Electronic supplementary information to accompany the manuscript:

An automated two-phase microfluidic system for kinetic analyses and the screening of compound libraries**

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Table of content of the electronic supplementary information:

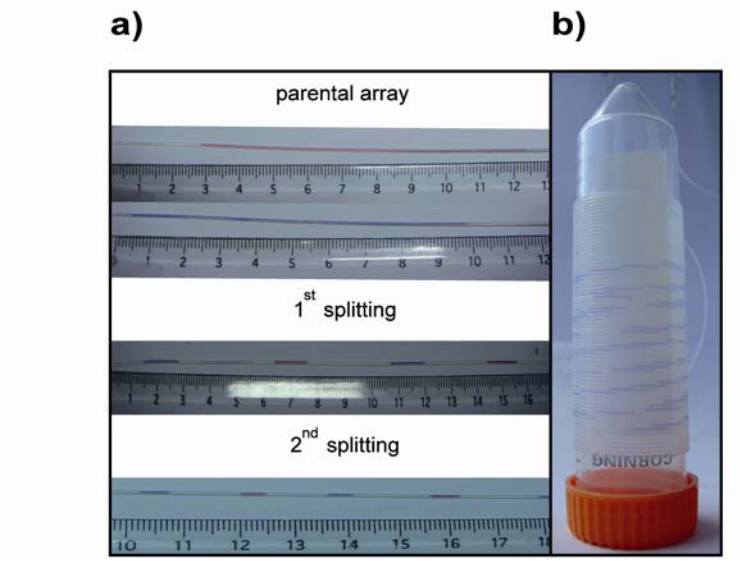

Fig. S1. Splitting and incubation of plug arrays. a) A parental array of alternating 8 µL plugs containing trypan blue (1 mM) and methyl red (50 mM) was split two consecutive times (1:8 and 1:2) to generate copies of the original plugs of 1 µL and 0.5 µL respectively. Analysis of the plug length revealed a coefficient of variation (CV) of 9.38% after splitting. b) Incubation of plugs in a length of tubing attached to a solid support.

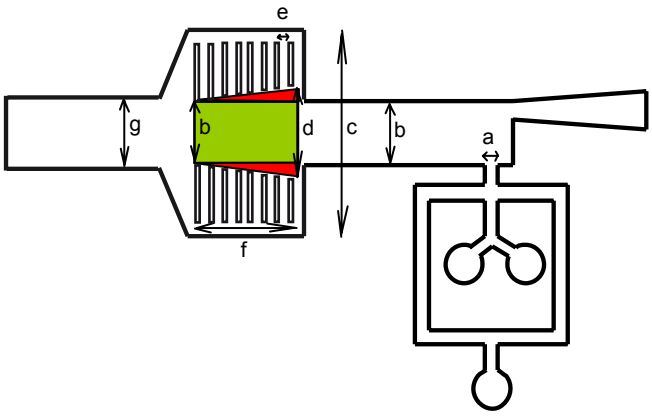

Fig. S2. Dimensions of the merging device. The drop maker has a width of 100 µm (a). while the fluidic channels have a width of 1000 µm (b), dilating to a total width of 3500 µm (c) in the pillar chamber (1426 µm between the pillars; d). This merging element has a length of 3225 µm (f) and consists of pillars having a width of 75 µm (with e = 75 µm) (f). The downstream channel has a width of 1800 µm (g). The total area of the chamber between the two pillar arrays can be divided into a rectangle (green colour) with an area of 3225 µm x 1000 µm and two equal triangles (red colour, up and down of the rectangle) having a total area of 3225 µm x 213 µm. For a channel depth of 225 µm, this corresponds to a chamber volume ($V_{chamber}$) of $V_{chamber}$ = 225 µm x (3225 µm x 1000 µm + 3225 µm x 213 µm) = 880 nL

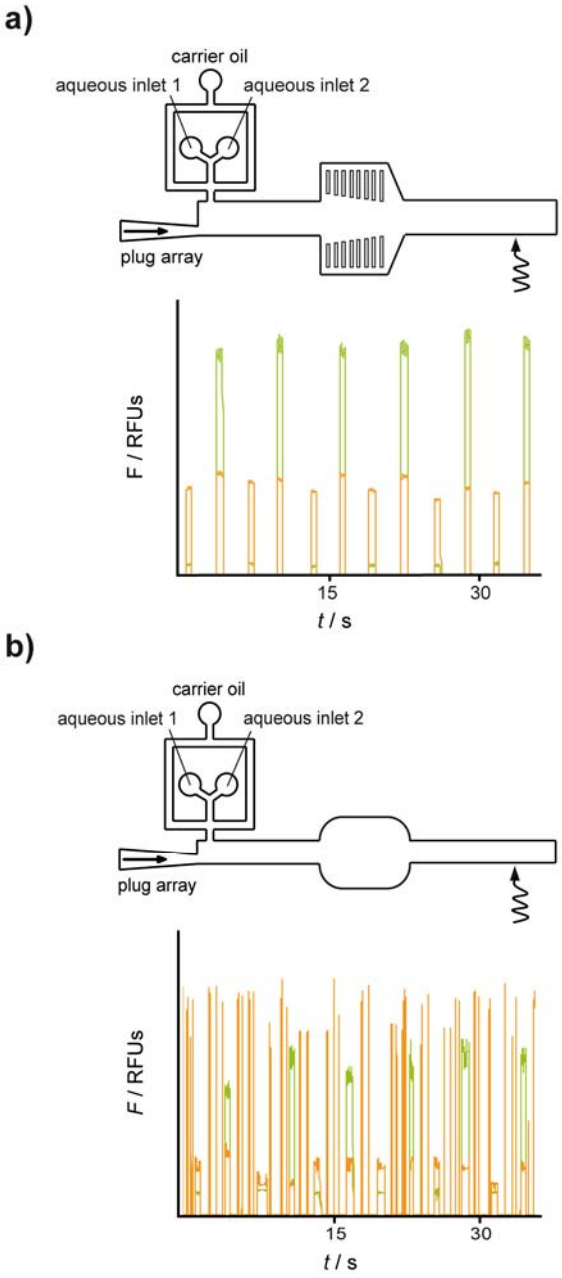

Fig. S3. Addition of further reagents to a pre-formed array of plugs. Droplets containing 400 µM resorufin (orange fluorescent) were passively fused with alternating plugs containing PBS and PBS plus 1 µM fluorescein (green fluorescent) respectively. Subsequently the orange and green fluorescence signal of each individual plug was determined (as indicated by the colour of the time trace). a) Performing the fusion step in a pillar chamber allows the reliable fusion of all substrate drops with the passing plugs. b) Performing the fusion step in a conventional chamber results in the release of free substrate drops in between the plugs.

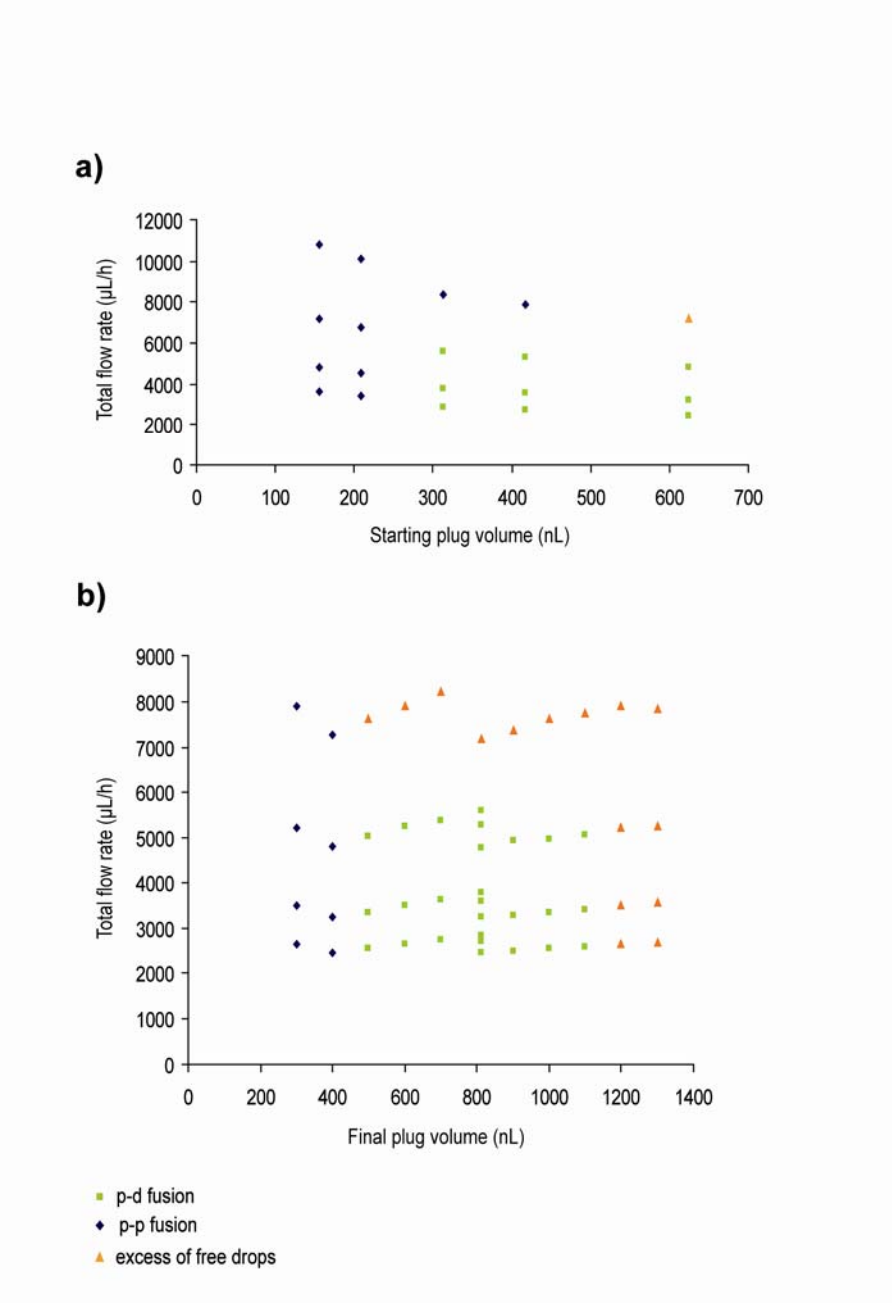

Fig. S4. Characterization of the merging device. a) Fusion efficiency for plug-drop mergers of constant final volume (V_{total}) at different total flow rates (Y-axis). Plugs of different starting volumes (X-axis) were injected into the device and the relative aqueous flow rates (QA_{plugs}:QA_{drops}) were adjusted to obtain a resulting plug-drop merger with a volume of 813 nL (see Material and Methods for details how the corresponding flow rates were obtained). The plug-drop fusion process (p-d fusion) was considered successful (indicated by green points), as long as two consecutive plugs did not fuse (p-p fusion; indicated by blue squares) and no free droplets (indicated by orange triangles) were released from the pillar chamber. b) Fusion efficiency for plug-drop mergers of different final volumes (V_{total}; X-axis) plotted

against the total flow rate (Y-axis). The indicated final volumes were obtained using relative volume fractions (V_{plug}:V_{drops}) for which successful plug-drop fusion had been demonstrated (green points in a)).

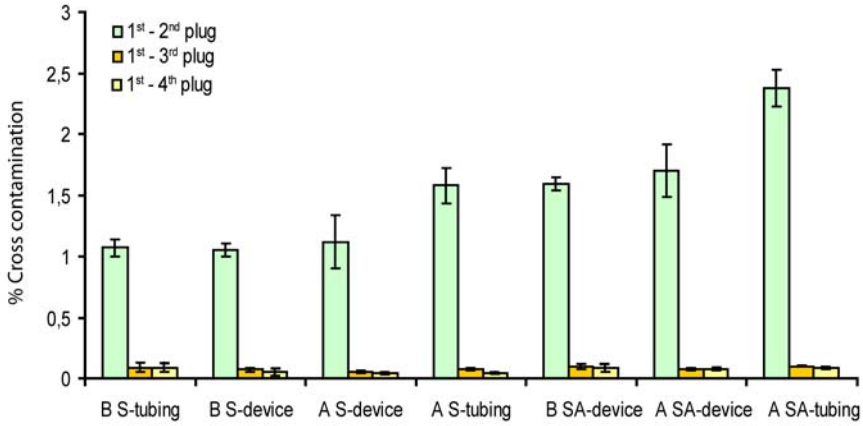

***Fig. S5*.** Cross-contamination between plugs**.** Arrays of plugs in which the first plug contains 30 µM fluorescein in PBS followed by three plugs containing PBS alone were generated. Subsequently, fluorescence measurements were performed before and after each manipulation step, either using transparent PFTE-tubing (tubing) or on-chip (device). B S = before splitting; A S = after splitting; B SA before substrate addition and A SA = after substrate addition. Error bars represent the mean standard deviation from six experiments.

Table S1. Standard deviation of the plug length (variation of the peak width for fluorescence signals from plugs containing 30 µM fluorescein) after different manipulation steps and corresponding plug volumes.

	Plug generation	Plug splitting	Substrate addition
Resulting plug length	7.1 ± 0.2 cm	9 ± 0.6 mm	11.5 ± 1 mm
Resulting plug volume	5 ± 0.15 µL	625 ± 44 nL	813 ± 69 nL
Coefficient of variation	3%	7%	8.5%

4. Supplementary movies

***M1*.** Injection of an array of plugs into a microfluidic chip using vertical inlets. The ports for the inlets were punched from the top, orthogonal to the channels, using a 0.75 mm-diameter Harris Uni-Core biopsy punch.

***M2*.** Injection of an array of plugs into a microfluidic chip using horizontal inlets. The ports for the inlets were punched from the sides (in line with the channels) using a 0.75 mm-diameter Harris Uni-Core biopsy and tubes connected with Loctite 351 glue.

***M3*.** Addition of further compounds to pre-formed plugs on-chip. Drops containing assay compounds (stained with trypane blue) are generated and continuously flushed into the pillar chamber, where they are trapped due to the drainage of the carrier phase between the pillars and coalesce spontaneously due to the absence of surfactant. When a plug containing the test compound arrives in the chamber it fuses with the coalesced drops containing the assay compounds, resulting in insufficient drainage of the carrier phase between the pillars and release of the merged plug. Subsequently a new cycle of the process is initiated.