



## Cell-Free System for Combinatorial Discovery of Enzymes Capable of Transforming Biomass for Biofuels

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**WARF: P08301US02**

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**The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a cell-free system that can be used to discover and produce target polypeptides capable of transforming biomass into fermentable sugars for use in the efficient production of biofuels.**

### Overview

Biofuels produced from biomass provide a promising alternative to fossil fuels. Biomass is an inexpensive, readily available and renewable resource. However, the process of converting biomass into biofuels is difficult and costly. Biomass consists of insoluble polysaccharides such as cellulose that are difficult to break down into fermentable sugars like glucose.

Many combinations of enzymes and proteins that naturally deconstruct cellulose and other biomass components have been identified, but efficient methods of generating target polypeptides that can be used in the production of biofuels from biomass have not been developed.

### The Invention

UW-Madison researchers have developed compositions and methods that expand the ability to make, express and identify target polypeptides, including enzymes capable of enhancing the deconstruction of biomass into fermentable sugars.

This approach uses a cell-free system to express enzymes and other polypeptides in a combinatorial manner. Because the system is cell-free, the enzymes can be assayed without intermediate cloning steps or purification of the protein products. This system also is more reliable than conventional methods for analyzing biomass transformation because it does not utilize living systems, which could rapidly consume soluble sugars.

Specifically, the system comprises a cell-free extract for synthesizing the target polypeptide, a nucleotide sequence that encodes a fusion protein containing a cohesion domain and a biomass binding domain, and a nucleotide sequence that encodes a second fusion protein containing a dockerin domain and a polypeptide capable of catalyzing biomass transformation. The system also may include additional polypeptides and fusion proteins. The target polypeptides may be synthesized in the presence of different types of biomass to determine their effects on biomass deconstruction.

### Applications

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- Facilitates high throughput, combinatorial analysis of existing, newly discovered and engineered versions of enzymes capable of cellulose degradation
- Directly assesses biomass deconstruction through the ability to incorporate this material into assays
- Identifies enzymes capable of enhancing the degradation of different types of biomass
- Allows target genes to be evaluated in multiple expression systems and multiple classes of enzyme architectures
- Offers increased reliability over non-analytical determinations of biomass transformation due to lack of competing cellular reactions.
- Quantitative detection and product analysis can be performed without obtaining purified preparations of target polypeptides.
- Capable of determining pH, ionic strength, solvent and thermal stability of target polypeptides
- Enables rapid evaluation of the known natural biological contexts for biomass deconstruction
- Allows testing of target polypeptides from thermophilic as well as temperate environments
- Suitable for many biomass types, including cellulose, hemicelluloses, lignin, pectin, starch and chitin

## Additional Information

### For More Information About the Inventors

- [Brian Fox](#)

### Tech Fields

- [Clean Technology.: Biobased & renewable chemicals & fuels](#)

For current licensing status, please contact Jennifer Gottwald at [jennifer@warf.org](mailto:jennifer@warf.org) or 608-960-9854

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