Code Condor - Conservation Genetics MiniLab

Student Guide

Cat# M3027TAE

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Laboratory Safety

1. Wear lab coats, gloves, and eye protection whenever possible.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

MiniLab Overview

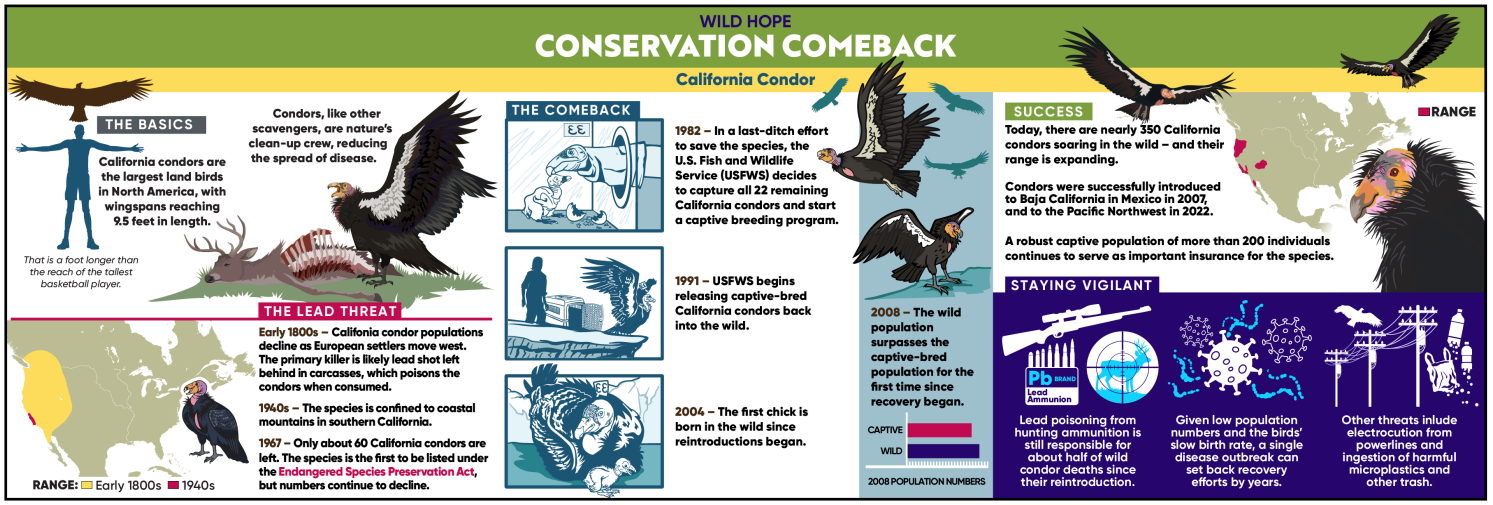
The Conservation Crisis

The California Condor is one of nature's greatest survivors. This massive scavenger once soared across the skies of British Columbia, the American Southwest, the Pacific Coast, and as far east as Florida. For many indigenous cultures, the condor is a sacred symbol. It is also one of the oldest bird species on Earth, having survived the Ice Age thousands of years ago.

But as humans spread across North America, life became increasingly difficult for condors. Because they are scavengers — animals that feed on dead animals — they were especially vulnerable to human-caused threats. Condors that fed on animals killed by hunters would accidentally swallow lead bullet fragments, causing lead poisoning. They also consumed plastic waste and absorbed dangerous chemicals like Polychlorinated Biphenyls (PCBs, man made chemicals) and dichlorodiphenyltrichloroethane (DDT, an insectide) from the bodies of dead marine animals. On top of all this, avian influenza posed yet another serious threat to their already shrinking population.

By the 1980s, the condor was on the verge of extinction. What followed was one of the most ambitious wildlife rescue efforts in history — a partnership between indigenous communities, conservation organizations, and government agencies working together to save the species.

In this lab, we will look at the history of the condor captive breeding program — a program where scientists had to make tough decisions without the help of modern genetic tools. We will then explore how today's DNA technology can help scientists make smarter breeding decisions and protect a keystone species while maintaining healthy genetic diversity.



https://www.wildhope.tv/article/the-california-condor/

Day 1: Introduction - California Condor Recovery Program

The California condor recovery program is a premier conservation success, led by a landmark partnership between indigenous peoples, nonprofits, and global government agencies.

The Crisis: A Race Against Time

The California condor has the distinction of being among the first species ever protected by the United States federal government, officially listed as endangered in 1967 under the precursor to the modern Endangered Species Act (ESA). Despite this early protection, the population plummeted to just 22 birds by 1982. Condors were dying primarily from lead poisoning (eating fragments of lead ammunition in animal carcasses), habitat loss, and the lingering effects of DDT, which caused eggshell thinning. To prevent total extinction, the high-stakes decision was made to capture every remaining wild bird and begin a conservation breeding program.

The Recovery (1987-Present)

- **Captive Breeding:** The last wild bird was caught in 1987. Of the original 22, only 14 founders successfully produced offspring, divided into three distinct genetic groups, or clans, based on where they were captured (Sespe, Bitter Creek, and Santa Barbara).
    - **Double Clutching:** To speed up their slow natural reproductive cycle (condors typically lay an egg every two years - a K-selected species), managers removed the first egg of the season to an incubator, tricking the parents into laying a second. This doubled the number of chicks produced each year.
    - **Puppet Rearing:** To prevent the incubated chicks from imprinting on humans, managers used lifelike condor-headed hand puppets for feeding. This ensured the birds remained wild and wary of people upon release.
- **Reintroduction:** In 1992, the first captive-bred birds were released back into the wild. Today, there are distinct populations in Central and Southern California, Arizona, Utah, and Baja California.
- **Status Today:** As of 2026, the population exceeds 600 birds, with nearly 400 flying free. Every wild bird is monitored via numbered wing-tags and GPS/VHF transmitters, allowing biologists to track their movements and quickly locate birds for health checks.

Current and Emerging Threats

- **Lead Poisoning:** It remains the #1 cause of death for wild condors. Many birds require chelation therapy (medical treatment to scrub lead from their blood) to survive.
- **DDT Legacy:** Scavenging on marine mammal carcasses exposes coastal condors to DDE, a byproduct of DDT stored in blubber. This continues to cause eggshell thinning in coastal nests today.
- **Avian Flu (H5N1):** Following a major 2023 outbreak, the program began a vaccination campaign to protect wild flocks from this new viral threat.
- **Genetic Diversity:** With only 14 founders, managing inbreeding and rare genetic conditions like chondrodystrophy is essential to ensure the population remains viable.

Genetic Management of the California Condor

The genetic management of the California condor is a global model for using molecular biology to pull a species back from the brink of extinction.

The Bottleneck & Historical Diversity

Ancient DNA shows condors were once abundant and diverse. The 20th-century crash created a severe population bottleneck. Modern genomes show long, identical DNA segments present before the bottleneck, indicating inbreeding and lack of genetic diversity.

Building the Pedigree (1980s-2010s)

- **Ancestral Ties:** Early DNA fingerprinting profiles estimated relatedness and identified genetic clans that aligned with the founders' original geographic homes.
- **Genetic Sexing:** Since condors are monomorphic (males and females look identical), DNA testing is the only way to identify sex for proper breeding pairs.
- **Parentage Confirmation:** Researchers eventually used microsatellites (repeating DNA sequences) to verify every chick's parents. This corrected the family tree, revealed "extra-pair copulations," and allowed managers to minimize individual relatedness to the population (mean kinship).

The Genomic Revolution (2020s-Present)

In this ongoing revolution, every new sequence is a step closer to solving the species' greatest mysteries.

- **Whole Genome Sequencing:** Mapping 1.2 billion pairs revealed 4 million SNPs (Single Nucleotide Polymorphisms) - one-letter DNA changes used as unique genetic tags.
- **SNP Analysis:** By scanning thousands of SNPs simultaneously, researchers are developing the capability to monitor health and parentage with near 100% accuracy.
- **Parthenogenesis:** During routine parentage checks, scientists found two male chicks genetically identical to their mothers, with no paternal DNA - the first documented virgin births in a bird species where females had access to mates.
- **Genetic Linkage Maps:** This "DNA GPS" shows exactly where genes sit on chromosomes, helping scientists identify healthy genes hitched to dangerous ones.

The Unsolved Mystery: Chondrodystrophy

Chondrodystrophy is a generic term for abnormal bone growth or dwarfism. While often non-lethal in dogs and humans, it is fatal in condors and prevents hatching. It remains a primary genetic challenge as the specific condor substitution is not yet definitively identified.

- **Inheritance:** It is likely autosomal recessive, meaning an affected embryo (aa) must inherit a copy of the mutated allele from each parent (Aa) to be affected.
- **The Chicken Model:** Chickens and condors share a common avian ancestor and many

highly conserved genes. A substitution in the chicken aggrecan (ACAN) gene causes a similar condition - a strong candidate to use as a proxy model for condors since the actual gene has not yet been identified in condors.

- **Management**: To protect the gene pool, known carriers (Aa) are kept in the breeding program but never paired with one another to reduce the risk of affected embryos.

Genetic Load

The comparison of an organism's fitness compared to a reference species genotype is called the Genetic Load. If you accumulate too many variant mutations that are not advantageous, your fitness is decreased. If you accumulate variant mutations there are advantageous to your survival, your fitness is increased. Population size is a critical starting point when considering genetic load. In the case of California Condors, scientists had only 22 birds to work with to rebuild an entire species. Every breeding decision carried enormous weight, requiring a careful balance between eliminating harmful mutations and preserving genetic diversity.

Removing a harmful mutation from the gene pool may seem straightforward, but the tradeoffs are complex. Doing so can reduce genetic diversity, increase inbreeding, introduce the risk of other mutations, or lower overall viability. Every strategic choice has consequences, some that enhance the population's fitness, and some that can result in a decrease in fitness.

Digital Karyotyping & Avian Sexing

Why Sexing Matters in Conservation

Because California condors do not show sexual dimorphism (males and females look exactly the same), it is critical to identify their sex for conservation breeding management. If you just make a best guess and accidentally pair two females or two males together, no chicks will be produced. Scientists would stain chromosomes and sort them to make a karyotype, an organized profile of the organism's chromosome. This portfolio can show the sex of the organism in question. When it comes to birds, their biological sex system is different than the human XY system. Birds follow the ZW system. Males are homogametic (ZZ), possessing two identical Z chromosomes. Females are heterogametic (ZW), possessing one Z and one W chromosome. This means the mother's gametes determine the biological sex of the chick.

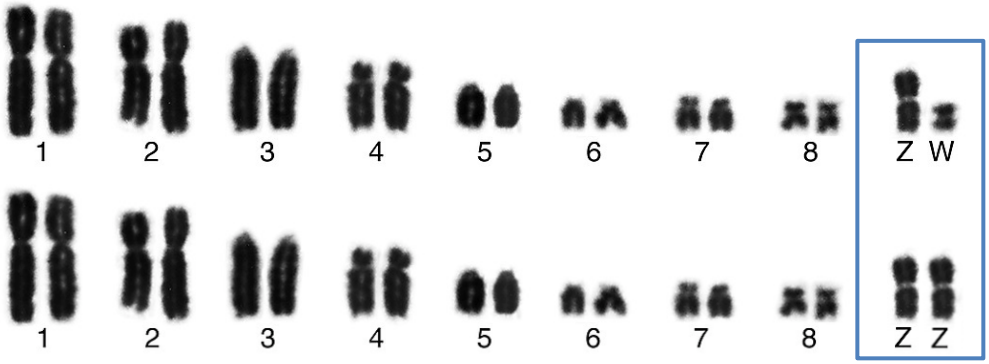


Figure 1. Sorted chromosomes. Top sort shows Z and W chromosomes, indicating this bird is a female (ZW). Bottom sorts shows Z and Z, indicating the bottom bird is a male (ZZ).

https://commons.wikimedia.org/wiki/File:Karyotype_of_chicken_%28Gallus_gallus%29.png

Pedigree Logic & Forensic Tracing

The Family Blueprint: What is a Pedigree?

A pedigree is a genetic map that can track a specific trait across multiple generations. We can use a pedigree like a historical audit to see how the predicted chondrodystrophy (a) allele has been inherited from one generation to the next.

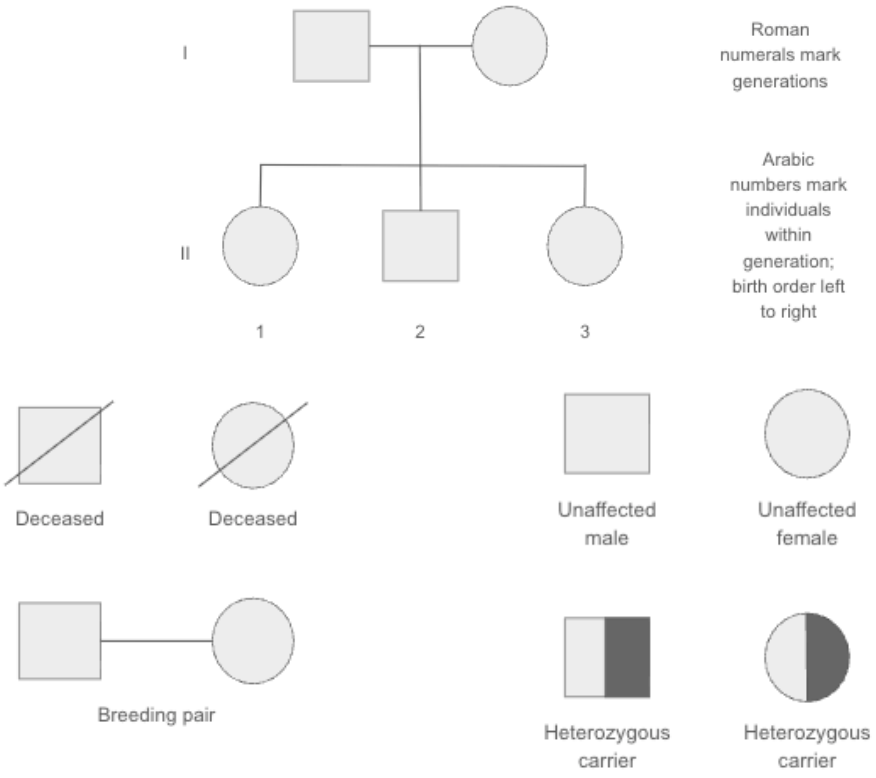
The Rule of the Recessive Trait (a)

- AA (Clear): Homozygous Dominant. These birds do not carry the chondrodystrophy gene.
- Aa (Carrier): Heterozygous. These birds are healthy but carry one mutated copy of the gene.
- aa (Affected): Homozygous Recessive. The embryo dies before hatching.

The Forensic Hunt: Identifying Obligate Carriers vs Suspect Carriers

Since chondrodystrophy is a recessive trait, it remains hidden until an affected embryo (aa) is produced. When this occurs, we apply the logic of autosomal recessive forensic tracing:

- If an embryo is affected (aa), BOTH biological parents must have contributed an (a) allele. We define these parents as "obligate" carriers - individuals proven to be (Aa) by the lineage of their offspring, even before a DNA test is performed.
- Any healthy offspring of an obligate carrier is considered a "suspect" carrier - there is a statistical probability they inherited the (a) allele (see next page), but only a DNA test or their producing an (aa) chick of their own would confirm their status.



Pedigree hierarchy and explanation of symbols and labels.

The Punnett Square as a Management Tool

The Math of Survival

Punnett squares calculate the mathematical probability for each potential offspring. In a small population like that of the California condor, these percentages represent the literal survival of the species. While a square shows four boxes, they do not represent four specific chicks; they represent the odds for every single egg laid.

The Probability of the Egg: Pairing Carriers (Aa x Aa)

- The Phenotype (Visible): We expect a 3:1 ratio of healthy (hatched) chicks to affected (failed to hatch) embryos.
- The Genotype (Hidden): Among those that hatch and appear healthy, the math changes. Because we know they are not (aa), we eliminate that box. This leaves three remaining possibilities: one (AA) and two (Aa), making the chance of being a carrier 2 out of 3.

The 67% Suspect Rule

This creates the "suspect" carrier status. Without DNA testing, any healthy bird born to two carrier parents has a 2/3 (67%) chance of being a carrier, and a 1/3 (33%) chance of being clear - a non-carrier. In conservation management, we must assume these birds are carriers until proxy molecular evidence proves they are clear.

Male: Carrier

Female: Carrier	A	a
A	AA	Aa
a	Aa	aa

- 2 out of 3 living offspring have a chance of being a carrier, 67%
- 1 out of 3 living offspring have a chance of being clear, 33%

Male: Clear

Female: Clear	A	A
A	AA	AA
A	AA	AA

- All offspring are clear, 100%

Male: Clear

Female: Carrier	A	A
A	AA	AA
a	Aa	Aa

- 2 out of 4 living offspring have a chance of being a carrier, 50%
- 2 out of 4 living offspring have a chance of being a clear, 50%

- **Teacher Note:** Students often want to say siblings have a 50% or 75% chance of being carriers. Because we are looking at living, healthy birds, the (aa) (dead) box in the Punnett Square is impossible and must be removed from the denominator, leaving a 2 out of 3 probability.

Student Worksheet Day 1

Part 1: Bottleneck & Discovery

1. Define chondrodystrophy in terms of inheritance and lethality. How does a bird "become" chondrodystrophic, and what is the physical result for the embryo?

2. Why is identifying a hidden recessive allele (a) more critical in a tiny, bottlenecked population than in a large, wild population?

Part 2: Digital Karyotyping and Sex Determination

Our Studbook is incomplete. We cannot choose breeding pairs if we don't know who is male and who is female.

Task: Use the Google Slides karyotype activity to analyze your assigned birds.

- ZZ = Male
- ZW = Female

Sample Prediction Table

Bird ID	Sex Genotype	Chondrodystrophy Genotype Prediction (fill after Part 3)

Task: Now that you have determined your birds' sex, add them to the Studbook, along with your classmates' results. Use the updated Studbook to fill in the Pedigree with the correct shapes for all bird IDs (male - square, female - circle).

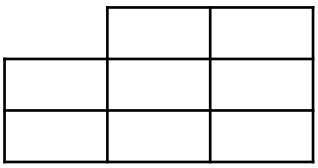
Part 3: Pedigree Audit

The rule of the recessive trait (a) outlines the possible genotypes as clear (AA), carrier (Aa), and affected (aa). Your mission is to use the appearance of affected embryos to work backward and trace the lethal allele through the pedigree.

Task: Egg 21 from Founders 3 and 4 failed to hatch. A necropsy confirms chondrodystrophy (aa). The hidden allele has officially been found. Update your pedigree and Sample Prediction Table on page 10 of student guide if relevant.

1. What are the parent's genotype and are they an obligate or suspect carrier?

2. Draw a Punnett square below using these parents' genotypes.



3. Egg 21 has two viable siblings, males 11 and 16. What is their carrier status, obligate or suspect?

4. What is the probability of AA vs Aa for Egg 21's siblings?

Task: Egg 31 from Birds 11 and 20 failed to hatch. A necropsy confirms chondrodystrophy (aa). Update your pedigree and Sample Prediction Table on page 10 of student guide if relevant.

5. What are the parent's genotype and are they an obligate or suspect carrier?

6. The Clan A grandparents are Founders 1 and 2. Are they an obligate or suspect carrier?

7. What is the probability of AA vs Aa for the Founders 1 and 2 ?

8. Why can we deduce obligate carriers but never confirm clear birds (AA) or suspect carriers (Aa) using a pedigree audit alone? What is the "missing piece" of evidence we need, and why is it critical we have this information for a conservation breeding program?

Part 4: Breeding Proposals

The 0% Lethal Probability Rule: In a K-selected species like the California condor, every reproductive cycle is a high-stakes investment. We must maximize genetic diversity while ensuring every egg has a 100% chance of being a healthy hatchling.

9. Assigned Bird Profile

- Bird ID: _____
- Chromosomal Sex: [ ] Female [ ] Male
- Carrier Status: [ ] Obligate [ ] Suspect [ ] Clear

10. Identify two potential mates for your assigned bird using the Studbook and Pedigree.

- Mate Option A (Safe Bet): Choose a bird with no affected embryos in their family tree.
  - Mate ID: _____
  - Justification: Why is this pairing likely "safe"?

- Mate Option B (Calculated Risk): Choose a bird that is an obligate or suspect carrier.
  - Mate ID: _____
  - Justification: Why might a biologist choose this bird despite their carrier status? What must your own bird's genotype be to make this pairing "safe"?

11. If you choose Mate Option B, and both birds turn out to be carriers (Aa), what is the statistical probability (%) that their first egg will be clear, a carrier, or affected? Explain why this pairing is considered a management failure - even if a healthy chick is successfully hatched?

As Lead Analysts, your claims today are what we test tomorrow using molecular techniques involving PCR, RFLP and gel electrophoresis!

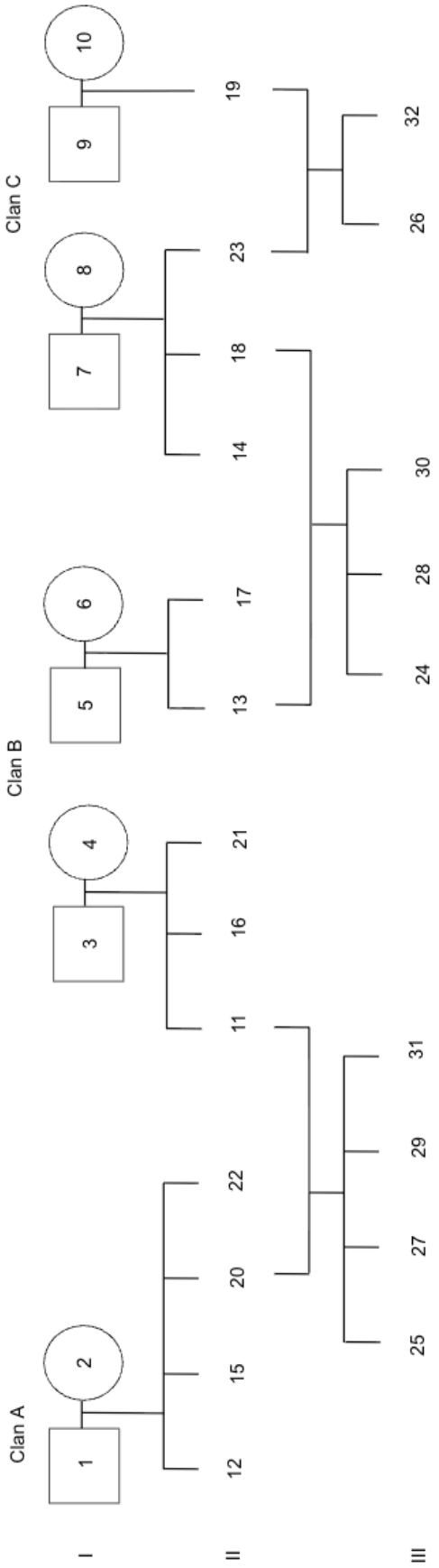
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Studbook

Bird ID	Generation	Sire	Dam	Clan	Sex (M/F)	Sex Genotype	Chondrodystrophy Genotype	Notes
1	I	—	—	A	Male			
2	I	—	—	A	Female			
3	I	—	—	B	Male			
4	I	—	—	B	Female			
5	I	—	—	B	Male			
6	I	—	—	B	Female			
7	I	—	—	C	Male			
8	I	—	—	C	Female			
9	I	—	—	C	Male			
10	I	—	—	C	Female			
11	II	3	4	B				
12	II	1	2	A				
13	II	5	6	B				
14	II	7	8	C				
15	II	1	2	A				
16	II	3	4	B				
17	II	5	6	B				
18	II	7	8	C				
19	II	9	10	C				
20	II	1	2	A				
21	II	3	4	B				
22	II	1	2	A				
23	II	7	8	C				
24	III	13	18	B/C				
25	III	11	20	A/B				
26	III	23	19	C/C				
27	III	11	20	A/B				
28	III	13	18	B/C				
29	III	11	20	A/B				
30	III	13	18	B/C				
31	III	11	20	A/B				
32	III	23	19	C/C				

**Simulated pedigree for educational use only; inspired by but not reflective of actual CA condor studbook data*

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Day 2: The Molecular Mission

We have reached the limit of what the Studbook and Pedigree can tell us. To save the California condor, we must move from probability to certainty and use molecular tools to look directly at their DNA. First, we must find the genes of interest in their DNA.

PCR - The Molecular Xerox Machine

The California condor genome contains roughly 1.2 billion base pairs of DNA. If we want to find one specific predicted mutation or identify a bird's sex, we can't look at all of it - that would be like trying to find one typo in a library of 1,000 books.

We use PCR to zoom in and make millions of identical copies of a specific region. This process is called amplification. Once we have millions of copies, the DNA fragments can be separated and visualized gel electrophoresis. See Appendix B for more details.

Targeting the Target

This lab utilizes two PCR product results:

- **The Sexing Test:** We amplified a specific region on the Z and W chromosomes. The region is larger on the W (329bp) than the Z (250bp), making it easy to differentiate on a gel.
- **The Genotyping Test:** While the exact substitution in condors is being mapped, researchers use the chicken aggrecan (ACAN) gene as a proxy. In chickens, a nearly identical condition called nanomelia is caused by a single G to T substitution. This change creates a premature stop codon, resulting in a non-functional protein that prevents skeletal development. Because the specific substitution in the California condor has not yet been definitively identified, we are using the chicken ACAN gene as a proxy to test the accuracy of our genomic model. We amplified a 950bp region of the chicken ACAN gene. We need a way to tell the difference between the genes that have the substitution and those that do not. Luckily, we have a restriction enzyme, BsaBI that can help.

Restriction Enzymes: Molecular Scissors

Bacteria produce restriction enzymes as immune scouts to scan and cut viral DNA. Scientists now use over 3,000 different enzymes (each recognizing a specific sequence) for many molecular applications.

How RFLP Works

RFLP uses these molecular scissors to cut DNA and reveal hidden substitution. This process is called restriction digestion. BsaBI looks for the palindrome GATNNNNATC recognition site (N is any nucleotide) and cuts at GATNN. GATNNNNATC become 2 pieces, GATNN and NNATC.

The Proxy Model Mutation

In this hypothetical model, a single G to T substitution in the chicken ACAN gene creates this exact cutting site. We can use BsaBI to find these sites if they exist in our ACAN PCR product by incubating them together.

- Healthy Allele (A): BsaBI finds no cutting site and leaves the 950bp fragment whole.

5'-GAGGGTTATC - 3'— No matching sequence, BsaBI does not cut

5' ————————GAGGGTTATC————————————————-3'
950 bp long

- Mutant Allele (a): The substitution creates the recognition site. BsaBI cuts the strand, creating two smaller fragments, 250 and 700bp

5'-GATGGTTATC-3' - matching sequence found. Sequence is cut between GATGG and TTATC

5' ————————GATGG/TTATC————————————————-3'

5' ————————GATGG TTATC————————————————-3'
250 bp long 700 bp long

The Molecular Sieve: Gel Electrophoresis (see Appendix A for more information)

DNA is negatively charged so it will travel towards the positive end of an electrical field. The smaller pieces travel faster than longer pieces. These properties allow us to perform gel electrophoresis which is the process of separating molecules via electrical current through an agarose gel. We will use gel electrophoresis to visualize both the PCR products for sexing and ACAN genotyping. Below is an example of where we expect our bands to be.

Example gel of Sex and Chondrodystropy Carrier Status

Sex Determination Samples		MiniOne Marker	Chondrodystrophy Carrier Status Samples		
ZZ	ZW		AA	Aa	Aa
1	2	3	4	5	6
		2000bp			
		1000bp			
		500bp			
		300bp			
		100bp			

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Part I: Electrophoresis

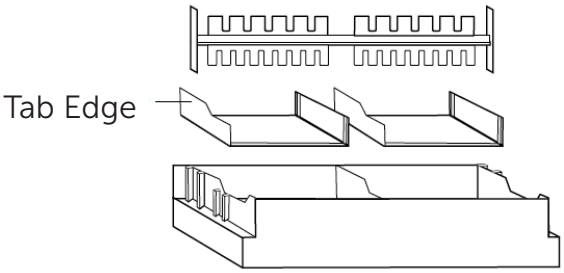
Materials

1 Minione® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (2%)
9 DNA samples
Running buffer (135 mL)
1 micropipette (2-20µL)
9 pipette tips

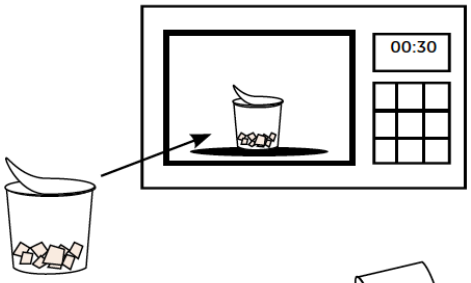
Lane #	What DNA is being loaded?	Sample Label	Volume
1	DNA for sex determination	Sex sample, S#	10 µL
2		Sex sample, S#	10 µL
3		Sex sample, S#	10 µL
4		Sex sample, S#	10 µL
5	MiniOne Marker	M	10 µL
6	DNA for disease marker genotyping	Disease sample, C#	10 µL
7		Disease sample, C#	10 µL
8		Disease sample, C#	10 µL
9		Disease sample, C#	10 µL

How to Cast a Gel

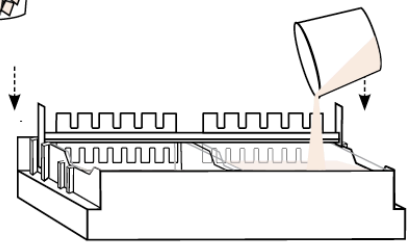
1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.



2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.

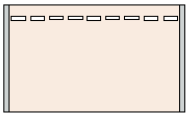


3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.



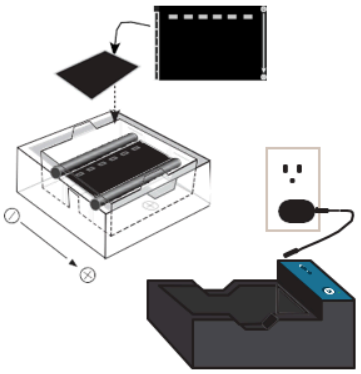
DO NOT disturb the gel until time is up.

4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.



How to Load a Gel

1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**

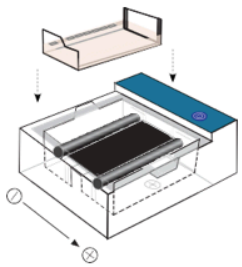


2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

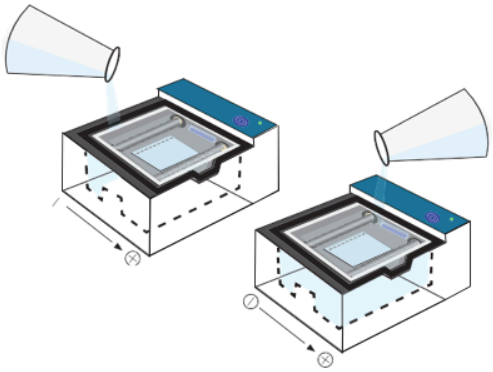
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.

Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

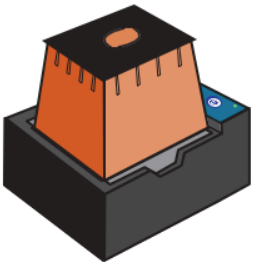


5. Turn the low intensity blue LED on by pressing the button on the carriage.



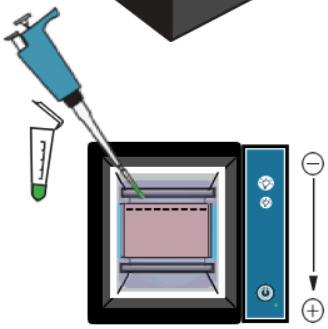
6. Measure 135 mL of running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**

7. Place photo hood on the carriage.



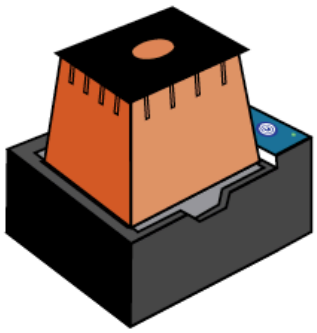
8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



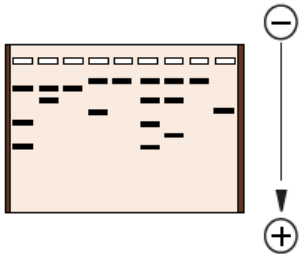
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image



1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   The green power LED will not turn on if:
   - **The tank is not properly placed inside the carriage.**
   - **There is no buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage.**
   - **There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne Electrophoresis Instruction Manual**



2. Have students periodically check the migration of the bands (~every five minutes).

3. Allow the gel to run **25 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.



4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.

5. Clean up. Follow teacher's instructions on disposal and clean up.

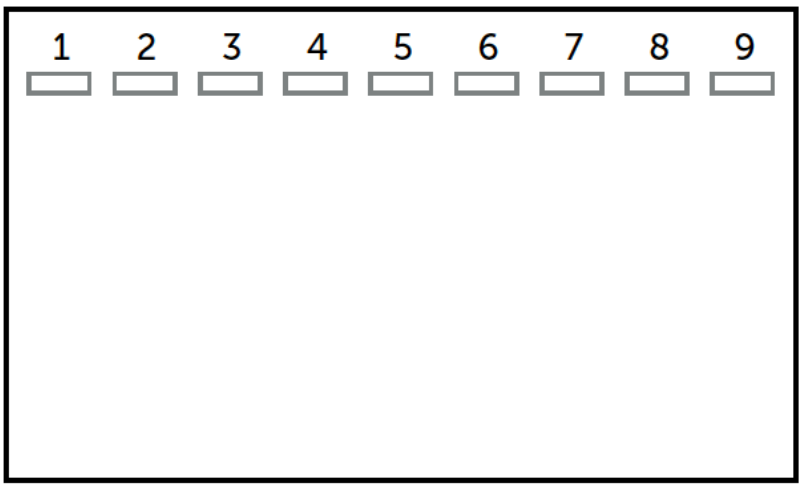
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____
Lane 7: _____
Lane 8: _____
Lane 9: _____

Student Worksheet Day 2

Part 5: Gel Analysis

Draw your expected sex determination bands in the Gel Prediction Table below. Remember, birds are ZW (female) or ZZ (male). Our primers target both chromosomes but produce different fragment sizes: Z (250 bp) and W (329bp).

Gel Prediction Table (record Bird ID in third row as you load your samples)								
1	2	3	4	5	6	7	8	9
Sex Determination Samples				MiniOne Marker	Chondrodystrophy Carrier Status Samples			
				2000bp 1000bp 500bp 300bp 100bp				

1. Why is it standard protocol to confirm a bird's karyotype via DNA even if the Studbook already has a sex recorded? What is the risk if the physical records don't match the genomic code?

Now look at the Bird IDs assigned to your station. Refer to the Studbook and Pedigree to see if your samples are obligate or suspect carriers. Fill in the Gel Prediction Table with predicted genotype bands ONLY if your group contains any obligate carriers.

2. If your test bird is a suspect carrier, which pattern are you MOST likely to see in your gel today? Why?

3. Why is it scientifically valid to use a chicken genomic model to study a lethal substitution in a California condor?

4. If the BsaBI enzyme finds no recognition sites in a DNA sample, what does that tell us about that bird's genetic status?

5. In a carrier (Aa), the enzyme will encounter two different versions of the gene. One version is "clear" (ignored by the enzyme) and one is mutated (cut by the enzyme). Why is it critical that the enzyme leaves the healthy allele whole?

6. In this proxy model, the substitution is a G to T. If the actual condor substitution turns out to be a C to A change instead, would our current BsaBI enzyme still be a useful diagnostic tool? Why or why not?

7. If the real substitution is found at the 475bp mark (exactly in the middle of a 950bp fragment), how would the (aa) pattern in your Reference Key change?

Part 6: Data Analysis & Management Verdict

Task: Observe the bands in your gel. Identify your results and update your Gel Prediction Table, Studbook, and Pedigree.

1. Did your physical gel match your Gel Prediction Table? If you saw a band you didn't expect (or a band was missing), what lab error or biological factor might explain the discrepancy?

Task: Refer back to your Breeding Proposal in Part 4. Now that you have molecular confirmation, you must make a final executive decision for this bird's future.

- Bird ID: _____
- Confirmed Sex: [ ] Female [ ] Male
- Carrier Status:
  - [ ] Green Light: Verified clear (AA). Eligible for all pairings.
  - [ ] Yellow Light: Confirmed carrier (Aa). Restricted to (AA) mates only.

2. Your bird's genetic status is now a permanent part of the species' history. If the 0% Lethal Probability Rule (AA x Aa) is followed for the next 50 years, explain how this changes the genetic load of the population. Why is masking the lethal allele (a) more beneficial for long-term survival than trying to purge it entirely? Think about how many original founders there were.

Appendix A - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Appendix B - Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called primers to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called Polymerase Chain Reaction (PCR) – *polymerase* because of the enzyme that is used to copy DNA and *chain reaction* because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded

DNA. As with ice, this is sometimes called melting, but is commonly referred to as denaturation. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to encourage primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called annealing, is typically between 45 and 65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that nonspecific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called extension, results in a new copy attached to the template as double-stranded DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75 - 80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. E. coli, the original source for the polymerase used in PCR, thrives at 37.5°C, the same temperature as the gut of mammals.

Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace E. coli polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

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US 11,879,117, US 11,879,118