Supplementary Information: Fast size-determination of intact bacterial plasmids using nanofluidic channels

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Materials and Methods

Nanofluidic chips

Nanofluidic chips were fabricated from a silicon wafer with 2 μm thermal oxide in clean-room facilities at Chalmers University of Technology. Nanochannels were created by electron-beam lithography (JBX-9300FS/JEOL Ltd) and reactive ion etching (Plasmalab 100 ICP180/Oxford Plasma Technology). Microchannels were made by optical lithography (MA 6/Suss MicroTec) and subsequent reactive-ion etching. Inlets were created by optical lithography and deep reactive-ion etching (STS ICP/STS). The wafer was sealed with a 175 μm thick Pyrex lid through fusion bonding (AWF 12/65/Lenton) and diced into 25x25 mm^2 chips (DAD3350/Disco). The methods are further described in reference [1].

Plasmid preparation

The total plasmid content from clinical bacterial isolates 008, 033, 071, 163 and 299 was extracted using Qiagen Plasmid Midi Kit (Qiagen, GmbH, Hilden) from overnight bacterial cultures grown in LB-broth. Plasmid R100 was extracted using Qiagen Plasmid Mini Kit (Qiagen, GmbH, Hilden). All plasmid DNA was eluated and stored in Tris buffer (10 mM Tris HCl, pH 8.5) at -20 °C until used in experiment.

Experimental setup

The extracted plasmid DNA was stained with the fluorescent dye YOYO-1 (Invitrogen) at a staining ratio of 1 dye per 10 basepairs and initially mixed at high ionic strength (5xTBE, Tris-Borate-EDTA, Medicago, diluted with mQ from 10x tablets) for fast equilibration of YOYO on DNA [2]. An oxygen scavenger, beta-mercaptoethanol (BME, Sigma, Aldrich), was added (3 %, v/v) to reduce photonicking. After about 20 minutes at room temperature, the sample was diluted with mQ to 0.2x TBE buffer to a final DNA concentration of 0.4 μM (bp) before it was introduced into a nanofluidic chip. The chip was pre-wetted with 0.2x TBE buffer with 3 % BME (v/v). The design of the chip, schematically depicted in Fig. 1A in the paper, consists of a set of nanochannels (150x150 nm^2) connected by microchannels (50x1 μm^2) to sample loading reservoirs. The sample was loaded into one of the reservoirs and directed, by pressure-driven flow (nitrogen gas), through the microchannel to the nanochannel

system. A large number of DNA-molecules were forced into the nanochannels simultaneously to enable visualization of several single molecules in one field of view. The plasmid samples were imaged using an epi-fluorescence microscope (Zeiss AxioObserver.Z1) equipped with a high quantum yield EMCCD camera (Photometrics Evolve) and a 100x oil immersion objective with high numerical aperture (NA 1.46) from Zeiss. Stacks of 200 images were recorded for each field of view at an approximate rate of 7 frames per second, using an exposure time of 100 ms.

Data analysis

For data analysis, a custom-written MatLab-based program was used. The stacks of images for each molecule were converted into kymographs (time traces) and the "instantaneous" extensions of the molecule at different times obtained by fitting the difference of two sigmoidal functions to each time frame, *i.e.*, to a functional form (Equation 1):

$$I(x) = a + f[\tanh((x-b)d) - \tanh((x-c)e)] \quad (1)$$

Here, a represents the mean background intensity and $f+a$ is the maximum intensity. The end positions are b and c respectively, and the sigmoidal shapes are determined by d and e. From the fits to the function above, the "instantaneous" extension, L, is obtained as $L = c - b$. The extension is obtained as the mean of the instantaneous extensions over 200 images, and the fluctuation of the molecule length given by the standard deviation of the "instantaneous" extensions. In Fig. S1 we show a representative kymograph (from isolate 163), where the marked ends were obtained using the method described above.

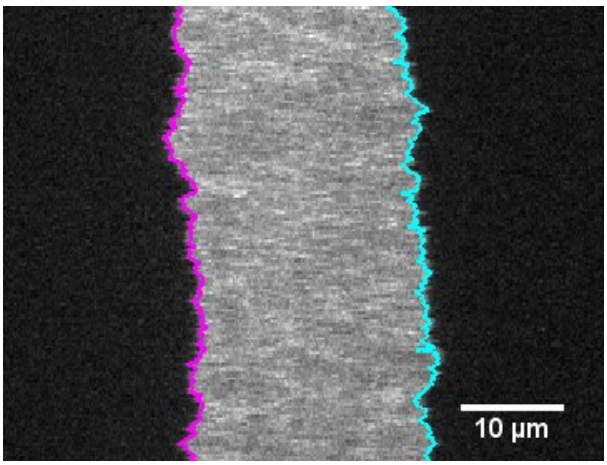

Figure S1: *Illustration of the result of the end-detection method used in this study: to each time frame we fit the difference of two sigmoidal functions. The mid-points of these functions are identified as molecule ends (cyan- and magenta-colored marks).*

Repeated experiments

Table S1 summarizes the data from nanofluidics measurements with two independent measurements of each isolate. As a comparison, the plasmid sizes obtained from S1/PFGE experiments are shown.[3] The actual S1/PFGE gel images can be found in Reference 3.

Table S1. Detected plasmids and their sizes in five bacterial isolates, from two independent experiments, with repeated experiments in grey font.

Isolate	Molecular extension (pixels)[a]	Plasmid size (kbp)[b]	Plasmid size S1/PFGE (kbp)[c]
008	82.3±3.0	156±5.7	151
	55.9± 1.2	106±2.3	103
	24.5±0.4	46±0.7	42
	78.7±2.8	149±5.3	
	51.6±5.3	98±10.0	
	24.2±0.9	46±1.7	
033	99.6±2.0	188±3.7	182
	98.0±2.7	185±5.1	
071	43.8±2.1	83±3.9	68
	39.1±3.9	74±7.4	
163	65.7±1.8	124±3.4	121
	30.5±0.5	58±0.9	55
	68.8±1.4	130±2.6	
	31.7±0.6	60±1.1	
299	64.5±1.9	122±3.6	121
	48.9±3.0	92±5.6	95
	-	-	19[d]
	3.8±0.6[e]	7±1.2	8
	68.4±1.4	129±2.6	
	55.3±3.1	105±5.9	

[a] Mean and standard deviation of measured extensions of 12-130 individual plasmids per isolate. [b] Conversion from pixels to kbp using plasmid R100 as reference. [c] from [3]. [d] This plasmid was identified with S1/PFGE, but not with 454-pyrosequencing data [3]. [e] Based on three molecules only.

Intensity colormaps

Colormaps were constructed to justify the choice of the threshold value of 400 for the fluorescence emission intensity and 10% for the normalized standard deviation of the extension to sort circular plasmids from linear DNA. Figure S4 shows the same data as in Figure 2 but with the standard deviation divided by the mean extension on the y-axes and emission intensities higher than 400 (a.u.) marked in red.

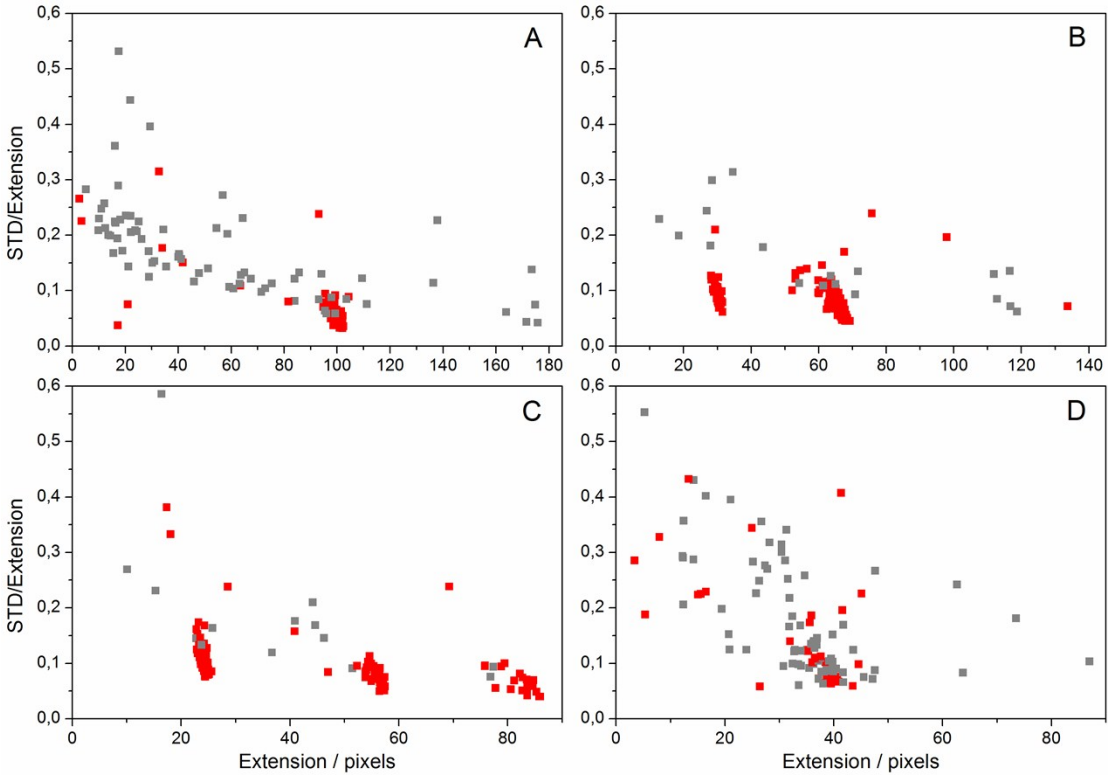

Figure S2: *Data from Figure 2 in the paper re-plotted with the standard deviation, divided by the mean extension for each molecule, on the y-axes. Each square represents an individual molecule with values of the emission intensity that are higher than 400 (a.u.) marked in red.*

Standard error of the mean

For independent observations, the standard error of the mean extension should decrease with the number of measurements (N) as $1/\sqrt{N}$. Here, we investigated data points that, with the help of cut-offs in standard deviation of the extension and emission intensity, were designated to three different plasmids from two different isolates (033 and 163). Figure S3 shows that the standard error of the mean indeed decreases as approximately $1/\sqrt{N}$ for N larger than about 10-20 molecules. We note that the absolute values for the error are really small, and for N > 20 always smaller than 1%.

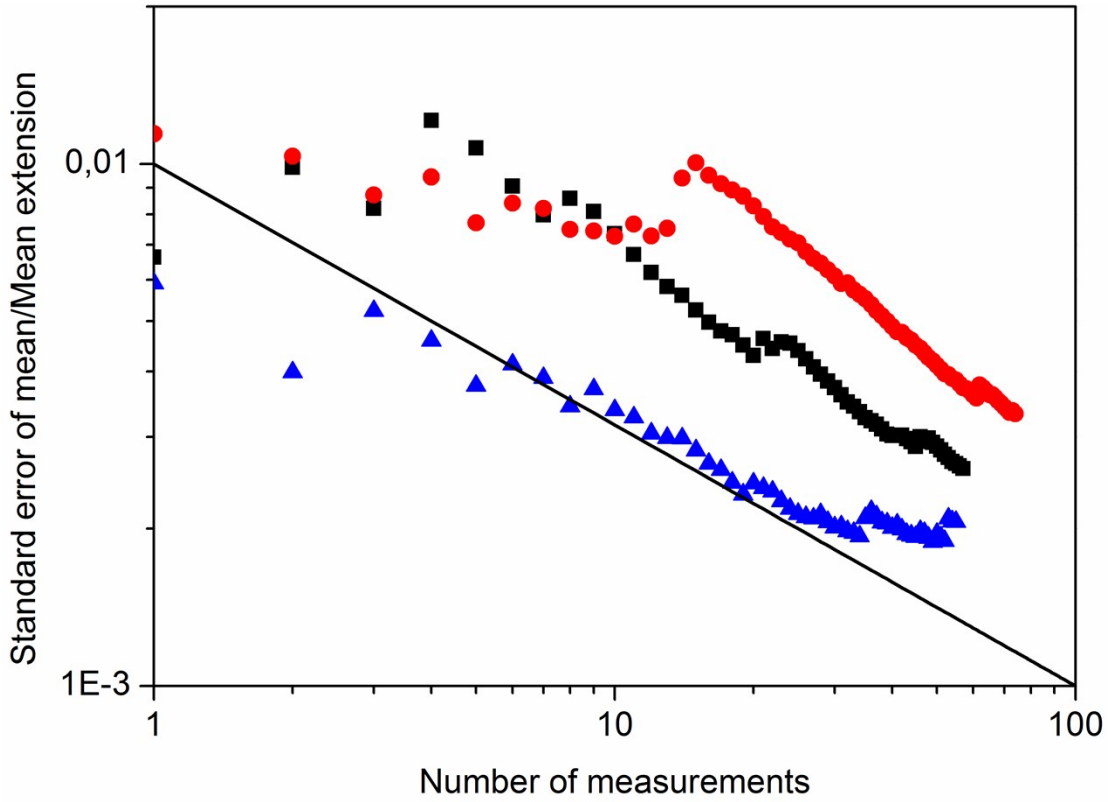

Figure S3: *Log-log plot of the standard error of the mean divided by the mean extension of each molecule, as a function of the number of measurements. Three examples from two different isolates are shown: isolate 033 (black), the long plasmid in isolate 163, (red) and the short plasmid in isolate 163, (blue). The order of the measurements within each data set was randomized. The black line shows the expected decay independent observations, the function $1/\sqrt{N}$, scaled with a factor of 0.01.*

References

[1] F. Persson and J. O. Tegenfeldt, *Chem. Soc. Rev.*, 2010, **39**, 985.
[2] L. Nyberg, F. Persson, B. Åkerman, and F. Westerlund, Nucleic Acids Res., 2013, 41, e184–e184.
[3] A. Brolund, O. Franzén, Ö. Melefors, K. Tegmark-Wisell, and L. Sandegren, *PLoS One*, 2013, **8**, e65793.