

**Supplementary materials for**

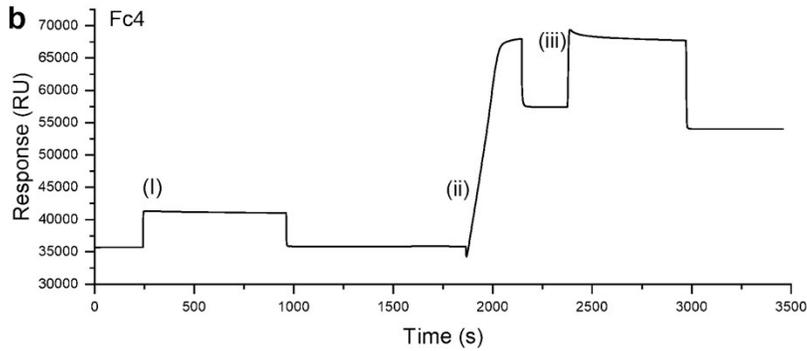
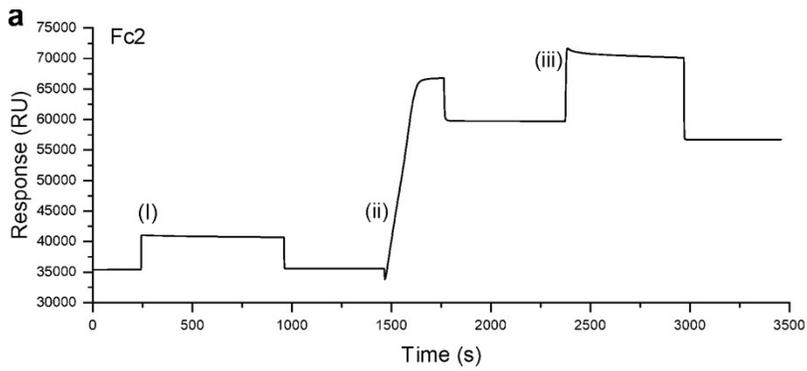
**Data storage based on the absence of nucleotides using a bacteriophage abortive infection system reverse transcriptase**

**SM include:**

Supplementary Figure 1

Supplementary Table 1

Supplementary Table 2



**Supplementary Figure 1: Amine-coupling immobilization profile of the bacteriophage abortive infection system reverse transcriptase AbiK on a CM5 chip. a**, Immobilization of AbiK on flow cell 2. **b**, immobilization of AbiK on flow cell 4. (i) surface activation using a mixture of 1-ethyl-3-(3-dimethylpropyl)-carbodiimide and N-hydroxysuccinimide, (ii) AbiK coupling, and (iii) surface blocking by ethanol amine. AbiK was injected at 50  $\mu\text{g}/\text{mL}$  in sodium acetate buffer (pH 5.0). Next, 21,160 response units (RU) and 18,150 RU of AbiK were immobilized on flow cell 2 (Fc2) and flow cell 4 (Fc4), respectively.

**Supplementary Table 1:** The three-nucleotide code used to encode information using the bacteriophage abortive infection system reverse transcriptase AbiK

<b>Three-nucleotide mix</b>	<b>Number assigned to the combination</b>
ATC	1
ATG	2
TCG	3
ACG	4

<b>Letter of the alphabet</b>	<b>Numerical code</b>
A	123
B	124
C	132
D	134
E	142
F	143
G	213
H	214
I	231
J	234
K	241
L	243
M	312
N	314
O	321
P	324
Q	341
R	342
S	412
T	413
U	421
V	423
W	431
X	432
Y (Q+Q)	341341
Z (X+X)	432432
space	dNTP mix

**Supplementary Table 2:** Nucleotide sequences of synthetic DNA fragments produced by the bacteriophage abortive infection system reverse transcriptase AbiK. The DNA segments are color-coded according to the nucleotide triplet: ATC in green, TCG in blue, ACG in orange, and ATG in red. Shaded in gray is the DNA region containing the nucleotide base guanine, which was inserted between the sequences encoding the word “DNA.” The section of DNA corresponding to the three letters (“D,” “N,” and “A”) or the word “DNA” is underlined; the corresponding numerical code with the codec is shown in Supplementary Table 1. Each sequence is shown together with the Microsynth identifier, raw sequences, and quality of the sequencing reads in Supplementary Data 1.

Sequence number	Microsynth identifier	Sequence (5'-3')	Numeric code	Word/letters
1	@27c19470 -5460-4ade- b32a- 6485c2a985 5b	<u>AATATCAAAAAATATCCAACAAAATATACTCACACATATAAACCTGTGTG</u> <u>TTGCGTGG</u> <u>AAAGAGAAAGAAGAAAGAGCGCGCGCTGTGCGTCCCTCGC</u> <u>TGTGTCA</u> <u>CAAAAATACCCAATACTCAAATAATAAACAAAATCAATAAAATAA</u> <u>AAAGGAAGAACGAAAGAGCGAAGAGAGAGAAAGAAAAGAGAAAGAAGA</u> <u>ATCTTTTACAACACCCAAAATTAATACCCATATATCAAACCATATATAAACA</u> <u>CAACATAAA</u> <u>GAGTGAGAGAGTTTGAAGGAGAAGGAAGGAAGGATGGATA</u> <u>AGAATGGGAGAGAGAAATGAATAGGTAGAAATAATTGGGAAAAAGAAT</u> <u>AAAAAGAAGTTAGAATAAAGATGAAGTAAAAGTTGAGAAGAGAGAGAAA</u> <u>GTGAAATGAGAGGGGAGGGAGAATAAAGAGAGTAAAAGGAG</u> <u>CGCGG</u> <u>CTAG</u>	<u>13431412</u> <u>3</u>	DNA
2	@6fbfdb44- ad7a-4b6e- b329- c68b1f100f be	CATCAATAAAAAACAAAAACACCCAACAATAAAAAAAAAATATAAATAAA AACAAAAATATCAAAAAACCTTTACATAAATAAAAACTATTAACCTTA AAACTCATACAAA <u>TTGCGTGTGCTGTGCGCGCT</u> <u>GGGGGGGGGGGGG</u> <u>GG</u> <u>ACATT</u> <u>TTACATACCAAACTTATCCAACCTACCATTATTCAACTACCAATCCACAAA</u> <u>TACCAACCTTAATCACAAACCAATAAAAACTACCACAGGTTGCGTGGCGTCC</u> <u>GCGCGTGTGCTGCGGGG</u> <u>AGTTGTCTGTTCGCGCTCGTGCCGACGCCACGA</u> <u>GACCTATAGTAGTTTTATTGGTTTTATAATTGTGGTATTGTCTAGAATTG</u> <u>GTCATTGATAAGATGGTTAAGTTGGATATGTTTTAATGT</u>	13-GGG- <u>1342</u>	D
3	@4653de13 -20da-47f6- b9e9- a51304021a 5c	AATAAAAAATACCAAAACATAAATACTCACAAAAAAACAAAAAAACAA ACTATTAATAAAAAACCCAAACCATCATAAAAACA <u>G</u> <u>CAGAGAAACGCAGC</u> <u>AGGG</u> <u>TTCTC</u> <u>CAAAAATAAAACCCCAATTTA</u> <u>G</u> <u>AACATACACACTCCAATCAA</u> <u>AACACCAAAATTC</u> <u>AAACACATAAAACATCCACCCCTTCATTCAAACCTATAA</u> <u>TAATCTATTAACATTACACATACACAATCATTTTACAATATATACATAAATA</u> <u>CAACACA</u> <u>ACTAAATACA</u> <u>ACTTATATTACA</u> <u>ACTACCAACACACACAAAGCCCA</u> <u>AACCAACACATTATTA</u> <u>ACTAATTCTAA</u> <u>CAAAAAATATACCAACCTAAAAT</u> <u>AAAACAAAAA</u> <u>CAAAA</u> <u>ACTTAAATTA</u> <u>ACCAATCCAAATCAATTTAATATACT</u> <u>ATAATCACATCCACCTCTTAAATACAATAAACATTTAACACACACCAACTCTC</u> <u>CATCTCTAAATCATA</u> <u>CACTAATTAATTATA</u> <u>CCAATAATAATTC</u> <u>CACTAAACAT</u> <u>GGT</u> <u>ACCTGGGCTGCCGGGCGCGCTTGTGCGTCTG</u> <u>ACGAAGGGGCAAG</u> <u>GGAGGGAGCGGGCGGTGTGGGGCGCCGTTGCGTGTGTC</u> <u>CCCGCCGG</u> <u>TGGCTGCTAAACAAAATAA</u> <u>AAAAATAAAAAATATTCAA</u> <u>AATCCAAAAAAT</u> <u>AATATTTACACATAACCATAT</u> <u>G</u> <u>AACTAAA</u> <u>ACTCTACCCTCATCAAACAAAA</u> <u>CATAAAAAAAATACAAAAACCTACCATATACAAAAATCCAAATAAATC</u> <u>ATAAAAAATAATATTA</u> <u>ACCAAACTATCATACACAACCAATAACACCTCCTA</u> <u>TTAAAAAA</u> <u>AAACCAATAACAATAAACACCTCC</u> <u>GAAAAGAGAGAAGCGGAA</u> <u>CCTTTACCCCTCAACATAACCAAAAA</u> <u>CAAAATCCCTTATAGTATGATATCTA</u> <u>AAA</u> <u>CCTCCTTATA</u> <u>ACAAAAAA</u> <u>AAATCTTA</u> <u>ACCACAAAAAACTATACA</u> <u>TATAAATCCATCATACCAATAAAAA</u> <u>ACTCTCAACTTCTACAAACAAATAAAAA</u> <u>TTACAAATCAAAATAAAAA</u> <u>ACACCACAACCCAAATTA</u> <u>CCATAATAACAAC</u> <u>ACGCCGACAAGCCGCGCCCGGGCAGCCAGAGAAATTTATGTTAGTGG</u>	<u>14134314</u> <u>1214232</u>	DN and 2/3 A

AATTAATTTATTGGTATAGTTAATTAGTGTATGATTTAGAGATGGGTATTA  
 GTTGGTGTGTGTTAAATGTTATTGTATTTAGAGGTGGATGTGTGATTAAT  
 AGTTATATTAATGATTGGATTGGTTAATTTAAAGTTTTGTTTTGTTTT  
 ATTTTAGGGTAGATCTTTTTGTTTAGAATTAGTTAATGTGTTGGTTGGGGT  
 TTTGGGTGTGTTGGTAGTTGTAATATAAGTTGATTTTCGTTGTGTTGATG  
 TATATATTGAAAATGATTGTGTATGTGTGAATGTTAATAGATTATTATCGT  
 AAATGAAGGGGTGGATGTTTTATGTGTTGAATTTTGGTGTGTTGATTTTGG  
 GAGAGTGTGAATGTTGAAATTTGGGGTTATTTTGGAGAACCTGCTGCG  
 TTTCTCTGCTGTTTTATGATGGTTTGGGTGTTTATTAATAGTTGTTTTTT  
 GTTTTTTTTGCCTAAGTTAGTTATCTTTTGGTGAT

- |   |  |   |                    |                         |
|---|--|---|--------------------|-------------------------|
| 4 | <b>@2fc4e38e-<br/>fd28-4457-<br/>8056-<br/>bf6193a7fdf<br/>a</b> | <p>CTTGCCGGGGTTTGGGGCGTCCGCGGGGGTGGCGGGGCCGGGGCGGTTG<br/>       GCGTGCCGGGCGCGTCCGTCGTCGCGCGCGCGTCTTGCCGCGGTTTTGGT<br/>       CGTTGGGCGTGGGGCGCTCGTGCGCGTGTGAGCAGGGACGAGAGCGC<br/>       GACAAGAGAAGAGAAAACCAAAAGAGACGAAGAGAAAGGAGCGGGGAA<br/>       AGACGGGCGCAAAAAGGAGAGAGCAAGAAGGGAGAAAGGAGAAAAC<br/>       ACACAAAAAAGAACCAGAGGGGAAAGAACGAGAACGAACCGCAC<br/>       CGGAAAGCGCACAAAATATAACAAAAATCTAACACTAACTATAAAACA<br/>       CAACAACCTCACTACACCTTAAAACCACTAAAACAAATAAAAAATACAAT<br/>       AAACACTTAATCAAAAAAAAACCTACCACATAAACATTAAAAATATAC<br/>       TTCTATATTAATAAAAAACAACAATAAAAAAACGAAGAGAAAGAACG<br/>       GGACAAAAACCAACAAAATATAACCTTATTCAACCAAATCGGGTTAAA<br/>       AGGTTATGGAGTATAATAAGATACGTCGGTGTGGTGGTGGTCCGGGGCG<br/>       TGGCGCTGGCCGCGCGCGGACCCACCGCCACCGACATATCTTATTATA<br/>       CTCATAACCTTTAAACCGATTGGTTGAATAAGGTTTATTTTTGTTGGT<br/>       TTTTGTCCGTTTCTCTCGTTTTTTCAGGTACTCTTTAATTAATATAGAA<br/>       GTATATTTTTAATGTGATGTGGTAGTTTTTGGATAAAAATTTTTATTGTA<br/>       TTTTTATTGTTAGTGGGTAAGAGTGTAGTGAGAGTTGTTGTGTTTTAT<br/>       AGTTAGTGTAGAAATTTTTGTTTATTTTTGTGCGCTTCCGTGTGGGTCC<br/>       GTTCTCGTTCTGCCGTTCTTTTTTGTGTGTTTCTCCTTCTCCTTCTCCTC<br/>       CTCCTTCTTTTTGCGCCGCTCTTCCCGCTCCTTCTCTCGTCTCTTTGG<br/>       TTTTCTTCTCTCTGTGCGCTCTGCTCTGCTTGGACACGCGCACGAGC<br/>       GCCCGCAACAAACCAAAACCGCGCA</p> | 34141234<br>123234 | 2/3N<br>and A           |
| 5 | <b>@8679d127<br/>-966d-40ec-<br/>bf4c-<br/>4526686d2e<br/>d2</b> | <p>GACCTACAAAACCATATAAACATACATATAATACAACAACATAAAAATTA<br/>       CCCAAATCTAACTTAATCTCTCCCCACATTTAACACCAATATTATAAAAT<br/>       TCCACTAAATATAACGGCGCGCCGTGTGCGCGTGTGCCGTGGGGGCGCTG<br/>       CTCAACTATAAACAATCAATAAACACCCCATATCATTTCAATAACATCATACTA<br/>       CCAATCCATTACCACACCAACTAAAACATATTATACCATATAACAAACATAA<br/>       ATAAATCTACATCCAACGAAAGAAGGGGAAAACGAAAAGCGAAAAAAG<br/>       AGAAAGCACACGAAGAGAAGCGAGGGGGCGAAATCTAAACATATCTACAA<br/>       AAAAAATAAACCCAAAATATAACCTAACCTCTATCTAAAAACAACAAAC<br/>       CACAACCTACCAAAAATAAATCAAAACCACTAACAAATAACAAACAAA<br/>       ATATATTTGTGTTATATGAGTTCCGGAAGTATATTTAGAAATGGGCCCTC<br/>       ACTCTTTTCGTGTGCTTTCTTTTTTTCGCTTTTCGTTTCCCTCTTTTCG<br/>       TTGGATGTCAGATTTATTTATGTTTGTATATGGTATAATATGTTTATGTTGG<br/>       TGTGGAATGGATTGGTAGTATAATGTAAGTAAATAAATAATGGGGTGT<br/>       TTATTGATTGTTATA</p>  | 13141212           | N and<br>2/3 of<br>an A |
| 6 | <b>@71f23883-<br/>34ab-4c44-<br/>a1e6-<br/>a1e809471d<br/>1e</b> | <p>CCGGGCAAAAGGACACCACTTACTTAATCTACAATTTCTCTCTCTCTTT<br/>       CTACCCCTTCATCAATGCTCTTCAACGCAACAGCAACGCCACGGCGG<br/>       AATTAATTACTTAATTCGCGCGTGGCGTTGCTCTGTTGCGTGAAGAGGCAT<br/>       TGATGGAAGGGGTAGAAGAGAGAATGAGGAAATGTGTTGACGGTGGT<br/>       CCTTTTGGCGGGGCGCGCGGGGGGCAAAAATCTATTCTACTTTCTAA<br/>       CTATCTATATACCAAAAATACAAACCTGGAACTTTTCTACCAACCCCTAAC</p>   | 41413231<br>42     | N                       |

ACAATGCCTCTTCAACGCAACACAGCAACGCCACGGCGGAATTAAGTAATT  
 AATTGTTATGGATTTTTAGTTTGTATTAATGGTGTGGTATTTTTATGGGT  
 GTATTTTTATGTTTGTGGAGGATTTTTAGTGAGTTATTGTAGTGGAGGGT  
 TTGATAGTGTATA

7	@6d18625c-507c-44ff-800a-c2fce6644846	CCTCAAAAAAAAAATAAAAAAAAAATTAATAATCCACCCAATCTCATCCCACTCTA ATCAAAAAAAAAAAAAAAAAACCCCAACATAAAAAACCATCTCTCAAAACAAT AAAATAAAAAACAAAATAAAAAATAAAAAATTCACAAAAAACACTAAACACAC AAAAAACAACCAAAAAACAAAAACAAAAATACGGAGAAGCAAACACAAAG CGCGCAGAAAAAAAAAAACAAATTTAAAAATCAACATAATATAATCAAAAAA AAAAAACAACAAAATAAACATTTAAAAACAAACAAAAATAACAAATACCCAC AATCCACAAATTACAAGGGAAGATGGTTGCATGGTATGT	1412	2/3 of N and 2/3 of A
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9	@96c85ce0-307a-426e-94c4-5a584458e348	CAAATAAAAAATAAAAAACCAACAAAACACTCACAAAACAAAAATAATACAA AAACAATCTTTCATCAAATAACCAATATAAACTAACCATAACGGCGTTG CGCGAGAGAAAAGGACAAGGGAGGAAGAACAGGCGGCCCGAGGCACGAG CAAGGGGAGGGCGGGGGGCGAGCAACAGGAAACGGGCCAGAGGAGAA GGGCAGGGTGGGCTGTTGCCGTGCGCTGGGCCCTAGCTCGTCCGGTGTGG GCGCTGCCGGCGGGGTGCGTGGTATCTATTCAACATAAAACAAAACATTATA CTTATCACCTAAAAACTATAACTAATACACACACCTATCATTATACTCCTA AACCCCTCTCGTATACCAACCAACGACGCAGAAAGAGGAACGGGAGAAAA ACGCGGGAGGAGCAGCAAAGGAGGAACAGACAGGAGCAAAGAGAGCA GCGAAGAGAAGGCAGGAACCGAAAAAGCTAAACAACAAATCAAATTACCT CAAAAAAAACCTTTAACATATAAACTACAAAAAAAACCTAAACCGAAGAG AATGGAAGATGTTTTGGATGCGAAAGAGAAAACAGCAGAGAGGGCGCGC AACCCACACAAAGCCGCCAAAGCGAACGGGGGCATTAAATAACTACC TCAAATACAACCATAACGAAGAGGAAGAAGCGAGAGGGCAACAAGAAGAG GAGAAAGGAGGAAAGAGCAAAAAAGAGAACAAAGAAAAAAGAAGAGA AAAGAAGAAAGCAAGGGCAAAAAACAGACGACAAGGACGCGAGAAG GCAGAAGGCAATAACACATAAAAAACCTCTAAAATCACAAAGTTCCCAAA ACAGAAAAATGTTGAATAATGGATGAGAAGAAAAAGGGAGGAAGATTGA TCTGCCGGTGCCTTGTGCTGGCGGGAAACGAGCACACGCCGAGCAAGT TACAGATGCCGGCAGAACGGGATATG	13431412 4141234	D, N and 2/3 of A
10	@9dd8ac2b-8e8c-4423-adc5-d36b0218d9cb	CCGGTGACAAATAAAAACTCAATATAAACAATTAACCAAAAAATATCACT TTTTAATAAAAAACAACAAACAACTAACAAATCTTCTAAAAAACACCTTT CAATAAATAAAATCTAAAAACTTTAAATAAAACTATCAAATCCCAATATCG AGAGAGAGGACGGGAAAAGGGCATTGTTGAATTTTTGTTGTTGGTTAATTT TTAAAGTTTTGTTTTTATGGCTTTTTCTCGTTCTCAAGTGTGTGT	314232	N
11	@fd939ea1-889d-4f4b-9d8d-56f737ca43fe	CCTATAAAAAAAATTATAACTACTAATCAATAAACACATTTACCATCTCCA AAATAACCCAAACAAAACAAACATCATACATCTAAAACCTCAACCAACCTTC TTCAAAAACACACTATCATTAAACACCTTCAACTAAAAAAGGGGAAATG AAGGTGAGAGGGAGTGGATTCAAGAGCCGAGCTTGCCAAGTAACTATGG TAAAGAGGCAGAGTTTCAAAGAGCGCAGCATACGACACGCGAGCAGTCCC GGCTTCTCGTCCGTTTTGGTAAATTTGGTGGGATTATTGAGTAGTTGGAT	13424324 1	D

ATTTTGAAAGAGTATTTTTTGGATAAATGATGGGTTGTGTGTTAGTGATA  
GTAATGATGATTGTTTAGGCAAGCAAGCGCGAGGCCAGCTTTCCTATCTT  
CATTTTTTCTATTATTCTATCTTCTCCATTTTTTTAGATT

- |    |                                       |  |        |                   |
|----|---------------------------------------|--|--------|-------------------|
| 12 | @2cc48fef-3059-4a59-86ad-dfe92473d4a0 | <p>AATCAATATCATCCAAAACAAAATAAATTAATCAAAAAACAAAAACAAA<br/>ACAAATTAACAAAAATCACATTTCCAAACTAACCTATAAATACAACAAC<br/>ATTAATAATTACCAACTAAATCAACTACAACCAAACTAAATTAACAAA<br/>ACTTAAAACAAATTTTATGCTTTGCTGTGCCGTCTCGTCGCGGTGCGGCGCT<br/>CGCAAAGAAAAGCGCAAAAGCAAACAAAGGAGAAAGAGAGAAAGAAGA<br/>ACGCACAGGGCGAGAACGAAAAGCGAGAGCAAGGGGAGAGAGAGCGCT<br/>GCCGTTTGGTGTGTCGGGTGCTGTATATAGATATTGTTTTCGGTGTCGTG<br/>GTT</p>  | 134323 | D                 |
| 13 | @e8b63abc-adcd-4e8c-be66-e06fe415077a | <p>ACTACTTCAAAAATATCTACATACACAAATACAAAACCTCCACTAAAACTAAA<br/>AAAAATACAAACCCCTTCAATTCAAACCTAAACCAAAAAAACACCACCA<br/>ATAATCTCATTCCAAAAAAAATATAAATACATCACGACAAAACCGGGA<br/>AAAGCAACGCGAAGAGAAAAGAAGAAAACCGAAAAGAAAAGAGAGCAAA<br/>GAACAAGCGAAAAGAAGGAAAGGAGCAATCATTAAACAAACCAATAAAAA<br/>AAAAACCTAAATAATCACAAACCTCAAATAATTAECTACTATAATTCACAAT<br/>TAAAAAATTTAATCAAAAAATAAGGAGTATAGGTAGGATATGGGGAATA<br/>GAAGAGAAGTAGTGGATAAGTGAAGAGGAGTAAGTAAAAGTTAATAAGT<br/>AGTGAGGAGGTGAGAAGGGAGGGTGAATGTTTCTGCGTGTGTTGTT<br/>GCTTGCGCTGT</p> | 14123  | 2/3 of N<br>and A |
| 14 | @6412fafa-19e2-4486-8fba-d3b432232338 | <p>ACGGAGGACACCAAGACCACACGAGGCGTCTCTTTTTCTACTTCTATCTT<br/>ATCTTTACATCATTCA GATTGTGTGTTATTTTGTGATTGTTTGTGTTGG<br/>ATGGTTTGATTTATTGTGTGTTGGTTGTAGTTTGAATTGGTTTGATTTTA<br/>GATATTCCGGCTCCCTTTTTGCTGTGCCTCGGTCTTCTTTTTTCGCTGTC<br/>GTCCTTTTTCCGGAAATATGATTGTTGTGAGTGTATTATTGATCGCCGGC<br/>TGCTCTTTCCGGTTTGCCGTCTCCTTTTTGTCCTCGGCGGCTTCGCCTTCG<br/>CTTCTCTCTTTGCCCTCT</p>   | 412323 | A                 |