Global Stewardship of Recombinant Plasmid Sequences
A Win-Win Approach to Enhancing Biosecurity and Biological Sciences Research

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Abstract

Recombinant laboratory plasmids (RLPs) are common in biological research and freely shared among academic research laboratories (ARLs), a practice required by many research funding agencies. However, the generation of accurate, reproducible results in experiments utilizing RLPs can be hampered by a lack of accompanying sequence information and metadata. This culture of RLP sharing without knowledge of sequence or etiology is accepted by publishers, not regulated by governments, and outside the realm of bio-industry. In addition, no centralized infrastructure currently exists to collate such data, which at the moment is fragmented across companies, non-profits, and governments and thus is not easily accessed or enacted toward threat assessment. The ubiquity, free exchange, and dual-use risk of RLPs exemplifies a biosecurity threat and elevates the need to characterize their composition to facilitate improved biorisk management by the academic community.

A number of common sense solutions are available to create a culture that addresses the biosecurity gap posed by RLP sharing. Culture shift in RLP management will require new norms, effective data management for collation of RLP sequences and metadata, and an incentive structure that encourages sequencing by stakeholders. The next generation of researchers must initiate and champion this shift with support from funding agencies and endorsement from governments and international organizations. Coordination of efforts and stakeholders will require international public/private collaboration, a structure that will be critical to ensure widespread utility as well as the ability of lower-resourced partners to participate, contribute, and benefit.
Introduction

The design and generation of unique nucleotide sequences has led to innovation across a variety of fields including chemistry, biology, and engineering. In this regard, academic research laboratories (ARLs) are a titan of innovation through the development of recombinant laboratory plasmids (RLPs), nucleotide circles with applications such as protein expression, gene regulation, and genetic engineering. However, global development and distribution of RLPs has occurred without oversight of appropriate sequence identity characterization. This knowledge gap has ramifications for the integrity of laboratory results, and poses a significant risk to biosecurity.

Because of the cost of nucleotide sequencing\(^1\) and the lack of a standard for providing sequence and metadata as supplement for manuscripts in scientific publishing, researchers infrequently sequence, track, or create off-site stocks of their RLPs. The resulting lack of sequence data presents hurdles to research innovation including: a) issues with troubleshooting, b) difficulty in replacing lost samples, c) reduced reproducibility, and d) inability to identify the source of the parent RLP to address the abovementioned issues. Further exacerbating these problems, funding agencies also do not require sequencing and encourage plasmid sharing between laboratories.\(^2\) Currently, there are a variety of non-academic organizations dedicated to accumulating RLPs designed/developed in ARLs to more easily facilitate resource sharing.\(^3\) The sequence and metadata accompanying the RLPs in these repositories relies either completely on data provided by the depositor,\(^4\) partial validation of “key regions” by the company,\(^5\) or full-length sequencing by the company.\(^6\) While ARLs largely buy into this resource-sharing ethos, it is currently unknown to what degree ARLs (nationally and internationally) are susceptible to scientific risk from lack of full sequence validation and origin information of their RLPs.\(^7\)

There is also dual-use biosecurity risk from recombinant DNA technologies applied to altering or generating biological threat agents.\(^8\) National norms toward the proper control of these resources are variably implemented, and international norms have yet to be established.\(^9\) This is concerning due to the relative ease and increasing ubiquity of gene synthesis technology,\(^10\) which exhibits that the culture of RLP sharing could serve as a mechanism for accidental or nefarious opportunism. Researchers may share research materials with those who intend to promulgate harm (unknowingly or otherwise), and current regulatory and enforcement institutions have little to no

\(^1\) NHGRI, “DNA Sequencing Costs: Data.”
\(^4\) ASU Biodesign Institute, “Plasmid Submission.”
\(^5\) Addgene, “Deposit Plasmids.”
\(^6\) Genscript, “MolecularCloud Plasmid Platform.”
\(^7\) “Personal Communication with James Diggans.”
\(^9\) NTI, “Biosecurity Innovation and Risk Reduction Initiative”; Carter, Morse, and Yassif, “Proposed Global Norms for Microbiology, Synthetic Biology, and Emerging Biotechnologies.”
way to respond to or trace such events. Additionally, ARLs have not developed codes of conduct toward screening of the sequences they develop, distribute, and receive.

No international body responsible for oversight of such risks currently exists.\(^{11}\) While the Joint External Evaluation (JEE) process can critically appraise biosecurity risks emanating from inadequate laboratory practices and infrastructure while assessing national laboratory biosafety and biosecurity capacity, it does not explicitly address RLP sharing.\(^{12}\) However, knowledge gaps in ARL RLP sequences and origin metadata provide a tangible example of a biosecurity risk that could be mitigated while addressing JEE biosecurity-related indicators. To this point, countries with some laboratory capacity but with indications of poor biosafety and biosecurity systems and practices\(^{13}\) could be viewed as more susceptible to accidental or intentional release of biologicals, particularly those involved in ongoing conflicts.\(^{14}\) Additionally, many countries with strong leadership in biotechnology innovation have yet to evaluate their biosafety and biosecurity system capacities,\(^{15}\) and there is still room for improvement by countries that have been assessed as highly capable.\(^{16}\)

These gaps in biosecurity policy and norms, paired with the specific risks associated with RLPs, highlight the need for change at both national and international scales. It is imperative that parties in the molecular biology, genetic engineering, and microbiology spaces generate new norms that encourage increased responsibility and transparency to address biosecurity and research risks from the current ad hoc approach to RLP management.

**Recommendations**

The time is ripe for implementing global stewardship of RLPs, as rapid innovation in technology since 2000 has dramatically decreased the cost of nucleotide sequencing.\(^{17}\) Establishment of global norms and/or requirements in RLP sequence validation, reporting, and data deposition requires leadership by young professionals in research, government, national and international funding agencies, and the biotechnology industry. To achieve this, we propose the creation of an organizing non-profit entity, modeled on established organizations that leverage public/private partnerships.\(^{18}\) This organization will have responsibility for central coordination and leveraging of an international coalition of biosecurity stakeholders (e.g. academic centers, funding agencies, governments) who can facilitate public/private funding acquisition, RLP data collection and management, development and implementation of norms, and national and international governance.

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11 “Personal Communication with Matthew Watson.”
14 JEE scores in P.6.1 and P.6.2 of a selection of countries in ongoing conflicts: Burkina Faso (1,2), Cameroon (2,1), Chad (1,1), Congo (1,2), Iraq (2,3), Mali (1,2), Mauritania (1,2), Myanmar (2,1), Niger (1,1), Nigeria (1,1), Pakistan (2,2), Somalia (1,1), Sudan (2,2).
17 NHGRI, “DNA Sequencing Costs: Data.”
Our proposed recommendation of how to achieve these goals involves addressing the main barriers to implementation through: a) incentivizing ARLs to participate, b) developing norms for RLP stakeholders to enhance global biosecurity, and c) establishing data governance structures that enable forensic investigations. Here, we provide targeted, stakeholder-specific recommendations toward the development of international oversight and expectations of ARLs producing, procuring, and handling RLPs and their associated sequences.

**Actions for Next-Generation Researchers:**
- Be catalysts for change by setting standards of responsibility toward RLPs via professional societies, encouraging advocacy from leaders in the field, and through the peer review process.
  - Seek mentorship from laboratories that encourage colleagues to submit RLPs to open repositories.\(^\text{19}\)
  - Work toward establishment of screening standards for ARL RLPs, using those of the International Gene Synthesis Consortium (IGSC) as a template.\(^\text{20}\)
  - Become involved in biosecurity-minded organizations such as the InterAcademy Partnership,\(^\text{21}\) the International Federation of Biosafety Associations,\(^\text{22}\) and the NextGen Global Health Security Network.\(^\text{23}\)
- Create a new culture of voluntary reporting of RLP sequences and metadata as part of the publication process and submit physical samples and sequence data to existing repositories to facilitate rapid detection and origin determination of biological threats.\(^\text{24}\)
- Self-impose budgeting for RLP sequence validation as part of the grant-writing process.
- Call for and be actively engaged in the formation of a public/private coalition toward stewardship of building an international RLP sequence identification framework.

**Actions for Next-Generation Professionals in Government and Non-Government Funding Agencies:**
- Support the formation of a coordinating entity and global coalition to create an open-source, international repository of full-length RLP sequences.
  - Partners will contribute to a regional pool of funds to increase collective capacity for supporting sequencing. Contributing partners have priority for fund access, while a dedicated funding proportion is allocated to support low-resourced regions. This type of collaboration aligns with recommendations of international biosecurity-minded organizations.\(^\text{25}\)

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\(^{19}\) IFBA, “Global Mentorship Program.”

\(^{20}\) “Personal Communication with James Diggans.”

\(^{21}\) IAP, “InterAcademy Partnership.”

\(^{22}\) IFBA, “Global Mentorship Program.”

\(^{23}\) GHSA, “Next Generation Network.”


Develop capabilities for rapid sequence and origin identification of emerging or potential biothreats using the repository as a template for screening.\textsuperscript{26} 
- Impose requirements for sequence identification as part of each nation’s biological research grant process and set expectations to budget for these costs in future grant applications.
- Prioritize funding of machine learning-based nucleic acid forensics\textsuperscript{27} for detection and response,\textsuperscript{28} either through existing mechanisms or through the establishment of new ones.
  - Work with research trainees to advocate for piloting these efforts in grants targeted for students, postdoctoral researchers, and new investigators.
- Recruit and foster expertise and leadership towards developing best practices for ARL use and distribution of RLPs, similar to those suggested for gene synthesis providers,\textsuperscript{29} that promote new standards in laboratory biosecurity capacities as evaluated by the JEE\textsuperscript{30} and in line with GHSA recommendations.\textsuperscript{31}

**Actions for Next-Generation Biotechnology Stakeholders:**
- Insist sequencing companies offer incentives for ARL RLP sequencing, such as discounts on future orders or premium services, focusing first on newly-formed laboratories.
  - Financing will be negotiated through the international coalition’s dedicated funding with the goal of providing sequencing services to as many researchers as possible.
  - If relevant, contributors will be members of the IGSC to ensure best practices in nucleic acid screening for biosecurity risks.\textsuperscript{32}
- Existing repository services should work with the international coalition to contribute their current sequences and metadata.
  - In return, the coalition will contribute funding to enhance organizational capabilities in adhering to screening guidelines,\textsuperscript{33} defray costs for establishing physical banking of RLPs, and encourage researchers to utilize these services as part of the above proposed guidelines and funding requirements.
- Providers of biotechnology resources should offer adequate biosecurity measures for sequence screening\textsuperscript{34} as recommended in the GHSA.\textsuperscript{35}

\textsuperscript{27} Nielsen and Voigt, “Deep Learning to Predict the Lab-of-Origin of Engineered DNA.”
\textsuperscript{29} HHS, “Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA.”
\textsuperscript{32} IGSC, “International Gene Synthesis Consortium.”
\textsuperscript{33} HHS, “Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA”; IGSC, “Harmonized Screening Protocol v2.0.”
\textsuperscript{34} Carter, Morse, and Yassif, “Proposed Global Norms for Microbiology, Synthetic Biology, and Emerging Biotechnologies”; “Personal Communication with James Diggans.”
\textsuperscript{35} GHSA, “Global Health Security Agenda: Action Package Detect-1.”
Conclusion

As the field of synthetic biology continues to accelerate, the international community must react quickly to adapt norms and policies to meet emerging biosecurity issues. Here, we propose tangible solutions to biosecurity problems associated with RLP technologies that simultaneously promote a culture of biosecurity norms among ARLs and the biotechnology field more broadly. Additionally, these practices promote high-quality research and will contribute to development of capacities across sectors for, and by, the next generation of professionals who will shape the future of the biosecurity landscape. Our vision for an international public/private partnership would stimulate goodwill and good governance among academic, government, and industry stakeholders and would facilitate development of norms and policies heralded by the next generation of professionals that will be formative for tomorrow’s biosecurity community. With these benefits in place, we envision the next generation of scientists and policy makers as a more cohesive, informed, and secure global collaborative.

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https://www.cdc.gov/globalhealth/security/pdf/ghsa-action-packages_24-september-2014.pdf%0A


