

# dnarXiv: storing information on DNA molecules

## 1. Abstract

The dnarXiv project aims to explore data storage on DNA molecules. This kind of storage has the potential to become a major archive solution in the mid- to long term. Our main objective in this project is to develop a large-scale multi-user DNA-based data storage system that is reliable, secure, efficient, affordable and with random access. For this we will consider two key promising biotechnologies: enzymatic DNA synthesis and DNA nanopore sequencing. We will propose advanced solutions in terms of coding schemes (i.e., source and channel coding) and data security (i.e., data confidentiality/integrity and DNA storage authenticity), that consider the constraints and advantages of the chemical processes and biotechnologies involved in DNA storage.

## 2. General context and motivations

The current data explosion era is bringing new challenges in data storage and leading research to alternative emerging technologies. The need to explore innovative solutions is now undeniable because the available data storage systems have grossly been outpaced by the ever-increasing data generation.

Currently, data archives (or archival storage) serve as a way of reducing primary storage consumption and related costs. This kind of storage protects information that is not needed for everyday operations but may have to be accessed occasionally. Devices for archival storage require large capacity and excellent durability. These are increasingly difficult to attain in modern memories, leading to the study of alternative technologies based on biochemical or molecular storage. Figure 1 presents a comparison of the current data storage technologies: DNA or molecular storage is largely superior to optical, tape, solid state drives (SSD or flash) and hard disk drives (HDD). In terms of data density, DNA is  $10^6$  times superior to HDD and, in terms of durability, DNA is at least 10 to 20 times superior to all the other technologies. Furthermore, storage on DNA has a very low energy consumption and technical means already exist to store DNA at room temperature for long term in databank [34]. Another advantage is the lack of foreseeable technical obsolescence: DNA is a universal and a fundamental data storage mechanism in biology since the beginning of life.

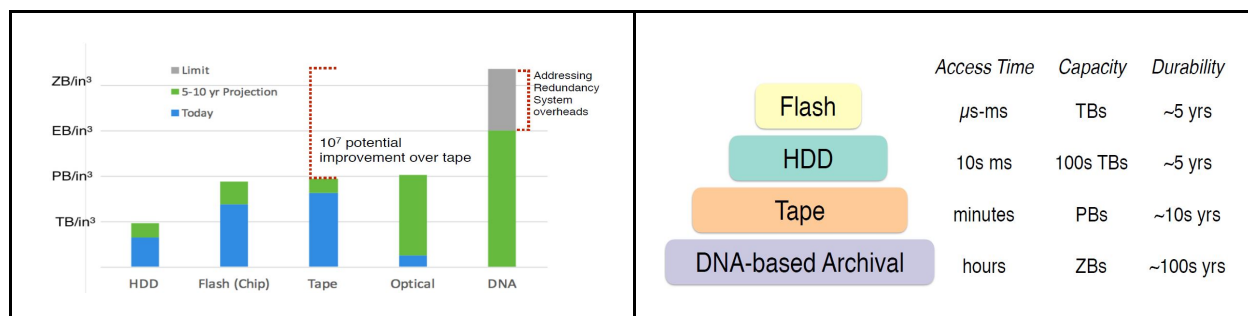


Figure 1. Comparison of current technologies and DNA in terms of storage density, access time, capacity and durability.  $1 \text{ ZB} = 10^3 \text{ EB} = 10^6 \text{ PB} = 10^9 \text{ TB}$  @ image Microsoft Research & University of Washington

Since 2012, several studies have demonstrated that digital data can be written in DNA, stored, and accurately read, thanks to the latest advances of biotechnologies in DNA synthesis (i.e., writing DNA) and sequencing (i.e., reading DNA). Different strategies concerning information and coding theory, bioinformatics and computer architectures, molecular and synthetic biology, have already been explored (mostly by American and British teams). However DNA storage is still in its infancy and we believe it is the right time to actively contribute to this emerging field. The dnarXiv project aims to propose original strategies for efficient DNA data storage, to lay the foundations of this promising technology and to present an end-to-end prototype. The project will also include cybersecurity in molecular data storage because to the best of our knowledge, security aspects such as confidentiality, traceability and integrity have not yet been explored in DNA storage. However, this is an important issue to explore as experts claim that, for security strategies to be efficient, they must be considered at the initial steps of any new technology.

The goal of this project is the design of an efficient, reliable, secure and affordable end-to-end prototype for random-access large-scale multi-user DNA-based data storage. For this, we plan to include the most promising related biotechnologies: enzymatic DNA synthesis and nanopore sequencing. Among all prior work, only [1] considered these two technologies together to store a few bytes of information, with an uncontrolled synthesis method and poor correction schemes. In the dnarXiv project, we plan to introduce original approaches including: (1) the design of a secured DNA-based file server with the potential to store huge quantities of information while guaranteeing confidentiality and integrity of data; (2) the use of innovative methods for enzymatic DNA synthesis; (3) the design of advanced source and channel codes for high data density and excellent error performance.

### 3. State of the art

Even if the basic concept of using DNA for data storage dates back to the mid- 1960s, it was necessary that DNA sequencing and synthesis technologies became mature enough for this concept to become feasible. It was in the early 2010s that two different teams outlined practical architectures for DNA data storage [2][3] by storing a few hundreds of kilobytes of data. In [2] the authors achieved a density of 700 TB/gram; in [3] the density was increased to 2 PB/gram thanks to source coding schemes. Other DNA storage systems were reported in [4-7] (among others).

The major steps of DNA storage are described in Figure 2, where digital data is transformed into DNA data to be synthesized, cloned and stored within a biological cell (in vivo) or, more commonly, stored in vitro (frozen in solution or dried down for protection from the environment). In [4] the authors estimate that a single physically isolated DNA pool can store on the order of  $10^{12}$  bytes. To read the requested data, the corresponding DNA pool is physically retrieved and thanks to random access a specific data item can be read without having to read all the data in the pool. DNA sequencing produces a set of reads that correspond to the molecules detected by the sequencer. These reads then provide the original digital data with a success rate that depends on the sequencing coverage and the error rate experienced throughout the process.

In May 2019, the authors in [8] summarized the main recent and ongoing projects on DNA storage ([3-7] among others). Currently, the most advanced team in the field is the one of Microsoft Research and University of Washington (Seattle). Their last published work [4] describes how they stored 35 distinct variable-size files (over 200 MB of data) and recovered each file individually without errors, which represents an outstanding major achievement. The synthesized DNA (around

10 million strands) was provided by the company Twist Bioscience (California, US) [12]. This company has developed a proprietary silicon-based synthetic DNA manufacturing process that allows to miniaturize the chemistry necessary for DNA synthesis. However, chemical DNA synthesis still presents drawbacks (throughput and cost) and fails to meet future market needs. Fortunately, enzymatic-based synthesis methods [1][9][10] are currently under development. They should allow the synthesis of much longer DNA fragments at higher throughputs (~10 times faster) and lower costs. Our partner company DNA Script is a top key player in this field and is currently developing a DNA printer based on enzymatic synthesis that will be commercialized in 1 to 3 years. They recently raised 35 M€ [13] and are on the way to disrupt the DNA synthesis market worldwide. Some concurrent companies are Nuclera Nucleics (UK, enzymatic-based synthesis), Evonetix (UK, silicon-based chemical synthesis) and Molecular Assemblies (CA, US, enzymatic-based synthesis). Thanks to the dnarXiv project, we could become the French counterpart to the American team (i.e. Microsoft / University of Washington / Twist Bioscience), proposing alternative approaches for efficient DNA storage.

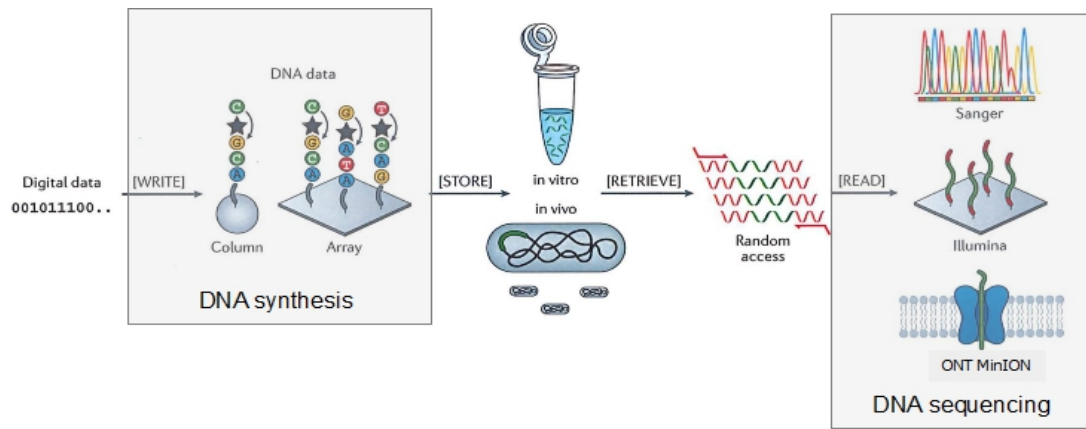


Figure 2. Steps in the DNA data storage chain [8]. Three different sequencing technologies are represented: Sanger, Illumina and Oxford Nanopore Technologies MinION. @ modified image from [8]

The other key biotechnology in DNA storage is DNA sequencing, which is now a well-known process as it has been used in most genomic studies for decades. Each generation of sequencing technology has raised a specific challenge (see Figure 2). After first-generation Sanger sequencing (originally from the seventies), next-generation Illumina technologies [14] have led to an impressive decrease of the sequencing costs over the last decade. However, this technology cannot read long strands of nucleotides. The devices must read short fragments and then combine the data together to retrieve the original sequence. In this project, we focus on the most recent sequencing devices (i.e. third generation) that are based on chemically modified biological nanopore channels. These high-throughput devices can decipher billions of nucleotides in a few hours at a very low cost. The nanopore sequencing principle [11] is based on the detection of changes in an ionic current when a DNA sequence passes through a nanoscale hole. Each k-mer (i.e., ordered vector of k nucleotides) causes a different current level because of the different atomic structure of nucleotides. This makes the identification possible.

In 2014, Oxford Nanopore Technologies (ONT) introduced the MinION sequencer [15] which contains hundreds of nanoscale holes, allowing this process to be parallelized to increase throughput. This results in read lengths from several hundred nucleotides to hundreds of thousands

of nucleotides. Other advantages of the MinION are: it directly connects to a PC via a USB3 port, it weighs ~90 g, and costs are for consumable reagents only. However, even if current trends are making prices to continue to decrease and reading throughputs to continue to increase, the main drawback is still its high error rates.

## 4. Project Overview

The general DNA storage pipeline that we have in mind is depicted in Figure 3. It primarily aims to demonstrate the feasibility of an affordable and reliable multi-user file server using DNA molecules as storage medium.

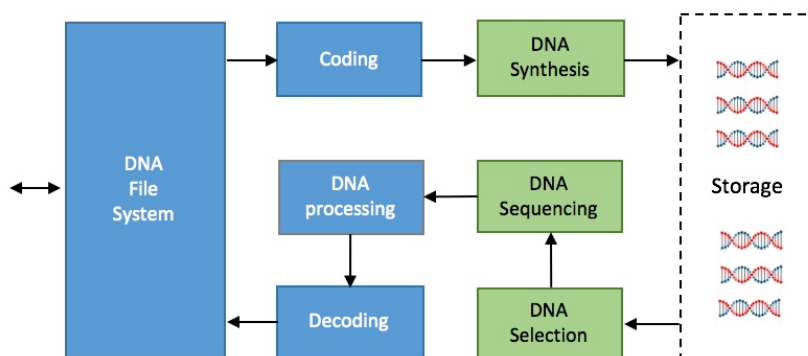


Figure 3. DNA-based file server.

The DNA File System block acts as a front end to organize the information. Input data are split into small pieces of information according to synthesis and sequencing constraints. Typically, today DNA synthesis technologies can only provide short oligonucleotide sequences (a few hundred nucleotides) which could be the equivalent of magnetic disk sectors. The information is then encoded into the DNA alphabet and each small DNA sequence has its own barcode (a specific DNA word). As the next steps, especially DNA sequencing, are error prone, powerful error-correcting codes need to be included. The DNA synthesis block then transforms DNA text into DNA molecules that are finally stored under the required conditions.

The read operation first extracts a subset of short DNA molecules according to their barcode, duplicates/amplifies them and concatenates this subset to form long DNA molecules (DNA Selection module). Duplicating the DNA molecules brings the redundancy that is mandatory for the sequencing step which generates texts of poor quality. The long molecules are then sequenced and sent to the DNA processing module which uses bioinformatics methods to perform sequence assembly. The Decoding module corrects the errors and restores the fragmented information. Finally, the DNA File System module re-organizes these pieces of information to retrieve the initial one.

The originality of our proposal compared to prior work is the combination of enzymatic DNA synthesis, nanopore DNA sequencing, and the introduction of security methods. The two biotechnologies are currently the most promising in the emerging DNA storage context. Thanks to advanced source and channel coding schemes, we will improve their reliability as well as the global efficiency of the system. Regarding security aspects, we will explore methods to guarantee confidentiality, integrity and authenticity of data. These are currently open problems in DNA storage and our work could definitely be pioneer in this field.

Based on the constraints of the biotechnologies, the dnarXiv project will explore how to set up a complete DNA storage system. Our project will have a large experimental part in order to practically demonstrate the feasibility of our approach. Even if, being realistic, we may not be able to propose a full operational system at the end of the project, we will definitely contribute and provide answers to several key issues: how such a system can be designed, what are the main bottlenecks, which are the impacts of the biotechnology constraints on upstream and downstream numerical processing, how security aspects (confidentiality, integrity...) can be directly integrated into the biotechnology processes... among others.

## 5. Research axes

The dnarXiv project is structured in three main research axes that are closely related:

1. Biotechnologies: DNA synthesis and sequencing
2. Error correcting code
3. Security

The constraints imposed by the biotechnological limitations will significantly determine the design of source and channel codes, as well as the way to secure and control the information. Alternatively, biomolecular properties may advantageously be exploited to provide new security mechanisms. A detailed description of the three research axes follows:

### 1. Biotechnologies: DNA synthesis and sequencing

This axis aims to investigate how enzymatic synthesis and nanopore sequencing technologies can be efficiently exploited for DNA storage. It also aims to identify constraints that need to be considered for efficient coding and security methods.

From one side, enzymatic synthesis technologies generate short DNA strands ranging from a few tens nucleotides today to a few hundred in a near future. On the other side, sequencing technologies based on nanopore are now able to decipher long DNA molecules (ranging from a few hundred to  $10^6$  bases). The challenge is to conciliate these two technologies and to exploit the best of both. The scenario we have in mind is that the files to archive will be fragmented into  $N$  short pieces of information, each one being supported by one DNA molecule (DNA\_1 to DNA\_N) corresponding to the enzymatic synthesis device capacity. Each DNA fragment will be tagged specifically and all DNA fragments will be pooled together to create the library. Schematically, trillions of short DNA fragments present in many copies will coexist in the same DNA pool.

At this point, many questions arise: how to recover this information or how to extract only a specific part? How to duplicate this information? What are the biotechnology protocols to set up? What are the impacts on the coding and security processes? The various wet lab experiments we did during the exploratory action were very informative. As an example, the MinION behaves poorly on homopolymer sequences. Thus, a strong constraint, at the coding stage, was to generate DNA fragments without stretches of identical nucleotides in order to minimize the error rate. Similarly, selecting the right DNA fragments to retrieve a specific file implies to use PCR (Polymerase Chain Reaction) amplification. This protocol works with pairs of primers (ordered vector of  $k$  nucleotides with  $k \sim 20$ ) that have to be very specific. In other words, these words ( $k$ -mers) must not be present inside the DNA fragments that support the useful information. This is another example of the constraint that the coding step has to consider.

Currently, different protocols and biotechnological tools can extract a subset of DNA fragments and arrange them into long DNA molecules, suitable for nanopore sequencing. Each of them has its own advantages and drawbacks with different impacts on the upstream and downstream data processing. Investigating various biotech scenarios together with their associated processing counterpart is the heart of this research axe.

## **2. Error correcting codes**

To enhance the reliability of the DNA storage file server we are going to explore several ideas to overcome synthesis and sequencing errors. First, for synthesis errors, we are going to develop a new error model that includes the characteristics of the DNA Script enzymatic-based methods and then design specific codes. For this, we will closely collaborate with this partner to identify differences, in terms of errors, between chemical and enzymatic synthesis. So far, it is well known that dominant error events in chemical synthesis are simple substitutions and error rates mainly depend on the cost of the technology. Sequencing methods using Polymerase Chain Reaction (PCR) amplify these substitutions errors by creating many copies of the synthesized sequence. Moreover, with high-throughput sequencing (i.e. nanopore), synthesis errors propagate through a number of reads produced through sequencing. These issues have been addressed in [16] with the introduction of DNA profile codes. Single insertion/deletion errors can also be introduced during synthesis. Tenengolts codes are well adapted for this kind of errors and can be directly encoded into the DNA sequence. Also, the problem of reconstructing the DNA sequence from deletions/insertions followed by PCR techniques has been considered in [17] and [18].

Errors inherent to nanopore sequencing correspond to single or burst deletions, insertions and substitutions. In the context of DNA storage, we can make the system more robust using the principles of channel coding. From this perspective, prior works include [19], where the authors propose asymmetric codes to deal with substitution errors characterized by the impulse response distributions of the sequencer output signals. These errors are in fact considered asymmetric because some substitutions are much more likely than others (e.g., nucleotide A is more likely to be substituted by a T than by a G). In [20] codes in the Damerau distance are introduced to correct single or block transposition errors combined with deletions. Other significant work [21] addresses the problem of fast translocation speeds of DNA molecules across the nanopore, which leads to burst deletions. All these kinds of codes offer firm error correction capability and the associated decoding algorithms use bounded distance decoding, based on taking a hard decision at the input of the decoder. At this step, we have several promising ideas: we plan to use the information before the basecalling step to exploit the channel information and introduce soft decoding algorithms (which are known to be much more performant than hard algorithms). In this context, we introduced a nanopore channel model in [24].

In [22][23] we collaborated with UCLA (Prof. Dolecek) to propose innovative deduplication techniques for data storage in the context of edit errors (i.e., insertions and deletions). Data deduplication is an emerging technology that improves storage utilization and offers an efficient way of handling data replication. The principle is that redundant data blocks are removed and replaced with pointers to a unique data copy. Before [22][23][24], the problem of deduplication had mostly been considered by computer scientists and large gains had been obtained for archival storage backup systems. Our work explored information/coding theoretic approaches and led to some interesting primary results. In the dnarXiv project we plan to extend these approaches to the DNA storage context, which is based on a quaternary alphabet and a particular edit channel model.

Finally, with Prof. Wang (Univ. California Irvine), we are currently studying advanced coded modulation schemes that exploit the ionic current changes in the nanopore. As the current signal is a function of k-mers, the use of modulation schemes of order of 2k should provide significant gains. Thanks to the dnrXiv project we will be able to quantify the obtained practical gains on the DNA storage chain.

### **3. DNA storage security**

Nowadays, security of data and systems is crucial in every application domain. In the past, security has always been considered after the development and deployment of novel technologies (e.g. IOT). However, to avoid limitations and constraints for users, security should be included in the early steps of the development of upcoming technologies. Data storage on DNA molecules is obviously concerned by security and, to the best of our knowledge, no prior work has focused on this problem as a whole. In the dnrXiv project, we are going to explore security mechanisms that are highly transparent and flexible for the users.

Since the 90's, several works have explored the link between DNA and security: how to exploit the random nature of the human genome to encrypt data [26][27] or how to encrypt data into synthetic or living cells DNA. For instance, in [28] the authors first convert binary data into DNA bases using an alphabet substitution strategy based on a secret dictionary. In [29] the complete encryption algorithm is biologically made. In the literature, DNA cryptography also includes different steganography approaches [30][31]. The idea is to dissimulate sensitive data into DNA. In [32], user information is converted into DNA strands that are then mixed with distracter DNA strands into a microdot. DNA primers play an important role so as to make possible the extraction of the secret DNA strands from the DNA pool.

In the dnrXiv data storage system, several security issues need to be considered. Even if the protection of the stored data in terms of integrity and confidentiality is a major concern, it is by far not the only one. The complete DNA storage pipeline has to be analyzed. To the best of our knowledge, [33] constitutes the very first work analyzing security issues in the synthesis, sequencing and processing of DNA. They demonstrate that it is possible to generate fake DNA data which – when sequenced and processed – can give an attacker the possibility to execute arbitrary code remotely, thus compromising a computer system using biological or synthetic DNA samples. In this context, new security questions arise: how to control the access to the data stored on DNA molecules or the authenticity of the DNA storage (is this my data?); has the data been altered with the addition of, for instance, fake DNA data?; could some pieces of information leak when sequencing synthetic DNA... among many other questions. Identifying such security issues and proposing counter solutions gives to dnrXiv the potential to significantly contribute in this field.

As exposed in Fig. 3, the DNA storage pipeline includes two parts. The first one, in blue in Fig. 3, regroups all digital functionalities, notably those in charge of the digital data encoding/decoding. The second part, in green, represents the biochemical functions including DNA synthesis and sequencing. If data security in the digital domain is now well known and can be easily achieved (e.g., addition of cryptographic chips to hard-disk-drives), this is not the case in the biochemical domain. In the dnrXiv project, we first aim to conduct a complete security analysis of the DNA storage pipeline, identifying threats and the security goals to attain. Secondly, we will develop novel DNA based security functionalities to respond to these needs. Our idea is not to develop these new mechanisms alone but at the interface with the digital domain so as to re-enforce data storage security and to go beyond solutions proposed for hard drives. To do so, we will take advantage of

DNA based steganographic solutions [30][31]. To be specific, our first research challenge will be on how to define DNA primers in order to make extremely difficult for an attacker to identify the information of one user. Primers are the keys to select specific fragments. If they are not known, the amplification step before sequencing is impossible and, by extension, the reading operation. There are many ways to play with primers and, consequently, to control access to the information. We will also explore how DNA cryptography can be deployed. Moreover, we will also work on DNA support authentication so as to be sure of its origin and that no extra-information can be added by an attacker without being detected.

Note that, by working with primers from a security point of view, we are clearly collaborating with the two other research axes. Our security solutions will have to take into account data fragmentation into small DNA strands (Axis 1 – system file management) and the constraints of DNA synthesis and data encoding strategies (Axis 2 – source and channel data encoding).

To conclude, this research axis will potentially lead to two main contributions: (1) A deep security analysis of the complete DNA storage pipeline, from the digital to the biochemical domains, with the goal to identify the security risks for both the stored data and the DNA material; (2) Novel security DNA storage mechanisms that take advantage of biotechnologies for access control and confidentiality of data, as well as providing authentication tools to DNA storage.

## **6. Research teams, permanent researchers and partners**

### **GenScale IRISA/INRIA**

The expertise of this team is bioinformatics with a specific focus on the treatment of sequencing data. The team develops fast algorithms with low memory footprint. Furthermore, for the past year, GenScale has been investing in the nanopore sequencing technology. The arrival of E. Roux in 2018 (INRIA delegation in the team, biologist by training) and the purchase of the ONT MinION (one of the first in the Rennes area) has allowed GenScale to perfectly master this technology.

### **IAS Lab-STICC Lorient**

The IAS group is known for its expertise in error-correcting codes for communications (LDPC codes, Turbo codes, Cortex codes...). This group developed one of the first practical implementations of a non-binary LDPC decoder during the FP7 DAVINCI project. Since then, they have been contributing to code design, coded modulations, applications and implementation of high-throughput decoders for non-binary codes.

### **LaTIM Inserm UMR 1101 / IMT Atlantique**

This group has an expertise on the security of medical data by means of mechanisms like watermarking, crypto-watermarking, digital content forensics and processing of encrypted data in domains like Big Health Data, Genetics (CominLabs PRIVGEN project) and connected medical implants. In this project, LaTIM will take advantage of the knowledge acquired during the PRIVGEN project where they developed several DNA data watermarking modulations that can be extended to synthetic DNA. LaTIM will contribute to identify and to address innovative security issues for DNA Storage, taking advantage of the expertise of the other dnarXiv partners on DNA synthesis.



## **Institute for genetics and development in Rennes (IGDR)**

IGDR is a multidisciplinary Research Institute on Biology (CNRS-UR1) aiming towards a quantitative and dynamic understanding of life. IGDR is composed of research teams exploring the fundamentals of gene expression, the structural basis of the function of biomolecules and their integration in the cellular processes involved in organism development, physiology and pathology. The IGDR will provide full access to its wet lab space and facility thereby providing the context to implement DNA library preparation, analysis and sequencing.

## **DNA Script**

DNA Script is a French company developing a solution for fast and inexpensive access to synthetic DNA. They are currently developing a DNA printer system based on innovative synthesis technology, using polymerization enzymes to replace the traditional organic chemistry reagents used for 50 years for DNA synthesis. This company will contribute to the dnarXiv project bringing their tools and expertise on enzymatic DNA synthesis. Ideally, our collaboration should lead to a synthesis channel model that allows the design of specific error correcting codes. Also, they will be involved in the generation of the DNA strands for the different experimental dnarXiv platforms.

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