Biotechnology Explorer™

Fish DNA Barcoding Kit

Quick Guide

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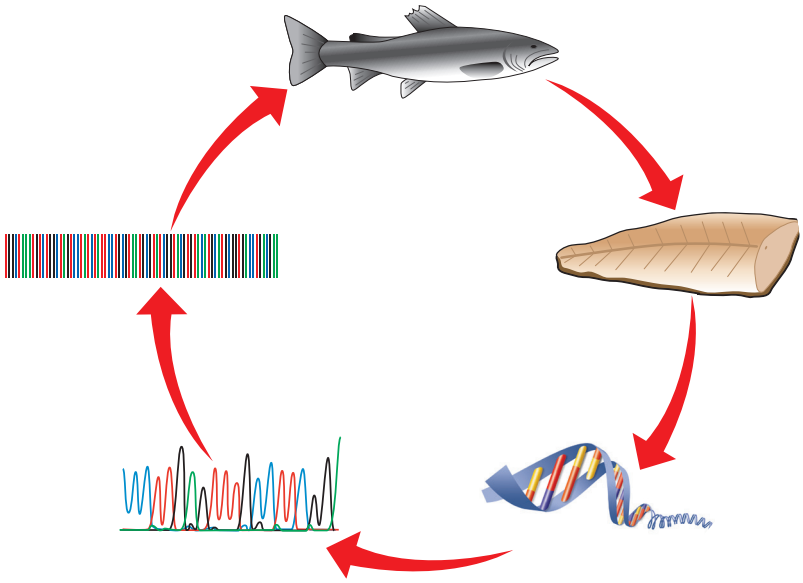
Catalog #166-5100EDU



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Quick Guide

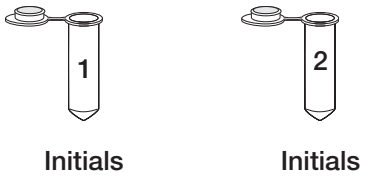
Lesson 1: DNA Extraction

Preparing Fish Samples

1. Label one capped 2 ml microcentrifuge tube for each of your fish samples (that is, “1” for fish sample 1, “2” for fish sample 2, etc.). Also label with your initials.

   Fish 1_____

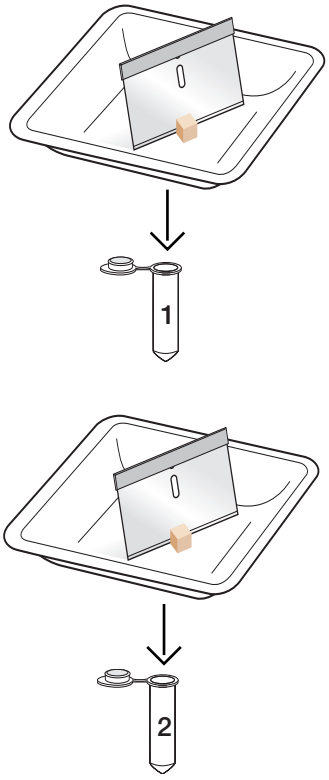
   Fish 2_____



2. Cut a piece of fish muscle up to 100 mg in mass, approximately the size of a pencil eraser-head, from your first fish sample. Place the piece in a new weigh boat and slice it with a razor blade or cutting implement until finely minced. Transfer the sample into the appropriately labeled microcentrifuge tube.

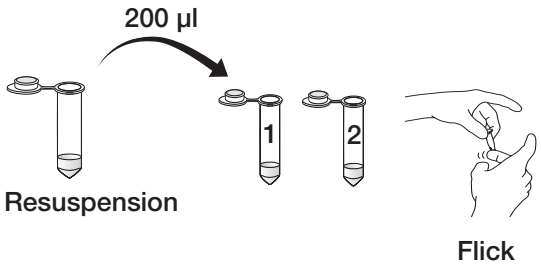
3. **Properly discard the razor blade or cutting implement.** If wearing gloves, change gloves before handling the next piece of fish. If not, wash hands thoroughly.

4. Using a new razor blade or cutting implement, cut a piece of fish muscle up to 100 mg in mass, approximately the size of a pencil eraser-head, from your second fish sample. Place the piece in a new weigh boat and slice it with a razor blade until finely minced. Transfer the sample into the appropriately labeled microcentrifuge tube. Properly discard the razor blade or cutting implement.

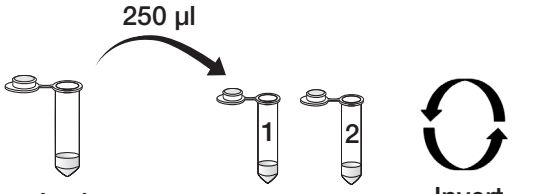


Extracting DNA from fish samples

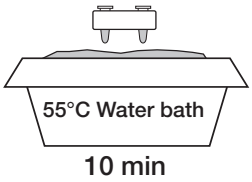
1. Add 200 µl of **Resuspension** to your two microcentrifuge tubes containing minced fish and flick the tubes several times to ensure full submersion of the fish sample in the resuspension solution.



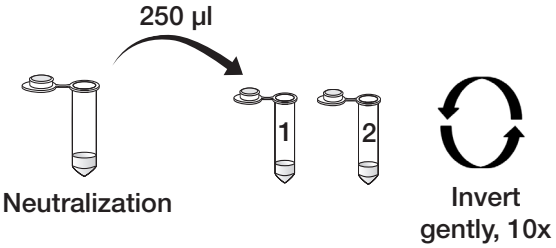
2. Add 250 µl of **Lysis** to each tube and mix gently by inverting tubes 10 times to mix contents. **Do not vortex!** Vortexing may shear genomic DNA, which can inhibit PCR amplification.



3. Incubate samples at 55°C for 10 min. The samples do not need to be shaken during incubation.

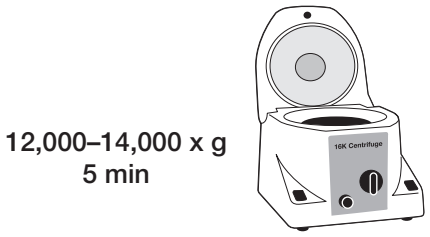


4. Add 250 µl of **Neutralization** to each microcentrifuge tube and mix gently by inverting tubes 10 times to mix contents (do not vortex). A visible cloudy precipitate may form.



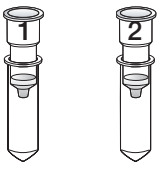
5. Centrifuge the tubes for 5 min at top speed (12,000–14,000 x g) in the microcentrifuge. A compact pellet will form along the side of the tube. The supernatant contains the DNA.

   If there are a lot of particulates remaining in the supernatant after centrifugation, centrifuge the tubes for 5 additional min.

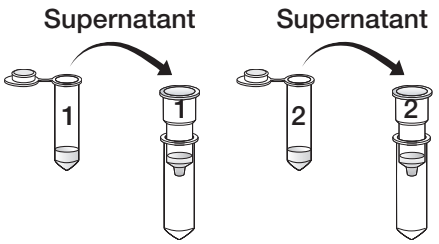


6. **Snap (do not twist!)** the bottoms off of the spin columns and insert each column into a capless 2 ml microcentrifuge tube.

7. Label one spin column **1** for Fish 1 and a second spin column **2** for Fish 2. Also label the columns with your initials.

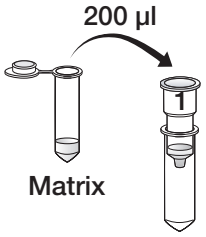


8. Transfer the entire supernatant (500–550 µl) of each fish sample from step 5 into the appropriately labeled spin column. Try not to get any of the particulates into the spin column because they will clog the column and prevent you from continuing.

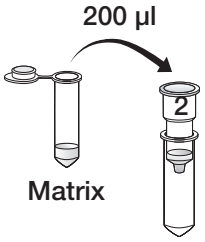


9. Thoroughly mix the tube labeled **Matrix** by vortexing or repeatedly shaking and inverting the tube to make sure particulates are completely resuspended before use.

10. Add 200 µl of thoroughly resuspended **Matrix** to the first column containing fish extract and pipet up and down to mix.

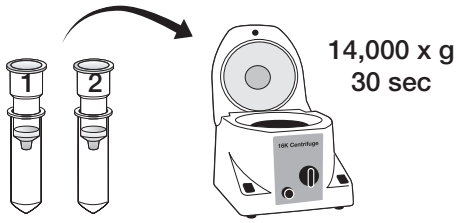


11. Using a new pipet tip, add 200 µl of thoroughly resuspended **Matrix** to the second column containing fish extract and pipet up and down to mix.



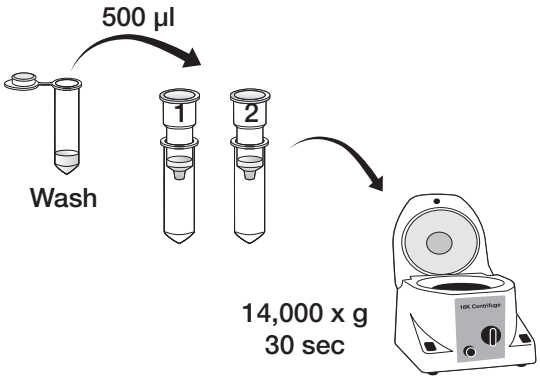
12. Centrifuge the columns for 30 sec at full speed.

Take care to spin the column for only 30 sec. Drying the matrix completely at this point will result in loss of DNA.



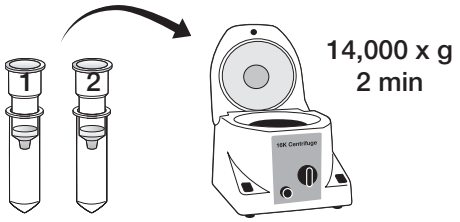
13. Remove the spin column from the 2 ml microcentrifuge tube, discard the flowthrough at the bottom of the 2 ml tube, and replace the spin column in the same tube. Add 500 µl of **Wash** and wash the matrix by centrifugation for 30 sec.

Take care to spin the column for only 30 sec. Drying the matrix completely at this point will result in loss of DNA.



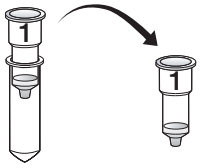
14. Repeat step 13 to wash samples again.

15. Remove the spin column from the 2 ml microcentrifuge tube, discard the flowthrough at the bottom of the 2 ml tube, and replace the spin column in the same tube. Centrifuge columns for a full 2 min to remove residual traces of ethanol and dry out the matrix.

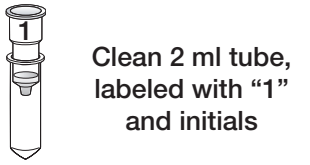


16. Label two clean 2 ml capless microcentrifuge tubes with your fish sample name and your initials.

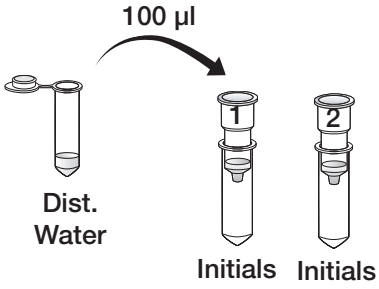
17. When your 2 min spin is completed, remove the spin columns and discard the 2 ml microcentrifuge wash tubes.



18. Place the spin column for each sample into a new capless 2 ml microcentrifuge tube from step 16.

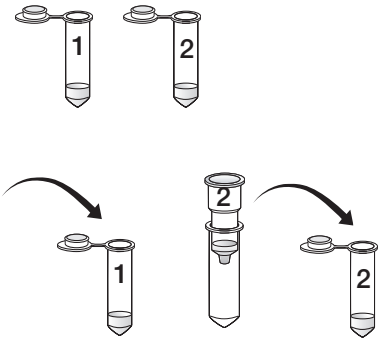


19. Using a fresh pipet tip for each sample, add 100 µl of distilled water to each spin column, being careful not to touch the resin. Elute the DNA by centrifuging for 1 min at full speed.



20. Label two clean 2 ml microcentrifuge tubes (with caps) **Fish 1** and **Fish 2** and your initials.

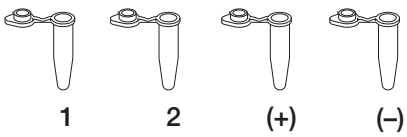
21. Transfer the eluted DNA into the appropriately labeled 2 ml microcentrifuge tube with caps and store the DNA at 4°C until you are ready to proceed.



Quick Guide

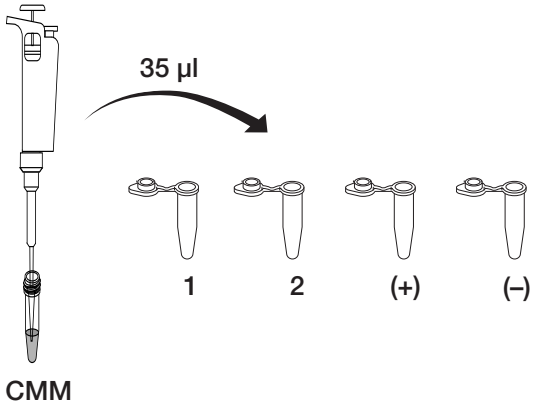
Lesson 2: PCR Amplification of DNA

1. Label four PCR tubes with your initials and the sample name (1 for fish sample 1, 2 for fish sample 2, (+) for the PCR positive control DNA, (–) for the PCR negative control). Keep the tubes on ice for the remaining steps.

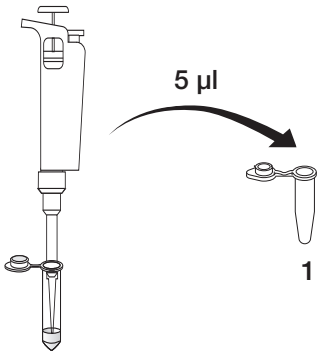


2. Using a fresh aerosol filter pipet tip each time, add 35 µl of **CMM** (COI master mix) reaction mix to each PCR tube, capping each tube immediately after the addition of liquid.

3. Using a fresh aerosol filter pipet tip for each tube, add 5 µl of the appropriate DNA sample directly into the CMM liquid in each PCR tube as indicated by the labels on the tubes, and pipet up and down to mix. Recap each tube immediately after adding DNA.



Tube Name	Master Mix DNA
1	35 µl CMM, 5 µl fish sample 1
2	35 µl CMM, 5 µl fish sample 2
(+)	35 µl CMM, 5 µl (+) sample
(–)	35 µl CMM, 5 µl (–) sample



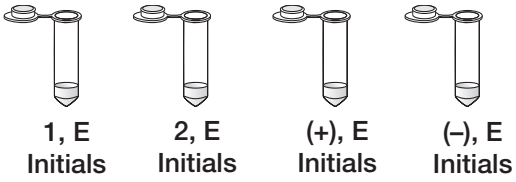
4. When instructed, place the PCR tubes in the thermal cycler and run the program with the following cycling conditions:

    1. 94°C – 2 min
    2. 94°C – 30 sec
    3. 55°C – 2 min
    4. 72°C – 1 min
    5. Repeat steps 2–4 35x
    6. 72°C – 10 min
    7. 4°C – hold

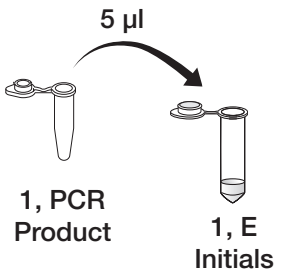
    Store tubes at 4°C after thermal cycling is complete.

Preparing PCR Samples for Electrophoresis and Sequencing

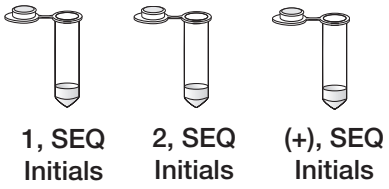
1. Label four 2 ml microcentrifuge tubes with both your initials and **E**. E stands for electrophoresis. Now label one of these tubes **Fish 1**, one tube **Fish 2**, one tube **(+)**, and one tube **(–)**.



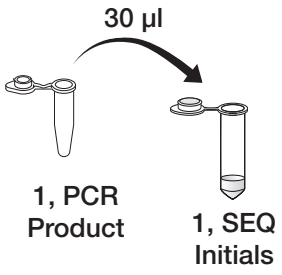
2. Remove 5 µl from each PCR reaction and deposit into the 2 ml microcentrifuge tube corresponding to that sample.



3. Label three 2 ml microcentrifuge tubes with both your initials and **SEQ**. SEQ stands for sequencing. Now label one of these tubes **Fish 1**, one tube **Fish 2**, and one tube **(+)**. You will not be sequencing your negative control sample.



4. Remove 30 µl from each PCR reaction and deposit into the 2 ml microcentrifuge tube corresponding to that sample.



5. Store all samples at 4°C until you are ready to proceed with electrophoresis and sequencing.

Quick Guide

Lesson 3: Gel Electrophoresis

1. Retrieve the 5 µl samples of PCR products (4 samples) from 4°C. To each one, add 5 µl of sterile water. Use a new pipet tip each time.

2. Add 2 µl of UView™ 6x loading dye to each sample, using a new pipet tip each time. Mix samples well and pulse-spin.

3. Set up your gel electrophoresis apparatus as instructed.

4. Load the agarose gel in the following lane order and volumes, using a new pipet tip each time:

   Lane Sample

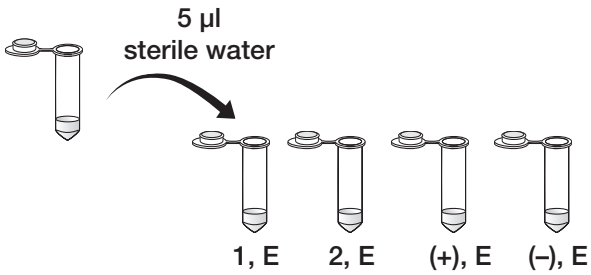
   1 – EMPTY
   2 – EMPTY
   3 – 20 µl PCR molecular weight ruler
   4 – 12 µl (+) E
   5 – 12 µl (–) E
   6 – 12 µl 1 E
   7 – 12 µl 2 E
   8 – EMPTY

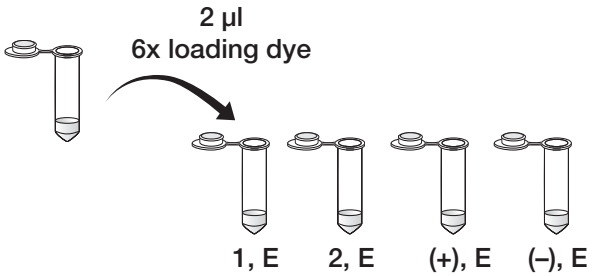
5. Ask your instructor whether the electrophoresis buffer your electrophoresis units contain is 0.25x TAE or 1x TAE.

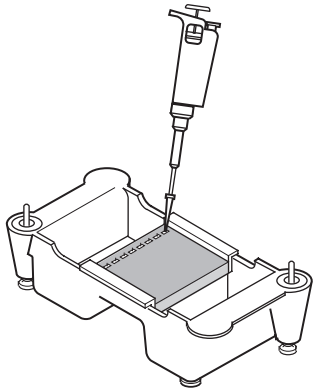
   If your buffer is 0.25 x TAE, run the gel at 200 V for 20 min.

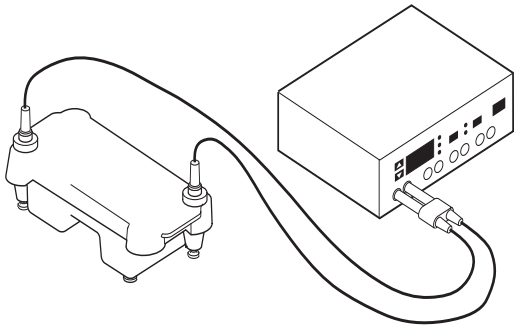
   If your buffer is 1x TAE, run the gel at 100 V for 30 min.

6. Visualize the gel on a UV transilluminator or imaging system. No gel staining is required as the loading dye contains a fluorescent compound that will allow visualization of DNA with UV light.









Quick Guide

Lesson 4: Sequencing

1. Parafilm your capped **Fish 1 SEQ, Fish 2 SEQ,** and **(+) SEQ** tubes thoroughly to prevent leakage while shipping.

2. Record the sample names on your tubes and make sure these match the names your instructor is submitting to the sequencing facility. This is the only way you can identify the correct sequencing data file for each sample.

3. Give your samples to your instructor for shipment to the sequencing facility.

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