

## Supporting Information

## Fabrication of Defined Polydopamine Nanostructures by DNA Origami-Templated Polymerization

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Table S1. The list of staple DNA sequences

#### References

### **Experimental Procedures**

#### Materials and instruments

All solvents and chemicals were purchased from commercial sources and were used without further purification. DNA staple strands and G-quadruplex containing staple strands (G4 staple) were either synthesized by 12-Column DNA Synthesizer from POLYGEN GmbH and purified by Agilent 1260 Infinity HPLC system with Agilent Eclipse XDB-C18 column or purchased from Sigma-Aldrich. Agarose gel electrophoresis was performed using Bio-Rad Mini-Sub Cell GT horizontal electrophoresis system. Bio-Rad MyCycler™ Thermal Cycler was used for annealing of MP13mp18 phage DNA and DNA staple strands to form DNA nanotile. Concentration of DNA nanotile was determined by Spark <sup>®</sup> 20M with Nanoquant plate<sup>TM</sup>.

#### Fabrication of DNA nanotile with G-quadruplex staple

DNA nanotile with G4 staples at different positions were prepared respectively by mixing M13MP18 phage DNA of 7k nt with desired staple strands and G4 staple strands in folding buffer (5 mM Tris, 1 mM EDTA, 5 mM NaCl, and 12 mM MgCl<sub>2</sub>, pH 8.0) and annealing from 65 °C to 20 °C over 2 h, followed by a purification with polyethylene glycol (PEG) precipitation method<sup>[1]</sup>. Briefly, the reaction solution containing DNA nanotile and excess of staple strands were mixed with 15% PEG8000 (w/v), 5 mM Tris, 1 mM EDTA, and 505 mM NaCl and centrifuged at 12000 rpm, at room temperature (RT) for 25 min. The supernatant was removed and the pellet was dissolved in 1 x TAE / Mg buffer (20 mM Tris, 1 mM EDTA, 12 mM MgCl<sub>2</sub>, pH 7.8).

#### Hemin binding assay

G4 DNA nanotile with square domain (50 nM) was added to a 384 well UV transparent plate. Hemin (1 µM) was mixed with G4 DNA nanotile solution. Immediately after mixing, the absorbance spectrum was measured every minute for a duration of 2 hours using a Tecan Spark® 20M plate reader. The absorbance spectrum of Hemin (1 µM) alone was also measured.

#### The ABTS assay of G4/hemin DNAzyme on DNA nanotile

G4 DNA nanotile with square domain (3.5 nM) in different buffer composition (20 mM Tris, 1 mM EDTA, 12 mM MgCl<sub>2</sub>, pH adjusted to 7.8 or pH 5.3 by addition of acetic acid) was mixed with hemin (70 nM) for 30 min at rt. 97.9 µL of the solution was added to a 384 well UV transparent plate. To G4/hemin DNA nanotile solution was added 1.1 uL of a freshly prepared 50 mg/ml ABTS solution and 1 uL of 0.1M H<sub>2</sub>O<sub>2</sub>. Immediately after H<sub>2</sub>O<sub>2</sub> addition, the absorbance spectrum was measured every minute for a duration of 1 hours using a Tecan Spark® 20M plate reader. The same procedure was used each for G4 DNA nanotile only (3.5 nM), G4/hemin (70 nM), and hemin (70 nM) only. For the low ionic strength buffer, (0.3 mM Tris, 0.2 mM acetic acid, 0.06 mM EDTA,  $0.6 \text{ mM MgCl}_2$ , 10 mM KCl, pH 5.3) was used.

#### Preparation of polydopamine on G4/hemin DNA nanotile

G4 DNA nanotile with square domain (3.5 nM) was mixed with hemin (100 nM) in TAE / Mg / K (0.3 mM Tris, 0.2 mM acetic acid, 0.06 mM EDTA, 0.6 mM MgCl<sub>2</sub>, 10 mM KCl, pH 5.3) for 30 min at room temperature before polymerization of polydopamine. To G4/hemin DNA nanotile solution was added 1 uL of freshly prepared dopamine (1 M) and  $H_2O_2$  (1M), and the reaction solution was incubated at rt for 3h. The samples were purified by size exclusion chromatography (800 µL volume of Sephacryl S-400, GE Healthcare) equilibrated with a reaction buffer in Ultrafree-MC-DV (Millipore).

#### Transformation of DNA nanotile to tube

To DNA nanotile with stripy G4/hemin pattern solution (0.5 pmol) was added a set of folding DNA strands (250 pmol each) and the mixture was incubated at 32 degrees for overnight. The obtained DNA tube was purified again with PEG precipitation method.

#### Kinetics of polydopamine formation on G4/hemin DNA nanotile

G4 DNA nanotile with square domain (3.5 nM) or G4 staple (70 nM) in the decreased ionic strength buffer (pH 5.3) was mixed with hemin (70 nM) for 30 min at rt. 98  $\mu$ L of the solution was added to a 384 well UV transparent plate. To G4/hemin DNA nanotile solution was added 1 uL of a freshly prepared 1M dopamine solution and 1 uL of 1M H<sub>2</sub>O<sub>2</sub>. Immediately after H<sub>2</sub>O<sub>2</sub> addition, the absorbance spectrum was measured every 5 minutes for a duration of 12 hours using a Tecan Spark® 20M plate reader.

#### Extraction of polydopamine nanostructure via acid treatment

The sample solution of purified polydopamine grown DNA nanotile was added to freshly cleaved mica surface and incubated for 5 min at room temperature to deposit DNA nanotile structure on mica surface. After imaging the polydopamine / DNA nanotile by AFM, the remaining solution on mica was removed and 1 M HCl solution was added to the mica surface. After 10 seconds, HCl solution was removed and the buffer was added again. The AFM measurement was performed at the same area as before acid treatment.

#### Atomic force Microscopy (AFM)

Imaging was performed with a Bruker Dimension FastScan Bio AFM equipped with the ScanAsyst mode. The sample solution was deposited onto freshly cleaved mica surface, and left for 5 min at room temperature to allow adsorption of the DNA nanotile structures. After addition of 70 µL of 1 x TAE / Mg buffer, the sample was scanned with the scan rates between 1 and 3 Hz. Several AFM images were acquired at different areas of the mica surface to ensure the reproducibility of the results. All images were analyzed by using the NanoScope Analysis 1.50 and Gwyddion 2.38 software.

#### Agarose gel electrophoresis

5 uL of sample (3 nM) was mixed with 6 x Loading buffer and run with 0.8 % agarose gel in 0.5 x TBE / Mg for 150 min in ice bath. After running, the gel was stained by SYBR Gold for 30 min and the image was taken by G: Box Chemi (Syngene).

#### Transmission electron microscopy (TEM)

5 uL of sample (1 nM) was applied on carbon coated copper grid with hydrophilic treatment. After 10 minutes incubation, the remaining solution was removed and the sample grid was stained with 2 % uranyl formate solution for 20 seconds. The stained grid

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was washed with filtered water for three times and dried in air. Imaging was done with JEOL 1400 instrument and obtained images were analyzed by ImageJ software.

### **Results and Discussion**



Figure S1. Proposed mechanism of polydopamine formation. Through the oxidation, isomerization, oligomerization and self-assembly polydopamine is formed via covalent bond, charge transfer,  $\pi$ -interactions and so on<sup>[2]</sup>.



Figure S2. Design of G4/hemin DNA nanotile. Schematic of DNA nanotile<sup>[3]</sup> and the orange circles at the bottom figures indicate the positions of G4 staple DNA sequences.



Figure S3. G4/hemin DNA nanotile. (a) Schematic illustration of DNA nanotile with 20 nm square G4/hemin domain and (b) agarose gel electrophoresis image of 1: M13MP18, 2: G4 DNA nanotile before purification, and 3: G4 DNA nanotile after purification. The staple DNA sequences were sufficiently removed after the purification. (c) AFM images before and after hemin incorporation. There was no clear difference observed before and after DNAzyme activation. Scale bar: 100 nm.



Figure S4. UV-Vis spectrum of hemin incorporation to G4 DNA nanotile. In the presence of G4 DNA nanotile, the hemin Soret band was immediately shifted from 400 nm to 405 nm due to hemin binding to G4 domain for activating DNAzyme<sup>[4]</sup>.



Figure S5. ABTS assay of G4/hemin DNA nanotile in different buffer compositions. It was observed that, under pH 7.8, the oxidation of ABTS will not last more than 400 seconds possibly due to the loss of DNAzyme activity, although potassium ion addition improved until certain extent (black and green). On the other hand, the improved activity was observed under acidic pH (5.3) condition (red line). By lowering the ionic strength (yellow line), the decreased DNAzyme activity was observed, but still higher than pH 7.8 condition.



Figure S6. No polydopamine formation on DNA nanotile under the high ionic strength buffer condition.



Figure S7. Nanomechanical property mapping of polydopamine DNA nanotile and G4 DNA nanotile.



**Figure S8 Proposed mechanism of polydopamine formation on DNA nanotile.** Under high ionic strength condition, cations (Tris, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>) shielded negative charges of the DNA backbone so that after dopamine molecules were oxidized to dopaminochrome on DNA origami, it diffused back into solution and spontaneously self-polymerized in solution. On the other hand, under low ionic strength condition, the locally concentrated dopamine, dopaminochrome, and formed oligomers facilitate the formation of polydopamine on DNA nanotile.



Figure S9. Tracking of polydopamine formation with free DNAzyme. (a) Absorbance spectra of dopamine polymerization on G4/ hemin DNA nanotile and G4/hemin over 3.5 hours (inset: absorbance spectra of polydopamine. dopamine [2 mg/ml] polymerized in 10 mM Tris buffer for 3.5 hr). Initial increase around 320 nm and 480 nm attributed to dopaminochrome and oligmers, respectively. Later increase in baseline (broadband absorbance) attributed to polydopamine formation. Representative wavelength at 700 nm chosen to avoid contributions from early oxidation products. (b) Dopaminochrome, oligomers, and polydopamine formation by G4/hemin DNA nanotile (3.5 nM containing 70 nM G4/hemin or free G4/hemin (70 nM) are tracked by UV-Vis spectroscopy (the solid lines: G4/hemin DNA nanotile, dashed lines: free G4/hemin). In case of free G4/hemin molecules, the production of dopaminochrome and oligomers were lower than G4/hemin DNA nanotile. Additionally, the peak corresponding to polydopamine (700 nm, green line) didn't increase over the time (c) The schematic illustration of the reaction with free G4/hemin. Free DNAzyme can oxidize dopamine molecules to dopaminochrome. After further isomerization and oxidization, oligomers are formed, however polydopamine is not formed due to the limited production of oligomers.



Figure S10. Polymerization of L-dopa on G4/hemin DNA nanotile. L-dopa is a derivative of dopamine having carboxylic acid group (left). Under pH 5.3 condition, it shows neutral charge which hinder its interaction with negatively charged DNAzyme domain resulting in no polymerization (right).



Figure S11. Polymerization of dopamine on G4 DNA nanotile (without hemin). Without hemin, no polydopamine was formed due to a lack of DNAzyme activity.



Figure S12. The effects of H<sub>2</sub>O<sub>2</sub> concentration and reaction time to the growth of polydopamine on G4/hemin DNA nanotile. Means + S.E., n = 28



Figure S13. Cross patterning of polydopamine nanostructure on DNA nanotile. Scale bar: 100 nm.



Figure S14. Polydopamine DNA tube with striped G4/hemin. (Top) Schematic Illustration of tube formation and subsequent polydopamine formation. (Bottom) AFM height image and cross section of polydopamine DNA nanotube with stripy G4/hemin before (left) and after polydopamine formation (right).



Figure S15. AFM images of polydopamine DNA nanotile with horizontal line. (Upper) AFM image after 90 min reaction time (left) and 180 minutes reaction time (right). Tile structure, partially folded structure, and folded structure were indicated by T, P, and F, respectively. The percentages of different conformation after 1.5 h and 3 h reaction time was calculated from counting 92 structures. (Bottom) Dimension and cross section of (partially) folded polydopamine DNA nanotile.



100 nm

Figure S16. TEM images of polydopamine DNA nanotile with horizontal line before and after 180 minutes reaction. Scale bar: 100 nm. In TEM image, the folding was more enhanced possibly due to the drying effect.



Figure S17. Polydopamine DNA nanotile with vertical line. There was no folding observed after 180 minutes reaction time.



Figure S18. AFM image of extracted polydopamine nanostructures after HCI treatment. The polydopamine nanostructures were indicated by red arrows.

#### Table S1. The list of staple DNA sequences

No.	Sequence
1	CAAGCCCAATAGGAACCCATGTACAAACAGTT
2	AATGCCCCGTAACAGTGCCCGTATCTCCCTCA
3	TGCCTTGACTGCCTATTTCGGAACAGGGATAG
4	GAGCCGCCCCACCGGGAACCGCGACGGAAA
5	AACCAGAGACCCTCAGAACCGCCAGGGGTCAG
6	TTATTCATAGGGAAGGTAAATATTCATTCAGT
7	CATAACCCGAGGCATAGTAAGAGCTTTTTAAG
8	ATTGAGGGTAAAGGTGAATTATCAATCACCGG
9	AAAAGTAATATCTTACCGAAGCCCTTCCAGAG
10	GCAATAGCGCAGATAGCCGAACAATTCAACCG
11	CCTAATTTACGCTAACGAGCGTCTAATCAATA
12	TCTTACCAGCCAGTTACAAAATAAATGAAATA
13	ATCGGCTGCGAGCATGTAGAAACCTATCATAT
14	СТААТТТАТСТТТССТТАТСАТТСАТССТБАА
15	GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGG

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16	I GCTCATTTTCGCATTAAATTTTTGAGCTTAGA
17	AATTACTACAAATTCTTACCAGTAATCCCATC
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25	GAACGTGGCGAGAAAGGGAAGGGAACAAACTAT
26	TAGCCCTACCAGCAGAAGATAAAAACATTTGA
27	
28	
29	CTGAAACAGGTAATAAGTTTTAACCCCTCAGA
30	
31	
32	GTTTGCCACCTCAGAGCCGCCACCGATACAGG
33	GACTTGAGAGACAAAAGGGCGACAAGTTACCA
34	AGCGCCAACCATTTGGGAATTAGATTATTAGC
35	GAAGGAAAATAAGAGCAAGAAACAACAGCCAT
36	GCCCAATACCGAGGAAACGCAATAGGTTTACC
37	ATTATTTAACCCAGCTACAATTTTCAAGAACG
38	TATTTTGCTCCCAATCCAAATAAGTGAGTTAA
39	GGTATTAAGAACAAGAAAAATAATTAAAGCCA
40	TAAGTCCTACCAAGTACCGCACTCTTAGTTGC
41	ACGCTCAAAATAAGAATAAACACCGTGAATTT
42	AGGCGTTACAGTAGGGCTTAATTGACAATAGA
43	ATCAAAATCGTCGCTATTAATTAACGGATTCG
44	CTGTAAATCATAGGTCTGAGAGACGATAAATA
45	CCTGATTGAAAGAAATTGCGTAGACCCGAACG
46	ACAGAAATCTTTGAATACCAAGTTCCTTGCTT
47	TTATTAATGCCGTCAATAGATAATCAGAGGTG
48	AGATTAGATTTAAAAGTTTGAGTACACGTAAA
49	AGGCGGTCATTAGTCTTTAATGCGCAATATTA
50	GAATGGCTAGTATTAACACCGCCTCAACTAAT
51	
52	CCCTCAGAACCGCCACCCTCAGAACTGAGACT
53	CCTCAAGAATACATGGCTTTTGATAGAACCAC
54	TAAGCGTCGAAGGATTAGGATTAGTACCGCCA
55	CACCAGAGTTCGGTCATAGCCCCCGCCAGCAA
56	TCGGCATTCCGCCGCCAGCATTGACGTTCCAG
57	AATCACCAAATAGAAAATTCATATATAACGGA
58	TCACAATCGTAGCACCATTACCATCGTTTTCA
59	ATACCCAAGATAACCCACAAGAATAAACGATT
60	ATCAGAGAAAGAACTGGCATGATTTTATTTTG
61	TTTTGTTTAAGCCTTAAATCAAGAATCGAGAA
62	AGGTTTTGAACGTCAAAAATGAAAGCGCTAAT
63	CAAGCAAGACGCGCCTGTTTATCAAGAATCGC
64	AATGCAGACCGTTTTTATTTTCATCTTGCGGG
65	
66	AATGGTTTACAACGCCAACATGTAGTTCAGCT
67	TAACCTCCATATGTGAGTGAATAAACAAAATC
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69	GCGCAGAGATATCAAAATTATTTGACATTATC
70	AACCTACCGCGAATTATTCATTTCCAGTACAT
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82	ACGCAAAGGTCACCAATGAAACCAATCAAGTT
83	TTATTACGGTCAGAGGGTAATTGAATAGCAGC
84	TGAACAAACAGTATGTTAGCAAACTAAAAGAA
85	CTTTACAGTTAGCGAACCTCCCGACGTAGGAA
86	GAGGCGTTAGAGAATAACATAAAAGAACACCC
87	TCATTACCCGACAATAAACAACATATTTAGGC
88	CCAGACGAGCGCCCAATAGCAAGCAAGAACGC
89	AGAGGCATAATTTCATCTTCTGACTATAACTA
90	TTTTAGTTTTCGAGCCAGTAATAAATTCTGT
91	TATGTAAACCTTTTTTAATGGAAAAATTACCT
92	TTGAATTATGCTGATGCAAATCCACAAATATA
93	GAGCAAAAACTTCTGAATAATGGAAGAAGGAG
94	TGGATTATGAAGATGATGAAACAAAATTTCAT
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104	TACATACATTTTGACGGGAGAATTAACTACAGGGAA
105	GCGCATTATTTTGCTTATCCGGTATTCTAAATCAGA
106	TATAGAAGTTTTCGACAAAAGGTAAAGTAGAGAATA
107	TAAAGTACTTTTCGCGAGAAAACTTTTTATCGCAAG
108	ACAAAGAATTTTATTAATTACATTTAACACATCAAG
109	AAAACAAATTTTTTCATCAATATAATCCTATCAGAT
110	GATGGCAATTTTAATCAATATCTGGTCACAAATATC
111	AAACCCTCTTTTACCAGTAATAAAAGGGATTCACCAGTCACACGTTTT
112	CCGAAATCCGAAAATCCTGTTTGAAGCCGGAA
113	CCAGCAGGGGCAAAATCCCTTATAAAGCCGGC
114	GCATAAAGTTCCACACAACATACGAAGCGCCA
115	GCTCACAATGTAAAGCCTGGGGTGGGTTTGCC
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123	TTTTAATTGCCCGAAAGACTTCAAAACACTAT
124	AAGAGGAACGAGCTTCAAAGCGAAGATACATT
125	GGAATTACTCGTTTACCAGACGACAAAAGATT
126	GAATAAGGACGTAACAAAGCTGCTCTAAAACA
127	CCAAATCACTTGCCCTGACGAGAACGCCAAAA
128	CTCATCTTGAGGCAAAAGAATACAGTGAATTT
129	AAACGAAATGACCCCCAGCGATTATTCATTAC
130	CTTAAACATCAGCTTGCTTTCGAGCGTAACAC
131	TCGGTTTAGCTTGATACCGATAGTCCAACCTA
132	TGAGTTTCGTCACCAGTACAAACTTAATTGTA
133	CCCCGATTTAGAGCTTGACGGGGAAATCAAAA
134	GAATAGCCGCAAGCGGTCCACGCTCCTAATGA
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142	TATATTTTAGCTGATAAATTAATGTTGTATAA
144	TCAATTCTTTTAGTTTGACCATTACCAGACCG
145	CGAGTAGAACTAATAGTAGTAGCAAACCCTCA
146	GAAGCAAAAAAGCGGATTGCATCAGATAAAAA
147	

148	CCAAAATATAATGCAGATACATAAACACCAGA
149	CATTCAACGCGAGAGGCTTTTGCATATTATAG
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151	AGTAATCTTAAATTGGGCTTGAGAGAATACCA
152	GCGAAACATGCCACTACGAAGGCATGCGCCGA
153	ATACGTAAAAGTACAACGGAGATTTCATCAAG
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156	TGTAGCATTCCACAGACAGCCCTCATCTCCAA
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166	GGTAGCTAGGATAAAAATTTTTAGTTAACATC
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169	TCCATATACATACAGGCAAGGCAACTTTATTT
170	TACCTTTAAGGTCTTTACCCTGACAAAGAAGT
171	CAAAAATCATTGCTCCTTTTGATAAGTTTCAT
172	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAA
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174	TTTCAACTATAGGCTGGCTGACCTTGTATCAT
175	CCAGGCGCTTAATCATTGTGAATTACAGGTAG
176	CGCCTGATGGAAGTTTCCATTAAACATAACCG
177	TTTCATGAAAATTGTGTCGAAATCTGTACAGA
178	ATATATTCTTTTTCACGTTGAAAATAGTTAG
179	AATAATAAGGTCGCTGAGGCTTGCAAAGACTT
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181	ACCCAAATCAAGTTTTTTGGGGTCAAAGAACG
182	TGGACTCCCTTTTCACCAGTGAGACCTGTCGT
183	TGGTTTTTAACGTCAAAGGGCGAAGAACCATC
184	GCCAGCTGCCTGCAGGTCGACTCTGCAAGGCG
185	
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193	TGCAACTAAGCAATAAAGCCTCAGTTATGACC
194	TTTTTGCGCAGAAAACGAGAATGAATGTTTAG
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196	ACTGGATAACGGAACAACATTATTACCTTATG
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198	CGATTTTAGAGGACAGATGAACGGCGCGACCT
199	CTTTGAAAAGAACTGGCTCATTATTTAATAAA
200	GCTCCATGAGAGGCTTTGAGGACTAGGGAGTT
201	ACGGCTACTTACTTAGCCGGAACGCTGACCAA
202	AAAGGCCGAAAGGAACAACTAAAGCTTTCCAG
203	GAGAATAGCTTTTGCGGGATCGTCGGGTAGCA
204	ACGTTAGTAAATGAATTTTCTGTAAGCGGAGT
205	TTTTCGATGGCCCACTACGTAAACCGTC
206	TATCAGGGTTTTCGGTTTGCGTATTGGGAACGCGCG
207	GGGAGAGGTTTTTGTAAAACGACGGCCATTCCCAGT
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213	TAAATATTTTTTGGAAGAAAAATCTACGACCAGTCA

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214	GGACGTTGTTTTCATAAGGGAACCGAAAGGCGCAG
215	ACGGTCAATTTTGACAGCATCGGAACGAACCCTCAG
216	CAGCGAAAATTTTACTTTCAACAGTTTCTGGGATTTTGCTAAACTTTT
217	AACATCACTTGCCTGAGTAGAAGAACT
218	TGTAGCAATACTTCTTTGATTAGTAAT
219	AGTCTGTCCATCACGCAAATTAACCGT
220	ATAATCAGTGAGGCCACCGAGTAAAAG
221	ACGCCAGAATCCTGAGAAGTGTTTTT
222	TTAAAGGGATTTTAGACAGGAACGGT
223	AGAGCGGGAGCTAAACAGGAGGCCGA
224	TATAACGTGCTTTCCTCGTTAGAATC
225	GTACTATGGTTGCTTTGACGAGCACG
226	GCGCTTAATGCGCCGCTACAGGGCGC
T1	AATAATAATAATAATCAAGCCCAATAGGAACCCATGTACAAACAGTT
T25	AATAATAATAATAATGAACGTGGCGAGAAAGGAAGGGAACAAACTAT
T27	CAAGCCCACTGGTAATATCCAGAACGAACTGA
T28	CCGCCAGCCACCCTCATTTTCCTATTATT
T51	CTCAGAGCCATTGCAACAGGAAAAATATTTTT
T52	GGAAATACACCGCCACCCTCAGAACTGAGACT
T75	CCCTCAGACTACATTTTGACGCTCACCTGAAA
T76	GAAATGGATACTCAGGAGGTTTAGCGGGGTTT
T99	TATCACCGTTATTTACATTGGCAGACATTCTG
T132	GAACGTGGGTCACCAGTACAAACTTAATTGTA
T133	TGTAGCATTAGAGCTTGACGGGGAAATCAAAA
T156	CCCCGATTTCCACAGACAGCCCTCATCTCCAA
T157	CGTAACGACTAAATCGGAACCCTAGTTGTTCC
T180	GTAAAGCATCTAAAGTTTTGTCGTGAATTGCG
T181	ACGTTAGTCAAGTTTTTTGGGGTCAAAGAACG
T204	ACCCAAATAAATGAATTTTCTGTAAGCGGAGT

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