

1 DNA Storage Designer: A practical and holistic design

2 platform for storing digital information in DNA sequence

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11 Abstract:

12 DNA molecules, as natural information carriers, have several benefits over conventional digital storage
13 mediums, including high information density and long-term durability. It is expected to be a promising
14 candidate for information storage. However, despite significant research in this field, the pace of development
15 has been slow due to the lack of complete encoding-decoding platform and simulation-evaluation system.
16 And the mutation in DNA sequences during synthesis and sequencing requires multiple experiments, and
17 wet experiments can be costly. Thus, a silicon-based simulation platform is urgently needed for promoting
18 research. Therefore, we proposed DNA Storage Designer, the first online platform to simulate the whole
19 process of DNA storage experiments. Our platform offers classical and novel technologies and experimental
20 settings that simulate three key processes: encoding, error simulation, and decoding for DNA storage system.
21 First, 8 mainstream encoding methods were embedded in the encoding process to convert files to DNA
22 sequences. Secondly, to uncover potential mutations and sequence distribution changes in actual experiments
23 we integrate the simulation setting for five typical experiment sub-processes (synthesis, decay, PCR,
24 sampling, and sequencing) in the error simulation stage. Finally, the corresponding decoding process realizes
25 the conversion of DNA sequence to binary sequence. All the above simulation processes correspond to an

26 analysis report will provide guides for better experiment design for researchers' convenience. In short, DNA
27 Storage Designer is an easy-to-use and automatic web-server for simulating DNA storage experiments, which
28 could advance the development of DNA storage-related research. And it is freely available for all users at:
29 <https://dmci.xmu.edu.cn/dna/>.

30 **Keywords:** webserver, DNA storage, simulation, encoding, DNA sequence

31 **Author summary**

32 DNA storage technology is an emerging and promising storage technology. At the same time, DNA storage
33 is an interdisciplinary technology that requires researchers to know both computer cryptography and
34 biological experiments knowledge. However, DNA storage experiments are costly and lengthy, many studies
35 have been prevented by the lack of a comprehensive design and evaluation platform to guide DNA storage
36 experiments. Herein, we introduce DNA Storage Designer, the first integrated and practical web server for
37 providing the simulation of the whole process of DNA storage application, from encoding, error simulation
38 during preservation, to decoding. In the encoding process, we not only provided the coding DNA sequences
39 but also analyzed the sequence stability. In the error simulation process, we simulated as many experimental
40 situations as possible, such as different mutation probabilities of DNA sequences due to being stored in
41 different bacteria hosts or different sequencing platforms. The platform provides high freedom in that users
42 could not only encode their files and conduct the entire operation but also could upload FASTA files and
43 only simulate the sustaining process of sequences and imitate the mutation errors together with distribution
44 changes of sequences.

45 **1. Introduction**

46 In the era of data explosion, traditional storage methods are fast approaching a critical limit in their
47 storage capacities (1) that are estimated to fail to satisfy global demand in 2040 (2). What's more, the
48 life expectancy of conventional mediums is rather short, even for magnetic tapes, which are utilized for
49 long-term storage currently and are copied every five years for data security (3). Thus, a recent
50 impressive development in the fields of biology and computer science involves using DNA sequences

51 to address these issues. DNA holds an estimated information density of about 4.6×10^8 GB/mm³, about
52 6 orders of magnitude greater than the maximal density of even the most advanced magnetic tape storage
53 system (4). Meanwhile, it could be stable for thousands of years under optimal conditions (3). High
54 information density and long-term durability, together with other fabulous characteristics like its
55 potentially low maintenance cost and environmental friendliness make up the expectation to let it
56 provide wide practicality in the future. Thus, research in this area is widely carried out, and the
57 researchers have prompted several novel technologies for the whole workflow (5-7).

58 Akin to classical electronic memory, a DNA-based data storage system generally involves three major
59 steps: encoding, storage, and decoding. Unlike the binary number system of the computer, it is known-
60 to-all that the DNA sequence has four different bases (ie. adenine, (A), thymine (T), cytosine (C),
61 guanine(G)). Thus, the start, encoding, is to convert a binary data stream into sequences of quaternary
62 DNA bases using a predeveloped coding schema (5). Once these DNA sequences are obtained, the next
63 step is to synthesize them to get real DNA strands through wet experiments and store them in oligo
64 pools. Depending on the experimental needs, the obtained DNA strands might also undergo additional
65 steps such as storage, PCR (Polymerase Chain Reaction), sampling, and sequencing. Finally, to retrieve
66 the data, a corresponding decoding method is required to convert information back to binary form.

67 Nonetheless, when it comes to actual implementation, numerous intricacies warrant careful
68 consideration. To begin with, a primary technical obstacle pertains to the selection of an appropriate
69 encoding method. There are many encoding methods prompted, but each way possesses unique
70 characteristics. For example, Church et al. (7) introduced additional limitations to reduce homopolymers
71 and repeat sequences, at the expense of lower information density, while Erlich and Zielinski (8)
72 approach the theoretical maximum information capacity per nucleotide of DNA. What's more, during
73 in vitro experiments of real application, error probability changes at all stages depending on the choices
74 and settings of experiments. As an instance, Bornhol et al. (9) reported that Illumina sequencing led to
75 an error rate of about 1%. And Organick et al. (10) found a higher error rate of up to 10% introduced by
76 Nanopore sequencing. It shows the truth that different choices may result in varying outcomes, making
77 it essential to choose carefully. Apart from synthesis, each parameter chosen for each step might lead to

78 mutations or changes in the distribution of DNA strands, which could cause information errors or even
79 losses. Given these complexities, it is crucial to carefully consider factors such as the cost of experiments
80 and the specificities of various documents when selecting encoding methods and experimental settings.
81 At the same time, no simulation and evaluation platform has been reported. Due to the high cost of wet
82 experiments, a silicon-based platform is urgently needed to assist in designing the workflow.
83 Herein, we introduce DNA Storage Designer, a practical and holistic web server that offers a
84 comprehensive simulation of the entire process of DNA storage application, ranging from encoding,
85 error simulation during preservation, to decoding. The encoding process embeds 8 mainstream
86 encoding-decoding methods including Church's code (7), DNA fountain code (8), Yin-yang code (11),
87 and so forth (6, 12-14). During the error simulation stage, our server is equipped with key experimental
88 conditions for DNA storage applications, which allows users to effortlessly configure experiments
89 without the need for complex parameters. DNA Storage Designer grants users immense flexibility,
90 enabling them not only to encode their files and simulate the entire process but also to upload FASTA
91 files and solely simulate the sustaining process of sequences while mimicking the mutation errors along
92 with distribution changes of sequences. It also gives thorough guidelines and simulated feedback based
93 on user settings so that users could adjust their experimental plan according to the report of the website.

94 **2. Design and implementation**

95 Figure 1 presents a schematic workflow of the whole process of DNA Storage Designer. Which
96 proposed to transfer files into DNA sequences and simulate the workflow of the whole system to guide
97 the design of the experiment. The whole process consists of three parts, encoding, error simulation and
98 decoding.

99

100 **Fig. 1. Schematic workflow of the whole process of DNA Storage Designer.** First, various types of
101 files can be uploaded to the website, the website will convert the file into binary information. After that,
102 according to the encoding method and verify code selected by the user, each segment bit sequence will
103 be converted into a DNA sequence after connecting the address sequence. The platform then simulated

104 possible sequence error scenarios in the five necessary processes in DNA sequence storage experiments.
105 Finally, the simulated sequences will be decoded to the initial digital information of the file. Each
106 process has a corresponding analysis report.

107 **2.1 Encoding**

108 The process of encoding is exemplified in Figure 1. A digital file uploaded by users, with file types
109 ranging from images, PDF, text, video, audio, exe, and others, is transformed into a bit matrix, where
110 the length and encoding schema are also solicited from the users but the index length is calculated out
111 and fixed according to the size of the uploaded file.

112 Initially, users should choose the encoding method to use. Currently, DNA Storage Designer provides
113 8 popular encoding methods, which vary from each other. Vanilla code is the most basic one, it simply
114 transforms the data according to the naive rules: 00 → A, 01 → C, 10 → G, 11 → T. Church's code (7)
115 encodes two bits per base, and Erlich (8) approach the theoretical maximum information capacity per
116 nucleotide of DNA. Goldman et al. (12) utilize single DNA sequences to represent files with no
117 homopolymers. Ping et al. (11) encode two binary bits into one double-stranded DNA molecule. Zan's
118 code (6) is proposed to only store English text, which uses a robust code book for common symbols in
119 English. These methods hold different features, users could freely choose from based on their
120 requirements and experiment settings. For the script of the encoding method, we refer to Chmaeleo (15),
121 which is a robust library for DNA storage coding schemes. The details of encoding methods could be
122 found on the "Method" page on the web server.

123 Then, to fix errors in the process of reading and writing DNA sequences and improve data recovery
124 capabilities, verify code can be added optionally. Hamming code (16) and Reed-Solomon code (17) are
125 provided. Next, users could set the segment length through the selection dot bar. The selection of
126 fragment lengths is meticulously designed, taking into consideration a range of factors, such as file size,
127 verification codes, and encoding methods. However, in general, it needs to meet the limitations of the
128 current synthesis technology.

129 After selections are done, the file consisting of DNA segments is finally outputted and the corresponding
130 report with the basic information of the file, together with evaluated metrics including guanine-cytosine
131 (GC) content, repeated subsequences length, homopolymer length and the minimum free energy are
132 directly given. Among them, GC content is a crucial indicator of DNA strand stability. It must fall within
133 a certain interval to minimize the probability of secondary structure formation and to ensure uniform
134 sequence coverage in the sequencing (18). Similarly, homopolymer length affects the accuracy of
135 synthesis and sequencing (19) whereas minimum free energy could measure the quality of DNA
136 sequences (4). Thus, we believe that these evaluation metrics could show the quality of generated
137 sequences and provide design guidance for researchers.

138 **2.2 Error simulation**

139 The second step of the workflow is error simulation. The simulation service enables users to replicate
140 potential errors that may arise during wet experiments, guiding designing and adjusting experiments
141 better. It encompasses the five stages of DNA storage, synthesis, storage decay, PCR, sampling, and
142 sequencing. As shown in Table 1, we performed full-flow simulations for these five processes while
143 taking into account sequence errors in different experimental situations. As each stage involves high-
144 throughput data, both in-sequences and within-sequences errors can arise, when in-sequences errors
145 might cause information error, within-sequences errors refer to the distribution changes of sequences
146 that could lead to information loss. As proposed and validated by Yuan et al. (20), we utilize the
147 binomial distribution to model the sequence distribution change and within-sequence errors of each
148 stage.

149 **Table 1. Available methods in DNA Storage Designer**

| Process | Situation |
|-----------|---|
| Synthesis | ErrASE (column-synthesized oligos) |
| | Muts (column-synthesized oligos) |
| | Consensus shuffle (column-synthesized oligos) |
| | Oligo (microarray-based oligo pools) |
| | Hybridization (microarray-based oligo pools) |

| | |
|------------|---|
| | Nuclease (microarray-based oligo pools) |
| | NGS (microarray-based oligo pools) |
| | ErrASE (microarray-based oligo pools) |
| | D melanogaster(in-vivo) |
| | H sapiens(in-vivo) |
| | M musculus(in-vivo) |
| | S cerevisiae(in-vivo) |
| | E coli(in-vivo) |
| Decay | pH=8, temperature=293.5 (in-vitro) |
| | pH=8, temperature=253.5 (in-vitro) |
| | pH=8, temperature=193.5 (in-vitro) |
| | pH=7, temperature=293.5 (in-vitro) |
| | pH=7, temperature=253.5 (in-vitro) |
| | pH=7, temperature=193.5 (in-vitro) |
| PCR | <i>Taq</i> |
| | <i>Pfu</i> |
| | <i>Pwo</i> |
| | <i>Phusion</i> |
| Sampling | ramdom |
| Sequencing | illumina (single-end) |
| | illumina (paired-end) |
| | Nanopore (1D) |
| | Nanopore (2D) |
| | PacBio |

150 Source: Data based on Kosuri and Church (2014); An et al. (2014) and Schwarz et al. (2020)

151 During the synthesis process, some molecules might not be able to be synthesized successfully, some
152 might be synthesized many more times than others, which causes an imbalanced sequence number
153 distribution. What's more, varying from different methods, the error rates and spectra are different, the
154 available ones on our website are shown in Table 1, with published error information for different
155 combinations of synthesis methods and error correction methods. The storage simulation simulates
156 mutations depending on host methods and adjustable time intervals, in vitro depurination rates gained
157 using the equation described by (21) or the Kimura model of molecular evolution (22). We provide 5
158 common host organisms, both Eukaryotic and Prokaryotes are considered, and 6 in-vitro experiments
159 conditions that are commonly applied (Table 1). Also, users could simulate storage situations using the

160 binary erasure channel or the additive white Gaussian noise channel. The polymerase used and the
161 number of simulated PCR cycles determines the PCR error rates. We offer selections for the
162 polymerases Taq, Pfu, Pwo and Phusion that (23) have characterized. Before sequencing, a proportion
163 of DNA strands should be sampled from the main oligo pools, only random sequences could proceed.
164 Thus, the sample ratio is the key parameter of this stage, and no within-sequences error will be
165 introduced in this stage. In real experiments and applications, to read the data out, sequencing is a must.
166 For this web server, we provide 3 kinds of prevailing sequencing platforms, Illumina (24), Nanopore
167 and PacBio (25) with corresponding methods, in total, 6 kinds of choices. It is mentionable that
168 substitution is the main error that occurs in this stage, especially the pair-to-pair ones, TAC-TGC and
169 CG-CA, for example.

170 The report of error simulation stage consists of three parts, Steps review, Sequence distribution and
171 Error counts. Steps review utilizes pie charts to uncover the distribution of different error types of
172 corresponding chosen methods. During the whole process, the number of sequences, causes of errors
173 and proportions of different types of errors change from time to time. Therefore, in Sequence
174 distribution part, we count and compare the numbers of DNA strands with errors and the left 100%
175 correct DNA strands for each stage using a stacked column chart, as well as show the changes in the
176 strand numbers that contained different types of mutations using line charts. Because the effects of
177 occurring errors are cumulative, it is reasonable that as the simulation proceeds, both the percentage of
178 strands with errors and the average error number for all strands will increase. Thus, we count the number
179 of strands with a different number of errors for the different stages in Error Counts section, hoping to
180 help users to define which stage will cause most errors and might adjust their choices.

181 **2.3 Decoding**

182 The last stage is to decode the DNA sequences according to the reverse rules of the encoding ones.
183 However, simulated DNA strands usually have a lot of redundancy, many of which have mutated errors,
184 causing trouble with clustering and decoding. So, we embedded two clustering algorithms, CD-HIT
185 (26) and Starcode (27), to de-redundancy and correct the data. To be specific, take CD-HIT as an

186 example, it outputs a clustered file, documenting the sequence 'groups' for each non-redundant
187 sequence representative. Similarly, Starcode clustering is based on all pairs search within a specified
188 Levenshtein distance (allowing insertions and deletions), followed by a clustering algorithm: Message
189 Passing, Spheres or Connected Components.
190 Then, the clustered sequences will be decoded to obtain file binary bits (or character) information.
191 Subsequently, the verification code and index code will be removed. Finally, we analyze the recovery
192 information of bit fragments in the report. Similar to the report of the encoding stage, this report would
193 also contain the basic information of users' choices, clustering time and decoding time, as well as final
194 results about sequence numbers and recall rate.

195 **3. Usage and experiment**

196 **3.1 Demo Usage**

197 To effectively showcase the capabilities of the DNA Storage Designer, we conducted a case study based
198 on the Example file - a 140KB jpg image for Monet Claude's Impression-Sunrise (Figure 2A, B). Upon
199 uploading the file, we fix the segment length at 122 nt and set the hamming code as the verify code
200 (Figure 2D). Encoded by Ping et al. (Figure 2C), the report first gives out basic file information and
201 then evaluates several encoding results using a table with three diagrams.

202 **Fig. 2. The demo run with example image file.** (A) the demo file: impression-sunrise.jpg; (B) The
203 website provides a function for users to upload files; (C) User-defined encoding method selection; (D)
204 semi-screenshot for segment length and verify code choose which means the length of bits (01), the bits
205 sequences will be encoded as a DNA sequence; (E) An example of a bit sequence encoded as a DNA
206 sequence and the run button.

207 As shown in Table 2, the information is about how many nucleotides and sequences are generated,
208 together with information density and so on, which first and foremost enables researchers to understand

209 the overall situation of the encoded DNA strands. Next, we analyzed the encoded DNA sequence, as
210 shown in Figure 3, sequence stability demonstrated by sequence minimum free energy and two diagrams
211 display the situation of GC content, repeated sequences of randomly sampled 1000 sequences
212 respectively. Besides, users can download the coding sequence from the report page in which the name
213 corresponding to each sequence is the bit sequence it encodes.

214 **Table 2. Sample encode information report for *impression-sunrise.jpg*.**

| Name | Information |
|------------------------------|----------------------|
| Encode method | Ping, Zhi, et al. |
| Segment length | 122 bits |
| Index length | 14 bits |
| Verify method | Hamming code |
| Verify code length | 8 bits |
| Encode segment length | 144 bits |
| Segment number | 8032 |
| Encoding time | 6.53 s |
| Single DNA length | 144nt |
| DNA sequence number | 8031 |
| Nucleotide counts | 1156464 nt |
| Information density | 0.847 bits/nt |
| Physical information density | 9.56E+22 petabyte/ug |

215 **Fig. 3. Analysis results of encoding DNA sequences for demo file** (A) The distribution of sequences
216 minimum free energy which reflects the stability of the coding sequence; (B) Statistics on the GC
217 content of coding DNA sequences; (C) Statistics on the repeated sequences of coding DNA sequences,
218 each column represents the number of corresponding repeated nucleotides contained in the 1000 DNA
219 sequence.; (D) Users can get all encoded DNA sequences by clicking the download button.

220 Then, we move on to the error simulation part. Under this tab, users can adjust their experimental
221 settings on the computer, conduct simulation experiments, modify experimental parameters based on
222 the calculated sequence analysis results, and then conduct offline experiments. To simplify, we directly
223 press the “Default” button, which could automatically run the simulation process based on default
224 settings for users, to conduct the demonstration. Users could also definitely adjust each parameter one

225 by one carefully for actual usage (Supplementary Figure 1). Upon completing all selections, users can
226 access the report page, which comprises two main sections. The simulation result provides an overview
227 of the simulated sequence situations. During the simulation, variations in sequence distribution, density,
228 and error occurrence are observed across different stages. To better comprehend these changes, we
229 present “Sequence distribution” and “Error count” diagrams in Figure 4A-B, which demonstrate the
230 change tendency of the sequences at each stage of the simulation.

231 **Fig. 4. Statistical results of simulated errors in coding DNA sequences for demo file** (A) The
232 number of correct sequences and incorrect sequences. And the number of different error types
233 (insert/delete/substitute) in the error sequence; (B) Statistics of sequence errors for five separate
234 experimental procedures. Users can understand the proportion of DNA strands with n errors in all
235 strands and the changes in the proportion over time.

236 Finally, we use Starcode method to decode our simulated sequences above and analyze the recovery
237 information of bit fragments in the report (Table 3). It is mentionable that the recall rate refers to the
238 ratio of correct sequence recall ratio and the recall segment bits number stands for the ratio to the
239 encoded counterpart respectively. These two proxies highly depend on the parameters of the simulation
240 part. Here, because the default sampling ratio is 0.005%, and the file size is relatively small, the results
241 could not cover all the information in the file and the recall rates are low.

242 **Table 3. Decode information table for *Impression-Sunrise.jpg*.**

| Name | Information |
|--------------------------------|-------------|
| Decode time | 1.04 s |
| Clustering method | starcode |
| Clustering time | 0.29 s |
| Encode DNA sequence number | 8031 |
| Simulation DNA sequence number | 1197 |
| Clustering DNA sequence number | 1028 |
| Recall DNA sequence number | 492 |
| Recall rate | 6.13 % |
| Encode segment bits number | 8032 |
| Decode segment bits number | 1974 |

| | |
|----------------------------|---------|
| Recall segment bits number | 1693 |
| Recall bits rate | 21.08 % |

243 **3.2 Experiments and Illustration**

244 To illustrate how our website displays variations when users select different options and how these
245 selections impact the final results, we counted several experiments. We tested 3 files ('impression-
246 sunrise.jpg', 'So far away.mp3' and 'Winmine.exe') with 7 encoding methods, except Zan's code,
247 which is primarily intended for English texts.

248 The experiment results, as depicted in Figure 5, were plotted with randomly selected 1000 encoded
249 DNA sequences from each of the three files. Compared to Vanilla code, all encoding methods exhibit
250 improved GC content, with medians and ranges almost falling within acceptable intervals. Except for
251 the Ping et al. (2022) code, which limits the range of GC content to 40% to 60%, the range of GC content
252 in sequences encoded by other methods varies from file to file (Figure 5A). The minimum free energy
253 of a DNA strand is the minimum of the Gibbs standard free energy of all feasible secondary structures.
254 Strands with low MFE are more susceptible to secondary structures and, consequently, are more stable.
255 While it has been reported that DNA sequences with stable secondary structures may pose challenges
256 to sequencing or amplification during random access or backup of stored information (28-30) holds the
257 view that a more stable strand may result in greater storage durability under appropriate conditions.
258 Therefore, it is essential to strike a balance between stability and other factors. For the three files, Ping
259 et al. (11), Church et al. (7), and Goldman et al. (12) encoding methods lead to lower MFE, while the
260 others exhibit higher MFE (Figure 5B). Our website also provides users with an option to view the
261 length of repeated sequences in encoded results. Except for Vanilla coding, all other proposed methods
262 impose certain constraints. Among them, Goldman's method does not allow repeated sequences, and
263 the other three methods (George's, Grass's and Blawat's) do not appear length repeat sequence greater
264 than 3 (Figure 5C-E). However, Ping's and Erlich's methods will produce repeat sequences of 4 bases.

265 **Fig. 5. The encoding result of three files ('impression-sunrise.jpg', 'So far away.mp3',**
266 **Winmine.exe'). (A) the range of GC content of the encoded DNA sequences from the three files; (B)the**
267 **distribution of minimum free energy (kcal/mol) of the encoded DNA sequences from the three files; (C)**
268 **the repeat sequence number in 'impression-sunrise.jpg' encoded DNA sequences. Each coloured bar**
269 **indicates how many repeat sequences of the corresponding length in 1000 randomly selected DNA**
270 **sequences; (D) the repeat sequence number in "So far away.mp3" encoded DNA sequences; (E) the**
271 **repeat sequence number in "Winmine.exe" encoded DNA sequences;**

272 The selection of different methods, platforms, and parameters affects the result of error simulation and
273 decoding obviously. For example, to deal with the problem we mentioned in the last part of Section 3.1,
274 we could simply increase the sampling ratio to 100 %, and the recall rate and recall bits rate become
275 34.93 % and 72.22 % directly. Further, when we increase the sequencing depth from 1 to 5, the rates
276 become 68.8% and 91.81% respectively. Also, users are encouraged to try different combinations of
277 platforms, technologies as well as parameter settings to find the most suitable solution for their own
278 files and application.

279 **4. Conclusion**

280 We proposed DNA Storage Designer, a practical and user-friendly web server that requires no
281 programming knowledge. It is the first all-in-one platform to integrate the three basic processes,
282 encoding, error simulation and decoding for DNA storage system. We embed 8 popular encoding
283 methods that transfer digital information into DNA sequences, users could freely choose and directly
284 transfer files into DNA sequences. The chosen encoding methods are mainly from high-impact journals
285 such as Science, Nature, Nature Computational Science, Briefings in Bioinformatics etc., corresponding
286 decoding processes are also included. What's more, to simulate the real experiments of DNA storage,
287 we also utilize the widely employed wet experiments settings in related technologies together with their
288 error rates and spectra, to provide in-silicon evaluated results, reports and guides for experimental
289 design. Also, the five stages, synthesis, storage, PCR, sampling and sequencing are optional. Therefore,

290 users could decide which stages to simulate on their own. For data recovery, we utilize 2 mainstream
291 verify codes and cluster tools to de-redundancy the sequences and help to conduct the decoding process.

292 In general, our contribution could be summarized as:

293 • It is the first practical web server to simulate the whole process of DNA storage application, from
294 encoding, and error simulation to decoding. Users could use this website to go through the whole process
295 as well as design and modify their experiment based on the feedback.

296 • It incorporates 8 encoding methods together with 2 mainstream verify codes, which is currently the
297 most inclusive one. It also has 2 cluster tools for de-redundancy purposes during decoding processes.

298 • It also holds a high level of usability that detailed instructions and explanations are given on the
299 website. What's more, each step has examples button and default settings, so users could start and use
300 it easily.

301 In short, users can use our website quickly and well and are given the high degree of freedom they could
302 upload files to go through the whole process but also can only simulate their own FASTA file or decode
303 the files. Although DNA storage systems are not competitive for commercial use due to the limitation
304 of current synthesis technology, it is expected that the costs for synthesis will drop significantly soon.
305 Nevertheless, DNA storage systems allow easy and low-cost copying of media, in contrast to
306 conventional storage systems (31). We believe that our website could provide great help for researchers
307 and DNA storage will be implemented into daily life in the feature.

308 **Supporting information:**

309 **S1 Fig. Semi-screenshot of parameters for user-defined simulation error.** (A) Semi-screenshot of
310 “choose the simulation steps”. All processes, except for synthesis, are optional and can be freely
311 combined to customize the experiments to specific requirements. (B) semi-screenshot of choose
312 ‘Synthesis’ parameters; (C) semi-screenshot of choose ‘Decay’ parameters; (D) semi-screenshot of
313 choose ‘Sampling’ parameters; (E) semi-screenshot of choose ‘sequencing’ parameters;

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321 **References**

- 322 1. Schwarz M, Welzel M, Kabdullayeva T, Becker A, Freisleben B, Heider D. MESA: automated assessment of
323 synthetic DNA fragments and simulation of DNA synthesis, storage, sequencing and PCR errors. *Bioinformatics*.
324 2020;36(11):3322-6.
- 325 2. Song LF, Geng F, Gong ZY, Chen X, Tang JJ, Gong CY, et al. Robust data storage in DNA by de Bruijn graph-
326 based de novo strand assembly. *Nat Commun*. 2022;13(1).
- 327 3. Dong YM, Sun FJ, Ping Z, Ouyang Q, Qian L. DNA storage: research landscape and future prospects. *Natl Sci
328 Rev*. 2020;7(6):1092-107.
- 329 4. Hofacker IL. Vienna RNA secondary structure server. *Nucleic Acids Research*. 2003;31(13):3429-31.
- 330 5. Ping Z, Ma D, Huang X, Chen S, Liu L, Guo F, et al. Carbon-based archiving: current progress and future
331 prospects of DNA-based data storage. *Gigascience*. 2019;8(6).
- 332 6. Zan X, Yao X, Xu P, Chen Z, Xie L, Li S, et al. A Hierarchical Error Correction Strategy for Text DNA Storage.
333 *Interdiscip Sci*. 2022;14(1):141-50.
- 334 7. Church GM, Gao Y, Kosuri S. Next-Generation Digital Information Storage in DNA. *Science*.
335 2012;337(6102):1628-.
- 336 8. Erlich Y, Zielinski D. DNA Fountain enables a robust and efficient storage architecture. *Science*.
337 2017;355(6328):950-4.
- 338 9. Bornhol J, Lopez R, Carmean DM, Ceze L, Seelig G, Strauss K. A DNA-Based Archival Storage System. *AcM
339 Sigplan Notices*. 2016;51(4):637-49.
- 340 10. Organick L, Ang SD, Chen YJ, Lopez R, Yekhanin S, Makarychev K, et al. Random access in large-scale DNA
341 data storage. *Nat Biotechnol*. 2018;36(3):242-8.
- 342 11. Ping Z, Chen S, Zhou G, Huang X, Zhu SJ, Zhang H, et al. Towards practical and robust DNA-based data
343 archiving using the yin-yang codec system. *Nature Computational Science*. 2022;2(4):234-42.
- 344 12. Goldman N, Bertone P, Chen S, Dessimoz C, LeProust EM, Sipos B, et al. Towards practical, high-capacity,
345 low-maintenance information storage in synthesized DNA. *Nature*. 2013;494(7435):77-80.
- 346 13. Grass RN, Heckel R, Puddu M, Paunescu D, Stark WJ. Robust chemical preservation of digital information on
347 DNA in silica with error-correcting codes. *Angew Chem Int Ed Engl*. 2015;54(8):2552-5.
- 348 14. Blawat M, Gaedke K, Hutter I, Chen XM, Turczyk B, Inverso S, et al. Forward Error Correction for DNA Data
349 Storage. *Procedia Comput Sci*. 2016;80:1011-22.
- 350 15. Ping Z, Zhang H, Chen S, Zhuang Q, Zhu SJ, Shen Y. Chamaeleo: a robust library for DNA storage coding
351 schemes. *bioRxiv*. 2020:2020.01. 02.892588.
- 352 16. Hamming RW. Error detecting and error correcting codes. *The Bell system technical journal*. 1950;29(2):147-
353 60.
- 354 17. Reed IS, Solomon G. Polynomial codes over certain finite fields. *Journal of the society for industrial and
355 applied mathematics*. 1960;8(2):300-4.

356 18. Jensen MA, Fukushima M, Davis RW. DMSO and Betaine Greatly Improve Amplification of GC-Rich
357 Constructs in De Novo Synthesis. *Plos One*. 2010;5(6).

358 19. Xu C, Zhao C, Ma B, Liu H. Uncertainties in synthetic DNA-based data storage. *Nucleic Acids Res*.
359 2021;49(10):5451-69.

360 20. Yuan L, Xie Z, Wang Y, Wang X. DeSP: a systematic DNA storage error simulation pipeline. *BMC
361 Bioinformatics*. 2022;23(1):185.

362 21. An R, Jia Y, Wan BH, Zhang YF, Dong P, Li J, et al. Non-Enzymatic Depurination of Nucleic Acids: Factors
363 and Mechanisms. *Plos One*. 2014;9(12).

364 22. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies
365 of nucleotide sequences. *J Mol Evol*. 1980;16(2):111-20.

366 23. McInerney P, Adams P, Hadi MZ. Error Rate Comparison during Polymerase Chain Reaction by DNA
367 Polymerase. *Mol Biol Int*. 2014;2014:287430.

368 24. Schirmer M, D'Amore R, Ijaz UZ, Hall N, Quince C. Illumina error profiles: resolving fine-scale variation in
369 metagenomic sequencing data. *Bmc Bioinformatics*. 2016;17.

370 25. Weirather JL, de Cesare M, Wang Y, Piazza P, Sebastian V, Wang XJ, et al. Comprehensive comparison of
371 Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. *F1000Res*.
372 2017;6:100.

373 26. Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide
374 sequences. *Bioinformatics*. 2006;22(13):1658-9.

375 27. Zorita E, Cusco P, Filion GJ. Starcode: sequence clustering based on all-pairs search. *Bioinformatics*.
376 2015;31(12):1913-9.

377 28. Behjati S, Tarpey PS. What is next generation sequencing? *Archives of Disease in Childhood-Education and
378 Practice*. 2013;98(6):236-8.

379 29. Kieleszawa J. Fundamentals of sequencing of difficult templates--an overview. *J Biomol Tech*.
380 2006;17(3):207-17.

381 30. Cao B, Zhang XK, Wu JQ, Wang B, Zhang Q, Wei XP. Minimum Free Energy Coding for DNA Storage. *Ieee
382 T Nanobiosci*. 2021;20(2):212-22.

383 31. Lochel HF, Welzel M, Hattab G, Hauschild AC, Heider D. Fractal construction of constrained code words for
384 DNA storage systems. *Nucleic Acids Res*. 2022;50(5):e30.

385

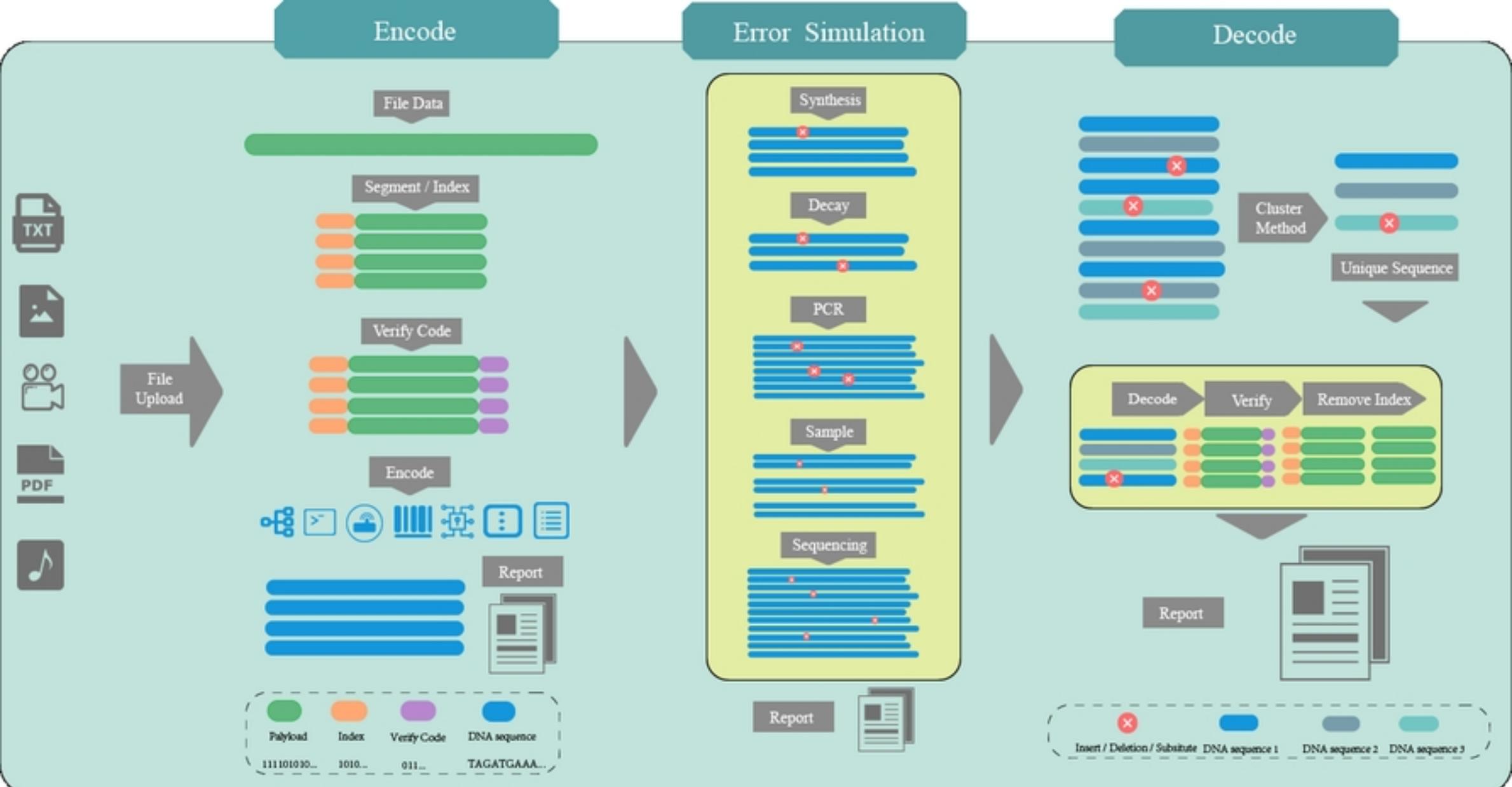
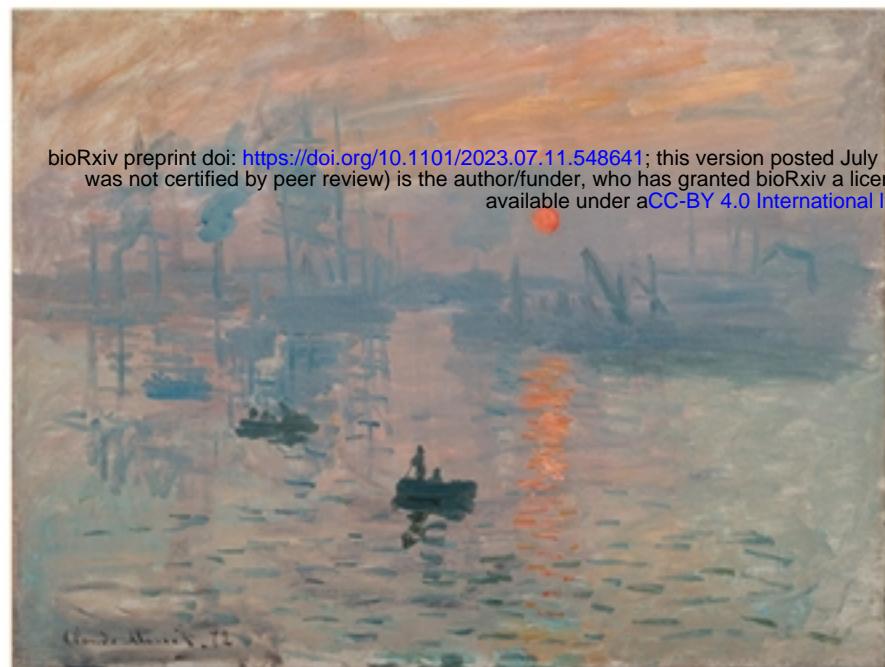


Figure1

A



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B

Upload the storage file

Tips: (a) the first and second methods require a file size large than 100KB. (b) the eighth method encodes English in text and does not support other languages. (c) the maximum file size is 6 MB. Compressed files such as doc or pdf will take longer to encode.



Let's start!

Support file types: jpg, png, txt, mp4, mp3, exe...



impression-sunrise.jpg

D

Verify method

- None
- Hamming code
- Reed Solomon code

method details please click the [method paper](#)

Segment length



Index length : 14 bits



C

Encode method

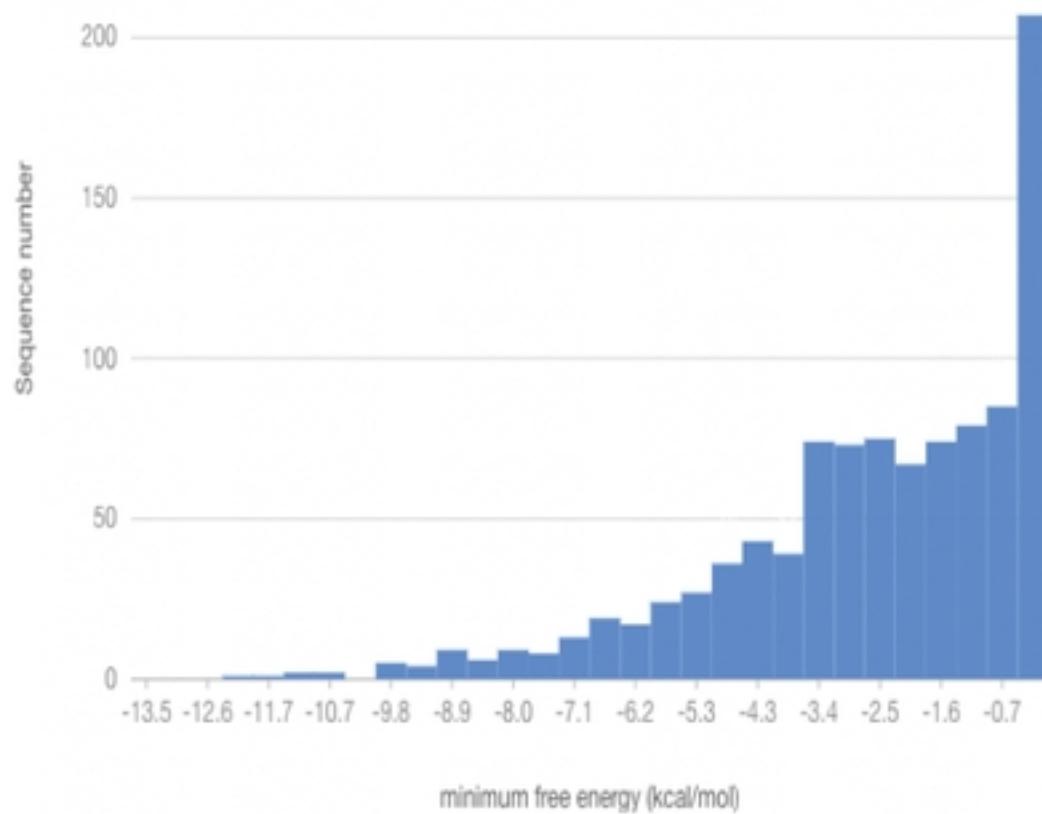
- Vanilla code
- Ping, Zhi, et al. "Towards practical and robust DNA-based data archiving using the yin-yang codec system." *Nature Computational Science* 2.4 (2022): 234-242.
- Erlich, Yaniv, and Dina Zielinski. "DNA Fountain enables a robust and efficient storage architecture." *Science* 355.6328 (2017): 950-954.
- George M. Church, et al. "Next-generation digital information storage in DNA." *Science* 337.6102 (2012): 1628-1628.
- Goldman, Nick, et al. "Towards practical, high-capacity, low-maintenance information storage in synthesized DNA." *Nature* 494.7435 (2013): 77-80.
- Grass, Robert N., et al. "Robust chemical preservation of digital information on DNA in silica with error-correcting codes." *Angewandte Chemie International Edition* 54.8 (2015): 2552-2555.
- Blawat, Meinolf, et al. "Forward error correction for DNA data storage." *Procedia Computer Science* 80 (2016): 1011-1022.
- Zan, Xiangzhen, et al. "A hierarchical error correction strategy for text DNA storage." *Interdisciplinary Sciences: Computational Life Sciences* 14.1 (2022): 141-150.

Figure2

Sequence minimum Free Energy

A

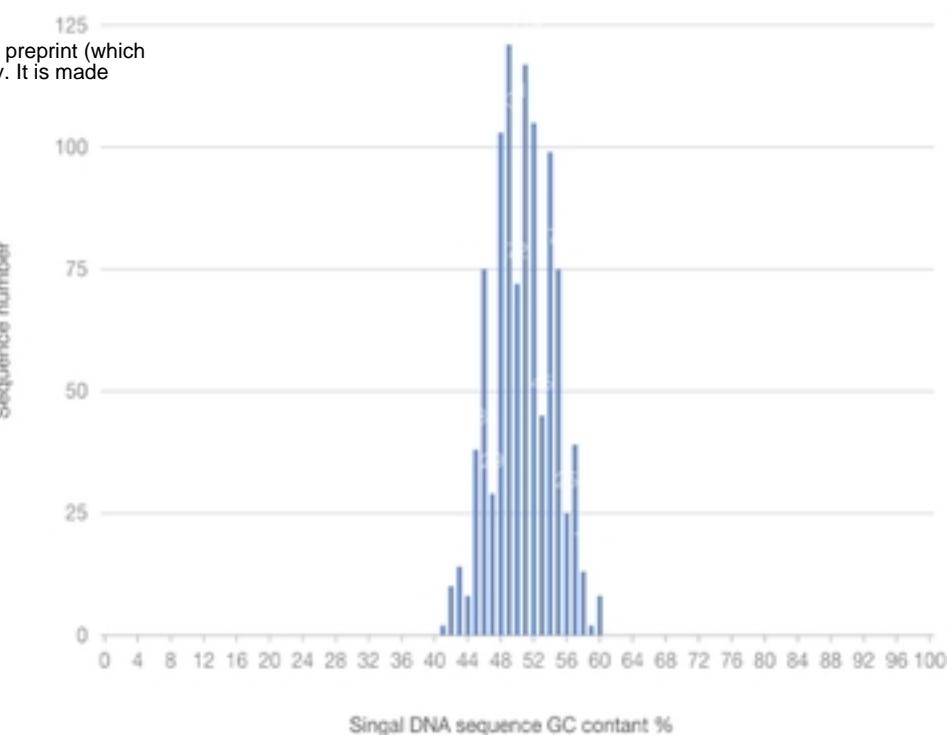
bioRxiv preprint doi: <https://doi.org/10.1101/2023.07.11.548641>; this version posted July 12, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



The sequence average minimum free energy is : -2.62 kcal/mol

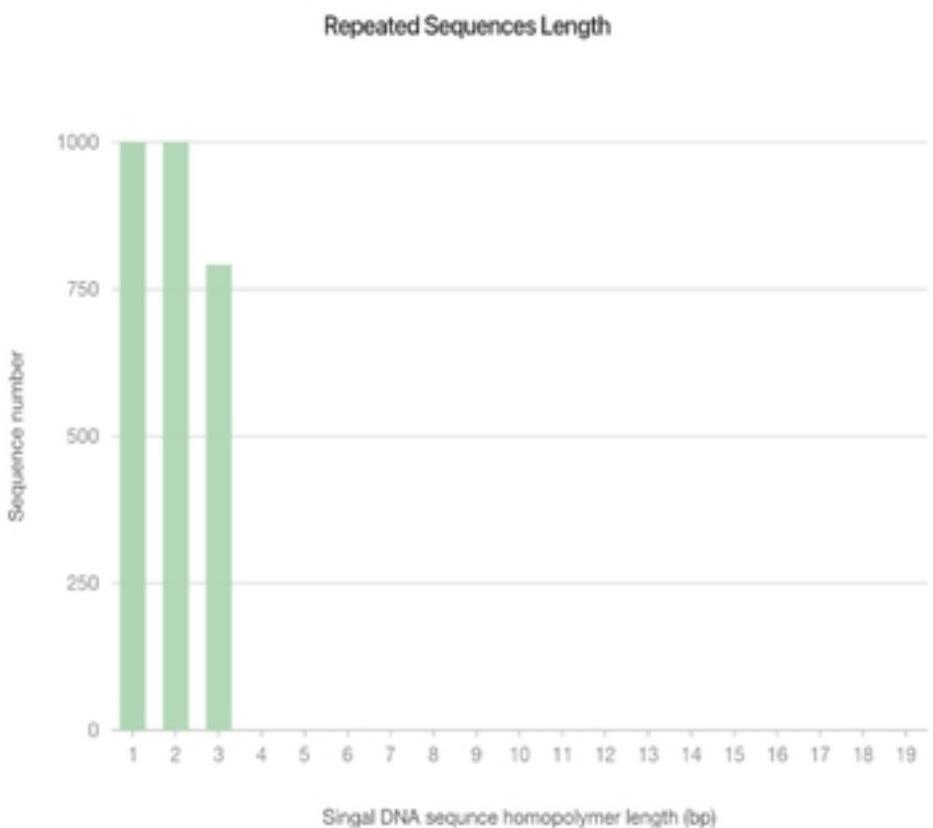
The minimum free energy (MFE) of a DNA sequence is the minimum of the Gibbs standard free energy of all possible secondary structures. Therefore, the quality of DNA sequences can be measured by calculating the MFE of each sequence. Here we calculated the minimum free energies of randomly selected 1000 encoded DNA sequences by RNAfold.

B



Because the ratio of GC-content is crucial to the stability of DNA sequence. We counted the GC content of each encoded DNA sequence. The x-axis is the percentage of GC content, and the y-axis is the number of corresponding sequences.

C



The presence of repetitive sequences affects the accuracy of synthesis and sequence sequencing during DNA storage. So, we counted the number of repeats in the encoded DNA sequence. The x-axis is the length of the repeated sequence, and the y-axis is the corresponding number.

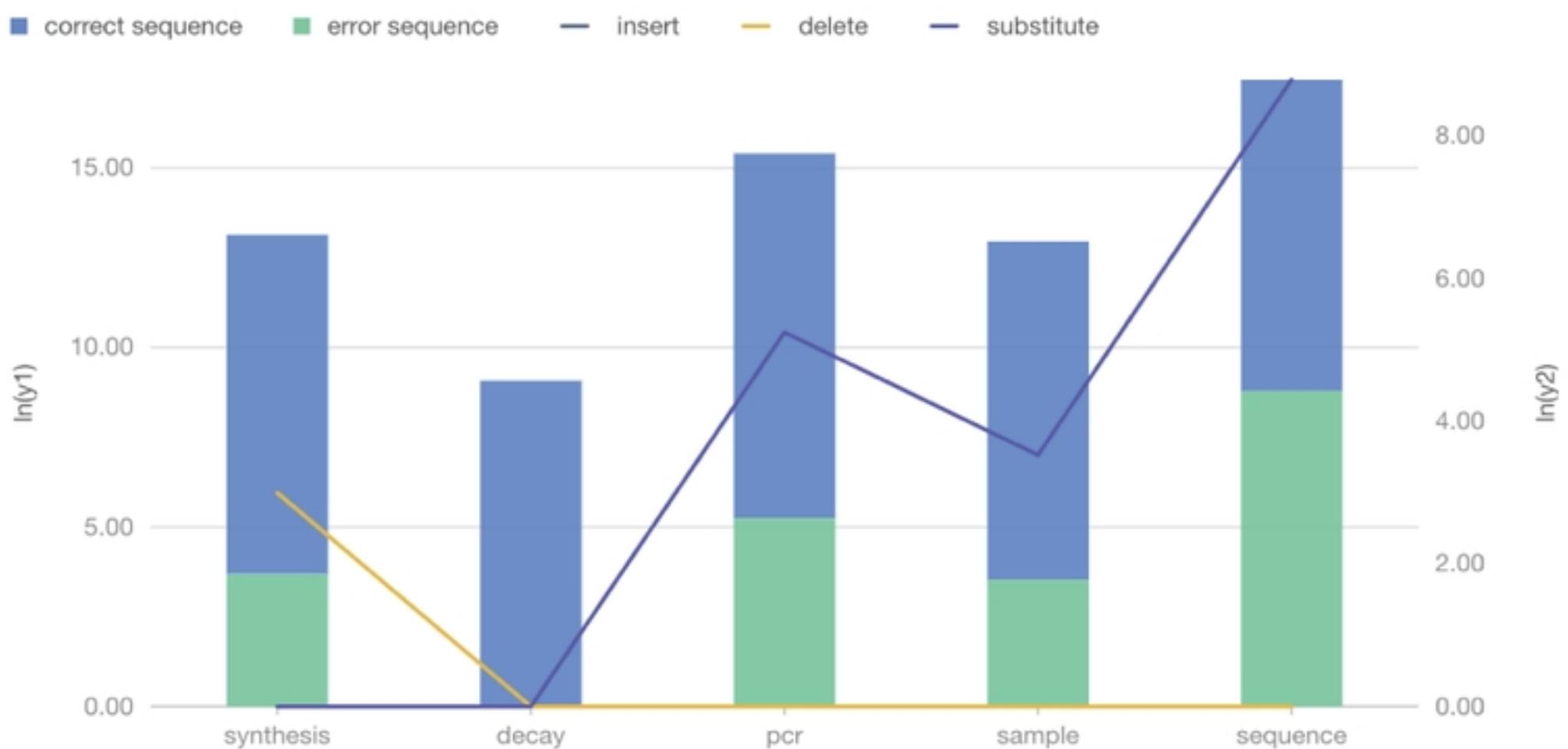
Download

Next

Figure3

A

Sequences distribution



After simulation, the number of strands in oligo pool is : 12387

B

Error counts

Error number in a strand:

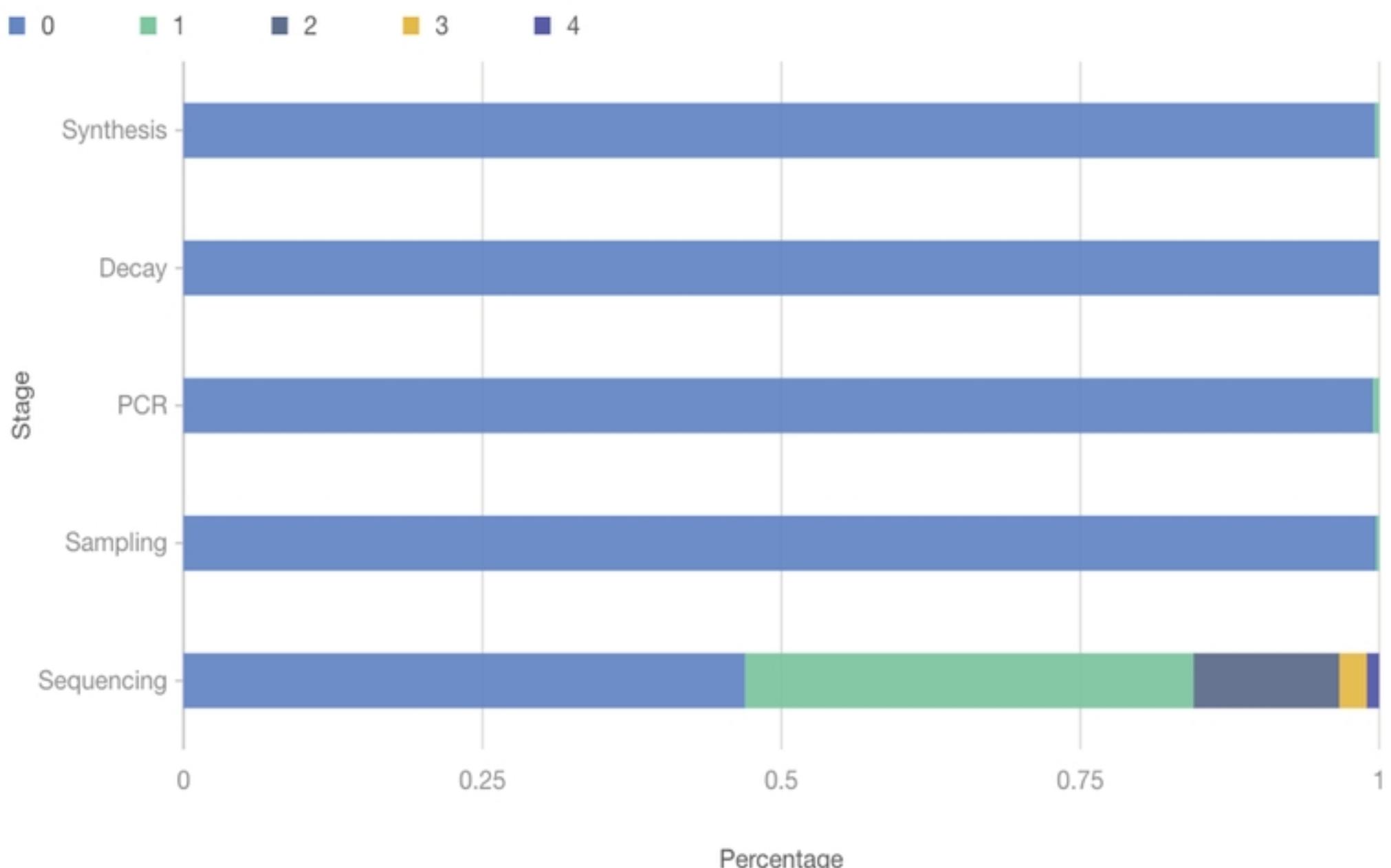


Figure4

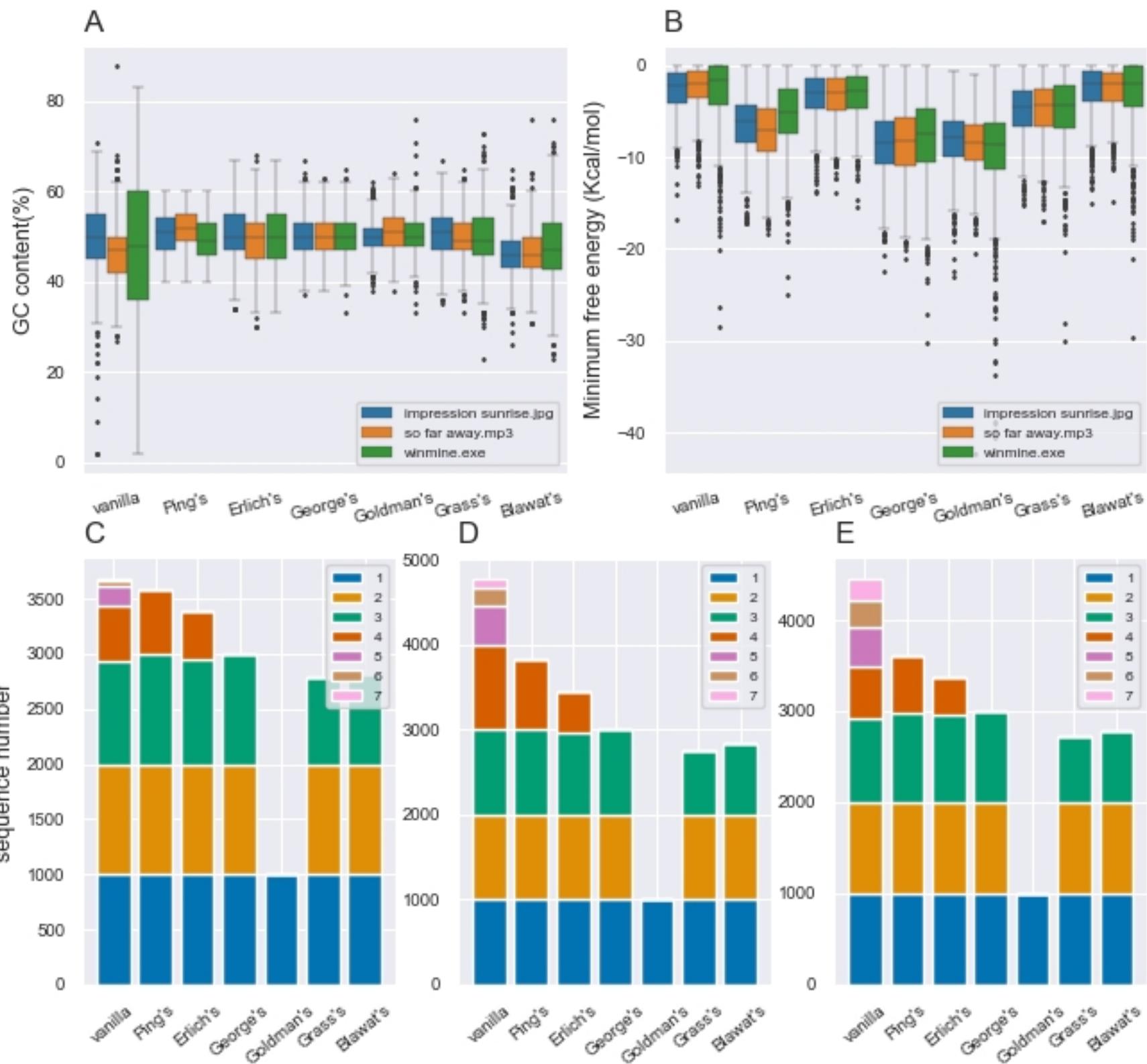


Figure5