Supplementary Information for

Electrochemiluminescence on Digital Microfluidics for microRNA Analysis

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Immobilization of probe sequence on magnetic particles.

See main text for materials and vendors. Magnetic particles were vortexed in their original vial, and then a 1 mL aliquot was transferred to an Eppendorf vial and exposed to a magnet for 1-2 minutes. When the particles were settled near the magnet, the supernatant was decanted. The particles were then washed in 1 mL of B&W (1x) buffer by re-suspending followed by separation of particles using the magnet. This procedure was repeated three times prior to immobilization. For probe sequence (ss-143) immobilization, the particles were first re-suspended in 2 mL B&W (2×) buffer at a density of 5 mg/mL, and then this suspension was mixed with a 2 mL aliquot of 1 µM ss-143 in the same vial. The mixture was allowed to incubate for 15 min at room temp with gentle rotation. The ss-143 modified particles were separated by placing the vial on a magnet for 2-3 min followed by separation from the supernatant solution. After separating the particles, the immobilization was confirmed by evaluating the supernatant using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA); the absence of an absorbance peak at 260 nm confirmed ss-143 immobilization. Finally, ss-143-modified beads were washed three times (as above), and then suspended in 2 mL hybridization buffer (at a density of 5 mg/mL or 3–3.5 x 10^5 beads/µL) and stored at 4^oC.

Fabrication of DMF Bottom-Plates.

Digital microfluidic device bottom-plates were fabricated in the Toronto Nanofabrication Centre (TNFC) cleanroom facility. Chromium-on-glass substrates (Telic Co., Santa Clarita, CA), coated with Parylene-C and Teflon-AF, were identical to those reported previously (Ng et al. 2012), featuring an array of 80 actuation electrodes (2.2 × 2.2 mm ea.), 4 reagent reservoir electrodes (16.4 × 6.7 mm ea.), and 4 waste reservoir electrodes (16.4 × 6.4 mm ea.).

Fabrication of DMF Top-Plates.

Digital microfluidic device top plates were fabricated in the Toronto Nanofabrication Centre (TNFC) cleanroom facility from indium tin oxide (ITO) coated glass substrates (Delta Technologies Ltd, Stillwater, MN) in three stages, using an adaptation of methods described previously (Shamsi et al. 2013). Briefly, seven isolated regions of ITO were created by photolithography and wet-etching, including six electrochemiluminescence (ECL) electrodes (four 800 μm diameter circular electrodes and two 560 x 560 μm square electrodes) and the seventh was the remainder of the irregularly shaped ITO, which serves as the ground-electrode for droplet actuation. Each ECL electrode was connected to a contact pad on the edge of the substrate. Next, a spin-coat/lift-off process [described previously (Eydelnant et al. 2012)] was used to apply a patterned coating of Teflon-AF to the patterned ITO surface. When complete, the surface had a global Teflon-AF coating punctuated with six apertures positioned over the ECL electrodes (matching the shapes and dimensions of the electrodes).

The square ECL electrodes, which served as counter/pseudoreference electrodes (CE/RE), were then modified by electroplating gold (from a 20 μL aliquot of 2 mM $KAuCl_4$) and then silver (from a 20 μL aliquot of 0.3 M $AgNO_3$ in 3 M NH_4OH). Both electrodepositions were performed at room temperature by applying –1.0 V potential for 20 s against an external platinum counter/reference electrode using an EmStat potentiostat (PalmSens BV, Utrecht, the Netherlands). The circular ITO ECL electrodes (which served as working electrodes, WE) were not modified.

Device assembly and operation.

Two-plate DMF devices were assembled with an ITO-glass top-plate and a chromium-glass bottom-plate using spacers of two pieces of Scotch double-sided tape (3M, St. Paul, MN)

with a total distance of 180 μm between two plates. Unit droplet volumes (covering one actuation electrode) on these devices were ~900 nL. Droplet movement and magnetic particle control were managed using a custom built instrument described in detail elsewhere (Choi et al. 2013). Briefly, the instrument includes a step-motor-controlled permanent magnet that is moved close to the DMF device (to enable magnetic particle separation from droplets) and away from the device (to enable particle suspension and manipulation in droplets). The instrument also includes a step-motor-controlled H10682-110 PMT with 5 V power supply (Hamamatsu Photonics, Hamamatsu City, Japan) which is moved close to the device (and shutter opened) when optical measurements are collected, and away from the device (and shutter closed) when droplets are manipulated. The instrument is housed in a light-tight aluminum enclosure, with a camera and LED source used to observe assay progress when the PMT is not engaged. All droplet movement, magnet movement, and PMT movement steps are pre-programmed and controlled using the open-source MicroDrop software described in detail elsewhere (Fobel et al. 2013).

Table S1. Oligonucleotide sequences.

ID	Sequence	Base length	Type
ss-143	Biotin5′-GAG-CTA-CAG-TGC-TTC-ATC-TCA-3′	21-mer	Probe
ds-143	3′-CTC-GAT-GTC-ACG-AAG-TAG-AGT-5′	21-mer	Complementary
143-m1	3′-CTC-GAT-GTC-ACG-AAG-TA**A**-AGT-5′	21-mer	Single mismatch
143-m2	3′-CTC-**A**AT-GTC-A**A**G-AAG-TAG-AGT-5′	21-mer	Double mismatch
nc-145	3′-T-CCC-TAA-GGA-CCC-TTT-TGA-CTC-G-5′	23-mer	Non-complementary

Supplementary Bibliography

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