Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A DNA Minimachine for Selective and Sensitive Detection of DNA

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1. Materials and Methods

Materials and instrumentation. DNAse/RNAse-free water was purchased from Thermo Fisher Scientific, Inc. (Pittsburgh, PA) and used for all the stock solutions of oligonucleotides. MQ water was purchased from Millipore RiOs-DI 3 Smart and used for buffers and solutions. Fluorogenic substrates (**F_sub**) was synthesised and HPLC purified by TriLink BioTechnologies, Inc. (San Diego, CA). All other oligonucleotides (see Table S1 for sequences) were obtained from Integrated DNA Technologies, Inc. (Coralville, IA). The oligonucleotides were dissolved in DNAse/RNAse-free water and stored at –20 °C.

The fluorescence intensities were measured at 517 nm (excitation wavelenght at 485 nm). Excitation and emission slits were both 10 nm (Cary Eclipse Fluorescence Spectrophotometer). The data were processed by using Microsoft Excel. The plasmid containing HPV-16 genome was kindly gifted by Svetlana V. Vinokurova, a Head of Laboratory of Molecular Biology of Viruses, National Medical Research Center of Oncology N.N. Blokhin, Moscow, Russian Federation. The full genome of human papilloma virus type 16 (HPV16) was cloned in vector p114 (analog pBR322). The plasmid has resistant to Ampicillin.

Clinical samples of human DNA samples were isolated from cell scrapings cervical canal in Laboratory of diagnostics «Medical center Garmonia». Material were collected with special brushes (DNA probes) by scraping and washed into a tube containing sterile saline in an amount of 1.5 ml. The samples were centrifuged at 13,000 rpm 10 min, the supernatant was removed, leaving a volume of 50 μL. Precipitation resuspension on a vortex, isolation of DNA were performed according to manufacturer's instructions by using the GS-plus sample Kit (DNA-technology, Russia).

2. Detailed Experimental Procedures

2.1. Assembling of DMM1. Stock solutions of DMM1 were prepared by annealing 100 nM of each of the three tile strands, **T1**, **T2**, **T3** (Table S1) in the reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO) followed by addition of 20 nM **DZ_a-1**. For all experiments we used 10 nM of DMM1 containing 2 nM **DZ_a-1** strand. These concentrations of DMM1 and **DZa** were found to be optimal in terms of **HPV-45** analyte dependent response over the background (data not shown).

2.2. General fluorescent assay for measuring limit of detection (LOD). Each sample prepared in 120 μL of reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO) contained 200 nM **F_sub**, 2 nM **DZ_a-1** , 10 nM **DZ_b** and 0, 5, 10, 25, 50 or 100 pM DNA analytes. All samples were incubated at 50 °C for 60 or 180 min followed by fluorescent measurement at 517 nm (λex = 485 nm).

3. Table S1. Sequences of oligonucleotides

Name	Sequence 5‘ ✂ 3‘[a-b]	Purification[b]
HPV-180	5’-GGTATGGCAA TACTGAAGTG GAAACTCAGC AGATGTTACA GGTAGAAGGG CGCCATGAGA CTGAAACACC ATGTAGTCAG TATAGTG**G**TG GAAGTGGGGG TGGTTGC AGT CAGTACAGTA GTGGAAGTGG GGGAGAGGGT GTTAGTGAAA GACACACTAT ATGCCAAACA CCACTTACAA	SD
HPV-45	5’- TGA GA CTGAAACACC ATGTAGTCA GTATAGTGGTG GAAGTG GGGG	SD
HPV-180-mm	GGTATGGCAA TACTGAAGTG GAAACTCAGC AGATGTTACA GGTAGAAGGG CGCCATGAGA CTGAAACACC ATGTAGTCA GTATA GTG **A** TG GAAGTG GGGG TGGTTGCAGT CAGTACAGTA GTGGAAGTGG GGGAGAGGGT GTTAGTGAAA GACACACTAT ATGCCAAACA CCACTTACAA	
F_sub	AAG GTT^{FAM} TCC TCg uCCC TGG GCA-BHQ1	HPLC
DZ_a-1 (for DMM1)	**CCCC** CAC TTC CA**C** CAC TATAC ACA ACG A GAG GAA ACCTT	SD
DZ_a-2 (for DMM2)	CAC TTC CA**C** CAC TATAC ACA ACG A GAG GAA ACCTT	SD
DZ_b	TGC CCA GGG A GGC TAG CT TGA CTA CAT GGT GTT TCA GTC TCA	SD
DZ_a (for mm)	CAC TTC CA **T** CAC TATAC *ACA ACG A GA* G GAA ACCTT	
DZ_a-A	CAC TTC CA**A** CAC TATAC ACA ACG A GAG GAA ACCTT	
DZ_a-G	CAC TTC CA**G** CAC TATAC ACA ACG A GAG GAA ACCTT	
T1	TG TGAATC CTGAC GCTCTG GCTAC CAGTT CAGTG TCAATG CTCAC CGTCTC GCATC CAGAA CTGAG ACTT AGC	SD
T2	TGC CCA GGG A GGC TAG CT T GAC TAC ATG GTG TTT TTT GAGACG GTGAG CATTGA CACTG AACTG GTAGC CAGAGC GTCAG GATTCA CA TTT TACTG AGTGC AACCA	SD
T3	CAGTC TCATG GCGCC CTTCTA TTT G CTAAGT CTC AG TTCTG GATGC	SD
T3_no_arm	GCT AAG TCT CAG TTC TGG ATGC	SD
T2_no_arm	TGC CCA GGG A GGC TAG CT T GAC TAC ATG GTG TTT TTT GAGACG GTGAG CATTGA CACTG AACTG GTAGC CAGAGC GTCAG GATTCA CA	SD
Primer1_1261-1340	TTG TAA GTG GTG TTT GGC ATA	SD
Primer2_1261-1340	GGT ATG GCA ATA CTG AAG TGG	SD

[a]BHQ-1 – Black Hole Quencher1, [b]SD, standard desalting; positions with variations in nucleotides are bold underlined

4. PCR

4.1 PCR optimization

a) For dsDNA amplicon

HPV1261-1340 DNA ($1 \cdot 10^{-11}$ M) was added to the samples and PCR amplified (CFX96 Touch Real-Time PCR Bio-Rad) using the following temperature profiles: initial denaturation 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 47 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min (1 cycle). For Limit-of-Detection (LOD) experiments 1.8 ng– 72 ng of HPV1261-1340 was used. Negative control PCR «no DNA» contained all the components except the plasmid. Reaction mixture consists of buffer $(NH_4)_2SO_4$, $MgCl_2$, primers (Primer1_1261-1340; Primer2_1261-1340), Taq – polymerase, water (Thermoscientific EP0072).

b) For plasmid – derived dsDNA amplicon

Plasmid HPV_16 (10^{-11} M) (initial concentration is 300 ng/μL and 0.5 μL was added to PCR) was added to the samples and PCR amplified (CFX96 Touch Real-Time PCR Bio-Rad) using the following temperature profiles: initial denaturation 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 47 °C for 30 s, extension at 72 °C for 11 min, and final extension at 72 °C for 5 min (1 cycle). For experiments was used 6 nM and 32 nM. Negative control PCR «no DNA» contained all the components except the plasmid. Reaction mixture consists of buffer $(NH_4)_2SO_4$, $MgCl_2$, primers (Primer1_1261-1340; Primer2_1261-1340), Taq – polymerase, water (Thermoscientific EP0072).

c) Human samples of dsDNA amplicons Each sample of human DNA was PCR amplified (CFX96 Touch Real-Time PCR Bio-Rad) using Primer1_1261-1340 and Primer2_1261-1340 (Table S1) and the following temperature profiles: initial denaturation 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 47 °C for 30 s, extension at 72 °C for 8 min, and final extension at 72 °C for 5 min (1 cycle) in the presence. For experiments were used 15 μL. Negative control PCR «no DNA» contained all the components except the plasmid. Reaction mixture consists of buffer $(NH_4)_2SO_4$, $MgCl_2$, primers (Primer1_1261-1340; Primer2_1261-1340), Taq – polymerase, water (Thermoscientific EP0072).

Characteristic of human samples:

Number	Type of virus	Genom-equals/ml	Add to PCR, μL
I	mixture of HPV viruses 16,31,33,35,52,58	6,0	15
II	mixture of HPV viruses 16,31,33,35,52,58	3,3	25

5. Figure S1. DMM1 in complex with Human Papilloma Virus (type 16)

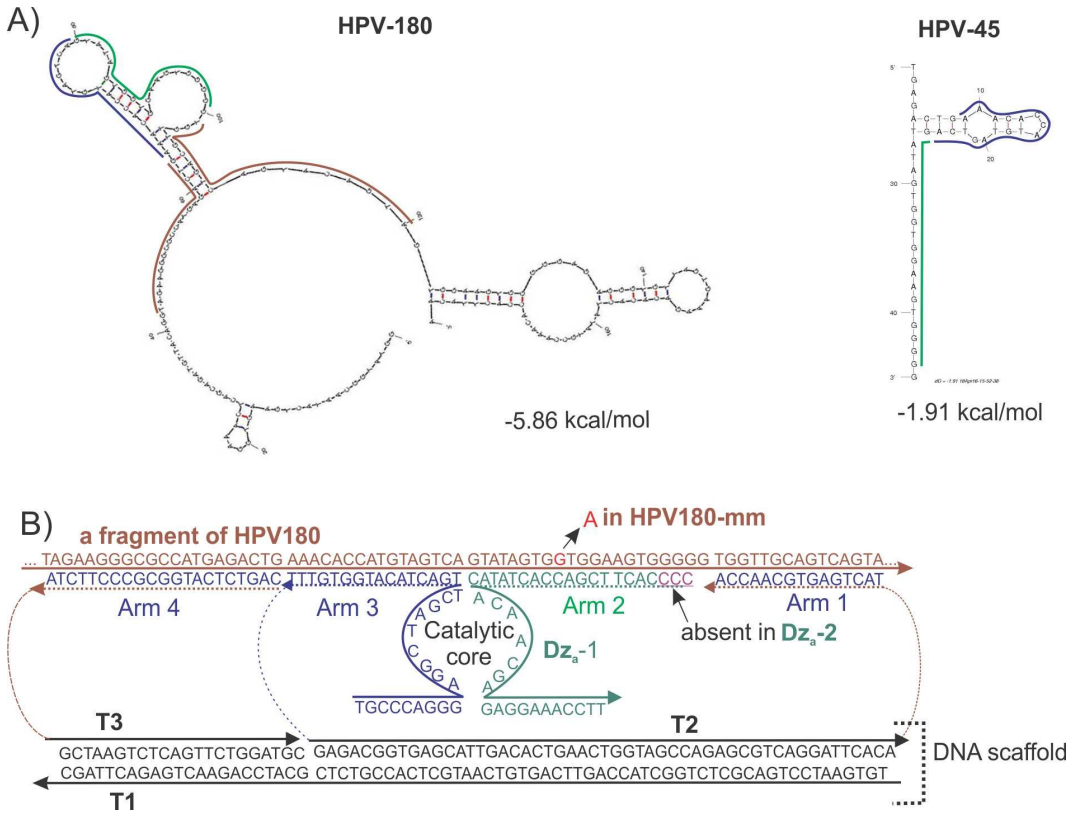


Figure S1. Design of a DNA Minimachine (DMM1) for the detection of folded DNA. A) Secondary structure of a long **HPV-180** and short **HPV-45** DNA analytes predicated by Mfold at 50°C in the 120 mM Na^+, 50 mM Mg^{2+}. B) Predicted structure a DNA - Minimachine in complex with a recognized DNA analyte.

6. Analysis of DMM1 association in agarose gel (Figure S2).

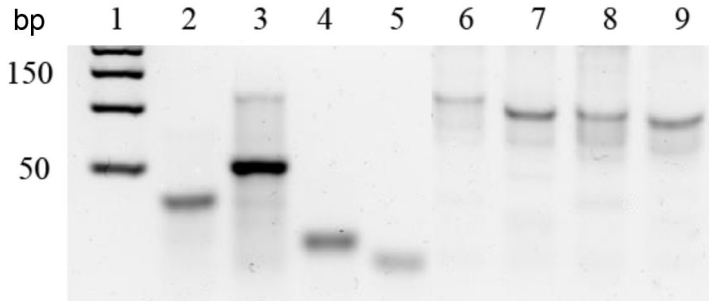


***Figure S2*. Analysis of DMM1 association in agarose gel.** 1 – Ladder, 2 – Tile stand **T1**; 3 – **T2**; 4 – **T3**, 5 – **DZ**$_a$; 6 – DMM1 (**T1**, **T2**, **T3** strands annealed), 7 – DMM1, no arm 4 (**T1**, **T2** and **T3_no_arm** strands annealed); 8 – DMM1, no arm 1 (**T1**, **T3** and **T2_no_arm** strands annealed), 9 – DMM1 no Arm 1 and 4 (**T1**+ **DZ**$_a$**-1** + **T2_no_arm** + **T3_no_arm**). The assembling of DMM1 was described in Detailed Experimental Procedures (2.1). The samples were separated in 2.0 % agarose gel at 75 V during 120 min followed by staining in SYBR Gold Nucleic Acid Gel Stain for 15 min.

7. Limits of Detection of short HPV-45 and long HPV-180 analytes by binary sensor BiDZ1 and DNA-nanomachine (DMM1) (Figure S3, S4)

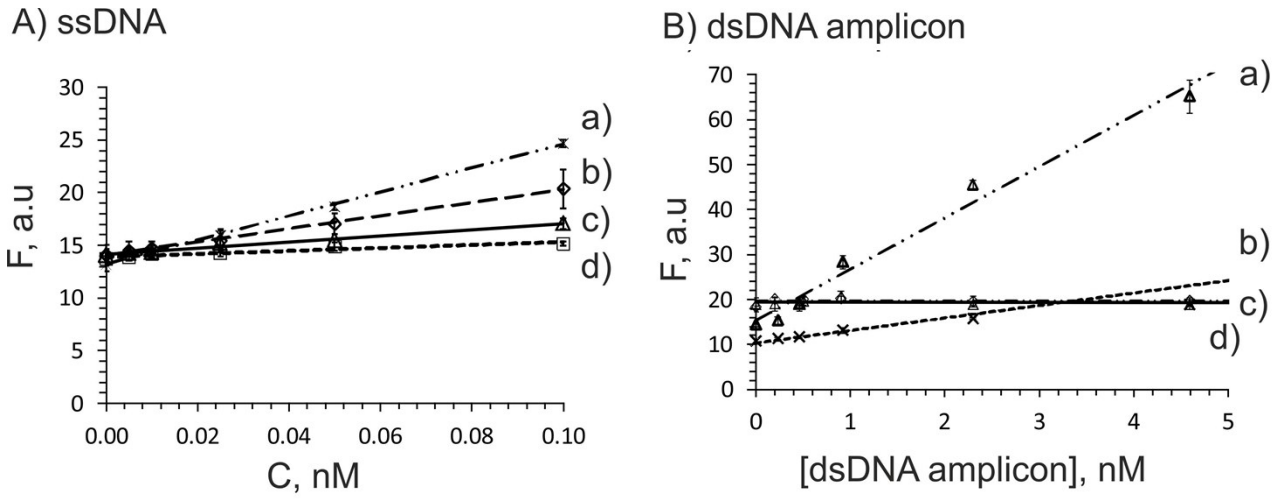
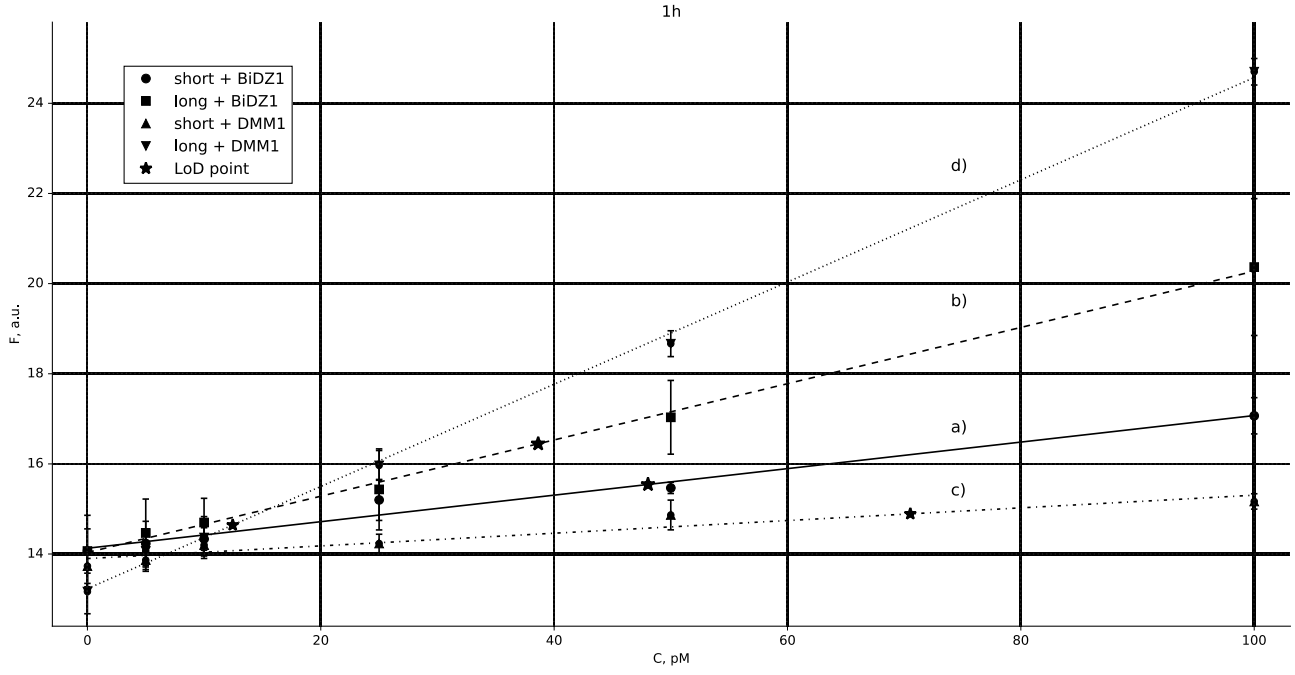


Figure S3. **DMM1 can detect single stranded and dsDNA amplicons with high sensitivity**. The dependence of the fluorescent response of DZ sensors on analyte concentrations. A) ssDNA: (a) long HPV-180 with DMM1 (10 nM) (b) Long HPV-180 with BiDZ1 (See detailed Experimental Procedures (2.2); (c) Short HPV-45 with BiDZ1 d) Short HPV-45 with DMM1 (10 nM). Samples were incubated 60 min at 50 °C. B) dsDNA amplicons: (a) DMM1 with dsDNA after 60 min; (b) BiDZ1 with dsDNA amplicons after 60 min; (c) DMM1 with dsDNA after 5 min; (d) BiDZ1 after 5 min.

A



B

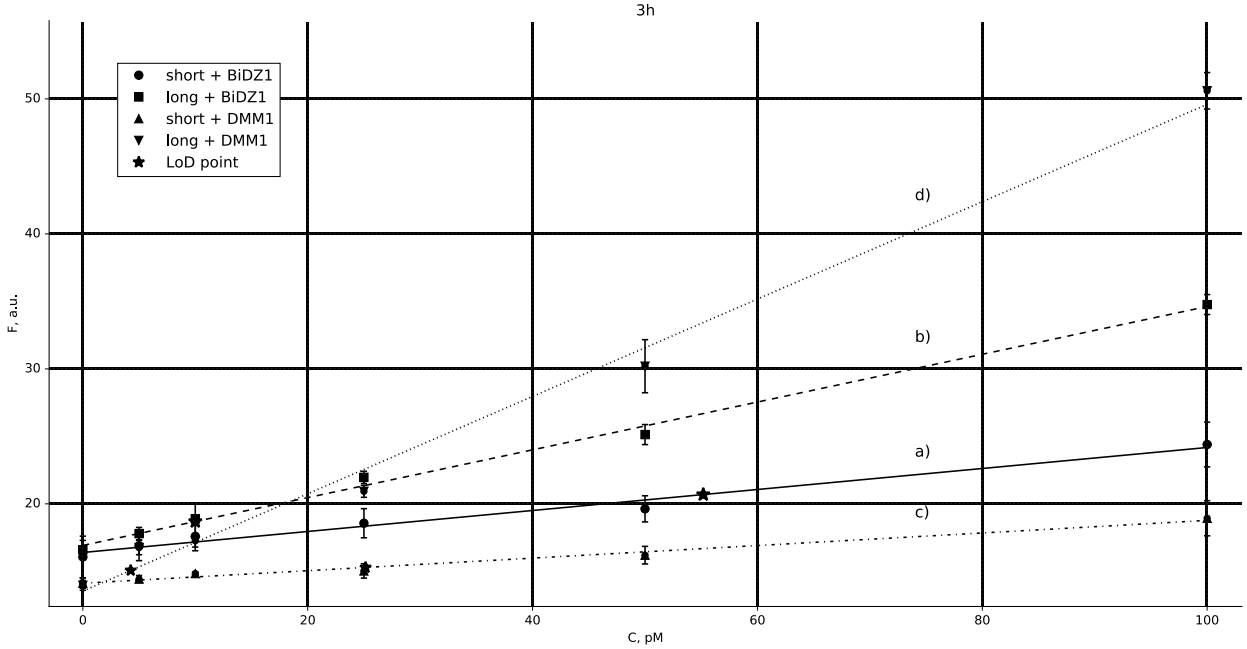
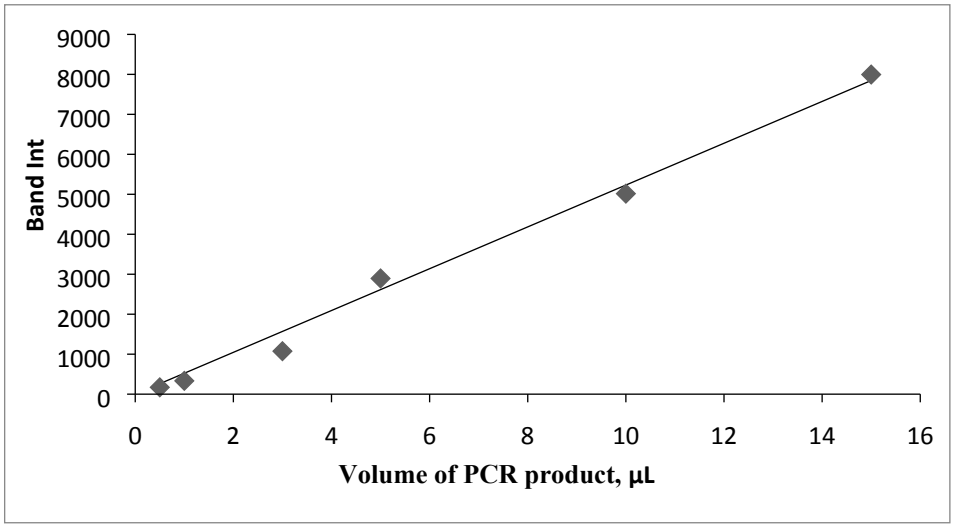


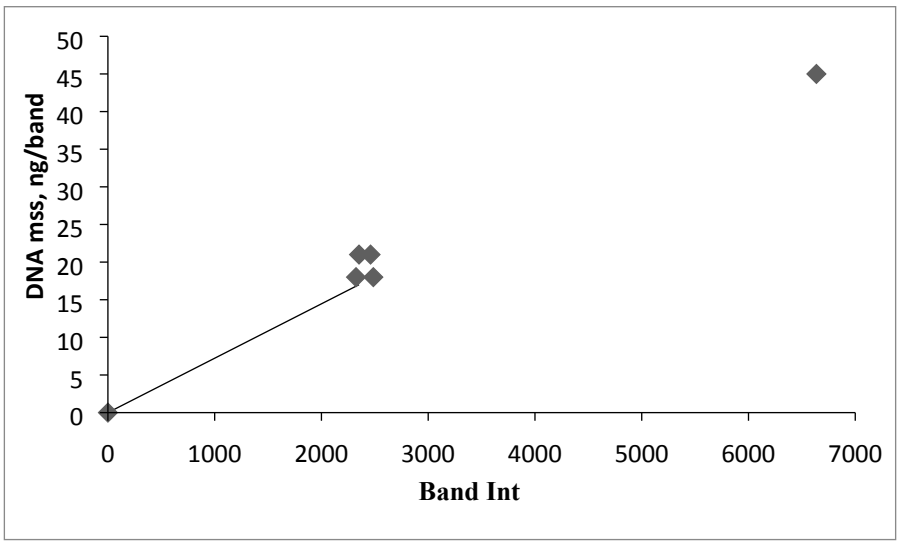
Figure S4. **Limits of detection of BiDZ1 and DMM1 for short HPV-45 and long HPV-180 analytes (A) 60 min and (B) 180 min assay.** The signal is shown for binary sensor (BiDZ1) with short HPV-45 (a), BiDZ1 with long HPV-180 (b), DNA – minimachine (DMM1) and short HPV-45 (c), and DMM1 with long HPV-180 (d). Reaction mixtures contained 200 nM F_sub, 2 nM **DZ_a-1** and 10 nM **DZ_b** (a) and (b) or DNA- nanomachine 10 nM (c) and (d). Samples were incubated at 50°C in the reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO) with different concentrations of analytes. Fluorescent intensities were measured at 517 nm (excitation at 485 nm) after 60 (A) and 180 (B) min. Limit of detection values are shown in Table 1 of the main text. The data of 3 independent experiments with the standard deviations is presented

8. Gel electrophoresis and DNA quantification (Figure S5)

8.1 Analysis of dsDNA and plasmid amplicons by agarose gel electrophoresis

PCR samples were analyzed in a 2,0 % agarose gel using a Thermoscientific EasyCast B1a mini gel electrophoresis system. DNA ladder GeneRuler 50 bp and 6×dye DNA Loading Dye was purchased from Thermo Scientific. The samples were run at 75 V for 120 min followed by staining in SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Molecular Probes, Inc. (Eugene, OR) for 15 min. The volume of samples were 0.2; 0.5; 1; 2; 3; 4; 5 µL (Fig. S5), respectively. For analysis of concentrations were used gel densitometry.





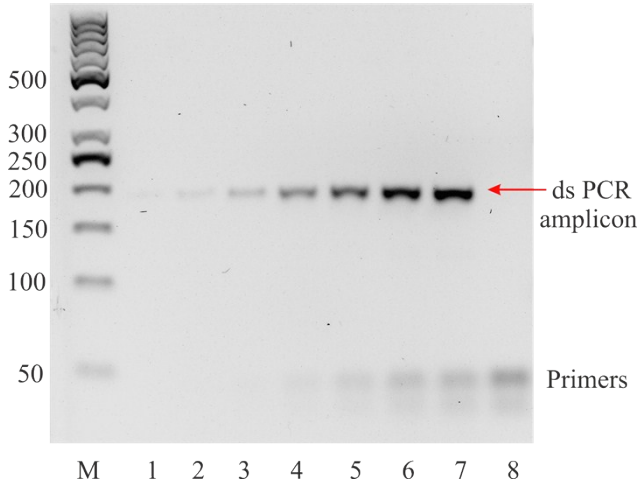


Figure S5. **Agarose gele electrophoresis analysis of plasmid dsDNA amplicons.** Marker (M) - ladder (Thermoscientific GeneRuler 50 bp). Lanes 1-7: amplification of product with various volumes: 0,5; 1; 3; 5; 10; 15; 20 µL. Lane 8: PCR "no DNA" negative control. According to our estimation, concentration of dsDNA amplicon was 32.7 nM.

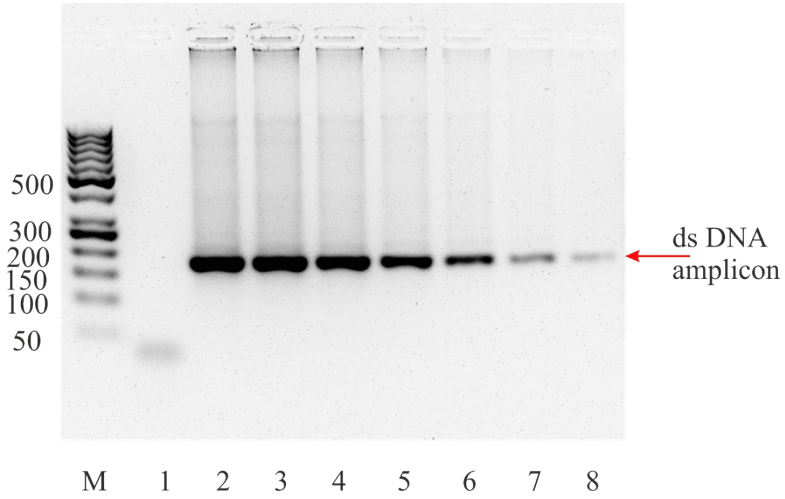


Figure S6. **Agarose gele electrophoresis analysis of plasmid dsDNA amplicons.** Marker (M) - ladder (Thermoscientific GeneRuler 50 bp). Lane 1: PCR "no DNA" negative control. Lanes 2-8: amplification of product with various volumes: 5; 4; 3; 2; 1; 0.5; 0.2 µL. Also we estimated concentration of plasmid dsDNA amplicon 6 nM (1 µL).

9. The dependence fluorescent signal on the presence of Arms 1 and 4 (Figure S6)

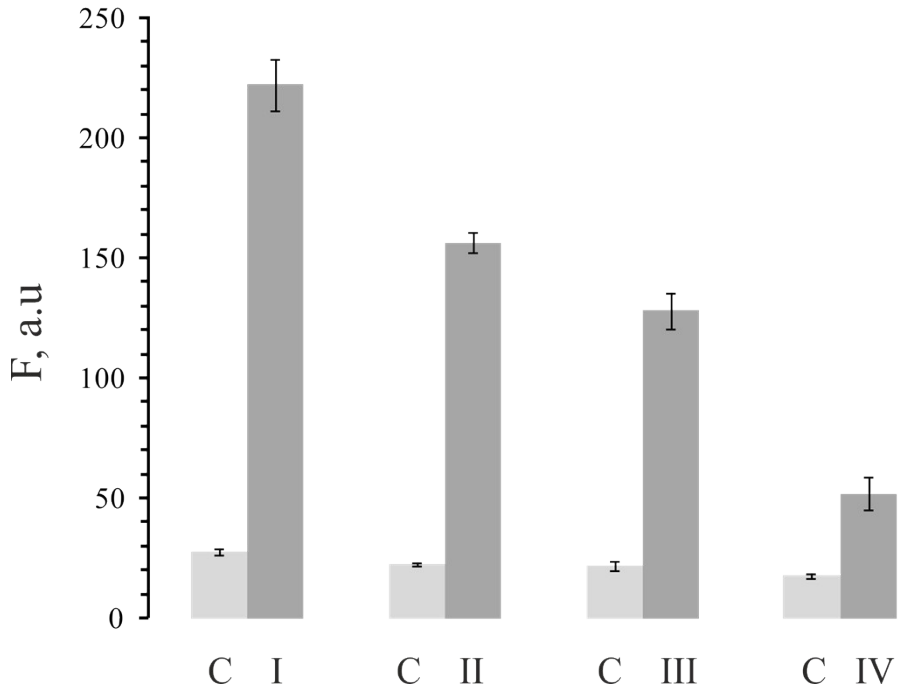


Figure S7. **Dependence fluorescent signal of structure of DMM1.** I - DMM1 (all tiles T1, T2, T3, DZ_a-1), II - DMM1 (all tiles, but instead of T3 was used *T3 no arm*); III – DMM1 (all tiles, but instead of T2 was used *T2 no arm*), IV – DMM-1 (T1+ DZ_a-1 +*T2 no arm*+*T3 no arm*). The time of incubation was 1 hr in present of 20 µL (5.5 nM, 72 ng) of PCR dsDNA amplicon at 50 °C. The fluorescence of the samples was measured after 1 hour at 517 nm, upon excitation at 485 nm.

10. Selectivity of DMM1 approach (Figure S9)

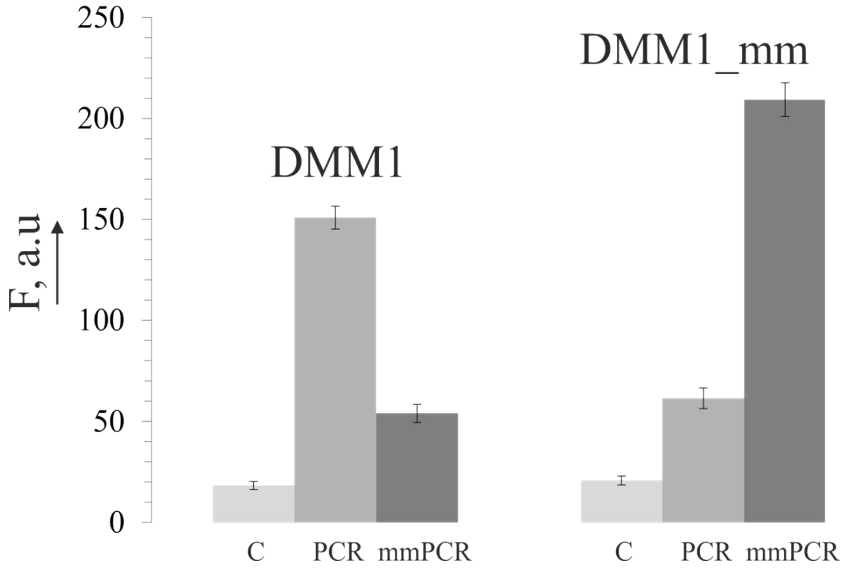


Figure S8. **DMM1 detects dsDNA amplicon with high selectivity.** Selectivity of **DMM1 and DMM1_mm** and different **analytes (dsPCR and dsPCR_mm)** 5.5 nM (20 µL, 72 ng). **DMM1** was as described in **Detailed Experimental Procedures 2.1**; **DMM1_mm** sensor used DZa stands with single base mismatch (see Table S1 **DZ_a for mm**); Fluorescence were measured at 517 nM, after 60 min at 50 °C. The data of 3 independent experiments with the standard deviations is presented. Importantly, in this case, we have partly selectivity for mmPCR that is why we did redesign the Dza. The better result was shown in the main article (see Figure 4, with see Table S1 **DZ_a-2 for DMM2**). Selectivity factor (SF) were found to be 73.4% and 73.7 % for DMM1 and DMM1_mm, respectively. SFs were calculated using a formula $SF=(1-(F_{ns}-F_o)/(F_s-F_o))\times 100\%$, where F_0, F_s, F_{ns} are fluorescence intensities of the probe in the absence of or in the presence of specific or non-specific analyte, respectively.

11. DMM1 discriminates different mistmatches both in ssDNA and dsDNA amplicon.

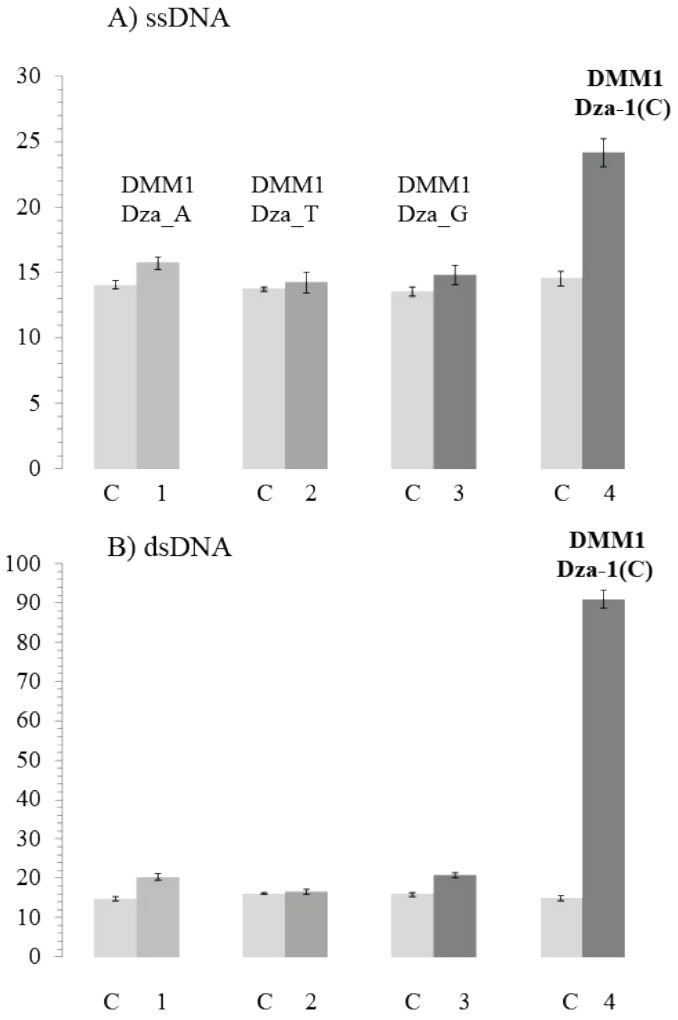


Figure S9. **Fluorescent signalling in the perscence of three missmatch variations between DDM1 and both ssDNA and dsDNA amplicon.**

A) Differentiation of mismatches in ssDNA analyte: Samples contained 200 μM **F-sub**, 10 nM **DMM1** with 2 nM DMM1 with 2 nM of different DZa strands in the absence (C) or prescence (1, 2, 3 and 4) of 0.1 nM of HPV180 analyte. 1: DZa –T ; 2: DZamm here is inacated as DZa –T; 3: DZa –G; 4: DMM1+ DZa -1 (fully matched)

B) Differentiation of mismatches in dsDNA amplicon: Samples contained 200 μM F-sub, 10 nM DMM1 with 2 nM of different $\mathbf{DZ}_a$ strands in the absence (C) or prescience (1, 2, 3 and 4) of 10 μL of 5.5 nM dsDNA amplicon. 1: $\mathbf{DZ}_a$ –T **; 2: $\mathbf{DZ}_a$mm** here is inacated as $\mathbf{DZ}_a$ –T; 3: $\mathbf{DZ}_a$ –G; 4: DMM1+ $\mathbf{DZ}_a$ -1 (fully matched)

All samples were incubated 60 min at 50 ^oC in reaction buffer followed by ffluorescence measurements at 517 nM (λex=385 nm). The data of 3 independent experiments with the standard deviations is presented.