LABEL-FREE DNA QUANTIFICATION VIA A 'PIPET, AGGREGATE AND BLOT' (PAB) APPROACH ON FILTER PAPER

Jingyi Li[1, 4], Qian Liu[1, 4], James P. Landers[1, 2, 3, 4]
Departments of [1]Chemistry, [2]Mechanical and Aerospace Engineering, [3]Pathology, [4]Center For Microsystems For The Life Sciences, University of Virginia, Charlottesville, VA 22904, USA

ABSTRACT

We present a new label-free method to quantify DNA for genetic analysis with the potential point-of-care analysis. The method requires only a simple protocol and cost-effective materials that are readily available; a pipettor, pipet tips and filter paper. An image analysis algorithm is developed to define a calibration curve, with which the DNA content in unknown samples can be quantified. Integration of this method with DNA extraction and PCR could promote the development of cost-effective microfluidic systems for point-of-care testing.

KEYWORDS: DNA, Quantification, Label-free, Point-of-care

INTRODUCTION

Genetic analysis has greatly promoted the development of clinical diagnosis and forensic applications. A typical experimental process includes DNA preparation and quantification, amplification by polymerase chain reaction (PCR), and subsequent detection. The success of amplification often depends on the quality of prepared DNA, specifically purity and concentration, consequently, a quantification step between DNA preparation and amplification is critical.

Towards point-of-care testing, paper-based microfluidic systems have been emerging due to their high portability and low cost and requirements for power. Lab-on-paper devices for cell lysis and DNA extraction have been reported recently [1], which could be combined with microfluidic PCR systems for rapid sample-to-result tests. However, the lack of a simple DNA quantification method at the point-of-care may compromise the success rate of PCR and hinder further analysis.

At MicroTAS 2011, we reported the pinwheel assay (recently published [2]) with a twist on the concept that involved preparing the aggregates in an 'image-ready' form via a 'Pipet, Aggregate and Blot' (PAB) approach on filter paper, as an alternative 'label-free' QUALITATIVE DNA detection modality [3]. Here, we extend the approach to a label-free DNA QUANTIFICATION method, which readily quantifies nanogram-scale samples prior to downstream analysis.

EXPERIMENTAL

The protocol of PAB assay includes: (1) pipetting silica-coated superparamagnetic microparticles in 8 M GuHCl and DNA sample, (2) promote aggregation of DNA and silica particles by exposure to a magnetic field, and (3) dispensing the pipetted volume (blotting) onto filter paper for image analysis (**Figure 1**).

RESULTS AND DISCUSSION

The particles transform from a dispersed state to tight aggregates upon mixed with DNA, as illustrated in the images of negative (**Figure 2A**) and positive controls (**Figure 2B**) and corresponding saturation histograms. **Figure 3A** shows the change of histogram as DNA concentration varies, and the total number of

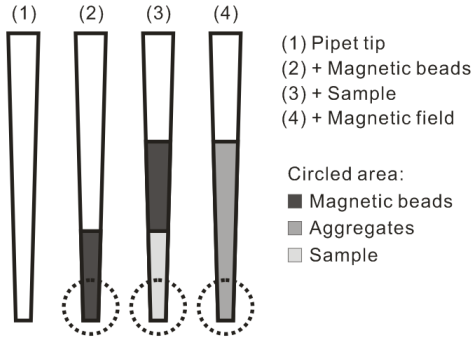

Figure 1. *General experimental procedure of the pinwheel assay via the PAB approach. [3]*

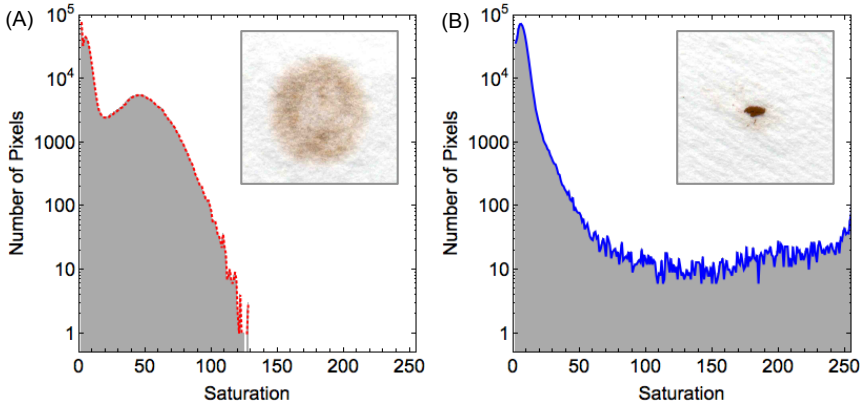

Figure 2. *(A) and (B) illustrate the scanned images of magnetic beads blotted on filter paper without and with DNA respectively. The saturation histograms in HSB (hue-saturation-brightness) color space denote the differences between the two samples.*

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

pixels representing particles and aggregates (i.e., dark area) decreases as DNA-particle interaction increases which, with constant bead concentration, is solely a function of DNA mass (**Figure 3B**). The sensitivity appears to increase (6-fold) proportionally with a decrease in bead size (8-fold), providing a simple way of tuning the standard curve (**Figure 4**).

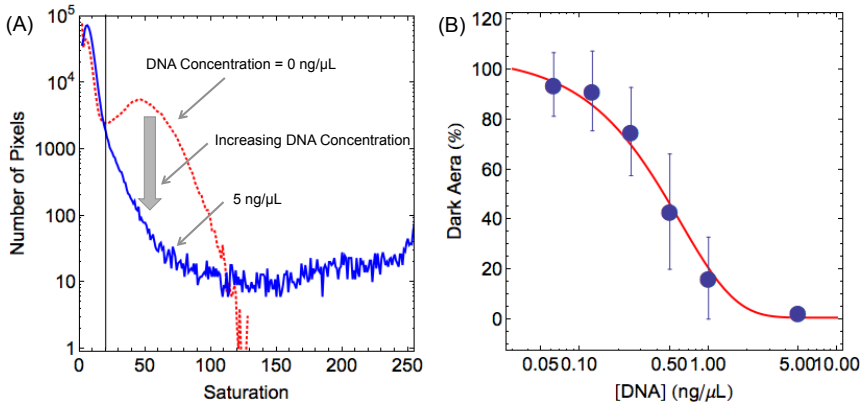

Figure 3. Algorithm and standard curve for DNA quantification. *(A) The change of saturation histogram at various [DNA] illustrates dispersed beads converting to tight aggregates. A threshold [2] was set by a negative control to define 'Dark Area'. (B) The dark area values were normalized with the negative control and correlated with [DNA]. Error bars denote standard deviation (n=14).*

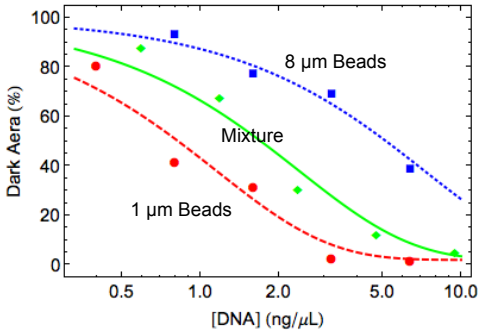

Figure 4. Adjusting the sensitivity by varying the size of beads. *The standard curves of 1 μm beads and 8 μm beads are shown in red and blue respectively. The green curve represents the standard curve of a bead mixture with 50% 1 μm beads and 50% 8 μm beads.*

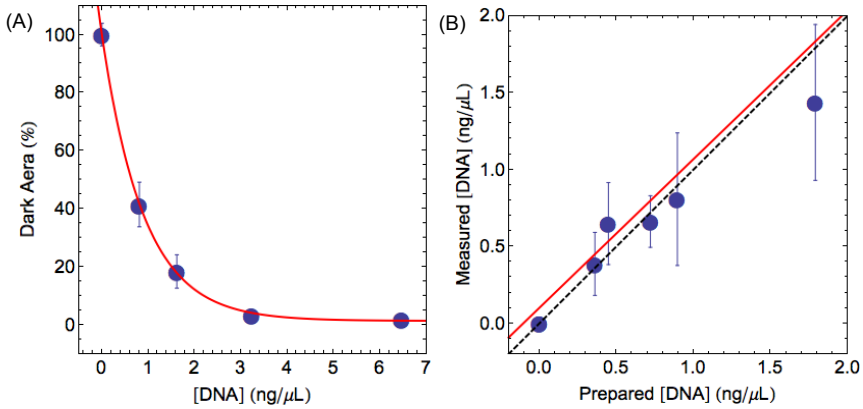

Figure 5. Quantification of human genomic DNA extracted from blood samples. *(A) A standard curve was generated with serially diluted DNA samples. (B) The DNA concentrations of six samples were measured with the PAB assay and compared with the prepared concentration based on UV-Vis spectroscopy. Error bars denote the standard deviation of four replicates. The red line represents a linear fit on the data points, while the black dashed line shows the theoretical correlation y = x.*

Human genomic DNA samples were extracted from blood, quantified with the PAB assay, and amplified with PCR for short tandem repeat (STR) analysis, a finicky assay that is sensitive to the input mass of DNA. The PAB quantification results are comparable to those from UV-Vis spectroscopy (**Figure 5**) and, most importantly, only the sample with appropriate DNA concentration yielded successful PCR, as shown in the electropherogram (**Figure 6**). Consistent with the concept of simplicity, image data was acquired with a photo scanner for high resolution and image

quality, while a cellphone camera also suffices the need of quantification with enhanced portability and lower power consumption (**Figure 6**).

Table 1. Application for short tandem repeat (STR) analysis. *The PCR reaction forSTR analysis typically requires 0.5 - 2 ng/μL DNA. Three DNA samples were measured with the PAB assay, and Sample C was determined as > 2.83 pg/μL because the result locates outside the dynamic range shown in Figure 5A. A comparison of the electropherograms shows that the interpretable result was only generated from the sample with appropriate DNA concentration.*

Sample	[DNA] (ng/μL)			Capillary Electrophoresis Results after PCR
	Pinwheel (n=3)		UV-Vis Spectroscopy	
	Mean	Standard Deviation		
A	0.67	0.46	0.11	Insufficient Amplification
B	1.24	0.49	1.08	All Peaks Correctly Identified
C	> 2.83	0.45	6.47	Off-scale peaks ("pull-up")

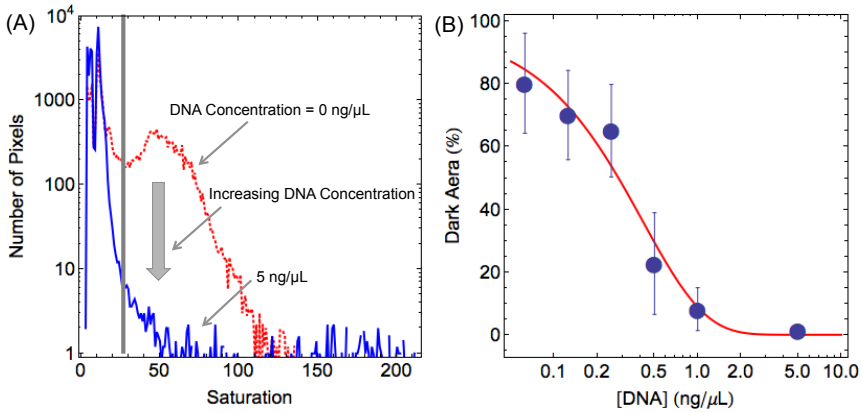

Figure 6. Towards a portable assay for point-of-care applications. *The images of blotted beads on filter paper can also be acquired by a camera phone, which still yield quantitative results (A) histogram (B) calibration curve. Error bars denote the standard deviation of four replicates.*

CONCLUSIONS

In conclusion, we report a paper-based label-free DNA quantification method with minimal footprint, cost, and power consumption. The assay provides quantitative information on DNA concentration in a simple and fast fashion, which can interface with lab-on-paper devices for DNA extraction with microchip PCR. This could advance the development of cost-effective microfluidic systems for point-of-care testing in resource-limited regions.

REFERENCES

[1] V. Govindarajan, *et al*, *A low cost point-of-care viscous sample preparation device for molecular diagnosis in the developing world; an example of microfluidic origami*, Lab Chip, 12, 174 (2012).

[2] D. C. Leslie, J. Li, *et al*, *New Detection Modality for Label-Free Quantification of DNA in Biological Samples via Superparamagnetic Bead Aggregation*, J. Am. Chem. Soc. 134, 5689−5696 (2012).

[3] J. Li, *et al*, *Pinwheel Assay Via a 'Pipet, Aggregate and Blot' (PAB) Approach on Filter Paper*, Proc. Micro Total Analysis Systems Conferences, 1959-1961 (2011).

CONTACT

James P. Landers, tel: 1-434-243-8658; jpl5e@virginia.edu