Electronic Supplementary Material (ESI) for Analyst.


## Supplementary information

### Microchip design

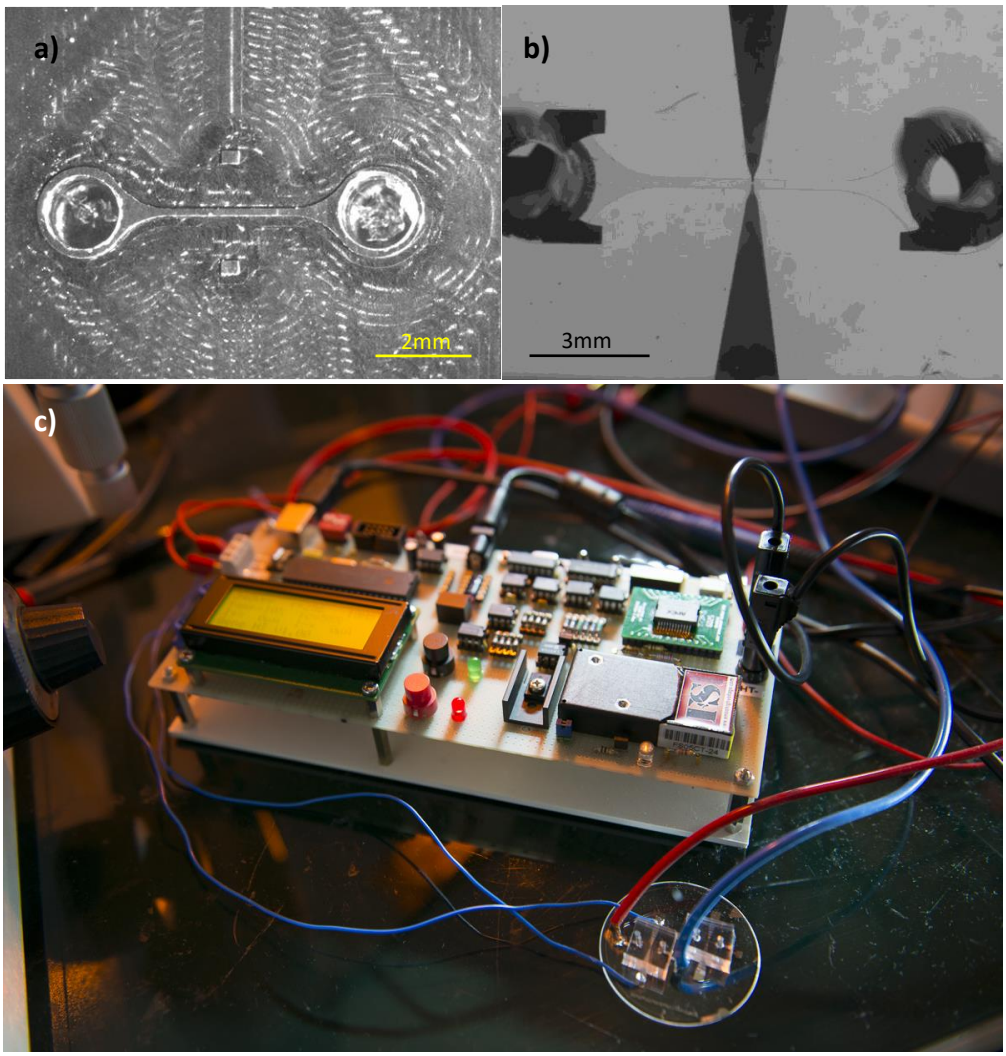


**Fig. S1** a) Picture of the micro-milled mould. b) Picture of the final microfluidic chip, after bonding of the PDMS replica on the patterned glass slide. c) Picture of the microfluidic chip and the HVPS.

### Excitation electronics

The schematics of the HVPS could be found below (Figure S2).

| SW2 | 1 | 2 |
|---|---|---|
| run | ON | ON |
| debug | ON | – |
| prog | – | – |

SW-DIP2 SW3 SW1 SW_PUSH D1 1N4148 RAZ C1 1µF P1 HE10-10 PWR_FLAG C7 10nF 22K R4 +5V

U4 PIC18F4455-DIP40
RA0/AN0 2 MESI1
RA1/AN1 3 MESI2
RA2/AN2/Vref-/CVref 4
RA3/AN3/Vref+ 5
RA4/T0CKI/C1OUT/RCV 6
RA5/AN4/SS/HLVDin/C2OUT 7 CS
RE0/AN5/CK1SSP 8 CLKL
RE1/AN6/CK2SPP 9 DINL
RE2/AN7/OESSP 10 CSL
RC0/T1OSO/T13CKI 15 PB_ON
RC1/T1OSI/CCP2/UOE 16 LEDR
RC2/CCP1/P1A 17 LEDV
Vusb 18
RC4/D-/VM 23
RC5/D+/VP 24
RC6/TX/CK 25 SS>
RC7/RX/DT/SDO 26 SDO>
RB0/AN12/INT0/FLT0/SDI/SDA 33 SDI<
RB1/AN10/INT1/SCK/SCL 34 SCL>
RB2/AN8/INT2/VMO 35 INT-MES<
RB3/AN9/CCP2/VPO 36
RB4/AN11/KBI0/CSSPP 37
RB5/KBI1/PGM 38
RB6/KBI2/PGC 39
RB7/KBI3/PGD 40
RD0/SPP0 19
RD1/SPP1 20
RD2/SPP2 21
RD3/SPP3 22
RD4/SPP4 27
RD5/SPP5/P1B 28
RD6/SPP6/P1C 29
RD7/SPP7/P1D 30
MCLR/Vpp/RE3 1
VDD 11 VDD 32
VSS 12 VSS 31
OSC1/CLKI 13
OSC2/CLKO/RA6 14
20MHz X1 C4 15pF C6 15pF

test1 C11 220nF R15 470 J1 USB DIS-HT

SW2 PB_ON R1 10K

D2 SRD R2 3.3K LEDR
D3 SGD R3 1K LEDV

U7 PIC16F1825
SCL> 10 RC0
SDO> 9 RC1
SDI< 8 RC2
SS> 7 RC3
INT-MES< 6 RC4
5 RC5
VDD 1
VSS 14
RA0 13
RA1 12
RA2 11
MCLR/RA3 4 RAZ
RA4 3
RA5 2
C16 10nF
X2 8MHz C23 15pF C24 15pF

U16B 4050 5 6
U16C 4050 6 7
P4 CONN_3 1 2 3 SYNC CLK GND

U10 LTC1452 C19 10nF
U9 LTC1451 C17 10nF
CLK Din CS Dout VCC Vout GND REF DIP8
CLKL DINL CSL
SIG1 signal 0-4.8V max
GND1
level out 0-2.4V

U16A U16D U16E U16F 4050 74HC4050 portes non utilisées

S1 FDCC2004C FLYYBW-51LK
GND VCC VLCD RS R/W CS D0 D1 D2 D3 D4 D5 D6 D7
LED+ 15 LED- 16
10K RV1
JE9013 Q3 R23 10K RV2 10K

100nF C2 C3 10nF +24V
U1A U1B LM358
BD237 Q1 JP1 JUMPER
U3 FS05CT +IN -IN +OUT -OUT CT ERR LOG5V DIS
+130V -130V +HT -HT
332K R7 1% 332K R9 1% 13.3K R8 1% 13.3K R10 1%
DIS-HT R5 10K Q2 JE9012 D4 SBC R6 330

CON1 24V PWR_FLAG TSR-2450 U2 VI VO GND C5 10µF
U5 ICL7660 CAP+ CAP- V+ VOUT LV OSC GND C8 10µF C9 10nF C10 10µF -5V

U14 U13 MCP4151-104 CS SCK SDI VSS PA PW PB
pot. signal pot. ref 100K
U15A U15B LM1458 C25 C26 C27 C29 10nF

R27 10K R24 10K R25 10K R26 10K U12A LM1458 C21 C22 10nF
4.8V cc
R11 1K R12 1K PA343 U6A R16 47.5K R19 100 C12 C13 10nF R22 301 R29 39
P2 HT+
U11 HCPL-7800 U8 IV0505S +Vin -Vin +Vout -Vout R21 470 C28 C18 C20 10nF
MESI1 MESI2
R31 10K R18 10K R28 10K R30 10K U12B LM1458
R13 1K R14 1K PA343 U6B R17 47.5K R20 100 C14 C15 10nF
P3 HT-

| KENIUM | | |
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| Title: Module excitation HT | | |
| Size: A3 | Date: 26 feb 2014 | Rev: 1.0 |
| KiCad E.D.A. | | Id: 1/1 |

**Oligonucleotide sequences**

List of the oligonucleotides used for SDA-HRCA. Underlined sequences with the same colour are identical. Sequences with the same colour are complementary. Nicking sequences are in italic and the nicked bond is bolded. Phos- indicates a 5' phosphorylation and * indicates a phosphorothiate bond. N is a random nucleotide.

| Name | Length | Sequence |
|---|---|---|
| ldh1 | 165nt | 5'-AGGTAATGGTGCAGTAGGTTCAAGCTACGCATTTTCATTAGTGAACCAAAGCATTGTTGATGAATTAGT CATCATTGATTTAGACACTGAAAAAGTTCGAGGAGATGTTATGGATTTAAAACATGCCACACCATATTCTCC AACAACAGTTCGTGTGAAAGCTGG |
| mecA | 169nt | 5'-TAATAGCAATACAATCGCACATACATTAATAGAGAAAAAGAAAAAAGATGGCAAAGATATTCAACTAA CTATTGATGCTAAAGTTCAAAAGAGTATTTATAACAACATGAAAAATGATTATGGCTCAGGTACTGCTATC CACCCTCAAACAGGTGAATTATTAGCACTT |
| Extension Primer 1 | 45nt | 5'-GCATAATACTACCAGTCT***CT***CAGCAAGCTACGCATTTTCATTAG |
| Extension Primer 2 | 44nt | 5'-TAGAATAGTCGCATACTT***CT***CAGCCATAACATCTCCTCGAACT |
| Bump Primer 1 | 19nt | 5'-AGGTAATGGTGCAGTAGGT |
| Bump Primer 2 | 17nt | 5'-CCAGCTTTCACACGAAC |
| RCA Padlock | 90nt | 5'-Phos-AGTCATCATTGATTTNNNNNNNNNNNNATCGACTGAGTGCTANNNNNNNNNNTAAGCGTTT CCCAAANNNNNNNNNNTTGTTGATGAATT |
| HRCA Primer 1 | 15nt | 5'-ATCGACTGAGTGC*T*A |
| HRCA Primer 2 | 15nt | 5'-TTTGGGAAACGCT*T*A |

**Signal acquisition and wavelet treatment**

Wavelet treatment was performed using the wavelet package in Matlab.

By trial and error, we validated the use of the Bior2.2 wavelet shape. In order to scan the complete conductometric signal, a collection of wavelets $\varphi_{j,k}$ were generated from the mother-wavelet $\varphi$.

$$\varphi_{j,k}(t) = 2^{-j/2}\varphi(2-j \quad t-k)$$

With $j,k \in \mathbb{N}$, where $k$ controlled the temporal position of the wavelet and $j$ the spatial width and amplitude of the wavelet. Discrete coefficients were then obtained by convolution with the temporal signal $x(t)$ to treat:

$$d_x(j,k) = \int x(u)\varphi^*_{j,k}(u)du$$

Each collection of coefficients $d_x(j,k)$ with a fixed spatial width $j$ reflects the contribution of peaks with a similar width to the wavelet $\varphi_{j,k}$. This transformation could be inverted:

$$x(t) = \sum_{k,j} d_x(j,k)\dot{\varphi}_{j,k}(t)$$

Where $\dot{\varphi}_{j,k}$ is the dual wavelet of $\varphi_{j,k}$. A detail signal $S_j(t)$ containing only the contribution of these peaks can be reconstructed using the following formula:

$$S_j(t) = \sum_{k} d_x(j,k)\dot{\varphi}_{j,k}(t)$$

The conductometric signal was then divided between several details signals $S_j(t)$ as seen on Figure S4:

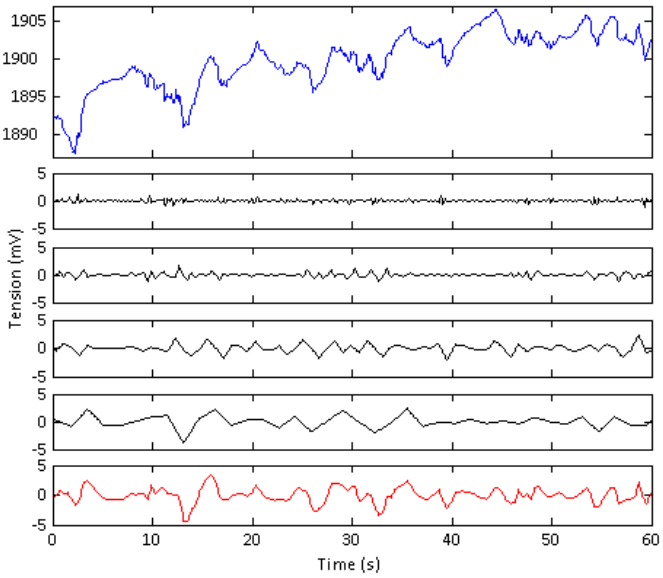


**Fig. S3** From top to bottom: raw signal (in mV) from a conductometric measurement of Lambda-DNA aggregation (blue). Detail signals associated: $S_2(t)$, $S_3(t)$, $S_4(t)$, $S_5(t)$ (black). Reconstructed signal $x_w(t)$ (red)

The EHDA-related contributions were mainly distributed between the 2nd, 3rd, 4th and 5th detail signals, while the electronic noise was present in the 1st detail signal and the slow baseline shifts was recovered above the 6th detail signal. A filtered signal $x_w(t)$ was thus reconstructed with the physically relevant detail signals only:

$$x_w(t) = \sum_{i=2}^{i=5} S_i(t)$$

Further treatments were needed to remove transient artefacts in the wavelet-treated signal. Each measurement was divided into 10 segments $x_w(t_s \rightarrow t_{s+1})$. To quantify both the quantity and the amplitude of the filtered peaks, we calculate the average of the absolute value of the filtered signal. A partial quantification parameter for the segment $s$ is:

$$Q_s = \langle |x_w(t_s \rightarrow t_{s+1})| \rangle$$

The quantification parameter for the whole filtered signal is:

$$Q = \langle |x_w(t_0 \rightarrow t_f)| \rangle$$

If a segment had a quantification parameter higher than 12% (value validated with optical controls) of $Q$, it would be removed and the quantification parameter would be updated to:

$$Q = \langle |x_w(t_0 \rightarrow t_s \,\&\, t_{s+1} \rightarrow t_f)| \rangle$$

The algorithm was looped until no segment was removed and the previous quantification parameter was kept.

**Gel electrophoresis protocol**

The amplification products were analyzed by agarose gel electrophoresis, concentration 4% in a TAE 1X buffer. The gel was premixed with a final concentration of 1X SyBr Gold before casting. 0.5µl of SDA sample and 25bp ladder (from Invitrogen) were mixed with 1µl of Blue/Orange 6X loading dye (from Promega) and 4.5µl of TAE buffer. 5µl of mix was deposited in the gel wells. Electrophoresis was performed during 1h at 100V. Imaging of the gel was done in an Amersham 600 Imager.

**SDA and HRCA scheme**

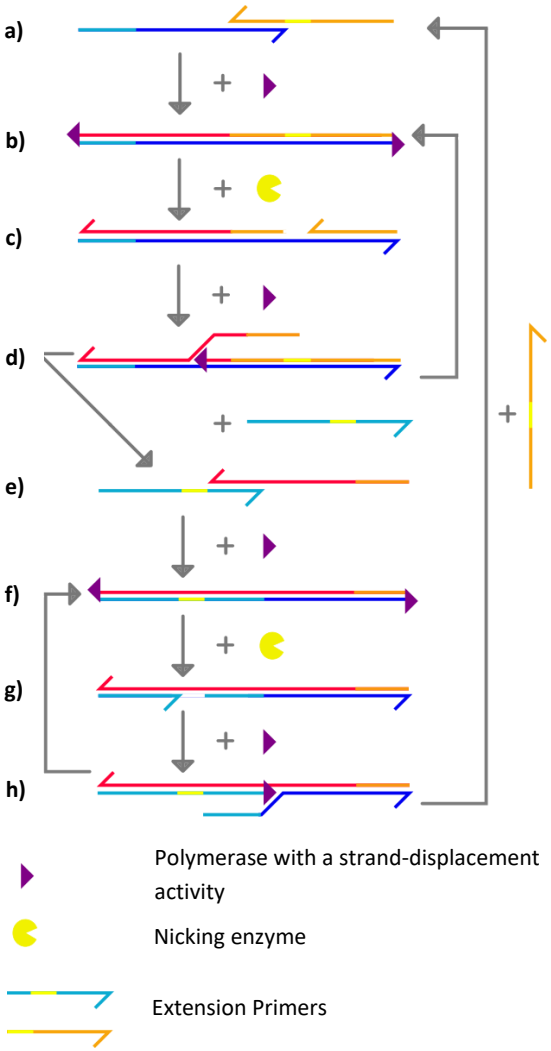


**Fig. S4** SDA scheme. 3' ends are indicated with arrows. a) & e) Primer-target hybridization. b) & f) Primer-target elongation. c) & g) Nicking. d) & h) Elongation from the downstream strand and strand-displacement of the upstream strand.

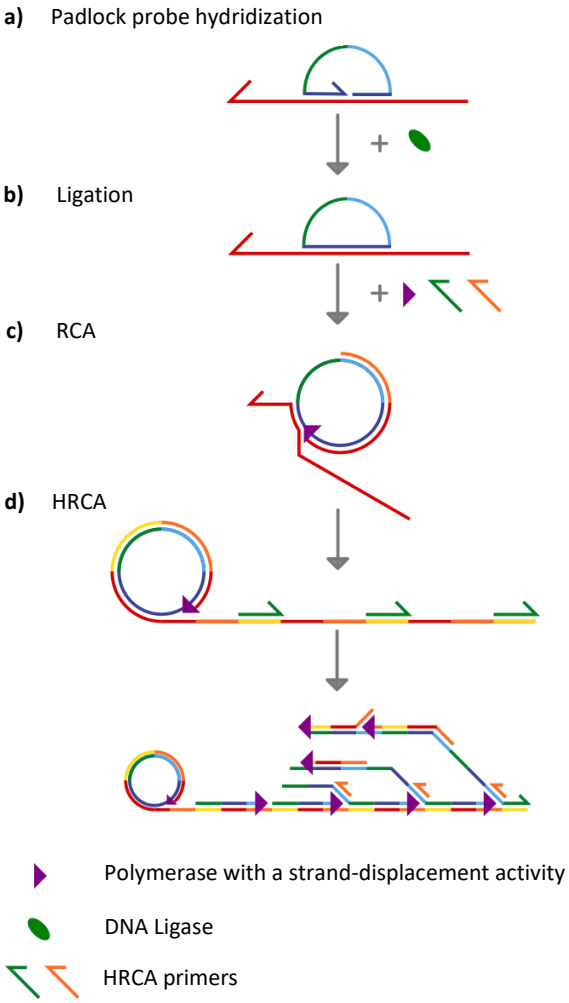


**Fig. S5** HRCA scheme. 3' ends are indicated with arrows. a) Hybridization of the RCA Padlock probe with the SDA single-stranded amplicon. b) Ligation of the RCA Padlock probe into a DNA circle. c) Priming of the circle with a primer, and displacement of the SDA amplicon. d) Branching of the RCA multimer with the primers