



Code Condor - Conservation  
Genetics MiniLab  
Student Guide

Cat# M3027TAE

Version 042326



## Table of Contents

Laboratory Safety	2
MiniLab Overview and The Investigation	3
Tech Note A: California Condor Recovery Program	4
Tech Note B: Genetic Management of the California Condor	5
Tech Note C: Digital Karyotyping & Avian Sexing	7
Tech Note D: Pedigree Logic & Forensic Tracing	8
Tech Note E: The Punnett Square as a Management Tool	9
Student Worksheet Day 1	10-12
Studbook and Pedigree	13-14
Tech Note F: PCR (Polymerase Chain Reaction)	15
Tech Note G: Restriction Fragment Length Polymorphism	16
Tech Note H: Gel Electrophoresis	17
Day 2 - Electrophoresis Protocol	18
Gel Analysis	22
Student Worksheet Day 2	23-27

## Laboratory Safety

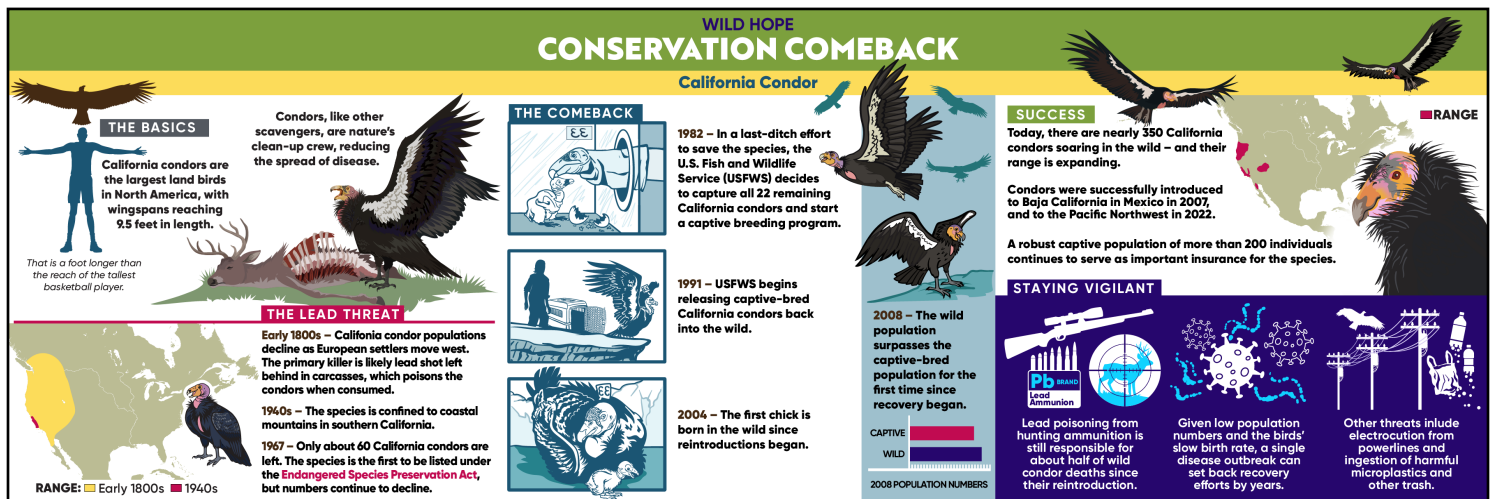
1. Wear lab coats, gloves, and eye protection whenever possible.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.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

## MiniLab Overview

### The Conservation Crisis

The California condor recovery program is a premier conservation success, led by a landmark partnership between indigenous peoples, nonprofits, and global government agencies. As a sacred symbol to indigenous cultures and a legendary survivor of the Ice Age, this giant scavenger represents a biological treasure whose recovery honors our shared heritage and global responsibility to nature.

Genetic screening is a vital management necessity for this conservation breeding program. The population carries a lethal, autosomal recessive skeletal disorder called chondrodystrophy. Because carriers (Aa) appear perfectly healthy, the mutation can stay hidden for generations, only appearing when two carriers are paired. To save the species from this genetic landmine, students must transition from historical studbook tracing to modern genomic screening.



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

### The Science: Comparative Genomics

While the exact mutation 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 G to T transversion. This mutation creates a premature stop codon, resulting in a non-functional protein that prevents skeletal development. By using the chicken as a comparative model, students validate a PCR-RFLP test to identify hidden carriers before they are paired in the breeding program.

### Lab Context

This investigation centers on the detection and management of a lethal autosomal recessive trait within a bottlenecked population. Students act as conservation geneticists, using molecular tools to identify hidden carriers and drafting breeding recommendations based on Mendelian probability.

## Tech Note A: 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.

## Tech Note B: 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 runs of homozygosity (long, identical DNA segments) present before the bottleneck, indicating inbreeding and lack of genetic diversity.

### Building the Pedigree (1980s-2010s)

- **Ancestral Ties:** Early molecular markers (DNA fingerprinting) 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 mean kinship (individual relatedness to the population).

### The Genomic Revolution (2020s-Present)

An ongoing revolution where 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 mutation 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 mutation in the chicken aggrecan (ACAN) gene causes a similar condition - a strong candidate to use as a proxy model for 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 that are advantageous to your survival, your fitness is increased. Population size is a critical starting point when considering genetic load. With California Condors, scientists had only 22 birds to work with for rebuilding 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.

## Tech Note C: Digital Karyotyping & Avian Sexing

### The Genetic Blueprint: ZZ vs ZW

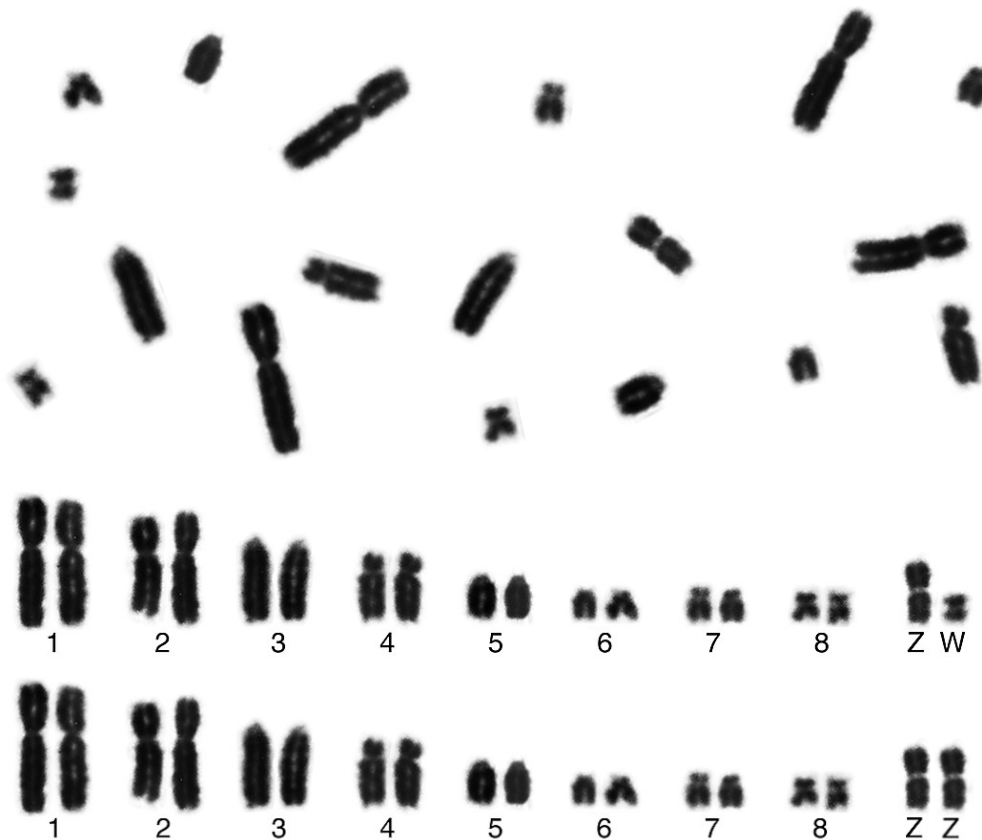
In the human world, biological sex is determined by the XY system, where males are XY and females are XX. However, birds follow the ZW system. Males are homogametic (ZZ), possessing two identical Z chromosomes. Females are heterogametic (ZW), possessing one Z and one W chromosome. This means the mother's gametes determine the biological sex of the chick.

### The Karyotype

A karyotype is an organized profile of an organism's chromosomes. To capture this view, scientists collect live samples, such as blood, feather pulp, or eggshell lining, and grow the cells in a nutrient-rich culture. Once cells begin to divide, researchers add a chemical like colchicine to stop the process during metaphase, when chromosomes are most condensed. These cells are dropped onto a slide, causing them to burst and spread the chromosomes. Scientists then stain and photograph the spread to sort the chromosomes by size and shape.

### 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.



[https://commons.wikimedia.org/wiki/File:Karyotype\\_of\\_chicken\\_%28Gallus\\_gallus%29.png](https://commons.wikimedia.org/wiki/File:Karyotype_of_chicken_%28Gallus_gallus%29.png)

## Tech Note D: 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 traveled through the population.

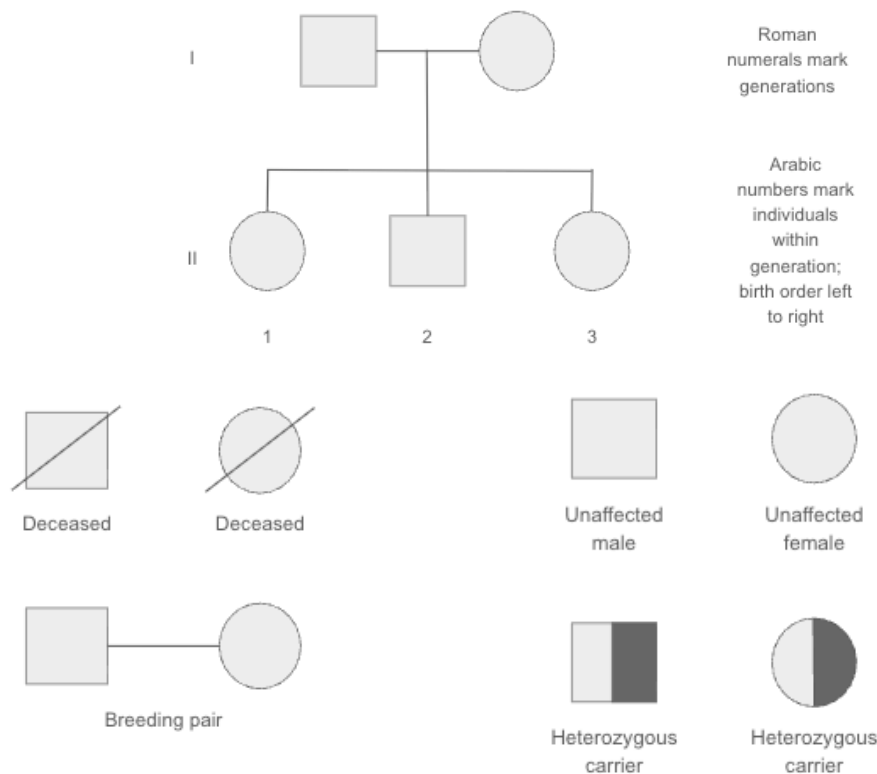
### The Rule of the Recessive Trait (a)

- 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.

## Tech Note E: 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 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.

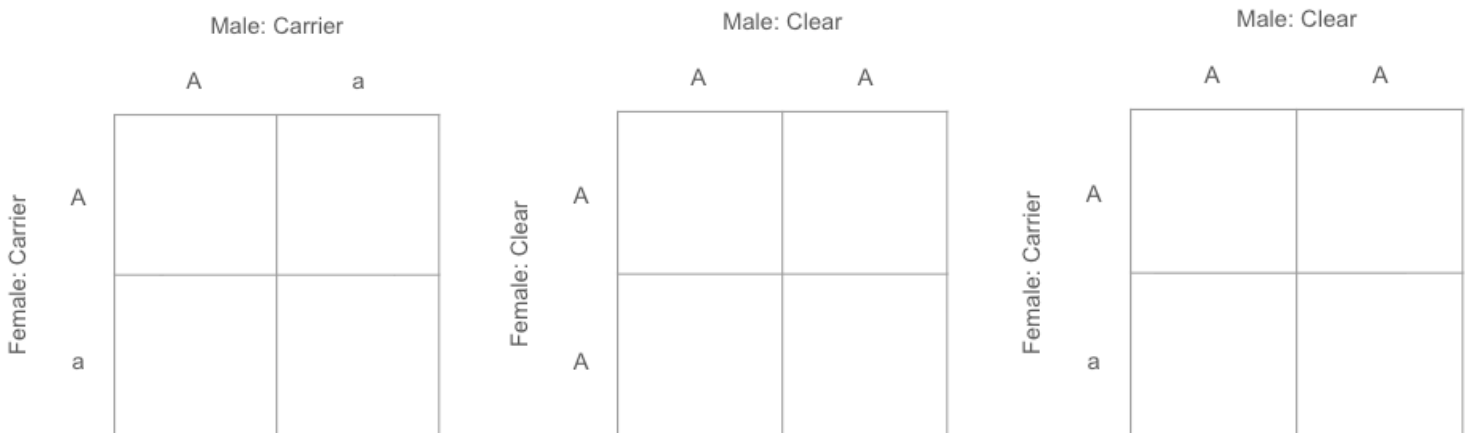
### The Probability of the Egg: Pairing Carriers (Aa x Aa)

- 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.

- Schrödinger's Condor: Much like the famous physics thought experiment, a "suspect" carrier exists in two states at once - carrier and clear - until we "open the genetic box" later in this lab.



## 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

See Tech Notes D & E in the Conservation Science Handbook for more information.

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: When News Alert 1 is issued by your teacher, complete the forensic trace and update your Sample Prediction Table on pg 10 if relevant.

- Affected embryo information
  - Embryo ID: \_\_\_\_\_ Genotype: \_\_\_\_\_
- Update the parent information.
  - Parent ID: \_\_\_\_\_ Genotype: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - Parent ID: \_\_\_\_\_ Genotype: \_\_\_\_\_ Carrier Status: Obligate or Suspect
- Draw a Punnett Square below using these parents' genotypes and calculate the mathematical probability (%) that healthy siblings of the affected embryo are hidden carriers.
  - Sibling ID: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - Sibling ID: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - (AA) probability for all siblings: \_\_\_\_\_
  - (Aa) probability for all siblings: \_\_\_\_\_


Task: When News Alert 2 is issued by your teacher, complete the forensic trace and update your Sample Prediction Table on pg 1 if relevant.

- Affected embryo information
  - Embryo ID: \_\_\_\_\_ Genotype: \_\_\_\_\_
- Update the parent information.
  - Parent ID: \_\_\_\_\_ Genotype: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - Parent ID: \_\_\_\_\_ Genotype: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - Clan A Grandparent ID: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - Clan A Grandparent ID: \_\_\_\_\_ Carrier Status: Obligate or Suspect
  - (AA) probability for both grandparents: \_\_\_\_\_
  - (Aa) probability for both grandparents: \_\_\_\_\_

1. 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: Preparing for the Molecular Mission

See Tech Notes F, G, & H in the Conservation Science Handbook for more information.

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. Because the specific mutation in the California condor has not yet been definitively identified, we are using the chicken aggrecan (ACAN) gene as a proxy to test the accuracy of our genomic model. For this mission, your DNA samples have already been amplified via PCR and digested with the BsaBI restriction enzyme. Your task tomorrow is to run the gel electrophoresis to visualize the results.

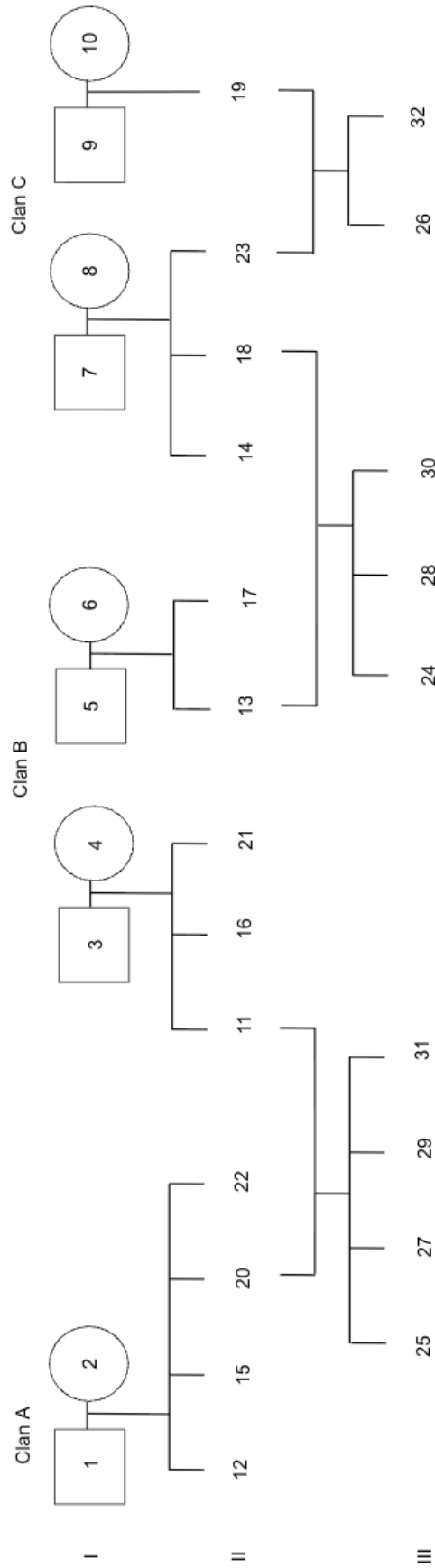
1. Why is it scientifically valid to use a chicken genomic model to study a lethal mutation in a California condor?
2. If the BsaBI enzyme finds no recognition sites in a DNA sample, what does that tell us about that bird's genetic status?
3. In a carrier (Aa), the enzyme will encounter two different 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?
4. 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?

## 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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## Tech Note F: PCR (Polymerase Chain Reaction)

### The Molecular Xerox Machine

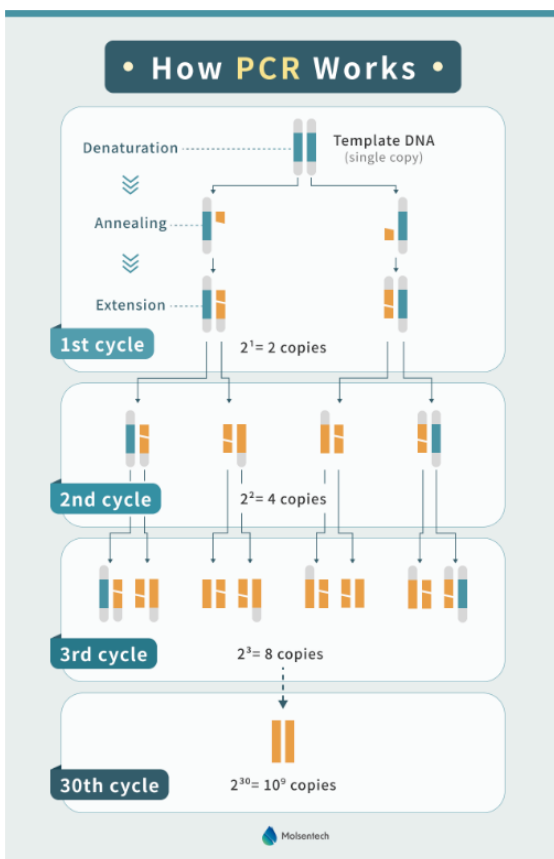
The California condor genome contains roughly 1.2 billion base pairs of DNA. If we want to find one specific predicted mutation or identify a bird's sex, we can't look at all of it - that would be like trying to find one typo in a library of 1,000 books.

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 become dense enough for us to actually see via gel electrophoresis.

### The Three-Step Cycle

PCR happens in micro-tubes placed in a thermal cycler (a machine that changes temperature rapidly). Each cycle has three stages, repeated ~30 times:

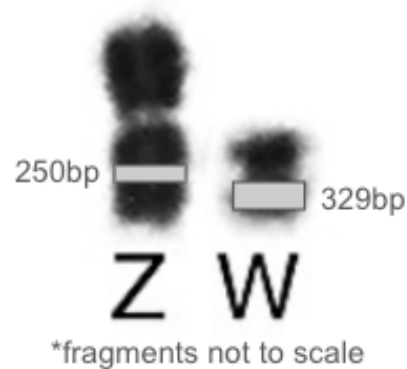
1. Denaturation (95C): High heat unzips the double-stranded DNA into two single strands.
2. Annealing (50-65C): DNA "bookmarks" called primers stick to the start and end of our target region.
3. Extension (72C): The Taq polymerase enzyme finds the primers and builds two copies of the target region.



### 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 than the Z, making it easy to differentiate on a gel.



- The Genotyping Test: We amplified a 950bp region of the chicken aggrecan (ACAN) gene (our proxy for the condor mutation). Before the gel is run, this DNA is incubated with a restriction enzyme (see next page).

<https://www.molsentech.com/post/pcr-vs-qpcr>

## Tech Note G: Restriction Fragment Length Polymorphism

### The Tool: 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 mutations. This process is called restriction digestion - the biochemical "breaking down" of a DNA molecule by an enzyme. We used BsaBI, which looks for the palindromic GAT-NNNN-ATC recognition site (N is any nucleotide).

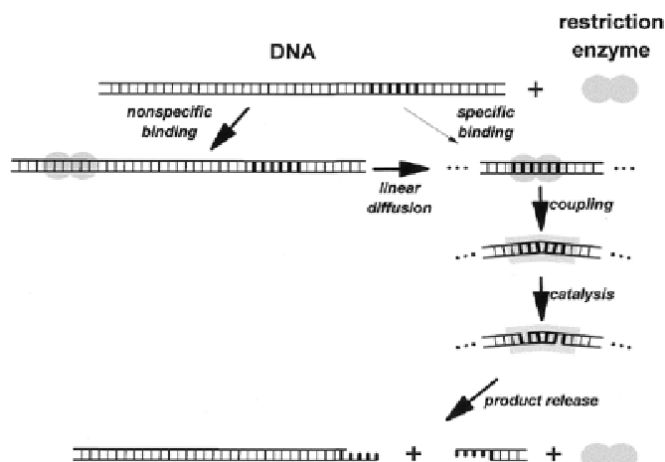
1. Scanning and Recognition: BsaBI scans the DNA until it finds the recognition site.
2. Binding: BsaBI acts as a homodimer; two identical protein subunits "grab" each strand of the DNA helix.
3. Cleavage: The enzyme catalyzes the hydrolysis of the phosphodiester bond, making two blunt-end incisions through the DNA strands.

### The Proxy Model Mutation

In this hypothetical model, a single G to T transversion in the chicken aggrecan (ACAN) gene creates this exact cutting site.

- Healthy Allele (A): BsaBI finds no cutting site and leaves the 950bp fragment whole.
- Mutant Allele (a): The mutation creates the recognition site. BsaBI cuts the strand, creating two smaller fragments.

By running these digested samples on a gel, the resulting band count tells us the bird's predicted genotype.



Pingoud, A., & Jeltsch, A. (2001). Structure and function of type II restriction endonucleases. *Nucleic acids research*, 29 18, 3705-27.

## Tech Note H: Gel Electrophoresis

### The DNA Race: Sorting by Size

Once we have used PCR to amplify our target regions and RFLP to digest the products, we must visualize the results. Because DNA is invisible, we use electrophoresis to sort fragments by their physical length.

- The Matrix: We load DNA into a sponge-like "sieve" called agarose gel, derived from seaweed
- The Buffer: The gel is submerged in a specialized electrophoresis buffer. This salt-rich solution conducts the electrical current and maintains a stable pH.
- The Charge: DNA has a negative charge. When the power is turned on, the buffer allows the current to pull the DNA through the gel toward the positive electrode.
- The Sieve: Small fragments (like a 100bp piece) weave through the gel quickly. Large, bulky fragments (like a 1000bp piece) move much slower.

### The Visualization: GelGreen & Blue Light

To see the DNA, we add a safe fluorescent dye called GelGreen. This dye binds to the DNA fragments. When the blue LED light hits the dye, it glows bright green.

### The Result: Banding Patterns

After 20-30 minutes, the fragments have separated enough to create distinct banding patterns. Each "band" you see is a collection of millions of identical DNA molecules that traveled to the exact same spot in the gel. By comparing these bands to a DNA Marker (a "ruler" with known sizes), we can predict the genotype and biological sex of every bird in our study using the chicken aggrecan (ACAN) gene proxy system.

## Part I: Electrophoresis

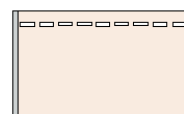
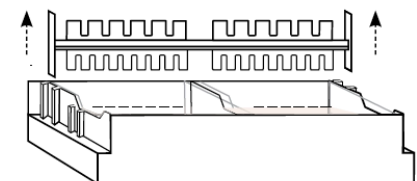
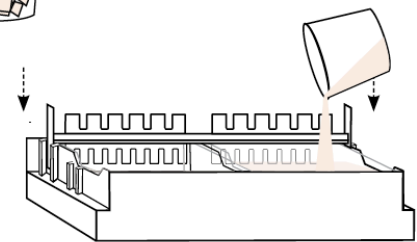
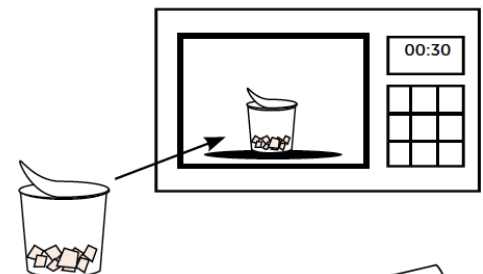
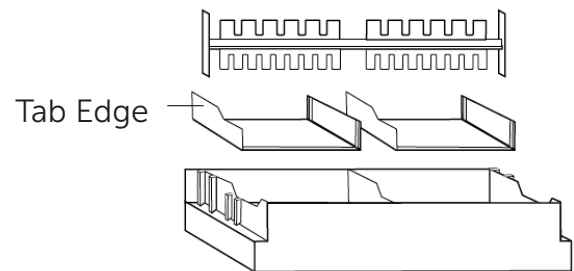
### Materials

- 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

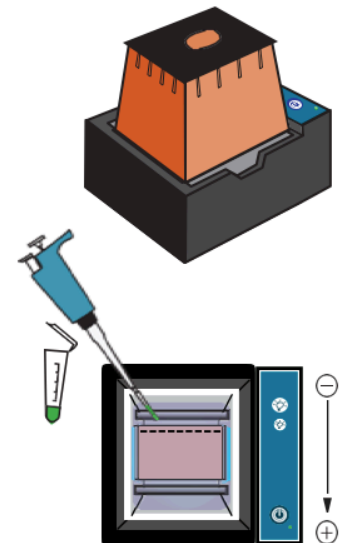
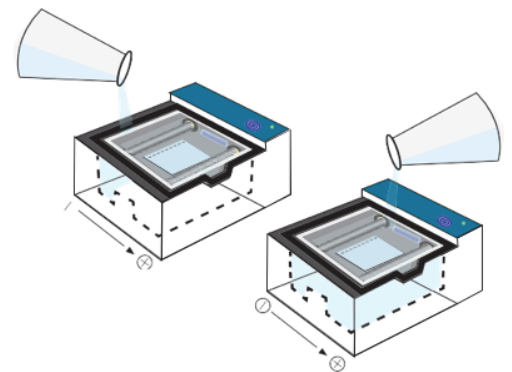
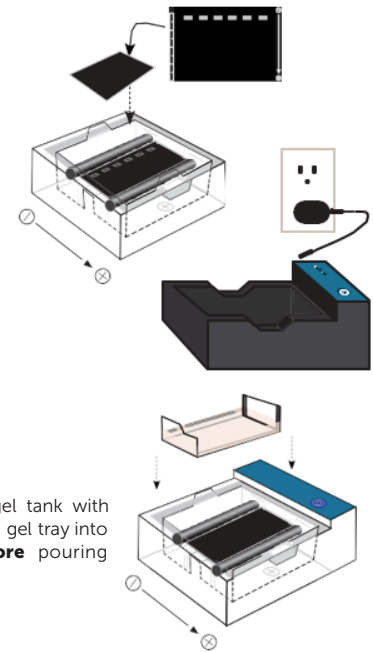
### How to Cast a Gel

- 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.
  - 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.
  - 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.**
- 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.
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  $\mu\text{L}$  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.

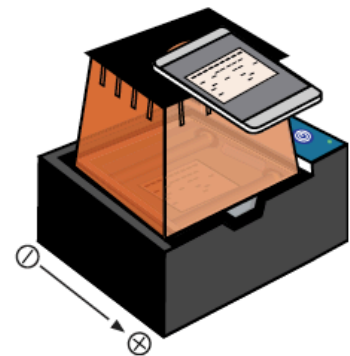
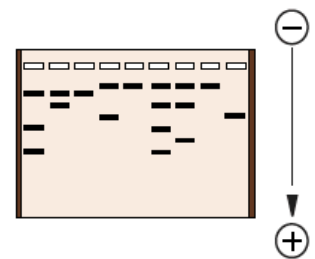
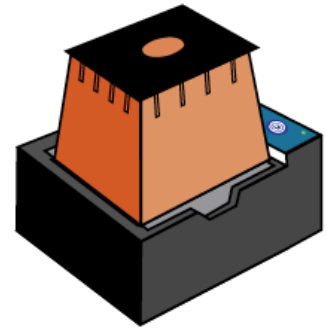
**The green power LED will not turn on if:**

- 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

1	2	3	4	5	6	7	8	9
								

Lane 1: \_\_\_\_\_

Lane 2: \_\_\_\_\_

Lane 3: \_\_\_\_\_

Lane 4: \_\_\_\_\_

Lane 5: \_\_\_\_\_

Lane 6: \_\_\_\_\_

Lane 7: \_\_\_\_\_

Lane 8: \_\_\_\_\_

Lane 9: \_\_\_\_\_

## Student Worksheet Day 2

### Part 1: Mission Launch & Lab Loading

Consult the Code Condor Lab Protocol at your station. Once your samples are successfully loaded and the power is on, return to this worksheet.

Today, we move from paper records to molecular evidence. Because the specific mutation in the California condor has not yet been definitively identified, we are using the chicken aggrecan (ACAN) gene as a proxy to test the accuracy of our genomic model.

Research Note: This is an ongoing investigation in conservation genetics. While we use this proxy today, the real condor mutation remains a mystery. One day, a researcher using molecular tools like these - perhaps even someone in this room - will be the one to solve this puzzle.

1. Once the power is on, observe the electrodes. Why does the DNA travel toward the positive electrode?

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				

### Part 2: Sex Determination Prediction

See Tech Notes F & H in the Conservation Science Handbook for more information.

**Task:** Based on the chromosomal sex identified on Day 1 for each Bird ID (check the Studbook or Pedigree), draw your expected sex determination bands in the Gel Prediction Table (pg 1). Remember, birds are ZW (female) or ZZ (male). Our primers target both chromosomes but produce different fragment sizes: Z (250 bp) and W (329bp).

1. Between the Z (250bp) and W (329bp) fragments, which one will have a harder time “weaving” through the gel matrix? Why? Based on your answer, which band do you predict will travel further from the starting well?

### Part 3: RFLP Sequence Audit & Prediction

See Tech Note G in the Conservation Science Handbook for more information.

While the specific condor mutation is still unknown, we are using the chicken aggrecan (ACAN) gene as a proxy - here there is a known 950bp fragment we amplified via PCR and digested with the BsaBI restriction enzyme. Restriction enzymes act like immune scouts that recognize and cut up specific DNA sequences. BsaBI specifically looks for this code: **GATNNNNATC** (where N stands for any nucleotide).

#### DNA Zoom

**Task:** Analyze the zoomed in portion of the DNA sequence of the ACAN gene proxy below to find the enzyme cutting site.

- Find the Mutation: Circle the single letter that has changed from a G in the healthy allele to a T in the mutant allele.
- Identify the Site: Draw a box around the 10-letter sequence in the mutant allele that now matches the **GATNNNNATC** code.

Mark the Cut: Draw a vertical line (|) through the mutant allele where the enzyme will make its cut (directly in the middle of the **GATNNNNATC** section).

Healthy Allele (A):

.....T G G T C A G A G G G T T A T C G T A C G G T C G G C C T G A...  
bp: 690                  695                  700                  705                  710                  715                  720

Mutant Allele (a):

.....T G G T C A G A T G G T T A T C G T A C G G T C G G C C T G A...  
bp: 690                  695                  700                  705                  710                  715                  720

1. In this proxy model, the mutation is a G to T transversion. If the actual condor mutation 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?

### Diagnostic Challenge

**Task:** Based on your DNA Zoom, annotate these scaled maps of the total 950bp ACAN fragment. Draw an "X" at the correct bp mark - **only** if the mutation is present and the enzyme makes a cut.

Bird X: Homozygous Dominant (AA)

- Allele 1 (A): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
  - Allele 2 (A): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
- 0 100 200 300 400 500 600 700 800 900bp
- Band Count: \_\_\_\_\_ List sizes: \_\_\_\_\_

Bird Y: Heterozygous Carrier (Aa)

- Allele 1 (A): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
  - Allele 2 (a): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
- 0 100 200 300 400 500 600 700 800 900bp
- Band Count: \_\_\_\_\_ List sizes: \_\_\_\_\_

Bird Z: Homozygous recessive (aa)

- Allele 1 (a): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
  - Allele 2 (a): [|-----|-----|-----|-----|-----|-----|-----|-----|-----|---] 950bp
- 0 100 200 300 400 500 600 700 800 900bp
- Band Count: \_\_\_\_\_ List sizes: \_\_\_\_\_

### Carrier Status Reference Key

**Task:** Before you can diagnose your bird(s), you must define what the three possible chondrodystrophy carrier status results look like on a gel. Use your calculations from the Diagnostic Challenge to draw predicted bands:

MiniOne Marker	Chondrodystrophy Carrier Status Samples		
	AA	Aa	aa
2000bp			
1000bp			
500bp			
300bp			
100bp			

**Task:** 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 on page 1 with predicted genotype bands ONLY if your group contains any obligate carriers.

1. If your test bird is a suspect carrier, which pattern from your Carrier Status Reference Key above are you MOST likely to see in your gel today? Why?
  
2. We used the chicken aggrecan (ACAN) gene as a proxy today because the condor mutation is still unknown. If the real mutation 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 4: Breeding Proposals

See Tech Note E in the Conservation Science Handbook.

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.

#### 3. Assigned Bird Profile

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

#### 4. 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"?

5. If you choose Mate Option B, and both birds turn out to be carriers (Aa) once the gel runs, 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?

### Part 5: Data Analysis & Management Verdict

Turn on the blue light on your MiniOne Electrophoresis System.

**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.

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S Y S T E M S

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