

The Biosecurity Landscape of Genome Engineering: Inscripta Perspective

Executive Summary

Inscripta's Onyx™ platform enables the production of large cell libraries of thousands of variants using a process called digital genome engineering. Inscripta's digital genome engineering technology is a high-throughput editing approach that allows for genome engineering at an unprecedented scale. Such genome engineering technology has enormous potential to enable innovation that will improve medical care, food supply, agriculture, and sustainable materials and fuels. As such, it is gaining visibility and is a growing contributor to the bioeconomy.

As a company, we are committed to responsible, ethical use of the technology we are creating. We have developed and implemented a biosecurity system designed to identify biothreat scenarios and aim to be leaders in ensuring that biosecurity is a priority for the growing genome engineering community.

The genome engineering community, through due diligence and education, can ensure that society recognizes that benefits far outweigh potential risks posed by the editing of organisms, and that any risks are being actively mitigated. An industry-wide proactive stance will build societal trust that is required for the technology to thrive.

The ability to generate enormous phenotypic diversity reduces barriers to biological discoveries and production advances. However, it also presents biosecurity screening challenges since the potential functional impacts of edits on an organism are extensive and often difficult to predict. We believe the genome engineering community could solve problems and meet resource needs by working together in conjunction with industry, academic research partners, and government agencies. We emphasize the collective strength of an ongoing coalition in promoting biosecurity and public trust. To further this goal, Inscripta would like to engage the genome engineering community through an active convening of members of the community to collaboratively address challenges and set biosecurity standards while continuing open dialogue across the broader synthetic biology world.

This document describes Inscripta's mission, challenges, and recommendations pertaining primarily to genome engineering for microbes. A follow-on document will be dedicated to mammalian genome engineering and the associated biosecurity and bioethical challenges.

Inscripta's Approach to Responsible Microbial Genome Engineering Biosecurity

The Onyx platform allows customers to design edits and carry out editing reactions in their own laboratory on their microbial genome of choice, initially approved strains of *E. coli* and *S. cerevisiae*, with plans to include other microbes. This customer empowerment is a major advantage of our platform and facilitates high-impact discoveries and rapid product optimization. To prevent potential biorisk resulting from customer-directed organism edits, Inscripta proactively implemented upfront customer and sequence screening processes. As the use of our technology expands, we will continue to optimize our process, and we encourage the genome engineering community to join together to develop genome engineering industry-wide definitions of best practices.

Biorisk in Genome Engineering and Community-Based Strategy to Minimize

The categories of biorisk scenarios resulting from genome engineering have been enumerated previously.^(1,2) These include malicious intent, reckless use, and accidental biothreats. Malicious intent is the least likely, but also the highest risk. Reckless use of the technology is a scenario of concern as genome engineering diffuses into a broad range of end-users, some with a limited biology background. Lastly, a major concern in genome engineering space are accidental creation of novel biothreat agents. Mitigating the actual risks as well as managing public perception will be key to safeguarding the bioeconomy as genome engineering increases its contribution.^(3,4)

The potential benefits of collaborative biosecurity problem solving in synthetic biology have been previously described.⁽⁵⁾ As Inscripta addresses the unique biosecurity challenges invoked by genome engineering, we have encountered multiple gaps in resources. We believe it will be important for the International genome engineering community to define a set of standards and share resources to address this issue. We encourage and welcome structured communication across the genome engineering community to solve technology-specific problems, and believe that open dialogue across the entire synthetic biology world is critical to share information and stay ahead of new threats.

In addition, Inscripta realizes that avoidance of biorisk and building public trust are essential to the broad adoption of genome engineering. Inscripta would like to engage the greater genome engineering community to help educate society in the virtues of genome engineering, and help reassure everyone that our industry is working together to minimize biorisk from edited organisms.

Genome Engineering Biosecurity Considerations Overlap with Gene Synthesis, yet also Present Unique Challenges.

Inscripta values open dialogue across the realm of synthetic biology, while also seeing a need for formation of smaller “break-out” groups to address novel challenges posed by specific technologies. Biosecurity considerations for gene synthesis and genome engineering have both shared and unique features requiring biosecurity processes and standards that are tailored to the specific needs of these groups. As a baseline, to avoid biothreat scenarios, all synthetic biology companies should screen customers and reagents, identify potential biothreats, and include proper controls to avoid unauthorized use of enzymes or instruments. However, divergences between gene synthesis and genome engineering impact biosecurity requirements such as the sequence entities that must be screened as well as organismal context. See Table 1 for a layout of intersections and divergences in biosecurity considerations between gene synthesis and genome engineering, followed by an elaboration on some of the challenges primarily unique to genome engineering.

Biosecurity processes have been established for the gene DNA synthesis community, and there are ongoing efforts to improve and expand these practices.^(6,7,8) An International Gene Synthesis Consortium, IGSC, was established in 2009 to discuss ongoing biosecurity challenges and standardized processes to ensure safe use of the products of gene synthesis. This industry-led, biosecurity-focused consortium model could be valuable as an initial framework to the genome engineering community as well, although we believe additional biosecurity measures will be necessary to ensure the safe and ethical use of genome engineering. For example, while many biosecurity needs are shared across synthetic biology, the technical challenges of genome engineering differ to the extent that a focus on the distinct requirements of genome engineering will be needed to set appropriate standards, create relevant test sets, and share resources to ensure proper implementation.

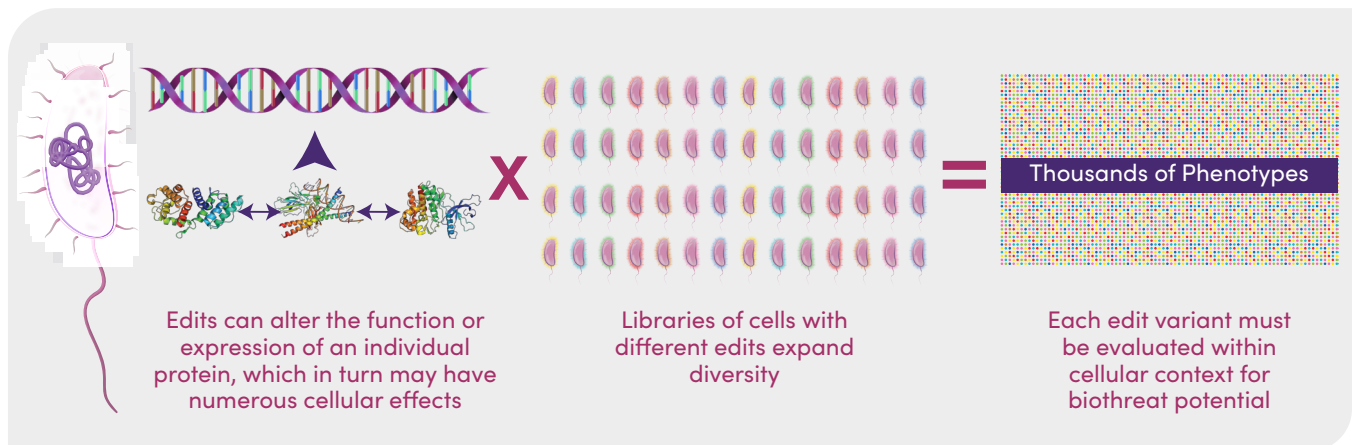
Table 1: Lists overlaps and distinctive requirements for biosecurity for Gene Synthesis vs Genome Engineering. This list is not comprehensive and not all unique features are entirely exclusive.

Features Shared Across Synthetic Biology	Features Primarily Unique to Gene Synthesis	Features Primarily Unique to Genome Engineering
<ul style="list-style-type: none"> • Customer Screening completed upfront of order delivery • Broad biothreat space includes sequence predicted to produce products that may cause harm to humans, animals, agriculture, ecology, economy, or infrastructure • Need process for reporting of predicted nefarious intent 	<ul style="list-style-type: none"> • Small oligos can be ordered separately, assembled • Potential to reconstruct pathogenic virus • Genetic recoding complicates computational screening • Benchtop DNA synthesizers transition actual synthesis into customers labs 	<ul style="list-style-type: none"> • Edits must be considered in genomic and cellular context <ul style="list-style-type: none"> - Regulatory sequences - Protein interactions • Accidental gain of biorisk is challenging to predict • Curated, ground truth test sets will be complex to create • Scale issues are a major challenge for massively parallel genome editing • Multiple edits per cell (combination editing) will add additional complexity to predicting potential threats and increase the computational burden

Unique Genome Engineering Biosecurity Considerations and Challenges:

The key strength of genome engineering, the enormous scale of phenotypic diversity that may be probed, also underlies its major biosecurity challenges, conceptualized in Figure 1.

Figure 1: The functional effect of a given edit may alter multiple cell processes, and the phenotypic diversity resulting from a typical experiment may be enormous.



Edits need to be considered in the context of the genome including protein interactions and potential impacts of genetic regulatory elements. Mutations, insertions, and deletions need to be considered in context of surrounding sequences as well as the context within the entire genome. Edits that may cause resistance or virulence-supporting phenotypes (e.g. secretion) in a benign organism may not have the same impact as in a pathogenic organism. Edits to proteins in pathways can affect the function of other proteins in the pathway that have genes far removed from the edits, requiring a systems biology approach to evaluate biothreat potential of such functional changes. The impact of edits in regulatory regions will be especially challenging, as the effects on multiple loci may not be predictable. It will be critical for biosecurity screening to evaluate edited regions in context to determine intrinsic and heterologous genes that may be affected by promoter and/or enhancer modifications.

Scale issues are a computational challenge for massively parallel genome editing. The range of potential functional impacts of edits on an organism are extensive and can have complex effects on phenotype. Currently, Inscripta's Onyx platform creates libraries of up to 10,000 designs with one edit per cell, but planned developments to the technology will enable "combinatorial editing", i.e., combinations of edit types and locations in a single cell. Such combinatorial editing will cause an exponential increase in predicted products or interactions to screen, and require new research into how to practically evaluate edits in the context of other edits and the organism as a whole.

Additional Resources Needed to Develop Requirement - Meeting Genome Engineering Biosecurity Systems

As we actively develop our biosecurity system based on the requirements we initially identified, we also recognize areas exist where additional resources are needed to better meet future applications. These gaps are not straightforward to fill, and we encourage the genome engineering community to work together, bringing in academic, industry and government partners and support from each to confer and discuss next steps.

As a start, we have identified three general categories of resource gaps:

- 1. Threat definition and curation of databases:** For threat characterization in genome engineering space, we need data sources that include information on edits that convert sequences to a biothreat status
- 2. Sequence test sets:** High quality sequence test sets are needed to test screening algorithms against biosecurity editing requirements, and these require sets of ground truth test data.
- 3. Algorithmic support:** As genome engineering technology expands to allow multiple edit types and genomic locations in individual cells, the logarithmic nature of the detection requirements will be computationally burdensome. A systems biology approach using appropriate algorithmic predictions will be needed to evaluate the enormity of edit variant combinations within genomic context.

See **Appendix A** for example strategies to fill gaps.

Summary of Recommendations for the Genome Engineering Community

Inscripta aims to encourage collaborative efforts amongst members of the genome engineering community to form an entity that interacts regularly. The goals of this entity will be to communicate the truth about the benefits and risks of genome engineering, to collectively identify and address challenges, define standards, acquire and share resources, and propose government support. See **Appendix B** for detailed suggestions.

Conclusions

Inscripta acknowledges the need for forward-thinking approaches to biosecurity for genome engineering and is developing process to ensure biosecurity for genome engineering. We are committed to responsible, ethical use of this powerful technology. We also aim to engage the community to help ensure this responsibility and prioritization of biosecurity is upheld across the genome engineering industry as it continues to expand. Lastly, we strongly believe that collaborative biosecurity dialogue must be actively ongoing within the genome engineering community as well as be engaged with the broader synthetic biology community.

Appendix A: Recommended Strategies to help fill Resource Gaps for Genome Engineering Biosecurity Maturation

The general categories of genome engineering-specific resource gaps are threat definition test sets, and algorithmic support. This is not exclusive.

- 1. Defining genome engineering biothreat categories and assembling datasources:** Databases containing ‘sequences of concern’ exist, and are being leveraged in Inscripta’s biosecurity screening. We lack data sources that include information on edits that convert sequences to a biothreat status. A microbial precedent does exist in that CARD, a Comprehensive Antimicrobial Database, includes single-nucleotide variants leading to resistance. Edits that change function of other categories of genes are not readily compiled, and furthermore, the genomic context needs to be considered. A strategy to assemble a ‘threat’ set against which to screen may be a challenge for government service labs, academia or industry. A tangible result would be curated databases that include a range of microbial edit impacts upon gene functions.
- 2. Test data to train threat models:** Testing screening algorithms against biosecurity editing requirements need a set of ground truth test data. This does not exist, to our knowledge, and will be challenging to create. We promote a government involvement to assist by funding service labs to create complex, curated test sets including contrived edits and chimeric genomes. Test sequences will ideally include curated sets of ‘ground truth’ edited versions of genes in genomic context and threat prediction guidelines for genome engineering purposes.
- 3. Algorithm assistance to address Combinatorial Edits:** Currently Inscripta technology aims to make a single edit per cell, yet as technology advances to multiple edit types and genomic locations in individual cells, the logarithmic nature of the detection requirements will be challenging. Algorithms that can cost and time efficiently evaluate the biothreat potential of all combinations are not yet available and will require advanced computational skills. This need may be satisfied best through competitive academic research funding.

Appendix B: Recommendations for a Genome Engineering Consortium to collectively address Biosecurity challenges:

We recommend a consortium that holds regular meetings with strategies and accountability and provides community biosecurity guidelines. A proposed name for such a consortium is International Genome Engineering Consortium, as we envision it to be analogous to the International Genome Synthesis Consortium. Members will include any entity with interest in the use or outcome of genome engineering.

A primary goal of the consortium ought to be defining Biosecurity requirements that encompass the applications of this industry, and establish standard practices that will effectively meet the requirements. Requirements would include scope of biothreat space against which to screen, entities that need to be screened, etc. Early on, the consortium ought to identify a process of certification and work within these guidelines.

As the consortium defines standards, it will undoubtedly encounter resource gaps, such as described above. As a group, the consortium would be better positioned to inform policy makers on issues related to genome engineering and to seek funding to address biosecurity needs. An initial step may be for participating members to hold a workshop, inviting a broad range of genome engineering stakeholders and government representatives. The asks of the participants could be to clearly define resource needs, and strategize projects that would effectively solve practical problems related to increasing biosecurity.

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