THREE-PLASMID (SUMO) YEAST VECTOR SYSTEM FOR HIGH-LEVEL FUNCTIONAL EXPRESSION OF VALUE-ADDED CO-PRODUCTS IN AN INDUSTRIAL SACCHAROMYCES CEREVISIAE STRAIN ENGINEERED FOR XYLOSE UTILIZATION AND METABOLIC CORRECTION ENZYMES

S.R. Hughes¹, D. Sterner², P.F. Dowd³, R. Hector⁴, K.M. Bischoff⁴, N. Qureshi⁵, S.S. Bang⁵, N. Grynvalsky⁶, T. Chakrabarty⁶, E. Johnson⁵, X.L. Li⁴, J.A. Mertens⁴, R.J. Caughey⁷, S. Liu¹, J.O. Rich¹, T.R. Butt⁸, J. LaBaer⁹, and M.A. Cotta⁴

¹Bioproducts and Biocatalysis Research, National Center for Agricultural Utilization Research, ARS-USDA, 1815 N. University Street, Peoria, IL 61604, USA
²Progenra, Inc., 271A Great Valley Parkway, Malvern, PA 19355, USA
³Crop Bioprotection Research, National Center for Agricultural Utilization Research, ARS-USDA, 1815 N. University Street, Peoria, IL 61604, USA
⁴Fermentation Biotechnology Research, National Center for Agricultural Utilization Research, ARS-USDA, 1815 N. University Street, Peoria, IL 61604, USA
⁵South Dakota Sch. Mine & Tech., 501 E. Saint Joseph St, Rapid City, SD 57701, USA
⁶Arryx, Inc., 316 North Michigan Avenue, Suite CL20, Chicago, IL 60601, USA
⁷Univ. of IL College of Medicine at Peoria, One Illini Drive, Peoria, IL 61656, USA
⁸LifeSensors, Inc., 271 Great Valley Parkway, Suite 100, Malvern, PA 19355, USA
⁹Harvard Institute of Proteomics, 320 Charles Street, Cambridge, MA 02141, USA

The three-plasmid yeast expression system utilizing the portable small ubiquitin-like modifier (SUMO) vector set combined with the efficient endogenous yeast protease rapidly produces large amounts of soluble functional protein. It provides high levels of expression for three different proteins simultaneously. This system is used to express a peptide of potential commercial value in an industrial Saccharomyces cerevisiae strain also engineered to express xylose utilization and metabolic correction enzymes. The xylose isomerase (XI) gene for pentose utilization is expressed using the first of three SUMO vectors. A value-added co-product is expressed in the second vector. The test product here is the putative bioinsecticidal peptide, lycotoxin-1 (Lyt-1). The third vector is employed to express metabolic correction genes to enhance pentose utilization. Engineering of the yeast strain involved three steps. First, using a novel PCR assembly strategy we cloned the XI open reading frame (ORF) into the URA-selectable SUMO vector and placed it into the S. cerevisiae strain. Second, using amino acid scanning mutagenesis we generated a library of mutagenized Lyt-1 ORFs, cloned it into the TRP-selectable SUMO vector, and placed it into the XI-yeast. Third, the Yersinia pestis xylulokinase gene was cloned into the LEU-selectable SUMO vector and placed into the Lyt-1-XI-yeast. Yeast strains expressing xylulokinase and Lyt-1 in addition to XI showed improved growth on xylose compared to XI-yeast.

Contact: Stephen R. Hughes, Bioproducts and Biocatalysis Research, NCAUR-ARS-USDA, 1815 N. University Street, Peoria, IL 61604, USA. Tel: 309-681-6176. E-mail: Stephen.Hughes@ars.usda.gov.