Agarose Gel Electrophoresis
Student Sheet

Agarose Gel Preparation and Running for: ______________________ Lab

Materials: check your workstations to make sure all supplies are present before beginning to prepare your gels.

<table>
<thead>
<tr>
<th>Student Workstation</th>
<th>Common Workstation</th>
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<tbody>
<tr>
<td>• 1 gel casting tray and comb</td>
<td>• agarose</td>
</tr>
<tr>
<td>• 1 small beaker or flask</td>
<td>• 1X electrophoresis buffer</td>
</tr>
<tr>
<td>• 1 graduated cylinder</td>
<td>• DNA stain such as GelGreen™</td>
</tr>
<tr>
<td>• 1 protective mitt</td>
<td>• weigh paper/boats &amp; scoopula</td>
</tr>
<tr>
<td>• 1 electrophoresis unit with power supply</td>
<td>• electronic balance</td>
</tr>
<tr>
<td>• p20 micropipette and tips</td>
<td>• microwave</td>
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For today’s lab you will need to make ______(#) of ______% agarose gel(s). you will use the comb with ____ teeth and the comb will be placed at the end or in the middle (circle one).

1. Obtain ______mL of ______ buffer to use for gel preparation and for filling the chamber of your electrophoresis unit. ▲ Caution: You must use the same type of electrophoresis buffer to pour and run your gels.

2. The volume of each gel is ~ ______ mL, so you will make ______mL of a ______% agarose solution in ______ buffer.

To prepare the gel:

3. Weigh out ______g of powdered agarose and place in a beaker or flask that has a volume more than 2X the total volume of your final agarose solution.

4. Gently pour ______mL of ______ buffer over the powdered agarose and swirl to mix.

5. Heat the agarose solution in a microwave in 30-second intervals and mix by swirling the flask after each interval until the solution is clear. ▲ Caution: The solution can boil over easily! In order to prevent burns be sure to use a protective mitt when handling the flask containing the agarose solution.

6. Check carefully to make sure that all the suspended particles have dissolved. Once the agarose is completely dissolved, remove the flask from the microwave. ▲ Caution: The flask will be hot. Use a protective mitt to prevent burns.

7. Let the solution cool until you can comfortably touch the flask with your hand for at least 10-15 seconds.

8. Add a DNA stain such as GelGreen™. You will need to add 1 μL of stain for every 10 mL of gel mix. So you will add ______ μL.

9. Once the molten agarose is just cool enough to touch, (but before it begins to solidify!) carefully pour about ______mL of molten agarose into the gel casting tray and immediately insert the comb in the gel. (Today you will use the comb with ____ teeth and the comb will be placed at the end or in the middle). ▲ Tip: Use a pipette tip to pop any bubbles that form while pouring the gel.

10. Allow the gel to cool and set for approximately 20 minutes or until it has become opaque and hardened.

9/23/19
To run the gel:

11. Once the gels have completely cooled, remove the combs. Place each gel (still in the casting tray) into the electrophoresis unit. **Tip:** Be sure to orient the gel such that the wells formed by the combs are closest to the negative electrode. Because DNA is negatively charged, it will migrate away from the negative electrode and towards the positive electrode. Dye molecules maybe positively or negatively charged so the wells are placed in the center of the gel.

12. Fill the electrophoresis unit with enough 1X buffer to just cover the agarose gel. The running buffer should cover the gel in the electrophoresis apparatus by only 3–5 mm. **Tip:** Too much buffer will decrease DNA mobility and cause band distortion.

13. Before loading the DNA samples into the wells, a loading dye must be present in each of your samples. Check to see if your samples are colored. If not consult your teacher. The loading dye serves three purposes:
   1. it provides a visible dye that helps with gel loading
   2. dye molecules moves through the gel much like small DNA molecules, so it allows you to gauge how far the molecules have moved and prevents you from running your molecules through and then off of the gel
   3. it contains glycerol, so after adding it your sample is heavier than water and will settle to the bottom of the gel well, instead of diffusing in the buffer

14. Plan the order of your samples. **Tip:** record this information. Often the first lane of the gel is used for the molecular weight markers or DNA ladder. A DNA ladder is a collection of many DNA molecules, each of which is a known size. This allows for easy determination of the fragment size of your samples by visual reference.

15. Using a micropipette, load ______ μL of sample.
   - Visually inspect the tip of your pipette to ensure that there are no bubbles in the sample.
   - Place the tip over the desired well and lower the tip until it just breaks the surface of the liquid.
   - With the tip over the well, slowly release the sample.
   - Avoid dispensing air bubbles into the well and do not release pressure on the pipet until the tip has been removed from the fluid.

16. Quickly load all lanes of the gel. **Tip:** Do not allow the gel to sit with DNA samples in the wells for very long before turning on the power. If you do, the DNA molecules will begin to diffuse through the gel and give you fuzzy bands.

17. Close the gel tank, switch on the power-source and run the gel at about ______ V.

18. After just one minute, check that your electrophoresis is underway by looking at the electrodes and confirming that they are releasing gas bubbles. In just a few minutes, you should see the dye moving out of the wells and toward the positive electrode.

   You will probably notice that there are twice as many bubbles on the negative electrode (the cathode) than at the positive electrode (the anode). These bubbles are hydrogen (cathode) and oxygen (anode) gas resulting from the electrolysis of water.