Docket #: S19-301

Methods for Isolating Cell Types and RNA from Fixed Plant Tissue with Increased Yield and Stability

A novel method for dissociating plant tissues for biochemical and genomic analysis that accommodates harsher digestion conditions with increased tissue dissociation efficiency and quality of isolated RNA. Unlike conventional cell-wall digesting enzymes, this dual part process both increases the yield of dissociated cells and preserves isolated RNA. Part one of the method fixates of plant tissue for digestion, releasing up to 9x more cells than solution methods while retaining their morphology and stability. Harsher digestion conditions can also be used with fixation allowing for more tissue types. The second component of this method overcomes degradation of RNA by digestion enzymes. The process for producing Rnase-free digesting enzymes results in 5% RNA degradation per minute as compared to ~40% with the original enzymes.

Stage of Research

Proof of concept

Applications

- · Isolation of specific plant cell types
- RNA sequencing
- Proteomics
- Genome sequencing
- DNA methylation analysis

Advantages

- Dissociation of previously inaccessible tissues and cell types
- Increased stability and maintained morphology for easier identification of cell types
 - 80-100% of cells dissociated vs 1% using unfixed tissue
- High quality RNA sequencing including single cell

Patents

• Published Application: 20210285852

• Issued: <u>11,519,831 (USA)</u>

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