OVERVIEW

This activity is part of a series of activities and demonstrations focusing on various aspects of the human immunodeficiency virus (HIV) life cycle.

HIV is a retrovirus. Retroviruses are distinguished from other types of viruses by two key steps in the replication cycle: **reverse transcription** and **integration**. After HIV infects a host cell, reverse transcription results in the production of a double-stranded DNA copy of the single-stranded HIV RNA genome. Integration results in the insertion of the HIV DNA into a region of the host genome.

In this activity, students model the integration step. It may be helpful for students to complete the reverse transcription activity before completing this activity.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Viruses can only replicate within host cells.
- The replication cycle of retroviruses is distinguished from that of other viruses by two key steps: reverse transcription and integration.
- Following infection, the genetic material of a retrovirus becomes part of the host cell genome.

Students will be able to:

- Model how viral RNA is integrated into host cell DNA by using different colors of plastic pop beads.
- Apply concepts related to DNA and RNA synthesis to viral replication processes.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (2013)	HS-LS-1.1; HS-LS-1.2; HS-LS-3.1
AP Biology (2013)	3.A.1; 3.C.3
IB Biology	6.3

KEY TERMS

Genome, DNA, RNA, reverse transcriptase, integrase, transcription, translation

TIME REQUIREMENTS

This activity is designed to be completed in a 50-minute class period.

SUGGESTED AUDIENCE

This activity is appropriate for all levels of high school biology.

PRIOR KNOWLEDGE

Students should know that viruses consist of a nucleic acid surrounded by a protein coat and that they infect different types of cells. It would be helpful for students to understand that some viruses use their own enzymes, as well as host cell enzymes, to reproduce.

MATERIALS

For this activity, you will need pop beads of three different colors to represent:

- the double-stranded DNA genome of the host cell (about 40 to 50 beads per student or group)
- the single-stranded RNA genome of the virus (about 5 to 10 beads)
- the double-stranded DNA copy of the virus's genome (double the number of beads used for the RNA)

TEACHING TIPS

Introduction

- In this activity, students will watch an animation of the HIV life cycle (http://www.hhmi.org/biointeractive/hiv-life-cycle). The reverse transcription and integration of the viral genome are demonstrated at about time 1:32, followed by viral protein synthesis, viral assembly, and budding. The HIV life cycle is also shown in the **Click and Learn** "*Virus Explorer*" (http://www.hhmi.org/biointeractive/virus-explorer) by clicking on the HIV 3-D model and then selecting the replication cycle illustration. Spend some time in class reviewing the HIV life cycle to ensure that students understand the different steps. [For additional background on retroviruses, students can also complete the **HHMI "Click & Learn"** resource **"Retroviruses and Viral Diversity"** (http://www.hhmi.org/biointeractive/retroviruses-and-viral-diversity).]
- HIV infects immune cells. If students are not familiar with different types of immune cells, you can show them a short 42-second clip from Lecture 1 of the **HHMI Holiday Lecture "AIDS: Evolution of an Epidemic"** (http://www.hhmi.org/biointeractive/aids-evolution-epidemic). Click on *Chapter 14, "Cells of the immune system,"* and watch from time 19:13 to 19:55. This chapter provides an overview of the immune system and its associated cells. HIV infects helper T cells by binding to the CD4 receptor on these cells.
- A more extensive review of the immune system is available as a "**Click and Learn**" resource entitled "*Cells of the Immune System*" (http://www.hhmi.org/biointeractive/cells-immune-system). This Click and Learn offers a student worksheet that can be completed as homework.

Procedure

- Students will need to work through the **Click and Learn** "*Virus Explorer*" (http://www.hhmi.org/biointeractive/virus-explorer) to complete questions 1–5 in the student worksheet before doing the viral DNA integration activity. This **Click and Learn** and the three questions can be completed as homework before class.
- Provide students with three different colors of pop beads and ask them to make models that represent single-stranded viral RNA, double-stranded viral DNA, and double-stranded host cell DNA genomes. Students will need at least 40 to 50 pop beads of one color (for the host DNA), 5 to 10 for the viral RNA genome, and 10 to 20 for the DNA copy of the viral genome. Students may work individually, in pairs, or in small groups.
- As an alternative to using pop beads, it's also possible to use pipe cleaners and regular pony beads.
- When reviewing student work, be sure that the single-stranded viral RNA model is the same length as the double-stranded viral DNA copy and that both viral genome models are shorter than the host cell DNA pop-bead strands. Ask students to try again if their models do not show this distinction. Point out to students that these are relative sizes. The actual human genome has over 3 million nucleotides, whereas the HIV RNA genome is less than 10,000 nucleotides long.
- One point that you may want to mention to students, depending on their level, is that when the reverse transcriptase synthesizes the double-stranded DNA molecule, the resulting molecule actually isn't identical to the RNA genome; the process generates a slightly longer molecule with long terminal repeats (LTR) at each end. The LTR sequence is not found in the RNA genome but is generated during the process of reverse transcription. (This step is not included in the animations.) The LTR is important for expression of the viral genes.
- Students may have noticed in the animation that each HIV particle contains two copies of the HIV genome. Each copy is a single-stranded RNA molecule. Human cells also have two copies of the genome; each copy is a double-stranded DNA molecule.

Extension activities

- As an extension activity, show a short 58-second clip from Lecture 2 of the HHMI Holiday Lecture "AIDS: Evolution of an Epidemic" (http://www.hhmi.org/biointeractive/aids-and-hiv-life-cycle). Click on *Chapter 5, "How viruses cause disease,"* and watch from time 5:57 to 6:55. Dr. Bruce Walker explains how a virus "acts as a commando that takes over a machine tool factory and turns it into a bomb factory." Ask students how HIV does that based on what they learned through this activity, using the integration of the viral genome as an example.
- After completing this activity, students may find it interesting to research antiretroviral drugs. Discuss with students how understanding the mechanism by which a virus replicates can help identify drug targets. Explain to them that a variety of antiretroviral drugs are available and the

drugs can be effective by targeting different parts of the replication process. Students may be particularly interested in the drugs that target integrase since they now have a better understanding of the process of integration. Three such drugs that prevent the virus from integrating into the host DNA are raltegravir, elvitegravir, and dolutegravir.

ANSWER KEY

1. Draw a diagram and explain the basic characteristics of HIV.

The diagram should include the envelope, envelope proteins, capsid, and RNA genome. They could also include the reverse transcriptase and integrase enzymes.

2. Define the term "genome" and describe the HIV genome.

Answers may vary but should include the information that genomes are made of nucleic acids and that genes are regions of DNA that encode proteins essential for function. A genome typically contains all of the information needed to build and maintain the organism. The HIV genome is a single-stranded RNA molecule.

3. What is the host cell for HIV? How are the composition and size of the host cell genome different from those of HIV?

HIV infects human immune cells, and more precisely helper T cells. The human genome consists of double-stranded DNA. It is made of DNA and not RNA, it is double-stranded rather than single-stranded, and it is much bigger than the HIV genome.

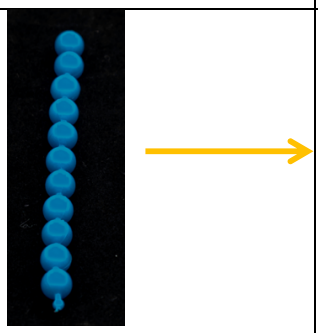
4. What is the enzyme responsible for reverse transcription? Is it a viral or host enzyme?

The enzyme responsible for reverse transcription is called reverse transcriptase. It is a viral enzyme that is released into the host cell after infection.

5. What is the enzyme responsible for the insertion of the viral DNA into the host genome? Is it a viral or host enzyme?

The viral enzyme integrase is involved in the insertion of a copy of the viral DNA into the host cell genome.

Table 1. Models of Host Cell Genome, HIV Genome, and Reverse-Transcribed Viral DNA.

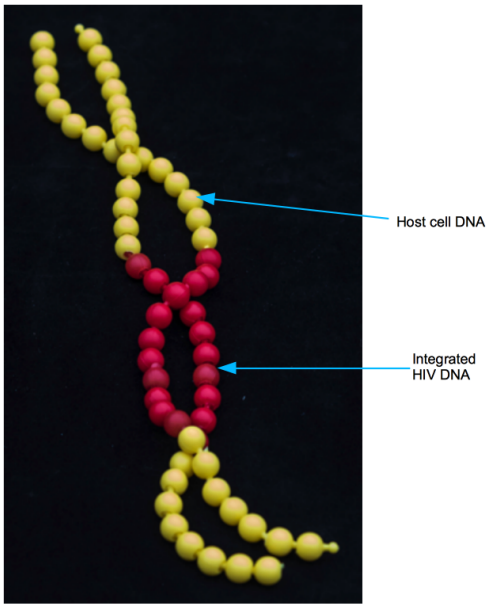
Model	Host Cell Genome	HIV genome	DNA copy of HIV genome
Draw a diagram of the model you constructed			
Characteristics (RNA or DNA; single- or double-stranded; relative size)	Double-stranded DNA Genome size = relatively large	Single-stranded RNA Genome size = relatively small	Double-stranded DNA Genome size = relatively small

8. Compare the three molecules you constructed. What are the key similarities and differences?

The human genome is many times larger than the HIV genome. The viral genome consists of single-stranded RNA, while the human host cell genome is composed of double-stranded DNA. DNA is composed of the nucleotides adenine, guanine, cytosine, and thymine. The HIV RNA genome is composed of the nucleotides adenine, guanine, cytosine, and uracil in place of thymine. Reverse transcription results in a double-stranded DNA copy of the viral genome. This molecule is like the host cell genome except much smaller in size. Students may also point out that each genome contains unique genes that encode proteins for their viral or cellular function. However, the viral genome does not code for all the proteins the virus needs to reproduce; for example, the virus relies on the host cell machinery to produce its proteins.

9. After the viral DNA is produced, the next step in the HIV life cycle is the insertion of viral DNA into the host cell genome. Use the double-stranded viral DNA and the host DNA pop-bead models to construct a

model showing how the viral genome is integrated into the host genome. Draw a diagram of your pop-bead construction below. (Make sure to label your diagram.)



10. Describe the steps involved in the integration process. (Think about what you had to do with the beads.)

The steps are breaking (or cleaving) the double-stranded host DNA and then splicing in the viral DNA. More advanced students will know that chemical reactions are involved in breaking bonds between nucleotides and then creating those bonds.

11. Describe what happens to the viral DNA now that it is integrated into the host cell genome.

The HIV genome will be transcribed into mRNA using the cellular enzyme RNA polymerase. Upon transcription, the viral mRNA encoding its structural proteins will be translated, processed in the cytoplasm, and transported to the cell membrane, which is where new viral particles containing single-stranded HIV genomes will bud from the host cell.

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