Master Thesis

DNA as a storage - Error Detection based on Cyclic Codes

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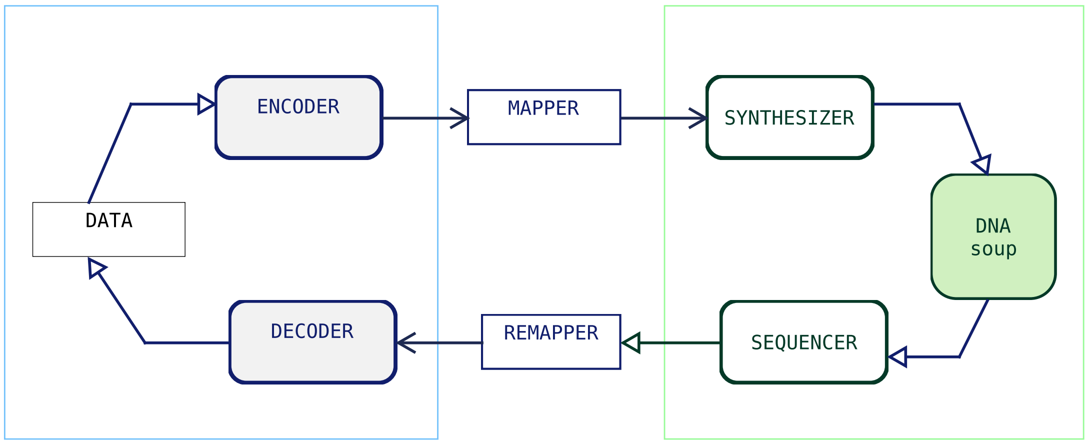

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Abstract

Due to the constant information generation, data centers are taking more and more space. State of the art technology allows storing data on multiple servers using cloud computing management. One of the alternate storage mediums that scientists considered is synthesized DNA molecules. Due to the properties of DNA, such as capacity (1 gram can store 2.1 Zettabytes of data), stability, and self-assembly, it is considered a storage medium. This thesis provides a comprehensive guide for electrical engineers to the topic of DNA as data storage and covers basics of biology, synthesis, sequencing algorithms and specifics of DNA channel.

The thesis also discusses challenges second-generation sequencers (devices for reading DNA back) are facing. The accuracy of DNA sequencers to read back DNA sequence without errors increases (98%), however, they are still imposing problems when sequencing long strands of DNA sample by using reads longer than 300 characters. To tackle the problem, this work introduces a unique process of encoding data. As a consequence, collected back together, it is possible to detect erroneous parts of sequenced reads (chunks of DNA sequences) and assemble DNA sequence.

The thesis finalizes with an empirical analysis of 3000 experiments on the randomized payload (randomly generated messages) and provides the comparison of coding based DNA storage and state-of-the-art sequencing approach.

Title page image: Basic schema of DNA as a storage. Schema is component based, where blue part corresponds to Error Control Codes (storage) and green corresponds to Biology (storage medium).

Acknowledgements

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Contents

Notations

x	scalar variables: italic lower case letters
$\underline{x}$	single vector: underline lower case letters
$\mathbf{x}$	set of vectors: boldface upright lower case letters
x_k	scalar elements of a vector : element index is a subscript
$\underline{x}_k$	vector elements in a set : element index is a subscript
$[x_1, x_2, .., x_k]$	vector values: square brackets represent vector values
$\{\underline{x}_1, \underline{x}_2, .., \underline{x}_k\}$	set of vectors: curly braces represent set items
$\mathbb{F}_4$	finite field with four elements : typeface upper case letters with subscript
$p(x)$	continuous function
$\underline{\underline{X}}$	matrix variables: double underlined upper case letters
$\underline{\underline{X}}_{r,c}$	scalar elements of a matrix: row and column indices are subscripts
$\underline{a} * \underline{b}$, $5.1 * 10^{-9}$	cross product of two vectors or scientific notation of numbers
$\underline{\underline{H}} * \underline{c}$	matrix - vector multiplication
`a = 15`	source code : typewriter font
`WorkflowManager`	class definition : camel case notation
`detect()`	method definition : lower case notation
`SYNTHESIZER`	constant variables : upper case notation

1. Introduction

This chapter serves gives brief description of state-of-the-art research in the field of DNA as a storage. Motivation, aims of this work and thesis structure are described in this chapter.

1.1. Motivation

In today's world of constant information generation, more and more data centres are built. Moreover, it is predicted that by 2025 global datasphere will hit 175 billion terabytes [1]. Even though there is tremendous development in cloud technology (distributed systems or modern software-defined networking), they are notably capacious. There are some aspects of risk involved in keeping data centers secure and safe: environmental changes, animals, insects and security breaches.

One of the alternate storage mediums that scientists considered is synthesized DNA molecules [2]. Recent research showed experimentally that it is indeed possible to store data in DNA. As an example, research teams from the University of Illinois were able to store the files in a jar with synthetic DNA molecules [3]. Additionally, researchers at Microsoft and the University of Washington have demonstrated the first fully automated read-only DNA system in artificially created - synthetic DNA [4]. Following is a direct quote from Microsoft team:

"This is a key step towards moving new technology from research laboratories to commercial data centres."

The main reason why scientists decided to look at DNA as an alternate storage medium is due to the properties it possesses. Unique properties of DNA (in terms of storage) are:

- DNA molecules can potentially store the entire volume of global digital information in about 9 litres of DNA solution for millennia as it is described in the article from technology magazine Habr. The capacity of DNA where one cell with certain DNA and mass of 3 pgrams stores 6.4 Gigabases of data [3] (which makes 1 gram equivalent to $1-2$ Zettabytes, depending on encoding scheme);
- Stability is another property that DNA possesses [5]. It is a great medium for long-term storage. Research conducted in ETH Zurich states that if synthetic DNA molecules are saved in a specific material and at a certain low temperature, then the information will be stored for millions of years [5].

- Finally, self-assembly potential - DNA is proven as the building blocks of small self-assembly based chips [6].

Interestingly enough in human body DNA in a certain way is used as a hereditary data storage. In nature, DNA molecules carry genetic instructions that control the functioning of living organisms [7].

1.2. Aim of this work

Despite research in both theoretical and experimental worlds, there is no clear step-by-step guide of how exactly DNA storage works, what components are necessary to create even simulation of the working system. Additionally, there is no comprehensive description of biological components and sequencing algorithms for reading DNA back and assembling it for electrical engineers. The thesis provides compiled comprehensive guide for electrical engineers to jump-start the topic of DNA as storage and basic prerequisite biology and computer science. Additionally, the thesis provides a virtual simulation of the system on python.

The popularity of modern second-generation sequencers (sequencer reads DNA from the unknown sample of molecules) increases and almost every sequencing lab is using them. Even though the accuracy of DNA sequencers to read back genome without errors increases (98%), they are still imposing problems when sequencing long strands of DNA sample by using reads longer than 300 characters. This work also introduces a unique way of encoding data, such that when collected back together, it is possible to detect erroneous parts of sequenced DNA. The empirical analysis was conducted on toy-parameter example, where parameters were assumed to be proportional to real-world parameters.

A thesis serves as a comprehensive guide for communication specialists or bioinformaticians on basics of DNA as a storage.

1.3. Chapters review

This work is divided into six parts. Chapter 1 provides a quick introduction to DNA as a storage - motivation in Section 1.1 behind considering DNA as a storage medium, goal of this work in Section 1.2 and project structure in Section 1.3.

Chapter 2 provides comprehensive introduction to biology, that is required for this work. Chapter 2 describes unique properties in Section 2.1 of DNA, introduction to DNA for electrical engineers in Section 2.2, biological synthesis in Section 2.3 and sequencing in Section 2.4. Chapter is finalized by putting biological/biochemical interface altogether in Section 2.5.

Chapter 3 provides all necessary background information in error control codes required for assembling model of DNA as a storage system. Chapter starts with brief introduction to coding theory in Section 3.1, follows by brief description of error control code types in Section 3.2, finite fields and mapping technique from quartenary system to DNA in Section 3.3, brief description of error detection techniques and cyclic codes in Section 3.4. Chapter is finalized by putting error control codes altogether in Section 3.5.

Chapter 4 introduces schematics of full blown DNA as a storage system. Chapter introduces the schematics of DNA as a storage in Section 4.1; follows by description of tools used for project in Section 4.2, concrete schemas of each package implemented for bioinformatics in Subsection 4.2.1, ECC in Subsection 4.2.2 and helper interfaces in Subsection 4.2.3, and workflow manager class Subsection 4.2.4. Chapter proceeds with description of sequencing as a computational problem in Section 4.3, where sequencing is first associated with exploding newspaper problem in Subsection 4.3.1 and then exploited using graph algorithms, such as shortest common superstring and de Bruijn graph in Subsection 4.3.2. Chapter is finalized by introducing ECC detection mechanism for erroneus reads - this Section 4.4 also serves as opening for the next chapter .

Chapter 5 continues the topic of error detection techniques for sequencing. The chapter introduces the differences and similarities between codewords and reads in Section 5.1, follows by the introduction of headers and proto messages in Section 5.2, where codewords are adapted to reads format for further assembly. The chapter proceeds with an assembly of the genome using valid codewords in Section 5.3, where reads are represented as codewords with a header for providing overlap. The chapter is finalized by presenting an empirical analysis of the system (simulated mini-system/”toy parameterized” system) in Section 5.4 that provides additional components to the initial setup in Subsection 5.4.1 and the comparison of coding based DNA storage and state-of-the-art sequencing approach in Subsection 5.4.2. Chapter finalizes with conclusion and topics for further discussion in Section 5.5.

Chapter 6 finalizes the thesis by discussing the future outlook of the DNA as storage and contribution of the work.

2. Biology for Electrical Engineers

Before going into the schematics and details of DNA as storage, it is necessary to understand the motivation behind using DNA and basic terminology from classic and synthetic biology. This chapter will describe significant reasons behind choosing DNA as a storage medium and will serve as a sufficient head start in biology for electrical engineers.

2.1. Properties of DNA

In today's world of constant information generation, data centers are built. Moreover, at the rate that data gets generated, it is predicted that by 2025 global datasphere will hit 175 billion terabytes. Additionally, the problem with state-of-the-art data storage technologies is a lifetime of solid-state drives and hard disks. Researchers decided to consider DNA as one of the possible storage mediums.
The work discusses DNA as a storage medium due to the unique properties of DNA. Unique properties of DNA are:

- capacity, where one cell with certain DNA and mass of 3 pgrams stores 6.4 Gigabases of data (which makes 1 gram equivalent to $1-2$ Zettabytes);
- stability - it was possible to read out the DNA of mammoth;
- self-assembly potential - DNA is proven as building block of small self-assembly based chips.

Interestingly in the human body, DNA in a certain way is used as a data storage. It carries genetic information - instructions to carry out vital biological processes.

2.2. Introduction to DNA

DNA (deoxyribonucleic acid) - is a *macromolecule (biopolymer)* that consists of smaller molecules (monomers) - nucleotides [8]. Nucleotides consist of three main parts: nitrogenous base, sugar base and phosphate group. Electrical engineers and channel coding specialists are interested only in nitrogenous bases. Nitrogenous base is a molecule that contains nitrogen and chemical base; nitrogenous bases are building blocks of DNA and for electrical engineers they are simply considered to be characters/symbols from alphabet of {**A**,**C**,**G**,**T**} - {Adenine, Cytosine, Guanine, Thymine}.

DNA sequence or strand - is *one side of DNA*, sequence of characters composed from nucleic acids alphabet. The reason why it is comfortable to use DNA for data storage is the ease of mapping from the quaternary system to an array of characters (A, C, T, G) and vice versa. Each molecule of DNA is a double helix, shown in Figure 2.1 formed from two complementary strands of nucleotides bonds (chemically hydrogen bonds) between G-C and A-T base pairs.

So in summary, *error control coders* consider single strand of DNA molecule - sequence, that is an ordered combination of 4 bases T,A,G,C, e.g., ACCTGAT. Figure 2.2 demonstrates schematic representation of DNA molecule.

Figure 2.1.: DNA Molecule: double helix

2.3. Synthesis

Synthesis is a biological paradigm aimed to develop new *biological* components, tools, and structures, or to reinvent structures already present in nature [9]. Basically example of synthesis is to create synthetic molecules out of a certain sequence of DNA. In other words, here, we are trying to create DNA physically from its sequence. **Synthesizer** a device that makes short, artificial oligonucleotides with any desired sequence of nucleotide bases. Let us decompose this definition.

Oligonucleotide is a sequence, that is an ordered combination of 4 bases T,A,G,C, e.g.: ACCTGAT .

- According to the definition of a synthesizer, we pass the oligonucleotide sequence as an *input* to the system.
- In turn, using certain chemicals(phosphoramidite is a basis of reaction) machine creates artificial DNA molecules - by adding a complementary strand to the system. As a result, we get DNA molecules, that structurally are similar to the cell DNA molecule.

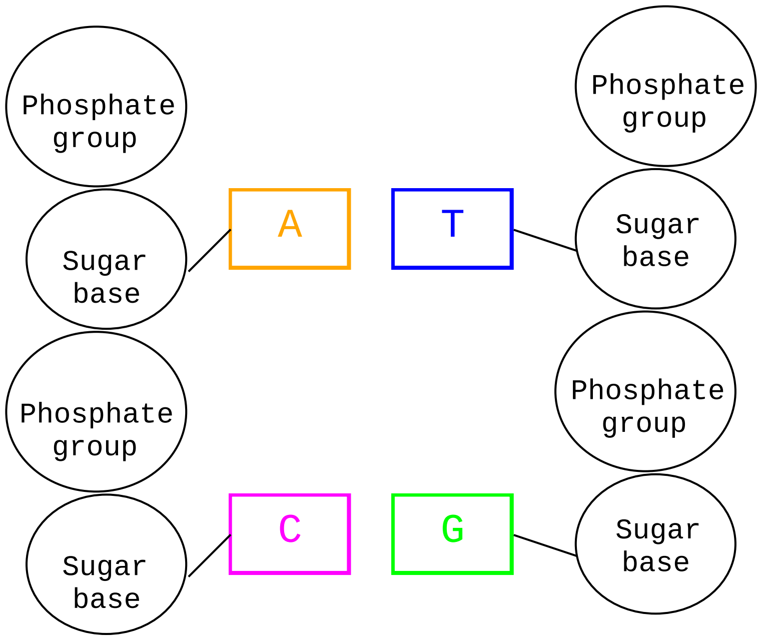


Figure 2.2.: DNA Structure: schematic visualization of DNA molecule

The only difference is cell DNA originates in the cell, synthetically produced DNA originates from phosphoramidite. Figure 2.3 depicts schematic representation of synthesizer.

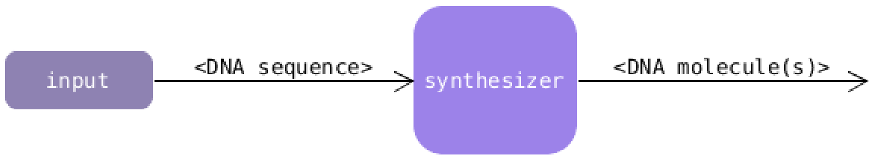


Figure 2.3.: Schematic representation of synthesizer

2.4. Sequencing

DNA sequencing is the process of determining the sequence of nucleotides (*A*s,*C*s,*G*s and *T*s) in piece of DNA [10]. An example of sequencing is to read the DNA sequence out of a certain collection of molecules. **Sequencer** is a device that automates the process of DNA sequencing. The process behind the sequencing is both biological and computational. This chapter will concentrate on sequencer as a black box and in chapter three computational part will be discussed in detail.

Following list describes steps necessary to take in order to assemble genome [11]. Step-by-step schema is shown in Figure 2.4.

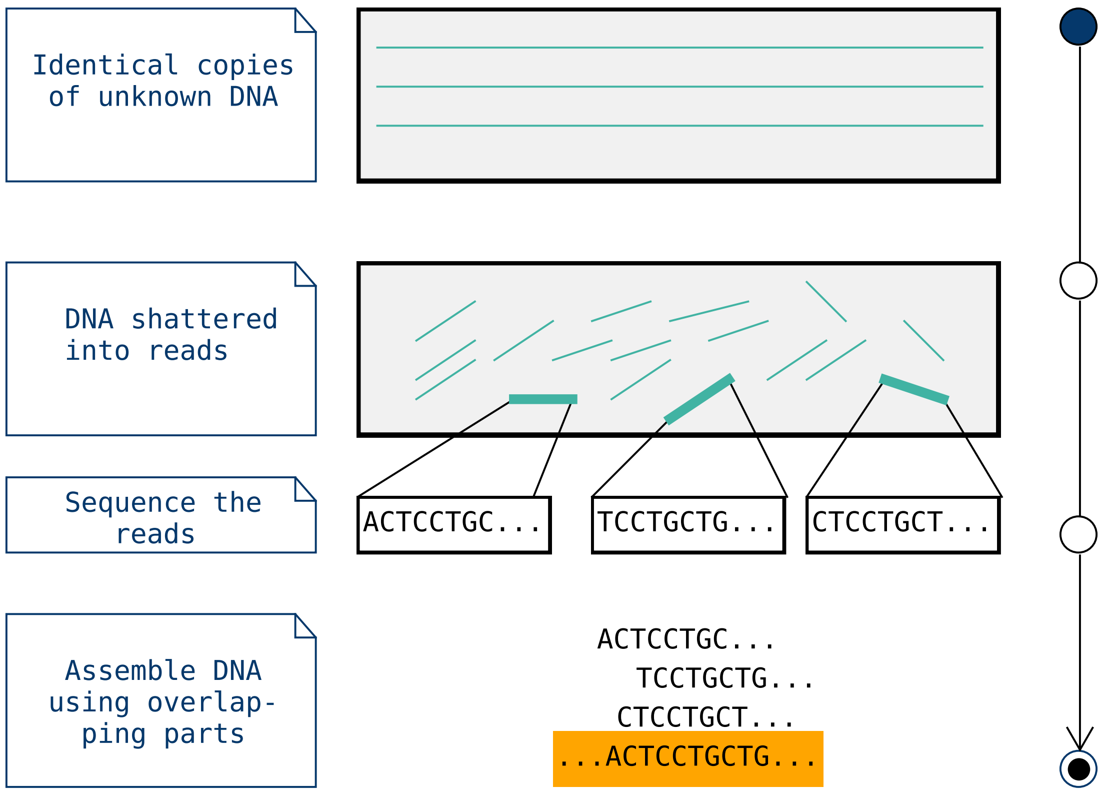


Figure 2.4.: Step-by-step representation of sequencer process. Assembly is a multiple-step process that has a biological layer and computational layer. In biological layer scientists are collecting reads together; in the computational layer using overlapping parts and techniques from graph theory the sequence is assembled.

- As an input sequencer takes a biological sample containing cells in our case molecules with certain DNA and creates multiple copies. Notice that goal of sequencer is to assemble the DNA sequence, so sequencer has no idea about the information stored in DNA.
- Using biochemical methods, the researchers are breaking DNA into fragments, and then sequence the fragments to produce **reads**. **Reads** are usually DNA sequences of length $20-300$ ($20-40$ are called ultrashort, $100-300$ - long reads).
- Now that the reads are given algorithm assembles the reads into one sequence (please notice the order of the reads is unknown).

DNA assembly is one of the most challenging parts of bioinformatics, and the research is still ongoing.

2.5. Putting biological part altogether

From the previous subchapters it can be seen that synthetic biology can be used as a certain biochemical interface. We can consider **synthesizer** as an **input** for creating molecules and **sequencer** as an **output** for reading back the the DNA sequence from the molecule.

However, one component that an interface system lacks is certain physical medium, where DNA molecules are contained. State-of-the-art technologies at the moment suggest a certain jar with chemicals for storing molecules and call it **DNA soup** [12].

Let us put together the system that will serve as an interface. In the next chapters, we are going to incorporate channel coding into our system for completeness of DNA as storage. Figure 2.5 demonstrates synthetic biochemical interface.

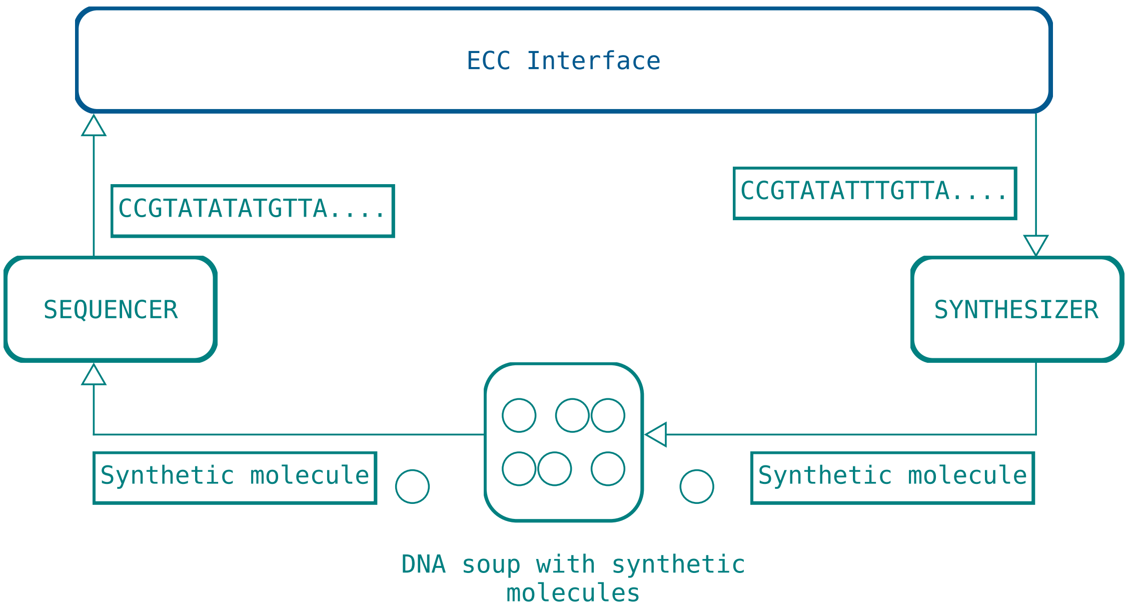


Figure 2.5.: Biochemical interface for DNA as a storage altogether

3. Error Control Codes

First chapter described biological interface of DNA as a storage work. This chapter will concentrate on error control codes area, which deals with data storage techniques. Techniques include error detection and correction.

3.1. Introduction

Coding theory is the process of converting information from a form convenient for direct use to a form convenient for transmission, storage, automatic processing and safety from unauthorized access. Several applications of coding theory exist cryptography, data compression, data storage and networking [13].

This chapter is going to concentrate on data storage. In order to proceed to the biochemical interface, the information must be **encoded** in a way that conveniently fits schema. Additionally, to the encoding process, it is necessary to establish the **mapping** schema between quaternary DNA alphabet and $\{0,1,2,3\}$. Next section covers **finite fields** since in case of DNA alphabet is quaternary. Since the work is concentrated mainly on errors, that occur during sequencing, penultimate section concentrates on **detecting the errors**.

3.2. Forward error correction

Forward error correction (FEC) - is a technique of noise-resistant coding and decoding, which allows correcting errors by the preemtive method. FEC is used to correct failures and errors during data transfer by transmitting redundant service information, on the basis of which the original content can be restored. The given system can correct one error and detect three errors [14]. Error-correcting code is a technique for adding redundancy to a message in a way such that it can be recovered using various mathematical methods [15]. Mainly there are two types of error-correcting codes:

1. Algebraic Codes: Given information is split into small pieces, where each piece corresponds to a message. Messages are consequently encoded into codewords (blocks). Examples of algebraic codes are Reed-Solomon codes, Hamming codes, Golay codes, CRC codes and. These types of codes are based on Boolean algebra and also referred to as block codes.

2. Convolutional codes: here the codes may operate on potentially infinite sequences of elements and not on finite blocks. However, there is no specific algebraic theory behind the construction of convolutional codes. Examples of techniques used on convolutional codes are the Viterbi algorithm.

This work concentrates on **algebraic/block codes**.

3.3. Finite Fields and Mapping

Finite field is a mathematical term and has applications in many subjects such as control coding, cryptography and computer science [16]. Basically as a name suggests, it is a *finite set* of symbols that consists of its own arithmetic, specifically *addition* and *multiplication*, by following some defined rules. Addition and multiplication behave in finite fields similar to real and rational numbers; difference is that in former case addition multiplication are carried out by modulo. It is worth mentioning that finite field has additive and multiplicative neutral elements, which are normally 0 and 1.

Galois field is a finite field where the arithmetic is carried out by **modulo**. So basically, we construct the *addition* and *multiplication* tables, where the operation is carried out between corresponding elements. After the latter as soon as the value of element is greater than the number of field elements, e.g. size is prime, we determine the value by performing modulo operation - $mod(p)$. For example in $\mathbb{F}_3$ - field with 3 elements or $mod(3)$. Each element has additive and multiplicative inverses except for 1 and 0.

In case of DNA as a data storage we have quartenary system - alphabet of four characters, which will be mapped to {A,C,G,T}. In order to create such a system it is necessary to look at Galois fields. According to *Galois theory* any field of power q and prime p can be constructed if there is given an irreducible polynomial *p(x)* of order *q*. In our case in order to construct $\mathbb{F}_p$q , where **p = 2 q = 2**; one should use the following polynomial: $p_22(x) = x^2 + x + 1$. By using a given polynomial one can list all the possible elements (4 elements in a field) that will appear in a given field. As a consequence this polynomial will serve for a modulo operation as : $mod(p_4(x))$ while performing the operations like multiplication and addition. In fact all elements of this field will be constructed from a given polynomial and as a consequence we will have a field with 4 elements, that are listed below in list : $0 = 0, 1 = 1, 2 = x, 3 = x + 1$. Tables 3.1 and 3.2 demonstrate addition and multiplication operations in $\mathbb{F}_4$.

3.4. Error Detection

The **error detection** step we cover here is going to be executed right before the computational procedure in sequencing, to detect the errors in reads - purify data. In this work we decided to use **cyclic codes** and **CRC** polynomial since they have specific properties that are comfortable

+	0	1	2	3
0	0	1	2	3
1	1	0	3	2
2	2	3	0	1
3	3	2	1	0

Table 3.1.: Addition table

*	0	1	2	3
0	0	0	0	0
1	0	1	2	3
2	0	2	3	1
3	0	3	1	2

Table 3.2.: Multiplication table

to use correctly for DNA as storage.

Cyclic codes are a subclass of linear codes, that satisfy the following condition: given a codeword $[c_1, c_2, ..., c_i, ...c_n]$ that belongs to cyclic code; then any other element obtained by a cyclic shift $[c_2, c_2, ..., c_i, ...c_n, c_1]$ of that codeword also belongs to that code [17]. All cyclic shifts of a codeword belong to cyclic code. As a consequence of the cyclic property, these codes have a significant number of structural facilities that can be used in the implementation of encoding and decoding operations. A large number of algorithms for current encoders and decoders of hard decisions were made using cyclic codes, making it possible in practical communication systems to build block codes of considerable length with a large number of codewords.

A **cyclic redundancy check (CRC)** is an error-detecting code commonly used in digital networks and storage devices to detect accidental changes to raw data [18]. Usually, the data that is sequenced from reads has certain error probability, CRC codes will help us detect them. There is a particular mathematical procedure that needs to be done in order to achieve detection. We must first have the **generator matrix** and proceed to multiply each message with the latter.
Let us see step-by-step **encoding** and **error detection**. It is worth mentioning that cyclic codes have the generator polynomials. Based on the example that is conducted on our simulation generator polynomial is

$$x^4 + x^3 + x^2 + 2x + 3,$$

and our code is $[\mathbb{F}_4; 15; 11]$ (n-codeword length, k-message length), where $n = 15$ and $k = 11$.

- Generator matrix from that polynomial and code parameters $[\mathbb{F}_4; 15; 11]$ is as follows:

$$\underline{\underline{\mathbf{G}}} = \begin{pmatrix} 1 & 1 & 1 & 2 & 3 & \dots & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 2 & 3 & \dots & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 2 & 3 & \dots & 0 \\ \vdots & 0 & 0 & \ddots & 0 & 0 & 0 & 0 & \vdots \\ 0 & \dots & 1 & 2 & 3 & 2 & 3 & 0 & 0 \\ 0 & 0 & \dots & 1 & 2 & 3 & 2 & 3 & 0 \\ 0 & 0 & 0 & \dots & 1 & 2 & 3 & 2 & 3 \end{pmatrix}$$

- Additionally for further processing, it is necessary to bring the matrix to reduced echelon form in order to get the **systematic** generator matrix:

  $$\underline{\underline{G}} = [\underline{\underline{I}}_k | \underline{\underline{P}}],$$

  where k is message length, $\underline{\underline{I}}$ - $k \times k$ identity matrix and $\underline{\underline{\mathrm{P}}}$ - is **parity**.

- Now to encode each message into codeword following equation is used:

  $$\underline{c}_i = \underline{m}_i * \underline{\underline{G}},$$

  where i serves as a counter element.

After performing procedure described above we have set of codewords - $\{\underline{c}_1, \underline{c}_2, ..., \underline{c}_i, ...\}$. Now, suppose there has been distortion (in our case in reads might be erroneus) and erroneus codewords must be discarded. Procedure is as follows:

- First of all it is necessary to transform *systematic generator* matrix to **parity check matrix**. In order to do so, it is necessary to use following equation:

  $$\underline{\underline{H}} = [-\underline{\underline{P}}^{\mathrm{T}} | \underline{\underline{I}}_{n-k}].$$

- Next we shall multiply each codeword from $\{\underline{c}_1, \underline{c}_2, ..., \underline{c}_i, ...\}$ by parity check matrix:

  $$\underline{s}_i = \underline{\underline{H}} * \underline{c}_i,$$

  where **s** is a **syndrome**. One of two possibilities happen:

  – $\underline{s}_i = 0_v \Rightarrow \underline{c}_i$ is indeed a codeword;

  – $\underline{s}_i \neq 0_v \Rightarrow \underline{c}_i$ is not a codeword.

This work is mainly concentrated on detecting the erroneus codewords and discarding them for further processing (sequencing and decoding).

3.5. Putting error control codes altogether

From previous subsections we have gathered enough knowledge to put the error control coding system together. Figure 3.1 displays our system in more details. Process of storage is as follows:

- Source contains certain payload, let us say e.g. set of messages.
- The messages - $\mathbf{m} = \{\underline{m}_1, \underline{m}_2, \underline{m}_3, ..., \underline{m}_i\}$ gets encoded into codewords $\mathbf{c} = \{\underline{c}_1, \underline{c}_2, \underline{c}_3, ..., \underline{c}_i\}$ through **encoder** using techniques described in previous subsection.
- Codewords $\mathbf{c} = \{\underline{c}_1, \underline{c}_2, \underline{c}_3, ..., \underline{c}_i\}$ are passed to biochemical interface (ref. chapter one), where they are distorted; we might think of biochemical interface as a **channel**.
- Sequenced data is mapped from DNA alphabet (As,Cs,Gs,Ts) to $\mathbb{F}_4$ and is passed through **decoder**, where codewords $\mathbf{c} = \{\underline{c}_1, \underline{c}_2, \underline{c}_3, ..., \underline{c}_i\}$ are decoded to message estimate $\mathbf{m} = \{\underline{m'}_1, \underline{m'}_2, \underline{m'}_3, ..., \underline{m'}_i\}$.

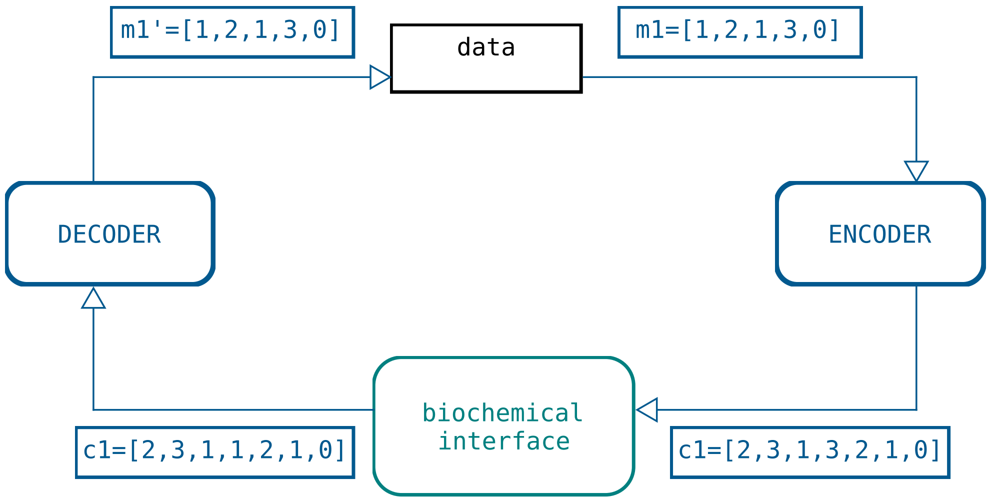


Figure 3.1.: Basic workflow of DNA as a data from Error Control Codes perspective. Here $\underline{m}_1$ is a message vector, $\underline{c}_1$ is codeword vector and $\underline{m'}_1$ is message estimate.

Technique described above is useful for delivery of information through unreliable channels.

Errors and erasures in the DNA system are described in the list below [19]:

- Either molecule is not synthesized at all, or it is synthesized more often than others (usually each molecule is synthesized once). Contemporary synthesizers typically generate multiple thousands of copies of the chain, which might contain several kinds of errors.
- Molecules in DNA soup itself might decompose, which results in loss of information/-molecules.

- When the molecules are extracted from the jar, depending on distribution, the only fraction is sequenced.
- Sequencing DNA drives to nucleotide insertion, deletion or substitution in particular DNA molecules.

This work tackles the type of errors that appear during sequencing and introduces coding scheme in between biological and computational layers.

4. Workflow of DNA as a storage

Chapters before have covered main building blocks of this work: error control codes and bioinformatics. From error control coding point of view, the main components are encoder, channel and decoder. From a biological point of view, the main components are synthesizer, DNA soup/channel and sequencer. This chapter is going to cover the whole system taking into account both areas.

Additionally, the package structure of the simulation/setup and computational layer in the sequencing problem are discussed in this chapter. Additional layer in between biological and computational parts of the assembly problem is introduced.

4.1. Schema of DNA as a storage

The best way to display the whole DNA as a storage system is to visualize it. Figure 4.1 displays the whole process. Let us go through once more through the main parts of system and the parts this work is going to concentrate on. Figure 4.1 has step numbers around each component, which correspond to the list below in process [20]:

1. Given certain payload - data in form of $\mathbb{F}_4$ messages - $\mathbf{m} = \{\underline{m}_1, \underline{m}_2, \underline{m}_3, ..., \underline{m}_i\}$.
2. In this step the payload m is **encoded** into $\mathbf{c} = \{\underline{c}_1, \underline{c}_2, \underline{c}_3, ..., \underline{c}_i\}$.
3. In order to synthesize the codewords first it is necessary to **map** them to quarternary alphabet - $\{A, C, G, T\}$. In this step, our approach is also to concatenate the codewords together into one sequence and then pass them to the synthesizer.
4. After concatenating codewords into one sequence, we **synthesize** the sequence into molecule/molecules (depending on the machines used in the process).
5. This is the illustration of a jar, the so-called **DNA soup**, where synthetic DNA molecules are floating around; however, the order of molecules is undefined. Please note here DNA breakage, which is equivalent to symbol/block deletion or even losses of whole sequences might happen!

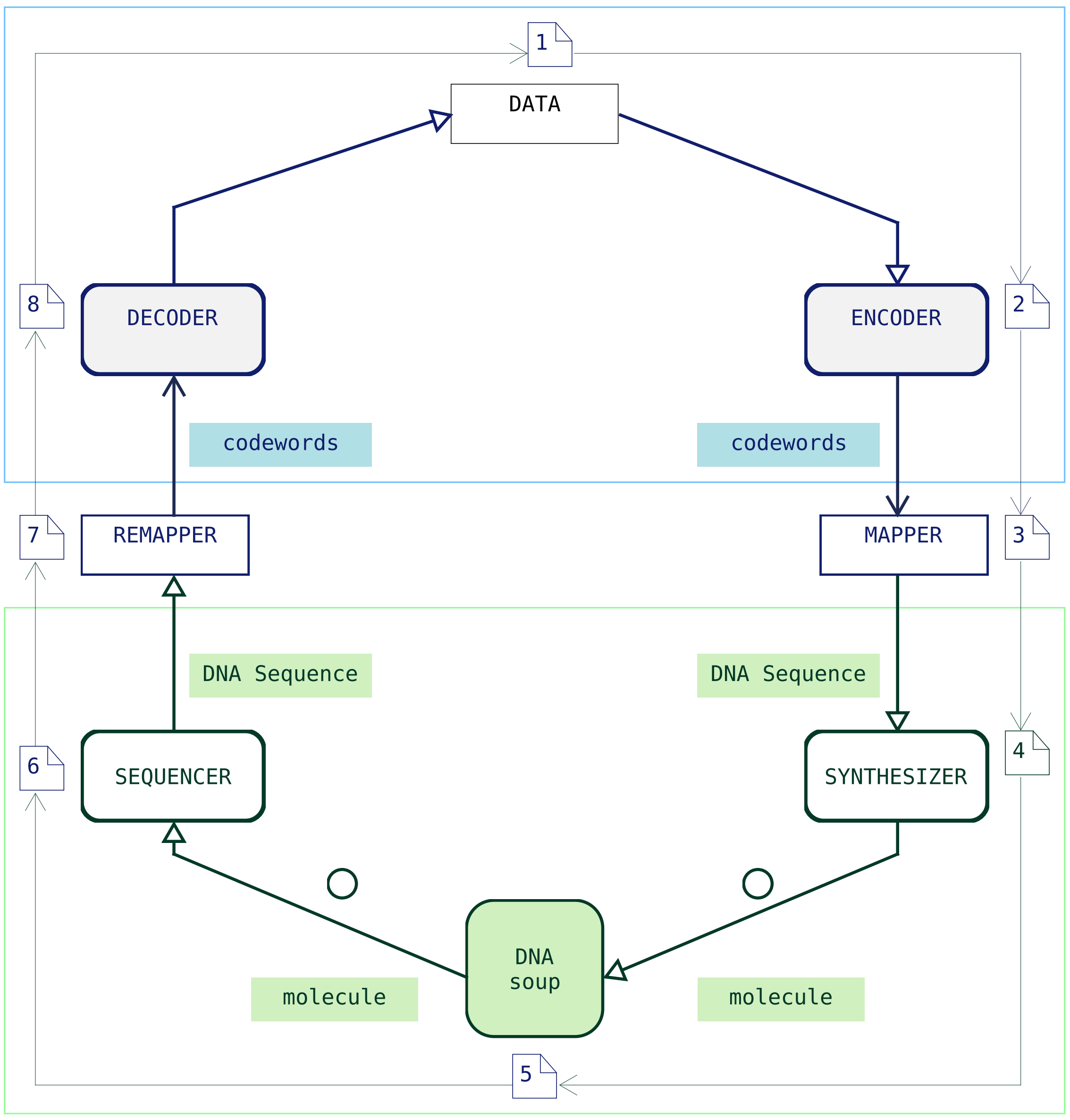


Figure 4.1.: Basic workflow of DNA as a data from both biological and ECC perspectives. As it was described before - messages stands for set of messages, codewords for set of codewords, and DNA soup is jar with encoded molecules.

6. Molecule(s) is picked randomly uniformly and passed to be **sequenced**. Please note this is the point where the errors/insertions/deletions or substitutions may happen. Here error control codes detect erroneous reads.
7. Here the sequenced data - DNA sequence is **re-mapped** back into $\mathbb{F}_4$ in order to be passed to decoder.
8. Finally by decoding the codewords into messages, where each message is estimate value $\underline{m'}_1 = [m_{11}, m_{12}, m_{13}, ..., m_{1i}, ...]$.

Errors in strands appear in synthesis, storage (to a smaller extent due to decay of DNA) and sequencing steps. Synthesis adds largely deletion and likely insertions. Given that NGS (next-generation sequencing) technology is used for gathering DNA strand, step 6 includes substitution and very few insertions or deletions.

The goal of this work is to introduce a particular way of minimizing errors that occur during step 6 of the process in a sequencer state. The issues that occur during sequencing are erroneous reads that cannot be reconstructed using one of the classical algorithms for assembly. Even though there are specific correction approaches for the assembly, they are heavily heuristic. We decided to approach the problem as error control coders by introducing intermediate steps in the beginning and in between sequencing steps.

In the next two sections, package structure and sequencing problem are going to be discussed. Third and fourth sections of this chapter will explain sequencing problem in greater detail, including algorithms, since it is necessary to understand the way assembly process works computationally and why there is a necessity for detecting the errors.

4.2. Tools used for DNA as a storage

As a programming language in this work, we decided to utilize python for two reasons: ease of use and possibility to reuse algorithms for bioinformatics. This section gives a brief description of **structural viewpoint** or in other words, class diagrams of setup for simulation. Nevertheless, before it is necessary to understand the philosophy of our approach to simulation.

- **Python**: As a programming language Python was a choice. Python is dynamically typed PL and has the garbage-collection capability. Furthermore, it supports various paradigms, such as object-oriented and functional programming [21].
- **UML2.0**: The most important reason why UML2.0 has been chosen as a tool is the ability to maintain a complex system with the help of structured documentation and capability to translate at least the structure to any object-oriented programming language [22]. UML2.0 has viewpoints and views for modelling the software [23]. Viewpoints

are structural or behavioural entities (in a given context viewpoint are types of diagrams) that encompass the views, where UML 2.0 diagrams represent the latter.

- **OOP**: Object-oriented programming (OOP) is a concept that is designed to facilitate the development of complex systems by introducing new concepts that are closer to the real world than functional and procedural programming languages [24]. In the case of DNA as a storage system, as shown in Figure 4.1 is component-based. The usage of OOP, in this case, helps organize components as classes. Each of those classes is going to have a certain set of properties and methods. Classes are going to be low coupled (degree of binding between classes is low) and highly-cohesive (each of those classes has one specific goal). Additionally, one controller class is managing the workflow of DNA storage is going to be appended to the overall system.

All the schemas and models in the next subsections are represented as **class diagrams** from a structural viewpoint. A detailed description of the methods used in the system and corresponding classes are provided in the appendix.

4.2.1. Error control codes package

For error control codes package we have two classes: **Encoder** and **Detector**(since our Channel is special, we put it in helpers package) as shown in Figure 4.2. Each one of those classes has corresponding methods that serve specific purpose; every method will be described in corresponding figure (for detailed overview please refer to code listings in appendix).

- **Encoder** - contains three methods: `construct_generator_matrix()`, `encode()` and `encode_messages()`. Decription of methods and attributes is shown in Figure 4.2.
- **Detector** - contains three methods: `check_whether_codeword()`, `get_h_matrix()` and `perform_calculation_to_checks`. Decription of methods and attributes is shown in figure 4.2.

4.2.2. Bioinformatics package

For bioinformatics package we have two classes: **Sequencer** and **Synthesizer** as shown in Figure 4.3. Each one of those classes has corresponding methods that serve synthesis and sequencing purpose - in other words biological layer; every method will be described in corresponding figure (for detailed overview please refer to code listings in appendix).

- **Synthesizer** - contains two methods: `join_for_synthesis()` and `map_codewords()`. Decription of methods and attributes is shown in Figure 4.3.
- **Sequencer** - contains three methods: `remap_codewords()`, `create__n_mers()` and `assemble_DNA(method_name)`. Decription of methods and attributes is shown in Figure 4.3.

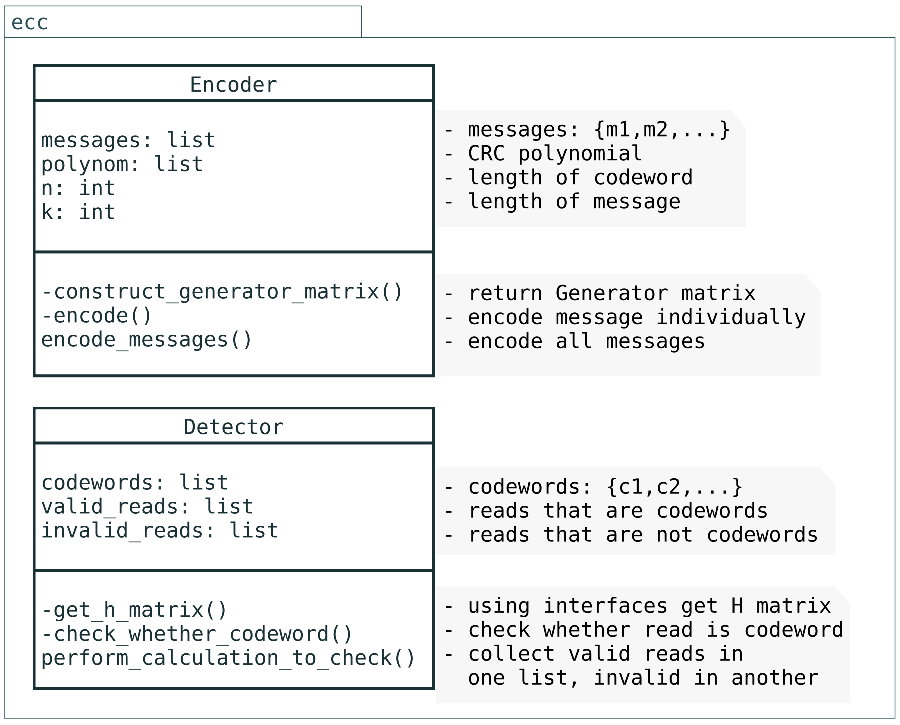

ecc
Encoder
messages: list
polynom: list
n: int
k: int
- messages: {m1,m2,...}
- CRC polynomial
- length of codeword
- length of message
-construct_generator_matrix()
-encode()
encode_messages()
- return Generator matrix
- encode message individually
- encode all messages
Detector
codewords: list
valid_reads: list
invalid_reads: list
- codewords: {c1,c2,...}
- reads that are codewords
- reads that are not codewords
-get_h_matrix()
-check_whether_codeword()
perform_calculation_to_check()
- using interfaces get H matrix
- check whether read is codeword
- collect valid reads in one list, invalid in another

Figure 4.2.: Error control codes package; contains two classes only: Encoder and Detector.

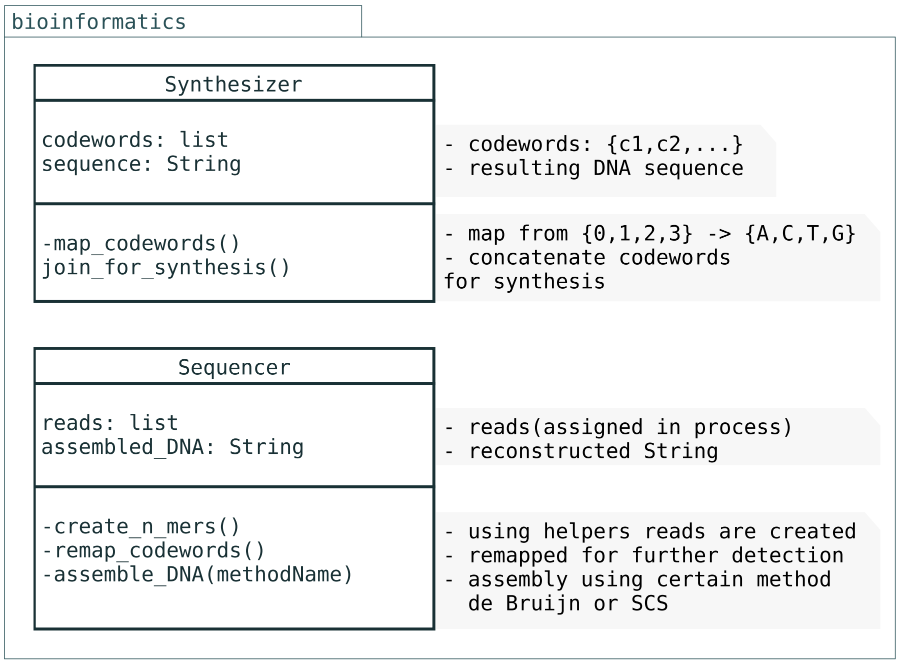


Figure 4.3.: Bioinformatics package; contains two classes only: Synthesizer and Sequencer.

4.2.3. Helpers package

One more package worth mentioning is **helpers** package, where all the necessary intermediate interfaces are stored. Intermediate interfaces and classes include **F_Four** ($\mathbb{F}_4$), **Mapper** and **Channel**, that imitates our distortion: where character selected at a random position gets distorted. Last but not least since our math is executed in F_Four, operations are prototyped in *math_helpers.py* file - it contains methods necessary to execute e.g. matrix_vector multiplication in $\mathbb{F}_4$. Figure 4.4 describes helpers folder.

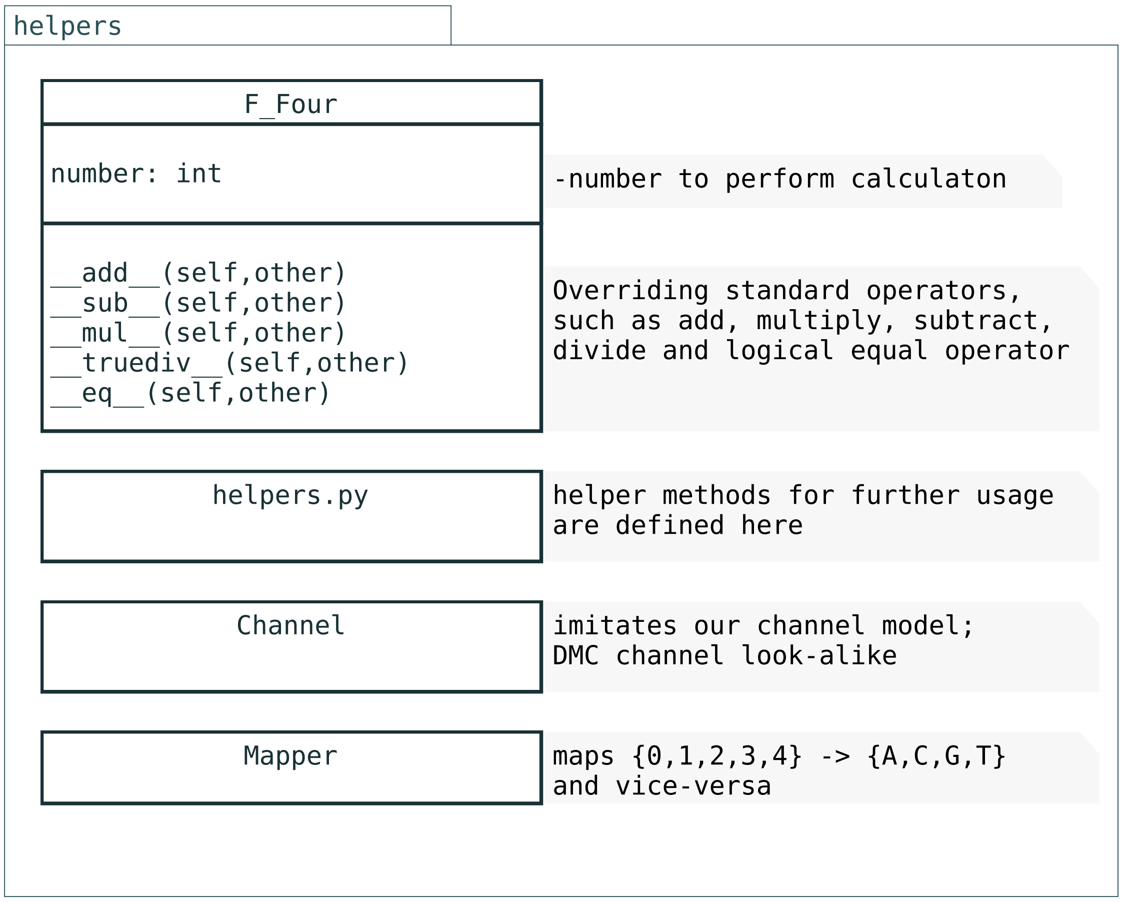

helpers
F_Four
number: int
-number to perform calculaton
__add__(self,other)
__sub__(self,other)
__mul__(self,other)
__truediv__(self,other)
__eq__(self,other)
Overriding standard operators, such as add, multiply, subtract, divide and logical equal operator
helpers.py
helper methods for further usage are defined here
Channel
imitates our channel model; DMC channel look-alike
Mapper
maps {0,1,2,3,4} -> {A,C,G,T} and vice-versa

Figure 4.4.: Helpers package; contains three classes and one file: Mapper, Channel, F_Four and *math_helpers.py*.

4.2.4. Workflow manager

WorkflowManager is the main class, where the whole process is being executed. WorkflowManager encompases all the components described above in one manager class where it is easy to control the system and where all those classes are mapped to individual methods. According to main principles from object-oriented programming our components/classes must be highly cohesive, low coupled. Each method demonstrated in Figure 4.5 is responsible for one task displayed in our previous schema. WorkflowManager was designed, taking into account those tasks and principles and is shown in Figure 4.5.

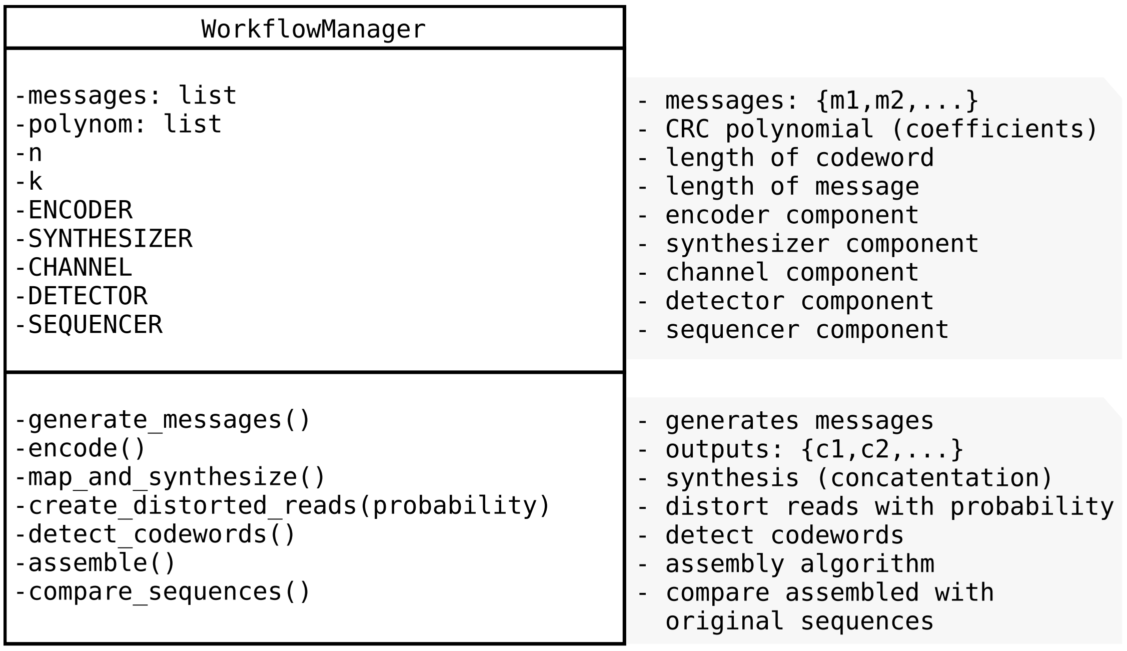


Figure 4.5.: WorkflowManager class; manages all necessary components using packages described above.

4.3. Sequencing as a computational problem

Suppose there is a DNA sample which should be sequenced. As it was described in chapter one in order to sequence certain DNA sample, researchers first shatter it into multiple pieces - **reads**. They do so because biologically it is tough to sequence the long DNA strand - certain techniques used in the labs are still limited, and there is much research on that topic. Biologists sequence reads and collects them in a certain database (normally they are plain text files,e.g., *.fasta, .fastq*) [25]. Now it is up to computer scientists to collect the reads back, and output sequenced DNA. Before looking at the algorithms for sequencing, it is required to understand the data/reads saved in a database.

4.3.1. Exploding newspaper

To simplify the sequencing algorithm and problem even further bioinformatician Pavel Pevzner associated it with exploding newspapers problem [26]. Everything described here is purely theoretical and serves for the explanation. Illustrative depiction of the problem is displayed in Figure 4.6.

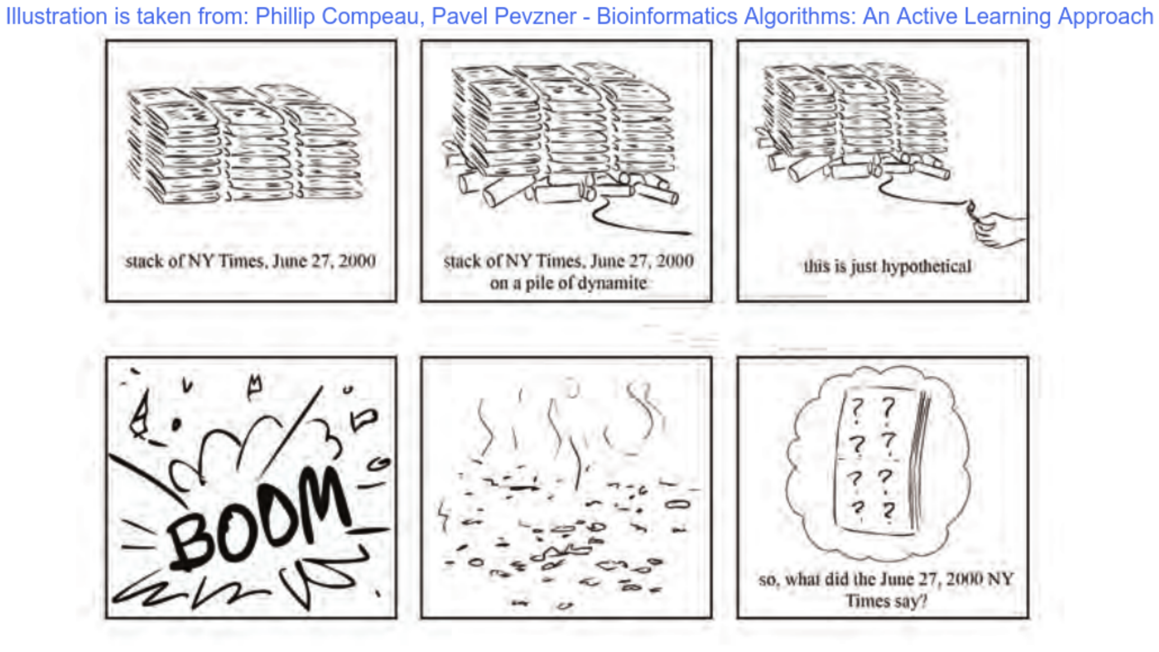


Illustration is taken from: Phillip Compeau, Pavel Pevzner - Bioinformatics Algorithms: An Active Learning Approach
stack of NY Times, June 27, 2000
stack of NY Times, June 27, 2000 on a pile of dynamite
this is just hypothetical
BOOM
? ? ? ? ? ? ? ?
so, what did the June 27, 2000 NY Times say?

Figure 4.6.: Exploding newspaper problem: On the top left it is seen that hundreds of copies of the newspaper are stacked. In the top middle placed under dynamite. Out of pieces, we must recollect the picture shown below on the right.

On the top left, there are hundreds of copies of the newspaper. Next, we place dynamite and start the fire. The explosion proceeds right after setting dynamite on fire, and then there are parts of all of the copies on the ground. The task of exploding newspaper is to recollect one copy of the newspaper. This process describes our DNA sequencing problem: parts of the text are our reads and newspaper is DNA strand.

Now the question is: how to go from little pieces of newspaper to one full copy? Answer to that question is simple: **overlapping parts** as shown in Figure 4.7 . As it is depicted certain parts of the text in newspaper are overlapping, e.g. yet named or is welcomed.

When the DNA sample is shattered we get the very similar picture: if there are enough shattered parts the DNA sequence or **genome** can be recollected.

Illustration is taken from: Phillip Compeau, Pavel Pevzner-Bioinformatics Algorithms: An Active Learning Approach

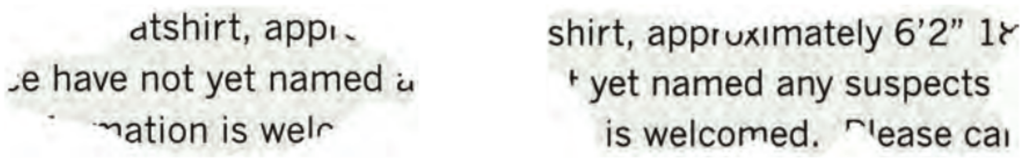


Figure 4.7.: Exploding newspaper problem: single pair of overlapping parts. Newspaper is being recollected from multiple pairs of the parts and as displayed if there are enough parts we can recollect the copy accordingly.

The task is not as simple as it seems since we have to take into account that reads are chaotically distributed when passed through an algorithm. Determining the order of nucleotides in a DNA sequence, or genome sequencing presents a fundamental task in bioinformatics. There are certain algorithms, specifically graph algorithms that are responsible for that.

4.3.2. Shortest common superstring and de Bruijn graph

As it was outlined in previous subchapter our data is very much alike the pieces of newspaper with many copies. There are many algorithms on this topic and it is a major research area. Sequencing the genome from those reads is called **DNA assembly** problem. Before going into graph algorithms used in this work we must first note that CS researchers decided to shatter those reads further into **k** pieces and called them *k-mers* as shown in Figure 4.8. However in our case since they are not very long and modern sequencers can output the reads with same length we will not shatter them further and call them ***n-mers***.

A little refresher on definitions for graph algorithms for definitions is shown in Figure 4.9. Algorithms that we are going to concentrate on are **SCS - shortest common superstring** and **de Bruijn assembly**:

- **SCS**:Formulation of shortest common superstring [27] is shown in Figure 4.10: Researchers decided to approach this problem using **directed overlap graphs**. As it was described above we must base our algorithms on overlapping parts, so overlap graph is one of those approaches. Furthermore it is necessary to find the shortest path in that overlap graph, depending on whether $n-mers$ have enough overlapping parts. Additionally, we assume that each **vertex** of overlap graph is actually an $n-mer$. Sometimes it is referred to as **travelling salesman** problem, where one should find **Hamiltonian path** [28].

  Definition 4.1 *A **Hamiltonian path** is a path that passes through every vertex exactly once.*

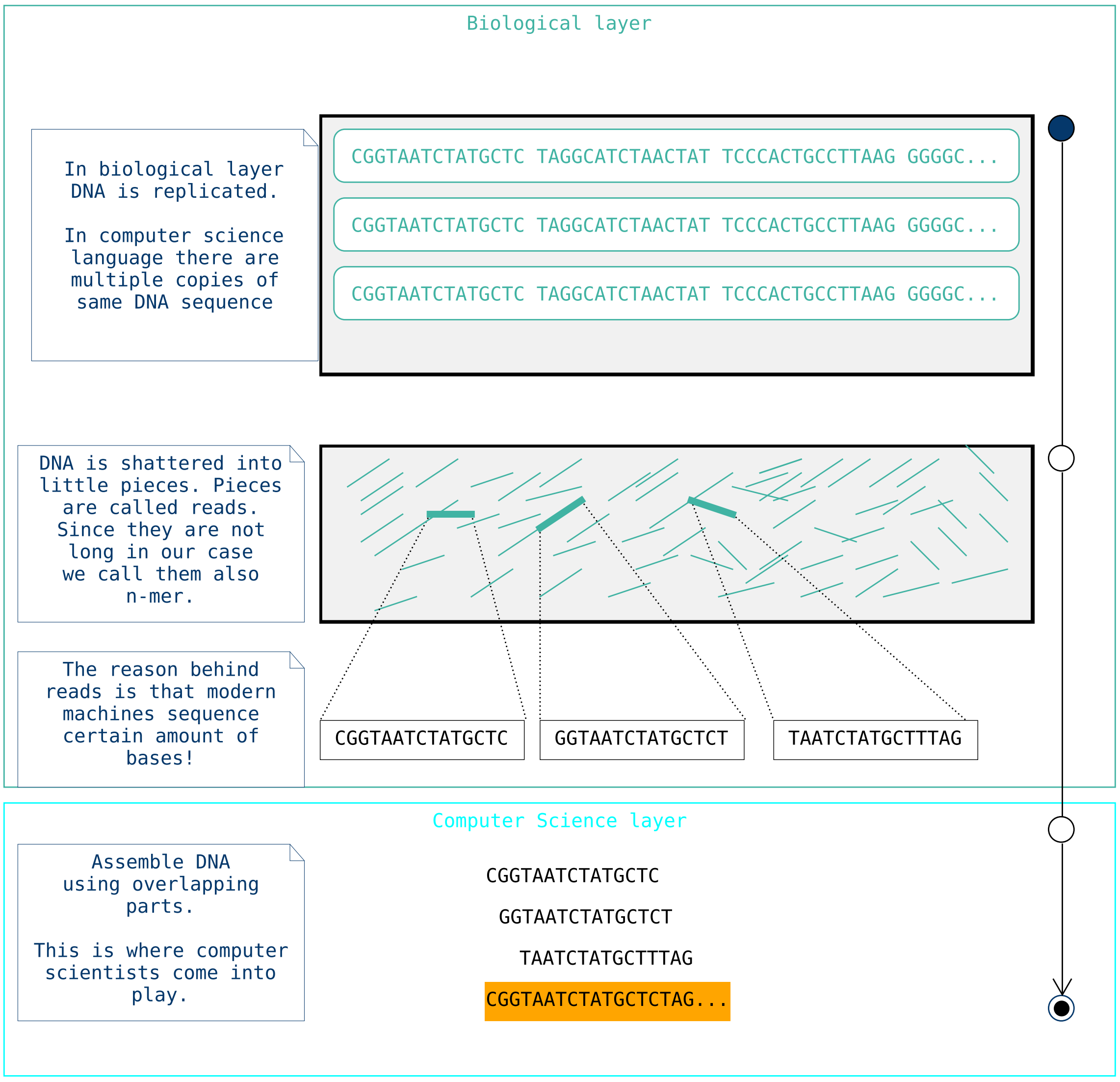

Biological layer
In biological layer DNA is replicated.
In computer science language there are multiple copies of same DNA sequence
CGGTAATCTATGCTC TAGGCATCTAACTAT TCCCACTGCCTTAAG GGGGC...
CGGTAATCTATGCTC TAGGCATCTAACTAT TCCCACTGCCTTAAG GGGGC...
CGGTAATCTATGCTC TAGGCATCTAACTAT TCCCACTGCCTTAAG GGGGC...
DNA is shattered into little pieces. Pieces are called reads. Since they are not long in our case we call them also n-mer.
The reason behind reads is that modern machines sequence certain amount of bases!
CGGTAATCTATGCTC
GGTAATCTATGCTCT
TAATCTATGCTTTAG
Computer Science layer
Assemble DNA using overlapping parts.
This is where computer scientists come into play.
CGGTAATCTATGCTC
GGTAATCTATGCTCT
TAATCTATGCTTTAG
CGGTAATCTATGCTCTAG...

Figure 4.8.: Sequencing explained in detail. As it can be seen on figure in biological layer we sequence reads (shattered parts) individually and save them to .fastq or .fasta file. Afterwards we pass it to one of the assembly algorithms where they are collected into one string.

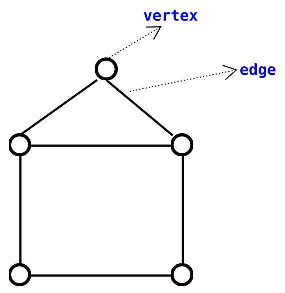
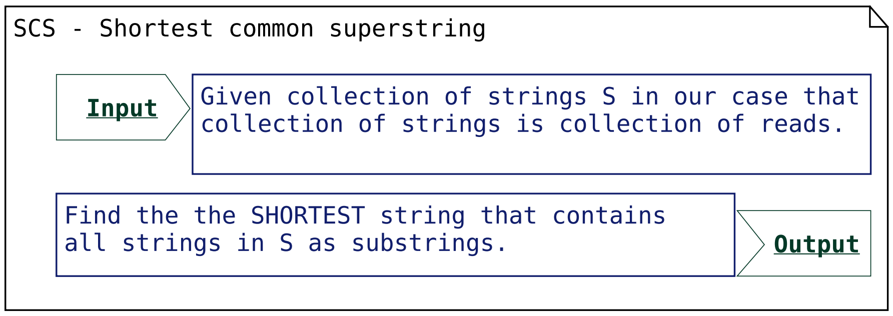


Figure 4.9.: Definitions from graph theory.



SCS - Shortest common superstring

Input

Given collection of strings S in our case that collection of strings is collection of reads.

Find the the SHORTEST string that contains all strings in S as substrings.

Output

Figure 4.10.: Assembly algorithm formulation. Problem formulation is taken from prof. Ben Langmead's slides.

However the problem is **NP-hard** (non-deterministic polynomial-time hardness) [29], so we will optimize the performance by minimizing the accuracy and using **greedy** version of SCS.

Greedy SCS algorithm is as follows (approach to solution is taken from *prof. Ben Langmead's* slides [30]):

1. `overlap(a, b, min_length=3)`: Return length of longest suffix of `a` matching a prefix of `b` that is at least `min_length` characters long. If no such overlap exists, return 0.

2. `pick_maximal_overlap(reads,k)`: Return a pair of reads from the list with a maximal suffix/prefix overlap >= k. Returns overlap length 0 if there are no such overlaps.

3. `greedy_scs(reads,k)`: Greedy shortest-common-superstring merge method-repeat until no edges (overlaps of length >= k) remain.

- **de Bruijn assembly**: De Bruijn assembly is very similar to the shortest common superstring. Formulation of the problem is the same as for SCS (see Figure 4.10) [31].

Difference between SCS and de Bruijn assembly is that in latter case we must find **Eulerian** path [32].

Definition 4.2 *An **Eulerian path** is a path that passes through every edge exactly once. If it ends at the initial vertex then it is an **Euler cycle**.*

Difference between these definitions is that one of them covers all the vertices, the other covers all the edges! Condensed version of the algorithm's pseudocode is as follows:

1. `dB := DeBruijnGraphGenerator(reads)` - generate de Bruijn graph where each vertex is $n-1$ - mer and each edge is n -mer.
2. `path := EulerianPath(dB)` - find Eulerian path in generated de Bruijn graph.
3. `reconstructedString := PathToDNASequence(path)` - method that converts path to DNA sequence.

The algorithms presented above work with certain precision and depending on the length of overlaps and repetiteveness of certain reads in genome the outcome may alter.

4.4. Error detection for sequenced reads

In the above sections, we have seen how the DNA as a storage system is organized as a whole and what are concrete algorithms for sequencing. We have also investigated each component in previous chapters. In this chapter we are going to introduce additional helper interface - the coding scheme for sequencing.

Current state of the art technologies does not have a particular way of correcting errors in detecting erroneous reads. They just go through sequencer-generated data, count the most frequently occurring patterns and assume latter is erroneous.

Our approach is to introduce **error detection layer** in between biological and computational layers. Figure 4.11 demonstrates the intermediate layer introduced for sequencing process. The idea for detecting erroneous reads is to set the length of the reads to the length of codewords and later detect the erroneous reads using channel coding and error detection techniques.

If we think about the reads in terms of codewords then we may detect erroneous reads using coding techniques introduced in chapter two. However, we will lose overlapping codewords and algorithms - SCS and de Bruijn assembly will both fail to assemble the codewords. Let us think of the statement made above in terms of example.

- Given collection of seven messages $\mathbf{m} = \{\underline{m}_1, \underline{m}_2, \underline{m}_3, ..., \underline{m}_7\}$ and say $k = 11$.

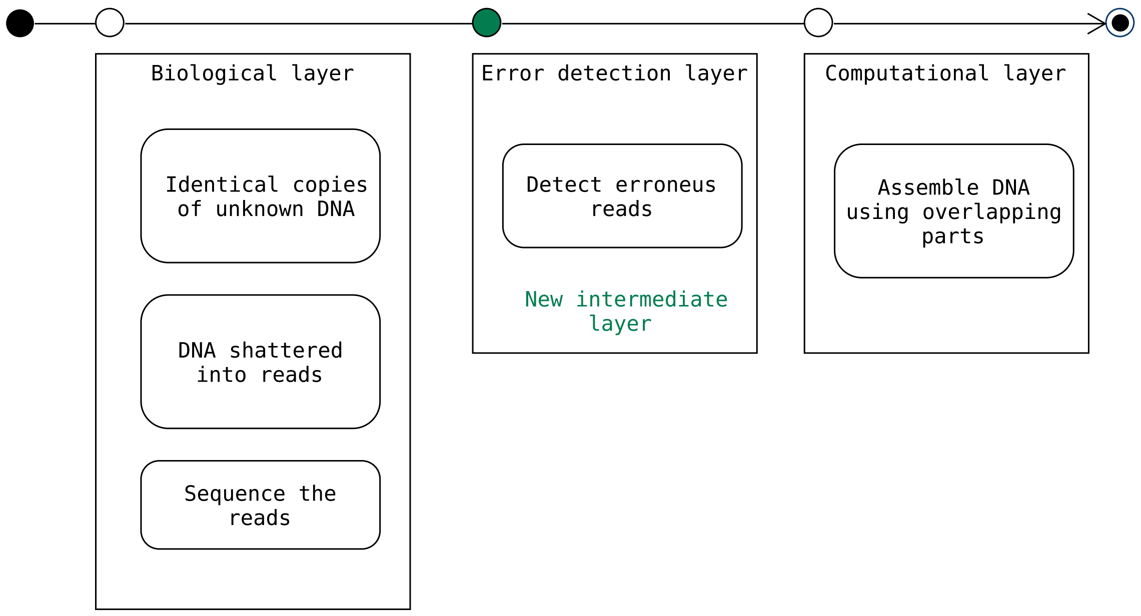


Figure 4.11.: Depiction of intermediate layer introduced for sequencing process in DNA as storage. Reads are being identified to be erroneous - discard the erroneous reads and keep the correct reads.

- After proper encoding we end up with corresponding collection of seven codewords $\mathbf{c} = (\underline{c}_1, \underline{c}_2, \underline{c}_3, ..., \underline{c}_7)$, with $n = 15$. Notice there are only *seven codewords* each of length 15.
- We map those codewords to DNA, concatenate them and synthesize them. Length of full synthetic **genome** will be $7 * 15 = 105$; in other words, it is necessary to reconstruct a full synthetic sequence of length 105.
- Now on sequencer side, there is a collection of reads with length $n = 15$, the unknown genome of length 105 and a way to detect whether reads are **erroneus** or not. Nevertheless, there occurs a problem, which is demonstrated in Figure 4.12.
- As it can be seen on Figure 4.12 ideally reads are assembled as shown on the left part of the figure, such that when fed into one of the assembly algorithms, it can reconstruct the genome.
- However what happens in the codewords-reads case is that every 15th read is codeword and algorithm ends up with an insufficient amount of information. It causes problems in both graph algorithms introduced in the sections above.

To investigate the problem further let us consider basic sequencing setup where reads are distributed in a way normally required for de Bruijn graph or shortest common superstring. The

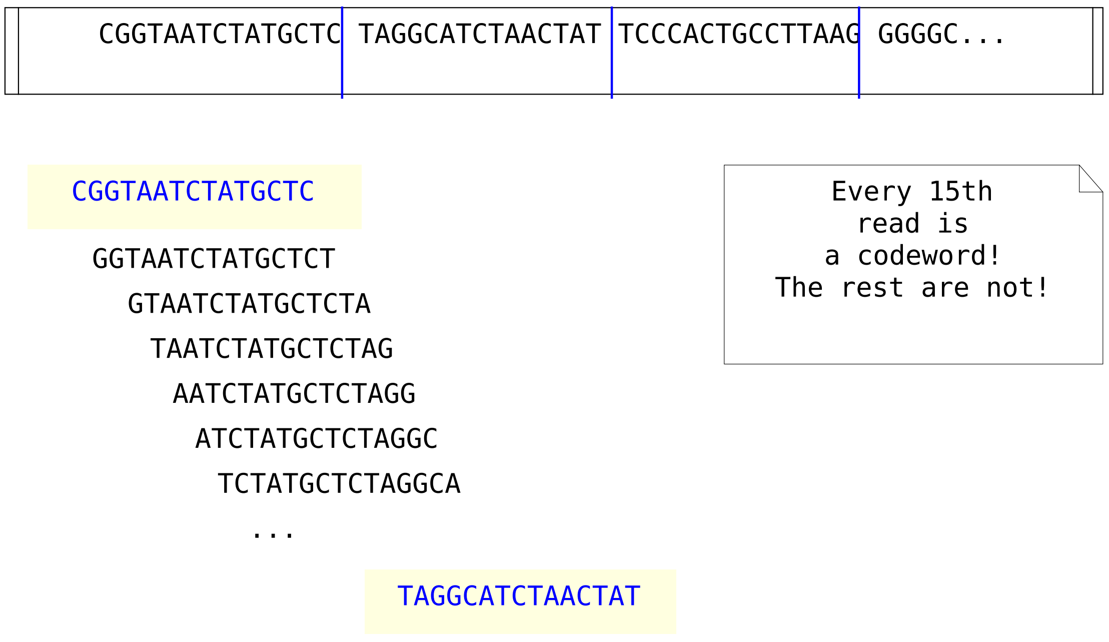


Figure 4.12.: DNA sequence assembly from reads vs codewords. The figure demonstrates problems introduced by error control codes while discarding the possible correct reads.

way those algorithms assemble reads together is by *"gluing"* reads using overlaps. Basic terminology for the overlaps and the codewords are shown in Figure 4.13.

In order to connect two pieces together, **suffix** of one read must be equal to **prefix** of the next one. According to example shown in Figure 4.13 if we completely assign reads to codewords then all overlapping parts (from our initial setup only 7 codewords are given, which are concatenated together), because they were not transferred at first place. SCS algorithm is based on overlapping parts between the reads. Given there are no overlaps between c1 and c2 it is impossible to reconstruct the DNA sequence. As a conclusion, it is not possible for SCS algorithm to reconstruct the DNA sequence out of codewords only.

Next chapter addresses how this problem may be solved by adding redundancy in front of original messages for providing enough overlap to be detected. Additionally, it will be required to make certain modifications to original simulation to adjust to a new setup.

Since the simulation mechanism of the system is based on main OOP principles it will be straightforward to incorporate changes, only by adding few goal-oriented methods to controller class - `WorkflowManager`. For random data generation, it is necessary to incorporate an additionally `RandomGenerator` class, where data is randomly generated.

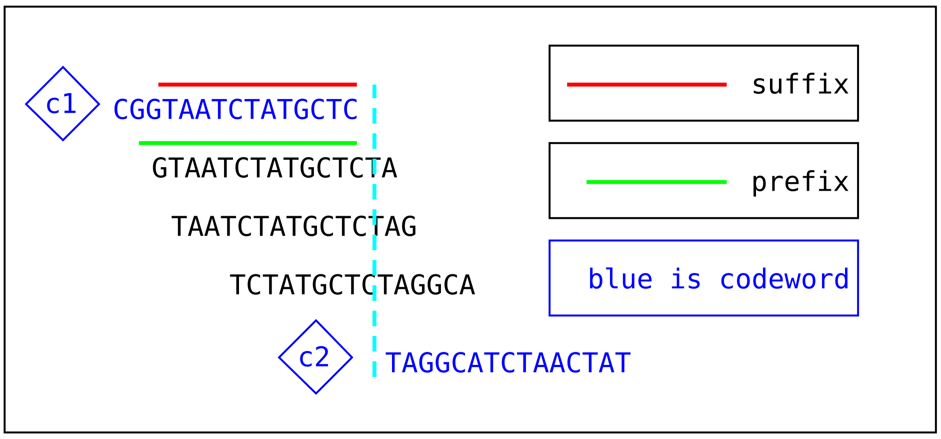


Figure 4.13.: Basic terminology in sequencing: **suffix** and **prefix**. Figure also demonstrates why it is impossible to recollect DNA sequence from **codewords** only. Interpretation is the fact that c1 and c2 do not have any overlap, if the rest of reads are thrown away from system.

5. Coding based DNA as a storage

In the last chapter we introduced an intermediate **error detection** layer between **biological** and **computational** layers in order to detect erroneous reads. However, the insufficient amount of reads (as it was agreed to make them the same length as codewords) is a major issue in the system.

As it was discussed in the previous chapter two algorithms are used for DNA assembly: shortest common superstring and de Bruijn assembly. The primary driving variable for those algorithms is based on **overlap** and the proposed codeword - read approach only took every **n**th read as a codeword. In this chapter, the read-codeword difference and similar concepts are going to be explained in greater detail. Additionally, a new method of appending the redundancy to the beginning of the message is going to be introduced.

5.1. Reads vs. Codewords

This section will revisit the reads as a codewords concept. As it is shown in Figure 5.1 reads have certain overlapping parts; codewords have no overlaps at all. Codewords appear at every **n**th position with a guarantee of no error. The problem in this case is that assembly algorithm is not going to work since no overlap is preserved.

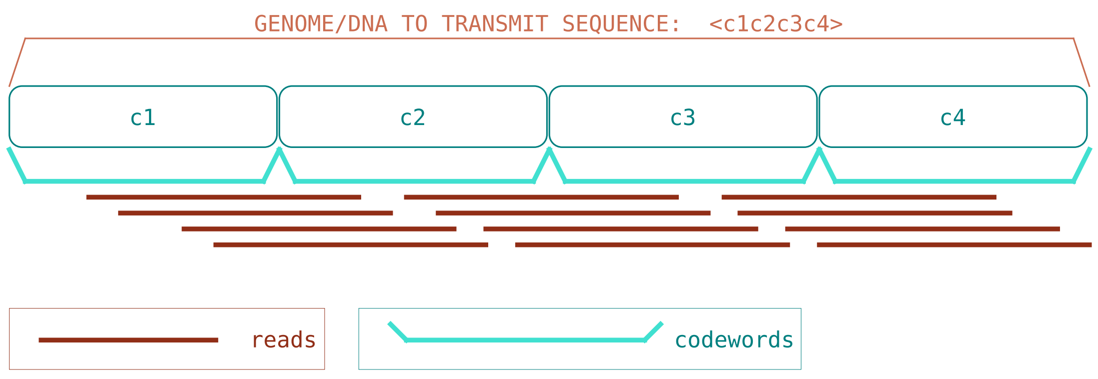


Figure 5.1.: Illustration of the fundamental difference between codewords and reads. The DNA sequence is a concatenation of all codewords.

Even though the DNA sequence consists of all **codewords concatenated together**, it is still unknown in which order they have been transmitted/stored due to the type of channel (all chemicals in one jar). That is the reason why reads must be in a structured manner that involves particular overlapping schema as it is shown in state-of-the-art sequencing technologies. On the other hand, it is known that sequencers are not very accurate, and errors may occur during the sequencing process in reads. For example, one of the most prominent situations that may happen is incorrectly identified symbols in the biological layer, which results in a sequence having erroneous reads.

The approach to the given problem in this thesis is introducing a header to the beginning of the actual payload. By adding a certain static header of length s we guarantee that there are at least s overlapping parts and at the same time the reads are not erroneous. By exploiting the shifting property of **cyclic codes**, some overlap will be preserved. In the next chapter, we will introduce the concept of **header**-which is a static sequence at the beginning of the payload, and **proto messages**-which will, in turn, be our payload.

5.2. Concept of headers and proto messages

The section will introduce the approach to sequencing the reads without errors; the explanation is based on a toy parameterized example as the initial setup.

Given a DNA as a storage setup with four messages of length 5 and CRC polynomial with corresponding generator matrix $\underline{\underline{G}}$. The list of all necessary steps to review in conventional (current state-of-the-art) DNA as storage system is as follows:

- Messages $\mathbf{m} = \{\underline{m}_1, \underline{m}_2, \underline{m}_3, \underline{m}_4\}$ are transferred to encoder. As it can be seen in Figure 5.2 the length of each message is 7, e.g. $\underline{m}_2 = [m_{21}, m_{22}, m_{23}, m_{24}, m_{25}, m_{26}, m_{27}]$.

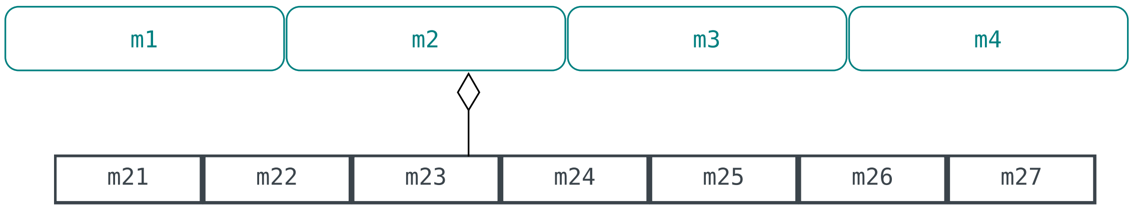


Figure 5.2.: Four messages that consist of five symbols each.

- Messages get encoded using systematic encoder (systematic $\underline{\underline{\mathbf{G}}}$ matrix) and set of codewords $\mathbf{c} = \{\underline{c}_1, \underline{c}_2, \underline{c}_3, \underline{c}_4\}$ with length 9 is generated.

- Let us closely inspect codewords transferred in our schema. As it can be seen in Figure 5.3, each codeword consists of 9 symbols $\underline{c}_2 = [c_{21}, c_{22}, c_{23}, c_{24}, c_{25}, c_{26}, c_{27}, c_{28}, c_{29}]$.

- But what if one of the symbols is shifted, e.g., $\underline{c}_2 = [c_{29}, c_{22}, c_{23}, c_{24}, c_{25}, c_{26}, c_{27}, c_{28}, c_{21}]$? Due to shifting property of cyclic codes used in this work, modified version of c_2 would still be recognized as a codeword.

- ***Idea* 5.1** *By exploiting the shifting property of cyclic codes it is possible to organize the codewords in such a way that detector recognizes overlaps of certain length as valid codewords and preserve them for later assembly.*

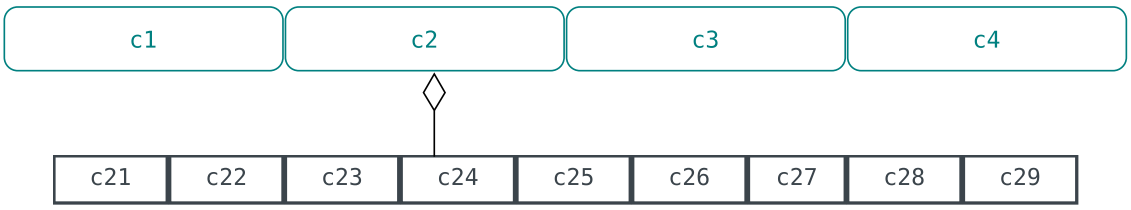


Figure 5.3.: Four codewords each consists of seven symbols.

- Let us adjust the setup to the proposed idea: we call original payload(information to be transferred) is updated to **proto messages** and the **header** is added to the beginning of our proto messages. The updated schema of messages is shown in Figure 5.4.
  - **Header** is a statically defined sequence of length s that is concatenated in **front** of the payload.
  - Message length - k is equal to
  $$k = s + k',$$
  where s is **header** length and k' is length of **proto messages**.

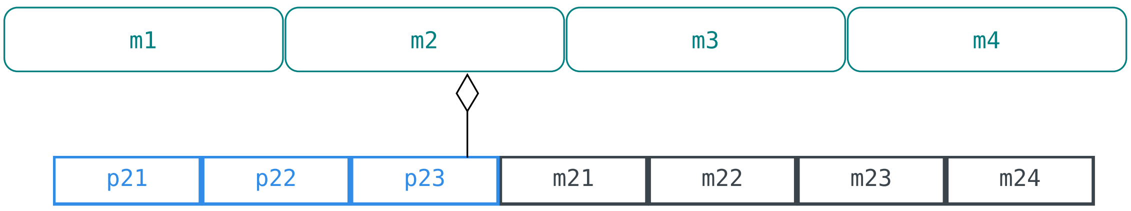


Figure 5.4.: The first three symbols are **header** and last four symbols are **proto messages**. As it can be seen now message is $\underline{m}_2 = [p_1, p_2, p_3, m_{21}, m_{22}, m_{23}, m_{24}]$,where $s = 3$, $l = 4$ and $k = 7$.

    - In the case of the concrete example $s = 3$, $k' = 4$ and $k = 7$. Three symbols in the beginning provide enough overlap in order to assemble the DNA sequence.

- Given that messages are encoded using CRC polynomial and dimensions of generator matrix are $7x9$, resulting codewords will be of length 9. Schema of resulting codewords is shown in Figure 5.5.
    - Using strategy described above codeword length - n is

$$n = s + n',$$

where s is **header** length. For concrete example as a result we get $s = 3$, $n' = 6$ and $n = 9$.

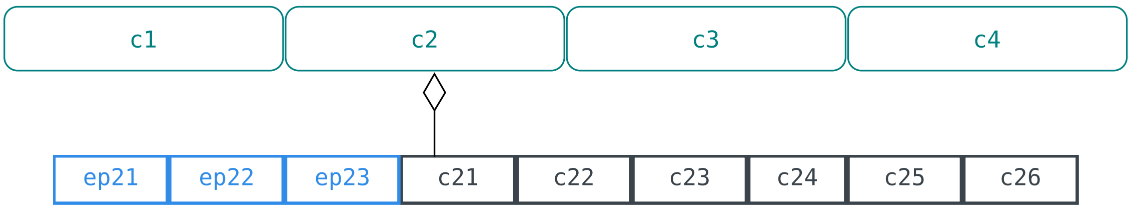


Figure 5.5.: The first three symbols are **header** and last four symbols are **pcodewords**. As it can be seen now codeword is $\underline{c}_2 = [ep_1, ep_2, ep_3, c_{21}, c_{22}, c_{23}, c_{24}, c_{25}, c_{26}]$

The section presented a particular technique of header introduction and gave intuition on why the proto messages' approach provides overlap. The next section illustrates how exactly the algorithm perceives the data containing valid reads. Later sections will empirically prove this in terms of a toy example.

5.3. Assembling DNA sequence using valid codewords

In order to provide enough overlap between codewords, we introduced the concept of proto messages. Why does the concept of proto messages work in the task of assembling DNA sequence? Let us answer the question using a concrete setup and example described below. Everything that has been introduced until this section:

- As it was described in Chapter 3, we introduced the concept of **error control codes** layer between biological and computational layers in DNA sequencing. We enforced the length of reads to be the same as codewords - which is why we called them **n-mers** and tried the conventional approach of transferring messages.
- Codewords that have been transferred without error were recognized as valid. Since the overlaps are needed for sequencing algorithms to work, codewords alone do not fit as data.

- In the previous section, the concepts of header and proto message were introduced.
- The previous section also described a useful property of cyclic codes that, in turn, led to the concept of proto messages.

Now that we have the setup described above with proto messages and the ECC layer in sequencing, let us discuss how they work. The updated schema is shown in Figure 5.6.

- Proto messages ($k = 7$) are encoded into sequence of $n = 9$ codewords, with length of header being $s = 3$. Codewords are concatenated together to form a DNA sequence: `<c1c2c3c4>` and passed to the synthesizer in order to get molecules for storage.
- As it is seen in Figure 5.7 two codewords are concatenated together:

  `c2c3=ep1ep2ep3c21c22c23c24c25c26ep1ep2ep3c31c32c33c34c35c36`,

  and are part of the DNA sequence.

  - On the sequencer's **biological** layer reads are sequenced individually at a length $n = 9$. In this case, Figure 5.7 displays all the toy parametered example reads, that have been sequenced; as it can be seen 3 of them have errors, specifically $r5$,$r7$ and $r8$ (according to research paper [19] errors are normally closer to the end of sequence).
  - On the sequencer's **ECC** layer, erroneus reads are going to be detected using parity check matrix and discarded and valid reads are going to be preserved.
  - We take into account that guaranteed minimum overlap is going to be equal to 3 (since the header's length is $s = 3$), and all the other reads are discarded from the beginning. As it can be seen in Figure 5.8 by giving up certain overlap, we get ideal (valid) reads.

- In the previous chapter, we also introduced algorithms for sequencing that are based on overlapping parts of reads. Since the overlap between reads is **enough** for assembly, it is possible to utilize the de Bruijn graph or shortest common superstring to assemble a DNA sequence from codewords. Reads that are recognized as valid codewords are shown in Figure 5.8.
- SCS is the algorithm of choice since our toy example only consists of $9 * 4 = 36$ symbols, and for de Bruijn assembly, it is recommended to use the overlap of $n - 1$.
- s overlaps between reads/codewords are enough to reconstruct the sequence. Utilizing them algorithm reconstructs DNA sequence from the reads, and assembled sequence is later transferred to **decoder**.

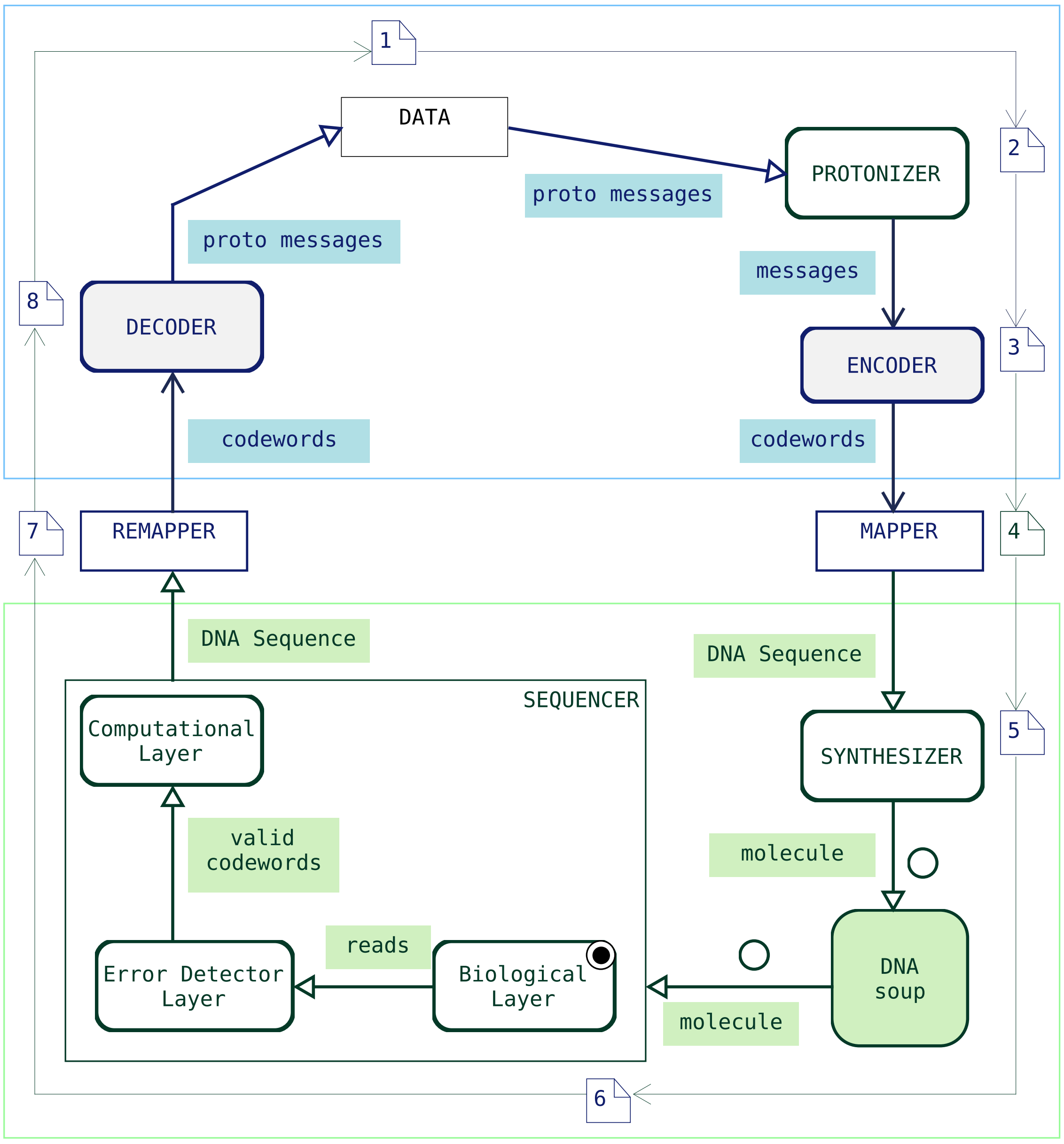


Figure 5.6.: Depiction of updated schema of DNA as a storage. As it can be seen on the figure new stages have been added to our initial schema of DNA as a storage. Here we have **PROTONIZER** and **Error Detector Layer** in sequencing.

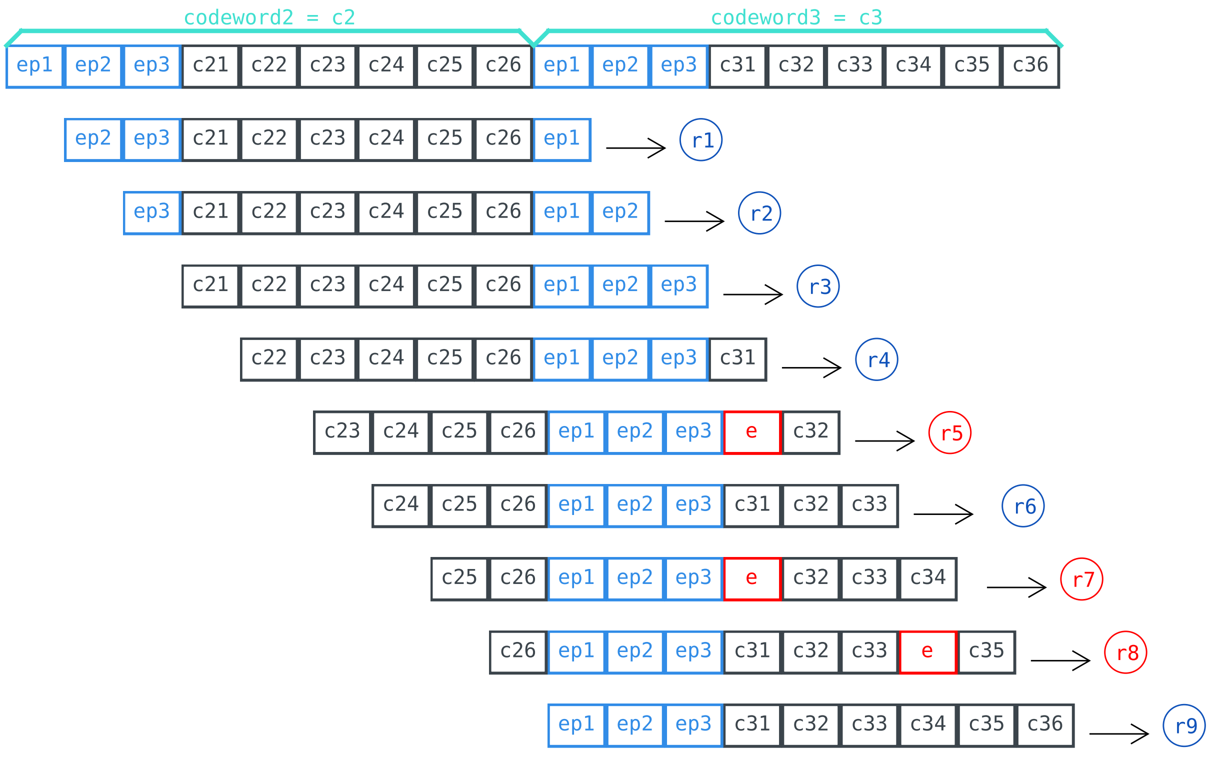

codeword2 = c2
codeword3 = c3
ep1 ep2 ep3 c21 c22 c23 c24 c25 c26 ep1 ep2 ep3 c31 c32 c33 c34 c35 c36
ep2 ep3 c21 c22 c23 c24 c25 c26 ep1 → r1
ep3 c21 c22 c23 c24 c25 c26 ep1 ep2 → r2
c21 c22 c23 c24 c25 c26 ep1 ep2 ep3 → r3
c22 c23 c24 c25 c26 ep1 ep2 ep3 c31 → r4
c23 c24 c25 c26 ep1 ep2 ep3 e c32 → r5
c24 c25 c26 ep1 ep2 ep3 c31 c32 c33 → r6
c25 c26 ep1 ep2 ep3 e c32 c33 c34 → r7
c26 ep1 ep2 ep3 c31 c32 c33 e c35 → r8
ep1 ep2 ep3 c31 c32 c33 c34 c35 c36 → r9

Figure 5.7.: Depiction of individual reads after sequencing unknown DNA sequence. Please note that the order of the reads is not defined; they are pretty much scattered. For better demonstration order of the reads is preserved. It is also seen that out of nine reads, three are erroneous, and six are valid.

As has been described in this section, we concentrated mainly on why the concept of proto messages works. The next section will experimentally make a comparison between the state-of-the-art/no coding implementation of DNA storage and the header/proto message approach.

Regardless of changes in simulation required to implement, old components remain in the system; instead, it is necessary to add new components like **Protonizer**. Also, notice that we are working on a ”toy parameterized” example, and the length of messages is $k = 11$, length codewords $n = 15$. Additionally, it is required to perform a certain number of repetitive experiments. So, random data generation has also been automated in order to achieve such a result. The next section will describe the updated structure of source code, initial setup, and experiments themselves. Conclusively, an average of 3000 experiments will be taken, and the application will produce comparative analysis with plots.

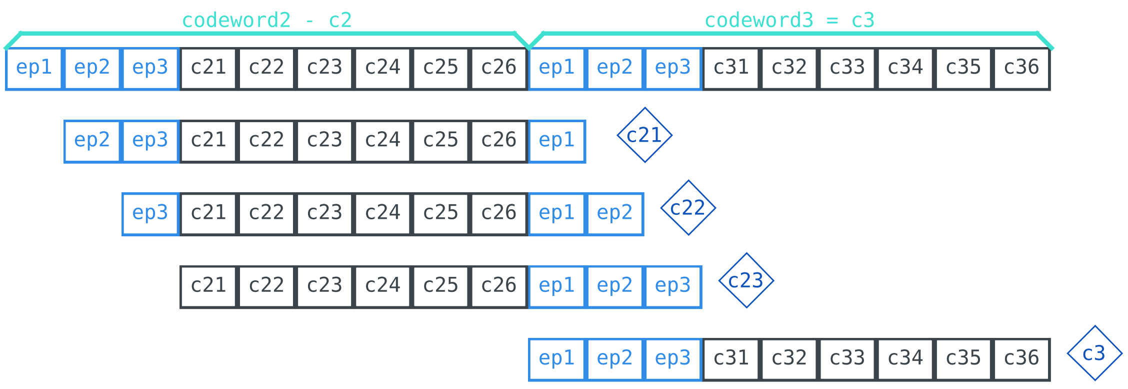

codeword2 - c2
codeword3 = c3
ep1 ep2 ep3 c21 c22 c23 c24 c25 c26 ep1 ep2 ep3 c31 c32 c33 c34 c35 c36
ep2 ep3 c21 c22 c23 c24 c25 c26 ep1
c21
ep3 c21 c22 c23 c24 c25 c26 ep1 ep2
c22
c21 c22 c23 c24 c25 c26 ep1 ep2 ep3
c23
ep1 ep2 ep3 c31 c32 c33 c34 c35 c36
c3

Figure 5.8.: Depiction of valid codewords preserved during sequencing. We discarded certain reads, however with a guarantee of assembly from **valid** reads, and discarding **erroneus** ones.

5.4. Empirical analysis on toy example

This section analyzes empirically the concepts discussed in previous sections by bringing a concrete working example of assembly from codewords on a simulated system. First, modifications to the original setup are going to be discussed. Then experiments are performed by simulating *substitutions* from sequencing errors and employing a particular randomized message generator. Conclusively, comparative graphs of both approaches are being generated.

5.4.1. Updated setup

As it was described in a prior section, a particular randomized message generator is going to be included in a system. The idea behind a randomized message generator is to produce a certain number of messages with defined length and automate the process. Figure 5.9 illustrates a definition of the message generator.

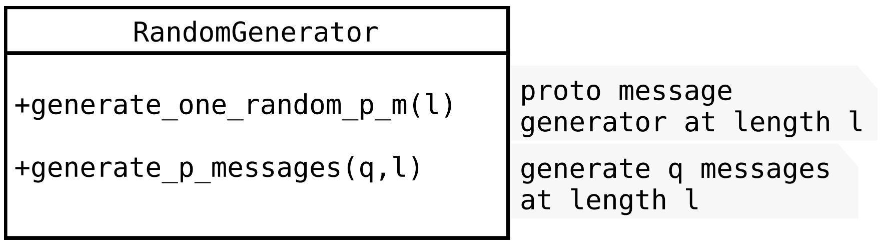

RandomGenerator
+generate_one_random_p_m(l)
+generate_p_messages(q,l)
proto message generator at length l
generate q messages at length l

Figure 5.9.: Random proto message generator

In addition to the randomized proto messages generator, it is required to add one more component - Protonizer. Protonizer appends a unique pre-defined sequence to the beginning of proto messages, which in turn results into `<[static_head|proto_message]>`. Figure 5.10 displays the definition of Protonizer class.

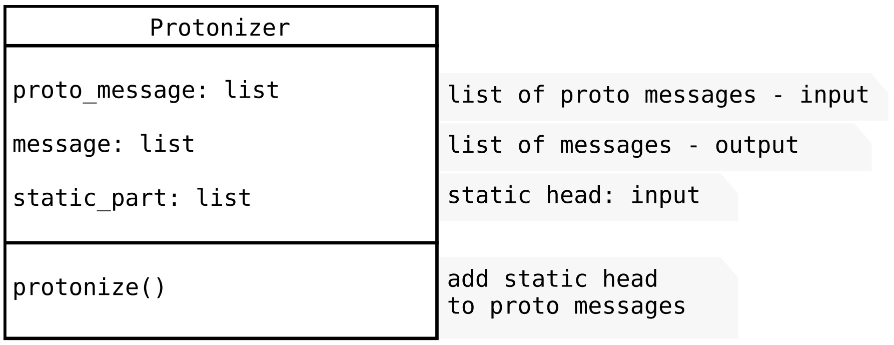

Protonizer
proto_message: list
message: list
static_part: list
protonize()
list of proto messages - input
list of messages - output
static head: input
add static head to proto messages

Figure 5.10.: Protonizer component - outputs messages given proto messages

Finally, it is essential to implement the channel that simulates errors introduced to the system by Sequencer at reads generation step. In order to be able to simulate such a system, it is required to revisit the error types introduced at the sequencing step. Errors introduced during DNA sequencing depend on the technology employed.

This thesis mostly took the approach from Illumina's single-read sequencing technology [33]; instructions, databases, and algorithms are available from open-source manuals on Illumina's website. Algorithms used are de Bruijn assembly and shortest common superstring. Errors introduced in sequencing are typically substitutions, and much less likely deletions and insertions. However, according to research papers, there are certain properties of the DNA sequence in the sequencing process, that increase error probability (mostly deletions and substitutions). Properties of the undefined DNA sequence are specifically the GC content and homopolymer stretches (same-symbol data, e.g., CCCCCCC). In the case of this work, the examples that are tested are based on substitutions only. Algorithm for substitution-based error introduction is shown in Listing 5.1.

```
def distort_reads_strong_version(reads,p):

    #INPUT: reads - reads/codewords to be distorted
    #INPUT: p - probability given at single simulation
    #normally probability increases

    #iterate over all reads/codewords
    for i in length_Of_Reads:
        #iterate over symbols in particular codeword
        for j in single_codeword:

            #pick randomly value between 0 and 1
            s=pick randomly from [0 and 1]

            #if random value is less than probability
            #at single simulation
            if s<p:
                swap character to random
                from given to random in [A,C,T,T]
```

Listing 5.1: Channel algorithm: working principle is very similar to Digital Memoryless Channel

The above paragraphs, specified components that have been added to the updated system of DNA as storage. In the workflow manager, an additional method for the Protonizer element was added. Now that all components of the system are present, it is possible to proceed to the comparison of coding based DNA storage and state-of-the-art sequencing approach.

5.4.2. Simulations performed on toy parameterized example

Now that the system for experiments is all set, it can be collected together, and empirical analysis can be performed. In order to produce a comparative analysis with plots, additional `plt` library from *Matplotlib* [34] is utilized.

Rules established for experiments are:

- Number of experiments is chosen to be 3000.
- At every experiment, a random set of proto messages is generated first for state-of-the-art (standard sequencing) approach and then the static header approach. Parameters are as follow:
  - **Lengths**: The length of a single proto message is 7; the length of a single message with static header is 11; the length of the codeword is equal to 15. Codewords and headers are not involved in experiments for standard sequencing.
  - **Quantity**: Quantity of proto messages per experiment is agreed to be 7.
  - **CRC Code Polynomial**: Polynomial for cyclic code is $x^4+x^3+x^2+2x+3$. Polynomial is not involved in experiments for standard sequencing.
  - **Header**: Header is chosen to be $[1,2,1,1,2]$.
- At every experiment, error probability starts from 0 and raises to 1.0 with an interval of 0.05. Even though the likelihood of substitution errors is not as high as shown in setup, it has been decided to test all the cases on a toy parameterized example.

- All the parameters are assumed to be proportionate to state-of-the-art sequencing technologies and can be adjusted to a given real-world example.

Listing 5.2 shows the header/proto messages approach section of tests for updated workflow. Listing 5.3 shows the state of the art approach (it can be perceived as a continuation of proto messages part). As can be seen, the distinction between the listings is that in the conventional sequencing approach, there are five steps to perform, whereas the proto message/header has eight steps.

```
from workflow_manager import WorkflowManager
import matplotlib.pyplot as plt
from bioinformatics.work_without_ecc import *
from helper import *

#error probabilities from 0->1
ERROR_PROBABILITIES = [0,0.05,0.1,0.15,0.2,0.25,0.35,0.4,
0.45,0.5,0.55,0.60,0.65,0.70,0.75,0.8,0.9,1.0]

#number of experiments
N_E=3000

#sets figure size to 12x25
plt.figure(figsize=(12, 25))

#coefficients of CRC code
POLYNOM = [1,1,1,2,3]

#static part set to [1,2,1,1,2]
S_PART=[1,2,1,1,2]

#codeword length set to be 15
N=15

#iterate through number of experiments
for t in range(N_E):

    #generate proto messages: 7 per experiment
    proto_messages = generate_messages(7,7)

    #1) manager takes proto messages, polynomial and static part
    #2) joins them with static part
    #3) encodes messages
    #4.1) performs simulation of mapping(0,1,2,3->A,C,T,G)
    #4.2) performs virtual synthesis -> simple concatenation
    #5) passes through channel for substitutions
    #6) detects erroneus sequences and discards them
    #7) assembles using shortest common superstring
    #8) #errors_in_assembly/#of_total_bases

    wm = WorkflowManager(proto_messages,POLYNOM,S_PART,N)
    wm.generate_messages()
    wm.encode()
    wm.map_and_synthesize()

    #iterate through error probabilites
    for probability in ERROR_PROBABILITIES:

        #create distorted reads depending on error probability
        #at 0 there are no substitutions
        wm.create_distorted_reads(probability)

        #detect correct codewords
        wm.detect_codewords()

        #assemble sequence using shortest common superstring
        wm.assemble()
```

Listing 5.2: 3000 simulations on toy parametered example using coding theory with header/proto message approach.

```
from workflow_manager import WorkflowManager
import matplotlib.pyplot as plt
from bioinformatics.work_without_ecc import *
from helper import *

#error probabilities from 0->1
ERROR_PROBABILITIES = [0,0.05,0.1,0.15,0.2,0.25,0.35,0.4,
0.45,0.5,0.55,0.60,0.65,0.70,0.75,0.8,0.9,1.0]

#number of experiments
N_E=3000

#sets figure size to 12x25
plt.figure(figsize=(12, 25))

#iterate through number of experiments
for t in range(N_E):

    #generate proto messages: 7 per experiment
    proto_messages = generate_messages(7,7)

    #1) manager takes proto messages
    #2.1) performs simulation of mapping(0,1,2,3->A,C,T,G)
    #2.2) performs virtual synthesis -> simple concatenation
    #3) passes through channel for substitutions
    #4) assembles everything in the end
    #5) #errors_in_assembly/#of_total

    wmsoa = WorkflowManagerForSOA(proto_messages)
    wmsoa.map_and_synthesize()
    #iterate through error probabilites
    for probability in ERROR_PROBABILITIES:

        #create distorted reads depending on error probability
        #at 0 there are no substitutions
        wmsoa.create_distorted_reads(probability)

        #assemble sequence using shortest common superstring
        wmsoa.assemble()

        #proportion of errors to the initial length of DNA sequence
        e_b = wm.number_of_erroneus_bases()
```

Listing 5.3: 3000 simulations on toy parametered example without using coding theory and headers.

From the listings described above, Figure 5.11 is generated.

- Independent variable (x) is the probability of substitution errors - variates from 0 to 1 (maximum probability of failure).
- The dependent variable in the figure describes the proportion of quantity of erroneous symbols to the length of DNA. Each point in the plot constitutes a cumulative average of 3000 experiments.
- As it is shown in the figure at some point, the approach proposed in this thesis for the particular case of 3000 experiments until 50% substitution error probability achieves a lower error rate in assembly. Starting from 53% performance of the static header approach worsens. Eventually, at 80%, graphs meet, and the conventional method has a better outcome.

5.5. Conclusion

As it can be seen in Figure 5.11, the approach introduced in this thesis offers specific improvements. However, it is necessary to take into account that the model presented here was produced on a toy parameterized example, where parameters are assumed to be proportional to the real-world application of sequencing. There are also certain facets in the biological layer in the sequencing step that were not taken into account in this thesis. The situation was assumed to be ideal, where substitution errors only were tested.

It is also necessary to note that the field the thesis dealt with is purely based on mathematical models. There are only three labs that implemented DNA as a storage system experimentally. This work can serve as a good starting point for electrical engineers to learn about DNA as a storage and certain sequencing algorithms. Using chapter four it is possible to implement a real-world component-based system for DNA as a storage.

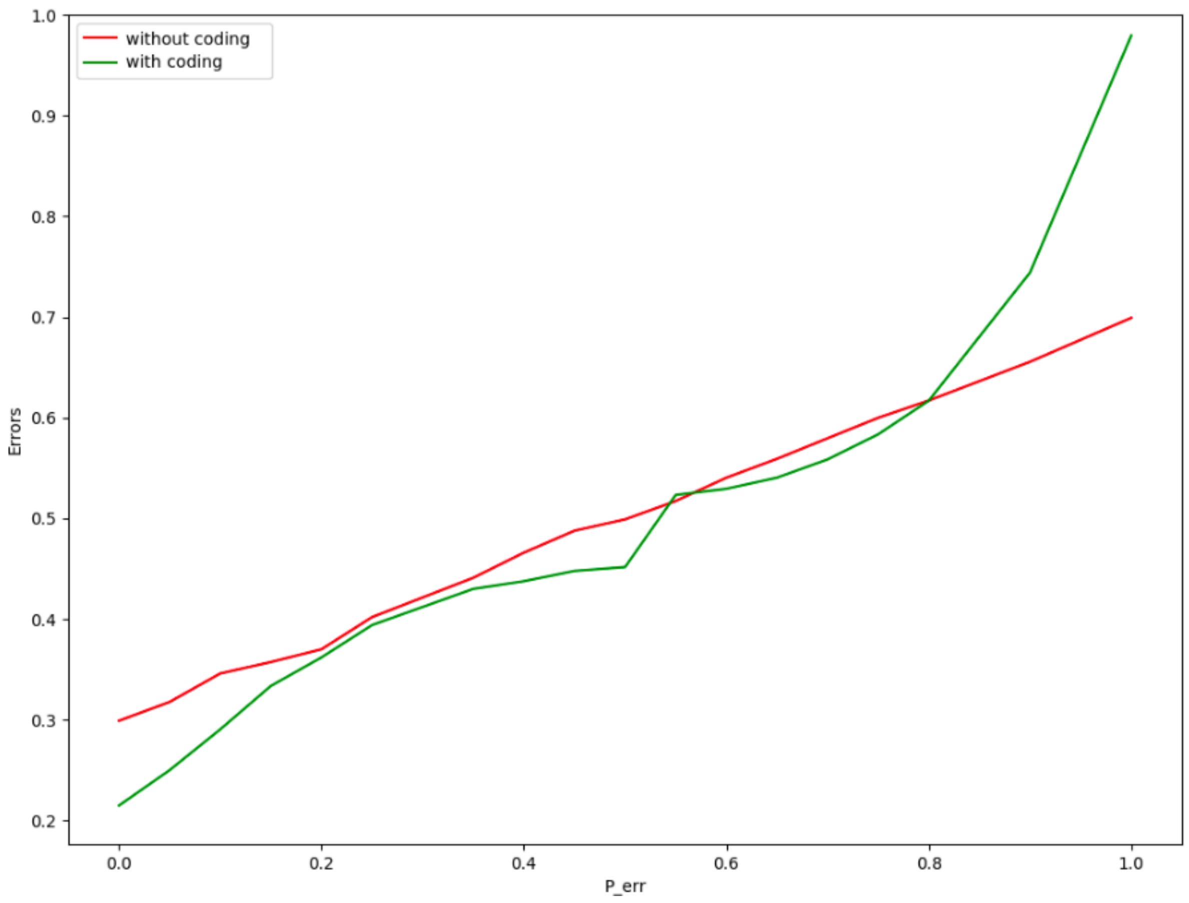


Figure 5.11.: Comparative plot of two different approaches to DNA as a storage for 3000 experiments. Red graph corresponds to conventional approach. Green plot corresponds to graph message/header approach. Parameters of setup are: $k' = 7$, $k = 11$, $n = 15$, generator polynomial is $x^4 + x^3 + x^2 + 2x + 3$ and header is $[1,2,1,1,2]$. Independent variable (x) is the probability of substitution errors - variates from 0 to 1 with interval 0.05. The dependent variable (y) describes the proportion of quantity of erroneous symbols to the length of originally synthesized DNA. Until 50% of substitution errors probability header/proto-message approach performs better in assembly (achieves a lower error rate) than state-of-the-art method. Eventually, at 80%, graphs meet, and the conventional method has a better outcome.

6. Future outlook

In this thesis, we compiled literature about DNA as storage and covered the basics of sequencing algorithms and biology for electrical engineers. Additionally, we provided a particular technique for detecting erroneous reads and preserving overlaps for assembly of DNA sequence.

There are many various tests and experiments are left for the future. Even though a particular implementation of simulation is provided, real-world parameters and components are needed in order to conduct more in-depth experiments. Furthermore, there are many other concepts, such as quality of reads (coverage) and approaches for sequencing problems (e.g., overlap layout consensus and hybrid methods) that can be discussed and tested. Another topic to be discussed is the biochemical aspect of the project, such as synthesis and chemical contents of DNA soup, DNA decays (result in loss of molecules). Chapter four of this thesis can lead to the creation of a lab dedicated specifically to DNA as storage.

The derived topic from this work could be an in-depth analysis of the header-based approach for de Bruijn sequencing or experiments using physical sequencers and synthesizers on a header-based approach.

A. Appendix

In course of the thesis, extensive simulation of DNA as storage system was developed. It is worth mentioning that mentioned below codes were developed by author of thesis [35].

In chapter four, we briefly introduced the way codes are organized. The appendix covers the functionality of methods and attributes in the listings format and provides documentation of codes attached to the thesis.

A.1. Error control codes package

There are two classes in error control codes package: **Encoder** and **Detector** (since the **Channel** involves biological facets, it is located in helpers package). Each of those classes has corresponding methods that serve a specific purpose designed for tasks from error control codes. Every method is described in corresponding code listings: for Encoder see Listing A.1, for Decoder see Listing A.2. Please note, blue italic font represents comments in python.

```
class Encoder(object):
        """
         constructor in Python language:
         messages: collection of messages to be encoded
         polynom: CRC polynomial to generate G-matrix with
         k: length of each message
         n: length of each codeword
        """
        def __init__(self,messages,polynom,k,n):
                ...

        #private helper method constructs generator matrix
        def __construct_generator_matrix():
                ...
                return generator_matrix

        #private encode method
        #encodes single message out of collection
        #of messages and returns codeword
        def __encode():
                ...
                return codeword

        #using helper methods encodes all the messages;
        #uses codewords attribute to assign result
        def encode_messages():
                ...
                self.codewords = result
```

Listing A.1: Description of methods and attributes from Detector class; implementation is omitted.

```
class Detector():

    #codewords: array of codewords fed in our system
    def __init__(self, codewords):
        ...

    #returns h_matrix using cross-class interfaces
    def __get_h_matrix():
        ...
        return h_matrix

    #checks whether each individual read is codeword
    #returns True or False
    def __check_whether_codeword(self):
        ...
        return status

    #method used for all codewords
    #collects valid codewords to
    def perform_calculation_to_check(self):
        ...
```

Listing A.2: Description of each method from Detector class.

A.2. Bioinformatics package

There are two classes in bioinformatics package: **Synthesizer** and **Sequencer**. Each of those classes has corresponding methods that serve a specific purpose in bioinformatics. Every method is described in corresponding code listings: for Synthesizer see Listing A.3, for Sequencer see Listing A.5.

```
#synthesis in simulation is simple string concatenation
class Synthesizer(object):
        """
         codewords: codewords to map and synthesize/concatenate
         sequence: resulting sequence after synthesis
        """
        def __init__(self,codewords):
                ...

        #private helper method constructs generator matrix
        def __map_codewords():
                ...
                return sequence_in_string

        #concatenates strings together and returns sequence
        def join_for_synthesis():
                ...
                return self.sequence
```

Listing A.3: Description of methods and attributes from Synthesizer class.

```
class Sequencer(object):
        """
         reads: list of Strings
         assembled_DNA: assembled DNA - String value
        """
        def __init__(self,reads):
                ...

        #create n-mers from reads
    def create_n_mers(self,n):
        ...
        return n_mers



    #method_name: String for de Bruijn or SCS
    #assign assembled_DNA to result
    def assemble_DNA(self,method_name):
        ...
        self.assemble_DNA=result
```

Listing A.4: Description of each method from Sequencer class.

A.3. Helpers package

There are four files in helpers package: **Channel**, **mapper.py**, **math_helpers.py** and **F_Four**. Channel and F_Four are classes; math_helper.py and mapper are simply a collection of methods. Channel contains methods that simulate errors that occur during sequencing and distorts reads correspondingly. F_Four is a class aimed to simulate $\mathbb{F}_4$ by overriding standard operators (plus, minus, multiply, divide, and equals). The file mapper.py is a python script that contains two methods to map $A, C, T, G <-> 0, 1, 2, 3$. The file math_helpers contains methods necessary for performing sophisticated mathematical operations. A brief description of each of the files is shown in the following listings correspondingly. For Channel see Listing 5.1, for F_Four see Listing A.6, for mapper see Listing A.7 and for math_helpers see Listing A.8.

```
class Channel:
        """
         synthesized_dna: String value - holds DNA sequence
         reads: String array - reads are output value
        """
        def __init__(self,synthesized_dna):
                ...

        #private helper method constructs generator matrix
        def __generate_reads():
                ...
                self.reads=result

        #private distortion method
        #distorts reads and returns array
        def _distort_reads_strong_version():
                ...
                return distored_reads

        #using helper methods assign reads
        #to distorted_reads
        def get_distorted_reads():
                ...
                self.reads = result
```

Listing A.5: Description of each method from Channel class.

```
class F_Four:

        elements = {0:[0,0],
                        1: [0,1],
                        2: [1,0],
                        3: [1,1]}

        #n: desired element for performing calculations
        def __init__(self,n):
                ...

        #helper method to perform calculations
        def _perform_calculation(self,other,SIGN):
                ...
                return result

        #overloaded methods are below
        def __add__(self,other):
                return self._perform_calculation(other,"+")

        def __sub__(self,other):
                return self._perform_calculation(other,"-")

        def __mul__(self,other):
                return self._perform_calculation(other,"*")

        def __truediv__(self,other):
                return self._perform_calculation(other,"/")

        def __eq__(self,other):
                return self.n == other.n
```

Listing A.6: Description of each method from F_Four class.

```

#maps dna sequence to information (from ACGT->0123)
def re_mapper(dna_seq):
        ...
        return information

#maps information to dna sequence (from 0123->ACGT)
def mapper(information):
        ...
        return dna_seq
```

Listing A.7: Description of each method from mapper script. This script contains only 2 methods.

```
"""
Helper Math functions:

This script compiles all mathematical operations used in this work.
Below is the list of methods and their description:

    * matrixmult - multiply two matrices and return the result in F_Four
    * transpose - transpose matrix and return result in F_Four
    * vec_mat - multiply a vector by matrix  and return the result in
      F_Four
    * rref - bring matrix to reduced row-echelon form (helper function) -
      a pass-by-value principle
    ---------------------------------------------------------
"""
```

Listing A.8: Description of each method from math_helper script. This listing contains only comments: method declarations are omitted.

A.4. WorkflowManager class

WorkflowManager is a python class aimed to collect the system and execute operations by collecting all the components together. The class has eight methods that correspond to steps from DNA as a storage schema. As shown in chapter 5, during empirical analysis, certain steps were skipped, since the class is heavily based on separate components. WorkflowManager is shown in Listing A.9.

```
class WorkflowManager(object):
    """
    proto_messages: original payload (array of messages)
    polynom: polynomial for cyclic code
    s_part: static part, if equals to empty list - skip
    n: codeword and read length
    """

    def __init__(self,proto_messages,polynom,s_part,n):
        ...

    #generates messages from proto_messages by appending static part
    #if s_part is empty the step simply assignes messages to proto
    def generate_messages(self):
        ...
        self.messages = result

    #constant ENCODER encodes all the messages
    def encode(self):
        ...


    #maps {0,1,2,3}->{A,C,T,G} and synthesizes
    def map_and_synthesize(self):
        ...

    #creates distorted reads from synthesized DNA
    def create_distorted_reads(self,probability):
        ...

    #detects valid codewords saves them for further assembly
    def detect_codewords(self):
        ...

    #if previous error control codes steps are performed
    #   uses valid codewords to assemble sequence
    #else uses proto messages to assemble sequence
    def assemble(self):
        ...

    #compares original and assembled sequence from previous step
    #and counts number of mismatches
    def compare_sequences(self):
        ...
        return count_of_mismatches
```

Listing A.9: Description of WorkflowManager class.

Usage of WorkflowManager is straight-forward. First, it is necessary to instantiate Workflow-Manager and, depending on the task, call the necessary methods. Example of usage is shown below:

```
wm=WorkflowManager(proto_message,polynom,s_part,n)
wm.generate_messages()
wm.encode()#can be ommited in state-of-the-art experiments
wm.map_and_synthesize()
wm.create_distorted_reads()
wm.detect_codewords()#can be ommited in state-of-the-art experiments
wm.assemble()
```

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Erklärung

Hiermit erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie alle wörtlich oder sinngemäß aus anderen Werken übernommenen Aussagen als solche gekennzeichnet habe.

Die Arbeit wurde bisher keiner anderen Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht. Das elektronische Exemplar stimmt mit den gedruckten Exemplaren überein.

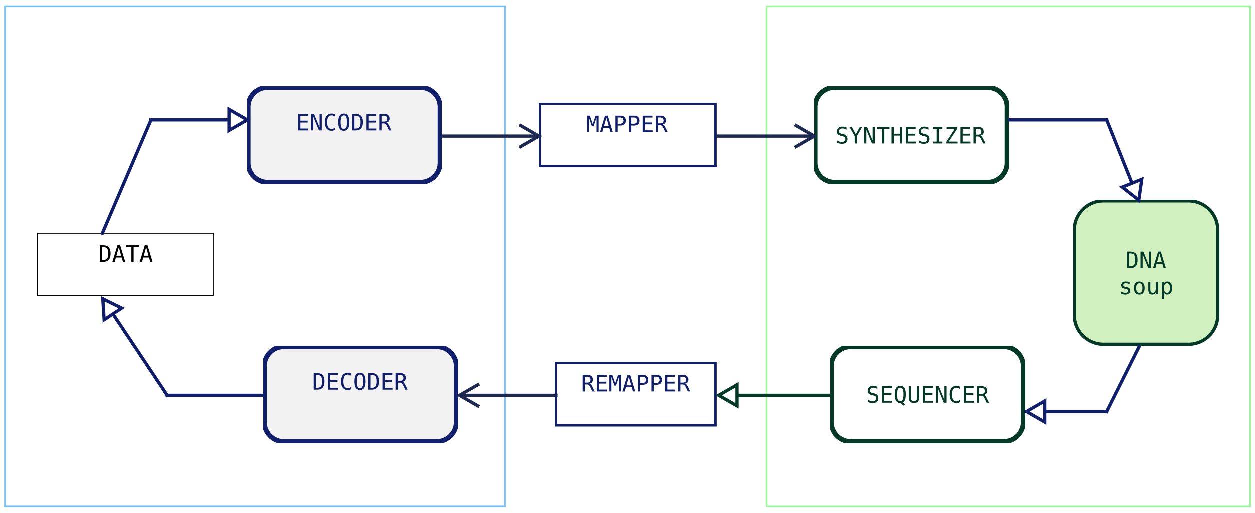
Stuttgart, 03. Juli 2020

Fikrat Talibli

Master Thesis

DNA as a storage - Error Detection based on Cyclic Codes

Fikrat Talibli



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Abstract

One of the alternate storage mediums that scientists considered is synthesized DNA molecules. This thesis provides a comprehensive guide for electrical engineers to the topic of DNA as data storage and specifics of DNA channel. The thesis finalizes with an empirical analysis of 3000 experiments on the randomized messages and provides the comparison of coding based DNA storage and state-of-the-art sequencing approach.

submitted to

University of Stuttgart
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