Breaking Down the Building Blocks: Innovation in DNA

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Next Generation Sequencing (NGS), or DNA sequencing, is fundamentally changing research and clinical approaches to human health. As a technology, NGS has advanced dramatically in speed, cost, and accuracy in the last decade. This has allowed it to gain widespread adoption in academic and research institutions and to find new and emerging applications in clinical settings. Today, NGS is driving innovations in disparate fields, from non-invasive prenatal testing (NIPT) to early cancer screening and detection (ECD). New sequencing technologies continue to emerge, further fueling the cycle of innovation. As a whole, NGS companies are poised to take advantage of underpenetrated markets with transformational technology. However, similar to the biotech boom of the last decade that promised (and at times delivered) similarly life-changing innovations, NGS comes with its fair share of risk, with equally-sized reward.

Background

Each human cell contains DNA, which contains the information necessary to sustain an organism. Information in DNA is stored in four chemical bases: adenine, guanine, cytosine, and thymine. Human DNA typically contains around three billion base pairs, and about 99% of the sequence of bases is uniform across humans, with the remaining 1% differentiating person from person.[1] In the cell, DNA is packaged into roughly 20,000 to 25,000 genes (averaging out to about 1,000 genes per chromosome), which are the basic unit of hereditary genetics. The DNA is

[1] National Library of Medicine

then spliced into RNA to encode for protein production. The genes that make it to the final RNA are known as *exons*, which is collectively known as the *exome*, while the other portion is "non-coding," collectively known as *introns*, but some of which may have yet undiscovered functions.[2] These genes are then packaged into 23 pairs of chromosomes, of which 22 are called *autosomes* (identical between male and female), and the remaining pair codes for biological sex.[3] As such, DNA contains the entire foundation of biological organisms, and sequencing it can yield significant research and clinical information on the state of an organism.

History

NGS as a technology is known as "Next Generation" because it is preceded by dideoxy sequencing technology first pioneered in the 1970s by Frederick Sanger and his colleagues at the University of Cambridge, England. Sanger sequencing became the first commercial sequencing method available, but it was limited in its application because it could only sequence very short DNA strands, between 100 and 1000 base pairs.[4] Continuous use of Sanger sequencing for longer DNA sequences is called "shotgun sequencing," named due to the random nature of using Sanger sequencing to achieve a consensus longer read. In the following years, Sanger sequencing powered innovative research, including the breakthrough Human Genome Project that sequenced a "complete" human genome for the first time (which was prematurely announced complete in an event in 2000 featuring Bill Clinton and Tony Blair raising their arms together symbolizing a chromosome). While Sanger sequencing was remarkably innovative for its time, earning Sanger himself a Nobel Prize in chemistry, it had its share of drawbacks as a first generation technology. Despite being remarkably fast, Sanger sequencing had limited sensitivity due to its single-

[2] Ibid.
[3] Ibid.
[4] Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics*, *107*(1), 1–8. https://doi.org/10.1016/j.ygeno.2015.11.003

fragment workflow. It was also incredibly cost inefficient for sequencing larger amounts of DNA, with 10 ng of DNA yielding just ~1 kb (or 1000 bases) of data while a modern Illumina AmpliSeq workflow can yield ~300 kb from the same 10 ng of DNA.[5] The Human Genome Project was a 13-year-long initiative that cost just under $3 billion, while today, using NGS technology, companies are pushing towards a commercial genome costing under $600 with turnarounds of just days.

Following the initial success of Sanger sequencing, several companies made attempts to create the second generation of sequencing technology. Second generation sequencing technology introduced the concept of sample prep and amplification, where the number of copies of certain target genes are increased without proportional increases in the number of copies of other genes.[6] Sequencing based on this fragmentation and amplification and the fluorescent chemical tags used to identify the different bases became known as Sequencing by Synthesis (SBS), and earned the moniker "short read sequencing" in recent years due to the short strand lengths the technology can process. SBS then relies on parallel reads of fragmented DNA which are stitched together to create a consensus sequence of nucleotides. One of the earliest innovators in the space was 454 Life Sciences, which launched one of the first second generation sequencers using pyrosequencing, a form of SBS chemistry that uses visible light detection and pyrophosphate, allowing data output of up to 20 Mb per run by 2005.[7] While improvements were made to the 454 systems, Illumina gradually established market dominance with technology it acquired from Solexa in 2006, which used a different form of SBS chemistry, allowing for

[5] "NGS vs. Sanger Sequencing," Illumina, accessed March 14, 2022, https://www.illumina.com/science/technology/next-generation-sequencing/ngs-vs-sanger-sequencing.html.
[6] Kulski, Jerzy. "Next-Generation Sequencing — An Overview of the History, Tools, and "Omic" Applications" In Next Generation Sequencing: Advances, Applications and Challenges, edited by Jerzy Kulski. London: IntechOpen, 2016. 10.5772/61964
[7] Slatko, B. E., Gardner, A. F., & Ausubel, F. M. (2018). Overview of Next-Generation Sequencing Technologies. *Current protocols in molecular biology*, *122*(1), e59. https://doi.org/10.1002/cpmb.59

higher output at a lower reagent cost.[8] Today, Illumina's suite of sequencers — including the MiSeq, NextSeq, and NovaSeq instruments with outputs of more than 500 Mb per run — account for a near-monopoly in the clinical market, with competition like BGI Group (originally Beijing Genomics Institute) kept at bay temporarily thanks to IP protection. While SBS chemistry has dramatically increased the throughput of DNA sequencing, the fragmentation and amplification of DNA in the sample prep process also had its drawbacks, including amplification bias, which results in duplicate data readouts.

Second generation sequencing methods continue to be widely used, but a few competitors have emerged as the third generation of sequencing technology. These competitors, including Oxford Nanopore and Pacific Biosciences (PacBio), have created brand new chemistries to process longer read lengths to code regions of the genome that were previously inaccessible via short read sequencing. Oxford Nanopore is perhaps the most differentiated from short read sequencing methods, with reads generated from much longer DNA strands (usually 25,000 base pairs in length or more) which are only limited in length by the quality of the library. This allows for "ultra-long reads," but consequently suffers from a relatively high error rate in comparison to the consensus accuracy achieved by SBS chemistry.[9] PacBio's Single-Molecule Real-Time, or SMRT, technology is fundamentally more similar to short read since it also relies on fluorescent tags to identify bases.[10] As a result, PacBio is capable of longer read lengths than traditional short read with a slightly higher error rate, but not quite capable of the read lengths of Oxford Nanopore. Both Nanopore and SMRT technologies have found utility in the research setting, particularly in rare diseases previously thought to have no genetic identifiers when analyzed by

[8] Ibid.
[9] Amarasinghe, S.L., Su, S., Dong, X. *et al.* Opportunities and challenges in long-read sequencing data analysis. *Genome Biol* **21,** 30 (2020). https://doi.org/10.1186/s13059-020-1935-5
[10] Slatko, B. E., Gardner, A. F., & Ausubel, F. M. (2018). Overview of Next-Generation Sequencing Technologies. *Current protocols in molecular biology*, *122*(1), e59. https://doi.org/10.1002/cpmb.59

short read sequencing. Oxford Nanopore has continued to innovate on their consumables while maintaining their highly differentiated chemistry. With the announcement of their Kit 12 and R10.4 consumables, Oxford Nanopore now claims capability of greater than 99.99% raw read accuracy, significantly improving on their prior technology. Similarly, PacBio announced data for their novel Sequencing by Binding (SBB) chemistry, claiming better accuracy and consistency versus existing SBS chemistries. While both companies have improved on both their cost efficiency and accuracy, their much higher sequencing cost and comparably higher error rates versus legacy short read sequencing have thus far prevented them from finding significant clinical usage, though that may change as both companies continue to innovate on their chemistries and consumables.

Applications

For now, short read sequencing continues to dominate the clinical market and has found tremendous success in the diagnostics space. Several common "applications" now include NIPT, organ transplant, and even oncology. Of these applications, NIPT and organ transplant are relatively straightforward, while oncology can be split into a few distinct specialties.

NIPT is a long-established application for NGS, with many medical guidelines already including NGS-based screening for average risk populations. Tests like Panorama from Natera and Harmony from Roche are well-validated in trisomy 21 (Down Syndrome), one of many fetal chromosomal diseases aggregately known as aneuploidies.[11] Previous methods of testing for trisomy 21 — amniocentesis, triple screen, and chorionic villus sampling being the most notable — had far lower PPV (Positive Predictive Value) and NPV (Negative Predictive Value) for the

[11] Lee, D. E., Kim, H., Park, J., Yun, T., Park, D. Y., Kim, M., & Ryu, H. M. (2019). Clinical Validation of Non-Invasive Prenatal Testing for Fetal Common Aneuploidies in 1,055 Korean Pregnant Women: a Single Center Experience. *Journal of Korean medical science*, *34*(24), e172. https://doi.org/10.3346/jkms.2019.34.e172

condition than modern NGS-based blood tests, and they also had a low, but non-zero, risk of miscarriage.[12] On the other hand, Natera's Panorama, for example, demonstrated >99% sensitivity and specificity to trisomy 21, thus generating a >99.99% negative predictive value. meaning that a low-risk test result is accurate 99.99% of the time. Similarly strong performance indicators are observed for these NIPT tests in other common aneuploidy conditions like trisomy 18 and trisomy 13. It should be noted that these blood-based NIPT tests are a form of liquid biopsy (LBx) and are screening tests; they are meant to be an early indicator for fetal abnormalities that should be confirmed via an invasive diagnostic test if necessary. This distinction is most important when discussing NIPT screening tests with lower sensitivities, including for conditions like microdeletions that are notoriously difficult to screen for due to the variability of genetic mutations that can cause the condition.

Organ transplant is another area where NGS-based testing is gaining traction. All cells typically have proteins known as antigens, which trigger immune responses. In the case of an organ transplant, the body will recognize the antigens secreted by the donor organ as "foreign," thus triggering a negative immune response that leads to rejection, or damage to the transplanted organ. However, there are cases of organ rejection where the immune response is not as obvious, leading physicians to miss the opportunity to medicate patients and prevent significant damage to the organ. Some tests, like Natera's Prospera, act much like traditional organ transplant diagnostics in detecting early signs of rejection, but with higher sensitivity. Others, like Verici Dx's Clarava, focus on predicting immune response prior to transplant, which helps physicians find better organ matches. In both cases, the test improves patient outcomes over traditional

[12] Richard S Olney et al., "Chorionic Villus Sampling and Amniocentesis: Recommendations for Prenatal Counseling," Centers for Disease Control and Prevention (Centers for Disease Control and Prevention, July 15, 1995)

testing methods via a non-invasive blood draw, making it easier for physicians to work the tests into their standard workflow.

In the field of **oncology**, NGS has found a strong foothold in what are called "companion diagnostics" (CDx). The US FDA defines CDx as a test that "helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks," which positions CDx as a key product in therapy selection.[13] Essentially, CDx helps physicians identify patients who would more likely benefit from specific therapies and also helps monitor the patient's response to treatment through the treatment cycle. CDx is a relatively crowded space, with a wide variety of worldwide participants, but one notable leader is Guardant Health, whose suite of Guardant360 CDx products are widely adopted in cancer centers.

There are also a few emerging applications within oncology that are gaining more market attention, including Minimal Residual Disease monitoring (MRD) and Early Cancer Screening and Detection (ECD). MRD makes use of circulating tumor DNA (ctDNA), which is DNA that tumors shed into the bloodstream and is then picked up by NGS tests via blood draw to monitor cancer recurrence. Currently, the majority of MRD testing is done in colorectal cancer (CRC), where most of the data have been generated. In an ideal world, an MRD test would be able to be used to determine risk of recurrence after a single test, allowing the physician to determine the next course of treatment. Unfortunately, the data that have been generated so far (which does not yet include any large-scale trials) seem to suggest that the sensitivity of a single MRD test post-treatment with curative intent is around 50%, which is far too low, and it only moves into the

[13] Center for Devices and Radiological Health, "Companion Diagnostics," U.S. Food and Drug Administration (FDA), accessed March 14, 2022, https://www.fda.gov/medical-devices/in-vitro-diagnostics/companion-diagnostics.

~90% range after serial testing. Even so, MRD has found its way into physician workflows, usually as an indicator for physicians to escalate treatment instead of de-escalate treatment. Familiar names like Natera and Guardant have received very positive reimbursement decisions from insurance payors in the United States for MRD, and they are working towards expanding data generation into additional indications beyond CRC.

While MRD may have a more niche market, ECD has immediate clinical utility. The ability to screen for and detect cancer in its very early stages allows for patients to undergo treatment when the outlook for survival is far more positive. A key example here is Exact Sciences' Cologuard test, which makes use of NGS to screen for CRC in stool samples. Cologuard made its way into national cancer guidelines after the readout of its large-scale Deep-C trial, which yielded 92% sensitivity and 87% specificity to CRC, and 42% sensitivity to precancerous lesions. Stool sampling was intended to help drive adoption of screening tests for CRC given that many patients did not want to undergo colonoscopy, but the market penetration has thus far been slow. However, a new modality for CRC screening in LBx may help drive further market penetration, with market research suggesting that blood-based testing is greatly preferred over both stool-sampling and colonoscopy. There are a plethora of competitors in the CRC LBx screening space, including Guardant and even Exact themselves. Guardant is potentially the leader here, as their 12,000 patient cohort ECLIPSE trial expected to read out in mid-2022 has the potential to cause massive disruption in the space.

These single-indication tests are not the only competitors in ECD. There are a few companies competing in multi-cancer early detection, or MCED. GRAIL's Galleri test is able to detect 50 different types of cancer (albeit with lower sensitivity), and international competitors like Burning Rock Biotech are continuing to generate data towards a 22-cancer test. Admittedly,

these MCED tests are still having trouble penetrating physician workflows, particularly due to their cost and questions surrounding the proper workup cascade after either positive or negative results. Nevertheless, MCED is an exciting new area for NGS, and it is very much still in its early stages.

Market size/participation

There is no doubt that NGS is a multi-billion dollar industry, both for the high-growth testing providers and the instruments that power those innovations. Oncology is perhaps the most obvious high-growth market given the costs associated with cancer treatment, both successful and unsuccessful. For example, the current total addressable market (TAM) in MRD, which is already a fairly niche area, is around $20 billion. ECD further expands the TAM to varying degrees depending on the indication. CRC, a very high incidence cancer, represents another $20 billion of TAM, with a comparably-sized market in lung cancer and additional incremental contributions from other, less pervasive, indications. These applications are fueled by the machines and consumables in the background. Illumina, the largest provider of NGS machines and consumables, recognized more than $1 billion of revenues in each quarter of 2021. While impressive, the potential market is magnitudes larger. A 2021 estimate on the market for sequencers from Decibio found a potentially $100 billion TAM in research and population sequencing, expanding up to potentially more than $300 billion when including applications like infectious disease, oncology, and immunology.

It is important to note that Illumina is the exception to the rule. For the most part, NGS is still an emerging subsector of healthcare, and it carries a lot of the same risks as emerging biotech and information technology companies. Transformational technology will always have steeper valuations in the market represented by higher market capitalization. The higher the

market caps rise, the harder they can fall. However, all three sectors (NGS, biotech, and information technology) are characterized as having a highly positive performance-to-price ratio. This is important to note because these high valuations are not solely sustained by the promise of technology, but they are also supported by strong current growth in the business. Natera, for example, recorded revenue growth of 29.3% and 60% over 2020 and 2021, respectively, while Guardant also saw >27% growth each year over the same timespan. Machine-makers had a more tumultuous two-year span with COVID affecting lab staffing and therefore instrument usage, but they managed to turn things around in 2021. Illumina saw 40% growth year-over-year in 2021, while PacBio, a "third generation" sequencing competitor, recognized 65% growth year-over-year and its seventh consecutive quarter of sequential growth.

Exome Asset Management has long been a participant in the NGS market since our inception, with a focus on finding the best-positioned companies that can deliver the most transformative products. We recognize the risks associated with these high-growth, high-risk names, and we anchor the portfolio with stable large-cap names in tools. NGS performed well in 2020 and 2021, but it had a rough start to 2022. Macro fears over interest rate hikes and general de-risking of portfolios to favor stable large cap names hit the healthcare sector at large, causing steep selloffs in less mature names through the first few months of 2022. The selloff continued despite positive news (pre-announced earnings, new product announcements, etc.) coming out of many players participating in the JP Morgan conference in January, reflecting the macro, sector-agnostic dip in the market. Furthermore, the acceleration in the Russia-Ukraine conflict has caused additional volatility, characterized by large intra- and inter-day swings. We also note that the Omicron surge in late-December and early-January significantly disrupted operations for several research-oriented companies, which added to uncertainty in the outlooks for 2022 and led

to more conservative guidance. This cumulation of factors has resulted in relatively significant multiple compression in the sector, with analyst ratings changing accordingly. We identify this contraction in valuation across the board as a buying opportunity, particularly due to the wealth of major catalysts throughout 2022, headlined by Guardant's ECLIPSE readout in Q2 or Q3. We also see continued strength in core markets as NGS continues to find new market opportunities and penetrate the clinical care spectrum. Recent guideline changes for NIPT, ongoing large-scale clinical trials in organ transplant, and beneficial Medicare reimbursement changes in oncology are fueling broader interest in the field and encouraging adoption. These next few months represent an exciting time for NGS, with a growing competitive landscape driving innovation at every turn and with ample opportunity for new applications to emerge.

Discussion on Selected Players

Starting with the instrument providers, the most prominent companies include Illumina, BGI, Oxford Nanopore, and PacBio, all of which were briefly discussed earlier. The table below details the relevant differences in technology by error rate and read length. This table also

	Oxford Nanopore	PacBio	Illumina	Ion Torrent
Read length	Variable (200 bp up to Mbps)	Up to 20 kb	Up to 600 bp (2x300 PE)	Up to 400 bp (SE)
SNV error rate	1%-5%	0.1%	<0.1%	<0.1%
Indel error rate	5%-10%	4%	<0.1%	1%

Source: Piper Sandler

includes Ion Torrent, a business unit of Thermo Fisher.

Illumina: The Giant in the Sequencing World

Illumina is one of the longest-tenured machine providers in NGS and a dominating force in short read sequencing. Founded in 1998, Illumina became the giant it is today thanks to the acquisition of Solexa, which came with the technology and patent estate that would form the backbone of its product offerings to this day. Today, Illumina has a comprehensive product offering spanning low-throughput benchtop sequencers (MiSeq, MiniSeq, iSeq), mid-throughput sequencers (NextSeq), and high-throughput sequencers (NovaSeq). The price of these sequencers ranges from about $130,000 for the MiSeq to nearly $1,000,000 for the NovaSeq, with comparably scaled consumable prices between $600 and $17,000.[14] Despite the higher upfront capital cost, Illumina's price per Gb of data generated is very low, with NovaSeq generating 1 Gb of data for less than $10. As of late 2020, Illumina had over 17,000 active sequencers placed in a variety of both research and clinical settings. This large installed base of instruments is reflected in Illumina's financials, as it achieved remarkably robust growth in 2021 with over $4.5 billion in total revenues. Of this $4.5 billion, instrument placements accounted for 17% of total revenues, or about $750 million, in 2021. Illumina, as a razor-razorblade business, further converts instrument placements into consumables sales. Consumables represented 71% of total 2021 revenues, or $3.2 billion, reflecting strong usage over the installed base during the year. On top of their instrument and consumable business, Illumina also recently announced the acquisition of GRAIL, a leading liquid biopsy MCED service company, a transaction pending regulatory review. GRAIL was previously spun out from Illumina in 2016. Although GRAIL did

[14] Illumina, "Illumina Introduces the NovaSeq Series-a New Architecture Designed to Usher in the $100 Genome," Illumina Introduces the NovaSeq Series-a New Architecture Designed to Usher in the $100 Genome | Business Wire, January 9, 2017; "Illumina Sequencing Pricing," Illumina Sequencing Pricing - | University of Utah, May 21, 2021, https://uofuhealth.utah.edu/huntsman/shared-resources/gba/htg/pricing/.

not meaningfully contribute revenues in 2021, MCED is one of the most exciting developments in oncology diagnostics, and it has the potential to change the patient care landscape.

ILMN Revenue Breakdown

3/16/2022

Year	Instrument Placements	Instrument Revenues	Consumable Revenue	Service and Other Revenue
2018	2615	$ 572.0	$ 2,177.0	$ 584.0
2019	1763	$ 537.0	$ 2,392.0	$ 614.0
2020	2050	$ 431.0	$ 2,304.0	$ 504.0
2021	3239	$ 751.0	$ 3,217.0	$ 558.0

Source: Illumina IR

BGI: China-Based Cost Competitor

BGI Group is a key competitor to Illumina, with operations primarily outside of the United States. Formerly known as the Beijing Genomics Institute, BGI was a contributor to the Human Genome Project and continues to be active in genetic research along with their commercial activities. BGI sequencers are marketed under the MGI subsidiary with three major products: the mid-throughput DNBSEQ-G50, the high-throughput DNBSEQ-G400, and the ultra-high-throughput DNBSEQ-T7. BGI Group's full financial situation is unclear, with only BGI Genomics, a sequencing services provider, publicly listed. BGI has had trouble getting its sequencing products into the United States, particularly because of ongoing patent disputes with Illumina that have locked them out of the market until at least 2023. BGI has also come under fire for its close ties to the Chinese government, with recent accusations that the company shared NIPT testing data with government agencies without patient permission. Nevertheless, BGI is

able to be highly competitive on cost, which poses a serious threat to Illumina's current dominance once it is able to break into the Western markets.

Oxford Nanopore: Exploratory Long Read Technology with Immense Promise

Oxford Nanopore boasts one of the most differentiated sequencing technologies commercially available today. Far different from the predominant SBS chemistry, nanopore sequencing makes use of a protein nanopore through which DNA strands are passed base-by-base to deliver data in real-time. Originally spun out of the University of Oxford in 2005, Nanopore's technology has been available since the introduction of the MinION in 2014, but the company only publicly listed in London in late 2021. Nanopore technology specializes in sequencing ultralong-length DNA, but research papers have shown that Nanopore also has the ability to sequence much shorter fragments.[15] Other unique advantages to the technology are its speed and native (or unamplified) reads, which have allowed it to establish the current official world record time for genome sequencing. However, the DNA input requirement is also quite high; Nanopore has product enhancements in its pipeline that will attempt to address this issue, including some that make use of DNA amplification more commonly seen in SBS-based systems. Long read as a segment has had trouble with adoption in clinical practice given that the current technologies suffer from higher error rates and their much higher costs, which are vital to adoption in clinical practice. Nanopore, for example, has kits that enable up to Q50 coverage, or 99.999% accuracy, but real-world data suggests much lower average accuracy.[16] Nanopore technology also struggles with indels, which occur when a base is missing from the sequence,

[15] Roberts, H.E., Lopopolo, M., Pagnamenta, A.T. *et al.* Short and long-read genome sequencing methodologies for somatic variant detection; genomic analysis of a patient with diffuse large B-cell lymphoma. *Sci Rep* **11,** 6408 (2021). https://doi.org/10.1038/s41598-021-85354-8

[16] Weirather, J. L., de Cesare, M., Wang, Y., Piazza, P., Sebastiano, V., Wang, X. J., Buck, D., & Au, K. F. (2017). Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. *F1000Research*, *6*, 100. https://doi.org/10.12688/f1000research.10571.2

and other systematic, non-random errors when assembling the DNA sequences. Despite difficulty penetrating the clinical side, long read has found success in research, with substantial presence in academic laboratories and government-sponsored sequencing projects. Oxford Nanopore upgraded its 2021 guidance more than once, due in part to a population sequencing project in Abu Dhabi that contributed meaningful revenue late in the year. Nanopore has also benefitted from its range of smaller, more portable product offerings, including the MinION and GridION. Nanopore has emphasized the convenience aspect of these instruments, with tablet and smartphone compatibility in their pipeline. On top of these research-oriented portable instruments, Nanopore offers the high-throughput PromethION instrument, which accommodates the most complex applications. Apart from the standard razor-razorblade business model for instruments, Nanopore also offers so-called "starter packs," which allow customers to rent a sequencer and pay a different price for the consumables. This service model helps expose additional customers to the technology without the potentially burdensome upfront cost of purchasing a sequencer, and it may ultimately drive conversion towards instrument purchases. Nanopore instrument cost ranges from around $1,500 for the most basic Flongle to around $286,000 for the most high powered PromethION. Consumable costs vary depending on the instrument, and can range from $90 per flow cell to more than $600 per flow cell. Stated cost per Gb of data generated ranges widely, though PromethION is estimated to be able to generate 1 Gb of data for around $10.

PacBio: More Accurate Long Reads at a Cost

PacBio is the other major long read competitor, albeit with chemistry more closely aligned with short read technologies. Unlike Nanopore, the original PacBio technology had a strict focus on long read sequencing, but the company recently acquired the short read firm

Omniome and now plans on integrating that technology into an "all-in-one" sequencer package. As a long read technology, PacBio sequencers are subject to many of the same pros and cons as Oxford Nanopore's. Two key differences (apart from the chemistry) are the speed and error rate. PacBio reads are slower than Nanopore reads, but make up for it with a much lower error rate. PacBio used to be known for having a much higher error rate, but the introduction of Circular Consensus Sequencing (CCS) protocol, which sequences the same DNA strand multiple times to achieve a "consensus sequence," meaningfully improved error rates, and is now the hallmark of their HiFi sequencing, with consistent Q30 coverage, or 99.9% accuracy. On the cost front, PacBio primarily ships the Sequel IIe system, which has a list price of about $495,000 but an ASP of closer to $400,000. Consumables for the system typically cost about $1,300, though bulk buying will yield better per unit pricing. At a cost per Gb level, Sequel IIe is able to deliver 1 Gb of data for under $30. Despite a lower raw error rate compared to Nanopore and continued iteration on both the technology front and the cost front, PacBio's technology has not yet gotten to a point where it is viable for current clinical applications. Current PacBio systems allow for the sequencing of a few hundred complete genomes per year, while clinical applications would need the capacity to run more than a few thousand complete genomes per year. Management has said that will be a key focus moving forward.

Exact Sciences: CRC Screening First Mover Looking Over its Shoulder

Exact Sciences was an early entrant in ECD with its Cologuard fecal screen for CRC, with a recommended re-testing interval of three years. While case control data, or data collected from predetermined disease and non-disease groups, were very strong with 98% sensitivity, 90% specificity, and 57% sensitivity to precancerous lesions, the full Deep-C trial readout suffered from some data degradation, ending with 92% sensitivity, 87% specificity, and 42% sensitivity

to precancerous lesions.[17] Still, the data were promising enough to warrant FDA approval and inclusion into clinical guidelines. Exact is now working on their improved Cologuard 2.0, which had a recent data readout at ASCO GI showing 95.2% sensitivity, 92.4% specificity, and 57.2% sensitivity for precancerous lesions.[18] The most notable statistic was 83.3% sensitivity for high-grade dysplasia, the most dangerous precancerous lesion. While these performance improvements are meaningful, Exact will still have to address their most pressing issue — lack of patient compliance — which has prevented increasing adoption. This gap in compliance represents an opportunity for next-gen technology, namely LBx, to significantly drive up screening rates for CRC. In response, Exact has diversified its screening portfolio and even plans on running a liquid biopsy sub-study during its Blue-C trial for Cologuard 2.0. The market competitiveness of different CRC screening methods will depend on both cost and patient compliance. Cologuard has a substantially lower list price compared to colonoscopy ($600 vs. $2,200) with full Medicare coverage and higher patient compliance, which helped it take initial market share in the space.[19] LBx methods have yet to be approved, so their ability to compete on cost is to be determined. On top of their CRC-centric products, Exact acquired Thrive in late 2020 for more than $2 billion. Thrive's primary product is CancerSEEK, a LBx MCED test that has already completed a large-scale trial, positioning Exact well for potential high growth in the future.

[17] "Exact Sciences' Deep-C Study Results Published in New England Journal of Medicine," Exact Sciences Corporation, accessed March 16, 2022, https://investor.exactsciences.com/investor-relations/press-releases/press-release-details/2014/Exact-Sciences-DeeP-C-Study-Results-Published-in-New-England-Journal-of-Medicine/default.aspx.

[18] "Exact Sciences Presents Data Showing Improved Accuracy of Second-Generation Cologuard® Test and Progress toward an Even Better Colorectal Cancer Screening Solution for Patients," Exact Sciences Corporation, accessed March 16, 2022, https://investor.exactsciences.com/investor-relations/press-releases/press-release-details/2022/Exact-Sciences-Presents-Data-Showing-Improved-Accuracy-of-Second-generation-Cologuard-Test-and-Progress-Toward-an-Even-Better-Colorectal-Cancer-Screening-Solution-for-Patients/default.aspx.

[19] "Digestive Diseases," Mayo Clinic (Mayo Foundation for Medical Education and Research), accessed March 16, 2022, https://www.mayoclinic.org/medical-professionals/digestive-diseases/news/cologuard-primed-to-change-landscape-of-crc-screening/mac-20429632.

Natera: Reproductive Health Powerhouse Expanding Horizontally

Natera has established a technology platform based on cell-free DNA (cfDNA), or DNA that circulates in the bloodstream. While Natera initially started their product portfolio in NIPT and carrier screening (84% of product revenues in 2021), they have since built out product pipelines in organ health and oncology (3% and 13% of product revenues respectively in 2021). Natera today is one of the leaders in NIPT in the United States, and according to their estimates, likely accounts for ~40% of all domestic NIPT testing.[20] Pricing for these tests is not particularly transparent. Average selling price (ASP) for NIPT and carrier screening seems to be around $310 and $410 respectively, though the price to consumers meaningfully increases in non-reimbursed segments since original list prices for these tests are in the thousands of dollars. Natera has also made significant strides in oncology with their Signatera MRD test. The Signatera assay was a first-mover in MRD, and recently secured reimbursement at $3,500 per test from MolDX, the administrator for Medicare, for recurrence monitoring purposes.[21] MRD remains a complicated field, with usage in very niche areas at the moment, and a variety of competing approaches. Natera's tumor-informed approach gives each patient a custom panel of genes, but requires tumor tissue to be sequenced first in order to find the appropriate gene mutations. Clinicians still need to see more large-scale trials run for MRD to assess its clinical benefit, and companies in the field continue to generate validation data to try to expand into additional indications. Natera has also mentioned plans to move into ECD, and they have accordingly signed a partnership with Aarhus University in Denmark to gain access to a large biobank, which will allow them to begin running samples to validate their assay.

[20] Natera IR

[21] "Moldx: Minimal Residual Disease Testing for Cancer," CMS.gov Centers for Medicare & Medicaid Services, accessed March 16, 2022, https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=38779&ver=4&keyword=minimal+residu&keywordType=starts&areaId=all&docType=F%2CP&contractOption=all&sortBy=relevance&bc=1.

Guardant Health: Oncology Franchise Positioning into Screening

Guardant Health, a company associated to two former members of the Stanford Genome Technology Center, is a leading LBx company primarily involved in oncology. Guardant's portfolio is anchored by their Guardant360 lineup of CDx products, which covers all solid cancers and all 55 genes recommended by the National Comprehensive Cancer Network (NCCN). Guardant360 previously received CMS's ADLT designation which reimbursed the test at $5,000 and is expected to receive final coverage pricing from CMS in early 2022.[22] Apart from their CDx products, Guardant is also heavily involved in MRD, where their REVEAL assay competes head-to-head with Natera's Signatera assay at the same $3,500 price point, as well as in ECD, where their SHIELD test has a first-mover advantage in LBx screening. Guardant's REVEAL MRD assay takes a simpler tumor-naïve approach, which is a very large panel with all of the most common genes to be tested. Guardant's ECLIPSE trial for CRC screening, the first large-scale prospective trial for LBx screening, is slated to read out mid-2022. Clinicians have suggested that liquid biopsy would meaningfully impact screening rates given a successful trial, and Guardant is possibly best-positioned to take advantage of the still very underpenetrated screening market.

New Horizon Health: China Market Leader in Self-Sampling Screening

New Horizon Health is the first cancer screening company to be listed in Hong Kong. NHH's primary products include their stool-sampling Coloclear NGS screening test, the self-sampling Pupu Tube FIT test also for CRC, and the recently-introduced self-sampling UU Tube

[22] "Guardant Health Receives ADLT Status from CMS for FDA-Approved guardant360® Cdx Test," Guardant Health, Inc. - Guardant Health Receives ADLT Status from CMS for FDA-Approved Guardant360® CDx Test, accessed March 16, 2022, https://investors.guardanthealth.com/press-releases/press-releases/2021/Guardant-Health-Receives-ADLT-Status-from-CMS-for-FDA-Approved-Guardant360-CDx-Test/default.aspx.

screen for H. Pylori. New Horizon was the first cancer screening company to be listed on the Hong Kong stock exchange, and is a market leader in China.

Burning Rock: Moving China Towards MCED

Burning Rock Biotech is a China-based US-listed ADR with a broad oncology-based product pipeline. Their current keystone product line of MCED tests is still in development, with readouts beginning later in 2022. Management has also indicated ongoing R&D efforts in MRD. In addition to the longer-term pipeline, Burning Rock also has a range of CDx products currently marketed in China as laboratory developed tests (LDTs), though the company is trying to transition volume away from their central lab into in-hospital sequencing facilities.

Genetron: Liver Cancer Focus Moving to New Indications

Genetron is another China-based US-listed ADR, with a pipeline focused on liver cancer. Their cornerstone product is HCCScreen, an NGS-based liver cancer screening test that has had a significant amount of data generated already and is currently offered as an LDT in China. On top of their core product, Genetron also recently announced a PCR-based assay also targeting liver cancer called HCCScan, which is intended to take advantage of excess PCR capacity in Chinese hospitals post-COVID. Other diagnostic pipeline assets include a MRD product for various solid tumors that may have some data towards the end of 2022. Another key contributor to revenues is the S5 sequencer, which is a licensed and rebranded version of Thermo Fisher's Ion GeneStudio S5 sequencer.

Conclusion

Based on the discussion above, we believe that NGS as a whole is well-positioned to experience substantial growth in the near- and long-term. In particular, a representative group of more than 20 companies across smid- and large-caps grew their revenues an estimated 28% in 2021 (see table on next page). We think that they will continue to see more than 20% growth in 2022 with possibility for even more upside, which should continue on for some time as NGS applications open up extremely underpenetrated markets like cancer screening. Most of the companies in this representative group are still losing money. Profits are dominated by Illumina, the dominant sequencer provider, and by "tools companies" Thermo-Fisher, Danaher, Qiagen and Sartorius Stedim. Excluding these five participants, the remaining 15 or so lost an estimated aggregate of $3.0 billion in 2021. In addition to ongoing R&D expenses in the aggregate for the remaining 15 or so of about $2.2 billion, these same companies also spent a considerable $4.4 billion in SG&A due to commercial buildout as their products begin to make their way into the broader market. Altogether, these two operating expenses of $6.6 billion were more than 2x the net losses they sustained over the course of the year. These are promising signs for corporate performance as spending begins to level off with the appropriate infrastructures built out, and high-growth end markets in sight.

24 Selected NGS Companies

Revenues (USD, millions)	Q4 2020	FY 2020	Q1 2021	Q2 2021	H1 2021	Q3 2021	Q4 2021	H2 2021	FY 2021	Revenue Growth Q/Q	Revenue Growth Y/Y	Market Cap 3/7/22
Genomics												
EXAS	$466	$1,491	$402	$435	$837	$456	$474	$930	$1,767	2%	18%	$12,504
GH	$78	$287	$79	$92	$171	$95	$108	$203	$374	38%	30%	$5,551
NTRA	$112	$391	$152	$142	$294	$158	$173	$331	$625	54%	60%	$5,021
TWST	$32	$90	$28	$31	$59	$35	$38	$73	$132	17%	47%	$2,682
NVTA	$100	$280	$104	$116	$220	$114	$126	$241	$460	26%	65%	$1,825
ADPT	$30	$98	$38	$39	$77	$40	$38	$77	$154	25%	57%	$1,662
PSNL	$20	$79	$21	$22	$43	$22	$21	$43	$86	2%	9%	$405
VCYT	$35	$118	$37	$55	$92	$60	$67	$128	$220	95%	87%	$1,769
CSTL	$17	$63	$23	$23	$46	$24	$25	$49	$94	45%	50%	$1,050
GTH*	$21	$67	$14	$22	$36	$24	$25	$48	$84	15%	25%	$245
BNR*	$21	$68	$16	$20	$36	$20	$24	$43	$79	13%	16%	$757
Subtotal	$934	$3,032	$914	$996	$1,910	$1,048	$1,118	$2,166	$4,076	20%	**34%**	$33,471
Large Cap Tools												
TMO	$10,550	$32,218	$9,906	$9,273	$19,179	$9,330	$10,702	$20,032	$39,211	1%	22%	$208,713
DHR	$6,760	$22,284	$6,858	$7,218	$14,076	$7,229	$8,148	$15,377	$29,453	21%	32%	$187,673
DIM FP	$633	$2,181	$790	$840	$1,629	$892	$890	$1,782	$3,411	41%	56%	$31,357
QGEN	$571	$1,870	$567	$567	$1,135	$535	$582	$1,117	$2,252	2%	20%	$9,902
Subtotal	$18,514	$58,553	$18,121	$17,898	$36,019	$17,986	$20,322	$38,308	$74,327	10%	**27%**	$437,644
Proteomics												
TXG	$112	$299	$106	$116	$222	$125	$144	$269	$490	28%	64%	$7,762
QTRX	$26	$87	$27	$25	$53	$28	$30	$58	$111	16%	28%	$1,021
SEER	$0	$1	$0	$1	$1	$2	$3	$5	$7	933%	857%	$814
OLK*		$54							$95		76%	$2,406
BLI	$22	$64	$19	$19	$38	$24	$23	$48	$85	7%	33%	$399
Subtotal	$160	$504	$152	$162	$314	$180	$200	$380	$788	25%	**56%**	$12,402
NGS Sequencers												
ILMN	$953	$3,239	$1,093	$1,126	$2,219	$1,108	$1,198	$2,306	$4,525	26%	40%	$49,937
PACB	$27	$79	$29	$31	$60	$35	$36	$71	$131	33%	65%	$2,320
OMIC	$0	$0	$0	$0	$0	$0	$0	$0	$0		0%	$559
ONT LN*		$149			$77			$88	$165		11%	$4,227
Subtotal	$980	$3,467	$1,122	$1,157	$2,356	$1,143	$1,234	$2,465	$4,821	26%	**39%**	$57,043
Grand total	$20,589	$65,556	$20,309	$20,212	$40,598	$20,356	$22,874	$43,318	$84,011	11%	**28%**	$540,559

*based on guidance/estimates

**exchange rates used as of 3.9.22

Private Companies

AnchorDx

Freenome

Qitan Diagnostics

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