FUTURELAB+

AG/ENVIRONMENTAL

*Community Science*

# Tech 3: Digital PCR

Developed in partnership with:

**Discovery Education and Ignited**

# In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →



*This document is separated into two sections,* For Teachers [T] *and* Student Resources [S]*, which can be printed independently.*

*Select the appropriate printer icon above to print either section in its entirety.*

*Follow the tips below in the Range field of your Print panel to print single pages or page ranges:*

Single Pages (use a comma): T3, T6

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**Cover Image**
Bacteria in a water sample is a potential source of environmental DNA (eDNA).

AG/ENVIRONMENTAL / COMMUNITY SCIENCE

# Technical (Tech) Lesson 3: Digital PCR

## DRIVING QUESTION

*How does digital PCR work as a DNA identification technique?*

## OVERVIEW

DNA technology has advanced to the point where we can analyze DNA found in very small concentrations. The ability to study small amounts of DNA has opened the door to research in many domains, including studying ancient DNA (aDNA) in fossils, preserved specimens, or human remains, and examining trace amounts of environmental DNA (eDNA) in soil and water samples. One of the tools allowing for this analysis is digital PCR (dPCR). In dPCR, a sample of interest is divided into many smaller samples for PCR analysis, allowing researchers to amplify even tiny amounts of the DNA of interest.

In this lesson, students will begin by learning about eDNA and aDNA, and the challenges in collecting and analyzing those forms of DNA. Students will then examine dPCR as a potential technology tool for analyzing eDNA and aDNA. Students will examine several research-based scenarios where dPCR was used to collect data to address a community issue, and will develop a general understanding of the types of community issues that could be investigated with dPCR. In the final portion of the lesson, students will add what they learned about the process of digital PCR to the Decision Tree Assessment.

## ACTIVITY DURATION

One class session
(45 minutes)

## ESSENTIAL QUESTIONS

***How can digital PCR be used to answer questions related to DNA identification?***

***How does digital PCR compare to other DNA identification techniques?***

***How does digital PCR connect to other DNA tools you have learned about, such as Sanger Sequencing and PCR?***

## OBJECTIVES

*Students will be able to:*

**Explain** the purpose of digital PCR.

**List** types of information that can be gathered using digital PCR.

**Describe** the pros and cons of digital PCR as a DNA identification technique.

| *Materials* |
| --- |
| **Digital PCR Scenario Cards** |
| **Digital PCR Scenario Analysis Capture Sheet** |
| **Technology Overview Capture Sheet, Part 1: DNA Identification** |

# Pedagogical Framing

***Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.***

***Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.***

***Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.***

## SOCIAL-EMOTIONAL LEARNING

Students must use self-discipline and self-motivation to stay on task and manage their time when working on the scenarios. Students need to communicate clearly with their groups and with other groups to produce the decision tree and provide feedback. This lesson also asks students to listen actively, cooperate, work collaboratively to problem-solve and negotiate conflict constructively, and seek or offer help when needed. Students will work on making a reasoned judgment on the technique to use after analyzing information, data, and facts.

## CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson connects to real-world issues identified by students in their own communities, as dPCR will be an option to include in their final proposal. The different scenarios also are reflective of diverse identities because they represent communities across the United States and the world.

## ADVANCING INCLUSIVE RESEARCH

Digital PCR enables researchers to analyze ancient DNA. In this lesson, students will discuss how utilizing dPCR with diverse communities enriches and provides robust information about how various historical communities lived. In order for scientists to advance projects that address issues from all communities, it is important to ensure marginalized communities are represented.

## COMPUTATIONAL THINKING PRACTICES

Students will understand how automation works in the process of dPCR and must extract key information about dPCR to complete the lesson.

## CONNECTION TO THE PRODUCT LIFE CYCLE

In this lesson, students compare traditional PCR and digital PCR, and understand how these technologies could help solve varied environmental challenges. This connects to the **develop** stage of the product life cycle as students think about different DNA sequencing techniques they could later apply to their product design.

# Have you ever wondered…

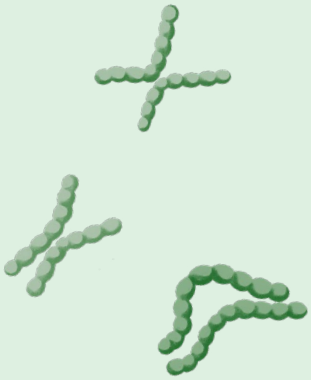
***Can we analyze DNA from fossils?***

DNA degrades very quickly in samples, with some estimates suggesting that DNA has a half-life of approximately 521 years (source: *The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils*). Even so, DNA fragments can be recovered from some preserved remains, such as frozen mammoth carcasses. Digital PCR technology is a tool that can be used to amplify these fragmented DNA remains. It allows researchers to recreate some or most of the genomes of those species. While finding dinosaur DNA in amber-encased insects is not scientifically possible (sorry, Jurassic Park!), we could someday use aDNA samples to bring back more recently extinct species, like the wooly mammoth or the passenger pigeon.

## MAKE CONNECTIONS!

### *How does this connect to the larger unit storyline?*

This is the third lesson in a series on major technologies used in DNA identification. Students will be selecting among these technologies as they prepare their final artifact proposals.



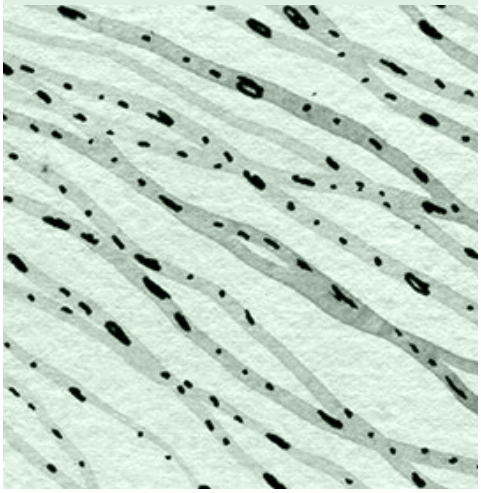
### *How does this connect to careers?*

***Fish and wildlife managers*** maintain healthy ecosystems and assure the continued success of fish and wildlife populations. These managers are increasingly using eDNA analysis to track population movements, collect population data on endangered or cryptic species, and monitor the spread of invasive species.

***Water quality specialists*** monitor water supplies and assure that those supplies meet relevant laws and guidelines for safe water quality. Water quality specialists may work for municipal or regional water management groups, sewer treatment facilities, or in parks and natural areas. Water quality specialists can use eDNA to monitor population sizes, track endangered species, or even check for potential disease organisms in the water supply.

### *How does this connect to our world?*

Scenario cards are based on real-life applications of the technology. Digital PCR will be one of the options students can select when designing their final artifact for the unit.



# Day 1

## LEARNING OUTCOMES

*Students will be able to:*

**Explain** the purpose of digital PCR.

**List** types of information that can be gathered using digital PCR.

**Describe** the pros and cons of digital PCR as a DNA identification technique.

# Procedure

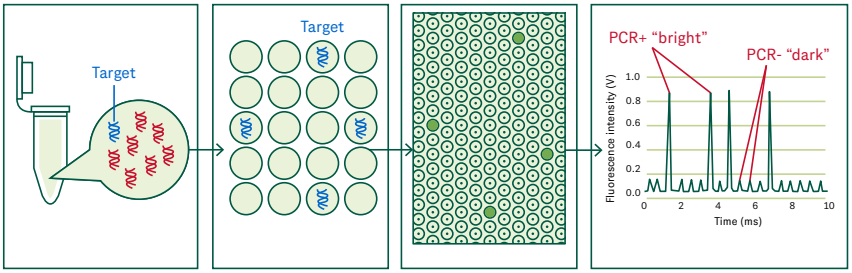
**Teacher Note >** *This lesson could be expanded to two days if you would like to examine examples of aDNA or eDNA in more detail, or have student groups examine more of the dPCR scenarios. Students could also be asked to use different resources (online simulations, video explanations, etc.) about dPCR to construct their own Venn diagrams individually or in small groups. If expanding to two days, you could also ask students to explain the difference between PCR and digital PCR to a friend or family member. Students can then give the family member a short quiz to see how well they explained the topic.*

**Whole Group (15 minutes)**

**Teacher Note >** *To start this lesson, you will project images of DNA-based reconstructions of ancient humans. An excellent example image to use is a reconstruction of a Stone Age girl based on DNA found in ancient birch pitch "chewing gum." An image of the gum itself and a discussion of the techniques used to sequence DNA from the gum is available in A 5700 year-old human genome and oral microbiome from chewed birch pitch. An Internet search for "Stone Age girl chewing gum" will locate an artist's interpretation of what the girl may have looked like based on the DNA results.*

1 In their journals, have students reflect on the images with the following prompt: What would be challenging about extracting DNA from ancient chewing gum?

2 Ask a few students to share their ideas with the class. Explain that the PCR technology they have used to this point is difficult to use when the DNA of interest is found in a small amount in the sample. Use this as a point of review by asking students to list all the things they remember about regular PCR from the Solution Seeking Microbe Unit and Alternative Proteins Unit. Use the image below to illustrate how digital PCR (dPCR) technology separates a sample into many individual components to allow for the detection of very small amounts of target DNA. Consider finding a video on digital PCR for more technical support before presenting to students.

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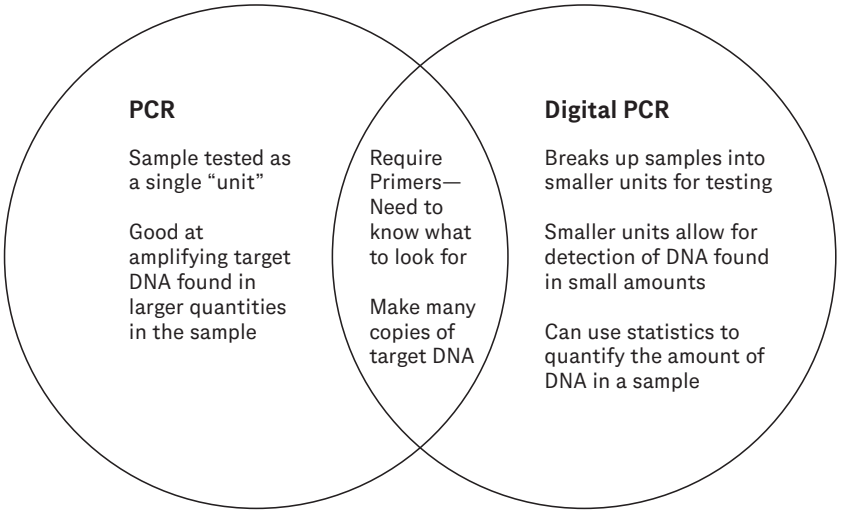
## Day 1

*Continued*

# Procedure

3 Draw or project the Venn diagram below to illustrate the major differences between PCR and digital PCR.




4 Use examples to introduce the terms ancient DNA (aDNA) and environmental DNA (eDNA). Potential examples can be found in the resource banks from Lesson 1. Ask students to predict why these types of DNA might be hard to analyze using regular PCR.

a*DNA is often degraded and found in very low amounts. eDNA is also found in small amounts, especially if looking at trace material left behind by a rare species or in a place like an ocean or river where DNA is easily diluted.*

**Small Group (15 minutes)**

1 Divide students into groups of four. Give each group a copy of the *Digital PCR Scenario Analysis Capture Sheet* and one of the *Digital PCR Scenario Cards* outlining an example of an issue investigated using digital PCR technology.

2 Direct groups to assign roles to each group member and work to answer the questions on the capture sheet, based on their scenarios.

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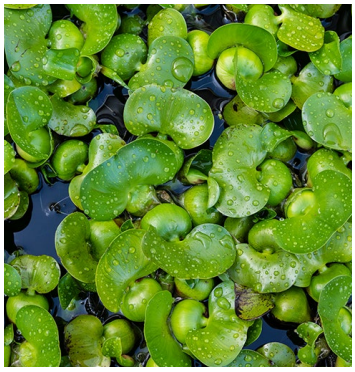
## Day 1

*Continued*



# Procedure

**Whole Group (10 minutes)**

1. The spokesperson for each group should share the group's scenario and answers with the class in a quick whip-around summary. As each group shares, record the overall topic (invasive species, species reintroduction, etc.) from each scenario and the reason for choosing dPCR to address the topic. Record this information on the board, a poster, or other document visible to the class. This step will allow students to develop an overall sense of the types of questions that can be answered with dPCR, and will aid them in completing the dPCR section of the *Technology Overview Capture Sheet, Part 1: DNA Identification*.

2. Lead a quick discussion of student responses.

   **a.** Based on these scenarios, what can we conclude about when or why digital PCR is used instead of PCR?

   **b.** What topics covered in the scenarios do we see in our communities? How could this technology be used to address current issues?

3. Evaluate student understanding of digital PCR to decide the appropriate next steps. If students fully understand the concepts, the next section can be used as a summative assessment and can be evaluated at the end of the unit. If not, use the *Technology Overview Capture Sheet, Part 1: DNA Identification* as a formative assessment to check student understanding after the sharing and reflection.

**Individual Work (5 minutes)**

Direct students to locate the Technology (Tech) Lesson 2—Digital PCR section of *Technology Overview Capture Sheet, Part 1: DNA Identification*. Either individually or with a partner, students should complete the digital PCR section of the worksheet.

**Teacher Note >** *The Technology (Tech) Lesson 2—Digital PCR section of Technology Overview Capture Sheet, Part 1: DNA Identification can be assigned as homework if time is pressing.*

# National Standards

## Next Generation Science Standards

**LS-1-1 From Molecules to Organisms: Structures and Processes**
Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

**ETS1-2 Engineering Design**
Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.

**Science and Engineering Practices**
Engage in argument from evidence
Compare and evaluate competing arguments or design solutions in light of currently accepted explanations, new evidence, limitations (e.g., trade-offs), constraints, and ethical issues.

## Career and Technical Education (CTE)

**A 1.0**
Define and assess biotechnology and recognize the diverse applications and impact on society.

**A1.6**
Explore and outline the various science and non-science fields and careers associated with biotechnology.

**A3.0**
Demonstrate competencies in the fundamentals of molecular cell biology, including deoxyribonucleic acid (DNA) and proteins and standard techniques for their purification and manipulation.

**A4.0**
Recognize basic concepts in cell biology and become familiar with the laboratory tools used for their analysis.

**A5.2**
Use a variety of methods, including literature searches in libraries, computer databases, and online for gathering background information, making observations, and collecting and organizing data.

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# National Standards

| | |
|---|---|
| **CTE**<br>*Continued* | **A8.7**<br>Determine which equipment is appropriate to use for a given task and the units of measurement used. |
| | **4.5**<br>Research past, present, and projected technological advances as they impact a particular pathway. |

## Digital PCR Scenario Analysis Capture Sheet

**ANSWER KEY** **Do not share with students**

**Directions**

*Your group will be analyzing a scenario wherein digital PCR technology is used to solve a problem or answer a question. Your group will work together to answer the questions on this and the next page, and will present your findings to the class.*

*Before beginning this task, assign each group member one of the following roles:*

| | |
|---|---|
| Reader | Reads the scenario card aloud for the group to start the task; locates information on the card as needed during the discussion. |
| Recorder | Records the group's responses to the questions below. |
| Spokesperson | Shares the group's responses with the class. |
| Manager | Assures all group members participate in the discussion; manages group time. |

*The answer key on the following page contains responses for all four questions for each scenario card, organized into a table.*

*Continues next page >*

## Digital PCR Scenario Analysis Capture Sheet

**ANSWER KEY** **Do not share with students**

*Continued*

| *Scenario Card* | 1<br>What community issue or problem is discussed in this scenario? | 2<br>Does this scenario involve analysis of aDNA samples, eDNA samples, or both? | 3<br>What community issue or problem is discussed in this scenario? | 4<br>What community issue or problem is discussed in this scenario? |
|---|---|---|---|---|
| *Stocking Rivers for Recreational Fishing* | Want to determine if wild fish were breeding with non-native fish stocked in the river. | aDNA (from preserved scales of fish) | They expected to find only very low levels of the target DNA, if any at all. Traditional PRC would not be sensitive enough. | The native fish had genes in common with the stocked fish that the historical fish did not, indicating mating between the two. |
| *Tracking Invasive Species and Saving Endangered Ones* | Want to track the population of invasive snakes that eat local endangered species. | eDNA (from local water and soil samples) | They wanted a precise count of the invasive snake species. Traditional DNA cannot be used to quantify DNA amounts. | A local group is aiming to raise funds to track the snake population. |
| *Debating the Status of the Gray Wolf* | Want to determine wolf population targets (population numbers that would list them as endangered or not). | aDNA (from wolves killed in late 19th and early 20th centuries) | They wanted to quantify the genetic diversity of the samples. Traditional DNA cannot be used to quantify. | There is less genetic diversity in current wolf populations than historical ones, suggesting that today's wolf population is smaller. |
| *Monitoring Orca Whales* | Want a non-invasive way to learn more about orca populations. | eDNA (from specific ocean areas) | Whale DNA would exist at very low levels in the ocean samples. Traditional PRC would not be sensitive enough. | Digital eDNA will enable this group to gather more information about orca populations. |
| *Stopping Harmful Algae Blooms* | Want to determine if algae blooms have increased over time. | Both eDNA and aDNA (from core samples of sediment from the lake) | DNA from algae blooms would only exist in very low levels in the core samples. Traditional PRC would not be sensitive enough. | Algae blooms have become larger and more frequent in recent years and contain more toxic algal species. |
| *Restoring an Iconic Species* | Want to know if the land iguana on Isla Seymour Norte is similar to the historical Isla Baltra population, and use it to restore that population. | Both aDNA (from museum samples of land iguanas from Isla Baltra) and eDNA (from soil on Isla Seymour Norte) | The amount of land iguana eDNA on Isla Seymour Norte would be very small. Traditional PCR would not be sensitive enough. | The land iguana on Isla Seymour Norte are similar to historical Isla Baltra populations. |
| *Searching for Extraterrestrial Life* | Want to determine if life has existed on Mars. | Both eDNA and aDNA (from soil samples from Mars) | They are looking for trace elements of any nucleic acid. Traditional PCR would not be sensitive enough. | These samples will hopefully be returned to Earth to test. |
| *Identifying and Protecting Unique Species* | Want to determine if the Sierra Nevada red fox is its own distinct species. | Both aDNA (from museum specimens) and eDNA (from soil near existing red fox populations) | The amount of red fox eDNA would be very small. Traditional PCR would not be sensitive enough. | The Sierra Nevada red fox is genetically distinct from other red foxes. |
| *Tracking Parasites* | Want to track fluke populations and prevent them from expanding. | eDNA (from water samples of fishing locations) | The amount of fluke eDNA would be very small. Traditional PCR would not be sensitive enough. | Existing measures to prevent fluke expansion are working. |

## Technology Overview Capture Sheet

*Part 1: DNA Identification*

**ANSWER KEY** **Do not share with students**

**Directions**

*After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.*

*This answer key is for the current lesson, Tech Lesson 3. Students should keep this sheet and others for use in Lesson 9.*

| Tech Lesson 3 | | Digital PCR | |
|---|---|---|---|
| **Describe** | Summarize how this technique works. | Digital PCR is a technique used to detect low levels of DNA or other nucleic acid.<br>It can also be used to quantify the amount of DNA found in a sample.<br>It can be used to detect the presence of species (find a needle in a haystack). | |
| **Discuss** | List the pros and cons you identify for the technique. | Pros | Cons |
| | | Only need a small amount of DNA | Specialized and expensive equipment |
| | | Can be used in large bodies of water where DNA could be diluted | Can only do a few samples at a time (96) |
| | | Accurate in detecting species | Need to know what kind of DNA you want to detect |
| | | It can detect low levels of the target DNA sequence | Can be inhibited by mutations because primers don't work |
| **Support** | Provide examples. | Finding invasive species in bodies of water or plant matter<br>Accurately determining amount of virus in a sample (can be used to correlate viral symptoms with amount of virus in the infected individual)<br>Detecting SARS-CoV-2 in sewage<br>Other examples not mentioned in the lesson do exist. | |

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## Digital PCR Scenario Cards

**Directions**
*Select one of these digital PCR examples to complete the Digital PCR Scenario Capture Sheet.*

### Stocking Rivers for Recreational Fishing

Source: Hansen, M.M. and Mensberg, K.D. (2009). *Admixture analysis of stocked brown trout populations using mapped microsatellite DNA markers: indigenous trout persist in introgressed populations.* Biology letters, 5,5.

#### Background Information

Recreational, or sport, fishing is a multi-billion dollar industry in the United States. An estimated 50 million people participate in recreational fishing every year. In many localities, fishery managers will "stock" rivers, streams, and lakes with popular fish species to increase the chance of catching a fish. Stocking is when fish are reared in a facility and then released into the wild. While many stocking programs use the same species of fish naturally found in those waters, other programs use species that are easier to grow and maintain. In cases where non-native fish are used, there is the possibility that the stocked fish will displace native species. In cases where the introduced species is a close relative of a wild one, there is also concern that the two species will interbreed, creating a hybrid form that could cause the wild species to become functionally extinct.

*Stocking brook trout in Vermont.*

#### Application of Digital PCR

Communities around one river system wanted to find out whether a native fish species was interbreeding with the non-native fish species stocked each year in the river basin. They located dried fish scales from fish caught before stocking started (before 1960) from local fishermen, museum specimens, and local college collections. In several locations along the river, they caught fish samples and clipped a small fin sample, before releasing the fish back into the stream. DNA was extracted and analyzed with digital PCR for differences in 50 microsatellite DNA markers. The results showed many populations of the native fish had gene forms from the introduced fish. The historical fish samples did not show those genetic markers.

*Close up of a brook trout.*

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## Digital PCR Scenario Cards

*Continued*

### Tracking Invasive Species and Saving Endangered Ones

Source: Hunter, M. E., Oyler-McCance, S. J., Dorazio, R. M., Fike, J. A., Smith, B. J., Hunter, C. T., Reed, R. N., & Hart, K. M. (2015). *Environmental DNA (eDNA) Sampling Improves Occurrence and Detection Estimates of Invasive Burmese Pythons.* PLOS ONE, 10(4), e0121655.

#### Background Information

The Burmese python and other species of constrictor snakes are invasive species in the Florida Everglades. These large snakes prey upon many native species that are already vulnerable due to habitat loss, climate change, and past hunting pressures. Tracking these elusive snakes is incredibly difficult due to their cryptic coloring and elusive nature.

*The invasive Burmese python.*

#### Application of Digital PCR

Communities around the Florida Everglades want to protect native bird species, as well as the small population of Florida panthers, from invasive snakes. Various groups in the area have tried using tracker dogs, attractive lures, remote sensing equipment, and even decoy snakes to try to locate and remove these snakes. None of these techniques have been able to detect the snakes in large numbers. A local group has proposed raising funds to collect water and soil samples from multiple locations in the area to test for DNA left behind in shed snake skin or feces. The results would be used to estimate the population size of the snakes in various locations and to track whether the snakes are moving into new areas. The community hopes this will allow them to better target areas for snake removal.

*The Florida panther (*Puma concolor coryi*) is highly endangered. Invasive pythons can kill young kits.*

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## Digital PCR Scenario Cards

*Continued*

### Debating the Status of the Gray Wolf

Source: Leonard J.A., Vilà C. and Wayne R.K. (2005). *Legacy Lost: genetic variability and population size of extirpated US grey wolves (Canis lupus).* Mol Ecol., 14(1).

Leonard, J.A. and Wayne, R.K. (2008). *Native Great Lakes wolves were not restored*. Biology letters, 4(1).

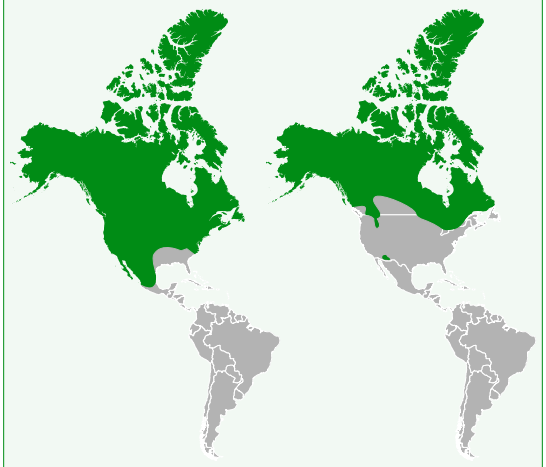
#### Background Information

Gray wolves once roamed across the majority of North America, before large-scale eradication programs led to their extinction throughout most of their historic range. Small populations of the gray wolf remain in a few parts of the United States, including in the northern Great Lakes region. Historical prejudices against wolves, human population movements into remaining wolf territory, and the lack of information about wolf interactions with livestock make for contentious debate around the appropriate population size and management practices for wolves in these areas.

*The gray wolf population used to extend throughout most of North America.*

#### Application of Digital PCR

Communities throughout the Great Lakes region have debated what the appropriate population size is for wolves in the area. The US Fish and Wildlife Service set a goal of between 1,250 and 1,400 wolves for the area, up from a low of 350 wolves in the 1960s. Wolf populations in the region now range from 2,00–3,000, which led to this population being removed from the endangered species list. The population size goal was partially based on an estimate of the historic number of wolves in the area. This estimate, in turn, was based on the amount of genetic diversity in DNA samples from wolves in the current population. To check whether the historic population estimate was correct, DNA was extracted from the remains of wolves that were killed in the late 19th and early 20th century. By examining this DNA, scientists found that the older wolves had more than twice the genetic diversity than the current population, which suggested that the historic wolf population size was significantly larger than the estimates. Stakeholders in the debate over wolf conservation are now examining these data to argue whether to restore endangered species protection to these wolves.



*Past (left) and present (right) distribution of gray wolves in North America.*

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## Digital PCR Scenario Cards

*Continued*

### Monitoring Orca Whales

Source: Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). *Environmental DNA (eDNA) From the Wake of the Whales: Droplet Digital PCR for Detection and Species Identification*. Frontiers in Marine Science, 5.

#### Background Information

Orcas, sometimes called "killer whales," are found throughout the world's oceans. Pods of these large, social, and charismatic animals are popular with whale watchers and are integral to many Indigenous cultures in coastal areas. While whale watching is a multi-billion dollar worldwide industry, data on the population of orcas is limited. The International Union for Conservation of Nature has listed the conservation status of orcas as data-deficient, as there is not even enough information to determine whether all orcas belong to the same species.

*Orca off the coast of Canada.*

#### Application of Digital PCR

A local group wants to help monitor the population of whales found off the coast of their community. However, the usual methods of tracking individual whales requires people to approach whales closely and collect a sample with a small biopsy dart, which is stressful for the whales. The group decides instead to collect sea water samples from selected areas and extract whale DNA from those samples. This method provides a way to track individual whale movements without causing added stress to the whale population.

*Collecting seawater sample.*

*Continues next page >*

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## Digital PCR Scenario Cards

*Continued*

### Stopping Harmful Algae Blooms

Source: Cao, X., Xu, X., Bian, R., Wang, Y., Yu, H., Xu, Y., Duan, G., Bi, L., Chen, P., Gao, S., Wang, J., Peng, J., & Qu, J. (2020). *Sedimentary ancient DNA metabarcoding delineates the contrastingly temporal change of lake cyanobacterial communities*. Water Research, 183, 116077.

#### Background Information

Algae blooms are explosive growths in different types of algae or cyanobacteria species in a waterway. Algae blooms frequently stink and make waterways unusable for recreational or other purposes. In addition, the fast-growing algae can block sunlight to lower reaches of the body of water, and can deplete dissolved oxygen, leading to the death of fish.

*Algae bloom in Lake Erie along Catawba Island in Ohio.*

#### Application of Digital PCR

Each summer a local freshwater lake becomes engulfed in blue-green cyanobacteria blooms. Some residents blame runoff from fertilizers for triggering the blooms, while other people argue that the blooms are part of the natural annual cycle of the lake. To answer this question, the community collects sediment core samples from several locations in the bottom of the lake. These sediment cores act as time capsules, preserving small amounts of DNA from whatever was found in the lake at different periods of time. By analyzing the core samples for different species of cyanobacteria, the community found that algae blooms had become larger and more frequent in recent years and that more recent algae blooms contain larger amounts of several algal species known to be toxic to animals if ingested.

*Scientist collecting a sediment core sample.*

*Continues next page >*

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## Digital PCR Scenario Cards

*Continued*

### Restoring an Iconic Species

Source: Hofkin, B.V., Wright, A., Altenbach, J., Rassmann K., Snell H.M., Miller, R.D., Stone, A.C., and Snell, H.L. (2003). *Ancient DNA gives green light to Galápagos Land Iguana repatriation*. Conservation Genetics, 4.

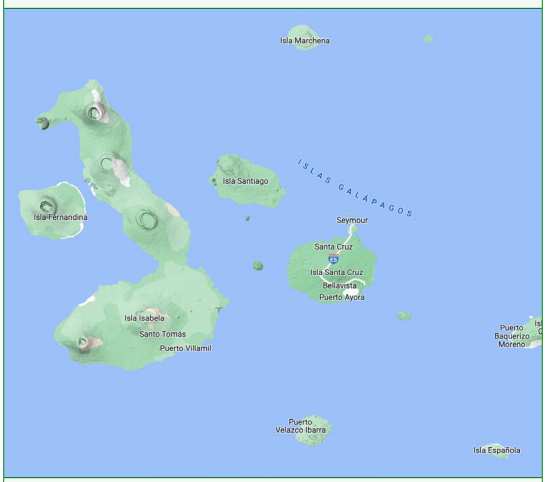
#### Background Information

The Galapagos Islands are home to many strange and wonderful species and are famous for their role in the development of Darwin's ideas about natural selection. Among its many famous species is the land iguana. Like many species in the Galapagos, important genetic differences exist between populations on different islands.

*Land iguana in the Galapagos.*

#### Application of Digital PCR

The last land iguanas on Isla Baltra were killed in the 1940s. Local people have wanted to re-establish a population on the island that would be as much like the extinct population as possible. Historical records suggest that a small group of Isla Baltra land iguanas were moved to Isla Seymour Norte in the 1930s. DNA samples were extracted from museum samples of land iguanas on Isla Baltra and were compared to DNA samples from living land iguanas on Isla Seymour Norte. The results indicated that a number of living land iguanas were very similar to the Isla Baltra iguanas and could be used to recolonize the island.




*Map of the Ecuadorian Galapagos Islands.*

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## Digital PCR Scenario Cards

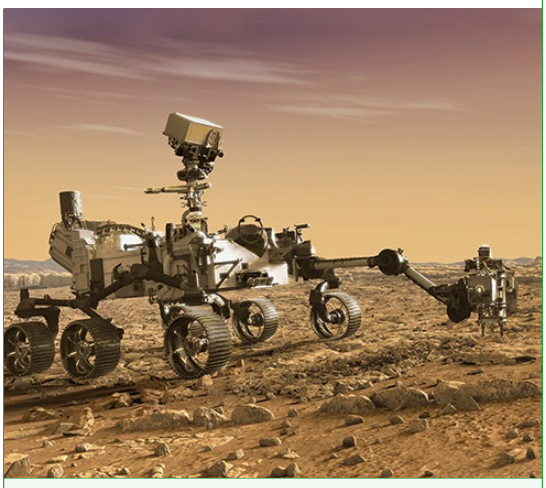
*Continued*

### Searching for Extraterrestrial Life

Source: Willerslev, E., and Cooper, A. (2005). *Ancient DNA*. Proceedings. Biological sciences, 272(1558).

#### Background Information

Many people have wondered if Earth is the only planet in the universe where life exists. As we develop more sophisticated tools to explore further out into space, we may soon be able to search nearby locations like Mars for remains of nucleic acids that would indicate past life.



*Mars Perseverance Rover.*

#### Application of Digital PCR

Humans have sent several uncrewed spacecraft to Mars to study its chemistry and geology. The Perseverance rover, launched in 2020, also includes equipment for collecting samples that will hopefully be returned to Earth to test for remains of ancient life. The extremely cold temperatures found in the Martian permafrost and near the polar regions are excellent for preserving nucleic acids like DNA, with some estimates indicating that a 100 base pair fragment could survive for $3.1 \times 10^{21}$ years in those conditions.

*The surface of Mars; "the red planet."*

*Continues next page >*

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## Digital PCR Scenario Cards

*Continued*

### Identifying and Protecting Unique Species

Source: Perrine, J.D., Pollinger, J.P., Sacks, B.N., Barrett, R.H., and Wayne, R.K. (2007). *Genetic evidence for the persistence of the critically endangered Sierra Nevada red fox in California*. Conservation Genetics. 8.

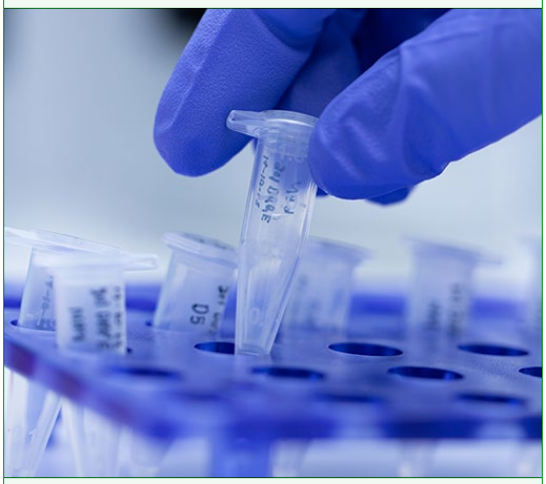
#### Background Information

Red foxes (*Vulpes vulpes*) are common throughout North America. A small number of unique red foxes live in the Sierra Nevada and Cascade ranges of California and Oregon. These foxes are called Sierra Nevada red foxes or High Sierra foxes (Vulpes vulpes necator). Populations of Sierra Nevada red foxes are isolated from other foxes and are found in very low numbers—less than 29 are left in the Sierra Nevada population.

*Sierra Nevada Red Fox.*

#### Application of Digital PCR

Many people want to see the Sierra Nevada red fox protected under the Endangered Species Act. To qualify for protection, people need to show that the Sierra Nevada red fox is really a distinct type of fox, and not just regular red foxes that have recently become isolated in mountainous areas. To investigate this question, DNA was isolated from historic museum specimens of the Sierra Nevada red fox and compared to DNA from modern Sierra Nevada foxes and modern red foxes from other locations. This DNA analysis showed that the Sierra Nevada red fox population has been genetically distinct from other red foxes since the end of the last major period of glaciation in North America.



*Loading test tubes with sampled DNA.*

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## Digital PCR Scenario Cards

*Continued*

### Tracking Parasites

Source: Rusch, J.C., Hansen, H., Strand, D.A., Markussen, T., Hytterød, S. and Vrålstad, T. (2018). *Catching the fish with the worm: a case study on eDNA detection of the monogenean parasite Gyrodactylus salaris and two of its hosts, Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss)*. Parasites Vectors 11.

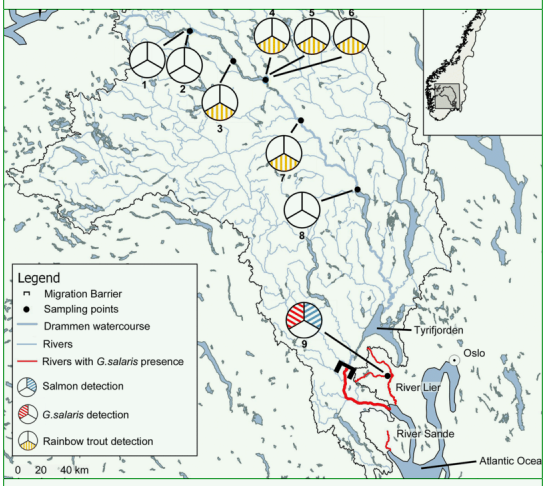
#### Background Information

Several species of fish, including Atlantic salmon and rainbow trout, can be infected by small fluke worms, called *Gyrodactylus salaris*. These flukes attach to the skin of the fish and release digestive enzymes that break down the outer layer of the skin. The fluke then ingests the mush of semi-digested skin and mucus. The fish is left with large open wounds that can easily become infected with other diseases.

*Atlantic salmon.*

#### Application of Digital PCR

Fishing communities in Norway want to remove this fluke from their waterways and prevent the movement of flukes upstream to new locations. To do this, they need a way to monitor areas for the presence of the fluke, which is not visible without a microscope. Traditional monitoring protocols required people to catch and euthanize fish from the waterways and then examine tissues from the fish for infection. Because this method takes an enormous amount of time and results in the deaths of many healthy fish, the community instead decided to test for the presence of fluke DNA in water samples. Based on this sampling, the community was able to confirm that current barriers to fluke movement were effective.




*Map of the Norwegian waterways sampled for fluke.*

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## Digital PCR Scenario Analysis Capture Sheet

**Directions**

*Your group will be analyzing a scenario wherein digital PCR technology is used to solve a problem or answer a question. Your group will work together to answer the questions on this and the next page, and will present your findings to the class.*

*Before beginning this task, assign each group member one of the following roles:*

| | |
|---|---|
| Reader | Reads the scenario card aloud for the group to start the task; locates information on the card as needed during the discussion. |
| Recorder | Records the group's responses to the questions below. |
| Spokesperson | Shares the group's responses with the class. |
| Manager | Assures all group members participate in the discussion; manages group time. |

1. What community issue or problem is discussed in this scenario?

2. Does this scenario involve analysis of aDNA samples, eDNA samples, or both?

*Continues next page >*

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## Digital PCR Scenario Analysis Capture Sheet

*Continued*

3. Why was digital PCR used to analyze these samples instead of traditional PCR?

4. What were the results of the DNA identification? How did those results help address or answer the initial issue or problem in the scenario?

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## Technology Overview Capture Sheet

*Part 1: DNA Identification*

**Directions**

*After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.*

| Tech Lesson 3 | | Digital PCR | |
|---|---|---|---|
| **Describe** | Summarize how this technique works. | | |
| **Discuss** | List the pros and cons you identify for the technique. | Pros | Cons |
| **Support** | Provide examples. | | |