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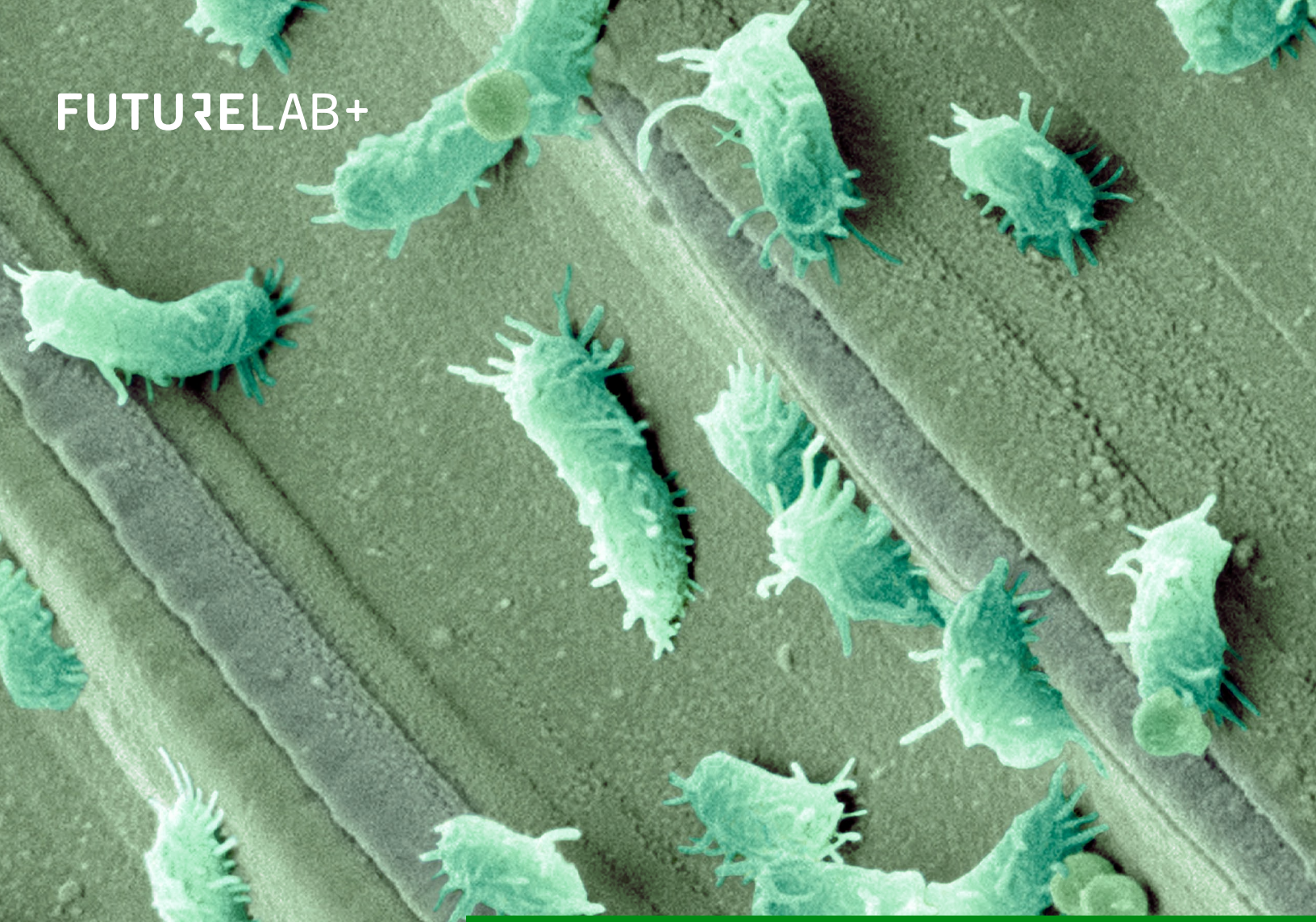

AG/ENVIRONMENTAL

Community Science

Community Applications of DNA Identification

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.*

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

AG/ENVIRONMENTAL / COMMUNITY SCIENCE

Community Applications of DNA Identification

DRIVING QUESTION

How can individuals and communities use the power of DNA identification tools to solve local problems?

OVERVIEW

Community science seeks to leverage the knowledge and power of local communities to answer scientific questions. Community science encompasses a range of activities, from groups collecting field data as part of a large scale study to individuals analyzing data through a gamified website. While some community science initiatives are organized by more traditional science stakeholders, such as universities and government researchers, other projects are coordinated by local communities to address issues deemed locally important. In this unit, students will identify local issues that could be addressed with a community science approach and will create a proposal for how DNA identification technologies could be used to develop a solution for those issues. Throughout this unit, students will consider issues of access to new technologies and learn how these technologies can be used to empower communities to make positive changes.

In this lesson, students will learn what community science entails, and will answer journal prompts about what constitutes a community. Students will then spend time collecting samples or data for a current community science project. With that concept as a lens, they will then use a Jigsaw to learn about how DNA technologies are being used in environmental, health and safety, and personal interest issues. Students will learn about novel ways DNA is being used to solve problems and create a topic summary board to share. After a class discussion, they will be presented with example learning artifacts for the unit.

ACTIVITY DURATION

Five class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

What is a community?

What is community science?

What are some ways DNA is being used to solve issues?

How do communities use DNA identification technologies to solve local issues?

OBJECTIVES

Students will be able to:

Explain the role of the community in community science.

Identify an area of interest in which DNA identification technology can be used to solve problems.

Materials

- **Communities Journal, Part 1**
- **Community Science Example: Young Sleuths Target Sushi**
- **Community Science Resource Packet**
- **Community Science Topic Overview Capture Sheet**
- **Community Science Topic Summary Board Template (optional)**
- **Learning Artifact Graphic Organizer: Coyote Example**
- **Learning Artifact Graphic Organizer**
- **Final Topic Interest Survey**

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 as they individually research examples and share their findings in a Jigsaw format. In the lesson, students will use compassion and empathy when examining different examples of DNA identification applications. In journal activities, students will reflect on their roles in different communities and will begin to identify a social problem or concern in the community. By reflecting on community needs, they will recognize how critical thinking skills are useful both inside and outside of school, and reflect on their roles in promoting community well-being.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

Students will encounter a variety of examples of how DNA technology can be used to solve problems in many diverse community settings. At the end of the lesson, students have the opportunity to select a topic for their own community science proposal that best suits their needs and interests. This proposal selection directly connects their learning to a real-world problem, allowing students to consider solutions to those issues and see themselves as agents of change. They will then draw on their own life experiences within that community to shape their proposals and guide their work throughout the unit.

ADVANCING INCLUSIVE RESEARCH

In this unit, students are asked to identify, research, and engage with community members in order to solve a problem using DNA identification technology. By definition, community science promotes inclusion, and this unit brings biotechnology and community science together to solve locally relevant issues. In this lesson, students explore several examples of how different communities have employed DNA technology to solve problems. Through this exploration, students will see how the science of DNA identification can be applied to situations beyond commercial and academic settings. They will then use this knowledge in future lessons to develop a proposal that addresses a specific issue in their own community, and create a pitch to engage all stakeholders in their community in their project proposals.

COMPUTATIONAL THINKING PRACTICES

Students use the skill of decomposition to break examples of community problems into component parts and extract key information.

CONNECTION TO THE PRODUCT LIFE CYCLE

In this lesson, students identify applications of DNA technology and begin the process of brainstorming how that technology could be employed in their own communities. This lesson connects to the **discovery** phase of the product life cycle.

Have you ever wondered…

Can DNA be used to solve problems?

DNA identification technologies are becoming more reasonably-priced, more accessible, and more accurate. Thanks to these advancements, the use of DNA to solve problems is more feasible than ever.

What are some problems DNA can solve?

Because all living things have DNA, the uses for DNA identification technologies are numerous. It can be used in the environment, food and safety, and human interest, as well as in many other areas. If a living thing needs to be quantified or identified, it can be done with DNA.

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

This is the introduction to the unit. Students will be introduced to ideas for how DNA identification can be used to solve community problems.

How does this connect to careers?

Business development managers find new leads, markets, and collaborations to expand businesses. They develop an organization's approach to gaining new customers through building relationships and analyzing potential competition. They often use tools, such as consumer input, data analytics, and financial and business planning, to develop business opportunities.

Environmental scientists use DNA identification technology to examine environmental DNA (eDNA) samples to monitor populations and identify pollution.

Food scientists use DNA identification technology to track potential disease outbreaks and assure the safety of the food supply.

How does this connect to our world?

DNA identification technology is used to address many questions related to human health and safety, environmental issues, and human interests. This lesson introduces students to examples of how diverse communities have used this technology to answer questions and solve problems.

Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

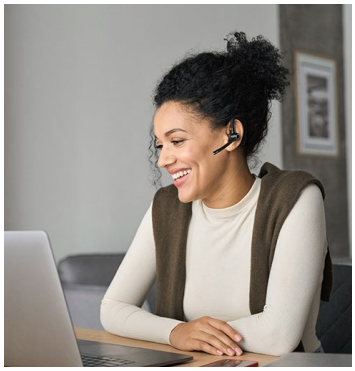
Identify the communities in which they belong.

Explain the role of the community in community science.

Teacher Note > *Before beginning this lesson, select a community science initiative in which your students can participate within the available time frame. Ideally, the community science initiative would focus on DNA technology, but any community science initiative that is engaging and accessible could be used. Alternatively, you could provide students with a list of possible options for projects and have students select one in which to participate. Depending on the project selected, students may need more than 30 minutes to complete data collection and submit their results. You could also include time for students to analyze some of the data produced by the initiative. Below are a few resources that can be used to identify and select a community science initiative.*

- *CALeDNA in California*
- *Phylo DNA Puzzle*
- *SciStarter*
- *CitizenScience.gov*
- *Zooniverse.org*
- *iNaturalist—A Community for Naturalists*

Teacher Note > *This would be a great opportunity to bring in a local scientist from a university or museum to provide a relevant local connection. In addition, there are several different opportunities to bring scientists into your classroom via virtual format (e.g., Skype a Scientist). If this is not possible, follow the lesson below to engage students.*



Individual Work (10 minutes)

Start the day by passing out the *Communities Journal, Part 1*, in which students answer the following questions in a Think-Pair-Share format:

- To what communities do you belong?
- What values and issues are important to those communities?
- Whether drawing from a previous unit in this class or from your own personal experience, what is a community struggle with which you identify?
- What are some connections between what you have learned about biotechnology and the communities to which you belong?

Teacher Note > *Students will refer to their answers as a part of PD Lesson 1.*

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Day 1

Continued

Procedure

Whole Group (30 minutes or more, depending on project selected)	
1	Students participate in an existing community science initiative following the guidelines for that project.
2	Engage students in a short Wrap-up discussion and save notes on the board, or collect a quick Exit Ticket.
	a. How are communities and science related?
	b. What does the term "community science" mean to you?
	c. Why do you think the project we engaged in used community science to collect data? What were the benefits and drawbacks of using community science?

Day 2

Procedure

LEARNING OUTCOMES

Students will be able to:

Analyze how DNA identification technology has been used to solve problems in different communities.

INDUSTRY AND CAREER CONNECTION

This is a good time to call out to students two careers that play roles similar to ones they'll be acting in during this lesson: environmental and food scientists. Environmental scientists use DNA identification technology to examine environmental DNA (eDNA) samples to monitor populations and identify pollution. Food scientists use DNA identification technology to track potential disease outbreaks and assure the safety of the food supply.

Whole Group (10 minutes)

1. Introduce the driving question for this lesson: How can individuals and communities use the power of DNA identification tools to solve local problems?

2. Revisit student responses from the Exit Ticket/Wrap-Up discussion from Day 1. Emphasize the benefits and drawbacks of community science and how students would define community science.

3. Project *Community Science Example: Young Sleuths Target Sushi* as an example of community science to pique student interest.
   - **a.** How does this example connect to a local community? What communities are involved?
   - **b.** How is this an example of community science?

4. Show students the three major topic areas that will be used throughout this unit:
   - **a.** Environmental Issues
   - **b.** Food and Safety Issues
   - **c.** Human Interest (Direct to Consumer) Issues

5. Ask students to share some of the community issues they wrote about yesterday. Into which topic area would those issues fit.

Individual Work (35 minutes)

1. Assign students to one of the three topic areas previously mentioned, or have them select the one in which they are most interested.

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Day 2

Continued

Procedure

2	Students will use the *Community Science Resource Packet* to gain an understanding of how DNA identification technology has been used. This packet of materials has both video and article resources in the three topic areas listed above. Students should *not* read or watch all of these—they should select (or be assigned to) specific resources to gain a global understanding of the breadth of problems that can be addressed with DNA identification tools within the topic area they selected. Invite students to complete Part 1 of the *Community Science Topic Overview Capture Sheet* to summarize their findings.
3	Exit Ticket: What was the most interesting example you learned about today and why?

Teacher Note > *If time allows, have students share their Exit Ticket responses with a partner.*

Days 3–4

Procedure

LEARNING OUTCOMES

Students will be able to:

Analyze how DNA identification technology has been used to solve problems in different communities.

Small Group (20 minutes)

Place students into small groups of three who explored the same topic area in the previous day's lesson. Students engage in a discussion about what they learned using the reflection questions on Part 2 of the *Community Science Topic Overview Capture Sheet*. Each group creates a Community Science Topic Summary Board to share the big ideas of their exploration with others.

Teacher Note > *A Community Science Topic Summary Board Template is provided, but you may also choose to create a topic summary board using chart paper or another digital platform of your choice.*

Small Group (20 minutes)

1	Create Jigsaw groups with at least one student from each topic area. Jigsaw groups share information from student topic summary boards.
2	After viewing each summary board, have students journal in a notebook or on paper as determined by the teacher about "opportunities and obstacles" for each topic. This will be useful when they complete the *Final Topic Interest Survey* during Day 5.

Whole Group (10 minutes)

Lead a short discussion to draw connections between the issues students identified in their communities and the ideas they just explored.

- What examples did you learn about that connect to the issues you identified?
- How could each topic area be used as a community science initiative for our communities?

Day 5

Procedure

LEARNING OUTCOMES

Students will be able to:

Identify an area of interest in which DNA identification technology can be used to solve problems.

INDUSTRY AND CAREER CONNECTION

In this activity, students are playing the role of a Business Development Manager who develops an organization's approach to gaining new customers through building relationships and analyzing potential competition. They often use tools such as consumer input, data analytics, and financial and business planning to develop business opportunities.

Whole Group (30 minutes)	
1	Introduce students to the final learning artifact for this unit: a funding proposal for a community science initiative in one of their own communities.
2	Show students the example of the DNA analysis of coyote attacks, shown on the *Learning Artifact Graphic Organizer: Coyote Example*. Using the Think-Pair-Share routine for each box in the graphic organizer, students individually reflect on the prompt, discuss their ideas with a partner, and then share their thoughts with the class.
3	After the Think-Pair-Share round for each prompt, record student responses on the *Learning Artifact Graphic Organizer: Coyote Example*. Display the completed graphic organizers in the room for reference throughout the unit.

Teacher Note > *Use the Learning Artifact Graphic Organizer as an optional display on butcher paper throughout the unit.*

Individual Work (15 minutes)	
1	Each student completes the *Final Topic Interest Survey*.
2	Use the results of the survey to create the student groups that will work together for the remainder of the unit on the learning artifact.

National Standards

Next Generation Science Standards	**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** Asking questions and defining problems Evaluate a question to determine if it is testable and relevant Obtaining, evaluating, and communicating information Critically read scientific literature adapted for classroom use to determine the central ideas or conclusions and/or to obtain scientific and/or technical information to summarize complex evidence, concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.
Career and Technical Education (CTE)	**A1.0** 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. **A5.1** Use the Internet and World Wide Web to collect and share scientific information. **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. **7.2** Explain the importance of accountability and responsibility in fulfilling personal, community, and workplace roles.

Learning Artifact Graphic Organizer Example

ANSWER KEY **Do not share with students**

Directions
Read the article, then reflect on each prompt and discuss your ideas with a partner.

Example	*Coyote attacking several San Francisco residents*
Article	*Search For Coyote Continues After Several Attacks In The San Francisco Bay Area*
What communities were involved?	San Francisco residents (East Bay communities of Moraga and Lafayette) Project Coyote - non-profit involved in wildlife conservation US Fish and Wildlife Service
What was the problem they were trying to solve?	Several different individuals in the community have reported being attacked by a coyote since July. Witness account says that the coyote does not seem scared of humans.
How did they use DNA to solve this problem?	They collected DNA samples from the bite wounds and clothing of victims. DNA was analyzed and found to all be from a single coyote.
How did they get community members involved?	Local news and police collected information on initial attacks. News coverage encouraged people to be alert to coyote attacks and shared information on who to contact if attacked. Conservation groups educated people about what to do in the case of a coyote encounter. Local police and US Fish and Wildlife Service are working on trapping the individual coyote involved. Community members circulated photos of the coyote for recognition.

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Communities Journal, Part 1

Directions
Answer the following questions, then share your responses with a partner. Responses may vary.

1. To what communities do you belong?

2. What values and issues are important to those communities?

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Communities Journal, Part 1

Continued

3. Whether drawing from a previous unit in this class or from your own personal experience, what is a community struggle with which you identify?

4. What are some connections between what you have learned about biotechnology and the communities to which you belong?

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Communities Journal, Part 2: Proposal Development

Directions
Answer the following questions, then share your responses with a partner. Responses may vary.

1. Which topic area did you choose (environment, food and safety, human interest)?

2. How does this topic fit into one or more of the communities to which you belong?

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Communities Journal, Part 2: Proposal Development

Continued

3. What concerns do people in those communities have related to that topic?

4. What problems exist that need to be solved?

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Community Science Example: Young Sleuths Target Sushi

Directions

Read this example of a community science project, and then participate in a class discussion about a community using science to solve a problem.

Source: *Young Sleuths' Last Target: Sushi. This Time: Tea. New York Times*

A recent study from *Scientific Reports*, a Nature Journal, found that a significant percentage of herbal teas contained ingredients not listed on the manufacturer's label. The researchers were three students (one freshman and two seniors) from Trinity School, a private school in Manhattan. These three students used DNA barcoding, a type of DNA sequencing, to test 70 tea products and 60 herbal products. Four percent of the 70 tea products they tested and 35 percent of the herbal products had unlisted ingredients, such as white goosefoot, Taiwanese cheesewood, and chamomile. The students teamed up with scientists at Rockefeller University to perform their DNA sequencing.

In 2008, a group of seniors from Trinity investigated sushi to see whether or not the sushi they sampled was mislabeled. Their findings were published in the New York Times, raising public concern and prompting an outcry. The findings from their DNA sequencing in tea were not as striking as their DNA sequencing for sushi, which revealed that Mozambique tilapia was mislabeled as white tuna. Both of these studies raise consumer concern, especially allergy concerns. Whether the manufacturers knowingly mislabeled ingredients in their product remains unclear, but the researchers speculated that the mislabeled ingredients may come from harvesting and processing raw plant material and cross-contamination. To protect the identities of well known brands and avoid any legal implications, the researchers declined to name any of the brands.

The researchers also found previously undocumented genetic differences in Indian and Chinese teas. The students conducted the research with $5,000 of equipment bought online. The students extracted and amplified DNA with the help of Dr. Mark Stoeckle, an adjunct member of the Rockefeller's Program for the Human Environment. They furthermore collaborated with researchers at the New York Botanical Garden who performed most of their DNA analysis before sending the sequencing data to the students. Students then compared their data to reference sequences compiled in online databases.

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Community Science Resource Packet: Environment

Directions

Use this Resource Packet to gain an understanding of how DNA identification technology has been used in environmental projects. You should not read or watch all of the articles and videos in this packet. Instead, explore a few resources to gain an understanding of the breadth of problems that can be addressed with DNA identification tools.

All the resources listed below are about the Environment. Use the Subtopic and Title to identify a few you would like to explore. Videos and Articles are linked. Here, each Article Title links to a Summary, and a link to the full text is provided on that page if you prefer to read it in its entirety.

In this section, environmental DNA is abbreviated eDNA.

Videos

Subtopic	Title and Link
Tracking Wildlife	*Tracking Snow*
Measuring Biodiversity	*Dam Biodiversity! Citizen Scientists use eDNA to Detect Wildlife in Farm Dams*
Measuring Biodiversity	*eDNA: How Scientists See Hidden Animals*
Using eDNA	*Working with Environmental DNA (eDNA)*

Articles

Subtopic	Title and Link to Summary
Tracking Wildlife	*No Place to Hide*
Tracking Wildlife	*Researchers Track Sharks and Whales Using DNA in Seawater Samples*
Tracking Wildlife	*Researchers Detect Land Animals Using DNA in Nearby Water Bodies*
Monitoring Pollinators	*DNA Traces on Wild Flowers Reveal Insect Visitors*
Tracking Wildlife	*Recreating Fish Migration Written through Environmental Genomics*
Tracking Wildlife	*How DNA from Snow Helps Scientists Track Elusive Animals*
Tracking Wildlife	*Rare Carnivore Detections from Environmental DNA in Snow*

Articles

Subtopic	Title and Link to Summary
Using eDNA	*National Genomics Center for Wildlife and Fish Conservation*
Tracking Wildlife	*The Rangewide Bull Trout eDNA Project*
Tracking Wildlife	*Asian Carp Early Detection*
Tracking Wildlife	*Tracking the Elusive Burmese Python with DNA Clues in the Dirt*
Tracking Wildlife	*Using Environmental DNA for Burmese Python Detection Probabilities and Range-Delimitation in Southern Florida*
Using eDNA	*Black and White and Shed All Over: How eDNA Analysis Can Help to Answer Your Species Questions*
Measuring Biodiversity	*Environmental DNA: An Emerging Tool for Understanding Aquatic Biodiversity*
Measuring Biodiversity	*Researchers Sequence DNA in Sewage Samples*
Monitoring Pollution	*New DNA Sequencing Technology Aids Fecal Pollution Management*
Monitoring Pollution	*Microbial Source Tracking: How Did that Get in There?*
Monitoring Pollution	*Decoding the DNA of Wastewater*
Tracking Wildlife	*Tracking Fish with eDNA*
Using eDNA	*1000 Rivers Fish eDNA Project*

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Community Science Resource Packet: Environment

Continued

Subtopic	Tracking Wildlife
Title	No Place to Hide
Full Text	*Claire Asher, The Scientist, May 2017*

Asian carp are invading rivers in the United States, wreaking havoc as they outcompete native species. Asian carp are plankton-eaters that devour as much as 20 percent of their bodyweight daily. They additionally represent as much as 97 percent of the total biomass in the Illinois and Mississippi rivers and are likely to soon begin populating the Great Lakes. The Great Lakes hold 21 percent of the world's surface fresh water and are home to highly productive fisheries and popular recreation areas. If Asian carp are introduced into this region, they could disrupt a $7 billion dollar fishing industry.

Carp belong to the *Cyprinidae* family and were introduced to the United States in the 1970s. Since then, invasive carp have spread to more than 20 states. Molecular methods have been used to track these fish in an attempt to slow their invasion. These methods include collection of environmental DNA (eDNA), which can consist of anything from skin cells to fecal matter, to offer scientists a snapshot of which types of organisms have recently passed through a body of water.

Surveying eDNA has allowed researchers to detect early signs of carp invasion in Lake Michigan. In a study conducted by Christopher Jerde and colleagues at the University of Notre Dame, they extracted eDNA from more than 2,800 two-liter water samples collected across the Great Lakes Basin. Using mitochondrial DNA with markers specific to carp, they were able to detect carp in the Chicago Area Water Way System, the only continuous connection between Lake Michigan and the Mississippi River basin, in an effort to contain their invasion. eDNA has been shown to be highly location-specific with prior data showing that DNA from introduced bass were undetectable 50 meters downstream from where they introduced it. In conjunction with traditional surveys, the US Fish and Wildlife Service surveys the Great Lakes annually for Asian carp and their tributaries.

While Asian Carp are undoubtedly wreaking havoc within native ecologies, they are not the fastest-growing threat facing our waterways. The single-celled algae *Didymosphenia geminata*, also referred to as Rock Snot, has been causing algal blooms in many river and stream systems in the Northeastern United States. Scientists have used eDNA to track the algal distribution in Maryland and Pennsylvania. Sequencing eDNA revealed similarities between the US population of *Didymo* algae and published sequences from elsewhere in the world, indicating that recent blooms are being caused by invading non-native strains. Building off these findings, states such as Maryland have taken simple measures to slow the spread of *Didymo* to new sites, including restricting the use of felt-soled waders by fishers.

eDNA can also be used to track elusive species that are otherwise inaccessible to scientists, including the only cave-dwelling salamander in Europe, *Proteus anguinus*, whose habitat primarily consists of flooded underground caves. eDNA was used by Judit Voros of the Hungarian Natural History Museum to identify the presence of this Salamander species in five new locations, as well as confirm their habitation in ten known cave systems.

These eDNA tracking methods have also enabled scientists to keep tabs on the endangered Yangtze Finless Porpoise, a species whose population is declining due to the construction of dams, high boat traffic, and habitat destruction. Their population is estimated to be around 1,040 in the wild, and they are difficult to locate due to the Yangtze's murky water.

eDNA is a very cost-effective method of studying rare and difficult-to-track species as it provides the opportunity to sequence all DNA in a provided sample. These findings can then be compared with public databases, which can be used to identify entire global communities. The next steps will be developing large-scale environmental DNA monitoring schemes that can track ecological communities through space and time that were previously unimaginable. eDNA technology will one day allow scientists to automatically determine species abundance and population structure, and to reconstruct evolutionary history.

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Community Science Resource Packet: Environment

Continued

Subtopic	Tracking Wildlife
Title	Researchers Track Sharks and Whales Using DNA in Seawater Samples
Full Text	*Jef Akst, The Scientist, January 2019*

Marine ecologist Kevin Lafferty from the University of California Santa Barbara found dozens of dying black abalone (*Haliotis cracherodii*) in his open-water enclosures. He was trying to test whether this critically endangered species was resistant to Rickettsiales-like prokaryote (RLP), a bacterium that nearly wiped out the black abalone along the southern Pacific coast. To identify where the pathogen was coming from, he sampled water near the outflow pipe of a farm growing red abalone, another species affected by the disease, and found samples containing the bacterium's DNA. He found that red abalone can survive RLP infection, likely due to an adaptation or to a bacteriophage living in their bodies. Using what he learned from sampling environmental DNA (eDNA) in ocean water to study marine species, he was able to analyze fragments of mitochondrial and nuclear genomes in the environment.

Geneticist Eske Willerslev and colleagues from the University of Copenhagen were the first to publish findings on the use of eDNA in a marine ecosystem. Their study of water samples collected off the northern coast of Denmark led to the discovery of mitochondrial DNA from four bird species and over a dozen species of fish. Since their discovery, scientists have taken advantage of developments in sequencing technology to characterize genes, aid species identification, and survey biodiversity. Lafferty collected seawater off the shore of Santa Barbara in an effort to test and sample mitochondrial DNA from great white sharks (Carcharodon carcharias). They further developed species-specific primers to test for great white shark mitochondrial DNA. Their initial results validated that there were sharks where they are known to swim. Their data will allow them to develop a heat map for the whole West Coast to show where great white hotspots are based on time and space using water samples. Coupling eDNA studies with models of ocean current speed and direction could improve predictions of where the DNA comes from.

As the study of eDNA continues to expand, Lafferty suggests that analyzing shark DNA could help identify individual sharks that have attacked swimmers. Another possible application could allow researchers to assess genetic structures. In 2016, Thomsen and colleagues at the University of Copenhagen sampled water in the Persian Gulf, which is home to seasonal aggregations of whale sharks. Although their analyses could not reveal exactly how many individual sharks contributed to the mitochondrial DNA fragments, they were able to analyze differences in gene sequences to assess genetic diversity.

Kim Parsons, a molecular geneticist at NOAA Fisheries in Seattle, analyzed mitochondrial DNA fragments found in water samples collected from southeastern Alaskan waters. Her studies continue to add new samples in order to provide genetic snapshots of harbor porpoises in southeast Alaska, since it is difficult to obtain cetacean biopsies. As researchers continue to learn how to extrapolate more data from eDNA samples, their ability to study marine populations will improve. For example, scientists would like to be able to identify individuals from a sample of eDNA. Nuclear DNA might provide this ability, although there would need to be a control for the variability in rates of DNA shedding.

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Community Science Resource Packet: Environment

Continued

Subtopic	Tracking Wildlife
Title	Researchers Detect Land Animals Using DNA in Nearby Water Bodies
Full Text	*Nayanah Siva, The Scientist, April 2020*

Environmental DNA (eDNA) testing has become a powerful tool to unravel mysteries about endangered and elusive aquatic species. This technique looks for traces of DNA shed by fish and other organisms in water bodies. Scientists have adapted this technique to search for the presence of land-dwelling creatures. Scientists who sampled eDNA from streams and rivers in the UK, the Amazon, and the Atlantic Forest in Brazil, found evidence of 20 wild animals including red deer, mountain hares, pine martens, red foxes, and badgers. They also found evidence of endangered animals, such as water voles. These findings add to recent work by scientists who are using eDNA testing to identify mammals, insects, and birds that live on land.

Many groups are seeking to improve the methodology of analyzing and sampling land animal eDNA by detecting low concentrations of DNA in samples. A group in Australia was able to detect endangered Gouldian finch (*Erythrura gouldiae*) from eDNA traces in waterholes. In Japan, scientists were able to identify the Japanese marten (*Martes melampus*), red fox (*Vulpes vulpes*), and wolf (*Canis lupus familiaris*). Another study in Montana found evidence of snow lynx among other animals from collecting eDNA from snow tracks. Researchers in Denmark used eDNA collected from flowers to identify terrestrial arthropods that have visited the plants, including ladybirds, spiders, butterflies, and beetles.

In 2017, Allan McDevitt, a molecular ecologist at the University of Salford in the United Kingdom, realized that he could also use eDNA found in rivers to gather information on mammals that live on land. Whenever these animals interacted with these waterways (drinking from them, crossing them, or expelling waste into them), they would leave behind data in the form of eDNA that could be used by McDevitt to detect their presence in the surrounding area. In addition to learning about living populations, scientists can also collect eDNA from animals that died thousands of years ago thanks to DNA's tendency to bind to sediment in water. They used polymerase chain reaction (PCR) with mammal-specific primers to amplify sections of mitochondrial DNA. They then used DNA barcoding to identify taxa. An aim of this study is to compare the efficiency of eDNA testing in semi-aquatic or terrestrial mammals with traditional ecological techniques, such historical records, cameras, animal tracks, and fecal samples. The eDNA results aligned with their expectations for surveys of certain animals, which was then confirmed with traditional methods. In some cases, they found unexpected results. In one instance, they found water vole DNA in an area where the species had not been documented before. As such, they used cameras to confirm the presence of these animals and accuracy of eDNA testing.

After initial studies in the United Kingdom, the eDNA methodology was tested in the largest tropical rainforest, the Amazon, which comprises at least 10 percent of Earth's biodiversity. The researchers were able to detect many species, such as river dolphins, anteaters, and tapirs, but were not able to detect as many species as they anticipated. They believe this may be caused by water samples with low pH, which could degrade DNA faster than other conditions. In other instances, they were only able to detect the genus, but not the species of their samples. This is largely due to the reference DNA database, which may be limited in data and can be improved only through accumulating more data. The next stage of the field will be building reference libraries of these animals in biodiverse areas, which will only expand the applications and findings from eDNA technology.

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Community Science Resource Packet: Environment

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Subtopic	Monitoring Pollinators
Title	DNA Traces on Wild Flowers Reveal Insect Visitors
Full Text	*Aarhus University, Denmark, Science Daily, February 2019*

Environmental DNA (eDNA) can create a snapshot of all species inhabiting a particular ecosystem by providing DNA sequences in water and soil samples. Water samples pulled from oceans and lakes have revealed DNA traces from insects, amphibians, fish, and large aquatic mammals. Flower-rich grasslands like meadows, on the other hand, can contain eDNA from the hundreds of insect species that pollinate those flowers. Philip Francis Thomsen, an associate professor from Aarhus University, has conducted eDNA analyses of 50 flowers from seven different plant species, and his research revealed that some flowers had been visited by at least 135 different species of butterflies, moths, bees, flies, beetles, aphids, plant bugs, and spiders. The flowers therefore function as passive DNA collectors that store data about each flower-visiting insect. These findings open up new possibilities of studying interactions between plants and insects. This knowledge can be used in many research applications, including pest control. This method also has implications in the management of endangered species, such as wild pollinators, which is increasingly more important as many flower-visiting insects are becoming threatened. Populations of several wild bees and butterflies have decreased significantly in recent decades with many species becoming locally extinct. Thus, eDNA technology serves as a valuable tool to survey pollinators and better understand insect-flower interactions.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Recreating Fish Migration Written Through Environmental Genomics
Full Text	*Aggie Mika, The Scientist, July 2017*

Mark Stoeckle, a biologist from Rockefeller University, and his colleagues have used environmental DNA (eDNA) to investigate different forms of marine life inhabiting the lower Hudson and East Rivers. Free-floating DNA shed by fish and other organisms is known as environmental DNA (eDNA) and can be analyzed with a reference library of known genetic sequences. This can give scientists a comprehensive picture of what organisms presently inhabit a given environment.

Stoeckle and his team gathered one-liter samples of water from the Hudson Estuary and East River once every week for six months, and sequenced their samples for 18 species of fish.They amplified a specific region of ribosomal RNA in mitochondria that serves as a useful molecular signature distinct to each species. Beginning in early April, they detected an increase in eDNA for many fish, including Atlantic menhaden, tautog, and cunner, among others. Compared to traditional techniques, eDNA is less expensive and intrusive to the environment, and so can provide a noninvasive method of ecological assessment for aquatic biologists from a variety of disciplines.

Other researchers are also using eDNA to answer questions. Elizabeth Alter, a geneticist at the City University of New York, found that rocky habitats tend to give off strong DNA signals and can be used to detect hidden organisms. Jesse Ausubel from the Program for the Human Environment at Rockefeller University is a proponent of eDNA for tracking ecological dynamics as it can effectively track migrating fish. Jennifer Miksis-Olds, a biological oceanographer at the University of New Hampshire, used Stoeckle's eDNA findings and paired them with acoustic detection techniques to improve her study of the ecosystem.

The rise of eDNA research has been facilitated by an ever-expanding portfolio of reference genomes. The construction of reference libraries containing the genetic sequences for different species of fish and other aquatic organisms will improve eDNA analyses and will become more telling as the amount of data increases. eDNA is still a relatively new strategy and standardization is important to prevent cross-contamination and to allow for data comparisons between groups.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	How DNA from Snow Helps Scientists Track Elusive Animals
Full Text	*Annie Roth, National Geographic, November 2018*

Canadian lynx are scarcely seen, and little is known about their distribution. This lack of information has hindered efforts to conserve this animal, which is listed as threatened under the Endangered Species Act. Canadian Lynx are listed as threatened under the Endangered Species Act. Scientists have therefore relied on collection of environmental DNA (eDNA) left in the tracks these creatures have left in the snow, and were able to confirm the presence of lynx in the Northern Rockies using this method. eDNA collection will significantly improve wildlife survey accuracies in snowy environments in the future, along with aiding conservationists in identifying critical habitats for these threatened or endangered species.

Canadian lynx are found across northern North America. Tracking lynx has been possible by following snow tracks and setting up cameras in remote boreal forests in Montana and Idaho for years in hopes of detecting them. tUntil the prevalence of eDNA analysis, the only way scientists were able to confirm the presence of a lynx was by searching a habitat for scat (feces) shortly after a snowfall. Now, however, scientists are no longer limited to this; the lynx, like all other organisms, is constantly shedding genetic material in the form of hair, skin cells, and reproductive material, all of which can be used to collect genetic information. These sources of eDNA can then be compared against a database of known DNA sequences to identify the species from which it originated. According to Justine Smith, an ecologist and post-doctoral researcher at University of California, Berkeley, “environmental DNA provides unprecedented opportunities to get information on species distribution, abundance, and diet. It increases the geographic area that can be sampled and can improve accuracy in sampling assessments.” eDNA research is so successful that the team studying the Canadian Lynx is now able to isolate DNA concentrations as low as five cells per liter of snow. It is also ten times as cost-effective, and ten times faster than comparable methods of sampling.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Rare Carnivore Detections from Environmental DNA in Snow
Full Text	*United States Forest Service Research and Development (USDA), 2019*

Managing rare species is a conservation priority, but difficulties tracking rare animals and detection errors make population surveys a limited tool for researchers to use. False positives (misidentification) and false negatives (missed detection) are prevalent in surveys for rare species and can lead to incorrect inferences about their population status and distribution. Environmental DNA (eDNA) shed from organisms in their environment coupled with molecular biology techniques, such as quantitative polymerase chain reaction (qPCR) analyses, have become reliable methods for surveying rare species in terrestrial settings. The USDA Forest Service has used eDNA testing for three rare forest carnivores: Canada lynx (*Lynx canadensis*), fisher (*Pekania pennanti*), and wolverine (*Gulo gulo*). Researchers investigated the effectiveness of this method using snow samples collected within tracks in locations where animals had been photographed months earlier, and identified species from old hair samples collected during the summer prior. They found that their species-specific assays could effectively detect DNA of all three species from the snow-track assays, snow collected at camera stations, and overwintering samples that could not be identified with conventional lab techniques. The eDNA collected provided sufficient quantities of DNA for robust species detection. They assert that qPCR used to detect DNA has the potential to revolutionize winter surveys for rare species in terrestrial settings with great accuracy or in areas where winter access is not feasible. This cost-effective method can revolutionize winter rare carnivore surveys by providing highly specific and sensitive results.

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Community Science Resource Packet: Environment

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Subtopic	Using eDNA
Title	National Genomics Center for Wildlife and Fish Conservation
Full Text	*Rocky Mountain Research Station, United States Forest Service, 2022*

Tracking and surveying invasive or endangered species is an important job for conservationists, but can be difficult using traditional species monitoring, which has relied on physical observations in the field. These tasks can be labor-intensive, particularly in remote areas or when searching for rare organisms. The powerful approach of environmental DNA (eDNA) by water sampling to determine the presence of species has revolutionized wildlife and fish monitoring. Organisms continually shed cells containing their DNA into their surroundings. For example, DNA in skin cells shed from fish can be found in water and DNA in plant pollen can be found in the air. DNA is a robust molecule, which allows it to be collected in air, soil, and water samples and analyzed for the presence of species. Using computational and molecular techniques, such as quantitative polymerase chain reaction (qPCR) analyses, scientists can detect DNA from just a few cells in an environmental sample. It can reveal information about species presence more quickly and cost-effectively than traditional sampling techniques. It is particularly useful for detecting organisms in low abundance, such as threatened, endangered, and even invasive species. Scientists have used water samples to detect the spread of invasive feral pigs in the Southwestern United States and Burmese pythons in Florida, as well as the distribution of bull trout, invasive brook trout, Canada lynx, and wolverine. It is also an effective technique for aquatic and semi-aquatic animals, such as mollusks, and river otters. eDNA can be used to simply detect the presence or absence of a specific species, or it can be combined with high-throughput quantitative PCR (qPCR) to understand community diversity.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	The Rangewide Bull Trout eDNA Project
Full Text	*Rocky Mountain Research Station, United States Forest Service, 2022*

The bull trout is an endangered species found in waters across the northwestern United States. Bull trout have declined in many regions because of climate change, invasive species, and habitat degradation. Informed conservation planning relies on detailed information about the bull trout distribution in thousands of streams, but gathering this information is a timely and expensive task. To overcome this issue, scientists used predictions from a range-wide, spatial model of the location of natal (nursery) habitats, and water sampling using environmental DNA (eDNA) technology to detect their presence. According to the National Genomics Center for Wildlife and Fish Conservation, eDNA sampling is a reliable method of detecting bull trout populations, as well as the non-spawning habitats of both juvenile and adult fish. More importantly, these sampling methods are consistent and repeatable across wide ranges of this species' habitat.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Asian Carp Early Detection
Full Text	*Great Lakes Restoration Initiative, United States Geological Survey (USGS), October 2017*

Since 2010, control and prevention efforts have increased in the Great Lakes, Upper Mississippi River, and Ohio River basins to combat the invasive Asian carp. United States Geological Survey (USGS) scientists are using environmental DNA (eDNA) testing as an early detection method to enhance the ability of agencies to manage Asian carp and minimize their spread. Asian carp are especially difficult to detect because they are hard to catch when population numbers are small. Early detection is critical to initiate rapid or early response efforts to block population establishment.

The USGS is attempting to develop new methods to identify Asian carp populations while they are still small, such as loop-mediated isothermal amplification [LAMP], digital PCR [dPCR], quantitative PCR [qPCR], and high-throughput sequencing [HTS]. LAMP, dPCR, and qPCR are processes that generate multiple copies of a target DNA sequence found in the environmental sample, while HTS is a rapid method of determining the sequence of bases in a DNA molecule. eDNA has other potential applications also, including detection of spawning events, fish movement, and habitat utilization. Thanks to advancements in eDNA methodology, scientists have improved detection sensitivity, enhanced cost-effectiveness, decreased the number of false negatives, and minimized the time between the initial sampling and obtaining results.used to help recognize data gaps and inform future studies.

Additionally, scientists are looking to create a new generation of sequencing technology which rapidly and simultaneously sequences millions of individual DNA or RNA strands within a single sample. The US Fish and Wildlife Service is looking to use this technology to gather information on Asian Carp populations in the Great Lakes and Mississippi and Ohio Rivers, hoping to learn more about how this invasive species interacts with the environment.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Tracking the elusive Burmese python with DNA clues in the dirt
Full Text	*Erica Tennenhouse, Science, March 2018*

The Burmese python, a snake endemic to southern Florida, is historically very elusive and therefore difficult to study. However, scientists are starting to use environmental DNA (eDNA) found in soil samples in addition to waterborne samples in order to better study these animals. In lab settings, researchers determined that DNA could be gathered from soil samples within snake enclosures up to seven days following the snake's removal. Because of this, researchers have begun sampling soil along the Gulf Coast of Florida with the expressed intent of studying the invasive python species. This has led to improved methods of tracking Burmese pythons in and around their burrows, thus increasing scientists' ability to protect native species by controlling the population of these invasive snakes.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Using Environmental DNA for Burmese Python Detection Probabilities and Range-Delimitation in Southern Florida
Full Text	*Wetland and Aquatic Research Center, United States Geological Survey (USGS), March 2016*

Environmental DNA (eDNA) can be used to detect and determine range limits for invasive species, such as the Burmese python, which can assist researchers in managing and controlling their populations. Invasive species are easiest to control and eradicate while their population densities are low, and detecting the existence of a small population can be difficult when their habitats are difficult to access by humans. Current methods of detecting and controlling Burmese python populations yield low detection rates and have used less technical tools, such as trapping or visually searching for snakes. eDNA, conversely, has been shown to be quite effective at detecting invasive species where other methods have failed.

eDNA, which is essentially genetic material shed by living organisms in the form of skin or hair cells, fecal matter, and reproductive material, can be collected in water samples and used to identify a species. These eDNA identification methods have been found to be much more cost-effective and less time-consuming than the detection methods previously used for constrictor snakes. However, to truly understand the range limits and accurately predict occurrences of Burmese python populations, comprehensive studies must be conducted all along the northern limit of their habitat range (and invaded habitats). A droplet PCR platform (ddPCR) will be used to detect a single molecule of DNA from an environmental sample, which significantly enhances accuracy and precision compared to traditional eDNA detection methods. To detect individual species, three species-specific markers (two primers and a fluorescently labeled probe) are developed and added to the samples. Filtered surface water samples are then split into 20,000 PCR droplets, each containing the markers and, if present, a copy of the target species' DNA. If the DNA of a specific species is detected, the sample fluoresces, with more fluorescence corresponding to a larger number of detected DNA molecules in a sample.

eDNA analysis will ultimately improve management actions, making inferences about distribution and movement patterns of pythons, and developing long-term management strategies. Analysis of eDNA samples can aid researchers in inferring distribution and movement patterns of Burmese pythons, as well as assist in the creation of strategies to manage their populations. eDNA can also potentially be used as an early detection tool for giant constrictor snakes in previously unknown locations, providing an early warning system to ecologists that their populations may become invasive in the near future and aiding in the decision making process to control this population spread before it becomes unmanageable. More precise information on the presence of these snakes can inform assessment of risk to native species and potentially allow for targeted removal efforts prior to major ecological and economic impacts. eDNA testing can also help determine the effectiveness of short- or long-term control and eradication efforts.

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Community Science Resource Packet: Environment

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Subtopic	Using eDNA
Title	Black and White and Shed All Over: How eDNA Analysis Can Help to Answer Your Species Questions
Full Text	*Rocky Mountain Research Station, United States Department of Agriculture (USDA), May/June 2020*

Surveying invasive or endangered species in the landscape is an important job for conservation managers. Traditional species monitoring has relied on physical observations of organisms in the field, which often requires expertise in species identification. Surveying can be labor-intensive, particularly in remote areas or for organisms that are rare. Environmental DNA (eDNA) helps navigate some of these issues by relying on computational and molecular biology techniques to provide important information about species presence or absence. This technology has been used primarily to survey fish and amphibians, however this technique can be expanded to other aquatic taxa as well as semi-aquatic and terrestrial mammals.

eDNA study can be used in two different ways: a "targeted" approach that is attempting to isolate DNA from one species in particular, and a "nontargeted" approach which gathers data from many different types of species in a given sample.

The main shortcoming of nontargeted approaches, such as "metabarcoding" and "capture enrichment," is that they are not as sensitive for detection of each individual species. Scientists commonly use quantitative PCR (qPCR) for targeted analyses to answer questions about invasive species populations. Due to the shortcomings in both the targeted and nontargeted approaches, researchers are working to create a third eDNA study option that somewhat bridges the gap between the two: High throughput qPCR. A novel application of this technique is using the DNA left by an animal's foot in a snow track to identify the presence and spatial patterning of a species. In general, eDNA sampling is used solely to establish presence or absence of a species. eDNA testing can be useful for projects surveying endangered

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species, such as bull trout. Bull trout are a threatened species with a historical range that encompasses many waters across the northwestern United States. Bull trout have declined in many locations from habitat degradation, population isolation, invasive species, and climate change. Federal mandates require reliable and precise information regarding bull trout distribution in thousands of waterways. However, due to the difficulty in collecting bull trout, these surveys are costly. To aid in this effort, eDNA information is being compiled into a reference library called the eDNAtlas. The eDNAtlas supplements other traditional sources of bull trout sampling so that biologists have the best available information on where bull trout reside for project-level planning and Endangered Species Act consultation. Bull trout occupancy models that include eDNA sampling information provide managers with broad strategic insight on where they might get the greatest impact for various types of bull trout conservation efforts.

eDNA testing has also been used to confirm invasive species eradication of brook trout. Native to eastern North America, brook trout were introduced to the western United States in the mid-1800s and spread throughout the area. The introduction of these species negatively impacted native western species, such as cutthroat and bull trout. eDNA analysis has been an invaluable resource in the restoration of these species. Typically, a chemical removal is the method used to remove invasive species of trout where no native species remain. Tools such as eDNA can efficiently remove any guesswork about where the target species is. This can help managers more efficiently mark the area that needs treatmentat the beginning of the project, which will reduce the overall treatment area and project costs. Typically, it takes more than one chemical treatment to eradicate brook trout, causing managers to treat the entire system two or three times. eDNA is so sensitive that it can allow researchers to identify the areas where the target invasive species persist so they can reduce the treatment area for subsequent treatments. eDNA can then help verify the project's success by a lack of detection of the target species.

eDNA has also been used to track invasions of Northern Pike in collaboration with the Confederated Tribes of the Colville Reservation in Washington. This invasive species is known to spread upstream of the Grand Coulee Dam on the Columbia River. If these fish expand downstream of the dam, they will be in waters with populations of threatened and endangered salmon. Above the dam, managers can set gill nets, a lethal form of sampling, so that fish can be caught and eliminated at the same time. Yet, this technique is difficult because of the risk of inadvertently killing endangered species. The Confederated Tribes of the Colville Reservation have been using eDNA in conjunction with conventional sampling, such as gill netting, to locate areas where pike may have spread and monitor areas where they have not yet arrived. They have been using the results from eDNA to help inform and create policy and funding to reduce the spread of pike.

Although the majority of eDNA sampling occurs in fish, new applications have been developed for rare or recovering mammals, water birds, and amphibians. Other groups have designed similar eDNA tools to survey water bodies for invasive wildlife, such as feral swine and pythons. Many groups are working to establish a standard approach to sample all life in a stream simultaneously using nontargeted approaches. The problem is that 'metagenomics'—studying the genetic material from a community of organisms—is not as sensitive and there are both false positives and false negatives, making comprehensive analysis difficult. As community-level methods are being refined, there is room for improvement for more common, targeted eDNA approaches to further our ecological understanding beyond detection of invasive species. As eDNA technology continues to develop, we can use this data to gain better ecological understandings of how species interact.

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Community Science Resource Packet: Environment

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Subtopic	Measuring Biodiversity
Title	Environmental DNA: An Emerging Tool for Understanding Aquatic Biodiversity
Full Text	*Trey Simmons, National Park Service, Damian Menning and Sandra Talbot, United States Geological Survey, Alaska Park Science 19(1): 34-41, 2020*

Field surveys for aquatic organisms can provide information that is vital to the management of resources. Such surveys can be costly in both labor and funding, especially when the sample areas are large and remote. Characterizing biodiversity in aquatic biomes requires individual organisms to be captured and identified, which often requires experts to be present to identify them. The revolution of environmental DNA (eDNA) brings researchers closer to a more accurate surveying methodology for capturing the genetic composition of an environment. Analysis of eDNA using sophisticated genetic techniques has the ability to identify unseen species from material collected in water samples.

eDNA sources consist of skin cells, hair, reproductive secretions, and feces. On land, eDNA typically is found in soil, but in aquatic ecosystems, eDNA can actually be found in the water itself, in addition to the sediment beneath. Small samples of water are collected and taken to a lab to be analyzed. The quality of the eDNA recovered from a lake or stream depends on the density of organisms; larger numbers tend to generate more eDNA than rare species. Environmental factors play a role

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by affecting the persistence of eDNA over time, influencing the quantity that can be successfully recovered. For example, ultraviolet light rapidly degrades DNA, so eDNA will disappear more quickly in systems subject to sunlight exposure. eDNA tends to degrade faster in warmer temperatures, but other factors such as pH, salinity, and oxygen levels also likely influence degradation rates.

There are two main approaches to eDNA analysis. Most often, eDNA is used to detect the presence of individual species of interest, such as invasive, rare, and endangered species. Quantitative polymerase chain reaction (qPCR) is a powerful way to determine whether eDNA corresponding to a particular target sequence is present. In qPCR, researchers tend to look for short stretches of DNA that are unique to their target organism. Often, mitochondrial DNA is used, as animal cells contain more copies of it than of nuclear DNA. For plants, chloroplast DNA can be used. These unique sequences are targeted in qPCR to make millions of copies of DNA in a process known as amplification. If there is no eDNA in the sample, then no detectable copies will be found.

The other common method is to simultaneously detect many species in the same test from the same environmental sample. This is known as metabarcoding. Metabarcoding involves an initial PCR amplification of mitochondrial DNA, but uses target genes specific to a group of species using group-specific DNA sequences. The amplified products are pooled into a library, which is then compared to a reference library to identify species of known identity. If a genetic sequence gathered from the sample is either an exact match, or lies above a "threshold of similarity" (typically within 98–99 percent), it is recorded as a "hit," meaning that the DNA for that species was present in the sample originally provided.

The National Park Service and the US Geological Survey Alaska Science Center are working together to develop multiple metabarcoding tests in order to identify invasive species and assess existing aquatic communities. The first of these tests was designed to identify the presence of 37 different freshwater species known to be invasive to Alaskan ecosystems. The goal of the research was to allow freshwater fish surveys to be conducted using eDNA collected in water samples rather than rely on traditional surveying methods. They used a set of genetic markers from the mitochondrial 12S ribosomal RNA and cytochrome oxidase I (COI) genes to develop the test. The survey results indicated that this method was able to accurately identify all species already known to be present in each respective ecosystem, a finding which is consistent with other studies which have determined eDNA results to be more sensitive than attempting to capture fish to survey their populations.

A second metabarcoding project was designed to detect the presence of multiple aquatic invasive species. Invasive species are an enormous and growing problem that negatively impact native species, ecosystems, and infrastructure. A cornerstone of effective invasive species management is early detection, however detection of invasive species when they are still rare is extremely challenging using traditional methodology. Ongoing metabarcoding projects focus on native aquatic species including amphibians, birds, and mammals. This technology will provide a powerful tool to rapidly test for the presence of multiple species that are difficult or expensive to detect otherwise.

In short, eDNA is quickly emerging as an effective tool to detect aquatic species. Due to the novelty of these techniques, there is still a great deal of optimization that must be conducted, both in the field and in the laboratory, in order to maximize detectability and minimize false positives and negatives. By further improving the capabilities of this already incredible research tool, we will enhance our comprehension of aquatic ecosystems, and be able to monitor changes in those environments.

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Community Science Resource Packet: Environment

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Subtopic	Monitoring Pollution
Title	Researchers Sequence DNA in Sewage Samples
Full Text	*Sara Jerome, Water Online, April 2015*

Researchers are experimenting with new methods of tracking sewage that is dumped into waterways in order to analyze how much water pollution can be attributed to human fecal matter. Each city's water pollution has a distinct microbial character which can be studied to determine the health of that city's inhabitants. Every city has a distinct microbial character that reveals signs of health about individuals within a community. The research began with an effort to understand bacteria in the human gut, but with a much broader view to analyze the microbiomes of entire human communities. With the assistance of wastewater treatment plants from 71 US cities to collect more than 200 samples of sewage, Mitchell Sogin, a molecular evolutionary biologist at the Marine Biological Laboratory, and his colleagues were able to sequence DNA in the samples and determine their origin. They were able to show that geographically distributed US populations share a small set of bacteria whose members represent various community states within US adults. Cities were differentiated by their sewage bacterial communities and the community structures were good predictors of a city's estimated level of obesity. Their approach demonstrates the use of sewage as a means to sample the fecal microbiota from millions of people and its potential to describe microbial patterns associated with human demographics. Around 15 percent of the sewage DNA belongs to microbes from humans. Other research showed waste contains data about drug use, the spread of disease, and the general state of public health.

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Subtopic	Monitoring Pollution
Title	New DNA Sequencing Technology Aids Fecal Pollution Management
Full Text	*United States Environmental Protection Agency (US EPA) Risk Management Research News, Water Online, August 2011*

The Environmental Protection Agency (EPA) is using DNA sequencing to research fecal bacterial communities that have the potential to affect the US beef and dairy industries as well as future recreational water quality monitoring criteria. The types of bacterial colonies found in cattle intestines can be used as indicators of the fecal pollution contaminating recreational waters. When bovine fecal waste leaks into the environment, it can result in serious human health risks, such as releasing pathogenic microbes such as *E. coli, Campylobacter, Salmonella,* or *Cryptosporidium*. New DNA sequencing technology, called next-generation pyrosequencing, has revolutionized understanding of fecal bacterial community composition and variability. It allows for the cost-efficient processing of hundreds of thousands of sequence reads in a single trial, enabling the characterization of both abundant and rare community members. Over the course of 90 days, researchers from the EPA profiled the fecal microbial communities of six different feeding operations and obtained more than 634,000 high-quality DNA sequences from 30 beef cattle. This study–the largest bovine DNA sequencing effort to date–also allowed researchers to analyze the effects that different feeding practices may have on the proliferation and diversity of microorganisms that are commonly used as water quality indicators. Results showed that the structure of bovine fecal bacterial communities can shift dramatically across different feeding operations, suggesting that animal diet is an important factor in cattle microbiome structure. Other research projects are using pyrosequencing technology to produce detailed bacterial community profiles from drinking water distribution systems, wastewater treatment systems, sewage biosolids, and soils. Continued application of deep-sequencing approaches will help us better understand the ecology of these environments to determine how microorganisms impact human health and the environment.

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Subtopic	Monitoring Pollution
Title	Microbial Source Tracking: How Did That Get In There?
Full Text	*United States Environmental Protection Agency (US EPA), Water Online, September 2018*

Throughout the United States, there are an estimated 90 million illnesses every year caused by microbial contaminants in recreational waters attributed to human or animal fecal contamination. In fact, some beaches in the Great Lakes region can be declared unsafe for recreational use due to elevated levels of fecal contamination stemming from sewer overflows, septic system leaks, pet and local wildlife scat, or nearby agricultural practices. Understanding the sources of this water contamination can aid resource managers in mitigating the effects of this pollution, or preventing waterway contamination outright, thereby decreasing the risk of fecal pollution and minimizing community costs associated with the closing and reopening of beaches. The Environmental Protection Agency (EPA) has used host-associated quantitative Polymerase Chain Reaction (qPCR) analyses to measure fecal pollution levels and identify the source of pollution. qPCR allows researchers to make millions of copies of highly diluted fecal bacterial host-associated target genes found in contaminated water samples. The EPA is using host-associated qPCR methods to identify contamination from key animal groups potentially contaminating waters at three Great Lakes beach areas that tend to have high levels of fecal indicator bacteria. These water samples were analyzed for human, ruminant, avian, and canine fecal pollution levels. The results of these analyses are compared to local fecal and sewage samples to determine how well the qPCR methodology identifies fecal pollution sources. This research represents an important development of qPCR methodology to help local communities identify fecal pollution sources, clean-up contaminated beaches, and prevent future water contamination.

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Subtopic	Monitoring Pollution
Title	Decoding the DNA of Wastewater
Full Text	*James Workman, Source Magazine, July 2018*

The biological treatment of wastewater uses microorganisms to help break down sewage to recover clean valuable water. Microorganisms, such as bacteria, are species that consume pollutants, but often decrease the effectiveness of treatment plants. Furthermore, it is difficult to identify which bacteria are responsible for this effect. One consequence of not being able to identify bacterial species is bulking, which affects 30 percent of the world's activated sludge wastewater treatment plants. Bulking occurs when sludge does not settle after air has been pushed through the wastewater. It lowers the effluent quality and drives up costs yet it is common to the engineering, hydraulic, and microbiological dynamics of the process. Using DNA sequencing, microbiologist Halkjaer Nielsen has been able to identify bacteria in treatment plants to feed, nurture, and improve their performance. Nielsen and colleagues have used metagenomics to extract DNA from wastewater or sludge samples and conduct sequence analysis using bioinformatic tools. Nielsen's discoveries can lead to many benefits, such as less bulking and foaming, reduced need for chemicals and energy, maintained plant stability, better effluent quality, and higher recovery of nutrients, such as phosphorus. DNA analyses can serve as an early warning system for pathogens, and will help facilitate better control strategies. Last year, Nielsen's discovery of the *Comammox* bacteria helped oxidise ammonium directly to nitrate in one step, fundamentally changing our understanding of the nitrogen cycle in nature and engineered systems. There is a universal demand to decipher wastewater treatment. A microbiome has a particular metabolic function unique to the treatment plant, whether it is located in northern China, eastern Brazil, or southern Portugal. DNA sequencing allows researchers to identify the microbial components of wastewater and begin to answer many unanswered questions. DNA sequencing will help understand the divisions between 'natural' and 'man-made' water systems and can serve as an important diagnostic tool in many fields, with wastewater treatment being one.

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Community Science Resource Packet: Environment

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Subtopic	Tracking Wildlife
Title	Tracking Fish with eDNA
Full Text	*A Citizen Science Project at Cornell University*

For thousands of years, natural barriers have bounded where organisms live and helped define their habitats, creating dynamic, natural ecosystems. The globalization of people and products has introduced organisms to new environments where they could not previously be found. Species introduced to an area where they did not live previously are called "non-native"species. These non-native species will often be unable to reproduce in large enough numbers to affect the local ecosystem, and therefore will disappear without creating a permanent population or adversely affecting other species of flora and fauna. However, in rare cases, these species can outcompete the existing wildlife in the area due to either a lack of natural predators or an abundance of nutrition, which can have drastic effects on the environment. These non-native, disruptive species are referred to as "invasive" species by ecologists. Many types of organisms—including plants, animals, and microbes—can be invasive. Invasive fish species are problematic in coastal regions, as well as in the Great Lakes and surrounding areas. They can cause serious environmental problems and significant economic loss, disrupting the natural ecosystem and threatening the diversity of native aquatic species. The consequences affect a wide range of commercial, agricultural, aquacultural, and recreational activities.

Globally, the introduction of non-native species into new environments is considered a major threat to natural environments and global species diversity. Invasive fish species are problematic because of the ease and frequency of waterway contamination by non-native fish. Aquatic species can be introduced into new areas from ships dumping large amounts of water from onboard ballast tanks that they use to maintain stability. Globally, millions of tons of this "ballast water" are released daily, inadvertently introducing aquatic species into new environments. Commercial aquaculture and the aquarium trade have also contributed to the release of fish species into new environments. The invasive species being tracked in this project are sea lamprey, Asian carp, round goby, northern snakehead, white perch, Asian swamp eel, and tench. The introduction of these invasive species have contributed to the decline of important native fish. The project also looks for the presence of threatened, endangered, and declining native fish species, including the deep water cisco, American eel, brook trout, Atlantic sturgeon, lake sturgeon, shortnose sturgeon, and rock bass.

FishTracker is a citizen science project based at Cornell University. Its goal is to map the fish in New York State, both native and invasive, by gathering environmental DNA (eDNA) samples from local water sources. Each of these samples is tagged with GPS coordinates, and is analyzed using quantitative PCR (qPCR) to identify the many species of fish that inhabit the waters from which the sample was pulled. The data is compiled in a database, which students and teachers can analyze and study further.

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Community Science Resource Packet: Environment

Continued

Subtopic	Using eDNA
Title	1000 Rivers Fish eDNA Project
Full Text	*International Fish eDNA Project*

1000 Rivers Fish eDNA Project is a collaborative effort among researchers and communities throughout the British Isles, Canada, and Europe. The project aims to monitor rivers for signs of changes or decline in fish populations. This long-term monitoring data will help researchers and conservation managers address those changes and maintain river health.

Groups participating in the 1000 Rivers Project collect water samples from various waterways using a simple plastic pipette collection device. Water collected in the pipette is pushed through a filter, which captures cells shed by fish and other organisms into the water. The filtered cells are then sent to research labs, where DNA is extracted from the cells and analyzed to determine the composition of different fish species in the sample. Results of the DNA analysis are published in scientific journals, and a summary of fish species and population estimates are sent to participants.

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Community Science Resource Packet: Food and Safety

Directions

Use this Resource Packet to gain an understanding of how DNA identification technology has been used in food and safety projects. You should not read or watch all of the articles and videos in this packet. Instead, explore a few resources to gain an understanding of the breadth of problems that can be addressed with DNA identification tools.

All the resources listed below are about Food and Safety. Use the Subtopic and Title to identify a few you would like to explore. Videos and Articles are linked. Here, each Article Title links to a Summary, and a link to the full text is provided on that page if you prefer to read it in its entirety.

Videos

Subtopic	Title and Link
Health and Disease	*Monitoring Wastewater as an Early Indicator of COVID-19 Outbreak Dynamics*
Health and Disease	*DNA Detectives: Fighting Infectious Diseases Using Genome Science*
Food Safety	*GenomeTrakr: Transforming Food Safety*
Food Safety	*Solving Food Poisoning Investigations with Dna*
Food Safety	*CDC in Action: Foodborne Outbreaks*
Microbiome Testing	*Human Microbiome Project: Analyzing Microbes that Play a Role in Health and Disease*

Articles

Subtopic	Title and Link to Summary
Monitoring Wastewater	*How Safe Is the Dna in Your Poop From Unwanted Snooping?*
Monitoring Wastewater	*DNA in Wastewater Could Provide Clues to Help Community Health, Stanford Researchers Say*
Monitoring Wastewater	*Researchers Sequence DNA in Sewage Samples*
Monitoring Wastewater	*New DNA Sequencing Technology Aids Fecal Pollution Management*
Microbiome Testing	*Exploiting the Oral Microbiome to Prevent Tooth Decay: Has Evolution Already Provided the Best Tools?*
Microbiome Testing	*Dysbiosis of the Oral Microbiota Causes Gut and Health Problems*
Microbiome Testing	*Oral Microbiome Composition, but not Diversity, Is Associated with Adolescent Anxiety and Depression Symptoms*
Microbiome Testing	*Anxiety Might Be Alleviated by Regulating Gut Bacteria*
Health and Disease	*TCU Study Engages Tribal Communities in Genomics Research*

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Community Science Resource Packet: Food and Safety

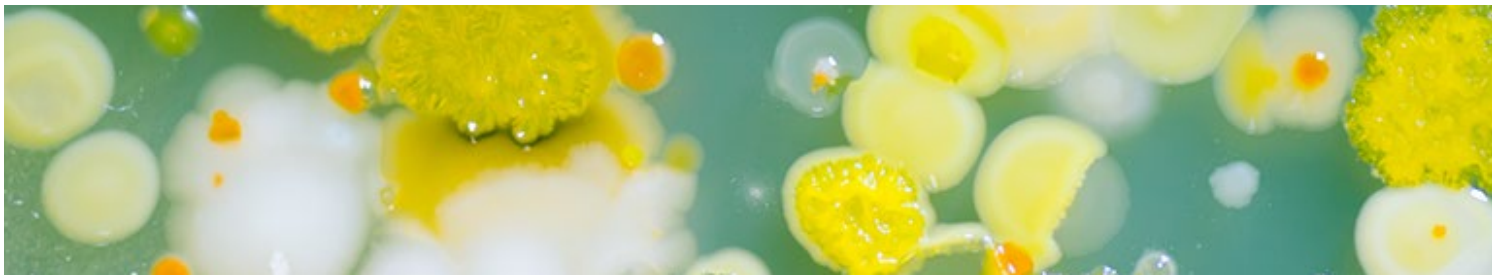
Continued

Subtopic	Monitoring Watewater
Title	How Safe Is the DNA in Your Poop from Unwanted Snooping?
Full Text	*Jennifer Tsang, Massive Science, September 2020*

In a 2014 study published by the American Society of Microbiology, Ryan Newton and colleagues from the University of Wisconsin-Milwaukee determined that they could use sewage microbiomes to predict obesity levels with 81–89 percent accuracy. The microbiome is a collection of microbes that live in the human gut and are thought to be linked to health. On average, 15 percent of DNA recovered from sewer samples came from human microbiomes, presenting itself as a valuable source of environmental DNA (eDNA). From the 200 samples originating from 71 cities, they noticed that there are about 60 types of bacteria common among cities. The abundance of less common microbes varied city to city, giving each place a unique signature. Humans excrete metabolites, vitamins, microbes, and cells containing genetic information that can be collected and sampled in wastewater treatment plants.

Wastewater epidemiologists have created methods of using waste to detect real-time outbreaks of disease, drug use, and more. Scientists have even been able to detect the genetic material of SARS-CoV-2, the virus that causes COVID-19, in sewage samples; in Italy, for example, traces of the virus were found in two different cities using this method. Researchers in Paris were able to track the rise and fall of SARS-CoV-2 infections via sewage, and Australian researchers leveraged viral RNA copies found in sewage to estimate the number of individuals infected among the population. Kendra Maas, a scientist at the University of Connecticut Microbial Analysis, Resources, and Services core facility, has created sewage monitoring for her own university in an effort to prevent outbreaks, as had been the case at University of Arizona when a positive wastewater sample was found in wastewater from campus housing. COVID-19 is not the only disease that can be monitored in wastewater. Poliovirus can be shed in fecal matter and cell-culture based methods of disease tracking have been used to track poliovirus since the 1940s. In 2013, wastewater surveillance detected poliovirus in Israel when no cases of the disease had been reported. Similarly, sewage monitoring also gave early signs of hepatitis A and norovirus outbreaks in Sweden.

Many believe that sewage monitoring may be more intrusive than beneficial to general health. At the University of Queensland, scientists were able to predict socioeconomic information using wastewater. They designed biomarkers to predict 37 characteristics from the Australian Census including age, education, and employment. Sewer surveillance can also detect illegal drug use, which countries such as China, Belgium, Spain, the Netherlands, and Germany have employed for epidemiological research. Even if sewage samples do not trace back to a single individual, wastewater data can trace to specific populations, such as neighborhoods. Some strategies to reduce unethical practices are aggregating samples from multiple sites, and removing names and locations of sampling sites. Wastewater-based epidemiology has the potential to be used as avaluable tool for surveying public health.



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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Monitoring Watewater
Title	DNA in Wastewater Could Provide Clues to Help Community Health, Stanford Researchers Say
Full Text	*Ula Chrobak, Stanford News, December 2016*

Wastewater is a valuable source of information encrypted in DNA and may hold a wealth of insight for public health officials, according to a team of researchers from Stanford University. Stanford professor of civil and environmental engineering, Craig Criddle, is working to track the DNA of pathogens in wastewater in an effort to improve early pathogen detection. Criddle and his team will be testing wastewater from the 7,000-member Stanford community. The researchers will test for pathogenic DNA from a list of bacteria and viruses, while also looking for new pathogens. The research will not only validate their tool to track disease, but will contribute to their growing dataset of microbial diversity. After validating the effectiveness of this method, this approach could make public health measures more proactive, allowing public health officials to improve responses to disease outbreaks. Used together with chemical testing, wastewater DNA analysis has the potential to be used for tracking antibiotic resistance.

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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Monitoring Watewater
Title	Researchers Sequence DNA in Sewage Samples
Full Text	*Sara Jerome, Water Online, April 2015*

Researchers have found new methods of determining if water pollution can be attributed to the introduction of human fecal matter by tracking the sewage dumped into waterways. Each city's microbial character is distinct and can reveal signs of the health of its individuals. The research began as an effort to understand bacteria that resides within the human digestive tract, but has expanded to include analysis of the microbiomes of entire human communities. With the assistance of wastewater treatment plants from 71 US cities, more than 200 sewage samples were collected. Mitchell Sogin, a molecular evolutionary biologist at the Marine Biological Laboratory, and colleagues were able to sequence DNA in the samples and determine their origin. They showed that geographically distributed US populations share a small set of bacteria whose members represent various communities of US adults. Cities were differentiated by their sewage bacterial communities and the community structures were good predictors of a city's estimated level of obesity. Similar studies have determined that human waste can be used to gather data regarding drug use, spread of infectious diseases, and general health of the populace.

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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Monitoring Watewater
Title	New DNA Sequencing Technology Aids Fecal Pollution Management
Full Text	*United States Environmental Protection Agency (US EPA) Risk Management Research, Water Online, August 2011*

The Environmental Protection Agency (EPA) is attempting to use DNA sequencing in order to learn more about fecal bacterial communities that may affect American beef and dairy industries, as well as to monitor recreational water quality. The bacteria present in cattle intestines can be indicators of the animals' health, and can also be used as indicators of fecal pollution in nearby water sources. When bovine fecal waste leaks into the environment, it can pose serious human health risks, such as releasing pathogenic microbes like *E. coli*, *Campylobacter, Salmonella,* or *Cryptosporidium*. New DNA sequencing technology, called next-generation pyrosequencing, has revolutionized scientists' understanding of fecal bacterial community composition and variability. Next-generation pyrosequencing allows for the cost-efficient processing of hundreds of thousands of sequence reads in a single trial, enabling the characterization of both abundant and rare community members together. Over the course of 90 days, they profiled six different feeding operations' fecal microbial communities and obtained more than 634,000 high-quality DNA sequences from the fecal samples of 30 beef cattle. This study–the largest bovine DNA sequencing effort to date–also allowed researchers to study the effects that different feeding practices may have on the proliferation and diversity of microorganisms that are commonly used as water quality indicators. Results showed that the structure of bovine fecal bacterial communities can shift dramatically across different feeding operations, suggesting that animal diet is an important factor in cattle microbiome structure. Other research projects have used pyrosequencing technology to produce detailed bacterial community profiles from drinking water distribution systems, wastewater treatment systems, sewage biosolids, and soils. Continued application of deep-sequencing approaches will help us better understand the ecology of these environments to determine how microorganisms impact human health and the environment.

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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Microbiome Testing
Title	Exploiting the Oral Microbiome to Prevent Tooth Decay: Has Evolution Already Provided the Best Tools?
Full Text	*Jonathon L. Baker and Anna Edlund, Frontiers in Microbiology January 2019*

The human immune system can influence the entire community of microbes (the microbiome) in the body, even altering the microbial community to benefit the human host. The immune system has evolved to tolerate, and even facilitate, maintenance of vastly diverse microbes that prevent the establishment of pathogens. Humans have a long history of coevolving with our resident bacteria, which suggests that ancient hominid microbiomes were much more stable and diverse than their contemporary counterparts. Two dietary shifts, caused by the development of agriculture and later by the Industrial Revolution, vastly increased human consumption of carbohydrates. This disrupted the homeostasis of the human oral microbiome. The earliest colonizers of the tooth surface are *Streptococci* bacteria, which bind to the naked tooth surface. Once bound, these bacteria create a surface to which other bacterial species can attach. In the absence of carbohydrates, these *Streptococci* bacteria tend to dominate the oral microbiome and are associated with good dental health. In a high-carbohydrate diet, particularly with a lack of oral hygiene, other bacteria colonize the oral cavity, creating acidic dental plaques that can cause tooth decay if not treated.

Tooth decay is caused, in part, by a highly processed Western diet, which has led to an increase in obesity, type 2 diabetes, cardiovascular disease, metabolic disorders, and even cancer. As with tooth decay, there is a growing body of evidence linking these conditions to diet by microbial studies. Education of the general public about the relationship among dietary habits, the microbiome, and overall health remains paramount.

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Community Science Resource Packet: Food and Safety

Continued

A return to a less processed or unprocessed diet is likely to have significant health benefits by supporting the mutualistic microbial profiles within our bodies.

Fluoride, including fluoridated toothpaste and drinking water, has been used to combat dental decay for more than 50 years. Fluoride prevents and treats dental decay by remineralizing the tooth enamel while impairing bacterial metabolism. While the efficacy of fluoride treatments is well-documented, the prevalence of tooth decay suggests that fluoride alone is insufficient to prevent dental disease. Other antimicrobial agents are available for dental use but are similarly broad-spectrum. As such, reengineering the oral microbiome is likely to generate a more positive outcome than its total destruction. The development of approaches to specifically alter the plaque composition and prevent outgrowth of pathogenic bacteria remains a highly attractive objective.

There are a few methods to accomplish this task. Prebiotics are foods or supplements that modulate the microbiome to benefit the host. Arginine has been demonstrated to be effective to prevent dental decay in clinical testing. In addition to creating a more alkaline pH, the breakdown of arginine produces ATP and a bioenergetic advantage for the beneficial *Streptococci* bacteria. Saliva provides binding sites and nourishment for specific bacterial species, which are largely benign or commensal. The flow of saliva and its component molecules have great influence over the taxa that are able to survive in the mouth, and tooth decay may be prevented simply by increasing salivary flow. Probiotics can modulate microbiome ecology by selectively adding or removing particular species from the oral community. Attempted probiotic strategies to prevent tooth decay either have sought to add health-associated microbes or to replace pathogenic ones with less pathogenic mutants. A number of studies have explored the use of *Lactobacillus* and *Bifidobacterium*, the bacteria traditionally used in probiotic formulations. Many of these bacteria are residents of the gut, meaning they are not well-adapted for long-term survival in the mouth where conditions are different. Species with a higher likelihood of outcompeting pathogenic bacteria are found in the healthy oral cavity. Studies in other environments have illustrated that the best probiotics for preventing the growth of pathogens both occupy the same ecological niche and produce compounds that directly target the pathogen. The other major strategy used in probiotic approaches is the displacement of pathogenic bacteria.

As opposed to adding species to the oral microbiome, some researchers have tried removing problematic bacterial species to restore a healthy oral microbiome. Specifically targeted antimicrobial peptides (STAMPS) are synthetic peptides consisting of targeting domains to kill targeted species. Several STAMP candidates have been shown to specifically target pathogenic bacteria without harming healthy ones, while also being able to enrich organisms associated with dental health. Small molecules have been proposed to specifically target pathogenic bacteria as well. These small molecules aim to disrupt the biofilm and membrane integrity of bacterial species, causing them to degrade. This is likely to allow for the rapid reformation of the problematic bacterial community, meaning treatment must be continuous. Using a bacteriophage, conversely, is a conceptually valid approach to fighting tooth decay bacteria, though it has received little attention. While in testing, few phages completely eliminated viable counts from single-species biofilms because the phage showed strict host specificity, which would make this approach a particular challenge for humans. Although several of these approaches have produced encouraging results, properly controlled vigorous human studies are still needed.

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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Microbiome Testing
Title	Dysbiosis of the Oral Microbiota Causes Gut and Health Problems
Full Text	*Leanne Edermaniger, Atlas Blog, March 2020*

The oral microbiota is composed primarily of bacteria, but also contains viruses and even fungi. Living in the mouth means that they have access to your gut via the digestive tract and can even access your bloodstream. The composition of the oral microbiome is similar in healthy people across different countries. Similar to gut microbiota, some oral bacteria are associated with a variety of diseases affecting not just the mouth, but the entire body.

The mouth is a complex environment because it is made of many structures for bacterial colonization. There are microenvironments, such as the surface of teeth, the tongue, hard and soft palates, where bacteria can colonize. The mouth is also relatively warm and a source of nutrients and water. The oral microbiome is the second most abundant microbiome besides the gut. Members of the oral microbiota, predominantly *Firmicutes, Proteobacteria,* and *Actinomycetes*, coexist in dense communities known as biofilms.

The gut microbiome is a complex ecosystem consisting mainly of bacterial cells, but also includes viruses, archaea, and fungi that work in harmony with the human body. It provides many benefits, such as strengthening the gut barrier, supporting the immune system, providing energy, and protecting you from optimistic pathogens. When the conditions in your gut are at their best, the bacteria are living in symbiosis. Some are harmless, while others are beneficial and can produce helpful metabolites that your immune system strong. When the gut microbiome is unbalanced, it is known as “dysbiosis” and can be linked to many health conditions. If dysbiosis occurs, pathogenic bacteria may dominate your gut or you may lack diversity of bacterial species, which is important to gut health. These microbes can release unhealthy metabolites and toxins, even triggering inflammation.

The human salivary gland is capable of producing up to 1.5 liters of saliva every day, which keeps the mouth hydrated and aids in the digestion of food. Saliva also contains oral bacteria, which can spread throughout the digestive tract. Some are killed by stomach acid, but others are acid-resistant. Some oral bacteria, such as *P. gigivalis*, are associated with gut dysbiosis. In the mouth, these cells cause gum disease and are linked to imbalances in the gut microbiome. This bacteria is particularly resilient to adverse conditions because they can withstand stomach acidity. The lining of the digestive tract is a barrier that enables nutrients to enter the bloodstream and be carried to other organ systems that require them, while simultaneously halting the spread of pathogens, toxins, and food particles from extending beyond the membrane and causing illness or injury. This is known as intestinal permeability and functions through tight-junction proteins. They act as gates and when healthy, they relax enabling nutrients to pass through them, but in unhealthy states, the gates or tight junctions may not close, allowing unwanted toxins to pass easily. One study with a single administration of *P. gingivalis* showed significant changes to the gut microbiome that affected the function and integrity of the gut barrier as a result of dysbiosis. Through impaired intestinal barrier function, endotoxemia occurs where substances called lipopolysaccharides (LPS) from bacteria enter the blood, causing an immune response and inflammation.

The development of obesity has also been linked to intestinal microbes. People who are overweight tend to have specific microbiomes that differ from healthy individuals and contain fewer lean microbes. Type 2 diabetes is one of the most common chronic diseases in the Western world. There is evidence to suggest that oral diseases are linked with type 2 diabetes. The oral microbiota is an important factor in the development of diabetes in that it affects the development of bones in the mouth. The gut microbiota is associated with an increased risk of colon cancer, so too is mouth bacteria. Some bacterial species, such as *Fusobacterium nucleatum*, are also a risk factor for developing colon cancer. Oral dysbiosis has been associated with other chronic diseases too, including rheumatoid arthritis and liver disease.

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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Microbiome Testing
Title	Oral Microbiome Composition, but not Diversity, Is Associated with Adolescent Anxiety and Depression Symptoms
Full Text	*Carra A. Simpson, et al, Physiology & Behavior, November 2020*

The co-occurrence of mental health disorders and oral disease is poorly understood. Depression is the leading cause of disability worldwide, while anxiety disorders are the sixth highest contributor to non-fatal health loss. Research in adults suggests this significant burden is further compounded by comorbid oral disease, particularly caries (cavities). Caries is a common disease that affects 2.3 million people globally. The co-occurence of depression, anxiety, and dental problems can begin as early as adolescence, a developmental period characterized by early inflammatory oral disease, caries, and high rates of emergent anxiety and depression. Adults with mental health disorders are at an increased risk for oral inflammation and poor general oral health.

However, this relationship has not been explored in adolescents. Mild oral inflammation in adolescents can progress to chronic and severe disease with profound lifelong consequences. Several mechanisms may underlie the connection between poor oral health and mental health disorders, including changes to the microenvironment in the oral cavity. Normally, the relationship between the oral microbiome and the human host is mutually beneficial: regular buffering by saliva prevents growth of pathogenic species, provides nutrients for benign, resident microorganisms, and secretes nitrates for anaerobic microbial respiration. Changes to the components of saliva can make the mouth vulnerable to invasions from pathogenic bacteria species, contributing to dental disease. Elevated levels of the stress hormone cortisol are associated with anxiety and depression; therefore high levels of cortisol are secreted from the salivary glands into the mouth, effecting those bacterial colonies. Moreover, cortisol can alter microbial gene transcription with changes resembling those observed in periodontitis, or gum disease. Immunological models also suggest that anxiety disorders disrupt neuroimmune pathways, often involving inflammation pathways. Both changes in the oral microbiome and anxiety and depression may cause an inflammatory response, which can be tracked with acute phase marker C-reaction protein (CRP).

To investigate the relationship between the adolescent microbiome and anxiety and depression, researchers analyzed the microbiome, cortisol, and CRP from 66 adolescents aged 14-18 years. Adolescents' anxiety and depression symptoms were not significantly associated with oral microbial diversity, but specific bacterial taxa were associated with anxiety and depression symptoms. Adolescents' oral microbiome was dominated by *Firmicutes, Bacteroidetes,* and *Actinobacteria*. This did not vary between people with anxiety or depression, although anxiety, inflammation, and poorer oral health were associated with the phylum Spirochaetes, specifically the order Pirochaetales, and the family *Spirochaetaceae*.

Existing studies have hypothesized that elevated salivary cortisol in mental health disorders is caused by dysregulated neuroendocrine activity and may contribute to changes in oral microbiome composition. The precise mechanisms by which cortisol may influence oral microbial composition is subject to ongoing investigation. Bacteria are dynamic organisms that can change when exposed to elevated cortisol levels, which has been supported by existing research showing that bacterial species *Fusobacterium* and *Leptotrichia* increased after cortisol exposure in vitro. However, neuroendocrine-bacteria associations in the oral cavity are not limited to cortisol. Numerous hormones released during the stress response have been associated with changes to microbial composition. It was hypothesized that inflammatory markers may also moderate the association between anxiety and depression symptoms and oral microbial composition. As observed with cortisol, inflammatory marker CRP moderated the relationship between anxiety symptoms and *Actinomycetaceae* taxa among others, and in particular *Streptococcaceae*, which was found in higher abundance in participants with high anxiety symptoms. The study revealed that specific bacterial taxa present in saliva were associated with adolescent anxiety and depression symptoms, and these relationships were moderated by salivary cortisol and CRP.

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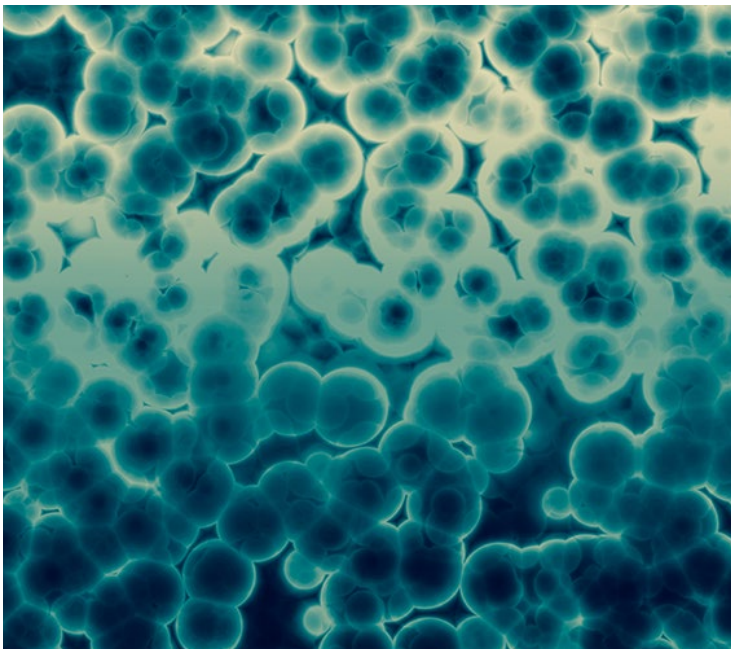
Community Science Resource Packet: Food and Safety

Continued

Subtopic	Microbiome Testing
Title	Anxiety Might Be Alleviated by Regulating Gut Bacteria
Full Text	*British Medical Journal, May 2019*

According to a study published in the journal of General Psychology, patients who experience anxiety may be able to improve their microbiota by either ingesting probiotic and non-probiotic food supplements. People with mental diseases and a variety of physical disorders can commonly exhibit anxiety symptoms, and research has indicated that digestive tract microbiota can aid in regulation of brain function through the "gut-brain axis." Researchers at Shanghai Jiao Tong University School of Medicine sought evidence to support that anxiety symptoms could be improved by regulating intestinal microbiota. Out of 21 studies conducted, 14 of those had chosen probiotics as their regulatory method, while the other seven used non-probiotic methods, such as dietary adjustment. Overall, 11 out of 21 studies yielded positive results on anxiety symptoms, indicating a possible correlation of effectiveness. Of the 14 studies that utilized probiotics as a source of intervention, more than a third of the studies found these probiotics to be effective at reducing anxiety symptoms, while 6 of the 7 studies that used non-probiotic methods as intervention for anxiety symptoms reported a successful result. Researchers suggested that the reason that the non-probiotic methods were so much more effective than probiotic methods was due to the fact that completely altering a diet could have a greater impact on the improvement of gut health than simply introducing specific types of bacteria with a probiotic supplement.



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Community Science Resource Packet: Food and Safety

Continued

Subtopic	Microbiome Testing
Title	TCU Study Engages Tribal Communities in Genomics Research
Full Text	*TCJ Staff, Tribal College Journal of American Indian Higher Education, April 2018*

Less than one percent of genomics research participants are of American Indian descent, reflecting a variety of shortcomings in sample groups to include researcher misconduct, under-recruitment, and a perceived lack of clinical unity. Despite this, many women of the Turtle Mountain Band of Chippewa Indians in Belcourt, North Dakota have participated in the Preeclampsia and Genetics Study since it began 15 years ago. Preeclampsia, a high blood pressure condition that develops during pregnancy, can be life-threatening to both mother and child. Dr. Lyle Best, an Indian Health Services family practitioner who began the Preeclampsia and Genetics Study at the Turtle Mountain Community College (TMCC), did so with the expressed intention of educating the public about the potential genetic associations of this condition. Since its inception, the lab has associated three different genetic variants of C-reactive protein (CRPs) to preeclampsia in Chippewa females, and has correlated hypertension in later stages of life to women who exhibited preeclampsia during pregnancy.

The key to such a long research presence has been an equitable relationship with the tribe. “Community-engaged research” methodologies ensuring that native communities are involved in every step of the research is relatively new in genomics. The lab updates the community on research findings through newsletters and radio programming, consults elders, and works with the tribe’s institutional review board, which oversees research. Developing the research and education capacity of tribal students is important to sustaining the relationship. The lab has facilitated the training of more than 40 students who had never been afforded an opportunity to work in a research environment. Because of educational opportunities like this, tribal students can attend conferences, receive mentorship from established educators, and co-author academic papers. Some students have even used these experiences to obtain graduate degrees. Due to the success of these programs, some tribes have begun to reconsider the potential benefits of genomic technologies and how they can positively affect their communities. For example, the Navajo Nation is considering amending their long-standing mandate that bans genomics research on tribal lands. Meanwhile, the lab at TMCC demonstrates the capacity of tribal colleges to advance Indigenous peoples’ health.

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Community Science Resource Packet: Human Interest

Directions

Use this Resource Packet to gain an understanding of how DNA identification technology has been used in human interest projects. You should not read or watch all of the articles and videos in this packet. Instead, explore a few resources to gain an understanding of the breadth of problems that can be addressed with DNA identification tools.

All the resources listed below are about Human Interest. Use the Subtopic and Title to identify a few you would like to explore. Videos and Articles are linked. Here, each Article Title links to a Summary, and a link to the full text is provided on that page if you prefer to read it in its entirety.

In this section, ancient DNA is abbreviated aDNA.

Videos

Subtopic	Title and Link
Pets	Search for "dog poop DNA testing" for a variety of videos on this topic.
Food and Supplement Labeling	*What's Really in Herbal Supplements?*

Articles

Subtopic	Title and Link to Summary
Using aDNA	*Indigenous Groups Look to Ancient DNA to Bring their Ancestors Home*
Using aDNA	*North America's Oldest Mummy Returned to Us Tribe After Genome Sequencing*
Using aDNA	*How Ancient DNA Can Help Recast Colonial History*
Pets	*What We Can—and Can't—Learn from Our Pets' DNA*
Pets	*DNA Testing Looks Into Dog Breeds and Cat History*
Food and Supplement Labeling	*FDA DNA Testing at Wholesale Level to Evaluate Proper Labeling of Seafood Species*
Food and Supplement Labeling	*Europe: DNA Shows Food Labeling Accuracy*
Food and Supplement Labeling	*Are Your Food and Vitamin Labels Lying to You?*
Food and Supplement Labeling	*New York Attorney General Targets Mislabeled Herbal Supplements*
Food and Supplement Labeling	*Herbal Supplements Are Often Not What They Seem*
Food and Supplement Labeling	*Herbal Supplements Often Contain Unlisted Ingredients*
Food and Supplement Labeling	*The DNA-Based Authentication of Commercial Herbal Products Reveals Their Globally Widespread Adulteration*
Pets	*Why You Can't Always Trust Pet Food Ingredient Labels*
Textile Labeling	*Keeping Synthetic Textiles Real and Sustainable*

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Using aDNA
Title	Indigenous Groups Look to Ancient DNA to Bring Their Ancestors Home
Full Text	*Nicky Phillips, Nature, April 2019*

Indigenous communities, such as the Yidinji community of Australia, are working together to sequence DNA from remains that were unrightfully taken from their homelands decades ago. Many ancestors were victims of dehumanizing practices in the nineteenth and early twentieth centuries when white collectors looted graves to sell the remains of Aboriginal people to museums in Australia, the United Kingdom, and other countries. It has been a longstanding goal of communities, such as the Yidinji, to return and rebury their ancestors. Before reburying the remains, scientists who have been analyzing the DNA of living community members have requested to sequence DNA found on the ancestral remains. Evolutionary geneticist David Lambert from Griffith University in Brisbane, Australia was able to extract DNA from a subject and determine that the ancestral remains were related to the Yidinji people. This sparked hope in the tribe, as the members recognized the ability of DNA evidence to allow them to return their ancestors to their native lands. In the past few decades, Indigenous peoples have demanded the return of sacred objects and human remains to their rightful tribes, yet the lack of DNA evidence has made identifying the specific tribe of origin difficult. Currently there are two teams, including Lambert's, who have partnered with Indigenous communities to create genomic maps that connect ancient and historical remains with present-day groups. The genomic databases will eventually help return remains to the right communities.

Australia is one of the countries where DNA research is being used to confirm the identity of Indigenous groups. DNA research has shown that Indigenous groups have lived on the continent for tens of thousands of years and in some places, it has been established that ancient individuals are closely related to present-day groups living in the same region. A possible application of this DNA research could be to provide genealogical information to the "stolen generations" of Aboriginal children who were removed from their families under Australian law prior to 1970–many of whom are still alive today. DNA database of Indigenous groups could be used to help individuals understand their genetic heritage and identify their homelands. Despite the promises of DNA technology, some Indigenous people fear that governments or scientists will misuse their genetic information as a result of their history of mistreatment.

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Community Science Resource Packet: Human Interest

Continued

European colonists' arrival to Australia in the late 1780s brought with it the exhumation of Indigenous remains; by the end of the nineteenth century, the remains of Indigenous Australians could be found in most major museums around the world. The lack of respect with which these remains were displayed has led to friction between the Indigenous peoples and those who collected the remains. Colonizing Europeans imposed their will over the Indigenous tribes, determining where they could live and work, who they were allowed to marry, and even if they had the "right" to keep their own children. Tribal groups were removed from their ancestral lands and systematically relocated onto reserves and missions. By the 1970s, Aboriginal groups were vehemently fighting for the return of their ancestors' remains. By the 1980s, the growing pressure prompted museums to introduce policies to return human remains and sacred objects to their communities. Tracking down the traditional owners of ancestral remains is important for Aboriginal people because it is part of reclaiming their identities after being forced to assimilate into colonized Australia. Because of this, Indigenous people have regained custody of more than 4,000 sets of remains from the United Kingdom, the United States, Canada, and European nations. However, several thousand sets of remains still exist in Australian museums as "Aboriginal" with no further identifying information.

In 2016 Lambert began working with elders from 11 Aboriginal groups, including the Yidinji and Paakantyi, to chart the continent's genetic history by collecting DNA samples and sharing the findings with other Indigenous Australians Lambert and evolutionary geneticist Eske Willerslev from the Natural History Museum of Denmark obtained mitochondrial genomes from all 27 remains and full or partial nuclear genomes from 10 of them. Mitochondrial DNA is genetically inherited maternally and is present in more copies in cells than nuclear DNA, providing them an easier template to use in their study. However, mitochondrial DNA was found to be limited in linking remains to contemporary groups. Nuclear DNA proved to be a richer source of ancestral information. In all 10 cases, the ancestral remains were closely related to Indigenous people in their study who came from the same geographic area. Lambert is now working with the Queensland Museum and Board of Indigenous Advisors to sequence around 300 unidentified remains housed in the museum. While the Indigenous advisors agree that genetic matching could be a powerful tool for identifying ancestral remains, they would like to see more proof of accuracy before using this technique more broadly. The risk of repatriation of remains to the wrong community can be reduced by combining genomic analysis and anthropological evidence.

The data generated from this project serves as a starting point for creating a service for present-day Indigenous people to compare their DNA against the reference library generated from ancestral DNA samples. This will allow people, including members of the Stolen Generations, to explore their genetics and reveal information about where their ancestors originated. The project has been largely embraced by the Aboriginal communities largely because they retain control. Of almost 180 families that have been approached, only two had decided not to participate. The results grant the community an opportunity to learn about the history of Australia and the relationships of different Indigenous groups.

The first map of Aboriginal groups was created in 2017, based on mitochondrial DNA from 111 different hair samples from three Indigenous communities. The evidence suggests that the first Australians emigrated to the continent from Asia approximately 50,000 years ago, a figure that is corroborated by archeological evidence and other genome studies. This study highlights the growing involvement of the Aboriginal people in science. For many years, science has kept them out and through their involvement, Indigenous groups can benefit from future studies using their data.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Using aDNA
Title	North America's Oldest Mummy Returned to US Tribe after Genome Sequencing
Full Text	*Ellen Callaway, Nature News, December 2016*

The 'Spirit Cave Mummy' is a human skeleton that was discovered in 1940 in northwest Nevada. Advances in sequencing have allowed scientists to analyze the 10,600 year old genome and give proper repatriation to the Fallon Paiute-Shoshone Tribe to which it is most closely related. The tribe has long argued that it should be given the remains for reburial, but the US government has strongly opposed repatriation. The genome of the Spirit Cave Mummy is significant because it helps reveal how humans settled in Northern America according to anthropological geneticist Jennifer Raff. This follows shortly after the US government's decision about another skeleton, an 8,500 year old human known as Kennewick Man, that has qualified for repatriation on the basis of genome sequencing. This, in turn, could benefit Native American tribes by returning ancient remains while providing genomic data of the people inhabiting the region.

The Spirit Cave Mummy is one of many skeletons in the Americas that are more than 10,000 years old. The skeleton was discovered by Archeologists Georgia and Sydney Wheeler in 1940. The skeleton was an adult male aged around 40 years at the time of death. He was found wrapped in a rabbit-skin blanket, wearing moccasins, and reed mats. He was found with cremated or partial remains of three other individuals. Radiocarbon dating conducted in the 1990s proved these remains to be much older than scientists initially believed them to be. Although the US Native American Graves Protection and Repatriation Act (NAGPRA) mandates that remains be returned to affiliated tribes, in 2000 the US government's Bureau of Land Management (BLM) decided against repatriation of the mummy's remains. The tribe sued, and a US District Court judge ordered the agency to reconsider the case in 2006. The mummy's remains, meanwhile, were stored in a Nevada museum, out of view and off-limits to most researchers except those who were attempting to determine its ancestry.

In 2015, an evolutionary geneticist named Eske Willerslev, conducted DNA sequencing on the mummy. Willerslev's research indicated that the remains from Spirit Cave are more genetically similar to North and South American Indigenous populations than any existing contemporary population. This is more than enough evidence to suggest these remains are Native American, though no findings can link them to any specific Indigenous group. Under the NAGPRA, remains can be returned as long as they have been linked to a specific geographic region.

The genome of a 12,600 year old skeleton from Montana, called the Anzick Child, is the only other published ancient genome from the Americas older than 10,000 years. The Spirit Cave and the Anzick Child both seem genetically closer to South American groups than to some North American groups, highlighting the value of sequencing ancient DNA to determine their origins. There are many other younger remains that are not clearly affiliated to any tribe, which now can be deemed Native American through ancient DNA sequencing and thus repatriated. However, through repatriating the ancient remains, scientists lose valuable information about the history of human occupation in the Americas. Further molecular studies can help identify details about the Spirit Cave individuals, such as foods they consumed and the diseases that affected them.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Using aDNA
Title	How Ancient DNA Can Help Recast Colonial History
Full Text	*Ed Young, The Atlantic, September 2019*

The island known as Puerto Rico was first colonized by various ethnic groups as early as 3,000 BC. In the 15th century, Europeans reached the island that was home to between 30,000 and 70,000 people known as the Taíno. Europeans colonized the island, implementing forced relocations, causing starvation, introducing diseases, and imposing slavery. As such, the number of Indigenous people plummeted along with their culture and influence. Colonial officials nearly erased their existence by recategorizing them as Christian converts, wives of colonists, Spanish, or "other." There is a growing narrative that the Indigenous peoples' culture was extinct, but Maria Nieves-Colón, an anthropological geneticist at Arizona State University, who grew up in Puerto Rico, believes otherwise. She grew up sharing oral histories about traditions from Native ancestors and perpetuates a counter-narrative in which Indigenous groups were not completely lost.

To support this idea, she and her team looked for genetic evidence of pre-colonization populations. Ancient DNA degrades rapidly in heat and humidity, making it especially difficult to obtain in this region. In 2001, Juan Carlos Martínez-Cruzado of the University of Puerto Rico analyzed contemporary Puerto Ricans' DNA and found substantial evidence of Native American ancestry in their mitochondrial genomes, a subset of DNA inherited maternally. His findings suggest that "the Taíno contribution to the current population is considerable." Interpreting the genomic data is difficult because of the forced relocations during colonization. When Indigenous ancestral evidence is found, they can only determine with confidence that it is Indigenous to the Americas.

Nieves-Colón has been analyzing tiny fragments of DNA from ancient remains. She and her colleagues have acquired 124 skeletal remains dated between 500 A.D. and 1300 A.D. They sequenced 45 mitochondrial genomes from the remains of people and partial nuclear genomes from two of them. This work showed strong connections between the Indigenous Puerto Ricans and Amazonian groups found in Venezuela and Colombia, and indicated the Indigenous Puerto Rican's likely originated from that area. If the narrative that native Puerto Ricans are extinct were correct, Nieves-Colón asserts that there would be no evidence in their genomic data. This data alone is sufficient to suggest that native Puerto Ricans did not disappear. There are ties to populations that are Indigenous to the island and are present in modern peoples.

This data guides new questions that Nieves-Colón continues to research, including how much Puerto Rican ancestry comes from precontact predecessors and whether those groups left traces of ancestry elsewhere in the Caribbean. The study of ancient DNA has been criticized as "modern capitalism" in what is described as researchers from Western countries collecting and analyzing samples without the consent of local communities. In response, many Indigenous scientists have fostered an inclusive environment to create opportunities for Indigenous researchers. The work of Nieves-Colón highlights the recognition of narratives and historical perceptions of Puerto Ricans as aspects of data and can use genomic data to support or refute their findings.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Pets
Title	What We Can—and Can't—Learn from Our Pets' DNA
Full Text	*Bethany Brookshire, Science News for Students, October 2019*

DNA testing is becoming increasingly popular in humans, but now DNA technology is being used to analyze DNA from dogs. DNA testing in dogs allows scientists to identify genetic traits in their DNA, what breeds a pet descends from, and even what region of the world its ancestors evolved from. Additionally, DNA testing has the capability to predict how a pet might behave and what diseases it may be at risk of developing. However, as DNA tests in pets are becoming more popular, scientists and veterinarians are concerned with the interpretations from at-home DNA tests. Many are concerned that people assume a DNA-based risk is an illness, when in fact the pet may not be sick.

The genome of a dog has 39 pairs of chromosomes, while a cat has 19 pairs of chromosomes. Sequencing the entire genome of pets is a long, expensive process, which caused scientists to begin looking for other ways to identify genetic differences. Scientists began to analyze single nucleotide polymorphisms, or SNPs ("snips"), which are responsible for traits, such as stripes or a solid coat. Many tests look for patterns in SNPs, which can then be used to determine a dog's breed or ancestry. Yet, these tests are limited in that there are many SNPs that are still not well studied.

Dog behavior is being analyzed using DNA sequencing technology. Elinor Karlsson, a geneticist at the University of Massachusetts Medical School, has identified candidate genes that cause obsessive-compulsive behavior in dogs. Yet, determining dog behavior genetically is difficult. Behavior is controlled by multiple genes, making it difficult to tease apart the function of individual genes. In order to do so, researchers would have to study tens of thousands of dogs. Karlsson founded Darwin's Ark, a company which offers genetic testing for your pet and tests every gene, not just SNPs. To reduce the likelihood of sequencing errors, Karlsson has created a sequencing database. Part of the DNA sequencing analysis relies on owner behavior and each pet owner fills out several surveys about his or her dog's personality to help identify candidate genes.

Another DNA test, EmBark, tests for more than 170 health conditions, looking for genetic variants associated with disease, such as risk of seizures, heart disease, and more. This is of interest to dog owners and breeders who want to know the genetic makeup of their pets. The same genetic testing is available for cats too, and can help identify genetic links to diseases in cats. While these tests can identify SNPs that put pets at risk of developing disease, certain SNPs, such as deletions or extra copies, are common in large populations, meaning that their presence does not mean the animal will develop a disease. A DNA test can only identify warnings of risks.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Pets
Title	DNA Testing Looks into Dog Breeds and Cat History
Full Text	*Bethany Brookshire, Science News for Students, October 2019*

There is a massive diversity of species in dogs, ranging from St. Bernards to Chihuahuas, which seemingly look unrelated. The differences in appearances are caused by tiny variations in their DNA and the order in which their nucleotides are constructed. Single differences in nucleotide sequences have a range of effects including curly hair instead of straight hair or short limbs instead of long. Scientists refer to these small but important changes as SNP ("snips") or single nucleotide polymorphisms. SNPs are places where one nucleotide has randomly substituted for another. Millions of SNPs appear within the DNA of every dog, cat, and human. Comparing the patterning of SNPs helps scientists identify the contribution of each SNP to the overall appearance of the animal. Scientists, such as Angela Hughes, an animal geneticist at Mars Petcare, analyze SNP patterns that characterize dog breeds. Using dogs that people have been specifically breeding for generations, Hughes and her team look for distinctive SNPs in their DNA. To identify a dog's lineage, they plug in 1,800 gene sequences each with its own SNP. A computer algorithm uses this data to find the best match between this pet and the known SNPs of purebred breeds. There are several other ways to test a dog's DNA. Adam Boyko founded a company called EmBark that similarly tests DNA using more than 200,000 different genetic fingerprints that sit close to each other on chromosomes. When animals mate, chunks of their chromosomes end up close to each other, allowing scientists to trace those chromosome segments back to the parents who passed them on.

While there are more researchers in dogs than cats, there are DNA tests for cats too. Grahan, a geneticist at the University of California, Davis, notes that there are fewer cat breeds and most cats are not really one "breed" or another. People who breed cats still want to know about their pet's family tree. A genetic test, such as Basepaws, allows cat owners to detect cat breeds in their pets. The test looks for SNPs similar to dog DNA testing. These DNA tests can not only inform owners about genetic characteristics such as fur length and coat color, but can also reveal a cat's geographic ancestors, giving owners the chance to learn about their pet's genetic makeup.

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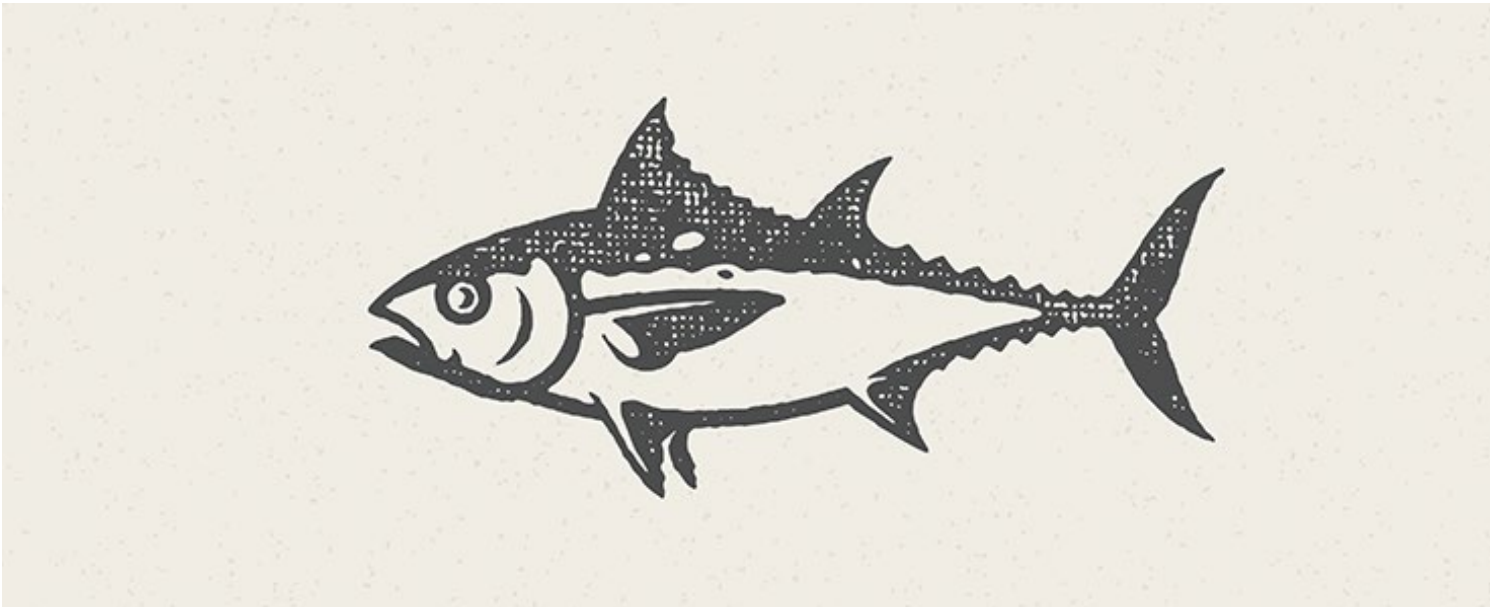
Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	FDA DNA Testing at Wholesale Level to Evaluate Proper Labeling of Seafood Species
Full Text	*US Food and Drug Administration (FDA, June 2018)*

The FDA developed the Seafood List to ensure that seafood is labeled truthfully with acceptable market names and to assist manufacturers in labeling seafood products. In recent years, there have been reports of seafood in the United States being labeled incorrectly. Consequently, the FDA started testing the DNA of fish with a history of being mislabeled, attempting to determine the accuracy of marketing claims. This testing focused on fish collected from US distribution chains and on seafood collected at import points.

The sampling efforts targeted seafood reported to be at higher risk of mislabeling or substitution. This includes cod, haddock, catfish, basa, swai, snapper, and grouper. The findings from their testing showed that fish species were labeled correctly 85 percent of the time. The FDA will use the results from their testing to guide future sampling, enforcement, and education efforts designed to ensure that seafood in the US market is labeled with acceptable market names for the species.



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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	Europe: DNA Shows Food Labeling Accuracy
Full Text	*FNI Team, Food News International, August 2016*

DNA testing is a useful tool to verify the ingredients listed on nutrition labels. While DNA sequencing technology is expensive and difficult to interpret, DNA sequencing technology can help reduce mislabeling and contamination in commercial foods. There have been several food scandals in the last decade in which melamine, a chemical found in plastics, has been added to infant formula and horsemeat has been sold as beef. As a result, consumers demand better assurance that foods are properly tested and labeled.

A student at the National Food Institute, Technical University of Denmark examined the feasibility of using whole genome sequencing to analyze the components of foods and determine if the product's label is accurate. In their study, whole genome sequencing was carried out on 22 burgers from around the world. The results were mapped against 15 reference libraries containing the DNA profiles of most living organisms. The study showed that this method has great potential as almost all components were identified correctly. An initial data analysis mistakenly showed that between 3–5 percent of DNA came from macaque monkeys, highlighting the shortcomings in this technology. This error shows that specialist knowledge is often required to analyze the results correctly. Furthermore, there is a need to expand the reference libraries and to improve both sequencing technology to yield longer reads and analysis methods. The study also found a considerable amount of *Bacillus cereus*, a bacteria known to cause food poisoningthough it might have grown on the sample during transit to the laboratory. This highlights the potential of this technology to expose mislabeled foods while also identifying contamination from microorganisms that can cause diseases.

While whole genome sequencing has displayed its usefulness in monitoring food fraud and contamination, the technology is still prohibitively expensive, and the data can still be difficult to accurately interpret. As the technology improves and becomes more cost effective, the National Food Institute expects that it will become a staple of the food industry.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	Are Your Food and Vitamin Labels Lying to You?
Full Text	*Catey Hill, Market Watch, February 2015*

DNA testing can be used to test whether the labels on foods and supplements are accurate. A recent study from the Attorney General of New York showed that supplements sampled from stores, such as GNC, Target, Walgreens, and Walmart, contained just 21 percent DNA from the herbs and plants listed on the label. Another study in 2013 published in the journal *BioMed Central (BMC) Medicine* supported these claims. In their study, they analyzed 44 bottles of herbal supplements from 12 different companies. They found that one-third of the supplements tested did not contain the supplement advertised. Many supplements contained ingredients, such as wheat and rice, that were not listed on the label. Similarly, a study from the Journal of American Dietetic Association found that the calorie content is 8 percent higher on average than what is listed on food labels and 18 percent higher than what is shown on restaurant menus. Both the manufacturer and lack of government regulation are responsible for these discrepancies. For naturally occurring vitamins, minerals, protein, carbohydrates, dietary fiber, polyunsaturated fats, monounsaturated fats, and potassium, the FDA requires that an item "must be present at 80 percent or more of the value declared on the label." If those nutrients are added to food, rather than naturally occurring, they must meet 100 percent of the label claim. Similarly with calories, sugars, total fat, saturated fat, cholesterol, and sodium, the ratio between the amount obtained through laboratory analysis and the amount listed on the label has to be less than 1.2× or less to be compliant with FDA regulations. Taken together, food manufacturers tend to put more food in the package than they state on the label. The majority of packaged foods in the United States are manufactured by corporations who have internal processes for checking label accuracy to prevent consumer backlash and legal issues. Mislabeling is more common in smaller companies, but the most alarming issues are found in supplements, which are less regulated. The FDA primarily regulates supplements as a way to prevent adverse effects. The supplement industry has supply chain issues as well. Manufacturers often purchase their supplies online and import them from other countries, and might not bother to verify their quality or efficacy upon arrival. Some smaller companies might not even be capable of conducting these tests. When ingredients are not listed, they can be deadly to those with allergies and sensitivities. DNA testing provides a solution to mislabeling and, as DNA testing becomes more accessible, can help improve the accuracy of nutrition labels and reduce consumer skepticism.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	New York Attorney General Targets Mislabeled Herbal Supplements
Full Text	*Hansi Lo Wang, National Public Radio (NPR), February 2015*

Major retailers Walmart, Target, GNC, and Walgreens have been accused of mislabeling herbal supplements by the New York State Attorney General's office. DNA testing showed that store brand supplements do not contain all the ingredients listed on the label. The New York State Attorney General's office has sent cease-and-desist letters to the four major retailers for the herbal supplements ginseng, St. John's Wort, *Echinacea*, and *Ginkgo biloba*. In their tests, they found that almost 80 percent of pills did not contain the key plant ingredient listed on the label, but rather included fillers, such as rice and beans and even wheat, mustard, or radish. This report calls into question the credibility of supplement manufacturers and consumer choices to trust the brands they purchase. However, executives in the herbal supplement industry have called into question the testing methods and results. They believe that the active chemical ingredients are still present but cannot be detected using the testing conducted by government officials. The testing methodology conducted by New York officials has not been disclosed publicly, which makes it difficult to interpret whether manufacturers in the supplement industry are accurately labeling their products. The issue can be attributed to the regulations of herbal supplements enforced by the US Food and Drug Drug Administration (FDA). FDA approval is not required for new supplements unless manufacturers claim that they contain a new ingredient, though more comprehensive DNA testing can help scientists and consumers better understand the ingredients listed.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	Herbal Supplements Are Often Not What They Seem
Full Text	*Anahad O'Connor, New York Times, November 2013*

Americans spend nearly $5 billion a year on unproven herbal supplements claiming to fight off colds, curb hot flashes, and even boost memory. However, DNA tests show that many pills considered to be healing herbs in fact do not contain the ingredients listed. DNA barcoding, a kind of genetic fingerprinting, has been used to uncover mislabeled commercial seafood. Canadian researchers are now using this technology to test 44 bottles of popular supplements from 12 companies. They discovered that pills labeled as herbs were often either diluted or replaced entirely by fillers such as soybeans, wheat, and rice. Consumer advocates and scientists alike have called into question these practices, while representatives from the supplement industry argue that this is not indicative of the industry as a whole or widespread.

In the study, researchers selected popular medicinal herbs from different brands in Canada and the United States to conduct their testing. They found that bottles of *Echinacea* supplements used to prevent and treat colds contained bitter weed, *Parthenium hysterophorus*, which is an invasive plant native to India and Australia that is linked to rashes, nausea, and flatulence. Two bottles of St. John's Wort, which several studies have shown to treat mild depression, contained no medicinal herb. Instead, the pills were nothing but rice or Alexandrian senna, an Egyptian yellow shrub that is a laxative. *Ginkgo biloba* supplements were found to be mixed with filler and black walnut, a potential hazard for people with allergies. Of the 44 herbal supplements tested, one-third showed outright substitution, meaning there was no trace of the plant advertised on the bottle. In some cases, fillers were the only plants detected in the bottle.

Because these findings rely on DNA testing, they offer perhaps the most credible evidence to date of adulteration, contamination, and mislabeling in the medicinal supplement industry. With that in mind, health professionals cannot easily recommend herbal supplements to consumers with confidence. Policing the supplement industry has been a difficult task. The US Food and Drug Administration (FDA) requires that companies test the products they sell to make sure that they are safe, but supplements are generally considered safe until proven otherwise. By law, they can only be pulled from shelves after causing serious injury, and they can be sold and marketed with little regulatory oversight. As FDA resources can be limited, the University of Guelph developed a system of DNA barcoding in the early 2010s. Instead of full genome sequencing, scientists began examining genes from a standardized region of every genome to identify species of plants and animals. These short sequences can be quickly analyzed, much like bar codes in a supermarket, to compare them to an electronic database. The reference library at the University of Guelph contains over 2.6 million barcode records for almost 200,000 species of plants and animals. While the testing is not foolproof, it can identify substances in a supplement, but it cannot easily detect chemical extracts without genetic material.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	Herbal Supplements Often Contain Unlisted Ingredients
Full Text	*Rachael Rettner, Live Science, October 2013*

Many supplements contain ingredients not listed on their labels. In the study published in the journal *BioMed Central (BMC) Medicine*, Steven Newmaster and colleagues at the University of Guelph found that nearly 60 percent of herbal products tested were found to contain substances not listed on the label. Almost a third of those products had the primary ingredient mislabeled or substituted with an alternate. More than 20 percent contained fillers, including wheat, soybeans, and rice. Unlisted ingredients post a health threat to consumers by not disclosing accurately the ingredients they contain. For example, one product labeled as St. John's Wort actually contained the laxative Senna alexandrina, which has known side effects of chronic diarrhea and liver damage. Other products contained walnut leaves, wheat, soybeans, and rice, which are problematic for people with allergies or those seeking gluten-free products. The findings in this study are consistent with earlier work. In a 2011 study, 131 herbal teas were tested and 33 percent were contaminated. Yet, the estimates from the new study should be interpreted with caution and warrants further research because the study tested only 12 out of thousands of companies in the herbal supplement industry.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Food and Supplement Labeling
Title	The DNA-Based Authentication of Commercial Herbal Products Reveals Their Globally Widespread Adulteration
Full Text	*Mihael Cristin Ichim, Frontiers in Pharmacology, October 2019*

Herbal products are perceived as low risk because they are natural and safe, yet the quality and composition of these products are ineffectively regulated by the FDA. The growing evidence for their lack of authenticity is a deep concern, but the scale of this issue is unknown. In the present study, Mihael Ichim at the National Institute of Research and Development for Biological Sciences in Romania analyzed 5,957 commercial herbal products sold in 37 countries, distributed in all six inhabited continents. His team's global survey shows that 27 percent of herbal supplements are adulterated and do not contain ingredients listed on their labels. The proportion of adulterated products varies between continents, beinghighest in Australia and South America, lower in Europe, North America, Africa, and lowest in Asia. The findings of their study confirm the large-scale presence of adulterated herbal products in the global market. These products contained undeclared contaminants, substitutes, filler species, or none of the labeled species. Global widespread adulteration is a serious threat to consumers' well-being and safety in spite of the herbal products' claimed or expected health benefits. The description of the current global situation serves as a useful resource for regulatory officials and consumers alike, highlighting the risks of adulterated herbal products to human health.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Pets
Title	Why You Can't Always Trust Pet Food Ingredient Labels
Full Text	*Herb Weisbaum, Today, October 2014*

According to a test performed at Chapman University, commercial pet foods may contain ingredients not listed on the label or mislabeled ingredients. Using DNA analysis, researchers were able to determine the types of meat the commercial foods contained. Almost 40 percent of the products had meat that was not listed on the label. Of the 52 samples, 31 were labeled correctly, 20 were potentially mislabeled, and one had a meat ingredient that could not be identified. It is difficult to know whether mislabeling is accidental or intentional without careful examination of suppliers and manufacturing plants, but DNA testing provides a useful tool to analyze the composition of commercial pet food. The Food and Drug Administration (FDA) regulates the product labeling of both human and pet food. It believes that "consumers should be able to trust that what is on the label is in the product." Pet foods do not require FDA approval prior to being marketed, though all ingredients must be listed on the label by common name. In 2012, ELISA technologies tested 21 commercial dog foods, and discovered 12 separate mislabeling instances. In the Chapman study, 16 out of 52 samples had a main ingredient of meat that was not found on the label, usually pork, which is a common food allergen for pets. A small amount of pig liver, if included in the product, would be more than enough to cause a catastrophic reaction in a dog or cat.

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Community Science Resource Packet: Human Interest

Continued

Subtopic	Textiles Labeling
Title	Keeping Synthetic Textiles Real and Sustainable
Full Text	*Applied DNA Sciences*

Textiles, such as the ones used in clothing, and other materials are produced around the world, and the environmental and safety regulations for textile manufacturing can differ significantly from one country to another. As demand for ethically and sustainably produced fabrics grows, companies need tools for tracking the sources of various materials used in their products. One method of tracking the source of these materials is DNA identification. Applied DNA Sciences is a company that uses DNA technology to help companies make sure the materials they are using are environmentally safe. This system, called the CertainT solution system, uses DNA to tag, test, and track textiles to confirm their genetic identity.

A unique molecular tag is added to raw materials, finished products, labels, and packaging. The materials are verified throughout the supply chain by confirming the presence of the unique molecular tag. In textiles, their sequencing technology can be applied to many materials, including leather, wool, cotton, hemp, viscose, thread, down, coatings, and recycled plastic fabric (RPET). This technology can confirm the sources of fabrics, and can verify claims made about a brand's product. For cotton textiles, the company can use DNA to distinguish between different types of cotton, while also testing for GMO cotton. The unique molecular tag can also be used with threads, printed inks, labels, and packaging.

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Community Science Topic Overview Capture Sheet

Part 1: Background Research

Directions

Complete the table below by selecting resources from the Resource Packet on your topic.

Topic

Resource Used			
What problem or issue was being addressed in this example? Source:			
How was DNA technology used to solve the problem or issue?			

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Community Science Topic Overview Capture Sheet

Part 2: Group Discussion

Directions

Discuss the following with your small group. Record your ideas on this sheet to use in the creation of your topic summary board.

1. What was the most interesting example that you learned about this topic?

2. *Science is a subject for all.* How does your research support or refute this statement?

Continues next page >

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Community Science Topic Overview Capture Sheet

Part 2: Group Discussion

Continued

3. Think about all the different examples you examined. Make a list of several general "subtopics" in the larger topic that have been addressed using DNA technology.

4. Which of the issues or problems from these examples also affect a community of which you are a part? How could you see one or more of these examples used in your community?

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Community Science Topic Summary Board Template

Directions

As a group, add text and images to share an overview of your exploration of Community Science topic with others. Groups can make a copy of this template or use another online tool to accomplish the task.

Topic

Team Members

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Learning Artifact Graphic Organizer: Coyote Example

Directions
Read the article, then reflect on each prompt and discuss your ideas with a partner.

Example	*Coyote attacking several San Francisco residents*
Article	*Search For Coyote Continues After Several Attacks In The San Francisco Bay Area*
What communities were involved?	
What was the problem they were trying to solve?	
How did they use DNA to solve this problem?	
How did they get community members involved?	

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Learning Artifact Graphic Organizer

Directions
Select and read an article, then, reflect on each prompt and discuss your ideas with a partner.

Example

Article

What communities were involved?	
What was the problem they were trying to solve?	
How did they use DNA to solve this problem?	
How did they get community members involved?	

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Final Topic Interest Survey

Directions
Complete this survey to share the topic you would like to focus on for the remainder of the unit with your teacher.

_____

What is your name?

Rank the topic areas from most interesting (1) to least interesting (3).

	1	2	3
Environmental Issues			
Food and Safety Issues			
Human Interest Issues			