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Expression and Purification of Recombinant α-Galactosidase in *Saccharomyces cerevisiae*

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Penicillium sp. α-Galactosidase cDNA was expressed in S. cerevisiae under the control of the yeast GAL10 promoters. S. cerevisiae cells carrying YEp-PGA were cultured in YPGal medium, and α-Galactosidase production was monitored. α-Galactosidase was secretes into the medium, and the activity reached about 63 U/ml of medium at 216 h. Little α-Galactosidase activity was detected in the periplasmic space or intracellular fractions throughout the culture period. No background activity was detected when the host cells carrying the expressionj vector YEp51 were cultured under the same conditions. Recombinant α-Galactosidase was purified to homogeneity by using three chromatographic steps. Starting from the 100 ml culture medium, 6.75 mg of the purified α-Galactosidase was obtained with 33% recovery.

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In vitro assay of neurofilament light chain self-assembly using truncated mutants

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Neurofilaments (NFs) are heteropolymers composed of light (NF-L), middle (NF-M), and heavy (NF-H) subunits, present in most neurons. NF-L polymerizes on its own to provide a scaffold on which regular NFs form via the cross-bridging of NF-M or NF-H. To clarify the mechanism of regulation of NF-L self-assembly, we developed an assay using truncated mutant NF-L fused to glutathione-S transferase (GST). Western immunoblotting data show that theGST-fused head-rod domains of NF-L are necessary and sufficient for detecting assembled NF-L. The levels of self-assembled NF-L subunits detected using GST fusion proteins were consistent with those detected by electron microscopy and turbidity assay. Our results collectively imply that GST-fused head-rod domains of NF-L are critical tools for analyzing NF-L self-assembly *in vitro*.