Resolving DNA in free solution

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DNA molecules are usually separated by gel electrophoresis. While gels help resolve DNA, the polymers bring problems to the analysis (e.g., difficulty in automating and poor reproducibility). One way to eliminate these problems completely is to separate DNA in free solutions. However, DNA fragments cannot normally be resolved in a free solution by electrophoresis because the electrophoretic mobilities of these molecules have similar charge-to-mass ratios, especially when the fragments are large.

Efforts have been invested in free-solution DNA separations and a number of approaches (e.g., high-performance liquid chromatography, end-labeled free-solution electrophoresis, entropic traps, and DNA prism) have been successfully developed. Very recently, a new technique, called bare narrow capillary-open tubular chromatography (BaNC-OTC), was developed and can resolve a wide range of sizes of DNA in a few minutes.

In this article, we review the significant progress on free-solution DNA separations, with emphasis on the most recent, advanced developments.

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Keywords: Bare narrow capillary-open tubular chromatography (BaNC-OTC); DNA prism; DNA separation; Electrophoresis; End-labeled free-solution electrophoresis (ELFSE); Entropic trapping; Free solution; High-performance liquid chromatography (HPLC); Lab-on-a-chip (LOC); Radial migration

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1. Introduction

DNA separation and analysis are important for molecular biological research. Deoxyribonucleic acid (DNA) is a large molecule that contains the genetic instructions used in developing all living organisms. Genes are the DNA segments that carry this genetic information. In a human body, there are about 20,000–25,000 genes that govern the biological processes throughout life (birth, growth, diseases, and aging until natural death) [1]. Analysis of DNA is essential to understand and, to a certain degree, to manipulate how DNA works (e.g., in everyday life, a doctor increasingly uses a patient's genetic information to diagnose a genetic disease, a court uses the comparison of the DNA of an accused with that found at the crime scene to decide on guilt or innocence, the Centers for Disease Control and Prevention (CDC) scientists (and their equivalents elsewhere) use the DNA fingerprints of a microorganism to identify the specific strain of a pathogen). In all these cases, DNA separation and analysis are performed.

DNA separations are usually carried out by electrophoresis. The study of DNA electrophoresis began in the early 1960s [2–4] when it was concluded that DNA fragments larger than ~400 base-pairs (bp) could not be resolved in free solutions. In the early 1970s, gel-electrophoretic techniques became commonly used for DNA separations [5–7] usually in a slab-gel format [8]. While good separations have been achieved, the drawbacks include excessive Joule heating, tedious and time-consuming manual operation, and low separation speed [9]. Further, the separated fragments need to be stained for detection. Compromises have to be made in choosing a staining agent (e.g., an otherwise attractive reagent, ethidium bromide, happens to be carcinogenic). Conventional slab-gel electrophoresis cannot usually resolve DNA fragments larger than 40~50 kbp [10,11]. Large fragments are often separated by pulsed-field gel electrophoresis (PFGE) [12], which is even slower (with running times of 10–200 h [13]).

In order to enhance the resolving power, reduce the separation time, and increase the throughput, many have resorted to capillary gel electrophoresis (CGE) for DNA separations [14,15]. A variant, capillary-array gel electrophoresis, uses parallel separation capillaries for greater throughput [16,17]. Sieving

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Table 1. Summary of major approaches used for free-solution DNA separations

Approach	Characteristics	DNA tested	Ref.
HPLC	Advantages: Excellent tool for DNA separation, purification and preparation; Good reproducibility and accurate quantitation; automated separation; Direct UV detection (elimination of DNA labeling/staining).	*Hin*dIII-cleaved DNA fragments (125 bp-23 kbp), 1 kbp ladder, oligonucleotide of 90-mer, *p*BR322-*Hae*III fragments	[22]
		(ion–exchange chromatography)	[36]
	Disadvantages: One-column/lane; limited resolution for DNA fragments larger than 10 kbp; Sequence-dependent retention times (in ion-exchange); column clogging (in gel permeation).	dsDNA (51-2176 bp) (Ion-pair RP chromatography)	[23]
		Restriction fragments, plasmids, RNA-DNA hybrids, mitochondrial DNA (size exclusion chromatography)	[24]
	Note: in hydrodynamic chromatography, long DNA fragments are eluted out sooner, while in most of other chromatographic modes, long DNA molecules are eluted out later.	λ DNA, λ/*Hin*dIII digest (slalom chromatography)	[25]
		Circular dsDNA molecules (3, 5 and 10 kbp) (hydrodynamic chromatography)	[26]
ELFSE	Advantages: high-resolution for short fragments; capable of DNA sequencing.	100 bp DNA ladder, DNA sample with 98, 127, 140, 225, 237, 271, 356, 367, and 878 bp	[29]
	Disadvantages: limited resolution for long (>1 kbp) fragments; requirement of drag-tag attachment.	A 5'-biotinylated 22-mer primer, ssDNA of M13mp18	[49]
		Three loci of the human p53 gene	[53]
		dsDNA of 50, 75, 100, and 150 bp	[54]
		ssDNA of 88, 134, 216, and 447 bases	[55]
		ssDNA of M13mp18	[52]
Entropy trapping	Advantages: integration with LOC devices; fast separation, minimal sample requirements.	T2 DNA (164 kbp) and T7 DNA (37.9 kbp), Mono Cut Mix sample, 5 kbp ladder sample	[31]
	Disadvantages: limited resolution; sophisticated fabrication processes; uncertain device/operation costs.	A low-molecular-weight DNA ladder containing 50, 150, 300, 500, and 766 bp	[61]
		A low-molecular-weight DNA ladder containing 50, 150, 300, 500, and 766 bp, λ DNA-*Hin*dIII digest containing 2322, 4361, 6,557, 9416, and 23130 bp.	[62]
DNA prism	Advantages: integration with LOC devices; fast separation, potential for DNA preparation. Disadvantages: limited resolution; sophisticated fabrication processes; uncertain device/operation costs.	BAC and PAC of 61, 114, 158, and 209 kbp	[32]
Radial migration	Advantages: use of commercial CZE instrument; having all advantages of CZE. Disadvantages: limited resolution.	A mixture of λ DNA (48 502 bp) and linear ϕX174 RF DNA (5386 bp)	[64]
BaNC-TOC	Advantages: integration with LOC devices; simple devices; capability of separating wide-size range of DNA in a single run; fast separation; minimal sample requirements; low operation cost.	ssDNA of 5, 10, 15 and 20 bases	[33]
		λ DNA, 1 kb ladders, 100 bp ladder, real-world genotyping samples (195&150 bp, 198&174 bp)	[33]
			[34]
		dsDNA of 75 bp-106 kbp	[34]
	Disadvantages: requirement of narrow (<2 μm-i.d.) capillaries; challenges in injecting samples reproducibly.	λ *Hin*dIII digest DNA, 100 bp DNA ladder	[79]
Other nanofabricated/ Nanostructure devices	Advantages: integration with LOC devices; fast separation; potential for integrated DNA analysis chip.	10 bp-100 bp DNA	[70]
		2 kbp–10 kbp DNA	[72]
		48.5 kbp and 1 kbp DNA	[73]
	Disadvantages: limited resolution; sophisticated fabrication processes; uncertain device/operation costs.		

matrices used in CGE are either cross-linked matrices in a single-use or limited-use capillary or replaceable polymeric matrices in capillaries that will be repeatedly refilled and reused. Both approaches have problems. Cross-linked polyacrylamide gels are difficult to prepare reproducibly [18,19]. The replaceable polymer matrices require high pressures for loading and replacement after each run [14,20,21].

All problems associated with polymeric matrices would naturally disappear if separations could be performed in free solutions. As previously stated, free-solution electrophoresis, which separates charged species based on their mass-to-charge ratios (m/z), cannot resolve DNA because all DNA molecules have similar m/z; their electrophoretic mobilities are virtually independent of size. Various attempts have been made to

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