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Research Article

Light-emitting diode induced fluorescence (LED-IF) detection design for a pen-shaped cartridge based single capillary electrophoresis system

CGE is a well-established separation technique for the analysis of biologically important molecules such as nucleic acids. The inherent high resolving power, rapid analysis times, excellent detection sensitivity, and quantification capabilities makes this method favorable compared to conventional manual polyacrylamide and agarose slab gel electrophoresis techniques. In this paper we introduce a novel single-channel capillary gel electrophoresis system with LED-induced fluorescence detection also utilizing a compact pen-shaped capillary cartridge design for automatic analysis of samples from a 96-well plate. To evaluate the suitability of the system, 1000 genomic DNA (gDNA) samples were analyzed in gel filled capillaries and detected by the microball ended excitation and emission optical fiber based LED-induced fluorescence detection system. Excellent migration time reproducibility of RSD $<0.75\%$ was obtained over the course of 1000 runs. The system rapidly distinguished between intact and degraded gDNA samples, therefore provided important information if they could be used for downstream quantitative PCR processing where high-quality intact gDNA was key. We envision that this novel system design will rapidly find new applications in both research and clinical diagnostic laboratories as a highly sensitive and easy to use bio-analytical approach.

Keywords:
Fluorescence detection / Genomic DNA purity / Light emitting diode / Quality control / Single-channel capillary gel electrophoresis
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1 Introduction

High-throughput DNA separation techniques are under rapid development, originally triggered by the Human Genome Project [1–3]. At the present time, most bioanalytical laboratories still utilize manual polyacrylamide and/or agarose slab gel based electrophoresis techniques for DNA fragment analysis, methods that are time consuming and labor intensive, also requiring improvements in terms of resolving power and throughput of analysis [3, 4]. CE, on the other hand, is rapidly becoming a more widely accepted separation technique in the biochemistry and molecular biology laboratories [5, 6]. Indeed, CE is now commonly accepted by the biotechnology industry, especially in nucleic acid-based testing, as a reliable, automated approach offering rapid separations with high sensitivity, excellent resolution, ruggedness and ease of operation. In addition to DNA analysis, CGE has also been widely applied for protein and carbohydrate analysis [7, 8]. Rapid separation of ssDNA or dsDNA fragments is a need in many molecular biology laboratories [9], including restriction fragment mapping, PCR product analysis, mutation detection and DNA sequencing. Combining the sensitivity of fluorescence detection with the versatility of a replaceable polymer matrix and the possibility of automation in both sample handling and data analysis, CGE has become an attractive alternative to traditional slab gel techniques [10, 11].

There are two main types of fluorescence detection systems for CE utilizing (i) on column irradiation of the capillary tubes and (ii) irradiation at a sheath-flow region at the end of the capillary. The first CE instrument with LIF detection was described by Zare and co-workers [12] with greatly improved limits of detections [13]. Later, Mathies et al. introduced a confocal fluorescence detection system [14, 15]. This method employed the very same lens set to focus the illuminating laser beam into the center of the capillary and collect the fluoresced light emitted by the fluorophore-labeled sample (either covalently or dynamically tagged). The confocal setting proved to be very efficient and relatively simple;

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Abbreviations: gDNA, genomic DNA; **LED-IF**, LED induced fluorescence

Colour Online: See the article online to view Figs. 1–3 in colour.

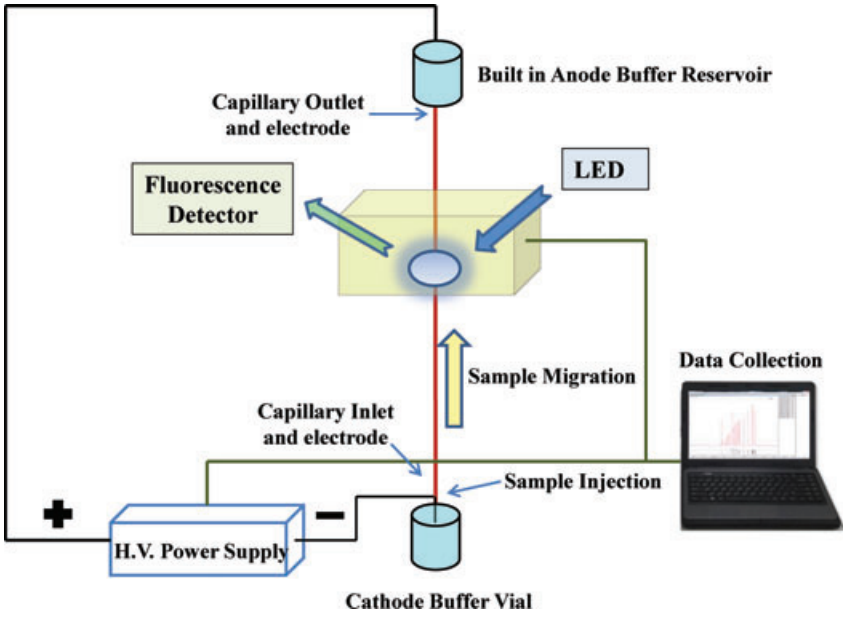

Figure 1. Schematic representation of the CGE instrument.

however, some reoptimization of the alignment was usually necessary on a daily basis [16, 17]. The sheath-flow cell and optical detection system has been reported by Dovichi and co-workers [18]. In their design, the sheath-flow fluorescence detection cell was applied between the end of the capillary and the buffer reservoir. Sheath-flow technology moved the irradiation/detection area from the capillary tubes thus provided very low background [19]. Recently emerging microfluidics approaches represent a new generation of capillary-based instruments for nucleic acid analysis also utilizing laser- (LIF) or LD induced fluorescence (LED-IF) as excitation sources. The latter one has recently been introduced as inexpensive and powerful alternative light source for fluorescence detection. New generation LEDs feature very high output power covering the entire visible wavelength range and even some of the UV region. These advantages combined with their very compact dimensions and low cost render them suitable for integration into small and compact instrument designs [20].

In this paper, we describe an efficient microball ended fiber optic based CE detection system with easy alignment for LED illumination. This novel single CGE system utilized a pen-shaped capillary cartridge and was applied for large-scale analysis of 1000 genomic DNA (gDNA) samples.

2 Materials and methods

2.1 Chemicals

For CGE separations the Qsep100 DNA-CE gel buffer and Qsep100 DNA-CE running buffer were used (BiOptic, New Taipei City, Taiwan). Other reagents and chemicals for sample preparation were purchased from Sigma-Aldrich (St. Louis, MO, USA). The GeneRuler 1 kb Plus DNA ladder (0.1 μg/μL, 75–20 000 bases) and GeneRuler 100 bp Plus DNA ladder (0.1 μg/μL, 100–3000 bases) were purchased from Biocenter (Szeged, Hungary). Precast agarose gels, as well as the separation and imaging apparatus for agarose slab gel electrophoresis analysis were from Life Technologies (Darmstadt, Germany). All buffers and reagents were filtered through 0.22-μm-pore size Acrodisc syringe filters (Millipore, Billerica, MA, USA) and degassed prior to use.

2.2 Sample preparation

For CGE, the gDNA samples were in TE buffer (10 mM tris, 1 mM EDTA, adjusted to pH 8.0 with HCl) and kept at −20°C until use. Before CGE analysis, the samples were diluted with HPLC-grade water (Sigma-Aldrich) to 25 ng/μL. The GeneRuler 1 kb Plus DNA molecular mass marker was diluted with HPLC water to 50 ng/μL final concentration. After dilution, all gDNA samples and DNA sizing ladders were aliquoted and kept at −20°C until use.

For agarose slab gel electrophoresis, the GeneRuler 1 kb Plus DNA ladder was diluted to a final concentration of 0.1 μg/μL. A total of 500 ng gDNA samples (in TE buffer: 10 mM tris, pH 8.0, 1 mM EDTA, adjusted to pH 8.0 with HCl) were applied into the sampling wells of the agarose gels.

2.3 Instrument design

In all experiments a novel single-channel CE system was used (Qsep100 DNA-CE unit, BiOptic) with real-time LED-induced fluorescent detection, utilizing a disposable, pen-shaped cartridge that incorporated a single separation capillary in a compact injection molded body with an integrated running gel-buffer reservoir, directly coupled to a modular nitrogen pressure source to accommodate separation

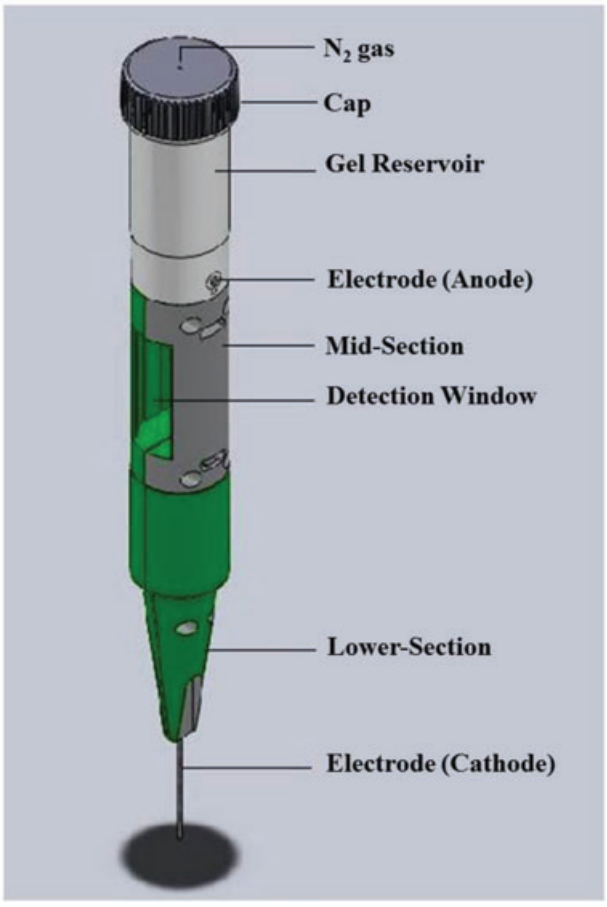

Figure 2. The front view of a pen-shaped capillary cartridge.

matrix replacement. A high-voltage power supply (EMCO, Sutter Creek, CA, USA) was used to deliver 500 V–20 kV of electrical potential to the capillary for injection and separations. The optical detection system comprised a super-bright royal blue LED (Cree XLamp, New York, NY) as excitation radiation source. The detection setup consisted of microball ended incident (excitation fiber) and output (emission fiber) optical fibers. The excitation fiber delivered the excitation light from an LED (505 nm with a FWHM of about 30 nm for dsDNA analysis) and the emission fiber collected the emission signal through the detection zone/window and transferred it to a high sensitivity multialkali PMT for data acquisition (Hamamatsu Photonics, Bridgewater, NJ, USA). The PMT detector had a build in emission filter (Long pass filter 590–650 nm) to improve detection sensitivity (Newport Co., Irvine, CA, USA). The fully automated CGE instrument also included an automated modular X-Y-Z translation stage mechanism with a hybrid linear actuator and stepper motor (Haydon Motion Solutions, Waterbury, CT, USA) for buffer and sample tray motion, which was able to accept either a 12-well sample strip or a 96-well microtiter plate interfacing with the disposable single-capillary gel-cartridge.

2.4 CGE

CGE separations were carried out in the Qsep100 DNA-CE single-channel CE unit (BiOptic) with a single-channel capillary cartridge, containing a 75 μm id bare fused silica capillary with the effective separation length of 11 cm (total length: 15 cm). Data analysis was performed using the Q-Analyzer software package (BiOptic). New capillaries were first filled with 5 mL 70°C MilliQ-grade water (Billerica,) and the capillary was rinsed for 500 s followed by the transfer of 5 mL Qsep100 DNA-CE gel buffer into the gel reservoir and purging for 2 × 1000 s. Prior to each injection the sieving matrix was replaced in the capillary by means of a 10 s purge step. The sample injection process was preceded by the introduction of an HPLC-grade water plug (4 kV for 3 s). The samples were introduced electrokinetically from a 96-well plate (4 kV for 6 s). All separations were carried out at ambient temperature applying 8 kV for 300 s.

2.5 Agarose slab gel electrophoresis

Agarose slab gel electrophoresis was carried out in an E-Gel iBas Power System (Life Technologies) for separation and the E-Gel Safe Imager Real-Time Transilluminator (Life Technologies) for real time viewing of sample migration. Precast agarose gels containing 2% agarose (2 × 8 wells, separation range: 100 bp–2 kb) E-Gel 2% double comb with Ethidium Bromide (Life Technologies) was applied for the separation of the gDNA samples and DNA molecular mass marker. The sizing ladder and gDNA samples were injected into the sample wells and electrophoresed in the E-Gel system for 8 min. The separated DNA bands were visualized by a blue light transilluminator in real time. Ultimately, the results were documented by an Alpha DigiDoc AD-1201 imaging system (Bio-Science, Budapest, Hungary) equipped with an Olympus digital camera (C-4000ZOOM) with appropriate filters (Olympus Hungary, Budapest, Hungary).

3 Results and discussion

The system described in this paper was developed for dsDNA fragment analysis using a compact pen-shaped capillary cartridge and microball ended optical fibers based LED-induced fluorescence detection setup. Figure 1 shows the block diagram of the instrument, including the high-voltage power supply, the separation platform and the principle of LED-IF. The system utilized electro-kinetic injection for dsDNA fragment introduction. As the lower electrode (the cathode) with its imbedded capillary tubing was immersed into the sample well, the applied voltage electrokinetically forced the negatively charged sample components (dsDNA fragments) into the capillary tubing, which then migrated and separated upstream within the separation gel-buffer system filled capillary toward the anode. As the fluorescently labeled gDNA fragments migrated through the detection area of the

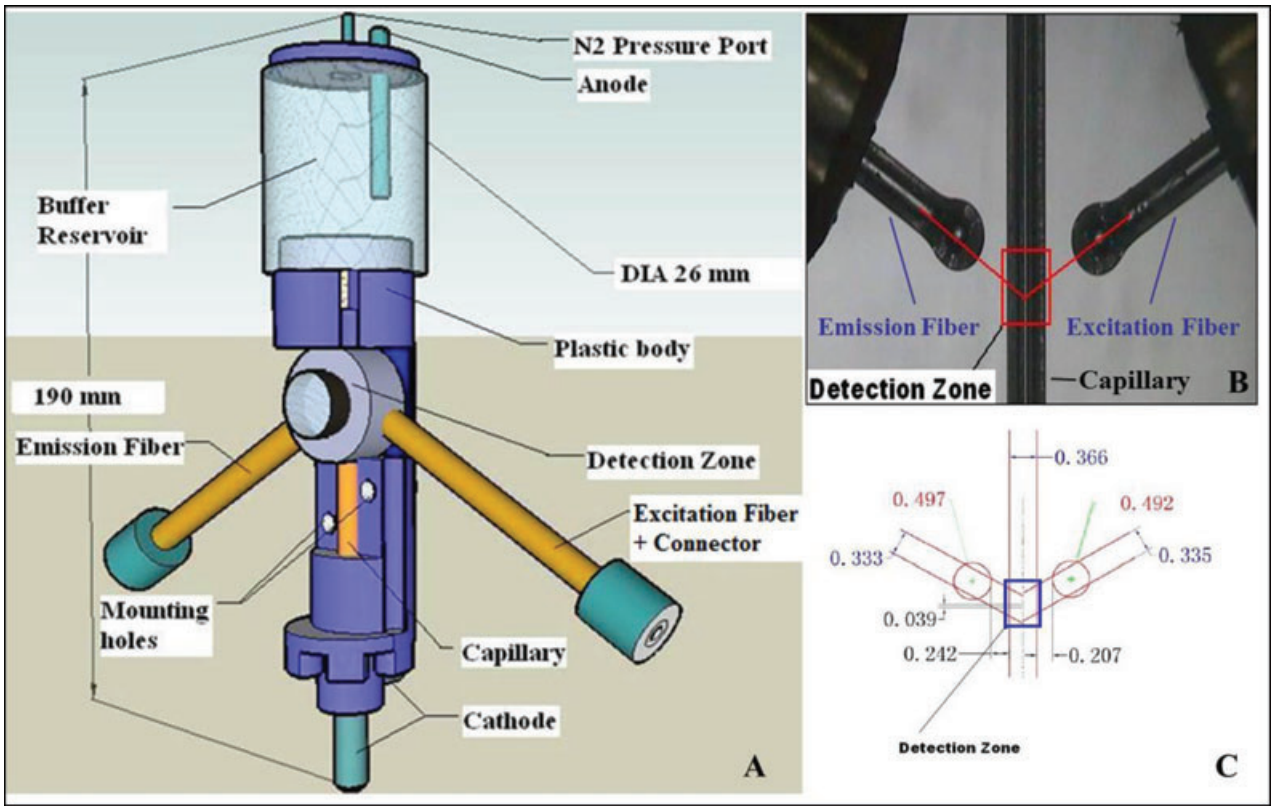

Figure 3. Detection optics design. (A) Schematic illustration of the detection section of the pen-shaped capillary cartridge incorporating the detection optic configuration, (B) The actual center plane sectional view at the detection region in the capillary cartridge in panel A, (C) Dimensions of the microball ended incident and output optical fibers as well as the separation capillary.

separation capillary, the excitation fiber transported the excitation light from the LED source and the emission fiber collected the fluorescent signal through the detection window and transferred it to the PMT for data acquisition.

3.1 The capillary cartridge design

Figure 2 shows the schematics of the pen-shaped cartridge design including a single separation capillary column, a lower (cathode) and an upper (anode) electrode, a detection window, and a gel reservoir connected to a nitrogen gas source. Application of nitrogen gas provided the required pressure to fill the capillary with the separation gel-buffer system. Depending on the viscosity of the separation matrix, pressures of up to 60 psi could be applied to the capillary through the top buffer reservoir. The inside wall of the separation column was dynamically coated by the separation matrix to suppress electroosmotic flow and prevent adsorption of the analyte molecules. The overall size of the cartridge was designed in a way that the length of the separation capillary was in the range of 15–20 cm (75 um id and 360 um od). The cartridge included the top and bottom electrodes, an exposed detection zone/window as well as an embedded radio frequency identification label to track the number of runs and provide identification labels for the cartridge type. The cartridge was made of injection molded plastic with stainless steel electrodes and did not include the detection optics. The external detection optics automatically clamped onto the capillary cartridge when it was installed into the holder, not requiring any fine alignment in respect to the detection zones. This approach provided simplicity in the capillary cartridge design and accommodated easy cartridge replacement. The sample handling module was designed to handle three axis of motion with two built in motors. One was a hybrid type providing dual motions of vertical and rotation (up and down) and the other motor was for the horizontal motion. The sample tray was designed to handle either single microfuge tubes or a 96-well tray.

3.2 The optical track design

Once the capillary cartridge was placed in the instrument, a pneumatically actuated fork assembly aligned the two microball ended excitation and emission fibers precisely to the detection zone of the fused silica capillary. Figure 3A illustrates the configuration of the detection optics. The incident radiation (from an LED) was provided to the detection zone and the output radiation was collected from the detection zone, using microball ended optical fibers as shown in Fig. 3B positioned at approximately 140° apart from each other to maximize detection sensitivity. Both excitation and

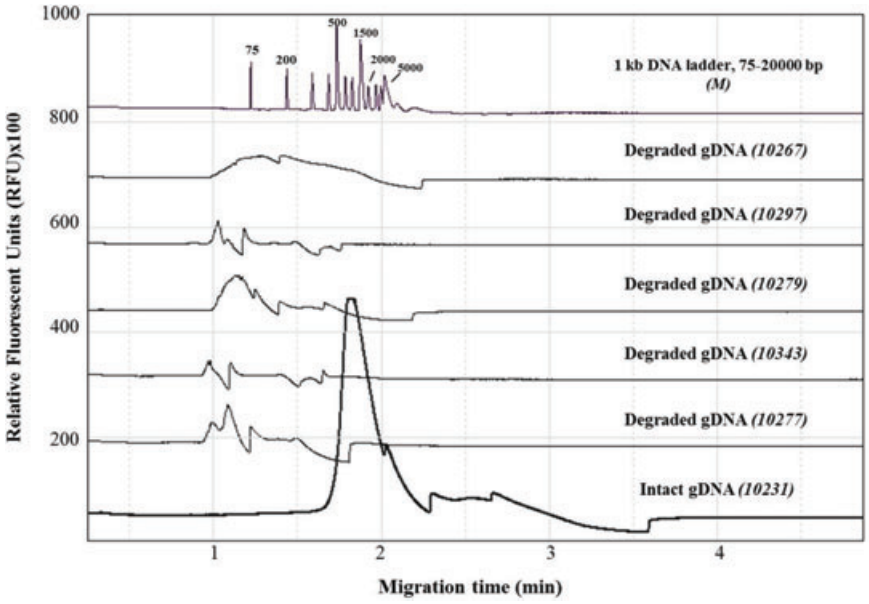

Figure 4. Representative electropherograms of selected gDNA samples. Upper trace: DNA sizing ladder (75 bases to 20 kilobases), Middle traces: degraded gDNA samples (sample IDs 10267, 10297, 10279, 10343 and 10277), Lower trace: intact gDNA sample (sample ID 10231). Conditions: injection: 4 kV/6 s; separation voltage 8 kV/300 s; capillary: 75-μm id, total length of 15 cm length (effective separation length: 11 cm); ambient temperature.

emission fibers with microball tips were positioned at the opposite sides of the separation capillary in a noncontact mode to reduce background fluorescence. To prevent any physical damage to either the capillary or the microballs, the tips of the microball end of the excitation and emission fibers were spaced at approximately 200–250 μm from the external surface of the separation capillary column. Both the excitation and emission fibers had 200-μm-diameter cores to guide light within an external cladding, and 350-μm-diameter microball shaped tips (i.e. the ratio of the fiber core diameter to the ball diameter was 1:1.75) as illustrated in Fig. 3C. A fiber optic fusion splicer was used to produce the microball lenses at one end of the excitation and emission collection fibers by heat melting. This microball lens produced a very robust optical fiber assembly and effective optical alignment with respect to the capillary tubing. By having the microball at the end of the fibers there were no additional micro lenses necessary simplifying in this way the optical alignment. The microballs also provided higher numerical aperture and concomitantly higher power density focused inside the capillary, and additionally provided strength to the tip of the fiber from not getting scratched or to brake if there was a mechanical impact to the component.

The angles of the excitation and emission fibers were about 70°. Compared with flat-end fibers (i.e. bare fiber, without microball lenses) the microball ended fibers provided good focusing of incident radiation for the excitation fiber and high collection efficiency (high numerical aperture, >0.3 NA) for the emission fiber. The distal output end of the excitation fiber allowed good coupling efficiency inside the separation channel to obtain high fluorescence detection sensitivity. The modular design and fiber optic coupling enabled easy exchange of the excitation radiation sources, for example, to a laser module or other type of light sources.

3.3 Large-scale gDNA analysis

To evaluate the suitability of the system, the degradation level of a thousand gDNA samples were analyzed to assess their quality. The ability to rapidly distinguish between intact and degraded gDNA samples was crucial for downstream quantitative PCR processing. Figure 4 compares the results of the analysis of representative intact and variously degraded gDNA samples. A DNA molecular mass marker in the base pair range of 75–20 000 bp was used for fragment size determination in a final concentration of 50 ng/μL (upper trace). Good, nondegraded intact gDNA samples featured a large peak at the 2000 bp range of the separation window (trace: intact gDNA, sample number: 10231). The variously degraded gDNA samples gave peak patterns at the lower bp range of several hundreds, i.e. strongly suggesting sample degradation (traces: degraded gDNA, sample numbers: 10267, 10297, 10279, 10343, 10277). For comparative purposes, Figure 5 shows the results of the analysis of the same gDNA samples on 2% agarose slab gel electrophoresis. Please note that compared to CGE, several degraded DNA fragments (sample numbers: 10343, 10297) could not be detected on agarose gels, while the degraded DNA peak patterns were clearly visible in CGE.

The migration time reproducibility of the separation system was assessed by a 10-run repeatability study. The 100 bp Plus DNA molecular mass marker (size range of 100–3000 bp) was used for this evaluation in a final concentration of 25 ng/μL. Each injection was from the same well of the 96-well plate. The results are summarized in Table 1, showing that the average migration times of the DNA sizing ladder fragments were between 88.22 and 143.22 s in the range of 100–3000 bases. The average percent RSD (RSD%) of the migration times for the fragments ranging from 100 to

Table 1. Ten-run migration time reproducibility study using the 100–3000 bp DNA fragment marker

	100 bp	200 bp	300 bp	400 bp	500 bp	600 bp	700 bp	800 bp	900 bp	1000 bp	1200 bp	1500 bp	2000 bp	3000 bp
First Run (s)	87.6	101.8	114.3	122.2	125.9	128.3	130.0	130.8	131.0	132.2	134.8	137.6	140.2	143.8
Second run (s)	88.6	102.5	114.8	122.7	126.4	128.8	130.4	131.2	131.9	132.7	135.2	137.9	140.4	144.0
Third run (s)	89.5	102.5	115.0	123.1	125.0	127.5	132.2	130.0	131.6	132.5	135.0	137.8	140.3	144.0
Fourth run (s)	88.9	102.9	115.3	123.4	127.2	129.7	130.3	132.2	132.9	133.6	136.1	138.9	141.3	143.0
Fifth run (s)	88.5	102.3	114.7	122.6	126.4	128.8	130.4	131.2	131.4	132.6	135.2	137.9	140.4	144.0
Sixth run (s)	88.2	101.9	114.2	122.1	125.8	128.2	129.9	130.6	131.7	132.0	134.5	137.2	139.7	143.3
Seventh run (s)	88.0	101.7	114.0	121.8	125.5	127.9	129.6	130.3	130.4	131.7	134.2	136.9	139.4	142.9
Eighth run (s)	87.9	101.7	114.0	121.8	125.5	127.9	129.5	130.3	131.0	131.7	134.2	136.9	139.3	142.9
Ninth run (s)	87.6	101.3	113.5	121.3	125.0	127.5	129.0	129.8	130.4	131.2	133.7	136.4	138.8	142.4
Tenth run (s)	87.4	101.0	113.2	121.0	124.7	127.1	128.7	129.4	130.0	131.8	133.3	135.9	138.3	141.9
tM (s)	88.2	102.0	114.3	122.2	125.7	128.2	130.0	130.6	131.2	132.2	134.6	137.3	139.8	143.2
SD	0.7	0.6	0.7	0.8	0.8	0.8	1.0	0.8	0.9	0.7	0.8	0.9	0.9	0.7
%RSD	*0.7*	*0.6*	*0.6*	*0.6*	*0.6*	*0.6*	*0.7*	*0.6*	*0.6*	*0.5*	*0.6*	*0.6*	*0.6*	*0.5*

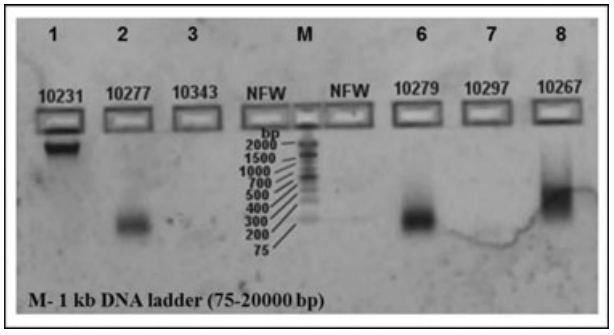

Figure 5. Analysis of the same gDNA samples as in Fig. 4 by agarose slab gel electrophoresis. Lanes 1: intact gDNA sample (sample ID 10,231); Lanes 2– 8: degraded DNA samples (sample IDs 10267, 10297, 10279, 10343, and 10277); M: DNA sizing ladder (75 bases to 20 kilobases). Conditions: gel composition: 2% agarose gel (2 × 8 wells); injection: 20 μL per well containing 500 ng gDNA; separation conditions: samples were electrophoresed on the E-Gel system power source for 8 min.

3000 base pair were between 0.5118–0.7477%, i.e. exhibited excellent reproducibility.

4 Concluding remarks

In this paper we introduced the design of a single capillary CGE instrument with a pen-shaped compact capillary-cartridge utilizing a novel microball ended fiber optic-based LED-based fluorescence detection setting. This automated system provided a good and easy-to-use alternative to labor-intensive slab gel electrophoresis systems, also featured high resolving power, excellent detection sensitivity and rapid analysis times for quantitative or qualitative analysis of ds-DNA fragments. The separation performance of the system was demonstrated by rapid and large-scale purity analysis of a thousand gDNA samples, exhibiting excellent migration time reproducibility (RSD <0.75%) and detection limit of 0.1 ng/μL in comparison to conventional agarose slab gel electrophoresis. The cost of analysis was in the range of $ 0.1–0.2 per sample, making it attractive for large-scale applications. The chemistry of the sieving matrix can also be modified for other applications such as protein analysis, immunoassays and carbohydrate profiling.

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The authors have declared no conflict of interest.

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