Amplified Detection of the Aptamer–Vanillin Complex with the Use of Bsm DNA Polymerase

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Table S1. Oligonucleotide sequences used in the work. The underlined sequence is a capture sequence.

| Sequence name | Sequence, 5'-3' direction | Comment |
|------------------|---|--|
| B1_bank | CGACCAGCTCATTCCTCA-N10- <u>GGAGTCTCGATG</u> -N40- GGATCCGAGCTCACCAGTC | Library for SELEX |
| B1_biotin | Bio-GTC-Spacer18-CATCGAGACTCC | Capture probe with biotin |
| B1 | CATCGAGACTCC | Capture probe without biotin |
| DP_biotin | biotin-GTC-spacer18-ACCACATCGAGACTCCTGTGTCCTTT | Bsm dehybridization probe with biotin |
| DP | ACCACATCGAGACTCCTGTGTCCTTT | Bsm dehybridization probe without biotin |
| Van_74 | CGACCAGCTCATTCCTCAGGAGAAACAT <u>GGAGTCTCGAT</u> <u>G</u> ATAGTAGGAGCGGCGGA ACGTAGGAAGAGAGGATGACGGAGGATCCGAGCTCACC AGTC | aptamer |
| FP | FAM- TCTTGGFCFCA <u>GGAGTCTCGATG</u> TGGTATTGTGTCCAAGA- BHQ1 | Fluorescence probe for Bsm DNA polymerase |
| PR | TCTTGGAC | Primer for Bsm DNA polymerase |



Figure S1. Stem-loop structure for the FP predicted by Mfold web (http://unafold.rna.albany.edu/?q=mfold).



Figure S2. Nondenaturating PAGE (photo without any modification) of PCR amplified (14 cycles) washout probes from magnetic beads modified with B1/Van_74 (**a**) and image analysis of phoresis with GelQuant.NET (**b**) for different incubation buffers: 1–Selection buffer with vanillin (1.5 mM), 2–Selection buffer, 3–Bsm buffer with vanillin (1.5 mM), this point doesn't correlate with other measurments, possible pipetting error, 4–Bsm buffer, 5–Bsm buffer with vanillin (1.5 mM), repeat, 6–Bsm buffer, repeat, 7–Bsm buffer with vanillin (1.5 mM), repeat, 8–Bsm buffer, repeat, 9–Selection buffer with vanillin (1.5 mM), repeat.



Figure S3. Nondenaturating PAGE (photo without any modification) of PCR amplified (14 cycles) washout probes from magnetic beads modified wirh DP/Van_74 (**a**) and image analysis of phoresis with GelQuant.NET (**b**) for different compositions: 1—Selection buffer, 2—Selection buffer, repeat, 3—Selection buffer, repeat, 4—Selection buffer with 1.5 mM of vanillin, 5—Selection buffer with 1.5 mM of vanillin, repeat.



Figure S4. Real time signal of the ISFET (in $\Delta \phi$) during Bsm DNA polymerase reaction in homogenous solution in Bsm buffer initiated (30–40 s) by addition of DP at different concentration (final concentration are marked on the picture); Slope ($\Delta \phi / \Delta t$) dependence on DP concentration calculated from real time signal curves is presented as insert. Reaction conditions: 0.2 mM dNTP, 0.05 pmol/µl FP, 1.6 pmol/µl PR, 0.1 U/µl BSM DNA polymerase.



Figure S5. Real time signal of the ISFET (in $\Delta \phi$): black line—Selection buffer with 0.2 mM dNTP, 0.05 pmol/µl FP, 1.6 pmol/µl PR, 0.1 U/µl BSM DNA polymerase, red line—Selection buffer with 0.2 mM dNTP, 0.05 pmol/µl FP, 1.6 pmol/µl PR, 0.1 U/µl BSM DNA polymerase during polymerase reaction initiated by addition of DP (35 s) at concentration of 3 × 10⁻⁸ M.



Figure S6. Real time signal of the modified with Van_74 ISFET (in $\Delta \phi$, with background subtraction): black line—Selection buffer with 0.2 mM dNTP, 0.05 pmol/µl FP, 1.6 pmol/µl PR, 0.1 U/µl BSM DNA polymerase during polymerase reaction initiated by addition of DP (32 s) to final concentration 3 × 10⁻⁸ M, red line—Selection buffer with 0.2 mM dNTP, 0.32 pmol/µl DP, 1.6 pmol/µl PR, 0.1 U/µl BSM DNA polymerase during polymerase reaction initiated by addition of FP (35 s) to final concentration of 4.8 × 10⁻⁸ M.