NATIONAL ACADEMIES Sciences Engineering Medicine

Genetic Biocontainment of Engineered Microbes for Environmental Release

Ashley Kim & Cole Mueth

Biotechnology Regulatory Fellowship Program

Committee on Science, Technology, and Law
National Academies of Sciences, Engineering, and Medicine

July 21st, 2026

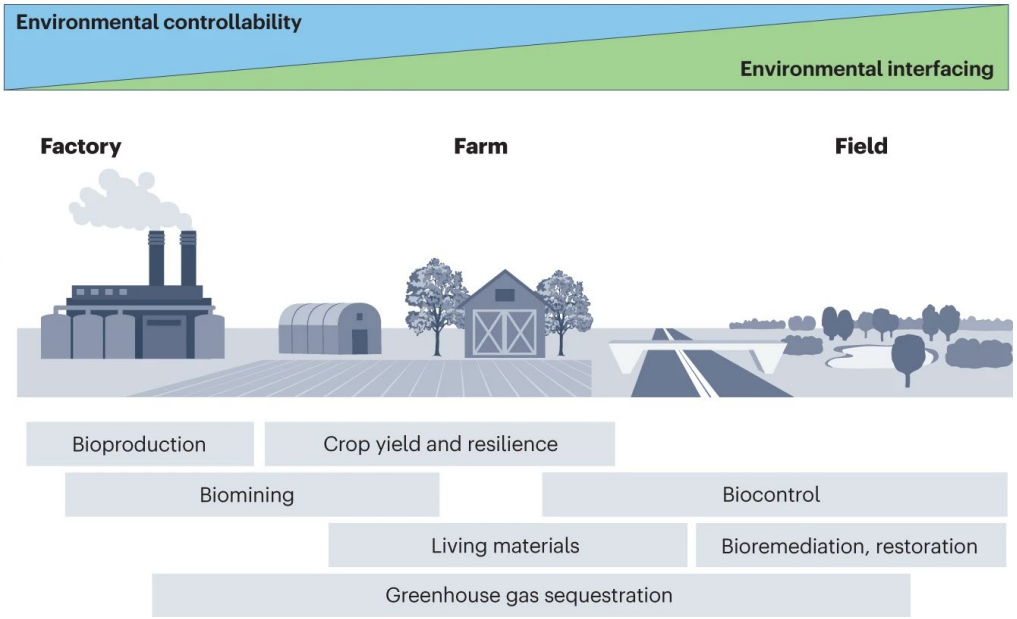
The containment continuum

Physical confinement:
infrastructure used to prevent organisms from escaping an enclosure

Biological containment:
genetic engineering used to limit the spread and persistence of organisms and their DNA

Examples: auxotrophy, kill switches, gene flow barriers

Most genetic approaches are designed for confined settings



Jones et al. 2024. *Nature Reviews Microbiology*

Asilomar, 1975

- Proposed risk-proportionate rDNA research enabled by physical/biological containment
- Influenced 1976 NIH Guidelines and (indirectly) 1986 Coordinated Framework
- Environmental release was a risk, not an application category
- Provisional, intended to be updated with new evidence

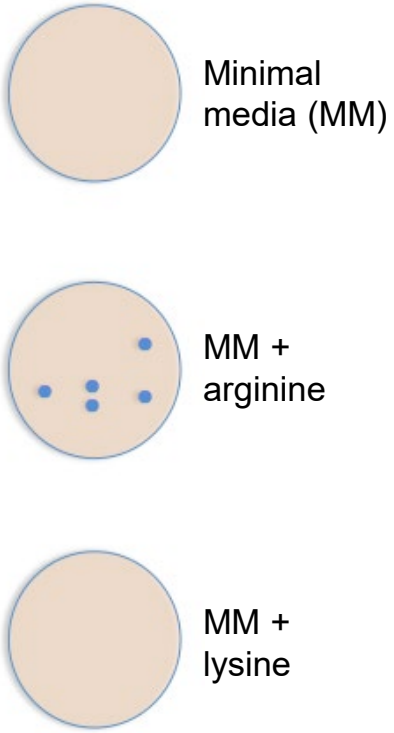
"The most significant contribution to limiting the spread of the recombinant DNAs is the use of biological barriers. These barriers are of two types:
(i) fastidious bacterial hosts unable to survive in natural environments, and
(ii) nontransmissible and equally fastidious vectors (plasmids, bacteriophages, or other viruses) able to grow only in specified hosts.
Physical containment…provides an additional factor of safety."

Berg et al. 1975 *PNAS*

Auxotrophy

- Strains are mutated/engineered to depend on an exogenous input
- Targets natural compounds (sugars, amino acids), or synthetic compounds (noncanonical amino acids, xenonucleic acids)
- Applies almost exclusively to confined settings
- Infeasible for EMERs because:
  - Difficulty of applying chemicals to landscapes/waterways
  - Uneven (bio)availability on soil/water/rock
  - ESH and regulatory issues with chemical application
  - Circumvented via horizontal gene transfer / cross-feeding

Arginine auxotroph
(requires arginine to grow)



Genetic biocontainment is seldom used in EMER R&D

Academia

- Few lab tests in soil or water†
- Few lab tests with environmentally relevant taxa††
- Only one experiment conducted outside†††

Developers

- No known EMERs include genetic biocontainment
- The only fully open release may have succeeded *because* there was no genetic biocontainment††††

Regulators

- Regulatory language focuses on physical, not genetic barriers
- Genetic biocontainment is not required for any known permitting process
- No quantitative standards for field performance

†Hartig et al. 2024, 2025; ††Halvorsen et al. 2022; †††Molina et al. 1998; ††††Peters et al. 1997, Chemla et al. 2025

Contributing reasons why genetic biocontainment is rarely used in EMERs

Lack of evidence of efficacy in environmental conditions

Limited relevance given widespread horizontal gene transfer

Undervalues trait x community ecology

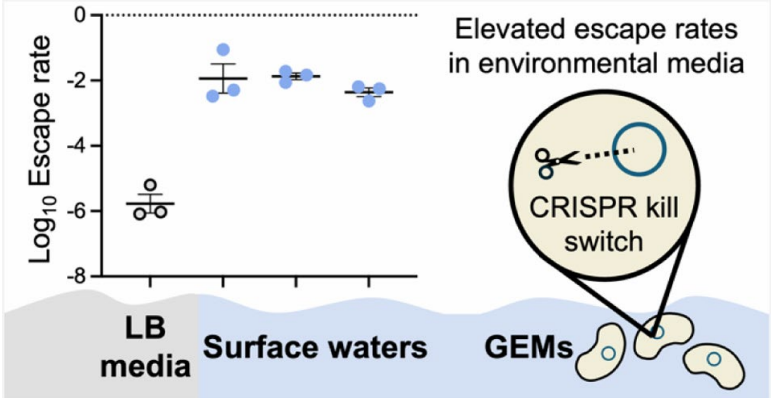
Triggers additional liabilities (environmental, regulatory, social)

Real-world efficacy of growth restriction techniques

- Performance falls by orders of magnitude in environmental substrates†
- Measured performance differs by orders of magnitude based on:

Containment target	Detection method
Organism	Survival ratio
DNA sequence	qPCR
Engineered trait	Reporter gene

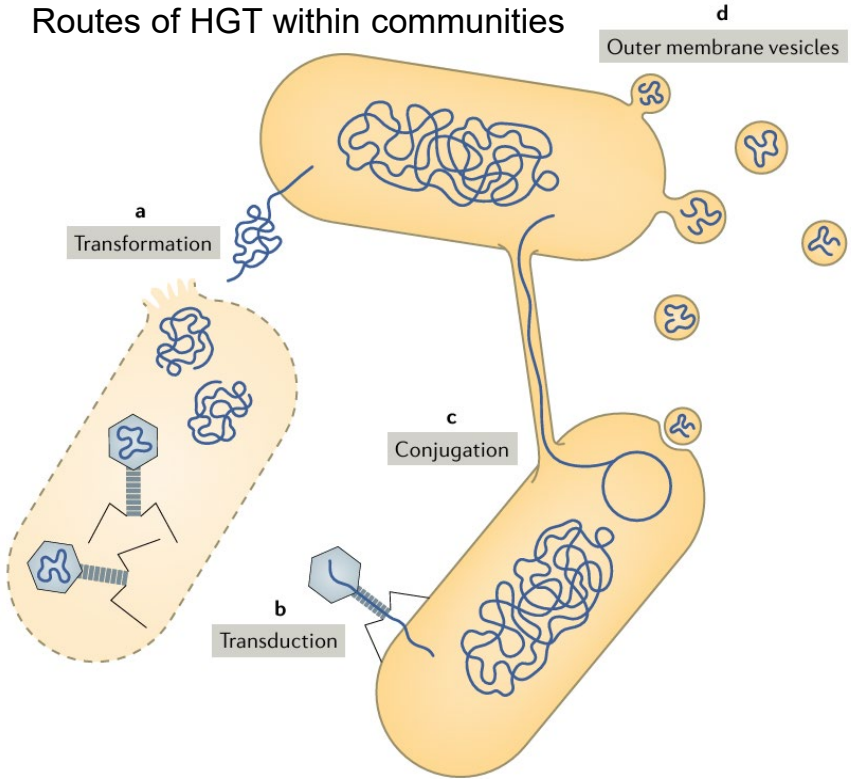
- Killing ≠ containment: dead cells leave environmental DNA for detection and potential HGT††



†Hartig et al. 2024; Rottinghaus et al. 2022
††Hartig et al. 2025

No gene flow barrier reliably prevents horizontal gene transfer

- Genetic approaches:
  - Chromosomal integration
  - Competence gene deletion
  - Genomic recoding
  - Toxin-antitoxin systems
- Some block one HGT route, but multiple routes operate in parallel, bidirectionally
- Containment module can dissociate from cargo genes, rendering it ineffective
- Most research measures HGT of the *containment module itself*, not the *primary engineered trait*



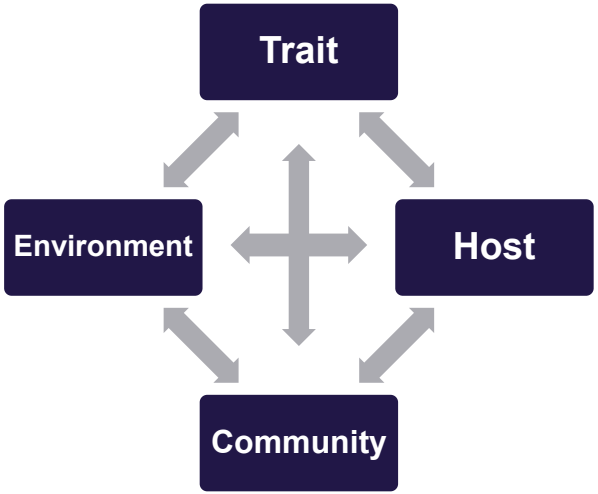
Brito 2021. *Nature Reviews Microbiology*

An ecological containment hypothesis

- Persistence *in situ* is related to *Trait x Host x Community x Environment*

- **For adaptive traits**: mutation, selection, and HGT eventually render genetic biocontainment *ineffective*
- **For non-adaptive traits**: competition, fitness disadvantage, and metabolic drag eventually render genetic biocontainment *irrelevant*

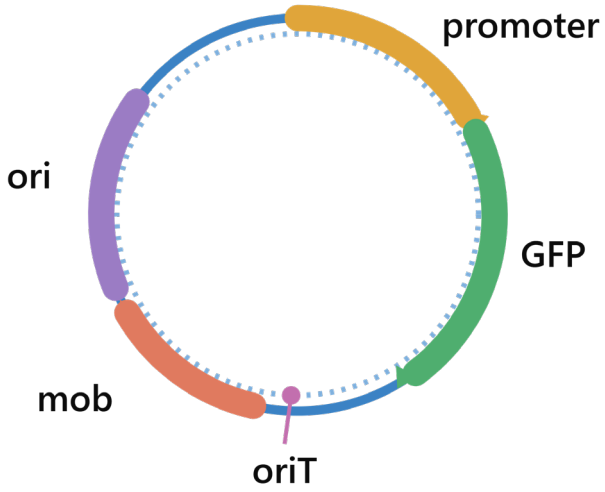
- Potentially stable traits are ecologically self-contained in a niche compatible with the developer's objective†
- Research is needed on trait persistence in:
  - Sterile and nonsterile environmental substrates
  - Small partly-enclosed field trials



†Vidiella and Solé. 2022. Ecological firewalls for synthetic biology. *iScience*

Horizon scan: Vector-based products

- Chassis-agnostic microbial biotechnology
- Mobile genetic elements could deliver traits to whole microbial communities†
- Genome editing can be performed on microbial consortia††
- Preliminary data indicate huge differences in transformability among genotypes
- How should these traits be monitored and evaluated?



†Marken et al. 2024. *LCSSP Caltech*, Box 4.2 by Bruce Hay

††Rubin et al. 2021. Species-and site-specific genome editing in complex bacterial communities. *Nature Microbiology*

Additional liabilities of genetic biocontainment

Environmental

- Biocontainment systems may contain genes for toxins, antibiotic resistance, nucleases, etc.
- Containment genes may dissociate and spread via HGT

Regulatory

- Each additional transgene is evaluated for safety, plant pest potential, etc.
- Biocontainment genes trigger the intergeneric rule, even if the primary trait would not

Social

- Genetic biocontainment is historically uncorrelated with commercial success or public acceptance†

Genetic biocontainment features may incur additional risks and complicate the path to regulatory approval

†George et al. 2025. *Arizona State University*

Regulatory Considerations

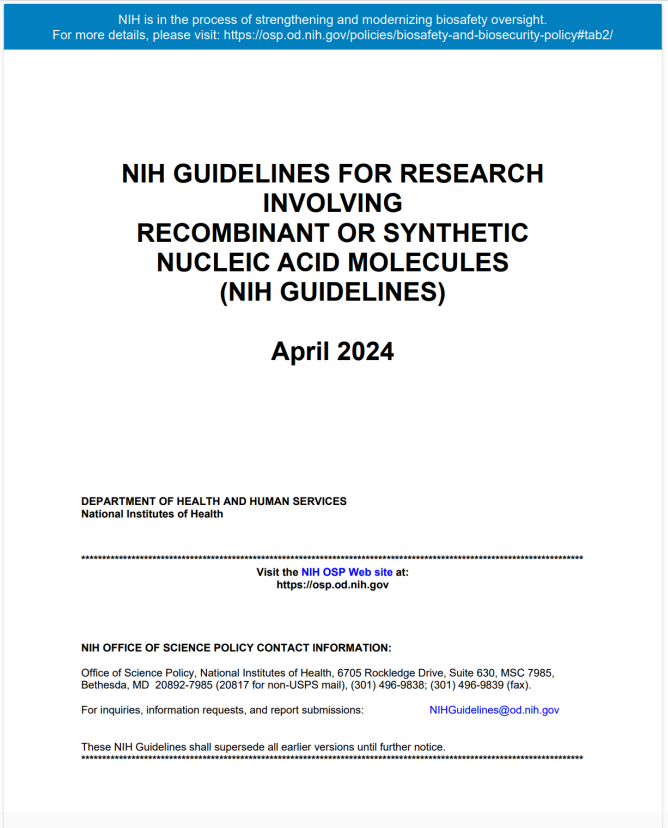
- Multiple pathways govern GEM environmental release: EPA (FIFRA, TSCA), USDA APHIS
- Existing containment definitions were developed largely for contained facilities with treatable waste streams
- No public dataset tracks post-release monitoring or containment failure, only the permit authorization decision itself

USDA eFile Interstate Movement and Release (2021-2026)

Total	Issued	Withdrawn	Other	CBI Masked
88	23	60	5	81

Quantitative Standards

The NIH 10^{-8} Benchmark



NIH is in the process of strengthening and modernizing biosafety oversight.
For more details, please visit: https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab2/

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (NIH GUIDELINES)

April 2024

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For inquiries, information requests, and report submissions: NIHGuidelines@od.nih.gov

These NIH Guidelines shall supersede all earlier versions until further notice.

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- NIH Guidelines set a quantitative containment target where escape rate below 1 in 10^8 under laboratory conditions
- No environmental release is allowed under NIH guidelines unless a relevant government agency is involved
- TSCA 6-log reduction benchmark: Requires a 99.9999% reduction in viable microorganisms for contained waste streams

Result: No written quantitative criteria exist to guide researchers testing biocontainment designs in the field

Conclusions

1. Genetic biocontainment technology has been under development since the advent of microbial engineering, but most techniques were designed as safeguards for physically confined settings, not environmental release.

2. Biocontainment of EMERs is not binary; it is probabilistic, time-dependent, and substrate-contingent. Escape frequency for a fixed genotype can shift by orders of magnitude between rich media and environmental substrates.

3. Multiple independent HGT modes operate bidirectionally at low but nonzero rates, and no biocontainment technique has demonstrated complete gene flow blockade.

Photo: BioConsortia

Conclusions

4. Growth-limiting biocontainment systems leave intact DNA that is PCR-detectable and available for HGT, creating discrepancies between viability-based and molecular abundance estimates that complicate measurement and compliance.

5. No containment technology has demonstrated durable performance under selection in field conditions. Ecological self-limitation of the engineered trait may be more robust than any genetic module, but it is application-specific and unavailable where the trait needs to persist without local selection pressure.

6. Jurisdiction for EMERs is spread across multiple agencies with overlapping authority but no unified mechanism for evaluating biocontainment in field trials.

Recommendations

Government

1. No mandate for genetic biocontainment technology

2. EMER field trials: agencies could increase coordination on shared risk assessment by harmonizing evaluation of persistence, dispersal, HGT, and off-site movement

3. Grant federal test-site access, such as DOE or defense land, to academic researchers and commercial developers to generate field-based evidence

4. Expand USDA's Biotechnology Risk Assessment Grants (BRAG) program funding for microbial applications

Recommendations

Industry

1. Treat genetic biocontainment as a design option rather than a default for market or regulatory acceptance

2. Prioritize generating field-specific evidence. Assess whether engineered traits confer a selective advantage in the target environment. Test HGT and persistence of the functional cargo.

3. Invest in monitoring and traceability for post-release accountability

4. Assess regulatory and market-access tradeoffs early

Recommendations

Academia

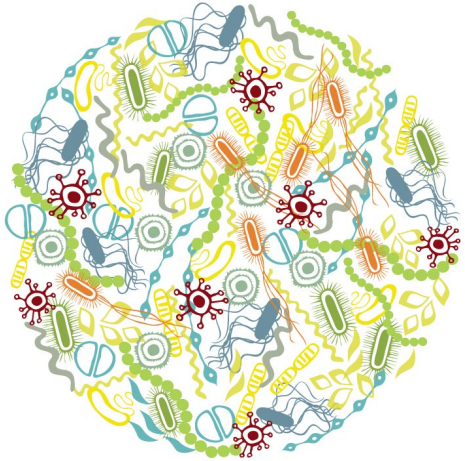
1. Test biological containment under environmental conditions using environmentally relevant taxa
2. Study trait ecology: selection and HGT of the cargo itself, not just containment genes in isolation
3. Bioengineering should incorporate quantitative microbial ecology as part of the design-build-test cycle
4. Develop a set of quantitative standards for measuring microbial persistence in field settings (survival, DNA, GE traits, etc.)
5. Engage regulators with evidence needs and help develop standards

Acknowledgements

Committee on Science, Technology, and Law
Steven Kendall
Stephanie Batchelor
Cristyl Watson
Karen Autrey
Office of Fellowships
Gordon and Betty Moore Foundation
Eric and Wendy Schmidt

Stakeholders
USDA
EPA
FDA
Academic Researchers
Industry Professionals

Thank You!



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