Review

A Framework for the Systematic Selection of Biosensor Chassis for Environmental Synthetic Biology

Swetha Sridhar,* Caroline M. Ajo-Franklin, and Caroline A. Masiello

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ABSTRACT: Microbial biosensors sense and report exposures to stimuli, thereby facilitating our understanding of environmental processes. Successful design and deployment of biosensors hinge on the persistence of the microbial host of the genetic circuit, termed the chassis. However, model chassis organisms may persist poorly in environmental conditions. In contrast, non-model organisms persist better in environmental conditions but are limited by other challenges, such as genetic intractability and part unavailability. Here we identify ecological, metabolic, and genetic constraints for chassis development and propose a conceptual framework for the systematic selection of environmental biosensor chassis. We identify key challenges with using current model chassis and delineate major points of conflict in choosing the most suitable organisms as chassis for environmental biosensing. This framework provides a way forward in the selection of biosensor chassis for environmental synthetic biology.

KEYWORDS: *chassis, biosensor, environmental, synthetic biology, marine, soil*
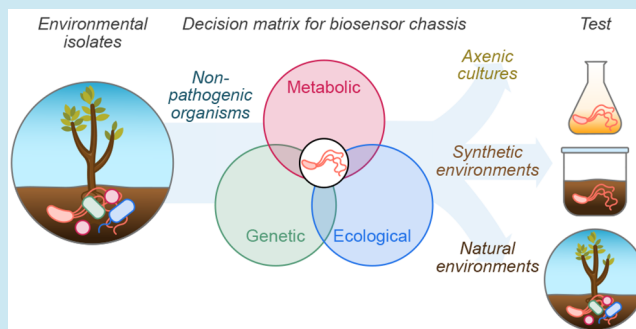
■ INTRODUCTION

The development of modular, controllable, and highly tunable genetic circuits in synthetic biology has opened new avenues of research in the environmental sciences.[1] The ability to sense and report microbial experiences allows for the design of biosensors—genetic circuits responsive to small molecule signals housed in a microbial host, or chassis. While the bulk of our efforts as a community have been dedicated to the design and optimization of biosensing circuits, far less attention has been devoted to optimizing the choice of organisms to host these circuits.[2]

Environmental biosensing poses unique restrictions on the choice of a chassis organism. Natural environments are spatially heterogeneous and temporally variable, and these variations severely impact the survival of non-native species.[3] Unlike bioproduction facilities or clinical laboratories, natural environments host a robust pre-existing microbiome. Furthermore, most terrestrial and marine environments have low nutrient concentrations with occasional bursts of organic matter that stimulate metabolic activity.[4] Deploying biosensors into the environment poses a significant risk due to the potential for uncontrolled proliferation and gene transfer into native species. These additional constraints make the choice of a chassis for environmental biosensing more challenging than for biosensing within bioproduction or clinical settings.

Much of the collective efforts of the synthetic biology community have been invested in optimizing genetic circuits in laboratory-domesticated *Escherichia coli*, with some tools being expanded into *Pseudomonas putida*,[5,6] *Bacillus subtilis*,[7] and *Saccharomyces cerevisiae*.[2,8] In 2016, Adams[2] suggested developing universal toolboxes for a select set of chassis— *P. putida*, *B. subtilis*, *Geobacillus*, cyanobacteria, and *Dein-ococcus*—to widely deploy for diverse applications in synthetic biology. We believe that advances over the past five years in rapid identification of genetically tractable members of a community,[9−11] broad-host range plasmids for non-model organisms,[5,12,13] and high-throughput chassis engineering techniques[14,15] position the field to take a more ecologically relevant, environment-centric approach for chassis selection.

Here, we advocate for selecting chassis that persist in the environment of interest by considering physical, chemical, and ecological factors that govern chassis persistence. We provide a conceptual framework for the systematic selection of biosensor chassis to host genetic circuits designed for environmental biosensing. We describe factors—genetic, metabolic, and ecological—that must be considered for a chassis organism to be a biosensor in the environment. We discuss how specific

chassis organisms meet these factors and outline constraints of current model chassis organisms. Lastly, we describe our vision for developing a range of chassis for synthetic biology.

ENVIRONMENTAL BIOSENSING POSES UNIQUE DESIGN CONSTRAINTS ON MICROBIAL CHASSIS

Biosensing in the environment poses unique constraints on the choice of a chassis organism. Biosensor persistence in the environment depends on the evolutionary stability of the sensing genetic circuit and the ability of the chassis organism to survive. Additionally, these requirements must be met in a specific ecological and metabolic context. Ecological factors in many environments are still unknown and challenging to characterize. Metabolic factors can also be unknown in most cases and often require rigorous characterization *ex situ*. Genetic tractability and access to curated genomic databases are not assured for most species from the environment, thereby narrowing down options for chassis organisms. These challenges add significant complexity to choosing a chassis. We posit here that, in environmental biosensing, choosing the right chassis organism is just as important as choosing ideal genetic elements, and a combination of safety, ecological, metabolic, and genetic factors must guide this choice.

Constraint 1: Do No Harm. Biosensing in the environment demands stringent requirements for safety. At minimum, this requirement eliminates the use of known pathogens as chassis. As a first step, we recommend following USDA restrictions on microorganisms that have been identified as plant pathogens and can adversely impact agriculture.[16] With the advancement of synthetic biology for non-model organisms, it will be essential to periodically revisit the constraints and impacts of engineered organisms in the environment.

The constraint of safety does not stop at the first step in chassis selection—after determining the ideal chassis for the environment of interest, safety must be revisited.[1] In particular, evolutionarily stable strategies for biocontainment must be adopted to ensure the safe deployment of any biosensor chassis in the environment. The NIH provides an escape frequency of 1 in 10^8 cells as a guideline to determine the success of biocontainment strategies.[17] Some successful biocontainment strategies developed include toxin−antitoxin systems,[18] auxotrophy,[19] inducible killswitches,[20−22] phage lytic systems,[23] sequence entanglement,[24] and xenobiology.[25−31] For environmental biosensor chassis, a multipronged approach combining different biocontainment strategies is essential to ensure stringent control on engineered organisms in the environment.[32,33]

Constraint 2: The Chassis Must Be Ecologically Persistent. To sense in the environment, the chassis organism must persist when exposed to biotic and abiotic stresses in the niche of interest without altering its ecological niche in deleterious ways. Organisms live in complex, multidimensional social hierarchies that regulate their survival and function.[34,35] As a consequence of this complexity, robust characterization of the organism's ecological niche is essential for its adoption as a chassis. For example, validating existing syntrophies of a potential chassis organism in complex communities is essential to establish culture protocols and ensure stable strain maintenance.[36−38] We summarize some methods of characterizing the ecological context of an organism below.

Interspecies microbial interactions are highly diverse and can be stimulated by the organisms themselves, other organisms in

their surroundings, or their environment.[39,40] These interactions are complex and hierarchical, making them challenging to emulate experimentally. However, the development of simulation toolboxes to represent the microbial interactome *in silico* has supported the prediction of interspecies interactions.[40−43] Advances in next-generation sequencing coupled with genome-scale metabolic models and constraint-based reconstruction and analysis present platforms for mining novel microbe−microbe interactions and predicting emergent interspecies interactions.[44−46] Insights from these models, while still predictive, can inform the ecological context of a potential chassis organism.

Ultimately, characterizing potential chassis organisms in their native ecological context will require experimental emulation of complex microbiomes and their environmental controls, which is a considerable challenge. Recreating the physical matrix of the organism is challenging due to the spatiotemporal heterogeneity and chemical complexity of natural environments.[1,47] Furthermore, recreating the biotic matrix is a considerable challenge as the biotic diversity of the environment of interest is unrepresentable *ex situ* due to limitations in sampling and culturing.[48,49] However, characterizing the ecological persistence of an organism may not be as insurmountable a challenge as replicating its native context. Benchtop incubation studies with a sample of the environment of interest offer a solution to characterizing the ecological persistence of a potential chassis *in situ*. Such incubation studies could include regular sampling and amplicon sequencing, or nondestructive reporters such as inducible volatiles and gas vesicles that act as indicators for chassis survival.[1,50] Such incubation experiments offer the closest analog to the environment of interest without reducing environmental complexity.

Constraint 3: The Chassis Must Be Metabolically Persistent. When choosing a chassis to answer an environmental question, we need to determine if the primary metabolism of the microbe is favorable in the environment of interest. For example, obligate aerobes may not persist in soils or sediments with oxygen gradients. Investigations into the primary metabolism of an organism can be performed using genome-scale metabolic modeling (GEMs). GEMs offer a method to interrogate an organism's metabolic potential and predict cellular growth on diverse substrates.[45,51,52] Additionally, if the microbe is predicted to host multiple functionally similar metabolic pathways, it is imperative to determine the physical conditions under which the microbe chooses one pathway over another. For example, purple nonsulfur bacteria from the marine column can be both autotrophs and heterotrophs and can switch between their metabolisms on the basis of their culture conditions.[53,54] Understanding this metabolic compartmentalization is imperative to formulating culture media under different conditions.

Along with a potential chassis' primary metabolic pathways, we need to know if there are any secondary metabolites produced that may either cross-react or interfere with the measurements of our chosen reporter proteins. For example, the production of a colored compound can interfere with colorimetric assays or the production of a native autoinducer can increase the noise in reporting assays. In addition, characterizing an organism's behavior under nutrient-deficient conditions and stress resilience could be central to its adoption as a biosensor chassis in natural environments.[55]
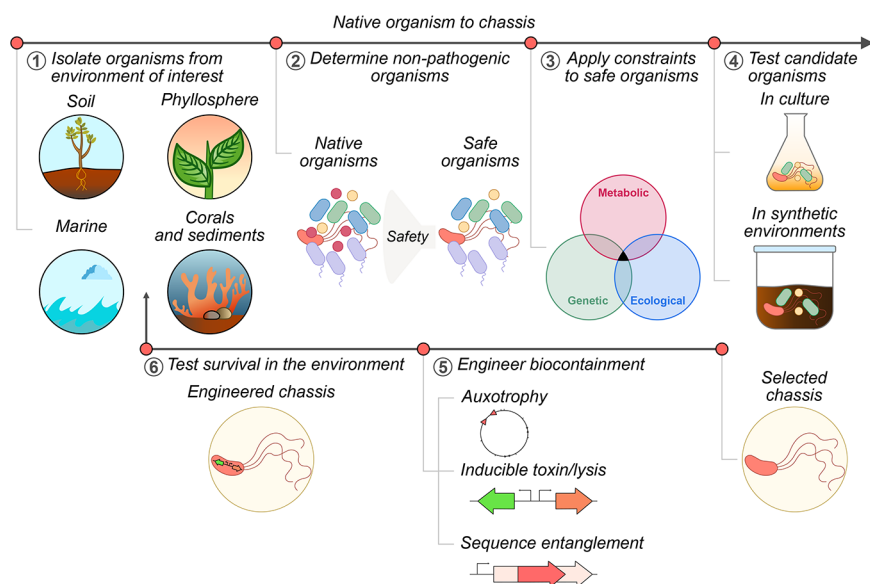
Figure 1. The journey from native organism to biosensor chassis. We recommend isolating organisms native to the ecosystems and environments of interest. After eliminating agricultural pathogens, a list of safe organisms will remain that can be narrowed down further based on our framework. This process results in a list of candidate chassis that, after sufficient biocontainment, can be tested in lab simulations of natural environments and evaluated for performance and persistence.

Constraint 4: The Chassis Must Be Genetically Tractable. To use a microbe as a chassis for genetic circuits, we need access to its fully sequenced and well-annotated genome. A well-annotated genome will help determine a microbe's most central pathways, its antibiotic resistance genes, and its defense mechanisms, such as encoded restriction enzymes.[56−64] Using a fully sequenced genome and comparative genomics, we can identify putative functions that the bacterium is associated with and characterize expected phenotypes that can be useful in designing cellular screening assays, such as response to an antibiotic or chemical.

Of equal importance is the need for robust DNA delivery protocols. Robust conjugation and transformation protocols allow the insertion of engineered genetic circuits into a chassis organism. Broad-host range genetic plasmid replication origins are the backbone for circuit engineering in non-model bacteria.[65] Additionally, methods for genomic integration of synthetic elements are necessary, especially in cases where reporter copy numbers need to be regulated or plasmid maintenance is less likely. Recent work has exploited diverse genetic tools to enable genomic integration in non-model bacteria—recombinase-based and recombinase-CRISPR-hybrids,[66−70] CRISPR-based,[71,72] retroelement-based,[73,74] transposase-based,[75,11,76] CRISPR-transposase hybrids,[77] integrative and conjugative elements,[10,78] and the novel CRISPR RNA-guided DNA integration.[79−82] These tools greatly support the engineering of non-model organisms, which results in novel engineerable chassis for biosensing.

While DNA delivery into non-model bacteria is a broadly discussed field of research,[83−85] we have distinct suggestions for plasmids that replicate in Gram-positive and Gram-negative bacteria. After gram verification, plasmid origins can be selected from Jain and Srivastava.[65] These broad-host range origins of replication are viable options, especially if these plasmids are stably maintained in an evolutionarily similar organism. Conjugative plasmid transfer has the maximum likelihood of success in most organisms that are culturable in

similar conditions as an *E. coli* donor. Electroporation, heat transformation, protoplast-mediated transformation, transduction, and induced natural competence have varied success rates and are highly organism-dependent.[83,85−87]

In addition to genetic tractability and engineerability, some aspects of cellular physiology and lifestyle must be characterized to select a chassis. Differentiated lifestyles, biofilm formation, spore formation, secretion systems, and membrane-bound transporters can be important for the choice of an organism as a chassis. Foley and Shuler[88] and Kim et al.[89] discuss the importance of a robust cell membrane. Characteristics such as surface colonization and hydrophobicity may be essential for biosensing in complex, rapidly fluctuating environments.[90]

■ CHALLENGES WITH CURRENT CHASSIS ORGANISMS

Escherichia coli has been the preferred chassis for synthetic biology as it offers desirable features that are essential for microbial chassis—aside from the ease of genetic manipulation, it cannot sporulate, making it easy to decontaminate from a surface with simple disinfection/sterilization protocols. Lab strains of *E. coli* have a doubling time of 20−30 min in aerobic rich media, which allows for rapid and facile engineering in lab time scales. *E. coli* has a simple cell structure with a fully sequenced chromosome and comprehensive metabolic databases that can better inform choices in circuit and chassis design. All these features make *E. coli* the standard proof-of-concept chassis organism.

Despite these salient features, *E. coli* has severe restrictions as a chassis for environmental synthetic biology. Environments are physicochemically complex with gradients in pressure, salinity, and pH that can impact chassis persistence. In such nonideal and uncontrollable conditions, the persistence of lab strains of *E. coli* is less likely. Furthermore, tools developed in *E. coli* are not easily portable into other potential chassis.[91,92] As Adams[2] noted, the adoption of different chassis organisms
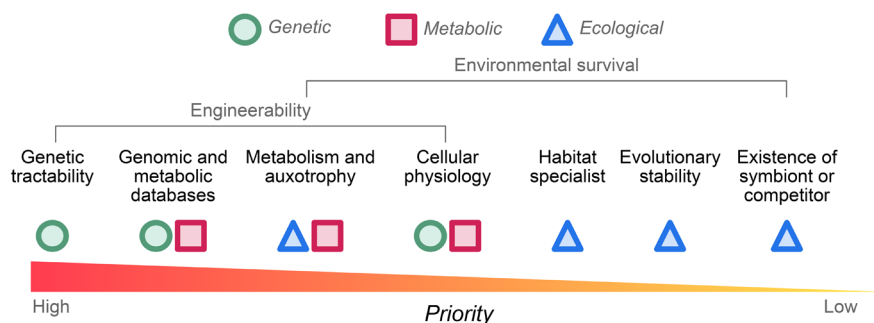
Figure 2. Prioritizing chassis constraints. It is challenging to resolve all our constraints in the selection of a chassis organism. In such cases, prioritizing some constraints over others can be a useful way forward. We recommend the above priority axis as a guide to negotiate the various constraints in the choice of a chassis organism.

for biosensor circuits is not as trivial as transferring circuits optimized in *E. coli* into the potential chassis. Expression and regulation of genetic circuits is a highly host-specific process, so much so that even with synthetic regulatory elements, we observe a difference in expression between different microbes.[91]

We do not recommend a minimalistic approach to chassis selection for biosensing applications in the environment. To expand the library of chassis organisms, *Pseudomonas putida* (soil), *Bacillus subtilis* (soil), and *Geobacillus* (hot springs) have been considered in the second generation of chassis organisms.[2] These organisms are viable chassis candidates due to existing synthetic biology toolkits, demonstrated genetic tractability, and reasonable growth and engineering conditions.[93,7,94] While these traits are desirable in a potential chassis organism, each microbe listed above suits a defined niche of applications and is severely restricted outside this niche without extensive and laborious genetic engineering. Thus, we envision additional chassis need to be developed for environmental biosensing.

■ A FRAMEWORK FOR CHOOSING ENVIRONMENTAL BIOSENSOR CHASSIS

Since each environment poses distinct constraints on the chassis, the ideal place to begin is the environmental problem of interest. Once the environmental context has been established, we can compile a list of native organisms from the relevant ecological niche. Next, we must eliminate pathogenic organisms from this list. Subsequently, using our constraints as a guide, we can shortlist some organisms that are amenable to engineering for biosensing. Lastly, shortlisted organisms can be tested in the laboratory using simulated environmental conditions. We illustrate our framework in Figure 1.

We recommend choosing chassis organisms that are habitat specialists in the environment of interest.[89] We make this recommendation considering the trade-off between challenges associated with engineering new tools in specialist organisms compared to challenges in engineering generalist organisms to survive in specific environmental conditions. Habitat specialists are well adapted to existing conditions in an environment. Specialists also fulfill a niche and are effective at competing with organisms in their niche. Since specialists are highly selective of their environment and growth substrates, choosing them may minimize the need for chassis engineering for survival in the environment of interest. For example, a bacterium that is known to colonize mineral-rich surfaces (a

habitat specialist) may have specific adaptations to tolerate mineral toxicity and can be used to address questions on the mineral surface but may not have a genetic toolbox. However, we anticipate that a bacterium from bulk soil (a habitat generalist) with a genetic toolbox would have to be engineered further to tolerate high mineral concentrations. This process can be extremely challenging as the mechanisms for mineral tolerance are complex, typically understudied, and require extensive testing after multiple genetic modifications to the generalist organism.

As an example of how to use this framework, we can consider the case of sensing volatile organic compounds in response to drying–wetting cycles in the rhizosphere. Hydrating a dry soil stimulates metabolic activity in the rhizosphere, which releases volatiles that are highly transient and challenging to measure.[95,96] These volatiles play central roles in interspecies communication and community structuring, thereby meriting study using nondestructive, unbiased detection methods.[96,97] Additionally, volatile concentrations are impacted by the soil matrix, meaning that analytically detected concentrations can be significantly different from bioavailable concentrations.[98] Biosensors offer a viable alternative to measuring such transient molecules *in situ*.[1,97] To thrive in the rhizosphere and measure transient signals, we need a chassis organism that is native to the rhizosphere. After eliminating environmental pathogens, we have two distinct ways to choose native organisms as chassis—either we select a host-specific plant growth promoting rhizobacterium (PGPR plu.), such as *Acinetobacter baylyi* (habitat specialist), or a relatively broad nonsymbiotic rhizosphere colonizer (habitat generalist), such as *Azospirillum brasilense*. In this case, both these species have sequenced genomes, phenotypic and metabolic characterization, and promising ecological roles that can be harvested for biosensing.[99,100] *Acinetobacter baylyi* ADP-1 has been engineered with synthetic biology tools and has been a species of interest due to its nutritional diversity and natural competence.[99] *Azospirillum brasilense* has a demonstrated ability to retain plasmids of varying origins and is widely studied.[100] Both these organisms are feasible chassis for rhizosphere volatile sensing.

As shortlisting one or a few chassis suitable for the environmental problem of interest may not always be feasible, an alternative approach is to explore non-native organisms that may survive the stringent conditions of the environment of interest. For example, soil and marine sediment environments often experience anaerobic conditions. Since the human intestinal tract is also anaerobic, we could consider obligate

anaerobes from this environment, such as *Bacteroides thetaiotaomicron*, as a potential chassis. This would require additional testing in the laboratory, as we do not know how *Bacteroides thetaiotaomicron* will affect ecological processes in the niche of interest, but it is worth considering as this organism has well-characterized synthetic genetic elements available for use.[101]

Significant knowledge gaps must be addressed before considering our recommendations. The journey of an organism from a natural microbial isolate to a widely adopted synthetic biology chassis requires robust bodies of work in genome sequencing and annotation, metabolic profiling and systems biology, and ecological characterization supported by experimental evidence, as discussed extensively by Liu and Deutschbauer,[102] Calero and Nikel,[103] and Venturelli et al.[3] Rigorous, actively curated genomic and metabolic databases, such as BioCyc (specifically EcoCyc and BSubCyc),[104,105] KEGG,[106] and modelSEED,[107] combined with standardized, community-level efforts in engineering multiple bacteria offer a targeted approach to adopting novel bacterial chassis with desirable phenotypes and diverse environmental niches.[108] However, these knowledge gaps are challenging to address each time a novel chassis is being considered for adoption. In such cases, we recommend consulting Figure 2, in which we parse our constraints into subconstraints and rank them in a qualitative priority order.

The rapid development of standardized, functionally characterized, and modular species-specific and broad host range toolkits will facilitate the adoption of novel chassis in environmental biosensing. Efforts in developing species-specific toolkits for *Vibrio natriegens*, *Pseudomonas putida*, etc. can be replicated in non-model novel chassis organisms with some modifications and prior characterization.[5,109] Broad host range tools help port synthetic biology parts into non-model organisms without known libraries.[11,110] Orthogonal transcription/translation tools can be a method to decouple circuit function from host regulatory machinery, especially when there is strong selection against synthetic circuits that compete for native replication resources.[111–113] Tools like UBER have been developed with robust broad-host range functionality.[114,115] However, more coordinated, community-wide efforts to develop toolkits for genome editing and stable cross-species expression in non-model bacteria will greatly accelerate progress in environmental biosensing.

In summary, we have outlined constraints for the systematic selection of biosensor chassis for environmental synthetic biology. We have articulated a conceptual framework to make this selection based on constraints that must be addressed. We offer some recommendations that can simplify the choice of a chassis organism and describe our vision for a new generation of biosensor chassis. With the systematic, standardized, and rapid development of robust tools for diverse bacteria, we can amplify the impact of synthetic biology tools in the Earth and environmental sciences to address novel questions in the face of a changing planet.

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(CRediT) Swetha Sridhar: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Visualization. Caroline M. Ajo-Franklin: Conceptualization, Writing - Review & Editing, Funding Acquisition. Caroline A. Masiello: Conceptualization, Writing - Review & Editing, Funding Acquisition

Notes

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■ REFERENCES

(1) Del Valle, I.; Fulk, E. M.; Kalvapalle, P.; Silberg, J. J.; Masiello, C. A.; Stadler, L. B. Translating New Synthetic Biology Advances for Biosensing Into the Earth and Environmental Sciences. *Front. Microbiol.* **2021**, DOI: 10.3389/fmicb.2020.618373.

(2) Adams, B. L. The Next Generation of Synthetic Biology Chassis: Moving Synthetic Biology from the Laboratory to the Field. *ACS Synth. Biol.* **2016**, *5*, 1328−1330.

(3) Venturelli, O. S.; Egbert, R. G.; Arkin, A. P. Towards Engineering Biological Systems in a Broader Context. *J. Mol. Biol.* **2016**, *428*, 928−944.

(4) Camenzind, T.; Hättenschwiler, S.; Treseder, K. K.; Lehmann, A.; Rillig, M. C. Nutrient limitation of soil microbial processes in tropical forests. *Ecol. Monogr. 88* **2018**, *88*, 4−21.

(5) Calero, P.; Jensen, S. I.; Nielsen, A. T. Broad-Host-Range ProUSER Vectors Enable Fast Characterization of Inducible Promoters and Optimization of p-Coumaric Acid Production in Pseudomonas putida KT2440. *ACS Synth. Biol.* **2016**, *5*, 741−753.

(6) Nikel, P. I.; de Lorenzo, V. Pseudomonas putida as a functional chassis for industrial biocatalysis: From native biochemistry to trans-metabolism. *Metab. Eng.* **2018**, *50*, 142−155.

(7) Liu, Y.; Liu, L.; Li, J.; Du, G.; Chen, J. Synthetic Biology Toolbox and Chassis Development in Bacillus subtilis. *Trends Biotechnol.* **2019**, *37*, 548−562.

(8) Lee, M. E.; DeLoache, W. C.; Cervantes, B.; Dueber, J. E. A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly. *ACS Synth. Biol.* **2015**, *4*, 975−986.

(9) Sheth, R. U.; Cabral, V.; Chen, S. P.; Wang, H. H. Manipulating Bacterial Communities by in situ Microbiome Engineering. *Trends Genet.* **2016**, *32*, 189−200.

(10) Brophy, J. A. N.; Triassi, A. J.; Adams, B. L.; Renberg, R. L.; Stratis-Cullum, D. N.; Grossman, A. D.; Voigt, C. A. Engineered integrative and conjugative elements for efficient and inducible DNA transfer to undomesticated bacteria. *Nat. Microbiol.* **2018**, *3*, 1043−1053.

(11) Ronda, C.; Chen, S. P.; Cabral, V.; Yaung, S. J.; Wang, H. H. Metagenomic engineering of the mammalian gut microbiome in situ. *Nat. Methods* **2019**, *16*, 167−170.

(12) Spangler, J. R.; Caruana, J. C.; Phillips, D. A.; Walper, S. A. Broad range shuttle vector construction and promoter evaluation for the use of Lactobacillus plantarum WCFS1 as a microbial engineering platform. *Synth. Biol.* **2019**, DOI: 10.1093/synbio/ysz012.

(13) Burbank, L.; Wei, W. Broad-Host-Range Plasmids for Constitutive and Inducible Gene Expression in the Absence of Antibiotic Selection. *Microbiol. Resour. Announc.* **2019**, DOI: 10.1128/MRA.00769-19.

(14) Serber, Z.; Dean, E. J.; Manchester, S.; Gora, K.; Flashman, M.; Shellman, E.; Kimball, A.; Szyjka, S.; Frewen, B.; Treynor, T.; Bruno, K. S. *Microbial strain improvement by a HTP genomic engineering platform.* US Patent, US20170159045A1, July 2, 2019.

(15) Tenhaef, N.; Stella, R.; Frunzke, J.; Noack, S. Automated Rational Strain Construction Based on High-Throughput Conjugation. *ACS Synth. Biol.* **2021**, *10*, 589−599.

(16) Casadevall, A.; Relman, D. A. Microbial threat lists: obstacles in the quest for biosecurity? *Nat. Rev. Microbiol.* **2010**, *8*, 149−154.

(17) *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*; National Institutes of Health, 2019.

(18) Zhou, Y.; Sun, T.; Chen, Z.; Song, X.; Chen, L.; Zhang, W. Development of a New Biocontainment Strategy in Model Cyanobacterium Synechococcus Strains. *ACS Synth. Biol.* **2019**, *8*, 2576−2584.

(19) Steidler, L.; Neirynck, S.; Huyghebaert, N.; Snoeck, V.; Vermeire, A.; Goddeeris, B.; Cox, E.; Remon, J. P.; Remaut, E. Biological containment of genetically modified Lactococcus lactis for intestinal delivery of human interleukin 10. *Nat. Biotechnol.* **2003**, *21*, 785−789.

(20) Chan, C. T. Y.; Lee, J. W.; Cameron, D. E.; Bashor, C. J.; Collins, J. J. Deadman" and "Passcode" microbial kill switches for bacterial containment. *Nat. Chem. Biol.* **2016**, *12*, 82−86.

(21) Stirling, F.; Bitzan, L.; O'Keefe, S.; Redfield, E.; Oliver, J. W. K.; Way, J.; Silver, P. A. Rational Design of Evolutionarily Stable Microbial Kill Switches. *Mol. Cell* **2017**, *68*, 686−697.

(22) Stirling, F.; Naydich, A.; Bramante, J.; Barocio, R.; Certo, M.; Wellington, H.; Redfield, E.; O'Keefe, S.; Gao, S.; Cusolito, A.; Way, J.; Silver, P. Synthetic Cassettes for pH-Mediated Sensing, Counting, and Containment. *Cell Rep.* **2020**, *30*, 3139−3148.

(23) Callura, J. M.; Dwyer, D. J.; Isaacs, F. J.; Cantor, C. R.; Collins, J. J. Tracking, tuning, and terminating microbial physiology using synthetic riboregulators. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 15898−15903.

(24) Blazejewski, T.; Ho, H.-I.; Wang, H. H. Synthetic sequence entanglement augments stability and containment of genetic information in cells. *Science* **2019**, *365*, 595−598.

(25) Schmidt, M. Xenobiology: A new form of life as the ultimate biosafety tool. *BioEssays* **2010**, *32*, 322−331.

(26) Wright, O.; Stan, G.-B.; Ellis, T. Building-in biosafety for synthetic biology. *Microbiology* **2013**, *159*, 1221−1235.

(27) Moe-Behrens, G. H. G.; Davis, R.; Haynes, K. A. Preparing synthetic biology for the world. *Front. Microbiol.* **2013**, DOI: 10.3389/fmicb.2013.00005.

(28) Torres, L.; Krüger, A.; Csibra, E.; Gianni, E.; Pinheiro, V. B. Synthetic biology approaches to biological containment: pre-emptively tackling potential risks. *Essays Biochem.* **2016**, *60*, 393−410.

(29) Schmidt, M.; de Lorenzo, V. Synthetic bugs on the loose: containment options for deeply engineered (micro)organisms. *Curr. Opin. Biotechnol.* **2016**, *38*, 90−96.

(30) Lee, J. W.; Chan, C. T. Y.; Slomovic, S.; Collins, J. J. Next-generation biocontainment systems for engineered organisms. *Nat. Chem. Biol.* **2018**, *14*, 530−537.

(31) Stirling, F.; Silver, P. A. Controlling the Implementation of Transgenic Microbes: Are We Ready for What Synthetic Biology Has to Offer? *Mol. Cell* **2020**, *78*, 614−623.

(32) Wright, O.; Delmans, M.; Stan, G.-B.; Ellis, T. GeneGuard: A Modular Plasmid System Designed for Biosafety. *ACS Synth. Biol.* **2015**, *4*, 307−316.

(33) Gallagher, R. R.; Patel, J. R.; Interiano, A. L.; Rovner, A. J.; Isaacs, F. J. Multilayered genetic safeguards limit growth of microorganisms to defined environments. *Nucleic Acids Res.* **2015**, *43*, 1945−1954.

(34) Braga, R. M.; Dourado, M. N.; Araújo, W. L. Microbial interactions: ecology in a molecular perspective. *Braz. J. Microbiol. Publ. Braz. Soc. Microbiol.* **2016**, *47* (Suppl 1), 86−98.

(35) Pacheco, A. R.; Segrè, D. A multidimensional perspective on microbial interactions. *FEMS Microbiol. Lett.* **2019**, *366*, fnz125.

(36) Morris, B. E. L.; Henneberger, R.; Huber, H.; Moissl-Eichinger, C. Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* **2013**, *37*, 384−406.

(37) Libby, E.; Hébert-Dufresne, L.; Hosseini, S.-R.; Wagner, A. Syntrophy emerges spontaneously in complex metabolic systems. *PLOS Comput. Biol.* **2019**, *15*, e1007169.

(38) Lewis, W. H.; Tahon, G.; Geesink, P.; Sousa, D. Z.; Ettema, T. J. G. Innovations to culturing the uncultured microbial majority. *Nat. Rev. Microbiol.* **2021**, *19*, 225−240.

(39) Stubbendieck, R. M.; Vargas-Bautista, C.; Straight, P. D. Bacterial communities: Interactions to scale. *Front. Microbiol.* **2016**, DOI: 10.3389/fmicb.2016.01234.

(40) Esvap, E.; Ulgen, K. O. Advances in Genome-Scale Metabolic Modeling toward Microbial Community Analysis of the Human Microbiome. *ACS Synth. Biol.* **2021**, *10*, 2121−2137.

(41) Baldini, F.; Heinken, A.; Heirendt, L.; Magnusdottir, S.; Fleming, R. M. T.; Thiele, I. The Microbiome Modeling Toolbox: from microbial interactions to personalized microbial communities. *Bioinformatics* **2019**, *35*, 2332−2334.

(42) Andrighetti, T.; Bohar, B.; Lemke, N.; Sudhakar, P.; Korcsmaros, T. MicrobioLink: An Integrated Computational Pipeline to Infer Functional Effects of Microbiome−Host Interactions. *Cells* **2020**, *9*, 1278.

(43) Diener, C.; Gibbons, S. M.; Resendis-Antonio, O. MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota. *mSystems* **2020**, *5*, e00606-19.

(44) Röttjers, L.; Faust, K. From hairballs to hypotheses−biological insights from microbial networks. *FEMS Microbiol. Rev.* **2018**, *42*, 761−780.

(45) Kumar, M.; Ji, B.; Zengler, K.; Nielsen, J. Modelling approaches for studying the microbiome. *Nat. Microbiol.* **2019**, *4*, 1253−1267.

(46) van den Berg, N. I.; Machado, D.; Santos, S.; Rocha, I.; Chacón, J.; Harcombe, W.; Mitri, S.; Patil, K. R. Ecological modelling approaches for predicting emergent properties in microbial communities. *Nat. Ecol. Evol.* **2022**, *6*, 855.

(47) McClain, M. E.; Boyer, E. W.; Dent, C. L.; Gergel, S. E.; Grimm, N. B.; Groffman, P. M.; Hart, S. C.; Harvey, J. W.; Johnston, C. A.; Mayorga, E.; McDowell, W. H.; Pinay, G. Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems. *Ecosystems* **2003**, *6*, 301−312.

(48) Pace, N. R. A Molecular View of Microbial Diversity and the Biosphere. *Science* **1997**, *276*, 734−740.

(49) Rappé, M. S.; Giovannoni, S. J. The Uncultured Microbial Majority. *Annu. Rev. Microbiol.* **2003**, *57*, 369−394.

(50) Becker, M. H.; Brophy, J. A. N.; Barrett, K.; Bronikowski, E.; Evans, M.; Glassey, E.; Kaganer, A. W.; Klocke, B.; Lassiter, E.; Meyer, A. J.; Muletz-Wolz, C. R.; Fleischer, R. C.; Voigt, C. A.; Gratwicke, B. Genetically modifying skin microbe to produce violacein and augmenting microbiome did not defend Panamanian golden frogs from disease. *ISME Commun.* **2021**, *1*, 57.

(51) O'Brien, E. J.; Monk, J. M.; Palsson, B. O. Using Genome-scale Models to Predict Biological Capabilities. *Cell* **2015**, *161*, 971−987.

(52) Lawson, C. E.; Harcombe, W. R.; Hatzenpichler, R.; Lindemann, S. R.; Löffler, F. E.; O'Malley, M. A.; García Martín, H.; Pfleger, B. F.; Raskin, L.; Venturelli, O. S.; Weissbrodt, D. G.; Noguera, D. R.; McMahon, K. D. Common principles and best

practices for engineering microbiomes. *Nat. Rev. Microbiol.* **2019**, *17*, 725−741.

(53) McEwan, A. G. Photosynthetic electron transport and anaerobic metabolism in purple non-sulfur phototrophic bacteria. *Antonie Van Leeuwenhoek* **1994**, *66*, 151−164.

(54) Hädicke, O.; Grammel, H.; Klamt, S. Metabolic network modeling of redox balancing and biohydrogen production in purple nonsulfur bacteria. *BMC Syst. Biol.* **2011**, *5*, 150.

(55) Brooks, S. M.; Alper, H. S. Applications, challenges, and needs for employing synthetic biology beyond the lab. *Nat. Commun.* **2021**, *12*, 1390.

(56) Stothard, P.; Wishart, D. S. Automated bacterial genome analysis and annotation. *Curr. Opin. Microbiol.* **2006**, *9*, 505−510.

(57) Aziz, R. K.; Bartels, D.; Best, A. A.; DeJongh, M.; Disz, T.; Edwards, R. A.; Formsma, K.; Gerdes, S.; Glass, E. M.; Kubal, M.; et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **2008**, *9*, 1−15.

(58) Vallenet, D.; Engelen, S.; Mornico, D.; Cruveiller, S.; Fleury, L.; Lajus, A.; Rouy, Z.; Roche, D.; Salvignol, G.; Scarpelli, C.; Medigue, C. MicroScope: a platform for microbial genome annotation and comparative genomics. *Database* **2009**, DOI: 10.1093/database/bap021.

(59) Richardson, E. J.; Watson, M. The automatic annotation of bacterial genomes. *Brief. Bioinform.* **2013**, *14*, 1−12.

(60) Jones, P.; Binns, D.; Chang, H.-Y.; Fraser, M.; Li, W.; McAnulla, C.; McWilliam, H.; Maslen, J.; Mitchell, A.; Nuka, G.; et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics* **2014**, *30*, 1236−1240.

(61) Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **2014**, *30*, 2068−2069.

(62) Shaffer, M.; Borton, M. A.; McGivern, B. B.; Zayed, A. A.; La Rosa, S. L.; Solden, L. M.; Liu, P.; Narrowe, A. B.; Rodríguez-Ramos, J.; Bolduc, B.; et al. DRAM for distilling microbial metabolism to automate the curation of microbiome function. *Nucleic Acids Res.* **2020**, *48*, 8883−8900.

(63) Segerman, B. The Most Frequently Used Sequencing Technologies and Assembly Methods in Different Time Segments of the Bacterial Surveillance and RefSeq Genome Databases. *Front. Cell. Infect. Microbiol.* **2020**, DOI: 10.3389/fcimb.2020.527102.

(64) Cantalapiedra, C. P.; Hernández-Plaza, A.; Letunic, I.; Bork, P.; Huerta-Cepas, J. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol. Biol. Evol.* **2021**, *38*, 5825−5829.

(65) Jain, A.; Srivastava, P. Broad host range plasmids. *FEMS Microbiol. Lett.* **2013**, *348*, 87−96.

(66) Santos, C. N. S.; Yoshikuni, Y. Engineering complex biological systems in bacteria through recombinase-assisted genome engineering. *Nat. Protoc.* **2014**, *9*, 1320−1336.

(67) Wang, G.; Zhao, Z.; Ke, J.; Engel, Y.; Shi, Y.-M.; Robinson, D.; Bingol, K.; Zhang, Z.; Bowen, B.; Louie, K.; Wang, B.; Evans, R.; Miyamoto, Y.; Cheng, K.; Kosina, S.; De Raad, M.; Silva, L.; Luhrs, A.; Lubbe, A.; Hoyt, D. W.; Francavilla, C.; Otani, H.; Deutsch, S.; Washton, N. M.; Rubin, E. M.; Mouncey, N. J.; Visel, A.; Northen, T.; Cheng, J.-F.; Bode, H. B.; Yoshikuni, Y. CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria. *Nat. Microbiol.* **2019**, *4*, 2498.

(68) Wang, B.; Zhao, Z.; Jabusch, L. K.; Chiniquy, D. M.; Ono, K.; Conway, J. M.; Zhang, Z.; Wang, G.; Robinson, D.; Cheng, J.-F.; Dangl, J. L.; Northen, T. R.; Yoshikuni, Y. CRAGE-Duet Facilitates Modular Assembly of Biological Systems for Studying Plant−Microbe Interactions. *ACS Synth. Biol.* **2020**, *9*, 2610−2615.

(69) Elmore, J. R.; Dexter, G. N.; Francis, R.; Riley, L.; Huenemann, J.; Baldino, H.; Guss, A. M.; Egbert, R. The SAGE genetic toolkit enables highly efficient, iterative site-specific genome engineering in bacteria. *bioRxiv*, June 28, 2020. DOI: 10.1101/2020.06.28.176339.

(70) Ke, J.; Robinson, D.; Wu, Z.-Y.; Kuftin, A.; Louie, K.; Kosina, S.; Northen, T.; Cheng, J.-F.; Yoshikuni, Y. CRAGE-CRISPR facilitates rapid activation of secondary metabolite biosynthetic gene clusters in bacteria. *Cell Chem. Biol.* **2022**, *29*, 696−710.

(71) Ao, X.; Yao, Y.; Li, T.; Yang, T.-T.; Dong, X.; Zheng, Z.-T.; Chen, G.-Q.; Wu, Q.; Guo, Y. A Multiplex Genome Editing Method for Escherichia coli Based on CRISPR-Cas12a. *Front. Microbiol.* **2018**, DOI: 10.3389/fmicb.2018.02307.

(72) Csörgő, B.; León, L. M.; Chau-Ly, I. J.; Vasquez-Rifo, A.; Berry, J. D.; Mahendra, C.; Crawford, E. D.; Lewis, J. D.; Bondy-Denomy, J. A compact Cascade−Cas3 system for targeted genome engineering. *Nat. Methods* **2020**, *17*, 1183.

(73) Farzadfard, F.; Lu, T. K. Genomically encoded analog memory with precise in vivo DNA writing in living cell populations. *Science* **2014**, *346*, 1256272.

(74) Farzadfard, F.; Gharaei, N.; Citorik, R. J.; Lu, T. K. Efficient retroelement-mediated DNA writing in bacteria. *Cell Syst.* **2021**, *12*, 860−872.

(75) Martínez-García, E.; de Lorenzo, V. Transposon-Based and Plasmid-Based Genetic Tools for Editing Genomes of Gram-Negative Bacteria. In *Synthetic Gene Networks*; Weber, W., Fussenegger, M., Eds.; Humana Press: Totowa, NJ, 2012 pp 267−283.

(76) Peters, J. E. Targeted transposition with Tn7 elements: safe sites, mobile plasmids, CRISPR/Cas and beyond. *Mol. Microbiol.* **2019**, *112*, 1635−1644.

(77) Chen, S. P.; Wang, H. H. An Engineered Cas-Transposon System for Programmable and Site-Directed DNA Transpositions. *CRISPR J.* **2019**, *2*, 376−394.

(78) Meng, D.; Mukhitov, N.; Neitzey, D.; Lucht, M.; Schaak, D. D.; Voigt, C. A. Rapid and simultaneous screening of pathway designs and chassis organisms, applied to engineered living materials. *Metab. Eng.* **2021**, *66*, 308.

(79) Klompe, S. E.; Vo, P. L. H.; Halpin-Healy, T. S.; Sternberg, S. H. Transposon-encoded CRISPR−Cas systems direct RNA-guided DNA integration. *Nature* **2019**, *571*, 219−225.

(80) Strecker, J.; Ladha, A.; Gardner, Z.; Schmid-Burgk, J.; Makarova, K. S.; Koonin, E. V.; Zhang, F. RNA-guided DNA insertion with CRISPR-associated transposases. *Science* **2019**, *365*, 48−53.

(81) Vo, P. L. H.; Ronda, C.; Klompe, S. E.; Chen, E. E.; Acree, C.; Wang, H. H.; Sternberg, S. H. CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering. *Nat. Biotechnol.* **2021**, *39*, 480−489.

(82) Rubin, B. E.; Diamond, S.; Cress, B. F.; Crits-Christoph, A.; Lou, Y. C.; Borges, A. L.; Shivram, H.; He, C.; Xu, M.; Zhou, Z.; Smith, S. J.; Rovinsky, R.; Smock, D. C. J.; Tang, K.; Owens, T. K.; Krishnappa, N.; Sachdeva, R.; Barrangou, R.; Deutschbauer, A. M.; Banfield, J. F.; Doudna, J. A. Species- and site-specific genome editing in complex bacterial communities. *Nat. Microbiol.* **2022**, *7*, 34−47.

(83) Freed, E.; Fenster, J.; Smolinski, S. L.; Walker, J.; Henard, C. A.; Gill, R.; Eckert, C. A. Building a genome engineering toolbox in nonmodel prokaryotic microbes. *Biotechnol. Bioeng.* **2018**, *115*, 2120−2138.

(84) Fatma, Z.; Schultz, J. C.; Zhao, H. Recent advances in domesticating non-model microorganisms. *Biotechnol. Prog.* **2020**, *36*, e3008.

(85) Riley, L. A.; Guss, A. M. Approaches to genetic tool development for rapid domestication of non-model microorganisms. *Biotechnol. Biofuels* **2021**, *14*, 30.

(86) Blokesch, M. Natural competence for transformation. *Curr. Biol.* **2016**, *26*, R1126−R1130.

(87) Dalia, T. N.; Hayes, C. A.; Stolyar, S.; Marx, C. J.; McKinlay, J. B.; Dalia, A. B. Multiplex Genome Editing by Natural Transformation (MuGENT) for Synthetic Biology in *Vibrio natriegens*. *ACS Synth. Biol.* **2017**, *6*, 1650−1655.

(88) Foley, P. L.; Shuler, M. L. Considerations for the design and construction of a synthetic platform cell for biotechnological applications. *Biotechnol. Bioeng.* **2010**, *105*, 26−36.

(89) Kim, J.; Salvador, M.; Saunders, E.; González, J.; Avignone-Rossa, C.; Jiménez, J. I. Properties of alternative microbial hosts used in synthetic biology: towards the design of a modular chassis. *Essays Biochem.* **2016**, *60*, 303−313.

(90) Inda, M. E.; Lu, T. K. Microbes as Biosensors. *Annu. Rev. Microbiol.* **2020**, *74*, 337−359.

(91) Cardinale, S.; Arkin, A. P. Contextualizing context for synthetic biology − identifying causes of failure of synthetic biological systems. *Biotechnol. J.* **2012**, *7*, 856−866.

(92) Jha, R. K.; Bingen, J. M.; Johnson, C. W.; Kern, T. L.; Khanna, P.; Trettel, D. S.; Strauss, C. E. M.; Beckham, G. T.; Dale, T. A protocatechuate biosensor for Pseudomonas putida KT2440 via promoter and protein evolution. *Metab. Eng. Commun.* **2018**, *6*, 33−38.

(93) Martin-Pascual, M.; Batianis, C.; Bruinsma, L.; Asin-Garcia, E.; Garcia-Morales, L.; Weusthuis, R. A.; van Kranenburg, R.; Martins dos Santos, V. A. P. A navigation guide of synthetic biology tools for Pseudomonas putida. *Biotechnol. Adv.* **2021**, *49*, 107732.

(94) Reeve, B.; Martinez-Klimova, E.; de Jonghe, J.; Leak, D. J.; Ellis, T. The Geobacillus Plasmid Set: A Modular Toolkit for Thermophile Engineering. *ACS Synth. Biol.* **2016**, *5*, 1342−1347.

(95) Kim, J.; Goldstein, A. H.; Chakraborty, R.; Jardine, K.; Weber, R.; Sorensen, P. O.; Wang, S.; Faybishenko, B.; Misztal, P. K.; Brodie, E. L. Measurement of Volatile Compounds for Real-Time Analysis of Soil Microbial Metabolic Response to Simulated Snowmelt. *Front. Microbiol.* **2021**, DOI: 10.3389/fmicb.2021.679671.

(96) Honeker, L. K.; Graves, K. R.; Tfaily, M. M.; Krechmer, J. E.; Meredith, L. K. The Volatilome: A Vital Piece of the Complete Soil Metabolome. *Front. Environ. Sci.* **2021**, DOI: 10.3389/fenvs.2021.649905.

(97) Redeker, K. R.; Cai, L. L.; Dumbrell, A. J.; Bardill, A.; Chong, J. P. J.; Helgason, T. Noninvasive Analysis of the Soil Microbiome: Biomonitoring Strategies Using the Volatilome, Community Analysis, and Environmental Data, in. *Adv. Ecol. Res.* **2018**, *59*, 93−132.

(98) Del Valle, I.; Webster, T. M.; Cheng, H.-Y.; Thies, J. E.; Kessler, A.; Miller, M. K.; Ball, Z. T.; MacKenzie, K. R.; Masiello, C. A.; Silberg, J. J.; Lehmann, J. Soil organic matter attenuates the efficacy of flavonoid-based plant-microbe communication. *Sci. Adv.* **2020**, DOI: 10.1126/sciadv.aax8254.

(99) Biggs, B. W.; Bedore, S. R.; Arvay, E.; Huang, S.; Subramanian, H.; McIntyre, E. A.; Duscent-Maitland, C. V.; Neidle, E. L.; Tyo, K. E. J. Development of a genetic toolset for the highly engineerable and metabolically versatile Acinetobacter baylyi ADP1. *Nucleic Acids Res.* **2020**, *48*, 5169−5182.

(100) Gullett, J.; O'Neal, L.; Mukherjee, T.; Alexandre, G. *Azospirillum brasilense*: Laboratory Maintenance and Genetic Manipulation. *Curr. Protoc. Microbiol.* **2017**, *47*, 3E.2.1−3E.2.17.

(101) Taketani, M.; Zhang, J.; Zhang, S.; Triassi, A. J.; Huang, Y.-J.; Griffith, L. G.; Voigt, C. A. Genetic circuit design automation for the gut resident species Bacteroides thetaiotaomicron. *Nat. Biotechnol.* **2020**, *38*, 962.

(102) Liu, H.; Deutschbauer, A. M. Rapidly moving new bacteria to model-organism status. *Curr. Opin. Biotechnol.* **2018**, *51*, 116−122.

(103) Calero, P.; Nikel, P. I. Chasing bacterial chassis for metabolic engineering: a perspective review from classical to non-traditional microorganisms. *Microb. Biotechnol.* **2019**, *12*, 98−124.

(104) Caspi, R.; Billington, R.; Fulcher, C. A.; Keseler, I. M.; Kothari, A.; Krummenacker, M.; Latendresse, M.; Midford, P. E.; Ong, Q.; Ong, W. K.; et al. The MetaCyc database of metabolic pathways and enzymes. *Nucleic Acids Res.* **2018**, *46*, D633−D639.

(105) Karp, P. D.; Billington, R.; Caspi, R.; Fulcher, C. A.; Latendresse, M.; Kothari, A.; Keseler, I. M.; Krummenacker, M.; Midford, P. E.; Ong, Q.; et al. The BioCyc collection of microbial genomes and metabolic pathways. *Brief. Bioinform.* **2019**, *20*, 1085−1093.

(106) Kanehisa, M.; Furumichi, M.; Tanabe, M.; Sato, Y.; Morishima, K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **2017**, *45*, D353−D361.

(107) Seaver, S. M D; Liu, F.; Zhang, Q.; Jeffryes, J.; Faria, J. P; Edirisinghe, J. N; Mundy, M.; Chia, N.; Noor, E.; Beber, M. E; Best, A. A; DeJongh, M.; Kimbrel, J. A; D'haeseleer, P.; McCorkle, S. R; Bolton, J. R; Pearson, E.; Canon, S.; Wood-Charlson, E. M; Cottingham, R. W; Arkin, A. P; Henry, C. S The ModelSEED Database for the integration of metabolic annotations and the reconstruction, comparison, and analysis of metabolic models for plants, fungi, and microbes. *Nucleic Acids Res.* **2021**, *49*, D575−D588.

(108) Karp, P. D.; Ivanova, N.; Krummenacker, M.; Kyrpides, N.; Latendresse, M.; Midford, P.; Ong, W. K.; Paley, S.; Seshadri, R. A Comparison of Microbial Genome Web Portals. *Front. Microbiol.* **2019**, DOI: 10.3389/fmicb.2019.00208.

(109) Tschirhart, T.; Shukla, V.; Kelly, E. E.; Schultzhaus, Z.; NewRingeisen, E.; Erickson, J. S.; Wang, Z.; Garcia, W.; Curl, E.; Egbert, R. G.; Yeung, E.; Vora, G. J. Synthetic Biology Tools for the Fast-Growing Marine Bacterium *Vibrio natriegens*. *ACS Synth. Biol.* **2019**, *8*, 2069−2079.

(110) Johns, N. I.; Gomes, A. L. C.; Yim, S. S.; Yang, A.; Blazejewski, T.; Smillie, C. S.; Smith, M. B.; Alm, E. J.; Kosuri, S.; Wang, H. H. Metagenomic mining of regulatory elements enables programmable species-selective gene expression. *Nat. Methods.* **2018**, *15*, 323.

(111) An, W.; Chin, J. W. Synthesis of orthogonal transcription-translation networks. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 8477−8482.

(112) de Lorenzo, V. Recombinant bacteria for environmental release: what went wrong and what we have learnt from it. *Clin. Microbiol. Infect.* **2009**, *15*, 63−65.

(113) Liu, C. C.; Jewett, M. C.; Chin, J. W.; Voigt, C. A. Toward an orthogonal central dogma. *Nat. Chem. Biol.* **2018**, *14*, 103−106.

(114) Kushwaha, M.; Salis, H. M. A portable expression resource for engineering cross-species genetic circuits and pathways. *Nat. Commun.* **2015**, *6*, 7832.

(115) Guseva, A.; Truong, A.; Sriram, S.; Anh, V. H.; King, S.; Huang, D.; Yoon, S.; Vittala, A.; McCormick, E.; Cohen, K.; Hellmer, V.; Bhakta, S.; Del Valle, I.; Zong, D.; Bluford, J.; Ykema, M. PORTAL: Portable Transcription-Translation System to Improve Cross-Species Genetic Circuit Reliability. *Rice 2018 IGEM.* https://2018.igem.org/Team:Rice.