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SUPPLEMENTARY INFORMATION

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A DNA-of-things storage architecture to create materials with embedded memory

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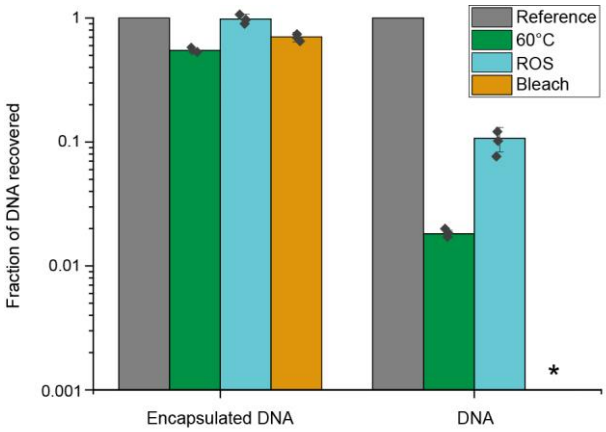
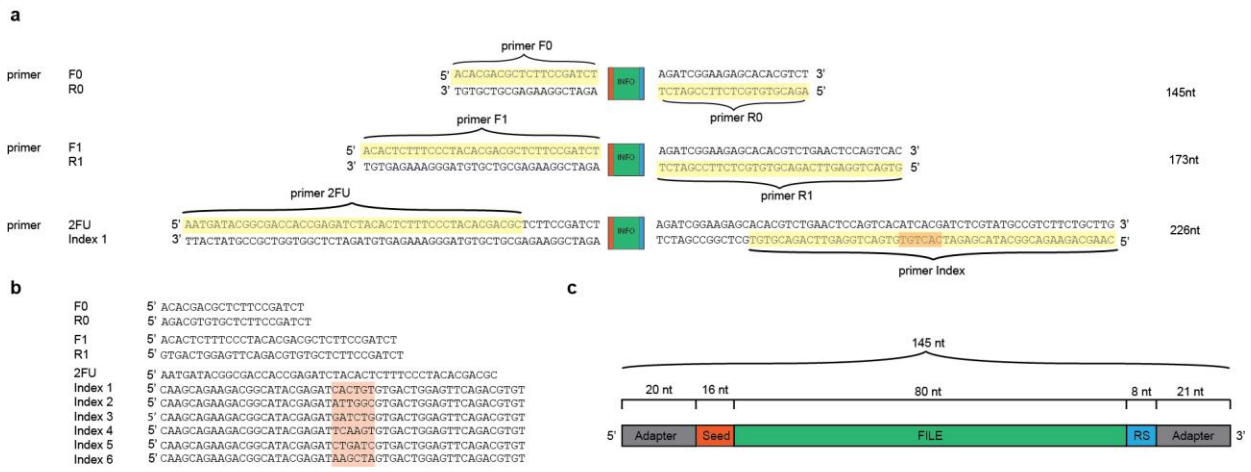


Supplementary Figure 1

The robustness of encapsulated DNA versus free DNA in the DoT architecture.

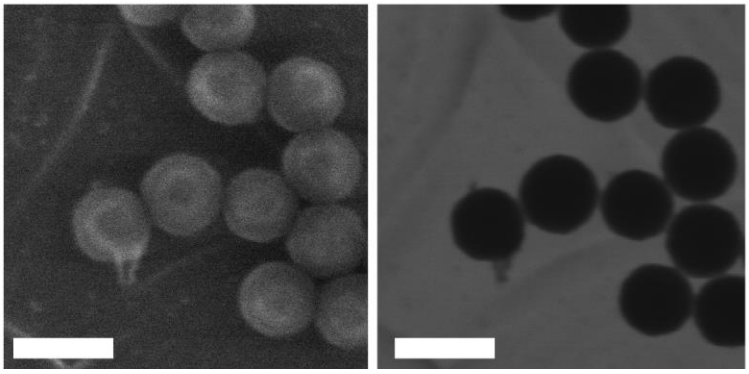
Chemical and thermal stability of the encapsulated DNA compared to non-encapsulated DNA. For the thermal stability assessment, solid state DNA and SPED encapsulated DNA were stored at 60°C for one week at 50% relative humidity. For stability against aggressive chemical species, DNA and encapsulated DNA were treated with bleach and reactive oxygen species (ROS) in aqueous solution. *denotes values < 10-5. Mean values and error bars of n=3 independent experimental experiments.



Supplementary Figure 2

The sequence design for the DoT experiments

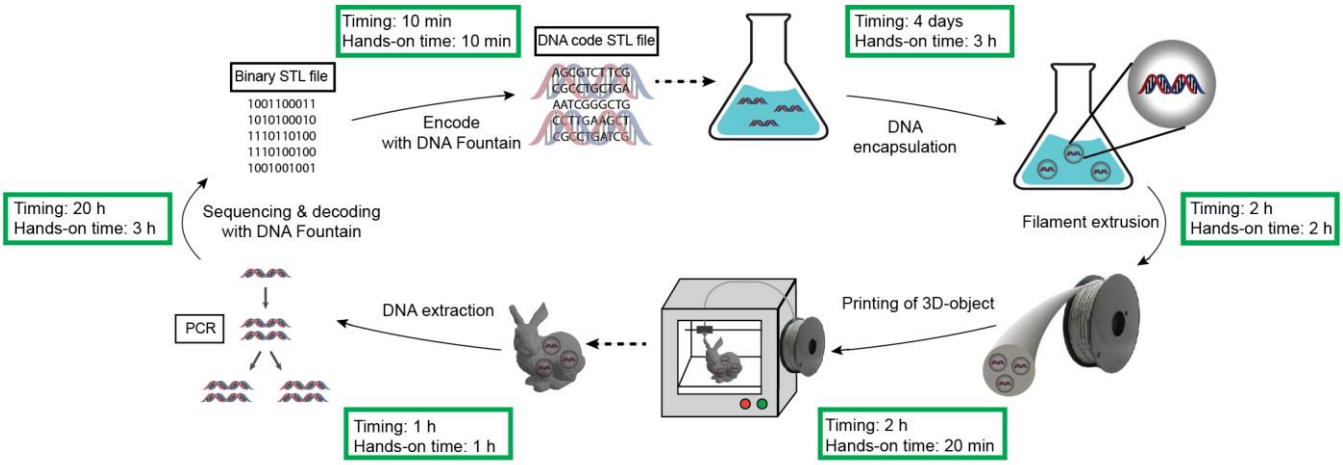
(a) primer design of sequencing adapters as used in DoT (b) list of primer and index sequences used in DoT (c) oligo architecture for each of 12,000 features Oligonucleotide sequences.



Supplementary Figure 3

SPED beads.

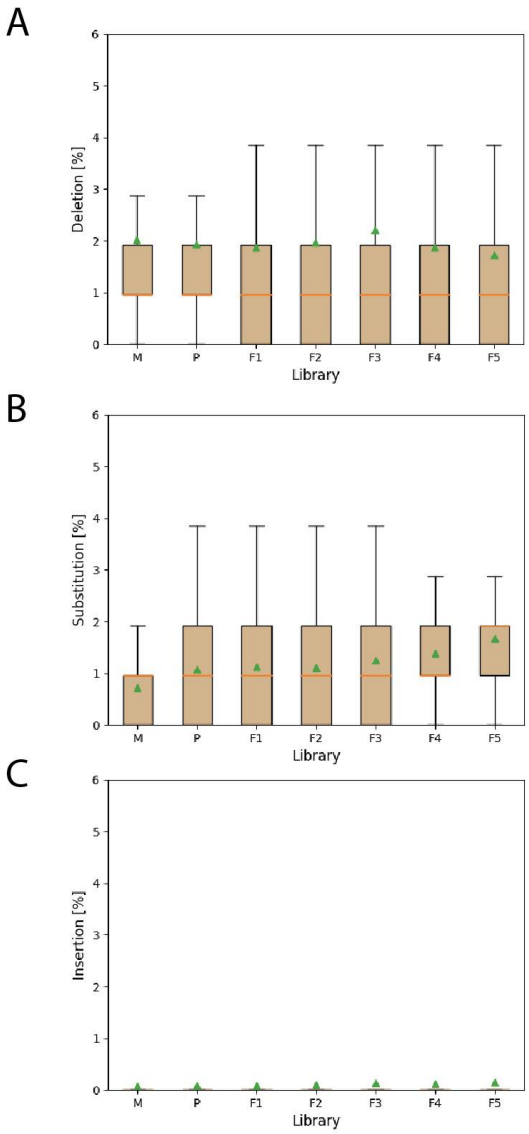
Scanning- (left) and scanning transmission (right) electron microscopy images of the SPEDs, which are about 160nm large particles comprising each ca. 60 DNA strands (DNA not visible). Scale bar = 200nm.



Supplementary Figure 4

Time estimates for each stage in the replicating experiments.

Handling and overall process timing for the individual steps in the formation and, reading of the DoT objects. Time estimation resulting from 6 independent experiments.

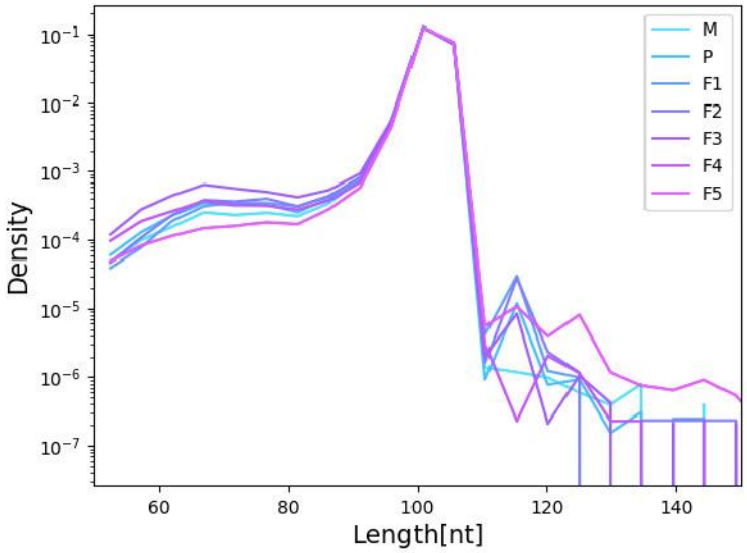


Supplementary Figure 5

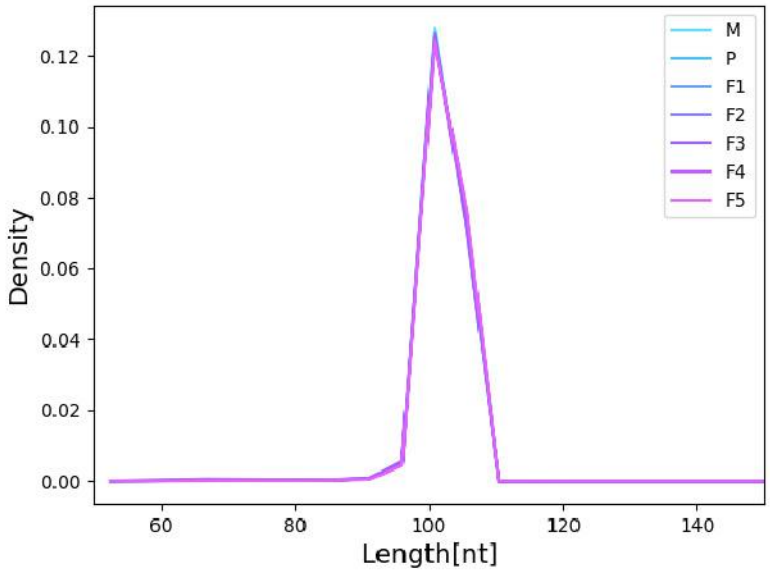
The error rates as a function of generation.

M: master DNA library before any incorporation to an actual filament or silica beads. P: Parent (generation number 1), F1-F5: all subsequent generations. Each plot shows the average (green triangle), median (orange), interquartile range (beige), and the 5%-95% percentile range (bars) (A) The frequency of deletions per synthesized nucleotide (B) The frequency of insertions per synthesized nucleotide (C) The frequency of substantiations per synthesized nucleotide.

A



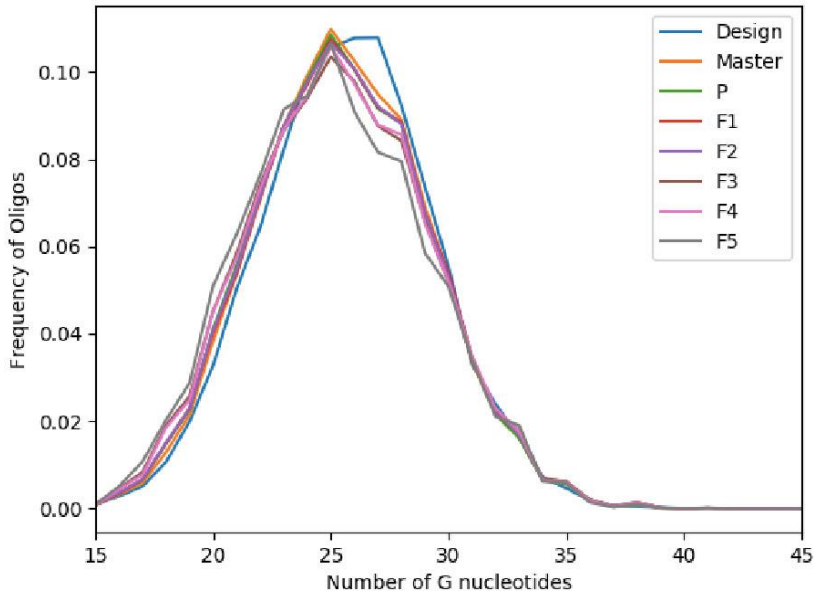
B



Supplementary Figure 6

The length distribution of oligos as a function of generation.

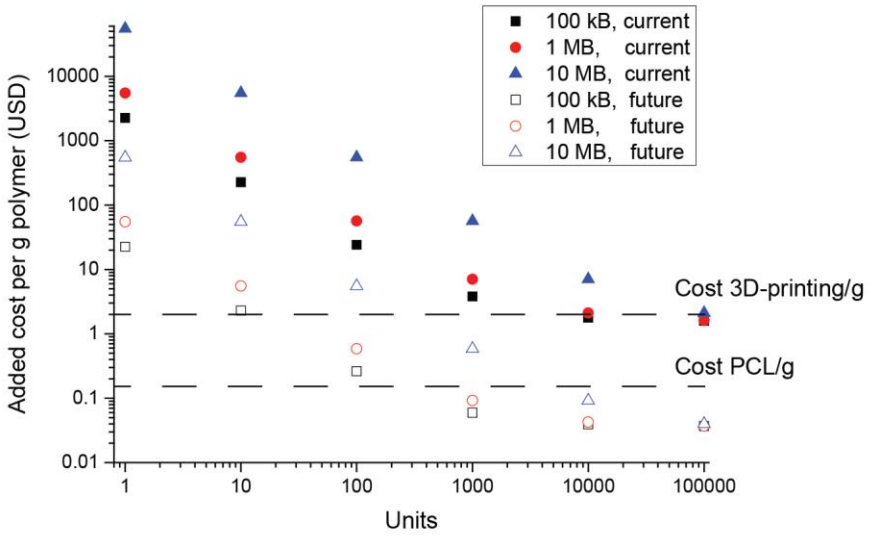
Each curves is a density plot of the oligo lengths. M: master DNA library before any incorporation to an actual filament or silica beads. P: Parent (generation number 1), F1-F5: all subsequent generations (A) logarithmic scale (B) regular scale.



Supplementary Figure 7

The distribution of sequence reads as a function of the original number of “G”s in the oligo in each generation.

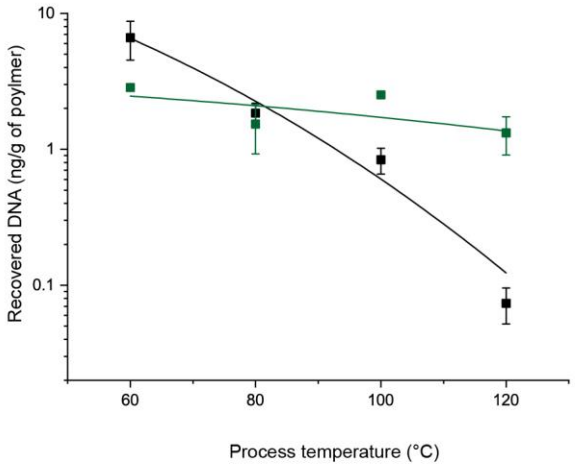
Design is the actual file sent to sequencing. Master is the master DNA library before any incorporation to an actual filament or silica beads. P-F5 are product generations as described in the main text.



Supplementary Figure 8

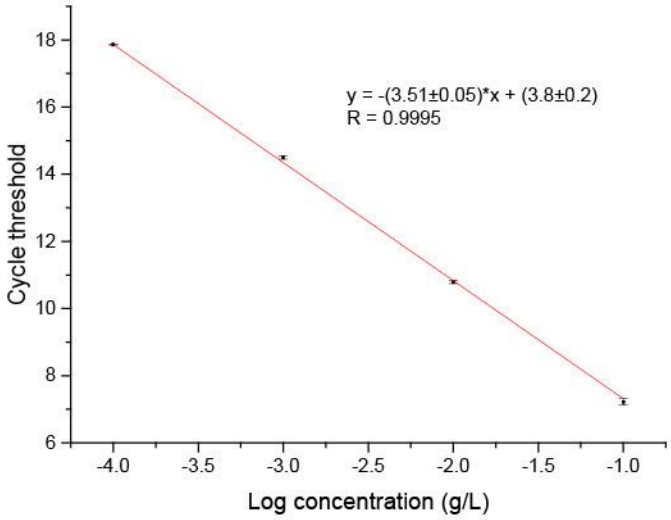
Cost of DoT.

Under the current scenario, the current laboratory scale costs were taken. For the future scenario, a 100 fold reduction in library cost was assumed, together with yield and cost optimization in product manufacturing (see discussion in Supplementary Note 6).



Supplementary Figure 9

Thermal stability of an encapsulated DNA file in PCL filament for different 3D printing (green) and filament extrusion (black) process temperatures. Error bars represent standard errors of experimental triplicates. The black and green lines represent a nonlinear regression for each dataset according to Arrhenius law for a first order decay reaction (see Supporting Information).



Supplementary Figure 10

qPCR cycle threshold as a function of logarithm of the SPED concentration.

Mean values and error bars (s.e.m) of n=3 independent experiments.

Supporting information

Supplementary Notes

Supplementary Note 1 DNA synthesis

The 12,000 DNA sequences were synthesized by CustomArray Inc. (Bothell, WA) on a 12K chip. We received the DNA in ssDNA form at a concentration of 46.93 ng/µL. The oligo pool was first amplified by PCR in order to dilute any fragmented features and to have sufficient material for silica encapsulation. For this, we diluted the DNA 1:46 and performed a standard PCR with the following reaction mixture in each 96-well: 10 µL master mix (Roche, Lightcycler 480 SYBR Green I Master Mix), 8 µL of MilliQ grade water, 1 µL of primer mix (primers F0 and R0 (Microsynth AG), each at 10 µM)[3] and 1 µL diluted DNA pool. Cycling parameters were 95°C for 15 s, 54°C for 30 s, 72°C for 30 s for 12 cycles. Once finished, 4 wells were pooled together (80 µL) and purified by QIAGEN QIAquick PCR Purification Kit with 150 µL elution buffer used per column. This yielded 1050 µL of 10 ng/µL DNA solution.

Supplementary Note 2 Particle synthesis

The synthesis of SPED was based on Paunescu et al.[8] 50 mg silica nanoparticles (microparticles GmbH, ⌀150 nm) in 1 mL ethanol were surface functionalized with 10 µL N-trimethoxysilylpropyl-N,N,N -trimethylammonium chloride (TMAPS, 50% MeOH, ABCR GmbH) for 12 h. The surface potential of the functionalized silica particles was measured to be +47 mV by a zeta potential analyzer (Zetasizer Nano, Malvern). To produce 1 batch of 1.75 SPED, 35 µL of particles (50 mg/mL) were added to 1050 µL of double stranded DNA (10-12 ng/µL). The amount of unbound DNA was determined by NanoDrop 2000c Spectrophotometer (0.7-1 ng/µL). On top of the DNA layer, silica was grown through Stöber synthesis. 0.5 µL of TMAPS and 0.5 µL of tetraethyl orthosilicate (TEOS, ≥90%, Aldrich) were added before shaking for 5 h. Then, 4 µL TEOS was added and the batch was shaken for 4 days. The DNA loading was determined by QUBIT Fluorometer DNA assay (ThermoFisher Scientific). To 100 µL of a particle suspension with known concentration, 3 µL of BOE were added. The DNA concentration of 5 µL of this suspension was then measured by QUBIT Fluorometer (0.744 ng/µL DNA in 0.35 g/L particle suspension, 0.2 wt% DNA loading on particles).

Supplementary Note 3 DNA quantification

Quantitative PCR was used to quantify DNA content and degradation. A dilution series from E-1 to E-4 g/L of SPED was measured to serve as a standard curve. To each dilution step, 10 µL BOE was added to 10 µL sample analogous to 10 mg of PCL. The DNA was then purified by QIAGEN QIAquick PCR Purification Kit with 50 µL elution buffer used per column. The reported values are averaged over triplicates (**Supplemental Figure 10)**

Supplementary Note 4 Mass of copy

An oligo consists of 145 base pairs and the full file is stored on 12’000 oligos. Taking 650 g/mol as the average weight per base pair, we can determine the minimum amount of DNA needed for one full copy: ((650 g/mol)/6.022e23)*145nt*12’000 = 1.88e-15 g DNA per copy.

Supplementary Note 5 Sequencing of Stanford Bunny file

Six DNA libraries from F0 to F5 were sequenced. The DNA was isolated by clipping 10 mg of PCL and dissolved in 250 µL THF and 10 µL BOE. The DNA was then purified by PCR Purification Kit (Qiagen). All libraries were first diluted to equal concentrations. This was done so that each sample went through the same number of PCR cycles and had the same amount of amplification errors introduced. The

DNA was then amplified with F1 and R1 primer with the following mix: 10 µL master mix, 8 µL of MilliQ water, 1 µL primer stock (F1 and R1 (Microsynth, Switzerland), 10 µM each), 1 µL DNA sample). The cycling parameter were as follows: 95°C for 15 s, 54°C for 30 s, 72°C for 30 s, 25 cycles. The DNA was extracted by gel electrophoresis. The band slightly below 200nt was cut out and purified with QIAquick Gel Extraction Kit (QIAGEN, Germany). Each library was indexed by a second PCR with the general forward primer 2FU and an indexed reverse primer. The qPCR mix with the following mix: 10 µL master mix, 8 µL of MilliQ water, 1 µL primer stock (2FU and indexed primer, 10 µM each), 1 µL of 1:10 diluted DNA sample). The cycling parameter were as follows: 95°C for 15 s, 53°C for 30 s, 72°C for 30 s, 10 cycles. Each qPCR product was purified by gel electrophoresis and the DNA concentration was measured by Qubit assay. All samples were pooled to a 1 nM library and then further diluted to 20 pM. Prior to sequencing 2x150bp on the iSeq 100 (illumina, San Diego CA), 2% of PhiX were added to ensure call diversity.

Supplementary Note 6 Economics and scalability of DoT

To put the economics of DoT into a broader perspective, we have performed cost per item calculations for two scenarios:

a) Current cost-structure of lab-scale experiments, 100 ppm particle loading

Upfront cost per library: 100 kB: 2250 USD*, 1 MB: 5500 USD*, 10 MB 55000 USD**:

Per 2 mg of SPED (typical lab-scale synthesis scale):

- 28 parallel PCR wells (0.5 USD per well)*
- 7 parallel DNA purification kits (2.4 USD per kit)*
- labscale pricing for chemicals for particle synthesis (0.2 USD per mg)*

At a typical loading of 100 ppm SPED in polymer, 2 mg of particles is sufficient for 20 g of polymer.

*Actual experimental costs
** Scaled from actual costs

b) Assuming future progress in DNA synthesis, and optimizations in DNA handling / purification steps, 10 ppm loading

Upfront cost per library: 100 fold cheaper than today[2]

Per 1g of SPED (typical scale-up):

- 2x increase in DNA quantity per unit cost PCR
- no post-PCR DNA purification (- USD)
- chemicals for particle synthesis (200 USD)

At a future loading of 10 ppm SPED, 1 g of particles is sufficient for 100 kg of polymer.

Supplementary Note 7 Thermal stability assays

DNA-infused PCL was extruded and 3D-printed at temperatures ranging from 60°C to 120°C. Of this extruded or printed material, 10 mg were clipped and dissolved in 250 µL THF, followed by purification with QIAGEN QIAquick PCR Purification Kit with 50 µL elution buffer used per column. Assessing long-term stability, 0.492 µg DNA and 3.5 µg SPED were dried in a vacuum centrifuge (Eppendorf Concentrator plus) and stored at 60°C and 50 %RH for 10 days and then re-suspended in 100 µL and

quantified by qPCR (10 µL master mix (Roche, Lightcycler 480 SYBR Green I Master Mix), 8 µL of MilliQ grade water, 1 µL of primer mix (primers F0 and R0 (Microsynth AG), each at 10 µM), 1 µL diluted DNA pool, Cycling parameters were 95°C for 15 s, 54°C for 30 s, 72°C for 30 s for 30 cycles).

	Encapsulated (Cycle threshold)	Non-encapsulated (Cycle threshold)
Control 5°C	6.98±0.03	11.47±0.08
60°C	9.158±0.001	18.922±0.004

Supplementary Note 8 Arrhenius fitting

For DNA decay we can assume a first order chemical degradation reaction, where the relative concentration (A/A_0) depends on the reaction rate (k) and reaction time (t),

$$\frac{A}{A_0} = e^{-k\,t}\,.$$

The temperature (T) dependence of the reaction rate is given by the Arrhenius law, involving a pre-exponential factor k_0, the activation energy (E_a) and the gas constant (R):

$$= k_0 e^{-\frac{E_a}{RT}}$$

The final equation for the relative concentration as a function of reaction temperature is obtained by inserting the Arrhenius law into the rate equation:

$$\frac{A}{A_0} = e^{-k_0 e^{-\frac{E_a}{RT}} t}$$

Nonlinear curve regression was performed for A/A_0 vs. T assuming constant k_0, E_a and reaction time t.

For half life we assume first order kinetics for DNA degradation:

$$\frac{A}{A_0} = e^{-k\,t}$$

with the relative DNA concentration (A/A_0), the reaction rate (k) and time (t). After storing the library at 60°C for 10 days, we recovered 65% of the initial DNA, allowing us to calculate k at T=60°C. By using the Arrhenius law and an activation energy of 158 kJ/mol[3] we can calculate k_0:

$$k = k_0 e^{-\frac{E_a}{RT}}$$

with the pre-exponential factor (k_0), activation energy (E_a) and the gas constant (R) Assuming a storage temperature of 20°C, we can calculate the rate constant with Arrhenius law. Finally we find the half life of encapsulated DNA at t=20°C by:

$$t_{\frac{1}{2}} = \frac{\ln(2)}{k(T)}$$

Supplementary Note 9 Chemical stability assays

Encapsulated and non-encapsulated DNA were subjected to radical treatment stability assay, where a combination of ascorbic acid, H_2O_2, and $CuCl_2$ produce reactive oxidative species and test the protective properties of SPED towards DNA oxidation[8]. 5 µL of DNA/encapsulated DNA were added to 2.5 µL L-ascorbic acid (20 mM), 12.5 µL H_2O_2 (20 mM) and 17.5 µL $CuCl_2$ (500 µM). After 10 minutes, the reaction was quenched by adding 17.5 µL of 100 mM EDTA and 20 µL of BOE. To measure the total amount of DNA, 50 µL of water and 20 µL BOE were added to 5 µL of DNA/encapsulated DNA. The DNA concentration of treated and untreated samples was measured by QUBIT assay. All reported values are averaged over quintuplets.

	Encapsulated (ng/µL)	Non-encapsulated (ng/µL)
Control	0.289±0.003	0.288±0.002
ROS assay	0.29±0.06	0.03±0.02

The DNA stability was also evaluated by subjecting it to household bleach. A bleach stock was prepared with 8.57 mL NaClO (14% activity), 1.164 mL NaOH 10 M and 30.266 mL H_2O. 143 µL of 100-fold diluted bleach stock was added to 143 µL of DNA (10 ng/ml) or SPED suspension (0.1 µg/mL) and incubated for 10 min. The reaction is quenched by adding 5 µL of thiosulfate (1.46 M). The unprotected DNA is directly measured by qPCR whereas to the SPED samples, 10 µL of BOE were added and then purified by QIAGEN QIAquick PCR Purification Kit with 50 µL elution buffer. The samples were quantified by qPCR (10 µL master mix (Roche, Lightcycler 480 SYBR Green I Master Mix), 8 µL of MilliQ grade water, 1 µL of primer mix (primers F0 and R0 (Microsynth AG), each at 10 µM), 1 µL diluted DNA pool, Cycling parameters were 95°C for 15 s, 54°C for 30 s, 72°C for 30 s for 30 cycles). All reported values are averaged over triplicates.

	Encapsulated (Cycle threshold)	Non-encapsulated (Cycle threshold)
Control	4.7±0.1	9.36±0.04
Bleach assay	5.26±0.05	29.6±0.9

Supplementary Note 10 Sequencing of Oneg Shabat movie file

10 mg of the PMMA-SPED glass was clipped and dissolved in 500 µL acetone (>99.8%, ProLabo). The sample is centrifuged at 15,000 rpm for 3 min and the supernatant is removed. The remaining SPED particles are resuspended in 500 µL acetone by vortexing and ultrasound for 1 min. The sample is again centrifuged at 15,000 rpm for 3 min, followed by removing the supernatant. This washing step is repeated once. Finally the particles are suspended in 50 µL MilliQ grade water and dissolved by adding 50 µL BOE. The DNA was then amplified with F1 and R1 primer with the following mix: 10 µL master mix, 8 µL of MilliQ water, 1 µL primer stock (F1 and R1 (Microsynth, Switzerland), 10 µM each), 1 µL DNA sample). The cycling parameter were as follows: 95°C for 15 s, 54°C for 30 s, 72°C for 30 s, 25 cycles. The DNA was extracted by gel electrophoresis. The band slightly below 200nt was cut out and purified with QIAquick Gel Extraction Kit (QIAGEN, Germany). The oligo pool was indexed by a second PCR with the general forward primer 2FU and an indexed reverse primer (TrueSeq, GCCAAT). The qPCR mix with the following mix: 10 µL master mix, 8 µL of MilliQ water, 1 µL primer stock (2FU and indexed primer, 10 µM each), 1 µL of 1:10 diluted DNA sample). The cycling parameter were as follows: 95°C for 15 s, 53°C for 30 s, 72°C for 30 s, 10 cycles. The qPCR mix was purified by gel electrophoresis and the DNA concentration was measured by Qubit assay. The resulting DNA pool was diluted to a 1 nM library for storage. The final sequencing concentration was 20 pM. Prior to sequencing 2x150bp on the iSeq 100 (illumina, San Diego CA), 2% of PhiX were added to ensure call diversity.