Automating protein production processes for drug discovery at Bayer Schering Pharma AG

One of the many functions of Bayer Schering Pharma's Berlin research facility is to provide recombinant protein tools for the company's lead discovery process. Scientists there have integrated two Freedom EVO® workstations into the protein production process, ranging from cloning and expression to purification, and including automation of GE Healthcare's MultiTrap® 96-well plates.

Technician Mario Mann loads samples onto the Freedom EVO 200

Bayer Schering Pharma AG, part of Bayer AG, develops new drugs and innovative therapies for improving healthcare in a range of specialist fields, with a research focus on oncology, cardiology, women's health and diagnostic imaging. Identifying and evaluating potential new drug candidates is a vital aspect of lead discovery, and the company's research center in Berlin supplies the protein tools that are essential to these processes.

"We perform more than a thousand protein expressions every year in order to deliver highly active protein preparations for high throughput screening and, in particular, to provide proteins for crystallization," said Dr Dieter Moosmayer from the Protein Supply function at the Bayer Schering Pharma research facility in Berlin. "The protein preparations are screened against our compound library to identify small molecule inhibitors. Selected hits are then co-crystallized with crystallization-grade protein batches to identify the binding mode and to allow structure-based optimization. Our research covers a variety of applications, but we have particular experience with human drug targets, such as kinases and nuclear steroid receptors. For instance, the kinase projects aim to replicate the success of Nexavar®, a b-Raf kinase inhibitor, which is approved for renal cancer and is a potential pan-tumor drug."

Being responsible for the whole protein production process, ranging from cloning of variants and mutations through to their expression and preparative purification, requires stringent analysis and quality control at every stage of the process. In order to deliver proteins within standard timeframes, the team switched to a multi-parallel approach a few years ago that included a Tecan Genesis liquid handling workstation. Dr Beate Müller-Tiemann explained, "To go from the initial cloning stages through to crystallization of the protein can take a couple of months. We do not have the time to fail, so we used a strategy of miniaturization, parallelization and automation that helped us to find the optimal approach for each individual target gene within given timeframes."

"A library of expression variants is initially developed by a team of structural

biologists and biochemists. These variants are then expressed in small-scale multi-parallel expression systems and the best candidates proceed to purification, where downstream processing of preparative multi-step chromatographic purification is initiated, again in a multi-parallel fashion, and finally up-scaled for structural determination."

The laboratory has recently upgraded its automated platform to include two Freedom EVO liquid handling workstations equipped with Te-VacS™ vacuum separation modules. "We decided to use Tecan instruments for these applications for two reasons," said Dr Arndt Schmitz. "Firstly, Tecan technology has an impressive history of compatibility with kits and procedures that are relevant to our applications and, secondly, the instrument software is very flexible, allowing integration with equipment from other manufacturers," he said. "The technical team at Tecan Germany is very knowledgeable and always available. Whenever we plan an upgrade, new script, or routine maintenance, the team is just a telephone call away. In addition, the training courses offered greatly aided our technicians Mario Mann, Ina Künkel, Andrè Hilpmann and Norbert Otto in setting up the automation here."

The laboratory's Freedom EVO 150 is dedicated to automating molecular biology processes, including complete processing of the Invitrogen Gateway® recombinant cloning technology, and preparation of DNA minipreps. "The workstation is able to support complex chains of scripts such as Gateway® cloning, transforming and plating. That openness and flexibility was of particular importance to us," Dr Schmitz said. "Performing thousands of tests on genetically modified organisms every year is an enormous task in terms of producing clones, transfection, purification and analysis."

The laboratory's Freedom EVO 200 has an integrated GENios™ microplate reader and automates protein purification steps, including processing MultiTrap™ plates from GE Healthcare, which are ready-to-use 96-well filter plates for high throughput screening, enrichment and purification of tagged proteins from cell extract before quantification. Fellow staff scientist Dr Guido Malawski initiated beta-testing of the MultiTrap™ plates on the Freedom EVO and found that automated processing of *E. coli* lysates containing HIS- or GST-tagged target proteins provided a highly reproducible yield of target proteins, with no significant cross-contamination. The high throughput expression and purification of kinases with MultiTrap™ plates gave increased protein production throughput for X-ray crystallography. Screening of expression and purification at an early stage minimizes the workload by identifying the most interesting expression variants on which scaled-up purification can focus[1].

Both Freedom EVO workstations have quickly become an important part of the facility's workflow. "We regard these instruments as our 'co-workers' and have even given them names," Dr Schmitz confessed. "The Freedom EVO 200 is named Marvin while our molecular biology robot is called Brad Pitt! We have implemented standard operating procedures and protocols with these systems, which allows everyone in the department to use them." He concluded: "Even today, we have not yet fully realized the potential of the systems; there is still plenty of room for growth."

Reference

1. *Malawski GA, Hillig RC, Monteclaro F, Eberspaecher U, Schmitz AAP, Crusius K, Huber M, Egner U, Donner P, Müller-Tiemann B (2006). Identifying protein construct variants with increased crystallization propensity - a case study. Protein Sci 15: 2718-2728*

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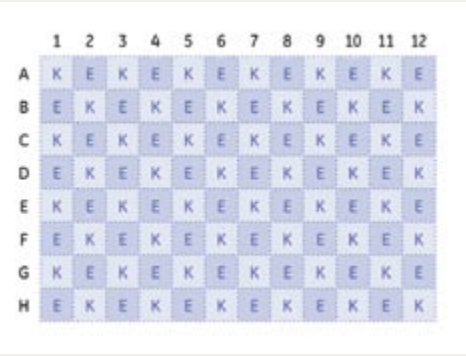

Fig 1A. To examine cross-contamination, clarified E. coli lysate containing histidine-tagged enhanced green fluorescent protein ($(His)_6$-EGFP, Mr 28 000) or histidine-tagged kinase ($(His)_6$-kinase, Mr 43 800) were applied in a chessboard scheme across one His MultiTrap™ HP plate, using a Tecan liquid handling workstation and the Te-VacS vacuum separation module. K: $(His)_6$-kinase E: $(His)_6$-EGFP.

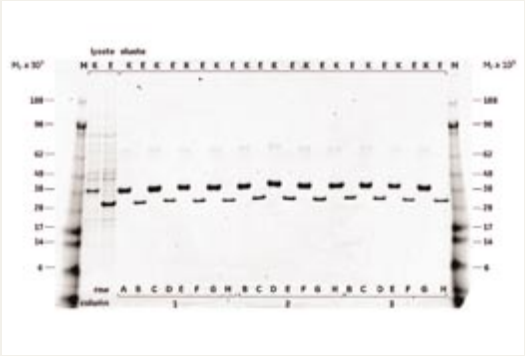

Fig 1B. Coomassie®-stained SDS-PAGE analysis (reduced conditions) of purified $(His)_6$-kinase and $(His)_6$-EGFP using His MultiTrap™ HP and a chessboard sample application scheme (Fig 1A). No significant cross-contamination was found. Lanes: M: molecular weight markers K: $(His)_6$-kinase (Mr 43 800) in lysate and eluates of 12 wells E: $(His)_6$-EGFP (Mr 28 000) in lysate and eluates of 12 wells.

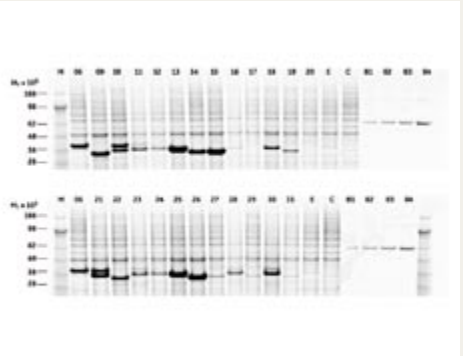

Figure 2. SDS-PAGE analysis of eluates of histidine-tagged truncated variants of kinase, expressed in a transient insect cell system for 72 h. Lanes 06-31: histidine-tagged variants of kinase, E: EGFP (untagged transfection positive control), C: non-transfected Hi5 cells, B1 to B4: 0.5, 0.75, 1.0 and 1.5 µg BSA, respectively.