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Synthetic Oligodeoxyribonucleotides and Human Health

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Abstract: The history of synthetic DNA includes the discovery of its structure and the development of techniques for its chemical synthesis. DNA synthesis story dates back to the work of E. J. Corey when he used the term synthons. At that time, this was a new but an important concept, never conceived before in the area of nucleic acid research. A synthon is defined as a hypothetical chemical fragment that can be used as a potential starting reagent in the synthesis of a target molecule. Synthons are created by breaking a target molecule into fragments through a series of logical disconnection involving deep chemistry. Therefore, synthon preceded the synthesis of DNA. The first successful synthetic DNA synthesis was reported by Khorana and his team.Fortunately, the discovery of DNA structure reported earlier by Watson and Crick turned out to be so accurate that it stood all the rigours and test of time. This discovery affected both pure and applied sciences changing the face of Biochemical, Biomedical and Biological researches in a big way. Today multibillion dollars Biotechnology industry is revolving around the synthetic DNA. Here, we discuss historical perspective of its synthesis with brief background information, highlighting commercially available synthesizers' and use of synthetic DNA in difference areas of biology, medicine and forensic sciences. We also propose a conceptual framework of cancer prognosis involving NSG DNA fingerprinting. Rampant use of synthetic DNA in conducting polymerase Chain Reaction (PCR) is all too well known to us, indeed a boon for human health care system. This is because PCR is helpful for DNA based disease diagnosis, identification and origin of samples in forensic cases, gene expression and regulation studies, characterization of microbiomes, genome analysis, species and gender identification and DNA fingerprinting. Conceptually, synthetic DNA may be used as chromosome specific marker if unique sequences are pre-recorded from such chromosomes. Synthetic DNA can be used to fish out transcribing sequences close to repeat element by employing minisatellite associated sequence amplification (MASA) by a simple PCR. Using single or multiple synthetic oligonucleotides, gene expression can be monitored and quantitated. This approach is useful both for genomic DNA and cDNA. Finally species and gender specific markers based on synthetic DNA may be developed for any species. Such markers are useful both for species identification in forensic cases and for identification of newer mutations. Accordingly, mutational load in any species may be uncovered. This approach is particularly useful for genes involved in causing human diseases. Similarly, screening of genomic and cDNA libraries may be conducted using synthetic DNA circumventing the use of radiolabelled genome derived cloned probes. Frequent use of PCR has enhanced the requirement of synthetic DNA for mining a large number of normal alleles and diseased genes. Synthetic DNA may be used for ascertaining the food adulteration, cell culture contamination, strain identification and for monitoring the success of bone marrow transplantation. Without synthetic DNA, sequencing reaction cannot be done. This paper is envisaged to impel the thoughts of both students and researchers enabling them to articulate their research dreams in a better and more logical manner. This will not only result in some original scientific contributions but also augment the much desired human health care system.

Keywords: Biomarkers, DNA, DNA based diagnosis, DNA sequencing, Genome analysis, Species specific probes, Synthetic solid matrix, Synthons.

DNA neither cares nor knows. DNA just is. And we dance to its music.

—Richard Dawkins (1995)

River Out of Eden: A Darwinian Life

River Out of Eden: A Darwinian View of Life is a 1995 popular science book by Richard Dawkins. The book is about Darwinian evolution and summarizes the topics covered in his

earlier books, The Selfish Gene, The Extended Phenotype and The Blind Watchmaker.

—Richard Dawkins (1995)

I. Introduction

The research advancement in one area affects the development and innovation in other areas facilitating the progress of interdisciplinary science. Since the discovery of double helix DNA (Watson and Crick, 1953), researcher examined this proposed structure from all possible angles only to confirm that the same is indeed highly accurate. Not only, it fulfills all the robust criteria, technical nuances of cell, packaging of chromosome, nucleus, duplication, replication, transcription, and translation but also accommodates newer emerging concepts such as mutational load, DNA roof reading, DNA repair and genome editing, copy number variation of genes, gene conversion, evolution of new genes and death of an old one. Synthetic DNA facilitates all these studies seamlessly (Katju *et al.,* 2003). This demonstrates its enormous applied potential in basic and advanced Biological, Biomedical and Forensic Researches. Indeed, visionary were the scientists who envisioned its enormous applications as a multidisciplinary tool. With the result, they undertook its chemical synthesis. This way, birth of synthetic DNA took place (Corey, 1967). A perusal of literature suggests that in the beginning, coupling of DNA used to take enormously long time. With the improved chemistry, automation coupled with use of computer and algorithm, DNA synthesis has become better, faster and more reliable. Earlier, during the synthesis, synthetic DNA used to carry large number of truncated molecules. However, soon this issue was resolved and now machine makes perfect Oligodeoxyribonucleotides without giving rise to any truncated molecules (Kaplan, 1985).

During the early days of synthetic DNA synthesis, the process was extremely slow, often taking several years to create even short DNA sequences as mentioned earlier. It was possible only to synthesize small fragments of DNA, due to limitations in chemical synthesis techniques and purification methods. Startlingly, the first synthetic gene, a yeast tRNA, took several years to create using the methods available at the time (Khorana *et al.,* 1972). Arthur Kornberg was the first to isolate DNA polymerase, the enzyme that assembles DNA from its components. Arthur Kornberg worked in Stanford University School of Medicine at Palo Alto, California. He was a Professor of Biochemistry there since 1959 until his retirement as a Professor Emeritus. Kornberg and Severo Ochoa of New York University were the first to demonstrate the mechanism of biological synthesis of DNA in a test tube (Kornberg, 1960). This work earned them Nobel Prize in 1959 in Physiology and Medicine (Friedberg, 2016).

Synthetic DNA synthesis began with early research by scientists like Har Gobind Khorana, who pioneered methods to chemically synthesize short DNA fragments (oligonucleotides) and eventually assembled them to create the first fully synthetic gene, a yeast tRNA advancing the field of Synthetic Biology (Khorana *et al.,* 1972). This process involved building synthetic DNA base-by-base using chemical reactions, allowing for precise control over its sequence (Li *et al.,* 2004).

The synthesis of synthetic DNA is most comprehensively discussed in Volume 200 of *Methods* in Enzymology, first published in 1991 (https://doi.org/10.1016/0014-5793(92)80485-Y) which focuses specifically on "Nucleic Acid Synthesis". This volume covers detailed protocols and techniques for enzymatic DNA synthesis, including methods for assembling long DNA fragments and generating custom sequences. Here we provide a brief description of this aspect and highlight the several types of commercially available DNA synthesizers.

II. Commercially Available DNA Synthesizers

Surprisingly, four months' time for a single base coupling at the outset has now reduced to less than a minute. All these became possible with the constant efforts towards the improvement of its chemistry and development of more powerful computer program. Now, the synthesis of oligonucleotide is algorithm driven and is fully automated. Dr. OLigo 768XLc is commercially available high throughput Oligo synthesizer (Fig. 1). This has the ability to modify the bases using altered chemistry or even label the four bases (ATCG) fluorescently. This advanced and flexible chemistry has made it more economical and has facilitated much wider use of synthetic DNA resulting in an impressive high throughput. Now, the highly reliable synthetic oligonucleotides are routinely used for a large number of biological and biomedical experiments.

Fig. 1

Fig. 1 represents an automated DNA synthesizer available commercially is catering to a large number research requirement

As an example, another automated DNA/RNA synthesizer is given here from other company as shown in Fig. 2.

Fig. 2

Fig. 2 represents the CS-OLIGO-D which is a fully automated DNA/RNA synthesizer that can be used for oligonucleotide synthesis at the pilot and commercial scale with capacity of 0.2 mmol to 7 mmol.

III. Pilot Scale

The CS-OLIGO-D is a fully automated DNA/RNA synthesizer that can be used for oligonucleotide synthesis at the pilot and commercial scale with capacity of 0.2 mmol to 7 mmol syntheses as mentioned earlier. This system includes a high load resin reaction vessel with patented 180º inversion mixing with flow through, while also allowing for traditional synthesis with recirculation.

As the situation stands now, several companies provide oligonucleotide DNA automated synthesizers, which are listed hereunder.

- *Agilent Technologies:* A leading company in the oligonucleotide synthesis market.
- *Integrated DNA Technologies (IDT):* A leading company in the oligonucleotide synthesis market.
- *Thermo Fisher Scientific:* A leading company in the oligonucleotide synthesis market.
- *Twist Bioscience:* A company that offers automated high-throughput DNA synthesis and assembly.
- *Biolytic Lab Performance Inc.:* A company that offers the Oligo 48, a medium throughput oligo synthesizer.
- *Sierra Bio Systems, Inc.:* A company that offers the Shasta Synthesizer, which is designed for production facilities.
- Other companies that provide oligonucleotide DNA automated synthesizers include:
  - ATD Bio Ltd.
  - Biano Science GmbH
  - BioS pring GmbH
  - Bioneer Corporation
  - Creative Biogene
  - Eurofins Genomics
  - GenScript
  - Kaneka Eurogentec S.A.
  - LGC Biosearch Technologies

It is envisaged that these brief overviews of the machine, material and methods would impel the mind of researchers to efficiently manage the research and innovation in clinical and non-clinical set up broadening the scopes of human health care system.

IV. Applications of Synthetic DNA

There are two types of DNA synthesis: natural and synthetic. DNA synthesis performed outside of a cell (synthetic DNA) has various important applications across different fields of science and technology as mentioned earlier. Synthetic DNA synthesis allows scientists to create DNA molecules without a template and of any desired sequence complexity including that of modified bases of a gene. Thus, synthetic DNA could have sequence complexity that is not found in nature. This provides working flexibilities with any kind of biological system. Some of the key uses of synthetic DNA are discussed here.

V. Biotechnology

A simple definition of Biotechnology is the *Technology Based on Biology.* Biotechnology deals with animal, human, plants, marine, forest, river, agriculture or any ecosystem that harbor living being. Understanding different aspects of the species and genetically manipulating their genomes to have better results are the key of Biotechnology. This branch of science is popular because it has the applied potential in almost every sub-discipline of biology. For most of the biotechnology experiments, synthetic DNA is routinely used. Biotechnology uses Recombinant DNA Technology and Genetic Engineering. Thus, engineered genome is the result of the Biotechnology. For example, use of Agricultural Biotechnology provides better and more yield of crops and insect resistant plants and proteins for therapeutic purposes. Much improved varieties of fruits have been developed with the help of biotechnology circumventing the traditional Mendelian selection. Following are some more uses:

- *Gene Cloning:* DNA synthesis is used to create copies of specific DNA fragments or genes for further study.
- *Site-Directed Mutagenesis:* Researchers can introduce specific mutations into a DNA sequence for functional studies of a gene.
- *Constructing Plasmids and Vectors:* DNA synthesis is crucial for creating customized plasmids and vectors used in Genetic Engineering and Molecular Biology experiments.
- *Creation of New Restriction Sites in the Plasmid:* In certain instances, newer cloning sites may be needed. This can be created using synthetic DNA facilitating the efficient cloning experiments.
- *Development of Species Specific Probes:* In a dispute when it is not clear if the origin of meat is cattle or buffalo, goat or sheep for example, the species specific probes are highly useful to resolve all such issues. Similarly, species specific probes are equally useful for ascertaining the origin of tissue samples of highly endangered species in case of wildlife forensics (Ogden *et al.,* 2009).

VI. DNA Fingerprinting

In 1984, Alec Jeffreys discovered the technique of DNA fingerprinting in the Department of Genetics at the University of Leicester, Leicester, England, UK (Jeffreys *et al.,* 1985). Soon after, an Indian scientist, then working at the Max Planck Institute of Immunobiology, at Freiburg, Germany reported human DNA fingerprinting using short synthetic Oligodeoxyribonucleotides probes specific for simple repeat (Ali *et al.,* 1986).

In order to understand, the basis and genesis of DNA fingerprinting, one needs to understand the basic concepts of human genetics at the molecular level. Human have 46 seemingly similar looking chromosomes except in male, we see one X and one Y chromosome. Thus, males have XY chromosomes instead of XX which we see in the normal females. Notwithstanding apparently this simple scheme of chromosome arrangement in the human genome, we have very high level of minute organizational variations at the DNA level. This organizational variation makes all of us unique. This uniqueness is faithfully maintained including those of identical twins (Yadav *et al.,* 2014). It is largely believed and agreed upon that identical twins have all the Physical, Physiological and Genetical attributes exactly identical. However, DYZ1 arrays from the human Y chromosome discriminate between the identical twins. Thus, it would be more apocopate to refer such twins as monozygotic twins rather than identical twins (Yadav *et al.,* 2014).

VII. Organizational Variations of (Minisatellite) Sequences

Jeffreys discovered that certain DNA sequences, called minisatellites vary from person to person. He used a genome derived cloned probe to bind to these sequences, creating a unique pattern of dark bands. He and his team developed a variation of the process for forensic use, called "genetic profiling" or DNA fingerprinting. It is also called genome individualization (Ramel *et al.,* 1997).

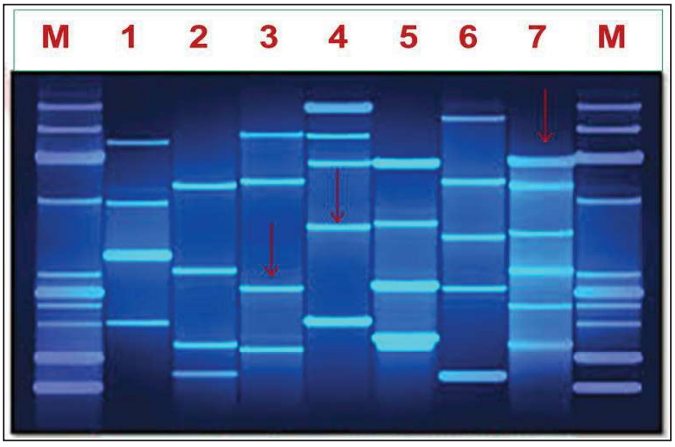

Fig. 3

Fig. 3 shows a simple scheme of actual DNA fingerprint. Lanes on both extreme sides are marked "M" denoting molecular size maker. Lanes 1-7 have individual human DNA amplified by a single STR primer by PCR. This STR is present in all the individuals but the position is different. This variation results in generation of bands (arrow) unique to every single individual. This DNA fingerprinting (Fig. 3) is largely based on PCR using STR primers. This is relatively simpler method compared to the traditional ones adopted earlier by Alec Jeffreys.

In the beginning, Alec Jeffreys conducted human DNA fingerprinting in a different way without using PCR. That involves DNA digestion with restriction enzyme (usually, 4 or 6 base cutters) followed by long agarose gel electrophoresis. When once DNA is fully resolved, the same is depurinated and transferred onto the nylon membrane overnight. Following morning, the membrane is subjected to UV fixation (Wilkie *et al.,* 1997). This way the DNA binds with the nylon membrane and is now ready for hybridization with radiolabelled minisatellite probes. The membrane then was hybridized with radiolabelled genome derived cloned probe specific for hypervariable regions of the genome. Upon exposure to the X-ray film, several monomorphic and polymorphic bands were detected (Fig. 4). The next step was to establish that the bands were statistically unique. Indeed, he did prove that no

two individuals are alike with respect to such band profile. In the beginning, genome derived cloned probes were used for this purpose. Later on, short synthetic DNA specific to VNTR loci or STR was used for DNA fingerprinting (Ali *et al.,* 1986). Today, we have several well defined, refined and reliable probes available for DNA fingerprinting for a large number of species. In addition, oligoprobes may be developed based on the specific requirement.

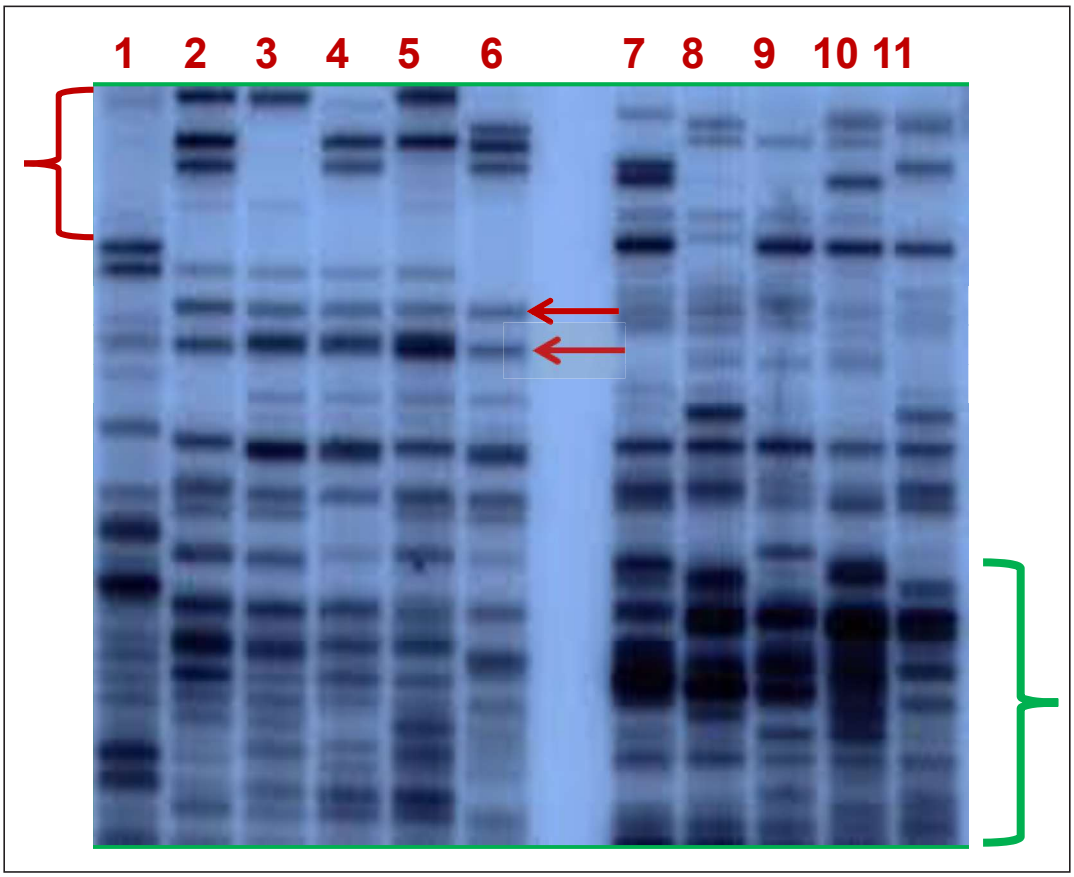

Fig. 4

Fig. 4 shows an X-ray autoradiogram showing DNA fingerprint of 11 samples. No two individuals have identical band patterns demonstrating the power of genetic heterogeneity useful for forensic identification. The regions encompassing *polymorphic* (see top left { brace bracket) and *monomorphic* bands (red arrows) are highlighted. Such autoradiograms are generated by restriction digestion of sample DNA followed by agarose gel electrophoresis, transfer of the same onto the nylon membrane and after UV fixation of DNA, hybridization with radiolabelled probes. Finally, the membrane is exposed to X-ray film and images of the bands are recorded. By changing the enzyme, the band patterns may be altered. Similarly, by changing the probe, band patterns would be different. The choice of restriction enzyme and probe is empirically adjusted and best possible enzyme–probe combination is used. This requires training, experiences, understanding the nuances of deep biology and desire to achieve the flawless result. Statistically, taking into consideration the global population, the overall band patterns have to be unique. Thus, no two individual would be similar with respect to their band profiles. This is the basis and genesis of DNA fingerprinting.

Now conventional agarose gel-based DNA fingerprinting has gone to a next level of automation. While the principle remains the same the results appear in new avatar. It may be noted that the present example cited here is similar to an ideal condition of DNA profiling. Notwithstanding the globally acknowledged power of DNA fingerprinting technology, there are instances when results remain inconclusive. This is due to several reasons of which one is less than ideal condition of the DNA collected from the scene of crime (Raymond *et al.,* 2009). The other problem could be DNA degradation of multiple mixtures. Yet another problem is deliberate mixing of the DNA and last but not least, faulty reaction where amplification does not take place despite correct template of DNA. We will refrain from these nuances because that is not our goal in the present paper. However, we will briefly discuss the automation part of this technology.

Automation of DNA Fingerprinting (DNF)

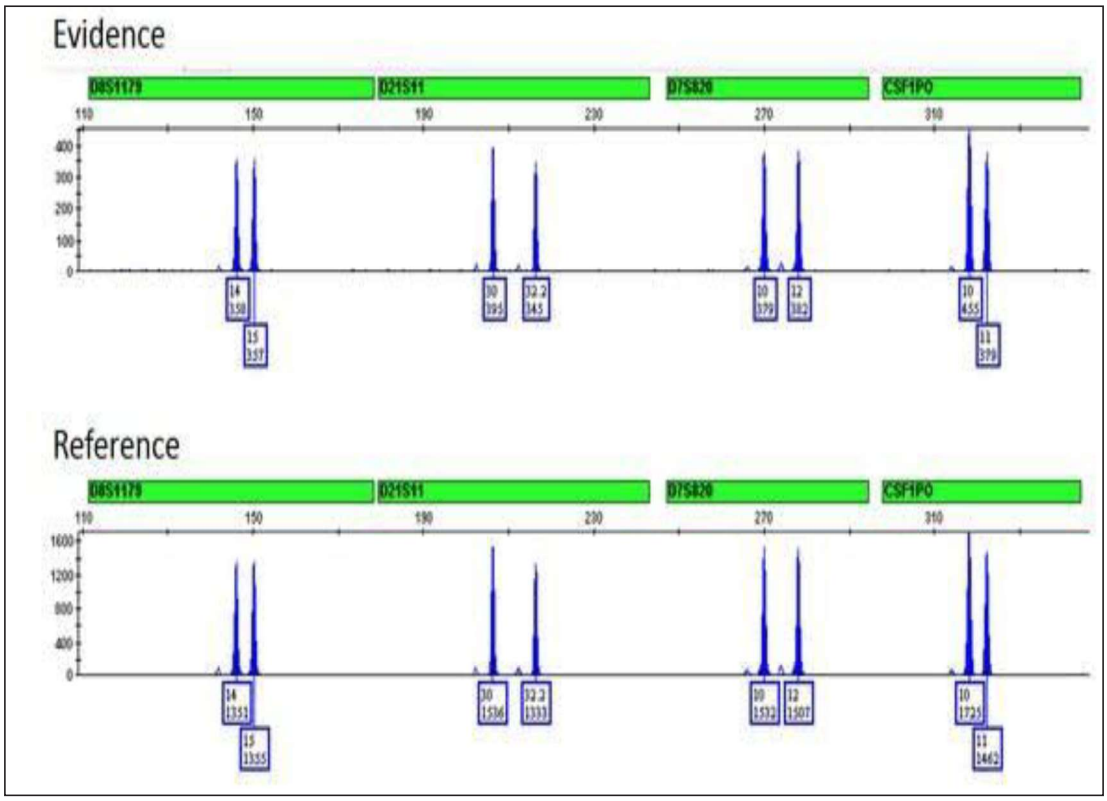

Fig. 5

Fig. 5 shows analysis of two samples that were used for DNA sequencing (https://www.apmreports.org/story/2016/09/13/dna-match).

The top image (evidence) represents a sample pattern taken from a crime scene. The bottom image is a "reference" sample, taken from a potential suspect. Of the 16 markers available for DNF, only four were used for this work. The peaks shown here are called alleles, and a typical person has two alleles for each marker, corresponding to one allele from each parent. From the Fig. 5, it is clear that the evidence sample profile is matching with the reference sample. This clear match is applicable to all the four markers. The samples line up visually, and the numbers that uniquely identify each allele — 14 and 15, 30 and 32.2, 20 and 12, 10 and 11 — are the same. It may be noted that all the three pictures shown here operate on the same principle. Statistically, no two samples would ever be identical unless they belong to identical twins. However, DYZ1 region discriminates that also as mentioned earlier. Even if a marker shows a perfect match, the other markers would vehemently revolt by showing mismatch because now we have more than 16 markers available to cover the entire global population. For such analysis, synthetic DNA is operating silently, diligently, faithfully and reliability, once again proving the prowess of oligonucleotides.

VIII. Applications and Impact of DNA Fingerprinting

DNA fingerprinting is used to solve crimes, determine paternity, identifying genetic diseases, success of bone marrow transplantation, establishing kinship, characterization of cell types and its mix up and many more. The first use of DNA fingerprinting in a criminal case was to help free an innocent man in 1986. Amongst other uses, DNA fingerprinting is used to identify genetic matches between tissue donors and recipients. It's also used to confirm pedigree in animals. DNA fingerprinting has revolutionized the way police solve crimes, and animal breeder propagate their livestock. Besides DNA fingerprinting, there are other applications of synthetic DNA as described hereunder.

A. Genome Editing

CRISPR-Cas9 Technology: Custom DNA synthesis is integral to the design and synthesis of guide RNAs and DNA repair templates for genome editing applications using CRISPR-Cas9 or other genome-editing techniques (Farboud *et al.,* 2015).

B. DNA Sequencing

- *Sequencing Controls:* Synthetic DNA is used as controls and standards in DNA sequencing experiments to validate the accuracy of the sequencing process. Artificial DNA synthesis permits the identification of novel biological systems. Sequencing is used for confirmation activities.

C. Diagnostic Applications

- *Probe Synthesis:* DNA probes used in various diagnostic techniques, such as fluorescence *in situ* hybridization (FISH) or Nested Polymerase Chain Reaction (nPCR), and Real Time PCR. These are often synthesized to specifically target and detect particular DNA sequences. In case of FISH, map position of a gene on the chromosome may be found out which otherwise is not feasible. For FISH, single or multiple fluorescently labelled synthetic DNA corresponding to different regions of the desired genes may be used. With this approach, use of genome derived cloned probed may be circumvented. Because all the flour bases (ATCG) may be fluorescently labelled, this enhances the signal intensity facilitating gene mapping, monitoring gene translocation, insertion, deletion and copy number variation. Fluorescence *in situ* hybridization (FISH) with labelled oligoprobes is yet another powerful tool for genome analysis.

D. Vaccine Development Research

- *Antigen Synthesis:* DNA synthesis is used to create synthetic genes that encode antigens for the development of vaccines. DNA fragments and genes can be used as *in vitro* transcription templates for mRNA production which in turn can be translated into proteins. Attempts are made to develop DNA vaccine where synthetic DNA is used. This approach is not only helpful for human system but also for livestock animal species.
- *Cancer Research:* In case of cancer research, FISH using synthetic DNA may uncover multiple mutation and progression of cancer during different stages of metastasis all the way to angiogenesis. Following this approach, metastasis may be monitored with respect to its progression across the spectrum of cells.

E. Therapeutic Proteins

- *Recombinant DNA Technology:* This involves inserting DNA that encodes the protein into bacterial or mammalian cells expressing the desired protein in those cells. The protein may then be purified for their subsequent therapeutic use. This approach is no longer a dream instead is used on regular basis augmenting the steps of gene therapy facilitating the concept of precision medicine (Bestor *et al.,* 1988).

F. Antibodies

- *Detection:* Antibodies can be used to detect newly synthesized DNA or proteins (Steinman *et al.,* 1976).

G. Drug Discovery Research

- *Library Synthesis:* DNA synthesis is employed to generate libraries of DNA sequences corresponding to functional genes. This can be screened for potential drug candidates or for high-throughput screening assays. This is just one example, but there are many more, including protein engineering and CRISPR library screening. Although with the advancement and availability of tools and techniques, Genomic or cDNA library construction and screening are often circumvented. However, in principle, genomic or cDNA library screening can be done using short synthetic DNA probe of about 17 bases long. Its 17 base long length has been empirically optimized and statistically proven. Thus, synthetic DNA of 17 bases complementary to any part of gene would prove to be unique. With this approach, the corresponding genes may easily be isolated from the genomic library (Ayon *et al.,* 2023). Further, signal enhancement is possible while screening the library by using several synthetic Oligodeoxyribonucleotides probes.
- *Cross Hybridization Studies:* Certain percentage of DNA from a species is shared by the genome of other species. This is due to slow and gradual evolution that leaves behind a trail of phylogeny. Thus, to ascertain if a given stretch of DNA is present in other species, that can be uncovered in a simple cross-hybridization experiment using dot blot approach. The technical approach is rather simple where 50-100 ng of DNA from different (any) species may be spotted on the nylon membrane. After UV fixation of the DNA, the membrane is hybridized with radio labelled oligonucleotide probes. After about 16 hr. hybridization, the membrane is washed repeatedly and exposed to X-film (Ali *et al.,* 1999). Within a day or two the signal will appear on the X-ray film. With the use of positive and negative controls on the same membrane, the authenticity of the signal may be established. An example of cross hybridization has been shown in the following diagrammatic illustration (Fig. 6).

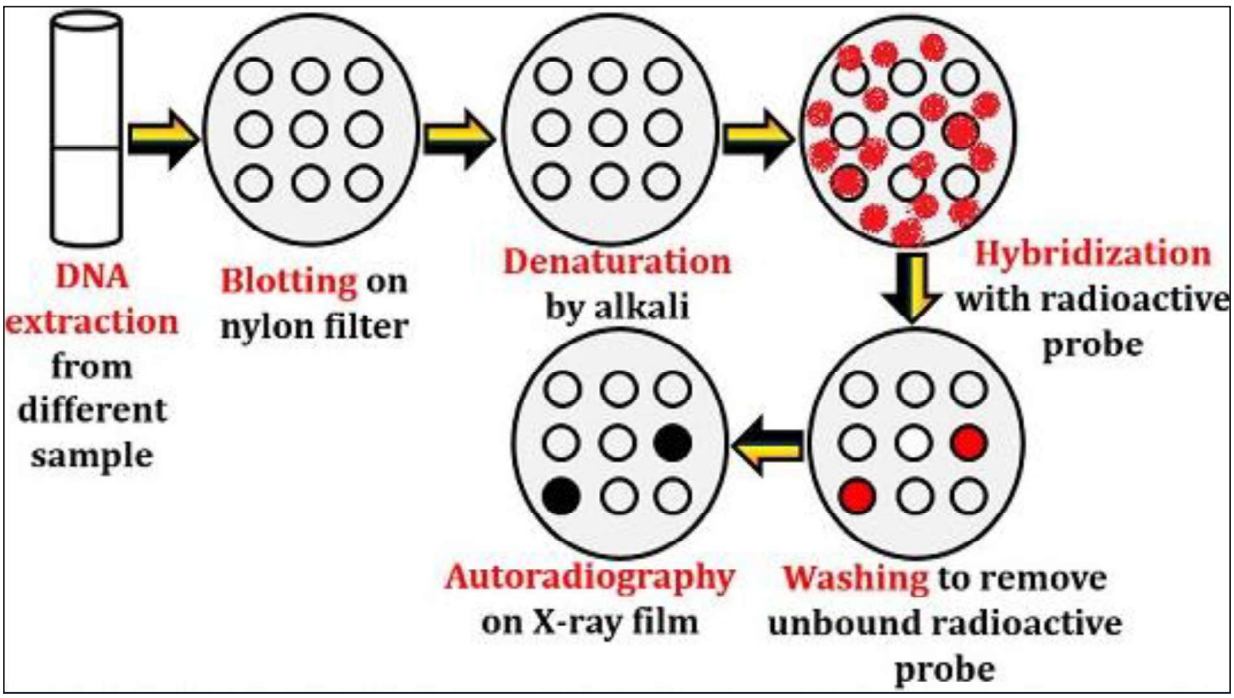

Fig. 6

Fig. 6 represents Diagrammatic illustration of Dot Blot Hybridization showing distinct spotted DNA samples (blotting on the nylon membrane) followed by alkali denaturation. The membrane is then hybridized with radiolabelled probes and exposed to X-ray film. The spotted samples may be DNA, cDNA, RNA, plasmid or LNA from any biological source including bacteria, viruses and plants. The visible black dots have the presence of the complementary sequence (shown red). This appears on the X-ray film after hybridization. A dot blot assay where darker signals are seen indicates higher concentration of the target nucleic acid. Thus, dot blot is used not only for the detection of corresponding DNA but also for its relative concentration. The approach has been used after refinement to ascertain the copy number of genes by using serial dilution of the target DNA. This simple approach can also be used to uncover the expressed genes if cDNA, bacterial DNA or viral DNA is spotted on the blot. Similarly, nucleic acids from pathogens showing signals after hybridization indicate presence of infection.

H. Synthetic Biology

- *Designing Synthetic Genomes:* Synthetic DNA synthesis plays a crucial role in the field of synthetic biology, where researchers design and engineer novel organisms with specific functions or traits (Heinemann *et al.,* 2006).
- *Gene Writing:* Genome engineering technology writes therapeutic code into a genome in order to treat a disease. This needs understanding, experience and knowledge of system biology to ensure that the synthetic genes will remain faithful and will not mutate and will not be affected by other genes due to gene–gene interaction (Hoose *et al.,* 2023).

I. Personalized Medicine

- *Customized DNA Constructs:* In personalized medicine, synthetic DNA can be used to create customized therapeutic constructs for personalized gene therapies or used as gene editing tools tailored to individual patients. It may be noted that newly synthesized genes and their insertion into the genome must ensure accurate or near accurate copies and their faithful expression in the genome. Gene expression may be monitored at mRNA or protein levels. Usually, mRNA expression is correlated with the expressed protein and vice–versa. However, in certain circumstances, mRNA level and that of resultant expressed proteins do not correlate (Saini *et al.,* 2020). This requires understating the causes to develop possible remedial measure. Failure of the eukaryotic transcriptional and translational machineries could be one reason. High turnover of mRNA but equally higher rate of degradation of proteins may be another reason. The mRNA isolation steps may be yet another reason because mRNA is prone to degradation.

J. Functional Genomics

- *Synthetic Genes for Functional Studies:* Synthetic DNA synthesis facilitates the creation of synthetic genes for studying their function or regulatory elements in cellular processes (Chang *et al.,* 2002).

K. Academic and Educational Purposes

- *Teaching and Training:* Synthetic DNA is often used in educational settings to teach molecular biology concepts and techniques. This encompasses replication, duplication, transcription, translation, alternate splicing, conceptual pleiotropism and aberration in Eukaryotic Transcriptional Machinery. In view of the large scale application of synthetic DNA in the area of modern biology, several companies have come up to assist researchers in doing their science. One such company is the Integrated DNA Technologies (IDT).

IX. Integrated DNA Technologies (IDT) and DNA Synthesis

Integrated DNA Technologies (IDT) is a leading provider of products and services related to DNA, including custom DNA synthesis.

Gene synthesis from IDT is an easy solution for researchers who want to bypass in-house cloning and instead wish to take up direct functional studies using 100% sequence-verified clonal DNA. Ordering from IDT is easy and includes guaranteed delivery dates. There is no minimum order requirement or additional charges for custom vectors.

The maximum length of synthetic DNA currently achievable is around 200 bases. This is due to limitations in chemical synthesis methods, where longer sequences result in significantly reduced yield and increased error rates. Most commercial DNA synthesizers can produce oligonucleotides up to this length. One may ask which make/model of the oligonucleotide synthesizer is the best. Based on current information, the "best" oligonucleotide synthesizer is widely considered to be the MerMade series from LGC Biosearch Technologies, particularly the MerMade 12 or MerMade 192X models, due to their high flexibility, ability to synthesize various types of oligonucleotides (DNA, RNA, LNA) using standard or modified chemistries, and medium to high throughput capabilities depending on the chosen model; making them suitable for a wide range of research and production needs.

DNA, RNA, and locked nucleic acid (LNA) are all nucleic acids that are made of nucleotides. *LNA is an RNA derivative* that has a high affinity for DNA and RNA. To be able to understand relevance of LNA, it is justified if we take conventional concept of DNA and RNA into consideration (Nielsen *et al.,* 2004).

A. DNA

- DNA is made of deoxyribose sugar, phosphate, and nitrogen-containing bases.
- The bases in DNA are adenine (A), cytosine (C), guanine (G), and thymine (T).
- In DNA, adenine always pairs with thymine (A-T), and guanine always pairs with cytosine (G-C). Any deviation from this scheme is deemed to be an aberration or mutation.

B. RNA

- RNA is made of ribose sugar, phosphate, and nitrogen-containing bases.
- The bases in RNA are adenine (A), cytosine (C), guanine (G), and uracil (U).
- In RNA, adenine always pairs with uracil (A-U). With this scheme, DNA is differentiated with RNA.
- RNA itself has its sub-types. This includes Messenger RNA (mRNA) copies portions of genetic code, a process called transcription, and transports these copies to ribosomes, which are the cellular factories that facilitate the production of proteins from this code. Transfer RNA (tRNA) is responsible for bringing amino acids, basic protein building blocks, to these protein factories, in response to the coded instructions introduced by the mRNA. This protein-building process is called translation. Finally, Ribosomal RNA (rRNA) which is a component of the ribosome factory needed for protein synthesis.

C. LNA

- LNA is an RNA derivative with a ribose ring that is "locked" into a conformation that's ideal for binding to DNA and RNA.
- LNA is used in therapeutics, diagnostics, and gene editing.
- LNA oligomers have been used to suppress transcription and telomerase activity.
- LNA-modified DNAzymes have been used to cleave RNA (Vester, B, 2004).

A locked nucleic acid (LNA) also known as bridged nucleic acid (BNA) is often referred to as inaccessible RNA. This is a modified RNA nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon.

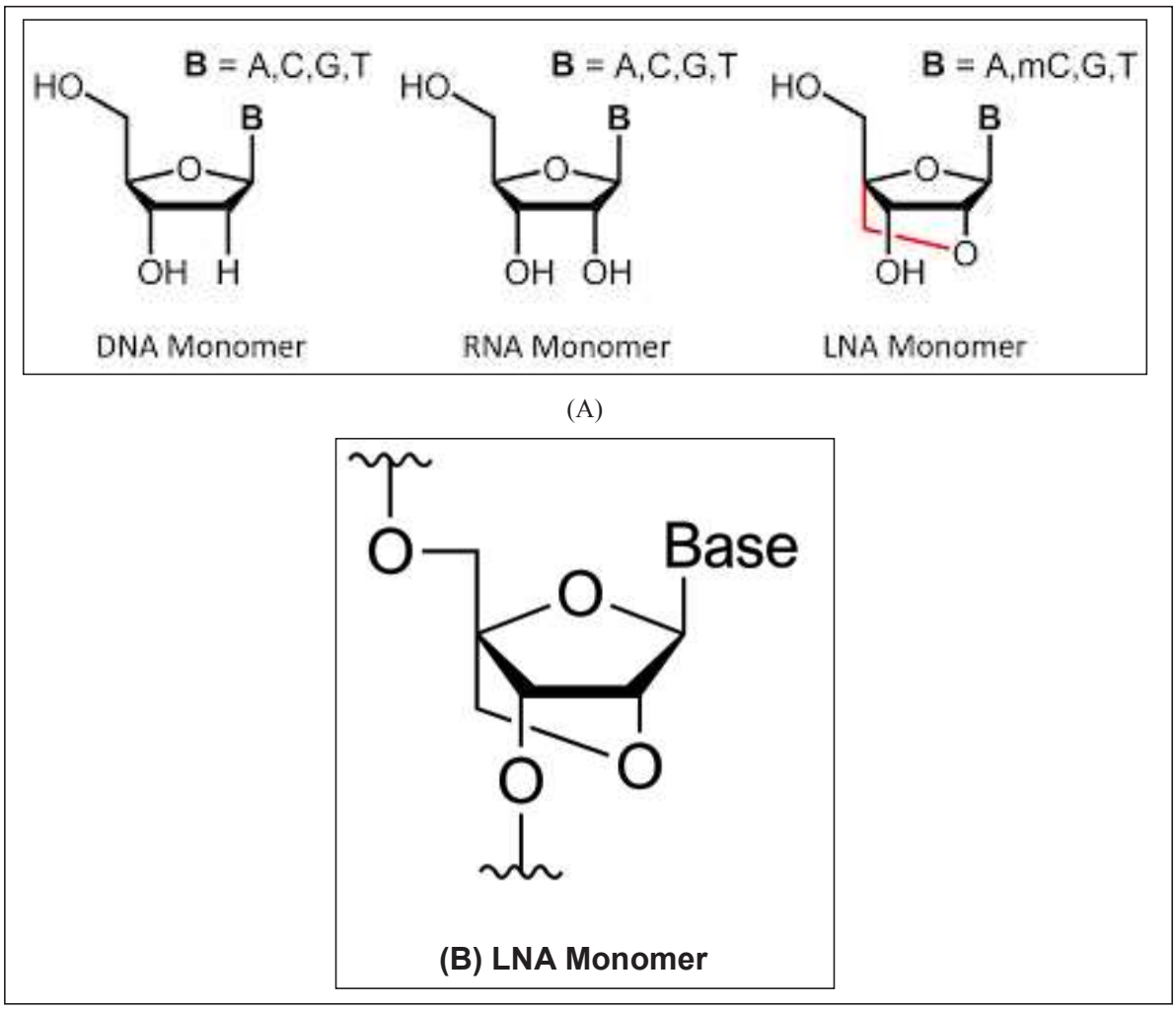

Fig. 7

Fig. 7 (A) shows Chemical structure of DNA, RNA and LNA. (B) Chemical structure of an LNA monomer with an additional bridge bond the 2' oxygen and the 4' carbon of the pentose.

The bridge "locks" the ribose in the 3'-*endo* (North) conformation, which is often found in the A-form duplexes. This structure provides for an increased stability against enzymatic degradation. LNA also offers improved specificity and affinity in base-pairing as a monomer or a constituent of an oligonucleotide. LNA nucleotides can be mixed with DNA or RNA residues in an oligonucleotide. LNA offers enhanced biostability compared to biological nucleic acids. LNA modified oligonucleotides have demonstrated improved thermodynamics in hybridization to RNA, ssDNA, and dsDNA. Compared to mRNA, LNA (Locked Nucleic Acid) is significantly more stable, often considered to be closer in stability to DNA due to its modified structure that enhances its binding affinity and resistance to degradation. This makes it a much more stable option for applications like molecular probes and antisense oligonucleotide therapy. It may be noted that locked nucleic acid (LNA) is not translated into protein, but it can inhibit gene expression and this way protein translation is obstructed. We have seen DNA, RNA, LNA, their chemical structures and commercial synthesis. Now we can elaborate a bit more of their synthesis.

We now take up the MerMade Series DNA synthesizer. The *MerMade* 12 Oligonucleotide synthesizer is designed to synthesize DNA, RNA and LNA oligonucleotides in a column format using standard or modified chemistries. However, there are other models in this make that can synthesize a large number of Oligonucleotide simultaneously.

Following are the key points of MerMade 12 Synthesizer

- Designed for the synthesis of DNA, RNA & LNA oligonucleotides.
- Capable of synthesizing 96 oligos in less than 2 hours.
- 12 Amidite ports for versatile synthesis.
- Scales range from 20 nanomole to 5 micromole.
- Fully-featured API for remote monitoring and control.
- Flexible and easy-to-use software running on Windows 7 and more.

- Mixed backbone capability (Phosphorothioate and Phosphodiester).
- Pre-loaded with standard protocols.

Versatility: This can synthesize diverse types of oligonucleotides with different chemical modifications.

Scalability: Offers various column configurations to suit different synthesis scales.

User-Friendly: Designed for ease of use and operation.

High Throughput: Capable of producing large quantities of oligonucleotides in a single run, especially with the MerMade 192X. This MerMade 192X is sufficient for all types of oligonucleotides (DNA, RNA, and modified ones). It fulfils the Synthesis scale criteria ensuring flexibilities as per the requirement (Rayner *et al.,* 1998).

The chemical synthesis of DNA oligonucleotides has become a feasible technology useful for modern molecular biology. We briefly reviewed the techniques and technologies that enable the synthesis of DNA oligonucleotides and their assembly into larger DNA constructs. The recent advancements has reduced synthesis cost and increased sequence fidelity. The development of lower-cost methods and production of high-quality synthetic DNA allows the exploration of large number of biological experiments facilitating broader understanding of normal and diseased genome. This is particularly useful for early diagnosis and development of subsequent remedial measures.

20th century was deemed to be the "century of the atom" in which discoveries on the physical and chemical properties of the elements led to breakthroughs such as atomic energy (and weaponry), medical diagnostics, computers, and the microchip to name just a few. These advances had a dramatic effect on our way of life and helped shape the promise and possibilities of science and technology. In the early part of the 21st century, we are witnessing what could very likely become known as the "century of DNA." As the score to life's intricate symphony, DNA provides nuances of biological functions. Synthetic DNA has made marked changes in our ability to understand, manipulate and engineer biological systems. These advancements have led to the development of ground-breaking technologies for the design, assembly, and manipulation of DNA encoded genes, materials, circuits, and metabolic pathways, which are allowing for an ever greater manipulation of biological systems and even entire organisms (Miller Jr. *et al.,* 2023).

X. Synthetic DNA and Next-Generation Sequencing (NGS)

We look at the conceptual framework of system biology and acknowledge the introduction of next-generation sequencing (NGS). This technology is capable of generating an estimated 15 petabases of sequence data per year worldwide. The current megagenomics era has led to the swelling of biological sequence repositories with DNA sequences isolated from every organism and environment imaginable. Associated improvements in bioinformatics techniques and software allow researchers to obtain, analyse, and manipulate these DNA sequences in ways easier than ever (Koren *et al.,* 2012). The ever-increasing availability of biological sequence information from all branches of the "tree of life" has deepened our understanding of biological systems and the interrelated nature of organisms at the genetic level.

XI. Synthetic DNA and Synthetic Biology

Synthetic biology is emerging as an important discipline impacting a number of academic and industrial applications including the creation of novel therapeutics, materials, bio sensing, and manufacturing capabilities. Although our current understanding of biological systems is seemingly vast, it is still far from complete. For example, we are not aware of the exact composition of cytoplasm across the spectrum of different species. We can construct a synthetic gene successfully but cannot express it unless we have right ambiance of cytoplasm. Do we expect all the cytoplasm even within the same species to be identical or even similar? We do not know. Work is needed along this line to resolve this issue. Currently, the engineering of biological systems requires a heavy dose of empirical trial and error to evaluate novel enzymes, expression systems, and molecular compartmentalization pathways for the desired function. Some molecules within the cell system last long whereas others have very short life. Such precise roistering of the molecules within the cytoplasm maintaining compartmentalization of biochemical reaction needs to be worked out.

Biological functions are encoded to a large part in DNA. Therefore, a gross simplification of biological engineering can be adapted to the design, production, and testing of DNA sequences where once again synthetic DNA comes into picture. As researchers seek to engineer biological systems with novel DNA sequences, the need therefore for custom synthetic DNA sequences has grown. This is particularly true when the sequences to be engineered are derived from metagenomic resources and not from a single organism. The synthesis of synthetic DNA is often referred to as "gene synthesis," which actually is the synthesis of gene-length pieces of DNA (250–2000 bp) directly from single-stranded synthetic DNA oligonucleotides (Hughes and Ellington, 2017).

XII. Entailment of Lower Cost Gene Synthesis

Lowering the cost of gene synthesis would invite the generation of larger datasets. Owing to reduced cost of gene construction, more constructs may be used to explore a large number of biological systems across the species. Thus, broadening the low cost synthetic DNA landscape would

enhance our understanding of a large number of biological phenomena.

Synthetic oligonucleotides are generally synthesized using variations of the phosphoramidite chemistry either on traditional column-based synthesizers or on microarray-based synthesizers that we briefly highlight here.

XIII. Phosphoramidite-Based Synthesis of Oligonucleotides

The phosphoramidite synthesis chemistry consists of a four-step chain elongation cycle. Here, one base per cycle is added onto a growing oligonucleotide chain attached to a solid support matrix (Fig. 8). *In the first step,* a dimethoxytrityl (DMT)-protected nucleoside phosphoramidite is attached to a solid support (usually contained within a synthesis column) which is deprotected by the addition of trichloroacetic acid. This activates the support-attached phosphoramidite for chain elongation with the next phosphoramidite monomer. *In the second step,* the next base of the sequence is added in the form of a DMT-protected phosphoramidite which is coupled to the 5′-hydroxyl group of the previous nucleoside phosphoramidite forming a phosphite triester. *Third*, any unreacted 5′-hydroxyl groups are capped by acylation to render any unextended sequences inert in subsequent rounds of the chain elongation cycle and thus reducing deletion errors in the finished oligonucleotide sequences. *In the fourth step,* the phosphite triester linkage between the monomers is converted to a phosphate linkage via oxidation with an iodine solution to produce a cyanoethyl-protected phosphate backbone. The synthesis cycle then repeats for the next base in the sequence via the removal of the 5′-terminal DMT protecting group. After the desired sequence has been synthesized from the 3′ to 5′, the oligonucleotide is chemically cleaved from the solid synthesis support and the protecting groups on the bases and the backbone are removed. This process is highly amenable to automation and forms the basis for oligonucleotide synthesizers. This can synthesize 96–1536 distinct oligonucleotides simultaneously at scales from 10 to 1000 nmol and cost ranging from $0.05 to $0.17 per base with error rates 0.5% or less.

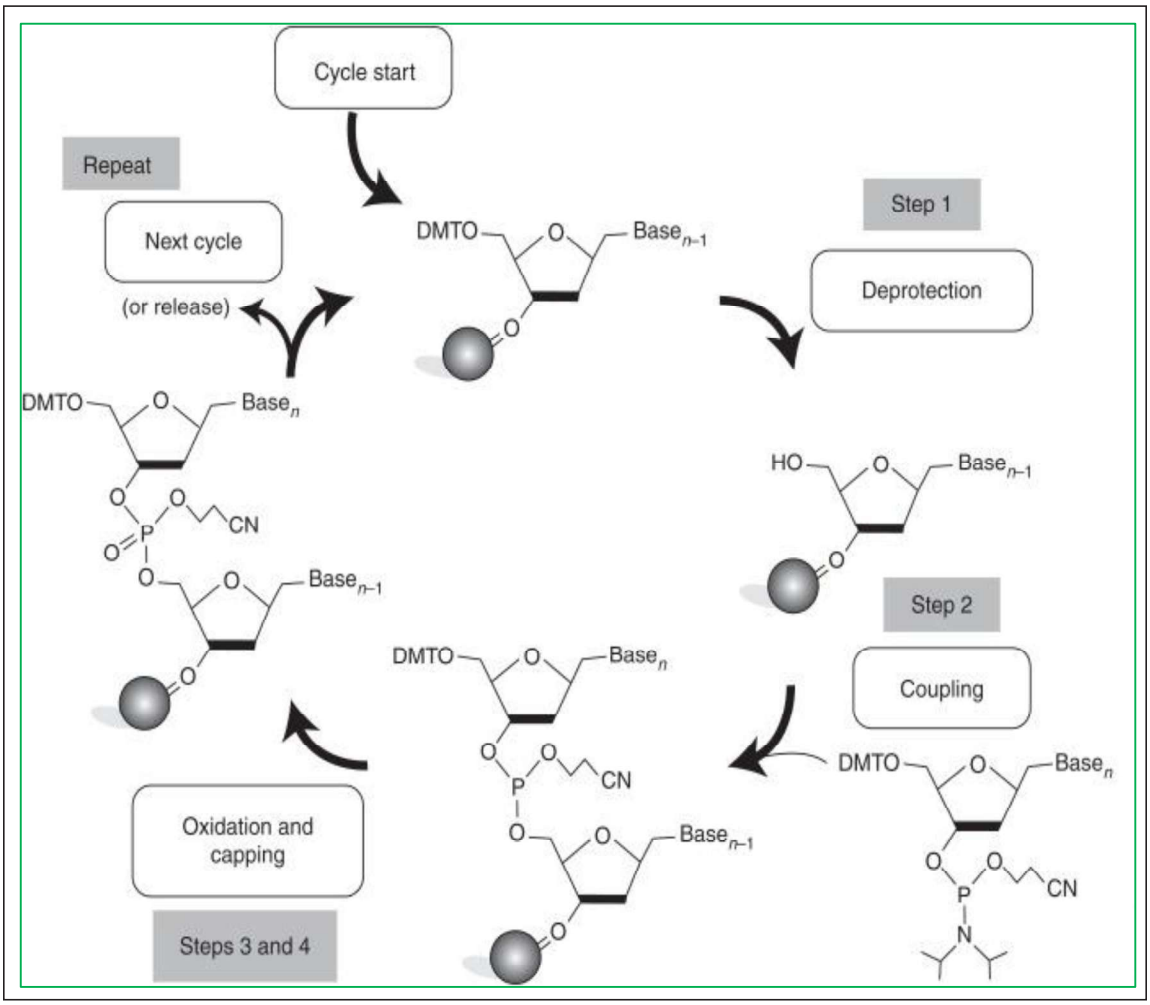

Fig. 8

Fig. 8 represents Phosphoramidite-based synthesis of oligonucleotides adopted from Cold Spring Harbor Publication. This process is the most commonly used for the synthesis of oligonucleotides.

In addition to Phosphoramidite-based synthesis of oligonucleotides, there are other methods such as column based and Microarray based oligonucleotide synthesis discussed briefly herein.

XIV. Column-Based Oligonucleotide Synthesis

This is the traditional method of synthesizing DNA using solid-phase phosphoramidite chemistry. The synthesis of a unique oligonucleotide sequence is done on the surface of controlled-porosity glass beads (CPG) contained with a synthesis column. During the synthesis process, the reagents flow through the column and across the packed CPG matrix and the oligonucleotide "grows" off from the bead surface. Only one sequence can be synthesized per column, although high-throughput synthesizers exist that can synthesize on multiple columns at once. A 96-column synthesis plate is shown as an example (Fig. 9A) (Brzezinska *et al.,* 2023).

XV. Microarray-Based Oligonucleotide Synthesis

In this method, microarray chips containing tens of thousands of distinct features synthesize unique oligonucleotide sequences at once with one unique oligonucleotide sequence synthesized per chip feature. On standard arrays, there are no physical barriers amongst these features. Therefore, following cleavage of the synthesized oligonucleotides from the chip surface as the end product is a pool of sequences containing every oligonucleotide synthesized on the array. Subsequent processing steps are required to "fish" the desired oligonucleotide sequences out of the synthesis pool for subsequent gene synthesis (Fig. 9B) (Koslov *et al.,* 2008)**.**

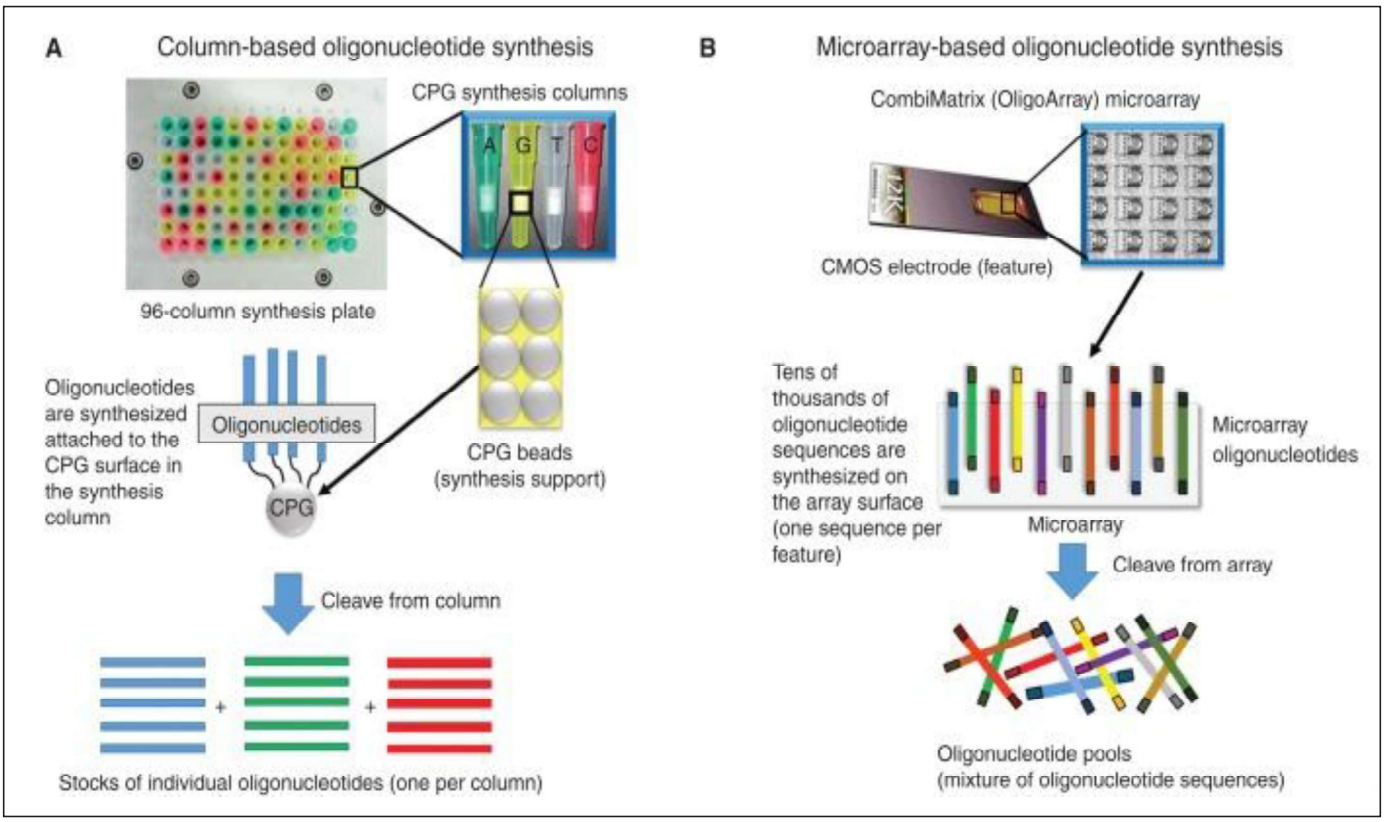

Fig. 9

Fig. 9 represents schemes showing Column-Based Oligonucleotide Synthesis and Microarray-based Oligonucleotide Synthesis, adopted from Cold Spring Harbor Laboratory Publication.

XVI. DNA Fingerprinting Test on NGS Library

We have seen DNA profiling earlier using different methods. Here we discuss DNA fingerprints from the NGS library mixture. Comparative analysis of the PCR based fingerprint to the original DNA fingerprint run in parallel, showed matching allelic markers between the two electropherograms, confirming proper sample identification. An occasional, small, non-specific peak could be seen (Fig. 10A, black arrow). However, it did not compromise the data comparison (Qin *et al.*, 2022).

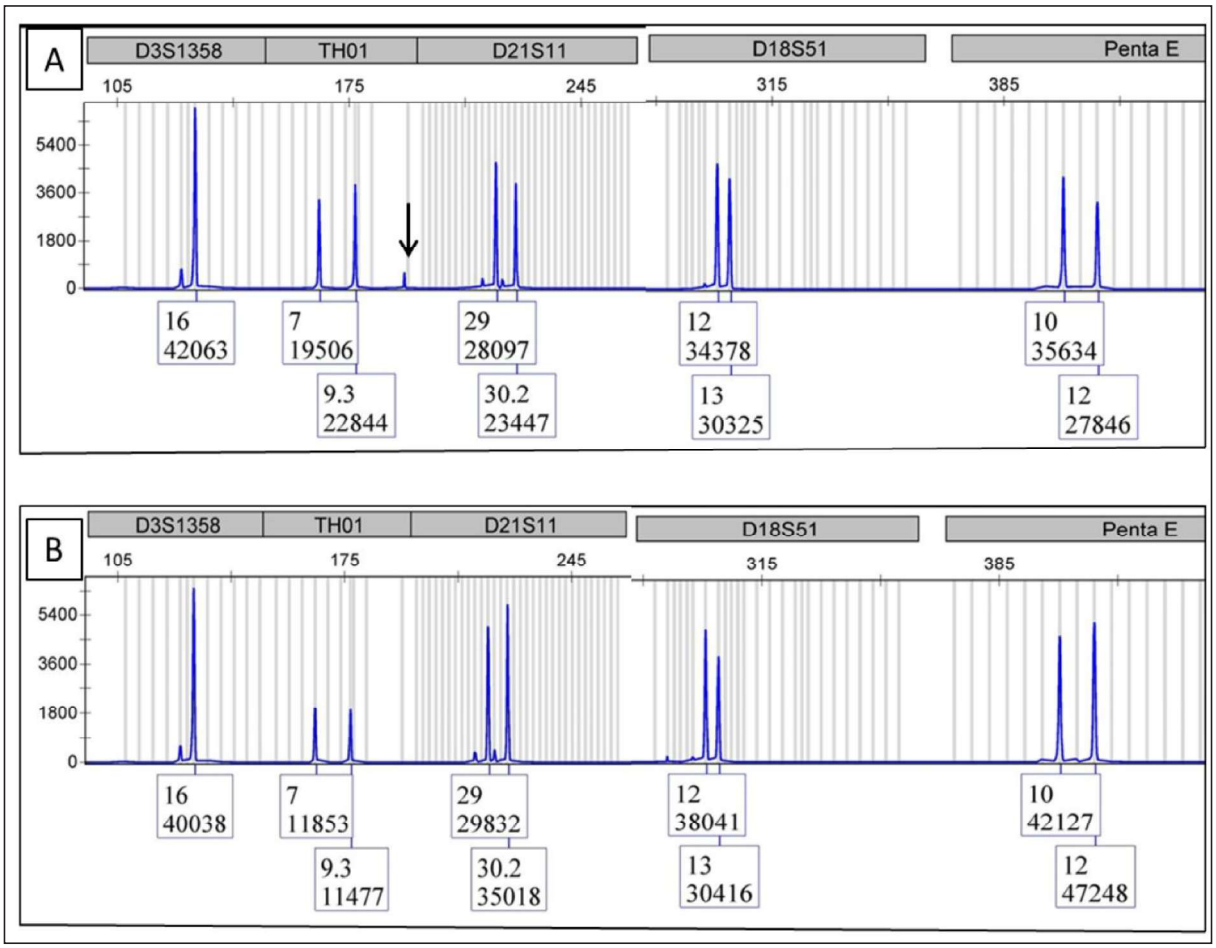

Fig. 10

Fig. 10 represents electropherograms from multiple DNA samples of the same category for which a total of five markers *(D3S1358, THO1, D21S11, D18S51 and Penta E)* were used for both the panels. The *"D3S1358"* marker refers to a specific genetic locus on chromosome 3, which is commonly used in DNA analysis for forensic identification and paternity testing; "D" signifies DNA, "3" indicates chromosome 3, "S" stands for "simple sequence repeat," and "1358" is the unique identifier for that specific location on the chromosome where the repeating sequence is found.

Here, (Fig. 10) DNA fingerprints are seen in the allelic forms. Panel (A) is the DNA fingerprint from the NGS library. The black arrow indicates a non-specific peak. (B) is the DNA fingerprint from the original sample. See the similar allelic patterns in both the panels indicating that even from the mixed samples, allelic profile can be matched with original samples.

XVII. DNA Fingerprinting Test on the Samples in the BCR/ABL1 Real-Time Assay

A BCR/ABL1 real-time assay, also known as BCR-ABL1 RT-qPCR, is a laboratory test that measures the amount of BCR-ABL1 RNA in a patient's blood. This test is used to monitor the effectiveness of treatment for chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). After reverse transcription and quantitative PCR, the PCR reaction mixture was tested using a DNA fingerprinting assay (Fig. 11) (Azad *et al.,* 2018).

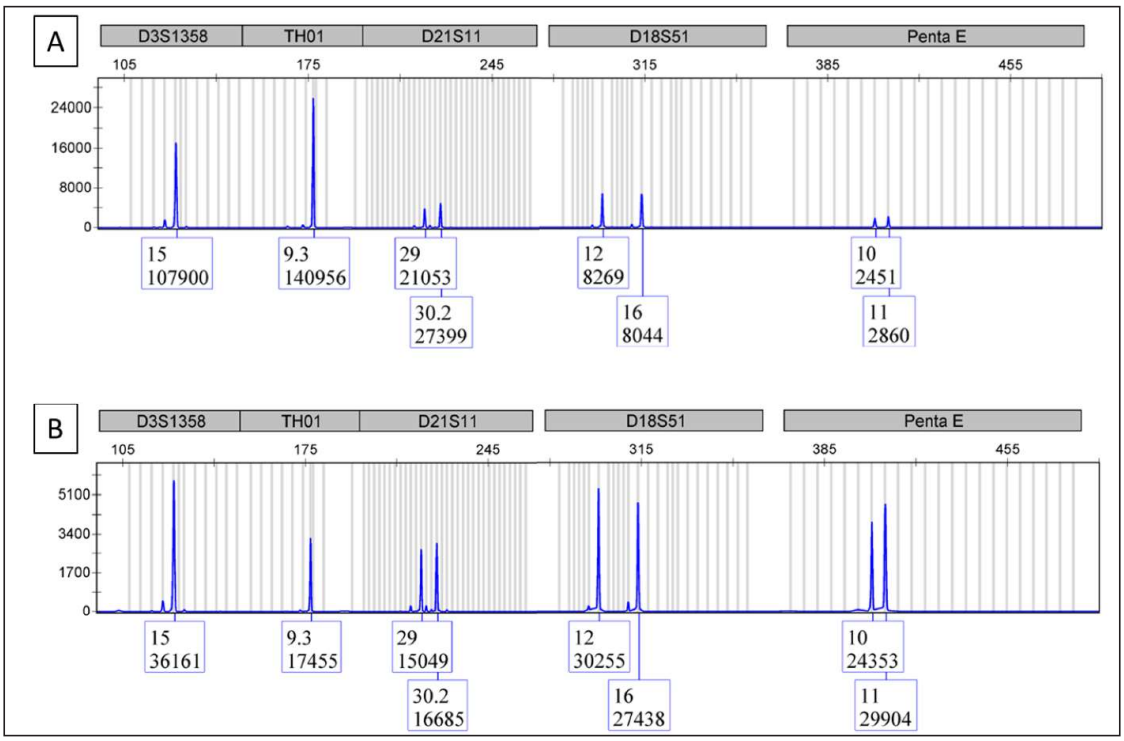

Fig. 11

Fig. 11 consists of the electropherograms from multiple samples of the same category to protect the DNA fingerprint of any one sample. (A) is the DNA fingerprint from the BCR/ABL1 real-time PCR reaction mixture. (B) is the DNA fingerprint from the original sample. The original DNA fingerprint (Fig. 11B), which was run in parallel, showed matching allelic markers between the two electropherograms, thus confirming proper sample identification.

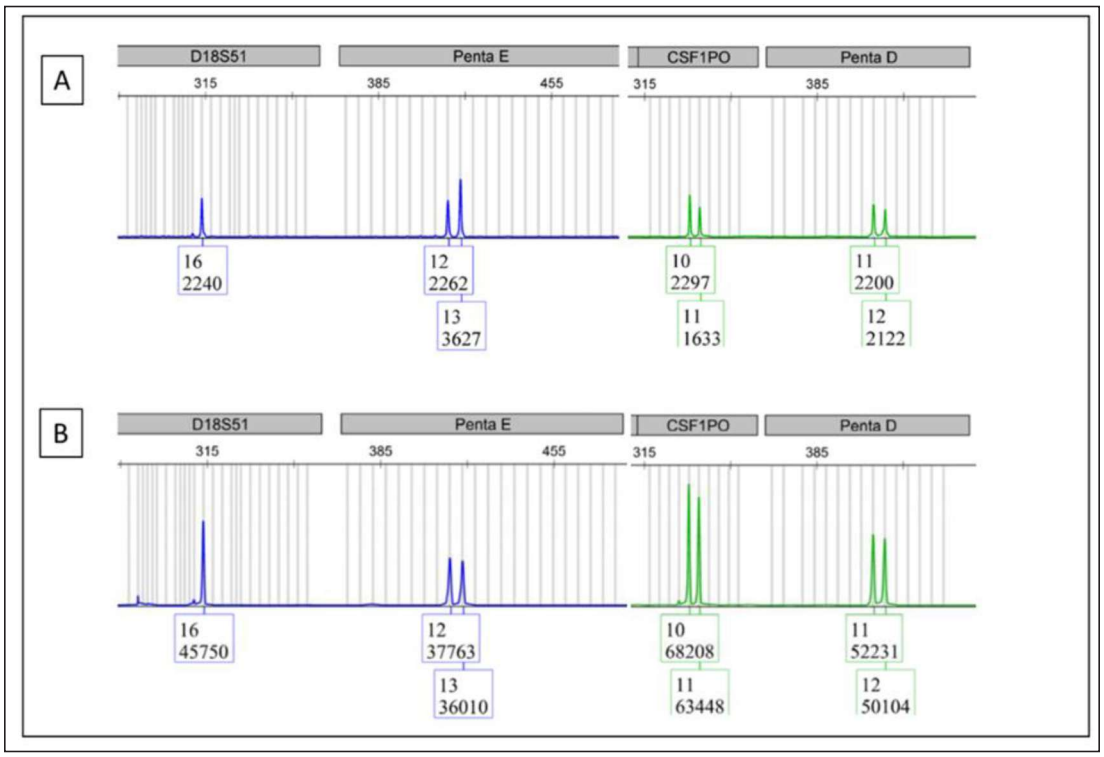

Fig. 12

Fig. 12 consists of the electropherograms from multiple samples of the same category to protect the DNA fingerprint of any one sample. (A) is the DNA fingerprint from a common reagent. (B) is the DNA fingerprint from the BCR/ABL1 real-time PCR reaction mixture with a high BCR/ABL1 level. In this experiment, four DNA samples and four markers were used. Note the allelic similarity between panel A and B suggesting the accurate identification of samples.

XVIII. Discussion

The DNA fingerprint markers are short nucleotide tandem repeats. Here we have shown genomic DNA contains the short tandem repeats and flanking genomic DNA, survived the NGS library preparation process

Although occasional small non-specific peaks can be present in the DNA fingerprints from the NGS library preparation, it will not compromise the DNA fingerprint results. If all the DNA fingerprint markers from the NGS library match the markers from the original sample, we know there is no sample ID error even though there is a random, small, non-specific peak.

Apart from checking samples, DNA fingerprinting technology is useful for checking lab reagents for contamination. There are instances when multiple samples from the patients in complete remission were noticed to have unexpected BCR/ABL1 positivity. In the QC process, DNA fingerprinting was used to test all the reagents used in this run. A common reagent used in the robotic nucleic acid (NA) extraction process tested positive (Fig. 12A). This common reagent is not supposed to have any human DNA in it. Therefore, a positive result indicates that it is contaminated by human DNA. The real-time PCR mixtures of all samples from that run were tested. A patient's sample with a high BCR/ABL1 level showed the same DNA fingerprint as that seen in the common reagent (Fig. 12B) indicating that this sample contaminated the common reagent and went on contaminating the other samples, which led to false positivity in the patients in complete remission.

As mentioned earlier, most standard DNA fingerprinting tests utilize around 13 to 24 markers depending on the application, with the most common being 13 markers for human identification in forensic analysis. However, more extensive tests can examine up to 68 markers for increased accuracy. Despite all the advances, DNA fingerprinting technology has not been fully exploited in the area of biomedical research and cancer biology. For example, every single cancer is expected to give rise a varying allelic profiles. Generation of such comprehensive allelic profile with all the 64 markers will uncover the overall genomic changes in a given sample of cancer. It is envisaged that no two cancer sample will have similar allelic profile. If in the process, a cancer specific pattern is obtained that may be used for diagnosis. Based on the allelic mining, advancement of cancer may be ascertained. This requires initial spade work and eventful refinement of the DNA profiling to be able to draw a meaningful conclusion. Finally, if all the cancer related data is subjected to AI with a question as to what all genetic alterations are common across the spectrum of different cancer, the answer if available will add a newer dimension to understanding of cancer, its diagnosis and prognosis.

XIX. Conclusions

The power of synthetic DNA lies in its design, understanding conceptual framework and chemistry of its conformational radii. Its rampant use shows that no modern Biological and Biochemical Research can be fathomed without the use of synthetic DNA. Scientific research spins around imagination, innovation, conjunctures, hypothesis, readiness to accept the failures and deep desire never ever to give up. We all know that failures are the pillars of success. However, we shy away when there is a need to construct these pillars. The purpose of this article is to apprise the readers about the gradual but powerful unfolding of the events developed on the real time basis by some of the best brains of 19th century involved in nucleic acid research. This has resulted in the development of several powerful platforms on which many new meaningful projects have been launched.

In short, DNA fingerprinting technology can be useful in detecting sample switch and sample contamination in both NGS and BCR/ABL1 assays. This method may potentially be a valuable technique used to monitor sample switch and sample contamination in other molecular assays. Finally, this approach may be used to explore allelic variation in different kind of cancer using both cDNA and genomic DNA. Large scale screening of all the 64 markers would establish their independent and collective informativeness. The large scale data so generated will pave the path for reliable predictive medicine and advanced diagnosis.

Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] E. J. Corey, "General methods for the construction of complex molecules," *Pure and Applied Chemistry*, 1967, doi: https://doi.org/10.1351/pac196714010019.

[2] H. G. Khorana, K. L. Agarwal, H. Büchi, M. H. Caruthers, N. K. Gupta, K. Klbppe, A. Kumar, E. Ohtsuka, U. L. Raj Bhandary, J. H. van de Sande, V. Sgaramella, T. Tebao, H. Weber, and T. Yamada, "Total synthesis of the structural gene for an alanine transfer ribonucleic acid from yeast," *Journal of Molecular Biology*, vol. 72, no. 2, pp. 209-217, 1972, doi: https://doi.org/10.1016/0022-2836(72)90146-5.

[3] J. Watson, and F. Crick, "Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid," *Nature,* vol. 171, pp. 737-738, 1953, doi: https://doi.org/10.1038/171737a0.

[4] S. Ali, Md. Asim Azfer, A. Bashamboo, P. K. Mathur, P. K. Malik, V. B. Mathur, A. K. Raha, and S. Ansari, "Characterization of a species specific repetitive DNA from a highly endangered wild animal *Rhinoceros unicornis* and assessment of genetic polymorphism by microsatellite associated sequence amplification (MASA)," *Gene*, vol. 4 no. 228, pp. 33-42, 1999. [This work was highlighted in Nature Biotechnology, November 1999 issue].

[5] A. Bashamboo, and S. Ali, "Minisatellite associated sequence amplification (MASA) of the hypervariable repeat marker 33.15 reveals a male specific band in humans," *Mol. Cell. Probes,* vol. 15, pp. 89-92, 2001.

[6] P. Yam, L. D. Petz, S. Ali, D. Stock, and R. B. Wallace, "Development of a single probe for documentation of chimerism following bone marrow transplantation," *Am. J. Hum. Genet*., vol. 441, pp. 867-888, 1987.

[7] V. Katju, and U. Bergthorsson, "Copy-number changes in evolution: Rates, fitness effects and adaptive significance," *Frontiers in Genetics*, vol. 4, pp. 273, 2013.

[8] B. E. Kaplan, "The automated synthesis of Oligodeoxyribonucleotides," *Trends in Biotechnology,* vol. 3, no. 10, pp. 253-256, 1985, doi: https://doi.org/10.1016/0167-7799(85) 90024-1.

[9] A. Kornberg, "Biologic synthesis of deoxyribonucleic acid: An isolated enzyme catalyzes synthesis of this nucleic acid in response to directions from pre-existing DNA," *Science,* vol. 131, no. 3412, pp. 1503-1508, 1960, doi: https://doi.org/10.1126/science.131.3412.1503.

[10] E. C. Friedberg, *Emperor of Enzymes: A Biography of Arthur Kornberg, Biochemist and Nobel Laureate*. World Scientific Publishing Company Pte Limited, 2016, pp. 338, ISBN: 9814699829; 9789814699822.

[11] X. Li, and D. R. Liu, "DNA-emplated organic synthesis: Nature's strategy for controlling chemical reactivity applied to synthetic molecules," *Angewandte Chemie International Edition*, vol. 43, no. 37, pp. 4848-4870, 2004.

[12] R. Ogden, N. Dawnay, and R. McEwing, "Wildlife DNA forensics—bridging the gap between conservation genetics and law enforcement," *Endangered Species Research*, vol. 9, no. 3, pp. 179-195, 2009.

[13] A. J. Jeffreys, V. Wilson, and S. L. Thein, "Individual specific 'fingerprints' of human DNA," *Nature,* vol. 316, pp. 76-79, 1985.

[14] S. Ali, C. R. Müller, and J. T. Epplen, "DNA fingerprinting by oligonucleotide probes specific for simple repeats," *Hum. Genet*., vol. 74, pp, 239-243, 1986.

[15] S. K. Yadav, A. Kumari, S. Javed, and S. Ali, "DYZ1 arrays show sequence variation between the monozygotic males," *BMC Genetics*, vol. 15, pp. 19-31, 2014.

[16] C. Ramel, "Mini-and microsatellites," *Environmental Health Perspectives*, vol. 105, (suppl 4), pp. 781-789, 1997.

[17] S. Wilkie, M. S. Clark, P. Leroy, M. Merlino, S. Nègre, J. C. Caissard, and M. Bernard, "Genomic DNA isolation, southern blotting and hybridization," in *Plant Molecular Biology - A Laboratory Manual*. Springer Berlin Heidelberg, 1997, pp. 3-53,

[18] J. J. Raymond, R. A. van Oorschot, P. R. Gunn, S. J. Walsh, and C. Roux, "Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes," *Forensic Science International: Genetics*, vol. 4, no. 1, pp. 26-33, 2009.

[19] T. Bestor, A. Laudano, R. Mattaliano, and V. Ingram, "Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells: The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases," *Journal of Molecular Biology*, vol. 203, no. 4, pp. 971-983, 1988.

[20] C. R. Steinman, U. Deesomchok, and H. Spiera, "Detection of anti-DNA antibody using synthetic antigens: Characterization and clinical significance of binding of poly (deoxyadenylate-deoxythymidylate) by serum," *The Journal of Clinical Investigation*, vol. 57, no. 5, pp. 1330-1341, 1976.

[21] N. J. Ayon, "High-throughput screening of natural product and synthetic molecule libraries for antibacterial drug discovery," *Metabolites,* vol. 13, no. 5, p. 65, 2023, doi: https://doi.org/10.3390/metabo13050625.

[22] B. Farboud, and B. J. Meyer, "Dramatic enhancement of genome editing by CRISPR/Cas9 through improved guide RNA design," *Genetics,* vol. 199, no. 4, pp. 959-971, 2015.

[23] R. J. A. Rocha, "Optimization of peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) for the

identification of microorganisms in food matrices," Doctoral dissertation, Universidade do Porto, Portugal, 2018.

[24] M. Heinemann, and S. Panke, "Synthetic biology—putting engineering into biology," *Bioinformatics*, vol. 22, no. 22, pp. 2790-2799, 2006.

[25] A. Hoose, R. Vellacott, M. Storch, P. S. Freemont, and M. G. Ryadnov, "DNA synthesis technologies to close the gene writing gap," *Nature Reviews Chemistry*, vol. 7, no. 3, pp. 144-161, 2023.

[26] M. Saini, A. N. Jha, R. Tangri, Md. Qudratullah, and S. Ali, "MN1 overexpression with varying tumor grade is a promising predictor of survival of glioma patients," *Human Molecular Genetics*, vol. 29, no. 21, pp. 3532-3545, 2020, doi: https://doi.org/10.1093/hmg/ddaa231.

[27] B. S. Chang, M. A. Kazmi, and T. P. Sakmar, "Synthetic gene technology: Applications to ancestral gene reconstruction and structure-function studies of receptors," in *Methods in Enzymology.* Academic Press, vol. 343, pp. 274-294, 2002.

[28] K. E. Nielsen, J. Rasmussen, R. Kumar, J. Wengel, J. P. Jacobsen, and M. Petersen, "NMR studies of fully modified locked nucleic acid (LNA) hybrids: Solution structure of an LNA: RNA hybrid and characterization of an LNA: DNA hybrid," *Bioconjugate Chemistry*, vol. 15, no. 3, pp. 449-457, 2004.

[29] B. Vester, and J. Wengel, "LNA (locked nucleic acid): High-affinity targeting of complementary RNA and DNA," *Biochemistry*, vol. 43, no. 42, pp. 13233-1324, 2004.

[30] W. B. Miller Jr, F. Baluška, and A. S. Reber, "A revised central dogma for the 21st century: All biology is cognitive information processing," *Progress in Biophysics and Molecular Biology*, vol. 182, pp. 34-48, 2023.

[31] S. Koren, M. C. Schatz, B. P. Walenz, J. Martin, J. T. Howard, G. Ganapathy, and A. M. Phillippy, "Hybrid error correction and de novo assembly of single-molecule sequencing reads," *Nature Biotechnology*, vol. 30, no. 7, pp. 693-700, 2012.

[32] R. A. Hughes, and A. D. Ellington, "Synthetic DNA synthesis and assembly: Putting the synthetic in synthetic biology," *Cold Spring Harbor Perspectives in Biology*, vol. 9, p. a023812, 2017, doi: https://doi.org/10.1101/cshperspect.a023812.

[33] J. Brzezinska, S. Trzciński, J. Strzelec, and M. K. Chmielewski, "From CPG to hybrid support: Review on the approaches in nucleic acids synthesis in various media," *Bioorg Chem.,* vol. 140, p. 106806, Nov. 2023, doi: https://doi.org/10.1016/j.bioorg.2023.106806.

[34] I. A. Koslov, F. Kaper, L. Zhou, and M. S. Chee, "Microarray based oligonucleotide synthesis," in *Nucleic Acids Symposium Series.* Oxford University Press, vol. 52, no. 1, pp. 723-723, Sept. 2008.

[35] D. Qin, M. Forster, S. M. Gandhi, R. Akabari, Z. Zheng, J. Lal, and K. Lovinger, "Usage of DNA fingerprinting technology to check sample error and contamination in molecular laboratories," *Current Issues in Molecular Biology*, vol. 44, no. 11, pp. 5543-5549, 2022.

[36] N. A. Azad, Z. A. Shah, A. A. Pandith, R. Rasool, and S. Jeelani, "Real-time quantitative PCR: A reliable molecular diagnostic and follow-up tool for 'minimal residual disease' assessment in chronic myeloid leukemia," *Bioscience Reports*, vol. 38, no. 5, BSR20180974, 2018.