

Supplementary Information

Tri-State Logic Computation by Activating DNA Origami Chain

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1. Additional AFM images of logic gate “AND”

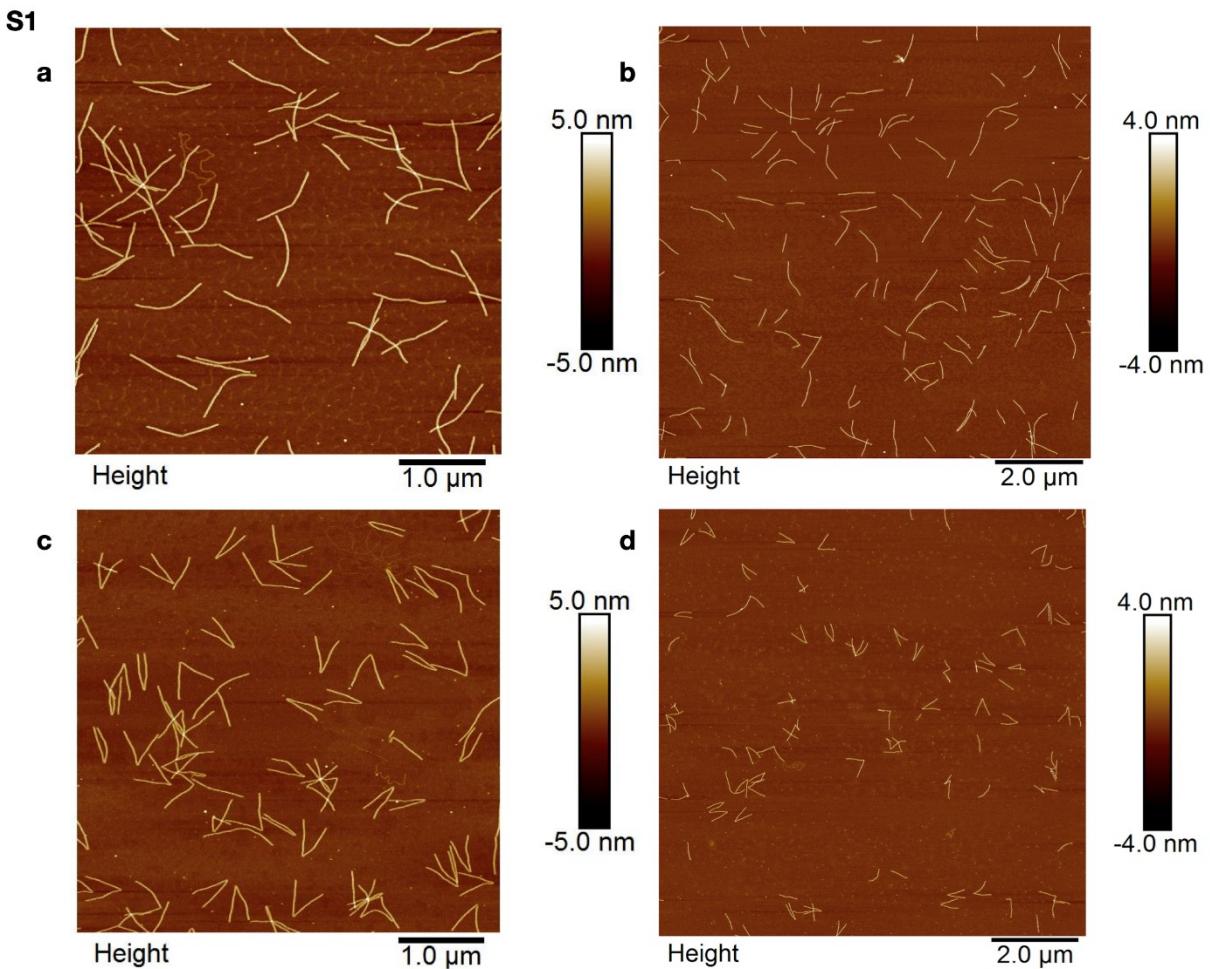


Figure S1. AFM images of logic gate “AND”. (a) and (b) are open geometry with output “0”. (c) and (d) are folded geometry with output “1”.

2. Additional AFM images of one-folding geometry in tri-state buffer logic gate

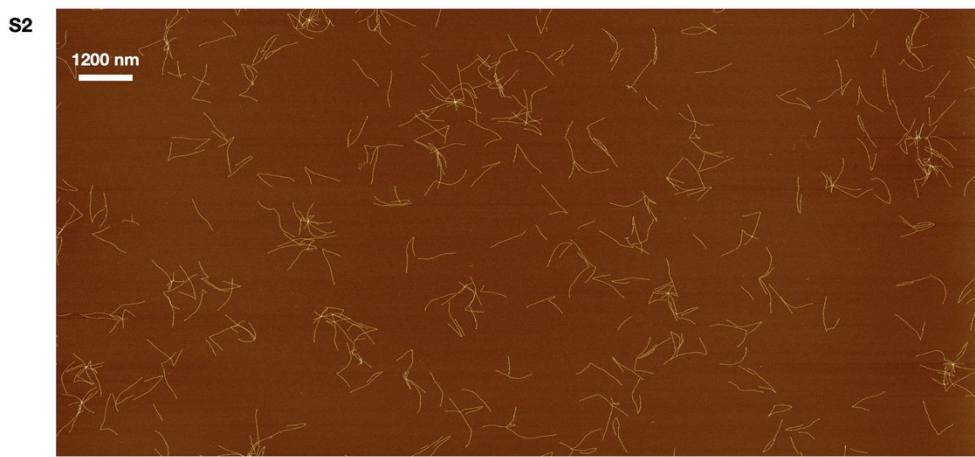


Figure S2. AFM images of one-folding geometry. Excluding non-desired monomer, dimer and non-specific aggregations, the percentage of trimer with one-folding geometry is at least 70% (Statistics was obtained from over 500 DNA origami, the AFM image in the figure is only a representative one). It is safe to set the threshold to be 50% to read the output to be “0”.

3. Additional AFM images of two-folding geometry in tri-state buffer logic gate



Figure S3. AFM images of two-folding geometry. Excluding non-desired monomer, dimer, and non-specific aggregations, the percentage of trimer with two-folding geometry is at least 70% (Statistics was obtained from over 500 DNA origami, the AFM image in the figure is only a representative one). It is safe to set the threshold to be 50% to read the output to be “1”. The entangled origami is ignored in statistics because it does not provide any clear view on individual’s geometry.

4. Additional AFM images of one-folding geometry in tri-state inverter logic gate

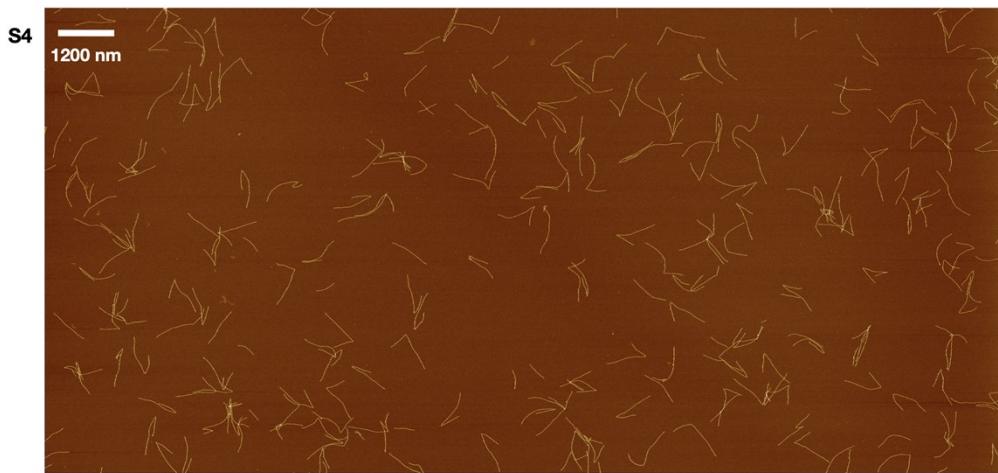


Figure S4. AFM images of one-folding geometry. Excluding non-desired monomer, dimer, and non-specific aggregations, the percentage of trimer with one-folding geometry is at least 70% (Statistics was obtained from over 500 DNA origami, the AFM image in the figure is only a representative one). It is safe to set the threshold to be 50% to read the output to be “0”.

5. Additional AFM images of two-folding geometry in tri-state inverter logic gate

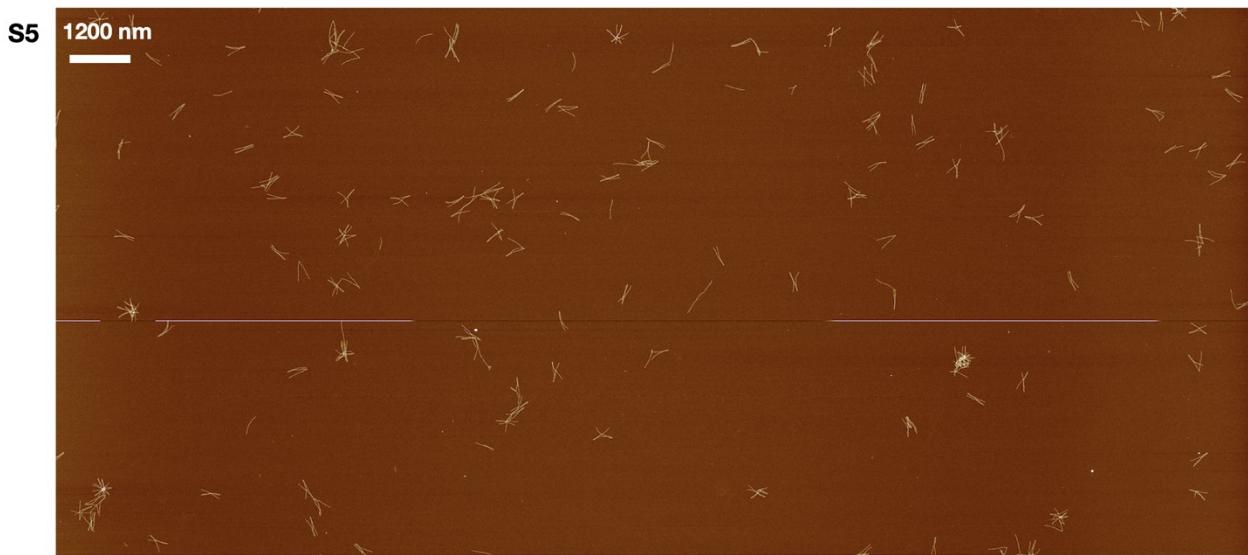


Figure S5. AFM images of two-folding geometry. Excluding non-desired monomer, dimer, and non-specific aggregations, the percentage of trimer with two-folding geometry is at least 70% (Statistics was obtained from over 500 DNA origami, the AFM image in the figure is only a representative one). It is safe to set the threshold to be 50% to read the output to be “1”.

6. Entropy Gain in Folding Left Hinge in Tri-state Buffer Design

In the presence of both enable and input strands in the free solutions, a partial duplex stem with green-green* would form, as the concentration of enable/input (50 nM) is 100 times more than that of the sticky ends on origami hinge (0.5 nM). Then the red* on this partial duplex stem may hybridize with the red on the left hinge, seemingly impeding the folding of left hinge. Let's call this is scenario 1(Fig. S6). However, the folding on the left hinge still forms because of lower Gibbs Free Energy in the folded state due to significantly higher local concentration of the hinge-sticky ends.

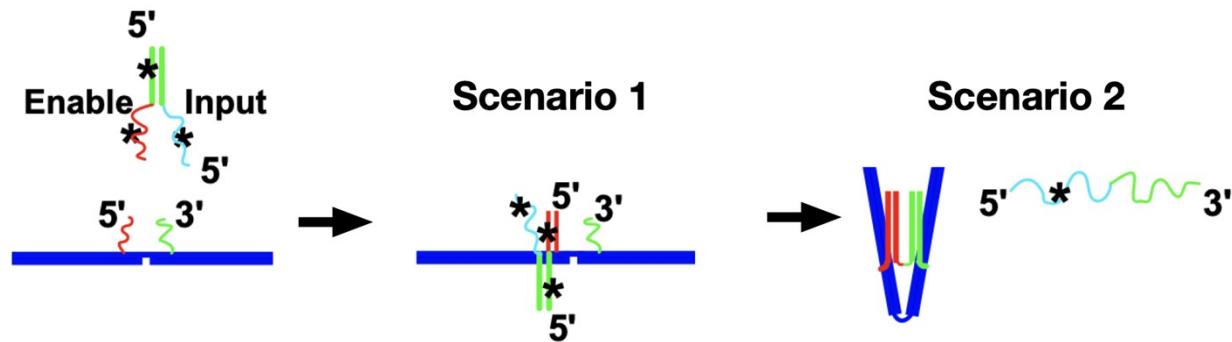


Fig. S6 Schematics of folding process by Enable/Input duplex.

In scenario 2 the folded state, a free enable strand hybridizes with the red and green sticky ends on the left hinge (Fig. S6). It was true that from the enthalpy-hybridization energy point of view, there was no reason for scenario 2 to displace scenario 1, if scenario 1 had taken placed first. However, the local concentration of sticky ends on the hinge is many orders of magnitude higher than that of the input/enable in free solutions, resulting in a great entropy gain to fold the hinge. The concentration of input strand is ~ 50 nM. Notably, one sticky end on the hinge is locally confined within ~ 5 nm³ after folded, giving a local concentration of ~ 0.01 M. Hence, the concentration ratio of the local sticky ends to free input in solution is $\sim 5*10^5$. The entropy linearly depends on the natural logarithm of relative concentrations. In our case, entropy change for transition from scenario 1 to scenario 2 is $\Delta S_{12} = k_B \ln(5 * 10^5) = 13 k_B$. Then $\Delta G_{12} = \Delta H - T\Delta S = -13k_B T$.

With Boltzmann distribution, Probability $\sim e^{-energy/kT}$, 13 k_BT lower in free energy give a factor of $5*10^5$ higher in probability for it to be in scenario 2 rather than in scenario 1.

The same reasoning also applies to the inverter logic gate in Figure 4 when both enable and input strands are present.

7. Factors Affecting Folding Angles

The folding angles in the two folding mechanisms(Fig.1c, d) by design might be a bit different, because the location of the sticky ends connected with the staple strands are different. It was also different that the left hinge was folded by one input strand, while the right hinge was folded by two input/enable strands. For 2-folding state, the angle of right hinge might tend to be more compact than the left hinge. There were many factors to vary the angles we've explored, such as changing the location design of the sticky ends on the hinge (longer or shorter distance away from the hinges vertex), different Mg²⁺ concentrations, the length of sticky ends, the concentration ratios of enable/input to origami, etc. The folding angles indeed seemed to be relevant to all these controlled factors. However, there were also some other not so easy to control factors involving in deposition of origami on the mica surfaces that as well slightly varied the folding angles more randomly, such as mica surface difference in different area, humidity or air flow speed when drying mica, AFM tips properties, etc. While these would be interesting, we have decided not to include the study of angles in this article. Because our major aim is to demonstrate the idea of tri-state logic using DNA origami. As long as the clear difference between folding and not-folding can be clearly told apart, the output signal is unambiguously generated.

8. Control Experiment for Folding Mechanisms

We designed control experiments with poly-T non-specific strands to substitute enable and input strands in the two folding mechanisms. When the substitute enable and input strands were added in the DNA origami dimer, no statistically meaningful number of hinges was found to be folded revealed by AFM imaging.

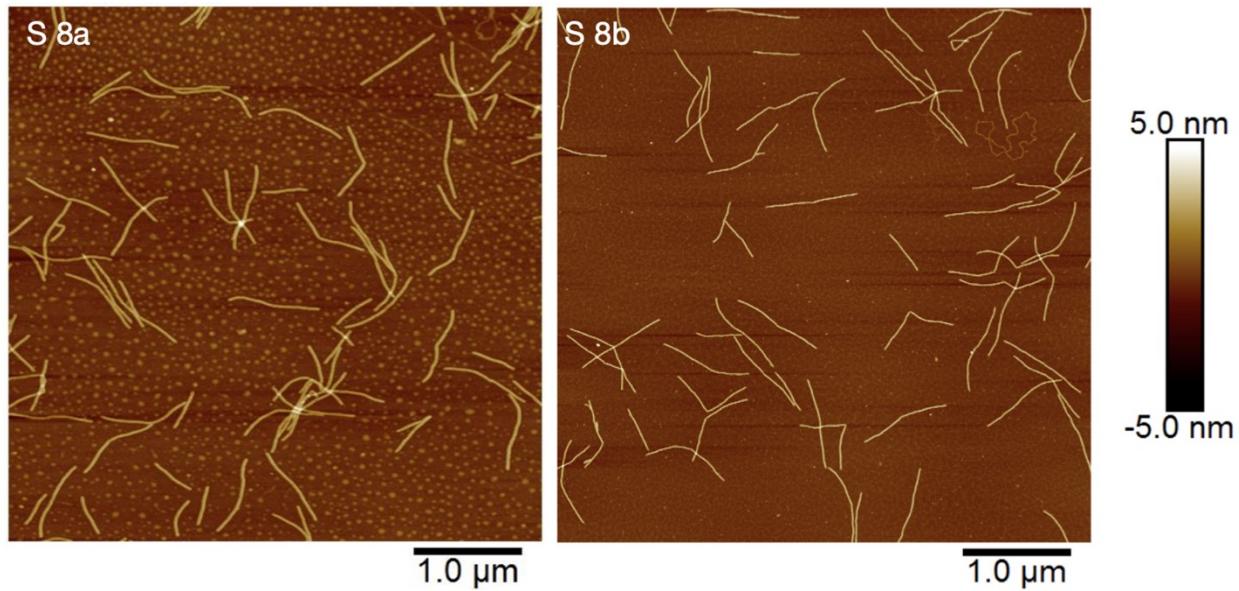


Figure S7. AFM images of control experiments. **a)** DNA origami dimer platform of folding mechanism 1 (as shown in Fig. 1c) was added with non-specific Poly-T strands as control to substitute the input to find no folding at the hinge. **b)** DNA origami dimer platform of folding mechanism 21 (as shown in Fig. 1d) was added with non-specific Poly-T strands as control to substitute the input to find no folding at the hinge.

9. DNA Sequences of 6HB DNA Origami and sticky ends

6HB DNA Origami uses scaffold DNA M13.

Staple strands:

6HBM13-

001 TTATCCGCTCACAAATTCCACACAACA

6HBM13-

002 TACGAGCCGGAAGCTCGAATTGTAA

6HBM13-

003 CGGGTACCGAGCATAAAGTGTAAAGCCTGG

6HBM13-

004 GGTGCCTAACATGAGTGCAAGCTTGCATGCC

6HBM13-

005 TAAAACGACGGCCAGTGAGCTAA

6HBM13-

006 CTCACATTAATTGCGTTGCGCTC

6HBM13-

007 CCTGTGTGCTTCCCAGTCACGACGTTGTGCAGGTCGACTTA

6HBM13-

008 ACTGCCCGAAATTGTCATGGTCATAGCTATCTAGAGGATCCC

6HBM13-

009 AGTTGGGTAACGCATGTGCTGCAAGGCGGTGCTGGCGAAAGG

6HBM13- CGCCAGGGTTTCCAGTCGGAAACCTGGCTATTACGCCATT

010
6HBM13-
011 GGGCCATCGCCATGTGCGGGCCTTCTCGTGCCGGAAGAT
6HBM13-
012 ACCAGGCAAAGCGACTCCAGCCAGCTTGGCCTCAAGCTGCA
6HBM13-
013 GGGAAGGATCGGCCACGACGACAGTATCCCGCACCGCTTAA
6HBM13-
014 TTAATGAGCGATCGTCAGGCTGCGAACGTCTGGTGCCGGAA
6HBM13-
015 CCGTGCATCTGCACTAGATGGCGCATCTGATAAGTCACGTT
6HBM13-
016 GACAGTTGAGGGAACGCGCGGGAGAGACCGTAATGGGTT
6HBM13-
017 GGCATTAAATGTGAACAAACGGCGGATTGGCGGTTCCAATAG
6HBM13-
018 AGCTTCATCAATGCCATCAAAAAATAATTTAATGCGTAT
6HBM13-
019 GATTCTCCAGGGTGTAAATCAGCTCATT CGCGTCTGGCTT
6HBM13-
020 TGGGCGCCGTGGGAGCGAGTAACAACCCTTCTCCTGTAGCC
6HBM13-
021 GTTAAAATCGCGTTGTAAACGTTAATAGTAAGCAAATATT

6HBM13-

022 AGATTAAATTTGGTTTCACGGAAGATTGTATCG

6HBM13-

023 AAAAAACTAGCATGAAGCCCCAAAAACACAGTGAGGGCTATC

6HBM13-

024 AACGGTAATCGTATCATTGCCTGAGAGTCTACAAAACGGGCA

6HBM13-

025 GTTGATAATTGCCCTATTTGAGAGATCTGGAGCAAACAGA

6HBM13-

026 ACAGCTGATCAGAACATCAATCATATGTACCTAGAGAACATCGATG

6HBM13-

027 TAAATTAATGCCAGTTCAACCGTTCTAGCCCACCATCAATAT

6HBM13-

028 GGGGAGAGGGTAGCTCACCGCCTGGCCAGACAGTCAAATCG

6HBM13-

029 GATTAAATGCAATGGTGAGAAAGGCCGGCTGAGAGTTTGCG

6HBM13-

030 CCCTCATATATTAAAGCCTTATTCAGTAATAACAGTTGCA

6HBM13-

031 AAAGATTGTCCACGAACATTATGACCCTACGCAAGGATAACA

6HBM13-

032 GCAAGCGCAAAAGGCCTGAGTAATGTGTAGAAATTTTAGAA

6HBM13- TAAAGCTAAATCTCCAATAAGCCTCAGAGAATTAGCAAAAT

033
6HBM13-
034 TTGGTTGTACCAAACTGGTTGCCCGAGGGCAAGGCAGGAAAGGT
6HBM13-
035 TAGAAAAGGTGGCACAATAATCATACACAGGCGAAGATACA
6HBM13-
036 GGGGCGCGAGCTAGGCAAATGGTCAATAGACCATTAAATCCT
6HBM13-
037 GTAGCATGGTGGTTAGTAGATTAGTTACCTGTTAGCTAC
6HBM13-
038 GTTGATTAACATCTCAATTCTACTAATTAATATTTCATTT
6HBM13-
039 AGTTGATTCCCACAAGTTCAATTCCATAAGAAGTACGGTGTC
6HBM13-
040 AAATTCTGCGAACGCCGAAATCGGCAAAAATATGCAACTATA
6HBM13-
041 TGTTGCGGATGGCCTAACATGTTAACCTTAAACTCC
6HBM13-
042 TAAGAGGTCATTGAGGTAGGATTAGAGCGGAAGCATAAATC
6HBM13-
043 ATATAATTAGCCCCAAAGCGAACCGACAGTACCTTAATAA
6HBM13-
044 AAAAGAAGCTGTAGTTAGAGCTTAATTGTCTGCTCCTTTGA

6HBM13-

045 ATATCGCGTTTGTAAAGCCGAAAGACTCTCAAAAAGATTAA

6HBM13-

046 TCAATTGAGCTTCAGATAGGGTTGAGTAGCGGATTGCATGA

6HBM13-

047 GAGAGAATGACCATATAGTCAGAAGCAAGTTGTTCTGCGGAA

6HBM13-

048 AGTCAGAAAACGGCATAAATATTCCAATACCAGTTG

6HBM13-

049 TTACCCTAGTCCACAGACTGGATAGCGTGAATCCCCCTCACC

6HBM13-

050 GAACAAGGACTATTAAATCAAAAATCAGTGAATGCTTAAAC

6HBM13-

051 AGAGGGGTAATCATGCAAAAGAAGTTGTAATAGCGAGAGG

6HBM13-

052 ATAGTAAAATGTTTATTAAAGAACGTGGATAAAAACCAACT

6HBM13-

053 CTAAGGAATTACGAGTTACCAGACGACGACTCCAAAGATT

6HBM13-

054 ACATAACGCCAATTGTTGAGATTAGGAAGGTAGAACGTCAA

6HBM13-

055 CTATCATAAAACCGAACACATTATTACATACCACATTCACT

6HBM13- AGGGCGAAACCCTCGGCATAGTAAGAGCAAACTAATGCAGAT

056
6HBM13-
057 TACGTTAATAAACAAACGTTGGGAAGAAAAACATTATACCACT
6HBM13-
058 CTACGAACTAACGGTCTATCACTAAATCTAAGAACTGGCTCA
6HBM13-
059 CATTGGGCTTGAGAACCTTATGCGATTGGAACCAAAGCTG
6HBM13-
060 ACGAGTAGTAAAGGTTCACTGAATAAGGACGTAACAAAGGG
6HBM13-
061 AATCATTGATTAGCATTACCCAAATCACTGCCCTGACGAG
6HBM13-
062 AGCCCCCGTGAATTGGTTAATTCAAAGAGAACACCAGA
6HBM13-
063 TAATCTTGACAAATGCTGACCTTCATCACTCCAGGCGCATAG
6HBM13-
064 TGGAACCGGATATTAGCTTGACGGGGAAACGGTGTACAGATT
6HBM13-
065 GCGCGCAGACGGTCAAGAGGACAGATGAAGCCGGCCATCGCC
6HBM13-
066 AGCCGGAACGAGTGAAATTGTGTCGAAATTGTATGAACGTG
6HBM13-
067 CTGACCAAGGAAGGAAGTACAACGGAGATCCGCGACCTGCC

6HBM13-

068 GCGAGAAACTTGAAATCATAAGGGAACCTCCATGTTACTT

6HBM13-

069 AGCGATTATACCACCACTCATCTTGACCGAAAGAATACACT

6HBM13-

070 GAAAGCGCGAACAGAACAGAAAGCGAAAGACGAAAGAGGGCAA

6HBM13-

071 AAGGAAGTTCCATGGCACCAACCTAAAGAGCAGGGAGCATCG

6HBM13-

072 ACTTTTCATGAAAGAGGGTAGCAACGGAAAGACCGCTAGG

6HBM13-

073 AATGCCACAAGTGTTCACCCCTCAGCAGCCTACAGAGGCTAG

6HBM13-

074 GCGCTGGCTACGAATAACGGTAAAATGGTGAGGACTAAAG

6HBM13-

075 TTAAAGGCCGCTAAGCTGAGGCTTGCAGACACCGATATATT

6HBM13-

076 AATTGCGGGATCGAGCGGTACGCTGCCGCCACGCATAGT

6HBM13-

077 GGAGGTGAATTCTATGACAACAACCATGCGTAACTCTCAA

6HBM13-

078 AGCTTGCTTCGTCAAGGCTCCAAAGGTTGAAAACACCACA

6HBM13- TAGTTGCCGCTTAAATAATTTTCACGAGCCTTAATTGAG

079
6HBM13-
080 CCCGCCGGCCGACATAAACAGCTTGATAAATATCGGTTATC
6HBM13-
081 GAACAACTAAAGTACGGAGTGAGAATAGCCTTAACAGTT
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082 AGGAATTGCGAATATGCCCGCTACAGGTTGCTAAACAAGA
6HBM13-
083 TCACGATCTAAAGTTTCTGTATGGGATGCGCGTACGTCACC
6HBM13-
084 ATAGTTAGCGTAAGCAAACACTACAAACGCCTGAGTTCTATGGT
6HBM13-
085 GTTAGTAACGAGCAATGTACCGTAACACTGTAGCATTCCAAG
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086 TGCTTGAAATGAATTTGTCGTCTTCCTCCAGACAGCCCTC
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6HBM13-

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6HBM13-

094 GTGAGGCTGAGACTACAGGAGGCCGATTACAGTTAATGCCGG

6HBM13-

095 CGAGTGTACTGGTACAGTGCCCGTATAAAAAGGGAAAGCGCA

6HBM13-

096 TGATGATACAGGGACTGAATTACCGTTGAATGGATTTAGA

6HBM13-

097 AGTGCCTGGTACGCCCTCATTAAAGCCACCAAGTAAGCGTCGC

6HBM13-

098 CAGGAACTGAGTAAATAAGTTAACGGTGATACATGGCTTT

6HBM13-

099 CTTGATATTACCCAGCAGGTCAAGACGATGGTTGACAGGAGGT

6HBM13-

100 ATAAACAAATAATCAGAATCCTGAGAACCGCCGCCAGCATC

6HBM13-

101 TGAGAACCGCCACCAACCACCAAGAGGTGTTAATCAA

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102
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6HBM13-

114 AAACGCAAAGACCGGTAGAAAATACATACGCAGTACATCACT

6HBM13-

115 AATAGAAGTAGAAGAAGACTCCTTATTACATAAAGGTGGCGA

6HBM13-

116 TGCCTGAAATTCATGTTATTTGTCACCGAACATATAAAAG

6HBM13-

117 ATACCCAAAAGAGAAAACGCAATAATAAACAGAAGGAAACC

6HBM13-

118 CAACTGGCATGATTAACCTAAACTATCGGAACAAAGTTACTC

6HBM13-

119 GAATGAAATAGCAAGTAAGCAGATAGCCGCCTGCCTAATAT

6HBM13-

120 GAGCAAGAACAGGGAGATAACCCACAATTGAGCGTGGTAAT

6HBM13-

121 CCTTTTACAATATAAGTCAGAGGGTAAGAATTGAGTTAAGG

6HBM13-

122 ATCCAGAAAGAAAATAGCTATCTTACCGCGGCCAATAATAA

6HBM13-

123 AGAATTAAC TGAGCGGAAGCGCATTAGAAAAATAACATAAAA

6HBM13-

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6HBM13- ACTATCCCAATCCAATGAAAATAGCAGCGAACAGCGCTAAC

125

6HBM13-

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6HBM13-

127 GTTTAACGAAATACTTTATCCTGAATCTAATTGCCAGTAG

6HBM13-

128 GCTCATGGTCAAAAAATAAGAAACGATTACAAAATAAAC

6HBM13-

129 TTGCTATTTGCCAGCCTAAATCAAGATTGCGGGAGGTTT

6HBM13-

130 AAACCCAGCTACAACATACATTGACGCAACCTCCGACTTT

6HBM13-

131 TGAAGCAAATCAGAGAGGCCTTTAGCGTCAATCGCATCGAG

6HBM13-

132 GCGCCAATAGCAAAGCAAGCCGTTTCCGCACTCTGAAA

6HBM13-

133 TATTCTATTAACATTATTAAACCAAGTAATTCATCGTATC

6HBM13-

134 TGGATTAAGAACGCTATAGAACGGCTATTGGGAATCATTACC

6HBM13-

135 TTTCCTTATCATAAAATCAATAATCGGCCCGAGCATGTAGA

6HBM13-

136 AATCCAAGAACGGTGGCAGATTACCCACATCCTAATTAGG

6HBM13-

137 AATGCAGAACGCGCGAAAAATAATATCCGTACACTACCGAC

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138 ATGTCAGCTAACCGTAAAGTAATTCTTATAAAGGACCACT

6HBM13-

139 TAAGTCCGGGACATCAGTAATAAGAGAAGTCCAGACGACGTA

6HBM13-

140 AATAAAATGAACAACGTAACTGTTATCAACAATGACAATAAACAC

6HBM13-

141 ATTAGGCAGAGAATAACAAACGCCAACATATGAGAATCGCCA

6HBM13-

142 TAGCATTTCGAGCTCTGGCCAACAGAGGTAGGGCTTAATGA

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6HBM13-

144 ACTAGAAAAAGCTTAAGGCGTTAAATATACCGACCCTCTG

6HBM13-

145 CTTACCAAGCGTAATTAATGGTTGAAAAGAACAAACACCAAG

6HBM13-

146 ACCTGAAGTATAAAATATCGTTATACATTGGAATCATAATT

6HBM13-

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6HBM13- CTTCTGACCTAAATGAATACTGGCACAAATCGCAAGACAATT

148

6HBM13-

149 AATTTAACCTCCGGCTGATGCAAATCCAGACAATATAAGACG

6HBM13-

150 AGAGACTACCTTCTGAAGAGTCATAGTCTTAGATTTTTGA

6HBM13-

151 ACTATATTAGTCTAACATAGCGATAGGAATTATCAAAAAA

6HBM13-

152 ATGGCTAGTAAATGCTTAGGTTGGGTATTATCATAGGTCTG

6HBM13-

153 TTAATTTCCTAAGTAAATCGTCGCTATAGAATAACCTTGC

6HBM13-

154 TCTAGAACATCCTTGATTAATGCGCGAACTATATATGTGAGTTA

6HBM13-

155 TTATTACATTAAACCACTACATAATCAGATAGCCATTCAATT

6HBM13-

156 AAAACAAAATTACTTACCTGAGCAAAAGCGAATTCTAAAAC

6HBM13-

157 TTTTTATTAAAAAAATCGCGCAGAGGAAGATGATGAACC

6HBM13-

158 ATCGCCAATGGAAAAATTCACTTGAATCGACAAACATCAAG

6HBM13-

159 TGATTGCTTGAATAACAATAACGGATTACTTTACATCGG

6HBM13-

160 TTATACCAAGTTACTACCGAACGAACCACAGTAACAGTACCC

6HBM13-

161 GACGTAAAACAGAACAGTCAGATGAATATAACCAGCAGGTTGGA

6HBM13-

162 AAATTATTGCAGAACTTCTGAATAATGCCTGATTAAGATAA

6HBM13-

163 TTTTCAGGTGAGGCTTCATCAATATAATGAAGGGTTAGAATA

6HBM13-

164 AACAGAGGTTAACATAAAGAAATTGCGCACCTACCATATCA

6HBM13-

165 TTCCTGATTATCTAAGCGGAATTATCATTAAAGAAACCACAG

6HBM13-

166 GAAGATGATGGCAAGGTCAAGTATTAAACATTGCGGAACAAGA

6HBM13-

167 AACGTATTAAATCCAGTAACATTATCATCCGCCTGGTCAATA

6HBM13- AATTCGACAACGGATACATTGAGGATTAGAGGCCAACAGTGCCACG

168 CAAGTTG

6HBM13- CAATCAATATCTGGTCAGTTGTAATTAAATGAGAGCAACAACTAATA

169 GAT

6HBM13-

170 TTTGCCCGAACGTTATGCAAATCAACAGTTAAACCCCT

6HBM13- ATCGAAAGGAATTGAGGAAGGGACTTACAAAC

171

6HBM13-

172 TTAGAAGTATTATTATCTAAAATATCCTCAAAT

6HBM13- TCACCTTGCTGAACTTAGGAGCACTCAGCAGCAAATGAAAAATCTAA

173 AGCA

Sequences Design of Sticky Ends, input and enable strands:

6H-D3-169-4T

TTTT CAATCAATATCTGGTCAGTTGTAATTTAAAAGTTG

6H-D3-168

AATTCGACAACCTGGATACATTGAGGAT

Ci-CN0-168+

CTCGTCCTATGTCCAGATTAA T

TAGATTAGAGCCCCAACAGTGCCACGCTGAGAGCAACAACTAA

6H-CN0-3T-173

GGTAAGGTGGTCGATACTCCTC TTT TCACCTTGCTGAACTTAGGAGCACT

6H-CN0-3T-174

CAGCAGCAAATGAAAAATCTAAAGCA TTT GGTATTGTTCCAGACGGT

6H-CN1-002

GAGGAGTATCGACCACCTTACC TACGAGCCGGAAGCTCGAATTCTGAA

6H-CN1-003

CATAAAAGTGTAAAGCCTGG ACCGTCTGGAACAATACC

Ci-CN2-008 (also CN1)

ACTGCCGAAATTGTCATGGTCATAGCTATCTAGAGGATCCC CGGGTACCGAG T
GAGCTGGAGAGTCTAATCTAA

Cir-Enable (strand 2*1*)

TTAGATTAGACTCTCCAGCTC TT TTAATCTGGACATAGGACGAG

6H-CN1-3T-173

TGCTGCTCCTAAATTCTCGTAT TTT TCACCTTGCTGAACCTTAGGAGCACT

6H-CN1-3T-174

CAGCAGCAAATGAAAAATCTAAAGCA TTT AATTAAGCAATTGGTAT

6H-CN2-002

ATACGAGAATTAGGAGCAGCA TACGAGCCGGAAGCTCGAATTGTAA

6H-CN2-003

CATAAAGTGTAAAGCCTGG ATACCAAATTGCTTAATT

Buf-CN2-008 (strand 3)

ACTGCCGAAATTGTCATGGTCATAGCTATCTAGAGGATCCC CGGGTACCGAG T
GAGCTAAAGTCGTGTGTTAAG

Cir-inp-Buf (strand 3*2)

CTTAACACACGACTTAGCTC TT GAGCTGGAGAGTCTAATCTAA

Inv-CN1-168+ (strand 41)

CCTAACTATCTTAGCCAATG CTCGTCCCTATGTCCAGATTAA T
TAGATTAGAGCCAACAGTGCACGCTGAGAGCAACAACCAA

Ci-inp-Inv (strand 1*4*)

TTAATCTGGACATAGGACGAG AA CATTGGCTAAAGATAGTTAGG

Cir-inp-Inv (strand 1*4*)

TTAATCTGGACATAGGACGAG CATTGGCTAAAGATAGTTAGG