# DNA sampling and research techniques

Studying DNA requires using a precise and complex, yet delicate process. First, DNA is extracted from cells in a sample from a mountain lion. Samples that work well for DNA extraction are blood, muscle, skin, saliva, and hair. Even feces from a mountain lion can be used to collect

*By Holly Ernest*

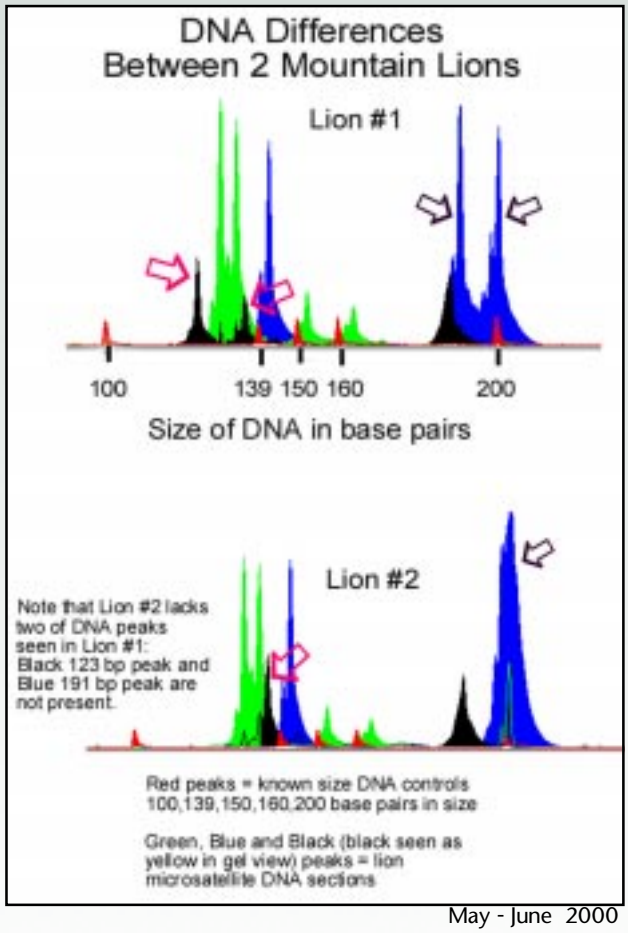
genetic information where the DNA comes from a few sloughed intestinal cells. A small amount of the sample, about the size of an aspirin tablet, is placed in a plastic tube, and heated with one milliliter (one fifth of a teaspoon) of chemicals which cause animal cells to burst open and release their DNA. The other cell contents are washed away, leaving a pure sample of DNA - *ALL* of the DNA from the sample.

Next we single out small sections of DNA that we are interested in. Remember that the DNA of one mountain lion is like a large library. To make sense of it, we need to examine one sentence out of one book at a time, but these sentences are so small that a process needed to be developed to allow us to detect them. Polymerase chain reaction (PCR), developed in the 1980s, allows geneticists to single out a small DNA sentence, label it with a fluorescent chemical, and make millions of copies of it so we can detect it. A tiny amount (one microliter which is 1000 times less than one milliliter) of the extracted DNA is placed in a tube with the building blocks of DNA: adenine, cytosine, guanine, and thymine (A, C, G, and T which are also the encoding molecules of DNA). Once duplication has occurred, tiny amounts of DNA, previously undetectable, can now give genetic information.

Included in the tube is an exact mixture of sterile distilled water, salts and buffers to supply the right pH and conditions to keep the DNA molecules happy. Also added to the mixture are fluorescently labeled DNA "primers", or probes that bind to specific segments of

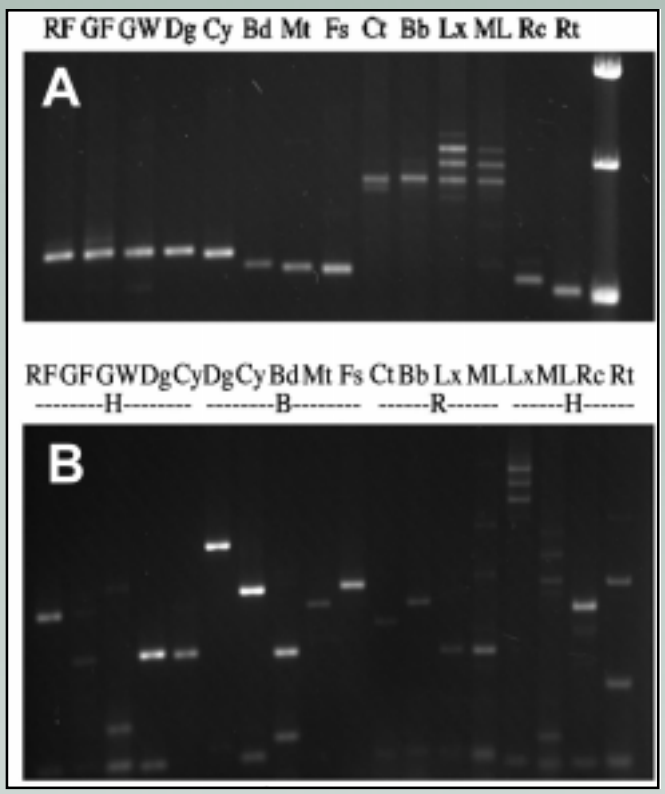
*Photo © Esther Rubin*

**Camera staged to photograph mountain lion at bighorn sheep kill site.**



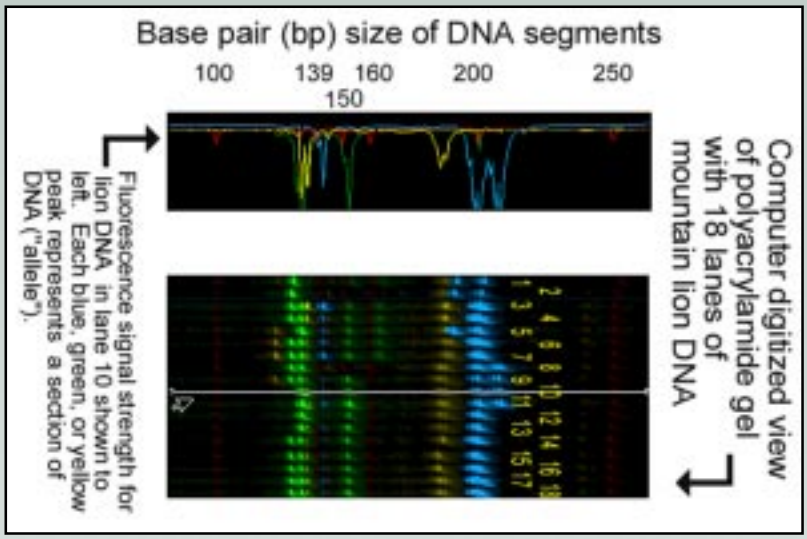

**A computer program translates the fluorescence information into graphsof DNA differences between 2 lions. Lion # 1 has one more black band (the yellow bands from the gel picture are shown as black peaks here) and one more blue band than lion #2.**

*Graphic courtesy of Holly Ernest.*




*Graphics courtesy of Holly Ernest*

**Mitochondrial DNA bands from multiple carnivore species. Top panel, closely related species such as mountain lion (ML) and lynx (Lx), or red and gray fox (RF, GF) usually generate similar sized DNA fragments. To differentiate these, the DNA is cut into unique sizes with one or a series of enzymes (bottom panel). Biologists compare the DNA profiles with those (from known control) individuals, allowing them to identify the species from which a sample was obtained. In some cases, including the mountain lion shown here, animals are "heteroplasmic" meaning more than one size class of DNA exists in an individual. This does not affect the ability to identify a species however. (Other species included in this figure gray wolf, dog, coyote, badger, marten, fisher, domestic cat, bobcat, raccoon, and ringtail.)**




**The computer gel image was produced from several samples of California mountain lion DNA. Each vertical column (lane) is a DNA sample. Red bands in each lane are known-size pieces of DNA that are run as size standards in the same lane as our mountain lion DNA. The blue, yellow, and green bands on the gel are several different sections of DNA that are used to tell one mountain lion from another. Smallest DNA segments are seen toward the bottom of the gel picture, with largest at the top.**

**DNA molecule helix.**

*Photo © Perkin-Elmer*

DNA. In much of the mountain lion genetic study that we do, we are looking for differences between individuals. Some DNA sections are the same (constant) between individuals, and some are different (variable). The variable regions are often located between regions that are constant among mountain lions. Fluorescently labeled DNA primers are made to bind to the constant regions on either side of a variable regions. The primers allow the last ingredient for this recipe, the enzyme Taq polymerase, to bind and produce an exact copy of the DNA segment. The PCR reaction requires very exact temperatures and times of heating and cooling to produce a copy of the DNA segment bound by the primers. Each of these cycles doubles the amount of DNA. After about 35 cycles and two hours, we have over a million fluorescently labeled DNA copies in 15 microliters of clear fluid.

A million copies of a piece of DNA sounds like a lot, but very sensitive detection equipment is needed to observe it. The fluorescent marker is invisible to the eye until a laser scans it. So that brings us to the DNA detection step. Less than one microliter of the PCR amplified fluorescently labeled DNA is loaded onto a very thin plate of Jell-O-like substance called polyacrylamide gel. The plate with its samples of DNA loaded is inserted into a laser scanner/light detector that is attached to a computer. An electrical current is applied to separate different DNA molecules by size. This is called gel electrophoresis. DNA is negatively charged, so it is attracted to a positive electrical charge located at the far end of the gel. Larger DNA molecules take longer to move through the gel than small ones. A laser aimed at the middle of the gel excites the fluorescent marker, causing it to emit a light of a certain color. The light is detected, and the information is transmitted to a computer where it can be studied and decoded.