Single-Stranded Templates as Railroad Tracks for Hierarchical Assembly of DNA Origami

Supporting Information

Janane F. Rahbani, John C. C. Hsu, Pongphak Chidchob and Hanadi F. Sleiman*

Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, H3A 0B8, Canada

Contents

I. Materials and instrumentation
II. Sequential growth of dsKL[10] backbone
III. Transformation protocol of pUC19-KL[10]
IV. Sequencing of pUC19-KL[10]
V. Magnetic beads separation of ssKL[10]
VI. Synthesis of dsKL[20], transformation and sequencing of pUC19-KL[20], single-strand conversion of dsKL[20]
VII. Preparation of the 3-tile system and related control experiments
VIII. Construction of the railroad track and related control experiments
IX. Additional AFM micrographs
X. Origami sequences
XI. References
I. Materials and Instrumentation:

A. Materials

Acetic acid, boric acid, EDTA, urea, magnesium chloride, GelRed, tris(hydroxymethyl)aminomethane (Tris), D(+) glucose, 2-betamercaptoethanol, were purchased from Aldrich. Nucleoside (1000 Å)-derivatized LCAACPG solid support with loading densities of 25-40 μmol/g, Sephadex G-25 (super fine DNA grade), and reagents for automated DNA synthesis were used as purchased from BioAutomation. Acrylamide (40%)/bis-acrylamide 19:1 solution and agarose were purchased from BioShop. All staple strands used for the assembly of origami were purchased from Bioneer. The scaffold M13mp18 single-stranded was purchased from New England Biolabs. AFM cantilevers were purchased from Asylum Research (model AC160TS) and RubyRed mica were ordered from Electron Microscopy Sciences. TBE buffer is composed of 90 mM Tris and boric acid and 1.1 mM EDTA, with a pH of ~8.3. TAMg buffer is composed of 45 mM Tris and 12.5 mM MgCl$_2$ with a pH of ~7.8 adjusted by glacial acetic acid. 1xTAE is composed of 45 mM Tris and 1 mM EDTA, with pH adjusted to 8.0 using glacial acetic acid. 1xOK buffer is composed of 50 mM Tris-HCl, 10 mM MgCl$_2$, 5 mM dithiothreitol (DTT), with a pH of 7.5. 1xQL buffer is composed of 66 mM Tris-HCl, 10 mM MgCl$_2$, 1 mM DTT, 1 mM ATP, 7.5% w/v PEG6000, with a pH of 7.6, and was made in-house as a 2x concentrate. 1xALK is composed of 30 mM NaOH and 1 mM EDTA. 1xSDA buffer is composed of 40 mM Tris, 10 mM MgCl$_2$, 50 mM NaCl, 5 mM DTT, 100 μg/mL bovine serum albumin (BSA), and 500 μM of each dNTP. 0.5xSSC buffer is composed of 75 mM NaCl and 7.5 mM sodium citrate, with a pH of 7.0.

Kits for Optikinase and Quick, T4 and T7 DNA ligase were purchased from New England Biolabs. A MyTaqTM HS Red PCR kit was purchased from CedarLane Laboratories. QIAquick Gel
Extraction and PCR purification kits from Qiagen were used for extraction or cleanup of PCR products. FastDigest® XbaI and EcoRI were purchased from Life Technologies. rSAP (Shrimp Alkaline Phosphatase) was purchased from New England Biolabs. Streptavidin Magnesphere® Paramagnetic Particles were purchased from Promega.

DH5α (Subcloning Efficiency Competent Cells) were purchased from Life Technologies. LB media is composed of 2.5 g BioTryptone, 2.5 g NaCl and 1.25 g Yeast Extract. PureLink Quick Plasmid DNA Mini/Maxi Prep Kits from Qiagen were used to isolate the plasmid.

B. Instrumentation

Standard automated oligonucleotide solid-phase synthesis was carried on a BioAutomation MerMade MM6 DNA synthesizer. UV-Vis quantifications were performed with a NanoDrop Lite Spectrophotometer. Polyacrylamide gel electrophoresis (PAGE) was carried out on a 20 x 20 cm vertical Hoefer 600 electrophoresis unit. Agarose gel electrophoresis (AGE) was performed on an Owl Mini gel electrophoresis unit. Thermal anneals, polymerase chain reaction (PCR), and enzymatic digestions were conducted using an Eppendorf Mastercycler Pro 96 well thermocycler. AFM was performed with a MultiMode™ MM8 SPM connected to a Nanoscope™ controller, from the Digital Instruments Veeco Metrology Group. The plasmids were sequenced using Sanger methods at McGill University Genome Center and Innovation Quebec.

II. Sequential growth of dsKL[10]

A. DNA synthesis
The sequences of each building block were generated by CANADA version 2.0 (available online at http://ls11-www.cs.uni-dortmund.de/molcomp/downloads/), a program that intends to minimize undesired secondary interactions, and idt DNA (Table S1). DNA synthesis was carried on a on a BioAutomation MerMade MM6 DNA synthesizer at 1 μmole scale. Deprotection and cleavage from the solid support was achieved through the addition of concentrated ammonium hydroxide (55°C, 14 hours). Crude products were then purified via polyacrylamide gel electrophoresis under denaturing conditions (4M urea). Following PAGE, the gel was visualized by UV light over a fluorescent TLC plate. The product was rapidly excised, then crushed and incubated in 11 mL of autoclaved water at 65°C overnight. Size exclusion chromatography (Sephadex G-25) was used to desalt the samples. Finally, strands were quantified via a NanoDrop Lite Spectrophotometer (OD260) and using IDT’s extinction coefficient at 260.

B. Temporal growth

Every strand having an internal 5’-terminus was phosphorylated by OptiKinase. The concentration of the strands was adjusted to 10 μM, with a 1xOptikinase buffer, 2.5 mM ATP, and 0.1 U/μL of OptiKinase. The mixture was incubated at 37°C for 30 min, then at 75°C for 10 min to inactivate the enzyme. It is worthy to mention that the efficiency of Optikinase is around 60% according to the manufacturer. KL[10] was synthesized following the Sequential Growth procedure by Hamblin et. al. It includes in-situ ligation, isolation of the dsKL[10] by native AGE, PCR enrichment and the separation of the non-nicked dsKL[10] via denaturing AGE. In PCR enrichment, PrimerL2eco and PrimerK1xbba were used for dsKL[10]; no additional restriction site was added through PCR.
Table S1. Duplex Seed Sequences for KL[10]

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5' -&gt; 3')</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1pxba</td>
<td>AATTAAGATAGCGCGGCTCTAGAGCGATATAATCTGGCTGCGCTTGAAACAACGGAAGGTCATGCTTTAGGA</td>
<td>73</td>
</tr>
<tr>
<td>K2pxba</td>
<td>TGACCTTCCGTTTTCAAGCGCAGCCAGATTATATCGCTCTAGAGCCGCGCCTATCTTAATT</td>
<td>63</td>
</tr>
<tr>
<td>K1</td>
<td>AATCTGGCTGCGCTTGAAACAACGGAAGGTCATGCTTTAGGA</td>
<td>43</td>
</tr>
<tr>
<td>K2</td>
<td>TGACCTTCCGTTTTCAAGCGCAGCCAGATTATATCGGATC</td>
<td>42</td>
</tr>
<tr>
<td>L1</td>
<td>ATCAAACCAAAGTTTCAGCAACAGGCCGTTAAGGATCAANAAGA</td>
<td>42</td>
</tr>
<tr>
<td>L2</td>
<td>CTTAACGGCCTGTGCTGAACCTTTGTTGATTTCTAACGGCCTGTGAACTTTTGGTTGATTTCTAAAGCA</td>
<td>42</td>
</tr>
<tr>
<td>L1peco</td>
<td>ATCAAACCAAAGTTTCAGCAACAGGCCGTTAAGGATCAAGAGACGTAGTCCGAATTACCTGCA</td>
<td>64</td>
</tr>
<tr>
<td>L2peco</td>
<td>TTGCAGGTGAAATTCGGACTACGTCTTTCTGATCCCTAAACGGCCTGTGCTGAAACTTTTGGTTGATTTCTAAAGCA</td>
<td>74</td>
</tr>
</tbody>
</table>

III. Transformation protocol of pUC19-KL[10]

First, dsKL[10] was amplified by PCR with annealing temperature 1-2 degree lower than that used in Sequential Growth. 1μg of dsKL[10] and 1μg of pUC19 were digested using FD EcoRI and FD XbaI (LifeTechnologies) for 1.5 hr at 37°C and purified using 2.5% and 1.2% (w/v) native agarose gel, respectively. The band from the gel was excised and the product was extracted by ethanol precipitation. Then 40ng of the digested backbone were ligated into 80ng of the digested plasmid using NEB T4 Ligase. We followed the manufacturer’s manual for overnight or 10 min ligation.
Finally, we transformed our insert into DH5α cells. Around 70 ng of ligated product was gently added to 50 μL aliquot of DH5α cells previously placed on ice. Then, the cells were heat shocked for 30 s at 42°C after 30 min incubation time. Lysogeny broth (LB) media was later added and the mixture was shaken at 225 rpm for 1 hour at 37°C. The transformation was plated on ampicillin-agar and incubated overnight at 37°C. Few colonies were picked from the ligation plate and each one was used to inoculate 3 mL of LB media containing ampicillin (100 µg/mL) at 225 rpm and 37°C. PureLink Quick Plasmid DNA Mini Prep Kit was used to isolate the plasmid containing our insert.

IV. Sequencing of pUC19-KL[10]

In order to examine the sequence of our backbone within the plasmid, we submitted our samples for Sanger Sequencing to McGill University Genome Center and Innovation Quebec.

The observed sequence was the following:

NNNNNNNNcgatataatctggctgcgttgaNNNacggaaggtcatgctgttNNNNNNNcaaccaaaagttcagcaacaggccgtta aggatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacaggccgttaaggatatcagaagaaatctggctgcgcttgaaacaacggaaggtcatgctttaggaatcaaccaaaagttcagcaacag...
gctgcgcttgaaacaacggaaggtcatgctttaggaatcaaaccaaagtccacagccgtaaggatcagaagacgtagtccgaattcactggc-3’

After determining the plasmid with perfect backbone sequence we wanted to improve further the yield of our product. We used 50ng of this plasmid from Miniprep solution to transform 50µL of DH5α, by means of standard protocol. The next day, we picked a single colony using a sterile pipette tip and dropped it into 4.5 mL of LB with ampicillin to inoculate the media for 6 hrs at 225rpm, 37°C. Then we poured the media into 300mL of LB with ampicillin to inoculate further overnight. Finally, we used Maxiprep (Qiagen) to prepare a bulk quantity of the plasmid from the rest of the media. The yield was 850 ng/µL.

V. Magnetic beads separation of ssKL[10]

To isolate dsKL[10] from pUC19-KL[10], two primers that bind the flanking region of ds[10] were designed: Primer pUC19-KL10-For (caggtcgactctagagcgatat) and primer pUC19-KL10-Rev (gccagtgaattcggactacg). Throughout this manuscript, when designing a new primer, we avoided using any sequence included within the repeating pattern of dsKL[10] since it can introduce non-specific binding. We followed the standard strategy for designing primers and synthesized them using DNA synthesizer. Optimal PCR conditions intend to improve the yield of the desired product and minimize the amount of contaminants. This is achieved by: (i) using the gradient function on the thermal cycler to find out the best temperature, (ii) varying the amounts of pUC19-KL[10] and primers. Table S2 summarizes the final quantities we used to extract ds[10] from pUC19-KL[10]. Note that the extension of the primers occurred at 63°C.
Table S2. Optimized PCR conditions to extract dsKL[10] from pUC19-KL[10]

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC19-KL[10] (20ng)</td>
<td>0.2</td>
</tr>
<tr>
<td>pUC19-KL10-For (0.5 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>pUC19-KL10-Rev (0.5 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Autoclaved H₂O</td>
<td>18.8</td>
</tr>
<tr>
<td>MyTaq 2×mix</td>
<td>20</td>
</tr>
</tbody>
</table>

Before starting the conversion of dsKL[10] to its single-stranded analogue ssKL[10], the PCR products were purified via PCR purification kit. Figure S1 lane 1 displays the successful isolation of dsKL[10] from the plasmid. In order to use our scaffolds in building higher-order DNA nanoarchitectures, we employed PCR followed by magnetic beads separation to convert double-stranded backbones to their single-stranded correspondents. To allow the binding of the double-stranded product to streptavidin-coated magnetic beads, we biotinylated the 5’ end of the antisense strand (the sense that will not be used in future experiments) by using a biotinylated reverse primer. However, prior to the incubation of the beads with dsKL[10], we pre-treated them with 30 mM NaOH solution for 2 hours. This step is supposed to cleave the weak interaction between biotin/streptavidin on the beads, hence minimizing byproducts. Later, dsKL[10] was added to the beads in 0.5×SSC buffer at pH=7 for 2 hours (gently inverted to maximize binding). The single-stranded scaffold was obtained upon denaturing dsKL[10] with a 20 mM solution of NaOH for 10 min. It is worthy to mention that both the concentration of the alkaline solution and the duration of incubation play a key role in determining the yield and the purity of ssKL[10]. Incubation for a shorter time results in a low yield of ssKL[10], whereas keeping the mixture in contact with NaOH for a longer time can break the remaining weak biotin-streptavidin interaction. Similarly, using a concentration higher than 30 mM might have a greater impact on biotin/streptavidin interaction. The supernatant containing the released ssKL[10] product was finally recovered by ethanol.
precipitation. Figure S1 lane 2 shows a discrete band between 200 and 300 bp that corresponds to ssKL[10]. Despite pre-treating the beads with NaOH, the non-penetrating band appearing in lane 2 is attributed to streptavidin proteins attached to 1-4 ssKL[10] or dsKL[10].

![Figure S1](image)

Figure S1. 2.5% AGE in TAE showing ds-KL[10] after PCR amplification (lane 1) and ss-KL[10] after magnetic beads separation.

VI. Synthesis of dsKL[20] and ssKL[20]

A. Synthesis of dsKL[20]

Adapted from the Golden Gate assembly, type II restriction enzyme BsmBI was used to help linking 2 fragments of dsKL[10]. Because dsKL[10] was already inserted into pUC19, we had to design 4 primers (Primer K1p-pUC19-For, PrimerL2p-In-Rev, PrimerK1p-In-For and PrimerL2p-pUC19-Rev) that introduce the corresponding complementary regions into dsKL[10] to synthesize dsKL[20]. As such, each primer was extended by 5 unique bases and a restriction site for the enzyme BsmBI (Figure S2). We made sure that all of the sticky ends are unique and do not overlap.
with each other besides than their own compatible ends. To generate the left fragment M, primer K1p-pUC19-For and primer L2p-In-Rev were used (In stands for the unique bases added to this primer allowing M to hybridize N). In a separate reaction mixture, primer K1p-In-For and primer L2p-pUC19-Rev were used to produce the right fragment N.

Figure S2. Scheme showing the generation of the two DNA fragments M and N via two PCR reactions.

dsKL[10] left fragment: M

TGCGTCCGTCTCGCGGAGTTAGAGCATAT

PrimeK1p-pUC19-For

TGCGTCCGTCTCGCGGAGTTAGAGCATAT

PrimerL2p-In-Rev

TGCGTCCGTCTCGCGGAGTTAGAGCATAT

PrimeK1p-In-For

TGCGTCCGTCTCGCGGAGTTAGAGCATAT

PrimerL2p-pUC19-Rev

TGCGTCCGTCTCGCGGAGTTAGAGCATAT
dsKL[10] right fragment: N
TGCGTCCGTCTCGTGCGACTCTTATAGGCGATATaatctggtgctgcttgaaacaacgggaaggtcatgtgttaggaatcagcagcagctagtattagcacaagctgtaaggtcatacgcgaagtttgagtttaggattggctggtcgcggggagccagcttcgggtgagctttaggcgcgggtgacgacagtgggtgggtttggtttttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttaggtagtttagg
employed for digestion. Later, digested pUC19 plasmid was mixed with M and N scaffolds in the presence of T7 ligase for 2 hours at room temperature. Table S5 summarizes the ligation conditions.

Table S4. Digestion conditions of pUC19 and M and N fragments with BsmBI

<table>
<thead>
<tr>
<th>Volume (µL)</th>
<th>pUC19 (1µg)</th>
<th>M (500ng)</th>
<th>N (500ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (Insert OR Plasmid)</td>
<td>14.12</td>
<td>3.75</td>
<td>4.06</td>
</tr>
<tr>
<td>10X Tango Buffer</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DTT (10mM)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>dH₂O</td>
<td>0.88</td>
<td>11.25</td>
<td>10.94</td>
</tr>
<tr>
<td>BsmBI</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table S5. Ligation conditions of pUC19 and M and N fragments with T7 ligase

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC19 (100ng)</td>
<td>2.84 (35.2 ng/µL)</td>
<td>0.877 (21.1 ng/µL)</td>
<td>0.894 (20.7 ng/µL)</td>
</tr>
<tr>
<td>ds[10]-M Insert (18.5 ng)</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ds[10]-N Insert (18.5 ng)</td>
<td>0.139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2X T7 Ligase Buffer</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7 Ligase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dH₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure S3. Native 2.5% AGE in TAE buffer displaying the comparison between the presence of ligase (Lanes 1 to 4) versus its absence (lanes 5 to 7). Lane 1: pUC19 + M + N, lane 2: M + N, lane 3: pUC19, lane 4: pUC19 (not purified), lane 5: pUC19 (not ligated), lane 6: M, lane 7: N and lane 8: pUC19 (not digested, nor ligated). Note that the all samples were digested except the ones in lane 8.

B. Transformation of dsKL[20] into pUC19

The ligation reaction (10 µL) was used without further purification for transforming 90 µL of MAX Efficiency DH5α cells (LifeTechnologies), following the standard protocol provided. Positive and negative controls consisting of pUC19 digested and pUC19 intact were transformed into the cells to compare with our product. Subsequent to picking the colonies from the ligation plates, each colony was inoculated overnight in a separate 3 mL of LB media containing ampicillin (100 µg/mL) at 225 rpm and 37°C. Finally, the plasmid was isolated using PureLink Quick Plasmid DNA Mini Prep Kit (eluted with 30 µL of EB) and the backbone dsKL[20] was screened
via BsmBI. Table S6 displays one example in which colony 1 was screened for dsKL[20]. The mixture was incubated at 37°C for 1h.

Table S6. Digestion conditions of pUC19-[KL]20 with BsmBI

<table>
<thead>
<tr>
<th></th>
<th>Colony 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (50ng)</td>
<td>1.088</td>
</tr>
<tr>
<td>10x Tango Buffer</td>
<td>1</td>
</tr>
<tr>
<td>DTT (10mM)</td>
<td>1</td>
</tr>
<tr>
<td>dH₂O</td>
<td>6.41</td>
</tr>
<tr>
<td>Esp3I (0.5U)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure S4. native AGE 2.5 % in TAE buffer showing one colony containing KL[20] (lanes 1 and 2) and another one that does not (lanes 3 and 4). Lanes 1 and 3: colonies were not treated with BsmBI, lanes 2 and 4: colonies were treated with BsmBI.

C. Sequencing of pUC19-KL[20]

The same procedure for sequencing pUC19-KL[10] was followed to test the sequence of pUC19-KL[20]. Primers pUC19-KL20BB-FOR (ataagggcgacacggaatg) and pUC19-KL20BB-REV (atcgcccttcacaacagt) were used to perform the experiment. Note that every primer is able to sequence about 500bp from each end of the insert region. The samples were submitted for Sanger
Sequencing to McGill University Genome Center and Innovation Quebec. From combining the two sequences given by the center, we found that our scaffold contains 1 substitution (highlighted in green) with respect to our theoretical expectations.

The observed sequence was:

cgtatcagagggcctttcgctcteggegcaggtcgtctactagacgcgtatatatcttggtgcgttgaagaacaacggaaggtcatgctttagg
aatcaaaacaaatgctcagcaacaggcgcgttaaggatcagaagaatacttggtgcgttgaagaacaacggaaggtcatgctttaggatcaaca
ccaagttcagcaacaggcgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagt
tcagcaacaggcgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagtgcag
cgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagtgcag
cgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagtgcag
cgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagtgcag
cgtttaaggatcagaagaatatctggctgctgttgaagaacaacggaaggtcatgctttaggatcaacaaccacagtgcag


We followed the same protocol described in section V to generate ss[20] using Primer pUC19-KL[20]BB-PCR-FOR (cgtatcagagggcctttc) and 5’ biotinylated Primer pUC19-KL[20]BB-PCR-REV (gcttacagacaagctgtgac). We incubated the double-stranded backbone for 3 hours instead of 2 in 0.5×SSC buffer, while gently inverting the mixture, to maximize binding. Because dsKL[20] is longer than dsKL[10], increasing the incubation time is expected to improve the binding of biotinylated dsKL[20] to the beads. Figure S5 lane 2 shows a discrete band around 500 bp that corresponds to ssKL[20]. It is noteworthy that improving the yield of ssKL[20] using this method is challenging. As described in the previous section, we attempted to increase the concentration of NaOH to enhance the separation of the double helix. However, an intense non-penetrating band was observed in the gel indicating the cleavage of biotin-streptavidin bond.
Alternatively, we optimized the conditions for using Lambda Exonuclease to generate ssKL[20] since it is faster and results in less byproducts (Figure S5). Table S7 summarizes the optimal conditions used to isolate ssKL[20] through Lambda Exonuclease method. Note that the same primers were used during PCR step before adding the exonuclease. In this case, the 5’end of the reverse primer was phosphorylated to facilitate the digestion of the antisense strand. The mixture was incubated at 37°C for 40 min then the enzyme was deactivated at 75°C for 15 min.

Table S7. Lambda Exonuclease digestion of dsKL[20]

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsKL[20] (180 ng/µL)</td>
<td>8.3 µL (60 ng/µL)</td>
</tr>
<tr>
<td>10x Lambda Exo Buffer</td>
<td>2.5 µL (1×)</td>
</tr>
<tr>
<td>λ Exo (5000 Units/mL)</td>
<td>7.5 µl (1.5 U/µL)</td>
</tr>
<tr>
<td>dH₂O</td>
<td>6.7 µL</td>
</tr>
</tbody>
</table>

Figure S5. 2.5% AGE gel in 1×TAE buffer displaying the mobility shift between dsKL[20] and ssKL[20]. (a) via magnetic beads separation and (b) via Lambda Exonuclease technique. Lane 1: dsKL[20] and lane 2: ssKL[20].
VII. Preparation of the 3-tile system and related control experiments

A. Tile assembly

The assembly of DNA tiles was based on the method reported by Rothemund.² The long circular single-stranded viral scaffold M13mp18 was folded into rectangular tile with the aids of multiple short staple single-stranded DNA. The sequences of staple strands required for different tiles are listed in final section.

DNA tiles were assembled in one-pot annealing at 1 nM M13mp18 scaffold and 10 nM each staple strands in 1xTAMg buffer (45 mM Tris, 20 mM acetic acid, 12.5 mM MgCl₂·6H₂O at pH ~8.0). Then, 500 µL samples were heated to and held at 95°C for 5 min and slowly annealed to 20°C (~1°C/min). To remove excess staple strands, the samples were purified with 100kDa Amicon centrifugal filters (Millipore). First, 500 µL samples were centrifuged at 6000 rpm at 4°C for 5 mins. Then, 400 µL 1xTAMg was added and the samples were centrifuged at 5000 rpm at 4°C for 5 mins. This filtration step was repeated two more time. Approximately 50-100 µL samples were recovered, which can be stored at 4°C up to a week before use.

To determine the concentrations of DNA tiles, absorbance at 260 nm was measured by Biotek Synergy HT plate reader. The extinction coefficient of different DNA tiles can be approximated by equation (1)³

$$\varepsilon = 6700ds + 10000ss$$  \hspace{1cm} (1)

where ds is the number of double stranded base pairs and ss is the number of single-stranded base. Using Beer-Lambert’s law (A₂₆₀ nm = εbc, b = 1 cm), the concentrations of DNA tiles can be calculated.
B. AGE and AFM characterization of the 3-tile system

We first attempted to increase a rigidity of single-stranded backbone, ss[10], by hybridizing K blocks of the backbones with its complements (K*). Briefly, ssKL[10] was mixed with single-stranded K* at 1:5 ratio in 1xTAMg, and the samples were annealed from 56°C to 20°C over 1 h. To construct 3-tile system, equimolar amounts of tile X, Y and Z (0.2 nM each) were mixed in 1xTAMg, which will give final tile concentration of 0.6 nM. Then, ssKL[10]/K* solution was added in 5 equimolar amount with respect to total tile concentration, i.e., 3 nM, before annealing from 44°C to 20°C over 45 min. Agarose gel electrophoresis (AGE) was used to characterize the products by mixing 20 µL samples with 4 µL 6X loading dye then running on 1% AGE at 80 V for 2.5 h. Lane 5 of Figure S7 shows a strong band correlated to the trimers, compared to a faint band representing individual tiles. Please see refer to Figure 2 on the main text for more quantitative analysis by AFM.
Figure S7. AGE 0.7% in TAMg. Lanes 1, 2 and 3 represent the individual tiles X, Y and Z. Lane 4: X + Y + Z and lane 5: X + Y + Z with ssKL[10] where the upper band represents the trimers, the middle band is attributed to individual tiles hybridized to ssKL[10]/K* and the lower band represents excess of ssKL[10]/K*.

To maximize the cohesion strength between DNA tiles, an assembly of DNA tiles containing 10 sticky ends (X10, Y10, Z10) was performed. In the absence of the backbone, there were tile monomer, band smearing, and non-penetrating materials, which were likely to be aggregations of DNA tiles (lane 4, Figure S8). Addition of the backbone did not improve the trimer yield as expected, even at higher backbone concentration (lane 5-7). Atomic force microscopy (AFM) revealed that tile aggregates were the major product. This is likely due to strong cohesion between tiles and various possible connections between tiles (e.g., linear dimer, staircase dimer), which can easily result in aggregation.
Since the backbone is shown to improve the trimer yield as observed by AFM, we then followed the yield of trimers with respect to the concentration of ssKL[10] scaffolds. An efficient binding of the tiles to the backbone will, in theory, directly translate to more efficient alignment of the tiles on the backbone. Therefore, the titration of ssKL[10]/K* to the mixture X+Y+Z was carried out. The gel mobility decreased with increasing backbone concentration. However, the gel mobility became unchanged at 5 equivalences of the backbone with respect to total tile concentration. Thus, we decided to choose 5 equivalences of the backbone for all experiments.

As one-pot assembly of 3-tile systems, which involved mixing together X, Y, Z and ssKL[10]/K*, was used in all previous experiment, we then investigated whether an order of addition of the tiles can improve the product formation. For example, X can bind first to the backbone and this preorganization may direct the binding of another two tiles to the backbone. As such, an assembly was performed in stepwise fashion: 1) annealing one of the tiles with the backbone from 44°C to 20°C, 2) adding the second tile and annealing from 44°C to 20°C, and 3) adding the third tile and annealing from 44°C to 20°C. In Figure S10, the stepwise assembly did not significantly improve the product formation (lane 6-9 VS lane 5). An exception was lane 8, which gave higher percentage of tile trimer, compared to other stepwise additions. The preorganization of the middle tile Y on the backbone seemed to be important for trimer formation. However, one-pot assembly showed significantly lower percentages of tile monomer and tile dimer.
Figure S10. Step of tile additions. Lane 1: X, lane 2: Y, lane 3: Z, lane 4: X+Y+Z, lane 5: (X+Y+Z) + (ssKL[10]/K*), lane 6: (X+(ssKL[10]/K*) + Z + Y, lane 7: (Z+(ssKL[10]/K*) + X + Y, lane 8: (Y+(ssKL[10]/K*) + X + Z and lane 9: (X+(ssKL[10]/K*) + Y + Z.

Subsequent to the AGE experiments showing that the stepwise assembly starting by tiles Y and the backbone, followed by the addition of X and Z was effective, we carried on AFM measurements to study the amount of trimers with respect to monomers and dimers (Figure S11). Interestingly, the sequential addition of the tiles to ssKL[10] did not improve the yield of trimers compared to the one-pot strategy. We think that the one-pot assembly provides more binding sites to the backbone (3 tiles binding the scaffold simultaneously), hence it amplifies the effect of ssKL[10] on the construction of higher-order architectures.
VIII. Construction of the railroad track and related control experiments

A. AGE characterization of the railroad track

The one-pot assembly of the 5-tile system follows the same protocol as the 3-tile system. ssKL[20] was first rigidified by hybridizing either K or L blocks of the backbones with their complements (K* or L*, respectively). We first attempted to hybridize one scaffold to tiles A, B, C, D and E, then examined the railroad system. Briefly, ssKL[20] was mixed with single-stranded K* or L* at 1:10 ratio in 1xTAMg, and the samples were annealed from 56°C to 20°C over 1 h. To construct 5-tile system, equimolar amounts of tiles A, B, C, D and E (0.2 nM each) were mixed in 1xTAMg, which will give final tile concentration of 1 nM. Then, ssKL[20]/K* and/or ssKL[20]/L* solutions were added each in 5 equimolar amount with respect to total tile concentration before annealing from 44°C to 20°C over 4 hours. AGE (Figure S12) was used to characterize the products by mixing 20 µL samples with 4 µL 6X loading dye then running on 0.7% AGE at 80 V for 2.5 h. Lane 9 corresponds to the nanostructure with ssKL[20]/K* only and lane 8 to the railroad track in the presence of ssKL[20]/K* and ssKL[20]/L*. The results validate the AFM data shown in Figure 3 where 2 scaffolds were needed to enhance the formation yield of pentamers. Compared to lane

Figure S11. AFM micrographs displaying the stepwise assembly in the absence and presence of ssKL[10] versus one-pot assembly, scale bar 500 nm.
7, the railroad track system helped minimize other contaminants such as dimers, trimers and so on.

![Figure S12. 1% AGE in 1×TAMg. Lane 1: A, lane 2: B, lane 3: C, lane 4: D, lane 5: E, lane 6: A + B + C + D, lane 7: A + B + C + D + E, lane 8: A + B + C + D + E + ssKL[20]/K* + ssKL[20]/L* and lane 9: A + B + C + D + E + ssKL[20]/K*.](image)

In an attempt to further increase the yield of pentamers, we only added ssKL[20]/K* to the 5 tiles during 44 to 20°C cycle. Then, we incubated the mixture with ssKL[20]/L* at room temperature for 2 hours. The AGE gel in Figure S13 shows no significant enhancement in the yield of pentamers. Again, we believe that both backbones are needed to better organize the pentamers by minimizing the non-desired interactions and maximizing the hybridizing sites between tiles and backbones.
B. AFM characterization of the railroad track

We examined the ability of ssKL[20] to organize pentamers and improve their yields at room temperature. Thus, we annealed/cooled the mixture containing A, B, C, D and E from 44°C to 20°C over 4 hours first, then added ssKL[20]/K* and ssKL[20]/L* at room temperature. Figure S14 displays a mixture of individual tiles, trimers, tetramers, pentamers and other misassembled nanostructures. Compared to the simultaneous addition of ssKL[20] to the tiles, the railroad track does not seem to improve the yield of pentamers significantly. Similar to the 3-tile system, we suppose that the interactions holding the pre-formed higher-order structures are difficult to re-arrange even after the addition of the backbones. As such, it is critical to add all the strands at the same time in order to promote the assembly of pentamers.
Next, we examined the organization of the 5 tiles using the same sticky ends between two tiles (with 16 bp complementary domains instead of 10). For example, the sequence of the 5 overhangs between tiles C and D is the same but different than the one between D and E. Similar to the 3-tiles system, we aimed at further decreasing the number of strands used and at increasing symmetry. However, the tiles did not arrange correctly and they aggregated instead (Figure S15). Furthermore, we increased the number of sticky ends, using the same sequences between the tiles, from 5 to 10. Figure S15 demonstrates the formation of aggregates on the mica surface. Thus, we decided to use 5 unique sticky ends to link the tiles.

It is noteworthy that in order to further grow the 1D tracks, a longer backbone is required. We are currently working on generating custom made DNA scaffolds with repetitive sequences of 4000 base pairs in length. Theoretically, higher-order assemblies of 1D and 2D DNA origami should be produced when these three main rules are respected: (i) the hybridization length between the backbone and each origami tile must be larger than the length of the lateral cohesion between the tiles; (ii) the pre-assembled individual origami tiles and the backbone must be annealed in a single
reaction mixture for at least 4 hours and (iii) the ratio of backbone to the origami tiles is critical to avoid secondary interactions and the growth of unwanted products.

![AFM micrographs](image)

**Figure S15.** AFM micrographs depicting the formation of aggregates between the tiles when using (a) 5 sticky ends and (b) 10 sticky ends, scale bar 500 nm.

### IX. Additional AFM micrographs

We present in this section additional AFM images that correspond to the railroad track system with ssKL[20]/K* and ssKL[20]/L* (Figure S17) and 3 tiles system with ssKL[10]/K* (Figure S16). The corresponding control experiments in the absence of the backbones are described in figures S18 and S19. These images were part of the data acquired to perform the statistical analysis on these nanostructures.

To perform the quantification on these images, we manually counted the number of trimers and pentamers versus all the other byproducts. For the 5-tiles system, the aim of our strategy was to improve the organization of pentamers by minimizing the non-desired interactions between the tiles and ss[20]. Interestingly, 5 tiles were successfully arranged by ss[10]/K* and ss[20]/L*
scaffolds up to 66% (69% if we considered not well aligned pentamers) compared to 19% (30% including all types of pentamers) in the absence of any backbone. Accordingly, the percentage of tetramers decreased from 31% to 10% and the amount of trimers was reduced from 10% to 7%. We have also found that the yield of monomers was reduced from 12% (in the absence of backbones) to 4% upon addition of ssKL[10]/K* and ssKL[10]/L*. The results suggest that the addition of 2 scaffolds is highly advantageous to assure the organization of the tiles in 1-D. By using the railroad track system, we think that applications requiring larger areas than a single origami can be achievable.

Figure S16. 3-tiles system
Figure S17. Railroad track system.

Figure S18. 3-tiles system in the absence of ssKL[10]/K*.
X. Origami sequences

Tables S8-S10 show lists of modified staple strands that replaced unmodified strands of the same number for generating different origami tiles. In general, the three-digit numbers or the numbers following the first letter indicate the position of the corresponding modified staple strands on origami tile. Other nomenclatures are described below.

**Staple strands that hybridize between the backbone and the origami tiles:**

1) Strands starting with letters X, Y and Z are staples that have single-stranded extension either on their 3’ or 5’ end to provide the hybridization between the backbone and origami tiles X, Y and Z (Figure S20).

2) Strands starting with letters A, B, C, D and E are staples that have single-stranded extension either on their 3’ or 5’ end to provide the hybridization between the backbone and origami tiles A, B, C, D and E (the same strategy as shown in Figure S20). In addition, the 10b abbreviation indicates that these staples are only used in the 5-tile system containing 10-base overhangs (see Table S10).
Figure S20. Scheme illustrating the staple-strand extension for hybridization of origami tiles to the backbone. The extension is highlighted in blue.

**Staples strands that have an internal hairpin:**

3) Strands starting with the letter H have an internal hairpin sequence (TCCTCTTTTGAGGAACAAGTTTTCTTGT). See Figure S21.
Staple strands for lateral cohesion between origami tiles:

4) Staples in the DNA tile’s corners (100, 111, 205 and 216) have a portion of their 5’ ends removed. This creates a set of strands starting with letter M. The purpose is to remove T4 spacer and to allow the extension of their neighboring staple strands for lateral cohesion between origami tiles. (i.e., M216 allows us to extend the 3’ end of staple 215; see Figure S22).

![Figure S22](image)

Figure S22. Scheme illustrating the modification of the strands involved in the lateral cohesion between the origami tiles. A portion of 5’ end of staple 216 was removed to allow the 3’ extension of staple 215, resulting in M216 (and 215AC).

5) A set of staples called ‘S’ strands have 12-bases deletion (8 bases of M13-binding segment and T4 spacer) on their 5’ ends. This allows us to extend the length of their neighboring staple strands for lateral cohesion (i.e., S103 allows us to extend the 3’ end of staple 102; see Figure S23).
Figure S23. Scheme illustrating the modification of the strands involved in the lateral cohesion between the origami tiles. A portion of 5’ end of staple 103 was removed to allow the 3’ extension of staple 102, resulting in S103 (and 102A).

6) Strands starting with three-digit number followed by one or two letters are staples that provide lateral cohesion between pairs of origami tiles (for example, 102A in Figure S23 and 214BC in Figure S24). Two modifications are required for these lateral cohesion staple strands (see the transformation of staple 102 to staple 102A in Figure S23):

a. Remove 12 bases on the 5’ end,

b. Extend the 3’ end for an additional 24 bases—8 bases bind to the M13 backbone and 16 bases overhang are for lateral cohesion.
Figure S24. Example of staple strands for lateral cohesion between origami tiles X, Y and Z.

Figure S25. Example of staple strands for lateral cohesion between origami tiles A, B, C, D and E.
Additional notes:

a. A and its complementary AC connect tile X and Y or tile A and B (Figures S24 and S25).

b. B and its complementary BC connect tile Y and Z or tile B and C (Figures S24 and S25).

c. C and its complementary CC connect tile C and D (Figures S25).

d. D and its complementary DC connect tile D and E (Figures S25).

e. The abbreviations A18 and AC18 indicate that the overhangs for lateral cohesion are 18 bases long, rather than 16 bases long (Figure S24).

f. The abbreviations 10 indicate that the overhangs for lateral cohesion are 10 bases long, rather than 16 bases long, and all have unique sequences (Figure S25).

Table S8. Modification of staple strands in 3-tile system. 5 stands for 5 sticky ends between the tiles and 10 stands for 10 sticky ends linking the tiles.

<table>
<thead>
<tr>
<th>Tile</th>
<th>Staple strand modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X54, X100</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
<tr>
<td></td>
<td>102-A18, 103, 104-A18, S105, 106-A18, S107, 108-A18, S109, 110-A18, M111</td>
</tr>
<tr>
<td>Y</td>
<td>Y52, Y132</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
<tr>
<td></td>
<td>102B, S103, 10B, S105, 106B, S107, 108B, S109, 110B, M111</td>
</tr>
<tr>
<td></td>
<td>M205, 206-AC18, S207, 208-AC18, S209, 210-AC18, S211, 212-AC18, S213, 214-AC18, S215</td>
</tr>
<tr>
<td>Z</td>
<td>Z178, Z202</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
<tr>
<td>X10</td>
<td>X54, X100</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
<tr>
<td></td>
<td>101-A18, 102-A18, 103-A18, 104-A18, 105-A18, 106-A18, 107-A18, 108-A18, 109-A18, 110-A18, M111</td>
</tr>
</tbody>
</table>
Table S9. Modification of staple strands in 5-tile system. The same sequences were employed in each sticky end linking the same tiles.

<table>
<thead>
<tr>
<th>Tile</th>
<th>Staple strand modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y10</td>
<td>Y52, Y132</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
<tr>
<td></td>
<td>M205, 206-AC18, 207-AC18, 208-AC18, 209-AC18, 210-AC18, 211-AC18, 212-AC18, 213-AC18, 214-AC18, 215AC-18, M216</td>
</tr>
<tr>
<td>Z10</td>
<td>Z178, Z202</td>
</tr>
<tr>
<td></td>
<td>H134, H136, H137, H139, H158, H160</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tile</th>
<th>Staple strand modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A78, A100, A111</td>
</tr>
<tr>
<td>B</td>
<td>B52, B132, B159, B73</td>
</tr>
<tr>
<td></td>
<td>102B, S103, 104B, S105, 106B, S107, 108B, S109, 110B, M111</td>
</tr>
<tr>
<td></td>
<td>M205, 206AC, S207, 208AC, S209, 210AC, S211, 212AC, S213, 214AC, S215</td>
</tr>
<tr>
<td>C</td>
<td>C132, C156, C135, C113</td>
</tr>
<tr>
<td>D</td>
<td>D52, D180, D135, D197</td>
</tr>
<tr>
<td></td>
<td>102D, S103, 104D, S105, 106D, S107, 108D, S109, 110D, M111</td>
</tr>
<tr>
<td></td>
<td>M205, 206CC, S207, 208CC, S209, 210CC, S211, 212CC, S213, 214CC, S215</td>
</tr>
<tr>
<td>E</td>
<td>E216, E205</td>
</tr>
<tr>
<td></td>
<td>206DC, S207, 208DC, S209, 210DC, S211, 212DC, S213, 214DC, S215</td>
</tr>
<tr>
<td>A10</td>
<td>A78, A100, A111</td>
</tr>
<tr>
<td>B10</td>
<td>B52, B132, B159, B73</td>
</tr>
<tr>
<td></td>
<td>M205, 206AC, 207AC, 208AC, 209AC, 210AC, 211AC, 212AC, 213AC, 214AC, 215AC, M216</td>
</tr>
<tr>
<td>C10</td>
<td>C132, C156, C135, C113</td>
</tr>
<tr>
<td>D10</td>
<td>D52, D180, D135, D197</td>
</tr>
<tr>
<td></td>
<td>M205, 206CC, 207CC, 208CC, 209CC, 210CC, 211CC, 212CC, 213CC, 214CC, 215CC, M216</td>
</tr>
<tr>
<td>Tile</td>
<td>Staple strand modifications</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>E10</td>
<td>E216, E205 206DC, 207DC, 208DC, 209DC, 210DC, 211DC, 212DC, 213DC, 214DC, 215DC, M216</td>
</tr>
</tbody>
</table>

Table S10. Modification of staple strands in 5-tile system. Each overhang (10 bases) is unique in this case.

<table>
<thead>
<tr>
<th>Tile</th>
<th>Staple strand modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5(10)u</td>
<td>100, 101, 102A10(1), S103, 104A10(2), S105, 106A10(3), S107, 108A10(4), S109, 110A10(5), S111</td>
</tr>
<tr>
<td>B5(10)u</td>
<td>B52, B132, B159, B73 100, 101, 102B10(1), S103, 104B10(2), S105, 106B10(3), S107, 108B10(4), S109, 110B10(5), S111 205, 206AC10(5), S207, 208AC10(4), S209, 210AC10(3), S211, 212AC10(2), S213, 214AC10(1), S215, 216</td>
</tr>
<tr>
<td>C5(10)u</td>
<td>C132-10b, C156-10b, C135-10b, C113-10b 100, 101, 102C10(1), S103, 104C10(2), S105, 106C10(3), S107, 108C10(4), S109, 110C10(5), S111 205, 206BC10(5), S207, 208BC10(4), S209, 210BC10(3), S211, 212BC10(2), S213, 214BC10(1), S215, 216</td>
</tr>
<tr>
<td>D5(10)u</td>
<td>D1-10b, D76-10b, D97-10b, D135-10b 100, 101, 102D10(1), S103, 104D10(2), S105, 106D10(3), S107, 108D10(4), S109, 110D10(5), S111 205, 206CC10(5), S207, 208CC10(4), S209, 210CC10(3), S211, 212CC10(2), S213, 214CC10(1), S215, 216</td>
</tr>
<tr>
<td>E5(10)u</td>
<td>E183-10b, E202-10b 205, 206DC10(5), S207, 208DC10(4), S209, 210DC10(3), S211, 212DC10(2), S213, 214DC10(1), S215, 216</td>
</tr>
</tbody>
</table>

Unmodified staple strands (1-216)

1. CAAGCCCAATAGGAACCACCATGTACAAACAGTT
2. AATGCCCGTAACAGTGCCCCGTATCTCCCTCA
3. TGCCCTTGACTGCCCTATTTTGGAAACAGGGATAG
4. GAGCGGGCCCACCACCCGGAACCACGCGAGGAAA
5. AACCCAGACCCCTAGAAACCGCAGGGGATAG
6. TTATTCATAGGGGAAGTTAAATATTCATTCAGT
7. CATACCCGAGGCTAAGTAAAGGCTTATCTTAAAG
8. ATTGAGGGTAAAGGATAATTATCAATCACTCCGG
9. AAAAGTAATATCTTACCGAAGCCCTTCCAGAG
GCAATAGCGCAGATAGCCGAACAATTCAACCG
CCTAATTTACGCTAACGAGCGTCTAATCAATA
TCTTACCGGCCAGTTACAAATAAATGAAAATA
ATCGGCTGCGAGCATGTAGAAACCTATCATAT
CTAATTTATCTTTCTTTATCTATTCATCCTGAA
GGTTATAGAAAAAGCCCTTTTAGAAGGGCCGG
GCTCATTTTCGCACTAAATTTTGAGCTTGA
TTAAGACGTGAAAAACATAGCGATAACAGTAC
TAGAACTCCGTGAAAGAGTCAATAGGAATCAT
CTTTACACAGATAATACAGAAAACAT
TTAACGTTCCGGGAAACAAATAATTTTCTT
CGACAACATAGTATTAGACTTTCAATACCGA
GGATTTAGGTTAAAATCTTTTGTGGTCAG
ACGAACAAACATCGCCCATTAAAATGTTGGT
GAACGTGGCGAGAAAGGAAGGGGAAACACTAT
TAGCCCTACGACGAGATAAAAAACATTTGA
CGGCCCTTGCTGTATATCCGAAAAAAGCTA
CTCAGAGCACCCACCCTCATTTTCTTTATT
CTGAAACAGGTAATAAGTTTTAACCCTCAGA
AGTGTACTTGAAAAATTAAGAGGCCGCCACC
GCCACCACTCTTTTCATAATCRAACCGTACC
GTTGCCCCACTAGCCGCCACCGATACAGG
GACTTGAGAGACAAAAAGGCCGAACAGTTACC
AGCAGCAAACATTGGAATTAGATATTAGC
GAAGGAAAATAAGAGCAAGAAAACACAGCCAT
GCCCAATACCGGAAAACGCAATAGGGTTACC
ATTATTTACCCGACTAATTTCCAGAACG
TATTTTGCTCCCAATCCAAATAAGTGAGTTAA
GGTATTAGAAGAAAGAAAAATATTAAAGC
TAAGTCCCTACAGTACCCGACCTCTTTAGTTGC
ACGTCAAATAAGAAATACCCGAGATTATTT
AGGCCTACAGTACGGCTTATAATCAGGAAT
ATCAAAAAAGTACGCTATTAAATTACGGATTG
CTGTAACATAGGGTATCGAGAGCGATAATA
80  TGCCCTTTAGTCAGACGATTGGCCTGCCAGAAT
81  CCGGAAACACACCACGGAAAATAAGTAAGACTCC
82  ACGCAAGGTCAACAAATGAACAAATCAAGTT
83  TTATTACGGTCAGAGGGTAATTGAATAGCAGC
84  TGAACAAACGTATGTTAGCAAACCTAAAAGAA
85  CTTTACAGTTAGCGACCCACCTCCTGGACGTAGGA
86  GAGGCAGTAGAGAATAAACAAAAGAACACCC
87  TCAATTACCGACAATAAAACACATATTTAGGC
88  CCAGACGAGCGCCCAATAGCAAGCAAGAACGC
89  AGAGGCATAATTTCATCTTCTGACTATAACTA
90  TTTTATTTTTTCGAGCCAGTAATAAAATTTCTGT
91  TATGTAAACCTTTTTTAATGGAAGAAAATACCT
92  TTGAATTATGCTGTAGGAAATCCACAATATA
93  GAGCAGGACATTTCGTAATAATGGAAGAAGGAG
94  GGGGTGATTTTATAAATCCTCATTAAATTTCTG
95  TCTAAGAGATAGATAAAACAAATTTTAGGAT
96  ATCAACAGTCGTATCATCTCCCTTGATTGTT
97  GGGGTGATTCTGACAGGACACCTCCTGTGCTCT
98  GCCAACAGTCACCTTGTGCAACCTGTTGGCAA
99  GAAATGGATTATTTACATCGGCCACATTCTG
100  TTTTATAAGTGATAGCCGGCGCTCGAG
101  AGGGTTGATTTTATAAATCCTCATTAAATTTCTG
102  ACAAAAATTTTATCGTAGCAGACATCGATAGC
103  AGCCACGGTTTTTTAAAGGTTGGCAACATAGTAA
104  TACATACATTTTGCGAGGAAATTAACCTACAGGGAA
105  GCCGATTATTGGTTTTTAATCCCTGATATGACAA
106  TATAGAAGTTTTTCGAGAAATTTAAATTGAGAATA
107  TAAGAGTTTTCGAGAAATTTAAATTGAGAATA
108  ACAAAAGATTTTATTAATTACATTTACACATCAAG
109  AAAACAAATTGTTTCCGATAATAATCAATTCTG
110  GATGGCAATTAAATCAGTAGATCCGTTACAAATATC
111  TAAAAGATTGTTGCAGAAAACATTTCATCGCAAG
112  ACAAAAGATTTTATTAATTACATTTACACATCAAG
113  AAAACAAATTGTTTCCGATAATAATCAATTCTG
114  CCTGAGGTGGGAAAAATTCCCTTTATAAAGGCGCA
115  GCATAAAAGTTCCACACACATACGAAGCGCA
ACGAGTAGTGACAAAGAACCGGATATACCAAGC
AGTAATCTTTAAATTGGCGTGGAGAATAACCA
GCCAAAACATGCCACTACGAAAGGCGATGCGCCA
ATACGTAAGGTACAGGAGATTTCATACAG
AAAAAGGACAACGACGCCACCGCGGTAAG
TGAGCATTACACAGAAGAGGAGGCTTACCAAA
GTAAGGCGGCTTTGAATTCGTATAACGC
CCCAGGTACTTTCCAGTGGGAAGACGGGAC
CAGCTGGCGGACGACGACTGATCGACCGAG
GTTGAGGAAAGGGGATGTGCCTAGAGGATC
CTTTATCCCACAAAACACGGAGACCGAGAG
AGAAAAGCAACATTAAATGTGAAGATCGACC
GCTGATTACAAGAGTCCACTATTGAGGTGCC
ACGCTACGTAGGATAAATTCTTTACCAA
TACATATCACAGTGTTACCCCAATTAGAGAG
CAAAATCATGGCTCTTTGATAAGTTTCAT
TTTCAGATCATGTTAGATTGATTGTTAA
AAAGATTGAGGGGATTAGTAAACACCATAT
TTTCAACTATAGGCTGGCTACATTGTACAT
CCAGGCTTAAATCGATTGAAATTCAGGTA
CGCCTGATGGAAGTGCTCCATTAAACATACAG
TTCATGAAAAATTGTGGCAATTGTTACAGA
ATATATTTCACGTTGAATACATG
AAATAAAGGTCGCTGAGGCTTGGCAAAAGCTT
CGTAAAGCTCTAAGTTTGTTGCTGAATGCG
ACCCAAATCAAGGGTCTGGGTTCAAGAAGCG
TGGACTCCCCTTTACCAGTGACCGTCGAGT
TGTTTTAAACGTCAGGCGGCAAGAACCATC
GCCAGCCTGCTCAGGCTGACCTCAGCAGCG
Modified staple strands

X54  TAAGCGTCGAAAGGATTAAGGTAGTACCCGCAATTATATCGCTCTAGAGCTGACCTGTTTTTTTT

X100 ATCTTTAACCGGCTTTGCTGTTTTTATAAGTATACGGCCCGGCGGTCGAG

Y52  CTTTAACGGCGCTTTGCTGACGTTTTTATAAGTATACCCGCAACTGAGCTG

Y132 TTTTTTTTTTTTTTCTGCTGACGTTTTTATAAGTATACCCGCAACTGAGCTG

Z178 ATATATTCTTTTTTCTGCTGAAATTCCCTCAGAACGCCCACTGATTTTTTTTT

Z202 AAAGGCAGAAAGAAACAACTAAAGCTTTCCCAGTTACTCTGATCTCTTTTTT

A78  GGAAAGCGGACCAGGCAGGATAAAGTGTATATAGGCGCTTCTGAGTACG

A100 ATATCGCTCTAGAGTCTTTTTTATAAGTGATAGCCCGGCGGTCGAG

A111 ACCAGTAATAAAAGGGATTACCAAGACTACAGCTCAGTTTTTTTAGTGAACGGGACTACG

B52  CTTCTAGTCTCTAAAGCGCCCTTTCCCCTGACGACGCTTCACCTCAGACTGAGCT

B73  GCCACGCTATACGTTGGCTGACAGCCTCTATTCTAGACGTATTCCGTTTTCT

B132 TTTTTTTTTTTTTTTCTGCTGAACTTTGGTTTGATTTTTGAGTTTCGTCACCAGTACAAACTTAATTGTA

B159 AGCTGATTACAAAGAGCTCCACTATTGAGTTTCGTCACCAGTACAAACTTAATTGTA

C113 CCAGAGAGCCAATCTCCCTTATAAAGGCGCTTTTGCCTTCTAGAGTACAGCTG

C132 ATCGCTCTAGAGACGGTGGTCTTGAGTTTCTGTCACAGTACAACCTTATTTTGA

C135 GAGTTGCACAGAGAAGGGGTTGTAACGCTTCTTCCGTTTTCTCAAGCCAGC

C156 GACTACGTCCTTCTAGTCTTTTTTTCTTATAGTTGACCTTTTCGCTACTCGACAGCTG

D52  GGTGCTGAACTTTTTGTTGATTTTTTTTCTGACAGTAAGTCGACGGCAACTGAGCT

D135 GAGTTGCACAGAGAAGGGGTTGTAACGCTTCTTCCGTTTTCTCAAGCCAGC

D180 ACCTGCTGTGCTGAACTTTGTTCGTAACGATCAAGTTTTTGTCGTGAATTTCG

D97  CTTAGGTAGTGAGCCGCTGTTGTTAAGCTTTTAAAGCATGACCTTCCGTTTTCAAGCGCAGC

E205 CTAGGTAGTGAGCCGCTGTTGTTAAGCTTTTAAAGCATGACCTTCCGTTTTCAAGCGCAGC

E216 AACTTTTAAAGTGCTTTTGGATTTTTGCTATTTTTTTCTAAAGGCGAGCATCAGTACG

C113-10b CCAGCAGGGGCAAAATCTCCCTTATAAAGGCGCTTTTGCCTTCTAGAGTACAGCTG

C132-10b CTAGGTAGTGAGCCGCTGTTGTTAAGCTTTTAAAGCATGACCTTCCGTTTTCAAGCGCAGC

C135-10b GAGTTGCACAGAGAAGGGGTTGTAACGCTTCTTCCGTTTTCTCAAGCGCAGC

C156-10b GACTACGTCCTTCTAGTCTTTTTTTCTTATAGTTGACCTTTTCGCTACTCGACAGCTG

D1-10b ACCTGCTGTGCTGAACTTTGTTCGTAACGATCAAGTTTTTGTCGTGAATTTCG

D76-10b TTCGATACCGTTTAACCGGCCTTTGAGTTTTAGCTTGGTTTTACGAGCTTTTTTTTTTTCT

D97-10b CTTAGGTAGTGAGCCGCTGTTGTTAAGCTTTTAAAGCATGACCTTCCGTTTTCAAGCGCAGC

D135-10b GAGTTGCACAGAGAAGGGGTTGTAACGCTTCTTCCGTTTTCTCAAGCGCAGC
E183-10b  TGGTTTTAACGTCAAAGGGCGAAGAACCATCTTTCGACCTGCGCGAGACGAA
E202-10b  AAAGGCCGAAAGGAACAACTAAAGCTTTCCAGTTGCTTACAGACAAGCTGTGACC
M100     TATAAGTATAGCCCGGCGTCGAG
M111     ACCAGTAATAAAAGGGATTCACCAGTCACACGTTTT
M205     CGATGGCCCACTACGTAAACCGTC
M216     AACTTTCAACAGTTCTGGGATTTTGCTAAACTTTT

101-A18  AGGGTTGAATAAAATCTCTACTATTATATCTCACAACACGCTCTCTCAAGTGAAT
102-A18  AATCAGTAGCAGACAGATCGGAGCGACCCGTTGCTTCAAGTGAAT
103-A18  TAAAGGTGGCAACATAGTAGAAAATACATACGTCTTCTCAGTGAAT
104-A18  GACCCGGAATTTAATCAAGGGAACGCATTACGCTCTCTCAGTGAAT
105-A18  GCTTATCCGTTATTTCAATAGATAGACCCGCTCTCTCAGTGAAT
106-A18  CGACAAAGGTAAAGTAGAGAATATTAAGTAGAACTACCGCTCTCAGTGAAT
107-A18  CGCGAGAAAATTTTTATCGCAAGACAAAGACGCTCTCTCAAGTGAAT
108-A18  ATTAATTACATTACACTCAAGGAAGAAAACGCTCTCTCAAGTGAAT
109-A18  TTCATCAATATAATCCTCATAGATGAGCGCTCTCTCAAGTGAAT
110-A18  AATCAATATCTGTTCACAATATCACAACCTCGCTCTCTCAAGTGAAT

206-AC18  CGGTGTGCTATTGCGAAACGGCGGAGGAGATTCTACTTTGAGAGAGCG
207-AC18  TGTAACAGGCAGGGCAATTCCAGTGACGTATTCTACTTTGAGAGAGCG
208-AC18  GTATGGGATAGTGCAAAAGCGCGGATTGACCTTCTACTTTGAGAGAGCG
209-AC18  GATGAACGGTTATCTGAGCAAAACAGAGATTATATTCTACTTTGAGAGAGCG
210-AC18  GTTTGTCAACAAAACAAGCATAAGGCTAATTCTACTTTGAGAGAGCG
211-AC18  CGTATGCTACATATTGCTGAAATATTGTCAATTCTACTTTGAGAGAGCG
212-AC18  CCTAACTCCCTCAATCTCGTCTAAATATTCTACTTTGAGAGAGCG
213-AC18  GGAGAAGAAAATCTACGAGCGAAGACGTTGATTTACTTTGAGAGAGCG
214-AC18  TCATAAGGGAAAGGCGAGACGGGTCAATTTCTACTTTGAGAGAGCG
215-AC18  GACAGCATCGGAACCAACCTCGTACGCAAGAAAATTTCTACTTTGAGAGAGCG

101A     AGGGTTGAATAAAATCTCTACTATTATATCTCACAACACGCTCTCTCAAGTGAAT
102A     AATCAGTAGCAGACAGATCGGAGCGACCCGTTGCTTCAAGTGAAT
103A     TAAAGGTGGCAACATAGTAGAAAATACATACGTCTTCTCAGTGAAT
104A     GACCCGGAATTTAATCAAGGGAACGCATTACGCTCTCTCAGTGAAT
105A  GCTTATCGGTATTCTAAATCGATATAGAAGATTACTTGAGAGAGCG
106A  CGAACAAAGGTTAAGTAgAGAATATAAGTACATTACTTTGAGAGAGCG
107A  CGCGAGAAAACCTTTTTATCGCAAGACAAAGAAATTACTTGAGAGAGCG
108A  ATTAATTACATTTAACACATCAAGAAAACAAATTTACTTTGAGAGAGCG
109A  TTCATCAATATAATCTTCTACATGATGATGAACTTTACTTGAGAGAGCG
110A  AATCAATATCTGGTACAAATATCAAACCCCTCTATTACTTTGAGAGAGCG

206AC  CGGTTCGTTTATTGGGAACGCCGCGGAGAGAGGCGCTCTCTCAAGTAAT
207AC  TGTTAAAACACGCCCATTTCCAAGTCACGAGCTGCCTCTCTCAAGTAAT
208AC  GATATGGGATTGTACCCAGACGGGAGTTGACCTCTCTCAAGTAAT
209AC  GATGAACGGTACTGTAAGCACAAGAAGAAATCCGCTCTCTCAAGTAAT
210AC  GTGTTTACCACAAAAAACGATAAAATCCTCTTTTCTCTCAAGTAAT
211AC  CTTGACTCAGCTCTACATGATTCTAAATGCTCTCTCTCAAGTAAT
212AC  CATTGCCAATACTTCAAGCTGTTTGACCTCTCTCTCAAGTAAT
213AC  AAGGAAAAATCTCAAGGGGACCGGAGGCGAGGTTGACCTCTCTCTCAAGTAAT
214AC  AGGTTGACCAAAACAGTATAGTACCCATGTTGACCTCTCTCTCAAGTAAT
215AC  GACAGCTACGCCAGAAAGCCCTCAGCAGCGAGCTCTCTCTCTCAAGTAAT

101B  AGGGTTAATAAAATCTCTATTAAATGATATCCTCAAAACAAAGCGTCCATTGAGTTA
102B  AATCAGTACGGACAGATCGATGACGACCGTACCCATGTTGACCTCTCTCAAGTAAT
103B  TAAAGGGTGGCAACCATAGAGAATACATACATACAGCGTCCATTGAGTTA
104B  GACGGGAGATAACTCAGGGGAACCGGATTAAGCTCCATTGAGTTA
105B  GCTTATCCGTTTATTGGGAACGCCGCGGAGAGAGGCGCTCTCTCAAGTAAT
106B  GATGAACGGTACTGTAAGCACAAGAAGAAATCCGCTCTCTCTCAAGTAAT
107B  GTGTTTACCACAAAAAACGATAAAATCCTCTTTTCTCTCAAGTAAT
108B  CTTGACTCAGCTCTACATGATTCTAAATGCTCTCTCTCAAGTAAT
109B  AATCAATATCTGGTACAAATATCAAACCCCTCTATTACTTTGAGAGAGCG
110B  AATCAATATCTGGTACAAATATCAAACCCCTCTATTACTTTGAGAGAGCG

206BC  CGGTTCGTTTATTGGGAACGCCGCGGAGAGAGGCGCTCTCTCAATGGACGCT
207BC  TGTTAAAACACGCCCATTTCCAAGTCACGAGCTGCCTCTCTCAATGGACGCT
208BC  GATATGGGATTGTACCCAGACGGGAGTTGACCTCTCTCAATGGACGCT
209BC  GATGAACGGTACTGTAAGCACAAGAAGAAATCCGCTCTCTCAATGGACGCT
210BC  GACAGCTACGCCAGAAAGCCCTCAGCAGCGAGCTCTCTCTCAATGGACGCT
107D  CGCGGAAAACCTTTTATCGCAAGACAAAGAATTACTACTGAGACATT
108D  ATTAATTACATTTAACACATCAAGAAAGAATTACTACTGAGACATT
109D  TTATCAATATAATCTCTATCGAGATGAGTACTACTGAGACATT
110D  AATCAATATCTGGTCAAAATATCAAAACCCTCTTTACTACTGAGACATT

206DC  CGGTTTTCGATTTGGGAAACGCGGCGGGGAGAGGATGCTTTGTAGTAA
207DC  TGGTTAAACGACGGCAATTCAGCTACGACGAATGTCTCAGTAGTAA
208DC  GTAATGGGATAGGTCAAAACGGCGGATTGACCAATGTCTCAGTAGTAA
209DC  GATGAACGGGAAATCGTGAGCAAACAGAGAATATCGTCTCAGTAGTAA
210DC  GGTTGTACAAAAAAGCATAAAGCTAAATCAATGTCTCAGTAGTAA
211DC  CTGTAGCTCAACATGATTGGCTGAAATATAAATGATTGTCTCAGTAGTAA
212DC  CATTGAATCCCCCTCAAATCGTCATAAATATTTATGTCTCAGTAGTAA
213DC  GAAAGGGAAAAAAGCTACGAGTTCAAGTTGGAATGCTCAGTCTAGTAA
214DC  TCATAAAGGGGAAAGCGAGGCAGACCGTCAAAATGTCTCAGTAGTAA
215DC  GACAGCATCGGAACGCAACCTCACGAGGAAAAAGTCTCAGTAGTAA

102A10(1)  AATCAGTAGCGACAGATCGATAGCAGCACCGTATTACTTGAG
104A10(2)  GACGGGAGAATTAACTACAGGGAAGCGCATTACGCTGCTGAG
106A10(3)  CGACAAAAGGTAAGTAGAGAATATAAAGTACGATCGTCCAG
108A10(4)  ATTAATTACATTTAACACATCAAGAAAACAAACGTACGTCCA
110A10(5)  AATCAATATCTGGTCAAAATATCAAAACCCTCCACAGGAAGCAT

214AC10(1)  TCATAAGGGGAAGCCGCGACGGCGATGCAAATCTCAAGTAAAT
212AC10(2)  CATTGAATCCCCCTCAAAATCGTCATAAATATTCTCAGCGAG
210AC10(3)  GGTTGTACAAAAAAGCATAAAGCTAAATCCCGACTGATCG
208AC10(4)  GTAATGGGATAGGTCAAAACGGCGGATTGACCTGGACGTACG
206AC10(5)  CGGTTTTCGATTTGGGAAACGCGGCGGGGAGAGGATGCTTTCTG

102B10(1)  AATCAGTAGCGACAGATCGATAGCAGCACCGTATTACTTGAG
104B10(2)  GACGGGAGAATTAACTACAGGGAAGCGCATTACTTGCCGAG
106B10(3)  CGACAAAAGGTAAGTAGAGAATATAAAGTACGATCTATCGAG
108B10(4)  ATTAATTACATTTAACACATCAAGAAAACAAACGTACGCTC
110B10(5)  AATCAATATCTGGTCAAAATATCAAAACCCTGTTGCTACGCG
214BC10(1)  TCATAAGGGAACCGAAAGGCGACAGCGGTCAAAATGGGACGCT
212BC10(2)  CATTGAATCCCCCTCAAATCGTCATAAATATTTCGCGGAAG
210BC10(3)  GGTGGTACAAAACAAACGCTAAAGCTAAAGCTGACGATC
208BC10(4)  GTAATTGGATAGTCAAACGCGATGGATGAGGTTC
206BC10(5)  CGGTGGTGATATTGGGAACGCGGGGAGAGGGCGTACGAAC

102C10(1)  AATCAATATCATCGTGCAATTGACAGTACCGGACGACGTC
104C10(2)  GACGGGAGAATTAACTACAGGGGAAGCGCATTACTATGCC
106C10(3)  CGACAAAAAGGTAAAGTAAAGAATAAAGTACGACAGGAT
108C10(4)  ATTAATTAGTTTACATCAAGAAGATGGAAGGATAGCTAC
110C10(5)  AATCAATATCATCGTGCAATTGACAGTACCGGACGACGTC

214CC10(1)  TCATAAGGGAACCGAAAGGCGACAGCGGTCAATCTTTGCGGGT
212CC10(2)  CATTGAATCCCCCTCAAATCGTCATAAATATTTCGCGGAAG
210CC10(3)  GGTGGTACAAAACAAACGCTAAAGCTAAAGCTGACGATC
208CC10(4)  GTAATTGGATAGTCAAACGCGATGGATGAGGTTC
206CC10(5)  CGGTGGTGATATTGGGAACGCGGGGAGAGGGCGTACGAAC

102D10(1)  AATCAATATCATCGTGCAATTGACAGTACCGGACGACGTC
104D10(2)  GACGGGAGAATTAACTACAGGGGAAGCGCATTACTATGCC
106D10(3)  CGACAAAAAGGTAAAGTAAAGAATAAAGTACGACAGGAT
108D10(4)  ATTAATTAGTTTACATCAAGAAGATGGAAGGATAGCTAC
110D10(5)  AATCAATATCATCGTGCAATTGACAGTACCGGACGACGTC

214DC10(1)  TCATAAGGGAACCGAAAGGCGACAGCGGTCAATCAGTAAA
212DC10(2)  CATTGAATCCCCCTCAAATCGTCATAAATATTTCGCGGAAG
210DC10(3)  GGTGGTACAAAACAAACGCTAAAGCTAAAGCTGACGATC
208DC10(4)  GTAATTGGATAGTCAAACGCGATGGATGAGGTTC
206DC10(5)  CGGTGGTGATATTGGGAACGCGGGGAGAGGGCGTACGAAC

S102  AATCAATATCATCGTGCAATTGACAGTACCGGACGACGTC
S103  TAAAGGATAGGAAATAGTAGAAAA
S104  GACGGGAGAATTAACTACAGGGGAAGCGCATTACTATGCC
S105  GCTTATCCGGTATTTTTCATCGGACGACGTC
XI. References