**California Condor Genetics Investigation**

**Purpose**: Students will use previously extracted condor DNA and the processes of PCR and gel electrophoresis to determine the gender of individual condors.

**Background:**

Historically, the California condor (Gymnogyps californianus), the largest bird in North America, ranged broadly along the Pacific coastline, from British Columbia, Canada all the way to the tip of Baja California, Mexico. But in the 20th century the condor was reduced to range from Southern California, Arizona (east of the Grand Canyon), to northern Baja California. With only about two dozen individuals left of the species, the condor nearly went extinct in the late 1980’s, primarily due to human impacts. California condors are scavengers, with specialized adaptations for feeding on animal carcasses, such as a sharp, curved beak for ripping tissue, a featherless head to promote hygiene, and specialized feet for efficiently walking on the ground. A primary mortality factor for the condors arises when an individual encounters a carcass that has been hunted with lead shot, leading to lead poisoning in the condor. This became widespread due to the fact that California condors, with a wingspan of three meters, can travel hundreds of miles in a single day, reaching flight speeds of up to 25 mph. Luckily in 2007, California Governor Arnold Schwarzenegger passed a law making hunting with lead shot anywhere in current condor range illegal. Additionally, these birds suffer from collisions with and electrocution by, power lines.

Partnering with several institutions in the late 1980’s (Peregrine Fund, US Fish and Wildlife, and the Los Angeles Zoo), San Diego Zoo Global started a captive breeding and reintroduction program, which has brought the number of California condors to more than 400, with approximately half released into the wild in Baja California, central California, and Arizona. Due to an autosomal recessive disease called Chondrodystrophy (discovered in the captive population), where chicks do not develop fully and die before or shortly hatching, strict management strategies were put in place. Using pedigree analysis, scientist can trace the history and future of that particular gene, ensuring that only healthy and non-carrier individuals are allowed to breed with one another. Individuals who are to be bred must have their gender determined using DNA extraction and PCR techniques, since males and females look identical.

California Condors, like all birds, possess no external genitalia. This species is also sexually monomorphic, exhibiting no size or color differences between males and females, making it impossible to determine gender just by looking at them! Scientist at the San Diego Zoo Institute for Conservation Research use DNA Technology to determine the gender of Condors soon after they hatch. By extracting DNA from the eggshell membrane, scientist can use the Nobel Prize-winning technology of PCR (Polymerase Chain Reaction) to make copies of a variable-sized gene on the sex chromosomes.

**Hypothesis:** Make a hypothesis as to the gender of your condor (male or female) and record it in your SNB. *(If/then!)*

**Pre-lab questions:**

1. Explain why the California Condor is endangered (at least 2 reasons!).
2. Why does DNA Technology have to be used to determine the gender of the California Condor?
3. Draw a labeled diagram of a micropipette and explain how it is used.
4. Why is PCR considered to be DNA replication in a test tube?
5. How specifically is PCR being used in this lab?
6. Explain how to identify the gender of a condor from its karyotype.
7. What is the relationship between the condor karyotype and the gel electrophoresis results?

**Day One: PCR**

**Materials:**

* 2-20µL Micropipette
* 2-20 µL Micropipette Tip Box
* 1 DNA tube
* 1 Primer Mix tube
* 1 PCR Pre-Mix tube (blue bottoms)
* Thermal Cycler\*
* Mini-Centrifuge\*

\* = per class

**Procedure:**

1. Add 18 ul of primer mix and 2 ul of condor DNA to the PCR tubes. (Note: the PCR tubes have a blue solution at the bottom that contains free nucleotides (the building blocks of DNA) as well as Taq Polymerase, a key enzyme.)
2. Mix the contents of the tube by flicking gently with your finger (see teacher demo).
3. Centrifuge the contents of the tube for 10 seconds.
4. Make sure the cap is securely in place and place the tube into the thermal cycler (PCR machine).
5. Label the tube with your group number by using a fine-tipped Sharpie marker.
6. Your teacher will turn on the thermal cycler to replicate your condor DNA in vitro.

**Day 2: Gel Electrophoresis**

**Materials:**

* 1 PCR product (amplified reaction tubes from Day 1)
* 1 2-20µL Micropipette
* 2-20 µL Micropipette Tip Box
* Gloves
* 1 E-Gel Safe Imager w/ UV shield\*
* 1 E-Gel I Base\*
* SYBR Sage Agarose gels\*

\* = per class

**Procedure:**

1. Your teacher will prepare the Gel Electrophoresis machine.
2. Load Gel with PCR products
	1. Put on gloves.
	2. Use the Micropipette to load all 20 µL of your PCR products into individual wells in the gel. *(Teacher note: If there are any empty wells after all students have loaded, load 20 uL of sterile water into these.)*
	3. Dispose of tip in designated “used tips” beakers.
	4. Record which Gel (there may be more than one being run each class) and lane your sample is loaded into in your SNB.
3. Once all the wells are all loaded, the teacher will start the program (apply the voltage) to start running the gel.
4. View the Gel: Students should observe the gel and make a sketch of the gel in the data section.
5. Interpret Results: There may be other banding present, but you should be able to identify the Z and/or W bands as the bands that moved down the farthest in each well (see example gel image). Use the banding pattern to determine if the condor is male or female (1 band: male, 2 bands: female).

**Data/Observations:**

Make sure to record your group #: and your Gel/Lane #

Draw a picture of your lane of the electrophoresis gel.

**Conclusion:** Evaluate your hypothesis.What was the sex of your condor? How do you know? Explain

**Reflection:** Reflect on the lab in a paragraph format that answers the following questions:

1. Discuss the possible errors that occurred that could have affected the outcome of your experiment.
2. Make suggestions for improving the lab, such as things you would do differently.
3. List one thing you learned from the lab.
4. Explain how the lab applies to a real life situation (in addition to the Condors).

**Lab Analysis Questions:**

1. Evaluate the importance of DNA biotechnology in the effort to conserve the California condor.
2. What was your favorite part of this lab and why?