

Supporting Information

Direct and real-time observation of rotary movement of a DNA nanomechanical device

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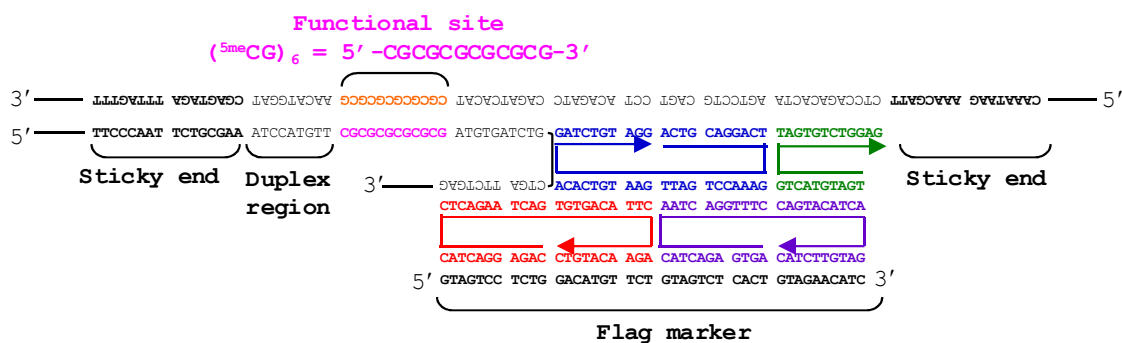
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Rotor



Stator

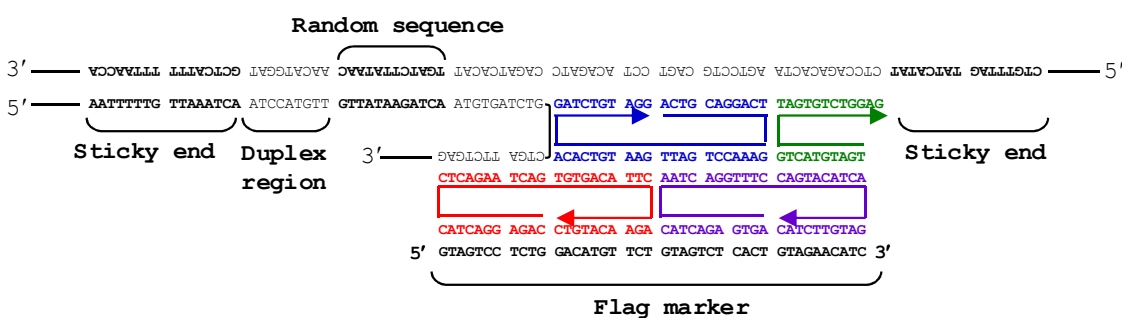


Figure S1. The designs of the functional rotary device and non-functional stator. The difference between the rotor and stator was the sequence at the functional site: $d(^{5\text{me}}\text{CG})_6$ was used for the rotor construction and random sequence was incorporated for stator.

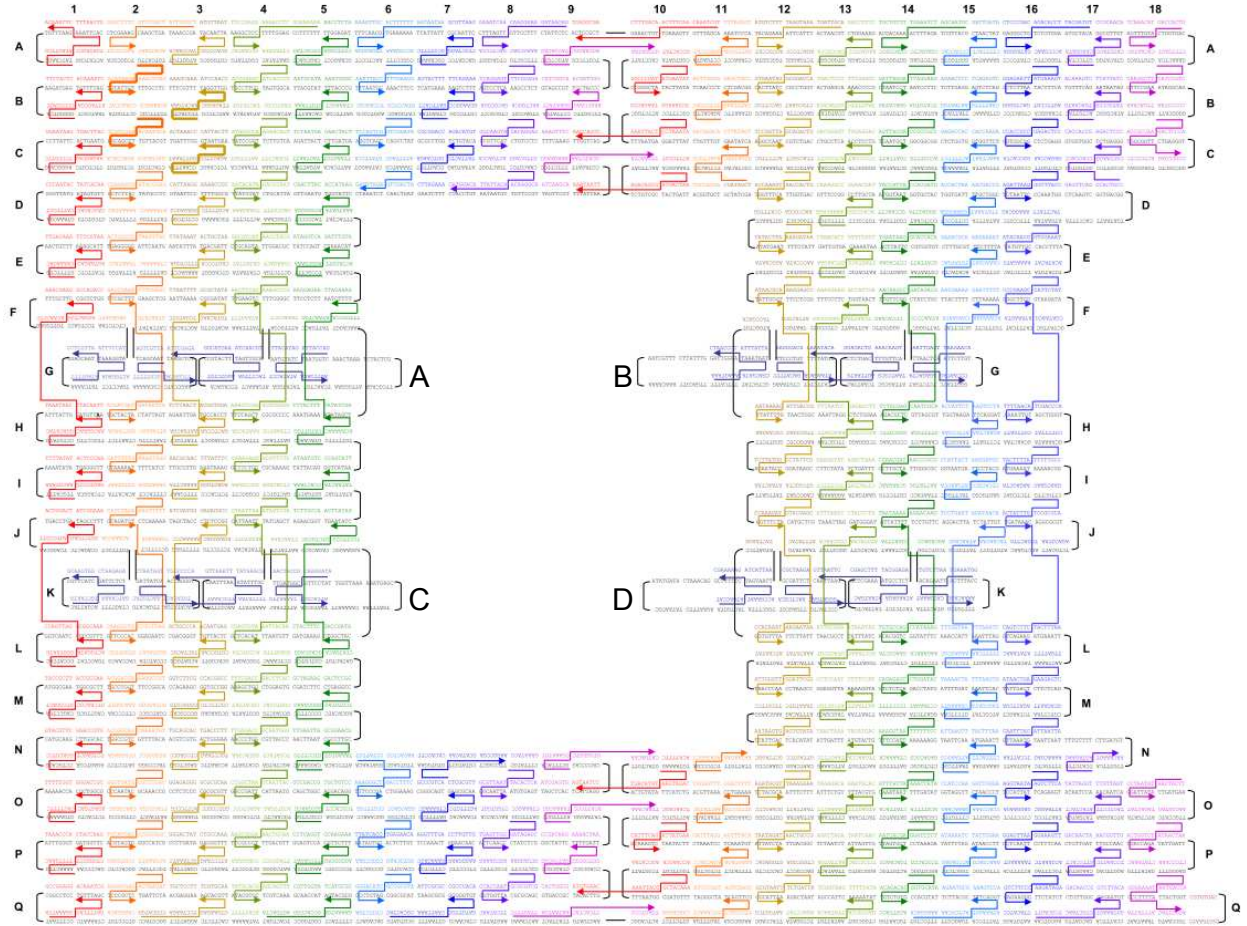


Figure S2. The design of the frame-shaped DNA origami. M13mp18 viral genome and staple strands are given in black and color fonts, respectively. The arrows indicate the staple strands and their direction. Staple strands indicated by bold arrows (top left corner) are hairpin-incorporated strands. A, B, C, and D at the middle of the frame are the loops where the rotor (A-B) and stator (C-D) can be connected.

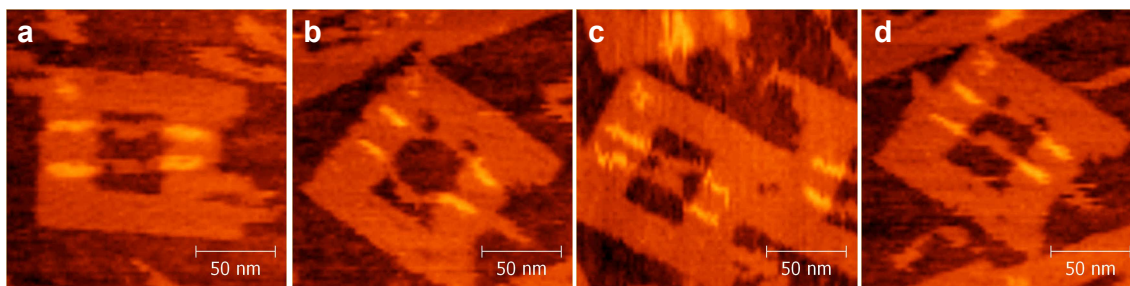


Figure S3. Representative AFM images used to calculate the yields of the relative orientations of the flags. a) Both rotor and stator flags face downward, b) stator flag face downward while rotor face upward, c) rotor face downward while stator moved to upward, d) both flags orient in upward direction. The yield data are given in main text Figure 2. $[\text{Mg}^{2+}] = 10 \text{ mM}$.

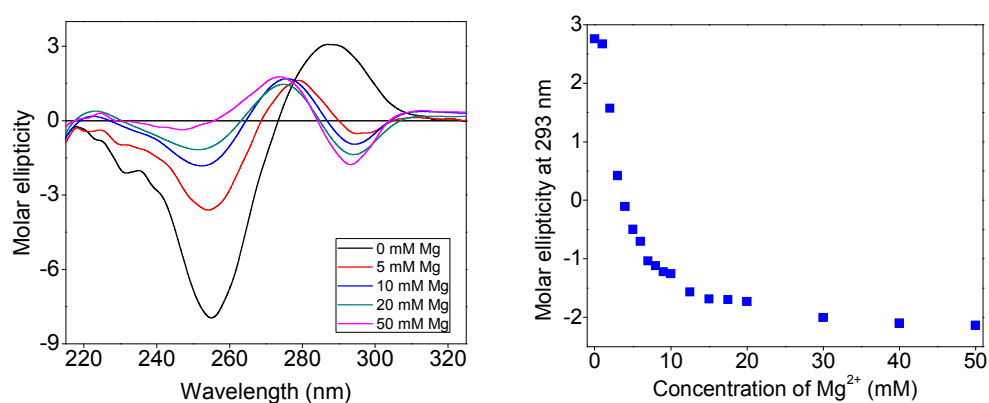


Figure S4. a) CD spectra of $\text{d}(\text{5meCG})_6$ in the absence and presence of various amounts of MgCl_2 . b) The plot of the concentration of MgCl_2 vs the molar ellipticity at 293 nm. Molar ellipticity in $10^5 \text{ deg cm}^2 \text{ dmol}^{-1}$. $[\text{dsDNA}] = 2 \text{ }\mu\text{M}$; $[\text{TrisHCl}] = 20 \text{ mM}$ (pH 7.6); $[\text{MgCl}_2] = 0\text{-}50 \text{ mM}$; path length = 1 cm.

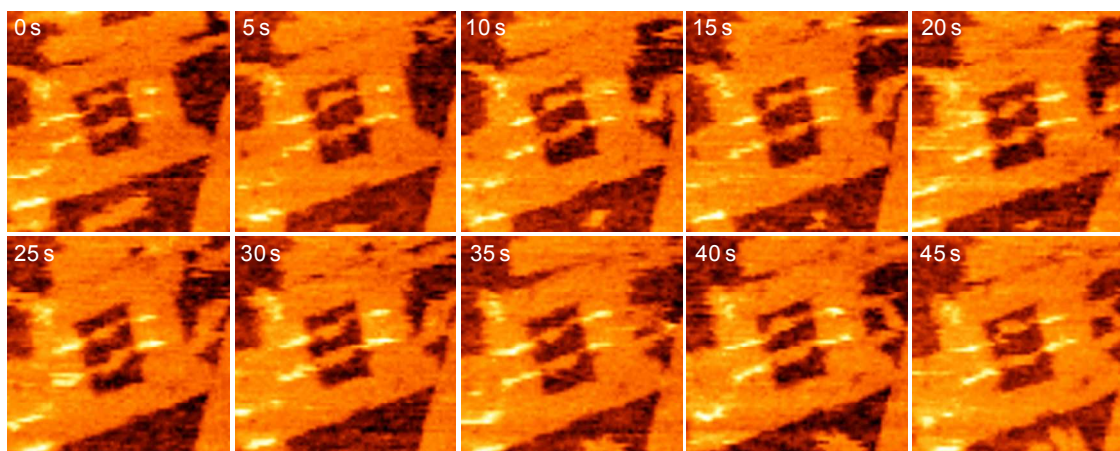


Figure S5. Time lapse HS-AFM images recorded at a scan speed of 0.2 frame/s. $[\text{TrisHCl}] = 20 \text{ mM}$ (pH 7.6); $[\text{Mg}^{2+}] = 10 \text{ mM}$; image size: $150 \times 150 \text{ nm}$.

Sequences used for the construction of rotor:

Name	Sequence (5' ----- 3')
3DTCO-1	ACTG CAGGACT GAAACCT GATT GAA TGTCACA GATCTGT AGG
3DTCO-2	CAGA GGACTAC CTCAGAA TCAG TGTGACA TTC AGA ACATGTC
3DTCO-3	AGTG AGACTAC AATC AGGTTTC CAGTACATCA GATGTTCTAC
3DTCO-4	GTAGTCC TCTG GACATGT TCT GTAGTCT CACT GTAGAACATC
3DTCO-5	TGATGTACTG TAGTGCTGGAG
3DBZ-S1	CAAATAAG AAACGATT CTCCAGACACTA AGTCCTG CAGT CCT ACAGATC CAGATCACAT XGXGXGXGXGXG AACATGGAT CGAGTAGA TTTAGTTT (X = ^{5me} C)
3DBZ-S2	TTCCCAAT TCTGCGAA ATCCATGTT XGXGXGXGXGXG ATGTGATCTG CTGA TTCTGAG (X = ^{5me} C)

Sequences used for the construction of stator:

Name	Sequence (5' ----- 3')
3DTCO-1	ACTG CAGGACT GAAACCT GATT GAA TGTCACA GATCTGT AGG
3DTCO-2	CAGA GGACTAC CTCAGAA TCAG TGTGACA TTC AGA ACATGTC
3DTCO-3	AGTG AGACTAC AATC AGGTTTC CAGTACATCA GATGTTCTAC
3DTCO-4	GTAGTCC TCTG GACATGT TCT GTAGTCT CACT GTAGAACATC
3DTCO-5	TGATGTACTG TAGTGCTGGAG
3DBZ-C1	CTGTTTAG TATCATAT CTCCAGACACTA AGTCCTG CAGT CCT ACAGATC CAGATCACAT TGATCTTATAAC AACATGGAT GCTCATT TTTAACCA
3DBZ-C2	AATTTTTG TTAAATCA ATCCATGTT GTTATAAGATCA ATGTGATCTG CTGA TTCTGAG

		Sequence of the DNA origami frame (5'-----3')
1	1A	CTCATCTTTTTTGCTTGATACCGATAGTGTGAATTCTTAAACA
2	1B	GAATAAGGTTTTTGACCCCCAGCGATTACTAAAACA
3	1C	CATAACCCTTTTCTTGCCCTGACGAGAACATTCAGT
4	1D	AAACAGTTTTTTTCGTTTACCAGACGACAACACTAT
5	1E	GAAGCAAATTTTCAGAAAACGAGAATGAAATGCTTT
6	1FH	TATATTTTCATACAGGCAAGGCAATTAACATCCAATAAATCTCCAACAGGTCAGGACCAGACCG
7	1I	TCAGGTCATTTTAAATGCAATGCCTGAGAACCTCA
8	1JL	TTGCCATGTAATGGGATAGGTCAAACGGCGGATTGACCTTGCTGAGAGTCTGGAAAGGCTA
9	1M	CTTGCATGTTTTTCAGGCTGCGCAACTGAAGCGCCA
10	1N	TGGTTTTTTTTCTGCAGGTCGACTCTGTGCCAAG
11	1O	ACCCAAATTTTTCTTTTACCAGTGAGACGCCAGGG
12	1P	GGAGGCCGTTTTCAAGTTTTTTGGGGTCGAACCATC
13	1Q	ATTAAAGGATTTTAGGCTAAACA
14	2A	TCGGTTTATCAGCTTGCTTTCGAGTGCGCCGA
15	2B-HP	CAATGACAGAGGCAAATCCTCTTTTGAGGAACAAGTTTTCTTGTAATAACATACCAAGC
16	2C-HP	GCGAAACAACGTAACATCCTCTTTTGAGGAACAAGTTTTCTTGTAAGCTGCTACACCAGA
17	2D	ACGAGTAGGAGGCATAGTAAGAGCGATAAAAA
18	2E	CCAAAATACATTGAATCCCCCTCACCATAAAT
19	2FH	CAAAAATCCGAGCTTCAAAGCGAATTAGAGAGATATAATGACTAATAGTAGTAGCAAGAATTAG
20	2I	CAAAATTAGGATAAAAAATTTTAGTAATGTGT
21	2JL	AGGTAAAGTTTTTGAGAGATCTACAGCAACAAGAAAAGCGATTCTCCGTGGGAACCGTTGGTG
22	2M	TAGATGGGTGCCGGAACAGGCATTGGGAAG
23	2N	GGCGATCGTGTAACACGACGGCCAAGAGGATC
24	2O	CCCGGTACGGTTTGCCTATTGGGCGGGCAAC
25	2P	AGCTGATTCGATGGCCCACTACGTGAGGTGCC
26	2Q	GTAAAGCATAGAATCAGAGCGGGAACAGGAAC
27	3A	AAACGAAAACAACCATCGCCACGTAATTGTA
28	3B-HP	CCAAATCAAAGTACAATCCTCTTTTGAGGAACAAGTTTTCTTGTCGGAGATTCCAACCTA
29	3C-HP	GGAATTACTAAATTGGTCCTCTTTTGAGGAACAAGTTTTCTTGCTTGAGATTCATTAC
30	3D	TAAATATTGCGAGAGGCTTTTGACGCCAAAA

31	3E	TTTAAATTAGGTCTTTACCCTGACAATCGTCA
32	3FH	CAACGCAAAGCAATAAAGCCTCAGAGGTGGCATCAATTCTCTGTAGCTCAACATGTATATCGCG
33	3I	GGTAGCTAATTCAAAGGGTGAGACTTTATTT
34	3JL	GCTTCTGGCGCATCGTAACCGTGCAGTAACAACCCGTCGCCCAAAACAGGAAGACCGGAGAG
35	3M	CACGACGTGTGCGGGCCTCTTCGCCCCGGCACC
36	3N	GGGAGAGGCCGAGCTCGAATTCGTTTCCCAGT
37	3O	TATCAGGGGCCCTTACC GCCTGGAACGCGCG
38	3P	TTCCTCGTCTAAATCGGAACCTAAAACCGTC
39	3Q	GGTACGCCAGAATCCTGAGAAGTGAACGTGCT
40	4A	AAAAAAGGCTCCAAAAGGAGCCTTCATAACCG
41	4B	ATATATTCTGCCACTACGAAGGCATGTATCAT
42	4C	CGCCTGATTGACAAGAACCGGATATGGTTTAA
43	4D	TTTCAACTTAATGCAGATACATAAAAAGAAGT
44	4E	TTTGCCAGGCGTCCAATACTGCGGTATTATAG
45	4FH	TCAGAAGCGCCCCGAAAGACTTCAATTTAAATATCGCAAATGGGGCGCGAGCTGAAAAGCATAAA
46	4I	GCTAAATCCTTTTTCGGGAGAAGCAAGGCCGG
47	4JL	AGACAGTCAGCTGATAAATTAATGTTGTATAAAAAATAATTAACATTAAATGTGAGCATCTGCCA
48	4M	GTTTGAGGCACTCCAGCCAGCTTTTATTACGC
49	4N	CAGCTGGCGGGTAACGCCAGGGTTAATCATGG
50	4O	TCATAGCTCATTAATGAATCGGCCCCCTGAGA
51	4P	GAGTTGCAAACGTCAAAGGGCGAAAAGGGAGC
52	4Q	CCCCGATTTTTCGAGGACGACGTATTTTTTATA
53	5A	ATACGTAAGGTCGCTGAGGCTTGATCTCCAA
54	5B	AGTAATCTAAATTGTGTCGAAATCCGGGTAAA
55	5C	CATTCAACTTAATCATTGTGAATTCATCAAG
56	5D	ACTGGATAAGGGGTAATAGTAAATTGAATACCA
57	5E	AAGAGGAAAAAGCGGATTGCATCATTATGTTTAG
58	5FH	CTGTAATAGGTTGTACCAAAAACAAGCTATATTTTCATTTGGTCAATAACCTGTTTAAAAGATT
59	5I	ACCGTTCTAAATCACCATCAATATAGACCTGA
60	5JL	GAAGATCGGGACGACGACAGTATCGTAGCCAGCTTTCATCCGCGTCTGGCCTTCTGATATTCA
61	5M	ATTAAGTTGAAAGGGGATGTGCTGGACGTTT
62	5N	GCCAGCTGGTTTCCTGTGTGAAAT

63	5O	TGGACTCCGCAAGCGGTCCACGCTCCTGTCTGT
64	5P	ATGGTTGCTAGAGCTTGACGGGGAAAAGAACG
65	5Q	ATCAGTGAGGCCACCGAGTAAAAGCGCGTACT
66	6A	AATAATAATTTTTTCACGTTGAAAAGGGAGTT
67	6B	AAAGGCCGGGAAGTTCCATTAAACGCGACCT
68	6C	GCTCCATGATAGGCTGGCTGACCTACCTTATG
69	6D	CGATTTTAATCAGTTGAGATTTAG
70	6O	TGTTATCCGCTCACAACTTCCAGTCGGGAAAGGTTTGCC
71	6P	CCAGCAGGACAAGAGTCCACTATTAAGCCGGC
72	6Q	GAACGTGGATGCGCCGCTACAGGGAGTCTGTC
73	7A	TTTCATGACTTTTGCGGGATCGTCGAATTGCG
74	7B	CCAGGCGCTTACTTAGCCGGAACGAAAGACTT
75	7C	AAAGATTCAGAACTGGCTCATTATGTACAGA
76	7N	ACTGCCCCGTTCCACACAACATACGAGCCGGAA
77	7O	AGTTTGACGAAAATCCTGTTTGATTGCGCTC
78	7P	CGCGCTTACGAGAAAGGAAGGAAGTTGTTC
79	7Q	CATCACGCAAATTAACCGTTGTAGACACCCGC
80	8A	GAGAATAGAAAGGAACAATAAGACCCTCAG
81	8B	CAGCGAAAAGAGGCTTTGAGGACTAGGCGCAG
82	8C	ACGGTCAAGAGGACAGATGAACGGACCAGTCA
83	8D	GGACGTTGACGGAACAACATTATTACAGGTAG
84	8O	GCATAAAGACTCACATTAATTGCGTGGTGGTT
85	8P	CCGAAATCCGAGATAGGGTTGAGTGAAAGCGA
86	8Q	AAGGAGCGGCTGCGCGTAACCAACCAATACTT
87	9A	ACGGCTACGACAGCATCGGAACGAAATAGGTG
88	9B	CTTTGAAATCATAAGGGAACCGAAGGGTAGCA
89	9C	ACGAACTAGGAAGAAAAATCTACGATCAAGTT
90	9N	GTGAGCTATGTAAAGCCTGGGGTGAACAGTAC
91	9O	GAATAGCCGGCAAAATCCCTTATAAAACAATT
92	9P	GCGGTCACGGCGCTAGGGCGCTGGAATCAAAA
93	9Q	CTTTGATTAGTAATAACATCACTTGCCTGAGT
94	10A	TGCTAAACAACCTTCAACAGTTTCAGCGGAGT

95	10B	TATCACCGTATAAGTATAGCCCCGGGCCAGAAT
96	10C	GGAAAGCGATAAATCCTCATTAACCTGACCAA
97	10D	TGCCTTTAAATCAGTAGCGACAGATTAATAAA
98	10O	CTTTTACACAGATGAATATACAGTCCTAATGA
99	10P	CGACAACCTAAGTATTAGACTTTACAATACCGA
100	10Q	ACGAACCAAAACATCGCCATTAAACAAGTGTA
101	11A	AGGGTTGATACTCAGGAGGTTTAGTGGGATTT
102	11B	ACAAACAACAGTCTCTGAATTTACCCGTCGAG
103	11C	TCGATAGCAGCACCGTGCGTCAGACTGTAGCGTGATATTC
104	11N	TTTAACGTTCTGGGAGAAACAATAA
105	11O	GGATTTAGCGTATTAAATCCTTTGTTTTCAGG
106	11P	TAGCCCTACCAGCAGAAGATAAAAAACATTTGA
107	11Q	AGAAGAACCTCAAATATCGGCCTTCGAACTGA
108	12A	ACGTTAGTAAATGAATTTTCTGTATACCGCCA
109	12B	CCCTCAGAACCAGGCGGATAAGTGCGTTCCAG
110	12C	TAAGCGTCGTCAGACGATTGGCCTCGTTTTCA
111	12D	TCGGCATTGTCACCAATGAAACCAGGTTTACC
112	12E	AGCGCCAAAATAGAAAATTCATATTTATAACGGA
113	12FH	ATACCCAACCGAGGAAACGCAATAACAGCCATGCGCATTAGCCAGTTACAAAATAAAGAACGC
114	12I	GAGGCGTTGCTTATCCGGTATTCTAATCAATA
115	12JL	ATCGGCTGCGAGCATGTAGAAACCGGAATCATCATATTTAAATAAGAATAAACACCTATAACTA
116	12M	CTGTAAATATATGTGAGTGAATAACGGATTTCG
117	12N	CTGTAAATATATGTGAGTGAATAACGGATTTCG
118	12O	CCTGATTGAAAGAAATTGCGTAGACCCGAAACG
119	12P	TTATTAATGCCGTCAATAGATAATCAGAGGTG
120	12Q	AGGCGGTCATTAGTCTTTAATGCGGCTGGTAA
121	13A	TGCTCAGTACCGCCACCCTCAGAACTTTCCAG
122	13B	TGAGGCAGATACATGGCTTTTGATCGGGGTTT
123	13C	CCGGAACTTCGGTCATAGCCCCCAGGAGGT
124	13D	TCACAATCAGACAAAAGGGCGACATAGCAAGG
125	13E	GAAGGAAAAAGAACTGGCATGATTTTATTTTG
126	13FH	TATAGAAGTTAGCGAACCTCCCGATTCCAGAGCCTAATTTGACGGGAGAAATTAAGTAGTTACCA

127	13I	CTAATTTATCTTTCCTTATCATTCAAATCAGA
128	13JL	TAACCTCCTGCTGATGCAAAATCCAGATAAAATAAGGCGTTAACAACGCCAACATGTAATCCCATC
129	13M	AAATCAATCGTCGCTATTAATTAATACCTTTT
130	13N	ACAGAAATCTTTGAATACCAAGTTCAGTACAT
131	13O	AGATTAGATTTAAAAGTTTGAGTACACGTAAA
132	13P	GAATGGCTAGTATTAACACCGCCTCAACTAAT
133	13Q	TATCCAGAACAATATTACCGCCAGATATTTTT
134	14A	CGTAACGATCTAAAGTTTTGTGTCGCCGCCACC
135	14B	CTCAGAGCGAAGGATTAGGATTAGGATACAGG
136	14C	AGTGTACTCCGCCGCCAGCATTGATTATTAGC
137	14D	GTTTGCCAGTAGCACCATTACCATTTCACCG
138	14E	ATTGAGGGACACCACGGAATAAGTAAGACTCC
139	14FH	TTATTACGGCAGATAGCCGAACAAGAACACCCGCCAATAACGCTAACGAGCGTCTCTTGCGGG
140	14I	AGGTTTTGGCGCCCAATAGCAAGCCAAGAACG
141	14JL	GGTATTAAGAACAAGAAAAATAATATTTAGGCCCAGACGAGAAATACCGACCGTGTATCGCAAG
142	14M	TAGAATCCCCTTTTTTAATGGAAAACAAAATC
143	14N	TAGAATCCCCTTTTTTAATGGAAAACAAAATC
144	14O	GCGCAGAGATATCAAAATTATTTGACATTATC
145	14P	ATTTTGCGTCTTTAGGAGCACTAAGCAACAGT
146	14Q	GCCACGCTATACGTGGCACAGACACCATTGCA
147	15A	CCTCAAGACACCACCTCATTTTCATAGTTAG
148	15B	CACCAGAGGGTAATAAGTTTTAACCTGAGACT
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150	15D	ACGCAAAGAGGGAAGGTAAATATTGCCAGCAA
151	15E	AAAAGTAACAGTATGTTAGCAAATAAAAGAA
152	15FH	TCATTACCAAGCCTTAAATCAAGATCCTGAATCTTACCAATAAGAGCAAGAAACATTTTAAAG
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155	15M	TTGAATTATTGAAAACATAGCGATGTGAATTT
156	15N	AACCTACCGCAATTATTTCATTTCAATTTTCAT
157	15O	CTAAAATAGAACAAAGAAACCACCGGGTTAG
158	15P	GCGTAAGAGAGAGCCAGCAGCAAAAAGGTTAT

159	15Q	ACAGGAAAAACGCTCATGGAAATAACCTGAAA
160	16A	TGTAGCATTCACAGACAGCCCTCAGGGATAG
161	16B	CAAGCCCATGAAAGTATTAAGAGGGGGGTCAG
162	16C	TGCCTTGACCTCAGAGCCGCCACCATCACC GG
163	16D	AACCAGAGCCATTGGGAATTAGAGACGGAAA
164	16E	TTATTCATTTTTTAAAGGTGGCAACATAGTAGAAA
165	16FH	TACATACATATCTTACCGAAGCCCATGAAATAGCAATAGCACCCAGCTACAATTTTTTAGTTGC
166	16I	TATTTTGCTTTTCCGTTTTTATTTTCATATCGAGAA
167	16JL	CAAGCAAGACGCGCCTGTTTATCAGTTCAGCTAATGCAGAAATTCATCTTCTGACCAAATATA
168	16M	TTAAGACGATTAATTACATTTAACAATTACCT
169	16N	TAGAATCCCCTTTTTTAATGGAAAAATTACCT
170	16O	GAGCAAAACTTCTGAATAATGGAAGAAGGAG
171	16P	CGGAATTATTGAAAGGAATTGAGGTGAAAAAT
172	16Q	CTAAAGCAAGATAGAACCCTTCTGCCTACATT
173	17A	CTGAAACAATAGGAACCCATGTACACAACGCC
174	17B	GCCACCACGTAACAGTGCCCGTATCTATTATT
175	17C	CCGTCACCGACTTGAGCCACCACCGGAACCGCCCTCAGA
176	17N	TGGATTATGAAGATGATGAAACAA
177	17O	ATCAACAGTCATCATATTCTTGATTGATTGTT
178	17P	GCCAACAGTCACCTTGCTGAACCTGTTGGCAA
179	17Q	TTGACGCTCAATCGTCTGAAATGGACATTCTG
180	18A	GTCACCAGTACAACTCGTAACAC
181	18B	TGAGTTTCTTTTCTGCCTATTTTCGGAACAAACAGTT
182	18C	AATGCCCTTTTACCCTCAGAACCGCCACTCCCTCAGAGCCGCC
183	18O	TTCATCAATATAATCCTATCAGAT
184	18P	GATGGCAATTTTAATCAATATCTGGTCACAAATATC
185	18Q	AAACCCTCTTTTACCAGTAATAAAAGGGATTATTTACATTGGCA
186	Connector-A 1	TTTTGATAAGAGGTCATACCTTTAATTGCTCC
187	Connector-A 2	ATTGCTGATTTTTCGGATGGCTTGTCTGGAAGTTTCATTGCAACTAAAGTACGGAGAGCTTA
188	Connector-A 3	GACCATTAGATACATTTCCATATAACAGTTGA
189	Connector-B 1	AAATGAAAATAGCAGCATTTATTTATCCCAATC
190	Connector-B 2	ACAGGGAACTTTACAGAGAGAATAGTAATTGAGCGCTAATTGAACAAAGTCAGAGGACATAAAA

191	Connector-B 3	ACAAGAATTGAGTTAAATCAGAGAGATAACCC
192	Connector-C 1	GTAATCGTAAACTAGAGAGAATCGATGAACG
193	Connector-C 2	TGATAATCCATGTCAATCATATGTAAACGTTAATATTTGCAAATATTTAAATTGACCCCGGT
194	Connector-C 3	ATAGGAACGCCATCAAGTTAAAATTCGCATTA
195	Connector-D 1	TACCAGTATAAAGCCAAATTACTAGAAAAAGC
196	Connector-D 2	AGAATCGCACGCTCAACAGTAGGGCAGTAATAAGAGAATAAGAGGCATTTTCGAGCCTTAATTG
197	Connector-D 3	GGTAAAGTAATTCTGTAAAGTACCGACAAAA