Supporting Information

Integration of a photocleavable element into DNA nanoswitches

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MATERIALS AND METHODS

Oligonucleotides:

Oligonucleotides were purchased from Integrated DNA Technologies (IDT) with standard desalting. M13 single strand was purchased from New England Biolabs (NEB). We have used the viral genome M13mp18 (7249 nt) for this and previous constructions of our nanoswitches, due to its commercial availability and frequent use in DNA origami. The DNA oligonucleotides with photocleavable linkers (PCL) were chemically synthesized at 1.0-μmol scales by solid phase synthesis using an Oligo-800 synthesizer (Figure S1). The PCL phosphoramidites were purchased from Glen research and used as 0.1 M solution in acetonitrile. All the other reagents are standard solutions obtained from ChemGenes Corporation. After synthesis, the oligos were cleaved from the solid support and fully deprotected with AMA (ammonium hydroxide:methylamine = 1:1) at 65 °C for 30 min. The amines were removed by Speed-Vac concentrator before purification. The DNA strands were purified by reverse phase HPLC using a Zorbax SB-C18 column at a flow rate of 6 mL/min. Buffer A was 20 mM triethylammonium acetate, pH 7.1; buffer B contains 50% acetonitrile in 20 mM triethylammonium acetate, pH 7.1. A linear gradient from buffer A to 80% buffer B in 25 min was used to elute the oligos. The purified samples were concentrated, desalted and lyophilized to dry before re-dissolving to working buffers. Synthesized strands were checked using denaturing polyacrylamide gel electrophoresis.

Linearization of M13 DNA:

5 μl of 100nM circular single-stranded M13 DNA, 2.5 μl of 10× Cut Smart buffer, 0.5 μl of 100 μM BtsCI restriction-site complementary-oligonucleotide and 16 μl of deionized water were mixed and annealed from 95 °C to 50 °C in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). 1 μl of the BtsCI enzyme (20,000 units/ml, NEB) was added to the mixture and incubated at 50 °C for 15 min. The mixture was brought up to 95 °C for 1 min to heat deactivate the enzyme followed by cooling down to 4 °C.

Construction of nanoswitches:

Linearized single-stranded M13 DNA (20 nM) was mixed with ten-fold excess of the backbone oligonucleotides, detector oligonucleotides and filler strands. The mixture was annealed from 90 °C to 20 °C at 1 °C min^{-1} in a T100™ Thermal Cycler (Bio-Rad, USA). The nanoswitches were LC-purified [1] after annealing to remove excess oligonucleotides. Purified constructs were diluted in 1× PBS. To form loops, the purified nanoswitches (~250 pM) were mixed with desired concentration of the input strands (typically at 25 nM) and incubated at room temperature.

UV irradiation:

Photo-cleavage was initiated by irradiating the samples with UV light at a wavelength of 254 nm (we also tested 365 nm). Samples (10-15 µl) were kept on ice in 0.2 ml tubes at a distance of 3 cm from the light source (Handheld UV light Spectroline EF 240C with an output of 4 Watts). Samples were irradiated for various durations for the time series experiment as mentioned in the text.

Gel electrophoresis:

Nanoswitches were run in 0.8% agarose gels, cast from molecular biology grade agarose (Fisher BioReagents) dissolved in 0.5× Tris-borate EDTA (TBE) (Ultra-pure grade, Amresco, Solon, OH, USA). Samples were mixed with a Ficoll-based loading solution (15% Ficoll, 0.1% bromophenol blue). Gels were typically run at 75 V (constant voltage) at room temperature. Samples were pre-stained by mixing 1× GelRed stain (Biotium, Fremont, CA, USA) with the samples before loading. Gels were imaged with a Bio-Rad Gel Doc XR+ gel imager and analyzed using ImageJ.

SUPPORTING REFERENCE

1. K. Halvorsen, M. E. Kizer, X. Wang, A. R. Chandrasekaran, M. Basanta-Sanchez, Shear dependent LC purification of an engineered DNA nanoswitch and implications for DNA origami. *Anal. Chem.*, 2017, 89, 5673-5677.

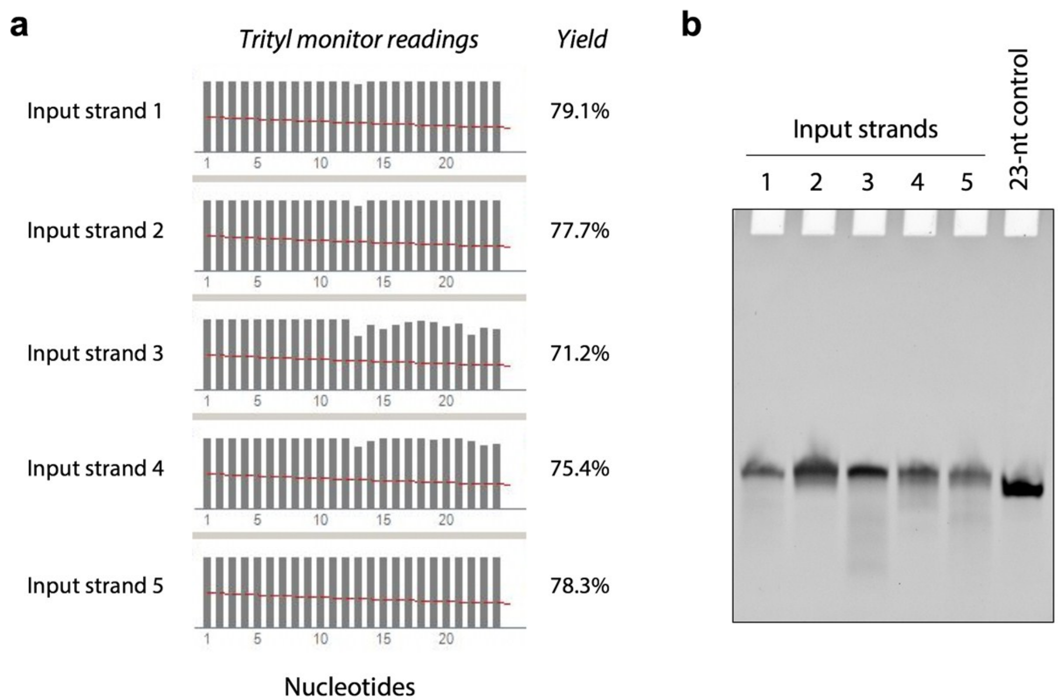

Figure S1. (a) Trityl readings of PCL-strands during synthesis and calculated yields. Synthesis was performed at 1 μmol scale. (b) Denaturing gel showing the PCL-strands purified after synthesis.

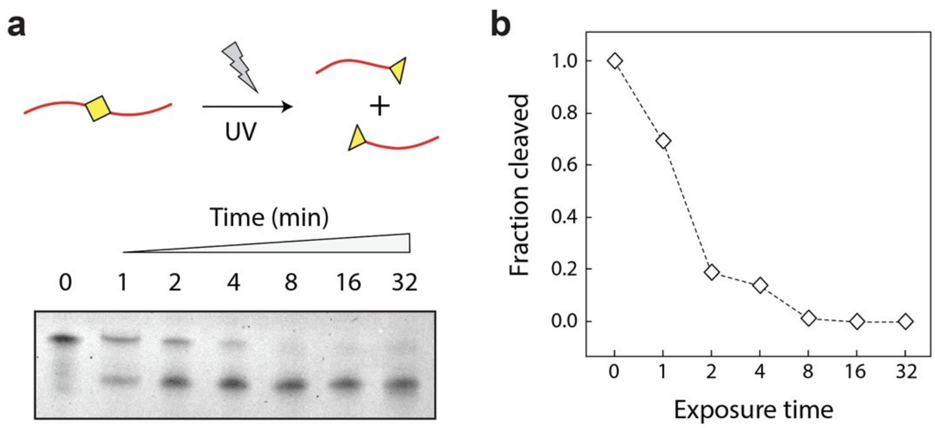

Figure S2. (a) UV exposure time series for cleavage of the PCL-containing strand. (b) Quantitative analysis of cleavage response over irradiation time.

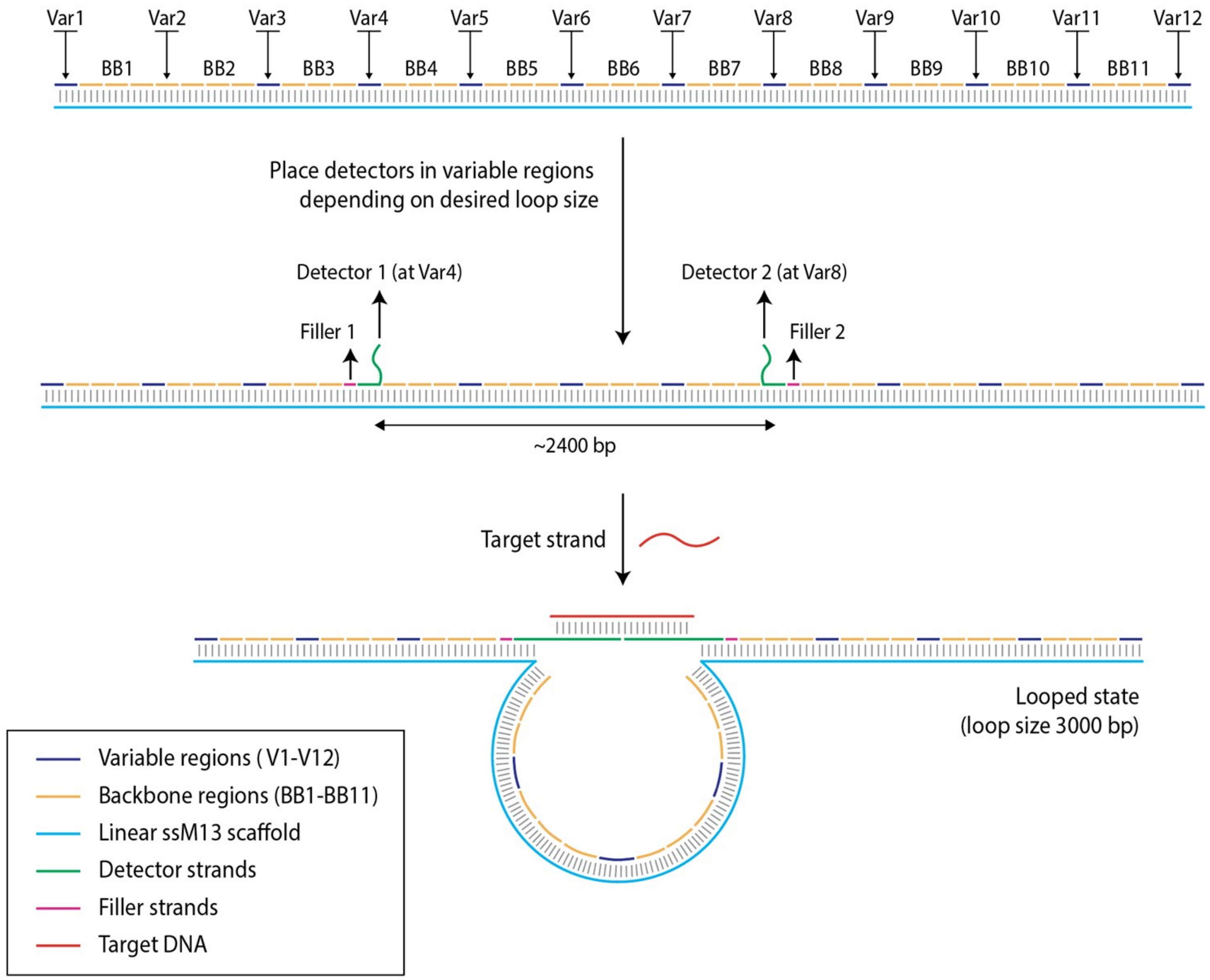

Figure S3. The nanoswitch is a duplex formed from linear M13 and short complementary backbone oligonucleotides. Twelve regions (60 nt each) are designated as “variable” regions. Two detectors containing single-stranded overhangs that complement the target can be inserted in place of two of the variable regions. The distance between the two detectors dictates the loop size and migration of the looped state on a gel.

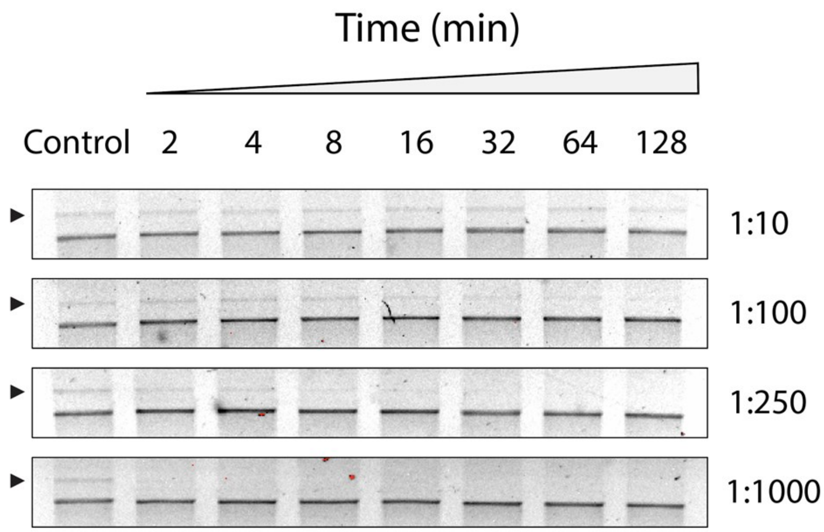

Figure S4. DNA strand displacement of the input strand resulting in unlooping of the nanoswitches. Gel results of unlooping with displacing strand at different ratios are shown. Looped bands are indicated by arrows.

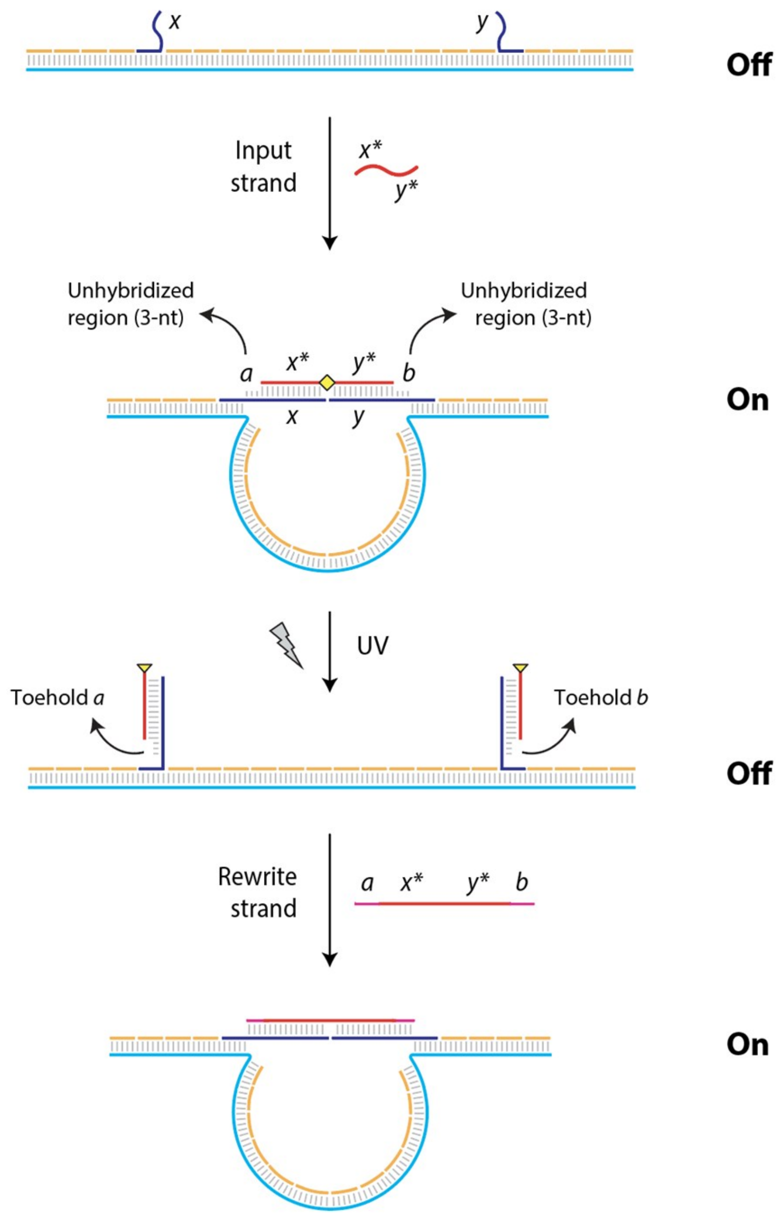

Figure S5. The detectors are 15-nt each and bind partially to the 24-nt target strand, leaving 3-nt single stranded regions on each detector. Once the PCL-containing target strand binds to the detectors, it triggers formation of the looped "on" state. On UV exposure, the target strand is cleaved, leading to unlooping of the nanoswitches ("off"). Addition of a 30-nt target strand that is fully complementary to both detectors displaces the cleaved targets strand from the detector re-forming the looped "on" state.

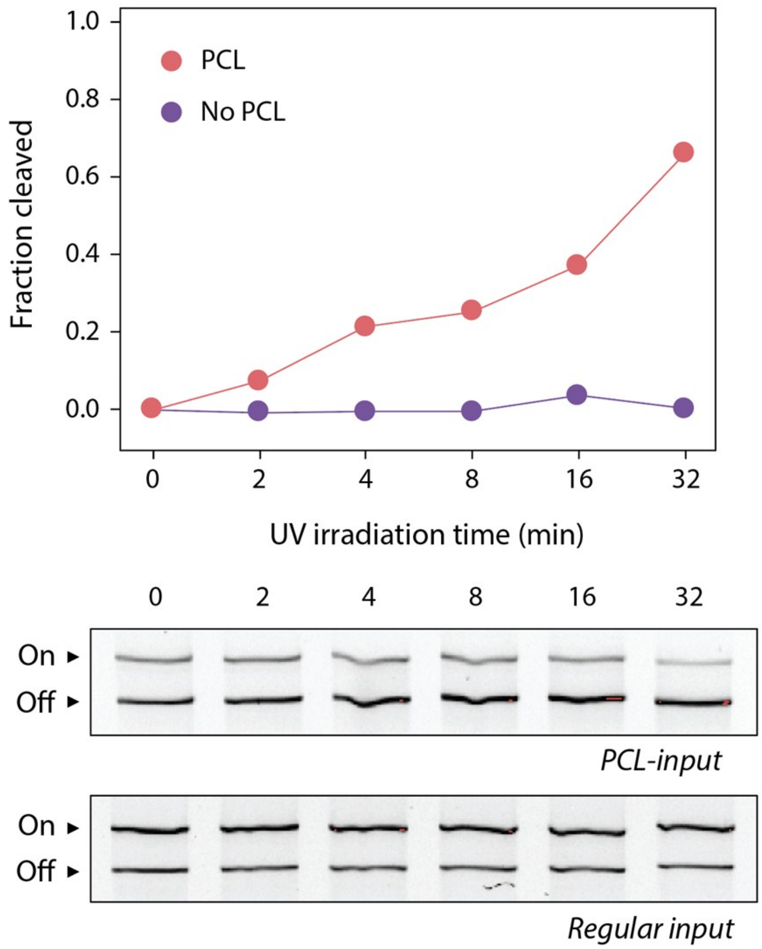

Figure S6. Photo-cleavage of looped nanoswitches using 365 nm UV light.

Complete list of all sequences used. All sequences are written from 5' to 3'.

Backbone oligonucleotides		
#	Sequence	Length
1	AGAGCATAAAGCTAAATCGGTTGTACCAAAAACATTATGACCCTGTAATACTTTTGCGGG	60
2	AGAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAGAACCCTCATATATTTTAAATGC	60
3	AATGCCTGAGTAATGTGTAGGTAAAGATTCAAAAGGGTGAGAAAGGCCGGAGACAGTCAA	60
4	ATCACCATCAATATGATATTCAACCGTTCTAGCTGATAAATTAATGCCGGAGAGGGTAGC	60
5	TATTTTTGAGAGATCTACAAAGGCTATCAGGTCATTGCCTGAGAGTCTGGAGCAAACAAG	60
6	AGAATCGATGAACGGTAATCGTAAAACTAGCATGTCAATCATATGTACCCCGGTTGATAA	60
7	TCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAA	60
8	TATTTTGTTAAAATTCGCATTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGA	60
9	ACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGTAGCCAGCTTTCATCAACATTAAAT	60
10	GGATAGGTCACGTTGGTGTAGATGGGCGCATCGTAACCGTGCATCTGCCAGTTTGAGGGG	60
11	ACGACGACAGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTTCCGGCACCGCTTCT	60
12	GGTGCCGGAAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGG	60
13	CGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGG	60
14	CGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGT	60
15	GCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC	60
16	GTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA	60
17	CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCAC	60
18	ATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCA	60
19	TTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTT	60
20	GTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGG	60
21	TTCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGT	60
22	TGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCG	60
23	AAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCAAATCAAGTTTTTT	60
24	GGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGC	60
25	TTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGG	60
26	CGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCT	60
27	TAATGCGCCGCTACAGGGCGCGTACTATGGTTGCTTTGACGAGCACGTATAACGTGCTTT	60
28	CCTCGTTAGAATCAGAGCGGGAGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGGA	60
29	ACGGTACGCCAGAATCCTGAGAAGTGTTTTTATAATCAGTGAGGCCACCGAGTAAAAGAG	60
30	TTGCCTGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATATCCAGAACAATATTAC	60
31	CGCCAGCCATTGCAACAGGAAAAACGCTCATGGAAATACCTACATTTTGACGCTCAATCG	60
32	TCTGAAATGGATTATTTACATTGGCAGATTCACCAGTCACACGACCAGTAATAAAAGGGA	60
33	CATTCTGGCCAACAGAGATAGAACCCTTCTGACCTGAAAGCGTAAGAATACGTGGCACAG	60
34	ACAATATTTTTGAATGGCTATTAGTCTTTAATGCGCGAACTGATAGCCCTAAAACATCGC	60
35	CATTAAAAATACCGAACGAACCACCAGCAGAAGATAAAACAGAGGTGAGGCGGTCAGTAT	60

36	TAACACCGCCTGCAACAGTGCCACGCTGAGAGCCAGCAGCAAATGAAAAATCTAAAGCAT	60
37	CACCTTGCTGAACCTCAAATATCAAACCCTCAATCAATATCTGGTCAGTTGGCAAATCAA	60
38	CAGTTGAAAGGAATTGAGGAAGGTTATCTAAAATATCTTTAGGAGCACTAACAACTAATA	60
39	GATTAGAGCCGTCAATAGATAATACATTTGAGGATTTAGAAGTATTAGACTTTACAAACA	60
40	CATTATCATTTTGCGGAACAAAGAAACCACCAGAAGGAGCGGAATTATCATCATATTCCT	60
41	GATTATCAGATGATGGCAATTCATCAATATAATCCTGATTGTTTGGATTATACTTCTGAA	60
42	TAATGGAAGGGTTAGAACCTACCATATCAAAATTATTTGCACGTAAAACAGAAATAAAGA	60
43	AATTGCGTAGATTTTCAGGTTTAACGTCAGATGAATATACAGTAACAGTACCTTTTACAT	60
44	CGGGAGAAACAATAACGGATTCGCCTGATTGCTTTGAATACCAAGTTACAAAATCGCGCA	60
45	GAGGCGAATTATTCATTTCAATTACCTGAGCAAAAGAAGATGATGAAACAAACATCAAGA	60
46	AAACAAAATTAATTACATTTAACAATTTCATTTGAATTACCTTTTTTAATGGAAACAGTA	60
47	CATAAATCAATATATGTGAGTGAATAACCTTGCTTCTGTAAATCGTCGCTATTAATTAAT	60
48	TTTCCCTTAGAATCCTTGAAAACATAGCGATAGCTTAGATTAAGACGCTGAGAAGAGTCA	60
49	ATAGTGAATTTATCAAAATCATAGGTCTGAGAGACTACCTTTTTAACCTCCGGCTTAGGT	60
50	GAAAACTTTTTCAAATATATTTTAGTTAATTTCATCTTCTGACCTAAATTTAATGGTTTG	60
51	AAATACCGACCGTGTGATAAATAAGGCGTTAAATAAGAATAAACACCGGAATCATAATTA	60
52	CTAGAAAAAGCCTGTTTAGTATCATATGCGTTATACAAATTCTTACCAGTATAAAGCCAA	60
53	CGCTCAACAGTAGGGCTTAATTGAGAATCGCCATATTTAACAACGCCAACATGTAATTTA	60
54	GGCAGAGGCATTTTCGAGCCAGTAATAAGAGAATATAAAGTACCGACAAAAGGTAAAGTA	60
55	ATTCTGTCCAGACGACGACAATAAACAACATGTTCAGCTAATGCAGAACGCGCCTGTTTA	60
56	TCAACAATAGATAAGTCCTGAACAAGAAAAATAATATCCCATCCTAATTTACGAGCATGT	60
57	AGAAACCAATCAATAATCGGCTGTCTTTCCTTATCATTCCAAGAACGGGTATTAAACCAA	60
58	GTACCGCACTCATCGAGAACAAGCAAGCCGTTTTTATTTTCATCGTAGGAATCATTACCG	60
59	CGCCCAATAGCAAGCAAATCAGATATAGAAGGCTTATCCGGTATTCTAAGAACGCGAGGC	60
60	ATTTTGCACCCAGCTACAATTTTATCCTGAATCTTACCAACGCTAACGAGCGTCTTTCCA	60
61	GAGCCTAATTTGCCAGTTACAAAATAAACAGCCATATTATTTATCCCAATCCAAATAAGA	60
62	AACGATTTTTTGTTTAACGTCAAAAATGAAAATAGCAGCCTTTACAGAGAGAATAACATA	60
63	AAAACAGGGAAGCGCATTAGACGGGAGAATTAACTGAACACCCTGAACAAAGTCAGAGGG	60
64	TAATTGAGCGCTAATATCAGAGAGATAACCCACAAGAATTGAGTTAAGCCCAATAATAAG	60
65	AGCAAGAAACAATGAAATAGCAATAGCTATCTTACCGAAGCCCTTTTTAAGAAAAGTAAG	60
66	CAGATAGCCGAACAAAGTTACCAGAAGGAAACCGAGGAAACGCAATAATAACGGAATACC	60
67	CAAAAGAACTGGCATGATTAAGACTCCTTATTACGCAGTATGTTAGCAAACGTAGAAAAT	60
68	ACATACATAAAGGTGGCAACATATAAAAGAAACGCAAAGACACCACGGAATAAGTTTATT	60
69	TTGTCACAATCAATAGAAAATTCATATGGTTTACCAGCGCCAAAGACAAAAGGGCGACAT	60
70	TCACCGTCACCGACTTGAGCCATTTGGGAATTAGAGCCAGCAAAATCACCAGTAGCACCA	60
71	TTACCATTAGCAAGGCCGGAAACGTCACCAATGAAACCATCGATAGCAGCACCGTAATCA	60
72	GTAGCGACAGAATCAAGTTTGCCTTTAGCGTCAGACTGTAGCGCGTTTTCATCGGCATTT	60
73	TCGGTCATAGCCCCCTTATTAGCGTTTGCCATCTTTTCATAATCAAAATCACCGGAACCA	60
74	GAGCCACCACCGGAACCGCCTCCCTCAGAGCCGCCACCCTCAGAACCGCCACCCTCAGAG	60
75	CCACCACCCTCAGAGCCGCCACCAGAACCACCACCAGAGCCGCCGCCAGCATTGACAGGA	60
76	GGTTGAGGCAGGTCAGACGATTGGCCTTGATATTCACAAACAAATAAATCCTCATTAAAG	60
77	CCAGAATGGAAAGCGCAGTCTCTGAATTTACCGTTCCAGTAAGCGTCATACATGGCTTTT	60
78	GATGATACAGGAGTGTACTGGTAATAAGTTTTAACGGGGTCAGTGCCTTGAGTAACAGTG	60

79	CCCGTATAAACAGTTAATGCCCCCTGCCTATTTCGGAACCTATTATTCTGAAACATGAAA	60
80	CCAGGCGGATAAGTGCCGTCGAGAGGGTTGATATAAGTATAGCCCGGAATAGGTGTATCA	60
81	CCGTACTCAGGAGGTTTAGTACCGCCACCCTCAGAACCGCCACCCTCAGAACCGCCACCC	60
82	TCAGAGCCACCACCCTCATTTTCAGGGATAGCAAGCCCAATAGGAACCCATGTACCGTAA	60
83	CACTGAGTTTCGTCACCAGTACAAACTACAACGCCTGTAGCATTCCACAGACAGCCCTCA	60
84	TAGTTAGCGTAACGATCTAAAGTTTTGTCGTCTTTCCAGACGTTAGTAAATGAATTTTCT	60
85	GTATGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGAGTGAGAATAGAAAGGAACA	60
86	ACTAAAGGAATTGCGAATAATAATTTTTTCACGTTGAAAATCTCCAAAAAAAAGGCTCCA	60
87	AAAGGAGCCTTTAATTGTATCGGTTTATCAGCTTGCTTTCGAGGTGAATTTCTTAAACAG	60
88	CTTGATACCGATAGTTGCGCCGACAATGACAACAACCATCGCCCACGCATAACCGATATA	60
89	TTCGGTCGCTGAGGCTTGCAGGGAGTTAAAGGCCGCTTTTGCGGGATCGTCACCCTCAGC	60
90	CTTTTTCATGAGGAAGTTTCCATTAAACGGGTAAAATACGTAATGCCACTACGAAGGCAC	60
91	CAACCTAAAACGAAAGAGGCAAAAGAATACACTAAAACACTCATCTTTGACCCCCAGCGA	60
92	TTATACCAAGCGCGAAACAAAGTACAACGGAGATTTGTATCATCGCCTGATAAATTGTGT	60
93	CGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGCGCAGACGGTCAATCATA	60
94	AGGGAACCGAACTGACCAACTTTGAAAGAGGACAGATGAACGGTGTACAGACCAGGCGCA	60
95	TAGGCTGGCTGACCTTCATCAAGAGTAATCTTGACAAGAACCGGATATTCATTACCCAAA	60
96	TCAACGTAACAAAGCTGCTCATTCAGTGAATAAGGCTTGCCCTGACGAGAAACACCAGAA	60
97	CGAGTAGTAAATTGGGCTTGAGATGGTTTAATTTCAACTTTAATCATTGTGAATTACCTT	60
98	ATGCGATTTTAAGAACTGGCTCATTATACCAGTCAGGACGTTGGGAAGAAAAATCTACGT	60
99	TAATAAAACGAACTAACGGAACAACATTATTACAGGTAGAAAGATTCATCAGTTGAGATT	60
100	TAAGAGCAACACTATCATAACCCTCGTTTACCAGACGACGATAAAAACCAAAATAGCGAG	60
101	AGGCTTTTGCAAAAGAAGTTTTGCCAGAGGGGGTAATAGTAAAATGTTTAGACTGGATAG	60
102	CGTCCAATACTGCGGAATCGTCATAAATATTCATTGAATCCCCCTCAAATGCTTTAAACA	60
103	GTTCAGAAAACGAGAATGACCATAAATCAAAAATCAGGTCTTTACCCTGACTATTATAGT	60
104	CAGAAGCAAAGCGGATTGCATCAAAAAGATTAAGAGGAAGCCCGAAAGACTTCAAATATC	60
105	GCGTTTTAATTCGAGCTTCAAAGCGAACCAGACCGGAAGCAAACTCCAACAGGTCAGGAT	60
106	TAGAGAGTACCTTTAATTGCTCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGC	60
107	TTAATTGCTGAATATAATGCTGTAGCTCAACATGTTTTAAATATGCAACTAAAGTACGGT	60
108	GTCTGGAAGTTTCATTCCATATAACAGTTGATTCCCAATTCTGCGAACGAGTAGATTTAG	60
109	TTTGACCATTAGATACATTTCGCAAATGGTCAATAACCTGTTTAGCTAT	49

Regions in bold indicate locations where detector strands are placed. Blue regions are single stranded extensions on detectors that are complementary to two halves of the input strands.

Variable sequences		
#	Sequence	Length
Var 1	AACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAGCAATAAAGCCTC	60
Var 2	GTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATG	60
Var 3	TTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGA	60
Var 4	**TCTGTCCATCACGCAAATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCAC**	60
Var 5	**ATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATTAATTTTAAAAGTTTGAGTAA**	60
Var 6	**TGGGTTATATAACTATATGTAAATGCTGATGCAAATCCAATCGCAAGACAAAGAACGCGA**	60
Var 7	**GTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCCTTAAATCAAGATTAGTTGCT**	60
Var 8	**TCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAATTATTCATTAAAGGTGAATTA**	60
Var 9	**GTATTAAGAGGCTGAGACTCCTCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGTA**	60
Var 10	AGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTACAGAGGCTTTGAGGACTAAAGA	60
Var 11	TAGGAATACCACATTCAACTAATGCAGATACATAACGCCAAAAGGAATTACGAGGCATAG	60
Var 12	ATTTTCATTTGGGGCGCGAGCTGAAAAGGTGGCATCAATTCTACTAATAGTAGTAGCATT	60

Address site oligos		
#	Sequence	Length
A0	CAATACTTCTTTGATTAGTAATAACATCACCTATGGATACGTTCT	45
A1	AGGTGCCTTATATTCATTCGACAACTCGTATTAAATCCTTTGCCC	45
A2	GGATATTCATTCCTGTGGGTTATATAACTATATGTAAATGCTGAT	45
A3	TTCAGTTACATGCGTGTTTTAGCGAACCTCCCGACTTGCGGGAGG	45
A4	GCACTAGTTTCTTAGTCAACCGATTGAGGGAGGGAAGGTAAATAT	45
A5	GTCCCTTATAGTTAGGTATTAAGAGGCTGAGACTCCTCAAGAGAA	45

Filler sequences		
#	Sequence	Length
F0	TCTGTCCATCACGCAAATTAACCGTTGTAG	30
F1	GAACGTTATTAATTTTAAAAGTTTGAGTAA	30
F2	GCAAATCCAATCGCAAGACAAAGAACGCGA	30
F3	TTTTGAAGCCTTAAATCAAGATTAGTTGCT	30
F4	TGACGGAAATTATTCATTAAAGGTGAATTA	30
F5	GGATTAGGATTAGCGGGGTTTTGCTCAGTA	30

Input strands		
#	Sequence	Length
i 0-1	TATAAGGCACCT(PCL)AGAACGTATCCA	24
i 0-2	GAATGAATATCC(PCL)AGAACGTATCCA	24
i 0-3	CATGTAACTGAA(PCL)AGAACGTATCCA	24
i 0-4	AGAAACTAGTGC(PCL)AGAACGTATCCA	24
i 0-5	ACTATAAGGGAC(PCL)AGAACGTATCCA	24

Other strands		
#	Sequence	Length
Rewrite strand	ACGCATGTAACTGAAAGAACGTATCCATAG	30
Toehold input	ACGCATGTAACTGAAAGAACGTATCCATAGGATCATCC	38
Eraser strand	GGATGATCCTATGGATACGTTCTTTCAGTTACATGCGT	38