The Center for Urban Responses to Environmental Stressors (CURES)

Announces a Request for Pilot Project Proposals

What is CURES?

The Center for Urban Responses to Environmental Stressors (CURES) is a cross-campus, community-engaged initiative that has been established to develop the leadership and research capacity to identify, evaluate, and mitigate Detroit’s environmental health challenges in close collaboration with the community and environmental policy makers. The ultimate goal of CURES is to be an active partner in the collective goal of creating a healthier Detroit.

CURES is focused on understanding how chemical and non-chemical stressors in the urban environment affect the health and well-being of Detroit’s vulnerable populations. Detroit is encumbered with an overabundance of industrial and post-industrial environmental toxicants, socioeconomic strains, violence, and housing decay. Identifying these health hazards and enhancing our understanding of how they impact our health is key to implementing effective remediation efforts. CURES is strategically designed to facilitate transdisciplinary research and training focused on: (1) the exposure to stressors that are especially prevalent in the urban industrialized environment, including chemical and non-chemical stressors, (2) the experiences of people who are particularly vulnerable to the adverse effects of such exposures (e.g., children, older adults, immigrants, and first responders), and (3) linking such environmental exposures to costly and serious public health disorders in Detroit's population.

The overall goals of CURES are (1) to provide leadership in environmental health science research by building capacity to enhance our understanding of the health effects from complex chemical and non-chemical urban environmental exposures, (2) to develop the next generation of environmental health researchers who are facile with transdisciplinary, translational team science, and (3) to interact with the community so as to leverage our research to contribute to a healthy Detroit.

What is the purpose of this RFA?

The guiding principal of CURES is to perform community-relevant research that addresses the most pressing environmental health concerns of Detroit’s urban community. CURES uses input from its Community Advisory Board to inform its research priorities, and the primary goal of the CURES Pilot Project Program is to develop research capacity and expertise to address those priorities.

This RFA seeks applications for research projects that are focused on understanding or mitigating the effects of the environment on human health. These research projects must make substantial use of the CURES facility cores. These cores have been specifically designed to provide state-of-the-art services to facilitate environmental health research.
**Time Line**

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
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<tbody>
<tr>
<td>November 2, 2015, 2:00 – 3:30PM</td>
<td>Informational Meeting at the Integrative Biosciences Center (IBio), 6135 Woodward Avenue, Conference Room 1A. Parking will be available in the IBio visitor’s lot, off of Burroughs.</td>
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<tr>
<td>November 17, 2015</td>
<td>Letters of intent (LOI) due by midnight</td>
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<td>November 24, 2015</td>
<td>Notification of successful LOIs and invitation to submit proposal</td>
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<tr>
<td>January 11, 2016</td>
<td>Full applications due by midnight</td>
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<tr>
<td>February 1, 2016</td>
<td>Announcement of awards; funding to begin as soon as possible afterward</td>
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**Submission of Information**

Submit Letters of Intent (LOI) in PDF format as an e-mail attachment to:

Robert Pearson  
Manager, IEHS Business Operations  
Phone (313) 577-6590  
Robert.L.Pearson@wayne.edu

**Questions**

If you have questions, please contact:

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What is the theme of this RFA?

This RFA seeks applications for research projects that are focused on understanding or mitigating the effects of the environment on human health. These research projects must make substantial use of the CURES facility cores.

- This year’s CURES Pilot Project Program will emphasize building the Center’s research capacity through use of its facility cores. By providing access to advanced technologies and expert services, the facility cores will enable CURES researchers to address research problems that would otherwise be beyond the scope of an individual research unit. Simultaneously, use of the CURES facility cores by pilot project researchers will enable the facilities to refine, enhance, and document their research-support capabilities. Therefore, this year’s Pilot Project Program will support research projects that substantially utilize the services of both of the CURES facility cores: (1) the Integrated Health Sciences Facility Core and (2) the Exposure Signatures Facility Core. Applicants will be required to develop their pilot project applications in consultation with the facility core leaders and must provide letters of support from these core leaders with their applications. The two facility cores are described in detail at the end of this document.

- The environment is broadly defined to include all types of environmental “stressors.” These include chemicals, such as environmental contaminants (e.g., heavy metals, air pollutants), ingredients in consumer products (e.g., phthalates, bisphenol A), and pharmaceuticals, as well as non-chemical stressors (e.g., noise, community violence, sleep deprivation, psychosocial stressors, and microorganisms).

- This RFA does not restrict the choice of topic that will be considered responsive. However, the applicant must provide a compelling justification for the selected topic, both in terms of the importance of the environmental health problem and the likelihood that the proposed research project will have a substantial impact in addressing the problem.

- CURES is committed to performing research that is relevant to the community. Therefore, while this RFA will consider all types of environmental health research project (e.g., fundamental mechanistic research, population-based research, community-engaged research), proposals that include partnerships with community members are always encouraged.

- The pilot project must be a research project that has the potential to generate peer-reviewed publications in high-impact journals and to be developed into a larger, longer-term project that is supported by extramural funding, in particular by the National Institute of Environmental Health Sciences.

- Applications that address environmental problems that are relevant to the strengths of current CURES investigators, the effects of environmental stressors on the immune system, metabolic disease, cancer, and mental health, are especially encouraged.
What are the terms of this RFA?

1. **Number of awards:** CURES plans to fund 3 to 4 pilot projects (depending on available funds).

2. **Funding time and amount:** Each pilot project will be funded for ~1.2 years at a total amount of up to $70,000 in direct costs. There will be no funds allocated for administrative and facility costs (indirect costs). Successful applicants will receive up to $30,000 as soon as they have completed all pre-award requirements (described below), expected to occur in early February, 2016. On approximately April 1, 2016, the projects will receive the remainder of the award.

3. **Eligible applicants:** Eligible applicants will include all current CURES members as well as non-CURES members at Wayne State University (WSU) and Henry Ford Health System (HFHS) who declare their willingness to join CURES and abide by its policies. Each Pilot Project PI must have a faculty or other appointment that would enable him/her to submit an extramural research grant application as a PI. A community member who participates on a community-engaged pilot project may have the role of either co-PI or co-investigator. Additional collaborating participants (e.g., co-investigators, collaborators, consultants) are encouraged.

   A list of current CURES members and members of the CURES Community Advisory Board can be found on the CURES website: http://iehs.wayne.edu/index.php

4. **Requirements:**
   
   1) The proposed research project must be responsive to this RFA – i.e., it must be a research project that addresses an environmental health problem of concern to the community, and it must substantially utilize the services of both of the CURES facility cores. The application must be developed with input from the facility core leaders and include letters of support from at least one co-leader of each facility core.
   
   2) The proposed research project must be of outstanding merit – i.e., there must be a high likelihood that the proposed research project will have a substantial impact in addressing the problem, and there must be a high likelihood that the pilot award will lead to extramural funding.
   
   3) While not absolutely required, another desirable characteristic of the proposed project will be the inclusion of a new investigator (as defined by NIH), since this will facilitate the career development mission of CURES. An NIH-defined new investigator is an investigator who has not yet been PI on a substantial NIH independent research award, such as an R01 grant. **We anticipate that at least one of the pilot projects that are funded will have a new investigator as PI.**

What may funds be used for?

Funds **may** be used as follows:

1. To purchase supplies, reagents, or equipment (clear justification required). Computers costing less than $5,000 and software fees are allowed.
2. For technical support salaries
3. For incentives for community partners and community research participants.
Funds **may not** be used as follows:
- For salary support of faculty
- For travel, except local travel (e.g., mileage for staff collecting environmental samples)

**How do I apply?**

The first step is to submit a letter of Intent (LOI) that is no more than 3 pages in length. The LOI should:

- Clearly explain why the proposed research is responsive to this RFA, including anticipated use of the two facility cores
- Include a “Statement of Impact” that explains why the project is significant and innovative, and why it will have a substantial impact on the field
- Identify the PI(s) and other participants and describe their positions, roles, and qualifications
- Provide a brief description of the project, including hypotheses, specific aims, and methods to be used
- Provide an estimate of the total budget and expected use of the funding (one paragraph)

LOIs will be reviewed by a subcommittee that is selected from the CURES Internal Advisory Board and Community Advisory Board. This committee will select those proposals that will be invited for submission of a full application. In the case of overlap among proposals, CURES may suggest collaboration. Details for preparing full applications will be provided to successful applicants when they are notified of their selection but the format will essentially be that of an NIH R03 with some additional requirements, as indicated:

a) Cover Page
b) Abstract and Personnel
c) Research sites
d) Biographical Sketches of key personnel
e) Other Support for key personnel
f) Available resources
g) Budget and Justification
h) Specific Aims (1 page)
i) Research Strategy (6 pages)
   a. Significance
   b. Innovation
   c. Approach (Preliminary Data should be incorporated into this section)
j) References Cited
k) Human, Vertebrate Animal, and Hazardous Materials Assurances of Compliance - Investigators using animals or human subjects in their research must obtain protocol approval from the Institutional Animal Care and Use Committee (IACUC) or the Human Investigation Committee (HIC), as applicable, before funds can be spent on activities that require such approval.
l) Plans for submission to external funding agencies
m) Letters of commitment from all project leaders.
n) Letters of support from facility core leaders.
o) Letters of support from the departmental chairs/directors of the PIs academic units. If you are the departmental chair, provide a letter from your Dean or Vice President.
Pre-award responsibilities. Successful applicants will be required to attend a pre-award meeting with the CURES Business Manager, at which they will be advised about account establishment and monitoring as well as the requirement to acknowledge support received from CURES in any publications that contain data that are generated under the pilot project award. Necessary IRB and/or IACUC approvals must be obtained as soon as practicable.

Award-time responsibilities

- PIs must cite the CURES Center Grant (P30 ES020957) on all publications that result from the pilot project award.
- PIs agree to meet with CURES program leaders to provide updates on their projects, as requested.
- PIs must provide written updates on their research progress for annual progress reports and meetings with the CURES External Advisory Board.
- PIs must present their research findings at one of the CURES Center-wide research meetings.
- Pilot project recipients will also be required to present their results at one of the CURES Community Outreach and Engagement Core’s Environmental Health Forums.

Post-award responsibilities. Upon completion of a project, the PIs will be required to submit a report that contains the following information:

1. A list of any publications (i.e., research articles, review articles, abstracts; submitted, in press, or published) or patents that resulted from the pilot project award.
2. A list of any grant applications submitted (funded, pending, or non-funded) that resulted from the pilot project award in which the applicant was listed either as PI or co-investigator.
3. A list of collaborations that developed as a result of the pilot project award.

Pilot project recipients will be expected to submit applications for extramural funding to continue their projects as soon as practicable.

Please adhere to the following formatting requirements when preparing LOIs:

- Font: Use an Arial, Helvetica, Palatino Linotype, or Georgia typeface, a black font color, and a font size of 11 points or larger. (A Symbol font may be used to insert Greek letters or special characters; the font size requirement still applies.)
- Type density, including characters and spaces, must be no more than 15 characters per inch. Type may be no more than six lines per inch.
- Use standard paper size (8 ½" x 11).
- Use at least one-half inch margins (top, bottom, left, and right) for all pages.
CURES Facility Cores

1. Integrative Health Sciences Facility Core (IHSFC)

Leaders:
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The purpose of the IHSFC is to facilitate the design, development and ethical achievement and dissemination of the translational research goals of the CURES program. The design of the core is to support the multidisciplinary collaborative research interest groups in their pursuit of understanding the complex role of chemical and non-chemical stressors as modifiers of human health in the urban environment. The IHSFC accomplishes this objective through 4 integrated core services:

1. Experimental Design and Biostatistics – Consultation support for environmental health science investigations focused, but not limited to, human subjects, human samples, human populations, and general study design and data analysis plans. This is done via four mechanisms: a) Statistical consultation on pilot grant applications to and funded by CURES, b) Experimental design consultation on pilot grant applications to and funded by CURES, including but not limited to issues pertaining to IRB, IACUC, biohazards, and translational GLP, c) Biostatistics methods development in research pertaining to environmental health, and d) Biostatistical education for CURES members and for the university at large. The IHSFC collaborates with the CURES Career Development and Pilot Project Programs to facilitate research progress (from pilot projects to K awards and R21 applications, and to covert R03 and R21 awards to R/U/P01 applications).

2. Health Models Development – Integrating exposure assessment with human health impact. Helping research teams to select the appropriate models to capture windows of heightened susceptibility to environmental contaminant exposure, the health impact of chronic low-level exposure to toxicants, exposure to complex mixtures of toxicants. (e.g., stem cell models, primary human cell culture models, access to specialized tissue banks such as the Michigan Neonatal Biobank).

3. Ethics and Research Integrity in Environmental Health Science – The protection and ethical treatment of patient subjects and samples is of paramount importance. These concerns expand and are integrated into our experimental design to acknowledge and address the appropriateness of certain levels of analysis that might have immediate or future implications for the safety and rights of an individual. The requirement for discussion of the propriety of proposed clinical translation goals serve not only to ensure the integrity of our research endeavor but serve as an educational opportunity for the career development of CURES program researchers. This developing topic will also cover emerging research ethics concerns.
with the expansion of “big data,” EMR, human biospecimens, stem cell models and geospatial analysis in environmental health research.

4. Bridging the Communication Gap – Integrating the Effectiveness of Research and Community Partnerships. Work closely with Community Outreach and Engagement Core to identify community partners for appropriate CURES research teams. Cross-train CURES researchers and community members to communicate topic-relevant data, research goals, and likely achievements in a responsible manner. Enhance and initiate national and global initiatives to educate and advance research consistent with the mission of the NIEHS.

2. Exposure Signatures Facility Core (ESFC)

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The ESFC provides CURES researchers with an interactive environment that allows them to integrate genomic, epigenomic, and proteomic technologies at all phases of investigation, from the design phase through generating preliminary data, to conducting the research and writing the methodologies, and finally data analysis and interpretation. “Exposure Signatures” are the quantifiable responses of a biological system to an environmental stressor, and include changes in gene expression, DNA methylation patterns, histone modifications, protein levels, and posttranslational modifications. The Core will contribute to advancing the research programs of Center members by refining existing methods, developing new genomics and proteomics methods, and educating CURES Center members about new technologies that quantify cellular changes in response to the environment. The ESFC unifies three existing facility cores at WSU – a Genomics Core (the Applied Genomics Technology Center), an Epigenomics Core, and a Proteomics Core, and provides a “one-stop-shop” to CURES members for biological and bioinformatics services.

Genomic Services (provided through the Applied Genomics Technology Center, Dr. Susan Land, Director)
The Applied Genomics Technology Center (AGTC) provides investigators with state-of-the-art genomic technologies and expert consultation services. To meet the needs of investigators, a meeting with the investigators and AGTC staff that includes bioinformaticians and statisticians, takes place to select the optimal technology platform, sample collection methods for the application and study design. This service is a no-cost design phase and has been shown to yield optimum workflows. The AGTC offers a full range of services starting with nucleic acid isolation through genetic analysis, data analysis, and validation studies. This comprehensive service allows the investigator to be involved in every stage of the experiment rather than merely shipping the sample to a third-party. The AGTC has multiple platforms to meet investigative needs. For example, an investigator who needs expression analysis has the options of running multiple array platforms, next generation sequencing, or quantitative RT-PCR (Illumina BeadArrays, Affymetrix GeneChips, Agilent SureSelect, Illumina RNA-Seq, Applied Biosystems 5'-nuclease assays and OpenArrays), depending on whether they need information on the entire known transcriptome, only a few known genes, or are undertaking discovery science. The optimal platform is also dependent on the sample quality, e.g., nucleic acid derived from formalin section is not suitable for all platforms because of strand breaks and crosslinking. The investigators choose how much of the workflow they will do and how much will be done by the AGTC. The AGTC then works with the investigator by generating preliminary data, providing methodology descriptions, analyzing cost, and performing high-throughput implementation technology. A genomics analyst group is available to incorporate seamless data management and analysis to genomic data generated by the AGTC or to work with the investigative team to transfer data in the correct format for their analysis software.

**Services and Technology:** AGTC services are divided into three categories:

1) Fully established services (e.g., DNA sequencing)

2) Fee-for-service with established prices, which may vary depending on sample type, quantity, and reagent availability, (e.g., genotyping)

3) Studies requiring new technology development. AGTC staff, in collaboration with investigators and data analysis groups, design experimental approaches to optimize results and provide investigators with a detailed cost analysis and documented methods. Investigators are encouraged to submit preliminary samples prior to an experiment in order to standardize methods and make modifications to optimize results.

Specific services include the following:

- **DNA Isolation** by magnetic bead technology (small scale, Qiagen EZ1 Advanced or larger scale QIAsymphony) or manual processing is available for the following samples: blood, buccal, saliva, frozen tissue, and formalin-fixed paraffin sections. Options include RNase treatment, glycogen addition for small samples, and additional protease treatment.

- **Next Generation Sequencing** utilizing an Illumina HiSeq 2500 provides both high output and rapid runs for discovery of genetic and epigenetic variation. Options include: library preparation for DNA-Seq, RNA-Seq, microRNA-Seq, ChIP-Seq, Exome-Seq, and Amplicon-Seq. Output from the HiSeq includes files containing the basecalls for each sample (*.bcl) which is demultiplexed (if necessary) and converted to *.fastq files using CASAVA 1.8. The quality of the sequencing run is determined using the FastQC software as well as the parameters from the First Base Report produced by the HiSeq. The reads are aligned to the reference genome using Novoalign or TopHat, which takes the *.fastq files as input and produces *.sam and *.bam files as output, respectively. The remaining analysis options depend on the requirements set forth by the investigator. From the aligned reads, we can determine differential expression (Cufflinks or Partek) or alternative splicing (Cufflinks or Partek), and do variant calling (samtools and GATK (Genome Analysis Toolkit)).
Next Generation Sequencing utilizing an Illumina MiSeq provides a rapid, low throughput system suitable for small genomes and targeted follow up. The MiSeq has automated data analysis capabilities.

DNA Sequencing (Sanger) utilizing an Applied Biosystems 3730. Options include: primer design, sample amplification, and amplicon purification before sequencing. Optimization of sequence reactions is available for hard to sequence templates such as GC rich regions.

Genotyping Services including primer and probe design, when the assays are not commercially available, assay optimization, troubleshooting, and limited analysis. Options for single nucleotide polymorphisms include 5’-nuclease assays (TaqMan, QuantStudio 12K Flex Real-Time PCR system - OpenArrays with fixed or custom content, digitalPCR, or single assays), GoldenGate (custom multiplexed panels up to 1536-plex and fixed content panels such as the Cancer Panel, Illumina iScan) and Infinium (fixed content panels of up to 1.2 M, Illumina iScan) assays, length polymorphisms (Applied Biosystems 3730), and SNP chips (fixed content, Affymetrix)

DNA Methylation Studies including bisulfite treatment of DNA, DNA sequencing (AB 3730), Illumina Infinium Methylation Bead Arrays (fixed and custom panels, Illumina iScan), Sequenom MassArray system and PyroMarkQ24 (Qiagen)

Somatic Mutation Analysis using the MassArray system (Sequenom) or PyroMarkQ24 (Qiagen).

RNA Isolation obtained from cell lines, blood, paraffin slices, and tissue is available. The quality of the RNA is determined using a 260nm/280nm (NanoDrop or DropSense Spectrophotomer) and the 28S/18S and RNA Integrity Number (RIN) (Agilent Bioanalyzer 2100 or TapeStation).

Expression Analysis options including quantitative RT-PCR (TaqMan, QuantStudio 12K Flex Real-Time PCR System - OpenArrays with fixed or custom content, digitalPCR, or single assays), SureScan arrays (Agilent), GeneChips (Affymetrix), Sentrix arrays (Illumina iScan), and DASL assays for RNA isolated from formalin-fixed paraffin tissues (Illumina iScan). Expression analysis can also be accomplished with next generation sequencing on both the Illumina HiSeq and MiSeq instruments.

Resources and Instrumentation:
- Qiagen EZ1 Advanced (2)
- Qiagen TissueLyser (2)
- Applied Biosystems 3730
- Applied Biosystems QuantStudio 12K Flex Real-Time PCR System with Automation Robot
- Illumina iScan with Tecan freedom evo robot customized to run Illumina protocols and an Illumina AutoLoader
- Affymetrix GeneChip Scanner 3000 with Autoloader, two GeneChip Fluidics Station 450, GeneChip Hybridization Oven 640, two Affymetrix workstations
- Agilent 2100 Bioanalyzer
- Agilent Tape Station
- NanoDrop ND-1000 Spectrophotometer (2)
- Trinean DropSense96 Spectrophotometer
- Eppendorf epMotion 5075 (3-two in prePRC labs and one in a postPRC lab)
• Covaris S2 (adaptive acoustic focused energy for shearing nucleic acid)
• Illumina HiSeq 2500
• Illumina MiSeq
• Fluidigm C1
• Leica DMi8 Fluorescent Microscope with automated focus and stage
• Primary Analysis Server (Dell R910 with 32 cores, 1TB memory, and 12 TB local storage)
• Server for Illumina Pipeline (Dell R900 with 16 cores, 64 GB memory, and 10 TB storage)
• Failover and Analysis Server (Dell R900 with 16 cores, 256 GB memory, and 30 TB storage)
• Testbed Server Dell R900 with 16 cores, 160 GB memory, and 30 TB storage)
• Sunfire X4540 with 40 TB storage and a X4550 with 96 TB storage for archiving at the CS Mott Center and one of each unit at WSU C&IT acting as a mirror
• Wayne State University grid (4,330 cores, 10+TB memory, and 40+TB storage)

**Genomics Data Management and Analysis** options are available for data generated within the Genomics Core. Next-generation sequencing (NGS) data are stored on local servers and backed-up by Wayne State University Computers and Information Technology (C&IT) personnel. The retention time for this data is currently three years for all data except next generation sequencing which is at least three months. Data analysis services are provided for Illumina Infinium HumanMethylation 450K BeadChips, SureScan arrays (Agilent), GeneChips (Affymetrix), Sentrix arrays (Illumina iScan), as well as NGS data (Illumina HiSeq 2000). The type and level of analysis is determined by the needs of the investigator and range from processing of raw data, to pathway analysis and variant detection. Our most frequently used analysis software includes Partek, OncoMine, GenomeStudio, IPA (Ingenuity Pathway Analysis), BioBase, and Genomatix.

**Proteomic Services** (Provided through the Proteomics Core, Dr. Paul Stemmer Director)
1. Protein identification using nano-LC/MS/MS instruments
2. Protein quantitation using spectral counting, isobaric tags and Stable Isotope Labeling with Amino acids in Cell culture (SILAC) with data acquired on the Orbi Elite, Q Exactive, or the LTQ and the Multiple Reaction Monitoring strategy using the TSQ Vantage system
3. Proteomic profiling using two-dimensional chromatographic separations or MuDPIT technologies
4. Analysis of post-translational modifications using nano-LC-MS/MS with fragmentation by ETD, higher energy collisional dissociation (HCD), or collision induced dissociation (CID).
5. Robotic protein digestion using the Genomic Solutions Investigator ProGest
6. Protein interaction and protease activity analysis by fluorescence polarization measurement using the Beacon 2000 fluorescence polarization system or the Tecan Polarion microplate reader
7. Peptide labeling and purification using an HPLC with UV and Fluorescence Detectors. Fluorescence measurement using a QM-6 spectrofluorometer equipped for simultaneous dual-emission recording and with removable dual-emission polarizers all controlled by software on an accompanying work station
8. Data Analysis using Mascot, Sequest, X!tandem, and Peaks algorithms with data compilation and secondary analysis using Scaffold.