CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Workflow for High-Throughput Screening of Enzyme Mutant Libraries Using Matrix-assisted Laser Desorption/Ionization Mass Spectrometry Analysis of E. coli Colonies

Background/Objective

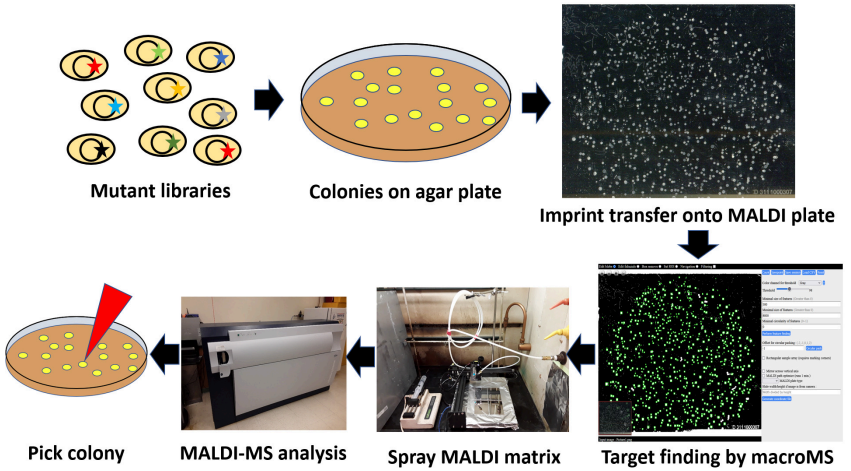
High-throughput molecular screening of microbial colonies and DNA libraries is critical to enable applications like directed evolution, functional genomics, microbial identification, and engineering microbial strains to produce high-value molecules. A promising complementary chemical screening approach is measuring products directly from microbial colonies via optically guided matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), which bypasses liquid culture preparation steps and takes approximately two seconds per sample. We describe a protocol combining a dedicated informatics pipeline and a sample preparation method that can process >3,000 colonies in under two hours.

Approach

- Microbial cells containing a mutant library were grown in agar colonies.
- Colonies were imprinted onto a MALDI target plate using a filter paper intermediate.
- An image of the MALDI target plate was analyzed by custom software, macroMS, to map individual colony locations and direct subsequent analysis of selected colonies.
- MALDI-MS analysis of the selected colonies was performed, and colonies showing the desired product profiles were found by data analysis and picked for downstream analysis.

Results

- This workflow screens thousands of colonies per day without requiring additional automation.
- The protocol was validated by two published projects, one modifying substrate specificity of thioesterase and one modifying regioselectivity of desaturase.



Overview of the MALDI-MS analysis pipeline of microbial colonies for screening mutant libraries.

Significance/Impacts

This workflow, with wide chemical coverage and high sensitivity, can efficiently screen mutant libraries for modifying a wide variety of enzymes or metabolic pathways that form compounds on colonies that are detectable by MALDI-MS.

Choe et al. 2023. "Workflow for High-Throughput Screening of Enzyme Mutant Libraries Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Analysis of *Escherichia coli* Colonies." *Bio-Protocol*. DOI: 10.21769/BioProtoc.4862.