Teacher's Guide

Materials:

- 200 or 500 ml beakers,
- powdered Bromothymol Blue (BTB) from Carolina Biological - 5g is $20 (enough for 10 labs)
- agar (not agarose), from Carolina Biological- 500 g bottle for $70 (for 10-12 labs)
- rulers,
- paring and / or plastic knives,
- NaOH,
- Safety Goggles
- 1 and 2 inch square ice cube trays (a 3 pack on Amazon.com is less than $20 and can be reused; each 2 inch tray makes 8 cubes, 1 inch trays make 15),
- sturdy cake pan (the more pans, the more agar you can make at one time),
- white vinegar (regular strength = 5%)
- white paper plates (to cut blocks on, measure on – avoiding mess).

Making the BTB agar

1. You can buy agar from Carolina Biological. Use about 10 g agar per 1000 ml water – mix and microwave or boil until dissolved (watch it - may overflow).
2. Add 0.1 g powdered BTB and mix. You are looking for a green-blue color. If not dark enough blue, add more BTB. If it is more green-yellow than blue, add NaOH until it turns dark blue (wear goggles/gloves with the NaOH)!
3. Pour the BTB agar into trays and cake pans – you want it at least 3 cm deep. Students will need to make $1cm^3$, $2cm^3$, 3 cm^3 cubes and a 1x1x8 cuboid, using agar from the cake pan to get these larger pieces.
    a. 2 liters generally makes 1- 9x13 cake pan with agar 3 cm deep.
    b. 1 liter will give you about 3 (2 inch square) ice cube trays of agar.
    c. Groups of 4 students, 8 groups per lab: you need three – 1 inch and three – 2 inch ice cube trays and 1 cake pans of agar (4 L) per lab.
    d. One 2 inch square cube can be used to make both the 1 cm^3 and 2 cm^3 block - make sure students understand this - don't waste BTB agar!
4. Cover and refrigerate overnight. Once set, you can pop them out of the ice cube trays and store in an air-tight container in fridge, so that you can re-use the trays to make more cubes. I've stored it at least 2 weeks in the fridge.
5. You can also use gelatin, such as "Knox Blox" made with 25% less water to keep it stiffer, and a red cabbage juice indicator.

Procedure:

1. With students working in pairs, each pair gets 1 – 2.5 cm^3 BTB agar cube and 200 ml vinegar. Without much explanation, watch what happens when you put the blocks in vinegar for ten minutes (remove after 10 minutes (diffusion will continue even after you remove the block from the beaker) and **Quickly** cut cubes in half and **Measure** the depth to which the acid diffused (your block will now be yellow on the outside and blue in the center – measure the distance, in mm, from the agar block surface to the start of the blue line).

2. Students are in groups of 4 – each group gets a couple of 1 inch cubes and a 2 inch cube and a larger piece to cut the 1x1x8cm piece. There will be some damage, so give a couple extra to each group. Not enough agar? Just do 2 cubes.
3. Students cut four cubes - they should be the following sizes:

1cm x 1cm x 1cm	**3cm x 3cm x 3cm**
2cm x 2cm x 2cm	**1cm x 1cm x 8cm**

4. Make hypotheses and predictions. (What happens to rate of diffusion as the size increases? Which cube will turn yellow first?)
5. Pour 200 ml of white vinegar (a weak acid) into a beaker or clear plastic cup. Gently (watch splashing) place the blocks into the acid all at the same time. Record the start time. You will wait for 10 minutes before taking the blocks out.
6. After **10 minutes** take all blocks out of the vinegar solutions and CUT them in ½ with a straight edge or paring knife. Diffusion will continue even after you remove the block from the beaker. **Quickly** measure the depth to which the acid diffused (your block will now be yellow on the outside and blue in the center – measure the distance, in mm, from the agar block surface to the start of the blue line). Also measure the shortest length of blue rectangle in the agar block, or measure the remaining area of blue (where diffusion hasn't reached yet). If you have enough time it is best to let all cubes turn completely yellow – and record how long it takes for each cube to turn yellow.
7. To test the effects of temperature on rate, repeat, use three 2.5 cm^3 cubes and three beakers with 200 ml of vinegar each – one with room temp vinegar, one with microwaved- warmed vinegar, one with refrigerator /freezer cold vinegar.
8. Cell Race: Allow students to design their own cell using a larger piece of BTB agar from the cake pan. Students will place their shape in a beaker of vinegar and time how long it takes to turn completely yellow. Instruct them to make a shape that maximizes diffusion, or has the highest ratio of mass / time. As time (and agar supply) permits, allow them to test, re-design, and test again.

Data Analysis: Graph temperature on the X- axis and depth of diffusion on the Y. Your graph will have three bars, one for each temperature. The independent variable is temperature, and the dependent variable is depth of diffusion. You should see the slowest diffusion in the cold vinegar, because molecules that are moving slowly register as cold, and slow moving molecules will diffuse more slowly.

Safety Considerations: Remind students not to eat or drink anything in lab. When making BTB agar, be careful of the hot plate as you heat the agar to boiling, and wear gloves and goggles when adding NaOH to the BTB agar (if necessary) to bring it to the appropriate color/pH. Grocery store vinegar is acetic acid, and is about 5% in concentration with a pH of 2.4. It is classified as a skin and eye irritant. You and the students should wear safety goggles while working with the vinegar (the BTB agar cubes are a little slippery and it may fall back into the beaker, splashing some out, as you try to remove the blocks). It is possible that a student may have an allergy to vinegar – check it out beforehand. Allergy symptoms generally include itchy watery eyes, and, if ingested, swelling of lips and / or hives and itching all over the body. Antihistamines will relieve this. Bromothymol blue doesn't have a hazard rating, though MSDS sheets show that it may be a skin irritant, but no risks are listed for it. Gloves aren't necessary, it does not stain the hands.