Name _____

PLANT PIGMENTS PHOTOSYNTHESIS

We will utilize the LabBench to learn more about plant pigments and how photosynthesis works. ***Some of the questions require you to think beyond the information posted on the LabBench Web site.***

Go to this Web address
http://www.phschool.com/science/biology_place/labbench/

Click on **Lab 4: Plant Pigments & Photosynthesis**.

A. PAPER CHROMATOGRAPHY

1. Walk through the paper chromatography exercise. Make sure to watch the little pigment migration animation.

2. Re-write the Rf formula and show your calculation for the green pigment in the black ink chromatogram.

3. Explain why the pigments migrate to different positions on the paper.

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   _____

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4. List the four pigments (name and color) on the green leaf chromatogram.

5. Why did we have to use a solvent like acetone rather than just plain water to separate the pigments?

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6. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.

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7. Which pigment do Photosystem 1 and 2 reaction centers use?

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8. What is the function of the other pigments and what is the adaptive advantage to a plant of having more pigments than chlorophyll?

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9. Do we see evidence, in everyday life, that these other pigments exist in leaves? Explain.

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10. Do the Self-Quiz and write out the answers below.

    1)

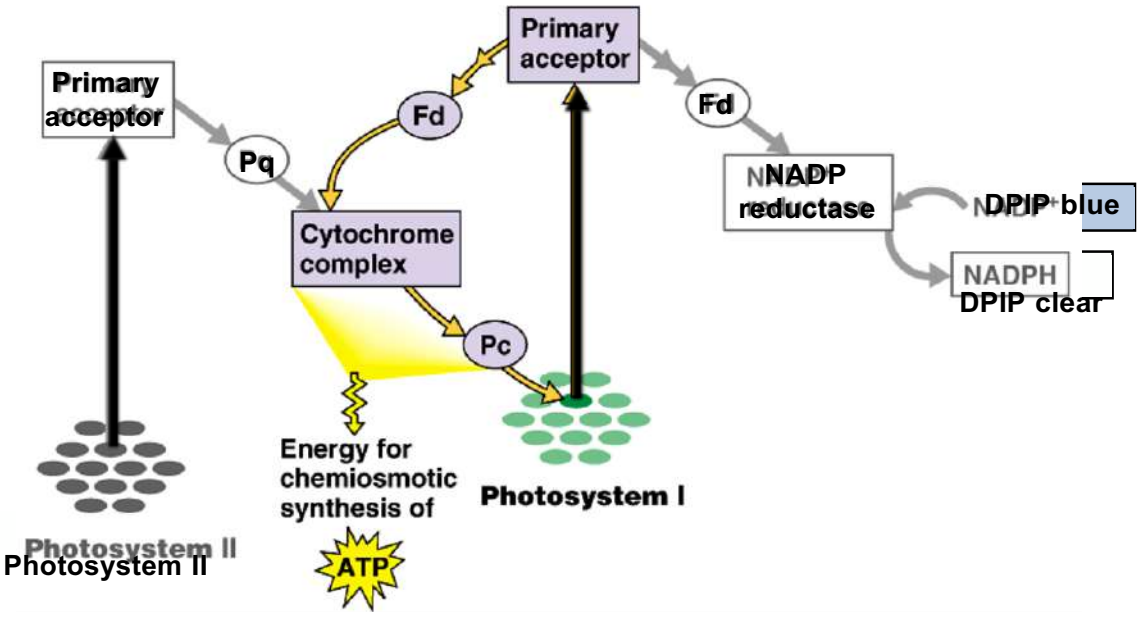
    2)

    3)

B. Photosynthesis Experiment

1. Walk through the Photosynthesis experiment exercise. This is an lab we do not do in class any longer, but I want you to be familiar with it and learn its lessons. Be sure to watch the animations on each page the first time they play; I have had some problems with them running when I hit replay.

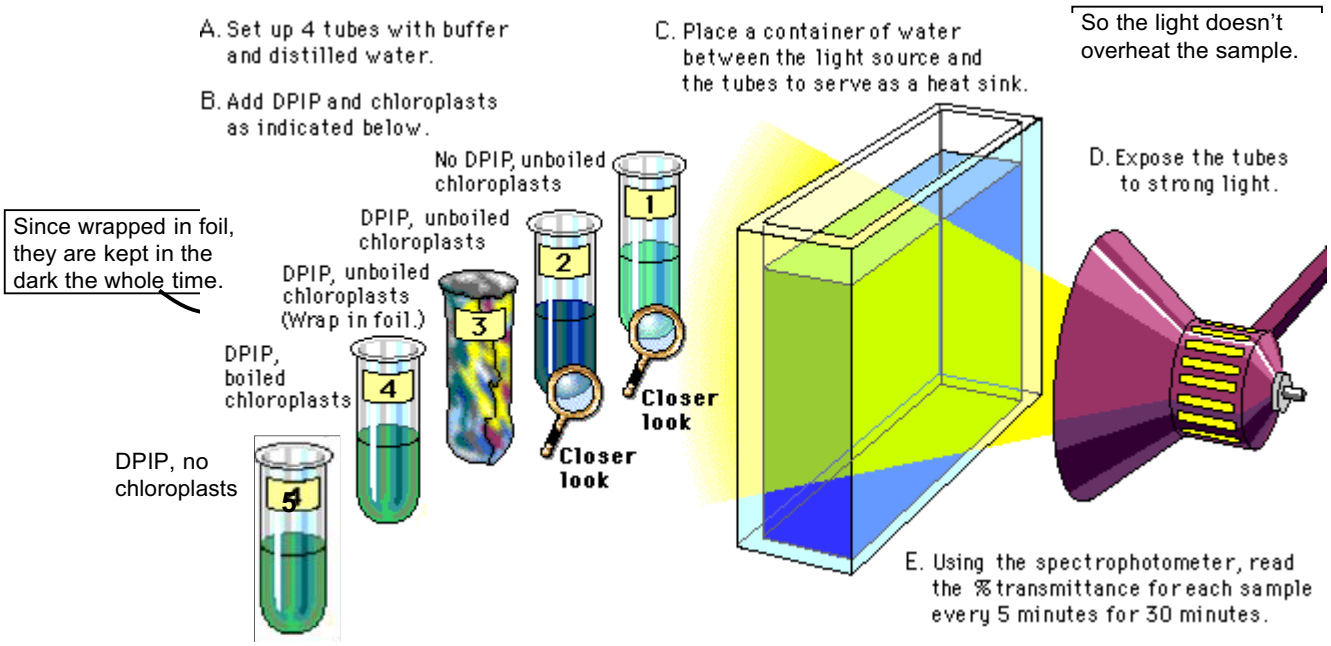
2. **DPIP**: Let me explain a bit, so I am sure you understand. In Photosystem 1 of the light reactions, plants normally use the excited electrons to reduce NADP to NADPH. And NADPH then goes off to the Calvin Cycle to help build sugars. In this experiment, we are going to remove the NADP from the system by isolating the chloroplasts from the rest of the leaf tissue and we will then replace the NADP with this chemical DPIP. So instead of reducing NADP, Photosysyem 1 is going to reduce DPIP. Why? Because when DPIP is reduced, it changes color. It is normally blue and when it is reduced, it becomes clear. So the color of our solution will be used as an indirect way to measure how much photosynthesis is occurring. The more photosynthesis, then the clearer the solution will be (DPIP will change from blue to clear). See the illustration below.



3. **Spectrophotometer**: We now just need a way to measure accurately the color changes of a solution and that's where the spectrophotometer comes in. It is an instrument that does just that—allows us to quantify color changes. When a solution is colored, it absorbs more light and let's less light to flow all the way through it (transmits less light). When a solution is clear, it absorbs less light and let's more light to flow all the way through it (transmits more light). So, in this experiment, as the DPIP goes from blue to clear, it will transmit more light in the spectrophotometer.

4. **Experimental Design**: The chloroplast solution is made by taking fresh spinach and blending it up in the an electric blender. This breaks up the leaf tissue and frees the chloroplasts from the cells. By doing that, we have extracted free but intact chloroplasts from the spinach leaf cells. We then put this chloroplast solution into separate test tubes and treat them in different ways. See below.

   With the help of the illustration below, list the **contents** of the five test tubes used in this experiment.



   a. **Test tube 1 (The "Blank"):** all solutions (buffer, unboiled chloroplasts, water) ex cept for DPIP, exposed to light. This tube is used just to calibrate the spectrophotometer device.

   b. **Test tube 2**: _____

   _____

   c. **Test tube 3**: _____

   _____

   d. **Test tube 4**: _____

   _____

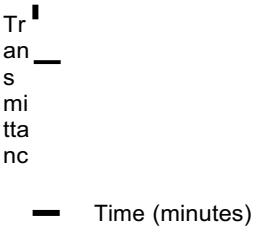
   e. **Test tube 5**: (not shown in original diagram, but added here):

   _____

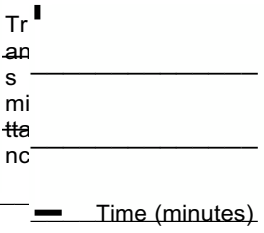
_____

5. Now for each test tube, (1) make a **prediction** as to whether the light-dependent reactions of photosynthesis will occur and explain what reasoning you used to make your prediction. Then, (2) make a **prediction** if the DPIP will remain blue or turn clear. Remember the DPIP takes the place of NADP. If you think NADP would be reduced to NADPH, then in this experiment the DPIP would be reduced and would turn from blue to clear. And (3) **draw a graph** of how the transmittance of the light through the solution in the test tube will change over time. Remember if photosynthesis occurs the DPIP will change from blue to clear and therefore let more light through the solution, which will then be measured as an increase in transmittance in the spectrophotometer. I will complete the first one as an example.

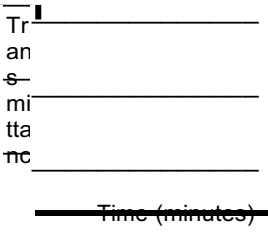
a. **Test tube 1 (The "Blank"):** Since this solution has unboiled chloroplasts and is exposed to the light, the light-dependent reactions should occur. However, there is no DPIP in the test tube, so the tube will not change color from blue to clear. This test tube will just be used as a way of showing the spectrophotometer what the unchanged, original color of the solutions was. It will be used to calibrate the machine.



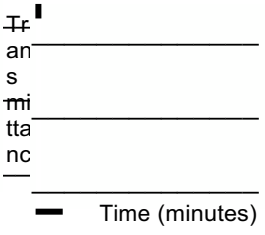
b. **Test tube 2**: _____



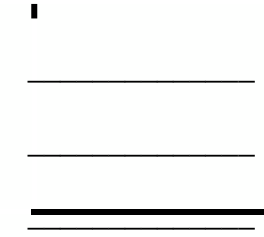
c. **Test tube 3**: _____



d. **Test tube 4**: _____



e. **Test tube 5**: _____



6. What is the function of DPIP in this experiment?

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7. What molecule found in chloroplasts does DPIP “replace” in this experiment?

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8. What is the source if the electrons that will reduce DPIP?

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9. What did the spectrophotometer measure in this experiment?

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10. What is the effect of keeping the sample in total darkness (the foil wrapped sample)?

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11. What is the effect of boiling the chloroplasts on photosynthesis and the reduction of DPIP?

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12. Identify which of the test tubes are **controls** and which are **experimentals**. Explain

a. **Test tube 1 (The "Blank"):** (no DPIP, unboiled chloroplasts, in the light)

CONTROL. This tube is used to calibrate the spectrophotometer, so it serves as a **negative control**, since we know it will not change color, because there is no DPIP present to be reduced.

b. **Test tube 2**: (DPIP, unboiled chloroplasts, in the light)

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c. **Test tube 3**: (DPIP, unboiled chloroplasts, in the dark)

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d. **Test tube 4**: (DPIP, boiled chloroplasts, in the light)

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e. **Test tube 5**: (DPIP, no chloroplasts, in the light)

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13. Do the Self-Quiz and record your answers below.

1)
2)
3)
4)
5)