

## Thermostable GlowTag (YFAST)

A new way to make enzymes tougher to survive and thrive at high temperatures

### Value Proposition

Scientists used a heat-loving microorganism called *Parageobacillus thermoglucosidasius* as a testing ground and created a modified version of a fluorogenic protein called YFAST that glows only when it folds correctly. The modified version called hsFAST is stable under heat and by attaching this glowing tag to other enzymes, we can quickly see which ones are working and stable just by checking if they glow.

### Technology Readiness Level 2-3

### IP Information

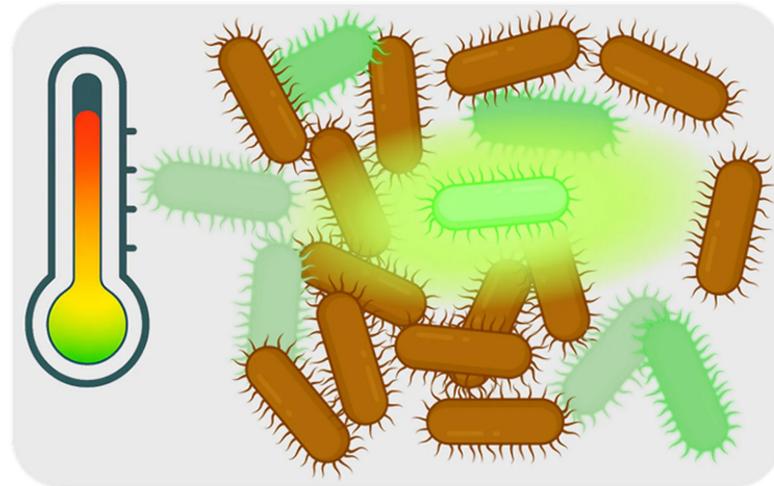
U.S. Patent Pending 19/284504

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### Overview

The enzyme engineering industry faces significant challenges, particularly in developing enzymes that can function reliably at high temperatures, which are common in industrial processes such as biofuel production, waste degradation and chemical manufacturing. Most available enzymes come from mesophilic organisms that degrade and become non-functional under heat, while naturally thermostable enzymes from thermophiles are rare, difficult to work with and limited in diversity. Additionally, current screening methods for identifying heat-resistant enzymes are slow, error-prone and produce many false positives, making the development process inefficient.



This illustration shows how a fluorogenic protein developed at Los Alamos makes its bacterial host glow green when the protein is produced properly, even at high temperatures. Credit to: Sang-Min Shin, LANL, created with BioRender.com

### Advantages

- Enables rapid, high-throughput screening at high temperatures.
- Introduces a thermostable fluorogenic reporter that provides an optical response that correlates to the quantity of the protein it is fused to.
- Improves screening accuracy by using direct selection rather than elimination-based methods, reducing false positives.
- Expands access to thermostable enzymes by allowing common mesophilic enzymes to be engineered for heat resistance.
- Allows for screening and optimization of proteins from thermophilic sources.
- Reduces storage and operational costs by producing enzymes that remain stable without refrigeration.

### Technology Description

This innovation introduces a novel high-throughput screening platform that uses a thermophilic organism (*Parageobacillus thermoglucosidasius*) combined with a specially engineered thermostable

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## Technology Description continued

fluorogenic reporter protein (hsFAST). This system enables scalable selection of enzyme variants that remain stable and functional at elevated temperatures. By allowing mesophilic enzymes to be re-engineered for high-heat environments, reducing reliance on cold storage, and streamlining the screening process with direct fluorescence-based selection, this technology overcomes key bottlenecks in enzyme development and opens the door to more robust, cost-effective and versatile enzymes for industrial and environmental applications.

## Market Applications

- Biofuels
- Waste Management
- Chemical and Pharmaceutical Manufacturing
- Food and Beverage Processing
- Textiles and Detergents

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**Contrary to some other thermostable fluorescent reporters such as TGP (thermal green protein, also developed at the Lab), the new reporter hsFAST can function in an oxygen-free environment, a characteristic highly relevant for applications in thermophiles, which mostly exist in anaerobic conditions.**

**Read the paper: <https://pubs.acs.org/doi/full/10.1021/acssynbio.5c00573>**