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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AP BIOLOGY AP CHEMISTRY AP PHYSICS APES HBIOLOGY BIOTECHNOLOGY SCIENCE CLUB 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:

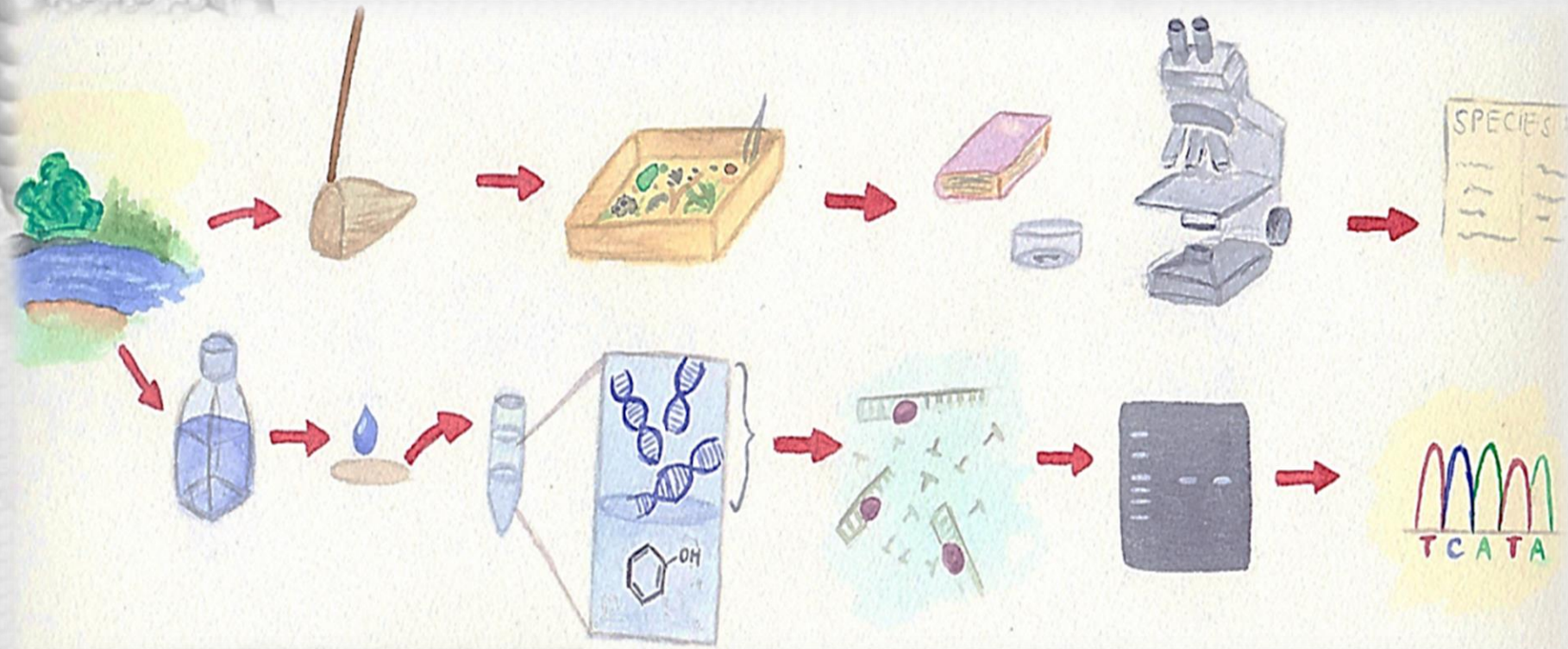
- Environmental DNA (eDNA)
- Marine polychaetes (fireworms)
- Neurospora crassa (fungus)
- CRISPR (clustered regularly inter-spaced short palindromic repeats)

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?

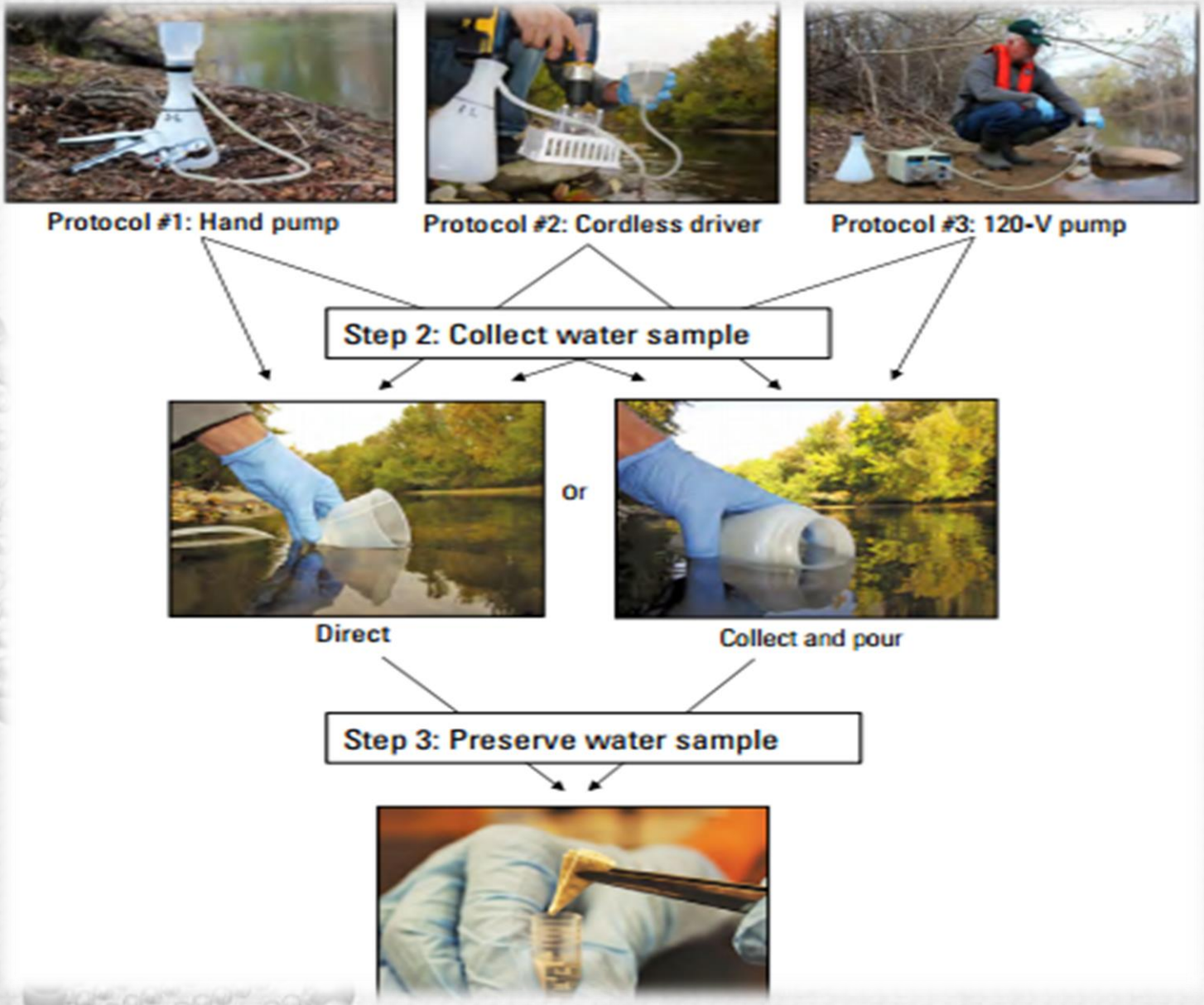
- Organisms naturally shed their DNA
- Skin cells, mucus, feces, carcasses, gametes, hair, etc.
- Material transported down waterway



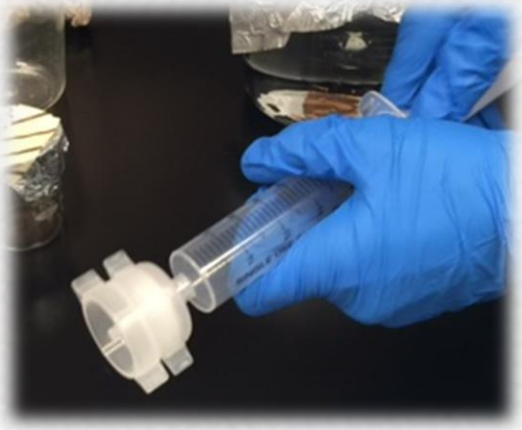
What is eDNA?

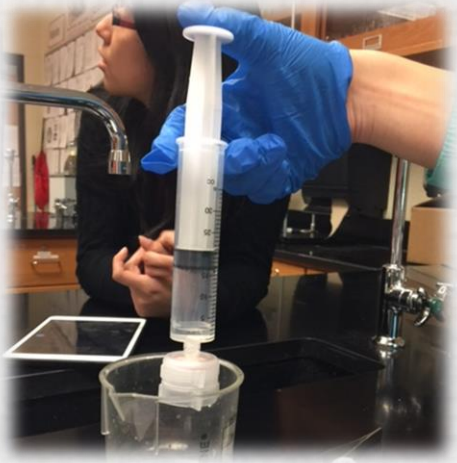
https://www.youtube.com/watch?v=KR4TEhFcZPc

How can I collect eDNA?



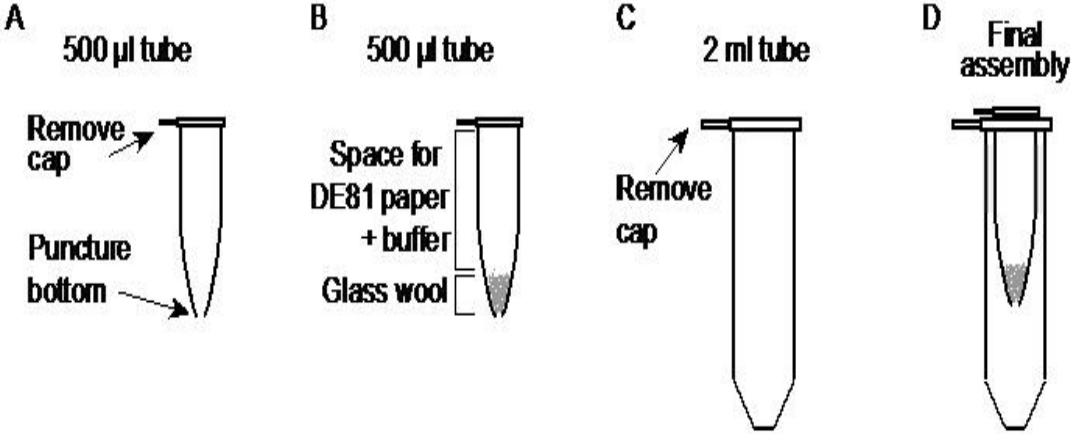
How Can I Collect eDNA?





How can I collect eDNA?

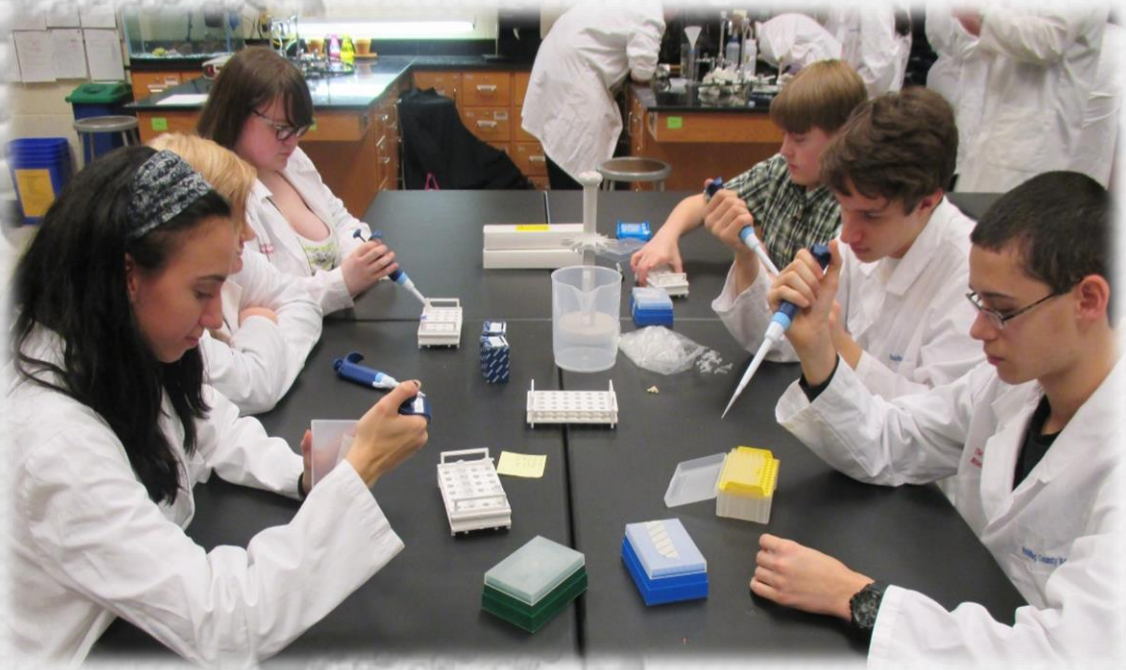
- Students used Qiagen Blood and Tissue Extraction Kits ($150/50 extractions)
- Silica-based column chromatography produces high yield genomic DNA
- Simple salt-based extractions will also yield enough DNA.



What can I do with eDNA?

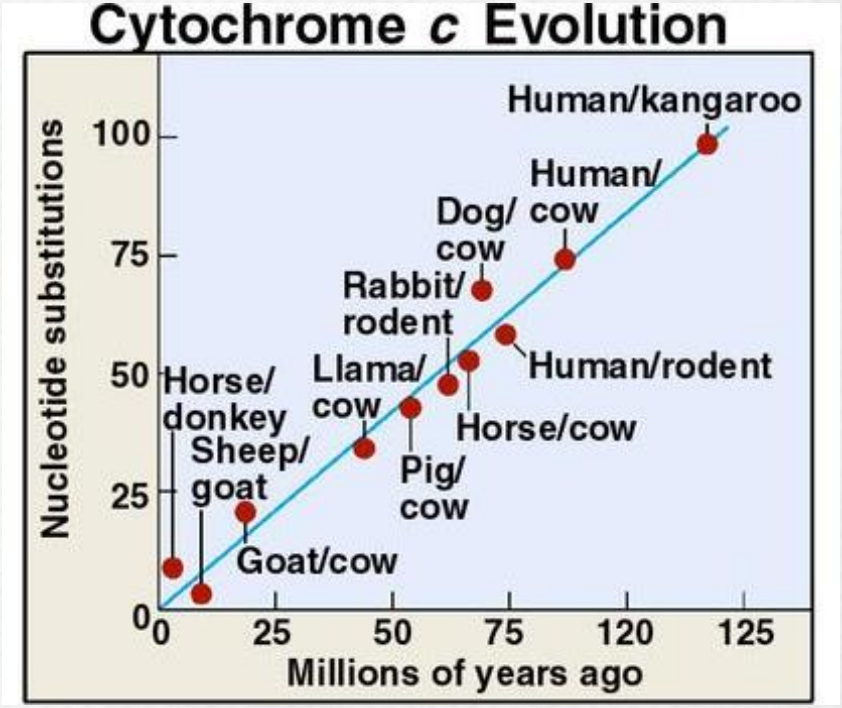
- Polymerase Chain Reaction can be used to amplify specific genes.

- 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.
- CO1 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)

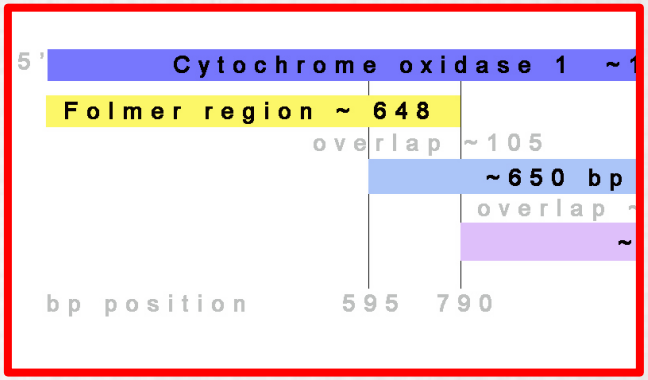
What can I do with eDNA?

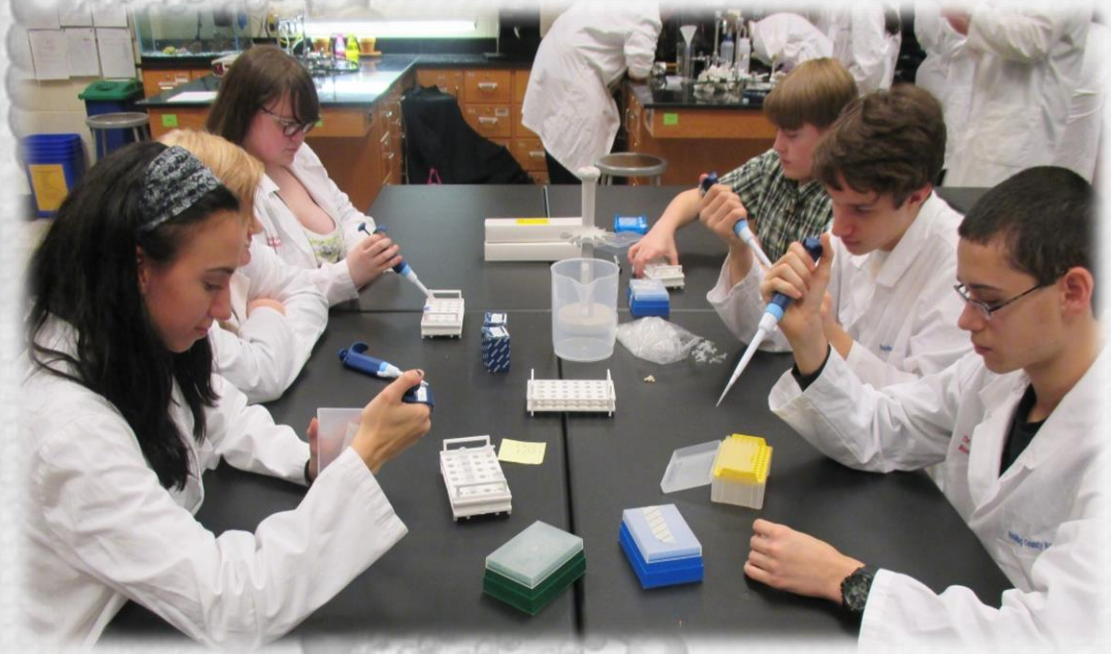
Cytochrome C Oxidase Subunit 1 (CO1)- DNA Barcoding

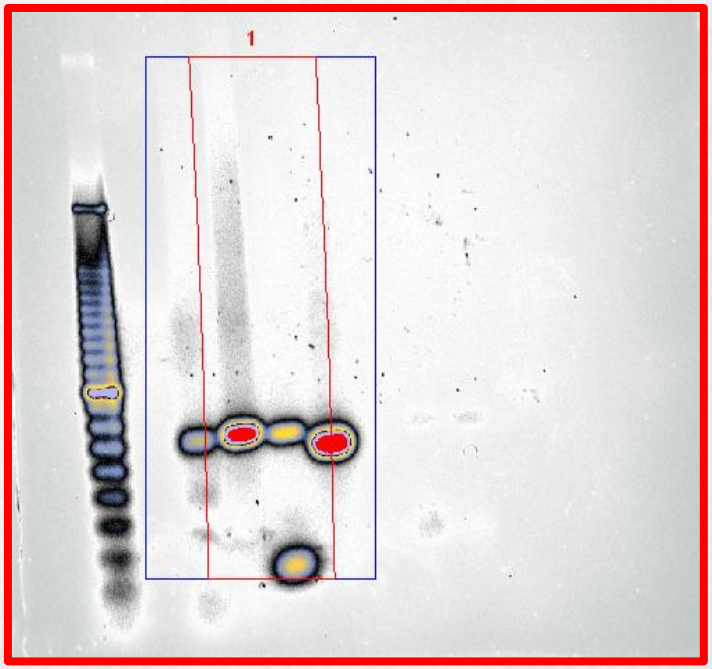
DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates

O. Folmer, M. Black, W. Hoeh,* R. Lutz, and R. Vrijenhoek+
Center for Theoretical and Applied Genetics, and Institute of Marine and Coastal Science, Rutgers University, New Brunswick, New Jersey 08903-231

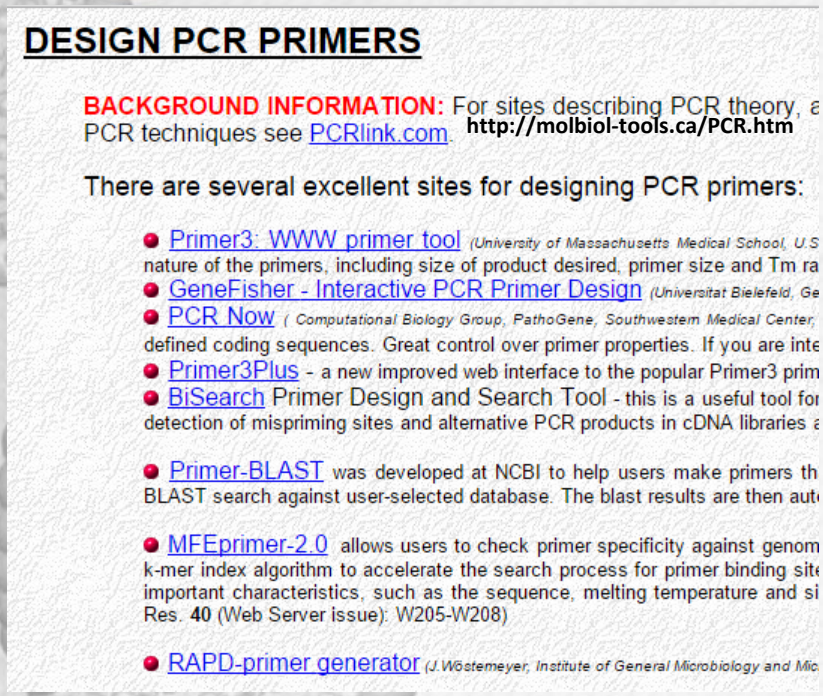
time depths likely to be found in our studies. We quickly became aware of the broad utility of these *COI* primers for broader systematic studies of metazoan invertebrates, including acoelomates, pseudocoelomates, and coelomate protostomes and deuterostomes.



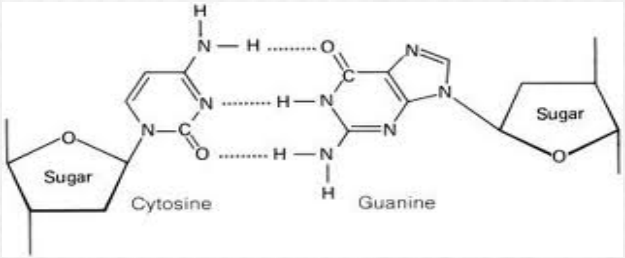




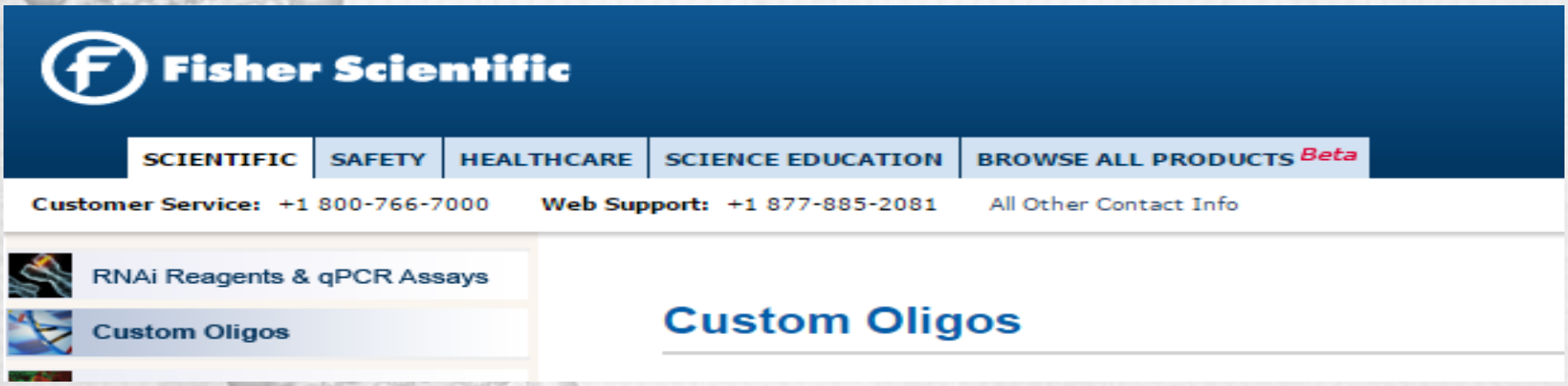
What can I do with eDNA?



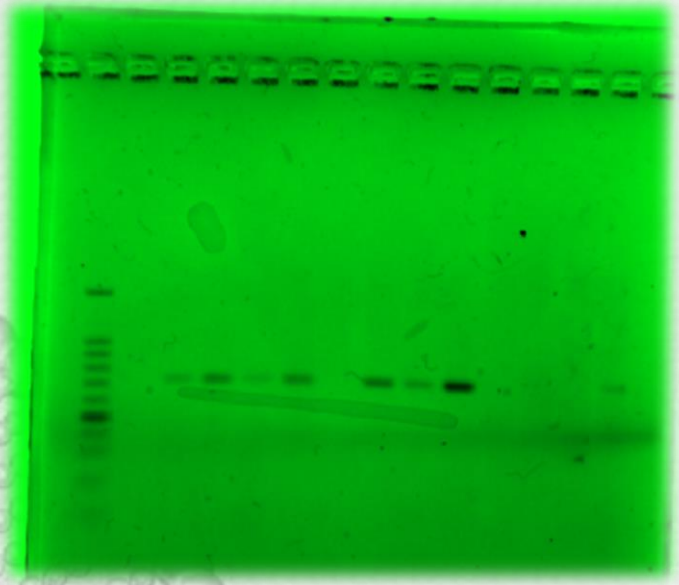
- **Primers are for the COI gene**
- **Primers have high guanine-cytosine content (40-60%)**
- **Use online primer generator**

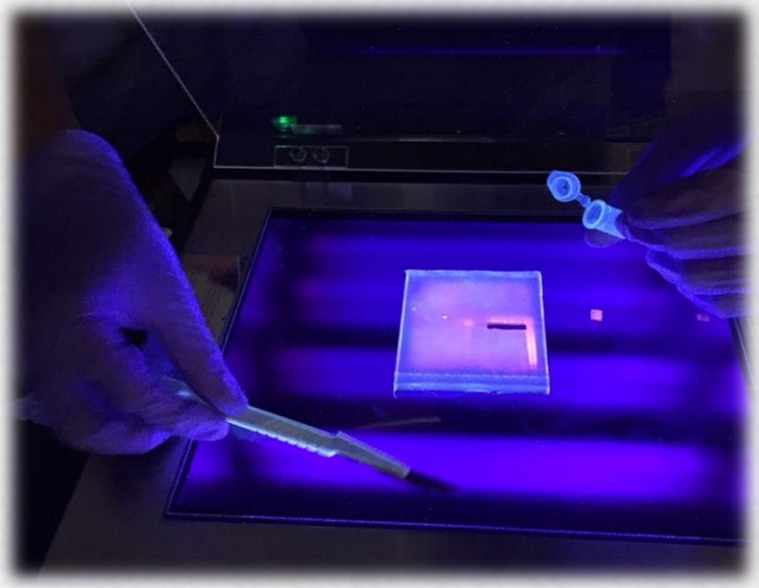


$= T_m$ (primer)



What can I do with eDNA?





Sequence File: COI-02-LCOI1490_R.seq

GENEWIZ®

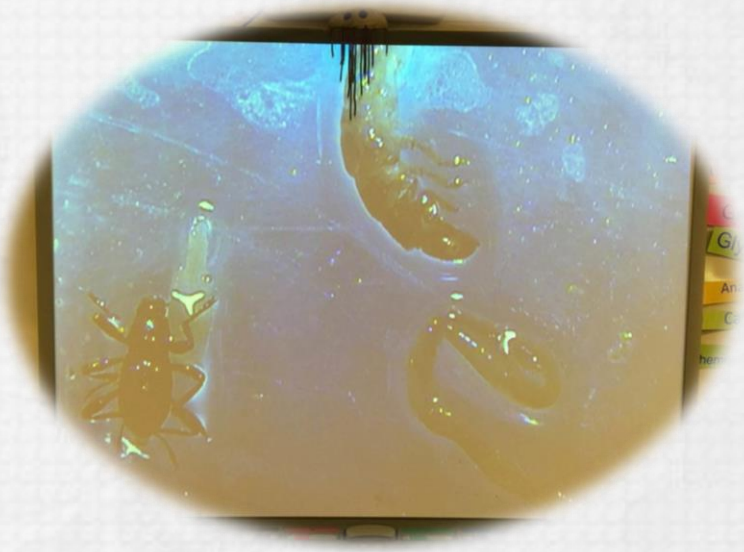
>COI-02-LCOI1490_R_G06.ab1

NNNNNNNNNNNNNNNNGCNTTCTCNGTTTTAGCAGGCCTTGTTGGTGGTTTGTTTTCTGTACTATTTCGTTTACAACTAG
CTTCTCCTGGTTCTGATTTTCTTGGTGGAGATCATCAGTTTTATAATGTATTAATTACTGCGCATGCGCTGATAATGGTT
TTCCTTATGATTATGCCAGCCTTATTTGGAGGGNCGGNTGNGGTTTGTTCCTTTAAAAAGAATTGAGCCAGAGGGGAATN
TTTATTTTAATTGTTGTGCAGAATATTTGAACTTTTTTTTCTTTCACTGGTTGTTGANTCTNACTTTTCATATNTTTGTA
ATGTTTATTTAAANTTTGAAGTTTATGAATTTANANATTAATTTTAAATTNNTATNNNAATGATATTAAAGTTCAGTATT
AAACATTTTTNAATATGATAAGNAAAATTTATTTCCTTCCTTTTTCACATGCATAAATTCACATTAATGAAATATTGAAT
TTTGNTTCATANCTNAATTCCAACTCTTTGGACNANANTTAANATCTCANATTAGCNTAAGCTTGNTCCACCCACCTCTA
TTCTCGGACTTTTCGTCTGNCNAGCCCTGNTTCCNNNTTTCCTCTTTCACATGGGCTGGAGNANGNNGAANNGATGCGCT
TNTNTCNAANCNTCNTTTTTATACNTAGNAAAATTNCNCGTNTNTAANNATNAATACTTATCTTTNATCNCTTNTNGATN
CNCCNNGANTGTACNTCCNCNNATTTTAAACTTCTNTNNNAAATTTNNGATTCNGTCCNCTCNAATTNNNNNNNCNNNNT
CNNNNNAANAAAAAAA

Why should I teach eDNA?

Pumpkinvine Creek, Paulding County GA

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



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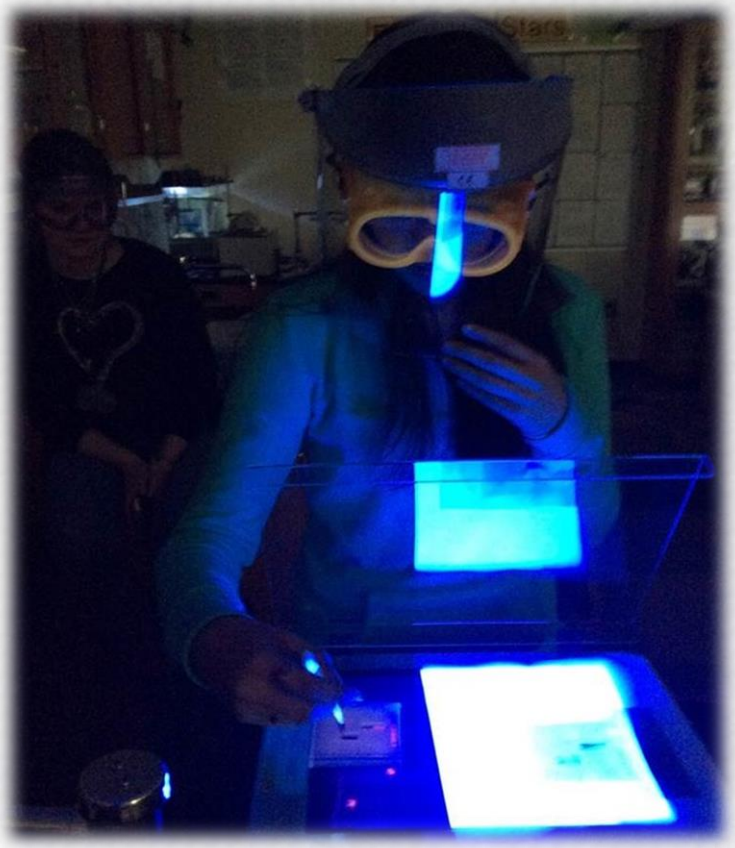
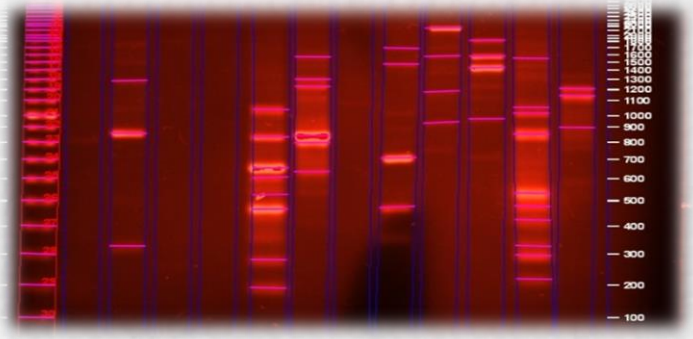




Why should I teach eDNA?

DNA Evidence Evaluation

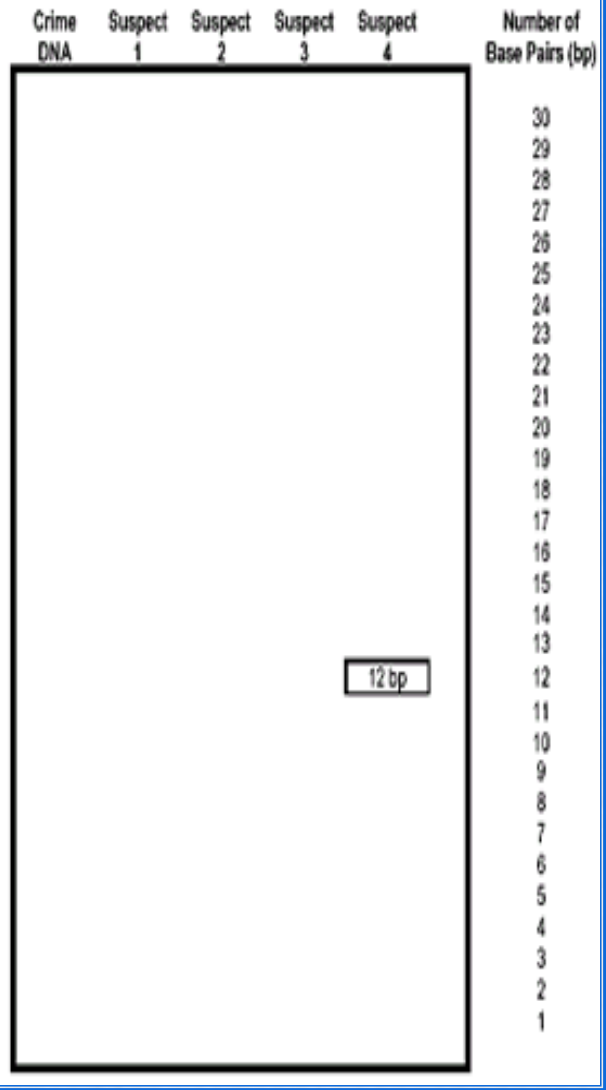
1. Turn your paper strips (DNA sequences) so that the side with the bases is facing you. The restriction enzyme cuts at ever point it finds C C G G, always cutting between the C and the G. Label the back of the slips with the suspect number so that you don't get them confused after cutting. Use scissors 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.



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® BIOLOGY FREE-RESPONSE QUESTIONS

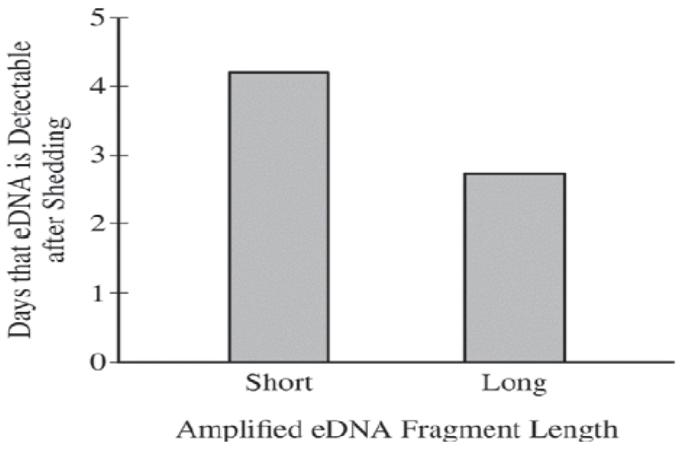


Figure 1. Detectability of eDNA fragments of varying lengths

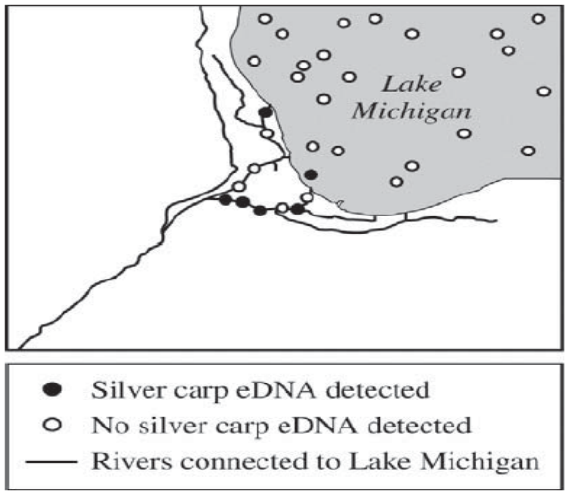
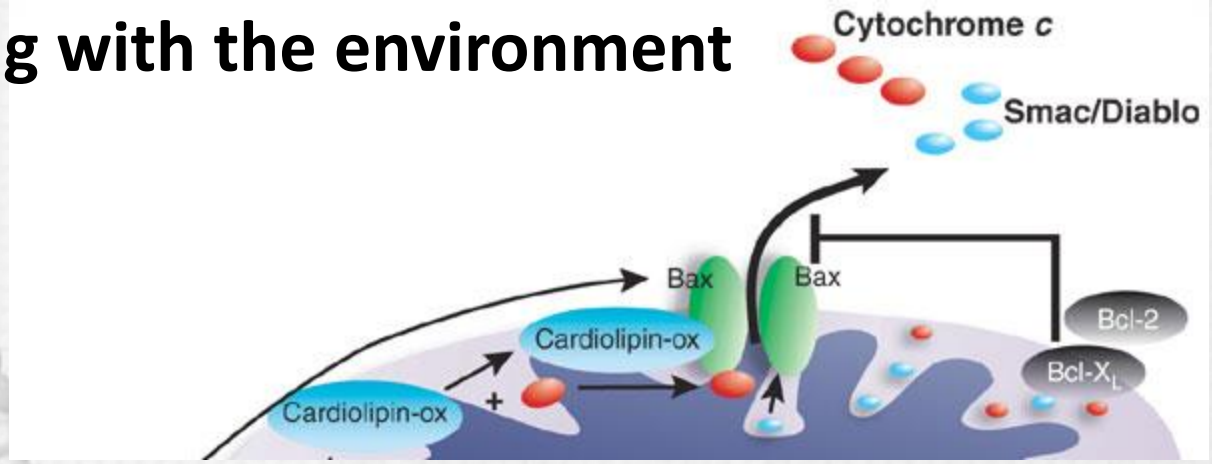


Figure 2. Map of the waterways that connect a nearby river system to Lake Michigan

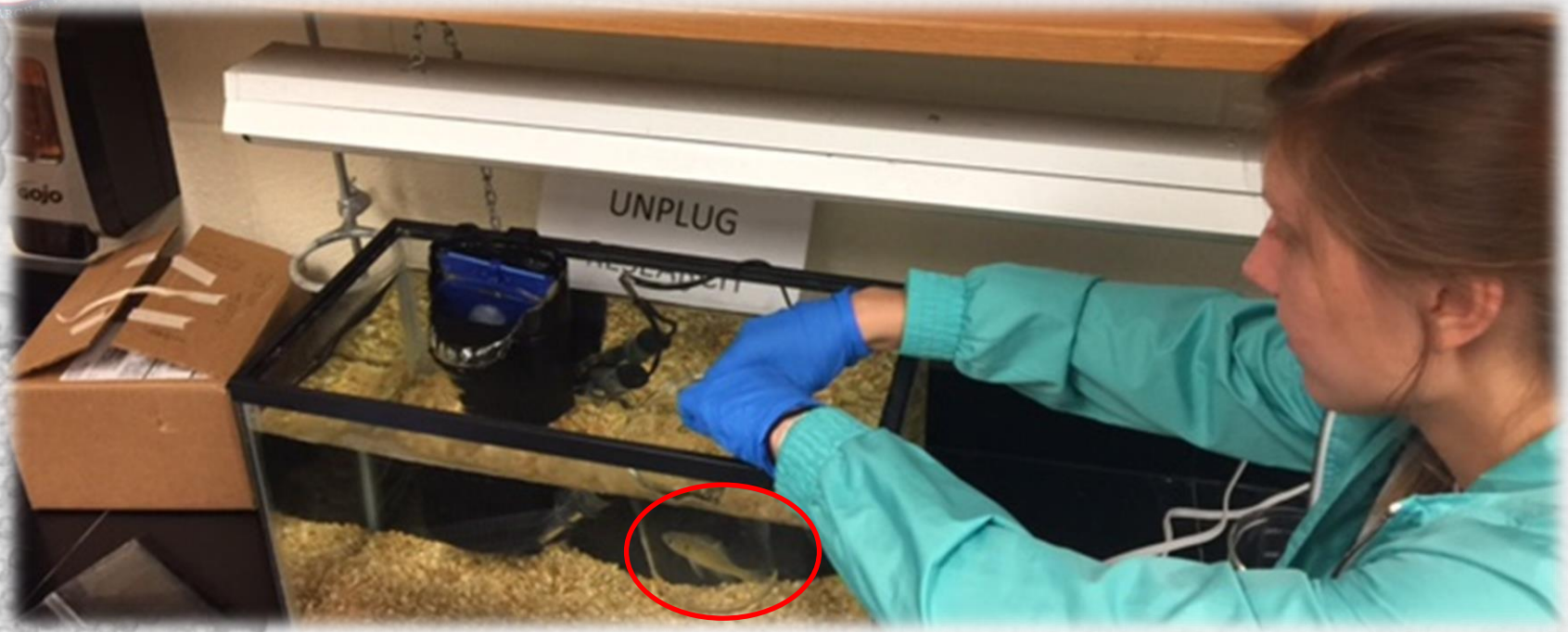
Why should I teach eDNA?

- 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?

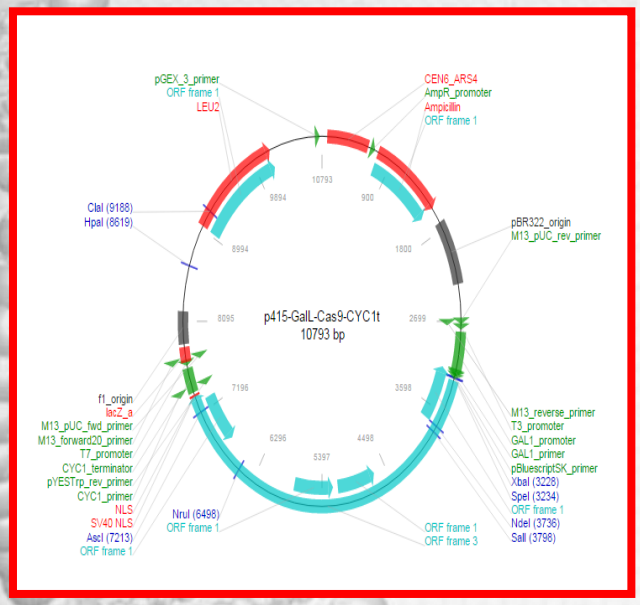


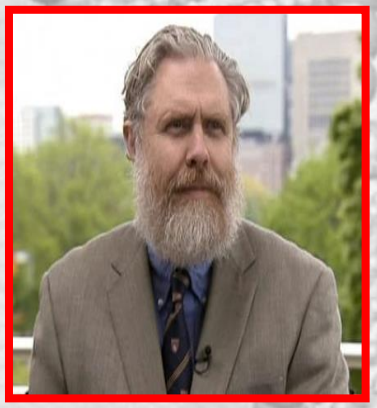
How can I start teaching with eDNA?

- 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!





George Church is a Professor of Genetics at Harvard Medical School

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.

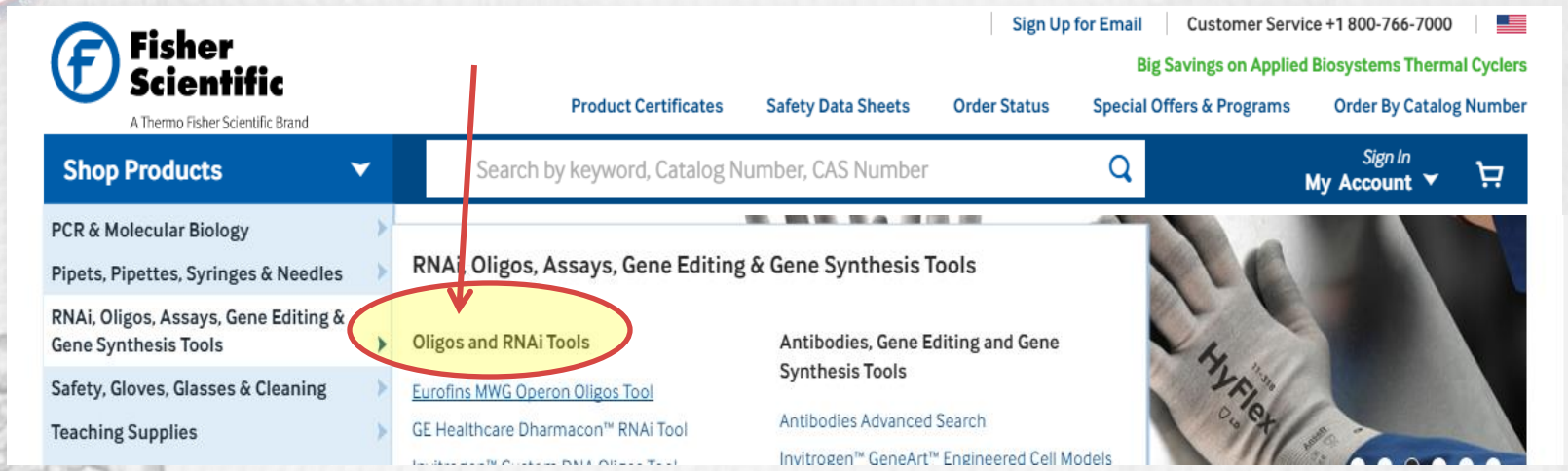
Project-based Learning with eDNA?

- 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

How can I start teaching with eDNA?

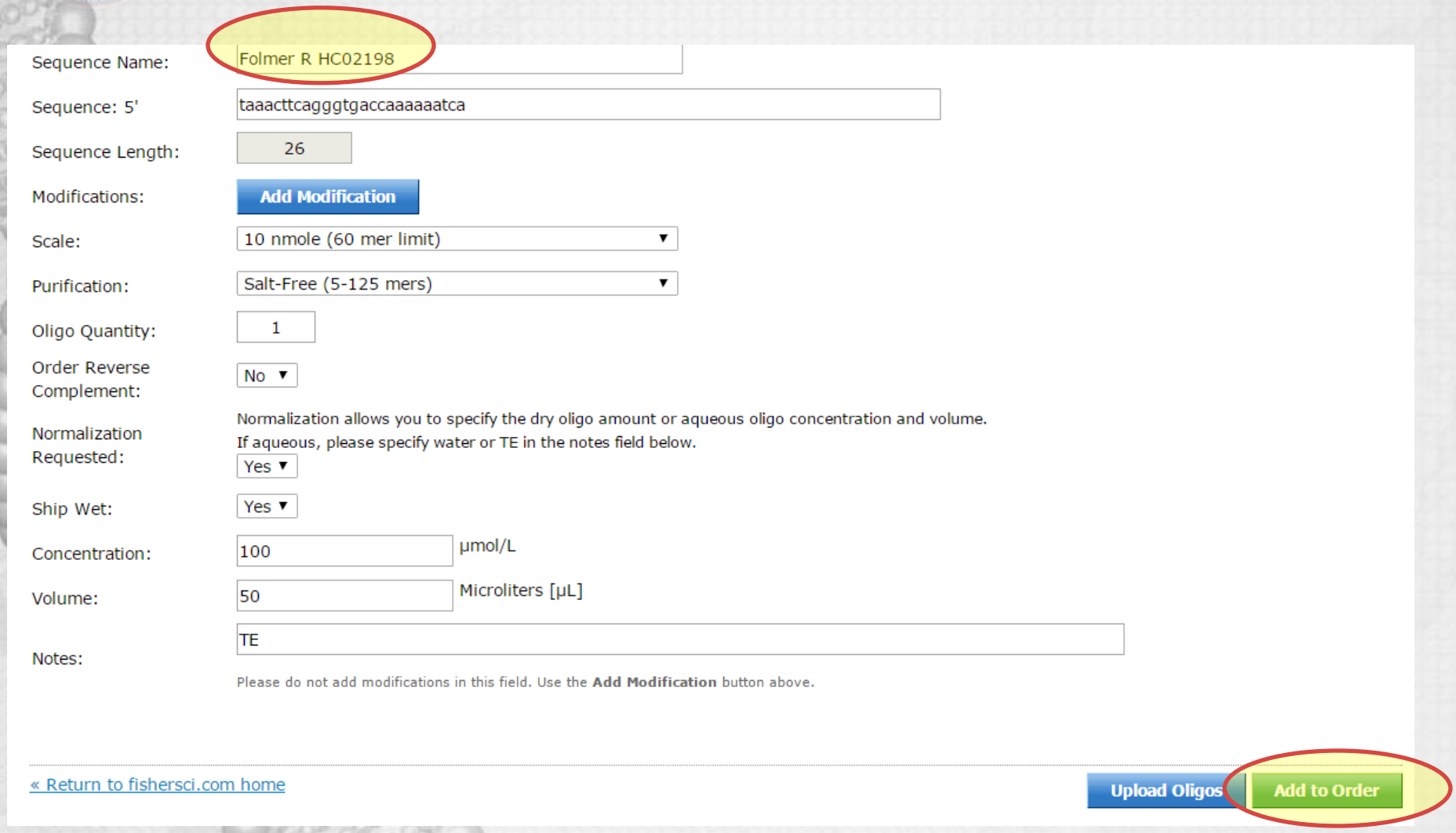
- 25mm Swin-Loc filter housing (from Whatman, #7011782)
- Ahlstrom Glass microfiber filters, 25mm (#101804)
- Sterile 50 mL Lauer-Loc syringes
- All of the above items are available through VWR

How can I start teaching with eDNA?



Sequence Name:	Folmer LCO1490
Sequence: 5'	ggtcaacaaatcataaagatattgg
Sequence Length:	25
Modifications:	Add Modification
Scale:	10 nmole (60 mer limit)
Purification:	Salt-Free (5-125 mers)
Oligo Quantity:	1
Order Reverse Complement:	No
Normalization Requested:	Normalization allows you to specify the dry oligo amount or aqueous oligo concentration and volume. If aqueous, please specify water or TE in the notes field below. Yes
Ship Wet:	Yes
Concentration:	100 µmol/L
Volume:	50 Microliters [µL]
Notes:	TE

How can I start teaching with eDNA?



How can I start teaching with eDNA?

- If thermal cycler available use the following profile:

35 cycles at:

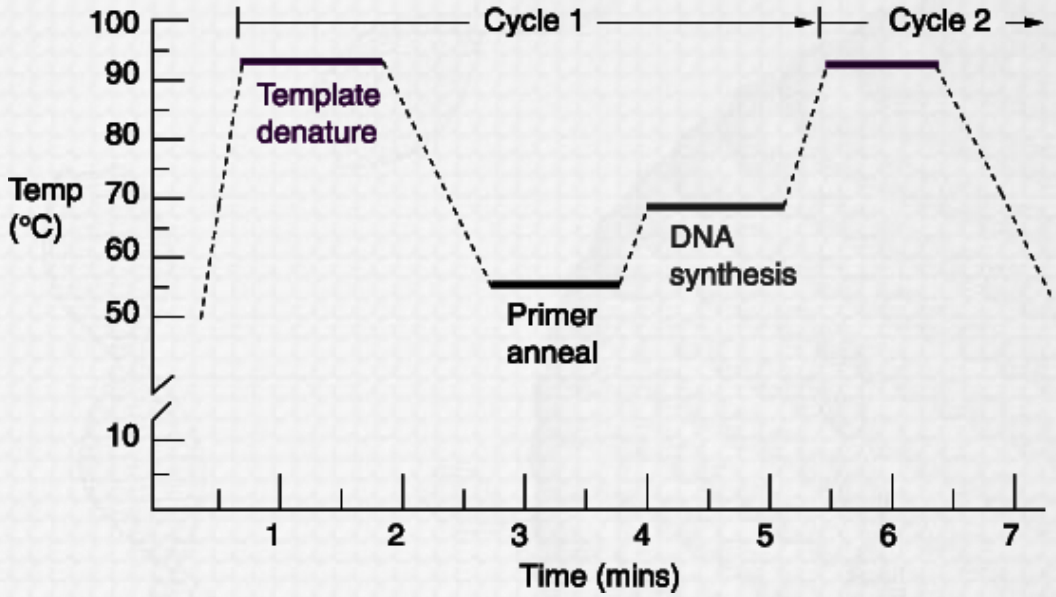
3:00 minutes at 95°C

1:00 minute at 95°C

1:00 minute at 40°C

1:30 minutes at 72°C

7:00 minutes at 72°C

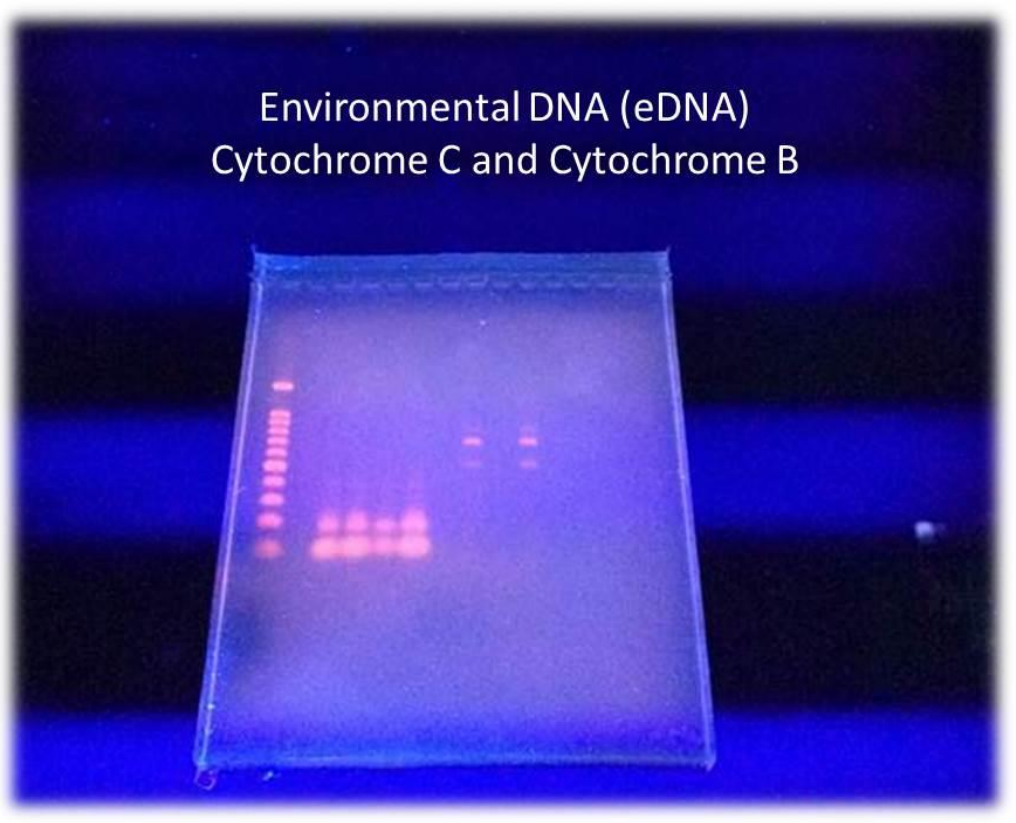


- If unable to quantify DNA, try varying amounts from 1µL- 5µL increasing in 0.5µL increments.

- Start with 2.5 µL of each primer and decrease in 0.5µL increments if needed.

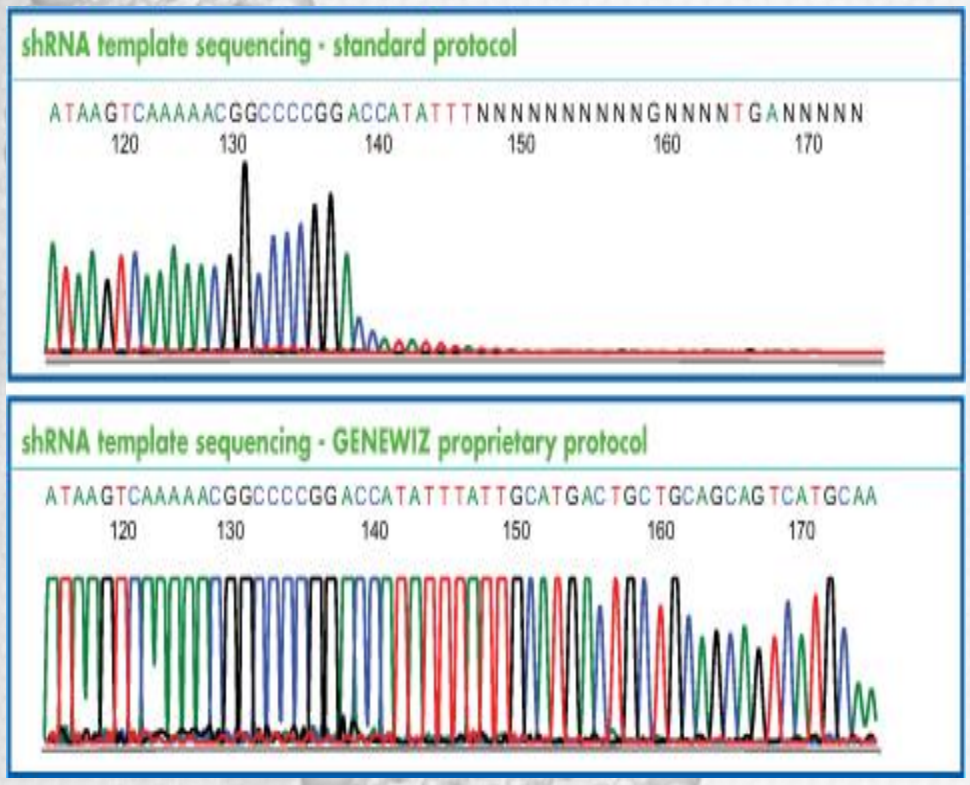
How can I start teaching with eDNA?

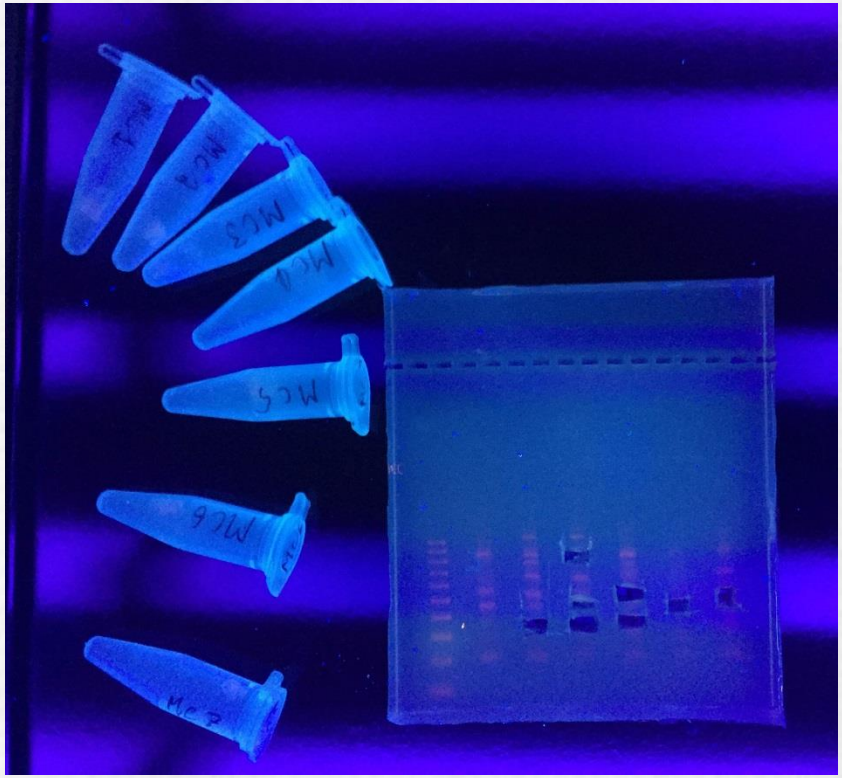
- Resolve DNA on a 1.5% agarose gel at 100 volts for 90 minutes.
- If purifying amplicons, use TBE gels and buffer.
- Stain using Fast Blast or SYBR green



How can I start teaching with eDNA?

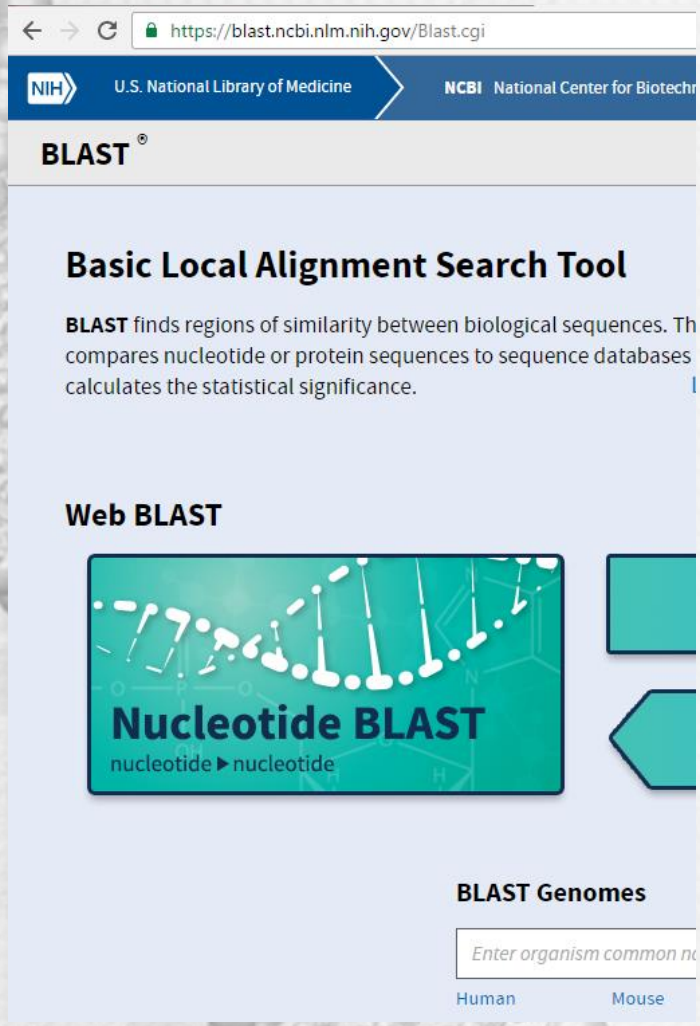
- Purify amplicons using QUIquick Gel Purification Kit (Qiagen; $100)
- Sequence genes using GENEWIZ Sanger Sequencing ($6 each; email me for protocol suggestions)





How can I start teaching with eDNA?

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



Resources for Teachers- eDNA

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

ARTICLE

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Environmental DNA reveals that rivers are conveyer belts of biodiversity information

Kristy Deiner[1,2], Emanuel A. Fronhofer[1,3], Elvira Mächler[1,3], Jean-Claude Walser[4] & Florian Altermatt[1,3]

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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