THREAT AGENT DEFEAT MODELING AND TESTING USING WMD SIMULANTS

CRISPR-Cas9-mediated Barcode Insertion Into Bacillus Thuringiensis For Surrogate Tracking

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The use of surrogate organisms can make it possible to conduct research on pathogens safely and in a broader set of conditions. Being able to differentiate between the surrogates used in the experiments and background contamination as well as between different experiments will further improve research efforts. One effective approach is to introduce unique genetic barcodes into the surrogate genome and track their presence using quantitative polymerase chain reaction (qPCR). In this report, we utilized the CRISPR-Cas9 methodology, which employs a single plasmid and transformation step, to insert five distinct barcodes into Bacillus thuringiensis, a well-established surrogate for Bacillus anthracis when Risk Group 1 organisms are needed. We subsequently developed qPCR assays for barcode detection and successfully demonstrated the stability of the barcodes within the genome through five cycles of sporulation and germination. Additionally, we conducted whole-genome sequencing on these modified strains and analyzed 187 potential Cas9 off-target sites. We found no correlation between the mutations observed in the engineered strains and the predicted off-target sites, suggesting this genome engineering strategy did not directly result in off-target mutations in the genome. This simple approach has the potential to streamline the creation of barcoded B. thuringiensis strains for use in future surrogate studies.