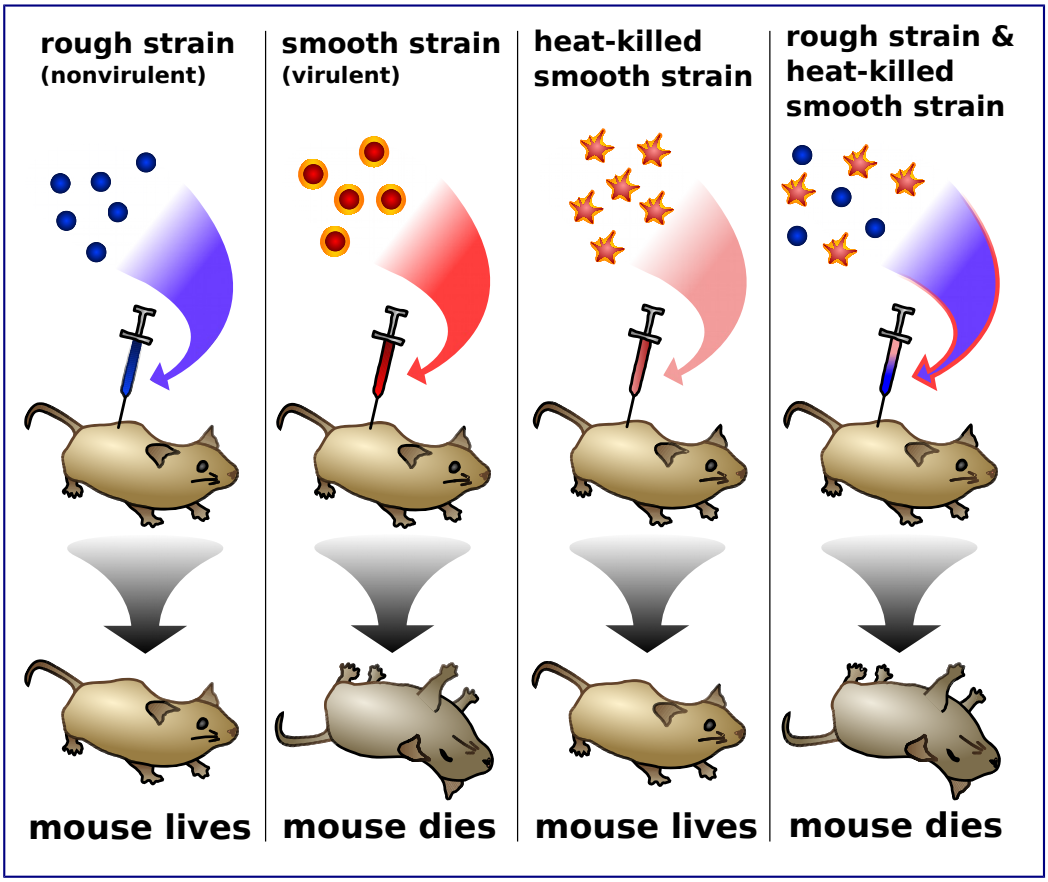
History of Genetic Transformation

Any uptake of genetic information from the external environment into cells that results in the expression of new traits is called **genetic transformation**. This process can occur naturally. Some bacteria are referred to as being "competent" to indicate that they are capable of taking DNA into the cell from the environment. This is referred to as **natural competence**. Bacteria are also capable of receiving DNA through the process of conjugation where plasmids from one bacteria are sent to another through the **conjugation pilus**. Other methods of introduction of foreign DNA include direct injection into the cytosol or through the use of viruses in a process called **transduction**. In eukaryotic cells, we refer to the introduction of DNA as **transfection**.

Frederick Griffith and the Transforming Agent

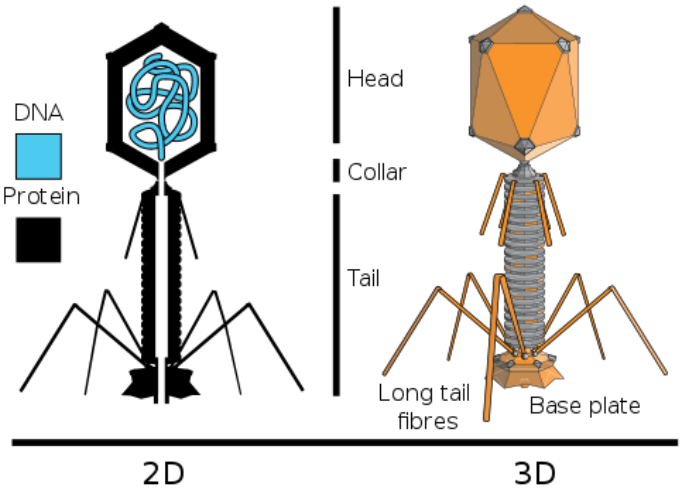
At the beginning of modern biology, the source of genetic material was not known to be DNA. In fact, many scientists thought DNA was too simple to perform this job. Scientists believed that proteins, with their 20 varied amino acids, were the carriers of genetic information. In an attempt to develop a vaccine for a bacterial induced pneumonia, Frederick Griffith was the first to describe the process of genetic transformation by accident in 1928. Griffith took a virulent strain of bacteria (smooth in appearance) that caused pneumonia and injected them into mice. This would result in death of the mice. He also observed that injection of a rough bacteria did not cause any disease. After heat-killing the smooth bacteria, he discovered that living bacteria of the virulent strain was required for the disease to progress. Finally, he observed that injecting the heat-killed virulent bacteria with living bacteria of the non-virulent strain resulted in pneumonia and death in the mice. From this experiment, a **transforming agent** with the capacity to pass on a trait was found to be within the contents of those dead cells. But no one knew this agent to be DNA at that point.



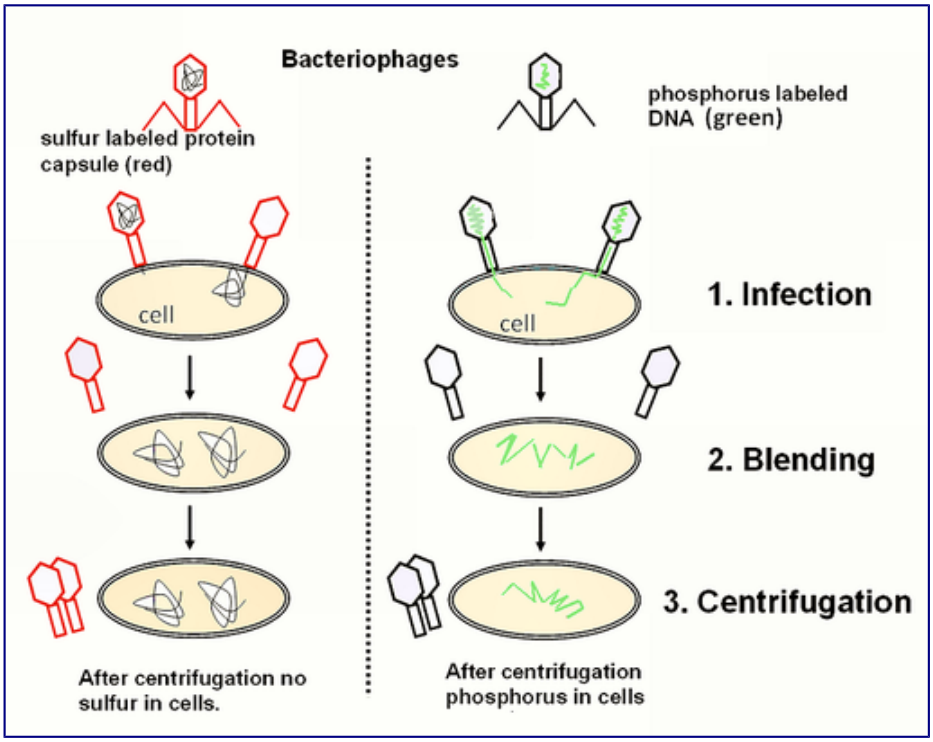
Jeremy Seto jseto@citytech.cuny.edu

Hershey and Chase

Hershey and Chase studied bacteriophage (phage=eater). Phage are bacterial viruses that infect bacteria and cause lysis of the cells. They have a very simple structure of a proteinaceous head/collar/tail and a DNA core. It was known that bacteria infected with phage were resistant to additional infection. In 1952 Hershey and Chase grew bacteriophage in conditions that would specifically label either the DNA or the protein with radioactivity. They subsequently took phage with radiolabeled DNA and infected bacteria. In parallel, they took phage with radiolabeled protein and infected another set of bacteria. After just enough time for infection, the bacterial cultures were placed into a blender to separate the bacteriophage from the bacteria.



T4 bacteriophage anatomy.
Credit: Adenosine, Pbroks13 (CC-BY-SA 2.5)



Solutions were centrifuged to isolate bacteria from the phage. Bacteria were radioactive only when the phage grown in conditions to radiolabel DNA infected the bacteria to indicate that DNA might be the transforming agent.

Questions to think about:

1. What isotope would be used to label protein?
2. What isotope would be used to label just DNA?

Jeremy Seto jseto@citytech.cuny.edu

Avery, McCarty and MacCleod

In 1944, Oswald Avery, Colin MacCleod and Maclyn McCarty repeated Griffith's experiment. Instead of using heat-killed bacteria, these scientists isolated protein, carbohydrates, lipids and nucleic acids from the virulent strain and co-injected with the non-virulent bacteria. Carbohydrate extracts were ineffective at transforming bacteria. Protein extracts were incapable of causing transformation. Lipid injections were unable to result in virulence. Only nucleic acid samples treated with RNase were capable of transforming bacteria. When co-injecting with DNase, bacteria were not transformed. Along with Hershey-Chase, this definitively illustrated that DNA was the transforming agent capable of transferring genetic information.

More Resources

- http://www.dnalc.org/view/16375-Animation-17-A-gene-is-made-of-DNA-.html

Jeremy Seto jseto@citytech.cuny.edu

Bacterial Transformation

Escherichia coli are commensal gram negative bacteria found in the guts of humans. They have the capacity to double every twenty minutes and make a favorable carrier of recombinant DNA. Plasmid DNA can be introduced into *E. coli* easily after making them competent. One method to achieve this is through chemical competence with heat shock. In this process, the bacteria are incubated in $CaCl_2$ solution on ice. The cold serves to slow down molecular motion of the plasma membrane while the Ca^{2+} ions remove the charge-charge repulsion between the phospholipids and the negatively charged DNA seeking to gain entry into the cell. Cells are placed for a short period of time at 42°C to induce **heat shock**. This heat shock results in the cell taking up the DNA. This method is very low efficiency so many bacteria do not take in any DNA. Cells are allowed to recover from heat shock at 37°C in rich nutrient broth to allow for the production of the antibiotic resistance proteins encoded on the vector as a selection marker. Transformed cells are then spread across an agar plate containing the antibiotic which will then kill all non-transformed cells. Only the bacteria containing the vector with the antibiotic resistance gene will survive and replicate to form small colonies on the surface of the agar.

- http://www.dnalc.org/view/15918-Transformation.html
- http://www.dnalc.org/view/15916-DNA-transformation.html

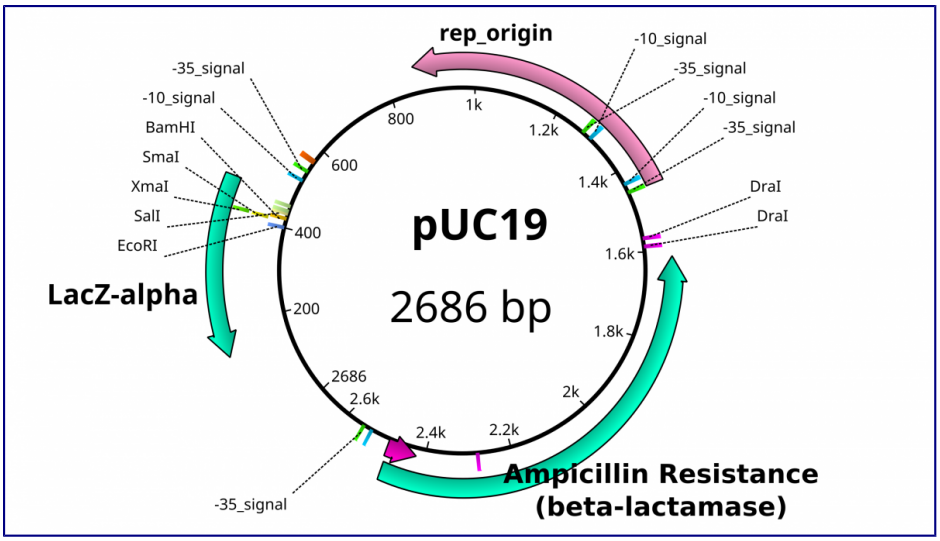
Exercise: Transformation of Bacteria with RE Identified Plasmids

1. Each group retrieves the 2 miniprepped plasmids from the previous week in the freezer and allow to thaw on ice.
2. Bring 2 agar plates to room temperature
    - 2 plates will contain antibiotic, X-Gal and arabinose
3. For each plasmid, obtain 250µl of transformation buffer (50mM $CaCl_2$) in microfuge tubes and place on ice for 10 minutes
4. Take an inoculating loop and remove a single colony of bacteria from a freshly streaked plate grown overnight
5. Swirl bacteria in each tube containing transforming solution to distribute bacteria throughout solution
6. Pipette 5 µl of plasmid into the tube and incubate on ice for 10 minutes
7. During this incubation, flip the warmed plates and label them with your group names.
8. Place transformation tubes into 42°C heatblock for 1 minute to heat shock the cells
9. Add 500µl fresh SOC media (or LB) and incubate at 37°C for 15 minutes.
10. Pipette 150µl of transformation solution onto each plate and spread across the plate.
11. Turn plates agar side up and place them into 37°C incubator overnight. (your instructor will retrieve them and place them into refrigerator)

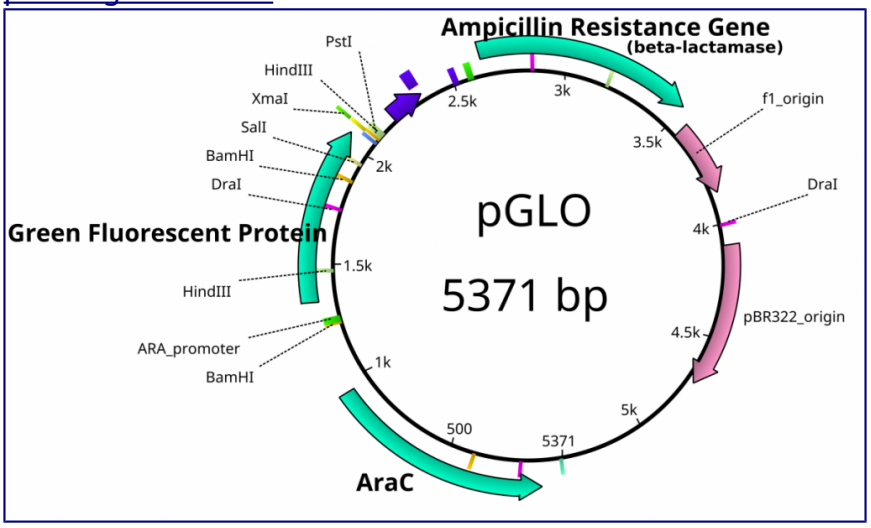
Hypothesize: What will I expect of my transformed cells?

From the previous lab, we can identify our plasmids. The plasmids are either pGlo, pUC18/19 or pUC18/19 with a 6kb insert disrupting the LacZ gene. pGlo contains a gene that encodes the protein GFP that will fluoresce green under UV light and is 5.4kb. pUC is typically 2.7kb in size. LacZ is a gene encoding the protein β-Galactosidase, the enzyme that hydrolyzes lactose into the monosaccharides galactose and glucose. X-Gal is a chemical resembling lactose, however upon hydrolysis, the molecule deposits a blue coloring into the cell.

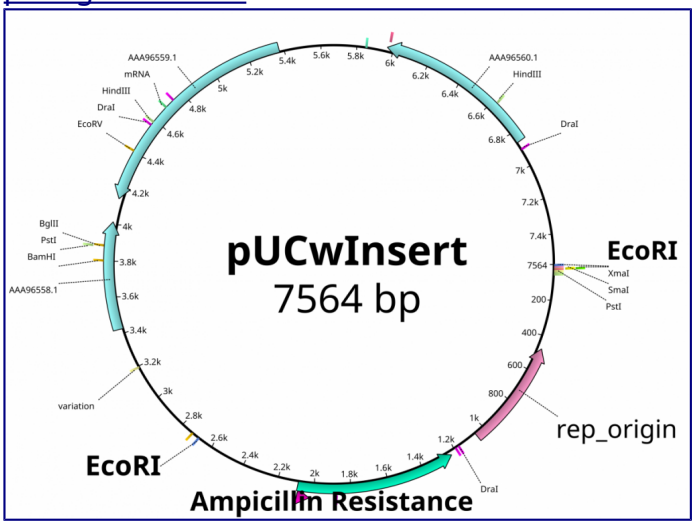
Jeremy Seto jseto@citytech.cuny.edu



- pUC19 genbank file



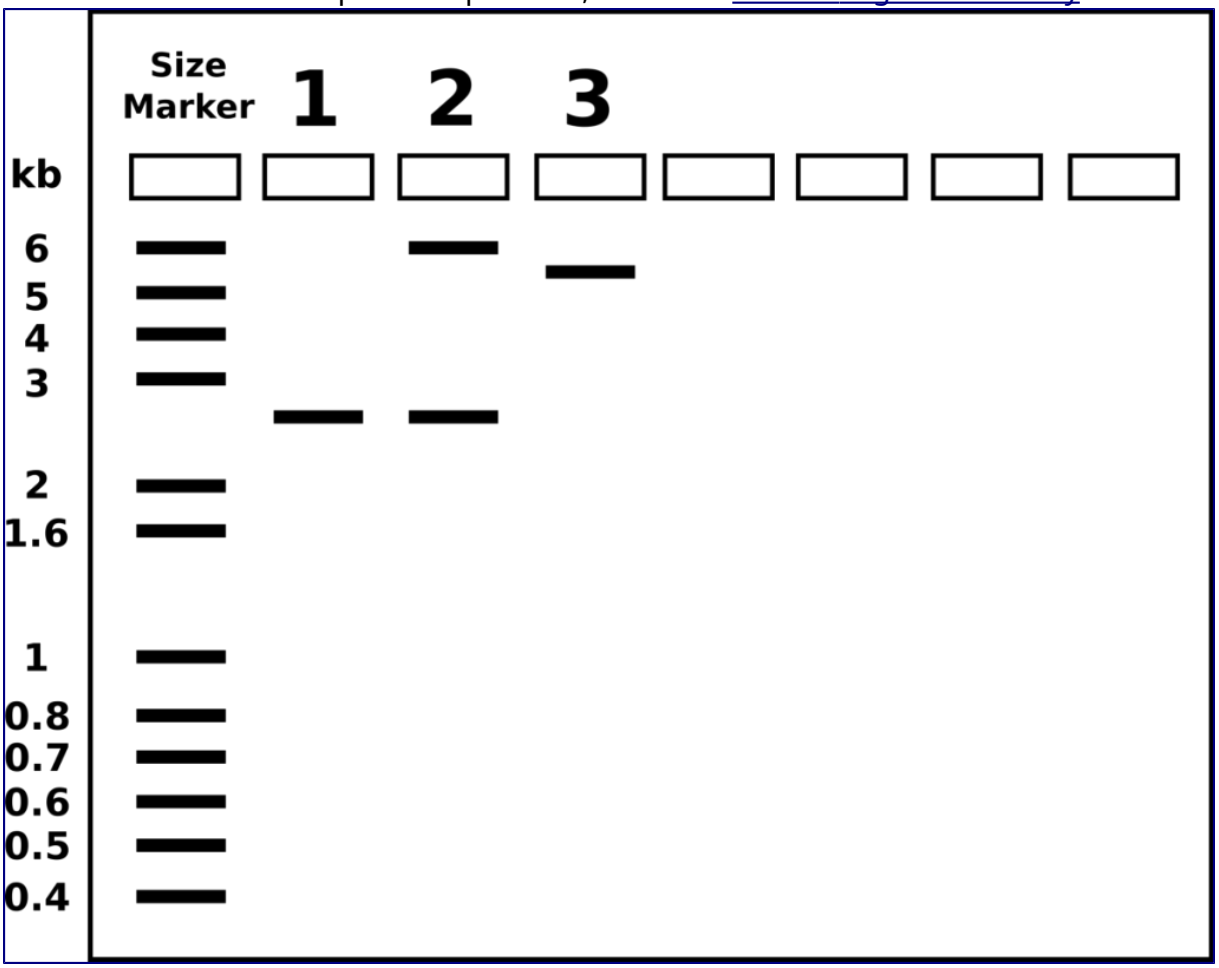
- pGlo genbank file



- pUC with insert file

Jeremy Seto jseto@citytech.cuny.edu

1. If the previously mentioned plasmids were digested by *EcoRI*, label the lanes below with the appropriate plasmid (pGlo, pUC, pUC-inserted)
2. Predict if your transformants will be green under UV, white in all conditions or blue.
3. For additional help on this problem, utilize the *In silico* digestion activity



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