Automated protein purification for efficient vaccine development

Biochemists at the Novartis Vaccines and Diagnostics Research Center in Siena, Italy, are processing vaccine candidates by automated purification of recombinant proteins from huge numbers of bacterial lysates on a Freedom EVO® platform, saving a lot of their time without compromising reproducibility, consistency or quality.

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The Protein Purification Group at the multi-disciplinary Novartis Vaccines and Diagnostics Research Center purifies proteins cloned from a range of bacteria. The research center is using 'reverse vaccinology', an approach that starts with *in silico* analysis of entire bacterial genomes, to identify hundreds of possible vaccine candidates, followed by cloning, expressing the proteins in *E. coli* and purifying the recombinant proteins, which are tested in screening studies using appropriate animal models. Dr Massimo Mariani, biochemist at the Protein Purification Group, explained: "We have about 10 different projects in vaccine development covering a diverse group of pathogenic bacteria, mainly meningococci, both human and animal streptococci, staphylococci, *Clostridium*, pneumococcus and pathogenic mutants of *E. coli*. All these projects generate a large number of recombinant proteins that require purification from bacterial lysates. Originally, we did this manually using affinity chromatography. This involved up to three operators who performed bacterial lysis, loading and washing columns, eluting and characterizing the eluted proteins, and determining concentrations as well as the degree of purity. This was tedious and time-consuming, taking almost a whole day, so we decided to look for an automated system for protein purification."

"We started to collaborate with Tecan in 2005 and, in just two months, were able to optimize our new Freedom EVO platform with tailored software to reproduce many of the steps that were previously performed manually. We still prepare the bacterial lysates manually, but all subsequent steps are automated, starting with loading of samples onto affinity chromatography columns, followed by washing and elution of each sample into two fractions, colorimetric quantification of each fraction using a Bradford assay, and preparation of samples for SDS-PAGE. On the next day, samples are analyzed by SDS-PAGE, giving information on molecular weights and protein profiles. The software script has the capability to enter our selection of fractions after gel electrophoresis for further analysis, and these fractions can be pooled if required. We run eight to 24 samples at a time, and the system can handle a variety of sample

The Freedom EVO platform at the Novartis Vaccines and Diagnostics Research Center

formats – 12 ml tubes, 1 ml columns and 24- and 96-well microtiter plates; the 96-well plates are used for the Bradford assay."

"Working closely with Tecan, we tailored the software so the procedure automatically stops at three points, during the loading, washing and elution steps. At these points, the system temporarily stops operating, and a warning on the screen prompts the operator to manually check that the columns are empty prior to loading samples, avoiding the risk of process failure due to occlusion of columns by overloading. When the samples are eluted from the columns and the Bradford assay begins, we can leave the Freedom EVO completely unattended because the samples are stored on a refrigerated plate on the Freedom EVO platform deck, to be collected during the next day. The automated procedure takes longer than the manual one – 11 hours compared to eight hours – for purification of 24 samples, but it requires far less operator time, as no human intervention is needed after elution of samples (Table). Yield and purity of proteins are similar between manual and automated procedures for both low and higher expression proteins."

Dr Vittoria Pinto, who has been involved in setting up the procedures on the Freedom EVO platform, said: "The system is fully reliable, we are very happy with its performance and our results, and they are consistent with those from our manual procedures. The local Tecan engineer comes regularly to give assistance when needed and we have developed a good working relationship with Tecan."

Massimo concluded: "The Tecan system has freed us from the tedium of manual protein purification, so we can do other experiments while the screening purification procedure is running on the platform. We are now investigating, in collaboration with Tecan, changing from the Bradford assay to the BCA protein assay, an alternative colorimetric method for protein quantitation that is faster and less affected by protein composition compared to the Bradford assay, with a greater linear range; this will further advance the automated technique, speeding up the procedure by a few hours."

Table: Comparison of manual and automated protein purification procedures.
Although the automated procedure takes longer, it saves considerable time for the operator.

Purification procedure step	Manual processing		Automated processing	
	Duration	No. of operators	Duration	No. of operators
Loading	1 h	1	1 h	0 [1]
Washing	1 h	1	1 h	0
Elution and fraction collection	1 h	1	1 h	0
Bradford assay	3 h	1	6 h	0 [1]
Sample preparation for SDS PAGE	2 h	1	2 h	0
Total for 24 samples	8 h	3	11 h	1

[1]Occasional attention for control of loading procedure and analytical results.

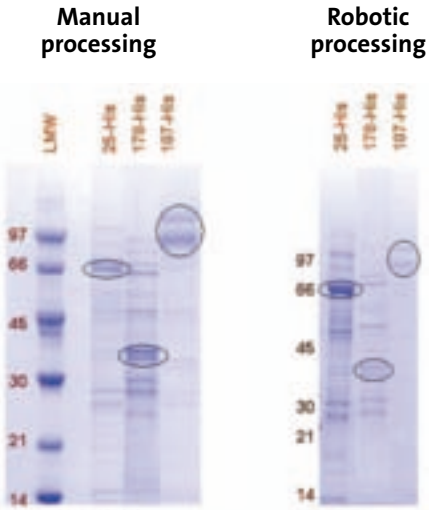

Figure: SDS PAGE of samples processed by manual and automated purification procedures. The protein profiles and yields are comparable between the two methods.