Supplementary Material For:

A device for automated hydrodynamic shearing of genomic DNA

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Materials and methods

Materials

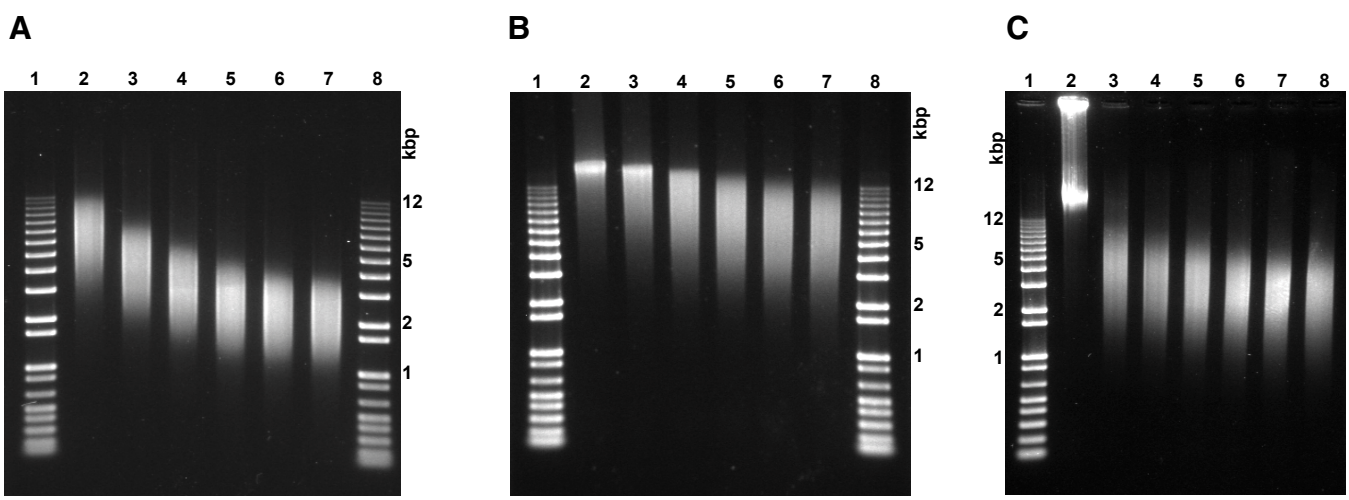
Our shearing device consists of a syringe pump with a built-in 9-port valve (Cavro XR syringe pump, part no. 729848, $2073; Tecan Systems, Inc., Männedorf, Switzerland) and a syringe (Cavro XLP syringe, 2.5 mL, part no. 734806, $60; Tecan) with a 1/4–28 fitting to the bottom port of the valve. The syringe pump is controlled by a computer via an RS232 interface using the PumpLink program provided by Tecan. The syringe pump plunger speed can be varied over a range of 1.2–1200 s/stroke, corresponding to 125–0.125 mL/min with a 2.5-mL syringe. All flow rates tested between 15 mL/min and 125 mL/min produced sheared fragments of predictable length. Shearing is accomplished with a removable stainless steel filter screen of 0.125-in diameter and of various pore sizes (0.5 µm, part no. 5SR2–10, $2 each; or 10 µm, part no. 10SR2–10, $1 each; VICI-Valco Instruments Co. Inc., Houston, TX, USA) housed in a standard filter adaptor (part no. ZUFR1C, $40; VICI-Valco). The thickness of each screen varies depending on the diameter of the type 316 stainless steel fibers used to construct the screen. The thickness of the 0.5 µm screen is 40 µm, while that of the 10 µm screen is 125 µm. All tubing used in the system is high-purity PFA Teflon tubing with 0.0625-in o.d. and 0.030-in i.d. (Cat. no. 1513, $1.80/foot, Upchurch Scientific, Oak Harbor, WA, USA). Tubing lengths between the sample and valve are generally <5 cm to minimize void volume. We recommend that users concerned about potential rust formation on stainless steel components consider an all-PEEK filter housing (Cheminert part no. ZU1FPK.5, $44; VICI-Valco) and replaceable PEEK-encapsulated titanium filter elements (Cheminert part no. C-F1.5TI, $4.50 each; VICI-Valco). The cost of our instrument is ~$2200 (computer not included):

Hydrodynamic shearing of genomic DNA with screens

DNA shearing with our device is a relatively simple 2-step process that can be carried out automatically in 20 min.

(1) Washing. After the chosen screen is inserted into the filter screen housing, the assembly was washed extensively, 6 times with 1.5 mL 0.5 M HCl followed by 6 times with 1.5 mL of 0.5 M NaOH. 500 µL air was drawn into the syringe to purge any wash solution remaining in the valve that may damage the DNA. To rinse the wash solution from the syringe and tubing, the system was washed 8 times with 2 mL dH_2O and then with TE buffer. About 50 µL TE buffer was left in the tubing so that no air was present in the system that could cause splashing of the sample onto the tube wall. The entire wash sequence is automated.

(2) DNA shearing. The DNA was sheared by pulling the sample into the syringe and then forced back into its original tube through the screen for a determined number of iterations at the desired speed. After the shearing, air is pulled into the syringe and used to purge the entire fluid sample into the collection tube. To produce DNA of various fragment lengths from the same genomic DNA, portions of the sample were sheared at incrementally higher speeds and output into tubes connected to the different ports of the valve. The plunger depth was adjusted to account for the decrease in sample volume, and the remainder of the sample was then sheared at

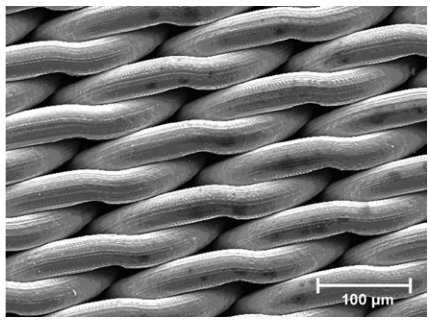


Supplementary Figure 1. Lengths and size distributions of the DNA fragments as a function of screen pore size, flow rate and number of iterations. (A) and (B) Gel images of human genomic DNA sheared with (A) 0.5-µm and (B) 10-µm screens at various flow rates. Lanes 1 and 8: 250 ng 1-kb Plus ladder. Lane 2–7: DNA sheared with a flow rate of 15, 25, 35, 45, 79, and 125 mL/min, respectively. (C) Effect of the number iterations. Human genomic DNA was sheared by increased number of iterations using a screen with pores of 0.5 µm diameter. Lane 1: 250 ng 1-kb Plus ladder. Lane 2: 250 ng genomic DNA. Lanes 3–7: DNA sheared with1, 3, 5, 10, 15 and 20 iterations, respectively.

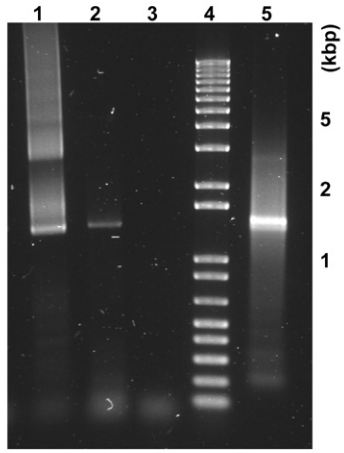
Table 1. Parts and Costs

Part	Cost ($USD)
Cavro XR Syringe Pump	2073.00
Cavro XLP Syringe	60.00
Filter Adaptor	40.00
PFA Teflon Tubing	1.80
0.5-µm filter	2.00
10-µm filter	1.00
Total	**2177.80**

The source DNA was human genomic DNA diluted to 30 µg/mL in a TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8). More than 90% of the DNA is longer than 50 kb in size. All solutions were prepared with 18.2 MΩ-cm H_2O (Milli-Q, Millipore Corp., Billerica, MA, USA) and filtered through a 0.22 µm cellulose nitrate filter (part no. 430758, $1.50 each, Corning Inc., Corning, NY USA) to prevent potential clogging by impurities.



Supplementary Figure 2. SEM image of a 10-µm screen. A representative section of a 10-µm screen from VICI-Valco (part no. 10SR2-10) were imaged at 274× magnification with an FEI **XL ESEM-FEG scanning electron microscope (FEI Company, Hillsboro, OR, USA). Note that the pores are of irregular geometry but they are very uniform in shape and size across the whole screen.**



Supplementary Figure 3. Elimination of cross-contamination. After washing the syringe and tubing and replacing the screen, no contamination was detected by PCR. Lane 1: PCR reaction with sheared DNA as template. Lane 2: PCR reaction with TE from tubing after washes. Lane 3: PCR reaction with TE from tubing after the washes and the replacement of the screen. Lane 4: 250 ng 1-kb Plus Ladder. Lane 5: sheared DNA spiked with 1600-bp fragment.

a higher speed. After each shearing experiment, the system was washed as described in step 1, above.

Characterization of fragment lengths and distributions

To quantify the effect of screen pore sizes and flow rates on fragment lengths and distributions, we sheared human genomic DNA using screens of two different pore sizes (0.5 µm and 10 µm) at 6 flow rates (15, 25, 35, 45, 79, and 125 mL/min). The initial sample used was 165 µL 30 µg/mL human genomic DNA (Cat. no. G304A, Promega Corp., Madison, WI, USA) in TE buffer. Shearing was performed as described in "Hydrodynamic shearing of genomic DNA with screens" section, above. A 15-µL aliquot was removed after shearing at each of the 6 flow rates. To determine the potential variation in performance with different batches of screens, 3 different screens of both 0.5- and 10-µm pore sizes were used to perform the shearing on 3 different days.

Elimination of contamination

To show that the system can be washed sufficiently to eliminate any residual DNA in the tubing and the syringe, we sheared a sample that had been spiked with a 1600-bp known DNA fragment. Forty microliters of 500-nM DNA was added to 10 µL of 163 ng/µL genomic DNA and 70 µL TE buffer. After shearing at 79 mL/min through a 0.5-µm screen, the sample was collected. The system was washed with NaOH and HCl solutions according to the procedure described in "Hydrodynamic shearing of genomic DNA with screens" section, above. Fifteen microliters of the final TE buffer rinse was saved and used as template in a PCR reaction with primers specific for the amplification of the spiked DNA. As a positive control, 0.2 µL of the sample was also used as a template for a PCR reaction. To see if the screen could be a source of contamination, the used screen was discarded and a new one placed into the housing. The system was rinsed with TE buffer again, and 15 µL of this final wash solution was used in the PCR reaction as well. Each PCR was carried out for 30 cycles using Phusion High-Fidelity Polymerase (New England Biolabs, Cat. No. F-530L) according to the manufacturer's instructions.

Analysis

The average size and range of the DNA fragments were analyzed by electrophoresis using 0.8% agarose gels. 250 ng 1-kb Plus DNA ladder (Cat. no. 10787018; Invitrogen, Carslbad, CA, USA) was also loaded onto the gel for quantification. Approximately 225 ng of sheared DNA was loaded in each lane and electrophoresis was carried out at 5V/cm for 60 min in TAE buffer (40 mM Tris, 20 mM acetic acid, and 2 mM EDTA, adjusted to pH 8.0 with NaOH). The gels were stained with SYBR Gold nucleic acid stain (Cat. no. S-11494, Invitrogen) in TAE buffer, and then imaged with a Gel Doc XR and a 12-bit camera system using the Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, CA, USA). After background subtraction, the raw intensity values at all the points in each lane were exported to Microsoft Excel (Microsoft Office 2003, Service Pack 3; Microsoft Corp., Redmond, WA, USA). To characterize each screen, data from 3 separate shearing runs with the same screen were averaged. An exponential equation relating fragment length and migration distance on the gel was established from the bands of the 1-kb Plus standard. That equation was used to assign a base pair value to each data point. By dividing the raw intensity values with their corresponding base pair number, the relative population of each fragment length was calculated. The average fragment length was calculated by dividing the sum of the raw intensity values by the sum of the relative population values. The relative population data was normalized by dividing each point by the maximum value in the lane and then plotted as a function of fragment length using Kaleidagraph (Synergy Software, Reading, PA, USA).

The range of fragment sizes for each screen (0.5 µm and 10 µm) at each speed (15, 25, 35, 45, 79, and 125 mL/min) was defined as the range of base pairs that encompassed 80% of the total fragments, with ~10% of fragments falling above and below this range. This was determined by calculating the area underneath the curve of the relative population versus length to find the lower and higher cutoffs. The percent range in Table 1 is the CV from 3 shearing runs. The coefficient is calculated by dividing the standard deviation by the mean value.