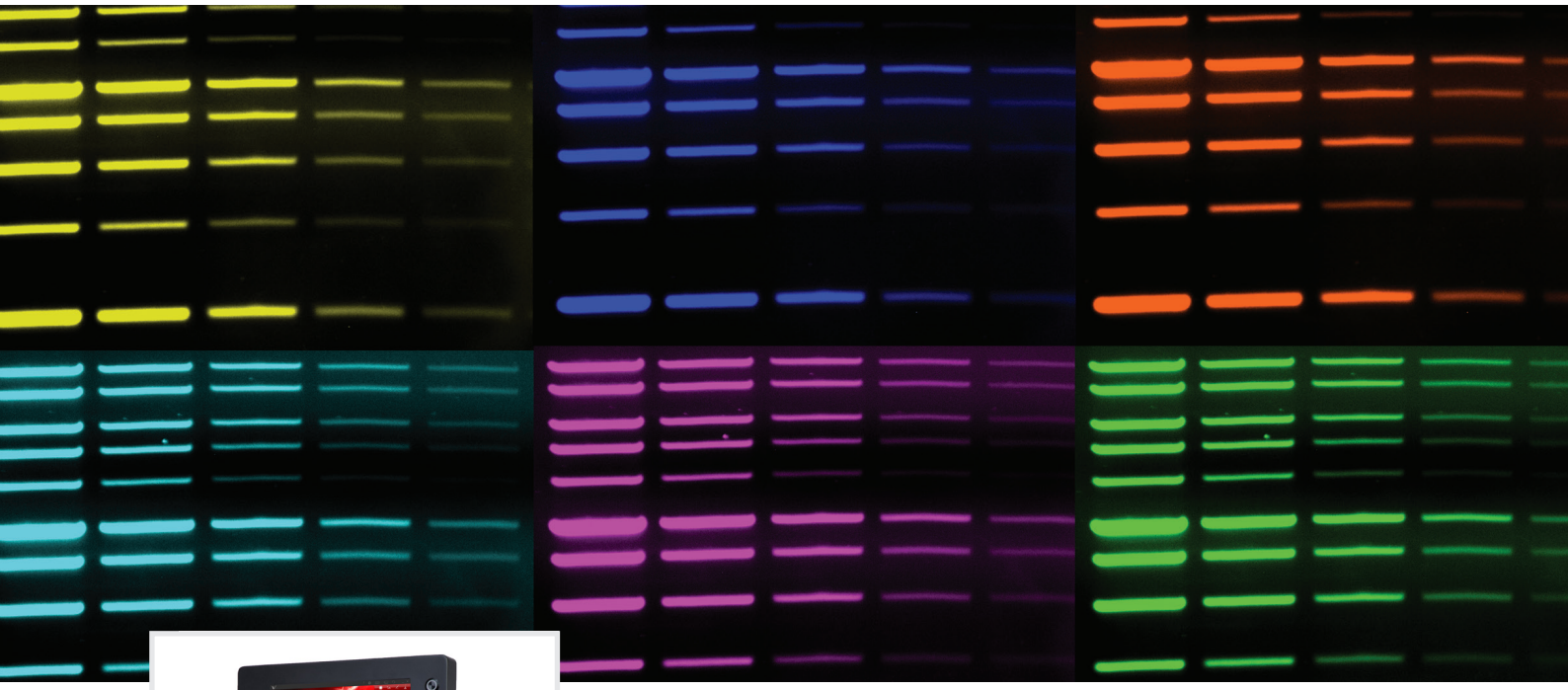

Task

Simplified high resolution imaging of nucleic acid

Solution

Analytik Jena's UVP GelSolo enables easy yet exceptional gel documentation with an intuitive user interface and a high resolution 5MP camera

Theory and Practice: Performing DNA Gel Electrophoresis

Introduction

Nearly 70 years since the discovery of the structure of DNA[1] and 60 years since researchers began investigating nucleic acid electrophoretic properties[2–6], nucleic acid gel electrophoresis remains a routine tool for many labs. From traditional molecular cloning and restriction mapping, to cleavage analysis of CRISPR-Cas9 targeted mutations[7], reliable documentation requires high-quality precision instrumentation—quality and precision are mainstays of Analytik Jena's 50 year imaging history.

The UVP GelSolo is an entry-level imaging system well-suited for any laboratory, and provides exceptional documentation of nucleic acid and protein gels, and beyond (contact our Applications Scientists for additional applications). Equipped with a 5 megapixel CMOS camera, the UVP GelSolo can capture the finest details in your samples. Below we provide a brief overview of DNA gel electrophoresis, along with some expert tips to get the most out of your DNA gels.

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Theory and Practice: Performing DNA Gel Electrophoresis

Electric Field

DNA gel electrophoresis involves the movement of charged particles through a gel matrix in a polar electrical field. Because the phosphate backbone of DNA is negatively charged, DNA uniformly migrates in the gel toward a positive electrode. Users can change the intensity of the electric field to suit their needs. As a general rule of thumb, 5-8 volts (constant voltage) should be applied to the gel for every centimeter between the negative and positive electrode. For example, 80-128 volts should be applied to a standard mini gel system measuring 16cm, and this value should be determined empirically for the best resolution.

Gel Matrix and Gel Casting

Agarose is the most common gel matrix used in nucleic acid electrophoresis. Agarose is a polysaccharide, which consists of repeating units of galactose and 3,6-anyhydrogalactose. The consistency of this structure engenders a uniform porosity throughout the gel. Combined with the uniform charge distribution across the DNA molecule, it is possible to precisely determine the size of DNA fragments mobilized through the gel. Mobility and resolution can be further adjusted by changing the concentration of agarose. Increasing the agarose concentration increases band resolution at low molecular weights—large DNA fragments experience more resistance by the agarose and travel slowly, devoting more of the gel to small band resolution. By contrast, decreasing the concentration of the agarose improves band resolution at high molecular weights (see Table 1).

Table 1: Agarose concentration and approximate DNA range resolution.

Concentration of agarose (%)	DNA size range (bp)
0.8	1000-7000
1	500-5000
1.5	300-3000
2	200-1500

When casting a gel, users need to consider three factors: 1) what buffer they are using, 2) what percent agarose do they need for band resolution, 3) and whether they are using pre- or post- stain for DNA visualization. In the case of a pre-stain, users will mix in agarose, with the electrophoresis buffer, and their DNA dye. This mixture is heated in a microwave until all the agarose has gone into solution (typically 1-2 minutes). Upon cooling to 55 °C, users then load the molten agarose in a gel casting tray. A comb is added to the gel casting tray, which creates wells once the gel matrix has solidified. Well size is an important consideration when trying to improve band resolution and is determined empirically.

Electrophoresis Buffers

There are several buffers used for gel electrophoresis. Continuous buffer systems, where the chemical makeup and concentration of buffer components are identical between the gel matrix and the tank, is most routinely used for double stranded DNA gel electrophoresis. Tris-acetate containing EDTA (TAE) is a common buffer. Buffer serves the primary purpose of "buffering" the pH of the electrophoretic system. In addition, it maintains the conductance of the systems, enabling DNA to migrate in the gel matrix. Without buffer, conductance is low, DNA will not migrate through the gel, and the system will overheat it. In the most severe cases, users who mistakenly use water or old buffer, both which are poor buffers, cause gels and/or the electrophoresis tank to melt. Lastly, EDTA, which chelates divalent cations, prevents the activity of nucleases, which can degrade DNA samples.

Visualization of DNA

There are several compounds used to visualize samples on a gel before and after electrophoresis. Loading dye enables researchers to see their samples as they run on the gel. Typically, the loading dye contains two dyes—bromophenol blue and xylene cyanol FF. These dyes give users the ability to indirectly visualize approximately how far their samples have run on a gel. These dyes do not bind to DNA and instead migrate separately through the gel. DNA binding dyes are numerous, for the purpose of this overview, we will discuss ethidium bromide.

Ethidium bromide is the longest running DNA binding dye used in laboratories. At the molecular level, it intercalates double stranded DNA and, when excited with UV light, will fluoresce. Ethidium bromide can be mixed in the gel matrix during gel casting. Alternatively, gels can be soaked in ethidium bromide upwards of 30 minutes for a post-stain. In recent times, ethidium bromide has fallen out of favor due to its mutagenic and moderately toxic properties.

Imaging on the UVP GelSolo with the Biotium 1kb DNA ladder

DNA Gel and Tank Conditions

DNA gel electrophoresis was performed with a 50 mL 0.8 % agarose gel using J.T. Baker low electroendosomosis agarose (Radnor, PA). 50X TAE electrophoresis buffer from Thermo Scientific (Waltham, MA), was used and diluted to 1X concentration, both for the gel and tank to create a continuous buffering system. A Biotium Ready-to-Use 1kb DNA ladder (Fremont, CA) was used as the DNA sample. 25 µg of ethidium bromide from ThermoFisher Scientific (Waltham, MA) was added to the gel to aid in visualization. In addition, 100 µg of ethidium bromide was added to the tank buffer. The gel was run at 100 V for 45 minutes and immediately visualized on a UVP GelSolo.

Image Capture and Sample Conditions

The UVP GelSolo comes equipped with blue and white epi-illumination. In addition, it comes with a 3 wavelength UV transilluminator (i.e. 254, 302, 365). For excitation of ethidium bromide, 302 nm UV light was used in combination with a 605 nm BP50 ethidium bromide emission filter. Exposure time was 1.3 s. The Biotium ladder was run on the gel in 1:2 dilution series from 200 ng-1.5 ng (Lanes 1-8, left to right, Figure 1). Lane 8 represents an approximate total of 1.5 ng—highlighting the exquisite detection capabilities of the UVP GelSolo.

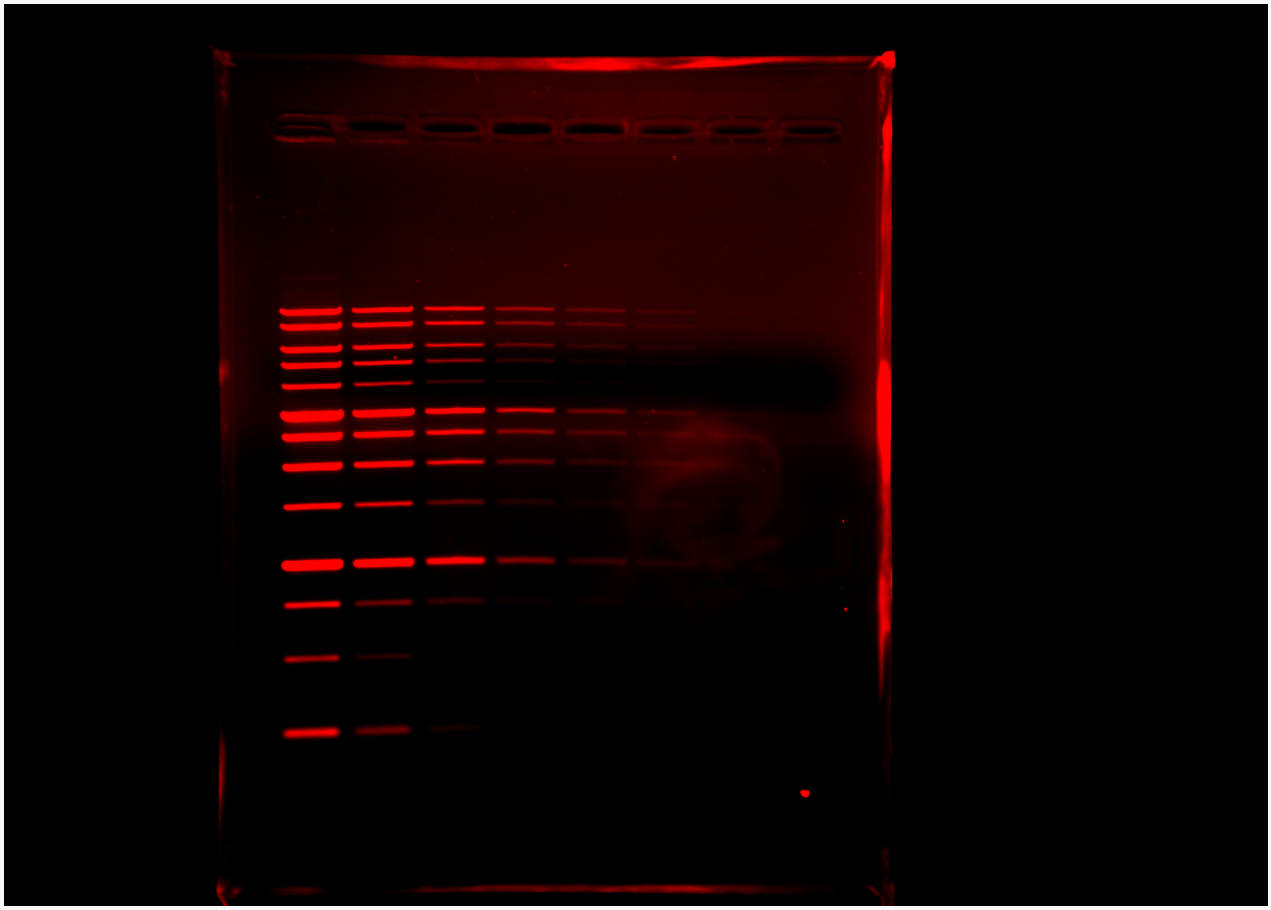

Figure 1: Agarose DNA Gel Electrophoresis using the UVP GelSolo. The Biotium ladder was run on the gel in 1:2 dilution series from 200 ng - 1.5 ng on 0.8 % agarose gel at 100 V for 45 minutes. Ethidium bromide was used for detection. Image was captured on the UVP GelSolo using 302 nm UV excitation and a 605 BP50 emission filter at 1.3 exposure. Image was pseudocolored with TexasRed.

Expert Tips

- Slide a color object underneath the gel tank positioned below the wells. This can enhance visualization of the wells ensuring all your sample makes it in the well. Alternatively, pipette a 1-2 µl of loading dye into each well.
- Cast as thin of a gel as possible. This can increase resolution as well as cut costs.
- When possible, avoid loading critical samples to outside wells. Smiling and frowning of bands occurs more often at the edges.
- It is okay to reuse buffer, but pay attention to heat generation during and after the run. If your gel and buffer are hot to the touch, it's time to replace the buffer.
- Add the same volume of 1x loading dye to your samples, to your empty wells, to ensure uniformity of the electric field.

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