SUPPLEMENTARY DATA

Supplementary Protocol S1: MDA in Shaken Emulsion Droplets

1. Immediately following preparation of 50 µL REPLI-g single cell reaction mixture (Qiagen, catalog no. 150343) in a PCR tube, add 50 µL of HFE-7500 fluorinated oil (3M, catalog no. 98-0212-2928-5) with 2% (w/w) PEG-perfluoropolyether amphiphilic block copolymer surfactant (RAN Biotechnologies, catalog no. 008-FluoroSurfactant-1G).
2. Hold PCR tube containing 100 µL combined mixture horizontally on a VWR Vortexer 2 (VWR, catalog no. 58816-123). Vortex for 10 seconds at 3000 rpm.
3. After vortexing hold PCR tube vertically. A white translucent emulsion should appear in the supernatant.
4. Incubate PCR tube for 16 hours at 30°C.
5. Following 16-hour incubation, heat PCR tube for 20 min at 70°C to inactivate Φ29 DNA polymerase.
6. Add 10 µL perfluoro-1-octanol (Sigma Aldrich, catalog no. 370533-5G) to supernatant. Pipet up and down vigorously and centrifuge briefly. This serves to destabilize the surfactant, thus coalescing the droplets.
7. Extract supernatant from PCR tube. DO NOT extract any of the oil phase.
8. Clean DNA using a DNA Clean and Concentrator -5 (Zymo Research, catalog no. D4004). Elute in 10 µL H_2O.

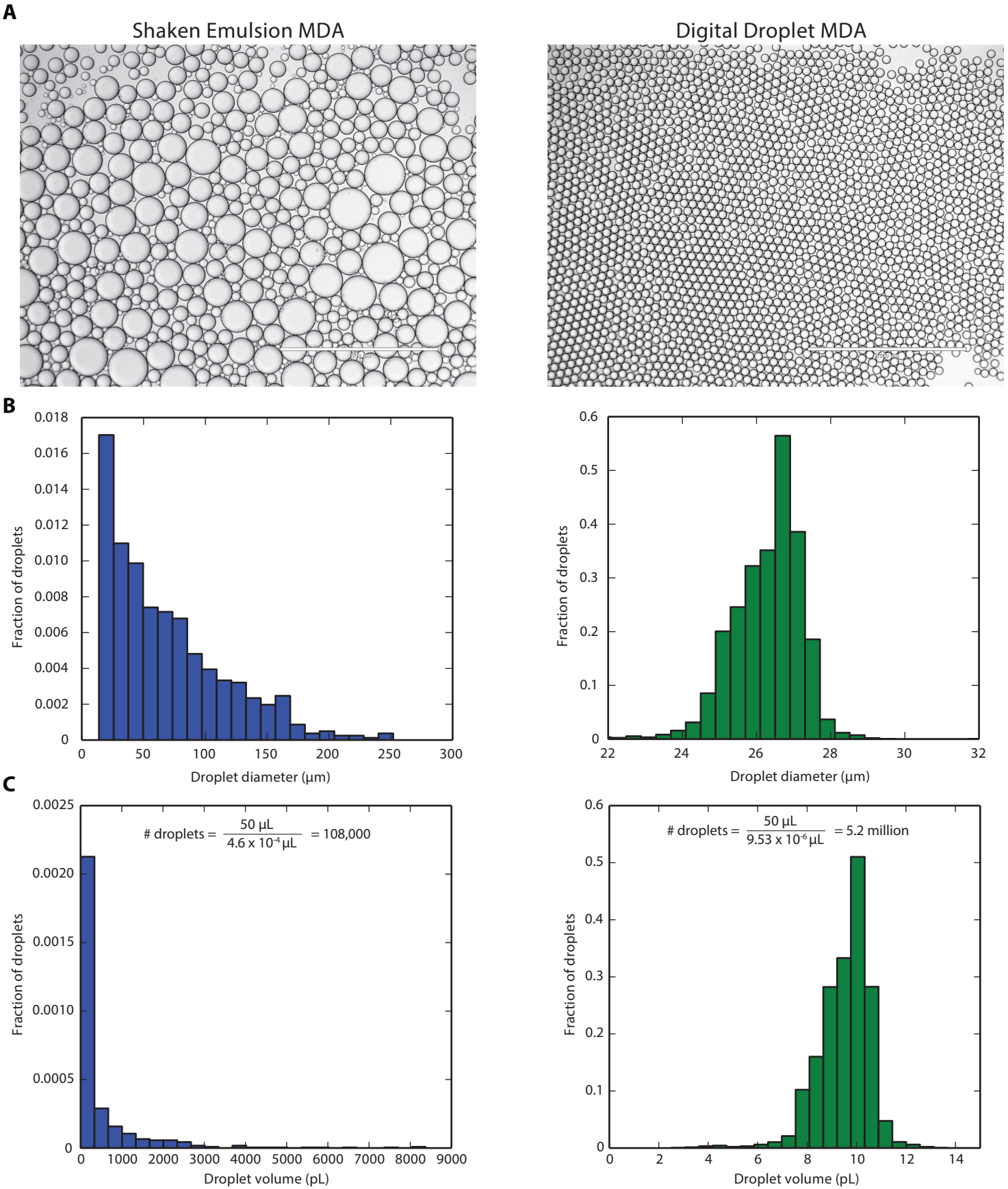
Supplementary Protocol S2: ddMDA in Monodisperse Microfluidic Emulsion Droplets

Fabricating PDMS Devices

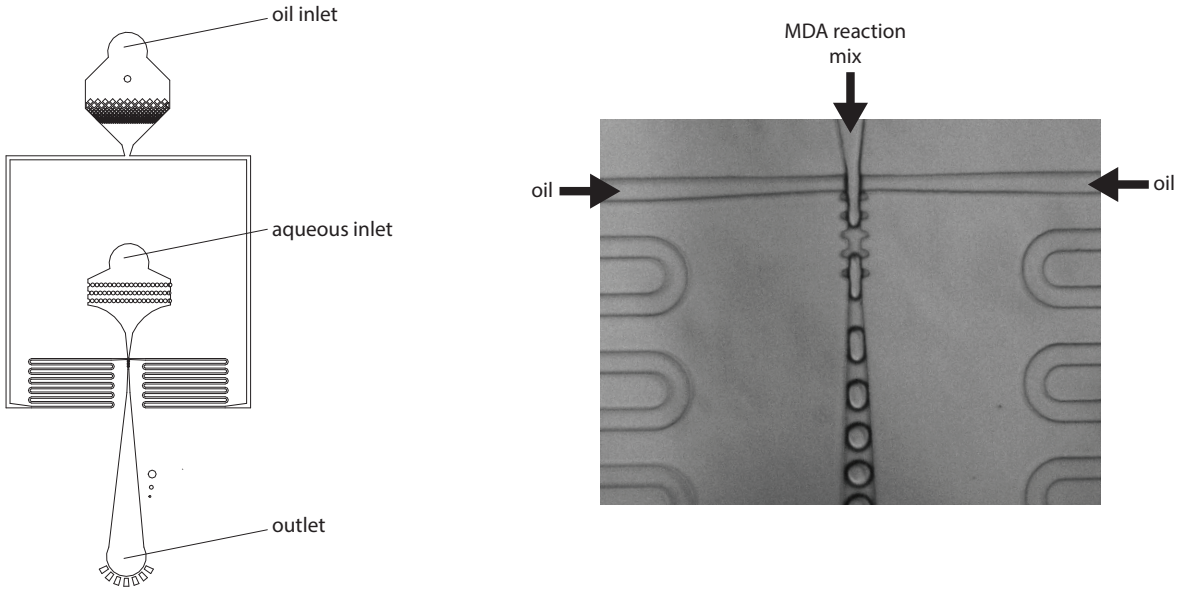
1. Create a device master by spin-coating a 20 µm-thick layer of photoresist (SU-8 3025, Microchem) onto a silicon wafer, followed by patterned UV exposure and resist development (1).
2. Combine 4 grams of Sylgard 184 Silicone Elastomer curing agent with 42 grams of Sylgard 184 Silicone Elastomer base (Dow Corning) in a plastic cup.
3. Use an electric mixer to mix curing agent and base until mixture is white and bubbly.
4. De-gas mixture by placing in a vacuum chamber for 20 min.
5. Pour 30 grams of newly formed PDMS over previously made photolithographically patterned layer of photoresist on silicon wafer.
6. Cure PDMS by placing in an 80°C oven for 3 hr.
7. Use a scalpel to cut out area of cured PDMS patterned by photoresist.
8. Use a 0.75 mm biopsy core (World Precision Instruments, catalog no. 504529) to punch holes in the inlet and outlet ports of the device (denoted in Supplementary Figure S2A).
9. Wash device with isopropanol and air-dry.
10. Bond device to a glass slide following a 30-second treatment of 1 mbar O_2 plasma in a 300 W plasma cleaner. Devices can also be bonded to tape (2).
11. Place bonded device in 80 °C oven for 30 min.
12. Using a syringe pre-loaded with Aquapel (PPG Industries) and connected to polyethylene micro tubing (Scientific Commodities, catalog no. BB31695-PE/2), flush all channels of device to make them hydrophobic.
13. Place flushed device in 80°C oven for an additional 10 min.
14. Carefully inspect device using a microscope for presence of non-bonded or obstructed channels.

Generating Monodisperse Droplets

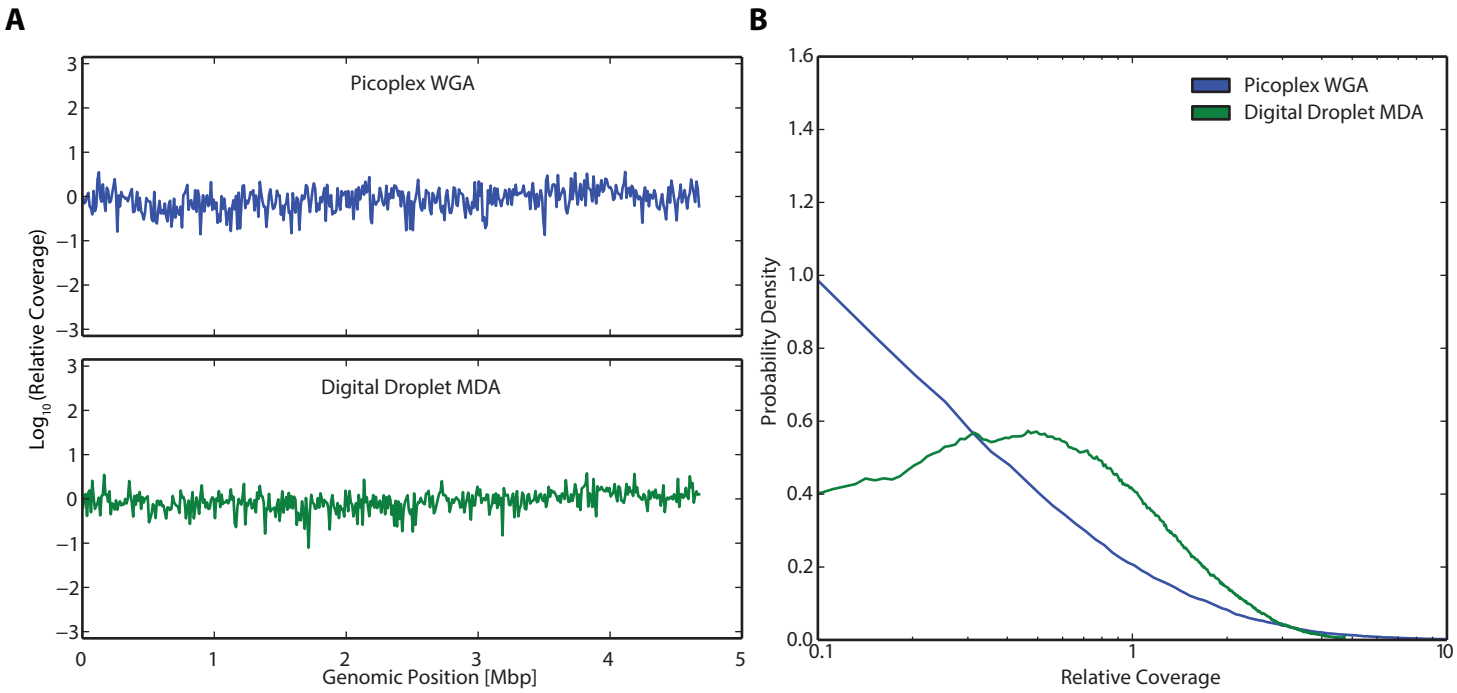
1. UV-treat the following for 30 min: polyethylene micro tubing, two 1 mL syringes, previously prepared microfluidic device, one PCR tube.
2. Pre-load one UV-treated syringe with at least 200 µL HFE-7500 fluorinated oil with 2% (w/w) PEG-perfluoropolyether amphiphilic block copolymer surfactant.
3. Pre-load second UV-treated syringe with 50 µL REPLI-g single cell reaction mixture back-filled with at least 200µL HFE-7500 fluorinated oil to prevent bottoming out of syringe.
4. Attach 8 inches of polyethylene micro tubing to syringe needles.
5. Place syringes in two syringe pumps (New Era, catalog no. NE-501) connected to a computer controlled with a custom pump control program (https://github.com/AbateLab/Pump-Control-Program).
6. Prime both syringes using the prime function in the pump control program.
7. Attach polyethylene micro tubing connected to oil syringe to the "oil inlet" denoted in Supplementary Figure S2A.
8. Attach polyethylene micro tubing connected to syringe with REPLI-g single cell reaction mixture to the "aqueous inlet" denoted in Supplementary Figure S2A.
9. Attach one 4-inch piece of tubing to device outlet denoted in Supplementary Figure S2A. Empty tubing into UV-treated PCR tube.
10. Set flow rate of syringe with REPLI-g single cell reaction mixture at 300 µL/hour and flow rate of oil syringe at 500 µL/hour.
11. Start flow program. Use a microscope to watch the formation of drops at the interface between oil and aqueous channels (see Supplementary Figure S2B).
12. Observe flow of droplets into outlet, through polyethylene micro tubing, and into PCR tube. Stop pump control program once entirety of 50 µL reaction mixture has been converted into droplets.
13. Incubate PCR tube for 16 hours at 30°C.
14. Following 16-hour incubation, heat PCR tube for 20 min at 70°C to inactivate Φ29 DNA polymerase.
15. Add 10 µL perfluoro-1-octanol to supernatant. Pipet up and down vigorously and centrifuge briefly. This serves to destabilize the surfactant, thus coalescing the droplets.
16. Extract supernatant from PCR tube. DO NOT extract any of the oil phase.
17. Clean DNA using a DNA Clean and Concentrator -5. Elute in 10 µL H_2O.



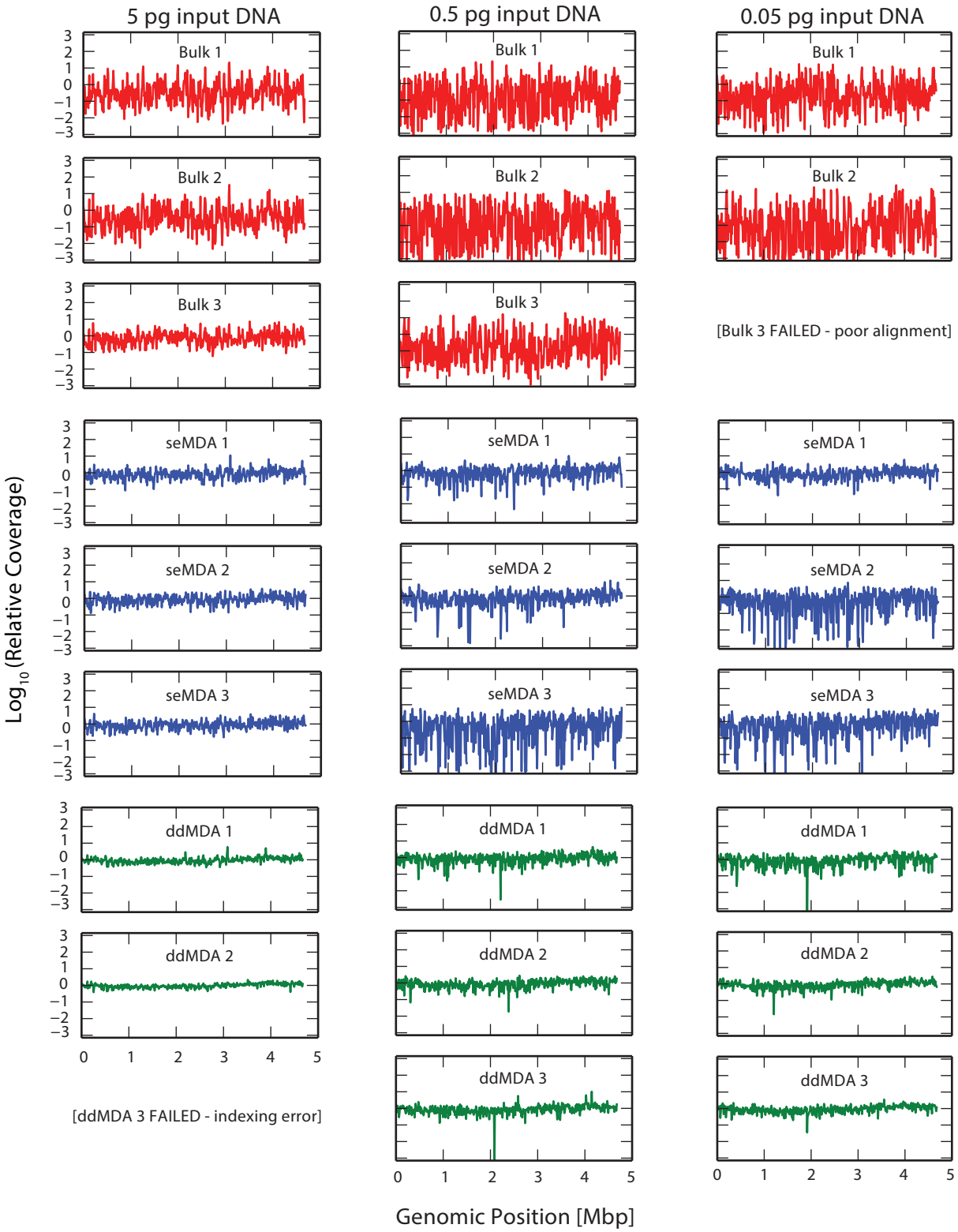
Supplementary Figure S1: Comparison of droplet size distribution between shaken emulsion MDA and ddMDA. (**A**) Bright field microscopy images of representative shaken emulsion and ddMDA reactions. Scale bar represented is 1000 μm. (**B**) Normalized diameter distribution of droplets measured in micrometers. (**C**) Normalized volume distribution of droplets measured in picoliters. For each method, an average drop size was calculated and a total drop number estimate was performed.



Supplementary Figure S2: Illustration and microscopy image of the microfluidic device used in this work. The device channels are 20 µm tall. The REPLI-g single cell reaction mixture enters through the aqueous inlet at 300 µL/hr and intersects with two channels containing HFE-7500 fluorinated oil with 2% (w/w) PEG-perfluoropolyether amphiphilic block copolymer surfactant entering from the oil inlet at 500 µL/hr. The intersection of these channels results in the production of monodisperse droplets, which are then exported through the outlet into a PCR tube.



Supplementary Figure S3: Comparison between PicoPLEX WGA and ddMDA. **(A)** Relative coverage as a function of genome position of 0.5 pg E. coli DNA amplified using the PicoPLEX WGA kit, compared to 0.5pg E. coli DNA amplified using ddMDA (replicated from Figure 3). Data points were consolidated into 10 kb bins. **(B)** Probability density as a function of relative coverage for PiocPLEX WGA and ddMDA. PicoPLEX WGA appears to have a greater proportion of bases with minimal coverage compared to ddMDA.



Supplementary Figure S4: Relative coverage of standard bulk MDA (red), shaken emulsion MDA (blue), and digital droplet MDA (green) of E. coli DNA amplified from 5 picograms (~1000 E. coli genomes), 0.5 picograms (~100 E. coli genomes), and 0.05 picograms (~10 E. coli genomes). Data points were consolidated into 10 kb bins. Two samples were excluded from the analysis: bulk MDA 3 had less than 5% of sequenced DNA aligned to the E. coli genome, while ddMDA 3 was not indexed properly and thus did not yield any sequencing data.

DROPOUT METRIC: $$\frac{\#\text{ of bases } < 0.10(\text{mean coverage of all reference bases})}{\text{genome length}}$$

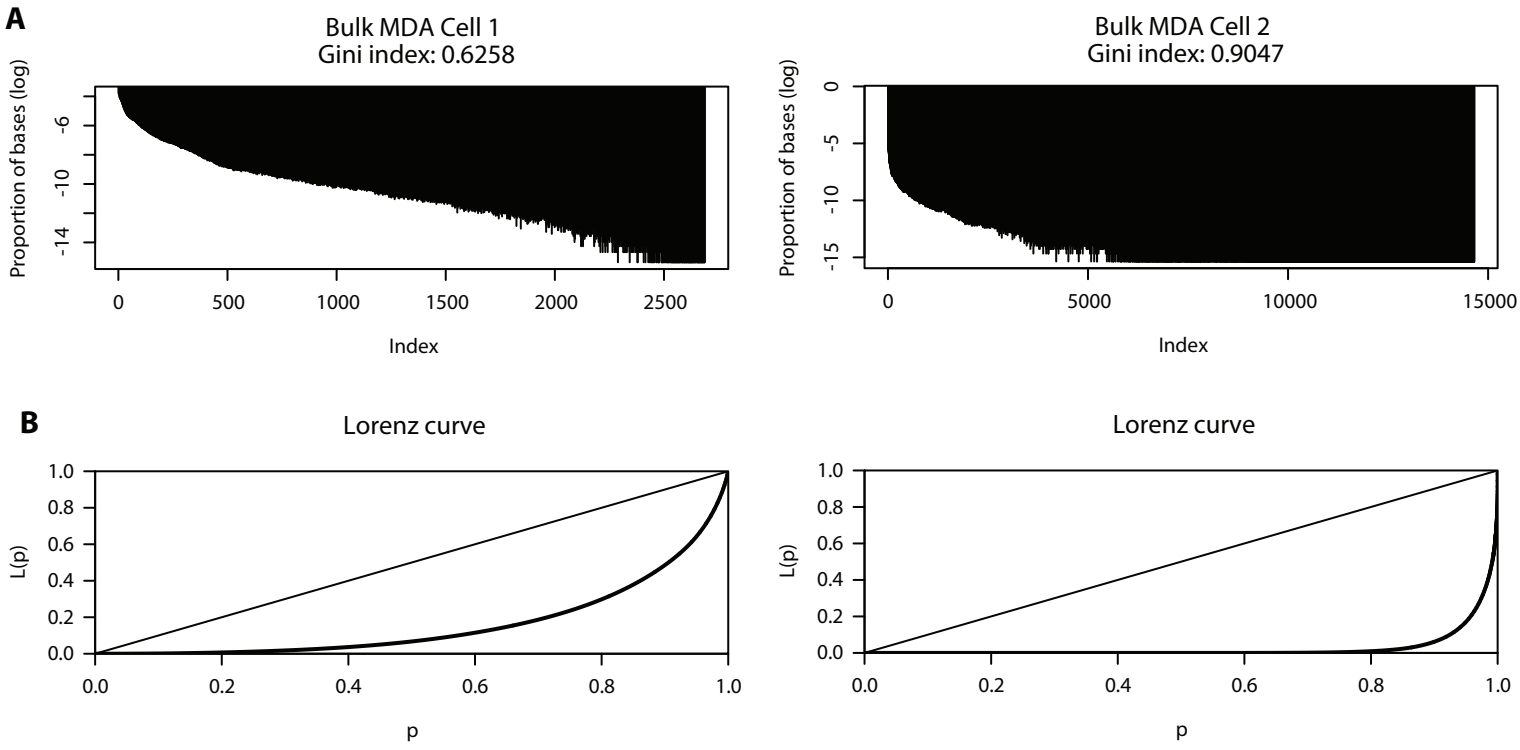
COVERAGE SPREAD: $$\sqrt{\frac{\sum[(RC_1)^2 + (RC_2)^2 + \cdots + (RC_{\text{genome length}})^2]}{\text{genome length}}}$$

ENTROPY: $$\sum_{i=1}^{1000} p_i \log_2 p_i$$

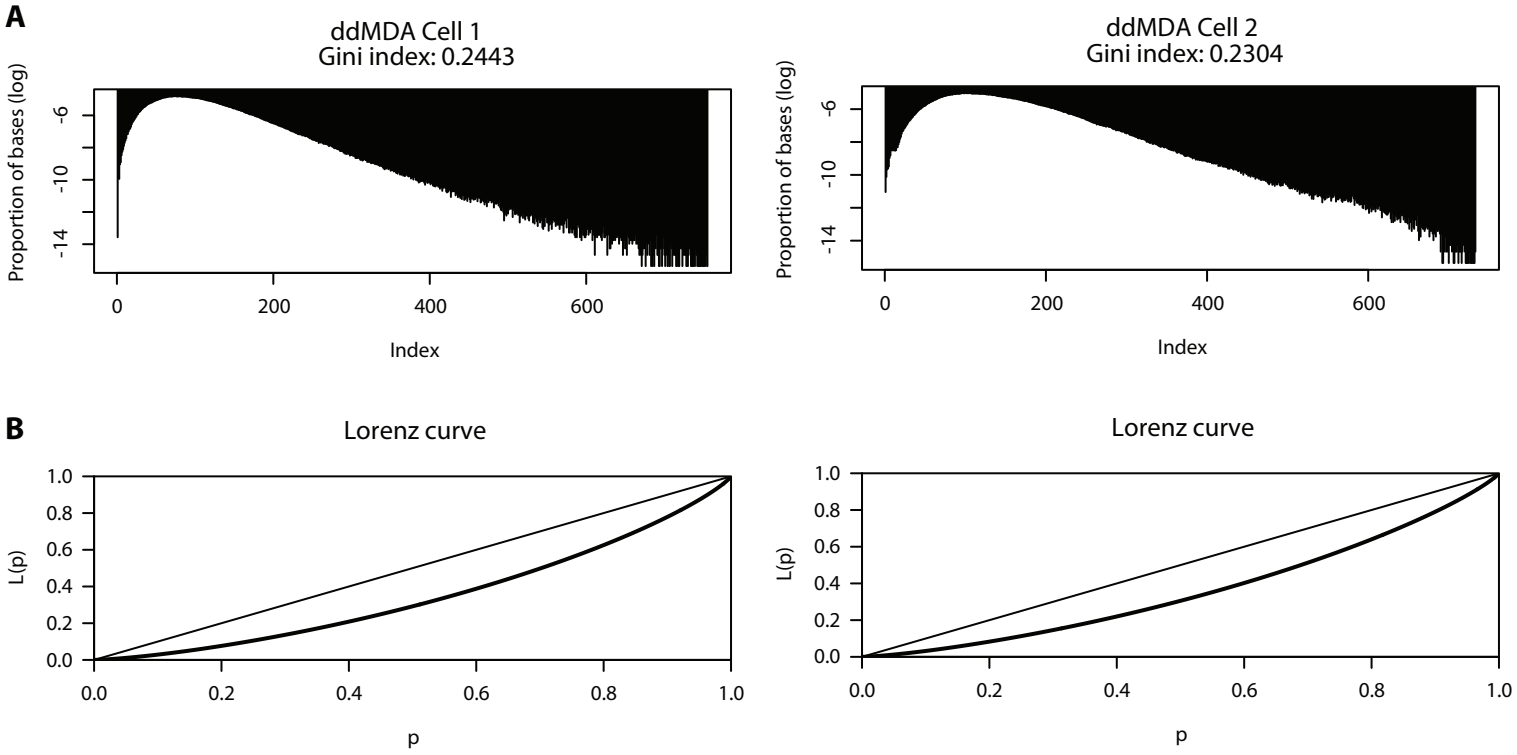
VARIABLES:

- $RC = \text{relative coverage} = \frac{\text{coverage of a given reference base}}{\text{mean coverage of all reference bases}}$ (3)
- p_i = probability of observing coverage of a given reference base in given bin

Supplementary Figure S5: Equations for the definitions described in Figure 4. Dropout metric represents the fraction of bases that are covered less than 10% of the mean coverage. Coverage spread is defined as the root mean square of the relative coverage. Informational entropy is defined as a sum of the product of the given probability with its base-2 logarithm.



Supplementary Figure S6: Statistical measures of coverage uniformity for two bulk MDA single-cell sequencing runs, calculated using htSeqTools (4). **(A)** Log probability mass functions of coverage for both samples. The Gini index, used to assess coverage unevenness, is also calculated. **(B)** Lorenz curves for both samples. The Lorenz curve shows the cumulative fraction of reads as a function of the cumulative fraction of the genome. Perfectly uniform coverage is represented by the diagonal line.



Supplementary Figure S7: Statistical measures of coverage uniformity for two ddMDA single-cell sequencing runs, calculated using htSeqTools (4). **(A)** Log probability mass functions of coverage for both samples. The Gini index, used to assess coverage unevenness, is also calculated. **(B)** Lorenz curves for both samples. The Lorenz curve shows the cumulative fraction of reads as a function of the cumulative fraction of the genome. Perfectly uniform coverage is represented by the diagonal line.

Supplementary References

1. Xia,Y. and Whitesides,G.M. (1998) Soft Lithography. *Annu. Rev. Mater. Sci.*, **28**, 153–184.

2. Thompson,C.S. and Abate,A.R. (2013) Adhesive-based bonding technique for PDMS microfluidic devices. *Lab Chip*, **13**, 632–5.

3. Ross,M.G., Russ,C., Costello,M., Hollinger,A., Lennon,N.J., Hegarty,R., Nusbaum,C. and Jaffe,D.B. (2013) Characterizing and measuring bias in sequence data. Genome Biol., 14, R51.

4. Planet E, Stephan-Otto C, Reina O, Flores O and Rossell D (2015). htSeqTools: Quality Control, Visualization and Processing for High-Throughput Sequencing data. R package version 1.16.0.