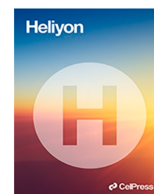
Heliyon 10 (2024) e26967

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Automated high-throughput DNA synthesis and assembly

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ARTICLE INFO

Keywords:
DNA synthesis
Microarray oligonucleotide synthesis
Enzymatic oligonucleotide synthesis
Automated DNA assembly
Synthetic biology

ABSTRACT

DNA synthesis and assembly primarily revolve around the innovation and refinement of tools that facilitate the creation of specific genes and the manipulation of entire genomes. This multifaceted process encompasses two fundamental steps: the synthesis of lengthy oligonucleotides and the seamless assembly of numerous DNA fragments. With the advent of automated pipetting workstations and integrated experimental equipment, a substantial portion of repetitive tasks in the field of synthetic biology can now be efficiently accomplished through integrated liquid handling workstations. This not only reduces the need for manual labor but also enhances overall efficiency. This review explores the ongoing advancements in the oligonucleotide synthesis platform, automated DNA assembly techniques, and biofoundries. The development of accurate and high-throughput DNA synthesis and assembly technologies presents both challenges and opportunities.

1. Introduction

The rapid expansion of synthetic biology has led to a heightened demand for DNA synthesis, particularly for applications in gene circuits, metabolic engineering, and genome synthesis [1]. DNA synthesis technology plays a pivotal role in the design, build, test, learn (DBTL) cycle of synthetic biology. High-throughput DNA synthesis not only enhances our comprehension of life but also empowers us to manipulate it more effectively. In 2002, scientists chemically synthesized a functional poliovirus genome of about 7500 bp [2], marking a major milestone in the field. Subsequently, in 2010, the Venter Institute synthesized the first self-replicating artificial cell, *Mycoplasma capricolum* [3]. More recently, in 2017, the Synthetic Yeast Genome Project (Sc2.0), an international collaboration, achieved the *de novo* design and chemical synthesis of six chromosomes (II [4], III [5], V [6], VI [7], X [8] and XII [9]) of the *S. cerevisiae* genome. The synthetic yeast strains were remarkably similar and consistent with the wild type. The current foundational strategies for synthetic genes or genomes encompass the following steps: (1) chemical synthesis of oligonucleotides; (2) synthesis of oligonucleotides into shorter double-stranded DNA (dsDNA); (3) assembly of short DNA fragments into larger constructs; (4) *in vivo* assembly of large DNA (>20 kb), typically using *in vivo* assembly in *E. coli*, *S. cerevisiae*, or other appropriate host cells.

The assembly of large DNA fragments from chemically synthesized oligonucleotides often involves a substantial amount of repetitive work. Therefore, the integration of automation technology has emerged as a pivotal and invaluable strategy for streamlining this process. Currently, the integration of conventional DNA synthesis and assembly methods with high-throughput automation techniques has proven effective in accelerating processing times, lowering error rates, and minimizing overall costs. This paper is dedicated to offering a comprehensive review of automated high-throughput DNA synthesis and assembly techniques.

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https://doi.org/10.1016/j.heliyon.2024.e26967
Received 23 April 2023; Received in revised form 20 February 2024; Accepted 22 February 2024
Available online 25 February 2024
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2. Automation of oligonucleotide synthesis

Oligonucleotides are linear polynucleotide fragments composed of multiple nucleotide residues linked by phosphodiester bonds. In the 1950s, Todd and Khorana et al. used phosphodiester [10], H-phosphonate [11] and phosphotriester [12] methods to synthesize oligonucleotides, enabling the *de novo* abiotic synthesis of DNA. In the 1980s, the automation and commercialisation of phosphoramidite chemistry led to the breakthrough development of oligonucleotide synthesis [13]. Today, oligonucleotides are used in a wide range of applications, including scientific research, clinical research, pharmaceutical development and the synthesis of molecular diagnostic probes.

2.1. Column-based oligonucleotide synthesizers

Oligonucleotide synthesis is a multi-step, continuous reaction in which nucleotide monomers are gradually added to solid-phase supports such as controlled pore glass (CPG) or polystyrene (PS) beads. Each cycle includes four critical reactions: deprotection, coupling, capping and oxidation. At the end of synthesis, oligonucleotides are released from the solid-phase support using ammonia or other alkaline conditions. Subsequently, oligonucleotides undergo purification through methods such as oligonucleotide purification columns (OPC), polyacrylamide gel electrophoresis (PAGE), or high-performance liquid chromatography (HPLC). In the 1980s, Applied Biosystems developed the first automated DNA synthesizer using this method, improving the accessibility of synthetic oligonucleotides [14,15]. Using the synthesis column as the synthesis support and the controlled porous glass as the reaction medium, the column synthesizer uses a computer-programmed solenoid valve to accurately control the flow of reaction reagents into the column, resulting in the synthesis of single-stranded DNA (ssDNA) [16,17].

Currently, commercial automated column oligonucleotide synthesis technology is relatively mature, and many oligonucleotide synthesizers have been built (Fig. 1A). Predominantly utilized synthesizers in this domain include the MerMade series, developed by BioAutomation, and the Dr.Oligo series, developed by Biolytic [17]. In 2017, Boles et al. presented a digital-to-biological converter (DBC) [18], which featured an oligonucleotide synthesizer (MerMade 192R) operated with Poseidon software, enabling the automated synthesis of oligonucleotides. The MerMade 192R Synthesizer has the capability to produce up to 192 oligonucleotides in a single DBC run, facilitating the construction of a 6 kb DNA fragment. In 1995, the Stanford Genome Technology Center (SGTC) developed the first 96-well automated multiplexed oligonucleotide synthesizer (96-AMOS). Subsequently, they integrated the 96-AMOS technology with the commercially available BioRAPTR Flying Reagent Dispenser, enabling high-throughput (up to 3456 wells) dispensing of aqueous buffers, diluents, and staining solutions. This improved system allows for the simultaneous synthesis of oligonucleotides of varying lengths, with an average yield of 3.5 nmol per well, obviating the need for post-synthesis amplification [19].

Automated column-based synthesizers have played a pivotal role in significantly enhancing both the efficiency and quality of oligonucleotide synthesis, establishing themselves as indispensable tools within various spheres of academic research and industrial applications. Advancements in DNA synthesis technology have resulted in an impressive increase in the synthesis throughput for synthetic oligonucleotides, reaching ranges of 96 to 1536. Furthermore, the cost for synthesizing sequences under 100 bases has decreased to a range of \$0.05 to \$0.15 per nucleotide, and the error rate has been markedly reduced to as low as one mutation per 200 base pairs or even less [20,21].

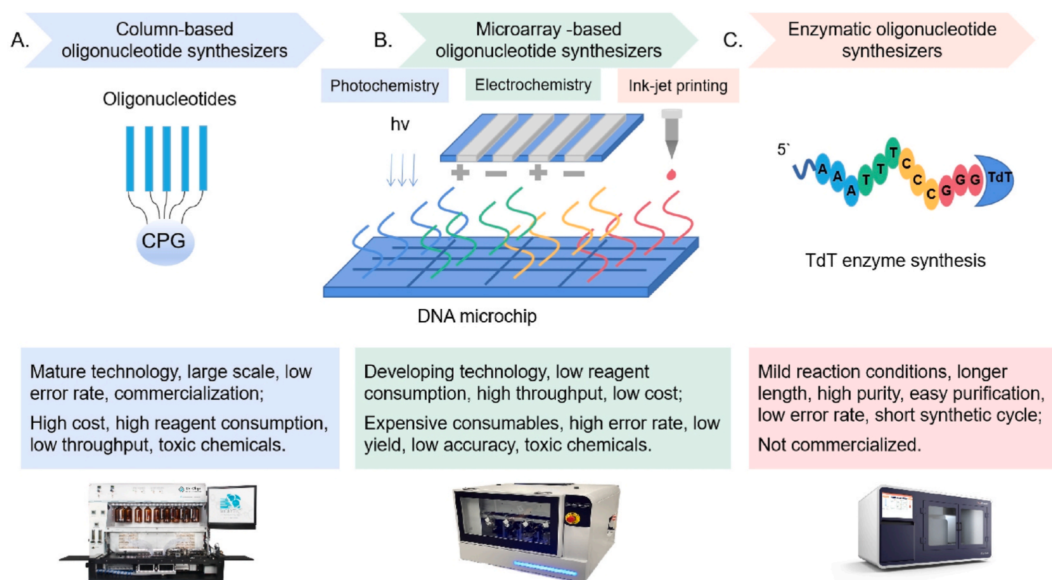

Fig. 1. Typical oligonucleotide synthesizers. A, Column-based oligonucleotide synthesizers. B, High-throughput microarray-based oligonucleotide synthesizers. C, Enzymatic oligonucleotide synthesizers.

However, despite the advancement in oligonucleotide synthesis, challenges persist when targeting oligonucleotides exceeding 200 base pairs, primarily due to an average reaction efficiency of less than 99.5% per cycle [1]. The practical synthetic limit typically hovers around 100 base pairs, a compromise struck between efficiency and cost considerations. Furthermore, the column-based oligonucleotide synthesis process necessitates the use of various organic solvents, resulting in the need for specialized waste liquid and gas management. Consequently, there is a pressing need to explore novel methodologies that can extend the length of synthetic oligonucleotides and discover approaches for oligonucleotide synthesis that do not rely on organic reagents.

2.2. *Microarray oligonucleotide synthesis*

Although column-based oligonucleotide synthesis has proven effective for synthesizing oligonucleotides at a medium to large scale (nmol to mmol), there remains a persistent need for higher-throughput approaches to align with DNA synthesis requirements and cost-efficiency goals. To address this challenge, researchers have turned their attention to DNA microarrays, which offer a cost-effective source of oligonucleotides. Microarray oligonucleotide synthesis is based on a surface of the silicon chip or glass slide, and grows in specific spots or volumes. DNA microarrays can generate large numbers of unique oligonucleotide sequences in a miniaturized and parallel manner, offering advantages in throughput, reagent consumption and cost [22–24]. These advantages are achieved by various mechanisms, including photolithography, electrochemical and array ink-jet printing, among others (Fig. 1B).

2.2.1. *Photolithography-based oligonucleotide synthesizers*

In the 1990s, Affymetrix developed a method for spatially localized polymer synthesis on surfaces using photo-activated chemistry [25,26]. This method laid the foundation for the development of array-based oligonucleotide synthesis and marked the beginning of DNA microarray synthesis. Subsequently, NimbleGen and LC Sciences used maskless techniques to guide the photochemistry using programmable micromirror devices [27,28]. In 2018, Hölz et al. used a photolithographic method for efficient reverse 5'→3′ synthesis of complex DNA arrays [29]. 2-(2-nitrophenyl)-propoxycarbonyl (NPPOC) was replaced by benzoyl-2-(2-nitrophenyl)-propoxycarbonyl (BzNPPOC), and the photolysis efficiency was increased by more than two times. Coupled with additional optimization of conjugation and oxidation reactions, the reverse synthesis efficiency of DNA oligonucleotide arrays is approximately 3 times higher. In 2021, Lietard et al. analyzed the error rate of DNA libraries synthesized by photolithography [30]. They identified the proportion of deletion, insertion and substitution errors, and found that the photolysis yield influenced the deletion rate. The study also illustrates the effects and limitations of optical defects, highlighting the importance of preventing internal reflections by absorbing ultraviolet light.

Affymetrix's technology relies on the precise control of light using photolithographic masks to selectively remove protective groups, enabling specific sites to engage in subsequent rounds of nucleotide coupling. This approach imparts a high degree of specificity, sensitivity, reproducibility, and a low false-positive rate to the oligonucleotide chip. Remarkably, this technology empowers the synthesis of up to 500,000 oligonucleotides on a single chip [16]. LC Sciences' DNA synthesis facility achieves high synthetic purity through advanced microarray synthesis technology (μParaflo®). This technology enables high-throughput parallel synthesis of custom DNA microarrays by integrating photo-generated acid (PGA) chemistry, digital photolithography (DLP) and advanced microarray technology. The microarray platform can synthesize thousands or more sequences per tube, with oligonucleotide lengths up to 150 bp, at a cost of less than $0.08 per base [16,31].

2.2.2. *Ink-jet printing based oligonucleotide synthesizers*

In 1996, Agilent developed a DNA synthesizer using non-contact industrial ink-jet printing, in which four bases are sprayed onto the chip one at a time as "ink", enabling in-situ printing of oligonucleotides [32]. Subsequently, Hughes and Lausted synthesized thousands of oligonucleotide arrays *in situ* using ink-jet printing [33,34]. In 2019, Li et al. introduced a microreactor chip for oligonucleotide synthesis. The chip uses fixed silica beads in microreactors. By increasing the surface area and enhancing the binding strength of the beads to the chip, they increased the yield of the oligonucleotide synthesis product [35].

Agilent took the pioneering initiative in oligonucleotide synthesis by employing ink-jet printing technology. Their chip substrate is composed of a glass plate, approximately the dimensions of a standard pathology slide. The latest SurePrint DNA chip synthesizes sequentially consistent, highly complex 30–230 bp oligonucleotides with 244,000 oligonucleotides in parallel (https://www.agilent.com.cn/). Based on the SurePrint oligonucleotide library, Coventry et al. discovered biophysical properties that can predict how proteins will interact [36]. Currently, Twist Bioscience also synthesizes oligonucleotides using ink-jet printing. Oligonucleotides with high uniformity, low error rate and up to 300 bp can be synthesized on a silicon-based platform. The silicon-based DNA synthesis platform boasts a capacity to produce over one million unique ssDNA oligonucleotides in a single run. Each chip comprises numerous discrete clusters, and within these clusters, 121 individually addressable surfaces exist, each with the capability to synthesize a distinct oligonucleotide sequence(https://www.twistbioscience.com/).

2.2.3. *Electrochemical array based oligonucleotide synthesizers*

Oligonucleotides can also be synthesized *in situ* using local electrochemical reactions, where electrochemical reactions are employed to generate acid for the removal of protective groups. The controlled coupling synthesis is then achieved using phosphoramidite monomers. In 2005, Egeland and colleagues successfully electrochemically synthesized a 16 bp oligonucleotide [37]. Furthermore, in 2007, CombiMatrix's oligonucleotide microarray platform contained 12,544 individually addressable microelectrodes in a semiconductor matrix. This innovation enabled semiconductor-based electrochemical acid production for the selective deprotection of nucleosides [38]. In 2017, Holden et al. fabricated DNA arrays directly on aminated polyethylene terephthalate (PET)

sheets. PET sheets are more robust, more flexible, and can tolerate the large number of chemical exposure steps required to synthesize relatively long oligonucleotides [39]. In 2018, Levrie et al. synthesized DNA directly on a chip using electrochemically modified gold electrodes as solid carriers [40].

CustomArray pioneered the electrochemical synthesis of oligonucleotides, using electricity as a reaction switch [37,38]. This method reduces the synthesis reaction to a micron-scale reaction hole, allowing the integration of tens of thousands of reaction points on a chip. While this approach excels in DNA quality compared to photochemical methods, the chip is entirely immersed in the reaction solution, and local acid diffusion can still affect nearby areas. Consequently, its actual efficiency is lower than ink-jet printing. CustomArray's high-throughput, electrochemical DNA synthesizer enables the simultaneous synthesis of 8.4 million unique oligonucleotides of up to 150 bp in length. DNA synthesis technology on complementary metal oxide semiconductor (CMOS) integrated circuit (IC) enables dynamic programming of microelectrode current/voltage optimization control with remarkable accuracy [41,42].

2.2.4. *Thermally controlled oligonucleotide synthesizers*

With the development of synthetic biology, some emerging microarray synthesizers have emerged. For example, Evonetix has

Table 1
Overview of different automated oligonucleotide synthesizer.

Type of synthesizer	Company and equipment	Characteristics	Advantage	Disadvantage	Reference
Column-based oligonucleotide synthesizers	BioAutomation (MerMade 4/6/12/48X/96E/192E/192R/192X)	≤192 column synthesizer, 0.02–200 μmol/column, 20 bp primers in 3.5 h	Mature technology, large scale, low error rate	High cost, toxic chemicals	18
	Biolytic (Dr. Oligo 96/192/384/768)	≤768 column synthesizer, 2 nmol to 5 μmol/column, a single 384-well plate in 6 min and 40 s	High quality, media flexibility, scalability, ultimate efficiency	High cost, toxic chemicals	http://www.biolytic.cn/
	Stanford Genome Technology Center (96-AMOS, 3456-AMOS)	≤3456 column synthesizer, 3.5 nmol/column	Large scale, high-throughput, low reagent consumption	Toxic chemicals	19
	Cytiva (ÄKTA Oligopilot)	8 oligonucleotides in 3–4 h	Large scale, sensor detection process	Low throughput, toxic chemicals	15
Microarray-based oligonucleotide synthesizers	Affymetrix (Oligo chip)	Up to 500,000 oligonucleotides in parallel	High-throughput, mature chip, low cost	Only synthetic oligonucleotide libraries	16
	LC Sciences	High synthetic purity, cost less than $0.08 per base, up to 150 bp, up to 4000 oligonucleotides in parallel	High-throughput, low reagent consumption, low cost	Complex chip processing, only synthetic oligonucleotide libraries	16, 31
	Agilent (SurePrint DNA chip)	30–230 bp, up to 244,000 oligonucleotides in parallel	High-throughput, low reagent consumption, low cost	Only synthetic oligonucleotide libraries	https://www.agilent.com.cn/
	Twist Bioscience	Up to 1 million oligonucleotides in parallel	High-throughput, good synthesis stability, low error rate	Low yield of single oligonucleotide	16
	CustomArray	Up to 150 bp, up to 8.4 million oligonucleotides in parallel	High-throughput, low reagent consumption, low cost	Poor synthesis stability, high error rate, only synthetic oligonucleotide libraries	37, 38
	Evonetix	Compatible with both phosphoramidite and enzymatic methods, *in situ* assembly and error correction	High-throughput, Efficient synthesis of repeat sequences and GC-rich	High reagent consumption, complex chip processing, low purity if not thermolysed	16, 43
	BGI	Up to 100,000 oligonucleotides in parallel	High-throughput, good synthesis stability, low error rate	Poor chip compatibility	16
Enzymatic oligonucleotide synthesizers	DNA Script (Syntax)	Up to 99.7% coupling efficiency, up to 150 bp, high-throughput benchtop synthesizers (96 oligonucleotides)	High accuracy, customized reagent kits	Long G-rich sequence secondary structure	15, 50, https://dnascript.com/
	Molecular Assemblies	Steric control of elongation and random polymerization	High accuracy	Limited range of cleavable terminators compatible with TdT	15
	Camena Bioscience	High sequence accuracy (85% full-length sequences)	High accuracy, avoids dependence on TdT	Complicated process, high cost	15
	Ansa Biotechnologies	Lengthen the single nucleotide within 10–20 s	Avoids difficulty in binding TdT to modified nucleic acid, low dependence on chemistry	Chemical scars, high consumption of TdT	15, 51

proposed a thermally controlled synthesis that is compatible with both phosphoramidite and emerging enzymatic methods [43]. By adjusting the temperature of the sites in the Evonetix microfluidic thermal array, it is possible to control the rate of chemical reactions used for oligonucleotide synthesis at each site in the array. A low melting point reheat blocking material is added to a closed chamber containing a specially designed large-scale addressable synthesis site, which is heated by using circuit signals to control whether the site is energized or not. Under the condition of heating, the special material can be adsorbed onto the corresponding site, preventing the subsequent incoming test agent from reacting with it. If further synthesis is needed, the material can be cleaned with a solvent to expose the site for the synthesis reaction [44]. Based on this technology, Evonetix is expected to offer a benchtop microarray synthesizer.

2.3. *Enzymatic oligonucleotide synthesizers*

Currently, column oligonucleotide synthesis is the main method for commercial oligonucleotide synthesis. However, it is characterized by limitations such as constrained throughput and elevated costs. Microarray oligonucleotide synthesis is typically used to form complex oligonucleotide libraries. Other problems include a higher error rate. In the synthesis cycle, oligonucleotide sequence depurination occurs spontaneously due to the oligonucleotide is exposed to the deprotection reagent. Misalignment of the reaction droplet, poor sealing of the reagent, or imprecise deprotection due to the beam shift in the photocontrol system can cause edge effects, resulting in base errors in the oligonucleotide sequences in neighboring regions [45]. Moreover, conventional chemical synthesis relies on organic reagents, which bear environmental implications. Consequently, there is a growing surge of interest in enzymatic oligonucleotide synthesis technology. In comparison to chemical synthesis, enzymatic oligonucleotide synthesis presents advantages, including milder reaction conditions and reduced by-product formation (Fig. 1C).

In 1959, Bollum described a ssDNA polymerase, terminal deoxynucleotidyl transferase (TdT), which is able to attach deoxynucleotide triphosphates (dNTPs) to the 3′ end of ssDNA in a template-independent manner [46,47]. Decades later, TdT was integrated into commercial Template-Independent Enzymatic Oligonucleotide Synthesis (TiEOS) methods [48]. This method synthesizes ssDNA on a solid-phase support using TdT and nucleoside 5'-triphosphates (NTPs). The NTPs are modified with a synthesis-interrupting “terminator”, which ensures that only one nucleotide is added during each reaction step. Subsequently, the terminator is removed, facilitating the addition of the next desired nucleotide in the oligonucleotide sequence [48,49].

Since 2013, the Molecular Assemblies, the DNA Script and the Nuclera Nucleics have established the TdT enzyme-based DNA synthesis technology [16]. These methodologies primarily involve the modification of nucleotide molecules, the chemical synthesis of nucleotide monomers featuring reversible terminating groups, and the subsequent addition of bases to the end of the synthesized sequence using TdT enzymes. Notably, DNA Script utilizes 3'–ONH_2–protected NTPs, while Molecular Assemblies and Nuclera Nucleics prefer azidomethyl terminators [15]. In 2020, DNA Script introduced Syntax, the pioneering DNA printer founded on Enzymatic DNA Synthesis (EDS), thereby enabling automated template-free synthesis [16]. Syntax comprises three essential components: (i) a highly engineered TdT enzyme, (ii) reversibly terminated nucleotides, and (iii) solid supports incorporating a short, predefined initiator DNA (iDNA). The system’s software automates the entirety of synthesis, post-processing and oligonucleotide concentration normalization, empowering users to commence their operations within a mere 15 min (https://dnascript.com/). The system allows parallel synthesis of 96 oligonucleotides of 60 bp within six to 7 h. DNA Script has achieved up to 99.7% coupling efficiency and has successfully synthesized a 280 bp sequence [50].

In 2018, Sebastian Palluk and colleagues introduced a novel oligonucleotide synthesis strategy using the template-independent polymerase, TdT [51]. They developed polymerase-nucleotide conjugates enabling reversible termination of chain elongation. The TdT-dNTP conjugate allows precise and quantitative extension of a single nucleotide within the primer in a brief 10–20 s. This innovative technology has been adopted by Ansa Biotechnologies. The following table lists the parameters for automated

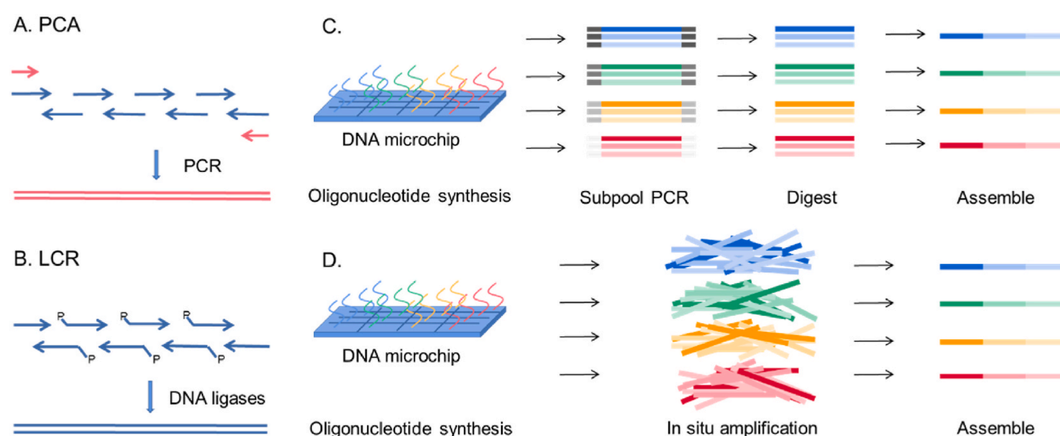

Fig. 2. Schematic representations of assembly methods for DNA synthesis. A, Polymerase cycling assembly (PCA). B, Ligase chain reaction (LCR). C, D, Different strategies for dealing with microarray oligonucleotide complexities.

oligonucleotide synthesizer (Table 1).

3. Automated DNA assembly

Currently, the length of *de novo* DNA synthesis is constrained, necessitating the assembly of longer genes or genomes from oligonucleotide fragments. DNA synthesis and assembly techniques are categorized by the methods they employ. Two commonly employed oligonucleotide assembly approaches are polymerase cycling assembly (PCA) [52,53] and ligase chain reaction (LCR) [54]. Methods to produce larger assemblies including overlap extension polymerase chain reaction (OE-PCR) [55], circular polymerase extension cloning (CPEC) [56,57], Golden Gate [58], Gibson [59,60], sequence and ligation independent cloning (SLIC) [61] and others. The assembly of longer DNA fragments is carried out *in vivo*, often utilizing organisms like *E. coli*, *B. subtilis*, and *S. cerevisiae*.

3.1. Oligonucleotide assembly methods

In 1985, the development of the polymerase chain reaction (PCR) created a favourable environment for DNA polymerase-based assembly technology [62,63]. PCA is a PCR-based method that uses partially overlapping fragments to synthesize dsDNA (Fig. 2A). This method involves cycling and amplification, with additional oligonucleotides or extension products, accommodating gaps between oligonucleotides [52]. In 2003, Smith and colleagues used PCA to assemble the complete phiX174 phage genome (5386 bp) in a mere 14 days [53]. LCR bears similarities to PCA but requires the 5′-end phosphorylation of spliced oligonucleotide chains. The 5′-phosphorylated oligonucleotide fragments are then connected into double-stranded DNA using DNA ligases (Fig. 2B). Furthermore, both OE-PCR and Gibson methods find application in oligonucleotide synthesis of single-stranded DNA (ssDNA). Zhang and colleagues introduced a straightforward, versatile, and effective gene synthesis approach founded on Sequence Overlapping Extended Polymerase Chain Reaction (OE-PCRs) [64]. In this method, they designed 54 bp oligonucleotides, including 18 bp overlapping sequences, to synthesize complete genes. On the other hand, Gibson and colleagues devised a one-step method for the direct assembly and cloning of oligonucleotides onto the plasmid backbone, both *in vitro* and *in vivo* [60,65–67].

DNA assembly involving larger pools of oligonucleotides is often less precise. To improve assembly accuracy, Kosuri et al. [68] and Quan et al. [69] used distinct methods for oligonucleotide classification within these pools. Kosuri et al. [68] employed PCR to amplify pre-designed barcodes, enabling the amplification of specific oligonucleotide subpools. Subsequently, they digested and removed the barcodes to facilitate DNA assembly (Fig. 2C). On the other hand, Quan et al. [69] utilized a custom ink-jet synthesizer to synthesize oligonucleotides within physically separated micropores. This approach allowed them to synthesize oligonucleotide subsets within different micropores, followed by *in situ* amplification and assembly (Fig. 2D).

3.2. DNA fragment assembly methods

While various DNA assembly methods are extensively reviewed elsewhere, our focus here is on two fundamental techniques

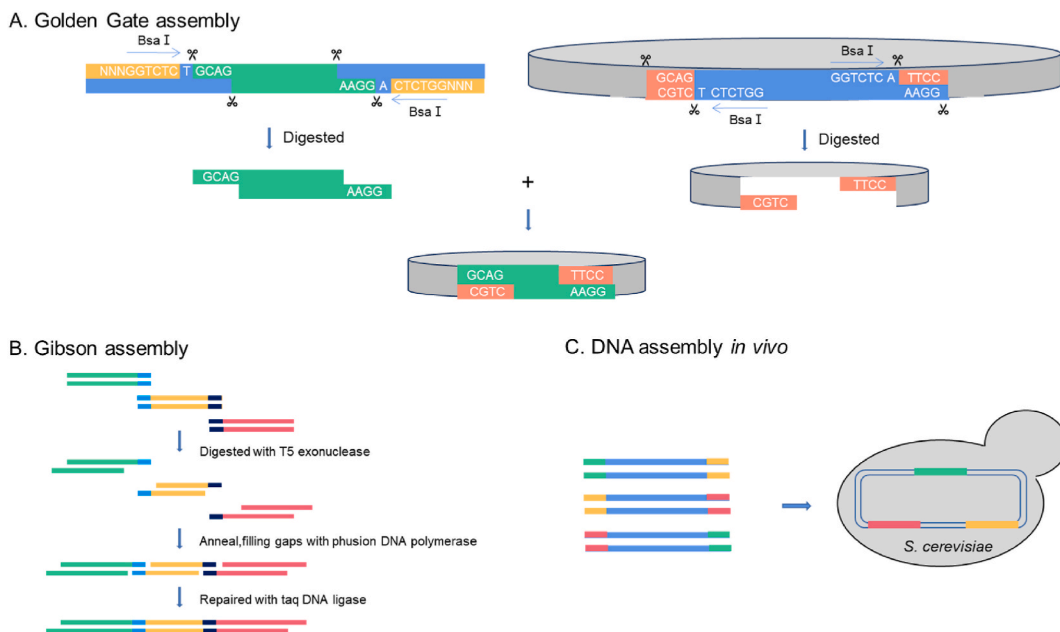

Fig. 3. Schematic representations of assembly methods for DNA assembly. A, Golden Gate assembly. B, Gibson. C, DNA assembly *in vivo*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

commonly employed in automated assembly: Golden Gate assembly and Gibson assembly. In 2008, Engler and colleagues introduced the concept of Golden Gate assembly [58]. This powerful DNA assembly method relies on type IIS restriction endonucleases (Fig. 3A). Distinct from enzymes with the same recognition and cleavage sites, type IIS enzymes have cleavage sites outside the recognition site and can be arbitrary. Type IIS enzymes generate cohesive ends, enabling the seamless joining of multiple fragments in a single step. On the other hand, Gibson assembly employs T5 exonuclease's 5′ exonuclease activity to digest DNA fragments. Subsequently, it fills gaps with Phusion DNA polymerase and repairs nicks using Taq DNA ligase (Fig. 3B). This method offers the advantages of rapid reaction times, straightforward operation, high scalability, and the capability to assemble longer DNA fragments of up to 100 kb [59]. In 2010, Gibson and colleagues used Gibson assembly to synthesize the entire 16.3 kb mouse mitochondrial genome from 600 overlapping 60-mers [60].

As DNA fragment lengths increase, *in vitro* manipulation becomes less reliable, and *in vivo* recombination systems are typically employed for splicing DNA fragments exceeding 20 kb. In gram-negative *E. coli*, the RecA recombination system serves as the endogenous recombination mechanism. Notably, the ET recombination method encompasses the RecET recombination system based on the Rac phage encoding and the Red recombination system based on λ phage encoding [70]. The gram-positive bacterium *B. subtilis* also possesses a robust *in vivo* recombination system. Extracellular DNA can be introduced into the cytoplasm in a single-stranded form and subsequently integrated into *B. subtilis* chromosomes through RecA-mediated homologous recombination [71–73]. *S. cerevisiae* exhibits the capacity to absorb and recombine DNA fragments, effectively assembling DNA fragments through homologous recombination (Fig. 3C) [3,74–79]. In 2016, Zhou and colleagues introduced Cas9-assisted homologous recombination assembly (CasHRA) technology, which enabled the assembly of a 1.03 Mb MGE-syn1.0 (minimal genome of *E. coli*) [80]. In recent years, *in vivo* assembly methods have been developed for various other microorganisms, including *Physcomitrella patens* [81], *Ralstonia*, *Pseudomonas putida*, *Lactobacillus plantarum*, and *Yersinia lipophilicity* [82].

3.3. Automated DNA assembly platform

In the field of synthetic biology, conducting large-scale trial-and-error engineering experiments presents formidable challenges that go beyond the capabilities of traditional, labor-intensive research methods. Consequently, the establishment of a high-throughput engineering research platform becomes imperative. Many companies and research institutions, both domestically and internationally, have developed automated DNA assembly platforms centered around automated synthetic biology technology. Examples of such platforms include iBioFAB, the Codex DNA Gibson assembly platform, Ginkgo Bioworks, the Edinburgh Genome Foundry, the Tianjin Institute of Industrial Biotechnology, the Shenzhen Synthetic Biology Research Center and so on. These platforms typically follow a common automated DNA assembly process, as depicted in Fig. 4. Here are some instances of automated synthetic biology facilities.

3.3.1. iBioFAB automation platform

In 2014, Dr. Zhao Huimin's research team developed FairyTALE, a high-throughput assembly platform built upon the principles of the Golden Gate method [83]. This platform leverages automated robotic liquid handling to synthesize hundreds of transcription

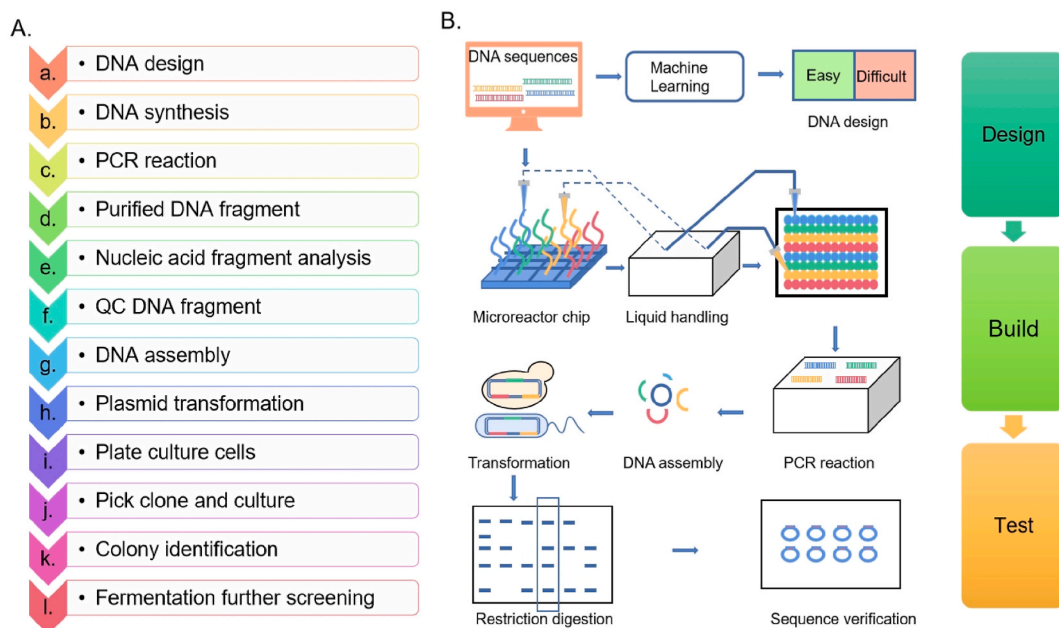

Fig. 4. An overview of the automated assembly DNA factory process. A. Workflow for high-throughput strain construction. B. The process of assembling DNA fragments in biofoundry.

activator-like effectors (TALEs) in a single day. The process encompasses plasmid construction, robotic assembly, lyophilization, Golden Gate reaction, *E. coli* transformation, polyclonal culture, and plasmid purification, resulting in an impressive 98% assembly efficiency and a material cost of $5 per gene [83]. It's essential to note that the platform's capability is primarily limited to assembling vectors containing a single TALE-*Fok*I monomer. Subsequently, they extended the synthesis process to the iBioFAB (Illinois Biofoundry for Advanced Biomanufacturing). Through automated procedures involving enzyme reactions, *E. coli* transformation, culture, plasmid extraction, enzyme digestion verification, and other steps, they managed to produce 400 pairs of transcription activator-like effector nucleases (TALENs) in a day, achieving a remarkable 96.2% assembly efficiency and a material cost of $2.1 per gene pair [84].

The iBioFAB is an automated workflow that encompasses both hardware and software components. Central to the iBioFAB is an articulated robotic arm capable of transporting samples along a dedicated track, facilitating movement from port to instrument. The iBioFAB system consists of three key elements: a central robotic platform, a modular computing framework, and an instrumentation component. This instrumentation component includes various robotic arms, a robotic liquid handler, Peltier temperature-controlled blocks, a microplate reader, incubators, shaking incubators, thermocyclers, a storage carousel, and more [84]. In a subsequent development, researchers devised an automated platform for multiplex genome-scale engineering in *S. cerevisiae*, effectively utilizing the iBioFAB for automated multiplex genome-scale engineering. Within each process module, a remarkable 192 transformations can be conducted simultaneously, achieving a transformation efficiency of 10^4 μg^{-1} plasmid DNA [85]. Furthermore, Zhao Huimin's team developed a general-purpose DNA assembly method based on *Pyrococcus furiosus* Argonaute (*Pf*Ago), an endonuclease similar to CRISPR-Cas. *PfAgo* demonstrates the capability to recognize and cleave nearly any arbitrary DNA sequence at elevated temperatures, guided by single-stranded DNA (ssDNA), thereby generating defined sticky ends of varying lengths [86]. This assembly method was subsequently integrated into the PlasmidMaker platform for plasmid construction, and the workflow was structured into three distinct phases: "design-build-test" (Fig. 4B).

3.3.2. Codex DNA Gibson assembly platform

In 2017, Gibson et al. reported a digital-to-biological converter (DBC) that can automatically assemble DNA starting from DNA sequence information [18]. DBC utilizes a system controller to integrate many pieces of software and equipment. First, the designed DNA sequence is entered into Archetype, which can automatically convert the input sequence into oligonucleotide sequence and write it to a specified folder. When the oligonucleotide file has been recognized, the DBC system controller sends it to the MerMade 192R oligonucleotide synthesizer. After synthesis, an automated pipetting system transfers the oligonucleotides to a thermocycler, where they are pooled, assembled, error corrected and reassembled into DNA amplicons. Finally, the DNA amplicons direct the production of RNA, proteins and viral particles. DBC's DNA assembly processes have been incorporated into the BioXp™ 3200, a commercially available automated DNA assembly system [87]. The BioXp™ 3200 has two modules, the assembly module and the assembly-and-cloning module. The assembly module is used to construct linear, blunt-ended, double-stranded DNA fragments overnight. On the other hand, the assembly-and-cloning module of the BioXp™ system extends its functionality to cloning DNA and collecting DNA clones via the Gibson assembly method. In 2020, Codex DNA launched the BioXp™ 3250 system, offering users the capability to assemble DNA sequences of up to 7000 bp with ease, eliminating the need for manually scripting genetic sequences for specific experiments on a computer in a few days (Fig. 5) [88]. In 2023, Codex DNA unveiled the BioXp™ 9600 system, representing the third generation of gene synthesizers, equipped with advanced throughput capabilities. This cutting-edge system is designed to accelerate the discovery and development of novel vaccines and biologics.

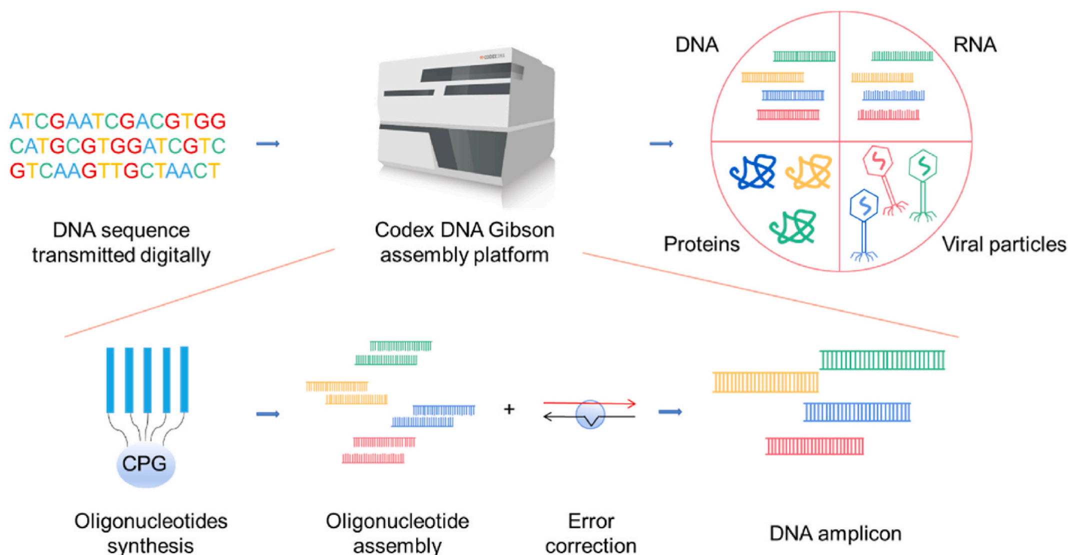

Fig. 5. Schematic diagram of Codex DNA Gibson assembly platform.

3.3.3. The Ginkgo Bioworks synthetic biology facility

Ginkgo Bioworks, a prominent commercial foundry, operates in two primary domains: the foundry and the codebase. The foundry encompasses biological factories where automated robots, along with proprietary software and data analysis, facilitate high-throughput, automated microbial construction and process data monitoring and analysis. The codebase, on the other hand, comprises the repository of synthetic biology knowledge and reusable biological data assets accumulated by the platform. It is responsible for various aspects of cell programming, such as designing, coding, and modifying strains, before transitioning them to the foundry for automated microbial cell production to meet specific requirements. Ginkgo Bioworks has developed multiple automated platforms for various tasks. For example, when Ginkgo Bioworks seeks to enhance pathway productivity through strain modification, it employs DNA synthesis, strain construction, and ultra high-throughput screening processes (Fig. 4A). Initially, DNA constructs are designed, synthesized, and strains constructed using the Inscripta Onyx platform. Next, automated colony pickers developed by kbiosystems are utilized to isolate individual colonies. These colonies are then cultured through an automated system developed in-house at Ginkgo and subsequently screened using ultra high-throughput acoustic ejection mass spectrometry from SCIEX. Finally, candidate strains are further screened using the Ginkgo Ambr 250 fermentation system. This comprehensive engineering process, from DNA design to screening data generation, is typically completed within approximately three months. Altogether, Ginkgo Bioworks leverages its DNA synthesis and high-throughput screening capabilities to synthesize 109 million base pairs and evaluate 2719 pathways, resulting in a total of 30,445 strain tests (https://www.ginkgobioworks.com/).

3.3.4. The Edinburgh Genome foundry

The Edinburgh Genome Foundry (EGF) represents a specialized research institution focused on the intricate task of assembling large DNA fragments, with a particular emphasis on those surpassing 5 kb in size. The core of EGF's capabilities resides in a highly automated robotic platform, distinguished as the most extensive integrated synthetic biology facility established within the academic realm. Notably, this platform is underpinned by the deployment of three autonomous robotic arms [89,90]. The EGF has developed and shared more than 20 industrial-grade software packages (https://cuba.genomefoundry.org/) for the design and construction of large DNA fragments and combinatorial DNA libraries, including sculpt a sequence, cloning simulation, design Golden Gate overhangs, transfer genbank features and so on. It is capable of handling more than 2000 DNA assembly reactions per week, a throughput 20 times that of a single researcher working manually [90,91]. EGF collaborates with Autodesk to develop Genetic Constructor, a user-friendly web-based tool for designing large DNA constructs and libraries. This platform offers a versatile robotic system for various DNA manipulation techniques like Golden Gate assembly, Gibson assembly, yeast recombination, and plasmid extraction, making it a valuable resource for research in genetic engineering and molecular biology [92].

Table 2
Parameters of the synthetic biology research related facilities.

Biofoundry	Assembly method	Core parameter	Estimated throughput	Success rate	Reference
Agile biofoundry	SLIC, Gibson, CPEC, and Golden Gate	J5 CAD software saves 3–8 times the time and 10–20 times the cost of the traditional combinatorial 200 plasmids.	>96 per day	>89% (random clones)	[93,95],
IBioFAB	Golden Gate	Multiple genome engineering can achieve three rounds of evolution in six weeks, which is 10 times more efficient than manual work.	400 per day	>96.2% (polyclonal cultures)	[84–86,95–97]
Amyris Living Foundry	Ligase Cycling Reaction (LCR)	One-step assembly of up to 20 DNA parts and up to 20 kb DNA constructs with very few single-nucleotide polymorphisms (<1 per 25 kb) and insertions/deletions (<1 per 50 kb).	>96 per day	>40%	[98–100]
Ginkgo Bioworks	Ink-jet printing oligonucleotide synthesizer	Construction 30,445 high-yield strains.	>Kilomega per year	About 95%	https://www.ginkgobioworks.com/
DAMP lab	Golden Gate	High-throughput DNA assembly and cloning.	>42 per day	>90%	[99,101]
Edinburgh Genome Foundry	Golden Gate, Gibson	Assembly large DNA fragments (>5 kb).	2000 per week	40–90%	[89–92]
London BioFoundry	BASIC	Automated design, build and verification of large DNA fragments using robotic equipment.	>96 per day	90–99%	[94,99]
Shenzhen Institute of Synthetic Biology		Three platforms, "synthetic test" platform, "user detector" platform, "design learning" platform.			[90]
Tianjin Institute of Industrial Biotechnology	Golden Gate, Gibson	Automated gene manipulation has been achieved in a wide range of model organisms, and high-throughput automated genome editing of 300 times per day has been achieved in some model organisms.	100–600 per day (single gene cloning) 100 per day (complex plasmid multi-modular assembly)	>90%	[102]

3.3.5. Other BioFoundries

In 2019, the Global Biofoundry Alliance (GBA) was established in Kobe, Japan. The consortium is a collaboration of 16 leading synthetic biology facilities, including iBioFAB, Edinburgh Genome Foundry, DAMP lab, SYNBIOCHEM, London DNA Foundry, Shenzhen Institute of Synthetic Biology and so on [91]. In addition to the previously mentioned biofoundries, there are other facilities dedicated to automated DNA synthesis and assembly. For instance, Nathan et al. from the US Department of Energy have developed the j5 computer-aided design (CAD) software. This tool automates the design of scarless, multi-part DNA assembly protocols, including methods like SLIC, Gibson, CPEC, and Golden Gate. Moreover, j5 facilitates the construction of scarless combinatorial DNA libraries. When working with combinatorial DNA libraries of approximately 200 plasmids, j5 is estimated to save 3 to 8 times the time and 10 to 20 times the cost compared to traditional methods [93]. The Baldwin team developed a DNA assembly using Biopart Assembly Standard for Idempotent Cloning (BASIC) on OpenTrons (DNA-BOT) platform at the London DNA Foundry, which supports automated high-throughput assembly of multi-fragments [94]. Using this technique, the team successfully constructed 88 plasmids simultaneously. The following table lists the parameters for automated DNA synthesis in biofoundries (Table 2).

Biofoundries typically encompass a central robotic platform, synthetic biology workflows, computer-aided design (CAD) software, and high-throughput instrumentation [95]. These facilities are highly automated, enabling the conversion of labor-intensive manual tasks into large-scale automated production processes. Biofoundries play a pivotal role in completing the DBTL cycle, facilitating remote customization, distributed design, and cost-efficient production of synthetic organisms [102]. They are suitable for both research and industrial applications [103]. These automated synthetic biofoundries signify a substantial advancement in the realm of synthetic biology, affording an unparalleled capacity to streamline intricate research procedures and foster innovation across various fields.

4. Conclusions and outlook

Currently, chemical synthesis remains the primary method for oligonucleotide production. However, as the demand for large-scale gene and genome synthesis in the field of synthetic biology continues to grow, the limitations of column synthesis have become more apparent. These limitations include the high cost of chemical reagents, numerous side reactions, and low throughput [104]. Efforts have been made to address these shortcomings by synthesizing thousands of oligonucleotides in parallel on chip supports or in microtiter wells [1]. Nevertheless, chip fabrication is a complex process, and microfluidic oligonucleotide synthesis is prone to higher error rates and challenges in isolation and purification. To enhance the sustainability of oligonucleotide synthesis, a shift from phosphoramidite-based chemicals to enzyme-catalyzed techniques has shown promise [51]. DNA enzymatic synthesis technology is currently undergoing rapid development, although there are still challenges to overcome in the synthesis process. Key goals include reducing costs, increasing throughput, and improving oligonucleotide length and fidelity. This necessitates the exploration of new enzymes, enzyme modification, and the development of artificial enzymes to create a more controllable strategy that can adapt to the automation of enzymatic synthesis [104].

Traditional DNA assembly methods are time-consuming and labor-intensive, making it challenging to ensure precision and reliability in synthesis. Introducing automated DNA synthesis technology has the potential to significantly boost efficiency, reduce costs, and advance synthetic biology. Currently, the synthesis and assembly of large DNA fragments exceeding 100 kb represent a significant challenge in the advancement of gene synthesis technology. Zurcher and colleagues have made notable progress in this field by developing continuous genome synthesis (CGS), which allowed them to successfully synthesize a 0.5 Mb segment of the *E. coli* genome within a relatively short period of 10 days [105]. Some polymeric sequences, long repeats and irregular DNA structures have a great influence on the assembly of Mb DNA during the assembly process [104]. As the number of DNA assembly layers increases, so does the need for quality control measures, including clone selection and sequencing. The use of next-generation sequencing techniques on substantial quantities of DNA can significantly reduce these costs [106,107]. The future direction in DNA assembly involves developing algorithms to optimize the synthesis strategy for genetic systems reaching millions of bases. This advancement necessitates the integration of DNA synthesis with high-throughput sequencing platforms, enabling a seamless process that encompasses synthesis, assembly, and the correction of sequencing errors [104].

Some DNA sequences with complex stricture can interfere with oligonucleotides synthesis process, such as hairpin structures [108], high guanine-cytosine (GC) regions [109] and large repeats [110]. Consequently, DNA synthesis and assembly are costly, time-consuming, and require considerable trial-and-error efforts. For instance, synthesizing the JCVI-1.0 genome by Gibson et al. took 15 years and $40 million [79,111]. To address these challenges, the thoughtful design of DNA fragments is essential. However, the design process is intricate. Various approaches, including custom synthesis [112], sequence rewriting [113] and machine learning models [114] can be utilized. Computational design aids in predicting synthesis challenges accurately, offering insights to save time and reduce costs. Additionally, establishing an easily accessible data-driven computing infrastructure is crucial in supporting biological DBTL processes.

DNA synthesis and assembly have a broad range of applications in genetic engineering, synthetic biology, drug development, molecular diagnosis, agricultural breeding, and data storage. In the context of COVID-19 prevention and control, synthetic DNA probes are crucial raw materials for nucleic acid detection kits. Moreover, synthetic DNA significantly expedites the development of biological antibody drug modifications, gene therapy, and oligonucleotide drugs. In agricultural breeding, DNA synthesis contributes to the development of genetically modified crops with enhanced resistance to pests and diseases, improved crop traits and quality, and increased crop yield, resistance, and nutritional value. Furthermore, DNA serves as an efficient storage medium, boasting high storage density, extended storage duration, minimal physical space requirements, and low maintenance costs. DNA synthesis technology, as the cornerstone of DNA storage processes, plays a pivotal role in the transition of DNA data storage from a concept to a practical

application [17].

Researchers in the fields of synthetic biology, automation, and information technology need to engage in interdisciplinary collaborations to design hardware systems that interface seamlessly with lab software. This synergy is imperative for the development of algorithms capable of simulating experimental processes and mitigating the occurrence of large-scale error testing. The primary aim of high-performance DNA synthesis is to achieve efficient, cost-effective, and high-quality DNA production at scale. This encompasses the sustainable, high-throughput, automated, and integrated synthesis of DNA. Such advancements are pivotal for enabling large-scale gene and genome synthesis. Furthermore, ongoing research is merited to improve the efficiency of automated large DNA fragment synthesis and to create automated genome synthesis platforms. These developments hold the potential to drive advancements in the fields of food and chemical production.

Data availability statement

All relevant data are included in the paper.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Yuxin Ma: Writing – original draft. **Zhaoyang Zhang:** Writing – original draft. **Bin Jia:** Writing – review & editing, Supervision, Funding acquisition. **Yingjin Yuan:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful for the financial support from the National Key Research and Development Program of China (No. 2018YFA0903700).

References

[1] S. Kosuri, G.M. Church, Large-scale de novo DNA synthesis: technologies and applications, Nat. Methods 11 (2014) 499–507, https://doi.org/10.1038/nmeth.2918.
[2] J. Cello, A.V. Paul, E. Wimmer, Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template, Science 297 (2002) 1016–1018, https://doi.org/10.1126/science.1072266.
[3] D.G. Gibson, J.I. Glass, C. Lartigue, V.N. Noskov, R.Y. Chuang, M.A. Algire, G.A. Benders, M.G. Montague, L. Ma, M.M. Moodie, et al., Creation of a bacterial cell controlled by a chemically synthesized genome, Science 329 (2010) 52–56, https://doi.org/10.1126/science.1190719.
[4] Y. Shen, Y. Wang, T. Chen, F. Gao, J. Gong, D. Abramczyk, R. Walker, H. Zhao, S. Chen, W. Liu, et al., Deep functional analysis of synII, a 770-kilobase synthetic yeast chromosome, Science 355 (2017) eaaf4791, https://doi.org/10.1126/science.aaf4791.
[5] N. Annaluru, H. Muller, L.A. Mitchell, S. Ramalingam, G. Stracquadanio, S.M. Richardson, J.S. Dymond, Z. Kuang, L.Z. Scheifele, E.M. Cooper, et al., Total synthesis of a functional designer eukaryotic chromosome, Science 344 (2014) 55–58, https://doi.org/10.1126/science.1249252.
[6] Z.X. Xie, B.Z. Li, L.A. Mitchell, Y. Wu, X. Qi, Z. Jin, B. Jia, X. Wang, B.X. Zeng, H.M. Liu, et al., "Perfect" designer chromosome V and behavior of a ring derivative, Science 355 (2017) eaaf4704, https://doi.org/10.1126/science.aaf4704.
[7] L.A. Mitchell, A. Wang, G. Stracquadanio, Z. Kuang, X. Wang, K. Yang, S. Richardson, J.A. Martin, Y. Zhao, R. Walker, et al., Synthesis, debugging, and effects of synthetic chromosome consolidation: synVI and beyond, Science 355 (2017) eaaf4831, https://doi.org/10.1126/science.aaf4831.
[8] Y. Wu, B.Z. Li, M. Zhao, L.A. Mitchell, Z.X. Xie, Q.H. Lin, X. Wang, W.H. Xiao, Y. Wang, X. Zhou, et al., Bug mapping and fitness testing of chemically synthesized chromosome X, Science 355 (2017) eaaf4706, https://doi.org/10.1126/science.aaf4706.
[9] W. Zhang, G. Zhao, Z. Luo, Y. Lin, L. Wang, Y. Guo, A. Wang, S. Jiang, Q. Jiang, J. Gong, et al., Engineering the ribosomal DNA in a megabase synthetic chromosome, Science 355 (2017) eaaf3981, https://doi.org/10.1126/science.aaf3981.
[10] A.M. Michelson, S.R. Todd, Synthesis of a dithymidine dinucleotide containing a 3': 5'-internucleotidic linkage, J. Chem. Soc. 2632–2638 (1955), https://doi.org/10.1039/JR9550002632.
[11] R.H. Hall, S. Todd, R.F. Webb, Mixed anhydrides as intermediates in the synthesis of dinucleoside phosphates, J. Chem. Soc. 3291–3296 (1957), https://doi.org/10.1039/JR9570003291.
[12] H.G. Khorana, W.E. Razzell, P.T. Gilham, G.M. Tener, E.H. Pol, Syntheses of dideoxyribonucleotides, J. Am. Chem. Soc. 79 (1957) 1002–1003, https://doi.org/10.1021/ja01561a065.
[13] S.L. Beaucage, M.H. Caruthers, Deoxynucleoside phosphoramidites—a new class of key intermediates for deoxypolynucleotide synthesis, Tetrahedron Lett. 22 (1981) 1859–1862, https://doi.org/10.1016/S0040-4039(01)90461-7.
[14] M.H. Caruthers, The chemical synthesis of DNA/RNA: our gift to science, J. Biol. Chem. 288 (2013) 1420–1427, https://doi.org/10.1074/jbc.X112.442855.
[15] A. Hoose, R. Vellacott, M. Storch, P.S. Freemont, M.G. Ryadnov, DNA synthesis technologies to close the gene writing gap, Nat. Rev. Chem 7 (2023) 144–161, https://doi.org/10.1038/s41570-022-00456-9.
[16] X.E. Jiang, Y. Wang, Y. Shen, The review of DNA synthesis technologies and instruments development, J. Integr. Technol. 10 (2021) 80–95.
[17] X. Huang, J. Dai, DNA synthesis technology: foundation of DNA data storage, Syn. Bio. J. 2 (2021) 335–353, https://doi.org/10.12211/2096-8280.2020-088.
[18] K.S. Boles, K. Kannan, J. Gill, M. Felderman, H. Gouvis, B. Hubby, K.I. Kamrud, J.C. Venter, D.G. Gibson, Digital-to-biological converter for on-demand production of biologics, Nat. Biotechnol. 35 (2017) 672–675, https://doi.org/10.1038/nbt.3859.

[19] M. Jensen, L. Roberts, A. Johnson, M. Fukushima, R. Davisa, Next generation 1536-well oligonucleotide synthesizer with on-the-fly dispense, J. Biotechnol. 171 (2014) 76–81, https://doi.org/10.1016/j.jbiotec.2013.11.027.
[20] B.I. Andrews, F.D. Antia, S.B. Brueggemeier, L.J. Diorazio, S.G. Koenig, M.E. Kopach, H. Lee, M. Olcrich, A.L. Watson, Sustainability challenges and opportunities in oligonucleotide manufacturing, J. Org. Chem. 86 (2021) 49–61, https://doi.org/10.1021/acs.joc.0c02291.
[21] R.T. Pon, S. Yu, Y.S. Sanghvi, Tandem oligonucleotide synthesis on solid-phase supports for the production of multiple oligonucleotides, J. Org. Chem. 67 (2002) 856–864, https://doi.org/10.1021/jo0160773.
[22] D.A. Lashkari, S.P. Hunicke-Smith, R.M. Norgren, T. Brennan, An automated multiplex oligonucleotide synthesizer: development of high-throughput, low-cost DNA synthesis, P. Natl. Acad. Sci. 92 (1995) 7912–7915, https://doi.org/10.1073/pnas.92.17.7912.
[23] J.H. Butler, M. Cronin, K.M. Anderson, G.M. Biddison, F. Chatelain, M. Cummer, D.J. Davi, L. Fisher, A.W. Frauendorf, F.W. Frueh, et al., In situ synthesis of oligonucleotide arrays by using surface tension, J. Am. Chem. Soc. 123 (2001) 8887–8894, https://doi.org/10.1021/ja003758r.
[24] E.M. LeProust, B.J. Peck, K. Spirin, H.B. McCuen, B. Moore, E. Namsaraev, M.H. Caruthers, Synthesis of high-quality libraries of long (150 mer) oligonucleotides by a novel depurination controlled process, Nucleic Acids Res. 38 (2010) 2522–2540, https://doi.org/10.1093/nar/gkq163.
[25] S.P.A. Fodor, J.L. Read, M.C. Pirrung, L. Strayer, A.T. Lu, D. Solas, Light-directed, spatially addressable parallel chemical synthesis, Science 251 (1991) 767–773, https://doi.org/10.1126/science.1990438.
[26] A.C. Pease, D. Solas, E.J. Sullivan, M.T. Cronin, C.P. Holmes, S.P. Fodor, Light-generated oligonucleotide arrays for rapid DNA sequence analysis, P. Natl. Acad. Sci. 91 (1994) 5022–5026, https://doi.org/10.1073/pnas.91.11.5022.
[27] S. Singh-Gasson, R.D. Green, Y. Yue, C. Nelson, F. Blattner, M.R. Sussman, F. Cerrina, Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array, Nat. Biotechnol. 17 (1999) 974–978, https://doi.org/10.1038/13664.
[28] X. Gao, P.E. Le, H. Zhang, O. Srivannavit, E. Gulari, P. Yu, C. Nishiguchi, Q. Xiang, X. Zhou, A flexible light-directed DNA chip synthesis gated by deprotection using solution photogenerated acids, Nucleic Acids Res. 22 (2001) 4744–4750, https://doi.org/10.1093/nar/29.22.4744.
[29] K. Hölz, J.K. Hoi, E. Schaudy, V. Somoza, J. Lietard, M.M. Somoza, High-efficiency reverse (5'→3') synthesis of complex DNA microarrays, Sci. Rep. 8 (2018) 15099, https://doi.org/10.1038/s41598-018-33311-3.
[30] J. Lietard, A. Leger, Y. Erlich, N. Sadowski, W. Timp, M.M. Somoza, Chemical and photochemical error rates in light-directed synthesis of complex DNA libraries, Nucleic Acids Res. 49 (2021) 6687–6701, https://doi.org/10.1093/nar/gkab505.
[31] X. Zhou, Q. Zhu, C. Eicken, N. Sheng, X. Zhang, L. Yang, X. Gao, MicroRNA profiling using μParaflo microfluidic array technology, Methods Mol. Biol. 822 (2012) 153–182, https://doi.org/10.1007/978-1-61779-427-8_11.
[32] A.P. Blanchard, R.J. Kaiser, L.E. Hood, High-density oligonucleotide arrays, Biosens. Bioelectron. 11 (1996) 687–690, https://doi.org/10.1016/0956-5663(96)83302-1.
[33] T.R. Hughes, M. Mao, A.R. Jones, J. Burchard, M.J. Marton, K.W. Shannon, S.M. Lefkowitz, M. Ziman, J.M. Schelter, M.R. Meyer, et al., Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer, Nat. Biotechnol. 19 (2001) 342–347, https://doi.org/10.1038/86730.
[34] C. Lausted, T. Dahl, C. Warren, K. King, K. Smith, M. Johnson, R. Saleem, J. Aitchison, L. Hood, S.R. Lasky, POSaM: a fast, flexible, open-source, inkjet oligonucleotide synthesizer and microarrayer, Genome Biol. 5 (2004) R58, https://doi.org/10.1186/gb-2004-5-8-r58.
[35] H. Li, Y. Huang, Z. Wei, W. Wang, Z. Yang, Z. Liang, Z. Li, An oligonucleotide synthesizer based on a microreactor chip and an inkjet printer, Sci. Rep. 9 (2019) 5058, https://doi.org/10.1038/s41598-019-41519-0.
[36] L. Cao, B. Coventry, I. Goreshnik, B. Huang, W. Sheffler, J.S. Park, K.M. Jude, I. Marković, R.U. Kadam, K.H.G. Verschueren, et al., Design of protein-binding proteins from the target structure alone, Nature 605 (2022) 551–560, https://doi.org/10.1038/s41586-022-04654-9.
[37] R.D. Egeland, E.M. Southern, Electrochemically directed synthesis of oligonucleotides for DNA microarray fabrication, Nucleic Acids Res. 33 (2005) e125, https://doi.org/10.1093/nar/gni117.
[38] A.L. Ghindilis, M.W. Smith, K.R. Schwarzkopf, K.M. Roth, K. Peyvan, S.B. Munro, M.J. Lodes, A.G. Stover, K. Bernards, K. Dill, et al., CombiMatrix oligonucleotide arrays: genotyping and gene expression assays employing electrochemical detection, Biosens. Bioelectron. 22 (2007) 1853–1860, https://doi.org/10.1016/j.bios.2006.06.024.
[39] M.T. Holden, M.C.D. Carter, S.K. Ting, D.M. Lynn, L.M. Smith, Parallel DNA synthesis on poly (ethylene terephthalate), Chembiochem 18 (2017) 1914–1916, https://doi.org/10.1002/cbic.201700321.
[40] K. Levrie, K. Jans, G. Schepers, R. Vos, P.V. Dorpe, L. Lagae, C.V. Hoof, A.V. Aerschot, T. Stakenborg, Direct on-chip DNA synthesis using electrochemically modified gold electrodes as solid support, Jpn. J. Appl. Phys. 57 (2018) 04FM01, https://doi.org/10.7567/jjap.57.04fm01.
[41] K. Maurer, N. Yazvenko, J. Wilmoth, J. Cooper, W. Lyon, D. Danley, Use of a multiplexed CMOS microarray to optimize and compare oligonucleotide binding to DNA probes synthesized or immobilized on individual electrodes, Sensors-Basel 10 (2010) 7371–7385, https://doi.org/10.3390/s100807371.
[42] J. Cooper, N. Yazvenko, K. Peyvan, K. Maurer, C.R. Taitt, W. Lyon, D.L. Danley, Targeted deposition of antibodies on a multiplex CMOS microarray and optimization of a sensitive immunoassay using electrochemical detection, PLoS One 5 (2010) e9781, https://doi.org/10.1371/journal.pone.0009781.
[43] A.J. Ferguson, M.J. Hayes, B.C. Kirkpatrick, Y. Lin, V. Narayan, A. Prak, Thermofluidic chip containing virtual thermal wells, Eng. Biol. 3 (2019) 20–23, https://doi.org/10.1049/enb.2018.5010.
[44] S. Crosby, Unlocking synthetic biology through DNA synthesis, Chim. Oggi 38 (2020) 22–24.
[45] S. Ma, N. Tang, J. Tian, DNA synthesis, assembly and applications in synthetic biology, Curr. Opin. Chem. Biol. 16 (2012) 260–267, https://doi.org/10.1016/j.cbpa.2012.05.001.
[46] F.J. Bollum, Thermal conversion of nonpriming deoxyribonucleic acid to primer, J. Biol. Chem. 234 (1959) 2733–2734.
[47] F.J. Bollum, Oligodeoxyribonucleotide-primed reactions catalyzed by calf thymus polymerase, J. Biol. Chem. 237 (1962) 1945–1949.
[48] M.A. Jensen, R.W. Davis, Template-independent enzymatic oligonucleotide synthesis (TiEOS): its history, prospects, and challenges, Biochemistry 57 (2018) 1821–1832, https://doi.org/10.1021/acs.biochem.7b00937.
[49] I. Sarac, M. Hollenstein, Terminal deoxynucleotidyl transferase in the synthesis and modification of nucleic acids, Chembiochem 20 (2018) 860–871, https://doi.org/10.1002/cbic.201800658.
[50] M. Eisenstein, Enzymatic DNA synthesis enters new phase, Nat. Biotechnol. 38 (2020) 1113–1115, https://doi.org/10.1038/s41587-020-0695-9.
[51] S. Palluk, D.H. Arlow, T. de Rond, S. Barthel, J.S. Kang, R. Bector, H.M. Baghdassarian, A.N. Truong, P.W. Kim, A.K. Singh, et al., De novo DNA synthesis using polymerase-nucleotide conjugates, Nat. Biotechnol. 36 (2018) 645–650, https://doi.org/10.1038/nbt.4173.
[52] W.P. Stemmer, A. Crameri, K.D. Ha, T.M. Brennan, H.L. Heyneker, Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides, Gene 164 (1995) 49–53, https://doi.org/10.1016/0378-1119(95)00511-4.
[53] H.O. Smith, C.A. Hutchison, C. Pfannkoch, J.C. Venter, Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides, P. Natl. Acad. Sci. USA. 100 (2003) 15440–15445, https://doi.org/10.1073/pnas.2237126100.
[54] L.C. Au, F.Y. Yang, W.J. Yang, S.H. Lo, C.F. Kao, Gene synthesis by a LCR-based approach: high-level production of leptin-L54 using synthetic gene in Escherichia coli, Biochem. Biophys. Res. Commun. 1 (1998) 200–203, https://doi.org/10.1006/bbrc.1998.8929.
[55] R.M. Horton, H.D. Hunt, S.N. Ho, J.K. Pullen, L.R. Pease, Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension, Gene 77 (1989) 61–68, https://doi.org/10.1016/0378-1119(89)90359-4.
[56] J. Quan, J. Tian, Circular polymerase extension cloning of complex gene libraries and pathways, PLoS One 4 (2009) e6441, https://doi.org/10.1371/journal.pone.0006441.
[57] J. Quan, J. Tian, Circular polymerase extension cloning for high-throughput cloning of complex and combinatorial DNA libraries, Nat. Protoc. 6 (2011) 242–251, https://doi.org/10.1038/nprot.2010.181.
[58] C. Engler, R. Kandzia, S. Marillonnet, A one pot, one step, precision cloning method with high throughput capability, PLoS One 3 (2008) e3647, https://doi.org/10.1371/journal.pone.0003647.
[59] D.G. Gibson, L. Young, R.Y. Chuang, J.C. Venter, C.A. Hutchison, H.O. Smith, Enzymatic assembly of DNA molecules up to several hundred kilobases, Nat. Methods 6 (2009) 343–345, https://doi.org/10.1038/nmeth.1318.

[60] D.G. Gibson, H.O. Smith, C.A. Hutchison, J.C. Venter, C. Merryman, Chemical synthesis of the mouse mitochondrial genome, Nat. Methods 7 (2010) 901–903, https://doi.org/10.1038/nmeth.1515.
[61] P.M. D'Agostino, T.A.M. Gulder, Direct pathway cloning combined with sequence- and ligation-independent cloning for fast biosynthetic gene cluster refactoring and heterologous expression, ACS Synth. Biol. 7 (2018) 1702–1708, https://doi.org/10.1021/acssynbio.8b00151.
[62] P.K. Saiki, S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, N. Arnheim, Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia, Science 4732 (1985) 1350–1354, https://doi.org/10.1126/science.2999980.
[63] P.K. Saiki, D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, H.A. Erlich, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase, Science 239 (1988) 487–491, https://doi.org/10.1126/science.2448875.
[64] P. Zhang, Y. Ding, W. Liao, Q. Chen, H. Zhang, P. Qi, T. He, J. Wang, S. Deng, T. Pan, et al., A simple, universal, efficient PCR-based gene synthesis method: sequential OE-PCR gene synthesis, Gene 524 (2013) 347–354, https://doi.org/10.1016/j.gene.2013.03.126.
[65] D.G. Gibson, Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides, Nucleic Acids Res. 37 (2009) 6984–6990, https://doi.org/10.1093/nar/gkp687.
[66] D.G. Gibson, Oligonucleotide assembly in yeast to produce synthetic DNA fragments, Methods Mol. Biol. 852 (2012) 11–21, https://doi.org/10.1007/978-1-61779-564-0_2.
[67] P.R. Dormitzer, P. Suphaphiphat, D.G. Gibson, D.E. Wentworth, T.B. Stockwell, M.A. Algire, N. Alperovich, M. Barro, D.M. Brown, S. Craig, et al., Synthetic generation of influenza vaccine viruses for rapid response to pandemics, Sci. Transl. Med. 5 (2013) 185, https://doi.org/10.1126/scitranslmed.3006368.
[68] S. Kosuri, N. Eroshenko, E.M. Leproust, M. Super, J. Way, J.B. Li, G.M. Church, Scalable gene synthesis by selective amplification of DNA pools from high-fidelity microchips, Nat. Biotechnol. 28 (2010) 1295–1299, https://doi.org/10.1038/nbt.1716.
[69] J. Quan, I. Saaem, N. Tang, S. Ma, N. Negre, H. Gong, K.P. White, J. Tian, Parallel on-chip gene synthesis and application to optimization of protein expression, Nat. Biotechnol. 29 (2011) 449–452, https://doi.org/10.1038/nbt.1847.
[70] Y. Zhang, J.P.P. Muyrers, G. Testa, A.F. Stewart, DNA cloning by homologous recombination in Escherichia coli, Nat. Biotechnol. 18 (2000) 1314–1317, https://doi.org/10.1038/82449.
[71] T. Iwata, S. Kaneko, Y. Shiwa, T. Enomoto, H. Yoshikawa, J. Hirota, Bacillus subtilis genome vector-based complete manipulation and reconstruction of genomic DNA for mouse transgenesis, BMC Genom. 14 (2013) 300, https://doi.org/10.1186/1471-2164-14-300.
[72] T. Yadav, B. Carrasco, E. Serrano, J.C. Alonso, Roles of Bacillus subtilis DprA and SsbA in RecA-mediated genetic recombination, J. Biol. Chem. 289 (2014) 27640–27652, https://doi.org/10.1074/jbc.M114.577924.
[73] T. Ogawa, T. Iwata, S. Kaneko, M. Itaya, J. Hirota, An inducible recA expression Bacillus subtilis genome vector for stable manipulation of large DNA fragments, BMC Genom. 16 (2015) 209, https://doi.org/10.1186/s12864-015-1425-4.
[74] D. Jovicevic, B.A. Blount, T. Ellis, Total synthesis of a eukaryotic chromosome: redesigning and SCRaMbLE-ing yeast, Bioessays 36 (2014) 855–860, https://doi.org/10.1002/bies.201400086.
[75] N.G. Kuijpers, D. Solis-Escalante, L. Bosman, M. van den Broek, J.T. Pronk, J.M. Daran, P. Daran-Lapujade, A versatile, efficient strategy for assembly of multi-fragment expression vectors in Saccharomyces cerevisiae using 60 bp synthetic recombination sequences, Microb. Cell Factories 12 (2013) 47, https://doi.org/10.1186/1475-2859-12-47.
[76] A. Hinnen, J.B. Hicks, G.R. Fink, Transformation of yeast, P. Natl. Acad. Sci. USA. 75 (1978) 1929–1933, https://doi.org/10.1073/pnas.75.4.1929.
[77] D.G. Gibson, G.A. Benders, K.C. Axelrod, J. Zaveri, M.A. Algire, M. Moodie, M.G. Montague, J.C. Venter, H.O. Smith, C.A. Hutchison, One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic Mycoplasma genitalium genome, P. Natl. Acad. Sci. USA. 105 (2008) 20404–20409, https://doi.org/10.1073/pnas.0811011106.
[78] C. Lartigue, S. Vashee, M.A. Algire, R. Chuang, G.A. Benders, L. Ma, V.N. Noskov, E.A. Denisova, D.G. Gibson, N. Assad-Garcia, et al., Creating bacterial strains from genomes that have been cloned and engineered in yeast, Science 325 (2009) 1693–1696, https://doi.org/10.1126/science.1173759.
[79] D.G. Gibson, G.A. Benders, C. Andrews-Pfannkoch, E.A. Denisova, H. Baden-Tillson, J. Zaveri, T.B. Stockwell, A. Brownley, D.W. Thomas, M.A. Algire, et al., Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome, Science 319 (2008) 1215–1220, https://doi.org/10.1126/science.1151721.
[80] J. Zhou, R. Wu, X. Xue, Z. Qin, CasHRA (Cas9-facilitated homologous recombination assembly) method of constructing megabase-sized DNA, Nucleic Acids Res. 44 (2016) e124, https://doi.org/10.1093/nar/gkw475.
[81] B.C. King, K. Vavitsas, N.K. Ikram, J. Schrøder, L.B. Scharff, J. Bassard, B. Hamberger, P.E. Jensen, H.T. Simonsen, In vivo assembly of DNA-fragments in the moss, Physcomitrella patens, Sci. Rep. 6 (2016) 25030, https://doi.org/10.1038/srep25030.
[82] Q. Pang, S. Ma, H. Han, X. Jin, X. Liu, T. Su, Q. Qi, Phage enzyme-assisted direct in vivo DNA assembly in multiple microorganisms, ACS Synth. Biol. 11 (2022) 1477–1487, https://doi.org/10.1021/acssynbio.1c00529.
[83] J. Liang, R. Chao, Z. Abil, Z. Bao, H. Zhao, FairyTALE: a high-throughput TAL effector synthesis platform, ACS Synth. Biol. 3 (2014) 67–73, https://doi.org/10.1021/sb400109p.
[84] R. Chao, J. Liang, I. Tasan, T. Si, L. Ju, H. Zhao, Fully automated one-step synthesis of single-transcript TALEN pairs using a biological foundry, ACS Synth. Biol. 6 (2017) 678–685, https://doi.org/10.1021/acssynbio.6b00293.
[85] T. Si, R. Chao, Y. Min, Y. Wu, W. Ren, H. Zhao, Automated multiplex genome-scale engineering in yeast, Nat. Commun. 8 (2017) 15187, https://doi.org/10.1038/ncomms15187.
[86] B. Enghiad, H. Zhao, Programmable DNA-guided artificial restriction enzymes, ACS Synth. Biol. 6 (2017) 752–757, https://doi.org/10.1021/acssynbio.6b00324.
[87] C. Alvarez, DNA assembly and cloning in an overnight run with the BioXp™ 3200 system, Nat. Methods 12 (2015), https://doi.org/10.1038/nmeth.f.390.
[88] https://www.the-scientist.com/features/2020-top-10-innovations-68176..
[89] R.Y. Hu, S.Y. Zhang, H.L. Meng, H. Yu, J.Z. Zhang, X.Z. Luo, T. Si, C.L. Liu, Y. Qiao, Machine learning for synthetic biology: methods and applications, Chin. Sci. Bull. 66 (2021) 284–299 (in Chinese).
[90] T. Tang, L.H. Fu, E.P. Guo, Z.S. Zhang, Z.N. Wang, C.F. Ma, Z.Y. Zhang, J.Z. Zhang, J.D. Huang, T. Si, Automation in synthetic biology using biological foundries, Chin. Sci. Bull. 66 (2021) 300–309, https://doi.org/10.1360/TB-2020-0498 (in Chinese).
[91] N. Hillson, M. Caddick, Y. Cai, J.A. Carrasco, M.W. Chang, N.C. Curach, D.J. Bell, R.L. Feuvre, D.C. Friedman, X. Fu, et al., Building a global alliance of biofoundries, Nat. Commun. 10 (2019) 2040, https://doi.org/10.1038/s41467-019-10079-2.
[92] M. Bates, J. Lachoff, D. Meech, V. Zulkower, A. Moisy, Y. Luo, H. Tekotte, C.J.F. Scheitz, R. Khilari, F. Mazzoldi, et al., Genetic constructor: an online DNA design platform, ACS Synth. Biol. 6 (2017) 2362–2365, https://doi.org/10.1021/acssynbio.7b00236.
[93] N.J. Hillson, R.D. Rosengarten, J.D. Keasling, J5 DNA assembly design automation software, ACS Synth. Biol. 1 (2012) 14–21, https://doi.org/10.1021/sb2000116.
[94] M. Storch, M.C. Haines, G.S. Baldwin, DNA-BOT: a low-cost, automated DNA assembly platform for synthetic biology, Synth. Biol. 5 (2020), https://doi.org/10.1093/synbio/ysaa010 ysaa010.
[95] J. Zhang, Y. Chen, L. Fu, E. Guo, B. Wang, L. Dai, T. Si, Accelerating strain engineering in biofuel research via build and test automation of synthetic biology, Curr. Opin. Biotechnol. 67 (2021) 88–98, https://doi.org/10.1016/j.copbio.2021.01.010.
[96] M. HamediRad, R. Chao, S. Weisberg, J. Lian, S. Sinha, H. Zhao, Towards a fully automated algorithm driven platform for biosystems design, Nat. Commun. 10 (2019) 5150, https://doi.org/10.1038/s41467-019-13189-z.
[97] B. Enghiad, P. Xue, N. Singh, A.G. Boob, C. Shi, V.A. Petrov, R. Liu, S.S. Peri, S.T. Lane, E.D. Gaither, et al., PlasmidMaker is a versatile, automated, and high throughput end-to-end platform for plasmid construction, Nat. Commun. 13 (2022) 2697, https://doi.org/10.1038/s41467-022-30355-y.
[98] S. de Kok, L.H. Stanton, T. Slaby, M. Durot, V.F. Holmes, K.G. Patel, D. Platt, E.B. Shapland, Z. Serber, J. Dean, et al., Rapid and reliable DNA assembly via ligase cycling reaction, ACS Synth. Biol. 3 (2014) 97–106, https://doi.org/10.1021/sb4001992.

[99] S. Chandran, Rapid assembly of DNA via ligase cycling reaction (LCR), Methods Mol. Biol. 1472 (2017) 105–110, https://doi.org/10.1007/978-1-4939-6343-0_8.
[100] C.J. Robinson, M.S. Dunstan, N. Swainston, J. Titchmarsh, E. Takano, N.S. Scrutton, A.J. Jervis, Multifragment DNA assembly of biochemical pathways via automated ligase cycling reaction, Methods Enzymol. 608 (2018) 369–392, https://doi.org/10.1016/bs.mie.2018.04.011.
[101] D.I. Walsh, M. Pavan, L. Ortiz, S. Wick, J. Bobrow, N.J. Guido, S. Leinicke, D. Fu, S. Pandit, L. Qin, et al., Standardizing automated DNA assembly: best practices, metrics, and protocols using robots, SLAS Technol 24 (2019) 282–290, https://doi.org/10.1177/2472630318825335.
[102] J.M. Cui, B.Z. Zhang, Y.F. Ma, X.F. Fu, M. Wang, C.L. Liu, Engineering platforms for synthetic biology research, Bull. Chin. Acad. Sci. 33 (2018) 1249–1257, https://doi.org/10.16418/j.issn.1000-3045.2018.11.013.
[103] J. Tellechea-Luzardo, I. Otero-Muras, A. Goñi-Moreno, P. Carbonell, Fast biofoundries: coping with the challenges of biomanufacturing, Trends Biotechnol. 40 (2022) 831–842, https://doi.org/10.1016/j.tibtech.2021.12.006.
[104] K. Peng, X. Lu, J. Cheng, Y. Liu, H. Jiang, X. Guo, Advances in technologies for de novo DNA synthesis, assembly and error correction, Syn. Bio. J. 1 (2020) 697–708, https://doi.org/10.12211/2096-8280.2020-034.
[105] J.F. Zürcher, A.A. Kleefeldt, L.F.H. Funke, J. Birnbaum, J. Fredens, S. Grazioli, K.C. Liu, M. Spinck, G. Petris, P. Murat, et al., Continuous synthesis of E. coli genome sections and Mb-scale human DNA assembly, Nature 619 (2023) 555–562, https://doi.org/10.1038/s41586-023-06268-1.
[106] S. Goodwin, J.D. McPherson, W.R. McCombie, Coming of age: ten years of nextgeneration sequencing technologies, Nat. Rev. Genet. 17 (2016) 333–351, https://doi.org/10.1038/nrg.2016.49.
[107] S. Ma, I. Saaem, J. Tian, Error correction in gene synthesis technology, Trends Biotechnol. 30 (2012) 147–154, https://doi.org/10.1016/j.tibtech.2011.10.002.
[108] M.J. Czar, J.C. Anderson, J.S. Bader, J. Peccoud, Gene synthesis demystified, Trends Biotechnol. 27 (2009) 63–72, https://doi.org/10.1016/j.tibtech.2008.10.007.
[109] R.A. Hughes, A.D. Ellington, Synthetic DNA synthesis and assembly: putting the synthetic in synthetic biology, Cold Spring Harbor Perspect. Biol. 9 (2017) a023812, https://doi.org/10.1101/cshperspect.a023812.
[110] C. Feng, M. Dai, Y. Liu, M. Chen, Sequence repetitiveness quantification and de novo repeat detection by weighted k-mer coverage, Briefings Bioinf. 22 (2021), https://doi.org/10.1093/bib/bbaa086 bbaa086.
[111] M. Baker, The next step for the synthetic genome, Nature 473 (2011) 403–408, https://doi.org/10.1038/473403a.
[112] M. Christen, S. Deutsch, B. Christen, Genome calligrapher: a web tool for refactoring bacterial genome sequences for de novo DNA synthesis, ACS Synth. Biol. 4 (2015) 927–934, https://doi.org/10.1021/acssynbio.5b00087.
[113] N. Ostrov, M. Landon, M. Guell, G. Kuznetsov, J. Teramoto, N. Cervantes, M. Zhou, K. Singh, M.G. Napolitano, M. Moosburner, et al., Design, synthesis, and testing toward a 57-codon genome, Science 353 (2016) 819–822, https://doi.org/10.1126/science.aaf3639.
[114] Y. Zheng, K. Song, Z.X. Xie, M.Z. Han, F. Guo, Y.J. Yuan, Machine learning-aided scoring of synthesis difficulties for designer chromosomes, Sci. China Life Sci. (2023), https://doi.org/10.1007/s11427-023-2306-x.