Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY DATA

1. PCR amplification and preparation of magnetically labelled single-stranded targets

1.1 ALU sequence

ALU247 F: P-5′-GTGGCTCACGCCTGTAATC-3
GGCCGGGCGCG**GTGGCTCACGCCTGTAATC**CCAGCACTTTGGGAGGCCGA

ALU115 F: P-5′-GTAGGTACCCTGAGGTCAGGAGTTC-3
GGCGGGCGGATCA**CCTGAGGTCAGGAGTTC**GAGACCAGCCTGGCCAACAT

GGTGAAACCCCGTCTCTACTAAAAATACAAAAATTAGCCGGGCGTGGTGG

ALU115 R: 3′-ACATTAGGGTCGATGAGCCC-5′-Biot
CGCGCGCC**TGTAATCCCAGCTACTCGGG**AGGCTGAGGCAGGAGAATCGCT

ALU247 R: 3′-GGTGACGTGA
TGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCGCG**CCACTGCACT**

GGTCGGAC-5′-Biot
CCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAA

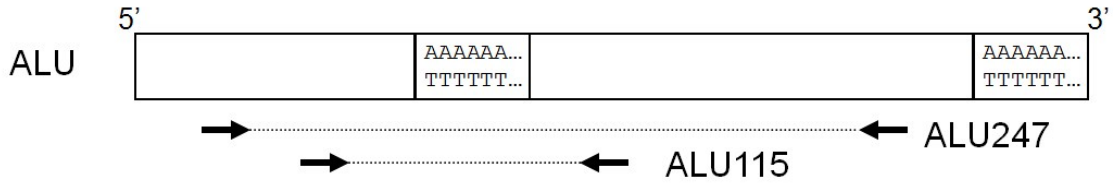

Fig. S1 – ALU sequence and Hybridization regions with the primers designed for ALU115 and ALU247.

1.2 Reaction mixture and PCR program

The reaction mixture for each ALU-qPCR consisted of 0.5 µM of forward and reverse primers; 1.25 mM $MgCl_2$; 2 µL of SYBR Green Mix and 2 µL of DNA template in a total reaction volume of 20 µL. Real-time PCR amplification was performed with precycling heat activation of DNA polymerase for 10 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 10 s, annealing at 68 °C for 4 s, and extension at 72 °C for 4 s in a Lighcycler 1.5, Roche Diagnostics. After the PCR and confirmation of size in a gel electrophoresis, the generated amplicons were purified from contaminants using the DNA Clean & Concentrator™-25 from Zymo research and diluted in TE (10 mM; pH 7.4) buffer for further use in labelling assays. The amount of generated fragments was quantified by UV absorption at 280 nm using a NanoDrop Spectrophotometer (NanoValue Plus from Bioxtra). A quantity

of 22 ng/µL for ALU115 and of 53 ng/µL for ALU247 was obtained, which corresponds to a concentration of approximately 0.6 µM and 0.7 µM respectively.

1.3 Preparation of Human Embryonic Kidney 293T cells culture and genomic DNA extraction (source of DNA for positive controls)

Human Embryonic Kidney (HEK) 293T cells (ATCC-LGC Nr: CRL-11268) were seeded in 10 mL of Dulbecco's Modified Eagle Medium (DMEM), supplemented with glucose (4.5 g/l), 10% fetal bovine serum (FBS) and 1% penicillin (100 µg/ml) supplied by Gibco, Invitrogen, on a Tissue Culture Flask of 75 cm^2 (T-75 Flask) to promote the growth of the cells in adherence until 80% confluence. After 48h culture at 37ºC with 5% CO_2-humidified atmosphere, the medium in which cells were settled was removed and replaced by 10 mL of phosphate buffer saline (PBS) (Gibco, Invitrogen) to wash the cells. Subsequently, the PBS was removed and the process was concluded with the addition of 4 mL of trypsin to resuspend the adherent cells. After 3 min of incubation at 37 ºC, 6 mL of DMEM was added to the cells to neutralize the trypsin. Cells were then harvested and centrifuged at 1000 rpm for 3 min. Following centrifugation, the supernatant was discharged and the pellet was resuspended in 10 mL of DMEM. The total cell number was subsequently estimated by the trypan blue exclusion method in a Neubauer-counting chamber and the correspondent desired amount of 3 x 10^6 cells were finally collected and subjected to genomic DNA (gDNA) extraction. The collected amount of cells (3 x 10^6) were transferred to a 1.5 mL *eppendorf* tube and subsequently centrifuged at 14000 x *g* for 30 seconds to pellet the cells. The supernatant was removed and 200 µL of PBS was added to wash the cells. The Genomic DNA extraction was then performed according to the Wizard Genomic DNA purification kit supplied by Promega (Section 3D, step 1, and step 4-14) [44]. The extracted gDNA was analyzed on a 0.7% agarose gel electrophoresis with 1x TAE buffer (40mM Tris-acetate, 1 mM EDTA, pH 8). The amount of the extracted gDNA was quantified by UV absorption at 280 nm using a NanoDrop Spectrophotometer (NanoValue Plus from Bioxtra). The human gDNA presents high molecular weight species usually in the order of 20-30 kb. As can be seen in Fig. S2 the extracted gDNA (lane 2) is placed above the 10 kb marker band suggesting that the extracted gDNA is not fragmented.

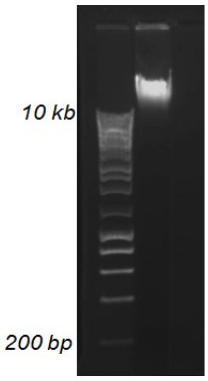

Fig. S2: gDNA analysis in a 0.7% agarose gel electrophoresis. 1-HypperLadder IV (ranging from 10kb to 200 bp). 2- gDNA extracted from HEK 293 T cells

1.4 Amplification curves and cp analysis

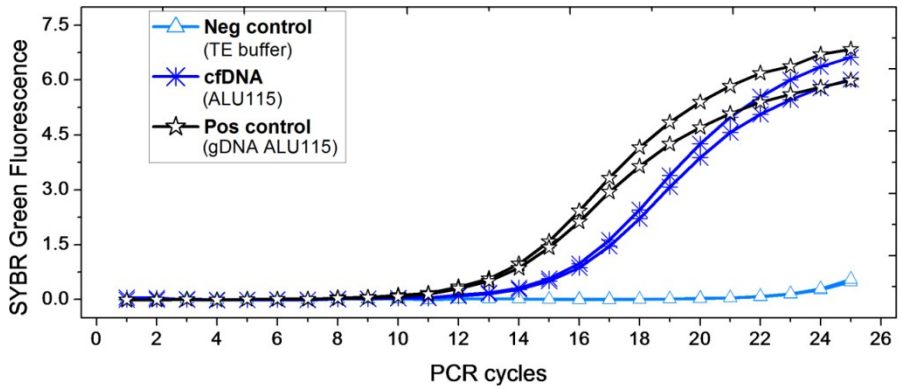

Fig. S3: ALU115 amplification by real-time PCR from a template of cfDNA isolated from plasma. Positive and negative controls were performed. The positive control contained 20 ng of genomic DNA extracted from HEK 293T cultured cells.

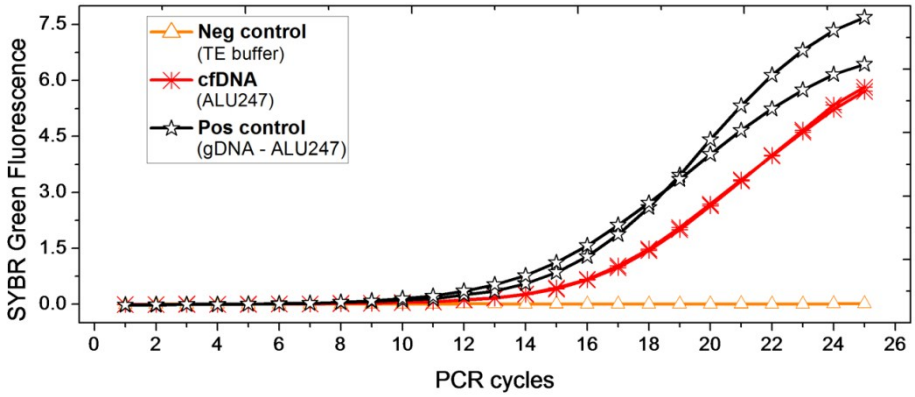

Fig. S4: ALU247 amplification by real-time PCR from a template of cfDNA isolated from plasma. Positive and negative controls were performed. The positive control contained 20 ng of genomic DNA extracted from HEK 293T cultured cells.

1.5 Lambda exonuclease digestion

The reaction mixtures for the digestion of the targets with lambda exonuclease consisted of 250 ng of DNA template ALU115 and 500 ng of ALU247 in separated sample solutions with 0.5 µL (2.5 Units) of Lambda exonuclease (supplied by New England Biolabs) and 1.25 µL Lambda exonuclease buffer 10x in a total reaction mixture of 12.5 µL. The digestion occurred for 30 min at 37 ºC and was followed by an inactivation step for 10 min at 70 ºC. The digestion was assessed on a 2% agarose gel electrophoresis.

1.6 Labelling of DNA targets with 250 nm magnetic beads

For the attachment of the generated amplicons with the designated beads (through streptavidin-biotin interaction), a few parameters were initially considered before proceeding with the conjugation. The maximum number of particles that could be accommodated on top of the sensors was calculated for a full coverage condition, i.e. when a monolayer of particles is formed on top of the sensors. Considering the dimensions of the sensors (80 x 2.5 μm^2) and the nominal diameter of the particles here investigated (250 nm), the maximum number of particles that could be accommodated on top of the sensors was approximately 3200. To allocate at least 3200 particles on top of each sensor, a concentration of at least 1.6 x 10^7 nanoparticles/µL had be introduced into the system, provided that the beads will spread through the whole length and width of the µ-channel used in this work. Additionally, considering that the 250 nm MPs have approximately 450 streptavidins available per particle for interaction (considering Micromod's technical data sheet), each sensor, in full coverage, will be able to detect in maximum, approximately 1.44 x 10^6 DNA molecules, if the beads were fully loaded. The provided calculations were accounted for the following procedures. From the stock suspension of beads (4.9 x 10^{11} nanoparticles/mL), 10 µL were collected in a clean sterile *eppendorf* and placed on a magnetic concentrator (DynaMagTM-2 supplied by Invitrogen) for around 1-2 min to attract the magnetic particles out of the initial preservative suspension. After, the supernatant was removed by aspiration with a Pasteur pipette while the eppendorf tube remained placed on the magnetic concentrator. The tube was then removed from the magnetic concentrator and the beads were washed with phosphate buffer 0.1 M with Tween20 at 0.02 % (v/v) and adjusted to pH 7.4 (PB buffer). This procedure was repeated for a total of 3 washes to fully remove the preservatives in the suspension. Finally, the magnetic nanoparticles were resuspended in a total volume of 100 µL in

PB buffer. A final concentration of 4.9 x 10^7 nanoparticles/µL was therefore obtained and left ready for the DNA labelling reaction. The labelling of the DNA targets occurred at a proportion of 1 µL of DNA to 10 µL of magnetic beads for 45 min at room temperature and under agitation. After incubation, the tubes where the reactions occurred were placed on the magnetic concentrator to capture the DNA targets that had bound to the nanoparticles through streptavidin-biotin interaction. The DNA molecules that have bound to the magnetic nanoparticles, at this step, are attracted to the magnet and are removed from the supernatant where remnant unbound DNA molecules reside. The supernatant was discarded and the magnetically labeled targets were rinsed in 10 µL of PB buffer for further detection with the proposed system.

2. Surface biochemistry assays

2.1 Composition of buffers

The buffers used in this work have the following compositions:

PB buffer – Phosphate buffer (0.1 M) with Tween20 at 0.02 % (v/v) and adjusted to pH 7.4.

TE buffer – Tris 10 mM with 1 mM *EDTA* (TE) and 0.1 mM KH_2PO_4, HCl and adjust to pH 7.4.

2.2 Spots of target ALU115 hybridized with SH-capture and SH-capture247

Optical images from which the surface coverage percentages were calculated using ImageJ are shown in Fig. S5 as an example of the formed spots for ALU115 when hybridized with SH-capture115 and SH-capture247.

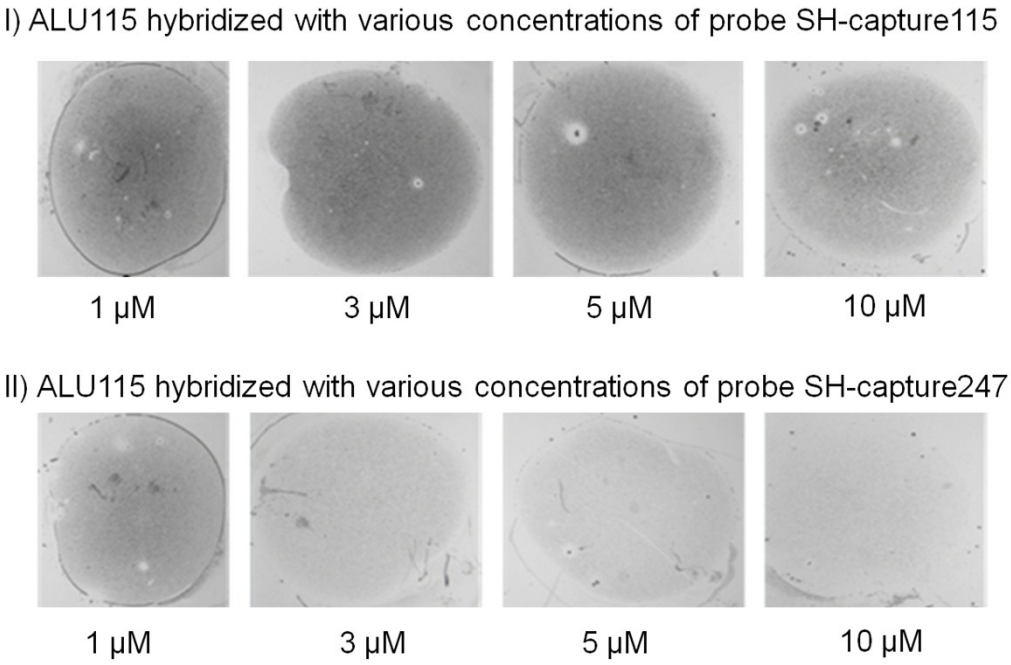

Fig. S5: Optical images in grey scale with a magnification of 40x showing different spot intensities for the hybridization of ALU115 with different concentrations of SH-capture115 and SH-capture247.

3. Biochip fabrication, integration with microfluidics and read-out of the sensors

3.1 Fabrication of the microfluidic module and integration with the biochip

Loading of samples and washing steps on the measurement platform were performed through a microfluidic module, made of polydimethilsiloxane (PDMS) with a U-shape and with 500 µm width and 100 µm height aligned with the sensors on the biochip through the integration in a polymethyl methacrylate (PMMA) module which simultaneously exerts the necessary pressure to ensure that the PDMS is reversibly bounded to the surface of the biochip during the measurement experiments (Fig S6).

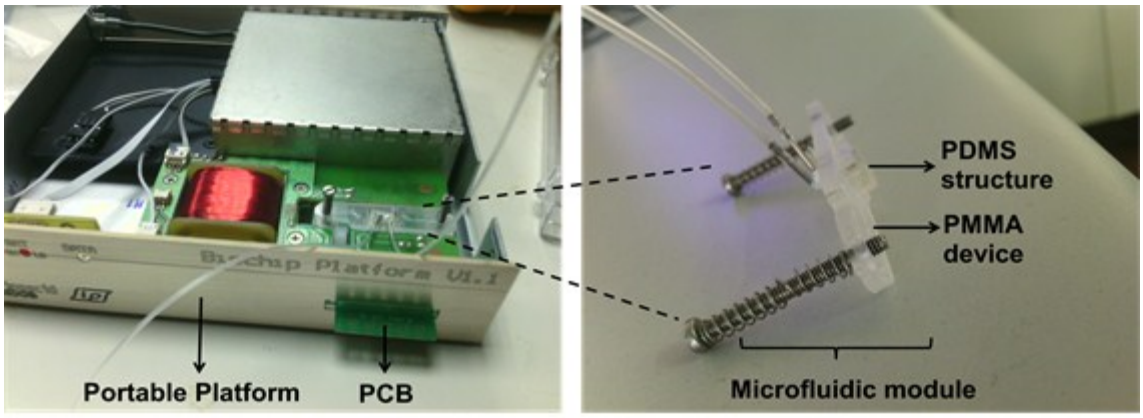

Fig. S6: PCB carrying the Biochip inserted in the Portable platform and integrated with the microfluidic module.

The microfluidic module allows to specifically control the flux entrance of fluids by connecting it through curved metallic pins with 0.8 µm of external diameter to a syringe pump system through inlet and outlet holes drilled on the PMMA. All the PMMA plates used, were micromachined using a CNC milling machine (TAIG Micro Mill from Super tech & Associates).

3.2 On-chip immobilization of SH-capture115 and SH-capture247

Droplets of 1 µL of SH-capture115 and SH-capture247 at a concentration of 5 µM (corresponding to $3x10^{12}$ DNA probes/ µL) were brought into contact with the biochip over the two columns of sensors, separately, before assembling with the microfluidic module. The droplets of the DNA probes occupy a surface area of approximately 1 mm^2. Considering the dimensions of the sensors (2.5 x 80 μm^2), the number of probes that are available for biointeraction over the sensors is approximately $6x10^8$ chains/sensor which is enough to capture all the beads in full saturation (3200 beads) even for the maximum DNA binding capacity of the particles over the sensors ($1.32x10^6$ DNA molecules per total number of beads).

3.3 Readout of the sensors

For readout of the sensors, initially PB buffer is loaded into the microfluidic channel, where it rests in contact with the sensors, in order to hydrate the previously immobilized DNA probes. Then, the baseline signal from each sensor (without particles) is acquired for 10-15 min until its stabilization. (II) The previously prepared DNA-beads conjugates are then injected into the microfluidic chamber, one concentration of each DNA target per chip, at a flow rate of 10 µL/min. (III) After injection, the conjugates are left to settle down and react inside the chamber with the immobilized DNA probes for 30 min, which in a experiment workflow corresponds to a decrease in the output signal. (IV) The

saturation signal is attained when the signal stabilizes meaning that a high number of particles, enough to saturate the sensor, are in contact or just over the surface. (V) Finally, the sensors are washed with PB buffer at a flow rate of 5 µL/min up to 50 µL/min to remove the unspecific, weakly bound DNA-beads conjugates. The washing step is stopped when the signal correspondent to the reference sensors (bioinert) had returned to the baseline, suggesting that all the non-attached beads had been removed from the channel. The final signal (Vbinding) of each sensor is finally acquired, which is proportional to the number of DNA-beads conjugates that had hybridized with the DNA probes immobilized on the gold pads on top of the sensors.

In Fig.S7 it is presented a group of five sensors exhibiting the presence of magnetic particles after detection of ALU115 (300 pM). The reference sensor is bioinert while the other four correspond to specific biointeraction between probe and target DNA after on-chip hybridization.

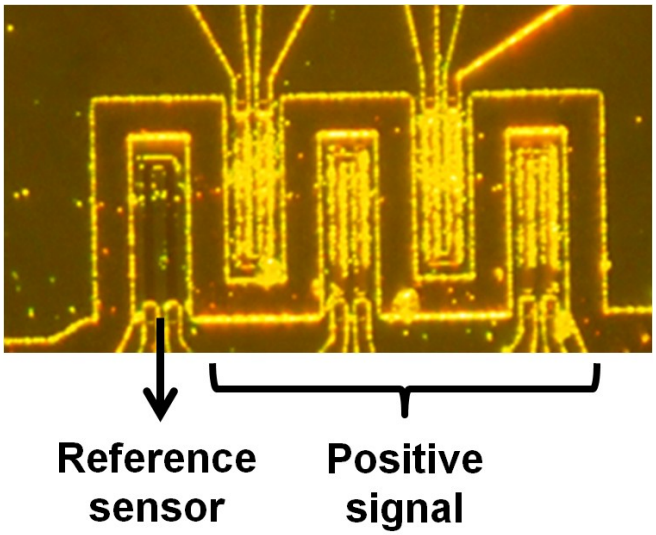

Fig. S7: Optical image (200 x magnification) of a group of five sensors. Four of the sensors show a density of particles after biorecognition of target DNA while the reference sensor is bioinert and therefore no particles are observed.