1 Robust data storage in DNA by de Bruijn graph-based 2 decoding

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11

12 Abstract

13 DNA data storage is a rapidly developing technology with great potential due to
14 its high density, long-term stability, and low maintenance cost. The major
15 technical challenges include various errors such as the indels, rearrangements,
16 and strand breaks that frequently arise during DNA synthesis, amplification,
17 sequencing, and preservation. Here, we report the development of a de Bruijn
18 graph-based, greedy path search algorithm (DBG-GPS) aiming to handle such
19 issues. We demonstrate that accurate data recovery could be achieved from

20 deep error-prone PCR products. The robustness of this method was further
 21 verified with one hundred parallel data retrievals from PCR products containing
 22 numerous rearrangements introduced by intended unspecific amplifications.
 23 With DBG-GPS, we successfully decoded the original information even after
 24 treating the DNA at 70°C for 65 days. DBG-GPS shows linear decoding
 25 complexity and is faster than the multiple alignment-based methods by two
 26 orders of magnitude, suited for large-scale data storage.

27 **Keywords**

28 Synthetic Biology, DNA data retrieval, DNA codec system, de Bruijn graph,
 29 Fountain codes

30 **Introduction**

31 DNA is the natural solution for the preservation of genetic information of all life
 32 forms on earth. Recently, million years old genomic DNA of mammoths was
 33 successfully decoded revealing its high stability as an information carrier under
 34 frozen conditions¹. Owing to its stability, high density, and low maintenance
 35 cost revealed by recent studies, DNA has been considered an ideal storage
 36 medium to meet the challenge of data explosion in the future²⁻⁹. Frequently
 37 occurring errors in DNA synthesis, amplification, sequencing, and preservation,
 38 however, challenge the reliability of data storage in DNA. Efforts to solve this
 39 problem led to the implementation of a codec system which requires two layers
 40 of error correction (EC) codes, the outer layer codes handling the strand
 41 dropouts and the inner layer codes dealing with intramolecular errors, ensuring

42 accurate information readouts¹⁰⁻¹³. Strand dropouts are erasures and
 43 therefore they can be well solved by erasure codes, e.g. Fountain,
 44 Reed-Solomon (RS) codes¹⁰⁻¹². By contrast, the performance of existing inner
 45 layer codec systems, e.g. multiple alignment-based methods, is less
 46 satisfactory as they are not efficient in correcting indel errors, and in particular,
 47 not capable of dealing with rearrangements and strand breaks. Highly robust
 48 inner codec systems remain to be explored.

49 Base-shift arises from indel is difficult to handle with traditional EC codes,
 50 which detect indels but cannot position them. A two-dimensional coding
 51 strategy with traditional EC codes can transform the indel errors into
 52 substitutions. In this strategy, a single indel in one dimension will lead to a
 53 series of ‘substitutions’ in another dimension (**Fig. S1**). Thus, this strategy only
 54 works with low indel rates. Increasing indels will lead to an overwhelming
 55 number of “substitutions” exhausting the column EC codes^{8,10}. Recently
 56 developed hash encoded, decoded by greedy exhaustive search (HEDGES)
 57 codes can be used to correct higher frequency of indels¹⁴. Based on the
 58 simulation, HEDGES codes require 40% redundancy codes for correction of 3%
 59 indel errors, which would significantly increase the cost of data writing. Strand
 60 rearrangements caused by unspecific amplifications are frequently
 61 encountered and could be a potential threat to data integrity especially when
 62 massive multiple retrievals from a large oligo pool are required. Currently,
 63 people optimize PCR conditions to avoid rearrangements to occur and use gel
 64 purification to physically exclude rearranged strands from the data pool. None
 65 of the existing methods can be used to correct rearrangements. DNAs are
 66 subjected to hydrolysis and degradation^{12,13} under certain conditions and in

67 long-term storage, highlighting the importance of data stability studies on
68 influences of harsh conditions, such as high temperature or UV exposure, and
69 methods of DNA protection^{8,14}. New coding methods that can tolerate
70 fragmented DNA strands are nevertheless critical for enhancing the
71 robustness of DNA data storage.

72 As the synthesis of long DNA is currently impractical, data storage in DNA
73 relies on a large number of fragments. Consequently, data decoding involves
74 the assembly of information pieces carried by individual DNA fragments, a
75 process similar to genome assembly from shotgun sequencing. De Bruijn
76 graph theory uses k -mer components to represent sequences. The analogy of
77 genome assembly to data decoding and the success of the application of De
78 Bruijn graph theory to genome assembly^{20,21} prompted us to investigate its
79 potentials in DNA data storage.

80 In this study, we report a strand reconstruction algorithm, de Bruijn
81 Graph-based Greedy Path Searching (DBG-GPS) that allows robust DNA data
82 storage. This algorithm handles indels, rearrangements, and strand breaks by
83 strand reconstruction from multiple, error-rich strand copies, resulting in
84 accurate and rapid data retrievals. Using this method, an up to 10% high indel
85 rate, intended rearrangements and forced DNA strand breaks were shown to
86 be efficiently corrected. DBG-GPS is faster than the clustering and multiple
87 alignment (CL-MA) based methods in decoding by two orders of magnitudes.
88 The linear decoding complexity revealed by simulation suggests that
89 DBG-GPS could be adapted to large-scale data storage.

90 Results

91 The great potentials of de Bruijn graph-based decoding

92 To inspect the potentials of de Bruijn graph theory in DNA data storage, we first
 93 analyzed effects of representative substitutions, indels, and strand breaks in
 94 de Bruijn graph as shown in **Fig. 1a**. The simple de Bruijn graph on the right
 95 was constructed from the four sequence copies of “AACGGCGGCTGT” on the
 96 left with a *k*-mer size of four (**Fig. 1a-i**). The sequence of “AACGGCGGCTGT”
 97 is the path of “ $a \rightarrow b \rightarrow c \rightarrow d \rightarrow e \rightarrow c \rightarrow f \rightarrow g \rightarrow h$ ” in the constructed graph. A
 98 substitution of “G” to “A” in sequence #2 will lead to a branch path, as shown in
 99 red color, and decreased coverages of the correct nodes (**Fig. 1a-ii**). With
 100 traditional EC codes, indels are much more difficult to handle than
 101 substitutions due to the base shift issue. The representative base indels in de
 102 Bruijn graph, however, are highly similar to substitutions (**Fig. 1a-iii**). Strand
 103 breaks also cause serious problems for traditional EC codes. With de Bruijn
 104 graph, the strand breaks only reduce the coverages of the correct nodes,
 105 without changes the graph structure. A greedy path search process, in
 106 combination with a path recognition mechanism, could decode the correct
 107 strand. In this way, the errors are transformed into a path search and selection
 108 problem by de Bruijn graph theory. With this mechanism, as long as the correct
 109 path, e.g. “ $a \rightarrow b \rightarrow c \rightarrow d \rightarrow e \rightarrow c \rightarrow f \rightarrow g \rightarrow h$ ” in **Fig. 1a-i**, is preserved, the
 110 corresponding DNA strand can be decoded theoretically. Thus, the robustness
 111 of the correct path determines the potentials of the graph theory-based
 112 decoding.

Clearly, the sequence error rates and copy number together determine the robustness of a strand path in de Bruijn graph. To further investigate how the sequence copies and error rates affect path robustness, we simulated DNA sequences with different error rates. Variant numbers of the simulated error-containing sequences were utilized for de Bruijn graph construction and path integrality analysis (**Fig. 1b, c**). The integrality of the correct path was tested by checking the existence of all k -mers in this path in the constructed graph. The rate of the sequences with a complete path in the graph was defined as ‘maximal strand decoding rate’ (S_m), which stands for the maximal strand decoding rate achievable by an actual de Bruijn graph-based decoder. Sequences with different types of errors were separately simulated. Twenty copies of sequences in a length of 320 bp containing different types and rates of errors were estimated for S_m values. As shown in **Fig. 1b**, the S_m values as expected are inversely related to the error rates but are similar regardless of the error types at the same error rates. Importantly, with merely twenty sequence copies, the sequence path in de Bruijn graph shows high robustness with an error rate of 5%, a common error level in reported DNA data storage studies^{11,12,15}. In practice, emerged errors are mixtures of different types. Therefore, we decided to investigate the sequence path robustness by simulations with sequences containing mixed errors. The simulations were performed with variable total error rates from 1% to 10%, among which the errors are consisted of substitutions (50%), insertions, and deletions (25% each), and variable strand copies from 5 to 100. As shown in **Fig. 1c**, even with a high error rate of 10%, $S_m > 0.99$ can still be obtained with sequence copies of 60 only (red dashed line), revealing the great potentials of de Bruijn graph-based decoding.

139   **Development of DBG-GPS for efficient strand reconstruction**

140   The decoding process requires three basic steps, de Bruijn Graph construction,
 141   greedy path search, and path recognition. The de Bruijn graph size is
 142   dependent on the total number of correct k -mers plus noise k -mers. The
 143   number of correct k -mers is fixed for the storage of any given volume of
 144   information. The number of noise k -mers becomes a determinant with a larger
 145   number increasing de Bruijn graph size and possible paths, leading to longer
 146   path search time. Hence, we propose to include an additional step to exclude
 147   noise k -mers to reduce search time, illustrated in **Fig. 2a**.

148   **Elimination of noise k -mers decreases the greedy path search** 149   **complexity**

150   With simulations, about thirty times enlargement of the de Bruijn graph size
 151   was observed when 4% random errors were introduced into 10,000 DNA
 152   strands with 100 sequence copies each (**Fig. S2a**). To quantify the noise
 153   k -mers in a de Bruijn graph, we introduce an indicator K_n , which is the ratio of
 154   noise k -mers and correct k -mers in a de Bruijn graph. Clearly, more strand
 155   copies and errors will introduce more noise k -mers to the de Bruijn graph,
 156   resulting in a larger K_n value. Monte Carlo simulations show a quick increase
 157   of K_n by increasing either the error rate or the sequence copies (**Fig. S2a**).
 158   The number of branch paths and decoding time increase exponentially with
 159   error rates or strand copy numbers (**Fig. S2b**).

160   DNA data operations introduce a large number of errors. The occurrence of
 161   each error is however a low probability event. Therefore, the coverage of noise

162 *k*-mers is generally much lower than that of the correct ones. Elimination of the
 163 *k*-mers with only one occurrence significantly reduced branch paths not
 164 affecting the integrity of correct paths (**Fig. S2b**). We next attempted to
 165 mathematically describe the validity of using coverage as an indicator to
 166 recognize noise *k*-mers. Monte Carlo simulations were performed with 10,000
 167 DNA strands, in which a Poisson model was employed for the simulation of the
 168 copy number distribution of each strand. The expectation value (λ) of strand
 169 copies was pre-set to be 50 and the error rate being 4%. The resulting
 170 coverage distribution of noise *k*-mers and correct *k*-mers were further analyzed.
 171 A coverage cut-off of 8 was found effective in the removal of most noise *k*-mers
 172 (**Fig. S2c**).

173 **Path recognition by CRC codes and both-way search strategy**

174 Noise *k*-mers cannot be completely removed solely by the elimination of low
 175 coverage *k*-mers. Branch paths still exist due to the remaining noise *k*-mers. A
 176 method to recognize the correct path is thus required. Traditional EC codes
 177 including cyclic redundancy check (CRC), RS, and hash codes are choices for
 178 this purpose. We failed to find a proper hash function that can generate short
 179 keywords. RS codes, optimized for correcting erasures, are not suited for path
 180 recognition, which does not involve erasures. CRC codes were chosen
 181 because of the efficiency of these codes in error detection instead of error
 182 correction. The effectiveness of CRC codes in path recognition was well
 183 demonstrated by intensive (one million) Monte Carlo simulations.

184 To further reduce the greedy path search time, we proposed a both-way
 185 search strategy that allows simultaneous search from both ends of DNA

186 strands. In this strategy, an additional ‘anchor’ element was designed for each
 187 DNA strand. The anchor corresponds to the index located on the other end of
 188 the DNA strand (**Fig. 2b**), and is generated from the index by a specific
 189 function. A given index processed by the function generates a specific number,
 190 represented by the anchor sequence. For proof-of-concept, the pseudorandom
 191 function of Python is used for the generation of the anchors from the indexes
 192 as seeds. Pseudorandom function generates an identical ‘random’ value with
 193 the same seed. Thus, informed with indexes, the greedy path search engine
 194 calculates corresponding anchors, and carries out the both-way search
 195 strategy. The both-way search strategy significantly reduced the decoding time,
 196 with effects more obviously observed when sequence error rates are high. Up
 197 to 60% reduction was evident with an error rate of 10% and a copy number of
 198 100, while decoding accuracy was well maintained (**Fig. S3**).

199 **Integration of DBG-GPS with outer erasure codes and** 200 **performance test**

201 DBG-GPS is designed as an inner decoding mechanism for the error-free
 202 reconstruction of DNA strands. It can be easily combined into the decoding
 203 process of outer erasure codes, e.g. fountain codes or RS codes, as shown by
 204 **Fig. 2c**. Python implementation of DBG-GPS using fountain codes as outer
 205 codes is available at
 206 [https://switch-codes.coding.net/public/switch-codes/DNA-De-Bruijn-Decoding/](https://switch-codes.coding.net/public/switch-codes/DNA-De-Bruijn-Decoding/git/files)
 207 [git/files](https://switch-codes.coding.net/public/switch-codes/DNA-De-Bruijn-Decoding/git/files).

208 To systematically analyze the decoding accuracy of our Python implementation
 209 of DBG-GPS, Monte Carlo simulations of 10,000 DNA strands (each strand

with 60 copies) containing various error rates ranging from 1% to 10% were performed and the results were subjected to decoding using the Python implementation and even at 10% of error, decoding accuracy was still remarkably satisfactory with the overall S_r higher than 96.7% (**Tab. S2**). We next tested the decoding speed of DBG-GPS in comparison with CL-MA based methods. To the best of our knowledge, the CL-MA based methods are the only methods that have been used to calculate the consensus sequence from multiple error-rich strand copies^{12,17}. To compare the decoding speed of DBG-GPS with CL-MA based methods, DNA fountain codes were used to generate 10,000 DNA droplets and fifty sequence copies for each droplet. 4% errors (2% substitutions, 1% insertions and 1% deletions) were randomly introduced. A total of 500,000 error-rich sequences were then subjected to strand decoding by DBG-GPS and CL-MA respectively. Considering the complexity of the clustering process for the CL-MA-based decoding, we fed the Muscle 3.8.31¹⁶ program with pre-clustered reads. For decoding speed comparison, estimation based on the previously reported clustering time in literature was used¹⁴. Since the multiple alignment process was performed by a standalone program (Muscle 3.8.31¹⁶), there will be 10,000 times of IO (input and output) with the file system during strand decoding by CL-MA. To estimate the IO time, one sequence of the DNA droplet was repetitively submitted to Muscle for multiple alignment analysis for 10,000 times. This process is iterated three times and the average time is subtracted from CL-MA based decoding time. The k -mer counting step of DBG-GPS was performed with JellyFish 2.3. As shown in **Tab. 1**, DBG-GPS shows 103 times faster than the CL-MA based methods. Importantly, in the range of data scale from 10 to 10,000 DNA droplets, DBG-GPS exhibited linear decoding complexity (**Fig.**

236 **S5)**, suggesting that this method is suited for large-scale data storage.

237 Large-scale simulations were then used to assess the capacity of DBG-GPS
 238 with big data sets. An Ubuntu server 20.10 installation disk image (~ 1 GB)
 239 was used as the input file to generate DNA droplets. Each DNA droplet was set
 240 to carry 60 bytes of information with a total DNA length of 316bp, around the
 241 technical limits of current DNA synthesis technologies. Ten million DNA
 242 droplets, representing a data scale of 600 megabytes (MB) were simulated.
 243 With an expected copy number of fifty, error rate of 4% (approximately 2%
 244 substitutions, 1% insertions, 1% deletions), a 155GB sequence file was
 245 generated. Loading the *k*-mer counting results of Jellyfish on a computation
 246 workstation equipped with 640GB memories, the Python script encountered
 247 memory limitations. We had to reduce the DNA droplet number to five million,
 248 representing a data size around 300MB. Preliminary decoding simulations on
 249 this data scale revealed a problem of long decoding time. Detailed analysis
 250 showed that this is due to repeated presentations of same *k*-mers located in
 251 different strands. We then solved the problem using a combination of two
 252 strategies, with the first employing a filtering process during the production of
 253 DNA droplets to exclude seriously entangled DNA strands, and the second
 254 setting an upper boundary for path number in greedy path search. The greedy
 255 path search engine aborts search if the number of found paths exceeding this
 256 boundary. Five million DNA droplets were simulated three times independently.
 257 On average, 4,971,880 DNA droplets ($S_r = 99.44\%$) were recovered, proving
 258 the great capacity of DBG-GPS.

259 **Experimental verification of the robustness of DBG-GPS**

260 To verify the robustness of DBG-GPS, a 314 KB zipped file, including two text
 261 files and four jpeg pictures, was encoded into 12,000 DNA strands, each in a
 262 length of 200 bp using fountain as outer codes. Each strand encodes thirty
 263 bytes of data, three bytes of CRC codes, four bytes of index codes, and four
 264 bytes of anchor codes (**Fig. 2b**). The overall strand redundancy was designed
 265 as 10.5% based on the expectation of more than 92% of the strands could be
 266 correctly decoded. A final concentration of 2 ng/μl designed DNA strands were
 267 synthesized by Twist Bioscience (**Fig. 3a**), and used as templates for PCR
 268 amplification and data retrievals. Different types of errors, including indels,
 269 substitutions, rearrangements, and strand breaks were intentionally introduced
 270 by three harsh experiments (**Fig. 3b-d**), followed by decoding to examine the
 271 robustness of DBG-GPS.

272 **Data retrievals with deep error-prone PCR products**

273 A serial of six error-prone PCR (ePCR), with each reaction containing 30
 274 thermal cycles, was performed using an error-prone PCR Kit to amplify the
 275 ssDNA library introducing large numbers of substitution errors, estimated 8
 276 errors per 1,000 bp per amplification cycle base on the manufacturer's
 277 instruction. All six ePCR products, ePCR#1-6, were sequenced by Illumina
 278 technology. Due to unspecific amplifications, in addition to substitution errors,
 279 ePCR#2-6 products contain DNAs of different sizes, evident by a smear from
 280 100 to 4,000 bp on agarose gel (**Fig. 4a**). As some of these DNA sequences
 281 were too long to be sequenced by Illumina platform, these PCR products were
 282 digested with DNase I to a target length of 200 bp. After digestion, all

283 fragments were collected for sequencing. The sequencing raw reads were
284 subjected to DBG-GPS based decoding.

285 Regardless of rough amplification conditions expected to introduce a large
286 number of errors, strikingly the S_m values of all six samples were higher than
287 99% (**Fig. 4b**), with the S_m values of ePCR#1-3 being 100%, indicating perfect
288 reservation of all strand paths even after 90 cycles of ePCR. S_r values in a
289 range of 93.2-99.8% were obtained for the six samples, ensuring reliable data
290 decoding by the outer fountain codes. Decrease of S_r was observed with
291 increased ePCR cycles. More PCR errors were expected to increase branch
292 paths, raising the necessary cutoff for noise k -mers elimination. More correct
293 k -mers were consequently discarded by mistake, resulting in a lower S_r value.

294 To further test the robustness of DBG-GPS, sequencing data with low
295 coverage ranging from 1 to 256 was obtained by random sub-sampling on the
296 sequencing results of ePCR#1 and ePCR#2. The k -mer dropout rates (K_d),
297 noise k -mer indicator (K_n) and strand recovery rate (S_r) were calculated and
298 plotted against coverage values (**Fig. 4c**). As expected, with the same
299 coverage, both K_n and K_d values of ePCR#2 are always higher than
300 ePCR#1, consistent with accumulating errors in each additional round of
301 ePCR. Upon increasing coverage, the K_n values of both samples reflecting
302 the numbers of noise k -mers increased quickly, while the K_d values reflecting
303 the dropouts of correct k -mers decreased quickly. As shown in **Fig. 4c**, the two
304 samples can be reliably decoded at coverage as low as 16 and 24.

305 Multiple data retrieval and accelerated aging test

306 Multiple data retrievals from storage media are required in practice. Thus, data
 307 robustness sustainable during multiple retrievals needs to be tested for our
 308 algorithm. Till now, only a limited number (<10) of multiple retrievals from
 309 high-quality PCR products have been reported for DNA data storage. To mimic
 310 the required multiple retrievals in practice and further demonstrate the
 311 robustness of our method, 100 times parallel data retrievals from
 312 PCR-amplified ssDNA library were carried out (**Fig. 3**), in which massive
 313 unspecific amplifications indicated by smears from 100 to 8,000 bp on agarose
 314 gel were observed (**Fig. 5a**). The S_m values of all retrievals were still
 315 remarkably high in the range from 99.44% to 99.78% (**Fig. 5b**). The S_r values
 316 ranged from 98.45% to 99.02%, ensuring accuracy in these data retrievals.

317 The CL-MA based codec systems highly rely on intact DNA strands for
 318 decoding, whereas DBG-GPS relies on short k -mers. Thus DBG-GPS likely
 319 tolerates DNA degradation. To test this hypothesis, accelerated aging
 320 experiments were performed as shown in **Fig. 3c**. The master pool was first
 321 PCR-amplified followed by incubation at 70°C for a prolonged period of time up
 322 to 65 days. Agilent 2100 Bioanalyzer was used to analyze the samples. A 14
 323 days of incubation caused the complete disappearance of the 200bp peak
 324 prominent in the original sample, indicating that the integrity of the DNA strand
 325 has been severely damaged (**Fig. S6**). Such degree of degradation would
 326 have made it impossible to recover the encoded data using the previously
 327 reported codec systems. By contrast, DBG-GPS based decoding enabled high
 328 strand recovery rates, ensuring accurate data retrievals from all samples that

329 have been heat-treated for 7, 14, 30 and even 65 days. A high S_r value of
 330 98.18% was obtained with the sample treated at 70°C for 65 days (**Fig. 5c**).
 331 This S_r value is only slightly lower than that of the control stored at -20°C
 332 (99.40%). Data retrievals from accelerated aged DNAs have been
 333 reported^{10,18,19}. In these studies, the authors tested the effects of different
 334 methods for the preservation of data encoded DNA, among which embedment
 335 in silicon beads was shown to be the best allowing recovery of information
 336 after treatment at 70°C for 7 days. According to the authors, this was thermally
 337 equivalent to storing information on DNA at 9.4°C for 2000 years¹⁰. Based on
 338 this estimation, our DBG-GPS algorithm can be used to retrieve data from a
 339 DNA solution preserved at 9.4°C for 18,571 years.

340 Discussion

341 Data robustness, density and low-cost are important parameters, determining
 342 the technical advancements of a storage medium. In general, higher data
 343 robustness requires more redundancy codes, which decrease information
 344 density and cost effectivity. Based on information theory, redundancy codes in
 345 the form of “data repetition” are unfavorable since production of additional data
 346 copies on traditional media is cost-ineffective and time-consuming. By contrast,
 347 data repetition in the form of DNA strand copies can be obtained inexpensively
 348 and speedily. The traditional definition of “coding efficiency” needs to be
 349 adapted to DNA data channel. With current technologies, producing a single
 350 copy of DNA data is not even doable. It has been reported that serial dilutions
 351 to reduce the copy numbers of information-bearing DNA strands till the
 352 average copy number below ten resulted in massive strand dropouts, making

reliable data retrieval impractical²⁰. Even if a single copy of DNA data is obtainable, it may be damaged due to DNA degradation under certain conditions or during long-term storage. Taken together, the design for DNA storage should be based on the reality and necessity, the presence of more than one copies of DNA strands, and not too many copies (below 100) to maintain high information density. In other words, “multicopy” is crucial for the robustness of DNA data storage and should ideally be maintained in a reasonable range. A new theory to design EC codes taking this unique “multicopy” feature into consideration will help solving the main technical issue in DNA storage, the error-free decoding of DNA sequences in a completely different fashion.

To the best of our knowledge, CL-MA based methods are the only reported methods used for DNA decoding from error-containing multicopies. These methods are computation-intensive, and are not robust when dealing with strand rearrangements and breaks. In this study, we developed a strand reconstruction algorithm based on the de Bruijn graph theory and tested its robustness. We were able to accurately and rapidly decode DNA data from error-rich strand copies using this algorithm. The errors include indels, rearrangements and strand breaks. It is worth noting that the encoded information from a DNA solution that has been incubated at 70°C for 65 days can be accurately recovered with our algorithm. The degree of damage to DNA under this heating condition was estimated to be equivalent to that under 9.4°C for more than 18,000 years¹⁰. Such unprecedented robustness highlights the importance of our method and suggests the great potential of de Bruijn graph theory in DNA data channel. Our algorithm is presumably compatible with a

378 variety of DNA preservation, e.g. silicon beads¹⁰, nanoparticles¹⁸, and alkaline
379 salts¹⁹, to further enhance data stability.

380 The Python codes implemented in this study use a simple hash dictionary for
381 the retrieval of *k*-mers. C++ implementation with a more advanced *k*-mer data
382 structure^{21,22} is expected to significantly reduce the memory cost, as well as
383 decoding time. In data decoding from 50 sequence copies containing 4%
384 errors, the performance of DBG-GPS is superior to that of the CL-MA based
385 methods in speed by one hundred fold. A higher ratio of noise *k*-mers is
386 expected to decrease decoding speed and accuracy with the DBG-GPS
387 method. Future incorporation of EC codes to help identify and exclude noise
388 *k*-mers prior to the greedy path search is expected to further improve the
389 decoding speed and accuracy, which is particularly important in the extreme
390 case of high error rate.

391 **Methods**

392 **Library design, PCR and Sequencing**

393 A DNA library of 12,000 oligonucleotides in length of 200 nt was synthesized
394 by Twist Biosciences. Error-prone PCR was performed with Controlled
395 Error-prone PCR Kit, TIANDZ, CAT#:160903-100. Thermo cycle parameters
396 were 94°C for 3 mins, 94°C for 1min, 45°C for 1min, 72°C for 1min, 30 cycles.
397 Six serial error-prone PCR were performed to introduce high rates of errors.
398 The first round ePCR utilized 0.6 µl samples of the master pool as templates.
399 The five following ePCR use 1 µl products of the previous round ePCR as
400 templates. All PCR experiments were performed with the following primers: P1

401 5'-CCTGCAGAGTAGCATGTC-3', P2 5'-CGGATGCATCAGTGTCTAG-3'. The
 402 PCR products were sequenced by Tianjin Novogene Sequencing Center &
 403 Clinical Lab. Sequencing libraries were generated with purified PCR products
 404 using Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA)
 405 following manufacturer's recommendations and index codes were added. For
 406 the products of the second-round error-prone PCR with very large DNA
 407 strands, DNase digestion is applied to break the long strands into small
 408 fragments around 200~300 bp. After digestion, all fragments are collected for
 409 further library construction using the protocol as just described. The library
 410 quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and
 411 Agilent Bioanalyzer 2100 system. All the libraries were sequenced on Illumina
 412 HiSeq platform and 150 bp paired-end reads were generated. For preparation
 413 of the 100 PCR samples for parallel retrievals, we first performed one round of
 414 PCR amplification with Taq DNA polymerase with a total volume of 100 μ l.
 415 Then, 100 parallel PCR amplification was performed using 1 μ l of the first
 416 round PCR reaction mixtures without purification. The purification step is
 417 removed in purpose of enhancing unspecific amplification. The 100 PCR
 418 samples were then sequenced by Illumina sequencing platform. All the Taq
 419 DNA Polymerase based PCR amplifications, except the error-prone PCRs,
 420 used Vazyme 2X Rapid Taq Master Mix (CAT#: P222-03).

421 ***k*-mers counting and elimination of low coverage *k*-mers**

422 The python implementation provided in this study used a simple hash
 423 dictionary structure for counting of *k*-mers, which is not memory efficient. Thus,
 424 although the scripts implemented with functions to read the common

nucleotide sequence files, *i.e.* FastQ or FastA, and count the k -mers accordingly, for large data sets, we strongly suggest to use a professional k -mer counting system, *e.g.* Jellyfish²¹, which is much more memory efficient and supports multi-cores. To count k -mers by the python scripts, please call the following functions of DeBruijnGraph() object: for reading and counting of FastQ file, use DeBruijnGraph-openFastQ('file name'); for reading and counting of Fasta file, use DeBruijnGraph-openFasta('file name').

For elimination of low coverage k -mers, call function DeBruijnGraph-remove_low_cov_kmers(n), then all k -mers with coverages lower than n will be removed. For usage of Jellyfish for counting of k -mers and dumping of k -mers satisfying the criteria, please refer to the Jellyfish manual, which is available at (<http://www.cbcb.umd.edu/software/jellyfish/>). The dumped k -mers can be introduced to the DeBruijnGraph() object using DeBruijnGraph-openDump('file name') function for further decoding test.

Setting of k -mer size

Obviously, with a specific k -mer size, the data scale can be decoded is limited. For example, it is clear that only small amount of data can be decoded with an extremely short k -mer size of three. However, the mathematical answer to this question is yet unsolved. In de Bruijn Graph theory, for each k -mer, the front $k-1$ bases were used for positioning (*i.e.* indexing), and the one base at the terminal was used for extension of path, *i.e.* for encoding of data. Thus, we estimate the decoding capacity (p) of k -mers with a specific size of k as follows:

$$p = 4^{k-1} \times 2bits$$

448 Where 4^{k-1} is the combination number of possible indexes with a length of
 449 $k - 1$, 2 bits stands for the encoding capacity of one base at the tail. Based on
 450 this formula, the decoding capacities of different k -mer sizes were calculated
 451 and listed in **Tab. S3**. As shown in the table, the decoding capacity is estimated
 452 to be around 57.6 PB with a k -mer size of 30, and 6×10^5 EB for a k -mer size of
 453 40, indicating a capable method for large-scale data decoding. Additionally, it
 454 should be noticed that, due to the repeated presentations of identical k -mers in
 455 different DNA strands, the actual data scale can be higher than this estimation
 456 theoretically.

457 **Details of greedy path search**

458 The greedy search process was illustrated in **Fig. S4**. The details are
 459 described as follows: **Step 1**, Select an index, *e.g.* 562451, and calculate the
 460 anchor value by the Python pseudorandom function using the index value as
 461 seed in case of both-way search mode. An anchor value of 47736 is obtained
 462 in the case of 562451. **Step 2**, Encode the index into DNA string based on the
 463 corresponding binary string, *i.e.* $562451 = 10001001010100010011 =$
 464 ATATATGGGATCAGAA. Similarly, the anchor of 47736 is encoded into DNA
 465 string of ATATATATGCGTTTGG in case of both way search. The greedy path
 466 search starts with ATATATGGGATCAGAA as an initial path. Similarly, the tail
 467 search start with ATATATATGCGTTTGG as an initial path in the case of
 468 both-way search. **Step 3**. Judgement step. This step is for the selection of an
 469 end with few path numbers for extension of paths. **Step 4**, Extension of paths
 470 on the end with fewer path numbers. **Step 5**, Judgment step. This step is to

471 judge if the path search has finished. **Step 6**, This step is specifically required
 472 for both-way search mode. The head paths are fused with tail paths if the
 473 overlap sequences are identical. For one-way search, this step is not required.
 474 **Step 7**, Encode the complete paths into binary strings. Perform parity checks
 475 and drop the wrong routes using the embedded CRC codes. For other EC
 476 codes applied, use the checking mechanism correspondingly. **Step 8**,
 477 Judgement step. Check if there is only one path passed the CRC checking. If it
 478 is true, choose it as decoded strands, otherwise, mark a missing strand for this
 479 index. If multiple routes or no routes pass the CRC codes, the
 480 searching-based decoding fails. Only in the case of one route passed the CRC
 481 checking, the decoding succeeds. **Step 9**, Judgement step. Judge if the
 482 search is finished. If not, iterate *step 1* to *step 9* until all indexes are searched
 483 for possible strands. If yes, finish the inner decoding process. This algorithm is
 484 implemented in the `deBruijnGraph.py` object by Python. The `deBruijnGraph()`
 485 object was implemented to search in both-way mode by default. Switch the
 486 value of `DeBruijnGraph()` `->dual_search` to 'False' to active the one-way
 487 search mode.

488 Error analysis in the concept of de Bruijn graph theory

489 Although distribution profiles of error rate and copy number among different
 490 strands shape the DNA data quality, they cannot reflect the data quality
 491 straightforwardly. Additionally, the sequence error rate is complicated for
 492 accurate accounting. For better estimation of the qualities of DNA data and
 493 simplification of the error analysis process in the concept of de Bruijn graph
 494 theory, here we introduce two indicators, *i.e.* K_d (in range of 0 to 1) and K_n

(in range of one to 4^k), which together can well define the data quality. The K_d stands for dropout rate of all correct k -mers and K_n is the ratio of noise k -mers and correct k -mers. DNA data with a K_d close to 1 and a K_n close to 0 stands for high-quality data with a high data coverage rate and low noises. Other than error rate which is complicated for accounting, K_d and K_n can be easily calculated by the following formulas:

$$K_d = K_{lost} / K_{ori} \quad 1$$

$$K_n = K_{all} / K_{corr} \quad 2$$

In formula 1, K_{ori} stands for the number of k -mers in the original encoded sequences and K_{lost} stands for the number of k -mers that presented in the original encoded sequences but not presented in the sequencing results. In formula 2, K_{all} stands for the number of k -mers in the sequencing results and K_{corr} stands for the number of correct k -mers, *i.e.* k -mers that presented in the original encoded sequences. DNA data with a K_d close to 1 and a K_n close to 0 stands for a high-quality data with high data integrity and low noises. The maximal strand decoding rate S_m is determined by K_d . With specific K_d , the actual strand decoding rate S_r is affected by the value of K_n . The greedy path search becomes extremely slow when decoding DNA data with higher K_n values, due to too many possible paths for each strand. The DBG-GPS will stop decoding if the path number exceeds a specific threshold. In this proof-of-concept study, we use 100 for fast decoding, 1,000 for high-quality decoding.

517   **The fountain codes**

518   The outer fountain codes used in this study is modified from
 519   <https://github.com/dbieber/fountaincode>. DNAFountain and DNADroplet
 520   objects were implemented for the handling of DNA strings. The assignment
 521   mechanism of the random degrees and chunk indexes of the Droplet object
 522   was modified to support DBG-GPS based decoding. Luby transform is applied
 523   for the generation of random degrees. Specific index encoded in the DNA
 524   Droplet is used for the selection of a degree from a pre-generated degree table.
 525   This index is also used as seed for the generation of the random strand
 526   combinations. The decoding process of the original Glass object has been
 527   modified to improve its performance.

528   **Details of the simulation experiments**

529   Simulations for investigation of the potentials of de Bruijn graph were
 530   performed with 10,000 DNA strands generated by the fountain codes with a
 531   strand length of 320 bp. For de Bruijn graph construction, a *k*-mer size of 21
 532   was applied. The simulation studies on the both-way search strategy are
 533   performed with 10,000 DNA droplets. 100 sequence copies with random base
 534   errors in rates of 10%. For the large-scale simulations, an Ubuntu Server 20.10
 535   live install disk image (Link provided in **Supplementary materials**) was used
 536   as input file for the generation of random DNA droplets. Each DNA droplet was
 537   set to encode 60 bytes of data and two bytes of CRC codes. The length of
 538   index and anchor are both set to four bytes. Primers of P1
 539   5'-CCTGCAGAGTAGCATGTC-3' and P2 5'-CGGATGCATCAGTGTCAG-3'
 540   were added to the ends. This will result with a DNA droplet in length of 300 bp,

541 around the theoretical limits of current DNA synthesis technologies. To avoid
542 entangled DNA strands, a filter process that always discards the DNA droplets
543 contains a $k-1$ mer that already presents more than four times was applied to
544 the droplet generating stage. For counting of k -mers by Jellyfish 2.3, a k -mer
545 size of 24, and k -mer coverage cut-off of 5.

546 **Data Availability**

547 All data files and raw sequencing data that support the findings of this study
548 have been deposited in public cloud server. The links are provided in
549 supplementary materials.

550 **Code availability**

551 All the original algorithms proposed in this study were implemented with
552 Python and the source codes are available at
553 <https://switch-codes.coding.net/public/switch-codes/DNA-De-Bruijn-Decoding/>
554 [git/files](#).

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563 **Competing interests**

564 Li-Fu Song, Ying-Jin Yuan and Feng Geng have filed a patent covering the
565 encoding structure and the de Bruijn graph based decoding process (CN
566 110190858 A).

567 **Author contributions**

568 LFS and Y.Y. conceived the study. LFS proposed the idea, wrote the Python
569 codes, designed the experiments, performed the simulations, and analyzed
570 the data. FG helped with experiment design and data analysis. ZYG performed
571 the error-prone PCR experiments. BZL and YJY edited the manuscript and
572 supervised the whole work.

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654 **Fig. 2 | Proposed four-step framework of DBG-GPS and its integration with outer erasure codes.** **a**, Proposed four-step
 655 framework of DBG-GPS. **b**, Proposed structure of DNA strands for DBG-GPS. There are four major parts including: A1 – The
 656 index/seed of the DNA strand; A2 – Data payload for encoding of data; A3 – Cyclic Redundancy Check codes (CRC) for selection of
 657 the correct path; A4 – Anchor codes which can be calculated by specific function based on the value of A1. A4, Anchor codes
 658 (optional) for both-way search; **c**, Integration of DBG-GPS with outer erasure codes. DBG-GPS is a full resolution for inner code
 659 decoding.

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662 **Fig. 3 | Experimental verification of the robustness of DBG-GPS.** **a**, A
663 314kb file is wrote on DNAs to generate a “Master Pool”. **b**, Serial deep
664 error-prone PCR to introduce base errors. **c**, Multiple data retrievals with
665 intended unspecific amplifications to introduce strand rearrangements. **d**,
666 Accelerated aging experiments to introduce strand breaks.

669 **Fig. 4 | Reliable data recovery with deep error-prone PCR products. a,**
 670 Agarose electrophoresis analysis of the serial error-prone PCR (ePCR)
 671 products. **b,** Reliable strand reconstruction with low quality ePCR products.
 672 The dashed red line stands for the decoding lower boundary of the outer
 673 fountain codes in this case study. **c,** Low coverage decoding by random
 674 sub-sampling. Sequencing reads of ePCR#1 and ePCR#2 are used for
 675 random sub-sampling individually. For each coverage tested, 12 independent
 676 sub-sampling simulations were performed and the averages values were
 677 presented. The standard deviations are presented in **Tab. S5** for clarity.
 678 Coverages in range of 1 to 256 were considered. The average strand recovery
 679 rates (S_r), k -mer dropout rates (K_d) and K_n values were presented. The dashed
 680 curves are the strand recovery results without elimination of low coverage
 681 k -mers. The horizontal dashed red line stands for the decoding limits by the
 682 outer Fountain codes.

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685 **Fig. 5 | Multiple retrieval and accelerated aging tests. a,** Agarose
 686 electrophoresis analysis of the parallel PCR products for multiple data
 687 retrievals. Twenty representatives out of 100 samples are shown. M stands for
 688 the DNA Marker. **b,** Strand decoding results of the 100 multiple retrievals with
 689 DBG-GPS. **c,** Strand decoding rates by DBG-GPS from accelerated aged DNA
 690 samples.

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693 **Tab. 1 | Comparison of the DBG-GPS and CL-MA based methods in**
 694 **decoding speed.**

DBG-GPS		CL-MA	
Stages	Time (s)	Stages	Time (s)
<i>k</i>-mer counting (<i>Jellyfish 32 threats</i>)	0.63 ± 0.04	Clustering²³ (<i>32 threats</i>)	0.36
Strand decoding (<i>Python Script</i>)	36.84 ± 0.91	Multi-alignment (<i>Muscle¹⁶, IO time</i> <i>subtracted</i>)	3874.48 ± 30.82
		Consensus calling (<i>Majority voting¹³</i>)	15.42 ± 0.40
Total	37.47 ± 0.95	Total	3890.26 ± 31.22

695   **Supplementary materials**

696   **Fig. S1 | A two-dimensional coding strategy of traditional EC codes**
 697   **handles the base shift problem of indel inefficiently.**

698   **Fig. S2 | Path search complexity caused by base errors can be**
 699   **remarkably released by elimination of low coverage k -mers.**

700   **Fig. S3 | A both-way search strategy reduces decoding time.**

701   **Fig. S4 | Details of DBG-GPS algorithm.**

702   **Fig. S5 | Linear decoding complexity of DBG-GPS.**

703   **Fig. S6 | Agilent 2100 analysis of the accelerated aged samples.**

704   **Tab. S1 | Computational estimation of the maximal strand decoding rates**
 705   **(S_m) of different types and rates of errors with 20 strand copies.**

706   **Tab. S2 | Strand decoding performance of four-step DBG-GPS algorithms**
 707   **estimated by Monte Carlo simulations.**

708   **Tab. S3 | Theoretical decoding capacities of DBG-GPS with different**
 709   **k -mers sizes.**

710   **Tab. S4 | Details of strand recovery results of deep ePCR experiments.**

711   **Tab. S5 | Details of the sub-sampling simulation results with ePCR#1 and**
 712   **ePCR#2.**

713