

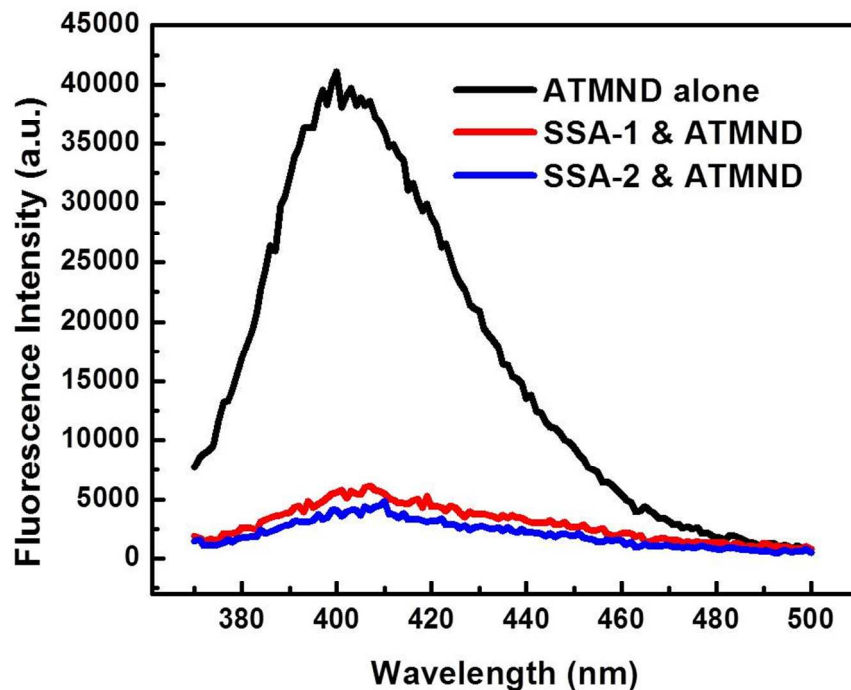
*In Vitro* Selection of Shape-Changing DNA  
Nanostructures Capable of Binding-Induced Cargo  
Release

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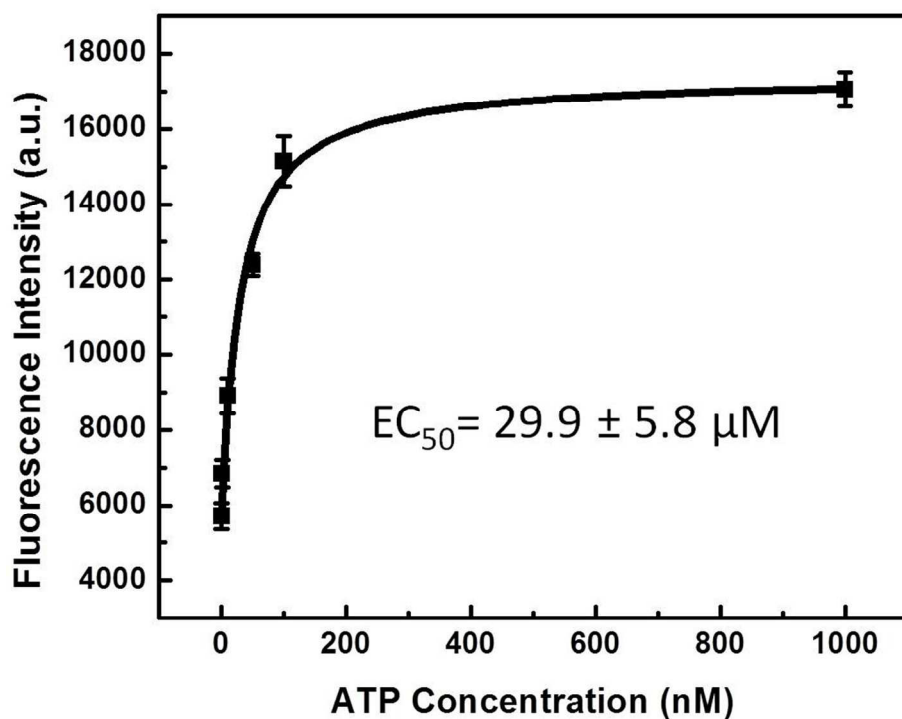
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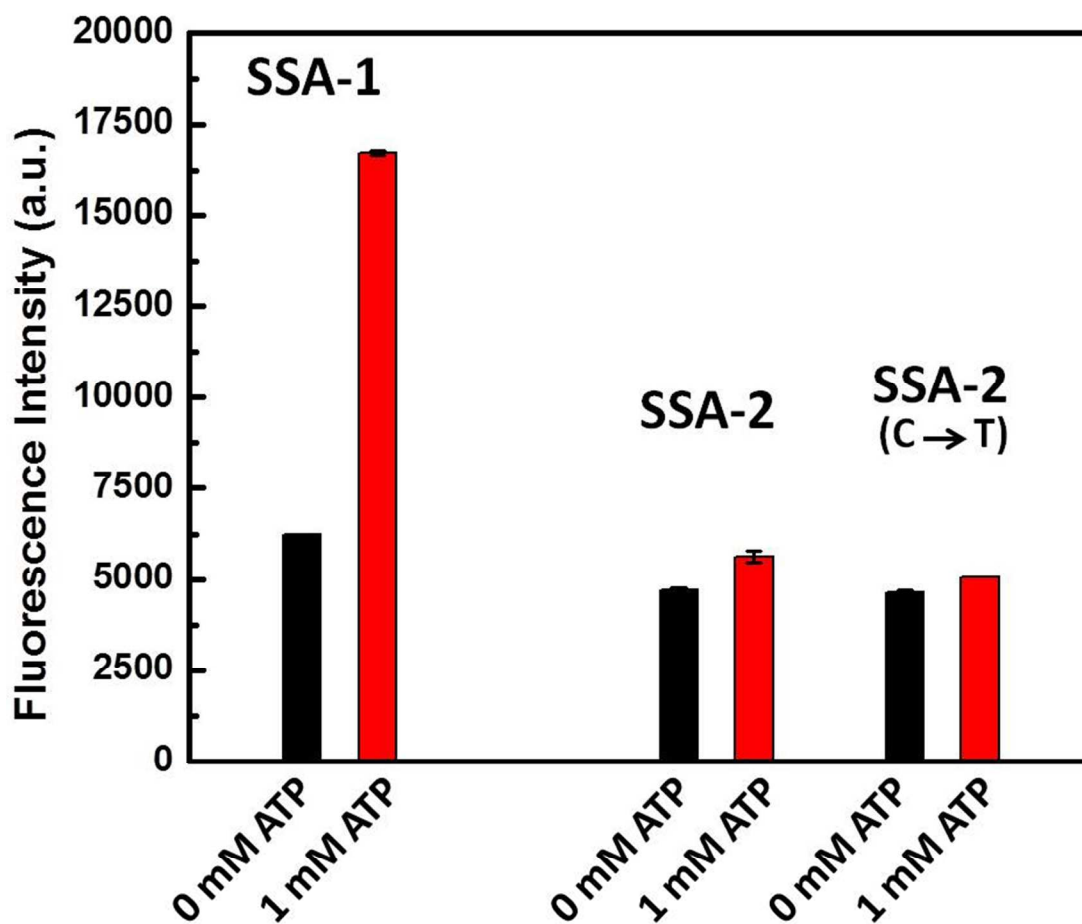
## SUPPORTING INFORMATION



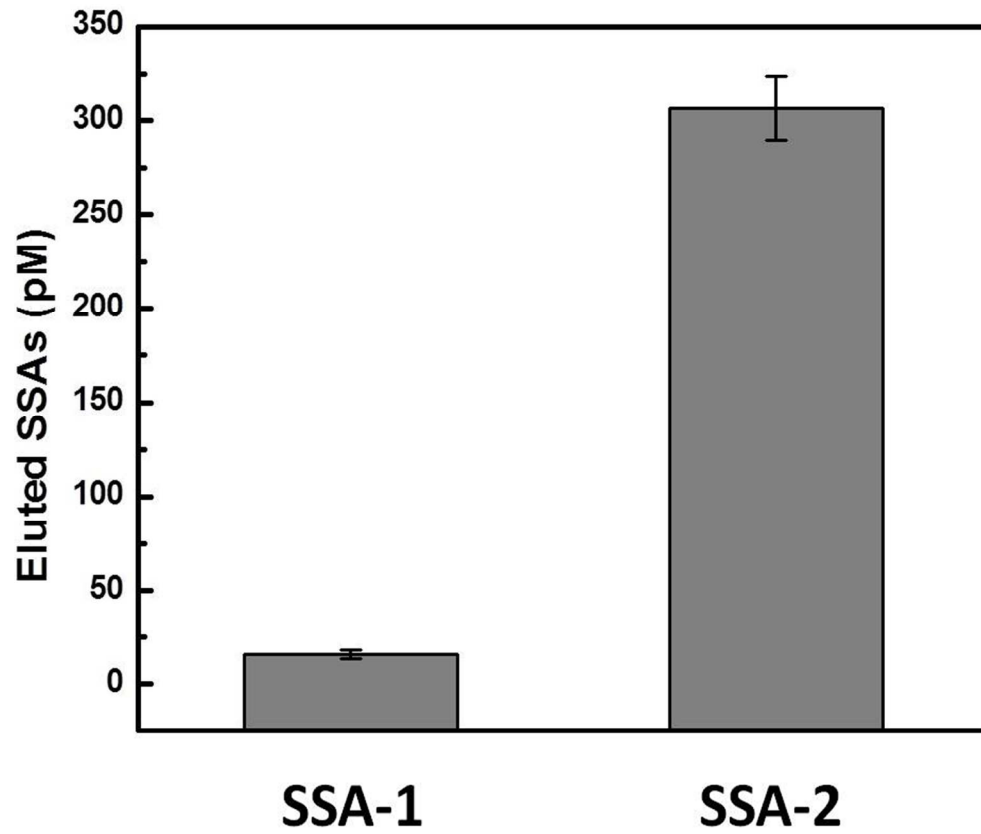
**Figure S1.** Loading into the abasic site of SSA/cargo strand duplex structures efficiently quenches ATMND fluorescence. Duplex-loaded ATMND yields 7- and 9-fold lower fluorescence intensities with SSA-1 (red) and SSA-2 (blue), respectively, compared to free ATMND in solution (black).



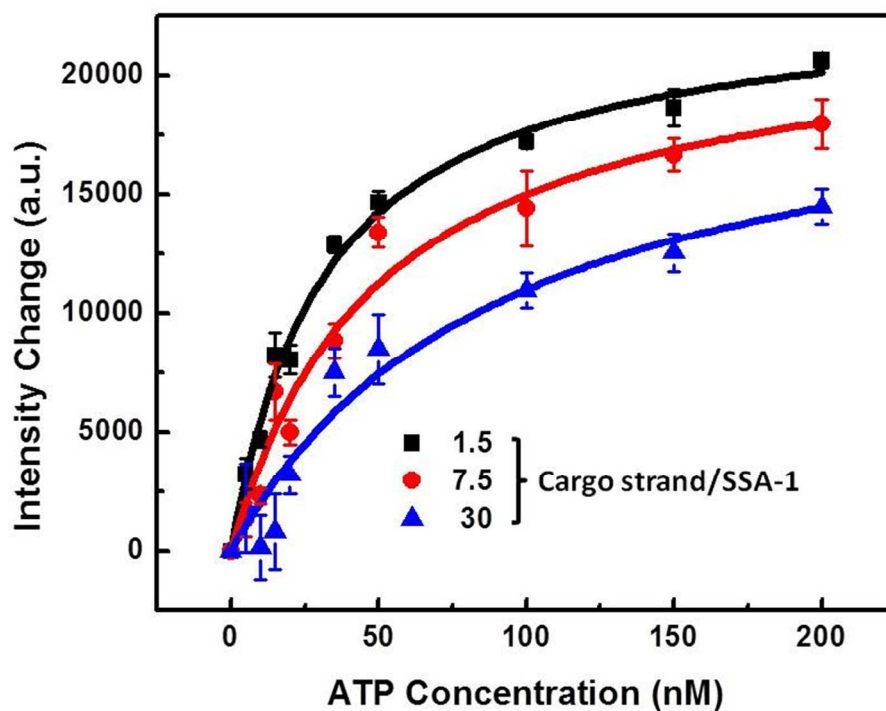
**Figure S2.** Calibration curve for SSA-1 structure-switching derived from fluorescence intensities detected at increasing ATP concentrations yields an  $EC_{50}$  value of  $29.9 \pm 5.8 \mu M$  at  $5 \mu M$  of SSA-1/cargo strand (1:1.5 in ratio) concentration.



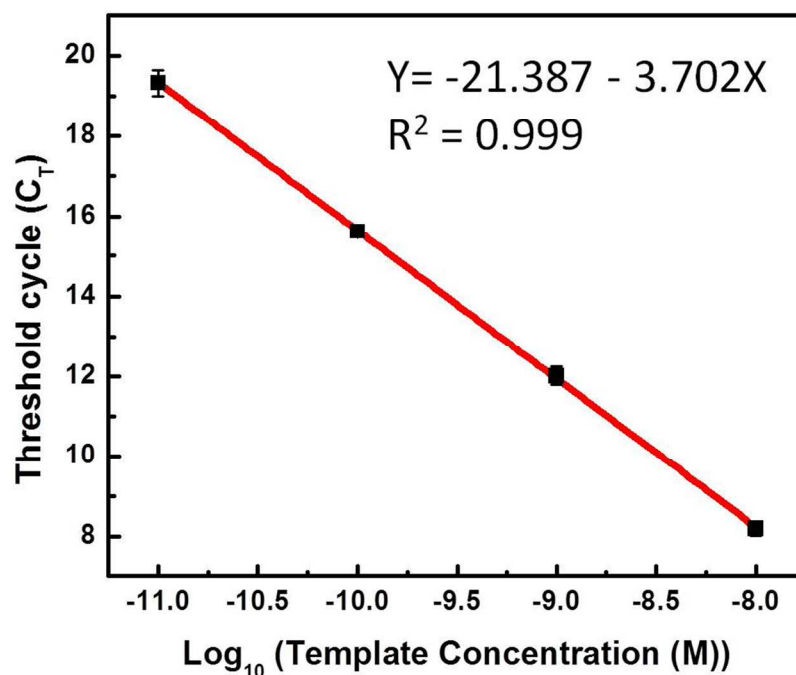
**Figure S3.** Comparison of ATP-induced cargo release by SSAs in solution. When challenged with 1 mM ATP, SSA-1 generates a strong fluorescent signal (170% increase relative to no ATP), indicating efficient cargo release as a result of binding-induced structure-switching. By comparison, SSA-2 and SSA-2 where the T-to-C mutation is reverted achieve only limited ATMND release in solution (15% and 9% increase, respectively). These results indicate that this mutation is not responsible for the difference in cargo release, which may instead be attributable to SSA-2's dependence on the presence of a solid support.



**Figure S4.** Comparison of SA-binding between the two SSAs. We incubated both SSAs at 100 nM concentration with SA-coated beads, and heat eluted bound DNAs after thorough washing. Eluted DNAs were quantified *via* RT-PCR, and we observed that SSA-2 binding to SA beads was ~20-fold greater than for SSA-1.



**Figure S5.**  $EC_{50}$  of SSA-1 in response to ATP is dependent on cargo strand concentration. As the cargo strand:SSA-1 ratio increases,  $EC_{50}$  also increases; For cargo strand:SSA-1 ratios of 1.5 (black), 7.5 (red) and 30 (blue) with a 30 nM concentration of SSA-1, we observed  $EC_{50}$  values of  $31.8 \pm 4.8 \mu\text{M}$ ,  $50.2 \pm 10.8 \mu\text{M}$  and  $92.0 \pm 31.7 \mu\text{M}$ , respectively.



**Figure S6.** Threshold cycle ( $C_T$ ) values of SSAs released from magnetic beads after binding to ATP. The template concentration and  $C_T$  were fitted with linear regression. We calculated a PCR efficiency of 86.3%.

**Table S1.** Sequences of DNA molecules used in these experiments

Name	Sequence	Description
ssDNA library	5'-AGCAGCACAGAGGTCAGATG-N <sub>17</sub> -TTTTGTGGGTAGGGCGGGTTGGTTTT-N <sub>17</sub> -CCTATGCGTGCTACCGTGAA-3'	DNA sequence with two randomized 17-base recognition domains
FP	5'-AGCAGCACAGAGGTCAGATG-3'	Forward primer
RP	5'-TTCACGGTAGCACGCATAGG-3'	Reverse primer
RP-biotin	5'-biotin-TTCACGGTAGCACGCATAGG-3'	Biotinylated reverse primer for ssDNA generation
FP-complementary	5'-CATCTGACCTCTGTGCTGCT-3'	Blocking strand of forward primer binding site.
M12-T12-biotin	5'-biotin-TTTTTTTTTTTTAACCCGCCCTAC-3'	Biotinylated cargo strand with T <sub>12</sub> extension
M12	5'-AACCCXCCCTAC-3'	Cargo strand with an abasic site



**Table S2.** Selection conditions during ten rounds of SSA selection.

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
SA			1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M
BSA			1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M	1 $\mu$ M
GTP							1 mM	1 mM	1 mM	1 mM
ATP	1 mM	1 mM	1 mM	0.8 mM	0.4 mM	0.2 mM	0.1 mM	70 $\mu$ M	20 $\mu$ M	20 $\mu$ M

**Table S3.** 50 cloned sequences from the SSA library synthesized from machine-mixed nucleotides.

	1	10	20	30	40	50	60	70	80	90	100									
1	AGCAGCACAGAGG	TCAGAGTA	AAA	CCAGGAG	AGA	GGGG	AGGG	GGG	GG	CA	GACAAAGGAG	CA	CCCA	CCG	CG	CA	CCG	GAA		
2	AGCAGCACAGAGG	TCAGAGTA	AACA	AAGA	AGG	GGGG	AGGG	GGG	GG	CA	AGAA	AAAA	CA	CCCA	CCG	CG	CA	CCG	GAA	
3	AGCAGCACAGAGG	TCAGAGTA	AAAA	AG	GGAGG	GGGG	AGGG	GGG	GG	CA	AAAA	AG	AAAA	CA	CCCA	CCG	CG	CA	CCG	GAA
4	AGCAGCACAGAGG	TCAGAGTA	CC	AGCA	GG	GGGG	AGGG	GGG	GG	CA	AGAG	AGAG	AGAG	CA	CCCA	CCG	CG	CA	CCG	GAA
5	AGCAGCACAGAGG	TCAGAGTA	CC	AG	GGGA	GGGG	AGGG	GGG	GG	CA	AGAG	AGAG	AGAG	CA	CCCA	CCG	CG	CA	CCG	GAA
6	AGCAGCACAGAGG	TCAGAGTA	GGG	GAAGAC	G	GGGG	AGGG	GGG	GG	CA	AAAA	CC	GG	CA	CCCA	CCG	CG	CA	CCG	GAA
7	AGCAGCACAGAGG	TCAGAGTA	AGGGG	AGAA	GAAA	GGGG	AGGG	GGG	GG	CA	AA	CC	GG	CA	CCCA	CCG	CG	CA	CCG	GAA
8	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
9	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
10	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
11	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
12	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
13	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
14	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
15	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
16	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
17	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
18	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
19	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
20	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
21	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
22	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
23	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
24	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
25	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
26	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
27	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
28	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
29	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
30	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
31	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
32	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
33	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
34	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
35	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
36	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
37	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
38	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
39	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
40	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
41	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
42	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
43	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
44	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
45	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
46	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
47	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
48	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
49	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA
50	AGCAGCACAGAGG	TCAGAGTA	AG	CA	AG	GGGG	AGGG	GGG	GG	CA	AG	AG	AG	CA	CCCA	CCG	CG	CA	CCG	GAA