Supporting Information For

An Integrated Microfluidic Processor for DNA-Encoded Combinatorial Library Functional Screening

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Experimental Procedures

Combinatorial ligation of bead-specific barcodes and encoding regions. Wells of filtration microtiter plates were wetted (DCM, 100 µL) then HDNA-functionalized photolabile Glu-pepstatin (10 µm, 0.22 mmol/g, 10 mg; 160 µm, 0.41 mmol/g, 0.5 mg; DMF) and HDNA-functionalized photolabile N-Acetyl-Glu resin (10 µm, 0.22 mmol/g, 10 mg; 160 µm, 0.41 mmol/g, 0.5 mg; DMF) were transferred as aliquots into separate, clean wells (1 mg/well, 10 wells). Plate wells were washed (1:1 DMF:H_2O, 1 x 150 µL; BTPWB, 4 x 150 µL), sealed with foil adhesive, incubated in UV-free environment with shaking (60 min, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of **≈0001[±]** (1.2 nmol), appropriate OP stock **≈11xx[±]** (1.2 nmol, **Supporting Information T2**) and T4 DNA ligase (216 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL, 10 wells) into a collection tube (15-mL conical) and mixed with DMF (6 mL). Pooled resin samples were mixed, transferred as equal aliquots into separate clean wells (1 mg/well, 10 wells), washed (DMF, 3 x 150 µL) sealed, and incubated in UV-free environment with shaking (14 h, RT, 700 rpm). Plate wells were washed (1:1 DMF:H_2O, 1 x 150 µL; BTPWB, 4 x 150 µL), sealed, incubated in UV-free environment with shaking (60 min, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stock **≈22xx[±]** (1.2 nmol, **Supporting Information T2**) and T4 DNA ligase (108 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking

(4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL, 10 wells) into a collection tube (15-mL conical), and mixed with DMF (6 mL). Pooled resin samples were mixed, transferred as equal aliquots into separate clean wells (1 mg/well, 10 wells), washed (DMF, 3 x 150 µL; BTPWB, 3 x 150 µL), sealed, incubated in UV-free environment with shaking (1 h, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stock **≈13xx[±]** (1.2 nmol, **Supporting Information T2**) and T4 DNA ligase (108 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL, 10 wells) into a collection tube (15-mL conical), and mixed with DMF (6 mL). Pooled resin samples were mixed, transferred as equal aliquots into separate clean wells (1 mg/well, 10 wells), washed (DMF, 3 x 150 µL) sealed, and incubated in UV-free environment with shaking (14 h, RT, 700 rpm). Plate wells were washed (1:1 DMF:H_2O, 1 x 150 µL; BTPWB, 4 x 150 µL), sealed, incubated in UV-free environment with shaking (60 min, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stock **≈24xx[±]** (1.2 nmol, **Supporting Information T2**) and T4 DNA ligase (108 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by

transferring each sample well (DCM, 4 x 100 µL, 10 wells) into a collection tube (15-mL conical), and mixed with DMF (6 mL). The pooled photolabile Glu-pepstatin and photolabile N-acetyl-Glu samples were transferred as equal aliquots into separate clean wells (Glu-pepstatin, 9 wells; N-acetyl-Glu, 12 wells), washed (DMF, 3 x 150 µL; BTPWB, 3 x 150 µL), sealed, incubated in UV-free environment with shaking (1 h, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stocks (**Supporting Information T2**) **≈15xx[±]** (1.2 nmol), **≈26xx[±]** (1.2 nmol), and T4 DNA ligase (216 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL) into a collection tube (15-mL conical), and mixed with DMF (6 mL). Pooled resin samples were mixed, transferred as equal aliquots into separate clean wells (Glu-pepstatin, 9 wells; N-acetyl-Glu, 12 wells), washed (DMF, 3 x 150 µL) sealed, and incubated in UV-free environment with shaking (14 h, RT, 700 rpm). Plate wells were washed (1:1 DMF:H_2O, 1 x 150 µL; BTPWB, 4 x 150 µL), sealed, incubated in UV-free environment with shaking (60 min, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stocks (**Supporting Information T2**) **≈17xx[±]** (1.2 nmol), **≈28xx[±]** (1.2 nmol), and T4 DNA ligase (216 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL) into a collection tube (15-mL conical), and mixed with DMF (6 mL). Pooled resin samples were mixed, transferred as

equal aliquots into separate clean wells (Glu-pepstatin, 9 wells; N-acetyl-Glu, 12 wells), washed (DMF, 3 x 150 µL; BTPWB, 3 x 150 µL), sealed, incubated in UV-free environment with shaking (1 h, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL).

An encoding solution consisting of appropriate OP stocks (**Supporting Information T2**) **≈19xx[±]** (1.2 nmol), **≈2Axx[±]** (1.2 nmol), and T4 DNA ligase (216 U) was prepared in BTPLB (150 µL) for each resin sample and added to the appropriate wells. The plates were sealed and incubated with shaking (4 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL) into a collection tube (15-mL conical), and mixed with DMF (6 mL). Resin was centrifuged (2 min, 10,000 RCF), supernatant was removed, the resin was resuspended (DMF, 1 mL) and stored protected from light (4 °C).

Equal aliquots of photolabile Glu-pepstatin and photolabile N-acetyl-Glu samples were transferred into separate clean wells (1 mg, 4 wells each), washed (DMF, 3 x 150 µL; BTPWB, 3 x 150 µL), sealed, incubated in UV-free environment with shaking (14 h, RT, 700 rpm), and washed (BTPLB, 1 x 150 µL). A ligation solution consisting of reverse primer module stock **≈0B02[±]** (1.2 nmol, **Supporting Information T2**) and T4 DNA ligase (108 U) was prepared in BTPLB (150 µL) for each resin sample and added to wells. The plates were sealed and incubated with shaking (8 h, RT, 700 rpm). Plate wells were washed (BTPWB, 3 x 150 µL; 1:1 H_2O:DMF, 1 x 150 µL; DMF, 3 x 150 µL; DCM, 2 x 150 µL), then the split aliquots for each resin sample were pooled by transferring each sample well (DCM, 4 x 100 µL per well) into a collection tube (15-mL conical) and mixed with DMF (3 mL). DNA-encoded photolabile Glu-pepstatin (**1**) and DNA-encoded photolabile N-acetyl-Glu resin (**2**) resin samples were centrifuged (2 min, 10,000 RCF), the supernatant removed, the resin was resuspended (DMF, 1

mL) and stored protected from light (4 °C).

qPCR mixture contained *Taq* (0.05 U/μL), oligonucleotide primers 5′-GCCGCCCAGTCCTGCTCGCTTCGCTAC-3′ and 5′-GTGGCACAACAACTGGCGGGCAAAC-3′ (0.3 μM each), and SYBR Green (0.2X, Life Technologies) in GC-PCR buffer (1X). Resin particles (HDNA-functionalized photolabile Glu-pepstatin, HDNA functionalized photolabile N-acetyl-Glu) in BTPWB (50 beads/μL, 2 μL) were added to separate amplification reaction wells (20 μL, 5 replicates each). Each resin supernatant (2 μL) was added to respective negative control reaction wells (20 μL, 2 replicates). Template standards (100 amol, 10 amol, 1 amol, 100 zmol, 10 zmol, 1 zmol, 100 ymol, and 10 ymol in BTPWB) were added to separate reaction wells (20 μL). The plate was thermally cycled (96 °C, 10 s; [95 °C, 8 s; 72 °C, 24 s] x 32 cycles; 72 °C, 120 s; C1000 Touch Thermal Cycler, Bio-Rad) with fluorescence monitoring (CFX-96 Real-Time System, Bio-Rad). Samples were quantitated using single-threshold Cq determination mode (400 RFU). Supernatant background was subtracted from respective sample measurements. Background-subtracted replicates were averaged and %RSD calculated.

Table T1

Overhangs

Overhang #	Sequence
≈X1XX[+]	/5Phos/ATGG
≈X1XX[-]	/5Phos/TGA
≈X2XX[+]	/5Phos/TCA
≈X2XX[-]	/5Phos/AAC
≈X3XX[+]	/5Phos/GTT
≈X3XX[-]	/5Phos/TAG
≈X4XX[+]	/5Phos/CTA
≈X4XX[-]	/5Phos/GAA
≈X5XX[+]	/5Phos/TTC
≈X5XX[-]	/5Phos/GCG
≈X6XX[+]	/5Phos/CGC
≈X6XX[-]	/5Phos/TAC
≈X7XX[+]	/5Phos/GTA
≈X7XX[-]	/5Phos/CCA
≈X8XX[+]	/5Phos/TGG
≈X8XX[-]	/5Phos/AGA
≈X9XX[+]	/5Phos/TCT
≈X9XX[-]	/5Phos/CTT
≈XAXX[+]	/5Phos/AAG
≈XAXX[-]	/5Phos/AGGC

Coding Sequences

Identifier #	Sequence	Identifier #	Sequence
≈1X01[+]	AAGAGGCA	≈2X01[+]	CCTCCTAA
≈1X01[-]	TGCCTCTT	≈2X01[-]	TTAGGAGG
≈1X02[+]	ACGGAGCA	≈2X02[+]	AACCTCAA
≈1X02[-]	TGCTCCGT	≈2X02[-]	TTGAGGTT
≈1X03[+]	ACGAGATT	≈2X03[+]	ATTCTCGG
≈1X03[-]	AATCTCGT	≈2X03[-]	CCGAGAAT
≈1X04[+]	AAGGAGGT	≈2X04[+]	AACCCTAC
≈1X04[-]	ACCTCCTT	≈2X04[-]	GTAGGGTT
≈1X05[+]	AGAAAGCA	≈2X05[+]	GACTCCGC
≈1X05[-]	TGCTTTCT	≈2X05[-]	GCGGAGTC
≈1X06[+]	ATAGAGCC	≈2X06[+]	CATTTCAA
≈1X06[-]	GGCTCTAT	≈2X06[-]	TTGAAATG
≈1X07[+]	CAGAAGGA	≈2X07[+]	CCCTCCGG
≈1X07[-]	TCCTTCTG	≈2X07[-]	CCGGAGGG
≈1X08[+]	GAGGAACA	≈2X08[+]	CGTTCCTG
≈1X08[-]	TGTTCCTC	≈2X08[-]	CAGGAACG
≈1X09[+]	TGAAGGAA	≈2X09[+]	TTCTTCAT
≈1X09[-]	TTCCTTCA	≈2X09[-]	ATGAAGAA
≈1X10[+]	TTGAGGAT	≈2X10[+]	TCTCCTCC
≈1X10[-]	ATCCTCAA	≈2X10[-]	GGAGGAGA

Primers

PCR Primer #	Sequence
≈0001[+]	/5Phos/GCCGCCCAGTCCTGCTCGCTTCGCTAC
≈0001[-]	/5Phos/CCATGTAGCGAAGCGAGCAGGACTGGGCGGCGG
≈0B01[+]	/5Phos/GCCTGTTTGCCCGCCAGTTGTTGTGCCAC
≈0B01[-]	/5AmMC6/GTGGCACAACAACTGGCGGGCAAAC
≈0B02[+]	/5Phos/GCCTCCCAAACNNNNNNNNGTTTGCCCGCCAGTTGTTGTGCCAC
≈0B02[-]	GTTTGGG

Table T2

Bead Type		Filter Microplate Column											
		1	2	3	4	5	6	7	8	9	10	11	12
1	OP1	≈1101[±]	≈1102[±]	≈1103[±]	≈1104[±]	≈1105[±]	≈1106[±]	≈1107[±]	≈1108[±]	≈1109[±]	≈1110[±]		
	OP2	≈2201[±]	≈2202[±]	≈2203[±]	≈2204[±]	≈2205[±]	≈2206[±]	≈2207[±]	≈2208[±]	≈2209[±]	≈2210[±]		
	OP3	≈1311[±]	≈1302[±]	≈1303[±]	≈1304[±]	≈1305[±]	≈1306[±]	≈1307[±]	≈1308[±]	≈1309[±]	≈1310[±]		
	OP4	≈2401[±]	≈2402[±]	≈2403[±]	≈2404[±]	≈2405[±]	≈2406[±]	≈2407[±]	≈2408[±]	≈2409[±]	≈2410[±]		
	OP5	≈1501[±]	≈1501[±]	≈1501[±]	≈1502[±]	≈1502[±]	≈1502[±]	≈1503[±]	≈1503[±]	≈1503[±]			
	OP6	≈2601[±]	≈2602[±]	≈2603[±]	≈2601[±]	≈2602[±]	≈2603[±]	≈2601[±]	≈2602[±]	≈2603[±]			
	OP7	≈1705[±]	≈1705[±]	≈1705[±]	≈1706[±]	≈1706[±]	≈1706[±]	≈1707[±]	≈1707[±]	≈1707[±]			
	OP8	≈2805[±]	≈2806[±]	≈2807[±]	≈2805[±]	≈2806[±]	≈2807[±]	≈2805[±]	≈2806[±]	≈2807[±]			
	OP9	≈1908[±]	≈1908[±]	≈1908[±]	≈1909[±]	≈1909[±]	≈1909[±]	≈1910[±]	≈1910[±]	≈1910[±]			
	OPA	≈2A08[±]	≈2A09[±]	≈2A10[±]	≈2A08[±]	≈2A09[±]	≈2A10[±]	≈2A08[±]	≈2A09[±]	≈2A10[±]			
2	OP1	≈1101[±]	≈1102[±]	≈1103[±]	≈1104[±]	≈1105[±]	≈1106[±]	≈1107[±]	≈1108[±]	≈1109[±]	≈1110[±]		
	OP2	≈2201[±]	≈2202[±]	≈2203[±]	≈2204[±]	≈2205[±]	≈2206[±]	≈2207[±]	≈2208[±]	≈2209[±]	≈2210[±]		
	OP3	≈1301[±]	≈1302[±]	≈1303[±]	≈1304[±]	≈1305[±]	≈1306[±]	≈1307[±]	≈1308[±]	≈1309[±]	≈1310[±]		
	OP4	≈2401[±]	≈2402[±]	≈2403[±]	≈2404[±]	≈2405[±]	≈2406[±]	≈2407[±]	≈2408[±]	≈2409[±]	≈2410[±]		
	OP5	≈1505[±]	≈1505[±]	≈1505[±]	≈1506[±]	≈1506[±]	≈1506[±]	≈1507[±]	≈1507[±]	≈1507[±]	≈1508[±]	≈1508[±]	≈1508[±]
	OP6	≈2605[±]	≈2606[±]	≈2607[±]	≈2605[±]	≈2606[±]	≈2607[±]	≈2605[±]	≈2606[±]	≈2607[±]	≈2605[±]	≈2606[±]	≈2607[±]
	OP7	≈1701[±]	≈1701[±]	≈1701[±]	≈1702[±]	≈1702[±]	≈1702[±]	≈1703[±]	≈1703[±]	≈1703[±]	≈1704[±]	≈1704[±]	≈1704[±]
	OP8	≈2808[±]	≈2809[±]	≈2810[±]	≈2808[±]	≈2809[±]	≈2810[±]	≈2808[±]	≈2809[±]	≈2810[±]	≈2808[±]	≈2809[±]	≈2810[±]
	OP9	≈1904[±]	≈1904[±]	≈1904[±]	≈1905[±]	≈1905[±]	≈1905[±]	≈1906[±]	≈1906[±]	≈1906[±]	≈1907[±]	≈1907[±]	≈1907[±]
	OPA	≈2A01[±]	≈2A02[±]	≈2A03[±]	≈2A01[±]	≈2A02[±]	≈2A03[±]	≈2A01[±]	≈2A02[±]	≈2A03[±]	≈2A01[±]	≈2A02[±]	≈2A03[±]

Figure S1. Time-dependent droplet fluorescence intensity histogram with UV photocleavage. Transient histogram plots visualize *hν*SABR assay performance over the course of the analysis. Droplets were loaded with model library, irradiated with UV light to cleave compound from the bead, incubated and detected by LIF for sorting. Droplet events (# droplets) are binned by droplet fluorescence (300 bins) in 1-min windows. Assay fluorescence emission (λ_{em} = 520 nm) is compared to a hit threshold (1000 counts, blue line) to trigger sort events. Internal standard (phychoerythrin) is introduced only from aqueous input AQ1, and its emission (λ_{em} = 570 nm) tracks AQ1:AQ2 flow consistency through the experiment. Out of the total droplets detected (532597 droplets), there were 1064 hit droplets with fluorescence under the inhibition threshold.

Figure S1

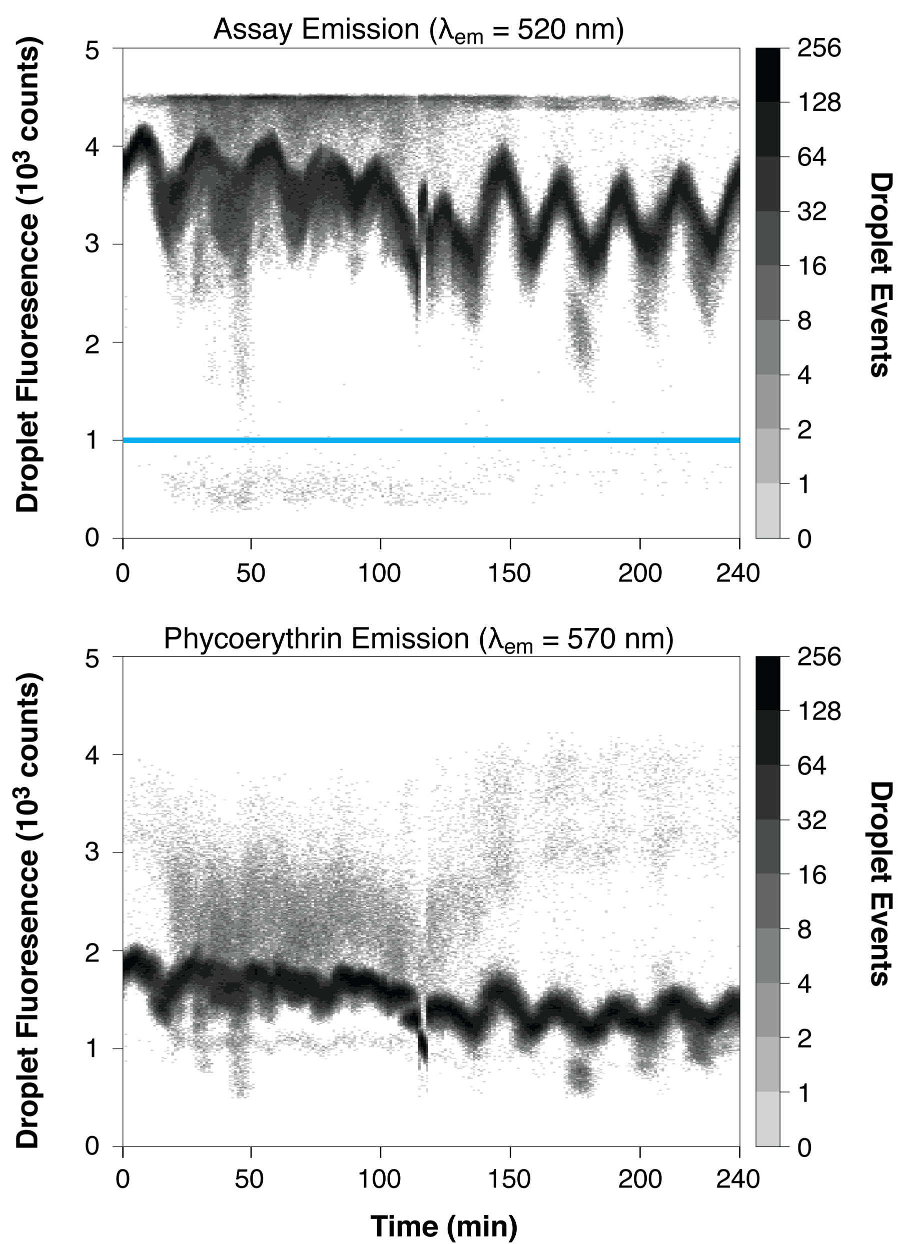

Figure S2. Time-dependent droplet fluorescence intensity histogram with no UV photocleavage. Transient histogram plots visualize *hv*SABR assay performance over the course of the analysis. Droplets were loaded with model library beads without UV irradiation to prevent compound cleavage from bead, incubated and detected by LIF for FADS. Droplet events (# droplets) are binned by droplet fluorescence (300 bins) in 1-min windows. Assay fluorescence emission (λ_{em} = 520 nm) is compared to a hit threshold (1000 counts, blue line) to trigger sort events. Internal standard (phychoerythrin) is introduced only from aqueous input AQ1, and its emission (λ_{em} = 570 nm) tracks AQ1:AQ2 flow consistency through the experiment. Out of the total droplets detected (596,998 droplets), no droplets were detected under the inhibition threshold.

Figure S2

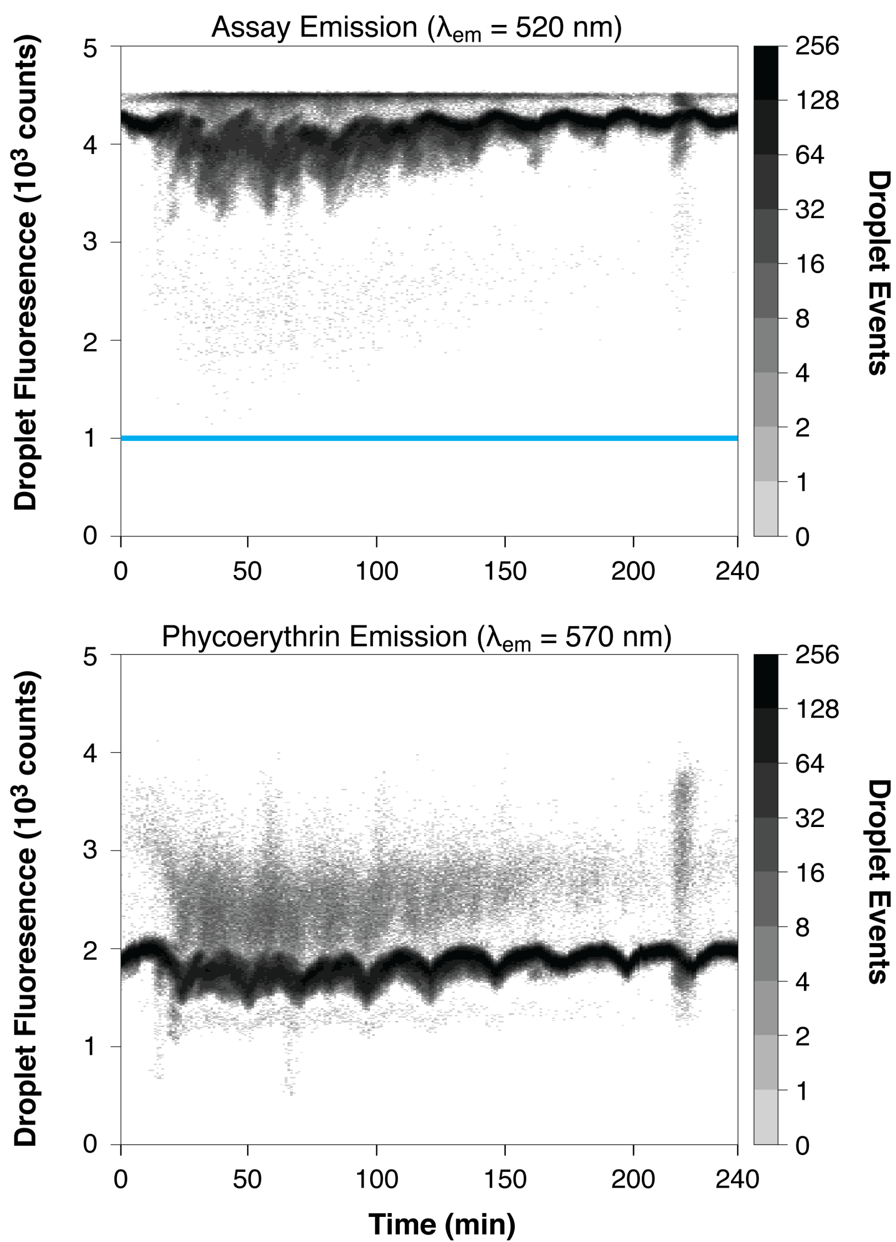

Figure S3. qPCR analysis of FACS counted bead lots. Calibration standards containing positive control bead **1** lots (30, 100, 300, 1000, 3000 beads) were prepared by FACS in triplicate, and analyzed by qPCR. Samples were quantitated using single-threshold Cq determination mode (400 RFU). Background-subtracted replicates were averaged and %RSD calculated for total DNA template (blue bars, %RSD) and PCR amplification efficiency (molecules/bead, orange circles).

Figure S3