

Carolina Biological Supply Company

Introduction to Electrophoresis



Objectives

- To learn about and practice gel electrophoresis
- To separate the components of a mixture using gel electrophoresis

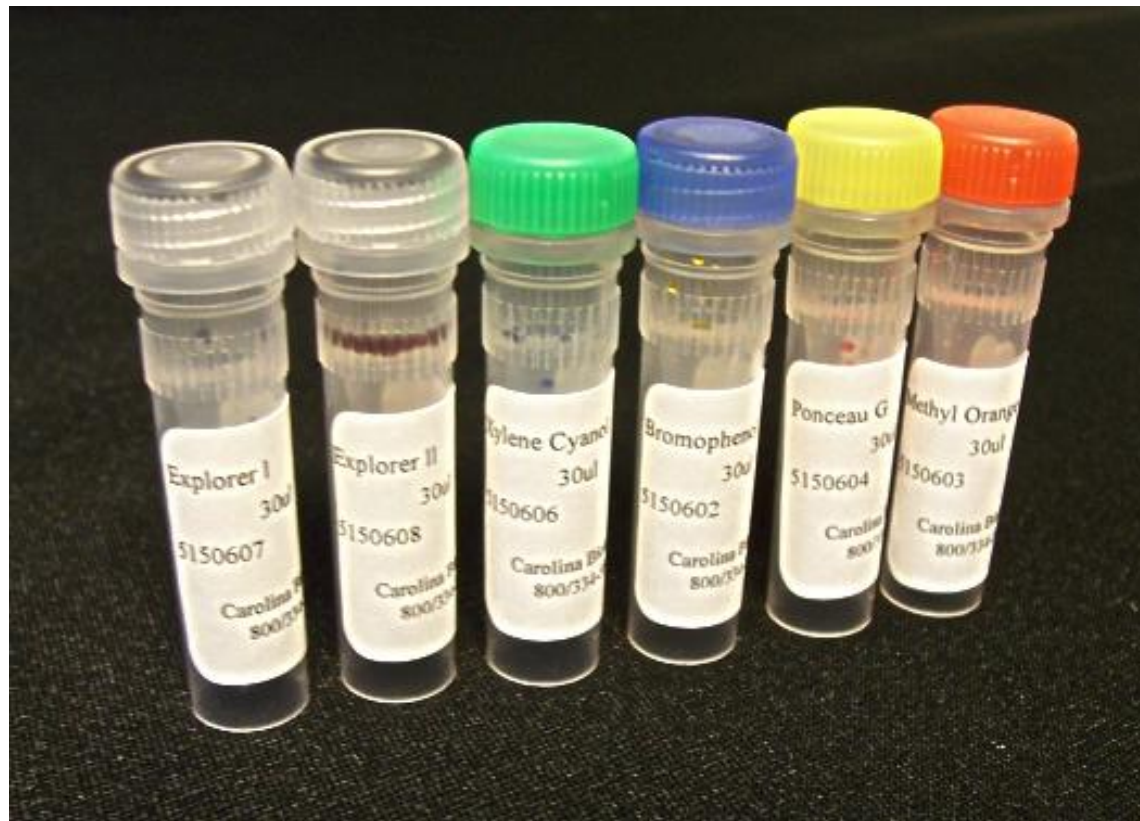
Agenda

- Quick instructions about how to load gels
- Loading and running gels
- Background information on gel electrophoresis
- Resources available from Carolina
- Analysis of gel results
- Evaluation forms and giveaways

Materials

- Set of 4 individual dyes and 2 unknown dye mixtures
- Agarose
- Gel tray and comb
- Carbon fiber electrodes
- Buffer
- Gel-loading device with tips
- Power supply or 9-V batteries
- Electrical leads with alligator clips

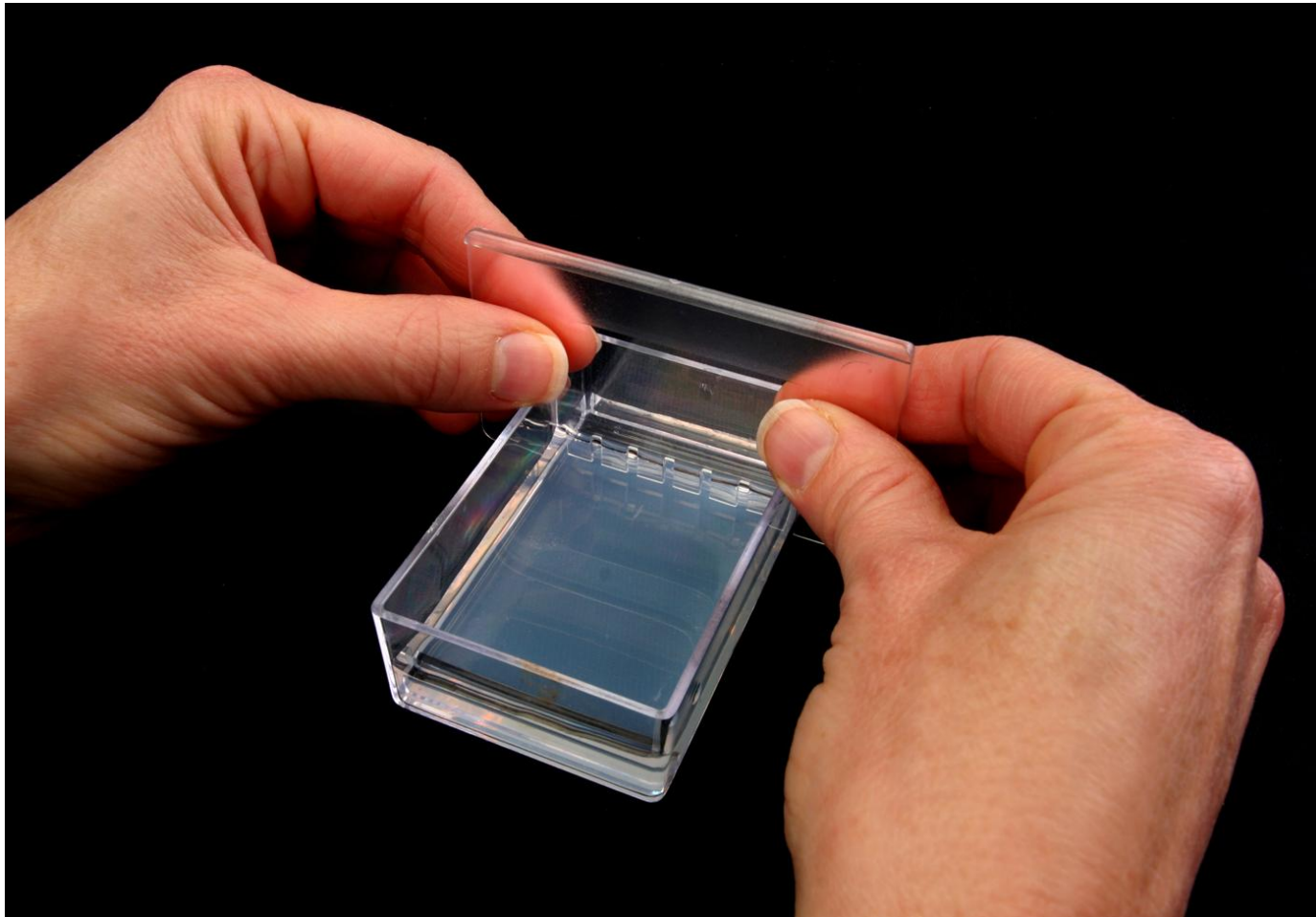
Samples of Dyes



Pour Buffer into the Gel Box



Carefully Remove the Comb



Draw the Sample into the Micropipet Tip



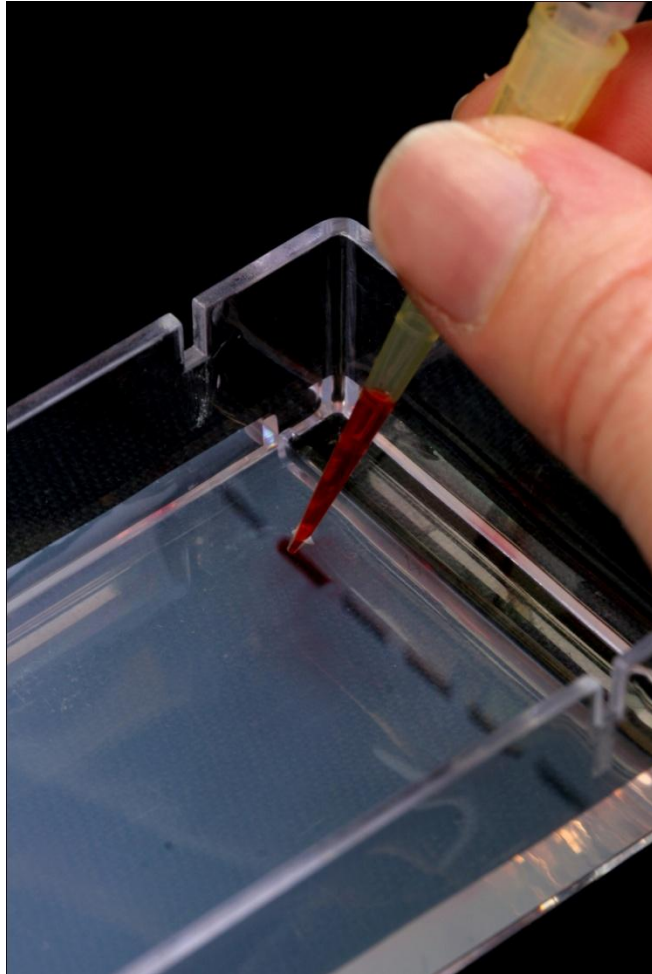
Pull up the plunger slowly; only a little bit of movement is needed to draw up 20 μL .

Loading the Gel



Gently depress the plunger with the finger of one hand. Steady the tip with the other hand.

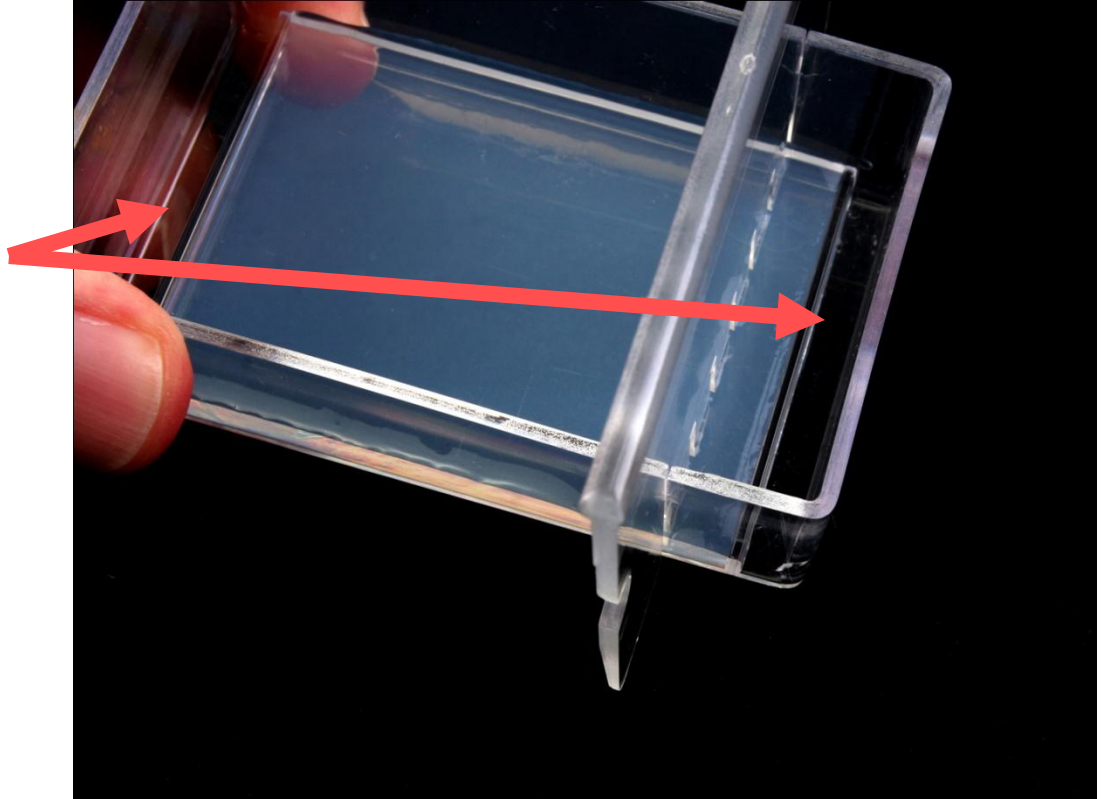
Loading the Gel



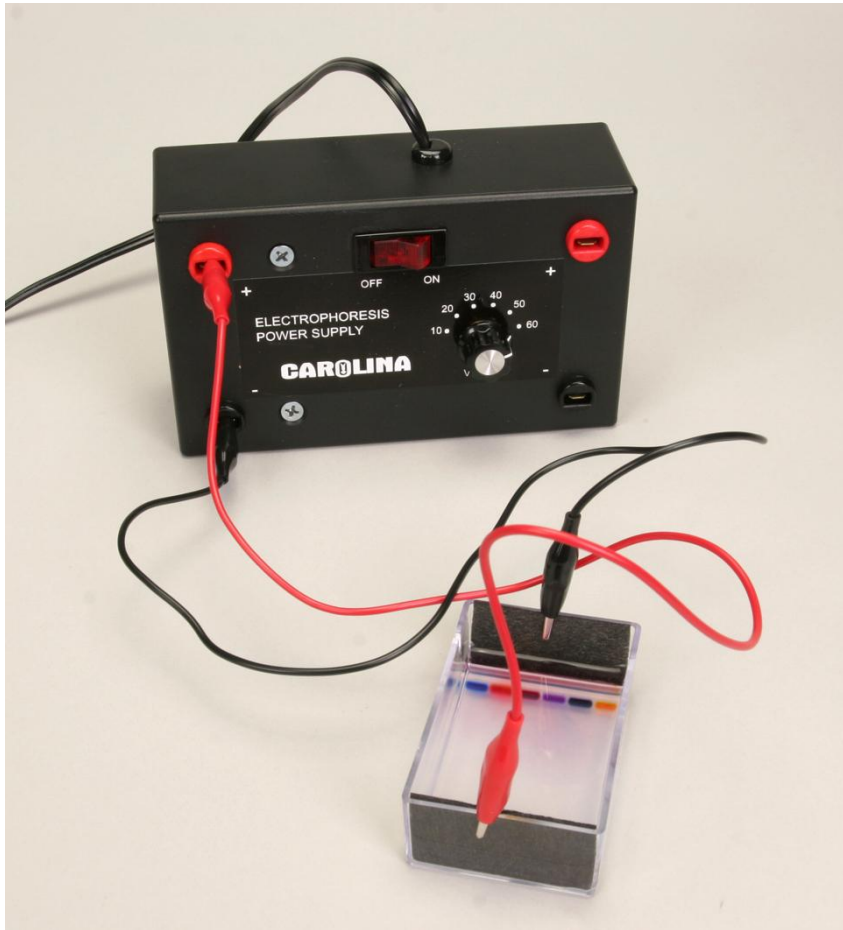
- Make sure there are no air bubbles in the pipet tip.
- Do not puncture the bottom of the well.
- Make sure the pipet tip rests over and just inside the well.
- Expel the sample slowly.
- Load 1 sample per well.

Placing the Carbon Fiber Electrodes

The carbon fiber electrodes rest in the narrow spaces indicated by the arrows.



Hooking Up to Power Supplies

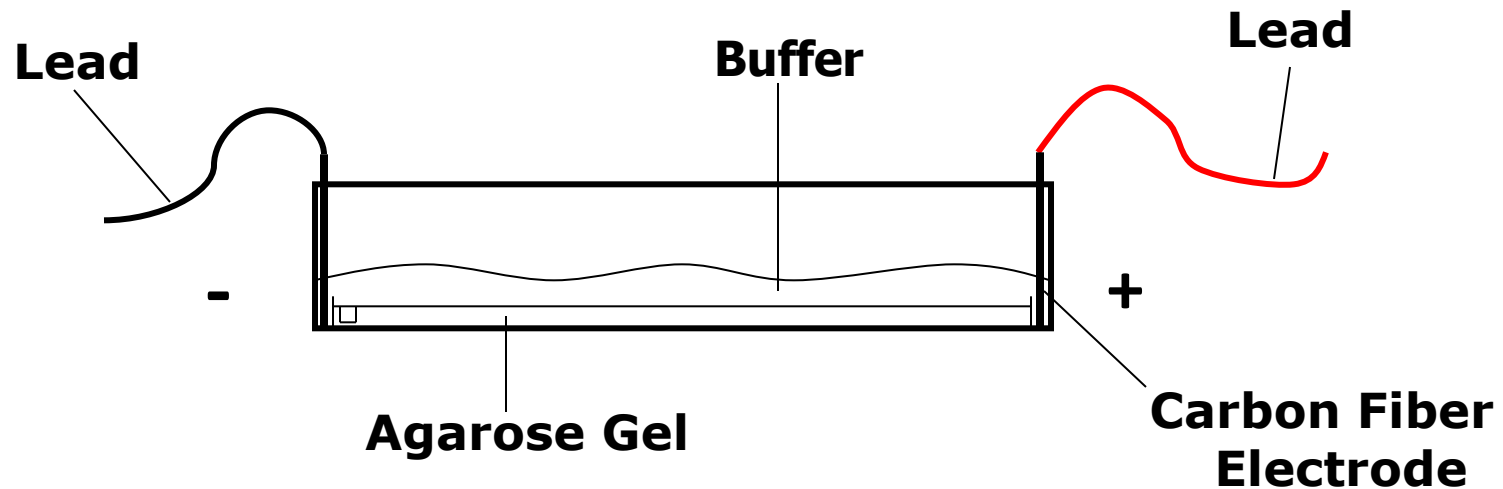


- Use leads to hook gel box to power supply
- Run at 50 V

Dyes Analyzed

- Bromphenol blue
- Methyl orange
- Ponceau G
- Xylene cyanol
- Explorer Mix 1 ??????
- Explorer Mix 2 ??????

Gel Electrophoresis



Gel—Matrix through which molecules to be separated are run; can be made of agarose or acrylamide.

Buffer—Ions in the buffer carry the current. The most commonly used buffers for DNA are:

TBE—tris/boric acid/EDTA

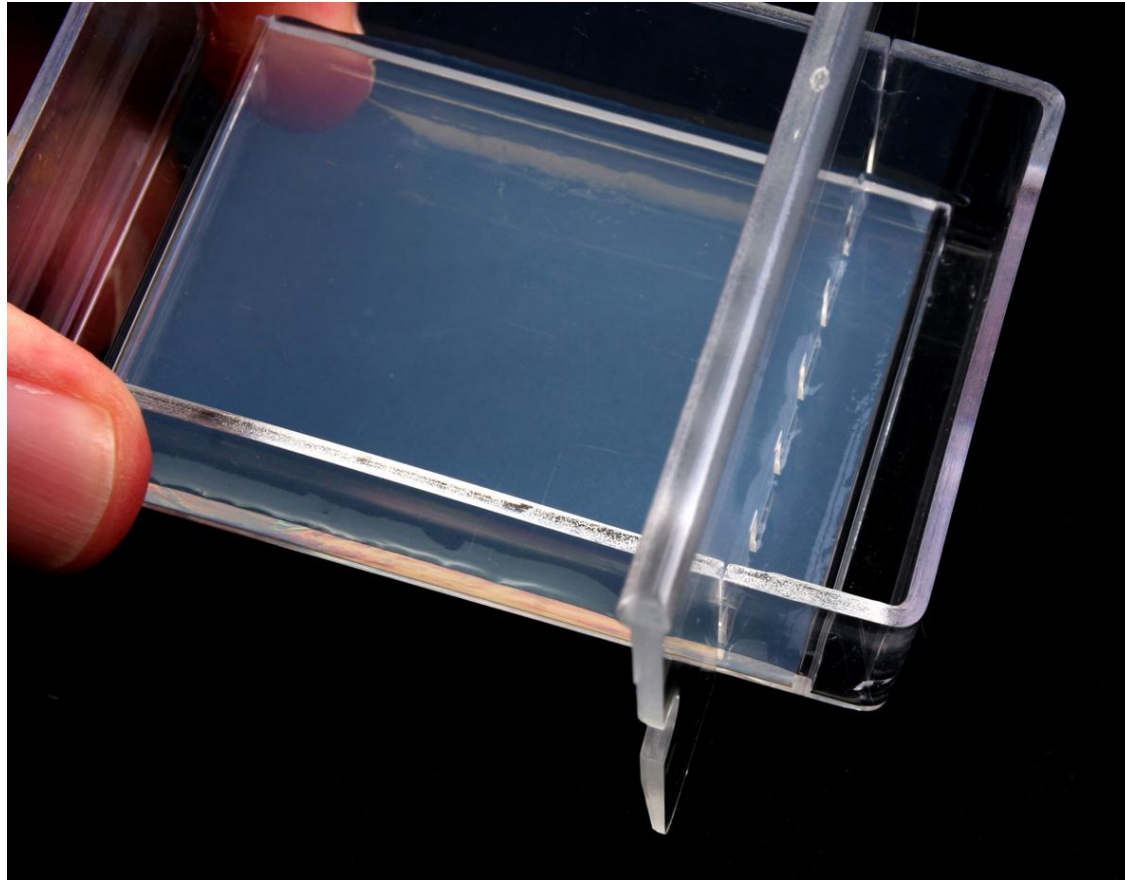
TAE—tris/acetic acid/EDTA

Pour the Gel



- Use about 10 mL of agarose
- Gel solidifies in 10–15 min

Gel Becomes Cloudy Once Hardened



Other Basic Electrophoresis Concepts

- The percentage gel used depends on the size of the components being separated.
- Small gels require less voltage than large gels to run at the same given speed.
- Once current is no longer applied, DNA/dyes start to diffuse.

Common Mistakes

- Not seating the comb completely
- Making up the gel with water instead of buffer
- Not melting and mixing the agarose powder completely
- Switching the leads
- Loading before pouring buffer over the gel

Internet Resources

<http://www.dnalc.org>

Animations and much more

<http://www.dnai.org/index.html>

DNA interactive site with simulations,
lesson plans, and more

<http://gslc.genetics.utah.edu/units/biotech>

Genetic Science Learning Center with
electrophoresis simulations, etc.

<http://www.zoo.utoronto.ca/able>

Select "Proceedings" for lab instructions

Results of Dyes Analyzed

- Bromphenol blue
- Methyl orange
- Ponceau G
- Xylene cyanol
- Explorer Mix 1: ponceau G
xylene cyanol
- Explorer Mix 2: ponceau G
bromphenol blue
methyl orange

National Science Education Standards

“Students at all grade levels and in every domain of science should have the opportunity to use scientific inquiry and develop the ability to think and act in ways associated with inquiry including using appropriate tools and techniques to gather data.”

Exploring Electrophoresis Series

- Kits contain inexpensive electrophoresis apparatus that can be run using batteries
- Inexpensive power supplies are also available
- Setup to be used by 5 groups of students
- Refills available
- All components can be purchased separately

Exploring Electrophoresis Series

- **Catalog no. 211002** Exploring Electrophoresis of Dyes Kit
- **Catalog no. 211006** Exploring Electrophoresis of DNA Kit
- **Catalog no. 211010** Exploring Restriction Analysis and Electrophoresis of DNA Kit
- **Catalog no. 211014** Exploring Electrophoresis and Forensics Kit
- **Catalog no. 211018** Nature's Dice—A Genetic Screening Simulation Kit

All components of these kits can be purchased separately.

Carolina Free Resources



**Carolina offers many free resources
to help support teachers.**

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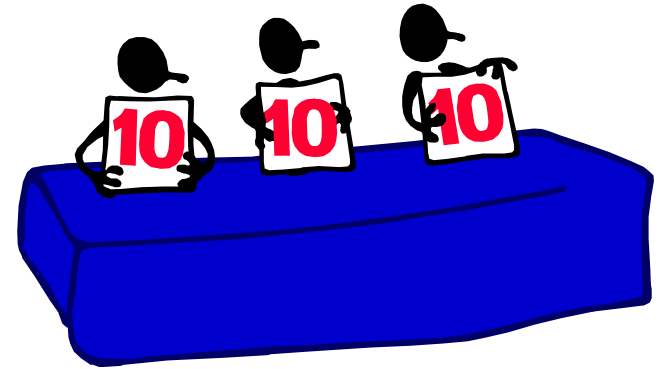


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Evaluations: Share Your Thoughts!

Scale = 1 to 10

- **10 = Outstanding**
- **9 = Above Average**
- **8, 7 = Average**
- **6, 5, 4 = Below Average**
- **3, 2, 1 = Well Below Average**



Please provide comments!

Carolina Biological Supply Company

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our training program.**

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check out our Web site,
www.carolina.com.**

Enjoy the rest of the conference!