What is Electrophoresis?

Electrophoresis is a technique that allows us to separate DNA, RNA or proteins according to their size.

What do I need to separate a mixture of DNA molecules?

In addition to your DNA sample, you will need:

- Gel Loading Solution – includes glycerol to help DNA samples enter into the wells and a visible dye to monitor migration through the gel.
- Agarose – a polysaccharide used as the separation matrix.
- Electrophoresis Buffer – contains ions necessary to conduct an electrical current, maintains pH of experiment.
- Horizontal electrophoresis apparatus – holds the buffer and the gel, has positive and negative electrodes.
- Power supply – generates the current necessary to move DNA through gel.
- Micropipet – used to transfer samples into wells.
- A special stain that allows us to visualize DNA.

How does electrophoresis separate DNA fragments?

The mixture of DNA molecules is added into depressions (or “wells”) within a gel, and then an electrical current is passed through the gel (Figure 1A). Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 1B).

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete “bands” within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 1C).
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).

2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).

3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).

4. **COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.

5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.

6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.

7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

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**Table A**

<table>
<thead>
<tr>
<th>Size of Gel Casting Tray</th>
<th>Concentrated Buffer (50X)</th>
<th>Distilled Water</th>
<th>Amt of Agarose</th>
<th>Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 ml</td>
<td>29.4 ml</td>
<td>0.23 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>7 x 10 cm</td>
<td>1.0 ml</td>
<td>49.0 ml</td>
<td>0.39 g</td>
<td>50 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.2 ml</td>
<td>58.8 ml</td>
<td>0.46 g</td>
<td>60 ml</td>
</tr>
</tbody>
</table>

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**Caution! Flask will be HOT!**
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2. MIX agarose powder with 1X buffer in a 250 ml flask (see Table A).

3. DISSOLVE agarose powder by boiling the solution. MICROWAVE the solution on high for 1 minute. Carefully REMOVE the flask from the microwave and MIX by swirling the flask. Continue to HEAT the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).

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6. POUR the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes.

7. REMOVE end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

8. PLACE gel (on the tray) into electrophoresis chamber. Completely COVER the gel with 1X electrophoresis buffer (See Table B for recommended volumes).

9. LOAD entire sample volumes into wells in consecutive order.

10. PLACE safety cover. CHECK that the gel is properly oriented. Remember, the samples will migrate toward the positive (red) electrode.

11. CONNECT leads to the power source and PERFORM electrophoresis (See Table C for time and voltage guidelines).

12. After electrophoresis is complete, REMOVE the gel and casting tray from the electrophoresis chamber and proceed to STAINING & VISUALIZATION.

**Table A**

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
<th>Dilution 50X Conc. Buffer + Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+ &amp; M12 (new)</td>
<td>300 ml</td>
<td>6 ml + 294 ml</td>
</tr>
<tr>
<td>M12 (classic)</td>
<td>400 ml</td>
<td>8 ml + 392 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml + 980 ml</td>
</tr>
</tbody>
</table>

**Table B**

<table>
<thead>
<tr>
<th>Electrophoresis Model</th>
<th>Min. / Max.</th>
<th>Min. / Max.</th>
<th>Min. / Max.</th>
<th>Min. / Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>150 V</td>
<td>15 / 20 min.</td>
<td>20 / 30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>M12 (new)</td>
<td>125 V</td>
<td>20 / 30 min.</td>
<td>30 / 35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>M12 (classic) &amp; M36</td>
<td>75 V</td>
<td>35 / 45 min.</td>
<td>55 / 70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>

**Table C**

**Reminder:** Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Wear gloves and safety goggles
EDVOTEK® Quick Guide: Agarose Gel Electrophoresis

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