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Yeast Expression Platform for the Massive Production of Recombinant Proteins and Their Industrial Applications

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The technology for the production of recombinant proteins has been recognized as an essential field not only for the biopharmaceuticals but also for the functional genomics in post-genome era. In vast quantities of genomic information, numerous uncovered proteins with values for human drugs and industrial enzymes are waiting for their characterizations and proper applications. Unfortunately, however, about 20~30% of tested proteins could be produced under the current protein expression systems using recombinant hosts such as bacteria, yeast and mammalian cells [1]. Thus, post-genomic biology requires novel expression strategies and improved throughput for the breakthrough of the current limitations. To this end, an improved yeast expression platform has been developed for the efficient production of massive recombinant proteins in a high-throughput manner.

A GRAS yeast *Saccharomyces cerevisiae* has an excellent protein secretion pathway and post-translational modification function necessary for higher eukaryotic proteins. During the last two decades, *S. cerevisiae* has been frequently chosen for the secretory production of numerous recombinant proteins. But the yield of secreted target proteins was generally low and unpredictable [2]. In fact, there has been limited number of vector systems available with secretion signals from several known proteins, such as mating factor alpha, invertase, acid phosphatase, and killer toxin etc. Such could not completely support the secretion of massive target proteins having different structures and physicochemical properties. For the improvement of the yeast secretion systems, more signals from different types of secretory protein and screening methods of an optimal signal from the pool of secretion signals for each target protein, are required. There are about 600 ORFs among 6,000 in the yeast genome encoding secretory proteins which pass the ER. Apparently, they all have different secretion signals. To utilize all of them for the secretion of different target proteins, genome-wide TFP (translational fusion partner) library containing signal sequences and fusion partners have been constructed from the yeast genomic and cDNA library using a secretion reporter, invertase. Simple insertion of target gene into TFP library vectors through *in*

vivo recombination and a positive selection on selection media could find an optimal fusion partner in a high throughput manner. The technology could rapidly screen an optimal TFP capable of inducing hyper-secretion of target proteins, especially proteins that are difficult to produce using conventional recombinant production methods. It could greatly improve the secretion level of several low-yield proteins up to several hundred folds. The platform technology will be useful for the production of massive recombinant proteins of bio-industry and basic research in the post-genome era. Several industrial applications of recombinant proteins produced up to grams per liter, especially for bioenergy field will be presented.

References

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2. Mattanovich *et al*., Stress in recombinant protein producing yeasts. *J. Biotechnol*. 113, 121, 2004.