Lab 9: Bacterial Transformation & Spectrophotometry, Part 1

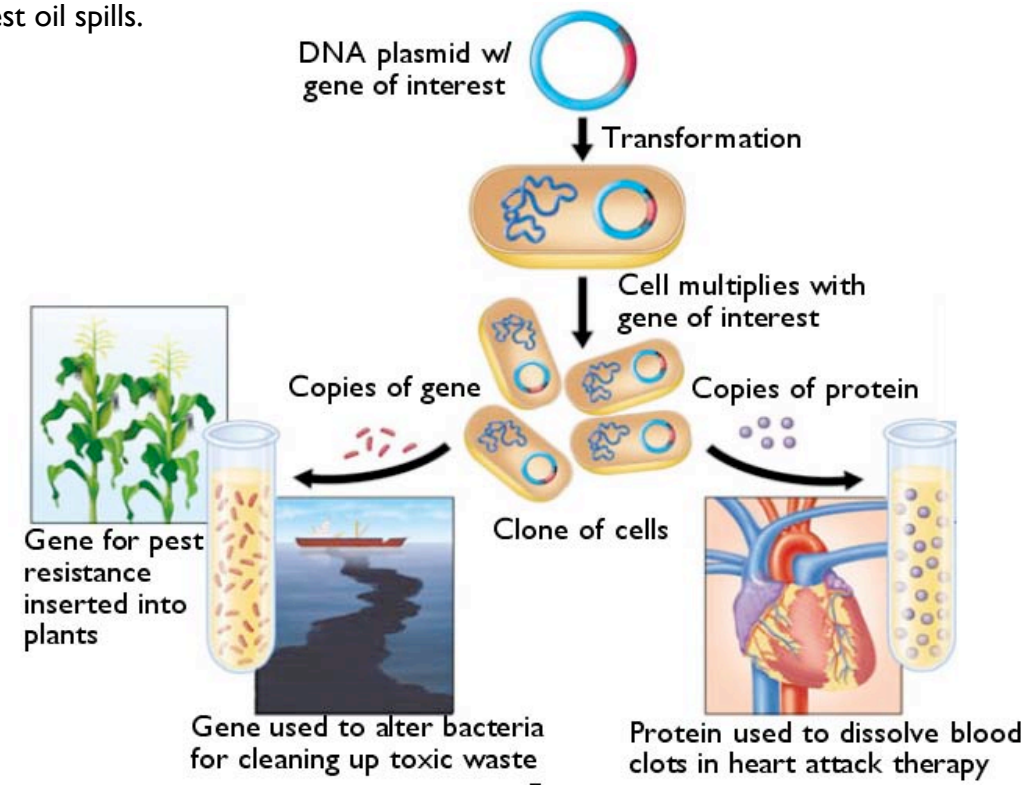
Activity 9a
Bacterial Transformation, Part 1

Purpose and Background

In this lab, you will perform a procedure known as genetic transformation. Transformation is defined as the insertion of a gene into an organism in order to change the organism's trait(s).

Genetic transformation is used in many areas of biotechnology.

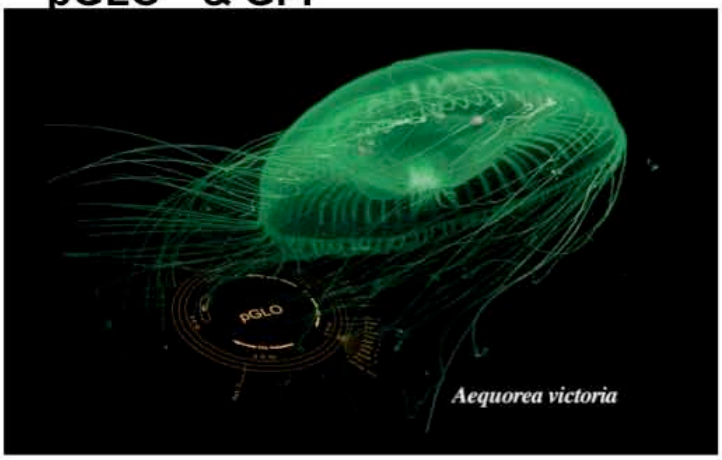
- In medicine, gene therapy involves transforming a sick person's cells with healthy copies of the defective gene that causes the disease.
- In research, bacteria are transformed with genes encoding human proteins for biomanufacturing production or for further study of these proteins.
- In agriculture, genes coding for traits such as frost-, pest-, or spoilage-resistance can be genetically transformed into plants.
- In bioremediation, bacteria can be transformed with genes that enable them to digest oil spills.



We will be transforming bacteria with a gene that codes for Green Fluorescent Protein (GFP). This gene originally came from the bioluminescent jellyfish *Aequorea Victoria*. GFP is the protein that causes this jellyfish to glow in the dark. If your transformation is successful, the transformed bacteria expressing the GFP protein will glow a brilliant green color under ultraviolet light.

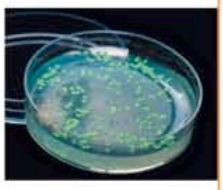
Before beginning the laboratory activities, we will first learn more background on transformation in general and our particular experiment in a Powerpoint lecture (see next 4 pages for notes).

pGLO™ & GFP



Uses of Green Fluorescent Protein

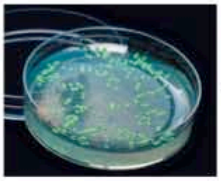
- GFP is a visual marker
- Study of biological processes (example: synthesis of proteins)
- Localization and regulation of gene expression
- Cell movement
- Cell fate during development
- Formation of different organs
- Screenable marker to identify transgenic organisms

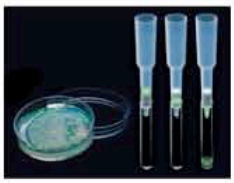


- Transformation is a natural process that bacteria have evolved in order to obtain DNA from their environment.
- Use of the procedure enables scientists to insert genes by recombinant techniques and place the plasmid into a bacteria for expression

Timeline for Transformation

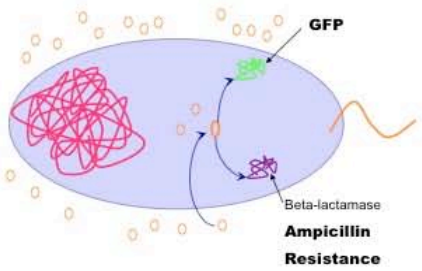
- Background
- Transform bacteria with pGLO plasmid
- Purify GFP using column chromatography *(later lab)*





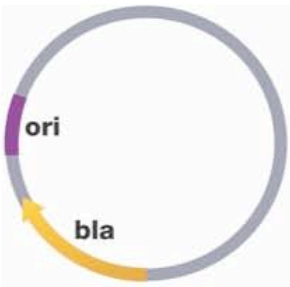
What is Transformation?

- Uptake of foreign DNA, often a circular plasmid



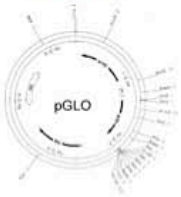
What is a plasmid?

- A circular piece of autonomously replicating DNA
- Originally evolved by bacteria
- May express antibiotic resistance gene or be modified to express proteins of interest

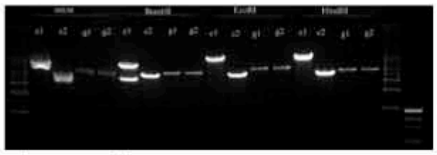


The Many Faces of Plasmids

Scanning electron micrograph



Graphic representation

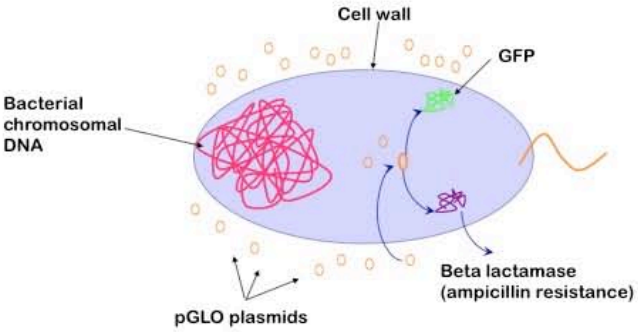


Agarose gel

pGLO Plasmid Map

- Beta Lactamase (*bla*)
  - Ampicillin resistance
- Green Fluorescent Protein (GFP)
  - *Aequorea victoria* jellyfish gene
- araC regulator protein
  - Regulates GFP transcription
- ori
  - Allows plasmid to replicate in bacterial cell

Bacterial Transformation



Transcriptional Regulation

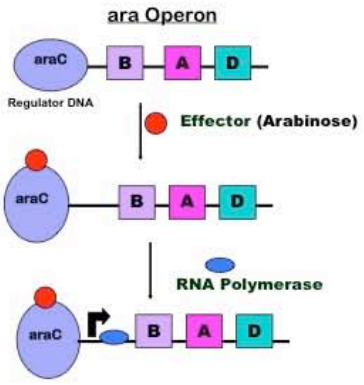
(DNA ⇨ RNA)

- Arabinose operon
  - Regulates expression of the GFP gene on the pGLO plasmid

Transcriptional Regulation

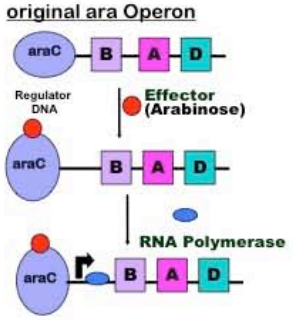
When the sugar arabinose is not present, the araC operon is **OFF**

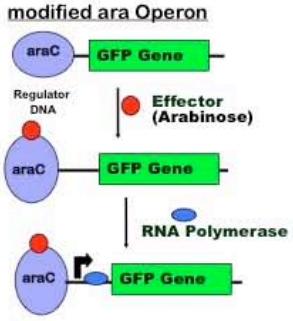
When the sugar arabinose is present, the araC operon is **ON**



GFP Gene Regulation in pGLO

Genes normally regulated by the araC operaon ("B" "A" and "D") have been replaced with the gene for GFP. Now, addition of arabinose turns the araC operon on, turning on expression of the GFP gene.





Methods of Transformation

- Electroporation
  - Electrical shock makes cell membranes permeable to DNA
- Calcium Chloride/Heat-Shock
  - Chemically-competent cells uptake DNA after heat shock

Transformation Procedure - *summary*

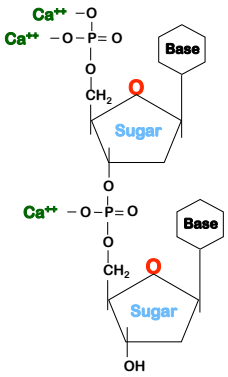
- Suspend bacterial colonies in Transformation solution
- Add pGLO plasmid DNA
- Place tubes in ice
- Heat-shock at 42°C and place back on ice
- Incubate with nutrient broth
- Streak plates

Reasons for Performing Each Transformation Step

1. **Transformation solution = $CaCl_2$**

Positive charge of Ca^{++} ions shields negative charge of DNA phosphates to help DNA get into cell

Cl^- ions enter cell with water, causing cell to swell and have tiny holes in membrane--allow DNA to get in



Reasons for Performing Each Transformation Step, *continued*

2. **Incubate on ice**

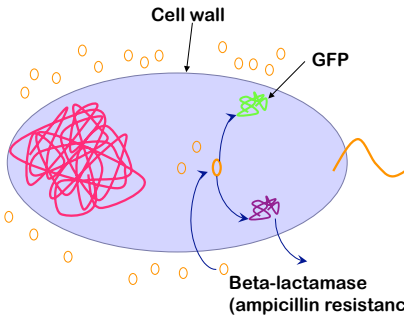
slows fluid cell membrane

3. **Heat-shock**

Increases permeability of membranes

4. **Nutrient broth incubation**

Allows beta-lactamase expression

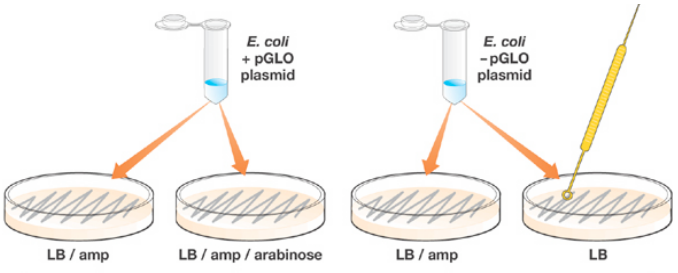


What is Nutrient Broth?

- Luria-Bertani (LB) broth
- Medium that contains nutrients for bacterial growth and gene expression
  - Carbohydrates
  - Amino acids
  - Nucleotides
  - Salts
  - Vitamins

Grow?
Glow?

- Follow protocol
- On which plates will colonies **grow**?
- Which colonies will **glow**?

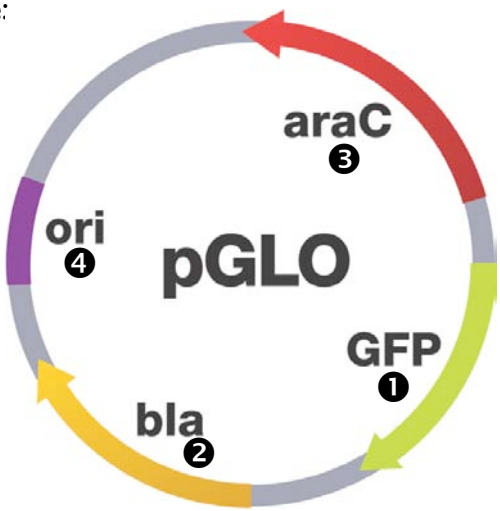


Procedure

The step-by-step procedure for performing your transformation is outlined with illustrations in the quick guide on the next two pages. Before beginning the transformation procedure, go over the quick guide and make sure you understand every step. If you have any questions, be sure to ask your instructor in lab.

To review a few key points from the Powerpoint notes:

- The plasmid that you will be transforming into the *E. coli* bacterial cells is the pGLO plasmid. Plasmids are made of DNA.
- The pGLO plasmid contains four regions that are important for the functioning of the plasmid inside bacterial cells. These four regions are:



  ❶. The gene encoding the protein GFP (Green Fluorescent Protein)

  ❷. The gene encoding the beta-lactamase enzyme, breaks down ampicillin and allows bacteria containing the pGLO plasmid to be resistant to the ampicillin antibiotic.

  ❸. The *araC* regulatory region that controls the expression of GFP. Under the control of the *araC* regulatory region, the GFP protein will only be expressed when arabinose is added to the medium.

  ❹. The origin of replication (*ori*), which allows the plasmid to be replicated inside bacterial cells.

- You will be transforming the pGLO plasmid into the bacterial cells using the calcium chloride/heat shock method.

In your lab notebook, **diagram your plates**, recording:

- which plates received bacteria +pGLO and which received bacteria -pGLO
- whether plates contained ampicillin, arabinose, etc.

Also, be sure to record any notes about how you carried out the transformation in your lab notebook (exactly how long your heat shock was, how long your incubations were on ice, in the 37° incubator, etc.) That way, if you find next week that your transformation results weren't as good as you expected, you might be able to figure out what could be improved if you were to repeat the experiment.

After you have finished setting up the transformation, label your plates, tape them together into one stack, and put them in the incubator upside down to grow.

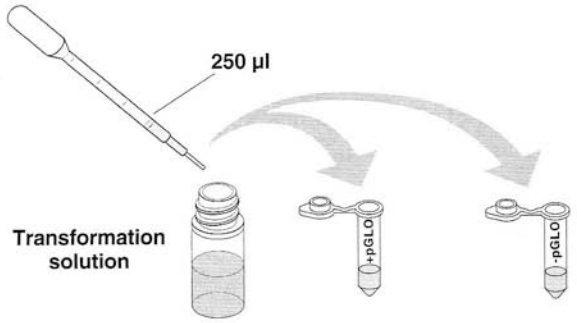
Finally, given what you have learned about bacterial transformation and the regulation of the GFP gene by arabinose, record some predictions in your lab notebook as to what results do you expect for each plate. Next week, we will examine our results and determine our transformation efficiency.

Transformation Kit—Quick Guide

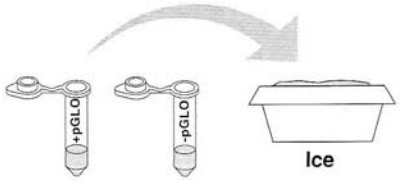
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.

(use P-1000 & sterile blue tip)

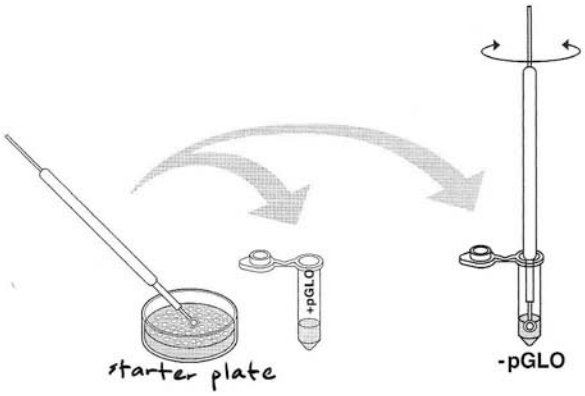
2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaC1_2$).



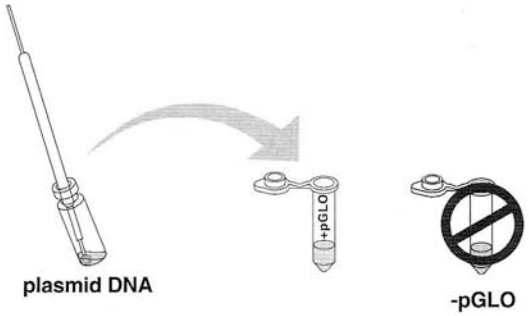
3. Place the tubes on ice.



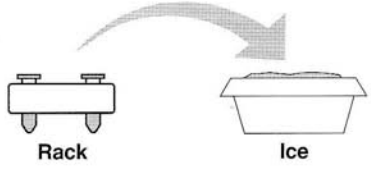
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the -pGLO tube.



5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?

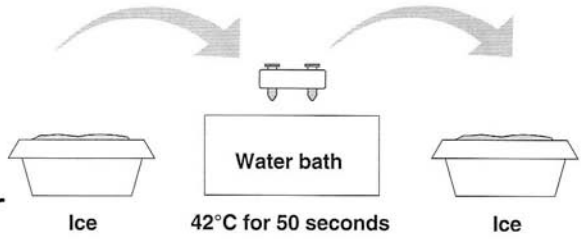


6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



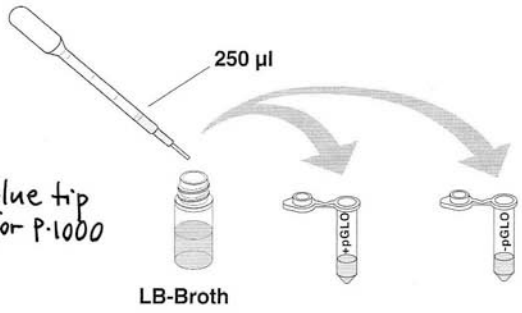
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows: Label one LB/amp plate: +pGLO; Label the LB/amp/ara plate: +pGLO; Label the other LB/amp plate: -pGLO; Label the LB plate: -pGLO.

8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.



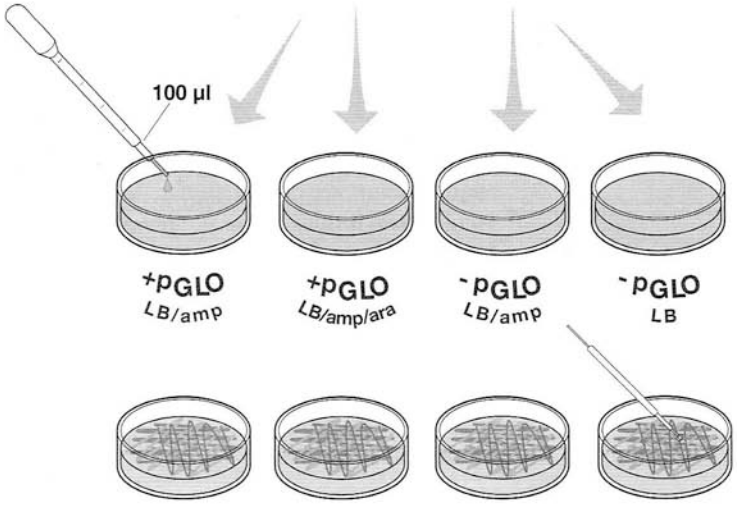
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile ~~pipet~~, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at ~~room temperature~~. 37°C (incubator)

blue tip for P-1000



10. Tap the closed tubes with your finger to mix. Using a new sterile ~~pipet~~ for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate plates.

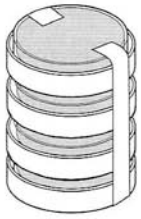
blue tip for P-1000



(or spreader)

11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.

12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack upside down in the 37°C incubator until the next day.



Activity 9b
Learning to Use the Spectrophotometer

Purpose

In this activity you will familiarize yourself with one type of spectrophotometer and determine the wavelength ranges for colors of visible light produced by the spectrophotometer.

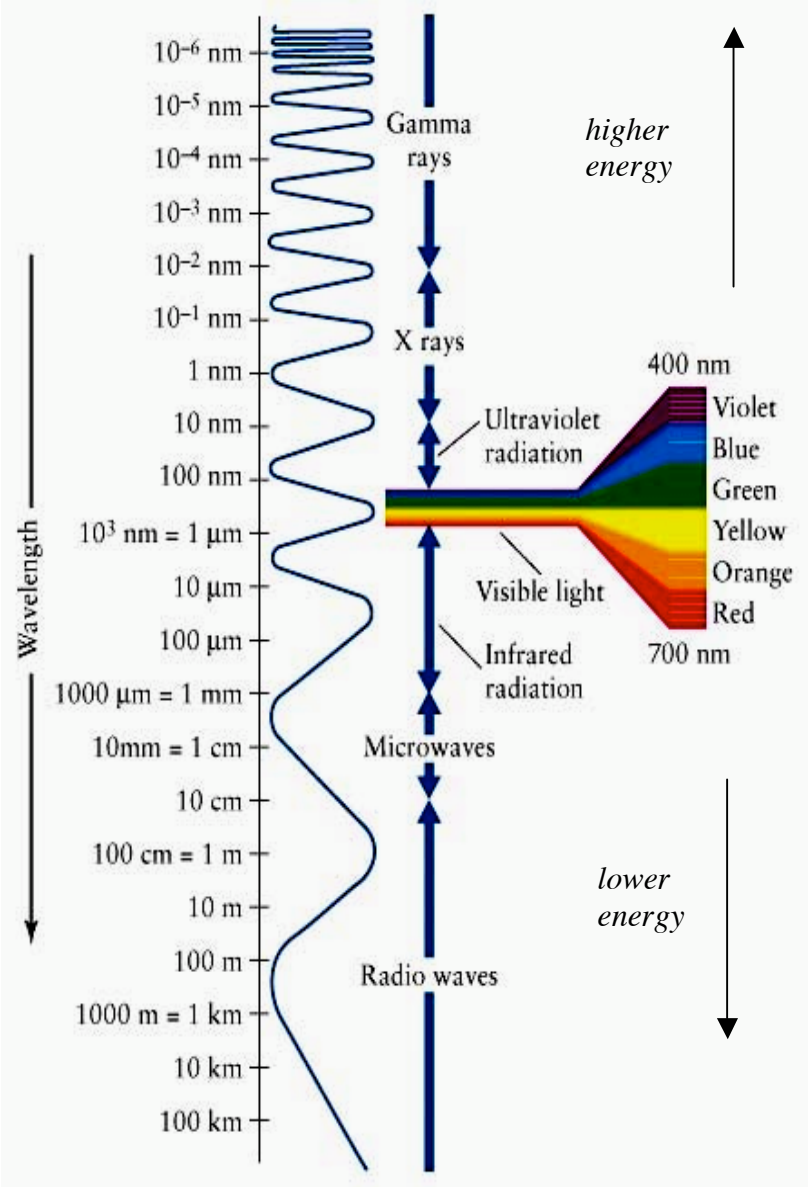
Background

A **spectrophotometer** is a laboratory instrument that uses light to study molecules that are in solution. To understand how to use the spectrophotometer (aka *spec*), we need to learn a little bit about light.

All light energy travels in waves. The distance between each wave crest is called the **wavelength**. The wavelengths of different types of light energy determines their properties. The shorter the wavelength, the more energy that type of light carries.

The range of wavelengths of visible colored light is called the **visible light spectrum**, and extends from 400 – 700 nm (see figure). We can't actually see the light waves, but the cells in our eyes have photoreceptors that can detect light waves between 400 – 700 nm.

White light contains all the wavelengths of visible light. Molecules can either absorb or transmit part or all of this light energy. Substances appear to be a certain color because of the light energy they do *not* absorb. For example, plants appear green because the pigment molecules in their leaves absorb all *except* green visible light waves.



Procedure

1. Familiarize yourself with the model of spectrophotometer in the lab. Refer to the "Spectrophotometer Quick Guide" on page 49 when you are using the spec in lab.
2. Cut a strip of white filter paper to fit into a glass spec cuvette. Then, insert it into the tube. Gently, place the cuvette in the sample holder so the inside of the fold faces the light source (see Figure 9.1).
3. Set the mode to "transmittance." Leave the sample holder open and cup your hands around the opening. Look through your hands into the sample holder.
4. Set the spec for 600 nm and adjust the tube and the Transmittance/Absorbance control so that you see the maximum amount of orange light on the paper.
5. Turn the wavelength knob slowly in both directions and record the range of wavelengths for each different color.
6. Record your data in a data table similar to Table 9.1 below.

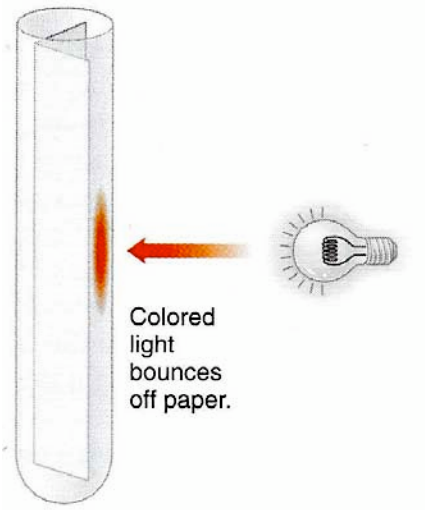


Figure 9.1: Filter paper in cuvette. Light of a specific wavelength reflects off the filter paper. By turning the wavelength knob, you can determine the color of each wavelength.

Table 9.1 Wavelength ranges for colors of visible light

Color	***Wavelength range (nm)***
red	
orange	
yellow	
green	
blue	
purple	

Activity 9c
Using the Spectrophotometer to Study Molecules

Purpose

In this activity you will learn how to measure the absorbance of molecules in solution at different wavelengths using a spectrophotometer. In this activity, three different colored solutions are studied to determine which wavelengths of light they absorb. The data you collect will then be graphed to create an absorbance spectrum for each solution tested. By graphing your data, you will learn about the relationship between the color of light and the ability of different molecules to absorb light energy.

Background

When molecules absorb wavelengths of light in the UV and visible range of the light spectrum, this absorbance can be detected by a spectrophotometer. In order to study molecules with a spec, a technician shines a light on a sample containing molecules in solution and measures the absorbance of light by the molecules. Absorbance is measured in absorbance units (au). The maximum amount of absorbed light that can be detected by a typical VIS (visible light) spec has a value of 2 au; therefore, all the absorbance values fall between 0 and 2 au.

Procedure

1. Measure the absorbance of each dye solutions at different wavelengths in the visible light spectrum (from 400 nm – 620 nm). Record your data in your lab notebook in data tables similar to Tables 9.2, 9.3, and 9.4 (see next page).
   - NOTE: We may be using the older VIS (visible light) spectrophotometers for this activity that were used earlier in Activity 9b). There is also a possibility that we will be able to use digital UV/VIS spectrophotometers, which will provide data much more quickly. Depending on which equipment is available, your instructor will give you more specific instructions about which cuvettes to use and how to operate the spectrophotometer in class.
   - Regardless of which spectrophotometer we use, you will always use water as your "blank" in this experiment; the blank is used to calibrate the spectrophotometer before taking measurements.
2. When you have finished recording all of the data, graph the absorbance spectra for the three dyes. The X axis will have wavelength values from 400 – 620 nm, and the Y axis will have absorbance units (au). You can create one graph with three different lines (one for each color dye). Each line on the graph represents the absorbance spectrum for that color dye.
3. What do you notice about the minimum absorbance values (lambda$_{min}$, or λ_{min}) for each dye in terms of where they fall in the visible light spectrum (you can refer back to Activity 9b for the wavelength ranges of each color in the visible light spectrum)?

Table 9.2
Absorbance of Red dye at Different Wavelengths

Red dye

Wavelength	Abs
400	
410	
420	
430	
440	
450	
460	
470	
480	
490	
500	
510	
520	
530	
540	
550	
560	
570	
580	
590	
600	
610	
620	

Table 9.3
Absorbance of Green dye at Different Wavelengths

Green dye

Wavelength	Abs
400	
410	
420	
430	
440	
450	
460	
470	
480	
490	
500	
510	
520	
530	
540	
550	
560	
570	
580	
590	
600	
610	
620	

Table 9.4
Absorbance of Blue dye at Different Wavelengths

Blue dye

Wavelength	Abs
400	
410	
420	
430	
440	
450	
460	
470	
480	
490	
500	
510	
520	
530	
540	
550	
560	
570	
580	
590	
600	
610	
620	

VIS Spectrophotometer quick guide

1) with sample holder empty:
   - set mode to "transmittance"
   - set wavelength;
   - check filter;
   - set left knob (#10 on diagram below) to 0% T

2) Insert blank (wipe fingerprints!) into sample holder:
   - set right knob to 100% T

3) Insert sample:
   - change mode to abs and read the value

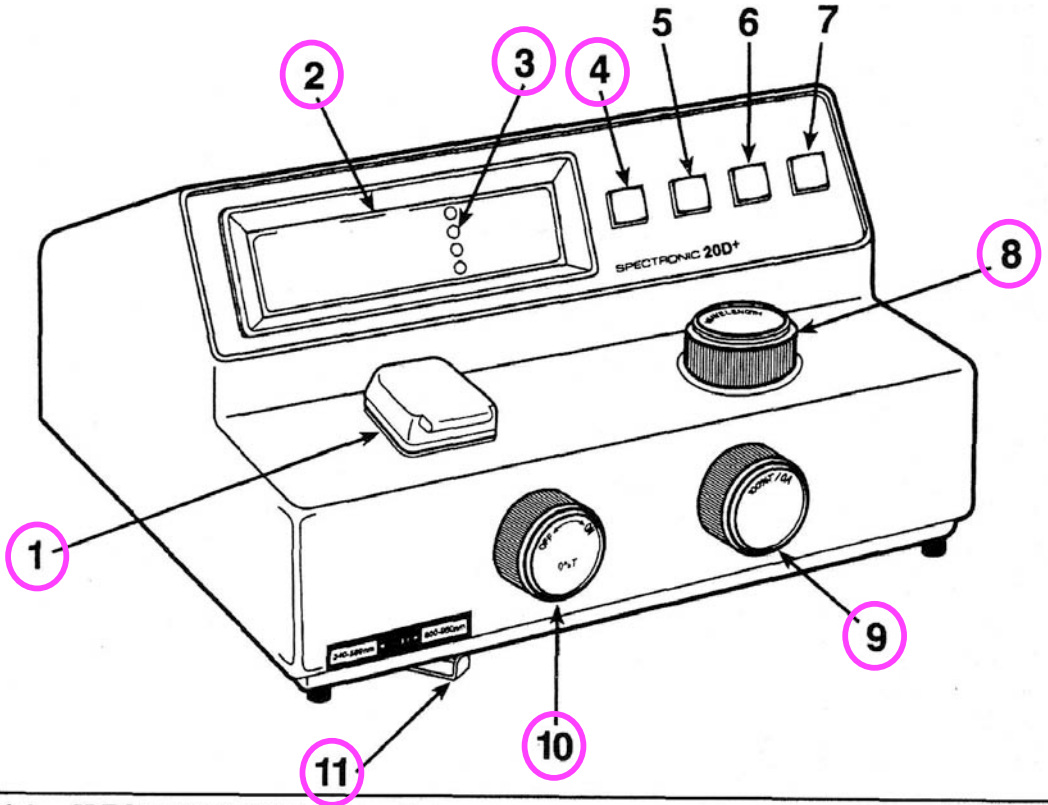


Figure 1-2 SPECTRONIC 20D+ spectrophotometer

KEY

1. Sample compartment
2. Digital readout
3. Mode indicators
4. Mode selection
5. Decrease
6. Increase
7. Print
8. Wavelength control
9. Transmittance/Absorbance control (100%T/0A)
10. Power switch/Zero Control
11. Filter lever

Lab 9 Homework

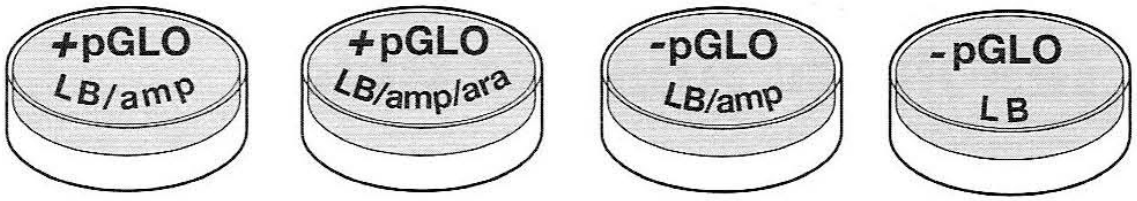
Name: _____

1. On which plate(s) would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed on your starter plate?
   Explain your predictions.

2. If there are any genetically transformed bacterial cells, on which plate(s) would they be located?
   Explain your prediction.

3. For each of the four plates you set up (labeled below), predict:
   a) whether there will be bacterial growth or not
   b) if there are bacteria growing, will they glow green or not?

Explain your predictions.



4. Given your data from Activity 9b, what are the approximate wavelengths of the following colors?

   a) blue-green:

   b) greenish-yellow:

   c) red-orange:

5. Looking at the absorbance spectrum for the red sample, does the red dye absorb red light or non-red light the most? What about the blue and green spectra?

6. Given your data and what you have learned about light absorption, what color light would you expect purple grape juice to absorb?