THOMSON INSTRUMENT COMPANY Solutions At Work

bioGRAMMATICS *Expression in the Language of Life*

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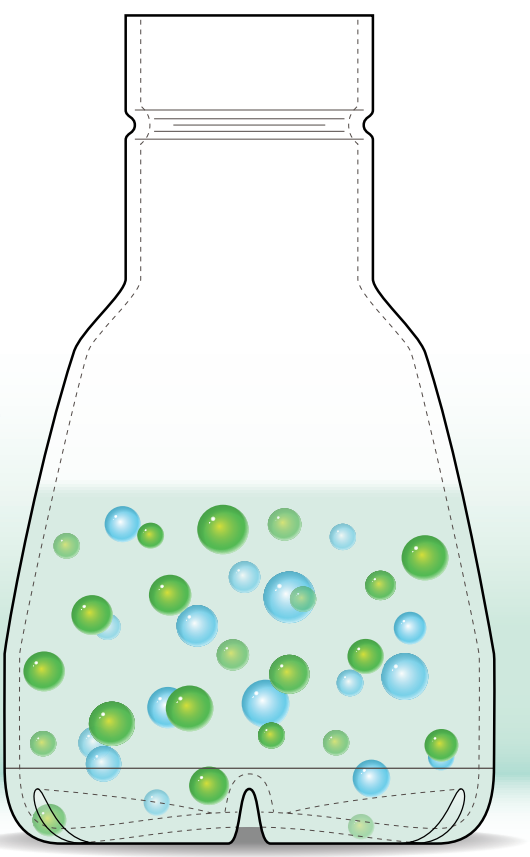
ULTRA YIELD™ FLASK & PICHIA PASTORIS

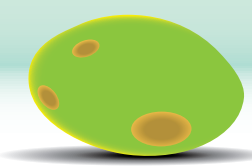
PATENTED

- Higher Cell Density
- Shorter Generation
- Longer Log Phase



The Pichia Pastoris Strain

was constructed by homologous integration of a single human α-galactosidase expression cassette into the genome. Expression was driven by a proprietary BioGrammatics constitutive promoter which shows strong expression when using glucose (dextrose) as a carbon source for growth.



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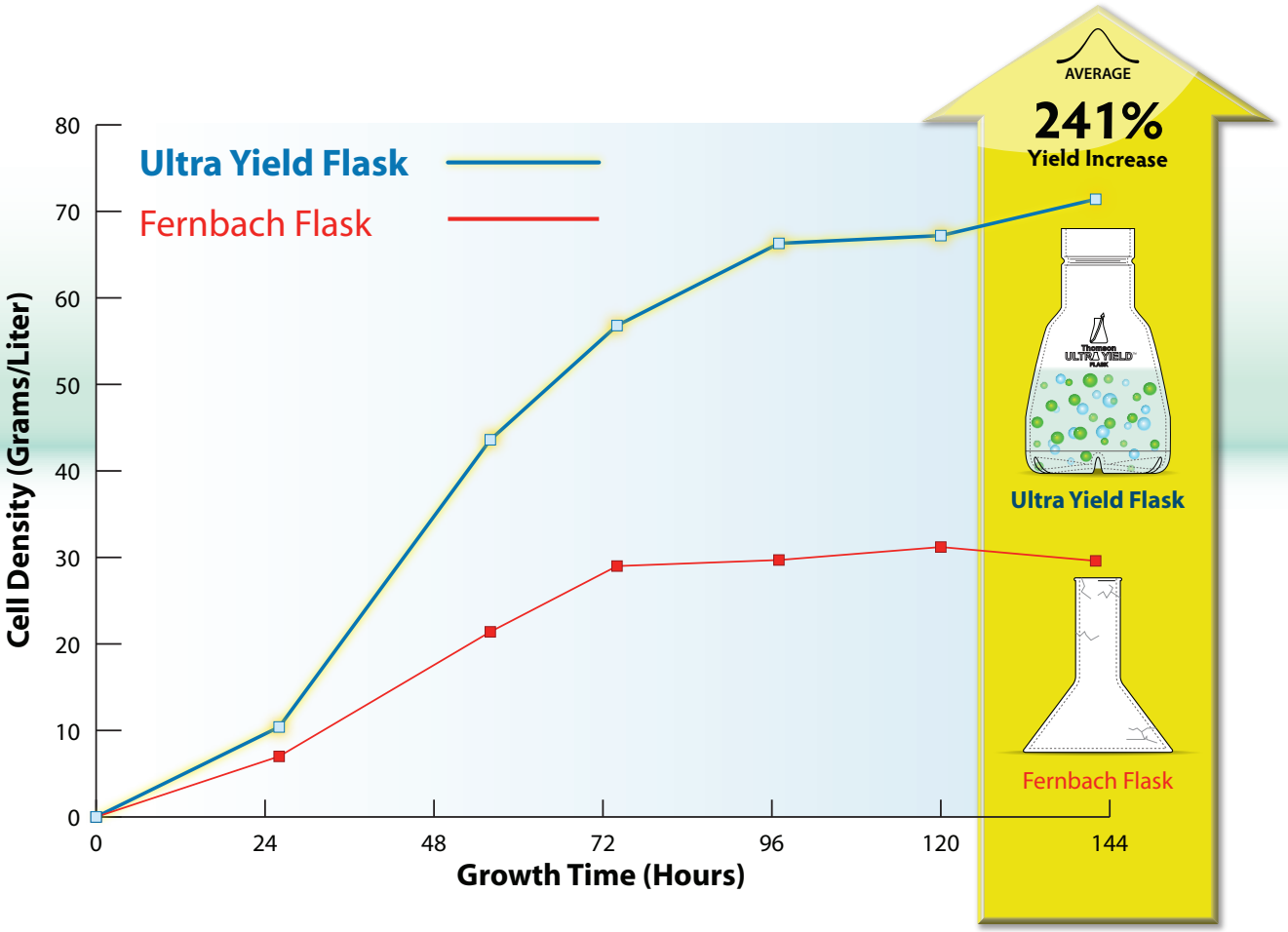
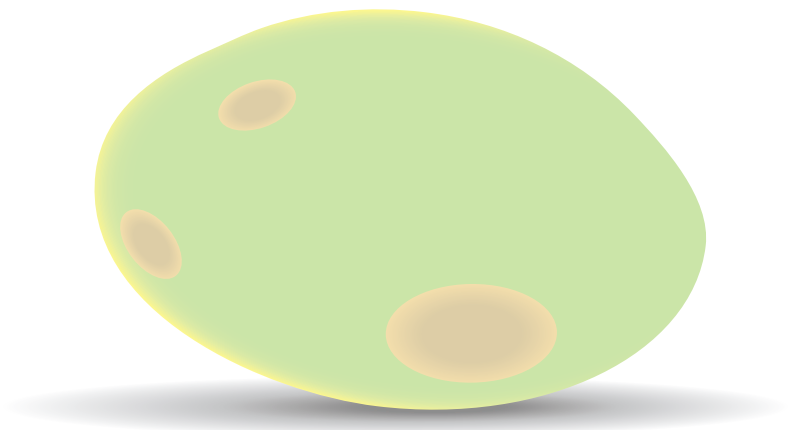
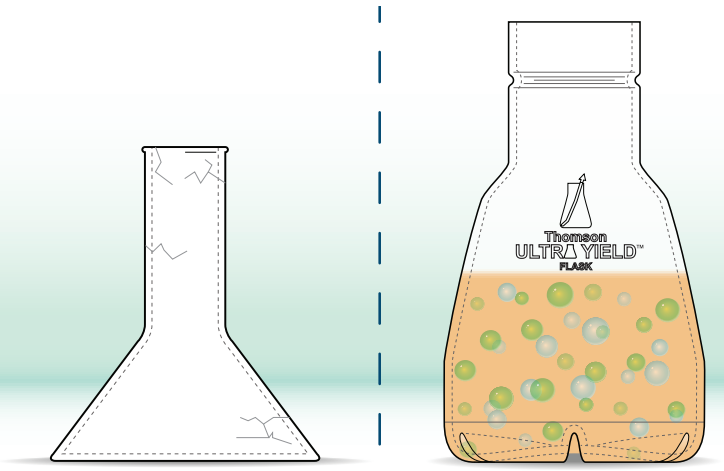


Growth Of Pichia Pastoris

strain expressing secreted human α-galactosidase. An equal volume (5mL) of a starter culture grown in YPD medium was innoculated into 2 liters of BMDY (BMY medium supplemented with 20g/L dextrose). The 2 liters of culture was equally divided between a 2.8 liter Fernbach flask and a 2.5 liter Thomson Ultra Yield Flask. Both flasks were capped with appropriate caps. Cultures were grown at 20°C in an Innova® 4430 incubator, shaken at 200rpm. Samples were removed at approximately 24hr intervals and wet cell mass determined. At each sampling point, 10g of dextrose was supplemented into each flask. Cell density at stationary phase was approximately 2x higher in the Ultra Yield Flask than in the Fernbach Flask.





Amazing Results!

Fernbach Flask

Ultra Yield Flask

Hours Growth: 26 56 74 97 120 142 | 26 56 74 97 120 142

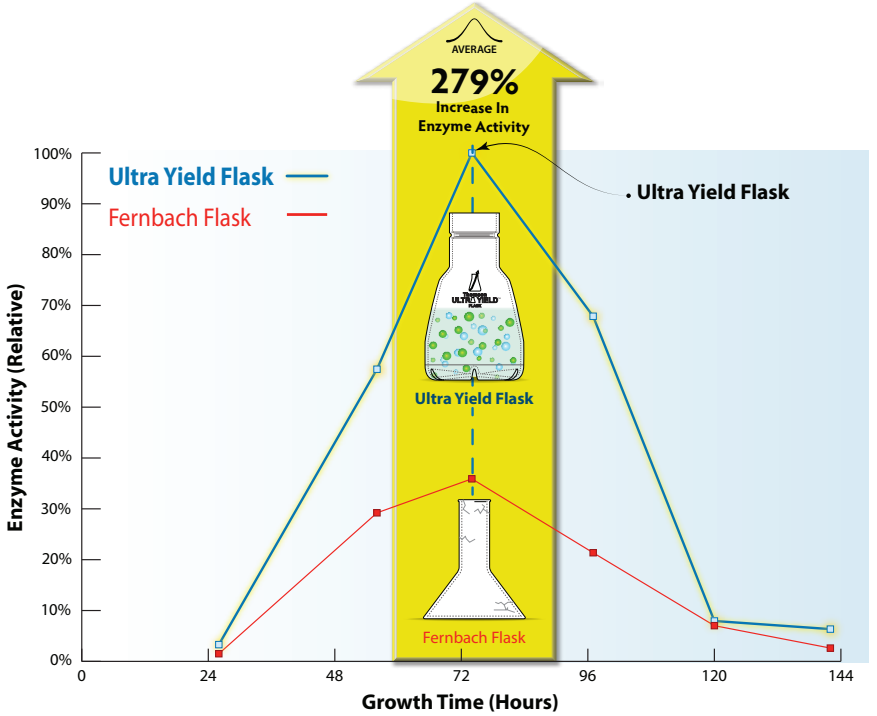
Replicate 1

Replicate 2

Blank

Enzymatic Activity Of Pichia

pastoris strain expressing secreted human α-galactosidase. The supernatant from each sampled time point was assayed for α-galactosidase using α-X-Gal as the enzymatic substrate. 150 µl of each supernatant was transferred to a 96 well plate in duplicate and supplemented with α-X-Gal. Supernatants were incubated at 37°C for 90 min to allow substrate cleavage. Samples were quantitated using a plate reader with a 650 nm filter set.



Enzymatic Activity

of Pichia pastoris strain expressing secreted human α-galactosidase. Replicates from the 96 well plate shown in the above graph were averaged and normalized to the highest activity sample (Ultra Yield Flask, 74 hr timepoint).

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Conclusion

Growth under identical conditions resulted in higher cell density as the strain reached stationary phase in the Ultra Yield Flask. Cells had a shorter generation time and continued to double for a longer period of time when grown in the Ultra Yield Flask. The higher cell density as the cells reached stationary phase resulted in the secretion of more human α-galactoside into the culture medium. In both flasks, degradation of the enzyme started to occur once the cells reached stationary phase.

For more information about the vector / strain system, contact BioGrammatics, Inc. at: info@biogrammatics.com

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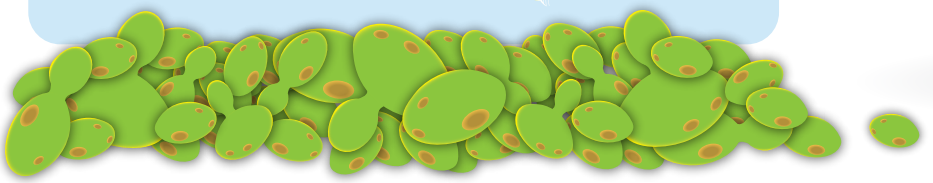
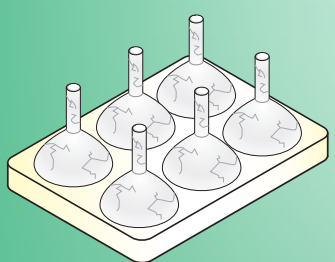


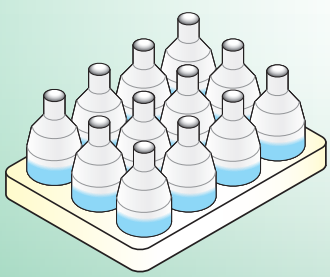
Ultra Yield Flask

Ultra Yield Flask 2.5L

Case Qty: 6: **Part No. 931136-B**

Double Shaker Capacity with Ultra Yield Flasks



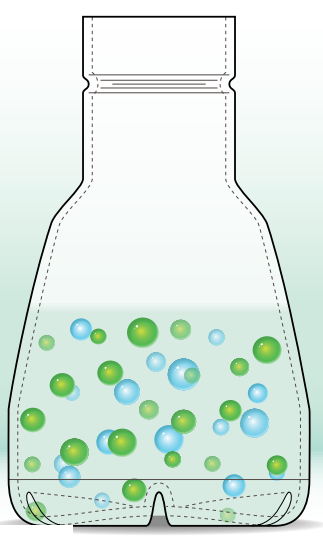


X6 BAD

Fernbach Flask

X12 GOOD

Ultra Yield Flask



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