# Quicker, cheaper methods to create test tube proteins

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(Phys.org)—Over the past decade, researchers have become increasingly interested in cell-free protein synthesis techniques as a means to produce proteins in the biotechnology and medical industries. Taking place in a test tube rather than a cell, these methods give researchers more control over the protein synthesis process. But many researchers have not explored these approaches due to the specialized equipment required and limited throughput.

Two papers, published in the September 2012 issue of *BioTechniques: The International Journal of* Life Science *Methods*, seek to resolve these issues and create wider adoption of cell-free protein synthesis techniques .

Although taking place outside the cell, cell-free protein synthesis still requires the use of cellular extracts that are typically obtained using specialized equipment, such as high-pressure or bead-mill homogenizers, to lysis—or breakdown—the cells, equipment that many labs do not have access to.

In the first paper, "Streamlined extract preparation for Escherichia coli -based cell-free protein synthesis by sonication or bead vortex mixing," Bradley C. Bundy, assistant professor of chemical engineering at Brigham Young University in Provo, Utah, and colleagues tested several low-cost alternatives to the traditional homogenizers. The team found that two alternatives, sonication and bead vortex mixing, were effective in obtaining E. coli cell extracts.

"Sonication kind of surprised us because a previous paper reported that sonication didn't work in their hands," said Bundy. "However, we found that if we sonicated even longer than needed for efficient lysis, we were able to reproducibly produce viable extract for our cell-free system. That was really exciting."

In addition, the group used the simple method of shake flask fermentation to grow their E. coli cells prior to cell lysis. This approach eliminates the need for specialized, large-scale fermentors. By simplifying the extract preparation method and providing alternative lysis options using less expensive equipment, Bundy and colleagues hope that more researchers will be able to quickly and effectively perform cell-free protein synthesis. In addition, lysis by sonication lends itself to high-throughput applications due to the availability of 96-well plate sonication systems.

In the other paper, "Cell-free synthesis of functional and endotoxin-free antibody Fab fragments by translocation into microsomes," Helmut Merk, a researcher at the RiNA Netzwek RNA-Technologien GmbH in Berlin, Germany, and colleagues from RiNA GmbH and Qiagen Gmbh in Hilden, Germany, describe a cell-free system to synthesis antigen-binding fragments (Fab) of antibodies. Over the past 10 years, RiNA has developed several other cell-free protein synthesis systems including a prokaryotic Fab-producing system.

While Fab fragments are valuable biochemistry tools due to their binding properties, use has been limited because of the time and labor required to produce these fragments.

Previously described cell-free systems for Fab production have been based on bacteria or other prokaryotic cells, where the protein output included inactive fragments and toxins, unwanted byproducts that have to be removed to prevent inaccurate results.

Merk's group developed a system based on cells from the insect Spodoptera frugiperda, which produced Fab fragments that could be directly applied to cell assays without purification.

"If you think in terms of automation, purification is a difficult step to automate," said Merk. "Our insect cell system is easier to automate compared to an E. coli system because there's no need for purification."

To achieve such a high percentage of active Fab fragment synthesis, the researchers directed synthesis to microsomes that were already present in the insect cells by a signal peptide.

"This is similar to the natural way, which is used by B lymphocytes to produce antibodies. So, why not go this way?" said Merk. "We were surprised that it worked this well."

"There's a lot of potential for cell-free systems, and we're going to see more and more research in this area, especially for synthetic biology applications" said Bundy. "It commonly takes many man-years to effectively perform synthetic biology in vivo. However, the open cell-free environment enables a researcher to directly control the mixing and matching of biological functions to rapidly optimize product production and reduce costs. "

**More information:** Shrestha, P., T.M. Holland, and B.C. Bundy. 2012. Streamlined extract preparation for Escherichia coli-based cell-free protein synthesis by sonication or bead vortex mixing. *BioTechniques* 53:163-174. www.biotechniques.com/article/113924

Merk, H., C. Gless, B. Maertens, M. Gerrits, and W. Stiege. 2012. Cell-free synthesis of functional and endotoxin-free antibody Fab fragments by translocation into microsomes. *BioTechniques* 53:153-160. www.biotechniques.com/article/113904

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