Supporting Information

DNA nanoswitch barcodes for multiplexed biomarker profiling

Arun Richard Chandrasekaran,[1]* Molly MacIsaac,[2,3,4] Javier Vilcapoma,[1]
Clinton H. Hansen,[2,3,4] Darren Yang,[2,3,4] Wesley P. Wong[2,3,4]* and Ken Halvorsen[1]*

[1]The RNA Institute, University at Albany, State University of New York, New York, NY, USA.
[2]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA 02115.
[3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115.
[4]Department of Biological Chemistry and Molecular Pharmacology, Blavatnik Institute, Harvard Medical School, Boston, MA 02115.

Email: arun@albany.edu, wesley.wong@childrens.harvard.edu, khalvorsen@albany.edu

MATERIALS AND METHODS

Materials

Oligonucleotides (including RNA, biotin-modified DNA and digoxygenin-modified DNA) were purchased from Integrated DNA Technologies (IDT) with standard desalting. M13 circular DNA and BtsCI enzymes were purchased from New England Biolabs (NEB). GelRed nucleic acid stain was purchased from Biotium, Fremont, CA, USA. Molecular biology grade agarose was purchased from Fisher BioReagents.

Linearization of M13 DNA

5 μl of 100 nM 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) 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.

Antibody coupling

Antibodies were coupled to oligonucleotides as previously described (Hansen et al, PNAS 2017, 114, 10367-10372). In brief, sandwiching antibodies against PSA (Medix Biochemica Anti-h PSA 8301 and Anti-h PSA 8311) were buffer exchanged in 1× PBS using two Zeba columns (Thermo Fisher Scientific) to remove storage sodium azide. 60 μL of washed PSA antibodies at ~4.0 μM were mixed with an equimolar amount of DBCO-PEG4-NHS ester linker (Sigma) in 40 mM, pH 8.6 HEPES buffer and incubated at room temperature for 30 min. Then, 5-fold excess of azide-modified oligonucleotides (Integrated DNA technologies) were added and incubated for 90 min at room temperature. Antibody-oligo conjugates were then purified from excess oligonucleotides and linkers using the Thunder-Link Conjugate Clean Up Reagent (Innova Biosciences) and resuspended in 40 μL of 1× PBS. Antibody coupling and purification were confirmed using 4-20% TBE polyacrylamide gels (Bio-Rad) stained with Krypton Fluorescent Protein Stain (Thermo Fisher Scientific).

Construction of nanoswitches

For genotyping, biotin and digoxygenin nanoswitches, linearized single-stranded M13 DNA (20 nM) was mixed with 10-fold excess of the backbone oligonucleotides and detector strands and annealed from 90 °C to 20 °C at 1 °C min^{-1} in a thermal cycler. Following construction, the nanoswitches were purified using HPLC to remove excess oligonucleotides or used unpurified after dilution in 1× PBS to a concentration of 400 pM. For PSA nanoswitches, linearized single-stranded M13 DNA (20 nM) was mixed with 10-fold excess of the backbone oligonucleotides and annealed from 90 °C to 20 °C at 1 °C min^{-1} in a thermal cycler. 80-fold excess of the conjugated antibody-oligonucleotide detectors were added when the hybridization protocol reached 37 °C. Following construction, the nanoswitches were purified with BluePippin using 0.75% agarose, 1-50kb gel cassette (Sage Science) to remove excess oligonucleotides and antibody-oligo conjugates. The PSA nanoswitches were stored undiluted in protein lobind tubes (Eppendorf) at 4 °C.

Nanoswitch characterization

A typical reaction contained 160 pM nanoswitch with 2.5 nM DNA targets in a 10 μl reaction. Samples were incubated at room temperature for 1 h. For sensitivity experiment, the target DNA was spiked into a 500 nM solution of off-target "blocking" oligos to minimize loss to the tubes. Microcentrifuge tubes and pipette tips were additionally pre-incubated in blocking oligo solution to minimize loss of the target DNA. The nanoswitch/DNA target solution was incubated overnight at 20 °C in a solution containing 1× PBS and 10 mM $MgCl_2$. For specificity experiments, the final concentration of target strands were 2.5 nM and incubation time was 1 h at 20 °C in a solution containing 1× PBS. For the time series experiment (Figure S5), we performed the reaction in reverse, starting with the longest time point followed by shorter time points until time 0. Samples were mixed with gel stain and loading dye, and immediately loaded in gels. For detecting double stranded DNA, the duplexes were heated at 90 °C for 3 minutes and added to DNA nanoswitches, and incubated at 20 °C for 1 h.

Nanoswitch barcode operation for gene identification and biomarker detection

Nanoswitch mixture was prepared by mixing equimolar amounts of individual nanoswitches (6 nanoswitches for multiplexed gene analysis and 4 nanoswitches for mixed multiplexing). For barcoded detection, a typical reaction contained final concentrations of ~160 pM nanoswitch mixture, 1× PBS and 2.5 nM single stranded DNA corresponding to gene fragments. For barcodes of mixed biomarkers, typical concentrations were ~1 nM anti-digoxygenin, ~0.8 nM RNA, ~2 nM DNA and ~0.8 nM streptavidin. For concentration series, different concentrations of the biomarkers were added as indicated in Figure 4 and Figure S9.

Biomarker panel detection in serum

Nanoswitch mixture was prepared by mixing miR-30c, PSA, and miR-141 nanoswitches to a final molar ratio of 1:2:3 respectively. EDTA was added to 20% FBS to a final concentration of 100 mM. For serum detection, a typical 11 μL reaction contained 6 μL of 20% FBS with 100mM EDTA, biomarkers at a final concentration of 2.5 nM, 1× PBS, and 2 μL of nanoswitch mixture (final concentrations of 30 pM, 60 pM, and 90 pM). The biomarkers used were PSA antigen (Biospacific, cat#: J63011, high purity) and ssDNA sequences of miR-141 and miR-30c (Integrated DNA technologies). Samples were incubated at room temperature for 90 minutes.

Multiplexed sensitivity test

Nanoswitch mixture was prepared by mixing PSA and miR-141 nanoswitches (of different loop sizes) to a final molar ratio of 1:2 respectively. Final reaction volume of 15 μl contained 3 μl nanoswitch mixture (final concentrations of ~100 pM PSA nanoswitch and ~200 pM miR-141 nanoswitch), 100 mM $MgCl_2$, 1× PBS, 95 pM PSA and varying concentrations of miR-141 (Figure S11). Target microRNA was spiked into a 500 nM solution of off-target "blocking" oligos to minimize loss to the tubes. Microcentrifuge tubes and pipette tips were additionally pre-incubated in blocking oligo solution to minimize loss of the target microRNA. Samples were incubated overnight at 20 °C. Samples were pre-stained by mixing 1× GelRed stain and a Ficoll based loading dye (15% Ficoll, 0.1% bromophenol blue) before loading. 10 μl of the samples was loaded and run on an unstained agarose gel.

Gel electrophoresis

Nanoswitches were run in 0.8% agarose gels, cast from molecular biology grade agarose dissolved in 0.5× Tris-borate EDTA (TBE). Characterization gels of individual nanoswitches were typically run at 75 V (constant voltage) at room temperature and barcode gels (multiplexing) were run at 55 V (constant voltage) at 4 °C. Samples were pre-stained by mixing 1× GelRed stain and a Ficoll based loading dye with the samples before loading. 10 μl of the samples were loaded and run on an unstained agarose gel. Gels were imaged with a Bio-Rad Gel Doc XR+ gel imager and analyzed using ImageJ. For the prostate cancer biomarker panel, nanoswitches were run in 0.8% agarose gels at 70 V (constant voltage) at room temperature. After incubation, samples were diluted by adding 1× volume of 0.5× TBE. Samples were then pre-stained with 1× GelRed stain and a Ficoll based loading dye (Promega) and 0.25 μL of 1% Coomassie Brilliant Blue G-250 were added before loading. Gels were imaged with an Invitrogen iBright FL1000 Imaging System and analyzed using ImageJ. Median filter in ImageJ was used to remove noise in gel images in Figure 5c.

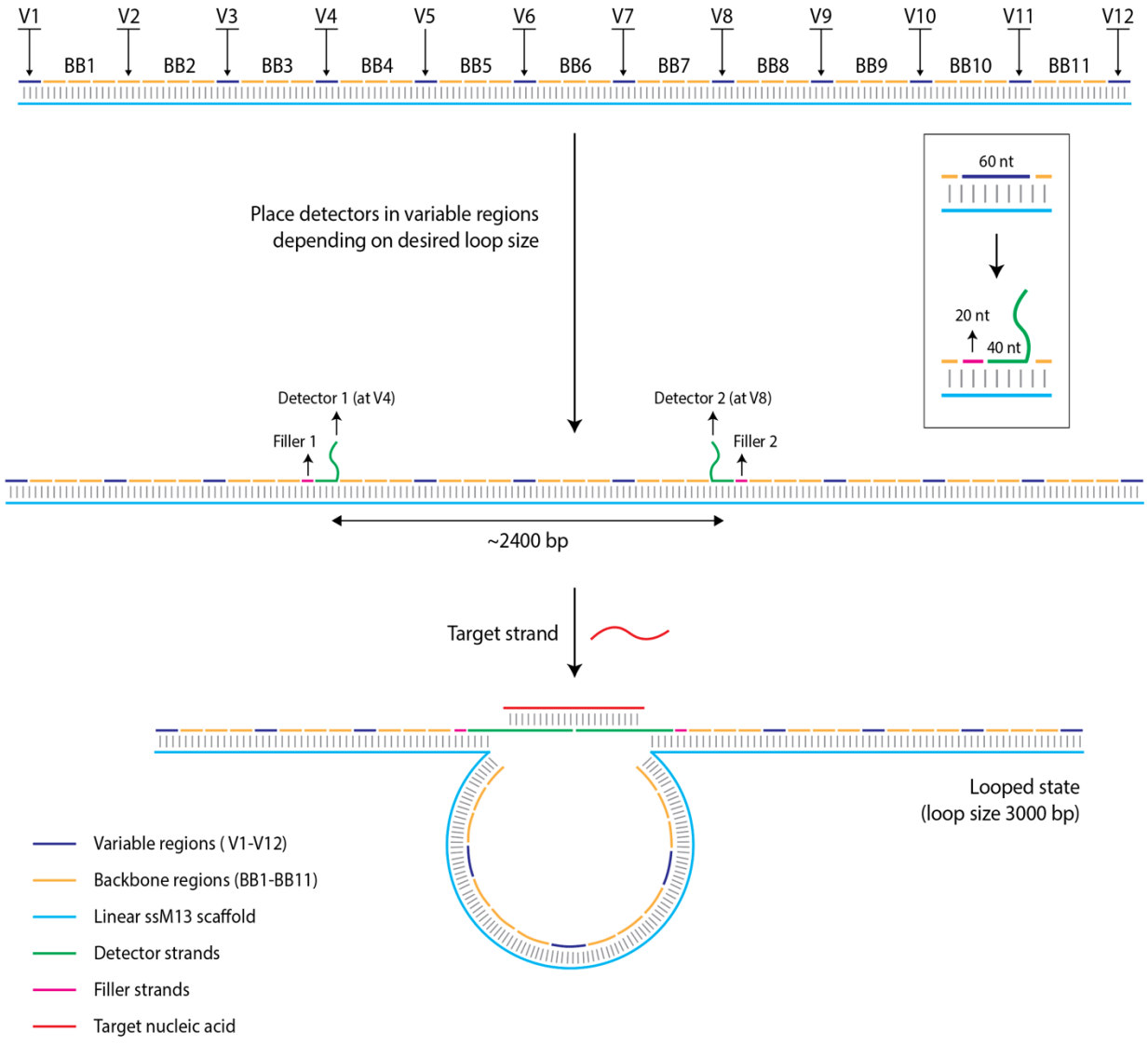

Figure S1. The nanoswitch is a duplex formed from linear M13 and short complementary backbone oligonucleotides. Twelve regions (60 nt each) are designated as “variable” regions. Two detectors containing single-stranded overhangs that complement the target can be inserted in place of two of the variable regions. The distance between the two detectors dictates the loop size and migration of the looped state on a gel.

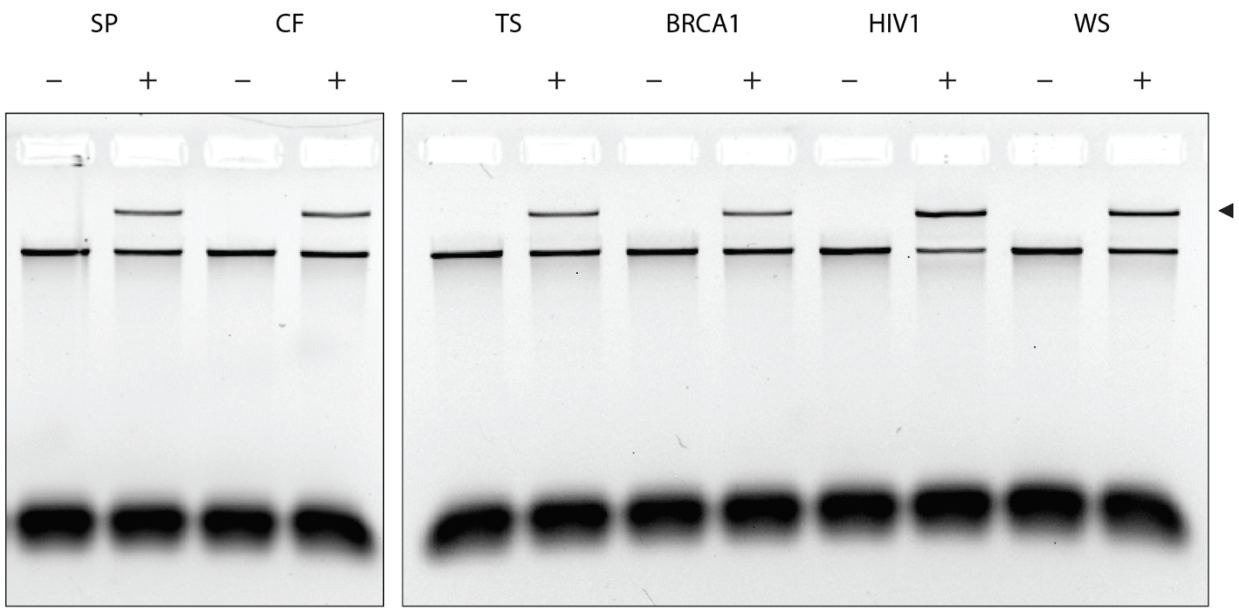

Figure S2. Detection of different gene fragments using DNA nanoswitches. Gel shown here is the full image of the gel shown in Figure 2b.

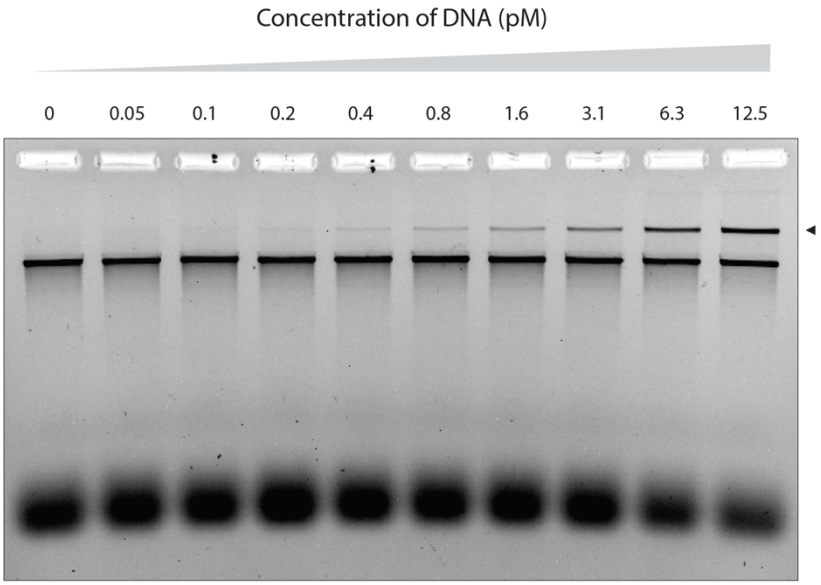

Figure S3. Sensitivity of DNA nanoswitch assay for cystic fibrosis gene fragment. Full image of gel shown in Figure 2c.

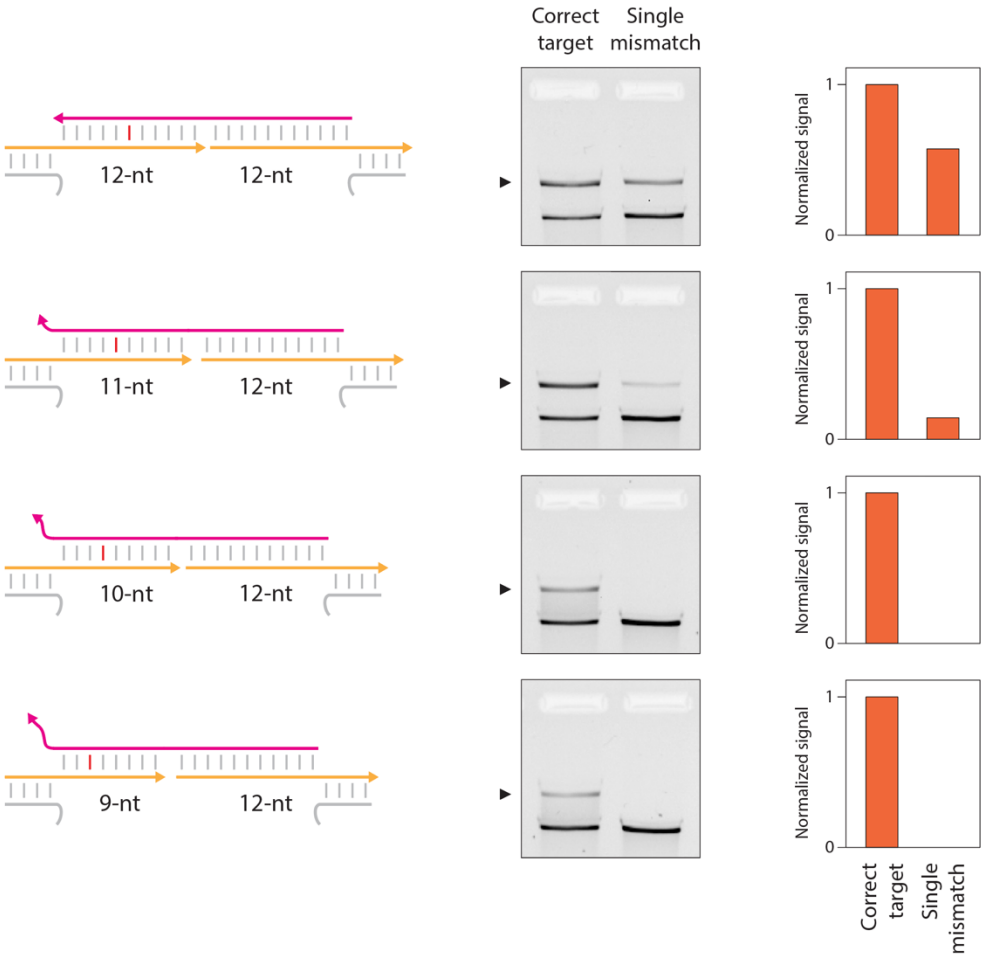

Figure S4. Specificity of DNA nanoswitch assay demonstrated with cystic fibrosis gene fragment. With detector length that complements the whole target sequence, a single mismatched target yielded 40% signal compared to a correctly matched target. On reducing the length of the detector, the specificity to discriminate a single mismatch increased, with no signal for the mismatched target using 10-nt and 9-nt detector length. The 9-nt detector length was used for the specificity image shown in Figure 2d.

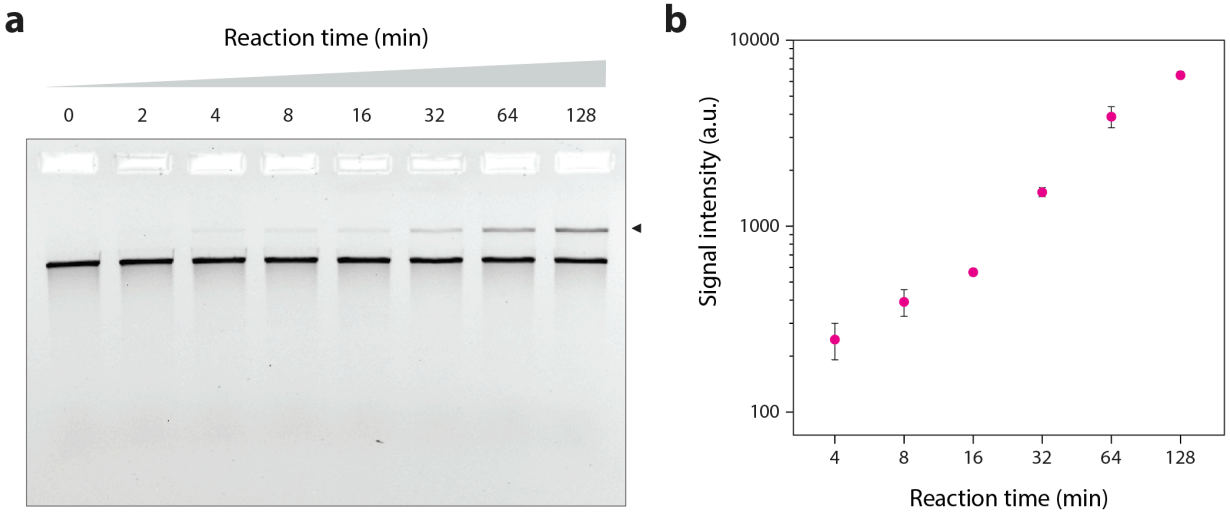

Figure S5. Time series of detection of a 1 nM cystic fibrosis gene fragment.

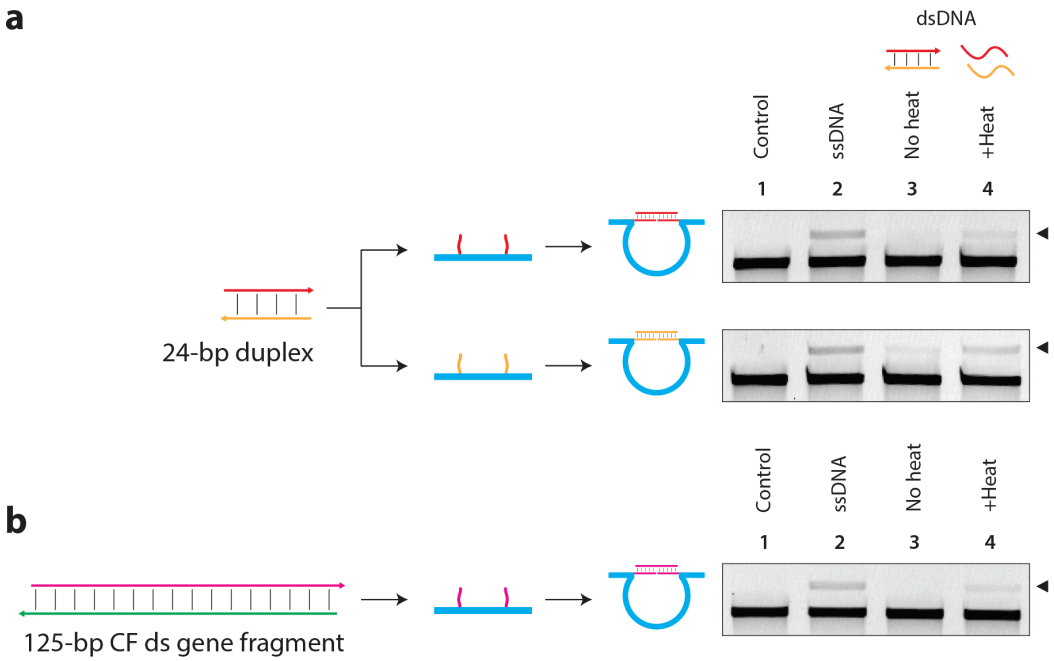

Figure S6. Detection of double stranded fragments using DNA nanoswitches. (a) Detection of each strand of a 24-bp duplex using corresponding DNA nanoswitches. (b) Detection from a double stranded 125-bp cystic fibrosis gene fragment.

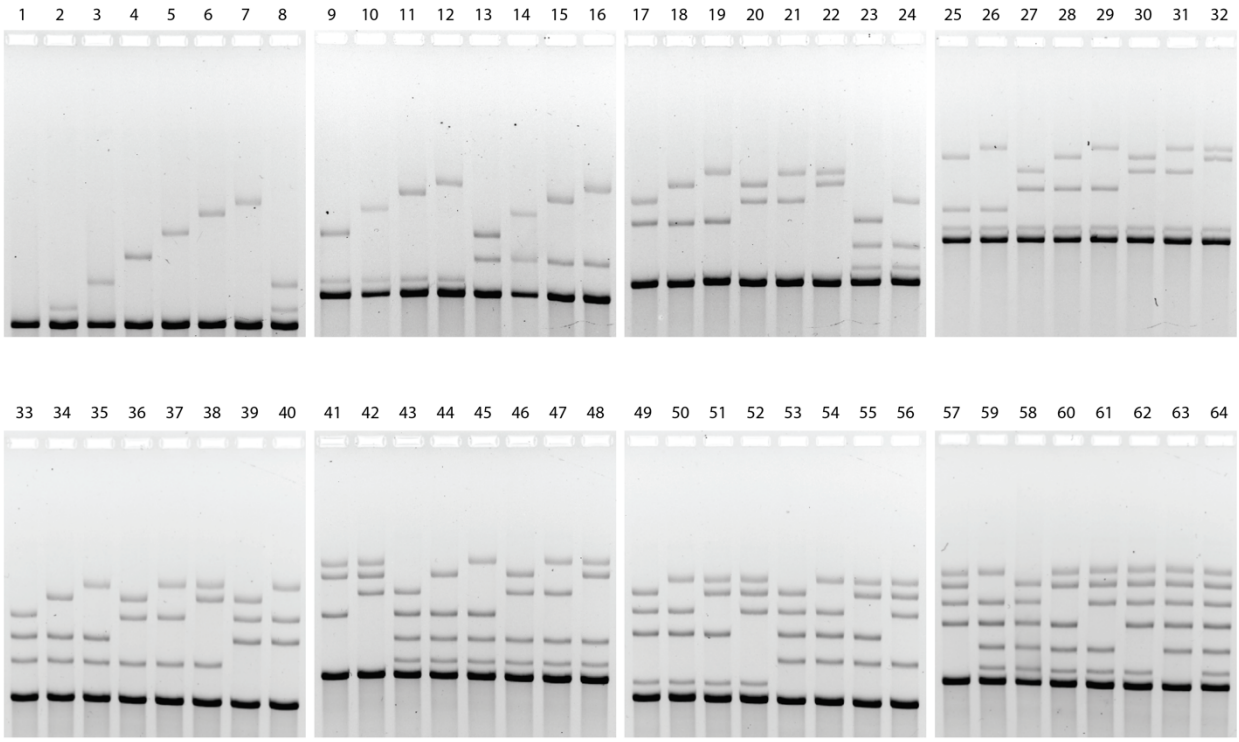

Figure S7. Full set of DNA nanoswitch gene barcodes. Gel images show all combinations of six different gene fragments. Full gel images of results shown in Figure 3d.

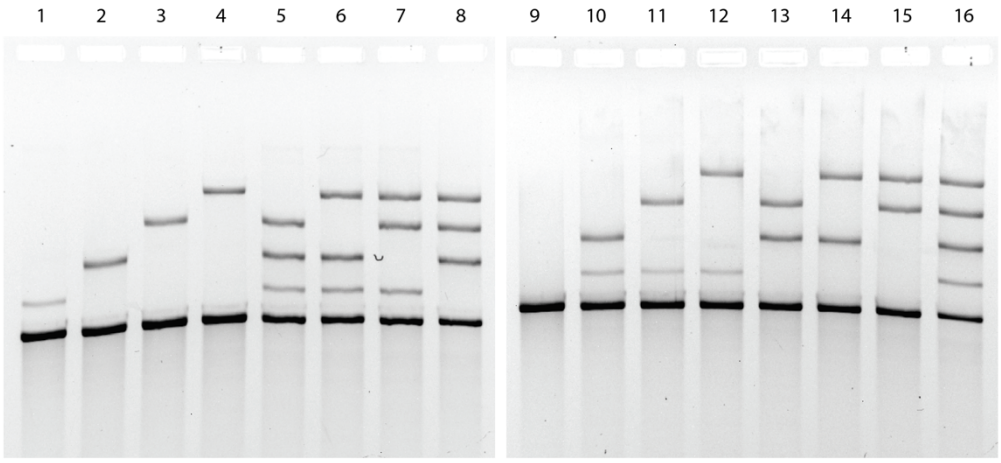

Figure S8. Full set of DNA nanoswitch barcodes for multiplexed detection of different types of biomarkers (anti-digoxygenin antibody, RNA, DNA and streptavidin). Full gel images of results shown in Figure 4e.

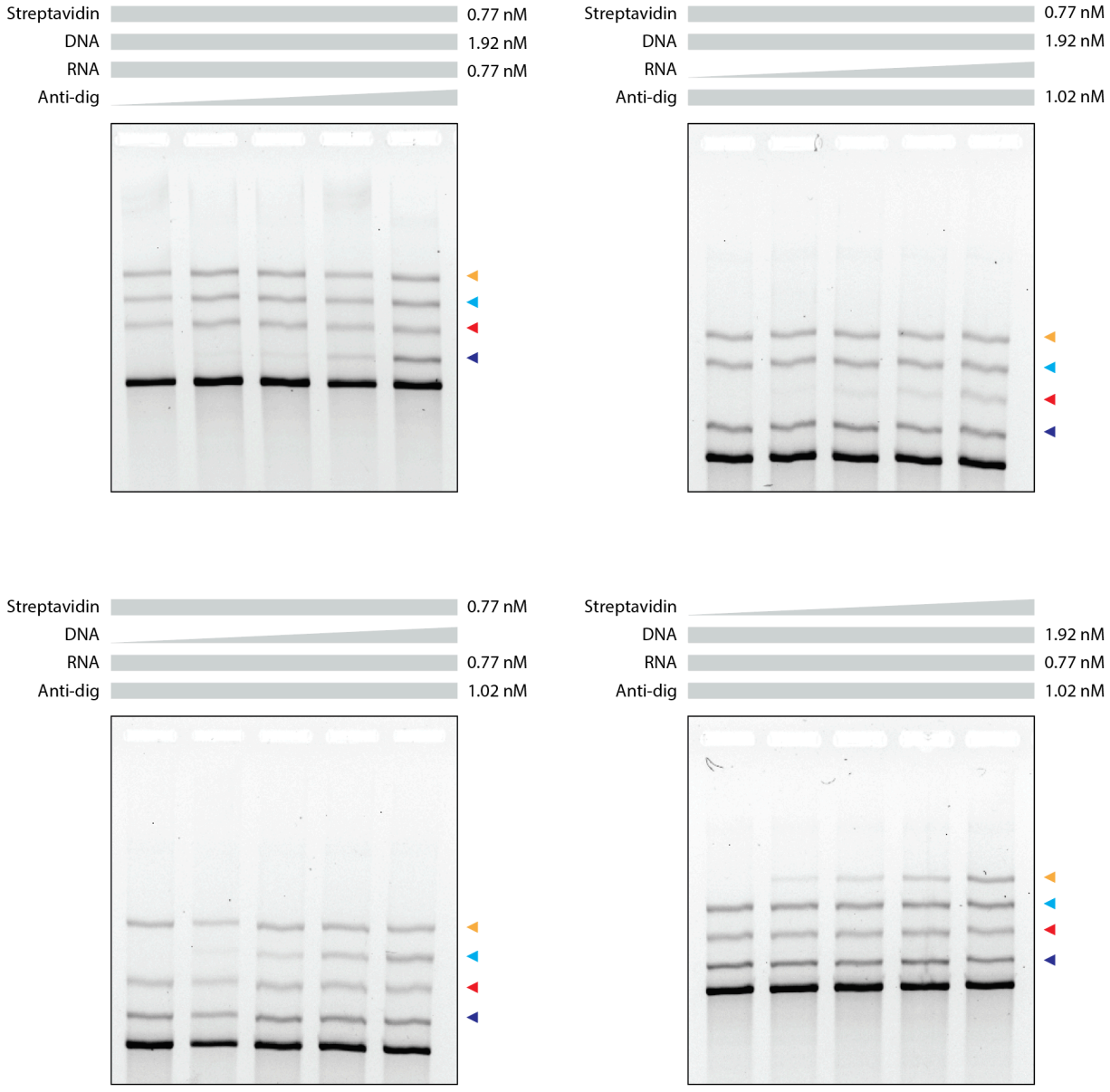

Figure S9. Multiplexed concentration series of different biomarkers. Full gel images of results shown in Figure 4f. Constant concentrations of target molecules are indicated above the gel images. The varying concentrations of a single target is shown in Figure 4f and Figure S10.

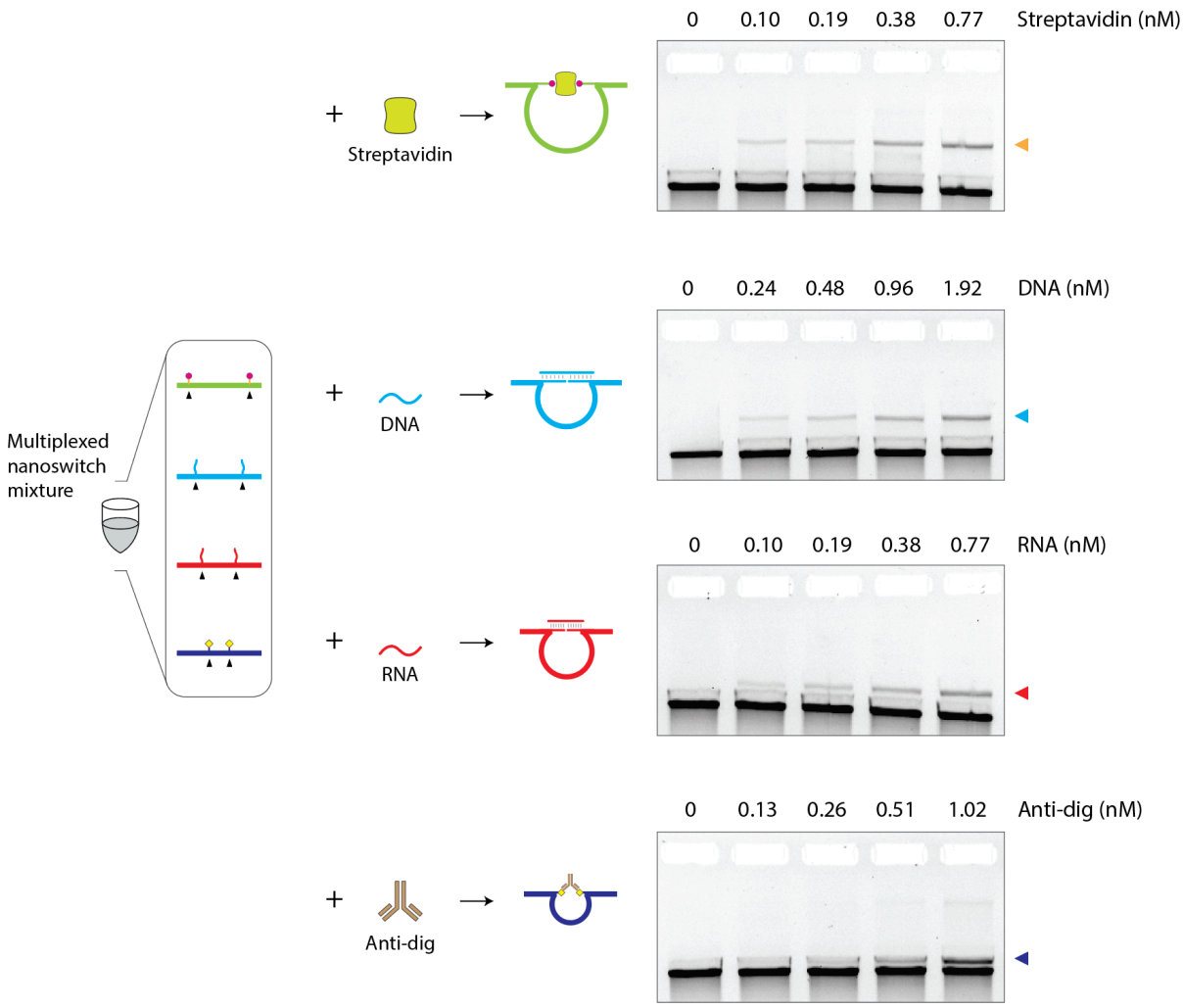

Figure S10. Concentration series of individual biomarkers using a nanoswitch mix.

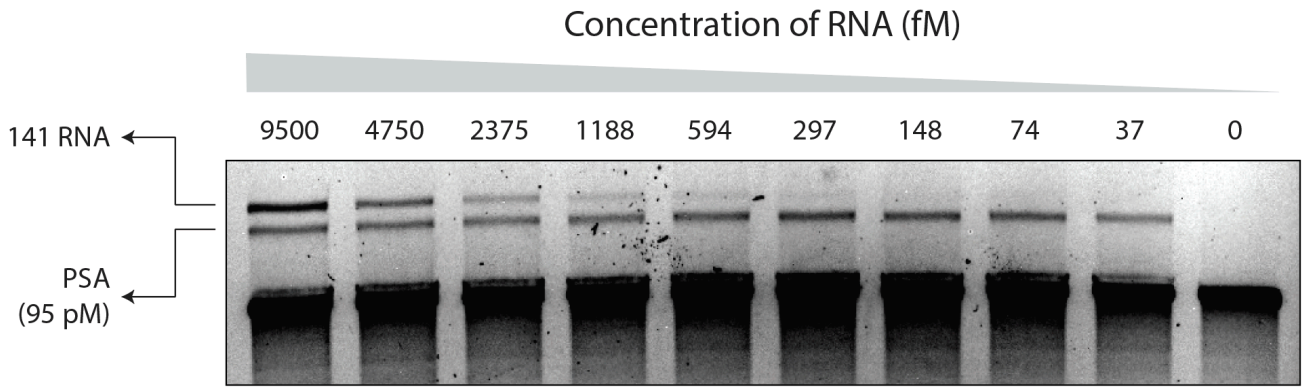

Figure S11. Clinical level sensitivity of microRNA 141 (varying concentrations) and PSA (~95 pM) in a multiplexed assay.

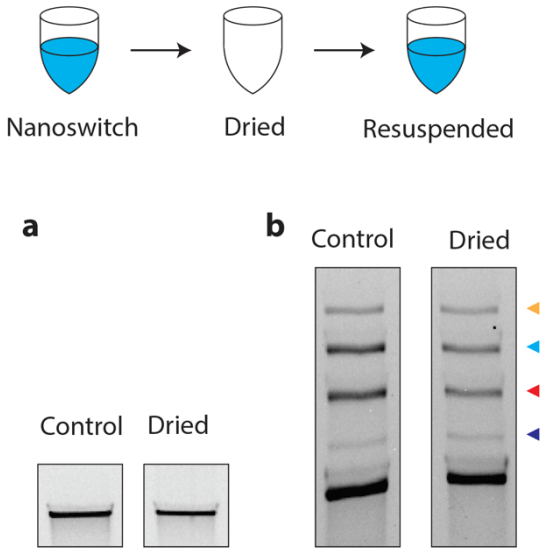

Figure S12. Stability and functionality of dried nanoswitches. (a) Nanoswitch mix is stable after drying and resuspending. (b) A dried and resuspended nanoswitch mixture can detect the four different target biomolecules.

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

Backbone oligonucleotides		
BB#	**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 and green regions are single stranded extensions on detectors that are complementary to two halves of the input strands (color coded in target sequences). For nanoswitch construction and detector placement, refer to Figure S1.

Variable sequences		
#	**Sequence**	**Length**
V1	AACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAGCAATAAAGCCTC	60
V2	GTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATG	60
V3	TTCTTTTCACCAGTGAGACG**GGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGA**	60
V4	TCTGTCCATCACGCAAATTA**ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCAC**	60
V5	**ATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATT**AATTTTAAAAGTTTGAGTAA	60
V6	**TGGGTTATATAACTATATGTAAATGCTGATGCAAATCCAA**TCGCAAGACAAAGAACGCGA	60
V7	**GTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCC**TTAAATCAAGATTAGTTGCT	60
V8	**TCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT**TATTCATTAAAGGTGAATTA	60
V9	**GTATTAAGAGGCTGAGACTCCTCAAGAGAAGGATTAGGAT**TAGCGGGGTTTTGCTCAGTA	60
V10	**AGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTACA**GAGGCTTTGAGGACTAAAGA	60
V11	TAGGAATACCACATTCAACTAATGCAGATACATAACGCCAAAAGGAATTACGAGGCATAG	60
V12	ATTTTCATTTGGGGCGCGAGCTGAAAAGGTGGCATCAATTCTACTAATAGTAGTAGCATT	60

Detector sequences for characterization (4-8 loop size)		
#	**Sequence**	**Length**
V4 SP 40-15	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACTCACAATTAGATCCT	55
V8 SP 15-40	GTAACTTACACATGATCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	55
V4 CF 40-12	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACGTCGTCTGCTGC	52
V8 CF 12-40	TGCTGCTGCTGCTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	52
V8 CF 11-40	TGCTGCTGCTGTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	51
V8 CF 10-40	TGCTGCTGCTTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	50
V8 CF 9-40	TGCTGCTGCTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	49
V4 TS 40-11	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACGAACCGTATAT	51
V8 TS 10-40	CCTATGGCCCTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	50
V4 BRCA 40-13	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACCTTCCAACAGCTA	53
V8 BRCA 12-40	TAAACAGTCCTGTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	52
V4 HIV 40-12	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACAGTCAGTGTGGA	52
V8 HIV 11-40	AAATCTCTAGCTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	51
V4 WS 40-17	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACCATCTTCAAATCCATCT	57
V8 WS 17-40	TCTTTTCATTCCACTTTTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	57

Detector sequences for gene barcodes (different loop sizes)		
Name	**Sequence**	**Length**
V4 SP 40-15	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACTCACAATTAGATCCT	55
V5 SP 15-40	GTAACTTACACATGAATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATT	55
V4 CF 40-12	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACGTCGTCTGCTGC	52
V6 CF 12-40	TGCTGCTGCTGCTGGGTTATATAACTATATGTAAATGCTGATGCAAATCCAA	52
V4 TS 40-11	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACGAACCGTATAT	51
V7 TS 10-40	CCTATGGCCCGTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCC	50
V4 BRCA 40-13	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACCTTCCAACAGCTA	53
V8 BRCA 12-40	TAAACAGTCCTGTCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	52
V4 HIV 40-12	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACAGTCAGTGTGGA	52
V9 HIV 11-40	AAATCTCTAGCGTATTAAGAGGCTGAGACTCCTCAAGAGAAGGATTAGGAT	51
V3 WS 40-17	GGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGACATCTTCAAATCCATCT	57
V10 WS 17-40	TCTTTTCATTCCACTTTAGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTACA	57

Detector sequences for mixed multiplexing barcodes (different loop sizes)		
Name	**Sequence**	**Length**
V4 dig	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCAC-dig	40
V5 dig	dig-ATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATT	40
V4 RNA	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACCGCCAATATTT	51
V6 RNA	ACGTGCTGCTATGGGTTATATAACTATATGTAAATGCTGATGCAAATCCAA	52
V4 DNA	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACCCAACAACAT	50
V7 DNA	GAAACTACCTAGTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCC	51
V4 biotin	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCAC-biotin	40
V8 biotin	biotin-TCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAAT	40
V4 miR-30c	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACTCCAACACTGT	51
V5 miR-30c	ACTGGAAGATGATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATT	51
V4 miR-141	ACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCACGCTGAGAGTGTA	52
V7 miR-141	GGATGTTTACAGTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCC	51
BB37 PSA1	CAATCAATATCTGGTCAGTTGGCAAATCAA-ab1	30
V7 PSA2	ab2-GTTTTAGCGAACCTCCCGACTTGCGGGAGG	30

Filler sequences		
Name	**Sequence**	**Length**
V3 filler	TTCTTTTCACCAGTGAGACG	20
V4 filler	TCTGTCCATCACGCAAATTA	20
V5 filler	AATTTTAAAAGTTTGAGTAA	20
V6 filler	TCGCAAGACAAAGAACGCGA	20
V7 filler	TTAAATCAAGATTAGTTGCT	20
V8 filler	TATTCATTAAAGGTGAATTA	20
V9 filler	TAGCGGGGTTTTGCTCAGTA	20
V10 filler	GAGGCTTTGAGGACTAAAGA	20
BB37 filler (PSA)	CACCTTGCTGAACCTCAAATATCAAACCCT	30
V7 filler (PSA)	TTTTGAAGCCTTAAATCAAGATTAGTTGCT	30

Target sequences		
Name	**Sequence**	**Length**
SP DNA	TCATGTGTAAGTTACAGGATCTAATTGTGA	30
CF DNA	GCAGCAGCAGCAGCAGCAGACGAC	24
TS DNA	GGGCCATAGGATATACGGTTC	21
BRCA1 DNA	CAGGACTGTTTATAGCTGTTGGAAG	25
HIV1 DNA	GCTAGAGATTTTCCACACTGACT	23
WS DNA	AAAGTGGAATGAAAAGAAGATGGATTTGAAGATG	34
CF 1 mismatch	GCAGCACCAGCAGCAGCAGACGAC	24
CF 2 mismatch	GCTGCACCAGCAGCAGCAGACGAC	24
CF 3 mismatch	GCTGGACCAGCAGCAGCAGACGAC	24
RNA (Fig 4)	UAGCAGCACGUAAAUAUUGGCG	22
DNA (Fig 4)	TAGGTAGTTTCATGTTGTTGG	21
miR-141 DNA	CATCTTCCAGTACAGTGTTGGA	22
miR-30c DNA	TGTAAACATCCTACACTCTCAGC	23
miR-141 RNA	CAUCUUCCAGUACAGUGUUGGA	22

Other strands		
Name	**Sequence**	**Length**
Blocking oligos	ACGGTCTCATGGCCCTTCAATC	22
BtsCI cut site oligo	CTACTAATAGTAGTAGCATTAACATCCAATAAATCATACA	40