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

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## 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).

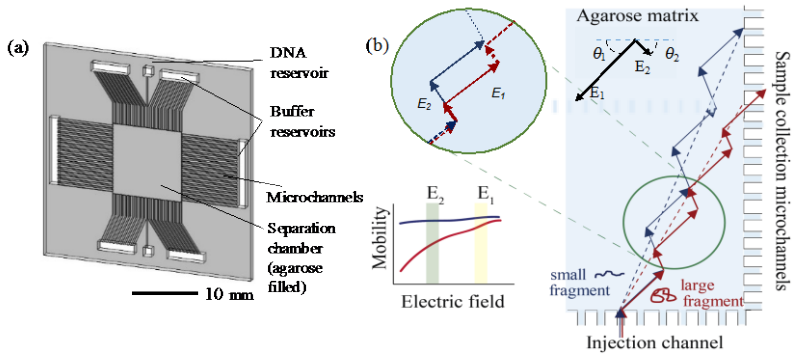


***Figure 1: (a)*** *Schematic illustration of the microchip and its components.* ***(b)*** *Orthogonal electric fields $E_1$ and $E_2$, angles $\theta_1$ and $\theta_2$, and separation mechanism based on the field-dependent mobility (FDM), where large DNA moves slower in the low field $E_2$..*

## RESULTS AND DISCUSSION

The migration angle $\emptyset$ with respect to the horizontal axis can be approximated by $\emptyset = \text{atan}\left(\frac{\sin\theta_1+\left(\frac{E_2\mu_2-2fL}{E_1\mu_1-2fL}\right)\sin\theta_2}{\cos\theta_1-\left(\frac{E_2\mu_2-2fL}{E_1\mu_1-2fL}\right)\cos\theta_2}\right)$, showing a combination of FDM ($E\mu$ terms) and the "switchback" mechanism as used in the DNA prism [2,3] ($2fL$ terms). Here $\mu_1$ and $\mu_2$ are the fragment mobilities at $E_1$ and $E_2$, $\theta_1$ and $\theta_2$ 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 $E\mu >> 2fL$, $\emptyset$ is a only function of $\mu_2/\mu_1$ (FDM), and since $\mu_2/\mu_1$ 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 $\theta_1$, 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 $\emptyset_0 = \text{atan}\left(\frac{E_1\sin\theta_1+E_2\sin\theta_2}{E_1\cos\theta_1-E_2\cos\theta_2}\right)$, 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 $2fL \sim E\mu$, occurring

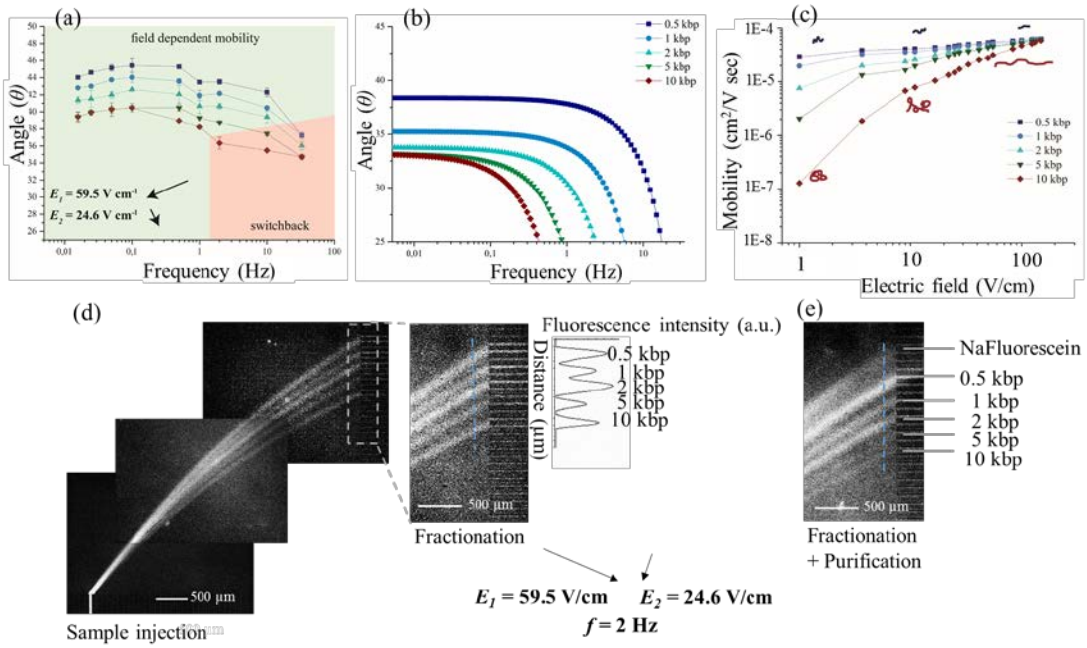


***Figure 2:*** ***(a)*** *Frequency spectra of deflection angle θ for the fragments when different $E_1$ and $E_2$ were applied.* ***(b)*** *Calculated angle-frequency plot based on Eq.1 for the separation obtained applying $E_1$=59.5 V/cm and $E_2$=24.6 V/cm.* ***(c)*** *Mobility of individual DNA 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 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/µl), 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.

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