Chapter 4

Introducing Students to Conservation Genetics Using Sturgeon Caviar and Other Fish Eggs

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Contents

Introduction

This laboratory exercise is based on one that was originally presented at the 21st Annual Association of Biology Laboratory Education Conference at the University of Nebraska-Lincoln in June, 1999 (Nolan et al. 2000) It has been successfully adapted by Claire Leonard at William Patterson University (Leonard and Nolan, 2000) and by Nancy Rosenbaum at Yale University (personal communication).

Most natural history museums have as one of their missions uncovering relatedness among species. Traditionally, scientists have used measurements of physical characteristics, embryology and the fossil record to help determine this relatedness. Many scientists are now using molecular biology tools in concordance with these traditional approaches.

Scientists that work in the Molecular Systematics Laboratory at the American Museum of Natural History in New York City are interested in uncovering relatedness among many different types of species. Molecular approaches have been used to assist in the construction of a "family tree" (phylogeny) of the 25 sturgeon and paddlefish species (Order Acipenseriformes). These are the caviar-producing species. Mitochondrial DNA genes have been sequenced for each of these species, and primers for amplifying these genes have been synthesized (Birstein and DeSalle 1998. These sequences have been used to develop species-specific genetic tags.

Cullen (1999) describes the decline of several species of caviar producing sturgeon for commercial purposes in the Caspian Sea. American caviar producing species are also in decline in areas such as the Hudson River. There is currently a moratorium on sturgeon fishing in the Hudson River for the next forty years (Waldman, personal communication). All twenty-five species of sturgeons and paddlefishes (order Acipensiformes) are threatened by over fishing and habitat degradation (Birstein et al., 1998). Most species of sturgeon do not reproduce until they are approximately fifteen years old, which means that sturgeons are very slow-growing fish. Unfortunately, they are often harvested before they are even at this reproductive age, which contributes even more to their decline in abundance. The three species that produce the caviar that is most often found in U.S. delis (and now over the Internet) are the beluga (*Huso huso*), the sevruga (*Acipenser stellatus*) and the osetra (*Acipenser gueldenstaedti*).

As caviar commands a high price in the marketplace, these fish are sometimes illegally caught. All sturgeon species were placed on the Convention for Trade in Endangered Species (CITES) list in 1998. Worse, sometimes caviar from the more "commonly available" sturgeon are replaced by even rarer species.

DeSalle et al. (1996) reported that of twenty-three lots of caviar purchased in Manhattan delis and two lots from Russia, five were incorrectly labeled, according to species-specific primers that have been developed. This was ascertained from DNA that was isolated from a single egg. Sometimes a species of sturgeon that produces "cheaper" caviar is substituted for a species that produces more expensive caviar. Quick methods of species identification of sturgeon as well as other organisms may reveal other misidentifications as well. Ultimately this work may put pressure on sellers and consumers alike to conserve our natural resources.

When female sturgeon are caught, they are first stripped of their roe. The fish eggs are packed carefully in salt to add to the flavor and so that they will keep for several months in an unopened jar. After the salt is added, the roe is kneaded in a certain fashion – this is the "art" to packing caviar (Cullen, 1999). When the jars are opened, the eggs should keep for several weeks if tightly covered. (We have isolated DNA from caviar that has been several months old – if tightly covered and refrigerated the eggs appear to keep their shape and integrity, which is necessary to isolate DNA from an intact egg.

In order to make this lab more investigative, the instructor may purchase the caviar initially and invite the students to bring in fish eggs from other sources, including their own refrigerators and supermarkets. Frozen herring eggs (Nolan, personal communication) and freshly-laid zebra fish eggs have been used (Leonard and Nolan, 2000). Rosenbaum (personal communication) has also tested caviar purchased via the Internet and from local delis, and has found that some of the caviar has been "mislabeled" through this exercise.

Materials

Caviar technically means "sturgeon eggs", the color of which is always black. Paddlefish, now raised commercially, yield eggs that are brown. Many varieties of fish, such as red salmon, eggs may say "caviar" on the jar. Whitefish eggs are also black. Sturgeon caviar comes in three commercial varieties. Sevruga and osetra are the least expensive, and cost about $25 for a half ounce each. Beluga caviar is twice as expensive. You may buy caviar from delis or specialty import food stores. Caviar is also now being sold over the Internet. Two sites are <http://www.petrossian.com> and <http://www.markys.com>. One-half ounce should yield hundreds of eggs, which are a little larger than the head of a pin.

Other fish eggs (salmon, osetra or beluga caviar, paddlefish) can be purchased for use for this experiment by

the instructor, or can be provided by the students.

- Gloves---small, medium and large sizes
- DNAzol---available from Modern Research Center Inc., 5645 Montgomery Rd., Cincinnati, Ohio 45212 Phone: 888-841-0900, Fax: 841-0080
- 100% ethanol
- 70% ethanol
- DNA grade water—FisherBiotech---Fisher Scientific---cat. # BP2470-1
- distilled water
- PCR Super mix-- this comes with Taq polymerase, a buffer, dNTP's, and 15 mM magnesium chloride---Qiagen Science, Inc., 19399 Germantown Rd., Germantown, MD 20874 Phone: 800-426-8157, Fax: 800-718-2056

Primers are designed with the sequences ordered from the 5' to the 3' ends. In this exercise, we will use three primers. B72 and S2A should amplify a 150 base pair fragment of a cytochrome b gene that has been sequenced and found to be in all sturgeon. When S2 is used with S2A, only a cytochrome b gene fragment (approximately the same size) specific to sevruga sturgeon (*Acipenser stellatus*) should be amplified. We ordered ours from Qiagen Operon. The sequences are e-mailed to the company. There is a set-up fee and a cost per base.

Primer 1 (B72) GCCTACGCCATTCTCCG
Primer 2 (S2A) CCTCCAATTCATGTGAGTACT
Primer 3 (S2) GGAGTCCTAGCCCTCCTG

Usually the primers come lyophilized in nanomole concentrations**.** These need to be reconstituted in RNase-free water to a 25 µmole concentration. For example, if the total amount on your primer vial is listed as 70 nanomoles, then use the following formulation:

25 µM = 25 µmoles per liter = 25 nanomoles per ml.

$$\frac{70 \text{ nmoles}}{X \text{ ml}} = \frac{25 \text{ nmoles}}{\text{ml}}$$

$$x = 2.8 \text{ ml}$$

- Reconstitute primer vial with 2.8 ml of RNase-free water.
  For this PCR reaction, the primer will be diluted from 1:25, depending on which protocol you use.
- agarose
- 20X TBE buffer pH 8.0 (Tris-boric acid-EDTA buffer) can be purchased from Carolina Biological--1-800-334-555--cat. # 219027---$25 for 500 ml--or made up yourself---see recipe below---you would first make up a 10X solution, and then dilute to 1X as needed)
- To make one liter of 10X TBE buffer:pH 8.0 add the follwoing to 700 ml of distilled water in a 2-liter flask:
  1 g of NaOH
  108 g. Of Tris base
  55 g boric acid
  7.4 g of EDTA
  Stir to dissolve; bring to volume. (Micklos and Freyer, 1990)
- NaOH (to adjust pH of TBE buffer to pH 8.0 if necessary)
- Hydrochloric acid (to adjust the pH)
- To make 100 ml of 6X loading dye, dissolve:
  0.25 g bromophenol blue

0.25 g xylene cyanol
in 49 ml of water. Stir in 50 ml of glycerol. (Micklos and Freyer, 1990)

- 0.025% methylene blue or ethidium bromide (5 mg/ml)--HANDLE WITH CAUTION!!--(see Micklos and Freyer (1990) to learn how to handle this mutagen)
- mineral oil
- ice bath
- minicentrifuge
- micropipettors (1-20 µl and 1000 µl)
- micropipettor tips (1-20 µl and 1000 µl)
- (some labs have micropettors that range from 0-10 µl; if this is the case, than an additional tip size would be needed)
- microfuge tubes (0.5 µl and 1.5 µl)
- Polymerase Chain Reaction (PCR) machine

or

- water baths set at the appropriate temperatures for manual PCR (this is untested for this particular laboratory investigation)
- gel electrophoresis set ups (trays, combs, gel chambers, power supplies)
- trays for staining with methylene blue and/or ethidium bromide
- Plastic Rubber-maid type containers with covers are good
- photography equipment (optional)
  (In this laboratory both Polaroid and a Kodak Digital Science EDAS 120 photography set-up in use with a MacIntosh computer are used for photography).
- A Polaroid specifically for use with UV can be purchased from Carolina Biological---cat. # 213699---$450
- light box--UV for viewing ethidium bromide stained gels and
- white light for viewing methylene blue stained gels

Notes to Instructor

Level of difficulty: Upper undergraduate genetics course
Time required to prepare and set up: five to eight hours
Time required for students to perform exercise: Two hours to isolate DNA and to make dilutions for the control primers; three hours for the PCR reaction to run; one hour to run the gel. A repeat of the above times for the species-specific primers

We suggest that you take two weeks to complete this exercise; one week to do the control, and the second week to do the species-specific reactions.

You will need to make a list of the total number of spots that are available on your PCR machine(s). Each student (if working individually) will need three spots; however, I suggest that one dilution (1:10) will probably work. Once you have the number of spots matched to the number of students you have, make a similar list for the number of reactions, number of students, and wells on a gel available based on type of comb and number of gel boxes.

The recipe for the reaction mix as given in the protocol is for one student. You need to multiply these amounts by the number of students you have, plus extra for negative controls. The number of negative controls will be determined by the number of wells you have on your gel, and how many students are using each gel.

This experiment has been successfully tested with the species-specific *Acipenser stellatus* primers. There are primers available for the *Huso huso* caviar, but this caviar is twice as expensive as the sevruga, and therefore probably not practical for this exercise. Also, the osetra primers are not considered to be species-specific enough for this exercise, as they will hybridize to species closely related to the *Acipenser gueldenstaedti*. The most precise way to determine whether a certain caviar egg is from a specific species is to sequence the DNA, which goes beyond the scope of this exercise.

The students should see nothing in their negative control lane and a nice, 150 base-pair band in their sample lanes. If they get this result, then they have successfully amplified and visualized a 150 bp fragment of a sturgeon cytochrome b mitochondrial DNA gene. This fragment may vary in size, according to species used. The William Paterson students also amplified a 150 base-pair fragment of DNA isolated from zebra fish and Romanoff whitefish eggs. The students did not obtain amplification of a 150 base pair fragment with a sample of salmon eggs or a second sample of whitefish, but this was attributed to poor egg quality. The sevruga species-specific primers should yield a 150 bp band with the S2 and S2A primers only. The William Paterson students did not obtain amplification using the sevruga-specific primers with any sample except with one exception – the zebra fish eggs, which was an interesting and unusual finding. The large smudges that you may see at the bottom of the lanes are primer-dimers, which occur when unutilized primers hybridize with each other.

Student Outline

Protocol--Week One

Isolation of DNA from single caviar eggs

Lysis and homogenization step
DNAzol, which contains proprietary chemicals, was used to isolate the DNA. A good biochemical techniques book (Robyt and White, 1987 is one example) should describe how traditional ingredients such as detergents and ethanol work in lysing cells and precipitating DNA.)

1. Add 50 µl of DNase free sterile water to each of two 1.5 ml microfuge tubes.
2. Add one sturgeon egg from a microfuge tube marked “S” for sevruga to the first tube with a yellow 20 µl pipette tip, and “smash open” against the side of the tube. You should see white material oozing from the egg. Repeat with an egg from a microfuge tube marked with the initial of the second type of fish you are testing—paddlefish, unknown caviar, salmon, herring, etc.
3. Add 1 ml of DNAzol to each tube.
4. Mix by inverting tube several times.
5. Centrifuge at high speed in a microfuge (14,000 RPM) for 10 minutes.
6. Transfer the supernatants to fresh tubes. This step removes insoluble cell fragments, partially hydrolyzed RNA, and excess polysaccharides from the lysate/homogenate. Discard the tubes with the pellets.
7. Add 500 µl of absolute ethanol to each tube of supernatant and mix well by inverting tubes 5-8 times. Store at room temperature for 1-4 minutes. Make sure that the DNAzol and the ethanol mix well to form a homogenous solution. The solution will be cloudy. This will precipitate the DNA.

8. Centrifuge at high speed (14,000 RPM) for 2 minutes. Discard the supernatant.
9. Rinse the precipitate with 1 ml of 70% ethanol or isopropanol. Centrifuge for 2 minutes at 14,000 RPM.. Discard the supernatant. Repeat this wash step.
10. Allow to air dry until there are no drops of alcohol left--this step may be aided with a hair dryer at a low setting. (You will most likely NOT see a pellet!)
11. Resuspend each pellet in 200 µl of DNase-free sterile water.

PCR Amplification

Polymerase Chain Reaction Background
Polymerase chain reaction (PCR) is a technique used to produce large amounts of a specific DNA fragment. A low concentration of target DNA is incubated with two primers (short stretches of single-stranded DNA) that are complementary to the sequences that flank each end of the target DNA.

For example: If your original DNA strand is:
5' PO_4 ATGGTCC…….ACATGCG-OH 3'
3' OH TACCAGG……TGTACGC-PO_4 5'
then you can design primers as depicted in the box below.

5'PO_4-	ATGGTCC.....ACATGCG-OH	3'
	TGTACGC	5'Primer 1
5' Primer 2	ATGGTCC	
3'OH-	TACCAGG.....ATGGTCC-PO_4	5'

Primers sequences are always listed in a 5' to 3' order. Thus, Primer 1 would be written: 5'CGCATGT 3' and Primer 2 would be written: 5'CCTGGTA 3'.

In addition to the target DNA and primers, the reaction mixture contains *Taq* polymerase, the four deoxynucleotide building blocks of DNA (DNTP's) and the cofactor $MgCl_2$. During PCR, the reaction is

- heated to 94°C to denature the target DNA (two strands separate),
- cooled to 48-55°C to allow the primers to anneal to the denatured DNA and then
- heated to 72°C for *Taq* polymerase to extend the strands of DNA during synthesis.

During replication, enzymes move along the template strands from the 3' to the 5' direction. *Taq* DNA polymerase functions best at 72°C as it was isolated from a thermophilic bacterium, *Thermus aquaticus*, that grows in hot springs in temperatures above 65°C. The *Taq* polymerase is so stable that it can undergo repeated heating and cooling without a loss of function.

The reaction mixture is placed in a thermal cycler that automatically heats and cools according to a programmed sequence for 33 cycles. The product of one cycle of synthesis becomes the template for another cycle and the amount of DNA doubles with each cycle. Since the cycle is repeated thirty-three times, the original copy of DNA is replicated 2^{33}.

1. Since you are not sure of the quantity of DNA that you have isolated, and the PCR reaction is DNA concentration-dependent, it will be necessary for you to do a dilution series of your DNA. (You need to see how many PCR machines you have, or how many spots you have available to you on a PCR machine to see if it is practical for your class to do the amplifications of every dilution. If you don't have enough room in the machines, pick a 1:10 dilution to run of your DNA to do your PCR reaction on.
2. Set up two 1.5 ml tubes for your sevruga DNA and label them 1:10, 1:100. Repeat for the other fish DNA.
3. Place one µl of your DNA in each tube. Add the appropriate amount of RNAse-free water to make the dilution. (Check with your instructor first to make sure that you are adding the correct amount of water.)
4. To set up the PCR mix you will do the following:
   - Add 1 µl of the appropriate dilution to a 0.5 ml (PCR) tube. You should have three PCR tubes: one for the undiluted DNA, and the other two for the two dilutions that you made.
   - Next, add 24 µl of the PCR reaction mix that has been prepared by the instructor and that is sitting on ice.

   The control reaction mix has been prepared to contain the following ratios of reactants:
   22 µl PCR supermix, containing water, Taq polymerase,
   PCR buffer supplemented with 15 mM magnesium chloride,
   DNTP's (10 mM each),
   1 µl of primer 1 (B72) and
   1 µl of primer 2 (S2A)
5. Spin tubes briefly at top speed in the microfuge for a few seconds.
6. Add two drops of mineral oil from a dropper bottle to each tube (if your PCR tubes do not have caps).
7. Spin tubes briefly at top speed in the microfuge for a few seconds.
8. Place your PCR tubes in the PCR machine and run it under the following parameters:
   - 94^{o} 1 min.
   - 48^{o} 1 min.
   - 72^{o} 1 min. 35 cycles
9. When the samples are finished, they can be placed at 4^{o} C until you have time to complete the electrophoresis.

Electrophoresis

Background

DNA is a negatively charged organic acid and in the presence of an electric current will move towards a positive pole. In gel electrophoresis, fragments of DNA are placed at one end of a gel (1.5% agarose in 1 X TBE [Tris base, EDTA, Boric Acid] Buffer). The gel is placed in an electrophoresis chamber filled with 1 X TBE buffer. Current passes through electrodes at each end of the chamber and the negatively charged DNA moves through the gel towards the positive electrode. The invisible DNA band is monitored by the dye bromophenol blue, which was added to

the sample. The dye does not interact with the DNA but migrates independently towards the positive pole.

> "During electrophoresis, DNA fragments sort by size in the agarose gel. The porous cell matrix acts as a molecular sieve through which smaller molecules can move more easily than larger ones; thus the distance moved by a DNA fragment is inversely proportional to its molecular weight. In a given period of time, smaller fragments migrate relatively far from the origin compared to larger fragments. An agarose matrix can efficiently separate larger DNA fragments ranging in size from 100 nucleotides to more than 50,000 nucleotides. Adjusting the agarose concentration can separate DNA fragments in different size ranges. A low concentration (down to 0.3%) produces a loose gel that separates larger fragments, whereas a high concentration (up to 2%) produces a stiff gel that resolves small fragments." (Micklos and Freyer, 1990)

1. Place 0.75 g of agarose into a 125 ml Erlenmeyer flask. Add 50 ml of 1X TBE buffer.
2. Microwave until boiling (about a minute and a half). Alternatively, heat to boiling on a hot plate. Make sure that all the agarose is dissolved, but don't burn it. (What percentage gel would this be?)
2. Let the gel cool at room temperature till hot but not burning to the touch. (Touch the flask to your cheek!)
3. Cast the gel according to the directions that come with your gel tray--you can use tape to seal the ends, or some trays come with rubber "gaskets" that fit over the ends and effectively seal it. Place your tray on a level surface. Place a 8-12 well comb into the tray. Pour the gel quickly, and push any bubbles to the side with a yellow micropipet tip.
4. The gel should be hardened after about 20 minutes – it will turn opaque and you may see wavy lines running through it.
5. Pull out the comb by wiggling it gently as you pull – this takes a little practice. Take off the tape or the rubber gaskets.
6. Place the gel into an electrophoresis chamber. Cover with 1X TBE buffer. You should just barely cover the gel – the less buffer, the less resistance to the current. As you increase the amount of buffer, you will notice that by switching to the "current" or "ampere" setting, the number of amperes increases, which is undesirable because this could create too much heat and cause your gel to melt.
7. If you like, you may practice pre-loading the gel with loading dye, and then run the dye a few centimeters through the gel by turning on the voltage to 125 V.
8. Steady the pipet over the well using two hands with your elbows on the bench.
9. Be careful not to punch the pipet tip through the bottom of the gel.
10. Gently depress the pipet plunger to expel the sample into the appropriate well. If the tip is centered over the well, the solution will sink to the bottom of the well. Leave the plunger depressed as you withdraw the pipet tip – if you let the plunger come up, you will withdraw the sample.
11. Prepare to load the gel. Take a piece of parafilm around 10-cm square and place it down flat on the lab bench.
12. Pipet 2 µl of dye as dots spaced apart on the parafilm – one for each sample that you will load.

13. Spin tubes briefly at top speed in the microfuge for a few seconds and take off as much mineral oil as possible with a micropipette. You must do this very slowly and carefully in order to avoid taking out your sample along with the oil.
14. Make a key of the order of your samples that you will be loading into your gel in your notebook.
15. Add 8-10 µl of each sample to the blue loading dye dot and mix by pipetting up and down once. Take the sample from the bottom of the tube so that you won't get any mineral oil in your sample. (The mineral oil won't mix with the dye.)
16. Carefully add your samples to the wells. Your lab instructor will load 10 µl of a reference ladder showing base pair sizes from 100 to 1000 bp at 100 bp intervals. Close the top of the chamber and connect the electrical leads red-red and black-black. Negative DNA 'runs red' towards the red + pole. Turn on the power supply and set the voltage at 125 volts.
17. You will notice that the loading dye will separate out into a dark blue and a light blue band. The dark blue band travels faster and co-migrates with a DNA fragment of a certain base-pair size. The light blue band co-migrates with a DNA fragment of another sized fragment. Let the gel run for 20 minute to a half-hour, or until the dark blue band has traveled three-quarters the length of the gel.
18. Turn off the power supply, remove the gel tray, and carefully slide into a staining chamber.
19. Your instructor will put your gel into a staining tray that contains 10 µl of 5 mg/ml solution of ethidium bromide to 250 ml of tap water. CAUTION!! Since ethidium bromide intercalates between the bases of DNA, it is a potential carcinogen. Handle with care. Omit this step if you are using the methylene blue method of staining.
20. If you want to use methylene blue for staining, cover the gel with 0.025 % methylene blue.
21. The ethidium bromide gel may be ready for photography after fifteen minutes of staining. View on UV light box with protective face shield or box cover, and determine if bright clear bands are present. If the bands are not differentiated enough from the background, destain by leaving in the distilled water for an hour to several hours.
22. If you used methylene blue, leave the gel in this solution for a half hour. Destain by pouring off methylene blue into a storage container, and running under tap water for a few minutes. Leave it in tap water for several hours; the bands will become visible after several hours to overnight of destaining.

Photography

A. Digital photography

If you have a digital camera, follow the directions for taking a picture. If you save the image as a TIF or PIC file, it will use up less room on your disc than the Kodak graphics save. Then you may print a copy of your photo, using Adobe graphics program.

B. Polaroid photography

For UV photography of ethidium bromide stained gels, use Polaroid high-speed film Type 667 (ASA 3000). Set camera aperture to f/8 and shutter speed to B. Depress shutter for a 2-3 second time exposure.

For white light photography of methylene blue stained gels, use Polaroid Type 667 film, with an aperture of f/8 and a shutter speed of 1/125 second. (You may have to play with these settings!)

Results

You should see nothing in your negative control lane and a nice, 150 base-pair band in your sample lanes. If you get this result, then you have successfully amplified and visualized a 150 bp fragment of a sturgeon cytochrome b mitochondrial DNA gene! This is your control; you will now repeat the species-specific experiment substituting the S2 primer for the B7-2 primer.

Week Two. Species-specific DNA PCR amplification

You will now take your DNA from both the sevruga caviar and the other fish egg and test it with the sevruga species-specific primers (S2 and S2A). You may pick the results from the dilution that gave you the brightest band for the control primers. This set of primers should give a positive amplification for any egg that is from the species *Acipenser stellatus*, (the sevruga caviar). Repeat from Step 5 under "PCR Amplification, substituting one tube for the three tubes with the three different dilutions. Follow the protocol through the "Eletrophoresis" and "Photography" sections. Note that the primers and the PCR reaction conditions are different. Note your results.

The experimental or species-specific reaction mix has been prepared to contain the following ratios of reactants:

- 22 µl PCR supermix, containing water, Taq polymerase,
- PCR buffer supplemented with 15 mM magnesium chloride,
- DNTP's (10 mM each),
- 1 ul of primer 1 (S2) (Note that this primer is different from that in control experiment above) and
- 1 ul of primer 2 (S2A)

The **PCR reaction conditions** are slightly different for the sevruga species-specific reactions:

- 94^{o} 1 min.
- 55^{o} 1 min.
- 72^{o} 1 min. 35 cycles

If the species is not *Acipenser stellatus* (or sevruga), there should be no bands that will light up. Make sure that you set up your gel so that you have one lane that contains some positive control DNA (from Week One).

Primers that are sturgeon species-specific are still being sought in order to differentiate endangered from non-endangered sturgeon species. You have just taken a step closer to becoming a wildlife forensic scientist!

Lab Report

Include the following in your report.

A. Attach a copy of the gel, label the PCR Mix A or B (it's significance) and fish analyzed.

1. Were all of the samples sturgeon or paddlefish? Discuss.

   Hint: what did the bands using B72/S2A primers (PCR Mix A) tell you?

2. How were eggs from sevruga sturgeon differentiated from other sturgeon? Did all the sevruga samples have bands? Did any other fish have bands using these primers? Explain.
3. You have a sample of eggs that you think are from beluga sturgeon. How could you determine if they were beluga eggs?
4. How did you follow the progress of the electrophoresis and how could you tell when to stop the electrophoresis?
5. How was the DNA visualized on the photograph? Why should you wear gloves and be extremely careful when handling the gel?
6. What types of controls would you have for this experiment?
7. One eggs of each fish was used to isolate DNA. How many copies of the genome does this represent? In this experiment, which DNA was amplified by PCR?

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