

# High Throughput Screening of Mutant Libraries for Producing Medium Chain Fatty Acids Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Analysis of Microbial Colonies

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**Project Goals:** The creation of a mass spectrometry workflow for high throughput screening of microbial colonies to produce medium chain fatty acids and medium chain fatty alcohols.

## Abstract

Fatty acids and fatty alcohols are value-added compounds used as industrial chemicals and biofuels. There is an unmet need for medium-chain variants of these compounds because of a lack of supply and their greater utility. One issue slowing biocatalyst research to produce enhanced organisms for their production is the lack of high throughput analytical screening methods. We developed a high throughput mass spectrometry (MS) workflow for screening up to 10,000 mutants per day. A custom software suite<sup>1</sup> and a unique sample preparation workflow<sup>2</sup> enable the high throughput workflow. The workflow tested microbial colonies grown on Petri dish instead of liquid culture, greatly simplifying and accelerating the chemical screen for the compounds. Random mutant library of several thioesterases were screened, revealing enzyme variants showing modified substrate specificity towards producing medium-chain fatty acids<sup>3</sup>. The workflow can be applied to screen projects for a wide variety of compounds that are detectable by matrix-assisted laser desorption / ionization MS, and the workflow is easily adaptable by other labs due to the simplicity of the required experimental tools.

## Publications

<sup>1</sup> Choe, K., Xue, P., Zhao, H., & Sweedler, J. V. (2021). maroMS: Image-guided analysis of random objects by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 32(5), 1180–1188. <https://doi.org/10.1021/jasms.1c00013>

<sup>2</sup> Choe, K., Sweedler, J. V. Phenotyping by Testing Microbial Colonies Using Image Guided Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry. *Bio-protocol (in preparation)*

<sup>3</sup> Jindra, M., Choe, K., Chowdhury, R., Kong, R., Ghaffari, G., Sweedler, J.V., Maranas, C.D.,

Pfleger, B.F. Achieving exclusive chain-length acyl-ACP thioesterase selectivity with a novel colony screening technique. *Metabolic engineering (in preparation)*

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