

# Introduction to the Molecular Lab

## Purpose

To learn how to perform DNA analysis at the virtual Molecular lab bench.

## Connections to What You Already Know About in Life

You've heard about how scientists can use DNA analysis to begin to understand the complexities of an organism's genes. However, since DNA is so small that you can't see it without special microscopes, it is very hard to understand how testing DNA can be so important. Is our DNA really that different from that of any other organism? Isn't it all made of the same basic building blocks? Molecular Biologists are working very hard to unlock the answers to those questions, to help everyone to understand more about our own bodies and the structures of all of the living things around us.

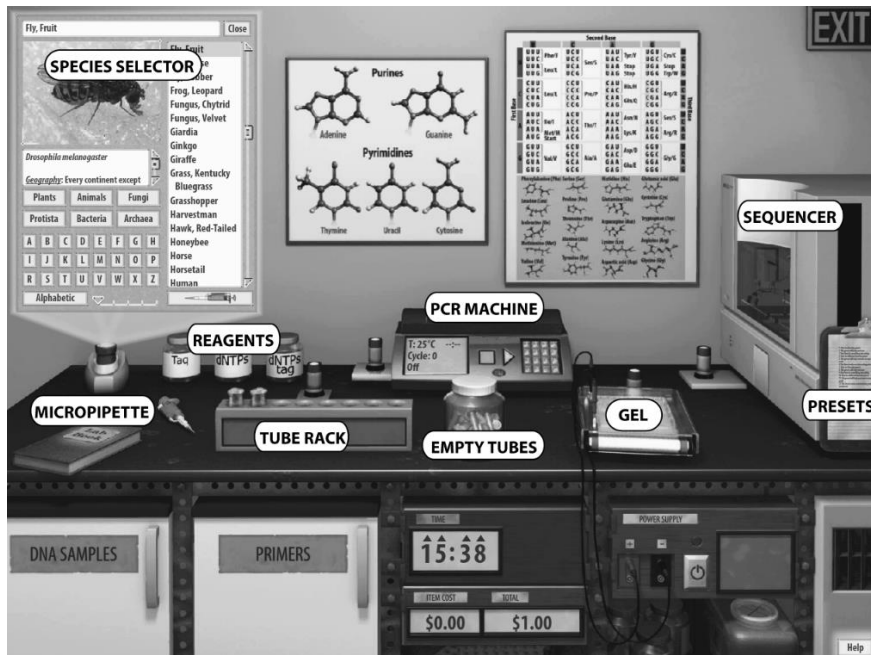
## Vocabulary

Gel electrophoresis, primer, sequencing

## Background

The Molecular lab bench offers virtual DNA samples of all of the species featured in Virtual Biology, along with real-world molecular biology tools that allow you to peer into and compare the DNA. This activity will introduce you to the Molecular bench and its capabilities. As you become more familiar with the Molecular bench, you will use it to determine relatedness of different species to produce DNA profiles like those used in modern forensic science.

Unlike the other four areas of Virtual Biology, this particular lab bench has components that you must use carefully and precisely to perform experiments successfully, just like in a real molecular biology lab. The labeled image below can be used as a map of this particular virtual lab bench.



One of the first things to do in most molecular activities is to set up the tubes in which your DNA samples will be processed. You can grab a microcentrifuge tube from the jar of empty tubes, drag it to the tube rack, label it, and load the DNA sample into it. (You can also double-click on the jar to make new tubes appear in the rack.) Getting DNA samples begins with selecting a species. If you select a species in the species selector and then click on the micropipette icon just below the species list, the micropipette on the bench will automatically load with a sample of that species' DNA. (Other worksheets will describe in detail how to handle the pipette.) You will also use the pipette to add reagents to perform polymerase chain reaction (PCR), a step that is necessary to run the DNA sequencer or gel electrophoresis. PCR amplifies, or makes many copies of, a specific part of a DNA sequence. The available PCR reagents include the polymerase enzyme "Taq", DNA building blocks called "dNTPs" (deoxynucleotide triphosphates), and tagged DNA building blocks called "dNTPs tag."

To determine which genes you will amplify in PCR, you will use primers, which select certain DNA sequences for amplification. The small projector directly behind the tube rack is a primer selector, where you select primers to target specific DNA fragments or genes. The worksheets will instruct you on which primers to use for specific experiments or investigations. Once your sample is ready, you can drag the tube to the PCR machine and run PCR to make copies of the particular gene you want to analyze.

Once you've completed PCR, you can pipette the amplified sample into the gel and run gel electrophoresis. This process will show you the relative molecular sizes of the DNA fragment(s) you have copied, and it will allow you to make a forensic DNA profile of an individual that can then be compared with another individual's profile. If gel electrophoresis is not necessary for a given activity, you can drag the microcentrifuge tube straight to the DNA sequencer. The sequencer spells out the nucleotide sequence of the fragment. The large digital clock below the bench can be used to track the duration of certain procedures and to advance time so that you don't have to sit around waiting for processes like PCR, gel electrophoresis, or sequencing to complete.

As you go through any procedure at this lab bench, you will notice the readout beneath the clock logging expenses. This will give you a rough idea of the costs of certain consumable materials, such as tubes, reagents, and primers, in such a lab.

The red lab book, found on the other end of the bench, is the place where you can save your work and do things like sequence comparisons—aligning sequences from different species and seeing where the sequences differ. Remember that anything saved to the lab book will remain available during your current session in Virtual Biology, but once you exit the main lab room the lab book will be cleared. To save your lab book work or saved media so you can revisit them at your next Virtual Biology session, click on the lab book's File menu and select *Save File*. This will save a lab book file on your local drive that can then be opened in Virtual Biology later on. You could even submit a lab book to your teacher so he or she can see your work, such as a gel you produced or a series of sequences you compared.

The Molecular bench also has a clipboard of presets, which are predetermined lab set-ups that let you dive right into a particular activity without having to start from scratch or do a lot of prep work. In this introductory activity you will use three such presets for a hands-on preview of what the virtual Molecular lab can do.



## Pre-Lab Question

1. Name a process that must be completed before performing gel electrophoresis and nucleotide sequencing.

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## Procedure Section 1

1. Start *Virtual Biology* and select *Introduction to the Molecular Lab* from the list of assignments. The lab will open on the Molecular lab bench.
2. Go the clipboard and select *Introduction to Molecular: Part A* from the list. You will see two microcentrifuge tubes appear in the tube rack. Roll over each tube to see its label. If you lift up a tube you will see a pale blue fluid inside. These tubes contain a mixture of DNA samples and reagents.
3. Click on the small projector behind the tube rack to see the list of primers that are available for use on the common house fly, which should be selected in the species selector. In other lab activities you may have to select specific primers from this list and add them to the tubes using the pipette. In this case, the primers have already been added to the tubes for you. These particular primers will target a gene called ND1 for amplification during PCR, hence the *ND1* labels on the tubes.
4. To see how PCR works, drag the tubes one at a time to the white lid of the PCR machine. The lid will open, and you will be able to drop each tube into an open slot. Once both tubes are in the machine, click on the lid to close it and then click the green arrow on the face of the machine to start the PCR process. At any time during the process, you can open the PCR controls projector (directly to the left of the PCR machine) and click on the DNA icon to watch animations of the process. The standard 35 cycles of PCR take a little over three hours in real life. Here you can click on the arrows of the main clock (beneath the lab bench) to advance time and complete PCR in a matter of seconds instead of hours.
5. Your targeted genes have now been amplified, which means you have many copies of the flies' ND1 genes that can now be used for gel electrophoresis or sequencing.
6. When finished with an experiment you can dispose of used microcentrifuge tubes and gels by dragging them to the red disposal bin beneath the right side of the bench. You can also clear the entire lab at any time by double-clicking on the same red disposal bin.

## Questions

1. The tubes have DNA from what two species?

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2. Keeping track of which arrows you click, advance time until the display on the face of the machine reads *Complete*. Which arrows did you click to advance time far enough?

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## Procedure Section 2

1. Click on the preset called *Introduction to Molecular: Part B* on the Clipboard. You will see that the two tubes are back in the tube rack. Some of the amplified genes have been loaded into the wells of the gel. In some activities you will be instructed on how to do this yourself, by using the pipette to transfer samples from the tubes over to the wells of the gel. In this case, this step has been done for you and gel electrophoresis has already been run. Click on the small projector behind the gel to see the two fly ND1 fragments in the gel under UV light. Note the red light on the power supply beneath the bench, which indicates that the process is complete.
2. The fruit fly's sample was loaded into Well 4, and the house fly's sample was loaded into Well 7. At the Molecular bench of Virtual Biology, wells 1 and 10 are always loaded with DNA ladders, or standard samples of DNA with known fragment lengths that allow for comparisons with the experimental samples. (The ladders in both wells are identical.) To see the approximate molecular weight of the fly samples' ND1 fragments, you can click on the circular icon in the top right of the gel window. This is the horizontal tool. Roll over the gel and you will see a dotted line that marks the estimated size of the fragment (in base pairs, or bp), which in the gel appears as a band. The larger the fragment, the shorter the distance it will have traveled from the well toward the far end of the gel.
3. A given band may appear relatively faint and gray, or it may be quite thick and white. A faint band indicates that there were not very many DNA fragments of that size in the portion of the sample that was loaded into the well. A thick, opaque band indicates that many fragments of that particular size were in the sample.
4. To measure approximate size of the fragments that accumulated as a particular band in the gel, place the horizontal tool in the middle of the white band you're analyzing.

## Questions

3. What is the size, in base pairs, of the largest DNA fragment in the DNA ladders in lanes 1 and 10?

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4. In this case, how large are the ND1 fragments of these two fly species, which appear in lanes 4 and 7?

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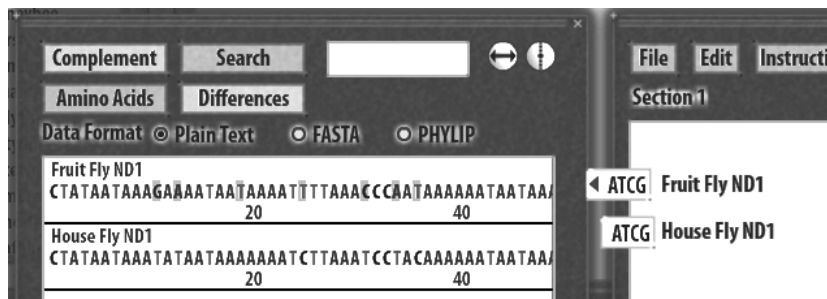
## Procedure Section 3

1. Click on the preset called *Introduction to Molecular: Part C*. In other activities, you will have to run the sequencer by dragging the tubes into the sequencer one at a time, starting the sequencer, and labeling the sequences. This preset has had the two fly ND1 genes sequenced and labeled. Click on the small projector next to the sequencer. A window will open, revealing the two sequences of the flies' respective ND1 genes. Click on either of them to see the titles. If you want, you can click on the titles and edit them using the keyboard.
2. In the sequencer window, you can do a rough comparison of the nucleotide sequences and see how long these particular genes are by scrolling to the right. The numbers below the sequences are in



intervals of 20 nucleotides. So, in the case of these flies' ND1 genes, you can see that both are indeed around 940 base pairs in length.

- Click on either of the sequences so that it's highlighted in yellow. Click the *Save* button at the top of the window. You have now saved this sequence to the lab book. (The highlighted lab book icon at the left end of the sequence indicates that the sequence is saved; a faded icon means it is not saved.) Save the other sequence. On the right-hand menu page of the lab book you will see the titles of the two fly sequences. If you click on either *ATCG* icon, that sequence will open on the left-hand page.
- When in the lab book, you have a narrower view of the sequences but you still have the same scroll bar at the bottom for moving along their full lengths. Saving the sequences to the lab book also allows you to make more direct comparisons of the sequences. Click on the *ATCG* icon of the Fruit Fly ND1 sequence. Then, click on the sequence on the left-hand page so that it is highlighted in yellow. Now, right-click (or control+click) on the highlighted sequence and select *Copy Data* from the menu that appears. You've now got a copy of that sequence that you can paste into the same page as the other sequence. To try this, click on the *ATCG* icon of House Fly ND1, right-click somewhere below that sequence on the left-hand page, and choose *Paste Data*. You should now see both sequences on the same page. (If you want to rename the page to reflect the inclusion of the other sequence, just click on the page's title and rename it.) Next, click on one sequence, hold down the control button, and click on the other sequence. Both sequences should now be highlighted. Click the *Differences* button. This will cause nucleotides that don't match between the two sequences to be highlighted (faint gray) in one of them, like this:



## Questions

- Is one of the flies' ND1 genes longer than the other's? If so, which is longer?

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- Within the first 60 bases of the fruit fly and house fly ND1 gene sequences, how many differences are there between the two species? (Hint: Use the scroll bar to move along the length of the sequences and count the number of gray-shaded letters between the left end and the "60" mark.)

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7. What can the number of nucleotide differences between two genes like these tell you about how closely related two different species are? Explain your reasoning.

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