Tools for Next-Generation Pichia Engineering
Technology #16982

Applications

This technology allows rapid genome engineering and inducible protein expression in the yeast *Komagataella pastoris* (*Pichia pastoris*).

Problem Addressed

The advent of protein-based therapeutics has led to an increased need for large scale production of recombinant proteins. One of the widely used yeast species for commercial protein production is *Pichia pastoris*. Protein production using eukaryotes such as yeast allows proper disulfide bond formation and glycosylation, which is not possible in bacteria such as *E.coli*. Additionally, *Pichia pastoris* has several advantages over the yeast *S. cerevisiae* for protein production including growth in minimal medium, the ability to use methanol as a carbon source, and the ability to grow at very high density, all of which lead to more efficient protein production. However, the molecular toolkit for *Pichia pastoris* has lagged significantly behind those available in *E.coli* and *S. cerevisiae*. The inventors of this technology have expanded the *Pichia pastoris* toolkit to include recombinase-based site-specific genome engineering and an inducible expression system to drive high levels of recombinant protein expression.

Technology

The first new tool these Inventors have developed is a recombinase-based method of genome engineering. This technology utilizes strains of *Pichia pastoris* in which an attP sequence has been inserted into a defined location in the *Pichia pastoris* genome. The two vector components of this tool are a recombinase vector, which encodes an integrase enzyme, and a transfer vector containing the DNA sequence of the protein to be produced and a flanking attB sequence. When the recombinase vector and transfer vector are electroporated into *Pichia pastoris* strains containing an attP “landing site” the recombinase enzyme catalyzes the sequence-specific integration of the transfer vector at this site. This is a more efficient than current *Pichia pastoris* genome engineering techniques because all transformants contain the desired integration, integration copy number is controlled, very large constructs can easily be integrated, and the integration is scarless.

The second new tool is a technique to rapidly induce high levels of recombinant protein expression in *Pichia pastoris*. Currently, the major commercially utilized inducible promoter for *Pichia pastoris* is methanol inducible. However, this is not easily scaled and there is a significant level of background expression without induction. This technology utilizes a zinc-finger (ZF) DNA binding domain fused to a fragment of the human estrogen receptor and an effector domain that enhances transcription. Under normal growth conditions, this fusion protein is sequestered in the cytoplasm. When beta-estradiol is added to the *Pichia pastoris* culture, the fusion protein translocates to the nucleus, where the ZF DNA binding domain binds specifically to activate expression of a recombinant protein of interest. This is an improvement over traditional protein expression systems because the inducible system decouples culture growth and product accumulation, which allows initial biomass gain without the potential of product accumulation slowing growth or affecting culture viability.
Additionally, this system is both safer and more sensitive than traditional methanol inducible systems.

**Advantages**

- Genome engineering tool for *Pichia pastoris* that allows rapid, efficient integration of any size of plasmid DNA
- Inducible expression system for *Pichia pastoris* that drives high levels of recombinant protein expression and decouples biomass gain from product formation

**Categories For This Invention:**

- Life Sciences
- Biotechnology
- Industrial/Energy
- Other (Biotechnology)
- Research Tools
- Expression Systems
- Protein & Protein Chemistry
- Vector & Plasmid

**Intellectual Property:**

Tools for next-generation pichia engineering
PCT 2018-013551

**Inventors:**

Timothy Lu
Pablo Perez-Pinera

**Publications:**

Synthetic biology and microbioreactor platforms for programmable production of biologics at the point-of-care
Nature Communications
2016