# Environment and Natural Resources Trust Fund

2024 Request for Proposal

## General Information

**Proposal ID:** 2024-208

**Proposal Title:** Sequencing and Portable Device to Detect Invasive Species

## Project Manager Information

**Name:** Tianhong Cui

**Organization:** U of MN - College of Science and Engineering

**Office Telephone:** (612) 626-1636

**Email:** cuixx006@umn.edu

## Project Basic Information

**Project Summary:** This project is to use high throughput sequencing to characterize the invasive species constituency and use this information to develop a low-cost, easy-to-use, point-of-collection portable device to detect invasive species.

**Funds Requested:** $500,000

**Proposed Project Completion:** June 30, 2027

**LCCMR Funding Category:** Aquatic and Terrestrial Invasive Species (D)

## Project Location

**What is the best scale for describing where your work will take place?**

Statewide

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

During the Project and In the Future

## Narrative

**Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Invasive insects, animals, pathogens, and plants are non-native species that threaten people, wildlife, and natural environments and cost more than $3 billion per year in Minnesota. Detecting invasive species and enacting countermeasures is crucial to avoid widespread distribution with associated fiscal and natural habitat ramifications. Natural and manmade pathways exist for the introduction and establishment of invasive species. Natural pathways include environmental effects such as wind currents and waterborne debris that introduce species to a new habitat. Manmade pathways can be intentional or unintentional such as with movement of pet or horticulture species or as an indirect byproduct of human behavior and mobility. Early detection and monitoring are crucial to prevent future invasive species invasions and expanded spread of existing species. A promising approach to accomplish this is analysis of environmental nucleic acid (DNA & RNA) using next generation, high throughput sequencing. Key bottlenecks to realizing the potential of this approach is a significant lag time from sample collection to results and the requirement of dedicated trained laboratory personnel and infrastructure. There is an urgent need for comprehensive and widespread ability to detect and monitor invasive species without need for complicated infrastructure. The proposed portable device will fulfil the requirements.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

Our proposed solution is to use the power of waterborne based epidemiology (WBE) and high throughput sequencing to characterize the invasive species constituency and use this information to develop low-cost, easy-to-use, point-of-collection methods for detection across a broad geographic region. We will apply our expertise in WBE built upon our CDC National Wastewater Surveillance System laboratory that tracks organisms of public health relevance in wastewater for 60% of the population of Minnesota. Because wastewater contains plant and animal materials to runoff and groundwater, we predict that wastewater analysis for invasive species nucleic acid (DNA and RNA) will be valuable as an early warning system for identifying and tracking invasive species. Many detection methods are biased for defined species, and we will use our unique capabilities of high throughput sequencing to characterize prevalent invasive species in an unbiased manner. We will then apply our expertise in device manufacturing to develop high throughput laboratory and point-of-collection based methodologies with short test turnaround times to allow for expansive testing, tracing, and monitoring of invasive species that represent a risk to natural resources. Based on micro- and nano-manufacturing technologies, we will design, fabricate, and test a new portable PRC device to detect invasive species.

**What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

Characterizing invasive species in an unbiased fashion represents a powerful approach for creating an early warning system that will protect natural resources. Invasive organisms shed DNA and RNA into water and this project will focus on applying cutting-edge, nucleic acid sequencing for identifying and characterizing invasive species. We will leverage this information to create a point-of-collection portable device that will enable widespread screening with rapid turnaround times to results. Our chief outcomes will be comprehensive sequence analyses and portable device design as part of an invasive species detection and tracking program for protecting, preserving, and conserving the state's natural resources.

## Activities and Milestones

### Activity 1: To perform high throughput sequencing for the characterization of watershed metagenomes for proactive invasive species detection and identification

**Activity Budget:** $166,506

**Activity Description:**
Metagenome, or 'shotgun' sequencing, identifies nucleic acids (DNA and RNA) in an unbiased fashion. Because invasive species shed DNA into water, we will use this method in samples from our wastewater detection program that obtains samples from 44 sites twice per week across the state of Minnesota. Samples will be processed, sequenced, and analyzed with genome databases to identify and characterize prevalent invasive species. Our hypothesis is that because wastewater contains material from plants, animals, and water sources (run off, lakes, rivers, and groundwater) that it will contain the nucleic acid of invasive species. We are presently surveilling wastewater for human pathogens and therefore have an integrated sample collection and processing procedure in place. Therefore, we can rapidly institute invasive species monitoring into this existing infrastructure (Fig. 1a in the attached one-page visualization). Should wastewater samples show a low abundance, we will isolate environmental DNA from key entry portals for invasive species (e.g., shipping ports) in collaboration with Dr Timothy LaPara, a wastewater treatment biologist. This work will be performed by PhD level scientists, wastewater laboratory scientific staff, and undergraduate students. The multidisciplinary expertise of this team is required to accomplish the goals and provides scientific and budgetary justification.

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| Sequencing and identification of invasive species from wastewater samples | June 30, 2025 |
| Bioinformatics pipeline development for streamlined analysis | June 30, 2026 |
| Sample collection and processing | June 30, 2027 |

### Activity 2: Design, fabrication, and evaluation of a low-cost portable device for invasive species detection

**Activity Budget:** $333,494

**Activity Description:**
Many take home tests (e.g., pregnancy, COVID, etc.) are based on lateral flow assay technology (LFA) and allows test turnaround times in a matter of minutes. We have used this technology to develop an ultrasensitive detection platform for RNA and DNA and will develop tests specific for high priority invasive species identified in Activity 1. Toward economizing and expanding our testing footprint and capabilities, we will pursue the fabrication and development of a point-of-collection (POC) device (Fig. 1b in the attached one-page visualization) that will contain three functional modules: 1) pre-packaged washing and isolation buffer for nucleic acid obtainment; 2) internal reaction chamber with graphene heating strips for isothermal nucleic acid amplification; 3) integrated LFA test strip for endpoint, visual readout of invasive species nucleic acid. This device will have a small footprint, will require minimal training, and can be used across broad geographic areas with minimal power requirements. We envision that this device can force multiply current inspection practices (e.g., boat launch visual inspection, etc.) by offering ultra-high sensitivity and short turnaround times. The optimized device will be evaluated and optimized by the testing wastewater samples with comparison to conventional PCR-based nucleic acid testing methods.

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| Developed FTA paper-based sampling device for nucleic acid isolation from water | December 31, 2024 |
| Optimize detection device using synthetic and control samples | June 30, 2025 |
| Size optimization of sampling devices and on-site detection devices to ensure functionality | December 31, 2025 |
| Integration of the whole monitoring platform | June 30, 2026 |
| Improved device for high performance | December 31, 2026 |
| Evaluated device in real wastewater | June 30, 2027 |

## Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|---|---|---|---|
| Mark Osborn | Medical School, University of Minnesota | Co PI and Co Project Manager. He is responsible for identification of invasive species using DNA sequencing, design of PCR protocols, evaluation and comparison of the portable devices with respect to the standard PCR tests in his laboratory. | Yes |

## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?**

This project will leverage our existent and unique sequencing capabilities and integrated waterborne surveillance infrastructure to identify priority invasive species for development of a low-cost, accurate, and monitoring device for detection of invasive species in water. The knowledge learned throughout the project will provide a solid foundation for further research and development efforts that would lead to the eventual implementation of this sensing technique for broader monitoring of Minnesota's watersheds. In addition, we plan to file patents on the proposed pathogen sensor for commercialization in the future and apply for funding from agencies such as NSF, EPA, USGS, and NIH.

## Other ENRTF Appropriations Awarded in the Last Six Years

| Name | Appropriation | Amount Awarded |
|---|---|---|
| Develop Small and Inexpensive Purification System for Community Drinking Water | M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04e | $425,000 |
| Develop Inexpensive Energy from Simple Roll-to-Roll Manufacturing | M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 07c | $300,000 |

## Project Manager and Organization Qualifications

**Project Manager Name:** Tianhong Cui

**Job Title:** Distinguished McKnight University Professor

**Provide description of the project manager's qualifications to manage the proposed project.**

Job Title (Cui): Distinguished McKnight University Professor at the University of Minnesota. Dr Cui is a Professor in Mechanical Engineering and an Affiliate Senior Member of the graduate faculty in Department of Electrical & Computer Engineering. He joined the faculty of the University of Minnesota in 2003. He is a Fellow of American Society of Mechanical Engineering. He is also a member of European Academy of Sciences and Arts. Dr. Cui is an international leading expert on micro sensors and advanced manufacturing. He has 368 archived publications in scientific journals and prestigious conferences. He is the founding Executive Editor-in-Chief for a Nature journal, Microsystems & Nanoengineering. He has received awards including the STA & NEDO Fellowships in Japan, the Alexander von Humboldt Fellowship in Germany, the Richard & Barbara Endowed Chair and the Distinguished McKnight University Professorship from the University of Minnesota, the Blaise Pascal Chair Visiting Professorship in France (named after famous French scientist Blaise Pascal), the Distinguished Visiting Fellowship from the Royal Academy of Engineering and the Global Chair at the University of Bath in UK, a JSPS faculty fellowship at the University of Tokyo, and numerous best paper awards. Dr. Cui will serve as the PI & project manager, responsible for overseeing the project, all reports, and deliverables. He will closely work with Dr. Osborn on development of portable devices for detecting invasive species.

Job Title (Osborn): Associate Professor, Medical School, University of Minnesota. Dr Osborn joined the faculty of the Department of Pediatrics in 2009 and has employed novel DNA binding proteins for the rapid detection of nucleic acids using lateral flow immunoassay (LFA) technology. He oversees the UMN:MDH Wastewater Surveillance Laboratory that is part of the CDC National Wastewater Surveillance System. He has more than 50 publications and invited lectures and serves on numerous scientific advisory and editorial boards.

**Organization:** U of MN - College of Science and Engineering

**Organization Description:**
The University of Minnesota is the State's flagship institution of higher education and is a Land Grant University. The proposed work will be performed at the University of Minnesota Technology Integration & Advanced Nano/Microsystems Laboratory (TIAN Lab) and Medical School facilities. The TIAN Lab is located in the Mechanical Engineering Building. Professor Tianhong Cui is the director of TIAN Lab equipped with the state-of-the-art instruments and facilities to conduct the proposed research, with a variety of fabrication and characterization equipment and tools, sufficient for Professor Cui and his postdoc to design, fabricate, characterize and analyze the proposed monitoring device in wastewater. Some fabrication work will be partially done in Minnesota Nano Center, a state-of-the-art facility for research in nanoscience and applied nanotechnology.

Dr Osborn has laboratory space in the McGuire Translational Research Facility and the Mayo Building on the East Bank. Each laboratory space has dedicated molecular equipment to include thermocyclers, clean hoods for nucleic acid isolation, quantitative real time PCR instruments and an Illumina NextSeq 2000 capable of metagenome sequencing at high sensitivity, resolution, and fidelity. He has also developed a broadly applicable nucleic acid detection methodology using lateral flow assays that synergizes with Dr Cui's expertise.

## Budget Summary

| Category / Name | Subcategory or Type | Description | Purpose | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | $ Amount |
|---|---|---|---|---|---|---|---|---|
| **Personnel** | | | | | | | | |
| Tianhong Cui | | Principal Investigator | | | 26.9% | 0.24 | | $58,446 |
| Mark Osborn | | Co-Principal Investigator | | | 26.9% | 0.15 | | $30,015 |
| Amber McElroy | | Research Scientist with expertise on PCR | | | 26.9% | 0.12 | | $14,862 |
| Christine Henzler | | Technician to help with the experimental set-up for sequencing | | | 26.9% | 0.12 | | $5,755 |
| Post Doctoral Associate | | Research on DNA/RNA sequencing | | | 20.4% | 3 | | $213,048 |
| Natalia Mancipe | | Technician on sequencing of DN/RNA | | | 26.9% | 0.12 | | $18,149 |
| | | | | | | | **Sub Total** | **$340,275** |
| **Contracts and Services** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Equipment, Tools, and Supplies** | | | | | | | | |
| | Tools and Supplies | Sequencing reagents, silicon and glass wafer, polymer materials, chemicals, etc. | To do sequencing of DNA/RNA, and to develop portable devices for invasive species | | | | | $123,725 |
| | | | | | | | **Sub Total** | **$123,725** |
| **Capital Expenditures** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Acquisitions and Stewardship** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Travel In Minnesota** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Other | 30 in-state travels | These trips are for field tests of portable PCR devices. | | | | | $6,000 |
| | | | | | | | **Sub Total** | **$6,000** |
| **Travel Outside Minnesota** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Printing and Publication** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Other Expenses** | | | | | | | | |
| | | Scientific Services at Minnesota Nano Center and Characterization Facility at the University of Minnesota | To fabricate and characterize the portable PCR devices | | | | | $30,000 |
| | | | | | | | **Sub Total** | **$30,000** |
| | | | | | | | **Grand Total** | **$500,000** |

## Classified Staff or Generally Ineligible Expenses

| Category/Name | Subcategory or Type | Description | Justification Ineligible Expense or Classified Staff Request |
|---|---|---|---|

## Non ENRTF Funds

| Category | Specific Source | Use | Status | Amount |
|---|---|---|---|---|
| **State** | | | | |
| | | | **State Sub Total** | **-** |
| **Non-State** | | | | |
| In-Kind | Unrecovered F&A | Support of Mechanical Engineering and Medical School facilities where research will be conducted. | Secured | $275,000 |
| | | | **Non State Sub Total** | **$275,000** |
| | | | **Funds Total** | **$275,000** |

## Attachments

### Required Attachments

*Visual Component*

File: da70cae3-599.pdf

*Alternate Text for Visual Component*

High throughput sequencing for identification of invasive species in wastewater and portable device for point-of-collection detection and analysis of invasive species in wastewater...

### Optional Attachments

*Support Letter, Photos, Media, Other*

| Title | File |
|---|---|
| UMN SPA Support Letter | b3ce46f2-e7f.pdf |

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

**Does your project have potential for royalties, copyrights, patents, or sale of products and assets?**

Yes

**Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?**

Yes

**Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?**

No

**Does your project include original, hypothesis-driven research?**

No

**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration

**Does your project include the design, construction, or renovation of a building, trail, campground, or other capital asset costing $10,000 or more?**

No

**Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services, as defined in Minnesota Statutes section 299C.61 Subd.7?**

No