Alix

VENTURES

Market Deep Dive Report

DNA Writing

August 2020

Contents

1. Summary **3**

2. Market Overview **3**

2.1 Major Stakeholders 4

2.3 Pitchbook Market Statistics: Gene Synthesis 5

3. Technology Overview **6**

3.1 DNA Writing in Synthetic Biology 6

3.2 Phosphoramidite Chemistry - Traditional DNA Synthesis 7

3.3 Microchip-based DNA Synthesis 8

3.4 Oligonucleotide Assembly - Gene Synthesis 9

3.5 Late Stage Privates & Publics 10

4. Key Trends, & Future Development **12**

4.1 Key Trends 12

4.2 Future Developments 14

5. Opportunities **15**

5.1 Emerging Technologies 15

5.2 Emerging Applications 16

5.3 Startups to Watch 16

5.4 Industry Challenges 18

6. Conclusions **19**

6.1 Vertical Strengths 19

6.2 Vertical Weaknesses 19

6.3 Opportunity Cost of Capital 19

6.4 Investment Theses 20

7. References & Further Reading **21**

1. Summary

DNA writing technology has become a crucial requirement for research in synthetic biology and biology more broadly. It is an enabler for functions such as heterologous gene expression, vaccine development, gene and cell therapies, molecular engineering, and cloning. These tools are applicable across a wide variety of industries including drug discovery, pharmaceuticals, food and agriculture, chemicals, and energy production. Despite their prevalence in modern biology research, there has been little to no innovation in oligonucleotide synthesis methods in particular. Time consuming chemical synthesis methods have dominated the market and are limited in terms of cost, speed, and accuracy, particularly for larger sequences of DNA. Instead of performing multi-step chemical oligo synthesis in-house, researchers have turned to oligonucleotide and gene synthesis service companies who synthesize and ship DNA to labs according to their specifications. Turnaround time is a major bottleneck for synthetic biology, as it may take 1-4 weeks to receive DNA from one of said services companies. Turnaround time typically increases with DNA length and complexity.

Recent advancements in DNA reading and editing have resulted in immense growth of the synthetic biology and gene therapy markets. Advancements in DNA writing, particularly in oligonucleotide synthesis where innovation has been most lacking, would be similarly transformational for synthetic biology research. Faster, less expensive, more accurate synthesis would dramatically increase the rate at which researchers can design, build, test, and iterate synthetic biology systems and enable previously inaccessible applications including DNA storage, DNA origami, and gene therapy. Emerging technologies including microfluidic-based and enzymatic-based synthesis may pave the way for these advancements. Recently, early-stage startups developing these technologies are departing from the traditional biomanufacturing-as-a-service business models of current major players in the space. Instead, these companies are focusing on the development of desktop platforms to automate synthesis and assembly in labs. These technologies could transform the industry by revolutionizing the speed at which research can occur.

Overall, this report finds the DNA writing space an attractive market for investment given that new companies can develop novel synthesis technologies that not only rival but triumph against traditional synthesis methods in terms of cost, accuracy, and speed. These enhanced capabilities will incite further growth in the market by enabling research in a multitude of industries and applications.

2. Market Overview

The global synthetic biology market was valued at $5.2 billion in 2019 and is projected to grow to $18.9 billion by 2025 at a CAGR of 28.8%. Growth in this market is largely attributed to increased R&D funding for several applications of synthetic biology including but not limited to

drug discovery, pharmaceuticals, cell and gene therapies, agriculture, chemicals, and energy production. The vast majority of capital invested in the synthetic biology market, that is 97.56% of funding, goes to companies in the medical and healthcare sectors, particularly for those enabling genome engineering and gene therapies. Recent advancements in DNA sequencing and editing, most notably in terms of the CRISPR toolbox, have led to an increased demand for synthetic DNA, synthetic RNA, and synthetic genes. Following these developments, in 2019, oligonucleotides (oligos) and synthetic DNA accounted for the largest share of the market (in 2014, the global market for oligos was $241 million, and the global market for genes was $137 million). Despite this, high cost of biomanufacturing has been a limited factor for growth in this sector.

2.1 Major Stakeholders

Synthetic DNA and RNA are required for nearly ubiquitous laboratory tasks including PCR, Real-Time PCR, DNA sequencing, site directed mutagenesis, single-nucleotide polymorphism assays, and microarrays. DNA writing technologies have become essential to the workflows of many biology researchers to enable such functions as heterologous gene expression, vaccine development, gene and cell therapies, molecular engineering, and cloning. For cloning applications, the use of synthetic genes for transfer from one organism to another compared to the use of natural genes is often less prone to error, particularly for sequences of large lengths. In addition, the use of synthetic genes opens up new opportunities for researchers to study sequences that do not exist already in nature, or perhaps exist only in different forms. These capabilities are especially useful for development of diagnostic tools as well as drug discovery and development. Large biotech companies including Ginkgo Bioworks, Zymergen, and Gigagen rely heavily on DNA synthesis technologies.

In lieu of performing time consuming DNA cloning and assembly functions in house, many researchers in academic labs, pharmaceutical companies, and biotech companies have turned to biomanufacturing-as-a-service companies as sources of synthetic DNA. These commercial service alternatives are typically more cost-effective, more accurate, and have shorter turnaround times which range from 7 - 28 days depending on the gene length and sequence. Some of the major players that operate in the gene synthesis services space are Integrated DNA Technologies, Twist Biosciences, Codex, Biomatik, and GeneWiz. Contract Research Organizations (CROs) such as Eurofins and GenScript are also large providers of gene synthesis technologies, however gene synthesis tends not to be their core business. In addition to gene synthesis, these vendors offer reagent services, antibody drug development services, clinical diagnostic testing services, and other biological products.

There are few do-it-yourself gene synthesis technologies on the market, however due to high cost of equipment and comparable turnaround time, the lab-as-a-service model is more popular. Codex, formerly known as SGI-DNA, the synthesis division of Synthetic Genomics, is one of such companies that offers a benchtop DNA printer (BioXp 3200), an automated

platform for building libraries, fragments, and clones. Additionally, they offer gene synthesis services and DNA assembly reagents which are useful for researchers performing procedures with complex DNA assembly steps. These hybrid companies support many customers across a variety of industries including pharma, industrial chemicals, agricultural biology, and academic research.

2.3 Pitchbook Market Statistics: Gene Synthesis

- **Quick stats**
    - No. Companies: 26
    - No. Deals: 74
    - No. Investors: 80
    - No. Exits: 15
    - Largest deal: $450 M (GeneWiz, Merger/Acquisition)
- Deal count (TTM): 5
- Most active VCs by deal count: Kleiner Perkins, Alloy Ventures, Civilization Ventures, Flagship Pioneering, HBM Partners, Khosla

Recent Deals

Company Name	Deal Size (M)	Deal Date	Deal Type	Investors
Ansa Biotechnologies		01-Jan-2020	Early Stage VC	Fifty Years
Ansa Biotechnologies	4.73	09-Jul-2020	Seed Round	
Blue Heron Biotechnology		06-Aug-2019	Merger/Acquisition	Eurofins Genomics
Evonetix	29.81	02-Mar-2020	Early Stage VC	Cambridge Consultants (Raymond Edgson), Civilization Ventures, Data Collective (Armen Vidian), Draper Esprit (LON: GROW) (Vishal Gulati), Foresite Capital Management (James Tananbaum), Morningside Group, Providence Investment Company Limited, Rising Tide Fund
SynHelix		10-Dec-2019	Early Stage VC	Advent France Biotechnology(Alain Huriez)

3. Technology Overview

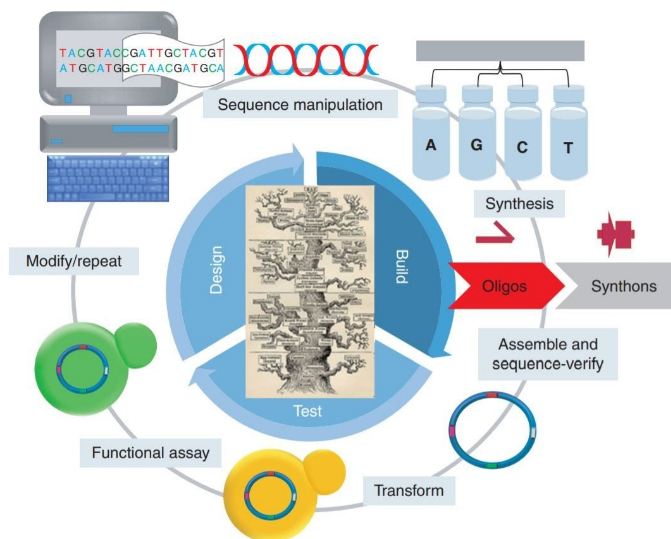
3.1 DNA Writing in Synthetic Biology

Across academic and industrial applications the standard synthetic biology workflow remains the same. First, researchers identify genetic sequences of interest and design a biological circuit to accomplish their desired task, typically through the use of computer software. The resulting constructs are often divided into smaller segments called synthons which are easier to synthesize. Synthons are assembled into larger DNA assemblies and then cloned into an expression vector. Once these vectors have been sequence-verified to ensure the introduction of the desired sequences, they can be transformed into a cell. Finally, these engineered cells are assayed for function. Oftentimes, this cycle requires many iterations before the transformation produces the desired result.

Beginning with oligonucleotide synthesis, DNA writing is a critical component of the synthetic biology test cycle. Oligonucleotides are short nucleic acid polymers, typically composed of 13-25 nucleotides, which are the building blocks of genes, longer pieces of DNA ranging from 250 to 2000 bp. The costs associated with gene synthesis are traditionally attributed to the reagents required, namely the oligonucleotides components and the enzymes used to bind them together in the correct sequence. As such, the cost of gene synthesis is tied to the cost of oligonucleotide synthesis. However, the longer the desired piece of DNA, the more difficult it is to synthesize accurately. The central goal of the DNA synthesis technology is to develop techniques that are ever cheaper, faster, and more accurate for longer sequences.

Design Considerations for DNA Writing Platforms

- Decrease turnaround time
- Decrease cost
- Increase yield
- Increase accuracy
- Increase or retain accuracy for longer sequences
- Increase or retain accuracy for longer assemblies
- Allow for corrections to be made if necessary
- Reduce reagent consumption
- Improve robustness
- Increase throughput and ensure fidelity

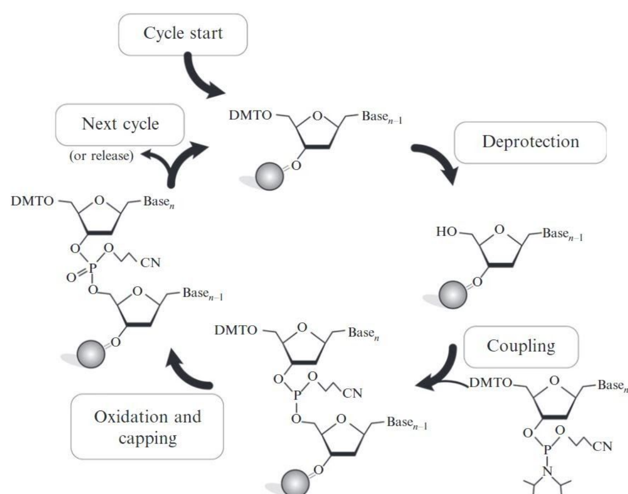


Synthetic Biology Workflow

3.2 Phosphoramidite Chemistry - Traditional DNA Synthesis

Since its advent in the 1980s, phosphoramidite synthesis, also known as solid-phase synthesis, has been the standard. For this method, activated DNA nucleoside monomers known as phosphoramidites serve as the building blocks for synthesis. To begin, a solid support matrix made from controlled pore glass (CPG) or polystyrene (PS) beads is packaged into a flow-through column with necessary reagents. The oligonucleotide chain is formed in the 3'-5' direction by a 4 step process (shown below) which includes deprotection, coupling, capping, and oxidation. The cycle is repeated for each nucleotide in the sequence. Once completed, the oligonucleotide is cleaved from the solid support, detritylation, and deprotected. The end result is a single-stranded DNA molecule. With the solid-phase method, each oligonucleotide is synthesized individually in a separate column or well. For more information about synthesis chemistry, see here.

Oligonucleotides up to about 100 nt in length can be synthesized using the solid-phase method with 99% coupling efficiency and error rates 0.5% or below. At this length, phosphoramidite synthesis produces high fidelity products with high accuracy and high yield (pmol scale). However, for oligonucleotides 150 nt or greater, synthesis becomes highly problematic. Both yield and accuracy decrease with increasing oligonucleotide length. In fact, IDT, one of the largest suppliers of oligonucleotides, recommends further purification for oligos greater than 40 bases and does not guarantee efficiency for oligos greater than 100 bases. The phosphoramidite method is associated with higher costs and is thus most applicable for the synthesis of small DNA products which require high accuracy. Additional challenges associated with phosphoramidite synthesis are the production of toxic by-products and the requirement of post-synthesis purification and processing steps.



Phosphoramidite Synthesis Cycle

3.3 Microchip-based DNA Synthesis

Microchip-based synthesis technologies are becoming more commonplace alternatives to the phosphoramidite method. They make use of light-directed synthesis on microarrays or microfluidics to selectively deprotect photolabile nucleoside phosphoramidites. Light-based chemistries can be controlled in such a way to allow for parallel synthesis of thousands of strands on a single microchip. Light can be directed by a number of methods which include photolithography masking, digital photolithography, ink-jet printing-based techniques, and micromirror control. Despite slight variations in outcome based on the chosen modality, in general microchip-based synthesis methods are advantageous to solid-phase synthesis in terms of cost and multiplexing capabilities. The cost of these array-based platforms ranges from $0.00001 to $0.001 per nucleotide, because less reagent is required than for phosphoramidite chemistry. With microchip-based synthesis, oligonucleotides up to 200 bt in length can be synthesized which further adds to the appeal of these methods.

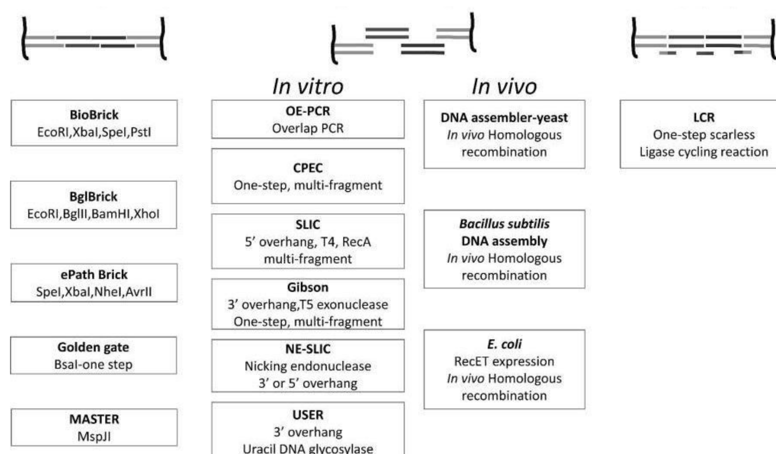
The core limitations associated with microchip-based synthesis technologies are accuracy and yield. The ability to synthesize oligonucleotides in parallel allows for up to 1,000,000 different sequences to be produced per chip. However, the yield for any one specific sequence tends to be 2 to 4 orders of magnitude lower than that resulting from solid-phase methods (typically only 10^7 to 10^8 molecules per sequence). Yield at this scale may be insufficient for gene assembly reactions; as such PCR is required for amplification. These oligos must also be cleaved off the chip in a single mixture, which adds to the complexity of possible interactions occurring within the mixture. Furthermore, the oligos synthesized tend to be relatively low quality as errors in synthesis are more common. Optimizing the parameters of the reaction can be effective in improving the quality of DNA product, however, continued improvement in microchip design, reagents, and procedures will be required for high fidelity, low-cost oligonucleotide synthesis for longer sequences.

3.4 Oligonucleotide Assembly - Gene Synthesis

Once single stranded oligonucleotides (60-100 nt in length) have been successfully synthesized, they can be assembled into larger, double stranded DNA constructs called synthons (200-2000 bp in length). In accordance with the synthetic biology workflow described above, these synthons are then sequence verified and can be further assembled to form even larger constructs. The longest piece of DNA synthesized in the lab was a 582,970 base pair M. genitalium bacterial genome composed of many commercially available DNA fragments. Early oligonucleotide assembly methods involved the use of thermostable ligases to bind oligonucleotide fragments together. Usually accompanied by a single PCR step, ligation-mediated assembly allows for ligation of longer oligonucleotides with few errors and high cost savings.

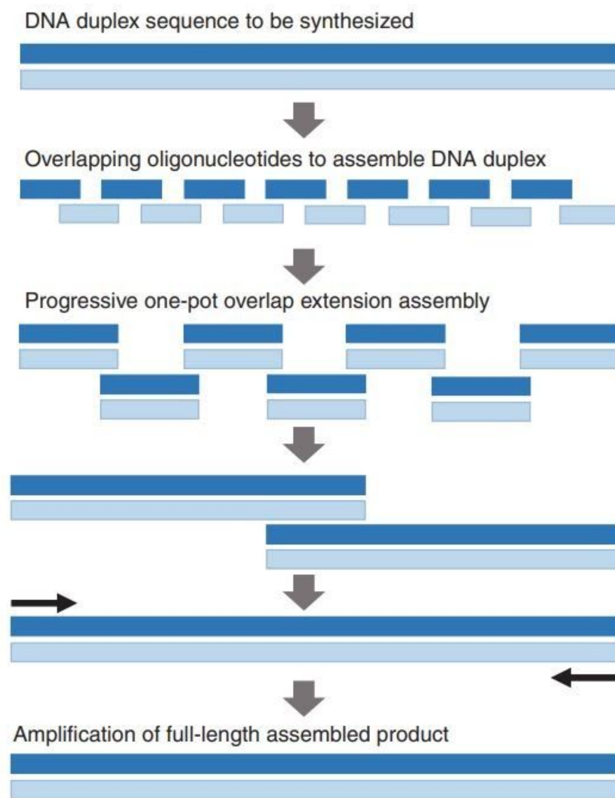
Today, the most common forms of DNA assembly are PCR-based methods which can be accomplished as "one pot", or single-step interactions. These methods make use of thermostable DNA polymerase to stitch together oligonucleotides designed and synthesized to contain complementary overlapping sequences. Of these methods, polymerase cycle assembly (PCA) and its variations are popular for synthon assembly. For PCA, the desired double stranded DNA is divided into smaller oligonucleotide fragments between 60 and 120 nt which contain 15 to 25 nt overlaps with adjacent fragments. Polymerase is used to extend these fragments into the full length construct which is used as a template for a second PCR reaction. The end result is amplification of only the full length, desired sequence and the excess PCR primer by-product which can be removed by gel purification.

In stark contrast to oligo synthesis methods, many novel forms of DNA assembly are continually being developed. In fact, as of 2015, 15 distinct assembly methods had emerged as standards which make use of restriction enzymes, recombinases, ligases, and long overlaps. A diagram of popular DNA assembly methods is shown below.



DNA Assembly Methods

Although there are many adaptations to this traditional PCA methodology, most DNA assembly techniques rely on the same core processes. The differences generally arise from how the oligonucleotide fragments or primers are designed to be assembled together. Generally, PCR-based techniques are versatile and low cost, however there are a number of challenges that may prohibit timely and accurate oligonucleotide assembly. Parameters including annealing temperature, PCR conditions, and primer concentrations must be optimized according to the oligonucleotide length, concentration, and characteristics. Oligonucleotide sequences with high GC content, secondary structures, and repetitive sequences are often more difficult to assemble using PCR-based methods. Finally, as is the case with oligonucleotide synthesis, the error rate increases with the number or oligos assembled and size of oligos used.



PCR-based Oligonucleotide Assembly

3.5 Late Stage Privates & Publics

Twist Bioscience: Twist was founded in 2013 by Emily Leproust, Bill Banyai, and Bill Peck, pioneers in high-throughput DNA synthesis due to their development of innovative silicon microchip technology. The company raised $369.16M before it IPOed in 2018 (current market cap: 2.49B). Twist is a well-known manufacturer of high quality, high fidelity synthetic DNA that fits into the biomanufacturing-as-a-service model.

- Location: San Francisco, CA
- Synthesis Method: Semiconductor-based silicon microchip technology; synthesis and assembly occurs on a single chip
- Price: starting at $0.07 per base
- Maximum Gene length: 1800 bp
- Turnaround time: 7 - 10 days

Integrated DNA Technologies (IDT): IDT was founded in 1987 and is currently one of the largest suppliers of nucleic acid products. In addition to manufacturing custom oligonucleotides for customers, it has developed proprietary technologies for next generation sequencing, targeted sequencing, genotyping, and library preparation for applications such as cancer research and screening, genotyping, and synthetic biology. The company raised $133M before it was acquired by Danaher in April 2018.

- Location: Skokie, IL
- Synthesis Method: Chemical synthesis
- Price: $0.17 per base
- Maximum Gene Length: 3,000 bp
- Turnaround time: 10-15 days

GenScript: GenScript was founded in 2002 and is the largest global provider of gene synthesis with 25% market share. Additionally, the company owns Legend Biotech, a multinational biopharmaceutical company developing cell-based therapies (currently focused on CAR-T in multiple myeloma). Before it IPOed in 2015 (current market cap: $32.21B), GenScript raised $165 M.

- Location: Piscataway, NJ
- Synthesis Method: Chemical synthesis
- Price: starting at $0.23 per base
- Length: up to 8,000 bp
- Turnaround time: 4 (rush synthesis, small gene) - 25 days

GENEWIZ: GENEWIZ was founded in 1999 and was acquired in 2018 by Brooks Automation for $450M. In addition to gene synthesis services, GENEWIZ offers services for next generation sequencing, plasmid preparation, molecular genetics, cloning, and clinical services. The company supports research in adeno-associated virus (AAV) services for gene delivery, cancer, food and agriculture, infectious disease, synthetic biology, genomics, and genetic engineering.

- Location: South Plainfield, NJ
- Price: $0.21 per base pair
- Length: up to 10,000 bp
- Turnaround time: 8-40 business days

Agilent: Agilent is a leading life science research and development company that spun out of Hewlett-Packard in 1999. It's current market cap is $29.74 B. Agilent supports customers across a variety of industries including diagnostics, genomics, biopharmaceuticals, chemicals, energy, and agriculture. Agilent provides custom oligonucleotide libraries and manufacturing services to early and late stage life science companies.

- Location: Santa Clara, CA
- Synthesis Method: SurePrint Inkjet Printer; microarray technology

Eurofins Genomics (Blue Heron): Eurofins is a Belgian life sciences company founded in 1987 that provides bioanalytical testing services for food, environment, and pharmaceutical products to clients in a variety of industries. They offer a wide range of services for genomics which include DNA & RNA oligonucleotide synthesis, DNA sequencing, next generation sequencing, gene synthesis, and genotyping. Furthermore, they plasmid preparation, mutagenesis, and cloning services. Eurofins IPOed in 1997 (current market cap: 10.475B) and has raised over $800M to date.

- Location: Luxembourg
- Synthesis Method: Chemical synthesis
- Price:
- Length: up to 10,000 bp
- Turnaround time: 6-12 days

Gen9 (acquired 2017): Gen9 was founded in 2009 by academic co-founder George Church. Gen9 developed automated BIOFAB technology that was particularly adept at high quality manufacturing of long length clonal DNA. The company raised $55.15M before being acquired by Ginkgo Bioworks in 2017 which will perform DNA synthesis in-house using Gen9's technology.

- Location: Cambridge, MA
- Synthesis Method: Microarray-based multiplex technology
- Price: $0.03 per base
- Maximum Gene Length: up to 10,000 bps

	Twist	**IDT**	**GenScript**	**GENEWIZ**	**Agilent**	**Eurofins**
Method	Silicon microchip	Chemical	Chemical	Chemical	Microarray	Chemical
Price per	$0.07	$0.17	$0.23	$0.21	-	-

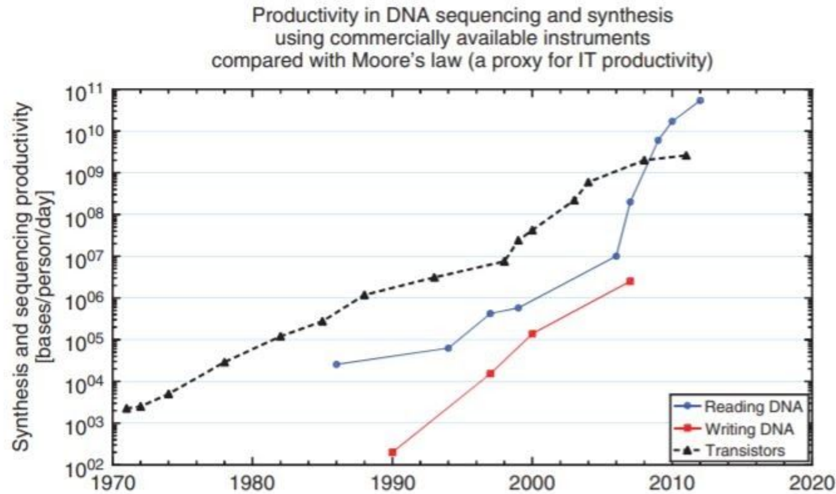
base						
Max Gene Length	1800 bp		8,000 bp	10,000 bp	-	10,000 bp
Time	7-10 days	10-15 days	4 - 25 days	8 - 40 days	-	6 - 12 days

4. Key Trends, & Future Development

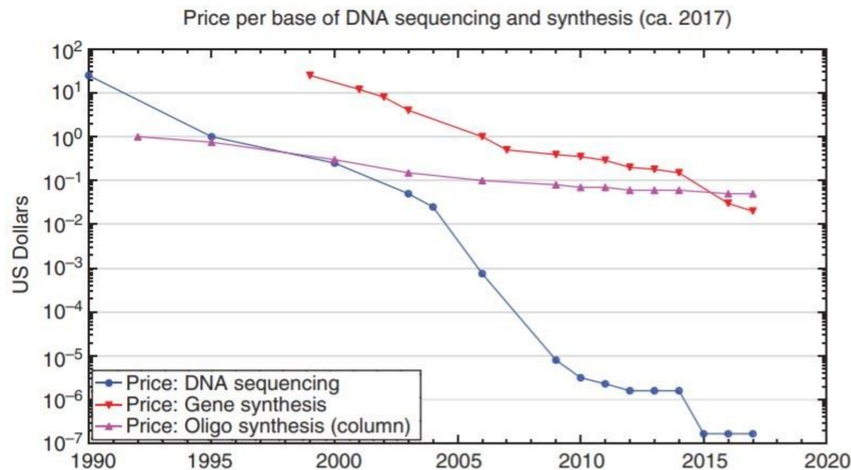
4.1 Key Trends

The origin of oligonucleotide synthesis can be traced back to 1955, when Michelson and Todd first demonstrated successful directed chemical synthesis of a dinucleotide. As discussed earlier in this paper, the advent of phosphoramidite synthesis as well PCR in the 1980s set the stage for modern chemical synthesis and assembly techniques. However, apart from these key innovations, until recently, DNA synthesis has remained rudimentary and limited in terms of cost, turnaround time, accuracy, and robustness. Instead, DNA sequencing has flourished.

Since the 1990s, DNA reading technologies have been core drivers of the synthetic biology and genomics markets. Driven by competition in terms of company and technology diversity, the cost, speed, and efficacy of sequencing instruments have improved rapidly. Analysis of cost and productivity of DNA sequencing and synthesis is commonly performed through the lens of Moore's Law, an economic observation that has become the business model for the semiconductor industry. Moore's Law predicts that the number of transistors per square inch of an integrated circuit would double every year and that the cost per component is inversely proportional to the number of transistors. For biological technologies, time is instead related to synthesis and sequencing productivity. Shown below are plots that illustrate the relationship between productivity and cost of DNA sequencing and gene synthesis as they compare to Moore's Law.



*Sequencing and synthesis compared to Moore's Law. ** synthesis data only available for column-based technologies*



DNA sequencing and synthesis cost over time

Whereas DNA sequencing technologies have improved in productivity at a growth rate that has surpassed Moore's Law, synthesis technologies have lagged behind. Furthermore, whereas the cost of DNA sequencing has decreased rapidly in accordance with Moore's Law, DNA writing has remained relatively stagnant in comparison. Notably, gene synthesis has decreased in cost at a faster rate than oligo synthesis. This can be attributed to the diversity of approaches to gene synthesis compared to a lack of commercial innovation in oligo synthesis technology where chemical synthesis prevails as the standard. This suggests that decreases in cost and increasing in productivity in the future will be linked to new technological approaches to oligo synthesis.

More recently, microchip-based DNA synthesis is becoming an increasingly prevalent method for oligonucleotide synthesis. Companies such as Gen9, Codon Devices, and Cambrian

Genomics entered the space with alternative synthesis technologies, however, all have gone bankrupt or have been acquired in recent years. Currently, Twist Bioscience is the frontrunner in the development of commercially competitive synthesis technology. In addition to its biomanufacturing-as-a-service play, Twist is exploring products for developing antibody libraries for drug development and DNA storage. Despite success in this arena, microchip-based synthesis is generally less accurate than chemical synthesis, particularly for oligo lengths greater than 1500 bps. Until further technological advances can be made, chemical synthesis will remain as the standard method for gene synthesis.

4.2 Future Developments

Emerging technological advances in DNA writing including microfluidic and enzymatic synthesis methods (discussed below) will contribute to competition in the space and drive down the cost of DNA synthesis as it did for DNA sequencing. Continued progression of this trend will be dependent on an increase in the size of the market for oligonucleotides and gene synthesis products. Therefore, synthesis technologies must be made available to customers at a price point and accessibility that is advantageous over in-house cloning techniques. In pursuit of this market, recently there have been a number of startups focusing on the development of desktop synthesizers comparable conceptually to Illumina's DNA reading platform. If successful, these technologies will democratize DNA writing, and increase accessibility of not only oligonucleotides, but also of larger gene constructs enabling functions such as gene editing, therapeutics development, personalized therapies, and manufacturing.

As gene construction is made less expensive and the synthetic biology workflow is shortened, data production will rapidly increase as will the demand for high-throughput DNA reading, writing, and editing platforms. For DNA synthesis to continue to be profitable as prices decrease, companies may explore partnerships to expand into other markets enabled by more accessible synthesis which may include DNA storage, DNA origami nanoparticles, personalized medicine, and the development of biological libraries as assets. These areas of opportunity will be explored further in the following section.

5. Opportunities

5.1 Emerging Technologies

Microfluidics Approach

Microfluidics synthesizers are emerging technologies that are advantageous compared to conventional DNA synthesis methods in terms of cost, programmability, and robustness. These technologies often make use of traditional phosphoramidite chemistry in such a way to allow for a reduction of the amount of reagents required for synthesis of the same quantity of oligos. In

these microfluidic synthesizers, reagents and raw materials are shuttled to multiple parallel reactions through the microfluidic architecture directed by a series of programmable valves. This allows for controlled mixing of materials that results in the production of more high-quality oligos and less waste. Turnaround time for gene synthesis can be drastically reduced via an automated microfluidic system due to the enhanced parallelization of reactions as well as the reduced need for manual multi-step protocols. However, existing microfluidic solutions are limited in regards to accuracy and oligo length due to their reliance on chemical synthesis principles.

Similar to some forms of existing microarray systems, experimental microfluidic synthesizers are robust enough to support assembly and cloning of synthetic DNA. This development is particularly interesting because these capabilities, combined with increased speed and decreased cost, could enable researchers to efficiently and reliably perform these tasks in-house with a microfluidic desktop system. Such a system would drastically increase the speed of the synthetic biology workflow as well as enable faster development of cell and gene therapies.

While microfluidic synthesizers improve upon speed, volume, and programmability limits of existing DNA writing techniques, technological development of these systems has been limited primarily to academic labs. Notably, Elegen is an up-and-coming seed stage life science company developing a hi-plex microfluidic synthesizer. However, most startups in the space have chosen to focus on other synthesis methods such as microarray synthesis and enzymatic synthesis.

Enzymatic De Novo DNA Synthesis

Enzymatic synthesis is a novel technique that is growing in popularity among early stage DNA synthesis companies including Ansa Biotechnologies, DNAScript, and Molecular Assemblies. Enzymatic synthesis is based on the activity of the polymerase terminal deoxynucleotidyl transferase (TdT) which can extend single-stranded DNA from in the 5' to 3' direction in a sequence-independent manner. Jay Keasling's lab at UC Berkeley is a key pioneer in this field and performed one of the first proof-of-concept studies for TdT-enabled de novo DNA synthesis. Currently enzyme-written DNAs are not yet commercially available nor can they rival chemical synthesis in length or efficiency. However, enzymatic synthesis may allow for faster, cheaper, and longer oligonucleotide synthesis in the future.

5.2 Emerging Applications

DNA Storage: DNA writing is a core enabler of DNA storage technologies which have been touted as the next frontier of digital information storage. It's four-letter code and stability allow DNA to accurately be used to store massive amounts of data for a long period of time. For

example, according to research from the Church Lab at Harvard, a single E. coli bacterium has a storage capacity of 10^19 bits per cubic centimeter, millions of times more data per volume than a conventional system could store. Despite these significant advantages, the cost of chemically synthesizing DNA is about $3,500 per 1 megabyte of information, prohibitively high for DNA to be used as a widespread storage medium. The development of low-cost, accurate, high-fidelity DNA writing platforms would be revolutionary for DNA storage applications.

Personalized Cancer Treatment: The concept of personalized cancer treatment has long been a goal of medicine. Both in vitro and in vivo gene delivery methods are being explored for the development of cell-specific therapy and cancer vaccines, however regulatory concerns, gene delivery technologies, and gene construction have all been challenges for researchers and physicians. More robust DNA synthesis platforms with shorter turnaround times would increase the speed of research in the space and increase the diversity of therapeutic gene constructs. A lack of robust delivery technology is a core limiting factor for personalized cancer treatment and gene therapy more broadly.

DNA Origami Nanoparticles: DNA origami nanoparticles are emerging technologies which take advantage of the molecular interactions within DNA to form highly organized and complex structures. Nanoparticle assembly and conjugation to other biological and therapeutic structures can allow for increased functionality and specificity of systems in electronics, bioelectronics, and medicine. Increased ability to synthesize DNA and control its assembly will democratize and enable research in DNA origami platforms.

5.3 Startups to Watch

Ansa Biotechnologies: Ansa is developing a novel DNA synthesis technology using polymerase-nucleotide conjugates to extend DNA molecules in such a way that it is faster, cheaper, and more accurate than existing methods. The company was founded in 2018 and has since raised $4.73 M in Seed funding from Fifty Years. The technology is licensed from Jay Keasling's lab at UC Berkeley and is the first of its kind to be published in Nature.
Location: San Francisco, CA

ATUM (formerly DNA2.0): ATUM is a biotechnology company founded in 2003 which offers a pipeline of tools for protein and strain engineering and production for the research community. These tools are designed for functions such as protein synthesis, protein engineering, cell line development, antibody services, and gene design and synthesis. ATUM has built gene optimization (GeneGPS) and vector design (VectorGPS) ML algorithms to aid in gene construction. The company raised $1.1 M in grant funding from NSF in 2011.
Location: Newark, CA

Codex DNA: Codex DNA is a manufacturer of synthetic biology equipment. Contrary to the common biomanufacturing-as-a-service model, the company is developing a DNA printer

(BioXp 3200) to produce high quality synthetic DNA. This technology will enable researchers to build genes, clones, and libraries overnight. Currently, the system allows for synthesis of 32 complex constructs ranging from 300 to 3600 bp. Additionally, Codex DNA offers reagents for cloning, amplification, and expression, as well as variant library services. The company was founded in 2013 and most recently raised an estimated $18 million of Series A1 venture funding in a round led by Northpond Ventures in 2019, putting the company's pre-money valuation at $57 million.
Location: San Diego, CA

DNAScript: DNA Script is the developer of an enzymatic DNA printer (called SYNTAX) designed for genome-scale DNA synthesis. Terminal Deoxynucleotidyl Transferase (TdT) is the core enzymatic engine of the printer which allows for controlled addition of single bases to oligo strands. The SYNTAX printer has also been designed to quantify and normalize oligo products. DNA Script was founded in 2014 and most raised $38.31 M in Series B funding in 2019, putting the company's pre-money valuation at $98.17 M. The round was led by Life Science Partners. Other investors include M Ventures, Idinvest Partners, Sofinnova Partners, Kurma Partners, Illumina Ventures, and Bpifrance.
Location: Le Kremlin-Bicêtre, France

Elegen: Elegen is a stealth-mode life sciences company founded in 2017 that is developing the world's first hi-plex microfluidic synthesizer. The company's mission is to deploy this technology in a new generation of benchtop DNA synthesizers aimed at enabling and accelerating biomedical advances.
Location: San Carlos, CA

Evonetix: Evonetix is a developer of a desktop platform for DNA synthesis, integrating synthesis of DNA on a chip. The company was founded in 2015 and most recently raised $29.81 M of Series B funding in a round led by Foresite Capital Management. Other investors include Cambridge Consultants, Data Collective, Draper Esprit, Morningside Group, Rising Tide Fund, Providence Investment Company Limited and Civilization Ventures.
Location: Cambridge, UK

Molecular Assemblies: Molecular Assemblies is a developer of an enzymatic, platform-independent DNA synthesis technology capable of synthesizing strands that are 10 to 50x larger than existing methods. The company was founded in 2013 and has most recently raised $12.2 M of Series A venture funding in a deal led by iSelect Fund. Other investors include Agilent Technologies, Alexandria Venture Investments, and Keshif Ventures.
Location: San Diego, CA

Nuclera Nucleics: Nuclear Nucleics is developing a DNA synthesis platform based on enzymatic synthesis principles. Their mission is to provide high quality genes and proteins at the benchtop. The company was founded in 2013 and has raised $0.84 M in grants. The company was a part of the first cohort of Bio-start in 2017.

Location: Cambridge, UK

5.4 Industry Challenges

1. **Technical Challenges:** In addition to the technical design considerations and limitations described earlier in this paper, there are several technical challenges which remain key barriers to success in DNA synthesis.
    a. Synthesis Fidelity and Error Correction: Errors can occur during both the synthesis and assembly stages of gene construction. For current chemical synthesis techniques, insertions and deletions are the most common errors which occur at frequencies up to 0.5% per position. Furthermore, enzymatic extension by DNA polymerases are prone to errors and as such, premiums are charged for high fidelity assembly methods. Combined, these sources of error lead to rates of about 1-10 mutations per kilobase of synthetic DNA. Methods must be developed to predict and/or eliminate errors in DNA writing to increase overall throughput and fidelity.
    b. Purification: Many existing DNA writing methods produce many variants of gene sequences and additional byproducts. Output must be purified for identification and oftentimes amplification of the desired sequence or sequences. These additional steps introduce more opportunities for error and increase the complexity of experiments that researchers must perform. Methods must be developed to account for this purification step or eliminate it altogether.

2. **Market Challenges:** The current market is populated by large, entrenched players who have operated successfully using the biomanufacturing-as-a-service model despite technical limitations and bottlenecks. Smaller companies must quickly become superior in terms of accuracy, speed, and turnaround time to be competitive with the big players. Acquisition and collaboration may be a preferable alternative.

3. **Ethical, Biosecurity, and Regulatory Challenges:** Concerns related to ethics and biosecurity are potential limiting factors for the growth of the market for synthetic DNA. Particularly in regards to dual-use research, that which has the potential to be used for nefarious purposes, governments are increasingly concerned about safety and biosecurity risks. Currently, the United States' strategy is to explicitly engage and encourage innovation, however in a regulated environment. For synthetic biology and gene therapy applications, researchers must comply with guidelines set by regulatory bodies that are guided by the EPA and FDA. However, an approval framework exists for both biological products and gene and cell therapies which will set the precedent for innovation in these sectors.

6. Conclusions

6.1 Vertical Strengths

- There is a large market for biological production (and DNA writing) spanning industries including chemistry, food and agriculture, medicine, and basic sciences research.
- Advances in DNA reading have set the stage for the DNA writing field in terms of demand.
- Increasing competition in the DNA writing space is expected to lead to increased productivity and decreased price of oligonucleotide and gene synthesis.
- Improvements to the speed, cost, and accuracy increase the accessibility of DNA writing and reduce barriers to entry for startup companies, small labs, and independent entrepreneurs.
- The market is expected to rapidly expand due to increased capabilities and applications enabled by better DNA writing platforms (i.e. DNA storage, novel cell and gene therapies, DNA origami etc.).

6.2 Vertical Weaknesses

- Until recently, there has been little technological development in the field. This has allowed entrenched players to dominate the market based on development of 35 year old technology.
- Currently, emerging technology for DNA synthesis falls short of the “better, faster, cheaper” requirement for synthesis platforms. Further, technological development is necessary to accomplish these goals to scale.
- Falling prices of DNA writing may limit profit margins and incentives for DNA synthesis companies to invest in developing new technologies.

6.3 Opportunity Cost of Capital

DNA writing is one of the most fundamental components of modern biology labs and is a core enabler of a multitude of verticals and application areas. Advances in the speed and cost of DNA synthesis would dramatically shorten the synthetic biology workflow and thus increase the speed at which innovation in these areas can occur. Based on the success of large oligonucleotide and gene synthesis companies despite the high costs of synthesis, the demand for synthetic DNA has been validated. Improvements in ease and accessibility of obtaining synthetic DNA will democratize research, and serve as a driving force for continued market growth. Advancements in DNA reading and editing have dramatically altered the synthetic biology space. DNA writing is the next frontier for synthetic biology and its applications.

6.4 Investment Theses

This report finds DNA writing a high-impact vertical with significant market opportunity. Specifically, companies that are able to address the following will have ample ground to build category defining leadership.

1. **Core technological innovation with highly defensible IP:** Because synthesis technology has remained more or less the same for over 35 years, the market is primed for startups to become leaders in the field based on a core technological innovation. Despite some variations in existing synthesis methods among the major players in the space, they've coalesced around the same chemical processes and synthetic DNA offerings. Departures from traditional methods and development of highly defensible IP will allow new companies to quickly become leaders in the space.

2. **Better, faster, cheaper oligonucleotide synthesis:** While there has been some development of novel gene assembly methods in recent years, the creation of gene constructs is largely dependent on the speed and quality of the production of smaller oligonucleotide components. The key to unlocking the potential of the DNA writing space is improving oligonucleotide synthesis specifically; that is first synthesizing smaller sequences of DNA better, faster, and cheaper to then better enable assembly.

3. **Automated platform technologies (Desktop):** Of the above goals of the DNA writing space, speed of synthesis is one of the most limiting bottlenecks. Among early-stage startups, there is an emerging trend towards all-in-one automated desktop synthesizers. Automation of the synthesis process such that it can be accomplished in-house may be paramount in reducing turnaround time for oligonucleotides and genes. In addition to companies that can accomplish better, faster, and cheaper oligonucleotide synthesis, companies that can automate other steps in the synthetic biology workflow (such as assembly, cloning, and screening) are high potential.

7. References & Further Reading

1. DNA Synthesis: Tackling the Main Bottleneck in Biology Research
2. DNA writers attracts investors
3. Competition and the Future of Reading and Writing DNA
4. Chemical Synthesis and Purification of Oligonucleotides
5. Synthetic DNA Synthesis and Assembly: Putting the Synthetic in Synthetic Biology
6. Gene Synthesis: Methods and Applications (Chapter 12)
7. The sequence of sequencers: The history of sequencing DNA
8. Recent advances in DNA assembly technologies
9. A microfluidic oligonucleotide synthesizer