Supplementary Information

Microfluidic long DNA sample preparation from cells

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Diffusion Characterization

The diffusion time for cell lysis and DNA purification was estimated by scaling up the results for fluorescein (MW = 332 g/mol, $D = 4.25 \times 10^{-10}$ m^2/s in water at 25 °C)[?] migration across MCF-7 cells embedded in 0.2 wt% agarose in the device using the diffusion model, given by

$$t \approx \frac{y^2}{D} \tag{1}$$

From the experimental image at t = 10 min (Fig. S1), it can be seen that fluorescein radially diffuses 1.5 mm to the bottom channel through the gel. To reach the reservoirs, the molecule needs to migrate 3 mm in either direction in the gel. However, during cell lysis and DNA purification, both fluid channels are filled with the reagent of interest, and the migration distance is reduced by a factor of 2. The biggest molecule in the lysis solution is Proteinase K (MW = 18.5 kDa[?]). The diffusivity of a larger 40 kDa growth factor in less porous Type I collagen at 37 °C is $D = 5 \times 10^{-11}$ m^2/s.[?] By correcting for the difference in diffusivity, migration length and boundary conditions, we obtain a lysis time of <90 minutes. Based on our conservative estimate, the lysis time is chosen to be 1.5 hours to ensure complete protein digestion in the entire gel. It is assumed that peptide sizes after protease digestion are similar to fluorescein, since Proteinase K cleaves human histone H3.1 (MW = 15404 g/mol) at 67 sites, with the largest peptide mass being 828 Da (analyzed by PeptideCutter tool from UniProt).[?] The digested proteins and cellular debris are quickly eliminated from the gel. However, to completely remove Proteinase K from the gel, the washing time is also selected as 1.5 hours.

Concentration measurements

The DNA and protein concentration in the output of our device was measured by fluorometry in the Qubit fluorometer (Table S2). The readings were scaled based on the measurements of the control solution (Table S1). Based on the known control solution concentration, the DNA scaling factor is 2.76 and the protein scaling factor is 3.16. The device DNA:protein ratio was compared with the ratio for DNA prepared by conventional plug lysis at a DNA concentration of 2.2 ng/μL.

Extraction Efficiency

To calculate the percentage recovery from the device, we compared the amount of DNA recovered with the input DNA amount in terms of the total number of cells in the active gel region of the device. The cells were loaded at a seed density of 1500 cells/μL. The volume of the gel channel is 6 mm × 1 mm × 0.1 mm. The electric field in the 5 mm deep reservoir is very weak, and we assume that only the DNA from the reservoir cells that are in the same z-plane as the gel channel (100 μm depth) experience significant electric field. The active volume of the two reservoirs is $2 \times \pi \times 1$ mm^2 × 0.1 mm. This gives 1842 diploid human cells corresponding to 12.34 ng DNA. The percentage recovery from the device is thus 81.4%.

Notes on Supplemental Video

Video1 is a 5× faster than the real-time movie of DNA extraction out of the agarose. The electrophoresis pulsing is done as 10 V for 18 s followed by 0 V for 2 s. The movie captures close to three full periods of the pulsing. The ROI is 322 μm × 322 μm.

Notes on Supplemental Molecular Combing files

The molecular combing image files are available from the University of Minnesota Digital Conservancy at:
http://hdl.handle.net/11299/200704
In the zip file, MC1 - MC11 are stitched jpeg images that span several fields of view across different combing microchannels, showcasing DNA extracted from the device. While MC1 - MC10 are DNA extracted from blind experiments, MC11 has combed DNA from an extraction experiment with visualization. Individual images were captured with a 100x oil immersion objective, giving a pixel conversion ratio of 15.86 px/μm. The combing calibration factor is 3.13 kbp/μm. The files are named as 'MCX_aaaaa.jpg', where 'X' goes from 1 to 11 and 'aaaaa' is the number of horizontal pixels spanned by each stitched image.

Table. S1 Qubit readings of DNA and protein concentration (ng/μL) of the control solution containing YOYO-labeled λ DNA at a concentration of 16.67 ng/μL and human histone H4 at a concentration of 16.67 ng/μL

Concentration (ng/μL)	Run 1 (DNA)	Run 2 (DNA)	DNA	Run 1 (Protein)	Run 2 (Protein)	Protein
Reading 1	42	57.2		53.1	52.9	
Reading 2	41.1	55.2		52.8	52.3	
Reading 3	33.9	46.9		52.7	52.5	
Average	39	53.01	46.01	52.9	52.6	52.75

Table. S2 Qubit readings of DNA and protein concentration (ng/μL) of samples prepared in the device and by conventional plug lysis

Concentration (ng/μL)	Device 1 (DNA)	Device 2 (DNA)	Device 3 (Protein)	Device 4 (Protein)	Plug (Protein)
Reading 1	1.87	1.81	35.9	23.7	44.3
Reading 2	1.85	1.88	34.9	23.5	43.7
Reading 3	1.84	1.89	34.7	21.6	43.1
Average	1.85	1.86	35.2	22.9	43.7
Scaled Average	0.67	0.67	11.14	7.25	13.83

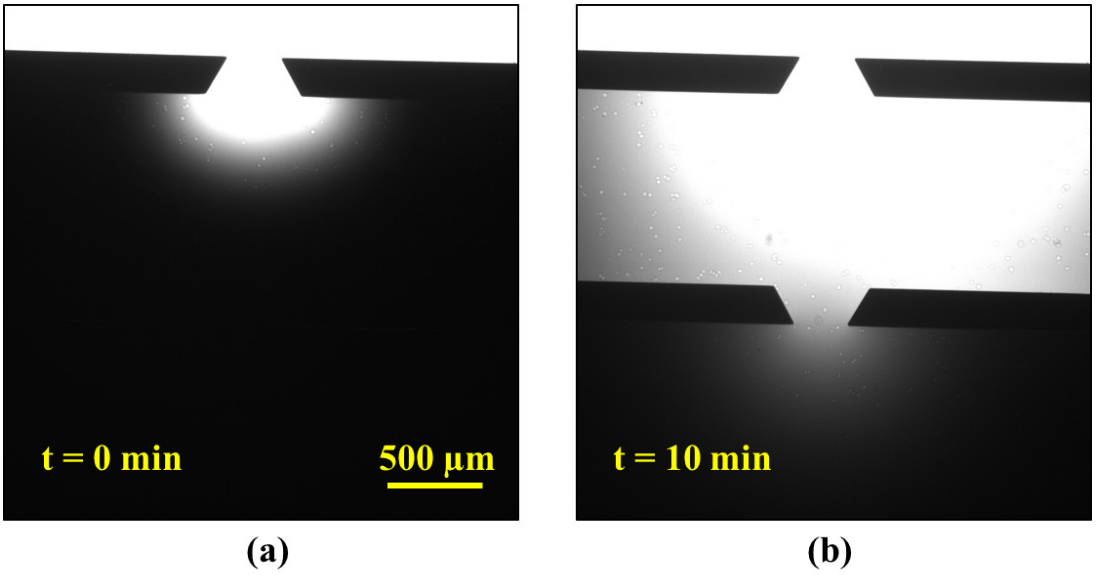

Fig. S1 Diffusion characterization experiment to study migration of fluorescein from the top fluid channel through the 0.2 wt% gel. The two trapezoids, 1 mm wide gel channel and 1.5 mm wide bottom fluid channel are filled with MCF-7 cells encapsulated in agarose. Diffusivity of fluorescein in water at 25 °C is 4.25×10^{-10} m^2/s.

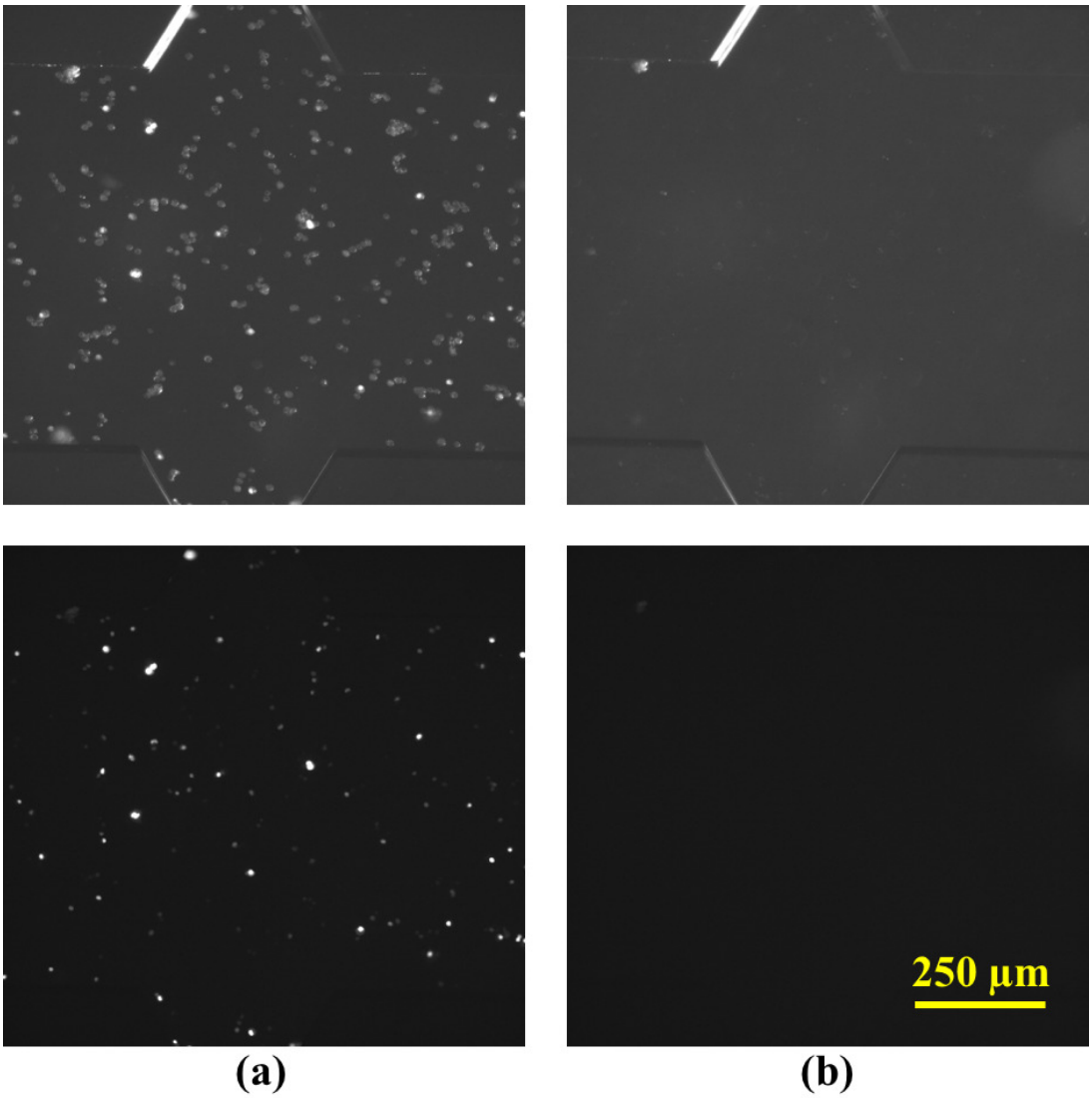

Fig. S2 Verification of cell lysis and histone digestion. (a) Transfected cells with GFP-labeled histones before cell lysis. Top bright-field image shows intact cells and bottom fluorescent image highlights intact histones. (b) Images of the gel after the 1.5 hour lysis step using the lysis solution without including YOYO. Top bright-field image shows complete lysis of cells and bottom fluorescent image shows complete digestion of histones.

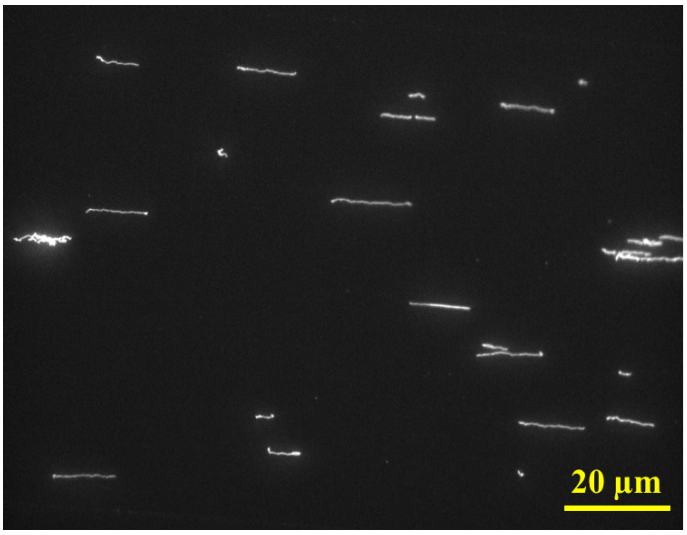

Fig. S3 Molecular combing of λ DNA (48.5 kbp) to calibrate pixel length to base content. Calibration factor is 3.13 kbp/µm.

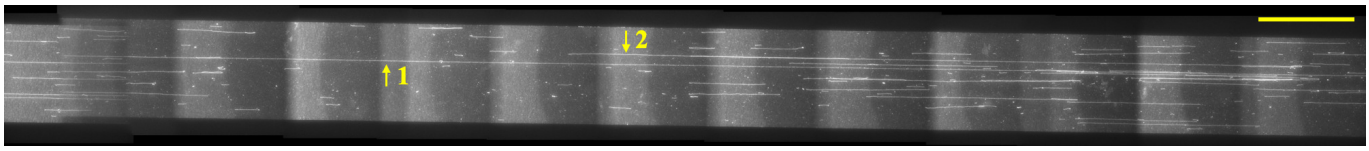

Fig. S4 Molecular combing of DNA recovered from the device during a blind extraction run. Molecule 1 is 4070 kbp long and molecule 2 is 2470 kbp long. The stitched image covers 13 ROIs of an Andor Zyla camera at 100x magnification. Scale bar represents 300 kbp or 96 µm.

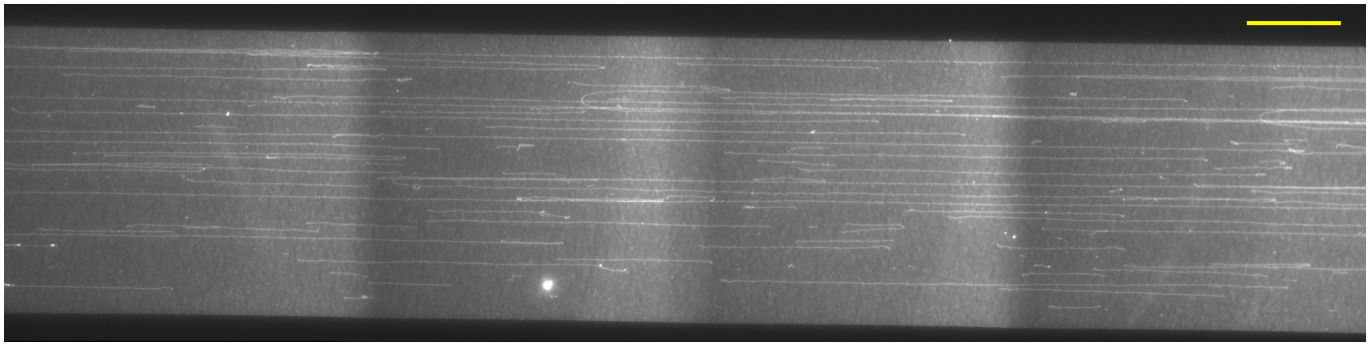

Fig. S5 Molecular combing of DNA recovered from the device during blind extraction at a pulsed 10 V applied potential. Scale bar represents 100 kbp or 32 µm.