DNAdots™

Simple explanations of modern genetic technologies

miniPCR bio®

Environmental DNA (eDNA)

What it is:

Getting DNA from soil, water, and even from thin air

Imagine you want to study a particular animal, but this animal is difficult to find or catch. You might trace their tracks to determine where they've been, or study their feces to learn what they eat. Biologists have always chosen to study some organisms in this way, sometimes to avoid disturbing the organisms, sometimes because it's too difficult to get any closer to them.

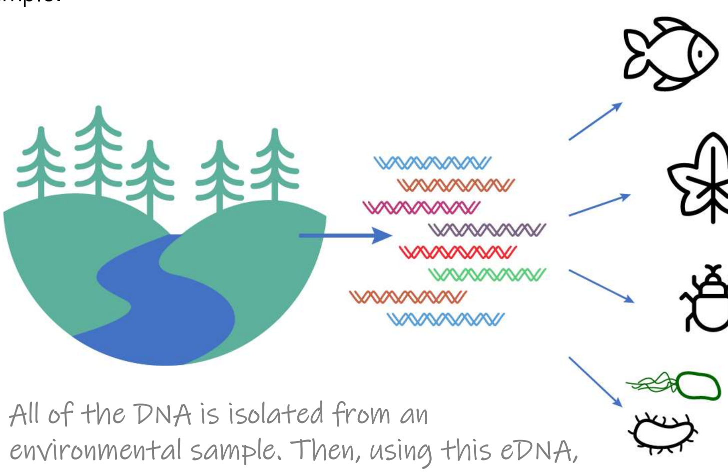
But scientists are now taking advantage of the fact that organisms leave behind more than their tracks; they leave DNA practically everywhere they go, even in the air that surrounds them. By developing methods to extract DNA directly from soil, water, and air samples, scientists can study organisms without ever even seeing them. We call DNA collected from the environment in this way *environmental DNA*, or *eDNA*.

eDNA can answer a wide variety of scientific questions. Often it is used in *metabarcoding* studies; these studies try to identify large collections of organisms that live in a particular place from a single eDNA sample. Or it can help scientists track specific organisms, for example to see if an endangered species lives in a particular area. While the nature of eDNA studies can differ, what unites eDNA as a field is the way scientists get the DNA—directly from the soil, water, or even air and ice, and not from a tissue sample.

How it works:

It's all in the filter

Organisms' cells are constantly turning over and being left behind. Skin, hair, scales, and leaves are all constantly being shed, bringing DNA with them. DNA can also be shed when an organism defecates (poops), secretes mucus, reproduces sexually (especially aquatic organisms or plants that release pollen), or dies and decomposes. And of course, many organisms (e.g., bacteria and fungi) live and die directly in the soil, water, and even air.



All of the DNA is isolated from an environmental sample. Then, using this eDNA, scientists can learn about the organisms that were present in that environment.

DNA is a relatively stable molecule, so when DNA enters the environment, it tends to stay there, at least for a while. How long can depend on the environment. Heat, UV radiation, environmental chemicals, and DNA-digesting enzymes called *nucleases* are all present in the environment and will break down DNA over time. This means that in some environments DNA is relatively short lived, so its presence indicates organisms that have been there recently. In others, DNA may last for thousands or sometimes even millions of years, giving scientists a deep record of life in a particular place.

In an environmental sample, there is typically far less DNA than is present in a biological tissue sample. This means that scientists have had to develop new ways of isolating DNA that may be present in low concentration. For soil samples, chemical buffers are often added to help release DNA from any cells that are available. Then, the sample is passed through a series of filters and columns that isolate the DNA, concentrate it, and remove contaminants. For water or air samples, where DNA may be in extremely low overall concentration, filters are first used to concentrate biological molecules. Large volumes of water are typically passed through extremely fine filters, and for airborne eDNA, vacuum pumps push air through concentrating filters, capturing airborne particles. eDNA is then extracted from the material left on these filters.

DNA that is gathered in an eDNA sample is likely to be an extremely complex mix from all sorts of different organisms. This means that even with concentrating filters, the DNA for any one particular type of organism or gene will usually still be relatively rare. For this reason, a common next step is to amplify DNA sequences of interest using PCR. The sequences produced by PCR will then be read by DNA sequencers and analyzed to identify what organisms or genes were in the sample—and therefore present in the environment.

Opening new areas to genetic research:

Aquatic, ancient, microbial, oh my!

eDNA analysis has been described as a game changer for aquatic and marine studies. Not only are organisms that live underwater inherently more difficult to catch and study, but molecules are constantly moving through water by diffusion. This means that a relatively small sample of water is likely to contain virtually all of the DNA that can be found in a particular area. With new portable DNA technologies (like miniPCR® thermal cyclers) and DNA sequencers, biologists can often accurately identify all the organisms living in a particular body of water, from microorganisms to fish and plants, all in a matter of hours without ever leaving the field.

eDNA has allowed microbiologists to study organisms like bacteria and archaea in ways that were previously not possible. Traditional microbiology methods involve isolating and culturing organisms (growing them in the lab). But many species of microbes are simply not well suited to growing in a lab. Using eDNA means that scientists can study all sorts of things about bacteria and other microbes without ever getting them to grow on a Petri dish. They can even study which biochemical pathways may be active in an ecosystem by surveying which genes they find in the environment.

In some environments, eDNA can last a very, very long time. For example, in marine sediments that lack oxygen, biological molecules don't easily decompose; similarly, samples that are frozen in permafrost or ice cores can be preserved for thousands of years. Using eDNA, scientists have been able to sequence the DNA of organisms found in these ancient samples, allowing them to reconstruct ancient environments with far more detail and accuracy than was ever possible before. It's a great reminder that the tracks we leave behind can be much more than just footprints.

Learn more:

- "What is eDNA?" Monterrey Bay Aquarium Research Institute mbari.org/what-is-edna/
- Stokstad, Erik. "DNA Pulled from Thin Air Identifies Nearby Animals." Science.org, July 20, 2021 LINK
- eDNA Project: Sampling Soil for Antibiotic Resistance—an authentic lab activity from miniPCR bio™ LINK

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Questions

Review:

1. Why might a scientist choose to study eDNA instead of directly collecting samples from organism?
2. What is metabarcoding?
3. What is a major difference between collecting eDNA and collecting DNA directly from a biological tissue?
4. Why has eDNA been so transformative for researchers who study aquatic organisms?
5. Why is it easier to study some microorganisms using eDNA than it is to study them in a lab?

Critical thinking:

1. One of the more common uses of eDNA is to perform metabarcoding studies. Before eDNA, what would a researcher need to do to gather the type of data a metabarcoding experiment can give you?
2. An interesting use of eDNA has been to collect samples from tracks that scientist find in the snow. What type of information might you get from the eDNA found in a track that may not be available just by finding the footprint? How might an eDNA sample from a footprint in the snow differ from a footprint left in mud?

Discussion:

1. In 2020, using eDNA, researchers in Brazil found DNA from a frog that was thought to be extinct for more than 50 years. Still, no one has seen the frog alive. How much effort should be put into protecting organisms that are found only through eDNA studies compared to animals that we can track more traditionally?

Answer key: For answers, instructors may email dnadots@minipcr. Please include the DNAdots subject, as well as your name, school, and title in the body of the email.