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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY (DARPA)
13.B Small Business Technology Transfer (STTR) Program
Proposal Submission Instructions

1.1 Introduction:

DARPA's mission is to prevent technological surprise for the United States and to create technological surprise for its adversaries. The DARPA SBIR and STTR Programs are designed to provide small, high-tech businesses and academic institutions the opportunity to propose radical, innovative, high-risk approaches to address existing and emerging national security threats; thereby supporting DARPA's overall strategy to bridge the gap between fundamental discoveries and the provision of new military capabilities.

The responsibility for implementing DARPA's Small Business Technology Transfer (STTR) Program rests with the Small Business Programs Office.

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Offerors responding to the DARPA topics must follow all the instructions provided in the DoD Program Solicitation. Specific DARPA requirements in addition to or that deviate from the DoD Program Solicitation are provided below and reference the appropriate section of the DoD Solicitation.

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DARPA STTR 13.B Topic Index

ST13B-001	**Advanced Tools for Mammalian Genome Engineering**
ST13B-002	Quantum Dot Mid-Wave Infrared Focal Plane Array
ST13B-003	Multiferroic Materials for RF Applications
ST13B-004	Data-Parallel Analytics on Graphics Processing Units (GPUs)

DARPA STTR 13.B Topic Descriptions

ST13B-001 TITLE: Advanced Tools for Mammalian Genome Engineering

TECHNOLOGY AREAS: Biomedical

OBJECTIVE: Improve the utility of Human Artificial Chromosomes (HACs) by developing new selectable metabolic markers for use in human cells, new high-fidelity methods for inserting DNA constructs of at least 50,000 base pairs (bp) in length into defined genomic loci, and new methodologies for facile intercellular genome transplantation.

DESCRIPTION: The ability to deliver exogenous DNA to mammalian cell lines is a fundamental tool in the development of advanced therapeutics, vaccines, and cellular diagnostics, as well as for basic biological and biomedical research. Current approaches to genetic engineering of mammalian cells rely on gene transfer methods such as plasmids, adenovirus-, lentivirus-, and retrovirus-vectors, cDNA, and minigene constructs. While these tools do provide the basic ability to deliver DNA to mammalian cells, there are several shortcomings associated with these state-of-the-art techniques. These include random DNA insertion into the host genome, variation in stable integration sites between cell lines, variation in the copy number and expression level of DNA that is delivered, limitations on the number and size of DNA constructs that can be delivered, and immunological responses to foreign DNA. Coupled with the significant time that is required to obtain useable engineered cell lines, these factors severely limit the scale and scope of research that can be performed and the applications that can be pursued.

One recently developed method for gene transfer that has the potential to address many of these shortcomings is the use of human artificial chromosomes (HACs). HACs possess several ideal properties, including very large DNA delivery capacities, stable, episomal maintenance within the cell, and lack of immunogenicity. Additionally, HACs can be designed to contain specific DNA sequences, such as integration sites, making them ideal for the creation of a completely engineerable platform. Although HACs show significant potential as a gene delivery vehicle, several technical hurdles remain that have prevented wide adoption of the technology. First, while HACs have the capacity to contain extremely large segments of DNA (potentially up to or surpassing 1,000,000 bp), currently molecular biology techniques are limiting in the amount of DNA that can be inserted into a DNA vector. It is typically difficult to insert more than 20,000 bp of DNA into a vector, negating much of the advantage that HACs possess as a delivery platform. Second, few selectable markers exist that are suitable for use in human cell lines, limiting the ability to screen for insertion or maintenance of the delivery platform. Third, methods utilized to transfer HACs between cell lines for vector delivery are extremely technically challenging, requiring highly specialized knowledge in order to be able to work with existing HAC vectors.

This solicitation is focused on improving the utility of HACs as a DNA delivery platform by developing technologies to address several key technical hurdles associated with current HAC vectors. These includes development of new selectable metabolic markers suitable for use in human cell lines, new high-fidelity methods for inserting DNA constructs at least 50,000 bp in length into defined genomic loci, and new methodologies for facile intercellular genome transplantation. A successful technology will be able to integrate into existing HAC vectors and will be capable of being readily transitioned to academic, government, and commercial researchers, all of whom rely on the ability to deliver DNA to mammalian cell lines.

PHASE I: Determine the technical feasibility of a new approach that focuses on addressing ONE of the following technical challenges:

a) The development of at least 3 new selectable markers based on metabolism for use in delivery of a HAC. Each metabolic marker should allow for the identification and selection of cells that have been transfected with the HAC vector, and should be broadly useful across human cell lines with minimal or no genetic manipulation of the host cell line required. Appropriate metabolic markers should be identified,

methods for genetic selection should be detailed, and an analysis of cost for selection and maintenance of cell lines using each marker should performed.

b) The ability to stably integrate at least 50,000 bp of DNA into at least 10 different targeted genomic loci. Methods for insuring single copy integration at each site should be described and the ability to test for proper integration site targeting and specificity should be addressed. Performance goals of the new approach for maximum size limit of DNA that can be integrated and the percentage of correct cells that are achieved per transformation event should be established.

c) The development of a methodology for the rapid and facile shuttling of chromosomes or chromosome-sized DNA (e.g. HACs) between at least 10 different cell lines. The possibility of truncation, rearrangement, or fragmentation during chromosome transfer should be addressed and risk mitigation strategies described as appropriate. Performance goals for the frequency of chromosome transfer should be established.

For the selected challenge, develop an initial concept design and describe an approach for transitioning this technology from a laboratory benchtop to an established commercial protocol.

PHASE I deliverables will include: a technical report detailing experiments and results supporting the feasibility of the approach, and defined milestones and metrics as appropriate for the selected technical challenge. Also included with the PHASE I deliverables is a PHASE II plan for transitioning initial proof-of-concept experiments to protocols that are sufficiently robust and reproducible that they are viable as commercial technologies. The plan should include a detailed assessment of the potential path to commercialization, barriers to market entry, and collaborators or partners identified as early adopters for the new system.

PHASE II: Finalize the experimental approach from PHASE I and initiate the development and production of the technology to address the selected technical challenge. Establish appropriate performance parameters through experimentation to determine the efficaciousness, robustness, and fidelity of the approach being pursued. Develop, demonstrate, and validate the reagents and protocols necessary to meet the key metrics as defined for the selected technical challenge. PHASE II deliverables include a prototype set of reagents, a detailed technical protocol sufficient to allow replication of results in an outside laboratory, and valid test data, appropriate for a commercial production path.

PHASE III: The successful development of technologies for rapid introduction of large DNA vectors into human cell lines will enable the ability to engineer much more complex functionalities into human cell lines than are currently possible. This capability may support a number of DoD challenges, including the development of complex, multifunctional cell-based sensors for chem/biodefense applications, the simultaneous encoding of thousands of prophylactic or therapeutic antibodies for on-demand production of next-generation disease prevention and treatment, and the creation of complex cell lines that can be rapidly reconfigured to produce large volumes of a given vaccine. The biotechnology and pharmaceutical sectors are heavily reliant on the ability to rapidly manipulate and introduce DNA into human cell lines. The successful development of technologies that allow for improved human cell line generation has significant potential to rapidly transition to commercial use, enabling biologically based production of new protein-based therapeutics, new systems for vaccine development and production, and new platforms for small molecule drug screens that provide a more specific and sophisticated testing environment. Many of these applications are currently inaccessible due to the limitation of existing DNA delivery technologies and have the potential to be transformative if the technologies described herein are developed.

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KEYWORDS: Bioengineering, Biology, Biotechnology, Cell Biology, Chromosome, Cytology, Gene Therapy, Synthetic Biology

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