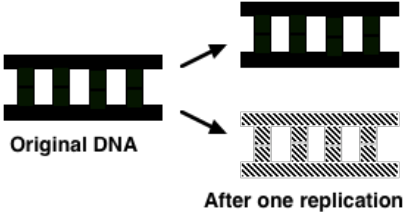
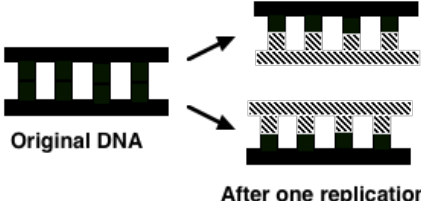



PULSE-CHASE PRIMER: THE MESELSON-STAHL EXPERIMENT

INTRODUCTION

In the 1950s, James Watson and Francis Crick suggested a mechanism for the replication of DNA, which they called the "Semiconservative Model of Replication." During replication, each of the two strands of DNA would act as a template for the synthesis of a new strand. The two new DNA molecules would consist of one strand from the original molecule and a newly synthesized strand. At the time, scientists were considering three possible replication models. They were:

Type of Replication	Composition of DNA Molecules Before and After
(a) Conservative Replication – the original DNA molecule remains intact and a new DNA molecule is synthesized that contains no part of the original. It is a completely new molecule.	 <p>Original DNA</p> <p>After one replication</p>
(b) Semiconservative Replication – each of the two DNA molecules is composed of one strand from the original molecule and one newly synthesized strand.	 <p>Original DNA</p> <p>After one replication</p>
(c) Dispersive Replication – each of the two DNA molecules is composed of sections of the original DNA and newly synthesized DNA randomly interspersed along each strand. Note that even though the illustrations look like there is a pattern, in dispersive replication, the original and newly synthesized nucleotides would be randomly interspersed.	 <p>Original DNA</p> <p>After one replication</p>

In 1958, Matthew Meselson and Franklin Stahl conducted their now-famous **pulse-chase experiment** to determine which of these three models was correct.

The key to the Meselson-Stahl experiment was the use of the nonradioactive isotopes of nitrogen, ^{14}N and ^{15}N , in the media for growing *E. coli* bacteria. As *E. coli* reproduced, new DNA molecules were synthesized incorporating these isotopes. Those *E. coli* grown in the presence of ^{15}N created "heavy" DNA. After many generations, all DNA in the *E. coli* cells was heavy. Cells grown in the presence of ^{14}N produced "light" DNA and after many generations, all DNA in the *E. coli* cells was light.

Meselson and Stahl took samples from each culture, extracted the DNA, mixed equal amounts together, and centrifuged the mixture. The light and heavy DNA separated. Molecules of different weight accumulated at different depths. The heavy DNA formed a band at a lower point in the mixture than where the light DNA formed a band. The difference in density of the DNA was enough to allow it to separate. (See **Figure 1**.)

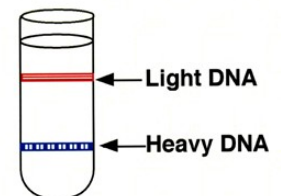


Figure 1: Result of the centrifugation of light and heavy DNA

THE PULSE PHASE

Using this technique, Meselson and Stahl grew *E. coli* on a medium containing ^{15}N for many generations. This ensured that all of the DNA would be labeled with ^{15}N . As the bacteria grew and reproduced, they incorporated the ^{15}N isotope. This is referred to as the **pulse** phase of the experiment (i.e., the pulse is exposing the cells to a particular version of a compound). Meselson and Stahl next took some of these bacteria, prepared the DNA as before, and centrifuged it. Because all DNA was labeled with ^{15}N , a single band was formed. This is "Generation Zero."

- Choose one colored pencil. Using **Figure 1** as a reference, indicate the location of the band for heavy DNA (^{15}N) in Generation Zero in the centrifuge tube represented to the right.



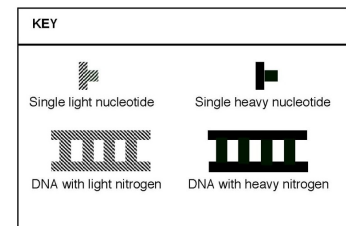
Generation Zero

THE CHASE PHASE

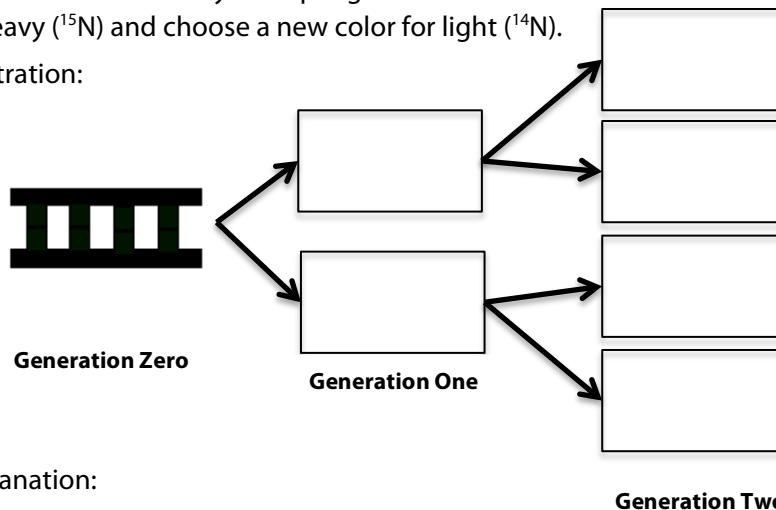
Next, the bacteria with heavy DNA were moved to a culture medium containing ^{14}N . This step marks the beginning of the **chase** phase of the experiment (i.e, exposing cells to a different version of the same compound). After 20 minutes (the time it takes for *E. coli* to grow and produce the next generation), a sample was prepared for centrifugation. This was identified as "Generation One." Another sample was taken after another 20 minutes had passed. This was "Generation Two," and so on.

QUESTIONS

- If DNA replication is **semiconservative**, use the key provided to illustrate the arrangement of light and heavy isotopes of nitrogen in the DNA molecules formed in Generation One and in Generation Two. Assume that each bacterium divided exactly once per generation. Use the same color as earlier for heavy (^{15}N) and choose a new color for light (^{14}N).

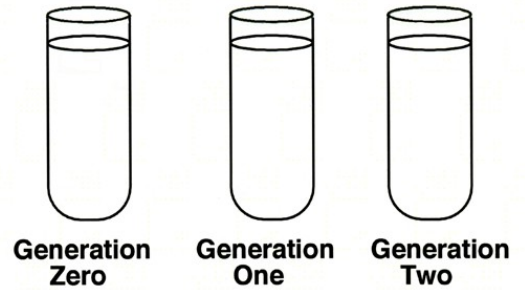


- Illustration:



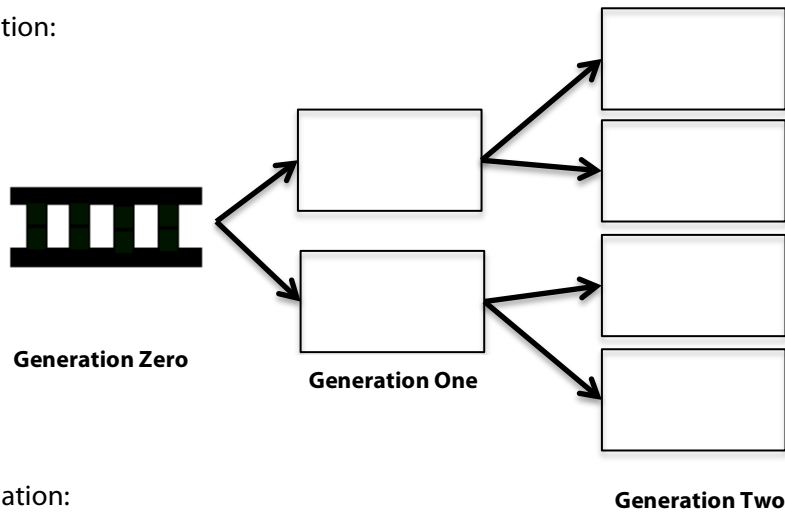
- Explanation:

- c. Using **Figure 1** as the standard, sketch where the bands of DNA would collect in the tubes for Generations Zero, One, and Two if DNA replication is **semiconservative**.



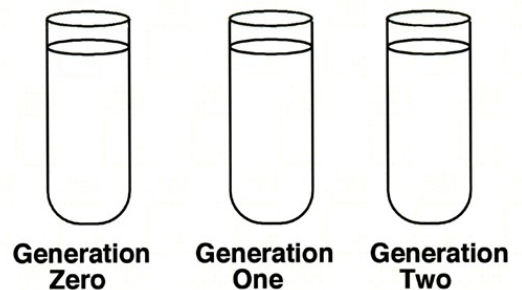
3. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **conservative**.

- a. Illustration:



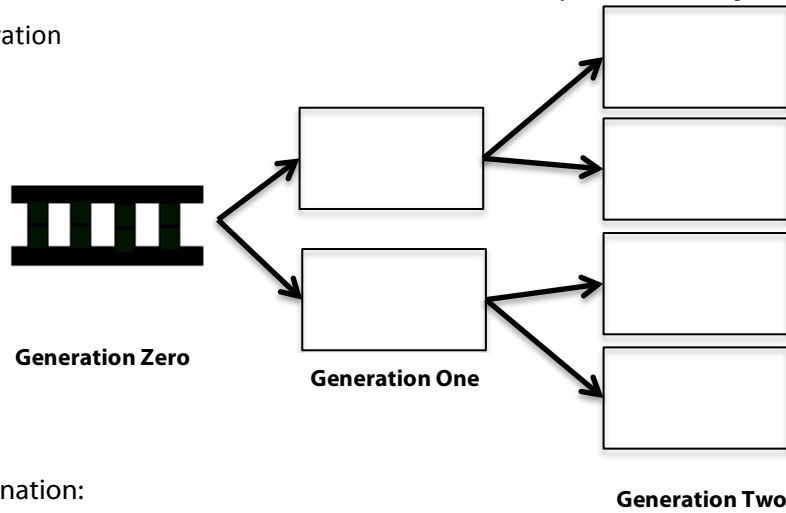
- b. Explanation:

- c. In the tubes to the right, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the **conservative** model of DNA replication.



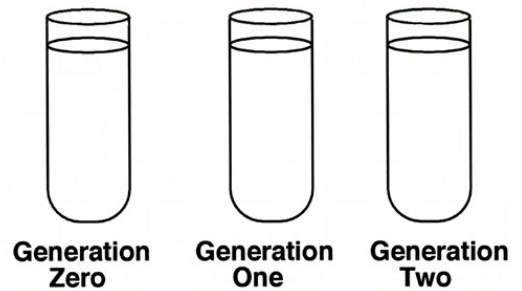
4. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **dispersive**.

a. Illustration



b. Explanation:

- c. In the tubes to the right, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the **dispersive** model of DNA replication.



RESULTS OF THE MESELSON AND STAHL EXPERIMENT

Now that you know the predicted results of the various replication models, evaluate the data from Meselson and Stahl. Their first replication in the ^{14}N medium produced a band of mid-weight hybrid (^{14}N and ^{15}N) DNA. The second replication in the ^{14}N medium produced both light (^{14}N) DNA and hybrid (^{14}N and ^{15}N) DNA.

5. Which model of replication did the actual results of the Meselson-Stahl experiment support? Explain your answer.