Introduction:

Desktop oligosynthesizers allow for the rapid, customizable, and in-lab synthesis of nucleic acid sequences to include both natural sequences and customized synthetic sequences. Previously, researchers have relied on specialized vendors for nucleic acid synthesis, but the introduction of desktop oligosynthesizers has enabled end users to generate synthetic nucleic acid products without reliance on a third party. However, while desktop oligosynthesizers can expedite research, they pose a risk of misuse if exploited to synthesize harmful pathogen or toxin sequences without the oversight of an external vendor (Carter, 2023). Traditional sequence screening methods are becoming inadequate as modified synthetic sequences can evade detection, thereby increasing biosecurity risk (Diggans, 2019; Langenkamp, 2024). These risks underscore the need for proactive governance surrounding the use of desktop oligosynthesizers.

Regulatory frameworks have evolved significantly since the 1975 Asilomar Conference, which established foundational principles for recombinant DNA research (Berg, 1975). Guidelines from the National Institutes of Health (NIH) and the Center for Disease Control and Prevention (CDC) in the US and World Health Organization (WHO) globally expanded biosafety and biosecurity standards but were mainly designed for known biological agents and traditional practices. While these guidelines provide a general roadmap for biosecurity in relation to synthetic biology research, they rely heavily on institutional and/or country-specific regulations for their implementation and lack any recommendations surrounding the use of desktop oligosynthesizers (NIH Guidelines, 2024; Biosafety in Microbiological and Biomedical Laboratories (BMBL) – 6th Edition, 2020; WHO Laboratory Biosafety Manual (LBM), 2020, WHO Laboratory Biosecurity Guidance, 2024). The Screening Framework Guidance for Providers and Users of Synthetic Nucleic Acids released by the Department of Human and Health Services (HHS) and Framework for Nucleic Acid Synthesis Screening released by the National Science and Technology Council (NSTC) and the Office of Science and Technology Policy (OSTP), hereby referred to as the OSTP Framework, provides the guidelines on the use of desktop oligosynthesizers within the United States (HHS Guidance, 2023 and OSTP Framework, 2024). The OSTP Framework outlines screening requirements for providers and mandates key requirements for benchtop equipment manufacturers, transitioning from voluntary to mandatory compliance.

As of 2025, the U.S. administration is proposing to expand the governmental oversight to manage risks associated with artificial intelligence (AI) and synthetic biology applications that may extend to desktop oligosynthesizers (Executive Order 14292; NIH Biosafety Modernization Initiative). While these efforts aim to meet future needs, the current OSTP Framework has several gaps for desktop oligosynthesizer users. These include fragmented guidelines, inconsistent

enforcement, voluntary self-reporting instead of mandatory compliance, data privacy concerns, limited legal frameworks, etc. Furthermore, the OSTP Framework only applies to federally funded institutions and lacks clarity on resale or secondary transfer of desktop oligosynthesizers. A 2025 article by Gillum and Moritz dives deep into the current state of guidelines, known and perceived gaps, and operational challenges related to synthetic nucleic acid oversight. If desktop oligosynthesizers become more prevalent across research institutes, addressing these gaps will be critical to prevent misuse of these tools. Our case study highlights some of these gaps and discusses the importance of collaboration with multiple stakeholders to implement biosafety and biosecurity measures surrounding the use of desktop oligosynthesizers within an institute.

Case Study – R&D and Bioinformatics Workflow:

In 2024, a client-funded biodefense research and development (R&D) program tasked our scientists with identifying, designing, and creating molecular guide RNAs (gRNAs) for a CRISPR-based pathogen detection and the corresponding positive control target material. The goal of this work was to create a capability for rapid assay reconfigurability for the detection of emerging and/or new pathogens while complying with client-specific requirements to maintain the security of any developed sequences. One of the key benefits of this capability is the ability to create and validate a newly developed assay without having to receive actual pathogen or target material for use as a positive control. Client requirements also included using original in-house software solutions for the project. This culminated in a unique set of considerations during the development of the work from both operational security and biosecurity perspectives.

The central challenge presented by this work was the on-site synthesis of the positive control target sequence material using a desktop oligosynthesizer. While gRNAs were below the OSTP Framework's 200 nucleotide screening size threshold, the pathogen target sequence material could range into the 200 – 300 nucleotide sequence lengths. This presented an important biosecurity concern as novel pathogens may be assumed to have at least a biological Risk Group 3 status with a high likelihood of containing sequence of concern (SOC) elements. As this positive control material was in the hundreds of bases, it was therefore beholden to the OSTP Framework.

Our multi-disciplinary team of scientists included R&D biologists, bioinformaticians, and credentialed biosafety experts charged with creating these gRNAs and positive control material in-house using a desktop oligosynthesizer to support the rapid development and validation of these assays. Traditionally, sequences chosen for pathogen detection typically target virulence factors or other uniquely identifiable regions of specificity that may have high overlap with the SOCs. SOCs alone are not a critical biosecurity risk but they provide a foundational

element for developing pathogenic organisms when combined with the necessary expertise, resources, and malicious intent. In the absence of specific OSTP Framework guidelines for this case study, well-intentioned printing of gRNA sequences and the corresponding positive control material for assay development presented a potential biosafety and biosecurity risk. With these risks in mind the work described in this case study was held to the following considerations: the OSTP Framework, best biosafety and biosecurity practices, and client-specific security requirements.

Within the OSTP Framework list of definitions, we identified ourselves as both a "non-traditional provider" and a "customer" of synthetic nucleic acid products. As our institute did not provide synthetic nucleic acid products as a client service, there were no established in-house synthetic nucleic acid screening procedures. While there are several open-source SOC screening tools, total transparency in the employed methodology was not a guarantee. The primary concern with the use of these open-source screening tools was the disclosure of the developed sequences – here, in a biodefense capacity, but could easily be extended to other propriety design concerns. Therefore, creating an in-house bioinformatics SOC screening workflow that was most compliant with the aforementioned considerations and limited to in-house analysis was critical.

The OSTP Framework requires the screening of products that are greater than 66 amino acids or 200 nucleotides across the sequence, which will lower to 50 nucleotides in October 2026. To follow best practices, our devised workflow screens across a 50-nucleotide sliding window against a Select Agent reference database curated in house (Figure 1). As no publicly available centralized SOC or Select Agent specific database exists, our database was purpose-built for this workflow. To guarantee coverage of all possible SOCs in the database, records were curated from NCBI at the taxonomic level. While not all sequences included in the database are relevant, this approach ensured that all SOCs defined by the OSTP Framework were included. Due to a short development timeline, the minimum screening requirements as outlined by the OSTP Framework were satisfied using the nucleotide BLAST database alone. In this framework, a "best match" as qualified by the OSTP Framework was chosen to be the BLASTn alignment with minimum number of base pair mismatches to database target sequence.

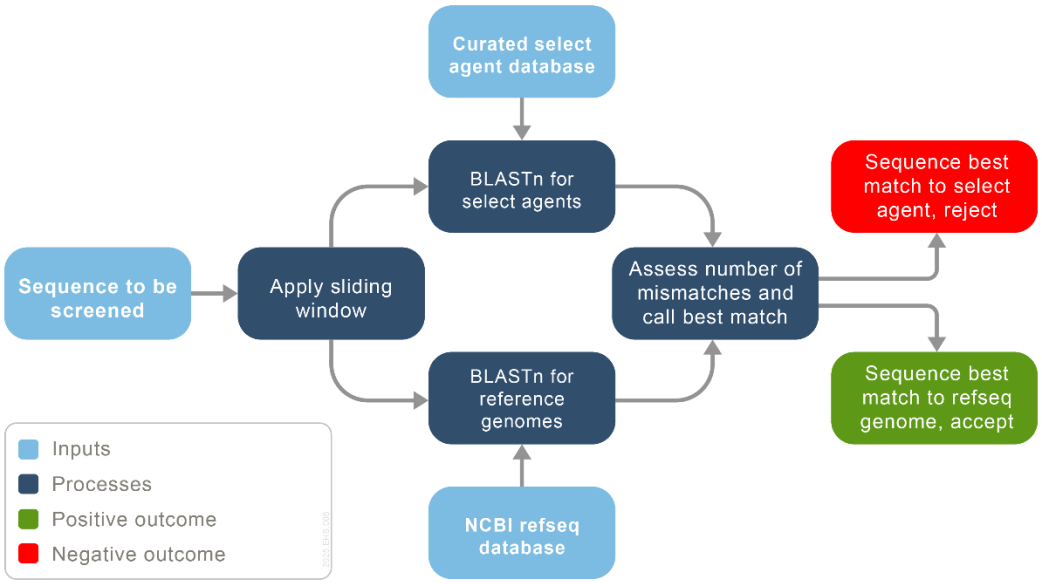

Figure 1: ***Sequence of Concern Screening Workflow.*** *Sequences to be screened are broken down into component 50 nucleotide sliding window segments. Each sub-sequence is then BLAST queried against two databases: NCBI's RefSeq database and an in-house curated sequences of concern database using available NCBI records. BLASTn outputs are then compared to evaluate mismatches to best alignments in both databases, before calling "best match" to the database with the minimum number of base pair mismatches.*

The next step was creating an in-house biosafety reporting workflow where our R&D scientists and biosafety SME ensured that scope of the project was evaluated across all applicable regulations affecting such work. A new biosafety reporting workflow was developed and implemented across our institute for work involving desktop oligosynthesizers and printing non-SOC or SOC sequences.

Case Study – The Biosafety Workflow:

The internal biosafety approval process for the proposed project considered risk assessment, threat parameters, and control measures based on the 2024 NIH Guidelines, the OSTP Framework, and Select Agent Regulations and required extensive in-depth discussions and collaboration between internal stakeholders. Biosafety risk assessment procedure followed WHO LBM's classic risk assessment matrix used by biosafety professionals and identified gaps in the following areas:

1. Use of a desktop oligosynthesizer in-house for a biodefense project and potential use of SOCs for assay development;
2. Institute fell under the definition of a "non-traditional provider" and a "customer" of synthetic nucleic acid products;
3. Lack of in-built synthetic nucleic acid screening tools within the commercially purchased desktop oligosynthesizer;

4. Biosecurity issues related to the use of open-source screening tools for a biodefense project; and
5. Fragmented guidelines and lack of appropriate oversight and enforcement for individual laboratories using an in-house desktop oligosynthesizer.

Figure 2 summarizes the risk assessment matrix and risk mitigation & control strategies considered and implemented at our institute. Figure 2A is the standard risk assessment matrix used by biosafety professionals to identify and categorize risk potential. Figure 2B shows how an initial risk assessment can classify the proposed work at a higher risk category. Applying appropriate mitigation strategies can reduce the same work to a lower risk category. In this case study, the initial risk assessment puts the overall scope of work into a "Medium High" category for biosafety and biosecurity risks in the absence of appropriate control measures. Therefore, several internal control measures were identified and implemented to bring the scope of work into a "Low-Medium" category:

1. Eliminate use of SOCs for assay development and select non-SOCs as assay targets for this research effort. Future work requiring SOCs will benefit from the other implemented control measures listed below, in Figure 2, and as discussed in lessons learned section;
2. Adhere to all available guidelines and regulations to meet requirements for non-traditional providers and customers of synthetic nucleic acid product;
3. Build an in-house synthetic nucleic acid SOC screening tool prior to printing the non-SOC synthetic nucleic acid sequences and share all screening data with biosafety SME. Established screening tool will be used for future projects that require in-house printing of any nucleic acid sequences including SOCs;
4. Create customized biosafety approval process prior to project initiation to ensure regulatory oversight; and
5. Develop multi-tier quality review process to ensure self-regulation.

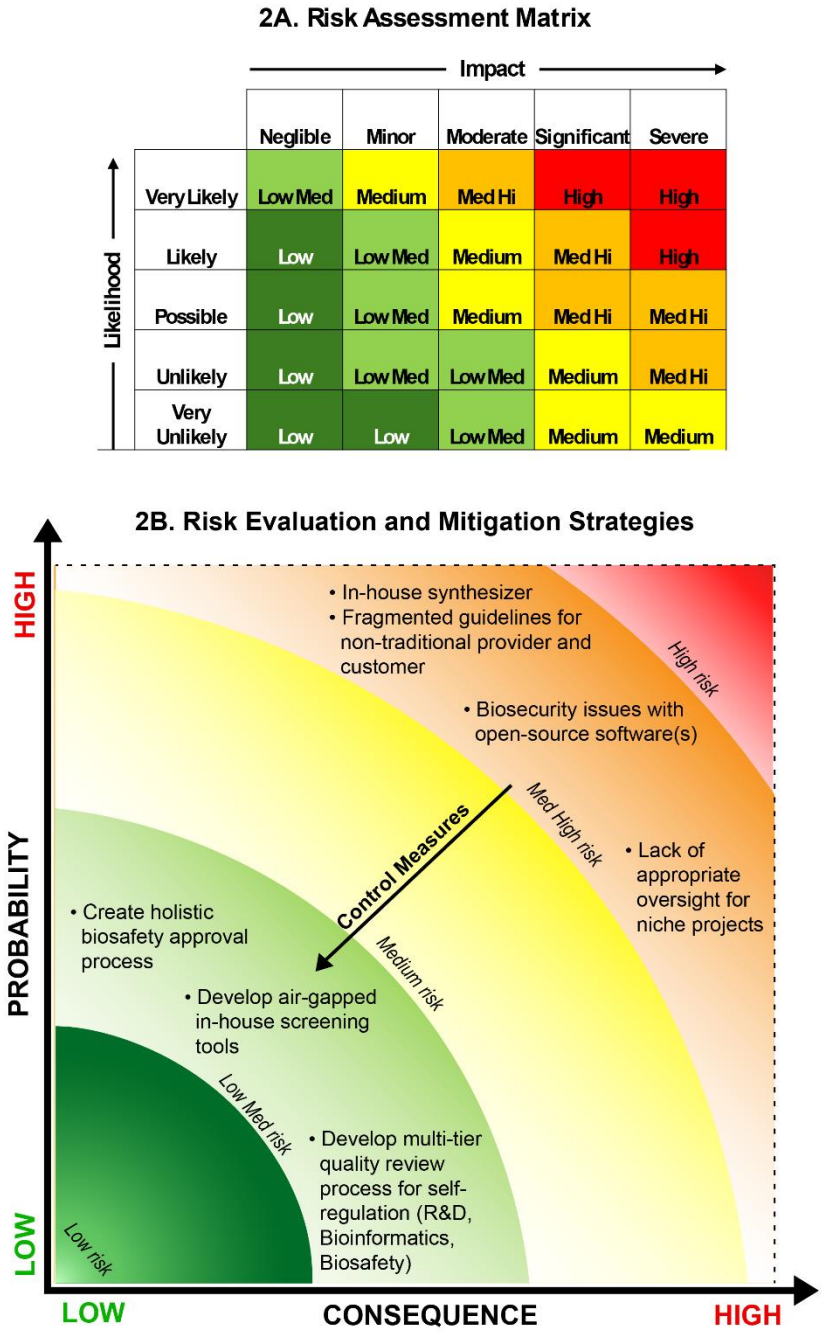

Figure 2: ***Biosafety and biosecurity risk evaluation and mitigation.*** *Figure 2A: the classic biosafety risk assessment matrix. Figure 2B: risk evaluation and risk mitigation strategies taken as control measures to reduce the overall risk to an acceptable level.*

Through this work, the internal biosafety approval process was revised to include questions and information specific to in-house printing of synthetic nucleic acid sequences, screening results, review and evaluation of the wet-laboratory work, review and evaluation of staff competency conducting the laboratory work, and multi-tier approval as a part of quality review process for self-regulation and oversight. A holistic biosafety approval process specifically for desktop oligosynthesizers and SOC screening is now a core capability that will support future research needs.

Case Study – Gaps and Lessons Learned:

Our case study, using a desktop oligosynthesizer, was performed between July – October, 2024. Operational challenges and short timelines required our team to quickly coordinate and collaborate with multiple stakeholders to create a customer, institutional biosafety, and guidelines compliant solution. Through this work, three central challenges were identified: (1) implementing current guidelines into an operational SOC screening tool with best practice standards, (2) the absence of centralized Select Agent reference database, and (3) establishing a reportable framework for institutional biosafety review.

(1) The OSTP Framework provide guidelines for manufacturers, providers, and customers of synthetic nucleic acid. However, we found two gaps in these guidelines: a. lack of specific guidelines for those that fall under the "non-traditional providers" and "customer" roles and b. lack of enforcement guidelines when the study was conducted in 2024. Our team interpreted the available HHS Guidance and the OSTP Framework guidelines as literal and comprehensive requirements to develop an in-house screening tool and implemented processes for self-regulation.

(2) There is a lack of a centralized Select Agent sequence reference database that researchers can screen against SOCs. The curation and synthesis of our Select Agent database was therefore critical for the development of this screening tool. While the SOCs are clearly outlined in the OSTP Framework, there were challenges with data availability and the specificity of the designations. A centralized list of references would ensure consistent standards for screening.

For example conotoxin is designated as a select agent withan IUPAC amino acid sequence. In the current guidelines, either a nucleotide screening approach or an amino acid screening approach is considered sufficient for evaluation. Conotoxins are made from complex clusters of genes known as gene superfamilies that are often challenging to find labeled within a publicly available database (Robinson 2014). An update to the select agent list to describe conotoxins as strings of nucleotides in addition to the current listing of amino acids would enhance the usability of the framework. Specific sequences for other SOCs would improve future development, time to answer, and answer confidence. Another challenge is

sequences that have not been characterized recently, such as a pathogen listed on the USDA Plant Protection And Quarantine (PPQ) Select Agents and Toxins and Commerce Control List (CCL) *Sclerophthora rayssiae* var. *zeae*, which has no specific Genbank sequences and has not had new sequencing studies in decades (Magill, 2017). Finally, guidelines in the OSTP Framework are not always unambiguous such as "Botulinum neurotoxin producing species of *Clostridium*". It is an open question to which Clostridium spp. has the capacity, which has been found in at least seven different Clostridium spp., to produce the Botulinum neurotoxin (Smith, 2018). When considering gene transfer potential, it is ambiguous which Clostridium spp. should be included under the guidelines, and it may be better to just consider all *Clostridium* a risk. These examples underscore how the absence of a central, authoritative Select Agent reference database can hinder consistent and precise SOC screening.

(3) HHS Guidance and the OSTP Framework lack adequate details on oversight and enforcement. When evaluating project with newer technologies, the biosafety personnel at U.S. research institutions will need to take a proactive approach. They will need to learn about current tools and emerging technologies, understand the risk and requirements for responsible usage, and be prepared to establish unique workflows for approval and oversight. At our institute, the R&D scientists, bioinformatics scientists, and biosafety officer worked closely together to create a final solution that met all needs, guidelines, and regulations. We formalized these considerations within our biosafety review framework. As a result, all work involving desktop oligosynthesizers and printing of synthetic nucleic acids is reviewed and approved by the Biosafety Officer and Biosafety Review Committee, when appropriate.

Conclusion:

This case study illustrates how an in-laboratory desktop oligosynthesizer can serve as an asset for biodefense research with specific security requirements, demonstrating how existing technology can be leveraged to address mission-critical challenges. We find it important to share how institutions can use existing guidelines in a real-world case scenario and evaluate available literature to develop workflows. Overall, our effort highlights the practical challenges faced by researchers and institutions when implementing fragmented guidelines. It also demonstrates an effective workflow to bridge those gaps while maintaining biosafety and biosecurity standards and best practices, and establishes a scalable capability for future multi-stakeholder projects in fast-paced research environments.

The gaps and lessons identified in this study reveal certain challenges faced by an institute to meet biosafety and biosecurity guidelines and regulations. These should be taken into consideration when looking ahead to a future in which AI-driven design tools will likely amplify both the capabilities and the risks associated

with the use of desktop oligosynthesizers and synthetic biology in general. AI can enable rapid and customizable biodesign of novel nucleic acids for printing as well as enable bad actors to create sequences which can bypass SOC screening tools and regulatory oversight, increasing biosecurity risk (Wittman, 2025; Groff-Vindman, 2025). This risk highlights the need for coordinated, forward-looking governance to ensure that the expanding availability of desktop oligosynthesizers strengthens, rather than compromises global biosecurity. The U.S. government initiatives and several published reports provide recommendations on prioritization of better detection algorithms, automated compliance reporting to and from centralized agencies, privacy-preserving infrastructure, and creation of new entity for oversight, among others (Mackelprang, 2025; Langenkamp, 2024; Gillum, 2025; NSCEB, 2025). We hope that the new guidelines and regulations will anticipate future emerging risks, address technology-specific vulnerabilities, and harmonize global standards to ensure that emerging technologies are used responsibly and safely.

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