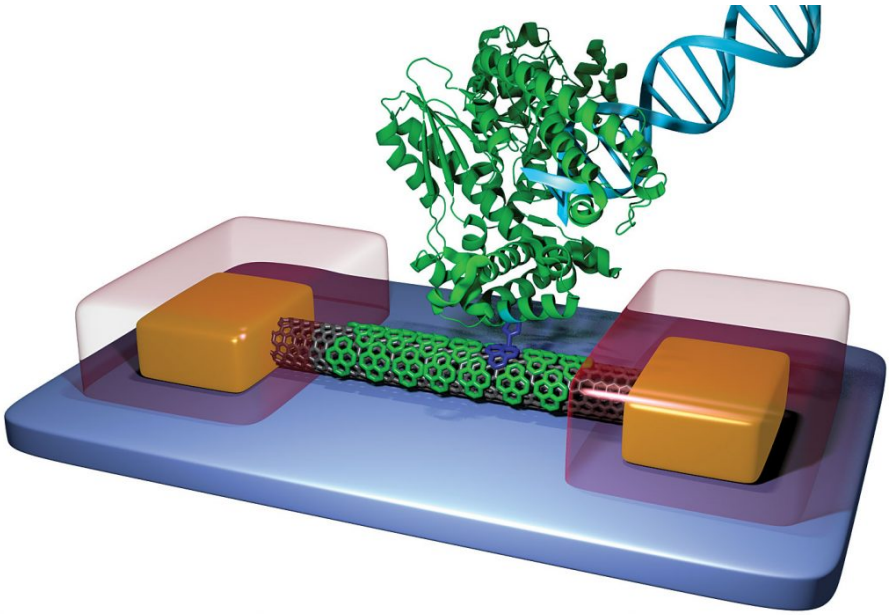


Roswell's integrated CMOS chip wafer is fabricated at Taiwan Semiconductor Manufacturing Company. Roswell's strategy is to take advantage of the CMOS scaling roadmap to lower the cost of DNA sequencing.

Although Roswell may be the first company openly talking about using molecular electronics technology to sequence DNA, the enabling discovery was made about six years ago in a laboratory at the University of California, Irvine (UCI).

Philip Collins, PhD, a physicist at UCI, tells *GEN* what happened when his group, in collaboration with Gregory Weiss, PhD, professor in the chemistry and molecular biology and biochemistry departments at UCI, published a paper in 2012. “For the next two years,” he recalls, “we had companies and potential startups and investors marching through campus, all asking the same question: ‘Have you tried to do DNA sequencing with this?’”

The paper, published in *Science*, was entitled, “Single Molecule Lysozyme Dynamics Monitored by an Electronic Circuit.” It described a molecular electronics approach in which a transducer for protein motion is obtained by tethering a single lysozyme molecule to a carbon nanotube. This approach, readers understood, could be used to build electronic devices capable of monitoring single enzymes. The work was an immediate sensation. Three years later, it was followed by a proof-of-concept paper from the same team, which described how a system similar to the lysozyme-monitoring system was used to measure the incorporation of nucleotides by a DNA polymerase.



Phil Collins, PhD, a physicist at the University of California, Irvine, developed a molecular electronics technology for sequencing DNA. This technology links a DNA polymerase to a nanoscale circuit. In this image, the polymerase is shown attached to a carbon nanotube transistor. As the polymerase moves base-by-base along the DNA strand, the transistor emits a signal stream that imparts sequence information.

Due to academic restrictions, Collins is limited to unique collaborations with companies. He has chosen to ally with Illumina on a project that has continued for six years and is funded by an NIH grant. The purpose of the collaboration, according to the grant abstract, is to "investigate a new, all-electronic sequencing methods that have the potential to become the next transformative step for DNA sequencing."

Collins says that molecular electronics is still very much in a proof-of-concept stage. Many wrinkles will have to be ironed out before the technology becomes both technologically and commercially competitive. "That's the way all up-and-coming technologies have to get started," he points out. The goal of his collaboration with Illumina is "to investigate whether this molecular electronics scheme really has legs" by "asking questions about accuracy and scaling and whether we can produce 'proof of concepts' that would make Illumina believe that this is going to be the new frontier for sequencing."

Baker tells *GEN* that this is the best way to approach a new sequencing technology, as there is "a big gulf between what can be demonstrated in a laboratory and what makes a good product." He adds that "it is very frequently underestimated how difficult it is to turn a good idea, even a very clever idea, into a real product." He adds that there are challenges at every step of the way, from manufacturing to commercialization, and that success stems from predicting as many of them as possible.

As one of the first employees at Illumina, Baker notes that the company has demonstrated its talent for taking an academically derived idea and turning it into a product. He cites the example of the original technology that Illumina was built on, the pioneering "beads in well" technology that allowed for the construction of the first microwell arrays. This innovation was developed in the laboratory of David Walt, PhD, who was at Tufts University at the time. (He has since moved to the Wyss Institute, Harvard Medical School.)

There is no question in Collins' mind that we will have commercial DNA sequencers that are based on molecular electronics, confirming that Roswell is not the only startup based in molecular electronics. "There is," he insists, "no scientific barrier to doing this." That said, Collins is less strident on timing, adding that a decade may pass before the UCI technology results in a commercial platform. Given the time frame that other

sequencing technologies have taken to move from concept to benchtop—such as nanopore technology, which took roughly 20 years from proof-of-concept to nature—10–15 years does not seem an unrealistic expectation. Collins notes that Illumina, unlike startups, has a long-term focus. “A venture capital startup needs a timeline, and that is tough to have when you are working with a brand-new technology.”

Stuart Lindsay, PhD, director of the Center for Single Molecule Biophysics in the Biodesign Institute at Arizona State University, was a reviewer of Collins’s original paper. Like Collins, he is confident that the future of DNA sequencing is in molecular electronics. Lindsay goes so far as to suggest that Illumina might have merged with PacBio to acquire technology for electronic sequencing. “PacBio has a lot of both intellectual property and expertise in the area of tethering polymerases,” he notes. “So, it’s not a wild stretch of the imagination to see how its technology could transfer to a platform that is read out electrically.” Both Illumina and PacBio would not comment on the alleged reason for the merger beyond the publicly disclosed press release.

Lindsay, who has had some of his own technology licensed by Roche, would be “very surprised” if there wasn’t very heavy investment in the Collins approach. The Roswell approach is based on the original observation made by Collins, Lindsay notes. Although Linday recognizes that there are differences in the details, he “doesn’t see much daylight in what Roswell are doing and the Collins technology.” The big distinction is that instead of using Collins’ carbon nanotube, Roswell uses proprietary molecular wires. Roswell claims that its system is much more scalable and better suited for mass production. Merriman says that “there is no good way to mass produce high-density carbon-nanotube-based chips.”

Merriman doesn’t feel a lot of competitive pressure coming from similar technologies, and Mola points out that, based on patents, Roswell is “the pioneer in this field.” The company has secured four patents and says that it has roughly 50 more in the pipeline. By amassing intellectual property, the company is progressing toward its goal of owning molecular electronics for biosensing.

Although Lindsay has a hunch that Illumina has licensed Collins' intellectual property or has an agreement with exclusive rights to it, UCI would not comment, citing that it does not share the names of its licensees, unless licensees agree to be named.

Lindsay guesses that there will be commercial machines in 3–5 years and that the market will be dominated by this technology in 10. "If it works out, it is going to be, by far, the most economical, rapid method of acquiring DNA sequence data," he says, and that "folks who ignore this technology are doing so at their own peril."

The sequencing question for the next generation

The evolving race to drive down the cost of genomic sequencing is definitely one to watch, but for many, the big question isn't about the generation of sequencing data. Instead, it's about the data's interpretation and use. Smith says that true "disruption" in genomic sequencing won't come from a new sequencing technology. He sees more disruptive potential in ideas for channeling the deluge of sequencing data. Such ideas, he suggests, will change our society as we know it.

Chris Dwan, a genomics consultant who helped establish the IT infrastructure at the New York Genome Center, says that "sequencing is no longer the dominant part of sequencing-based projects and is certainly not the most interesting part of these projects." The more information we get, the more flexible sequencing will have to become.

New companies will be charged with making sequencing work underwater, or in space, predicts Dwan, and it will be up to a new generation of inventors to put that technology into applications that are truly innovative. For example, Dwan hears murmurs about sequencing-based technologies that won't bother emitting reads. Instead, detectors might turn an indicator blue to indicate the presence of a particular DNA sequence. Embeddable sequencing technologies could transform how sequencing is used for health. They could also lead to limitless and almost unimaginable applications in our everyday life.

If it were up to Pearson, whatever comes next would not fall under the “meaningless marketing speak” that constitutes the fossilized term “next-generation sequencing.” He suggests that we turn toward using more concrete, descriptive labels such as short-read, long-read, etc.—names that mean something to a listener. Regardless of what we call it, the next disruption will shift our focus from “how we do it” to “what we can we do with it,” bringing us squarely into the next generation of sequencing.

Practical Medical Utility of Sequencing

Effective health maintenance with personalized disease prevention and management requires accurate sequencing data at a price that supports adoption in routine medical practice.

BGI offers multiple DNA sequencing platforms that incorporate the company’s DNBseq DNA Nanoball technology. In this photo, a BGISEQ-500 instrument is shown at the BGI laboratory in Shenzhen, China.

According to Rade Drmanac, PhD, chief science officer, BGI, the company’s DNBseq™ next-generation sequencing (NGS) technology addresses those requirements by “eliminating amplification errors through truly PCR-free NGS with high efficiency and base-calling accuracy enabled by DNA Nanoballs, which are smaller but brighter than PCR clusters.” The latest generation of the technology generates 60 highly accurate human genomes per day with a single instrument, he adds.

Another limitation to the medical value of current routine NGS technology is the limited read length that prevents complete and accurate haplotype-resolved assembly of individual genomes, notes Drmanac. Single Tube Long Fragment Read (stLFR) technology employs novel barcoding techniques to obtain sequences from ~100-kb-long genomic DNA molecules using accurate and efficient but relatively short NGS reads, he continues.

“DNBseq and stLFR technologies were invented by Complete Genomics, now part of MGI (BGI’s life sciences product company),” explains Drmanac. ”Combined, these innovations are poised to provide the ‘perfect’ (phased de novo) individual genome sequence at a cost that enables genome-based population-wide personalized healthcare and advanced biodiversity research.”

Targeted Sequencing Adopts CRISPR

Widely used as a gene editing tool, the CRISPR-Cas system has recently proven to be an extremely versatile selection method for high-throughput targeted sequencing. By coupling sequence-specific guide RNAs with Cas enzymes, researchers can target up to thousands of selected regions in genomic DNA or RNA, and then manipulate those genomic elements in various ways.

For instance, targets can be specifically excised and then size-selected, or isolated using affinity-based means, and then sequenced on short- or long-read platforms. To facilitate the use of this increasingly popular technique, Arbor Biosciences recently introduced myNGS Guides, custom libraries of guide RNAs for pairing with Cas enzymes in CRISPR-driven targeted sequencing, according to Jacob Enk, PhD, senior scientist at the company.

“A variety of Cas enzymes are now thoroughly characterized, varying by site recognition requirements, processivity, and cleavage site morphology. This presents a virtually limitless toolkit for highly specific target manipulation,” says Enk. “When coupled with just a few, or thousands, of site-specific guide RNAs, highly complex but site-specific effects can be achieved in a simple, single reaction. Continued adoption across biotech will undoubtedly hone these techniques into routine use, adding yet another example to CRISPR-Cas system’s portfolio of transformative impacts.”