Supporting information to accompany the manuscript

Supporting Note: comparison of the volume of assay reagents consumed and reagent costs in microtiter plates and in the droplet-based microfluidic system

The assay miniaturization in the droplet-based microfluidic system significantly decreased the volume of reagents used and hence the reagent costs. Using single molecule PCR to amplify 10^6 genes at $\lambda = 0.15$ requires 6.67×10^6 reactions. This corresponds to 16.7 µL of PCR reagents in the droplet-based microfluidic system (2.5 pL per droplet) compared to 33.4 litres of PCR reagents in microtiter plates (5 µL per well), a reduction in volume of 2 million-fold. The price of a Phusion High Fidelity kit (500 x 50 µL reactions) is $681. Therefore, it would cost $0.91 million to amplify 10^6 genes by single molecule PCR in microtiter plates and only $0.46 using the droplet-based microfluidic system.

Similarly, 6.67×10^6 IVTT reactions require 133.4 µL of reagents in the droplet-based microfluidic system (20 pL per droplet) compared to 6.67 litres of reagents in a microtiter plate format (1 µL per well), a reduction in volume of 50,000-fold. The price of an IVTT kit (50 x 50 µL reactions) is $560. Therefore, it would cost $1.5 million in microtiter plates and only $30 using the droplet-based microfluidic system.

Finally, when coupling PCR and IVC experiments, as in this study, the cost of reagents to screen 10^6 genes can be estimated at $2.41 million in classical microtiter plate format, while the same experiment performed using the microfluidic approach would cost slightly less than $31, an overall reduction of reagents costs of almost 10^5-fold.

Supporting Figures

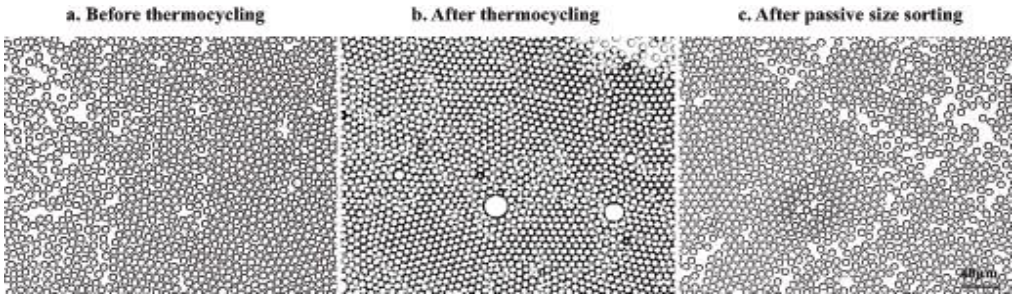

Figure S1. Micrographs of PCR emulsions. Before thermocycling (**a**), after thermocycling (**b**) and after thermocycling and passive size sorting.

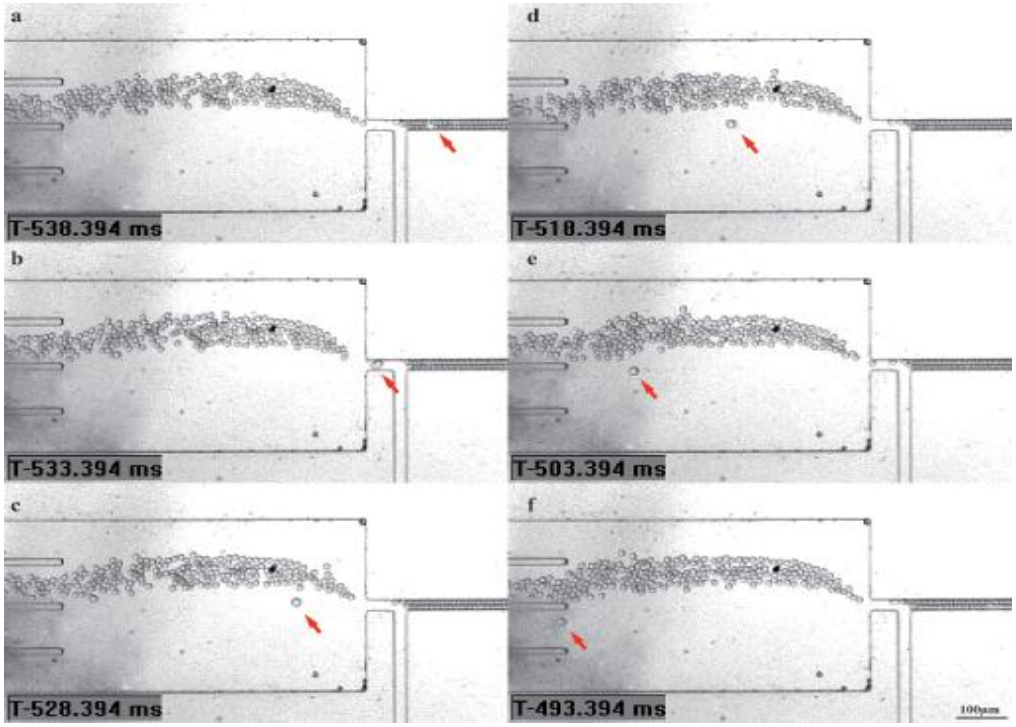

Figure S2. Micrographs of the passive sorting device. Red arrows indicate the path of a coalesced droplet that is separated from the unfused droplets. Time intervals are indicated in the gray boxes.

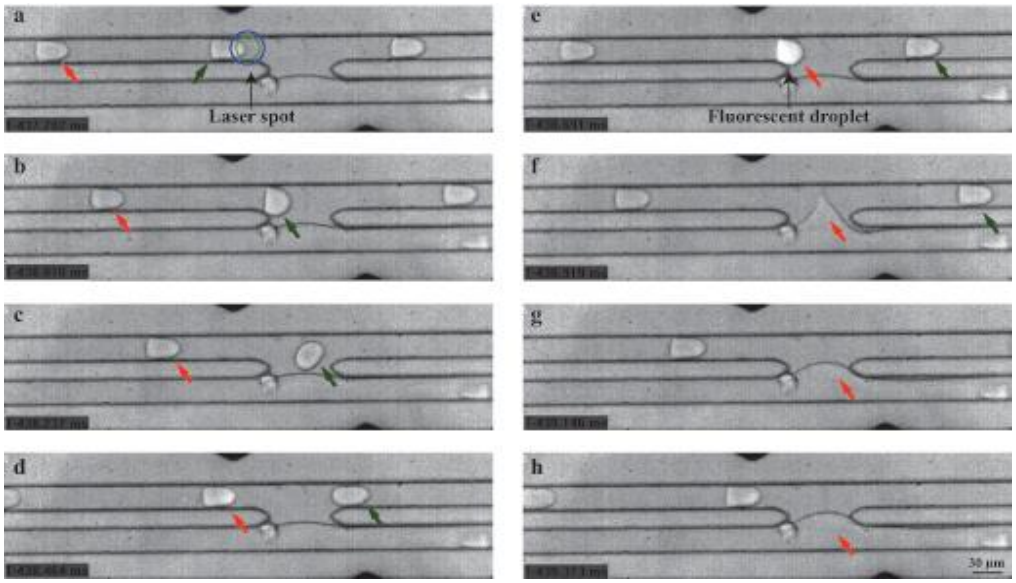

Figure S3. Micrographs of the sorting device in operation. Red arrows indicate the path of a sorted (high green fluorescence) droplet and green arrows the path of an unsorted (low green fluorescence) droplet. The green laser spot overlapped by the blue laser spot is indicated in image **a** and the detection of the fluorescent droplet is shown in image **e**. The low fluorescence droplet does not interact with the aqueous stream (**c**) while the high fluorescence droplet is electrocoalesced with the aqueous stream after the electrodes are triggered on droplet fluorescence (**f**). The time sequence is shown in the gray boxes.

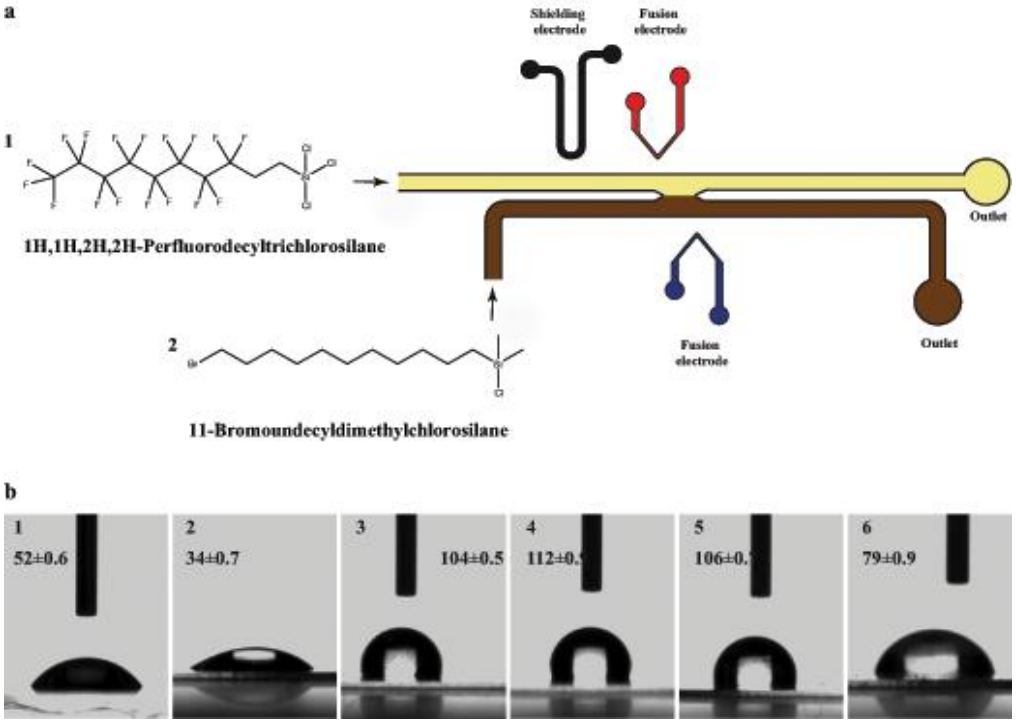

Figure S4. Surface treatment of the sorting device and static contact angle measurement. a, Two different coating agents were used for the treatment of the device. Compound **1** was used for the treatment of the region in contact with the reinjected emulsion (color coded in yellow) and compound **2** for the treatment of the region in contact with the aqueous phase (color coded in brown). **b**, Static contact angle measurement of water droplet in air using different coating reagents. Silanes **1** and **2** were grafted to a glass slide after activation of the slide with oxygen plasma. Contact angles (presented on the pictures) were measured between the substrate (glass slide) and a 2 µl droplet of water or HFE-7500. **Picture 1**: a droplet of water on an untreated glass slide. **Picture 2**: a droplet of water on a glass slide activated by the oxygen plasma. **Picture 3**: a droplet of HFE-7500 on an activated glass slide treated with a solution of **2** at 1% in cyclohexane. **Picture 4**: a droplet of water on an activated glass slide treated with a solution of **1** at 1% in HFE-7500. **Picture 5**: a droplet of water on an activated glass slide treated with a solution of **2** at 1% in cyclohexane. **Picture 6**: The glass slide is identical to picture 5 and the droplet is supplemented with 1% of Pluronic F68. All contact angle values were averaged from twenty measurements.

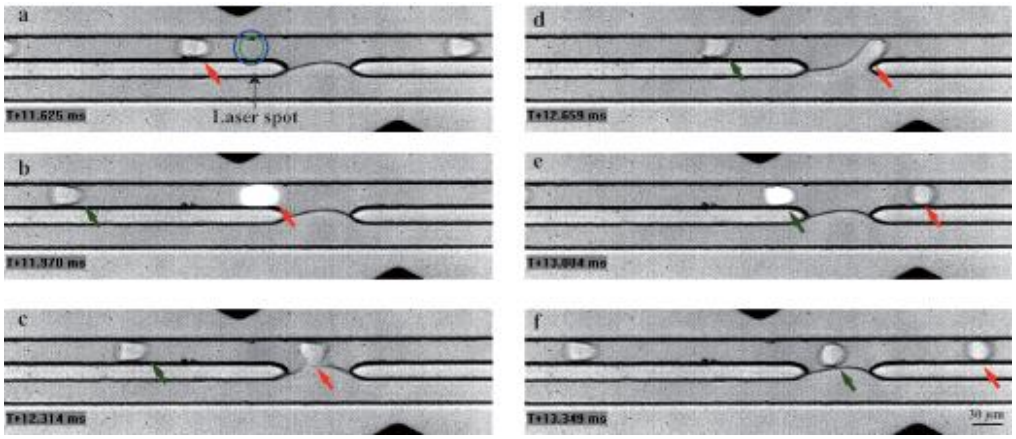

Figure S5. Micrographs of the sorting device showing the formation of a transient bridge between a droplet and the continuous aqueous stream. Red arrows indicate the path of a sorted (high green fluorescence) droplet and green arrows the path of an unsorted (low green fluorescence) droplet. Although the low-fluorescence droplet is excited by the laser in panel **e,** it is not sorted because the fluorescence is below the threshold. The sorting rate is 2500 Hz and positive droplets were not

efficiently extracted due to partial electrocoalescence with the aqueous stream. In panel d we can observe the formation of a bridge between the continuous phase and the inefficiently sorted droplet. The time sequence is shown in the gray boxes.

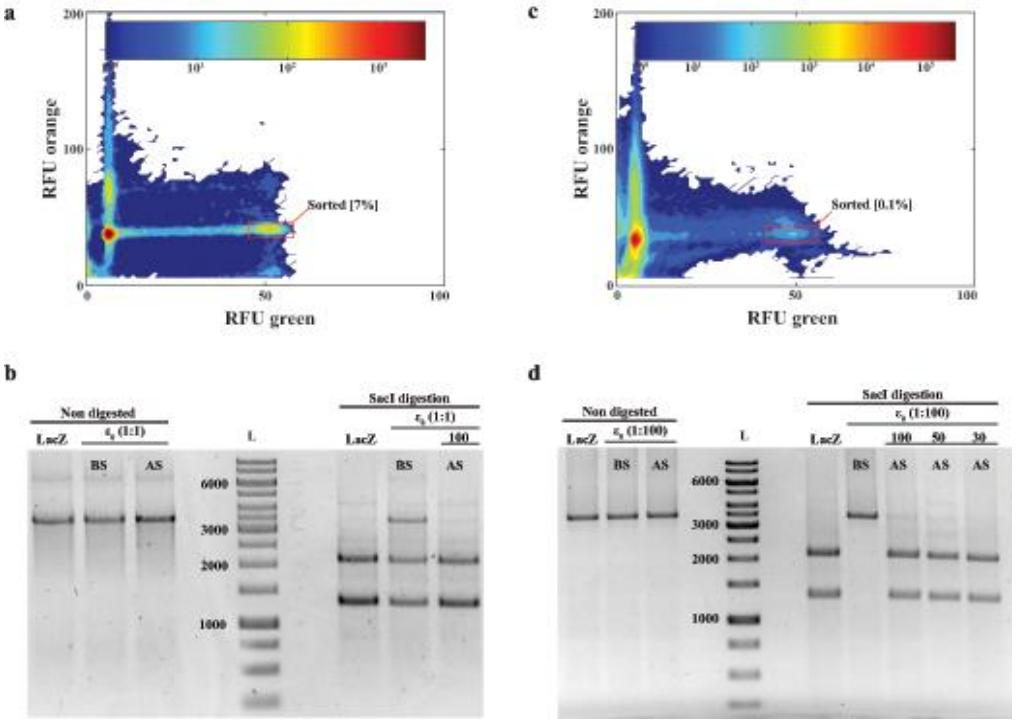

Figure S6. Selection of droplets based on β-galactosidase activity. Mixtures of *lacZ* and *lacZmut*, expressing active β-galactosidase and inactive variant, respectively, at starting ratios (ε_0) of 1:1 (**a**, **b**) or 1:100 (**c**, **d**) were compartmentalized in 2.5 pL droplets at λ = 0.15, together with Dextran-Texas Red (orange fluorescent) and amplified by PCR. After gene amplification and size fractionation, these droplets were fused with 20 pL droplets containing an IVTT system and FDG (100 µM), which is transformed into galactose and fluorescein (green fluorescence) by β-galactosidase. 2-D contour plots representing green and orange fluorescence after IVTT (**a**, **c**).The high green fluorescent populations containing active β-galactosidase (red box) were sorted and analyzed. DNAs recovered from the emulsions before (BS) and after the sort (AS) were amplified by PCR, digested by a restriction enzyme SacI and analyzed on an agarose gel stained with ethidium bromide. The initial DNA ratios between *lacZ* and *lacZmut* (ε_0) are indicated. L = size marker (1kb DNA ladder; Fermentas, Villebon sur Yvette, France). The recovered DNA was amplified by PCR, digested with SacI and analyzed on an agarose gel stained with ethidium bromide (**b**, **d**), With ε_0 of 1:1, 100 droplets were sorted and analyzed (**b**). With ε_0 of 1:100, 100, 50 and 30 droplets were sorted and analyzed (**d**).

lacZ:*lacZmut* starting ratio (ε_0)	*lacZ*:*lacZmut* final ratio (ε_1)	Theoretical enrichment factor (η_m)	Experimental enrichment factor (η_{exp})	Number of droplets Sorted
1	13.84	13	14	100
0.1	5.89	74	59	100
0.05	5.48	141	110	100
0.01	5.02	674	502	100
0.01	5.07	674	507	50
0.01	6.6	674	660	30

Table S1. Predicted (η_m) and experimentally obtained (η_{exp}) enrichment values after one round of sorting as a function of lambda and the starting ratio of *lacZ* and *lacZmut* DNA. All selections were performed with a mean number of template DNA molecules per droplet (λ) of 0.15.

Name	Sequence	Application
LMB2-9E	5'-GCATTTATCAGGGTTATTGTC-3'	Pre-amplification before encapsulation
LMB2-10E	5'-GATGGCGCCCAACAGTCC-3'	Amplification of single DNA molecules in droplets
LMB2-11E	5'-GCCCGATCTTCCCCATCGG-3'	Amplification of DNA after sorting
PIVB-1	5'-GCGTTGATGCAATTTCT-3'	Pre-amplification before encapsulation
PIVB-4	5'-TTTGGCCGCCGCCCAGT-3'	Amplification of single DNA molecules in droplets
PIVB-8	5'-CACACCCGTCCTGTGGA-3'	Amplification of DNA after sorting

Table S2. Oligonucleotide primers used during selection.

Electronic Supplementary Material (ESI) for Lab on a Chip

Captions for Supporting Movies

Supporting Movie S1. Formation of monodisperse 2.5 pL droplets by hydrodynamic flow-focusing.

Supporting Movie S2. Passive droplet size sorting based on Pinched Flow Fractionation (PFF).

Supporting Movie S3. Pairing and electrocoalescence of 2.5 pL droplets containing amplified DNA with 20 pL droplets containing an IVTT system.

Supporting Movie S4. Sorting of droplets by fluorescence-activated electrocoalescence.