



# How Teachers Can Implement Environmental DNA (eDNA) Technology in the Classroom



**Marc Pedersen**  
**Paulding County High School**  
**Academy of Science, Research, & Medicine**



# Resources for Teachers- eDNA

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ABOUT



Students in the Biotechnology Pathway are conducting original and innovative research and are required to submit their findings for peer-reviewed publication in the [Journal of Emerging Investigators](#) by the end of their senior year. We have identified several model systems and techniques for our lab. Although some students are conducting research outside of these areas, we have chosen to provide details on our identified specialty areas of research:

- Environmental DNA (eDNA)
- Marine polychaetes (fireworms)
- *Neurospora crassa* (fungus)
- CRISPR (clustered regularly inter-spaced short palindromic repeats)

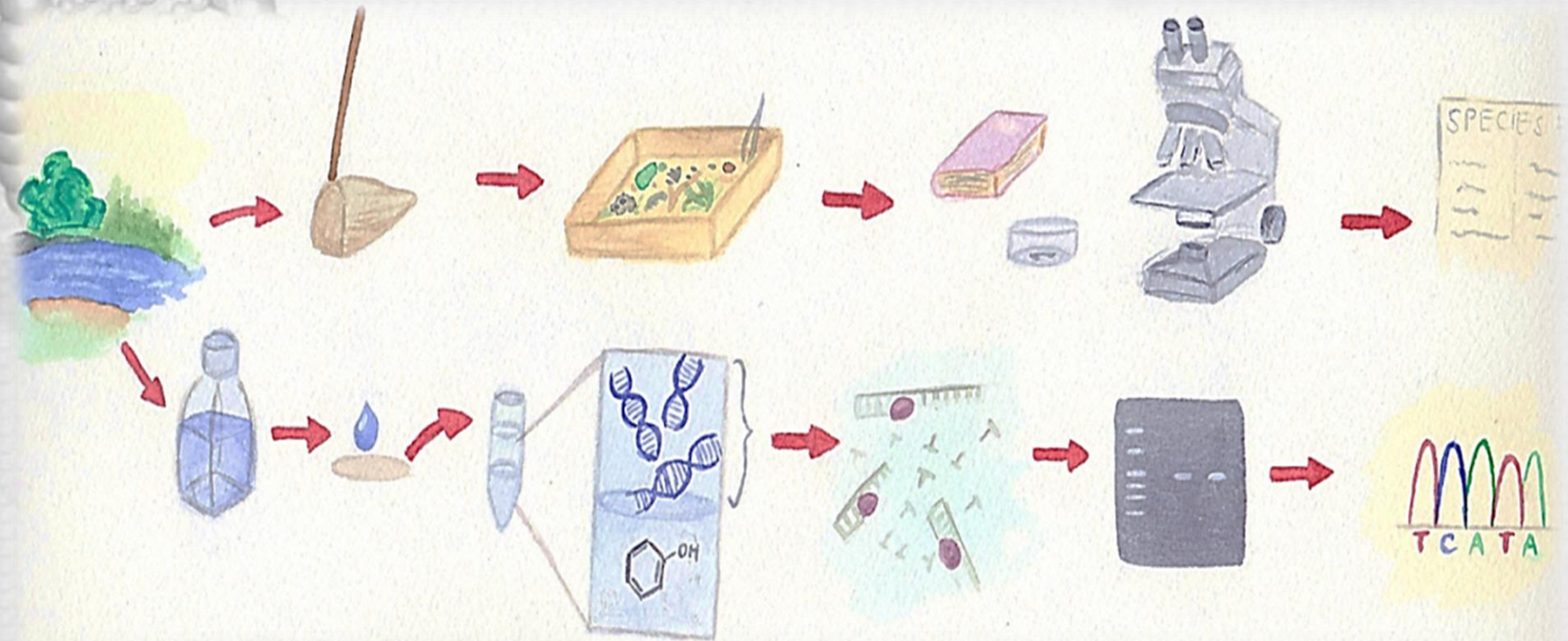
## ***Environmental DNA (eDNA)***

Environmental DNA (eDNA) is naturally released into an aquatic system in the form of skin cells, mucus, feces, carcasses, gametes, and hair. This type of DNA can be collected from water samples and isolated using special filters that capture the



# What is eDNA?

- Organisms naturally shed their DNA
- Skin cells, mucus, feces, carcasses, gametes, hair, etc.
- Material transported down waterway





# What is eDNA?

Working with environmental DNA (eDNA)

VIDEO



01:20



<https://www.youtube.com/watch?v=KR4TEhFcZPc>



# How can I collect eDNA?



Protocol #1: Hand pump



Protocol #2: Cordless driver



Protocol #3: 120-V pump

Step 2: Collect water sample



Direct

or



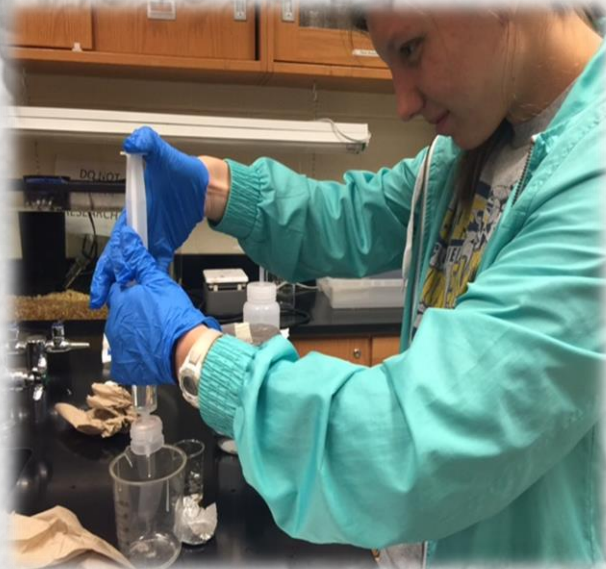
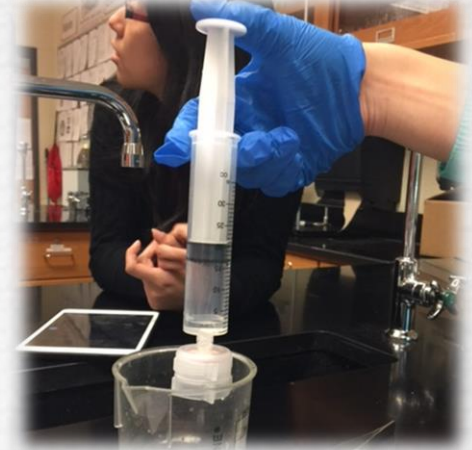
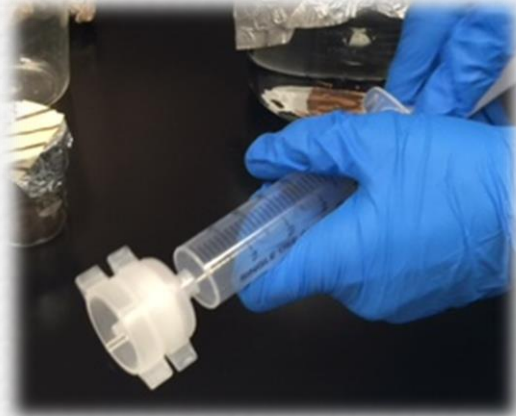
Collect and pour

Step 3: Preserve water sample





# How Can I Collect eDNA?

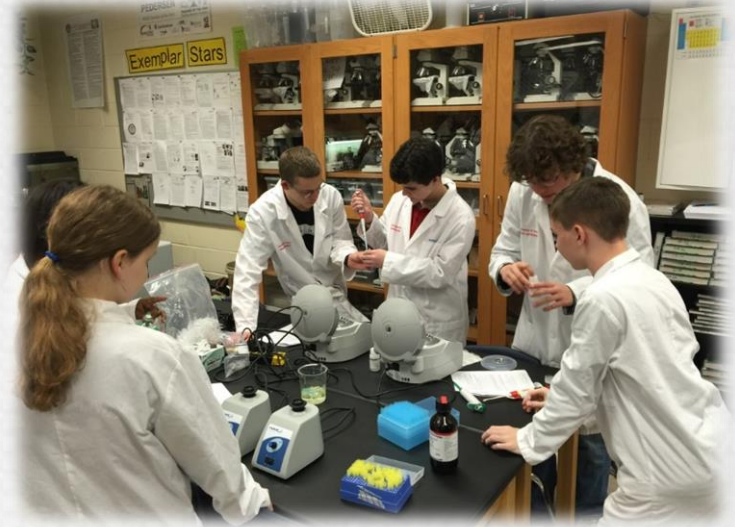




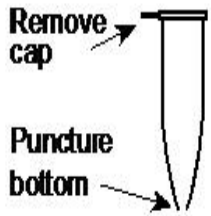


# How can I collect eDNA?

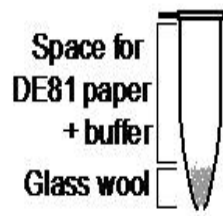
- Students used Qiagen Blood and Tissue Extraction Kits (\$150/50 extractions)
- Silica-based column chromatography produces high yield genomic DNA
- Simple salt-based extractions will also yield enough DNA.



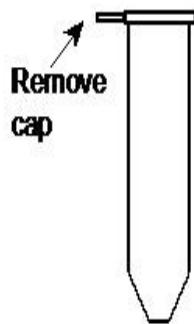
A 500  $\mu$ l tube



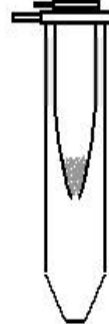
B 500  $\mu$ l tube



C 2 ml tube



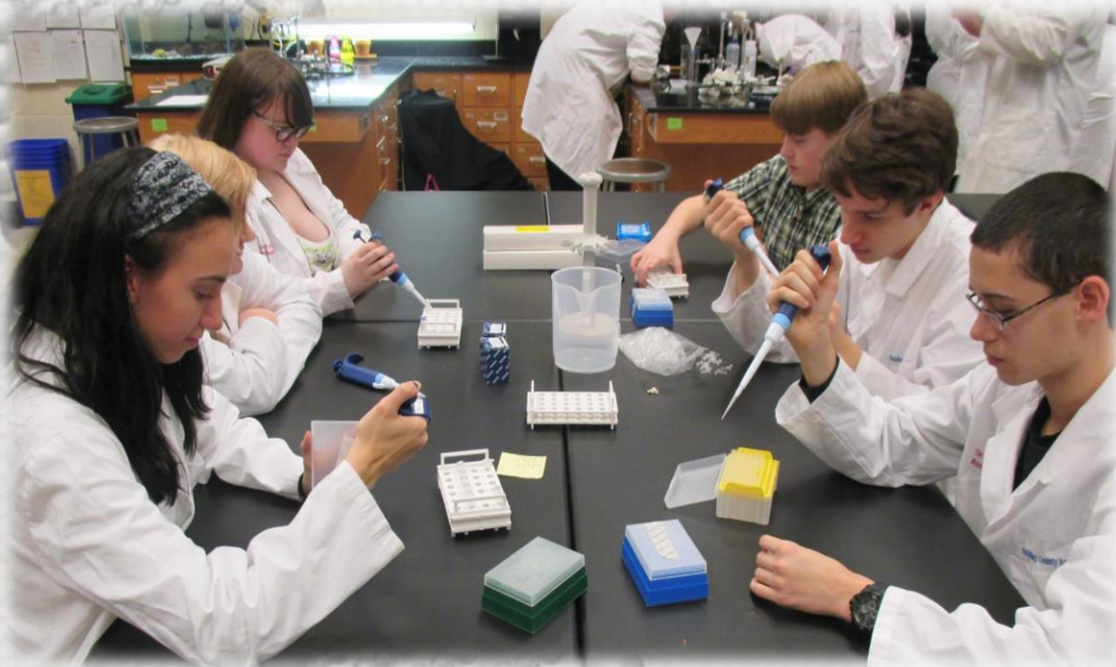
D Final assembly





# What can I do with eDNA?

- Polymerase Chain Reaction can be used to amplify specific genes.
- Thermal cyclers are becoming much more affordable (ex., T100 and miniPCR)





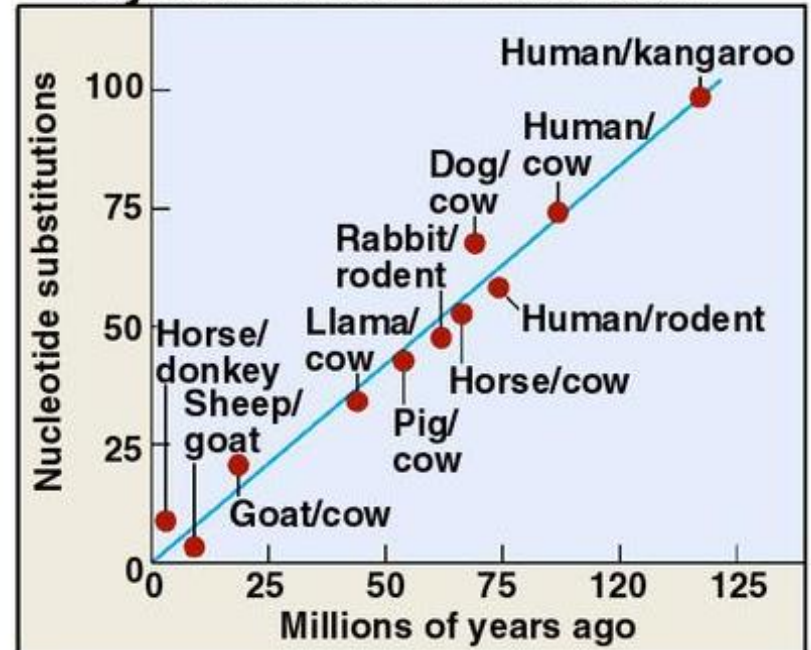


# What can I do with eDNA?

- The Cytochrome c oxidase 1 (COI) gene is highly conserved across species where energy is generated from mitochondria.
- COI gene can be used to identify individuals belonging to the same species, as well as to distinguish between individuals from different species.
- The rate that the gene sequence changes over time is slow enough so that it's likely to be identical in the same species, but fast enough so that it's different between species.

**Cytochrome C Oxidase  
Subunit 1 Gene (COI)**

### Cytochrome c Evolution









# What can I do with eDNA?

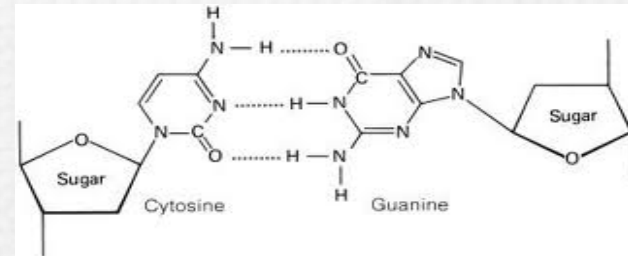
## DESIGN PCR PRIMERS

**BACKGROUND INFORMATION:** For sites describing PCR theory, and PCR techniques see [PCRLink.com](http://molbiol-tools.ca/PCR.htm). <http://molbiol-tools.ca/PCR.htm>

There are several excellent sites for designing PCR primers:

- [Primer3: WWW primer tool](#) (University of Massachusetts Medical School, U.S. nature of the primers, including size of product desired, primer size and T<sub>m</sub> range)
- [GeneFisher - Interactive PCR Primer Design](#) (Universität Bielefeld, Germany)
- [PCR Now](#) (Computational Biology Group, PathoGene, Southwestern Medical Center, defined coding sequences. Great control over primer properties. If you are interested in coding sequences, this is a great tool.)
- [Primer3Plus](#) - a new improved web interface to the popular Primer3 program
- [BiSearch](#) Primer Design and Search Tool - this is a useful tool for detection of mispriming sites and alternative PCR products in cDNA libraries
- [Primer-BLAST](#) was developed at NCBI to help users make primers through a BLAST search against user-selected database. The blast results are then automatically filtered to show only primer pairs that amplify a single region of the target sequence.
- [MFEprimer-2.0](#) allows users to check primer specificity against genome databases using a k-mer index algorithm to accelerate the search process for primer binding sites. It also checks for important characteristics, such as the sequence, melting temperature and self-complementarity. (Web Server issue: W205-W208)
- [RAPD-primer generator](#) (J. Wöstemeyer, Institute of General Microbiology and Microbiology)

- Primers are for the COI gene
- Primers have high guanine-cytosine content (40-60%)
- Use online primer generator



= T<sub>m</sub>  
(primer)



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RNAi Reagents & qPCR Assays

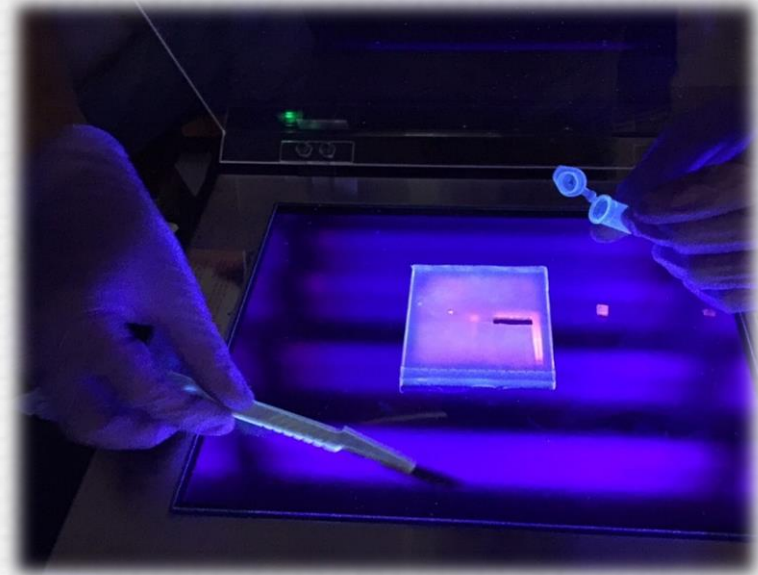
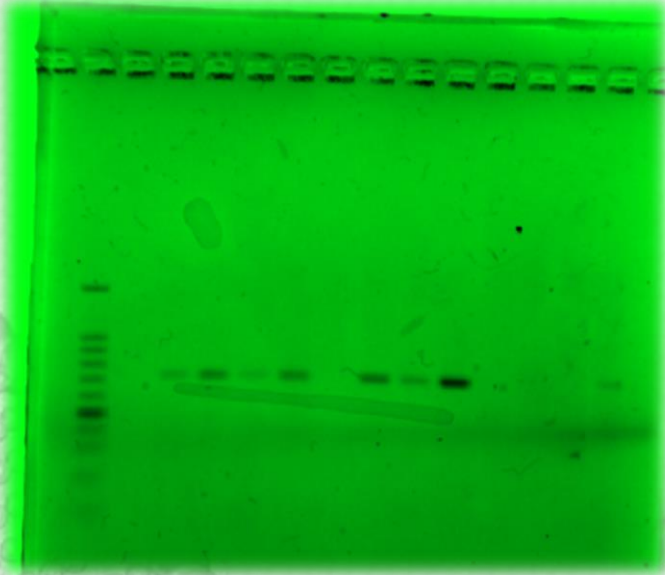


Custom Oligos

## Custom Oligos



# What can I do with eDNA?



Sequence File: COI-02-LCOI1490\_R.seq



>COI-02-LCOI1490\_R\_G06.ab1

```
NNNNNNNNNNNNNNNNNNNGCNTTCTCNGTTTTAGCAGGCCTTGTGGTGGTTTGTTTTCTGTACTATTTTCGTTTACAACCTAG  
CTTCTCCTGGTTCTGATTTTCTTGGTGGAGATCATCAGTTTTATAATGTATTAATTACTGCGCATGCGCTGATAATGGTT  
TTCTTATGATTATGCCAGCCTTATTTGGAGGGNCGGNTGNGGTTTGTTCCTTTAAAAAGAATTGAGCCAGAGGGGAATN  
TTTTTTTTAATTGTTGTGCAGAATAATTTGAACTTTTTTTTCTTTCACCTGGTTGTTGANTCTNACTTTTTCATATNTTTGTA  
ATGTTTATTTAAANTTTGAAGTTTATGAATTTANANATTAATTTTAAATNNNTATNNNAATGATATTTAAAGTTCAGTATT  
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TTCTCGGACTTTTTCGTCTGN CNAGCCCTGNTTCCNNNTTTCTCTTTCACATGGGCTGGAGNANGNNGAANNNGATGCGCT  
TNTNTCNAANCNTTTTTTATACNTAGNAAAAATTNCNCGTNTNTAANNATNAATACTTATCTTTNATCNCTTNTNGATN  
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CNNNNNAANAAAAAAA
```

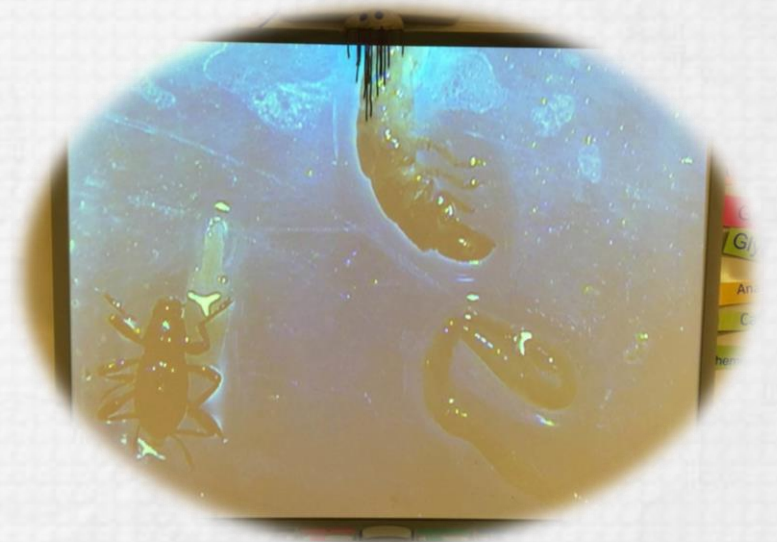




# Why should I teach eDNA?



- We need new ways to teach DNA and DNA technology.
- Technology is constantly evolving, so let's teach what is new and innovative.

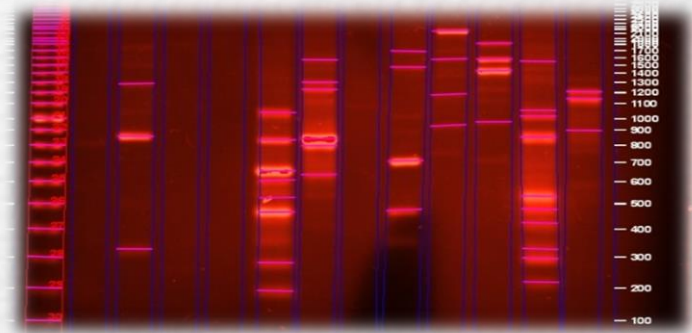
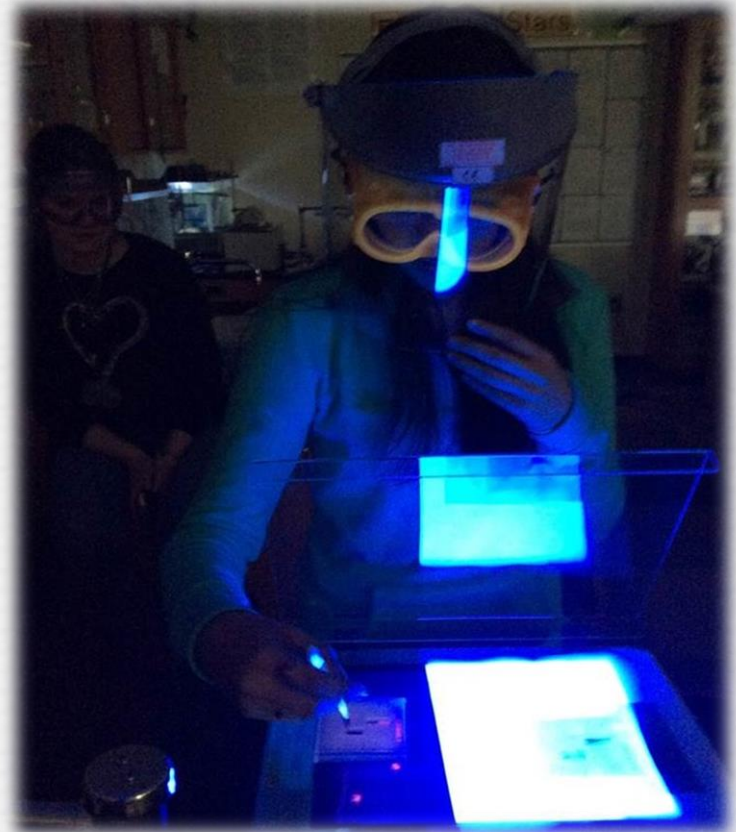


Pumpkinvine Creek, Paulding County GA





# Why should I teach eDNA?







# Why should I teach eDNA?

## DNA Evidence Evaluation

1. Turn your paper strips (DNA sequences) so that the side with the bases is facing you. The restriction enzyme cuts at every point it finds C C G G, always cutting between the C and the G. Label the back of the strips with the suspect number so that you don't get them confused after cutting. Use scissors to cut the DNA sequence at the C C G G points.
2. Count the number of base pairs (bp) in each piece of DNA that you created. Record the base pair number on the back side of the DNA fragment.
3. Make an enlarged chart like the one shown. Your teacher will give you paper for this. Use a ruler to ensure that the lengths are uniform.
4. Tape your DNA fragments to the chart, using the base pair numbers as a guideline for fragment placement.
5. Compare the crime scene DNA to the suspects and indicate on your chart, which suspect is guilty of eating the cheese.

Crime DNA	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Number of Base Pairs (bp)
					30
					29
					28
					27
					26
					25
					24
					23
					22
					21
					20
					19
					18
					17
					16
					15
					14
					13
					12
					11
					10
					9
					8
					7
					6
					5
					4
					3
					2
					1

12 bp



# Why should I teach eDNA?

- The concept of eDNA aligns with numerous Georgia standards, College Board Big Ideas, and Next Generation Science Standards.
- Biology, AP Biology, Forensics, Environmental Science, AP Environmental Science, Biotechnology

## 2016 AP<sup>®</sup> BIOLOGY FREE-RESPONSE QUESTIONS

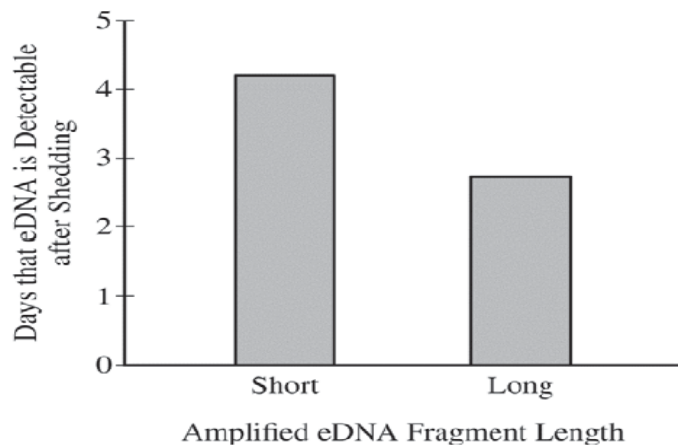


Figure 1. Detectability of eDNA fragments of varying lengths

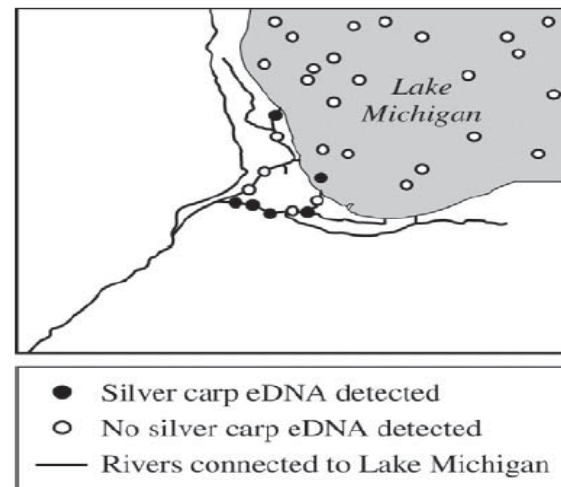


Figure 2. Map of the waterways that connect a nearby river system to Lake Michigan

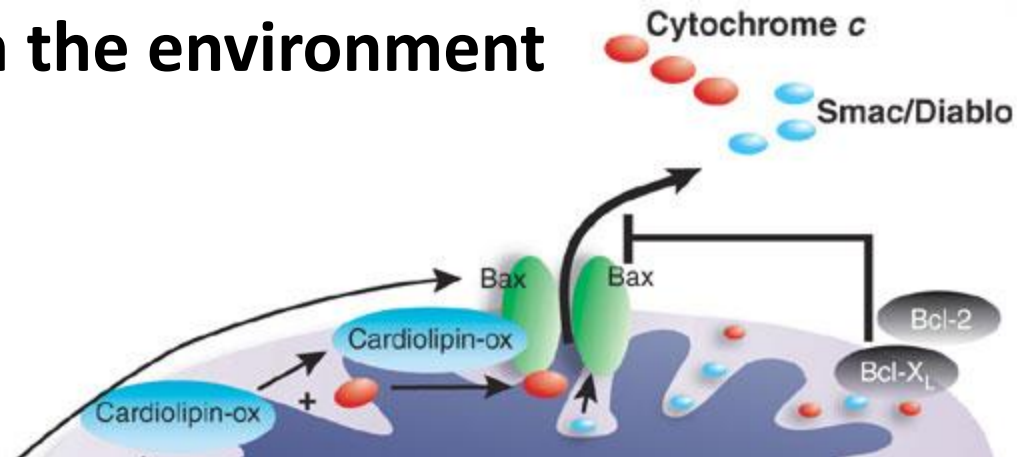




# Why should I teach eDNA?

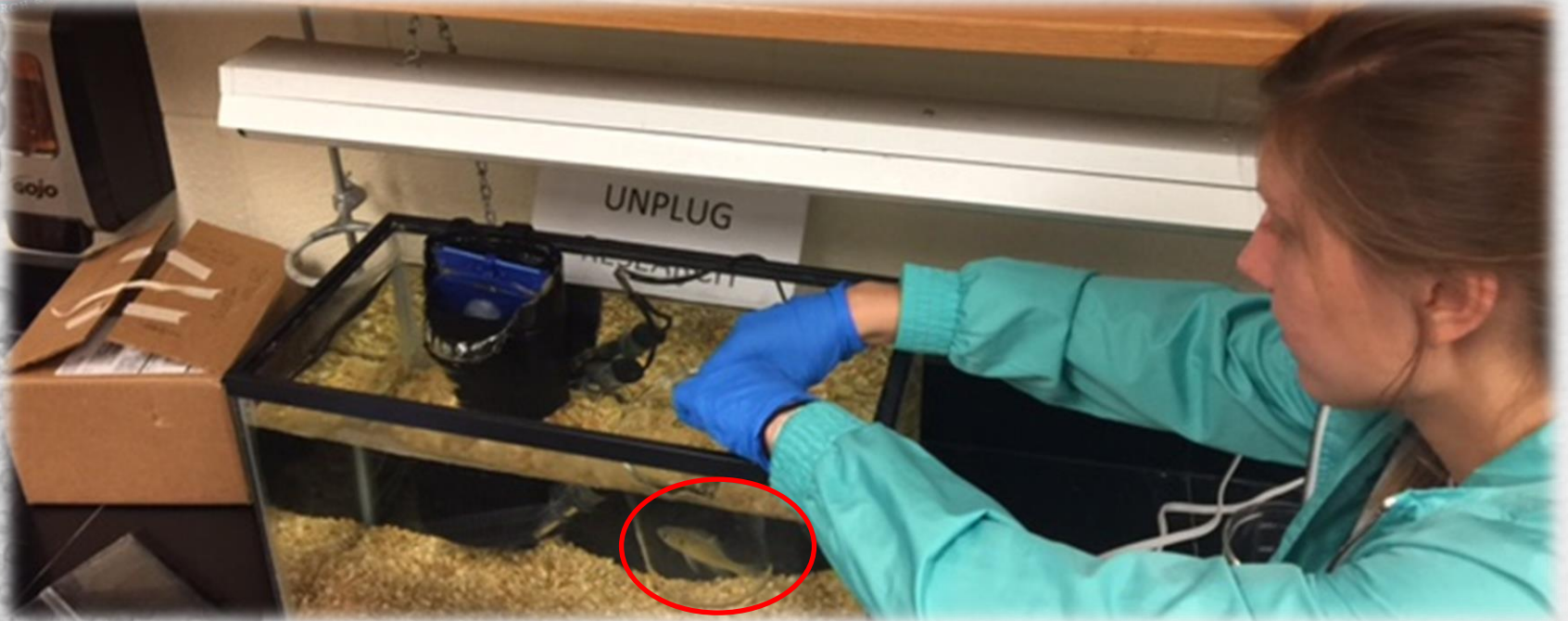
- Standards dealing with DNA and DNA technology
- Standards dealing with cell structure and function
- Standards dealing with classification and evolution
- Standards dealing with ecosystems and biomes
- Standards dealing with the environment

**Where do I start?**





# How can I start teaching with eDNA?







# How can I start teaching with eDNA?

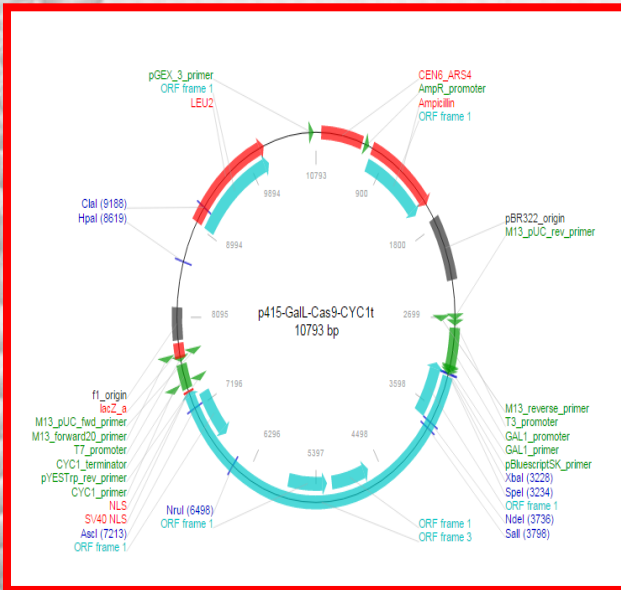


- Identify your teaching goals based on the standards and the level of students you are targeting.
- Even if you do not have the equipment, it is still possible to explore the idea of eDNA.
- Form partnerships with local colleges and universities.
- Seek funding through grants.



# How can I start teaching with eDNA?

- Think BIG- anything is possible!



Dear Dr. Church,

Sorry to bother you as we know you are very busy. We are a group of high school students at the Paulding County Academy of Science, Research and Medicine, the county's only magnet school. We are conducting a research project in our Honors Scientific Research Class on the viability of transforming *Neurospora crassa* with the CRISPR cas system. In our research, we came across the paper "Efficient Gene Editing in *Neurospora crassa* with CRISPR technology" and with slight modification, have decided to attempt to replicate the results using a different knockout strand of *Neurospora* known as His 3. We read that you donated the p415-Gall-Cas9-CYC1t plasmid that the researchers used to transform into CRISPR. We are hoping that you would be willing to send any materials like plasmids containing CRISPR, or the sequences and maps so we could reconstruct them here. We are more than happy to pay for any materials or information if that is an issue. If it is too much to ask, is there any guidance or another person you know that can help us in any way in replicating this experiment. We know how busy you are and any way you can help us would make a difference in the science we are doing and would be greatly appreciated.

Thank you for your time.



**George Church is a Professor of Genetics at Harvard Medical School**





# Project-based Learning with eDNA?

- Detect and monitor extirpated species of mussels using eDNA
- Georgia DNR and KSU provide positive controls
- Professor at Cornell helps to design species-specific primers

Dr. Ensign Speaks to Magnet-Raccoon Creek Watershed (eDNA)





# How can I start teaching with eDNA?

- 25mm Swin-Loc filter housing (from Whatman, #7011782)
- Ahlstrom Glass microfiber filters, 25mm (#101804)
- Sterile 50 mL Lauer-Loc syringes
- All of the above items are available through VWR







# How can I start teaching with eDNA?

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  - Antibodies Advanced Search
  - Invitrogen™ GeneArt™ Engineered Cell Models

Sequence Name:

Sequence: 5'

Sequence Length:

Modifications:

Scale:  ▼

Purification:  ▼

Oligo Quantity:

Order Reverse Complement:  ▼

Normalization Requested:  ▼

Ship Wet:  ▼

Concentration:  μmol/L

Volume:  Microliters [μL]

Notes:



# How can I start teaching with eDNA?

Sequence Name:

Folmer R HC02198

Sequence: 5'

taaacttcagggtgaccaaaaaatca

Sequence Length:

26

Modifications:

[Add Modification](#)

Scale:

10 nmole (60 mer limit) ▼

Purification:

Salt-Free (5-125 mers) ▼

Oligo Quantity:

1

Order Reverse

No ▼

Complement:

Normalization

Requested:

Yes ▼

Ship Wet:

Yes ▼

Concentration:

100

μmol/L

Volume:

50

Microliters [μL]

Notes:

TE

Please do not add modifications in this field. Use the **Add Modification** button above.

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[Upload Oligos](#)

[Add to Order](#)





# How can I start teaching with eDNA?

- If thermal cycler available use the following profile:

**35 cycles at:**

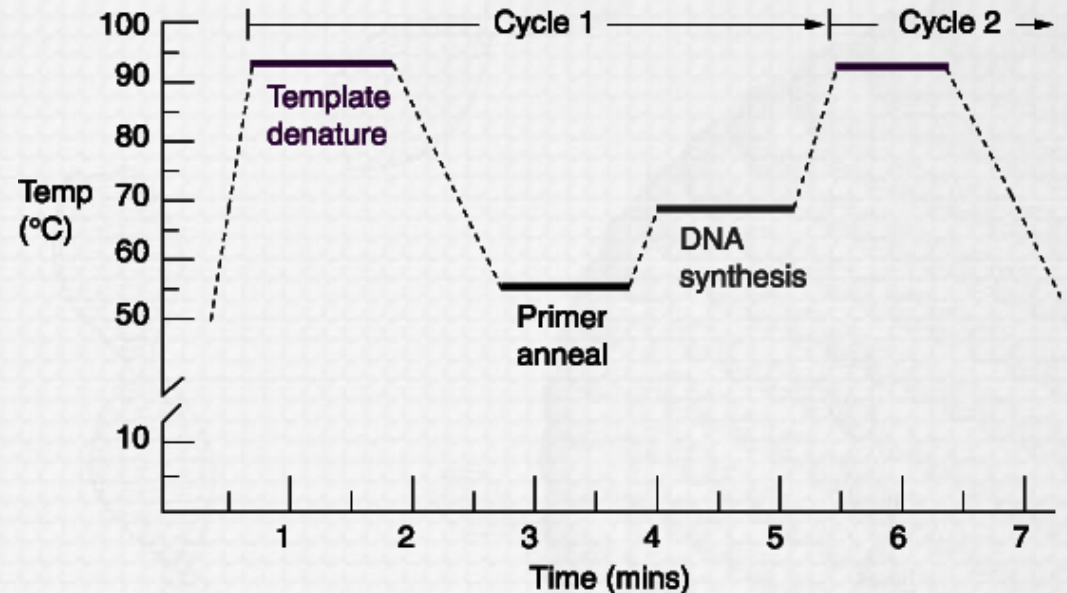
**3:00 minutes at 95°C**

**1:00 minute at 95°C**

**1:00 minute at 40°C**

**1:30 minutes at 72°C**

**7:00 minutes at 72°C**

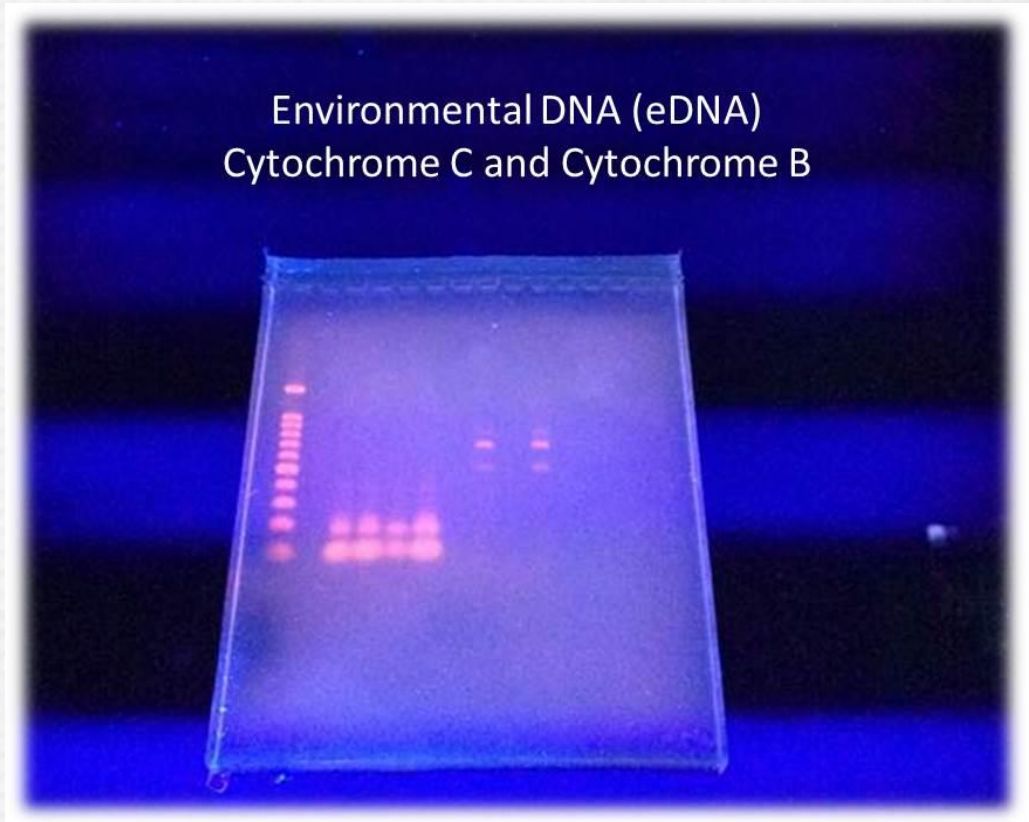


- If unable to quantify DNA, try varying amounts from 1 $\mu$ L- 5 $\mu$ L increasing in 0.5 $\mu$ L increments.
- Start with 2.5  $\mu$ L of each primer and decrease in 0.5 $\mu$ L increments if needed.



# How can I start teaching with eDNA?

- Resolve DNA on a 1.5% agarose gel at 100 volts for 90 minutes.
- If purifying amplicons, use TBE gels and buffer.
- Stain using Fast Blast or SYBR green





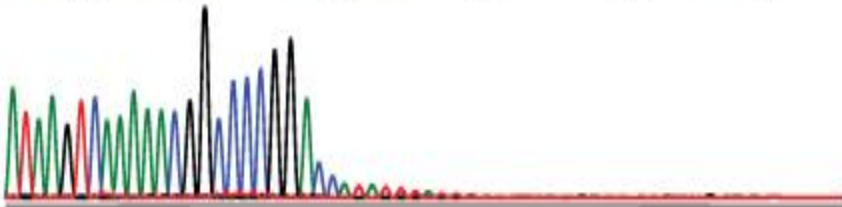


# How can I start teaching with eDNA?

- Purify amplicons using QIquick Gel Purification Kit (Qiagen; \$100)
- Sequence genes using GENEWIZ Sanger Sequencing (\$6 each; email me for protocol suggestions)

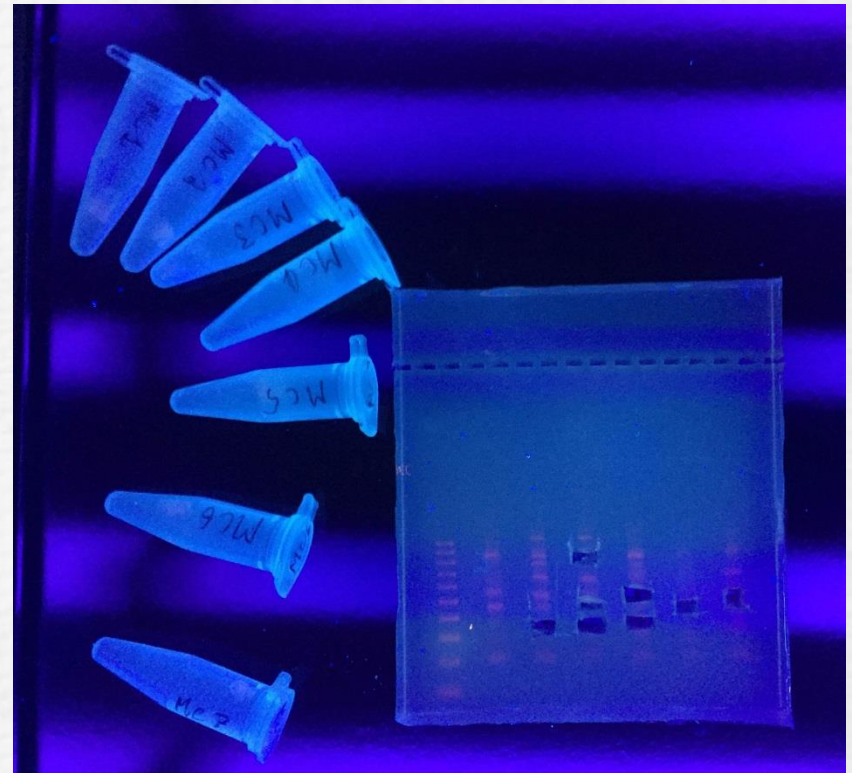
## shRNA template sequencing - standard protocol

ATAAGTCAAAAACGGCCCCGGACCATATTTNNNNNNNNNGNNNNNTGANNNNN



## shRNA template sequencing - GENEWIZ proprietary protocol

ATAAGTCAAAAACGGCCCCGGACCATATTTATTGCATGACTGCTGCAGCAGTCATGCAA





# How can I start teaching with eDNA?

- Use BLAST to identify species, which could be another AP biology lab).

https://blast.ncbi.nlm.nih.gov/Blast.cgi

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology

## BLAST<sup>®</sup>

### Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. It compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.

### Web BLAST

#### Nucleotide BLAST

nucleotide ► nucleotide

#### BLAST Genomes

Enter organism common name

Human Mouse



Sequence File: LM02-CytB\_F.seq

>LM02-CytB\_F\_B12.ab1

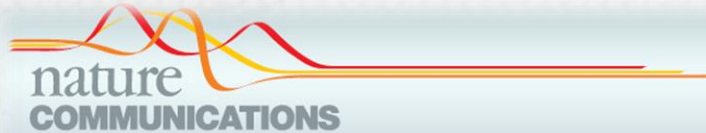
```
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CAN'TGGCTGNNCCGACGCGGNNCANN'TGTTGATTGGCTAANGGGNAGNNCNACCGGCAACCCACCGGCATCCG
GGTNCGGGCANACTGGGCAGTCGGAGACNAGACCGGNANNCTAGCGNANTGTATGGCACGGCAAATGACTATNN
TCTGGNCCAAGTGGGNNNNNGCNCNNTGGNNNNNGGCCNTCTANCNCCNNNNCCNCTTAANNANGNNNN
```





# Resources for Teachers- eDNA

- Review the current literature and do not hesitate to email professionals for advice.



## ARTICLE

Received 6 Nov 2015 | Accepted 12 Jul 2016 | Published 30 Aug 2016

DOI: [10.1038/ncomms12544](https://doi.org/10.1038/ncomms12544)

OPEN

## Environmental DNA reveals that rivers are conveyer belts of biodiversity information

Kristy Deiner<sup>1,2</sup>, Emanuel A. Fronhofer<sup>1,3</sup>, Elvira Mächler<sup>1,3</sup>, Jean-Claude Walser<sup>4</sup> & Florian Altermatt<sup>1,3</sup>

DNA sampled from the environment (eDNA) is a useful way to uncover biodiversity patterns. By combining a conceptual model and empirical data, we test whether eDNA transported in river networks can be used as an integrative way to assess eukaryotic biodiversity for broad spatial scales and across the land–water interface. Using an eDNA metabarcode approach, we detect 296 families of eukaryotes, spanning 19 phyla across the catchment of a river. We show for a subset of these families that eDNA samples overcome spatial autocorrelation biases associated with the classical community assessments by integrating biodiversity information over space. In addition, we demonstrate that many terrestrial species are detected; thus suggesting eDNA in river water also incorporates biodiversity information across terrestrial and aquatic biomes. Environmental DNA transported in river networks offers a novel and spatially integrated way to assess the total biodiversity for whole



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- CRISPR (clustered regularly inter-spaced short palindromic repeats)

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