GENETIC ENGINEERING TECHNOLOGIES AND DETECTION OF GENE EDITING

Determining The Prevalence Of Crispr Off-target Effects In Bacteria: Can We Detect If An Organism Has Been Genetically Engineered?

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Tools for genetic engineering have expanded rapidly in recent years, driven in large part by the development of CRISPR gene editing. This powerful, dual-use technology has been used to modify a variety of organisms, from bacteria to humans. While CRISPR has been touted for its simplicity, specificity, and versatility, CRISPR off-target effects—unintended modifications elsewhere in the genome—have been reported. Extensive work has gone into understanding these off-target effects in higher organisms due to the potential ramifications when applying CRISPR to human gene therapy, but this area has remained relatively unexplored in bacteria. As the barriers to genome editing in diverse bacterial species continue to drop at an accelerated rate, there is a growing need to be able to detect genetically-modified organisms, especially those created using techniques like CRISPR that lack traditional markers at the point of editing.

Here, we describe work to understand the off-target effects of CRISPR editing in bacteria as a potential method to identify signatures of engineered threats. We developed an algorithm to identify putative neutral insertion points in bacterial genomes, and applied this algorithm to 10 common synthetic biology chassis organisms. The sites identified in E. coli were experimentally validated and used to construct novel selection strains with engineered on- and off-target sites to aid in quantifying potentially rare off-target effects in vivo. In parallel, we applied an in vitro sequencing approach to assess off-target effects using purified molecular components. This knowledge of rates and identities of signatures of CRISPR editing in bacteria has the potential to enable detection of CRISPR-engineered threats and inform software tools being developed to identify genetically-modified organisms.

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