

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.

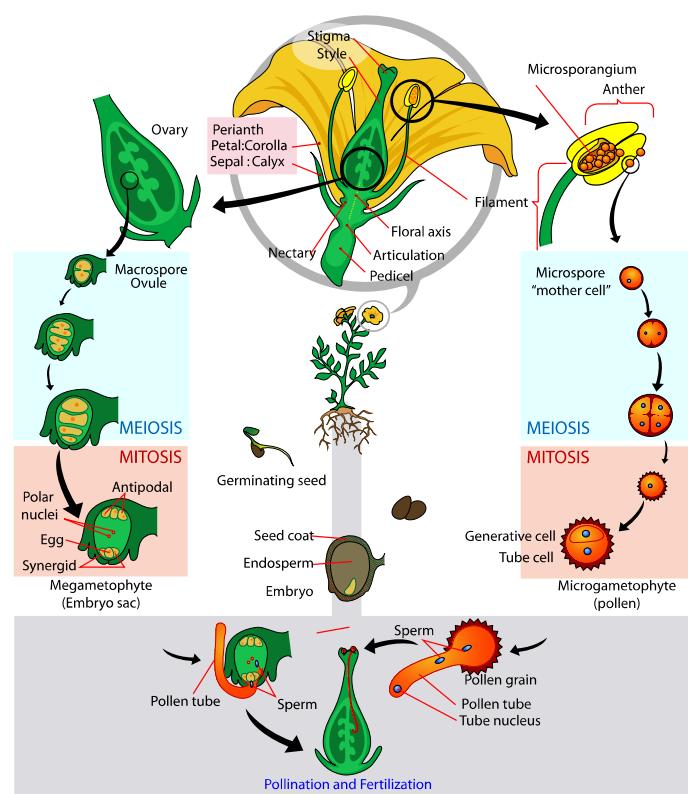


The pea plant (*Pisum sativum*) offered a great advantage of being able to control the fertilization process and having large quantities of offspring in a short period of time. In a simple experiment of tracking the passage of a single trait (**monohybrid cross**) like flower color through multiple generations 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 (F₁)**. In the first generation, all flowers are purple. Permitting self-pollination generates a **Second Filial Generation (F₂)**. 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: [Bmdavill \[CC-BY-SA 4.0\]](#)



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 F₁ plants with the re-emergence in the F₂ 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 F₁ 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 ($\frac{1}{2}$ -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:

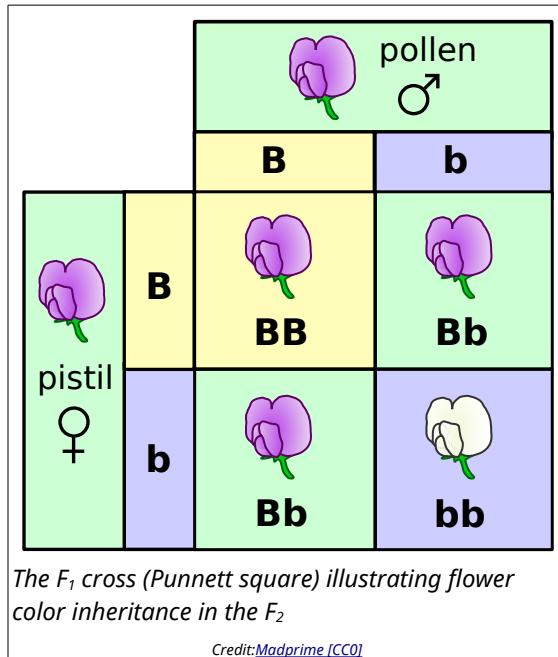
1. Law of Segregation

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

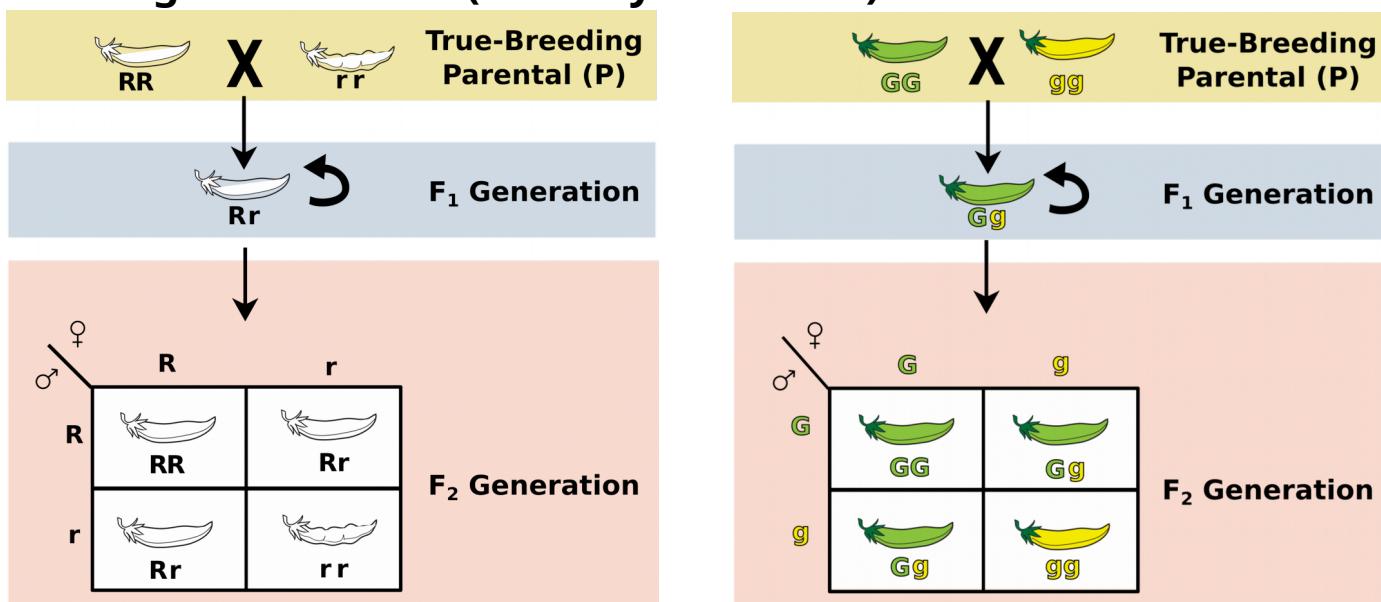
2. Law of Dominance

- 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.

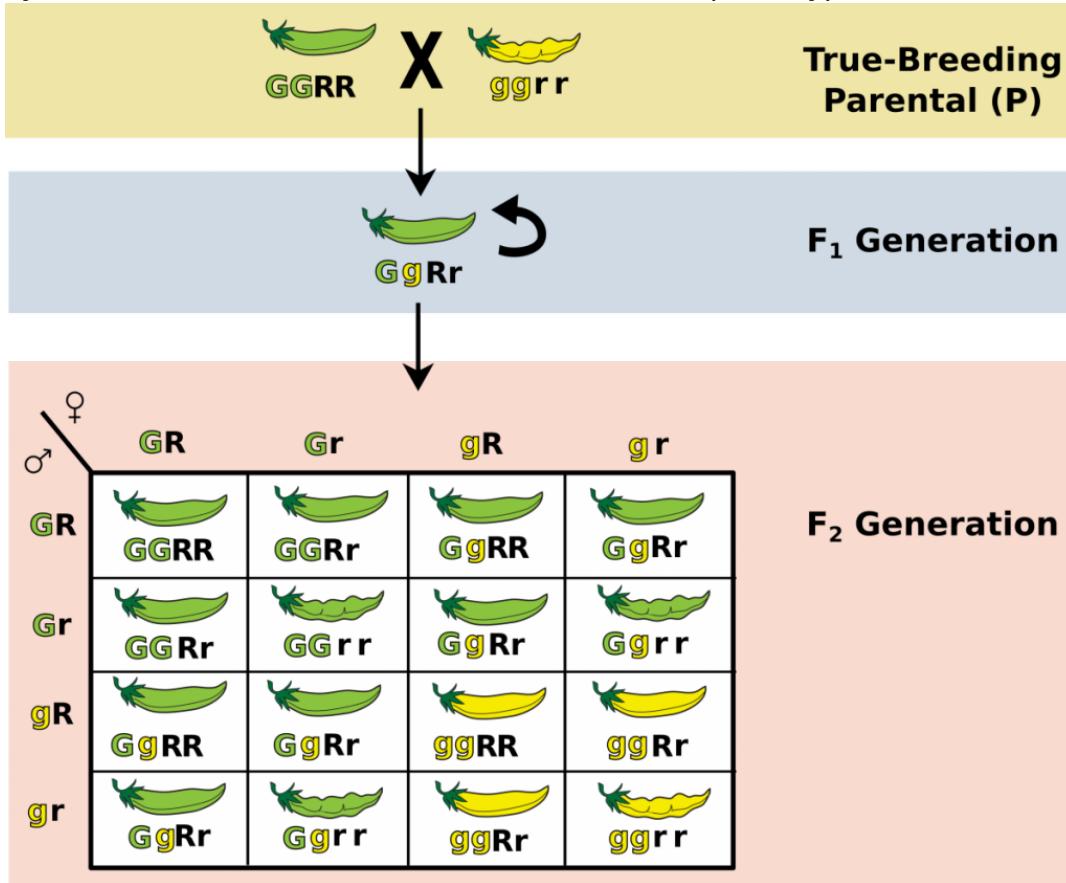


The Single Trait Cross (Monohybrid Cross)



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

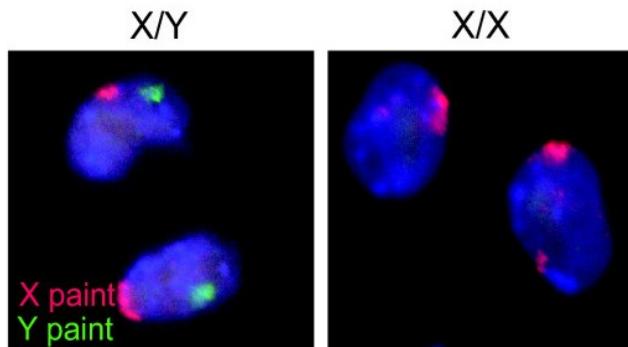
The dihybrid cross revealed another law of inheritance to Mendel. By observing the 9:3:3:1 ratio, Mendel concluded that traits were not tied to each other. That is to say, if a pea pod was yellow, it could still be either smooth or wrinkled in texture. This lack of linkage between genes yielding different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

Sex-Linked Genes



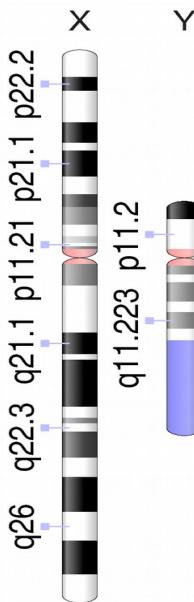
For the most part, mammals have gender determined by the presence of the Y chromosome. This chromosome is gene poor and a specific area called sex determining region on Y (**SRY**) is responsible for the initiation of the male sex determination. The X-chromosome is rich in genes while the Y-chromosome 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.



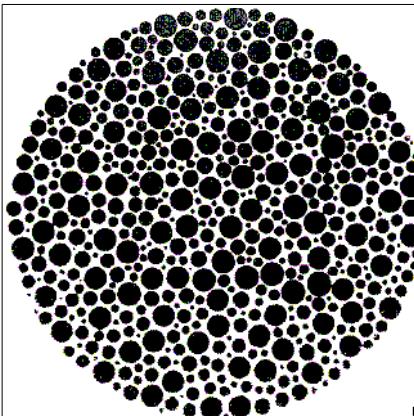
The male cells have an X and a Y while the female cells have X and X combination.

Credit: Janice Y Ahn, Jeannie T Lee [CC BY 2.0]



The human X and Y chromosomes

Ishihara tests (Activity)



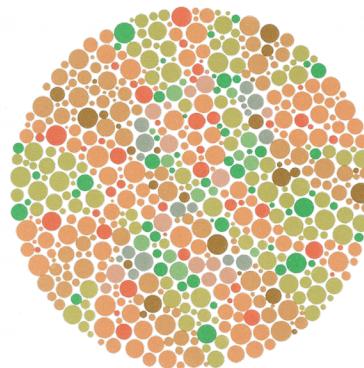
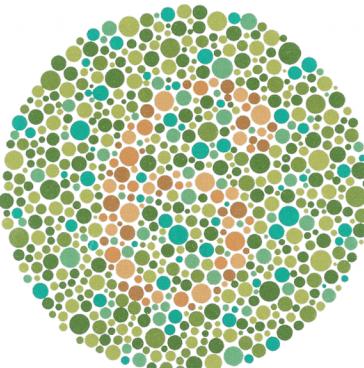
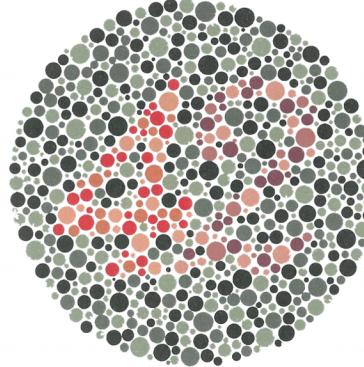
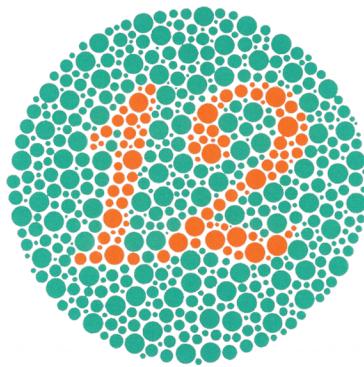
Monochromic representation of Ishihara test to a color blind individual as it emerges to something visible to a color-sighted individual

<https://commons.wikimedia.org/wiki/File:Ishihara-12.gif>

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.

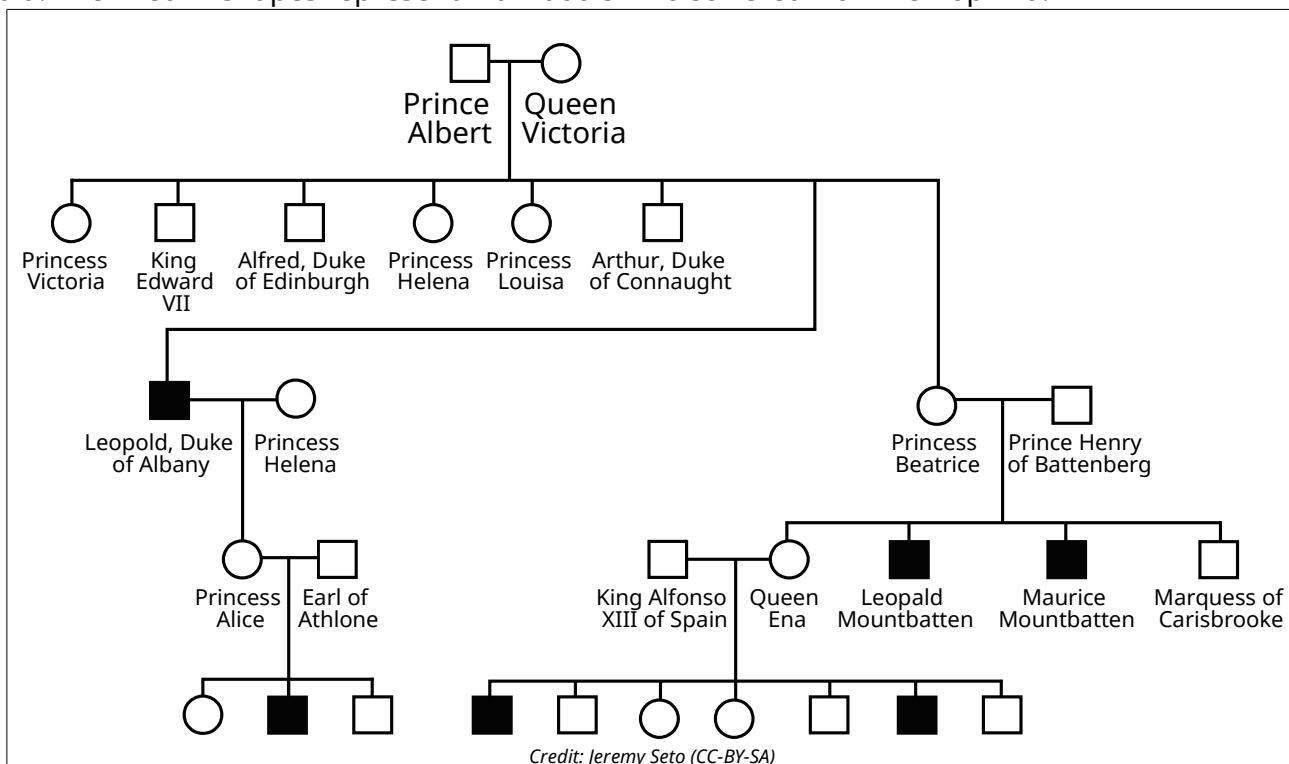
Dr. Shinobu Ishihara published his test for color perception in 1917 and this test is widely used to detect deficits in color perception. Below are examples of Ishihara plates. Record the number that you perceive in each plate and discuss with the rest of the class.

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.



1. From the pedigree above, what can you say about this form of hemophilia with respect to dominance?
2. From this pedigree, can you comment on the probable chromosome where the deficiency occurs?
3. 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.
4. 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?
5. 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 \[CC-BY-SA-3.0\]](#)

While the genetic information for 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

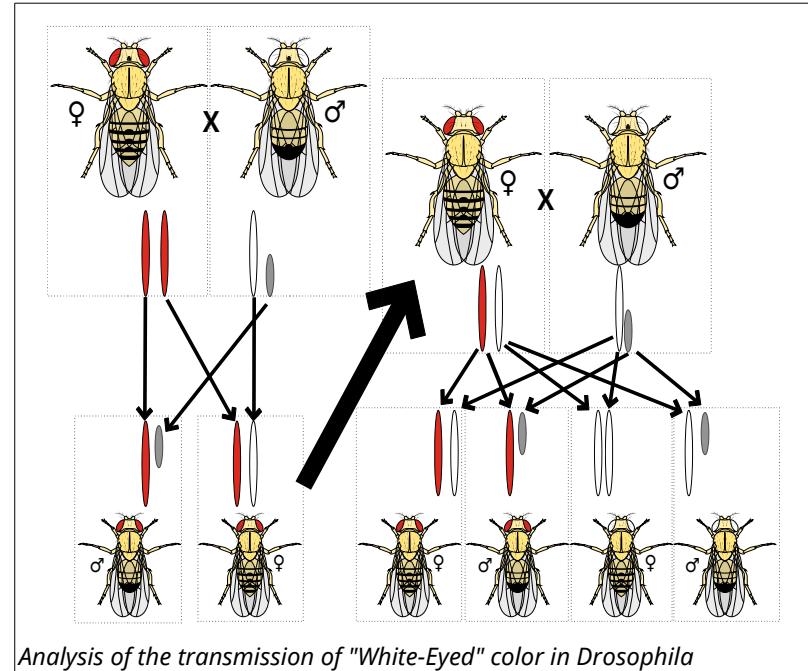
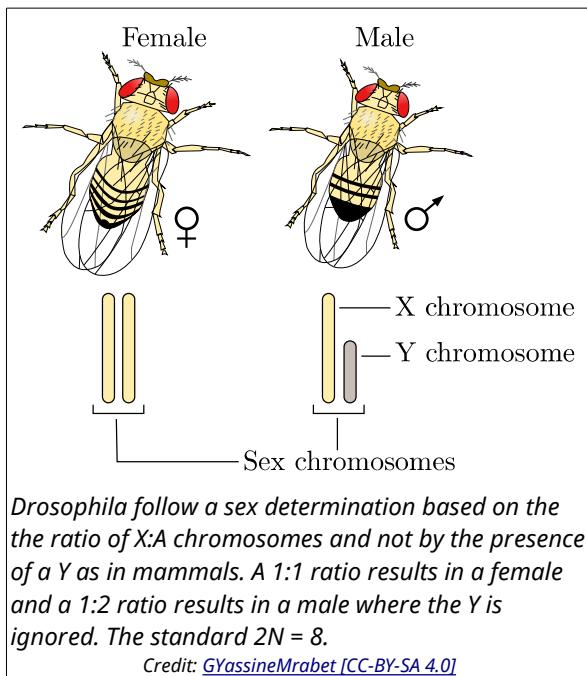


Eye colors (clockwise): brown, cinnabar, sepia, vermillion, white, wild. Also, the white-eyed fly has a yellow body, the sepia-eyed fly has a black body, and the brown-eyed fly has an ebony body. White-eyed flies have a gender imbalance and occur mostly in males.

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.



Morgan received the [Nobel Prize in Physiology or Medicine](#) in 1933 for his inference of chromosomes being a physical mechanism for packaging genetic information in the cells.

- **Morgan TH.** [THE ORIGIN OF FIVE MUTATIONS IN EYE COLOR IN DROSOPHILA AND THEIR MODES OF INHERITANCE](#). *Science*. 1911 Apr 7;33(849):534-7

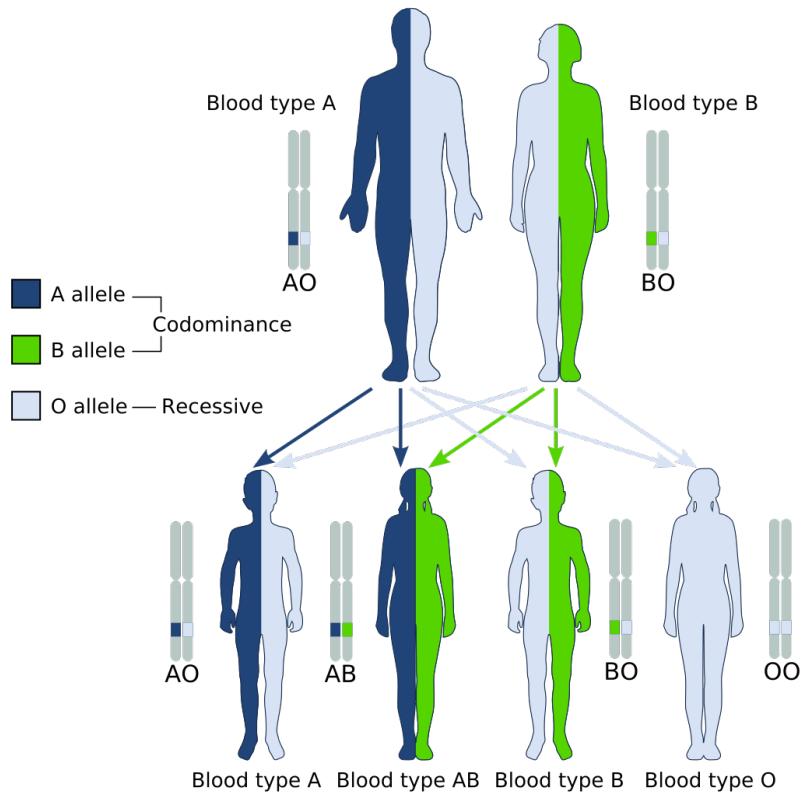
Non-Mendelian Genetics

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				None

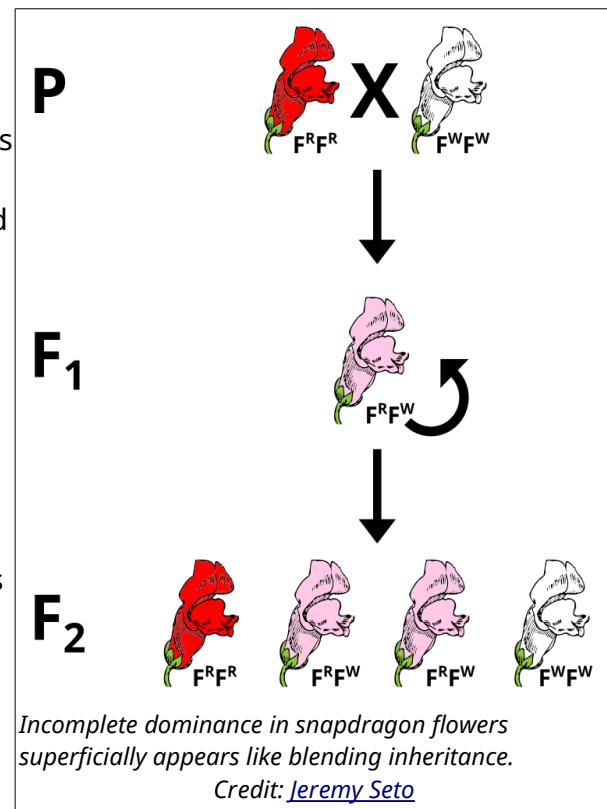
Three alleles exist in the ABO system: A, B and O. This results in four blood types: A, B, O and the blended AB.



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



Interplay of multiple enzymes in a biochemical pathway will alter the phenotype. Some genes will modify the actions of another gene.

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.

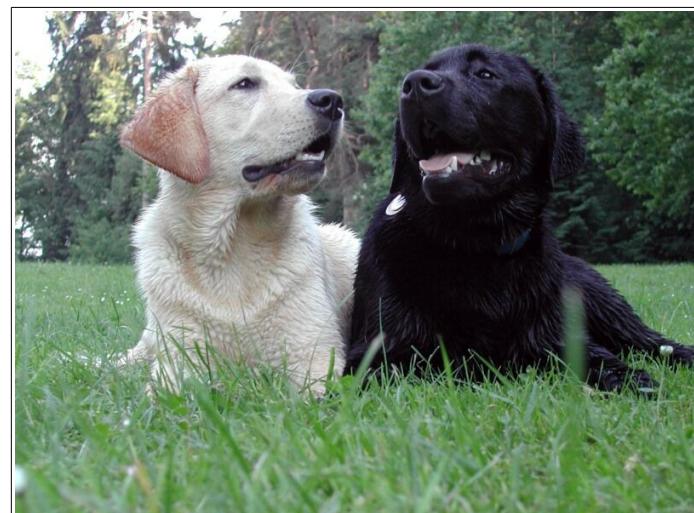


Chocolate lab (top), Black lab (middle), Yellow lab (bottom)
coat colorations arise from the interaction of 2 gene loci, each
with 2 alleles.

Credit: [Erikeltic](#) / CC-BY-SA 3.0]



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

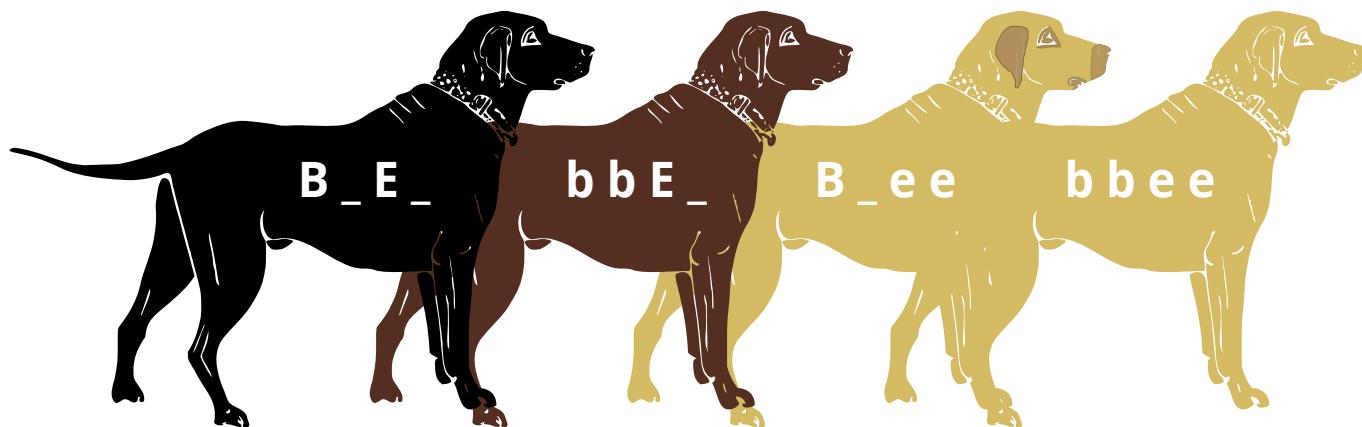


Black lab (EE or Ee) and Yellow lab (ee)
Credit: [Public Domain](#)

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 \(CC-BY-SA 3.0\)](#)

Using Cytobrush

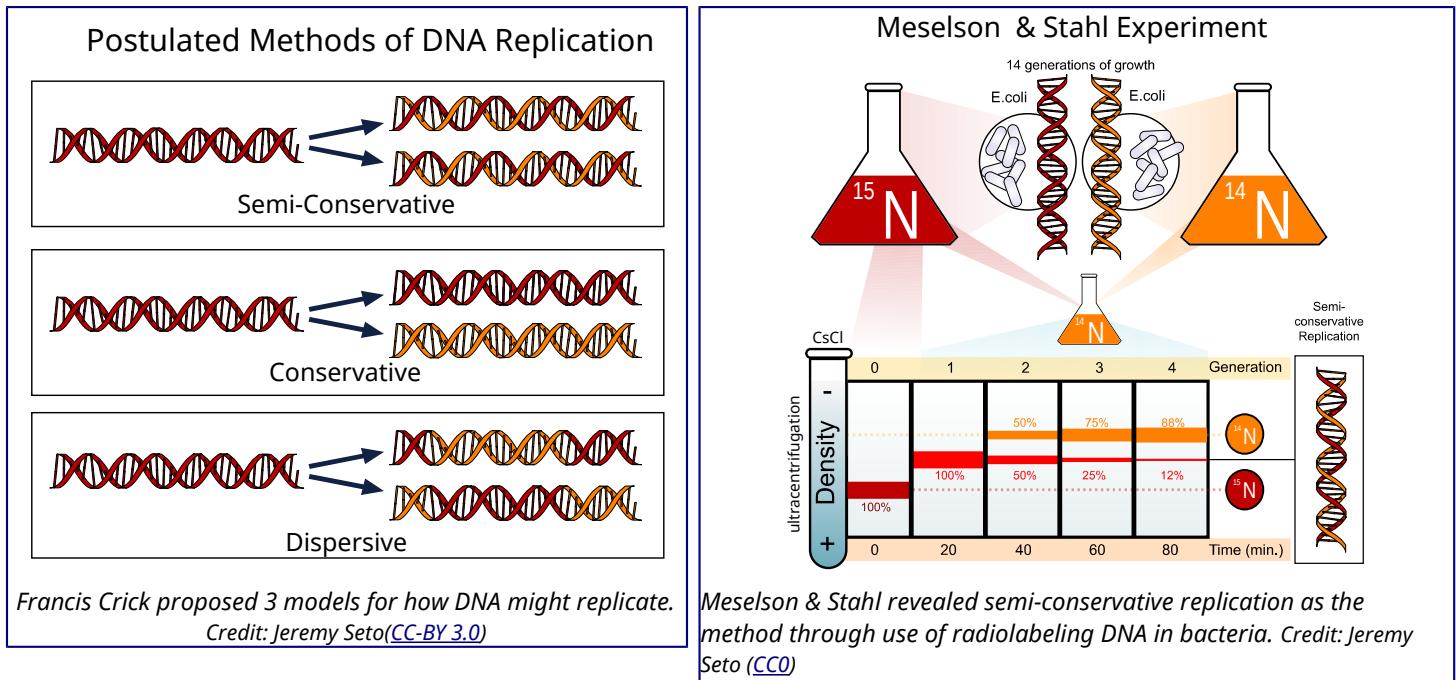
1. Use sterile cytobrush and insert into mouth
2. brush cytobrush on inside of cheek 25 times
3. Swirl cytobrush in 100 µl of Chelex suspension (10% w/v)
4. Place centrifuge tube with Chelex and cell suspension on 100 °C heat block for 10 minutes
5. Centrifuge tubes at maximum speed for 5 minutes
6. DNA is in the supernatant. (avoid beads at bottom)
7. Store DNA in -20 °C

PCR with PCR Beads

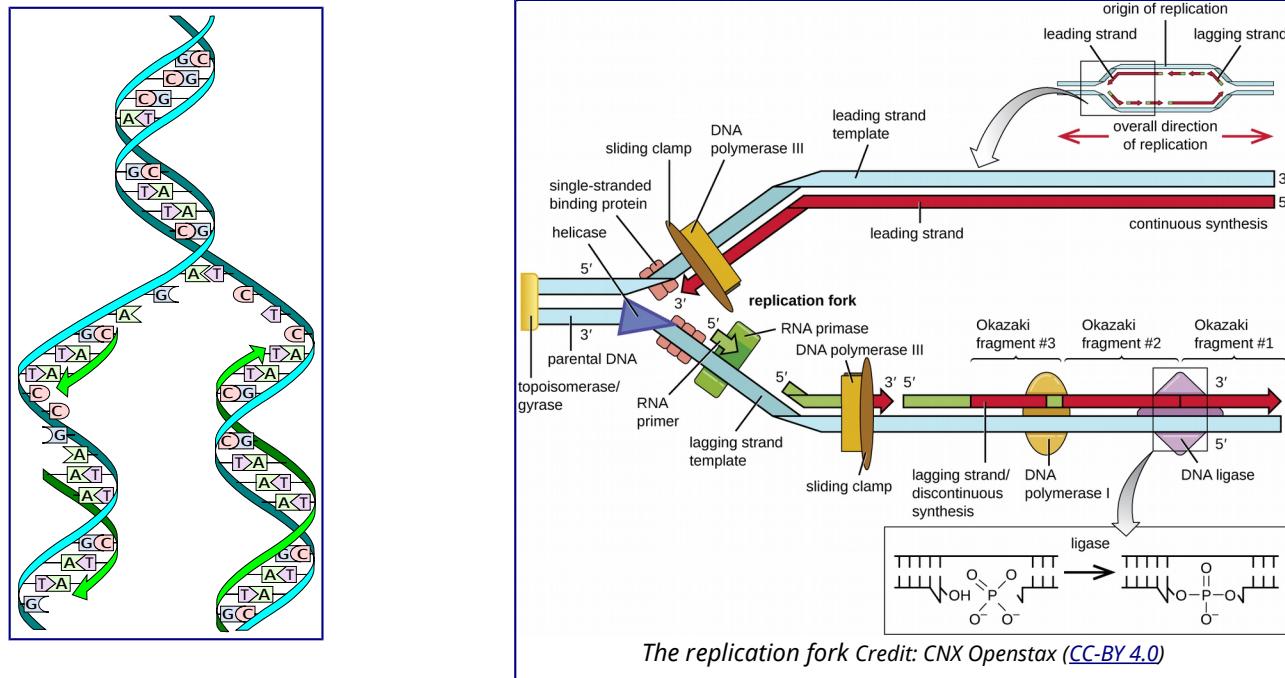
1. Add 22 µl of primer mix (forward and reverse) to beads
2. Ensure that the bead is dissolved
3. Add 3 µl of DNA

DNA Replication

Meselson & Stahl Experiment

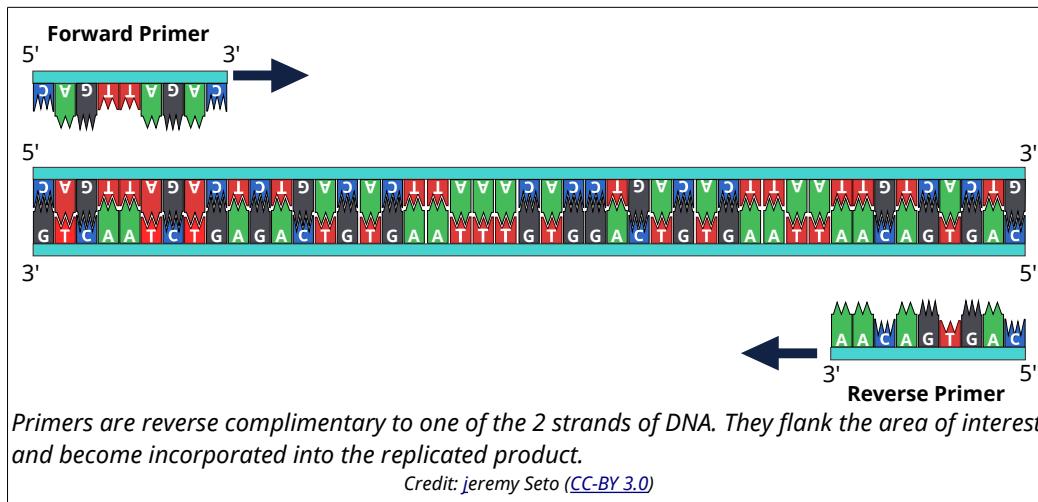


Replication Machinery



Polymerase Chain Reaction (PCR)

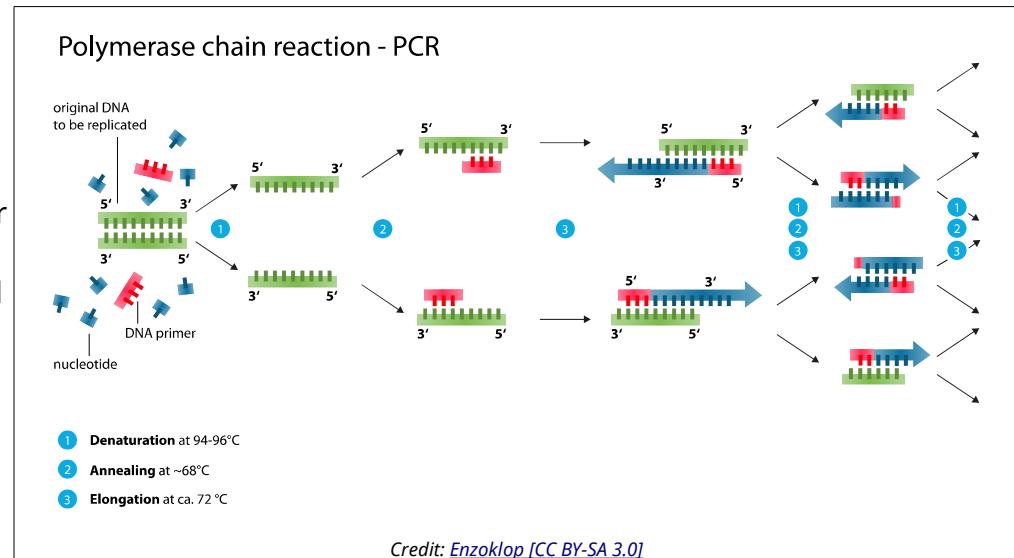
The **Polymerase Chain Reaction (PCR)** is a method of rapidly amplifying or copying a region of DNA in a tube. As the name implies, the technique uses a thermostable **DNA Polymerase** enzyme to mimic in a tube what happens within a cell during DNA replication. The chain reaction permits us to rapidly 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^{2+} , 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^{30} or 1,073,741,824 copies. This is over a billion copies in a few hours of time.

