Benchmark

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3D-printed assistive pipetting system for gel electrophoresis for technicians with low acuity vision

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ABSTRACT

In molecular biology laboratories, many tasks require fine motor control and high acuity vision. For example, lab technicians with visual impairment experience difficulty loading samples into the small wells of a horizontal agarose gel. We have developed a 3D-printable gel loading system which allows technicians with low-contrast vision to load gels correctly. It includes a casting tray, a bridge, and a modified comb. The system provides a high-contrast visual field to improve visibility, and the bridge allows pipette tips to be inserted at the correct location and only to the correct depth. The necessary computer files for printing this device are freely available to increase the accessibility of molecular biology laboratories to people with visual impairment.

METHOD SUMMARY

We have produced a system for people with visual impairment to successfully load an agarose gel for gel electrophoresis. This system can be produced using a 3D printer. There are three components: a casting tray, a bridge, and a modified comb. The bridge fits into side notches in the casting tray and is suspended above the tray. The comb is inserted into the bridge while the molten gel is poured. When the gel cools, the comb is removed leaving the bridge in place. Pipettes can then only be inserted in the correct location and to the correct depth to load the wells. Importantly, the bridge and tray are contrasting colors to aid in visual discernment.

KEYWORDS:

3D printing ● gel electrophoresis ● visual aid ● visual impairment

Gel electrophoresis has become an essential technique in molecular biology, medical diagnostics, and forensic laboratories. The concept of separating biologic material by size and charge using an electric field dates back to, at least, the early 1900's [1]. Arne Tiselius pioneered moving boundary electrophoresis of serum proteins in the 1930's [2]. A breakthrough occurred when Oliver Smithies ran human plasma samples though a starch substrate, which allowed for stable separation of defined protein components and was the initial implementation of slab-gel electrophoresis [3]. This more recognizable method of gel electrophoresis now commonly uses polyacrylamide and various forms of agarose as substrates for separation of proteins and nucleic acids. The procedure typically consists of aqueous biologic samples loaded into small, 1–5 mm^3 wells on one zone of a gel submerged in aqueous salt buffer. An applied electric field causes charged particles within the sample to exhibit an electrophoretic shift toward the oppositely charged electrode. As the sample must travel through pores within the substrate, smaller fragments will migrate quicker than larger, causing components to become distributed by size.

Precision in loading samples into the wells within a gel is essential, and involves utilizing fine motor skills to pipette small volumes of nucleic acids or protein samples into a translucent gel. Puncturing the gel or improper pipetting can lead to loss of sample or uninterpretable results. The task of loading a gel requires the user to have vision capable of low-contrast, high-acuity detection and close-range depth perception. When working with technicians and volunteers with even moderate visual impairment, it was clear that the task of loading a gel was challenging to unsurmountable. Previous efforts to increase accessibility of the laboratory to people with visually impairment included handmade parts which were difficult to reproduce [4]. As part of an ophthalmology department, our goal is both to research vision and benefit the lives of visually impaired people. In the interest of making the laboratory as accessible as possible, we have designed a 3D-printed pipette guide to aid in pipetting samples into a horizontal agarose gel.

Input from lab technicians with low-acuity vision was reviewed, and a list of common difficulties was determined. The most important design criteria were the prevention of gel puncture, the ability for the pipette tips to be lowered sufficiently into the well, and the ability to see which wells had already been filled were considered critical.

Because transparent casting trays and translucent gels provide very little visual contrast, the first issue to be addressed was the low-contrast loading environment. To achieve a visual scene with higher contrast we designed a casting tray in a light, opaque material

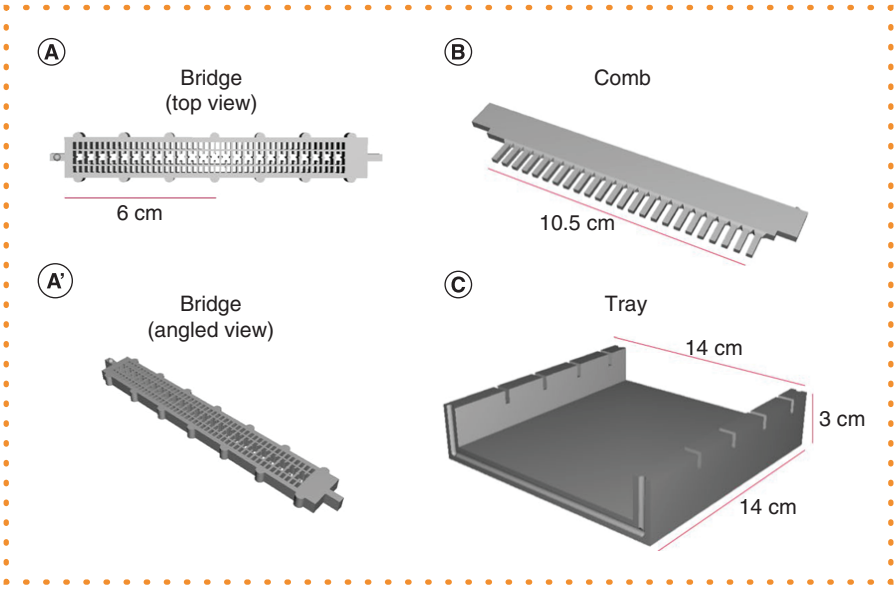

Figure 1. Components of the pipette guide system. (A) The bridge allows for pipette tips to only be inserted in the correct positions and to the correct depth for the wells. **(B)** The comb is designed to be inserted through the bridge. **(C)** The tray is of the correct dimensions to accept four bridge constructions.

with a separate bridge that could be constructed using a contrasting printing material (Figure 1). The bridge is suspended over the gel and running buffer, resting in notches on the walls of the casting tray. We tried multiple color combinations and found that when making the tray an opaque bright color (white) and the bridge a dark color (black) or bright contrasting color (orange), the user does not need to rely on visually discerning the wells of the gel (Figure 2). Next, we addressed a method by which the pipet tips could be lowered to the correct depth. Considering standard diameter and length of 10–200 μl pipet tips, we designed holes in the bridge with apertures which allowed conical pipet tips to descend only to the correct depth before becoming lodged in the holes of the bridge (Figure 2D). Small stabilizing tabs inside the holes restrict rotation and ensure the correct angle of the tip into the well. Two stabilizing arms at the ends were designed to ensure a snug fit in the tray notches, but it may be helpful to sand down the sides of the stabilizing arms for easier disassembly. We also designed a third piece, a casting comb which would create wells of the appropriate size directly below the holes in the bridge (Figure 1B & 2A–C). The comb was designed so that it could be inserted through the bridge while an agarose gel cooled and then removed leaving the bridge in place (Figure 2B & C). Last, we wished to allow visibility of the gel beneath while maintaining contrast between the bridge and the tray. For this we designed an open grid in the dark bridge so that one can visually confirm which wells have been successfully loaded (Figure 1A).

The casting comb is designed to create wells that end 1 mm above the bottom of the casting tray. It has 24 teeth spaced identically to the holes in the bridge. Both the comb and bridge have been designed with bumps on one end which can be aligned for correct use. The holes in the bridge have a 30 degree inward draft to create a better fit for conical pipette tips and are designed so that the pipette tip will only extend 11 mm beneath. To determine the proper radius of each hole, we took the desired length of 11 mm and measured the radius of each pipette tip at that point. Because the holes have a draft of 30 degrees, that effectively decreases the radius of the holes by 0.15 mm, so the actual radius of guide holes is 0.15 mm less than the radius of the pipette tips at 11 mm from the end. The spacing of the holes aligns with the comb, so that a 12-multichannel pipette will fit into the guide with the tips at the same depth. The bridge is printed with a full-thickness hollow grid pattern to allow monitoring of the gel underneath while still maintaining a strong contrast with opaque material above (Figure 1A).

The comb is printed horizontally to ensure structural strength, as it is more difficult to break the comb's teeth when the grain of direction of each layer runs longitudinally along the tooth. Small reinforcement structures were added at the base of each tooth to more evenly distribute pressure where they connect with to the body. The casting comb is 136 mm long at its longest part, and is 2.6 mm thick at thickest point. Including the body and the teeth, the comb is 25.5 mm wide. Resulting wells are $10.5 \times 1.7 \times 2.2$ mm.

The casting tray contains notches to accommodate 4 combs/bridges. We found that the best visual contrast for technicians with visual impairment was achieved when the tray was printed in white and the bridge printed in black, or similarly dark material (Figure 3). The material used for all parts was PETG, a widely available and commonly used 3D printing material that is compatible with most common printers. Its extruding temperature, the temperature at which it is extruded from the printer's nozzle, is around 250°C, which is higher than any expected temperature that would be experienced during the cooling/solidification of agarose. Unlike PLA, a material commonly used for prototyping, PETG is water resistant and is not biodegradable [5].

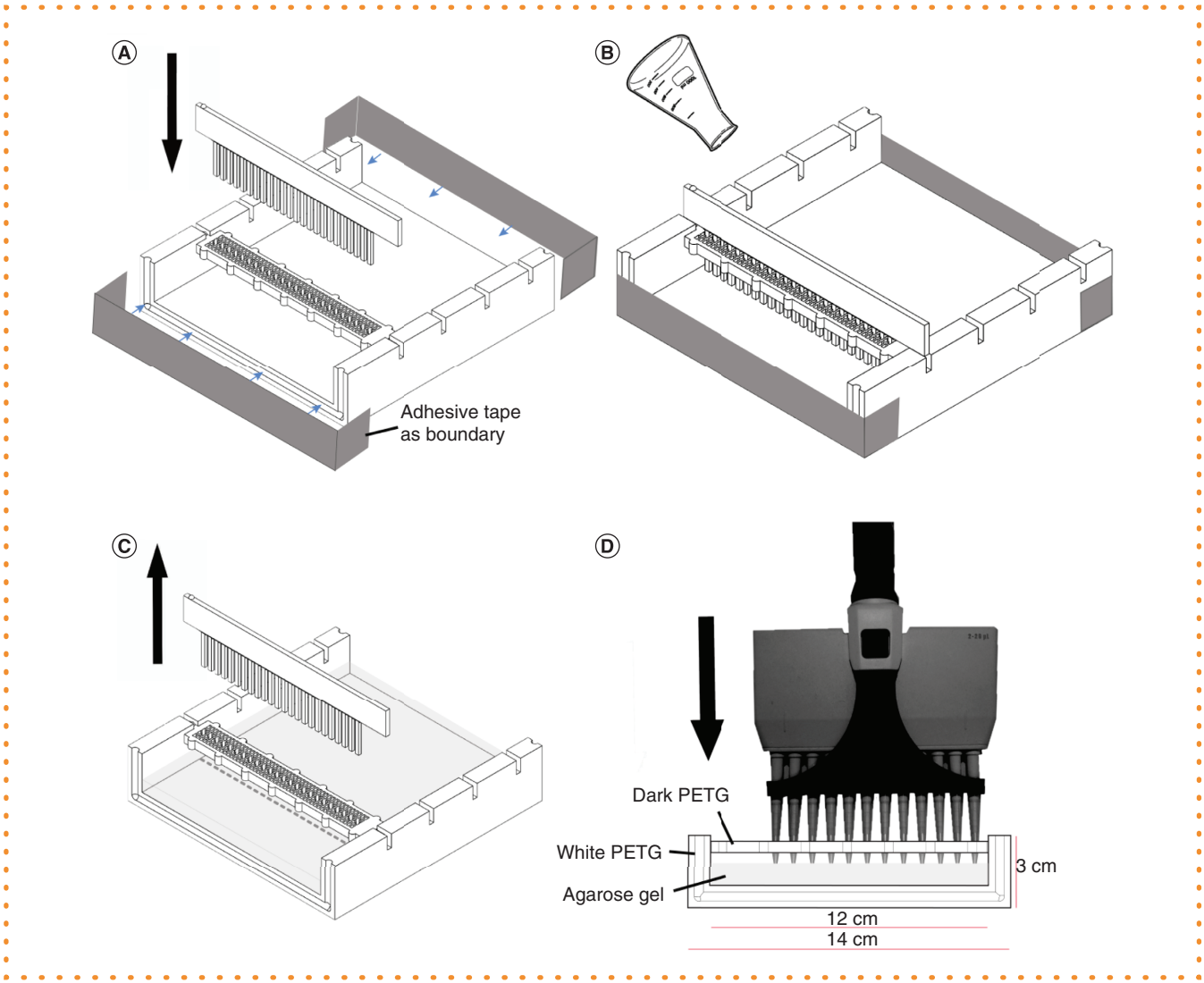

Figure 2. Schematic of how to assemble and use the system. (A) Adhesive tape can be applied to the open sides of the tray to form a temporary boundary. The comb is inserted into the bridge. **(B)** The molten gel is poured into the intact system. **(C)** The comb is removed leaving the bridge in place. Adhesive tape boundary has also been removed. **(D)** Front view of a micropipette with 10–200 μl tips inserted into the system. A single or multichannel micropipette can be used.

The printer used for modeling was an Anycubic i3 Mega (www.anycubic.com), and the software used to design the system was Solidworks (www.solidworks.com). The printer was a Fused Deposition Modeling (FDM) printer which is typically less expensive than Stereolithography Apparatus (SLA) printers which offer higher resolution. However, with a nozzle diameter of 0.4 mm, the resolution was sufficient for this project and should be compatible with all FDM printers. The settings used were 0.2 mm layer height, 3% infill density, 1.2-mm wall thickness, 250°C printing temperature, 86°C bed temperature, 20% fan speed for the whole print, 0.4 mm Z-distance for the supports, a skirt to help build plate adhesion, and a printing speed of 70 mm/second. Printing all three parts required 7 h and 56 min, and used 126 g of PETG for approximately $3.15 of materials. If one decides to only print the bridge and comb, it would take 1 h and 19 min and weigh 17 g, costing around $0.42.

To use, first insert the bridge (or multiple bridges) into the notches of the casting tray (Figure 2A). The comb can be inserted into the bridge prior to adding the molten gel matrix. Standard laboratory tape or autoclave tape can be used to create a boundary at the open ends of the tray prior to adding gel matrix (Figure 2A). Alternatively, grooves have been added to the open sides of the tray if one wishes to fashion a rubber gasket to secure into a molding scaffold. However, we have found adhesive tape to be a suitable temporary wall during the gel cooling period (Figure 2A & B). Once the gel has been poured and cooled, the comb can be removed as would a standard comb, leaving the bridge in place (Figure 2C). Adhesive tape must be removed prior to running the gel electrophoresis.

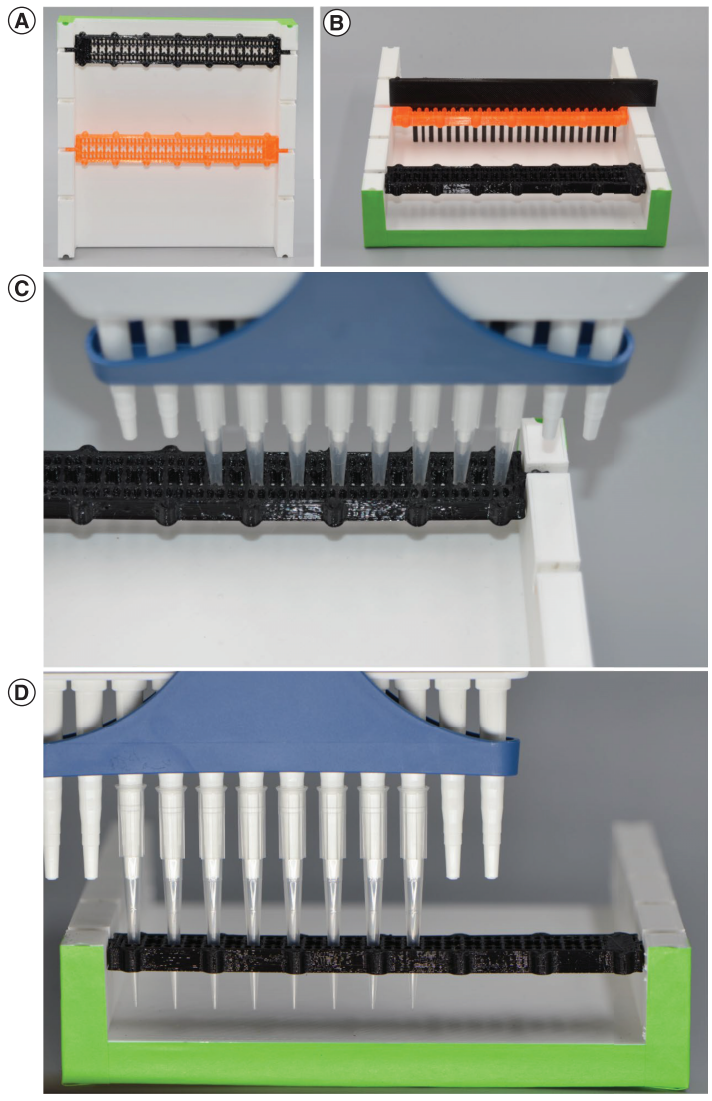

Figure 3. Photographs of the completed system. (A) The white casting tray with a black bridge and an orange bridge placed into positions 1 and 3 photographed from the top. **(B)** The same set-up as **(A)** photographed from the front with a comb inserted into the orange bridge. Green adhesive tape was applied to the edge of the casting tray for visualization in the photos. **(C)** Black bridge with a multichannel pipette in loading position from an angled-top view. **(D)** Black bridge and white tray with a multichannel pipette in loading position from a front view.

An advantage of this system is that any lab with access to a FDM 3D printer can create their own. The cost of printing one should be low, and many universities have facilities with 3D printing capabilities. Modifications to the design files provided here can change the dimensions to fit already existing running tanks. Alternately, creating a custom running tank would only require a power supply, electrodes and a tank of sufficient size to submerge the gel in the casting tray while separating positive and negative electrode baths. Because these materials are often available in most laboratories, a system by which visually impaired technicians can participate in molecular biology should be feasible even for laboratories with modest budgets.

We see this as only a first step in making the molecular biology laboratory more accessible to people with visual impairment. In the future, we plan to continue to design freely available laboratory aids, software, and techniques with visual impairment in mind. We also hold as a priority the open-source nature of anything we design so that laboratories of all budgets can benefit from the products and will allow people with visual impairment to contribute more easily to the molecular biology endeavor. Additionally, the exciting advancement of nano-level materials will offer new levels of product design incorporating magnetic and biologic elements in the future [6–9].

Author contributions

AV Huynh designed the devices, performed the work, and wrote the manuscript. P Stein designed the device and performed the work. ED Buhr supervised the project and wrote the manuscript.

Financial & competing interests disclosure

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