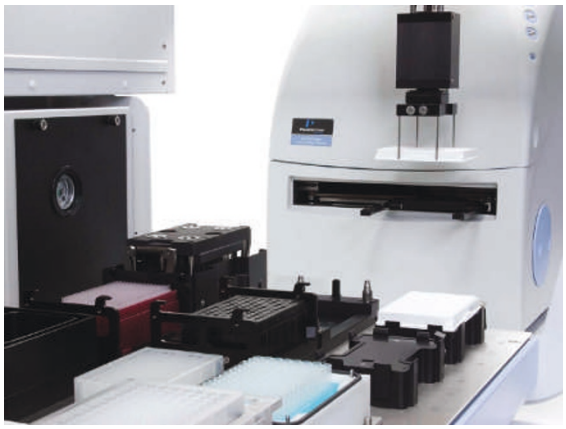
tion of His or GST fusion proteins by magnetic or filtration-based affinity purification protocols, all in a 96-well format. The kits have been validated on both PerkinElmer and Tecan robotic liquid handlers.

Other providers of reagents for high-throughput protein purification include GE Healthcare of Little Chalfont, UK, with its Tricorn High Performance Columns designed for high-resolution purification of proteins, peptides and other biomolecules. Sartorius of Göttingen, Germany, introduced the Vivapure 8-to-96 well cobalt chelate kit for the simultaneous purification of multiple His-fusion proteins by affinity chromatography. And St Louis-based Sigma-Aldrich's HIS-Select iLAP Plates are plates coated with cell-lysis reagents and a HIS-Select nickel chelate matrix allowing for cell lysis, protein capture, and assay of a His-fusion protein in a single well.

As well as purification kits, several companies offer products to help screen the outcome of each step in the purification pipeline. Novagen's RoboPop Solubility Screening Kit contains a filtration plate that retains insoluble inclusion bodies while allowing soluble proteins to be collected for rapid quantitation and analysis. "You can screen expression conditions for soluble protein before you proceed to purification," says Grabski. In January, the company released the iFold Protein Refolding System 1 to screen different refolding conditions in parallel. The system includes inclusion-body purification reagents and 92 different refolding buffers. "Structural proteomics groups have harvested most of the low-hanging fruits, so now they will have to focus on difficult proteins that are insolubly expressed," says Grabski. Another product for monitoring the purification procedure is the Protein 200-HT2 assay of Agilent Technologies (Palo Alto, California), which allows the identification, sizing and quantification of proteins from 14 kD to 200 kD in size. "It allows researchers to replace SDS–PAGE procedures in the lab," says product manager Carsten Buhlmann. Finally, some reagents and instruments have been designed to help researchers hone in, for example, on medically relevant targets, which can then be purified and studied (see "Target Identification").



PERKINELMER

Mix and match: PerkinElmer's JANUS workstation.

Getting automated

The available reagents and kits can be used to purify several hundred proteins in a 96-well format at considerable speed. However, the tedious and error-prone nature of manually performed high-throughput operations calls for automation of the process. "When a robot is doing the pipetting there are no mistakes, so you can have greater consistency and reproducibility," says David Daniels, applications marketing manager for Beckman Coulter in Fullerton, California. Liquid handlers perform all the steps of protein purification from loading cell cultures to obtaining a protein in solution, in under four hours.

Two leading liquid handlers are Beckman Coulter's Biomek 3000 and Biomek FX instruments, with plate-deck configurations that can handle 10 and 18 plate positions, respectively. The Biomek 3000 will process two 96-well plates in 2–3 hours; the FX twice as many.

Promega in Madison, Wisconsin, automated high-throughput protein purification using its MagneHis Protein Purification System and the Biomek FX instruments. The MagneHis reagent contains a cell-lysis solution that allows resuspension and lysis of bacterial cell pellets without sonication and centrifugation. Magnetic pre-charged nickel particles are then used to isolate His-tagged proteins from the cell lysate. The MagneHis particles bound to the target proteins are captured on a MagnaBot 96

CELL OR CELL-FREE?

Only a fraction of proteins can be overproduced in *E. coli* in sufficient yield and without the formation of inclusion-body aggregates or the proteolytic degradation of expressed proteins. Alternative expression systems include cell cultures from eukaryotic organisms, such as insect cells, and cell-free, *in vitro* protein expression.

The latter is the focus of John Markley's group at the Center for Eukaryotic Structural Genomics at the University of Wisconsin, Madison. Collaborating with Ehime University and CellFree Sciences, both in Japan, Markley and colleagues developed a pipeline using wheat germ cell-free protein translation as a way to produce proteins for nuclear magnetic resonance (NMR) structure determination.

Cell-free systems simplify the purification efforts, as "only the protein of interest is expressed and labelled, thus the background is cleaner", says Markley (pictured). For the NMR studies, his group has been getting twice as many folded proteins using the cell-free system than with expression in *E. coli*. The cell-free method also requires smaller volumes, avoiding lengthy concentration steps, and it lends itself to the labelling of proteins, which is a requirement for NMR. "But there is a steep learning curve for learning how to do a cell-free system," says Dmitriy Vinarov, who is responsible for high-throughput production at the centre. One downside of cell-free systems is the expense of the reagents, especially when success rates are not high. "About 79% of human proteins will be expressed, about one half of those will produce protein in sufficient quantities for structural studies, and half of those will remain stably folded for NMR studies," says Markley.

R. DAVIES

Cell-free and cell-based systems are not mutually exclusive. "Each has unique advantages and capabilities so we will continue to do both," explains Markley. By using a new cloning system from Promega the researchers have been able to transfer target cDNAs from cell-free to cell-based expression systems to achieve greater flexibility. "We still have a lot to learn. It is working quite well for us but we still have improvements to make," says Vinarov. "The technology is not quite primetime."

Cell-free systems are available from companies such as Roche Applied Science, QIAGEN and Invitrogen, as well as from CellFree Sciences. L.B.

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