

SIMULTANEOUS DNA PURIFICATION AND FRACTIONATION IN AGAROSE GEL ON THE MICRO-SCALE

Burcu Gumuscu, Albert van den Berg, and Jan C.T. Eijkel

BIOS Lab-on-a-Chip Group, MESA+ Institute for Nanotechnology and MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, The Netherlands

ABSTRACT

We report a new and simple approach for preparative purification and fractionation of sub-10-kbp DNA molecules in a microfluidic device. Agarose gel with 1.2% concentration is used as the separation matrix. A 0.5-10 kbp DNA ladder is fractionated and separated from other ionic species in continuous flow within 2 minutes by periodically switching between two orthogonal electrical fields of strongly differing magnitude. The high-resolution separation is based on the variation in field-dependent mobility (FDM) for differently-sized DNA fragments.

KEYWORDS: DNA fractionation, continuous flow, biased reptation, field dependent mobility.

INTRODUCTION

In gel electrophoresis, DNA exhibits a field-dependent mobility (FDM).[1](Fig. 2c) FDM has been used for concentrating DNA fragments in the SCODA method[4,5], but its potential for fractionation has thus far been overlooked.

EXPERIMENTAL

The microchip (Fig. 1a) consists of a 10 mm by 10 mm chamber of 20 μm height, which is connected to buffer reservoirs via microchannels (50 μm x 10 mm, 50 μm periodicity) on four sides. Microchannels enable the application of uniform electric fields over the separation chamber.[6] Fractionation of 0.5-10 kbp DNA molecules was achieved by periodically applying orthogonal DC electric fields E_1 and E_2 at frequencies between 0.016 and 33 Hz (Fig.2a-b). Optimal fractionation was obtained using $E_1=59.5$ V/cm and $E_2=24.6$ V/cm at 2 Hz (Fig.2a).

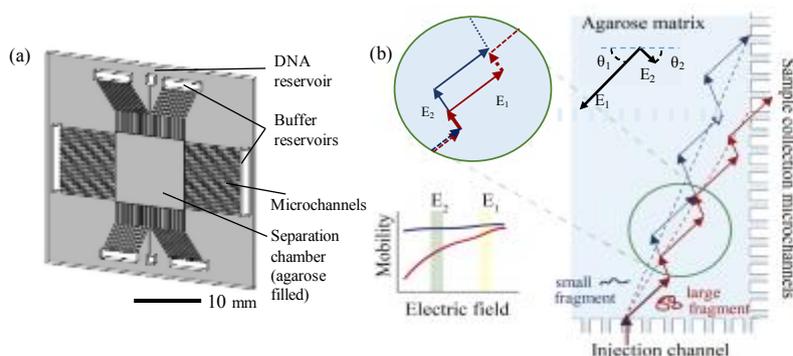


Figure 1: (a) Schematic illustration of the microchip and its components. (b) Orthogonal electric fields E_1 and E_2 , angles θ_1 and θ_2 , and separation mechanism based on the field-dependent mobility (FDM), where large DNA moves slower in the low field E_2 .

RESULTS AND DISCUSSION

The migration angle ϕ with respect to the horizontal axis can be approximated by
$$\phi = \text{atan} \left(\frac{\sin \theta_1 + \left(\frac{E_2 \mu_2 - 2fL}{E_1 \mu_1 - 2fL} \right) \sin \theta_2}{\cos \theta_1 - \left(\frac{E_2 \mu_2 - 2fL}{E_1 \mu_1 - 2fL} \right) \cos \theta_2} \right),$$
 showing a combination of FDM ($E\mu$ terms) and the ‘switchback’ mechanism as used in the DNA prism [2,3] ($2fL$ terms). Here μ_1 and μ_2 are the fragment mobilities at E_1 and E_2 , θ_1 and θ_2 the angles as indicated in Fig. 1b, f the applied frequency, and L the DNA contour length.[3] Fig. 2c shows the measured DNA mobilities, and Fig. 2b plots this equation using measured mobilities. At low frequencies where $E\mu \gg 2fL$, ϕ is a only function of μ_2/μ_1 (FDM), and since μ_2/μ_1 is smaller for large fragments than for small fragments (Fig. 2c), separation then occurs by FDM (Fig. 2b). Fig. 1b illustrates the FDM mechanism, with long fragments following a trajectory closer to θ_1 , and short fragments following a larger angle path. For non-reptating ionic species the $2fL$ term drops, and since $\mu_2 = \mu_1$ at all applied fields they migrate at an angle $\phi_0 = \text{atan} \left(\frac{E_1 \sin \theta_1 + E_2 \sin \theta_2}{E_1 \cos \theta_1 - E_2 \cos \theta_2} \right)$, larger than all DNA fragments. Fig. 2e demonstrates the separation of NaFluorescein from all DNA fragments. The ‘Switchback’ mechanism contributes to the separation only when $2fL \sim \mu E$, occurring

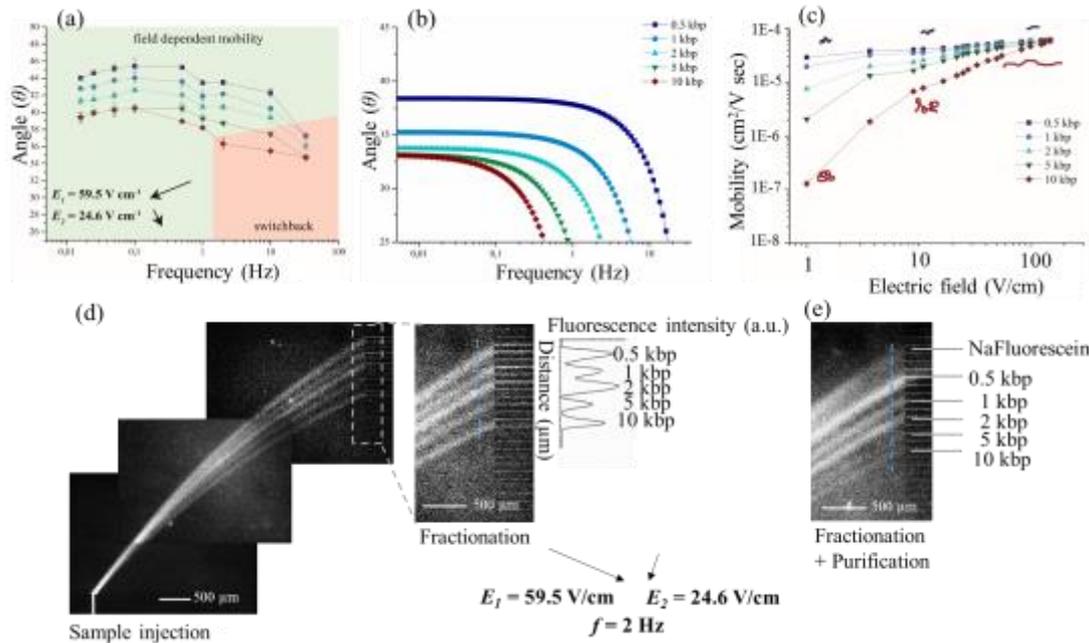


Figure 2: (a) Frequency spectra of deflection angle θ for the fragments when different E_1 and E_2 were applied. (b) Calculated angle-frequency plot based on Eq.1 for the separation obtained applying $E_1 = 59.5$ V/cm and $E_2 = 24.6$ V/cm. (c) Mobility of individual DNA fragments as a function of electric field. The red and dark blue figures present the molecular conformation of large and small fragments at low and high electric fields. (d) Fluorescence image of fractionating 0.5–10 kbp DNA in a agarose sieving matrix. (e) Fluorescence image of fractionating and purifying 0.5–10 kbp DNA molecules from Fluorescein sodium salt (NaFluorescein).

at frequencies above 0.2 Hz for 10 kbp fragments (Fig.2c).[2,3] We maximally exploited the FDM mechanism by applying orthogonal electrical fields of strongly different magnitude. Device throughput is 0.18 ng molecules/hour at the DNA input concentration used (12.5 ng/ μ l), comparable to that of previously-reported micromachined devices.[2,6,7]

CONCLUSION

The microfluidic device performs purification and high-resolution fractionation of DNA molecules, yet is easy to fabricate and operate. This simple and flexible gel technology offers great promise for addressing second-generation sequencing challenges, including low-cost and high-resolution purification and fractionation of DNA sizes of interest. Furthermore, the separation matrix can easily be modified for protein gel electrophoresis by replacing agarose with polyacrylamide.

ACKNOWLEDGEMENTS

This work was funded by the Dutch network for Nanotechnology, NanoNext NL, in the subprogram “Nanofluidics for Lab-on-a-chip”.

REFERENCES

- [1] J. L. Viovy, *Rev. Mod. Phys.*, 72, 813, 2000.
- [2] Y. Zeng, M. He, D. J. Harrison, *Angew. Chem. Int. Ed.*, 47, 6388, 2008.
- [3] Z. Chen, K. D. Dorfman, *Phys. Rev. E*, 87, 012723, 2013.
- [4] J. Pel, D. Broemeling, L. Mai, H.L. Poon, G. Tropini, R.L. Warren, R.A. Holt, A. Marziali, *Proc. Natl. Acad. Sci.*, 106, 14796, 2009.
- [5] K.D. Dorfman, S.B. King, D.W. Olson, J.D.P. Thomas, D.R. Tree, *Chem. Rev.*, 113, 2584, 2013.
- [6] L.R. Huang, J.O. Tegenfeldt, J.J. Kraeft, J.C. Sturm, R.H. Austin, E.C. Cox, *Nature Biotechnol.*, 20, 1048, 2002.
- [7] J. Fu, R.B. Schoch, A.L. Stevens, S.R. Tannenbaum, J. Han, *Nature Nanotech.*, 2, 121, 2007.

CONTACTS

*B.Gumuscu; phone: +31653888927; b.gumuscu@utwente.nl J.C.T. Eijkel; phone: +31534892839; j.c.t.eijkel@utwente.nl.