Supplementary Information for

High information capacity DNA-based data storage with augmented encoding characters using degenerate bases

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This file includes:

Definition of information capacity

The parameter, information capacity, is defined as the 'Bytes per bases[1]', 'The input information in bits divided by the number of synthesized DNA nucleotides, bits per nucleotide[2] ' or 'Bits per base[3]' in previous reports. Even though the unit for the term is slightly different between literature, the parameter is used to determine how much data can be encoded in the DNA sequence, not physical molecules, before synthesis of the DNA. Therefore, it is a value that can intuitively convey the performance of a data to DNA encoding algorithm. Moreover, current DNA synthesis methods synthesize more than billions of molecules per sequence, rather than as a single molecule, the parameter is directly related to the amount of use of the synthesizer and is related to the cost as written in this paper.

The value that reflects the number of physical molecule used is described as the "physical density" (ratio of the number of bytes encoded to the weight of the DNA library, Pbyte/g or Pbyte/mm^3) in both previous researches and our manuscript. The additional use of the parameter "physical density" is necessary to use multiple molecule copies when storing information (single molecule-based DNA-based data storage is not possible yet), due to errors or dropouts of DNA molecules that can occur during DNA synthesis, amplification and sequencing. For example, Erlich *and* Zielinski[2] experimentally showed that each designed oligonucleotide should be represented by at least ~1300 molecule copy on average to recover the data.

DNA synthesis

For the large-scale DNA synthesis, most-used synthesis method is the column-based, such as the Mermade platform from Bioautomation (https://bioautomation.com). Using the platform, only the increment of the number of the nozzle, which releases phosphoramidites on the column while synthesis, would be needed to synthesize degenerate base and no additional synthesis cost is not needed when the platform is set. The oligo provider, such as the one we used (Macrogen, https://www.macrogen.com) usually has the setup of the machine and therefore additional cost is not needed.

For the array synthesis, which used for synthesizing pooled oligonucleotide library, there are several methods used such as semiconductor-based (Customarray, http://www.customarrayinc.com), photolithography-based (Nimblegen), or inkjet-based (Agilent(https://www.genomics.agilent.com/article.jsp?pageId=2011) and Twist Bioscience (https://twistbioscience.com)). Among these technology, the inkjet-based array synthesis would be applied to the introduced platform, with the increment of the nozzle. However, for other methods, use of the degenerate base while synthesis could result the increment of the steps of the cycles while synthesis and the cost decrement would not be expected.

Data to DNA encoding: encoded files

For the first demo, we encoded a text file(txt) describing a brief introduction and member list of the laboratory to which the corresponding author belongs (Supplementary Fig. 1).

For the second demo, we encoded a thumbnail image of Hunminjeongum Manuscript (or Hunminjeongum Haerye, Supplementary Fig. 2), which is the UNESCO memory of the world registered documented heritage submitted by Republic of Korea in 1997(http://www.unesco.org/new/en/communication-and-information/memory-of-the-world/register/full-list-of-registered-heritage/registered-heritage-page-8/the-hunmin-chongum-manuscript/). Please see the link for further detail. Image file was resized to 692 × 574 and the file size was 135,393 bytes.

Data to DNA encoding ← 数据到DNA

We encode the data as DNA by using the process introduced by Grass et al.[4].

For the first demo:

1. Binary data was extracted from the file and the total data was grouped as 19bits. The 19bits were transformed into 2 DNA codons of Supplementary Table 1.

2. DNA sequence was fragmented as 42nt.

3. Addresses were attached. Address digits were transformed to DNA codons as described in Supplementary Table 2.

For the second demo:

1. Binary data was extracted from the image and the total data was fragmented after each 7bit was grouped. Fragmented length was 37 X 7 bit.

2. Reed-Solomon redundancy fragments were added.

3. Addresses were attached

4. Digits were transformed to DNA codons as described in Supplementary Table 2, 3

The encoded information is divided into fragments of 111 nt, and an address composed of non-degenerative nucleotides of a length of 9 nt is assigned thereto. Each fragment is supplemented with an adapter for amplification and sequencing, and the entire fragment is 160 nt in length. We also add Reed-Solomon based redundancy block in order to cope with errors that may occur during DNA synthesis, storage, and sequencing (Supplementary Fig. 4). Depending on the ratio of the size of the Reed-Solomon block to the information block size, the error correction capability and the data density are traded-off. We designed 9 redundancy fragments in 118 information fragments to correct 3.5% false information or 7% missing information in maximum.

Data to DNA encoding: Reed-Solomon error correction

We only use the outer error correction code (Supplementary Fig. 4). Reed Solomon codes with parameters (n-total length, k-message length) follow the relation:

$2e + f \leq n-k$, e: number of errors f: number of erasures

For all Reed-Solomon error correction, we use base 128 number system (or see each 7bit as digit) to match with the codon with degenerate bases.

4183 fragments in total were generated from the data fragmentizing and address attaching process. Fragments were divided into 35 blocks of 118 fragments and one block of 53 fragments. We added 9 redundancy fragments in the blocks of 118 fragments. Also, 5 redundancy fragments were added in block of 53 fragments. Finally, 4503 fragments in total were generated.

Data to DNA encoding: Address attaching

Each fragment was matched to addresses consisting of three digits of 48 base number system, which is matched with codon without degenerative bases (Supplementary Table 2). For the second demo, the system can encode about 110,000 fragments, which is about 3 MB of encoding capability. To encode data in few gigabytes, additional space of 6nt (or 2 more digit) is needed. For the second demo, address sequence was reversed, to avoid error in cluster identification during Illumina sequencing due to the homo polymer sequence.

Data to DNA encoding: Codon table

1. We have created a codon table with a degenerative sequence added. The sequence of the last position of the 3 nt codons was not the same as that of the front sequence in order to avoid homopolymer of 4 base pairs or more. The W and S sequences correspond to both A, T and C, G respectively. This gives us total 750 codons (Supplementary Table 1) for

the first demo and 132 codons for the second demo (Supplementary Table 3). Data digits generated in previous step were transformed using the table.

2. Each fragment was matched to addresses consisting of three 48 codons without degenerative (Supplementary Table 2). Address digits generated in the previous step were transformed using the table.

Data to DNA encoding: Adaptor sequence

The adapter sequence was made by trimming the sequencing primer of the Illumina system.

Forward adaptor: ACACGACGCTCTTCCGATCT

Reverse adaptor: AGATCGGAAGAGCACACGTC

Synthesized oligonucleotide pool quantification

qPCR was utilized for quantification of synthesized DNA oligonucleotide pool. Samples were analyzed by qPCR (FAST 7500, Applied Biosystems) using a KAPA SYBR® FAST qPCR Master Mix (2X) Kit. Sample mix of 10 µL master mix, 7 µL of PCR grade water, 1 µL of a 10 µM primer stock of forward and reverse each, 1 µL oligo pool solution was used. We followed standard thermal protocol from the manual. Sequences of the forward and reverse primer are:

Multiplexing Read 1 Sequencing Primer (Forward)

5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT

Multiplexing Read 2 Sequencing Primer (Reverse)

5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Relative sample quantification was accomplished by interpolation from a standard curve, generated from DNA samples of known concentration. The synthesized DNA library consisted of 1974204 molecules per microliter (438 molecules per fragment). Reported values are averaged from the three replicates (standard deviation: 81969). We used 1ul sample of pooled oligonucleotide synthesized.

DNA to Data decoding: Overview

1. Pair-end reads of the raw Fastq file were stitched using the PEAR.
2. NGS reads with the appropriate lengths were filtered.
3. Duplicated reads were removed.
4. Reads were categorized by addresses.
5. Representing sequence (include degenerate base) was figured.
6. The DNA codon was transformed to digit
7. Error correction using Reed-Solomon code was performed

DNA to Data decoding: length filtering, duplication removing, categorizing reads by address

1. Pair-end reads of the raw Fastq file were stitched using the PEAR. assembly length value (-m, -n) were specified as 47 and 43 for the first demo, since the designed length of the DNA is shorter than its default value.

2. For the first demo, reads were filtered based on the quality score in address position (Q-score over 40).
3. Only NGS reads with the appropriate length (45 for the first demo, 120nt for the second) were used for decoding.
4. Reads were categorized by address. The address sequences were decoded using Table S2.
5. Duplicated reads were removed

Supplementary Table 4 describes the number of reads obtained in step 1, 3, 5 in Raw NGS data. To see the assignment error between the address, we checked the false assignment rate from the data of first demo. After we categorized the read by address, we checked the whether the read is appropriately categorized by comparing the original design. In this step, if the read is more than 50% different from the design (in the case of degenerate base, all the combination in the base are considered when compared), we considered this as the false assign due to the crosstalk. The average rate of false assignment is 0.18%, and the maximum value was 0.93%.

DNA to Data decoding: Transform the DNA codon to digit

1. DNA sequences were divided into 3nt and transformed to the 7bit digit, by following the codon listed in Supplementary Table 1 or 3.

If there was no codon matched to the digit, it was categorized as 'erased' for further error correction in next step.

DNA to Data decoding: Error correction using Reed-Solomon code

We corrected error by process introduced from Grass et al[4].

1. For the 2.0 bits/character model, outer Reed-Solomon code was used for error correction (Supplementary Fig. 4).

Binomial distribution confirmation for Supplementary Fig. 6

1. Fragments with more than 50 read calls were selected. After that, 50 reads were randomly sampled per fragment.
2. If we draw a histogram of the elements that form the degenerate base (Supplementary Fig. 6, blue line), the histogram could be fit into the binomial distribution (red line), which follows the equation:

$$P(x) = p^x(1-p)^{n-x}\binom{n}{x}$$

in which, n is 50. Also, from the fitted distribution, we could extract value p, which was used from the simulation.

Calculation of physical density

Physical density of the DNA-based data storage is ratio of the number of bytes encoded to the weight of the DNA library. The calculation method is,

$$Physical\ density\ (Pbyte/g) = \frac{Quantity\ of\ stored\ data}{Weight\ of\ DNA\ molecule\ used\ (g/single\ molecule) * number\ of\ molecules\ used\ for\ storage}$$

From this, we calculate the physical density of our result as;

1) Molecular weight of DNA molecule used: (length of oligonucleotide) * 303.7 + 79 (g/mol) :
   - ⇨ For experiment used 15 encoding characters: 85 nucleotides
   - ⇨ For experiment used 6 encoding characters: 160 nucleotides
   - ⇨ Gram per mole was converted to gram per single molecule
2) Number of molecules used for storage:
   - ⇨ For experiment used 15 encoding characters: 40 (library number) * 800 (molecular copy)
   - ⇨ For experiment used 6 encoding characters: 4503 (library number) * 438 (molecular copy)
3) Quantity of stored data:
   - ⇨ For experiment used 15 encoding characters: 854 bytes
   - ⇨ For experiment used 15 encoding characters: 135.4 Kbytes

Cost projection and calculation of information capacity

1. If 15 encoding characters are used for data storage, 750 3-base-codons are generated, by using the method described above. Also, for the case using 26 encoding characters, 1932 codons could be generated. Here, the information capacity is obtained as:

$$\frac{\log_2(Number\ of\ codons)}{3}\ bits/character$$

2. The capacity should be multiplied by the reduction factor of the information capacity due to the address used in the design. The reduction factor is:

$$\frac{148\ \text{(length excluding addresses)}}{160\ \text{(length}\ including\ addresses)}$$

3. The resulting value is multiplied by factor of 1 / 1.1 to give the error correction of 10%. This gives us the information capacity described in the text.
4. To reflect the length of the adapter for PCR amplification, the resulting value should be multiplied by:

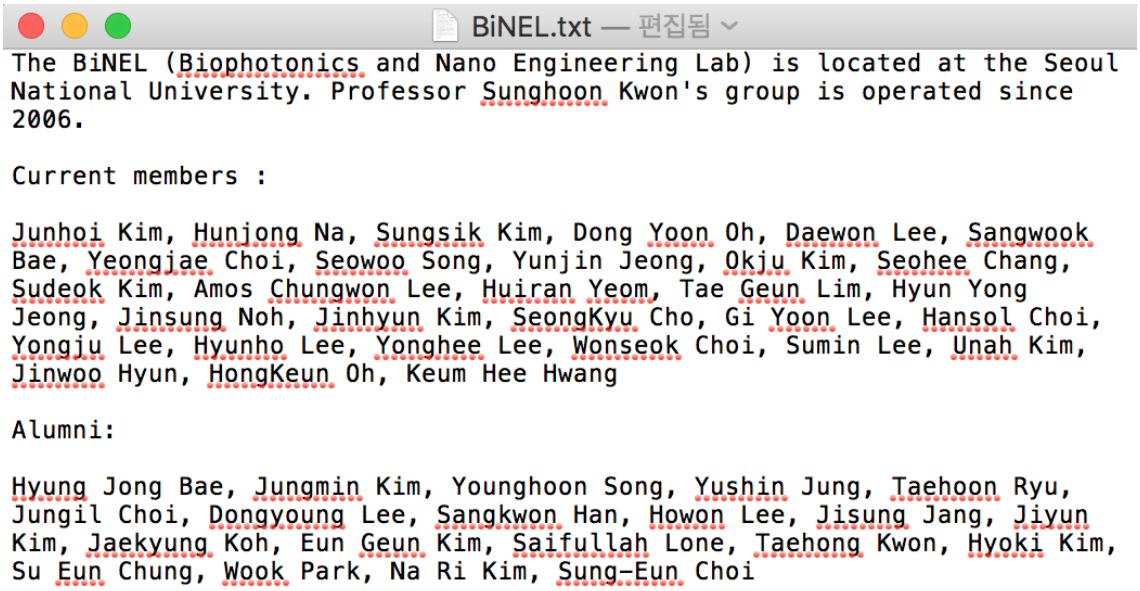
$$\frac{160\ \text{(length used for information storage)}}{200\ \text{(full length when adapter is used)}}$$

5. The number of nucleotides storing 1MB is calculated by:

$$\frac{1\text{MB}}{\text{Information capacity reflecting adapter}}$$

The information capacity of other studies follows the values summarized in previous studies.

The estimated number of nucleotides is multiplied by the synthesis cost per nucleotides to get the price described in the manuscript. Also, NGS cost was estimated using the estimated number of nucleotides and the NGS coverage used in previous studies.



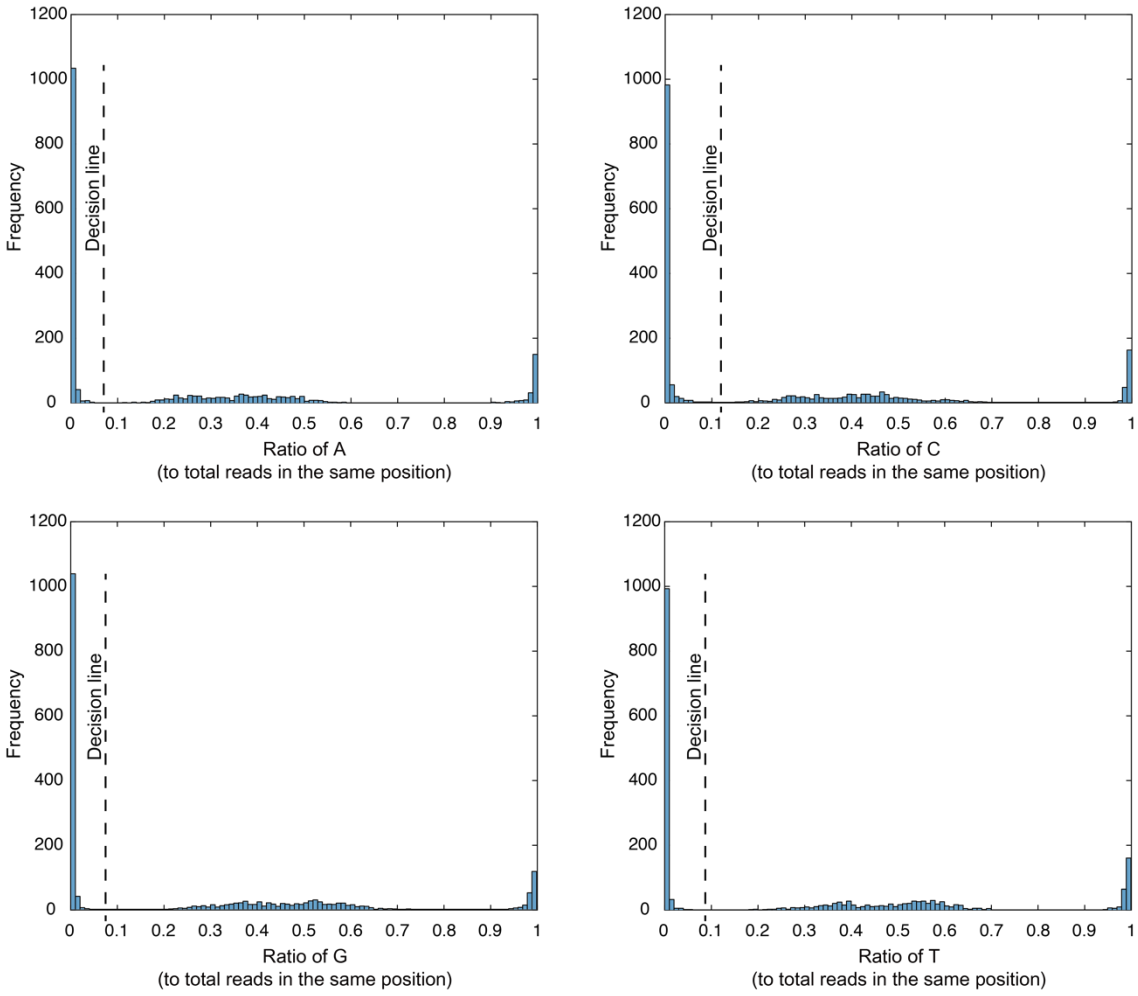
Supplementary Figure S1

The text file used for encoding in the first demonstration. The content of the text file is a member list of the research group.

Supplementary Figure S2

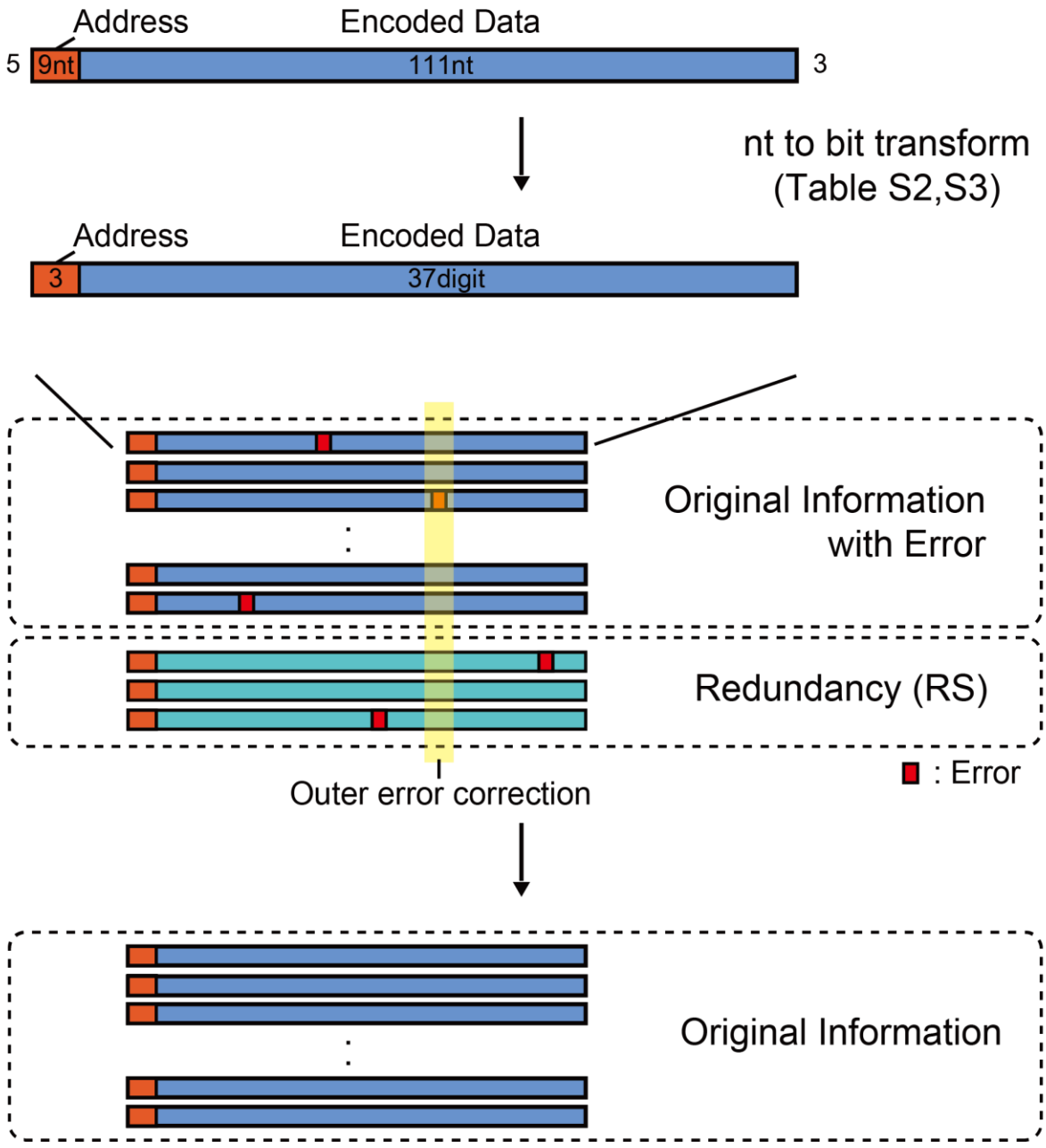
The thumbnail image of Hunminjeongum Manuscript (or Hunminjeongum Haerye), which is the UNESCO memory of the world registered documented heritage submitted by Republic of Korea in 1997.
(**http://heritage.go.kr/heri/cul/culSelectDetail.do?region=1&searchCondition=&searchCondition2=&s_kdcd=11&s_ctcd=00&ccbaKdcd=11&ccbaAsno=00700000&ccbaCtcd=11&ccbaCpno=1111100700000&ccbaCndt=&stCcbaAsno=70&endCcbaAsno=70&stCcbaAsdt=&endCcbaAsdt=&ccbaPcd1=99&culPageNo=1&chGubun=&header=view&returnUrl=%2Fheri%2Fcul%2FculSelectViewList.do&sCond=**)

This image was originally posted by the Cultural Heritage Administration of the Republic of Korea under the Korea Open Government License (https://www.mcst.go.kr/kor/s_open/kogl/koglType.jsp?pTab=05) type 1.



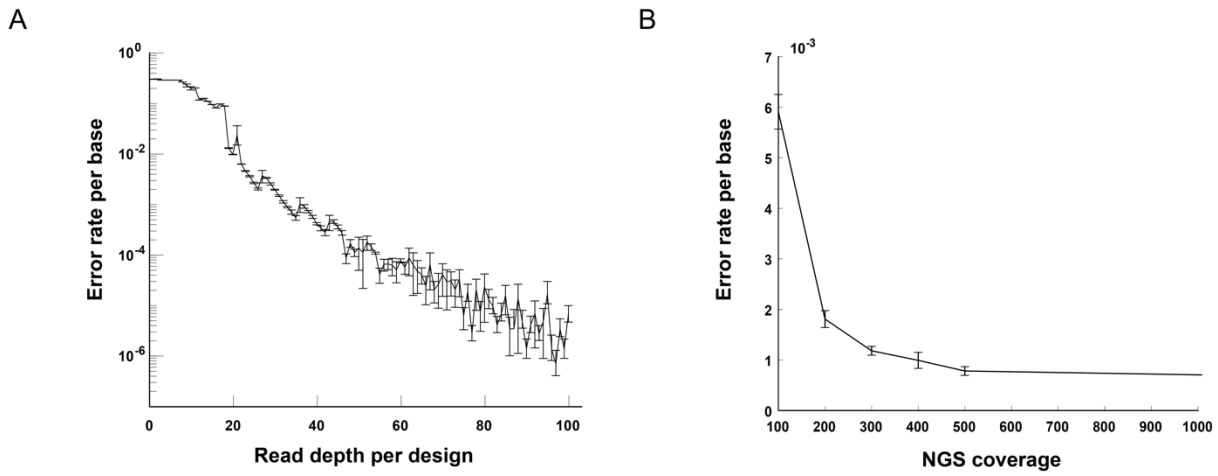
Supplementary Figure S3

The histogram of the ratio of base in a position in the sequence.



Supplementary Figure S4

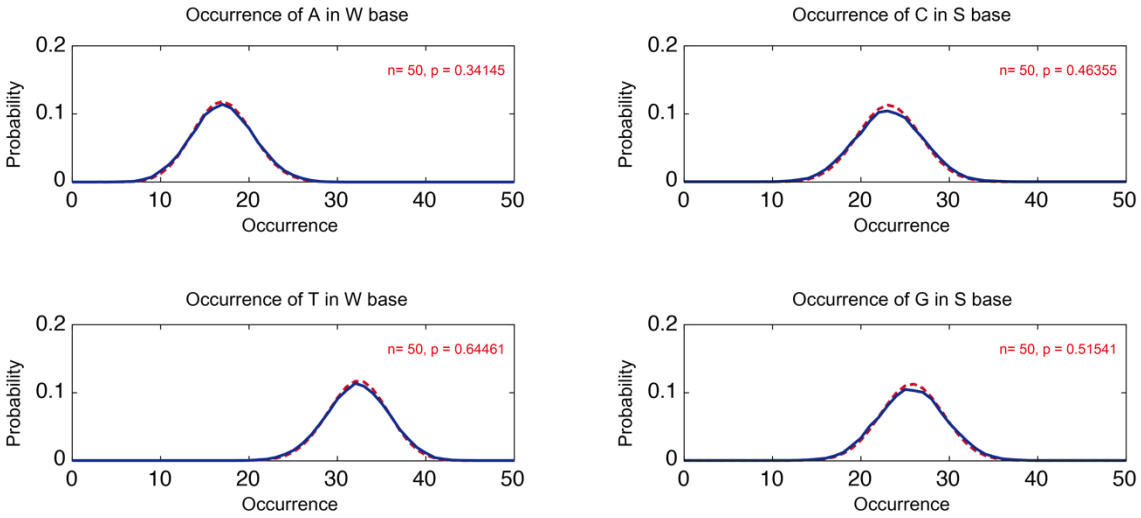
Structure of the data fragment (without adaptor) and error correcting scheme for the 2.0 bits/character model.



Supplementary Figure S5

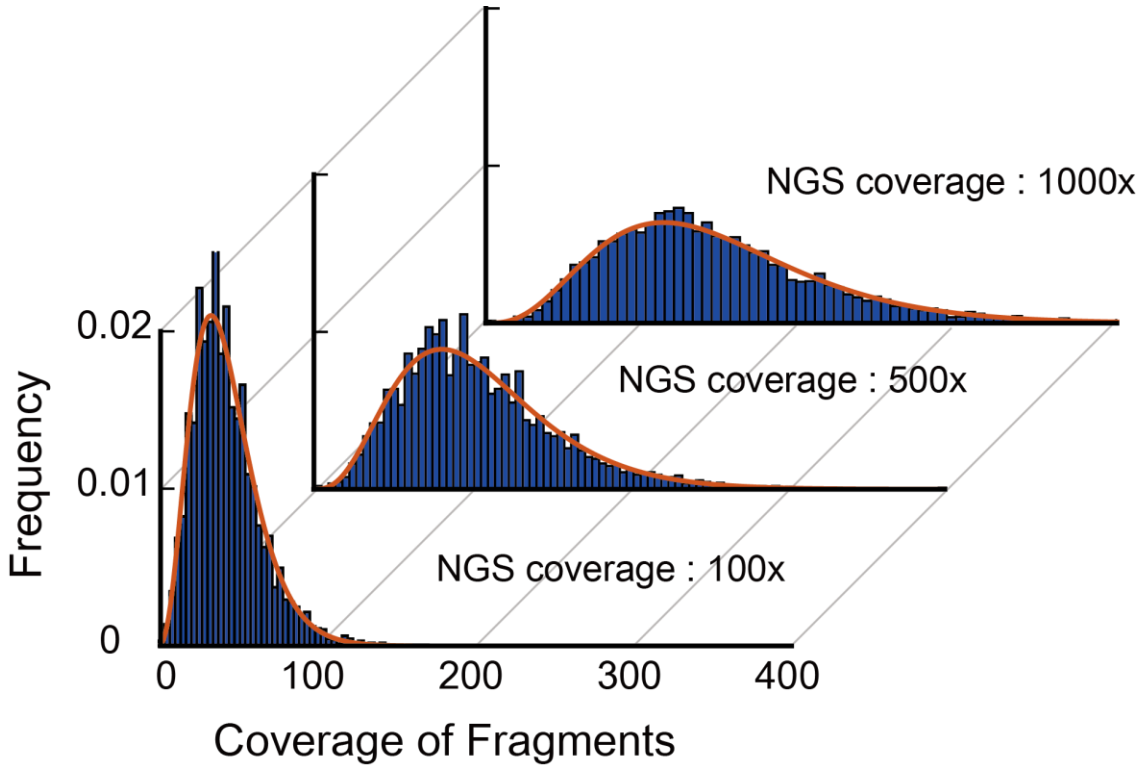
A. The error rate per sequenced base-pairs according to depth of different designs, from which the reads were randomly and uniformly sampled (See supplementary information for details). The standard deviations were obtained by repeating the random sampling 10 times. Bars represent one standard deviation from the mean efficiency.

B. The error rate of synthesized base pairs in fragments of specific average coverage over the total fragments. The standard deviations were obtained by repeating the random sampling 10 times. Bars represent one standard deviation from the mean efficiency.



Supplementary Figure S6

Occurrence of original nucleotide that comprises a degenerate base. Blue: histogram, Red: Fitted binomial graph. This is experimental data from the second demonstration where we used W and S.



Supplementary Figure S7

Profile of uneven representation of fragments due to the PCR bias, which was obtained from the second experiment using six encoding alphabets. Red, negative binomial fit following previous reports

Supplementary Table S1

Codon table with degenerative sequence used for data encoding.

ACA	ZTA	OYA	USA	DIA	BXA
CCA	XTA	PYA	ISA	NIA	VXA
TCA	AGA	ZYA	OSA	UIA	DXA
GCA	CGA	XYA	PSA	IIA	NXA
RCA	TGA	AKA	ZSA	OIA	UXA
YCA	GGA	CKA	XSA	PIA	IXA
MCA	RGA	TKA	ABA	ZIA	OXA
KCA	YGA	GKA	CBA	XIA	PXA
WCA	MGA	RKA	TBA	APA	ZXA
SCA	KGA	YKA	GBA	CPA	XXA
HCA	WGA	MKA	RBA	TPA	AAC
BCA	SGA	KKA	YBA	GPA	CAC
VCA	HGA	WKA	MBA	RPA	TAC
DCA	BGA	SKA	KBA	YPA	GAC
NCA	VGA	HKA	WBA	MPA	RAC
UCA	DGA	BKA	SBA	KPA	YAC
ICA	NGA	VKA	HBA	WPA	MAC
OCA	UGA	DKA	BBA	SPA	KAC
PCA	IGA	NKA	VBA	HPA	WAC
ZCA	OGA	UKA	DBA	BPA	SAC
XCA	PGA	IKA	NBA	VPA	HAC
ATA	ZGA	OKA	UBA	DPA	BAC
CTA	XGA	PKA	IBA	NPA	VAC
TTA	AYA	ZKA	OBA	UPA	DAC
GTA	CYA	XKA	PBA	IPA	NAC
RTA	TYA	ASA	ZBA	OPA	UAC
YTA	GYA	CSA	XBA	PPA	IAC
MTA	RYA	TSA	AIA	ZPA	OAC
KTA	YYA	GSA	CIA	XPA	PAC
WTA	MYA	RSA	TIA	AXA	ZAC
STA	KYA	YSA	GIA	CXA	XAC
HTA	WYA	MSA	RIA	TXA	ATC
BTA	SYA	KSA	YIA	GXA	CTC
VTA	HYA	WSA	MIA	RXA	TTC
DTA	BYA	SSA	KIA	YXA	GTC
NTA	VYA	HSA	WIA	MXA	RTC
UTA	DYA	BSA	SIA	KXA	YTC
ITA	NYA	VSA	HIA	WXA	MTC

OTA	UYA	DSA	BIA	SXA	KTC
PTA	IYA	NSA	VIA	HXA	WTC

STC	KRC	YWC	GUC	CZC	XAT
HTC	WRC	MWC	RUC	TZC	ACT
BTC	SRC	KWC	YUC	GZC	CCT
VTC	HRC	WWC	MUC	RZC	TCT
DTC	BRC	SWC	KUC	YZC	GCT
NTC	VRC	HWC	WUC	MZC	RCT
UTC	DRC	BWC	SUC	KZC	YCT
ITC	NRC	VWC	HUC	WZC	MCT
OTC	URC	DWC	BUC	SZC	KCT
PTC	IRC	NWC	VUC	HZC	WCT
ZTC	ORC	UWC	DUC	BZC	SCT
XTC	PRC	IWC	NUC	VZC	HCT
AGC	ZRC	OWC	UUC	DZC	BCT
CGC	XRC	PWC	IUC	NZC	VCT
TGC	AKC	ZWC	OUC	UZC	DCT
GGC	CKC	XWC	PUC	IZC	NCT
RGC	TKC	ADC	ZUC	OZC	UCT
YGC	GKC	CDC	XUC	PZC	ICT
MGC	RKC	TDC	APC	ZZC	OCT
KGC	YKC	GDC	CPC	XZC	PCT
WGC	MKC	RDC	TPC	AAT	ZCT
SGC	KKC	YDC	GPC	CAT	XCT
HGC	WKC	MDC	RPC	TAT	AGT
BGC	SKC	KDC	YPC	GAT	CGT
VGC	HKC	WDC	MPC	RAT	TGT
DGC	BKC	SDC	KPC	YAT	GGT
NGC	VKC	HDC	WPC	MAT	RGT
UGC	DKC	BDC	SPC	KAT	YGT
IGC	NKC	VDC	HPC	WAT	MGT
OGC	UKC	DDC	BPC	SAT	KGT
PGC	IKC	NDC	VPC	HAT	WGT
ZGC	OKC	UDC	DPC	BAT	SGT
XGC	PKC	IDC	NPC	VAT	HGT
ARC	ZKC	ODC	UPC	DAT	BGT
CRC	XKC	PDC	IPC	NAT	VGT
TRC	AWC	ZDC	OPC	UAT	DGT
GRC	CWC	XDC	PPC	IAT	NGT
RRC	TWC	AUC	ZPC	OAT	UGT
YRC	GWC	CUC	XPC	PAT	IGT
MRC	RWC	TUC	AZC	ZAT	OGT

PGT	IMT	NVT	VOT	HAG	WTG
ZGT	OMT	UVT	DOT	BAG	STG
XGT	PMT	IVT	NOT	VAG	HTG
ART	ZMT	OVT	UOT	DAG	BTG
CRT	XMT	PVT	IOT	NAG	VTG
TRT	AST	ZVT	OOT	UAG	DTG
GRT	CST	XVT	POT	IAG	NTG
RRT	TST	AUT	ZOT	OAG	UTG
YRT	GST	CUT	XOT	PAG	ITG
MRT	RST	TUT	AXT	ZAG	OTG
KRT	YST	GUT	CXT	XAG	PTG
WRT	MST	RUT	TXT	ACG	ZTG
SRT	KST	YUT	GXT	CCG	XTG
HRT	WST	MUT	RXT	TCG	AYG
BRT	SST	KUT	YXT	GCG	CYG
VRT	HST	WUT	MXT	RCG	TYG
DRT	BST	SUT	KXT	YCG	GYG
NRT	VST	HUT	WXT	MCG	RYG
URT	DST	BUT	SXT	KCG	YYG
IRT	NST	VUT	HXT	WCG	MYG
ORT	UST	DUT	BXT	SCG	KYG
PRT	IST	NUT	VXT	HCG	WYG
ZRT	OST	UUT	DXT	BCG	SYG
XRT	PST	IUT	NXT	VCG	HYG
AMT	ZST	OUT	UXT	DCG	BYG
CMT	XST	PUT	IXT	NCG	VYG
TMT	AVT	ZUT	OXT	UCG	DYG
GMT	CVT	XUT	PXT	ICG	NYG
RMT	TVT	AOT	ZXT	OCG	UYG
YMT	GVT	COT	XXT	PCG	IYG
MMT	RVT	TOT	AAG	ZCG	OYG
KMT	YVT	GOT	CAG	XCG	PYG
WMT	MVT	ROT	TAG	ATG	ZYG
SMT	KVT	YOT	GAG	CTG	XYG
HMT	WVT	MOT	RAG	TTG	AMG
BMT	SVT	KOT	YAG	GTG	CMG
VMT	HVT	WOT	MAG	RTG	TMG
DMT	BVT	SOT	KAG	YTG	GMG
NMT	VVT	HOT	WAG	MTG	RMG
UMT	DVT	BOT	SAG	KTG	YMG

MMG	RHG	TOG	ACR	ZTR	OIR
KMG	YHG	GOG	CCR	XTR	PIR
WMG	MHG	ROG	TCR	AYR	ZIR
SMG	KHG	YOG	GCR	CYR	XIR
HMG	WHG	MOG	RCR	TYR	AAY
BMG	SHG	KOG	YCR	GYR	CAY
VMG	HHG	WOG	MCR	RYR	TAY
DMG	BHG	SOG	KCR	YYR	GAY
NMG	VHG	HOG	WCR	MYR	RAY
UMG	DHG	BOG	SCR	KYR	YAY
IMG	NHG	VOG	HCR	WYR	MAY
OMG	UHG	DOG	BCR	SYR	KAY
PMG	IHG	NOG	VCR	HYR	WAY
ZMG	OHG	UOG	DCR	BYR	SAY
XMG	PHG	IOG	NCR	VYR	HAY
AWG	ZHG	OOG	UCR	DYR	BAY
CWG	XHG	POG	ICR	NYR	VAY
TWG	AIG	ZOG	OCR	UYR	DAY
GWG	CIG	XOG	PCR	IYR	NAY
RWG	TIG	AZG	ZCR	OYR	UAY
YWG	GIG	CZG	XCR	PYR	IAY
MWG	RIG	TZG	ATR	ZYR	OAY
KWG	YIG	GZG	CTR	XYR	PAY
WWG	MIG	RZG	TTR	AIR	ZAY
SWG	KIG	YZG	GTR	CIR	XAY
HWG	WIG	MZG	RTR	TIR	AGY
BWG	SIG	KZG	YTR	GIR	CGY
VWG	HIG	WZG	MTR	RIR	TGY
DWG	BIG	SZG	KTR	YIR	GGY
NWG	VIG	HZG	WTR	MIR	RGY
UWG	DIG	BZG	STR	KIR	YGY
IWG	NIG	VZG	HTR	WIR	MGY
OWG	UIG	DZG	BTR	SIR	KGY
PWG	IIG	NZG	VTR	HIR	WGY
ZWG	OIG	UZG	DTR	BIR	SGY
XWG	PIG	IZG	NTR	VIR	HGY
AHG	ZIG	OZG	UTR	DIR	BGY
CHG	XIG	PZG	ITR	NIR	VGY
THG	AOG	ZZG	OTR	UIR	DGY
GHG	COG	XZG	PTR	IIR	NGY

UGY	DUY	BGM	SPM	KCK	YOK
IGY	NUY	VGM	HPM	WCK	MOK
OGY	UUY	DGM	BPM	SCK	KOK
PGY	IUY	NGM	VPM	HCK	WOK
ZGY	OUY	UGM	DPM	BCK	SOK
XGY	PUY	IGM	NPM	VCK	HOK
ARY	ZUY	OGM	UPM	DCK	BOK
CRY	XUY	PGM	IPM	NCK	VOK
TRY	ATM	ZGM	OPM	UCK	DOK
GRY	CTM	XGM	PPM	ICK	NOK
RRY	TTM	AKM	ZPM	OCK	UOK
YRY	GTM	CKM	XPM	PCK	IOK
MRY	RTM	TKM	AAK	ZCK	OOK
KRY	YTM	GKM	CAK	XCK	POK
WRY	MTM	RKM	TAK	AMK	ZOK
SRY	KTM	YKM	GAK	CMK	XOK
HRY	WTM	MKM	RAK	TMK	ACW
BRY	STM	KKM	YAK	GMK	CCW
VRY	HTM	WKM	MAK	RMK	TCW
DRY	BTM	SKM	KAK	YMK	GCW
NRY	VTM	HKM	WAK	MMK	RCW
URY	DTM	BKM	SAK	KMK	YCW
IRY	NTM	VKM	HAK	WMK	MCW
ORY	UTM	DKM	BAK	SMK	KCW
PRY	ITM	NKM	VAK	HMK	WCW
ZRY	OTM	UKM	DAK	BMK	SCW
XRY	PTM	IKM	NAK	VMK	HCW
AUY	ZTM	OKM	UAK	DMK	BCW
CUY	XTM	PKM	IAK	NMK	VCW
TUY	AGM	ZKM	OAK	UMK	DCW
GUY	CGM	XKM	PAK	IMK	NCW
RUY	TGM	APM	ZAK	OMK	UCW
YUY	GGM	CPM	XAK	PMK	ICW
MUY	RGM	TPM	ACK	ZMK	OCW
KUY	YGM	GPM	CCK	XMK	PCW
WUY	MGM	RPM	TCK	AOK	ZCW
SUY	KGM	YPM	GCK	COK	XCW
HUY	WGM	MPM	RCK	TOK	AGW
BUY	SGM	KPM	YCK	GOK	CGW
VUY	HGM	WPM	MCK	ROK	TGW

GGW	CXW	XAS	PWS	IGH	NTV
RGW	TXW	ATS	ZWS	OGH	UTV
YGW	GXW	CTS	XWS	PGH	ITV
MGW	RXW	TTS	AZS	ZGH	OTV
KGW	YXW	GTS	CZS	XGH	PTV
WGW	MXW	RTS	TZS	AAB	ZTV
SGW	KXW	YTS	GZS	CAB	XTV
HGW	WXW	MTS	RZS	TAB	ACD
BGW	SXW	KTS	YZS	GAB	CCD
VGW	HXW	WTS	MZS	RAB	TCD
DGW	BXW	STS	KZS	YAB	GCD
NGW	VXW	HTS	WZS	MAB	RCD
UGW	DXW	BTS	SZS	KAB	YCD
IGW	NXW	VTS	HZS	WAB	MCD
OGW	UXW	DTS	BZS	SAB	KCD
PGW	IXW	NTS	VZS	HAB	WCD
ZGW	OXW	UTS	DZS	BAB	SCD
XGW	PXW	ITS	NZS	VAB	HCD
ASW	ZXW	OTS	UZS	DAB	BCD
CSW	XXW	PTS	IZS	NAB	VCD
TSW	AAS	ZTS	OZS	UAB	DCD
GSW	CAS	XTS	PZS	IAB	NCD
RSW	TAS	AWS	ZZS	OAB	UCD
YSW	GAS	CWS	XZS	PAB	ICD
MSW	RAS	TWS	AGH	ZAB	OCD
KSW	YAS	GWS	CGH	XAB	PCD
WSW	MAS	RWS	TGH	ATV	ZCD
SSW	KAS	YWS	GGH	CTV	XCD
HSW	WAS	MWS	RGH	TTV	ACU
BSW	SAS	KWS	YGH	GTV	CCU
VSW	HAS	WWS	MGH	RTV	TCU
DSW	BAS	SWS	KGH	YTV	GCU
NSW	VAS	HWS	WGH	MTV	RCU
USW	DAS	BWS	SGH	KTV	YCU
ISW	NAS	VWS	HGH	WTV	MCU
OSW	UAS	DWS	BGH	STV	KCU
PSW	IAS	NWS	VGH	HTV	WCU
ZSW	OAS	UWS	DGH	BTV	SCU
XSW	PAS	IWS	NGH	VTV	HCU
AXW	ZAS	OWS	UGH	DTV	BCU

VCU	HYU	WAI	MRI	RTO	TKO
DCU	BYU	SAI	KRI	YTO	GKO
NCU	VYU	HAI	WRI	MTO	RKO
UCU	DYU	BAI	SRI	KTO	YKO
ICU	NYU	VAI	HRI	WTO	MKO
OCU	UYU	DAI	BRI	STO	KKO
PCU	IYU	NAI	VRI	HTO	WKO
ZCU	OYU	UAI	DRI	BTO	SKO
XCU	PYU	IAI	NRI	VTO	HKO
ATU	ZYU	OAI	URI	DTO	BKO
CTU	XYU	PAI	IRI	NTO	VKO
TTU	AIU	ZAI	ORI	UTO	DKO
GTU	CIU	XAI	PRI	ITO	NKO
RTU	TIU	AGI	ZRI	OTO	UKO
YTU	GIU	CGI	XRI	PTO	IKO
MTU	RIU	TGI	AUI	ZTO	OKO
KTU	YIU	GGI	CUI	XTO	PKO
WTU	MIU	RGI	TUI	AGO	ZKO
STU	KIU	YGI	GUI	CGO	XKO
HTU	WIU	MGI	RUI	TGO	APO
BTU	SIU	KGI	YUI	GGO	CPO
VTU	HIU	WGI	MUI	RGO	TPO
DTU	BIU	SGI	KUI	YGO	GPO
NTU	VIU	HGI	WUI	MGO	RPO
UTU	DIU	BGI	SUI	KGO	YPO
ITU	NIU	VGI	HUI	WGO	MPO
OTU	UIU	DGI	BUI	SGO	KPO
PTU	IIU	NGI	VUI	HGO	WPO
ZTU	OIU	UGI	DUI	BGO	SPO
XTU	PIU	IGI	NUI	VGO	HPO
AYU	ZIU	OGI	UUI	DGO	BPO
CYU	XIU	PGI	IUI	NGO	VPO
TYU	AAI	ZGI	OUI	UGO	DPO
GYU	CAI	XGI	PUI	IGO	NPO
RYU	TAI	ARI	ZUI	OGO	UPO
YYU	GAI	CRI	XUI	PGO	IPO
MYU	RAI	TRI	ATO	ZGO	OPO
KYU	YAI	GRI	CTO	XGO	PPO
WYU	MAI	RRI	TTO	AKO	ZPO
SYU	KAI	YRI	GTO	CKO	XPO

AAP	ZCP	OOP	UGZ	DXZ	BTX
CAP	XCP	POP	IGZ	NXZ	VTX
TAP	AMP	ZOP	OGZ	UXZ	DTX
GAP	CMP	XOP	PGZ	IXZ	NTX
RAP	TMP	ACZ	ZGZ	OXZ	UTX
YAP	GMP	CCZ	XGZ	PXZ	ITX
MAP	RMP	TCZ	ASZ	ZXZ	OTX
KAP	YMP	GCZ	CSZ	XXZ	PTX
WAP	MMP	RCZ	TSZ	AAX	ZTX
SAP	KMP	YCZ	GSZ	CAX	XTX
HAP	WMP	MCZ	RSZ	TAX	AWX
BAP	SMP	KCZ	YSZ	GAX	CWX
VAP	HMP	WCZ	MSZ	RAX	TWX
DAP	BMP	SCZ	KSZ	YAX	GWX
NAP	VMP	HCZ	WSZ	MAX	RWX
UAP	DMP	BCZ	SSZ	KAX	YWX
IAP	NMP	VCZ	HSZ	WAX	MWX
OAP	UMP	DCZ	BSZ	SAX	KWX
PAP	IMP	NCZ	VSZ	HAX	WWX
ZAP	OMP	UCZ	DSZ	BAX	SWX
XAP	PMP	ICZ	NSZ	VAX	HWX
ACP	ZMP	OCZ	USZ	DAX	BWX
CCP	XMP	PCZ	ISZ	NAX	VWX
TCP	AOP	ZCZ	OSZ	UAX	DWX
GCP	COP	XCZ	PSZ	IAX	NWX
RCP	TOP	AGZ	ZSZ	OAX	UWX
YCP	GOP	CGZ	XSZ	PAX	IWX
MCP	ROP	TGZ	AXZ	ZAX	OWX
KCP	YOP	GGZ	CXZ	XAX	PWX
WCP	MOP	RGZ	TXZ	ATX	ZWX
SCP	KOP	YGZ	GXZ	CTX	XWX
HCP	WOP	MGZ	RXZ	TTX	AZX
BCP	SOP	KGZ	YXZ	GTX	CZX
VCP	HOP	WGZ	MXZ	RTX	TZX
DCP	BOP	SGZ	KXZ	YTX	GZX
NCP	VOP	HGZ	WXZ	MTX	RZX
UCP	DOP	BGZ	SXZ	KTX	YZX
ICP	NOP	VGZ	HXZ	WTX	MZX
OCP	UOP	DGZ	BXZ	STX	KZX
PCP	IOP	NGZ	VXZ	HTX	WZX

SZX
HZX
BZX
VZX
DZX
NZX
UZX
IZX
OZX
PZX
ZZX
XZX

Supplementary Table S2

Codon table without degenerative sequence used for address encoding.

0	ACA	12	AAC	24	AAT	36	AAG
1	CCA	13	CAC	25	CAT	37	CAG
2	TCA	14	TAC	26	TAT	38	TAG
3	GCA	15	GAC	27	GAT	39	GAG
4	ATA	16	ATC	28	ACT	40	ACG
5	CTA	17	CTC	29	CCT	41	CCG
6	TTA	18	TTC	30	TCT	42	TCG
7	GTA	19	GTC	31	GCT	43	GCG
8	AGA	20	AGC	32	AGT	44	ATG
9	CGA	21	CGC	33	CGT	45	CTG
10	TGA	22	TGC	34	TGT	46	TTG
11	GGA	23	GGC	35	GGT	47	GTG

Supplementary Table S3

Codon table with degenerative sequence W and S, used for data encoding.

Data	Codon	Data	Codon	Data	Codon	Data	Codon
1	ACA	33	TTC	65	WGT	97	ACW
2	CCA	34	GTC	66	SGT	98	CCW
3	TCA	35	WTC	67	AST	99	TCW
4	GCA	36	STC	68	CST	100	GCW
5	WCA	37	AGC	69	TST	101	WCW
6	SCA	38	CGC	70	GST	102	SCW
7	ATA	39	TGC	71	WST	103	AGW
8	CTA	40	GGC	72	SST	104	CGW
9	TTA	41	WGC	73	AAG	105	TGW
10	GTA	42	SGC	74	CAG	106	GGW
11	WTA	43	AWC	75	TAG	107	WGW
12	STA	44	CWC	76	GAG	108	SGW
13	AGA	45	TWC	77	WAG	109	ASW
14	CGA	46	GWC	78	SAG	110	CSW
15	TGA	47	WWC	79	ACG	111	TSW
16	GGA	48	SWC	80	CCG	112	GSW
17	WGA	49	AAT	81	TCG	113	WSW
18	SGA	50	CAT	82	GCG	114	SSW
19	ASA	51	TAT	83	WCG	115	AAS
20	CSA	52	GAT	84	SCG	116	CAS
21	TSA	53	WAT	85	ATG	117	TAS
22	GSA	54	SAT	86	CTG	118	GAS
23	WSA	55	ACT	87	TTG	119	WAS
24	SSA	56	CCT	88	GTG	120	SAS
25	AAC	57	TCT	89	WTG	121	ATS
26	CAC	58	GCT	90	STG	122	CTS
27	TAC	59	WCT	91	AWG	123	TTS
28	GAC	60	SCT	92	CWG	124	GTS
29	WAC	61	AGT	93	TWG	125	WTS
30	SAC	62	CGT	94	GWG	126	STS
31	ATC	63	TGT	95	WWG	127	AWS
32	CTC	64	GGT	96	SWG	128	CWS

Supplementary Table S4

Number of NGS read that acquired from each step.

	3.37 bits/character (85nt)		2 bits/character (160nt)	
Before Assemble	162707	100%	5847136	100%
Assemble	158260	97%	5660429	97%
Length filter	127082	78%	2928269	50%
Heterogeneous reads	26675	16%	1083343	19%

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