



# General Biology 1 Lab Manual

Open Educational Resource



## NEW YORK CITY COLLEGE OF TECHNOLOGY

Department of Biological Sciences

### BIO1101L

<https://openlab.citytech.cuny.edu/bio-oer>



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v18.02



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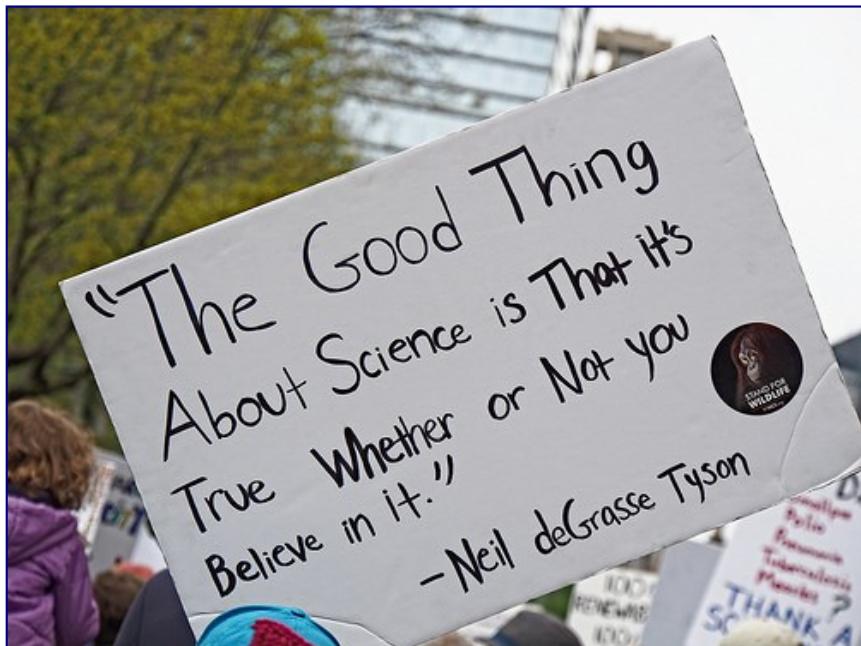
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## The Scientific Method

Scientists use a methodology for systematically investigating natural phenomena. This method uses existing information or observations to acquire new information or validate previous knowledge. These knowledge types come from empirical (experiential) or measured information. Empirical and measured data (or knowledge) are referred to as **observations**.

While **empirical** data comes from experiences, science has developed into a mode of inquiry using **experimentation**. Experimental science uses the pre-existing base of knowledge to ask a testable question called a **hypothesis**.

As a youngster, we're incorrectly taught that a hypothesis is *an educated guess*. Formulating previous observations and measurements into a cohesive line of inquiry requires no guessing. People often have "theories" on something, when they actually have hypotheses based on their observations and assumptions.



## Experimental Science

Hypothesis testing is the means by which experimental science is conducted. Experimental science is designed to enhance the understanding of a problem and removing biases from the interpretation. The goal of hypothesis testing is to try every way possible to disqualify the validity of the hypothesis. By doing so, the experimenter removes any biases in the experimental design. If the experimenter is unable to invalidate the hypothesis, the hypothesis becomes more valid and better able to act as a predictor of phenomena.

Experiments utilize **controls**. In a controlled experiment, there is a positive and negative control. These controls act as references in the experiment. A **positive control** is an experimental condition where the expected outcome that is tested will be produced. This control is necessary to assess the validity of a test or treatment. There can be multiple instances used as a positive control to examine the sensitivity of the experiment. A **negative control** is an experimental condition where the expected outcome is known not to occur. This type of control sometimes comes in the form of a sham or mock treatment such as giving someone a sugar pill (a **placebo**).

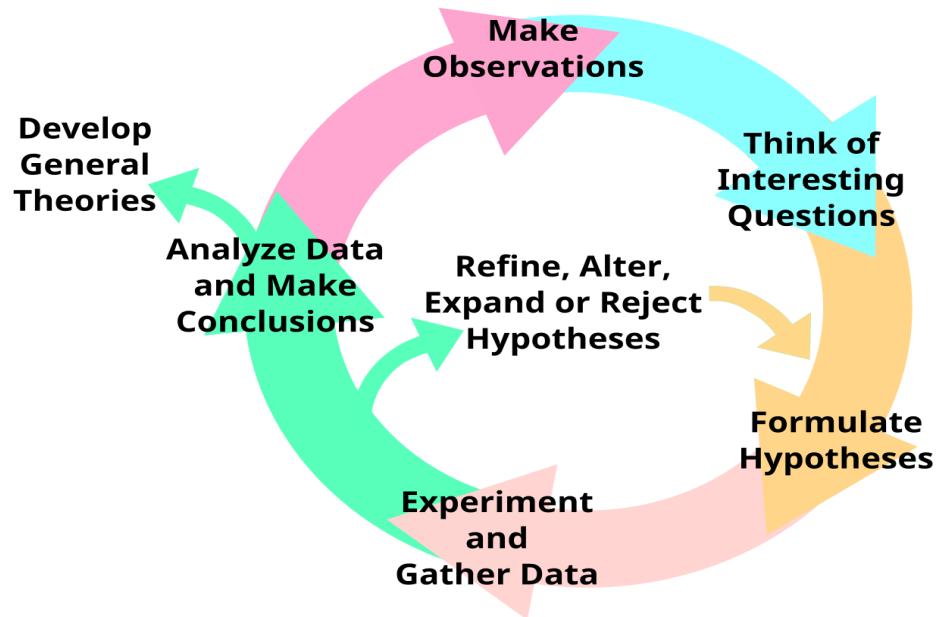
Through the use of experimental science and hypothesis testing, an increased refinement of existing knowledge can aid in designing new hypotheses. Hypothesis testing is re-iterative. That is to say, we use new knowledge to continue to enhance our understanding of the universe.

### Theories

A **scientific theory** comes from repeated substantiation of multiple tested hypotheses. That is to say, confirmed hypotheses, observations and experiments permit scientists to formulate a cohesive idea that integrates multiple substantiated pieces of evidence. As with hypotheses, theories are designed to be predictive and falsifiable. In the common language, we often hear the word theory to mean a conjecture, and as already discussed, conjectures based on evidence can be formulated into testable hypotheses.

When a theory is accepted by a predominant population of the specialists, it is referred to as a

## The Scientific Method Revision and Extension of Knowledge



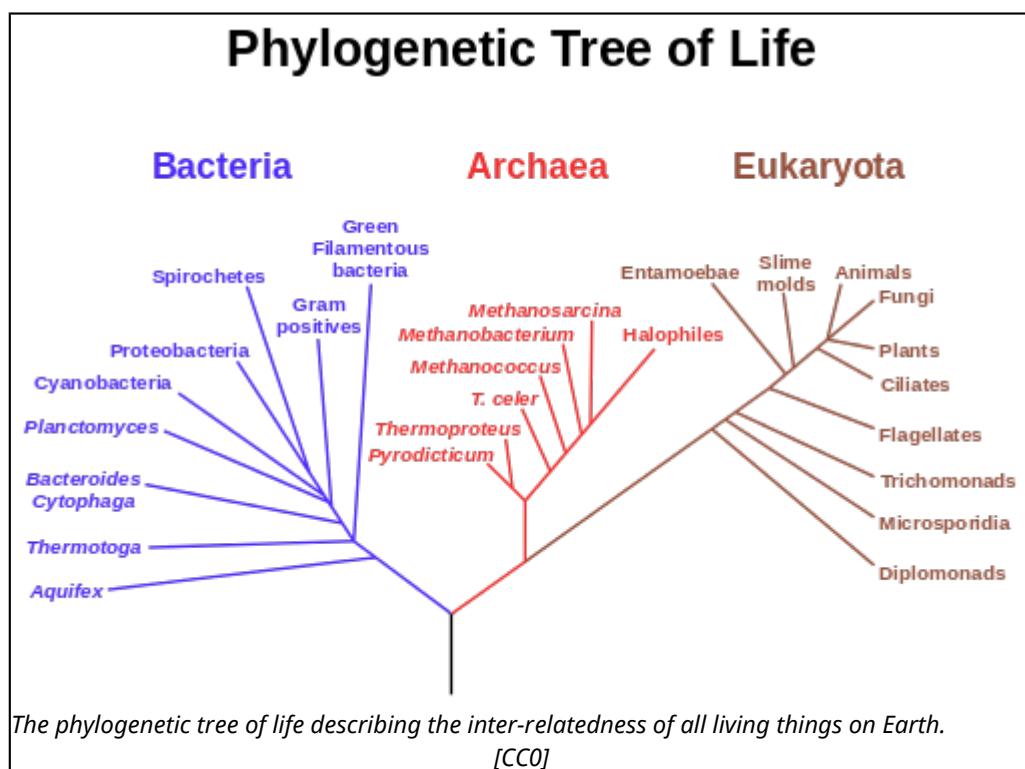
*Hypothesis testing is a continuous and repeated process designed to increase knowledge.*

*Credit: Jeremy Seto [CC-BY-NC-SA]*

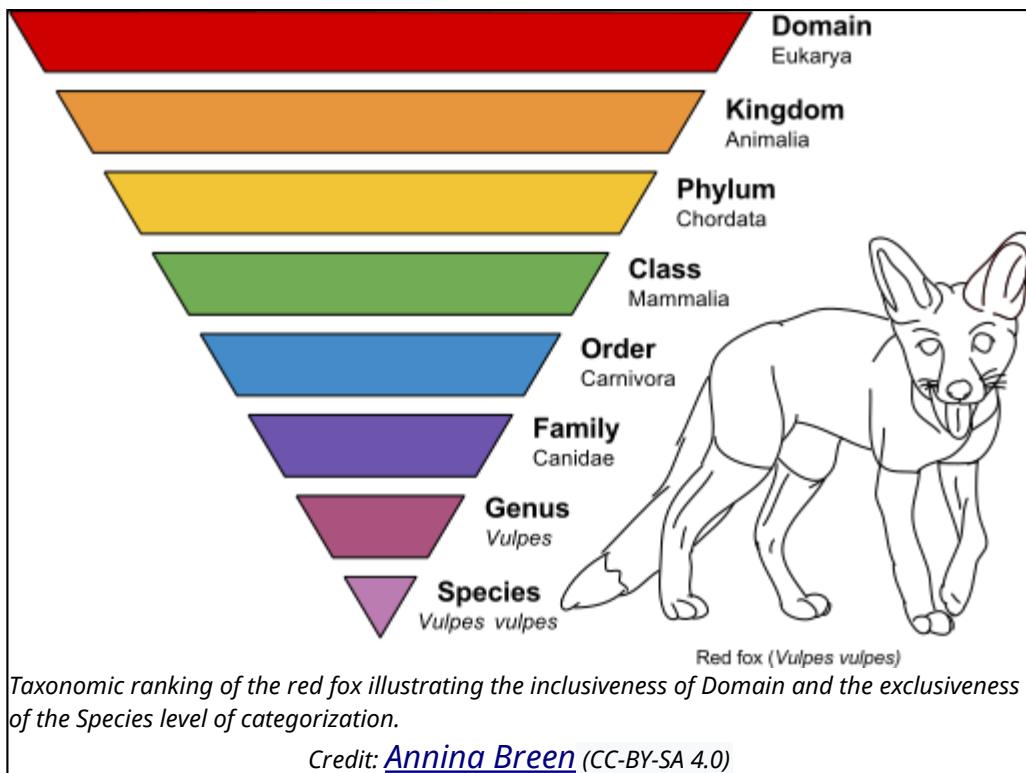
Numerous tested hypotheses have been confirmed that lead to the understanding of natural selection as a method of evolution. This theory allows scientists to understand the underlying relatedness of all living things on the planet. Additionally, it unifies the disparate fields of Biology that can utilize the theory in a predictive manner. It is therefore also referred to as a **unifying principle** of Biology.

## Classification of Life

All living things on Earth share a relationship. The rules that govern life processes can be generalized across all **organisms** (living things), as well as non-living biological entities (viruses). The relatedness of organisms is often visualized as a **phylogenetic tree**. This tree is a hierarchical classification system that groups organisms together based on common features that is used to name them and is referred to as **taxonomy**. The most broad category is called a **domain**.

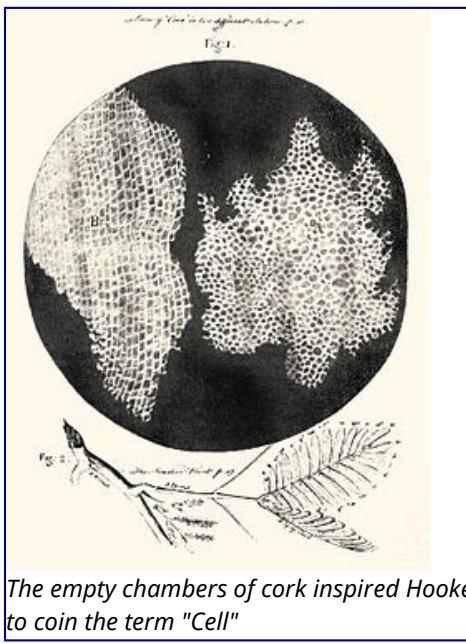
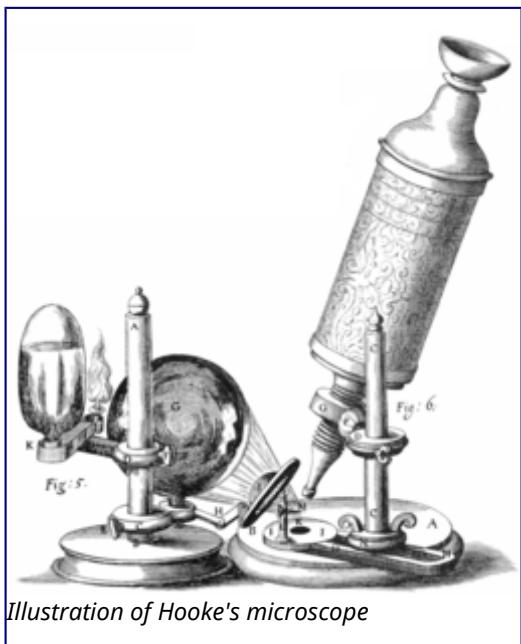


Three domains exist: **Archaea**, **Bacteria** and **Eukarya**. Archaea and Bacteria are also grouped together as **Prokaryotes** (*pro-* before; *karya-* nucleus). Eukarya (*eu-* true; *karya-* nucleus), or eukaryotes, is a group of organisms that have nuclei. The second most inclusive or broad category is **Kingdom**. Humans are in the Kingdom of animals. The third most inclusive or broad category is **Phylum**. Humans are in the phylum called chordata. Each level of organization can be further subdivided and you may be more familiar with the subphylum called vertebrata. Within this division, humans fall in the **class** of mammals. Amongst the mammals, humans are in the **order** of primates. Humans are categorized into a narrower group of organisms in the **family** of great apes or hominids. Within this family, humans fall into the **genus** of *Homo*. Biologists use a method of identifying specific organisms called binomial nomenclature. Binomial nomenclature uses the most specific groupings of taxonomy (genus and **species**) as a two part name. While humans are of the species *sapiens*, the species name of humans using binomial nomenclature is *Homo sapiens*.



# The Light Microscope

## Hooke's Cell



In 1665, Robert Hooke published *Micrographia*, a book that illustrated highly magnified items that included insects and plants. This book spurred on interest in the sciences to examine the microscopic world using lenses but is also notable for Hooke's observations of cork where he used the word "cell" in a biological sense for the first time.

## The father of Microbiology: van Leeuwenhoek



The Dutch tradesman Antonie van Leeuwenhoek used high power magnifying lenses to examine the parts of insects and to examine the quality of fabric in his drapery business. He began to experiment with pulling glass to generate lenses and developed a simple microscope to observe samples. Using a simple single lens with a specimen mounted on a point, he was able to identify the first microscopic "animalcules" (*little animals*) that will be later known as protozoa (*original animals*).

Though van Leeuwenhoek's apparatus was simple, the magnifying power of his lenses and his curiosity enabled him to perform great scientific observations on the the microscopic world. He was ridiculed for fabricating his observations of protists at first. Ever the scientist, van Leeuwenhoek examined samples of his own diarrhea to discover *Giardia intestinalis*. While he did not make the connection of the causative nature of this microorganism, he described the details of the way this organism could propel itself through the medium in great detail.



Modern micrograph of *Giardia intestinalis* (Kingdom Protista)

Credit: Doc. RNDr. Josef Reischig, CSc. (CC BY-SA 3.0)

## Using the Light Microscope

[https://www.youtube.com/watch?v=lo2aC\\_m2vyo](https://www.youtube.com/watch?v=lo2aC_m2vyo)

Unlike van Leeuwenhoek's single lens microscope, we now combine the magnifying power of multiple lenses in what is called a compound microscope.

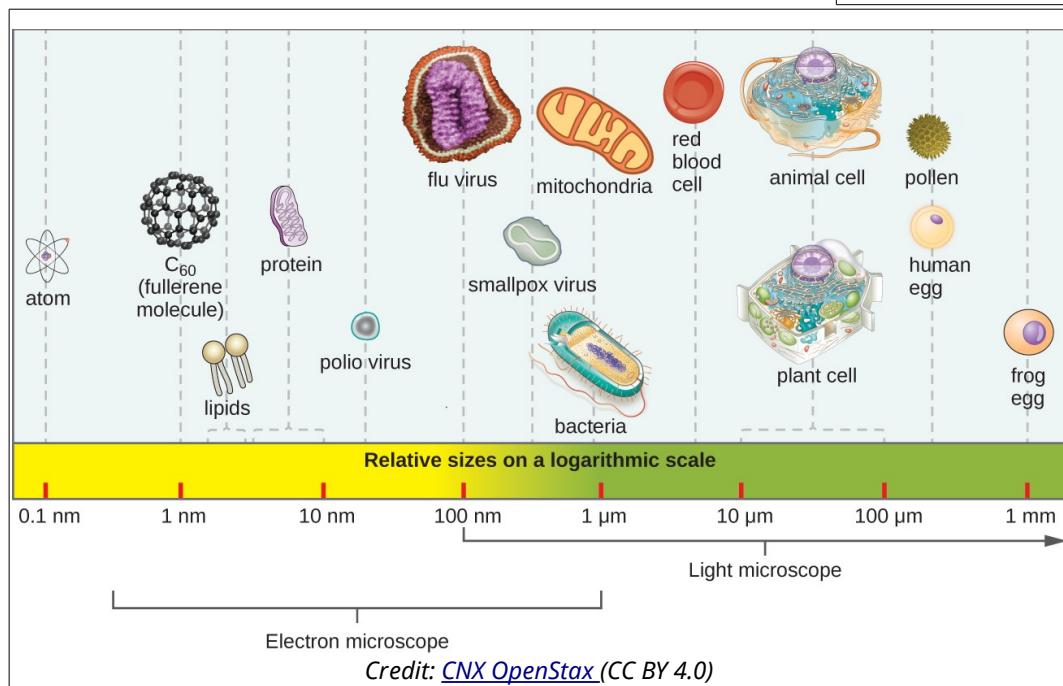


1. Ocular lens or eyepiece
2. Nose Piece/ Lens Carousel
3. Objective lens
4. Course Focus Knob
5. Fine Focus Knob
6. Stage
7. lamp
8. condenser
9. stage control

## Microscopic World



## Scale



- Discover more about scale and microscopy at this link  
<http://learn.genetics.utah.edu/content/cells/scale/>

## Magnification

**Magnification** is the process of enlarging the appearance of an object. We calculate the magnification of an object by indicating the fold change in size. So if something appears to be double the size of the real item, then it is obviously magnified 2X. Because there is a magnification by the eye-piece (**ocular lens**), as well as the **objective lenses**, our final magnification of an item is the product of those two lenses.

The lowest magnification objective lens (usually 4X or 5X) is referred to as a **scanning lens**. There is also usually a low power lens at 10X and a higher magnification lens at 40X. There may be a higher magnification lens at 100X but these usually require oil to function properly and are often reserved for microbiology labs.

- What is the power of the ocular lens?
- We can calculate that as:

$$\text{Magnification}_{\text{total}} = \text{Magnification}_{\text{objective}} \times \text{Magnification}_{\text{ocular}}$$

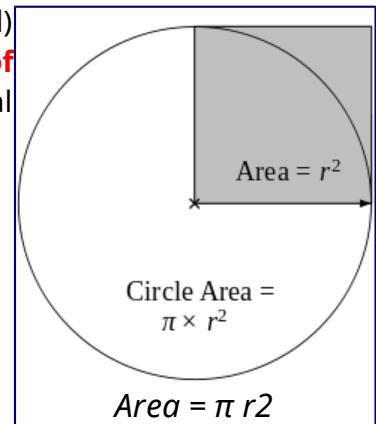
- With this in mind, fill in the following table:

	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion	100X		

## Field of View (FOV)

In a microscope, we ordinarily observe things within a circular space (or field) as defined by the lenses. We refer to this observable area as the **field of view (FOV)**. Understanding the size of the FOV is important because actual sizes of object can be calculated using the Magnification of the lenses.

FOV can be described as the area of a circle:



What are the effects of magnification on FOV?



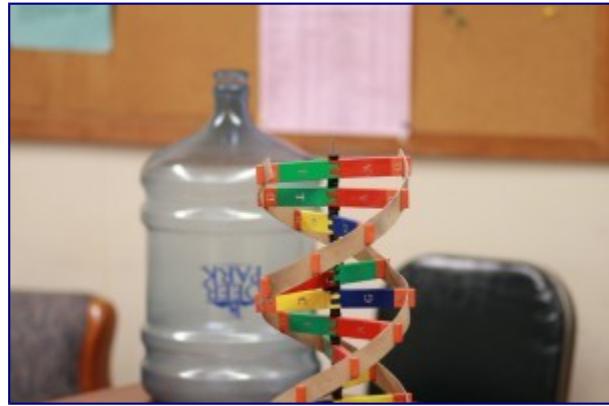
1) Lowest Magnification



2) Low Magnification



3) High Magnification



4) Highest Magnification

In image 1, we can see a model of DNA on a table with a water bottle and a large area of the room. Image 2 displays less of the room in the background but the DNA model is larger in appearance because the magnification is greater. In image 3, we no longer see evidence of a door and the DNA model is much larger than before. In image 4, we no longer see the table the model and water bottle rest upon. While the last image is largest, we see less of the surrounding objects. We have higher magnification at the cost of field of view. FOV is inversely related to the magnification level.

## Field of View Calculation

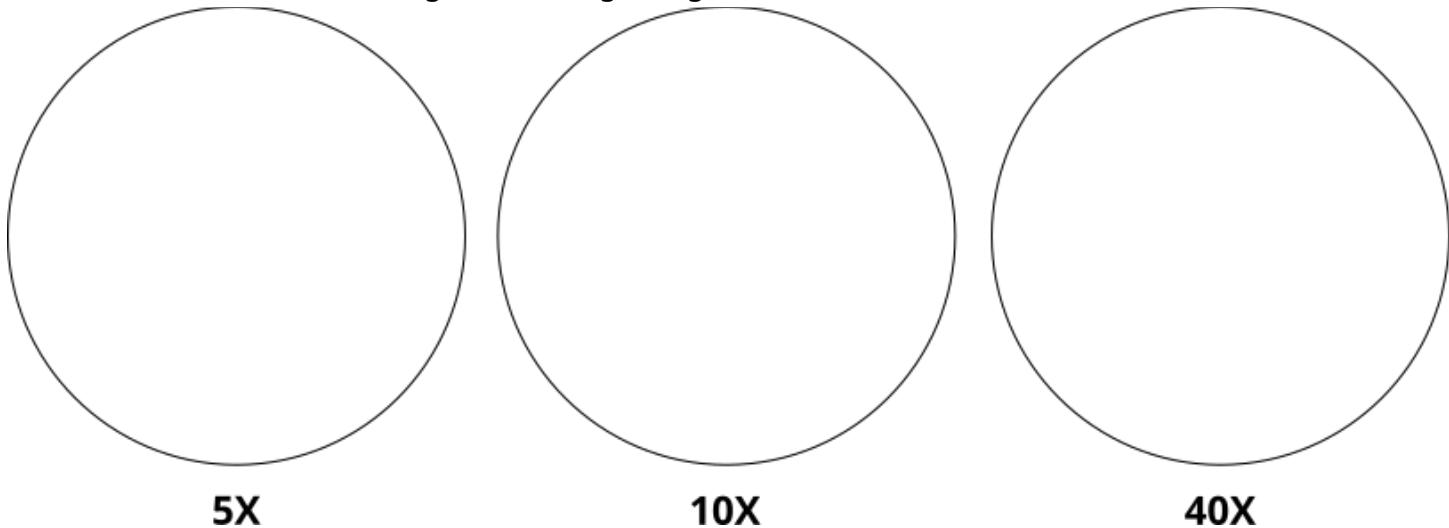
1. Examine a ruler under scanning magnification
  - Measure the diameter in mm
  - diameter= \_\_\_\_\_
  - radius= \_\_\_\_\_
  - Calculate the field of view at this magnification= \_\_\_\_\_

2. Examine a ruler under low magnification (10x)
  - Measure the diameter in mm
  - diameter= \_\_\_\_\_
  - radius= \_\_\_\_\_
  - Calculate the field of view at this magnification= \_\_\_\_\_

3. What is the relationship in the between the magnification and field of view?
4. What is the proportion of change in field of view when doubling the magnification?

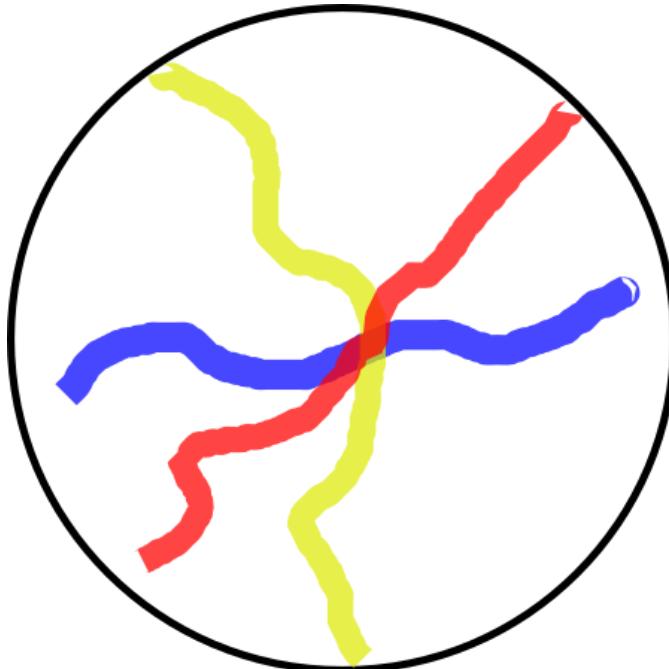
## The Letter "e"

1. Orient a slide with the Letter "e" so that it is read as an "e" without magnification
2. Draw the "e" at scanning, low and high magnification



## Depth of field

1. Examine the slide of colored threads under scanning power so the cross-point of the threads is at the center of the field



- 

2. Raise the magnification to the low power objective

- What do we notice about the threads and the focus?
- How can we explain this observation with respect to the threads?
- Close the diaphragm so allow a pinpoint of light through the slide. What effect does this have on the image?

We notice that when we observe 3 overlapping threads of different color under a microscope, we can focus on one thread at a time. Similarly, when we zoom in a great deal on the DNA model below, we notice that the print on the water bottle is not sharp.

We know that the water bottle is **behind** the DNA molecule. Under the microscope, the threads of differing color are also stacked on top of each other. We recognize that they are in different planes because they are three dimensional. Each thread has depth and do not occupy the same exact space. If we focus on the print of the water bottle on the image above, we would no longer see the lettering on the DNA molecule sharply. We refer to this concept as **Depth of Field (DOF)**. Under the microscope, at a low magnification, we can make out fewer finer details. However, most items appear on the same plane in this case and or comparably sharp. But as we increase the magnification and see finer details, the distances between the various planes in view become more apparent. We can see a similar phenomenon at low magnification of the DNA model. At

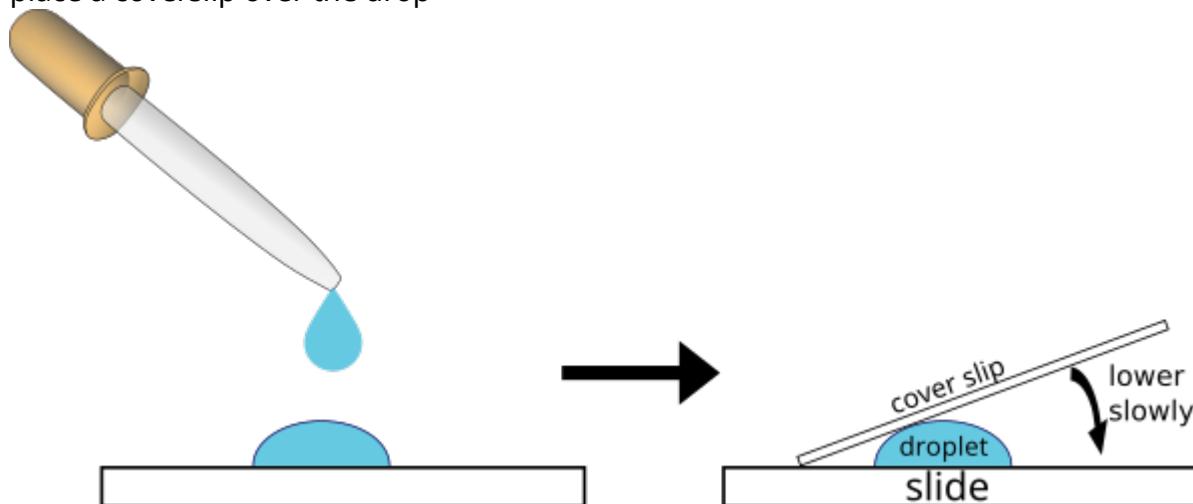


*Highest Magnification with shallow depth of field.  
Notice how the label on the water bottle is blurry while the lettering on the DNA model is sharp.*

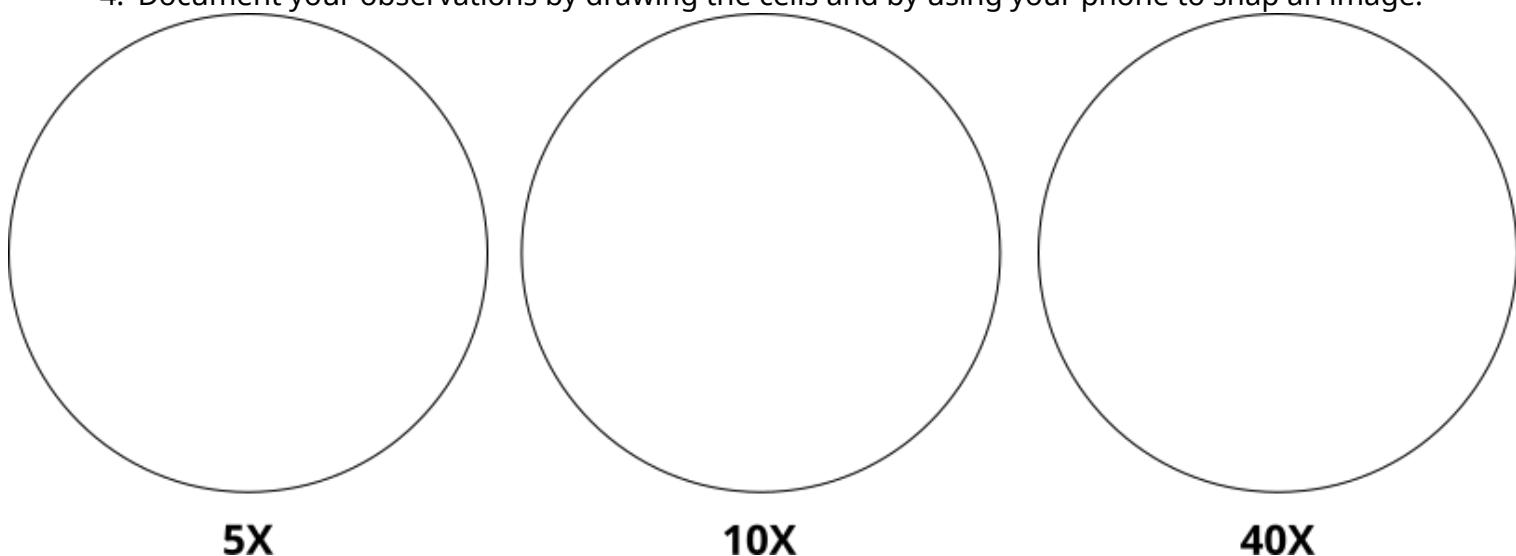
the low magnification, we may not be able to read the print on the water bottle, but the bottle and DNA molecule are of a similar distance from our view that the small difference in apparent depth is not as noticeable. We can still draw on other visual cues to know that the bottle is behind the model, but the sharpness of both items are equivalent.

## Examining Cells

1. Choose a prepared slide of a Protist (*Euglena, Amoeba, Paramecium*)
2. Prepare a wet mount of a drop of pond water and place a cover slip over the drop.
3. Swab the inside of your cheek
  1. Roll the swab across a slide
  2. Drop some methylene blue onto the slide
  3. place a coverslip over the drop



4. visualize and draw your cheek cells
4. Document your observations by drawing the cells and by using your phone to snap an image.



## Real biological examples

We can see the concepts of FOV and DOF in the following pictures.



In an even more extreme close-up (higher magnification), we would have difficulty focusing on both the eyes and beak since there is depth and distance between those features.

## How do we use microscopes

In our lab, we look at some pond water. What do we see? Why is this significant? How does the microscope help us study these items? What is the utility of the the concepts of magnification, FOV and DOF when we use microscopes to study biological samples?

# The Metric System

The **metric system** is an internationally agreed upon measurement system based on decimals or powers of 10. Scientists use a refined version called the **International System of Units** (abbreviated **SI**). In biology, you will often find a need to describe measurements of length, volume, mass, time, temperature or amount of substance.

## International System of Units

BASIC SI UNITS		
MEASURE	SI UNIT	SYMBOL
length	meter	m
mass	kilogram	kg
time	second	s
temperature	Kelvin ( <i>Celsius is used in Biology</i> )	K (°C)
quantity	mole	mol
current	Ampere	A
luminosity	candela	cd

## Metric Units:

- length: meter (**m**)
- volume: liter (**L**)
- mass: gram (**g**)
- time: second (**s**)
- temperature: Celsius (**°C**)
  - Kelvin (**K**) is a unit of thermodynamic temperature and is the SI unit. The Kelvin scale is the same as the Celsius or centigrade scale but offset by 273.16
  - Biology uses Celsius predominantly because of the range in which organisms live.
- amount of substance: mole (**mol**)
  - A mole is a number representing  $6.022 \times 10^{23}$  of something
  - Just as a pair of shoes equals 2 shoes, a mole of shoes is  $6.022 \times 10^{23}$  shoes
  - Just as a dozen eggs equals 12 eggs, a mole of eggs is  $6.022 \times 10^{23}$  eggs

METRIC PREFIXES IN EVERYDAY USE				
PREFIX	SYMBOL	SCIENTIFIC NOTATION	FACTOR	
tera	T	$10^{12}$	1 000 000 000 000	
giga	G	$10^9$	1 000 000 000	
mega	M	$10^6$	1 000 000	
kilo	k	$10^3$	1 000	
hecto	h	$10^2$	100	
deca	da	$10^1$	10	
<b>BASE UNIT</b>	(none)	$10^0$	1	
deci	d	$10^{-1}$	0.1	
centi	c	$10^{-2}$	0.01	
milli	m	$10^{-3}$	0.001	
micro	μ	$10^{-6}$	0.000 001	
nano	n	$10^{-9}$	0.000 000 001	
pico	p	$10^{-12}$	0.000 000 000 001	

## Strategy for conversions

1. What unit is being asked for?
  - $500\text{ml} = \underline{\hspace{2cm}}\text{L} \rightarrow \text{liters}$
2. What unit are you starting from?
  - $500\text{ml} = \underline{\hspace{2cm}}\text{L} \rightarrow \text{milliliters}$
3. Which unit is larger? By how much is that unit larger?
  - Liters are the larger unit. Liters are  $1,000X (10^3)$  greater than milliliters.
4. Which direction are we moving?
  - Since we are moving to a larger unit, our value will be smaller. In this case, the value is smaller by  $1,000X$
  - In other words, the value is  $1/1000$  or  $0.001$  the value.
  - So what is the answer?

## Factoring Out

Using the idea of factors of ten, you can assess the difference of the two units and cancel out the original unit algebraically to reach the desired final unit.

- $500\text{ml} = \underline{\hspace{2cm}}\text{L}$
- $1\text{ml} = \frac{1}{1000}\text{L}$  OR,  $\frac{1\text{L}}{1000\text{ml}}$ 
  - which states 1000 milliliter in every 1 liter
- $500\text{ml} \times \frac{1\text{L}}{1000\text{ml}} = \frac{500\text{L}}{1000} = 0.5\text{L}$ 
  - pay attention to the units and how we've canceled out the ml in the numerator of 500ml and in the denominator in the conversion of 1L in 1000ml

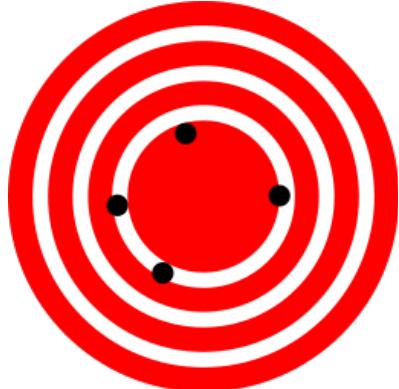
## Additional Resources

[https://youtu.be/w0nqd\\_HXHPQ](https://youtu.be/w0nqd_HXHPQ)

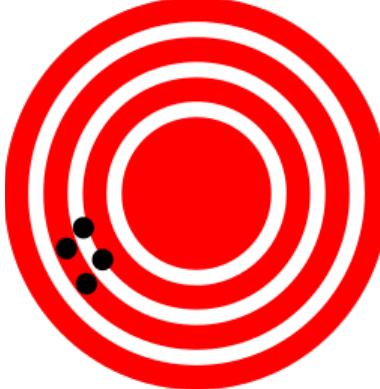
## ACCURACY and PRECISION

**Accuracy** refers to how closely a measured value agrees with the correct or target value.

**Precision** refers to how closely individual measurements agree with each other and reflects a repeatability in those measurements.



*This illustrates accuracy.  
Measurements are on target.*



*This illustrates precision.  
Measurements are very close  
to each other and repeatable.*

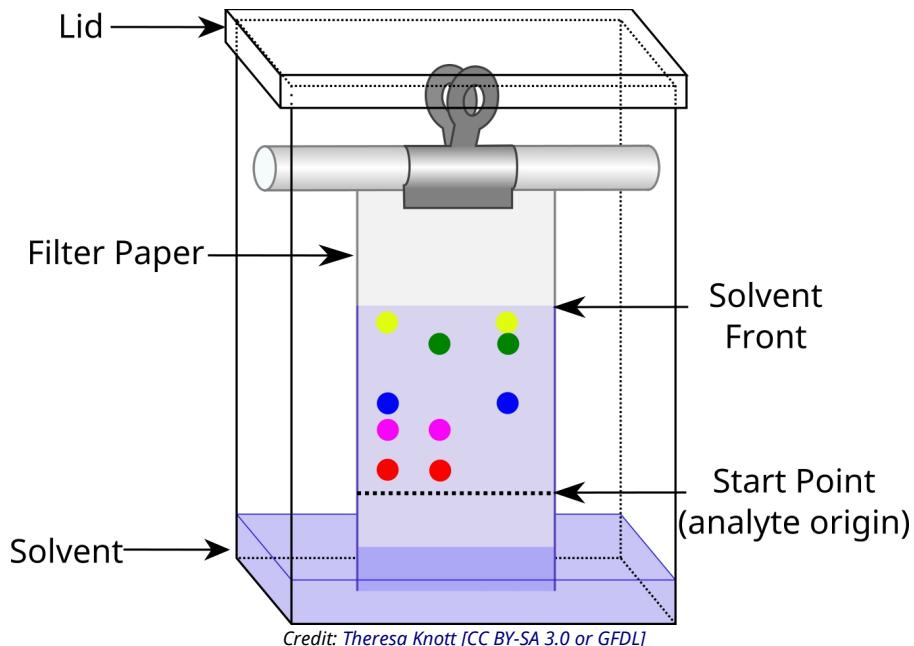


*This illustrates Accuracy AND  
Precision. Each measurement  
is on target and also highly  
repeatable.*

Instruments have a finite amount of accuracy and it is important to report measurements within that level of accuracy. **Significant figures**, report the number of digits that are known to some degree of confidence with the measuring device. With increased sensitivity of the equipment , the number of significant figures increases.

# Chromatography

Chromatography is a collective term for a set of analytical techniques used to separate mixtures. *Chroma* means color and *graph* means to write or draw. Paper chromatography is an analytical technique used to separate mixtures of chemicals (sometimes colored pigments) using a partitioning method. The paper in this method is called the **stationary phase** because it does not move and serves as a substrate or surface for the separation. **Analytes** (substances being analyzed) are separated from each other based on a **differential affinity** to a solvent. The solvent dissolves and carries the analytes along the matrix of the stationary phase. Since the solvent moves through a wicking action, it is called the **mobile phase**.



The distance that the analyte migrates along the paper related to the total distance that the solvent or mobile phase moves is called the **Retention Factor** or  $R_f$ .

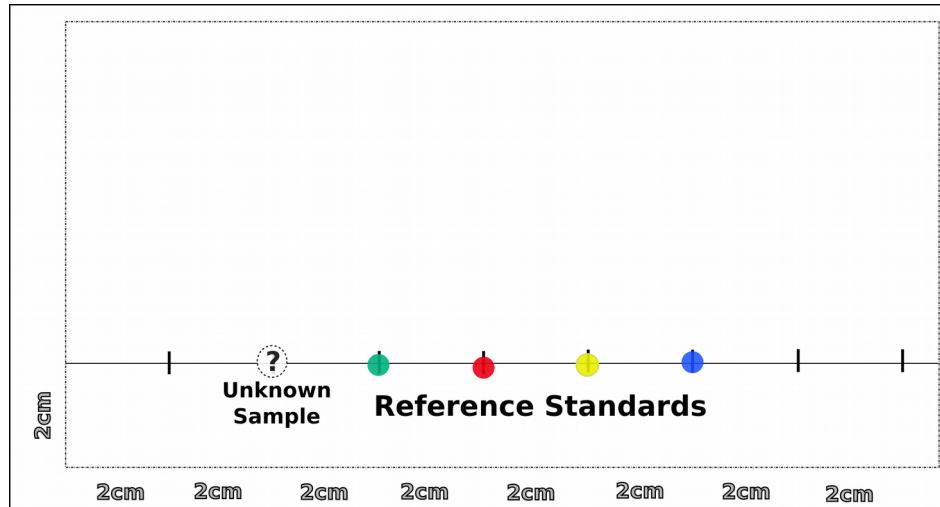
$$R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$$

## Are the food colorings used in colored candy the same as the the FD&C approved chemicals?

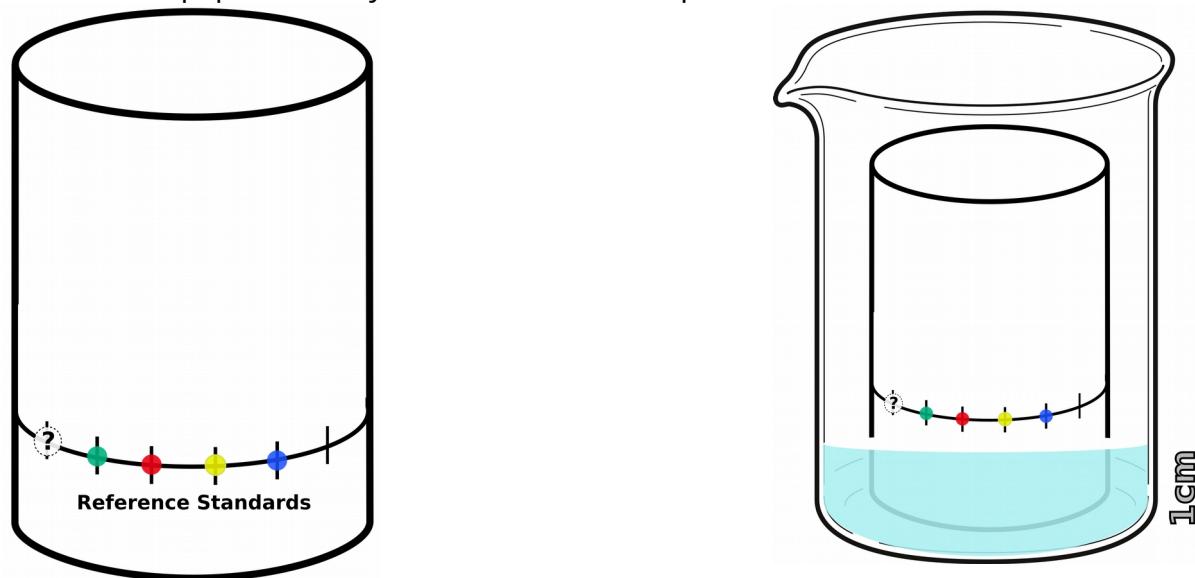
How many colored spots do you expect to see for each reference standard?

1. Obtain a 25 cm square piece of chromatography paper that will fit into the beaker that will serve as the chromatography chamber.
2. Draw a pencil line across the lower end of the chromatography paper about 2 cm from the bottom.
3. Draw additional vertical tick marks along this line every 2 cm
4. Place colored candy in a flask with 2 ml ethanol until the color dissolves into the solution
5. Using an applicator, create a very small spot on a tick mark and allow to dry

6. repeat application on the spot to make a very small and dark spot
7. Continue to spot reference standards along other tick marks. These reference standards are food coloring.



8. Place approximately 1 cm of mobile phase solution (a very polar salt water solution) into the beaker
9. Roll the filter paper into a cylinder and fix with staples



10. place cylinder into the beaker and cover for 20 minutes **or** until the mobile phase reaches 2 cm from the top of the paper.
11. Mark the final distance of the mobile phase and dry the filter
12. Measure the distance of each spot from the starting point
  1. Each spot is a separate analyte.
  2. Some spots separate into multiple analytes.
  3. Measure **EACH** one
13. Measure the distance from the starting point to the final point that the solvent reached.
14. Calculate  $R_f$  values and tabulate results.

**Table of Reference Standards**

Reference Standard	Distance (cm)	R <sub>F</sub>
<b>Green</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Red</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Yellow</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Blue</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =

**Table of Unknowns**

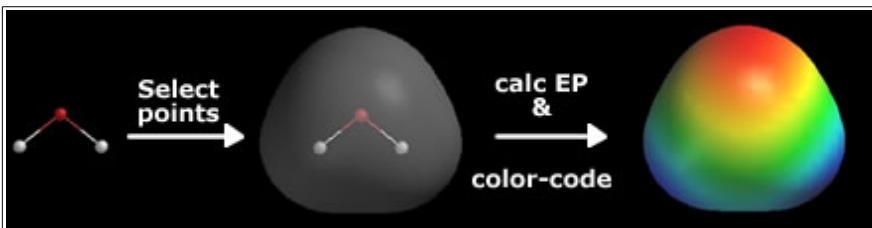
Unknown	Distance (cm)	R <sub>F</sub>
<b>Brown Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Blue Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Red Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Green Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Orange Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =
<b>Yellow Candy</b>	D <sub>1</sub> =	R <sub>F1</sub> =
	D <sub>2</sub> =	R <sub>F2</sub> =
	D <sub>3</sub> =	R <sub>F3</sub> =

**Reflect**

1. Compare and average the R<sub>F</sub> values of each analyte across the entire class
2. Did you predict the number of spots that would appear from each analyte (reference or candy)?
3. Assuming all the dye molecules are of the same mass, what influenced the migration patterns of each spot?
4. Were the colors used in the candy the same as the references?
5. What does it mean if the candy color didn't match anything from the food colors from the cake decorating set used as references?

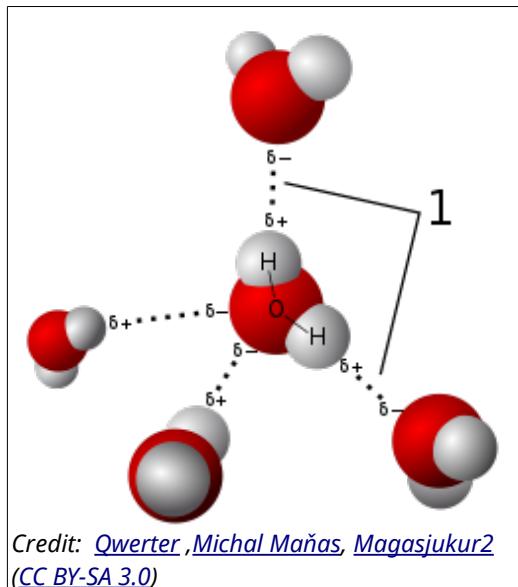
# Polar Covalent Bonds

$\text{H}_2\text{O}$  is a polar covalent molecule. The bonds between the H atoms and the O atom arise from sharing electrons. These shared electrons form to satisfy the **octet rule**. However, Oxygen is a "selfish" sharer. This **electronegative** aspect of Oxygen means that the electrons of the  $\text{H}_2\text{O}$  molecule preferentially associate near the Oxygen atom, creating partial charges. We indicate this by placing a  $\delta^-$  near the O and  $\delta^+$ 's near the H atoms. These partial charges make the  $\text{H}_2\text{O}$  **polar**.



The electron cloud around a water molecule lingers around the oxygen molecule to render it partially negative. Red illustrates the partial negative end of the molecule while blue indicates the partial positive.  
Credit: [FrozenMan](#) (CC0)

Because of this polarity, H<sub>2</sub>O molecules arrange in a highly structured way. Use the following [simulation to explore polarity of molecules](#).

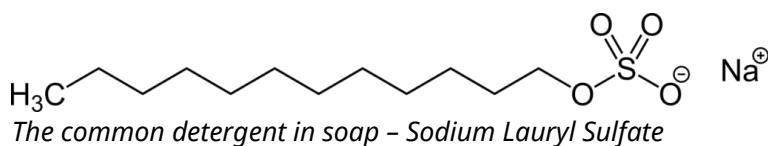


Credit: [Qwerter](#), [Michal Maňas](#), [Magasjukur2](#)  
(CC BY-SA 3.0)

These weak associations that arise from the polar:polar attractions ([CC BY-SA 3.0](#)) are referred to as **Hydrogen Bonds (H-bonds)**. While independently weak, the summation of all the H-bonds are very strong. These associations give rise to the special properties of water: **surface tension, cohesion, adhesion, high specific heat capacity**.

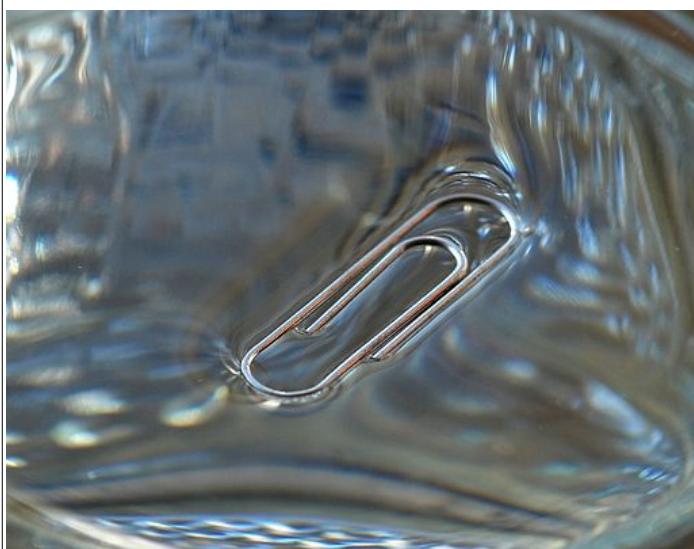
### **• Hydrogen Bond Simulation**

Polar materials mix with polar materials. Things that can dissolve in water are also polar and referred to as being **hydrophilic** (*hydro-* water, *philic-* liking). Non-polar substances do not interact or mix with polar solvents and are referred to as **hydrophobic** (*hydro-* water, *phobic-* hating). Since carbon and hydrogen share electrons equally, organic compounds are non-polar. Oil is a hydrocarbon that does not mix well with water or vinegar. Vinegar, however is a polar compound that interacts with water. Detergents are called **amphiphilic** (*amphi-* both; *philic-* liking) because they have portions that are non-polar and portions that are very polar. Detergents can therefore dissolve in hydrocarbons and water. Water alone cannot effectively remove oil from your skin, but a detergent can dissolve the oil and carry it away in water.



## Surface Tension

Surface tension presents as an invisible film that encompasses the surface of water. The attractive



This paperclip would sink if it broke through the surface of the water. Credit: [Alvesgaspar](#) (CC BY-SA 3.0)

forces arising from the intermolecular **cohesion** holds the surface of water together.



These water striders are not on top of the water because they are light. They have not broken through the surface of the water and is therefore, on top of the water.

Credit: [Markus Gayda](#) (CC-BY-SA-3.0)

## Solutions

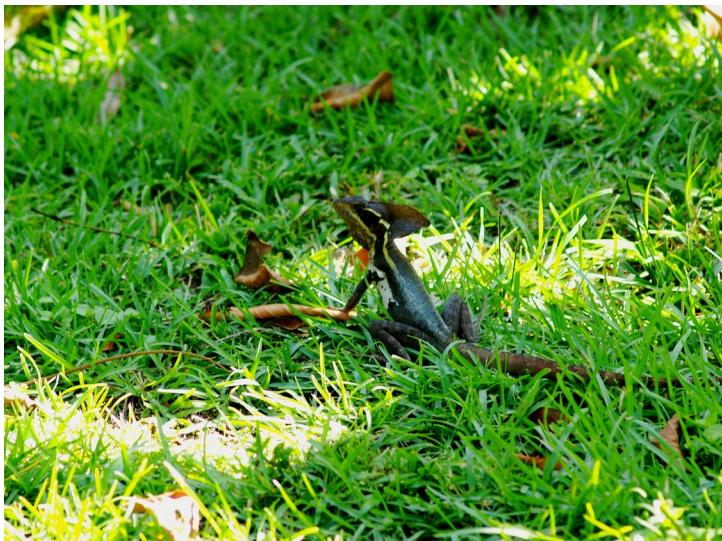
Water is an excellent solvent of other polar compounds. Table salt (NaCl) ionizes readily in water. The  $\delta^-$  O associate around  $\text{Na}^+$  while the  $\delta^+$  H associate with the  $\text{Cl}^-$ . If NaCl is dissolved in  $\text{H}_2\text{O}$ , what do you think happens to the intermolecular interactions between water molecules? What do you think would happen to the H-bonds? Would you expect there to be a difference in the surface tension? How do you think this explains the difference of boiling or freezing?

## Running on Water



*Basiliscus plumifrons (green basilisk)*

<https://flic.kr/p/7BTyCX>



*Basiliscus basiliscus (common basilisk)*

<https://flic.kr/p/7BXkv7>

Lizards of the genus [Basiliscus](#) have the nickname "Jesus Christ lizard" for their very special adaptation regarding water.

In the face of danger, these lizards run on their hind legs across water to escape predation. Their hind legs have long toes that help in increasing surface area to distribute their weight so they can propel themselves on the surface of the water. They do not sink because the surface tension of the water is not broken by the large surface covered by their feet. After about 4.5m, they lose sufficient momentum to propel themselves on the water surface and break through. H-bonds enable this adaptation.

<http://youtu.be/45yabrnryXk>

## What if?

The namesake of these lizards walked on water but was also able to turn water into wine. Wine is a solution of ethanol (11%) in water. Ethanol has a polar end and a non-polar end.

- How successful would the basilisk run on wine?
- Why?
- How could you test this without necessarily using a lizard?

## Acids and Bases

We can call any compound that adds H<sup>+</sup> ions (a free proton) into solution an **acid**. Along with this, we would expect that any compound that would decrease the concentration of free H<sup>+</sup> of a solution as a **base**. pH is the power of H<sup>+</sup> of a solution. We define this power as a **molar concentration** of H<sup>+</sup> in solution. This concentration invariably ends up being a relatively small number (though great in absolute numbers) and is expressed as a decimal number. Because the range of the concentrations is so great, we express these numbers as logarithmic numbers to avoid writing many 0's after the decimal and to facilitate communicating the concentration. Since these numbers are so (relatively) small, we use the negative logarithm to describe this concentration.

Mathematically defined,  $pH = -\log_{10}[H^+]$

The pH scale ranges so that anything below pH 7 is acidic and anything above pH 7 is alkaline. So a smaller number is more acidic. But didn't we just state that something acidic contains more H<sup>+</sup> ions? Remember, because we are dealing with a negative Logarithm, this means the concentration is higher.

## Logarithmic Scales

If we have a quantity that is 10<sup>2</sup>, we know that translates into 100. Just as if we have a quantity of 10<sup>4</sup>, we know that translates into 10000. Just as it becomes inconvenient to keep writing all those 0's, it's really impractical to write many many 0's after a decimal. It's really hard to talk about too! So we likewise will express numbers like 0.0001 as 10<sup>-4</sup>. A logarithm is the reverse function of an exponent. Therefore:

$$\log_{10}(0.0001) = -4$$

$$\log_{10}(10^{-4}) = -4$$

So how do we define a solution that is pH 2? Well, we already decided that this solution is below pH 7 — making it an acid. But what does this mean in terms of H<sup>+</sup> ion concentration?

Let's work this out algebraically:

•  $pH = -\log_{10}[H^+]$  Let's bring the (-) over to the other side

•  $-pH = \log_{10}[H^+]$  Now let's reverse the Log → base 10

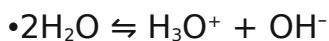
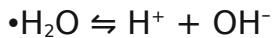
•  $10^{-pH} = [H^+]$  Plug in the pH → molar concentration of  $[H^+]$

As we can now see, a solution of pH 2 is acidic because the molar concentration of  $[H^+]$  is  $10^{-2}$  mole/L or 0.01M

## Dissociation of ions: That number is small!

It's not a small number. Remember that a mole is  $6.022 \times 10^{23}$ . That's a very large number! Think about it! A solution of pH 4 is acidic, but if we plug in the formula, we realize that this is equal to 0.0001M  $H^+$  – less than pH 2 at 0.01M!

But let's compare it to the  $[H^+]$  content of  $H_2O$ . Now I'm going to sound crazier! Water can be thought of as being in an equilibrium where some of the molecules are ionizing and deionizing. We can express this in 2 ways:



So at any given point, a liter of  $H_2O$  at neutral pH (7) has  $10^{-7}$  moles of  $H^+$  ions. Incidentally, it also has  $10^{-7}$  moles of  $OH^-$  in solution. The second expression indicates the formation of a hydronium ion ( $H_3O^+$ ) instead of a free proton in solution. So something that is pH 2 is a stronger acid than pH 4, right? Nope. That just indicates the amount of free protons in solution. It is more acidic but acid strength means something else. When we talk about **strong acids**, it means that it is more likely to donate a proton to the solution because it is more likely to ionize. Let's look at the following:



If this dissociation is very high, then we say that it is a strong acid. Similarly, a compound like NaOH readily dissociates completely in solution and provides  $OH^-$  ions that can

readily remove H<sup>+</sup> from solution -a strong base! We speak of dissociation in terms of rates and we express this as the acid dissociation constant, K<sub>a</sub>. This is calculated using the concentrations of [H<sup>+</sup>] (proton), [A<sup>-</sup>] (conjugate base) and [HA] (non-dissociated) at equilibrium:

$$\bullet K_a = \frac{[H^+][A^-]}{[HA]}$$

Just like the orders of magnitude we have when discussing pH, the rates of dissociation are more conveniently communicated on a logarithmic scale.

$$\bullet pK_a = -\log_{10} K_a$$

Think about it this way, if the concentration of the dissociated ions is very high, the numerator in the rate is very high → K<sub>a</sub> is great. In other words, at equilibrium, the dissociation reaction looks more unidirectional than bi-directional as the compound is readily ionized:

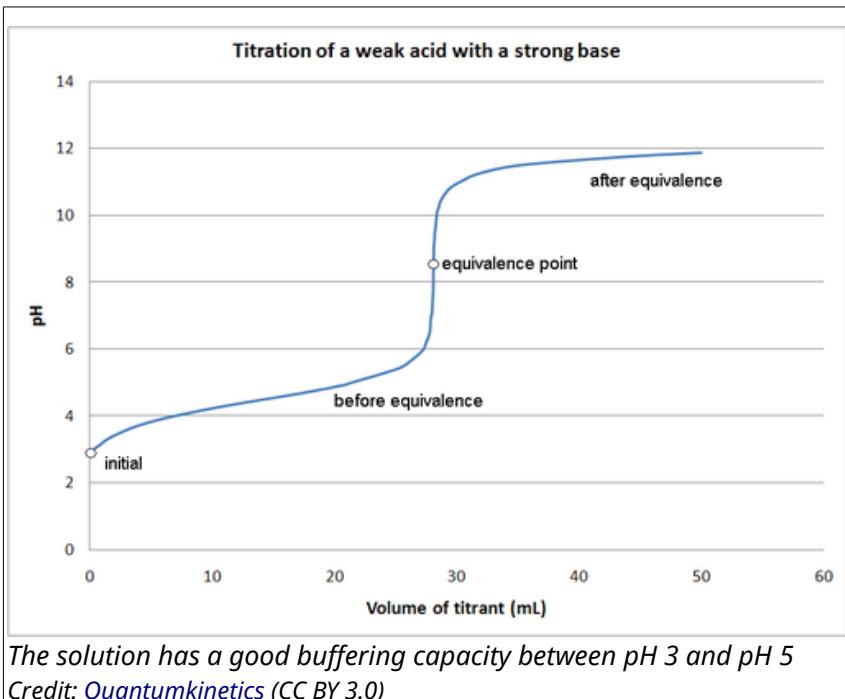


On this scale, we refer to anything with a pK<sub>a</sub> < -2 as a strong acid since it will readily dissociate in solution. This form of the dissociation constant is extremely useful in estimating the pH of **buffered solutions** and for finding the equilibrium pH of the acid-base reaction (between the proton and the conjugate base). We can estimate the pH by utilizing the Henderson-Hasselbalch Equation:

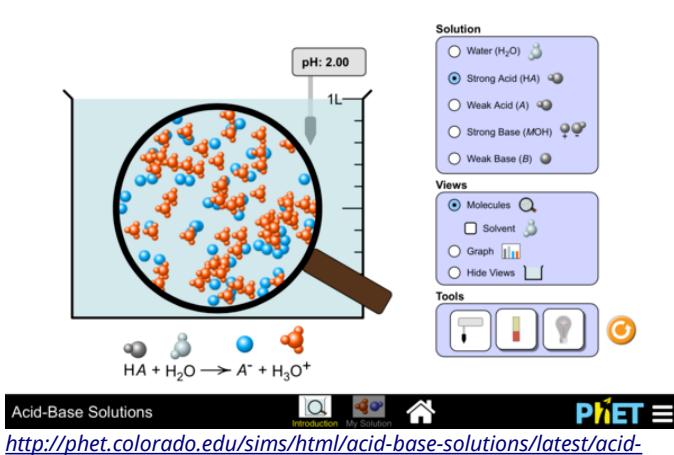
$$\bullet pH = pK_a + \log_{10} \left( \frac{[A^-]}{[HA]} \right)$$

## Buffered Solutions

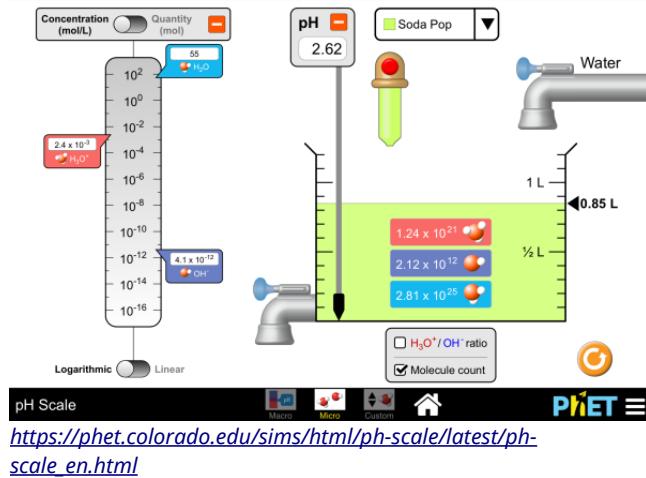
A **buffer** is something that resists change. A **buffered solution** is one that consists of a weak acid or weak base that will control the pH of a solution. Imagine a buffer to be a reservoir of available H<sup>+</sup> or OH<sup>-</sup> ions. If a buffered solution is pH 2, adding a basic solution to it will not cause a drastic change to the pH because the reservoir of H<sup>+</sup> will continuously neutralize the base. Eventually, this store or reservoir of H<sup>+</sup> will be depleted. When this happens, the pH will suddenly change. The range in which acid or base is added without a significant change in pH is called the buffered zone or the **buffering capacity**. When this store of H<sup>+</sup> or buffering capacity is expended, we have reached the **equivalence point** that describes the point at which the base has completely neutralized the weak acid.



## Acids and Bases Simulation



## pH simulation



## pH of common items

Use the table below to indicate whether an item is acid or base and what you predict the pH to be. We can determine these in class through measuring.

Solution	Acid or Base	Predicted pH	Actual pH
Coffee			
Cola			
Distilled H <sub>2</sub> O			
Detergent			
Bleach			
Apple Juice			
Antacid Solution			

## Inquire!

- What is the mechanism of action for antacids?
- Do they all antacids have the same efficacy?

## Explore: Determine the pH of common solutions

Use the table below to indicate whether an item is acid or base and what you predict the pH to be. We can determine these in class through measuring. Using the pH meter, measure the pH and validate your predictions

Solution	Acid or Base	Predicted pH	Actual pH
Coffee			
Cola			
Distilled H <sub>2</sub> O			
Detergent			
Bleach			
Apple Juice			
Antacid Solution			

## Discovering Buffers

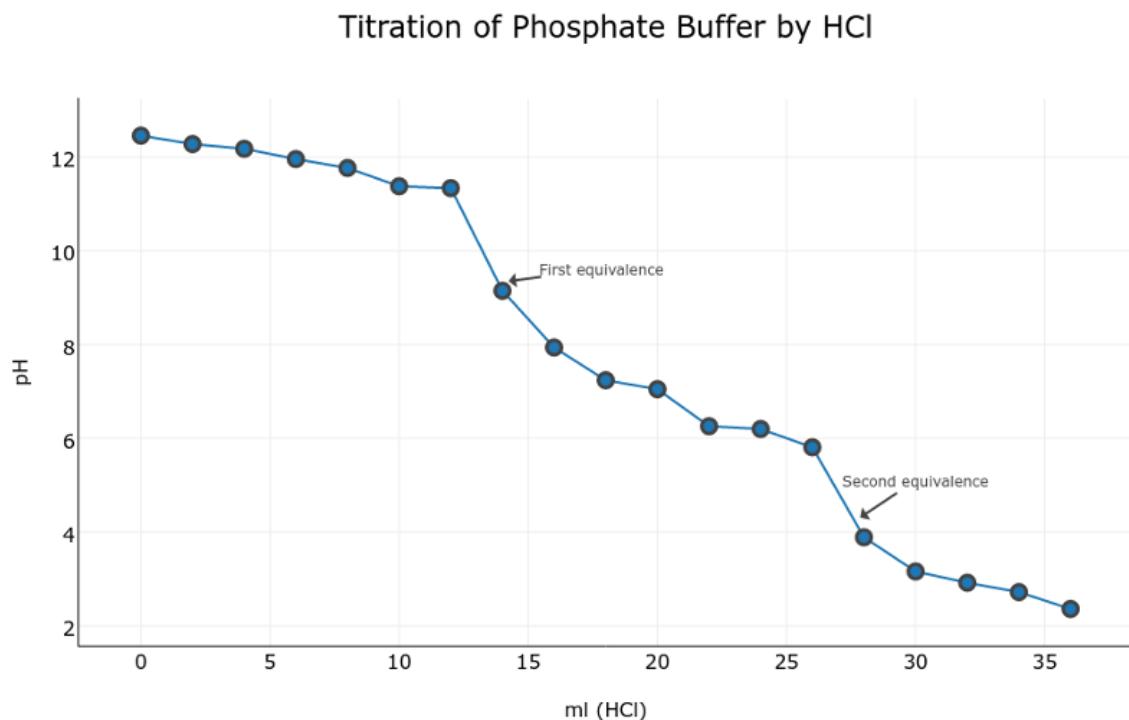
1. Take a small beaker of distilled water and determine its pH. Add one drop of strong acid or of strong base to the water and observe the pH reading on the meter.
2. Now take a small sample of either the standard pH buffer or of any other buffer solution in the lab and add a drop of either strong acid or strong base. Does the reading on the pH meter remain constant or does it change rapidly as it did with the distilled water?

## Titration of Acetic Acid

1. Place 50 ml of 0.1M acetic acid into a 250ml beaker
2. Add 5 drops of phenolphthalein indicator and swirl briefly
3. Determine the initial pH with probe
4. Fill a buret with 0.1 N NaOH
5. Add 3 ml volumes NaOH and swirl flask briefly
6. Record the pH and observe the color of the solution
7. Continue to add increments of 3 ml (with swirling) and record the pH until the pH reaches 10.
8. Using a computer, plot pH readings on the y-axis and the volume of base on the x-axis. You can try to use [Plot.ly](#)

# Scatterplot Tutorial

pH	ml (HCl)
choose as x	choose as x
choose as y	choose as y
12.46	0
12.28	2
12.18	4
11.96	6
11.77	8
11.38	10
11.34	12
9.15	14
7.94	16
7.24	18
7.05	20
6.26	22
6.2	24
5.81	26
3.89	28
3.16	30
2.92	32
2.72	34
2.36	36



- [Download the data](#) to try in Plot.ly
- Follow this tutorial on using [Plot.ly](#) to generate a graph. However, do not draw a trendline.
- <https://youtu.be/bxGFS-RukZY>

# Titration of Antacid Inquire!

- What pH do you predict an antacid to be?
- Do you expect the antacid to be a strong acid, weak acid, strong base or weak base?
- Does brand of antacid matter in the effectiveness?

## Activity

1. Retrieve an antacid tablet from the instructor
2. Crush the antacid in 100 ml of distilled water
3. Transfer 50 ml of the solution to clean 250 ml beaker
  - Share remaining 50 ml with another group (ensure it is a different tablet)
  - if available, add 5 drops of bromocresol purple (0.04% solution) to each sample
4. Measure the pH of the antacid solutions and observe color (if using bromocresol purple)
5. Determine if you want to titrate with an acid or with a base
6. Titrate each antacid solution with either 0.1N HCl or 0.1N NaOH in 2ml increments (you must decide)
  - if using bromocresol purple, stop when yellow is reached

## Indicator References

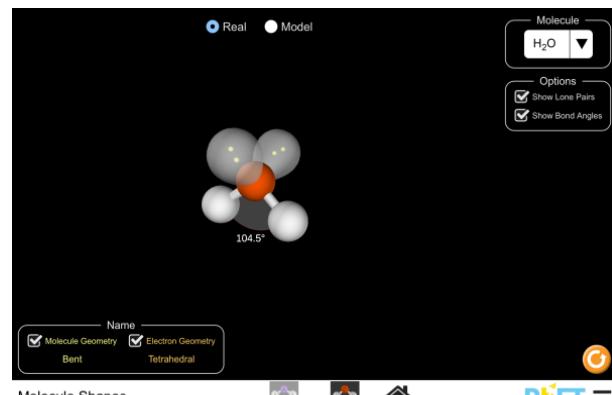
Bromocresol purple (pH indicator)	Phenolphthalein (pH indicator)
below pH 5.2      above pH 6.8	below pH 8.2      between pH 10.0 and 13.0
5.2 $\rightleftharpoons$ 6.8	colorless $\rightleftharpoons$ fuchsia

## Questions for reflection

1. What is a buffer?
2. What is the biological significance of a buffer?
3. Explain differences between strong and weak acids and bases.
4. What are the differences between acids and bases in terms of  $[H^+]$  and  $[OH^-]$ .
5. Why is pH 7 designated as neutral?
6. How does the antacid work?
7. How much difference in  $[H^+]$  is there between pH 4 and pH 8?

## Understanding Chemistry in 3D

1. Click on the image to launch the simulation
2. Choose "Real Molecules"
3. Click on "Show Bond Angles"
  - you may also de-select "Show Lone Pairs"
4. Drag the atoms to understand the 3-D shape
5. Choose  $\text{CH}_4$  from the drop-down menu of molecules
  - $\text{CH}_4$  is methane, the simplest organic molecule
  - notice the restraints on the physical space occupied by the atoms as you drag the atoms around
  - remember these bond angles and constraints when we explore organic chemistry
  - toggling between "Model" and "Real" will illustrate if the position of the atoms are influenced by other factors we don't see
6. Choose  $\text{NH}_4$  from the drop-down menu
  - $\text{NH}_4$  is ammonia, a polar solvent
  - $\text{NH}_4$  is **NOT** an organic molecule
  - Toggle the lone pairs to see how these electrons play a role in the positioning of atoms
7. Choose  $\text{CO}_2$  from the drop-down
  - explore this non-organic molecule to visualize the effects of double bonds on geometry



## Organic Chemistry

Living things are composed of **organic molecules** primarily made up of the elements carbon and hydrogen. Molecules of hydrogen and carbon (referred to as **hydrocarbons**) have the property of being **non-polar**. Yet 70- 90% of cells are composed of water (a **polar** compound). Polar substances mix with other polar substances. Likewise, non-polar substances interact with other non-polar compounds. Polar and non-polar compounds are immiscible (unable to mix).

## So how do cells keep from falling apart in a water environment?

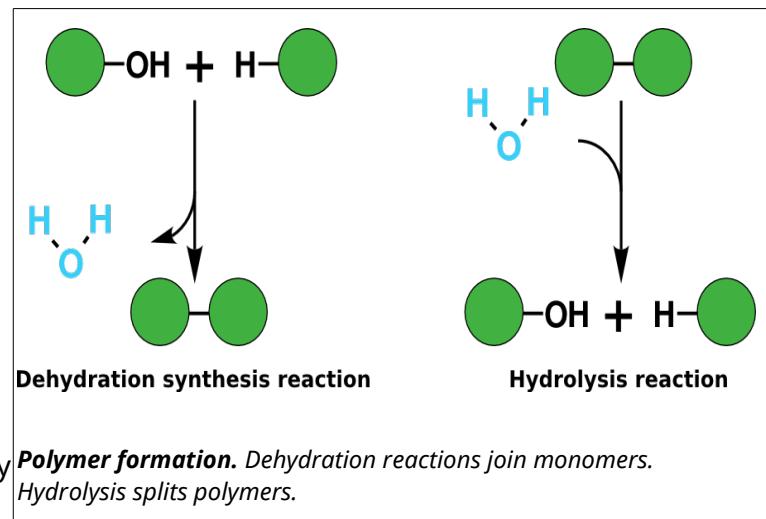
**Functional groups** are clusters of atoms in a group that impart a new “function” to the compound they are attached to. Hydrocarbons in cells have functional groups attached to them that permit them to interact with the water environment of the cell. These functional groups also define the type of molecule it is based on the characteristics of those groups.

Group Name	Structure	Compound Name	Property
Hydroxyl	$\text{R}-\text{OH}$	alcohol	polar
Carbonyl	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{R}_1 \end{array}$	Aldehyde ( $\text{R}_1=\text{H}$ ) Ketone ( $\text{R}_1=\text{C}$ )	polar
Carboxyl	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{OH} \end{array} \rightleftharpoons \begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{O}^- \end{array} \text{H}^+$	acid	polar acidic
Amino	$\begin{array}{c} \text{H} \\   \\ \text{R}-\text{N}-\text{H} \\   \\ \text{H} \end{array} \rightleftharpoons \begin{array}{c} \text{H} \\   \\ \text{R}-\text{N}^+-\text{H} \\   \\ \text{H} \end{array}$	amine	polar basic
Sulfhydryl	$\text{R}-\text{SH}$	thiol	polar
Phosphate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{O}-\text{P}-\text{OH} \end{array} \rightleftharpoons \begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{O}-\text{P}-\text{O}^- \end{array} \text{H}^+$		polar acidic

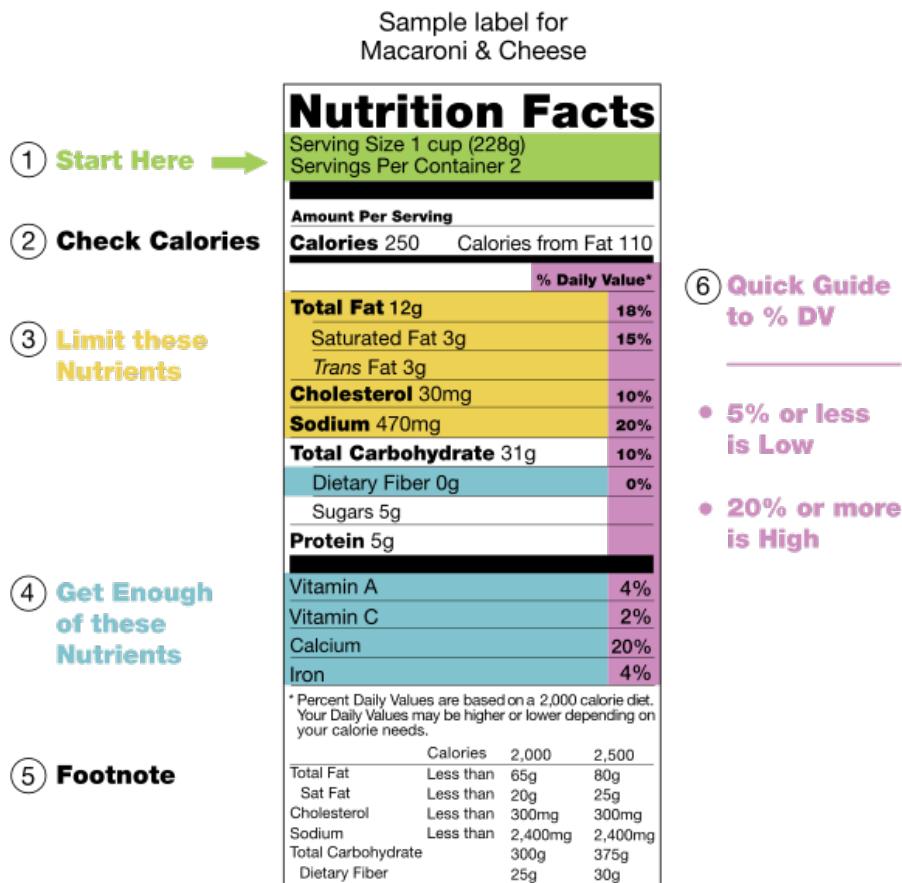
**Functional Groups.** Functional groups commonly found in organic compounds. R is a placeholder chemical that can be anything (like a hydrocarbon chain). The functional groups in this table are those that add polar or charged properties to the hydrocarbon chains. Bi-directional arrows indicate that those functional groups dynamically ionize and reach an equilibrium in solution.

## How are macromolecules assembled?

The common organic compounds of living organisms are **carbohydrates, proteins, lipids, and nucleic acids**. Each of these are macromolecules or **polymers** made of smaller subunits called **monomers**. The bonds between these subunits are formed by a process called **dehydration synthesis**. This process requires energy; a molecule of water is removed (dehydration) and a covalent bond is formed between the subunits. Because a new water molecule is formed, this is also referred to as *condensation*. The opposite where water and energy are used to break apart polymers into simpler monomers is called **hydrolysis** (*hydro-* water, *lysis-* to break or split).



## Where do we find macromolecules?

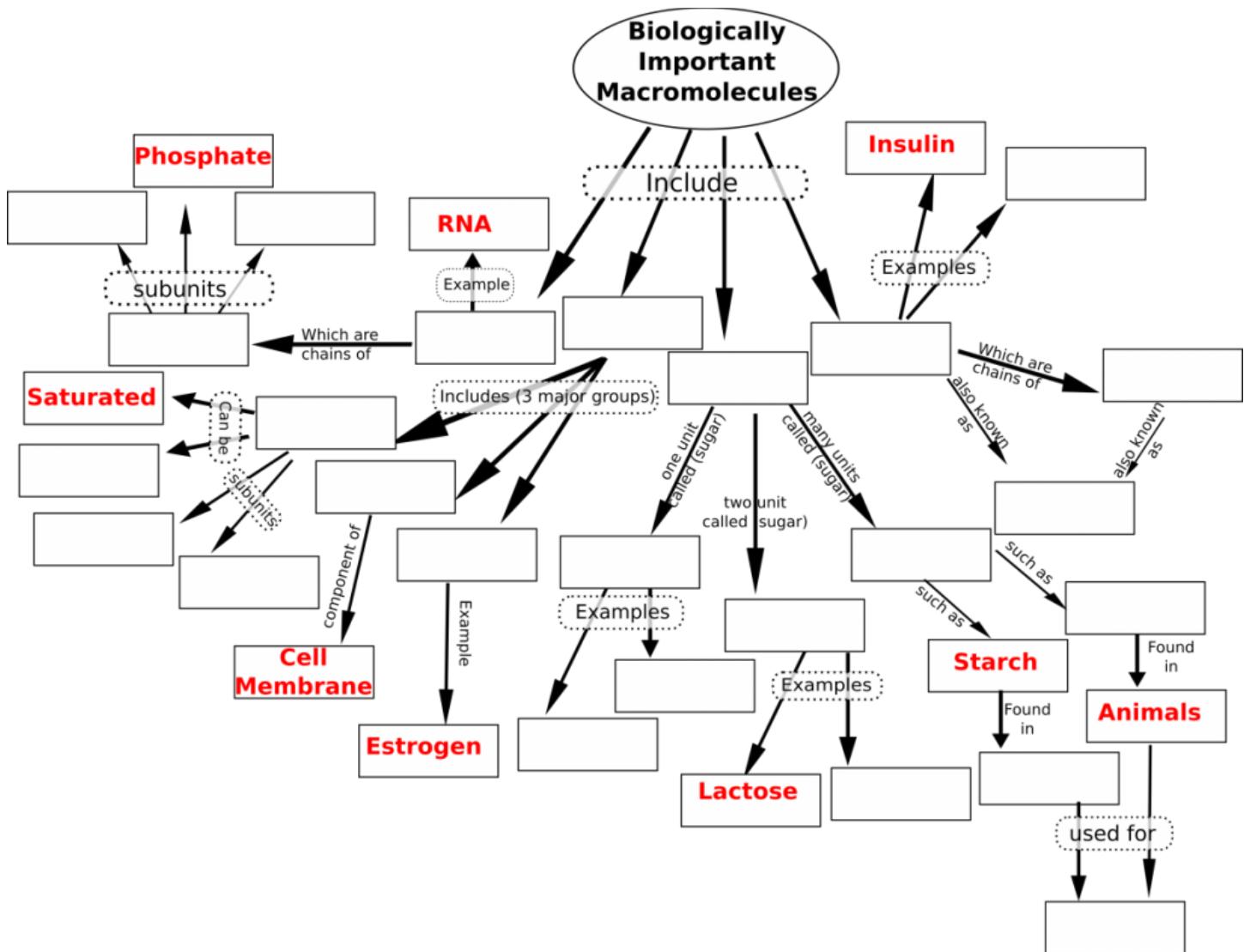


## Additional Resources

- Carbohydrates  
<http://www.visionlearning.com/en/library/Biology/2/Carbohydrates/61>
- Lipids <http://www.visionlearning.com/en/library/Biology/2/Lipids/207/reading>
- Fats and Proteins <http://www.visionlearning.com/en/library/Biology/2/Fats-and-Proteins/62>

## Vocabulary

- carbohydrates
- reducing sugar
- polymer
- monomer
- monosaccharide
- disaccharide
- polysaccharide
- aldehyde
- ketone
- lipid
- Dehydration synthesis
- hydrolysis
- reduction
- oxidation

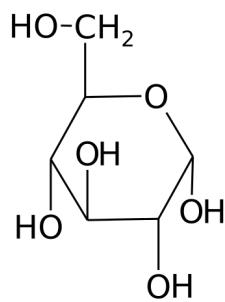


## Introduction: Carbohydrates

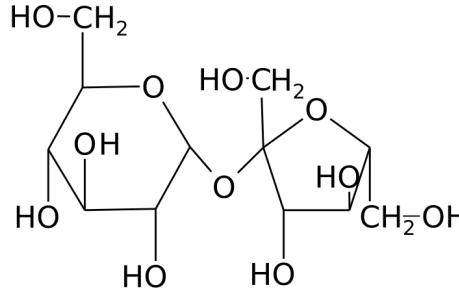
**Carbohydrates** serve 2 major functions: energy and structure. As energy, they can be simple for fast utilization or complex for storage. Simple sugars are monomers called **monosaccharides**. These are readily taken into cells and used immediately for energy. The most important monosaccharide is glucose ( $C_6H_{12}O_6$ ), since it is the preferred energy source for cells. The conversion of this chemical into cellular energy can be described by the equation below:



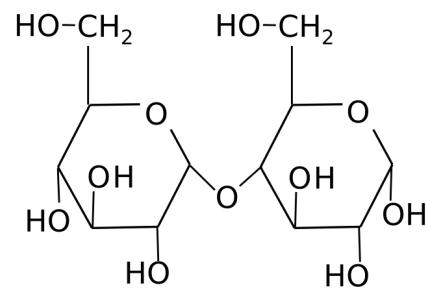
Long polymers of carbohydrates are called **polysaccharides** and are not readily taken into cells for use as energy. These are used often for energy storage. Examples of energy storage molecules are: amylose or **starch** (plants) and **glycogen** (animals). Some polysaccharides are so long and complex that they are used for structure like **cellulose** in the cell walls of plants. Cellulose is very large and practically indigestible, making it unsuitable as a readily available energy source for cells.



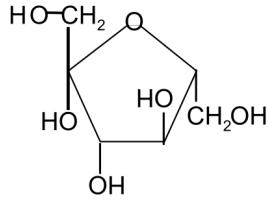
Glucose, a monosaccharide



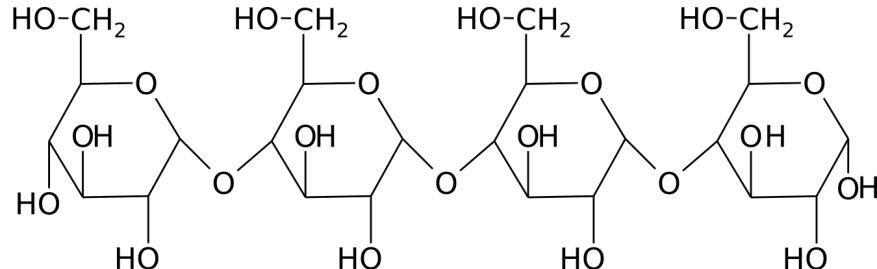
Sucrose, a disaccharide



Maltose, a disaccharide



Fructose, a monosaccharide

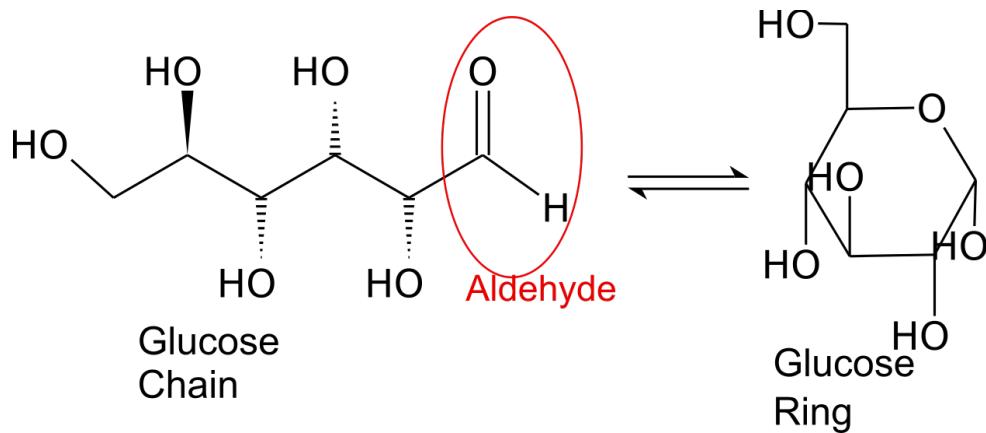
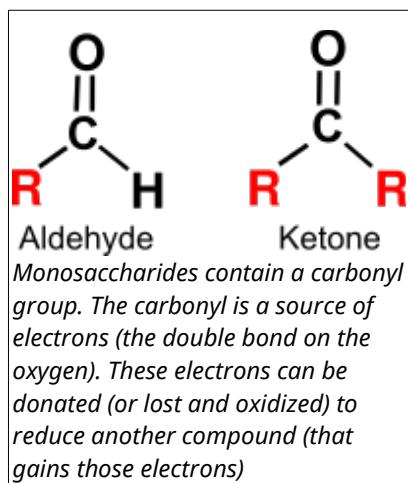


Amylose (Starch), a polysaccharide

**Carbohydrates:** Carbohydrates are composed of sugar units referred to as -saccharides.

Many monosaccharides such as glucose and fructose are **reducing sugars**, meaning that they possess free **aldehyde** or **ketone** groups that reduce weak oxidizing agents such as the copper in Benedict's reagent. The double bond in the carbonyl group is a source of electrons that can be donated to something else. That is to

say, those electrons can be “lost” by the sugar and “gained” by another chemical. **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric ( $Cu^{2+}$ ) ions to cuprous oxide ( $Cu^+$ ) at basic (high) pH. Cuprous oxide is green to reddish orange. Roughly speaking, **reduction** is a type of chemical reaction that is paired with **oxidation**. In oxidation/reduction reactions (**RedOx**), some chemical loses electrons (oxidized) to another chemical that gains them (reduced). We remember whether a compound is reduced or gained by using the mnemonic: LEO goes GER or Loss of Electrons is Oxidation & Gain of Electrons is Reduction.



Monosaccharides are capable of **isomerizing**. This means they alternate in structure from a linear chain to a ring form in solution. In the chain form, the aldehyde is free to donate (lose) electrons to reduce another compound. When monosaccharides undergo dehydration synthesis to form polymers, they can no longer isomerize into chains with free aldehydes and are unable to act as reducing sugars. Green color indicates a small amount of reducing sugars, and reddish orange color indicates an abundance of reducing sugars. Non-reducing sugars produce no change in color (i.e., the solution remains blue).

**Oxidized Benedict's reagent ( $Cu^{2+}$ ) + Reducing sugar ( $R-C=O$ )  
(blue)**

Heat  
High pH

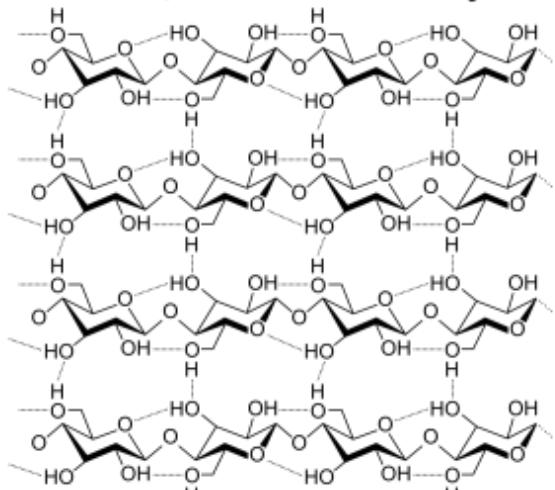
**Reduced Benedict's reagent ( $Cu^+$ ) + Oxidized sugar ( $R-COOH$ )  
(green to reddish orange)**

Note:  $Cu^{2+}$  has fewer electrons than  $Cu^+$ .

## Structural Carbohydrates

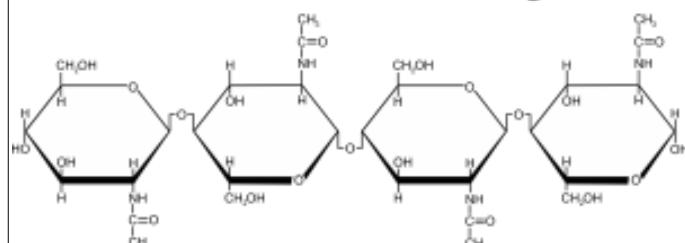
In food, more complex carbohydrates are derived from larger polysaccharides. These larger carbohydrates are fairly insoluble in water. Dietary fiber is name given to indigestible materials in food most often derived from the complex carbohydrates from vegetable material. Some of this material serves the plants as a structural component of the cells and is completely insoluble. **Cellulose** is the major structural carbohydrate found in plant cell walls. Similarly, animals and fungi have structural carbohydrates that are composed of the indigestible compound called **chitin**. We will not be testing for these items.

### Cellulose (structural carbohydrate)



Cellulose is a complex carbohydrate of glucose molecules. It is the major structural component of plant cell walls. Its structural durability is enhanced by intramolecular hydrogen bonds.

### Chitin (animal and fungi)



Chitin is a structural carbohydrate found in animal shells or fungi cell walls. The polymer contains amide groups that differentiates it from other carbohydrates composed of



A cicada molting from its shell made of chitin.  
Credit: [Jodelet / Lépinay](#) (CC BY-SA 2.0)

## Test your knowledge

- <http://www.visionlearning.com/en/library/Biology/2/Carbohydrates/61/quiz>

## Materials

- |   |  |
|---|--|
| <ul style="list-style-type: none"> <li>• potato juice</li> <li>• apple juice</li> <li>• urine sample 1</li> <li>• urine sample 2</li> <li>• reducing sugar solution</li> <li>• starch solution</li> <li>• Benedict's Reagent</li> </ul> | <ul style="list-style-type: none"> <li>• Sucrose solution</li> <li>• glucose solutions</li> <li>• distilled water</li> <li>• hot plates</li> <li>• beakers of water</li> <li>• test tubes</li> <li>• test tube rack</li> </ul> |
|---|--|

## Stop and Think:

- Use your senses and previous observations/experiences about the qualities of the experimentals.
- Formulate some hypotheses about the carbohydrate content of the experimentals or unknowns.
  - Identify if the sample is experimental or control before making hypothesis

## QUESTION: Are there simple reducing sugars in my juice? Are there simple reducing sugars in my urine?

Diabetes mellitus is a disease that refers to the inability of the cells to take in glucose. The word diabetes refers to urination and mellitus refers to sweetness. Since the cells of diabetics cannot remove glucose from the blood, there is an excess of glucose circulating that is eliminated in the urine. The traditional method of diagnosing someone with diabetes mellitus was to taste the sweetness of the patient's urine. Let's use Benedict's test for the detection process instead of the unhygienic alternative.

Make a hypothesis and ask what we would predict from a Benedict's test if testing a urine sample of someone with diabetes mellitus.

## Benedict's Test For Reducing Sugars

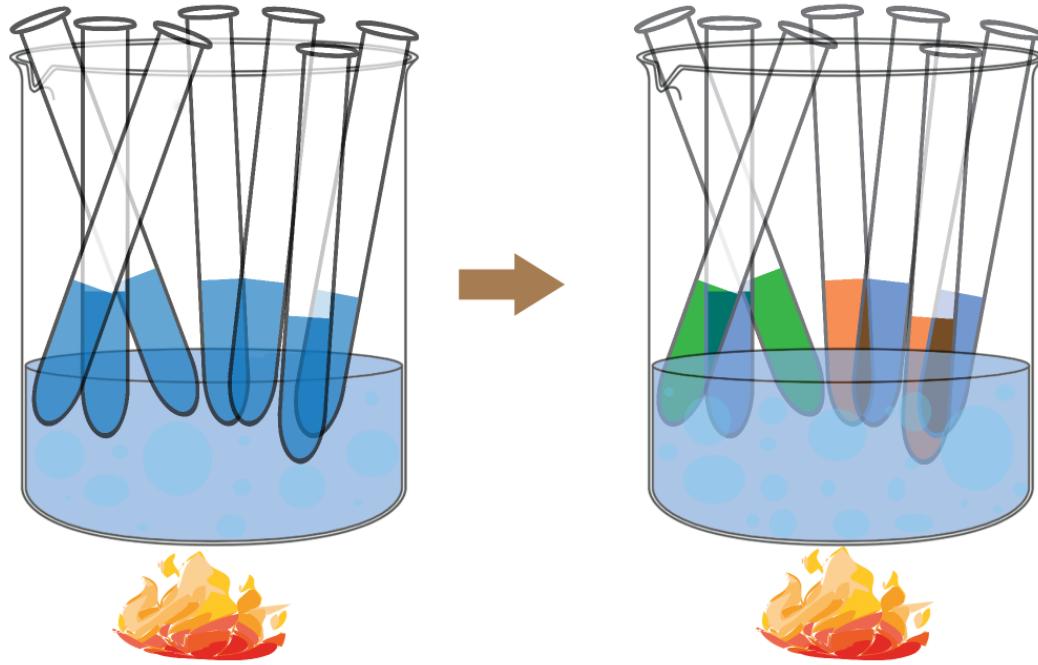
1. Obtain 9 test-tubes and number them 1-9.
2. Add to each tube the materials to be tested. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Indicate in the table whether the the sample you are testing is positive control, a negative control or an experimental.
4. Before you begin the heating of the samples, use predict the color change (if any) for each sample. (use the sample type to aid in your prediction)
5. Add 40 drops (or 2 ml) Benedict's solution to each tube.
6. Place all of the tubes in a boiling water bath for 3 min or until a noticeable color change and observe colors during this time.

7. After 3 min, remove the tubes from the water bath and let them cool to room temperature. Record the color of their contents in the Table.

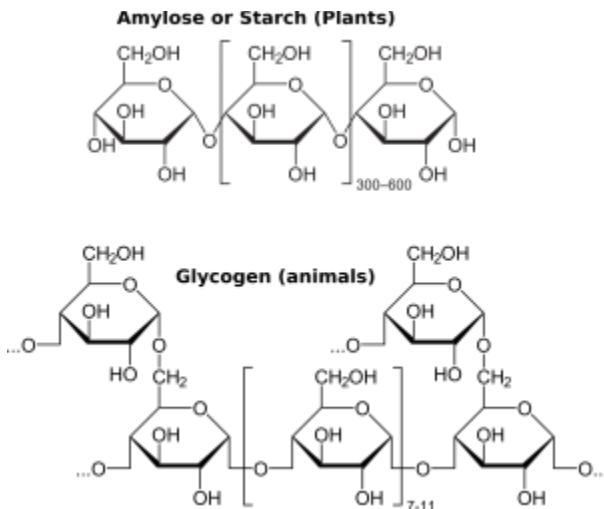
**Table: Solution And Color Reactions For Benedict's Test For Reducing Sugars**

Tube	Solution	Type of Sample (unknown, + control, - control)	Hypothesized Color	Benedict's Test Color
1	10 drops apple juice			
2	10 drops potato juice			
3	10 drops sucrose solution			
4	10 drops glucose solution			
5	10 drops distilled water	- Control	Blue	
6	10 drops reducing sugar	+ Control	Red	
7	10 drops starch solution			
8	10 drops Urine sample 1			
9	10 drops Urine sample 2			

Boil solutions until color changes



## Iodine Test for Starch



Carbohydrates that are used for energy storage are not reducing sugars since they are polymers that lack free aldehydes. Plant cells store energy in the form of starches like amylose or pectin. Since these molecules are larger than monosaccharides or disaccharides, they are not sweet to the taste and are not very soluble in water. **Iodine** (iodine-potassium iodide, I<sub>2</sub>KI) staining distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose --- iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with other carbohydrates that are not coiled, and remains yellowish brown. Therefore, a **bluish black color** is a positive test for starch, and a **yellowish brown** color (i.e., no color change) is a negative test for starch.

Notably, glycogen, a common energy storage polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

### Activity: Iodine Test For Starch

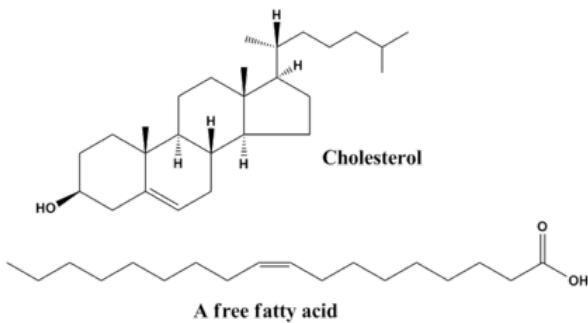
- Obtain 7 test-tubes and number them 1-7.
- Hypothesis Testing: Indicate in the table if the sample is experimental or control. Predict your expected color changes for each sample.
- Add to each tube the materials to be tested as indicated in the table below. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
- Add 10 drops of iodine to each tube. This test does **NOT** require boiling.
- Record the color of the tubes' contents in the table below.

**Table: Solution And Color Reactions For Iodine Test For Starch**

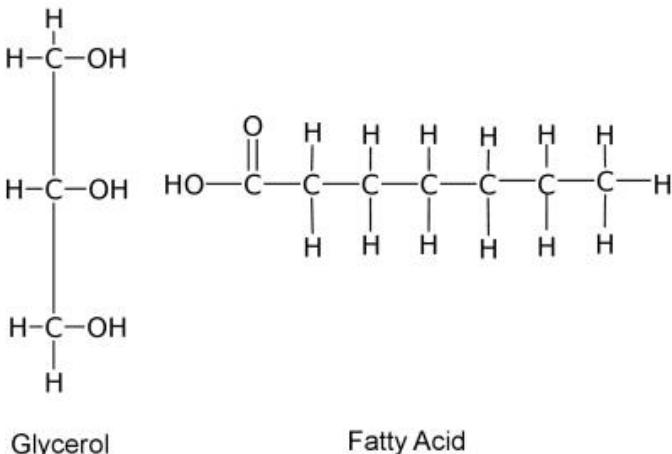
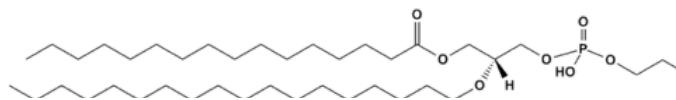
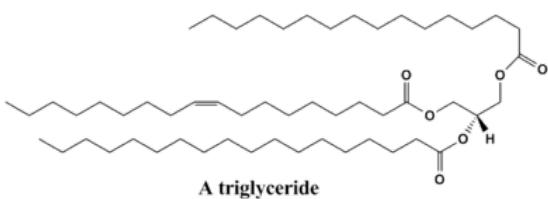
Tube	Solution	Type of Sample (unknown, + control, - control)	Hypothesized Color	Iodine Test Color
1	20 drops apple juice			
2	20 drops potato juice			
3	20 drops sucrose solution			
4	20 drops glucose solution			
5	20 drops distilled H <sub>2</sub> O	- Control	Yellow	
6	20 drops reducing sugar			
7	20 drops starch solution	+ Control	Black-Purple	

## Questions for Reflection

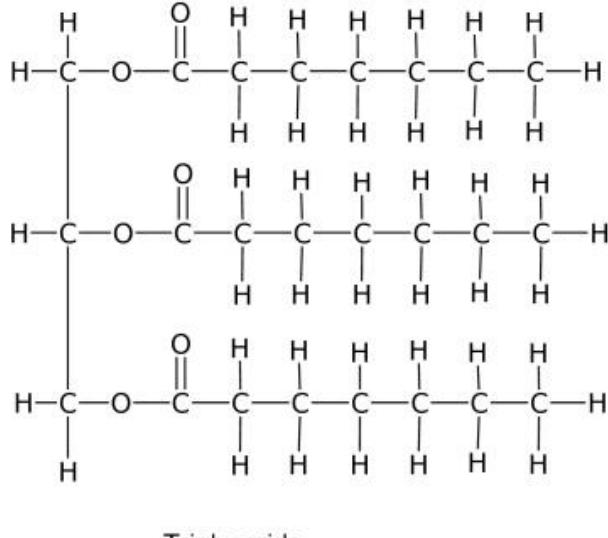
1. In the Benedict's test, which of the solutions is a positive control? Which is a negative control?
2. Which is a reducing sugar, sucrose or glucose?
3. Which contains more reducing sugars, potato juice or onion juice?
4. Is there a difference between the storage of sugars in onions and potatoes?
5. Which patient sample likely comes from a diabetic patient and how do we know this?
6. In the Iodine test, which of the solutions is a positive control? Which is a negative control?
7. Which is more positive for the iodine test: onion juice or potato juice?
8. What can you infer about the storage of carbohydrates in onions? In potatoes?
9. Describe the half reaction  $\text{Cu}^{2+} \rightarrow \text{Cu}^+$  as oxidation or reduction.
10. Describe the half reaction  $\text{Cu}^+ \rightarrow \text{Cu}$  as oxidation or reduction.



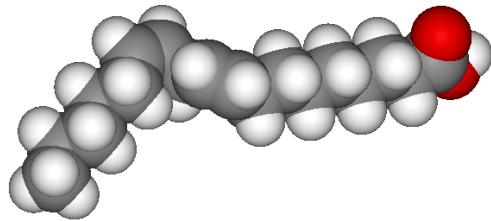
**Lipids** are the class of macromolecules that mostly serve as long-term energy storage. Additionally, they serve as signaling molecules, water sealant, structure and insulation. Lipids are insoluble in polar solvents such as water, and are soluble in nonpolar solvents such as ether and acetone.



Fats or triglycerides are made of glycerol and three fatty acid chains. They form through 3 dehydration synthesis reactions between a hydroxyl of the glycerol and the carboxyl group of the fatty acid.



## Saturated versus Unsaturated fats



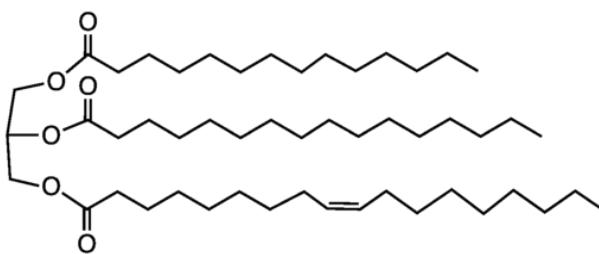
A

A saturated fatty acid. The molecule takes up little space in three dimensions. Many molecules can stack upon each other. Saturated fats are solid at room temperature.

polyunsaturated fatty acid. A kink from the double bond increases the amount of three dimensional space that the molecule fills. Unsaturated fats tend to be liquid at room temperature.



A trans fatty acid. Despite an unsaturated bond, the molecule fills as much space as a saturated fatty acid and is solid at room temperature. Trans fats usually arise from artificial saturation techniques.



Butterfat is almost completely saturated. Notice how molecules can stack very closely. Because butterfat can stack together very closely, it is dense and found as a solid at room temperature.



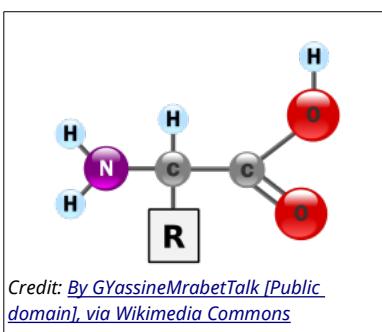
Because butterfat can stack together very closely, it is dense and found as a solid at room temperature.  
Credit: Steve Karg (CC BY 2.5)

## Testing for Lipids

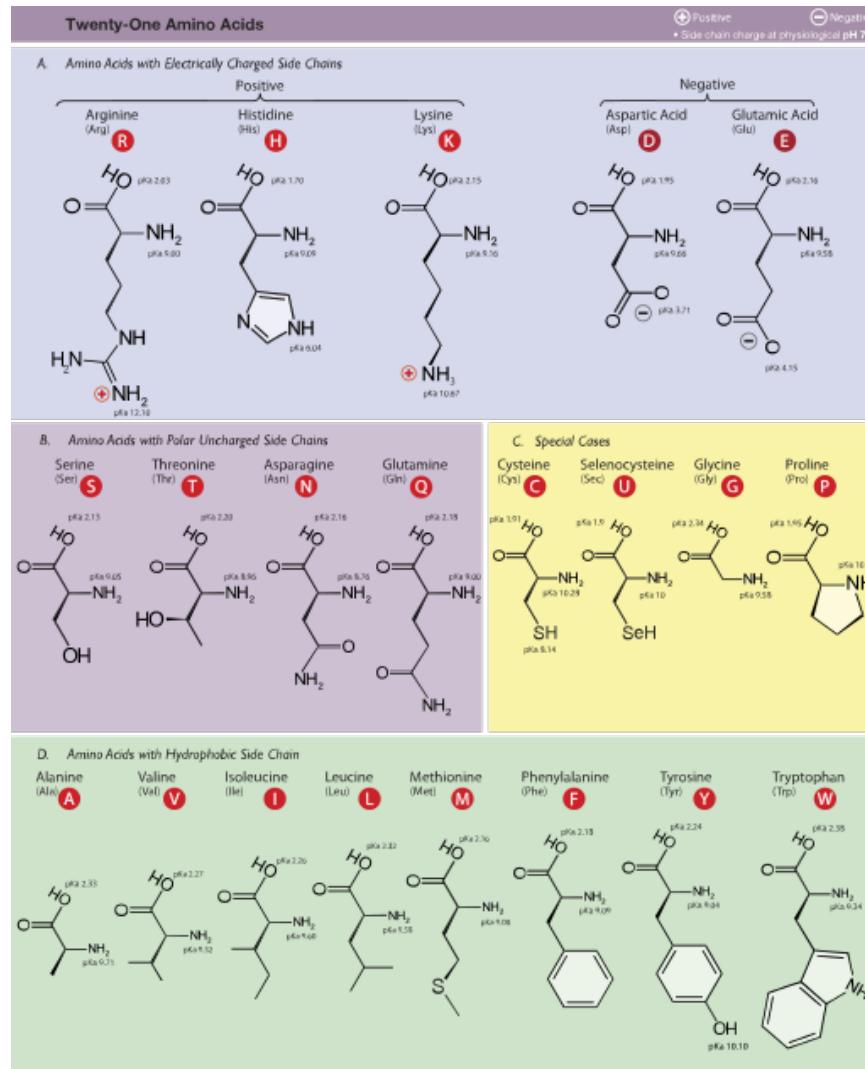
Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Oil Red O or Sudan IV.

Tube	Solution	Control (+/-) or Experimental	Reaction Description
1	1 ml vegetable oil + water		
2	1 ml vegetable oil + Oil Red O		
3	1 ml water + Oil Red O		
4	1 ml Lipid Solution + Oil Red O		

## Proteins are polymers of Amino Acids

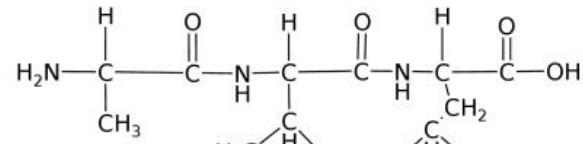
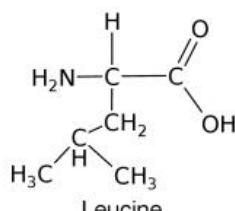
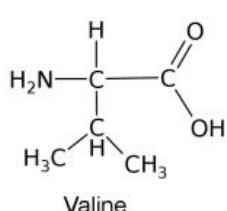
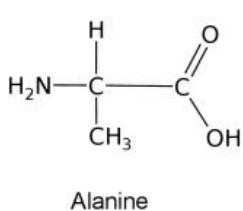


Proteins provide much of the structural and functional capacity of cells. Proteins are composed of monomers called amino acids. **Amino Acids** are hydrocarbons that have an **amino group** ( $\text{-NH}_2$ ) and an acidic **carboxyl group** ( $\text{-COOH}$ ). The R group represents a hydrocarbon chain with a modification that alters the properties of the amino acid. 20 universal amino acids are used to construct proteins. The variation in functional groups along the amino acid chain gives rise to the functional diversity of proteins.



20 amino acids and their properties. A 21st amino acid on this table represents the non-universally found selenocysteine. Credit: By Dancojocari CC BY-SA 3.0 or GFDL, via Wikimedia Commons

Monomers bond together through a dehydration synthesis reaction between adjacent amino and carboxyl groups to yield a **peptide bond**.



Three Common Amino Acids

*Three amino acids bound into a tripeptide.*

Tripeptide  
The beginnings of a protein

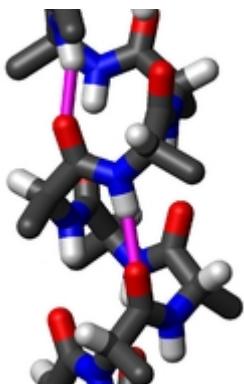
## How amino acids interact with each other and the environment

Use the following simulation to test how a polypeptide chain will fold based on the type of solution it is in and the composition of the amino acids.

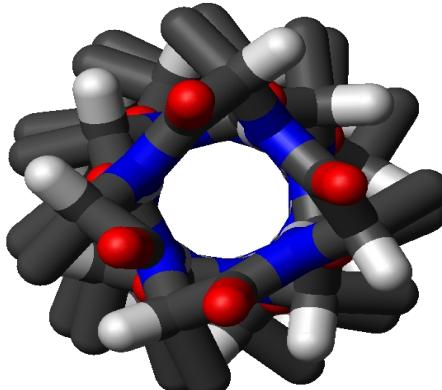
- [Protein Folding Simulation](#) (CC BY 4.0 Concord Consortium)

## Levels of structure

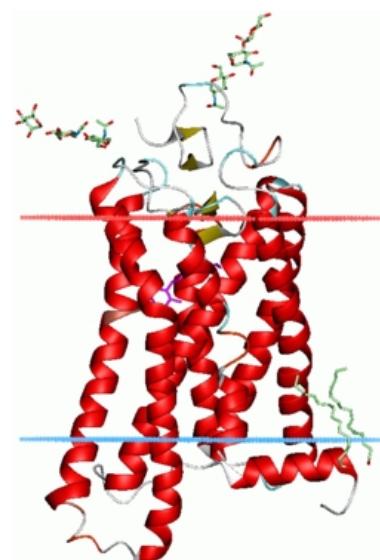
- **Primary Structure (1°):** The sequence of amino acids read from the Amino or N-terminal end of the molecule to the Carboxyl or C-terminal end
  - Tyr-Cys-Arg-Phe-Leu-Val-....
- **Secondary Structure (2°):** local three-dimensional structures that form from interactions of amino acids, like hydrogen bonding
  - **Alpha Helix** - coils occurring from the H-bonds between N-H and C=O groups along the backbone of the protein



Side view of  $\alpha$ -helix illustrating H-bonds in magenta between carboxyl oxygen (red) and amine nitrogen (blue). Credit: By WillowW [GFDL or CC-BY-SA-3.0, via Wikimedia Commons]

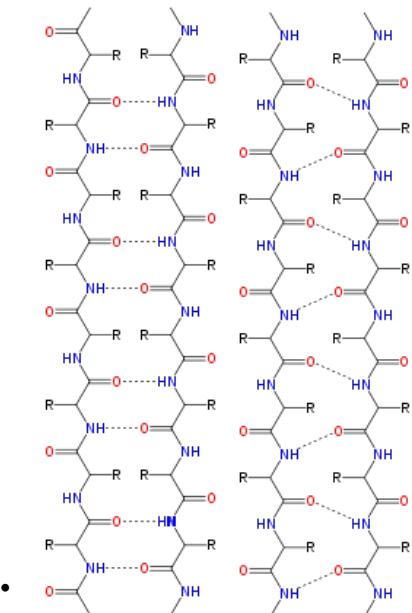


Top-down view of an  $\alpha$ -helix  
Credit: By WillowW [assumed GFDL or CC-BY-SA-3.0, via Wikimedia Commons]

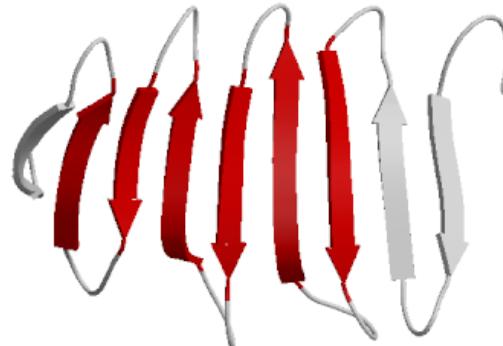


Side view of ribbon diagram of  $\alpha$ -helices traversing a membrane.  
Credit: By Andrej Lomize [GFDL or CC-BY-SA-3.0, via Wikimedia Commons]

- **Beta Sheets** - laterally connected strands or sheets of amino acids occurring from the H-bonds between N-H and C=O groups along the backbone of the protein



- Credit: [Fvasconcellos](#) [Public domain], via Wikimedia Commons
- Credit: [Fvasconcellos](#) [Public domain], via Wikimedia Commons



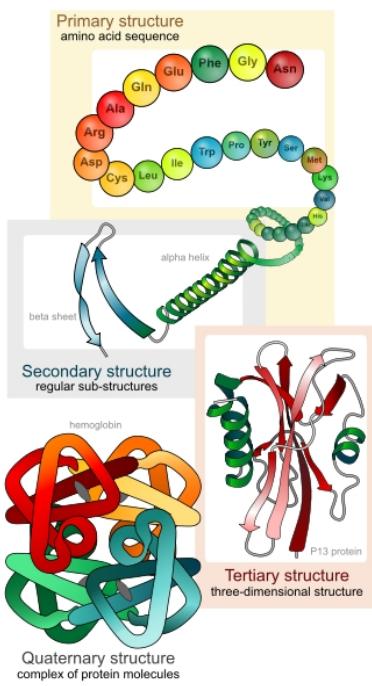
Ribbon diagram of  $\beta$ -sheets

Credit: [Xenonblast](#) [Public domain], via Wikimedia Commons

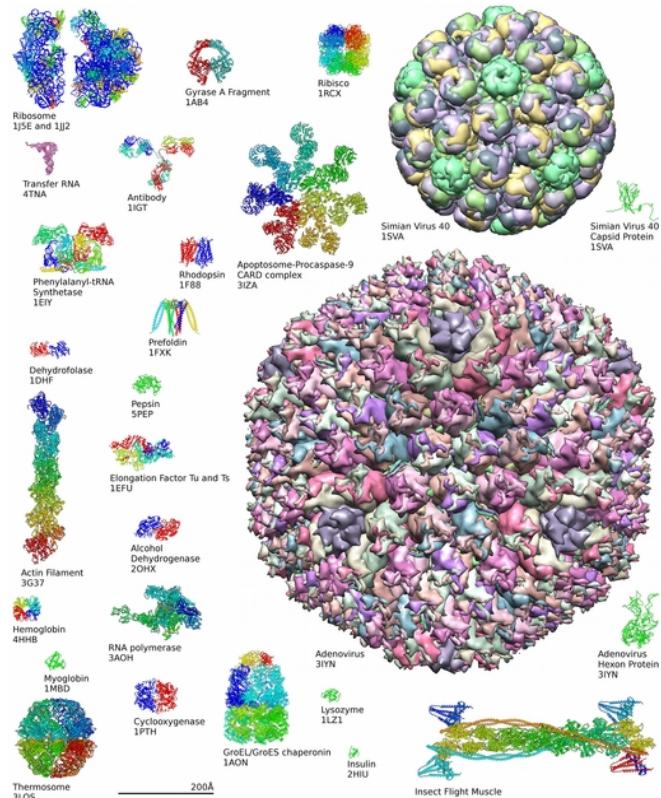
- **Tertiary structure (3°)**: overall 3-D structure of the peptide chain
- **Quaternary structure(4°)**: multimeric protein structure from assembling multiple peptide subunits

## Diversity of Proteins

Learn more about complexity of protein structures at the [Protein Data Bank](#)



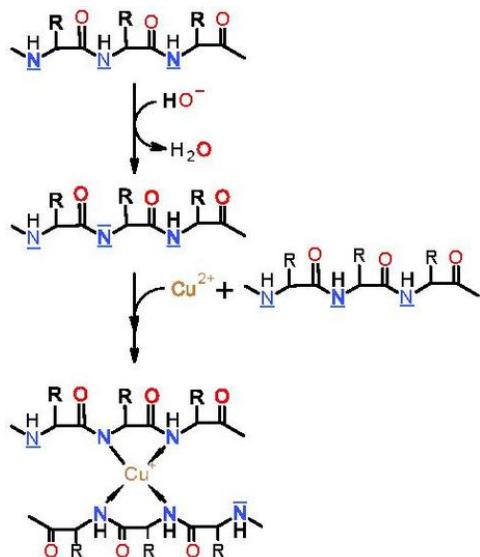
Credit: [LadyofHats](#) [Public domain], via Wikimedia Commons



Credit: [Axel Griewel](#), CC BY-SA 3.0, via Wikimedia Commons

## Protein Detection Theory

Proteins can be detected through the use of the **Biuret test**. Specifically, peptide bonds (C-N bonds) in proteins complex with  $\text{Cu}^{2+}$  in Biuret reagent and produce a violet color. A  $\text{Cu}^{2+}$  must complex with four to six peptide bonds to produce a color; therefore, free amino acids do not positively react. Long polypeptides (proteins) have many peptide bonds and produce a positive reaction to the reagent. **Biuret reagent** is an alkaline solution of 1%  $\text{CuSO}_4$ , copper sulfate. A violet color is a positive test for the presence of protein, and the intensity of color is proportional to the number of peptide bonds in the solution.



*By Ebuxbaum (Own work) [CC BY-SA 3.0]*



## Biuret Test

1. Examine the table below. Indicate if the sample is a negative control, positive control or an experimental.
2. Predict the color change of the solution.
  - Formulate a hypothesis about the components of the experimentals
3. Obtain 6 test tubes and number them 1-6.
4. Add the materials listed in the table.
5. Add 3 drops of Biuret reagent (1.0%  $\text{CuSO}_4$  with NaOH) to each tube and mix
6. Record the color of the tubes' contents in Table

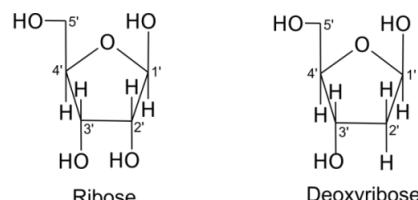
Tube	Solution	Control (+/-) or Experimental	Predicted Color Change	Actual Color Change
1	2 ml urine sample 1			
2	2 ml urine sample 2			
3	2 ml egg albumin			
4	2 ml amino acid solution			
5	2 ml distilled water			
6	2 ml protein solution			

## Conclusions about the Urine Samples

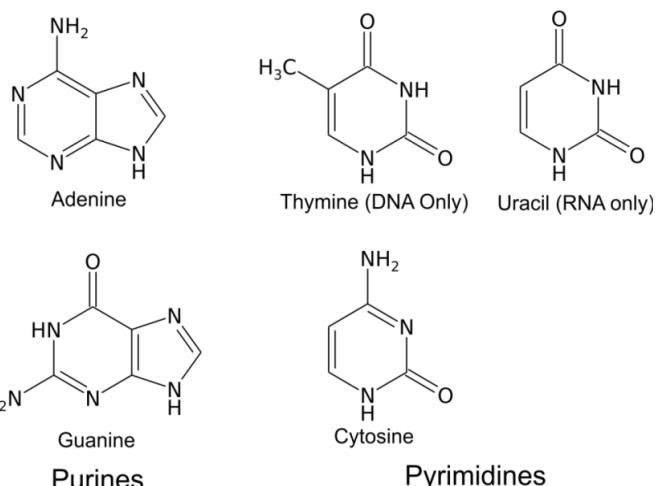
Based on the results of the Benedict's test and the Biuret test, can we make any conclusions?

## Introduction: NUCLEIC ACIDS

DNA and RNA are nucleic acids and make up the genetic instructions of an organism. Their monomers are called **nucleotides**, which are made up of individual subunits. Nucleotides consist of a 5-Carbon sugar (a **pentose**), a charged phosphate and a **nitrogenous base** (Adenine, Guanine, Thymine, Cytosine or Uracil). Each carbon of the pentose has a position designation from 1 through 5. One major difference between DNA and RNA is that DNA contains deoxyribose, and RNA contains ribose. The discriminating feature between these pentoses is at the 2' position where a hydroxyl group in ribose is substituted with a hydrogen.

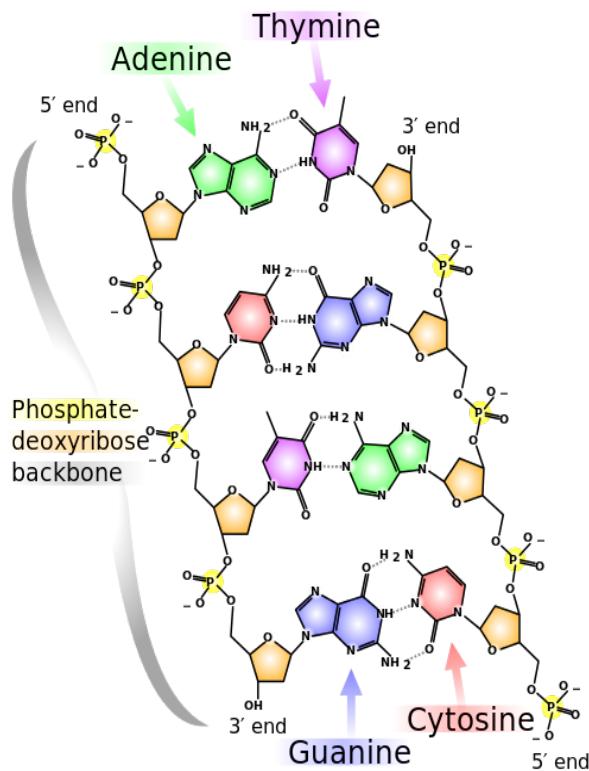


The Two Sugar Subunits Of Nucleic Acids



### The Five Nitrogenous Bases of Nucleic Acids

Nucleic acids are composed of linked nucleotides. DNA includes the sugar, deoxyribose, combined with phosphate groups and combinations of thymine, cytosine, guanine, and adenine. RNA includes the sugar, ribose with phosphate groups and combinations of uracil, cytosine, guanine, and adenine.



DNA has a double helical structure. Two anti-parallel strands are bound by hydrogen bonds.

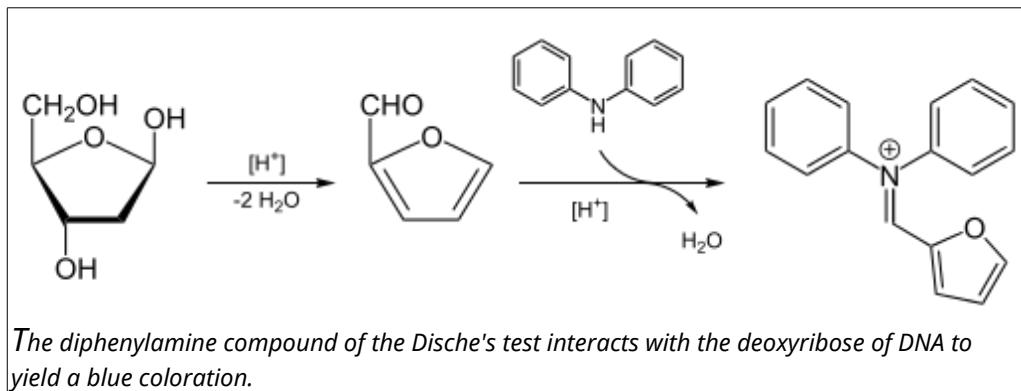
DNA is a double helical molecule. Two anti-parallel strands are bound together by hydrogen bonds. Adenine forms 2 H-bonds with Thymine. Guanine forms 3 H-bonds with Cytosine. This AT & GC matching is referred to as **complementarity**. While the nitrogenous bases are found on the interior of the double helix (like rungs on a ladder), the repeating backbone of pentose sugar and phosphate form the backbone of the molecule. Notice that phosphate has a negative charge. This makes DNA and RNA, overall negatively charged.

## Nucleic Acids: DNA extraction and Dische's Diphenylamine test (Activity)

### Prelab Questions

1. What are fruits?
  - a. Where do they come from?
  - b. What are they made of?
2. Use phylogeny to classify plants (DKPCOFGS)
3. Where is DNA located within the fruits? Where is it located in you?
4. Why would you want to extract DNA from an organism?
5. What class of molecule is DNA?

DNA can be identified chemically with the **Dische diphenylamine test**. Acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA. The Dische's Test will detect the deoxyribose of DNA and will not interact with the ribose in RNA. The amount of blue corresponds to the amount of DNA in solution.

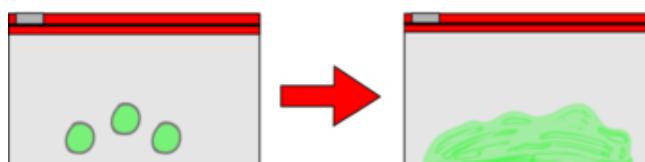


### Methods: Extraction of DNA from fruit

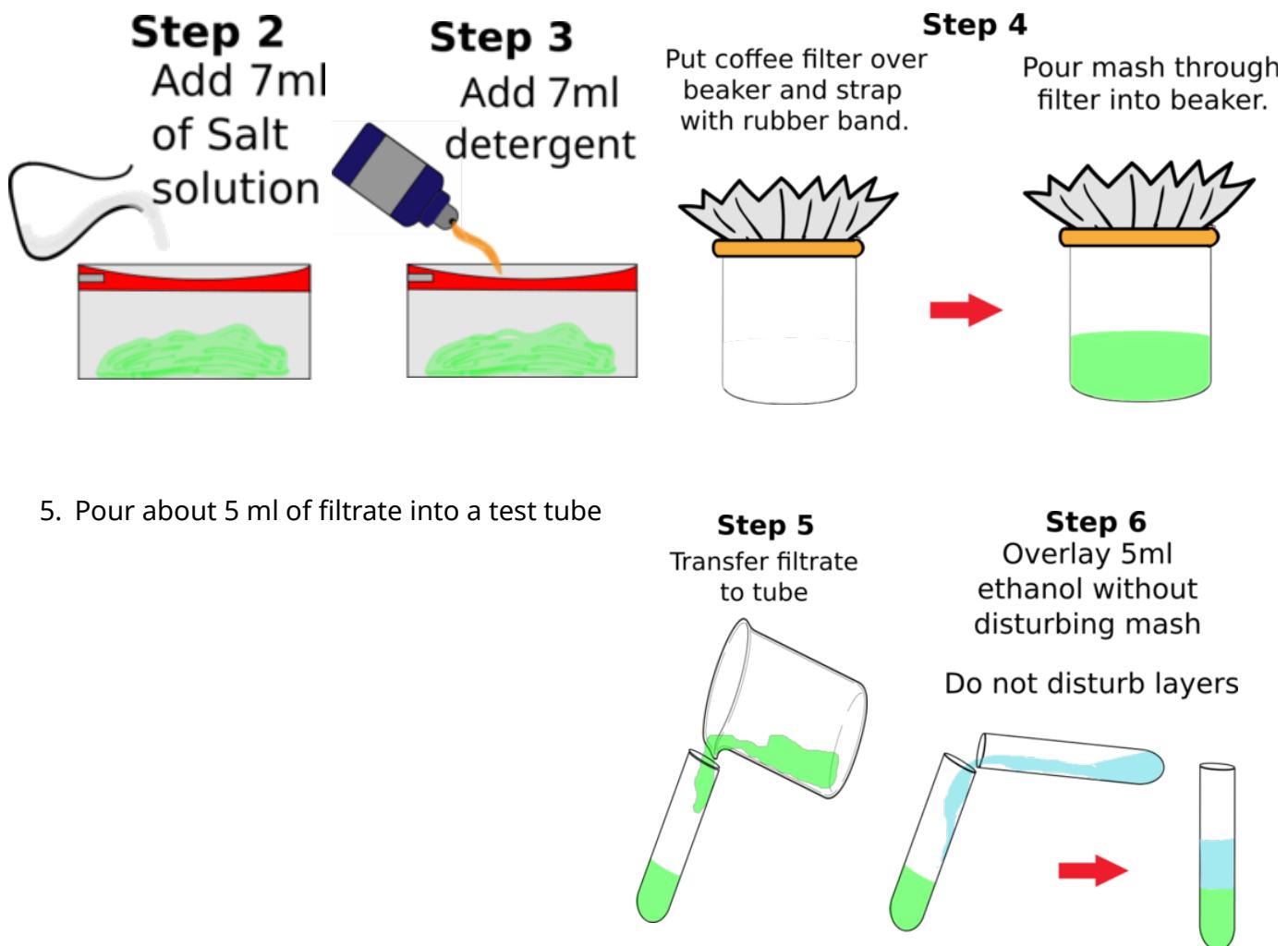
1. Mash about 10g or 3cm of over-ripe banana OR 3 grapes OR 1 strawberry in zip-top bag

#### Step 1

Crush fruit to  
destroy cells



2. Add 7ml of salt solution
  - The salt solution helps the DNA to aggregate (clump together).
3. Add 7 ml of liquid detergent and mix
  - dissolves the lipids in the cell and nuclear membranes
  - releases DNA into the salt solution
4. Place a coffee filter over a cup or beaker and fasten with an elastic band



5. Pour about 5 ml of filtrate into a test tube

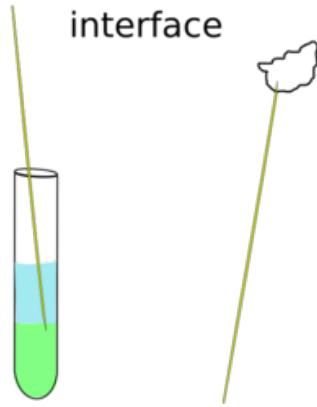
6. Slowly pour an **EQUAL** volume of cold ethanol down the side of tube to form a layer on top of the fruit fluid.

- carefully run the alcohol down the side to form a separate layer on top of the fruit solution
- Do not mix the alcohol and banana solution.
- Ice-cold 100% ethanol works best

7. Spool the DNA: use a plastic loop or glass rod to gently swirl at the interface of the two solutions

**Step 7**

Use rod to spool  
DNA at  
interface

**Step 8**

Transfer DNA  
to empty tube



- the interface is where the two solutions meet
- DNA is not soluble in alcohol
- bubbles may form around a wooly substance (this is the DNA)

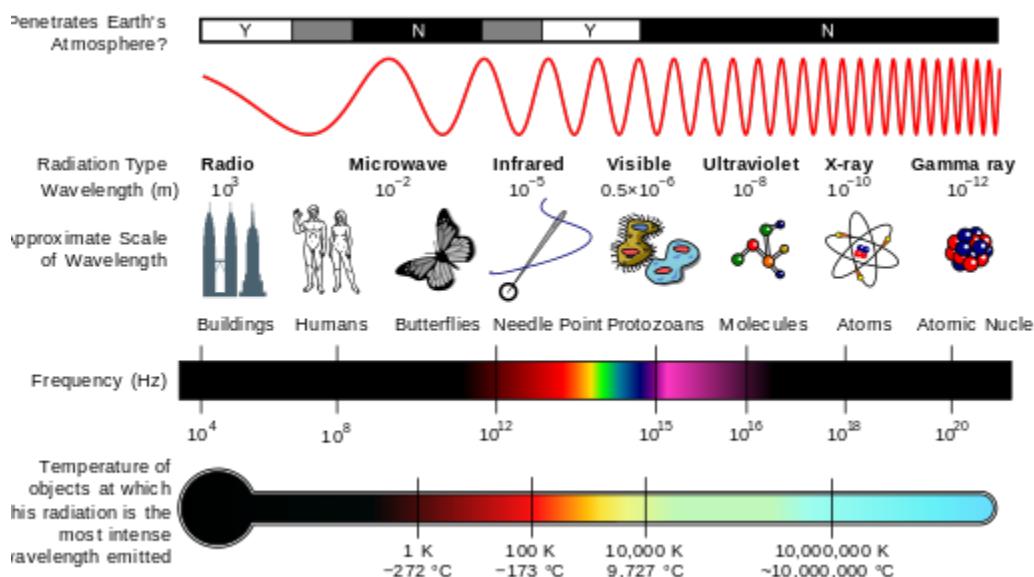
8. Transfer the DNA

## Dische Diphenylamine Test For DNA

1. Obtain 3 test tubes and number them 1-3.
2. Suspend the spooled DNA in 3 ml of distilled water. MIX.
3. Add to tubes:
  1. 2 ml of DNA solution
  2. 1 ml of DNA solution with 1 ml H<sub>2</sub>O
  3. 2 ml of H<sub>2</sub>O
4. Add 2 ml of the Dische's diphenylamine reagent to each tube and mix thoroughly.
5. Place in a boiling water bath for 10 minutes.
6. Evaluate your results. A clear tube indicates no nucleic acids. A blue color indicates the presence of DNA. A greenish color indicates the presence of RNA.

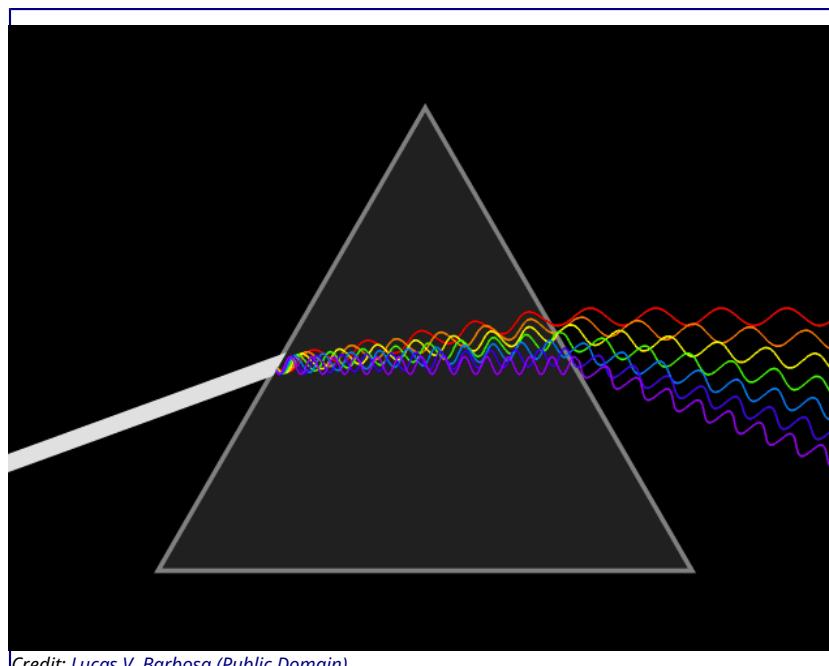
## Properties of Light

Light is a type of energy that travels as a wave-particle. The **wavelength** of light is the distances between peaks in the waves as light travels. Wavelengths are measured in nanometers (nm) and different wavelengths of light represent differing colors. White light is a mixture of the visible light **spectrum**. Light of long wavelengths (infra-red) and very short wavelengths (ultra violet) are invisible to humans but can be observed by other organisms. As wavelength decreases, the energy of the light is increased.



Credit: By Inductiveload, NASA GFDL or CC-BY-SA-3.0

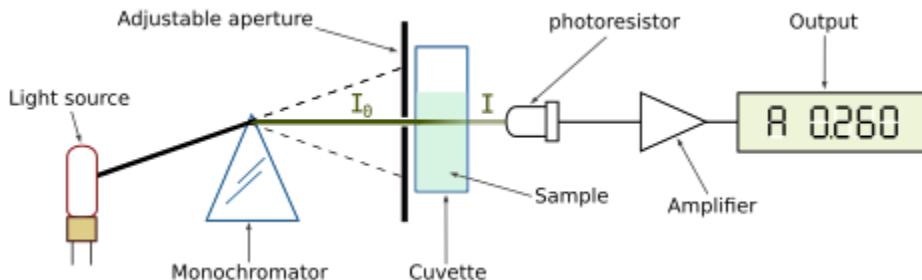
Diffraction of light through a prism exposes the components wavelengths of light.



Credit: Lucas V. Barbosa (Public Domain)

## Spectrophotometry

**Spectrophotometers** (*spectro*-image/color ; *photo*-light ; *meter*-measure) are used for chemical analysis of solutions based on properties of absorption or transmission.



*Schematic of a spectrophotometer. The monochromator is a prism that splits the light. A single wave-length of light is focused through the aperture to pass through the solution in the cuvette.*

Credit: [GyassineMrabetTalk](#) [GFDL or CC BY-SA]

**Transmittance** refers to the amount of light that passes through the solution.

$$T = \frac{I}{I_0}$$

Transmittance of a light source through a cuvette. The intensity of light,  $I_0$ , decreases as it passes through the solution. The light detected by the sensor,  $I$ , reflects the transmittance of the solution. If light is being absorbed by chemicals in the solution, this results in a lower transmission. **Absorbance** is therefore inversely related to transmittance as expressed by the equation:

$$A = -\log_{10} T$$

Follow the virtual demonstration at: <http://www.virtual-labs.leeds.ac.uk/pres/spectrophotometry/> (CC-BY-NC-SA) for a more in-depth explanation of spectrophotometry.

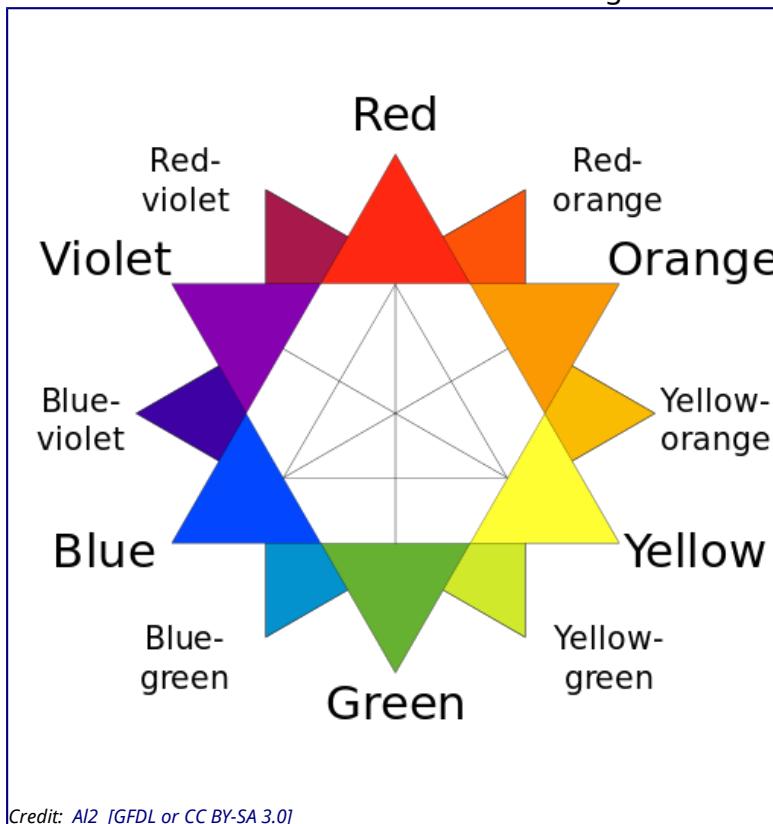
## Beer's Law

**Beer's Law** is a relationship between the concentration or amount of a dissolved substance in a solution that is reducing the amount of transmitted light due to the absorption of the radiant energy. **Lambert's Law** states that the reduction of transmittance was related to the length of the path of light. As the light path increases through a substance, there is a reduction in transmittance. Collectively, these ideas are referred to as **Beer-Lambert Law**, but most observers will control the path length and simply refer to it as Beer's Law.

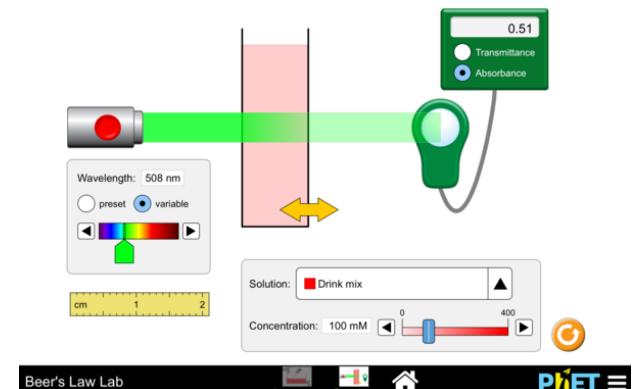
## Exploring Beer's Law (virtual)

Use the [link below](#) to launch a simulation where you can alter the properties involved in spectrophotometry and examine the Beer-Lambert Law. Increase and decrease the concentration slider in the simulation:

1. What happens to the contents in the cuvette?
2. How does this change the Transmittance and Absorbance readings?
3. Click "variable" and use the slider. What happens to the readings when the Wavelength of the laser is a similar color as the solution in the cuvette?
4. Consult the color star below and find the color wavelength that is opposite of the color of the solution. Set the laser to this color using "variable" and the slider.



Credit: [Al2 \[GFDL or CC BY-SA 3.0\]](#)



[Follow link to launch simulation](#)

1. What is the effect on Transmittance and Absorbance with this color?
2. Using the previous observations (using variable wavelength slider), how would you use the relationship on Transmittance/Absorbance to best measure the concentration of a solution?

## Experimental Background

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

## Dilute BSA Standards

1. Label 9 tubes 1-9
2. Combine the components of the table below to generate appropriate concentration of solutions

	BSA Standard Dilution						Blank	Unknowns	
	1 1.0 mg/ml	2 0.8 mg/ml	3 0.6 mg/ml	4 0.4 mg/ml	5 0.2 mg/ml	6 0.1 mg/ml	7 0 mg/ml	8 ? mg/ml	9 ? mg/ml
ml BSA	1	0.8	0.6	0.4	0.2	0.1	0	-	-
ml H <sub>2</sub> O	0	0.2	0.4	0.6	0.8	0.9	1	-	-
ml unknown	-	-	-	-	-	-	-	1	1
ml Biuret	4	4	4	4	4	4	4	4	4

3. Place tube 7 (**blank**) into a cuvette and measure absorbance (**A**) in the spectrophotometer at 540 nm
4. Calibrate the spectrophotometer to read 0 at  $A_{540\text{nm}}$
5. Sequentially read each sample at  $A_{540\text{nm}}$  and record values in table
6. Plot each BSA dilution in a spreadsheet program like Excel as a scatterplot
7. Generate best-fit line for these standards with the equation of the line
8. Use the equation of the line to estimate the concentration of the unknown sample.

Tube #	BSA (mg/ml)	$A_{540}$
1	1.0	
2	0.8	
3	0.6	
4	0.4	
5	0.2	
6	0.1	
7	0.0	
Do not plot the values below. Use the plot to estimate the concentrations.		
8	?	
9	?	

## Curve Fitting

Run the [simulation](#) to understand how you can use the standard dilution series to estimate your sample concentrations.

## Scatterplot Tutorial

[Use the tutorial](#) and watch at 1.25X to plot your own data.

## Experimental Background

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

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1. Label 9 tubes 1-9
2. Combine the components of the table below to generate appropriate concentration of solutions

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	1 1.0 mg/ml	2 0.8 mg/ml	3 0.6 mg/ml	4 0.4 mg/ml	5 0.2 mg/ml	6 0.1 mg/ml		8 ? mg/ml	9 ? mg/ml
ml BSA	1	0.8	0.6	0.4	0.2	0.1	0	-	-
ml H <sub>2</sub> O	0	0.2	0.4	0.6	0.8	0.9	1	-	-
ml unknown	-	-	-	-	-	-	-	1	1
ml Biuret	4	4	4	4	4	4	4	4	4

3. Place tube 1 (**1mg/ml**) into a cuvette for measuring absorbance (**A**) in the SpectroVis Plus. This will find the peak absorbance value.
4. The instructor will begin to set-up the units for distribution
5. Enter the LabQuest 2 application and press on the green Start button to generate a full spectrum
  - tap on the file cabinet icon to store this data
6. On the Meter Screen, tap on **Mode**
  1. Change the mode to "Events with Entry"
  2. Enter the Name: Concentration
  3. Enter Units: mg/ml
  4. Select OK
  5. If message appears about saving run, choose Discard
7. Sequentially read each sample at the stored wavelength (between **A<sub>540nm</sub>-A<sub>600nm</sub>**) and record values in table below

1. Plot each BSA dilution in [plot.ly](#) as a scatterplot
2. Generate best-fit line for these standards with the equation of the line
3. Use the equation of the line to estimate the concentration of the unknown sample.

Tube #	BSA (mg/ml)	$A_{540}$
1	1.0	
2	0.8	
3	0.6	
4	0.4	
5	0.2	
6	0.1	
7	0.0	
Do not plot the values below. Use the plot to estimate the concentrations.		
8	?	
9	?	

## LabQuest2 and SpectroVis Tutorial

<https://youtu.be/8ZVYkMW03Rs>

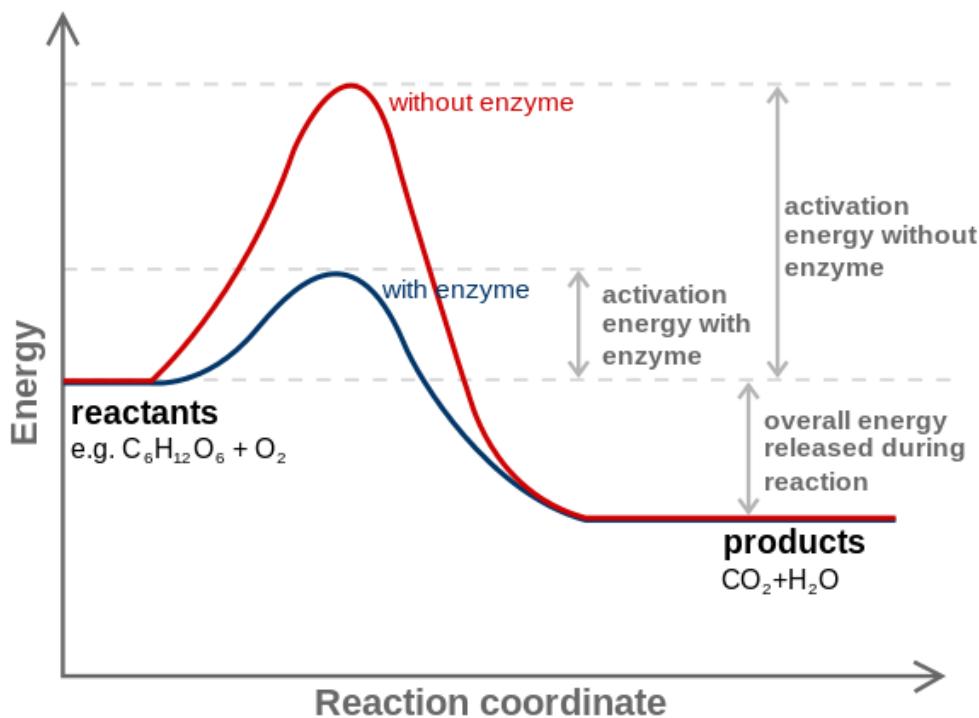
## Scatterplot Tutorial

You can watch this tutorial at 1.25X and pause when needed.

<https://youtu.be/jeRMxXvbI7g>

## Energy and catalysts

In Biological systems, **energy** is roughly defined as the capacity to do work. Molecules are held together by electrons. Breaking and building these bonds requires an input of energy. The energy needed to initiate such reactions is referred to as **activation energy ( $E_A$ )**. Sometimes the necessary energy to initiate a reaction is so great, that it greatly limits the likelihood of the reaction ever occurring. **Catalysts** are chemicals that take part in facilitating reactions by reducing the energy of activation. If the activation energy is reduced, the likelihood of a reaction occurring is greatly enhanced. In cells, the catalysts are often made of proteins and called **enzymes**.



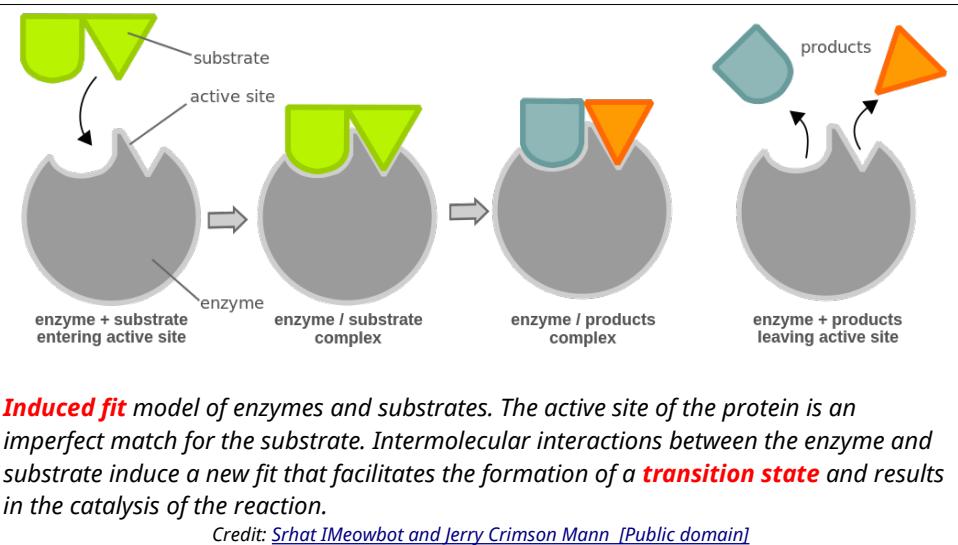
Reaction coordinate of an **exothermic** reaction with and without an enzyme. The enzyme reduced the  $E_A$  to facilitate the likelihood that the reaction occurs. This **catabolic** reaction breaks complex things down, thus increasing entropy and releasing energy into the system.

Credit: [\[en:User:\]](#) /GFDL or CC-BY-SA-3.0

## Enzymes

Reactants in enzymatic reactions are called **substrates**. They have an imperfect fit to a binding domain of the enzyme called the **active site**. Substrate binding to this active site induces a change in the shape of the protein that coordinates the substrate into a transition state that will reduce the amount of  $E_A$  required for the reaction to go to completion. The **induced fit** of the protein also aids in coordinating other cofactors or **coenzymes** that will aid in the reaction.

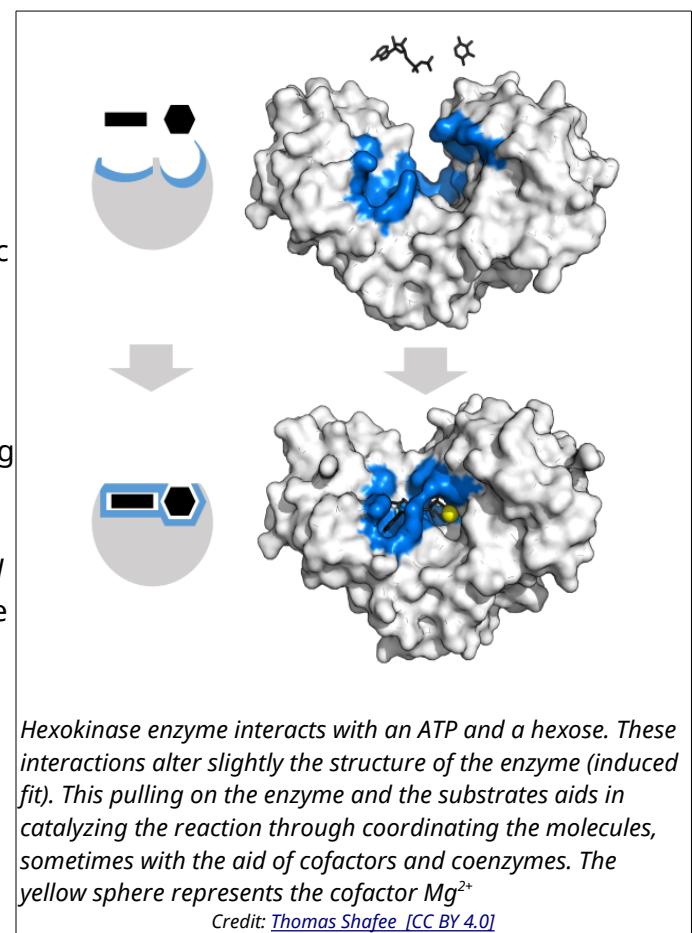
The reaction follows the standard flow where the Enzyme (E) and the Substrate (S) interact to form an Enzyme-Substrate Complex (ES). The ES then dissociates into Enzyme and the resultant Product (P)



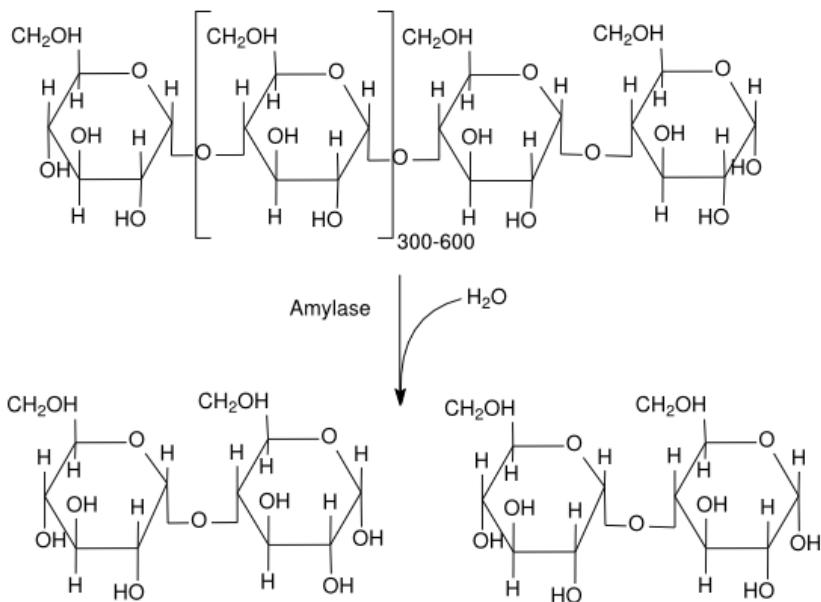
The induced fit of the enzyme-substrate complex coordinates the transition state to facilitate the reaction. This induced fit occurs through non-covalent means that result in a tugging on the molecules (an application of energy) while molecules are coaxed into the reactions.

Coenzymes can be covalently linked to amino acid side chains of the enzyme and are also referred to as **prosthetic groups**. While prosthetic groups are organic in nature, they may also involve the coordination of metal ions, like the heme group which binds to iron. These prosthetic groups enhance the repertoire of the amino acids to provide additional functioning to the entire protein. Early coenzymes were described as being vital to normal functioning and were characterized as organic molecules with amine groups. Because of this coincidence, they were referred to as **vitamins** (for **vital amines**) though not all vitamins have amine groups. The trace metal ions that work with these groups are also required and represent the minerals on food items.

<https://youtu.be/yk14dOOvwMk>



## The Enzyme



**Amylase** is an enzyme that breaks down amylose (starch) into glucose molecules.

1. What test can be used to indicate the presence of Starch?
  2. What test can be used to indicate the presence of glucose?
  3. What is the role of an enzyme in a chemical reaction and what is it made of?
  4. What parameters would influence the ability of the enzyme to facilitate the rate of the reaction?



*Salivary amylase is produced in the mouth, where digestion begins.*



Pancreatic amylase is produced in the pancreas and is supplied to the duodenum of the small intestines.



## Collect Data from the Following Experiments and Plot the results

1. Using a [plot.ly](#), plot each experimental dataset on the same chart. (4 charts)
2. Log-in to Plot.ly using Google, Twitter or Facebook credentials
3. Create scatterplots. Calculate the line of best fit of each data condition.
4. The slope represents the activity of the enzyme in each condition. What is the unit of this activity?

## Effect of Temperature on Enzyme Activity

1. Add 5 ml of H<sub>2</sub>O to a tube (this is the BLANK)
2. Add 5 ml of starch (substrate) to 3 separate tubes
  - one on ice (0°C), one on the bench (25°C) and one in a 40°C water bath
3. Add 2 drops of iodine to each tube and mix: Blank, 0°C, 25°C, 40°C
4. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm
5. Read each tube in the spectrophotometer. This is time 0 min.
6. Add 35 drops of amylase solution to each tube simultaneously, mix and ensure incubation is occurring at the correct temperature.
7. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer and immediately return the tubes to the proper temperature.
8. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	0°C % Transmittance	20°C % Transmittance	40°C % Transmittance
0			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			
22			

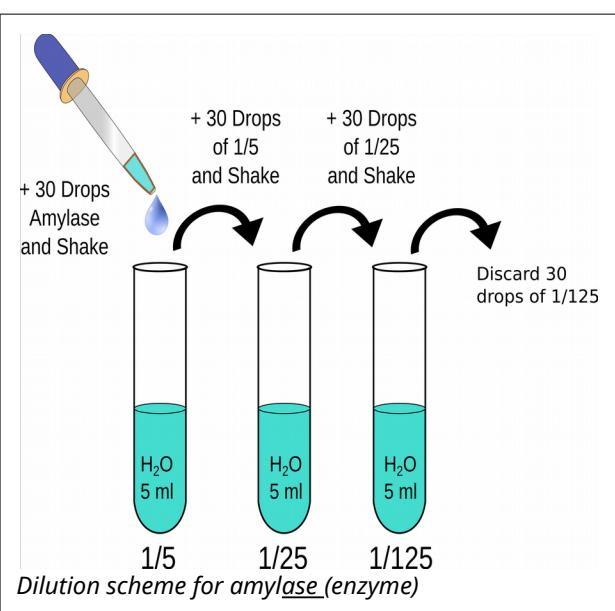
## Effect of pH on Enzyme Activity

1. Add 5 ml of water to an empty tube (this is the BLANK)
2. To 3 separate tubes, add 2.5 ml of buffer pH 3, pH 5 or pH 7
  1. Add 2.5 ml of starch (substrate) to each of these tubes (excluding BLANK)
3. Add 2 drops of iodine to each tube and mix: Blank, pH 3, pH 5, pH 7
4. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm
5. Read each tube in the spectrophotometer. This is time 0 min.
6. Add 35 drops of amylase solution to each tube simultaneously, mix to homogeneity.
7. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.
8. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	pH 3 % Transmittance	pH 5 % Transmittance	pH 7 % Transmittance
0			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			
22			

## Effect of Enzyme Concentration on Enzyme Activity

1. Add 5 ml of water to an empty tube (this is the BLANK)
2. Add 5 ml of pH 7 buffer to 3 separate tubes
3. Follow the dilution scheme below:
4. In 4 separate tubes, ADD 4 ml of starch solution label them 1x, 1/5x, 1/25x, 1/125x
5. Add 2 drops of iodine to each starch tube and the Blank
6. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm
7. Read each tube in the spectrophotometer. This is time 0 min
8. Add 35 drops of diluted amylase solutions to the appropriately labeled tubes simultaneously and mix.
9. Each tube receives a different Amylase dilution
10. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.

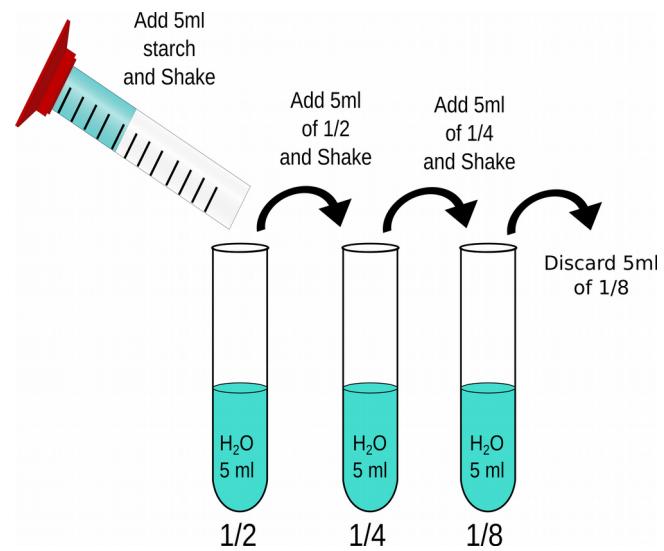


11. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	1X % Transmittance	1/5X % Transmittance	1/25X % Transmittance	1/125X % Transmittance
0				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				
22				

## Effect of Substrate Concentration on Enzyme Activity

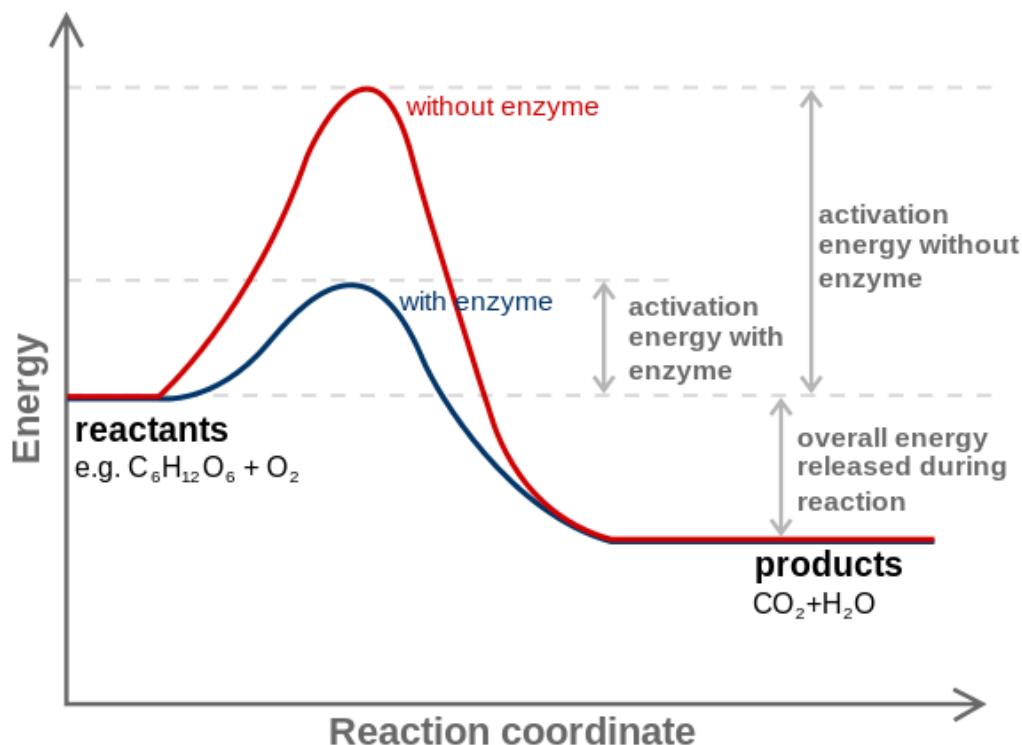
1. Add 5 ml of water to an empty tube (this is the BLANK)
2. Add 5 ml of pH 7 buffer to 3 separate tubes
3. Follow the dilution scheme below:
4. In 4 cuvettes, ADD 4 ml of the diluted starch solution
5. label them 1x, 1/2x, 1/4x, 1/8x
6. Add 2 drops of iodine to each starch tube and the Blank
7. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm
8. Read each tube in the spectrophotometer. This is time 0 min
9. Add 35 drops of amylase solution to each tube simultaneously and mix.
10. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.
11. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.



Time (min)	1X % Transmittance	1/2X % Transmittance	1/4X % Transmittance	1/8X % Transmittance
0				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				
22				

## Energy and catalysts

In Biological systems, **energy** is roughly defined as the capacity to do work. Molecules are held together by electrons. Breaking and building these bonds requires an input of energy. The energy needed to initiate such reactions is referred to as **activation energy ( $E_A$ )**. Sometimes the necessary energy to initiate a reaction is so great, that it greatly limits the likelihood of the reaction ever occurring. **Catalysts** are chemicals that take part in facilitating reactions by reducing the energy of activation. If the activation energy is reduced, the likelihood of a reaction occurring is greatly enhanced. In cells, the catalysts are often made of proteins and called **enzymes**.

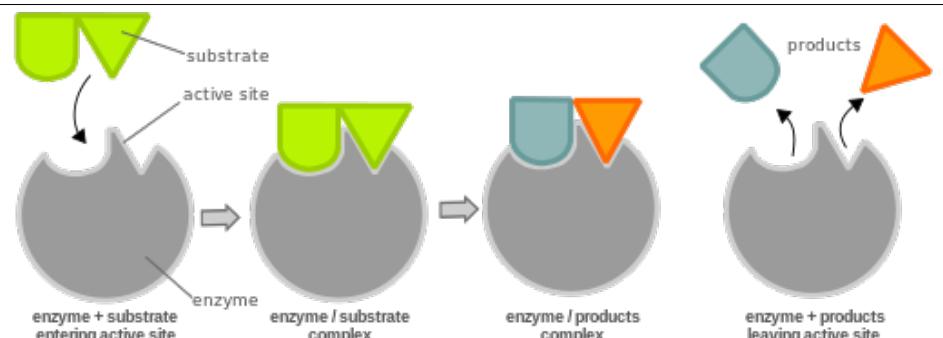


Reaction coordinate of an **exothermic** reaction with and without an enzyme. The enzyme reduced the  $E_A$  to facilitate the likelihood that the reaction occurs. This **catabolic** reaction breaks complex things down, thus increasing entropy and releasing energy into the system.

## Enzymes

Reactants in enzymatic reactions are called **substrates**. They have an imperfect fit to a binding domain of the enzyme called the **active site**. Substrate binding to this active site induces a change in the shape of the protein that coordinates the substrate into a transition state that will reduce the amount of  $E_A$  required for the reaction to go to completion. The **induced fit** of the protein also aids in coordinating other cofactors or **coenzymes** that will aid in the reaction.

The reaction follows the standard flow where the Enzyme (E) and the Substrate (S) interact to form an Enzyme-Substrate Complex (ES). The ES then dissociates into Enzyme and the resultant Product (P)

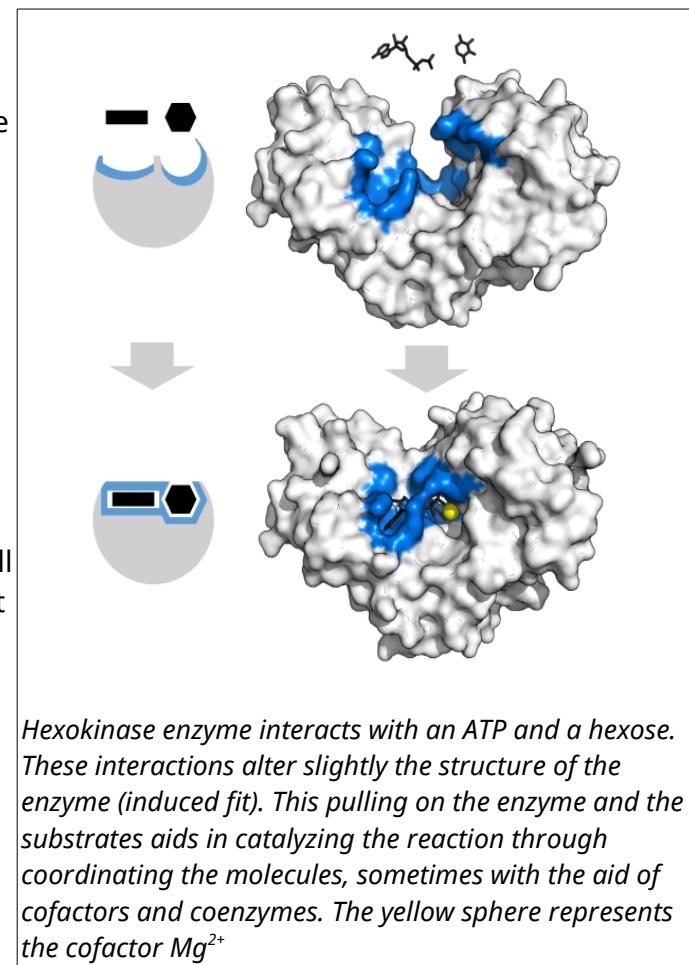


**Induced fit** model of enzymes and substrates. The active site of the protein is an imperfect match for the substrate. Intermolecular interactions between the enzyme and substrate induce a new fit that facilitates the formation of a **transition state** and results in the catalysis of the reaction.

The induced fit of the enzyme-substrate complex coordinates the transition state to facilitate the reaction. This induced fit occurs through non-covalent means that result in a tugging on the molecules (an application of energy) while molecules are coaxed into the reactions.

Coenzymes can be covalently linked to amino acid side chains of the enzyme and are also referred to as **prosthetic groups**. While prosthetic groups are organic in nature, they may also involve the coordination of metal ions, like the heme group which binds to iron. These prosthetic groups enhance the repertoire of the amino acids to provide additional functioning to the entire protein. Early coenzymes were described as being vital to normal functioning and were characterized as organic molecules with amine groups. Because of this coincidence, they were referred to as **vitamins** (for *vital amines*) though not all vitamins have amine groups. The trace metal ions that work with these groups are also required and represent the minerals on food items.

<https://youtu.be/yk14dOOvwMk>



## Enzyme kinetics of Turnip Peroxidase

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a strong oxidizing agent that can damage cells and is formed as a by-product of oxygen consumption. Fortunately, aerobic cells contain peroxidases that break down peroxide into water and oxygen. This enzyme reduces hydrogen peroxide into  $\text{H}_2\text{O}$ .



This exercise uses turnip extract as a source of peroxidase. This turnip extract requires a source of electrons (a reducing agent) in order for the reaction to occur. In this case, a colorless organic compound called **guaiacol** is used. Guaiacol is oxidized in the process of converting the peroxide and becomes brown. Enzymatic activity can then be traced using a spectrophotometer to measure the amount of brown being formed.

### Set-up and Calibrate LabQuest with SpectroVis Plus

1. Set-up a cuvette to serve as a Blank
  1. Add 10 drops 0.02% hydrogen peroxide
  2. Add 5 drops 0.2% guaiacol
  3. Add 20 drops of pH 7 buffer
  4. Add 10 drops extraction buffer
2. Connect a LabQuest2 to a SpectroVis plus and start the data collection program
3. Calibrate the SpectroVis
  1. Change Mode
    1. Select "Time-Based" from the dropdown menu
      - Rate: 0.5 samples/s
      - Interval: 2 s/sample
      - Duration: 200 s
    2. OK
  2. Press Play button (green arrow)
    1. Perform Warm-up for 90 seconds
      1. Choose "Finish Calibration"
      2. Press "OK"
    2. Press Stop button "Red Square"
4. Select "Meter Icon" (top left most of display)
  1. Press the large red area showing the current absorbance reading
  2. Choose "Change Wavelength"
  3. Set Wavelength to 500nm
5. Proceed to the individual exercises

## Effect of pH on Peroxidase Activity

1. Set-up cuvette for pH 3
  1. Add 10 drops 0.02% hydrogen peroxide
  2. Add 5 drops 0.2% guaiacol
  3. Add 20 drops of pH 3 buffer
  4. Add 10 drops turnip extraction last
  5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
  6. Press play button to begin recording data (choose “discard data” if prompted)
    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
    4. Press OK
    5. Press the “File Cabinet” icon to temporarily save the run
    6. Press the “Play” button
    7. Continue with calibration, then skip the warm-up
    8. Repeat the exercise with the next condition and press “Finish Calibration” when the next condition is ready for reading
2. Sequentially repeat the experiment exchanging the pH buffer with pH 5, 7, 10



Credit: [Nadina Wiórkiewicz](#)

## Effect of Temperature on Peroxidase Activity

1. Buffers are incubated on ice (0°C), at room temperature (20°C), in 40°C or 60°C
2. Set-up cuvette for 0°C
  1. Add 10 drops 0.02% hydrogen peroxide (incubated at 0°C for 10 minutes)
  2. Add 5 drops 0.2% guaiacol
  3. Add 20 drops of extraction buffer at 0°C
  4. Add 10 drops turnip extraction (incubated at 0°C for 10 minutes) last
  5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
  6. Press play button to begin recording data (choose “discard data” if prompted)
    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
    4. Press OK
    5. Press the “File Cabinet” icon to temporarily save the run
    6. Press the “Play” button
    7. Continue with calibration, then skip the warm-up
    8. Repeat the exercise with the next condition and press “Finish Calibration” when the next condition is ready for reading
3. Sequentially repeat the experiment exchanging the buffers stored at 20°C, 40°C, 60°C

## Effect of Substrate Concentration on Peroxidase Activity

1. Set-up cuvette for 1X substrate
  1. Add 10 drops 0.02% hydrogen peroxide
  2. Add 5 drops 0.2% guaiacol
  3. Add 20 drops of extraction buffer
  4. Add 10 drops turnip extraction last
  5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
  6. Press play button to begin recording data (choose “discard data” if prompted)
    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
    4. Press OK
    5. Press the “File Cabinet” icon to temporarily save the run
    6. Press the “Play” button
    7. Continue with calibration, then skip the warm-up
    8. Repeat the exercise with the next condition and press “Finish Calibration” when the next condition is ready for reading
2. Sequentially repeat the experiment with differing amounts of buffer and peroxide:
  1. Repeat the experiment with 0.2X substrate and export data to USB drive
  2. Repeat the experiment with 2X substrate and export data to USB drive
  3. Repeat the experiment with 3X substrate and export data to USB drive

	<b>0.2X substrate</b>	<b>1X substrate</b>	<b>2X substrate</b>	<b>3X substrate</b>
<b>Drops Peroxide</b>	2	<b>10</b>	20	30
<b>Drops Guaiacol</b>	5	<b>5</b>	5	5
<b>Drops Extraction Buffer</b>	28	<b>20</b>	10	0
<b>Drops Extract</b>	10	<b>10</b>	10	10

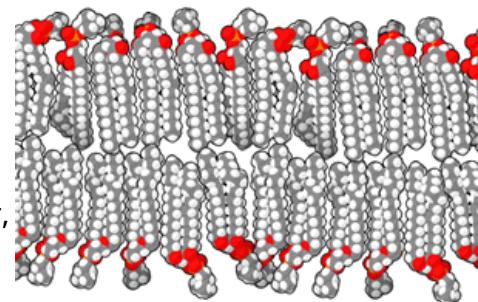
## Effect of Enzyme Concentration on Peroxidase Activity

1. Set-up cuvette for 1X enzyme
  1. Add 10 drops 0.02% hydrogen peroxide
  2. Add 5 drops 0.2% guaiacol
  3. Add 20 drops of extraction buffer
  4. Add 10 drops turnip extraction last
  5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus
  6. Press play button to begin recording data (choose “discard data” if prompted)
    1. After 200s, remove SpectroVis from USB
    2. Insert a USB flash drive and wait 1 minute
    3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name
    4. Press OK
    5. Press the “File Cabinet” icon to temporarily save the run
    6. Press the “Play” button
    7. Continue with calibration, then skip the warm-up
    8. Repeat the exercise with the next condition and press “Finish Calibration” when the next condition is ready for reading
2. Sequentially repeat the experiment with differing amounts of buffer and extract:
  1. Perform the experiment on 1X enzyme and export data to USB drive
  2. Repeat the experiment with 0.2X enzyme and export data to USB drive
  3. Repeat the experiment with 2X enzyme and export data to USB drive
  4. Repeat the experiment with 3X enzyme and export data to USB drive

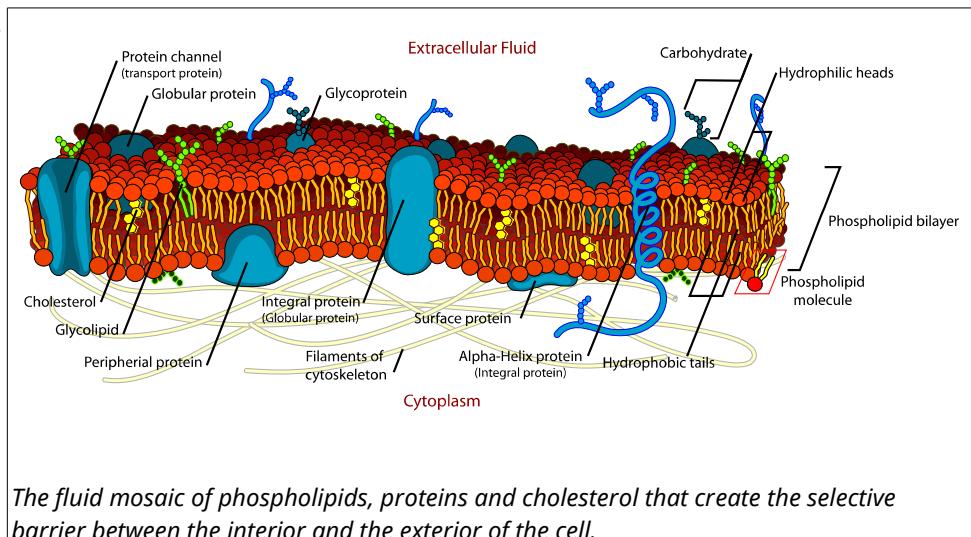
	0.2X enzyme	1X enzyme	2X enzyme	3X enzyme
Drops Peroxide	10	10	10	10
Drops Guaiacol	5	5	5	5
Drops Extraction Buffer	28	20	10	0
Drops Extract	2	10	20	30

## Understanding Membranes

The cell membrane is the barrier that separates the cytoplasm from the external world. The cell membrane consists primarily of phospholipids in a bilayer. Phospholipids are amphipathic with a polar head (phosphate group) and a hydrophobic tail (2 hydrocarbon chains). Due to the chemical properties of the heads being attracted to water and the tails having a desire to avoid water, phospholipids self assemble into micelles. Cell membranes form from a phospholipid bilayer where the lipid tails interact with each other and the phosphate heads face the external water environment or the internal cytoplasm of the cell.

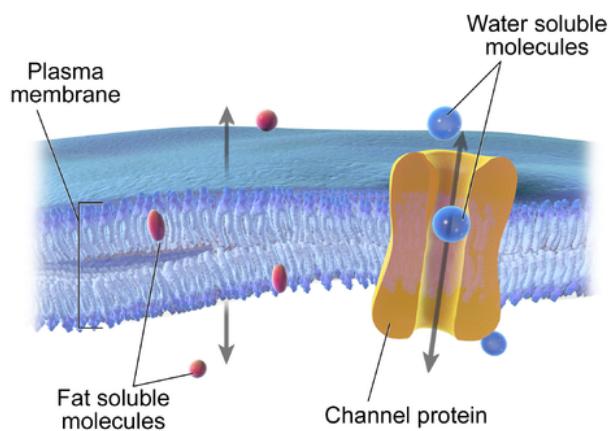


The cell membrane does not solely consist of phospholipids but also have proteins and cholesterol inserted into the bilayer. As the image of the bilayer above indicates, the molecules are constantly moving and flow in a lateral motion. Cholesterol modulates the fluidity of this motion. Proteins associated with the membrane may sit on either side (**peripheral proteins**) of the membrane or pass through both layers of the membrane



(**transmembrane proteins**). The model that describes the components of the cellular membrane is referred to as the **Fluid Mosaic Model**. This model states that the cell membrane is a mosaic of 1)Phospholipids 2)Proteins 3) cholesterol that move about in a side to side motion.

Small uncharged molecules pass through the double layer of phospholipids. Polar, charged or large molecules have great difficulty passing through the membrane and require the aid of transmembrane proteins. An example of a transmembrane protein that facilitates movement of a polar substance is aquaporin, which permits the free movement of water.

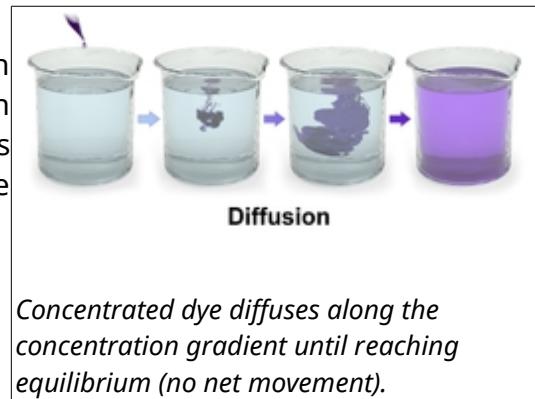


## Diffusion Across the Plasma Membrane

## Diffusion

**Diffusion** is the net movement of a substance from high concentration to low concentration. This difference in concentration is referred to as a **concentration gradient**. This movement does not require any external energy, but uses the free energy intrinsic to the system.

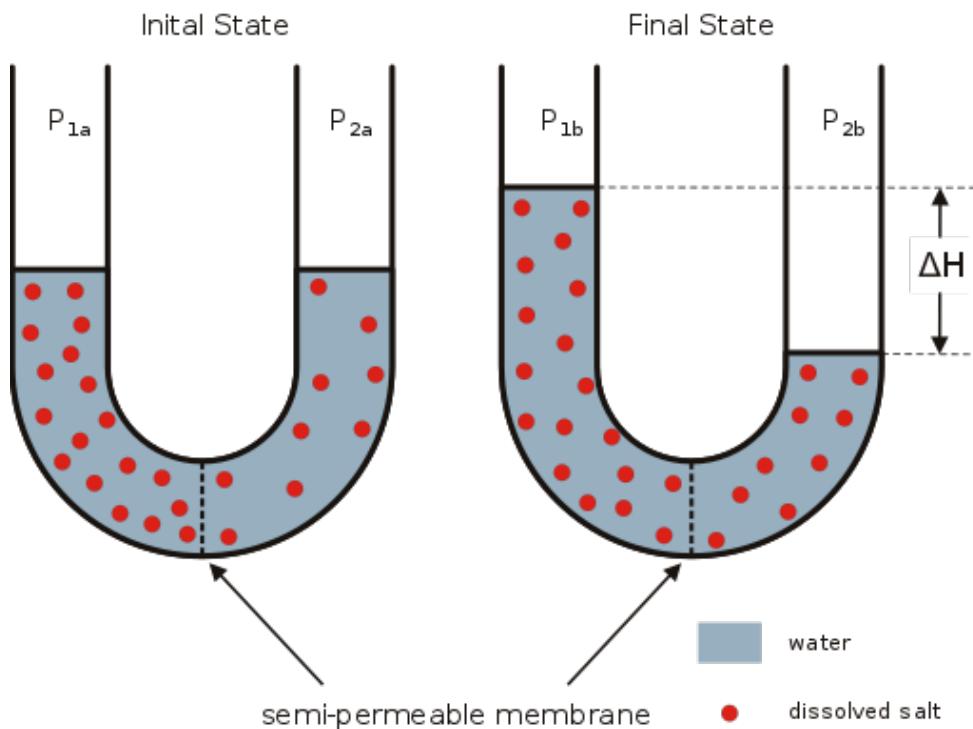
- Temperature Effects on Diffusion
  - [Temperature/Diffusion Simulation](#)
- Molecular Mass Effects on Diffusion
  - [Size/Mass/Diffusion Simulation](#)



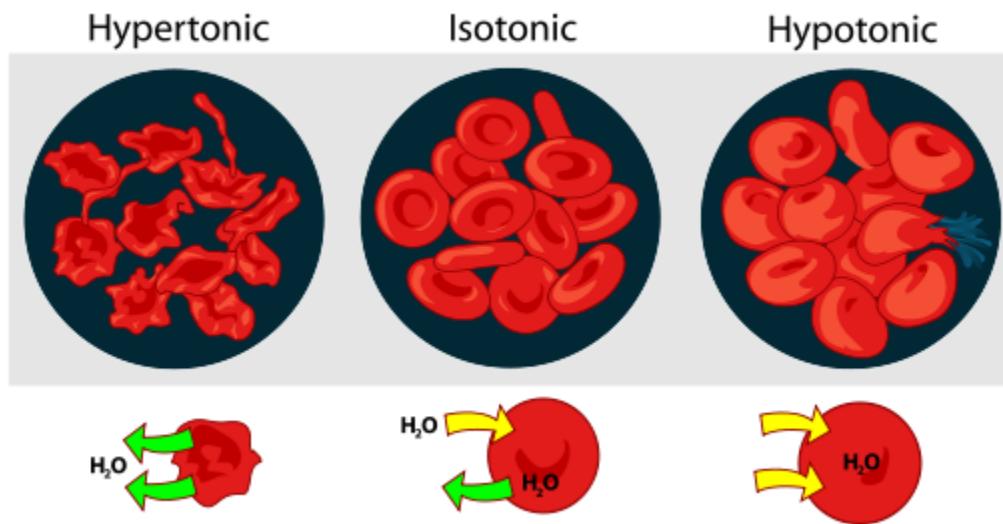
## Osmosis

**Osmosis** is a special case of diffusion. Instead of observing the net change in solute, osmosis follows the net movement of solvent across a **semipermeable membrane**. Since a semi-permeable membrane permits specific things to pass through, some solutes are partitioned.

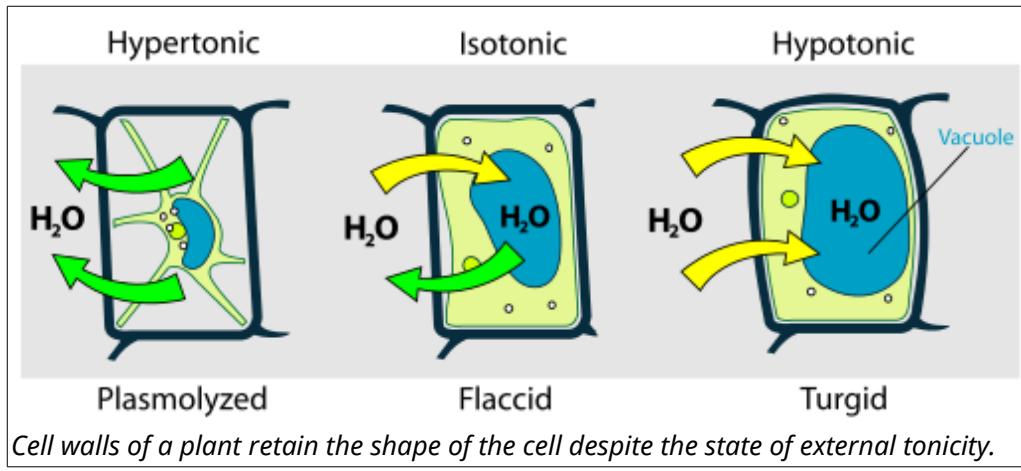
- [Simulation of diffusion through a semipermeable membrane](#) (CC-BY 4.0 Concord Consortium)



A cell lacking a cell wall is affected greatly by the tonicity of the environment. In a **hypertonic** solution where the concentration of dissolved solute is high, water will be drawn out of the cell. In a **hypotonic** solution where the concentration of dissolved solute is lower than the interior of the cell, the cell will be under great **osmotic pressure** from the environmental water moving in and can rupture.



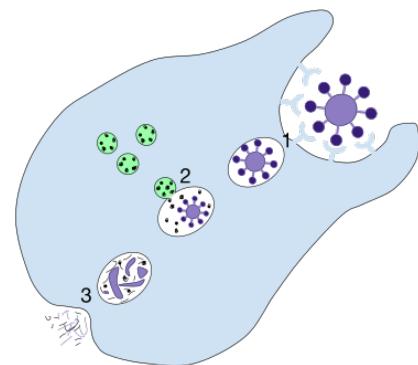
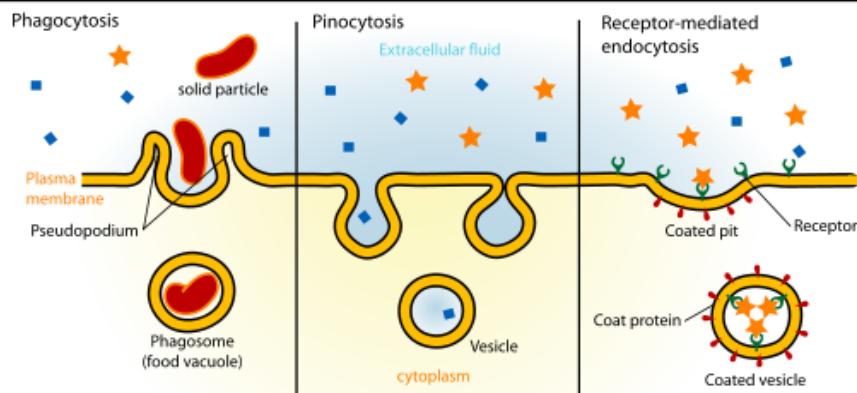
Plants have rigid cell walls composed of cellulose. These cell walls permit for maintenance of cellular integrity when the external environment is **hypotonic** (less dissolved substances). In this situation, the water moves into the cell. Without the cell wall, the cell would burst open from the excessive water pressure entering the cell. This state of swelling is referred to as turgid, resulting from **turgor pressure**.



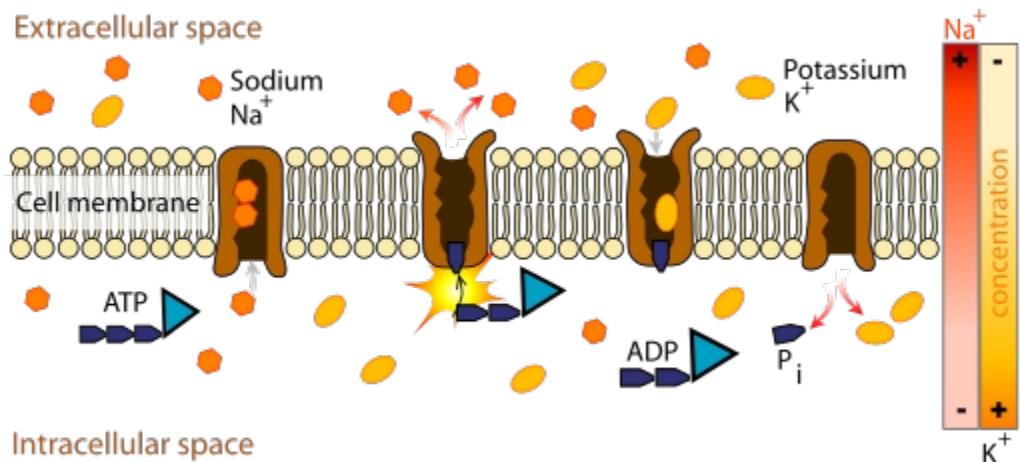
When the exterior environment is **hypertonic**, (greater amount of dissolved substances), the reverse condition occurs whereby the cellular fluid exiting the cell reduces the size of the cytoplasm. This condition is referred to as **plasmolysis**

## Active Transport Mechanisms

### Endocytosis



### Extracellular space



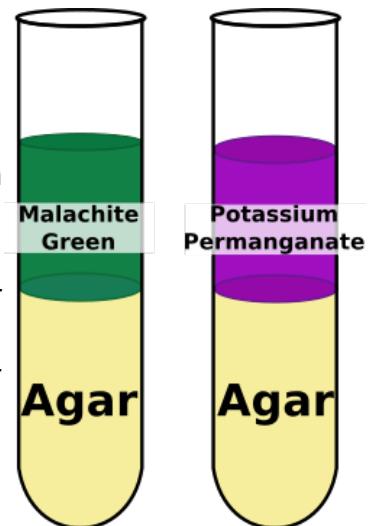
## Further Reading

- <http://www.visionlearning.com/en/library/Biology/2/Membranes-I/198/reading>
- <http://www.visionlearning.com/en/library/Biology/2/Membranes-II/204>
- [http://www.biologycorner.com/bio1/notes\\_diffusion.html](http://www.biologycorner.com/bio1/notes_diffusion.html) (CC-BY-NC)

## Activity: Do larger things diffuse faster?

Agar is a gelatinous substance derived from a structural carbohydrate found in seaweed. It is often used in cooking as a vegetarian alternative to gelatin and can be used as a thickener. Microbiologists pour plates of agar containing nutrients in order to isolate and grow bacteria and other microbes. As with gelatin, the long fibery nature of this structural carbohydrate permits it to be melted and tangled together in a mesh-like network where the spaces between molecules are filled with solution. Altering the amount of fluid solution will change the pores between fibers. More fluid will create a looser gel that has larger spaces between molecules. Reducing the fluid solution volume will conversely create a stiffer gel with smaller spaces between fibers.

1. Take 2 tubes of agar and a solution of Malachite green (365 g/mole) and a solution of Potassium permanganate (164 g/mole)
2. Mark the top of the agar on the outside of the tube (the starting point)
3. Add 10 drops of malachite green to one tube and 10 drops of Potassium permanganate to the other
4. Take note of the time
5. At 20 minute intervals, measure the distance from the top that the agar has moved. Do this for at least 1 hour.
6. Plot the data and compare the trends. Describe the rate of diffusion for each.



**Hypothesize** which solution will move faster through the agar and provide a reason.

Diffusion speed of dye molecules		
Dye	Molecular Weight	Hypothesis (fast or slow diffusion)
Malachite Green	365 g/mole	
Potassium Permanganate	164 g/mole	

## Conclude:

- Which solution actually moved faster?
- Did this meet your expectations?
- Propose a reason why a certain dye moved faster.
- [Review through simulation](#)

## Activity: What makes my Gummy Bear swell faster?

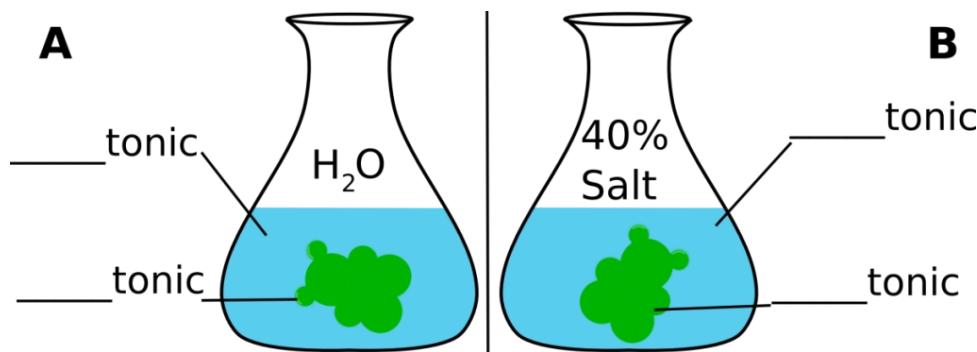
We're all familiar with gelatin (like the Jello brand). Gummy candies are made of gelatin. Gelatin is a protein that exists as long fibers. When gelatin is dissolved in a liquid and cooled, the gelatin fibers tangle together in a mesh-like network. The space in between the gelatin molecules is filled with the fluid it was dissolved in. Gummy candies are considerably more firm than the gelatin molds we have as desserts because they contain a lot less fluid. Nonetheless, gummy candies are filled with a sugary solution with coloring. Like a cell, a gummy candy placed in solution will be affected by the properties of osmosis when submerged in different solutions.

### Stop and think

- Is distilled water hypertonic, hypotonic or isotonic compared to the sugar solution inside a gummy candy?
- Based on your answer, hypothesize if a gummy candy submerged in distilled water or 40% salt solution will swell faster? Label the diagram below with your hypothesis.

### Procedures

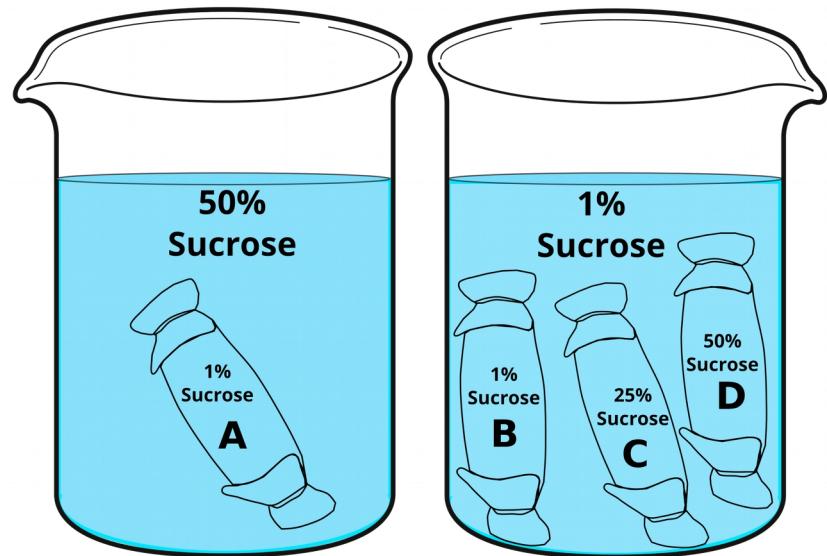
- Obtain 2 gummy bears and place them in 2 different small flasks.
- Drown 1 bear in distilled water and drown the other in 40% salt solution.
- At the end of the lab session, remove the bears from solution and document the size difference with your mobile phone.



Condition	Tonicity Inside Bear Relative to the Solution	Tonicity Outside relative to the Bear	Hypothesis about swelling
A			
B			

## Observe Osmosis Along A Free Energy Gradient

1. Obtain four pieces of water-soaked dialysis tubing 15 cm long and eight pieces of string. Seal one end of each tube by tying it into a knot.
2. Open the other end of the tube by rolling it between your thumb and finger.
  1. write A, B, C, D on 4 pieces of paper
  2. insert the labels into individual bags
3. Fill the bags with the contents shown in the figure below with 10 ml of solution.
  1. **Bag A** 10ml 1% Sucrose
  2. **Bag B** 10 ml 1% Sucrose
  3. **Bag C** 10 ml 25% Sucrose
  4. **Bag D** 10 ml 50% Sucrose
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 gram.
6. Record the weights in **Data Table1: Weight of Dialysis Bags**.
7. Place bags B, C, and D in a beaker or large bowl filled with 1% sucrose. Record the time.
8. Place bag A in an empty beaker and fill the beaker with just enough 50% sucrose to cover the bag. Record the time.
9. Remove the bags from the beakers at 10-minute intervals for the next hour, blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers.
  - o For each 10-minute interval record the total weight of each bag and its contents in **Data Table 1**.
  - o Then calculate and record in **Data table 2: Change in Weight of Dialysis Bags** the change in weight from the initial weight.



### Stop and Think:

- Define the tonicity of the solution inside the bag relative to the outside
- Based on your definitions, **hypothesize** the direction the solution will move (in or out of the bag) and fill in the table below

### Hypothesized Movement of Solution based on Tonicity

Bag	Tonicity Inside Bag Relative to the Solution	Tonicity of Outside Solution relative to the Bag	Hypothesized solution movement (in, out, none)
A			
B			
C			
D			

**Data Table 1: Weight of Dialysis Bags**

Bag	0 Min	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min
A							
B							
C							
D							

**Data Table 2: Net Weight Change of Dialysis Bags**

Bag	0 Min (WT <sub>0</sub> -WT <sub>0</sub> )	10 Min (WT <sub>10</sub> -WT <sub>0</sub> )	20 Min (WT <sub>20</sub> -WT <sub>0</sub> )	30 Min (WT <sub>30</sub> -WT <sub>0</sub> )	40 Min (WT <sub>40</sub> -WT <sub>0</sub> )	50 Min (WT <sub>50</sub> -WT <sub>0</sub> )	60 Min (WT <sub>60</sub> -WT <sub>0</sub> )
A	0						
B	0						
C	0						
D	0						

### Present your data:

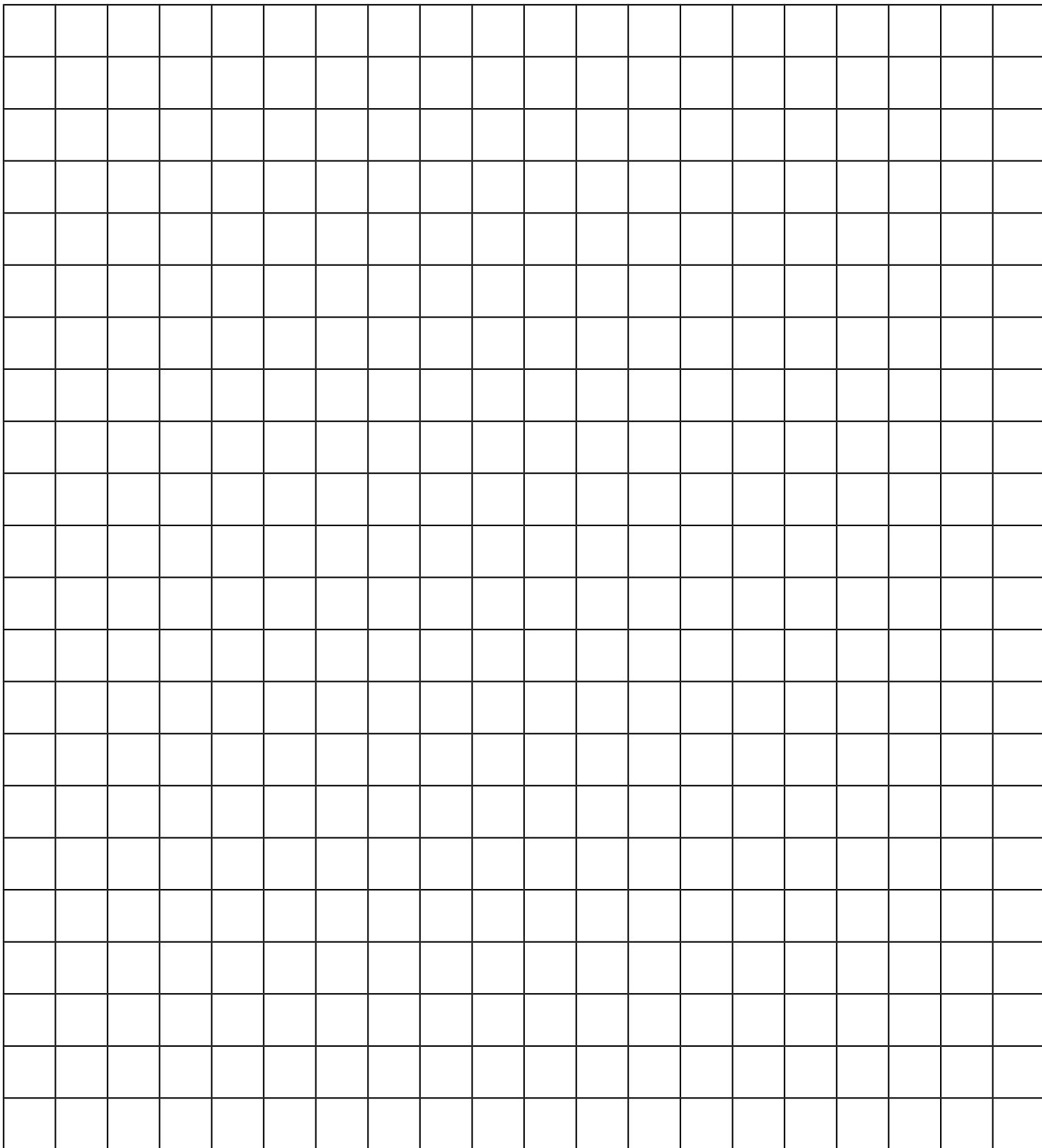
- Plot your data using only the Change in Weight. (subtract the Initial Weight at 0 minutes from the Total Weight at each time point)

$$\text{Change in Weight} = \text{Total Weight}_{\text{current\_time}} - \text{Initial Weight}_{\text{time}_0}$$

- Using a computer, create a scatterplot of the data from Table 2 and calculate the equation of the line

### Conclude:

- Did your results match your hypotheses?
- What do the slopes of the lines generated from plotting Change Weight indicate to you?
- Can you analyze and articulate in words what has occurred with respect to these slopes?



## Why are cells so small?

1. Take 3 blocks of agar of different size (1cm, 2cm, 3cm) → these are our cell models
2. Measure the length, width and height of each cube using a ruler
3. Calculate the area of each face of the cubes and add all the areas together for a single cube
  - a cube has 6 faces → the total surface area is the same as the area of one side multiplied by 6
4. Calculate the volume of each cube
5. Report the surface area-to-volume in the table below

**Data Table: Calculating Surface Area-to-Volume Ratio**

Cell Model (cube)	Length	Width	Height	Total Surface Area	Volume of cell	Surface Area: Volume
1						
2						
3						

### Stop and think:

- Which cube has the greatest surface area:volume ratio?
- Which cube has the smallest surface area:volume ratio?
- **Hypothesize:** In an osmosis or diffusion experiment, which cube size would have the greatest diffusion rate?

### Procedures:

1. Each group will retrieve three agar cubes: A 3cm cube, a 2cm cube, and a 1cm cube.
2. Pour 200mL of 0.1M NaOH into your beaker.
3. Immerse your 3 cubes in the NaOH, noting the time.
4. Let the cubes soak for approximately 10 minutes.
5. Periodically, gently stir the solution, or turn the cubes over.
6. After 10 minutes, remove the diffusion solution
7. Blot the cubes with a paper towel.
8. Promptly cut each cube in half and measure the depth to which the pink color has penetrated. Sketch each block's cross-section.
9. Record the volume that has remained white in color.
10. Do the following calculations for each cube and complete the following data table:

## Data Table: Calculation of Diffusion Area-to-Volume

Cube Size	Cube volume (cm <sup>3</sup> ) $V_{total}$	Volume white (cm <sup>3</sup> ) $V_{white}$	Sketch of each Cube	Volume of the diffused cube ( $V_{total} - V_{white}$ ) $V_{diffused}$	Percent Diffusion ( $V_{diffused}/V_{total}$ ) % Diffused	Surface Area: Volume (from previous table)
1cm						
2cm						
3cm						

## Conclude:

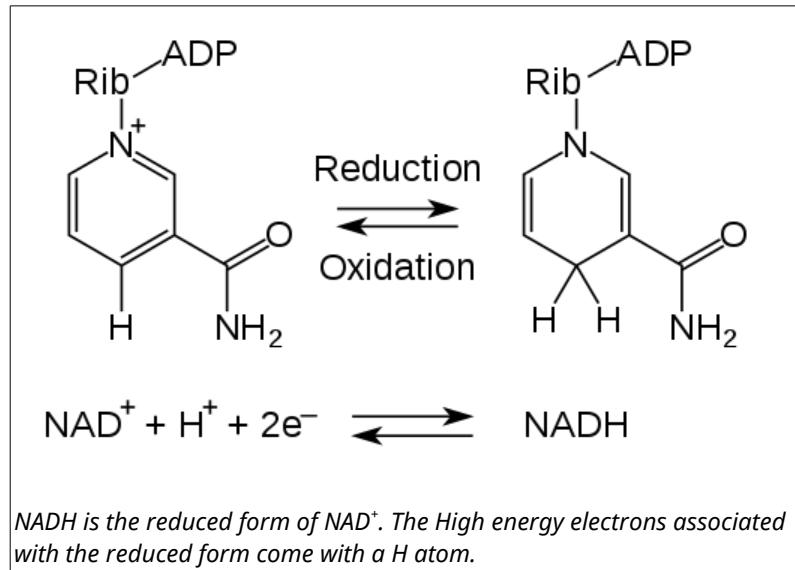
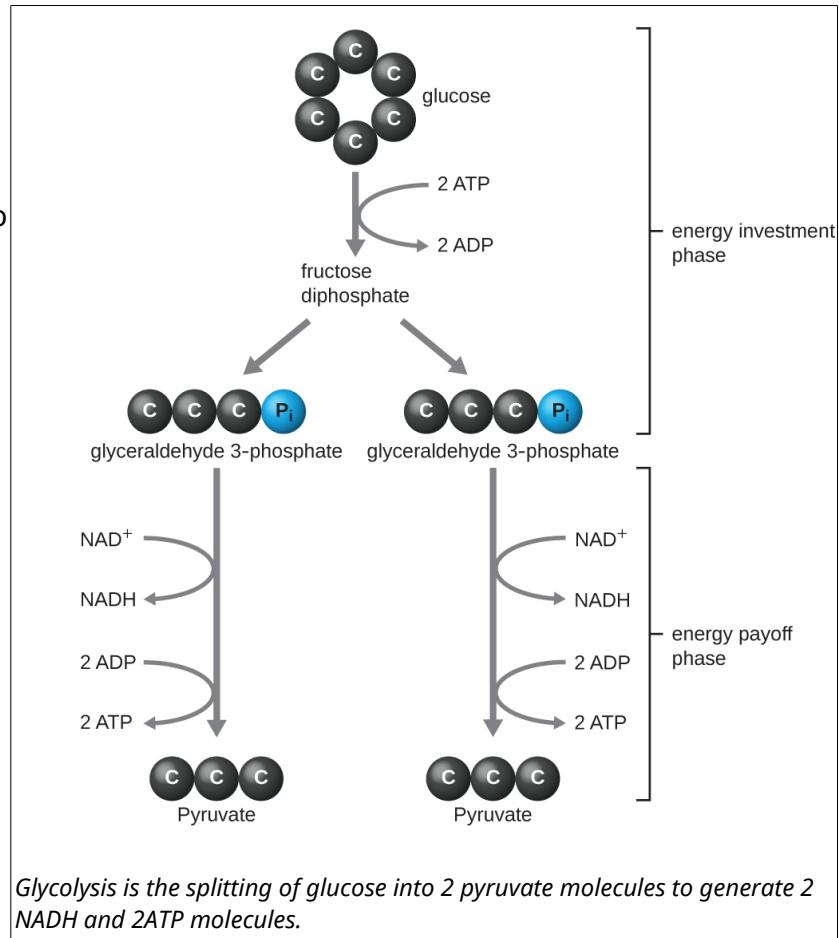
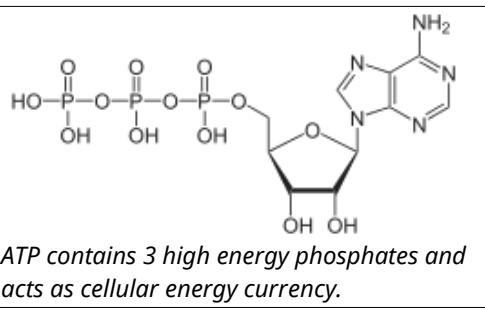
- Which cube had the greatest percentage of diffusion?
- Did this meet your expectations with your hypothesis?
- If you designed a large cell, would it be a large sphere or something long and flat?

Energy is stored in the bonds of the carbohydrates. Breaking these bonds releases that energy. Crushing sugar crystals creates tiny electrical fields that give off invisible ultraviolet light. The wintergreen chemical (methyl salicylate) gets excited by these excited electrons and fluoresces in a visible blue wavelength. This phenomenon is called triboluminescence.

### Video Demonstration of Energy Release from Crushing Sugar

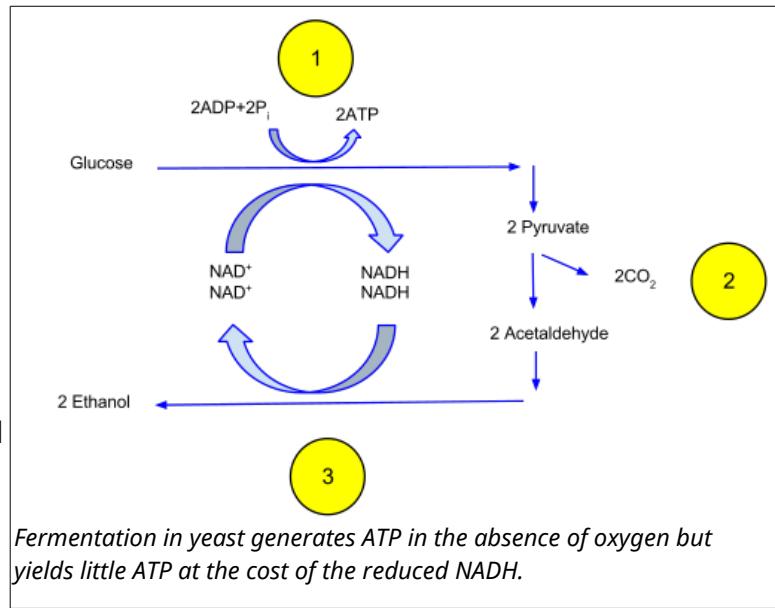
## Glycolysis

Glucose is the preferred carbohydrate of cells. **Glycolysis** (*glyco* - sugar; *lysis* - splitting) is a universal process of all cells that occurs in the cytosol whereby the glucose (a 6-carbon sugar) is split into two pyruvate (a 3-carbon molecule) molecules to generate ATP and reduced NADH. **ATP** (adenosine triphosphate) is the energy currency of the cell that stores chemical energy in 3 high energy phosphate bonds. **NADH** (reduced nicotinamide adenine dinucleotide) is a high energy electron carrier that acts as a coenzyme in reactions and as a rechargeable battery of sorts. The uncharged state that is not carrying high energy electrons is called **NAD<sup>+</sup>**.



## Fermentation

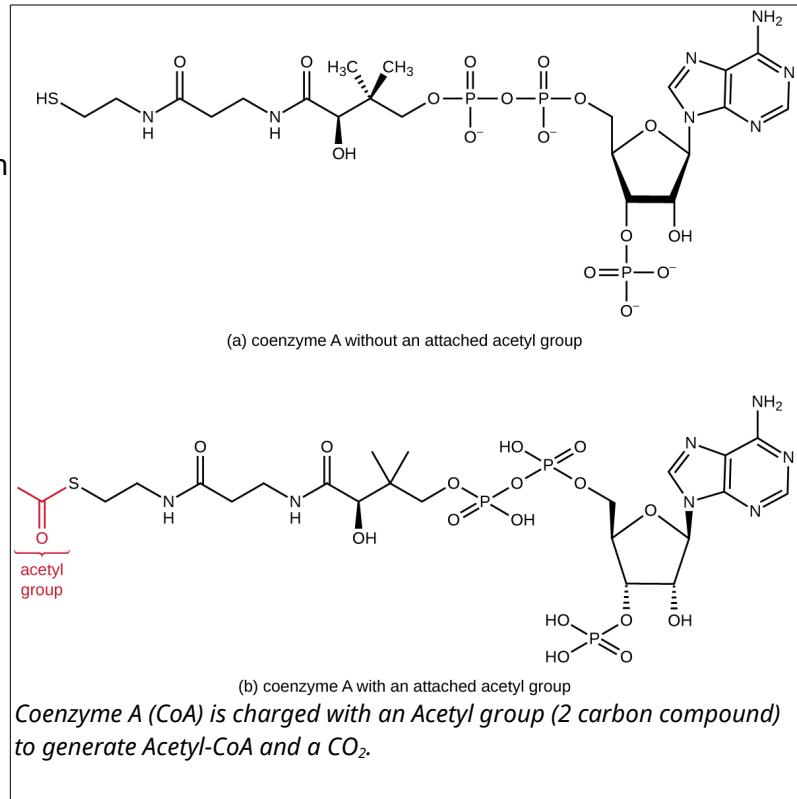
In the absence of oxygen, cells may decide to utilize the pyruvate from glycolysis to rapidly generate additional ATP molecules in a process called fermentation. **Fermentation** is the anaerobic process of reducing pyruvate to generate ATP. This process uses the NADH generated from glycolysis as the reducing agents. Fermentation is a familiar process that occurs in yeast to generate ethanol. In other organisms, like humans, fermentation results in the production of lactic acid. Both lactic acid and ethanol are toxic, but this aids the cells in generating ATP when energy is required rapidly. Fermentation also generates CO<sub>2</sub> as a waste molecule as pyruvate is broken down into a 2-carbon compound.



## The Preparatory Reaction

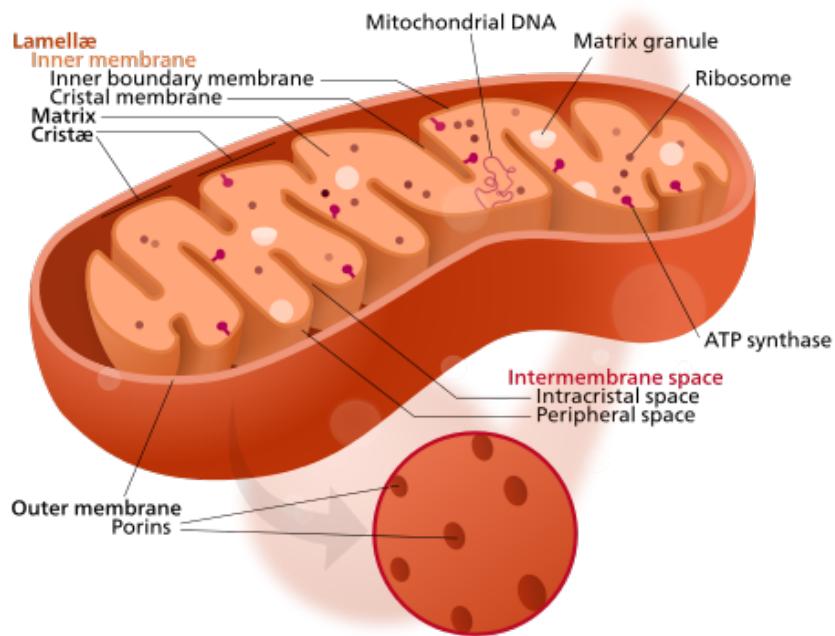
In the presence of O<sub>2</sub>, aerobic organisms will use a reaction of pyruvate decarboxylation in the cytosol. This reaction generates a molecule of **Acetyl-CoA** from the Coenzyme A which can enter the mitochondria.

When there is an excess of carbohydrates, the Acetyl-CoA is used as a starting point for long-term energy storage in lipid synthesis.

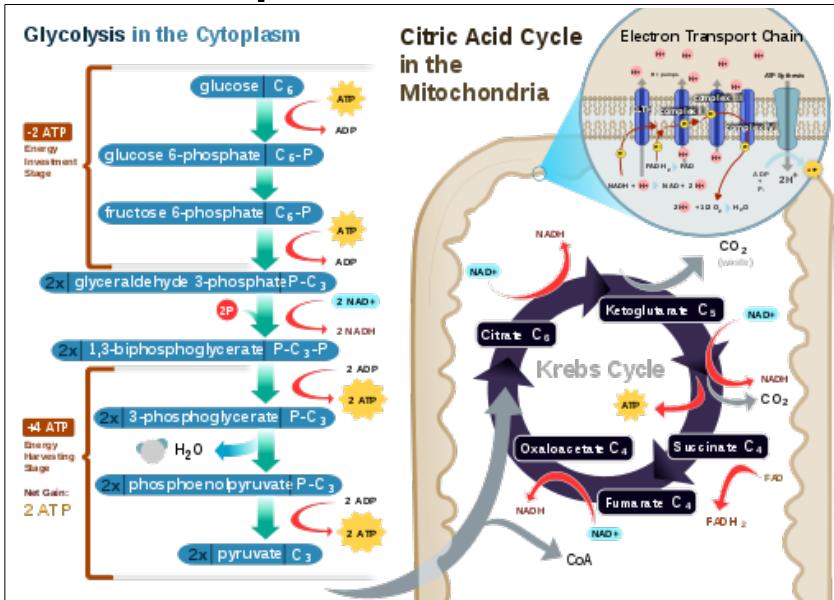


## Mitochondria

Mitochondria are the power station of eukaryotic cells. They are derived from a process described by the **endosymbiotic theory** whereby aerobic prokaryotes were engulfed by a protoeukaryote. In this mutualistic arrangement, the prokaryote detoxified the deadly O<sub>2</sub> gas in the environment and used it to fully break down glucose to yield many ATP molecules. Evidence for this theory comes from the independent replication of the mitochondria, the bacterial-like mitochondrial DNA, the bacterial-like mitochondrial ribosomes, the bacterial lipids found in the inner membrane and the eukaryotic nature of the outer membrane. Mitochondria are genetically similar to bacteria of the order *Rickettsiales*. Some bacteria of this order are still free-living and some are intracellular pathogens.



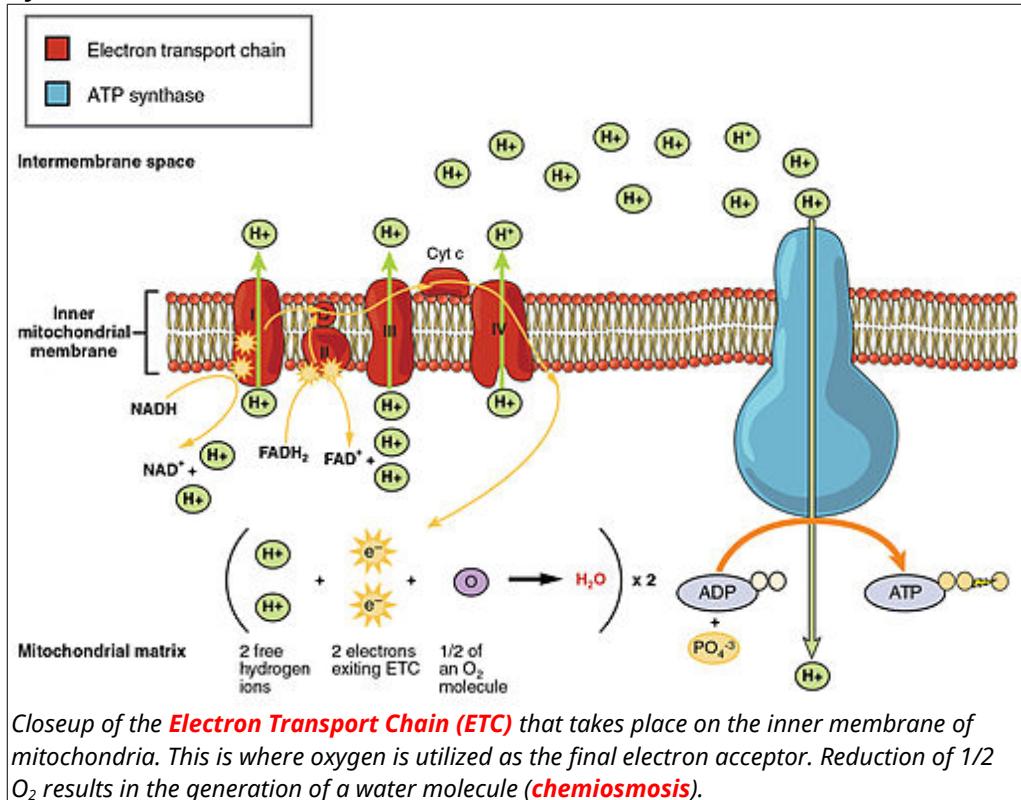
## Aerobic Respiration

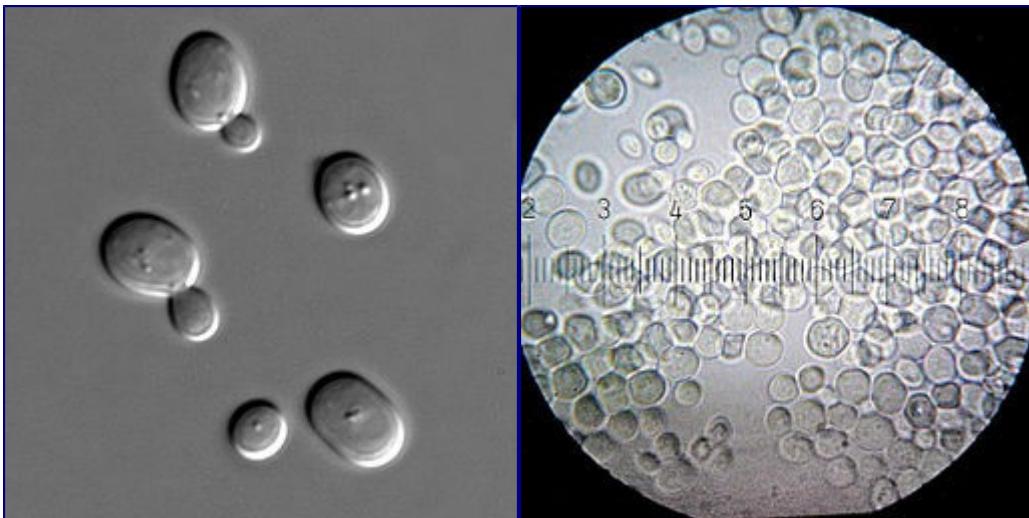


Cellular Respiration. Left side is glycolysis (anaerobic). The Right side is what occurs in the presence of oxygen in eukaryotes. The aerobic reactions occur inside the mitochondria after being fed Acetyl-CoA molecules from the cytoplasmic preparatory reaction.

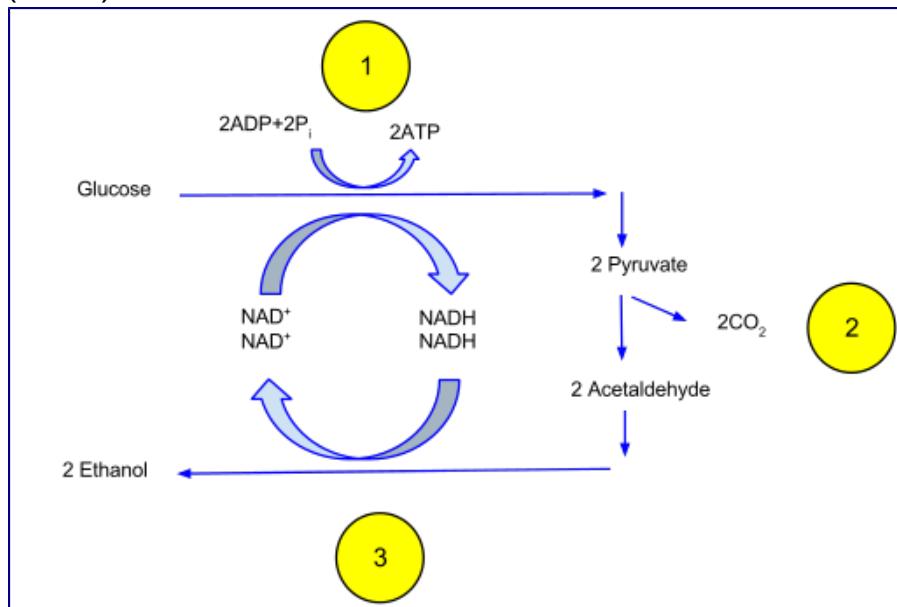
Acetyl-CoA enters the mitochondrial matrix where it is used in the **Krebs Cycle** (aka Tricarboxylic acid cycle (TCA), aka Citric acid cycle). For each pyruvate, there are 2 turns of the cycle where additional NADH and another high energy electron carrier **FADH<sub>2</sub>** (flavin adenine dinucleotide) are generated. The electrons stored by NADH and FADH<sub>2</sub> are transferred to proteins called **cytochromes** that have metal centers for conducting these electrons. In the process of moving these electrons, the cytochromes in this **Electron Transport Chains** (ETC) power the movement of protons into the intermembrane space. The terminus of these electrons is an O<sub>2</sub> molecule that is reduced into 1/2 H<sub>2</sub>O molecules. This apparent movement of water molecules

from the chemical synthesis is termed **chemiosmosis**. A channel in the membrane called **ATP synthase** acts as a gateway for the H<sup>+</sup> back into the matrix, but uses this motion to convert ADP into ATP.

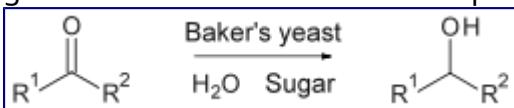




Yeast are single-celled fungi. The species called *Saccharomyces cerevisiae* is commonly called Baker's or Brewer's yeast. Like other eukaryotes with mitochondria, yeast can use oxygen to generate ATP in the process of **oxidative phosphorylation**. These yeast are **facultative aerobes** which means they can also switch to an anaerobic mechanism of ATP production called fermentation. In all organisms, the process of glycolysis occurs anaerobically in the cytoplasm to produce two pyruvate molecules from a single glucose. This process produces 2 new ATP molecules and reduced nicotinamide adenine dinucleotide (NADH).



**Fermentation** is an anaerobic process that occurs in the cytoplasm and quickly generates an additional ATP through the reduction of pyruvate. NADH is the source of electrons in this process that is oxidized to  $\text{NAD}^+$ . Many organisms will ferment to generate lactic acid and  $\text{CO}_2$  from the pyruvate in order to generate ATP. Yeast fermentation produces **ethanol**.



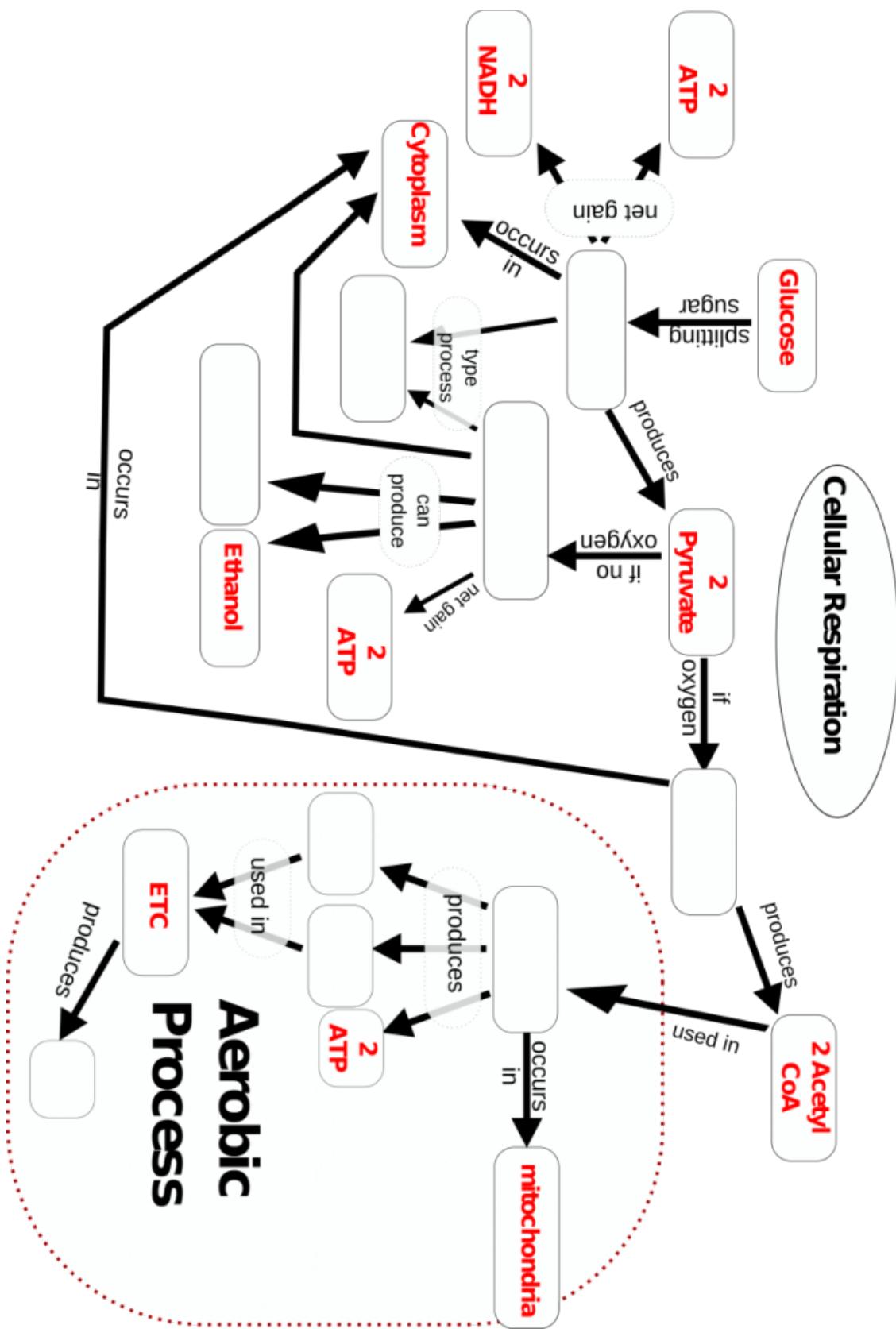
## Fermentation Activity

1. Mix the solutions in the table below in a fermentation tube
2. Eliminate the air bubble in the sealed end of the fermentation tube
3. Place rubber stopper into the open end of the fermentation tube and place at the appropriate temperature for an hour
4. Predict the amount of CO<sub>2</sub> generated in the last column of the table using +, - or +++
  - after an hour, measure the head space created by the bubbles and compare with your predictions

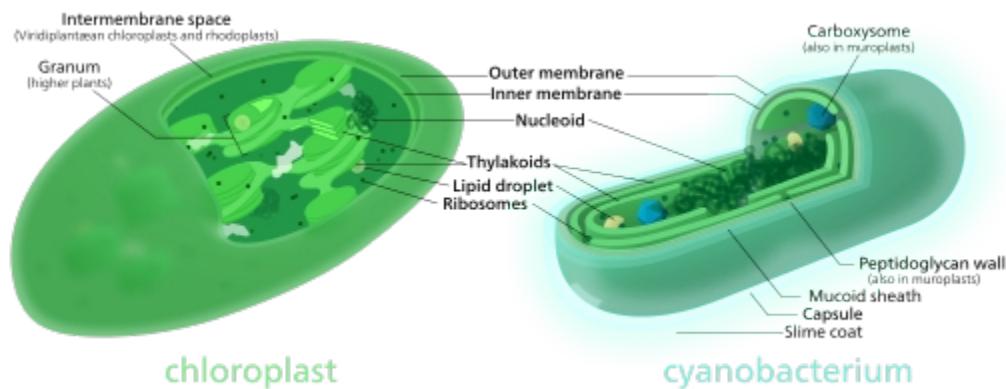
TUBE	YEAST	SUGAR	ADDITIVE	TEMP.	CO <sub>2</sub> generation
1	10 ml	None	20 ml H <sub>2</sub> O	25°C	
2	10 ml	10 ml glucose	10 ml H <sub>2</sub> O	25°C	
3	10 ml	10 ml glucose	10 ml H <sub>2</sub> O	37°C	
4	10 ml	10 ml lactose	10 ml H <sub>2</sub> O	37°C	
5	10 ml	10 ml sucrose	10 ml H <sub>2</sub> O	37°C	
6	10 ml	10 ml maltose	10 ml H <sub>2</sub> O	37°C	
7	10 ml	10 ml glucose	10 ml 0.1M MgSO <sub>4</sub>	37°C	
8	10 ml	10 ml glucose	10 ml 0.1 M NaF	37°C	
9	10 ml	10 ml lactose	10 ml H <sub>2</sub> O + LactAid	37°C	

## Questions to direct hypothesis formation

1. What is the preferred energy source of the all cells?
2. What types of sugars are being used in each tube (monosaccharide, disaccharide, etc)?
3. What effect should temperature have on the fermentation reactions?
4. What does CO<sub>2</sub> indicate in these tubes?
5. What do you think the additives do? What effect will they have?

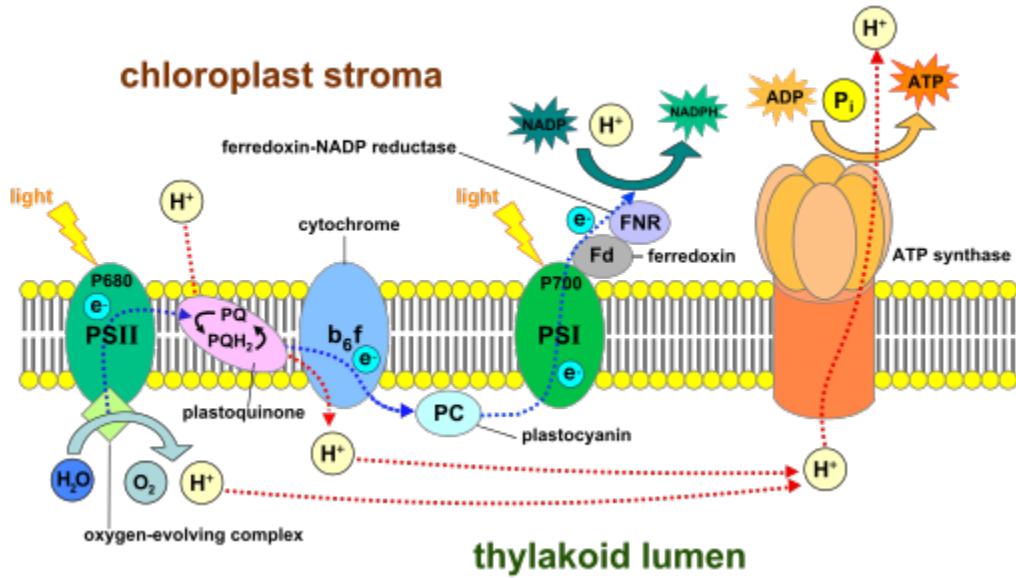


## Chloroplasts



**Chloroplasts** arose through a second endosymbiotic event in plants and various protists. These light harvesting organelles share similarity in structure and genome to photoautotrophic cyanobacteria.

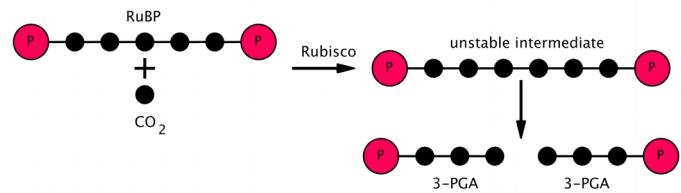
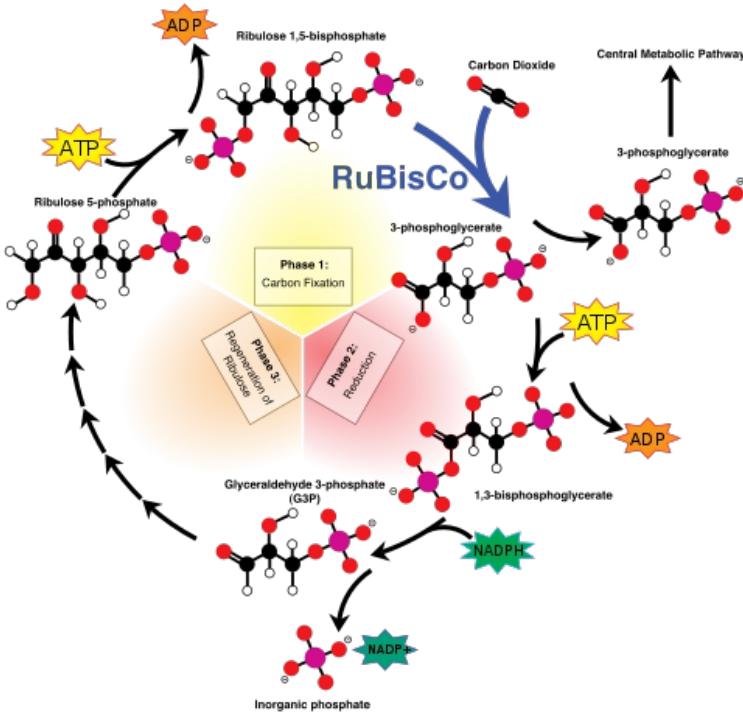
## Light Harvesting



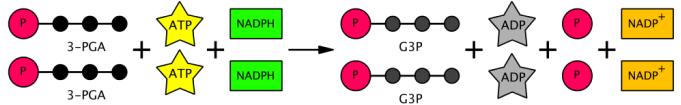
The thylakoid membranes of chloroplasts and cyanobacteria provide additional surface area for energy capture of light to occur. The light-dependent reactions in chloroplasts utilize two protein complexes referred to as **Photosystem I** (PSI) and **Photosystem II** (PSII) located on the thylakoid membranes. At the center of each photosystem complexes are **photopigments** optimized to absorb specific wavelengths of light. When light is absorbed in a photosystem, an electron is excited and transferred to the electron transport chain. In PSII, the electron is regenerated by splitting of two water molecules into  $4\text{H}^+ + 4\text{e}^- + \text{O}_2$ . As the electrons move through the ETC, protons are pumped into the thylakoid space. The ETC leads to the reduction of a high energy electron carrier  $\text{NADP}^+$  to **NADPH**. Since this pathway uses consumes water in a chemical reaction, the apparent loss of water in the thylakoid space is referred to as chemiosmosis.

PSI is also known as the cyclic pathway since the excited electron runs through a closed circuit of the ETC to regenerate the lost electron. This closed circuit also generates a proton gradient through powering of a proton pump but does not lead to the reduction of NADPH. As with the ETC-powered proton pump in mitochondria, the proton gradient is used to power ATP-synthase in producing ATP molecules.

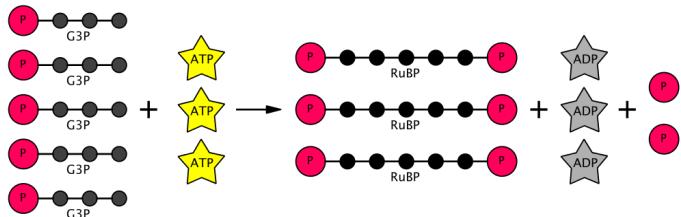
## Light Independent Reactions



1: Carbon fixation by RuBisCO



2: Reduction by NADPH



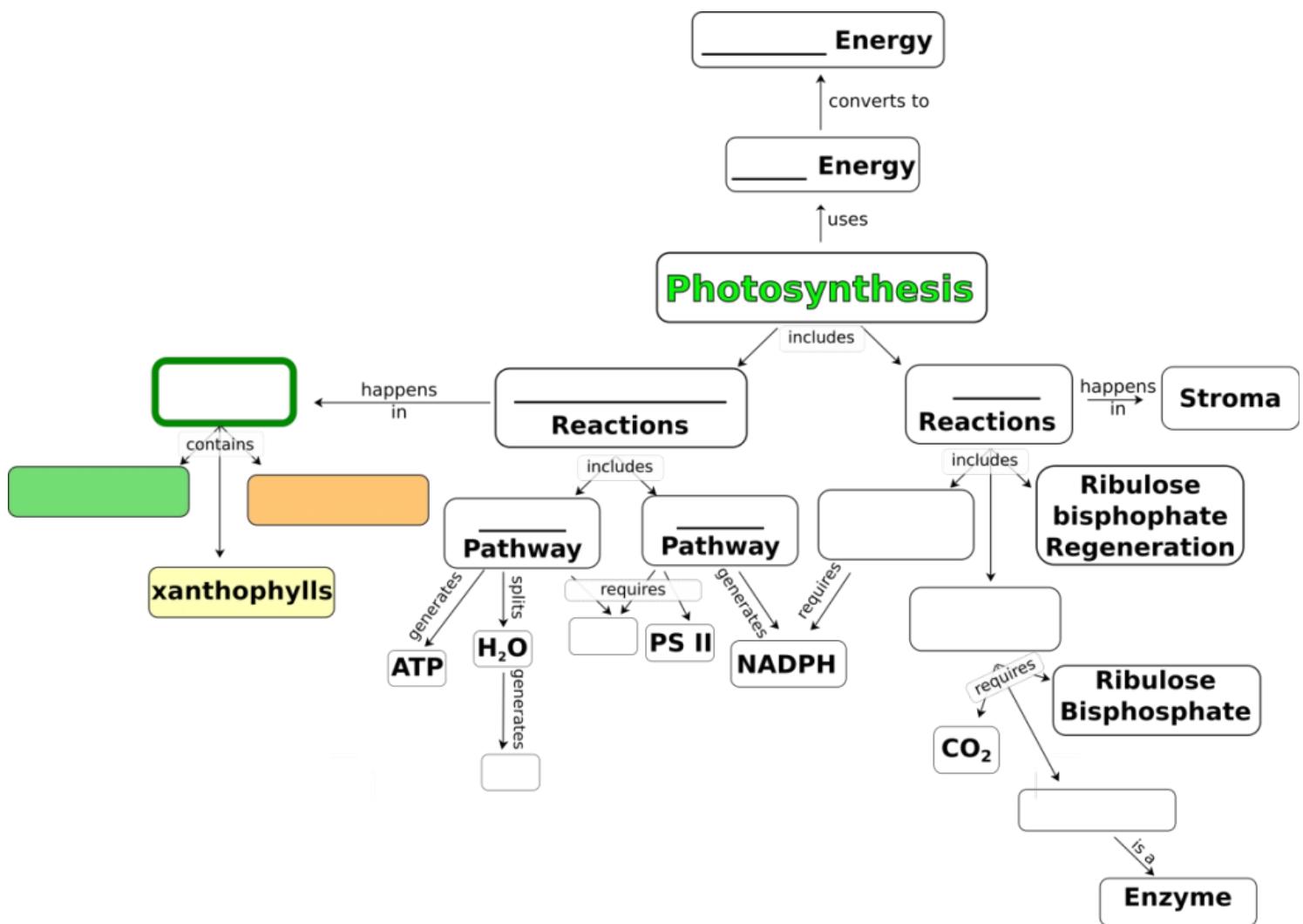
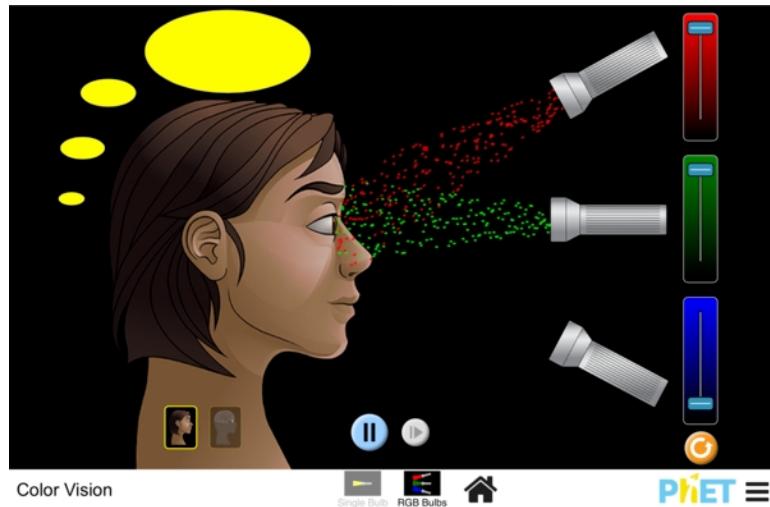
3: Ribulose ,5-bisphosphate regeneration

The light independent reactions are also known as the dark reactions or **Calvin Cycle** and utilize the ATP and NADPH from the light-dependent reactions to fix gaseous CO<sub>2</sub> into carbohydrate backbones.

Photosynthesis is often simplified into  $6\text{CO}_2 + 6\text{H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$ . However, the true product is 3-phosphoglycerate that can be used to generate longer carbohydrates like glucose. The starting point of **carbon fixation** is the carbohydrate Ribulose 1,5-bisphosphate. The enzyme Ribulose Bisphosphate Carboxylase (**RuBisCO**) captures a CO<sub>2</sub> molecule onto Ribulose 1,5-bisphosphate to generate 2 molecules of 3-phosphoglycerate which can enter the process of **gluconeogenesis** to generate glucose. ATP from the light reactions can then facilitate the conversion of 3-phosphoglycerate to 1,3 bisphosphoglycerate which can be reduced by NADPH to glyceraldehyde-3-phosphate (G3P). G3P can then be used to regenerate Ribulose 1,5-bisphosphate.

## Exploring light

Run the simulation below to understand how white light, specific wavelengths of light and filtered light work.



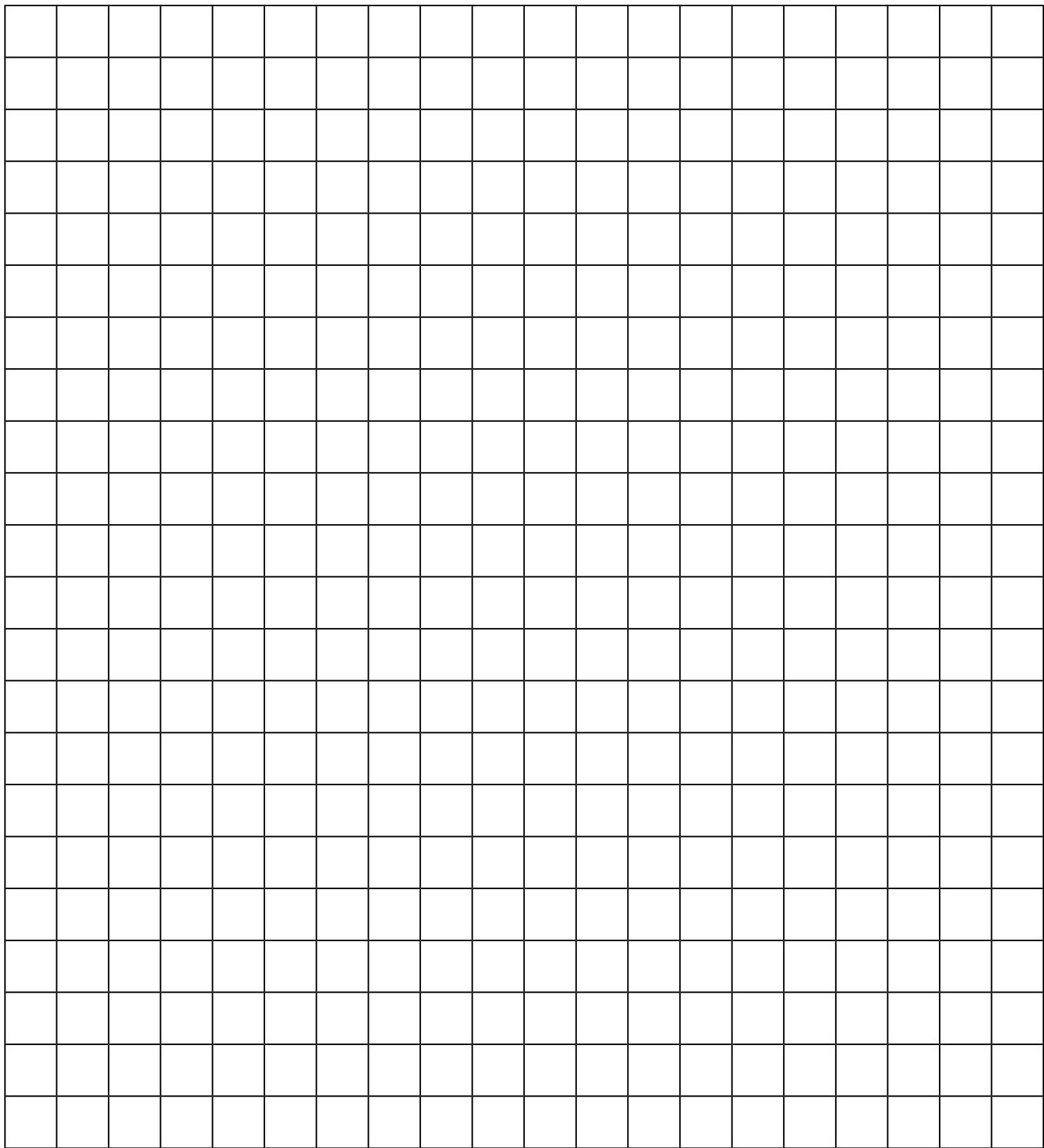
## Prelab Exercise

1. Fill the *Color* field in the table below
2. Use [plot.ly](#) to create a **line** graph with the 3 samples below (A, B, C)
  - plot % Reflectance on the Y-axis and Wavelength (nm) on the X-axis

Color	nm	% Reflectance		
		A	B	C
	400	68	92	78
	425	40	71	77
	450	90	38	51
	475	97	49	57
	500	100	92	45
	525	100	100	66
	550	96	97	100
	575	98	96	100
	600	96	98	100
	625	97	80	100
	650	79	71	100
	675	56	96	100
	700	88	100	100

## Stop and Think: Reflectance

A sign of plant health is viewed through the near infra-red. While we cannot see this spectrum of light with our eyes, we can use other sensors to detect this light. Compare the images of the Black & White with the Infra-red image. What differences can you see in the 2 images that will help you understand how this is a useful measure of plant health? How do you think this corresponds to the table above?





*The English Garden*

[Reflectance Slider shows an overlay of the next images](#)



*The English Garden (black & white)*



*The English Garden (near infra-red)*

Visible light wavelengths (between 400nm-700nm) are strongly absorbed by the pigments in leaves (Chlorophylls, Xanthophylls, Carotenoids). These pigments utilize the energy of these wavelengths to take part in the light reactions. The cellular structure of leaves do not absorb wavelengths longer than these wavelengths ( $>700\text{nm}$  in the infra-red range). By comparing the amount of visible light to the amount of near infra-red light that are reflected, one can gauge the relative health of leaves, forests or jungles. This is the rough description of the Normalized Differential Vegetation Index (NDVI) that scientists use in conjunction with satellite imagery to assess the health of vegetation.

## The Role of Light in Carbohydrate Synthesis

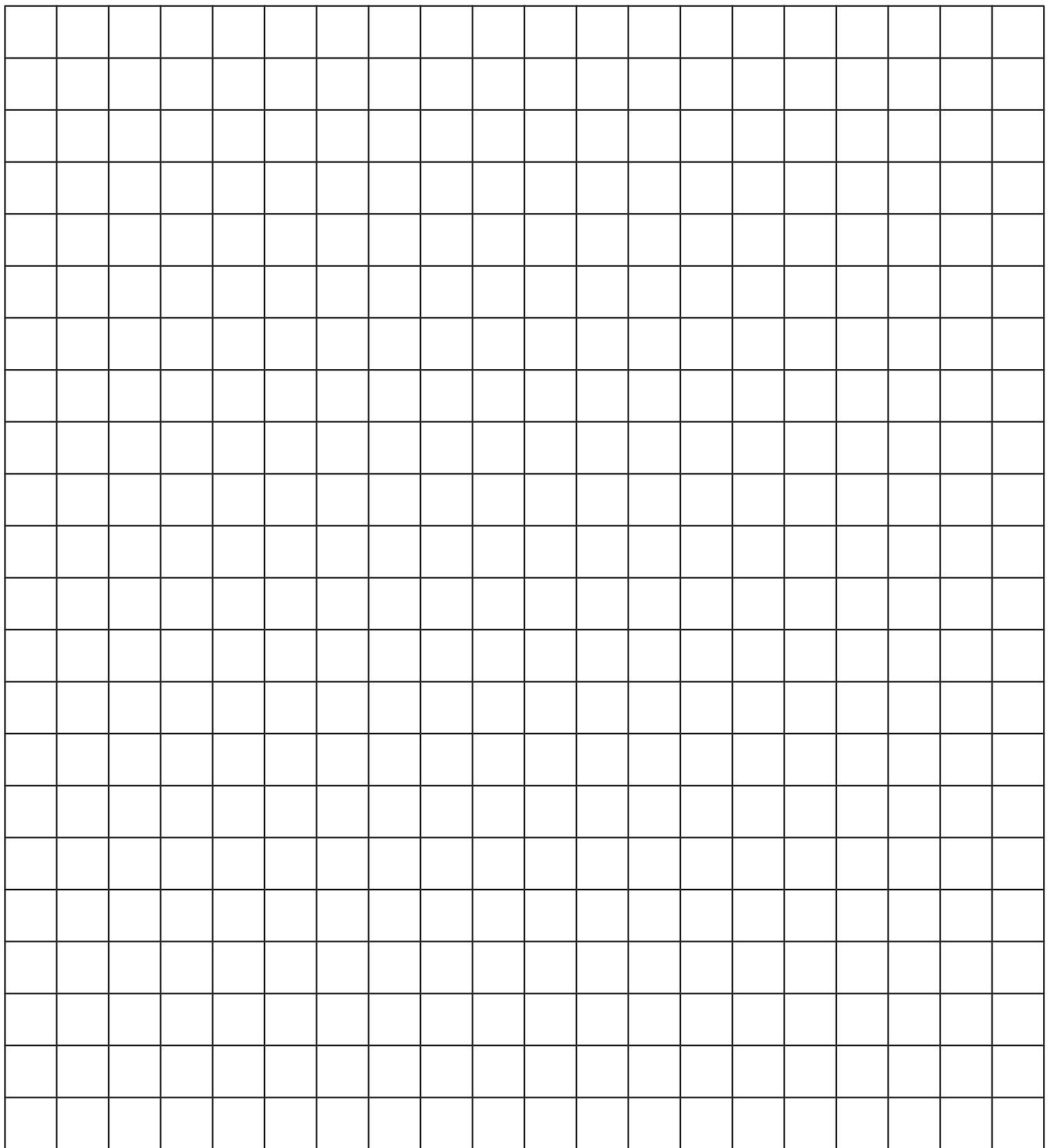
1. Pick a leaf from a geranium exposed to light and one kept in the dark for 48 hours.
  - keep the stem on the leaf grown in light
  - remove the stem from the leaf grown in the dark
2. Hydrolyze the cell walls of the geranium leaves by boiling in a water bath for 5 minutes or until it looks like over-cooked vegetables)
3. "Bleach" the leaves by removing the pigments. Place the leaves in hot alcohol for 7 minutes or until they turn white.
  - Save this green solution for Absorbance Spectrum exercise
4. Remove the leaves and place it in a petri dish.
5. Add iodine to the dish. If starch is present, the leaf will turn a deep bluish-black color.
6. Photograph the leaf with your phone to document the effects of light on carbohydrate storage.

## Measuring Absorbance using the LabQuest2

1. Connect the Spectrovis to the LabQuest2
2. Turn on the Labquest2 units
3. Choose the Labquest app
4. Select the icon that looks like X|Y
5. Press the green Play button on the bottom left
6. Press OK to calibrate
7. Let the machine calibrate for 90 seconds
8. Choose "Finish calibration"
9. Insert the Geranium pigment from the bleaching reaction
  1. Do NOT use Acetone in these plastic cuvettes since it will frost over the plastic
10. Press the Red Stop button
11. Students should record the absorbance values at every 10 nm from 380nm-700nm
12. The professor will prepare *Spirulina* extract diluted in ethanol in a cuvette and obtain the continuous absorbance spectrum.
13. Plot Relative Absorbance against *wavelength* using a line graph and compare the absorption spectrum of the extracts.
  1. Relative Absorbance sets the maximum value in each dataset as a denominator
  2. Every value is divided by this maximum value

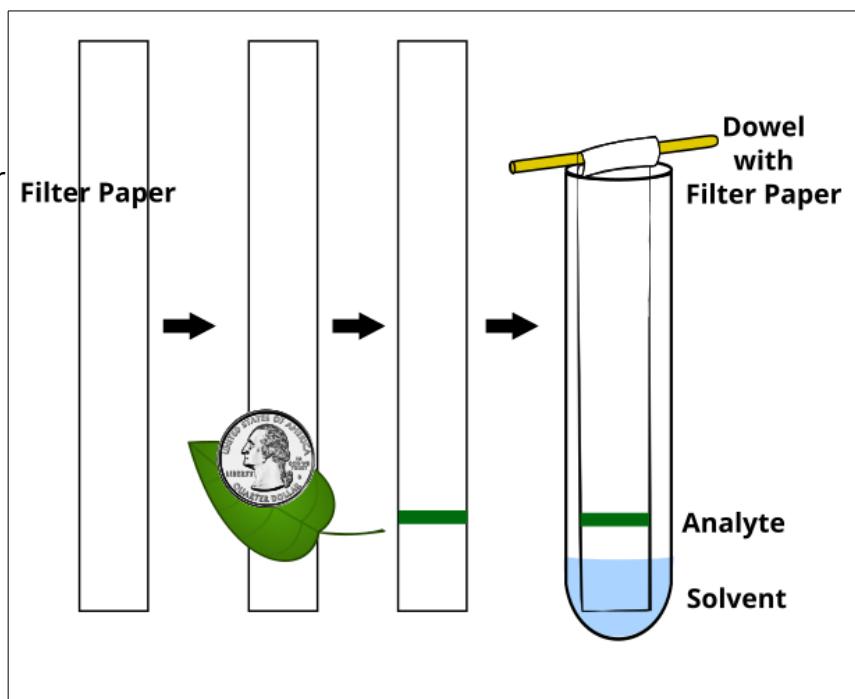
## Measuring Absorbance using Spectronic (alternative method)

1. Create a blank and blank (Ethanol) the Spectronic at 400nm set to Absorbance
2. Place cuvette with Geranium Bleaching solution into the Spectronic and read the absorbance value
3. Continue to read absorbance values at 25nm intervals until reaching 700nm
4. Plot Relative Absorbance against *wavelength* using a line graph and compare the absorption spectrum of the extracts.
  1. Relative Absorbance sets the maximum value in each dataset as a denominator
  2. Every value is divided by this maximum value



## Extract and separate the pigments

1. Lay a strip of filter paper on the bench
2. about 2 cm from the bottom of the strip, place a fresh spinach leaf and rub a coin across the leaf to transfer pigment to the strip
3. The instructor will be provided with a spoonful of *Spirulina* powder that has been soaked in 10ml acetone overnight.
  - on a separate strip, the instructor will apply the *Spirulina* extract approximately 2cm from the bottom of the strip
4. Suspend the strips by a dowel or paper clip in a tube with about 3ml chromatography solution (2 *isooctane*: 1 *acetone*: 1 *diethyl ether*).
5. Develop the strips until the solvent reaches about 2 cm from the top

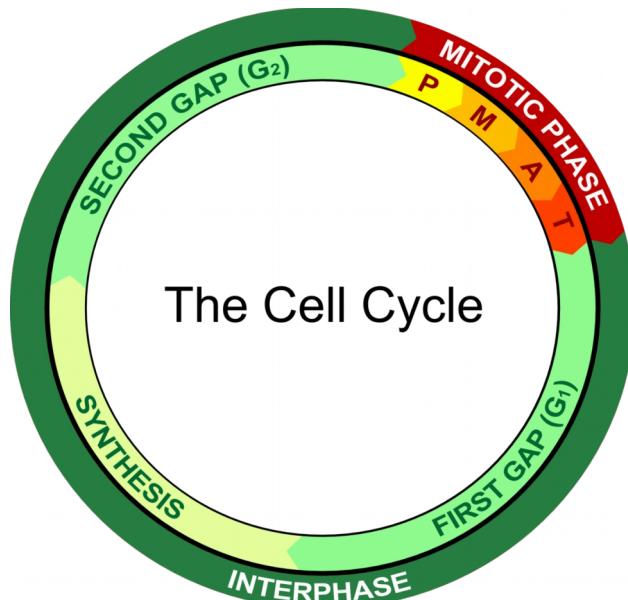


## Chromatography Analysis

1. How many different pigments separate from the spinach extract? From the spirulina?
2. Are all pigments represented between the two extract?
3. The mobile phase is non-polar, what are the properties of each pigment?
4. Measure the  $R_f$  of each pigment.

## Introduction: The Cell Cycle and Mitosis

The cell cycle refers to the a series of events that describe the metabolic processes of growth and replication of cells. The bulk of the cell cycle is spent in the “living phase”, known as **interphase**. Interphase is further broken down in to 3 distinct phases: G<sub>1</sub> (Gap 1), S (Synthesis) and G<sub>2</sub> (Gap 2). G<sub>1</sub> is the phase of growth when the cell is accumulating resources to live and grow. After attaining a certain size and having amassed enough raw materials, a checkpoint is reached where the cell uses biochemical markers to decide if the next phase should be entered. If the cell is in an environment with enough nutrients in the environment, enough space and having reached the appropriate size, the cell will enter the S phase. **S phase** is when metabolism is shifted towards the replication (or synthesis) of the genetic material. During S phase, the amount of DNA in the nucleus is doubled and copied exactly in preparation to divide. The chromosomes at the end of G<sub>1</sub> consist of a single **chromatid**. At the end of S phase, each chromosome consists of two identical **sister chromatids** joined at the **centromere**. When the DNA synthesis is complete, the cell continues on to the second growth phase called G<sub>2</sub>. Another checkpoint takes place at the end of G<sub>2</sub> to ensure the fidelity of the replicated DNA and to re-establish the success of the cell's capacity to divide in the environment. If conditions are favorable, the cell continues on to mitosis.



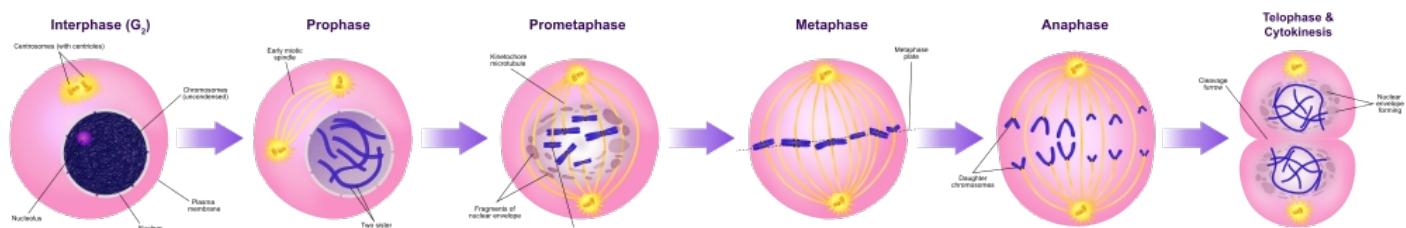
By [LadyofHats](#) [Public domain], via Wikimedia Commons



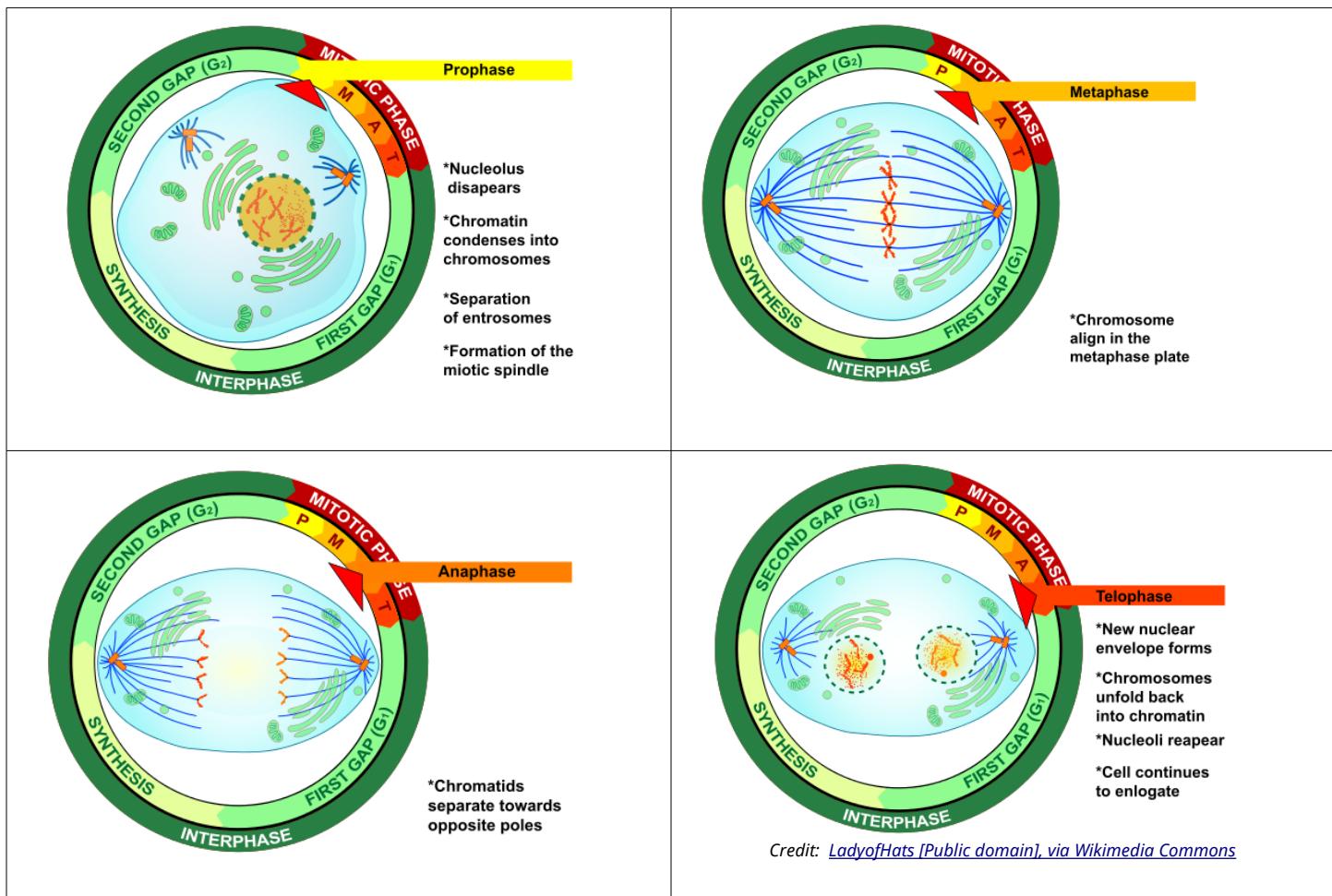
Credit: [TheAlphaWolf](#) [CC BY-SA 3.0], via Wikimedia Commons

**Mitosis** is the process of nuclear division used in conjunction with cytokinesis to produce 2 identical daughter cells. **Cytokinesis** is the actual separation of these two cells enclosed in their own cellular membranes. Unicellular organisms utilize this process of division in order to reproduce asexually. Prokaryotic organisms lack a nucleus, therefore they undergo a different process called binary fission. Multicellular eukaryotes undergo mitosis for repairing tissue and for growth. The process of mitosis is only a short period of the lifespan of cells. Mitosis is traditionally divided into four stages: **prophase**, **metaphase**, **anaphase** and **telophase**. The actual events of mitosis are not discreet but occur in a continuous sequence - separation of mitosis into four stages is merely convenient for our discussion and organization. During these stages important cellular structures are synthesized and perform the mechanics of mitosis. For example, in animal cells two microtubule organizing centers called **centrioles**

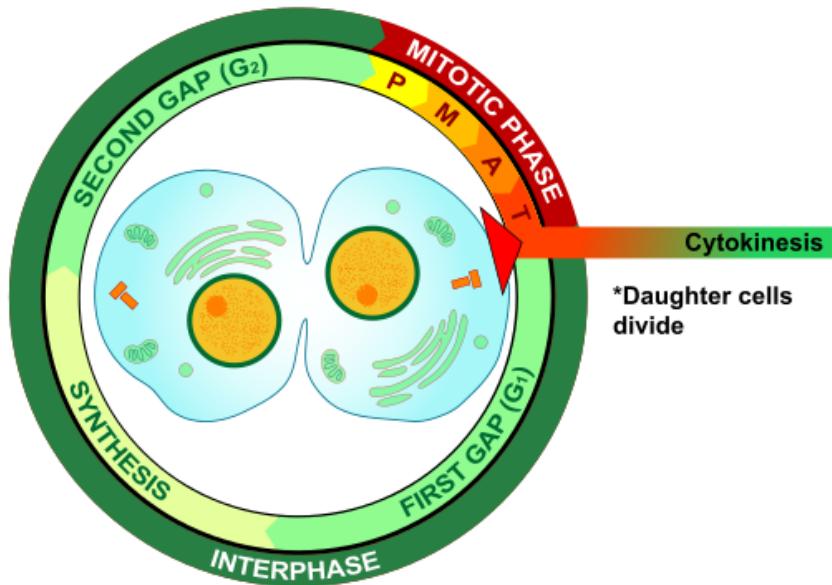
replicate. The pairs of centrioles move apart and form an axis of proteinaceous microtubules between them called **spindle fibers**. These spindle fibers act as motors that pull at the centromeres of chromosomes and separate the sister chromatids into newly recognized chromosomes. The spindles also push against each other to stretch the cell in preparation of forming two new nuclei and separate cells. In animal cells, a contractile ring of actin fibers cinch together around the midline of the cell to coordinate cytokinesis. This cinching of the cell membrane creates a structure called the **cleavage furrow**. Eventually, the cinching of the membrane completely separates into two daughter cells. Plant cells require the production of new cell wall material between daughter cells. Instead of a cleavage furrow, the two cells are separated by a series of vesicles derived from the Golgi. These vesicles fuse together along the midline and simultaneously secrete cellulose into the space between the two cells. This series of vesicles is called the **cell plate**.



Credit: Ali Zifan [CC BY-SA 4.0], via Wikimedia Commons



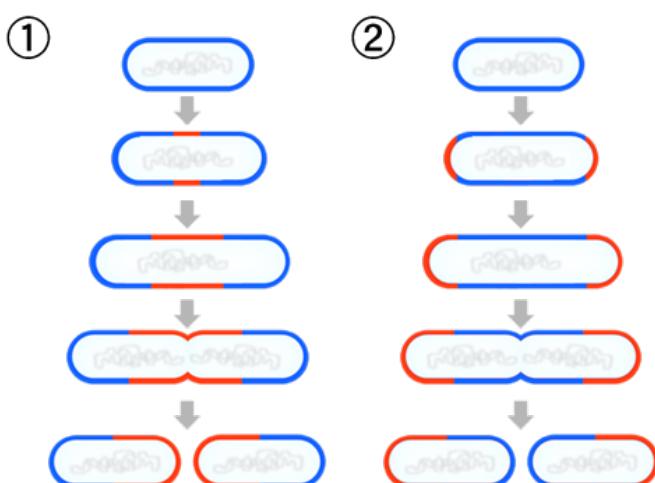
Credit: LadyofHats [Public domain], via Wikimedia Commons



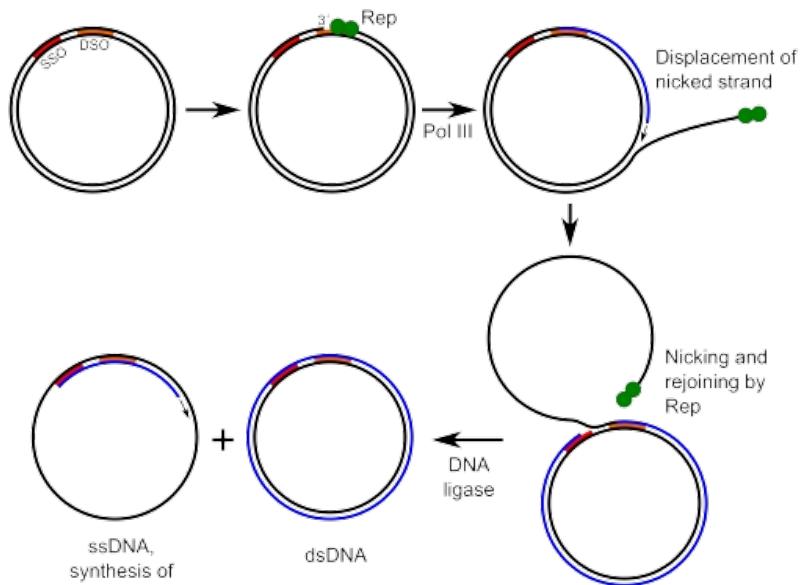
Credit: [LadyofHats \[Public domain\], via Wikimedia Commons](#)

## Binary Fission

Mitosis refers to nuclear division, which happens to coincide with cytokinesis. Prokaryotes do not have nuclei, therefore they do not undergo a process of mitosis. Instead, prokaryotes undergo the process of binary fission. Growth of new membranes during the cellular growth and expansion in a prokaryote occurs by 2 mechanisms **1)** along the center of the cell or **2)** apically.



Credit: [Y tambe GFDL or CC-BY-SA-3.0, via Wikimedia Commons](#)



Credit: [Tobias Vornholt \[CC BY-SA 4.0\], via Wikimedia Commons](#)

## Introduction: Meiosis

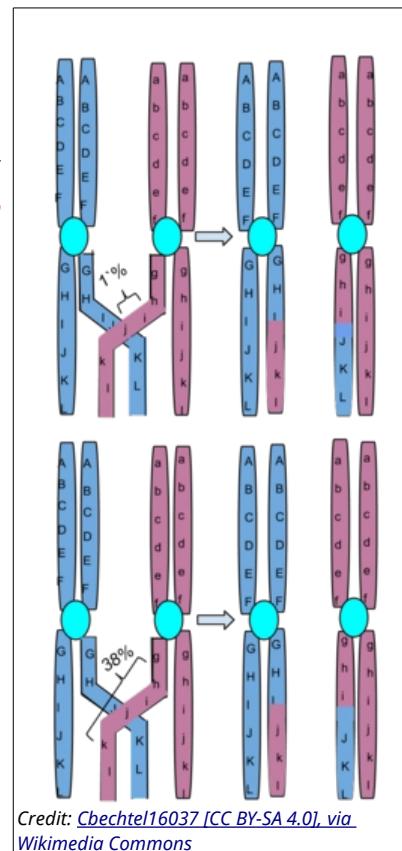
Meiosis is a process of nuclear division that reduces the number of chromosomes in the resulting cells by half. Thus, meiosis is sometimes called "reductional division." For many organisms the resulting cells become specialized "sex cells" or **gametes**. In organisms that reproduce sexually, chromosomes are typically **diploid (2N)** or occur as double sets (**homologous pairs**) in each nucleus. Each homolog of a pair has the same sites or loci for the same genes. You might recognize that you have one set of chromosomes from your mother and the remaining set from your father. Meiosis reduces the number of chromosomes to a **haploid (1N)** or single set. This reduction is significant because a cell with a haploid number of chromosomes can fuse with another haploid cell during sexual reproduction and restore the original, diploid number of chromosomes to the new individual. In addition to reducing the number of chromosomes, meiosis shuffles the genetic material so that each resulting cell carries a new and unique set of genes in a process of **independent assortment**.

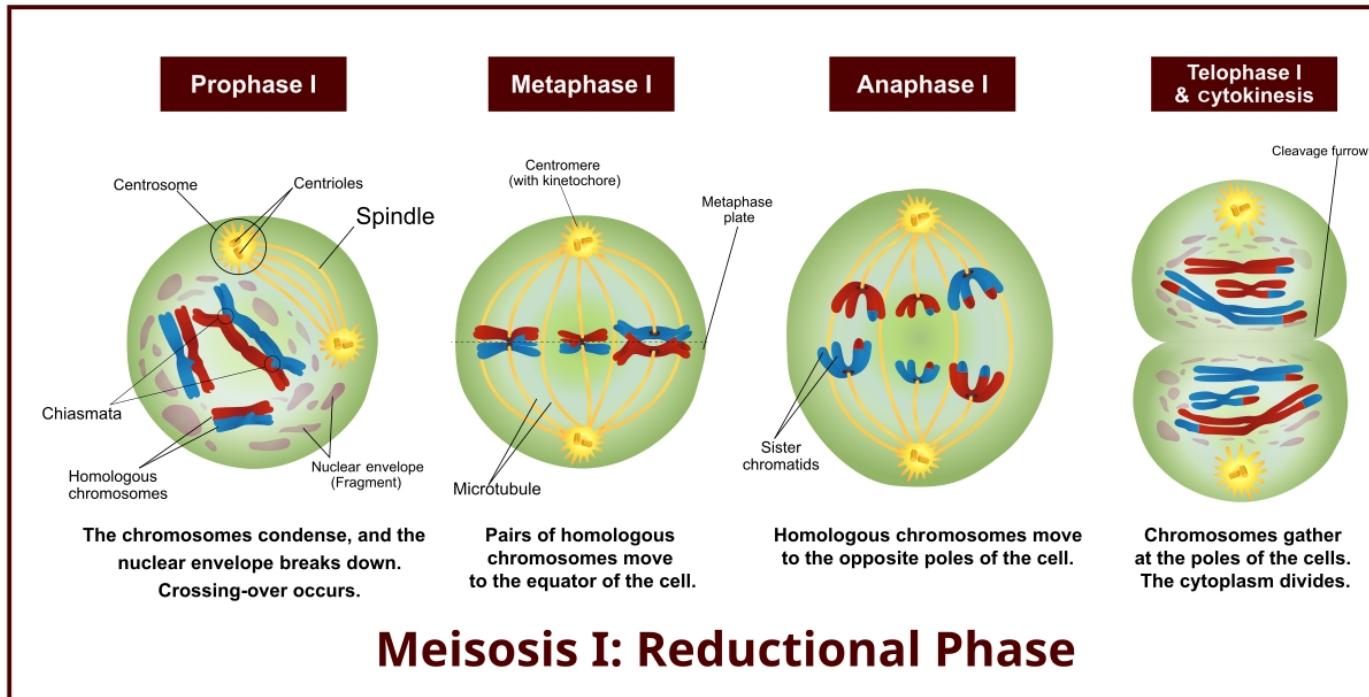
As in mitosis, meiosis is preceded by replication of each chromosome to form two chromatids attached at a centromere. However, reduction of the chromosome number and production of new genetic combinations result from two events that don't occur in mitosis. First, meiosis includes two rounds of chromosome separation. Chromosomes are replicated before the first round, but not before the second round. Thus, the genetic material is replicated once and divided twice. This produces half the original number of chromosomes.

Second, during an early stage of meiosis each chromosome (comprised of two chromatids) pairs along its length with its homolog. This pairing of homologous chromosomes results in a physical touching called **synapsis**, during which the four chromatids (a tetrad) exchange various segments of genetic material. This exchange of genetic material is called **crossing-over** and produces new genetic combinations. During crossing-over there is no gain or loss of genetic material. But afterward, each chromatid of the chromosomes contains different segments (alleles) that it exchanged with other chromatid.

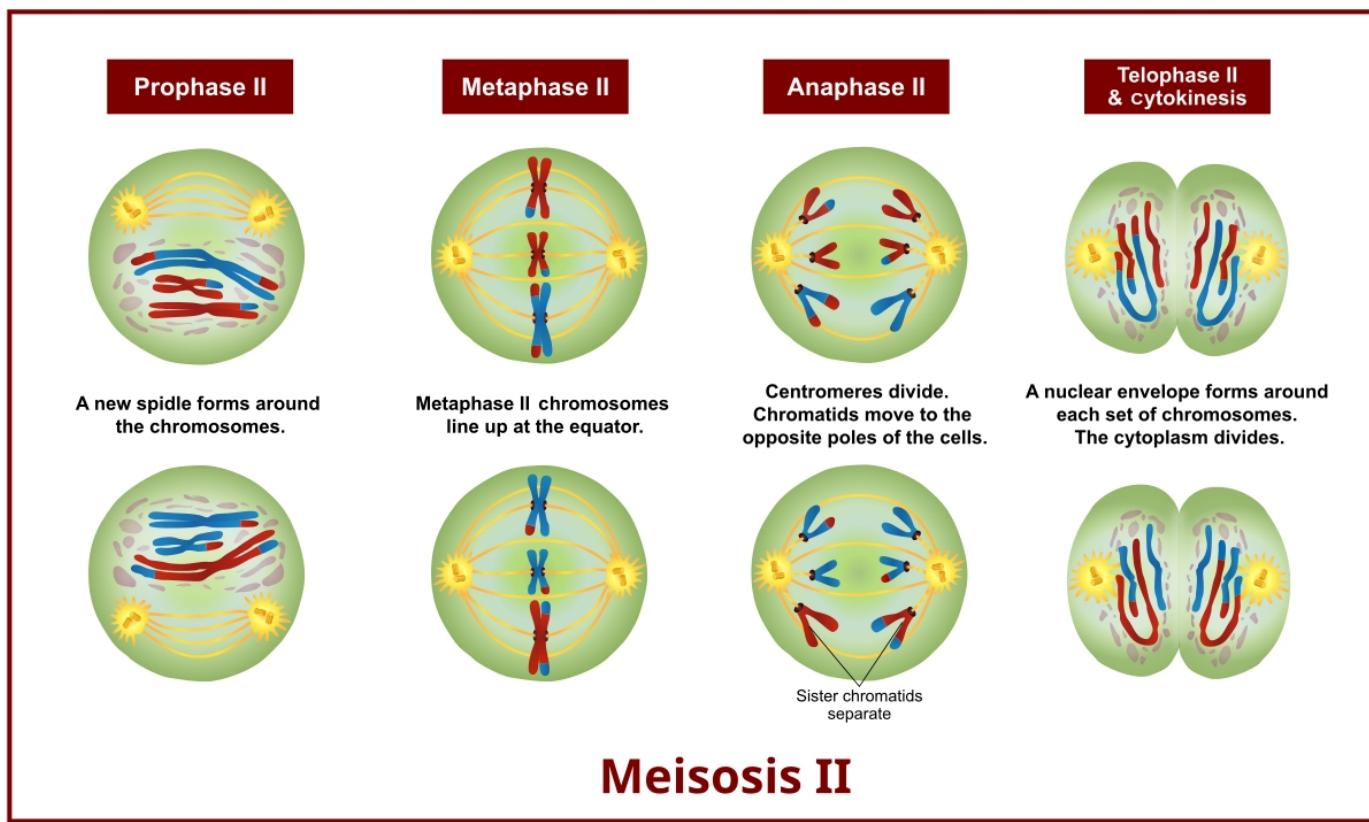
## Stages and Events of Meiosis

Although meiosis is a continuous process, we can study it more easily by dividing it into stages just as we did for mitosis. Indeed, meiosis and mitosis are similar, and their corresponding stages of prophase, metaphase, anaphase, and telophase have much in common. However, meiosis is longer than mitosis because meiosis involves two nuclear divisions instead of one. These two divisions are called Meiosis I and Meiosis II. The chromosome number is reduced (**reductional division**) during Meiosis I, and chromatids comprising each chromosome are separated in Meiosis II. Each division involves the events of prophase, metaphase, anaphase, and telophase.





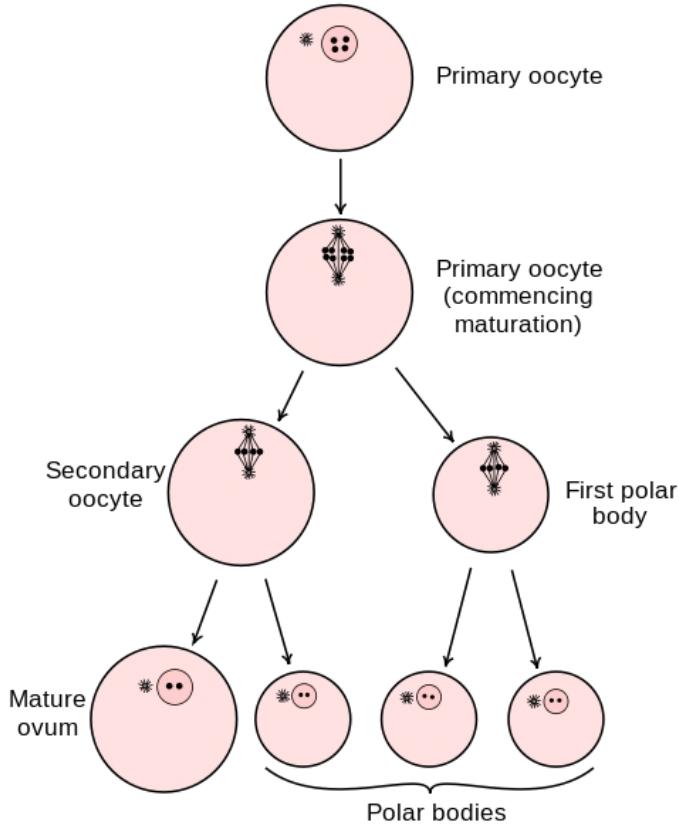
## Meiosis I: Reductive Phase



## Meiosis II

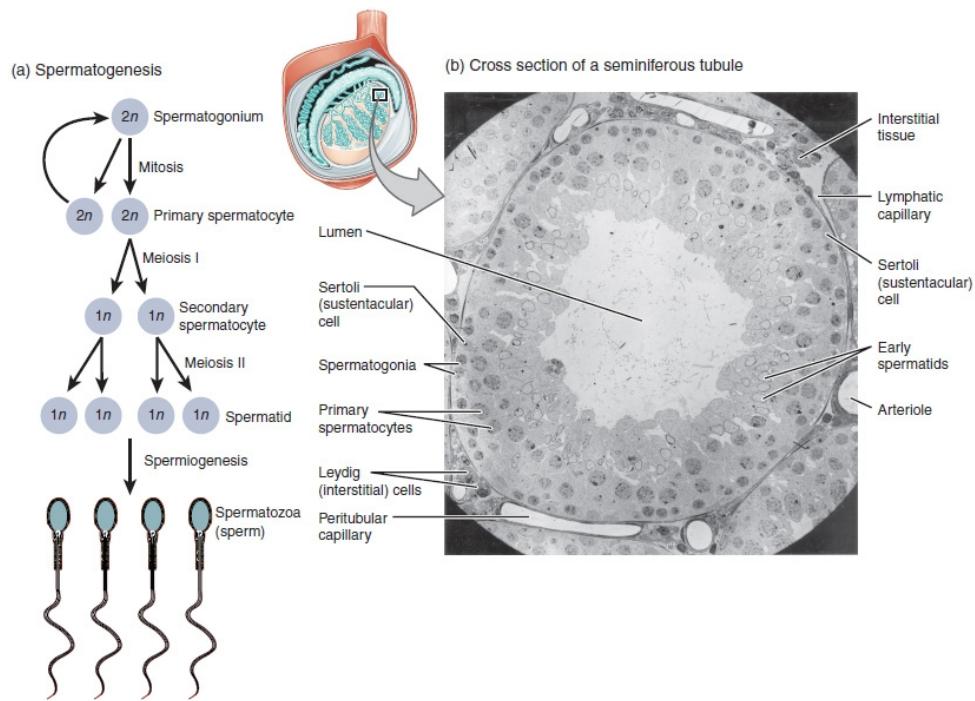
Credit: Ali Zifan [CC BY-SA 4.0], via Wikimedia Commons

## Oogenesis



Credit: [Henry Vandyke Carter \[Public domain\], via Wikimedia Commons](#)

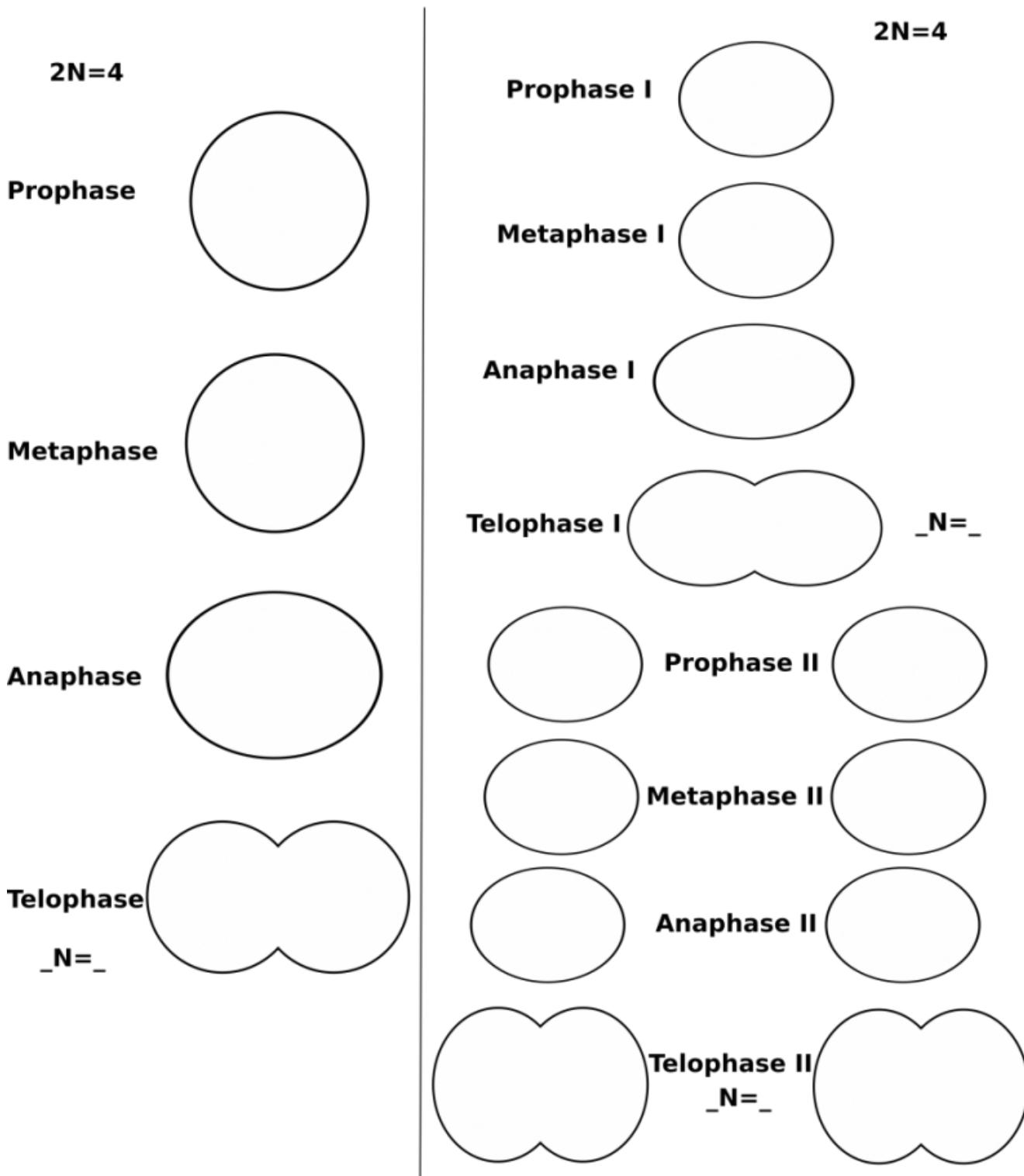
## Spermatogenesis



Credit: [OpenStax College \[CC BY 3.0\], via Wikimedia Commons](#)

# Comparing Mitosis and Meiosis

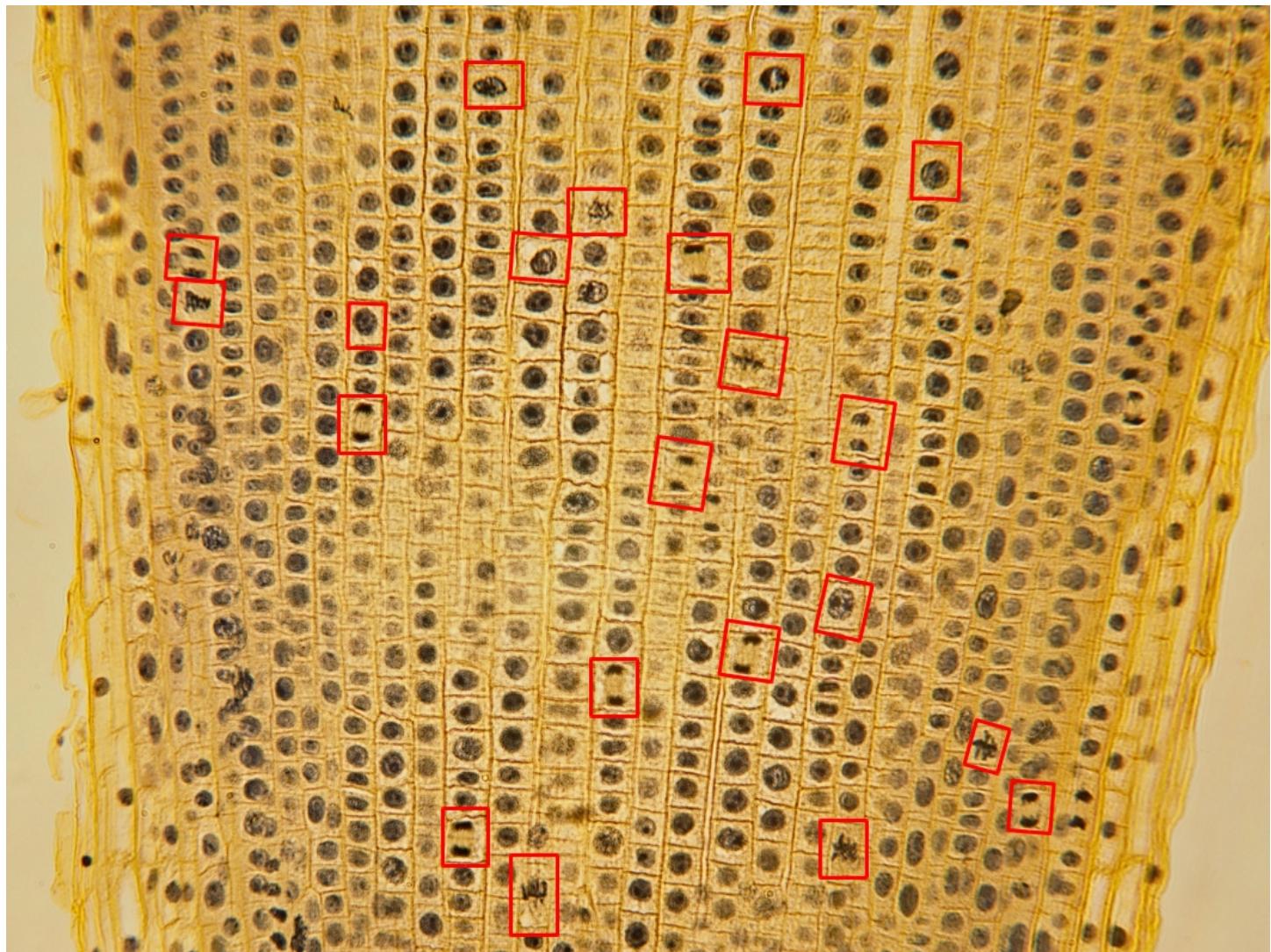
Using your pop bead knowledge, illustrate the phases below.  
Note the ploidy where indicated ( $_N=_$ )



## Summary of Cell Division

	<b>Mitosis</b>	<b>Meiosis</b>
Number of Cells at start		
Number of Cells at end		
Number of Cell divisions		
Chromosome number (N)	Start: _____ End: _____	Start: _____ End: _____
Number of chromosomes in the cell at start of process (human cell)		
Number of chromosomes in the cell at end of process (human cell)		
Daughter cells (N or 2N)		
Daughter cell genetics (identical or non-identical)		
Purpose of division		

## Identifying mitotic phases



*Identifying phases of mitosis in onion root tip. Click on the image to view larger image. [The original figure without red boxes](#)*

## Activity: Estimating the Time Spent in the Phases of Mitosis

Cells in your body reproduce at different rates. Skin cells reproduce frequently (about once per day); liver cells reproduce rarely (about once per year). Some specialized cells like nerve and muscle cells almost never reproduce and are in a special stage called G<sub>0</sub>. The whole process of mitosis, prophase to telophase, takes approximately 90 min. In plants, an area of rapid growth is the tips of roots. This exercise uses onion root tips to illustrate the amount of time spent in each phase of mitosis.

1. Work as a team to look at onion root tips under the microscope. This area of the root is undergoing rapid cell reproduction.
  - If time is short, use the slides below
    - [slide 1](#) ; [slide 2](#) ; [slide 3](#) ; [slide 4](#) ; [slide 5](#) ; [slide 6](#) ; [slide 7](#)
2. Identify the phases of the cell cycle for 20 randomly chosen cells. Record this information in the table.
3. Trade results with 3 other people
4. In an onion root tip, the entire cell cycle takes about 12 hours or 720 minutes
5. Calculate the percentage of time spent in each phase by counting the total number of cells in each phase (total in interphase, in prophase, etc.) and dividing each by the total number of cells you counted.
6. Multiply the percentage of time in each phase by the total time of the cell cycle (720 minutes) and this gives you an estimate of the time spent in each phase.

### Number of Cells in each phase

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
You (25)						
Partner 1 (25)						
Partner 2 (25)						
Partner 3 (25)						
Totals						

### Estimate of time spent in each phase of the cell cycle

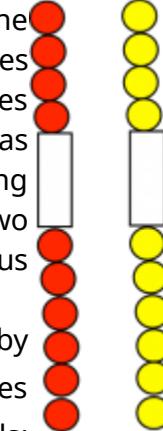
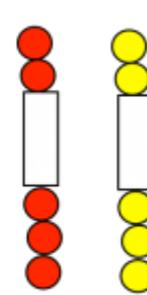
	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
% of cells in each phase						100%
Time estimate						720 minutes

### Test yourself at home

Use the following resource [https://bio.rutgers.edu/~gb101/lab2\\_mitosis/section1\\_frames.html](https://bio.rutgers.edu/~gb101/lab2_mitosis/section1_frames.html) to test yourself and practice without a microscope.

## Mitosis Simulation with Pop-it Beads

Pop-beads are small, colored beads that can be joined together to simulate chromosome strands. We will use the pop-it beads to simulate the process that chromosomes undergo during cell division. Imagine that the beads represent long stretches of DNA that comprises the genetic instructions for the cell. Start with a cell with a chromosome number of 4 (4 chromosomes, or 2 homologous pairs). We will use red to identify the chromosome from the mother and yellow for the chromosome from the father.

1. Make 4 chromosomes: two long chromosomes (one red and one yellow) and two short chromosomes (one red, one yellow). The two long chromosomes should each have the same number of beads, as should the two short chromosomes. The two long chromosomes are one homologous pair; the two short chromosomes are the second homologous pair.  

2. *Simulate S phase:* Replicate your chromosomes by making an identical set of pop-it bead chromosomes (you should have a total of eight pop-it bead strands; four long and four short). Attach the identical replicas (chromatids) by their magnetic centromeres.  

3. *Simulate Mitosis:* Move the “chromosomes” through each of the four stages of mitosis. Draw and label the pop- bead chromosomes for ONE of the phases on a separate sheet. (It is not necessary to draw each individual bead.)
4. Draw a large circle on the paper to represent the cell and the nucleus with a pencil.
5. Place your chromosomes in this and walk through each stage of Meiosis.
6. Erase the outlines as you go along and re-draw the boundaries of nuclei.
7. Document each stage with your cell phone
  1. Prophase
  2. Metaphase
  3. Anaphase
  4. Telophase

## Meiosis Simulation with Pop-it Beads

Repeat exercise but simulating Meiosis

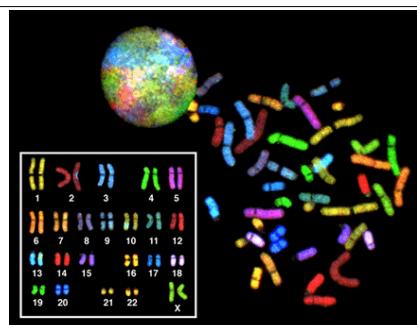
## Chromosomal Features

Chromosomes in Interphase are not visible individually. In preparation for nuclear division (mitosis or meiosis), they begin to organize tighter and condense in preparation for movement to subsequent daughter nuclei. The animation below illustrates the process of histone packaging and the molecular visualization of DNA replication.

**Histones** are proteins that aid in packaging of the chromosomes into organized coils that give rise to the recognizable chromosomes during metaphase.

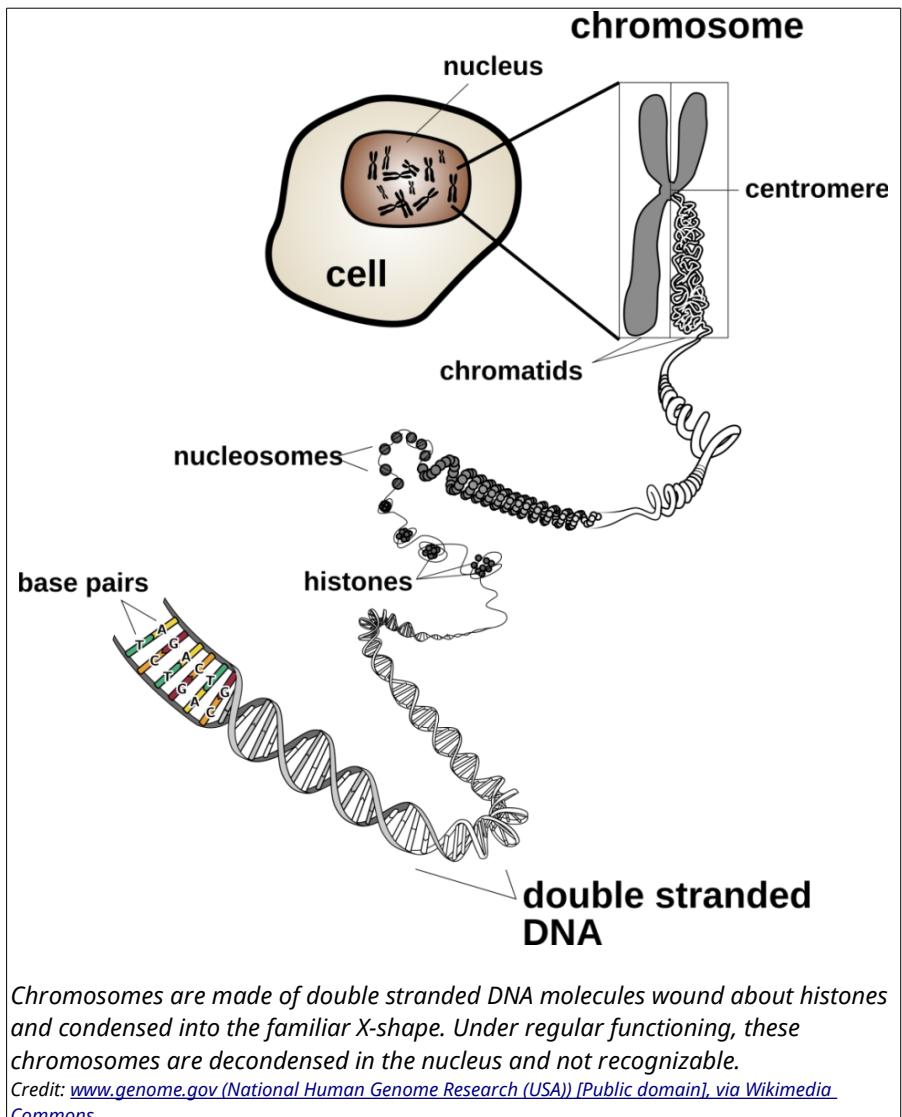
<https://youtu.be/OjPcT1uUZiE>

Large-scale genomic rearrangements result in genetic abnormalities. Biologists utilize a technique called a **chromosome spread** followed by a **karyotype** or **karyogram**. To make a chromosome spread, one blocks the progression of mitosis at metaphase where chromosomes are condensed into the structures we are familiar. A karyotype analysis is an arrangement of the chromosome spread into the homologous pairs of chromosomes.



A "spectral" karyotype of a female nucleus. Each homologous pair is "painted" to differentiate them.

Credit : National Human Genome Research Institute [Public domain], via Wikimedia Commons



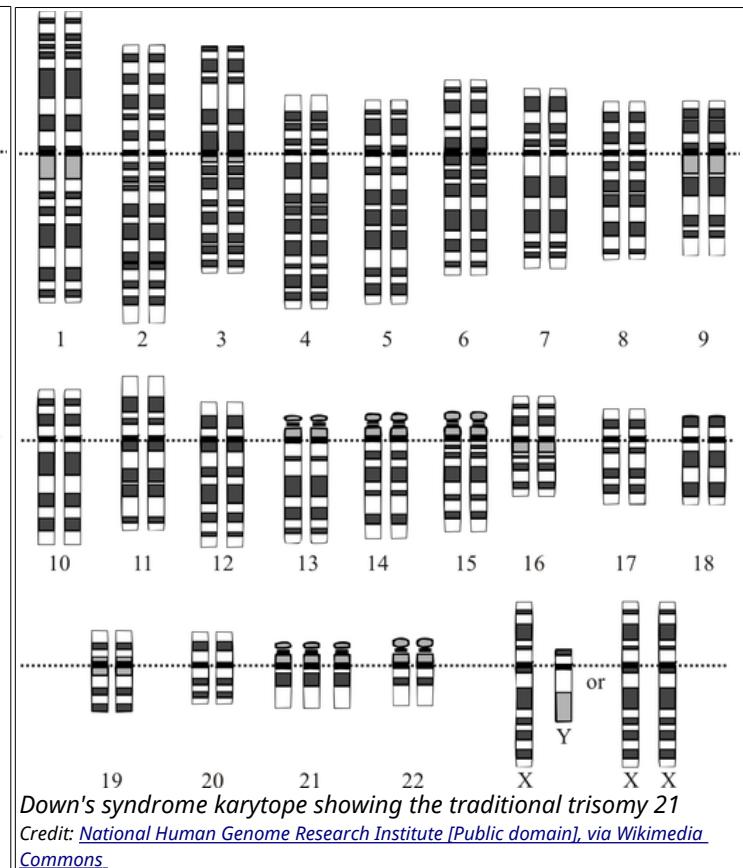
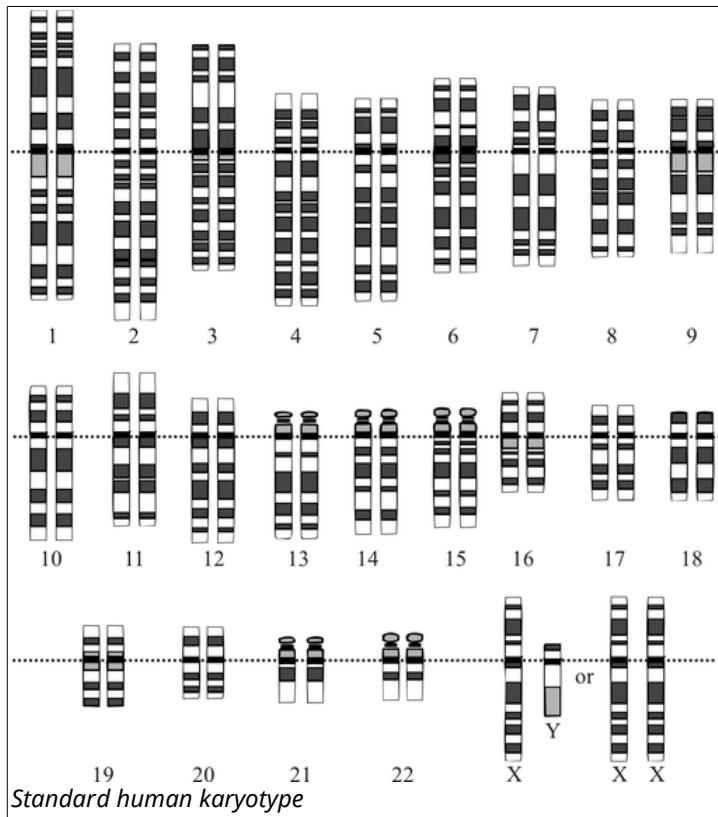
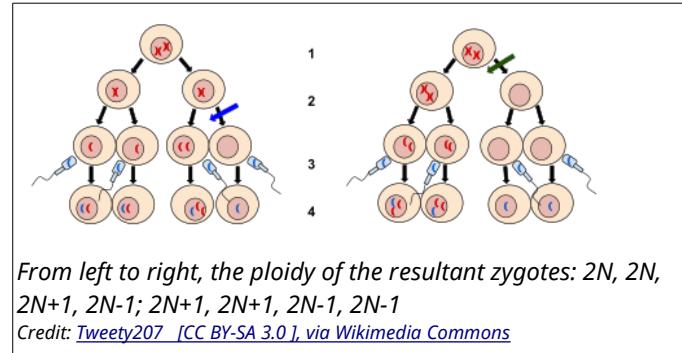
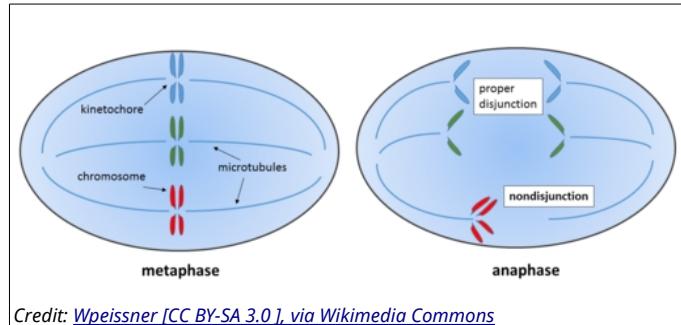
*Chromosomes are made of double stranded DNA molecules wound about histones and condensed into the familiar X-shape. Under regular functioning, these chromosomes are decondensed in the nucleus and not recognizable.*

*Credit: [www.genome.gov](http://www.genome.gov) (National Human Genome Research (USA)) [Public domain], via Wikimedia Commons*

Events associated with the improper separation of chromosomes during metaphase results in an alteration of chromosome number in the subsequent generation of cells. Using the Pop-beads, we can understand better how the timing of these events will lead to differences in the karyotype.

## Abnormal Karyotypes

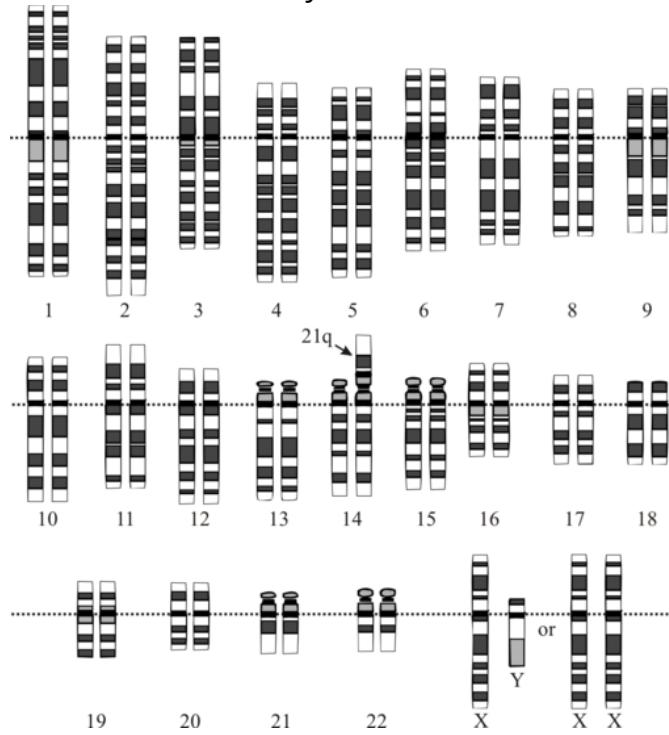
Down's Syndrome is a common genetic abnormality referred to as Trisomy 21. Instead of having the complement of 46 chromosomes of 22 homologous pairs plus 2 sex chromosomes, there are 47 chromosomes consisting of an additional Chromosome 21.



The appearance of extra or missing chromosomes arises during meiosis in an event called **nondisjunction**. After fertilization, a zygote with an improper chromosome complement occurs.

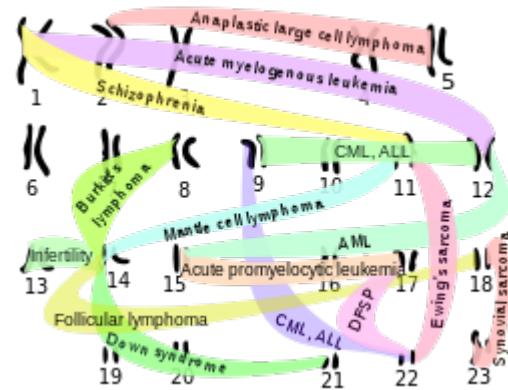
## Translocation

**Translocation** is the movement of a piece or a whole chromosome onto another chromosome. The acrocentric nature of Chr 21 and its small size makes it prone to an event called **Robertsonian Translocation** whereby two acrocentric chromosomes fuse.



Down's Syndrome from a 14:21 translocation.

Credit: National Human Genome Research Institute [Public domain], via Wikimedia Commons



Abnormalities in mitosis also occur and can result in diseases from translocations.

Credit: Häggström, Mikael (2014). "Medical gallery of Mikael Häggström 2014". Wikijournal of Medicine 1 (2). DOI:10.15347/wjm/2014.008. ISSN 2002-4436. Public Domain.

## Polyplody

Some organisms and cells have entire sets of chromosomes additional to the standard  $2N$  diploid. Cells that have extra sets in the formula of  $3N$  are called **triploid**. If they are  $4N$ , they are called **tetraploid**. This is different than the case of Down's syndrome, which has a chromosome complement of  $2N+1$ . Any time there are abnormal numbers of chromosomes, cells are referred to as **aneuploid**. A special case of aneuploid occurs from having entire sets more of chromosomes - **polyploid**. Plants are especially robust in the regard of polyploidy and often have different species arise in such a way. Some plants become sterile in the case of polyploidy and will not produce seeds properly.

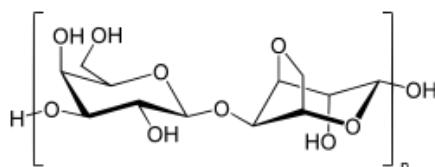


*Wild bananas or plantains (*Musa acuminata* or *Musa balbisiana*) are deemed inedible because of their large seeds.*

*Credit: Warut Roonguthai [GFDL or CC BY-SA 3.0], via Wikimedia Commons*

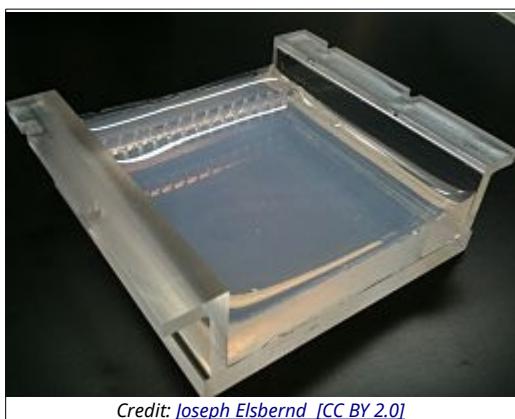
Have you ever seen a banana with these large seeds? The answer is most likely "No!" since these are not regarded as being edible. However, due to selective breeding practices, most edible plantains and bananas are hybrids of the two species *Musa acuminata* or *Musa balbisiana* that are  $3N$  or  $4N$ . In this case, the fruit are sterile and the seeds don't develop. Other seedless fruit are also developed this way and require propagation through clonal means.

## Agarose Gel Electrophoresis



Credit: Yikrauul [Public domain]

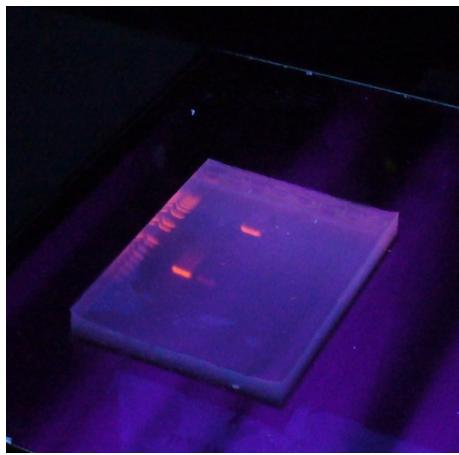
**Agarose** is a linear carbohydrate polymer purified from the cell walls of certain species of algae. Agar is a combination of the crude extract that contains agarose and the smaller polysaccharide agarpectin. When dissolved and melted in liquid, agarose strands become tangled together to form a netting that holds the fluid in a gel. Reduction of the fluid creates a higher percentage gel that is firmer and contains smaller pores within the netting.



Credit: Joseph Elsbernd [CC BY 2.0]

Placing a **comb** within the melted agarose creates spaces that allow for the insertion of samples when the gel is solidified. Molecules can traverse through the pores as they are drawn by electrical currents. Charged compounds will migrate towards the electrode of opposite charge but migration rate will be influenced by the size of the molecules. Smaller compounds can easily traverse through the webbing while larger items are retarded by the pore size. [Follow this simulation](#) to get a better idea of how we use **Agarose Gel Electrophoresis** in molecular biology to study DNA fragments.

DNA molecules are not readily visible when **resolved** (separated) on an agarose gel. In order to visualize the molecules, a DNA dye must be administered to the gel. In research labs, a **DNA intercalating agent** called Ethidium Bromide is added to the molten gel and will bind to the DNA of the samples when run. Ethidium Bromide can then be visualized on a UV box that will fluoresce the compound and reveal bands where DNA is accumulated. Since Ethidium Bromide is known as a carcinogen, teaching labs will use a safer DNA intercalating agent known as Sybr Green. This can be visualized in a similar fashion, but will fluoresce a green color instead.



Credit: TransControl [GFDL or CC-BY-SA-3.0]



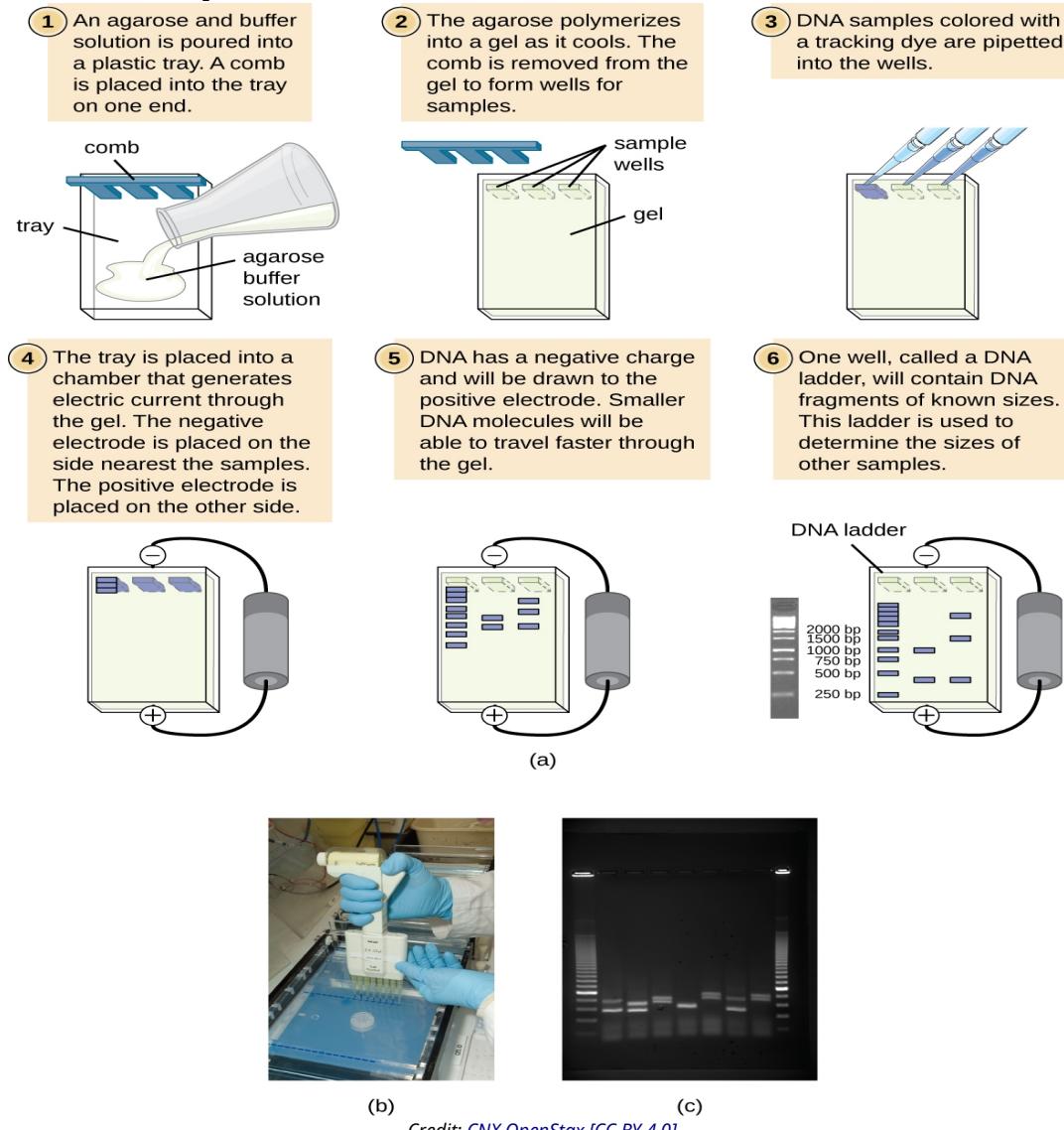
Credit: By Maryfilip98 [CC BY-SA 4.0]

(**TBE**) or Tris-Acetate-EDTA (**TAE**). Regardless of buffer solution, the buffer provides necessary electrolytes for the current to pass through and maintain the pH of the solution.

DNA samples are prepared in a buffer similar to the solution that it will be run in to ensure that the phosphate backbone of the DNA remains deprotonated and moves to the positive electrode.

Additionally, **glycerol** or another compound is added to this buffer in order for the solution to sink into the wells without spreading out. A dye is often included in this loading buffer in order to visualize the loading in the wells and to track the relative progression of gel.

## Agarose Gel Set-up



[https://upload.wikimedia.org/wikipedia/commons/8/84/Agarose\\_Gel\\_Electrophoresis -- Assembling\\_the\\_Rig\\_and\\_Loading-Running\\_the\\_Gel.webm](https://upload.wikimedia.org/wikipedia/commons/8/84/Agarose_Gel_Electrophoresis -- Assembling_the_Rig_and_Loading-Running_the_Gel.webm)

## External Resources

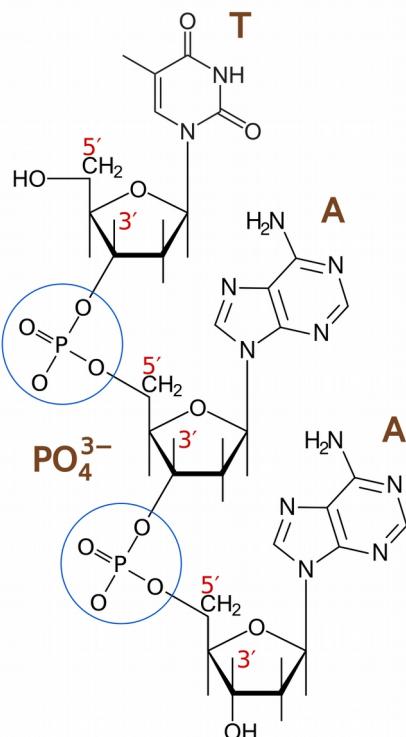
- [Gel Electrophoresis Simulation](#)
- The Structure of DNA <http://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397>
- <http://learn.genetics.utah.edu/content/science/forensics/>

## Electrophoresis of Dyes (activity)

1. Prepare a 1% agarose gel by adding 60ml Tris-Borate-EDTA buffer (TBE) to 0.6g agarose in an erlenmeyer flask
2. Place flask in microwave or on heat until agarose is melted
  - stop periodically and swirl solution and do not permit to boil over
3. Assemble the casting tray by blocking the ends with tape or plastic gaskets
4. Place the comb into the center of the casting tray
5. You may place the casting trays inside a refrigerator and pour the solution into the tray
6. Wait until the gel is solidified
7. Carefully separate the gaskets from the tray
8. Remove the comb and place the casting tray into an electrophoresis chamber
9. Cover the gel with TBE buffer
10. Using a micropipettor, load 40-50 $\mu$ l dye samples sequentially into the wells
11. Cover the electrophoresis chamber with the lid and ensure good contact between electrodes
  - It is conventional that the **POSITIVE** side of the tank is nearest to you
  - With the **POSITIVE** side nearest to you, load the samples from left to right
12. Set the power supply to 100-120V and press the Run button (you should see bubbles at each electrode) and allow to run for at least 40 minutes
13. After 40 minutes, stop the current and remove the gel in casting tray
14. Place tray on a white background and document your gel

## Activity Follow-up

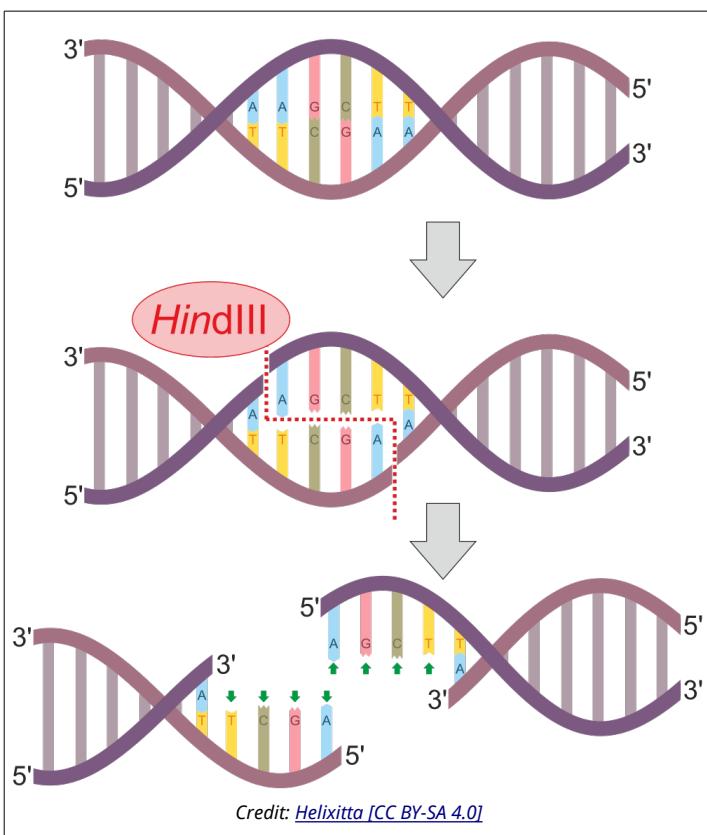
1. What colors were the dyes originally before loading into the wells?
2. How many separate bands of dye are in each well following the run?
3. What does it mean that there are multiple bands in a lane? What does it mean that there is only one band in a lane?
4. What does the length of migration illustrate to us about the properties of the dye molecules?
5. In which direction did the dye molecules migrate? What does the direction of migration indicate about the analytes?
6. Are there lanes where there are multiple bands of the **SAME** color?



Credit: G3pro [GFDL or CC-BY-SA-3.0]

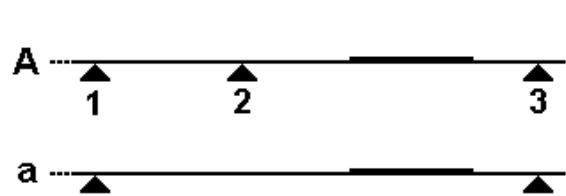
DNA can be cut by **restriction endonucleases (RE)**. Endonucleases are enzymes that can hydrolyze the nucleic acid polymer by breaking the **phosphodiester** bond between the phosphate and the pentose on the nucleic acid backbone. This is a very strong covalent bond while the weaker hydrogen bonds maintain their interactions and double strandedness.

As the name implies, restriction endonucleases (or restriction enzymes) are “*restricted*” in their ability to cut or digest DNA. The restriction that is useful to biologists is usually **palindromic** DNA sequences. Palindromic sequences are the same sequence forwards and backwards. Some examples of palindromes: RACE CAR, CIVIC, A MAN A PLAN A CANAL PANAMA. With respect to DNA, there are 2 strands that run antiparallel to each other. Therefore, the reverse complement of one strand is identical to the other. Molecular biologists also tend to use these special molecular scissors that recognize palindromes of 6 or 8. By using 6-cutters or 8-cutters, the sequences occur throughout large stretches rarely, but often enough to be of utility.



Restriction enzymes hydrolyze covalent phosphodiester bonds of the DNA to leave either “sticky/cohesive” ends or “blunt” ends. This distinction in cutting is important because an *EcoRI* sticky end can be used to match up a piece of DNA cut with the same enzyme in order to glue or ligate them back together. While endonucleases cut DNA, **ligases** join them back together. DNA digested with *EcoRI* can be ligated back together with another piece of DNA digested with *EcoRI*, but not to a piece digested with *SmaI*. Another blunt cutter is *EcoRV* with a recognition sequence of GAT | ATC.

**Restriction fragment length polymorphism (RFLP)** is a technique that exploits variations in DNA sequences. DNA from differing sources will have variations or **polymorphisms** throughout the sequence. Using **Restriction Enzymes**, these differences in sequences may be teased out. However, if one were to take the entirety of the human genome and chop it up with a restriction enzyme, many indecipherable fragments would be made. In fact, the resulting agarose gel would simply show a large smear of DNA. RFLP analysis requires that a **probe** to a specific area of DNA be used to identify specific locations. Agarose gels would be transferred to a membrane or filter where they would be **hybridized** to these radioactive probes.

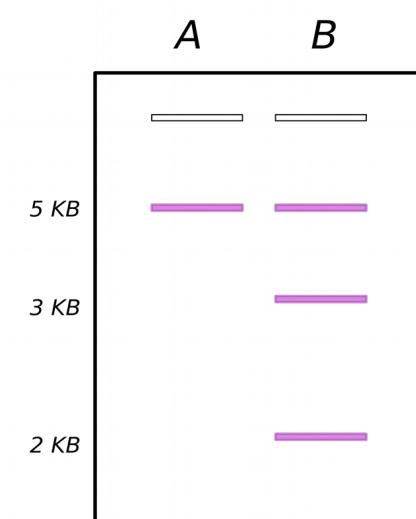


*Homologous chromosomes with restriction sites noted by triangles. the rectangle sitting on the chromosomes correspond to a probe locus.*

Credit: [PaleWhaleGail \[CC BY-SA 3.0 or GFDL\]](#)

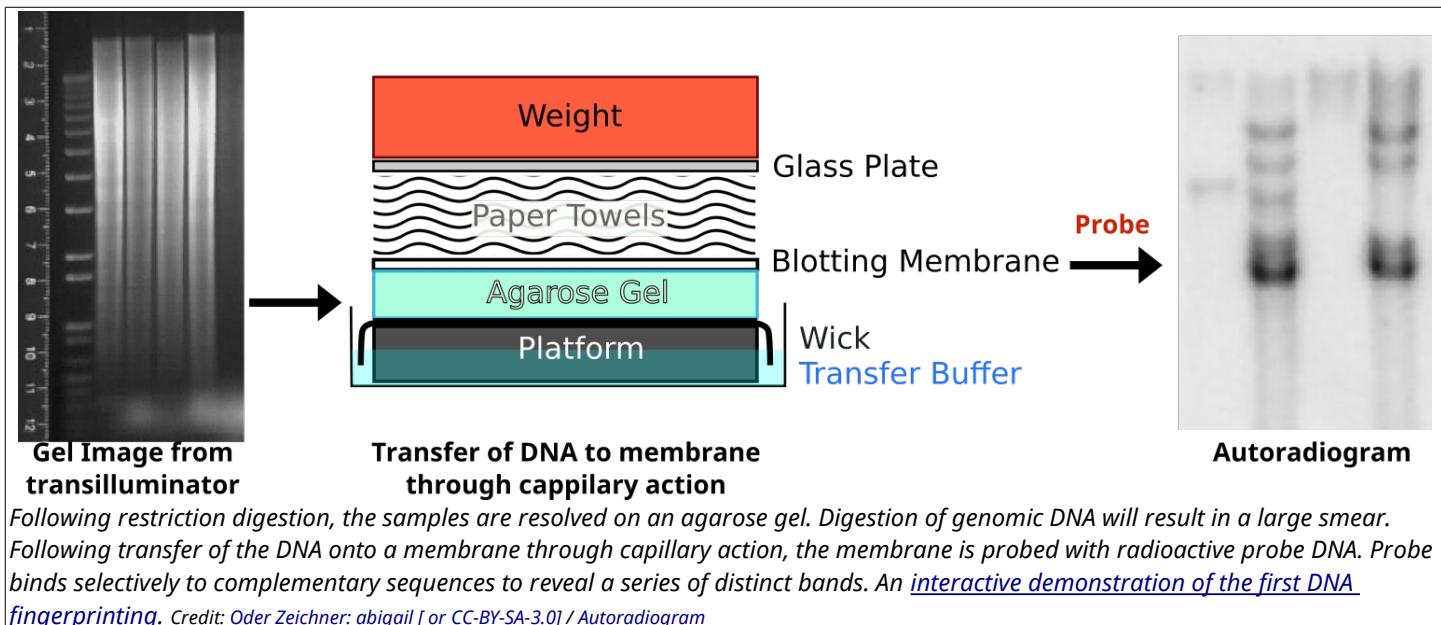
RFLP analysis was designed for forensic science to discriminate between people. Since people are 2N, they have pairs of homologous chromosomes with the same loci. However, these loci may contain different alleles. In this case, the phenotype for these alleles is the actual sequence that may or may not contain restriction sites. The presence or absence of a restriction site may arise from **single nucleotide polymorphisms (SNPs)** that reveal the natural variation between people.

The schematic below illustrates a comparison of restriction profiles between two sources. Note that the probe overlaps a restriction site in one of the alleles. This probe will be able to bind to both fragments given sufficient sequence overlap. Upon resolving on an agarose gel, genomic DNA that does not hybridize with the probe will obscure the locus of interest as a large smear. A filter is placed on top of the agarose and pressed against it to transfer the DNA in a process called **Southern Blotting**. Following a lengthy transfer, the filter is denatured to and incubated with the radioactive probe. To visualize this probe hybridization, film is exposed to the filter and processed.



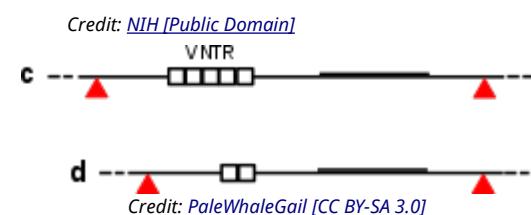
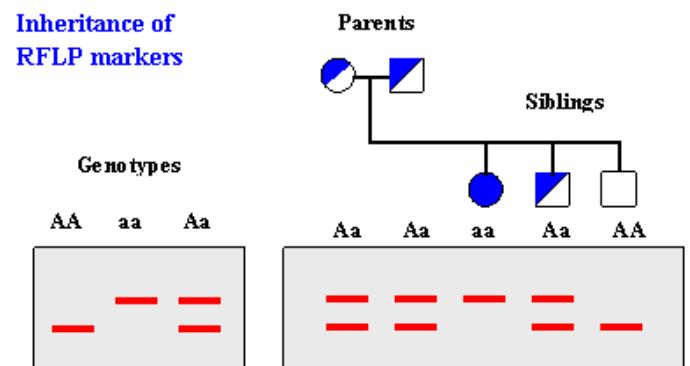
*Sample A only reveals one band after processing because this person is homologous for the same allele. Sample B is heterozygous and reveals three bands.*

Credit: [Retama \[GFDL or CC BY-SA 4.0-3.0-2.5-2.0-1.0\]](#)



RFLPs represent inheritable markers and can reveal relationships between different individuals. A pedigree can illustrate the relationship of the inherited alleles. The technique can be more informative if using multiple probes simultaneously for different loci or to use **multi-locus probes** that hybridize to multiple locations.

While RFLPs can arise from SNPs, they may also be caused by the expansion or contraction of repeated elements between restriction sites. These repeated elements of DNA are referred to as Variable Number Tandem Repeats (VNTR) and illustrate polymorphisms that normally occur in non-coding regions of the genome.



## Related Pages

- [Restriction Enzymes](#)
- [Analyzing DNA](#)
- [VNTR](#)
- [Genetics](#)

## External Resources

- [Using Tandem Repeats for DNA Fingerprinting](#) (interview)
- [The first DNA fingerprint](#) (flash simulation)

## DNA Fingerprinting (activity)

1. The origin of the DNA samples for this exercise will be explained by the Instructor as numerous scenarios may be used
2. Prepare a 1% agarose gel by adding 60ml Tris-Borate-EDTA buffer (TBE) to 0.6g agarose in an erlenmeyer flask
3. Place flask in microwave or on heat until agarose is melted
  - stop periodically and swirl solution and do not permit to boil over
4. Assemble the casting tray by blocking the ends with tape or plastic gaskets
5. Place the comb into the casting tray at the **NEGATIVE** end
6. The instructor will add 6 $\mu$ l Sybr Safe to his/her own gel solution at this time
7. You may place the casting trays inside a refrigerator and pour the solution into the tray
8. Wait until the gel is solidified
9. Carefully separate the gaskets from the tray ensuring not to tear apart the wells made by the comb
10. Remove the comb and place the casting tray into an electrophoresis chamber
11. Cover the gel with TBE buffer
12. Using a micropipettor, load 40-50 $\mu$ l dye samples sequentially into the wells
13. Cover the electrophoresis chamber with the lid and ensure good contact between electrodes
  - It is conventional that the **POSITIVE** side of the tank is nearest to you
  - With the **POSITIVE** side nearest to you, load the samples from left to right
14. Set the power supply to 100-120V and press the Run button (you should see bubbles at each electrode) and allow to run for at least 40 minutes
15. After 40 minutes, stop the current and remove the gel in casting tray
16. Slide the gels into the staining solution if they do not include Sybr Safe for visualization the subsequent meeting time
17. The instructor will slide the gel onto a UV transilluminator behind a shield and show the results to the class
  - Document the findings of the gel by photographing with your phone
  - The instructor will discuss the results and ask for you to interpret the findings

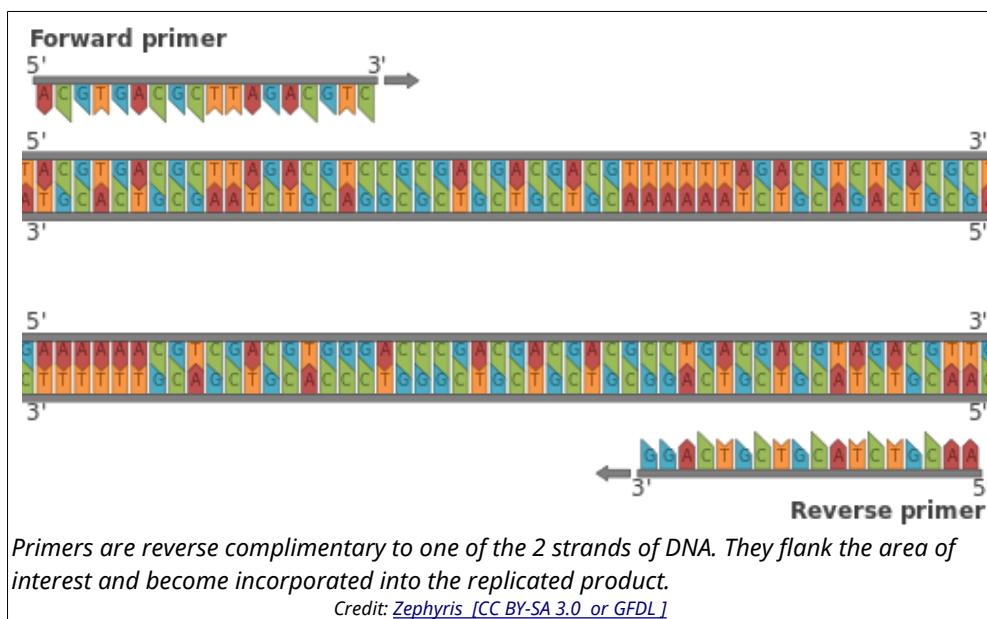
## Activity Follow-up

1. Why are the samples loaded at the negative side of the gel?
2. What is the role of the dye in these samples? Should we be alarmed that the samples are all the same color?
3. What does it mean that there are multiple bands in a lane? What does it mean that there is only one band in a lane?
4. What can we conclude from the banding patterns in this forensics or paternity case? Is this sufficient data for these conclusions?

## DNA Replication

### Polymerase Chain Reaction (PCR)

The **Polymerase Chain Reaction (PCR)** is a method of rapidly amplifying or copying a region of DNA in a tube. As the name implies, the technique uses a thermostable **DNA Polymerase** enzyme to mimic in a tube what happens within a cell during DNA replication. The chain reaction permits us to rapidly copy DNA from very minute source material in an exponential way. This technique is used in forensic science, genetic testing and cloning of rare genes. Because of the exponential copying process, a stray cell left behind can provide enough genetic material to make billions of copies of this DNA. The process of PCR can be observed in an animation found at Cold Spring Harbor Laboratory's DNA Learning Center website (<http://www.dnalc.org/resources/3d/19-polymerase-chain-reaction.html>).

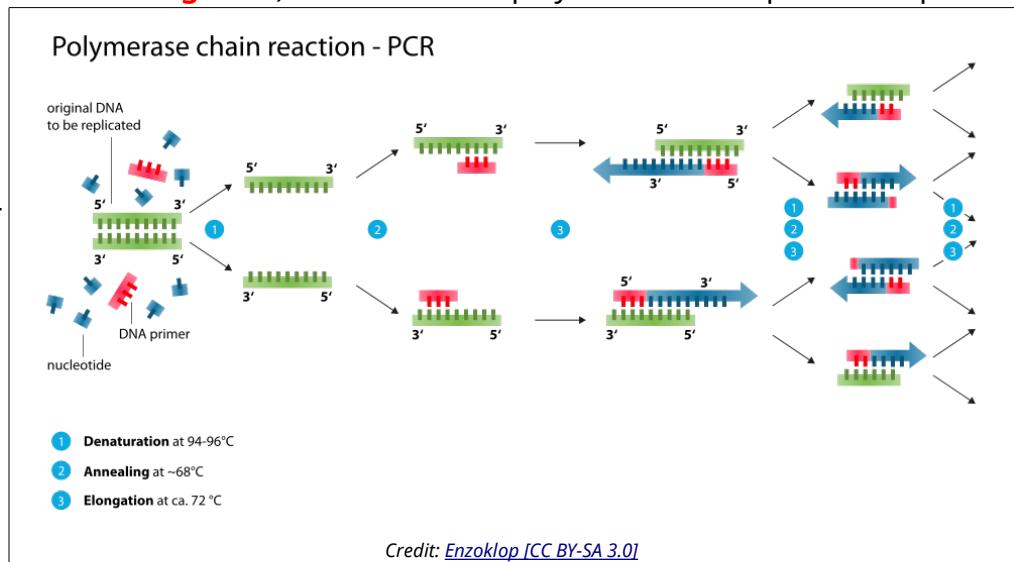


As with any DNA replication process, one needs to start off with a **template**. The template is the source material that is meant for duplication. In this process, scientists are not interested in copying the entirety of the genome, just a small segment of interest. DNA polymerases require primers to begin the polymerization process. **Primers** are designed as small oligonucleotide segments that flank the area of interest. These are short strands of DNA that reverse complement to the DNA area of interest so that the DNA polymerase has a starting point and is guided only to the DNA segment of interest. These primers tend to be about 18-24 bases long.

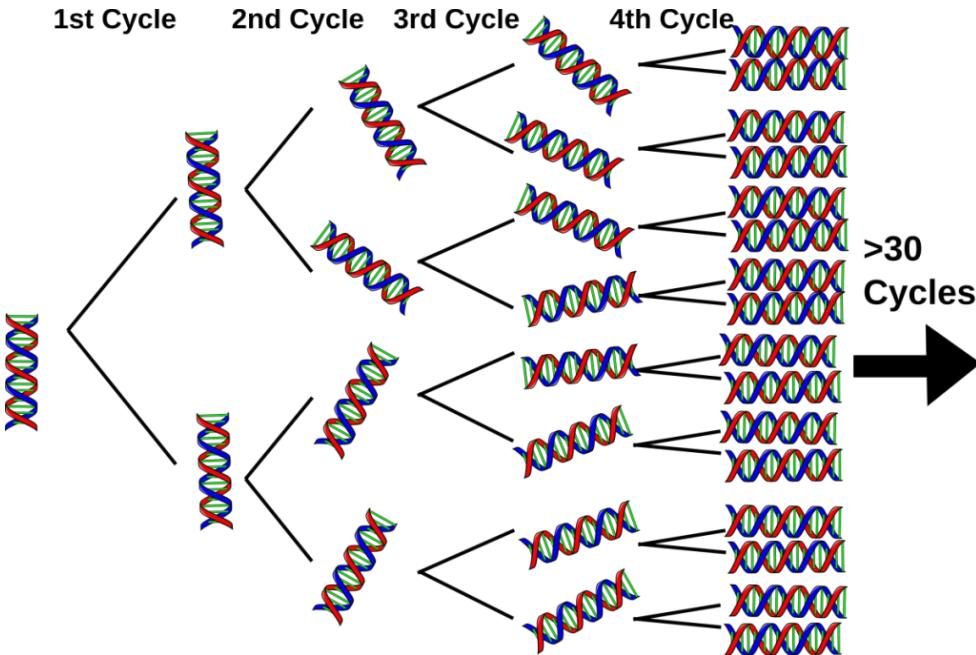
However, a double stranded DNA molecule is already base paired together into a double helix so our primers can not interact. The first step of PCR is to separate the double-stranded DNA molecule by **denaturing** the H-bonds using high heat (95°C). The primer concentrations are much higher than the original template. The next step of PCR is called **annealing**. During this step, the temperature is reduced to a temperature of about 55°C. This temperature is still hot by our standards, but is necessary to enhance the stringency of the correct base pairing of the primers to their targets on the template. The DNA Polymerase used in this process is derived from a bacteria that lives in very high temperatures

and does not denature as other proteins would under such conditions (thermostable). The original enzyme was isolated from an organism called *Thermus aquaticus*, so we call the enzyme Taq polymerase or just **Taq** for short. This bacteria lives in hot springs where the temperatures are about 50°C but it thrives at a range between 50-80°C. The temperature is raised again to a higher temperature of 72°C for the polymerase to **extend** (also called **elongation**) or continue the polymerization step from the primer.

Within this tube are all the components for the polymerase to act appropriately including buffer to maintain the pH, divalent cations like Mg<sup>2+</sup>, primers and the supply of nucleotide monomers – **dNTPs** or deoxynucleoside triphosphates (dATP, dCTP, dTTP, dGTP).



PCR is accomplished by cycling rapidly between these three steps: denature, anneal, extension. The rate limiting step is the extension which limits the length of DNA to be copied. If the original template is only a single copy, then after the completion of a cycle, we would have 2 copies. The subsequent cycle would have 4 copies, then 8, then 16, 32, and so on. The doubling process is exponential so from 1 copy undergoing 30 cycles; we would have 2<sup>30</sup> or 1,073,741,824 copies. This is over a billion copies in a few hours of time.



## Polymorphisms

The difference in nucleotide sequences between humans lies between 0.1-0.4%. That means that people are greater than 99% similar. But when you look around the room at your classmates, you can see that that small difference amounts to quite a bit of variation within our species. The bulk of these differences aren't even within the coding sequences of genes, but lie outside in regulatory regions that change the expression of those genes. Imagine if there were mutations to the coding sequences, this could be very deleterious to the well-being of the organism. We say that the coding sequences of genes that ultimately lead to proteins has a **selective pressure** to remain the same. The areas outside of the coding sequences have a reduced and sometimes non-existent selection pressure. These areas are allowed to mutate in sequence and even expand or contract. Areas of changes or differences are called **polymorphic** (many forms). If you were to read a repetitive set of sequences and count the repetition, you'd make mistakes and lose count. Likewise, DNA polymerase will make errors or stutter in areas of repetitiveness and produce polymorphic regions.

## Tandem Repeats

A type of polymorphism occurs due to these repeats expanding and contracting in non-coding regions. These regions are called variable number tandem repeats (**VNTRs**) or sometimes short tandem repeats (**STRs**). Any region or location on a chromosome is referred to as **locus** (loci for plural). Scientists use polymorphic loci that are known to contain VNTRs/STRs in order to differentiate people based on their DNA. This is often used in forensic science or in maternity/paternity cases. Any variation of a locus is referred to as an **allele**. In standard genetics, we often think of an allele as a variation of gene that would result in a difference in a physical manifestation of that gene. In the case of STRs, these alleles are simply a difference in number of repeats. That means the length of DNA within this locus is either longer or shorter and gives rise to many different alleles. VNTRs are referred to as **minisatellites** while STRs are called **microsatellites**.

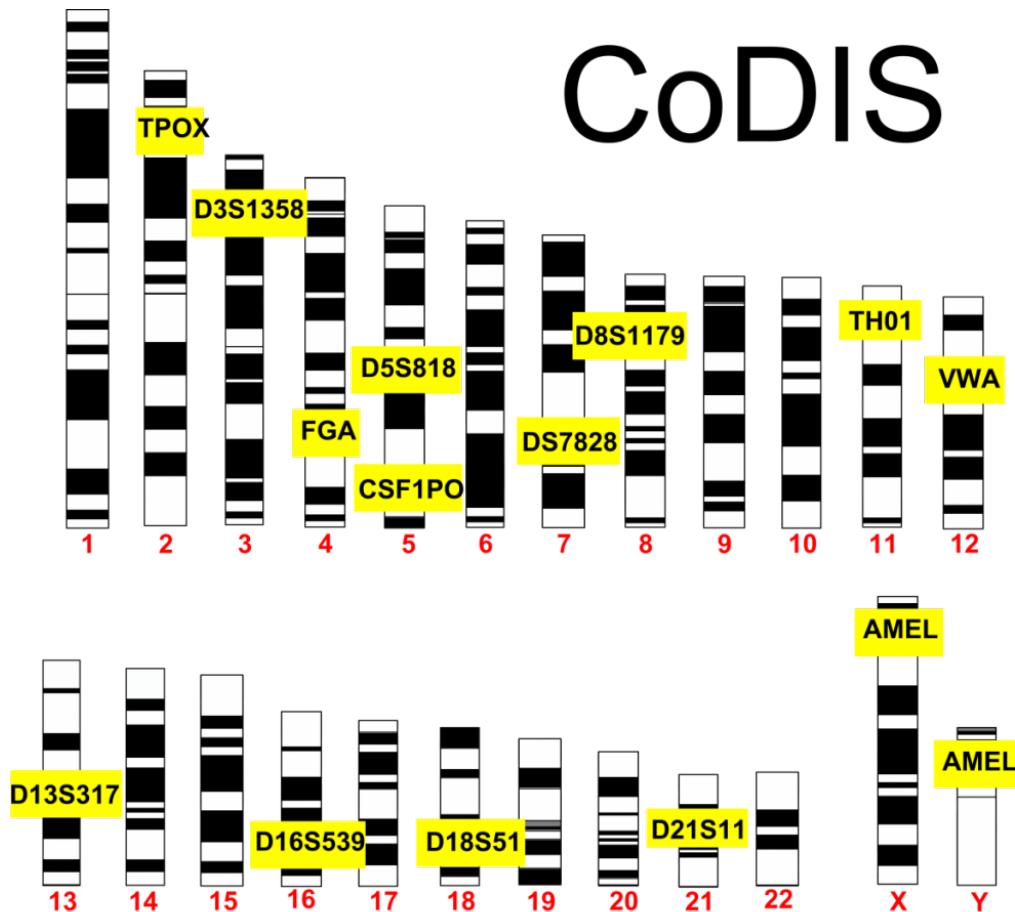
## CoDIS

The FBI and local law enforcement agencies have developed a database called the Combined DNA Index System (**CoDIS**) that gathers data on a number of STRs. By establishing the number of repeats of a given locus, law enforcement officials can differentiate individuals based on the repeat length of these alleles. CoDIS uses a set of 13 loci that are tested together. As you



Credit: Shruthi.n.christ [CC BY-SA 4.0]

would imagine, people are bound to have the same alleles of certain loci, especially if they were related. The use of 13 different loci makes it statistically improbable that 2 different people could be confused for each other. Think about this in terms of physical traits. As you increase the number of physical traits used to describe someone, you are less likely to confuse that person with someone else based on those combinations of traits. Using the CoDIS loci increases the stringency since there are many alleles for each locus. The thirteenth locus in CoDIS (called AMEL) discriminates between male and female.



**CoDIS STRs:** The FBI utilizes 13 different loci to discriminate between people. AMEL discriminates by gender and is located on the X & Y.

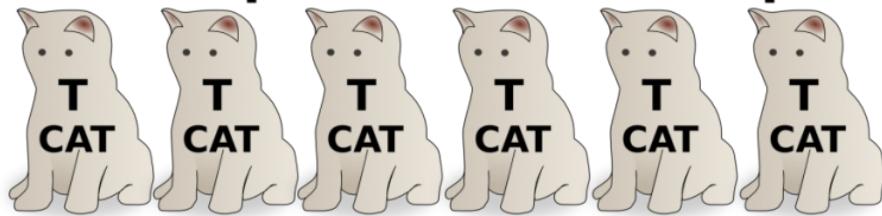
## Crime Scene Investigation

This lab uses a CoDIS locus called TH01. TH01 is a locus on chromosome 11 that has a repeating sequence of TCAT. There are reported to be between 3-14 repeats in this locus. With the exception of X and Y in a male, all chromosomes have a homologous partner. Therefore, each individual will have 2 alleles for each CoDIS locus.

**The TH01 locus contains repeats of TCAT.**

CCC **TCAT** **TCAT** **TCAT** **TCAT** **TCAT** **TCAT** **TCAT** AAA

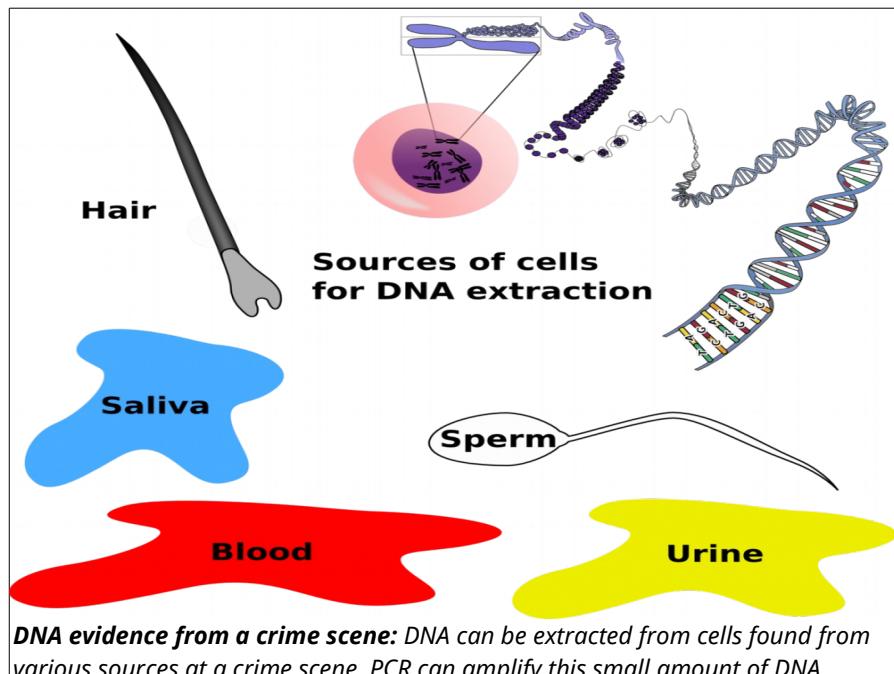
**This example has 6 TCAT repeats.**

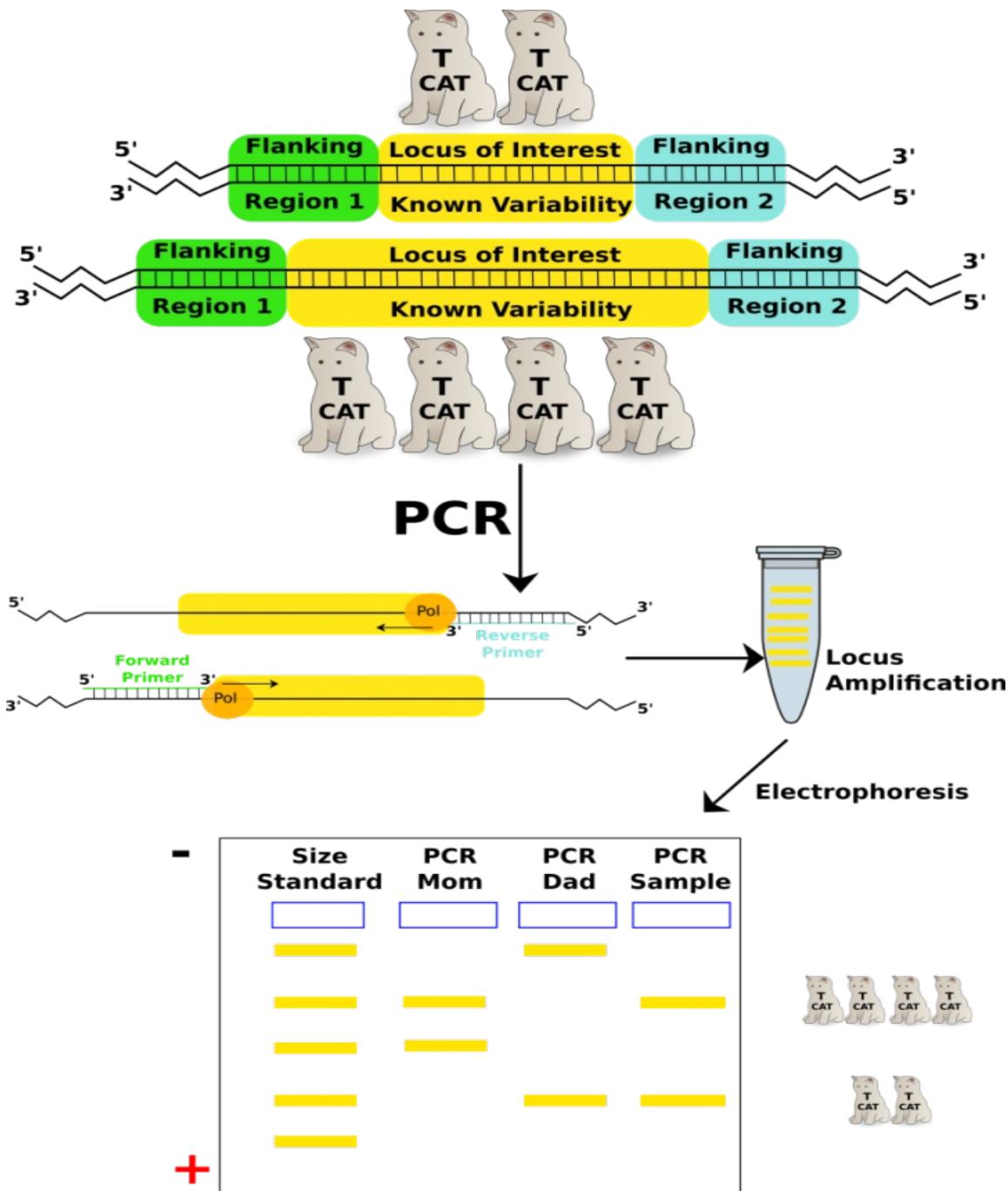


**TH01 STR:** Outside of the STR, there is flanking areas of known sequence. The primers that amplify TH01 in PCR recognize these flanking sequences to amplify the TCAT repeats.

At a crime scene, criminals don't often leave massive amounts of tissue behind. Scant evidence in the form of a few cells found within bodily fluids or stray hairs can be enough to use as DNA evidence. DNA is extracted from these few cells and amplified by PCR using the specific primers that flank the STRs used in CoDIS.

Amplified DNA will be separated by gel electrophoresis and analyzed. Size reference standards and samples from the crime scene and the putative suspects would be analyzed together. In a paternity test, samples from the mother, the child and the suspected father would be analyzed in the same manner. A simple cheek swab will supply enough cells for this test.





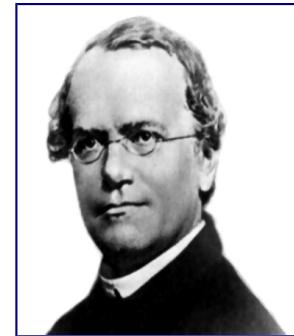
**TH01 locus used in a Paternity/Maternity test:** Individual PCR reactions are run for each sample (mom, dad, child). The TH01 primer pair specifically amplifies the locus. Each amplified sample is run on the same gel to resolve the different alleles of TH01 from each individual. From this test the sample could be the offspring from these 2 parents but use of more STRs would make it more definitive. Count the TCATs.

## External Resources

- Flash animation walking through what a STR is <http://www.dnalc.org/view/15981-DNA-variations.html>

## Writing the Rules of Heredity

In the mid 1800's, an Augustinian friar named Gregor Mendel formalized quantitative observations on heredity in the pea plant. He undertook hybridization experiments that utilized purebred or **true breeding** plants with specific qualities over many generations to observe the passage of these traits. Some of these physical traits included: seed shape, flower color, plant height and pod shape.

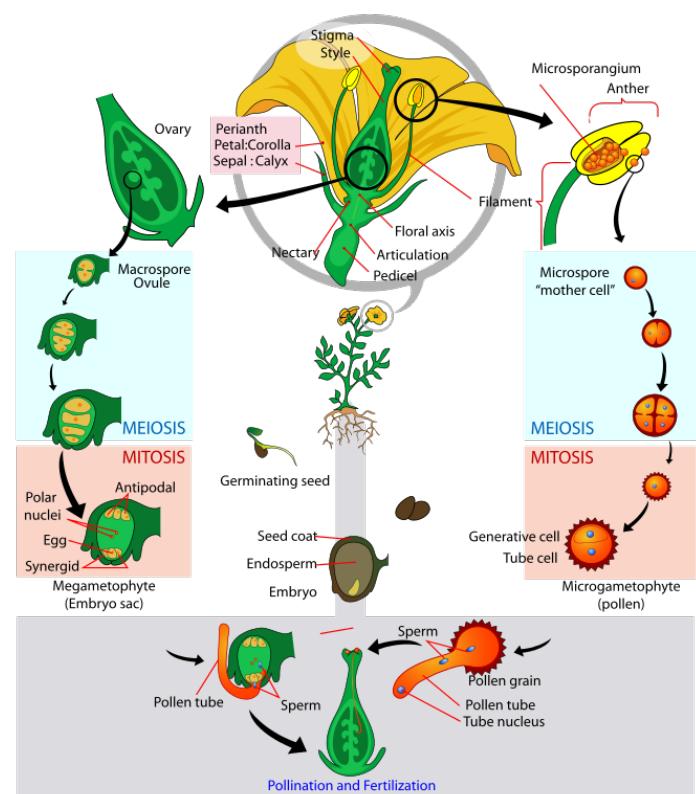


The pea plant (*Pisum sativum*) offered a great advantage of being able to control the fertilization process and having large quantities of offspring in a short period of time. In a simple experiment of tracking the passage of a single trait (**monohybrid cross**) like flower color through multiple generations he was able to formulate rules of heredity. In this case, pea plants either produced white flowers or purple flowers for many generations (true breeding purple flower or true breeding white flower). These true breeding plants are referred to as the **Parental Generation (P)**. By removing the male parts of the pea flower (anthers containing pollen), Mendel was able to control for self-pollination. The hybridization came from applying the pollen from one true breeding plant to the female part (the pistil) of the opposite true breeding plant. The subsequent offspring are referred to as the **First Filial Generation (F<sub>1</sub>)**. In the first generation, all flowers are purple. Permitting self-pollination generates a **Second Filial Generation (F<sub>2</sub>)**. This generation sees the re-emergence of the white flowered plants in an approximate ratio of 3 purple flowered to 1 white flowered plants.



*Pea flowers (Pisum sativum)*

Credit: [BmdavII /GFDL or CC BY-SA 4.0-3.0-2.5-2.0-1.0](#)



*Male and female parts of flowers. Mendel removed the anthers containing pollen to prohibit self-pollination and selectively applied the pollen to stigmas in order to control the "hybridization".*

Credit: [LadyofHats Mariana Ruiz \[Public domain\]](#)

The loss of one variant on the trait in the  $F_1$  plants with the re-emergence in the  $F_2$  prompted Mendel to propose that each individual contained 2 hereditary particles where each offspring would inherit 1 of these particles from each parent. Furthermore, the loss of one of the variants in the  $F_1$  was explained by one variant masking the other, as he explained as being **dominant**. The re-emergence of the masked variation, or **recessive** trait in the next generation was due to the both particles being of the masked variety. We now refer to these hereditary particles as **genes** and the variants of the traits as **alleles**.

Seed		Flower		Pod		Stem	
Form	Cotyledons	Color		Form	Color	Place	Size
Grey & Round	Yellow	White		Full	Yellow	Axial pods, Flowers along	Long (6-7ft)
White & Wrinkled	Green	Violet		Constricted	Green	Terminal pods, Flowers top	Short (1-1ft)
1	2	3		4	5	6	7

Credit: [LadyofHats \[Public Domain\]](#)

## Mendel's Rules of Segregation and Dominance

The observations and conclusions that Mendel made from the monohybrid cross identified that inheritance of a single trait could be described as passage of genes (particles) from parents to offspring. Each individual normally contained two particles and these particles would separate during production of gametes. During sexual reproduction, each parent would contribute one of these particles to reconstitute offspring with 2 particles. In the modern language, we refer to the genetic make-up of the two "particles" (in this case, alleles) as the **genotype** and the physical manifestation of the traits as the **phenotype**. Therefore, Mendel's first rules of inheritance are as follows:

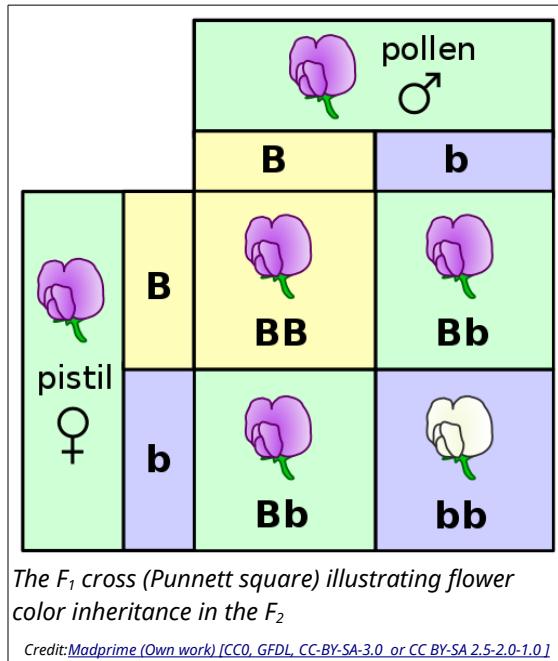
### 1. Law of Segregation

- During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene

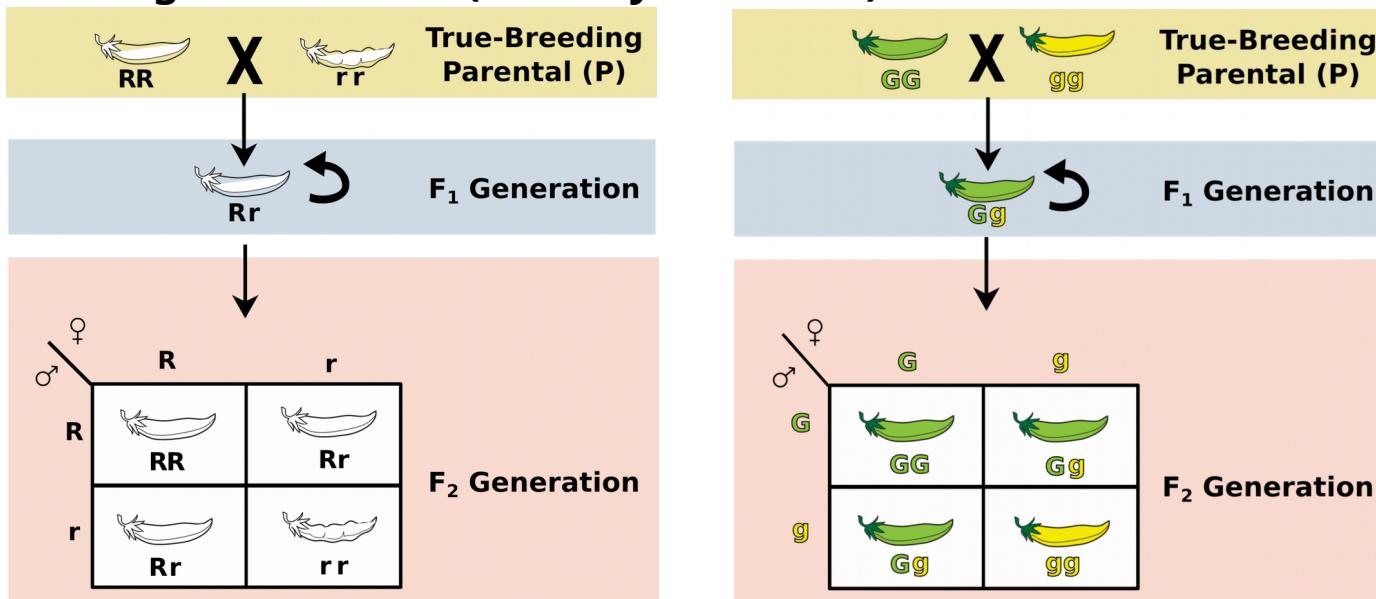
### 2. Law of Dominance

- An organism with at least one dominant allele will have the phenotype of the dominant allele.
- The recessive phenotype will only appear when the genotype contains 2 recessive alleles. This is referred to as **homozygous recessive**
- The dominant phenotype will occur when the genotype contains either 2 dominant alleles (**homozygous dominant**) or one dominant and one recessive (**heterozygous**)

The Punnett Square is a tool devised to make predictions about the probability of traits observed in the offspring in the  $F_2$  generation and illustrate the segregation during gamete formation.



## The Single Trait Cross (Monohybrid Cross)



## Corn Coloration in an F<sub>2</sub> Population (activity)



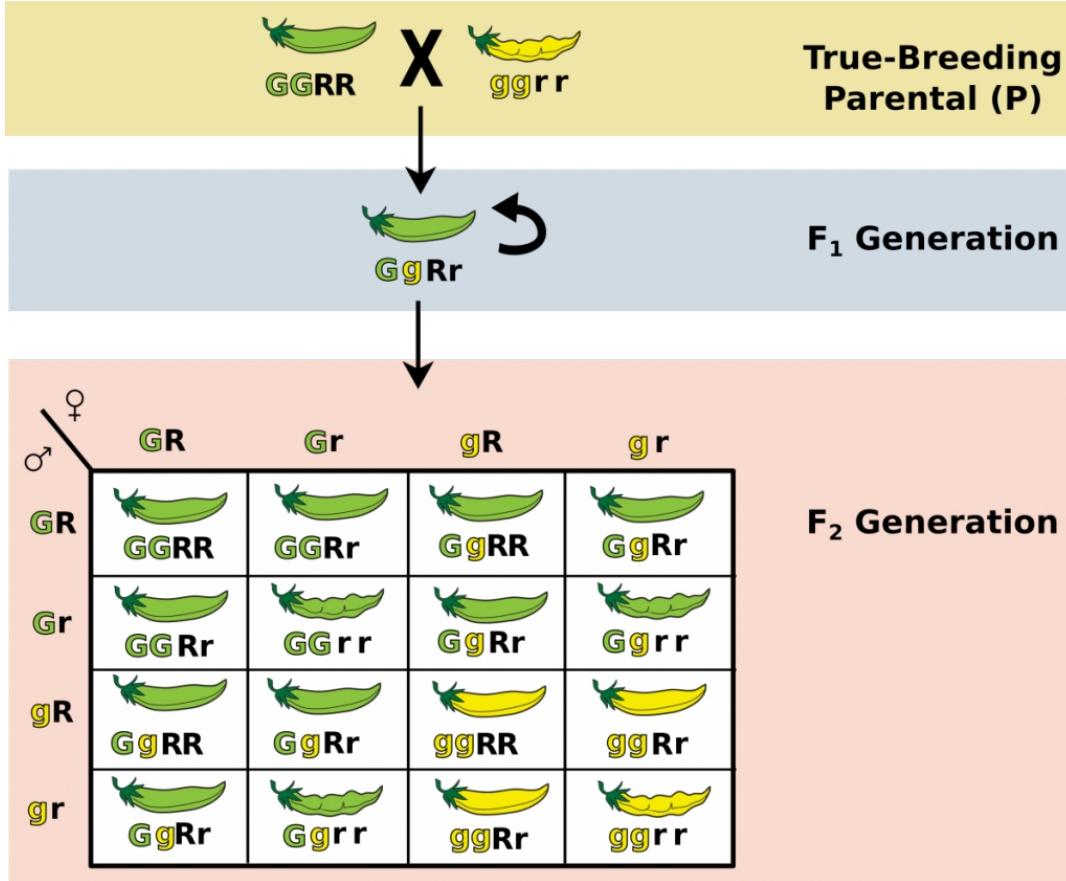
*Credit: Jeremy Seto CC-BY-NC-SA*

A corn cob contains hundreds of kernels. Each kernel is a seed that represents an individual organism. In the cob, we can easily see kernel color as a phenotype.

1. Retrieve an F<sub>2</sub> corn cob
2. Count a total of 100 kernels
  1. Tally the number of Yellow Kernels within that 100 (in the dried state, anything yellow or honey colored counts as yellow)
  2. Tally the number of Purple Kernels within that 100 (in the dried state, purple colored kernels may appear brown)
  3. Ignore any speckled kernels that may have yellow and purple within them
3. Compare numbers with the class as a whole
4. From the numbers:
  1. Is there a dominant color?
  2. Which is dominant, if there is?
  3. Create a Punnet square to illustrate the expected number of each color in a simple dominant:recessive paradigm.

## The Two Trait Cross (Dihybrid Cross)

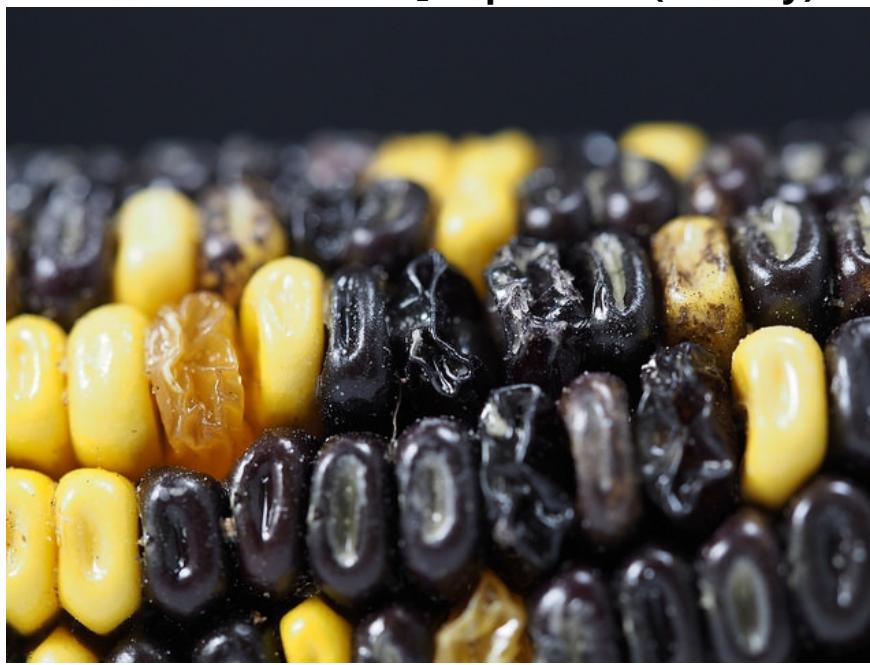
Mendel continued his experimentation where he looked at two traits. These two trait crosses are called **dihybrid crosses**. While the monohybrid cross would yield 3:1 ratios of the phenotypes, the dihybrid crosses would yield 9:3:3:1 ratios of all the combinations of each phenotype.



## Mendel's Rule of Independent Assortment

The dihybrid cross revealed another law of inheritance to Mendel. By observing the 9:3:3:1 ratio, Mendel concluded that traits were not tied to each other. That is to say, if a pea pod was yellow, it could still be either smooth or wrinkled in texture. This lack of linkage between genes yielding different characteristics was dubbed the **Law of Independent Assortment**. Genes for different traits can segregate independently during the formation of gametes.

## Kernel Coloration and Texture in an F<sub>2</sub> Population (activity)



Credit: [Jeremy Seto CC-BY-NC-SA](#)

1. Retrieve a dihybrid F<sub>2</sub> corn cob
2. Count a total of 200 kernels
  1. Tally the number of Yellow Kernels that are rounded and smooth in texture
  2. Tally the number of Yellow Kernels that are shriveled and wrinkly in texture (honey colored)
  3. Tally the number of Purple Kernels within that rounded and smooth in texture
  4. Tally the number of Purple Kernels within that are shriveled and wrinkly in texture
  5. Ignore any speckled kernels that may have yellow and purple within them
3. Compare numbers with the class as a whole
4. Each kernel constitutes an individual organism (a seed that can give rise to a whole new plant). From the numbers:
  1. Is there a dominant texture (smooth or shriveled)?
  2. Which is dominant, if there is?
  3. Is there a color that always pairs with a texture or do these characteristics *assort independently*?
  4. Create a Punnet square to illustrate the expected number of each color/texture combination in a simple dominant:recessive paradigm.

## Genetics leaves a bad taste in my mouth... or not

Some of our personal preferences arise from the way we were brought up. Culture plays a role in our likes and dislikes. Likewise, our experiences play a role in how we respond to certain stimuli. Another major factor that plays a role into our preferences comes wired in our genome. The DNA in our cells is the instruction manual for who we are. We are programmed to seek out things of a nutritive values in order to acquire raw materials like carbohydrates, proteins and lipids. In our search for nutritive compounds we have learned to avoid things that don't taste good. Bitter things have a tendency to be associated with toxic compounds in nature. When eating a food item for the first time, molecules hit our tongue and stimulate multiple sensations: sweet, sour, salty, savory and bitter. Attributed to these multiple taste types are a diverse family of receptors that bind to the molecules that result in our perception of these sensations. Something bitter might make us learn to avoid this food item in the future.

One type of bitter receptor senses the presence of a chemical called phenylthiocarbamide (**PTC**). This chemical chemically resembles toxic compounds found in plants but is non-toxic. The ability to taste PTC is comes from the gene called *TAS2R38*. This gene encodes a protein that on our tongues that communicates the bitterness of this chemical. There are two common alleles of this gene with at least five more uncommon variants. Within the two common forms, a **single nucleotide polymorphism (SNP)** is responsible for changing one amino acid in the receptor. It's this difference of one amino acid that results in the ability of the receptor to either respond or not respond to PTC. We inherit one copy of the gene from our father and one copy from our mother. Based on how our parents gametes were formed and what alleles we received during the fertilization event determines how we respond to this chemical. Because we each have 2 copies of this gene, we can utilize simple Mendelian genetics to understand which allele is dominant or recessive.

1. Place a piece of "Control" paper on the tongue and indicate if there is a taste
2. Place a piece of "PTC" paper on the tongue and indicate if there is a taste and the taste severity
3. Fill out the table for the class to identify how many non-tasters, tasters or super-tasters there are.
4. Indicate if you believe the trait is dominant or recessive (ability to taste or not taste)
5. Assign a descriptor allele for the dominant (a capital letter) or the recessive (a lowercase letter) and draw a Punnet square for the F<sub>2</sub> generation of 2 Heterozygous parents.
6. Compare the class tally of tasters and non-tasters in the class and discuss with your instructor if there is a clear dominance of this trait.

**Table: PTC Tasting Tally**

Phenotypes	Number	% Total
<b>PTC Tasters</b> (Dominant or Recessive)		
<b>PTC Non-Tasters</b> (Dominant or Recessive)		
<b>Total</b>		

### Questions:

1. How do you explain the presence of those who can't taste PTC, those who can taste it and those who really can't stand the taste of it?
2. This chemical is non-toxic and doesn't exist in nature. Do you think there is a **selective pressure** that confers an advantage to those who do taste it?

## Exercise: Coding Bitterness

Prior to this exercise, review the [Central Dogma](#).

The full coding sequence of [\*\*TAS2R38\*\*](#) is 1,002 bases (334 amino acids) long. A segment of the gene is shown below where the SNP (in red) occurs. Variant 1 is the version of the gene that encodes for the ability to taste PTC. Variant 2 is the version of the gene that is unable to bind to PTC. This SNP mutation is called a **missense** mutation because it changes the amino acid. Some mutations cause the insertion of a premature stop codon. This **nonsense mutation** results in a truncated protein and can be disastrous to the function. We already know that the simple substitution of one nucleotide translates to a change in one amino acid and determines the ability to taste PTC. Imagine if a large group of amino acids from the protein was missing.

With template strand ("Complement") information:

1. Write the sequence of the coding strand.
2. Write the sequence of the mRNA
3. Use the Genetic Code Chart to translate the amino acid sequence

### Variant 1

**Coding Strand : 5'-**

**Complement : 3'-TTC TCC GTC **CGT** GAC TCG-5'**

**mRNA : 5'-**

**Amino Acid :**

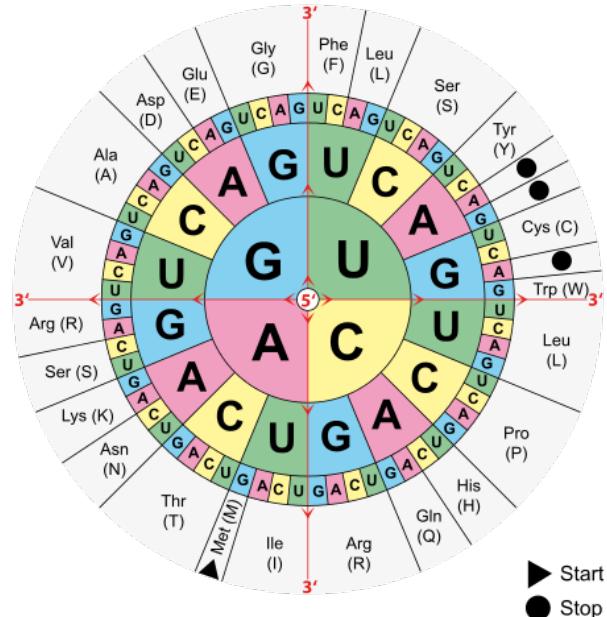
### Variant 2

**Coding Strand : 5'-**

**Complement : 3'-TTC TCC GTC **GGT** GAC TCG-5'**

**mRNA : 5'-**

**Amino Acid :**



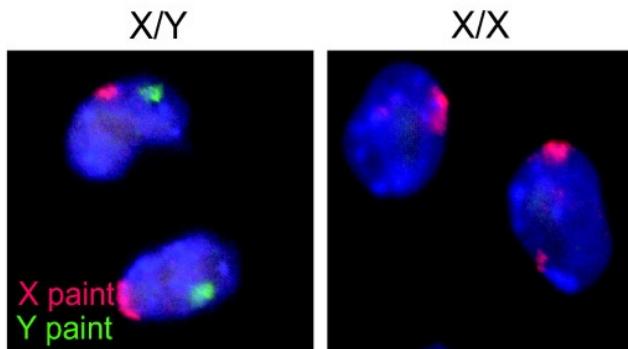
Credit: [Mouagip \[Public domain\]](#)

## Sex-Linked Genes



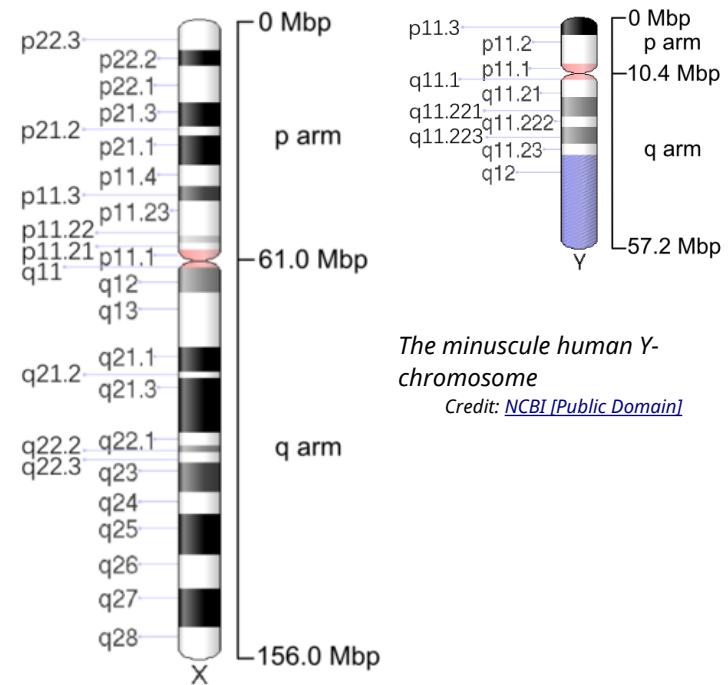
For the most part, mammals have gender determined by the presence of the Y chromosome. This chromosome is gene poor and a specific area called sex determining region on Y ([SRY](#)) is responsible for the initiation of the male sex determination. The X-chromosome is rich in genes while the Y-chromosome is a gene desert. The presence of an X-chromosome is absolutely necessary to produce a viable life form and the default gender of mammals is traditionally female.

Chromosomal painting techniques can reveal the gender origin of mammalian cells. By using fluorescent marker sequences that can hybridize specifically to X or Y chromosomes through Fluorescence In Situ Hybridization (FISH), gender can be identified in cells.



The male cells have an X and a Y while the female cells have X and X combination.

Credit: [Janice Y Ahn, Jeannie T Lee \[CC BY 2.0\]](#)



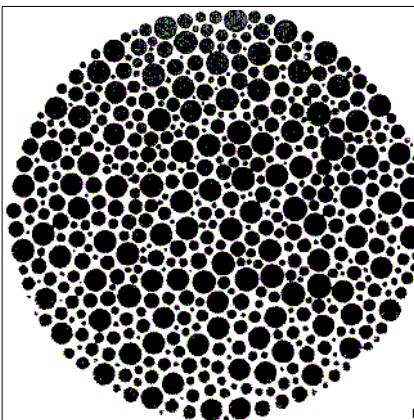
*The minuscule human Y-chromosome*

Credit: [NCBI \[Public Domain\]](#)

*The human X-chromosome*

Credit: [NCBI \[Public Domain\]](#)

## Ishahara tests (Activity)



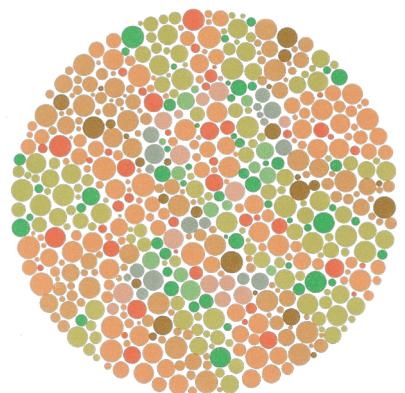
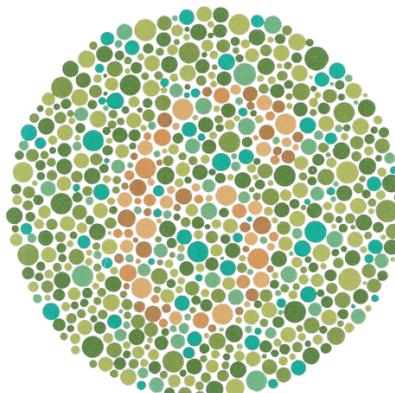
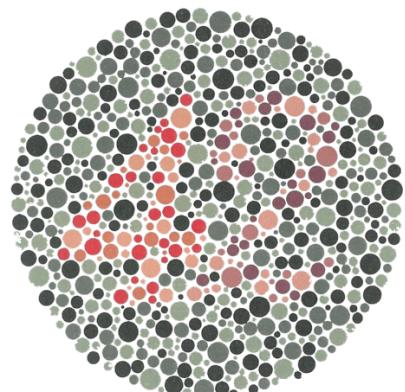
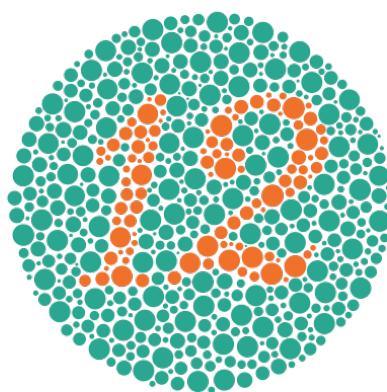
*Monochromatic representation of Ishihara test to a color blind individual as it emerges to something visible to a color-sighted individual*

Credit: [\[Public domain\]](#)

The genes encoding photoreceptor proteins for the long wave-length (reds) and middle wave-lengths (greens) reside on the X chromosome at Xq25. Since the Y-chromosome is not homologous, any mutation to either of these genes that render them non-functional results in an inability to perceive either of those colors. Men are more susceptible to the condition of red-green colorblindness since they are **hemizygous**. This means that there is no corresponding gene that could complement a deficient red or green photoreceptor gene.

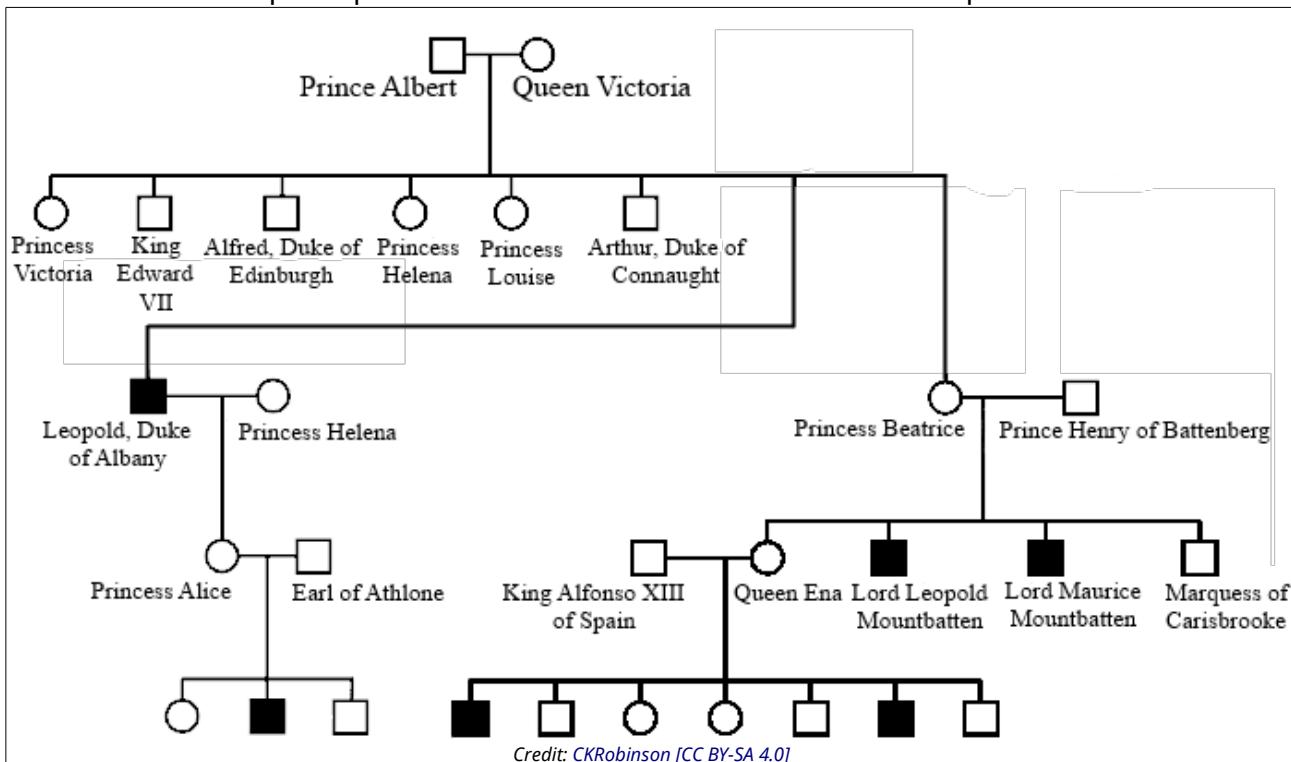
Dr. Shinobu Ishihara published his test for color perception in 1917 and this test is widely used to detect deficits in color perception. Below are examples of Ishihara plates. Record the number that you perceive in each plate and discuss with the rest of the class.

1. As you go through the plates above, note the number that you see (if any).
2. The genes for the Red and Green receptors are on the X-chromosome, who are most affected by mutation? Create a Punnet Square to illustrate how this works.
3. Can women be color-blind for red/green?
4. Humans have 3 color light receptors and have trichromatic vision. Some women are described as possibly having tetrachromatric vision (seeing 4 colors) and being able to discriminate colors invisible to the rest of us. Describe a mechanism for why this could happen. Why is there a possible gender bias?



## The case of Queen Victoria

**Hemophilia** literally translates to *blood loving*. This is a description of a series of disorders where an individual has an inability to clot blood after a cut. In modern times, clotting factors may be administered to an afflicted individual, but a prior treatment involved blood transfusions. A very famous family had a genetic predisposition to hemophilia and due to the proliferative nature of this family, we have some statistical power to verify predictions on the probabilities of passing the disease state. Below is a partial pedigree for Victoria, Queen of the United Kingdom of Great Britain and Ireland and Empress of India. The filled in shapes represent individuals who suffered from hemophilia.



- From the pedigree above, what can you say about this form of hemophilia with respect to dominance?
- From this pedigree, can you comment on the probable chromosome where the deficiency occurs?
- Assign genotypes for Prince Albert and Queen Victoria and perform a Punnet Square to illustrate if their offspring reflect your statements on dominance and chromosome location.
- Albert and Victoria were 1st cousins. Do you believe this had anything to do with the propagation of this disease? What does your Punnet Square tell you?
- Highlight the definitive carriers of the disease gene in the pedigree above.

## Additional Resources

- Full case study can be acquired at the [National Center for Case Study Teaching in Science](#).
- Human [Factor IX mRNA](#) sequence

## X-inactivation

The mammalian X-chromosome contains significantly more genetic information than the Y-chromosome. This gene dosage is controlled for in females through a process called **X-inactivation** where one of the X-chromosomes is shut down and highly condensed into a **Barr body**. Inactivation of the X-chromosome occurs in a stochastic manner that results in females being cellular mosaics where a group of cells have inactivated the paternal X-chromosome and other patches of cells have inactivated the maternal X-chromosome. The most striking example of **mosaicism** is the calico cat. A calico cat (tortoise shell cat) is always a female. One of the genes that encodes coat color in cats resides on the X-chromosome and exist as either orange or black alleles. Due to the stochastic inactivation, the patterning of orange and black fur is a distinctive quality of calicos.



Credit: [Howcheng \[GFDL CC-BY-SA-3.0 or CC BY-SA 2.5\]](#)

While the genetic information for the the orange or black coat color exists in all cells, they are not equally expressed. This type of heritable trait in spite of the presence of the genetic material (DNA) is called **epigenetic** to imply that it is "above" (epi) genetics .

## Drosophila: Thomas Hunt Morgan

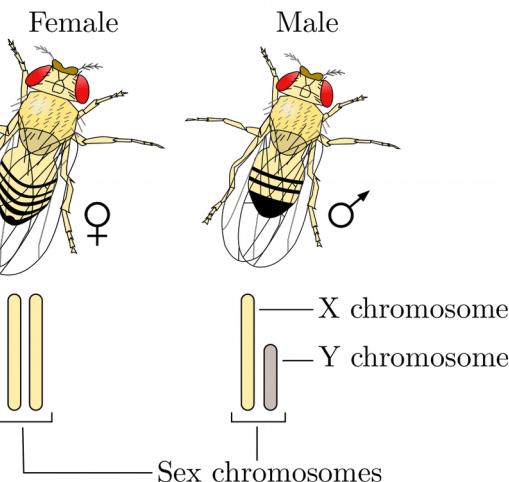


*Eye colors (clockwise): brown, cinnabar, sepia, vermillion, white, wild. Also, the white-eyed fly has a yellow body, the sepia-eyed fly has a black body, and the brown-eyed fly has an ebony body. White-eyed flies have a gender imbalance and occur mostly in males.*

Credit: [\[Public Domain\]](#)

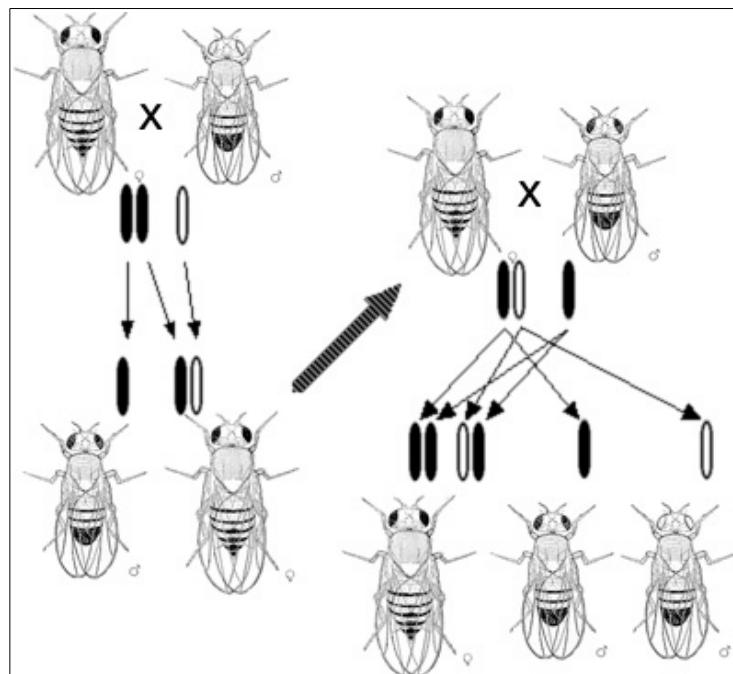
Around 1908, Thomas Hunt Morgan began to explore the genetics of what was to become a model organism, *Drosophila melanogaster* (Fruit fly). This small organism had a relatively short life cycle, great fecundity and was easily managed. From these flies that normally have red eye coloring, he and his students found white-eyed mutants. The lab noted that white-eyed flies were almost exclusively male. This gender imbalance lead Morgan to believe that the trait was sex-linked. In 1911, Morgan published a paper that described the inheritance patterns of 5 eye-colors in *Drosophila* ([Morgan, 1911](#)).

While DNA was not yet known as the source of genetic information, Morgan's studies revealed that the location of genes most likely resided on the chromosomes. By cataloging many mutations in the lab, he was able to construct a map of gene locations. His 1922 paper specifically stated that some traits were sex-linked and therefore residing on the sex chromosome. When performing crosses of white-eyed males to wild-type females, he continued to find white-eyed trait only in males. However, in the subsequent cross of females from that generation with white-eyed males, the presence of white-eyed males and females were revealed. This indicated that the white-eyed trait was recessive and resided on the X chromosome.



*Drosophila follow a sex determination based on the ratio of X:A chromosomes and not by the presence of a Y as in mammals. A 1:1 ratio results in a female and a 1:2 ratio results in a male where the Y is ignored. The standard 2N = 8.*

Credit: [GYassineMrabet \[GFDL or CC BY-SA 4.0-3.0-2.5-2.0-1.0\]](#)



*Analysis of the transmission of "White-Eyed" color in Drosophila*

Credit: [\[Public Domain\]](#)

## Non-Mendelian Genetics

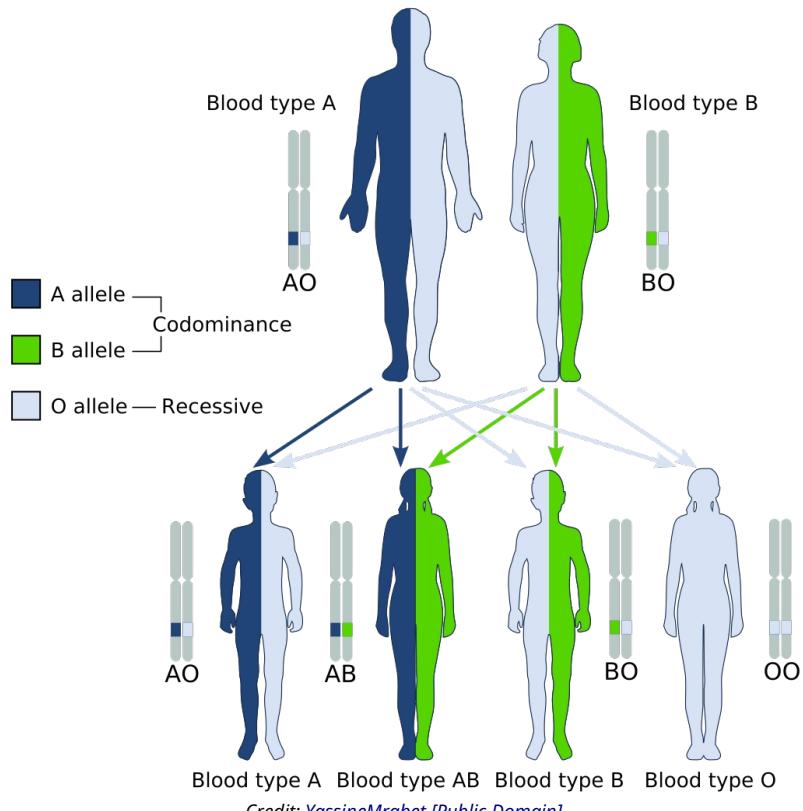
### Co-Dominance and multiple alleles

Co-dominance is said to occur when there is an expression of two dominant alleles. The prototypical case for this is the human ABO blood grouping.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma			None	
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

Credit: [InvictaHOG \[Public Domain\]](#)

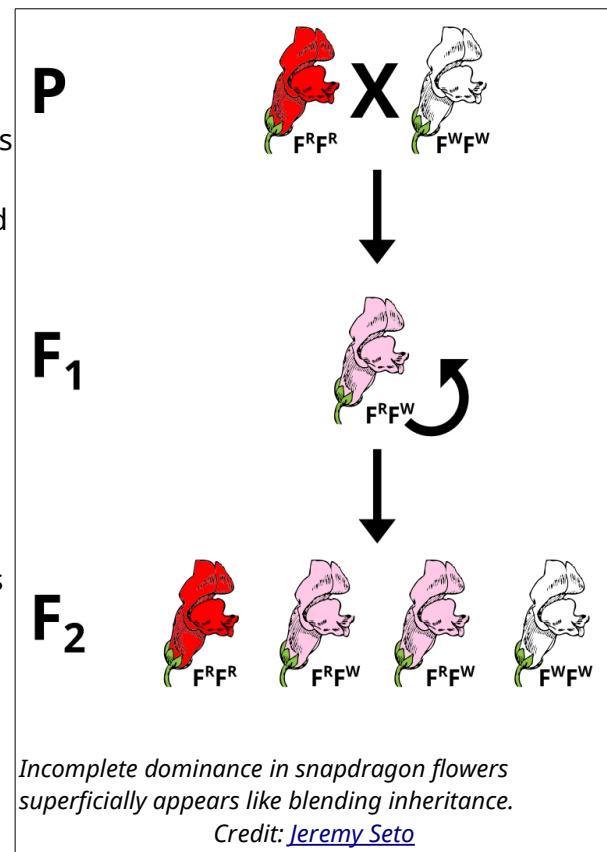
Three alleles exist in the ABO system: A, B and O. This results in four blood types: A, B, O and the blended AB.



## Incomplete Dominance

During Mendel's time, people believed in a concept of blending inheritance whereby offspring demonstrated intermediate phenotypes between those of the parental generation. This was refuted by Mendel's pea experiments that illustrated a Law of Dominance. Despite this, non-Mendelian inheritance can be observed in sex-linkage and co-dominance where the expected ratios of phenotypes are not observed clearly. **Incomplete dominance** superficially resembles the idea of blending inheritance, but can still be explained using Mendel's laws with modification. In this case, alleles do not exert full dominance and the offspring resemble a mixture of the two phenotypes.

The most obvious case of a two allele system that exhibits incomplete dominance is in the snapdragon flower. The alleles that give rise to flower coloration (Red or White) both express and the heterozygous genotype yields pink flowers. There are different ways to denote this. In this case, the superscripts of R or W refer to the red or white alleles, respectively. Since no clear dominance is in effect, using a shared letter to denote the common trait with the superscripts (or subscripts) permit for a clearer denotation of the ultimate genotype to phenotype translations.



## Problem: Incomplete Dominance

If pink flowers arose from blending inheritance, then subsequent crosses of pink flowers with either parental strain would continue to dilute the phenotype. Using a Punnet Square, perform a test cross between a heterozygous plant and a parental to predict the phenotypes of the offspring.

## Epistasis and Modifier Genes



Genes do not exist in isolation and the gene products often interact in some way. **Epistasis** refers to the event where a gene at one locus is dependent on the expression of a gene at another genomic locus. Stated another way, one genetic locus acts as a modifier to another. This can be visualized easily in the case of labrador retriever coloration where three primary coat coloration schemes exist: black lab, chocolate lab and yellow lab.

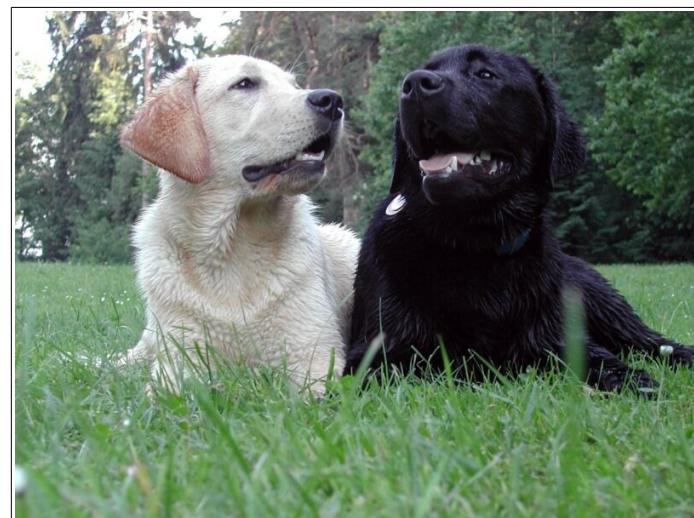


Chocolate lab (top), Black lab (middle), Yellow lab (bottom) coat colorations arise from the interaction of 2 gene loci, each with 2 alleles.

Credit: [Erikeltic](#) | CC BY-SA 3.0 or GFDL



Black lab (BB or Bb) and Chocolate lab (bb)  
Credit: [dmealiffe](#) | CC BY-SA 2.0

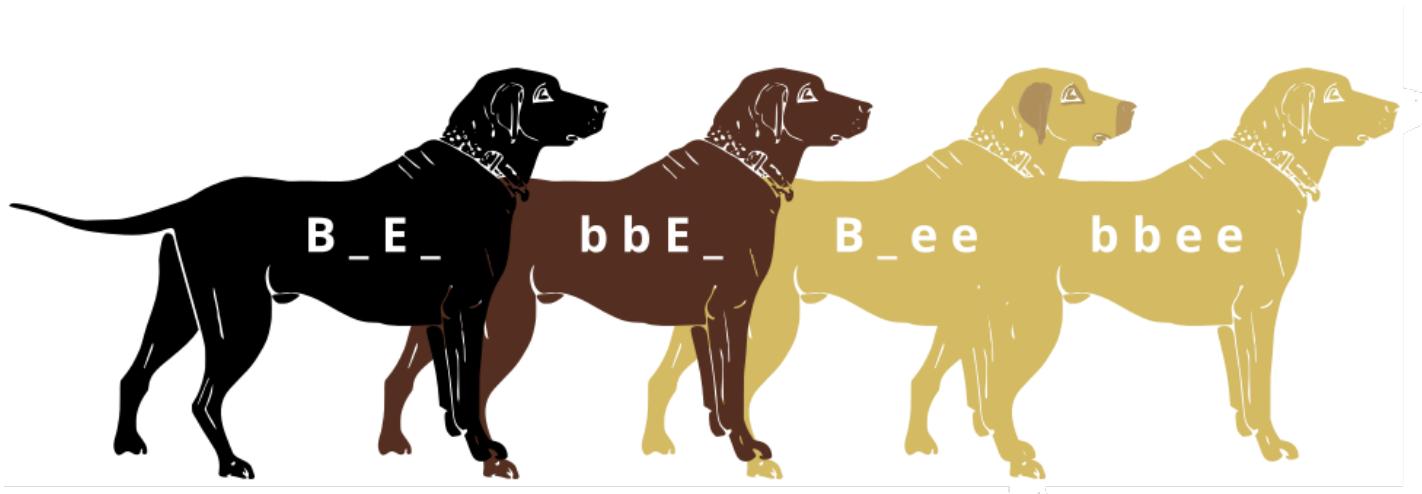


Black lab (EE or Ee) and Yellow lab (ee)  
Credit: [Public Domain](#)

Two genes are involved in the coloration of labradors. The first is a gene for a protein called TYRP1, which is localized to the melanosomes (pigment storing organelles). Three mutant alleles of this gene have been identified that reduce the function of the protein and yield lighter coloration. These three alleles can be noted as "**b**" while the functioning allele is called "**B**". A heterozygous (Bb) or a homozygous dominant individual will be black coated while a homozygous recessive (bb) individual will be brown.

The second gene is tied to the gene for Melanocortin 1 Receptor (MC1R) and influences if the eumelanin pigment is expressed in the fur. This gene has the alleles denoted "**E**" or "**e**". A yellow labrador will have a genotype of either *Bbee* or *bbEE*.

The interplay between these genes can be described by the following diagram:



Black lab (*B\_E\_*), Chocolate lab (*bbE\_*), Yellow lab with dark skin where exposed (*B\_ee*) and Yellow lab with light skin where exposed.

Credit: [Jeremy Seto](#)

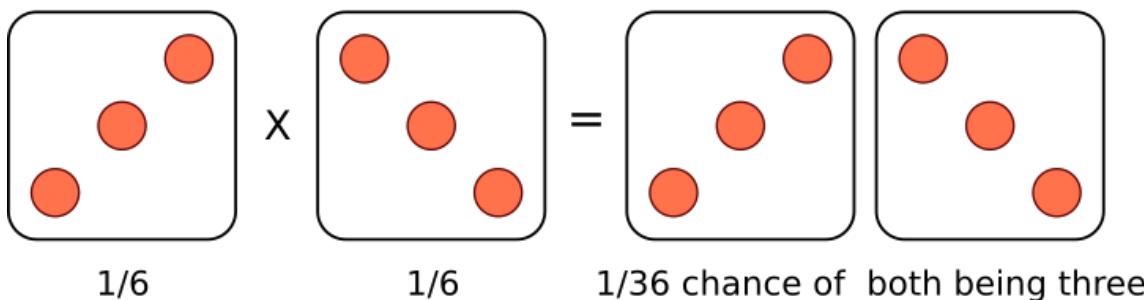
## Mendel's Observations

Parental Cross	F <sub>1</sub> Phenotypes	F <sub>2</sub> Phenotypes	F <sub>2</sub> Ratios
Round x Wrinkled Seeds	All Round	5474 Round: 1850 Wrinkled	2.96:1
Yellow x Green Seeds	All Yellow	6022 Yellow: 2001 Green	3.01:1
Purple x White Flowers	All Purple	705 Purple: 224 White	3.15:1
Tall x Dwarf Plants	All Tall	787 Tall: 227 Dwarf	2.84:1

## Probability: Past Punnett Squares

Punnett Squares are convenient for predicting the outcome of monohybrid or dihybrid crosses. The expectation of two heterozygous parents is 3:1 in a single trait cross or 9:3:3:1 in a two-trait cross. Performing a three or four trait cross becomes very messy. In these instances, it is better to follow the rules of probability. **Probability** is the chance that an event will occur expressed as a fraction or percentage. In the case of a monohybrid cross, 3:1 ratio means that there is a 3/4 (0.75) chance of the dominant phenotype with a 1/4 (0.25) chance of a recessive phenotype.

$$1/6 \times 1/6 = 1/36$$



A single die has a 1 in 6 chance of being a specific value. In this case, there is a 1/6 probability of rolling a 3. It is understood that rolling a second die simultaneously is not influenced by the first and is therefore independent. This second die also has a 1/6 chance of being a 3.

We can understand these rules of probability by applying them to the dihybrid cross and realizing we come to the same outcome as the 2 monohybrid Punnett Squares as with the single dihybrid Punnett Square.

Round = R  
Wrinkled = r

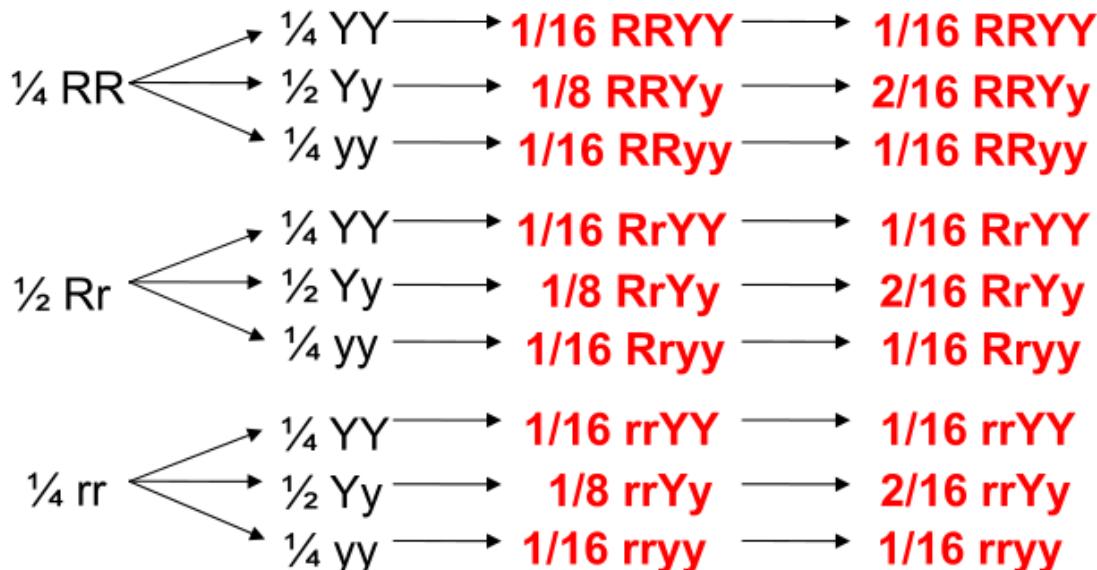
	R	r
R	RR	Rr
r	Rr	rr

Yellow = Y  
Green = y

	Y	y
Y	YY	Yy
y	Yy	yy

$\frac{1}{4}$  RR  $\frac{1}{2}$  Rr  $\frac{1}{4}$  rr

$\frac{1}{4}$  YY  $\frac{1}{2}$  Yy  $\frac{1}{4}$  yy



This forked line method of calculating probability of offspring with various genotypes and phenotypes can be scaled and applied to more characteristics.

## The Chi-Square Test

The  $\chi^2$  statistic is used in genetics to illustrate if there are deviations from the expected outcomes of the alleles in a population. The general assumption of any statistical test is that there are no significant deviations between the measured results and the predicted ones. This lack of deviation is called the **null hypothesis ( $H_0$ )**.  $\chi^2$  statistic uses a distribution table to compare results against at varying levels of probabilities or **critical values**. If the  $\chi^2$  value is greater than the value at a specific probability, then the null hypothesis has been rejected and a significant deviation from predicted values was observed. Using Mendel's laws, we can count phenotypes after a cross to compare against those predicted by probabilities (or a Punnett Square).

p-Value for $\chi^2$ test				
DF	0.1	0.05	0.01	0.005
1	2.706	3.84	6.64	7.88
2	4.605	5.99	9.21	10.60
3	6.251	7.82	11.35	12.94
4	7.779	9.49	13.28	14.86
5	9.236	11.07	15.09	16.75
6	10.65	12.59	16.81	18.55
7	12.02	14.07	18.48	20.28
8	13.36	15.51	20.09	21.96
9	14.68	16.92	21.67	23.59
10	15.99	18.31	23.21	25.19

In order to use the table, one must determine the stringency of the test. The lower the p-value, the more stringent the statistics. **Degrees of Freedom (DF)** are also calculated to determine which value on the table to use. Degrees of Freedom are the number of classes or categories there are in the observations minus 1.  $DF=n-1$

In the example of corn kernel color and texture, there are 4 classes: Purple & Smooth, Purple & Wrinkled, Yellow & Smooth, Yellow & Wrinkled. Therefore,  $DF = 4 - 1 = 3$  and choosing  $p < 0.05$  to be the threshold for significance (rejection of the null hypothesis), the  $\chi^2$  must be greater than 7.82 in order to be significantly deviating from what is expected. With this dihybrid cross example, we expect a ratio of 9:3:3:1 in phenotypes where 1/16th of the population are recessive for both texture and color while 9/16th of the population display both color and texture as the dominant. 3/16th will be dominant for one phenotype while recessive for the other and the remaining 3/16th will be the opposite combination.

With this in mind, we can predict or have expected outcomes using these ratios. Taking a total count of 200 events in a population,  $9/16(200)=112.5$  and so forth. Formally, the  $\chi^2$  value is generated by summing all combinations of:

**(observed-expected)<sup>2</sup>/expected**

## Chi-Square Test: Is this coin fair or weighted? (activity)

1. Everyone in the class should flip a coin 2x and record the result (assumes class is 24)
2. Fair coins are expected to land 50% heads and 50% tails
  - 50% of 48 results should be 24
  - 24 heads and 24 tails is already written in the "Expected" column
3. As a class, compile the results in the "Observed" column (total of 48 coin flips)
4. In the last column, subtract the expected heads from the observed heads and square it, then divide by the number of expected heads
5. In the last column, subtract the expected tails from the observed tails and square it, then divide by the number of expected tails
6. Add the values together from the last column to generate the  $\chi^2$  value
7. Compare the value with the value at 0.05 with DF=1
  - there are 2 classes or categories (head or tail), so DF = 2 - 1 = 1
  - Were the coin flips fair (not significantly deviating from 50:50)?

Heads or Tails	Expected Number	Observed Number	$\frac{(\text{Observed-Expected})^2}{\text{Expected}}$
Heads	24		
Tails	24		
$\chi^2 = \sum \frac{(\text{Observed-Expected})^2}{\text{Expected}} \rightarrow$			

Let's say that the coin tosses yielded 26 Heads and 22 Tails. Can we assume that the coin was unfair? If we toss a coin an odd number of times (eg. 51), then we would expect that the results would yield 25.5 (50%) Heads and 25.5 (50%) Tails. But this isn't a possibility. This is when the  $\chi^2$  test is important as it delineates whether 26:25 or 30:21 etc. are within the probability for a fair coin.

## Chi-Square Test of Kernel Coloration and Texture in an F<sub>2</sub> Population (activity)

1. From the counts, one can assume which phenotypes are dominant and recessive
2. Fill in the "Observed" category with the appropriate counts
3. Fill in the "Expected Ratio" with either 9/16, 3/16 or 1/16
4. The total number of counted event was 200, so multiply the "Expected Ratio" x 200 to generate the "Expected Number" fields
5. Calculate the (Observed-Expected)<sup>2</sup>/Expected for each phenotype combination
6. Add all (Observed-Expected)<sup>2</sup>/Expected values together to generate the  $\chi^2$  value and compare with the value on the table where DF=3
7. Do we reject the Null Hypothesis or were the observed numbers as we expected as roughly 9:3:3:1?
  - What would it mean if the Null Hypothesis was rejected? Can you explain a case in which we have observed values that are significantly altered from what is expected?

Phenotype	Expected Ratio (9/16, 3/16, 1/16)	Expected Number (Total # x Ratio)	Observed Number	$\frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$
Purple & Smooth				
Purple & Wrinkly				
Yellow & Smooth				
Yellow & Wrinkly				
$\chi^2 = \sum \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}} \longrightarrow$				

## Hardy-Weinberg Principle

The **Hardy-Weinberg principle** is a mathematical model used to describe the equilibrium of two alleles in a population in the absence of evolutionary forces. This model was derived independently by G.H. Hardy and Wilhelm Weinberg. It states that the allele and genotype frequencies across a population will remain constant across generations in the absence of evolutionary forces. This equilibrium makes several assumptions in order to be true:

1. An infinitely large population size
2. The organism involved is diploid
3. The organism only reproduces sexually
4. There are no overlapping generations
5. Mating is random
6. Allele frequencies equal in both genders
7. Absence of migration, mutation or selection

As we can see, many items in the list above can not be controlled for but it allows for us to make a comparison in situations where expected evolutionary forces come into play (selection etc.).

## Hardy-Weinberg Equilibrium

The alleles in the equation are defined as the following:

- Genotype frequency is calculated by the following:

$$\text{genotype frequency} = \frac{\# \text{ individuals of given genotype}}{\text{total } \# \text{ individuals in population}}$$

- Allele frequency is calculated by the following:

$$\text{allele frequency} = \frac{\# \text{ of copies of an allele in a population}}{\text{total } \# \text{ of alleles in population}}$$

- In a two allele system with dominant/recessive, we designate the frequency of one as **p** and the other as **q** and standardize to:

$$p = \text{Dominant allele frequency}$$

$$q = \text{recessive allele frequency}$$

- Therefore the *total frequency of all alleles* in this system equal 100% (or 1)

$$p + q = 1$$

- Likewise, the *total frequency of all genotypes* is expressed by the following quadratic where it also equals 1:

$$p^2 + 2pq + q^2 = 1$$

- This equation is the Hardy-Weinberg theorem that states that there are no evolutionary forces at play that are altering the gene frequencies.

## Calculating Hardy-Weinberg Equilibrium (activity)

This exercise refers to the [PTC tasting exercise](#). One can test for selection for one allele within the population using this example. Though the class size is small, pooling results from multiple section can enhance the exercise. Remember to surmise the dominant/recessive traits from the class counts.

Phenotype	Genotype	Genotype Frequency
<b>Super Taster</b>		
<b>Taster</b>		
<b>Non-taster</b>		

1. What is the recessive phenotype and how can we represent the genotype?
2. What is the dominant phenotype and how can we represent the genotypes?
3. What is the frequency of recessive genotype? ( $q^2$ )
4. What is the frequency of the recessive allele? (q)
5. What is the frequency of the dominant allele? ( $p=1-q$ )
6. Use Hardy-Weinberg to calculate the frequency of heterozygotes in the class. ( $2pq$ )
7. Use Hardy-Weinberg to calculate the frequency of homozygotes in the class. ( $p^2$ )
8. Using an aggregate of multiple section, compare the local allelic and genotypic frequencies with what the Hardy-Weinberg would predict.
9. With this small number in mind, we can see that there are problems with the assumptions required for this principle. The instructor will perform the following simulation in class to illustrate the effects on multiple populations with the effects of selection and /or population limitations. A coefficient of fitness can be applied to illustrate a selective pressure against an allele.
  - [Population Genetics Simulation of Alleles](#)
10. In the case of a selective pressure, a **fitness coefficient (w)** can be introduced. A research article <http://www.jci.org/articles/view/64240> has shown that the Tas2R38 receptor aids in the immune response against *Pseudomonas*. Imagine a situation where there is an epidemic of antibiotic resistant *Pseudomonas*. This would show that the dominant allele will have a selective advantage.
  - Modify the fitness coefficient in the Population Genetics Simulator and describe the effects this would have over many successive generations.

# Lab Reports

## Title

A description of the main idea or question of the lab. This can also highlight a key finding or question.

## Abstract

A brief summary of the main question, methods and findings. This is usually the last thing written but the first thing presented in order to grab the attention of the reader.

A rough breakdown of an abstract would contain about:

- 3 sentences worth of introduction with the key question
- 2 sentences of major methodology
- 3-6 sentences of the major results and conclusions drawn from them

Lengths will vary, but using this framework, you will not deviate too far from having a reader lose interest.

## Introduction

Introduce the background that is relevant for forming the hypotheses being tested. What were the previous observations or prior knowledge used to come to these ideas? State the actual hypotheses to be tested and how it will further the understanding of the issue.

## Materials and Methods

This section is a little like a cooking recipe. The main steps taken should be summarized as a standard prose in a manner that anyone could follow and repeat. This is written in the past tense and 3rd person. Do not write in the first person as \*you\* have nothing to do with the experiments. Explain "What was done with which reagents?"

## Results

This section is descriptive of what was observed. Figures and tables serve as a summary of the results to illustrate the data. They also serve as guides to outline the text of the section. Slowly describe each figure or table. Expand these points into sentences and paragraphs. Present the data as fully as possible, including stuff that at the moment does not quite make sense. This is written in the past tense and 3rd person. Conclusions are not provided in this section as they are made from analyzing the information and synthesizing the results.

## Discussions

Discussions are the conclusions made through analyzing the results. At this time, you will be able to re-emphasize the original hypotheses made in the introduction. Indicate whether or not the hypotheses were demonstrated sufficiently. If this is not the case, offer alternatives and interpretations. Can you improve or modify your hypotheses? Explain how multiple lines of evidence corroborate each other and help to further the understanding of the problem.

## References

Prior knowledge requires demonstration of strength or validity. The references should be from primary sources and should illustrate the point of the statement. The section is presented in numerous formats as a bibliography, but citations are inserted near the text where knowledge or statements are displayed. Use a reference Manager to insert citations and format the bibliography. An excellent free reference manager with a plug-in to common word processors is [Zotero](#).

## Additional resources

- [MIT OCW](#)

