The National Institute for Innovation in Manufacturing Biopharmaceuticals

Optimize Long-read Next Generation Sequencing (NGS)-based Methods for Confirmation of Viral Genome and Detection of Viral Genome Variants
Request for Application

RFA Release Date: March 4, 2025

Submission due Date: April 3, 2025

Target Decision Date: April 30, 2025

NIIMBL

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1. Executive Summary

The mission of the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) is to accelerate biopharmaceutical manufacturing innovation, support the development of standards that enable more efficient and rapid manufacturing capabilities, and educate and train a world-leading biopharmaceutical manufacturing workforce. NIIMBL is pleased to announce this Request for Application (RFA) to participate in the Viral Vector Program's initiative on characterizing long-read NGS capabilities. Questions can be sent to projectcalls@niimbl.org.

Funding Opportunity Title: RFA: Optimize Long-read Next Generation Sequencing (NGS)-based Methods for Confirmation of Viral Genome and Detection of Viral Genome Variants

Applications must be submitted via the NIIMBL Proposal Submission Hub. Applications received after the deadline (see Table 1), or otherwise non-compliant with the submission requirements, will not be considered.

The RFA concludes with a decision by the NIIMBL Viral Vector Program Leadership Team to fund or not to fund applications. Awarded projects will be expected to complete contracting within 90 days of formal notification of NIIMBL's intent to fund. NIIMBL reserves the right to rescind offers of funding to awardees.

Table 1. Timeline

EVENT	DATE
Submission Due by 5:00 pm ET	April 3, 2025
Application Review Period:	April 7 – 30, 2025
Award Decisions Announced	May 2, 2025
Estimated Project Start Date	August 1, 2025

Total Amount to be Awarded:

NIIMBL intends to fund up to 1 project through this RFA, with the selected project eligible to receive funding up to $400,000. Cost share requirements are described in the Submission Requirements section.

2. Project Requirements

Background

The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) is announcing a funding opportunity for the analysis of AAV vector genomes via long-read NGS (next generation sequencing). This funding opportunity is facilitated through the NIIMBL Viral Vector Program (VVP). The VVP consists of subject matter expert representatives of NIIMBL member organizations.

Motivations for the study: There is a need for reproducible assays to assess the quality of AAV-based gene therapy vectors. Next generation sequencing (NGS) is a promising technology for sequence confirmation of viral genome and detection of contaminating DNA.

Objectives of the study: NIIMBL seeks to fund a team to determine the capabilities of long-read NGS based methods to serve as characterization and potentially quality control methods for AAV-based viral vectors. Such capabilities have the potential to improve modality and process understanding, translating to lower risks and greater patient outcomes in AAV vectored gene therapies. Prospectively, this study may be used to inform a future inter-laboratory study for this NGS application.

Specific area of Interest and Expected Outcomes

The goal of this project is to evaluate and optimize long-read next generation sequencing (NGS) as a reliable and comprehensive analytical method for assessing the quality of adeno-associated virus (AAV) products, focusing on its ability to detect and quantify genome variants, structural features, and contaminating DNA with high accuracy and precision. For the purposes of this document, long-read sequencing refers to a technology capable of producing read lengths greater than 1,000 base pairs.

The team is expected to evaluate the suitability and, if needed, optimize long-read NGS-based methods for confirmation of viral genome and detection of viral genome variants (e.g. sequence variants, truncations, insertions) and contaminating DNA

- The participating team will collaborate closely with NIIMBL at regularly scheduled updates (monthly), sharing their best methods, protocols, and results with subject matter experts within the VVP.
- At the end of the period of performance, a workshop will be organized to facilitate extensive discussions among the participating team, the VVP, and NIIMBL staff. The workshop will focus on the approaches, methods, results, and challenges.
- By accepting NIIMBL funding, participants agree to make their protocol, analysis, and data available to NIIMBL and the community. A protocol sufficient for facilitating a method transfer to a qualified testing lab is envisioned.
- Companies choosing to participate may retain any preexisting methods and algorithms as proprietary. However, the freedom for which the protocol can be

published, distributed, and applied to clinical and commercial drug development will be a criterion for the selection of funding.

- The team is expected to describe types of study materials required (e.g. genome length, variants, purification level, etc). to demonstrate the performance of the method. Procurement of the required research materials should be included in the team's requested budget. Limited materials may be provided from NIIMBL stocks upon request.

Project Constraints

- Proposals must be consistent with NIIMBL Bylaws and should be labeled as NIIMBL Confidential.
- Proposals will be accepted with the following constraints:
    - A maximum $400,000 of NIIMBL funding per project
    - All project team members must be NIIMBL members before the final award decision date
    - A maximum 9-month period of performance

Proposal Narrative

Describe the technical approach that will be used to evaluate the suitability of a method for long read NGS-based characterization of AAV vectors, and if needed, the approach for optimization. Include the types of pre-treatment, extraction, library preparation, sequencing, and analysis that will be carried out. Also include the anticipated read-out of the method – what information will be provided and how will it be used to characterize AAV vectors. If orthogonal methods need to be applied or optimized to evaluate long read NGS, please describe those. Also, if specific test articles or reference materials are required for the evaluation, please describe.

Specific questions which should be addressed should include, but don't have to be restricted to:

- What is the method capability to detect and quantify variants (single nucleotide, insertions, truncations) and impurities (residual host cell or plasmid DNA)?
    - Assay parameters to be evaluated as appropriate: accuracy, precision, LLOQ, dynamic range.
    - Evaluation/Identification of method artifacts (e.g., ability for method to discriminate between partial genomes/truncations and sample prep induced fragmentation)
    - Evaluation of method biases that impact accuracy (e.g., impact of length of DNA)

- What is the accuracy for base calling of the technique? How accurate is long read NGS at homopolymer regions, genome ends, inverted terminal repeats (ITRs), secondary structures, structural variants etc.?

The technical approach should be specifically related to the tasks and milestones outlined on the Gantt chart (Appendix C). Proposers should also describe the success criteria / evaluation approach for the project, including metrics for measuring project success. Deliverables must be specific and quantitative whenever possible.

Description of Capabilities

This RFA seeks to fund a single organization to accelerate funding and contracting. This section should be used to describe the capabilities at your organization that enable the work to be carried out, including personnel, instrumentation, and facilities. Identify the Principal Investigator and any other senior/key personnel that will play a leadership role on the project. Each of these individuals should have a Biosketch (Appendix A). Include considerations for how the team will communicate, maintain timelines, stay within budget, and how decisions will be made.

Description of Study Outputs and Commitments

Briefly describe the proposed study deliverables and method transfer:

<u>Deliverables</u>

- Define the scope of procedures (e.g., Sample Prep, Reagents, Data Analysis) to be furnished for use within the NIIMBL Viral Vector Program only
- Define the scope of procedures (e.g., Sample Prep, Reagents, Data Analysis) to be furnished for publication and distribution by the NIIMBL Viral Vector Program
- Indicate any concerns and constraints related to sharing protocols, data, results, methods, algorithms, and approaches.
- Describe a supportive data package demonstrating method performance

<u>Method Transfer</u>

- Indicate the capacity for supporting an analytical method transfer to NIIMBL or a qualified testing lab following the study
- Indicate the capacity for participation in a future interlaboratory study evaluating the furnished procedures

2.1 Eligibility Criteria

Membership

Proposers need to be a federal employee or NIIMBL member. Non-NIIMBL organizations should submit a partially executed NIIMBL Membership Agreement by 5:00pm EST two weeks prior to the submission due date 04Apr25. Information on how to join NIIMBL is available at: https://www.niimbl.org/membership/

Cost Share

There is no minimum cost share commitment required. Proposers must offer and document their cost share commitment consistent with their Membership tier requirements. Applicants should be aware that the institutional cost share requirements for NIIMBL member organizations vary based on institution type (e.g., industry, academic/non-profit organization) and tier level.

For Delaware based organizations requesting State of Delaware cost share support, additional review and approval is required. Proposers are encouraged to include confirmation of the support (Appendix I).:

Delaware: Contact Marta Rosario (martar@udel.edu) by 5:00 p.m. Eastern Time on two weeks before due 04Apr25, to request state cost share. The request should include a 1-paragraph description of the project, title, partners, and budget narrative.

Federal Agency Participation

NIIMBL Solicitations are open to Federal proposers. NIIMBL welcomes and encourages the participation of Federal employees. Federal employees may suggest a project that NIIMBL should undertake as a community, participate on a project team, or lead a project, as appropriate, within the mission and constraints of their agency. Participation in a NIIMBL project must be compatible with agency missions and any constraints related to accepting resources from NIIMBL. In general, NIIMBL will try to accommodate the unique needs of Federal proposers in this process to reduce barriers to participation. Federal employees should review the Guide for Information for Federal Stakeholders.

Human Subjects Activities are not expected and will require prior written approval before work can begin.

Vertebrate Animal Activities are not expected and will require prior written approval before work can begin.

3. Application Instructions

3.1 General Instructions

Submissions
Applications must be submitted via the NIIMBL Proposal Submission Hub and must be received no later than the submission deadline in Table 1. Applications received after the deadline, or otherwise not compliant with the requirements of this solicitation, will not be considered.

Confidentiality
Teams are expected to mark their submissions as "NIIMBL Confidential," in accordance with the NIIMBL Bylaws, limiting access to NIIMBL members or Federal representatives. The exception is the Proposal Abstract, which will be released to the public if an award is made.

3.2 Application

The proposal narrative must be no more than 4 pages.

Applications will be evaluated by the Viral Vector Program Leadership Teams, composed of subject matter experts from member organizations and federal stakeholders.

Submissions that do not adhere to the formatting and length limits for the narrative and required appendix documents will be considered non-compliant and will not receive a technical review.

The application must address and include the following:

1. Application Narrative must be no more than 4 pages.
2. Abstract (200 words max; not counted towards narrative page count)
3. Executive Summary (up to 1 page; not counted towards narrative page)
4. Required Appendices (not counted towards narrative page count)

Required Appendices-

Appendix A	Biosketches 2-pages
Appendix B	N/A
Appendix C	Project Plan – **see template**
Appendix D	Budget & Justification (.xlsx & .docx – **see templates**)
Appendix E	N/A
Appendix F	References
Appendix G	List of Acronyms
Appendix H	Project Partner Organization Identification Form – see templates
Appendix I	Cost Share Confirmation Support

Table 2. Summary of Application submission documents.

	Constraints
Abstract	200 words
Application Narrative	Single-spaced 1-inch margins 11-point Arial font (or equivalent) Maximum of [4]-pages File Type: .pdf only
Required Appendices	No page limits. Adhering to templates provided at: NIIMBL.org File Type: consistent with templates

Abstract
The abstract must include the PI, co-PI names and organizational information, and a brief description of the proposal. The abstract will be released to the public if an award is made; therefore, proposers are expected to ensure that it does not contain any confidential or proprietary information.
NOTE: The Abstract should be included in the pdf file of your proposal documents. You will also be required to copy and paste the Abstract into a text field in the Submission Hub. The names and organizations are not included in the 200-word count.

Application Narrative: All documents listed above should be included in one .pdf file with the exception of Appendices C, and D, which should be uploaded separately in their appropriate file format.

This section should be used to describe the capabilities at your organization that enable the work to be carried out, including personnel, instrumentation, and facilities. Identify the Principal Investigator and any other senior/key personnel that will play a leadership role on the project. Each of these individuals should have a Biosketch (Appendix A). Include considerations for how the team will communicate, maintain timelines, stay within budget, and how decisions will be made.

- Technical Approach
- Description of Capabilities
- Description of Outputs

3.3 Required Submission Appendices

Appendix A: Biosketches

Provide biosketches for the PI and all named senior/key personnel. Biosketches are limited to two pages each.

Appendix C: Project Plan

The project plan should include a Gantt Chart detailing the major tasks, and deliverables for the proposed project. It will visually show how the work will be

completed over time, with a minimum of 1-month increments. **The Period of performance is not to exceed 9 months**. The Gantt chart should be submitted as a .pdf, or .xlsx file.

Appendix D: Budget & Justification

The Budget should be broken into requested funding segments that align with the Gnatt Chart in the Project Plan. The budget should include the NIIMBL funding request and cost share commitment and be submitted as an xls. File. The budget justification should be a written narrative explaining in detail the need for the identified cost categories.

Appendix F: References

Provide a complete list of references cited in the project proposal. If references are not used, indicate N/A.

Appendix G: List of Acronyms

Provide a complete list of acronyms used in the project proposal. If acronyms are not used, indicate N/A.

Appendix H: Project Partner Organization Identification Form

All organizations requesting NIIMBL funding and committing cost share are required to complete and submit either a Subrecipient Commitment Form or a Letter of Intent.

If your organization is a federal agency or is a participant in the Federal Demonstration Partnership (FDP) Clearinghouse, your organization should submit a Letter of Intent.

Templates can be found on the NIIMBL website NIIMBL.org.

4. Application Review and Evaluation

4.1 NIIMBL Review Process

Applications must comply with the requirements outlined in this RFA. All formatting requirements, administrative requirements, terms and conditions, and other requirements will be assessed for completeness.

Automatic rejection will occur if the submission is received after the published deadline or if the proposer did not meet the deadline to submit a membership request.

NIIMBL will review applications to ensure suitability of the work within the project description (see Section 2 of this RFA). Applications will be provided to subject-matter experts in the Viral Vector Program for review.

4.2 NIIMBL Acceptance Criteria

NIIMBL Subject-Matter Expert Review Panel

Proposals will undergo a merit review by a panel of subject-matter experts, and will be assessed using the following criteria:

1. The proposal's ability to address the defined need outlined in the project description
2. The proposal team's capability to provide a solution as demonstrated by team PI/member qualifications, available technical infrastructure, and past contributions to NIIMBL projects
3. The freedom with which the study output can be published, distributed, and incorporated into pre-clinical, clinical, and commercial process development
4. Budget and cost share commitment

5. Reporting

Project reporting requirements will be outlined in the Subaward contract.

6. Abbreviated List of Acronyms

1. AAV Adeno-Associated Virus
2. BRL: NIIMBL Biomanufacturing Readiness Level
3. Co-PI: Co-Principal Investigator
4. CQA: Critical Quality Attribute
5. FDP: Federal Demonstration Partnership
6. FFRDC: Federally Funded Research and Development Centers
7. IP: Intellectual Property
8. ITR: Inverted Terminal Repeats
9. LLOQ – Lower Level of Quantification
10. NGS – Next Generation Sequencing
11. NIIMBL: National Institute for Innovation in Manufacturing Biopharmaceuticals
12. PI: Principal Investigator
13. RFA: Request for Application
14. SC: Steering Committee
15. VVP: Viral Vector Program