# Optical imaging of individual biomolecules in densely packed clusters

Mingjie Dai,    Ralf Jungmann,    Peng Yin

## Supplementary Tables

1

**Supplementary Table S1 │ List of staple sequences for self-assembly of the rectangular DNA origami nanostructure**

All staple strands for self-assembly of the rectangular DNA origami nanostructure, used as a template for assembly of various imaging standard samples. The colours of staples match those in Supplementary Figure S3. Grey: unmodified staple strands. Light orange and crimson: strands with biotin extension for surface fixation, and strands with modified wiring pattern to accommodate those orange ones.

| Strand ID | Sequence | Colour | Notes |
|---|---|---|---|
| 0[47]1[31] | AGAAAGGAACAACTAAAGGAATTCAAAAAAA | | Structure staples |
| 0[79]1[63] | ACAACTTTCAACAGTTTCAGCGGATGTATCGG | | Structure staples |
| 0[111]1[95] | TAAATGAATTTTCTGTATGGGATTAATTTCTT | | Structure staples |
| 0[143]1[127] | TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA | | Structure staples |
| 0[175]0[144] | TCCACAGACAGCCCTCATAGTTAGCGTAACGA | | Structure staples |
| 0[207]1[191] | TCACCAGTACAAACTACAACGCCTAGTACCAG | | Structure staples |
| 0[239]1[223] | AGGAACCCATGTACCGTAACACTTGATATAA | | Structure staples |
| 0[271]1[255] | CCACCCTCATTTTCAGGGATAGCAACCGTACT | | Structure staples |
| 1[32]3[31] | AGGCTCCAGAGGCTTTGAGGACACGGGTAA | | Structure staples |
| 1[96]3[95] | AAACAGCTTTTTGCGGGATCGTCAACACTAAA | | Structure staples |
| 1[160]2[144] | TTAGGATTGGCTGAGACTCCTCAATAACCGAT | | Structure staples |
| 1[224]3[223] | GTATAGCAAACAGTTAATGCCCAATCCTCA | | Structure staples |
| 2[47]0[48] | ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT | | Structure staples |
| 2[79]0[80] | CAGCGAAACTTGCTTTCGAGGTGTTGCTAA | | Structure staples |
| 2[111]0[112] | AAGGCCGCTGATACCGATAGTTGCGACGTTAG | | Structure staples |
| 2[143]1[159] | ATATTCGGAACCATCGCCCACGCAGAGAAGGA | | Structure staples |
| 2[175]0[176] | TATTAAGAAGCGGGGTTTTGCTCGTAGCAT | | Structure staples |
| 2[207]0[208] | TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG | | Structure staples |
| 2[239]0[240] | GCCCGTATCCGGAATAGGTGTATCAGCCCAAT | | Structure staples |
| 2[271]0[272] | GTTTTAACTTAGTACCGCCACCCAGAGCCA | | Structure staples |
| 3[32]5[31] | AATACGTTTGAAAGAGGACAGACTGACCTT | | Structure staples |
| 3[96]5[95] | ACACTCATCCATGTTACTTAGCCGAAAGCTGC | | Structure staples |
| 3[160]4[144] | TTGACAGGCCACCACCAGAGCCGCGATTTGTA | | Structure staples |

| | | | |
|---|---|---|---|
| 3[224]5[223] | TTAAAGCCAGAGCCGCCACCCTCGACAGAA | | Structure staples |
| 4[47]2[48] | GACCAACTAATGCCACTACGAAGGGGGTAGCA | | Structure staples |
| 4[79]2[80] | GCGCAGACAAGAGGCAAAAGAATCCCTCAG | | Structure staples |
| 4[111]2[112] | GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA | | Structure staples |
| 4[143]3[159] | TCATCGCCAACAAAGTACAACGGACGCCAGCA | | Structure staples |
| 4[175]2[176] | CACCAGAAAGGTTGAGGCAGGTCATGAAAG | | Structure staples |
| 4[207]2[208] | CCACCCTCTATTCACAAACAAATACCTGCCTA | | Structure staples |
| 4[239]2[240] | GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT | | Structure staples |
| 4[271]2[272] | AAATCACCTTCCAGTAAGCGTCAGTAATAA | | Structure staples |
| 5[32]7[31] | CATCAAGTAAAACGAACTAACGAGTTGAGA | | Structure staples |
| 5[96]7[95] | TCATTCAGATGCGATTTTAAGAACAGGCATAG | | Structure staples |
| 5[160]6[144] | GCAAGGCCTCACCAGTAGCACCATGGGCTTGA | | Structure staples |
| 5[224]7[223] | TCAAGTTTCATTAAAGGTGAATATAAAAGA | | Structure staples |
| 6[47]4[48] | TACGTTAAAGTAATCTTGACAAGAACCGAACT | | Structure staples |
| 6[79]4[80] | TTATACCACCAAATCAACGTAACGAACGAG | | Structure staples |
| 6[111]4[112] | ATTACCTTTGAATAAGGCTTGCCCAAATCCGC | | Structure staples |
| 6[143]5[159] | GATGGTTTGAACGAGTAGTAAATTTACCATTA | | Structure staples |
| 6[175]4[176] | CAGCAAAAGGAAACGTCACCAATGAGCCGC | | Structure staples |
| 6[207]4[208] | TCACCGACGCACCGTAATCAGTAGCAGAACCG | | Structure staples |
| 6[239]4[240] | GAAATTATTGCCTTTAGCGTCAGACCGGAACC | | Structure staples |
| 6[271]4[272] | ACCGATTGTCGGCATTTTCGGTCATAATCA | | Structure staples |
| 7[32]9[31] | TTTAGGACAAATGCTTTAAACAATCAGGTC | | Structure staples |
| 7[56]9[63] | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG | | Structure staples |
| 7[96]9[95] | TAAGAGCAAATGTTTAGACTGGATAGGAAGCC | | Structure staples |
| 7[120]9[127] | CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA | | Structure staples |
| 7[160]8[144] | TTATTACGAAGAACTGGCATGATTGCGAGAGG | | Structure staples |
| 7[184]9[191] | CGTAGAAAATACATACCGAGGAAACGCAATAAGAAGCGCA | | Structure staples |
| 7[224]9[223] | AACGCAAAGATAGCCGAACAAACCCTGAAC | | Structure staples |
| 7[248]9[255] | GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA | | Structure staples |
| 8[47]6[48] | ATCCCCCTATACCACATTCAACTAGAAAAATC | | Structure staples |

| | | | |
|---|---|---|---|
| 8[79]6[80] | AATACTGCCCAAAAGGAATTACGTGGCTCA | | Structure staples |
| 8[111]6[112] | AATAGTAAACACTATCATAACCCTCATTGTGA | | Structure staples |
| 8[143]7[159] | CTTTTGCAGATAAAAACCAAAATAAAGACTCC | | Structure staples |
| 8[175]6[176] | ATACCCAACAGTATGTTAGCAAATTAGAGC | | Structure staples |
| 8[207]6[208] | AAGGAAACATAAAGGTGGCAACATTATCACCG | | Structure staples |
| 8[239]6[240] | AAGTAAGCAGACACCACGGAATAATATTGACG | | Structure staples |
| 8[271]6[272] | AATAGCTATCAATAGAAAATTCAACATTCA | | Structure staples |
| 9[32]11[31] | TTTACCCCAACATGTTTTAAATTTCCATAT | | Structure staples |
| 9[64]11[63] | CGGATTGCAGAGCTTAATTGCTGAAACGAGTA | | Structure staples |
| 9[96]11[95] | CGAAAGACTTTGATAAGAGGTCATATTTCGCA | | Structure staples |
| 9[128]11[127] | GCTTCAATCAGGATTAGAGAGTTATTTTCA | | Structure staples |
| 9[160]10[144] | AGAGAGAAAAAAATGAAAATAGCAAGCAAACT | | Structure staples |
| 9[192]11[191] | TTAGACGGCCAAATAAGAAACGATAGAAGGCT | | Structure staples |
| 9[224]11[223] | AAAGTCACAAAATAAACAGCCAGCGTTTTA | | Structure staples |
| 9[256]11[255] | GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA | | Structure staples |
| 10[47]8[48] | CTGTAGCTTGACTATTATAGTCAGTTCATTGA | | Structure staples |
| 10[79]8[80] | GATGGCTTATCAAAAAGATTAAGAGCGTCC | | Structure staples |
| 10[111]8[112] | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | | Structure staples |
| 10[143]9[159] | CCAACAGGAGCGAACCAGACCGGAGCCTTTAC | | Structure staples |
| 10[175]8[176] | TTAACGTCTAACATAAAAACAGGTAACGGA | | Structure staples |
| 10[207]8[208] | ATCCCAATGAGAATTAACTGAACAGTTACCAG | | Structure staples |
| 10[239]8[240] | GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA | | Structure staples |
| 10[271]8[272] | ACGCTAACACCCACAAGAATTGAAAATAGC | | Structure staples |
| 11[32]13[31] | AACAGTTTTGTACCAAAAACATTTTATTTC | | Structure staples |
| 11[64]13[63] | GATTTAGTCAATAAAGCCTCAGAGAACCCTCA | | Structure staples |
| 11[96]13[95] | AATGGTCAACAGGCAAGGCAAAGAGTAATGTG | | Structure staples |
| 11[128]13[127] | TTTGGGGATAGTAGTAGCATTAAAAGGCCG | | Structure staples |
| 11[160]12[144] | CCAATAGCTCATCGTAGGAATCATGGCATCAA | | Structure staples |
| 11[192]13[191] | TATCCGGTCTCATCGAGAACAAGCGACAAAAG | | Structure staples |
| 11[224]13[223] | GCGAACCTCCAAGAACGGGTATGACAATAA | | Structure staples |

| | | | |
|---|---|---|---|
| 11[256]13[255] | GCCTTAAACCAATCAATAATCGGCACGCGCCT | | Structure staples |
| 12[47]10[48] | TAAATCGGGATTCCCAATTCTGCGATATAATG | | Structure staples |
| 12[79]10[80] | AAATTAAGTTGACCATTAGATACTTTTGCG | | Structure staples |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | | Structure staples |
| 12[143]11[159] | TTCTACTACGCGAGCTGAAAAGGTTACCGCGC | | Structure staples |
| 12[175]10[176] | TTTTATTTAAGCAAATCAGATATTTTTTGT | | Structure staples |
| 12[207]10[208] | GTACCGCAATTCTAAGAACGCGAGTATTATTT | | Structure staples |
| 12[239]10[240] | CTTATCATTCCCGACTTGCGGGAGCCTAATTT | | Structure staples |
| 12[271]10[272] | TGTAGAAATCAAGATTAGTTGCTCTTACCA | | Structure staples |
| 13[32]15[31] | AACGCAAAATCGATGAACGGTACCGGTTGA | | Structure staples |
| 13[64]15[63] | TATATTTTGTCATTGCCTGAGAGTGGAAGATT | | Structure staples |
| 13[96]15[95] | TAGGTAAACTATTTTTGAGAGATCAAACGTTA | | Structure staples |
| 13[128]15[127] | GAGACAGCTAGCTGATAAATTAATTTTTGT | | Structure staples |
| 13[160]14[144] | GTAATAAGTTAGGCAGAGGCATTTATGATATT | | Structure staples |
| 13[192]15[191] | GTAAAGTAATCGCCATATTTAACAAAACTTTT | | Structure staples |
| 13[224]15[223] | ACAACATGCCAACGCTCAACAGTCTTCTGA | | Structure staples |
| 13[256]15[255] | GTTTATCAATATGCGTTATACAAACCGACCGT | | Structure staples |
| 14[47]12[48] | AACAAGAGGGATAAAAATTTTTAGCATAAAGC | | Structure staples |
| 14[79]12[80] | GCTATCAGAAATGCAATGCCTGAATTAGCA | | Structure staples |
| 14[111]12[112] | GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | | Structure staples |
| 14[143]13[159] | CAACCGTTTCAAATCACCATCAATTCGAGCCA | | Structure staples |
| 14[175]12[176] | CATGTAATAGAATATAAAGTACCAAGCCGT | | Structure staples |
| 14[207]12[208] | AATTGAGAATTCTGTCCAGACGACTAAACCAA | | Structure staples |
| 14[239]12[240] | AGTATAAAGTTCAGCTAATGCAGATGTCTTTC | | Structure staples |
| 14[271]12[272] | TTAGTATCACAATAGATAAGTCCACGAGCA | | Structure staples |
| 15[32]17[31] | TAATCAGCGGATTGACCGTAATCGTAACCG | | Structure staples |
| 15[96]17[95] | ATATTTTGGCTTTCATCAACATTATCCAGCCA | | Structure staples |
| 15[160]16[144] | ATCGCAAGTATGTAAATGCTGATGATAGGAAC | | Structure staples |
| 15[224]17[223] | CCTAAATCAAAATCATAGGTCTAAACAGTA | | Structure staples |
| 16[47]14[48] | ACAAACGGAAAAGCCCCAAAAACACTGGAGCA | | Structure staples |

| | | | |
|---|---|---|---|
| 16[79]14[80] | GCGAGTAAAAATATTTAAATTGTTACAAAG | | Structure staples |
| 16[111]14[112] | TGTAGCCATTAAAATTCGCATTAAATGCCGGA | | Structure staples |
| 16[143]15[159] | GCCATCAAGCTCATTTTTTAACCACAAATCCA | | Structure staples |
| 16[175]14[176] | TATAACTAACAAAGAACGCGAGAACGCCAA | | Structure staples |
| 16[207]14[208] | ACCTTTTTATTTTAGTTAATTTCATAGGGCTT | | Structure staples |
| 16[239]14[240] | GAATTTATTTAATGGTTTGAAATATTCTTACC | | Structure staples |
| 16[271]14[272] | CTTAGATTTAAGGCGTTAAATAAAGCCTGT | | Structure staples |
| 17[32]19[31] | TGCATCTTTCCCAGTCACGACGGCCTGCAG | | Structure staples |
| 17[96]19[95] | GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC | | Structure staples |
| 17[160]18[144] | AGAAAACAAAGAAGATGATGAAACAGGCTGCG | | Structure staples |
| 17[224]19[223] | CATAAATCTTTGAATACCAAGTGTTAGAAC | | Structure staples |
| 18[47]16[48] | CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA | | Structure staples |
| 18[79]16[80] | GATGTGCTTCAGGAAGATCGCACAATGTGA | | Structure staples |
| 18[111]16[112] | TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC | | Structure staples |
| 18[143]17[159] | CAACTGTTGCGCCATTCGCCATTCAAACATCA | | Structure staples |
| 18[175]16[176] | CTGAGCAAAAATTAATTACATTTTGGGTTA | | Structure staples |
| 18[207]16[208] | CGCGCAGATTACCTTTTTTAATGGGAGAGACT | | Structure staples |
| 18[239]16[240] | CCTGATTGCAATATATGTGAGTGATCAATAGT | | Structure staples |
| 18[271]16[272] | CTTTTACAAAATCGTCGCTATTAGCGATAG | | Structure staples |
| 19[32]21[31] | GTCGACTTCGGCCAACGCGCGGGGTTTTTC | | Structure staples |
| 19[96]21[95] | CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC | | Structure staples |
| 19[160]20[144] | GCAATTCACATATTCCTGATTATCAAAGTGTA | | Structure staples |
| 19[224]21[223] | CTACCATAGTTTGAGTAACATTTAAAATAT | | Structure staples |
| 20[47]18[48] | TTAATGAACTAGAGGATCCCCGGGGGGTAACG | | Structure staples |
| 20[79]18[80] | TTCCAGTCGTAATCATGGTCATAAAAGGGG | | Structure staples |
| 20[111]18[112] | CACATTAAAATTGTTATCCGCTCATGCGGGCC | | Structure staples |
| 20[143]19[159] | AAGCCTGGTACGAGCCGGAAGCATAGATGATG | | Structure staples |
| 20[175]18[176] | ATTATCATTCAATATAATCCTGACAATTAC | | Structure staples |
| 20[207]18[208] | GCGGAACATCTGAATAATGGAAGGTACAAAAT | | Structure staples |
| 20[239]18[240] | ATTTTAAAATCAAAATTATTTGCACGGATTCG | | Structure staples |

| | | | |
|---|---|---|---|
| 20[271]18[272] | CTCGTATTAGAAATTGCGTAGATACAGTAC | | Structure staples |
| 21[32]23[31] | TTTTCACTCAAAGGGCGAAAAACCATCACC | | Structure staples |
| 21[56]23[63] | AGCTGATTGCCCTTCAGAGTCCACTATTAAAGGGTGCCGT | | Structure staples |
| 21[96]23[95] | AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC | | Structure staples |
| 21[120]23[127] | CCCAGCAGGCGAAAAATCCCTTATAAATCAAGCCGGCG | | Structure staples |
| 21[160]22[144] | TCAATATCGAACCTCAAATATCAATTCCGAAA | | Structure staples |
| 21[184]23[191] | TCAACAGTTGAAAGGAGCAAATGAAAAATCTAGAGATAGA | | Structure staples |
| 21[224]23[223] | CTTTAGGGCCTGCAACAGTGCCAATACGTG | | Structure staples |
| 21[248]23[255] | AGATTAGAGCCGTCAAAAAACAGAGGTGAGGCCTATTAGT | | Structure staples |
| 22[47]20[48] | CTCCAACGCAGTGAGACGGGCAACCAGCTGCA | | Structure staples |
| 22[79]20[80] | TGGAACAACCGCCTGGCCCTGAGGCCCGCT | | Structure staples |
| 22[111]20[112] | GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT | | Structure staples |
| 22[143]21[159] | TCGGCAAATCCTGTTTGATGGTGGACCCTCAA | | Structure staples |
| 22[175]20[176] | ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA | | Structure staples |
| 22[207]20[208] | AGCCAGCAATTGAGGAAGGTTATCATCATTTT | | Structure staples |
| 22[239]20[240] | TTAACACCAGCACTAACAACTAATCGTTATTA | | Structure staples |
| 22[271]20[272] | CAGAAGATTAGATAATACATTTGTCGACAA | | Structure staples |
| 23[32]22[48] | CAAATCAAGTTTTTTGGGGTCGAAACGTGGA | | Structure staples |
| 23[64]22[80] | AAAGCACTAAATCGGAACCCTAATCCAGTT | | Structure staples |
| 23[96]22[112] | CCCGATTTAGAGCTTGACGGGGAAAAAGAATA | | Structure staples |
| 23[128]23[159] | AACGTGGCGAGAAAGGAAGGGAAACCAGTAA | | Structure staples |
| 23[160]22[176] | TAAAAGGGACATTCTGGCCAACAAAGCATC | | Structure staples |
| 23[192]22[208] | ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG | | Structure staples |
| 23[224]22[240] | GCACAGACAATATTTTTGAATGGGGTCAGTA | | Structure staples |
| 23[256]22[272] | CTTTAATGCGCGAACTGATAGCCCCACCAG | | Structure staples |
| 1[64]4[64] | TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGAGGTCAATC | | Biotin helper strand |
| 1[128]4[128] | TGACAACTCGCTGAGGCTTGCATTATACCAAGCGCGATGATAAA | | Biotin helper strand |
| 1[192]4[192] | GCGGATAACCTATTATTCTGAAACAGACGATTGGCCTTGAAGAGCCAC | | Biotin helper strand |
| 1[256]4[256] | CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGGGAACCAG | | Biotin helper strand |
| 15[64]18[64] | GTATAAGCCAACCCGTCGGATTCTGACGACAGTATCGGCCGCAAGGCG | | Biotin helper strand |

| 15[128]18[128] | TAAATCAAAATAATTCGCGTCTCGGAAACCAGGCAAAGGGAAGG | | Biotin helper strand |
|---|---|---|---|
| 15[192]18[192] | TCAAATATAACCTCCGGCTTAGGTAACAATTTCATTTGAAGGCGAATT | | Biotin helper strand |
| 15[256]18[256] | GTGATAAAAAGACGCTGAGAAGAGATAACCTTGCTTCTGTTCGGGAGA | | Biotin helper strand |
| 4[63]6[56] | Biotin-TTTTATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA | | Biotin-labelled strand |
| 4[127]6[120] | Biotin-TTTTTTGTGTCGTGACGAGAAACACCAAATTTCAACTTTAAT | | Biotin-labelled strand |
| 4[191]6[184] | Biotin-TTTTCACCCTCAGAAACCATCGATAGCATTGAGCCATTTGGGAA | | Biotin-labelled strand |
| 4[255]6[248] | Biotin-TTTTAGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA | | Biotin-labelled strand |
| 18[63]20[56] | Biotin-TTTTATTAAGTTTACCGAGCTCGAATTCGGGAAACCTGTCGTGC | | Biotin-labelled strand |
| 18[127]20[120] | Biotin-TTTTGCGATCGGCAATTCCACACAACAGGTGCCTAATGAGTG | | Biotin-labelled strand |
| 18[191]20[184] | Biotin-TTTTATTCATTTTTGTTTGGATTATACTAAGAAACCACCAGAAG | | Biotin-labelled strand |
| 18[255]20[248] | Biotin-TTTTAACAATAACGTAAAACAGAAATAAAAATCCTTTGCCCGAA | | Biotin-labelled strand |

8

**Supplementary Table S2 │ List of DNA-PAINT extension sequences for self-assembly of 10 nm spaced test standard samples**

**Table S2a │ Sequences for 10 nm spaced two-lines sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 7[160]8[144] | TTATTACGAAGAACTGGCATGATTGCGAGAGG | TTATTACGAAGAACTGGCATGATTGCGAGAGGTTATACATCTA | two-lines |
| 9[160]10[144] | AGAGAGAAAAAAATGAAAATAGCAAGCAAACT | AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTTATACATCTA | two-lines |
| 10[111]8[112] | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGTTTATACATCTA | two-lines |
| 11[160]12[144] | CCAATAGCTCATCGTAGGAATCATGGCATCAA | CCAATAGCTCATCGTAGGAATCATGGCATCAATTATACATCTA | two-lines |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATTATACATCTA | two-lines |
| 13[160]14[144] | GTAATAAGTTAGGCAGAGGCATTTATGATATT | GTAATAAGTTAGGCAGAGGCATTTATGATATTTTATACATCTA | two-lines |
| 14[111]12[112] | GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | GAGGGTAGGATTCAAAAGGGTGAGACATCCAATTATACATCTA | two-lines |
| 15[160]16[144] | ATCGCAAGTATGTAAATGCTGATGATAGGAAC | ATCGCAAGTATGTAAATGCTGATGATAGGAACTTATACATCTA | two-lines |
| 16[111]14[112] | TGTAGCCATTAAAATTCGCATTAAATGCCGGA | TGTAGCCATTAAAATTCGCATTAAATGCCGGATTATACATCTA | two-lines |
| 18[111]16[112] | TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC | TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCCTTATACATCTA | two-lines |

**Table S2b │ Sequences for 10 nm spaced two-targets sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 0[239]1[223] | AGGAACCCATGTACCGTAACACTTGATATAA | AGGAACCCATGTACCGTAACACTTGATATAATTATACATCTA | marker |
| 0[47]1[31] | AGAAAGGAACAACTAAAGGAATTCAAAAAAA | AGAAAGGAACAACTAAAGGAATTCAAAAAAATTATACATCTA | marker |
| 11[160]12[144] | CCAATAGCTCATCGTAGGAATCATGGCATCAA | CCAATAGCTCATCGTAGGAATCATGGCATCAATTATACATCTA | two-targets |
| 14[111]12[112] | GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | GAGGGTAGGATTCAAAAGGGTGAGACATCCAATTATACATCTA | two-targets |
| 21[224]23[223] | CTTTAGGGCCTGCAACAGTGCCAATACGTG | CTTTAGGGCCTGCAACAGTGCCAATACGTGTTATACATCTA | marker |
| 21[32]23[31] | TTTTCACTCAAAGGGCGAAAAACCATCACC | TTTTCACTCAAAGGGCGAAAAACCATCACCTTATACATCTA | marker |

**Table S2c | Sequences for 10 nm spaced densely labelled grid sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 5[160]6[144] | GCAAGGCCTCACCAGTAGCACCATGGGCTTGA | GCAAGGCCTCACCAGTAGCACCATGGGCTTGATTATACATCTA | 24-pt-grid |
| 8[239]6[240] | AAGTAAGCAGACACCACGGAATAATATTGACG | AAGTAAGCAGACACCACGGAATAATATTGACGTTATACATCTA | 24-pt-grid |
| 8[207]6[208] | AAGGAAACATAAAGGTGGCAACATTATCACCG | AAGGAAACATAAAGGTGGCAACATTATCACCGTTATACATCTA | 24-pt-grid |
| 8[175]6[176] | ATACCCAACAGTATGTTAGCAAATTAGAGC | ATACCCAACAGTATGTTAGCAAATTAGAGCTTATACATCTA | 24-pt-grid |
| 8[111]6[112] | AATAGTAAACACTATCATAACCCTCATTGTGA | AATAGTAAACACTATCATAACCCTCATTGTGATTATACATCTA | 24-pt-grid |
| 8[79]6[80] | AATACTGCCCAAAAGGAATTACGTGGCTCA | AATACTGCCCAAAAGGAATTACGTGGCTCATTATACATCTA | 24-pt-grid |
| 9[160]10[144] | AGAGAGAAAAAAATGAAAATAGCAAGCAAACT | AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTTATACATCTA | 24-pt-grid |
| 12[239]10[240] | CTTATCATTCCCGACTTGCGGGAGCCTAATTT | CTTATCATTCCCGACTTGCGGGAGCCTAATTTTTATACATCTA | 24-pt-grid |
| 12[207]10[208] | GTACCGCAATTCTAAGAACGCGAGTATTATTT | GTACCGCAATTCTAAGAACGCGAGTATTATTTTTATACATCTA | 24-pt-grid |
| 12[175]10[176] | TTTTATTTAAGCAAATCAGATATTTTTTGT | TTTTATTTAAGCAAATCAGATATTTTTTGTTTATACATCTA | 24-pt-grid |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATTATACATCTA | 24-pt-grid |
| 12[79]10[80] | AAATTAAGTTGACCATTAGATACTTTTGCG | AAATTAAGTTGACCATTAGATACTTTTGCGTTATACATCTA | 24-pt-grid |
| 13[160]14[144] | GTAATAAGTTAGGCAGAGGCATTTATGATATT | GTAATAAGTTAGGCAGAGGCATTTATGATATTTTATACATCTA | 24-pt-grid |
| 16[239]14[240] | GAATTTATTTAATGGTTTGAAATATTCTTACC | GAATTTATTTAATGGTTTGAAATATTCTTACCTTATACATCTA | 24-pt-grid |
| 16[207]14[208] | ACCTTTTTATTTTAGTTAATTTCATAGGGCTT | ACCTTTTTATTTTAGTTAATTTCATAGGGCTTTTATACATCTA | 24-pt-grid |
| 16[175]14[176] | TATAACTAACAAAGAACGCGAGAACGCCAA | TATAACTAACAAAGAACGCGAGAACGCCAATTATACATCTA | 24-pt-grid |
| 16[111]14[112] | TGTAGCCATTAAAATTCGCATTAAATGCCGGA | TGTAGCCATTAAAATTCGCATTAAATGCCGGATTATACATCTA | 24-pt-grid |
| 16[79]14[80] | GCGAGTAAAAATATTTAAATTGTTACAAAG | GCGAGTAAAAATATTTAAATTGTTACAAAGTTATACATCTA | 24-pt-grid |
| 17[160]18[144] | AGAAAACAAAGAAGATGATGAAACAGGCTGCG | AGAAAACAAAGAAGATGATGAAACAGGCTGCGTTATACATCTA | 24-pt-grid |
| 20[239]18[240] | ATTTTAAAATCAAAATTATTTGCACGGATTCG | ATTTTAAAATCAAAATTATTTGCACGGATTCGTTATACATCTA | 24-pt-grid |
| 20[207]18[208] | GCGGAACATCTGAATAATGGAAGGTACAAAAT | GCGGAACATCTGAATAATGGAAGGTACAAAATTTATACATCTA | 24-pt-grid |
| 20[175]18[176] | ATTATCATTCAATATAATCCTGACAATTAC | ATTATCATTCAATATAATCCTGACAATTACTTATACATCTA | 24-pt-grid |
| 20[111]18[112] | CACATTAAAATTGTTATCCGCTCATGCGGGCC | CACATTAAAATTGTTATCCGCTCATGCGGGCCTTATACATCTA | 24-pt-grid |
| 20[79]18[80] | TTCCAGTCGTAATCATGGTCATAAAAGGGG | TTCCAGTCGTAATCATGGTCATAAAAGGGGTTATACATCTA | 24-pt-grid |

**Supplementary Table S3 | List of DNA-PAINT extension sequences for self-assembly of 20 nm square grid, 5 nm triangular grid, and "Wyss!" letter pattern samples**

**Table S3a | Sequences for 20 nm square grid sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 4[239]2[240] | GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT | GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTTATCTACATA | 20-nm-grid |
| 4[175]2[176] | CACCAGAAAGGTTGAGGCAGGTCATGAAAG | CACCAGAAAGGTTGAGGCAGGTCATGAAAGTTATCTACATA | 20-nm-grid |
| 4[111]2[112] | GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA | GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATTATCTACATA | 20-nm-grid |
| 4[47]2[48] | GACCAACTAATGCCACTACGAAGGGGGTAGCA | GACCAACTAATGCCACTACGAAGGGGGTAGCATTATCTACATA | 20-nm-grid |
| 12[239]10[240] | CTTATCATTCCCGACTTGCGGGAGCCTAATTT | CTTATCATTCCCGACTTGCGGGAGCCTAATTTTTATCTACATA | 20-nm-grid |
| 12[175]10[176] | TTTTATTTAAGCAAATCAGATATTTTTTGT | TTTTATTTAAGCAAATCAGATATTTTTTGTTATCTACATA | 20-nm-grid |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATTATCTACATA | 20-nm-grid |
| 12[47]10[48] | TAAATCGGGATTCCCAATTCTGCGATATAATG | TAAATCGGGATTCCCAATTCTGCGATATAATGTTATCTACATA | 20-nm-grid |
| 20[239]18[240] | ATTTTAAAATCAAAATTATTTGCACGGATTCG | ATTTTAAAATCAAAATTATTTGCACGGATTCGTTATCTACATA | 20-nm-grid |
| 20[175]18[176] | ATTATCATTCAATATAATCCTGACAATTAC | ATTATCATTCAATATAATCCTGACAATTACTTATCTACATA | 20-nm-grid |
| 20[111]18[112] | CACATTAAAATTGTTATCCGCTCATGCGGGCC | CACATTAAAATTGTTATCCGCTCATGCGGGCCTTATCTACATA | 20-nm-grid |
| 20[47]18[48] | TTAATGAACTAGAGGATCCCCGGGGGGTAACG | TTAATGAACTAGAGGATCCCCGGGGGGTAACGTTATCTACATA | 20-nm-grid |

**Table S3b | Sequences for 5 nm triangular grid sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 7[120]9[127] | CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA | CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGATGCTCGGA | 5-nm-lattice |
| 7[96]9[95] | TAAGAGCAAATGTTTAGACTGGATAGGAAGCC | TAAGAGCAAATGTTTAGACTGGATAGGAAGCCTGCTCGGA | 5-nm-lattice |
| 7[56]9[63] | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAGTGCTCGGA | 5-nm-lattice |

11

| | | | |
|---|---|---|---|
| 9[128]11[127] | GCTTCAATCAGGATTAGAGAGTTATTTTCA | GCTTCAATCAGGATTAGAGAGTTATTTTCATGCTCGGA | 5-nm-lattice |
| 9[96]11[95] | CGAAAGACTTTGATAAGAGGTCATATTTCGCA | CGAAAGACTTTGATAAGAGGTCATATTTCGCATGCTCGGA | 5-nm-lattice |
| 9[64]11[63] | CGGATTGCAGAGCTTAATTGCTGAAACGAGTA | CGGATTGCAGAGCTTAATTGCTGAAACGAGTATGCTCGGA | 5-nm-lattice |
| 10[111]8[112] | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGTTGCTCGGA | 5-nm-lattice |
| 10[79]8[80] | GATGGCTTATCAAAAAGATTAAGAGCGTCC | GATGGCTTATCAAAAAGATTAAGAGCGTCCTGCTCGGA | 5-nm-lattice |
| 10[47]8[48] | CTGTAGCTTGACTATTATAGTCAGTTCATTGA | CTGTAGCTTGACTATTATAGTCAGTTCATTGATGCTCGGA | 5-nm-lattice |
| 11[128]13[127] | TTTGGGGATAGTAGTAGCATTAAAAGGCCG | TTTGGGGATAGTAGTAGCATTAAAAGGCCGTGCTCGGA | 5-nm-lattice |
| 11[96]13[95] | AATGGTCAACAGGCAAGGCAAAGAGTAATGTG | AATGGTCAACAGGCAAGGCAAAGAGTAATGTGTGCTCGGA | 5-nm-lattice |
| 11[64]13[63] | GATTTAGTCAATAAAGCCTCAGAGAACCCTCA | GATTTAGTCAATAAAGCCTCAGAGAACCCTCATGCTCGGA | 5-nm-lattice |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATGCTCGGA | 5-nm-lattice |
| 12[79]10[80] | AAAATTAAGTTGACCATTAGATACTTTTGCG | AAAATTAAGTTGACCATTAGATACTTTTGCGTGCTCGGA | 5-nm-lattice |
| 12[47]10[48] | TAAATCGGGATTCCCAATTCTGCGATATAATG | TAAATCGGGATTCCCAATTCTGCGATATAATGTGCTCGGA | 5-nm-lattice |
| 13[128]15[127] | GAGACAGCTAGCTGATAAATTAATTTTTGT | GAGACAGCTAGCTGATAAATTAATTTTTGTTGCTCGGA | 5-nm-lattice |
| 13[96]15[95] | TAGGTAAACTATTTTTGAGAGATCAAACGTTA | TAGGTAAACTATTTTTGAGAGATCAAACGTTATGCTCGGA | 5-nm-lattice |
| 13[64]15[63] | TATATTTTGTCATTGCCTGAGAGTGGAAGATT | TATATTTTGTCATTGCCTGAGAGTGGAAGATTGCTCGGA | 5-nm-lattice |
| 14[111]12[112] | GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | GAGGGTAGGATTCAAAAGGGTGAGACATCCAATGCTCGGA | 5-nm-lattice |
| 14[79]12[80] | GCTATCAGAAATGCAATGCCTGAATTAGCA | GCTATCAGAAATGCAATGCCTGAATTAGCATGCTCGGA | 5-nm-lattice |
| 14[47]12[48] | AACAAGAGGGATAAAAATTTTTAGCATAAAGC | AACAAGAGGGATAAAAATTTTTAGCATAAAGCTGCTCGGA | 5-nm-lattice |
| 16[111]14[112] | TGTAGCCATTAAAATTCGCATTAAATGCCGGA | TGTAGCCATTAAAATTCGCATTAAATGCCGGATGCTCGGA | 5-nm-lattice |
| 16[79]14[80] | GCGAGTAAAAATATTTAAATTGTTACAAAG | GCGAGTAAAAATATTTAAATTGTTACAAAGTGCTCGGA | 5-nm-lattice |
| 16[47]14[48] | ACAAACGGAAAAGCCCCAAAAACACTGGAGCA | ACAAACGGAAAAGCCCCAAAAACACTGGAGCATGCTCGGA | 5-nm-lattice |

12

**Table S3c | Sequences for "Wyss!" letter pattern sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 0[47]1[31] | AGAAAGGAACAACTAAAGGAATTCAAAAAAA | AGAAAGGAACAACTAAAGGAATTCAAAAAAATGCTCGGA | Wyss!-pattern |
| 0[111]1[95] | TAAATGAATTTTCTGTATGGGATTAATTTCTT | TAAATGAATTTTCTGTATGGGATTAATTTCTTTGCTCGGA | Wyss!-pattern |
| 1[32]3[31] | AGGCTCCAGAGGCTTTGAGGACACGGGTAA | AGGCTCCAGAGGCTTTGAGGACACGGGTAATGCTCGGA | Wyss!-pattern |
| 1[96]3[95] | AAACAGCTTTTTGCGGGATCGTCAACACTAAA | AAACAGCTTTTTGCGGGATCGTCAACACTAAATGCTCGGA | Wyss!-pattern |
| 3[32]5[31] | AATACGTTTGAAAGAGGACAGACTGACCTT | AATACGTTTGAAAGAGGACAGACTGACCTTTGCTCGGA | Wyss!-pattern |
| 3[96]5[95] | ACACTCATCCATGTTACTTAGCCGAAAGCTGC | ACACTCATCCATGTTACTTAGCCGAAAGCTGCTGCTCGGA | Wyss!-pattern |
| 5[32]7[31] | CATCAAGTAAAACGAACTAACGAGTTGAGA | CATCAAGTAAAACGAACTAACGAGTTGAGATGCTCGGA | Wyss!-pattern |
| 5[96]7[95] | TCATTCAGATGCGATTTTAAGAACAGGCATAG | TCATTCAGATGCGATTTTAAGAACAGGCATAGTGCTCGGA | Wyss!-pattern |
| 7[32]9[31] | TTTAGGACAAATGCTTTAAACAATCAGGTC | TTTAGGACAAATGCTTTAAACAATCAGGTCTGCTCGGA | Wyss!-pattern |
| 7[56]9[63] | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAGTGCTCGGA | Wyss!-pattern |
| 7[96]9[95] | TAAGAGCAAATGTTTAGACTGGATAGGAAGCC | TAAGAGCAAATGTTTAGACTGGATAGGAAGCCTGCTCGGA | Wyss!-pattern |
| 9[32]11[31] | TTTACCCCAACATGTTTTAAATTTCCATAT | TTTACCCCAACATGTTTTAAATTTCCATATTGCTCGGA | Wyss!-pattern |
| 9[64]11[63] | CGGATTGCAGAGCTTAATTGCTGAAACGAGTA | CGGATTGCAGAGCTTAATTGCTGAAACGAGTATGCTCGGA | Wyss!-pattern |
| 9[96]11[95] | CGAAAGACTTTGATAAGAGGTCATATTTCGCA | CGAAAGACTTTGATAAGAGGTCATATTTCGCATGCTCGGA | Wyss!-pattern |
| 14[47]12[48] | AACAAGAGGGATAAAAATTTTTAGCATAAAGC | AACAAGAGGGATAAAAATTTTTAGCATAAAGCTGCTCGGA | Wyss!-pattern |
| 14[79]12[80] | GCTATCAGAAATGCAATGCCTGAATTAGCA | GCTATCAGAAATGCAATGCCTGAATTAGCATGCTCGGA | Wyss!-pattern |
| 3[160]4[144] | TTGACAGGCCACCACCAGAGCCGCGATTGTA | TTGACAGGCCACCACCAGAGCCGCGATTGTATGCTCGGA | Wyss!-pattern |
| 5[160]6[144] | GCAAGGCCTCACCAGTAGCACCATGGGCTTGA | GCAAGGCCTCACCAGTAGCACCATGGGCTTGATGCTCGGA | Wyss!-pattern |
| 6[111]4[112] | ATTACCTTTGAATAAGGCTTGCCCAAATCCGC | ATTACCTTTGAATAAGGCTTGCCCAAATCCGCTGCTCGGA | Wyss!-pattern |
| 7[160]8[144] | TTATTACGAAGAACTGGCATGATTGCGAGAGG | TTATTACGAAGAACTGGCATGATTGCGAGAGGTGCTCGGA | Wyss!-pattern |
| 8[111]6[112] | AATAGTAAACACTATCATAACCCTCATTGTGA | AATAGTAAACACTATCATAACCCTCATTGTGATGCTCGGA | Wyss!-pattern |
| 9[128]11[127] | GCTTCAATCAGGATTAGAGAGTTATTTTCA | GCTTCAATCAGGATTAGAGAGTTATTTTCATGCTCGGA | Wyss!-pattern |
| 9[160]10[144] | AGAGAGAAAAAAATGAAAATAGCAAGCAAACT | AGAGAGAAAAAAATGAAAATAGCAAGCAAACTTGCTCGGA | Wyss!-pattern |
| 10[111]8[112] | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGTTGCTCGGA | Wyss!-pattern |

13

| | | | |
|---|---|---|---|
| 11[128]13[127] | TTTGGGGATAGTAGTAGCATTAAAAGGCCG | TTTGGGGATAGTAGTAGCATTAAAAGGCCGTGCTCGGA | Wyss!-pattern |
| 11[160]12[144] | CCAATAGCTCATCGTAGGAATCATGGCATCAA | CCAATAGCTCATCGTAGGAATCATGGCATCAATGCTCGGA | Wyss!-pattern |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATGCTCGGA | Wyss!-pattern |
| 13[128]15[127] | GAGACAGCTAGCTGATAAATTAATTTTTTGT | GAGACAGCTAGCTGATAAATTAATTTTTTGTTGCTCGGA | Wyss!-pattern |
| 17[96]19[95] | GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC | GCTTTCCGATTACGCCAGCTGGCGGCTGTTTCTGCTCGGA | Wyss!-pattern |
| 18[111]16[112] | TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC | TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCCTGCTCGGA | Wyss!-pattern |
| 20[79]18[80] | TTCCAGTCGTAATCATGGTCATAAAAGGGG | TTCCAGTCGTAATCATGGTCATAAAAGGGGTGCTCGGA | Wyss!-pattern |
| 20[111]18[112] | CACATTAAAATTGTTATCCGCTCATGCGGGCC | CACATTAAAATTGTTATCCGCTCATGCGGGCCTGCTCGGA | Wyss!-pattern |
| 2[271]0[272] | GTTTTAACTTAGTACCGCCACCCAGAGCCA | GTTTTAACTTAGTACCGCCACCCAGAGCCATGCTCGGA | Wyss!-pattern |
| 4[271]2[272] | AAAATCACCTTCCAGTAAGCGTCAGTAATAA | AAAATCACCTTCCAGTAAGCGTCAGTAATAATGCTCGGA | Wyss!-pattern |
| 6[271]4[272] | ACCGATTGTCGGCATTTTCGGTCATAATCA | ACCGATTGTCGGCATTTTCGGTCATAATCATGCTCGGA | Wyss!-pattern |
| 8[271]6[272] | AATAGCTATCAATAGAAAATTCAACATTCA | AATAGCTATCAATAGAAAATTCAACATTCATGCTCGGA | Wyss!-pattern |
| 10[271]8[272] | ACGCTAACACCCACAAGAATTGAAAATAGC | ACGCTAACACCCACAAGAATTGAAAATAGCTGCTCGGA | Wyss!-pattern |
| 16[271]14[272] | CTTAGATTTAAGGCGTTAAATAAAGCCTGT | CTTAGATTTAAGGCGTTAAATAAAGCCTGTTGCTCGGA | Wyss!-pattern |
| 18[271]16[272] | CTTTTACAAAATCGTCGCTATTAGCGATAG | CTTTTACAAAATCGTCGCTATTAGCGATAGTGCTCGGA | Wyss!-pattern |
| 6[143]5[159] | GATGGTTTGAACGAGTAGTAAATTTACCATTA | GATGGTTTGAACGAGTAGTAAATTTACCATTATGCTCGGA | Wyss!-pattern |
| 6[175]4[176] | CAGCAAAAGGAAACGTCACCAATGAGCCGC | CAGCAAAAGGAAACGTCACCAATGAGCCGCTGCTCGGA | Wyss!-pattern |
| 8[143]7[159] | CTTTTGCAGATAAAAACCAAAATAAAGACTCC | CTTTTGCAGATAAAAACCAAAATAAAGACTCCTGCTCGGA | Wyss!-pattern |
| 9[192]11[191] | TTAGACGGCCAAATAAGAAACGATAGAAGGCT | TTAGACGGCCAAATAAGAAACGATAGAAGGCTTGCTCGGA | Wyss!-pattern |
| 10[143]9[159] | CCAACAGGAGCGAACCAGACCGGAGCCTTTAC | CCAACAGGAGCGAACCAGACCGGAGCCTTTACTGCTCGGA | Wyss!-pattern |
| 11[192]13[191] | TATCCGGTCTCATCGAGAACAAGCGACAAAAG | TATCCGGTCTCATCGAGAACAAGCGACAAAAGTGCTCGGA | Wyss!-pattern |
| 12[175]10[176] | TTTTATTTAAGCAAATCAGATATTTTTTGT | TTTTATTTAAGCAAATCAGATATTTTTTGTTGCTCGGA | Wyss!-pattern |
| 13[192]15[191] | GTAAAGTAATCGCCATATTTAACAAAACTTTT | GTAAAGTAATCGCCATATTTAACAAAACTTTTTGCTCGGA | Wyss!-pattern |
| 16[143]15[159] | GCCATCAAGCTCATTTTTTAACCACAAATCCA | GCCATCAAGCTCATTTTTTAACCACAAATCCATGCTCGGA | Wyss!-pattern |
| 18[175]16[176] | CTGAGCAAAAATTAATTACATTTTGGGTTA | CTGAGCAAAAATTAATTACATTTTGGGTTATGCTCGGA | Wyss!-pattern |
| 3[224]5[223] | TTAAAGCCAGAGCCGCCACCCTCGACAGAA | TTAAAGCCAGAGCCGCCACCCTCGACAGAATGCTCGGA | Wyss!-pattern |
| 8[207]6[208] | AAGGAAACATAAAGGTGGCAACATTATCACCG | AAGGAAACATAAAGGTGGCAACATTATCACCGTGCTCGGA | Wyss!-pattern |
| 8[239]6[240] | AAGTAAGCAGACACCACGGAATAATATTGACG | AAGTAAGCAGACACCACGGAATAATATTGACGTGCTCGGA | Wyss!-pattern |

14

| 9[224]11[223] | AAAGTCACAAAATAAACAGCCAGCGTTTTA | AAAGTCACAAAATAAACAGCCAGCGTTTTATGCTCGGA | Wyss!-pattern |
|---|---|---|---|
| 10[207]8[208] | ATCCCAATGAGAATTAACTGAACAGTTACCAG | ATCCCAATGAGAATTAACTGAACAGTTACCAGTGCTCGGA | Wyss!-pattern |
| 12[207]10[208] | GTACCGCAATTCTAAGAACGCGAGTATTATTT | GTACCGCAATTCTAAGAACGCGAGTATTATTTTGCTCGGA | Wyss!-pattern |
| 14[239]12[240] | AGTATAAAGTTCAGCTAATGCAGATGTCTTTC | AGTATAAAGTTCAGCTAATGCAGATGTCTTTCTGCTCGGA | Wyss!-pattern |
| 15[224]17[223] | CCTAAATCAAAATCATAGGTCTAAACAGTA | CCTAAATCAAAATCATAGGTCTAAACAGTATGCTCGGA | Wyss!-pattern |
| 16[239]14[240] | GAATTTATTTAATGGTTTGAAATATTCTTACC | GAATTTATTTAATGGTTTGAAATATTCTTACCTGCTCGGA | Wyss!-pattern |
| 18[207]16[208] | CGCGCAGATTACCTTTTTTAATGGGAGAGACT | CGCGCAGATTACCTTTTTTAATGGGAGAGACTTGCTCGGA | Wyss!-pattern |
| 18[239]16[240] | CCTGATTGCAATATATGTGAGTGATCAATAGT | CCTGATTGCAATATATGTGAGTGATCAATAGTTGCTCGGA | Wyss!-pattern |

15

**Supplementary Table S4 | List of DNA-PAINT extension sequences for self-assembly of three-colour 20 nm grid marker and 5 nm triangular grid samples**

The colours of staples in the following two tables (S4a, S4b) match those in Fig. 6 and Supplementary Figure S24.

**Table S4a | Sequences for three-colour 20 nm grid drift and alignment marker sample**

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 0[175]0[144] | TCCACAGACAGCCCTCATAGTTAGCGTAACGA | TCCACAGACAGCCCTCATAGTTAGCGTAACGATGCTCATTT | colour-1 |
| 2[79]0[80] | CAGCGAAACTTGCTTTCGAGGTGTTGCTAA | CAGCGAAACTTGCTTTCGAGGTGTTGCTAATGCTCATTT | colour-1 |
| 2[207]0[208] | TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG | TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCGTGCTCATTT | colour-1 |
| 7[160]8[144] | TTATTACGAAGAACTGGCATGATTGCGAGAGG | TTATTACGAAGAACTGGCATGATTGCGAGAGGTGCTCATTT | colour-1 |
| 10[79]8[80] | GATGGCTTATCAAAAAGATTAAGAGCGTCC | GATGGCTTATCAAAAAGATTAAGAGCGTCCTGCTCATTT | colour-1 |
| 10[207]8[208] | ATCCCAATGAGAATTAACTGAACAGTTACCAG | ATCCCAATGAGAATTAACTGAACAGTTACCAGTGCTCATTT | colour-1 |
| 10[271]8[272] | ACGCTAACACCCACAAGAATTGAAAATAGC | ACGCTAACACCCACAAGAATTGAAAATAGCTGCTCATTT | colour-1 |
| 15[160]16[144] | ATCGCAAGTATGTAAATGCTGATGATAGGAAC | ATCGCAAGTATGTAAATGCTGATGATAGGAACTGCTCATTT | colour-1 |
| 18[79]16[80] | GATGTGCTTCAGGAAGATCGCACAATGTGA | GATGTGCTTCAGGAAGATCGCACAATGTGATGCTCATTT | colour-1 |
| 18[207]16[208] | CGCGCAGATTACCTTTTTTAATGGGAGAGACT | CGCGCAGATTACCTTTTTTAATGGGAGAGACTTGCTCATTT | colour-1 |
| 18[271]16[272] | CTTTTACAAAATCGTCGCTATTAGCGATAG | CTTTTACAAAATCGTCGCTATTAGCGATAGTGCTCATTT | colour-1 |
| 2[271]0[272] | GTTTTAACTTAGTACCGCCACCCAGAGCCA | GTTTTAACTTAGTACCGCCACCCAGAGCCATGCTCATTT | colour-1 |
| 4[47]2[48] | GACCAACTAATGCCACTACGAAGGGGGTAGCA | GACCAACTAATGCCACTACGAAGGGGGTAGCATCGTCATTT | colour-2 |
| 4[111]2[112] | GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA | GACCTGCTCTTTGACCCCCAGCGAGGGAGTTATCGTCATTT | colour-2 |
| 4[175]2[176] | CACCAGAAAGGTTGAGGCAGGTCATGAAAG | CACCAGAAAGGTTGAGGCAGGTCATGAAAGTCGTCATTT | colour-2 |
| 4[239]2[240] | GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT | GCCTCCCTCAGAATGGAAAGCGCAGTAACAGTTCGTCATTT | colour-2 |
| 12[47]10[48] | TAAATCGGGATTCCCAATTCTGCGATATAATG | TAAATCGGGATTCCCAATTCTGCGATATAATGTCGTCATTT | colour-2 |

# SUPPLEMENTARY INFORMATION

| | | | |
|---|---|---|---|
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATCGTCATTT | colour-2 |
| 12[175]10[176] | TTTTATTTAAGCAAATCAGATATTTTTTGT | TTTTATTTAAGCAAATCAGATATTTTTTGTTCGTCATTT | colour-2 |
| 12[239]10[240] | CTTATCATTCCCGACTTGCGGGAGCCTAATTT | CTTATCATTCCCGACTTGCGGGAGCCTAATTTTCGTCATTT | colour-2 |
| 20[47]18[48] | TTAATGAACTAGAGGATCCCCGGGGGGTAACG | TTAATGAACTAGAGGATCCCCGGGGGGTAACGTCGTCATTT | colour-2 |
| 20[111]18[112] | CACATTAAAATTGTTATCCGCTCATGCGGGCC | CACATTAAAATTGTTATCCGCTCATGCGGGCCTCGTCATTT | colour-2 |
| 20[175]18[176] | ATTATCATTCAATATAATCCTGACAATTAC | ATTATCATTCAATATAATCCTGACAATTACTCGTCATTT | colour-2 |
| 20[239]18[240] | ATTTTAAAATCAAAATTATTTGCACGGATTCG | ATTTTAAAATCAAAATTATTTGCACGGATTCGTCGTCATTT | colour-2 |
| 3[160]4[144] | TTGACAGGCCACCACCAGAGCCGCGATTTGTA | TTGACAGGCCACCACCAGAGCCGCGATTTGTATCCTGATTT | colour-3 |
| 6[79]4[80] | TTATACCACCAAATCAACGTAACGAACGAG | TTATACCACCAAATCAACGTAACGAACGAGTCCTGATTT | colour-3 |
| 6[207]4[208] | TCACCGACGCACCGTAATCAGTAGCAGAACCG | TCACCGACGCACCGTAATCAGTAGCAGAACCGTCCTGATTT | colour-3 |
| 6[271]4[272] | ACCGATTGTCGGCATTTTCGGTCATAATCA | ACCGATTGTCGGCATTTTCGGTCATAATCATCCTGATTT | colour-3 |
| 14[271]12[272] | TTAGTATCACAATAGATAAGTCCACGAGCA | TTAGTATCACAATAGATAAGTCCACGAGCATCCTGATTT | colour-3 |
| 19[160]20[144] | GCAATTCACATATTCCTGATTATCAAAGTGTA | GCAATTCACATATTCCTGATTATCAAAGTGTATCCTGATTT | colour-3 |
| 22[79]20[80] | TGGAACAACCGCCTGGCCCTGAGGCCCGCT | TGGAACAACCGCCTGGCCCTGAGGCCCGCTTCCTGATTT | colour-3 |
| 22[207]20[208] | AGCCAGCAATTGAGGAAGGTTATCATCATTTT | AGCCAGCAATTGAGGAAGGTTATCATCATTTTCCTGATTT | colour-3 |
| 22[271]20[272] | CAGAAGATTAGATAATACATTTGTCGACAA | CAGAAGATTAGATAATACATTTGTCGACAATCCTGATTT | colour-3 |
| 11[160]12[144] | CCAATAGCTCATCGTAGGAATCATGGCATCAA | CCAATAGCTCATCGTAGGAATCATGGCATCAATCCTGATTT | colour-3 |
| 14[79]12[80] | GCTATCAGAAATGCAATGCCTGAATTAGCA | GCTATCAGAAATGCAATGCCTGAATTAGCATCCTGATTT | colour-3 |
| 14[207]12[208] | AATTGAGAATTCTGTCCAGACGACTAAACCAA | AATTGAGAATTCTGTCCAGACGACTAAACCAATCCTGATTT | colour-3 |

## Table S4b │ Sequences for three-colour 5 nm triangular grid sample

| Strand ID | Replace this sequence | With this sequence | Notes |
|---|---|---|---|
| 9[64]11[63] | CGGATTGCAGAGCTTAATTGCTGAAACGAGTA | CGGATTGCAGAGCTTAATTGCTGAAACGAGTATGCTCATTT | colour-1 |
| 9[96]11[95] | CGAAAGACTTTGATAAGAGGTCATATTTCGCA | CGAAAGACTTTGATAAGAGGTCATATTTCGCATGCTCATTT | colour-1 |
| 9[128]11[127] | GCTTCAATCAGGATTAGAGAGTTATTTTCA | GCTTCAATCAGGATTAGAGAGTTATTTTCATGCTCATTT | colour-1 |

| | | | |
|---|---|---|---|
| 10[47]8[48] | CTGTAGCTTGACTATTATAGTCAGTTCATTGA | CTGTAGCTTGACTATTATAGTCAGTTCATTGATGCTCATTT | colour-1 |
| 10[79]8[80] | GATGGCTTATCAAAAAGATTAAGAGCGTCC | GATGGCTTATCAAAAAGATTAAGAGCGTCCTGCTCATTT | colour-1 |
| 10[111]8[112] | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT | TTGCTCCTTTCAAATATCGCGTTTGAGGGGGTTGCTCATTT | colour-1 |
| 16[47]14[48] | ACAAACGGAAAAGCCCCAAAAACACTGGAGCA | ACAAACGGAAAAGCCCCAAAAACACTGGAGCATGCTCATTT | colour-1 |
| 16[79]14[80] | GCGAGTAAAAATATTTAAATTGTTACAAAG | GCGAGTAAAAATATTTAAATTGTTACAAAGTGCTCATTT | colour-1 |
| 16[111]14[112] | TGTAGCCATTAAAATTCGCATTAAATGCCGGA | TGTAGCCATTAAAATTCGCATTAAATGCCGGATGCTCATTT | colour-1 |
| 7[96]9[95] | TAAGAGCAAATGTTTAGACTGGATAGGAAGCC | TAAGAGCAAATGTTTAGACTGGATAGGAAGCCTCGTCATTT | colour-2 |
| 13[64]15[63] | TATATTTTGTCATTGCCTGAGAGTGGAAGATT | TATATTTTGTCATTGCCTGAGAGTGGAAGATTTCGTCATTT | colour-2 |
| 13[96]15[95] | TAGGTAAACTATTTTTGAGAGATCAAACGTTA | TAGGTAAACTATTTTTGAGAGATCAAACGTTATCGTCATTT | colour-2 |
| 13[128]15[127] | GAGACAGCTAGCTGATAAATTAATTTTTGT | GAGACAGCTAGCTGATAAATTAATTTTTGTTCGTCATTT | colour-2 |
| 7[56]9[63] | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG | ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAGTCGTCATTT | colour-2 |
| 7[120]9[127] | CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA | CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGATCGTCATTT | colour-2 |
| 14[47]12[48] | AACAAGAGGGATAAAAATTTTTAGCATAAAGC | AACAAGAGGGATAAAAATTTTTAGCATAAAGCTCGTCATTT | colour-2 |
| 14[79]12[80] | GCTATCAGAAATGCAATGCCTGAATTAGCA | GCTATCAGAAATGCAATGCCTGAATTAGCATCGTCATTT | colour-2 |
| 14[111]12[112] | GAGGGTAGGATTCAAAAGGGTGAGACATCCAA | GAGGGTAGGATTCAAAAGGGTGAGACATCCAATCGTCATTT | colour-2 |
| 11[64]13[63] | GATTTAGTCAATAAAGCCTCAGAGAACCCTCA | GATTTAGTCAATAAAGCCTCAGAGAACCCTCATCCTGATTT | colour-3 |
| 11[96]13[95] | AATGGTCAACAGGCAAGGCAAAGAGTAATGTG | AATGGTCAACAGGCAAGGCAAAGAGTAATGTGTCCTGATTT | colour-3 |
| 11[128]13[127] | TTTGGGGATAGTAGTAGCATTAAAAGGCCG | TTTGGGGATAGTAGTAGCATTAAAAGGCCGTCCTGATTT | colour-3 |
| 12[47]10[48] | TAAATCGGGATTCCCAATTCTGCGATATAATG | TAAATCGGGATTCCCAATTCTGCGATATAATGTCCTGATTT | colour-3 |
| 12[79]10[80] | AAATTAAGTTGACCATTAGATACTTTTGCG | AAATTAAGTTGACCATTAGATACTTTTGCGTCCTGATTT | colour-3 |
| 12[111]10[112] | TAAATCATATAACCTGTTTAGCTAACCTTTAA | TAAATCATATAACCTGTTTAGCTAACCTTTAATCCTGATTT | colour-3 |

18

**Supplementary Table S5 │ Sequence for M13mp18 phage single-stranded DNA scaffold**

```
TTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATC
GCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGAACCAC
CATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC
GGCTCGTATGTTGTGTGGAATGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTT
TTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
GCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATC
CCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTCCTATTGGTTAAAAAATGAGC
TGATTTAACAAAAATTTAATGCGAATTTTAACAAAATATTAACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTA
CCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGTAGATCTCTCAAAAATAGCTACCCTCTCCGGCATTAATTTATCAGCTAGAACGGTTGAATATCATATTGATGGTGATTTGACTGT
CTCCGGCCTTTCTCACCCTTTTGAATCTTTACCTACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCAAAAGTATTACAGGGTCATAATGTTT
TTGGTACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAATTCTTTGCCTTGCCTGTATGATTTATTGGATGTTAATGCTACTACTATTAGTAGAATTGATGCCACCTTTTCAGCTCGCGCCCCAAAT
GAAAATATAGCTAAACAGGTTATTGACCATTTGCGAAATGTATCTAATGGTCAAACTAAATCTACTCGTTCGCAGAATTGGGAATCAACTGTTATATGGAATGAAACTTCCAGACACCGTACTTTAGTTGCATATTTAAAACA
TGTTGAGCTACAGCATTATATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGAGCAATTAAAGGTACTCTCTAATCCTGACCTGTTGGAGTTTGCTTCCGGTCTGGTTCGCTTTGAAGCTCGAA
TTAAAACGCGATATTTGAAGTCTTTCGGGCTTCCTCTTAATCTTTTTGATGCAATCCGCTTTGCTTCTGACTATAATAGTCAGGGTAAAGACCTGATTTTTGATTTATGGTCATTCTCGTTTTCTGAACTGTTTAAAGCATTT
GAGGGGGATTCAATGAATATTTATGACGATTCCGCAGTATTGGACGCTATCCAGTCTAAACATTTTACTATTACCCCCTCTGGCAAAACTTCTTTTGCAAAAGCCTCTCGCTATTTTGGTTTTTATCGTCGTCTGGTAAACGA
GGGTTATGATAGTGTTGCTCTTACTATGCCTCGTAATTCCTTTTGGCGTTATGTATCTGCATTAGTTGAATGTGGTATTCCTAAATCTCAACTGATGAATCTTTCTACCTGTAATAATGTTGTTCCGTTAGTTCGTTTTATTA
ACGTAGATTTTTCTTCCCAACGTCCTGACTGGTATAATGAGCCAGTTCTTAAAATCGCATAAGGTAATTCACAATGATTAAAGTTGAAATTAAACCATCTCAAGCCCAATTTACTACTCGTTCTGGTGTTTCTCGTCAGGGCA
AGCCTTATTCACTGAATGAGCAGCTTTGTTACGTTGATTTGGGTAATGAATATCCGGTTCTTGTCAAGATTACTCTTGATGAAGGTCAGCCAGCCTATGCGCCTGGTCTGTACACCGTTCATCTGTCCTCTTTCAAAGTTGGT
CAGTTCGGTTCCCTTATGATTGACCGTCTGCGCCTCGTTCCGGCTAAGTAACATGGAGCAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAATCTCCGTTGTACTTTGTTTCGCGCTTGGTATAATCGCTGGGG
GTCAAAGATGAGTGTTTTAGTGTATTCTTTTGCCTCTTTCGTTTTAGGTTGGTGCCTTCGTAGTGGCATTACGTATTTTACCCGTTTAATGGAAACTTCCTCATGAAAAAGTCTTTAGTCCTCAAAGCCTCTGTAGCCGTTGC
TACCCTCGTTCCGATGCTGTCTTTCGCTGCTGAGGGTGACGATCCCGCAAAAGCGGCCTTTAACTCCCTGCAAGCCTCAGCGACCGAATATATCGGTTATGCGTGGGCGATGGTTGTTGTCATTGTCGGCGCAACTATCGGTA
TCAAGCTGTTTAAGAAATTCACCTCGAAAGCAAGCTGATAAACCGATACAATTAAAGGCTCCTTTTGGAGCCTTTTTTTTGGAGATTTTCAACGTGAAAAAATTATTATTCGCAATTCCTTTAGTTGTTCCTTTCTATTCTCA
CTCCGCTGAAACTGTTGAAAGTTGTTTAGCAAAATCCCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCTAACTATGAGGGCTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTA
CTGGTGACGAAACTCAGTGTTACGGTACATGGGTTCCTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGAT
ACACCTATTCCGGGCTATACTTATATCAACCCTCTCGACGGCACTTATCCGCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCTCTTAATACTTTCATGTTTCAGAATAATAGGTTCCG
AAATAGGCAGGGGCATTAACTGTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCAGTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTT
TCCATTCTGGCTTTAATGAGGATTATTTGTTTGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGGTGGCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGC
GGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAA
AGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTG
ATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTC
TTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAATCATGCCAGTTCTTTTGGGTATTCCGTTATTATTGCGTTTCCTCGGTTTCCTTCTGGTAACT
TTGTTCGGCTATCTGCTTACTTTTCTTAAAAAGGGCTTCGGTAAGATAGCTATTGCTATTTCATTGTTTCTTGCTCTTATTATTGGGCTTAACTCAATTCTTGTGGGTTATCTCTCTGATATTAGCGCTCAATTACCCTCTGA
CTTTGTTCAGGGTGTTCAGTTAATTCTCCCGTCTAATGCGCTTCCCTGTTTTTATGTTATTCTCTCTGTAAAGGCTGCTATTTTCATTTTTGACGTTAAACAAAAAATCGTTTCTTATTTGGATTGGGATAAATAATATGGCT
GTTTATTTTGTAACTGGCAAATTAGGCTCTGGAAAGACGCTCGTTAGCGTTGGTAAGATTCAGGATAAAATTGTAGCTGGGTGCAAAATAGCAACTAATCTTGATTTAAGGCTTCAAAACCTCCCGCAAGTCGGGAGGTTCGC
TTTGGAATGATAAGGAAAGACACGCCGATTATTGATTGGTTTCTACATGCTCGTAAATTAGGATGGGATATTATTTTTCTTGTTCAGGACTTATCTATTGTTGATAAACAGGCGCGTTCTGCATTAGCTGAACATGTTGTTTAT
TGTCGTCGTCTGGACAGAATTACTTTACCTTTTGTCGGTACTTTATATTCTCTTATTACTGGCTCGAAAATGCCTCTGCCTAAATTACATGTTGGCGTTGTTAAATATGGCGATTCTCAATTAAGCCCTACTGTTGAGCGTTG
GCTTTATACTGGTAAGAATTTGTATAACGCATATGATACTAAACAGGCTTTTTCTAGTAATTATGATTCCGGTGTTTATTCTTATTTAACGCCTTATTTATCACACGGTCGGTATTTCAAACCATTAAATTTAGGTCAGAAGA
TGAAATTAACTAAAATATATTTGAAAAAGTTTTCTCGCGTTCTTTGTCTTGCGATTGGATTTGCATCAGCATTTACATATAGTTATATAACCCAACCTAAGCCGGAGGTTAAAAAGGTAGTCTCTCAGACCTATGATTTTGAT
AAATTCACTATTGACTCTTCTCAGCGTCTTAATCTAAGCTATCGCTATGTTTTCAAGGATTCTAAGGGAAAATTAATTAATAGCGACGATTTACAGAAGCAAGGTTATTCACTCACATATATTGATTTATGTACTGTTTCCAT
TAAAAAAGGTAATTCAAATGAAATTGTTAAATGTAATTAATTTTGTTTTCTTGATGTTTGTTTCATCATCTTCTTTTGCTCAGGTAATTGAAATGAATAATTCGCCTCTGCGCGATTTTGTAACTTGGTATTCAAAGCAATCA
GGCGAATCCGTTATTGTTTCTCCCGATGTAAAAGGTACTGTTACTGTATATTCATCTGACGTTAAACCTGAAAATCTACGCAATTTCTTTATTTCTGTTTTACGTGCAAATAATTTTGATATGGTAGGTTCTAACCCTTCCAT
TATTCAGAAGTATAATCCAAACAATCAGGATTATATTGATGAATTGCCATCATCTGATAATCAGGAATATGATGATAATTCCGCTCCTTCTGGTGGTTTCTTTGTTCCGCAAAATGATAATGTTACTCAAACTTTTAAAATTA
ATAACGTTCGGGCAAAGGATTTAATACGAGTTGTCGAATTGTTTGTAAAGTCTAATACTTCTAAATCCTCAAATGTATTATCTATTGACGGCTCTAATCTATTAGTTGTTAGTGCTCCTAAAGATATTTTAGATAACCTTCCT
CAATTCCTTTCAACTGTTGATTTGCCAACTGACCAGATATTGATTGAGGGTTTGATATTTGAGGTTCAGCAAGGTGATGCTTTAGATTTTTCATTTGCTGCTGGCTCTCAGCGTGGCACTGTTGCAGGCGGTGTTAATACTGA
CCGCCTCACCTCTGTTTTATCTTCTGCTGGTGGTTCGTTCGGTATTTTTAATGGCGATGTTTTAGGGCTATCAGTTCGCGCATTAAAGACTAATAGCCATTCAAAAATATTGTCTGTGCCACGTATTCTTACGCTTTCAGGTC
AGAAGGGTTCTATCTCTGTTGGCCAGAATGTCCCTTTTATTACTGGTCGTGTGACTGGTGAATCTGCCAATGTAAATAATCCATTTCAGACGATTGAGCGTCAAAATGTAGGTATTTTCCATGAGCGTTTTTCCTGTTGCAATG
GCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTTCTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCTACAACGGTTAATTTGCGTGATGGACAGACTCTTTTACTCGGTGG
CCTCACTGATTATAAAAACACTTCTCAGGATTCTGGCGTACCGTTCCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCATAGTAC
GCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTC
```

**Supplementary Table S6 | List of PAINT docking and imager strand sequences**

| Strand | Sequence |
|---|---|
| Docking strand for 10 nm test patterns | 5'– Staple – TTATACATCTA – 3' |
| Docking strand for 20 nm square grid sample | 5'– Staple – TTATCTACATA – 3' |
| Docking strand for 5 nm triangular grid and "Wyss!" letter pattern sample | 5'– Staple – TGCTCGGA – 3' |
| Docking strand for three-colour imaging samples - colour 1 | 5'– Staple – TGCTCATTT – 3' |
| Docking strand for three-colour imaging samples - colour 2 | 5'– Staple – TCGTCATTT – 3' |
| Docking strand for three-colour imaging samples - colour 3 | 5'– Staple – TCCTGATTT – 3' |
| Imager strand for 10 nm test patterns | 5' – CTAGATGTAT – Cy3b |
| Imager strand for 20 nm square grid sample | 5' – TATGTAGATC – Cy3b |
| Imager strand for 5 nm triangular grid and "Wyss!" letter pattern sample | 5' – TCCGAGC – Cy3b |
| Imager strand for three-colour imaging samples - colour 1 | 5' – AAATGAGC – Cy3b |
| Imager strand for three-colour imaging samples - colour 2 | 5' – AAATGACG – Cy3b |
| Imager strand for three-colour imaging samples - colour 3 | 5' – AAATCAGG – Cy3b |

20