Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Disc structure

The DVD-based bioanalytical platform consists on a multi-layer disc, as it is described in Figure SI.1. The bottom layer is a standard DVD disc, composed of two thick-polycarbonate substrates and a middle layer of highly reflective metallic material. The upper layer is the microfluidic substrate composed by pressure sensitive adhesive foil and a disc-shape polycarbonate plastic. The holes, channels, and reservoirs have been fabricated on this substrate. Finally, the inlet holes (sample/reagent loading) and outlet holes (waste removing) are sealed with an inert tape.

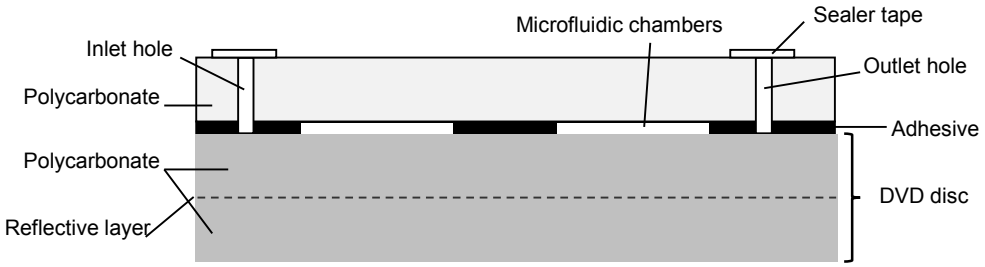

Figure SI.1: Scheme of the optical disc containing microfluidic structures.

Solid-phase amplification on disc

The isothermal amplification was based on solid-phase recombinase polymerase amplification (RPA) in the reaction chamber. For that, the forward primer (FP-I) was immobilized on the DVD following a microarray format via streptavidin-biotin interaction. The sample and other reaction components were dispensed in the liquid phase to the reaction chamber by centrifugation. This reaction mixture included digoxigenin-labelled forward primer (Dig-FP-S) and the reverse primer (RP). Table SI.1 shows the oligonucleotide sequences of primers used.

Table SI.1: List of oligonucleotides. RP: reverse primer, FP-I: forward primer immobilized on DVD surface, FP-S: forward primer in solutions, C: controls.

Gen	Use	Sequence 5'-3'	Len	Tm	ref
Control					
	C-	[BtnTg]-T10-AGGGTCGTACACCGGCTGTAATCAAA	46	75.9	own
	C+	[DIG]-T10-TTTTTGTCATGGGCCTCGTGTCGGAAAACC-[BtnTg]	40	81.0	own
Endogenous					
Le1	FP-I	[BtnTg]-T10-CGAAGCTGGCAACGCTACCGGTT	33	74.1	[a]
Le1	FP-S	[DIG]-TCCACCCCCATCCACATTT	19	59.2	[a]
Le1	RP	GGCATAGAAGGTGAAGTTGAAGGA	24	58.8	[a]
adh1	FP-I	[BtnTg]-T10-CCTCACCAGTTACGAAACCAATCGATCCAA	35	67.1	own
adh1	FP-S	[DIG]-CGTCGTTTCCCATCTCTTCCTCC	23	64.2	[a]
adh1	RP	CCACTCCGAGACCCTCAGTC	20	63.5	[a]
Lat52	FP-I	[BtnTg]-T10-ACTCTCTTTGCAGTCCTCCCTTGGG	25	66.8	own
Lat52	FP-S	[DIG]-AGACCACGAGAACGATATTTGC	22	58.4	[b]
Lat52	RP	TTCTTGCCTTTTCATATCCAGACA	24	57.6	[b]
Screening					
p35S	FP-I	[BtnTg]-T10-ATATAGAGGAAGGGTCTTGCGAAGGATA	35	64.8	own
p35S	FP-S	[DIG]-CCACGTCTTCAAAGCAAGTGG	21	59.8	[c]
p35S	RP	TCCTCTCCAAATGAAATGAACTTCC	25	59.7	[c]
tNOS	FP-I	[BtnTg]-T10-GCGTATTAAATGTATAATTGCGGGACT	27	63.7	own
tNOS	FP-S	[DIG]-GCATGACGTTATTTATGAGATGGG	24	59.3	[c]
tNOS	RP	GACACCGCGCGCGATAATTTATCC	24	64.4	[c]
Construct					
Bt-11	FP-I	[BtnTg]-T10-TTCTGGGTTACTCAAGCAGTTGTATGG	30	66.6	own
Bt-11	FP-S	[DIG]-AAAAGACCACAACAAGCCGC	20	58.4	[a]
Bt-11	RP	CAATGCGTTCTCCACCAAGTACT	23	62.9	[a]

[BtnTg]: Biotin with triethylene glycol spacer; T10: Thymine tail (10 nucleotides); [Dig]: digoxigenin

Then, the initial amplification (in solution) was followed by the extension of immobilized primers (solid-phase reaction) and the formation of attached digoxigenin-labelled product (Dig-product). As the FP-I was designed to be specific for a region located within the first product, the heminested approach led to a shorter immobilised product than that formed in the liquid phase. Selectivity and sensitivity of this amplification method is improved compared to conventional solid-phase approaches [d]. Figure SI.2 shows the amplification principle on disc.

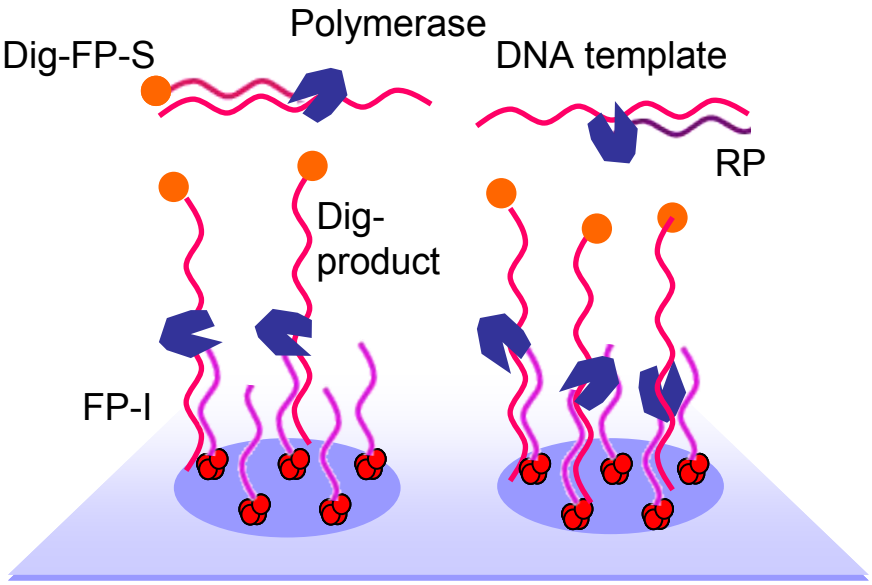

Figure SI.2: Scheme of solid-phase isothermal amplification on disc based on RPA.

[a] International Organization for Standardization, Geneva, Switzerland (2005) ISO 21570:2005. Foodstuffs-methods of analysis for the detection of genetically modified organisms and derived products-quantitative nucleic acid based methods

[b] L. Yang, A. Pan, J. Jia, J. Ding, J. Chen, H. Cheng, C. Zhang, D. Zhang. *J. Agr. Food Chem.*, 2005, **53**, 183-190.

[c] International Organization for Standardization, Geneva, Switzerland (2005) ISO 21569:2005. Foodstuffs-methods of analysis for the detection of genetically modified organisms and derived products-qualitative nucleic acid based methods.

[d] S. Santiago-Felipe, L. A. Tortajada-Genaro, S. Morais, R. Puchades, A. Maquieira, *Sensor. Actuat. B-Chem.*, 2014, **204**, 273-281.