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Simultaneously Sizing and Quantitating Zepto-Mole DNA at High-Throughput in Free Solution

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Abstract

Determining the sizes and measuring the quantities of DNA molecules are fundamental tasks in molecular biology. DNA sizes are usually evaluated by gel electrophoresis, but this method cannot simultaneously size and quantitate a DNA at low zepto-mole levels. We have recently developed a new technique, called Bare Narrow Capillary-Hydrodynamic Chromatography or BaNC-HDC, for resolving DNA based on their sizes without using any sieving matrices. In this report, we utilize BaNC-HDC for measuring the sizes and quantities of DNA fragments at zepto-mole to several-molecule levels. DNA ranging from a few base pairs to dozens of kilo base pairs are accurately sized and quantitated at a throughput of 15 samples per hour, and each sample contains dozens of DNA having different lengths. BaNC-HDC can be a cost-effective means and an excellent tool for high-throughput DNA sizing and quantitation at extremely low quantity level.

Keywords

DNA sizing; gel-free DNA separation; microchip; hydrodynamic chromatography; high-throughput

Introduction

Size and quantity are two basic parameters to characterize DNA, and these values are usually measured via slab gel electrophoresis,^[1] pulsed-field gel electrophoresis (PFGE)^[2], or capillary gel electrophoresis.^[3] However, none of these methods are capable of simultaneously measuring the size and quantity of DNA at low zepto-mole levels. Single-molecule microscopy^[4] can be used for measuring the size of a large DNA, but preparing samples for these measurements is tedious and time-consuming, and therefore the throughputs are low and costs are high. Flow cytometry has been utilized for single-molecule DNA sizing, but its resolving power is limited.^[5] This technique is impractical for

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sizing small DNA fragments because signals from small fragments are indistinguishable from background signals.

In our lab, we have employed a narrow open capillary (of 1–10 μm i.d.) for separating DNA in a gel-free solution;^[6] DNA from a few base pairs (bp) to more than one hundred kilo base pairs (kbp) have been nicely resolved. Similar work has also been performed in other labs.^[6c,7] Because a Bare Narrow Capillary was utilized as the separation column, and the primary separation mechanism is based on HydroDynamic Chromatography,^[8] we call this technique BaNC-HDC. When two DNA fragments are confined inside a narrow capillary and transported under a pressure-driven flow, these molecules move as particles. The larger fragment has a greater effective diameter ($2r$) and cannot access to the capillary wall (the slow-moving region) as close as the smaller fragment (with an effective diameter of $2r'$; $r' < r$) can, and therefore it moves faster than the smaller one. [Detailed transport mechanisms can be found in the literature.^[9]] DNA sizes can thus be determined based on the relative velocities of these molecules. Recently, we automated the separation system and improved injection reproducibility.^[10] In this work, we develop a splitting-based microchip injector so that we can adjust the injection volumes (from sub-pL to several pL) conveniently. We incorporate this injector with BaNC-HDC for high-throughput DNA sizing and quantitation. Importantly, with this scheme, we can inject a sample while a separation is being performed. Using this system, DNA sizes ranging from a few base pairs to dozens of kilo base pairs are accurately measured, and DNA quantities as low as a few molecules can be reliably determined; both the size and quantity information can be obtained simultaneously in a single run. To demonstrate the feasibility of this setup for real-world applications, we size the fragments of digested λ -DNA and budding yeast strains. The setup is capable of analyzing these samples without additional sample treatments or purifications.

Results and Discussion

In this experiment, we aimed at developing an injection scheme which is capable of successively delivering pL-to-fL DNA samples into a BaNC-HDC system. Since such low-volume injection valves are commercially unavailable, we turned to flow splitters which have been used in chromatography.^[11] A flow splitter usually has a single restriction capillary (RC) and therefore a fixed flow splitting ratio. To improve its versatility, we attached an RC selector so that we could select among six different restriction capillaries; which enabled us to select an appropriate injection volume for BaNC-HDC analysis. Figure 1A presents a schematic diagram of the injection scheme. As the eluent was driven through the 60-nL injection valve to the microfabricated Chip-T, it was split into two streams; one stream went to the narrow capillary column while the other went through a restriction capillary (RC) to a waste reservoir. Because the flow resistance through the narrow capillary column was fixed, a specific flow splitting ratio was determined by selecting a specific restriction capillary via the RC selector.

Figure 1B presents an image of a Chip-T incorporated with capillaries. The microchannels were fabricated using standard photo-lithographic technologies and had circular cross-section profiles. First, “T” grooves were created on two glass wafers using HF etching. Because the line-width of the channel pattern on the photomask was 10 μm and HF etching

was isotropic, the grooves had a semicircular profile after being etched to a depth of 190–200 μm . Round channels (with 380–400 μm diameter) were formed as the two etched wafers were face-to-face aligned and bonded. The narrow capillary column (47-cm and 200- μm -o.d.), the capillary (6-cm-long, 100 375- μm -o.d.) between the injector and Chip-T, and the auxiliary capillary (AC) (15-cm-long, 100- μm -i.d., and 375 connected to Chip-T at positions 1, 2, and 3, respectively. Capillary-to-Chip connections were secured using epoxy adhesive. The other end of AC was connected to an RC selector (six-position selector, also from VICI). The six restriction capillaries (RC_1 – RC_6) had the same i.d. but different lengths (3.5, 6.5, 13, 22, 33, and 44 cm, respectively). As the RC selector connected AC to RC_1 , RC_2 , RC_3 , RC_4 , RC_5 , and RC_6 , the flow splitting ratio was measured to be 1.05×10^4 , 1.40×10^4 , 2.14×10^4 , 3.53×10^4 , 7.06×10^4 , and 1.40×10^5 , corresponding to an injection volume of 5.7, 4.3, 2.8, 1.7, 0.85, and 0.43 pL, respectively.

In this experiment, injection volumes from 430 fL to 5.7 pL were tested. Obviously, the volume range can be extended in both directions by employing different restriction capillaries. Figure 2 presents typical BaNC-HDC chromatograms for DNA from 75 bp to 20 kbp as the injected sample volume varied from 5.7 pL to 0.85 pL. As expected, the resolutions improved with the decreasing sample volume. Fifteen fragments were baseline resolved when the injection volume was 1.7 pL or smaller. The linear relationship between the peak area and the injection volume was satisfactory, with the regression coefficients for all trend-lines greater than 0.985.

To boost the sample throughput, we used this apparatus to inject samples successively, without regenerating/reconditioning the capillary column. Figure 3a presents the results when five DNA samples with total DNA concentrations of 5, 10, 20, 50, and 100 ng/ μL were injected in this fashion and resolved. Please note: there appears only one trace in this figure, but it contains five consecutive chromatograms.

Figure 3b presents the fluorescence signal (peak area) as function of DNA concentration for all DNA fragments; linear regressions produced excellent linear coefficients ($R^2 > 0.985$). However, the slope varied from one fragment to another, presumably due to bonding variations between DNA and YOYO-1. Because DNA having similar sizes usually had similar slopes, we developed an approximate method for determining quantities. For example, after we measure the peak area of a DNA fragment having a size of b bp, we first identify two calibration curves in Figure 3b for two fragments having sizes of a and c bp; a and c are the closest to b , but $a < b < c$. Because we know the calibration curve for the a -bp DNA is $Y = m_a X$ (where Y the fluorescence signal and X is the DNA concentration) and the calibration curve for the c -bp DNA is $Y = m_c X$, the calibration curve for quantitating the b -bp DNA should be

$$Y = \left(\frac{b-a}{c-a} m_c + \frac{c-b}{c-a} m_a \right) X \quad (1)$$

As can be seen from Figure 3a, a sample throughput of more than 10 samples per hour was achieved. Please note that there were only 8 molecules in the first peak (20 kbp), and 15, 22, 115, 38, 51, 79, 403, 194, 280, 1180, 472, 633, 953, and 2483 molecules in the following

(10, 7, 5, 4, 3, 2, 1.5, 1, 0.7, 0.5, 0.4, 0.3, 0.2, and 0.075 kbp, respectively) peaks of the first chromatogram. The maximum sample throughput of this analysis depends on the time interval between the first peak and the last peak in one chromatogram. Under the conditions legended in Figure 3a, fragments of 50 kbp and 20 kbp could be baseline resolved (see Figure S1), while fragments of 50 kbp and 106 kbp were eluted out but unresolved (data not shown) at ~6.2 min. That is an indication that all fragments larger than 50kbp will have a similar retention time (i.e., these fragments enter a so-called “constant mobility region”^[9]). When fluorescein (smaller than any DNA fragment) was included in this sample, it was eluted out at ~9.5 min. The velocity of fluorescein had a value very close to that of the eluent. This corresponded to a time interval of ~3.5 min between the largest (the fastest moving) and the smallest (the slowest moving) DNA. If we leave 0.5 min gap between two consecutive chromatograms (to prevent chromatograms from overlapping), we can analyze DNA at a throughput of 15 samples per hour. Obviously, this throughput can be increased considerably if baseline resolutions were not required.

To demonstrate the utility of this method for sizing DNA in real-world samples, we digested λ -DNA using restriction enzyme *Hind* III, a common method to prepare restriction enzyme digests. We also amplified two DNA tandem repeats from two *Saccharomyces cerevisiae* (*S. cerevisiae*) strains (CAT-1 and BG-1) via polymerase chain reactions; *S. cerevisiae* strains are used to initiate the fermentation process in sugar mills. Characterizations of the λ -DNA digests and the DNA tandem repeats are presented in Figure S2 and S3 we then injected a DNA size standard (Sample I), the λ -(Sample II), the locus G4 CAT-1 of *S. cerevisiae* and the locus G4 BG-1 of *S. cerevisiae* (Sample III), and the locus G4 BG-1 of *S. cerevisiae* (Sample IV); three repetitive injections were performed for each sample. We then injected twice the DNA size standard (Sample I') again. The elapsed times between two successive injections were (4.5 min), corresponding to a gap of at least one minute between consecutive chromatograms and a throughput of 13.3 samples per hour. These results are presented in Figure 4. The insets show expanded views of the first chromatograms of all samples; all peaks were baseline resolved.

To validate the method for measuring the lengths of DNA fragments, we use the first chromatogram in Figure 4 to establish a relationship between the relative mobility of a DNA and its length based on an HDC quadratic model as we described previously.^[9] Relative mobility was defined as the ratio of the velocity of a DNA fragment to the average velocity of the eluent. The fragment velocity was calculated by dividing the effective capillary length by its retention time, while the eluent velocity was obtained by measuring its flow rate and dividing the measured flow rate by the narrow capillary cross-section area. As presented in Figure 5, the curve-fitting generated an excellent correlation coefficient ($R^2=0.9997$).

$$Y_i = 1 + 2 * \left(81 * \frac{L_i^{0.65}}{R} \right) - \left(81 * \frac{L_i^{0.65}}{R} \right)^2 - 0.0132 * L_i \quad (2)$$

where Y_i is the relative mobility of DNA fragment i ($Y_i = v_i/v_0$, where v_0 and v_i are the transport velocities of the eluent and DNA fragment i), L_i is the length of the fragment in kbp, and R is the radius of the bore of the narrow capillary. We then measured the Y_i values for the fragments in all consecutively injected samples, substituted the Y_i values into

Equation 2, and computed the L_i . These results are listed in Table 1; excellent length accuracies (with single digit percentage error) were obtained.

To validate the method for quantitating DNA, we measured the peak areas for all peaks in Figure 4, calculated the DNA concentrations in original samples based on the calibration curves in Figure 3b, and computed the number of molecules in all peaks using these calibration curves. These results are also presented in Table 1. There were only hundreds to thousands of DNA molecules in each peak. In general, the relative quantitation errors were around or less than 10%.

Conclusions

In conclusion, we have demonstrated the feasibility of utilizing BaNC-HDC for high-throughput sizing and quantitation of DNA at zepto-mole to several-molecule levels using a single open capillary column without any sieving matrices or wall coatings. Both sizing and quantitation accuracies are excellent (errors are generally at the single-digit-percentage level), and sample throughputs of more than a dozen have been achieved. Additional advantages of BaNC-HDC are the low waste generation (pL/min effluent to waste), the low consumable costs (the expenses of a few nL eluent and a few pL sample per assay), and readiness for automation. It should be pointed out that, although DNA at the single molecule level can be resolved and quantitated by BaNC-HDC, a lot more molecules have been utilized to carry out these separations. The current injection scheme has used less 0.01% of the sample; most of it has been flushed into the waste. To realize the full benefit of this technique, however, we will need to improve the efficiency of the sample injection scheme. Since narrow capillaries can be conveniently fabricated on a chip^[12] and single DNA can be captured inside microfabricated channels,^[13] we can “inject” the captured DNA at 100% efficiency for BaNC-HDC separation. An immediate application of BaNC-HDC could be for rapid tracing the origin of a foodborne bacterium. Restriction fragment length polymorphism (RFLP) and PFGE pattern are widely used for this work.^[14] A “rapid” RFLP-PFGE protocol takes ~24–28 h, and further reduction of the analysis time is challenging because PFGE itself takes 18–19 h.^[14b] A RFLP-BaNC-HDC protocol could shorten the analysis time to 4–5 h; this difference could play a vital role in controlling an infectious disease.

Experimental Section

Reagents and materials

Fused-silica capillaries were products of Polymicro Technologies (Phoenix, AZ). GeneRuler™ 1-kb plus DNA ladder (SM1331) was purchased from Fermentas Life Sciences Inc. (Glen Burnie, MD), and YOYO-1 was from Molecular Probes (Eugene, OR). Ammonium acetate, concentrated hydrochloric acid, ethylenediaminetetraacetic acid (EDTA), fluorescein, sodium hydroxide, and tris(hydroxymethyl)aminomethane (Tris) were obtained from Fisher Scientific (Fisher, PA).

Preparation of eluent and standard DNA samples

10 mM TE buffer, composed of 10 mM Tris-HCl and 1.0 mM Na₂EDTA at pH 8.0, was used as the eluent, and it was prepared using DDI water from a NANO pure infinity ultrapure water system (Barnstead, Newton, WA). Before being used, the solution was filtered through a 0.22- μ m filter (VWR, TX) and vacuum-degassed. The stock solution of 100 ng/ μ L 1-kb Plus DNA ladder was prepared by mixing 39 μ L 10 mM TE buffer, 10 μ L 500 ng/ μ L DNA, and 1 μ L YOYO-1. Working standard DNA solutions were made by diluting the stock solution with DDI water at the ratio as needed. Eluent and DNA samples were stored at 4°C.

Fluorescence dye-DNA intercalation

A stock solution of 100 ng/ μ L 1-kb plus DNA ladder was prepared by mixing 39 μ L of 10 mM TE buffer (pH=8.0), 10 μ L of 500 ng/ μ L DNA, and 1 μ L of 1 mM YOYO-1 in DMSO. Working solutions were made by diluting the stock solution with DDI water. The eluent solution and DNA samples were stored at 4°C.

Digestion of λ -DNA

λ -DNA was purchased from New England Biolabs (Ipswich, MA, USA). A restriction enzyme, *Hind* III (New England Biolabs, Ipswich, MA), was used to digest λ -DNA; 10 activity units of *Hind* III for 2.5 μ g λ -DNA in a 50 μ L reaction at 37°C overnight. After the digestion products were characterized by agarose gel electrophoresis (see results in Figure S2), the sizes and quantities of the DNA fragments were measured by BaNC-HDC without any further purification.

Preparation of DNA tandem repeats

Bioethanol *S.cerevisiae* strains, CAT-1 and BG-1, were kindly supplied by Drs. Ana Teresa B. F. Antonangelo and Debora Colombi at San Paulo State University in Brazil. We first grew these strains in 10 ml yeast peptone dextrose (YPD) medium for 12~16 h at 30°C until A600 of culture reached to 0.6–0.8. DNA of yeast cultures were extracted using Yeast Genomic DNA Purification Kit (AMRESCO, LLC, Solon, OH). The amplification of tandem DNA marker, G4, was conducted following the method as described in the literature.^[15] Briefly, 50 μ L polymerase chain reaction (PCR) solution contained 100 ng genomic DNA, 10 μ L of 5 $\times$ Reaction Buffer, 800 μ MdNTP mix (200 μ M each), 0.2 μ M of each forward and reverse primer for locus G4 (forward primer: 5'-AACCCATTGACCTCGTTACTATCGT-3'; reverse primer: 5'-TTTCGATGGCTCTGATAACTCCATTC-3'), 5 units of *Tfi* DNA polymerase (Invitrogen, Carlsbad, CA), and 1.5 mM of MgCl₂. PCR reaction was proceeded by denaturing at 94°C for 5 min, cycling temperatures for 14 cycles from 94°C for 15 s, to 60°C for 30 s (this temperature was decreased by 1°C for every cycle), and to 72°C for 30 s, cycling temperatures for 25 cycles from 94°C for 15 s, to 48°C for 30 s, and to 72°C for 30 s, and maintaining the temperature at 72°C for 5 min. The amplified products were analyzed by slab gel electrophoresis (see results in Figure S3).

Apparatus

FigureS4 presents the apparatus we used for high-throughput sizing and quantitation of DNA. The apparatus consisted of a pressure chamber, a pL-to-fL sample injection scheme, a bare narrow open capillary column, and a laser-induced fluorescence (LIF) detector. As pressurized air was introduced to the pressure chamber, the eluent in the solution vial was pressurized into the BaNC-HDC system. A pL-to-fL sample containing a few to a few hundred of DNA molecules was injected into the narrow capillary column for BaNC-HDC separations. The resolved DNA fragments were monitored by the LIF detector.

The LIF detector was built in-house. Briefly, a 488-nm laser beam from an argon ion (Spectra-Physics, Salt Lake City, UT, USA) was reflected by a dichroic mirror (Q505LP, Chroma Technology, Rockingham, VT, USA) and focused onto the separation capillary through an objective lens (206 and 0.5 NA, Rolyn Optics, Covina, CA, USA). The fluorescence from the narrow capillary was collimated by the same objective lens, passed through the dichroic mirror, a band-pass filter (532 nm), and a 2-mm pinhole, and finally collected by a photosensor module (H5784-01, Hamamatsu, Japan). The amplified signal from the photosensor module was acquired by an ADC card DAQCard-6062E (National Instrument, Austin, TX, USA) and processed using an in-laboratory written LabView program.

BaNC-HDC separation

Because pL-to-fL injection valves are not commercially available, a splitting injection scheme was used in this work. Referring to Figure 1A, with a commercial injector (C14W. 16, VICI, Houston, TX), 60-nL DNA sample was introduced into the BaNC-HDC system. As the sample solution passed the cross section, a slight portion (<0.01%) of the sample was split into the separation capillary while most of it was flushed away through the stream selector. The sample volume injected into the separation capillary was determined by the splitting ratio, which was determined by the selected restriction capillary. In high-throughput analysis, DNA samples were injected periodically and the injection frequency was ~5 min per injection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. a) Danna K, Nathans D. Proc. Natl. Acad. Sci. U. S. A. 1971; 68:2913–2917. [PubMed: 4332003] b) Kaplan DA, Russo R, Wilcox G. Anal. Biochem. 1977; 78:235–243. [PubMed: 848745]
2. a) Schwartz DC, Cantor CR. Cell. 1984; 37:67–75. [PubMed: 6373014] b) Wagner L, Lai E. Electrophoresis. 1994; 15:1078–1083.

3. a) Swerdlow H, Gesteland R. Nucl. Acids Res. 1990; 18:1415–1419. [PubMed: 2326186] b) Hunkapiller T, Kaiser RJ, Koop BF, Hood L. Science. 1991; 254:59–67. [PubMed: 1925562] c) Ruiz-Martinez MC, Berka J, Belenkii A, Foret F, Miller AW, Karger BL. Anal. Chem. 1993; 65:2851–2858. [PubMed: 8250265]
4. a) Perkins TT, Smith DE, Chu S. Science. 1997; 276:2016–2021. [PubMed: 9197259] b) Walter NG, Huang C-Y, Manzo AJ, Sobhy MA. Nat. Methods. 2008; 5:475–489. [PubMed: 18511916]
5. Goodwin PM, Johnson ME, Martin JC, Ambrose WP, Marrone BL, Jett JH, Keller RA. Nucl. Acids Res. 1993; 21:803–806. [PubMed: 8451182]
6. a) Wang X, Wang S, Veerappan V, Byun CK, Nguyen H, Gendhar B, Allen RD, Liu S. Anal. Chem. 2008; 80:5583–5589. [PubMed: 18500828] b) Wang X, Veerappan V, Cheng C, Jiang X, Allen RD, Dasgupta PK, Liu S. J. Am. Chem. Soc. 2009; 132:40–41. [PubMed: 20014789] c) Liu L, Veerappan V, Pu Q, Cheng C, Wang X, Lu L, Allen RD, Guo G. Anal. Chem. 2014; 86:729–736. [PubMed: 24274685]
7. Liu KJ, Rane TD, Zhang Y, Wang T-H. J. Am. Chem. Soc. 2011; 133:6898–6901. [PubMed: 21504160]
8. Small H. J. Colloid Interface Sci. 1974; 48:147–161.
9. Wang X, Liu L, Pu Q, Zhu Z, Guo G, Zhong H, Liu S. J. Am. Chem. Soc. 2012; 134:7400–7405. [PubMed: 22512501]
10. Zhu Z, Chen H, Wang W, Morgan A, Gu C, He C, Lu JJ, Liu S. Angew. Chem., Int. Ed. 2013; 52:5612–5616.
11. a) Bonn J, Redeby J, Roeraade J. Anal. Chem. 2009; 81:5327–5332. [PubMed: 19563212] b) Vazquez MM, Blanco MEV, Lorenzo SM, Mahia PL, Fernandez EF, Rodriguez DP. J. Chromatogr., A. 2001; 919:363–371. [PubMed: 11442043]
12. a) Lee C, Yang E-H, Myung NV, George T. NanoLett. 2003; 3:1339–1340. b) Menard LD, Ramsey JM. NanoLett. 2011; 11:512–517.
13. a) Xu Y, Vaidya B, Patel AB, Ford SM, McCarley RL, Soper SA. Anal. Chem. 2003; 75:2975–2984. [PubMed: 12964741] b) Witek MA, Llopis SD, Wheatley A, McCarley RL, Soper SA. Nucl. Acids Res. 2006; 34:e74. [PubMed: 16757572] c) Park DSW, Hupert ML, Witek MA, You BH, Datta P, Guy J, Lee JB, Soper SA, Nikitopoulos DE, Murphy MC. Biomed. Microdev. 2008; 10:21–33. d) Witek MA, Hupert ML, Park DSW, Fears K, Murphy MC, Soper SA. Anal. Chem. 2008; 80:3483–3491. [PubMed: 18355091] e) Kriukiene E, Labrie V, Khare T, Urbanaviciute G, Lapinaite A, Koncevicius K, Li D, Wang T, Pai S, Ptak C, Gordevicius J, Wang S-C, Petronis A, Klimasauskas S. Nat. Commun. 2013; 4:2190. [PubMed: 23877302]
14. a) Olive DM, Bean P. J. Clin. Microbiol. 1999; 37:1661–1669. [PubMed: 10325304] b) Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Foodborne Pathog. Dis. 2006; 3:59–67. [PubMed: 16602980] c) Alaidan A, Bohol MF, Al-Thawadi SI, Al-Suwaine A, Al-Qahtani AA, Al-Ahdal MN. J. Microbiol. Methods. 2009; 78:238–241. [PubMed: 19446583]
15. Antonangelo ATBF, Alonso DP, Ribolla PEM, Colombi D. Yeast. 2013; 30:307–317. [PubMed: 23765797]

Figure 1.

Injection scheme for pL-to-fL sample injection. A. Overall schematic of the injection scheme. B. Image of the microfabricated Chip-T (the inset shows an expanded view of the capillary-joint region). AC had an i.d. of and a length of ~20 cm. RC₁, RC₂, RC₃, RC₄, RC₅, and RC₆ 20 μm and lengths of 3.5, 6.5, 13, 22, 33, and 44 cm, respectively. By switching the RC selector to RC₁ (RC₂, RC₃, RC₄, RC₅, RC₆), a sample pL (0.85, 1.7, 2.8, 4.3, or 5.7 pL) was automatically injected into the narrow capillary column.

Figure 2.

Effect of the injection volume on BaNC-HDC separation. The number on the left on each chromatogram indicates the volume of sample injected into the narrow capillary column. The number close to each chromatogram indicates the peak identification (the length of the DNA). The sample contained fifteen DNA fragments, and the total concentration of DNA was 10 ng/ μ L (1.6 ng/ μ L for the 1.5 kbp fragment, 1.5 ng/ μ L for the 0.5, and 5 kbp fragments, 0.5 ng/ μ L for the 0.075, 0.2, 0.3, 0.4, 0.7, and 1 kbp fragments, and 0.4 ng/ μ L for the 2, 3, 4, 7, 10, and 20 kbp fragments, respectively). narrow capillary column had an i.d. of 2 μ m, an o.d. of 200 length of 47 cm (41-cm effective length). The pressure applied for BaNC was 360 psi, and the eluent was 10 mM tris-EDTA buffer at pH 8.0.

Figure 3.

Quantitating DNA with BaNC-HDC. (a) A trace of BaNC chromatograms for successive injections. The injection volume was 0.85 pL. The concentration on each chromatogram indicates the concentration of the DNA sample. The sample injection frequency was not accurately controlled, but it was ~5 min per injection. All other conditions were presented in Figure 2; (b) Calibration curves for DNA having different lengths. Peak areas at different concentrations were obtained from Figure 3a. The trend-lines had linear regression coefficients in the range of 0.985–0.991.

Figure 4.

BaNC-HDC for high-throughput DNA sizing and quantitation. Sample I: 1 kbp plus DNA ladder at the total concentration of 10 ng/ μ L, and the concentrations of individual fragments were presented in Figure 2. Sample II: digested λ -DNA at the total concentration of 10 ng/ μ L (the individual fragment concentrations were 4.8, 1.9, 1.4, 0.90, 0.48 and 0.42 ng/ μ L for 23.13, 9.42, 6.56, 4.36, 2.32 and 2.03 kbp DNA, respectively). Sample III and Sample IV: locus G4 CAT-1 and BG-1 of *S. cerevisiae* strain. Sample I' – identical to sample I. All other conditions were as in Figure 2.

Figure 5.
Curve-fitting results between DNA relative mobility and fragment length. All data were obtained from Figure 4.

Table 1

DNA length and quantity measurement results.

Sample	Length, kbp		Conc., ng/ μ L		# of Molecules, 10^3	
	Theo[a]	Mea[b]	Theo	Mea	Theo	Mea
1-kbp Plus DNA Ladder	20	19.5 $\pm$ 0.3	2	2.2 $\pm$ 0.1	0.79	05
	10	10.2 $\pm$ 0.1	2	2.2 $\pm$ 0.1	1.6	1.7 $\pm$ 0.1
	7	7.29 $\pm$ 0.04	2	2.5 $\pm$ 0.2	2.3	2.8 $\pm$ 0.2
	5	4.95 $\pm$ 0.02	7.5	7.8 $\pm$ 0.3	12	12.2 $\pm$ 0.1
	4	3.93 $\pm$ 0.03	2	2.3 $\pm$ 0.1	3.9	4
	3	2.88 $\pm$ 0.02	2	2.1 $\pm$ 0.1	5.3	4.5 $\pm$ 0.2
	2	1.91 $\pm$ 0.03	2	2.1 $\pm$ 0.1	7.9	5.6 $\pm$ 0.3
	1.5	1.46 $\pm$ 0.02	8	7.9 $\pm$ 0.3	42	8.1 $\pm$ 0.4
	1	1.02 $\pm$ 0.02	2.5	2.6 $\pm$ 0.1	20	42 $\pm$ 2
	0.7	0.73 $\pm$ 0.01	2.5	2.7 $\pm$ 0.1	28	20 $\pm$ 1
λ -DNA Digests	0.5	0.53 $\pm$ 0.01	7.5	7.8 $\pm$ 0.3	120	30 $\pm$ 1
	0.4	0.42 $\pm$ 0.01	2.5	2.6 $\pm$ 0.1	49	123 $\pm$ 5
	0.3	0.32 $\pm$ 0.01	2.5	2.7 $\pm$ 0.1	66	52 $\pm$ 1
	0.2	0.22 $\pm$ 0.01	2.5	2.6 $\pm$ 0.1	99	71 $\pm$ 3
	0.075	0.09 $\pm$ 0.02	2.5	2.5 $\pm$ 0.1	260	104 $\pm$ 5
						270 $\pm$ 10
	23.13	22 $\pm$ 2	4.8	5.1 $\pm$ 0.2	1.6	1.8 $\pm$ 0.1
	9.42	9.47 $\pm$ 0.04	1.9	2.2 $\pm$ 0.1	1.6	1.9 $\pm$ 0.1
	6.56	6.76 $\pm$ 0.01	1.4	1.6 $\pm$ 0.1	1.6	1.9 $\pm$ 0.1
	4.36	4.57 $\pm$ 0.03	0.90	0.71 $\pm$ 0.05	1.6	1.3 $\pm$ 0.1
Tandem Repeats	2.32	2.20 $\pm$ 0.02	0.48	0.44 $\pm$ 0.03	1.6	1.5 $\pm$ 0.1
	2.03	1.96 $\pm$ 0.03	0.42	0.39 $\pm$ 0.03	1.6	1.5 $\pm$ 0.1
	0.2–0.3	0.27 $\pm$ 0.02	n.a.[c]	0.13 $\pm$ 0.04	n.a.	16 $\pm$ 1
	0.3–0.4	0.39 $\pm$ 0.02	n.a.	0.55 $\pm$ 0.06	n.a.	4 $\pm$ 1

Sample	Length, kbp		Conc., ng/ μ L		# of Molecules, 10^3	
	Theo ^[a]	Mea ^[b]	Theo	Mea	Theo	Mea
	0.3–0.4	0.38 $\pm$ 0.01	n.a.	0.76 $\pm$ 0.07	n.a.	11 $\pm$ 1

[a] Theoretical values.

[b] Measured values.

[c] Not available.