

## Supplementary Materials for

### **A DNA Nanoscope that identifies and precisely localizes over a hundred unique molecular features with nanometer accuracy**

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## Supplementary Materials and Methods

### DNA origami design, manufacture and purification

**Design:** The rectangular DNA origami nanostructure used in all experiments was designed using the cadnano software (27), according to principles laid out in (28). The origami is 18 helices tall and 376 base pairs wide, and is ‘twist corrected’ (28) to promote flatness and minimize stress. Assuming a helix width of 2 nm and a helix to helix spacing of 1 nm, the structure’s height is calculated to be  $18 \times 2 \text{ nm} + 17 \times 1 \text{ nm} = 53 \text{ nm}$ . Assuming a rise of 0.34 nm per base pair, the structure’s width is calculated to be  $376 \times 0.34 \text{ nm} = 127.84 \text{ nm}$ . The origami is composed of a scaffold strand (M13mp18 single-stranded DNA, New England Biolabs (NEB), catalog no. N4040S) and 216 ‘blunt’ staple oligos (Integrated DNA Technologies (IDT)). See Supplementary Fig. 6 for the scaffold routing and Supplementary Table 2 for ‘blunt’ staple oligo sequences. Extended staple oligos (IDT) composed of a ‘blunt’ staple followed by a barcoded handle domain are used to recruit barcoded molecular ruler primers (IDT) via hybridization. The sequences for extended staple oligos are listed in Supplementary Table 3 and the sequences for barcoded molecular ruler primers are listed in Supplementary Table 4.

**Manufacture of randomly tagged DNA origami patterns:** Random sparse tagging was achieved by setting up a winner take all competition for staple incorporation at every target site, by supplying both the barcoded ‘handle’ staple and the corresponding barcode-less ‘blunt’ staple. At every target site, either the handle staple or the corresponding blunt staple can incorporate into the DNA origami, but not both. In any particular copy of the DNA origami, a site was successfully tagged when the handle staple won the competition, allowing recording primer recruitment at that site. Conversely, the site was not tagged when the blunt staple won the competition. The average density of tagged points on a DNA origami depends on the relative propensity of incorporation of these competing staples. The relative propensity, and hence the average density of tagged points on a DNA origami, was tuned by varying the relative concentrations of competing staples. The locations of the barcoded handle staples for each pattern are presented in Supplementary Fig. 7, 8 and 9.

The scaffold strand (5 nM final concentration) was combined with: (i) all 216 ‘blunt’ staple oligos (50 nM final concentration of each oligo, see Table S2 for sequences), (ii) the appropriate subset (depending of the pattern being tagged, see Supplementary Fig. 7, 8 and 9 and Supplementary Table 3) of barcoded ‘handle’ staple oligos (5 nM final concentration of each

oligo) and (iii) corresponding appropriate subset of barcoded primers of type a and a\* (5 nM final concentration of each oligo, see Supplementary Table 4 for sequences) in 1X TE Mg buffer (pH 7.4, 10 mM Tris-HCl, 0.1 mM EDTA, 10 mM MgSO<sub>4</sub>). The mixture was then cooled from 90 °C to 60 °C at the rate of 1 min/°C and then from 60 °C to 50 °C at the rate 10 min/°C and finally from 50 °C to 25 °C at the rate of 1 min/°C. Folded origami was stored at 4 °C for up to one week prior to purification.

**Purification:** DNA origami patterns were purified by agarose gel electrophoresis to eliminate aggregated structures and unbound oligos. 20 µL of folded DNA origami was mixed with 4 µL of 6X loading dye and loaded per lane in a 8 cm tall and 6 cm wide 1 % agarose gel (UltraPure agarose, Thermo Fisher Scientific, catalog no. 16500100) pre-stained with 1X SYBR Gold. The gel was run at 80 V for 2 hours on an Amersham instrument to separate well-folded DNA origami from excess oligos and aggregates. The band corresponding to well-folded DNA origami was visualized under a blue-light transilluminator (Safe Imager, Thermo Fisher Scientific) and excised with a clean razor blade. The gel slice was transferred to a freeze-n-squeeze DNA gel-extraction spin column (Bio-Rad, catalog no. 7326165), crushed with a clean pestle, kept at -20 °C for 10 min and then centrifuged at 2,000 g for 4 min. The flow through containing purified DNA origami was diluted 10-fold in 1X TE Mg buffer and 50 µL aliquots were stored at -20 °C for recording experiments.

#### DNA nanoscope recording experiment

**1. Prepare fluid-exchange reaction chamber:** A thin layer of mica of dimensions 25 mm X 75 mm is peeled from a mica sheet (Muscovite Mica V-1 Quality, Electron Microscopy Sciences, catalog no. 71855-05-10) using sticky tape (3M Scotch Clear Magic Tape, 25 mm wide). The mica sheet stuck to the tape is then affixed to a sticky bottomless six-channel slide (sticky-slide VI 0.4, ibidi, catalog no. 80608) to assemble fluid-exchange reaction chambers for recording experiments. Each channel is used to perform an independent DNA nanoscope recording experiment.

**2. Deposit origami on surface:** 50 µL of frozen DNA origami aliquot is thawed and heated to 42 °C for 2 min, to dissociate any aggregates. It is then added to a reaction chamber and allowed to bind to the mica surface for 10 min. The chamber is then washed twice with 50 µL of 1X TE Mg to remove unbound origami. The exposed, unbound mica surface is then passivated with a BSA solution (50 µg/mL, NEB) for 5 min and further washed with 1X TE Mg and a magnesium-

supplemented 1X Thermopol buffer (20 mM Tris-HCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 10 mM KCl, 7 mM MgSO<sub>4</sub>, 0.1% Triton®-X-100, pH 8.8 @ 25 °C, NEB, catalog no. B9004S).

**3. Perform molecular ruler reaction:** 50  $\mu\text{L}$  of the recording mix, consisting of 100 nM extension hairpin type ‘a’, 100 nM extension hairpin type ‘a\*’, 0.08 U/ $\mu\text{L}$  Bsm DNA polymerase LF (*ThermoFisher Scientific*, catalog no. EP0691), 100  $\mu\text{M}$  dNTP solution mix (NEB, catalog no. N0447S), 1X Thermopol buffer and 5 mM MgSO<sub>4</sub>, is added to the reaction chamber and the slide kept at 37 °C for 3 hours. After 3 hours, the supernatant containing distance records is collected for further processing.

#### PCR amplification of distance records

Distance records were amplified by PCR. For PAGE analysis, the PCR reaction mix contained distance records (1  $\mu\text{L}$  per 20  $\mu\text{L}$  reaction mix), fluorescent PCR primer 1 (0.3  $\mu\text{M}$ , IDT. See Supplementary Table 1 for sequence), fluorescent PCR primer 2 (0.3  $\mu\text{M}$ , IDT. See Supplementary Table 1 for sequence), AccuPrime *Pfx* Reaction Mix (1X), AccuPrime *Pfx* DNA polymerase (0.025 U/ $\mu\text{L}$ , *ThermoFisher Scientific*, catalog no. 12344024) and EvaGreen fluorescent nucleic acid dye (1X, Biotium, catalog no. 31000-T). For next-gen sequencing analysis, the PCR mix contained inner primers (10 nM each, IDT), outer primers (0.3  $\mu\text{M}$  each, IDT) and the other PCR mix components listed above. The outer primers are sequencing barcodes used to multiplex multiple libraries (i.e. distinct DNA nanoscope experiments) on the same sequencing chip. The inner primers contain sequences common to the ends of all barcoded recording primers. The sequence information for these can be found in table S5.

Temperature cycling for the PCR mix had (i) an initial denaturation step of 95 °C for 2 min, followed by (ii) 25 to 35 cycles of denaturation at 95 °C for 15s and primer binding and extension at 60 °C for 45s and (iii) a final extension at 65 °C for 2 min. The progress of the PCR amplification was monitored on a real time PCR machine. The number of temperature cycles was chosen to allow the signal to plateau, indicating completion of the PCR reaction.

#### PAGE characterization of distance records for calibration experiments

The length distribution of the distance records for each calibration distance was characterized using PAGE. 10  $\mu\text{L}$  of PCR-amplified distance records were mixed with 10  $\mu\text{L}$  of 2X denaturing loading dye (95 % formamide, 10 mM NaOH, 0.025 % bromophenol blue, 1 mM EDTA), heated to 95 °C for 2 min and then loaded into individual lanes of an 8 cm X 8 cm 4 % denaturing polyacrylamide gel (4 % v/v acrylamide/bis-acrylamide (19:1), 7.4 M Urea, 0.5X TBE). The gel

was electrophoresed at a voltage of 180 V for 30 min at 50 °C. The gel was then stained with a SYBR Gold (1:10,000 v/v) solution for 10 min and then imaged on a Typhoon FLA 9500 gel scanner (General Electric) in the red (635 nm emission laser and 665 nm low-pass red filter) and blue channels (473 nm emission laser and 510 nm low-pass blue filter). Gel images were analyzed with the Fiji image processing software package.

#### Purification of distance records for next-gen sequencing

Distance records were purified to remove short-length spurious distance records prior to sample preparation for next-gen sequencing. The purification steps were as follows:

1. **Column concentration:** The post-PCR distance records were cleaned and concentrated using a bind-wash-elute procedure on a silica membrane column using the MinElute PCR purification kit (Qiagen, catalog no. 28004) and it's associated standard protocol. The input was 100 µL of post-PCR mix and the elution volume was 10 µL in buffer EB (10 mM Tris-Cl, pH 8.5).
2. **PAGE purification:** Concentrated samples were mixed with 10 µL of 2X denaturing loading dye (95 % formamide, 10 mM NaOH, 0.025% bromophenol blue, 1 mM EDTA), heated to 95 °C for 2 min and then loaded into individual lanes of an 8 cm X 8 cm 4 % denaturing polyacrylamide gel (4 % v/v acrylamide/bis-acrylamide (19:1), 7.4 M Urea, 0.5X TBE). The gel was electrophoresed at a voltage of 180 V for 60 min to 80 min at 50 °C to allow the short-length spurious distance records to diffuse out of the gel into the surrounding running buffer. The appropriate time to run the gel to allow this to happen was determined by monitoring, in adjacent lanes, the migration of fluorescent fiduciary bands that co-migrate with short-length spurious distance records.
3. **PAGE concentration:** After the short-length spurious distance records were discharged into the surrounding running buffer, the desired distance records, which range in size from ~150 b to ~1 kb and were distributed over a large gel volume, were concentrated by reversing the current flow, as follows. First, the running buffer was replaced to prevent the spurious records from re-entering the gel. Next, 50 µL of a dense 15 % denaturing polyacrylamide gel (15 % v/v acrylamide/bis-acrylamide (19:1), 7.4 M Urea, 0.5X TBE) was pipetted into each lane of the gel and allowed to polymerize. The voltage across the gel terminals was now reversed and the gel run at 180 V for 60 min to 80 min at 50 °C to allow the desired distance records to collect in the dense gel, concentrating them. The dense gel was excised with a razor blade. A clean 0.5 mL tube was pierced at the bottom with a 20-gauge syringe needle and placed into a 1.5 mL tube,

setting up a gel-shredder tube-in-tube widget. The excised gel from each lane was placed in the gel-shredder tube-in-tube widget and centrifuged at 14,000 g for 2 min. The disintegrated gel collects in the 1.5 mL tube, and the 0.5 mL tube is discarded. 50 µL of buffer EB is added to the 1.5 mL tube, which is then heated to 65 °C for 5 min followed by freezing at –20 °C for 10 min. The frozen pellet is transferred to a Freeze ‘N Squeeze gel extraction spin column (Biorad, catalog no. 7326165) and centrifuged at 14,000 g for 3 min to collect ~ 50 µL of solution containing desired distance records. The solution is finally cleaned and concentrated using a bind-wash-elute procedure on a silica membrane column using the MinElute PCR purification kit (Qiagen, catalog no. 28004) and it’s associated standard protocol. The final elution volume is 10 µL in buffer EB (10 mM Tris-Cl, pH 8.5). The concentration is measured on a NanoDrop 2000 (ThermoFisher) using OD260 absorption.

#### Library preparation for next-gen sequencing

Purified distance records were prepared for next-gen sequencing using the Oxford Nanopore SQK-LSK109 ligation sequencing kit. The protocol titled ‘*1D Genomic DNA by Ligation*’ (see Oxford nanopore website) was used to prepare samples. A total of 0.5 pmole of purified distance records were used in the library preparation. Sometimes samples from two different experiments (i.e. distinct patterns) were combined, in which case we used 0.25 pmole of sample from each experiment. Briefly, the protocol involves a combined step of DNA repair and end-prep with the NEBNext FFPE DNA Repair Mix (New England Biolabs, catalog no. M6630S) and the NEBNext Ultra II End Repair / dA-Tailing Module (New England Biolabs, catalog no. E7546) respectively. This is followed by cleanup with the Agencourt AMPure XP beads (2X bead to sample ratio). Then, sequencing adaptors are ligated using the NEBNext Quick T4 DNA Ligase (New England Biolabs, catalog no. E6056) followed by a final round of bead clean-up. The sample is then mixed with loading beads, added to a ‘primed’ flow cell (FLO-MIN106) (see protocol for details) and sequenced for between 12 hours to 24 hours, producing around 10 million to 15 million raw reads. The sample can be split across multiple flow cells if more reads are required.

#### Data analysis workflow

We use Oxford Nanopore’s Guppy basecalling software (v3.2.1) to (1) read sequence information from raw sequencing data and then use MATLAB scripts to (2) demultiplex reads from different experiments, (3) extract the lengths of the distance records and assign them to

their appropriate target-pair, (4) infer the distance for each target-pair from all assigned distance records, and finally (5) reconstruct the underlying geometry from pairwise distance measurements. The MATLAB scripts referenced below can be found at [github.com/nikhil314/DNA-Nanoscope](https://github.com/nikhil314/DNA-Nanoscope). Details of the process are as follows:

**1. Basecalling using Guppy (v3.2.1):** Guppy is a base-calling software provided by Oxford Nanopore that can be downloaded and run on local machines. It takes raw sequencing data in the FAST5 file format and produces sequences in the FASTQ file format. Guppy was run on Windows Server 2008 machines with either 12 or 16 CPU cores. The process took about 40min to base-call 1 million reads on our computing setup. The command used to perform base-calling was:

```
guppy_basecaller.exe --input_path <input_path_fast5> --save_path  
<output_path_fastq> --num_callers 12 --cpu_threads_per_caller 4 --  
qscore_filtering -q 50000 -c dna_r9.4.1_450bps_fast.cfg
```

where:

<input\_path\_fast5> is the path of the raw reads in FAST5 format,  
<output\_path\_fastq> is the path where base-called reads are stored in FASTQ format,  
--num\_callers specifies the number of parallel base-calling instances to run,  
--cpu\_threads\_per\_caller specifies the number of threads used by each instance of the base-calling software,  
--qscore\_filtering specifies that the reads be filtered by quality (default is to pass all reads with q\_score above 7),  
-q specifies number of reads to write per fastq file, and  
-c specifies the configuration file.

**2. Demultiplex reads from different experiments:** The ends of the reads are scanned for sequencing barcodes (using local sequence alignment) and sorted into subdirectories based on barcodes identified. The MATLAB command used was:

```
sort_barcoded_reads(fastq_dir,ONT_barcodes)
```

where:

fastq\_dir is the path of the fastq sequence files and

ONT\_barcodes specifies the sequencing barcodes and their reverse complements.

The output is written into fastq files with reads that are sorted into sub-directories corresponding to the identity of the sequencing barcodes.

**3. Extract record lengths and assign to correct target pair:** Reads are scanned to identify the unique staple barcode sequences associated with each distance record. The length of the repeat region between the barcode sequences is extracted and assigned to the appropriate target pair. The following MATLAB command was executed for each directory containing reads from the same experiment:

```
pairwise_record_list = extract_pairwise_record_lengths(target_barcodes, path,  
library_size, color_length)
```

where:

`target_barcodes` specifies the staple barcode sequences,

`path` specifies the path of the fastq reads,

`library_size` specifies the number of sequencing libraries that were combined for a single run,

`color_length` specifies the length of the auxiliary tag sequence, and

`pairwise_record_list` is the output, a matrix of size ( $n, n, 2001$ ) where  $n$  is the number of target points and cell  $(i, j, k)$  holds the number of distance records of length  $k$  bases (only counting the repeat region) between points  $i$  and  $j$ . All distance records of length  $> 2000$  are stored in the slice  $(:, :, 2001)$ .

`pairwise_record_list` variables from different sequencing runs of the same experiment were combined by simply adding them.

**4. Infer the measured distance between every target pair:** The distribution of distance record lengths between every pair of points was examined to identify the major peak (in base pairs), which was then converted into a distance (in nanometers) by applying the calibration function.

The MATLAB function used was:

```
[pairwise_distances, pairwise_peak_heights] =  
finddist_geometry(pairwise_records_list, calibration_fun)
```

where:

`pairwise_records_list` is the output of `extract_pairwise_record_lengths` (see previous step),

`calibration_fun` is a `cfit` object that holds the calibration function of the ruler, which maps bases to nanometers,

`pairwise_distances` is one output, an array of measured distances for each pair of points, and

`pairwise_peak_heights` is the peak height (in bases) corresponding to the distances measured. It is a measure of the confidence in the measurement.

**5. Reconstruct geometry from pairwise distance measurements:** The distance measurements are integrated into a coherent embedding of the targets in the 2D Euclidean plane, using the following MATLAB function:

```
[theta, prune, score] = solveDGP(pairwise_distances, pairwise_peak_heights,  
opt_threshold)
```

where:

`pairwise_distances` and `pairwise_peak_heights` are the outputs from `finddist_geometry` (see previous step),

`opt_threshold` is a parameter used to prune unreliable measurements and to generate weights for measurements reflecting their reliability (see supplementary text for details on how `opt_threshold` is auto-set),

`theta` is a list of coordinates, specifying the final embedding,

`prune` is a logical-valued array indicating which target points were dropped from the final reconstruction, and

`score` is a measure of the internal consistency of the embedding. See supplementary text S2D.

The final embedding is compared to the designed pattern by superimposing them to minimize the RMSD (root-mean-square deviation). The MATLAB script used is:

```
[theta_translated, lrms] = superimpose(theta, theta_designed, prune)
```

where:

`theta` and `prune` are the outputs from `solveDGP`,

`theta_designed` contains a list of coordinates specifying the designed pattern,

`theta_translated` is the superimposition of the final embedding that minimizes the RMSD between the designed and reconstructed pattern, and

`lrms` is the corresponding RMSD.

## **Supplementary Notes**

### 1. Design of molecular ruler primers and extension hairpins

Two main considerations went into the design of molecular ruler primers and extension hairpins. First, we reasoned that even weak secondary structure in the growing primers ('a a ...a' and 'a\* a\* ... a\*') would result in a high propensity intra-molecular reaction where the primer folds back on itself. Normally such a state is transient and would resolve itself. However, in the presence of DNA polymerase in the molecular ruler reaction mixture, the primer could extend back on itself, proving fatal to the ruler reaction. This led us towards adopting a two letter code for the sequence domains 'a' and 'a\*'. The second consideration was the number of bases added to the primer at every growth step. Here, we reasoned that the fewer the number of bases added at each step, the more gradual the growth of the primer and consequently more precise the ruler. These two considerations, unfortunately, are in tension with each other. A two-letter code results in a relatively weak binding interaction between a primer and an extension hairpin. Experiments showed that a toehold binding interaction of at least 12 bases (of an {A, T} alphabet) is necessary for the extension reaction to proceed with reasonable efficiency, under standard reaction conditions. In conventional PER repeat extension reactions, the extension sequence at each step is identical to the toehold sequence (for example, 's' is extended to 's s'). Thus, a conventional PER implementation would result in the addition of 12 bases at every step. However, we engineered a system where a 12 base toehold only adds a 4 base repeat at every step (Supplementary Fig. 2), by making the toehold sequence itself a repeat. In particular we chose 'a' = 'r r r' where 'r' = 'AAAT'. Thus, a toehold sequence of a (= 'r r r') was extended by the sequence 'r' at every step of the extension reaction. Correspondingly, we chose 'a\*' = 'r\* r\* r\*' and extended it by 'r\*' at every step. The complete sequences for the primers and extension hairpins are listed in Supplementary Table 1.

### 2. Inferring geometry from pairwise distance measurements

The question of inferring geometry from pairwise distance measurements is modeled as an embedding problem in two-dimensional Euclidean space. Each target point  $i$  of the pattern is parameterized with X and Y co-ordinates as  $(x_i, y_i)$ . We look for an embedding that minimizes the error between the experimentally measured distances and the Euclidean embedded distances. Not every measurement made by the ruler is equally reliable. We observed a clear positive correlation between the height of the major peak (in units of number of reads) and the accuracy

of the measurement. Therefore, we developed an algorithm that assigns weights to the various measurements according to the height of the corresponding major peak. The embedding algorithm proceeds in three stages – pruning, producing an initial embedding and finally, refining the embedding.

**A. Pruning:** A threshold parameter (auto-tuned, as explained below) was used to filter measurements. All measurements corresponding to peak heights less than the threshold were marked unreliable and pruned. All measurements corresponding to peak heights greater than the threshold were marked equally reliable and retained. In rare cases, some points were left with very few (less than three) associated reliable distance measurements as a result of this pruning. Such points were dropped from the reconstruction by deleting all associated distance measurements.

**B. Initial embedding:** We used a robust facial reduction algorithm (18) to obtain an initial embedding. This initial embedding has been shown to work well as an initial solution for generating embeddings using nonlinear optimization approaches, making it less likely that the optimization process is trapped in local minima or at saddle points.

**C. Refining the embedding:** In refining the embedding, we assigned each measurement a weight in the range [0,1] to denote its reliability. Measurements with corresponding peak heights less than the threshold parameter are assigned a weight of zero (i.e. pruned). Measurements with corresponding peak heights greater than the threshold parameter were assigned a positive weight  $w_{ij}$  as described in the below equations. In particular the objective function  $J$ , which we seek to minimize, is defined as:

$$J = \sum_{i=1}^n \sum_{j=1}^{i-1} \frac{w_{ij}}{W} (d_{ij}^{measured} - d_{ij}^{embedded})^2$$

where:

$\{1, 2, \dots, n\}$  are the uniquely identified points that make up the pattern,

$w_{ij} = \max\left(\frac{2}{1 - e^{-k(p_{ij}-t)}} - 1, 0\right)$  is the weight assigned to the measured distance between points  $i$  and  $j$ . The smoothness parameter  $k = 0.8$ ,  $p_{ij}$  is the peak height and  $t$  is the threshold

parameter. Note that  $w_{ij} \in [0,1]$ ,

$W = \sum_{i=1}^n \sum_{j=1}^{i-1} w_{ij}$  is the sum of all weights, used to normalize the weights,

$d_{ij}^{measured}$  is the experimentally measured distance between points  $i$  and  $j$  and,

$d_{ij}^{embedded} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$  is the embedded Euclidean distance.

The optimization is performed using the fminunc MATLAB function, which implements a quasi-Newton algorithm to find the minimum of an unconstrained multivariable function.

**D. Choosing the threshold parameter:** The threshold parameter was auto-set, without any *a priori* knowledge of the geometry of the designed pattern, as follows. The embedding was performed for all integer values of the threshold parameter  $t$  from 0 to 100 and an embedding score was calculated as  $score = J + \alpha r$

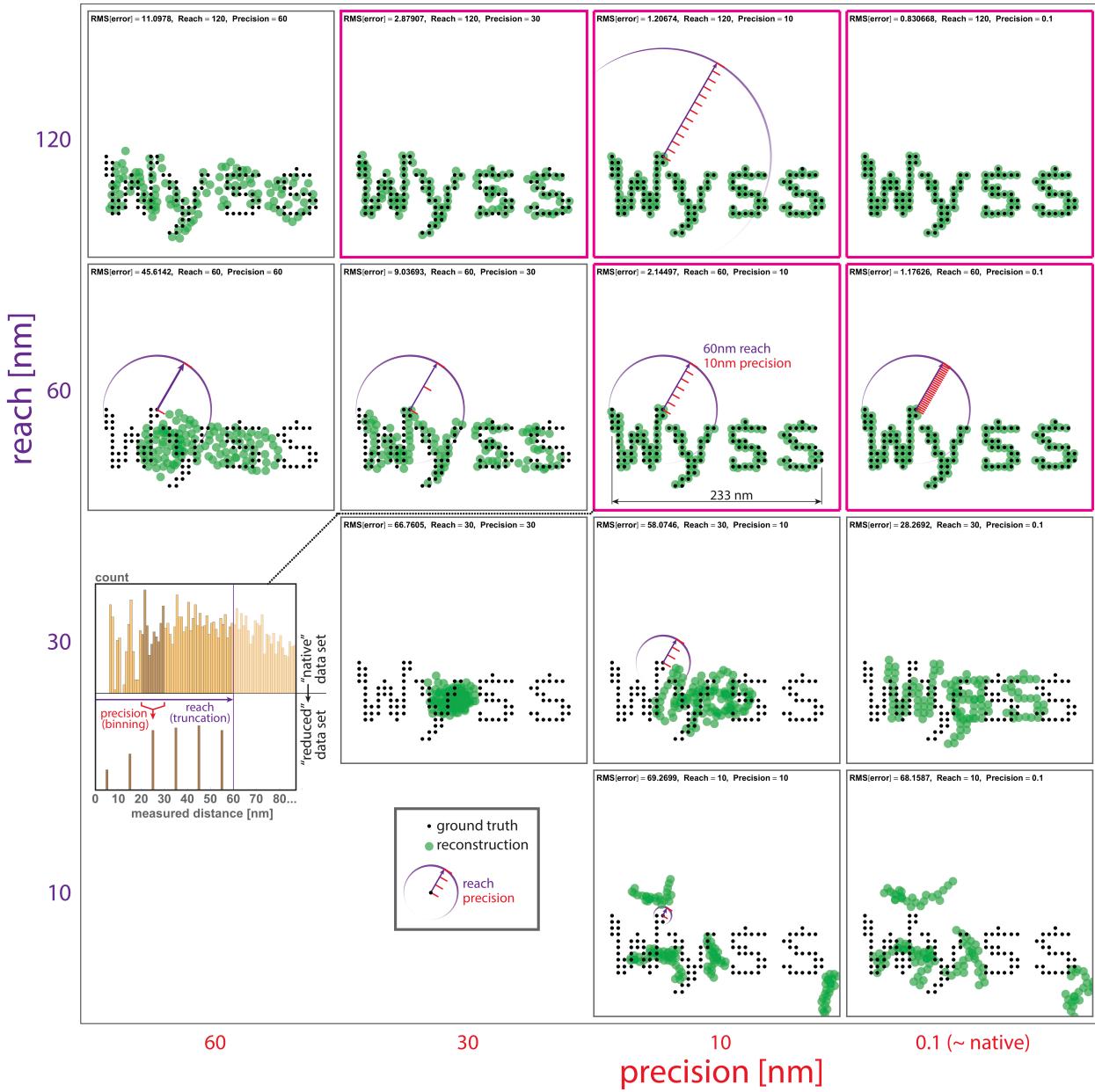
where:

$J$  is the objective function, defined above,

$\alpha$  is the penalty applied for pruning points. We empirically set  $\alpha = 0.5$  for all patterns except for the Wyss pattern, for which  $\alpha = 0.25$  and,

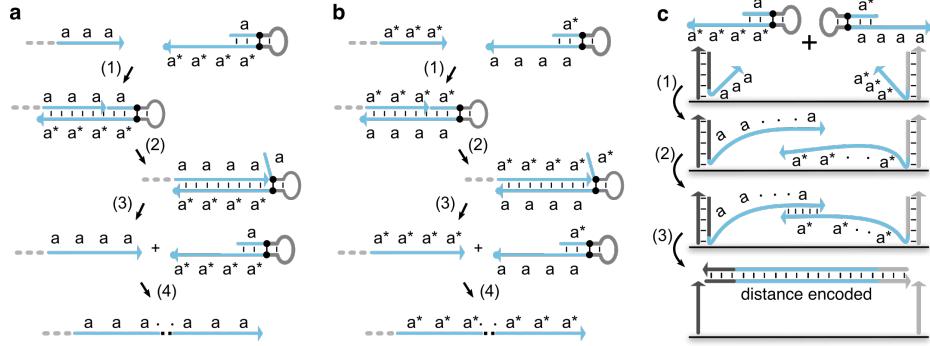
$r$  is the number of points pruned.

The optimum threshold was chosen to be the one that minimized  $score$ , reflecting the best agreement between the measured and embedded distances. Note that the embedding score cannot take negative values. The term  $J$  captures the agreement between the measured distances and the embedded distances for all non-pruned points. The penalty term for pruned points,  $\alpha r$ , is necessary to prevent the algorithm from trivially achieving  $J = 0$  by setting the threshold to a very high value that prunes all points because all weights  $w_{ij}$  are set to zero.

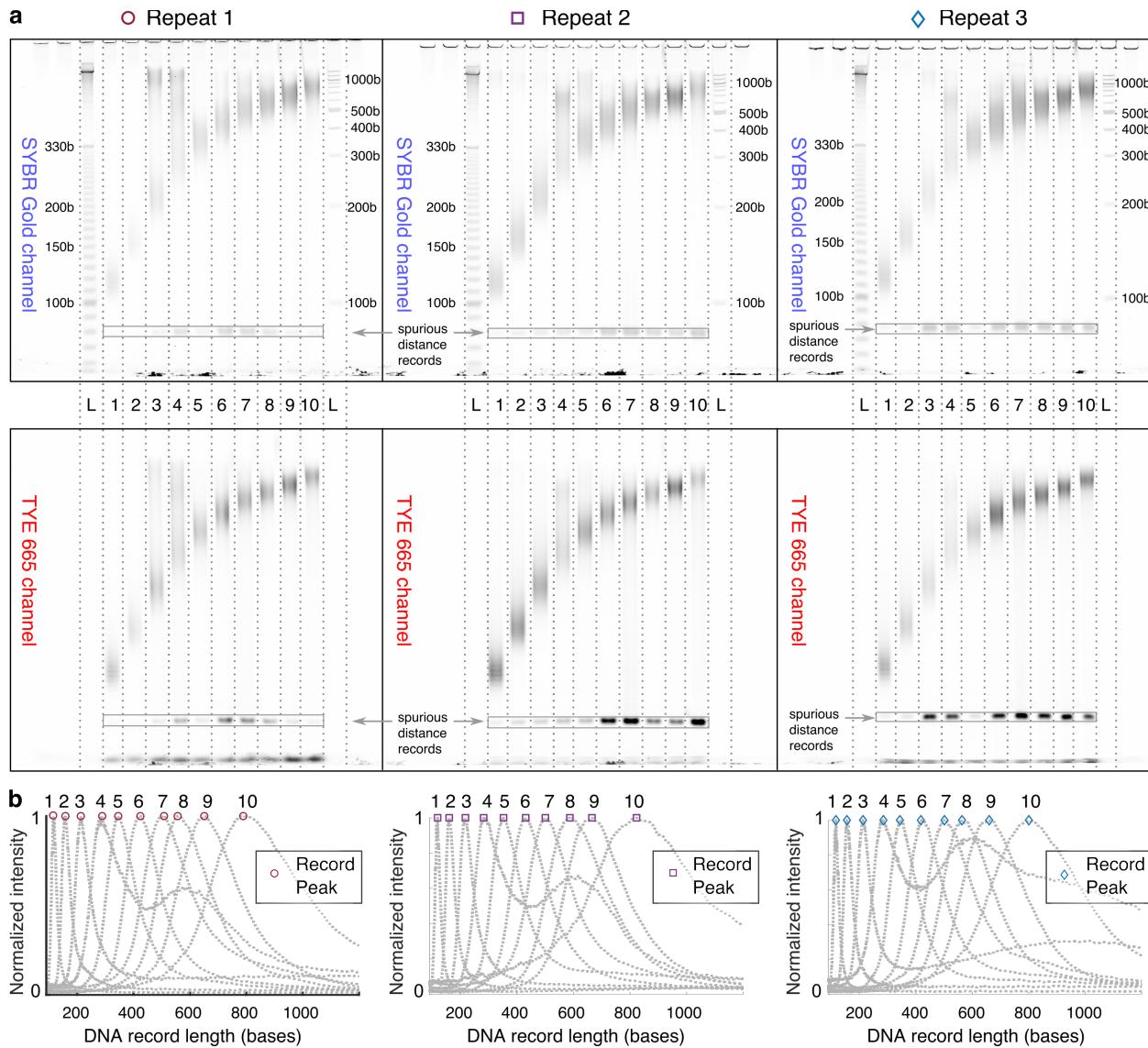


**Supplementary Fig. 1.** Effect of ruler precision and reach in reconstruction. Shown are results of a completely *in silico* study simulating diminished ruler precision and reach. A base “ground truth” geometry (“Wyss”) comprising 121 points and spanning 233 nm was designed. Distance measurements for each pair (i.e., post sequencing and processing) were generated by modifying known (ground truth) values about a normal distribution of error with a fixed coefficient of variation (CV) of 5%. Measurements were then degraded by deteriorating precision and/or limiting reach. “Precision” is effectively the width of bins used to group similar measurements (histogram inset), while “reach” is the length of the farthest distance that results in a measurement. Reconstructions follow the same computational process (a reconstruction minimizing total discrepancy<sup>2</sup> between reads) as in the main text. Results show that limiting precision (60 nm), even at high (120 nm) reach, results in high RMSD. Similarly, high precision (0.1 nm, virtually without binning) yielded poor results if reach was limited to nearest neighbors

(10 nm). Only with reasonable precision of 10 nm and reach of 60 nm (1/4 of total pattern span, enough to cross gaps within and between letter components) did reconstructions fall near RMSD = 2 nm. Further precision or reach improvements helped, but minimally. Pink outlines denote reconstructions near or below RMSD = 2nm.

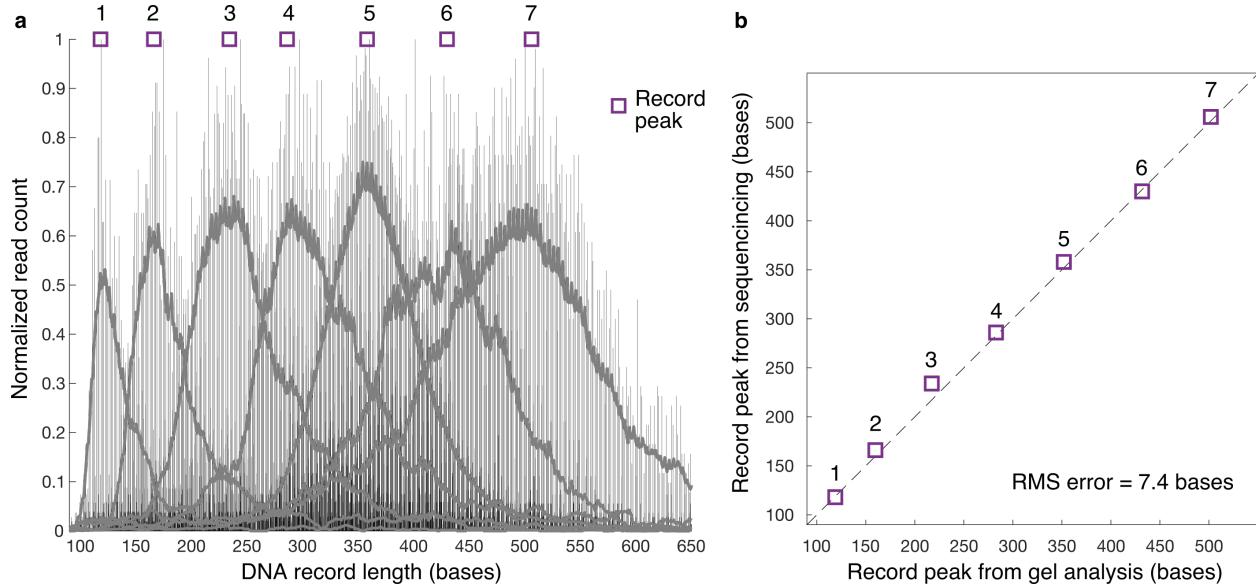


**Supplementary Fig. 2.** The full molecular ruler mechanism. Note that unlike the simplified version depicted in Fig. 2, the primer actually binds to the hairpin using a 12 base domain ‘a a a’. The rest of the details of the mechanism are identical. **a.** A primer exchange reaction (PER) cascade repeatedly adds the four base sequence domain ‘a’, as follows. (1) The recording primer hybridizes to a PER hairpin, (2) a strand displacing DNA polymerase (Bsm large fragment) extends the primer into the stem of the hairpin and in the process copies domain ‘a’. A ‘stopper’, a non-canonical base pair modification (isoG-isoC) on the template that is not recognized by the DNA polymerase, blocks further extension. The polymerase dissociates from the hairpin. (3) The recording primer is only weakly bound to the hairpin and also dissociates. (4) The above sequence of reactions repeat, adding domain ‘a’ every time. **b.** In the same manner, a complementary PER cascade repeatedly adds the four base sequence domain ‘a\*’. **c.** A double-stranded DNA ‘distance record’ is generated as follows. Consider two DNA labeled targets with recording primers hybridized to them. (1) The primers take part in PER reaction cascades, as described in parts A and B, adding sequence repeats of ‘a’ and ‘a\*’ respectively. (2) The extended primers hybridize, (3) copy each other with the aid of the polymerase, are displaced from the targets and released into solution, making a distance record. The whole process is isothermal and autonomous.

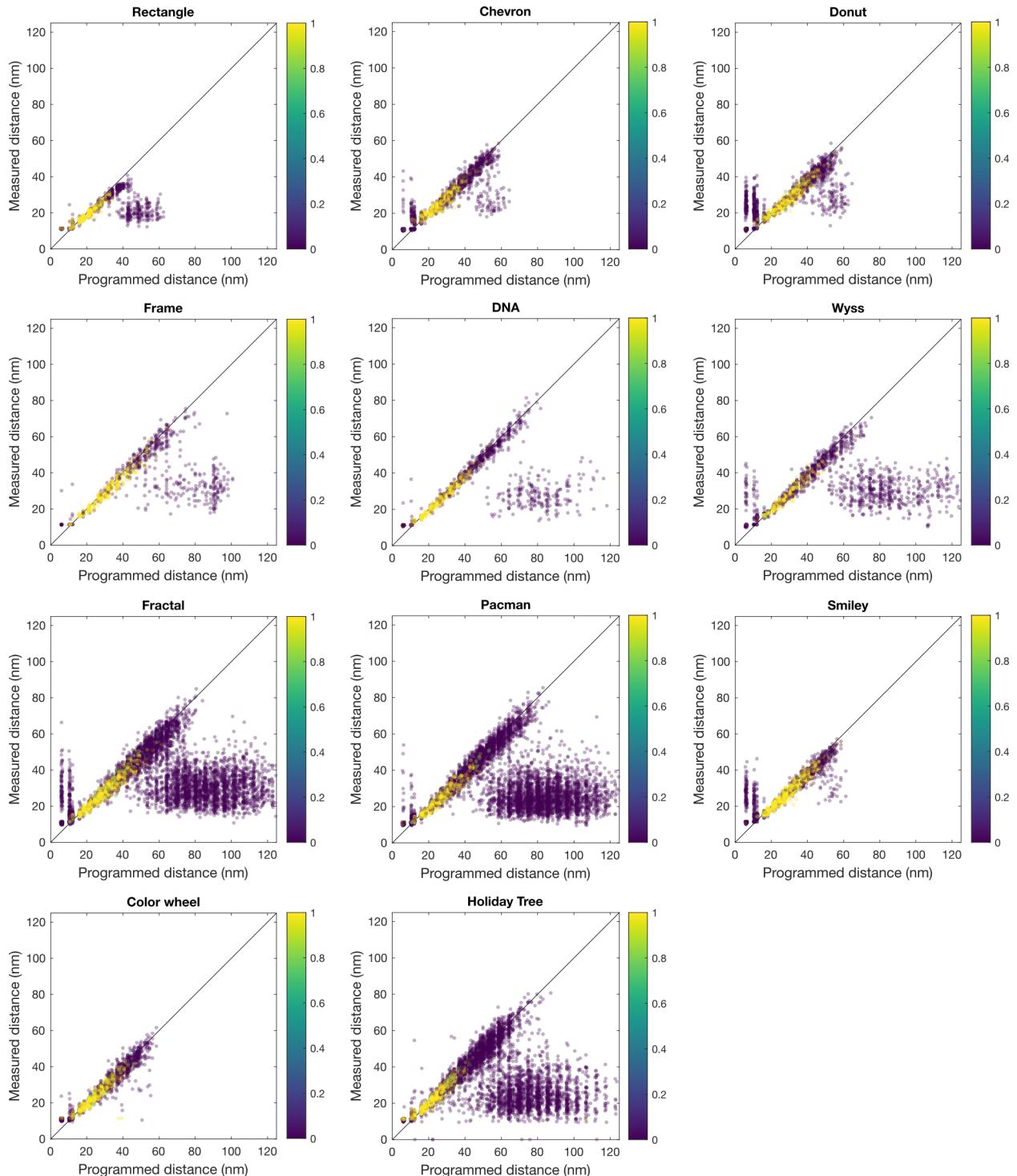


**Supplementary Fig. 3.** Molecular ruler calibration experiment. The experimental setup is described in Fig. 3. **a.** PAGE characterization of distance records. After PCR, distance records were size separated on a denaturing PAGE (see Materials and Methods section for details). The gels were imaged in two non-overlapping optical channels. Solid lines indicate gel boundaries and dotted lines are lane guides. SYBR Gold is known to stain DNA in a sequence and length dependent manner, confounding estimation of relative quantities of distance records of various lengths. Instead, the relative molar ratio of distance records of various lengths was estimated using the TYE665 channel. The TYE665 intensity should be proportional to the molar amount of the distance record, since it is introduced in an equimolar ratio by conjugation of the dye to the PCR primers. The SYBR Gold channel is used to track the ladder and hence estimate the absolute length of the distance records. **b.** Length distribution of distance records for various calibration distances. Each distance measurement reveals a distinct length distribution of distance records. The distance records are skew-normal distributed, with longer distances resulting in longer records that are more broadly distributed. The peak of the distribution was used as an archetype to generate a calibration curve. Gel profiles corresponding to lane 4 show a minor

peak, which we believe is due to either a defect in manufacturing DNA origami or contamination. This peak is ignored.

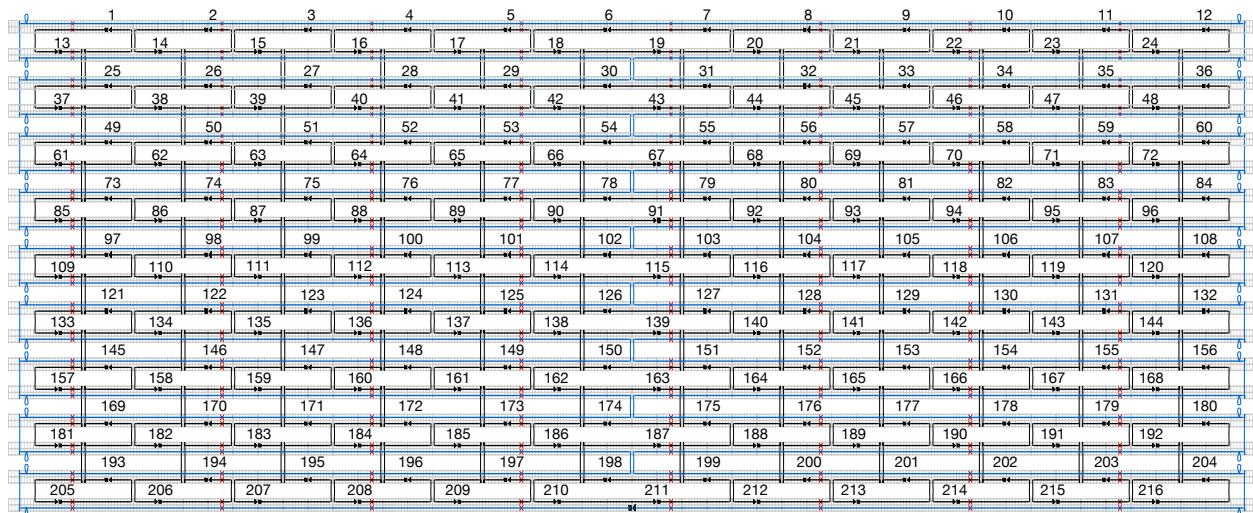


**Supplementary Fig. 4.** Characterization of the lengths of distance records by sequencing. Records from ruler experiments corresponding to seven different calibration distances were barcoded using barcoded PCR primers, pooled and then read using next-generation DNA sequencing. The programmed calibration distances are 1 = 21.4 nm, 2 = 32.0 nm, 3 = 42.8 nm, 4 = 53.4 nm, 5 = 63.9 nm, 6 = 74.8 nm and 7 = 85.3 nm **a.** The height of the bars equals the number of reads, normalized relative to the count of the most read record. The length includes primer regions, 32 bases each, flanking the variable length repeat region. The gray ‘outline’ curves are the moving average traces (span = 8) of the record lengths, shown here to allow the reader to discern records belonging to different calibration distances and their corresponding peaks. Next-gen sequencing sampled a few thousand reads for every pair of distances, while gel electrophoresis looks at billions of records in aggregate and hence results in smoother profiles (Fig. S3). **b.** A comparison of the peak locations characterized by gel electrophoresis (X-axis) versus peak locations obtained from next-generation DNA sequencing shows no significant systematic biases in sampling distance records with next-gen sequencing. The typical sequencing depth used in this work (about a few hundred sequences per pairwise distance) is inadequate to accurately sample record distributions corresponding to longer calibration distances (8, 9 and 10 in Fig. 3) and hence these are not included in this figure. The absence of accurate measurements of these longer distances from our sequencing data did not preclude us from successfully reconstructing patterns with points that span longer distances.

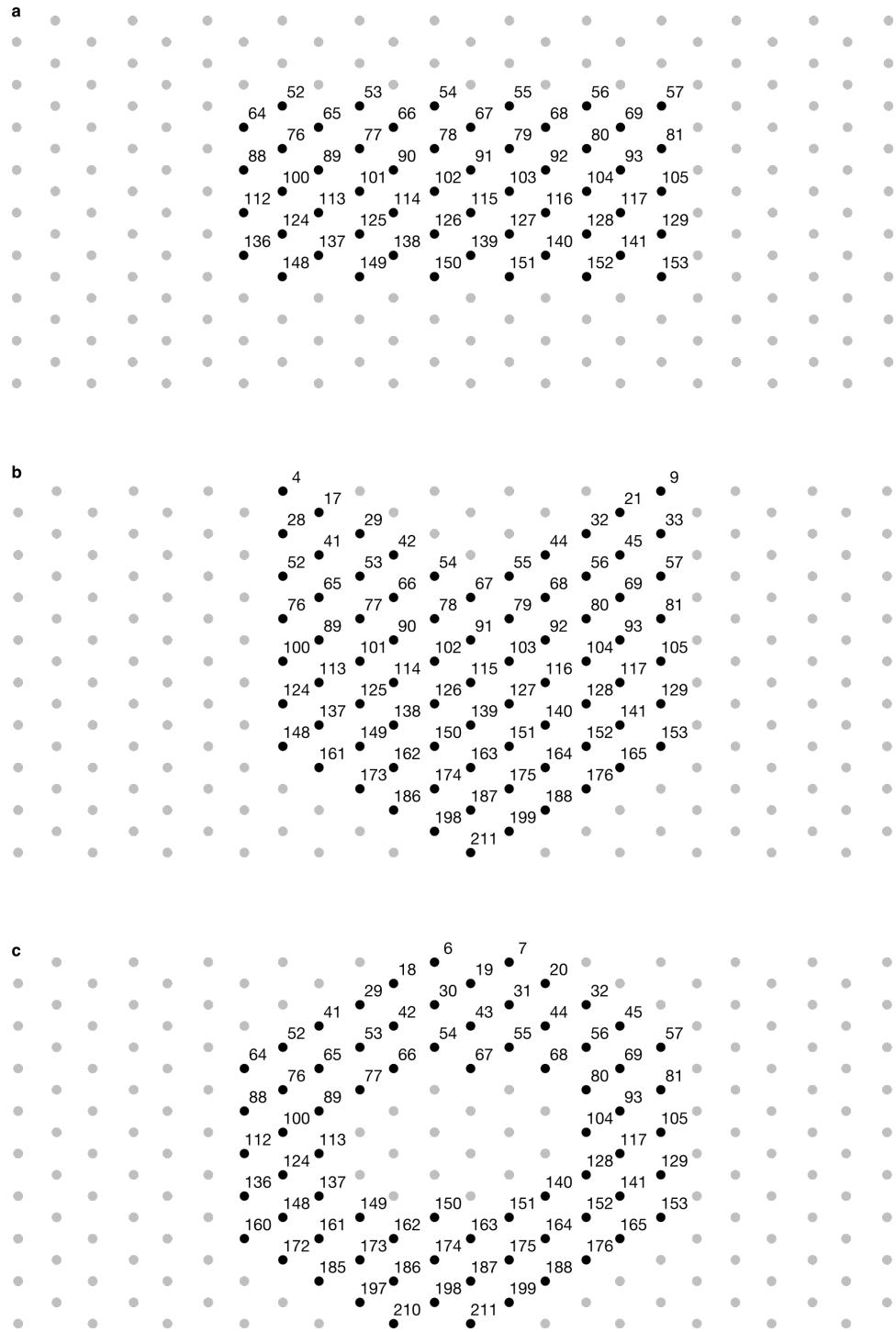


**Supplementary Fig. 5.** Programmed distances versus measured distance for each pattern from Fig. 4. Each dot within one plot corresponds to a unique pair of points. The color of the dot indicates the weight assigned to that measurement by the reconstruction algorithm (see Supplementary Note 2 for details). The overall quality of the reconstructions exceeds the average accuracy of the measurements made because of network effects. The plots indicate that points that are farther apart from each other are more likely to have an unreliable ruler measurement.

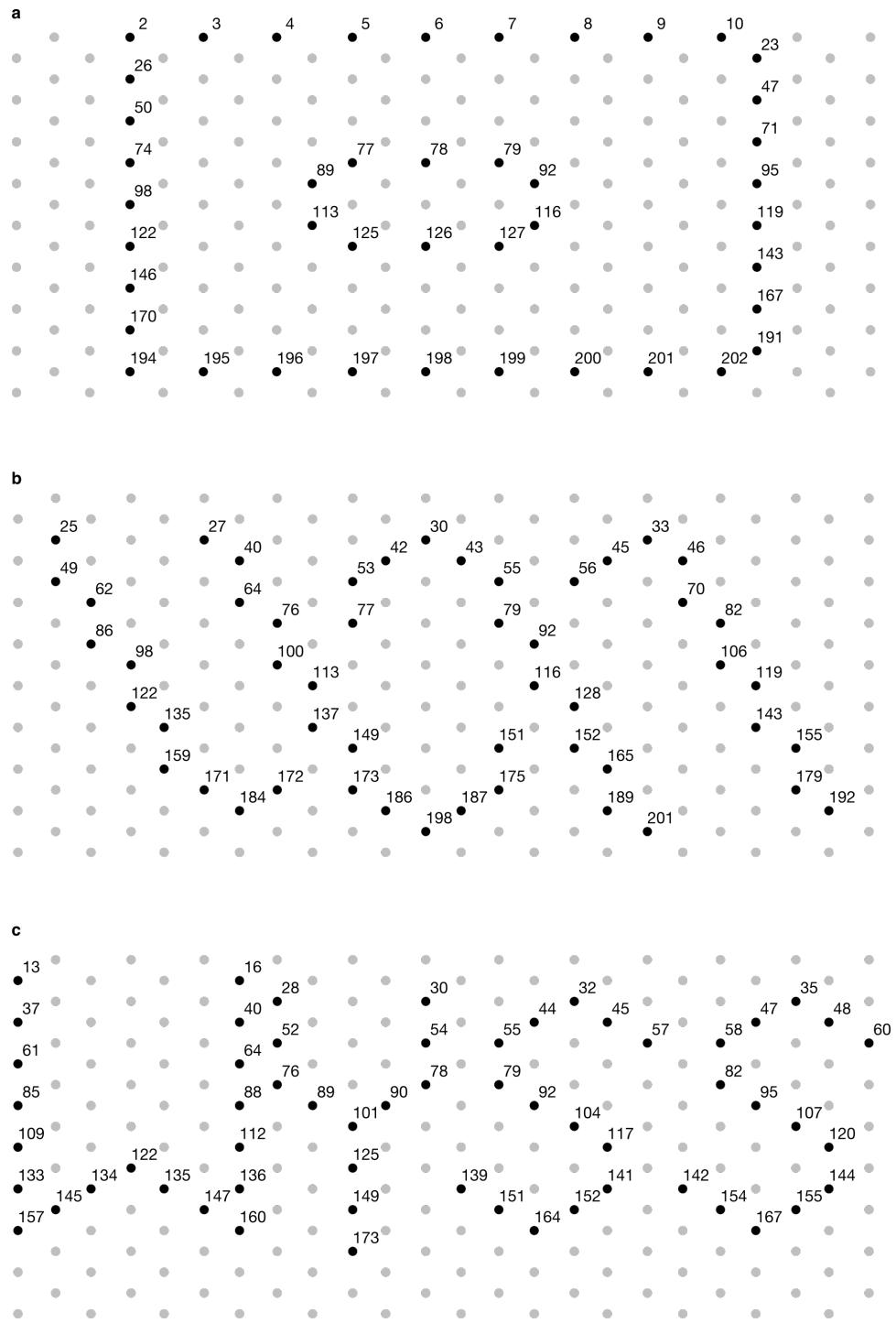
This is a result of two factors. One, fewer records are generated for longer distances because the efficiency of successfully creating records drops with increasing distance, likely limited by insufficient primer extensions due to either deleterious reactions and/or insufficient reaction time. Two, the longer the distance being measured, the more the number of sequence reads required to accurately sample the distribution of distance records. This is because the lengths of distance records are more broadly distributed as the distance being measured increases. We expect deeper sequencing to yield accurate distance measurements over longer distances.



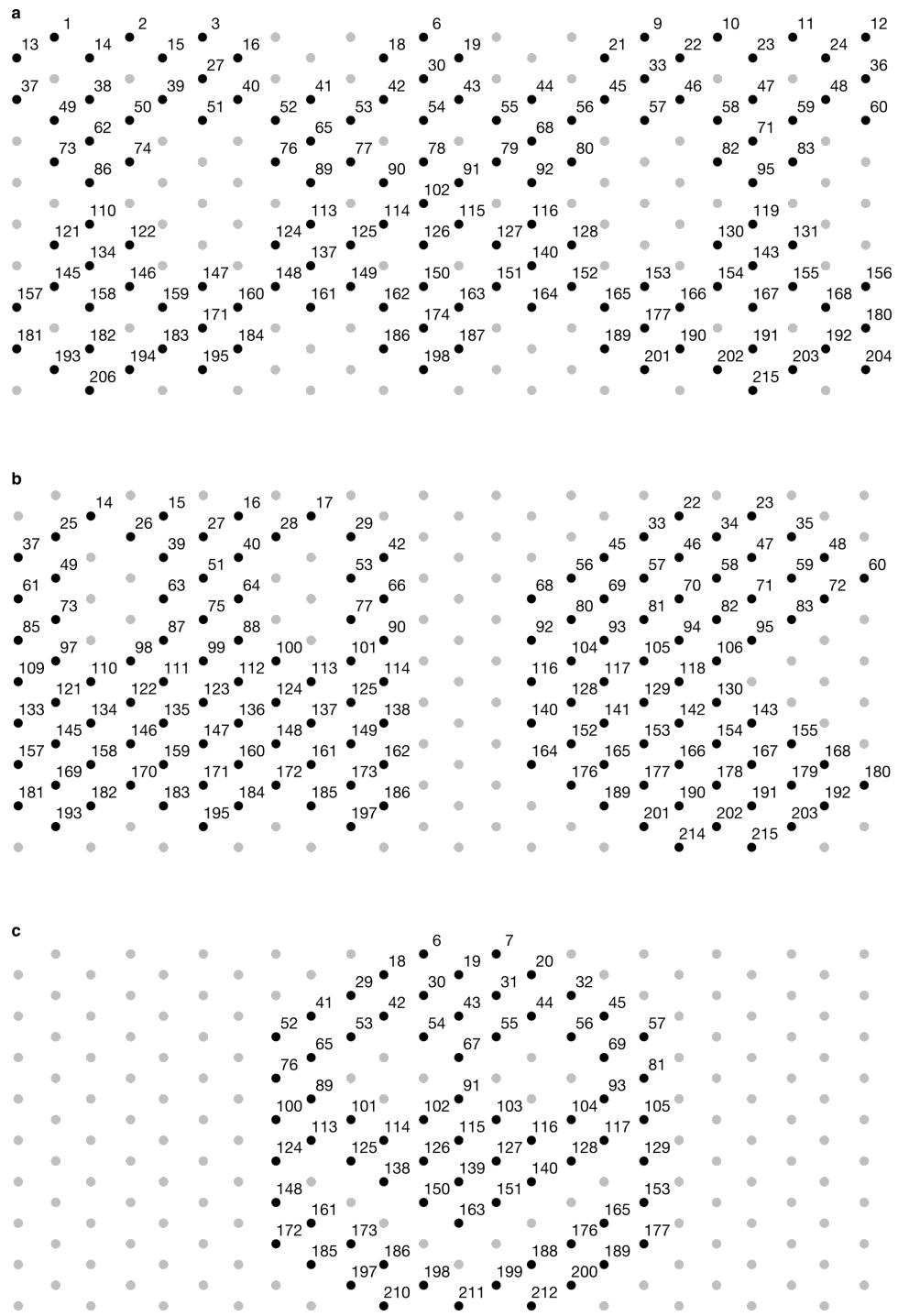
**Supplementary Fig. 6.** Scaffold and staple diagram for the DNA origami used in this work. The scaffold strand (M13mp18) is depicted in blue and the staple strands in black. Arrows at the end of strands represent 3 prime ends and the blocks at the other end represent 5 prime ends. The red crosses represent ‘skipped’ bases in the cadnano design software, corresponding to twist corrections (see Materials and Methods) made to minimize strain and promote flatness. The numeric labels correspond to the three prime ends of the staple strands. They are also the sequence IDs for blunt staples, extended staples and recording primers in the corresponding supplementary tables. The ‘blunt’ staple sequences are listed in Table S2, extended staple sequences in Table S3 and recording primer sequences in Table S4.



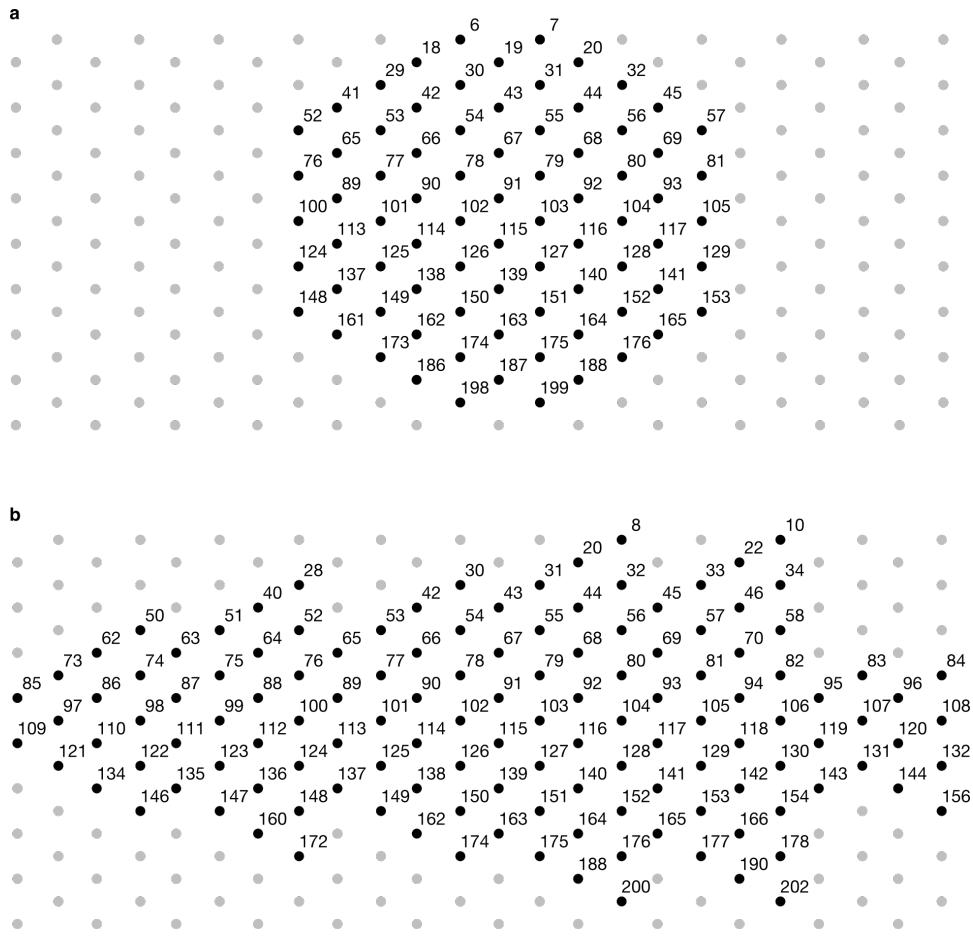
**Supplementary Fig. 7.** Pattern diagrams. Grey dots are 3' ends of blunt staples and black dots are 3' ends where either a blunt or extended staples may be incorporated. Numeric labels indicate the unique identity of every position in an origami. **a.** Rectangle. **b.** Chevron. **c.** Donut.



**Supplementary Figure 8.** Pattern diagrams. Grey dots are 3' ends of blunt staples and black dots are 3' ends where either a blunt or extended staples may be incorporated. Numeric labels indicate the unique identity of every position in an origami. **a.** Frame. **b.** DNA. **c.** Wyss.



**Supplementary Figure 9.** Pattern diagrams. Grey dots are 3' ends of blunt staples and black dots are 3' ends where either a blunt or extended staples may be incorporated. Numeric labels indicate the unique identity of every position in an origami. **a.** Fractal. **b.** Pacman. **c.** Smiley.



**Supplementary Figure 10.** Pattern diagrams. Grey dots are 3' ends of blunt staples and black dots are 3' ends where either a blunt or extended staples may be incorporated. Numeric labels indicate the unique identity of every position in an origami. **a.** Color wheel. **b.** Holiday tree.

Strand	Sequence (5' to 3')
Recording primer 'a'	TCGTGCGAGTATAGAAAGTGAGGGATTAATGGAAATAAATAAAT
Recording primer 'a*'	TCTACCCCATGAAGAGTAAATAGGTTGTGGGAATTATTTATTT
PER growth hairpin 'a'	AAAT/iisodG/mCGCCCGCCACTAGCAGGGCmG/iMe-isodC/ATTATTTATTATTT/3InvdT/
PER growth hairpin 'a*'	ATTT/iisodG/mCGCCCGCCACTAGCAGGGCmG/iMe-isodC/AAATAAATAAATAAAT/3InvdT/
PCR Primer 1	/TYE665/TCGTGCGAGTATAGAAAGTGAGGGATTAATGG
PCR Primer 2	/TYE665/TCTACCCCATGAAGAGTAAATAGGTTGTGGGA

**Supplementary Table 1.** Growth hairpins, recording primers and fluorescent PCR primers for the molecular ruler calibration experiments described in Fig. 3 and Fig. S3.

<b>Location ID</b>	<b>Blunt origami staple sequences, 5' to 3'</b>
1	AACCATCGCGTAACACTGAGTTGCCACCT
2	TTGATACCACAACGCCTGTAGCACGCCACC
3	GCTTGCTTCATAGTTAGCGTAACGAGGTGTAT
4	AAAAGGAGGTCTTCCAGACGTTACGGAGAGGG
5	TTCACGTTATGGGATTGGCTATTGCT
6	CATGAAAGTATTAAGAGGCTGAGACTCCTCA
7	GCGTTTGCAGAGCCACCACCGGAATTCTGAAA
8	CATCGGCACGCCACCCCTCAGAACTAATGCC
9	TTGCCTTACCAACCCCTCAGAGCCGGTGCCTTG
10	AGCACCGTCCAGAGCCGCCAGAGTGTACT
11	CCGGAAACAGGCAGGTCAAGCAGAAAGCGTC
12	ATCACCAAGCAAACAAATAATCCTGAAAGCGC
13	CAGAGCCACCAACCTCATTTCATAGGAACC
14	CTCAGAACGCCACCCCTCAGAACCGTCACCAG
15	CACCGTACTCAGGAGGTTAGTACTTCCACAG
16	TTGATATAAGTATAGCCCGAATATCTAAAG
17	CAGTACCAGCGGATAAGTGCCTGTAAATGA
18	AGAGAAGGATTAGGATTAGCGGGGAAACAACCTT
19	AACAACTATTCAAGCGGAGTGAGAAAAATCAC
20	CCCTGCCTATTCGGAACCTATTACCGCCTCC
21	AGTAACAGTGCCGTATAAACAGTCGCCACCC
22	GGTAATAAGTTAACGGGTCACCACCAAGA
23	ATACATGGTTTGATGATAACAGGCATTGACA
24	AGTCTCTGAATTACCGTTCCAGTTGGCCTT
25	ACCGAACTAAAGGCCGCTTGCATGACAAC
26	GGAACGAGCAGCGAAAGACAGCAAAACAGC
27	AAATCCGCAACGGCTACAGAGGCTTTATCA
28	GATTGTATTTTACGAGGAAGTAAGGCTCC
29	ATTATACCAAATACGTAATGCCATAATT
30	CGGAACCCATCTTTCATAATCTAGAAAGG
31	ACGTAGAAAGAAACGCAAAGACACCTTATTA
32	TTAAGACTTTGTACAATCAATGCCTT
33	ATAAACGGTACCGCGCAAAGAAATCAAGT
34	AGTTACCACAACCGATTGAGGGAGTCGATAGC
35	TTTTTAAGCGGAAATTATTCAATTAGCAAGG
36	TGAAATAGCGTCACCGACTTGAGCCCAGCAAA
37	CATGTACCCACGCATAACCGGAGGCTTGCA
38	TACAAACTGATAGTTGCCCGACAGGATCGTC

<b>Location ID</b>	<b>Blunt origami staple sequences, 5' to 3'</b>
39	ACAGCCCTTCGAGGTGAATTCTTCGGAACG
40	TTTGTCCCTTAATTGTATCGTTGAGGAC
41	ATTTCTGGAAAATCTCAAAAAATTCCATTA
42	TCAACAGTAAGGAATTGCGAATAACTACGAAG
43	ACACTAAACTAAAACGAAAGAGGCGTGGCAAC
44	CTCAGAGCTTCGGTCATAGCCCCACGGAAT
45	TCAGAGCCAGCGTCAGACTGTAGCAGAAAATT
46	ACCACCAAATCAGTAGCGACAGCAAAAGGG
47	GGAGGTTGGTCACCAATGAAACCAGGAAGGTA
48	GATATTCATAGCACCATACCATTAAAGGTGA
49	CCAAAAGGATAGGCTGGTGACCTATAAGGGA
50	ATACCACATGACAAGAACCGATCTAGCC
51	GGTAGAAAAACGTAACAAAGCTGCTGTGTCG
52	TAAAACGAGGCTTGCCCTGACGAGACAACGGA
53	GTCAGGACAGTAAATTGGGTTGCCAGCG
54	ATATAAAAATACATACATAAAGAAAAGAAT
55	TACAAAATTAAGAAACGATTTTTGTTAGCAA
56	ACGAGCGTAAAATAGCAGCCTGGCATGA
57	TACAATTTAAAAACAGGGAAGCGAACGCAAT
58	AATCAAGATTAACCTGAACACCCCTGCCAACAA
59	CCTCCGAAATTGAGCGCTAATAGAACCCC
60	GTATTCTACAAGAATTGAGTTAACGAAACAA
61	GGGAGTTGACCAACTTGAAAGGTACAGAC
62	ACCCCTCAGGCAGACGGTCAATCTCATCAAG
63	AGGGTAGCGACCTGCTCCATGTTAATTCTTA
64	TAAAGACTCATGCCCTGATAAAATCATTCAAG
65	AAACGGGTAAAGCGCGAACAAAGTAAACACCA
66	GCACCAACACACTCATTTGACCAGATGGTT
67	ATGCGATTACTTTAACATTGTGATTATCCCA
68	AAGTTTATCCTTATTACCGAGTATGTTAACG
69	CATATGGTAATACCCAAAAGAACCTACAGAGA
70	CGACATTGAAGGAAACCGAGGACATTAGAC
71	AAATATTGAAAAAGTAAGCAGATAGAACAAAGT
72	ATTATCACCAATAGCTATCTTACCTCAGAGAG
73	CCTTTAACAGACGACGATAAAAACATAACG
74	AGCAAACCTTTGCAAAAGAACGTTAGGA
75	TAATTGAAATAGTAAAATGTTATTACAA
76	GGAAGCCAATACTGCGGAATCGTTACGTTAA

<b>Location ID</b>	<b>Blunt origami staple sequences, 5' to 3'</b>
77	AAGCAAAGATCCCCCTCAAATGCTATAACCA
78	ATCCAAAAAACAGCCATATTATATTACCTT
79	ACAATAAAGTTATCAACAATAGATTGCCAGT
80	ACAAAAGGAAAATAATATCCCATAACGCTA
81	CGAGCCAGGTAGAAACCAATCAATCACCCAGC
82	CGCCAACACTTATCATTCCAAGAAAAGCCTTA
83	AGGGCTTATACCGCACTCATCGATAGCGAA
84	TCTTACCATTATTTCATCGTAGCTTATCCG
85	CAGGCAGCAATTACGAGGCATAGATAACCCCT
86	AGTAATCTTCAACTAATGCAGATCCAAAATA
87	CCCAAATCGATTCATCAGTTGAGATTTGCCA
88	TGAATAAACTAACGGAACAACAGACTGGAT
89	GAACGAGTGTGGGAAGAAAAATCCATAAATA
90	TAATTCATTAAGAACTGGCTATTAAACA
91	ATCAGGTCAACGAGAATGACCATAATGCAGAA
92	TCAAAAATCTTCCAGAGCCTAATTAAGTCCT
93	GAATAACATATCCTGAATCTTACCCCTAATTT
94	GGGAGAATTAGTTGCTATTTGAATCGGCT
95	CAGAGGTCTTGGGGAGGTTTGCGGGTATT
96	ATAACCCAAGAACGCGAGGCCTTGAACAAGC
97	TTTGGGGATTGCTGAATATAATGAGAGAGTA
98	TTGTACCATTTAAATATGCAACTGACCGGA
99	AATAAAGCAAGTTCATCCATATTGCGCTTT
100	CAGGCAAGATTCTGCGAACGAGTAGATTAAGA
101	ATAGTAGTTAGATACATTCGCTAGTCAG
102	CGCGCCTCAACATGTTAGCTAAATCAAAA
103	ACCTCCGGTGCTGATGCAAATCCAAGACGACG
104	CAAAATCACCGAGAAAACCTTACCG
105	AGACGCTGTAATTCATCTTGAGGCATT
106	ATCCTTGATTGAAATACCGACCGTTAACAA
107	AAATCGTCTAATAAGAATAAACCAACAGT
108	AATATATGCTAGAAAAAGCCTGTTATACAAAT
109	CGTTTACTGCTCCTTTGATAATGGCTTA
110	GCGAGAGGCCAACAGGTAGGATTCTGTAGCT
111	GAGGGGGTGCTCAAAGCGAACCAAAAGTACG
112	AGCGTCCGAAAGACTTCAAATAAACAGTTG
113	TTCATTGACGGATTGCATCAAAAGATTTAGT
114	GTTCAGAATTACCTGACTATTAATGGTCA

<b>Location ID</b>	<b>Blunt origami staple sequences, 5' to 3'</b>
115	GCGAGCTGTTAGCTATTTCAATACTAT
116	GAACAAGATAAAAGTAATTCTGTCCATCGAAG
117	ACGAGCATTAATAAGAGAATATAATCAAATAT
118	GTCTTCTGTAATTAGGCAGACCTAAATT
119	AAACCAAGATTGAGAATGCCATAGTGATAAA
120	AAGCCGTTGATAAAGCCAACGCTACCGGAAT
121	TTTTGTTATATTTAAATGCAAGTAATACT
122	AACGTTAAAGGTAAAGATTCAAAAAATCGG
123	GAAGATTGGAGACAGTCAAATCACAAATTAAGC
124	CGGTTGATCAACC GTCTAGCTGAAAATCATA
125	TCGTAAAAGGGTAGCTATTTTCTACTA
126	ATGTAAACTTAGGTTGGGTATTTGGGGC
127	TTCAGGTTCTTTACATCGGGAGACCTTTA
128	CGTAAAAC CCTGATTGCTTGAAAATTAT
129	GTTAGAACCGCGCAGAGCGAATTAGATTA
130	TGTTGGACTGAGCAAAGAAGATCCCTAGA
131	AGATGATGAGAAAACAAAATTAAC TTCTGT
132	GAGCGGAACATTGAAATTACCTTCAAAATC
133	GAGCTTAAGAAGC TTTATTCTTTAGA
134	CAACATGTAACATTATGACCC TTGCCTGAG
135	GTGCTGGCTCAGAGCATAAAGCTAGGGTGAG
136	ATTCCCAGCAAAGAATTAGCAACATCAATA
137	TTGACCATAGCATT AACATCCAATTAAATTAA
138	ATAACCTGAAAAGGTGGCATCAATGAGAGATC
139	TGGAGCAACTATCAGGT CATTGCCATACAGTA
140	ACAAAGAATAGGTCTGAGAGACTAAACAATAA
141	ATTTTAGTAGAAGAGTC AATAGTGACCAAGT
142	TAATGGTAAACATAGCGATAGCATTCAATT
143	TAAGGCGTGTATTAAATTAAATTGATGAAAC
144	CATAATTATGAGTGAATAACCTTGTACATT
145	AAGGCGATCGCGTCTGGCCTTCCTGCATTAAA
146	TTACGCCAACATTAAATGTGAGAATTGTA
147	GGAAGGGCGGATTCTCCGTGGGAAAAAACAG
148	GCCATTGCCGTAAATGGGATAGGTATGTACCC
149	CACCGCTTGGCGCATCGTAACCGACGGTAA
150	ACAGTACTAACGTCAGATGAATTGAGAGTC
151	TGGTCAGTAAGGTTATCTAAAATACGTAGATT
152	GAACCTCACA ACTAATAGATTAGATTGCA

Location ID	Blunt origami staple sequences, 5' to 3'
153	GCAAATGATACTTGAGGATTAAATGGAAGG
154	GCCTGCAAACAAACAATTGACAAATCCTGAT
155	AAAACAGATGCCGAACGTTATTGATTATC
156	AAAAATACGTAACATTATCATTTCACCAGAAG
157	ACCCTCAAAATCAGCTCATTTCATCAAA
158	TAATGTGTTATTTGTTAAAATTCTGTAGCCAG
159	AAAGGCCGTATAAGCAAATATTACCGAGTAAC
160	TGATATTAATCAGAAAAGCCCCAACGGC
161	TGCCGGAGCTAGCATGTCAATCATCACGTTGG
162	TACAAAGGACAAGAGAATCGATGATGCATCTG
163	CAGGAAGAAGGGGACGACGACAGTTGAAAGGA
164	CGGATTGAGAAATAAGAAATTGTCTTAGG
165	TACAAAATCTACCATATCAAATTAGCCGTCA
166	CAATTACTTATACTTCTGAATAGAAGTATT
167	AAACATCAGCAATTCAATCATACTCGTATT
168	AAACATTTTATCATCATATTCCAATTAA
169	CTGATTGCGACGCCAGTGCAAGTGTGCTGC
170	GTTTTCTCGACTCTAGAGGATTCGCTA
171	AGAGGC GGCAATTGTAATCATGAACGTGTTG
172	GCTGCATTTGTGAAATTGTTATGGCAAAGC
173	CCCGCTTTACAACATACGAGCCGTTCCGG
174	ATTGAGGTGGCAAATCACAGTATCGGCCT
175	TTGCAACATGACGCTCAATCGTCTCAATATC
176	GGTAATATATTGGCAGATTCAACCCCTTGCT
177	GAGTAGAAATAAAAGGGACATTCTAGCCAGCA
178	CTTCTTGAAACCCCTCTGACCTGATTAACACC
179	GTCCATCAGGCACAGACAATTAGAAGAT
180	TAATCAGTTCTTAATGCGCGAACTCGCCATT
181	AATAATTAAAGTTGGTAACGCACGACGTT
182	CTTCATCGCTGGCGAAAGGGGACTTGCATG
183	AACCCGTCGATCGGTGGGGCTCCCCGGGT
184	GGATTGACCATTGAGCTGCGCGTCATAGC
185	TGTAGATGCTGGTGCCGAAACCACCGCTCAC
186	CCAGTTGTCGCACTCCAGCCAGCGAAGCATA
187	CTAACTCAAGCCTGGGTGCCTAAGAAATACC
188	AGCACTAAAATATCAAACCCCTCAAGAAATGGA
189	ATAGATAAAAATCTAAAGCATCAAGTCACAC
190	AGACTTCAAGTGCCACGCTGAGGGCCAACA

Location ID	Blunt origami staple sequences, 5' to 3'
191	AAATCCTTGGTGAGGCGGTCACTAAAGCGTAA
192	AAGTTTGACGAACGAACCACCAAGCTTGAATG
193	CCACGCTGGTTGCCAGCAGGGGCAACAG
194	CTGTTGATGGTGGTCCGAAATCAGGGTG
195	TCCCTTATAAAATCAAAGAATAGCGCGCGGGG
196	GGGTTGAGTGGTGTCCAGTTGTGCGGCCA
197	GTCCACTATTAAAGAACGTGGACCTCACTG
198	TACATTGGAAAAACGCTCATGTGAGTGAG
199	CGCTGGCAAGTGTAGCGGTACGGCCAGCCA
200	AACCACACACCCGCCGCCTACCTTGCT
201	CTACAGGGCGCGTACTATGGTTGCACTTGCCT
202	GCACGTATAACGTGCTTCCTCGTAGCAATA
203	AGAGCGGGAGCTAACACAGGAGGCAGAGTCT
204	GGGATTTAGACAGGAACGGTACGTGTTTTA
205	GTAAAACCCCTTCACCGCCTGGCAAGCGGT
206	CCTGCAGGTTTCACCAAGTGGAGACCGAAGATC
207	ACCGAGCTTTGCGTATTGGGCGCCGGCAAAA
208	TGTTTCCAATGAATCGGCAACCGAGATA
209	AATTCCACCCAGTCGGAAACCTGGAACAAGA
210	AAGTGTAACTTAATTGCGTTGCGTCCAACGT
211	CAAAGGGCGAAAACCGTCTATCACCGCTAGGG
212	TTATTTACCCAGAACAAATTACCCCTGCGCGT
213	GACCAAGTAGAACTCAAACATCGGATGCGCCG
214	GAGATAGATTAGTAATAACATCTTGACGA
215	GAATACGTGCCAAATTAAACCGTTGTTAGAATC
216	GCTATTAGGAGGCCACCGAGTAAACGATTAAA

**Supplementary Table 2.** Blunt staple sequences for DNA origami used in all the experiments.

Location ID	Extended staple sequence with barcoded handle, 5' to 3'
1	AACCATCGCGTAACACTGAGTTGCCACCCCTCCTACGCTGGTATAGACT
2	TTGATACCACAACGCCGTAGCACGCCACCCATTGCACCTCCAGTAA
3	GCTTGCTTCATAGTTAGCGTAACGAGGTGTATTAGACCACTAGCGTTAGAC
4	AAAAGGAGGTCTTCCAGACGTTACGAGAGGGAGCTACAAGAATGACTGACT
5	TTCACGTTATGGGATTTGCTATTTGCTAGGTTATTGACGATGGATA
6	CATGAAAGTATTAAGAGGCTGAGACTCCTCACGCAAGGTTACGTGTAATGA
7	GCGTTTGCAGAGCCACCACCGAATTCTGAAACTCTTAGGTGGACAACCTACC
8	CATCGGCACGCCACCCCTCAGAACTAATGCCCTCATCTCATCCTGCATTGT
9	TTGCCTTACCCACCCCTCAGAGCCGGTGCCTTGATCCAGAGGTATATCAGTCC
10	AGCACCGTCCAGAGCCGCCAGAGTGTACTCTCTTCGTAGGTGACATC
11	CCGGAAACAGGCAGGTAGACGAAAGCGTCCGATAGCGTTGAACAAGAA
12	ATCACCAAGCAAACAAATAATCCTGAAAGCGCGGTACTCTGCAGGATCATA
13	CAGAGCCACCACCCCTCATTTCATAGGAACCGGCTGAAATTGCTGAGGTTAG
14	CTCAGAACGCCACCCCTCAGAACCGTCACCAAGGAATTAGCGTATTCCGCTTA
15	CACCGTACTCAGGAGGTTAGTACTTCCACAGTGAGACTAACAGATGAGGT
16	TTGATATAAGTATAGCCGGAATATCTAAAGCCAGATATTGTTCTCCGGTT
17	CAGTACCAAGCGGATAAGTGCCTGTAATGACCTAGTTGCATAATGTCCTC
18	AGAGAAGGATTAGGATTAGCGGGAAACAACATTGTTACACCGTTAGAGGTTCA
19	AACAACATTAGCGGAGTGAGAAAAATCACCACAATCCTATCAGTTGGTT
20	CCCTGCCTATTCGGAACCTATTACCGCCTCCAACTAATGGCATTAGTTTC
21	AGTAACAGTCCCCGTATAAACAGTCGCCACCCGAATAGAGACTTACGTGGCA
22	GGTAATAAGTTAACGGGTCACCACAGAAGGTGAAGTTGTGCATAGT
23	ATACATGGCTTTGATGATAACAGGCATTGACATAGTCTCGGAGCGTATAGTG
24	AGTCTCTGAATTACCGCTTCCAGTTGGCTTAATGGTTACAAGGTAGTT
25	ACCGAACTAAAGGCCGTTTGCATGACAACAGACTTCAACCTGTTACGAAGCAA
26	GGAACGAGCAGCGAAAGACAGCAAAACAGCTTCAACCTGTTACGAAGCAA
27	AAATCCGCAACGGCTACAGAGGCTGTTATCAGAACTCTGCGTTCCAATT
28	GATTTGTATTTCATGAGGAAGTAAGGCTCCGTAGTCATTGAAAGCTGC
29	ATTATACCAAATACGTAATGCCATAATTATAACCTCACGACTCACTAA
30	CGGAACCCATTTCTAAATCTAGAAAGGACAACCGATAACAAGGATG
31	ACGTAGAAAGAACGAAAGACACCCATTAGGACCGTAAGTAACCATTG
32	TTAAGACTTTGTCACAATCAATGCGTTTGTGTGACGAGTACCATCTAG
33	AATAACGGTTACCGCGCAAAGAAATCAAGTGTAGAGTCATTGACGTACC
34	AGTTACCACAACCGATTGAGGGAGTCGATAGCTACGTGTTACTTCCGAT
35	TTTTAAGCGGAAATTATTCAATTAGCAAGGCAGGCCGGATAGTACAGTTAG
36	TGAAATAGCGTCACCGACTTGAGGCCAGCAAAATAAGGCACCTCTTACT
37	CATGTACCCCACGCATAACCGAGGCTGCAATATCCTCCAGGTCACTAA
38	TACAAACTGATAGTTGCGCCAGAGGATCGTCTGAGACACTTACAATCCGG

<b>Location ID</b>	<b>Extended staple sequence with barcoded handle, 5' to 3'</b>
39	ACAGCCCTTCGAGGTGAATTCTTCGGAACGAGAGGCATATGAGGTAATCG
40	TTTGTCCCTTAATTGTATCGTTGAGGACTAGATCACCCTAGCAACTT
41	ATTTCTGGAAAATCTCCAAAAATTCCATTACCTCTGGATCAATAGGAAG
42	TCAACAGTAAGGAATTGCGAATAACTACGAAGGAAGTGTGTTGCAAGTATT
43	ACACTAAACTAAACGAAAGAGGCGTGGCAACTTAACGGTGTGTTGATAGGT
44	CTCAGAGCTTCGGTCATAGCCCCACGGAATCTCATTGTCTGGACACTAGG
45	TCAGAGCCAGCGTCAGACTGTAGCAGAAAATTAAATTCATCGCATCCACTGAG
46	ACCACCAAATCAGTAGCGACAGCAAAAGGGTTGGCATCTTAAGAGACTGG
47	GGAGGTTGGTCACCAATGAAACCAGGAAGGTAGGTTCCATGTTGATACTCGA
48	GATATTCA TAGCACCAATTACCAATTAAAGGTGACGCACATAAGTCCTTATCCT
49	CCAAAAGGATAGGCTGGCTGACCTATAAGGAGTCGTACCGACATTACCTGGATGA
50	ATACCACATGACAAGAACCGGATCTAGCCTTCCACAGTAGCGATAACTA
51	GGTAGAAAAACGTAACAAAGCTGCTTGTCGCTTACAGCACGTTGGTGTAA
52	TAAAACGAGGCTTGCCCTGACGAGACAACGGACTTATGATCGTAGACCGTGG
53	GTCAGGACAGTAAATTGGGCTTGCCCAGCGGAGTATTCCACACGATTGTT
54	ATATAAAAATACATACATAAAGAAAATTAGGAACGACTCTCTTCG
55	TACAAAATTAAAGAACGATTTTTGTAGCAACATGCAATAGAGTTGTCGAT
56	ACGAGCGTAAAATAGCAGCCTGGCATGAACTACCTTGTAATTGGCT
57	TACAATTAAAAACAGGGAAAGCGAACGCAATCTAATGTCGACAACGACGAC
58	AATCAAGATTAACTGAACACCCCTGCCAACAACTCTAACCTACTCGTAGTCG
59	CCTCCGAAATTGAGCGCTAATAGAACCCCTGAGTCGATTGGATTCAA
60	GTATTCTACAAGAATTGAGTTAAGAGAAACAAACGAATACCAACTGGTTGT
61	GGGAGTTGACCAACTTGAAAGGTACAGACTGTACTATGCCCTGAATCCA
62	ACCCCTCAGCGCAGACGGTCAATCTCATCAAGGGTAACTAAGCCGTGAGATG
63	AGGGTAGCGACCTGCTCCATGTTAATTCAATTATTCCCTGGCTATTCTAAC
64	TAAAGACTCATCGCCTGATAAAATCATTCAAGGCTCTATCTTACATCCGACG
65	AACGGGTAAAGCGCAGAACAAAGTAAACACCACACAGTTACGTTATTGGTG
66	GCACCAACACACTCATTTGACCAGATGGTTGAGAGAATGTTCTGAACGTG
67	ATGCGATTACTTTAATCATTGTGATTATCCCAGGTTACAAATAGAGCGACTA
68	AAGTTTATCCTTATTACGAGTATGTTAACGTGTCGAGGTATCTCAACAAAG
69	CATATGGTAATACCCAAAAGAACTTACAGAGAGCTGTTGAACATACCAGAA
70	CGACATTGAAGGAAACCGAGGGACATTAGACCCCTCTGAACAAACTGAGCTT
71	AATATTGAAAAGTAAGCAGATAGAACAAAGTAACAGGTAGGTAATAACCGG
72	ATTATCACCAATAGCTATCTTACCTCAGAGAGGGCAATGACTCAATAAGTCG
73	CCTTAATCAGACGACGATAAAAACATAACGCGGTGTTCAATAGACGTATC
74	AGCAAACCTTTGCAAAAGAAGTTAGGAGGACTATTGGTACTCAGAT
75	TAATTCGAAATAGTAAAATGTTATTACATCAACTACGTCCATCAACAC
76	GGAAGCCAATACTGCGGAATCGTTACGTTAAGAACTGACAATCACTCTGTT

<b>Location ID</b>	<b>Extended staple sequence with barcoded handle, 5' to 3'</b>
77	AAGCAAAGATCCCCCTCAAATGCTATACCAAGAGAGCAACCCCTCATATAG
78	ATCCAAAAAACAGCCATATTATATTACCTTGCATAAGCAGGGTTCTAGTG
79	ACAATAAAAGTTATCAACAATAGATTGCCAGTGAAGACCATCTAGAACCTGA
80	ACAAAAGGAAAATAATATCCCATAACGCTAGAACTAGGCGATAGTCTTGC
81	CGAGCCAGGTAGAACCAATCAATCACCCAGCTGGCTAATGTAATCACCCT
82	CGCCAACACTTATCATTCAAGAAAAGCCTTATAACAGCTTCGTTCAATCCT
83	AGGGCTTATACCGCACTCATCGATAGCGAATATCGTACTCGAACACAAAT
84	TCTTACCATTTATTTCATCGTAGCTTATCCGAGCGAGTGTCTATTGTCAC
85	CAGGCGCAATTACGAGGCATAGATAACCCCTAATCCGAACCAATGTTCT
86	AGTAATCTTCAACTAATGCAGATCCAAAATACACAACGTAAAGCGTACAAC
87	CCCAAATCGATTCATCAGTTGAGATTTGCCAATTCCGATCTAATCGTCTA
88	TGAATAAAACTAACCGAACACAGACTGGATTCAAGGTACTATGAGCTTGAG
89	GAACGAGTGTGGGAAGAAAAATCCATAAATACACGTAAGTCGTTACATG
90	TAATTCATTAAGAACTGGCTATTTAAACAGGAGACTTACCGATTGCGAA
91	ATCAGGTCAACGAGAATGACCATAATGCAGAATGAGTGGTGTATTGTC
92	TCAAAAATCTTCCAGAGCTTAATTAAGTCCTAGGCGTACTTTCTCGATAG
93	GAATAACATATCCTGAATCTTACCCCTAATTTGATTGAAGGTAACCAAGTGA
94	GGGAGAATTAGTTGCTATTTGAATGGCTTAGTGAATCCACATGCAACT
95	CAGAGGGCTTGGGGAGGTTTGGGTATTGTACTGGAGCAATCTAGTG
96	ATAACCCAAGAACGCGAGGCGTTGAACAAGCCTCAAGCTATCCACATAACC
97	TTTGGGGATTGCTGAATATAATGAGAGACTAACTTACGTCTTCGAGACAA
98	TTGTACCATTTAAATATGCAACTGACGGACTCAATCATCAATTGGTGGT
99	AATAAAGCAAGTTCAATTCCATATTGGCTTTACTGGACGGAGTGTAAAT
100	CAGGCAAGATTCTGCGAACGGAGTAGATTAAAGAGCAATCTTAGACAGTACCGT
101	ATAGTAGTTAGATAACATTCGCATAGTCAGCCATCAGAATTGTGAGTTCT
102	CGCGCCTCAACATGTTCAAGCTAAATCAAATAGTCCTCATGGTGTCTTATA
103	ACCTCCGGTGCTGATGCAAATCCAAGACGACGTGGAGGTAGGACGTAGTG
104	CAAAATCACGCGAGAAAATTAGTACCGCTTCGGCTTCTAGTGTAAAGC
105	AGACGCTGTAATTTCATCTTGAGGCATTGGTACAATGCTCTCAATGT
106	ATCCTTGATTGAAATACCGACCGTTTAACAACATACGCACGAGGTG
107	AAATCGTCTAAATAAGAATAAACCAACAGTGTGATGATTGCTTACATCG
108	AATATATGCTAGAAAAAGCCTGTTACAAATTGCGAACTTATTACACCTCG
109	CGTTTACTGCTCCTTTGATAAAATGGCTTACCGATAACCGTCTTGATGAC
110	GCGAGAGGCCAACAGGTCAGGATTCTGTAGCTCCTACGTTATCTGCAAGGAT
111	GAGGGGGTGCTTCAAAGCGAACCAAAAGTACGTTCTAGCGCAGATCAGTT
112	AGCGTCCGAAAGACTTCAAATAAACAGTTGGAAGGTTACTTCCAGACCTC
113	TTCATTGACGGATTGCATAAAAAGATTAGTTATGTCAGTGGATACTGGT
114	GTTCAGAATTACCTGACTATTAAATGGTCATTAACACGCTTGGTTCGT

Location ID	Extended staple sequence with barcoded handle, 5' to 3'
115	GCGAGCTGTTAGCTATATTCATAACTATCTCGGAAGTTCTCGTAGTAT
116	GAACAAGATAAAAGTAATTCTGTCCATCGCAAGAGTCAGAACATCGATCCAC
117	ACGAGCATTAAATAAGAGAATATAATCAAATATCGAGTCACATCAAGGACATT
118	GTCTTCTGTAATTAGGCAGACCTAATTGTACAAGTCGAAACCTACCG
119	AAACCAAGATTGAGAATGCCATAGTGATAAAGCTACGATACTGCTGAACTC
120	AAGCCGTTGTATAAAGCCAACGCTACCGGAATCGACACGTATAGTCTGTCTA
121	TTTTGTTATATTTAAATGCAAGTAATACTGACATCGTTTGAGGTCTT
122	AACGTTAAAGGTAAGATTCAAAAAATCGGAGCGTATTAGGGATCTTACT
123	GAAGATTGGAGACAGTCAAATCACAAATTAGCTAAGCGTACTTGGCTAGA
124	CGGTTGATCAACC GTCTAGCTGAAAATCATAGACTGTTCTGGACTGATAT
125	TCGTAAAAGGGTAGCTATTTTCTACTACGGATAGAAGGAACACTACA
126	ATGTAACCTAGGTTGGTTATTTGGGGCGAGCTAGTCCTAGTGGTTAG
127	TTCAGGTTCTTTACATCGGGAGACCTTTATGTAGCGGATTATCAATGCG
128	CGTAAAACCTGATTGCTTGAAAATTATTTAGCTGTTCGATGATT
129	GTTAGAACCGCCAGAGGCGAATTTAGATTAACCATTCTCGTAGTACCT
130	TGTTGGACTGAGCAAAAGAAGATCCCTAGATTCACTACTCCCTACGA
131	AGATGATGAGAAAACAAAATTAACTTCTGTCTGTCACCATCTAGTACGTT
132	GAGCGGAACATTGAAATTACCTTCATAAAATCTGTAACCTGGAATACGGTT
133	GAGCTTAAGAACGCTTATTCCTTTAGATGCGTATTCTCTCTGTGTT
134	CAACATGAAAACATTATGACCCCTGCCTGAGTGTATCACC GGATACCTC
135	GTGCTGGCTCAGACCATAAGCTAGGGT GAGAATGGACCGTCATTAGAAC
136	ATTCCCAGCAAAGAATTAGCAACATCAATAGAATAGTTGGCCTGAATCTT
137	TTGACCATAGCATTAAACATCCAATTAAACCGCTACTAGCGATGTTAT
138	ATAACCTGAAAAGGTGGCATCAATGAGAGATCCGAGGTTAGGTATTAGAGG
139	TGGAGCAACTATCAGGT CATTGCCATACAGTATAAGATCCGATACCATAGCG
140	ACAAAAGAATAGGTCTGAGAGACTAAACAATAAGCTGGAACAAGTCTGTTATG
141	ATTTTAGTAGAAGAGTCATAGTGACCAAGTCCATATAAGGAAGCAGAGGC
142	TAATGGTAAACATAGCGATAGCATTCCATGACTTACGATGAGTGG
143	TAAGGC GTCTATTAATTAACTTGTGAAACAGGTATTCACTGTGGTGTTC
144	CATAATTATGAGTGAAATAACCTTGTACATTCTCAGTAGACAATCTCGCT
145	AAGGC GATCGCGTCTGGCCTCCTGCATTAAACATACTACAGTCCATGTGCG
146	TTACGCCAACATTAAATGTGAGAATTGTAAACTCACTCCGTCAATTG
147	GGAAGGGCGGATTCTCCGGAAAAAAACAGGGCTATCAATAGACTCCTCG
148	GCCATT CGCCGTAAAGGATAGGTATGTACCC TATACTGTGTC ACTCGTAG
149	CACCGCTTGGCGCATCGTAACCGACGGTAAGATCCGCTATTCTATTCCGA
150	ACAGTACTAACGTCAAGATGAATTGAGAGTCATACGTTCCGGAGATGTA
151	TGGTCAGTAAGGTTATCTAAAATCGTAGATTGGTGAATAGGGGTGATTGAT
152	GAACCTCACAACTAATAGATTAGATTGCAACGCTTAGATGTAACACAGA

Location ID	Extended staple sequence with barcoded handle, 5' to 3'
153	GCAAATGATACTTGAGGATTAAATGGAAGGCTTCGGTATTTGGTCATCGA
154	GCCTGCAAACAAACAATTGACAAATCCTGATATTGACCTACCACATGAGTA
155	AAAACAGATGCCGAACGTTATTGATTATCCATTGTAGGCCTGGAGAAT
156	AAAAATACGTAACATTATCATTTCACCAGAAGGCGTACCTAACATAGGCTC
157	ACCCCTAAATCAGCTCATTTCCATCAAACATGTGATACCTTGACACAT
158	TAATGTGTTATTGTTAAAATTGTAAGCCAGTTGCATCACTTCATCAGGTA
159	AAAGGCCGTATAAGCAAATTTACGAGTAACCTCAAGCACATGACCTTATG
160	TGATATTAATCAGAAAAGCCCCAAACGGCGTATTGTCATCCGTACAG
161	TGCCGGAGCTAGCATGTCAATCATCACGTTGGTGCCTACTGTTCCCTCATGG
162	TACAAAGGACAAGAGAATCGATGATGCATCTGGAGTTACCTTCGAGTGT
163	CAGGAAGAAGGGGACGACGACAGTTGAAAGGATAGGTCTGTTAGCAACAA
164	CGGATTCGAGAAATAAGAAATTGTCTTAGGTATAGAGGAGCCAACCACTA
165	TACAAAATCTACCATATCAAATTAGCCGTCAAGATGGTATTGGGAATGGATG
166	CAATTACTTATACTTCTGAATAGAAGTATTGGTTGAGAAATACCGTCTT
167	AAACATCAGCAATTCAATACTCGTATTCACAACAGTGTGAGCTAT
168	AAACATTTTATCATATTCTTAATTCATACTCGTATTCACAACAGTGTGAGCTAT
169	CTGATTGCGACGCCAGTGCAAGTGTGCTGCGTTCATATCTGACCTCTT
170	GTTTTCTTCGACTCTAGAGGATTCGCTACTCATGGAATTACGTCATG
171	AGAGGCAGCGAATCGTAATCATGAACGTGATATGGTCAGGAGTATTGT
172	GCTGCATTTGTGAAATTGTTATGGCAAAGCGCATTACATCATGGCCTAG
173	CCCGCTTACAACATACGAGCCGTTCCGGCAACTCGAGGAGAACCAT
174	ATTGAGGTGGCAAATCAACAGTATCGGCTGGTCGGTCTTATTCGACATG
175	TTGCAACATGACGCTCAATCGTCTTCAATATCGTTGTTGTTACACACAC
176	GGTAATATATTGGCAGATTCCCCCTGCTAACGTGGAGTCCGTATATC
177	GAGTAGAAATAAAGGGACATTCTAGCCAGCATAGGACCATGGTATCTTAGG
178	CTTCTTGAAACCCCTCTGACCTGATTAACACCGGATTGTTACTCCGAGTAGG
179	GTCCCATCAGGCACAGACAATATTAGAAGATGAATCAAGCTGCAATAGTGT
180	TAATCAGTTCTTAATGCGCGAACCTGCCATTACAATTACGACGCATTAGG
181	AATAATTAAAGTGGTAACGACGCTTAGAACACTGCTGTTGTTGT
182	CTTCATCGCTGGCGAAAGGGGACTTGCAATGCAATTCCAGTAACGGCATAG
183	AAACCGTCGATCGGTGCGGGCTCCCCGGTTCTGATGTTGGACTTGTGA
184	GGATTGACCATTCAAGGCTGCGCGTACAGCGTATCCACTACCGTTCT
185	TGTAGATGCTGGTGGCGAAACCACCGCTACAACATATCACCTCTGTGTGA
186	CCAGTTGTCGCACTCCAGCCAGCGAACGATAGATAAGAGGCCACTTATGT
187	CTAACTCAAGCCTGGGGTGCCTAAGAAATACCGGTCTACTATCATGTGGCTT
188	AGCACTAAAATATCAAACCCCTCAAGAAATGGATATTGGACACTAAGCTCGTT
189	ATAGATAAAAATCTAAAGCATCAAGTCACACCCTCTCAATTGCTTGTCA
190	AGACTTCAAGTGCCACGCTGAGGGCCAACATCTGTATTCCAACACTGGAG

Location ID	Extended staple sequence with barcoded handle, 5' to 3'
191	AAATCCTTGGTGAGGCCTCAGTAAAGCGTAACCAACTCTCTTAGTGTGCT
192	AAGTTGACGAACGAACCACCAGCTTGAATGGAGGACTAGAAAGTCGTTGT
193	CCACGCTGGTTGCCAGGGCAACAGTCTCGGTGAATAAGTCACAC
194	CTGTTGATGGTGGTCCGAAATCAGGGTGGTGAACCTATTGGCTGTAG
195	TCCCTTATAAATCAAAGAACAGCAGGGGGAGGATTGGTACCGGATAAT
196	GGGTTGAGTGTGTTCCAGTTGTCGTGCCAGTACGTGGTTCACTAACCAT
197	GTCCACTATTAAAGAACAGTGGACCTCACTGTGACGGTAGAGTACATTG
198	TACATTGGAAAAACGCTCATGTGAGTGAGTAGTGTCTTAGGTACATCC
199	CGCTGGCAAGTGTAGCGGTACGCCAGCCACTGACCTGTATTGTCCACTG
200	AACCACACACCCGCCGCCTACCTGCTCATCTAGCAATCTACGCACG
201	CTACAGGGCGCGTACTATGGTGCACTTGCCCTGGATCTGAAAAGAACATGTGC
202	GCACGTATAACGTGCTTCCTCGTAGCAATACAATACGCGACTCTGCTATT
203	AGAGCGGGAGCTAACACAGGAGGCAGAGTCTCTGAGGGATCTTCTACAG
204	GGGATTAGACAGGAACGGTACGTGTTAAACTGCACATGTGCTAAG
205	GTAAAACCTTCACCGCCTGGCAAGCGGTGACCGACAGTTACTCATG
206	CCTGCAGGTTCACCGTGGAGACCGAATCGATACATTGCGTACGACATT
207	ACCGAGCTTGGCTATTGGCGCCGGAAAAGTATGACACCGAGCAATTCT
208	TGTTCCAATGAATCGGCCAACCGAGATACTAGCTCTAATATCGAACGGT
209	AATTCCACCCAGTCGGAAACCTGGAACAAGAGCTATGACAACCGCAGTATA
210	AAGTGTAACTTAATTGCGTGCCTGCGTCAACGTGCATTGTTGTAGACAAGT
211	CAAAGGGCGAAAACCGCTATCACGCTAGGGAACTGATTGAAACAACGGTC
212	TTATTACCCAGAACAAATTACCGCTGGCGTGTGGAACATTCAATGACAGG
213	GACCAGTAGAACTCAAACATCGGATGCGCCGGAATCAATGTTCAAGTGGT
214	GAGATAGATTAGTAATAACATCTTGACGAGATCTGTTCACGAAGTCTCC
215	GAATACGTCGAAATTAAACGTTGTTAGAATCTGCATGGTAGATCTTCTCC
216	GCTATTAGGAGGCCACCGAGTAAACGATTAAACCAACGATACAACACGTTCAAT

**Supplementary Table 3.** Staples extended with barcoded handles for recruiting barcoded recording primers. These are used in experiments described in Fig. 4. The location IDs correspond to the numeric position labels in Fig. S6.

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
1	GTCTTGAGCAAATAGCAGGTGACAAGTC TATACCAGCGTAGGAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTC TATACCAGCGTAGGAGATTATTTATTT
2	GTCTTGAGCAAATAGCAGGTGACATTAC TGGAGGAGTGCATAAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTAC TGGAGGAGTGCATAAGATTATTTATTT
3	GTCTTGAGCAAATAGCAGGTGACAGTCT AACGCTAGTGGCTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTCT AACGCTAGTGGCTAAAATTATTTATTT
4	GTCTTGAGCAAATAGCAGGTGACAAGTC AGTCATTCTGTAGCTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTC AGTCATTCTGTAGCTATTATTTATTT
5	GTCTTGAGCAAATAGCAGGTGACATATC CATCGTCAATAAGCTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTATC CATCGTCAATAAGCTATTATTTATTT
6	GTCTTGAGCAAATAGCAGGTGACATCAT TACACGTAAACCTTGCATAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCAT TACACGTAAACCTTGCATAATAAATAAAT
7	GTCTTGAGCAAATAGCAGGTGACAGGTA GTTGTCCACCTAAGAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTA GTTGTCCACCTAAGAGATTATTTATTT
8	GTCTTGAGCAAATAGCAGGTGACAACAA TGCAGGATGAGATGAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA TGCAGGATGAGATGAGATTATTTATTT
9	GTCTTGAGCAAATAGCAGGTGACAGGAC TGATATACCTCTGGATAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAC TGATATACCTCTGGATATTATTTATTT
10	GTCTTGAGCAAATAGCAGGTGACAGATG TCACCTACGAAGAGAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGATG TCACCTACGAAGAGAGATTATTTATTT
11	GTCTTGAGCAAATAGCAGGTGACATTCT TGTTCGAACGCTATCGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTCT TGTTCGAACGCTATCGATTATTTATTT
12	GTCTTGAGCAAATAGCAGGTGACATATG ATCCTGCAGAAGTACCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTATG ATCCTGCAGAAGTACCAAATAAATAAAT
13	GTCTTGAGCAAATAGCAGGTGACACTAT CAAGCGAATTCAAGCCAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAT CAAGCGAATTCAAGCCATTATTTATTT
14	GTCTTGAGCAAATAGCAGGTGACATAAG CGGAATACGCTAATTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTAAG CGGAATACGCTAATTCAAATAAATAAAT
15	GTCTTGAGCAAATAGCAGGTGACAACCT CATCTTGTCTAGTCTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACCT CATCTTGTCTAGTCTCAATTATTTATTT
16	GTCTTGAGCAAATAGCAGGTGACAACCC GGAGAACAAATATCTGGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAACC GGAGAACAAATATCTGGATTATTTATTT
17	GTCTTGAGCAAATAGCAGGTGACAGAGG ACATTATGCAACTAGGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAGG ACATTATGCAACTAGGAAATAAATAAAT
18	GTCTTGAGCAAATAGCAGGTGACATGAA CCTCTAACGGTGTACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTGAA CCTCTAACGGTGTACAAATAAATAAAT
19	GTCTTGAGCAAATAGCAGGTGACAACCC AACTGATAGGATTGTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAACC AACTGATAGGATTGTGATTATTTATTT
20	GTCTTGAGCAAATAGCAGGTGACACGAA CTGAATGCCATTAGTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAA CTGAATGCCATTAGTTATTTATTT
21	GTCTTGAGCAAATAGCAGGTGACATGCC ACGTAAGTCTCTATTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTGCC ACGTAAGTCTCTATTCAAATTATTT
22	GTCTTGAGCAAATAGCAGGTGACAACCTA TGCACAAACTCACCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACTA TGCACAAACTCACCAAATAAATAAAT
23	GTCTTGAGCAAATAGCAGGTGACACACT ATACGCTCCGAGACTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACT ATACGCTCCGAGACTAAAATAAATAAAT
24	GTCTTGAGCAAATAGCAGGTGACAGAAC TACCTTGTGAACCATTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAAC TACCTTGTGAACCATTAAATAAATAAAT
25	GTCTTGAGCAAATAGCAGGTGACAACAA TGGTCTACCTAGACTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA TGGTCTACCTAGACTCAAATAAATAAAT
26	GTCTTGAGCAAATAGCAGGTGACATTGC TTCGTAACAGGTTGAAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTGC TTCGTAACAGGTTGAAAAATAAATAAAT
27	GTCTTGAGCAAATAGCAGGTGACAGAAAT TGGAACGACAGAGTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAAT TGGAACGACAGAGTCAAATAAATAAAT
28	GTCTTGAGCAAATAGCAGGTGACAGCAG CTTCAAAATGCACTACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGCAG CTTCAAAATGCACTACAAATAAATAAAT
29	GTCTTGAGCAAATAGCAGGTGACATTAG TGAGTCGTGAGGTTATAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTAG TGAGTCGTGAGGTTATAAATAAATAAAT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
30	GTCTTGAGCAAATAGCAGGTGACACATC CTTGTATCCGGTTGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCATC CTTGTATCCGGTTGATTATTTATTT
31	GTCTTGAGCAAATAGCAGGTGACACGAA TGGTTACTTACGGTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAA TGGTTACTTACGGTCATTATTTATTT
32	GTCTTGAGCAAATAGCAGGTGACACTAG ATGGTACTCGTCACACAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAG ATGGTACTCGTCACACATTATTTATTT
33	GTCTTGAGCAAATAGCAGGTGACAGGTA CGTGCAATGACTCTACAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGGTA CGTGCAATGACTCTACATTATTTATTT
34	GTCTTGAGCAAATAGCAGGTGACAATCG CAAGAAGTAACACGTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGATCG CAAGAAGTAACACGTAAATTATTTATTT
35	GTCTTGAGCAAATAGCAGGTGACACTAA CTGTAATCCGCCCTGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAA CTGTAATCCGCCCTGATTATTTATTT
36	GTCTTGAGCAAATAGCAGGTGACAAGTA AGAGGAGTGCCTTATTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTA AGAGGAGTGCCTTATTATTTATTTATTT
37	GTCTTGAGCAAATAGCAGGTGACATTAA GTGACCTGGAGGATATAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTTAA GTGACCTGGAGGATATATTATTTATTT
38	GTCTTGAGCAAATAGCAGGTGACACCGG ATTGTAAGTGTCTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCGG ATTGTAAGTGTCTCAATTATTTATTT
39	GTCTTGAGCAAATAGCAGGTGACACGAT TACCTCATATGCCCTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAT TACCTCATATGCCCTCAATTATTTATTT
40	GTCTTGAGCAAATAGCAGGTGACAAAGT TGCTAGTGGTGTCTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGT TGCTAGTGGTGTCTAAATTATTTATTT
41	GTCTTGAGCAAATAGCAGGTGACACTTC CTATTGATCCGAGAGGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCTTC CTATTGATCCGAGAGGATTATTTATTT
42	GTCTTGAGCAAATAGCAGGTGACAAATA CTTGCCAACACACTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAATA CTTGCCAACACACTCAAATTATTTATTT
43	GTCTTGAGCAAATAGCAGGTGACAACCT ATCAACACACCCTTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGACCT ATCAACACACCCTTAAATTATTTATTT
44	GTCTTGAGCAAATAGCAGGTGACACCTA GTGTCCAGACAATGAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCCTA GTGTCCAGACAATGAGATTATTTATTT
45	GTCTTGAGCAAATAGCAGGTGACACTCA GTGGATGCGATGAATTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCTCA GTGGATGCGATGAATTATTTATTTATTT
46	GTCTTGAGCAAATAGCAGGTGACACCCAG TCTCTTAAGATGCCAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCAG TCTCTTAAGATGCCAAATTATTTATTT
47	GTCTTGAGCAAATAGCAGGTGACATCGA GTATCACATGGAACCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTCGA GTATCACATGGAACCAATTATTTATTT
48	GTCTTGAGCAAATAGCAGGTGACAAGGA TAAGGACTTATGTGCGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGGA TAAGGACTTATGTGCGATTATTTATTT
49	GTCTTGAGCAAATAGCAGGTGACATCAT CCAGGTTAATGACGACAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTCAT CCAGGTTAATGACGACATTATTTATTT
50	GTCTTGAGCAAATAGCAGGTGACATAGT TATCGCTACTGTGAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTAGT TATCGCTACTGTGAAAATTATTTATTT
51	GTCTTGAGCAAATAGCAGGTGACATTAC ACCAACGTGCTGTAAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTTAC ACCAACGTGCTGTAAGATTATTTATTT
52	GTCTTGAGCAAATAGCAGGTGACACCCAC GGTCTACGATCATAAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCAC GGTCTACGATCATAAGATTATTTATTT
53	GTCTTGAGCAAATAGCAGGTGACAACACA ATCGTGTGGAATACTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAACAA ATCGTGTGGAATACTCAATTATTTATTT
54	GTCTTGAGCAAATAGCAGGTGACACGAG AAGAGAGTCGTTCTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAG AAGAGAGTCGTTCTAAATTATTTATTT
55	GTCTTGAGCAAATAGCAGGTGACAATCG ACAACTCTATTGATGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGATCG ACAACTCTATTGATGATTATTTATTT
56	GTCTTGAGCAAATAGCAGGTGACAAGCC AATTACACAAGGTAGTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGCC AATTACACAAGGTAGTATTATTTATTT
57	GTCTTGAGCAAATAGCAGGTGACAGTCG TCGTTGTCGACATTAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGTCG TCGTTGTCGACATTAGATTATTTATTT
58	GTCTTGAGCAAATAGCAGGTGACACGAC TAGGAGTAGGTTAGGAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAC TAGGAGTAGGTTAGGAAATTATTTATTT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
59	GTCTTGAGCAAATAGCAGGTGACATTGA ATCGCAAATCGACTAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTGA ATCGCAAATCGACTCAATTATTATTATT
60	GTCTTGAGCAAATAGCAGGTGACAACAA CCAGTATGGTATTCTGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA CCAGTATGGTATTCTGATTATTATTATT
61	GTCTTGAGCAAATAGCAGGTGACATGGA TTCAAGGCATAGTACAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTGGA TTCAAGGCATAGTACAATTATTATTATT
62	GTCTTGAGCAAATAGCAGGTGACACATC TCACGGCTTAGTTACCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATC TCACGGCTTAGTTACCAATTATTATT
63	GTCTTGAGCAAATAGCAGGTGACAGTTA GGAATGAGCCAAGGAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTTA GGAATGAGCCAAGGAAAATAAAAT
64	GTCTTGAGCAAATAGCAGGTGACACGTC GGATGTAAGATAGAGCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGTC GGATGTAAGATAGAGCAATTATTATT
65	GTCTTGAGCAAATAGCAGGTGACACACC AATAACGTGAACGTGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACC AATAACGTGAACGTGATTATTATTATT
66	GTCTTGAGCAAATAGCAGGTGACACACG TTCAGAACATTCTCTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACG TTCAGAACATTCTCTCAATTATTATT
67	GTCTTGAGCAAATAGCAGGTGACATAGT CGCTCTATTGTGAACCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTAGT CGCTCTATTGTGAACCAATTATTATT
68	GTCTTGAGCAAATAGCAGGTGACACTTG TTGAGATACTCGACAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTTG TTGAGATACTCGACAAAATAAAAT
69	GTCTTGAGCAAATAGCAGGTGACATTCT GGTATGTTCAACAAGCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTCT GGTATGTTCAACAAGCAATTATTATT
70	GTCTTGAGCAAATAGCAGGTGACAAAGC TCAGTTGTTCAGAGGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGC TCAGTTGTTCAGAGGATTATTATT
71	GTCTTGAGCAAATAGCAGGTGACACCGG TTATTACCTACCTGTTAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCGG TTATTACCTACCTGTTATTATTATT
72	GTCTTGAGCAAATAGCAGGTGACACGAC TTATTGAGTCATTGCCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAC TTATTGAGTCATTGCCATTATTATT
73	GTCTTGAGCAAATAGCAGGTGACAGATA CGTCTATTGAACACCGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGATA CGTCTATTGAACACCGATTATTATT
74	GTCTTGAGCAAATAGCAGGTGACAATCT GAGTACCGAATAGTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGATCT GAGTACCGAATAGTCAAATTATTATT
75	GTCTTGAGCAAATAGCAGGTGACAGTGT TGATGGACGTAGTTGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTGT TGATGGACGTAGTTGAAATTATTATT
76	GTCTTGAGCAAATAGCAGGTGACAAACA GAGTGATTGTCAGTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAACA GAGTGATTGTCAGTCATTATTATT
77	GTCTTGAGCAAATAGCAGGTGACACTAT ATGAGGGGTTGCTCTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAT ATGAGGGGTTGCTCTCAATTATTATT
78	GTCTTGAGCAAATAGCAGGTGACACACT AGAACCTGCTTATGCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACT AGAACCTGCTTATGCAATTATTATT
79	GTCTTGAGCAAATAGCAGGTGACATCAG GTTCTAGATGGTCTCTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCAG GTTCTAGATGGTCTCTCAATTATTATT
80	GTCTTGAGCAAATAGCAGGTGACAGCAA GACTATCGCCTAGTCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGCAA GACTATCGCCTAGTCATTATTATT
81	GTCTTGAGCAAATAGCAGGTGACAAGTG GTGATTACATTAGCAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTG GTGATTACATTAGCAATTATTATT
82	GTCTTGAGCAAATAGCAGGTGACAAGGA TTGAACGAAGCTGTAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGGA TTGAACGAAGCTGTAAATTATTATT
83	GTCTTGAGCAAATAGCAGGTGACAATTG TGTTCGAGTACGATAAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGATTG TGTTCGAGTACGATAATTATTATT
84	GTCTTGAGCAAATAGCAGGTGACAGTAC GAATAGACACTCGCTGAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTAC GAATAGACACTCGCTGATTATTATT
85	GTCTTGAGCAAATAGCAGGTGACAAGAA CATTGGTTCGGATTGAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGAA CATTGGTTCGGATTGAAATTATTATT
86	GTCTTGAGCAAATAGCAGGTGACAGTTG TACGCTTACGTTGCAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTTG TACGCTTACGTTGATTATTATT
87	GTCTTGAGCAAATAGCAGGTGACATAGC ACGATTAGATCGGAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTAGC ACGATTAGATCGGAATAAAATAAAAT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
88	GTCTTGAGCAAATAGCAGGTGACACTCA AGCTCATAGTACCTGAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTCA AGCTCATAGTACCTGAATTATTTATTT
89	GTCTTGAGCAAATAGCAGGTGACACATG TAAGACGACTTACGTGAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATG TAAGACGACTTACGTGATTATTTATTT
90	GTCTTGAGCAAATAGCAGGTGACATTG CAATGGATAAGTCTCCAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCG CAATGGATAAGTCTCCATTATTTATTT
91	GTCTTGAGCAAATAGCAGGTGACAGACA CAATACACACCACCAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGACA CAATACACACCACCAAAATTATTTATTT
92	GTCTTGAGCAAATAGCAGGTGACACTAT CGAGAAAAGTACGCTAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAT CGAGAAAAGTACGCTATTATTTATTT
93	GTCTTGAGCAAATAGCAGGTGACATCAC TGGTTACCTTCAATCAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCAC TGGTTACCTTCAATCAAATTATTTATTT
94	GTCTTGAGCAAATAGCAGGTGACAAGTT GCATGTGGATTCACTAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTT GCATGTGGATTCACTAATTATTTATTT
95	GTCTTGAGCAAATAGCAGGTGACACACT AGATTGCTCCAGTACAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACT AGATTGCTCCAGTACAAATTATTTATTT
96	GTCTTGAGCAAATAGCAGGTGACAGGTT ATGTGGATAGCTTGAGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGGTT ATGTGGATAGCTTGAGATTATTTATTT
97	GTCTTGAGCAAATAGCAGGTGACATTGT CTCGAAAGACGTAAGTAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTGT CTCGAAAGACGTAAGTATTATTTATTT
98	GTCTTGAGCAAATAGCAGGTGACAACCA CCAATTGATGATTGAGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACCA CCAATTGATGATTGAGATTATTTATTT
99	GTCTTGAGCAAATAGCAGGTGACAATTAA CACTCCGTCCAAGTAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGATTA CACTCCGTCCAAGTAAATTATTTATTT
100	GTCTTGAGCAAATAGCAGGTGACAACGG TACTGTCTAACGATTGCAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACGG TACTGTCTAACGATTGCAATTATTTATTT
101	GTCTTGAGCAAATAGCAGGTGACAAGAA CTCACAAATTCTGATGGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGAA CTCACAAATTCTGATGGATTATTTATTT
102	GTCTTGAGCAAATAGCAGGTGACATATA AGCACCATGAGGACTAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGTATA AGCACCATGAGGACTAATTATTTATTT
103	GTCTTGAGCAAATAGCAGGTGACACACT ACGTCCCTATACCTCAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACT ACGTCCCTATACCTCAATTATTTATTT
104	GTCTTGAGCAAATAGCAGGTGACAGCTT ACACTAGAAGCCGAAGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGCTT ACACTAGAAGCCGAAGATTATTTATTT
105	GTCTTGAGCAAATAGCAGGTGACAACAT TGAGAGCATTGTACAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAT TGAGAGCATTGTACCAATTATTTATTT
106	GTCTTGAGCAAATAGCAGGTGACACACC TCGTAGTGCCTTATAGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACC TCGTAGTGCCTTATAGATTATTTATTT
107	GTCTTGAGCAAATAGCAGGTGACACGAT GTAGCAACGAATCATCAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAT GTAGCAACGAATCATCAATTATTTATTT
108	GTCTTGAGCAAATAGCAGGTGACACGAG GTGTAATAAGTTGCAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAG GTGTAATAAGTTGCAATTATTTATTT
109	GTCTTGAGCAAATAGCAGGTGACAGTC TCAAGACGGTTATCGGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTCA TCAAGACGGTTATCGGATTATTTATTT
110	GTCTTGAGCAAATAGCAGGTGACAATCC TTGCAGATAACGTAGGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGATCC TTGCAGATAACGTAGGATTATTTATTT
111	GTCTTGAGCAAATAGCAGGTGACAAACAT GATCTGCGCTAACGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGAACT GATCTGCGCTAACGAAATTATTTATTT
112	GTCTTGAGCAAATAGCAGGTGACAGAGG TCTGGAAGTAACCTCAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAGG TCTGGAAGTAACCTTCATTATTTATTT
113	GTCTTGAGCAAATAGCAGGTGACAAACCA AGTATCCACTGACATAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACCA AGTATCCACTGACATAATTATTTATTT
114	GTCTTGAGCAAATAGCAGGTGACAACGA ACCTAAAGCGTGTAAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGACGA ACCTAAAGCGTGTAAAATTATTTATTT
115	GTCTTGAGCAAATAGCAGGTGACAATAC TACGAGAACTTCCGAGAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGATAC TACGAGAACTTCCGAGATTATTTATTT
116	GTCTTGAGCAAATAGCAGGTGACAGTGG ATCGAATGTTCTGACTAAATAAAATAAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTGG ATCGAATGTTCTGACTATTATTTATTT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
117	GTCTTGAGCAAATAGCAGGTGACAAATG TCCTTGATGTGACTCGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAATG TCCTTGATGTGACTCGATTATTATT
118	GTCTTGAGCAAATAGCAGGTGACACGGT AGGTTCGAACCTGTACAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGGT AGGTTCGAACCTGTACATTATTATT
119	GTCTTGAGCAAATAGCAGGTGACAGAGT TCAGCAGTATCGTAGCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGAGT TCAGCAGTATCGTAGCATTTATTATT
120	GTCTTGAGCAAATAGCAGGTGACATAGA CAGACTATACGTGTCGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTAGA CAGACTATACGTGTCGATTATTATT
121	GTCTTGAGCAAATAGCAGGTGACAAAGA CCTCAAAACGGATGTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGA CCTCAAAACGGATGTCAATTATTATT
122	GTCTTGAGCAAATAGCAGGTGACAAGTA AGATCCCTAATACGCTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGTA AGATCCCTAATACGCTATTATTATT
123	GTCTTGAGCAAATAGCAGGTGACATCTA GCCAAGTACGCTTAGAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTCTA GCCAAGTACGCTTAGAATTATTATT
124	GTCTTGAGCAAATAGCAGGTGACAATAT CAGTCCAGGAACAGTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGATAT CAGTCCAGGAACAGTCATTATTATT
125	GTCTTGAGCAAATAGCAGGTGACATGTA GTGTTCCCTCTATCCGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGTGTA GTGTTCCCTCTATCCGATTATTATT
126	GTCTTGAGCAAATAGCAGGTGACACTAA CCACTAGGACTAGCTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAA CCACTAGGACTAGCTATTATTATT
127	GTCTTGAGCAAATAGCAGGTGACACGCA TTGATAATCCGCTACAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGCA TTGATAATCCGCTACAAATTATTATT
128	GTCTTGAGCAAATAGCAGGTGACAAATC ATCGACGAACAGCTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAATC ATCGACGAACAGCTAAATTATTATT
129	GTCTTGAGCAAATAGCAGGTGACAAGGA TACTACGAAGAATGGTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGGA TACTACGAAGAATGGTATTATTATT
130	GTCTTGAGCAAATAGCAGGTGACATCGT AAGGAAGTAGACTGAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGT AAGGAAGTAGACTGAAATTATTATT
131	GTCTTGAGCAAATAGCAGGTGACAAACG TACTAGATGGTGACAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAACG TACTAGATGGTGACAGATTATTATT
132	GTCTTGAGCAAATAGCAGGTGACAAACC GTATTCCAGGTTACAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAACC GTATTCCAGGTTACAAATTATTATT
133	GTCTTGAGCAAATAGCAGGTGACAAACA CAAGAGAGAACATCGAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAACA CAAGAGAGAACATCGAAATTATTATT
134	GTCTTGAGCAAATAGCAGGTGACAGAGG TATATCCGGTGATACAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGAGG TATATCCGGTGATACAAATTATTATT
135	GTCTTGAGCAAATAGCAGGTGACAGCTT CTAATGACGGTCCATTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGCTT CTAATGACGGTCCATTATTATTATT
136	GTCTTGAGCAAATAGCAGGTGACAAAGA TTCAGGCCAACATTCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGA TTCAGGCCAACATTCAAATTATTATT
137	GTCTTGAGCAAATAGCAGGTGACAATAA CATCGCTAGTAAGCGTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGATAAA CATCGCTAGTAAGCGTATTATTATT
138	GTCTTGAGCAAATAGCAGGTGACACCTC TAATACCTAACCTCGGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCCTC TAATACCTAACCTCGGATTATTATT
139	GTCTTGAGCAAATAGCAGGTGACACGCT ATGGTATCGGATCTAAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGCT ATGGTATCGGATCTAAATTATTATT
140	GTCTTGAGCAAATAGCAGGTGACACATA ACAGACTTGTCCAGCAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCATA ACAGACTTGTCCAGCATTATTATT
141	GTCTTGAGCAAATAGCAGGTGACAGCCT CTGCTTCCATTATGGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGCCT CTGCTTCCATTATGGATTATTATT
142	GTCTTGAGCAAATAGCAGGTGACACCCAC TCATCGTAAGTCATGGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCCAC TCATCGTAAGTCATGGATTATTATT
143	GTCTTGAGCAAATAGCAGGTGACAGAAC ACCACAGTGAATACTAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGGAAC ACCACAGTGAATACTATTATTATT
144	GTCTTGAGCAAATAGCAGGTGACAAGCG AAGATTGTCTACTGAGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGAGCG AAGATTGTCTACTGAGATTATTATT
145	GTCTTGAGCAAATAGCAGGTGACACGCA CATGGACTGTAGTATGAAATAAATAAT	TCCATCTTGTCTGTTAGCAAGCTGCGCA CATGGACTGTAGTATGATTATTATT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
146	GTCTTGAGCAAATAGCAGGTGACACGAA TTGACGGAAGTGAGTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAA TTGACGGAAGTGAGTTATTTATTTATTT
147	GTCTTGAGCAAATAGCAGGTGACACGAG GAGTCTATTGATAGCCAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGAG GAGTCTATTGATAGCCATTATTTATTT
148	GTCTTGAGCAAATAGCAGGTGACACTAC GAGTGACACAGGTATAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAC GAGTGACACAGGTATAATTATTTATTT
149	GTCTTGAGCAAATAGCAGGTGACATCGG AATAGAATAGCGGATCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCGG AATAGAATAGCGGATCATTTATTTATTT
150	GTCTTGAGCAAATAGCAGGTGACATACA TCTCCGGAACGTATGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTACA TCTCCGGAACGTATGAATTATTTATTT
151	GTCTTGAGCAAATAGCAGGTGACAATCA ATCACCCCTATTCCAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATCA ATCACCCCTATTCCAATTATTTATTT
152	GTCTTGAGCAAATAGCAGGTGACATCTG TGTTACATCTAACGCTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCTG TGTTACATCTAACGCTATTATTTATTT
153	GTCTTGAGCAAATAGCAGGTGACATCGA TGACCAAATACCGAAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCGA TGACCAAATACCGAAGATTATTTATTT
154	GTCTTGAGCAAATAGCAGGTGACATACT CATGTGGTAGGTCAATAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTACT CATGTGGTAGGTCAATTATTTATTT
155	GTCTTGAGCAAATAGCAGGTGACAATT TCCAAGGCCTACATGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATT TCCAAGGCCTACATGATTATTTATTT
156	GTCTTGAGCAAATAGCAGGTGACAGAGC CTATGTTAGGTACGCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGAGC CTATGTTAGGTACGCAATTATTTATTT
157	GTCTTGAGCAAATAGCAGGTGACAATGT GTCAAGGTATCACATGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATGT GTCAAGGTATCACATGATTATTTATTT
158	GTCTTGAGCAAATAGCAGGTGACATACC TGATGAAGTGATGCAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTACC TGATGAAGTGATGCAATTATTTATTT
159	GTCTTGAGCAAATAGCAGGTGACACATA AGGTCTATGTGCTGAAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATA AGGTCTATGTGCTGAAATTATTTATTT
160	GTCTTGAGCAAATAGCAGGTGACACTGT GACGGATGACAATACGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTGT GACGGATGACAATTACGATTATTTATTT
161	GTCTTGAGCAAATAGCAGGTGACACCAT GAAGGAAACAGTAGCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCAT GAAGGAAACAGTAGCAATTATTTATTT
162	GTCTTGAGCAAATAGCAGGTGACAGACA CTCGAAAAGGTAACCTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGACA CTCGAAAAGGTAACCTCAATTATTTATTT
163	GTCTTGAGCAAATAGCAGGTGACATTGT TGCTACAAACAGACCTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTTGT TGCTACAAACAGACCTATTATTTATTT
164	GTCTTGAGCAAATAGCAGGTGACATAGT GGTTGGCTCCTCTATAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTAGT GGTTGGCTCCTCTATAATTATTTATTT
165	GTCTTGAGCAAATAGCAGGTGACACATC CATTCCAATACCATCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATC CATTCCAATACCATCAATTATTTATTT
166	GTCTTGAGCAAATAGCAGGTGACAAAAGA CGGTATTCTCAACCGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGA CGGTATTCTCAACCGATTATTTATTT
167	GTCTTGAGCAAATAGCAGGTGACAATAG CTCAACACTGTTGTTGAAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATAG CTCAACACTGTTGTTGAAATTATTTATTT
168	GTCTTGAGCAAATAGCAGGTGACAACAT CCAAGACTCTAGGTGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAT CCAAGACTCTAGGTGAATTATTTATTT
169	GTCTTGAGCAAATAGCAGGTGACAAAAGA GGTCGAGATATGAACCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGA GGTCGAGATATGAACCAATTATTTATTT
170	GTCTTGAGCAAATAGCAGGTGACAGATC GACGTAATTCCATGAGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGATC GACGTAATTCCATGAGATTATTTATTT
171	GTCTTGAGCAAATAGCAGGTGACAACAA TACTCCTGCACCATATAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA TACTCCTGCACCATATTATTTATTT
172	GTCTTGAGCAAATAGCAGGTGACACTAG GACCATGATGTAATGCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAG GACCATGATGTAATTGCAATTATTTATTT
173	GTCTTGAGCAAATAGCAGGTGACAATGG ATTCTCCTCGAAGTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATGG ATTCTCCTCGAAGTTGATTATTTATTT
174	GTCTTGAGCAAATAGCAGGTGACACATG TCGAATAAGACCGACCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATG TCGAATAAGACCGACCAATTATTTATTT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
175	GTCTTGAGCAAATAGCAGGTGACAGTGT GTGTACAACAACAACCAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTGT GTGTACAACAACAACCATTATTATTATT
176	GTCTTGAGCAAATAGCAGGTGACAGATA TACGGAACTCCACGTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGATA TACGGAACTCCACGTTATTATTATTATT
177	GTCTTGAGCAAATAGCAGGTGACACCTA AGATACCATGGCTCTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCATA AGATACCATGGCTCTAATTATTATTATT
178	GTCTTGAGCAAATAGCAGGTGACACCTA CTCGGAGTAACAATCCAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCATA CTCGGAGTAACAATCCATTATTATTATT
179	GTCTTGAGCAAATAGCAGGTGACAACAC TATTGCAGCTTGATTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAC TATTGCAGCTTGATTCAATTATTATTATT
180	GTCTTGAGCAAATAGCAGGTGACACCTA ATGCGTCGTAATTGTAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCATA ATGCGTCGTAATTGTAATTGATTATTATT
181	GTCTTGAGCAAATAGCAGGTGACAACAA CACAAACAGCAGTTCTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA CACAAACAGCAGTTCTAATTATTATTATT
182	GTCTTGAGCAAATAGCAGGTGACACTAT GCCGTTACTGGAATTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAT GCCGTTACTGGAATTGATTTATTATTATT
183	GTCTTGAGCAAATAGCAGGTGACATACA CAAGTCCAACATCAGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTACA CAAGTCCAACATCAGAATTATTATTATT
184	GTCTTGAGCAAATAGCAGGTGACAAGAA CCGTGTAAGTGGATACAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGAA CCGTGTAAGTGGATACATTATTATTATT
185	GTCTTGAGCAAATAGCAGGTGACATCAC ACAGAACGGTGATAGTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTCAC ACAGAACGGTGATAGTTATTATTATTATT
186	GTCTTGAGCAAATAGCAGGTGACAACAT AAAGTGGCGCTTATCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAT AAAGTGGCGCTTATCAAATTATTATTATT
187	GTCTTGAGCAAATAGCAGGTGACAAGC CACATGATAGTAGACCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAAGC CACATGATAGTAGACCAAATTATTATTATT
188	GTCTTGAGCAAATAGCAGGTGACAACAG AGCTTAGTGTCCAATAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAACG AGCTTAGTGTCCAATAATTATTATTATT
189	GTCTTGAGCAAATAGCAGGTGACATGAC AAGCAAATTGAGAAGGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTGAC AAGCAAATTGAGAAGGATTATTATTATT
190	GTCTTGAGCAAATAGCAGGTGACACTCC AGTGTGGAATACAGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTCC AGTGTGGAATACAGAAAATAAATAAAT
191	GTCTTGAGCAAATAGCAGGTGACAAGCA CACTAAGAGAAGTTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGCA CACTAAGAGAAGTTGAAATTATTATTATT
192	GTCTTGAGCAAATAGCAGGTGACAACAA CGACTTTCTAGTCCTCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACAA CGACTTTCTAGTCCTCAAATTATTATTATT
193	GTCTTGAGCAAATAGCAGGTGACAGTGT GACTTATTCAACCGAGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGTGT GACTTATTCAACCGAGAATTATTATTATT
194	GTCTTGAGCAAATAGCAGGTGACACTAC AGCCGAATAGGTTCACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTAC AGCCGAATAGGTTCACATTATTATTATT
195	GTCTTGAGCAAATAGCAGGTGACAATTAA TCCGGTACCAATCCTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATTA TCCGGTACCAATCCTTATTATTATTATT
196	GTCTTGAGCAAATAGCAGGTGACAATGG TTAGTGAACCACGTACAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATGG TTAGTGAACCACGTACATTATTATTATT
197	GTCTTGAGCAAATAGCAGGTGACAACAGA ATGTACTCTACCGTCAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACGA ATGTACTCTACCGTCAATTATTATTATT
198	GTCTTGAGCAAATAGCAGGTGACAGGAT GACCTACAAGACACTAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGGAT GACCTACAAGACACTAAAATAAATAAAT
199	GTCTTGAGCAAATAGCAGGTGACACAGT GGACAATACAGGTCAAGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCACT GGACAATACAGGTCAAGAAAATAAATAAAT
200	GTCTTGAGCAAATAGCAGGTGACACGTG CGTAGATTGCTAGATGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGCAC ATTCTTTCAAGATCCAATTATTATTATT
201	GTCTTGAGCAAATAGCAGGTGACAGCAC ATTCTTTCAAGATCCAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCGTG CGTAGATTGCTAGATGATTATTATTATT
202	GTCTTGAGCAAATAGCAGGTGACAATAA GCAGAGTCGCGTATTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAATA GCAGAGTCGCGTATTGATTATTATTATT
203	GTCTTGAGCAAATAGCAGGTGACACTGG TAGGAAGATCCTCAGAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTGG TAGGAAGATCCTCAGAAAATAAATAAAT

Location ID	Barcoded recording primer type 'a'	Barcoded recording primer type 'a*'
204	GTCTTGAGCAAATAGCAGGTGACACTTA GACACATGTGCGAGTTAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCTTA GACACATGTGCGAGTTATTTATTTATTT
205	GTCTTGAGCAAATAGCAGGTGACACATG AGTAACGTGGTCACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCATG AGTAACGTGGTCACATTATTTATTT
206	GTCTTGAGCAAATAGCAGGTGACAATG TCGTACGCAATGTATCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAATG TCGTACGCAATGTATCATTTATTTATTT
207	GTCTTGAGCAAATAGCAGGTGACAAGAA TTGCTCGGTGTCAACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGAGAA TTGCTCGGTGTCAACATTATTTATTT
208	GTCTTGAGCAAATAGCAGGTGACAACCG TTCGATATTAGAGCTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACCG TTCGATATTAGAGCTGATTATTTATTT
209	GTCTTGAGCAAATAGCAGGTGACATATA CTGCGGTTGTCAAGCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGTATA CTGCGGTTGTCAAGCATTTATTTATTT
210	GTCTTGAGCAAATAGCAGGTGACAACCT GTCTACACAACAATGCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACTT GTCTACACAACAATGCATTATTTATTT
211	GTCTTGAGCAAATAGCAGGTGACAGACC GTTGTTCAATCAGTCACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGACC GTTGTTCAATCAGTCATTATTTATTT
212	GTCTTGAGCAAATAGCAGGTGACACCTG TCATTGAATGTTCCACAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGCCCTG TCATTGAATGTTCCACATTATTTATTT
213	GTCTTGAGCAAATAGCAGGTGACAACCA CTTGAACATTGATTGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGACCA CTTGAACATTGATTGATTATTTATTT
214	GTCTTGAGCAAATAGCAGGTGACAGGGAG ACTTCGTGAACAGATCAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGGAG ACTTCGTGAACAGATCATTATTTATTT
215	GTCTTGAGCAAATAGCAGGTGACAGGGAG AAGATCTACCATGCAAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGGGAG AAGATCTACCATGCAAATTATTTATTT
216	GTCTTGAGCAAATAGCAGGTGACAATTG AACGTGTTGTATCTGGAAATAAATAAAT	TCCATCTTGTCTGTTAGCAAGCTGATTG AACGTGTTGTATCTGGATTATTTATTT

**Supplementary Table 4.** Barcoded recording primers for pattern reconstruction experiments in Fig. 4.

Direction	Barcode ID	Inner Primers (5' to 3')	Outer Primers (5' to 3')
Forward	1	AAGAAAGTTGTCGGTGTCTTG GTCCTTGAGCAAATAGCAGGTGACA	AAGAAAGTTGTCGGTGTCTTG GTCCTTGAGCAAATAGCAGGTGACA
Forward	2	TCGATTCCGTTGTAGTCGTCTG GTCCTTGAGCAAATAGCAGGTGACA	TCGATTCCGTTGTAGTCGTCTG GTCCTTGAGCAAATAGCAGGTGACA
Forward	3	GAGCTTGTGTCCCAGTTACCA GTCCTTGAGCAAATAGCAGGTGACA	GAGCTTGTGTCCCAGTTACCA GTCCTTGAGCAAATAGCAGGTGACA
Forward	4	TTCGGATTCTATCGTGTTC GTCCTTGAGCAAATAGCAGGTGACA	TTCGGATTCTATCGTGTTC GTCCTTGAGCAAATAGCAGGTGACA
Forward	5	CTTGTCCAGGGTTGTAA GTCCTTGAGCAAATAGCAGGTGACA	CTTGTCCAGGGTTGTAA GTCCTTGAGCAAATAGCAGGTGACA
Forward	6	TTCTCGAAAGGCAGAAAGTAGC GTCCTTGAGCAAATAGCAGGTGACA	TTCTCGAAAGGCAGAAAGTAGC GTCCTTGAGCAAATAGCAGGTGACA
Forward	7	GTGTTACCGTGGGAATGAATC GTCCTTGAGCAAATAGCAGGTGACA	GTGTTACCGTGGGAATGAATC GTCCTTGAGCAAATAGCAGGTGACA
Forward	8	TTCAGGGAACAAACCAAGTTAC GTCCTTGAGCAAATAGCAGGTGACA	TTCAGGGAACAAACCAAGTTAC GTCCTTGAGCAAATAGCAGGTGACA
Forward	9	AACTAGGCACAGCGAGTCTTGG GTCCTTGAGCAAATAGCAGGTGACA	AACTAGGCACAGCGAGTCTTGG GTCCTTGAGCAAATAGCAGGTGACA
Forward	10	AAGCGTTGAAACCTTGTCC GTCCTTGAGCAAATAGCAGGTGACA	AAGCGTTGAAACCTTGTCC GTCCTTGAGCAAATAGCAGGTGACA
Forward	11	GTTTCATCTATCGGAGGGAA GTCCTTGAGCAAATAGCAGGTGACA	GTTTCATCTATCGGAGGGAA GTCCTTGAGCAAATAGCAGGTGACA
Forward	12	CAGGTAGAAAGAACGAGA GTCCTTGAGCAAATAGCAGGTGACA	CAGGTAGAAAGAACGAGA GTCCTTGAGCAAATAGCAGGTGACA
Forward	13	AGAACGACTTCCATA GTCCTTGAGCAAATAGCAGGTGACA	AGAACGACTTCCATA GTCCTTGAGCAAATAGCAGGTGACA
Forward	14	AACGAGTCTCTGGGACCC GTCCTTGAGCAAATAGCAGGTGACA	AACGAGTCTCTGGGACCC GTCCTTGAGCAAATAGCAGGTGACA
Forward	15	AGGTCTACCTCGCTAACACC GTCCTTGAGCAAATAGCAGGTGACA	AGGTCTACCTCGCTAACACC GTCCTTGAGCAAATAGCAGGTGACA
Forward	16	CGTCAACTGACAGTGG GTCCTTGAGCAAATAGCAGGTGACA	CGTCAACTGACAGTGG GTCCTTGAGCAAATAGCAGGTGACA
Forward	17	ACCCCTCAGGAAAGTAC GTCCTTGAGCAAATAGCAGGTGACA	ACCCCTCAGGAAAGTAC GTCCTTGAGCAAATAGCAGGTGACA
Forward	18	CCAAACCCAACA GTCCTTGAGCAAATAGCAGGTGACA	CCAAACCCAACA GTCCTTGAGCAAATAGCAGGTGACA
Forward	19	GTTCCCTCGTCAG GTCCTTGAGCAAATAGCAGGTGACA	GTTCCCTCGTCAG GTCCTTGAGCAAATAGCAGGTGACA
Forward	20	TTGCGTCCTGTTAC GTCCTTGAGCAAATAGCAGGTGACA	TTGCGTCCTGTTAC GTCCTTGAGCAAATAGCAGGTGACA
Forward	21	GAGCCTCTCAT GTCCTTGAGCAAATAGCAGGTGACA	GAGCCTCTCAT GTCCTTGAGCAAATAGCAGGTGACA
Forward	22	ACCACTGCCATGT GTCCTTGAGCAAATAGCAGGTGACA	ACCACTGCCATGT GTCCTTGAGCAAATAGCAGGTGACA
Forward	23	CTTACTACCCAGT GTCCTTGAGCAAATAGCAGGTGACA	CTTACTACCCAGT GTCCTTGAGCAAATAGCAGGTGACA
Forward	24	GCATAGTTCTGCAT GTCCTTGAGCAAATAGCAGGTGACA	GCATAGTTCTGCAT GTCCTTGAGCAAATAGCAGGTGACA
Reverse	25	GTAAGTTGGGTAT TCCATCTTGTCTGTTAGCAAGCTG	GTAAGTTGGGTAT GCAACGCAATG
Reverse	26	CATACAGCGACT TCCATCTTGTCTGTTAGCAAGCTG	CATACAGCGACT ACGCATTCTCAT
Reverse	27	CGACGGTTAGATT TCCATCTTGTCTGTTAGCAAGCTG	CGACGGTTAGATT CACCTCTTACA
Reverse	28	TGAAACCTAAGA TCCATCTTGTCTGTTAGCAAGCTG	TGAAACCTAAGA AGGCACCGTATC
Reverse	29	CTAGACACCTTGGG TCCATCTTGTCTGTTAGCAAGCTG	CTAGACACCTTGGG TGACAGACC

Direction	Barcode ID	Inner Primers (5' to 3')	Outer Primers (5' to 3')
Reverse	30	TCAGTGAGGATCTACTTCGACCCA TCCATCTTGTCTGTTAGCAAGCTG	TCAGTGAGGATCTACTTCGACCCA
Reverse	31	TGCGTACAGCAATCAGTTACATTG TCCATCTTGTCTGTTAGCAAGCTG	TGCGTACAGCAATCAGTTACATTG
Reverse	32	CCAGTAGAAGTCCGACAACGTCAT TCCATCTTGTCTGTTAGCAAGCTG	CCAGTAGAAGTCCGACAACGTCAT
Reverse	33	CAGACTTGGTACGGTTGGGTAACT TCCATCTTGTCTGTTAGCAAGCTG	CAGACTTGGTACGGTTGGGTAACT
Reverse	34	GGACGAAGAACTCAAGTCAAAGGC TCCATCTTGTCTGTTAGCAAGCTG	GGACGAAGAACTCAAGTCAAAGGC
Reverse	35	CTACTTACGAAGCTGAGGGACTGC TCCATCTTGTCTGTTAGCAAGCTG	CTACTTACGAAGCTGAGGGACTGC
Reverse	36	ATGTCCCAGTTAGAGGAGGAAACA TCCATCTTGTCTGTTAGCAAGCTG	ATGTCCCAGTTAGAGGAGGAAACA
Reverse	37	GCTTGCATTGATGCTTAGTATCA TCCATCTTGTCTGTTAGCAAGCTG	GCTTGCATTGATGCTTAGTATCA
Reverse	38	ACCACAGGAGGACGATAACAGAGAA TCCATCTTGTCTGTTAGCAAGCTG	ACCACAGGAGGACGATAACAGAGAA
Reverse	39	CCACAGTGTCAACTAGAGCCTCTC TCCATCTTGTCTGTTAGCAAGCTG	CCACAGTGTCAACTAGAGCCTCTC
Reverse	40	TAGTTGGATGACCAAGGATAGCC TCCATCTTGTCTGTTAGCAAGCTG	TAGTTGGATGACCAAGGATAGCC
Reverse	41	GGAGTTCGTCCAGAGAAGTACACG TCCATCTTGTCTGTTAGCAAGCTG	GGAGTTCGTCCAGAGAAGTACACG
Reverse	42	CTACGTGTAAGGCATAACCTGCCAG TCCATCTTGTCTGTTAGCAAGCTG	CTACGTGTAAGGCATAACCTGCCAG
Reverse	43	CTTTCGTTGTTGACTCGACGGTAG TCCATCTTGTCTGTTAGCAAGCTG	CTTTCGTTGTTGACTCGACGGTAG
Reverse	44	AGTAGAAAGGGTTCTTCCCACTC TCCATCTTGTCTGTTAGCAAGCTG	AGTAGAAAGGGTTCTTCCCACTC
Reverse	45	GATCCAACAGAGATGCCTTCAGTG TCCATCTTGTCTGTTAGCAAGCTG	GATCCAACAGAGATGCCTTCAGTG
Reverse	46	GCTGTGTTCCACTTCATTCTCCTG TCCATCTTGTCTGTTAGCAAGCTG	GCTGTGTTCCACTTCATTCTCCTG
Reverse	47	GTGCAACTTCCCACAGGTAGTC TCCATCTTGTCTGTTAGCAAGCTG	GTGCAACTTCCCACAGGTAGTC
Reverse	48	CATCTGGAACGTGGTACACCTGTA TCCATCTTGTCTGTTAGCAAGCTG	CATCTGGAACGTGGTACACCTGTA

**Supplementary Table 5.** Inner and outer PCR primers for DNA nanoscope experiments in Fig. 4 and Fig. S4. Inner and outer primers in the same row are designed to be used together to PCR barcoded recording primers. Any ‘forward’ inner and outer primer pair can be used with any ‘reverse’ inner and outer primer pair to generate a unique sequencing library. Multiple such distinct libraries can be combined and run on the same sequencing chip. The sequence of the outer primer is used to demultiplex libraries.

## References

27. S. M. Douglas, A. H. Marblestone, S. Teerapittayanon, A. Vazquez, G. M. Church, W. M. Shih, Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res.* **37**, 5001–5006 (2009).
28. P. W. K. Rothemund, in *IEEE/ACM International Conference on Computer-Aided Design, Digest of Technical Papers, ICCAD* (2005), vol. 2005, pp. 470–477.