#A039

# Future Fields EntoEngine™

## Revolutionizing Recombinant Protein Production

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FUTURE FIELDS

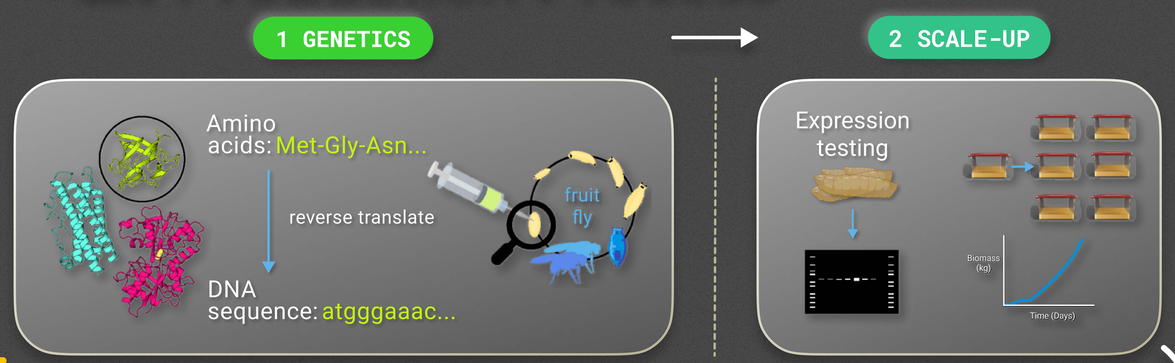
## 1. Introduction

Future Fields introduces the **EntoEngine™**, a breakthrough ***bioreactor-independent platform*** utilizing ***Drosophila melanogaster*** for scalable and robust recombinant protein production.

The expansive genetic toolkit for *Drosophila* allows for genetically stable, tissue-specific, and inducible expression to produce high-quality, bioactive proteins. Illustrated through a case study, our system seamlessly scales and produces functional protein with appropriate PTMs. This innovative approach has the potential to overcome recombinant protein expression difficulties associated with conventional systems.

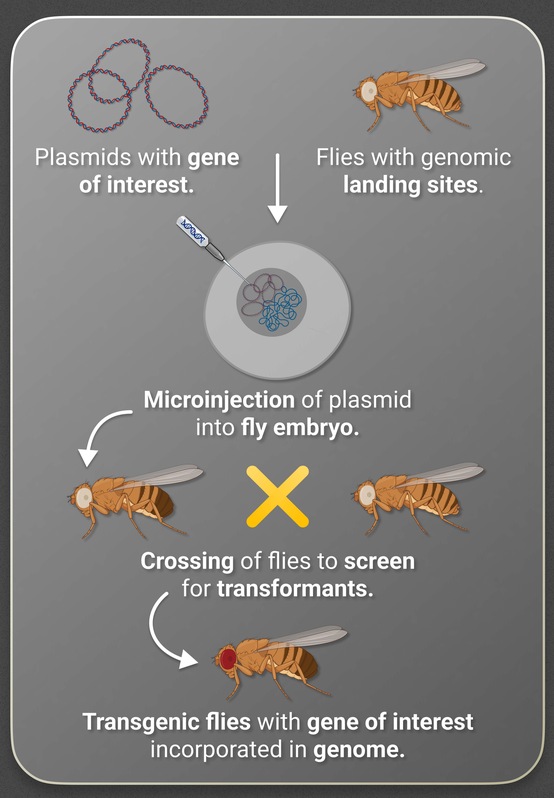
1. Tissue-specific expression
2. Post-translational modification
3. Stable expression
4. Modular scalability

## 2. Production Process

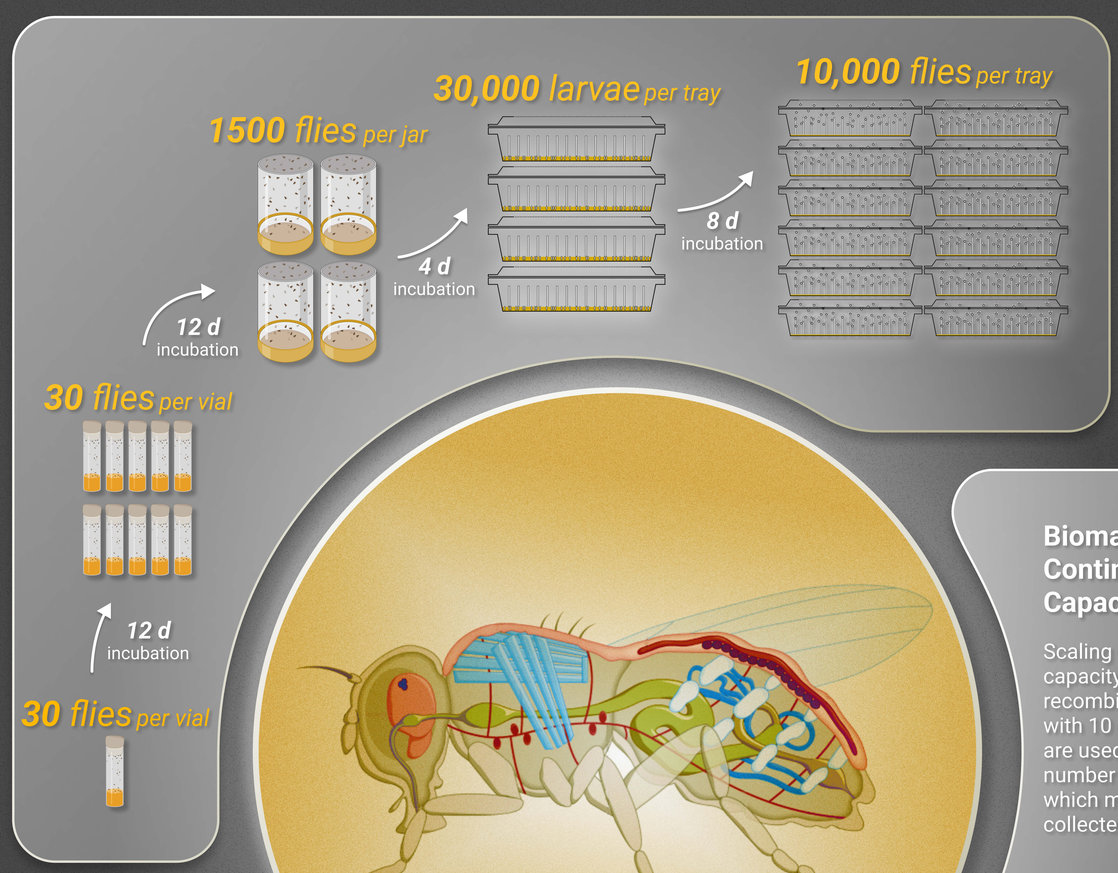


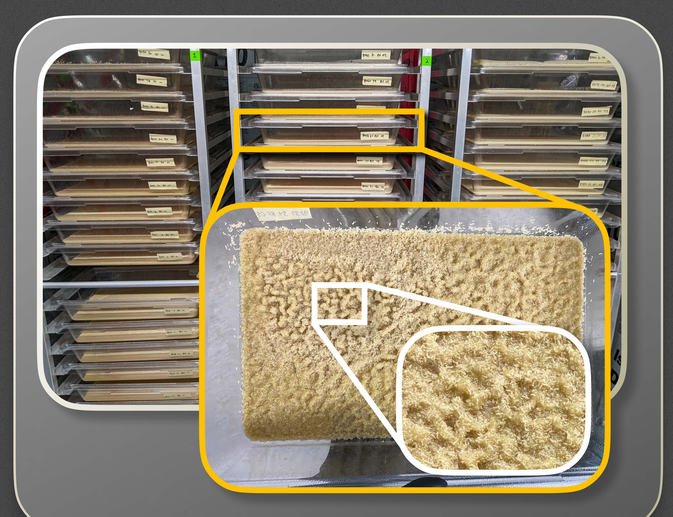

## 3. The EntoEngine™

### 3.1 Genetic Stability




### 3.2 Biomass Scale-up



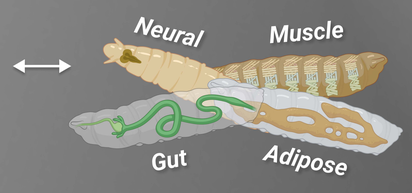





### 3.3 Tissue-specific Expression

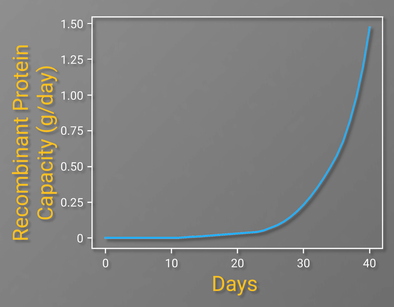
Flies have tissues **analogous** to most human organs.

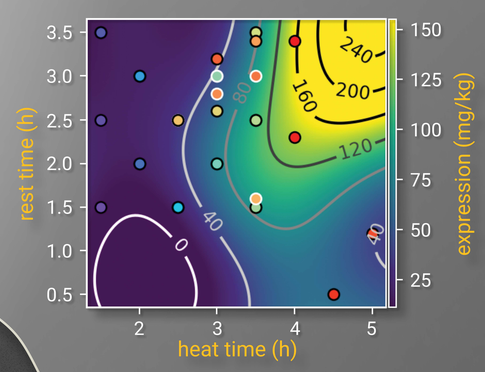
Proteins expressible in **many** different cell types.




### Biomass Scaling and Continuous Production Capacity

Scaling graph of production capacity of purified recombinant protein. Starting with 10 g of flies, their progeny are used to multiply the number of flies laying eggs, which mature into larvae and collected for biomass.




### Expression Level Optimization

Utilizing Design of Experiments (DOE) and Machine Learning, expression levels can be optimized by selecting different temperatures, heat times and rest times.

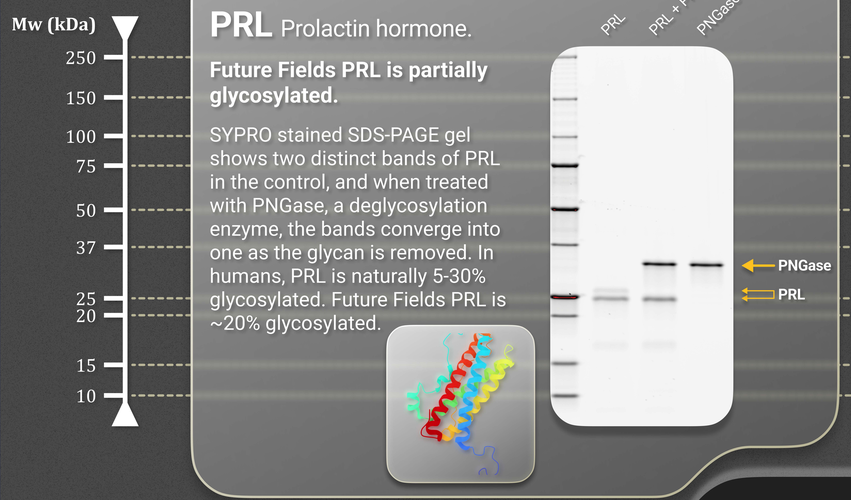
## 4. Case Studies

### PTMs

**PRL** Prolactin hormone.

**Future Fields PRL is partially glycosylated.**

SYPRO stained SDS-PAGE gel shows two distinct bands of PRL in the control, and when treated with PNGase, a deglycosylation enzyme, the bands converge into one as the glycan is removed. In humans, PRL is naturally 5-30% glycosylated. Future Fields PRL is ~20% glycosylated.

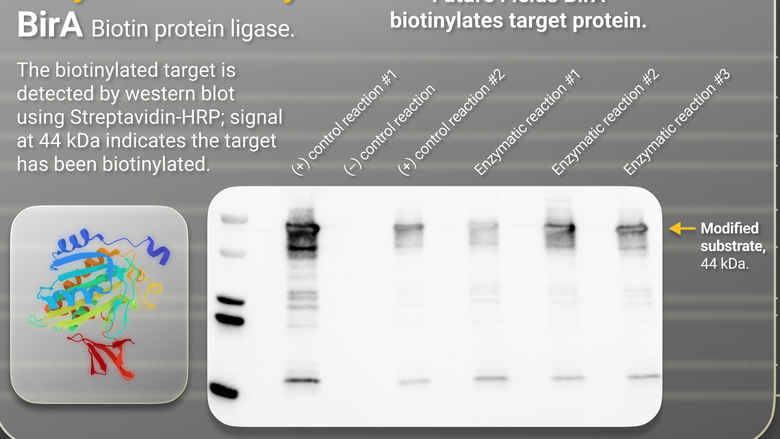



### Enzymatic Activity

**BirA** Biotin protein ligase.

The biotinylated target is detected by western blot using Streptavidin-HRP; signal at 44 kDa indicates the target has been biotinylated.

**Future Fields BirA biotinylates target protein.**



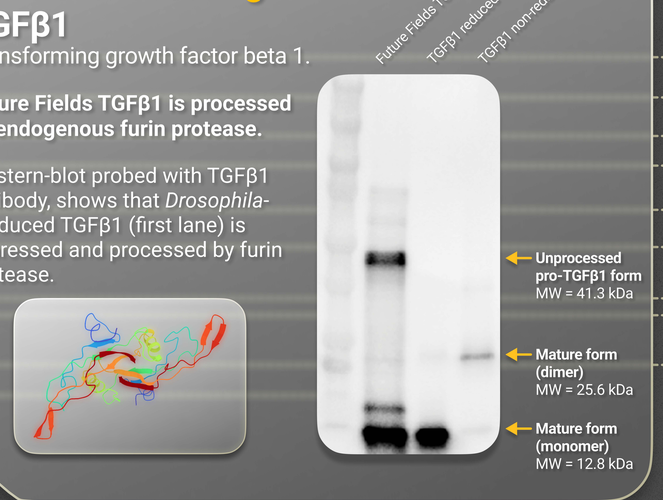

### Cellular Processing

**TGFβ1**

Transforming growth factor beta 1.

**Future Fields TGFβ1 is processed by endogenous furin protease.**

Western-blot probed with TGFβ1 antibody, shows that *Drosophila*-produced TGFβ1 (first lane) is expressed and processed by furin protease.

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