Name ______________________________________ Class _______ Date __________

Odd Fish Out

Use modern molecular biology lab techniques to analyze the relatedness of three fish species.

Molecular Systematics

Lab Benches Used

Molecular biology allows scientists to peer into the DNA of countless species. In this virtual lab activity, you will use molecular techniques such as polymerase chain reaction (PCR) and DNA sequencing to compare the DNA of three species of fish and determine their relatedness.

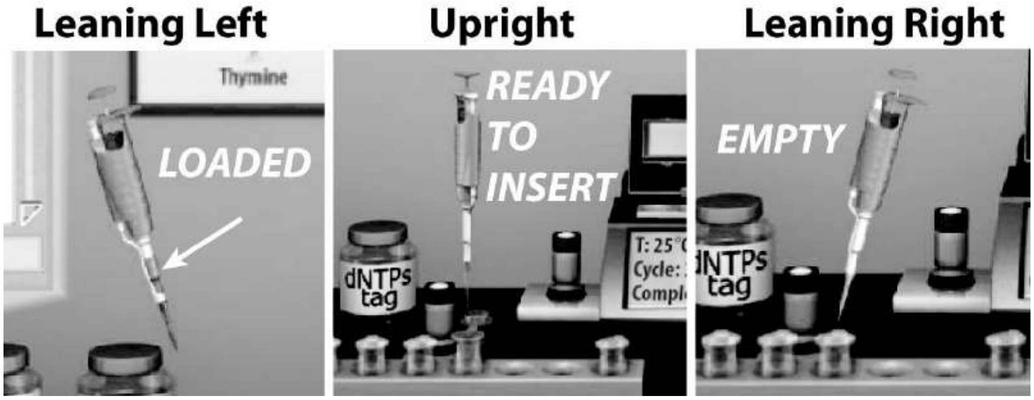
Enter the Virtual Bio Lab and select the title of this lab activity from the "DNA" menu on the whiteboard. You will be taken to the virtual Molecular lab bench.

Part A: DNA Preparation and Amplification

For the rainbow trout, brown trout, and common carp, use the following steps to produce multiple copies of a specific gene called 12S that all three species have in their genomes. The 12S gene codes for mitochondrial rRNA and is commonly used as a point of comparison between different species' DNA. Be sure to complete each of these steps for each fish species.

∓ **Grab and label a tube.** Drag a microcentrifuge tube from the white-capped tube jar to the tube rack. Click on the tube and then click in the blank face of the tube rack. The cursor will become a pen. Type in the species name as a label for that tube. Click "Enter" to complete the label.

∓ **Prepare DNA.** Open the Species Selector and click on the species name (Ex. "Trout, Rainbow"). Once selected, click on the pipette icon below the species list. You now have a pipette loaded with a DNA sample from that species. Drag that pipette over the correct tube until it straightens up. Click on the pipette and it will lean to the right, indicating that the sample has been inserted into the tube. Here is an image showing the three positions of the pipette and what they indicate:



You can lift the tube out of the rack to see that it now contains a liquid sample.

∓ **Add PCR reagents.** Once the tube has its DNA sample, use the pipette to load "Taq" and insert it into the tube. Do the same for "dNTPs tag."

∓ **Add primers.** Open the primer selector by clicking on the projector that's just behind the tube rack. You will need to select two specific primers for each species' DNA—a left primer and a right primer. Use the table below to guide your selections. (*Note:* If a primer doesn't appear in the virtual primer list, you can type or copy-and-paste it in manually. Once entered, click "add" to put it at the top of the list where it can then be selected. Manually added primers will be marked with an asterisk to help you find them.) Select a primer and then click on the pipette icon at the bottom of the primer selector window. You now have a pipette loaded with that specific primer. Insert the primer into the proper tube. Remember to add both the left and right primer for each species' sample. Otherwise, you will not be able to amplify the specific gene you are comparing across these species. Use the chart below.

Species	**Left Primer**	**Right Primer**
Rainbow trout	caaaggcttggtcctgactttacta	gttattccaagcgcaccttc
Brown trout	caaaggcttggtcctgactttact	gttattccaagcgcaccttccg
Common carp	caaaggcatggtcccgacc	ggtttgatccaagtgcactt

∓ **Run PCR.** Your DNA samples and reagents are now ready for the polymerase chain reaction in the PCR machine. Load each tube into the machine, close the lid, and click the green arrow to start the process. Because this process takes over three hours, use the arrows on the main clock to advance time by just over three hours, until the machine's display reads "complete." Open the PCR machine lid and move the tubes back to the main tube rack.

Part B: Sequencing

Now you will use the DNA sequencer to find the exact length and nucleotide sequence of each fish's 12S gene.

∓ **Load the sequencer.** Drag one tube to the sequencer. The drawer will open. Hold the tube over the visible slot and click to drop the tube in place. Click on the drawer to close it. The sequencer controls window will now be projected. Type in the name of the species you are sequencing. Click "Start" to begin sequencing. Again, this process takes a while, so use the lab clock beneath the lab bench to advance time until the sequencing is complete.

∓ **Save each sequence.** Click on the sequence to highlight it (yellow) and then click on the "Save" button. The titled sequence will now be saved to your lab book. The small lab book icon to the left of each sequence is gray when it isn't saved and red when it has been saved.

∓ **Remove and repeat.** Remove the sequenced sample from the drawer and repeat the sequencer steps for the other two samples. Be sure to title and save each sequence to the lab book.

Part C: Sequence Comparison

Now you will compare the three 12S gene sequences to see how closely related these species are. To do this, you will need to paste two of the sequences into the same lab book page as the third sequence. This will let you see all three in one space and quantify the different nucleotides between them.

∓ **Use copy and paste to organize your sequences.** When you click on a sequence title, that sequence will be displayed on the left-hand page. To compare all three, open one of them clicking on the ATCG icon, then click on the sequence (without clicking on a specific nucleotide) and use right-click "Copy Data." Now, open one of the other sequences, then right-click "Paste." You'll now see two sequences, one on top of the other. Now go to the third sequence, copy its data, and paste it below the other two on the same page.

∓ **Check alignment.** Once you have all three fish 12S gene sequences in one page, you can make sure they are aligned by seeing that each sequence's left-hand end begins with the same nucleotides. The double-headed arrow button can be clicked if you need to slide a sequence left or right relative to the others. Use the scroll bar at the bottom to scroll all the way to the far right, to the far right ends of the sequences.

1. About how long are these 12S genes in terms of the number of nucleotides?

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2. Is it surprising that these genes would be of such similar lengths despite being from different species? Explain.

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∓ **Highlight and compare.** Highlight any pair of sequences (use control-click to select more than one) and click on the "differences" button. This will highlight in gray all of the nucleotides in one sequence that differ from those in the other sequence. Be sure to compare all three possible pairs of species: rainbow trout with brown trout, rainbow trout with carp, and brown trout with carp. You can also highlight all three at once. Scan along the lengths of the compared sequences to see where and how often they differ.

3. Which pair appears to be most similar at this particular gene? Which pair is most different? Explain.

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4. Use the scroll bar to analyze where these genes differ. What do you notice?

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5. What is your overall hypothesis about the evolutionary relationships among these three fish species? Explain.

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Part D: Classification

Exit the Molecular lab bench and go to the Systematics bench on the left side of the main lab room. Click on the preset clipboard that's on the lab bench and select the preset called "Bony Fishes (Teleostei)." You will see the branch of the "6 Kingdoms" tree and the order, Teleostei, where the two trout and the common carp can be found. Click on this group to learn more about the evolutionary relationships among these species. At any point in the tree you can roll over a taxon (group name) and read the defining traits of that taxon in the Traits box beneath the lab bench. To see if a given branch beyond Teleostei still contains the three species you've been studying, keep an eye on the "Included" and "Excluded" thumbnail image menus below the trees window.

6. At which taxonomic levels do you find divergence among the three species?

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7. Which other species featured in the Virtual Bio Lab belongs to the same genus as one of the fish species you studied in this lab activity?

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8. Is your hypothesis about relatedness among these species supported by what you learned at the Systematics bench? Explain.

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9. If you were to analyze the DNA from the Chinook salmon, how close do you think its genome would match the other three species' genomes? Put your response in order of most to least related, and explain your reasoning.

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