BioSpotlight

Target practice

Gene targeting in human somatic cells can be challenging owing to the low rates of homologous recombination. Improving targeting rates requires a dependable and effective method to monitor the efficiency of homologous recombination. This is often accomplished through the use of reporter cells carrying a vector containing a reporter gene with inactivating mutations. Transduction of the cell with a targeting vector containing a partial sequence of the wild-type reporter gene results in correction of the inactivated reporter gene through homologous recombination. A major issue with this system, however, is unstable reporter gene expression, especially with viral promoters that are susceptible to epigenetic changes that reduce expression over time. To overcome this drawback, H. Konishi and colleagues at Aichi Medical University (Nagakute, Japan) describe in this month's issue their use of a reporter gene consisting of the fusion of the hygromycin B phosphotransferase (Hyg^R) gene with the 5' portion of the EGFP gene. Loss of Hyg^R-5' EGFP gene expression in a portion of reporter cells by epigenetic silencing will eliminate those cells under hygromycin selection. Homologous recombination of this fusion reporter gene with a targeting vector containing an EGFP

Representation of the reporter and targeting vectors.

gene without an authentic start codon results in a functional EGFP gene. When monitoring gene targeting efficiency, this novel reporter system accurately reproduced previously described data obtained with various targeting vectors. More importantly, reporter cell clones with the Hyg^R-5' EGFP gene retained their ability to monitor gene targeting efficiency after long-term culture (up to six months), whereas reporter cells with a nonfused 5' EGFP suffered significant reductions.

See “A system for the measurement of gene targeting efficiency in human cell lines using antibiotic resistance-GFP fusion gene” on page 141.

Cell-free for me

Cell-free protein synthesis (CFPS) provides researchers with an enhanced degree of control when optimizing synthesis conditions for a protein of interest. While a number of CFPS systems have been developed, there still exists a need to refine and improve these current protein synthesis techniques to enhance ease of use and application for high-throughput. The current issue of *BioTechniques* has two articles addressing different aspects of CFPS methodology, each focusing on robust modifications that greatly enhance the utility of CFPS systems. In the first article, B.C. Bundy and his colleagues at Brigham Young University examine preparation methods for cell extracts from *Escherichia coli*. Traditionally, *E. coli* cell extracts for use in CFPS are generated by using either a high-pressure homogenizer or a bead mill homogenizer, both of which require significant investment. Bundy and his co-authors realized that the adoption of CFPS approaches could be greatly expanded if extract preparation could be performed using equipment readily available in most biotechnology labs. The authors went about testing sonication, bead vortex mixing, freeze-thaw cycling, and lysosome incubation as methods to produce cell extracts from *E. coli*, finding

that sonication and bead vortex mixing were effective means to produce extracts that could be used in CFPS. In addition, the authors also found that by overexpressing RNA polymerase prior to cell lysis, purified polymerase did not have to be added to the CFPS reaction independently. These adaptations should make CFPS more accessible to life science researchers.

In the second article, H. Merk and his colleagues from RiNA Netzwerk RNA-Technologien and Qiagen in Germany detail their new approach for the CFPS synthesis of antigen binding fragments (Fab) and other disulfide containing proteins. Merk et al.'s system takes advantage of a cell lysate which is prepared with mildly reducing conditions to maintain vesicle trafficking activity from the endoplasmic reticulum along with signal dependent translocation into these vesicles with a redox potential based on reduced and oxidized glutathione. The system allows for monomeric immunoglobulin chains to be converted into active dimeric Fab joined through disulfide bonds. The unique aspect of this work is the fact that creation of disulfide bonds does not require supplementation with either protein disulfide isomerase or chaperones, and results in the generation of Fab antibodies that can be directly used

in cell assays without the need for further purification. The authors demonstrate the effectiveness of their new CFPS approach by synthesizing anti-lysosome and anti-CD4 Fab antibodies. Although the yield of synthesized Fab fragments is lower than traditional CFPS systems, the specific activity of these fragments was much higher. This new system should prove highly valuable to researchers seeking a high-throughput approach to generate antibody fragments or disulfide containing proteins.

See “Streamlined extract preparation for *Escherichia coli*-based cell-free protein synthesis by sonication or bead vortex mixing” on page 163 and “Cell-free synthesis of functional and endotoxin-free antibody Fab fragments by translocation into microsomes” on page 153.

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