Electronic Supplementary Material (ESI) for Lab on a Chip
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Supporting Information

To

Microfluidic Extraction, Stretching and Analysis of Human Chromosomal DNA from Single Cells

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Single Cell Real-Time PCR Analysis

Real-Time PCR was used to quantify DNA extracted from a single cell. Single cell genomic DNA was extracted as described in *Materials and Methods* in the main text. Standards were prepared from a batch sample of M0-91 cells. Cells were lysed using 1% SDS and proteinase K digestion. The sample was purified by phenol-chloroform extraction and quantified using the PicoGreen assay. Bulk DNA was diluted to 9.9, 6.6 and 3.3 pg in a total volume of 10 μL. A single cell whole genome amplification kit (Single Cell WGA kit, NEB, cat # E2620S/L) was used to amplify standards and single cell extractions and the reaction was monitored by SYBR Green I (Invitrogen) fluorescence (Figure S1). The crossing point (C_P) was determined by calculating the maximum in the second derivative on the amplification curve. Samples amplified using the WGA kit tend to show very early amplification on the real-time PCR instrument, making it difficult to obtain a good baseline to calculate a crossing point value. To delay the measured C_P value, samples were diluted two-fold prior to the amplification reaction. Although sample dilution delayed the C_P value of the single cell DNA samples and standards, it did little for the negative control sample (NTC: No Template Control, $C_P = \sim 14$). Crossing point values were plotted against their corresponding mass of M0-91 DNA and linear regression analysis performed. The mass obtained from the microfluidic single cell extraction was calculated from the fit results to be (8 ± 1) pg (Figure S2).

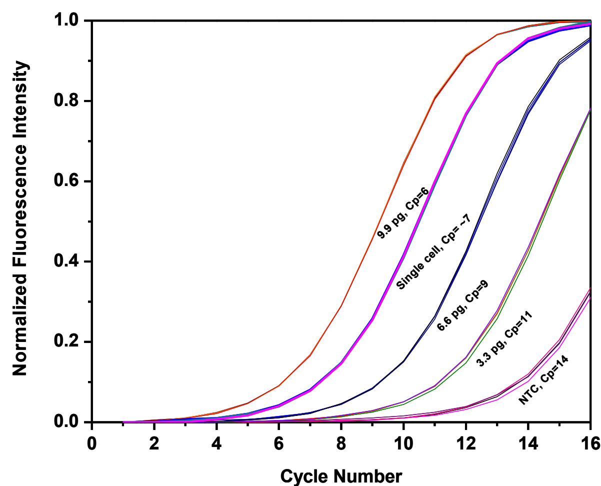

Figure S1. Real-Time PCR amplification curves.

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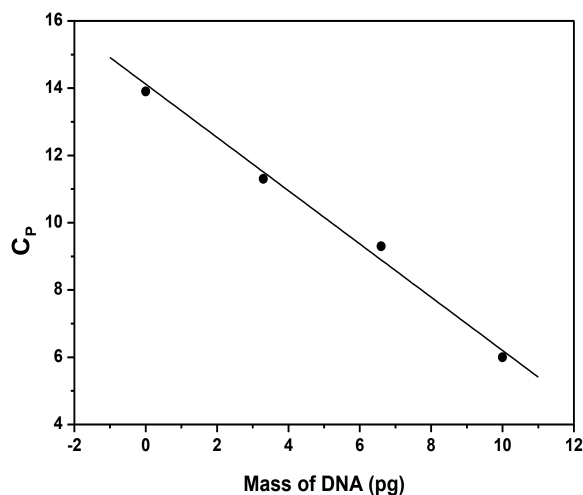
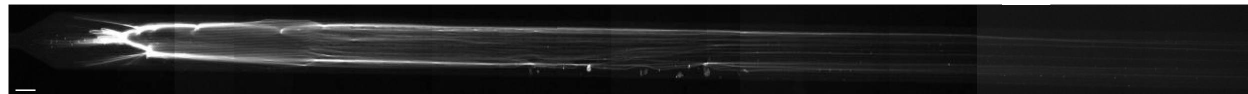


Figure S2. Linear regression fit to Cp vs mass data. Slope: – (0.8 ± 0.1) C_p/pg. Intercept: (14.1 ± 0.8) C_p. Single Cell C_P = 7.4 corresponding to (8 ± 1) pg, R^2 = 0.988.

Stretching Genomic DNA from a small population of cells

A small population of cells (~12) was trapped and lysed in the microfluidic pillar array. Stretched DNA strands were labeled with PicoGreen reagent and its resulting fluorescence imaged. Figure S3 shows a composite of many sections of the micofluidic channel with PicoGreen labeled DNA. A small population of cells was used to increase the fluorescence signal and facilitate imaging. Figure S3 A shows the first 11 mm of channel length. DNA strands can be observed up to ~20 mm into the channel (Figure S3 B).

A.



B.

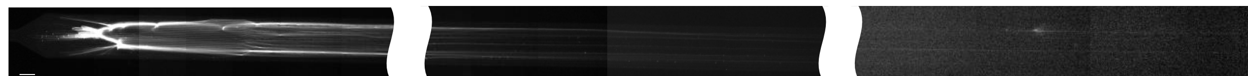


Figure S3. A. Fluorescence micrograph of chromosomal DNA extracted from 12 cells, labeled with PicoGreen fluorescent stain. The image spans approximately 11 mm. B. Fluorescence micrograph of the same 12 cells as in A, viewed further down the channel showing the DNA stretched up to ~20 mm into the channel. Scale bars represent 200 µm.