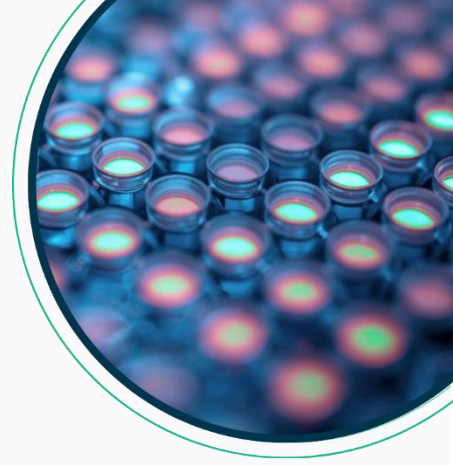




Alagene Pichia Screen™

Overcoming Screening Bottlenecks in *Pichia pastoris*: A *High-Throughput Episomal Platform for Large-Scale Variant Discovery in Pichia pastoris*

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Technical Brief

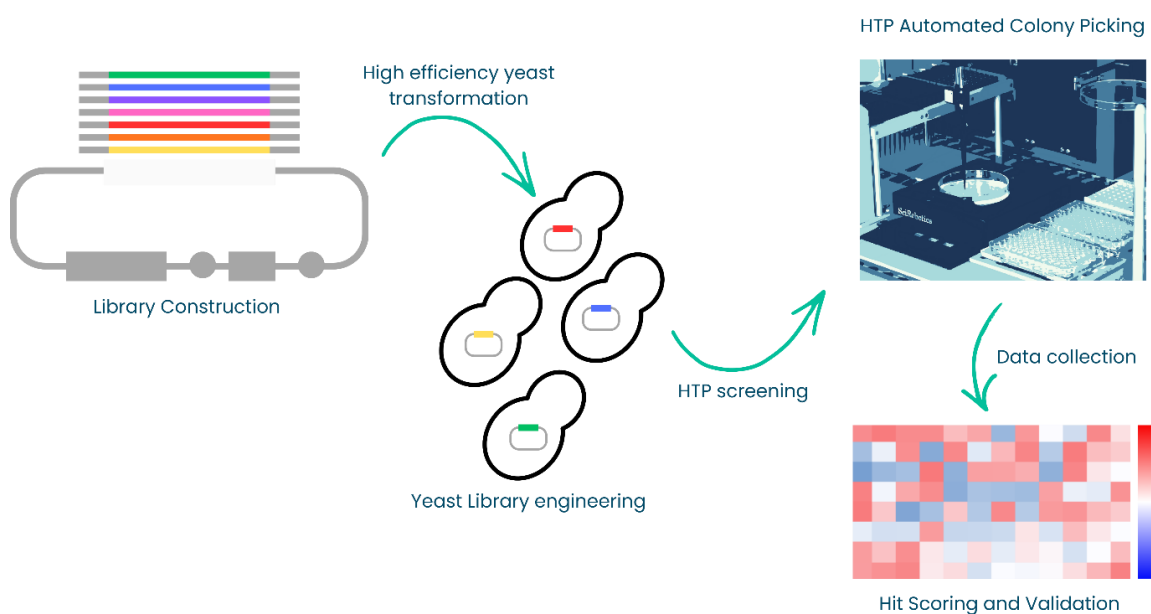
Accelerating Variant Screening in *Pichia pastoris*

One of the primary limitations in engineering *Pichia pastoris* for recombinant protein production is the inability to efficiently screen large genetic libraries.

While *Pichia* is widely used as a production host, traditional expression systems significantly constrain early-stage strain engineering and protein optimization workflows. Most established *Pichia* expression systems rely on chromosomal integration of expression constructs. While this approach provides stability for production strains, it creates significant bottlenecks during early-stage discovery and optimization workflows.

Integration-based systems typically exhibit variability in integration site and gene copy number, requiring extensive clone screening to identify high-performing strains. In addition, transformation efficiencies are often limited, restricting the size of variant libraries that can realistically be evaluated. These constraints slow down the design–build–test cycles required for modern protein engineering and synthetic biology workflows.

To address these limitations, Alagene has developed Alagene *Pichia* Screen™, an episomal plasmid-based expression platform specifically designed to enable rapid and scalable screening of large genetic libraries in *Pichia pastoris*, increasing the screening landscape from a few individual variants to hundreds or thousands of variants in a single screen. By allowing expression constructs to be maintained episomally during the screening stage, the system eliminates the need for genomic integration during early evaluation steps and significantly accelerates strain development workflows.



(1) Alagene *Pichia pastoris* library screening platform. Schematic overview of the Alagene *Pichia pastoris* screening workflow, highlighting high-efficiency transformation of large variant libraries, high-throughput screening, and selection of top candidates for development of stable production strains.

High Transformation Efficiency Enables Screening of Large Libraries

A central advantage of the episomal platform is its markedly higher transformation efficiency compared to traditional integrative expression systems. Higher transformation efficiency directly translates into the ability to introduce larger genetic libraries into *Pichia* populations.

This capability is critical for modern protein engineering approaches, where identifying optimal constructs often requires extensive variants screening of. The Alagene *Pichia* Screen™ episomal system enables the transformation and evaluation of large variant libraries within a single experimental cycle, dramatically expanding the design space that can be explored.

The ability to transform large numbers of cells with diverse constructs allows researchers to move beyond small-scale clone testing and instead perform library-level screening experiments, increasing the probability of identifying high-performing designs. As a result, development timelines can be significantly shortened, and optimization strategies that would otherwise be impractical in *Pichia* become feasible.

Faster Design–Build–Test Cycles

Because episomal constructs do not require chromosomal integration, the time required to evaluate new genetic designs is substantially reduced. Transformation and screening can be performed directly following plasmid construction, enabling rapid evaluation of new variants.

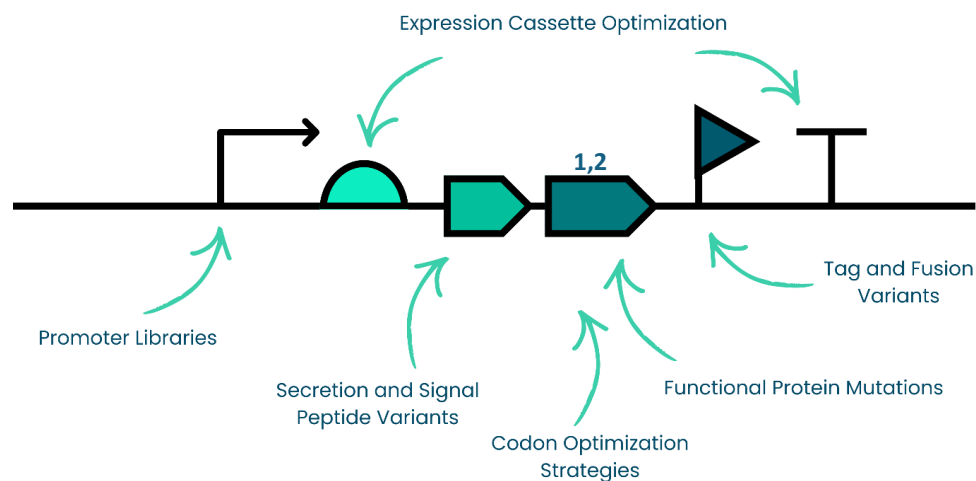
By eliminating integration and clone verification steps, this workflow enables efficient design–build–test cycles, allowing researchers to iterate across large variant sets and rapidly identify high-performing constructs.

Applications in Multi-Dimensional Expression Optimization

The Alagene episomal screening platform enables evaluation of a wide range of genetic design variables that influence recombinant protein expression and performance in *Pichia pastoris*. Because large variant libraries can be transformed and screened efficiently, the platform is particularly well suited for systematic optimization of expression constructs.

Examples of screening applications

libraries of genetic and protein design elements, including codon-optimized gene variants, promoter libraries, expression cassette components, secretion signal peptides, protein mutations, and fusion tags, can be assembled into large episomal plasmid libraries. Rapid transformation and high-throughput screening in *Pichia pastoris* enables identification of optimal expression configurations that maximize protein yield, secretion efficiency, and functional performance.



Promoter Libraries

Promoter strength plays a central role in determining recombinant protein expression levels. Screening promoter libraries allows identification of promoter variants that balance expression strength, metabolic burden, and protein folding capacity.



Expression Cassette Optimization

Expression levels can be strongly influenced by regulatory elements within the expression cassette. Libraries of regulatory variants can be evaluated to determine the optimal combination of genetic components for a given protein.



Secretion and Signal Peptide Variants

Efficient secretion is critical for many recombinant proteins produced in *Pichia*. Libraries of signal peptides or secretion signals can be evaluated to identify sequences that improve secretion efficiency for specific target proteins.



1. Codon Optimization Strategies

Different codon usage strategies can have significant effects on translation efficiency, protein folding, and secretion. The episomal platform enables parallel testing of multiple codon-optimized gene variants to identify sequences that maximize expression in *Pichia*.



2. Functional Protein Mutations

Protein engineering frequently requires screening large numbers of sequence variants to identify mutations that improve activity, stability, or binding properties. The episomal platform allows large mutant libraries to be expressed directly in *Pichia*, enabling functional screening in the same host used for downstream development.



Tag and Fusion Variants

Fusion tags, secretion enhancers, and stabilization domains can significantly influence protein yield and solubility. Libraries of fusion constructs can be rapidly evaluated to determine the most effective configuration for a given protein.

(2) Multi-dimensional screening enabled by the Alagene *Pichia* Screen™ episomal *Pichia* platform.

Next step: End-to-End work at Alagene

From High-Throughput Screening to Production Strain Development

While episomal expression provides significant advantages for rapid screening applications, its primary role within the Alagene workflow is to enable fast discovery and optimization of genetic constructs prior to production strain development. Alagene Pichia Screen™ allows efficient evaluation of large variant libraries, providing high-resolution data on how genetic design choices influence protein expression and performance in *Pichia pastoris*.

Importantly, the insights generated through large-scale screening directly inform the engineering of stable production strains. Once optimal genetic configurations have been identified, including gene variants, regulatory elements, and secretion signals, these constructs can be transferred into chromosomally integrated expression systems designed for industrial fermentation.

Alagene's capabilities enable the rapid transition from screening to production. Using the data generated from high throughput episomal screening, the most promising designs can be incorporated into genetically stable, high-performance, GRAS production strains optimized for large-scale protein manufacturing. This workflow combines the speed and scale of episomal library screening with the robustness and stability required for commercial bioproduction.

By coupling large-library screening capabilities with production strain engineering, Alagene enables a streamlined development pipeline that accelerates the path from genetic design to industrial protein production.