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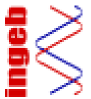

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Highly Sensitive Detection of DNA Fragments by an Inexpensive and Simple Method Fabricated In-House Blue LED Transilluminator

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Abstract

Agarose gel electrophoresis is a routinely employed experimental technique for visualizing nucleic acids, typically combined with ethidium bromide or some other molecular dye staining and a UV transilluminator. However, the utilization of a UV transilluminator is restricted due to high cost and safety considerations. In this study, an inexpensive and simple in-house blue Light Emitting Diode (LED) transilluminator was developed using a high-brightness blue LED, carbon resistor, and smartphone charging cable. Its sensitivity for DNA detection was compared with conventional methods. A detection limit of 4 ng/band was confirmed by observing a 100 bp DNA fragment, which is comparable to ethidium bromide staining and UV transilluminator. Additionally, the in-house blue LED transilluminator can be attached to existing electrophoresis chambers, allowing real-time observation of electrophoresis and offering a superior alternative to conventional methods. In conclusion, the developed in-house blue LED transilluminator demonstrates high sensitivity in detecting DNA fragments and holds potential for advancing low-cost research facilities.

Introduction

Agarose polymers are widely used in agarose gel electrophoresis for separation of nucleic acids and confirmation of existence of PCR products or genomic DNA due to their excellent molecular sieving ability. Ethidium bromide and a UV transilluminator are commonly used to observe nucleic acid fragments separated by electrophoresis. Ethidium bromide specifically binds to nucleic acids, has a high ability to detect nucleic acids, and the running cost is low (Lee et al., 2012). However, there are several problems in visualization of nucleic acids by this method. First, a UV transilluminator is expensive, requiring about $1,000 to purchase a new one. This may limit their availability to researchers at low-resource facilities. In addition, both UV light and ethidium bromide are toxic to organisms (Macgregor and Johnson, 1977; Hanawalt and Spivak, 2008). UV light also damages laboratory DNA samples because it induces cyclobutane pyrimidine dimers and (6-4) photoproducts (Lindahl and Wood, 1999). For this reason, blue and cyan LED transilluminators and optimized fluorescent reagents are now available (Huang and Fu, 2005). The LED transilluminator is a simple device consisting of an LED, a resistor, optical filters and a heat dissipation fan. Despite this, many products are in the same price range as a UV transilluminator, and there remains an unmet need for a low-cost device.

Recently, several in-house blue LED transilluminators have emerged in the field. Motohashi (2019) demonstrated the high-sensitivity detection of DNA using a commercial cyan LED desktop light and a shortpass filter, while Cunha et al. (2020) successfully developed a cost-effective device. These in-house transilluminators offer a more economical alternative to conventional commercial counterparts. Nonetheless, their production remains challenging for the inexperienced due to the requirement of specialized optical filters to select the wavelengths and programming skills to capture DNA images. Given the increasing significance of biotechnology, fostering opportunities for broader participation of researchers in life science investigations is anticipated to contribute not only to technology diffusion but also to advancements in public health and education. Therefore, the aim of this study was to develop an exceedingly simple, highly sensitive, and ultra-low-cost (approximately $20) blue LED transilluminator for DNA detection, utilizing a readily available commercial product from general tool stores, and subsequently evaluate its detection capabilities. The transilluminator was affixed to a submarine electrophoresis chamber to assess the feasibility of real-time electrophoresis observation.

Material and methods

Eleven high brightness bullet-type blue light-emitting diodes (LEDs) measuring 5 mm in diameter and emitting light within the emission wavelength of 460-463 nm with intensity of 14,000-16,000 millicandela (mcd) were meticulously soldered in a double-row configuration on a universal breadboard with a standard 2.54 mm pitch. Each blue LED was precisely coupled with a 56Ω, 1/4 W carbon film resistor with a 5% error tolerance, and the blue LED lights were connected in a parallel circuit as depicted in Figure 1. The positive and negative wires taken from a 5V, 2A USB charging cable for a smartphone were each connected to the LED circuit with solder. Finally, vinyl coated steel wire and bulldog binder clips were affixed to the blue LED lights and securely fastened to the black plastic desk organizer.

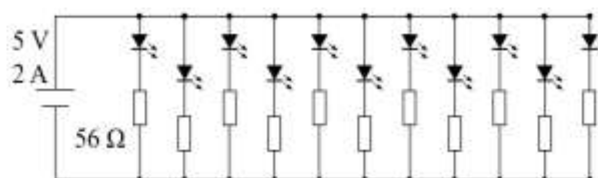

Figure 1. Circuit diagram of the blue LED light.

As typically observed in electrophoresis, larger DNA fragments exhibit distinct bands, while smaller fragments tend to be more obscured (Ordovas, 1998). In this study, a 100 bp DNA fragment was observed to prevent overestimation of detection sensitivity. To assess the sensitivity of DNA detection, ExcelBand 100 base pair (bp) DNA Ladder (SMOBiO, Taiwan) and 6×GR Green Loading Buffer GRG-1000 (BIO CRAFT, Japan) were employed. A 2% agarose gel (Agarose S, NipponGene, Japan) was used for the DNA detection experiment. In the experimental trials, varying volumes of the DNA ladder (including 6×Loading Buffer) in quantities of 6.0, 3.0, 1.8, 1.2, 0.6, 0.5, 0.4, and 0.3 μL were meticulously loaded onto a 2% agarose gel (Agarose S, NipponGene, Japan). The DNA concentration of the 100 bp fragment of the DNA ladder was 8.0 ng/μL as indicated by the manufacturer. Electrophoresis was performed in Trisboric acid-disodium EDTA (TBE) buffer at a constant voltage of 100 V for duration of 15 minutes.

For imaging the electrophoresis results, the Single-Lens Reflex (SLR) camera EOS kiss X7 (Canon, Japan) coupled with the Art 30 mm F1.4 DC HSM lens (SIGMA, Japan), or alternatively, the iPhone SE2 camera (Apple, USA), were employed. The SLR camera was configured with an aperture setting of f/13, ISO value of 100, and an exposure time of 20 seconds. Conversely, the default photo mode was utilized for imaging with the iPhone SE2 camera. All photographic captures were carefully acquired within a light-shielded room. Subsequently, images obtained from each camera underwent a 40% brightness enhancement and color inversion using the freely available image editor GIMP (version 2.10.8, https://www.gimp.org) to optimize visibility. To enable real-time observation of electrophoresis, a blue LED light was clipped to the side of the electrophoresis chamber, facilitating electrophoresis under the aforementioned conditions. The entire electrophoresis process was methodically recorded using the video recording functionality of the SLR camera. The video recording parameters remained consistent with those employed for image capture.

Results and Discussion

The materials for the devices were procured from Japanese e-commerce platforms and physical retail outlets (Table 1). The materials were easily obtainable without encountering any challenges. The total material cost amounted to approximately $20 (equivalent to 135 yen per dollar), representing a significant cost reduction compared to commercial blue LED transilluminators; ranging from 1/25 to 1/35 of their price. This in-house blue LED transilluminator stands as the most straightforward structure documented (Figure 2).

In order to assess the sensitivity of DNA detection, electrophoresis experiments were conducted using DNA ladder markers, and the resulting gels were captured using either an SLR camera or an iPhone SE2. Among the eight dilution stages, distinct DNA fragments were detectable up until the fifth dilution stage (Figure 3; lanes 1-5, 9-13). The sixth and seventh dilution stages exhibited indistinct DNA fragments, rendering them undetectable (Figure 3; lanes 6, 7), while no DNA fragments were detected in the eighth dilution stage (Figure 3; lanes 8-16). Considering the dilution conditions, the fifth dilution sample was estimated to contain 4.0 ng of a 100 bp DNA fragment.

To enable real-time observation of electrophoresis, an in-house blue LED light was affixed to the electrophoresis chamber, and the process was recorded using an SLR camera. The migration of 11 bands could be observed within approximately 15 minutes of electrophoresis. It is worth noting that a black film should be positioned beneath the gel when visualizing small quantities of DNA fragments.

These findings demonstrate the capability of the in-house blue LED transilluminator and DNA staining reagent to detect DNA fragments at a sensitivity of 4 ng/band.

Table 1. Materials constituting In-House Blue Transilluminators and their costs

Material	Product identifiers	Cost ($)
High brightness blue LED (bullet type)	ASIN: B01N4C1FFB	4.4
Universal breadboard	JAN: 4931442029303	2.0
Tinned copper wire	JAN: 4901087144419	1.3
Plastic desk organizer (black)	JAN: 4955959219309	0.8
Vinyl coated steel wire	JAN: 4550480115917	0.8
56 Ω 1/4W carbon film resistor 5% error	-	2.2
USB charging cable	JAN: 4549131697186	0.8
Orange translucent acrylic sheet	JAN: 4953463002066	6.3
Binder clip	JAN: 4549131025613	0.8
Solder Wire for Electrical Soldering	JAN: 4550480244884	0.8

ASIN (Amazon Standard Identification Number): A unique alphanumeric code assigned within the Amazon marketplace. JAN (Japanese Article Number): A barcode standard widely used in Japan.

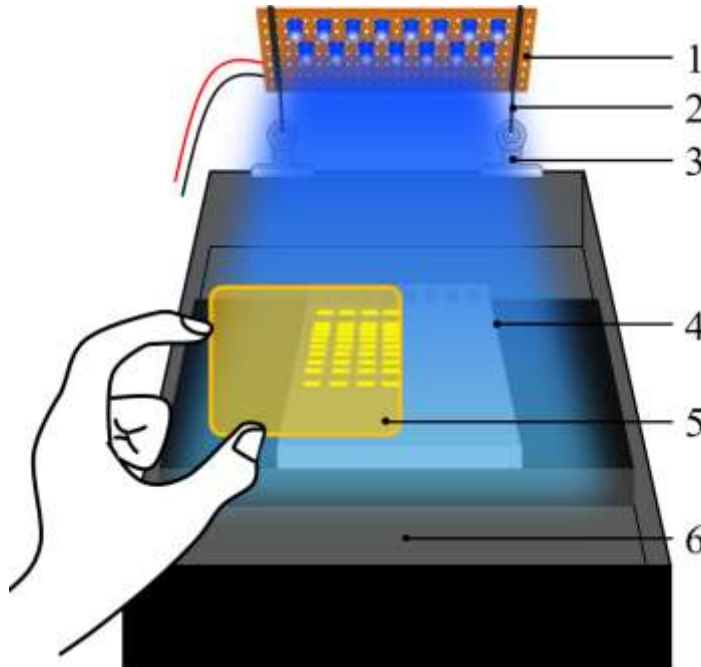

Figure 2. Schematic of the in-house blue LED transilluminator described in this study. 1: Blue LED light. 2: Vinyl coated steel wire. 3: Binder clip. 4: Agarose gel. 5: Orange translucent acrylic sheet. 6: Plastic desk organizer.

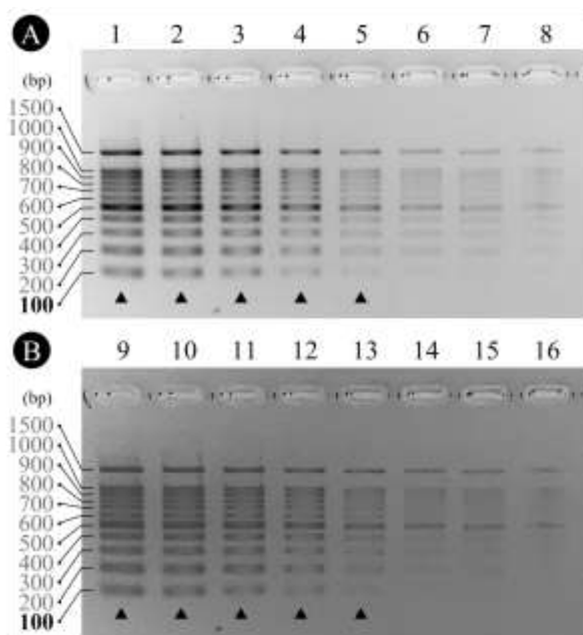

Figure 3. Agarose gel electrophoresis images of DNA ladder markers observed with an in-house blue LED transilluminator. A: agarose gel electrophoresis image taken with a SLR camera. B: agarose gel electrophoresis image taken with the iPhone SE2 camera function. Lanes 1-8 and 9-16 contain 6.0-0.3 μL of DNA ladder (including 6×Loading Buffer). Black triangles (▲) indicate clearly observed DNA fragments.

The detection limit of commonly used DNA detection methods involving ethidium bromide staining and UV ranges from approximately 1 ng to 10 ng, with slight variations across reports (Ordovas, 1998; Lee et al., 2012; O'Neil et al., 2018). Thus, the in-house blue LED transilluminator employed in this study exhibited comparable effectiveness to conventional DNA detection methods for detecting DNA on agarose gels. The electrophoresis images captured using the iPhone SE2 exhibited a darker background and lower contrast compared to those obtained with the SLR camera, making the latter more suitable for imaging electrophoresis gels. However, the detection limit of DNA using the iPhone SE2, at 4.0 ng/band, aligns with that of the SLR camera, making it a suitable and convenient imaging approach. Moreover, the iPhone SE2's shorter exposure time provides the advantage of rapid imaging of electrophoresis results.

Typically, electrophoresis procedures span from 15 to 60 minutes, making it challenging to monitor the progress and obtain real-time results. This limitation is addressed by the real-time electrophoresis chamber, although commercially available options come at a high cost. In contrast, the blue LED transilluminator utilized in this study functions as a versatile illumination device that can be easily affixed to any electrophoresis chamber, enabling real-time observation. An additional advantage of this experimental apparatus is its inherent safety as a DNA detection system, as it allows observation without the need for mutagenic reagents throughout the entire workflow. It is worth noting, however, that caution should be exercised to avoid direct eye contact with the high-brightness LED light source. Furthermore, the USB power supply compatibility of this device with commercially available mobile batteries (5 V, 2 A) enhances result verification independent of indoor conditions and enhances the method's portability and versatility.

Conclusion

An affordable, in-house, blue LED transilluminator, constructed from commercially available components exhibited comparable sensitivity to ethidium bromide staining in detecting DNA fragments. While a single-lens reflex camera proved superior for imaging electrophoresis results, basic photography was achievable using an iPhone SE2. This approach facilitates real-time electrophoresis observation when integrated with an electrophoresis chamber.

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Conflict of interest

The author declares no conflict of interest.

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