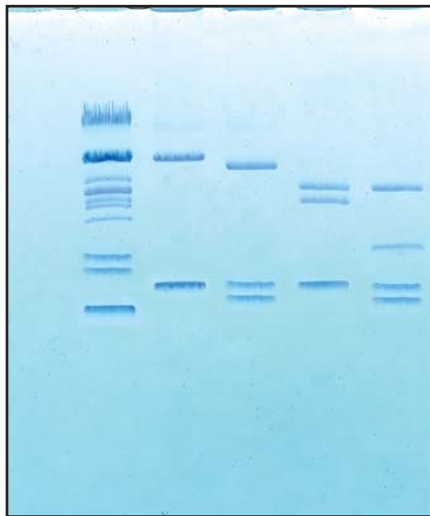




# Protocol for *Carolina*BLU™ DNA Stain



*CarolinaBLU*<sup>™</sup> stain was developed as a significant advance over the methylene blue staining protocol. It is provided at two concentrations.

*The Gel/Buffer stain is a 20× concentration* that can be used to add stain to the gel and/or the running buffer. It intercalates in the DNA, allowing students to see the larger bands of DNA during and immediately after electrophoresis, without the lengthy staining and destaining steps required for methylene blue.

*The Final Stain is a 1× concentration* used to stain gels after electrophoresis. Soaking the gel for 15–20 minutes in the Final Stain will more intensely stain the DNA bands and make them more conspicuous. Background stain in the gel can be removed by several washes in distilled/deionized water over a period of 30–40 minutes. This additional staining and destaining intensifies the staining of all the fragments, making the smallest bands more easily seen than is usually possible with other blue stains.

Even if the Gel/Buffer stain is not added to the agarose and running buffer, results using just the Final Stain compare well with results using methylene blue. Overall, the advantages of *CarolinaBLU* over methylene blue are the following:

- Immediate visualization of DNA.
- Greatly shortened staining and destaining times.
- Superior results (when used both during and after electrophoresis as described above).
- Longer storage life (bands are still visible on gels stored in a refrigerator in a sealed bag, plastic wrap, or covered tray for up to 8 weeks).

Recommended amounts of DNA are as follows (at a recommended concentration of 0.2 µg/µL):

Lambda DNA	0.8 µg
Predigested lambda DNA	1.5 µg
Plasmid DNA	2.0 µg

## Procedure

1. In a beaker, prepare the agarose solution required. Cool the molten agarose to about 60°C by placing the beaker in a 60°C water bath or allowing it to stand at room temperature for several minutes. Swirl the beaker occasionally so that the agarose will cool evenly throughout. The quantity of stain to add to the agarose and the buffer depends on

the voltage used for electrophoresis. At voltages less than 50, a slightly lower concentration is used than at voltages greater than 50. Refer to the following chart:

<b>Voltage</b>	<b>Agarose Volume</b>	<b>Stain Volume</b>
<50 Volts (1 drop/30 mL)	30 mL	40 $\mu$ L (1 drop)
	150 mL	200 $\mu$ L (5 drops)
	400 mL	520 $\mu$ L (13 drops)
>50 Volts (2 drops/50 mL)	50 mL	80 $\mu$ L (2 drops)
	300 mL	480 $\mu$ L (12 drops)
	400 mL	640 $\mu$ L (16 drops)

After adding the stain to the agarose, swirl to mix and immediately pour the gel. Gels may be prepared 1 day ahead of the lab, but, if stored longer, they tend to fade and lose their ability to stain bands during electrophoresis. If you do prepare them ahead of time, store them covered with a small amount of buffer (leaving masking tape in place), or store them covered in the gel box.

**Note: Do not** try using more stain than is recommended in the gel. Doing so leads to precipitation of the DNA in the wells and can create artifactual aggregated DNA bands in the agarose gel.

- Refer to the following chart for the quantity of stain to add to the 1 $\times$  electrophoresis buffer:

<b>Voltage</b>	<b>Buffer Volume</b>	<b>Stain Volume</b>
<50 Volts (12 drops/500 mL)	500 mL	480 $\mu$ L (12 drops)
	3 L	2880 $\mu$ L (72 drops)
>50 Volts (24 drops/500 mL)	500 mL	960 $\mu$ L (24 drops)
	2.6 L	5 mL (125 drops)

The dropper bottle provided delivers 40  $\mu$ L/drop. If a calibrated pipet is available, the dropper tip can be removed for quicker addition of larger volumes of stain. The volume of buffer and agarose required for some gel box options is listed in the following table.

Type of Gel Box	Volume of Buffer Required	Volume of Agarose Required
Mini Gel System Box	20 mL	30 mL/casting tray
Carolina Gel Box, 1 tray	250 mL	50 mL
Carolina Gel Box, 2 trays	450 mL	100 mL

While *Carolina*BLU is not toxic, we recommend that the students wear gloves to prevent staining their skin. If you want to reuse the buffer, we recommend that you use *Carolina*BLU only in the gel and as a final stain.

3. Load the DNA samples and apply current.
4. Following electrophoresis, remove the gel from the box. The DNA bands are best visualized when viewed against a white background or, even better, on a light box.
5. Place the gel in a staining tray. Cover the gel with the final *Carolina*BLU stain and allow it to sit for 15–20 minutes. Agitate the tray gently, if possible. Pour the stain back into the bottle. The stain can be reused up to eight times. Cover the gel with deionized water to destain. (Tap water contains chloride ions, which can partially remove the stain from the DNA bands and give inferior results.) Occasionally, gently agitate the gel. Change the water 3–4 times over the course of 30–40 minutes. The gel can be left at room temperature in a little water to destain fully overnight.
6. Once sufficiently destained, the gel can be removed and covered in plastic wrap or a plastic storage bag, or it can be left in the staining tray and covered with plastic wrap. The gel can be stored for 8 weeks in a refrigerator with no significant loss of staining.

To order call:

**800-334-5551 (US and Canada)**

**336-584-0381 (International)**

For technical help call:

**800-227-1150**

[www.carolina.com](http://www.carolina.com)

# Carolina Biological Supply Company

2700 York Road, Burlington, North Carolina 27215

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