Preparing the Gel Box and Pouring the Agarose Gel

Student Workstation	Quantity
Plastic chamber	1
8-well comb	1
Ruler	1
Molten agarose	50 ml
Marking pen	1

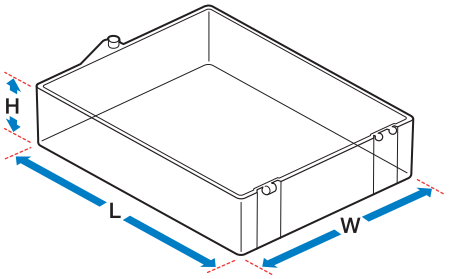
Protocol

1. Using a ruler, measure the length, width and height of the plastic box and record here.

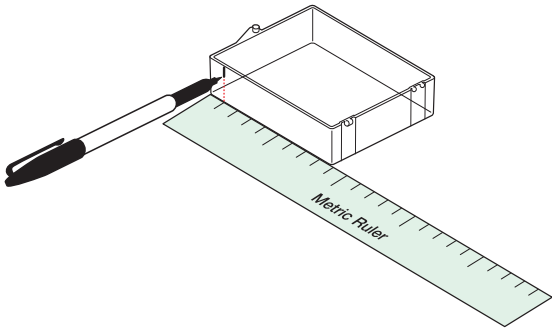
   Length L=_____ cm

   Height H=_____ cm

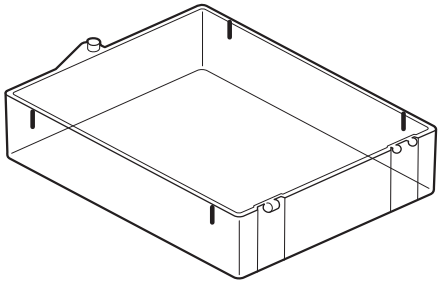
   Width W= _____ cm



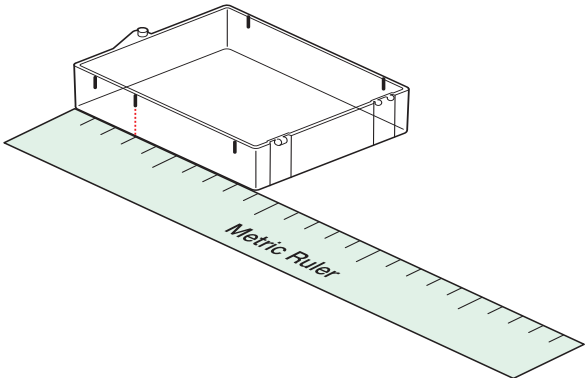
2. Measure a distance 1 cm from the end of the box on the longest side, and with a marking pen, make a dash on the outside of the box.



3. Repeat step 2 so that you have a mark 1 cm from the end of each of the longest sides of the box.

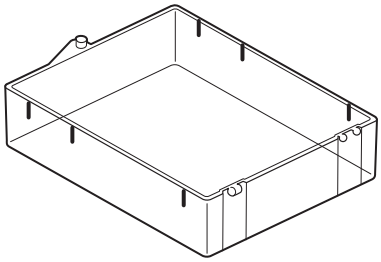


4. Measure a distance 3 cm from the end of the box on the longest side, and with a marking pen, make a dash on the outside of the box.

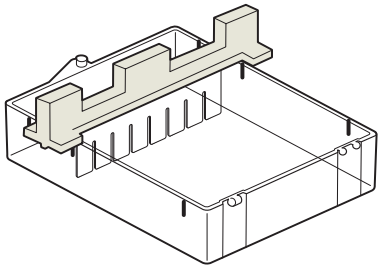


Protocol (cont.)

5. Repeat step 4 on the opposite side of the box. When you have finished, each side of the box should have three marks.

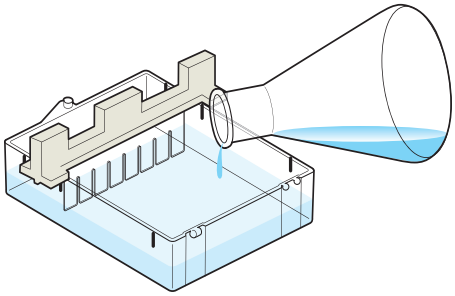


6. Place your 8-well comb on the marks that are 3 cm from the end. Make sure that the comb is centered so that none of the clear plastic well-formers touch the plastic box and that the comb is straight across the box.

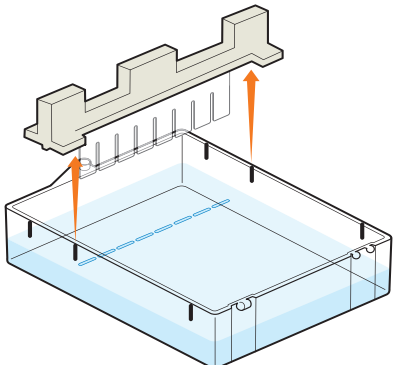


7. Carefully pour 50 ml of molten agarose into the box and allow the gel to solidify for 10–20 min. The gel will appear cloudy, or opaque, when ready to use.

   Caution: Always were protective gloves, goggles, and lab coat while preparing and casting agarose gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.



8. Carefully remove the comb from the solidified gel by pulling gently in an upward direction.



9. If you do not have sufficient time to proceed to Agarose gel electrophoresis, store the gel in the box, covered with 25 ml of 1x TAE buffer in a sealable plastic bag at room temperature for 1 day, or in the refrigerator (4°C) for up to 1 week before using them. Be sure to label your plastic bag.

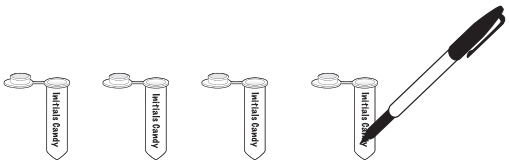
Dye Extraction From Candies

Student Workstation	Quantity
Dye extraction solution	2 ml
2 ml microcentrifuge tubes	4
Microcentrifuge tube rack	1
Marking pen	1
Plastic cups or small beakers	4
Eyedropper	1
Colored candies	4 varieties, 1–4 candies per variety*

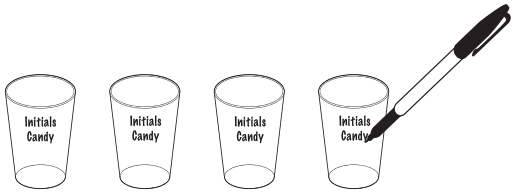
*Candy example: 3 green Skittles, 3 orange jelly beans, 4 Red Hots, 1 brown gumball

Protocol

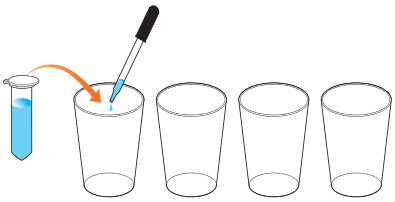
1. Label the four microcentrifuge tubes with your initials and the names and colors of the candies you are using.



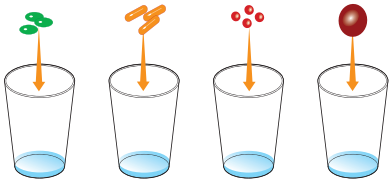
2. Label four cups with your initials and the names and colors of the candies you are using.



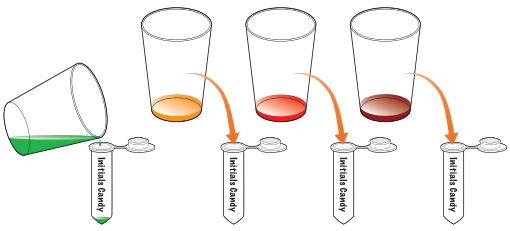
3. Using an eyedropper or pipet add 0.5 ml of dye extraction solution to each cup. Use the volume marks on the 2 ml microcentrifuge tube to measure the correct volume.



4. Place your candy into the appropriately labeled cup and swirl the candy in the dye extraction solution. If using a candy such as M&M'S or Skittles, just dissolve the color coating off until you get to the white layer of the candy. For all other candies, try to get as dark a solution of dye as possible.



5. Remove your candy from the cup. Pour the solution containing the dissolved colored candy coating into the appropriately labeled microcentrifuge tube.



Agarose Gel Electrophoresis

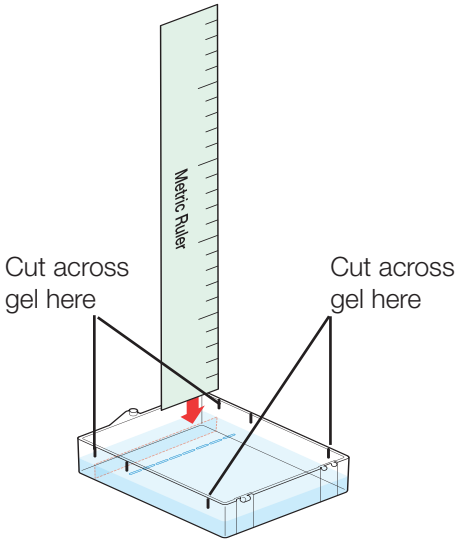
Student Workstation	Quantity
Agarose gel poured into plastic chamber	1
Plastic ruler	1
Paper clips	2
Black lead with alligator clips	1
Red lead with alligator clips	1
9 volt batteries	3-5
Blue 1 reference dye	15 µl
Yellow 5 reference dye	15 µl
Yellow 6 reference dye	15 µl
Red 40 reference dye	15 µl
1x TAE buffer	55 ml
Dyes extracted from candies from **Dye extraction from candies** activity	4 samples
2–20 µl adjustable-volume micropipet or 10 µl fixed-volume micropipet and 8 tips	1
Marking pen	1

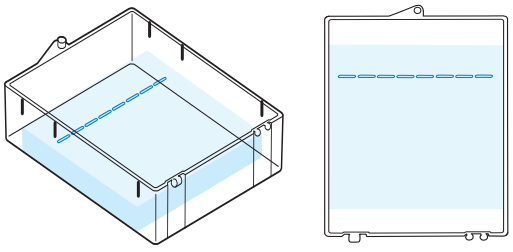
Protocol

1. Obtain your agarose gel in the plastic chamber. If you stored your gel after preparing it, pour off the 25 ml of 1x TAE buffer.

2. Using your ruler and following the marks you made one centimeter from the end of the box, cut a slab off the end of the gel using the end of a ruler. Press straight down through the gel to the box — do not slice across the gel. Loosen the slab by sliding the ruler between the end of the gel and the box end, then lift out the slab and discard.

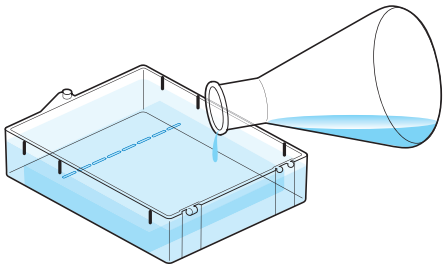
3. Repeat at the other end of the gel.



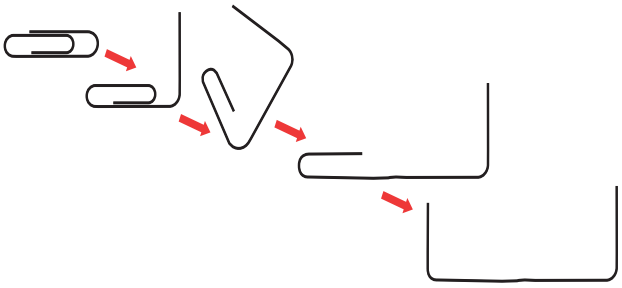

(Top view)

Protocol (cont.)

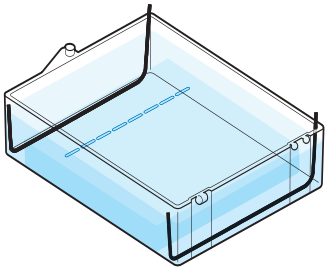
4. Add 55 ml of 1x TAE buffer to the box.



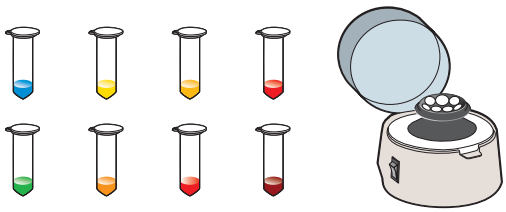
5. Construct your electrodes from two paper clips. Carefully straighten the paper clip and bend the two ends so they are perpendicular to the rest of the paper clip. Place your completed electrode on a flat surface. If it does not lie flat (in other words, if one of the angled pieces is not in the same plane as the rest of the electrode), hold the two ends and twist gently until the electrode will lie flat. The longer end will stick up above the gel box — this is where you will attach the alligator clip.



6. Place the electrodes into the gel box with the long ends on the same side. The electrodes should be as close to the end of the box as possible (as far away from the gel as possible).

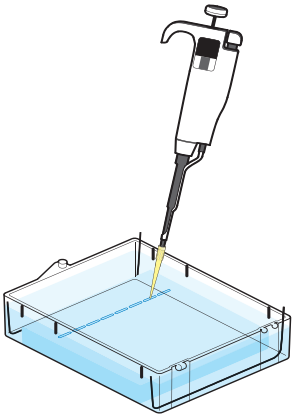


7. Prepare your extracted candy dye samples. If a centrifuge is available, pulse spin the microcentrifuge tubes in the centrifuge to bring all the liquid to the bottom of the tube and to settle any insoluble particles. Spin down your dye standard samples as well, if needed.



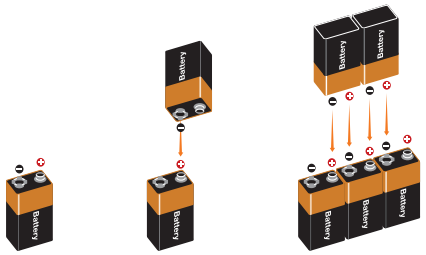
8. Using a separate tip for each sample, load 10 µl of each sample into 8 wells of the gel in the following order:

   Lane 1: Blue 1 reference dye
   Lane 2: Yellow 5 reference dye
   Lane 3: Yellow 6 reference dye
   Lane 4: Red 40 reference dye
   Lane 5: Candy 1 dye extract
   Lane 6: Candy 2 dye extract
   Lane 7: Candy 3 dye extract
   Lane 8: Candy 4 dye extract

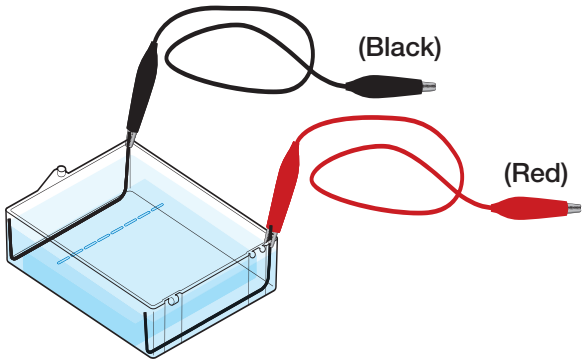


Protocol (cont.)

9. Assemble your battery tower by connecting negative nodes to positive nodes.

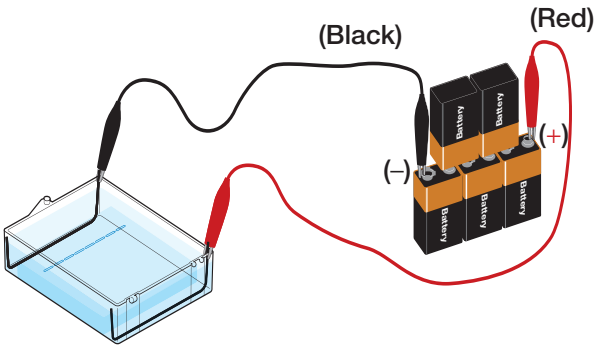


10. Attach the black alligator clip to the long end of the paper clip and box at the end of the box closest to the sample wells. Make sure the paper clip still remains on the bottom of the gel box under the buffer. Repeat the process for the red alligator clip and the electrode at the other end of the box.



11. When you are ready to begin your electrophoresis run, attach the free black alligator clip on your lead to the (–) terminal of your battery tower and the free red alligator clip on your lead to the (+) of your battery tower. You should notice bubbles coming off of the paper clip electrodes if the circuit is complete.

12. Allow your gel to run for 20 min. Disconnect the red and black alligator clips from the battery tower.



13. Take a photograph of the gel for your records.

Bio-Rad
Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin 6288 Rev A US/EG *Sig 1211*