Supporting Information

Controlled Nucleation and Growth of DNA Tile Arrays within Prescribed DNA Origami Frames and Their Dynamics

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Experimental Materials and Methods

Materials: All DNA helper strands used in the origami frame were purchased in 96-well plates from Integrated DNA Technologies, Inc. (www.IDTDNA.com), desalted, with concentrations normalized to 200 µM. Single stranded M13mp18 viral DNA and phi X 174 DNA were purchased from New England Biolabs, Inc. (NEB, catalog number: N4040S and N3023S). All DNA strands in the DNA origami frame were used without further purification.

All DNA strands used in the DX tiles were purchased from Integrated DNA Technologies, Inc. (www.IDTDNA.com) in the format of desalted dry powder. The tile strands were all purified using denaturing polyacrylamide gel electrophoresis (10% 19:1 acrylamide/bisacrylamide, containing 50% urea) in 1xTBE buffer (pH 8.0, 89 mM tris base, 89 mM boric acid, 2 mM EDTA). The bands corresponding to the full length strands were individually excised from the gel, chopped into small pieces, soaked in 500 µL elution buffer (500 mM NH₄OAc, 10 mM Mg(OAc)₂, and 2 mM EDTA) and then shaken overnight to allow the DNA strands to elute from the gel blocks into the solution. After filtering out the gel blocks, the solutions were then mixed with butanol to extract any organic residue. After removing the butanol layer, 1 mL of ethanol was mixed with each solution to precipitate the DNA molecules. The mixtures were kept at -20 °C to ensure rapid and complete DNA precipitation. Then the purified DNA strands were spun down using a centrifuge, and then dried under vacuum. The DNA strands were then reconstituted in pure water and their concentrations were measured by absorbance at 260 nm.

Assembly Procedure: The DNA origami frame structure was assembled by mixing M13mp18 DNA (10 nM) and phi X 174 DNA (10 nM) with the helper strands in a 1:1:30 molar ratio in 1xTAE/Mg²⁺ buffer (pH 8.0, 20 mM Tris base, 20 mM acetic acid, 2 mM EDTA, 12.5 mM Mg(OAc)₂). The final volume of the reaction was 100 µL. The solution was annealed in a PCR thermocycler with the temperature decreased from 90 °C to 70 °C at a rate of 1 °C every 5 minutes, from 70 °C to 40 °C at a rate of 1 °C every 15 minutes, then from 40 °C to 25 °C at a rate of 1 °C every 10 minutes, and finally kept at 4 °C. Following annealing, the origami frame was washed with 1xTAE/Mg²⁺ buffer three times and passed through a 100 kD MWCO Microcon centrifugal filter device (Amicon, catalog number: UFC510096) to remove the excess helper strands.

Each DNA DX tile was assembled by mixing all the strands in the tile in an equal molar ratio (1 mM) in 100 µL 1xTAE/Mg²⁺ buffer. The solution was annealed in a PCR thermocycler with the temperature decreased from 90 °C to 25 °C at a rate of 4 °C every 5 minutes, and then kept at 25 °C.

The DNA origami frame – DX tile 2D array hybrid was assembled by mixing 1 pmol of purified DNA origami frame (100 µL, 10 nM) with the solutions of the four DX tiles. The amount of each tile was 100 pmol (100 µL, 1 mM). The final 500 µL solution was incubated at 25 °C.
overnight. Then the mixture was concentrated to 100 µL using a 100 kD MWCO Amicon centrifugal filter device.

**Agarose Gel Electrophoresis Purification:** The assembled frame-array hybrid was loaded onto an agarose gel (0.3% agarose containing 0.5 µg/mL ethidium bromide, 1×TAE/Mg\(^{2+}\) buffer) and subjected to gel electrophoresis at 80 volts for one hour on an ice-water bath. The product band was excised from the gel and shredded. The shredded gel blocks were transferred into a Freeze 'N Squeeze DNA Gel Extraction Spin Column (Bio-Rad, catalog number: 732-6165) and centrifuged to recover the buffer containing the purified product. The product was then stored at 4 °C and characterized by AFM.

**Monomeric Avidin Resin Purification:** 100 µL Monomeric Avidin Resin (Thermo Scientific, catalog number: 53146) suspension was transferred into a SigmaPrep™ spin column (Sigma, catalog number: SC1000). The resin was washed with 1×PBS buffer once (Sigma, catalog number: P4417), then washed with 2 mM biotin solution to block the non-reversible binding sites, and finally regenerated with glycine solution. The resin and biotin modified DNA origami frame – 2D array hybrid were mixed and incubated for 30 minutes. The resin bound with the frame-array hybrid was then washed with 1×PBS buffer to remove the free 2D array and DX tiles. The purified frame-array hybrid was then displaced from the resin with 100 µL biotin (2 mM) solution. The solution containing the purified product was then stored at 4 °C and subjected to AFM characterization.

**AFM Imaging:** The AFM imaging was performed using a Dimension FastScan AFM (Bruker). The samples (2 µL to 5 µL) were deposited onto freshly cleaved mica (Ted Pella, Inc.) and left to adsorb for 2 min. Buffer (1×TAE/Mg\(^{2+}\), 100 µL) was added on top of the sample and the sample was imaged in ScanAsyst in Fluid mode, using ScanAssyst Fluid+ probes (Bruker).

**Fluorescence Kinetics:** The fluorescence kinetics experiments were performed using a Nanolog fluorometer (Horiba Jobin Yvon). The origami frame was purified with 100 kD MWCO Microcon centrifugal filter devices (Amicon, catalog number: UFC510096) to remove excess helper strands. The concentration of the origami stock solution was 10 nM. The concentration of each tile stock solution was 1 µM. The sample chamber of the fluorometer was preset at 21 °C. 2.4 µL of Tile C solution (labeled with Fluorescein), and 2.4 µL of Tile D solution were added to a 120 µL quartz fluorescence cuvette. 1×TAE/Mg\(^{2+}\) buffer was added to make the final volume 120 µL. To the reaction with tile/origami at a molar ratio of 100:1, 2.4 µL the purified origami solution was added. To the reaction with tile/origami at a molar ratio of 100:2 or 100:3, the volume of the origami stock solution added was doubled or tripled. The sample was placed in the fluorometer and the time dependence of the intensity was monitored. Then 2.4 µL of Tile A solution (labeled with a black quencher) and 2.4 µL of Tile B solution were added to the cuvette and mixed well. The fluorescence intensity was measured once every 30 seconds, with an integration time of 10 seconds. The fluorescence intensities were first corrected for the volume difference, to a total volume of 124.8 µL after the addition of Tile A and B and then the data
were corrected for photo bleaching using a control with the same concentration of Tile C and Tile A.

**Fluorescence Data:**

For each reaction, the first trace is the original data collected by the fluorometer. The second trace is the data after correcting for the volume change. The third trace is the data after correcting for photo bleaching. The fourth trace is the data after normalization, which was used to generate the plots shown in Figure 4C and Figure S11B.

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**Design of the DX Tiles**

**Figure S1.** The design of the four DX tiles. (A) Schematic design of the four tiles. The four tiles share the same sequences of Strands 2, 3, and 5. Each tile has a specific Strand 1 and 4. The sticky end pairing e.g. a, a’ are marked for each tile. (B) The detailed design of the four tiles. Each tile is four helical turns long. Strand 3 is 42 nts long. Strands 2 and 5 are both 37 nts long. Strands 1 and 4 are both 26 nts long.
PAGE Characterization of DX Tiles

**Figure S2.** Native polyacrylamide gel electrophoresis characterization of the formation of the four tiles. **Lanes 1 & 15:** 10 bp DNA marker. **Lane 2:** the core structure of the four tiles: Strand A2 + Strand A3 + Strand A5. (For Tile B, C, and D, the core structures all have the same sequences as Tile A). **Lane 3:** core + Strand A1. **Lane 4:** core + Strand A4. **Lane 5:** full Tile A (core + Strand A1 + Strand A4). **Lane 6-8:** the same combinations as Lanes 3-5 for Tile B. **Lane 9-11:** the same combinations as Lanes 3-5 for Tile C. **Lane 12-14:** the same combinations as Lanes 3-5 for Tile D.
**Design of the DNA Origami Frame**

**Figure S3.** Detailed design of the DNA origami frame. The origami frame is 210 nm wide, 60 nm and 95 nm tall (the two sides). The blue strand represents the phi X 174 scaffold and the red strand corresponds to the M13mp18 scaffold. The interior is decorated with sticky ends complementary to the sticky ends on Tiles A and B. At the outer ends of each helix, two extra thymine bases are added to prevent π-π stacking between origami.
**AFM Image of Empty Origami Frame**

**Figure S4.** AFM image of the empty origami frame. (A) Zoom-out AFM image of the empty origami frame. Most of the origami frames are well formed. There are several aggregated structures in the image that may be caused by crosslinking of multiple scaffold strands. (B) Zoom-in AFM image of selected well-formed empty origami frame. The scale bar is 100 nm.
Examination of the spontaneous formation of the DX tile arrays

Figure S5. Unregulated growth of 2D arrays of DX tiles. The four DX tiles were mixed together to a final concentration of 250 nM each. The mixture was incubated at 25 °C overnight and characterized by AFM. The four tiles form 2D arrays as designed.
Figure S6. Image of agarose gel electrophoresis showing the purification of the origami-2D array hybrid. **Lane 1**: 1kb DNA ladder. **Lane 2**: Empty origami frame without purification. The fastest intense band corresponds to the extra helper strands. The second fastest band corresponds to the empty origami frame. Upper faint bands are aggregated structures (see Figure S4). **Lane 3**: Origami frame and the four tiles incubated overnight at r.t. The faster band and the smear after it correspond to uncontrolled 2D tile-array of various sizes. The slower band corresponds to the origami-array hybrid, which runs faster than the empty origami frame in Lane 2, because once the frame is fully filled, the structure gets more solid. **Lane 4**: The four tiles incubated overnight at r.t. without the origami frame. The band and smear correspond to uncontrolled 2D tile-array of various sizes.
AFM Image of DNA Origami Frame – 2D Array Hybrid Purified by Agarose Gel Electrophoresis

Figure S7. AFM image of Frame-array hybrid purified by agarose gel electrophoresis. (A) Zoom-out AFM image of Frame-array hybrid purified by agarose gel electrophoresis. There were quite a few pieces of free 2D array of DX tiles that were not cleanly removed. Note that these 2D arrays had similar sizes as the frame-array hybrid, which mostly showed a filled interior. (B) Zoom-in AFM image of selected Frame-array hybrid purified by agarose gel electrophoresis. The scale bar is 100 nm.
Boitin Modified DNA Origami Frame – 2D Array Hybrid Purified with Monomeric Avidin Resin

Figure S8. AFM images of Boitin modified frame-array hybrid after purification with monomeric avidin resin. The origami frame was modified with biotin. When purifying with monomeric avidin resin, unmodified tiles and 2D arrays were washed away while the boitin modified frame-array hybrids were bound to the resin. The purified product was then washed off with excess biotin solution. (A) & (B) The AFM images show that using this purification method, fewer free 2D array residues remained. (C) Zoom-in AFM image of selected Frame-array hybrid purified with monomeric avidin resin. The scale bar is 100 nm.
**Figure S9.** AFM image of unpurified frame-array hybrid. Several, but not all of, distinguishable frame-array hybrid structures are marked in the image.
Defects of DNA Origami Frame – 2D Array Hybrid

**Figure S10.** Three major classes of defects in the frame-array hybrids. (A) The shrunken frame-array hybrid caused by sticky ends on tiles hybridizing with another row of non-neighboring tiles. (B) The widened frame-array hybrid caused by inserting one or two rows of tiles between neighboring rows. (C) The bent frame-array hybrid caused by association of sticky ends between non-neighboring columns of tiles. Each image in the figure is 610 nm × 610 nm.
Figure S11. FS-AFM images showing the dynamics of nucleation and growth of DX tiles into the DNA origami frame. (A) This is another example of the experiment shown in Figure 3. Each frame was collected over 87 seconds. Each frame is 287 nm × 287 nm. (B) The full set of images in Figure 3. Each frame was collected over 87 seconds. The scale bar is 100 nm.
Kinetics of the Nucleation Process of the Four Tiles

Figure S12. Characterization of the kinetics of the nucleation process. (A) The modification of the tiles with a fluorophore and dark quencher. The 5’ end of Strand A1 was modified with an Iowa Black Dark Quencher. The 3’ end of Strand C2 was modified with 6-FAM. Upon sticky end association in the tile array formation, the fluorophore and the quencher are brought into close proximity and fluorescence quenching is expected. (B) Normalized fluorescence decrease. The concentration of each of the tiles was 20 nM in all experiments. The legend indicates the molar ratio between the tiles and the origami frame. Each experiment was conducted in duplicate, the data of which coincided with each other. All curves shown are after correction for photo-bleaching. (C) Logarithm of the data in Panel B to the base e. The average of the curves of the reactions without origami seed in Panel B are subtracted from all other curves. Then ln(I/I_{ini}) is plotted against time. The data are then fit by Equation 5 in the main text.
DNA Sequences

Sequences of tile strands:

A1: AGGAACCATGAACCCCTGCAGCATGTC
A2: GCTGCAGGCGGAATCCGACCCTGTCCGTTGCACCATT
A3: GTGGGATTTCCGCTGGCTTGGCTTAGGTCACCACGCCACAGG
A4: ACTCAATGGTGACTAAACCTCTCTAAG
A5: AGGTTTAGTGACTCTTAGGCAAGCCAGTTTCATGG

B1: GTGATCCATGAACCCCTGCAGCAAG
B2=A2
B3=A3
B4: TAACGATGGTGACTAAACCTAAGCT
B5=A5

C1: TGAGTCCATGAACCCCTGCAGCAGCTT
C2=A2
C3=A3
C4: TTCCTATGGTGACTAAACCTGTTCT
C5=A5

D1: CGTTACCATGAACCCCTGCAGCAGT
D2=A2
D3=A3
D4: ATCACATGGTGACTAAACCTGACAT
D5=A5
Sequences of the helper strands and sticky end strands in the DNA origami frame:

**Helper 1**
GTATTAACCTCATTGCCTGAGTACGCCGTTGAGCAATACATTCTTTGATTTT

**Helper 2**
AGAGTCTGTCATCACGCAGGCACGGATGTTGAGCTCTTCTCTCTCC

**Helper 3**
CAGCAGAAGGCCCTGCTGGTATACGAGTGCTAA

**Helper 4**
AAACCGTCTACATTGAGGCCCTACCCAGGA

**Helper 5**
ACATCAGCCTCGCCAGCATGGCAAAAGGGCGAA

**Helper 6**
AAAGAACGTGGGACTCCAAGGTCAACAGGAAAA

**Helper 7**
TAGCTTTTGAAATACCTATTTTCTCATATT

**Helper 8**
TTGTTCCAGTTTGGAACAGAGGTGAGCTCT

**Helper 9**
CGTCGACCTGAAATGCAAATGTATTGAGT

**Helper 10**
ATCAAAGAATAGCCGAGATAGGCAATTGCA

**Helper 11**
TAGAACACAGTCACGACCGAAGTATCATTAA

**Helper 12**
CCTGTCTGATTGCTTCCGAAATCGGAAATATCAAAAGGGAAATTTTT

**Helper 13**
GTCACCCCGCCCCTAAAATCTCAGCAGAAAAT

**Helper 14**
TCCAGCCTGTATGTGGCAGAGCAGCCTCAAA

**Helper 15**
CCTAGCAGCAGAATGCAATAGGAGCGGAGCGAG

**Helper 16**
CCGCCTGCGCTGAGAGGTTGCAAATCGCGA

**Helper 17**
CGAGAATACTGATGCAATTAAAGGGCCCTTCA

**Helper 18**
AGTGAAGACGGGCAACAGCTGATGGCTACAT

**Helper 19**
CAGCTATTACCTGAGTATTTCCATTTTTCC

**Helper 20**
GGCTTTTGCCGTATGCTGGCAGTTTTGTTTGCAGAAACTGAAACGGACT

**Helper 21**
TAAAACGAGTCTATCTGGTCTTTGGGAGAG

**Helper 22**
GCATTAATGAACCGCGTGACCGGGCAATA

**Helper 23**
TTAGTTAATACCCAGAACTCAGGACATT

**Helper 24**
AAACCTCTGATAAAAACAGAGGTGAGAAAATGAAA

**Helper 25**
CAATTTAACTATAAAATATCCGAGCTCAAA

**Helper 26**
ACGCTCATAATGGCGCAGACGATGACTTGA

**Helper 27**
CAATGTGAGAAATAGTGGGAAATGTTGCTG

**Helper 28**
GATTCCGCTTTGAGTTCCGAAATATGAGGATT

**Helper 29**
TACCGCTTCTCCGGCGAATAATTTTCTGCAAAACTGAAACGGACT

**Helper 30**
TCTTTATGAAATACCTACCAGGCTTCCAC

**Helper 31**
GTGGTGCGAAAAAGTCTGAAACATGAACGTGAT

**Helper 32**
TAAATTTTCAAGAAAAAGTCTTGATCTCATT

**Helper 33**
GGAAACACGTGCGCAGAAGCTGAGTCAAGAATGCAATGAAAGAAAAACAC

**Helper 34**
AGTACGCTGACGATGACGCTTATACATCAAA

**Helper 35**
AAGATAGTGCTGAGGCCAGGCAGCGGTCG

**Helper 36**
CGAATTTGACATCACTTCTGCAAGGAACCCAC

**Helper 37**
TTGATACCCCTCAATATCTTGGCCTAAAA

**Helper 38**
GGGAGGAAAAACATTTGGAAGGAAATATTAGGCAT

**Helper 39**
GTTAACCTATTGACGTTTAAATATACATTCTTAGGAGCTAAGAAATA

**Helper 40**
GAAAATGTGCTAGGCGCCTGCAATGAAACAGAG

**Helper 41**
ACCTCACATTAGACTTTTTAAGAATATCCTGGCTTG

**Helper 42**
TGCGAAATAAAAAGTTTGTAGTTAAATGATTAG

**Helper 43**
GCCAGAGTGCTATCAAGGACCGGAATTCATACAAAAAGAAAAACACCAGAGTGAAC

**Helper 44**
ATAGCCAGGCATTAACCGTCAACGGTGTCTG

**Helper 45**
TTACAGTGCCAGAAACACATT
Helper 46  AATCTAAATCATTTCAATTACCTGTTAAGTGG
Helper 47  TATCAAACCAAGTTAAATATCGGACCTGA
Helper 48  GCAAATCACAATAACGGGATTCCCCATTAGTACG
Helper 49  GTAATCTAACAGTAACAGTACCTACCAACA
Helper 50  CTAATAGAGTAGATTTCAGGTTTTGGAAAGGACGTCAAATAGTCCGGAACAGC
Helper 51  TAAATTTACCATATAATTCCTGGAAGAAAG
Helper 52  TTAAGGGAACACAAACAGGCAATGATAG
Helper 53  TTTCGGAATCATCATCTTTGATTAAATTTA
Helper 54  CATTACCAGGCCGTGAGATGTACTCATTCTGAAGCACCACACAGAAACCTAGAGGAC
Helper 55  TATAACCTGTGTTGTCGTTCCGACGCATGA
Helper 56  CATTGAAAAATTAATACATTTGACAAAAAG
Helper 57  AGCAACAAAAATAATCTCTTAAATCGCAGAG
Helper 58  GTGTAACCTAGCAAAACCAACATGAAATTTACATC
Helper 59  ATGGCCGACCATTCAAAAGGATAAAACGGGTTAGA
Helper 60  CTCAAAGCGAACCACACAGGCAAATACAGTGA
Helper 61  TTTCAAGAAAAACATACCTTTTTTT
Helper 62  CTGGAAGACACATAAATCACCCTACTATGTGAG
Helper 63  TTCCGACGCAGAAGCAATACCCGGCTCCAA
Helper 64  AGATGGCGTTAGGAGTCCGGAGGGTGAGTCGGGATCGGAGG
Helper 65  CAAGTTAAAGAGCGTTGTCGAGCTAAACTGG
Helper 66  TAGCCATAAGTCAATAATACAAATGCAATTT
Helper 67  TAAATTAATCTTGCTTCTGAATATTACGAGAAT
Helper 68  TTTCATGGAACACGCTTTAGATTATTT
Helper 69  TGAATAACCTTTCCCTTGAATCTCATAATTACAG
Helper 70  AACAATTTGGCGGCTTTTTGACCTATCGGTA
Helper 71  AATCATAGAAAGATGCCATAGTGAATGAAACA
Helper 72  ATTAGAGCATGCCTACAGTATTGTGCTAT
Helper 73  TTAGACGCCTGAGGTCTGAGGCTTATCATC
Helper 74  CATCACCCTTGAATGGCGAGATTTGGTTAT
Helper 75  AGCAAGCGCCGGCTCATCGAGGACCAGCT
Helper 76  AACAAATATAACCCGGCTTTGATTATTTA
Helper 77  TCGCAAGAATGTAAATGCTGATGCTTAGGAAC
Helper 78  TTTCACCTTTTTTTATGTAATTATTT
Helper 79  ATAACTATCAAAGAACGCGAGAATGTTGCCAC
Helper 80  TTAGCCATTCAAGAAGTGCTTTTTATCAAG
Helper 81  ACCGACCGGACCTAATTAATGGACCTTTTT
Helper 82  ATCCCTTACCAAAATCAAGCACTAAATCCAA
Helper 83  TTTTCATCTTCTTTGTGATAAAATT
Helper 84  CAAGTCCATTTTATCAGTGCATCTCTTTCT
Helper 85  AACGCCCAGGCGAGAAGTAAATGACACC
Helper 86  CGCTCAAATAAAGAATAAAACCCGTTTTGAAAT
Helper 87  CGTTATACAAAAAGCCTGTTTAGTTCAGGACT
Helper 88  TTAAGGCGTTAAAGTACTGCCTTTTT
Helper 89  ATTACTAGAAATCTCTTACAGTATCTCTTTCT
Helper 90  GCACGCTCAGCGAGAAATCGCTCTTTT
Helper 91  GTAATTTCGCGCATTTTAACAAAACAAAGGCCA
Helper 92  AGTCTCATAGTGGCATTTTAGTAAATCATATG
Helper 93  TTAATTGAGAATGCCAGAGGCATT
Helper 94  GATTGCTCTTTTCATCTCGAGAATTGCAATAGCAG
Helper 95  TTGATTCTTGAAATGCCAGACAGAG
Helper 96  ACTGAACAGTAATGAGAATAGGCAAGC
Helper 97  AAAACAGGTCATAGTTCTTCAGCTCGTGCTGAAG
Helper 98  AAATAGCAGAAGATGATTCTTTCAGCTCGTGCTGAAG
Helper 99  ATATACCTGCTTTTCTGATTCAGATCGA
Helper 100  AGAAACAGGTTATTTATCAACAAATAGTATTTGCTGC
Helper 101  AACAGCCAAAAATAATATCCATAGACCTCGCAGATGCT
Helper 102  CCGATCTGAAATCAGGAACGAGCACTGCTCAAATT
Helper 103  AATCTCGGAAAACCTGCTTTGTCAGAATCGGCTCTTCCCTT
Helper 104  GCTCAAATGGAACGAGCATTTATTGATCAGCAG
Helper 105  TTTGCTCACTCTACGGGTTTACGCTCAGTACGAGAC
Helper 106  TTGATTTGGTCTTGGAATAATACCGTTTTT
Helper 107  AACCTCCCAGGAATCAGATCTAGCTCGTCATTT
Helper 108  CGGTATCTCAGAGGTTTACGCTCAGTACGAGAC
Helper 109  GCATCTGCTTTTTCATGCTAGCTCGTCAGTTTT
Helper 110  AACATACAAACCTGAGCTTTTCCGAAATATAG
Helper 111  AGAAATATCCTTTTCATGCTCAGTACGAGAC
Helper 112  TTTTTTCGAGCCCTGTAACAAAT
Helper 113  GACAAGAGAGAGGCGATAGACGGTCAGAGAG
Helper 114  CGACAATAGCCTTTTACAGATCAGAAGACAAATA
Helper 115  ACAGCAGCTTGTGTTGTCAGATCAGAAGACAAATA
Helper 116  TGAACAAATGTTATTATTATATCCCAAAAAAGTA
Helper 117  ACAGCAGCTGCTTTTTGCAAGAGGAGGAG
Helper 118  ATCATTTCCCCATTCAATCTTAACTCAGTACGTACCT
Helper 119  ATTTTCATGACTTGCGGGAGGTTTACTCAGTACG
Helper 120  ATAGCAATAGAAGAGCAGACGTCATCTGCTCAAG
Helper 121  GAGCCAATATTGGAGGTTGTCAATCTGAGCTCAGTACGAGATCAGCAGAAGCA
Helper 122  GAAATGTGCTTCACAGAATTGGGATGGCACAAG
Helper 123  TCAACCCGATAATTTGAGCCTAAAAATAGACAAATAGAAGACAAATA
Helper 124  GTTTACCCAAGAATTGGTATAAGATAACAAACA
Helper 125  ATTTTGTCAGAGAAACATGGAATTTAAATAGGAATA
Helper 126  AAAGAAACCCAGCCCTTTTTAAGTCCAATAA
Helper 127  GAAAATACGCCGAAACAAAGTTACCAAAAATA
Helper 128  ACTCTTAAAAACGCAATATAAACGCGAGGCTC
Helper 129  CCATTACACGTCAGAGCAGCCCTTTATGCAACCCA
Helper 130  GGGAGCAGCATATCACCATTATCAGATGAAAGCCT
Helper 131  GGTGTGCTCAGAAAAAGGAGAATCTTTTACG
Helper 132  TCCTAAAAATGCAGTTATCATCTGCTGCTTATC
Helper 133  GCAGCCAGTGAGAAGAGTAGAGAAAGGCAATGAAA
Helper 134  TTAGTCAGAGGGTTGAGGAGAGTT
Helper 135  ATAAACCCGCGCCAAAGACAAAAAGCAATTAAG
Helper 136  ATAGAGCACAATCAAATAGAAGAAAGGACCAT
Helper 137  TATCTTACGGAACAGCCAGGAAACCATGAG
Helper 138  AGCAGATAATACAAAGGTGGCAAAACGTC
Helper 139  ACCGGAGTATTACGAGTAGTTAGTCAAGCTTAAAT
Helper 140  TCACGAAACTTTCATCAGATGAAAGAATCTCAGGACTTTAGCGTCAGACTGTA
| Helper  | Sequence |
|---------|----------|
| 189     | CTGGTGCCAGGCTGCAGCAACTGTTATAGCTGT |
| 190     | CCCGGGTACCCAGGCTGCAATTCGTGAATCGTGCAGGCGAGCAGAGATCGTGT |
| 191     | CCTCAGGATCGCTATTACGCAGACAGGATC |
| 192     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 193     | CGGCCAGTTGCTCAGGAGGGAAGGCTTGACAGAGATCGTGT |
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| 196     | CCTCAGGATCGCTATTACGCAGACAGGATC |
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| 199     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 200     | GATGGTACAGGCTGCAATTCGTGAATCGTGCAGGCGAGCAGAGATCGTGT |
| 201     | CTGGTGCCAGGCTGCAGCAACTGTTATAGCTGT |
| 202     | CCCGGGTACCCAGGCTGCAATTCGTGAATCGTGCAGGCGAGCAGAGATCGTGT |
| 203     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 204     | CTGGTGCCAGGCTGCAGCAACTGTTATAGCTGT |
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| 207     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 208     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 209     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
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| 215     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 216     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 217     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
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| 219     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 220     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 221     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 222     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 223     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 224     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 225     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 226     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 227     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 228     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 229     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 230     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 231     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 232     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 233     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 234     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |
| 235     | TTGGCAATGGTTGGAGATGTCAGGCTAGAAGGCGAGGAAAGGCAAAGGCAGATCGTGT |

S21
| Helper 236 | GAATTAGCTAATCATACAGGCACATCAAT |
| Helper 237 | TTAAAACTCCCAAAAAATTAACGCTT |
| Helper 238 | TCTACAGTTGAGGGACATAAAAAGATGAACTT |
| Helper 239 | ATGGTCAGCAGCAGCTGGGAGATGTCAGGGAAG |
| Helper 240 | TTGGGGCGTAACCTGTTTAGCTATACGGAGAG |
| Helper 241 | TCTACTAATGACCATTAGATACAAGTTGATC |
| Helper 242 | TTTAGATTTTAGTTCAGTAGTACGTTT |
| Helper 243 | ACAGCAAGAGACCAGAATGAGGAAAGTAAATATG |
| Helper 244 | GCGATAACCGCTCATCTCGAAGGTTCGCAG |
| Helper 245 | CGCACAAGCCGGGATGATTGAAAATGTAATTGCT |
| Helper 246 | TCCCAATTTCATCTCATATAACGCTTTAA |
| Helper 247 | TTGGTCTGGAAGTCTGCGAAGCAGCGTT |
| Helper 248 | CGCTCGGAGATGAGAAGCTATGTAATAAG |
| Helper 249 | TCTACTAATGACCATTAGATACAAGTTGATC |
| Helper 250 | ATATGCAAAATTGCTCCTTTTGAAGCAAC |
| Helper 251 | TTGGGGCGTAACCTGTTTAGCTATACGGAGAG |
| Helper 252 | TCTACTAATGACCATTAGATACAAGTTGATC |
| Helper 253 | TTGGTCTGGAAGTCTGCGAAGCAGCGTT |
| Helper 254 | ACATACCAGCTGCTAAATGTTTAAGAGGT |
| Helper 255 | GAAGCCCAAGAGCCGCAACGTTCATGTAATAAG |
| Helper 256 | TCCCAATTTCATCTCATATAACGCTTTAA |
| Helper 257 | TTGGGGCGTAACCTGTTTAGCTATACGGAGAG |
| Helper 258 | ACATACCAGCTGCTAAATGTTTAAGAGGT |
| Helper 259 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 260 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 261 | ACTTTTTCTGCTGCTATGTCAGGAGCC |
| Helper 262 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 263 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 264 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 265 | ACTTTTTCTGCTGCTATGTCAGGAGCC |
| Helper 266 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 267 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 268 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 269 | ACTTTTTCTGCTGCTATGTCAGGAGCC |
| Helper 270 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 271 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 272 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 273 | ACTTTTTCTGCTGCTATGTCAGGAGCC |
| Helper 274 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 275 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 276 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 277 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 278 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 279 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 280 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
| Helper 281 | TGTAAAATGGAAAGGACGAGGAAAGCTTCCTTTA |
| Helper 282 | CAAAAAGAAGTCAGAAGCAAGCTGAGAG |
| Helper 283 | GCCTCTTCAAGGGGAGATGAGAAGCTATGTAATAAG |
Helper 284     AGAATCTCTACCATGAACAAAATGATGGCG
Helper 285     GCAAGGATCAAAGTAAGAGCTTCTTCAACAAG
Helper 286     CTCAGAGCATAGGAACCCATGTACGGAAGTAG
Helper 287     CTTTAAGCCCAACAGCCATATAAGTTCCAT
Helper 288     CAGTTTTTACTTTTGTTAAGCATAGCAAGGC
Helper 289     AAAAGTCGAGGTGGAATTTTTCTCGGTAAAC
Helper 290     TAAGGGAAACCGAACAAGATAATTTCCTGACT
Helper 291     GTGAGCATCTGCAACGGATCTTTCACGTAACAC
Helper 292     GGATTAAGTGGTTTTTAGTGAGTTAGGGATAG
Helper 293     GGCGTCGCTCCTAGACCTTTAGCATTTAGCCA
Helper 294     TTTTTGCGCCATTTTCGATTTAATTATTTTCCG
Helper 295     GTAACCTTTGTAATTCTGCTTTTATCGAGCTGC
Helper 296     CGACAGCTCATCCTGGACAGATTTCTTAAAA
Helper 297     TCTTTAAGCTCGTAGAACCAGTTGACAATG
Helper 298     CATATCTGTTCTGCTTCAATATCTCGATATA
Helper 299     AAGCAGTATCCCCACCTCCTAATCTGTTAAAG
Helper 300     CATTAAAGGATATCACAACAAAAAGCATAGAC
Helper 301     TATCAGCTTGCTTTCCAGGATATTGTGCTATT
Helper 302     CAGCCTGATTACCGATAGTTGCGCCGGTAAGTT
Helper 303     ACAACAACCATCAGCATAGCTTTGGAAC
Helper 304     TCCGCTCGTGAAGGTAATTCTCGGTAAAC
Helper 305     GCCGCTTTTTCGCGGATCGTCACCCGGCTACA
Helper 306     ATCAGGAACGAGGTAGCAACGGCTACTTCTGC
Helper 307     CATTAAAGGATATCACAACAAAAAGCATAGAC
Helper 308     TCCGGACATCATAAAGCCTCAAATATC
Helper 309     GCAGTCGGGCAAGAACATACGACTAAATCCT
Helper 310     ACAGAAATTCAGGCACAAAAAAGCATG
Helper 311     TATTATCTAGCCCTGCCCTATTTACTGATA
Helper 312     ACCATAAAGGATATTCCAAACAAAGCATG
Helper 313     AAATGAAGGCGCCGATAAAAGTGCAACGAACAA
Helper 314     ATTAGGTCGAACCTGACGAGTTGTATACGTGA
Helper 315     CTCCTACAGTCACCAGTGAAAAACCGATG
Helper 316     CCCCCAGATGTCACCAGTGAAACCGATG
Helper 317     GTTTCCAGCGATTTTGCTAAAACACTACAACGC
Helper 318     ATCTCTGATTTCATCCCCAGGTTATCTCGGT
Helper 319     AGCGTACCTTGAAATGAGAAGCTCAGTAATT
Helper 320     GAGCAGGAAGCGAAGCGAAATAAATAGCGAAG
Helper 321     ATAGCCAAAGCAGGATCATCCTCAAGAAAAGAT
Helper 322     ACACATATCTACAGGCAAGCAGATCTCAACATT
Helper 323     TTGGCCAAAGGAATAAACCCTCGGT
Helper 324     TACTGACGCACGAGACGACGAGAAAGATCC
Helper 325     TAACGGAATGAGAATTAGGAATACCTCAACAG
Helper 326     TCATCGATCAATATTACAGGTTCGGTTAA
Helper 327     AACTAATGAAATACTAGGTAAAATTTAAGCGT
Helper 328     TGTTGGGAAAGAAGCAGATACATAATT
Helper 329     TGTTCCAGATAAAATCCGAAATCATCCTCGGTACAG
Helper 330     GAGCTCAGACCTATTAGTGTGGAGTACGGAT
Helper 331     ATCCCAAAAGCAGTGCTGAAACAAACACCA
Sticky End Right 13  CGTTACACTTTCTACTGTCAGAGCTCTCATCGCCGACTTAG
Sticky End Right 14
TGAGTATAGACGCATGATTTTATATAGTAAATCCAGCTCCTTTAAAAATGCTGACCAA

Sticky End Up 1  TAAACGTTATTGCCCGGCGCCAGGTCCAGCTT
Sticky End Up 2  TTCCTCCGAAGAGCTACACAGTCCTTGACGAAATAA
Sticky End Up 3  AACTCGTATTCTGAATAATGGAAATCATGGAGCTGGCTTAG
Sticky End Up 4  ATACCCCGTGCCGAACCATTGGTTGATTATAATCTTTTGCGCGATTAAACT
Sticky End Up 5  GGGTGCGCATCAAAGCAATCGGCCGCAGCTT
Sticky End Up 6  TTCCTCGAGCAGCCTGATTAGCATGCCAGAGATTAATGCAACATC
Sticky End Up 7  TCAGGAACGTGGAACGACCAGCAATAAAGCTCTTCTTCTTAG
Sticky End Up 8  ATCACTGCTACAGGAAATGATTTTATAGTCTAAAGAAACCCGCGACAAAGGTACT
Sticky End Up 9  GTCAATATGCAAAATTAGCAACAGTGAGCTTT
Sticky End Up 10  TTCCTAGAGCTCCATGTCAATAGATGTGGGAGCAAAC

Sticky End Down 1  TGAGTGCAGCAGCGCCCTTTCTGTTGATAAAGCAAGCATACTCATAAGTCC
Sticky End Down 2  TTTCAGAGTAGAAACCAATCATAAGTGTAAAACTTACATAAATAGCCGCGTCTT
Sticky End Down 3  CGTTAAGTAAAGGTGCATTCCAAGTACCGCACTCGATTAGTTGCTATTTTGCGCGT
Sticky End Down 4  TAAATCAAATCGAGAACAAGCAAGCTGCAGGGAAATGCACAT
Sticky End Down 5  TGAGTGGCGGGCTAGTCAACCTCAGCACTAACCTTGCGAGCGCCCA
Sticky End Down 6  AAGAGCCATACCGCTGATCAAGAACTGTTCT
Sticky End Down 7  CGTTACGTGTTGGCAGTGAGCTTTATACCCAGAAGGGTGATTAGTGAGCATAC
Sticky End Down 8  CGTATTACGTGCGAGCATATATGGCTCAGGCTTTATAGTCAACCTTGAGT
Sticky End Down 9  CCAGCAGTCAGAATCGTGTAGGCTGACTCATATCTCAAATGGCCAGGGACAT

Sticky End-Scaffold Linker Left 1  CCATACAGCAGCGCC
Sticky End-Scaffold Linker Left 2  CAGTGACTCCGTCAGT
Sticky End-Scaffold Linker Left 3  GCGATACAGGCTACCA
Sticky End-Scaffold Linker Left 4  TGAGCGGCGTTCATGG
Sticky End-Scaffold Linker Left 5  GTGCGTGTGGACCACA
Sticky End-Scaffold Linker Left 6  CTGCGCTGGCTAAGGA
Sticky End-Scaffold Linker Left 7  GTATAAGTGTGTACCT
Sticky End-Scaffold Linker Left 8  TGAGCGGCGTTCATGG
Sticky End-Scaffold Linker Left 9  TTAAAGAGAAGCTTG
Sticky End-Scaffold Linker Left 10  GACTTCCGAGATCTAG
Sticky End-Scaffold Linker Left 11  GAGGAAAGTGCGAAC
Sticky End-Scaffold Linker Left 12  TGAAGCTTACGTTTGT
Sticky End-Scaffold Linker Left 13  TAGGCACATACGCTG

Sticky End-Scaffold Linker Up 1  GGACCTGGAAGATGTTAAGCTTGTCGCAGACTCTTGCGG
Sticky End-Scaffold Linker Up 2  CCAGCTCCCCACGCGATCCTCTCCTCCGCAGCGACTCGG
Sticky End-Scaffold Linker Up 3  GGCGCGACGACACTCCTGGCGTCCTCTAGGCTGCTCG
Sticky End-Scaffold Linker Up 4  AAGAGGCTCGATCAGTGGGTGCTCCTGACTGTAGCAG
Sticky End-Scaffold Linker Up 5  CCATGGTTATAGCGGTCATGAGCAGCAGGTGGAGCCTCT

Sticky End-Scaffold Linker Right 1  GCTCCTGAGTCCAGCA
Sticky End-Scaffold Linker Right 2  CCGGCGATATTGGCGCA
Sticky End-Scaffold Linker Right 3  ACATAAAGCCGCAAG
Sticky End-Scaffold Linker Right 4  GCTTGAACAGTGAT
Sticky End-Scaffold Linker Right 5  TCTCGGCTATAGGCTG
Sticky End-Scaffold Linker Right 6  AGTGCTCAATCCGAA
Sticky End-Scaffold Linker Right 7  GCCATTAGTCTCGGCC
Sticky End-Scaffold Linker Right 8  GGACTACCCGCTGTC
Sticky End-Scaffold Linker Right 9  CGGCCACGCCGCTAG
Sticky End-Scaffold Linker Right 10  TGTTCTGCTGCGCGT
Sticky End-Scaffold Linker Right 11  GCCATAAGCGAGAC
Sticky End-Scaffold Linker Right 12  GAGGACTGGAAGAGTG
Sticky End-Scaffold Linker Right 13  TCGGCGATGCGCTAT

Sticky End-Scaffold Linker Down 1  GCCCAAATTATCTCAGATCCGAGCAGGCAGGCGGCTGCGC
Sticky End-Scaffold Linker Down 2  GCATTCCAGATCGAGCGAGTCGTCGAGACCTACT
Sticky End-Scaffold Linker Down 3  AGTTCTTGAACAGAGGATGCTTCATCCTGAGGAGCGCCGC
Sticky End-Scaffold Linker Down 4  CCGGATTCATTTGACCAGTAGCTGACTGAGCCAACAG
Sticky End-Scaffold Linker Down 5  CCTGGCCACCCGACTCTGGTCAGACGCAGTGGAGCT

Helpers modified with biotin:

Biotin Helper 158  CAAAGCCTTTGCAATCCATCAAAACGTCAAGCATTCTTTTTTTTTTTTTTTTTTTTTTTT
Biotin Helper 159  AATTTACCAGGAGGGTGGAGCAGGACCAGAAGGGCAGCATTCTTTTTTTTTTTTTTTTTTTTTTT
Biotin Helper 161  TTGGCCTCTCCAGAATGGAAAGCGCCTTGAGGACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
Biotin Helper 162  ACTGGTAATTGAGCTTTTTTATAGTACAGTCTCCTGTTTTTTTTTTTTTTTTTTTTTTTTTT
Biotin 20A  [5' biotin]AAAAAAAAAAAAAAAAAAAAAA