

# Revealing Mitotype-Specific Lipid Profiles in Plant Cells via Targeted Mitochondrial Isolation

Mario Waespy<sup>1</sup>; [Magdalene Reinkensmeier](#)<sup>2</sup>; Viola Jeck<sup>2</sup>, Aiko Barsch<sup>2</sup>; Stefanie Wernisch<sup>2</sup>; Markus Lübeck<sup>2</sup>; Matthew Lewis<sup>2</sup>; Rita Groß-Hardt<sup>1</sup>

<sup>1</sup>University of Bremen, Bremen, Germany; <sup>2</sup>Bruker Daltonics GmbH & Co.KG, Bremen, Germany

## Short abstract

Mitochondria are essential organelles sustaining energy metabolism and signaling within cells. While traditionally viewed as a homogeneous network, recent evidence highlights substantial diversity between and within cells. To investigate the molecular basis of this diversity, we combined mRACE, a method for cell-type-specific isolation of mitochondria in *Arabidopsis thaliana*, with 4D-lipidomics profiling. Temperature-dependent distinct lipid signatures emerged between mesophyll and guard cell mitochondria, revealing cell-type-specific lipid remodeling as a new dimension of mitochondrial specialization in plants. Our results highlight the power of selective isolation combined with advanced mass spectrometry for elucidating organelle diversity.

## Introduction

Mitochondria are dynamic hubs of energy production, metabolic regulation, and signaling. Their function enables growth and survival across organisms. Traditionally viewed as part of relatively homogenous network, recent studies highlight the concept of mitochondrial diversity between and within cells, suggesting a division of labor among distinct mitochondrial subtypes (“mitotypes”). The molecular basis of this diversity, especially lipid composition, represents a radically new concept in molecular biology.

## Methods

To explore mitochondrial diversity, we established mRACE, a method for rapid cell-type-specific isolation of mitochondria using a chimeric protein (MTF) that facilitates targeting, visualization, and biotinylation of mitochondria [Hater et al., *Physiol. Plant.*, 2025]. Cell type specific promoter enable the expression of MTF and a biotin ligase in specific cell

types, while selective isolation was achieved via biotin-streptavidin binding, allowing for cell-type-specific analysis of mitochondria. We applied mRACE to isolate mitochondria from mesophyll cells, specialized for photosynthesis, and guard cells, which regulate stomatal aperture and gas exchange, present in leaves of *A. thaliana* grown under different temperatures. Isolated mitochondria underwent non-targeted lipidomics using LC-MS/MS with 4D-TIMS-PASEF acquisition (Bruker Daltonics). Lipidomics data were annotated and statistically evaluated with MetaboScape 2026 (Bruker Daltonics).

## **Results**

We analyzed gene activity profiles for five differentially enriched factors, finding that four promoter-reporter constructs exhibited differential activity, consistent with mass spectrometry data. Lipidomic profiling revealed distinct lipid signatures between mesophyll and guard cell mitochondria and across different temperature treatments, indicating cell-type-specific lipid remodeling. Differential enrichment of lipid classes reflected unique metabolic and respiratory demands of each cell type.

## **Conclusion**

Our study uncovers a new layer of molecular diversity in plants. Combining mRACE with 4D-lipidomics provides a framework to dissect mitochondrial diversity at high resolution: Lipid composition and ratio is temperature dependent and not uniform in mitochondria with different origin in plant but adapts to cellular identity and environment. Beyond plant biology, these approaches offer broad applicability for studying mitochondrial function in diverse systems, laying foundations for biomarker discovery and advanced tools to evaluate physiological performance and health.