Received: 24 October 2022 | Accepted: 27 October 2022

DOI: 10.1111/1462-2920.16267

CRYSTAL BALL

environmental microbiology Applied Microbiology International

Mark-recapture of microorganisms

John George McMullen II | **Jay T. Lennon**

Department of Biology, Indiana University, Bloomington, Indiana, USA

Correspondence
John George McMullen II and Jay T. Lennon, Department of Biology, Indiana University, 1001 E 3rd St., Bloomington, IN 47405, USA.
Email: jgmcmull@iu.edu and lennonj@indiana.edu

Funding information
National Aeronautics and Space Administration, Grant/Award Number: 80NSSC20K0618; National Science Foundation, Grant/Award Numbers: DBI-2022049, DEB-1934554; U.S. Department of Defense, Grant/Award Numbers: W911NF-14-1-0411, W911NF-22-1-0014

Fundamental questions in ecology and evolution require the quantitative tracking of individuals across space and time. For more than a century, mark-recapture techniques have been used to estimate demographic events and population dynamics of animal and plant species. Recent developments in molecular barcoding allow for the monitoring of microorganisms while meeting many of the key assumptions of traditional mark-recapture studies. Beyond applications related to the biosecurity of human pathogens, molecular barcoding is emerging as a cost-effective, versatile, and scalable tool to quantify species interactions, biogeographic ranges, and evolutionary dynamics in simple and complex communities. Although there are logistical and ethical issues related to the release of barcoded organisms, mark-recapture provides opportunities to test theory and better understand the evolutionary ecology of microorganisms in the wild.

Traditional mark-recapture—Mark-recapture has been used for more than a century to study animal and plant populations (Goudie & Goudie, 2007; Maunder, 2004). The primary purpose of mark-recapture is to estimate the size of a population in a given area where the total count of individuals cannot readily be determined. The basic approach involves capturing some portion of the population, marking those individuals, and releasing them back into the environment. After a subsequent recapture event, one can estimate the population size based on the ratio of unmarked and marked individuals (Figure 1A). By studying a population over time, mark-recapture can be used to make inferences about migration, survivorship, reproduction, and geographic range limits (Creel & Rosenblatt, 2013; Sandercock, 2003).

There are a number of important assumptions to consider when using mark-recapture. First, marked individuals are assumed to be an unbiased sample of the larger population. Second, markers should not be lost or misread over time, nor should they influence the performance of the marked organism, including their interactions with unmarked individuals (Lindberg, 2012). Third, marked and unmarked individuals should have an equal probability of being recaptured. Last, it is assumed that births, deaths, and migration events do not influence population size, unless mark-recapture is explicitly used to model population dynamics over time (Lindberg, 2012). While difficult to achieve in all instances, the general assumptions of mark-recapture should be accounted for whenever possible (Briggs et al., 2022).

Need for microbial mark-recapture—Mark-recapture has not explicitly been incorporated into the study of microorganisms (Douglas, 2018). While it is increasingly common to describe microbial population dynamics in natural settings with amplicon sequencing and shotgun metagenomics, these approaches are indirect and unable to efficiently track individual lineages and their associated genetic information (Bruger & Marx, 2018; Ellegaard & Engel, 2016). Although genome sequencing is becoming more affordable, it is not always effective for tracking low-frequency lineages or for quantifying statistically small yet biologically meaningful differences in fitness (Blundell & Levy, 2014; Bruger & Marx, 2018; Chubiz et al., 2012; Robinson et al., 2014). Mark-recapture methods would serve as a powerful tool for studying a range of biological phenomena using pre-defined lineages (Box 1).

Leveraging mark-recapture for microbial science—In microbiology, markers tend to involve

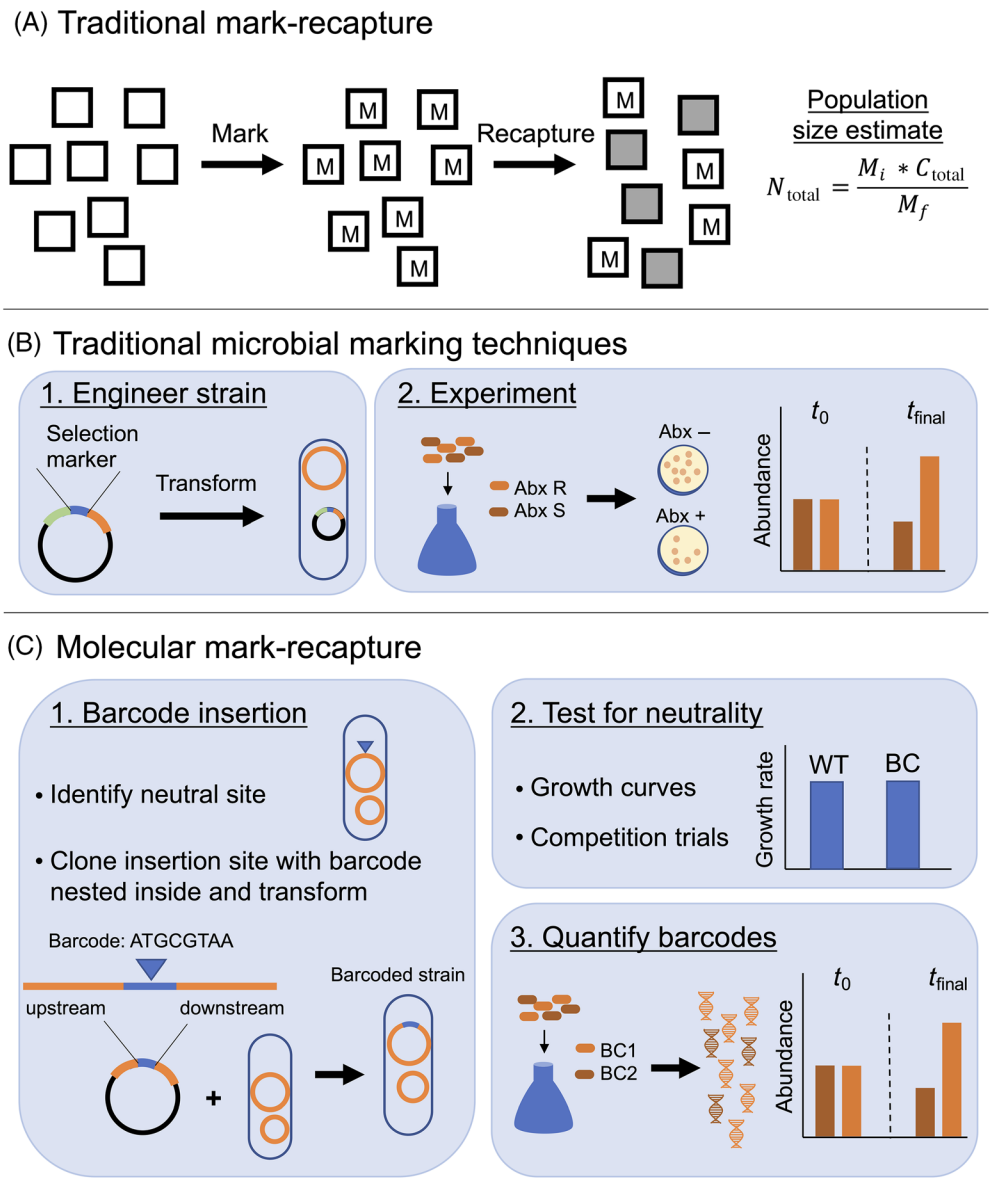

FIGURE 1 Strategies to mark and track organisms. (A) Traditional mark-recapture. Boxes indicate organisms that were collected in a particular area, which are then marked (*M*). The marking process allows researchers to come back to this area in a future resampling event to estimate the populations size based on the number of marked and new unmarked (grey) individuals captured. The simplest equation is the Lincoln-Petersen estimator, which uses the initial number of marked individuals (M_i), the final number of marked individuals captured (M_f), and the total number of marked and unmarked individuals in the recapture event (C_{total}) to estimate the population size (N_{total}) (Krebs, 1999). In the example, the population size is 16 individuals. (B) Traditional microbial marking techniques. A common approach is to engineer a strain, for example, with an antibiotic resistance selection cassette (Abx) encoded on a plasmid. This allows for identifying the number of colony-forming units (CFUs) that grow with and without the antibiotic to determine the changes in abundance over time in an experiment. (C) Molecular mark-recapture. The process of barcoding individual strains involves first identifying candidate neutral regions in a genome where a barcode (BC) can be integrated into the focal strain (Buckley et al., 2012). A key step in this process is to confirm that the barcode insertion has little to no effect on population fitness of the focal strain. Following this step, experiments can be performed with samples containing barcoded strain(s), and their abundance can be tracked over time with quantitative sequencing methods.

genomic modification or selection on traits that have a discernable phenotype (Figure 1B). Antibiotic resistance is a useful marker that can be developed through selection of spontaneous mutants or the insertion of drug-resistance cassettes either directly into the genome or by introducing cloned plasmids into a cell (Alseth et al., 2019; Gómez & Buckling, 2011; Reyrat et al., 1998). Another commonly used technique involves the insertion of fluorescent protein-encoding genes downstream of promoters, which can either be expressed constitutively or under a given set of conditions (Landete et al., 2016; Schlechter et al., 2018). These so-called reporter genes can be useful for tracking populations. For example, fluorescently labelled

BOX 1 Applications of microbial mark-recapture in microbial science

Competition-colonization trade-offs	Co-existence among strains in patchy environments can be mediated by spatial niche partitioning (Livingston et al., 2012). This is of particular interest for microbes that transition between a free-living phase in the environment and a host-associated phase (Bashey, 2015). Molecular barcoding has been used to understand the process of competitive colonization in a host by tracking barcoded lineages as they transition into the host environment, like pathogens in the mammalian gut (Abel et al., 2015; Hullahalli & Waldor, 2021), and have linked important traits associated with competitive colonization of a host, such as quantifying the degree of nitrogen fixation in barcoded rhizobia living inside nodules (Mendoza-Suárez et al., 2020).
Cross-feeding	Interactions between and within microbial species are commonly mediated by metabolite exchange (Douglas, 2020). Observing these processes can be challenging due to changes in flux and turnover of the metabolite pool. Molecular barcoding has been used to identify auxotrophies that promote cooperation through division of labour (Noto Guillen et al., 2021) as well as to identify cross-fed metabolites that mediate higher-order metabolic interactions in multi-species consortia that cannot be predicted from pairwise interactions (Morin et al., 2018). Much of the molecular barcoding work is done under simple laboratory conditions, but its application could be expanded to consider more complex environments and different taxa.
Dispersal	The active and passive movement of microorganisms is widespread in the wild (Barraud et al., 2015; Choudoir & DeAngelis, 2022; Shen et al., 2018). Dispersal has important implications for population dynamics and community assembly processes (Custer et al., 2022). Barcode labelling of individual microbial strains can enhance our mechanistic understanding on how dispersal impacts an organism's lifecycle, ecological interactions, and gene pool with direct source tracking that is well suited to be combined with current manipulation techniques (Vannette & Fukami, 2017).
Experimental evolution	A common use of molecular barcoding is to track individual lineages over time to observe changes in population genetics in experimental evolution assays. The number of lineages considered can be scaled to thousands of otherwise isogeneic cells with different barcodes to infer the distribution of fitness effects in a population under selection (Bruger & Marx, 2018). Over time as the genome changes with de novo mutations and gene flow, it is possible to follow the trajectory of each barcoded strain to track potential beneficial mutations. In simple laboratory experiments, processes like selective sweeps (Levy et al., 2015; Venkataram et al., 2016), clonal interference (Chubiz et al., 2012), genetic drift and lottery effects (Wright & Vetsigian, 2019), and the role of standing genetic variation on lineage trajectories (Jasinska et al., 2020) have been inferred. Important barcoded lineages identified can then be isolated and resequenced to determine the genetic basis for their rise in the population (Chubiz et al., 2012; Venkataram et al., 2016). Future applications could expand on experimental designs with more biologically and physically complex backgrounds.
Genotype-to-phenotype mapping	Molecular barcoding of forward genetic screens is a quantitative approach to identify the genetic basis for phenotypes of interest (Mutalik et al., 2020; Wetmore et al., 2015). By adding a barcode to each mutation made in a genome-wide mutagenesis screen, for example, it is possible to quantitatively track a pool of mutants with better precision than ever before to characterize changes in genotype–phenotype networks (Cain et al., 2020; Wetmore et al., 2015). For example, this approach has been used to identify candidate functions for proteins of unknown function in conjunction with high-throughput growth assays to identify metabolite transport enzymes that often are annotated with generalized functions as well as putative DNA repair enzymes (Price et al., 2018).
Invasion	The invasion of microorganisms into new environments involves several selective hurdles, including colonization and persistence in the new environment due to competition with resident taxa and other novel abiotic factors (Mallon et al., 2015). Apart from the fundamental knowledge that can be gained on community assembly and coalescence processes through source tracking of barcoded strain(s) (Rillig et al., 2015), there are numerous applications of this theory to microbial-mediated therapies in probiotics, biopesticides, biofertilizers, and bioremediation (Albright et al., 2020).
Mobile genetic elements	Although the tracking of subcellular features is not the typical focus of traditional animal and plant mark-recapture, microbes evolve on ecological time scales. Gene flow in a population or a community can be mediated by horizontal transfer of mobile genetic elements (Ma et al., 2019; Saak et al., 2020), which could be tracked among lineages using mark-recapture. This technique has been successfully used to determine population dynamics of influenza during host colonization to identify bottlenecking events impacting transmission (Varble et al., 2014).
Spatial organization	The spatial heterogeneity of microbial populations and communities is often ignored when sampling the bulk pool of nucleic acids, proteins, and metabolites in environmental systems. This results in the loss of spatial information that can be used to determine where and how microbial cells interact (Tropini et al., 2017). Combining tools like molecular barcoding with recent advances in fluorescence in situ hybridization could lead to the detection of the spatial organization of over 1000 distinct fluorophores and barcodes in a single sample (Shi et al., 2020). Together, this work can be combined with other emerging single-cell techniques (Sharma & Thaiss, 2020) to determine the rules of engagement across varying taxonomic scales and environments.

microbes can be detected with epifluorescent microscopy to visualize and quantify individual cells (Matthysse et al., 1996).

However, traditional microbial marking methods have drawbacks that violate critical assumptions of mark-recapture. Genetic modifications and selection procedures often affect survivorship and reproduction of marked individuals. It is costly to express fluorescent proteins and export toxic drugs across the cell membrane (Durão et al., 2018; Rang et al., 2003). The energetic requirements to maintain these markers can bias estimates of fitness and alter ecological interactions among taxa (Sugar et al., 2012). Another downside is that natural selection can lead to the loss of costly markers from some or all individuals in a focal population, preventing the reliable recapture of marked organisms (Schlechter et al., 2018).

Molecular barcoding provides a means to overcome some of the challenges that are commonly associated with traditional markers. It was first described almost 20 years ago for practical use in biosecurity and bioremediation (Block et al., 2004) before the technology was in place to attempt such a feat in a high-throughput quantitative manner (Shoemaker et al., 1996). The basic premise of barcoding involves the insertion of an artificial stretch of non-functional DNA with a unique sequence of nucleotides, which can be recaptured from a sample with different quantitative tools (Figure 1C). One important feature is that the DNA barcode is heritable, which enables the barcoded lineage to be tracked across generations. A second important feature is that, when carefully developed, barcodes can serve as effectively neutral markers. A short DNA barcode (~10 bp) can be inserted into a genome at a location that does not disrupt cellular functions or result in any phenotype that might be acted upon by natural selection, such as inside intergenic regions of the genome (Block et al., 2004; Buckley et al., 2012). A final feature of a DNA barcode is that it should be easy to detect and quantify. For example, molecular barcoding can be scaled with only one or a few barcoded strains monitored by quantitative polymerase chain reaction (Buckley et al., 2012) to thousands of barcoded lineages tracked with amplicon sequencing (Jasinska et al., 2020). With the approach of molecular barcoding, the evolutionary ecology of focal population(s) can more easily be studied in systems spanning degrees of complexity. For instance, barcoded strains can be introduced into communities with low versus high diversity to understand how biotic interactions modify microbial responses to environmental conditions (temperature, physical structure, pH, etc.).

Does barcoding meet the mark?—Although it has some advantages over conventional tools, it is important to evaluate whether or not molecular barcoding meets assumptions of mark-recapture. Below, we critically evaluate each of the major assumptions in turn.

1. *The marker is not lost*. One of the key assumptions of mark-recapture is that the marker is not lost during the lifespan of an individual. Similarly, the marker cannot be shared with other individuals of the population. In contrast to traditional mark-recapture, the molecular barcode cloned into a microorganism is vertically inherited over generations. Mutations will inevitably hit a barcoded region over extended periods of time, which may affect recovery. However, for most purposes, barcode insertion is generally stable over time, thus satisfying a major assumption of mark-recapture (Buckley et al., 2012),
2. *The marker is not misread*. In general, barcodes can be distinguished with a high degree of precision and accuracy. Additional steps can be taken to reduce the probability of misidentification. It is possible to engineer barcodes to differ from one another by manipulating the barcode length, nucleotide composition, and/or genomic location. In experiments that use multiple markers, minimizing sequence similarity among barcodes can improve barcode assignment accuracy as de novo mutations and sequencing errors can change nucleotide composition of the barcode (Ali et al., 2021; Buschmann, 2017).
3. *Marking does not influence an individual's performance*. If the barcode insertion has no phenotypic effects, then this assumption should be satisfied. One needs to be careful, however, since tests of barcode neutrality are likely to be performed under simple laboratory conditions (Figure 1C) and may not extend to more complex environmental settings.
4. *Marked and unmarked individuals have an equal chance of being recaptured*. In traditional mark recapture, population size is estimated by the ratio of marked and unmarked individuals. With molecular barcoding, unmarked individuals are excluded from microbial mark-recapture. Therefore, the recapture event will only quantify marked microorganisms. While this represents a departure from traditional mark-recapture, it also allows for more precise and sensitive quantification of barcoded individuals from biologically complex communities (e.g. mammalian gut).
5. *Demographic processes do not influence the population size estimate*. In traditional mark-recapture, the frequency of sampling is minimized so that births, deaths, and migration events do not interfere with population size estimates. With molecular barcoding, population size estimates of microorganisms are generally measured from a pool of individuals likely generated over multiple generations, especially in the laboratory where conditions favour growth and reproduction. However, in natural environments, microbes tend to have generation times that are orders of magnitude longer than

under optimal laboratory conditions (Gibson et al., 2018). Therefore, it may be possible to measure the fate of barcoded individuals within a generation under environmental conditions.

Ethical consideration of releasing barcoded microorganisms—Risks and regulations must be considered before releasing molecularly barcoded microorganisms into the environment (Saravanan et al., 2022). A primary concern is the spread of recombinant DNA to native microorganisms (i.e. horizontal gene transfer of recombinant DNA). In addition, genetically modified organisms can compete with and displace resident taxa, which could disrupt ecosystem processes (Myhr & Traavik, 1999). In order to safeguard against these outcomes, controlled studies are required to evaluate risks (Myhr & Traavik, 1999).

There are several strategies that can be employed to mitigate the risks associated with the release of barcoded microorganisms. Features of molecular barcoding are more appealing than alternative strategies relying on transgenes, such as fluorescent protein gene markers, to track microorganisms in nature. In particular, the design of the barcode may be one of the most important features as it is a small artificial stretch of non-functional DNA and is essentially junk DNA that should not pose any selective benefit for the microbe (Buckley et al., 2012; Levy et al., 2015) nor should it be beneficial to a novel host (i.e. plant, animal, or microbe). In fact, barcoding is proposed as a monitoring tool for genetically modified organisms and their transgenes (EFSA Scientific Committee et al., 2020; Wright et al., 2013). Combining the barcode with a suicide gene would reduce the risk of releasing the recombinant DNA by degrading cellular DNA (Li & Wu, 2009). Similarly, the barcode can be combined with a metabolic dependence on a synthetic metabolite, like a non-standard amino acid that does not exist in the natural environment, to limit population growth (Mandell et al., 2015). Furthermore, the use of biological barriers for containment (e.g. host cell containing a microbe) can limit the release of recombinant DNA by containing the barcode inside the cell (Moon et al., 2010). For example, barcoding intracellular symbionts of animals and plants would limit the release of recombinant DNA to microorganisms outside of the host (Arora & Douglas, 2017; Elston et al., 2022; Maire et al., 2021) and could prove to be a useful strategy combined with genetically modified hosts to limit the spread of vectored diseases and insect pests in medicine and agriculture (Raphael et al., 2014; Romeis et al., 2020). Similarly, encapsulating the barcode into an environmentally resistant cell state, such as an endospore, can further limit the spread of recombinant DNA in the environment (Qian et al., 2020).

In the future, society will be confronted with decisions related to challenges presented by climate change, food security, bioremediation, and emerging pathogens. The use of genetically modified organisms, including barcoded strains, may prompt scientists and governmental entities to reconsider in which cases the benefits of their release outweigh their risks in the face of these challenges.

Societal benefits of barcoding—Microbes are used for a wide variety of purposes like bioremediation of pollutants (Saravanan et al., 2022), probiotics in human health (Gosálbez & Ramón, 2015), and pest management of insects (Amarger, 2002), but each application faces similar challenges. In the laboratory, we can identify and even engineer microbes that are highly efficient at specific functions (e.g. oil degradation). However, when the microbe leaves the confines of the laboratory and is introduced to an ecosystem to execute a desired function, there are selective hurdles that must be overcome to ensure colonization and persistence in the new environment (Mallon et al., 2015). Barcoding would enable one to monitor establishment more easily and determine if modifications are required to make the microbial-mediated strategy more successful in future efforts.

Molecular barcoding could also be used with infectious laboratory strains to monitor their accidental release and dispersal (Block et al., 2004; Buckley et al., 2012). For example, debate among the public, scientists, politicians, and governmental regulatory agents focused on the origins of the COVID-19 pandemic, including a spillover event from an animal reservoir host and release from the laboratory (Maxmen & Mallapaty, 2021). In particular, the ever-growing concern of "lab-leaks" of pathogens, like SARS-CoV-2 (Maxmen & Mallapaty, 2021) and anthrax (Sahl et al., 2016), and genetically modified organisms, such as laboratory strains with knock-in mutations that increase the virulence of deadly pathogens (Dance, 2021; Kaiser, 2022), is under constant oversight to ensure the best policies are in place to reduce chances of release (Achenbach, 2022; Maher, 2012). Barcoding strategies could be developed in laboratory strains of concern to include one or more unique identifiers in the genome. By working with barcoded strains, scientists could abate public and political concerns about the accidental release of pathogens during biosecurity management efforts to determine provenance and evolutionary history of a contagion.

Microbial mark-recapture and beyond—Mark-recapture of microorganisms has the potential to change how scientists track organisms and lineages in environmental, engineered, and host-associated ecosystems. Molecular barcoding can meet most mark-recapture assumptions to address the study of a range of ecological and evolutionary phenomena in microbial science (Box 1) that have been unattainable owing to limitations of traditional approaches (Figure 1).

The technique of molecular barcoding can be used across the microbial tree of life; not only with bacteria, archaea, and microeukaryotes (Abel et al., 2015; Levy et al., 2015) but also with mobile genetic elements like viruses, plasmids, and transposons (Ma et al., 2019; Varble et al., 2014; Wetmore et al., 2015). With current technologies, molecular barcoding can only be used with culturable microorganisms that are amenable to genetic manipulation. However, given rapid advances in novel cultivation strategies (Chaudhary et al., 2019) along with efforts to expand the list of genetically tractable organisms (Yan & Fong, 2017) and development of CRISPR-mediated barcoding strategies (Kalhor et al., 2018; Kebschull & Zador, 2018), microbial mark-recapture should be applicable in biological systems spanning a range of complexity.

ACKNOWLEGEMENT

The authors thank Canan Karakoç, Brent Lehmkuhl, Emmi Mueller, Joy O'Brien, Daniel Schwartz, and Will Shoemaker for stimulating feedback and discussion.

AUTHOR CONTRIBUTIONS

John George McMullen II: Conceptualization (equal); writing – original draft (lead); writing – review and editing (equal). **Jay T. Lennon:** Conceptualization (equal); funding acquisition (lead); writing – review and editing (equal).

FUNDING INFORMATION

This work was funded by the National Science Foundation (DEB-1934554 and DBI-2022049 to Jay T. Lennon), the US Army Research Office Grants (W911NF-14-1-0411 and W911NF-22-1-0014 to Jay T. Lennon), and the National Aeronautics and Space Administration (80NSSC20K0618 to Jay T. Lennon).

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

John George McMullen II https://orcid.org/0000-0002-6675-3983
Jay T. Lennon https://orcid.org/0000-0003-3126-6111

REFERENCES

Abel, S., zur Wiesch, P.A., Chang, H.-H., Davis, B.M., Lipsitch, M. & Waldor, M.K. (2015) STAMP: sequence tag-based analysis of microbial population dynamics. *Nature Methods*, 12, 223–226.

Achenbach, J. (2022) NIH advisers urge tighter oversight of experiments on deadly viruses. *Washington Post*.

Albright, M.B.N., Sevanto, S., Gallegos-Graves, L.V. & Dunbar, J. (2020) Biotic interactions are more important than propagule pressure in microbial community invasions. *MBio*, 11, e02089-20.

Ali, S.A., Mittal, D. & Kaur, G. (2021) In-situ monitoring of xenobiotics using genetically engineered whole-cell-based microbial biosensors: recent advances and outlook. *World Journal of Microbiology and Biotechnology*, 37, 81.

Alseth, E.O., Pursey, E., Luján, A.M., McLeod, I., Rollie, C. & Westra, E.R. (2019) Bacterial biodiversity drives the evolution of CRISPR-based phage resistance. *Nature*, 574, 549–552.

Amarger, N. (2002) Genetically modified bacteria in agriculture. *Biochimie*, 84, 1061–1072.

Arora, A.K. & Douglas, A.E. (2017) Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. *Journal of Insect Physiology*, 103, 10–17.

Barraud, N., Kjelleberg, S. & Rice, S.A. (2015) Dispersal from microbial biofilms. *Microbiology Spectrum*, 3, MB-0015-2014.

Bashey, F. (2015) Within-host competitive interactions as a mechanism for the maintenance of parasite diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370, 20140301.

Block, S.M., Donoho, D., Hwa, T., Joyce, G., Nelson, D., Stearns, T. et al. (2004) *DNA barcodes and watermarks*. McLean, VA: The MITRE Corporation.

Blundell, J.R. & Levy, S.F. (2014) Beyond genome sequencing: lineage tracking with barcodes to study the dynamics of evolution, infection, and cancer. *Genomics*, 104, 417–430.

Briggs, E.L., Baranski, C., Münzer Schaetz, O., Garrison, G., Collazo, J.A. & Youngsteadt, E. (2022) Estimating bee abundance: can mark-recapture methods validate common sampling protocols? *Apidologie*, 53, 10.

Bruger, E.L. & Marx, C.J. (2018) A decade of genome sequencing has revolutionized studies of experimental evolution. *Current Opinion in Microbiology*, 45, 149–155.

Buckley, P., Rivers, B., Katoski, S., Kim, M.H., Kragl, F.J., Broomall, S. et al. (2012) Genetic barcodes for improved environmental tracking of an anthrax simulant. *Applied and Environmental Microbiology*, 78, 8272–8280.

Buschmann, T. (2017) DNABarcodes: an R package for the systematic construction of DNA sample tags. *Bioinformatics*, 33, 920–922.

Cain, A.K., Barquist, L., Goodman, A.L., Paulsen, I.T., Parkhill, J. & van Opijnen, T. (2020) A decade of advances in transposon-insertion sequencing. *Nature Reviews. Genetics*, 21, 526–540.

Chaudhary, D.K., Khulan, A. & Kim, J. (2019) Development of a novel cultivation technique for uncultured soil bacteria. *Scientific Reports*, 9, 6666.

Choudoir, M.J. & DeAngelis, K.M. (2022) A framework for integrating microbial dispersal modes into soil ecosystem ecology. *iScience*, 25, 103887.

Chubiz, L.M., Lee, M.-C., Delaney, N.F. & Marx, C.J. (2012) FREQ-Seq: a rapid, cost-effective, sequencing-based method to determine allele frequencies directly from mixed populations. *PLoS One*, 7, e47959.

Creel, S. & Rosenblatt, E. (2013) Using pedigree reconstruction to estimate population size: genotypes are more than individually unique marks. *Ecology and Evolution*, 3, 1294–1304.

Custer, G.F., Bresciani, L. & Dini-Andreote, F. (2022) Ecological and evolutionary implications of microbial dispersal. *Frontiers in Microbiology*, 13, 855859.

Dance, A. (2021) The shifting sands of "gain-of-function" research. *Nature*, 598, 554–557.

Douglas, A.E. (2018) What will it take to understand the ecology of symbiotic microorganisms? *Environmental Microbiology*, 20, 1920–1924.

Douglas, A.E. (2020) The microbial exometabolome: ecological resource and architect of microbial communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375, 20190250.

Durão, P., Balbontín, R. & Gordo, I. (2018) Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends in Microbiology*, 26, 677–691.

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. et al. (2020) Evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology. *European Food Safety Authority Journal*, 18, e06263.

Ellegaard, K.M. & Engel, P. (2016) Beyond 16 S rRNA community profiling: intra-species diversity in the gut microbiota. *Frontiers in Microbiology*, 7, 1475.

Elston, K.M., Leonard, S.P., Geng, P., Bialik, S.B., Robinson, E. & Barrick, J.E. (2022) Engineering insects from the endosymbiont out. *Trends in Microbiology*, 30, 79–96.

Gibson, B., Wilson, D.J., Feil, E. & Eyre-Walker, A. (2018) The distribution of bacterial doubling times in the wild. *Proceedings of the Royal Society: Biological Sciences*, 285, 20180789.

Gómez, P. & Buckling, A. (2011) Bacteria-phage antagonistic coevolution in soil. *Science*, 332, 106–109.

Gosálbez, L. & Ramón, D. (2015) Probiotics in transition: novel strategies. *Trends in Biotechnology*, 33, 195–196.

Goudie, I.B.J. & Goudie, M. (2007) Who captures the marks for the Petersen estimator? *Journal of the Royal Statistical Society*, 170, 825–839.

Hullahalli, K. & Waldor, M.K. (2021) Pathogen clonal expansion underlies multiorgan dissemination and organ-specific outcomes during murine systemic infection. *eLife*, 10, e70910.

Jasinska, W., Manhart, M., Lerner, J., Gauthier, L., Serohijos, A.W. R. & Bershtein, S. (2020) Chromosomal barcoding of E. coli populations reveals lineage diversity dynamics at high resolution. *Nature Ecology & Evolution*, 4, 437–452.

Kaiser, J. (2022) Was a study that created a hybrid COVID-19 virus too risky? Science.

Kalhor, R., Kalhor, K., Mejia, L., Leeper, K., Graveline, A., Mali, P. et al. (2018) Developmental barcoding of whole mouse via homing CRISPR. *Science*, 361, eaat9804.

Kebschull, J.M. & Zador, A.M. (2018) Cellular barcoding: lineage tracing, screening and beyond. *Nature Methods*, 15, 871–879.

Krebs, C.J. (1999) *Ecological methodology*, 2nd edition. Menlo Park, CA: Benjamin Cummings.

Landete, J.M., Medina, M. & Arqués, J.L. (2016) Fluorescent reporter systems for tracking probiotic lactic acid bacteria and bifidobacteria. *World Journal of Microbiology and Biotechnology*, 32, 119.

Levy, S.F., Blundell, J.R., Venkataram, S., Petrov, D.A., Fisher, D. S. & Sherlock, G. (2015) Quantitative evolutionary dynamics using high-resolution lineage tracking. *Nature*, 519, 181–186.

Li, Q. & Wu, Y.-J. (2009) A fluorescent, genetically engineered microorganism that degrades organophosphates and commits suicide when required. *Applied Microbiology and Biotechnology*, 82, 749–756.

Lindberg, M.S. (2012) A review of designs for capture–mark–recapture studies in discrete time. *Journal für Ornithologie*, 152, 355–370.

Livingston, G., Matias, M., Calcagno, V., Barbera, C., Combe, M., Leibold, M.A. et al. (2012) Competition–colonization dynamics in experimental bacterial metacommunities. *Nature Communications*, 3, 1234.

Ma, X., Liang, H., Cui, X., Liu, Y., Lu, H., Ning, W. et al. (2019) A standard for near-scarless plasmid construction using reusable DNA parts. *Nature Communications*, 10, 3294.

Maher, B. (2012) Bird-flu research: the biosecurity oversight. *Nature*, 485, 431–434.

Maire, J., Blackall, L.L. & van Oppen, M.J.H. (2021) Intracellular bacterial symbionts in corals: challenges and future directions. *Microorganisms*, 9, 2209.

Mallon, C.A., van Elsas, J.D. & Salles, J.F. (2015) Microbial invasions: the process, patterns, and mechanisms. *Trends in Microbiology*, 23, 719–729.

Mandell, D.J., Lajoie, M.J., Mee, M.T., Takeuchi, R., Kuznetsov, G., Norville, J.E. et al. (2015) Biocontainment of genetically modified organisms by synthetic protein design. *Nature*, 518, 55–60.

Matthysse, A.G., Stretton, S., Dandie, C., McClure, N.C. & Goodman, A.E. (1996) Construction of GFP vectors for use in Gram-negative bacteria other than *Escherichia coli*. *FEMS Microbiology Letters*, 145, 87–94.

Maunder, M.N. (2004) Population viability analysis based on combining Bayesian, integrated, and hierarchical analyses. *Acta Oecologica*, 26, 85–94.

Maxmen, A. & Mallapaty, S. (2021) The COVID lab-leak hypothesis: what scientists do and don't know. *Nature*, 594, 313–315.

Mendoza-Suárez, M.A., Geddes, B.A., Sánchez-Cañizares, C., Ramírez-González, R.H., Kirchhelle, C., Jorrin, B. et al. (2020) Optimizing Rhizobium-legume symbioses by simultaneous measurement of rhizobial competitiveness and N_2 fixation in nodules. *Proceedings of the National Academy of Sciences*, 117, 9822–9831.

Moon, H.S., Li, Y. & Stewart, C.N. (2010) Keeping the genie in the bottle: transgene biocontainment by excision in pollen. *Trends in Biotechnology*, 28, 3–8.

Morin, M., Pierce, E.C. & Dutton, R.J. (2018) Changes in the genetic requirements for microbial interactions with increasing community complexity. *eLife*, 7, e37072.

Mutalik, V.K., Adler, B.A., Rishi, H.S., Piya, D., Zhong, C., Koskella, B. et al. (2020) High-throughput mapping of the phage resistance landscape in *E. coli*. *PLoS Biology*, 18, e3000877.

Myhr, T. & Traavik, A.I. (1999) The precautionary principle applied to deliberate release of genetically modified organisms (GMOs). *Microbial Ecology in Health and Disease*, 11, 65–74.

Noto Guillen, M., Rosener, B., Sayin, S. & Mitchell, A. (2021) Assembling stable syntrophic *Escherichia coli* communities by comprehensively identifying beneficiaries of secreted goods. *Cell Systems*, 12, 1064–1078.e7.

Price, M.N., Wetmore, K.M., Waters, R.J., Callaghan, M., Ray, J., Liu, H. et al. (2018) Mutant phenotypes for thousands of bacterial genes of unknown function. *Nature*, 557, 503–509.

Qian, J., Lu, Z., Mancuso, C.P., Jhuang, H.-Y., del Carmen Barajas-Ornelas, R., Boswell, S.A. et al. (2020) Barcoded microbial system for high-resolution object provenance. *Science*, 368, 1135–1140.

Rang, C., Galen, J.E., Kaper, J.B. & Chao, L. (2003) Fitness cost of the green fluorescent protein in gastrointestinal bacteria. *Canadian Journal of Microbiology*, 49, 531–537.

Raphael, K.A., Shearman, D.C., Gilchrist, A.S., Sved, J.A., Morrow, J. L., Sherwin, W.B. et al. (2014) Australian endemic pest tephritids: genetic, molecular and microbial tools for improved Sterile Insect Technique. *BioMed Central Genetics*, 15, S9.

Reyrat, J.-M., Pelicic, V., Gicquel, B. & Rappuoli, R. (1998) Counterselectable markers: untapped tools for bacterial genetics and pathogenesis. *Infection and Immunity*, 66, 4011–4017.

Rillig, M.C., Antonovics, J., Caruso, T., Lehmann, A., Powell, J.R., Veresoglou, S.D. et al. (2015) Interchange of entire communities: microbial community coalescence. *Trends in Ecology & Evolution*, 30, 470–476.

Robinson, D.G., Chen, W., Storey, J.D. & Gresham, D. (2014) Design and analysis of Bar-seq experiments. *G3: Genes, Genomes, Genetics*, 4, 11–18.

Romeis, J., Collatz, J., Glandorf, D.C.M. & Bonsall, M.B. (2020) The value of existing regulatory frameworks for the environmental risk assessment of agricultural pest control using gene drives. *Environmental Science & Policy*, 108, 19–36.

Saak, C.C., Dinh, C.B. & Dutton, R.J. (2020) Experimental approaches to tracking mobile genetic elements in microbial communities. *Federation of European Microbiological Societies Microbiology Reviews*, 44, 606–630.

Sahl, J.W., Pearson, T., Okinaka, R., Schupp, J.M., Gillece, J.D., Heaton, H. et al. (2016) A *Bacillus anthracis* genome sequence

from the Sverdlovsk 1979 autopsy specimens. *MBio*, 7, e01501–e01516.

Sandercock, B.K. (2003) Estimation of survival rates for wader populations: a review of mark-recapture methods. *Wader Study Group Bulletin*, 13, 163–174.

Saravanan, A., Kumar, P.S., Ramesh, B. & Srinivasan, S. (2022) Removal of toxic heavy metals using genetically engineered microbes: molecular tools, risk assessment and management strategies. *Chemosphere*, 298, 134341.

Schlechter, R.O., Jun, H., Bernach, M., Oso, S., Boyd, E., Muñoz-Lintz, D.A. et al. (2018) Chromatic bacteria—a broad host-range plasmid and chromosomal insertion toolbox for fluorescent protein expression in bacteria. *Frontiers in Microbiology*, 9, 3052.

Sharma, P.V. & Thaiss, C.A. (2020) Host–microbiome interactions in the era of single-cell biology. *Frontiers in Cellular and Infection Microbiology*, 10, 569070.

Shen, D., Langenheder, S. & Jürgens, K. (2018) Dispersal modifies the diversity and composition of active bacterial communities in response to a salinity disturbance. *Frontiers in Microbiology*, 9, 2188.

Shi, H., Shi, Q., Grodner, B., Lenz, J.S., Zipfel, W.R., Brito, I.L. et al. (2020) Highly multiplexed spatial mapping of microbial communities. *Nature*, 588, 676–681.

Shoemaker, D.D., Lashkari, D.A., Morris, D., Mittmann, M. & Davis, R.W. (1996) Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy. *Nature Genetics*, 14, 450–456.

Sugar, D.R., Murfin, K.E., Chaston, J.M., Andersen, A.W., Richards, G.R., deLéon, L. et al. (2012) Phenotypic variation and host interactions of *Xenorhabdus bovienii* SS-2004, the entomopathogenic symbiont of *Steinernema jollieti* nematodes. *Environmental Microbiology*, 14, 924–939.

Tropini, C., Earle, K.A., Huang, K.C. & Sonnenburg, J.L. (2017) The gut microbiome: connecting spatial organization to function. *Cell Host & Microbe*, 21, 433–442.

Vannette, R.L. & Fukami, T. (2017) Dispersal enhances beta diversity in nectar microbes. *Ecology Letters*, 20, 901–910.

Varble, A., Albrecht, R.A., Backes, S., Crumiller, M., Bouvier, N.M., Sachs, D. et al. (2014) Influenza A virus transmission bottlenecks are defined by infection route and recipient host. *Cell Host & Microbe*, 16, 691–700.

Venkataram, S., Dunn, B., Li, Y., Agarwala, A., Chang, J., Ebel, E.R. et al. (2016) Development of a comprehensive genotype-to-fitness map of adaptation-driving mutations in yeast. *Cell*, 166, 1585–1596.e22.

Wetmore, K.M., Price, M.N., Waters, R.J., Lamson, J.S., He, J., Hoover, C.A. et al. (2015) Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons. *MBio*, 6, e00306–e00315.

Wright, E.S. & Vetsigian, K.H. (2019) Stochastic exits from dormancy give rise to heavy-tailed distributions of descendants in bacterial populations. *Molecular Ecology*, 28, 3915–3928.

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

Yan, Q. & Fong, S.S. (2017) Challenges and advances for genetic engineering of non-model bacteria and uses in consolidated bioprocessing. *Frontiers in Microbiology*, 8, 2060.

How to cite this article: McMullen, J.G. II & Lennon, J.T. (2023) Mark-recapture of microorganisms. *Environmental Microbiology*, 25(1), 150–157. Available from: https://doi.org/10.1111/1462-2920.16267