Supplementary Materials

Direct identification of the herpes simplex virus *UL27* gene through single particle manipulation and detection using a micromagnetic mirror array

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Table S1. Oligonucleotide probes and synthetic polynucleotides used in the detection of a fragment derived from the UL27 gene encoding the envelope glycoprotein B (gB) of herpes simplex virus type 1 with the magnetic bead aggregation assay. The T_m quoted for each oligonucleotide is calculated for a 50 nM concentration of oligonucleotide in 50 mM NaCl. The nearest neighbor and thermodynamic calculations are done as described by Breslauer et al., (1986) Proc. Nat. Acad. Sci.83:3746-50 but using the values published by Sugimoto et al., (1996) Nucl. Acids Res. 24:4501-4505.

Notation	Sequence (5' → 3')	T_m [°C]*
Biotin HSV gB(+)	Biotin-AACGCGTCCGTGGAGCGCATCAAGA	65.6
Dig-HSV-gB(-)	Digoxigenin-TCTTGATGCGCTCCACGGACGCGTT	65.6
HSV-1 KOS gB+	GCCGCGTTGCCATCGCGTGGTGCGAGCTACAGA ATCACGAGCTGACCCTGTGGAACGAGGCCCGCA AGCTGAACC	83.0
HSV-1 KOS gB-	GGTTCAGCTTGCGGGCCTCGTTCCACAGGGTCAG CTCGTGATTCTGTAGCTCGCACCACGCGATGGCA ACGCGGC	83.0
Biotin-HSV-1P1	TGGAACGAGGCCCGCAAGCTGAACC-Biotin	64.7
Biotin-HSV-1P2	Biotin-GCCGCGTTGCCATCGCGTGGTGCGA	69.5
Dig-HSV-1P1	DIG-GCCGCGTTGCCATCGCGTGGTGCGA	69.5

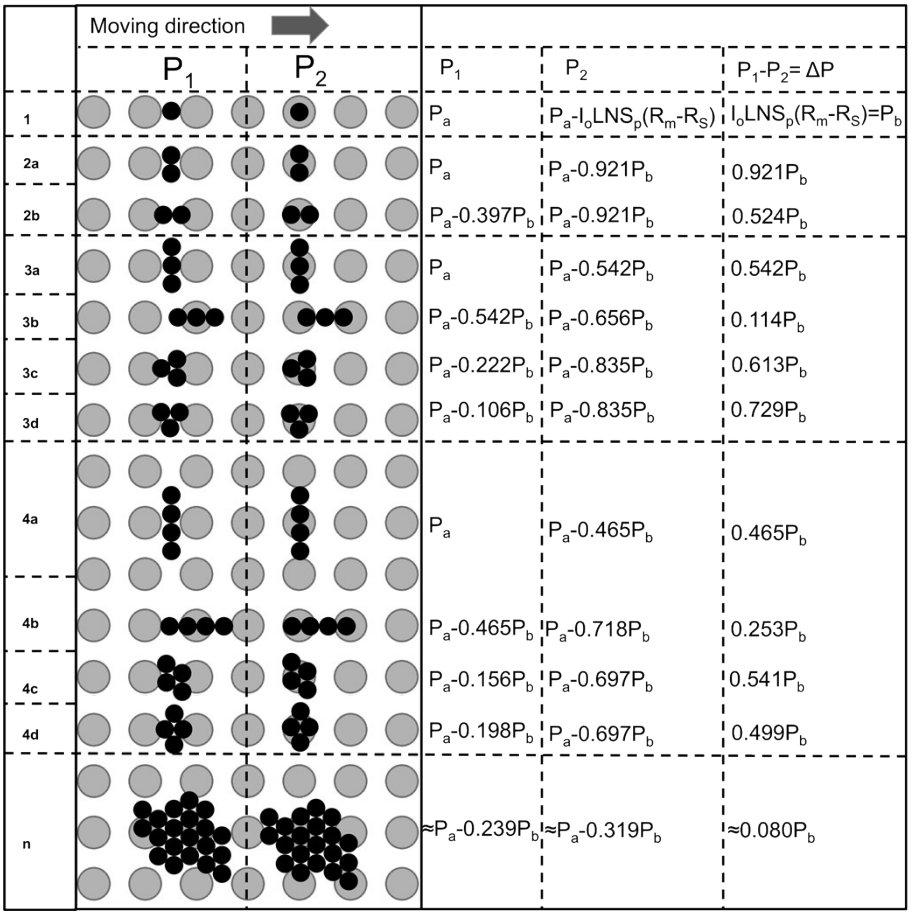
Table S.2. Antibodies used in this study. All antibodies used were purchased from Abcam®, Cambridge (UK) and used without purification.

Notation	Description
Anti-Digoxigenin antibody (Biotin)	Mouse monoclonal[BT.21H8] to Digoxigenin (Biotin)
Anti-Digoxigenin antibody	Mouse monoclonal [21H8] to Digoxigenin

Buffers used in this study

Notation	Description
Annealing Buffer (10x)	100 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1 M NaC
BP Buffer	B&W Buffer (1x) and PBST 1:1 (v/v)
B&W Buffer (2x)	10 mM Tris-HCL (pH 7.5), 1 mM EDTA, 2 M NaCl
PBS	137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4
TE Buffer	10 mM Tris (pH 7.5), 1mM EDTA

Table S3. Differential reflectivity, *ΔP*, of aggregates relative to mono-dispersed magnetic beads in specific orientations. P_1 and P_2 are calculated based on effective coverage of the micromagnets per SPM bead, and the SPM beads are assumed to have 100% adsorption of light and reflectivity is proportional to the reflective area (to simplify, assuming it equal to projected area of beads on MMA). P_1 corresponds to maximum reflectivity from MMA as the bead coverage on micromagnets was minimum. P_2 corresponds to minimum reflectivity from MMA as the bead coverage on micromagnets was maximum. The reflectivity of silicon substrate and micromagnets with area of N beads can be calculated by $I_oLNS_pR_m$ and $I_oLNS_pR_m$, respectively. P_a can be calculated by using reflectivity from all micromagnets and silicon substrate (the area on silicon substrate covered by beads-- $I_oLNS_pR_s$, should be excluded). $P_b = I_oLNS_p(R_m - R_s)$, is the difference of reflectivity between the situation when beads totally on micromagnets and the situation when beads totally on silicon substrate. P_b is proportional to the total projected area of the beads, NS_p. In the situation when beads partially on micromagents, the projected area of beads on micromagnets are calculated. For instance, in situation when dimmers are partially on micromagnets (as shown in 2b in Table 1), the smallest area of the beads on the micromagnets is $0.397NS_p$ at P1; the largest area of the beads on the micromagnets is $0.921NS_p$ at P_2. Therefore, ΔP is calculated as $0.524P_b$.



	P_1	P_2	$P_1-P_2=\Delta P$
1	P_a	$P_a-I_oLNS_p(R_m-R_S)$	$I_oLNS_p(R_m-R_S)=P_b$
2a	P_a	$P_a-0.921P_b$	$0.921P_b$
2b	$P_a-0.397P_b$	$P_a-0.921P_b$	$0.524P_b$
3a	P_a	$P_a-0.542P_b$	$0.542P_b$
3b	$P_a-0.542P_b$	$P_a-0.656P_b$	$0.114P_b$
3c	$P_a-0.222P_b$	$P_a-0.835P_b$	$0.613P_b$
3d	$P_a-0.106P_b$	$P_a-0.835P_b$	$0.729P_b$
4a	P_a	$P_a-0.465P_b$	$0.465P_b$
4b	$P_a-0.465P_b$	$P_a-0.718P_b$	$0.253P_b$
4c	$P_a-0.156P_b$	$P_a-0.697P_b$	$0.541P_b$
4d	$P_a-0.198P_b$	$P_a-0.697P_b$	$0.499P_b$
n	$\approx P_a-0.239P_b$	$\approx P_a-0.319P_b$	$\approx 0.080P_b$

Figure S.1. Optical response of the motion of the SPM beads on the MMA as a function of frequency and the complementary cumulative distribution function (CCDF) of the beads as a function of frequency. The NLM response of the SPM beads to frequency result in a Gaussian distrubiton. Equation (2) is a result of the cummulative nature of mobility of the beads. The cumulative distribution funciton is defined as

$$\Phi(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} e^{-t^2/2} dt = \frac{1}{2}[1 + \mathrm{erf}\left(\frac{x}{\sqrt{2}}\right)]$$

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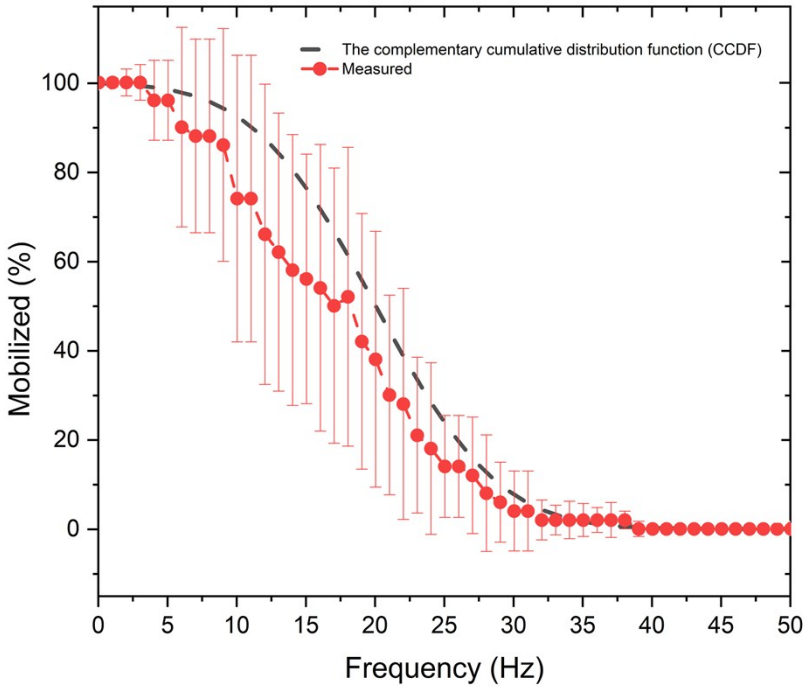

Figure S.2. Characterization of the sensitivity of the magnetic bead aggregation (MBA) assay for dsDNA 5' labeled with biotin and dig using *anti*-DIG and streptavidin magnetic beads. The biotin-25mer-dig dsDNA was mixed with the streptavidin and anti-dig beads at concentrations of 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M and 10^{-14} M. This reaction was performed with a 1:1 ratio of the streptavidin and anti-dig SPM beads was used at a total concentration of $7 \cdot 10^6$ beads/mL in BP buffer (concentration of *anti*-DIG on beads 10^{-8} M). The reactants were sonicated for 35 seconds and incubated for 30 minutes at room temperature. After incubation the bead solution was subjected to magnetic assisted aggregation and analyzed using the flow cytometer. The analyzed results are presented as the percentage of measured aggregates (100% all events, 10.000 events recorded) as a function of the concentration of dsDNA HSV gB+ on the beads. Control 1: $7 \cdot 10^6$ streptavidin beads functionalized with 10^{-8} M dsDNA Kos HSV 5' labeled with biotin and dig. Control 2: $7 \cdot 10^6$ *anti*-DIG on beads/mL with out any DNA on the beads.

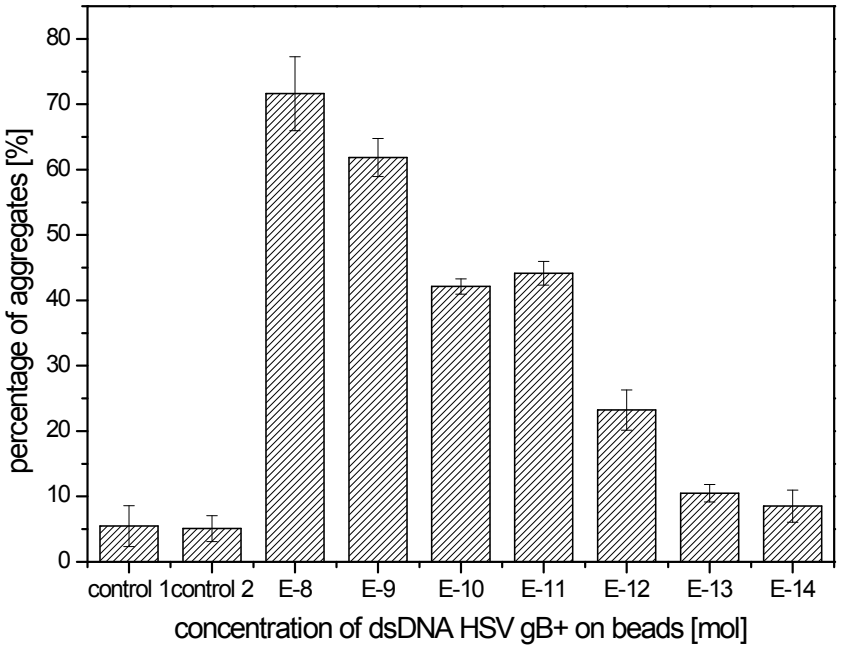

Figure S.3. Study of the sensitivity of the dsDNA MBA assay with different incubation periods. The biotin-25mer-dig dsDNA at a concentration of 10^{-8} M was reacted with streptavidin and *anti*-DIG beads for a defined period of time. For this MBA assay both kinds of beads were added in a 1:1 ratio at a total concentration of $7 \cdot 10^6$ beads/mL in BP buffer (concentration of *anti*-DIG on beads 10^{-8} M). The reagents were sonicated for 35 seconds and incubated for 2, 10, 20 and 30 minutes at room temperature. After incubation the bead solution was subjected to magnetic assisted aggregation, re-suspended and analyzed using the flow cytometer. The results are displayed is percentage of aggregates (10.000 events recorded) as a function of the incubation period. Control 1: $7 \cdot 10^6$ streptavidin beads functionalized with 10^{-8} M dsDNA Kos HSV 5' labeled with biotin and dig. Control 2: $7 \cdot 10^6$ *anti*-DIG on beads/mL with out any DNA on the beads.

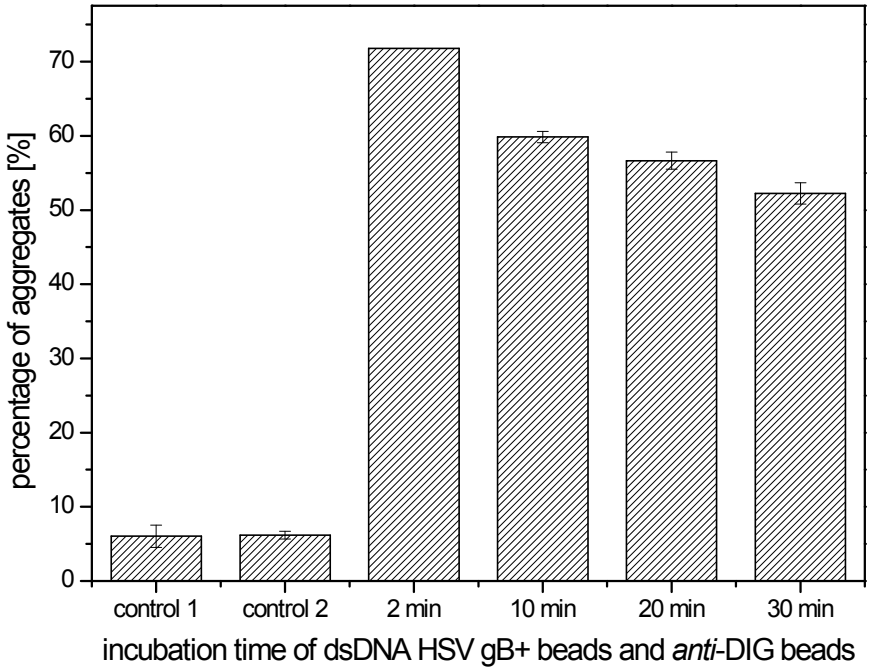

Figure S.4. Sensitivity of the dsDNA HSV aggregation assay with different ratios of *anti*-DIG and streptavidin coated magnetic beads. The biotin-25mer-dig dsDNA at a concentration of 10^{-8} M was reacted with streptavidin and *anti*-DIG beads for 30 minutes. The beads were mixed in ratios of 10:1, 8:1, 5:1, 4:1, 2:1, 1:1, 2:1, 4:1, 5:1, 8:1, and 10:1 streptavidin beads to *anti*-DIG beads (total concentration of $7 \cdot 10^6$ beads/mL in BP Buffer). The reactants were sonicated for 35 seconds and incubated for 30 minutes at room temperature. After incubation the bead solution was subjected to magnetic assisted aggregation, resuspended, and analyzed using the flow cytometer. The results are presented as the percentage of aggregates (10.000 events recorded) as a function of the ratio of streptavidin beads to *anti*-DIG beads. Control 1: $7 \cdot 10^6$ streptavidin beads functionalized with 10^{-8} M dsDNA Kos HSV 5' labeled with biotin and dig. Control 2: $7 \cdot 10^6$ *anti*-DIG on beads/mL with out any DNA on the beads.

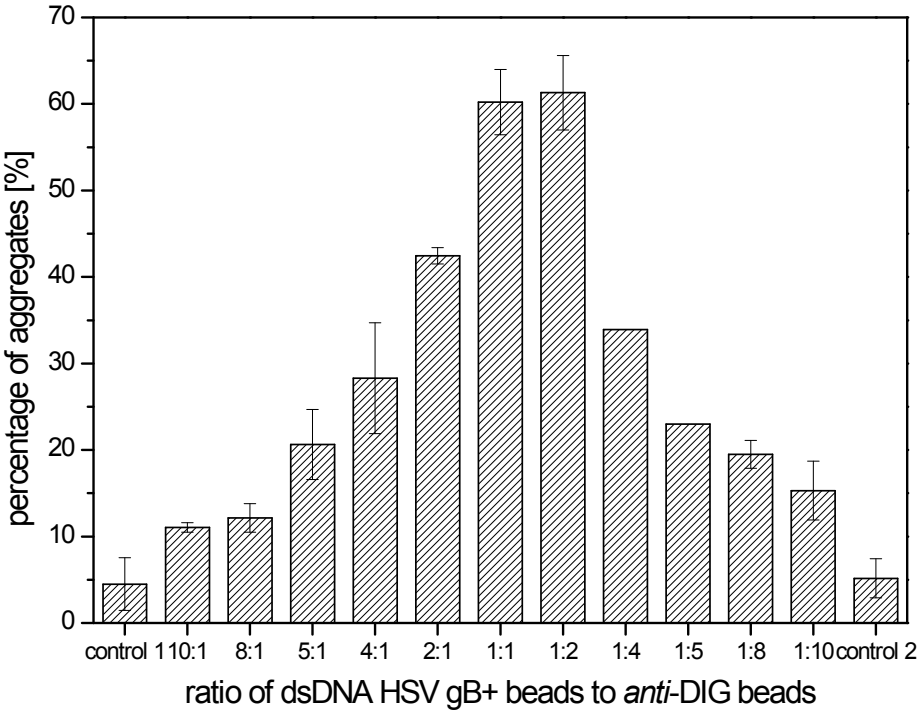

Figure S.5. Sensitivity of the dsDNA HSV MBA assay with addition of Salmon Sperm DNA. The biotin-25mer-dig dsDNA at a concentration of 10^{-8} M was reacted with streptavidin and *anti*-DIG beads for 30 min. For this MBA assay both kinds of beads were added in a 1:1 ratio at a total concentration of $7 \cdot 10^6$ beads/mL in BP buffer (concentration of *anti*-DIG on beads 10^{-8} M). In addition, salmon sperm DNA was added to the BP buffer at a concentration of 0, 2.5, 5 and 10 mg/m. The reagents were sonicated for 35 seconds and incubated for 30 minutes at room temperature. After incubation the bead solution was subjected to magnetic assisted aggregation, re-suspended and analyzed using the flow cytometer. The results are displayed is percentage of aggregates (10.000 events recorded) as a function of the incubation period.

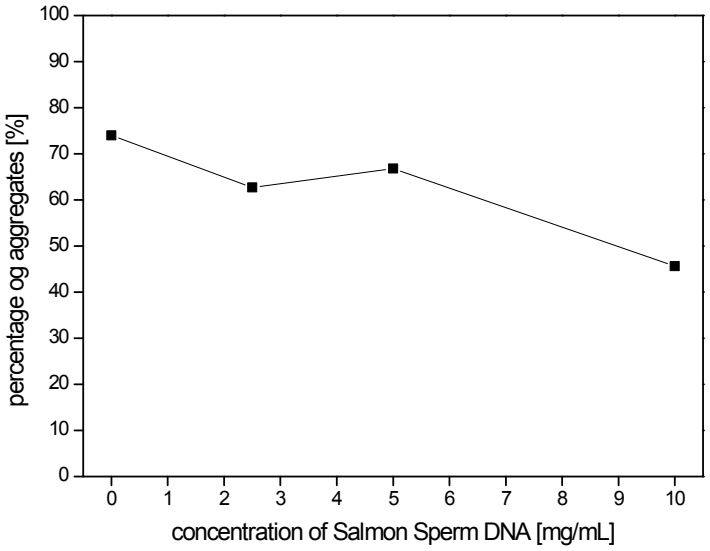

Figure S.6. Characterization of the sensitivity of the MBA assay for 75-mer synthetic fragment of the (-) strand of HSV KOS gene using 3'-labled biotin-HSV-1P1 and 5'-labeled dig-HSV-1P1. The beads were prepared by reacting 10^{-8} M 5'-labeled dig-HSV-1P1 with $3.5 \cdot 10^6$ beads/ml *anti*-DIG antibody SPM beads and 10^{-8} M 3'-labled biotin-HSV-1P1 with $3.5 \cdot 10^6$ beads/ml streptavidin SPM beads. The beads (at a concentrations of $7 \cdot 10^6$ beads/ml) were reacted with single stranded HSV-1 KOS gB- at different concentrations, i.e., 10^{-8}, 10^{-10}, 10^{-12}, 10^{-14} and 10^{-16} M, at 60 °C in 1x annealing buffer. Control 1: $3.5 \cdot 10^6$ beads/ml of 5'-labeled dig-HSV-1P1 with *anti*-DIG antibody and $3.5 \cdot 10^6$ beads/ml of 3'-labled biotin-HSV-1P1 with streptavidin.

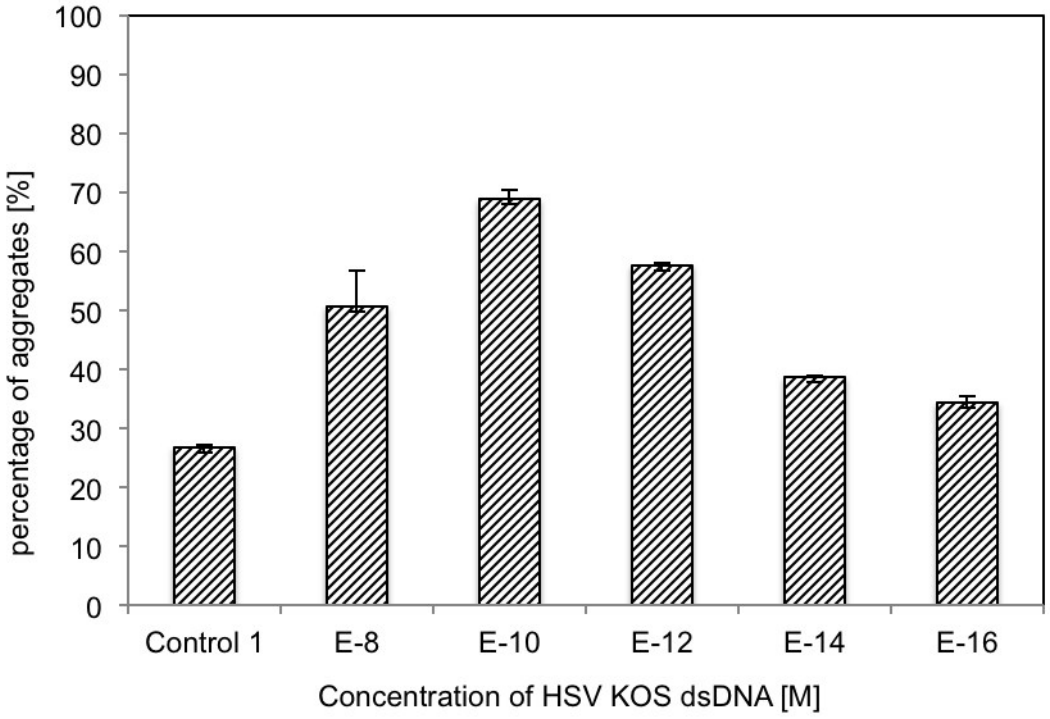

Figure S.7 Characterization of the sensitivity of the MBA assay for double stranded 75-mer HSV KOS gene using 3'-labled biotin-HSV-1P1 and 5'-labeled dig-HSV-1P1. The 3'-labled biotin-HSV-1P1 and 5'-labeled dig-HSV-1P1 were reacted at 10^{-8} M with the double stranded 75-mer synthetic HSV-1 KOS gene at different concentrations, i.e., 10^{-8}, 10^{-10}, 10^{-12}, 10^{-14} and 10^{-16} M, at 80 °C in 1x annealing buffer. The construct was reacted with *anti*-DIG and streptavidin beads at a concentration of $3.5 \cdot 10^6$ beads/mL at 23 °C to execute the aggregation assay. Control 1: $7 \cdot 10^6$ streptavidin beads/mL. Control 2: $7 \cdot 10^6$ *anti*-DIG on beads/mL.