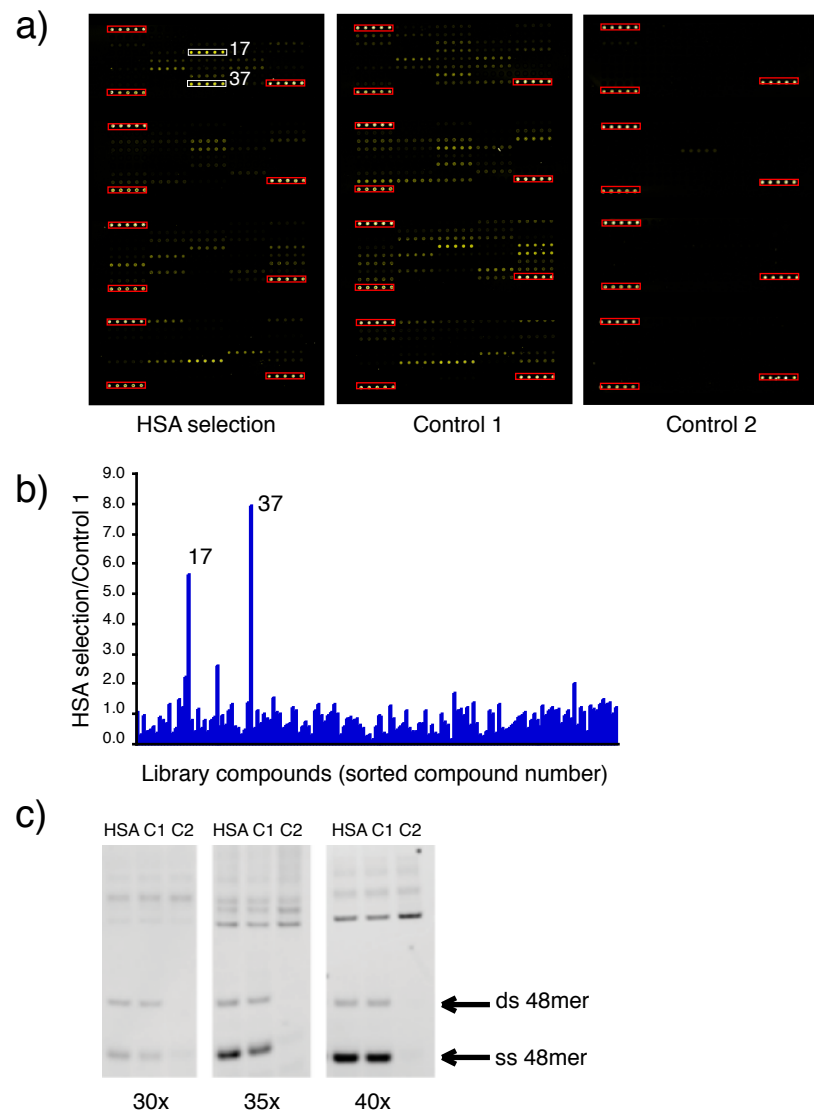


Supplementary Figure 1



Supplementary Figure 1: Control hybridization experiments.

a) Microarray decoding of ESAC affinity maturation selections. The first panel corresponds to a selection against HSA performed as in **Figure 3**; the second panel corresponds to a selection against HSA, in which the oligonucleotides had not been coupled to pharmacophores; the third panel represents a negative control hybridization, following a PCR reaction as in the first two panels, but omitting the template. A preferential enrichment of pharmacophores **17** and **37** is observed when comparing the first two panels. The third panel confirms the absence of contaminants in the microarray hybridization.

b) Ratios of the average microarray signal intensities for individual compounds, obtained by dividing the signal intensities for the compounds of the first two panels of **Supplementary Figure 1a**. The experiment confirms that the decoding signals of the pharmacophores which were selected preferentially [**17** and **37**] dominate the background signals which correspond to the amplification of residual DNA after 40 cycles of PCR.

c) After selection, the use of decoding PCR protocols with fewer rounds (30 or 35, rather than 40), provides a better discrimination between normal selections and negative control selections. The figure shows a gel electrophoretic analysis of PCR products obtained using fluorescent oligonucleotides as described in Methods; selections and controls (C1 and C2) were performed as described in **Supplementary Figure 1a**. For electrophoretic analysis, 2 μ l of the Cy3-labelled PCR products were applied on 20% TBE polyacrylamide gels (Novex) and run for 70 min at 200 V and 15 mA. Fluorescent single-stranded and double-stranded DNA bands were visualized with a DIANA III imager (Raytest). Higher molecular weight fluorescent DNA bands could be observed even in the absence of template DNA [C2], but did not affect microarray hybridization experiments [see third panel of **Supplementary Figure 1a**].