HR001120S0019-16

TITLE: Rapid, flexible de novo manufacturing of DNA molecules for synthetic biology and therapeutic applications

RT&L FOCUS AREA(S):
Biotechnology

TECHNOLOGY AREA(S):
Bio Medical, Chem Bio Defense

OBJECTIVE:
Develop a rapid and cost-effective de novo synthetic DNA manufacturing capability.

DESCRIPTION:
There is a critical DoD need to be able to rapidly and efficiently synthesize highly accurate kilobase (kb) pair length DNA constructs for medical countermeasure and synthetic biology applications. Several DARPA programs and technologies (ex: Living Foundries, PREPARE, P3) rely heavily on synthetic DNA and the timely generation, manipulation, and delivery of genetic constructs. Current synthetic DNA production is costly, time-consuming, and requires highly specialized technical expertise and equipment. Consequently, few commercial suppliers are capable of producing synthetic DNA at a length that is appropriate for DARPA technologies (i.e. >2,500 base pairs (bp)) in the days-long turnaround time required for rapid response. First, due to the limited capability base, commercial sources experience significant backlog in synthetic DNA production services, extending R&D timelines dependent on gene-encoded products, and increasing costs for the consumer. Second, current methods for synthesis or assembly of kilobase length constructs are often error prone, requiring manual purification and/or analytics steps to achieve the final product. Third, as demand for synthetic DNA production increases, any achieved throughput increases will need to maintain or even decrease the cost per base pair.
Phase I proposals should advance the science of de novo DNA synthesis and develop a platform capable of generating oligonucleotides of sufficient sequence accuracy, length, diversity, and quantity for downstream assembly into full-length fragments. To progress from Phase I to Phase II efforts, performers must demonstrate assembly of final product (>2,500 bp) from their synthesized oligonucleotides using either a commercially available methodology or a novel proprietary DNA assembly method. Direct to Phase II proposals must include development of both the de novo DNA synthesis AND assembly platforms.
Requirements for de novo DNA synthesis platform:

- Phase I: The de novo DNA synthesis platform must have the capacity to synthesize, in two weeks, a sufficient quantity of >150 bp oligonucleotides at >99% accuracy, which, when assembled, would generate at least 96 unique >2,500 bp fragments (for example, assembling 96 unique 2,500 bp sequences would require either 1,600 oligonucleotides of 150 bp, or 960 oligonucleotides of 250 bp);
- Phase I transition to Phase II: Assembly of final product must be demonstrated using either a commercially available assembly method or a novel, proprietary assembly method; 96 unique 2,500 bp products assembled at 99% accuracy after error correction methodologies are applied;
- Phase I and II: Product accuracy must be determined utilizing a sequencing technology;
- Phase I and II: Reagents must be non-hazardous and ideally aqueous;
- Phase II: Cost per nucleotide in final product must be < $0.03 per bp.

Table 1: De novo DNA manufacturing SBIR metrics Phase I (6 months) Phase I (11 months) Phase II (13 months) Phase II (24 months) Phase II (36 months)
Oligonucleotide length 150 bp 150 bp 150 bp 150 bp 150 bp Assembled DNA sequence length Unspecified; must simply demonstrate assembly 2,500 2,500 2,500 2,500 Number of DNA sequences Unspecified; must simply demonstrate assembly 96 96 192 288 Timeframe N/A 2 weeks 2 weeks 1 week 1 week Prototype status Air-gapped synthesis and assembly modules Air-gapped synthesis and assembly modules Air-gapped synthesis and assembly modules Alpha prototype Beta prototype Cost per nucleotide Unspecified Unspecified <$0.03 <$0.03 <$0.03 Accuracy of assembled product

N/A N/A >99% >99% >99%

PHASE I:

De novo DNA synthesis platform: Performers will develop a rapid de novo DNA synthesis platform capable of generating oligonucleotides greater than 150 bp long with an accuracy of >99%. The oligonucleotide products should be produced in sufficient quantities for downstream assembly and error-correction into larger templates, although innovation in DNA assembly is not required in Phase I. Specifically, the de novo DNA synthesis platform should produce enough product within a two week production timeframe to enable assembly of 96 unique >2,500 bp fragments. To transition to Phase II, performers must demonstrate the platform's capability of producing these oligonucleotides based on DARPA-defined targets in less than two weeks. Performers must additionally demonstrate assembly of the oligonucleotides into 96 unique >2,500 bp DNA products utilizing either a commercially available or novel, proprietary method.
Schedule/Milestones/Deliverables for de novo DNA synthesis platform Phase I deliverables: Basic prototype design of the de novo DNA synthesis platform and associated assembly method in a final report that must include: (1) prototype performance metrics; (2) results of the capability demonstration; (3) proposed methods to scale de novo DNA synthesis towards production levels capable of generating more than 192 unique sequences in one week; and (4) competitive assessment of the market.
Plans for Phase II should include optimization design goals and key technological milestones to scale no less than 192 or more unique 2,500 bp DNA molecules or equivalent oligonucleotides to assemble in one week.
Proposers interested in submitting a Direct to Phase II (DP2) proposal must provide de novo DNA synthesis AND assembly platform documentation to substantiate that the scientific and technical merit and feasibility described above has been met and describes the potential commercial applications. Documentation should include all relevant information including, but not limited to: technical reports, test data, prototype designs/models, and performance goals/results. For detailed information on DP2 requirements and eligibility, please refer to Section 4.2, Direct to Phase II (DP2) Requirements, and Appendix B of HR001120S0019.
Schedule/Milestones/Deliverables Phase I fixed payable milestones for this program should include:

- Month 1: Kickoff meeting and initial report on status of de novo DNA synthesis methodology and approach for meeting phase I requirements
- Month 6: Demonstrate ability to synthesize >150 base pair oligonucleotides at >99% accuracy; demonstrate initial assembly of those oligonucleotides into longer DNA molecules
- Month 11: Demonstrate ability to scale oligonucleotide synthesis for assembly into 96 unique DNA molecules greater than 2,500 base pairs in length in two weeks
- Month 12: Final Phase I Report summarizing de novo DNA synthesis approach; report summarizing ability to assemble 96 unique DNA sequences of greater than 2,500 base pairs in length utilizing either a commercially available or novel, proprietary methodology.

PHASE II:

De novo synthesis and assembly platform: Develop and demonstrate a flexible, multiplexed platform for the rapid de novo synthesis of DNA molecules based on the basic prototype developed during Phase I. The platform should enable scaled de novo synthesis of at least 192 unique gene sequences of at least 2,500 bp. At the end of Phase II, performers will demonstrate the feasibility of producing 192 unique DNA sequences based on DARPA-defined targets in one week. Sequences produced for each milestone in this phase must be more than 99% accuracy and less than $0.03 per nucleotide.
Phase II deliverables: Working prototype of the multiplexed system and a final report that includes: (1) system performance metrics; (2) results of the capability demonstration; and (3) projections for commercial scale manufacturing yield and costs.
Schedule/Milestones/Deliverables Phase II fixed payable milestones for this program should include:

- Month 13: Demonstrate ability to synthesize and assemble at least 96 unique DNA sequences greater than 2,500 base pair with an accuracy of more than 99% in two weeks and <$0.03 per nucleotide.
- Month 24: Demonstrate ability to synthesize and assemble at least 192 unique DNA sequences greater than 2,500 base pairs with an accuracy of more than 99% in one week and <$0.03 per nucleotide.
- Option Month 36: Demonstrate ability to synthesize greater than 288 unique DNA sequences

of greater than 2,500 base pairs in length on a beta prototype device with more than 99% accuracy and <$0.03 per nucleotide.

PHASE III DUAL USE APPLICATIONS:
The commercial applications of synthetic DNA include, but are not limited to, applications for synthetic biology, manufacturing of protein therapeutics, and drug discovery modalities. These technologies create potential for the use of DNA technologies in cancer immunotherapy. DoD/military applications include generation of synthetic biology components required to produce DoD relevant materials and for the manufacturing of DNA-encoded antibodies and vaccines to provide protection against infectious diseases.

REFERENCES:
1) [1] Randall A. Hughes and Andrew D. Ellington. Synthetic DNA Synthesis and Assembly: Putting the Synthetic in Synthetic Biology. Cold Spring Harb Perspect Biol. 2017 Jan 3;9(1)

2) [2] Palluk S. et. al. De novo DNA synthesis using polymerase-nucleotide conjugates. Nat Biotechnol. 2018 Aug;36(7):645-650. doi: 10.1038/nbt.4173. Epub 2018 Jun 18.]

KEYWORDS:
DNA, manufacturing, synthesis, synthetic biology, DNA assembly

TPOC USERS:
None