

High-level secretion of functional IgG, Fab and scFv antibody fragments and fusion proteins in *Saccharomyces cerevisiae*

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Recombinant monoclonal antibodies constituted over \$7 bi in sales in 2003 and are expected to grow to \$12 billion by 2010 . The cost of monthly treatment for antibody based drugs is between \$4,400-\$17,000 per patient. A critical factor in high cost of these drugs is that the cell culture production systems currently used for recombinant antibodies are not very efficient.

ApoLife Inc. will be presenting data on its cost effective system for the production of monoclonal antibodies (MAb) in *S. cerevisiae* (yeast). ApoLife's proprietary "Twin Cassette" system enables cloning of two chains of MAb into a single vector, which facilitates production of equivalent amounts of both H and L chains, and formation of correctly assembled, secreted, functional antibody molecules. Using proprietary yeast strains, the company has produced a humanized single chain scFv, Fab, and full length antibody molecules (IgG) and fusion proteins which are secreted into the media.

Various strategies to increase the yields of antibodies by fermentation manipulations, media formulations, plasmid selections and yeast strain selection will be presented

There are several advantages of the yeast system:

Yeast cells can be grown rapidly to high density in inexpensive media. Fed batch fermentation in yeast takes 7 days, versus 21 days in mammalian cells which minimizes the size of manufacturing facilities thus saving in capital expenditure. Recombinant products from yeast contain no known viruses, prions, or bacterial endotoxins. Presence of eukaryotic control mechanism in yeast allows proper folding and processing complex mammalian proteins correctly, resulting in full bioactivity.

Glycoproteins produced by mammalian cell cultures as well as yeast cultures show heterogeneous glycosylation patterns. However novel methods of addressing glycosylation issues are now available. These include genetically engineering the human glycosylation pathway into the yeast or enzymatically modifying expressed proteins *in vitro*.

The ease of strain manipulation and the short cycle time for fermentation, this system has advantages over existing cell culture system for antibody production as well as screening several early stage antibody candidates. Therefore, the system offers advantages over existing technologies from the early discover stage all the way through commercial manufacturing.

These factors highlight the advantages of a rapid, lower-volume yeast fermentation process over mammalian production, since the yeast system requires not only lower capital investment initially but can also be expanded more rapidly and cost effectively. We believe the yeast system described here has a potential for dramatic reductions in cost of commercial scale manufacturing of antibodies.

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